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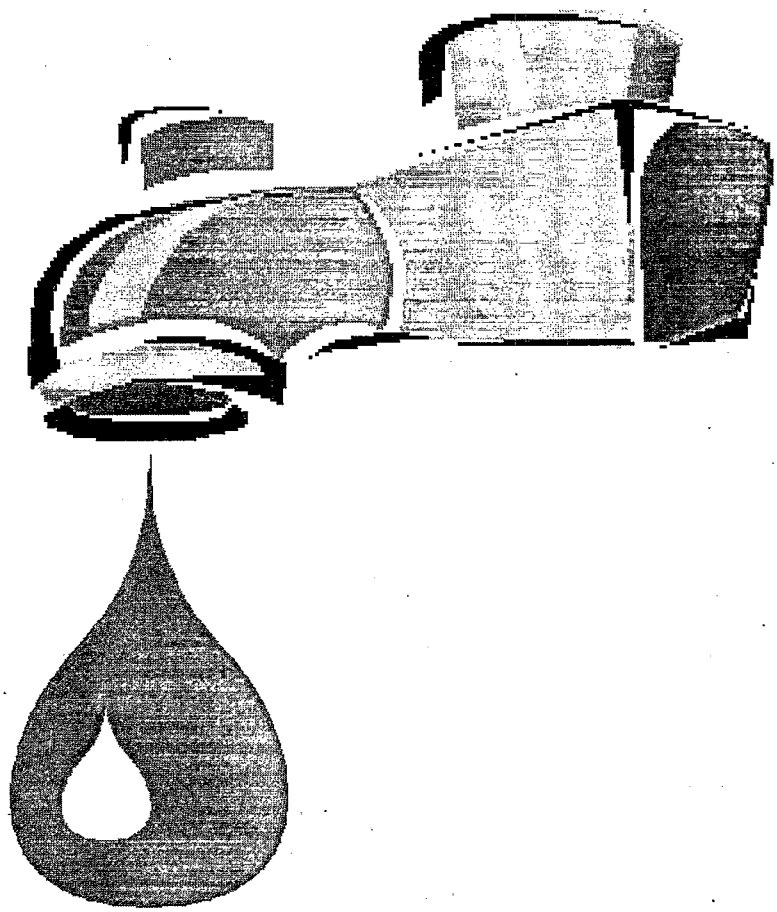
Methods for the Determination of Organic and Inorganic Compounds in Drinking Water

Volume 1



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METHODS FOR THE DETERMINATION OF ORGANIC AND INORGANIC COMPOUNDS IN DRINKING WATER

Volume I

Technical Support Center
Office of Ground Water and Drinking Water

and

National Exposure Research Laboratory
Office of Research and Development

U.S. Environmental Protection Agency
Cincinnati, Ohio

Printed on Recycled Paper

DISCLAIMER

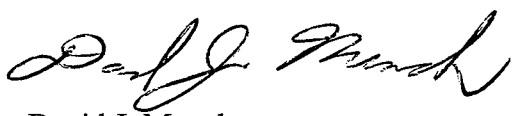
The methods contained in this manual were developed at either the Office of Research and Development's National Exposure Research Laboratory or the Office of Ground Water and DrinkingWater's Technical Support Center. All of these methods have been reviewed in accordance with the U.S. EPA's peer review requirements and cleared by EPA management for publication. Publication of these methods by the U.S. EPA does not infer anything about their status of approval for compliance monitoring. The user should refer to current drinking water regulations to determine which of these methods have been approved for compliance monitoring. In addition, mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

Seven methods for the analysis of organic compounds and four methods for the analysis of inorganic compounds in drinking water are contained in this manual. Many of these methods have either already been approved for drinking water compliance monitoring or for performing analysis required in the Unregulated Contaminant Monitoring Regulation. Other methods included in this manual may be approved for compliance monitoring at a future date or are useful for developing occurrence data. Methods for the analysis of inorganic and organic compounds have been combined in this manual to facilitate their timely publication. Most of these methods are also available at the Office of Water website, www.epa.gov/ogwdw/methods/sourcalt.html or at the Office of Research and Development website, www.epa.gov/nerlcwww/ordmeth.htm.

FOREWORD

Accurate and precise analytical measurements are essential to the determination of the quality and character of drinking waters. Both the Office of Ground Water and Drinking Water's Technical Support Center and the Office of Research and Development's National Exposure Research Laboratory are dedicated to developing new and innovative methods that provide the data quality required, while at the same time using technologies that can simplify analytical methods, and thereby reduce the costs of performing drinking water analyses. This manual was prepared to assemble under a single cover 7 new analytical methods for the determination of organic compounds and 4 new analytical methods for the determination of inorganic compounds in drinking water. We are pleased to provide this manual and believe that it will be of considerable value to both public and private analytical laboratories that wish to monitor for these compounds in drinking water.



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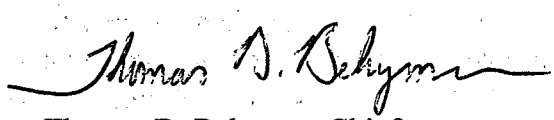

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National Exposure Research Laboratory

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METHOD 300.1 DETERMINATION OF INORGANIC ANIONS IN DRINKING WATER BY ION CHROMATOGRAPHY

Revision 1.0

September 1997

John D. Pfaff, USEPA, ORD, NERL (Method 300.0, 1993)

Daniel P. Hautman and David J. Munch, USEPA, Office of Water

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METHOD 300.1

DETERMINATION OF INORGANIC ANIONS IN DRINKING WATER BY ION CHROMATOGRAPHY

1. SCOPE AND APPLICATION

1.1 This method covers the determination of the following inorganic anions in reagent water, surface water, ground water, and finished drinking water. As a result of different specified injection volumes (See conditions in Tables 1A and 1B), these anions are divided between the common anions listed in Part A and the inorganic disinfection by-products listed in Part B. These different injection volumes are required in order to compensate for the relative concentrations of these anions in drinking water and maintain good chromatographic peak shape throughout the expected dynamic range of the detector. Bromide is included in both Part A, due to its importance as a common anion, as well as Part B due to its critical role as a disinfection by-product precursor.

PART A.-- Common Anions

Bromide	Nitrite
Chloride	ortho-Phosphate-P
Fluoride	Sulfate
Nitrate	

PART B.-- Inorganic Disinfection By-products

Bromate	Chlorite
Bromide	Chlorate

1.2 The single laboratory Method Detection Limits (MDL, defined in Sect. 3.11) for the above analytes are listed in Tables 1A, 1B and 1C. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample and the specific instrumentation employed.

1.2.1 In order to achieve comparable detection limits, an ion chromatographic system must utilize suppressed conductivity detection, be properly maintained and must be capable of yielding a baseline with no more than 5 nS noise/drift per minute of monitored response over the background conductivity.

1.3 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.4 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Sect. 9.4.1.

1.5 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must demonstrate the ability to generate acceptable results with this method, using the procedures described in Sect. 9.0.

1.6 Bromide and nitrite react with most oxidants employed as disinfectants. The utility of measuring these anions in treated water should be considered prior to conducting the analysis.

2. SUMMARY OF METHOD

2.1 A small volume of sample, 10 uL for Part A and 50 uL for Part B, is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, and conductivity detector.

2.2 The ONLY difference between Parts A and B is the volume of sample analyzed by the ion chromatographic system. The separator columns and guard columns as well as eluent conditions are identical.

3. DEFINITIONS

3.1 ANALYSIS BATCH -- A group of no more than 20 field samples (Field sample analyses include only those samples derived from a field sample matrix. These include the initial and duplicate field samples as well as all Laboratory Fortified Sample Matrices). The analysis batch must include an Initial Calibration Check Standard, an End Calibration Check Standard, Laboratory Reagent Blank, and a Laboratory Fortified Blank. Within an ANALYSIS BATCH, for every group of ten field samples, at least one Laboratory Fortified Matrix (LFM) and either a Field Duplicate, a Laboratory Duplicate or a duplicate of the LFM must be analyzed. When more than 10 field samples are analyzed, a Continuing Calibration Check Standard must be analyzed after the tenth field sample analysis.

3.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the surrogate analyte. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2.1 INITIAL CALIBRATION STANDARDS -- A series of CAL solutions used to initially establish instrument calibration and develop calibration curves for individual target anions.

3.2.2 INITIAL CALIBRATION CHECK STANDARD -- An individual CAL solution, analyzed initially, prior to any sample analysis, which verifies previously established calibration curves.

3.2.3 CONTINUING CALIBRATION CHECK STANDARD -- An individual CAL solution which is analyzed after every tenth field sample analyses which verifies the previously established calibration curves and confirms accurate analyte quantitation for the previous ten field samples analyzed.

3.2.4 END CALIBRATION CHECK STANDARD -- An individual CAL solution which is analyzed after the last field sample analyses which verifies the previously established calibration curves and confirms accurate analyte quantitation for all field samples analyzed since the last continuing calibration check.

3.3 FIELD DUPLICATES -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.5 LABORATORY DUPLICATE -- Two sample aliquots, taken in the laboratory from a single sample bottle, and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated specifically with the laboratory procedures, removing any associated variables attributed by sample collection, preservation, or storage procedures.

3.6 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added

in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.9 LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.10 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.11 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.12 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration that can be reported for an anion in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard and can only be used if acceptable quality control criteria for this standard are met.

3.13 PERFORMANCE EVALUATION SAMPLE (PE) -- A certified solution of method analytes whose concentration is unknown to the analyst. Often, an aliquot of this solution is added to a known volume of reagent water and analyzed with procedures used for samples. Results of analyses are used to determine statistically the accuracy and precision that can be expected when a method is performed by a competent analyst.

3.14 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.15 SURROGATE ANALYTE -- An analyte added to a sample, which is unlikely to be found in any sample at significant concentration, and which is added directly to a

sample aliquot in known amounts before any sample processing procedures are conducted. It is measured with the same procedures used to measure other sample components. The purpose of the surrogate analyte is to monitor method performance with each sample.

3.16 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 Interferences can be divided into three different categories: direct chromatographic coelution, where an analyte response is observed at very nearly the same retention time as the target anion; concentration dependant coelution, which is observed when the response of higher than typical concentrations of the neighboring peak overlap into the retention window of the target anion; and, ionic character displacement, where retention times may significantly shift due to the influence of high ionic strength matrices (high mineral content or hardness) overloading the exchange sites in the column and significantly shortening target analyte's retention times.

4.1.1 A direct chromatographic coelution may be solved by changing columns, eluent strength, modifying the eluent with organic solvents (if compatible with IC columns), changing the detection systems, or selective removal of the interference with pretreatment. Sample dilution will have little to no effect. The analyst must verify that these changes do not negatively affect performance by repeating and passing all the QC criteria in Section 9.

4.1.2 Sample dilution may resolve some of the difficulties if the interference is the result of either concentration dependant coelution or ionic character displacement, but it must be clarified that **sample dilution will alter your Minimum Reporting Limit (MRL)** by a proportion equivalent to that of the dilution. Therefore, careful consideration of project objectives should be given prior to performing such a dilution. An alternative to sample dilution, may be dilution of the eluent as outlined in 11.9.

4.1.3 Pretreatment cartridges can be effective as a means to eliminate certain matrix interferences. Prior to using any pretreatment, the analyst should be aware that all instrument calibration standards must be pretreated in exactly the same manner as the pretreated unknown field samples. The need for these cartridges have been greatly reduced with recent advances in high capacity anion exchange columns.

4.1.3.1 Extreme caution should be exercised in using these pretreatment cartridges. Artifacts are known to leach from certain cartridges

which can foul the guard and analytical columns causing loss of column capacity indicated by shortened retention times and irreproducible results. Frequently compare your calibration standard chromatograms to those of the column test chromatogram (received when the column was purchased) to insure proper separation and similar response ratios between the target analytes is observed.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in an ion chromatogram. These interferences can lead to false positive results for target analytes as well as reduced detection limits as a consequence of elevated baseline noise.

4.3 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems.

4.4 Any anion that is only weakly retained by the column may elute in the retention time window of fluoride and potentially interfere. At concentrations of fluoride above 1.5 mg/L, this interference may not be significant, however, it is the responsibility of the user to generate precision and accuracy information in each sample matrix.

4.5 Close attention should be given to the potential for carry over peaks from one analysis which will effect the proper detection of analytes of interest in a second, subsequent analysis. Normally, the elution of sulfate (retention time of 13.8 min.) indicates the end of a chromatographic run, but, in the ozonated and chlorine dioxide matrices, which were included as part of the single operator accuracy and bias study (See Table 2B), a small response (200 nS baseline rise) was observed for a very late eluting unknown peak at approximately 23 minutes. Consequently, a run time of 25 minutes is recommended to allow for the proper elution of any potentially interferant late peaks. It is the responsibility of the user to confirm that no late eluting peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

4.6 Any residual chlorine dioxide present in the sample will result in the formation of additional chlorite prior to analysis. If any concentration of chlorine dioxide is suspected in the sample, the sample must be purged with an inert gas (helium, argon or nitrogen) for approximately five minutes or until no chlorine dioxide remains. This sparging must be conducted prior to ethylenediamine preservation and at time of sample collection.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid -- When used to prepared a 25 mN sulfuric acid regenerant solution for chemical suppression using a Dionex Anion Micro Membrane Suppressor (AMMS).

6. EQUIPMENT AND SUPPLIES

6.1 Ion chromatograph -- Analytical system complete with ion chromatograph and all required accessories including syringes, analytical columns, compressed gasses and a conductivity detector.

6.1.1 Anion guard column: Dionex AG9-HC, 2 mm (P/N 52248), or equivalent. This column functions as a protector of the separator column. If omitted from the system the retention times will be shorter.

6.1.2 Anion separator column: Dionex AS9-HC column, 2 mm (P/N 52244), or equivalent. The microbore (2 mm) was selected in the development of this method as a means to tighten the bromate elution band and thus reduce the detection limit. An optional column (2 mm or 4 mm) may be used if comparable resolution of peaks is obtained, and the requirements of Sect. 9.0 can be met. The AS9-HC, 2 mm column using the conditions outlined in Table 1A and 1B produced the separation shown in Figures 1 through 4.

6.1.2.1 If a 4 mm column is employed, the injection volume should be raised by a factor of four to 40 uL for Part A anions and 200 uL for Part B anions in order to attain comparable detection limits. A four fold increase in injection volume compensates for the four

fold increase in cross sectional surface area of the 4 mm standard bore column over the 2 mm microbore column.

6.1.2.2 Comparable results can be attained using the Dionex, AS9-HC, 4 mm column. MDLs for the part B, inorganic disinfection by-products using this 4 mm column are displayed along with analysis conditions in Table 1C.

6.1.3 Anion suppressor device: The data presented in this method were generated using a Dionex Anion Self Regenerating Suppressor (ASRS, P/N 43187). An equivalent suppressor device may be utilized provided comparable detection limits are achieved and adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 5 nS per minute over the background conductivity.

6.1.3.1 The ASRS was set to perform electrolytic suppression at a current setting of 100 mA using an external source DI water mode. Insufficient baseline stability was observed using the ASRS in recycle mode.

6.1.4 Detector -- Conductivity cell (Dionex CD20, or equivalent) capable of providing data as required in Sect. 9.2.

6.2 The Dionex Peaknet Data Chromatography Software was used to generate all the data in the attached tables. Systems using a strip chart recorder and integrator or other computer based data system may achieve approximately the same MDL's but the user should demonstrate this by the procedure outlined in Sect. 9.2.

6.3 Analytical balance, ±0.1 mg sensitivity. Used to accurately weigh target analyte salts for stock standard preparation.

6.4 Top loading balance, ±10 mg sensitivity. Used to accurately weigh reagents to prepare eluents.

6.5 Weigh boats, plastic, disposable - for weighing eluent reagents.

6.6 Syringes, plastic, disposable, 10 mL - used during sample preparation.

6.7 Pipets, Pasteur, plastic or glass, disposable, graduated, 5 mL and 10 mL.

6.8 Bottles, high density polyethylene (HDPE), opaque or glass, amber, 30 mL, 125 mL, 250 mL. For sampling and storage of calibration solutions. Opaque or amber due to the photoreactivity of chlorite anion.

6.9 Micro beakers, plastic, disposable - used during sample preparation.

7. REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.2 Eluent solution : Sodium carbonate (CASRN 497-19-8) 9.0 mM. Dissolve 1.91 g sodium carbonate (Na_2CO_3) in reagent water and dilute to 2 L.

7.2.1 This eluent solution must be purged for 10 minutes with helium prior to use to remove dissolved gases which may form micro bubbles in the IC compromising system performance and adversely effecting the integrity of the data.

7.3 Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased as certified solutions or prepared from ACS reagent grade, potassium or sodium salts as listed below, for most analytes. Chlorite requires careful consideration as outline below in 7.3.5.1.

7.3.1 Bromide (Br^-) 1000 mg/L: Dissolve 0.1288 g sodium bromide (NaBr, CASRN 7647-15-6) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.2 Bromate (BrO_3^-) 1000 mg/L: Dissolve 0.1180 g of sodium bromate ($NaBrO_3$, CASRN 7789-38-0) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.3 Chlorate (ClO_3^-) 1000 mg/L: Dissolve 0.1275 g of sodium chlorate ($NaClO_3$, CASRN 7775-09-9) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.4 Chloride (Cl^-) 1000 mg/L: Dissolve 0.1649 g sodium chloride (NaCl, CASRN 7647-14-5) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.5 Chlorite (ClO_2^-) 1000 mg/L: Assuming an exact 80.0 % $NaClO_2$ is amperometrically titrated from technical grade $NaClO_2$ (See Sect. 7.3.5.1). Dissolve 0.1676 g of sodium chlorite ($NaClO_2$, CASRN 7758-19-2) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.5.1 High purity sodium chlorite ($NaClO_2$) is not currently commercially available due to potential explosive instability. Recrystallization of the technical grade (approx. 80%) can be

performed but it is labor intensive and time consuming. The simplest approach is to determine the exact % $NaClO_2$ using the iodometric titration procedure (Standard Methods, 19th Ed., 4500-ClO_2.C). Following titration, an individual component standard of chlorite must be analyzed to determine if there is any significant contamination (greater than 1% of the chlorite weight) in the technical grade chlorite standard from any of the Part B components. These contaminants will place a high bias on the calibration of the other anions if all four Part B components are mixed in an combined calibration solution. If these other anions are present as contaminants, a separate chlorite calibration needs to be performed.

7.3.6 Fluoride (F^-) 1000 mg/L: Dissolve 0.2210 g sodium fluoride (NaF, CASRN 7681-49-4) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.7 Nitrate (NO_3^--N) 1000 mg/L: Dissolve 0.6068 g sodium nitrate ($NaNO_3$, CASRN 7631-99-4) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.8 Nitrite (NO_2^--N) 1000 mg/L: Dissolve 0.4926 g sodium nitrite ($NaNO_2$, CASRN 7632-00-0) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.9 Phosphate (PO_4^{3-}-P) 1000 mg/L: Dissolve 0.4394 g potassium dihydrogenphosphate (KH_2PO_4, CASRN 7778-77-0) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.10 Sulfate (SO_4^{2-}) 1000 mg/L: Dissolve 0.1814 g potassium sulfate (K_2SO_4, CASRN 7778-80-5) in reagent water and dilute to 100 mL in a volumetric flask.

NOTE: Stability of standards: Stock standards (7.3) for most anions are stable for at least 6 months when stored at 4°C. Except for the chlorite standard which is only stable for two weeks when stored protected from light at 4°C, and nitrite and phosphate which are only stable for 1 month when stored at 4°C. Dilute working standards should be prepared monthly, except those that contain chlorite, or nitrite and phosphate which should be prepared fresh daily.

7.4 Ethylenediamine (EDA) preservation solution, 100 mg/mL: Dilute 2.8 mL of ethylenediamine (99%) (CASRN 107-15-3) to 25 mL with reagent water. Prepare fresh monthly.

7.5 Surrogate Solution: 0.50 mg/mL dichloroacetate (DCA) prepared by dissolving 0.065 g dichloroacetic acid, potassium salt (Cl_2CHCO_2K, CASRN 19559-59-2) in reagent water and dilute to 100 mL in a volumetric flask.

7.5.1 Dichloroacetate is potentially present in treated drinking waters as the acetate of the organic disinfection by product, dichloroacetic acid (DCAA). Typical concentrations of DCAA rarely exceed 50 ug/L, which, for this worst case example, would represent only a five percent increase in the observed response over the fortified concentration of 1.00 mg/L. Consequently, the criteria for acceptable recovery (90% to 115%) for the surrogate is weighted to 115% to allow for this potential background.

7.5.2 Prepare this solution fresh every 3 months or sooner if signs of degradation are present.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis, if required, and minimize waste disposal.

8.2 Special sampling requirements and precautions for chlorite.

8.2.1 Sample bottles used for chlorite analysis must be opaque to protect the sample from light.

8.2.2 When preparing the LFM, be aware that chlorite is an oxidant and may react with the natural organic matter in an untreated drinking water matrix as a result of oxidative demand. If untreated water is collected for chlorite analysis, and subsequently used for the LFM, EDA preservation will not control this demand and reduced chlorite recoveries may be observed.

8.3 Sample preservation and holding times for the anions that can be determined by this method are as follows:

PART A : Common Anions

Analyte	Preservation	Holding Time
Bromide	None required	28 days
Chloride	None required	28 days
Fluoride	None required	28 days
Nitrate-N	Cool to 4°C	48 hours
Nitrite-N	Cool to 4°C	48 hours
ortho-Phosphate-P	Cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days

PART B : Inorganic Disinfection By-products

Analyte	Preservation	Holding Time
Bromate	50 mg/L EDA	28 days
Bromide	None required	28 days
Chlorate	50 mg/L EDA	28 days
Chlorite	50 mg/L EDA, Cool to 4°C	14 days

8.4 When collecting a sample from a treatment plant employing chlorine dioxide, the sample must be sparged with an inert gas (helium, argon, nitrogen) prior to addition of the EDA preservative at time of sample collection.

8.5 All four anions, in Part B, can be analyzed in a sample matrix which has been preserved with EDA. Add a sufficient volume of the EDA preservation solution (Sect. 7.4) such that the final concentration is 50 mg/L in the sample. This would be equivalent to adding 0.5 mL of the EDA preservation solution to 1 L of sample.

8.6 EDA is primarily used as a preservative for chlorite. Chlorite is susceptible to degradation both through catalytic reactions with dissolved iron salts and reactivity towards free chlorine which exists as hypochlorous acid/hypochlorite ion in most drinking water as a residual disinfectant. EDA serves a dual purpose as a preservative for chlorite by chelating iron as well as any other catalytically destructive metal cations and removing hypochlorous acid/hypochlorite ion by forming an organochloroamine. EDA preservation of chlorite also preserves the integrity of chlorate which can increase in unpreserved samples as a result of chlorite degradation. EDA also preserves the integrity of bromate concentrations by binding with hypobromous acid/hypobromite which is an intermediate formed as by-product of the reaction of either ozone or hypochlorous acid/hypochlorite with bromide ion. If hypobromous acid/hypobromite is not removed from the matrix further reactions may form bromate ion.

8.7 Degradation of ortho-phosphate has been observed in samples held at room temperature for over 16 hrs (see table 3A). Therefore, samples to be analyzed for ortho-phosphate must not be held at room temperature for more than 12 cumulative hours.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The requirements of this program consist of an initial demonstration of laboratory performance, and subsequent analysis in each analysis batch (Sect. 3.1) of a Laboratory Reagent Blank, Laboratory Fortified Blank, Instrument Performance Check Standard, calibration check standards, Laboratory Fortified Sample Matrices (LFM) and either Field, Laboratory or LFM duplicate sample analyses. This section

details the specific requirements for each of these QC parameters. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of accuracy through the analysis of the QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 15% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.3 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of three to five times the estimated instrument detection limit.[6] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method over at least three separate days. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates].
S = standard deviation of the replicate analyses.

9.2.3.1 MDLs should be determined every 6 months, when a new operator begins work or whenever there is a significant change in the background, or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each analysis batch (defined Sect 3.1). Data produced are used to

assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.1.1 If conducting analysis for the Part B anions, EDA must be added to the LRB at 50 mg/L. By including EDA in the LRB, any bias as a consequence of the EDA which may be observed in the field samples, particularly in terms of background contamination, will be identified.

9.3.2 Laboratory Fortified Blank (LFB) -- The LFB should be prepared at concentrations similar to those expected in the field samples and ideally at the same concentration used to prepare the LFM. Calculate accuracy as percent recovery (Sect. 9.4.1.3). If the recovery of any analyte falls outside the required concentration dependant control limits (Sect. 9.3.2.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.2.1 If conducting analysis for the Part B anions, EDA must be added to the LFB at 50 mg/L. The addition of EDA to all reagent water prepared calibration and quality control samples is required not as a preservative but rather as a means to normalize any bias attributed by the presence of EDA in the field samples.

9.3.2.2 Control Limits for the LFB

Concentration range	Percent Recovery Limits
MRL to 10xMRL	75 - 125 %
10xMRL to highest calibration level	85 - 115 %

9.3.2.2.1 These control limits only apply if the MRL is established within a factor of 10 times the MDL. Otherwise, the limits are set at 85% to 115%.

9.3.2.3 The laboratory must use the LFB to assess laboratory performance against the required control limits listed in 9.3.2.2. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = x + 3S$$
$$\text{LOWER CONTROL LIMIT} = x - 3S$$

The optional control limits must be equal to or better than those listed in 9.3.2.2. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations monitored. These data must be kept on file and be available for review.

9.3.3 Instrument Performance Check Solution (IPC) -- The Initial Calibration Check Standard is to be evaluated as the instrument performance check solution in order to confirm proper instrument performance. Proper chromatographic performance must be demonstrated by calculating the Peak Gaussian Factor (PGF), which is a means to measure peak symmetry and monitoring retention time drift in the surrogate peak over time. Critically evaluate the surrogate peak in the initial calibration check standard, and calculate the PGF as follows,

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where: W(1/2) is the peak width at half height
W(1/10) is the peak width at tenth height

9.3.3.1 The PGF must fall between 0.80 and 1.15 in order to demonstrate proper instrument performance.

9.3.3.2 The retention time for the surrogate in the IPC must be closely monitored on each day of analysis and throughout the lifetime of the analytical column. Small variations in retention time can be anticipated when a new solution of eluent is prepared but if shifts of more than 2% are observed in the surrogate retention time, some type of instrument problem is present. Potential problems include improperly prepared eluent, erroneous method parameters programmed such as flow rate or some other system problem. The chromatographic profile (elution order) of the target anions following an ion chromatographic analysis should closely replicate the profile displayed in the test chromatogram that was shipped when the column was purchased. As a column ages, it is normal to see a gradual shift and shortening of retention times, but if after several years of use, extensive use over less than a year, or use with harsh samples, this retention time has noticeably shifted to any less than 80% of the original recorded value, the column may require

cleaning or replacement. Particularly if resolution problems are beginning to become common between previously resolved peaks. A laboratory must retain a historic record of retention times for the surrogate and all the target anions to provide evidence of an analytical columns vitality.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of the field samples within an analysis batch. The LFM sample must be prepared from a sample matrix which has been analyzed prior to fortification. The analyte concentration must be high enough to be detected above the original sample and should adhere to the requirement of 9.4.1.2. It is recommended that the solutions used to fortify the LFM be prepared from the same stocks used to prepare the calibration standards and not from external source stocks. This will remove the bias contributed by an externally prepared stock and focus on any potential bias introduced by the field sample matrix.

9.4.1.1 If the fortified concentration is less than the observed background concentration of the unfortified matrix, the recovery should not be calculated. This is due to the difficulty in calculating accurate recoveries of the fortified concentration when the native sample concentration is so high.

9.4.1.2 The LFM should be prepared at concentrations no greater than five times the highest concentration observed in any field sample. If no analyte is observed in any field sample, the LFM must be fortified no greater than five times the lowest calibration level which as outlined in 12.2 is the minimum reported level (MRL). For example, if bromate is not detected in any field samples above the lowest calibrations standard concentration of 5.00 ug/L, the highest LFM fortified concentration allowed is 25.0 ug/L.

9.4.1.3 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample. Percent recovery should be calculated using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where, R = percent recovery.
C_s = fortified sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample.

9.4.1.4 Until sufficient data becomes available (usually a minimum of 20 to 30 analysis), assess laboratory performance against recovery limits of 75 to 125%. When sufficient internal performance data becomes available develop control limits from percent mean recovery and the standard deviation of the mean recovery. The optional control limits must be equal to or better than the required control limits of 75-125%.

9.4.1.5 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem encountered with the LFM is judged to be matrix induced and the results for that sample and the LFM are reported with a "matrix induced bias" qualifier.

9.4.2 SURROGATE RECOVERY -- Calculate the surrogate recovery from all analyses using the following formula

$$R = \frac{SRC}{SFC} \times 100$$

where, R = percent recovery.
SRC = Surrogate Recovered Concentration
SFC = Surrogate Fortified Concentration

9.4.2.1 Surrogate recoveries must fall between 90-115% for proper instrument performance and analyst technique to be verified. The recovery of the surrogate is slightly bias to 115% to allow for the potential contribution of trace levels of dichloroacetate as the halogenated organic disinfection by-product (DBP) dichloroacetic acid (DCAA) Background levels of this organic DBP are rarely observed above 50 ug/L (0.05 mg/L) which constitutes only 5% of the 1.00 mg/L recommended fortified concentration.

9.4.2.2 If the surrogate recovery falls outside the 90-115% recovery window, a analysis error is evident and sample reanalysis is required. Poor recoveries could be the result of imprecise sample injection or analyst fortification errors.

9.4.3 FIELD OR LABORATORY DUPLICATES -- The laboratory must analyze either a field or a laboratory duplicate for a minimum of 10% of the collected field samples or at least one with every analysis batch, whichever is greater. The sample matrix selected for this duplicate analysis must

contain measurable concentrations of the target anions in order to establish the precision of the analysis set and insure the quality of the data. If none of the samples within an analysis batch have measurable concentrations, the LFM should be employed as a laboratory duplicate.

9.4.3.1 Calculate the relative percent difference (RPD) of the initial quantitated concentration (I_C) and duplicate quantitated concentration (D_c) using the following formula,

$$RPD = \frac{(I_C - D_c)}{([I_C + D_c]/2)} \times 100$$

9.4.3.2 Duplicate analysis acceptance criteria

Concentration range	RPD Limits
MRL to 10xMRL	+/- 20 %
10xMRL to highest calibration level	+/- 10 %

9.4.3.3 If the RPD fails to meet these criteria, the samples must be reported with a qualifier identifying the sample analysis result as yielding a poor duplicate analysis RPD. This should not be a chronic problem and if it frequently recurs (>20% of duplicate analyses) it indicates a problem with the instrument or individual technique.

9.4.4 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

9.4.5 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns, injection volumes, and/or eluents, to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Sect. 9.2 and adhere to the condition of baseline stability found in Sect. 1.2.1.

9.4.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should perform analysis of quality control check samples and participate in relevant performance evaluation sample studies.

10. CALIBRATION AND STANDARDIZATION

10.1 Establish ion chromatographic operating parameters equivalent to those indicated in Tables 1A or 1B if employing a 2 mm column, Table 1C if employing a 4 mm column.

10.2 Estimate the Linear Calibration Range (LCR) -- The LCR should cover the expected concentration range of the field samples and should not extend over more than 2 orders of magnitude in concentration (For example, if quantitating nitrate in the expected range of 1.0 mg/L to 10 mg/L, 2 orders of magnitude would permit the minimum and maximum calibration standards of 0.20 mg/L and 20 mg/L, respectively.) The restriction of 2 orders of magnitude is prescribed since beyond this it is difficult to maintain linearity throughout the entire calibration range.

10.2.1 If quantification is desired over a larger range, then two separate calibration curves should be prepared.

10.2.2 For an individual calibration curve, a minimum of three calibration standards are required for a curve that extends over a single order of magnitude and a minimum of five calibration standards are required if the curve covers two orders of magnitude. (For example, using the nitrate example cited above in section 10.2, but in this case limit the curve to extend only from 1.0 mg/L to 10 mg/L or a single order of magnitude. A third standard is required somewhere in the middle of the range. For the calibration range of 0.20 mg/L to 20 mg/L, over two orders of magnitude, five calibrations standards should be employed, one each at the lower and upper concentration ranges and the other three proportionally divided throughout the middle of the curve.)

10.3 Prepare the calibration standards by carefully adding measured volumes of one or more stock standards (7.3) to a volumetric flask and diluting to volume with reagent water.

10.3.1 For the Part B anions, EDA must be added to the calibration standards at 50 mg/L. The addition of EDA to all reagent water prepared calibration and quality control samples is required not as a preservative but rather as a means to normalize any bias attributed by the presence of EDA in the field samples.

10.3.2 Prepare a 10.0 mL aliquot of surrogate fortified calibration solution which can be held for direct manual injection or used to fill an autosampler vial. Add 20 uL of the surrogate solution (7.5) to a 20 mL disposable plastic micro beaker. Using a 10.0 mL disposable pipet, place exactly 10.0 mL of calibration standard into the micro beaker and mix. The calibration standard

is now ready for analysis. The same surrogate solution that has been employed for the standards should also be used in the section 11.3.2 for the field samples.

10.4 Using a 2 mm column, inject 10 uL (Part A) or 50 uL (Part B) of each calibration standard. Using a 4 mm column, inject 50 uL (Part A) or 200 uL (Part B) of each calibration standard. Tabulate peak area responses against the concentration. The results are used to prepare calibration curves using a linear least squares fit for each analyte. Acceptable calibration curves are confirmed after reviewing the curves for linearity and passing the criteria for the initial calibration check standard in section 10.5.1. Alternately, if the ratio of response to concentration (response factor) is constant over the LCR (indicated by < 15% relative standard deviation (RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve,

10.4.1 Peak areas are strongly recommended since they have been found to be more consistent, in terms of quantitation, than peak heights. Peak height can tend to be suppressed as a result of high levels of common anions in a given matrix which can compete for exchange sites. Using peak areas, it is the analyst responsibility to review all chromatograms to insure accurate baseline integration of target analyte peaks since poorly drawn baselines will more significantly influence peak areas than peak heights.

10.5 Once the calibration curves have been established they must be verified prior to conducting any sample analysis using an initial calibration check standard (3.2.2). This verification must be performed on each analysis day or whenever fresh eluent has been prepared. A continuing calibration check standard (3.2.3) must be analyzed after every tenth sample and at the end of the analysis set as an end calibration check standard (3.2.4). The response for the initial, continuing and end calibration check must satisfy the criteria listed in 10.5.1. If during the analysis set, the response differs by more than the calibration verification criteria shown in 10.5.1., or the retention times shift more than ± 5% from the expected values for any analyte, the test must be repeated, using fresh calibration standards. If the results are still outside these criteria, sample analysis must be discontinued, the cause determined and/or in the case of drift, the instrument recalibrated. All samples following the last acceptable calibration check standard must be reanalyzed.

10.5.1 Control limits for calibration verification

Concentration range	Percent Recovery Limits
MRL to 10xMRL	75 - 125 %
10xMRL to highest calibration level	85 - 115 %

10.5.1.1 These control limits only apply if the MRL is established within a factor of 10 times the MDL. Otherwise, the limits are set at 85% to 115%.

10.5.2 CALIBRATION VERIFICATION REQUIREMENT FOR PART B
As a mandatory requirement of calibration verification, the laboratory MUST verify calibration using the lowest calibration standard as the initial calibration check standard.

10.5.3 After satisfying the requirement of 10.5.2, the levels selected for the other calibration check standards should be varied between a middle calibration level and the highest calibration level.

11. PROCEDURE

11.1 Tables 1A and 1B summarize the recommended operating conditions for the ion chromatograph. Included in these tables are estimated retention times that can be achieved by this method. Other columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.2 are met.

11.2 Check system calibration daily and, if required, recalibrate as described in Sect. 10.

11.3 Sample Preparation

11.3.1 For refrigerated or samples arriving to the laboratory cold, ensure the samples have come to room temperature prior to conducting sample analysis by allowing the samples to warm on the bench for at least 1 hour.

11.3.2 Prepare a 10.0 mL aliquot of surrogate fortified sample which can be held for direct manual injection or used to fill an autosampler vial. Add 20 uL of the surrogate solution (7.5) to a 20 mL disposable plastic micro beaker. Using a 10.0 mL disposable pipet, place exactly 10.0 mL of sample into the micro beaker and mix. Sample is now ready for analysis.

11.3.2.1 The less than 1% dilution error introduced by the addition of the surrogate is considered insignificant.

11.4 Using a Luer lock, plastic 10 mL syringe, withdraw the sample from the micro beaker and attach a 0.45 um particulate filter (demonstrated to be free of ionic contaminants) directly to the syringe. Filter the sample into an autosampler vial (If vial is not designed to automatically filter) or manually load the injection loop injecting a fixed amount of well mixed sample. If using a manually loaded injection

loop, flush the loop thoroughly between sample analysis using sufficient volumes of each new sample matrix.

11.5 Using a 2 mm column, inject 10 uL (Part A) or 50 uL (Part B) of each sample. Using a 4 mm column, inject 40 uL (Part A) or 200 uL (Part B) of each sample. Tabulate peak area responses against the concentration. During this procedure, retention times must be recorded. Use the same size loop for standards and samples. Record the resulting peak size in area units. An automated constant volume injection system may also be used.

11.6 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.7 If the response of a sample analyte exceeds the calibration range, the sample may be diluted with an appropriate amount of reagent water and reanalyzed. If this is not possible then three new calibration concentrations must be employed to create a separate high concentration curve, one standard near the estimated concentration and the other two bracketing around an interval equivalent to ± 25% the estimated concentration. The latter procedure involves significantly more time than a simple sample dilution therefore, it is advisable to collect sufficient sample to allow for sample dilution or sample reanalysis, if required.

11.8 Shifts in retention time are inversely proportional to concentration. Nitrate, phosphate and sulfate will exhibit the greatest degree of change, although all anions can be affected. In some cases this peak migration may produce poor resolution or make peak identification difficult.

11.9 Should more complete resolution be needed between any two coeluting peaks, the eluent (7.2) can be diluted. This will spread out the run, however, and will cause late eluting anions to be retained even longer. The analysts must verify that this dilution does not negatively affect performance by repeating and passing all the QC criteria in Section 9. As a specific precaution, upon dilution of the carbonate eluent, a peak for bicarbonate may be observed within the retention time window for bromate which will negatively impact the analysis.

11.9.1 Eluent dilution will reduce the overall response of an anion due to chromatographic band broadening which will be evident by shortened and broadened peaks. This will adversely effect the MDLs for each analyte.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve for each analyte by plotting instrument response, as peak area, against standard concentration. Compute sample concentration by comparing sample response with the standard curve. If a sample has been diluted, multiply the response by the appropriate dilution factor.

12.2 Report ONLY those values that fall between the lowest and the highest calibration standards. Samples with target analyte responses exceeding the highest standard should be diluted and reanalyzed. Samples with target analytes identified but quantitated below the concentration established by the lowest calibration standard should be reported as below the minimum reporting limit (MRL).

12.3 Report results for Part A anions in mg/L and for Part B anions in ug/L.

12.4 Report NO_2^- as N
NO_3^- as N
$HPO_4^=$ as P
Br^- in mg/L when reported with Part A
Br^- in ug/L when reported with Part B

13. METHODS PERFORMANCE

13.1 Tables 1A, 1B, and 1C give the single laboratory (OW OGWDW TSC-Cincinnati) retention times, standard conditions and MDL determined for each anion included in the method. MDLs for the Part A anions were determined in reagent water on the 2 mm column (Table 1A). MDLs for the Part B anions were conducted not only in reagent water but also a simulated high ionic strength water (HIW) on the 2 mm column (Table 1B) and in reagent water on the 4 mm column (Table 1C). HIW is designed to simulate a high ionic strength field sample. It was prepared from reagent water which was fortified with the common anions of chloride at 100 mg/L, carbonate at 100 mg/L, nitrate at 10.0 mg/L as nitrogen, phosphate at 10.0 mg/L as phosphorous, and sulfate at 100 mg/L.

13.2 Tables 2A and 2B give the single laboratory (OW OGWDW TSC-Cincinnati) standard deviation for each anion included in the method in a variety of waters for the standard conditions identified in Table 1A and 1B, respectively.

13.3 Tables 3A and 3B shown stability data for the Part A and B anions, respectively. Each data point in these tables represent the mean percent recovery following triplicate analysis. These data were used to formulate the holding times shown in Sect. 8.3.

14. POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 Quantity of the chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036,
(202) 872-4477.

15. WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

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3. Dionex, System DX500 Operation and Maintenance Manual, Dionex Corp., Sunnyvale, California 94086, 1996.

4. Method Detection Limit (MDL) as described in "Trace Analyses for Wastewater," J. Glaser, D. Foerst, G. McKee, S. Quave, W. Budde, Environmental Science and Technology, Vol. 15, Number 12, page 1426, December, 1981.

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7. Hautman, D.P. & Bolyard, M. Analysis of Oxyhalide Disinfection By-products and other Anions of Interest in Drinking Water by Ion Chromatography. Jour. of Chromatog., 602, (1992), 65-74.

8. Standard Methods for the Examination of Water and Wastewater, Method 4500-ClO_2,C "Amperometric Method I" (for the determination of Chlorine Dioxide), 19th Edition of Standard Methods (1995).

17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1A. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS IN REAGENT WATER FOR THE COMMON ANIONS (PART A).

ANALYTE	PEAK # [1]	RETENTION TIME (MIN.)	MDL DETERMINATION		
			Fort Conc, mg/L	Number of Replicates	DI MDL mg/L
Fluoride	1	2.53	0.020	7	0.009
Chloride	2	4.67	0.020	7	0.004
Nitrite-N	3	6.01	0.010	7	0.001
Surrogate: DCA	4	7.03			
Bromide	5	8.21	0.040	7	0.014
Nitrate-N	6	9.84	0.010	7	0.008
ortho-Phosphate-P	7	11.98	0.040	7	0.019
Sulfate	8	13.49	0.040	7	0.019

Standard Conditions:

Ion Chromatograph: Dionex DX500
Columns : Dionex AG9-HC / AS9-HC, 2 mm
Detector: Suppressed Conductivity Detector, Dionex CD20
Suppressor: ASRS-I, external source electrolytic mode, 100 mA current
Eluent: 9.0 mM Na_2CO_3
Eluent Flow: 0.40 mL/min
Sample Loop: 10 uL

System Backpressure: 2800 psi
Background Conductivity: 22 uS

Recommended method total analysis time: 25 minutes

(1) See Figure 1

TABLE 1B. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS IN BOTH REAGENT WATER AND HIGH IONIC STRENGTH WATER FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B).

ANALYTE	PEAK # [1]	RETENTION TIME (MIN.)	MDL DETERMINATION			
			Fort Conc, ug/L	Number of Replicates	DI MDL ug/L	HIW[2] MDL ug/L
Chlorite	1	3.63	2.00	7	0.89	0.45
Bromate	2	4.19	2.00	7	1.44	1.28
Surrogate: DCA	4	7.28				
Bromide	5	8.48	2.00	7	1.44	2.51
Chlorate	6	9.28	2.00	7	1.31	0.78

Standard Conditions:

Ion Chromatograph: Dionex DX500
Columns : Dionex AG9-HC / AS9-HC, 2 mm
Detector: Suppressed Conductivity Detector, Dionex CD20
Suppressor: ASRS-I, external source electrolytic mode, 100 mA current
Eluent: 9.0 mM Na_2CO_3
Eluent Flow: 0.40 mL/min
Sample Loop: 50 uL

System Backpressure: 2800 psi
Background Conductivity: 22 uS

Recommended method total analysis time: 25 minutes

(1) See Figure 2 and 3

(2) HIW indicates High Ionic Strength Water which is a simulated drinking water prepared from reagent water and fortified with chloride at 100 mg/L, carbonate at 100 mg/L, nitrate at 10.0 mg/L as nitrogen, phosphate at 10.0 mg/L as phosphorous, and sulfate at 100 mg/L.

TABLE 1C. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS IN REAGENT WATER FOR THE INORGANIC DISINFECTION BY-PRODUCTS USING AN ALTERNATE 4 mm AS9-HC COLUMN (PART B).

ANALYTE	PEAK #	RETENTION TIME (MIN.)	MDL DETERMINATION		
			Fort Conc, ug/L	Number of Replicates	DI MDL ug/L
Chlorite	1	4.43	2.00	7	1.44
Bromate	2	5.10	2.00	7	1.32
Surrogate: DCA	4	8.82			
Bromide	5	10.11	2.00	7	0.98
Chlorate	6	10.94	2.00	7	2.55

Standard Conditions:

Ion Chromatograph:	Dionex DX500
Columns :	Dionex AG9-HC / AS9-HC, 4 mm
Detector:	Suppressed Conductivity Detector, Dionex CD20
Suppressor:	ASRS-I, external source electrolytic mode, 300 mA current
Eluent:	9.0 mM Na_2CO_3
Eluent Flow:	1.25 mL/min
Sample Loop:	200 uL

System Backpressure: 1900 psi
Background Conductivity: 21 uS

Recommended method total analysis time: 25 minutes

TABLE 2A. SINGLE-OPERATOR PRECISION AND RECOVERY FOR THE COMMON ANIONS (PART A).

ANALYTE	MATRIX	UNFORT MATRIX CONC., mg/L	FORT CONC mg/L	# OF REP	MEAN mg/L	MEAN %REC	SD (n-1)	%RSD
Fluoride	RW	<MDL[1]	2.00	9	1.79	89.7	0.02	1.18
	SW	0.139	2.00	9	1.75	80.4	0.01	0.56
	GW	0.280	2.00	9	1.97	84.3	0.02	0.85
	CDW	0.807	2.00	9	2.59	89.0	0.01	0.46
Chloride	RW	0.029	50.0	9	49.4	98.7	0.03	0.10
	SW	12.1	50.0	9	58.7	93.3	0.04	0.10
	GW	56.6	50.0	9	100.	---[2]	0.22	0.22
	CDW	16.0	50.0	9	64.9	97.8	0.11	0.16
Nitrite-N	RW	<MDL	1.00	9	0.851	85.1	0.00	0.51
	SW	<MDL	1.00	9	0.780	78.0	0.00	0.40
	GW	0.013	1.00	9	0.879	86.6	0.01	0.77
	CDW	<MDL	1.00	9	0.720	72.0	0.00	0.55
Bromide	RW	<MDL	0.500	9	0.480	96.1	0.00	0.92
	SW	0.028	0.500	9	0.469	88.1	0.00	0.94
	GW	0.153	0.500	9	0.634	96.3	0.00	0.52
	CDW	<MDL	0.500	9	0.431	86.2	0.01	1.28
Nitrate-N	RW	<MDL	10.0	9	9.50	95.0	0.01	0.14
	SW	2.12	10.0	9	10.9	87.7	0.03	0.30
	GW	0.016	10.0	9	9.64	96.3	0.03	0.27
	CDW	1.64	10.0	9	10.9	92.4	0.04	0.41
Phosphate-P	RW	<MDL	10.0	9	9.62	96.2	0.01	0.14
	SW	<MDL	10.0	9	8.70	87.0	0.02	0.18
	GW	<MDL	10.0	9	6.12	61.2	0.28	4.66
	CDW	<MDL	10.0	9	9.15	91.5	0.04	0.42
Sulfate	RW	<MDL	50.0	9	44.8	89.5	0.05	0.11
	SW	47.8	50.0	9	92.1	88.6	0.21	0.23
	GW	105	50.0	9	154	---[2]	0.60	0.39
	CDW	57.8	50.0	9	105	---[2]	0.33	0.32
Surrogate:	RW	----	5.00	9	5.12	102.3	0.50	0.49
	SW	----	5.00	9	5.09	102.3	1.12	1.09
	GW	----	5.00	9	5.16	101.8	0.67	0.66
	CDW	----	5.00	9	5.17	103.1	1.36	1.32

RW = Reagent Water
SW = Surface Water
GW = Ground Water
CDW = chlorine dioxide treated finished drinking water

(1) <MDL indicates less than method detection limit.
(2) Not calculated since amount fortified was less than unfortified native matrix concentration (See 9.4.1.1.).

TABLE 2B. SINGLE-OPERATOR PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B).

ANALYTE	MATRIX	UNFORT CONC. ug/L	FORT CONC ug/L	# OF REP	MEAN ug/L	MEAN %REC	SD (n-1)	%RSD
Chlorite	RW	<MDL[1]	100	9	96.2	96.2	0.95	0.99
			500	9	523	105	3.13	0.60
	HIW	<MDL	100	9	102	102	2.19	2.15
			500	9	520	104	3.64	0.70
	SW	<MDL	100	9	91.4	91.4	1.22	1.33
			500	9	495	99.0	7.54	1.52
	GW	<MDL	100	9	92.9	92.9	1.65	1.77
			500	9	490	98.1	3.40	0.69
	ClW	<MDL	100	9	87.4	87.4	0.59	0.68
			500	9	485	97.1	6.36	1.31
	CDW	292	100	9	396	---[2]	1.64	0.41
			500	9	811	104	4.00	0.49
	O3W	<MDL	100	9	84.4	84.4	0.46	0.54
			500	9	481	96.1	3.24	0.67
Bromate	RW	<MDL	5.00	9	5.04	101	0.45	8.86
			25.0	9	26.5	106	1.71	6.47
	HIW	<MDL	5.00	9	4.88	97.5	0.95	19.5
			25.0	9	25.6	102	1.37	5.37
	SW	<MDL	5.00	9	4.46	89.2	0.58	13.0
			25.0	9	26.3	105	1.10	4.18
	GW	<MDL	5.00	9	5.10	102	0.50	9.75
			25.0	9	22.2	88.9	1.29	5.81
	ClW	<MDL	5.00	9	4.63	92.6	0.77	16.7
			25.0	9	25.1	100	1.64	6.55
	CDW	<MDL	5.00	9	4.14	82.7	0.62	15.1
			25.0	9	25.1	101	1.28	5.09
	O3W	1.45	5.00	9	5.49	80.9	0.61	11.1
			25.0	9	24.1	90.6	1.13	4.69

RW = Reagent Water
HIW = High Ionic strength Water [see note (2) in Table 1B]
SW = Surface Water
GW = Groundwater
ClW = Chlorinated drinking water
CDW = Chlorine dioxide treated drinking water
O3W = Ozonated drinking water

(1) <MDL indicates less than method detection limit.
(2) Not calculated since amount fortified was less than unfortified native matrix concentration (See 9.4.1.1.).

TABLE 2B. SINGLE-OPERATOR PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B)(contd.).

ANALYTE	MATRIX	UNFORT CONC. ug/L	FORT CONC ug/L	# OF REP	MEAN ug/L	MEAN %REC	SD (n-1)	%RSD
Bromide	RW	<MDL[1]	20.0	9	20.9	104	0.80	3.82
			100	9	107	107	0.60	0.56
	HIW	3.24	20.0	9	21.8	92.5	0.79	3.63
			100	9	105	102	1.05	1
	SW	31.0	20.0	9	51.3	---[2]	0.97	1.9
			100	9	140.	109	1.88	1.35
	GW	151	20.0	9	172	---[2]	0.78	0.45
			100	9	265	---[2]	2.18	0.82
	ClW	16.3	20.0	9	39.3	115	0.64	1.62
			100	9	125	109	2.00	1.6
	CDW	11.5	20.0	9	34.4	115	0.76	2.22
			100	9	125	113	1.24	0.99
	O3W	39.8	20.0	9	65.4	---[2]	3.67	5.61
			100	9	153	113	1.00	0.65
Chlorate	RW	<MDL	100	9	98.3	98.3	0.80	0.82
			500	9	520	104	4.15	0.8
	HIW	<MDL	100	9	86.1	86.1	1.47	1.7
			500	9	502	100.	4.52	0.9
	SW	3.18	100	9	102	98.3	1.57	1.55
			500	9	513	102	7.11	1.39
	GW	<MDL	100	9	93.5	93.5	2.00	2.14
			500	9	510	102	3.84	0.75
	ClW	34.4	100	9	136	102	1.01	0.74
			500	9	549	103	3.11	0.57
	CDW	121	100	9	223	---[2]	3.20	1.44
			500	9	651	106	3.50	0.54
	O3W	6.15	100	9	106	100	1.20	1.13
			500	9	523	103	2.45	0.47

RW = Reagent Water
HIW = High Ionic strength Water [see note (2) in Table 1B]
SW = Surface Water
GW = Groundwater
ClW = Chlorinated drinking water
CDW = Chlorine dioxide treated drinking water
O3W = Ozonated drinking water

(1) <MDL indicates less than method detection limit.
(2) Not calculated since amount fortified was less than unfortified native matrix concentration (See 9.4.1.1.).

TABLE 2B. SINGLE-OPERATOR PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B)(contd.).

ANALYTE	MATRIX	FORT CONC mg/L	# OF REP	MEAN mg/L	MEAN %REC	SD (n-1)	%RSD
Surrogate: DCA	RW	5.00	9	5.11	102	0.93	0.91
(see NOTE below)				4.98	99.5	0.69	0.69
	HIW	5.00	9	5.00	100	0.79	0.79
				4.96	99.2	1.76	1.78
	SW	5.00	9	4.95	98.9	0.70	0.7
				4.99	99.8	1.60	1.61
	GW	5.00	9	5.12	102	0.50	0.49
				5.13	103	0.50	0.49
	ClW	5.00	9	5.15	103	1.73	1.68
				5.13	103	1.12	1.09
	CDW	5.00	9	5.01	100	1.02	1.02
				5.04	101	1.08	1.07
	O3W	5.00	9	4.99	99.8	0.70	0.7
				5.11	101	0.53	0.52

RW = Reagent Water
HIW = High Ionic strength Water [see note (2) in Table 1B]
SW = Surface Water
GW = Groundwater
ClW = Chlorinated drinking water
CDW = Chlorine dioxide treated drinking water
O3W = Ozonated drinking water

NOTE: The surrogate DCA was fortified at 5 mg/L but due to concerns about measuring trace concentrations of bromide with such high concentration of the neighboring surrogate peak, the recommended fortified concentration for the surrogate has been reduced to 1.00 mg/L.

TABLE 3A. STABILITY STUDY RESULTS FOR THE COMMON ANIONS (PART A).

ANALYTE	Preservative	Matrix	UNFORT CONC. mg/L	FORT CONC mg/L	Analyte % Recovery Day 0	Day 14	Day 28	See Note
Fluoride	None	RW	<MDL	2.00	89.8	88.3	88.4	
		SW	0.140	2.00	79.9	80.2	80.0	
		GW	0.280	2.00	84.7	87.8	87.0	
		CDW	0.929	2.00	82.9	83.6	81.6	
Chloride	None	RW	<MDL	50.0	98.8	99.1	98.1	
		SW	12.0	50.0	93.4	93.5	92.8	
		GW	56.6	50.0	87.6	87.6	86.5	
		CDW	16.0	50.0	97.9	98.4	97.8	
Nitrite-N	None	RW	<MDL	1.00	85.2	85.5	83.6	
		SW	<MDL	1.00	77.8	76.6	11.9	(1)
		GW	<MDL	1.00	88.2	85.4	56.1	(1)
		CDW	<MDL	1.00	71.9	71.7	73.9	(2)
Bromide	None	RW	<MDL	0.500	95.5	97.0	96.2	
		SW	0.028	0.500	87.5	88.3	86.7	
		GW	0.153	0.500	96.9	96.0	96.1	
		CDW	<MDL	0.500	85.7	87.1	89.2	(2)
Nitrate-N	None	RW	<MDL	10.0	94.9	94.7	94.2	
		SW	2.12	10.0	87.6	87.0	88.7	
		GW	<MDL	10.0	96.5	96.5	95.5	
		CDW	1.64	10.0	92.3	93.3	91.9	
Phosphate-P	None	RW	<MDL	10.0	96.3	95.8	95.2	
		SW	<MDL	10.0	86.9	86.4	85.1	
		GW	<MDL	10.0	62.8	93.1	89.5	(3)
		CDW	<MDL	10.0	91.6	91.4	90.8	
Sulfate	None	RW	<MDL	50.0	89.6	89.3	89.1	
		SW	47.8	50.0	89.0	89.0	88.1	
		GW	105	50.0	97.5	97.3	96.5	
		CDW	57.8	50.0	94.3	94.9	93.8	

NOTES:

(1) Degradation apparent.

(2) Analyte recovery will be adversely effected by reactions with free chlorine.

(3) Phosphate recovery on day 0 is believed to have been adversely effected by biological degradation since the sample sat in the autosampler for 18 hrs prior to analysis

TABLE 3B STABILITY STUDY RESULTS FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B).

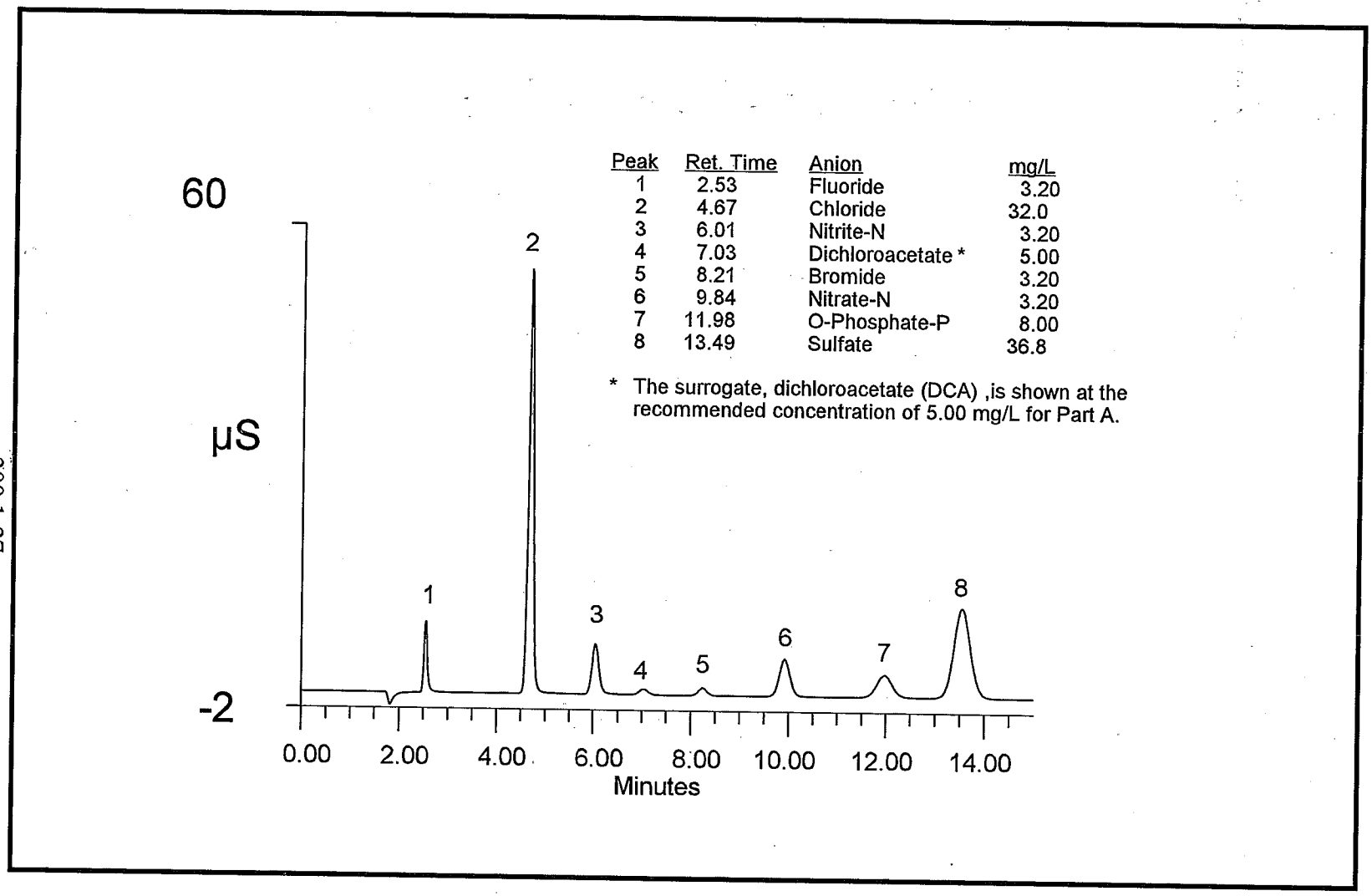
ANALYTE	Preservative	Matrix	UNFORT CONC. ug/L	FORT CONC ug/L	Analyte % Recovery Day 0	Day 3	Day 10	Day 30	See Note
Chlorite	None	RW	<MDL	500	99.8	100	104	94.3	
		HIW	<MDL	500	99.3	98.5	106	89.3	
		SW	<MDL	500	92	88.5	82.3	75.1	(1)
		GW	<MDL	500	93.9	94.5	96.1	91.7	
		ClW	<MDL	500	93.7	NA[1]	90.3	84.7	(2,3)
		CDW	286	500	98.6	101	91.7	77.5	(1,3)
		O3W	<MDL	500	10	NA	82.5	90.5	(2)
Chlorite	EDA	RW	<MDL	500	101	101	104	95.3	
		HIW	<MDL	500	98.4	98.7	104	95.4	
		SW	<MDL	500	98.3	97.3	97.8	92.7	
		GW	<MDL	500	97.7	97.1	97.5	92.6	
		ClW	<MDL	500	98.9	NA	96.9	92.6	(2)
		CDW	297	500	103	107	102	94.5	
		O3W	<MDL	500	105	NA	96.3	91.9	(2)
Bromate	None	RW	<MDL	25.0	93.6	94.1	110	96.1	
		HIW	<MDL	25.0	100	86.0	105	87.7	
		SW	<MDL	25.0	98.7	95.1	105	102	
		GW	<MDL	25.0	79.4	92.4	77.8	82.2	
		ClW	<MDL	25.0	102	NA	101	103	(2)
		CDW	<MDL	25.0	104	96.8	98.9	92.1	
		O3W	2.27	25.0	87.3	NA	84.3	99.9	(2)
Bromate	EDA	RW	<MDL	25.0	97.3	95.3	99.5	102	
		HIW	<MDL	25.0	86.9	86.1	107	91.2	
		SW	<MDL	25.0	100	104	103	94.9	
		GW	<MDL	25.0	83.2	101	88.4	88.3	
		ClW	<MDL	25.0	105	NA	101	102	(2)
		CDW	<MDL	25.0	117	97.3	98.1	83.9	
		O3W	2.32	25.0	92.6	NA	84.5	88.9	(2)

See bottom of next page for explanation of notes

TABLE 3B. STABILITY STUDY RESULTS FOR THE INORGANIC DISINFECTION BY-PRODUCTS (PART B)(contd.)

ANALYTE	Preservative	Matrix	UNFORT CONC. ug/L	FORT CONC ug/L	Analyte % Recovery Day 0	Day 3	Day 10	Day 30	See Note
Bromide	None	RW	<MDL	100	99.4	97.2	107	101	
		HIW	<MDL	100	102	103	105	105	
		SW	30.6	100	102	97.1	107	99.1	
		GW	149	100	97.7	95.3	109	100	
		ClW	4.73	100	8.9	NA(1)	37.1	11.4	(2,3)
		CDW	<MDL	100	5.78	21.3	38.8	51.3	(3)
		O3W	30.4	100	98.3	NA	120	108	(2)
Bromide	EDA	RW	<MDL	100	98.4	98.6	107	100	
		HIW	<MDL	100	104	103	106	105	
		SW	30.5	100	99.5	98.2	107	100	
		GW	149	100	100	97.0	114	97.7	
		ClW	11.9	100	101	NA	115	97.4	(2,3)
		CDW	6.14	100	101	96.5	119	110	(3)
		O3W	31.0	100	97.3	NA	122	102	(2)
Chlorate	None	RW	<MDL	500	102	102	105	97.4	
		HIW	<MDL	500	96.5	97.8	101	95.4	
		SW	5.84	500	99.8	97.8	100	96	
		GW	<MDL	500	99.5	98.7	101	99.8	
		ClW	37.8	500	102	NA	104	98.2	(2)
		CDW	125	500	102	99.9	104	99.6	
		O3W	8.34	500	100	NA	103	97.3	(2)
Chlorate	EDA	RW	<MDL	500	104	98.6	103	97.3	
		HIW	<MDL	500	97.3	103	100	95	
		SW	6.70	500	99.7	98.2	99.9	95.6	
		GW	<MDL	500	102	97.0	101	99.3	
		ClW	38.2	500	101	NA	102	96.1	(2)
		CDW	123	500	102	96.5	105	97.7	
		O3W	8.62	500	98.4	NA	103	96.4	(2)

NOTES:
(1) Degradation in the unpreserved matrix is apparent.
(2) NA indicates "NOT ANALYZED"
(3) Analyte recovery will be adversely effected by reactions with free chlorine.



Peak	Ret. Time	Anion	mg/L
1	2.53	Fluoride	3.20
2	4.67	Chloride	32.0
3	6.01	Nitrite-N	3.20
4	7.03	Dichloroacetate *	5.00
5	8.21	Bromide	3.20
6	9.84	Nitrate-N	3.20
7	11.98	O-Phosphate-P	8.00
8	13.49	Sulfate	36.8

* The surrogate, dichloroacetate (DCA) ,is shown at the recommended concentration of 5.00 mg/L for Part A.

Figure 1. Chromatogram showing separation of the Part A common anions on the AS9-HC column. See Table 1A for analysis conditions.

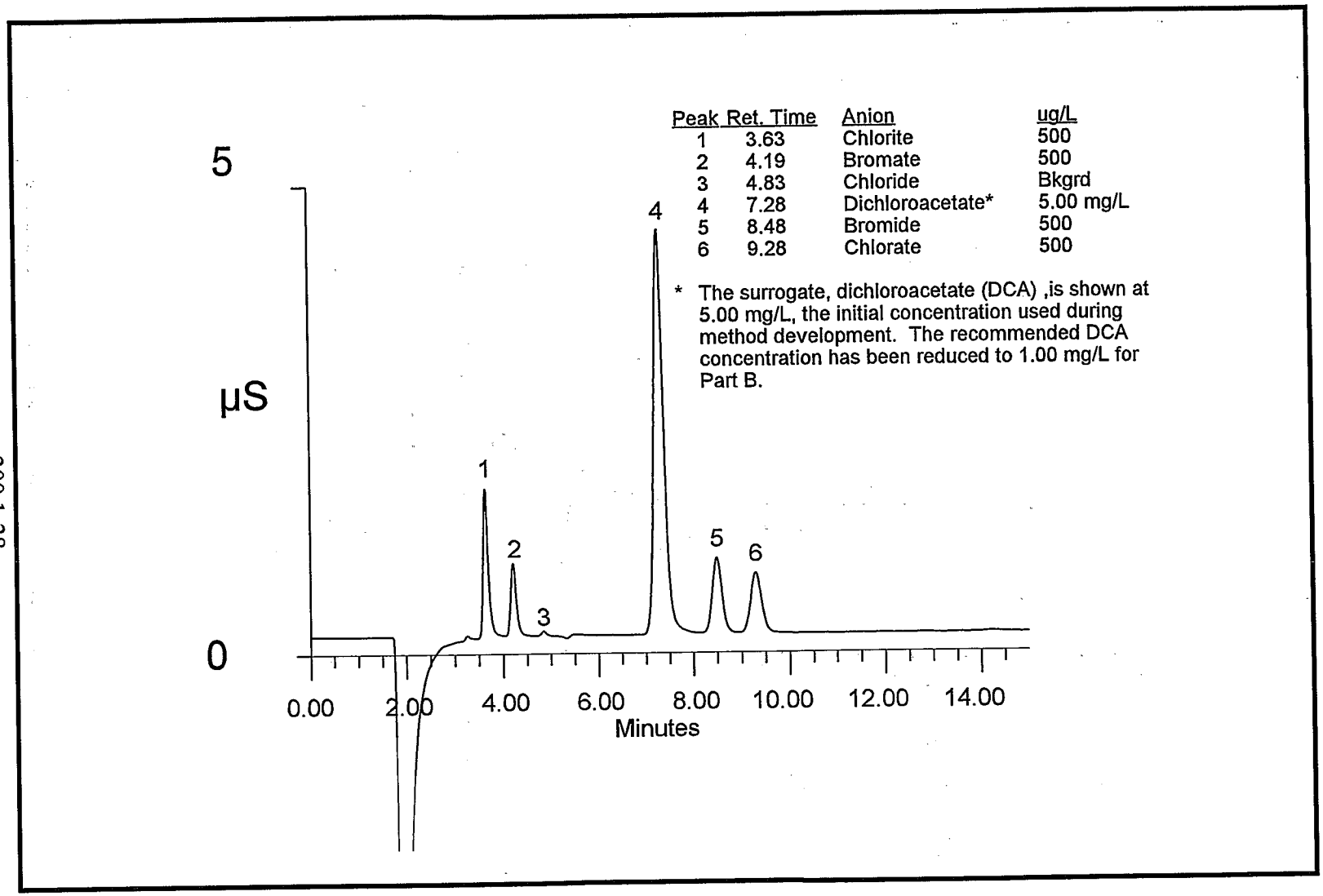


Figure 2 Chromatogram showing separation of the Part B inorganic DBPs and bromide on the AS9-HC column. See Table 1B for analysis conditions.

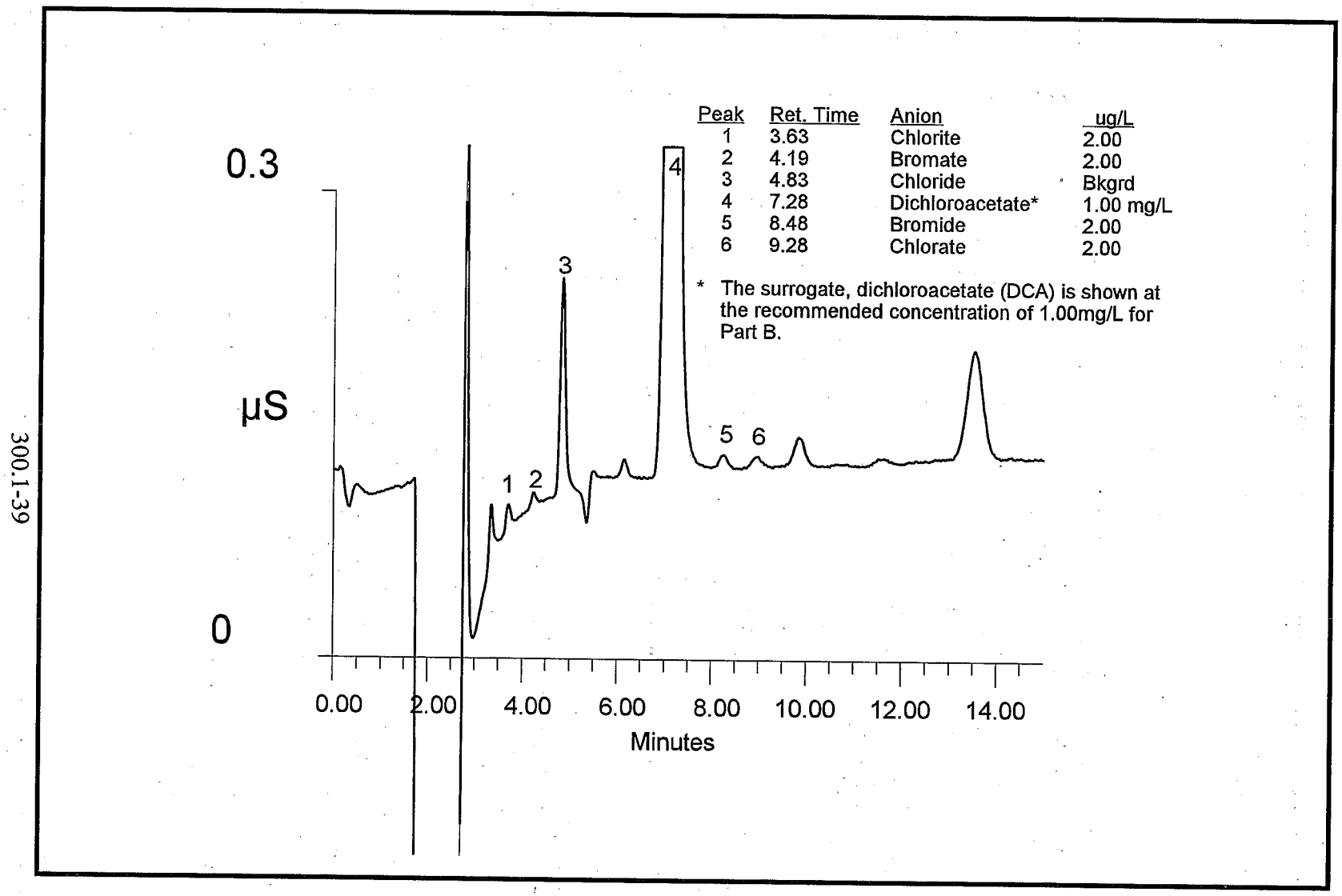
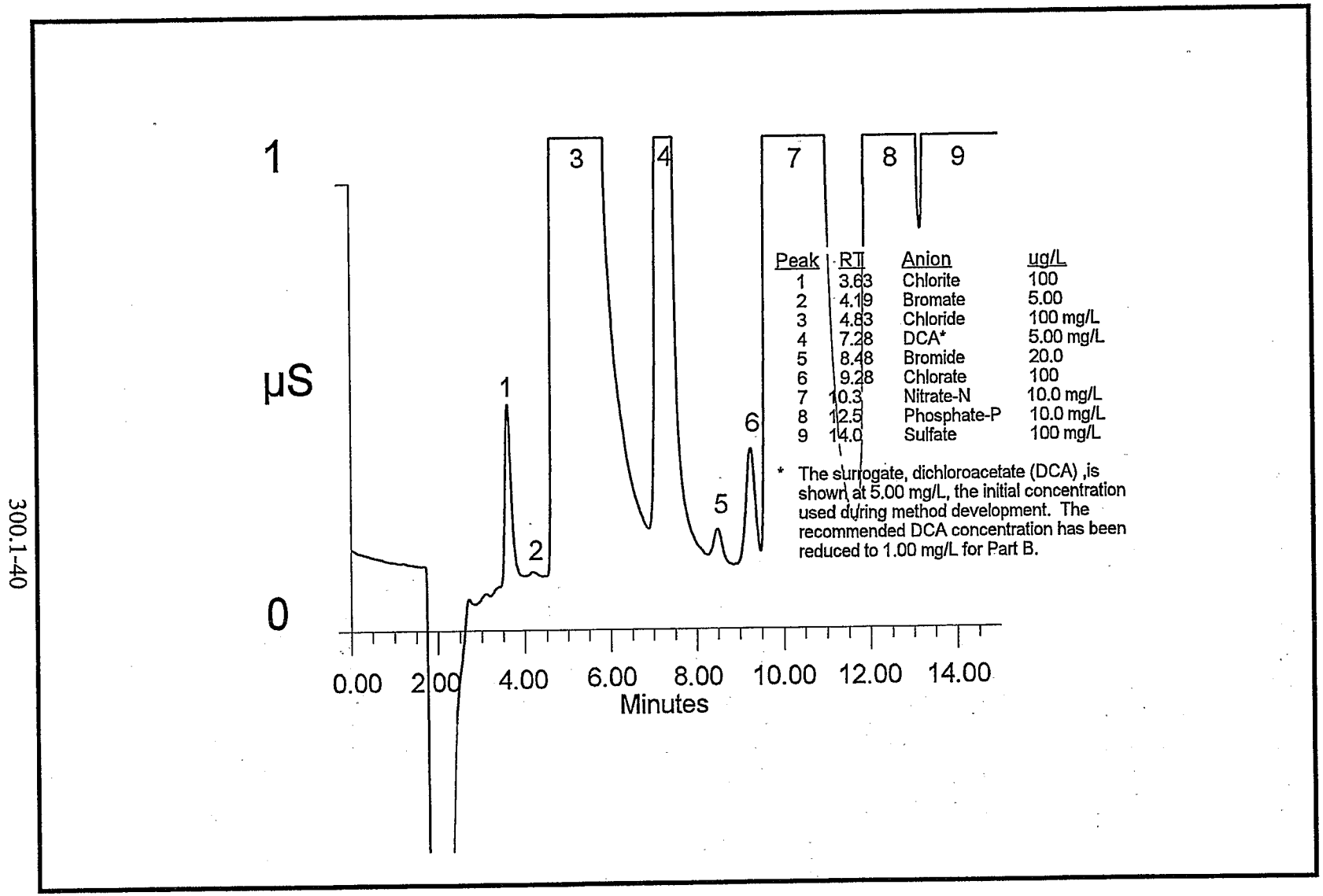


Figure 3. Chromatogram of the inorganic DBPs and bromide (Part B) during the MDL determination in reagent water. See Table 1B for analysis conditions.



300.1-40

Figure 4. Chromatogram of the inorganic DBPs and bromide (Part B) in high ionic strength water (HIW). See Table 1B for analysis conditions.

ERRATA SHEET TO U.S.EPA METHOD 300.1

April 27, 1999

The following were editorial changes which have been incorporated into U.S.EPA Method 300.1. These minor clarifications are incorporated into the body of this text as follows:

ERRATA #1 -
An additional sentence was added to Section 4.1.1 reiterating the analyst's responsibilities when incorporating any method change, including modifying eluent strength, or any other method parameter. The additional sentence states,

> "*...The analyst must verify that these changes do not negatively affect performance by repeating and passing all the QC criteria in Section 9.*"

On this same theme, section 11.9, was also further clarified and specific precautions were added as follows,

> "*...The analysts must verify that this dilution does not negatively affect performance by repeating and passing all the QC criteria in Section 9. As a specific precaution, upon dilution of the carbonate eluent, a peak for bicarbonate may be observed within the retention time window for bromate which will negatively impact the analysis.*"

ERRATA #2 -
An acronym in Section 9.3.2.2 for Laboratory Fortified Blank (LFB) was incorrectly identified as LRB. This typographical error was corrected.

ERRATA #3 -
Clarifications and corrections were made to Section 9.4.1.5, 9.4.3.2 and 9.4.3.3. These clarifications pertain to data reportability for Laboratory Fortified Sample Matrices (LFM) as well as to analysis continuation when Duplicate Sample QC acceptance criteria are not met.

Section 9.4.1.5 clarifies and now specifies how to report data when the LFM recovery falls outside the established control criteria by stating,

> "*...the recovery problem encountered with the LFM is judged to be matrix induced and the results for that sample and the LFM are reported with a "matrix induced bias" qualifier.*"

Section 9.4.3.2 required the correction of a typographical reference by removing "%Diff" in the duplicate sample acceptance criteria and replacing it with the defined RPD, indicating "relative percent difference".

Section 9.4.3.3, also had a "%Diff" reference corrected with RPD and included clarification regarding continuation of an analysis set when a duplicate analysis fails to meet the acceptance criteria. This section now reads,

> "*If the RPD fails to meet these criteria, the samples must be reported with a qualifier identifying the sample analysis result as yielding a poor duplicate analysis RPD. This should not be a chronic problem and if it frequently recurs, (>20% of duplicate analysis) it indicates a problem with the instrument or individual technique.*"

METHOD 314.0 DETERMINATION OF PERCHLORATE IN DRINKING WATER USING ION CHROMATOGRAPHY

Revision 1.0

November 1999

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METHOD 314.0

DETERMINATION OF PERCHLORATE IN DRINKING WATER USING ION CHROMATOGRAPHY

1. SCOPE AND APPLICATION

1.1 This method covers the determination of perchlorate in reagent water, surface water, ground water, and finished drinking water using ion chromatography.

1.2 The single laboratory reagent water Method Detection Limit (MDL, defined in Section 3.16) for the above analyte is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample and the specific instrumentation employed.

1.2.1 In order to achieve comparable detection limits, an ion chromatographic system must utilize suppressed conductivity detection, be properly maintained, and must be capable of yielding a baseline with no more than 5 nanosiemen (nS) noise/drift per minute of monitored response over the background conductivity.

1.3 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.4 When this method is used to analyze unfamiliar samples for perchlorate, anion identification should be supported by the use of a laboratory fortified matrix sample. The fortification procedure is described in Section 9.4.1.

1.5 Users of the method data should identify data quality objectives prior to analysis. Users of the method must demonstrate the ability to generate acceptable results, using the procedures described in Section 9.0.

1.6 This method specifies an IC column and analytical conditions which were determined to be the most effective for the widest array of sample matrices. Other IC procedures have been written which incorporate similar columns and conditions, such as hydroxide based mobile phases, low hydrophobicity IC columns, and measurement by suppressed conductivity detection.[1-5] During the development of this method, these other procedures, as well as the columns and conditions outlined in this method, were concurrently investigated with comparable results for test matrices with moderate levels of common inorganic background anions. These findings were consistent with those of the Inter-Agency Perchlorate Steering Committee, Analytical Subcommittee's Report,[6] published in 1998, which reported on the results of an interlaboratory validation of

these other Ion Chromatographic Methods. The columns and conditions identified in this method were recommended since they bore the greatest tolerance for the highest levels of common inorganic anion interference.

2. SUMMARY OF METHOD

2.1 A 1.0 mL volume of sample (see Note), is introduced into an ion chromatograph (IC). Perchlorate is separated and measured, using a system comprised of an ion chromatographic pump, sample injection valve, guard column, analytical column, suppressor device, and conductivity detector.

NOTE: This large sample loop (1.0 mL) can be made using approximately 219 cm (86 inches) of 0.03 inch i.d. PEEK tubing. The exact volume is not critical since all standards and samples will use the same sample loop. However, the volume should be verified to be within 5% of this volume by weighing the sample loop empty, filling the loop with deionized water and re-weighing the loop. The volume can then be approximated by assuming the density of water is 1.0 mg/uL.

3. DEFINITIONS

3.1 ANALYSIS BATCH -- A sequence of samples, which are analyzed within a 30 hour period and include no more than 20 field samples. An Analysis Batch must also include all required QC samples, which do not contribute to the maximum field sample total of 20. The required QC samples include:

- Instrument Performance Check Standard (IPC)
- Laboratory Reagent Blank (LRB)
- Initial Calibration Check Standard (ICCS)
- Laboratory Fortified Blank (LFB)
- Continuing Calibration Check Standard (CCCS), when the batch contains more than 10 field samples
- End Calibration Check Standard (ECCS)
- Laboratory Fortified Matrix (LFM)
- Either a Field Duplicate, a Laboratory Duplicate or a duplicate of the LFM
- (if pretreated samples are included in batch) Pretreated LRB
- (if pretreated samples are included in batch) Pretreated LFB
- (if pretreated samples are included in batch) Pretreated LFM, for each pretreated matrix.

NOTE: Every field sample analysis, including both diluted and pretreated field samples, but excluding any LFM or duplicate field sample analysis which qualify as QC samples, must be applied to the maximum of 20 total field samples permitted in an analysis batch.

3.1.1 A field sample(s), included in the analysis batch, can be reanalyzed following the ECCS provided the 30 hr time limit for the analysis batch has not expired. The laboratory can reanalyze that sample(s) but must initially conduct a second ICCS before the reanalysis and an ECCS after the final reanalysis. The ECCS must be completed within the 30 hr window.

3.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution(s) or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 INITIAL CALIBRATION STANDARDS -- A series of CAL solutions used to initially establish instrument calibration and develop calibration curves for individual target anions (Section 10.2).

3.4 INITIAL CALIBRATION CHECK STANDARD (ICCS) -- A CAL solution, which is analyzed initially, prior to any field sample analyses, which verifies the previously established calibration curve. The concentration for the initial calibration check standard MUST be at or below the MRL (Section 3.17) level.

3.5 CONTINUING CALIBRATION CHECK STANDARDS (CCCS) -- A CAL solution which is analyzed after every tenth field sample analyses, not including QC samples, which verifies the previously established calibration curve and confirms accurate analyte quantitation for the previous ten field samples analyzed. The concentration for the continuing calibration check standards should be either at a middle calibration level or at the highest calibration level (Section 10.3.2).

3.6 END CALIBRATION CHECK STANDARD (ECCS) -- A CAL solution which is analyzed after the last field sample analyses which verifies the previously established calibration curve and confirms accurate analyte quantitation for all field samples analyzed since the last continuing calibration check. The end calibration check standard should be either the middle or high level continuing calibration check standard (Section 10.3.2).

3.7 FIELD DUPLICATES (FD) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.8 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution containing a specific concentration of perchlorate and other test substances (namely chloride, sulfate and carbonate) used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.9 LABORATORY DUPLICATE (LD) -- Two sample aliquots (LD1 and LD2), taken in the laboratory from a single sample bottle, and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated specifically with the laboratory procedures by removing variation contributed from sample collection, preservation and storage procedures.

3.10 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water, or other blank matrix, to which a known quantity of perchlorate is added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.11 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental field sample to which a known quantity of perchlorate is added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical result (when compared to the result for the LFB). The background concentrations of perchlorate, in the sample matrix, must be initially determined in a separate aliquot and the measured value in the LFM corrected for this background concentration.

3.12 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, filtration and reagents that are used with other samples. The LRB is used to determine if perchlorate or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.13 LINEAR CALIBRATION RANGE (LCR) – The concentration range over which the instrument response is linear.

3.14 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.15 MATRIX CONDUCTIVITY THRESHOLD (MCT) – The highest permitted conductance of an unknown sample matrix, measured prior to conducting the analysis, which is used to determine when sample matrix dilution or pretreatment is required. The conductance of a sample matrix is proportional to the common anions present in the matrix (which contribute to the level of total dissolved solids [TDS]) which can greatly affect the integrity of this analysis. The value for this threshold is dependant on the conditions, hardware, and state of the hardware employed. Consequently, this threshold is not method defined and must be determined by the individual analytical laboratory during the Initial Demonstration of Capability (IDC) and confirmed in each analysis batch using the Instrument Performance Check (IPC) Solution. Matrix

conductivity is measured in microsiemens/cm (uS/cm) or microMhos/cm (uMhos/cm) which are considered equivalent terms.

3.16 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[7,8]

3.17 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard and can only be used if acceptable quality control criteria for this standard are met.

3.18 PEAK AREA TO HEIGHT RATIO (A/H) – The ratio of the peak area divided by the peak height which is used as a tool to monitor analytical performance. This ratio is used to establish and monitor the MCT and represents an objective means of assessing analytical performance when analyzing high conductivity matrices. A gradual distortion of the baseline is typically observed in the retention time window for perchlorate as the matrix conductivity increases (consistent with elevated levels of common anions) which will more significantly influence peak height relative to the influence on peak area. As the distortion of the baseline increases, this ratio increases, and the integrity of the measured perchlorate will be compromised.

3.19 PROFICIENCY TESTING (PT) or PERFORMANCE EVALUATION (PE) SAMPLE -- A certified solution of method analytes whose concentration is unknown to the analyst. Often, an aliquot of this solution is added to a known volume of reagent water and analyzed with procedures used for samples. Often, results of these analyses are used as part of a laboratory certification program to objectively determine the capabilities of a laboratory to achieve high quality results.

3.20 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.21 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing perchlorate which is either prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.22 TOTAL DISSOLVED SOLIDS (TDS) -- Both organic and inorganic constituent which are dissolved in a sample matrix and are not removed by particulate filtration..

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in an ion chromatogram. These interferences can lead to false positive results for the target analyte as well as reduced detection limits as a consequence of elevated baseline noise.

4.2 Interferences can be divided into three different categories: direct chromatographic coelution, where an analyte response is observed at very nearly the same retention time as the target anion; concentration dependant coelution, which is observed when the response of higher than typical concentrations of the neighboring peak overlap into the retention window of the target anion; and, ionic character displacement, where retention times may significantly shift due to the influence of high ionic strength matrices (high mineral content or hardness) overloading the exchange sites in the column and significantly shortening target analyte's retention times.

4.2.1 A direct chromatographic coelution may be solved by changing columns, eluent strength, modifying the eluent with organic solvents (if compatible with IC columns), changing the detection systems, or selective removal of the interference with pretreatment. Sample dilution will have little to no effect. The analyst MUST verify that these changes do not induce any negative affects on method performance by repeating and passing all the QC criteria as described in Section 9.

4.2.2 Sample dilution may resolve some of the difficulties if the interference is the result of either concentration dependant coelution or ionic character displacement, but it must be clarified that **sample dilution will alter your Minimum Reporting Limit (MRL)** by a proportion equivalent to that of the dilution. Therefore, careful consideration of project objectives should be given prior to performing such a dilution. An alternative to sample dilution, may be dilution of the eluent as outlined in Section 11.2.6.

4.2.3 Pretreatment cartridges can be effective as a means to eliminate certain matrix interferences. With any proposed pretreatment, the analyst must verify that the target analyte is not affected by monitoring recovery after pretreatment (additional pretreated LFM requirement see Section 11.1.4.6) and that no background contaminants are introduced by the pretreatment (additional pretreated LRB requirement see Sections 9.3.1.1 and 11.1.4.2). With advances in analytical separator column technology which employ higher capacity anion exchange resins, the need for these cartridges has been greatly reduced.

4.2.3.1 Extreme caution should be exercised in using these pretreatment cartridges. Artifacts are known to leach from certain cartridges which can foul the guard and analytical columns causing loss of column capacity indicated by shortened retention times and irreproducible results. Frequently compare your calibration standard chromatograms to those of the column test chromatogram (received when the column was purchased) or use calibration chromatograms generated when the column was initially installed, to insure proper separation and similar response ratios between the target analytes are observed.

4.2.3.2 If LRB background problems are encountered in the retention time window for perchlorate when these pretreatment cartridges have been employed, increase the initial reagent water rinse of the cartridge to approximately five times the volume specified by the manufacturer.

4.3 Sample matrices with high concentrations of common anions such as chloride, sulfate and carbonate can make the analysis problematic by destabilizing the baseline in the retention time window for perchlorate. This is evidenced by observing a protracted tailing following the initial elution of the more weakly retained anions (chloride, carbonate, and sulfate) which extends into the perchlorate retention time window. These common anion levels can be indirectly assessed by monitoring the conductivity of the matrix. Consequently, all sample matrices must be monitored for conductivity (Section 11.1.2) prior to analysis. When the laboratory determined Matrix Conductivity Threshold (MCT, see Section 9.2.8) is exceeded, procedures incorporating sample dilution and/or pretreatment must be performed as specified in Sections 11.1.3 and 11.1.4, respectively.

4.4 All reagent solutions (eluents, external water for ASRS suppressor, etc...) used by the instrument must be filtered through no larger than a 0.45 um nominal pore size membrane or frit to remove particulates and prevent damage to the instrument, columns and flow systems. Sample filtration must also be employed on every sample prior to analysis. This applies not only to field samples but also to the laboratory reagent blank (LRB) and laboratory fortified blank (LFB). The LRB and LFB samples function as controls and must be filtered to confirm no bias is attributable to the filtration.[5] Filter the samples through a membrane or frit with no larger than a 0.45 um nominal pore size. Syringe mounted, cartridge type, filters work well. Filters specifically designed for IC applications should be used.

4.5 Close attention should be given to the potential for carry over peaks from one analysis which will effect the proper detection of perchlorate in a second, subsequent analysis. It is the responsibility of the user to confirm that no late eluting peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are specifically listed below in Section 5.3 for hazardous materials.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable. Additional references on laboratory safety are available.[9-12]

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sodium Hydroxide (NaOH), used in the preparation of the eluent is considered caustic.

6. EQUIPMENT AND SUPPLIES

6.1 Ion chromatograph (IC) -- Analytical system complete with eluent reservoirs, an ion chromatographic pump, injection valves, both guard and analytical separator columns, suppressor, conductivity detector, and computer based data acquisition system.

6.1.1 Anion guard column -- Dionex AG16 4 mm (P/N 55377), or equivalent. This column functions as a protector of the separator column. If omitted from the system, the retention times will be shorter.

6.1.2 Anion separator column -- Dionex AS16, 4 mm (P/N 55376), or equivalent (see Sections 6.1.2.1 - 6.1.2.2). The AS16, 4 mm column using the conditions outlined in Table 1 produced the separations shown in Figures 1 through 4.

6.1.2.1 The development of this method included investigations into the performance of alternate 4 mm IC guard and analytical separator columns which have been used for the IC analysis of perchlorate and are specified in procedures external to the U.S.EPA.[1-5] These alternate guard /separator columns included the Dionex AG5 / AS5 and the Dionex AG11 / AS11. The AG5 / AS5 is currently specified in the standard operating procedure (SOP) for the IC analysis of perchlorate by the State of California, Department of Health Services.[1, 5] The AG11 / AS11 is used by several commercial labs conducting IC analysis for perchlorate and is recognized by California as an

acceptable alternate to the AG5 / AS5. [2-4] A multilab validation study included both of these analytical columns and indicated comparable results could be attained.[6] In U.S.EPA studies, both the AG5 / AS5 and the AG11 / AS11 performed well for reagent water and simulated drinking water samples with low to moderate common anion levels but as these levels increased, performance began to diminish for both columns. The AG16 / AS16 columns could tolerate much higher levels of these common anions and therefore it is recommended in this method as the column of choice. A summary of the results of examining these three columns for simulated matrices with various common anion levels is presented in Table 4.

6.1.2.2 Any alternate, equivalent column must be characterized as hydrophilic or conversely, must be rated as having low to very low hydrophobicity.[4] This is one characteristic that is consistent for the AS5, AS11 and AS16 analytical separator columns. This requirement for low hydrophobicity is to allow the efficient, reproducible and symmetrical band elution of polarizable anions, such as perchlorate. If the perchlorate analysis is attempted on a hydrophobic column, such as those typically used for the analysis of common anions,[13] poor performance will result due to very asymmetric, tailing peaks. Using a middle to high calibration standard, conduct a typical analysis. Any alternate column must be capable of yielding symmetrical peak elution for this perchlorate response as demonstrated by yielding a Peak Gaussian Factor of between 0.80 and 1.15 using the following equation,

$$PGF = \frac{1.83 \times W(\frac{1}{2})}{W(\frac{1}{10})}$$

where,
$W(\frac{1}{2})$ is the peak width at half height, and
$W(\frac{1}{10})$ is the peak width at tenth height.

NOTE: Values for $W(\frac{1}{2})$ and $W(\frac{1}{10})$ can be attained through most data acquisition software.

6.1.3 Anion suppressor device -- The data presented in this method were generated using a Dionex Anion Self Regenerating Suppressor (4 mm ASRS, ULTRA, P/N 53946). An equivalent suppressor device may be utilized provided comparable conductivity detection limits are achieved and adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 5 nS per minute over the background conductivity. Proper suppressor

performance is essential to analytical data reproducibility and sensitivity of the conductivity detector.

6.1.3.1 The ASRS was set to perform electrolytic suppression at a current setting of 300 mA using the external water mode. External water was delivered to the suppressor directly from a pressurized source at a flow rate of 5 mL/min

6.1.3.2 If pretreated samples (Section 11.1.4), or sample matrices which contain appreciable concentrations of transition metal cations (e.g., Fe or Al) are frequently analyzed, cationic components may bind to the suppressor membrane and over time effect suppressor performance. If the instrument begins to have problems with reduced peak response or asymmetrical perchlorate peaks, the suppressor membranes should be cleaned. As a quick and easy cleaning step, the manufacturer's ASRS "Quickstart" procedure for installing a new ASRS should be followed.[14] If this procedure does not correct the problem, follow the manufacturer's recommended cleaning procedure for removing metal contaminants.[15]

6.1.4 Detector -- Conductivity cell (Dionex CD20, or equivalent) capable of providing data as required in Section 9.2.

6.2 Data Acquisition System -- The Dionex Peaknet Data Chromatography Software was used to generate all the data in Tables 1 through 4. Other computer based data systems may achieve approximately the same performance but the user should demonstrate this by the procedures outlined in Section 9.

6.3 Conductivity Meter – Used to monitor sample matrix conductance which is directly related to the common anion levels in a matrix and used to determine if sample pretreatment is required. At a minimum, this meter should be capable of measuring matrix conductance over a range of 1 - 10,000 uS/cm.

6.4 Analytical balance -- Used to accurately weigh target analyte salt for stock standard preparation (±0.1 mg sensitivity).

6.5 Top loading balance -- Used to accurately weigh reagents such as sodium hydroxide solution in the preparation of eluents (±10 mg sensitivity).

6.6 Weigh boats -- Plastic, disposable - for weighing eluent reagents.

6.7 Micro beakers -- Plastic, disposable - used during sample preparation.

6.8 Syringes -- Plastic, disposable, 10 mL - used during sample preparation.

6.9 Pipets -- Pasteur, plastic or glass, disposable, graduated, 5 mL and 10 mL.

6.10 Bottles -- High density polyethylene (HDPE) or glass, amber or clear, 30 mL, 125 mL, 250 mL. For sampling and storage of calibration solutions. Stability studies presented by the Interagency Perchlorate Steering Committee for Analytical Methods [6] and confirmed at the EPA (see Table3A), indicate perchlorate is neither photoreactive nor prone to adsorption to the walls of either HDPE plastic or glass bottles.

6.11 Particulate filters -- 0.45 micron syringe filters, specifically designed for IC applications (Gelman IC Acrodisc, PN 4485, or equivalent). These cartridges are used to remove particulates from the sample matrix while loading the sample manually or if the autosampler employed does not filter the sample during loading.

6.12 Matrix pretreatment cartridges in the barium form -- (Dionex OnGuard-Ba cartridges, PN 046072, or equivalent.) These cartridges are conditioned according to the manufacturer's directions and are used to reduce the matrix levels of sulfate.

6.13 Matrix pretreatment cartridges in the silver form – (Dionex OnGuard-Ag cartridges PN 039637, or equivalent.) These cartridges are conditioned according to the manufacturer's directions and are used to reduce the matrix levels of chloride.

6.14 Matrix pretreatment cartridges in the hydrogen form -- Dionex OnGuard-H cartridges (PN 039596) or equivalent. These cartridges are conditioned according to the manufacturer's directions and are used to reduce cations in the sample matrix. This protects the analytical column by removing silver which has leached from the Ag cartridge and may indirectly minimize the effect of carbonate by removing the cationic counter ion.

7. REAGENTS AND STANDARDS

7.1 Reagent water -- Distilled or deionized water 17.8 Mohm or better, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.2 Eluent solution -- 50 mM sodium hydroxide (NaOH, [CASRN 1310-73-2]), dissolve 8.0 grams of 50% (W/W) sodium hydroxide in reagent water to a final volume of 2.0 L. **NOTE:** This eluent solution is specific to the columns listed in Table 1. Any alternate columns will likely have unique and specific conditions identified by the manufacturer.

7.2.1 Solutions of NaOH are very susceptible to carbonate contamination resulting from adsorption of carbon dioxide from the atmosphere. This contamination will result in poor reproducibility of perchlorate retention times, elevated

instrument background conductivity, and increased baseline noise/drift. Consequently, exposure to the atmosphere should be minimized by storing these eluent solutions in sealed reservoirs under low pressure (3 to 5 psi) helium. In addition, these solutions should be regularly prepared and held for no more than 5 days. When refilling the eluent reservoir, completely replace old eluent solution by emptying the old eluent, rinsing the reservoir with reagent water, and refilling with the freshly prepared eluent solution. With this eluent, the suppressed conductivity detector background signal should be between 2 - 5 uS.

7.2.2 This eluent solution must be purged for 10 minutes with helium prior to use. This effectively removes dissolved gases which may form micro bubbles in the IC, compromising system performance and adversely effecting the integrity of the data. Alternatively, an in-line degas apparatus may be employed.

7.2.3 A system or apparatus which automatically generates the hydroxide eluent (Dionex EG40, or equivalent) is an acceptable alternative to physically preparing this hydroxide eluent.

7.3 Perchlorate stock standard solution, 1000 mg/L (1 mg/mL) – A stock standard solution may be purchased as a certified solution or prepared from ACS reagent grade, sodium salt as listed below. (NOTE: Sodium perchlorate represents a molar weight fraction of 81.2 % perchlorate anion)

7.3.1 Perchlorate (ClO_4^-) 1000 mg/L -- Dissolve 0.1231 g sodium perchlorate ($NaClO_4$, CASRN [7601-89-0] in reagent water and dilute to 100 mL in a volumetric flask.

NOTE: Stability of standards -- Perchlorate stock standards, stored at room temperature, appear to be very stable and may be stable for an extended period of time. However, specified expiration dates should be marked on each prepared stock standard as part of any laboratory's quality control program. In this regard, it is recommended that stock standards for perchlorate be held for no more than 12 months and an expiration date should be clearly specified on the label.

7.4 Mixed Common Anion Stock Solution - containing the anions chloride, sulfate and carbonate each at 25 mg/mL anion concentration. This solution is used to prepare simulated common anion samples in the determination of the MCT (Section 9.2.8).

7.4.1 Dissolve the following salts in reagent water to a final volume of 25.0 mL:
1.0 g sodium chloride (NaCl, CASRN [7647-14-5]) = 0.61 g Cl^-
0.93 g sodium sulfate (Na_2SO_4, CASRN [7757-82-6]) = 0.63 g $SO_4^=$
1.1 g sodium carbonate (Na_2CO_3, CASRN [497-19-8]) = 0.62 g $CO_3^=$

7.5 Conductivity Meter Calibration Solution

7.5.1 Potassium Chloride (KCl), 745 mg/L (total salt weight) -- Dissolve 0.745 g potassium chloride (KCl, [CASRN 7447-40-7]) in reagent water and dilute to a final volume of 1.00 L in a volumetric flask. On a properly functioning and calibrated conductivity meter, the reference conductance for this solution is 1410 uS/cm at 25 °C.[16]

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples may be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and laboratory fortified matrix analysis, if required, and minimize waste disposal.

8.2 Samples do not need to be shipped iced or stored cold in a refrigerator but every effort should be taken to protect the samples from temperature extremes. A thermally insulated sampling kit, designed to fit sampling bottles securely during shipment, should be used to protect the samples from these temperature extremes.

8.3 Sample preservation and holding times for the anions are as follows:

Analyte	Preservation	Holding Time
Perchlorate	None required	28 days

NOTE: Perchlorate has been shown to be stable for more than 28 days[6] but extended holding time studies (beyond 35 days) were not conducted by EPA. Typically, when analytes are believed to be stable, a 28 day holding time is established as a sufficient time period to permit a laboratory to conduct the analysis.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The requirements of this program consist of an initial demonstration of laboratory capability, and subsequent analysis in each analysis batch (Section 3.1) of an Instrument Performance Check Standard (IPC), Laboratory Reagent Blank (LRB), Initial Calibration Check Standard (ICCS), Laboratory Fortified Blank (LFB), Continuing and End Calibration Check Standards (CCCS/ECCS), Laboratory Fortified Sample Matrix (LFM) and either a Field, Laboratory or LFM duplicate sample analysis. This section details the specific requirements for each of these QC parameters. The QC criteria discussed in the following sections are summarized in Section 17, Table 5

and 6. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF CAPABILITY

9.2.1 The Initial Demonstration of Capability (IDC) -- This is used to characterize instrument and laboratory performance prior to performing analyses by this method. The QC requirements for the IDC discussed in the following section are summarized in Section 17, Table 5.

9.2.2 Initial demonstration of low system background -- See Section 9.3.1.

9.2.3 Initial Demonstration of Accuracy (IDA) -- Prepare and analyze 7 replicate LFBs fortified at 25.0 ug/L. Calculate the mean measured concentration ($C_{\bar{x}}$) of the replicate values as follows.

$$C_{\bar{x}} = \frac{(C_1 + C_2 + C_3 + ...C_n)}{n}$$

where,

$C_{\bar{x}}$ = Mean recovered concentration of the replicate analysis.
$C_1, C_2, ...C_n$ = Recovered concentrations of the replicate 1,2...n.
n = 7

To pass the IDA, the value derived for $C_{\bar{x}}$ must be within ± 10% of the true value or between 22.5 ug/L and 27.5 ug/L.

9.2.4 Initial Demonstration of Precision (IDP) -- Using the data generated for Section 9.2.3, calculate the percent relative standard deviation (%RSD) of the replicate analysis, as indicated below. To pass the IDP, the %RSD must be less than 10%.

$$\%RSD = \frac{(S_{n-1})}{(C_{\bar{x}})} \times 100$$

where,

S_{n-1} = sample standard deviation (n-1) of the replicate analyses.
$C_{\bar{x}}$ = mean recovered concentration of the replicate analysis.

9.2.5 Quality Control Sample (QCS) – After calibration curves have initially been established or have been re-established, or as required to meet data quality needs, verify both the calibration and acceptable instrument performance with the preparation and analyses of an external/second source QCS. If the determined concentrations are not within ± 10% of the stated values,

performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the IDC or continuing with on-going analyses.

9.2.6 Method Detection Limit (MDL) – An MDL must be established using reagent water (blank) fortified at a concentration of three to five times the estimated instrument detection limit.[7, 8] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method over a three day period. These seven MDL replicate analyses may be performed gradually over three days or may represent data that has been collected, at a consistent MDL estimated concentration, over a series of more than three days. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S_{n-1})$$

where,

t = student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]

S_{n-1} = sample standard deviation (n-1) of the seven replicate analyses.

9.2.6.1 MDLs should be periodically verified, but MUST be initially determined when a new operator begins work or whenever there is a significant change in the background, or instrument response.

NOTE: Do not subtract blank values when performing MDL calculations.

9.2.7 Minimum Reporting Level (MRL) – The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should be established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal to noise ratio of five. Setting the MRL too low may cause repeated QC failure upon analysis of the ICCS. **Although the lowest calibration standard may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard.**

9.2.8 Matrix Conductivity Threshold (MCT) – The MCT is an individual laboratory defined value which must be determined by preparing a series of sequentially increasing, common anion fortified, reagent water samples each contain a constant perchlorate concentration. Initially, a reagent water prepared LFB, containing no common anions, must be analyzed which contains perchlorate at a suggested concentration of 25 ug/L perchlorate. Next, the series of sequentially

increasing anionic solutions are prepared, each containing perchlorate at a suggested concentration of 25 ug/L, which also containing the individual common anions of chloride, sulfate and carbonate, all included at uniform increasing concentrations of 200, 300, 400, 500, 600, 800, and 1000 mg/L for each anion. A concentration of 25 ug/L perchlorate has been suggested assuming the MRL has been set in the range of 3.0 ug/L to 5.0 ug/L. If a laboratory's MRL is higher, choose a perchlorate concentration for this exercise at approximately 5 times that MRL.

9.2.8.1 Prepare the mixed common anion stock solution (see Section 7.4) containing chloride, sulfate and carbonate, each at 25 mg/mL.

9.2.8.2 Prepare a perchlorate secondary stock dilution standard at 1.00 mg/L from the 1000 mg/L perchlorate stock standard (Section 7.3) by diluting 0.50 mL of the stock solution to a final volume of 500 mL.

9.2.8.3 Prepare the LFB at suggested perchlorate concentration of 25 ug/L by diluting 0.625 mL of the perchlorate secondary stock dilution standard (Section 9.2.8.2) to a final volume of 25.0 mL.

9.2.8.4 Next, prepare the series of common anion fortified reagent water samples by adding 0.20 mL, 0.30 mL, 0.40 mL, 0.50 mL, 0.60 mL, 0.80 mL, and 1.00 mL of the mixed common anion stock solution (Section 7.4) into separate 25 mL volumetric flasks. Next, add 0.625 mL of the perchlorate secondary stock dilution standard (Section 9.2.8.2) to each 25 mL volumetric flask and dilute to volume with reagent water to yield a final perchlorate concentration of 25.0 ug/L.

9.2.8.5 Measure and record the conductance of each of these prepared solutions on a calibrated conductivity meter (This meter must be calibrated as described in Section 10.4 prior to measuring conductance). To use as a relative reference conductance, the 400 mg/L mixed anion sample, which contains chloride at 400 mg/L, sulfate at 400 mg/L and carbonate at 400 mg/L, should display a conductance of between 3200 uS/cm and 3700 uS/cm.

9.2.8.6 Analyze each solution, recording the peak area to height (A/H) ratio and the quantified concentration of perchlorate. In many data acquisition and instrument control software, the peak area to height ratio is a definable parameter which can be specified for printout on the analysis report.

9.2.8.7 Both the A/H ratio and quantified perchlorate concentration for the LFB and the 200 mg/L mixed common anion solution should be reproducibly consistent but as the common anion levels increase, the

A/H ratio will also begin to increase as the peak height is distorted and reduced. As the peak is distorted, the area will also eventually begin to be distorted and the quantitated concentration will be reduced, but this is typically secondary, with the ratio of peak area to height initially predicting this pending quantitation problem.

9.2.8.8 Calculate the A/H ratio percent difference ($PD_{A/H}$) between the average A/H ratio for the LFB (A/H_{LFB}) and the average A/H ratios for each mixed common anion solutions (A/H_{MA}) using the following equation.

$$PD_{A/H} = \frac{|(A/H_{LFB} - A/H_{MA})|}{A/H_{LFB}} \times 100$$

9.2.8.9 As the conductivity of the matrices increase, the $PD_{A/H}$ will increase. The MCT is the matrix conductance where the $PD_{A/H}$ exceeds 20%. To derive the MCT, perform a linear regression on these data by plotting $PD_{A/H}$ (as the independent variable, x) versus the matrix conductance (as the dependent variable, y). The resulting regression data should yield an r^2 value of > 0.95. (See Figure 5) Record the "constant" (intercept value) and the "X-coefficient" (slope) and calculate the MCT as follows,

MCT = (20%) x (X-coefficient) + (constant)

NOTE: Be careful to consistently apply percentages as either whole numbers or as fractional values (20% = 0.20) for both the regression analysis and the MCT calculation.

9.2.8.10 As an alternate to the regression analysis, the laboratory can choose to establish their MCT at the conductance level of the highest mixed anion solution which yielded a $PD_{A/H}$ value below the 20 % threshold.

9.2.8.11 As a final procedure, the laboratory should confirm their perchlorate MRL in a mixed common anion solution which reflects a conductance near (within +/- 10%) that specified as the MCT. This solution must contain perchlorate, at the laboratory determined MRL, as well as the common anions chloride, sulfate and carbonate, prepared consistent with the instruction for the mixed anion solutions in this section and at a concentration estimated to generate a conductance near the MCT. The conductance of this solution must be measured at within ±10% of the MCT and following the analysis, the recovered perchlorate must be between 70 - 130% of the MRL concentration. If the MRL recovery

fails this criteria, the MCT should be lowered by 10% and this MRL verification must be repeated.

9.2.8.12 Prior to conducting any field sample analysis, the conductivity of that matrix must be determined. When the conductance of a field sample is above the MCT, sample dilution or pretreatment, as described in respective Sections 11.1.3 and 11.1.4 must be performed.

9.3 ASSESSING LABORATORY PERFORMANCE - The following items must be included in every analysis batch (Section 3.1).

9.3.1 Laboratory Reagent Blank (LRB) – An LRB must be prepared and treated exactly as a typical field sample including exposure to all glassware, equipment, solvents, filtration and reagents that are used with field samples. Data produced are used to assess instrument performance of a blank sample and evaluate contamination from the laboratory environment. Values that exceed ½ the MRL indicate a laboratory or reagent contamination is present. The source of the contamination must be determined prior to conducting any sample analysis. Any sample included in an automated analysis batch which has an invalid LRB, indicated by a quantitated perchlorate that exceeds ½ the MRL, must be reanalyzed in a subsequent analysis batch after the contamination problem is resolved.

9.3.1.1 When sample matrices have been pretreated to reduce the risk of high common anion interference (Section 11.1.4), a second LRB must be prepared, pretreated in exactly the same manner, and analyzed to confirm no background effects from the pretreatment process are present. If an analysis batch only contains pretreated samples, then only a pretreated LRB is required.

9.3.2 Instrument Performance Check (IPC) -- The MCT, which was determined as part of the IDC in Section 9.2.8, must be verified through the analysis of an IPC. The IPC is three tiered and is used to verify the state of the IC system, over time, to quantitate perchlorate in highly ionic matrices. This must be conducted with each analysis batch since over time, column performance can change.

9.3.2.1 Prepare a mixed common anion solution which reflects a conductance near (within +/- 10%) that specified as the MCT. This solution must be prepared consistent with the instruction in Section 9.2.8, and containing the common anions chloride, sulfate and carbonate as well as perchlorate at a suggested concentration of 25 ug/L. This perchlorate concentration has been specified assuming the MRL has been set in the range of 3.0 ug/L to 5.0 ug/L. If a laboratory's MRL is

higher, chose a perchlorate concentration for this exercise at approximately 5 times that MRL.

9.3.2.2 Confirm the conductance of the IPC and analyze it as the initial sample in the analysis batch. If, after several weeks of storage, the measured conductance of this solution has shifted by more than 10% from the original measured value, prepare a fresh IPC solution. Following the analysis, calculate the $PD_{A/H}$ (Section 9.2.8.8), by comparing the peak area to height ratio of this IPC mixed anion standard (A/H_{MA}) for this analysis batch to the value that was derived for the LFB (A/H_{LFB}) either in the original IDC or in the previous analysis batch. As the first tier criteria, the value for $PD_{A/H}$ must be less than 25% before proceeding with the analysis batch.

9.3.2.3 At the second tier criteria, the measured recovery for perchlorate in this IPC must fall between 80% and 120 % (20.0 ug/L to 30.0 ug/L for a 25 ug/L fortification).

9.3.2.4 As a third tier and final criteria for the IPC, the laboratory must closely monitor the perchlorate retention time for this analysis. Small variations in retention time can be anticipated when a new solution of eluent is prepared but if sudden shifts of more than 5% are observed in the perchlorate retention time, some type of instrument problem may be present. Potential problems include improperly prepared eluent, erroneous method parameters programmed such as flow rate or some other system problem. The observed retention time for perchlorate should closely replicate the times established when the column was originally installed. As a column ages, it is normal to see a gradual shift and shortening of retention times, but if after several years of use, extensive use over less than a year, or use with harsh samples, this retention time has noticeably shifted to any less than 80% of the original recorded value, the column requires cleaning (according to manufacturer's instructions) or replacement. A laboratory should retain a historic record of retention times for perchlorate to provide evidence of an analytical column's continued performance.

9.3.2.5 If any of the conditions defined in Section 9.3.2.2 through 9.3.2.4 are not met, the MCT must be repeated and revised to a more appropriate lower matrix conductivity threshold or the source of the problem must be determined and the IPC reanalyzed.

9.3.3 Laboratory Fortified Blank (LFB) – Prepare a secondary dilution stock using the same stock solution used to prepare the calibration standards. This separate,

secondary dilution stock is used as a concentrate to fortify the LFB and the LFMs (Section 9.4.1). An external source stock or QCS, which is used to verify the accuracy of the calibration curve when it was initially prepared (Section 10.2.5), should not be used to prepare this secondary dilution stock. Laboratories are required to analyze a LFB (filtered as if it were a field sample) with each analysis batch immediately following the ICCS. The LFB must be prepared with the same solution used to prepare the LFM and should be prepared at concentrations no greater than ten times the highest concentration observed in any field sample and should be varied to reflect the range of concentrations observed in field samples. By analyzing the LFB initially, a control check is performed on the concentrated solution used to prepare the LFM. If any deviations in the perchlorate concentration are present, it will be reflected in the LFB and not exclusively attributed to a matrix upon analysis of the LFM. Calculate accuracy as percent recovery (Section 9.4.1.3). The recovery for perchlorate must fall in the range of 85 - 115% prior to analyzing samples. If the LFB recovery for an analysis batch does not meet these recovery criteria the data are considered invalid, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3.1 When sample matrices have been pretreated to reduce the risk of high common anion interference (Section 11.1.4), a second LFB must be prepared, pretreated in exactly the same manner, and analyzed to confirm no background effects or recovery bias induced by the pretreatment are present. If an analysis batch only contains pretreated samples, then only a pretreated LFB is required.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY - The following must be included in every analysis batch (Section 3.1).

9.4.1 Laboratory Fortified Sample Matrix (LFM) – The laboratory must add a known amount of each target analyte to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater. Samples which exceed the MCT must either be diluted (Section 11.1.3) or pretreated to reduce the common anion levels (Section 11.1.3). Samples which are pretreated have additional LFM requirements described in Section 11.1.4.6, and must be fortified before pretreatment. For a LFM to be valid, the target analyte concentrations must be greater than the native level and should adhere to the requirement outlined in Section 9.4.1.2. It is recommended that the solutions used to fortify the LFM be prepared from the same stocks used to prepare the calibration standards and not from external source stocks. This will remove the bias contributed by an externally prepared stock and focus on any potential bias introduced by the field sample matrix.

9.4.1.1 The fortified concentration must be equal to or greater than the native sample concentration. Fortified samples that exceed the calibration range must be diluted to be within the linear range. In the event that the fortified level is less than the observed native level of the unfortified matrix, the recovery should not be calculated. This is due to the difficulty in calculating accurate recoveries of the fortified concentration when the native sample concentration to fortified concentration ratio is greater than one.

9.4.1.2 For normal drinking waters, the LFM typically should be prepared in the range of 20 - 50 ug/L. The LFM should not be prepared at concentration greater than ten times the highest concentration observed in any field sample and should be varied to reflect the range of concentrations expected in field samples.

9.4.1.3 Calculate the percent recovery for each target analyte, corrected for concentrations measured in the unfortified sample. Percent recovery should be calculated using the following equation:

$$\%REC = \frac{(C_s - C)}{s} \times 100$$

where,
%REC = percent recovery,
C_s = measured perchlorate in the fortified sample,
C = measured native perchlorate sample concentration, and
s = concentration equivalent of analyte added to sample.

9.4.1.4 Recoveries may exhibit a matrix dependence. If the recovery for perchlorate falls outside 80 - 120%, and the laboratory's performance for all other QC performance criteria is acceptable, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample and the LFM must be labeled suspect/matrix to inform the data user that the result is suspect due to matrix effects. Repeated failure to meet suggested recovery criteria indicates potential problems with the procedure and should be investigated.

9.4.2 FIELD, LABORATORY DUPLICATES OR DUPLICATE LFM – The laboratory must analyze either a field duplicate, a laboratory duplicate, or a duplicate LFM for a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater. The sample matrix selected for this duplicate analysis must contain measurable concentrations of the target

anions in order to establish the precision of the analysis set and ensure the quality of the data. Without prior knowledge or strong suspicion that an unknown sample has measurable perchlorate concentrations, the best alternative is to analyze a duplicate LFM.

9.4.2.1 Calculate the relative percent difference (RPD) of the initial quantitated concentration (I_C) and duplicate quantitated concentration (D_c) using the following formula.

$$RPD = \frac{|(I_C - D_c)|}{([I_C + D_c]/2)} \times 100$$

9.4.2.2 Duplicate analysis may exhibit a matrix dependance. If the RPD for the duplicate measurements of perchlorate falls outside ± 15% and if all other QC performance criteria are met, laboratory precision is out of control for the sample and perhaps the analytical batch. The result for the sample and duplicate should be labeled as suspect/matrix to inform the data user that the result is suspect due to a potential matrix effect, which led to poor precision. This should not be a chronic problem and if it frequently recurs (>20% of duplicate analyses), it indicates a problem with the instrument or individual technique that must be corrected.

9.4.3 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns (which meet the criteria in Section 6.1.2.2), injection volumes, and/or eluents, to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 9.2 and adhere to the condition of conductivity baseline stability found in Section 1.2.1.

9.4.4 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should perform analysis of quality control check samples and participate in relevant proficiency testing (PT) or performance evaluation (PE) sample studies.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required prior to the IDC and before any samples are analyzed, and is required intermittently throughout sample analysis to meet required QC performance criteria outlined in this method and

summarized in Table 6. Initial calibration verification is performed using a QCS as well as with each analysis batch using an initial, continuing (when more than 10 field samples are analyzed), and end calibration check standards. The procedures for establishing the initial calibration curve are described in Section 10.2. The procedures to verify the calibration with each analysis batch is described in Section 10.3.

10.2 INITIAL CALIBRATION CURVE

10.2.1 Establish ion chromatographic operating parameters equivalent to those indicated in Table 1.

10.2.2 Estimate the Linear Calibration Range (LCR) -- The LCR should cover the expected concentration range of the field samples and should not extend over more than two orders of magnitude in concentration. The restriction of two orders of magnitude is prescribed since beyond this it is difficult to maintain linearity throughout the entire calibration range.

10.2.2.1 If quantification is desired over a larger range, then two separate calibration curves should be prepared.

10.2.2.2 A minimum of three calibration standards are required for a curve that extends over a single order of magnitude and a minimum of five calibration standards are required if the curve covers two orders of magnitude.

10.2.2.3 Since the anticipated concentration range for perchlorate in actual field samples is expected to cover two orders of magnitude, the use of at least five calibration standards in the range 4 - 400 µg/L is recommended.

10.2.3 Prepare the calibration standards by carefully adding measured volumes of the stock standard (Section 7.3) to a volumetric flask and diluting to volume with reagent water.

10.2.4 Inject 1.0 mL of each calibration standard. Tabulate peak area responses against the perchlorate concentration. The results are used to prepare a calibration curve. Acceptable calibration is confirmed after reviewing the curve for linearity (second order fits are also acceptable) and passing the criteria for the initial calibration check standard in Section 10.3.1. Alternately, if the ratio of area to concentration (response factor) is constant over the LCR (indicated by < 15% relative standard deviation), linearity through the origin can be assumed and the average ratio or response factor can be used in place of a calibration curve.

10.2.4.1 Peak areas must be used as a measure of response since they have been found to be more consistent, in terms of quantitation, than peak heights. Peak height can tend to be suppressed as a result of high levels of common anions in a given matrix which can compete for exchange sites leading to peak broadening. Using peak areas, it is the analyst's responsibility to review all chromatograms to insure accurate baseline integration of target analyte peaks, since poorly drawn baselines will significantly influence peak areas.

10.2.5 After establishing or reestablishing calibration curves, the accuracy of this calibration must be verified through the analysis of a QCS or externally prepared second source. The QCS should be prepared at a concentration near the middle of the calibration curve. As specified in Section 9.2.5, determined concentrations must fall within ± 10% of the stated values.

10.3 CONTINUING CALIBRATION VERIFICATION -- Initial calibrations may be stable for extended periods of time. Once the calibration curve has been established it MUST be verified for each analysis batch, prior to conducting any field sample analysis using an Initial Calibration Check Standard. Continuing Calibration Check Standards and End Calibration Check Standards are also required as described in the sections below.

10.3.1 INITIAL CALIBRATION CHECK STANDARD (ICCS) – For each analysis batch the calibration must initially be verified prior to analyzing any samples. The lowest level standard used to prepare the linear calibration curve must be used. In cases where the analyst has chosen to set the MRL above the lowest standard, a standard at a concentration equal to the MRL is acceptable. Percent recovery for the ICCS must be in the range or 75 - 125% before continuing the analysis batch and conducting any sample analyses.

10.3.2 CONTINUING CALIBRATION CHECK/END CALIBRATION CHECK STANDARDS (CCCS/ECCS) -- Continuing calibration check standards MUST be analyzed after every tenth field sample analysis and at the end of the analysis batch as an end calibration check standard. If more than 10 field samples are included in an analysis batch, the analyst must alternate between the middle and high continuing calibration check standard levels.

10.3.2.1 The percent recovery for perchlorate in the CCCS/ECCS must be between 85 - 115%.

10.3.2.2 If during the analysis batch, the measured concentration for perchlorate in the CCCS or ECCS differs by more than the calibration verification

criteria shown above, or if the perchlorate peak retention time shifts outside the retention time window (as defined in Section 11.2.4), all samples analyzed after the last acceptable check standard are considered invalid and must be reanalyzed. The source of the problem must be identified and resolved before reanalyzing the samples or continuing analyses.

10.3.2.3 In the case where the end calibration fails to meet performance criteria, but the initial and middle calibration checks are acceptable, the samples bracketed by the acceptable calibrations may be reported. However, all field samples between the middle and end calibration checks MUST be reanalyzed.

10.4 CONDUCTIVITY METER CALIBRATION -- Prior to conducting the MCT and coinciding with each analysis batch, conductivity meter calibration must be verified or established using a standard KCl solution (Section 7.5).

10.4.1 Thoroughly rinse the conductivity electrode with reagent water. Place the electrode in the reagent water, turn on the meter and confirm the conductance of this blank is < 1 uS/cm.

10.4.2 Pour approximately 15 mL of the standard KCl solution (Section 7.5) into a plastic disposable micro beaker (Section 6.7) and place the electrode into the solution. The reference conductance for this solution is 1410 uS/cm at 25 °C.[16] The conductivity meter must yield a conductance between 1380 uS/cm and 1440 uS/cm to be in calibration.

10.4.3 If the conductivity meter fails calibration, recalibrate the unit per manufacture's instruction and repeat the procedure in 10.4.2 as if the standard solution were an unknown matrix.

11. PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 Samples do not need to be refrigerated but if samples are held refrigerated as a standard practice for sample control, ensure the samples have come to room temperature prior to conducting sample analysis.

11.1.2 MATRIX CONDUCTANCE VERIFICATION - Prior to conducting the analysis of a field sample matrix, the conductance of that matrix must be measured. Matrix conductivity is directly related to the common anion levels which, at high concentrations, can influence the integrity of the perchlorate analysis.

11.1.2.1 Verify conductivity detector calibration by following the procedure outlined in Section 10.4.

11.1.2.2 Pour approximately 15 mL of sample into a plastic disposable micro beaker (Section 6.7) and reseal the sample bottle to protect the sample integrity.

11.1.2.3 Place the electrode into the matrix and measure the conductivity.

11.1.2.4 If the conductance is less than the MCT, continue to Section 11.1.5.

11.1.2.5 If the conductance is greater than the MCT, the matrix requires dilution or pretreatment prior to analysis. The dilution procedure is found in Section 11.1.3. Pretreatment is described in Section 11.1.4.

11.1.2.6 Discard this aliquot of sample and be certain to thoroughly rinse the electrode with reagent water between each matrix conductivity measurement.

11.1.3 MATRIX DILUTION - If matrix conductivity is less than the MCT, go to Section 11.1.5.

11.1.3.1 A sample can be analyzed once diluted with reagent water to a conductance below the MCT. The exact magnitude of this dilution will adversely increase the MRL by an equivalent proportion.

11.1.3.2 Knowing the matrix conductance exceeds the MCT, estimate the proportion required for the dilution by dividing the measured matrix conductance by the MCT. Round up to the next whole number and dilute the sample by a proportion equivalent to this value. For example, if the established MCT is 6100 uS/cm and a sample reflecting a conductance of 8000 uS/cm was measured, dilute the sample with reagent water by a factor of 2.

11.1.3.3 Measure the conductance of the diluted sample to confirm it is now below the MCT. Analyze the sample as specified in Section 11.1.5 with the understanding that the MRL has now been elevated by a proportion equivalent to the dilution.

11.1.3.4 If perchlorate is measured above the elevated MRL, back calculate actual field sample concentration and report. If no perchlorate is measured above the elevated MRL and analysis or project objectives

required monitoring below the concentration of the elevated MRL, proceed to Section 11.1.4 and pretreat the matrix.

11.1.4 PRETREATMENT FOR MATRICES WHICH EXCEED THE MCT – If matrix conductivity is less than the MCT, go to Section 11.1.5. If sample dilution did not yield the required results, sample pretreatment should be employed. When the MCT is exceeded, it is most often due to a high levels of common anions (chloride, sulfate, and carbonate) in a particular matrix. If the analyst were to attempt the IC analysis of this particular matrix, the common anions present in the sample would distort the baseline and negatively affect the accurate quantitation of perchlorate. To effectively reduce a significant amount of these anions which contribute to the high conductivity reading, a series of pretreatment cartridges must be employed. For this pretreatment, three cartridges are attached in series in the following order: Ba, Ag, and H. It is recommended that all three cartridges be employed unless the analyst has specific knowledge that a matrix primarily has high levels of a specific common anion.

11.1.4.1 Individually and thoroughly rinse each pretreatment cartridge with reagent water in order to insure all residual background contaminants are removed from the cartridge. Perform this rinse per manufacturer's instructions.

11.1.4.2 Prior to pretreating any field samples, prepare and pretreat both an LRB and an LFB. These pretreated quality control samples are required when an analysis batch contains a matrix which must be pretreated. This pretreatment is conducted by placing the cartridges in the following prescribed series (–>Ba–>Ag–>H). The pretreated LRB and LFB are used to verify that no background interference or bias is contributed by the pretreatment. If a response is observed in the pretreated LRB, triple or quadruple the volume of reagent water rinse suggested by the manufacturer in Section 11.1.4.1 and repeat until a blank measures no more than ½ the MRL. If this additional rinsing procedure is required, it must be consistently applied to all the cartridges prior to conducting any matrix pretreatment.

11.1.4.3 Filter 3 mL of sample through the series of rinsed, stacked cartridges as an initial sample rinse (Ba, Ag and H) at a flow rate of 1.0 mL/ min or less (approximately one drop every 3 to 4 seconds). This flow rate is critical to the pretreatment and must be carefully followed. Discard this fraction and begin collecting the pretreated sample aliquot of collected sample.

11.1.4.4 When sufficient volume has been collected, measure the conductance of the pretreated sample aliquot being certain the conductivity meter's probe has been thoroughly rinsed and excess water has been shaken from the tip. If the conductance is now below the MCT, the sample is ready for analysis. If the conductance is still above the MCT, the flow rate through the pretreatment cartridge is likely too fast and the pretreatment should be repeated with new cartridges. In some instances, double pretreatment cartridges may need to be applied. When this pretreatment is performed properly, U.S.EPA has found 70% to 95% reduction in matrix conductance with good recoveries for perchlorate.

11.1.4.5 Place this aliquot of pretreated sample into an autosampler vial as described in Section 11.1.3.

11.1.4.6 In order to ensure data quality, all samples which fail the MCT and have been selected for pretreatment, as described in Section 11.1.4, must also be used to prepare an LFM. This LFM must be fortified with perchlorate at concentrations close to, but greater than, the level determined in the native sample prior to the pretreatment. Initially, the pretreated sample is analyzed and perchlorate level is determined. Then, a second aliquot of sample must be fortified with perchlorate, pretreated to reduce the high common anion levels, and analyzed to assess perchlorate recovery from that matrix. This additional QC is required to rule out matrix effects and to confirm that the laboratory performed the pretreatment step appropriately. **If the perchlorate recovery falls outside the acceptance range of 80 - 120% (Section 9.4.1.4), that particular sample should be reported as suspect/matrix.**

11.1.4.7 The pretreatments prescribed above are effective at reducing the chloride and sulfate content of a sample matrix but will not reduce matrix concentrations of other anions such as nitrate or phosphate.

11.1.5 Pour approximately 15 mL of sample into a micro beaker (Section 6.7) and reseal the sample bottle to protect the sample integrity. Using a Luer lock, plastic 10 mL syringe, withdraw approximately 10 mL of sample from the micro beaker and attach a 0.45 µm particulate filter (Section 6.11), which has been demonstrated to be free of ionic contaminants, directly to the syringe. Filter the sample into an autosampler vial or manually load the injection loop injecting a fixed amount of filtered, well mixed sample. If using a manually loaded injection loop, flush the loop thoroughly between sample analysis using sufficient volumes of each new sample matrix.

11.1.5.1 If the autosampler vials or vial caps are designed to automatically filter the sample matrix as the sample is loaded on the IC system, this filtration procedure can be omitted and the sample can be directly transferred to the autosampler vial.

11.2 SAMPLE ANALYSIS

11.2.1 Table 1 summarizes the recommended operating conditions for the ion chromatograph. Included in this table is the estimated retention time for perchlorate which has been achieved by this method. Other columns, chromatographic conditions or detectors may be used if the requirements of Sections 1.2.1, 6.1.2.2 and 9.2 are met.

11.2.2 Establish a valid initial calibration and verify this calibration by conducting a QCS as described in Section 10.2 and complete the IDC (Section 9.2). Initially, analyze the IPC solution, followed by the LRB. Then confirm the IC system calibration by analyzing an ICCS (Section 10.3.1) and, if required, recalibrate as described in Section 10.2. Lastly, analyze the LFB.

11.2.3 Inject 1.0 mL of each filtered sample. Use the same size loop for standards and samples. An automated constant volume injection system may also be used. Record the resulting peak size in area units and retention time for each analyte.

11.2.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards measured over several days. Three times the standard deviation of retention time may be used as a suggested window size but the retention time window should not extend beyond ± 5% of the retention time for perchlorate. The experience of the analyst should weigh heavily in the interpretation of these chromatograms.

11.2.5 If the response of a sample analyte exceeds the calibration range, the sample must be diluted with an appropriate amount of reagent water and reanalyzed. If this is not possible then three new calibration concentrations must be employed to create a separate high concentration calibration curve, one standard near the estimated concentration and the other two bracketing around an interval equivalent to approximately ± 25% the estimated concentration. The response generated by these three new high concentration calibration standards must not exceed the upper linear range for the conductivity detector. The latter procedure involves significantly more time than a simple sample dilution therefore, it is advisable to collect sufficient sample to allow for sample dilution and sample reanalysis, if required.

11.2.6 Should more complete resolution be needed between perchlorate and a coeluting, shoulder peak, the eluent (Section 7.2) may be diluted. This will spread out the peaks, causing later elution of perchlorate. Analysts are advised to carefully evaluate any of these eluent dilutions since when these eluent changes are incorporated, other coelutions may be encountered which were not initially evident. Additionally, the analyst must verify that this dilution does not negatively affect performance by repeating and passing all the QC criteria in Section 9, and by reestablishing a valid initial calibration curve (Section 10.2).

11.2.6.1 Eluent dilution will reduce the overall response of an anion due to chromatographic band broadening which will be evident by shortened and broadened peaks. This will adversely effect the MDLs for each analyte.

11.3 AUTOMATED ANALYSIS WITH METHOD 314.0

11.3.1 Laboratories conducting analyses on large numbers of samples often prepare large analysis batches that are run in an automated manner. When conducting automated analyses, careful attention must be paid to ensure sufficient volume of eluent in the reservoir is available to sustain extended operation. In order to ensure their data are of acceptable quality, laboratories must ensure that all QC performance criteria are met throughout the analysis batch through subsequent careful inspection of the data.

11.3.2 Analysis sequences must be carefully constructed to meet required QC specifications and frequency (Table 6). To help with this task, an acceptable sequence for a sample analysis batch, with all the method-required QC, is shown in Table 7. This schedule is included only as an example of a hypothetical analysis batch which contains normal sample matrices as well as samples which have failed the MCT. Within this analysis batch, references to exact concentrations for the ICCS, CCCS and ECCS are for illustrative purposes only.

11.3.3 Table 7 may be used as a guide when preparing analysis batches. Additional batches may be added sequentially on to the end of these types of schedules as long as all QC samples, which define an individual batch (IPC, LRB, ICCS, LFB, LFM, etc.) are individually reanalyzed with each successive serial batch and the QC criteria for these analyses are continually met (from the IPC through ECCS).

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify perchlorate in the sample chromatogram by comparing the retention time of a suspect peak within the retention time window to the actual retention time of a known

analyte peak in a calibration standard. If the perchlorate retention time has slightly shifted (generally towards shorter times) since the initial calibration, but is still within acceptance criteria and are reproducible during the analysis batch, the analyst should use the retention time in the daily calibration check standards to confirm the presence or absence of perchlorate anion.

12.1.1 If a low concentration of perchlorate is suspected in an unknown sample, but the retention time has drifted to the edge of the retention time window, a low level perchlorate LFM, prepared at nearly the same concentration as the suspect peak, should be prepared from this sample matrix to confirm the matrix induced retention time shift. If the fortified sample reveals a split or shouldering peak response, the low concentration in the unfortified sample is likely an interferant and should not be reported as perchlorate.

12.2 Compute sample concentration using the initial calibration curve generated in Section 10.2.

12.3 Report ONLY those values that fall between the MRL and the highest calibration standards. Samples with a perchlorate response which exceeds the highest calibration standard concentration must be diluted and reanalyzed. When this is not possible the alternate calibration procedures described in Section 11.2.5 must be followed. Samples with perchlorate identified but quantitated below the concentration established by the lowest calibration standard, may be reported as "trace present" above the MDL but below the minimum reporting limit (MRL) and therefore not reported as a quantitated concentration.

12.4 Report results in µg/L.

13. METHOD PERFORMANCE

13.1 Table 1 gives the standard conditions, typical retention time, single laboratory MCT and single laboratory MDL in reagent water, as determined for perchlorate. This retention time is graphically indicated in the chromatograms in Figures 1 through 4.

13.2 Table 2 shows the precision and accuracy of the perchlorate measurement at two fortified concentrations, in reagent water, simulated high ionic strength water (HIW), simulated high organic content water (HOW), ground water, untreated surface water and treated surface water. The mean perchlorate recovered concentration (accuracy relative to the fortified level) and the precision (expressed as %RSD of the replicate analysis) are tabulated. The HIW was designed to simulate a high ionic strength field sample and the HOW designed to simulate a high organic content field sample. The HIW was prepared from reagent water which was fortified with the common anions of chloride at 400 mg/L, carbonate at 600 mg/L, and sulfate at 500 mg/L. The HOW was prepared from reagent water fortified with 10.0 mg/L fulvic acid.

13.3 Table 3 shows the stability data for perchlorate held for 35 days and stored under various conditions. Conditions investigated included sample bottle construction (HDPE plastic vs. glass), storage condition (refrigerated vs. held at room temperature) and various matrices including some with a measured perchlorate concentration assumed to contain microbiological constituents acclimated to the presence of the anion. Matrices without perchlorate were fortified at 25 ug/L. Each data point in this table represents the mean percent recovery following triplicate analyses. These data were used to formulate the holding times shown in Section 8.3.

13.4 Table 4, in conjunction with the chromatograms overlaid in Figure 4 as well as the linear regression plots in Figure 5, show the results of the single laboratory MCT determination. The data presented in Table 4 and graphically illustrated in Figure 5, show results for not only the AS16 but also the AS11 and AS5. The chromatogram shown in Figure 4 were generated using the AS16 column.

14. POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasiblely reduced at the source, the Agency recommends recycling as the next best option.

14.2 Quantity of chemicals purchased should be based on expected usage during its shelf-life and the disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and

by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in Section 14.3.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS IN REAGENT WATER FOR PERCHLORATE.

Standard Conditions and Equipment[a]:

Ion Chromatograph:	Dionex DX500
Sample Loop:	1000 µL
Eluent:	50 mM NaOH
Eluent Flow:	1.5 mL/min
Columns :	Dionex AG16, 4 mm / AS16, 4 mm
Typical System Backpressure:	2600 psi
Suppressor:	ASRS ULTRA (P/N 53946), external water mode, 300 mA current
Detectors:	Suppressed Conductivity Detector, Dionex CD20 Background Conductivity: 2 - 3 µS
Determined MCT[b]:	6100 uS/cm

Recommended method total analysis time: 15 minutes (may be shortened to 12 minutes)

Analyte Retention Times and Method Detection Limits (MDLs):

		MDL DETERMINATION		
Analyte	Retention Time [c] (min.)	Fortified Conc. (µg/L)	# of Reps.	MDL (µg/L)
Perchlorate	10.1 ± 0.2	2.0	7	0.53

(a) Mention of trade names or commercial products does not necessarily constitute endorsement or recommendation for use.

(b) This was the single laboratory MCT determined for these conditions listed (See Table 4 and Figure 5 for more detail as well as data pertaining to the AS11 and AS5).

(c) Reference to chromatograms in Figure 1 through 4.

TABLE 2. SINGLE LABORATORY PRECISION AND RECOVERY FOR PERCHLORATE IN VARIOUS MATRICES

Matrix	Matrix Conductivity uS/cm	Unfortified Conc. (µg/L)	Fortified Conc. (µg/L)	# of Reps.	Mean (µg/L)	Mean %REC	SD(n-1)	%RSD
Reagent Water	~ 1	<MRL[a]	4.00	8	4.04	101%	0.43	10.6%
			25.0	8	26.2	105%	0.89	3.4%
Synthetic High Inorganic Water [b]	4200	<MRL	4.00	8	3.42	86%	0.27	7.9%
			25.0	8	24.1	96%	0.46	1.9%
Synthetic High Organic Water [c]	5.0	<MRL	4.00	8	3.84	96%	0.38	10.0%
			25.0	8	25.7	103%	1.12	4.4%
Ground Water (high TDS)	710	<MRL	4.00	8	4.22	106%	0.54	12.8%
			25.0	8	26.5	106%	0.62	2.3%
Untreated Surface Water	460	<MRL	4.00	8	4.46	112%	0.24	5.4%
			25.0	8	28.3	113%	0.29	1.0%
Chlorinated Surface Water	460	<MRL	4.00	8	4.18	105%	0.23	5.6%
			25.0	8	28.0	112%	0.63	2.3%

(a) <MRL = analyte was not detected above the laboratory minimum reporting level (MRL) of 4.0 ug/L.
(b) Synthetic High Inorganic Water was prepared from reagent water and contained synthetic high TDS or common anion levels of 400 mg/L chloride, 500 mg/L sulfate and 600 mg/L carbonate.
(c) Synthetic High Organic Water contained 10 mg/L fulvic acid (extracted and crystallized from untreated surface water) fortified into reagent water.

Note: These data were collected using the equipment and conditions listed in Table 1.

TABLE 3. STABILITY STUDY RESULTS FOR PERCHLORATE IN VARIOUS MATRICES

A. Stability when stored in various sampling bottles - All stored at room temperature

Matrix	Bottle type	Unfortified Conc.(µg/L)	Fortified Conc.(µg/L)	Analyte % Recovery Day 0	Day 7	Day 14	Day 28	Day 35
Reagent Water	Clear Glass	<MRL[a]	25.0	108%	101%	88%	91%	109%
Reagent Water	Amber Glass	<MRL	25.0	108%	101%	91%	90%	107%
Reagent Water	Opaque HDPE Plastic	<MRL	25.0	108%	97%	92%	89%	107%
Reagent Water	Translucent HDPE Plastic	<MRL	25.0	108%	105%	93%	90%	108%

B. Stability in various matrices under different storage conditions -
All samples stored in HDPE, opaque sampling bottles and fortified with 25.0 ug/L perchlorate.

Matrix	Storage Condition	Unfortified Conc.(µg/L)	Matrix Cond.uS/cm	Analyte % Recovery Day 0	Day 7	Day 14	Day 28	Day 35
Reagent Water	Room Temp.	<MRL	< 1	104%	104%	95%	87%	107%
	Refrigerated	<MRL		104%	102%	94%	88%	107%
Treated Surface Water #1	Room Temp.	<MRL	520	109%	105%	101%	99%	111%
	Refrigerated	<MRL		109%	102%	100%	97%	108%
Treated Surface Water #2	Room Temp.	<MRL	510	107%	115%	102%	99%	113%
	Refrigerated	<MRL		107%	113%	103%	99%	111%
Untreated Surface Water #1	Room Temp.	<MRL	470	110%	115%	110%	106%	110%
	Refrigerated	<MRL		110%	114%	110%	105%	111%
Untreated Surface Water #2	Room Temp.	<MRL	700	105%	112%	110%	104%	107%
	Refrigerated	<MRL		105%	112%	111%	103%	107%
Untreated Surface Water #3	Room Temp.	<MRL	920	109%	109%	113%	104%	110%
	Refrigerated	<MRL		109%	105%	111%	103%	105%
Untreated Surface Water #4	Room Temp.	<MRL	930	107%	105%	110%	106%	105%
	Refrigerated	<MRL		107%	106%	107%	105%	106%
Ground Water #1	Room Temp.	<MRL	1900	110%	113%	103%	101%	107%
	Refrigerated	<MRL		110%	112%	105%	103%	107%

C. Stability of native perchlorate in matrices stored under different storage conditions -
All samples stored in HDPE, opaque sampling bottles.

Matrix	Storage Condition	Matrix Cond. uS/cm	Measured concentration, ug/L Day 0	Day 7	Day 14	Day 28	Day 35
Ground Water #2 (with native ClO_4^-)	Room Temp.	603	1090	1110	1080	990	1100
	Refrigerated		1090	1110	1080	1010	1110
Ground Water #3 (with native ClO_4^-)	Room Temp.	960	1010	1040	1010	950	1000
	Refrigerated		1010	1040	1020	940	1030
Ground Water #4 (with native ClO_4^-)	Room Temp.	750	439	450	427	407	434
	Refrigerated		439	441	427	400	434

(a) <MRL = analyte was not detected above the laboratory minimum reporting level (MRL) of 4.0 ug/L.
Note: Each data point represented the average from triplicate analysis.

TABLE 4. SINGLE LABORATORY RESULTS FOR THE DETERMINATION OF MCT - Determination on the AS16, AS11 and the AS5.

AS16 Studies - Perchlorate fortified at 25 ug/L								
Sample	Conductivity uS/cm	RT min.	Measured ClO4-, ug/L	%Rec	Area	Height	A/H ratio	$PD_{A/H}$
LFB	< 1	10.3	25.3	101%	20268	1151	17.6	0.00%
MA(50)[a]	540	10.3	26.0	104%	20799	1135	18.3	4.07%
MA(100)	932	10.3	26.3	105%	21060	1144	18.4	4.54%
MA(200)	1770	10.2	26.2	105%	20998	1112	18.9	7.24%
MA(400)	3570	10.2	25.2	101%	20170	1028	19.6	11.4%
MA(600)	5010 [b]	10.2	24.2	97%	19307	954	20.2	14.9%
MA(800)	6450	10.1	25.1	100%	20038	932	21.5	22.1%
MA(1000)	7820	10.2	24.3	97%	19400	878	22.1	25.5%

AS11[c] Studies - Perchlorate fortified at 25 ug/L								
Sample	Conductivity uS/cm	RT min.	Measured ClO4-, ug/L	%Rec	Area	Height	A/H ratio	$PD_{A/H}$
LFB	< 1	8.9	25.0	100%	25213	1591	15.8	0.00%
MA(50)[a]	540	8.9	25.2	101%	25445	1515	16.8	5.98%
MA(100)	932	9.0	25.0	100%	25192	1486	17.0	6.98%
MA(200)	1770 [b]	9.0	24.1	96%	24340	1384	17.6	11.0%
MA(400)	3570	9.0	23.6	94%	23855	1243	19.2	21.1%
MA(600)	5010	9.0	22.7	91%	22922	1101	20.8	31.4%
MA(800)	6450	8.9	19.9	80%	20243	870	23.3	46.8%
MA(1000)	7820	8.8	17.0	68%	17407	678	25.7	62.0%

AS5[d] Studies - Perchlorate fortified at 25 ug/L								
Sample	Conductivity uS/cm	RT min.	Measured ClO4-, ug/L	%Rec	Area	Height	A/H ratio	$PD_{A/H}$
LFB	< 1	9.7	22.75	91.0%	30348	1780	17.0	0.00%
MA(50)[a]	540	9.7	24.89	99.6%	33505	1751	19.1	12.2%
MA(100)	932	9.7	23.72	94.9%	31776	1721	18.5	8.30%
MA(200)	1770 [b]	9.7	22.99	92.0%	30704	1591	19.3	13.2%
MA(400)	3570	9.6	23.51	94.0%	31474	1478	21.3	24.9%
MA(600)	5010	9.6	23.84	95.4%	31948	1441	22.2	30.0%
MA(800)	6450	9.6	21.01	84.0%	27792	1214	22.9	34.3%
MA(1000)	7820	9.6	22.95	91.8%	30650	1183	25.9	52.0%

(a) "MA" indicates mixed common anion solution with each anion (chloride, sulfate and carbonate) included in the sample matrix at the parenthetical mg/L concentration for each anion.

(b) If the regression analysis is not performed on these data, 5010 uS/cm, 1770 uS/cm and 1770 uS/cm would be the default MCT for the AS16, AS11 and AS5, respectively, as described in Section 9.2.8.10. See Figure 5 for a graphical representation of this data, applying a regression analysis of $PD_{A/H}$ vs matrix conductivity for the AS16, AS11 and AS5.

(c) AS11 conditions: See reference #2 and #3.

(d) AS5 conditions: See reference #1.

TABLE 5. INITIAL DEMONSTRATION OF CAPABILITY QC REQUIREMENTS.
Requirements prior to beginning any analysis batch

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.2 9.3.1	Initial Demonstration of Low System Background	Analyze a method blank (LRB) and determine that all target analytes are below ½ of the proposed MRL prior to performing the IDC.	The LRB concentration must be ≤½ of the proposed MRL.
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Analyze 7 replicate LFBs fortified with perchlorate at 25 ug/L. Calculate the mean recovered concentration ($C_{\bar{x}}$) See Equation in Section 9.2.3.	The $C_{\bar{x}}$ must be ± 10% of true value.
Sect. 9.2.4	Initial Demonstration of Precision (IDP)	Calculate percent relative standard deviation (%RSD)of IDA replicates. See Equation in Section 9.2.4.	The %RSD must be ≤10%
Sect. 9.2.5	Quality Control Sample (QCS)	Initially, upon reestablishing calibration or at least quarterly analyze a QCS from an external/second source.	The QCS must be ± 10% of the true value.
Sect. 9.2.6	Method Detection Limit (MDL) Determination	Select a fortifying level at 3-5 times the estimated instrument detection limit. Analyze 7 replicate LFBs over multiple days and calculate MDL using equation in Section 9.2.6 - do not subtract blank	
Sect. 9.2.7	Minimum Reporting Level (MRL)	An MRL should be established for perchlorate during the IDC.	The low CAL standard can be lower than the MRL, but the MRL MUST be no lower than the low CAL standard
Sect. 9.2.8	Matrix Conductivity Threshold (MCT)	Prepare a series of LFB samples, each containing a suggested perchlorate concentration of 25 ug/L, at sequentially increasing fortified levels of common anions. Measure sample conductance and analyze each, calculate average A/H ratios and $PD_{A/H}$ (using equation in Section 9.2.8.8). Perform linear regression to calculate MCT (using equation in Section 9.2.8.9) or follow step outlined in Section 9.2.8.10.	MCT, based upon linear regression, is point where $PD_{A/H}$ equals 20%. Alternatively, the MCT is set at the highest measured conductance observed in the last fortified MCT sample to yield a $PD_{A/H}$ value below 20%.
Sect. 9.2.8.11	MRL verification	Verify the MRL in a solution prepared at the MCT.	Prepared within ±10% of the MCT. Perchlorate recovery must be 70- 130% of the MRL.

TABLE 6. QUALITY CONTROL REQUIREMENTS (SUMMARY).
Requirements specific for each analysis batch

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.3	Sample Holding Time / Preservation / Storage	Perchlorate 28 days No Preservation technique required. Room Temperature adequate for shipping and storage.	Holding time must not be exceeded.
Sect. 10.2	Initial Calibration	Generate calibration curve. At least 5 calibration standards are recommended.	MRL MUST be no lower than the lowest calibration standard
Sect. 9.3.2	Instrument Performance Check (IPC)	Designed to verify Matrix Conductivity Threshold (MCT). Prepare mixed common anion solution at the MCT (prepared consistent with procedures in Section 9.2.8). Confirm the sample's conductance and analyze at the beginning of each analysis batch.	Prepared within ±10% of the MCT. IPC solution conductance verified to within ± 10% of original measured value (when originally prepared) $PD_{A/H}$, (when compared to the A/H_{LFB}) must be < 25%. Perchlorate quantitated between 80 -120% of fortified level. <5% shift in perchlorate retention time.
Sect. 10.3.1	Initial Calibration Check (ICCS)	With each analysis batch, initially verify calibration at the MRL by analyzing an initial low-level continuing calibration check standard (ICCS).	Recovery must be 75-125% of the true value.
Sect. 10.3.2	Continuing Calibration (CCCS) and End Calibration Checks (ECCS)	Alternately analyze separate mid and high level CCCS/ECCS after every 10 samples and after the last sample in an analysis batch.	Recoveries must fall between 85 - 115%
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	Include LRB with every analysis batch (up to 20 samples) Analyze prior to analyzing field samples	Perchlorate must be ≤ ½ MRL

TABLE 6. QUALITY CONTROL REQUIREMENTS (SUMMARY CONTINUED).
Requirements specific for each analysis batch

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.1.1	PRETREATED Laboratory Reagent Blank (LRB)	REQUIRED in any analysis batch which includes samples which have exceeded the MCT and have been pretreated in any way to reduce the common anion levels.	Perchlorate must be ≤ ½ MRL
Sect. 9.3.3	Labortary Fortified Blank (LFB)	Laboratory must analyze LFB in each analysis batch following the ICCS. Calculate %REC prior to analyzing samples. The concentration selected for the LFB in subsequent analysis batches should be varied throughout the calibration range.	Recovery for LFB MUST be 85 - 115% prior to analyzing samples. Sample results from batches that fail LFB are invalid.
Sect. 9.3.1.1	PRETREATED Laboratory Reagent Blank (LRB)	REQUIRED in any analysis batch which includes samples which have exceeded the MCT and have been pretreated in any way to reduce the common anion levels. Fortification must be made prior to pretreatment.	Recovery for pretreated LFB MUST be 85 - 115% prior to analyzing samples. Sample results from batches that fail a pretreated LFB are invalid.
Sect. 9.4.1	Laboratory Fortified Sample Matrix (LFM)	Must add known amount of perchlorate to a minimum of 5% of field samples or at least one within each analysis batch. LFM must be fortified above the native level and at no greater than 10 x the highest field sample concentration. Calculate target analyte recovery using formula (Sect. 9.4.1.3).	Recovery must be 80 - 120% If fortified sample fails the recovery criteria, label both as suspect/matrix.
Sect. 11.1.4.6	SPECIAL LFM for matrices requiring pretreatment	When a sample exceeds the MCT and pretreatment is employed to reduce the common anion levels, an additional LFM must be prepared from this matrix and subsequently pretreated exactly as the unfortified matrix.	Same criteria, recoveries must be 80 -120%.
Sect. 9.4.2	Field or Laboratory Duplicates or LFM Duplicate	Analyze either a field, laboratory or LFM duplicate for a minimum of 5% of field samples or at least one within each analysis batch. Calculate the relative percent difference (RPD) using formula in Section 9.4.2.1.	RPD must be ± 15%.
Sect. 6.1.2.2	ALTERNATE IC analytical column performance criteria	If a laboratory chooses an alternate analytical column for this analysis, it must be hydrophilic and pass the criteria for Peak Gaussian Factor (PGF) using equation (Sect. 6.1.2.2).	PGF must fall between 0.80 and 1.15.

TABLE 7. EXAMPLE SAMPLE ANALYSIS BATCH WITH QUALITY CONTROL REQUIREMENTS

Injection #	Sample Description	Acceptance Criteria
1	Instrument Performance Check Standard at MCT	$PD_{A/H}$ for IPC < 25%
2	Laboratory Reagent Blank (LRB)	≤ ½ MRL
3	ICCS at the MRL (4.0 µg/L)	3.00 to 5.00 µg/L
4	Laboratory Fortified Blank (LFB)	Recovery of 85 - 115%
5	Sample 1	normal analysis
6	Sample 1 - Laboratory Duplicate (LD) [a]	± 15 % RPD
7	Sample 2	normal analysis
8	Sample 2 - Laboratory Fortified Matrix (LFM) [a]	Recovery of 80 - 120%
9	Sample 3	normal analysis
10	Sample 4	normal analysis
11	Sample 5	normal analysis
12	Sample 6	normal analysis
13	Sample 7	normal analysis
14	Sample 8	normal analysis
15	Sample 9	normal analysis
16	Sample 10	normal analysis
17	CCCS (25.0 ug/L)	21.3 to 28.8 ug/L
18	Sample 11 (failed MCT, matrix conductance = 8000 uS/cm) - Analyzed diluted (Section 11.1.3) by factor of 2 or by 50% with reagent water (diluted matrix conductance = 3800 uS/cm).	MRL increases from 4 to 8 ug/L, noted in analysis report - sample found to contain 50 ug/L (measured at 25 ug/L in diluted sample)
19	Sample 12	normal analysis
20	Sample 13	normal analysis

CONTINUED TO NEXT PAGE

Injection #	Sample Description	Acceptance Criteria
21	Sample 14 (failed MCT, matrix conductance=15000 uS/cm) Analyzed diluted (Section 11.1.3) by a factor of 3 or by 33% with reagent water (Diluted matrix conductance = 4600 uS/cm)	MRL increases from 4 to 12 ug/L, noted in analysis report - No perchlorate > 12ug/L measured - project required monitoring to MRL - sample pretreatment is therefore required
22	Ba/Ag/H Pretreated LRB (Section 9.3.1.1)	≤ ½ MRL
23	Ba/Ag/H Pretreated LFB (Section 9.3.3.1)	Recovery of 85 - 115%
24	Sample 14 - Ba/Ag/H pretreated (Section 11.1.4), following pretreatment the matrix conductance = 230 uS/cm.	normal pretreated analysis perchlorate < MRL of 4.0 ug/L
25	Sample 14 [b] - pretreated LFM (Section 11.1.4.6)	Recovery of 80 - 120%
26	Sample 15	normal analysis
27	Sample 16	normal analysis
28	Sample 17	normal analysis
29	Sample 18	normal analysis
30	Sample 19[b]	normal analysis
31	ECCS (100 µg/L)	85.0 to 125 µg/L

[a] If no analytes are observed above the MRL for a sample, an alternate sample which contains reportable values should be selected as the laboratory duplicate. Alternately, the LFM can be selected and reanalyzed as the laboratory duplicate ensuring the collection of QC data for precision.

[b] Sample #19 (inj #30) was the final field sample permitted in this batch but 20 total field samples were analyzed. Sample #14 (inj #21 and #24) was analyzed both initially as a diluted sample and subsequently as a pretreated sample, therefore it accounted for two "field sample analyses" toward the maximum of twenty in an analysis batch (Section 3.1).

Note: Sample #11 and #14 illustrate examples of proper ways to handle sample matrices which exceed the MCT.

FIGURE 1. CHROMATOGRAM OF LOW LEVEL PERCHLORATE (4.0 ug/L) IN REAGENT WATER
(Conditions as indicated in Table 1)

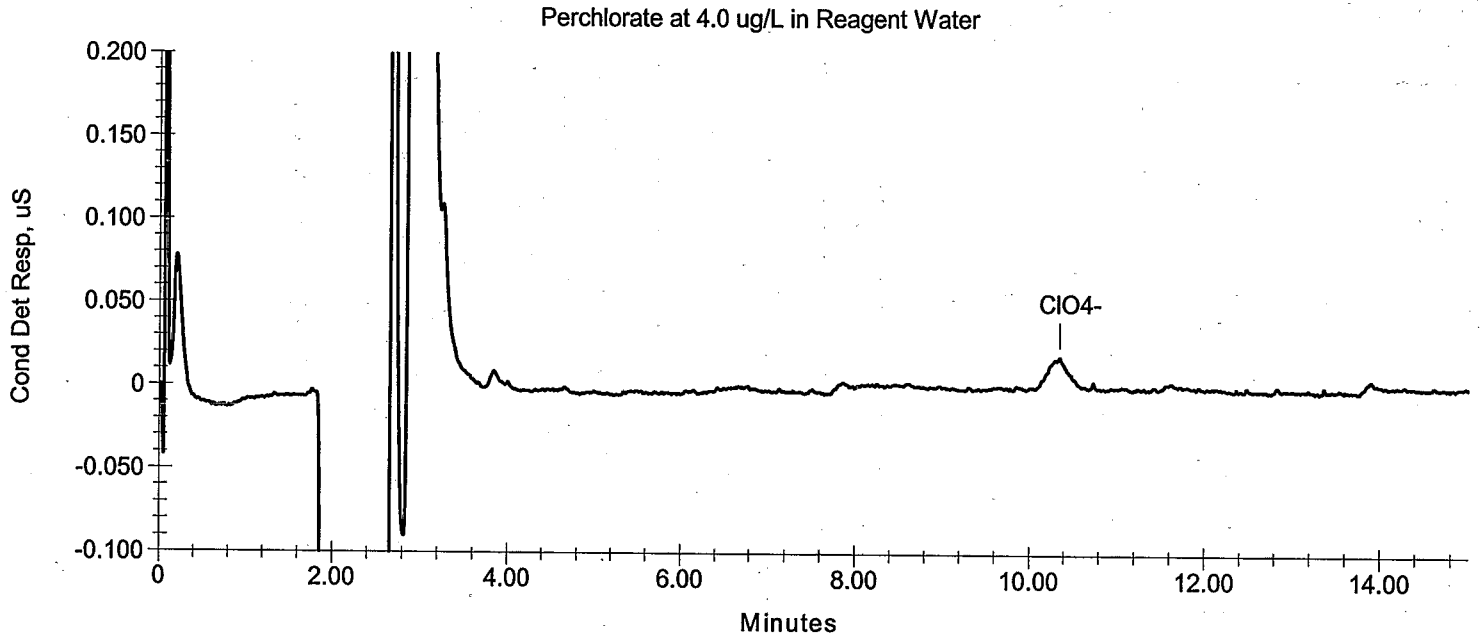


FIGURE 2. CHROMATOGRAM OF 25 ug/L PERCHLORATE IN REAGENT WATER
(Conditions as indicated in Table 1)

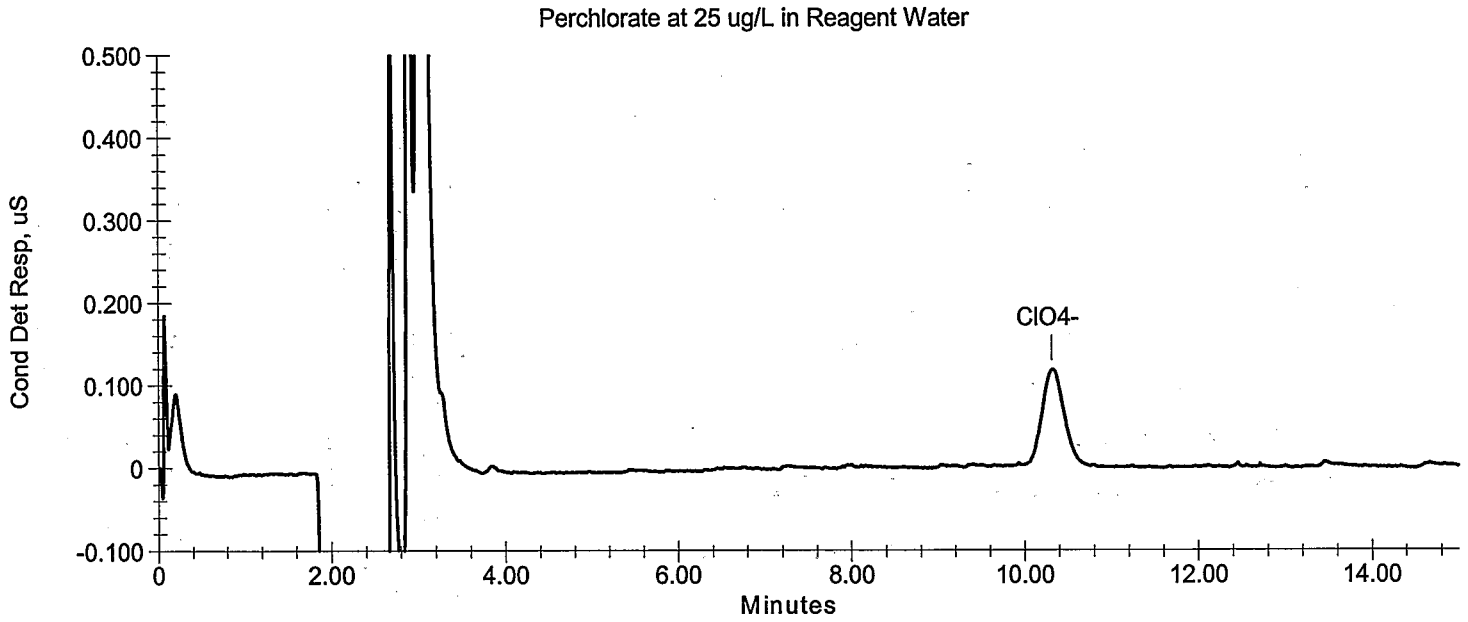


FIGURE 3. STACKED CHROMATOGRAMS INDICATING INFLUENCE OF HIGH CONCENTRATIONS OF COMMON ANIONS ON LOW CONCENTRATION MEASUREMENT OF PERCHLORATE AT 4.0 ug/L (Conditions as indicated in Table 1)

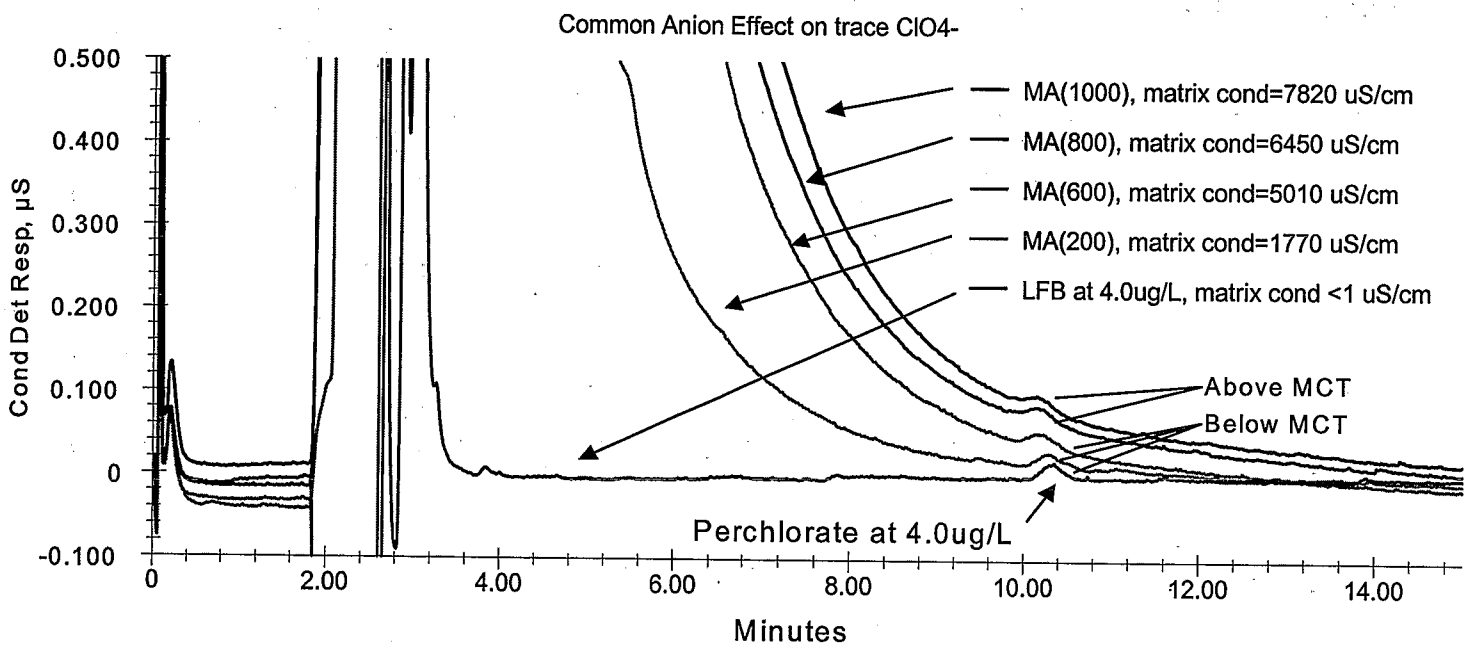


FIGURE 4. STACKED CHROMATOGRAMS INDICATING INFLUENCE OF HIGH CONCENTRATIONS OF COMMON ANIONS ON PERCHLORATE AT 25 ug/L DURING THE MCT DETERMINATION (Conditions as indicated in Table 1)

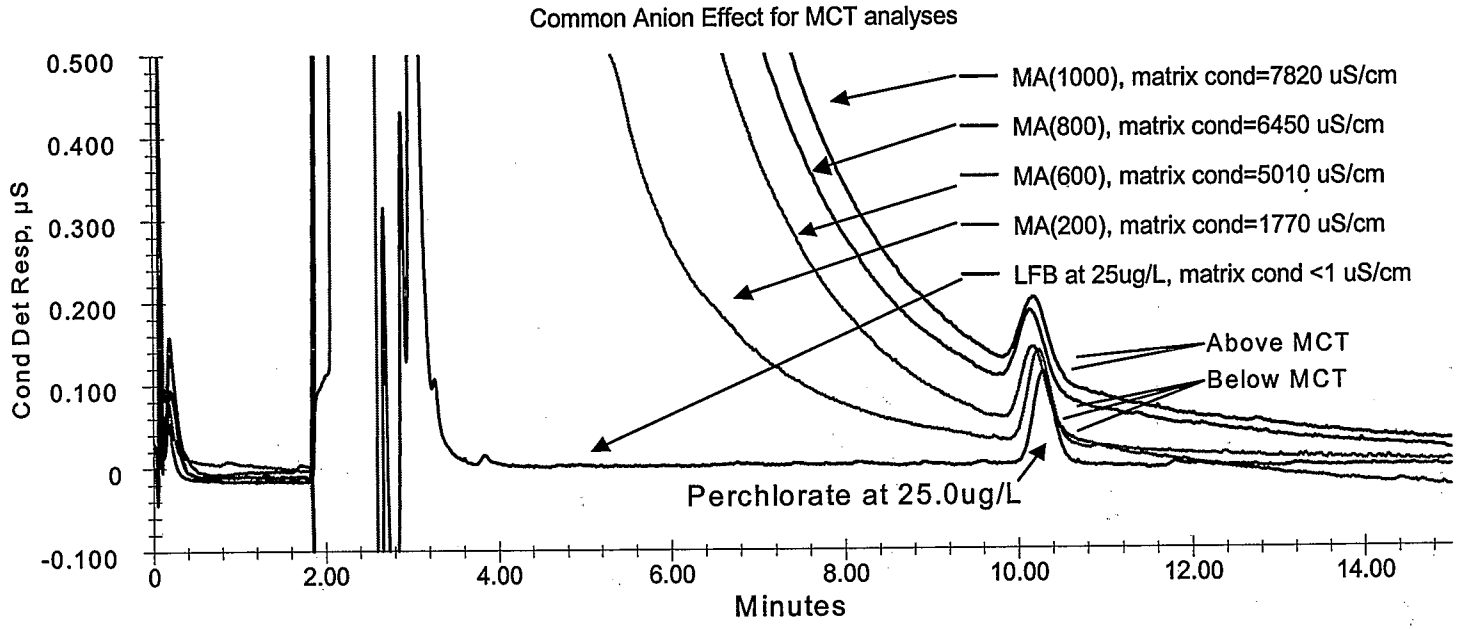
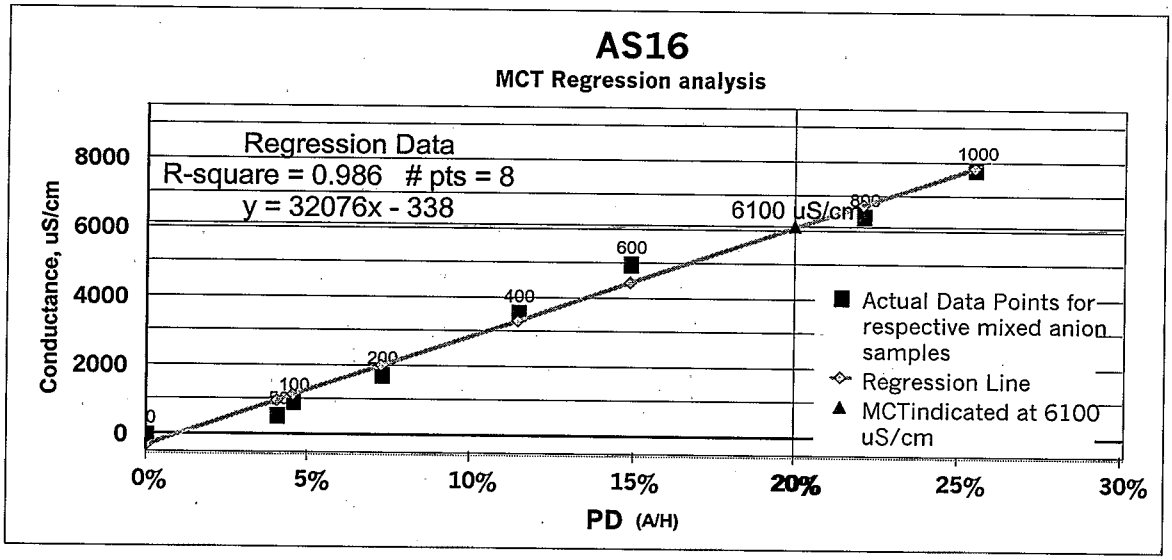
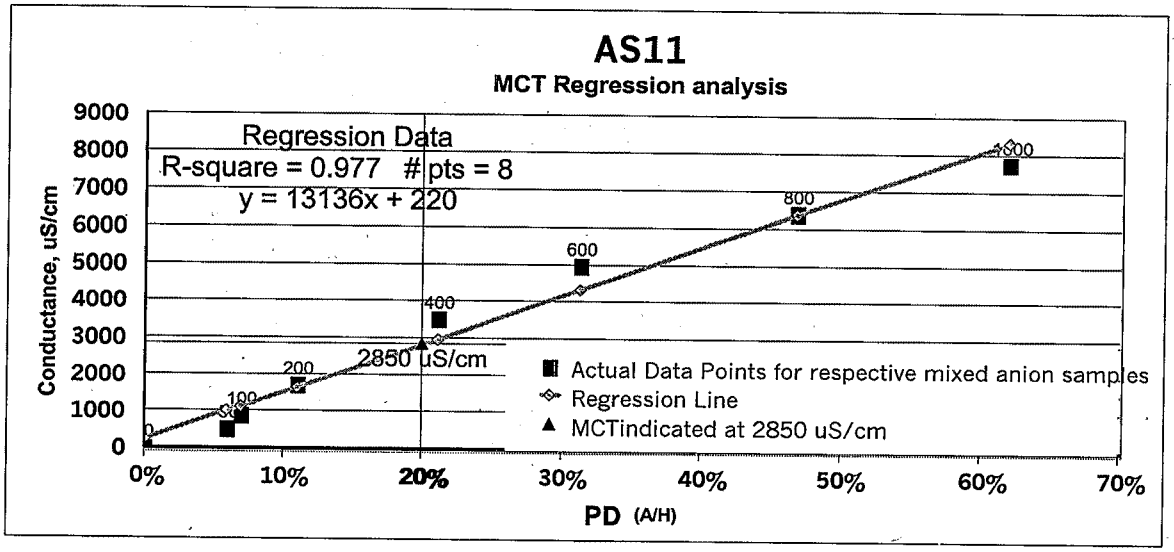
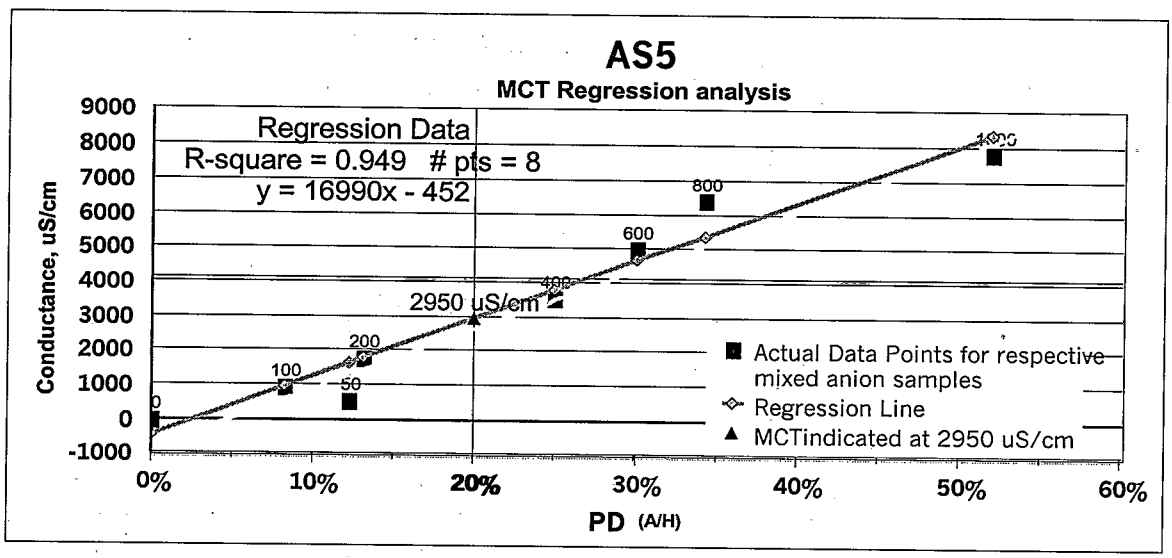


FIGURE 5. REGRESSION ANALYSIS OF THE MCT DETERMINATION DATA







METHOD 317.0 DETERMINATION OF INORGANIC OXYHALIDE DISINFECTION BY-PRODUCTS IN DRINKING WATER USING ION CHROMATOGRAPHY WITH THE ADDITION OF A POSTCOLUMN REAGENT FOR TRACE BROMATE ANALYSIS

Revision 1.0

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METHOD 317.0

DETERMINATION OF INORGANIC OXYHALIDE DISINFECTION BY-PRODUCTS IN DRINKING WATER USING ION CHROMATOGRAPHY WITH THE ADDITION OF A POSTCOLUMN REAGENT FOR TRACE BROMATE ANALYSIS

1. SCOPE AND APPLICATION

1.1 This method covers the determination of inorganic oxyhalide disinfection by-product anions in reagent water, surface water, ground water, and finished drinking water. In addition, bromide can be accurately determined in source or raw water and it has been included due to its critical role as a disinfection by-product precursor. Bromide concentration in finished water can differ significantly between preserved and unpreserved samples and should not be attempted due to numerous variables which can influence the concentration. Since this method, prior to the addition of the postcolumn reagent (PCR), employs the same hardware as EPA Method 300.1[1], the analysis of the common anions (using EPA Method 300.1, Part A[1]) can be performed using this instrument setup with the postcolumn hardware attached but "off-line" and with the appropriate smaller sample loop.

Inorganic Disinfection By-products by Conductivity Detection

Bromate (report values > 15.0 ug/L)	Chlorite
Bromide (source or raw water only)	Chlorate

Inorganic Disinfection By-product by Postcolumn UV/VIS Absorbance Detection

Bromate (report values > Minimum Reporting Limit (MRL) to 15.0 ug/L)

1.2 The single laboratory reagent water Method Detection Limits (MDL, defined in Section 3.14) for the above analytes are listed in Table 1. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample and the specific instrumentation employed.

1.2.1 In order to achieve comparable detection limits on the conductivity detector, an ion chromatographic system must utilize suppressed conductivity detection, be properly maintained and must be capable of yielding a baseline with no more than 5 nanosiemen (nS) noise/drift per minute of monitored response over the background conductivity.

1.2.2 In order to achieve acceptable detection limits on the postcolumn absorbance detector, the postcolumn reagent must be delivered pneumatically and some form of software signal filtering or smoothing of the absorbance signal from the absorbance detector must be incorporated.[2]

1.3 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.4 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Section 9.4.1.

1.5 Users of the method data should state the data quality objectives prior to analysis. Users of the method must demonstrate the ability to generate acceptable results with this method, using the procedures described in Section 9.0.

2. SUMMARY OF METHOD

2.1 A volume of sample, approximately 225 µL (see Note), is introduced into an ion chromatograph (IC). The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, conductivity detector, a postcolumn reagent delivery system (pneumatically controlled), a heated postcolumn reaction coil, and a ultraviolet/visible (UV/VIS) absorbance detector.[2,3]

NOTE: A 225 uL sample loop can be made using approximately 111 cm (44 inches) of 0.02 inch i.d. PEEK tubing. Larger injection loops may be employed.[4] The volume should be verified to be within 5% by weighing the sample loop empty, filling the loop with deionized water and re-weighing the loop assuming the density of water is 1 mg/uL.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A sequence of samples, which are analyzed within a 30 hour period and include no more than 20 field samples. An analysis batch must also include all required QC samples, which do not contribute to the maximum field sample total of 20. The required QC samples include:

- Laboratory Reagent Blank (LRB)
- Initial Calibration Check Standard (ICCS)
- Laboratory Fortified Blank (LFB)
- Continuing Calibration Check Standard (CCCS), when the batch contains more than 10 field samples
- End Calibration Check Standard (ECCS)
- Laboratory Fortified Matrix (LFM)
- Either a Field Duplicate (FD), a Laboratory Duplicate (LD) or a duplicate of the LFM

3.2 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution(s) or stock standard solutions and the surrogate analyte. The CAL

solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 INITIAL CALIBRATION STANDARDS – A series of CAL solutions (either individual or combined target analytes) used to initially establish instrument calibration and develop calibration curves for individual target anions (Section 10.2).

3.4 INITIAL CALIBRATION CHECK STANDARD (ICCS) – A CAL solution, (either individual or combined target analytes) which is analyzed initially, prior to any field sample analyses, which verifies previously established calibration curves. The concentration for the initial calibration check standard MUST be at or below the MRL (Section 3.15) level which is also the level of the lowest calibration standard (Section 10.3.1).

3.5 CONTINUING CALIBRATION CHECK STANDARDS (CCCS) – A CAL solution (either individual or combined target analytes) which is analyzed after every tenth field sample analyses which verifies the previously established calibration curves and confirms accurate analyte quantitation for the previous ten field samples analyzed. The concentration for the continuing calibration check standards should be either at a middle calibration level or at the highest calibration level (Section 10.3.2).

3.6 END CALIBRATION CHECK STANDARD (ECCS) – A CAL solution (either individual or combined target analytes) which is analyzed after the last field sample analysis which verifies the previously established calibration curves and confirms accurate analyte quantitation for all field samples analyzed since the last continuing calibration check. The end calibration check standard should be either the middle or high level continuing calibration check standard (Section 10.3.2).

3.7 FIELD DUPLICATES (FD) – Two separate field samples collected at the same time and place under identical circumstances and handled exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.8 INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) – A solution of one or more method analytes, surrogates, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.9 LABORATORY DUPLICATE (LD) – Two sample aliquots, taken in the laboratory from a single field sample bottle, and analyzed separately with identical procedures. Analysis of the initial sample (I_C) and the duplicate sample [(D_C) Section 9.4.3.1] indicate precision associated specifically with the laboratory procedures by removing variation contributed from sample collection, preservation and storage procedures.

3.10 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.11 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental field sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the field sample matrix contributes bias to the analytical result. The background concentrations of the analytes in the field sample matrix must be determined in a separate, unfortified aliquot and the measured values in the LFM corrected for background concentrations.

3.12 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is handled exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.13 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.14 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.[5]

3.15 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard and can only be used if acceptable quality control criteria for the ICCS are met.

3.16 PROFICIENCY TESTING (PT) or PERFORMANCE EVALUATION (PE) SAMPLE – A certified solution of method analytes whose concentration is unknown to the analyst. Frequently, an aliquot of this solution is added to a known volume of reagent water and analyzed with procedures used for samples. Often, results of these analyses are used as part of a laboratory certification program to objectively determine the capabilities of a laboratory to achieve high quality results.

3.17 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.18 SURROGATE ANALYTE – An analyte added to all samples, standards, blanks, etc., which is unlikely to be found at a significant concentration, and which is added directly in a known amount before any sample processing procedures are conducted (except in the procedure for the removal of chlorite as described is Section 11.1.4). It is measured with the same procedures used to measure other sample components. The purpose of the surrogate analyte is to monitor method performance with each sample.

3.19 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 Interferences can be divided into three different categories: direct chromatographic coelution, where an analyte response is observed at very nearly the same retention time as the target anion; concentration dependant coelution, which is observed when the response of higher than typical concentrations of the neighboring peak overlap into the retention window of the target anion; and, ionic character displacement, where retention times may significantly shift due to the influence of high ionic strength matrices (high mineral content or hardness) overloading the exchange sites on the column and significantly shortening target analyte's retention times.

4.1.1 A direct chromatographic coelution may be solved by changing columns, eluent strength, modifying the eluent with organic solvents (if compatible with IC columns), changing the detection systems, or selective removal of the interference with pretreatment. Sample dilution will have little to no effect. The analyst must verify that these changes do not induce any negative affects on method performance by repeating and passing all the QC criteria as described in Section 9.

4.1.2 Sample dilution may resolve some of the difficulties if the interference is the result of either concentration dependant coelution or ionic character displacement, but it must be clarified that sample dilution will alter your Minimum Reporting Limit (MRL) by a proportion equivalent to that of the dilution. Therefore, careful consideration of project objectives should be given prior to performing such a dilution. An alternative to sample dilution, may be dilution of the eluent as outlined in Section 11.2.6.

4.1.3 Pretreatment cartridges can be effective as a means to eliminate certain matrix interferences. With any proposed pretreatment, the analyst must verify that target analyte(s) are not affected by monitoring recovery after pretreatment. With advances in analytical separator column technology which employ higher capacity anion exchange resins, the need for these cartridges has been greatly reduced.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in an ion chromatogram. These interferences can lead to false positive results for target analytes as well as reduced detection limits as a consequence of elevated baseline noise.

4.3 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems.

4.4 Close attention should be given to the potential for carry over peaks from one analysis which will effect the proper detection of analytes of interest in a second or subsequent analysis. Normally, in this analysis, the elution of sulfate (retention time of 17.5 min.) indicates the end of a chromatographic run, but, in the ozonated and chlorine dioxide matrices, which were included as part of the single operator accuracy and bias study, a small response (200 nS baseline rise) was observed for a very late eluting unknown peak following the response for sulfate. Consequently, a run time of 25 minutes is recommended to allow for the proper elution of any potentially interferant late peaks. It is the responsibility of the user to confirm that no late eluting peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

4.5 Any residual chlorine dioxide present in the sample will result in the formation of additional chlorite prior to analysis. If residual chlorine dioxide is suspected in the sample, the sample must be purged with an inert gas (helium, argon or nitrogen) for approximately five minutes. This sparging must be conducted prior to ethylenediamine preservation and at the time of sample collection.

4.6 The presence of chlorite can interfere with the quantitation of low concentrations of bromate on the postcolumn UV/VIS absorbance detector. In order to accurately quantify bromate concentrations in the range 0.5 - 15.0 µg/L in this postcolumn system, the excess chlorite must be removed prior to analysis as outlined in Section 11.1.4.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established although the postcolumn reagent o-dianisidine, is listed as a potential human carcinogen. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 Each laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets (MSDS) should be made available to all personnel involved in the chemical analysis.

The preparation of a formal safety plan is also advisable. Additional references on laboratory safety are available.[6-9]

5.3 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1 Sulfuric acid – used to prepared a 25 mN sulfuric acid regenerant solution for chemical suppression using a Dionex Anion Micro Membrane Suppressor (AMMS) and for pretreatment for chlorite removal (Section 11.1.4)

5.3.2 Nitric acid – used to prepare the postcolumn reagent.

5.3.3 *o*-dianisidine [3, 3'- dimethoxybenzidine dihydrochloride (ODA)] – used as the postcolumn reagent.

6. EQUIPMENT AND SUPPLIES

6.1 Ion chromatograph – Analytical system complete with ion chromatographic pump and all required accessories including syringes, analytical columns, compressed gasses, suppressor, conductivity detector, mixing "tee", postcolumn reagent delivery system, reaction coil, reaction coil heater, UV/VIS absorbance detector (Figure 1) and a PC based data acquisition and control system.

NOTE: Because of its acidic nature and high salt content, the PCR MUST be flushed from the reaction coil upon completion of the final analysis and prevented from draining through the reaction coil by gravity once the system is shut down. This can be accomplished either manually or by incorporating a column switching valve in combination with a flush and close method in the schedule.

6.1.1 Anion guard column – Dionex AG9-HC 4 mm (P/N 51791), or equivalent. This column functions as a protector of the separator column. If omitted from the system the retention times will be shorter.

6.1.2 Anion separator column – Dionex AS9-HC column, 4 mm (P/N 51786), or equivalent (see Note). The AS9-HC, 4 mm column using the conditions outlined in Table 1 produced the separations shown in Figures 2 and 3.

NOTE: The use of 2 mm columns is not recommended. A 50 uL sample loop would be required with the 2 mm columns. This reduced injection volume would decrease the "on-column" bromate and negatively affect PCR reactivity and the subsequent absorbance response. As well, the 2 mm columns require a flow rate approximately 4 times less than the 4 mm columns. At the lower flow rates, band broading may become an issue and it would be difficult, if not impossible, to accurately maintain the appropriate reduced flow rate for the PCR.

6.1.3 Anion suppressor device – The data presented in this method were generated using a Dionex Anion Self Regenerating Suppressor (4 mm ASRS, P/N 46081). An equivalent suppressor device may be utilized provided comparable conductivity detection limits are achieved and adequate baseline stability is attained as measured by a combined baseline drift/noise of no more than 5 nS per minute over the background conductivity. The suppressor must be able to withstand approximately 80 - 120 psi back pressure which results from connecting the postcolumn hardware to the eluent out side of the suppressor.

6.1.3.1 The ASRS was set to perform electrolytic suppression at a current setting of 100 mA using the external water mode. Insufficient baseline stability was observed on the conductivity detector using the ASRS in recycle mode.

6.1.3.2 This method was developed as a multiple component procedure employing both suppressed conductivity and postcolumn UV/VIS absorbance detectors in series. If a laboratory is exclusively interested in monitoring trace bromate using the PCR and the UV/VIS absorbance detector, the suppressor may not be required. The performance data presented within this method for the PCR and UV/VIS absorbance detector, is based upon a suppressed mobile phase system. A laboratory must generate comparable data as a result of a complete IDC (Section 9.2) in order to demonstrate comparability of a non suppressed system.

6.1.4 Detector – Conductivity cell (Dionex CD20, or equivalent) capable of providing data as required in Section 9.2.

6.1.5 Detector – Absorbance detector (Dionex AD20 or equivalent with 10 mm cell pathlength, equipped with a tungsten source bulb, or equivalent and capable of measuring absorbance at 450 nm) capable of providing data as required in Section 9.2.

6.1.6 Postcolumn reagent delivery system (Dionex PC-10, or equivalent), pneumatically delivers the postcolumn reagent to mixing tee. The pressure settings will need to be established on an individual basis for each specific instrument configuration and at a level which yields the prescribed PCR flow rates.

6.1.7 Reaction Coil, 500 uL internal volume, knitted, potted or configured to fit securely in the postcolumn reaction coil heater. (Dionex P/N 39349, or equivalent).

6.1.8 Postcolumn Reaction Coil Heater, capable of maintaining 60°C. (Dionex PCH-2, or equivalent).

6.2 Data System – The Dionex Peaknet Data Chromatography Software was used to generate all the data in the attached tables. Other computer based data systems may achieve approximately the same MDLs but the user must demonstrate this by the procedure outlined in Section 9.2.

6.3 Analytical balance – Used to accurately weigh target analyte salts for stock standard preparation (±0.1 mg sensitivity).

6.4 Top loading balance – Used to accurately weigh reagents to prepare eluents (±10 mg sensitivity).

6.5 Weigh boats – Plastic, disposable - for weighing eluent reagents.

6.6 Syringes – Plastic, disposable, 10 mL - used during sample preparation.

6.7 Pipets – Pasteur, plastic or glass, disposable, graduated, 5 mL and 10 mL.

6.8 Bottles – High density polyethylene (HDPE), opaque or glass, amber, 30 mL, 125 mL, 250 mL, used for sample collection and storage of calibration solutions. Opaque or amber bottles are required due to the photoreactivity of the chlorite anion.

6.9 Micro beakers – Plastic, disposable - used during sample preparation.

6.10 Particulate filters – Gelman ion chromatography Acrodisc 0.45 micron (PN 4485) syringe filters or equivalent. These cartridges are used to remove particulates and $[Fe(OH)_{3(s)}]$ which is formed during the oxidation-reduction reaction between Fe (II) and ClO_2^-.

6.11 Hydrogen cartridges – Dionex OnGuard-H cartridges (PN 039596) or equivalent. These cartridges are conditioned according to the manufacturer's directions and are used to protect the analytical column and the suppressor membrane by removing excess ferrous iron [Fe (II)]. The ferrous iron is added to field samples to reduce chlorite levels prior to analysis of chlorine dioxide disinfected water samples.

7. REAGENTS AND STANDARDS

7.1 Reagent water – Distilled or deionized water 18 M Ω or better, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.2 Eluent solution – Sodium carbonate (CASRN 497-19-8) 9.0 mM. Dissolve 1.91 g sodium carbonate (Na_2CO_3) in reagent water and dilute to 2 L.

7.2.1 This eluent solution must be purged for 10 minutes with helium prior to use to remove dissolved gases which may form micro bubbles in the IC compromising system performance and adversely effecting the integrity of the data. Alternatively, an in-line degas apparatus may be employed.

7.3 Stock standard solutions, 1000 mg/L (1 mg/mL) – Stock standard solutions may be purchased as certified solutions or prepared from ACS reagent grade, potassium or sodium salts as listed below, for most analytes. Chlorite requires careful consideration as outlined below in Section 7.3.4.1.

7.3.1 Bromide (Br^-) 1000 mg/L – Dissolve 0.1288 g sodium bromide (NaBr, CASRN 7647-15-6) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.2 Bromate (BrO_3^-) 1000 mg/L – Dissolve 0.1180 g of sodium bromate ($NaBrO_3$, CASRN 7789-38-0) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.3 Chlorate (ClO_3^-) 1000 mg/L – Dissolve 0.1275 g of sodium chlorate ($NaClO_3$, CASRN 7775-09-9) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.4 Chlorite (ClO_2^-) 1000 mg/L – If the amperometric titration of the technical grade sodium chlorite ($NaClO_2$), specified in 7.3.4.1, had indicated the purity of the salt to be 80.0 % $NaClO_2$, the analyst would dissolve 0.1676 g of sodium chlorite ($NaClO_2$, CASRN 7758-19-2) in reagent water and dilute to 100 mL in a volumetric flask.

7.3.4.1 High purity sodium chlorite ($NaClO_2$) is not currently commercially available due to its potential explosive instability. Recrystallization of the technical grade (approx. 80%) can be performed but it is labor intensive and time consuming. The simplest approach is to determine the exact purity of the $NaClO_2$ using the iodometric titration procedure.[10] Following titration, an individual component standard of chlorite must be analyzed to determine if there is any significant contamination (greater than 1% of the chlorite weight) from chlorate, bromate or bromide (as other method target anions) in the technical grade chlorite standard.

NOTE: Stability of standards – Stock standards (Section 7.3) for most anions are stable for at least 6 months when refrigerated at <6°C. The chlorite standard is only stable for two weeks when stored refrigerated at <6°C and protected from light. Dilute working standards should be prepared monthly, except those that contain chlorite, which must be prepared every two weeks or sooner if signs of degradation are indicated by repeated QC failure.

7.4 Ethylenediamine (EDA) preservation solution, 100 mg/mL – Dilute 2.8 mL of ethylenediamine (99%) (CASRN 107-15-3) to 25 mL with reagent water. Prepare fresh monthly.

7.5 Surrogate Solution, 0.50 mg/mL dichloroacetate (DCA) – Prepare by dissolving 0.065 g dichloroacetic acid, potassium salt (Cl_2CHCO_2K, CASRN 19559-59-2) in reagent water and diluting to 100 mL in a volumetric flask.

7.5.1 Dichloroacetate is potentially present in treated drinking waters as the acetate of the organic disinfection by product, dichloroacetic acid (DCAA). Typical concentrations of DCAA rarely exceed 50 µg/L, which, for this worst case example, would represent only a five percent increase in the observed response over the fortified concentration of 1.00 mg/L. Consequently, the criteria for acceptable recovery (90% to 115%) for the surrogate is weighted to 115% to allow for this potential background.

7.5.2 Prepare this solution fresh every 3 months or sooner if signs of degradation are indicated by the repeated failure of the surrogate QC criteria.

7.5.3 If the analyst is exclusively interested in monitoring trace bromate using the PCR and the UV/VIS absorbance detector, the surrogate may be omitted since it only yields a signal on the conductivity detector. If the surrogate is removed, the laboratory must adhere to the alternate QC requirements found in Section 9.3.3.3 in order to monitor and demonstrate proper instrument performance.

7.6 Postcolumn reagent – The postcolumn reagent is prepared by adding 40 mL of 70% redistilled nitric acid (purity as 99.999+%, Aldrich, Cat. No. 22,571-1, Milwaukee, WI, or equivalent) to approximately 300 mL reagent water in a well rinsed 500 mL volumetric flask (see Note) and adding 2.5 grams of ACS reagent grade KBr (Sigma, Cat. No. P-5912, St. Louis, MO, or equivalent). Two-hundred-and-fifty milligrams of purified grade o-dianisidine, dihydrochloride salt [(ODA), (Sigma, Cat. No. D-3252, or equivalent)] are dissolved, with stirring, in 100 mL methanol (Spectrophotometric grade, Sigma, Cat. No. M-3641, St. Louis MO, or equivalent). After dissolution, the o-dianisidine solution is added to the nitric acid/KBr solution and diluted to volume with reagent water. The reagent is stable for up to one month.

7.6.1 The purity of all reagents employed in the preparation of the postcolumn reagent is critical. Some commercial manufacturers/suppliers of laboratory chemicals sell inferior grades of o-dianisidine dihydrochloride. ONLY the purified grade of this reagent is acceptable. The purified ODA dihydrochloride salt is a white, fine crystalline powder.

NOTE: All glassware used to prepare the postcolumn reagent must be thoroughly rinsed with reagent water prior to use. A champagne or light amber

coloration of the PCR reagent may be evident when freshly prepared. Over several hours, this slight coloration will fade. Consequently, the PCR must be prepared in advance and allowed to sit until it is clear, for a minimum of 4 hours (preferably overnight) prior to use. Occasionally, no matter how well all the glassware used to prepare the postcolumn reagent is rinsed, a darkly colored solution (oxidized ODA) may result. These solutions MUST be discarded. For this reason, it is recommended that the PCR be made in a series of 500 mL lots with dedicated glassware. The clear solution should be filtered using a 0.45 micron membrane to remove particulates before use.

7.7 Ferrous iron [1000 mg/L Fe (II)] solution – Dissolve 0.124 g ferrous sulfate heptahydrate ($FeSO4.7H2O$, Sigma, F-7002) in approximately 15 mL reagent water containing 6 uL concentrated nitric acid and dilute to 25 mL with reagent water in a volumetric flask (final pH ~2). The Fe (II) solution must be prepared fresh every two days.

7.8 Sulfuric acid (0.5 N) – Dilute 1.4 mL of concentrated sulfuric acid (Fisher Scientific Certified ACS Plus, A 300-500) to 100 mL.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and laboratory fortified matrix analysis, if required, and minimize waste disposal.

8.2 Special sampling requirements and precautions for chlorite.

8.2.1 Sample bottles used for chlorite analysis must be opaque or amber to protect the sample from light.

8.2.2 When preparing the LFM, be aware that chlorite is an oxidant and may react with the natural organic matter in an untreated drinking water matrix as a result of oxidative demand. If untreated water is collected for chlorite analysis, and subsequently used for the LFM, EDA preservation will not control this demand and reduced chlorite recoveries may be observed.

8.3 Sample preservation and holding times for the anions are as follows:

Analyte	Preservation	Holding Time
Bromate	50 mg/L EDA, refrigerate at <6°C	28 days
Chlorate	50 mg/L EDA, refrigerate at <6°C	28 days
Chlorite	50 mg/L EDA, refrigerate at <6°C	14 days
Bromide (source/raw water only)	EDA permitted, refrigerate at <6°C	28 days

NOTE: Samples for chlorite analysis must arrive at the laboratory within 48 hours of collection and must be received at 10°C or less.

8.4 When collecting a field sample from a treatment plant employing chlorine dioxide, the field sample must be sparged with an inert gas (helium, argon, nitrogen) prior to addition of the EDA preservative at time of sample collection.

8.5 All four anions (bromate > 15.0 ug/L) can be analyzed by conductivity, in a sample matrix which has been preserved with EDA. Add a sufficient volume of the EDA preservation solution (Section 7.4) such that the final concentration is 50 mg/L in the sample. This would be equivalent to adding 0.5 mL of the EDA preservation solution to 1 L of sample.

8.6 Chlorite is susceptible to degradation both through catalytic reactions with dissolved iron salts and reactivity towards free chlorine which exists as hypochlorous acid/hypochlorite ion in most drinking water as a residual disinfectant.[11] EDA serves a dual purpose as a preservative for chlorite by chelating iron as well as any other catalytically destructive metal cations and removing hypochlorous acid/hypochlorite ion by forming an organochloramine. EDA preservation of chlorite also preserves the integrity of chlorate which can increase in unpreserved samples as a result of chlorite degradation. EDA also preserves the integrity of bromate concentrations by binding with hypobromous acid/hypobromite ion which is an intermediate formed as a by-product of the reaction of either ozone or hypochlorous acid/hypochlorite ion with bromide ion. If hypobromous acid/hypobromite ion is not removed from the matrix, further reactions may form bromate ion.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The requirements of this program consist of an initial demonstration of laboratory capability (IDC), and subsequent analysis in each analysis batch (Section 3.1) of a Laboratory Reagent Blank (LRB), Initial Calibration Check Standard (ICCS), Laboratory Fortified Blank (LFB), Instrument Performance Check Standard (IPC), Continuing Calibration Check Standards (CCCS), Laboratory Fortified Sample Matrix (LFM) and either a Field, Laboratory or LFM duplicate sample analysis. This section details the specific requirements for each of these QC parameters for both the conductivity and absorbance detectors used in this application. Although this method involves both conductivity and absorbance detection, the MDLs and MRLs may differ but the QC requirements and acceptance criteria are the same for both detectors. The QC criteria discussed in the following sections are summarized in Section 17, Tables 4 and 5. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF CAPABILITY

9.2.1 The Initial Demonstration of Capability (IDC) – This is used to characterize instrument and laboratory performance prior to performing analyses by this method. The QC requirements for the IDC discussed in the following section are summarized in Section 17, Table 4.

9.2.2 Initial demonstration of low system background – Section 9.3.1.

9.2.3 Initial Demonstration of Precision (IDP) – For the 4 conductivity detector analytes, prepare 7 replicate LFBs fortified at a recommended concentration of 20 ug/L. For the absorbance detector, prepare 7 replicate LFBs fortified at a recommended concentration of 2.0 ug/L bromate. The percent relative standard deviation (RSD) of the results must be less than 20%.

9.2.4 Initial Demonstration of Accuracy (IDA) – Using the data generated for Section 9.2.3, calculate the average recovery. The average recovery of the replicate values must be within ± 15% of the true value.

9.2.5 Quality Control Sample (QCS) – After calibration curves have initially been established or have been re-established, on a quarterly basis or as required to meet data quality needs, verify both the calibration and acceptable instrument performance with the preparation and analyses of an external/second source QCS. If the determined concentrations are not within ± 20% of the stated values, performance of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the IDC.

9.2.6 Method Detection Limit (MDL) – MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of three to five times the estimated instrument detection limit.[4] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. The replicates must be prepared and analyzed over three days. Report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where, t = student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates], and
S = standard deviation of the replicate analyses.

9.2.6.1 MDLs should be periodically verified, but MUST be initially determined when a new operator begins work or whenever there is a significant change in the background, or instrument response.

NOTE: Do not subtract blank values when performing MDL calculations.

9.2.7 Minimum Reporting Level (MRL) – The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should be established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal to noise ratio of five. Setting the MRL too low may cause repeated QC failure upon analysis of the ICCS. Although the lowest calibration standard may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each analysis batch (Section 3.1). Data produced are used to assess contamination from the laboratory environment. Values that exceed ½ the MRL indicate a laboratory or reagent contamination is present. If a method analyte is observed in the LRB it must not exceed ½ the MRL. Analytes that exceed this level will invalidate the analysis batch for that method analyte in all corresponding field samples.

9.3.1.1 EDA must be added to the LRB at 50 mg/L. By including EDA in the LRB, any bias as a consequence of the EDA which may be observed in the field samples, particularly in terms of background contamination, will be identified.

9.3.1.2 When the PCR method is used for low level bromate analysis on samples from public water systems (PWSs) which employ chlorine dioxide disinfection, the matrix must be pretreated to remove the potentially interferant chlorite anion (Section 11.1.4). When these types of pretreated samples, or any type of pretreatment is applied to field samples included as part of an analysis batch, a second LRB must be prepared, pretreated and analyzed to confirm no background effects of the pretreatment are present. If the analysis batch contains only pretreated samples, then only a pretreated LRB is required.

9.3.2 Laboratory Fortified Blank (LFB) – Prepare a secondary dilution stock using the same stock solutions used to prepare the calibration standards and the LFM

fortification solution. Since calibration solutions are prepared in large volumes and can be used over an extended period of time, the integrity of the concentration of the solution used to fortify the LFM is checked by analyzing the LFB. The recovery of all analytes must fall in the acceptable recovery range, as indicated below, prior to analyzing samples. If the LRB recovery for an analysis batch does not meet these recovery criteria the data are considered invalid, and the source of the problem must be identified and resolved before continuing with analyses.

LFB Fortified Concentration range	LFB Percent Recovery Limits
MRL to 5 x MRL	75 - 125 %
5 x MRL to highest calibration level	85 - 115 %

9.3.2.1 EDA must be added to the LFB at 50 mg/L. The addition of EDA to all reagent water prepared calibration and quality control samples is required not as a preservative but rather as a means to normalize any bias attributed by the presence of EDA in the field samples.

9.3.3 Instrument Performance Check (IPC) – The Initial Calibration Check Standard (ICCS) is to be evaluated as the IPC solution in order to confirm proper instrument performance. As specified in Section 10.3.1, this must be done using the lowest calibration standard or the standard level established as the MRL. This analysis confirms the MRL and demonstrates proper chromatographic performance at the beginning of each analysis batch. Chromatographic performance is judged by calculating the Peak Gaussian Factor (PGF), which is a means to measure peak symmetry and monitoring retention time drift in the surrogate peak over time. If these criteria are not met, corrective action must be performed prior to analyzing additional samples. Major maintenance like replacing columns require rerunning the IDC (Section 9.2).

9.3.3.1 Critically evaluate the surrogate peak in the initial calibration check standard, and calculate the PGF as follows:

$$PGF = \frac{1.83 \times W(\frac{1}{2})}{W(\frac{1}{10})}$$

where, $W(\frac{1}{2})$ is the peak width at half height, and
$W(\frac{1}{10})$ is the peak width at tenth height.

NOTE: Values for $W(\frac{1}{2})$ and $W(\frac{1}{10})$ can be attained through most data acquisition software.

9.3.3.2 Small variations in retention time can be anticipated when a new solution of eluent is prepared but if sudden shifts of more than 5% are observed in the surrogate retention time, some type of instrument problem is present. Potential problems include improperly prepared eluent, erroneous method parameters programmed such as flow rate or some other system problem. The chromatographic profile (elution order) of the target anions following an ion chromatographic analysis should closely replicate the profile displayed in the test chromatogram that was shipped when the column was purchased. As a column ages, it is normal to see a gradual shift and shortening of retention times, but if after several years of use, extensive use over less than a year, or use with harsh samples, this retention time has noticeably shifted to any less than 80% of the original recorded value, the column requires cleaning or replacement; especially if resolution problems are beginning to become common between previously resolved peaks. A laboratory should retain a historic record of retention times for the surrogate and all the target anions to provide evidence of an analytical columns vitality.

9.3.3.3 If a laboratory chooses to monitor exclusively for trace bromate using PCR and the UV/VIS absorbance detector, and no other analytes are being monitored on the conductivity detector, the surrogate may be omitted from the procedure. In this case, no measurement of PGF is required. However, the laboratory must carefully monitor the bromate retention time in the ICCS as an alternate to the surrogate retention time and, in the same manner, adhere to those specifications set forth in Section 9.3.3.2. During the course of the analysis, bromate retention times in the CCCS and ECCS must also be closely monitored to be certain they adhere to the QC requirements set forth in Section 10.3.2.2.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) – The laboratory must add a known amount of each target analyte to a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater. Additional LFM requirements, as described in Section 9.4.1.5, apply when the PCR system is used for low level bromate in chlorine dioxide disinfected waters. For a LFM to be valid, the target analyte concentrations must be greater than the native level and must adhere to the requirement outlined in Section 9.4.1.2. It is recommended that the solutions used to fortify the LFM be prepared from the same stocks used to prepare the calibration standards and not from external

source stocks. This will remove the bias contributed by an externally prepared stock and focus on any potential bias introduced by the field sample matrix.

9.4.1.1 The fortified concentration must be equal to or greater than the native concentration. Fortified samples that exceed the calibration range must be diluted to be within the linear range. In the event that the fortified level is less than the observed native level of the unfortified matrix, the recovery should not be calculated. This is due to the difficulty in calculating accurate recoveries of the fortified concentration when the native sample concentration to fortified concentration ratio is greater than one.

9.4.1.2 The LFM should be prepared at concentrations no greater than ten times the highest concentration observed in any field sample and should be varied to reflect the range of concentrations observed in field samples. If no analytes are observed in any field sample, the LFM should be fortified near the MRL.

9.4.1.3 Calculate the percent recovery for each target analyte, corrected for concentrations measured in the unfortified sample. Percent recovery should be calculated using the following equation:

$$\%REC = \frac{(C_s - C)}{s} \times 100$$

where, %REC = percent recovery,
C_s = fortified sample concentration,
C = native sample concentration, and
s = concentration equivalent of analyte added to sample.

9.4.1.4 Recoveries may exhibit a matrix dependence. If the recovery of any analyte falls outside 75 - 125%, and the laboratory's performance for all other QC performance criteria are acceptable, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample and the LFM must be labeled suspect/matrix to inform the data user that the result is suspect due to matrix effects. Repeated failure to meet suggested recovery criteria indicates potential problems with the procedure and should be investigated.

9.4.1.5 When the PCR method is used for low level bromate analysis on field samples from PWSs which employ chlorine dioxide disinfection and

consequently contain chlorite, a LFM must be prepared, exclusively for trace bromate, for each of these field samples. Initially, the field sample is analyzed and chlorite, chlorate and bromide levels are determined. Then, a second aliquot of field sample is pretreated to remove chlorite, as described in Section 11.1.4, and analyzed to determine native bromate concentration. A third aliquot of the field sample then must be fortified with bromate, pretreated as described in Section 11.1.4 to remove chlorite, and analyzed to assess bromate recovery from that matrix. This additional QC is required to rule out matrix effects and to confirm that the laboratory performed the chlorite removal step (Section 11.1.4.1) appropriately. This LFM should be fortified with bromate at concentrations close to but greater than the level determined in the native sample. Recoveries are determined as described above (Section 9.4.1.3). Samples that fail the LFM percent recovery criteria of 75 - 125% must be reported as suspect/matrix.

9.4.2 SURROGATE RECOVERY – The surrogate is specific to the conductivity detector and shows no response on the postcolumn absorbance detector. Calculate the surrogate recovery for the conductivity detector from all analyses using the following formula:

$$\%REC = \frac{SRC}{SFC} \times 100$$

where, %REC = percent recovery,
SRC = surrogate recovered concentration, and
SFC = surrogate fortified concentration.

9.4.2.1 Surrogate recoveries must fall between 90-115% for proper instrument performance and analyst technique to be verified. The recovery range for the surrogate is extended to 115% to allow for the potential contribution of trace levels of dichloroacetate as a halogenated organic disinfection by-product (DBP) of dichloroacetic acid (DCAA). Background levels of this organic DBP are rarely observed above 50 µg/L (0.05 mg/L) which constitutes only 5% of the 1.00 mg/L recommended fortified concentration.

9.4.2.2 If the surrogate recovery falls outside the 90-115% recovery window, an analysis error is evident and sample reanalysis is required. Poor recoveries could be the result of imprecise sample injection or analyst fortification errors. If the second analysis also fails the recovery criterion, report all data for that sample as suspect.

9.4.2.3 If a laboratory chooses to monitor exclusively for trace bromate using PCR and the UV/VIS absorbance detector, and no other analytes are being monitored on the conductivity detector, the surrogate may be omitted from the procedure. In this situation, the laboratory MUST adopt the QC protocol outlined in Section 9.3.3.3.

9.4.3 FIELD OR LABORATORY DUPLICATES – The laboratory must analyze either a field or a laboratory duplicate for a minimum of 5% of the collected field samples or at least one with every analysis batch, whichever is greater. The sample matrix selected for this duplicate analysis must contain measurable concentrations of the target anions in order to establish the precision of the analysis set and insure the quality of the data. If none of the samples within an analysis batch have measurable concentrations, the LFM should be repeated as a laboratory duplicate.

9.4.3.1 Calculate the relative percent difference (RPD) from the mean using the following formula:

$$RPD = \frac{(I_C - D_C)}{([I_C + D_C]/2)} \times 100$$

where, RPD = relative percent difference
I_C = the initial quantitated concentration, and
D_C = the duplicate quantitated concentration

9.4.3.2 Duplicate analysis acceptance criteria.

Concentration range	RPD Limits
MRL to 5 x MRL	± 20 %
5 x MRL to highest calibration level	± 10 %

9.4.3.3 If the RPD for any target analyte falls outside the acceptance criteria (Section 9.4.3.2) and if all other QC performance criteria are met for that analyte, the result for the sample and duplicate should be labeled as suspect/matrix to inform the data user that the result is suspect due to a potential matrix effect, which led to poor precision. This should not be a chronic problem and if it frequently recurs (>20% of duplicate analyses), it indicates a problem with the instrument or analyst technique that must be corrected.

9.4.4 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns, injection volumes, and/or eluents, to improve the separations or lower the cost of

measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 9.2 and adhere to the condition of conductivity baseline stability found in Section 1.2.1.

9.4.5 It is recommended that the laboratory adopt additional quality assurance (QA) practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should perform analysis of quality control check standards and participate in relevant proficiency testing (PT) or performance evaluation (PE) sample studies.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required prior to the IDC and before any samples are analyzed, is required intermittently throughout sample analysis to meet required QC performance criteria outlined in this method and is summarized in Tables 4 and 5. Initial calibration verification is performed using a QCS as well as with each analysis batch using an initial, continuing (when more than 10 field samples are analyzed), and end calibration standards. The procedures for establishing the initial calibration curve are described in Section 10.2. The procedures to verify the calibration with each analysis batch is described in Section 10.3.

10.2 INITIAL CALIBRATION CURVE

10.2.1 Establish ion chromatographic operating parameters equivalent to those indicated in Table 1 and configured as shown in Figure 1.

10.2.2 Estimate the Linear Calibration Range – The linear concentration range is the concentration range over which the instrument response is linear. On the conductivity detector for the four target analytes (chlorite, bromate, bromide and chlorate) the linear range should cover the expected concentration range of the field samples and should not extend over more than two orders of magnitude in concentration. The restriction of two orders of magnitude is prescribed since beyond this it is difficult to maintain linearity throughout the entire calibration range.

10.2.2.1 If quantification is desired over a larger range, then two separate calibration curves must be prepared.

10.2.2.2 For an individual calibration curve, a minimum of three calibration standards are required for a curve that extends over a single order of magnitude and a minimum of five calibration standards are required if the curve covers two orders of magnitude. Because high

concentrations of chlorite can interfere with the postcolumn analysis of low levels of bromate, the conductivity and absorbance detectors must be calibrated separately.

10.2.2.3 Since the concentration ranges in actual field samples by conductivity detection for chlorite, bromide and chlorate are expected to cover two orders of magnitude, the use of at least five calibration standards in the range 5 - 500 µg/L is recommended. Bromate concentrations are expected to be significantly lower. It is suggested that the conductivity detector be calibrated using at least five bromate calibration standard levels in the range 5 - 100 µg/L. Additionally, report values for bromate by conductivity ONLY when they are measured by the PCR above 15.0 ug/L. The conductivity detector will observe a response for bromate at concentration below 15.0 ug/L but concentrations between 5.0 and 15.0 ug/L are within the calibrated range for PCR detection and will reflect far better precision and accuracy.

10.2.2.4 Although the bromate calibration curve for the absorbance detector extends over less than two orders of magnitude, the use of five calibration standards, containing only bromate in the range 0.5 - 15.0 µg/L, is recommended.

10.2.3 Prepare the calibration standards by carefully adding measured volumes of one or more stock standards (Section 7.3) to a volumetric flask and diluting to volume with reagent water. Prior to using mixed standards for calibration, it must be ensured that the individual calibration standards do not contain any appreciable concentrations of the other target analytes.

10.2.3.1 EDA must be added to the calibration standards at 50 mg/L. The addition of EDA to all reagent water prepared calibration and quality control samples is required not as a preservative but rather as a means to normalize any bias contributed by the addition of EDA to preserve the field samples.

10.2.3.2 Prepare a 10.0 mL aliquot of surrogate fortified calibration solution which can be held for direct manual injection or used to fill an autosampler vial. This is done by adding 20 µL of the surrogate solution (Section 7.5) to a 20 mL disposable plastic micro beaker. Next, transfer 10.0 mL of calibration standard into the micro beaker and mix. These volumes may be adjusted to meet specific laboratory autosampler volume requirements provided the fortified surrogate concentration is at the prescribed concentration of 1.0 mg/L. The calibration standard is now ready for analysis. The same surrogate

solution that has been employed for the standards should also be used in Section 11.1 for the field samples.

NOTE: This surrogate fortification procedure may be omitted if a laboratory chooses to monitor exclusively for trace bromate using PCR and the UV/VIS absorbance detector, and no other analytes are being monitored on the conductivity detector. In this situation, the laboratory must adopt the QC protocol outlined in Section 9.3.3.3.

10.2.4 Inject 225 µL of each calibration standard. Increased sensitivity for low level detection of bromate by PCR can be achieved by increasing the injected sample volume.[4] If the injection volume is increased special operating conditions must be used to insure proper chromatographic performance.[4]

10.2.5 Tabulate peak area responses against the concentration for the four target analytes, the surrogate from the conductivity detector and bromate from the postcolumn absorbance detector. The results are used to prepare calibration curves using linear regression analysis for each analyte on the conductivity detector and using a quadratic regression analysis for bromate on the absorbance detector.

10.2.5.1 Use of peak areas are strongly recommended since they have been found to be more consistent, in terms of quantitation, than peak heights. Peak height can tend to be suppressed as a result of high levels of common anions in a given matrix which can compete for exchange sites leading to peak broadening. Using peak areas, it is the analyst responsibility to review all chromatograms to insure accurate baseline integration of target analyte peaks, since poorly drawn baselines will more significantly influence peak areas than peak heights.

10.2.6 After establishing or reestablishing calibration curves, the accuracy of this calibration must be verified through the analysis of a QCS or an externally prepared second source standard. The QCS should be prepared at a concentration near the middle of the calibration and is best to be analyzed in triplicate. As specified in Section 9.2.5, determined concentrations must fall within ± 15% of the stated values.

10.3 CONTINUING CALIBRATION VERIFICATION – Initial calibrations may be stable for extended periods of time. Once the calibration curves have been established for both the conductivity and absorbance detectors, they must be verified for each analysis batch, prior to conducting any field sample analyses using an Initial Calibration Check Standard. Continuing Calibration Check Standards and End Calibration Check Standards are also required as described in the sections below.

10.3.1 INITIAL CALIBRATION CHECK STANDARD (ICCS) – The initial calibration must be determined to be valid each day prior to analyzing any samples. Since two detectors are incorporated in this method, this must be accomplished by using a mixed calibration check standard for the four conductivity analytes and a separate low level bromate initial calibration check standard for the absorbance detector. In both cases, the lowest level standard used to prepare the calibration curve must be used. In cases where the analyst has chosen to set the MRL above the lowest standard, a standard at a concentration equal to or below the MRL is acceptable. Percent recovery for the ICCS must be in the range or 75 - 125% before the analyst is allowed to analyze samples.

10.3.2 CONTINUING CALIBRATION CHECK/END CALIBRATION CHECK STANDARDS (CCCS/ECCS) – Continuing calibration check standards must be analyzed after every tenth field sample analysis and at the end of the analysis batch as an end calibration check standard. For the reasons noted above, two separate continuing and end calibration check standards must be incorporated. If more than 10 field samples are included in an analysis batch, the analyst must alternate between the middle and high continuing calibration check standard levels.

10.3.2.1 The percent recovery for the CCCS/ECCS must meet the following criteria:

Concentration range	Percent Recovery Limits
MRL to 5 x MRL	75 - 125 %
5 x MRL to highest calibration level	85 - 115 %

10.3.2.2 If during the analysis batch, the measured concentration on either detector differs by more than the calibration verification criteria shown above, or the retention times shift more than ± 2% from the last acceptable initial or continuing calibration check standard for any analyte, all samples analyzed after the last acceptable calibration check standard are considered invalid and must be reanalyzed. The source of the problem must be identified and resolved before reanalyzing the samples or continuing with the analyses.

10.3.2.3 In the case where the end calibration failed to meet performance criteria, but the initial and middle calibration check standards were acceptable, the samples bracketed by the acceptable calibration check standards may be reported. However, all field samples between the middle and end calibration check standards must be reanalyzed.

11. PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 For refrigerated or field samples arriving to the laboratory cold, ensure the samples have come to room temperature prior to conducting sample analysis by allowing the samples to warm on the bench for at least 1 hour.

11.1.2 Prepare a 10.0 mL aliquot of surrogate fortified sample which can be held for direct manual injection or used to fill an autosampler vial. This is done by adding 20 µL of the surrogate solution (Section 7.5) to a 20 mL disposable plastic micro beaker. Next, place a 10.0 mL aliquot of sample into the micro beaker and mix. These volumes may be adjusted to meet specific laboratory autosampler volume requirements provided the fortified surrogate concentration is at the prescribed concentration of 1.0 mg/L. The sample is now ready for analysis.

NOTE: The less than 1% dilution error introduced by the addition of the surrogate is considered insignificant. In addition, this surrogate fortification procedure may be omitted if a laboratory chooses to monitor exclusively for trace bromate using PCR and the UV/VIS absorbance detector, and no other analytes are being monitored on the conductivity detector. In this situation, the laboratory must adopt the QC protocol outlined in Section 9.3.3.3.

11.1.3 Using a Luer lock, plastic 10 mL syringe, withdraw the sample from the micro beaker and attach a 0.45 µm particulate filter (demonstrated to be free of ionic contaminants) directly to the syringe. Filter the sample into an autosampler vial (if vial is not designed to automatically filter) or manually load the injection loop injecting a fixed amount of filtered, well mixed sample. If using a manually loaded injection loop, flush the loop thoroughly between sample analysis using sufficient volumes of each new sample matrix.

11.1.4 CHLORINE DIOXIDE - TREATED WATERS CONTAINING CHLORITE – Treatment plants that use chlorine dioxide as part of their treatment process can produce high levels of chlorite in samples. Since chlorite can interfere with the postcolumn quantitation of low levels of bromate as described in Section 4.6, chlorite must be removed from these samples prior to analysis.[12] The oxidation-reduction reaction between ferrous iron and chlorite[13] is used to remove chlorite without any adverse affects on the bromate concentration.[14] The EDA stabilized sample is acidified to a pH of 5-6 (verified using pH test strips), ferrous iron solution is added and allowed to react for 10 minutes. The sample is then filtered using a 0.45 micron membrane to remove precipitated ferric hydroxide and the excess soluble iron is removed by passing the filtered sample through a

hydrogen cartridge [a solid phase extraction (SPE) clean-up cartridge in the H+ form, (Section 6.11)], prior to analysis. Prior to using any pretreatment, each lot of cartridges must be QC checked to insure proper analyte recoveries are maintained and laboratory reagent blanks are free from interferences. In addition, consistent lots of reagents, pretreatment cartridges, and membrane cartridges must be used throughout an entire analysis batch to maintain assured QC uniformity.

11.1.4.1 Place a 10 mL aliquot of sample in a 20 mL micro beaker and add 35 uL of 0.5 N sulfuric acid (Section 7.8). After mixing, verify the pH is between 5 and 6 using pH test strips, add 40 uL of ferrous iron solution (Section 7.7), mix and allow to react for 10 minutes. Filter the reaction mixture using a 0.45 micron particulate filter (Section 6.10) attached to a 10 mL syringe into the barrel of a second syringe to which a pre-conditioned hydrogen cartridge (Section 6.11) is attached. Pass the solution through a hydrogen cartridge at a flow rate of approximately 2 mL per minute. Discard the first 3 mL, and collect an appropriate volume (depending on autosampler vial size) for analysis. Add the respective volume of surrogate solution, depending on the volume collected, and the sample is ready for analysis.

NOTE: Pretreated samples can be held for no more than 30 hours after initial pretreatment. If this time has expired, the pretreatment steps must be repeated on a second aliquot of both the field sample matrix and the respective LFM.

11.1.4.2 In order to ensure data quality, all samples from PWSs which utilize chlorine dioxide which have been pretreated to remove chlorite, MUST also be used to prepare a pretreated LFM specific to trace bromate. This LFM should be fortified with bromate at concentrations close to but greater than the level determined in the native sample. Initially, the field sample is analyzed and chlorite, chlorate and bromide levels are determined. Then, a second aliquot of field sample is pretreated to remove chlorite, as described above and analyzed to determine native bromate concentrations. A third aliquot of the field sample then must be fortified with bromate, pretreated to remove chlorite, and analyzed to assess bromate recovery from that matrix. This additional QC is required to rule out matrix effects and to confirm that the laboratory performed the chlorite removal step appropriately. If the bromate recovery falls outside the acceptance range of 75 - 125% (Section 9.4.1.5), that particular sample should be reported as suspect/matrix.

11.1.4.3 Suppressor devices which have had long term exposure to iron cations may have reduced method performance in other applications, such as the determination of certain common inorganic anions. If reduced peak response is observed, particularly for fluoride or phosphate, the ASRS should be cleaned according to the manufacturer's recommendations.

11.2 SAMPLE ANALYSIS

11.2.1 Table 1 summarizes the recommended operating conditions for the ion chromatograph and delivery of the postcolumn reagent. Included in this table is estimated retention times that can be achieved by this method. Other columns or chromatographic conditions may be used if the requirements of Section 9.2 are met.

11.2.2 Establish a valid initial calibration as described in Section 10.2 and complete the IDC (Section 9.2). Check system calibration by analyzing an ICCS (Section 10.3.1) as part of the initial QC for the analysis batch and, if required, recalibrate as described in Section 10.3.

11.2.3 Inject 225 µL of each sample. Use the same size loop for standards and samples. An automated constant volume injection system may also be used.

11.2.3.1 Increased sensitivity for low level detection of bromate by PCR can be achieved by increasing the injected sample volume.[4] If the injection volume is increased (Section 10.2.4) special operating conditions must be used to insure proper chromatographic performance.[4]

11.2.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards measured over several days. Three times the standard deviation of retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.2.5 If the response of a sample analyte exceeds the calibration range, the sample must be diluted with an appropriate amount of EDA fortified reagent water and reanalyzed. If this is not possible then three new calibration concentrations must be employed to create a separate high concentration calibration curve, one standard near the estimated concentration and the other two bracketing around an interval equivalent to approximately ± 25% the estimated concentration. The latter procedure involves significantly more time than a simple sample dilution and, therefore, it is advisable to collect sufficient sample to allow for sample dilution and sample reanalysis, if required.

11.2.6 Should more complete resolution be needed between any two coeluting peaks, the eluent (Section 7.2) can be diluted. This will spread out the run, however, and will cause late eluting anions to be retained even longer. The analyst must verify that this dilution does not negatively affect performance by repeating and passing all the QC criteria in Section 9, and by reestablishing a valid initial calibration curve (Section 10.2). As a specific precaution, upon dilution of the carbonate eluent, a peak for bicarbonate may be observed by conductivity within the retention time window for bromate which will negatively impact the analysis.

11.2.6.1 Eluent dilution will reduce the overall response of an anion due to chromatographic band broadening which will be evident by shortened and broadened peaks. This will adversely effect the MDLs for each analyte.

11.3 AUTOMATED ANALYSIS WITH METHOD 317.0

11.3.1 Laboratories conducting analyses on large numbers of samples often prepare large analysis batches that are run in an automated manner. When conducting automated analyses, careful attention must be paid to all reservoirs to be certain sufficient volumes are available to sustain extended operation. Laboratories must ensure that all QC performance criteria are met as described in preceding sections to ensure their data are of acceptable quality.

11.3.1.1 Special attention must be made when the PCR reservoir is refilled. Since this is a pneumatically driven system, the baseline will require a minimum of ten minutes to restabilize after the reservoir has been refilled and the bottle repressurized.

11.3.2 Because this method has two detectors that require independent calibration, analysis sequences must be carefully constructed to meet required QC specifications and frequency (Table 5). To help with this task, an acceptable sequence for a sample analysis batch, with all the method-required QC, is shown in Table 6. This schedule is included only as an example of a hypothetical analysis batch where the analyst desires to collect data using both detectors. Within the analysis batch, references to exact concentrations for the ICCS, CCCS and ECCS are for illustrative purposes only. The analyses for sample #14 provides an example of the QC requirements for a complete conductivity and trace bromate PCR analysis of a sample from a PWS employing chlorine dioxide disinfection.

11.3.3 Table 6 may be used as a guide when preparing analysis batches.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspected analyte peak to the retention time of a known analyte peak in a calibration standard. If analyte retention times have shifted (generally towards shorter times) since the initial calibration, but are still within acceptance criteria and are reproducible during the analysis batch, the analyst should use the retention time in the daily calibrations to confirm the presence or absence of target analytes.

12.2 Compute sample concentration using the initial calibration curve generated in Section 10.2.

12.3 Report ONLY those values that fall between the MRL and the highest calibration standards. Samples with target analyte responses exceeding the highest standard must be diluted and reanalyzed. When this is not possible the alternate calibration procedures described in Section 11.2.5 must be followed. Samples with target analytes identified but quantitated below the concentration established by the lowest calibration standard may be reported as present, but below the minimum reporting limit (MRL), and consequently not quantitated.

12.3.1 Report bromate concentrations using the postcolumn UV/VIS absorbance detector when they fall between the MRL and 15.0 ug/L. When bromate concentrations exceed 15.0 ug/L, as detected by UV/VIS absorbance, either report by conductivity, calibrate the postcolumn UV/VIS absorbance detector to a higher bromate concentration, or dilute the sample.

12.4 Report results in µg/L.

12.5 Software filtering of the postcolumn UV/VIS absorbance signal is required to improve the precision of peak measurements, minimize non-random noise and improve peak appearance. Olympic smoothing (25 points, 5 seconds with 1 iteration) was chosen using peak area for quantitation because it was determined to have minimal effect on peak height and/or area.[2,15] The use of alternate smoothing routines is acceptable providing all QC criteria are met.

13. METHOD PERFORMANCE

13.1 Table 1 gives the standard conditions, typical retention times and single laboratory MDLs in reagent water, as determined for each of the inorganic oxyhalide DBPs and bromide. Included in this table is a comparison of the MDLs determined by conductivity both with and without the postcolumn UV/VIS absorbance system on-line. These data indicate that the postcolumn UV/VIS detector system has no effect on conductivity detector performance (careful attention must however be paid to insure backpressure on the suppressor is kept below 120 psi).

13.2 Table 2 shows the precision and accuracy of the trace bromate measurement, evaluated on both detectors, at two fortified concentrations, in chlorinated surface water, a simulated high ionic strength water (HIW) and a simulated high organic (HOW) content water. The mean bromate recovered concentration (accuracy relative to the fortified level) and the precision (expressed as %RSD of the replicate analyses) are tabulated. The HIW was designed to simulate a high ionic strength field sample and the HOW designed to simulate a high organic content field sample. The HIW was prepared from reagent water which was fortified with the common anions of chloride at 100 mg/L, carbonate at 100 mg/L, nitrate at 10.0 mg/L as nitrogen, phosphate at 10.0 mg/L as phosphorous, and sulfate at 100 mg/L.[1] The HOW was prepared from reagent water fortified with 1.0 mg/L fulvic acid.[1]

13.3 Table 3 gives the single laboratory standard deviation and precision (% RSD) for each anion included in the method in a variety of waters for the standard conditions identified in Table 1.[1,2]

13.4 Table 3A shows the stability data for the inorganic oxyhalide DBPs. Each data point in these tables represent the mean percent recovery following triplicate analyses. These data were used to formulate the holding times shown in Section 8.3.[1]

14. POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasiblely reduced at the source, the Agency recommends recycling as the next best option.

14.2 Quantity of the chemicals purchased should be based on expected usage during its shelf-life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any waste discharge permit and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from the American Chemical Society at the address listed in Section 14.3.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS IN REAGENT WATER FOR THE INORGANIC OXYHALIDE DISINFECTION BY-PRODUCTS AND BROMIDE.

Standard Conditions and Equipment[a]:

Ion Chromatograph:	Dionex DX500
Sample Loop:	225 µL
Eluent:	9.0 mM Na_2CO_3
Eluent Flow:	1.3 mL/min
Columns :	Dionex AG9-HC / AS9-HC, 4 mm
Typical System Backpressure:	2300 psi
Suppressor:	ASRS-I, external water mode, 100 mA current
Detectors:	Suppressed Conductivity Detector, Dionex CD20 Background Conductivity: 24 µS Absorbance Detector, Dionex AD20 (10 mm cell path) Set for absorbance at 450 nm (Tungsten lamp)
Postcolumn Reagent Flow:	0.7 mL/min
Postcolumn Reactor Coil:	knitted, potted for heater, 500 uL internal volume
Postcolumn Heater:	60° C

Recommended method total analysis time: 25 minutes

Analyte Retention Times and Method Detection Limits (MDLs):

		MDL DETERMINATION		
Analyte	Retention Time [b] (min.)	Fortified Conc. (µg/L)	# of Reps.	MDL (µg/L)
Chlorite[c]	4.20	2.0	8	0.45
Chlorite[d]	4.20	2.0	8	0.89
Bromate[c]	4.85	2.0	8	0.98
Bromate[d]	4.85	2.0	8	0.71
Bromate[e]	5.35	0.50	7	0.12
Surrogate: DCA[d]	8.50			
Bromide[c]	10.0	2.0	8	0.54
Bromide[d]	10.0	2.0	8	0.69
Chlorate[c]	11.0	2.0	8	0.92
Chlorate[d]	11.0	2.0	8	0.62

(a) Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
(b) Reference to chromatograms in Figure 2 and 3.
(c) Method 317.0 conductivity detection without PCR online.
(d) Method 317.0 conductivity detection with PCR online.
(e) Method 317.0 ONLY bromate by postcolumn UV/VIS absorbance detection.

TABLE 2. SINGLE LABORATORY PRECISION IN VARIOUS MATRICES FOR BROMATE BY CONDUCTIVITY AND ABSORBANCE DETECTION.

		PRECISION				
Matrix	Detection	Fortified Conc. (µg/L)	# of Reps.	Mean (µg/L)	SD (n-1)	% RSD
Reagent Water	Conductivity	0.50	8	< MRL[a]		< MRL
	Conductivity	5.0	8	4.8	0.420	8.83
	Absorbance	0.50	8	0.50	0.054	10.8
	Absorbance	5.0	8	5.4	0.110	2.10
Chlorinated Drinking Water	Conductivity	0.50	8	< MRL		< MRL
	Conductivity	5.0	7[b]	4.1	0.650	15.7
	Absorbance	0.50	8	0.53	0.050	2.77
	Absorbance	5.0	8	5.2	0.098	1.87
High Ionic Water	Conductivity	0.50	8	< MRL		< MRL
	Conductivity	5.0	8	4.5	0.960	21.4
	Absorbance	0.50	8	0.52	0.430	8.20
	Absorbance	5.0	8	5.1	0.199	3.90
High Organic Water	Conductivity	0.50	8	< MRL		< MRL
	Conductivity	5.0	8	5.1	0.199	3.90
	Absorbance	0.50	8	0.50	0.044	8.81
	Absorbance	5.0	8	5.2	0.014	2.61

[a] <MRL = analyte was not detected at or above the minimum reporting level.
[b] n = 8 one outlier reject using Dixon's Outlier Test.[16]

Standard Conditions: Same as listed in Table 1.

TABLE 3. SINGLE-LABORATORY PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS AND BROMIDE.[1,2]

Analyte	Matrix	Unfortified Conc. (µg/L)	Fortified Conc. (µg/L)	# of Reps.	Mean (µg/L)	Mean %REC	SD(n-1)	%RSD
Chlorite	RW	<MRL[a]	100	9	96.2	96.2	0.95	0.99
			500	9	520	105	3.13	0.60
	HIW	<MRL	100	9	102	102	2.19	2.15
			500	9	520	104	3.64	0.70
	SW	<MRL	100	9	91.4	91.4	1.22	1.33
			500	9	500	99.0	7.54	1.52
	GW	<MRL	100	9	92.9	92.9	1.65	1.77
			500	9	490	98.1	3.40	0.69
	ClW	<MRL	100	9	87.4	87.4	0.59	0.68
			500	9	490	97.1	6.36	1.31
	CDW	292	100	9	400	NC[b]	1.64	0.41
			500	9	810	104	4.00	0.49
	O3W	<MRL	100	9	84.4	84.4	0.46	0.54
			500	9	480	96.1	3.24	0.67
Bromate by Conductivity	RW	<MRL	5.0	9	5.05	101	0.45	8.86
			25	9	26.5	106	1.71	6.47
	HIW	<MRL	5.0	9	4.88	97.5	0.95	19.5
			25	9	25.5	102	1.37	5.37
	SW	<MRL	5.0	9	4.46	89.2	0.58	13.0
			25	9	26.3	105	1.10	4.18
	GW	<MRL	5.0	9	5.10	102	0.50	9.75
			25	9	22.2	88.9	1.29	5.81
	ClW	<MRL	5.0	9	4.63	92.6	0.77	16.7
			25	9	25.0	100.	1.64	6.55
	CDW	<MRL	5.0	9	4.14	82.7	0.62	15.1
			25	9	25.3	101	1.28	5.09
	O3W	1.45	5.0	9	5.50	80.9	0.61	11.1
			25	9	24.1	90.6	1.13	4.69

RW = Reagent Water
HIW = High Ionic Strength Water
SW = Surface Water
GW = Groundwater
ClW = Chlorinated Drinking Water
CDW = Chlorine Dioxide Treated Drinking Water
O3W = Ozonated Drinking Water

[a] <MRL = analyte was not detected at or above the minimum reporting level.
[b] NC = Not calculated since amount fortified was less than unfortified native matrix concentration (Section 9.4.1.1.).

TABLE 3. SINGLE-LABORATORY PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS AND BROMIDE (cont.).[1,2]

Analyte	Matrix	Unfortified Conc. (µg/L)	Fortified Conc. (µg/L)	# of Reps.	Mean (µg/L)	Mean %REC	SD(n-1)	%RSD
Bromide	RW	<MRL[a]	20.0	9	20.8	104	0.80	3.82
			100	9	110	107	0.60	0.56
	HIW	3.2	20.0	9	21.7	92.5	0.79	3.63
			100	9	110	102	1.05	1.00
	SW	31	20.0	9	51.0	NC[b]	0.97	1.90
			100	9	140	109	1.88	1.35
	GW	150	20.0	9	170	NC	0.78	0.45
			100	9	260	NC	218	0.82
	ClW	16	20.0	9	39.0	115	0.64	1.62
			100	9	130	109	2.00	1.60
	CDW	12	20.0	9	35.0	115	0.76	2.22
			100	9	130	113	1.24	0.99
	O3W	40.	20.0	9	65.0	NC	3.67	5.61
			100	9	150	113	1.00	0.65
Chlorate	RW	<MRL	100	9	98.3	98.3	0.80	0.82
			500	9	520	104	4.15	0.80
	HIW	<MRL	100	9	86.1	86.1	1.47	1.70
			500	9	500	100.	4.52	0.90
	SW	3.2	100	9	100	98.3	1.57	1.55
			500	9	510	102	7.11	1.39
	GW	<MRL	100	9	93.5	93.5	2.00	2.14
			500	9	510	102	3.84	0.75
	ClW	34	100	9	140	102	1.01	0.74
			500	9	550	103	3.11	0.57
	CDW	120	100	9	220	NC	3.20	1.44
			500	9	650	106	3.50	0.54
	O3W	6.2	100	9	110	100.	1.20	1.13
			500	9	520	103	2.45	0.47

RW = Reagent Water
HIW = High Ionic Strength Water
SW = Surface Water
GW = Groundwater
ClW = Chlorinated Drinking Water
CDW = Chlorine Dioxide Treated Drinking Water
O3W = Ozonated Drinking Water

[a] <MRL = analyte was not detected at or above the minimum reporting level.
[b] NC = Not calculated since amount fortified was less than unfortified native matrix concentration (Section 9.4.1.1.).

TABLE 3. SINGLE-LABORATORY PRECISION AND RECOVERY FOR THE INORGANIC DISINFECTION BY-PRODUCTS AND BROMIDE (cont.).[1,2]

Analyte	Matrix	Fortified Conc. (mg/L)	# of Reps.	Mean (mg/L)	Mean %REC	SD(n-1)	%RSD
Surrogate: DCA (see Note)	RW	5.0	9	5.1	102	0.93	0.91
				5.0	99.5	0.69	0.69
	HIW	5.0	9	5.0	100.	0.79	0.79
				5.0	99.2	1.76	1.78
	SW	5.0	9	4.9	98.9	0.70	0.7
				5.0	99.8	1.60	1.61
	GW	5.0	9	5.1	102	0.50	0.49
				5.1	103	0.50	0.49
	ClW	5.0	9	5.2	103	1.73	1.68
				5.1	103	1.12	1.09
	CDW	5.0	9	5.0	100.	1.02	1.02
				5.0	101	1.08	1.07
	O3W	5.0	9	5.0	99.8	0.70	0.7
				5.1	101	0.53	0.52

RW = Reagent Water
HIW = High Ionic Strength Water
SW = Surface Water
GW = Groundwater
ClW = Chlorinated Drinking Water
CDW = Chlorine Dioxide Treated Drinking Water
O3W = Ozonated Drinking Water

NOTE: The surrogate DCA was fortified at 5 mg/L but due to concerns about measuring trace concentrations of bromide with such high concentration of the neighboring surrogate peak, the recommended fortified concentration for the surrogate has been reduced to 1.00 mg/L.

TABLE 3A. STABILITY STUDY RESULTS FOR THE INORGANIC DISINFECTION BY-PRODUCTS AND BROMIDE.[1]

Analyte	Preservative	Matrix	Unfortified Conc. (µg/L)	Fortified Conc. (µg/L)	Analyte % Recovery Day 0	Day 3	Day 10	Day 30	See Note
Chlorite	None	RW	<MRL	500	99.8	100	104	94.3	
		HIW	<MRL	500	99.3	98.5	106	89.3	
		SW	<MRL	500	92.0	88.5	82.3	75.1	(a)
		GW	<MRL	500	93.9	94.5	96.1	91.7	
		ClW	<MRL	500	93.7	NA[(a)]	90.3	84.7	(b,c)
		CDW	290	500	98.6	101	91.7	77.5	(a,c)
		O3W	<MRL	500	10.0	NA	82.5	90.5	(b)
Chlorite	EDA	RW	<MRL	500	101	101	104	95.3	
		HIW	<MRL	500	98.4	98.7	104	95.4	
		SW	<MRL	500	98.3	97.3	97.8	92.7	
		GW	<MRL	500	97.7	97.1	97.5	92.6	
		ClW	<MRL	500	98.9	NA	96.9	92.6	(b)
		CDW	300	500	103	107	102	94.5	
		O3W	<MRL	500	105	NA	96.3	91.9	(b)
Bromate	None	RW	<MRL	25.0	93.6	94.1	110	96.1	
		HIW	<MRL	25.0	100.	86.0	105	87.7	
		SW	<MRL	25.0	98.7	95.1	105	102	
		GW	<MRL	25.0	79.4	92.4	77.8	82.2	
		ClW	<MRL	25.0	102	NA	101	103	(b)
		CDW	<MRL	25.0	104	96.8	98.9	92.1	
		O3W	2.3	25.0	87.3	NA	84.3	99.9	(b)
Bromate	EDA	RW	<MRL	25.0	97.3	95.3	99.5	102	
		HIW	<MRL	25.0	86.9	86.1	107	91.2	
		SW	<MRL	25.0	100.	104	103	94.9	
		GW	<MRL	25.0	83.2	101	88.4	88.3	
		ClW	<MRL	25.0	105	NA	101	102	(b)
		CDW	<MRL	25.0	117	97.3	98.1	83.9	
		O3W	2.3	25.0	92.6	NA	84.5	88.9	(b)

NOTES:
(a) Degradation in the unpreserved matrix is apparent.
(b) NA indicates "NOT ANALYZED"
(c) Analyte recovery will be adversely effected by reactions with free chlorine.

TABLE 3A. STABILITY STUDY RESULTS FOR THE INORGANIC DISINFECTION BY-PRODUCTS AND BROMIDE (cont.).[1,2]

Analyte	Preservative	Matrix	Unfortified Conc. (µg/L)	Fortified Conc. (µg/L)	Analyte % Recovery Day 0	Day 3	Day 10	Day 30	See Note
Bromide	None	RW	<MRL	100	99.4	97.2	107	101	
		HIW	<MRL	100	102	103	105	105	
		SW	31	100	102	97.1	107	99.1	
		GW	150	100	97.7	95.3	109	100.	
		ClW	4.7	100	8.90	NA[a]	37.0	11.4	(b,c,d)
		CDW	<MRL	100	5.78	23.1	39.0	51.3	(c,d)
		O3W	30	100	98.3	NA	120	108	(b,d)
Bromide	EDA	RW	<MRL	100	98.4	98.6	107	100.	
		HIW	<MRL	100	104	103	106	105	
		SW	31	100	99.5	98.2	107	100.	
		GW	150	100	100.	97.0	114	97.7	
		ClW	12	100	101	NA	115	97.4	(b,c)
		CDW	6.1	100	101	96.5	119	110	(c)
		O3W	31	100	97.3	NA	122	102	(b)
Chlorate	None	RW	<MRL	500	102	102	105	97.4	
		HIW	<MRL	500	97.0	97.8	101	95.4	
		SW	5.8	500	100.	97.8	100.	96.0	
		GW	<MRL	500	100.	98.7	101	99.8	
		ClW	38	500	102	NA	104	98.2	(b)
		CDW	130	500	102	99.9	104	99.6	
		O3W	8.3	500	100.	NA	103	97.3	(b)
Chlorate	EDA	RW	<MRL	500	104	98.6	103	97.3	
		HIW	<MRL	500	97.0	103	100.	95.0	
		SW	6.7	500	100.	98.2	100.	95.6	
		GW	<MRL	500	102	97.0	101	99.3	
		ClW	38	500	101	NA	102	96.1	(b)
		CDW	120	500	102	96.5	105	97.7	
		O3W	8.6	500	98.0	NA	103	96.4	(b)

NOTES:
(a) Degradation in the unpreserved matrix is apparent.
(b) NA indicates "NOT ANALYZED"
(c) Analyte recovery will be adversely effected by reactions with free chlorine.
(d) Measurement of Br^- is not in the scope (source/raw water only)

TABLE 4. INITIAL DEMONSTRATION OF CAPABILITY QC REQUIREMENTS.

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.2 9.3.1	Initial Demonstration of Low System Background	Analyze a method blank (LRB) and determine that all target analytes are below ½ of the proposed MRL prior to performing the IDC	The LRB concentration must be ≤½ of the proposed MRL
Sect. 9.2.3	Initial Demonstration of Precision (IDP)	Conductivity: analyze 7 replicate LFBs recommend fortify at 20 ug/L Absorbance: analyze 7 replicate LFBs recommend fortify with bromate at 2.0 ug/L	%RSD must be ≤20%
Sect. 9.2.4	Initial Demonstration of Accuracy (IDA)	Calculate average recovery of IDP replicates	Mean % recovery must be ± 15% of true value.
Sect. 9.2.5	Quality Control Sample (QCS)	Initially and at least quarterly analyze a QCS from an external/second source	QCS must be ± 20% of the true value
Sect. 9.2.6	Method Detection Limit (MDL) Determination	Select a fortifying level at 3-5 times the estimated instrument detection limit at or lower than the MRL. Analyze 7 replicate LFBs Calculate MDL using equation in Section 9.2.6 - do not subtract blank	
Sect. 9.2.7	Minimum Reporting Level (MRL)	MRLs MUST be established for all analytes during the IDC.	The low CAL standard can be lower than the MRL, but the MRL MUST be no lower than the low CAL standard

TABLE 5. QUALITY CONTROL REQUIREMENTS (SUMMARY).

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.3	Sample Holding Time / Preservation	Bromate 28 days, refrig. at <6°C / EDA Preservation Bromide 28 days, EDA Permitted Chlorate 28 days, refrig. at <6°C / EDA Preservation Chlorite 14 days, refrig. at <6°C / EDA Preservation	Holding time and temperature must not be exceeded. EDA added to all samples
Sect. 11.1.4.1 (specific to PCR)	Pretreated Sample (acidified/ Fe [II] added to remove chlorite) Holding Time	ONLY REQUIRED when samples containing chlorite are pretreated and PCR is employed to measure trace bromate in samples. MAXIMUM PRETREATED SAMPLE HOLDING TIME: 30 hours	Pretreated sample holding time must not be exceeded
Sect. 10.2	Initial Calibration	Conductivity: generate calibration curve using at least 5 standards Absorbance: generate calibration curve using at least 5 bromate standards	MRL MUST be no lower than the lowest calibration standard
Sect. 10.3.1	Initial Calibration Check	Daily, verify calibration of conductivity detector at the MRL by analyzing an initial low-level continuing calibration check standard (ICCS) and a separate low-level ICCS for the absorbance detector at the MRL.	Recovery must be 75- 125% of the true value on both detectors
Sect. 10.3.2	Continuing Calibration and End Calibration Checks	Alternately analyze separate mid and high level CCCS/ECCS after every 10 samples and after the last sample	MRL to 5 x MRL must have 75 - 125% recovery on both detectors For 5 x MRL to highest CCCS must have 85 - 115% recovery on both detectors
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	Include LRB with every analysis batch (up to 20 samples) Analyze prior to analyzing field samples	All analytes must be ≤ ½ MRL
Sect. 9.3.1.2 (specific to PCR)	PRETREATED Laboratory Reagent Blank (LRB)	REQUIRED in any analysis batch which includes samples which have been pretreated to remove chlorite prior to PCR measurement of trace bromate.	PCR measured bromate < ½ MRL
Sect. 9.3.2	Laboratory Fortified Blank (LFB)	Laboratory must analyze LFB in each analysis batch following the ICCS. Calculate %REC prior to analyzing samples	LFB recovery if fortified at conc. from MRL to 5X MRL must be 75 - 125%. For 5X MRL to highest CCCS must be 85 - 115%. Must have acceptable recoveries prior to analyzing samples. Sample results from batches that fail LFB are invalid

TABLE 5. QUALITY CONTROL REQUIREMENTS (SUMMARY CONTINUED).

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.3	Instrument Performance Check (IPC)	Calculate Peak Gaussian Factor (PGF) using equation (Sect. 9.3.3.1) and monitor retention time for surrogate in Initial Calibration Check Standard (ICCS) each day	PGF must fall between 0.80 and 1.15 Ret. Time (RT) for surrogate must remain 80% of initial RT when column was new
Sect. 9.4.1 Sect. 11.1.4.2	Laboratory Fortified Sample Matrix (LFM)	Must add known amount of each target analyte to a minimum of 5% of field samples or at least one within each analysis batch for both detectors LFM must be fortified above the native level and at no greater than 5 x the highest field sample concentration Calculate target analyte recovery using formula (Sect. 9.4.1.3) When field samples from chlorine dioxide plants which contain chlorite are pretreated prior to the PCR measurement of trace bromate, an additional LFM must be prepared for each pretreated field sample (Sect. 9.4.1.5)	Recovery should be 75 - 125% If fortified sample fails the recovery criteria, label both as suspect/matrix.
Sect. 9.4.2	Surrogate	Dichloroacetate is added to all blanks, samples and standards (if measuring by conductivity and absorbance) Calculate recovery using formula in Section 9.4.2	Surrogate recovery must be 90 - 115%. Samples that fail surrogate recovery must be reanalyzed. If second analysis fails label result as suspect/matrix
Sect. 9.4.3	Field or Laboratory Duplicates	Analyze either a field or laboratory duplicate for a minimum of 5% of field samples or at least one within each analysis batch for both detectors Calculate the relative percent difference (RPD) using formula in Section 9.4.3.1	The RPD for concentrations at MRL to 5 x MRL should be ± 20% on both detectors, and ± 10% on both detectors for concentrations at 5 x MRL to highest CCCS. If this range is exceeded, label both as suspect/matrix

TABLE 6. EXAMPLE SAMPLE ANALYSIS BATCH WITH QUALITY CONTROL REQUIREMENTS

Injection #	Sample Description	Acceptance Criteria
1	Laboratory reagent blank (LRB)	≤ ½ MRL
2	ICCS conductivity detector (5.0 µg/L)	3.75 to 6.25 µg/L
3	ICCS absorbance detector (0.5 µg/L)	0.375 to 0.625 µg/L
4	Laboratory Fortified Blank (LFB) - conductivity detector	± 25 % fortified level
5	LFB - absorbance detector	± 25 % fortified level
6	Field sample 1	
7	Field sample 1 - Laboratory Duplicate (LD) [a]	± 15 % RPD
8	Field sample 2	
9	Field sample 2 - Laboratory Fortified Matrix (LFM) [a] at concentrations specific for conductivity detector	± 25% fortified level
10	Field sample 2 - LFM specific for trace bromate on the absorbance detector	± 25% fortified level
11	Field sample 3	
12	Field sample 4	
13	Field sample 5	
14	Field sample 6	
15	Field sample 7	
16	Field sample 8	
17	Field sample 9	
18	Field sample 10	
19	CCCS conductivity detector (75.0 µg/L)	63.8 to 86.3 µg/L
20	CCCS absorbance detector (5.0 µg/L)	4.25 to 5.75 µg/L
21	Field sample 11	
22	Field sample 12	

23	Field sample 13	
24	Field sample 14 - (finished water from PWS using chlorine dioxide)	
25	Pretreated LRB (Section 9.3.1.2) using the acid/Fe(II) chlorite removal procedure (Section 11.1.4)	≤ ½ MRL
26	Field sample 14 [b] - (finished water from PWS using chlorine dioxide) pretreated with acid/Fe(II) (Section 11.1.4)	
27	Field sample 14 - (finished water from PWS using chlorine dioxide) LFM specific for trace bromate on the absorbance detector, pretreated with acid/Fe(II) (Section 11.1.4.2)	± 25% fortified level
28	Field sample 15	
29	Field sample 16	
30	Field sample 17	
31	Field sample 18	
32	Field sample 19[b]	
33	ECCS conductivity detector (500.0 µg/L)	425 to 575 µg/L
34	ECCS absorbance detector (15.0 µg/L)	12.8 to 17.3 µg/L

[a] If no analytes are observed above the MRL for a sample, an alternate sample which contains reportable values should be selected as the laboratory duplicate. Alternately, the LFM can be selected and reanalyzed as the laboratory duplicate ensuring the collection of QC data for precision.

[b] Field sample #19 was the final field sample permitted in this batch but 20 total field samples were analyzed.
Field sample #14 was analyzed both initially and as a acid/Fe (II) pretreated sample, therefore, it accounted for two "field sample analyses" toward the maximum of twenty in an analysis batch (Section 3.1).

System Configuration for EPA Method 317.0

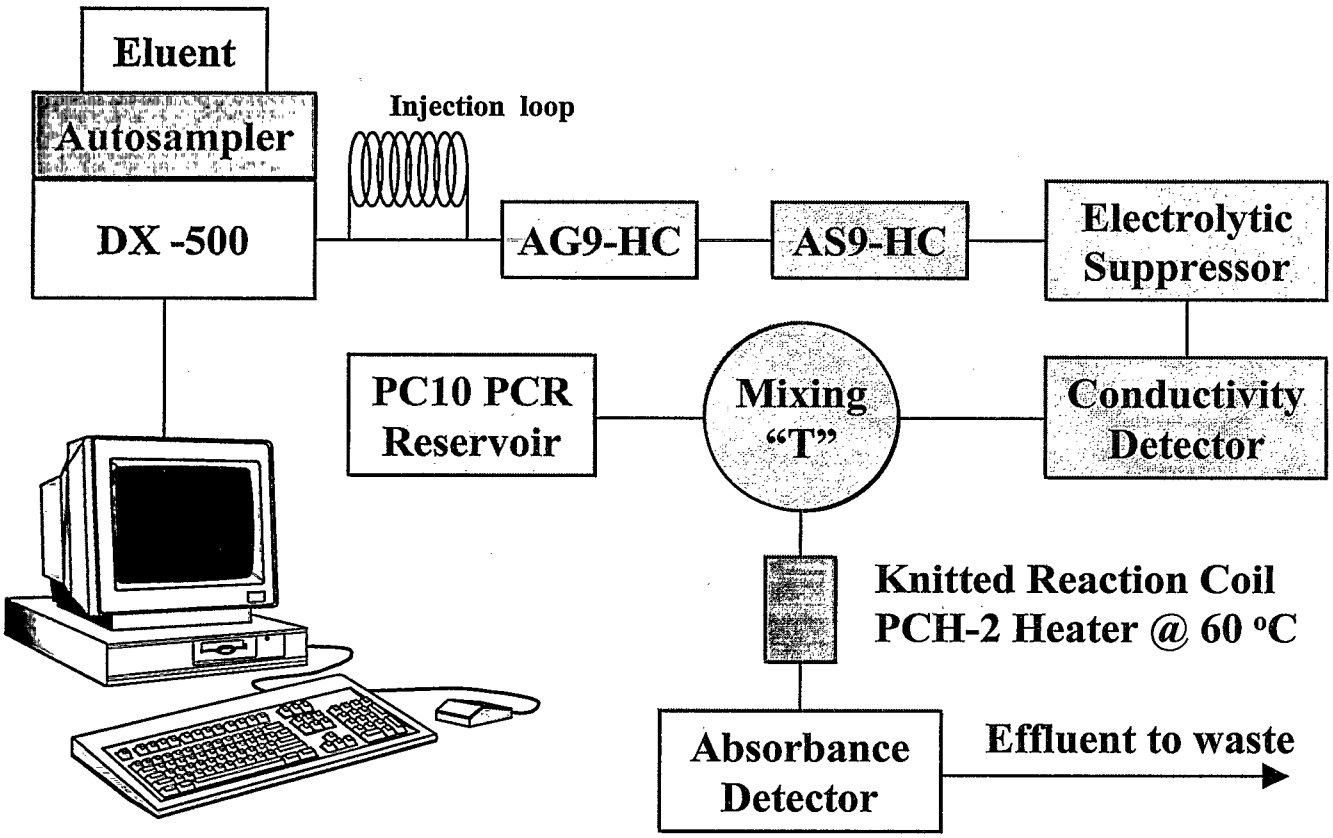
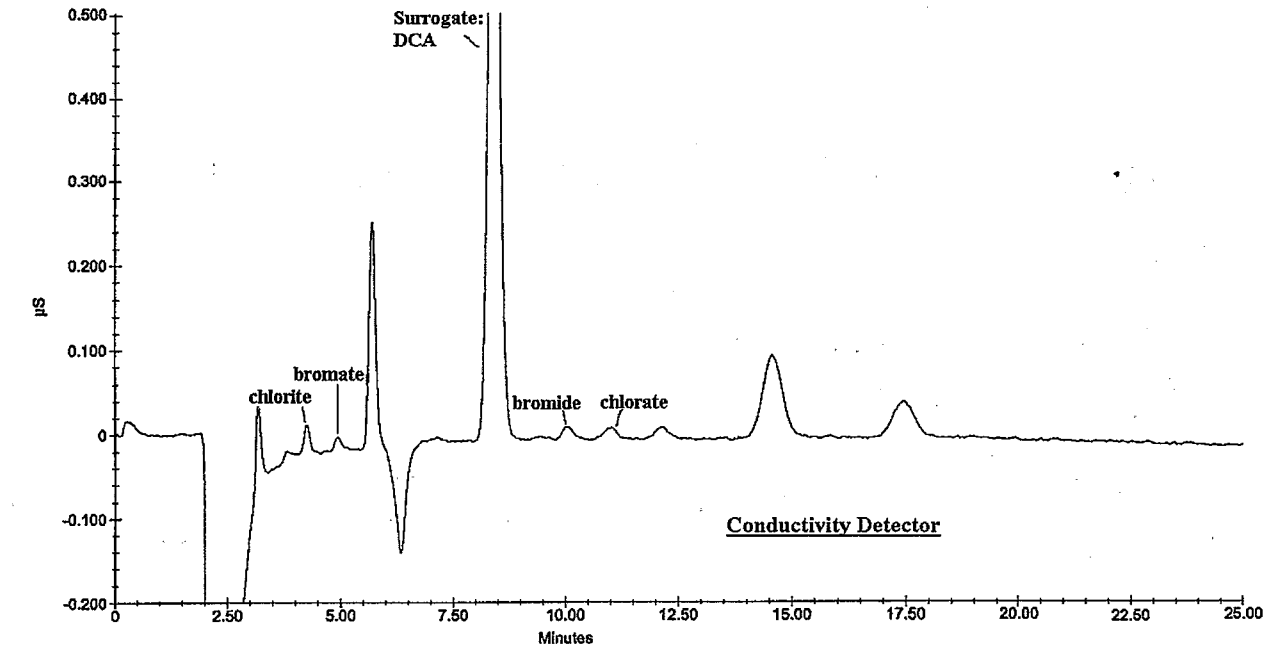


Figure 1: Schematic detailing the configuration of postcolumn hardware addition to an ion chromatograph. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. If the requirements found in Section 9 are met, equivalent products or hardware can be employed.

NOTE: In a typical Method 300.1 hardware configuration, a backpressure coil is included after the conductivity cell as part of the waste stream when this manufacturer's equipment is used. These backpressure coils are not required when the Method 317.0 instrument configuration is employed since the additional PCR system components, placed in-line, function in the same capacity and provide sufficient backpressure.



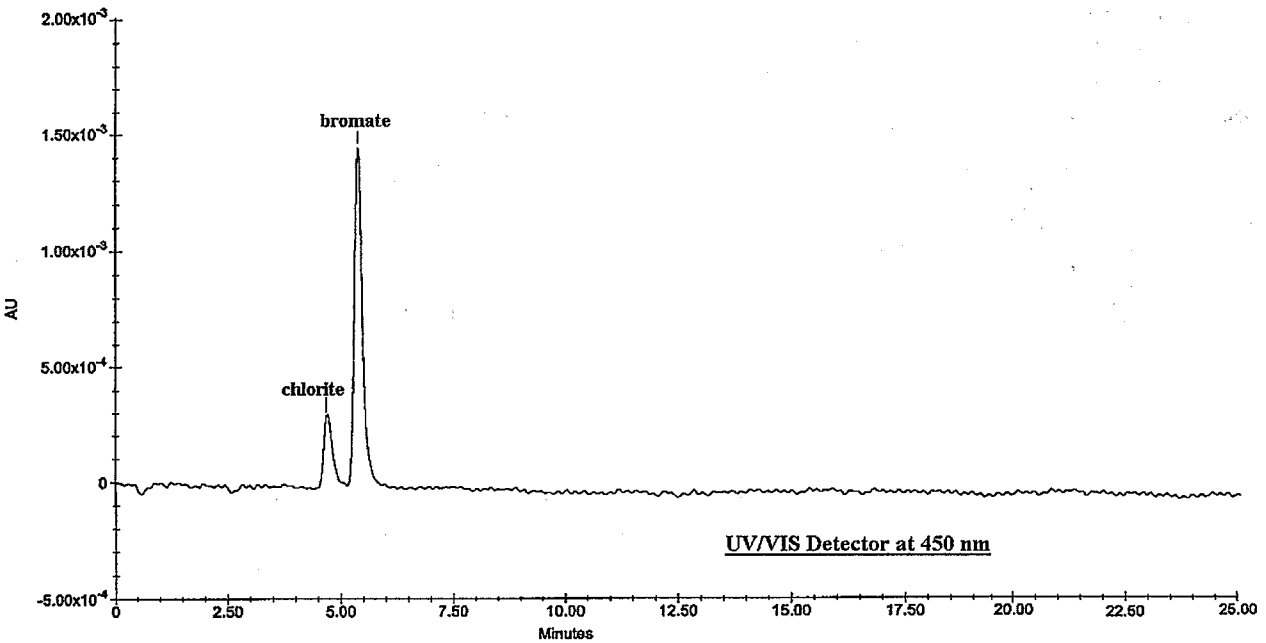


Figure 2: Reagent water fortified with inorganic disinfection by-products and bromide at 10 ug/L.

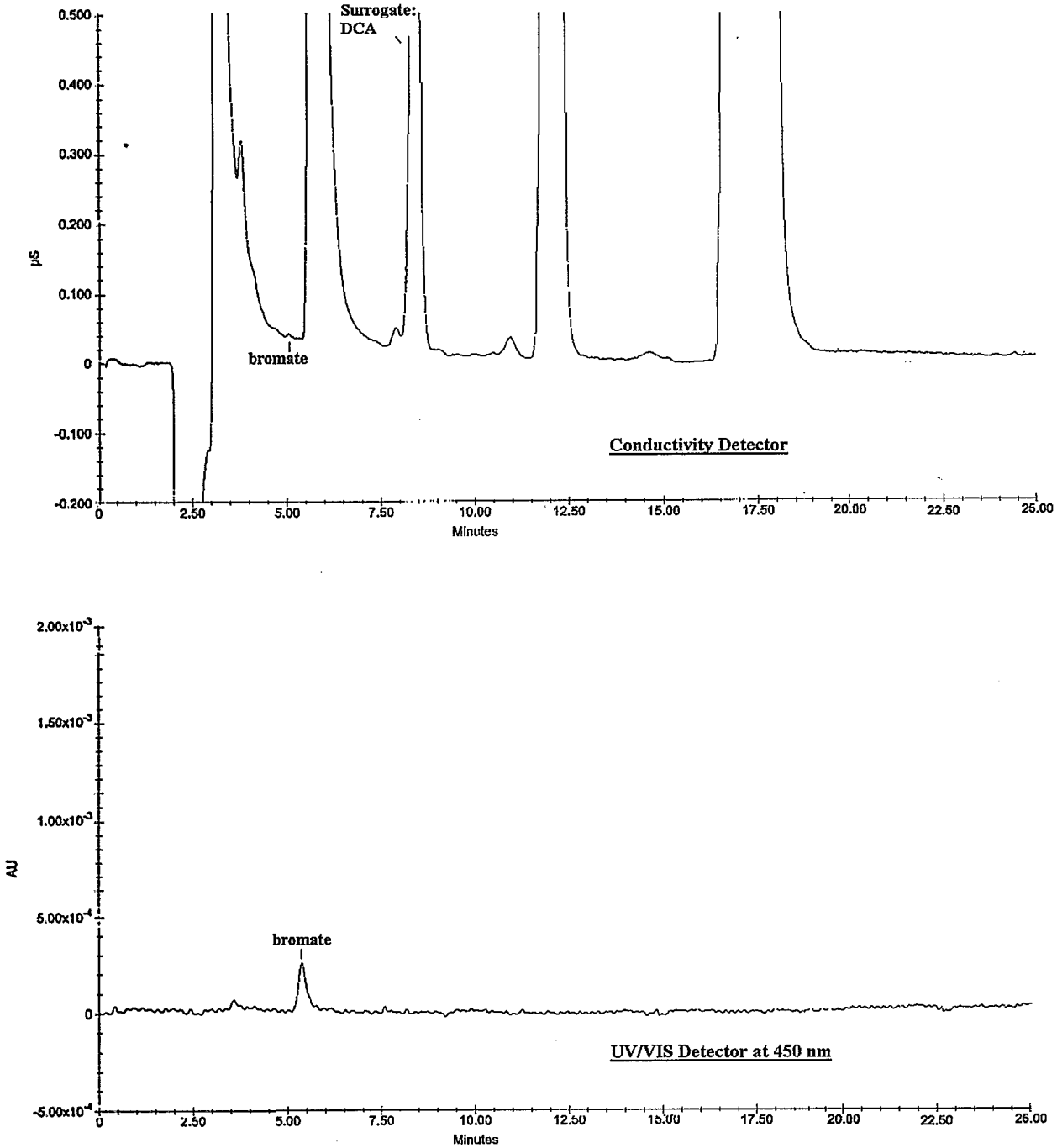


Figure 3: Chlorinated tap water fortified with bromate at 2.0 ug/L.

METHOD 321.8 DETERMINATION OF BROMATE IN DRINKING WATERS BY ION CHROMATOGRAPHY INDUCTIVELY COUPLED PLASMA/MASS SPECTROMETRY

Revision 1.0

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METHOD 321.8

DETERMINATION OF BROMATE IN DRINKING WATERS BY ION CHROMATOGRAPHY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1. SCOPE AND APPLICATION

1.1 This method provides a procedure for determination of bromate in drinking water.

Analyte	Chemical Abstract Services Registry Numbers (CASRN)
Bromate (BrO_3^-)	15541-45-4

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Safe Drinking Water Act (SDWA)], consult both the appropriate sections of the Code of Federal Regulation (Part 141 § 141.23 for drinking water), and the latest Federal Register announcements.

1.3 This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS), and the interpretation of spectral and matrix interferences. A minimum of six months experience with commercial instrumentation is recommended. It is also recommended that the analyst have experience in liquid chromatography and the use of ICP-MS as a chromatographic detector.

1.4 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must document and have on file the required Initial Demonstration of Performance data described in Section 9.2 prior to using the method for analysis.

2. SUMMARY OF METHOD

2.1 An aliquot of a finished drinking water is passed through a preparatory cartridge capable of removing the trisubstituted haloacetic acids which interfere with the analysis of bromate. The sample is then injected onto a column which separates the remaining brominated haloacetic acids and bromide from the bromate. The ICP-MS is interfaced to the ion chromatograph and both mass 79 and mass 81 are monitored in time as bromate elutes from the column. The resulting signal is integrated and a concentration determined from a calibration curve. Mass 79 is used for quantitation while mass 81 provides isotope ratio information which can be used to screen for potential polyatomic interferences.

2.2 Chromatography: The chromatographic separation is based on an anion exchange resin. The sample is injected on the column and the matrix and analyte partition themselves between the mobile phase and the stationary phase as they move along the column. Early eluting analytes spend most of their time in the mobile phase while the late eluting compounds spend a larger percentage of their time interacting with the stationary phase. The matrix anions can influence retention times by blocking the interaction of the stationary phase with the analytes producing a shift in the retention time.

Inductively Coupled Plasma Mass Spectrometer: The detection technique is based on the use of an ICP-MS for the detection of trace elements[1-3]. The chromatographic eluent is introduced by pneumatic nebulization into a radio frequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by the mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the mass spectrometer are detected by an electron multiplier or Faraday detector and the ion information is processed by the data system. Interferences relating to the technique (Sect. 4) must be recognized.

Although ICP-MS is typically used for multi-analyte determinations, it is used in 321.8 for species specific quantification. In this mode the signal response is recorded via chromatographic or time resolved software. The use of ion chromatography in combination with ICP-MS detection has been reported for the detection of bromate [4-7].

3. DEFINITIONS

3.1 CALIBRATION BLANK - A volume of reagent water pH adjusted (to 10) with the same base as in the calibration standards.

3.2 CALIBRATION STANDARD (CAL) - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration. This solution is pH adjusted to 10.

3.3 INSTRUMENT PERFORMANCE CHECK (IPC) SOLUTION - A solution of method analytes, used to evaluate the performance of the instrumental system with respect to defined set of method criteria. Within this method, the IPC is identical to the laboratory fortified blank.

3.4 DRIFT STANDARD- A calibration standard added to a post column sample loop (via a second valve) which is transported into the plasma when the sample is injected. This analyte does not traverse the column and is used to compensate for instrumental (ICP-MS) drift during the analysis of a set of samples.

3.5 LABORATORY DUPLICATES (LD1 and LD2) - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of a number of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.6 LABORATORY FORTIFIED BLANK (LFB) - An aliquot of LRB to which known quantities of the method analyte is added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) - An aliquot of an environmental sample to which known quantities of the method analyte is added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

3.9 LINEAR DYNAMIC RANGE (LDR) - The concentration range over which the instrument response to an analyte is linear.

3.10 METHOD DETECTION LIMIT (MDL) - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.11 QUALITY CONTROL SAMPLE (QCS) - A solution of the method analyte of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance.

3.12 STOCK STANDARD SOLUTION - A concentrated solution containing the method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.13 TUNING SOLUTION - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses.

3.14 WATER SAMPLE - For the purpose of this method, a sample taken from a finished drinking water supply.

4. INTERFERENCES

4.1 Several interference sources may cause inaccuracies or imprecisions in the determination of bromate by ICP-MS. These are:

4.1.2 Abundance sensitivity - This is a property defining the degree to which the "wings" of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and operating pressure. "Wing" overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the mass spectrometer operating conditions adjusted to minimize the effect.

This interference is relevant in this method given the large $^{40}Ar^{40}Ar^{+}$ dimer adjacent to mass 79 which is present in conventional ICP-MS. The extent to which the dimer contributes to the signal on mass 79 can be determined by scanning over masses 76-83 using 20 points per amu (skipping mass 80) using a 5mM HNO_3 solution(See Figure 1). The interference signal from the argon dimer is apparent by examining the background signal at mass 79.4 relative to 76.4, 77.4 and 78.4. With proper mass calibration and adequate abundance sensitivity, the signal on masses 76.4, 77.4, and 78.4 should be close to normal photon background. The signal on 79.4 commonly is elevated relative to the above masses. This elevated signal is caused by the mass spectrometers insufficient abundance sensitivity.

The signal should decrease as the mass decreases from 79.5 to 79.3 etc. To determine if the instrument has adequate abundance sensitivity the decrease (79.5 to 79.3) in this signal should be extrapolated to mass 79.0 at which point it should be no higher than twice the normal photon background(See Figure 1). The signal may increase as mass 79.0 is approached depending on the bromide contamination in the 5mM HNO_3. The resolution etc., should be adjusted to minimize the dimers contribution to mass 79.0.

> Note: If the decrease in the signal from 79.5 to 79.0 does not have an inflection point, this may indicate that the abundance sensitivity is insufficient to resolve $^{79}Br^{+}$ from $^{40}Ar^{40}Ar^{+}$

4.1.3 Isobaric polyatomic ion interferences are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use[8]. These ions are commonly formed in the plasma or interface system from support gases or sample components. The two polyatomics

which are inherent to conventional ICP-MS are the $^{40}Ar^{38}ArH$ and $^{40}Ar^{40}ArH$. $^{40}Ar^{40}ArH$ contributes to the background signal on mass 81. To minimize this, it is recommended that the sample flow rate remain on between injections. This will produce smoother baselines on mass 81 and thereby produce more reproducible integrations on mass 81 for the early eluting peaks.

The ICP-MS interferences which apply to the detection of bromate are listed in Table 1. These spectral interferences are common to the plasma or produced by matrix anions eluting from the column. These interferences are outside the retention window for bromate but may cause baseline shifts which will degrade integration precision.

4.1.4 Physical interferences are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Samples containing high concentration of chloride (which elutes immediately after bromate) may cause chromatographic baseline shift possibly from a small change in the $^{40}Ar^{40}ArH$ production during the chloride elution. In addition, the removal of high concentrations of sodium and potassium which may exist in the drinking water are removed by using an anion self regenerating suppressor. This minimizes their deposition on the sampling cone and thus improves long term stability.

> **Note**: The mobile phase in this method was chosen based on its ability to produce a stable baseline and sensitivity over a multiple week period. The long term stability of the instrument is monitored by injecting a post column drift standard with each chromatogram. The analyte concentration in a sample is corrected by using this drift standard in the same fashion that an internal standard is used in Method 200.8[9].

4.1.5 Baseline Drift - This results when a constituent from the sample matrix is not quantitatively removed from the column leading to a slow column bleed of the strongly retained species. If this is suspected, the column should be flushed according to the manufacturer's recommendations. A slowly rising baseline can be caused by trisubstituted brominated haloacetic acids.

4.1.6 Chromatographic Interferences - The known chromatographic interferences for the determination of bromate in drinking water via ICP-MS detection are listed in Table 1 and their approximate retention characteristics are reported in Figure 2. These known interferences have been chromatographically resolved using the procedure described in this method.

Given the diversity of environmental samples, the possibility of unidentified interferences exist. The following section is written to provide the analyst with some guidance if an interference is identified. The two possible interferences are chromatographic overlap with a bromine containing species or a co-eluting polyatomic. In the case of co-elution with a bromine containing anion it is recommended that the analyst try the following method modifications in the order presented in an attempt to resolve the co-elution.

1.) Use weaker mobile phases (i.e. lower NH_4NO_3 concentrations).
2.) Alternative columns.
3.) Pretreatment cartridge which selectively removes the interference.

This co-elution should be documented and the changes in the mobile and stationary phase should produce a method capable of meeting all requirements in Section 9.

In the case of a co-elution with a polyatomic interference (on mass 79), the recommendation to the analyst is to try the following method modifications in the order presented in an attempt to resolve the co-elution.

1.) Use mass 81 for quantitation.
2.) Use weaker mobile phases.
3.) Alternative columns
4.) Pretreatment cartridge to selectively remove the interference.

This co-elution should be documented and the changes in the mobile and stationary phase should produce a method capable of meeting all requirements in Section 9.

4.1.7 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.2 micron require filtration to prevent damage to instrument columns and HPLC pumping system.

4.1.8 The analyst should be aware of the potential for carryover peaks from one analysis which will effect the proper detection of bromate in the subsequent analysis. Carryover was not observed in the analysis listed in Table 3 using the column, mobile phase and flow rate reported in Table 2. However, the analyst should be aware of the potential for carryover peaks.

4.1.9 Retention time shifts in ion chromatography are possible do to weak eluent strengths and high ionic strength matrices. These shifts are minimized by the eluent system reported in table 2. However, the analyst should be aware of the potential for retention time shifts do to high ionic strength matrices. These effects can be minimized by dilution of the sample matrix.

5. SAFETY

5.1 The toxicity or carcinogenicity of bromate and reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method [10,11]. A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis. Specifically, concentrated nitric presents various hazards as it is moderately toxic and extremely irritating to skin and mucous membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing, and observe proper mixing when working with these reagents.

5.2 Analytical plasma sources emit radio frequency radiation, in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

5.3 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6. EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma mass spectrometer. This instrument must meet the following requirements:

6.1.1 An instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 0.75 amu peak width at 5% peak height is required. This instrument may be fitted with a conventional or extended dynamic range detection system. The abundance sensitivity must be greater than 1.0×10^6 on the low side of mass 80 or such that the dimer's ($^{40}Ar^{40}Ar^{+}$)low mass shoulder does not influence mass 79.

6.1.2 Radio-frequency generator compliant with FCC regulations.

6.1.3 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical.

6.1.4 A variable-speed peristaltic pump may be used to pump the drain of the spray chamber to waste.

6.1.5 A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber may be of benefit in reducing the water vapor entering the plasma and thereby minimizing the $^{40}Ar^{40}ArH^{+}$. A double-pass spray chamber is recommended to increase background stability on mass 81 if $^{40}Ar^{40}ArH^{+}$ is present in the spectrum generated while nebulizing the mobile phase.

6.1.6 If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise, changes in instrument response or damage to the multiplier may result. This may be true for samples containing bromide in the parts per million range.

6.1.7 A nebulizer with a low dead volume is recommended.

6.2 Ion Chromatograph. This instrument must meet the following specifications:

6.2.1 Eluent Pump - Programmable flow high pressure pumping system capable of delivering pressures up to 3000 psi and flow rates up to 1.5 ml/min.

6.2.2 Control Valves - Inert double stacked pneumatic operated 4 way valves capable of withstanding 3000 psi.

6.2.3 Sample Loops- narrow bore, high pressure tefzel® tubing or equivalent.

6.2.4 Tubing- narrow bore high pressure tefzel® tubing or equivalent.

6.2.5 Guard and Analytical Column - Dionex PA-100 or equivalent.

6.2.6 Suppressor - Dionex (ASRS) anion self regenerating suppressor or equivalent.

6.2.7 Pretreatment Cartridges - Dionex On-Guard-RP or equivalent

6.3 Analytical balance, with capability to measure to 0.1 mg, for use in weighing samples and preparing standards.

6.4 An air displacement pipette capable of delivering volumes ranging from 50 to 2500 µL with an assortment of high quality disposable pipet tips. Calibration of the pipette should be verified frequently by weighing aliquots of distilled deionized water using the analytical balance to assure precision and accuracy of the pipette.

6.5 Labware - For determination of bromate, plastic labware has been used exclusively and measurable concentrations of bromate in the blank have not been observed.

6.5.1 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with ETFE (ethylene tetrafluorethylene) screw closure, 125-mL to 250-mL capacities.

6.5.2 One-piece stem FEP wash bottle with screw closure, 125-mL capacity.

6.5.3 Syringes- 10cc Becton-Dickinson plastic syringes or equivalent.

7. REAGENTS AND STANDARDS

7.1 Reagents may contain elemental impurities that might affect the integrity of analytical data. Due to the high sensitivity of ICP-MS, high-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade. The acid used to prepare the 5mM HNO_3 in the mobile phase does contain some bromide background. Care should be taken to minimize this background intensity.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.2 Nitric acid (1+1) - Add 500 mL conc. nitric acid to 400 mL of reagent grade water and dilute to 1L.

7.1.3 Ammonium Nitrate - Fisher ACS certified or equivalent.

7.1.4 Sodium Hydroxide - Fisher 50/50 liquid mixture or equivalent.

7.2 Reagent water - All references to reagent grade water in this method refer to ASTM type I water (ASTM D1193)[12]. Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

7.3 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals. These standards have extremely low ionic strengths and should be pH adjusted to 10 (with NaOH)in order to minimize bromate interaction with the plastic sample loop tubing[6]. Replace stock standards when they can not be verified with QC standards.

7.3.1 Preparation of calibration standards - a fresh bromate standard should be prepared once a month or as needed. Dilute the stock bromate standard solution to levels appropriate to the operating range of the instrument using reagent water and adjust the pH to 10 using NaOH. The bromate

concentrations in the standards should be sufficiently high to produce good isotope ratio precision (<2%) and to accurately define the slope of the response curve. Depending on the sensitivity of the instrument, concentrations ranging from 10 µg/L to 50 µg/L are suggested.

Note: A blank and one calibration standard or multi-point calibration can be utilized to calibrate the response of the instrument.

7.4 Drift standard - The drift standard should be made such that its concentration is approximately five times higher than the bromate calibration standard. (This will compensate for the sample loop volume difference between the sample and the drift standard.) The drift standard concentration should be chosen based on analytical precision (< 5% rsd of replicate peak integration).

7.5 Blanks - The calibration blank is used to establish and verify the analytical calibration.

7.5.1 Calibration blank - Consists of reagent grade water which is pH adjusted to 10 with sodium hydroxide.

7.6 Tuning Solution - This solution is used for instrument tuning (lens, argon flows etc.), mass calibration and abundance sensitivity prior to analysis. The tuning solution should be approximately twice the bromate calibration standard concentration (producing greater than 30,000cps) and should be delivered to the plasma using a peristaltic pump at a flow rate of 1 mL/min. The instrument should be tuned for maximum signal-to-noise using mass 79. After tuning, the tuning solution should be analyzed by scanning over masses 76-84 using 20 points per amu (skipping mass 80). This data can be used to verify the mass calibration (mass shifts of greater than 0.1 amu should be corrected) and check the instrument sensitivity (approximately 35,000cps/100ppb bromate given the instrument conditions outlined in Table 2). The calibration blank or a 5mM HNO_3 solution should then be analyzed using the same scanning conditions to check the mass analyzer's abundance sensitivity. This preanalysis routine should be performed daily(see figure 1).

7.7 Quality Control Sample (QCS) - The QCS should be obtained from a source outside the laboratory. The concentration of the QCS solution analyzed will depend on the sensitivity of the instrument. To prepare the QCS dilute an appropriate aliquot bromate to a concentration which is approximately 75% of the concentration of the highest calibration standard. The QCS should be analyzed as needed to meet data-quality needs and a fresh solution should be prepared quarterly or more frequently as needed.

7.8 Laboratory Fortified Blank (LFB) - To an aliquot of Calibration Blank add an aliquot of the stock standard solution to prepare the LFB. The fortified concentration should

produce percent relative standard deviations of 4-7% on replicate determinations. The LFB must be carried through the same entire preparation scheme as the samples. This solution should be pH adjusted to 10 with sodium hydroxide.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 The pH of all aqueous samples should be tested immediately prior to analysis to ensure the sample has been properly preserved. If the sample requires a pH adjustment throughly mix the sample after the sodium hydroxide has been added. The pH of the sample must be adjusted to 10. This pH adjustment should be performed just prior to analysis. This pH adjustment assures the solubility of bromate within the sample loop[6].

8.2 If required by the data user, prepare a field blank using reagent water. Use the same sample containers (see section 6.5.1 for container recommendations)as used in sample collection.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory calibration blanks, fortified blanks and calibration solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data generated.

> **NOTE:** Because the sample preparation step of this method is limited to pH adjustment prior to analysis, the number of required solutions needed to verify data quality has been reduced. In this method the calibration blank (Section 7.5 and 9.3) is used to establish baseline calibration and is used to verify the absence of contamination. The laboratory fortified blank (Sections 7.8 and 9.3) is used to assess both method accuracy and instrument performance.

9.2 Initial Demonstration of Performance (mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration range and analysis of quality control sample) and laboratory performance (determination of method detection limit) prior to analyses conducted by this method.

9.2.2 Linear dynamic range - Linear dynamic range is detector or chromatographic resolution limited.

The useable linear range must be determined for the instrument configuration to be used.

Note: The linear dynamic range may be limited by the chromatographic resolution of bromate from bromoacetic acid or other interferences. In this situation, the linear calibration range is limited to a concentration of bromate which can be chromatographically resolved from bromoacetic acid. Given the experimental conditions in Table 2, the linear dynamic range was limited to 50µg/L based on chromatographic resolution.

9.2.3 Quality control sample (QCS) - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. To verify the calibration standards, the determined mean concentration from three analyses of the QCS must be within ± 10% of the stated QCS value. If the calibration standards cannot be verified, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.4 Method detection limit (MDL)- This should be established using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit[13]. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t)_{(n-1,1-alpha=0.99)} \times (S)$$

where:

$(t)_{(n-1,1-alpha=0.99)}$ = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t=3.14 for seven replicates].

n = number of replicates

S = standard deviation of the replicate analyses.

The MDL should be determined annually, when a new operator begins work or whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

The MDL for bromate using the conditions listed in Table 2 is 0.3 µg/L.

9.3 Assessing Laboratory Performance (mandatory)

9.3.1 Calibration blank - **Within this method a calibration blank and the**

laboratory reagent blank are operationally the same. The calibration blank will be used as the LRB and a fresh calibration blank should be made daily to verify the lack of contamination.
Analysis of the calibration blank can be used to verify the calibration baseline and to assess chromatographic carryover interference or contamination. The calibration blank should be analyzed as a sample. If the calibration blank produces an integrable signal for bromate, the laboratory should find the source of this problem prior to analyzing samples. The laboratory must complete the analysis of one calibration blank with each batch of 20 samples.

9.3.2 Laboratory fortified blank (LFB) - **Within this method the LFB is used to assess both laboratory and instrument performance.** The laboratory must analyze at least one LFB (Sect. 7.8) immediately after calibration and after each 10 samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - CB}{s} \times 100$$

where:

R	=	percent recovery.
LFB	=	laboratory fortified blank concentration.
CB	=	calibration blank concentration.
S	=	concentration equivalent of analyte added to fortify the CB solution.

If the recovery falls outside the required control limits of 85-115%, bromate is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data become available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent

twenty to thirty data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of

concentrations included in the LFB.
These data must be kept on file and be available for review.

> **Note:** Using the experimental conditions in Table 2, the average recovery of the LFB was 99.8% with a three sigma control limit of 10.2%

9.3.4 Instrument performance - For all determinations the laboratory must check instrument performance and verify that the instrument is properly calibrated on a continuing basis. This is accomplished via the recovery on the LFB (85-115%,section 9.3.2) and monitoring the instrument drift standard injected with each sample.

9.3.5 Instrument Drift Standard - The analyst is expected to monitor the response from the instrument drift standard(in each sample) throughout the sample set being analyzed. The absolute response of any one drift standard must not deviate more than 70-130% of the original response associated with the calibration blank. If deviations greater than these are observed, the reason for the drift should be investigated. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 The chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure is required.

9.4.2 The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The added bromate concentration must be the same as that used in the laboratory fortified blank. Over time all routine sample sources should be fortified.

9.4.3 Calculate the percent recovery for bromate, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Percent recovery may be calculated using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where: R = percent recovery.
C_s = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of bromate added to fortify the sample.

Note: The precision and recovery in six drinking water matrices are reported in Table 3.

9.4.4 If recovery falls outside the designated range and laboratory performance is shown to be in control, the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for the unfortified sample is suspect due to an uncorrected matrix effect.

10. CALIBRATION AND STANDARDIZATION

10.1 Operating conditions - Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements of this method and to maintain quality control data verifying instrument performance and analytical results. Instrument operating conditions which were used to generate precision and recovery data for this method are included in Table 2.

10.2 Precalibration routine - The following precalibration routine must be completed prior to calibrating the instrument.

10.2.1 Initiate proper operating configuration of instrument and data system. Allow a period of not less than 30 min for the instrument to warm up. Conduct mass calibration and resolution checks using the tuning solution. The tuning solution should be analyzed by scanning over masses 76-83 using 20 points per amu (skipping mass 80). For good performance, adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass. The abundance sensitivity must be checked by analyzing the calibration blank(See Figure 1).

10.3 Instrument drift - An instrument drift solution must be injected into the column effluent at the same time a sample is injected onto the column to verify instrument

drift throughout the analysis of a set of samples. This solution does not traverse the column. The correction factor is calculated by ratioing the drift standard response in the calibration solution to the drift standard response in the current sample. This

correction factor is then multiplied by the sample concentration. This is similar to an internal standard correction used in EPA Method 200.8[9].

> **Note:** The stability of the baseline on mass 81 is strongly influenced by the IC pump being turned on and off. Therefore it is recommended that this pump remain on throughout an analysis set.

10.4 Calibration - Prior to initial calibration, set up proper instrument software routines for the collection of time resolved data. (See Table 2 for the experimental parameters used to collect the data within the method.) The instrument must be calibrated using the calibration blank and a calibration standard prepared at one or more concentration levels. If single point calibration is used, the standard concentration should be near the determined upper linear range.

10.5 The rinse blank should be used to flush the IC injection loop and by-pass loops. This procedure is recommended between injections.

11. PROCEDURE

11.1 Aqueous Sample Preparation - The sample must be adjusted to pH 10 prior to analysis. The sample must be room temperature and pretreated with an on-guard RP cartridge prior to analysis. The RP cartridges were used according to the manufacturer's recommendations. Trisubstituted haloacetic acids (removed by the RP cartridge) can cause a slowly rising baseline because these trisubstituted haloacetic acids are strongly retained on the column.

11.2 The sample is injected onto the column at the same time the instrument drift standard is injected into the post column mobile phase. For sample and drift standard sample loop volumes see table 2.

11.3 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Sample data should be reported in units of µg/L for aqueous samples. Do not report bromate concentrations below the determined MDL. Drift standard correction should be applied to all sample concentrations.

12.2 For data values less than ten, two significant figures should be used for reporting element concentrations. For data values greater than or equal to ten, three significant figures should be used.

12.3 If additional dilutions were made to any samples, the appropriate dilution factor should be applied.

12.4 The primary quantitative isotope should be 79 because it has the most stable background and is less prone to the known interferences. Mass 81 should be monitored to verify that the bromide isotope ratio within the retention window is near unity. A ratio of the two isotopes can provide useful information for the analyst in detecting a possible spectral interference.

12.5 The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results.

13. METHOD PERFORMANCE

13.1 Instrument operating conditions used for single laboratory testing of the method are summarized in Table 2.

13.2 Data obtained from single laboratory testing of the method are summarized in Table 3 for six drinking water samples. The average concentrations reported in the second column are the native bromate concentrations. The percent relative standard deviations associated with the native concentrations are reported in the second column. The samples were then fortified with 25µg/L bromate. The average recovery and precision of this recovery is reported in the 4th and 5th columns respectively.

14. POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *"Less is Better: Laboratory Chemical Management for Waste Reduction"*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202)872-4477.

15. WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *"The Waste Management Manual for Laboratory Personnel"*, available from the American Chemical Society at the address listed in the Section 14.2.

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. SPECTRAL AND CHROMATOGRAPHIC INTERFERENCES

Spectral Interferences

Interferent Source	MASS 79	MASS 81
Plasma	$^{40}Ar^{38}ArH^+$	$^{40}Ar^{40}ArH^+$
Sulfate*		SO_3H^+
Phosphate**	PO_3^+	$PO_3H_2^+$
Potassium	$^{40}Ar^{39}K^+$	

* Determined in 300 µg/mL sulfate.
** Determined in 100 µg/mL phosphate.

Potential Chromatographic or Coelution Interferences

	Retention Time*
Bromate	230
Bromoacetic Acid	170
Dibromoacetic Acid	460
Bromochloroacetic Acid	400
Bromide	570
Phosphate Matrix (PO_3^+)	180
Sulfate Matrix (SO_3H^+)	400

*Based on experimental conditions listed in Table 2. Reported in seconds to the leading edge of the peak using the drift standard as t = 0.

TABLE 2. EXPERIMENTAL CONDITIONS FOR THE DETECTION OF BROMATE VIA ICP-MS

ICP-MS Experimental Condition and Detection Limit	
Instrument	Upgraded VG Elemental PQ1
Power	1.4 KW
Cool Gas	12.0 L/min
Aux Gas	1.2 L/min
Nebulizer Gas	0.957 L/min (Concentric)
m/z Monitored	79 and 81
Analysis Mode	Time Resolved or Chromatographic
Time Slice	0.4 seconds
Spray Chamber	5°C
Sensitivity (100µg/L BrO_3^-)	35,000cps m/z 79
Background ($5mM\ HNO_3 + 25mM\ NH_4NO_3$)	100 cps m/z=79; 2500cps m/z=81
Detection Limit	0.3µg/L
Chromatographic Experimental Conditions	
Chromatograph	Dionex Gradient GPM-2
Column	PA100 Guard and Analytical
Flowrate	1 mL/min.
Pretreatment Cartridge	On-Guard RP
Mobile Phase	$5mM\ HNO_3 + 25mM\ NH_4NO_3$ (Isocratic)
Sample Loop	580 µL (based on i.d. and length)
Drift Standard Loop	170 µL

TABLE 3. PRECISION AND RECOVERY DATA FOR BROMATE IN OZONATED DRINKING WATER*

WATER	AVERAGE CONCENTRATION ng/mL	%RSD Of AVG CONC.	AVG%** RECOVERY LFM	%RSD Of LFM RECOVERY
1	22.2	5.5	97	3.6
2	3.0	6.4	98	1.4
3	10.1	3.6	98	3.4
4	2.7	5.1	96	3.8
5	1.3	10.6	96	3.0
6	0.8	15.5	102	2.4

* n=5 for all analyses, determined using experimental conditions listed in Table 2.

** Fortified with 25µg/L Bromate

Figure 1: Abundance Sensitivity Considerations for Bromate Analysis

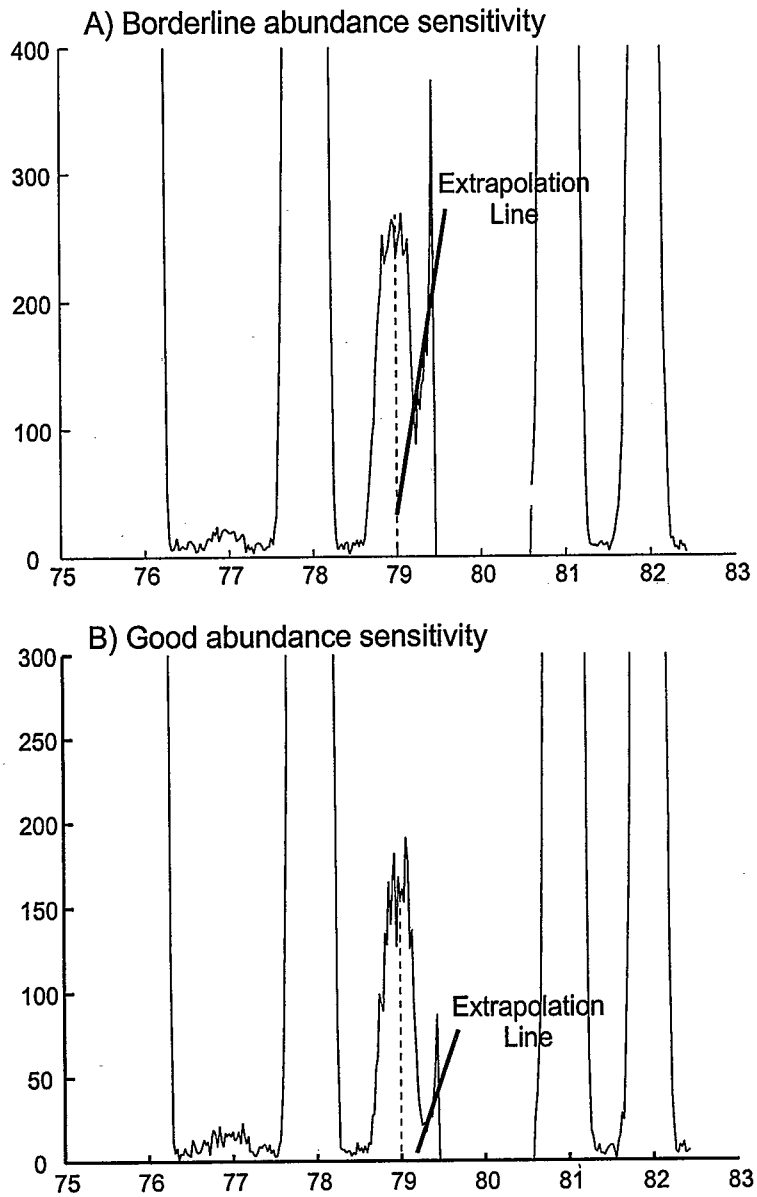
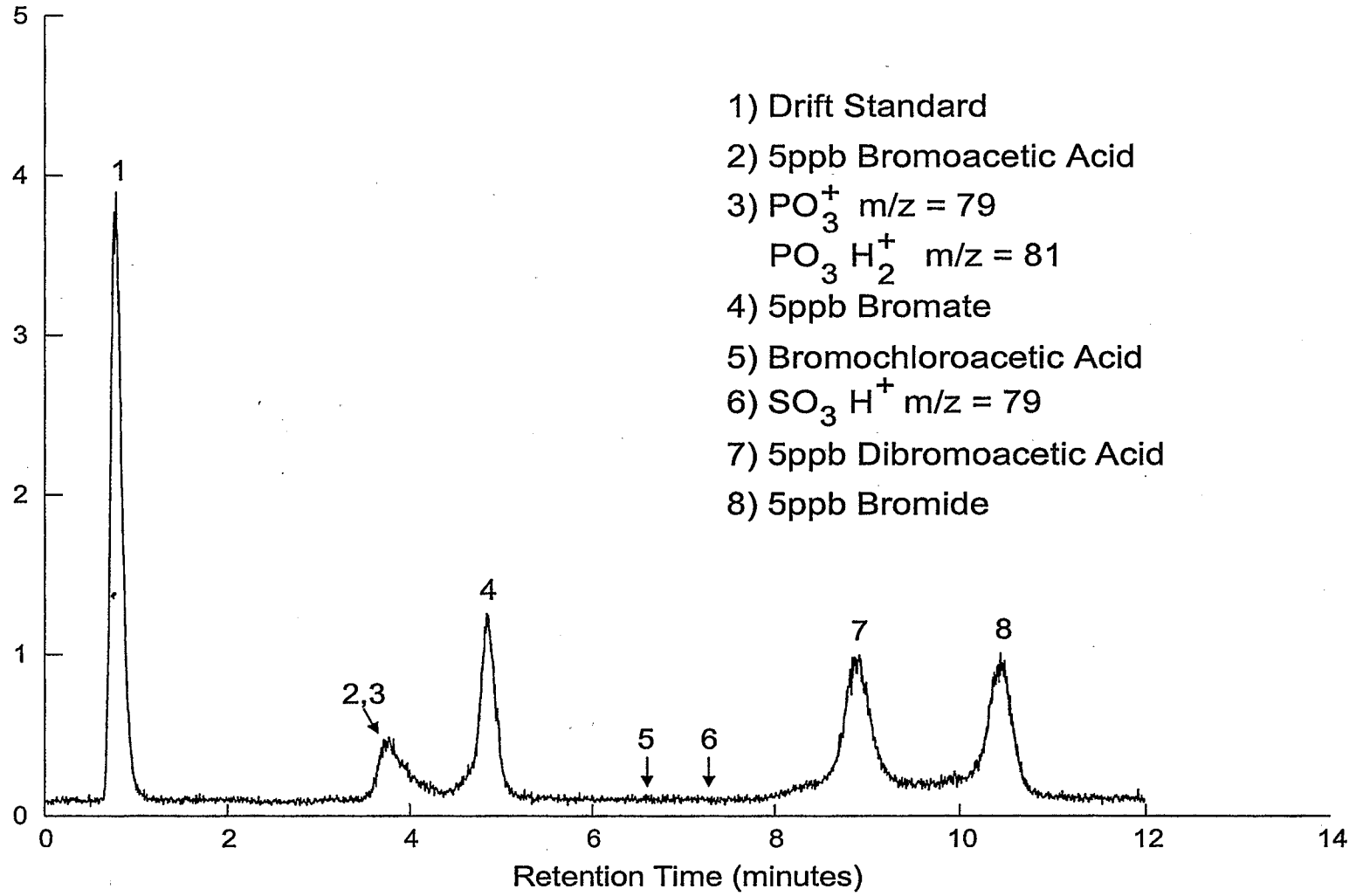


Figure 2: Sample Chromatogram Indicating Elution Order



METHOD 515.3 DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION, DERIVATIZATION AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION.

Revision 1.0

July 1996

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METHOD 515.3

DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION, DERIVATIZATION AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic (GC) method (1-12) applicable to the determination of the listed chlorinated acids in drinking water, ground water, raw source water and water at any intermediate treatment stage.

Analyte	Chemical Abstract Services Registry Number
Acifluorfen[a]	50594-66-6
Bentazon	25057-89-0
Chloramben	133-90-4
2,4-D	94-75-7
Dalapon	75-99-0
2,4-DB	94-82-6
Dacthal acid metabolites[b]	
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid	51-36-5
Diclorprop	120-36-5
Dinoseb	88-85-7
5-Hydroxydicamba	7600-50-2
4-Nitrophenol	100-02-7
Pentachlorophenol	87-86-5
Picloram	1918-02-1
2,4,5-T	93-76-5
2,4,5-TP (Silvex)	93-72-1

[a] The herbicide Lactofen will be quantitated as Acifluorfen as their structures represent different esters of the same carboxylate moiety.

[b] Dacthal monoacid and diacid metabolites as well as the parent di-ester included in method scope; Dacthal diacid used for validation studies.

1.2 This method is also applicable to the determination of salts and esters of analyte acids. The form of each acid is not distinguished by this method. Results are calculated and reported for each listed analyte as the total free acid.

1.3 Experimentally determined method detection limits (MDLs) (Section 9.4) for the above listed analytes are provided in Tables 2 and 3. Actual MDLs may vary according to the particular matrix analyzed and the specific instrumentation employed.

1.4 This method is designed for analysts skilled in liquid-liquid extractions, derivatization procedures and the use of GC and interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 9.3.

1.5 When this method is used for the analyses of waters from unfamiliar sources, it is strongly recommended that analyte identifications be confirmed by GC using a dissimilar column or by GC/MS if concentrations are sufficient.

1.6 When using the diazomethane derivatization procedure, it is recommended that only qualitative identification be performed for 4-nitrophenol and 5-hydroxydicamba. Examination of supporting data presented in Tables 2, 4, 6, 8, 10 and 12 shows control over precision has not been achieved for these method analytes, and quantitative identification is therefore not recommended.

1.7 When using the base-promoted methylation procedure, it should be noted that the esterification efficiences of dinoseb and picloram were found to be less than 50%. Although the supporting data presented in Tables 3, 5, 7, 9, 11 and 13 demonstrates that accurate and precise data can be obtained through the use of procedural standards, care should be exercised.

1.8 5-Hydroxydicamba was not recovered from chlorinated waters. The exact interaction between this compound and the free chlorine is not known. The extremely low recoveries of 5-hydroxydicamba found in Tables 8 and 9 serve to illustrate this. As noted, the matrix used to obtain these results was local chlorinated tap water that was first fortified and then dechlorinated. (Note that in further experiments, 5-hydroxydicamba was recovered in waters that were dechlorinated prior to fortification.)

2. SUMMARY OF METHOD

2.1 A 40-mL volume of sample is adjusted to pH 12 with 4N sodium hydroxide for one hour to hydrolyze derivatives. (NOTE: Since many of the analytes contained in this method are applied as a variety of esters and salts, it is imperative to hydrolyze them to the parent acid prior to extraction). The aqueous sample is then acidified and extracted with 4-mL of methyl-tert-butyl-ether (MtBE). The chlorinated acids that have been partitioned into the organic phase are then converted to their methyl esters by one of two derivatization techniques. The first uses

diazomethane as the methylating agent; the second is a base-promoted esterification procedure and involves the addition of tetramethylammonium hydroxide followed by the addition of methyl iodide. The target esters are then identified and measured by capillary column gas chromatography using an electron capture detector (GC/ECD). Analytes are quantitated using procedural standard calibration.

3. DEFINITIONS

3.1 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.2 SURROGATE ANALYTE (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.3 LABORATORY DUPLICATES (LD1 AND LD2) -- Two aliquots of the same sample designated as such in the laboratory. Each aliquot is extracted, derivatized and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 FIELD DUPLICATES (FD1 AND FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that are treated exactly as a sample including exposure to all glassware, equipment, solvents, preservation and other reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to

sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB should be treated like a sample including the addition of all preservation and other reagents. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, including the addition of all preservation and other reagents, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.10 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.11 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.12 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentration which is used to fortify an aliquot of reagent water or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.13 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) -- A solution of selected method analytes used to evaluate the performance of the instrumental system with respect to a defined set of method criteria.

3.14 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be detected, identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.15 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data including storage, spill, and handling precautions.

3.16 ESTIMATED DETECTION LIMIT (EDL) - Defined as either the MDL or a level of a compound in a sample yielding a peak in the final extract with a signal to noise (S/N) ratio of approximately 5, whichever is greater.

3.17 PROCEDURAL STANDARD QUANTITATION -- A quantitation method where aqueous calibration standards are prepared and processed (e.g. purged, extracted and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

3.18 CONTINUING CALIBRATION CHECK (CCC) -- A procedural calibration standard containing the method analytes, which is extracted, derivatized and analyzed to verify the accuracy of the existing calibration curve or response factors for those analytes.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.5. Subtracting blank values from sample results is not permitted.

4.1.1 Glassware must be scrupulously cleaned. (1) Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap water and reagent water. Drain and heat in an oven or muffle furnace at 400°C for 1 hr. Do not heat volumetric ware. (Thorough rinsing with reagent grade acetone may be substituted for the heating. Thermally stable materials such as PCBs may not be eliminated by this treatment.) After drying and cooling, store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Solvent blanks should be analyzed for each new bottle of solvent before use. An interference free solvent is a solvent containing no peaks yielding data at ≥ MDL (Tables 2 and 3) at the retention times of the analytes of interest. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Routine between-sample rinsing of the sample syringe and associated equipment with MTBE can minimize sample cross-contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

4.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Analyte identifications should be confirmed using the confirmation column specified in Table 1 or another column that is dissimilar to the primary column or by GC/MS if the concentrations are sufficient.

4.4 Interferences by phthalate esters can pose a major problem in pesticide analysis when using an electron-capture detector. These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates that are easily extracted or leached during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination. (2,3)

4.5 The presence of water may cause incomplete methylation when using the base-promoted esterification procedure. It is imperative to ensure that all reagents and glassware are completely free of water.

4.6 5-Hydroxydicamba was not recovered from chlorinated waters. The exact interaction between this compound and the free chlorine is not known. The extremely low recoveries of 5-hydroxydicamba found in Tables 8 and 9 serve to illustrate this. As noted, the matrix used to obtain these results was local chlorinated tap water that was first fortified and then dechlorinated. (Note that in

further experiments, 5-hydroxydicamba was recovered in waters that were dechlorinated prior to fortification.)

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (4-6) for the information of the analyst.

5.2 The toxicity of the extraction solvent, MTBE, has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. Therefore protective clothing and gloves should be used and MTBE should be used only in a chemical fume hood or glove box. The same precaution applies to pure standard materials.

5.3 Diazomethane is a toxic carcinogen which can explode under certain conditions. The following precautions must be followed:

5.3.1 Use the diazomethane generator behind a safety shield in a well ventilated fume hood. Under no circumstances can the generator be heated above 90°C, and all grinding surfaces such as ground glass joints, sleeve bearings and glass stirrers must be avoided. To minimize safety hazards, the diazomethane generator apparatus used in the esterification procedure (Section 11.2) produces micromolar amounts of diazomethane in solution. If the procedure is followed exactly, no possibility for explosion exists.

5.4 Methyl iodide is a toxic carcinogen. When handling, protective clothing and gloves should be worn, and this reagent should only be used in a fume hood or glove box.

6. APPARATUS AND EQUIPMENT

6.1 SAMPLE CONTAINERS -- Amber glass bottles, approximately 50 mL, fitted with teflon-lined screw caps.

6.2 EXTRACTION VIALS -- 60 mL clear glass vials with teflon-lined screw caps.

6.3 VIALS -- Autosampler, 2.0 mL vials with screw or crimp cap and a teflon-faced seal.

6.4 STANDARD SOLUTION STORAGE CONTAINERS -- 10-20 ml amber glass vials with teflon-lined screw caps.

6.5 GRADUATED CONICAL CENTRIFUGE TUBES WITH TEFLON-LINED SCREW CAPS -- 15-mL with 1 mL graduation markings.

6.6 BLOCK HEATER (or SAND BATH) -- Capable of holding screw cap conical centrifuge tubes in Section 6.5.

6.7 PASTEUR PIPETS -- Glass, disposable.

6.8 PIPETS -- 2.0 mL and 4.0 mL, type A, TD, glass.

6.9 VOLUMETRIC FLASKS -- 5 ml, 10 mL, 100 mL.

6.10 MICRO SYRINGES -- 10 µL, 25 µL, 50 µL, 100 µL, 250 µL, 500 µL and 1000 µL.

6.11 BALANCE -- analytical, capable of weighing to 0.0001 g.

6.12 DIAZOMETHANE GENERATOR -- See Figure 1 for a diagram of an all glass system custom made for these validation studies. A micromolar generator is also available from the Aldrich Chemical Company.

6.13 GAS CHROMATOGRAPH -- Analytical system complete with gas chromatograph equipped for electron-capture detection, split/splitless capillary or direct injection, temperature programming, differential flow control, and with all required accessories including syringes, analytical columns, gases and strip-chart recorder. A data system is recommended for measuring peak areas. An autoinjector is recommended for improved precision of analyses. The gases flowing through the electron-capture detector should be vented through the laboratory fume hood system.

6.14 PRIMARY GC COLUMN -- DB-1701 [fused silica capillary with chemically bonded (14% cyanopropylphenyl)-methylpolysiloxane)] or equivalent bonded, fused silica column, 30 m x 0.25 mm ID, 0.25 µm film thickness.

6.15 CONFIRMATORY GC COLUMN -- DB-5.625 [fused silica capillary with chemically bonded (5% phenyl)-methylpolysiloxane)] or equivalent bonded, fused silica column, 30m x 0.25mm ID, 0.25 µm film thickness.

7. REAGENTS AND STANDARDS

7.1 REAGENT WATER -- Reagent water is defined as a water in which an interference is not observed ≥ to the MDL of each analyte of interest.

7.1.1 A Millipore Super-Q water system or its equivalent may be used to generate deionized reagent water. Distilled water that has been passed through granular charcoal may also be suitable.

7.1.2 Reagent water is monitored through analysis of the laboratory reagent blank (Section 9.5).

7.2 SOLVENTS

7.2.1 METHYL tert-BUTYL ETHER (MtBE) -- High purity, demonstrated to be free from analytes and interferences, redistilled in glass if necessary.

7.2.2 ACETONE -- High purity, demonstrated to be free from analytes and interferences.

7.2.3 CARBITOL (DIETHYLENE GLYCOL MONOETHYL ETHER) -- High purity, demonstrated to be free from analytes and interferences.

7.2.4 ETHYL ETHER -- High purity, unpreserved, demonstrated to be free from analytes and interferences.

7.3 REAGENTS

7.3.1 SODIUM SULFATE, Na_2SO_4 -- (ACS) granular, anhydrous. If interferences are observed, it may be necessary to heat the sodium sulfate in a shallow tray at 400°C for up to 4 hr. to remove phthalates and other interfering organic substances. Alternatively, it can be extracted with methylene chloride in a Soxhlet apparatus for 48 hr. Store in a capped glass bottle rather than a plastic container.

7.3.2 ACIDIFIED SODIUM SULFATE -- Acidify by slurrying 500g of muffled sodium sulfate with enough ethyl ether to just cover the solid. Add 0.7 mL concentrated sulfuric acid dropwise while mixing thoroughly. Remove the ether under vacuum. Mix 1g of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. The pH must be below pH 4. Store at 100°C.

7.3.3 COPPER II SULFATE PENTAHYDRATE, $CuSO_4 5H_2O$ -- ACS reagent grade.

7.3.4 SODIUM HYDROXIDE, pellets -- ACS reagent grade.

7.3.5 POTASSIUM HYDROXIDE, pellets -- ACS reagent grade

7.3.6 SODIUM THIOSULFATE, $Na_2S_2O_3$ -- ACS reagent grade, used as a dechlorinating agent in this method.

7.3.7 DIAZALD -- ACS reagent grade.

7.3.8 SULFURIC ACID, CONCENTRATED -- ACS reagent grade

7.3.9 METHYL IODIDE -- ACS reagent grade

7.3.10 TETRABUTYLAMMONIUM HYDROXIDE -- ACS reagent grade. This reagent can be purchased as a 1.0M solution in methanol. It is important that it contain no water as moisture may result in incomplete methylation.

7.3.11 SILICA GEL -- ACS reagent grade. If interferences are observed, it may be necessary to heat this reagent at 100°C for 1 hour.

7.3.12 FLORISIL -- 60-100/PR mesh. Activate by heating in a shallow container at 150°C for at least 24 hours and not more than 48 hours.

7.4 SOLUTIONS

7.4.1 4N NaOH SOLUTION -- Dissolve 16g NaOH pellets in reagent water and dilute to 100 mL.

7.4.2 37% (w/v) KOH SOLUTION -- Dissolve 37g KOH pellets in reagent water and dilute to 100 mL.

7.4.3 DIAZALD SOLUTION -- Prepare a solution containing 5g Diazald in 50 mL of a 50:50 by volume mixture of ethyl ether and carbitol. This solution is stable for one month or longer when stored at 4°C in an amber bottle with a Teflon-lined screw cap.

7.5 STANDARDS

7.5.1 4,4'-DIBROMOOCTAFLUOROBIPHENYL, 99+% -- For use as the internal standard. Prepare a stock internal standard solution of 4,4'-Dibromooctafluorobiphenyl by accurately weighing approximately 0.0200 g of neat material. Dissolve the neat material in MtBE and dilute to volume in a 10 mL volumetric flask. Transfer the solution to an amber glass vial with a teflon-lined screw cap and store at 4°C. The resulting concentration of the stock internal standard solution will be approximately 2.0 mg/mL. Prepare a primary dilution standard at approximately 2.5 µg/mL by the addition of 12.5 µL of the stock standard to 10 mL of MtBE. Transfer the primary dilution to an amber glass vial with a teflon-lined screw cap and store at 4°C. Addition of 10 µL of the primary dilution standard to the final 1 mL extract results in a final internal standard concentration of 25 ng/mL. The solution should be replaced when ongoing QC indicates a problem. This compound has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the QC requirements in Section 9 are met.

7.5.2 2,4-DICHLOROPHENYLACETIC ACID, 99+% -- For use as a surrogate compound. Prepare a surrogate stock standard solution of 2,4-Dichlorophenylacetic acid by accurately weighing approximately 0.0100 g of neat material. Dissolve the neat material in acetone and dilute to volume in a 10 mL volumetric flask. Transfer the solution to an amber glass vial with a teflon-lined screw cap and store at 4°C. The resulting concentration of the stock surrogate solution will be approximately 1.0 mg/mL. Prepare a primary dilution standard at approximately 100 µg/mL by the addition of 1 mL of the stock standard to 10 mL of acetone. Transfer the primary dilution to an amber glass vial with a teflon-lined screw cap and store at 4°C. Addition of 10 µL of the primary dilution standard to the 40 mL aqueous sample results in a surrogate concentration of 25 µg/L. The solution should be replaced when ongoing QC indicates a problem. This compound has been shown to be an effective surrogate for the method analytes, but other compounds may be used if the QC requirements in Section 9 are met.

7.5.3 STOCK STANDARD SOLUTION (SSS) -- Prepare separate stock standard solutions for each analyte of interest at a concentration of 1-5 mg/mL in acetone. Method analytes may be obtained as neat materials or ampulized solutions (> 99% purity) from a number of commercial suppliers but ampulized solutions should not be used if the solvent is methanol. (7) These stock standard solutions should be stored at 4°C. They are

stable for at least one month but should be checked frequently for signs of evaporation.

7.5.3.1 For analytes which are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.01 to 0.05 grams of pure material in a 10.0 mL volumetric flask. Dilute to volume with acetone. Each compound's purity must be assayed to be 96% or greater.

7.5.3.2 Stock standard solutions for analytes which are liquid in their pure form at room temperature can be accurately prepared in the following manner.

7.5.3.3 Place about 9.8 mL of acetone into a 10.0 mL volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes to allow solvent film to evaporate from the inner walls of the volumetric, and weigh to the nearest 0.1 mg.

7.5.3.4 Use a 10 µL syringe and immediately add 10.0 µL of standard material to the flask by keeping the syringe needle just above the surface of the acetone. Be sure that the standard material falls dropwise directly into the acetone without contacting the inner wall of the volumetric.

7.5.3.5 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in milligrams per milliliter from the net gain in weight.

7.5.4 PRIMARY DILUTION STANDARD (PDS) – Prepare the primary dilution standard solution by combining and diluting stock standard solutions with acetone. This primary dilution standard solution should be stored at 4°C. It is stable for at least one month but should be checked before use for signs of evaporation. As a guideline to the analyst, the primary dilution standard solution used in the validation of this method is described below.

	Concentration,µg/mL
Acifluorfen	5.0
Bentazon	10.
Chloramben	5.0
2,4-D	10.
Dalapon	10.
2,4-DB	10.
Dacthal acid metabolites	5.0
Dicamba	5.0
3,5-Dichlorobenzoic acid	5.0
Diclorprop	10.
Dinoseb	10.
5-Hydroxydicamba	5.0
4-Nitrophenol	10.
Pentachlorophenol	1.0
Picloram	10.
2,4,5-T	2.5
2,4,5-TP (Silvex)	2.5

This primary dilution standard is used to prepare calibration standards, which comprise five concentration levels of each analyte with the lowest standard being at or near the MDL of each analyte. The concentrations of the other standards should define a range containing the expected sample concentrations or the working range of the detector.

7.5.5 CALIBRATION STANDARDS (CAL) – A five-point calibration curve is to be prepared by diluting the primary dilution standard into acetone at the appropriate levels. A designated amount of each acetone calibration standard is then spiked into separate 40 mL aliquots of reagent water to produce a calibration curve ranging from near the detection limit to approximately 10-20 times the lowest calibration level. These aqueous calibration standards should be treated like samples and therefore require the addition of all preservation and other reagents. They are extracted by the procedure set forth in Section 11. (The calibration standard solutions in acetone should be stored at 4°C. They are stable for at least one month but should be checked frequently for signs of evaporation.)

7.5.6 LABORATORY PERFORMANCE CHECK STANDARD (LPC) – The low level standard of the calibration curve can serve as the LPC standard. (Section 9.2)

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 SAMPLE VIAL PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices (8) using amber glass containers with TFE-lined screw-caps and capacities of at least 50 ml.

8.1.2 If residual chlorine is present, add 4 mg of sodium thiosulfate per 50 mL of sample to the sample bottle prior to collecting the sample.

8.2. SAMPLE COLLECTION

8.2.1 Fill sample bottles but take care not to flush out the sodium thiosulate. Because the target analytes of this method are not volatile, it is not necessary to ensure that the sample bottles are completely headspace free.

8.2.2 When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Remove the aerator so that no air bubbles can be visibly detected and collect samples from the flowing system.

8.2.3 When sampling from an open body of water, fill a 1-quart wide-mouth bottle or 1-liter beaker with sample from a representative area, and carefully fill sample vials from the container.

8.2.4 After collecting the sample in the bottle containing the sodium thiosulfate, seal the bottle and agitate by hand for 15 seconds.

8.2.5 Because of the several pH adjustments made to the samples in the course of this method, the addition of hydrochloric acid to the samples to retard biological activity has been omitted. However, the analyst should be aware of the potential for the biological degradation of the analytes.

8.3 SAMPLE STORAGE/HOLDING TIMES

8.3.1 Samples must be iced or refrigerated at 4°C and maintained at these conditions away from light until extraction. Synthetic ice (i.e. blue ice) is not recommended. Holding studies performed to date have shown that, in samples preserved with sodium thiosulfate, the analytes are stable for up to 14 days. (See Tables 16-19) Thus, once extracted, samples must be analyzed within 14 days. Since stability may be matrix dependent, the

analyst should verify that the prescribed preservation technique is suitable for the samples under study.

8.3.2 Extracts (Sections 11.2.7 and 11.3.11) must be stored at 4°C or less away from light in glass vials with Teflon-lined caps. Holding time studies indicate that the analytes are stable for up to 14 days in the extracts. (Tables 20-23)

9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. Minimum quality control requirements are monitoring the laboratory performance check standard, initial demonstration of laboratory capability, performance of the method detection limit study, analysis of laboratory reagent blanks and laboratory fortified sample matrices, determination of surrogate compound recoveries in each sample and blank, monitoring internal standard peak area or height in each sample, blank and CCC, and analysis of QC samples. Additional QC practices may be added.

9.2 LABORATORY PERFORMANCE CHECK STANDARD (LPC) – At the beginning of an analysis batch, prior to any calibration standard or sample analysis and after an initial solvent blank, a laboratory performance check standard must be analyzed. It is not necessary that a new check standard be extracted each day. This check standard ensures proper performance of the GC by evaluation of the instrument parameters of detector sensitivity, peak symmetry, and peak resolution. It also demonstrates that instrument sensitivity has not changed drastically since the analysis of the MDL study. Inability to demonstrate acceptable instrument performance indicates the need for re-evaluation of the instrument system. Criteria are listed in Table 14.

9.2.1 The sensitivity requirement is based on the EDLs published in this method. If laboratory EDLs differ from those listed in Tables 2 and 3, concentrations of the LPC standard may be adjusted to be compatible with the laboratory EDLs.

9.2.2 The compounds listed in Table 15 for the LPC may not be included in the analyses of a particular laboratory. Therefore, other analytes may be chosen as long as each of the parameters (detector sensitivity, peak symmetry and peak resolution) can be sufficiently evaluated.

9.2.3 If column or chromatographic performance cannot be met, a new column may need to be installed, column flows corrected or modifications made to the oven temperature program.

9.3. INITIAL DEMONSTRATION OF CAPABILITY (IDC)

9.3.1 Select a representative fortification concentration for each of the target analytes. Concentrations near those in Tables 6 and 7 are recommended. Prepare 4-7 replicate laboratory fortified blanks by adding an appropriate aliquot of the primary dilution standard or another certified quality control sample to reagent water. Analyze the LFBs according to the method beginning in Section 11.

9.3.2 Calculate the mean percent recovery and the standard deviation of the recoveries. For each analyte, the mean recovery value, expressed as a percentage of the true value, must fall in the range of 80-120% and the relative standard deviation should be less than 20%. For those compounds that meet these criteria, performance is considered acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be repeated using 4-7 fresh samples until satisfactory performance has been demonstrated. Maintain these data on file to demonstrate initial capabilities.

9.3.3 Furthermore, before processing any samples, the analyst must analyze at least one laboratory reagent blank to demonstrate that all glassware and reagent interferences are under control.

9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method, the quality of data should improve beyond those required here.

9.3.5 The analyst is permitted to modify GC columns, GC conditions, internal standard or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.1 through Section 9.3.4.

9.4 METHOD DETECTION LIMIT STUDY (MDL)

9.4.1 Prior to the analysis of any field samples, the method detection limits must be determined. Initially, estimate the concentration of an analyte which would yield a peak equal to 5 times the baseline noise and drift. Prepare seven replicate laboratory fortified blanks at this estimated concentration. Analyze the LFB's according to the method beginning in Section 11.

9.4.2 Calculate the mean recovery and the standard deviation for each analyte. Multiply the Student's t value at 99% confidence and n-1 degrees of freedom (3.143 for seven replicates) by this standard deviation to yield a statistical estimate of the detection limit. This estimate is the MDL.

9.4.3 MDL's should be recalculated after major changes in the chromatographic temperature program or stationary phase or after a change in instrument or detector.

9.5 LABORATORY REAGENT BLANKS (LRB) -- Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. The LRB must contain the preservation and other reagents added to the sample. If the LRB produces an interferant peak within the retention time window (Section 12.3) of any analyte that would prevent the determination of that analyte or a peak of concentration greater than the MDL for that analyte, the analyst must determine the source of contamination and eliminate the interference before processing samples. For the analyte(s) that failed to meet this criteria, concentrations in field samples are considered suspect.

9.6 LABORATORY FORTIFIED BLANK (LFB) – Since this method utilizes procedural calibration standards, which are fortified reagent water, there is no difference between the LFB and the continuing calibration check standard. Consequently, the analysis of an LFB is not required (Section 10.2).

9.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM)

9.7.1 The concentrations of the analytes in a given sample may be equal to or greater than the fortified concentrations. Subsequently, relatively poor accuracy and precision may be anticipated when a large background must be subtracted. For many samples, the concentrations may be so high that fortification may lead to a final extract with instrumental responses exceeding the working range of the electron capture detector. If this occurs, the extract must be diluted. In spite of these problems, sample sources should be fortified and analyzed as described below. By fortifying sample matrices and calculating analyte recoveries, any matrix induced analyte bias is evaluated.

9.7.2 The laboratory must add known concentrations of analytes to one sample per extraction set or a minimum of 10% of the samples, whichever is greater. The concentrations should be equal to or greater than the background concentrations in the sample selected for fortification. If the fortification level is less than the background concentration, recoveries are

not reported. Over time, samples from all routine sample sources should be fortified.

9.7.3 Calculate the mean percent recovery, R, of the concentration for each analyte, after correcting the total mean measured concentration, A, from the fortified sample for the back-ground concentration, B, measured in the unfortified sample, i.e.:

$$R = 100 (A - B) / C,$$

where C is the fortifying concentration. In order for the recoveries to be considered acceptable, they must fall between 70% and 130% for all the target analytes.

9.7.4 If a recovery falls outside of this acceptance range, a matrix induced bias can be assumed for the respective analyte and the data for that analyte must be reported to the data user as suspect due to matrix effects.

9.8 ASSESSING SURROGATE RECOVERY – The surrogate standard is fortified into the aqueous portion of all calibration standards, samples, QC samples and laboratory reagent blanks. The surrogate is a means of assessing method performance from extraction to final chromatographic performance.

9.8.1 When surrogate recovery from a sample, blank, QC sample or CCC is < 70% or > 130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.8.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.8.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable continuing calibration check standard. If the CCC fails the criteria of Section 10.2.1, recalibration is in order per Section 10.1. If the CCC is acceptable, it may be necessary to extract another aliquot of sample. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect.

9.9 ASSESSING THE INTERNAL STANDARD

9.9.1 The analyst must monitor the IS response (peak area or peak height) of all injections during each analysis day. A mean IS response should be determined from the five point calibration curve. The IS response for any run should not deviate from this mean IS response by more than 30%.

9.9.2 If a greater deviation than this occurs with an individual extract, optimize instrument performance and inject a second aliquot of that extract.

9.9.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.9.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, the analyst should check the calibration by analyzing the most recently acceptable CCC. If the CCC fails the criteria of Section 10.2.1, recalibration is in order per Section 10.1. If the CCC is acceptable, analysis of the sample should be repeated beginning with Section 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.10 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

9.11 The laboratory may adapt additional QC practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10. CALIBRATION AND STANDARDIZATION

10.1 INITIAL CALIBRATION CURVE

10.1.1 Establish GC operating parameters equivalent to the suggested specifications in Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other columns or conditions may be used if equivalent or better performance can be demonstrated.

10.1.2 Calibration standards at five concentrations are required. The lowest should contain the analytes at a concentration near to but greater than the EDL (Tables 2 and 3) for each compound. The others should be evenly distributed throughout the concentration range expected in the samples.

10.1.3 Inject 2 µL of each calibration standard extract and tabulate peak height or area response and concentration for each analyte and the internal standard.

10.1.4 Generate a calibration curve by plotting the area ratios (A_a/A_{is}) against the concentration ratios (C_a/C_{is}) of the five calibration standards where

A_a is the peak area of the analyte.
A_{is} is the peak area of the internal standard.
C_a is the concentration of the analyte.
C_{is} is the concentration of the internal standard.

This curve can be defined as either first or second order; the correlation coefficients must be greater than 0.95. Also, the working calibration curve must be verified daily by measurement of one or more calibration standards (Section 10.2). If the response for any analyte falls outside the predicted response by more than 30%, the calibration check must be repeated using a freshly prepared calibration standard. Should the retest fail, a new calibration curve must be generated.

10.1.5 Alternately, an average relative response factor can be calculated and used for quantitation. Relative response factors are calculated for each analyte at the five concentration levels using the equation below:

$$RRF = \frac{(A_a)(C_{is})}{(A_{is})(C_a)}$$

If the RRF value over the working range is constant (<20% RSD), the RRF can be assumed to be invariant and the average RRF used for calculations. Also, the average RRF must be verified daily by measurement of one or more calibration standards (Section 10.2). If the RRF for the continuing calibration standard deviates from the average RRF by more than 30%, the calibration check must be repeated using a freshly prepared calibration standard. Should the retest fail, a new calibration curve must be generated.

10.1.6 A data system may be used to collect the chromatographic data, calculate relative response factors, or calculate linear or second order calibration curves.

10.2 CONTINUING CALIBRATION CHECK (CCC)

10.2.1 At least one CCC must be extracted with each set of samples. A CCC must be analyzed at the beginning of each analysis set, after every tenth sample analysis and after the final sample analysis, to ensure that the instrument is still within calibration. These checks should be at two different concentration levels. Calculate analyte recoveries for all target analytes. In order for the calibration check to be considered valid and subsequently for the preceding ten samples to be considered acceptable with respect to calibration, recoveries must fall between 70% and 130% for all the target analytes. Additionally, the internal standard area must be within 30% of the mean IS response. (Section 9.9.1)

NOTE: Continuing calibration check standards need not all be different extracts but can be injections from the same extract as long as the holding time requirements for extracts (Sect. 8.3.2) are met. However, at least one must be extracted with each batch of samples.

10.2.2 If this criterion cannot be met, the continuing calibration check standard extract is re-injected in order to determine if the response deviations observed from the initial analysis are repeated. If this criterion still cannot be met, a CCC that has already been analyzed and has been found to be acceptable should be run. If this second CCC fails, then the instrument is considered out of calibration and needs to be recalibrated. Should all CCC's associated with a particular set of samples fail, the set of samples must be re-extracted.

11. PROCEDURE

11.1 SAMPLE EXTRACTION AND HYDROLYSIS

11.1.1 Remove the samples from storage (Sect. 8.3.1) and allow them to equilibrate to room temperature.

11.1.2 Place 40 mL of the water sample into a precleaned 60 mL glass vial with a teflon-lined screw cap using a graduated cylinder.

11.1.3 Add 10 µL of surrogate standard (100 µg/mL 2,4-Dichlorophenylacetic acid in acetone per Section 7.5.2) to the aqueous sample.

NOTE: After injection, cap the sample and invert once. This ensures that the fortification volume is mixed well with the water.

11.1.4 Add 1 mL of the 4N NaOH solution prepared in Section 7.4.1 to each glass vial. Check the pH of the sample with pH paper or a pH meter; if the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 4N NaOH solution. Let the sample sit at room temperature for 1 hour, shaking the contents periodically.

NOTE: Since many of the herbicides contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction. This step must be included in the analysis of all extracted field samples, LRBs, LFMs and calibration standards.

11.1.5 Adjust the pH to less than 0.5 by adding at least 2 mL of concentrated sulfuric acid. Cap, shake and then check the pH with a pH meter or narrow range pH paper.

11.1.6 Quickly add approximately 2 g of copper II sulfate pentahydrate and shake until dissolved. This colors the aqueous phase blue and therefore allows the analyst to better distinguish between the aqueous phase and the organic phase in this micro extraction.

11.1.7 Quickly add approximately 16 g of muffled sodium sulfate and shake for 3 to 5 minutes until almost all is dissolved. Sodium sulfate is added to increase the ionic strength of the aqueous phase and thus further drive the chlorophenoxy acids into the organic phase. The addition of this salt and the copper II sulfate pentahydrate should be done quickly so that the heat generated from the addition of the acid (Section 11.1.5) will help dissolve the salts.

11.1.8 Add exactly 4.0 mL MtBE and place on the mechanical shaker for 30 minutes. (If hand-shaken, two minutes is sufficient if performed vigorously).

11.1.9 Allow the phases to separate for approximately 5 minutes.

11.2 METHYLATION - DIAZOMETHANE

NOTE: It is not recommended that this method of derivatization be used if 4-nitrophenol and 5-hydroxydicamba are included in the target list.

11.2.1 Generation of Diazomethane.

11.2.1.1 Assemble the diazomethane generator (Figure 1) in a hood. The collection vessel is a 10 or 15-mL glass vial equipped with a teflon-lined screw cap and maintained at 0-5°C.

11.2.1.2 Add a sufficient amount of ethyl ether (approximately 7 ml) to tube 1 to cover the first impinger. Add 5 ml of MtBE to the collection vial. Set the nitrogen flow at 5-10 mL/min. Add 4 mL Diazald solution (Section 7.4.3) and 3 mL of 37% KOH solution (Section 7.4.2) to the second impinger. Connect the tubing as shown and allow the nitrogen flow to purge the diazomethane from the reaction vessel into the collection vial for 30 minutes. Cap the vial when collection is complete and maintain at 0-5°C. When stored at 0-5°C, this diazomethane solution may be used over a period of 48 hours.

11.2.2 Using a Pasteur pipet, transfer the sample extract (upper MtBE layer) to a 10 mL screw cap vial. Add 0.1 g acidified sodium sulfate and shake. This step is included to ensure the MtBE extract contains no water.

11.2.3 Using a Pasteur pipet, transfer exactly 3 mL of the dried MtBE extract to a 15 mL graduated conical centrifuge tube.

11.2.4 Add 250 µL of the diazomethane solution prepared in Section 11.2.1.2. to each centrifuge tube. The contents of the centrifuge tube should remain slightly yellow in color. If this is not the case, more diazomethane solution may be added, making sure to add the exact additional amount to every calibration standard, blank, QC sample and field sample. Let the esterification reaction proceed for 30 minutes.

11.2.5 Remove any unreacted diazomethane by the addition of 0.1 g silica gel. Effervescence (evolution of nitrogen) is an indication that excess diazomethane was present. Allow the extracts to sit for 0.5 hour.

11.2.6 Place a small plug of glass wool into a disposable Pasteur pipet. Fill the pipet with approximately 2 inches of florisil. (Section 7.3.12) (This step is the preparation of clean-up columns for the methylated extracts. One column should be prepared for each extract.)

11.2.7 Apply the methylated extract to the prepared clean-up column and collect the eluate in a 5 mL vial.

11.2.8 Transfer exactly 1.0 ml of the MtBE extract to an autosampler vial. A duplicate vial should be filled using the excess extract.

11.2.9 Add 10 µL of internal standard to the vial to be analyzed. (2.5 µg/mL 4,4'-Dibromooctafluorobiphenyl in MtBE per Section 7.5.1). Internal standard should be added to the duplicate vial before analysis.

11.2.10 Analyze the sample extracts as soon as possible. The sample extract may be stored up to 14 days if kept at 4°C or less. Keep the extracts away from light in amber glass vials with Teflon-lined caps.

11.3. METHYLATION - BASE-PROMOTED ESTERIFICATION

11.3.1 Using a Pasteur pipet, transfer approximately 3 mL of the sample extract (upper MtBE layer) to a 10 mL screw cap vial. Add 0.1 g acidified sodium sulfate and shake. This step is included to ensure that the MtBE extract contains no water.

11.3.2 Using a Pasteur pipet, transfer exactly 3 mL of the dried MtBE extract to a 15 mL graduated conical centrifuge tube.

11.3.3 Add 80 µL of the 1.0M solution of tetrabutylammonium hydroxide.

11.3.4 Add 40 µL of methyl iodide.

11.3.5 Cap the centrifuge tubes and place in the heating block (or sand bath) at 50°C and maintain for 1.5 hr. The vials must fit snugly into the heating block to ensure proper heat transfer. At this stage, methylation of the method analytes is attained and the tetrabutylammonium iodide by-product may be viewed as a precipitate.

11.3.6 Remove the centrifuge tubes from the heating block (or sand bath) and allow them to cool before removing the caps.

11.3.7 Place a small plug of glass wool into a disposable Pasteur pipet. Fill the pipet with approximately 2 inches of florisil. (Section 7.3.12) (This step is the preparation of clean-up columns for the methylated extracts. One column should be prepared for each extract.)

11.3.8 Apply the methylated extract to the prepared clean-up column and collect the eluate in a 5 mL vial.

11.3.9 Transfer exactly 1.0 ml of the MtBE extract to an autosampler vial. A duplicate vial should be filled using the excess extract.

11.3.10 Add 10 µL of internal standard to the vial to be analyzed. (2.5 µg/mL 4,4'-Dibromooctafluorobiphenyl in MTBE per Section 7.5.1). Internal standard should be added to the duplicate vial before analysis.

11.3.11 Analyze the samples as soon as possible. The sample extract may be stored up to 14 days if kept at 4°C or less. Keep the extracts away from light in amber glass vials with Teflon-lined caps.

11.4 GAS CHROMATOGRAPHY

11.4.1 Table 1 summarizes recommended GC operating conditions and retention times observed using this method. Figure 2 illustrates the performance of the recommended primary column with the method analytes. Figure 3 illustrates the performance of the recommended confirmation column with the method analytes. Other GC columns or chromatographic conditions may be used if the requirements of Section 9 are met.

11.4.2 Calibrate the system daily by either the analysis of a calibration curve (Section 10.1) or a continuing calibration check as described in Section 10.2.

11.4.3 Inject 2 µL of the sample extract. Record the resulting peak sizes in area or height units.

11.4.4 If the response for the peak exceeds the working range of the system, dilute the extract, add an appropriate additional amount of internal standard and reanalyze. The analyst must not extrapolate above or below the calibration range established.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify sample components by comparison of retention times to retention data from the calibration standard analysis. If the retention time of an unknown peak corresponds, within limits (Section 12.3), to the retention time of a standard compound, then the identification is considered positive. Calculate analyte concentrations in the samples and reagent blanks from the calibration curves generated in Section 10.1.

12.2 If an average relative response factor has been calculated, analyte concentrations in the samples and reagent blanks are calculated using the following equation:

$$C_a = \frac{(A_a)(C_{is})}{(A_{is})(RRF)}$$

12.3 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13. METHOD PERFORMANCE

13.1 In a single laboratory, accuracy and precision data were obtained at three concentrations in reagent water (Tables 5-8). The MDL and EDL data are given in Tables 2 and 3. In addition, recovery and precision data were obtained at a medium concentration for dechlorinated tap water (Tables 9 and 10), high ionic strength ground water (Tables 11 and 12) and high humectant reagent water (Tables 13 and 14).

14. POLLUTION PREVENTION

14.1 This method utilizes a micro-extraction procedure which requires the use of very small quantities of organic solvents. This feature reduces the hazards involved with the use of large volumes of potentially harmful organic solvents needed for conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1 Due to the nature of this method there is little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Sect. 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. RETENTION DATA AND CHROMATOGRAPHIC CONDITIONS OF ANALYTE METHYL DERIVATIVES

Analyte	Retention Time, min. Primary column	Confirmatory column
Dalapon	15.43	19.73
3,5-Dichlorobenzoic Acid	34.62	39.98
4-Nitrophenol	38.45	40.31
2,4-Dichlorophenylacetic Acid[b]	40.62	48.50
Dicamba	41.88	49.21
Dichlorprop	47.67	53.59
4,4'Dibromooctafluorobiphenyl[a]	49.13	55.88
2,4-D	49.83	54.60
Pentachlorophenol	50.18	58.13
Silvex	53.58	59.13
5-Hydroxydicamba	55.32	59.59
2,4,5-T	55.60	60.21
Chloramben	57.17	59.59
2,4-DB	57.38	62.77
Dinoseb	59.68	62.61
Bentazon	61.55	63.64
Dacthal acid metabolites	62.78	67.82
Picloram	65.77	66.70
Acifluorfen	72.57	75.60

Primary column: DB-1701, 30 m x 0.25 mm i.d., 0.25 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 290°C, Helium Linear Velocity = 24 cm/sec at 35°C, Splitless injection with 30 s delay

Program: Hold at 35°C for 10 min, ramp to 150°C at 5C°/min. and hold 10 min., ramp to 222°C at 4C°/min. and hold 5 min, ramp to 260°C at 5C°/min. and hold 6 min.

Confirmatory column: DB-5.625, 30 m x 0.25 mm i.d., 0.25 µm film thickness, Injector Temp. = 200°C, Detector Temp. = 290°C, Linear Helium Velocity = 25 cm/sec at 35°C, splitless injection with 30 s delay.

Program: Hold at 35°C for 10 min, ramp to 150°C at 5C°/min. and hold 10 min., ramp to 222°C at 4C°/min. and hold 5 min, ramp to 260°C at 5C°/min. and hold 6 min.

[a] Internal Standard

[b] Surrogate Compound

TABLE 2. ANALYTE ACCURACY AND PRECISION DATA AND METHOD DETECTION LIMITS[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 1 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Method Detection Limit[b], µg/L	Estimated Detection Limit[c], µg/L
Dalapon	1.25	1.60	0.31	19	0.97	1.25
3,5-Dichlorobenzoic Acid	0.625	0.804	0.060	7.5	0.19	0.625
4-Nitrophenol[d]	1.25	1.24	0.29	23	0.91	1.25
Dicamba	0.625	0.844	0.095	11	0.30	0.30
Dichlorprop	1.25	1.66	0.16	9.6	0.51	0.51
2,4-D	1.25	1.71	0.11	6.4	0.35	0.35
Pentachlorophenol	0.125	0.176	0.027	15	0.085	0.085
Silvex	0.314	0.409	0.046	11	0.14	0.14
5-Hydroxydicamba[d]	0.625	0.822	0.19	23	0.60	0.625
2,4,5-T	0.314	0.307	0.064	21	0.20	0.20
Chloramben	0.625	0.857	0.079	9.2	0.25	0.25
2,4-DB	1.25	1.61	0.21	13	0.66	1.25
Dinoseb	1.25	1.56	0.26	17	0.82	0.82
Bentazon	1.25	1.46	0.28	19	0.88	1.25
Picloram	1.25	1.31	0.33	25	1.0	1.0
Acifluorfen	0.625	0.720	0.15	21	0.47	0.47
Dacthal Acid Metabolites	1.25	1.47	0.20	14	0.63	0.63

[a] Produced by analysis of seven aliquots of fortified reagent water.
[b] The MDL is a statistical estimate of the detection limit. To determine the MDL for each analyte, the standard deviation of the mean concentration of the seven replicates is calculated. This standard deviation is then multiplied by the Student's t-value at 99% confidence and n-1 degrees of freedom (3.143 for seven replicates). The result is the MDL.
[c] The EDL is defined as either the MDL or a level of a compound in a sample yielding a peak in the final extract with a signal to noise (S/N) ratio of approximately 5, whichever is greater.
[d] Quantitation not recommended due to poor precision.

TABLE 3. ANALYTE ACCURACY AND PRECISION DATA AND METHOD DETECTION LIMITS[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 1 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Method Detection Limit[b], µg/L	Estimated Detection Limit[c], µg/L
Dalapon	1.25	1.37	0.17	12	0.53	1.25
3,5-Dichlorobenzoic Acid	0.625	0.781	0.041	5.2	0.13	0.625
4-Nitrophenol	1.25	1.07	0.21	20	0.66	1.25
Dicamba	0.625	0.689	0.065	9.4	0.20	0.20
Dichlorprop	1.25	1.55	0.13	8.4	0.41	0.41
2,4-D	1.25	1.39	0.11	7.9	0.36	0.36
Pentachlorophenol	0.125	0.108	0.0068	6.3	0.021	0.021
Silvex	0.314	0.326	0.023	7.1	0.072	0.072
5-Hydroxydicamba	0.625	0.708	0.068	9.6	0.21	0.21
2,4,5-T	0.314	0.255	0.052	20	0.16	0.16
Chloramben	0.625	0.644	0.044	6.8	0.14	0.14
2,4-DB	1.25	1.47	0.19	13	0.60	0.60
Dinoseb	1.25	1.06	0.24	23	0.75	1.25
Bentazon	1.25	0.927	0.16	17	0.50	1.25
Picloram	1.25	1.12	0.15	13	0.47	0.47
Acifluorfen	0.625	0.639	0.12	21	0.38	0.38
Dacthal Acid Metabolites	1.25	1.52	0.12	7.9	0.38	0.38

[a] Produced by analysis of seven aliquots of fortified reagent water.

[b] The MDL is a statistical estimate of the detection limit. To determine the MDL for each analyte, the standard deviation of the mean concentration of the seven replicates is calculated. This standard deviation is then multiplied by the Student's t-value at 99% confidence and n-1 degrees of freedom (3.143 for seven replicates). The result is the MDL.

[c] The EDL is defined as either the MDL or a level of a compound in a sample yielding a peak in the final extract with a signal to noise (S/N) ratio of approximately 5, whichever is greater.

TABLE 4. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 2 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	5.00	5.26	0.34	6.5	105
3,5-Dichlorobenzoic Acid	2.50	3.04	0.27	8.9	122
4-Nitrophenol[b]	5.00	5.69	0.96	17	114
Dicamba	2.50	2.62	0.068	2.6	105
Dichlorprop	5.00	5.97	0.18	3.0	119
2,4-D	5.00	6.34	0.20	3.2	127
Pentachlorophenol	0.500	0.524	0.012	2.3	105
Silvex	1.25	1.26	0.084	6.7	101
5-Hydroxydicamba[b]	2.50	2.32	0.45	19	93
2,4,5-T	1.25	1.30	0.065	5.0	104
Chloramben	2.50	2.51	0.13	5.2	100
2,4-DB	5.00	5.88	0.37	6.3	118
Dinoseb	5.00	5.55	0.21	3.8	111
Bentazon	5.00	5.09	0.18	3.6	102
Picloram	5.00	5.12	0.35	6.8	102
Acifluorfen	2.50	2.82	0.24	8.3	113
Dacthal Acid Metabolites	5.00	5.53	0.38	6.8	111

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Quantitation not recommended due to poor precision.

TABLE 5. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 2 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	5.00	6.37	0.67	10	127
3,5-Dichlorobenzoic Acid	2.50	3.26	0.18	5.4	131
4-Nitrophenol	5.00	5.24	0.29	5.5	105
Dicamba	2.50	2.75	0.22	8.2	110
Dichlorprop	5.00	5.70	0.29	5.1	114
2,4-D	5.00	5.59	0.33	5.9	112
Pentachlorophenol	0.500	0.520	0.065	12	104
Silvex	1.25	1.31	0.081	6.2	104
5-Hydroxydicamba	2.50	2.75	0.17	6.0	110
2,4,5-T	1.25	1.20	0.19	16	96
Chloramben	2.50	2.34	0.25	11	94
2,4-DB	5.00	5.46	0.24	4.4	109
Dinoseb	5.00	5.42	0.69	13	108
Bentazon	5.00	4.54	0.33	7.3	91
Picloram	5.00	4.28	0.52	12	86
Acifluorfen	2.50	2.58	0.50	19	103
Dacthal Acid Metabolites	5.00	5.16	0.66	13	103

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 6. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 4 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	10.3	0.27	2.6	103
3,5-Dichlorobenzoic Acid	5.00	4.63	0.24	5.2	93
4-Nitrophenol[b]	10.0	14.3	3.1	22	143
Dicamba	5.00	4.80	0.083	1.7	96
Dichlorprop	10.0	9.26	0.28	3.1	93
2,4-D	10.0	9.67	0.19	1.9	97
Pentachlorophenol	1.00	0.967	0.015	1.5	97
Silvex	2.50	2.43	0.032	1.3	97
5-Hydroxydicamba[b]	5.00	6.70	2.4	36	134
2,4,5-T	2.50	2.46	0.088	3.6	98
Chloramben	5.00	4.95	0.081	1.6	99
2,4-DB	10.0	9.67	0.22	2.3	97
Dinoseb	10.0	9.78	0.19	2.0	98
Bentazon	10.0	9.78	0.19	1.9	98
Picloram	10.0	10.2	0.25	2.4	102
Acifluorfen	5.00	5.15	0.40	7.8	103
Dacthal Acid Metabolites	10.0	8.85	0.31	3.5	88

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Quantitation not recommended due to poor precision.

TABLE 7. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 4 IN REAGENT WATER

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	8.37	0.88	10	84
3,5-Dichlorobenzoic Acid	5.00	4.55	0.16	3.5	91
4-Nitrophenol	10.0	9.52	1.0	11	95
Dicamba	5.00	4.55	0.14	3.0	91
Dichlorprop	10.0	8.87	0.18	2.0	89
2,4-D	10.0	9.09	0.38	4.2	91
Pentachlorophenol	1.00	0.870	0.16	19	87
Silvex	2.50	2.29	0.050	2.2	92
5-Hydroxydicamba	5.00	4.23	0.30	7.1	85
2,4,5-T	2.50	2.26	0.12	5.2	90
Chloramben	5.00	4.76	0.34	7.2	95
2,4-DB	10.0	10.2	0.34	3.3	102
Dinoseb	10.0	10.7	3.1	29	107
Bentazon	10.0	9.54	0.84	8.8	95
Picloram	10.0	9.60	0.44	4.6	96
Acifluorfen	5.00	5.27	0.46	8.7	105
Dacthal Acid Metabolites	10.0	8.17	1.2	15	82

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 8. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 3 IN DECHLORINATED TAP WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	8.18	0.93	11	109
3,5-Dichlorobenzoic Acid	<0.625	3.75	4.07	0.38	9.3	108
4-Nitrophenol[c]	<1.25	7.50	5.76	1.0	18	77
Dicamba	<0.30	3.75	3.91	0.16	4.0	104
Dichlorprop	<0.51	7.50	7.29	0.40	5.4	97
2,4-D	<0.35	7.50	7.00	0.38	5.4	93
Pentachlorophenol	<0.085	0.750	0.754	0.016	2.2	101
Silvex	<0.14	1.87	1.70	0.077	4.5	90
5-Hydroxydicamba[c]	<0.625	3.75	0.233	0.12	51	6[d]
2,4,5-T	<0.20	1.87	1.66	0.046	2.8	88
Chloramben	<0.25	3.75	3.93	0.25	6.4	105
2,4-DB	<1.25	7.50	7.51	0.70	9.3	100
Dinoseb	<0.82	7.50	8.02	0.32	4.0	107
Bentazon	<1.25	7.50	7.64	0.44	5.8	102
Picloram	<1.0	7.50	7.91	1.0	13	105
Acifluorfen	<0.47	3.75	3.97	0.38	9.7	106
Dacthal Acid Metabolites	<0.63	7.50	7.87	0.52	6.6	105

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Chlorinated surface water from a local utility to which sodium thiosulfate was added as the dechlorinating agent.

[c] Quantitation not recommended due to poor precision.

[d] As noted in Section 4.6, 5-Hydroxydicamba cannot be recovered from chlorinated waters.

TABLE 9. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 3 IN DECHLORINATED TAP WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	8.06	0.39	4.8	108
3,5-Dichlorobenzoic Acid	<0.625	3.75	4.18	0.54	13	111
4-Nitrophenol	<1.25	7.50	6.63	0.78	12	88
Dicamba	<0.20	3.75	3.69	0.14	3.6	98
Dichlorprop	<0.41	7.50	6.70	0.24	3.6	89
2,4-D	<0.36	7.50	6.85	0.55	8.0	91
Pentachlorophenol	<0.021	0.750	0.771	0.071	9.2	103
Silvex	<0.072	1.87	1.69	0.13	7.6	90
5-Hydroxydicamba	<0.21	3.75	0.180	0.048	26	5[c]
2,4,5-T	<0.16	1.87	1.55	0.19	12	83
Chloramben	<0.14	3.75	3.22	0.26	8.1	86
2,4-DB	<0.60	7.50	7.31	0.48	6.6	97
Dinoseb	<1.25	7.50	8.81	1.8	20	117
Bentazon	<1.25	7.50	6.98	0.58	8.3	93
Picloram	<0.47	7.50	7.11	0.74	10	95
Acifluorfen	<0.38	3.75	4.08	0.63	15	109
Dacthal Acid Metabolites	<0.38	7.50	6.71	0.70	10	90

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Chlorinated surface water from a local utility to which sodium thiosulfate was added as the dechlorinating agent.

[c] As noted in section 4.6, 5-Hydroxydicamba cannot be recovered from chlorinated waters.

TABLE 10. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 3 IN HIGH IONIC STRENGTH WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	5.98	0.47	7.8	80
3,5-Dichlorobenzoic Acid	<0.625	3.75	3.86	0.14	3.7	103
4-Nitrophenol[c]	<1.25	7.50	10.44	2.1	20	139
Dicamba	<0.30	3.75	3.80	0.098	2.6	101
Dichlorprop	<0.51	7.50	7.12	0.16	2.2	95
2,4-D	<0.35	7.50	6.98	0.14	2.0	93
Pentachlorophenol	<0.085	0.750	0.787	0.053	6.7	105
Silvex	<0.14	1.87	1.76	0.066	3.7	94
5-Hydroxydicamba[c]	<0.625	3.75	5.43	2.0	36	145
2,4,5-T	<0.20	1.87	1.72	0.044	2.6	92
Chloramben	<0.25	3.75	4.16	0.24	5.8	111
2,4-DB	<1.25	7.50	8.84	0.38	4.3	118
Dinoseb	<0.82	7.50	7.74	0.21	2.7	103
Bentazon	<1.25	7.50	7.83	0.44	5.6	104
Picloram	<1.0	7.50	7.29	0.39	5.4	97
Acifluorfen	<0.47	3.75	3.82	0.27	7.2	102
Dacthal Acid Metabolites	<0.63	7.50	7.73	0.28	3.7	103

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Chlorinated ground water from a water source displaying a hardness of 460 mg/L as $CaCO_3$.

[c] Quantitation not recommended due to poor precision.

TABLE 11. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 3 IN HIGH IONIC STRENGTH WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	7.33	0.65	8.9	98
3,5-Dichlorobenzoic Acid	<0.625	3.75	4.25	0.29	6.8	113
4-Nitrophenol	<1.25	7.50	7.90	0.51	6.4	105
Dicamba	<0.20	3.75	3.96	0.12	3.0	106
Dichlorprop	<0.41	7.50	7.11	0.74	10	95
2,4-D	<0.36	7.50	6.67	0.17	2.6	89
Pentachlorophenol	<0.021	0.750	1.00	0.068	6.8	134
Silvex	<0.072	1.87	1.80	0.081	4.5	96
5-Hydroxydicamba	<0.21	3.75	3.62	0.12	3.4	96
2,4,5-T	<0.16	1.87	1.65	0.074	4.5	88
Chloramben	<0.14	3.75	3.84	0.30	7.8	102
2,4-DB	<0.60	7.50	7.74	0.54	7.0	103
Dinoseb	<1.25	7.50	7.45	0.72	9.7	99
Bentazon	<1.25	7.50	7.88	0.46	5.8	105
Picloram	<0.47	7.50	6.15	0.63	10	82
Acifluorfen	<0.38	3.75	4.17	0.46	11	111
Dacthal Acid Metabolites	<0.38	7.50	6.82	0.39	5.7	91

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Chlorinated ground water from a water source displaying a hardness of 460 mg/L as $CaCO_3$.

TABLE 12. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY DIAZOMETHANE

LEVEL 3 IN HIGH HUMIC CONTENT WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	8.41	1.92	23	112
3,5-Dichlorobenzoic Acid	<0.625	3.75	4.46	0.38	8.6	119
4-Nitrophenol[c]	<1.25	7.50	6.21	2.4	39	83
Dicamba	<0.30	3.75	4.36	0.19	4.4	116
Dichlorprop	<0.51	7.50	9.22	0.69	7.5	123
2,4-D	<0.35	7.50	9.28	0.77	8.3	124
Pentachlorophenol	<0.085	0.750	0.797	0.020	2.5	106
Silvex	<0.14	1.87	1.96	0.048	2.5	104
5-Hydroxydicamba[c]	<0.625	3.75	2.52	1.4	55	67
2,4,5-T	<0.20	1.87	2.06	0.19	9.1	110
Chloramben	<0.25	3.75	3.86	0.29	7.6	103
2,4-DB	<1.25	7.50	9.10	0.36	3.9	121
Dinoseb	<0.82	7.50	8.66	1.0	12	115
Bentazon	<1.25	7.50	7.89	0.30	3.7	105
Picloram	<1.0	7.50	6.79	1.9	29	91
Acifluorfen	<0.47	3.75	3.67	0.53	14	98
Dacthal Acid Metabolites	<0.63	7.50	9.23	1.0	11	123

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Reagent water fortified at 1.0 mg/L with fulvic acid extracted from Ohio River water. Sample simulates high TOC matrix.

[c] Quantitation not recommended due to poor precision.

TABLE 13. ANALYTE ACCURACY AND PRECISION DATA[a]

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

LEVEL 3 IN HIGH HUMIC CONTENT STRENGTH WATER[b]

Analyte	Background Conc., µg/L	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	<1.25	7.50	6.82	1.1	16	91
3,5-Dichlorobenzoic Acid	<0.625	3.75	3.86	0.33	8.5	103
4-Nitrophenol	<1.25	7.50	7.04	0.49	7.0	94
Dicamba	<0.20	3.75	3.67	0.13	3.4	98
Dichlorprop	<0.41	7.50	7.24	0.52	7.2	97
2,4-D	<0.36	7.50	6.37	0.93	15	85
Pentachlorophenol	<0.021	0.750	0.736	0.12	16	98
Silvex	<0.072	1.87	1.77	0.19	11	95
5-Hydroxydicamba	<0.21	3.75	3.17	0.34	11	84
2,4,5-T	<0.16	1.87	1.49	0.29	20	79
Chloramben	<0.14	3.75	3.68	0.24	6.6	98
2,4-DB	<0.60	7.50	8.01	0.42	5.2	107
Dinoseb	<1.25	7.50	7.08	0.81	11	94
Bentazon	<1.25	7.50	7.50	0.44	5.9	100
Picloram	<0.47	7.50	5.66	1.0	18	75
Acifluorfen	<0.38	3.75	3.89	0.45	12	104
Dacthal Acid Metabolites	<0.38	7.50	6.93	0.53	7.6	92

[a] Produced by the analysis of seven aliquots of fortified reagent water.

[b] Reagent water fortified at 1.0 mg/L with fulvic acid extracted from Ohio River water. Sample simulates high TOC matrix.

TABLE 14. LABORATORY PERFORMANCE CHECK SOLUTION

PARAMETER	ANALYTE	CONC., µg/L IN WATER SAMPLE	ACCEPTANCE CRITERIA
INSTRUMENT SENSITIVITY	DINOSEB	2.50	DETECTION OF ANALYTE; S/N > 3[a]
CHROMATOGRAPHIC PERFORMANCE	4-NITROPHENOL	2.50	PGF BETWEEN 0.80 AND 1.15[b]
COLUMN PERFORMANCE	CHLORAMBEN 2,4-DB	1.25 2.50	RESOLUTION ≥ 0.50[c]

[a] S/N, a ratio of peak signal to baseline noise.

peak signal - measured as height of peak.
baseline noise - measured as maximum deviation in baseline (in units of height) over a width equal to the width of the base of the peak.

[b] PGF = Peak Gaussian Factor

$$PGF = \frac{1.83 \times W_{1/2}}{W_{1/10}}$$

where $W_{1/2}$ = the peak width at half height.
$W_{1/10}$ = the peak width at one-tenth height.

This is a measure of the symmetry of the peak.

[c] Resolution between two peaks is defined by the equation:

$$R = \frac{t}{W_{ave}}$$

where t = the difference in elution times between the two peaks.
W_{ave} = the average peak width of the two peaks (measurements taken at baseline).

This a measure of the degree of separation of two peaks under specific chromatographic conditions.

FIGURE 1. APPARATUS FOR GENERATION OF DIAZOMETHANE

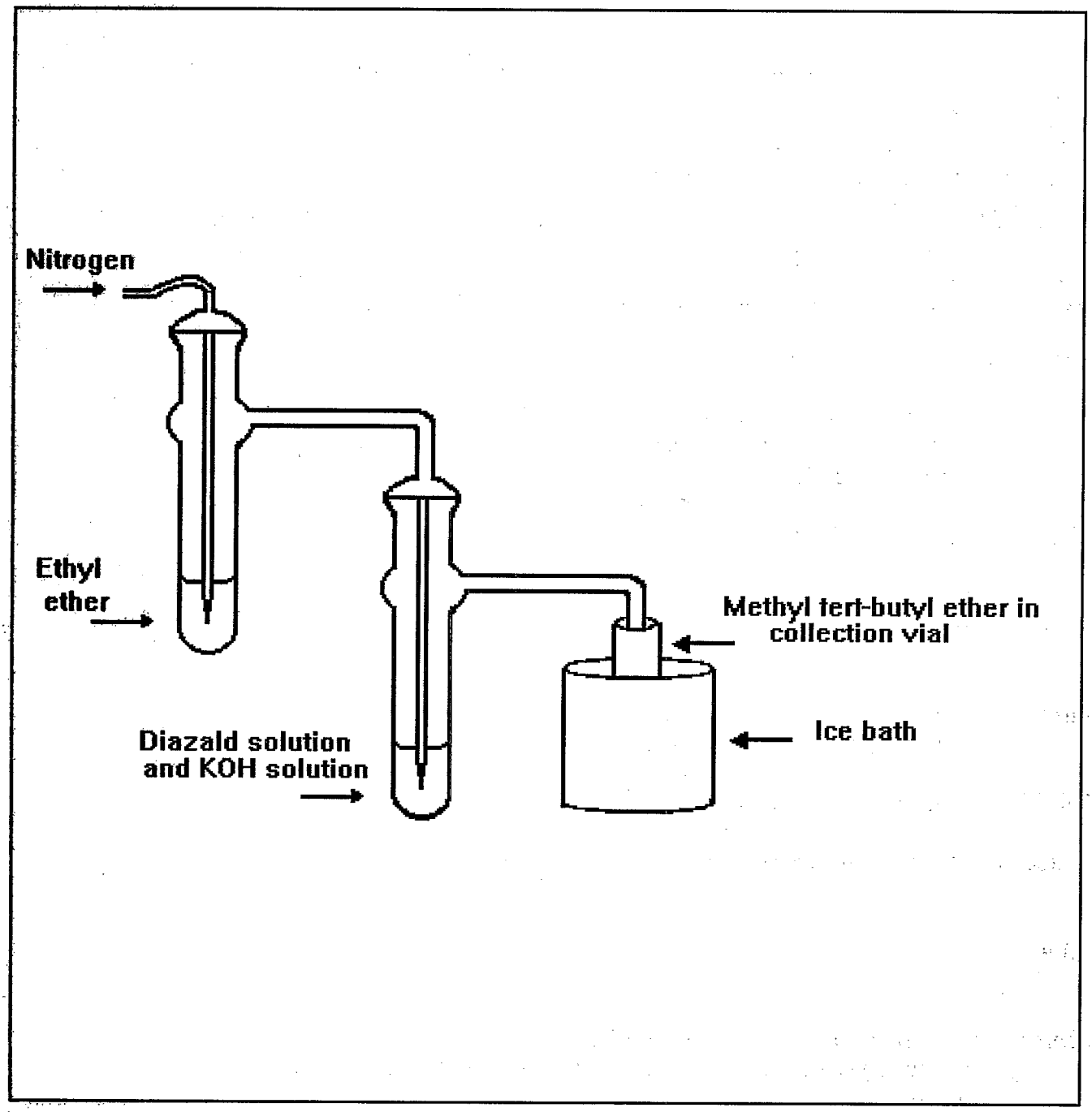

FIGURE 2. CHROMATOGRAM OF CHLOROPHENOXY HERBICIDES ON DB-1701. DERIVATIZATION BY DIAZOMETHANE (LEVEL 4 CALIBRATION)

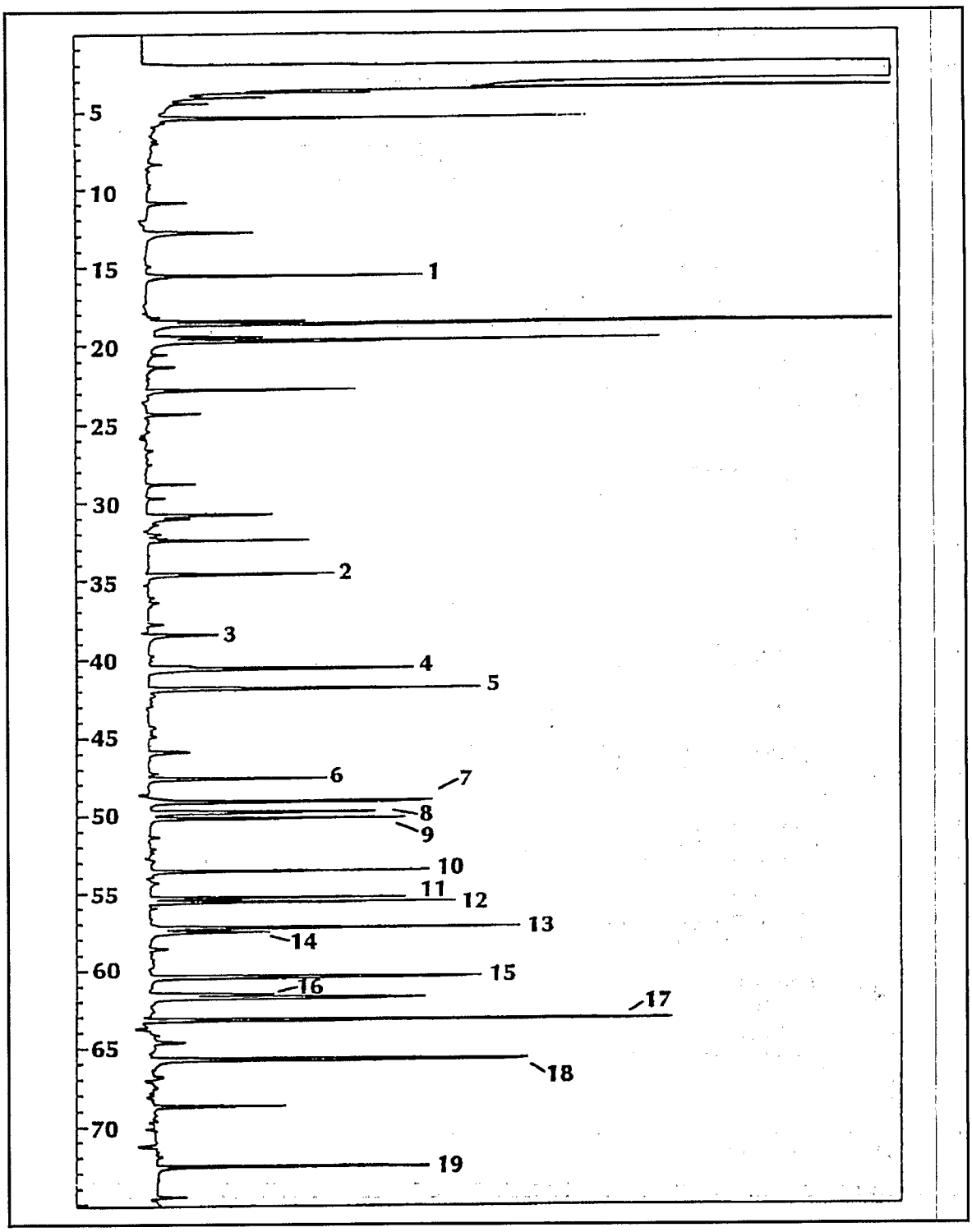

FIGURE 3. CHROMATOGRAM OF CHLOROPHENOXY HERBICIDES ON DB-5.625. BASE-PROMOTED DERIVATIZATION (LEVEL 2 CALIBRATION)

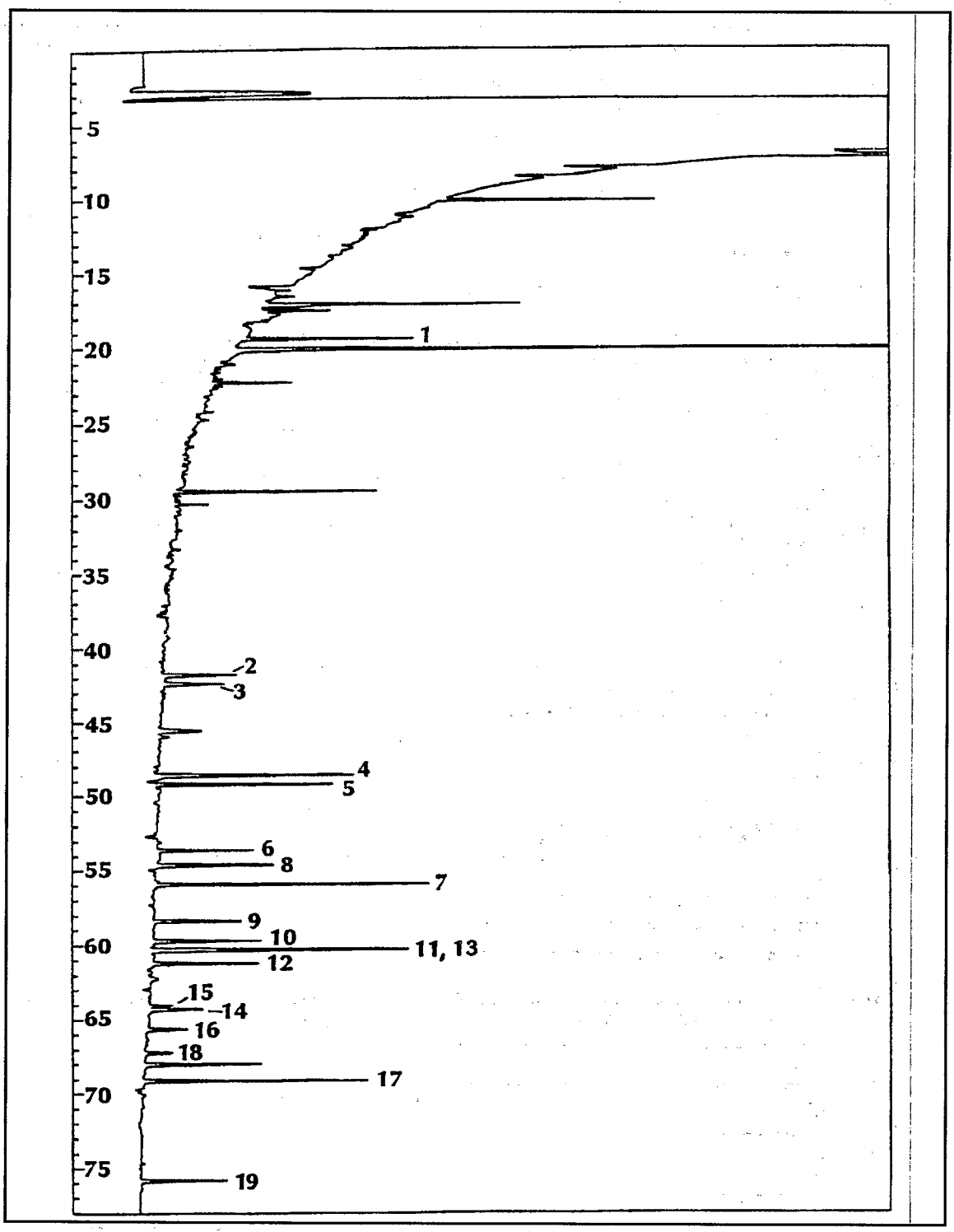

TABLE 15. KEY FOR PEAK NUMBERS DISPLAYED IN FIGURES 2 AND 3

Peak Number	Method 515.3 compound
1	Dalapon
2	3,5-Dichlorobenzoic Acid
3	4-Nitrophenol
4	2,4-Dichlorophenylacetic Acid (Surrogate)
5	Dicamba
6	Dichlorprop
7	4,4'-Dibromooctafluorobiphenyl (Internal Standard)
8	2,4-D
9	Pentachlorophenol
10	Silvex (2,4,5-TP)
11	5-Hydroxydicamba
12	2,4,5-T
13	Chloramben
14	2,4-DB
15	Dinoseb
16	Bentazon
17	Dacthal Acid Metabolites
18	Picloram
19	Acifluorfen

TABLE 16. HOLDING TIME STUDY FOR AQUEOUS SAMPLES

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 0

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	8.37	0.88	10	84
3,5-Dichlorobenzoic Acid	5.00	4.55	0.16	3.5	91
4-Nitrophenol	10.0	9.52	1.0	11	95
Dicamba	5.00	4.55	0.14	3.0	91
Dichlorprop	10.0	8.87	0.18	2.0	89
2,4-D	10.0	9.09	0.38	4.2	91
Pentachlorophenol	1.00	0.870	0.16	19	87
Silvex	2.50	2.29	0.050	2.2	92
5-Hydroxydicamba	5.00	4.23	0.30	7.1	85
2,4,5-T	2.50	2.26	0.12	5.2	90
Chloramben	5.00	4.76	0.34	7.2	95
2,4-DB	10.0	10.2	0.34	3.3	102
Dinoseb	10.0	10.7	3.1	29	107
Bentazon	10.0	9.54	0.84	8.8	95
Picloram	10.0	9.60	0.44	4.6	96
Acifluorfen	5.00	5.27	0.46	8.7	105
Dacthal Acid Metabolites	10.0	8.17	1.2	15	82

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 17. HOLDING TIME STUDY FOR AQUEOUS SAMPLES

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 7

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	9.48	1.4	15	95
3,5-Dichlorobenzoic Acid	5.00	4.98	0.19	3.8	100
4-Nitrophenol	10.0	9.65	0.38	4.0	97
Dicamba	5.00	4.99	0.052	1.0	100
Dichlorprop	10.0	9.86	0.16	1.6	99
2,4-D	10.0	9.76	0.19	2.0	98
Pentachlorophenol	1.00	1.10	0.15	14	110
Silvex	2.50	2.62	0.057	2.2	105
5-Hydroxydicamba	5.00	5.07	0.19	3.8	101
2,4,5-T	2.50	2.75	0.20	7.2	110
Chloramben	5.00	5.32	0.16	3.1	106
2,4-DB	10.0	10.3	0.22	2.1	103
Dinoseb	10.0	9.50	2.4	26	95
Bentazon	10.0	10.9	0.35	3.2	109
Picloram	10.0	11.5	2.4	21	115
Acifluorfen	5.00	5.92	0.38	6.4	118
Dacthal Acid Metabolites	10.0	10.0	0.57	5.7	100

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 18. HOLDING TIME STUDY FOR AQUEOUS SAMPLES

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 14

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	9.53	0.27	2.8	95
3,5-Dichlorobenzoic Acid	5.00	4.85	0.16	3.4	97
4-Nitrophenol	10.0	9.18	0.32	3.5	92
Dicamba	5.00	4.60	0.15	3.3	92
Dichlorprop	10.0	8.98	0.23	2.5	90
2,4-D	10.0	8.73	0.20	2.3	87
Pentachlorophenol	1.00	1.22	0.15	12	122
Silvex	2.50	2.42	0.066	2.7	97
5-Hydroxydicamba	5.00	4.26	0.066	1.6	85
2,4,5-T	2.50	2.28	0.12	5.2	91
Chloramben	5.00	4.65	0.12	2.7	93
2,4-DB	10.0	9.30	0.20	2.1	93
Dinoseb	10.0	10.1	1.2	12	101
Bentazon	10.0	9.92	0.33	3.3	99
Picloram	10.0	9.43	2.2	23	94
Acifluorfen	5.00	4.89	0.28	5.6	98
Dacthal Acid Metabolites	10.0	8.23	0.67	8.1	82

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 19. HOLDING TIME STUDY FOR AQUEOUS SAMPLES

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 21

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	9.65	1.3	13	97
3,5-Dichlorobenzoic Acid	5.00	5.32	0.49	9.2	106
4-Nitrophenol	10.0	9.32	0.81	8.7	93
Dicamba	5.00	4.64	0.27	5.8	93
Dichlorprop	10.0	9.34	0.46	4.9	93
2,4-D	10.0	8.35	0.13	1.6	84
Pentachlorophenol	1.00	1.10	0.071	6.5	110
Silvex	2.50	2.10	0.13	6.2	84
5-Hydroxydicamba	5.00	3.76	0.19	5.0	75
2,4,5-T	2.50	1.97	0.24	12	79
Chloramben	5.00	3.74	0.46	12	75
2,4-DB	10.0	8.32	0.38	4.6	83
Dinoseb	10.0	9.66	1.8	19	97
Bentazon	10.0	8.63	0.81	9.4	86
Picloram	10.0	11.4	2.9	25	114
Acifluorfen	5.00	4.20	0.43	10	84
Dacthal Acid Metabolites	10.0	8.22	0.78	9.5	82

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 20. HOLDING TIME STUDY FOR MTBE EXTRACTS

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 0

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	8.37	0.88	10	84
3,5-Dichlorobenzoic Acid	5.00	4.55	0.16	3.5	91
4-Nitrophenol	10.0	9.52	1.0	11	95
Dicamba	5.00	4.55	0.14	3.0	91
Dichlorprop	10.0	8.87	0.18	2.0	89
2,4-D	10.0	9.09	0.38	4.2	91
Pentachlorophenol	1.00	0.870	0.16	19	87
Silvex	2.50	2.29	0.050	2.2	92
5-Hydroxydicamba	5.00	4.23	0.30	7.1	85
2,4,5-T	2.50	2.26	0.12	5.2	90
Chloramben	5.00	4.76	0.34	7.2	95
2,4-DB	10.0	10.2	0.34	3.3	102
Dinoseb	10.0	10.7	3.1	29	107
Bentazon	10.0	9.54	0.84	8.8	95
Picloram	10.0	9.60	0.44	4.6	96
Acifluorfen	5.00	5.27	0.46	8.7	105
Dacthal Acid Metabolites	10.0	8.17	1.2	15	82

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 21. HOLDING TIME STUDY FOR MTBE EXTRACTS

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 7

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	9.67	1.6	17	97
3,5-Dichlorobenzoic Acid	5.00	5.50	0.60	11	110
4-Nitrophenol	10.0	10.7	1.0	9.7	107
Dicamba	5.00	5.31	0.18	3.4	106
Dichlorprop	10.0	10.4	0.50	4.8	104
2,4-D	10.0	10.4	0.66	6.3	104
Pentachlorophenol	1.00	1.21	0.23	19	121
Silvex	2.50	2.64	0.17	6.5	106
5-Hydroxydicamba	5.00	5.32	0.38	7.1	106
2,4,5-T	2.50	2.58	0.32	12	103
Chloramben	5.00	5.75	0.60	10	115
2,4-DB	10.0	11.0	0.24	2.1	110
Dinoseb	10.0	9.60	0.91	9.5	96
Bentazon	10.0	11.4	0.98	8.6	114
Picloram	10.0	13.8	1.3	9.6	138
Acifluorfen	5.00	6.27	0.88	14	125
Dacthal Acid Metabolites	10.0	10.8	1.1	10	108

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 22. HOLDING TIME STUDY FOR MTBE EXTRACTS

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 14

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	10.1	0.72	7.2	101
3,5-Dichlorobenzoic Acid	5.00	5.55	0.35	6.3	111
4-Nitrophenol	10.0	10.8	1.1	9.8	108
Dicamba	5.00	5.40	0.30	5.5	108
Dichlorprop	10.0	10.1	0.42	4.1	101
2,4-D	10.0	10.1	0.50	5.0	101
Pentachlorophenol	1.00	1.07	0.12	11	107
Silvex	2.50	2.57	0.16	6.2	103
5-Hydroxydicamba	5.00	5.13	0.40	7.8	103
2,4,5-T	2.50	2.43	0.21	8.6	97
Chloramben	5.00	5.40	0.68	12	108
2,4-DB	10.0	10.5	0.74	7.0	105
Dinoseb	10.0	9.68	2.0	21	97
Bentazon	10.0	10.8	1.2	11	108
Picloram	10.0	13.3	2.6	19	133
Acifluorfen	5.00	5.17	0.79	15	103
Dacthal Acid Metabolites	10.0	10.1	1.3	13	101

[a] Produced by the analysis of seven aliquots of fortified reagent water.

TABLE 23. HOLDING TIME STUDY FOR MTBE EXTRACTS

DERIVATIZATION BY TETRABUTYLAMMONIUM HYDROXIDE AND METHYL IODIDE

DAY 21

Analyte	Fortified Conc., µg/L	Mean Measured Conc., µg/L	Std. Dev., µg/L	Relative Std. Dev., %	Percent Recovery, %
Dalapon	10.0	10.8	1.8	16	108
3,5-Dichlorobenzoic Acid	5.00	5.72	0.88	15	114
4-Nitrophenol	10.0	10.1	1.7	16	101
Dicamba	5.00	5.13	0.50	9.7	103
Dichlorprop	10.0	10.4	0.71	6.8	104
2,4-D	10.0	9.51	0.85	9.0	95
Pentachlorophenol	1.00	1.06	0.27	26	106
Silvex	2.50	2.65	0.28	10	106
5-Hydroxydicamba	5.00	5.20	0.82	16	104
2,4,5-T	2.50	2.50	0.27	11	100
Chloramben	5.00	5.36	0.58	11	107
2,4-DB	10.0	11.1	1.2	11	111
Dinoseb	10.0	8.92	1.6	18	89
Bentazon	10.0	10.2	0.74	7.3	102
Picloram	10.0	12.6	1.0	8.1	126
Acifluorfen	5.00	5.67	0.71	12	113
Dacthal Acid Metabolites	10.0	14.0	1.1	7.9	140

[a] Produced by the analysis of seven aliquots of fortified reagent water.

METHOD 526. DETERMINATION OF SELECTED SEMIVOLATILE ORGANIC COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

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METHOD 526

DETERMINATION OF SELECTED SEMIVOLATILE ORGANIC COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

1. SCOPE AND APPLICATION

1.1 This is a gas chromatography/mass spectrometry (GC/MS) method for the determination of selected semivolatile organic compounds in raw and finished drinking waters. This method is applicable to the organic compounds listed below, which are efficiently extracted from water using a polystyrene divinylbenzene solid phase sorbent, and are sufficiently volatile and thermally stable for gas chromatography. Accuracy, precision, and method detection limit (MDL) data have been generated in reagent water, finished ground and surface water for the following compounds:

Analyte	Chemical Abstract Services Registry Number
Acetochlor	34256-82-1
Cyanazine	21725-46-2
Diazinon	61790-53-2
2,4-Dichlorophenol	120-83-2
1,2-Diphenylhydrazine	122-66-7
Disulfoton	298-04-4
Fonofos	944-22-9
Nitrobenzene	98-95-3
Prometon	1610-18-0
Terbufos	13071-79-9
2,4,6-Trichlorophenol	88-06-2

1.2 MDLs are compound, instrument, and matrix dependent. The MDL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined MDLs for the above listed analytes are provided in Section 17, Table 3. The MDL differs from, and is lower than, the minimum reporting limit (MRL)

(Sect. 3.17). Precision and accuracy were evaluated at 0.5 and 20 ug/L. Precision and accuracy data and sample holding time data are presented Section 17, Tables 4 through 8. Analyte retention times are in Section 17, Table 2.

1.3 This method is restricted to use by or under the supervision of analysts skilled in solid-phase extractions (SPE) and GC/MS analysis.

2. SUMMARY OF METHOD

2.1 A 1 liter water sample is passed through a SPE disk or cartridge containing polystyrenedivinylbenzene (SDVB) to extract the target analytes and surrogate compounds. The extract is dried by passing through a column of anhydrous sodium sulfate and is concentrated by blowdown with nitrogen to a volume of about 0.7 mL. Internal standards are added and the extract is diluted to a final volume of 1 mL. Components are separated chromatographically by injecting an aliquot of the extract onto a gas chromatograph equipped with a high resolution fused silica capillary column. The analytes pass from the capillary column into a mass spectrometer where the they are identified by comparing their measured mass spectra and retention times to reference spectra and retention times collected for the same compounds. Instrument specific reference spectra and retention times for analytes are obtained by the analyses of calibration standards under the same GC/MS conditions used for samples. The concentration of each identified component is measured by relating the MS response of the compound's quantitation ion to the internal standard's quantitation ion MS response.

3. DEFINITIONS

3.1 EXTRACTION BATCH – A set of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices, solvents, surrogate solution, and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Matrix, and either a Field Duplicate or Laboratory Fortified Matrix Duplicate.

3.2 ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check standards (CCC). Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.3 INTERNAL STANDARD (IS) – A pure analyte added to an extract or standard solution in a known amount and used to measure the relative responses of other method analytes and surrogates. The internal standard must be an analyte that is not a sample component.

3.4 SURROGATE ANALYTE (SUR) – A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in a known amount before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

3.5 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, sample preservatives, internal standards, and surrogates that are used in the extraction batch. The LRB is analyzed is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMD) – A second aliquot of the Field Sample used to prepare the LFM which is fortified, extracted and analyzed identically. The LFMD is used instead of the Field Duplicate to access method precision and accuracy when the occurrence of target analytes is low.

3.9 LABORATORY DUPLICATES (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, and storage procedures.

3.10 FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.11 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.12 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing method analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.13 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.14 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.15 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check standard integrity.

3.16 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.4). This is a statistical determination of precision. Accurate quantitation is not expected at this level.[1]

3.17 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest continuing calibration check standard for that analyte, and can only be used if acceptable quality control criteria for this standard are met.

3.18 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. Non-volumetric

glassware can be heated in a muffle furnace at 400 °C for 2 hours as a substitute for a solvent rinse. Volumetric glassware should not be heated in an oven above 120 °C.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for each target analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.4. **Subtracting blank values from sample results is not permitted.**

4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Water samples high in total organic carbon may have elevated baseline or interfering peaks.

4.4 Relatively large quantities of the buffer and preservatives (Sect. 8.1) are added to sample bottles. The potential for trace-level organic contaminants in these reagents exist. Interferences from these sources should be monitored by analysis of laboratory reagent blanks, particularly when new lots of reagents are acquired.

4.5 Benzophenone was an interferent recovered at low level from one manufacturer's lot of tris(hydroxymethyl)aminomethane hydrochloride. The spectra of benzophenone and 1,2-diphenylhydrazine are very similar, sharing the major m/z ions of 51, 77, 105, 152, and 182. At first glance, the benzophenone may appear to be a contaminant in the LRB. Given that the two compounds share common ions, their chromatographic peaks must be resolved.

4.6 During method development, one lot of anhydrous sodium sulfate was found to add an agent to the extract that, when injected, caused complete loss of prometon recovery and caused severe chromatographic tailing of the phenols. It is very important to check a new sodium sulfate lot before general use. When only one or two samples with the interfering agent were injected, recovery of good chromatographic performance was possible by removing the first meter of the capillary column and replacing the deactivated glass inlet liner.

4.7 Solid phase cartridges and disks and their associated extraction devices have been observed to be a source of interferences. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of solid phase extraction devices should be tested to ensure that contamination will not preclude analyte identification and quantitation.

4.8 Analyte carryover may occur when a relatively "clean" sample is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes and splitless injection port liners must be cleaned carefully or replaced as needed. After analysis of a sample, containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample.

4.9 Silicone compounds may be leached from punctured autosampler vial septa, particularly when particles of the septa sit in the vial. These silicone compounds should have no effect on the analysis, but the analyst should be aware of this potential problem.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of MSDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[2-4]

5.2 Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin and eyes, and care should be taken not to breath the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS – 1 liter glass bottles fitted with polytetrafluoroethylene (PTFE) lined screw caps.

6.2 VIALS – Various sizes of amber glass vials with PTFE lined screw caps for storing standard solutions and extracts. Crimp-top glass autosampler vials, 2 mL, with PTFE faced septa.

6.3 VOLUMETRIC FLASKS – Class A, suggested sizes include 1, 5, and 10 mL, for preparation of standards and dilution of extract to final volume.

6.4 GRADUATED CYLINDERS – Suggested sizes include 5, 10, and 250 mL.

6.5 MICRO SYRINGES – Suggested sizes include 25, 50, 100, 250, 500, and 1000 mL.

6.6 DRYING COLUMN – The drying column must be able to contain 5 to 7 g of anhydrous sodium sulfate. The drying column should not leach interfering compounds or irreversibly adsorb target analytes. Any small column may be used, such as a glass pipet with glass wool plug.

6.7 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for collection of the eluate from the cartridge or disk after extraction.

6.8 COLLECTION TUBES OR VIALS – 25 mL or larger, or other glassware suitable for collecting extract from drying tube. Conical centrifuge tubes, 50 mL, with graduations (VWR #: 21049-063) were used to develop this method.

6.9 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.10 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

6.10.1 SOLID PHASE EXTRACTION CARTRIDGES – 6 mL, packed with 500 mg (125 um d_p) of polystyrene divinylbenzene (SDVB) sorbent phase (Varian Bond Elut ENV phase; cat.#:1225-5011 or equivalent).

6.10.2 SAMPLE RESERVOIR AND TRANSFER TUBE– Sample reservoirs (VWR cat.#: JT7120-3 or equivalent) with a volume of about 75 mL are attached to the cartridges to hold the water sample. An alternative method is using transfer tubes (Supelco "Visiprep"; cat.#: 57275 or equivalent) which transfer the sample directly from the sample container to the SPE cartridge.

6.10.3 VACUUM EXTRACTION MANIFOLD – With flow/vacuum control (Supelco cat.#: 57044 or equivalent). The use of replaceable needles or valve liners may be used to prevent cross contamination.

6.10.4 REMOTE VACUUM GAUGE/BLEED VALVE ASSEMBLY – To monitor and adjust vacuum pressure delivered to the vacuum manifold (Supelco cat.#: 57161-U or equivalent)

6.10.5 An automatic or robotic system, designed for use with SPE cartridges, may be used as long as all quality control requirements discussed in Section 9 are met. Automated systems may use either vacuum or positive pressure to process samples and solvents through the cartridge. All extraction and elution steps must be the same as in the manual procedure. Extraction or elution steps may not be changed or omitted to accommodate the use of an automated system.

6.11 SOLID PHASE EXTRACTION (SPE) APPARATUS USING DISKS

6.11.1 SOLID PHASE EXTRACTION DISKS – 47 mm diameter and 0.5 mm thick, manufactured with a polystyrene divinylbenzene (SDVB) sorbent phase (Varian cat. #: 1214-5010 or equivalent). Larger disks may be used as long as the QC performance criteria outlined in Section 9 are met.

6.11.2 SPE DISK EXTRACTION GLASSWARE – funnel, PTFE coated support screen, PTFE gasket, base, and clamp used to support SPE disks and contain samples during extraction. May be purchased as a set (Fisher cat. #:K971100-0047 or equivalent) or separately.

6.11.3 VACUUM EXTRACTION MANIFOLD – Designed to accommodate extraction glassware and disks (Varian cat.#: 1214-6001 or equivalent).

6.11.4 An automatic robotic system for disks as described in Section 6.10.5.

6.12 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by blowdown with nitrogen using water bath set to 40°C (Meyer N-Evap, Model 111, Organomation Associates, Inc. or equivalent).

6.13 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of about 10 inches of mercury for cartridges. A greater vacuum of 15 to 25 inches of mercury may be used with disks.

6.14 GAS CHROMATOGRAPH/MASS SPECTROMETER (GC/MS) INSTRUMENTATION

6.14.1 FUSED SILICA CAPILLARY GC COLUMN – 30 m x 0.25 mm i.d. fused silica capillary column coated with a 0.25 um bonded film of poly(dimethylsiloxy)poly(1,4-bis(dimethylsiloxy)phenylene)siloxane (J&W DB-5MS or equivalent). Any capillary column that provides adequate resolution, capacity, accuracy, and precision as summarized in Section 17 may be used. A nonpolar, low-bleed column is recommended for use with this method to provide adequate chromatography and minimize column bleed.

6.14.2 GC INJECTOR AND OVEN – Capable of temperature programming and equipped for split/splitless injection. Target compounds included in this method are subject to thermal breakdown in the injector port, which increases when the injector is not properly deactivated or at excessive temperatures. The injection system must not allow analytes to contact hot stainless steel or other metal surfaces that promote decomposition. The

performance data in Section 17 was obtained by hot, splitless injection using a 4 mm i.d. glass, deactivated liner (Restek cat.#: 20772). Other injection techniques such as temperature programmed injections, cold on-column injections and large volume injections may be used if the QC criteria in Sections 9 and 10 are met. Equipment designed appropriately for these alternate types of injections must be used if these options are selected.

6.14.3 GC/MS INTERFACE – Interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, like jet separators, are acceptable as long as the system has adequate sensitivity and QC performance criteria are met.

6.14.4 MASS SPECTROMETER – The MS must be capable of electron ionization at a nominal electron energy of 70 eV to produce positive ions. The spectrometer must be capable of scanning at a minimum from 45 to 450 amu with a complete scan cycle time (including scan overhead) of 1.0 second or less. (Scan cycle time = total MS data acquisition time in seconds divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 1 when a solution containing approximately 5 ng of DFTPP is injected into the GC/MS. Use a single spectrum at the apex of the DFTPP peak, an average spectrum of the three highest points of the peak, or an average spectrum across the entire peak to evaluate the performance of the system. The scan time should be set so that all analytes have a minimum of five scans across the chromatographic peak. Seven to ten scans across chromatographic peaks are preferred.

6.14.5 DATA SYSTEM – An interfaced data system is required to acquire, store, and output mass spectral data. The computer software should have the capability of processing stored GC/MS data by recognizing a GC peak within a given retention time window. The software must allow integration of the ion abundance of any specific ion between specified time or scan number limits. The software must be able to calculate relative response factors, construct a linear regression or quadratic calibration curve, and calculate analyte concentrations.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents will conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available.

Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 HELIUM – 99.999% or better, GC carrier gas.

7.1.2 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes or interfering compounds at or above 1/3 the MRL for each compound of interest.

7.1.3 METHANOL (CAS# 67-56-1) – High purity, demonstrated to be free of analytes and interferences (B&J Brand GC$^{2®}$, Capillary GC/GC-MS Grade or equivalent).

7.1.4 ETHYL ACETATE (CAS# 141-78-6) – High purity, demonstrated to be free of analytes and interferences (B&J Brand GC$^{2®}$, Capillary GC/GC-MS Grade or equivalent).

7.1.5 METHYLENE CHLORIDE (CAS# 75-09-02) – High purity, demonstrated to be free of analytes and interferences (B&J Brand GC$^{2®}$, Capillary GC/GC-MS Grade or equivalent).

7.1.6 SODIUM SULFATE, ANHYDROUS (CAS# 7757-82-6) – Soxhlet extracted with methylene chloride for a minimum of four hours or heated to 400°C for two hours in a muffle furnace. One lot of "ACS grade" anhydrous sodium sulfate had a contaminant that degraded the capillary column so that analyte recoveries were unacceptable. An "ACS grade, suitable for pesticide residue analysis," or equivalent, of anhydrous sodium sulfate is recommended.

7.1.7 SAMPLE PRESERVATION REAGENTS – These preservatives are solids at room temperature and may be added to the sample bottle before shipment to the field.

7.1.7.1 BUFFER SALT MIX, pH 7 – The sample must be buffered to pH 7 with two components: 1) tris(hydroxymethyl)aminomethane, also called Tris, 0.47 g (CAS# 77-86-1, ACS Reagent Grade or equivalent); and 2) tris(hydroxymethyl)aminomethane hydrochloride, also called Tris HCl, 7.28 g (CAS# 1185-53-1, ACS Reagent Grade or equivalent). Alternately, 7.75 g of a commercial buffer crystal mixture, that is blended in proportion to the amounts given above, can be used.

7.1.7.2 L-ASCORBIC ACID (CAS# 50-81-7) – Ascorbic acid reduces "free chlorine" at the time of sample collection (ACS Reagent Grade or equivalent).

7.1.7.3 ETHYLENEDIAMINETETRAACETIC ACID, TRISODIUM SALT (Trisodium EDTA, CAS# 10378-22-0) – Trisodium EDTA is added to inhibit metal-catalyzed hydrolysis of analytes. The trisodium salt is used instead of the disodium salt because the trisodium salt solution pH is closer to the desired pH of 7 (Sigma cat.#: ED3SS or equivalent).

7.1.7.4 DIAZOLIDINYL UREA (DZU) (CAS# 78491-02-8) – DZU is added to inhibit microbial growth. DZU (Sigma cat.#: D-5146) is commonly used as a preservative in cosmetics such as skin lotion.

7.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should used standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTIONS – This method uses three internal standard compounds listed in the table below.

Internal Standards	CAS#	FW
acenaphthene-d_{10}	15067-26-2	164.3
phenanthrene-d_{10}	1517-22-2	188.3
chrysene-d_{12}	1719-03-5	240.4

7.2.1.1 INTERNAL STANDARD PRIMARY DILUTION STANDARD (500 ug/mL) – Prepare or purchase commercially the Internal Standard Primary Dilution Standard (PDS) at a concentration of 500 ug/mL. If prepared from neat or solid standards, this solution requires the preparation of a more concentrated stock standard similar to the procedure followed for the analyte stock (Sect. 7.2.3.1). The solvent for the Internal Standard PDS may be acetone or ethyl acetate. The Internal Standard PDS has been

shown to be stable for 1 year in amber glass screw cap vials when stored at -10°C or less.

7.2.1.2 INTERNAL STANDARD EXTRACT FORTIFICATION SOLUTION (50 ug/mL) – Dilute a portion of the Internal Standard PDS (500 ug/mL) (Sect. 7.2.1.1) to a concentration of 50 ug/mL in ethyl acetate and use this solution to fortify the final 1 mL extracts (Sect. 11.6). The Internal Extract Fortification Solution has been shown to be stable in amber glass screw cap vials for 6 months when stored at -10°C or less.

7.2.2 SURROGATE (SUR) ANALYTE STANDARD SOLUTIONS – The two surrogates for this method are listed in the table below.

Surrogates	CAS #	FW
1,3-dimethyl-2-nitrobenzene	81-20-9	151.2
triphenylphosphate	115-86-6	326.3

7.2.2.1 SUR STOCK SOLUTION (~ 4 to 10 mg/mL) – Surrogate Stock Solutions may be purchased commercially or prepared from neat materials. The solvent for the SUR Stock Solution may be acetone or ethyl acetate. These solutions have been shown to be stable for one year when stored in amber glass containers at -10°C or less.

7.2.2.2 SUR PRIMARY DILUTION STANDARD (SUR PDS) (500 ug/mL) – The 500 ug/mL SUR PDS may be purchased commercially or prepared by volumetric dilution of the SUR Stock Solutions (Sect. 7.2.2.1) in acetone or ethyl acetate The PDS has been shown to be stable for one year when stored in amber glass screw cap vials at -10°C or less. This solution is used to make the 50 ug/mL solution for sample fortification and also to prepare calibration solutions.

7.2.2.3 SUR SAMPLE FORTIFICATION SOLUTION (50 ug/mL) – Dilute the 500 ug/mL SUR PDS in methanol to make a 50 ug/mL sample fortification solution. Add 100 uL of this 50 ug/mL solution to each 1 liter water sample before extraction to give a concentration of 5 ug/L of each surrogate. This solution has been shown to be stable for six months when stored in amber glass screw cap vials at -10°C or less.

7.2.3 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat or solid standards or as commercially prepared ampulized solutions from a reputable standard manufacturer. Prepare the Analyte Stock and Primary Dilution Standards as described below.

7.2.3.1 ANALYTE STOCK STANDARD SOLUTIONS (1 to 10 mg/mL) – Analyte standards may be purchased commercially as ampulized solutions or prepared from neat materials. Stock standards have been shown to be stable for one year when stored in amber glass screw cap vials at -10°C or less.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARDS (200 ug/mL and 20 ug/mL) – Prepare the 200 ug/mL Analyte PDS by volumetric dilution of the Analyte Stock Standard Solution (Sect. 7.2.3.1) in ethyl acetate to make a 200 ug/mL solution. The 20 ug/mL Analyte PDS can be made by a volumetric dilution of the 200 ug/mL Analyte PDS in ethyl acetate. The Analyte PDSs are used to prepare calibration and fortification standards. They have been shown to be stable for six months when stored in an amber glass screw cap vial at -10°C or less. Check frequently for signs of evaporation, especially before preparing calibration solutions.

7.2.4 CALIBRATION SOLUTIONS – Prepare a calibration curve of at least 5 CAL levels over the concentration range of interest from dilutions of the Analyte PDSs in ethyl acetate. All calibration solutions should contain at least 80% ethyl acetate so that gas chromatographic performance is not compromised. The lowest concentration of calibration standard must be at or below the MRL. The level of the MRL will depend on system sensitivity. A constant concentration of each internal standard and surrogate (in the range of 2 to 5 ng/uL) is added to each CAL. For instance, for method development work, 50 uL of the 500 ug/mL Internal Standard PDS and 50 uL of the 500 ug/mL SUR PDS were added to each CAL level standard for final concentrations of 5 ug/mL. The calibration solutions have been shown to be stable for six months when stored in an amber glass screw cap vial at -10°C or less.

7.2.5 ANALYTE FORTIFICATION SOLUTIONS (0.05 to 5.0 ug/mL) – The Analyte Fortification Solutions contain all method analytes of interest in methanol. They are prepared by dilution of the Analyte PDSs (200 ug/mL or 20 ug/mL). These solutions are used to fortify the LFBs, the LFMs and LFMDs with method analytes. It is recommended that three concentrations be prepared so that the fortification levels can be rotated. The Analyte Fortification Solutions have been shown to be stable for six months when stored in an amber glass screw cap vial at -10°C or less.

7.2.6 GC/MS TUNE CHECK SOLUTION (5 ug/mL) – Prepare a Decafluorotriphenylphosphine (DFTPP) solution in methylene chloride. DFTPP is more stable in methylene chloride than in acetone or ethyl acetate. Store this solution in an amber glass screw cap vial at -10°C or less.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [5] using a 1 liter or 1 quart amber or clear glass bottle fitted with PTFE-lined screw-caps.

8.1.2 Preservation reagents, listed in the table below, are added to each sample bottle prior to shipment to the field.

Compound	Amount	Purpose
L-Ascorbic Acid	0.10 g/L	Dechlorination
Ethylenediaminetetraacetic acid trisodium salt	0.35 g/L	Inhibit metal-catalyzed hydrolysis of targets
Diazolidinyl Urea	1.0 g/L	Microbial inhibitor
***Tris(hydroxymethyl)aminomethane**	0.47 g/L	First component of pH 7 buffer mixture
***Tris(hydroxymethyl)aminomethane hydrochloride**	7.28 g/L	Second component of pH 7 buffer mixture

*Alternately, 7.75 g of a commercial buffer crystal mixture, that is blended in the proportions given in the table, can be used (Sect. 7.1.7.1).

8.1.2.1 Residual chlorine must be reduced at the time of sample collection with 100 mg of ascorbic acid per liter. Sodium thiosulfate and sodium sulfite cannot be used because they were found to degrade target analytes. In addition, while ammonium chloride is effective in converting free chlorine to chloramines, the chloramines also caused target compound loss.

8.1.2.2 Ethylenediaminetetraacetic acid, trisodium salt (trisodium EDTA) (0.35 g) must be added to inhibit metal-catalyzed hydrolysis of the target analytes, principally terbufos, disulfoton, diazinon, fonofos, and cyanazine.

8.1.2.3 Diazolidinyl urea (1.0 g) is added to inhibit microbial degradation of analytes. Diazolidinyl urea is used in cosmetics such as skin lotion. The antimicrobial activity of diazolidinyl urea has been proposed as due to protein alkylation of sulfhydryl groups and the ability to release formaldehyde.[6] Plate count studies conducted during method development indicated that it was effective in inhibiting microbial degradation for extended periods.

8.1.2.4 The sample must be buffered to pH 7 to reduce the acid and base catalyzed hydrolysis of target analytes. The pH buffer has two components: tris(hydroxymethyl)aminomethane (0.47 g) and tris(hydroxymethyl)aminomethane hydrochloride (7.28 g). A commercially prepared combination of these two compounds can be purchased as pre-mixed crystals. When using the pH 7 pre-mixed crystals, add 7.75 g per liter of water sample.

8.2 SAMPLE COLLECTION

8.2.1 When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1 L beaker with water sampled from a representative area, and carefully fill sample bottles from the container. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2.3 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.4 After collecting the sample, cap the bottle and agitate by hand until preservatives are dissolved. Keep the sample sealed from time of collection until extraction.

8.3 SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment and must not exceed 10°C during the first 48 hours after collection. Samples should be confirmed to be at or below 10°C when they are received at the laboratory. Samples stored in the lab must be held at or below 6°C until extraction, but should not be frozen.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Sect. 17, Table 7) indicated that most compounds listed in this method have adequate stability for 14 days when collected, dechlorinated, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.3. At 14 days, even the most unstable compound, terbufos, was recovered above 60 %. Water samples should be extracted as soon as possible, but must be extracted within 14 days. The extract storage stability study must be stored at 0°C or less and analyzed within 28 days after extraction. The extract storage stability study data are presented in Section 17, Table 8.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability (Sect. 17, Table 9), the determination of the MDL, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards (CCC), a Laboratory Fortified Blank (LFB), a Laboratory Fortified Sample Matrix (LFM), and either a Laboratory Fortified Sample Matrix Duplicate (LFMD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 9 and 10. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections and summarized in Section 17, Table 9.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solid phase extraction (SPE) cartridges or disks is used, it must be demonstrated that a laboratory reagent blank (Sect. 9.4) is reasonably free of contamination and that the criteria in Section 9.4 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare, extract, and analyze 4-7 replicate LFBs fortified near the midrange of the initial calibration curve according to the procedure described in Section 11. Sample preservatives as described in Section 8.1 must be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY – Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ±30% of the true value.

9.2.4 MDL DETERMINATION – Prepare, extract and analyze at least seven replicate LFBs at a concentration estimated to be near the MDL, over a period of at least three days (both extraction and analysis should be conducted over at least three days) using the procedure described in Section 11. The fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level. The appropriate concentration will be dependent upon the sensitivity of the GC/MS system being used. Sample preservatives as described in Section 8.1 must be added to these samples. Calculate the MDL using the equation

$$MDL = St_{(n-1,\ 1-alpha=0.99)}$$

where
$t_{(n-1,\ 1-alpha=0.99)}$ = Students t value for the 99% confidence level with n-1 degrees of freedom,
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations. The MDL is a statistical determination of precision only.[1] If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify GC columns, GC conditions, evaporation techniques, internal standards or surrogate standards, but each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.3 MINIMUM REPORTING LEVEL (MRL) – The MRL is a threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should be established at an analyte concentration that is either greater than three times the MDL or at an analyte concentration which would yield a response greater than a signal-to-noise ratio of five. **Although the lowest calibration standard for an analyte may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard.**

9.4 LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.1) of samples to determine the background system contamination. If the LRB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. If the target analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.5 CONTINUING CALIBRATION CHECK (CCC) – A CCC is a standard prepared with all compounds of interest which is analyzed during the analysis batch to ensure the stability of the instrument initial calibration. This calibration check is required at the beginning of each day that samples are analyzed, after every ten injections, and at the end of any group of sample analyses. See Section 10.3 for concentration requirements and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANK (LFB) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB should be rotated between low, medium, and high concentrations from day to day. The low concentration LFB must be as near as practical to, but no more than two times the MRL. Similarly, the high concentration LFB should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the low-level LFB analyses must be 50-160% of the true value. Results of the medium and high-level LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for target analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.7 MS TUNE CHECK – A complete description of the MS tune check is in Section 10.2.1. This check must be performed each time a major change is made to the mass spectrometer, and prior to establishing and/or re-establishing an initial calibration (Sect. 10.2). In this method daily DFTPP analysis is not required.

9.8 INTERNAL STANDARDS (IS) – The analyst must monitor the peak area of each internal standard in all injections during each analysis day. The IS response (as indicated by peak area) for any chromatographic run must not deviate by more than ±50% from the average area measured during the initial calibration for that IS. A poor injection could cause the IS area to exceed these criteria. Inject a second aliquot of the suspect extract to determine whether the failure is due to poor injection or instrument response drift.

9.8.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.8.2 If the internal standard area for the reinjected extract deviates greater than 50% from the initial calibration average, the analyst should check the continuing calibration check standards that ran before and after the sample. If the continuing calibration check fails the criteria of Section 9.5 and 10.3, recalibration is in order per Section 10. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.9 SURROGATE RECOVERY – The surrogate standards are fortified into the aqueous portion of all samples, duplicates, LRBs, LFMs and LFMDs prior to extraction. Surrogates are also added to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.9.1 When surrogate recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.9.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.9.3 If the extract reanalysis fails the 70-130% surrogate recovery criterion, the analyst should check the surrogate calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the 70-130% surrogate recovery criteria of Section 9.9.1, recalibration is in order. If the surrogate recovery of the calibration standard is acceptable, extraction of the sample should be repeated, provided the sample is still within the holding time. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect/surrogate recovery.

9.9.4 The surrogate, 1,3-dimethyl-2-nitrobenzene, is used to track the recovery of nitrobenzene. The other surrogate, triphenylphosphate, monitors the recovery of all the other target analytes. If nitrobenzene is not included on the target analyte list, then the first surrogate, 1,3-dimethyl-2-nitrobenzene, does not need to be analyzed.

9.10 LABORATORY FORTIFIED SAMPLE MATRIX AND DUPLICATE (LFM AND LFMD) – Analyses of LFMs (Sect. 3.7) are required in each extraction batch and are used to determine that the sample matrix does not adversely affect method accuracy. If the occurrence of target analytes in the samples is infrequent,

or if historical trends are unavailable, a second LFM, or LMFD, must be prepared, extracted, and analyzed from a duplicate field sample used to prepare the LFM to assess method precision. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate (Sect. 9.11). If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be established for each. Over time, LFM data should be documented for all routine sample sources for the laboratory.

9.10.1 Within each extraction batch, a minimum of one field sample is fortified as an LFM for every 20 samples extracted. The LFM is prepared by spiking a sample with an appropriate amount of Analyte PDS (Sect. 7.2.5). Select a spiking concentration that is at least twice the matrix background concentration, if known. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate spiking levels.

9.10.2 Calculate the percent recovery (R) for each analyte using the equation

$$R = \frac{(A - B)}{C} * 100$$

where: A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

9.10.3 Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 and 130%, except for low-level fortification near or at the MRL where 50 to 160% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.10.4 If an LFMD is analyzed instead of a Field Duplicate (Sect. 9.11), calculate

$$RPD = \frac{LFM - LFMD}{(LFM + LFMD)/2} * 100 \qquad (1)$$

the relative percent difference (RPD) for duplicate LFMs (LFM and LFMD) using the equation RPDs for duplicate LFMs should fall in the range of ±30% for samples fortified at or above their native concentration. Greater variability may be observed when LFMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ±50% for samples fortified at or above their native concentration. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.11 FIELD DUPLICATES (FD1 AND FD2) – Within each extraction batch, a minimum of one Field Duplicate (FD) or LFMD (Sect. 9.10) must be analyzed. FDs check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, a LFMD (Sect. 9.10) should be analyzed to substitute for this requirement. Extraction batches that contain LFMDs (Section 9.10) will not require the analysis of a Field Duplicate.

9.11.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} * 100 \qquad (2)$$

9.11.2 RPDs for duplicates should be in the range of ±30%. Greater variability may be observed when analyte concentrations are near the MRL. At the MRL, RPDs should fall in the range of ±50%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.12 QUALITY CONTROL SAMPLES (QCS) – Each time that new standards are prepared or a new calibration curve is run, analyze a QCS from a source different than the source of the calibration standards. The QCS may be injected as a calibration standard or fortified into reagent water and analyzed as a LFB. If the QCS is analyzed as a continuing calibration, then the acceptance criteria are the same as for the CCC. If the QCS is analyzed as a LFB, then the acceptance

criteria are the same as for an LFB. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable mass spectrometer tune and initial calibration is required before any samples are analyzed. After the initial calibration is successful, a continuing calibration check is required at the beginning and end of each period in which analyses are performed, and after every tenth sample. Verification of mass spectrometer tune must be repeated each time a major instrument modification is made or maintenance is performed, and prior to analyte calibration.

10.2 INITIAL CALIBRATION

10.2.1 MS TUNE/MS TUNE CHECK– Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet tuning requirements. Inject 5 ng or less of the DFTPP solution (Sect. 7.2.6) into the GC/MS system. Acquire a mass spectrum that includes data for m/z 45-450. Use a single spectrum at the apex of the DFTPP peak, an average spectrum of the three highest points of the peak, or an average spectrum across the entire peak to evaluate the performance of the system. If the DFTPP mass spectrum does not meet all criteria in Table 1, the MS must be retuned and adjusted to meet all criteria before proceeding with the initial calibration.

10.2.2 INSTRUMENT CONDITIONS – Operating conditions are described below. Conditions different from those described may be used if QC criteria in Section 9 are met. Different conditions include alternate GC columns, temperature programs, and injection techniques, such as cold on-column and large volume injections. Equipment designed for alternate types of injections must be used if these options are selected.

10.2.2.1 Inject 1 uL into a hot, splitless injection port held at 210°C with a split delay of 1 min. The temperature program is as follows: initially hold at 55°C for one minute, then ramp at 8°C/ min to 320°C. Total run time is approximately 33 min. Begin data acquisition at 3.5 minutes.

Note: The GC was operated in a constant flow rate mode at a rate of 1.4 mL per minute and an initial head-pressure of 12.2 psi.

10.2.2.2 Many of the target compounds exhibit decreased sensitivity for low-level injections due to degradation or irreversible adsorption in the injector port. Deactivated glass or quartz inlet liners are recommended.

10.2.2.3 MS Detection and Sensitivity – Acquire and store data from m/z 45 to 450 with a total cycle time (including scan overhead time) of 1.0 second or less. Adjust the cycle time to measure at least five or more spectra during the elution of each GC peak. Seven to ten scans across each GC peak are recommended. The GC/MS/DS peak identification software must be able to recognize a GC peak in the appropriate retention time window for each of the compounds in the calibration solution, and make correct qualitative identifications.

10.2.3 CALIBRATION SOLUTIONS – Prepare a set of at least 5 calibration standards as described in Section 7.2.4. The lowest concentration of the calibration standard must be at or below the MRL, which will depend on system sensitivity. Acceptable calibration over a large dynamic range, greater than about 50 fold range, may require multiple calibration curves.

10.2.4 CALIBRATION – The system is calibrated using the internal standard technique. Concentrations may be calculated through the use of average relative response factor (RRF) or through the use of a calibration curve. Calculate the RRFs using the equation

$$RRF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)}$$

where: A_x = integrated abundance (peak area) of the quantitation ion of the analyte,

A_{is} = integrated abundance (peak area) of the quantitation ion internal standard,

Q_x = quantity of analyte injected in ng or concentration units, and

Q_{is} = quantity of internal standard injected in ng or concentration units.

Average RRF calibrations may only be used if the RRF values over the calibration range are relatively constant (<30% RSD). Average RRF is determined by calculating the mean RRF of a minimum of five calibration concentrations.

10.2.5 As an alternative to calculating average RRFs and applying the RSD test, use the GC/MS data system software to generate a linear regression or quadratic calibration curve. The analyst may choose whether or not to force zero to obtain a curve that best fits the data. Examples of common GC/MS system calibration curve options are: 1) A_x / A_{is} vs Q_x / Q_{is} and 2) RRF vs A_x / A_{is}.

10.2.6 Acceptance criteria for the calibration of each analyte is determined by calculating the concentration of each analyte and surrogate in each of the analyses used to generate the calibration curve or average RRF. Each calibration point, except the lowest point, for each analyte must calculate to be 70-130 % of its true value. The lowest point must calculate to be 50-150% of its true value. If this criteria cannot be met, reanalyze the calibration standards, restrict the range of calibration, or select an alternate method of calibration. The data presented in this method were obtained using quadratic fit (RRF vs. amount). Quadratic fit calibrations should be used with caution, because the non-linear area of the curve may not be reproducible.

10.3 CONTINUING CALIBRATION CHECK (CCC) – The CCC verifies the initial calibration at the beginning and end of each group of analyses, and after every 10th sample during analyses. In this context, a "sample" is considered to be a field sample. LRBs, LFBs, LFMs, LFMDs and CCCs are not counted as samples. The beginning CCC for each analysis batch must be at or below the MRL in order to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL solutions to meet this requirement. Subsequent CCCs should alternate between a medium and high concentration.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Determine that the absolute areas of the quantitation ions of the internal standards have not changed by more than ± 50% from the areas measured during initial calibration. If any IS area has changed by more this amount, remedial action must be taken (Sect. 10.3.4). Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be ±30% of the true value. The calculated amount for the lowest calibration level for each analyte must be within ±50% of the true value. If these conditions do not exist, then all data for the problem

analyte must be considered invalid, and remedial action (Sect. 10.3.4) should be taken which may require recalibration. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored, with the following exception. If the continuing calibration fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular target compound, and field sample extracts show no detection for that target compound, non-detects may be reported without re-analysis.

10.3.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance such as cleaning an ion source, cleaning quadrapole rods, replacing filament assemblies, etc., require returning to the initial calibration step (Sect. 10.2).

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper preparation of laboratory glassware and sample containers (Sect. 4.1), and sample collection and storage (Sect. 8). This section describes the procedures for sample preparation, solid phase extraction (SPE) using cartridges or disks, and extract analysis.

11.2 SAMPLE BOTTLE PREPARATION

11.2.1 Samples are preserved, collected and stored as presented in Section 8. All field and QC samples must contain the preservatives listed in Section 8.1.2, including the LRB and LFB. Before extraction, mark the level of the sample on the outside of the sample bottle for later sample volume determination. If using weight to determine volume (Sect. 11.3.7), weigh the bottle with collected sample before extraction.

11.2.2 Add an aliquot of the surrogate fortification solution to each sample to be extracted. For method development work, a 100 uL aliquot of the 50 ug/mL SUR Sample Fortification Solution (Sect. 7.2.2.3) was added to 1 L for a final concentration of 5.0 ug/L.

11.2.3 If the sample is an LFB, LFM, or LFMD, add the necessary amount of analyte fortification solution. Swirl each sample to ensure all components are mixed.

11.2.4 Proceed with sample extraction using either SPE cartridges (Sect. 11.3) or disks (Sect. 11.4).

11.3 CARTRIDGE SPE PROCEDURE – The cartridge extraction procedure is carried out in a manual mode or by using a robotic or automatic sample preparation device. This section describes a SPE manual procedure using the equipment outlined in Section 6.10. The manual mode of sample addition to cartridges is performed with a large reservoir attached to the cartridge or with a transfer tube from the sample bottle to the cartridge. Cartridge extraction data in Section 17 was collected using the transfer tube option described below.

11.3.1 CARTRIDGE CLEANUP – Attach the extraction cartridges to the vacuum manifold. Rinse each cartridge with a 5-mL aliquot of ethyl acetate, allowing the sorbent to soak in the ethyl acetate for about 1 minute by turning off the vacuum temporarily. Repeat with a 5-mL aliquot of methylene chloride ($MeCl_2$). Let the cartridge vacuum dry after each flush.

11.3.2 CARTRIDGE CONDITIONING – This conditioning step is critical for recovery of analytes and can have a marked effect on method precision and accuracy. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the cartridge must not go dry until the last portion of the sample passes because analyte and surrogate recoveries may be affected. The analyst should note premature drying of the solid phase, because the sample may require re-extraction due to low surrogate recoveries.

11.3.2.1 CONDITIONING WITH METHANOL – Rinse each cartridge with a 5-mL aliquot of methanol (MeOH), allowing the sorbent to soak for about 30 seconds by turning off the vacuum temporarily. From this point on, do not allow the cartridge to go dry until after extraction is complete. Drain most of the MeOH without going below the top of the cartridge packing and rinse again with a 5-mL aliquot of MeOH.

11.3.2.2 CONDITIONING WITH REAGENT WATER – Drain most of the MeOH and rinse the cartridge with two consecutive 5-mL aliquots of reagent water, being careful not to allow the water level to go below the cartridge packing. Turn off the vacuum. Fill the cartridge to the top with reagent water and attach a reservoir or a transfer tube (Sect. 6.10.2).

11.3.3 CARTRIDGE EXTRACTION – Prepare samples, including the QC samples, as specified in Section 11.2. The sample may be added to the cartridge using either a large reservoir attached to the cartridge or using a transfer tube from the sample bottle to the cartridge.

11.3.3.1 SAMPLE ADDITION USING RESERVOIRS – Attach a reservoir to the conditioned cartridge from Section 11.3.2. Fill the reservoir with sample and turn on the vacuum adding additional aliquots of sample until the entire 1 L sample is processed. Adjust the vacuum so that the flow rate is about 20 mL/min. Do not let the cartridge packing go dry before all the sample has been extracted. After all of the sample has passed through the SPE cartridge, draw air through the cartridge for 10 minutes at full vacuum (minus 10 to 15 inches Hg). If the cartridge is dried for period much longer than 10 minutes, there may be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release vacuum.

11.3.3.2 SAMPLE ADDITION USING TRANSFER TUBES – Fit the PTFE transfer tube adapter securely to the conditioned cartridge. The screw on the adapter must be finger-tight, otherwise, air can leak and the cartridge may go dry. Place the weighted end of the transfer tube inside on the bottom of the sample bottle. Adjust the flow rate to about 20 mL/min. If the adapter is securely attached, the water level in the cartridge should drop only as much as the volume of the transfer tube and no more. Do not let the SPE sorbent go dry before all the sample has been extracted. After all the sample has passed through the SPE cartridge, draw air through the cartridge for 10 minutes at full vacuum (minus 10 to 15 inches Hg). If the cartridge is dried for a much longer period than 10 minutes, there may be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release vacuum.

11.3.4 CARTRIDGE ELUTION – Keep reservoirs or transfer tubes attached. Lift the manifold top, place collection tubes into the extraction tank, and insert valve liners into the collection tubes for extract collection. Add 5 mL of ethyl acetate (EtAc) to the empty sample bottle and rotate the bottle on its side, rinsing the inside of the bottle. If using the cartridge reservoir method, pour the EtAc from the bottle into the cartridge reservoir and draw enough of the EtAc through the cartridge to soak the sorbent. If using the transfer tubes method, pull the EtAc through the PTFE transfer tubes and draw enough of the EtAc through the tubes into the cartridge to soak the sorbent. Turn off the vacuum and vent the system and allow the sorbent to soak in EtAc for approximately 30 seconds. Start a low vacuum (minus 2-4 in Hg) and pull the ethyl acetate through in a dropwise fashion into the collection tube. Repeat rinse with 5 mL $MeCl_2$. Take off

reservoirs and transfer tubes and rinse the cartridge body with 2 to 3 mLs of 1:1 mixture of $MeCl_2$ and EtAc (1:1 $MeCl_2$/EtAc).

11.3.5 DRYING OF THE EXTRACT – Small amounts of residual water from the sample container and solid phase may form an immiscible layer with the solvent in the extract. Set up a drying column (Sect. 6.6) packed with about 5 grams of anhydrous sodium sulfate. Pre-rinse the sodium sulfate column with about 2 mL of 1:1 EtAc/$MeCl_2$. Place a clean collection tube that can hold at least 20 mL beneath the drying column. Add the extract to the column and follow with two, 3 mL aliquots of 1:1 EtAc/$MeCl_2$.

11.3.6 EXTRACT CONCENTRATION – Concentrate the extract to about 0.7 mL under a gentle stream of nitrogen in a warm water bath (at ~ 40°C). Do not blow down samples to less than 0.5 mL, because the most volatile compounds will show diminished recovery. Transfer the extract to a 1 mL volumetric flask and add the internal standard (method development used 100 uL of 50 ug/mL internal standard solution for an extract concentration of 5 ug/mL). Rinse the collection tube that held the dried extract with small amounts of EtAc and add to the volumetric flask to bring the volume up to the 1 mL mark. Transfer to autosampler vial.

11.3.7 SAMPLE VOLUME OR WEIGHT DETERMINATION – Use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction (Sect. 11.2.1). Determine volume to the nearest 10 mL for use in the final calculations of analyte concentration (Sect. 12.2). If using weight to determine volume, reweigh empty sample bottle. From the weight of the original sample bottle measured in Section 11.2.1, subtract the empty bottle weight. Use this value for analyte concentration calculations in Section 12.2.

11.4 DISK SPE PROCEDURE – The disk extraction procedure may be carried out in a manual mode or by using a robotic or automatic sample preparation device. This section describes the disk SPE procedure using the equipment outlined in Section 6.10 in its simplest, least expensive mode without the use of a robotics systems. The manual mode described below was used to collect data presented in Section 17.

11.4.1 SAMPLE PREPARATION – Prepare the sample as given in Section 11.2.

11.4.2 DISK CLEANUP – Assemble the extraction glassware onto the vacuum manifold, placing disks on a support screen between the funnel and base. Add a 5 mL aliquot of 1:1 mixture of ethyl acetate (EtAc) and methylene chloride ($MeCl_2$) (1:1 $MeCl_2$/EtAc), drawing about half through the disk,

and allowing the solvent to soak the disk for about a minute. Draw the remaining solvent through the disk to waste until the disk is dry of solvent.

11.4.3 DISK CONDITIONING – The conditioning step is critical for recovery of analytes and can have a marked effect on method precision and accuracy. If the disk goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the disk must not go dry until the last portion of the sample passes, because analyte and surrogate recoveries may be affected. The analyst should note premature drying of the solid phase, because the sample may require re-extraction due to low surrogate recoveries. During conditioning, it is not unusual for the middle of the solid phase disk to form a wrinkle. This typically does not adversely affect extraction.

11.4.3.1 CONDITIONING WITH METHANOL – Add approximately 10 mL methanol to each disk. Pull about 1 mL of MeOH through the disk and turn off the vacuum temporarily to let the disk soak for about one minute. Draw most of the remaining MeOH through the disk, but leave a layer of MeOH on the surface of the disk. The disk must not be allowed to go dry from this point until the end of the sample extraction.

11.4.3.2 CONDITIONING WITH WATER – Follow the MeOH rinse with two, 10 mL aliquots of reagent water, being careful to keep the water level above the disk surface. Turn off the vacuum.

11.4.4 DISK EXTRACTION – Add sample to the extraction funnel containing the conditioned disk and turn on the vacuum. Do not let the disk go dry before all sample has been extracted. Drain as much water from the sample container as possible. After all of the sample has passed, draw air through the disk by maintaining full vacuum (minus 10-15 in Hg) for 10 minutes. If the disk is dried for a period much longer than 10 minutes, there will be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release the vacuum.

11.4.5 DISK ELUTION – Detach the glassware base from the manifold without disassembling the funnel from the base. Dry the underside of the base. Insert collection tubes into the manifold to catch the extracts as they are eluted from the disk. The collection tube must fit around the drip tip of the base to ensure collection of all the eluent. Reattach the base to the manifold. Add 5 mL of ethyl acetate to the empty sample bottle and rinse the inside of the bottle. Transfer the ethyl acetate to the disk and, with vacuum, pull enough ethyl acetate into the disk to soak the sorbent, and

allow the solvent to soak the disk for about one minute. Pull the remaining solvent slowly through the disk into the collection tube. Repeat the rinse with 5 mL $MeCl_2$. Rinse the SPE funnel surface once with a 2-3 mL aliquot of 1:1 EtAc/$MeCl_2$. Repeat this last rinse of the SPE funnel. Detach glassware from manifold and remove collection tube from the manifold.

11.4.6 DRYING OF THE EXTRACT – Proceed with drying the extract, Section 11.3.5.

11.4.7 EXTRACT CONCENTRATION – Proceed with extract concentration, Section 11.3.6.

11.4.8 SAMPLE VOLUME OR WEIGHT DETERMINATION – Proceed with sample volume or weight determination, Section 11.3.7.

11.5 ANALYSIS OF SAMPLE EXTRACTS

11.5.1 Establish operating conditions as described in Section 10.2.2. Confirm that retention times, compound separation and resolution are similar to those summarized in Table 2 and Figure 1.

11.5.2 Establish a valid initial calibration following the procedures outlined in Section 10.2 or confirm that the calibration is still valid by running a CCC as described in Section 10.3. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2.

11.5.3 Analyze aliquots of field and QC samples at appropriate frequencies (Sect. 9) with the GC/MS system using the conditions used for the initial and continuing calibrations. At the conclusion of data acquisition, use the same software that was used in the calibration procedure to tentatively identify peaks in predetermined retention time windows of interest. Use the data system software to examine the ion abundances of components of the chromatogram.

11.5.4 COMPOUND IDENTIFICATION – Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base.

11.5.4.1 Establish an appropriate retention time window for each target analyte, internal standard and surrogate standard to identify them in QC and Field Samples chromatograms. Ideally, the retention time window should be based on measurements of actual

retention time variation for each compound in standard solutions collected on each GC/MS over the course of time. The suggested variation is plus or minus three times the standard deviation of the retention time for each compound for a series of injections. The injections from the initial calibration and from the Initial Demonstration of Capability may be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of an appropriate retention window size.

11.5.4.2 In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within an absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

11.5.5 EXCEEDING CALIBRATION RANGE – An analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, the extract may be diluted with ethyl acetate, with the appropriate amount of internal standard added to match the original level, and the diluted extract injected. Acceptable surrogate performance (Sect. 9.9) should be determined from the undiluted sample extract. Incorporate the dilution factor into final concentration calculations. The dilution will also affect analyte MRLs.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify method analytes present in the field and QC samples as described in Section 11.8.4. Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation.

12.1.1 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining plots of characteristic ions. When analytes coelute (i.e., only one GC peak is apparent), the

identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

12.1.2 In validating this method, concentrations were calculated by measuring the characteristic ions listed in Table 2. Other ions may be selected at the discretion of the analyst.

12.2 Calculate analyte and surrogate concentrations, using the multipoint calibration established in Section 10.2. Do not use daily continuing calibration check data to quantitate analytes in samples. Adjust the final analyte concentrations to reflect the actual sample volume or weight determined in Section 11.3.7 or 11.4.8. Field Sample extracts that require dilution should be treated as described in Section 11.5.5.

12.3 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND MDLs – Method performance data are summarized in Section 17. Method detection limits (MDLs) are presented in Table 3 and were calculated using the formula in Section 9.2.4. Single laboratory precision and accuracy data are presented for reagent water (Sect. 17, Tables 4A and 4B), chlorinated "finished" ground water (Sect. 17, Tables 5A and 5B), and chlorinated "finished" surface water (Sect. 17, Tables 6A and 6B).

13.2 POTENTIAL PROBLEM COMPOUNDS

13.2.1 MATRIX ENHANCED SENSITIVITY – Cyanazine, and to a lesser extent 2,4,6-trichlorophenol and prometon, tend to exhibit "matrix-induced chromatographic response enhancement."[7-11] Compounds that exhibit this phenomenon often give analytical results that exceed 100 % recovery in fortified extracts at low concentration and in continuing calibration checks. More frequent recalibration is required. It has been proposed that these compounds are susceptible to GC inlet absorption or thermal degradation so that analytes degrade more when injected in a "cleaner" matrix. The injection of a "dirty" sample extract coats surfaces with matrix components and "protects" the problem compounds from decomposition or adsorption. As a result, a relatively greater response is observed for analytes in sample extracts than in calibration solutions. This effect is minimized by using deactivated injection liners (Sect. 10.2.2.2). The analyst may also choose to condition the injection port after maintenance by injecting a few aliquots of a field sample extract prior to

establishing an initial calibration. Preparation of calibration standards in clean extracts is not allowed.

13.2.2 COMPOUND DEGRADATION – Method development work indicated that several of the target compounds were unstable when stored in water without preservation. There were various modes of loss. Hydrolysis of 1,2-diphenylhydrazine, terbufos, diazinon, disulfoton and cyanazine was accelerated at low and high pH. In addition, transition metal ions further catalyzed hydrolysis of terbufos, fonofos, and diazinon. Free chlorine and chloramines degraded 2,4-dichlorophenol, terbufos, fonofos, diazinon, and disulfoton. When water samples were not properly preserved, after three days, there was more than 80% loss of some targets, initially fortified at 5 ppb. Sample preservation conditions (Sect. 8) have been carefully chosen to minimize analyte degradation to acceptable levels during the 14 day sample holding time.

13.2.3 Inlet liners and/or capillary GC columns that develop active sites can cause a complete loss of prometon and excessive tailing of 2,4-dichlorophenol and 2,4,6-trichlorophenol peaks in the chromatogram.

13.3 ANALYTE STABILITY STUDIES

13.3.1 FIELD SAMPLES – Chlorinated surface water samples, fortified with method analytes at 5.0 ug/L, were preserved and stored as required in Section 8. The average of triplicate analyses, conducted on days 0, 3, 7, and 14, are presented in Section 17, Table 7. These data document the 14-day sample holding time. It is advisable to extract as soon as possible because some compounds exhibit significant losses by 7 days.

13.3.2 EXTRACTS – Extracts from the day 0 extract holding time study described above were stored below 0 °C and analyzed on days 0, 6, 13, 20, and 32. The data presented in Section 17, Table 8, document the 28-day extract holding time.

14. POLLUTION PREVENTION

14.1 This method utilizes solid phase extraction technology to remove the analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment involved with the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less Is Better: Laboratory Chemical Management for Waste Reduction" available form the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16 REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYL PHOSPHINE, (DECAFLUOROTRIPHENYL PHOSPHINE, DFTPP)

Mass (M/z)	Relative Abundance Criteria	Purpose of Checkpoint[1]
51	10-80% of the base peak	Low-mass sensitivity
68	<2% of Mass 69	Low-mass resolution
70	<2% of Mass 69	Low-mass resolution
127	10-80% of the base peak	Low- to mid-mass resolution
197	<2% of Mass 98	Mid-mass resolution
198	Base peak or >50% of Mass 442	Mid-mass resolution and sensitivity
199	5-9% of Mass 198	Mid-mass resolution and isotope ratio
275	10-60% of the base peak	Mid- to high-mass sensitivity
365	>1% of the base peak	Baseline threshold
441	Present and <Mass 443	High-mass resolution
442	Base peak or >50% of Mass 198	High-mass resolution and sensitivity
443	15-24% of Mass 442	High-mass resolution and isotope ratio

[1]All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization to fragmentation patterns.

TABLE 2. RETENTION TIMES (RTs), SUGGESTED QUANTITATION IONS (QIs), AND INTERNAL STANDARD REFERENCE

Peak #[a]	Analyte	Peak Label in Figure #1	RT[b] (min)	Quantitation Ion	IS# Ref.
1	Nitrobenzene	1	6.33	77	1
2	2,4-Dichlorophenol	2	7.70	162	1
3	2,4,6-Trichlorophenol	4	10.81	196	1
4	1,2-Diphenylhydrazine	6	15.08	182	2
5	Prometon	7	16.64	225	2
6	Terbufos	8	17.08	231	2
7	Fonofos	9	17.14	246	2
8	Diazinon	11	17.29	179	2
9	Disulfoton	12	17.51	88	2
10	Acetochlor	13	18.39	146	2
11	Cyanazine	14	19.73	225	2
12	Acenaphthene-d_{10} (IS#1)	5	12.88	164	-
13	Phenanthrene-d_{10} (IS#2)	10	17.20	188	-
14	Chrysene-d_{12} (IS#3)	16	24.98	240	-
15	1,3-Dimethyl-2-Nitrobenzene (SURR)	3	7.98	151	1
16	Triphenylphosphate (SURR)	15	24.29	326	3

a- Number refers to peak number in Figure 1.
b- Column: 30 m X 0.25 mm i.d. DB5-MS (J&W), 0.25 um film thickness.

TABLE 3. METHOD DETECTION LIMITS IN REAGENT WATER FOR SDVB DISK AND CARTRIDGE EXTRACTION PROCEDURES

Compound	Disk Extraction		Cartridge Extraction	
	Spiking Conc. (ug/L)	MDL[a] (ug/L)	Spiking Conc. (ug/L)	MDL[a] (ug/L)
Nitrobenzene	0.05	0.015	0.20	0.09
2,4-Dichlorophenol	0.05	0.012	0.20	0.04
2,4,6-Trichlorophenol	0.05	0.012	0.20	0.14
1,2-Diphenylhydrazine	0.20	0.028	0.20	0.10
Prometon	0.10	0.035	0.20	0.14
Terbufos	0.05	0.017	0.20	0.05
Fonofos	0.05	0.022	0.20	0.06
Diazinon	0.10	0.015	0.20	0.03
Disulfoton	0.10	0.024	0.20	0.05
Acetochlor	0.05	0.015	0.20	0.10
Cyanazine	0.05	0.025	0.20	0.09

[a]Method detection limits samples were extracted and analyzed over 3 days for 7 replicates following the procedure outlined in Section 9.2.4.

TABLE 4A. PRECISION, ACCURACY AND SENSITIVITY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN REAGENT WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration= 0.5 ug/L, n=7			Concentration = 20 ug/L, n=7	
	Mean % Recovery	%RSD[a]	S/N Ratio[b]	Mean % Recovery	%RSD[a]
Nitrobenzene	106	3.8	159	81.5	5.6
2,4-Dichlorophenol	114	1.9	71	97.6	4.3
2,4,6-Trichlorophenol	136	2.3	134	104	3.4
1,2-Diphenylhydrazine	121	2.5	25	103	3.7
Prometon	138	2.3	42	101	3.8
Terbufos	111	2.7	71	91.4	4.0
Fonofos	104	2.0	138	106	4.4
Diazinon	101	2.5	14	98.3	4.7
Disulfoton	105	2.4	103	95.0	4.8
Acetochlor	124	2.9	67	98.9	4.0
Cyanazine	153	2.5	10	104	4.6
1,3-Dimethyl-2-Nitrobenzene (SUR)[c]	86.2	2.6	NC	84.2	4.6
Triphenylphosphate (SUR)[c]	106	2.6	NC	105	5.2

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Signal-to-noise ratios were calculated for each peak by dividing the peak height for each compound by the peak-to-peak noise, which was determined for each component from the method blank over a period of time equal to the full peak width in the target analyte's retention time window.
[c]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 4B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN REAGENT WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n=7		Concentration = 20 ug/L, n=7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	86.3	6.9	73.4	3.7
2,4-Dichlorophenol	104	7.3	83.8	3.3
2,4,6-Trichlorophenol	129	3.8	92.9	3.0
1,2-Diphenylhydrazine	96.9	16	123	3.2
Prometon	148	3.2	100	0.8
Terbufos	115	2.8	85.0	1.8
Fonofos	101	3.8	90.1	1.9
Diazinon	104	3.4	91.1	1.4
Disulfoton	106	3.0	85.5	1.7
Acetochlor	125	3.0	91.3	1.2
Cyanazine	147	5.6	101	1.2
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	81.7	4.1	80.2	3.0
Triphenylphosphate (SUR)[b]	107	7.5	108	3.0

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 5A. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, AND 20 UG/L IN GROUND WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	110	4.7	83.5	5.3
2,4-Dichlorophenol	113	3.0	96.5	5.0
2,4,6-Trichlorophenol	148	2.0	103	4.1
1,2-Diphenylhydrazine	134	3.8	102	4.0
Prometon	152	2.4	101	3.8
Terbufos	119	1.6	93.4	3.5
Fonofos	105	2.4	105	3.7
Diazinon	110	1.5	97.6	3.7
Disulfoton	113	2.5	95.1	4.2
Acetochlor	130	2.1	98.3	3.7
Cyanazine	163	2.3	104	3.8
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	86.3	4.8	83.8	5.5
Triphenylphosphate (SUR)[b]	105	2.9	106	4.0

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 5B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, 5.0 AND 20 UG/L IN GROUND WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 5.0 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	114	5.4	87.7	3.9	84.1	1.9
2,4-Dichlorophenol	110	3.4	89.8	3.9	89.1	2.2
2,4,6-Trichlorophenol	136	4.1	102	3.8	98.3	1.4
1,2-Diphenylhydrazine	122	4.3	84.8	3.2	90.4	3.7
Prometon	144	3.3	103	2.9	100	2.3
Terbufos	120	3.0	87.1	4.2	84.9	2.4
Fonofos	107	3.0	87.9	3.5	90.8	2.4
Diazinon	105	2.4	88.7	3.1	91.6	2.3
Disulfoton	108	3.8	93.3	3.7	88.6	3.1
Acetochlor	129	4.5	103	3.7	94.2	1.1
Cyanazine	160	3.1	106	3.6	98.6	3.6
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	86.7	6.3	86.3	3.3	84.1	4.6
Triphenylphosphate (SUR)[b]	119	4.3	123	3.4	111	14

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 6A. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, 5.0 AND 20 UG/L IN SURFACE WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 5.0 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	105	3.8	72.4	4.4	76.7	4.1
2,4-Dichlorophenol	111	4.0	81.2	4.0	89.9	4.8
2,4,6-Trichlorophenol	143	2.8	90.8	4.2	92.4	4.0
1,2-Diphenylhydrazine	118	2.4	105	4.5	88.3	3.9
Prometon	136	3.6	102	3.1	89.5	3.9
Terbufos	109	3.0	85.7	4.6	80.2	3.4
Fonofos	98.9	3.8	83.0	4.2	89.1	4.0
Diazinon	102	3.6	83.7	3.5	86.1	4.1
Disulfoton	105	3.4	81.2	4.2	86.6	3.7
Acetochlor	124	3.4	89.2	3.6	89.4	3.4
Cyanazine	151	3.2	105	3.9	93.6	3.9
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	82.9	4.5	77.4	4.4	83.9	3.9
Triphenylphosphate (SUR)[b]	101	4.5	98.2	3.6	98.8	4.3

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 6B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN SURFACE WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	91.7	6.6	84.9	2.9
2,4-Dichlorophenol	113	4.9	89.7	2.2
2,4,6-Trichlorophenol	135	4.0	97.2	1.8
1,2-Diphenylhydrazine	107	9.9	93.7	2.3
Prometon	158	3.3	101	1.3
Terbufos	128	3.0	89.0	2.1
Fonofos	109	2.3	91.6	1.6
Diazinon	112	3.7	92.8	1.5
Disulfoton	109	3.0	90.0	1.6
Acetochlor	132	2.8	94.6	1.4
Cyanazine	158	1.7	99.9	1.0
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	83.0	5.3	85.1	3.8
Triphenylphosphate (SUR)[b]	104	1.9	99.1	1.60

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 7. SAMPLE HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 5 UG/L, AND PRESERVED ACCORDING TO SECTION 8.

Compound	Day 0 % Rec.	Day 3 % Rec.	Day 7 % Rec.	Day 14 % Rec.
Nitrobenzene	83.5	82.4	76.7	88.1
2,4-Dichlorophenol	97.2	92.6	86.9	98.1
2,4,6-Trichlorophenol	110	102	97.3	108
1,2-Diphenylhydrazine	98.8	93.9	87.5	97.2
Prometon	104	97.7	93.1	102
Terbufos	96.7	79.3	69.1	65.4
Fonofos	94.1	88.0	84.8	93.1
Diazinon	93.0	86.7	83.4	91.3
Disulfoton	90.8	84.7	81.5	89.1
Acetochlor	100	93.3	90.5	97.6
Cyanazine	126	119	112	125

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery]*100) for replicate analyses were all less than 13.8 %.

TABLE 8. EXTRACT HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 5 UG/L, AND PRESERVED ACCORDING TO SECTION 8.

Compound	Day 0 % Rec.	Day 6 % Rec.	Day 13 % Rec.	Day 20 % Rec.	Day 32 % Rec.
Nitrobenzene	83.5	84.7	85.2	85.5	80.5
2,4-Dichlorophenol	97.2	97.5	96.2	96.7	95.9
2,4,6-Trichlorophenol	110	110	108	107	112
1,2-Diphenylhydrazine	98.8	97.9	97.9	97.0	98.3
Prometon	104	104	103	103	101
Terbufos	96.7	97.5	97.9	98.6	89.5
Fonofos	94.1	95.0	94.3	95.3	97.3
Diazinon	93.0	93.0	93.6	94.3	93.0
Disulfoton	90.8	91.9	92.9	94.6	93.5
Acetochlor	100	100	99.8	99.8	98.7
Cyanazine	126	125	124	122	123
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	79.3	78.5	78.7	78.5	77.1
Triphenylphosphate (SUR)[b]	97.9	96.6	96.7	97.8	93.4

[a]Extracts were stored at less than 0 °C and reinjected periodically. Each table value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery]*100) for replicate analyses were all less than 5.7 %.

TABLE 9. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.1	Initial Demonstration of Low Method Background	Analyze LRB prior to any other IDC steps	Demonstrate that all target analytes are below $^1/_3$ the reporting limit or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantification of method analytes.
Section 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at midrange concentration.	%RSD must be ≤20%
Section 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery for replicates used in IDP	Mean recovery 70-130% of true value.
Section 9.2.4	Method Detection Limit (MDL) Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the MDL. Analyze the replicates through all steps of the analysis. Calculate the MDL using the equation in Section 9.2.4.	Note: Data from MDL replicates are not required to meet method precision and accuracy criteria. If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

TABLE 10. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2.1	MS Tune Check	Analyze DFTPP to verify MS tune each time the instrument is calibrated.	Criteria are given in Table 1.
Section 10.2	Initial Calibration	Use internal standard calibration technique to generate an average RRF or first or second order calibration curve. Use at least 5 standard concentrations.	When each calibration standard is calculated as an unknown using the calibration curve, the result must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value.
Section 9.4	Laboratory Reagent Blank (LRB)	Daily, or with each extraction batch of up to 20 samples, whichever is more frequent.	Demonstrate that all target analytes are below $^1/_3$ the method reporting limit or lowest CAL standard, and that possible interferences do not prevent quantification of method analytes. If targets exceed $^1/_3$ the MRL, results for all subject analytes in extraction batch are invalid.
Section 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a calibration standard at the beginning of each analysis batch prior to analyzing samples, after every 10 samples, and after the last sample. Low CCC - near MRL Mid CCC - near midpoint in initial calibration curve High CCC - near highest calibration standard	1) The result for each analyte must be 70-130% of the true value for all but the lowest standard. The lowest standard must be 50-150% of the true value. 2) The peak area of internal standards must be 50-150% of the average peak area calculated during the initial calibration. Results for analytes that do not meet IS criteria or are not bracketed by acceptable CCCs are invalid.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.6	Laboratory Fortified Blank (LFB)	One LFB is required daily or for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium, and high amounts.	Results of LFB analyses at medium and high fortification must be 70-130% of the true value for each analyte and surrogate. LFB Results of the low level LFB must be 50-160% of the true value.
Section 9.8	Internal Standard	Acenaphthene-d_{10} (IS#1) phenanthrene-d_{10} (IS#2), and chrysene-d_{12} (IS#3), are added to all standards and sample extracts.	Peak area counts for all ISs in LFBs, LRBs, and sample extracts must be within 50-150% of the average peak area calculated during the initial calibration. If ISs do not meet criteria, corresponding target results are invalid.
Section 9.9	Surrogate Standards	Surrogate standards, 1,3-dimethyl-2-nitrobenzene and triphenylphosphate, are added to all calibration standards and samples, including QC samples. If nitrobenzene is not to be included on the target list, then the surrogate 1,3-dimethyl-2-nitrobenzene is not required.	Surrogate recovery must be 70-130% of the true value. If surrogate fails this criterion, report all results for sample as suspect/surrogate recovery.
Section 9.10	Laboratory Fortified Sample Matrix (LFM) and Laboratory Fortified Matrix Duplicate (LFMD)	Analyze one LFM per analysis batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration. LFMD should be used in place of Field Duplicate if frequency of detects for targets is low.	Recoveries at mid and high levels not within 70-130% or low-level recoveries not within 50-150% of the fortified amount may indicate a matrix effect. Target analyte RPDs for LFMD should be ±30% at mid and high levels of fortification and ±50% near MRL.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.11	Field Duplicates (FD)	Extract and analyze at least one FD with each extraction batch (20 samples or less). A LFMD may be substituted for a FD when the frequency of detects for target analytes is low.	Target analyte RPDs for FD should be ±30% at mid and high levels of fortification and ±50% near MRL.
Section 9.12	Quality Control Sample (QCS)	Analyzed QCS quarterly.	Results must be 70-130% of the expected value.
Section 8.4	Sample Holding Time	14 days with appropriate preservation and storage	Sample results are valid only if samples are extracted within sample hold time.
Section 8.4	Extract Holding Time	28 days with appropriate storage	Sample results are valid only if extracts are analyzed within extract hold time.

FIGURE 1. EXAMPLE CHROMATOGRAM FOR METHOD 526. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 2.

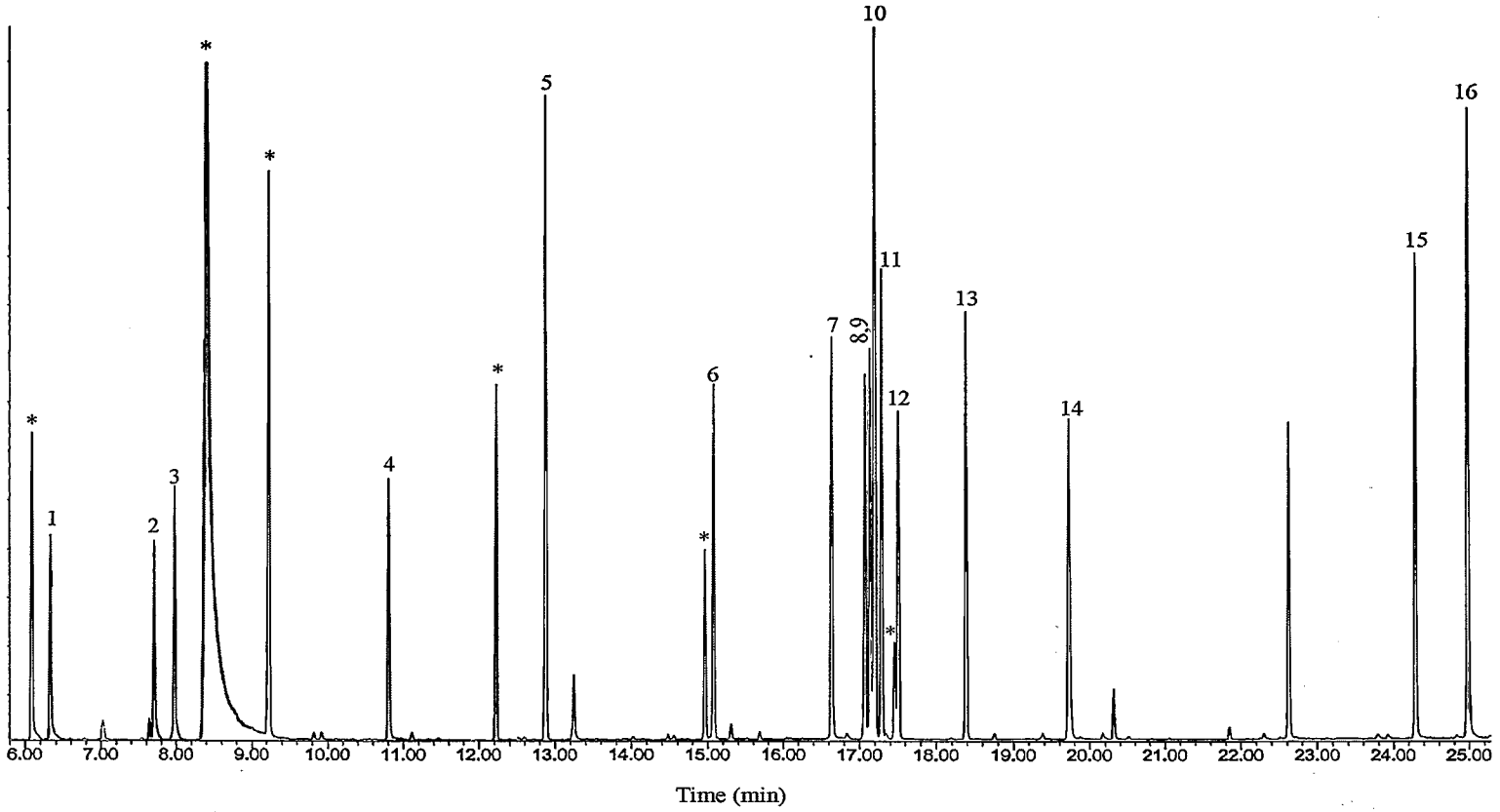

Figure 1: Total ion chromatogram of surface water sample extract with target compounds, internal standards, and surrogate standards fortified at 5 ppm level in extract. Peaks with asterisk (*) are interference associated with the use of diazolidinyl urea.

METHOD 528 DETERMINATION OF PHENOLS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

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METHOD 528

DETERMINATION OF PHENOLS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

1. SCOPE AND APPLICATION

1.1 This method provides procedures for the determination of phenols in finished drinking water. The method may be applicable to untreated source waters and other types of water samples, but it has not been evaluated for these uses. The method is applicable to a variety of phenols that are efficiently partitioned from the water sample onto a modified polystyrene divinylbenzene solid phase sorbent, and sufficiently volatile and thermally stable for gas chromatography. The method includes the following compounds:

ANALYTE	CAS NUMBER
phenol	108-95-2
2-chlorophenol	95-57-8
2-methylphenol (o-cresol)	95-48-7
2-nitrophenol	88-75-5
2,4-dimethylphenol	105-67-9
2,4-dichlorophenol	120-83-2
4-chloro-3-methylphenol	59-50-7
2,4,6-trichlorophenol	88-06-2
2,4-dinitrophenol	51-28-5
4-nitrophenol	93951-79-2
2-methyl-4,6-dinitrophenol	534-52-1
pentachlorophenol	87-86-5

1.2 Method detection limit (MDL) is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero (1). The MDL is compound dependent and is particularly dependent on extraction efficiency, sample matrix, and instrument performance. MDLs for method analytes range from 0.02-0.58 µg/L, and are listed in Table 1. The concentration calibration range demonstrated by this method is 0.1 µg/L to 15 µg/L for most analytes, and approximately 1.0 µg/L to 15 µg/L for 2,4-dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, and pentachlorophenol.

1.3 This method should be performed only by analysts with experience in solid phase extractions and GC/MS analyses.

2. SUMMARY OF METHOD

Analytes and surrogates are extracted by passing a 1 L water sample through a solid phase extraction (SPE) cartridge containing 0.5 g of a modified polystyrene divinyl benzene copolymer. The organic compounds are eluted from the solid phase with a small quantity of methylene chloride. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high resolution fused silica capillary column of a GC/MS system. Compounds eluting from the GC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a data base. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantitation ion(s) produced by that compound to the MS response of the quantitation ion(s) produced by a compound that is used as an internal standard. Surrogate analytes, whose concentrations are known in every sample, are measured with the same internal standard calibration procedure.

3. DEFINITIONS

3.1 ANALYSIS BATCH -- A set of samples analyzed on the same instrument during a 24 hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples

3.2 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 CONTINUING CALIBRATION CHECK (CCC) -- A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.4 EXTRACTION BATCH -- A set of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices and solvents, surrogate solution, and fortifying solutions. Required QC samples for each extraction batch include: Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Matrix, and either a Field Duplicate or Laboratory Fortified Matrix Duplicate.

3.5 FIELD DUPLICATES (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.6 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.7 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, including the use of sample preservatives, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMD) -- A second aliquot of the Field Sample, or duplicate Field Sample, that is used to prepare the LFM. The LFMD is fortified, extracted and analyzed identically to the LFM. The LFMD is used instead of the Laboratory Duplicate to assess method precision when the occurrence of target analytes are low.

3.10 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates, and sample preservatives that are used with other samples. The LRB is used to determine if

method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.11 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.12 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination (Section 9.2.4), and accurate quantitation is not expected at this level. [1]

3.13 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte, and can only be used if acceptable quality control criteria for the analyte at this concentration are met.

3.14 PEAK TAILING FACTOR (PTF) -- A calculated value that indicates the amount of peak tailing exhibited by a chromatographic peak. The value is calculated by dividing the peak width of the back half of the peak (at 10% peak height), by the peak width of the front half of the peak (at 10% peak height). The calculation is demonstrated in Figure 4.

3.15 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.16 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.17 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.18 SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in a known amount before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

4. INTERFERENCES

4.1 During analysis, major contaminant sources are reagents and SPE devices. Analyses of laboratory reagent blanks provide information about the presence of contaminants. Solid phase extraction devices described in this method have two potential sources of contamination, both the solid phase sorbent and the polypropylene cartridge that it is packed in. Brands and manufacturers lot numbers of these devices should be monitored and tracked to ensure that contamination will not preclude analyte identification and quantitation.

4.2 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes and splitless injection port liners must be cleaned carefully or replaced as needed. After analysis of a sample containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample.

4.3 Silicone compounds may be leached from autosampler vial septa by methylene chloride. This contamination of the extract will be enhanced if particles of the septa are introduced into standards and sample extracts by the needle used for injection. These silicone compounds should, in most cases, have no effect on the analysis. However, the analyst should be aware of this potential problem.

4.4 Airborne phenol may be a source of phenol contamination in samples and sample extracts. Samples should not be stored or extracted in areas where phenol is used for other laboratory operations.

4.5 2,3,4,5-Tetrachlorophenol is used as one of the internal standards for the quantitation of reactive and thermally labile phenols. Tetrachlorophenol isomers may be present at low levels (less than 4% total tetrachlorophenol) in pentachlorophenol used as a pesticide and wood preservative. However, occurrence of pentachlorophenol in U.S. drinking waters is rare, and measured concentrations are typically 1 µg/L or less. If a matrix interference with the internal standard is suspected, an alternate internal standard may be selected.

5. SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Each laboratory should maintain a file of applicable MSDSs. Additional references to laboratory safety are cited (2-4).

5.2 Some method analytes and solvents, including 2,4,6-trichlorophenol, pentachlorophenol, and methylene chloride have been classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.

6. <u>EQUIPMENT AND SUPPLIES</u> (All specifications are suggested. References to specific brands or catalog numbers are included for illustration only.)

6.1 GLASSWARE -- All glassware must be meticulously cleaned. This may be accomplished by washing with detergent and water, rinsing with water, distilled water, or solvents, air-drying, and heating (where appropriate) in a muffle furnace. Volumetric glassware should never be heated to the temperatures obtained in a muffle furnace.

6.2 SAMPLE CONTAINERS -- 1 L or 1 qt amber glass bottles fitted with polytetrafluoroethylene (PTFE) lined polypropylene screw caps. Amber bottles are highly recommended since some of the method analytes are sensitive to light and may degrade upon exposure. Clear glass bottles may be used if they are wrapped in foil, or samples are stored in boxes that prevent exposure to light. Although specific contamination problems from bottle caps were not observed during method development, phenolic resin bottle caps should be avoided.

6.3 VOLUMETRIC FLASKS -- various sizes.

6.4 LABORATORY OR ASPIRATOR VACUUM SYSTEM -- Sufficient capacity to maintain a vacuum of approximately 25 cm (10 in.) of mercury.

6.5 MICRO SYRINGES -- various sizes.

6.6 VIALS -- Various sizes of amber vials with PTFE lined screw caps for storing standard solutions and extracts.

6.7 DRYING COLUMN -- The drying tube should contain about 5 to 7 grams of anhydrous sodium sulfate to remove residual water from the extract. Any small tube may be used, such as a syringe barrel, a glass dropper, etc. as long as no particulate sodium sulfate passes through the column into the extract.

6.8 ANALYTICAL BALANCE -- Capable of weighing 0.0001 g accurately.

6.9 FUSED SILICA CAPILLARY GAS CHROMATOGRAPHY COLUMN -- Any capillary column that provides adequate resolution, capacity, accuracy, and precision can be used. Medium polarity, low bleed columns are recommended for use with this method to provide adequate chromatography and minimize column bleed.

Deactivated injection port liners are highly recommended. During the course of the development of this method, two columns were used. Although these are both polyphenylmethylsilicone columns, the exact phase is slightly different. Information on the exact composition of each phase is available from the manufacturers. Most of the work was performed with column 1. Any column which provides analyte separations equivalent to or better than these columns may be used. Example chromatograms are shown in Figs 1-3. Retention times are presented in Table 2.

6.9.1. Column 1- 30 m × 0.25 mm id fused silica capillary column coated with a 0.25 µm bonded film of polyphenylmethylsilicone, (J&W DB-5ms).

6.9.2 Column 2- 30 m × 0.25 mm id fused silica capillary column coated with a 0.25 µm bonded film of polyphenylmethylsilicone, (SGE BPX5).

6.10 GAS CHROMATOGRAPH/MASS SPECTROMETER/DATA SYSTEM (GC/MS/DS)--

6.10.1 The GC must be capable of temperature programming and should be equipped for split/splitless injection. The injection system must not allow the analytes to contact hot stainless steel or other metal surfaces that promote decomposition. Other injection techniques such as temperature programmed injections, cold on-column injections and large volume injections may be used if QC criteria in Section 9 and 10 are met. If an alternate injection technique is performed, the analyst will need to select an instrument configuration which has been specifically designed for that application. Performance data in Section 17 include data obtained both by hot, splitless injection and temperature programmed splitless injection.

6.10.2 The GC/MS interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, for example the open split interface, are acceptable if the system has adequate sensitivity.

6.10.3 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV to produce positive ions. The spectrometer must be capable of scanning at a minimum from 45 to 450 amu with a complete scan cycle time (including scan overhead) of 1.0 sec or less. (Scan cycle time = total MS data acquisition time in sec divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 3 when an injection of approximately 5 ng of DFTPP is introduced into the GC. A single spectrum at the apex of the chromatographic peak, or an average of the three spectra at the apex of the peak, or an average spectrum across the entire GC peak may be used to

evaluate the performance of the system. Background subtraction is permitted. The scan time must be set so that all analytes have a minimum of 5 scans across the chromatographic peak. Seven to ten scans across chromatographic peaks are recommended.

6.10.4 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored GC/MS data by recognizing a GC peak within any given retention time window. The software must also allow integration of the ion abundance of any specific ion between specified time or scan number limits, calculation of response factors as defined in Sect. 10.2.5 or construction of a linear regression calibration curve, and calculation of analyte concentrations.

6.11 VACUUM MANIFOLD -- A vacuum manifold (Supelco # 57030 and #57275) is required for processing samples through the extraction/elution procedure. An automatic or robotic sample preparation system designed for use with solid phase extraction cartridges may be utilized in this method if all quality control requirements discussed in Sect. 9 are met. Automated systems may use either vacuum or positive pressure to process samples and solvents through the cartridge. All extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system.

7. REAGENTS AND STANDARDS

7.1 HELIUM -- carrier gas, purity as recommended by the GC/MS manufacturer.

7.2 SOLID PHASE EXTRACTION CARTRIDGES -- Varian Bond Elut PPL or equivalent. Cartridges are inert non-leaching plastic, for example polypropylene, or glass, and must not contain plasticizers that leach into the methylene chloride eluant and prevent the identification and quantitation of method analytes. The polypropylene cartridges (6 mL volume) are packed with 0.5 g highly cross-linked, and chemically modified styrene divinyl benzene copolymer. The packing must have a narrow size distribution and must not leach interfering organic compounds into the eluting solvent.

7.3 SOLVENTS --

7.3.1 Methylene chloride, acetone, and methanol. High purity pesticide quality or equivalent.

7.3.2 Reagent water. Water in which an interference is not observed at >1/3 the MRL of any of the compounds of interest. Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon or by using a water purification system. Store in clean, narrow-mouth bottles with PTFE lined septa and screw caps.

7.4 HYDROCHLORIC ACID -- 6 N and 0.05 N.

7.5 SODIUM SULFATE, ANHYDROUS -- (Soxhlet extracted with methylene chloride for a minimum of 4 h or heated to 400°C for 2 h in a muffle furnace.)

7.6 STOCK STANDARD SOLUTIONS -- Individual solutions of surrogates, internal standards, and analytes, or mixtures of analytes, may be purchased from commercial suppliers or prepared from pure materials. To prepare stocks from neat materials, add 10 mg (weighed on an analytical balance to within 0.1 mg) of the pure material to 1.9 mL of methanol, methylene chloride, or acetone in a 2 mL volumetric flask, dilute to the mark, and transfer the solution to an amber glass vial. The solvent to be used is dependent upon the final use of the standard. In general, calibration standards and internal standards are prepared in methylene chloride, sample fortification solutions are prepared in methanol or acetone. Follow any specific instructions for each standard or standard mixture. If compound purity is confirmed by the supplier at >96%, the weighed amount can be used without correction to calculate the concentration of the solution (5 µg/µL). Store the amber vials at 0°C or less.

7.7 PRIMARY DILUTION STANDARD SOLUTION -- The stock standard solutions are used to prepare a primary dilution standard solution that contains multiple method analytes in methylene chloride. Aliquots of each of the stock standard solutions are combined to produce the primary dilution in which the concentration of the analytes is at least equal to the concentration of the most concentrated calibration solution, that is, 15 ng/µL. Store the primary dilution standard solution in an amber vial at 0°C or less, and check regularly for signs of degradation or evaporation, especially just before preparing calibration solutions. Mixtures of method analytes to be used as primary dilution standards may also be purchased from commercial suppliers.

7.8 CALIBRATION SOLUTIONS (CAL1 through CAL7) -- Prepare a series of seven calibration solutions in methylene chloride which contain analytes of interest at suggested concentrations of 15,10, 5, 2, 1, 0.5, and 0.1 ng/µL, with a constant concentration of each internal standard in each CAL solution (2-5 ng/µL is recommended). Surrogate analytes are also added to each CAL solution, and may be added at a constant concentration or varied concentrations (similar to those for method analytes), at the discretion of the analyst. CAL1 through CAL7 are prepared

by combining appropriate aliquots of a primary dilution standard solution (Sect. 7.7) and the fortification solution of internal standards and surrogates (Sect. 7.10). All calibration solutions should contain at least 80% methylene chloride to avoid gas chromatographic problems due to mixed solvents. Store these solutions in amber vials at 0°C or less. Check these solutions regularly for signs of evaporation and/or degradation.

NOTE: Because the MS sensitivity to analytes 9-12 (Table 2) is significantly less than compounds 1-8, it may be more convenient to prepare calibration solutions in which the concentrations of analytes 9-12 (Table 2) are higher than the concentrations of analytes 1-8. Use of this option is at the discretion of the analyst. Calibration requirements are specified in Sect. 10.

7.9 INTERNAL STANDARD SOLUTION(S) -- This method uses two internal standards: 1,2-dimethyl-3-nitrobenzene (IS#1) and 2,3,4,5-tetrachlorophenol (IS#2). The first internal standard, 1,2-dimethyl-3-nitrobenzene is used to monitor instrument sensitivity and is used to quantify analytes 1-8 in Table 2. The second internal standard, 2,3,4,5-tetrachlorophenol is used to quantify analytes 9-12 (Table 2). IS#2 was selected for its chemical similarity to these compounds which are susceptible to adsorption and/or thermal decomposition in the GC inlet. A full explanation of the use of 2,3,4,5-tetrachlorophenol to quantify these compounds is given in Section 13. If cold, on-column or temperature programmed injection techniques are used, acceptable performance may be obtained using only one internal standard (IS#1).

7.9.1 1,2-Dimethyl-3-nitrobenzene (Aldrich) -- 100 µg/mL in methylene chloride. Use 25 µL of this solution per 1 mL of sample extract for a final concentration of 2.5 µg/mL.

7.9.2 2,3,4,5-Tetrachlorophenol (Chem Service Inc.) -- 200 µg/mL in methylene chloride. Use 25 µL of this solution per 1 mL of sample extract for a final concentration of 5 µg/mL.

7.9.3 The internal standard solutions listed above can be made individually or together in one solution.

7.10 SAMPLE FORTIFICATION SOLUTIONS --

7.10.1 Surrogate fortification solutions --

7.10.1.1. 2-Chlorophenol-3,4,5,6-d_4 (Chem Service Inc.) -- 100 µg/mL in methanol. Use 20 µL of this solution per 1 L of water sample for a final concentration of 2 µg/L.

7.10.1.2 2,4-Dimethylphenol-3,5,6-d_3 (CDN Isotopes) -- 100 µg/mL in acetone. Use 20 µL of this solution per 1 L of water sample for a final concentration of 2 µg/L.

7.10.1.3 2,4,6-Tribromophenol -- 200 µg/mL in methanol. Use 25 µL of this solution per 1 L water sample for a final concentration of 5 µg/L.

7.10.2 Analyte fortification solution(s). This solution contains all method analytes of interest in methanol. These solutions are used to fortify LFBs and LFMs with method analytes. It is recommended that more than one concentration of this solution be prepared. During the method development, two solutions were used. One containing 100 µg/mL of each analyte, was used for higher concentration fortifications, and the other containing10 µg/mL of each analyte in methanol was used for lower level fortifications.

NOTE: Because the MS sensitivity to analytes 9-12 (Table 2) is significantly less than analytes 1-8, it may be more convenient to prepare analyte fortification solutions in which the concentrations of analytes 9-12 are higher than the concentrations of analytes 1-8. Use of this option is at the discretion of the analyst.

7.11 GC/MS TUNE CHECK SOLUTION -- Decafluorotriphenylphosphine (DFTPP), 5 µg/mL in methylene chloride. Store this solution in an amber vial at 0°C or less.

7.12 SODIUM SULFITE, ANHYDROUS -- Reducing agent used to reduce residual chlorine at the time of sample collection.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION -- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 2 min). Adjust the flow to about 500 mL/min and collect samples from the flowing stream. The sample should nearly fill the 1 L or 1 qt bottle, but does not need to be headspace free. Keep samples sealed from collection time until analysis. When sampling from an open body of water, fill the sample container with water from a representative area. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2 SAMPLE DECHLORINATION AND PRESERVATION -- All samples must be dechlorinated and acidified at the time of collection. Residual chlorine is reduced

by addition of 40-50 mg of sodium sulfite. It may be added as a solid to the sample bottles before the bottles are transported to the field. It is **very important** that the sample be dechlorinated prior to acidification. Wait until sodium sulfite is dissolved before acidification. Adding sodium sulfite and HCl (together) to the sample bottles prior to shipping bottles to the sampling site is not permitted. After dechlorination, samples are acidified to less than pH 2 with 6 N hydrochloric acid. The acid serves as a chemical and biological preservative. This pH is the same that is used in the sample extraction, and is required to support the recovery of several method analytes.

8.3 SAMPLE TRANSPORT AND STORAGE -- All samples should be iced during shipment and must not exceed 10° C during the first 48 hours. Samples should be confirmed to be at or below 10° C when they are received at the laboratory. Samples stored in the lab must be held at or below 6° C until extraction, but should not be frozen.

8.4 HOLDING TIME -- Results of holding time studies of all method analytes showed that all compounds are stable for 14 days in water samples when the samples are dechlorinated, preserved, and stored as described in Sect. 8.2 and 8.3. Therefore, samples must be extracted within 14 days of collection. Sample extracts may be stored at 0°C or less for up to 30 days after sample extraction. Data from holding time studies are shown in Tables 7 and 8.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include: the initial demonstration of laboratory capability (summarized in Table 9) followed by regular analyses of continuing calibration checks, laboratory performance check standards, laboratory reagent blanks, laboratory fortified blanks, and laboratory fortified matrix samples. An MDL must be determined for each analyte of interest. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs. The laboratory must maintain records to document the quality of the data generated. A complete summary of QC requirements is summarized in Table 10.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) -- Requirements for the initial demonstration of laboratory capability are described in the following sections and summarized in Table 9.

9.2.1 INITIAL DEMONSTRATION OF LOW CARTRIDGE EXTRACTION BACKGROUND AND SYSTEM BACKGROUND -- Before any samples are analyzed, or any time a new supply of solid phase extraction cartridges is received from a supplier, it must be demonstrated that a laboratory

reagent blank (LRB) is reasonably free of any contamination that would prevent the determination of any analyte of concern.

9.2.1.1 A source of potential contamination is the solid phase extraction cartridge which may contain phthalate esters, silicon compounds, and other contaminants that could interfere with the determination of method analytes. Although extraction cartridges are generally made of inert materials, they may still contain extractable organic material. If the background contamination is sufficient to prevent accurate and precise measurements, the condition must be corrected before proceeding with the initial demonstration.

9.2.1.2 Other sources of background contamination are solvents, reagents, and glassware. Background contamination must be reduced to an acceptable level before proceeding with the next section. Background from method analytes and interferences should be ≤ 1/3 the MRL.

9.2.2 INITIAL DEMONSTRATION OF PRECISION (IDP) -- Prepare 4-7 replicate LFBs fortified at 5-10 µg/L, or other mid-range concentration. Sample preservatives described in Sect. 8.2 must be added to these samples. Extract and analyze these replicates according to the procedure described in Section 11. The relative standard deviation (RSD) of the results of the replicate analyses must be less than or equal to 20% for all method analytes with the exception of phenol. The RSD for replicate analyses for phenol must be less than or equal to 30%.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY (IDA) -- Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within 70-130% of the true value, except for phenol. Phenol will typically be recovered less effectively than other method analytes. Because of its higher water solubility some breakthrough from the extraction cartridge does occur. The recovery limits for phenol are 50-150%.

9.2.4 MDL DETERMINATION -- Replicate analyses for this procedure should be done over at least 3 days (both the sample extraction and the GC analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs at a concentration estimated to be near the MDL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. Concentrations shown in the example data in Table 1 may be used as a guide, however the appropriate concentration will be dependent upon the

injection technique and the sensitivity of the GC/MS system used. Sample preservatives described in Sect. 8.2 must be added to these samples. Analyze the seven replicates through all steps of Section 11. Calculate the MDL using the following equation:

$$MDL = St_{(n-1,\ 1-\ alpha\ =\ 0.99)}$$

where:

$t_{(n-1,1-alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations.

9.2.5 The analyst is permitted to modify GC columns, GC conditions, extract evaporation techniques, internal standards or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.2.1 through 9.2.4.

9.3 MINIMUM REPORTING LEVEL (MRL) -- The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should be established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal-to-noise ratio of five. **Although the lowest calibration standard for an analyte may be below the MRL, the MRL for an analyte must never be established at a concentration lower than the lowest calibration standard for that analyte.**

9.4 LABORATORY REAGENT BLANKS (LRB) -- With each extraction batch, analyze a laboratory reagent blank to determine the background system contamination. If, within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analyses should be $\leq$ 1/3 the MRL. Any time a new batch of SPE cartridges is received, or new supplies of other reagents are used, repeat the demonstration of low background described in Sect. 9.2.1.

9.5 CONTINUING CALIBRATION CHECK (CCC) -- This calibration check is required at the beginning of each day that samples are analyzed, after every ten field

samples, and at the end of any group of sample analyses. See Sect.10.3 for concentration requirements and acceptance criteria.

9.6 MS TUNE CHECK -- This performance check consists of verifying the MS tune using the mass spectrum of DFTPP. A complete description of the check is in Sect. 10.2.1. This check must be performed each time a major change is made to the mass spectrometer, and each time analyte calibration is performed (i.e. average RFs are calculated, or first or second order calibration curves are developed).

9.7 PEAK TAILING FACTOR (PTF) -- This check consists of calculating the PTF as described in Sect. 10.2.3.1. and in Figure 4. This check must be performed once every 24 hr of instrument operation.

9.8 LABORATORY FORTIFIED BLANK (LFB) -- With each extraction batch, extract and analyze an LFB containing each analyte of concern. If more than 20 field samples are included in a batch, analyze a LFB for every 20 samples. The fortified concentration of the LFB should be rotated between low, medium, and high concentrations from day to day. The low concentration LFB must be as near as practical to the MRL. Results of LFB analyses corresponding to the lowest CAL point for an analyte must be 50-150% of the true value for all analytes. Results of LFB analysis from medium and high level concentrations must be 70-130% of the true value for all analytes except phenol. The acceptance limit for phenol is 50-150% of the true value.

9.9 INTERNAL STANDARD (IS) --The analyst must monitor the peak area of the 1,2-dimethyl-3-nitrobenzene (IS#1) in all injections during each analysis day. The IS#1 response (peak area) in any chromatographic run should not deviate from the response in the most recent CCC by more than 30%, and must not deviate by more than 50% from the area measured during initial analyte calibration. If the IS#1 area in a chromatographic run does not meet these criteria inject a second aliquot of that extract.

NOTE: The peak area of 2,3,4,5-tetrachlorophenol may not be consistent. It may vary depending upon the composition of the extract or standard being analyzed. See Section 13.2.1 for a detailed explanation.

9.9.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.9.2 If a deviation of greater than 30% is obtained for the reinjected extract, when compared to the most recent CCC, the analyst should check the calibration by reanalyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3.3, recalibration is

in order per Section 10. If the calibration standard is acceptable, extraction of the sample may need to be repeated provided the sample is still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.10 SURROGATE RECOVERY -- The surrogate standards are fortified into all calibration standards, samples, LFBs, LFMs, FDs, FRBs and LRBs. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.10.1 Surrogate recovery criteria are 70-130% of the fortified amount for 2-chlorophenol-3,4,5,6-d_4 and 2,4-dimethylphenol-3,5,6-d_3. The criteria for 2,4,6-tribromophenol is 60-130% of the fortified amount. When surrogate recovery from a sample, blank, or CCC does not meet these criteria, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. Correct any problems that are identified. If these steps do not reveal the cause of the problem, reanalyze the extract.

9.10.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.10.3 If the extract reanalysis fails the recovery criterion, the analyst should check the calibration by reanalyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3.3, recalibration is in order per Section 10. If the calibration standard is acceptable, it may be necessary to extract another aliquot of sample if sample holding time has not been exceeded. If the sample reextract also fails the recovery criterion, report all data for that sample as suspect.

9.11 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- Determine that the sample matrix does not contain materials that adversely affect method performance. This is accomplished by analyzing replicates of laboratory fortified matrix samples and ascertaining that the precision, accuracy, and method detection limits of analytes are in the same range as obtained with laboratory fortified blanks. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, matrix independence should be established for each. Over time, LFM data should be documented for all routine sample sources for the laboratory. A laboratory fortified sample matrix should be extracted and analyzed for each extraction batch. If more than 20 samples are processed in a batch, extract and analyze a LFM for every 20 samples. If the recovery data for an LFM does not meet the recovery criteria in Sect. 9.8, and LFBs show the laboratory

to be in control , then the samples from that matrix (sample location) are documented as suspect due to matrix effects.

9.11.1 Within each extraction batch, a minimum of one field sample is fortified as a LFM for every 20 samples analyzed. The LFM is prepared by spiking a sample with an appropriate amount of the fortification solution. The concentrations 5, 10, and 15 µg/L are suggested spiking concentrations. Select the spiking concentration that is closest to, and at least twice the matrix background concentration. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed, aids in the selection of appropriate spiking levels.

9.11.2 Calculate the percent recovery (R) for each analyte, after correcting the measured fortified sample concentration, A, for the background concentration, B, measured in the unfortified sample, i.e.,

$$R = \frac{(A-B)}{C} * 100$$

where C is the fortified concentration. Compare these values to control limits for LFBs (Sect. 9.8).

9.11.3 Recoveries may exhibit a matrix dependence. For samples fortified at or above their native concentration, recoveries should range between 70 and 130%, for all method analytes except phenol which should be recovered at 50-150%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

NOTE: Matrix effects are expected to be more likely with compounds 9-12 (Table 2) than other method analytes.

9.12 FIELD DUPLICATES (FD) -- Within each extraction batch, a minimum of one field sample should be analyzed in duplicate. Duplicate sample analyses serve as a check on sampling and laboratory precision. If analytes are not routinely observed in field samples, duplicate LFMDs should be analyzed to substitute for this requirement.

9.12.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) as shown below.

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} * (100)$$

9.12.2 Relative percent differences for laboratory duplicates and LFMDs should fall in the range of ± 30 %.

NOTE: Greater variability may be observed for target analytes with concentrations at the low end of the calibration range.

9.13 QUALITY CONTROL SAMPLE (QCS) -- Each time that new standards are prepared, analyze a QCS from an external source. If standards are prepared infrequently, analyze a QCS at least quarterly. The QCS may be injected as a calibration standard, or fortified into reagent water and analyzed as an LFB. If the QCS is analyzed as a calibration check standard, then the acceptance criteria are the same as for the CCC (Sect. 10.3.3). If the QCS is analyzed as a LFB, then the acceptance criteria are the same as for an LFB (Sect. 9.8). If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable mass spectrometer tune and initial calibration is required before any samples are analyzed. After initial calibration is successful, a continuing calibration check is required at the beginning and end of each period in which analyses are performed, and after every tenth sample. Verification of mass spectrometer tune must be repeated each time a major instrument modification or maintenance is performed, and prior to analyte calibration. A peak tailing factor check is required every day that samples are analyzed. In periods of continuous operation, the peak tailing factor must be performed every 24 hr.

10.2 Initial calibration

10.2.1 MS TUNE -- Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet tuning requirements. Inject 5 ng or less of DFTPP solution into the GC/MS system. Acquire a mass spectrum that includes data for m/z 45-450. If the DFTPP mass spectrum does not meet all criteria in Table 3, the MS must be retuned and adjusted to meet all

criteria before proceeding with calibration. A single spectrum at the apex of the chromatographic peak, or an average of the three spectra at the apex of the peak, or an average spectrum across the entire GC peak may be used to evaluate the performance of the system. Background subtraction is permitted. The tune check may be performed as a separate analysis, or for routine MS tune verification, DFTPP may be added to one or more of the CAL standards used for calibration verification, so that the tune check and calibration verification can be performed in a single analysis. DFTPP elutes shortly after pentachlorophenol on both of the columns cited in Sect.6.9.

10.2.2 ANALYTE CALIBRATION -- Inject an aliquot of a medium concentration calibration solution. For example, 2-10 µg/mL, and acquire and store data from m/z 45-350 with a total cycle time (including scan overhead time) of 1.0 sec or less. Cycle time must be adjusted to measure at least five or more scans during the elution of each GC peak. Seven to ten scans across each GC peak are recommended.

Chromatographic conditions used during method development are outlined below. These conditions were found to work well on the instrumentation used. Since some of the method analytes are vulnerable to active sites and thermal decomposition, optimum chromatographic conditions may vary with individual instrument design. Although the following conditions are recommended, GC conditions may be modified, if all performance criteria in Sections 9 and 10 are met.

10.2.2.1 The following parameters are suggested GC conditions for hot, splitless injection: injector temperature 200° C, carrier gas head pressure 12-15 psi. Inject at an oven temperature of 35° C and hold in splitless mode for 0.2 min. After 6 min, temperature program the GC oven at 8° C per min to 250° C. Start data acquisition at approximately 10 min. Example chromatograms are shown in Figures 1 and 2.

10.2.2.2 The following parameters are suggested injection conditions for temperature programmed splitless injection. Inject with the injector temperature at 25° C, program the injector at 200° C per min to 200° C. Hold in the splitless mode for 1.0 min. Use the same column temperature program as listed in Sect. 10.2.2.1. An example chromatogram is shown in Figure 3.

10.2.3 Performance criteria for the calibration standards. Examine the stored GC/MS data with the data system software.

10.2.3.1 PEAK TAILING FACTOR (PTF) -- Peak tailing can be a problem associated with phenols. The phenols most likely to tail are those with low acidity constants: 2,4-dinitrophenol, 4-nitrophenol, pentachlorophenol and 2-methyl-4,6-dinitrophenol. These compounds must exhibit a peak tailing factor of 5 or less at a concentration equivalent to 5-10 µg/L in a water sample (5-10 µg/mL in an extract or calibration standard). For example peak tailing factor calculations, see Fig.4. Peak tailing factors must be evaluated for the four analytes listed above each day that samples are analyzed. In periods of continuous instrument operation, verify acceptable PTFs every 24 hr. Peak tailing factors may be evaluated in either a CAL standard, LFB or LFM.

10.2.3.2 The GC/MS/DS peak identification software should be able to recognize a GC peak in the appropriate retention time window for each of the compounds in the calibration solution, and make correct identifications (Sect. 11.5).

10.2.4 If all performance criteria are met, inject an aliquot of an appropriate volume (usually 1-2 µL unless a large volume injector is used) of each of the other CAL solutions using the same GC/MS conditions.

10.2.4.1 Some GC/MS systems may not be sensitive enough to detect some of the analytes in the two lowest concentration CAL solutions (0.1 and 0.5 µg/mL). If this is the case, it is acceptable to calibrate using the remaining (higher concentration) points, as long as a minimum of 5 calibration points are used to generate the calibration curve or average response factor (RF) for each analyte. In addition, some GC/MS systems might reach signal saturation at the highest calibration concentration. If this is the case, it is acceptable to drop the highest point and calibrate on the remaining points, as long as at least 5 calibration concentrations are used to generate the calibration curve or average RF for each analyte. Points in the middle of the calibration range may not be dropped. Data outside of the established calibration range should never be reported.

10.2.5 Concentrations may be calculated through the use of average response factor (RF) or through the use of a calibration curve. Average RF calibrations may only be used if the RF values over the calibration range are relatively constant (<30% RSD).

Average RF is determined by calculating the mean RF of each calibration point, with a minimum of five calibration concentrations.

$$RF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)}$$

where:

A_x = integrated abundance (peak area) of the quantitation ion of the analyte.

A_{is} = integrated abundance (peak area) of the quantitation ion internal standard.

Q_x = quantity of analyte injected in ng or concentration units.

Q_{is} = quantity of internal standard injected in ng or concentration units.

10.2.6 As an alternative to calculating average RFs and applying the RSD test, use the GC/MS data system software to generate a linear regression or quadratic calibration curve. The analyst may choose whether or not to force zero, to obtain a curve that best fits the data. Examples of common GC/MS system calibration curve options are: 1) A_x /A_{is} vs Q_x /Q_{is} and 2) RF vs A_x /A_{is}.

10.2.7 Acceptance criteria for the calibration of each analyte is determined by calculating the concentration of each analyte and surrogate in each of the analyses used to generate the calibration curve or average RF. Each calibration point, except the lowest point, for each analyte must calculate to be 70-130 % of its true value. The lowest point must calculate to be 50-150% of its true value. If this criteria cannot be met, reanalyze the calibration standards, or select an alternate method of calibration. The data presented in this method were obtained using linear regression (RF vs A_x /A_{is}). Quadratic fit calibrations should be used with caution, because the non-linear area of the curve may not be reproducible.

10.3 CONTINUING CALIBRATION CHECK (CCC) -- The minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. (In this context, a "sample" is considered to be a field sample. LRBs, LFMs, LFBs and CCCs are not counted as samples.) The beginning CCC each day should be at or near the MRL in order to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL solutions to meet this requirement. Subsequent CCCs can alternate between a medium and high concentration standard.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Determine that the absolute areas of the quantitation ions of the internal standard 1,3-dimethyl-2-nitrobenzene has not changed by more than 30% from the areas measured in the most recent continuing calibration check, or by more than 50% from the areas measured during initial calibration. If this area has changed by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may include cleaning of the MS ion source, or other maintenance as indicated in Sect. 10.3.4. Major instrument maintenance requires recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be within 70-130% of the true value. The calculated amount for the lowest calibration point for each analyte must be within 50-150% of the true value. If these conditions do not exist, remedial action should be taken which may require recalibration. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored, with the following exception. **If the continuing calibration check in the middle or at the end of an analysis batch fails because the calculated concentration is >130% of the true value, and field sample extracts showed no detection of method analytes, non-detects may be reported without re-analysis.**

10.3.4 Some possible remedial actions are listed below. This list is not meant to be all inclusive. Major maintenance such as cleaning an ion source, cleaning quadrupole rods, replacing filament assemblies, etc. require returning to the initial calibration step (Sect. 10.2).

10.3.4.1 Check and adjust GC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.

10.3.4.2 Clean or replace the splitless injection liner; silanize a new injection liner.

10.3.4.3 Flush the GC column with solvent according to manufacturer's instructions.

10.3.4.4 Break off a short portion (about 1 meter) of the column from the end near the injector, or replace GC column. This action will cause a change in retention times.

10.3.4.5 Prepare fresh CAL solutions, and repeat the initial calibration step.

10.3.4.6 Clean the MS ion source and rods (if a quadrupole).

10.3.4.7 Replace any components that allow analytes to come into contact with hot metal surfaces.

10.3.4.8 Replace the MS electron multiplier, or any other faulty components.

11. PROCEDURE

11.1 CARTRIDGE EXTRACTION

11.1.1 This procedure may be performed manually or in an automated mode (Sect. 6.11) using a robotic or automatic sample preparation device. If an automatic system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system.

11.1.2 Mark the level of the sample on the outside of the sample bottle for later sample volume determination (Sect. 11.2). Verify that the sample is at pH 2 or less and is free of residual chlorine. If the sample is a LRB or LFB, add sodium sulfite and acidify following procedures in Sect.8.2. Add an aliquot of the surrogate fortification solution(s), and mix immediately until homogeneous. The resulting concentration of these compounds in the water should be 2-5 µg/L. If the sample is a LFB or LFM, add the desired amount of analyte fortification solution.

11.1.3 CARTRIDGE CLEAN-UP AND CONDITIONING -- Rinse each cartridge with three, 3 mL aliquots of methylene chloride. Let the cartridge drain dry after each flush. Then rinse the cartridge with three, 3mL aliquots of methanol, but DO NOT allow the methanol to elute below the top of the cartridge packing. From this point, do not allow the cartridge packing to go dry. Rinse with three, 3mL aliquots of 0.05 N hydrochloric acid, but before the dilute acid level drops below the top edge of the packing, turn off the vacuum. Add approximately 3 mL additional 0.05 N hydrochloric acid to the cartridge, attach the transfer tube, and turn on the vacuum, and begin adding sample to the cartridge.

11.1.4 Adjust the vacuum so that the approximate flow rate is 20 mL/min (50 min for a 1 L sample). After all of the sample has passed through the SPE cartridge, draw air or nitrogen through the cartridge for 15-30 min at high vacuum (10-15 in Hg). The cartridge packing should appear dry (light tan color) before continuing with the elution steps. It is important that the cartridge packing be dry, in order to obtain good recoveries. The drying time may vary, depending upon the strength of the vacuum source, and the number of cartridges being processed simultaneously. The color and appearance of the packing is the most reliable indicator of dryness. During the method development, drying for more than 60 minutes was not observed to have any negative effect upon the sample data.

NOTE: Samples with a high level of hardness and/or high TOC may exhibit a lower flow rate than "cleaner" samples at the same vacuum setting. This may be due to partial plugging of the solid phase. Fortified sample matrices of these types showed no loss of method performance.

11.1.5 Rinse the inside of each sample bottle with 8-10 mL methylene chloride and use vacuum to pull the solvent through the transfer tube and through the cartridge, collecting the solvent in a collection tube. Remove the transfer tubing from the top of the cartridge. Add 2-3 mL methylene chloride to the top of the cartridge with a disposable pipette. Pull this solvent through the cartridge at low vacuum, such that the solvent exits the cartridge in a dropwise fashion. Small amounts of residual water from the sample container and the SPE cartridge may form an immiscible layer with the eluate. Pass the eluate through the drying column (Sect. 6.7), which is packed with approximately 5 to 7 grams of anhydrous sodium sulfate, and collect in a clean collection tube. Wash the sodium sulfate with at least 2 mL methylene chloride and collect in the same tube. Concentrate the extract to approximately 0.9 mL in a warm (40°C) water bath under a gentle stream of nitrogen. Do not concentrate the extract to less than 0.5 mL, as this will result in losses of analytes. Add the internal standards (Sect 7.9). Adjust final volume to 1 mL. Make any volume adjustments with methylene chloride.

11.2 Fill the sample bottle to the volume mark noted in Sect.11.1.2. with tap water. Transfer the tap water to a 1000 mL graduated cylinder, and measure the sample volume to the nearest 10 mL. Record this volume for later analyte concentration calculations. As an alternative to this process, the sample volume may be determined by the difference in weight between the full bottle (before extraction) and the empty bottle (after extraction). Assume a sample density of 1.0.

11.3 Analyze an aliquot of the sample extract with the GC/MS system under the same conditions used for the initial and continuing calibrations (Sect. 10.2.2 and 10.3).

11.4 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks in predetermined retention time windows of interest. Use the data system software to examine the ion abundances of components of the chromatogram.

11.5 Identification of analytes. Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base. The GC retention time of the sample component should be within 1-2 sec of the retention time observed for that same compound in the most recently analyzed continuing calibration check standard. Ideally, the width of the retention time window should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of the chromatogram.

11.5.1 In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation. Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing the same ions contributed by more than one analyte. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining plots of characteristic ions for each tentatively identified component. When analytes coelute (i.e., only one GC peak is apparent), the identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound. In validating this method, concentrations were calculated by measuring the characteristic ions listed in Table 2. Other ions may be selected at the discretion of the analyst. If the response of any analyte exceeds the calibration range established in Section 10, dilute the extract,

add additional internal standard, and reanalyze. The resulting data should be documented as a dilution, with an increased MRL.

12.1.1 Calculate analyte and surrogate concentrations, using the multipoint calibration established in Sect. 10. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.2.

12.1.2 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for concentrations above 99 µg/L, two significant figures for concentrations between 1.0-99 µg/L, and one significant figure for lower concentrations.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND MDLs-- Single laboratory accuracy and precision data from both fortified reagent water and fortified matrices using hot, splitless injection are presented in Tables 4 and 5. Table 6 includes data from 2 matrices using temperature programmed splitless injection. Method detection limits (MDLs) are presented in Table 1 for both types of injectors used. MDLs were calculated using the formula in Section 9.2.4. Although the calculated MDLs using the two different types are not dramatically different, for compounds 9-12 (Table 2) the peak shapes are significantly better using temperature programmed injection, and the peak heights and areas are greater.

13.2 POTENTIAL PROBLEM COMPOUNDS –

13.2.1 2,4-Dinitrophenol, 4-nitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol and 2,4,6-tribromophenol have a tendency to exhibit a chromatographic phenomenon known as "matrix-induced chromatographic response enhancement" (5-8). Compounds that exhibit this phenomenon often give analytical results that exceed 100% recovery. The theory behind this phenomenon is that these compounds are susceptible to adsorption and/or thermal degradation in the GC inlet. The "cleaner" the matrix they are injected in, e.g. clean solvent, the more they degrade. When they are injected in a sample extract, matrix components in the sample extract "protect" these compounds from decomposition and a relatively greater response is observed. While most of the literature references to this phenomenon refer to organophosphate pesticides in river water and food samples, the effect seen during development of this method suggests the same type of problem occurs with these acidic phenols.

This method uses 2,3,4,5-tetrachlorophenol as the internal standard for quantifying these analytes. The chromatographic behavior of 2,3,4,5-tetrachlorophenol mimics these particular method analytes. Therefore its use as an internal standard helps maintain accurate measurement of these analytes. It should be noted however that these particular analytes will probably not be measured with the same level of precision and accuracy as other method analytes, but the precision and accuracy requirements should still be achievable.

13.2.2 The same compounds listed in sect. 13.2.1. also have a tendency to tail. QC criteria for peak tailing factors have been given in Sect. 10.2.3.1. During method development, significantly less peak tailing was observed using temperature programmed injection. Other measures shown to minimize peak tailing and improve peak shape are pressure pulsed injection, and increasing the GC oven temperature program rate. Pulsed injection is recommended on GCs which have that option available. A faster GC oven temperature program is recommended if there are no interferences, and if the minimum number of scans across all chromatographic peaks can be obtained. This is a function of how fast the MS can scan.

13.2.3 Phenol is very water soluble compared to other method analytes. Breakthrough experiments performed during method development indicate that some breakthrough from the SPE cartridge can be expected. Breakthrough can be minimized by monitoring the flow of the sample through the cartridge. In general, slower flow rates will minimize breakthrough. Precision and accuracy requirements in Sect. 10 should be achievable.

13.3 HOLDING TIME STUDY RESULTS –

13.3.1 Holding time studies for aqueous samples were conducted for a period of 35 days. Chlorinated surface water samples fortified with method analytes and preserved and stored according to requirements in Section 8, were analyzed on days 0, 7, 10, 15, 23, 28, and 35. Small, but statistically significant losses of 2-chlorophenol, o-cresol, and 2,4-dimethylphenol were observed beginning between day 15 and 23. Therefore the aqueous holding time was determined to be 14 days. Data from these studies are in Table 7.

13.3.2 Holding time studies for sample extracts were conducted for a period of 35 days. A single set of extracts were stored at 0°C, and analyzed on days 0, 14, 23, and 35. No significant losses were observed within this time frame. Therefore the extract holding time was established at 30 days. Data from these studies are in Table 8.

14. POLLUTION PREVENTION

14.1 This method utilizes SPE technology to remove the analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment when compared with the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less Is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. METHOD DETECTION LIMITS [a]

Analyte	Hot Splitless Injection [b]		Temperature Programmed Splitless Injection [c]	
	Spiking Conc. (µg/L)	MDL (µg/L)	Spiking Conc. (µg/L)	MDL (µg/L)
phenol	1.0	0.58	0.1	0.025
2-chlorophenol	0.1	0.020	0.1	0.041
2-methylphenol (o-cresol)	0.1	0.026	0.1	0.028
2-nitrophenol	0.1	0.026	0.1	0.044
2,4-dimethylphenol	0.1	0.026	0.1	0.034
2,4-dichlorophenol	0.1	0.027	0.1	0.046
4-chloro-3-methylphenol	0.1	0.036	0.1	0.042
2,4,6-trichlorophenol	0.1	0.046	0.1	0.024
2,4-dinitrophenol	1.0	0.31	0.5	0.22
4-nitrophenol	1.0	0.42	0.5	0.18
2-methyl-4,6-dinitrophenol	1.0	0.26	0.5	0.092
pentachlorophenol	1.0	0.25	0.5	0.081

a- data obtained using Column 1
b- n=7
c- n=8

TABLE 2. RETENTION TIMES (RTs) AND SUGGESTED QUANTITATION IONS (QIs)

Cmpd # [a]	Analyte	RTs (min) column 1 [b]	RTs (min) column 2 [c]	QIs (m/z)	IS Ref
1	phenol	11:00	12:38	94	1
2	2-chlorophenol	11:07	12:50	128	1
3	2-methylphenol (o-cresol)	12:52	14:27	107	1
4	2-nitrophenol	14:36	16:22	139	1
5	2,4-dimethylphenol	15:00	16:35	107	1
6	2,4-dichlorophenol	15:22	17:04	162	1
7	4-chloro-3-methylphenol	17:46	19:27	142	1
8	2,4,6-trichlorophenol	18:56	20:42	97	1
9	2,4-dinitrophenol	21:30	23:30	154,184[d]	2
10	4-nitrophenol	21:53	23:44	139	2
11	2-methyl-4,6-dinitrophenol	23:09	25:09	121,198[d]	2
12	pentachlorophenol	25:14	27:12	266	2
13	1,2-dimethyl-3-nitrobenzene (IS#1)	17:43	19:29	134	
14	2,3,4,5-tetrachlorophenol (IS#2)	22:09	24:02	232	
15	2-chlorophenol-3,4,5,6-d_4 (SURR)	11:04	12:47	132	1
16	2,4-dimethylphenol-3,5,6-d_3 (SURR)	14:59	16:34	110	1
17	2,4,6-tribromophenol (SURR)	23:37	25:37	330	2

a- Number refers to peak number in Figures 1-3.
b- Column 1- 30 m × 0.25 mm id DB-5ms (J&W), 0.25 µm film thickness.
c- Column 2- 30 m × 0.25mm id BPX5 (SGE), 0.25 µm film thickness.
d- Because the MS response to these compounds is low, and both the listed ions are near 100% ion abundance, the signal from both ions may be added together to increase sensitivity.

TABLE 3. ION ABUNDANCE CRITERIA FOR DECAFLUOROTRIPHENYLPHOSPHINE (DFTPP)

Mass (m/z)	Relative Abundance Criteria	Purpose of Checkpoint [a]
51	10-80% of the base peak	low mass sensitivity
68	<2% of mass 69	low mass resolution
70	<2% of mass 69	low mass resolution
127	10-80% of the base peak	low-mid mass sensitivity
197	<2% of mass 198	mid-mass resolution
198	base peak or >50% of 442	mid-mass resolution and sensitivity
199	5-9% of mass 198	mid-mass resolution and isotope ratio
275	10-60% of the base peak	mid-high mass sensitivity
365	>1% of the base peak	baseline threshold
441	Present and < mass 443	high mass resolution
442	base peak or >50% of 198	high mass resolution and sensitivity
443	15-24% of mass 442	high mass resolution and isotope ratio

a- All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization to fragmentation patterns.

TABLE 4. RESULTS FROM REPLICATE ANALYSES OF REAGENT WATER FORTIFIED WITH METHOD ANALYTES AT 1-10 µg/L [a] USING HOT SPLITLESS INJECTION

Analyte	Concentration= 10 µg/L [b], n=4		Concentration= 5 µg/L [c], n=4		Concentration= 1 µg/L [b], n=7	
	Mean % Recovery	RSD (%)	Mean % Recovery	RSD (%)	Mean % Recovery	RSD (%)
phenol	75.9	4.3	103	18	90.8	20
2-chlorophenol	91.3	2.6	86.7	4.3	89.7	7.9
2-methylphenol (o-cresol)	93.3	2.2	86.1	3.6	84.9	7.3
2-nitrophenol	97.4	3.6	97.8	4.8	75.0	11
2,4-dimethylphenol	91.0	1.7	74.7	8.5	76.3	8.0
2,4-dichlorophenol	94.3	2.9	94.4	1.8	88.2	4.8
4-chloro-3-methylphenol	96.5	1.9	92.3	4.2	90.8	8.4
2,4,6-trichlorophenol	89.9	3.4	91.5	4.7	92.9	11
2,4-dinitrophenol	107	3.6	104	6.0	61.8	16
4-nitrophenol	107	3.0	90.5	11	96.3	14
2-methyl-4,6-dinitrophenol	105	3.2	83.0	1.6	91.5	8.9
pentachlorophenol	103	1.9	86.2	9.8	103	7.6
2-chlorophenol-3,4,5,6-d4 (SURR)	93.4	1.8	84.9	6.6	95.9	11
2,4-dimethylphenol-3,5,6-d3 (SURR)	94.0	2.0	82.0	7.2	95.1	13
2,4,6-tribromophenol (SURR)	82.2	1.8	77.1	6.1	98.0	8.6

a- Surrogate concentrations in all sample are 5 µg/L for tribromophenol and 2 µg/L for the deuterated phenols.
b- Data obtained using Column 1.
c- Data obtained using Column 2

TABLE 5. ACCURACY [a] AND PRECISION DATA FOR METHOD ANALYTES FORTIFIED AT 10 µg/L IN THREE MATRICES USING HOT SPLITLESS INJECTION

ANALYTE	HARD GROUND WATER [b,c] n=4		CHLORINATED SURFACE WATER [c] n=4		SIMULATED HIGH TOC WATER [c,d] n=4	
	% Rec	RSD (%)	%Rec	RSD (%)	%Rec	RSD (%)
phenol	77.6	5.1	73.9	4.5	73.7	5.1
2-chlorophenol	91.2	2.6	88.6	3.0	85.8	5.2
2-methylphenol (o-cresol)	93.2	3.4	91.3	2.3	89.2	4.3
2-nitrophenol	102	2.5	99.4	2.1	99.0	2.8
2,4-dimethylphenol	86.3	2.0	86.6	1.9	83.2	5.3
2,4-dichlorophenol	94.8	1.2	93.3	1.4	90.0	5.1
4-chloro-3-methylphenol	98.5	1.6	97.5	1.4	94.6	3.9
2,4,6-trichlorophenol	95.5	3.9	92.7	3.1	89.1	4.1
2,4-dinitrophenol	117	4.2	118	2.3	121	1.0
4-nitrophenol	102	2.4	102	3.8	95.9	2.3
2-methyl-4,6-dinitrophenol	115	2.2	113	1.5	115	0.71
pentachlorophenol	110	4.0	108	5.1	105	1.8
2-chlorophenol-3,4,5,6-d4 (SURR)	93.0	2.4	91.5	1.6	89.1	5.3
2,4-dimethylphenol-3,5,6-d3 (SURR)	88.9	3.0	87.2	2.4	85.4	4.8
2,4,6-tribromophenol (SURR)	74.7	2.6	76.3	5.1	71.0	4.5

a- Accuracy is presented as % recovery
b- Hard municipal chlorinated ground water, 450mg/L hardness measured as calcium carbonate.
c- Data obtained using Column 1.
d- Simulated high organic content (high Total Organic Carbon) sample prepared by adding 10 mg/L humic acid (Fluka Chemical Corp., Milwaukee, WI) to reagent water.

TABLE 6. RESULTS OF REPLICATE ANALYSES IN TWO MATRICES USING TEMPERATURE PROGRAMMABLE INJECTION [a]

ANALYTE	Reagent Water Concentration= 0.5 µg/L, n=7		Reagent Water Concentration= 5 µg/L, n=4		Chlorinated Surface Water Concentration= 10 µg/L, n=5	
	% Recovery	RSD (%)	% Recovery	RSD (%)	% Recovery	RSD (%)
phenol	74.5	13	107	21	74.1	7.5
2-chlorophenol	88.3	11	95.5	7.7	94.8	4.3
2-methylphenol (o-cresol)	96.2	9.4	93.0	4.5	103	8.1
2-nitrophenol	89.1	8.9	102	6.8	94.5	7.4
2,4-dimethylphenol	82.0	8.2	85.5	8.8	93.8	9.8
2,4-dichlorophenol	83.0	8.3	100	7.3	93.7	2.9
4-chloro-3-methylphenol	79.0	6.6	101	7.5	96.6	2.9
2,4,6-trichlorophenol	78.9	8.1	102	10	90.7	3.3
2,4-dinitrophenol	63.8	22	95.4	6.6	113	4.7
4-nitrophenol	84.1	14	103	8.1	102	6.8
2-methyl-4,6-dinitrophenol	62.4	9.4	103	5.1	107	4.9
pentachlorophenol	72.0	7.2	88.0	7.2	93.8	6.8
2-chlorophenol-3,4,5,6-d4 (SURR)	79.1	7.6	87.6	3.9	84.7	10
2,4-dimethylphenol-3,5,6-d3 (SURR)	79.9	8.5	91.9	7.8	97.0	8.3
2,4,6-tribromophenol (SURR)	79.6	7.8	86.1	7.8	79.5	12

a- Data obtained using Column 1.

TABLE 7. RESULTS OF AQUEOUS HOLDING TIME STUDIES FOR METHOD 528 ANALYTES [a]

ANALYTE	DAY 0		DAY 7		DAY 10		DAY 15	
	% Rec	RSD (%)	% Rec	RSD (%)	% Rec	RSD (%)	% Rec	RSD (%)
phenol	74.5	1.9	77.0	3.8	74.3	3.4	74.3	3.5
2-chlorophenol	91.5	1.9	88.8	1.3	88.0	2.9	85.8	4.1
2-methylphenol (o-cresol)	99.9	2.8	96.0	1.2	94.3	2.6	93.0	2.8
2-nitrophenol	99.1	4.3	100	4.0	98.8	3.9	101	3.1
2,4-dimethylphenol	85.5	4.2	80.7	3.9	79.1	8.1	75.2	4.7
2,4-dichlorophenol	94.2	2.2	93.0	2.5	92.3	1.0	91.8	3.3
4-chloro-3-methylphenol	96.5	2.1	95.5	3.5	96.5	2.3	96.5	3.9
2,4,6-trichlorophenol	94.8	3.3	97.1	2.9	96.0	2.8	95.0	3.3
2,4-dinitrophenol	102.8	5.1	102	3.4	107	8.0	112	2.9
4-nitrophenol	100.1	3.2	100	4.2	101	4.9	96.2	3.3
2-methyl-4,6-dinitrophenol	96.1	5.1	109	8.7	113	5.8	115	5.0
pentachlorophenol	94.5	4.1	102	2.1	103	3.5	102	2.3

a- All analytes fortified into a chlorinated surface water at a concentration of 10 µg/L, dechlorinated and acidified according to Section 8, stored for 48 hr at 10° C, followed by storage at 6° C. For each time point, n=5.

TABLE 8. RESULTS OF EXTRACT HOLDING TIME STUDIES FOR METHOD 528 ANALYTES [a]

ANALYTE	DAY 0		DAY 14		DAY 23		DAY 35	
	% Rec	RSD (%)	% Rec	RSD (%)	% Rec	RSD (%)	% Rec	RSD (%)
phenol	74.5	1.9	74.2	3.8	72.7	3.4	74.9	3.5
2-chlorophenol	91.5	1.9	89.8	1.3	88.6	2.9	89.1	4.1
2-methylphenol (o-cresol)	99.9	2.8	97.5	1.2	98.4	2.6	96.6	2.8
2-nitrophenol	99.1	4.3	100	4.0	96.4	3.9	101	3.1
2,4-dimethylphenol	85.5	4.2	86.9	3.9	88.0	8.1	88.7	4.7
2,4-dichlorophenol	94.2	2.2	95.4	2.5	96.1	1.0	98.6	3.3
4-chloro-3-methylphenol	96.5	2.1	98.9	3.5	98.1	2.3	102	3.9
2,4,6-trichlorophenol	94.8	3.3	97.1	2.9	97.6	2.8	102	3.3
2,4-dinitrophenol	102.8	5.1	104	3.4	90.9	8.0	99.1	2.9
4-nitrophenol	100.1	3.2	91.1	4.2	89.3	4.9	96.3	3.3
2-methyl-4,6-dinitrophenol	96.1	5.1	108	8.7	98.2	5.8	110	5.0
pentachlorophenol	94.5	4.1	99.1	2.1	100	3.5	105	2.3

a- All extracts were from the Day 0 aqueous holding time samples, and were stored in amber vials at 0° C. For each time point, n=5.

TABLE 9. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low Method Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all target analytes are below 1/3 the MRL, and that possible interferences from extraction media do not prevent the identification and quantification of method analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at 5-10µg/L	RSD must be ≤20% for all analytes except phenol which must be ≤ 30%.
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP	Mean recovery 70-130% of true value, except phenol which is 50-150%
Sect. 9.2.4	Method Detection Limit (MDL) Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the MDL. Analyze the replicates through all steps of the analysis. Calculate the MDL using the equation in Section 9.2.4.	**Note:** Data from MDL replicates are <u>not required</u> to meet method precision and accuracy criteria. If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

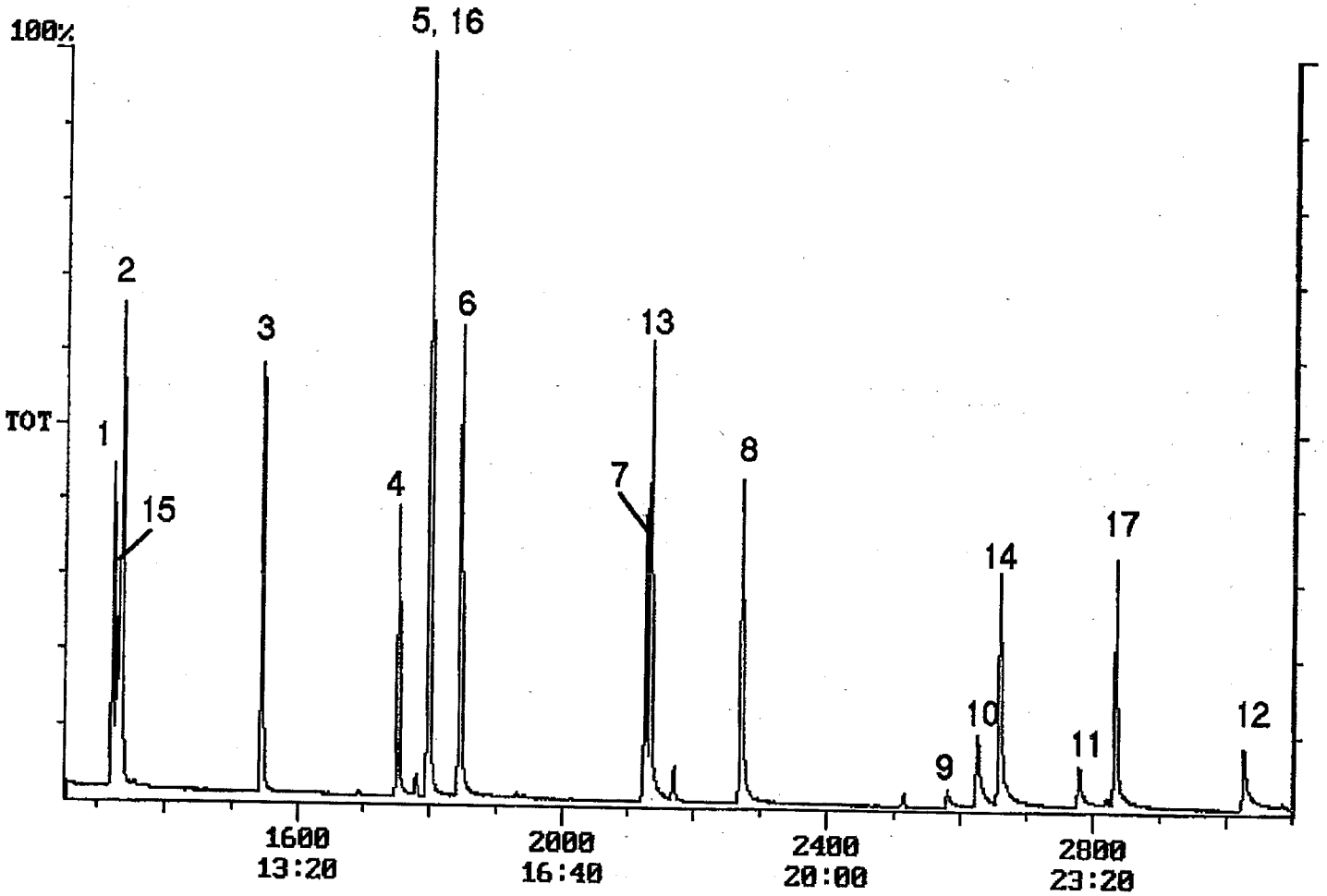
TABLE 10. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.4	Sample Holding Time	14 days, dechlorinated and acidified to pH≤2	Iced or refrigerated at 10°C or less for up to 48 hours, 6°C thereafter.
Sect. 8.4	Extract Holding Time	30 days	Stored at 0°C or less in amber vials.
Sect.9.11	Laboratory Fortified Sample Matrix (LFM)	Analyze one LFM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to the native concentration.	Recoveries not within 70-130% of the fortified amount may indicate a matrix effect.
Sect. 9.12	Field Duplicates	Analyze 1 FD for each 20 samples, or 1 per extraction batch, whichever is greater.	Suggested RPD±30%.
Sect. 9.13	Quality Control Sample (QCS)	Analyze QCS whenever new standards are prepared, or at least quarterly.	If analyzed as a calibration sample, CCC criteria apply. If analyzed as a LFB, those criteria apply.
Sect 9.2.1	Laboratory Reagent Blank (LRB)	Daily, or with each extraction batch of up to 20 samples, whichever is more frequent.	Demonstrate that all target analytes are below 1/3 the MRL, and that possible interference from extraction media do not prevent the identification and quantification of method analytes.

Sect. 9.8	Laboratory Fortified Blanks (LFB)	Analyze at least one LFB daily or for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value (except phenol) for each analyte and surrogate for all fortified concentrations greater than the lowest CAL point. Results of LFBs corresponding to the lowest CAL point must be 50-150% of the true value.
Sect. 9.9	Internal Standard	1,2-dimethyl-3-nitrobenzene (IS#1) and 2,3,4,5-tetrachloro-phenol (IS#2) are added to all standards and sample extracts.	Peak area counts for IS#1 in LFBs, LRBs and sample extracts must be within 70-130% of the peak area in the most recent CCC, and 50-150% of average area in the initial calibration.
Sect 9.10	Surrogate Standards	Surrogate standards are added to all standards, samples, LFBs, LFMs, FDs, LRBs, and LFBs.	Recovery for 2-chlorophenol-3,4,5,6-d_4 and 2,4-dimethylphenol-3,5,6-d_3 in all standards, LRB, LFB, LFM, FD and sample extracts must be 70-130% of the true value. Recovery for 2,4,6-tribromophenol must be 60-130%.
Sect. 10.2.1	MS Tune Check	Analyze DFTPP to verify MS tune before initial calibration and before every recalibration.	Criteria are given in Table 3.
Sect.10.2.2	Initial Calibration	Use internal standard calibration technique to generate an average RF or first or second order calibration curve. Use at least 5 standard concentrations that span the approximate range of 0.1- 15 µg/L.	When each calibration standard is calculated as an unknown using the calibration curve, the result must be 70-130% of the true value for all but the lowest standard. The lowest standard must be 50-150% of the true value.

Sect. 10.2.3.1	GC Performance-Peak Tailing Check	Calculate the peak tailing factor for compounds listed in the referenced section, at the beginning of each day during which samples are analyzed. In cases of continuous instrument operation, check peak tailing factors every 24 hr.	Peak tailing factor of 5 or less. (See Fig.4 for calculation of PTF.)
Sect. 10.3	Continuing Calibration Check	Verify initial calibration by analyzing a calibration standard prior to analyzing samples, after every 10 samples, and after the last sample. Always analyze a low concentration (near the MRL) CCC at the beginning of the analysis period.	The result for each analyte and surrogate must be 70-130% of the true value for all concentrations except the lowest CAL point for each analyte. The lowest CAL point for each analyte must be 50-150% of the true value. The peak area of IS#1 must be within 70-130% of the peak area in the most recent CCC, and 50-150% of the average peak area calculated during initial calibration.

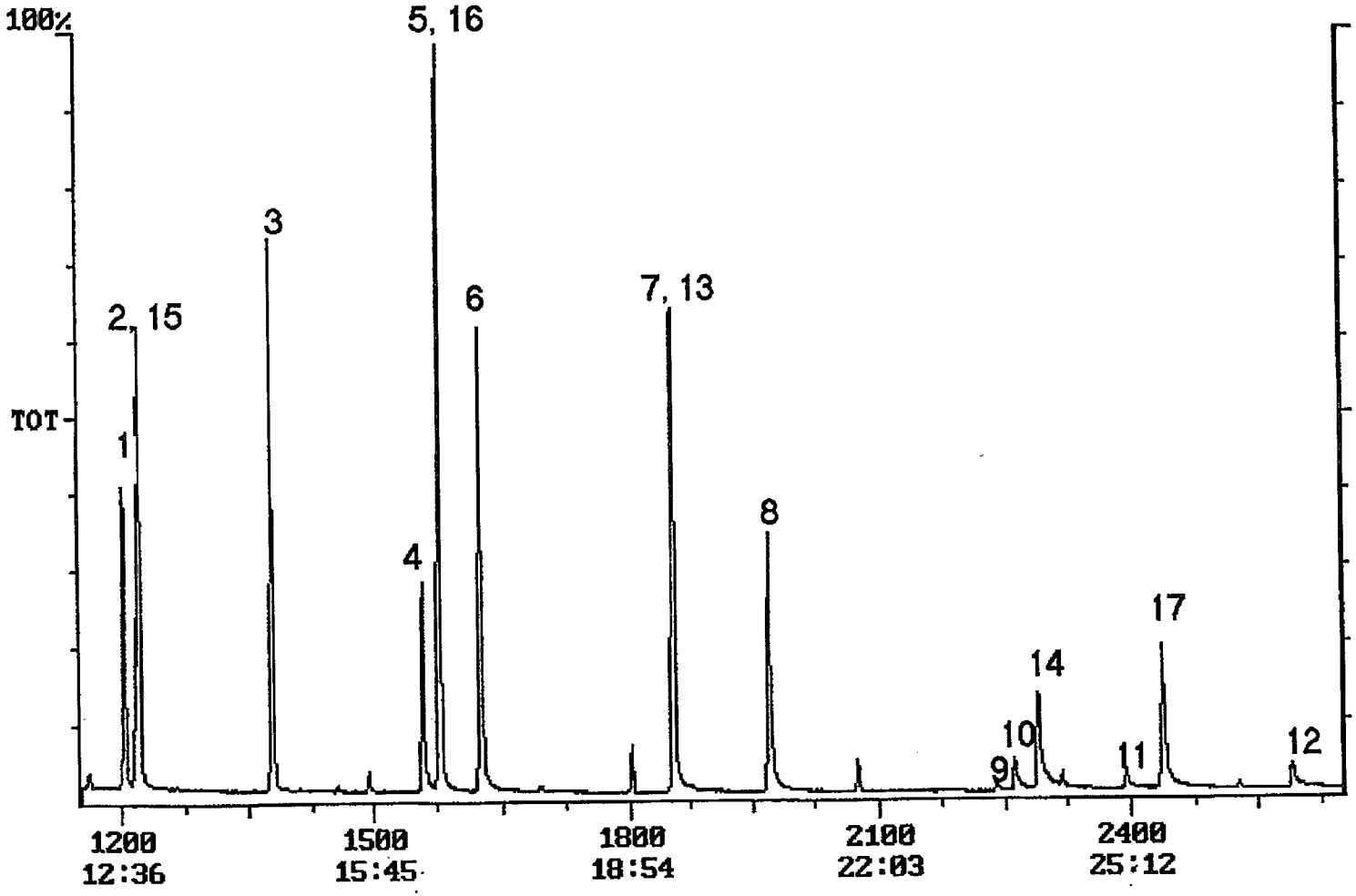
FIGURE 1.CHROMATOGRAM OF PHENOL STANDARD MIX (5 ng/ µL EACH ANALYTE) ON A DB-5ms COLUMN WITH HOT SPLITLESS INJECTION[a].



528-43

a- peak numbers refer to compounds as listed in Table 2.

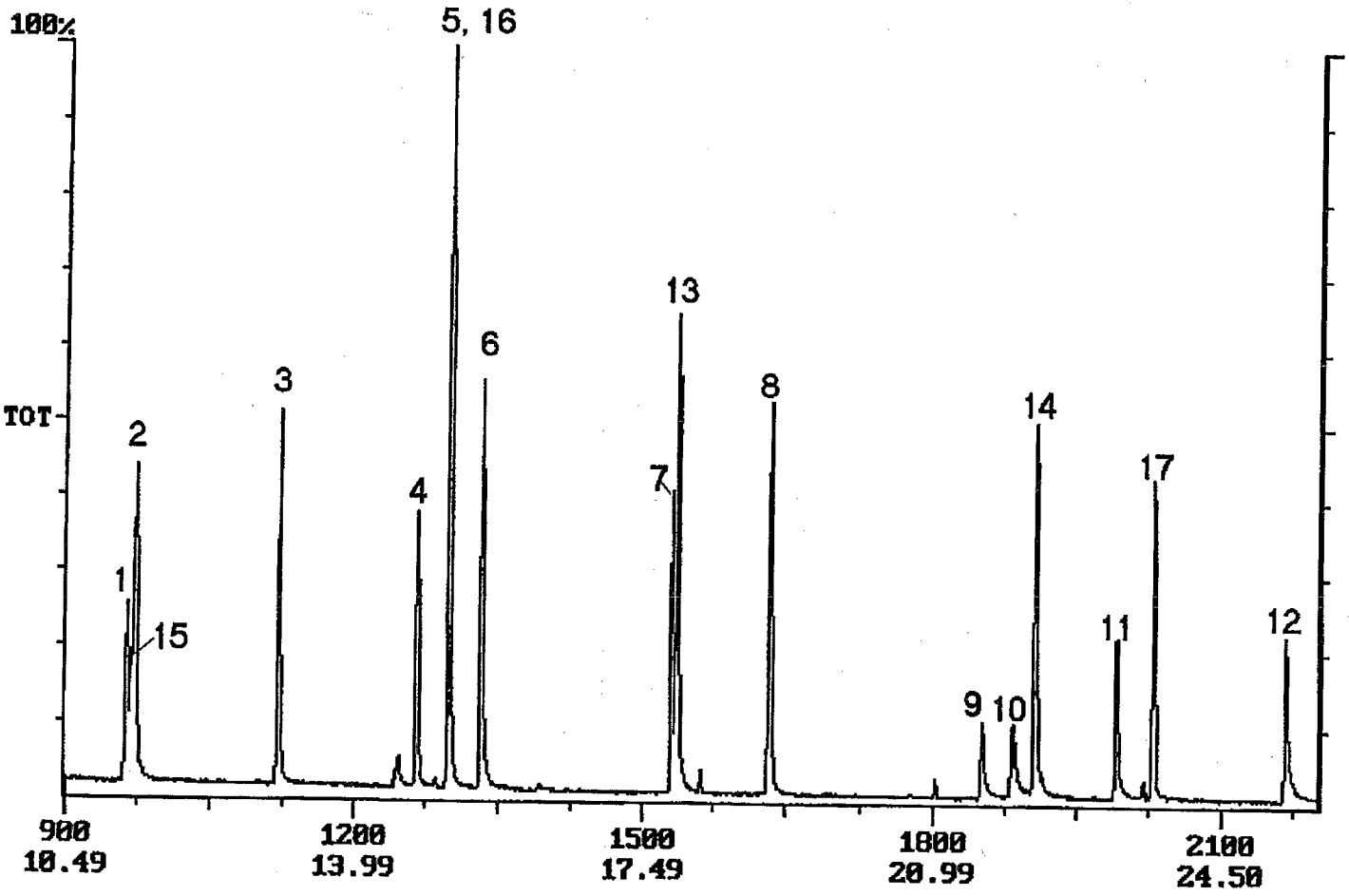
FIGURE 2.CHROMATOGRAM OF PHENOL STANDARD MIX (5 ng/ µL EACH ANALYTE) ON A BPX5 COLUMN WITH HOT SPLITLESS INJECTION. [a]



528-44

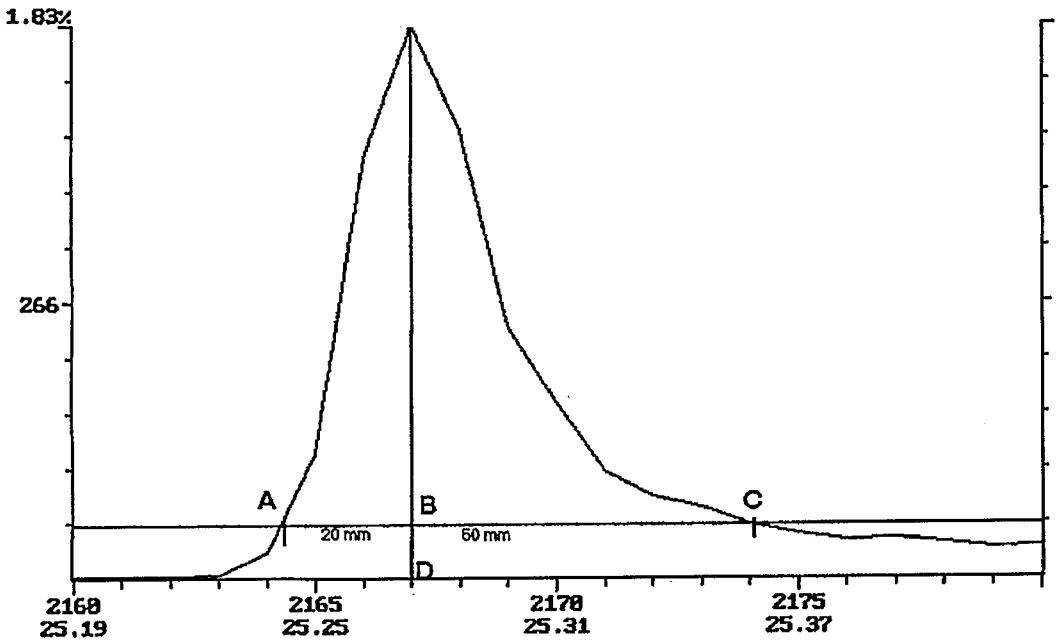
a- peak numbers refer to compounds as listed in Table 2..

FIGURE 3. CHROMATOGRAM OF PHENOL STANDARD MIX (5 ng/ µL EACH ANALYTE) ON A DB-5ms COLUMN WITH TEMPERATURE PROGRAMMED SPLITLESS INJECTION. [a]



a- peak numbers refer to compounds as listed in Table 2.

FIGURE 4. PEAK TAILING FACTOR (PTF) CALCULATION.



$$\text{Peak Tailing Factor} = \frac{BC}{AB}$$

BD = 10 % peak height

Note: the PTF should be calculated from the single ion chromatogram of the quantitation ion. This example is for the pentachlorophenol peak. The PTF = 2.5.

METHOD 532. DETERMINATION OF PHENYLUREA COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

Revision 1.0

June 2000

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METHOD 532

DETERMINATION OF PHENYLUREA COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method for the determination of phenylurea pesticides in drinking waters. This method is applicable to phenylurea compounds that are efficiently extracted from the water using a C_{18} solid phase cartridge or disk. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds in reagent water and finished ground and surface waters:

Analyte	Chemical Abstracts Service Registry Number
Diflubenzuron	35367-38-5
Diuron	330-54-1
Fluometuron	2164-17-2
Linuron	330-55-2
Propanil	709-98-8
Siduron	1982-49-6
Tebuthiuron	34014-18-1
Thidiazuron	51707-55-2

1.2 MDLs are compound, instrument, and matrix dependent. The MDL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined MDLs for the above listed analytes are provided in Section 17, Table 3. The MDL differs from, and is usually lower than (but never above), the minimum reporting limit (MRL) (Sect. 3.16). The concentration range for target analytes in this method was evaluated between 1.0 ug/L and 30 ug/L for a 500 mL sample. Precision and accuracy data and sample holding time data are presented in Section 17, Tables 4 - 9.

1.3 This method is restricted to use by or under the supervision of analysts skilled in solid phase extraction (SPE), and HPLC analysis.

2. SUMMARY OF METHOD

2.1 A 500 mL water sample is passed through a SPE cartridge or disk containing a chemically bonded C_{18} organic phase to extract the phenylurea pesticides and surrogate compounds. The analytes and surrogates are eluted from the solid phase with methanol, and the extract is concentrated to a final volume of 1 mL. Components are then chromatographically separated by injecting an aliquot of the extract into an HPLC system equipped with a C_{18} column and detected using a UV/Vis detector. Identification of target and surrogate analytes and quantitation is accomplished by comparison of retention times and analyte responses using external standard procedures. Sample extracts with positive results are solvent exchanged and confirmed using a second, dissimilar HPLC column that is also calibrated using external standard procedures.

3. DEFINITIONS

3.1 EXTRACTION BATCH – A set of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices and solvents, surrogate solution, and fortifying solutions. Required QC samples include: Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Matrix, and either a Field Duplicate or Laboratory Fortified Matrix Duplicate.

3.2 ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check standards (CCC). Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.3 SURROGATE ANALYTE (SUR) – A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

3.4 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, sample preservatives, and surrogates that are used in the extraction batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.5 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.7 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMD) – A second aliquot of the Field Sample used to prepare the LFM which is fortified, extracted, and analyzed identically. The LFMD is used instead of the Field Duplicate to access method precision and accuracy when the occurrence of target analytes is low.

3.8 LABORATORY DUPLICATES (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.9 FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage.

3.10 STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing method analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution or stock standard solution and the surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing one or more of the method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.14 QUALITY CONTROL SAMPLE (QCS) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check standard integrity.

3.15 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.4). Accurate quantitation is not expected at this level. [1]

3.16 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest continuing calibration standard for that analyte, and can only be used if acceptable quality control criteria for this standard are met.

3.17 MATERIAL SAFETY DATA SHEET (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.18 PEAK GAUSSIAN FACTOR (PGF) – The peak gaussian factor is calculated using the equation in Section 10.2.3.1. It provides a quantitative measure of peak asymmetry. A perfectly symmetric peak would have a PGF of 1. Poor peak symmetry can result in imprecise quantitation, degraded resolution and poor retention reproducibility. For this reason, columns and conditions that produce symmetric peaks are required.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 hours. Volumetric glassware should not be heated above 120 °C.

4.2 Method interferences may also be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for each analyte) under the conditions of the

analysis by analyzing laboratory reagent blanks as described in Section 9.3. **Subtracting blank values from sample results is not permitted.**

4.2.1 An extraneous peak was noted that elutes very near fluometuron on the confirmation column that can cause problems with quantitation if the chromatography is not fully optimized. No interferences were observed for the primary column analysis.

4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. Water samples high in total organic carbon may have an elevated baseline and/or interfering peaks.

4.4 Solid phase cartridges and disks and their associated extraction devices have been observed to be a source of interferences in other EPA organic methods. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of solid phase extraction devices should be monitored to ensure that contamination will not preclude analyte identification or quantitation.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining an awareness of OSHA regulations regarding the safe handling of the chemicals used in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[2-4]

5.2 Pure standard materials and stock standards of these compounds should be handled with suitable protection to skin and eyes, and care should be taken not to breath the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS – 500 mL amber or clear glass bottles fitted with PTFE (polytetrafluoroethylene) lined screw caps.

6.2 VIALS – Screw cap or crimp top glass autosampler vials with PTFE faced septa, amber or clear.

6.3 VOLUMETRIC FLASKS – Class A, suggested sizes include 1,5,and 10 mL.

6.4 GRADUATED CYLINDERS –Suggested sizes include 5,10, and 500 mL.

6.5 MICRO SYRINGES – Various sizes.

6.6 ANALYTICAL BALANCE – Capable of accurately weighing to the nearest 0.0001 g.

6.7 DISPOSABLE SYRINGES – 1 mL (B-D cat.#: 309602 or equivalent) size, used to filter sample extracts before analysis.

6.8 FILTERS – Disposable filters to filter sample extracts before analysis (Gelman 0.45 um Nylon Acrodisk cat.#: 4426 or equivalent).

6.9 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

6.9.1 EXTRACTION CARTRIDGES – 6 mL, packed with 500 mg (40 um d_p) silica bonded with C_{18} (Varian cat.#: 1210-2052 or equivalent).

6.9.2 SAMPLE RESERVOIRS – (VWR cat.#: JT7120-3 or equivalent) These are attached to the cartridges and water samples are poured into them, although they hold only 75 mL at one time. An alternative is a transfer tube system (Supelco "Visiprep"; cat. #: 57275 or equivalent) which transfers the sample directly from the sample container to the SPE cartridge.

6.9.3 VACUUM EXTRACTION MANIFOLD – With flow/vacuum control (Supelco cat.#: 57044 or equivalent). The use of replaceable needles or valve liners may be used to prevent cross contamination.

6.9.4 REMOTE VACUUM GAUGE/BLEED ASSEMBLY – To monitor and adjust vacuum pressure delivered to the vacuum manifold (Supelco cat.#: 57161-U or equivalent).

6.9.5 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for elution of the sample from the cartridge after extraction.

6.9.6 An automatic or robotic system designed for use with SPE cartridges may be used if all quality control requirements discussed in Section 9 are met. Automated systems may use either vacuum or positive pressure to process samples and solvents through the cartridge. All extraction and elution steps must be the same as the manual procedure. Extraction or elution steps may not be changed or omitted to accommodate the use of the automated system.

6.10 SOLID PHASE EXTRACTION (SPE) APPARATUS USING DISKS

6.10.1 EXTRACTION DISKS – 47 mm diameter, manufactured with a C_{18} bonded sorbent phase (Varian cat.#: 1214-5004 or equivalent). Larger disks may be used as long as the QC performance criteria outlined in Section 9 are met.

6.10.2 SPE DISK EXTRACTION GLASSWARE – Funnel, PTFE coated support screen, PTFE gasket, base, and clamp used to support SPE disks and contain samples during extraction. May be purchased as a set (Fisher cat# K971100-0047 or equivalent) or separately.

6.10.2 VACUUM EXTRACTION MANIFOLD – Designed to accommodate extraction glassware (Varian cat. #: 1214-6001 or equivalent).

6.10.3 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for collection of the eluent that drips from the disk extraction base.

6.10.4 An automated or robotic system may be used as specified in Section 6.9.6.

6.11 EXTRACT CONCENTRATION SYSTEM – To concentrate extracts in 15 mL conical tubes, the bottoms of which are submersed in a 40°C water bath, under a steady steam of nitrogen to the desired volume (Meyer N-Evap, Model III, Organomation Associates, Inc. or equivalent).

6.12 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a minimum vacuum of approximately 25 cm (10 in.) of mercury for cartridges. A greater vacuum of approximately 66 cm (26 in.) of mercury may be used with disks.

6.13 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) INSTRUMENTATION

6.13.1 HPLC SYSTEM – Capable of reproducibly injecting 20 or 10 uL aliquots, and performing binary linear gradients at a constant flow rate near 1.5 mL/min.

6.13.2 HPLC DETECTOR – A UV detector capable of collecting data at 240-245 nm. For the development of this method, a photodiode array detector was used. A LC/MS system may also be used.

6.13.3 PRIMARY COLUMN – An HPLC column (4.6 × 150 mm) packed with 3.5 um d_p C_{18} solid phase particles (Waters cat. # WAT200632). Any

column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

6.13.4 CONFIRMATION COLUMN – An HPLC column (4.6 × 150mm) packed with 5 d_p cyanopropyl stationary phase (Supelco "Supelcosil LC-CN" cat. # 58221-U). The secondary column must be chemically dissimilar to the primary column and must yield a different elution order for some compounds which will result in dissimilar retention times compared to the primary column.

6.13.5 HPLC DATA SYSTEM - A computerized data system is recommended for data acquisition and manipulation. The Waters Millennium software system was used to generate all primary column data contained in the Section 17 tables.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 REAGENT WATER – Purified water which does not contain any measurable quantities of any target analytes or interfering compounds at or above 1/3 the MRL for each compound of interest.

7.1.2 ACETONITRILE – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.3 METHANOL – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.4 ACETONE – High purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

7.1.5 PHOSPHATE BUFFER SOLUTION, 25 mM – Used for HPLC mobile phase. Add 100 mL 0.5 M potassium phosphate stock solution (Sect. 7.1.5.1) and 100 mL of 0.5 M phosphoric acid stock solution (Sect. 7.1.5.2) to reagent water and dilute to a final volume of 4 L . The pH should be about 2.4 and should be confirmed with a pH meter. Filter using a 0.45 um nylon filter.

7.1.5.1 POTASSIUM PHOSPHATE STOCK SOLUTION (0.5 M) – Weigh 68 g KH_2PO_4 (Monobasic Potassium Phosphate) and dilute to 1 L using reagent water.

7.1.5.2 PHOSPHORIC ACID STOCK SOLUTION (0.5M) – 34.0 mL of phosphoric acid (85%, HPLC grade in reagent water) diluted to 1 L with reagent water.

7.1.6 SAMPLE PRESERVATION REAGENTS

7.1.6.1 CUPRIC SULFATE, $CuSO_4 \cdot 5H_2O$ (ACS Grade or equivalent) – Added as a biocide to guard against potential degradation of method analytes by microorganisms (Sect. 8.1.2).

7.1.6.2 TRIZMA PRESET CRYSTALS, pH 7.0 (Sigma cat# T 3503 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]. Alternatively, a mix of the two components with a weight ratio of 15.5/1; Tris HCL/Tris may be used. These blends are targeted to produce a pH of 7.0 at 25°C in reagent water. Tris functions as a buffer, binds free chlorine in chlorinated finished waters, and prevents the formation of a copper-based precipitate.

7.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should used standard QC practices to determine when their standards need to be replaced.**

7.2.1 SURROGATE ANALYTES (SUR), MONURON (CAS #150-68-5) and CARBAZOLE (CAS# 86-74-8) – Monuron and carbazole were chosen as surrogates. Monuron is a phenylurea no longer in use in the U.S. that elutes early in the chromatogram. Carbazole elutes late in the chromatogram (Figure 1) and has been used as a surrogate in other EPA drinking water methods. Alternate surrogates may be selected if there is a problem with matrix interferences or chromatography. However, if an alternate surrogate is used, it must have similar chemical properties (structure, solubility, C_{18} retention, etc.) to the phenylureas, be chromatographically resolved from all target analytes and matrix interferences, and be highly unlikely to be found in any sample.

7.2.1.1 SUR STOCK SOLUTION (5 to 7 mg/mL) – Accurately weigh approximately 25 to 35 mg of the neat SUR to the nearest 0.1 mg into a tared, 5 mL volumetric flask. Dilute to the mark with the appropriate solvent: methanol should be used for monuron and acetonitrile for carbazole. Prepare each compound individually, as they will be combined in the SUR primary dilution standard. Stock solutions have been shown to be stable for 6 months when stored at -10°C or less. Laboratories should use standard QC practices to determine when their standards need to be replaced.

7.2.1.2 SUR PRIMARY DILUTION STANDARD (500 ug/mL) – Prepare the SUR Primary Dilution Standard (PDS) by dilution of the SUR stock standards. Add enough of each of the SUR stock standards to a volumetric flask partially filled with methanol to make a 500 ug/mL solution when filled to the mark with methanol. The PDS has been shown to be stable for 3 months when stored at -10°C or less.

7.2.2 ANALYTE STOCK STANDARD SOLUTION – Prepare analyte stock standard solutions for all compounds in methanol except thidiazuron and diflubenzuron. Thidiazuron and diflubenzuron should be prepared in acetone due to their limited solubility in methanol. Acetone elutes early in the chromatogram and should not interfere with compound quantitation as long as its volume is minimized as specified in this method. Method analytes may be obtained as neat materials or as ampulized solutions. Stock solutions have been shown to be stable for 6 months when stored at -10°C or less.

7.2.2.1 For analytes in their pure form that are soluble in methanol, prepare stock solutions by accurately weighing 25 to 35 mg of pure material to the nearest 0.1 mg in a 5 mL volumetric flask. Dilute to volume with methanol.

7.2.2.2 Thidiazuron and diflubenzuron should be dissolved in acetone. Accurately weigh neat material to the nearest 0.1 mg into volumetric flasks, but using smaller amounts than those used for other target analytes, approximately 10 to 12 mg. Thidiazuron is especially difficult to dissolve, 10 mg of pure material should dissolve in a 10 mL final volume of acetone. Sonnication may be used to help dissolve these compounds.

7.2.3 ANALYTE PRIMARY DILUTION STANDARD (PDS, 200 ug/mL and 10 ug/mL) – Prepare the Analyte PDS by dilution of the stock standards

(Sect. 7.2.2). Add enough of each stock standard to a volumetric flask partially filled with methanol to make a 200 ug/mL solution when filled to the mark with methanol. Once prepared, a dilution of the 200 ug/mL solution may be used to prepare a 10 ug/mL solution used for low concentration spiking. The PDSs can be used to prepare calibration and fortification solutions. Analyte PDSs have been shown to be stable for 3 months when stored at -10°C or less.

7.2.4 CALIBRATION SOLUTIONS – At least 5 calibration concentrations will be required to prepare the initial calibration curve (Sect. 10.2). Prepare at least 5 Calibration Solutions over the concentration range of interest, approximately 0.5-15 ug/mL, from dilutions of the analyte PDS in methanol. The lowest concentration of calibration standard must be at or below the MRL, which will depend on system sensitivity. In this method, 500 mL of an aqueous sample is concentrated to a 1 mL final extract volume. The calibration standards for the development of this method were prepared as specified below.

PREPARATION OF CALIBRATION (CAL) CURVE STANDARDS					
CAL Level	PDS Conc. (ug/mL)	Volume PDS Standard (uL)	Final Volume of CAL Standard (mL)	Final Conc. of CAL Standard (ug/mL)	Equivalent Conc. in 500 mL sample (ug/L)
1	10	25	1	0.25	0.50
2	10	50	1	0.50	1.00
3	200	5.0	1	1.00	2.00
4	200	25	1	5.00	10.0
5	200	50	1	10.0	20.0
6	200	75	1	15.0	30.0

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [5] using 500 mL amber or clear glass bottles fitted with PTFE lined screw caps.

8.1.2 Prior to shipment to the field, 0.25 g of cupric sulfate and 2.5 g of Trizma crystals (Sect. 7.1.6) must be added to each bottle for each 500 mL of sample collected. Alternately, the Tris buffer may be prepared by adding

2.35 g of Tris HCl and 0.15 g Tris to the sample bottle in addition to the 0.25 g of cupric sulfate. Cupric sulfate acts as a biocide to inhibit bacteriological decay of method analytes. Trizma functions as a buffering reagent, binds the free chlorine, and helps to prevent the formation of a precipitate. Add these materials as dry solids to the sample bottle. The stability of these materials in concentrated aqueous solution has not been verified.

8.2 SAMPLE COLLECTION

8.2.1 When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample from a representative area, and carefully fill sample bottles from the container. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2.3 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.4 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 minute. Keep samples sealed from collection time until extraction.

8.3 SAMPLE SHIPMENT AND STORAGE – All samples should be iced during shipment and must not exceed 10° C during the first 48 hours after collection. Samples should be confirmed to be at or below 10 °C when they are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but should not be frozen.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study of all method analytes indicated that all compounds are stable for 14 days in water samples that are collected, dechlorinated, preserved, shipped and stored as described in Sections 8.2 and 8.3. Samples must be extracted within 14 days. Sample extracts may be stored in methanol at 0°C or less for up to 21 days after extraction. Samples that are exchanged into reagent water/acetonitrile (60/40) for confirmational analysis may be stored 7 days at 0° or less; however, the combined extract holding time may not exceed 21 days.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability, the determination of the MDL, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards (CCC), a Laboratory Fortified Blank (LFB), a Laboratory Fortified Sample Matrix (LFM), and either a Laboratory Fortified Sample Matrix Duplicate (LFMD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 10 and 11. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections and summarized in Section 17, Table 10.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solid phase extraction (SPE) cartridges or disks is used, it must be demonstrated that a laboratory reagent blank is reasonably free of contamination and that the criteria in Section 9.4 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare, extract, and analyze 4-7 replicate LFBs fortified at 5 to 10 ug/L, or near the mid-range of the initial calibration curve, according to the procedure described in Section 11. Sample preservatives as described in Section 8.1.2 must also be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY – Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.4 MDL DETERMINATION – Prepare, extract and analyze at least 7 replicate LFBs at a concentration estimated to be near the MDL over at least 3 days (both extraction and analysis should be conducted over at least 3 days) using the procedure described in Section 11.. This fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level. The appropriate concentration will be dependent upon the sensitivity of the HPLC system being used. Sample preservatives as described in Section 8.1.2 must be added to these samples. Calculate the MDL using the equation

$$MDL = St_{(n-1,\ 1-alpha\ =\ 0.99)}$$

where

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations. This is a statistical determination based on precision only. [1] If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet method precision and accuracy criteria.

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify HPLC columns, HPLC detector, HPLC conditions, evaporation techniques, and surrogate standards, but each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.3 Minimum Reporting Level (MRL) – The MRL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL may be established at an analyte concentration either greater than three times the MDL or at a concentration which would yield a response greater than a signal to noise ratio of five. **Although the lowest calibration standard for an analyte may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard.**

9.4 LABORATORY REAGENT BLANK (LRB) – A LRB is required with each extraction batch (Sect. 3.1) of samples to determine any background system contamination. If within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes must be below $^1/_3$ the MRL. If the target analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.5 CONTINUING CALIBRATION CHECK (CCC) – A CCC is a standard prepared with all compounds of interest which is analyzed during an analysis batch to ensure the stability of the instrument initial calibration. See Section 10.3 for concentration requirements, frequency requirements, and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANKS – A LFB is required with each extraction batch. The fortified concentration of the LFB should be rotated between, low, medium and high concentrations from day to day. The low concentration LFB must be as near as practical to, but no more than two times the MRL. Similarly, the high concentration should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the LFB for the low level fortification must be 50-150% of the true value. The concentration determined for the medium and high LFBs must be 70-130% of the true value. If LFB results do not meet these criteria for target analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.7 SURROGATE RECOVERY – The surrogate standards are fortified into the aqueous portion of all samples, LRBs, and LFMs and LFMDs prior to extraction. They are also added to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.7.1 When surrogate recovery from a sample, blank, or CCC is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.7.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.7.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.7.1, recalibration is in order per Section 10.2. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect/surrogate recovery.

9.8 LABORATORY FORTIFIED SAMPLE MATRIX AND DUPLICATE (LFM AND LFMD) – Analysis of LFMs are required in each extraction batch and are used to determine that the sample matrix does not adversely affect method accuracy. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFM, or LMFD, must be prepared, extracted, and analyzed from a duplicate of the field sample used to prepare the LFM to assess method precision. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate (Sect. 9.8). If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources,

method performance should be established for each. Over time, LFM data should be documented for all routine sample sources for the laboratory.

9.8.1 Within each extraction batch, a minimum of one field sample is fortified as a LFM for every 20 samples extracted. The LFM is prepared by spiking a sample with an appropriate amount of the appropriate Analyte PDS (Sect. 7.2.3). Select a spiking concentration at least twice the matrix background concentration, if known. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate spiking levels.

9.8.2 Calculate the percent recovery (R) for each analyte using the equation

$$R = \frac{(A - B)}{C} * 100$$

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample, and
C = fortification concentration.

9.8.3 Analyte recoveries may exhibit a matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%, except for thidiazuron which should be recovered at 60 - 120%. For LFM fortification at the MRL, 50 to 150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.8.4 If a LFMD is analyzed instead of a Field Duplicate, calculate the relative percent difference (RPD) for duplicate LFMs (LFM and LFMD) using the equation

$$RPD = \frac{LFM - LFMD}{(LFM + LFMD)/2} * (100)$$

RPDs for duplicate LFMs should fall in the range of $\pm$ 30% for samples fortified at or above their native concentration. Greater variability may be observed when LFMs are spiked near the MRL. At the MRL, RPDs

should fall in the range of ± 50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.9 FIELD DUPLICATES (FD1 AND FD2) – Within each extraction batch, a minimum of one field duplicate (FD) or LFMD (Sect. 9.8) must be analyzed. FDs serve as a check the precision associated with sample collection, preservation, and storage, as well as laboratory procedures. If target analytes are not routinely observed in field samples, a LFMD should be analyzed to substitute for this requirement. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate.

9.9.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2)using the equation

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} * (100)$$

RPDs for duplicates should fall in the range of ± 30%. Greater variability may be observed when analyte concentrations are near the MRL. At the MRL, RPDs should fall in the range of ± 50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.10 QUALITY CONTROL SAMPLES (QCS) – Each time that new standards are prepared or a new calibration curve is run, analyze a QCS from a source different from the source of the calibration standards. The QCS may be injected as a calibration standard, or fortified into reagent water and analyzed as a LFB. If the QCS is analyzed as a continuing calibration check, then the acceptance criteria are the same as for the CCC. If the QCS is analyzed as a LFB, then the acceptance criteria are the same as for an LFB. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10. CALIBRATION AND STANDARDIZATION

10.1 After initial calibration is successful, a Continuing Calibration Check is required at the beginning and end of each analysis batch, and after every tenth sample (Sect. 10.3). Initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2 INITIAL CALIBRATION

10.2.1 Establish HPLC operating parameters equivalent to the suggested conditions in Section 17, Table 1. The system is calibrated using the external standard technique. For this method, a PDA detector was used and the analyte absorbance at 240 or 245 nm was used in order to maximize target compound signal relative to the background interferences. Other HPLC conditions may be used as long as all QC requirements in Section 9 are met.

10.2.2 Prepare a set of at least 5 calibration standards as described in Section 7.2.4. The lowest concentration of calibration standard must be at or below the MRL, which will depend on system sensitivity.

10.2.3 INJECTION VOLUME – Optimum injection volume for the primary column may vary between HPLC instruments when a sample is dissolved in an organic solvent such as methanol. Prior to establishing the initial calibration, the injection volume must first be determined. Peak asymmetry on the primary column was occasionally noted on one of the two instruments used to develop this method. This asymmetry only occurred with 20 uL injections. This phenomena is attributed to the difference in elutropic strength between the initial mobile phase composition (phosphate buffer/acetonitrile; 60/40) and the extract solvent composition (100% methanol). In all cases, the asymmetry was eliminated by reducing the injection volume to 10 uL. Prior to establishing the initial calibration curve, acceptable chromatographic performance is determined by calculating the Peak Gaussian Factor (PGF).

10.2.3.1 Section 17 (Table 3) lists MDLs on the primary column using two injection sizes: 20 uL and 10 uL. Inject a 20 uL aliquot of the medium level calibration standard on the primary column using the suggested conditions listed in Section 17, Table 1. Determine the PGF for the analyte fluometuron using the equation

$$PGF = \frac{1.83 \times W_{0.5}}{W_{0.1}}$$

where,
$W_{0.5}$ is the peak width at half height, and
$W_{0.1}$ is the peak width at tenth height.

If fluometuron has not been included in the calibration standards, one of the surrogate compounds may be substituted.

NOTE: Values for $W_{0.5}$ and $W_{0.1}$ can be attained via most data acquisition software packages. If these values are manually measured, the analyst should limit the retention time window to enlarge the peak of interest allowing accurate determination of the PGF. Inaccurate measurements may result when using a chromatogram of the entire analysis run.

10.2.3.2 If the PGF is in the range of 0.90 to 1.10, the peak shape is considered acceptable, and a 20 uL injection may be used. If not, injection volume should be reduced, and the PGF redetermined as described in Section 10.2.3.1.

10.2.4 Generate a calibration curve for each analyte by plotting the peak response (area is recommended) against analyte concentration. Both instruments used during method development yielded linear curves for the target analytes over the concentration range of interest. However, data may be fit with either a linear regression (response vs concentration) or quadratic fit (response vs concentration). Alternately, if the ratio of the analyte peak area to concentration (or response factor) is relatively constant (RSD < 30%) an average response factor may be used to calculate analyte concentration. Siduron separates into two isomers (labeled A & B in Sect. 17, Figure 1). The responses of the two isomers should be added together before plotting against the concentration.

10.2.5 Repeat steps 10.2.1 through 10.2.4 for the confirmation column. Laboratories may choose to wait to establish an initial calibration curve for the confirmation column until they have samples with positive results that require confirmation; however, this step must be successfully completed prior to confirming sample results.

10.3 CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. (In this context, a "sample" is considered to be a Field sample. LRBs, LFBs, LFMs, LFMDs and CCCs are not counted as samples.) The beginning CCC each day should be at or below the MRL in order to verify instrument sensitivity prior to any analyses.

Subsequent CCCs should alternate between a medium and high concentration standard.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ±50% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken which may require recalibration. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored.

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper preparation of laboratory glassware and sample containers (Sect. 4.1), and sample collection and storage (Sect. 8). This section details the procedures for sample preparation, solid phase extraction (SPE) using cartridges or disks, and extract analysis.

11.2 SAMPLE PREPARATION

11.2.1 Samples are preserved, collected and stored as presented in Section 8. All field and QC samples must contain the preservatives listed in Section 8.1.2, including the LRB and LFB. Determine sample volume. The sample volume may be measured directly in a graduated cylinder to the nearest 10 mL. To minimize the need to use a different graduated cylinder for each sample, an indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 10 g. After extraction, proceed to Section 11.3.5 for final volume determination. The LRB and LFB may be prepared by measuring 500 mL of reagent water into an erlenmeyer flask.

11.2.2 Add an aliquot of the SUR PDS (Sect. 7.2.1.2) to all samples and mix by swirling the sample. Addition of 10 uL of a 500 ug/mL SUR PDS to a 500 mL sample will result in a concentration of 10 ug/L.

11.2.3 If the sample is a LFB or LFM, add the necessary amount of analyte PDS. Swirl each sample to ensure all components are properly mixed.

11.2.4 Proceed with sample extraction. Refer to Section 11.3 if SPE cartridges are being used. Refer to Section 11.4 if SPE disks are being used.

11.3 CARTRIDGE SPE PROCEDURE – Proper conditioning of the solid phase can have a marked effect on method precision and accuracy. This section describes the SPE procedure using the equipment outlined in Section 6.9 in its simplest, least expensive mode without the use of the alternate transfer system or robotics systems (Sect. 6.9.2 and 6.9.6) This configuration was used to collect data presented in Section 17.

11.3.1 CARTRIDGE CONDITIONING – Once the conditioning of the cartridge is started, the cartridge must not be allowed to go dry until the last portion of the sample is filtered through it. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. However, if the cartridge goes dry during sample extraction, the analyte and surrogate recoveries may be affected. If this happens the analyst should make note of this as this sample may require re-extraction due to low surrogate recoveries.

11.3.1.1 CONDITIONING WITH METHANOL – Assemble the extraction cartridges into the vacuum manifold. Rinse each cartridge with two, 5 mL aliquots of methanol, allowing the sorbent to soak in the methanol for about 30 seconds by turning off the vacuum temporarily during the first rinsing. Do not allow the methanol level to go below the top of the cartridge packing.

11.3.1.2 CONDITIONING WITH REAGENT WATER – Follow the methanol rinse with two, 5 mL aliquots of reagent water being careful not to allow the water level to go below the cartridge packing. Turn off the vacuum. Add approximately 5 mL additional reagent water to the cartridge, and attach a reservoir (or transfer tube - Sect. 6.9.2) before adding sample to the cartridge.

11.3.2 CARTRIDGE EXTRACTION – Prepare samples, including QC samples, as specified in Section 11.2. The samples may be added to the cartridge using either a large reservoir attached to the cartridge or using a transfer tube from the sample bottle to the cartridge.

11.3.2.1 SAMPLE ADDITION USING RESERVOIRS – Attach a reservoir to the conditioned cartridge. Fill the reservoir (Sect. 6.9.2) with sample and then turn on the vacuum. Adjust the vacuum so that the approximate flow rate is about 20 mL/min (minus 9-10 in Hg.). Care must be taken to add additional aliquots of sample to the reservoir to keep the cartridge packing from going dry before all the sample has been extracted. Rinse the sample container with reagent water and add to the reservoir after

the last addition of sample, but before the cartridge goes dry. After all of the sample has passed through the SPE cartridge, detach the reservoir and draw air through the cartridge for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.3.2.3 SAMPLE ADDITION USING TRANSFER TUBES – If the sample transfer tubes are employed, make sure the transfer tube is attached to the conditioned cartridge before turning on the vacuum. Adjust the vacuum to a similar flow rate of approximately 20 mL/min (minus 9-10 in Hg). Rinse down the sample container with reagent water as it approaches dryness. After all of the sample has passed through the SPE cartridge, detach the transfer tube and draw air through the cartridge for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.3.3 CARTRIDGE ELUTION – Lift the extraction manifold top and insert collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridge. Add approximately 3 mL of methanol to the top of each cartridge. Pull enough of the methanol into the cartridge at low vacuum to soak the sorbent. Turn off the vacuum and vent the system. Allow the sorbent to soak in methanol for approximately 30 seconds. Start a low vacuum (minus 2-4 in Hg) and pull the methanol through in a dropwise fashion into the collection tube. Repeat this elution a second time with approximately 2 mL of methanol, and then a third time with approximately 1 mL.

11.3.4 EXTRACT CONCENTRATION – Concentrate the extract to about 0.5 mL in a warm water bath (at about 40^{0C}) under a gentle steam of nitrogen. Transfer to a 1 mL volumetric flask, rinsing the collection tube with small amounts of methanol. Adjust to volume with methanol. Filter the sample using a 1 mL syringe and filter (Sects. 6.7 & 6.8) into an appropriate autosampler vial.

11.3.5 SAMPLE VOLUME DETERMINATION – If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 10 mL. If using weight to determine volume, weigh the empty bottle to the nearest 10 g and determine the sample weight by subtraction of the empty bottle from the original weight (Sect. 11.2.1). In either case, the sample volume will be used in the final calculations of analyte concentration (Sect. 12.4).

11.4 DISK SPE PROCEDURE – Proper conditioning of the solid phase can have a marked affect on method precision and accuracy. This section describes the SPE procedure using the equipment outlined in Section 6.10 in its simplest, least expensive mode without the use of robotics systems (Sect. 6.10.4). This configuration was used to collect data presented in Section 17.

11.4.1 DISK CONDITIONING – Once the conditioning of the disk is started, the disk must not be allowed to go dry until the last portion of the sample is filtered through it. If the disk goes dry during the conditioning phase, the conditioning must be started over. However, if the disk goes dry during sample extraction, the analyte and surrogate recoveries may be affected. If this happens the analyst should make note of this as this sample may require re-extraction due to low surrogate recoveries.

11.4.1.1 CONDITIONING WITH METHANOL – Assemble the extraction glassware in the vacuum manifold, placing the disks on a support screen between the funnel and base. Rinse each disk with two, 10 mL aliquots of methanol to the funnel, allowing the sorbent to soak for about 30 seconds by pulling approximately 1mL through the disk and turning off the vacuum temporarily during the first rinsing. Draw the methanol through the disk until it is 3-5 mm above the disk surface, adding more methanol if needed to keep the methanol from going below this level.

11.4.1.2 CONDITIONING WITH WATER – Follow the methanol rinse with two, 10 mL aliquots of reagent water being careful to keep the water level at 3-5 mm above the disk surface. Turn off the vacuum.

11.4.2 DISK EXTRACTION – Prepare samples, including QC samples, as specified in Section 11.2. Fill the extraction funnel containing the conditioned disk with sample and turn on the vacuum. Care must be taken to add additional aliquots of sample to the funnel to keep the disk from going dry before all the sample has been extracted. Rinse the sample container with reagent water and add to the funnel after the last addition of sample, but before the disk goes dry. After all of the sample has passed through the SPE disk, draw air through the disk for 15 minutes at full vacuum (minus 10-15 in Hg). Turn off and release the vacuum.

11.4.3 DISK ELUTION – Detach the glassware base from the manifold without disassembling the funnel from the base. Dry the underside of the base. Insert collection tubes into the manifold to collect the extracts as they are eluted from the disk. The collection tubes must fit around the drip tip of the base to ensure the collection of all of the eluent. Reattach the base to

the manifold. Add approximately 5 mL of methanol to the top of each disk. Pull enough of the methanol into the disk to soak the sorbent. Turn off the vacuum and vent the system. Allow the sorbent to soak in methanol for approximately 30 seconds. Start the vacuum and pull the methanol through. Attempt to pull the methanol through in a dropwise fashion into the collection tube by slowly turning the valve (which controls the flow through the funnel) until the methanol starts eluting into the collection tube. Repeat this elution a second time with approximately 4 mL of methanol, and then a third time with approximately 3 mL.

11.4.4 EXTRACT CONCENTRATION – Proceed with extract concentration as in Section 11.3.4.

11.4.5 SAMPLE VOLUME DETERMINATION – Proceed with sample volume determination as in Section 11.3.5.

11.5 SOLVENT EXCHANGE FOR CONFIRMATION ANALYSIS – Samples that will be confirmed must be exchanged into reagent water/acetonitrile (60/40). To accomplish this, transfer the remaining 980 uL of the extract to a 1 mL volumetric (or other appropriate collection tube). Mark the sample volume. Take the extract to dryness in a warm water bath (at ~ 40°C) under a gentle steam of nitrogen. Reconstitute the residue with a mixture of reagent water/acetonitrile (60/40) to the mark made before the extract was taken to dryness. Care must be taken to redissolve the film as thoroughly as possible. Use of a vortex mixer is recommended. Transfer to an appropriate autosampler vial.

Note: Recovery experiments have been conducted to investigate the effect of drying time on compound recoveries by allowing the extract to sit an additional 1, 2, or 4 hours in the bath after being taken to dryness. These studies indicate that: 1) the best recoveries are obtained when extracts are reconstituted immediately after the methanol is removed; 2) recoveries decrease with increasing time in the bath; and 3) carbazole recovery is the most sensitive to the additional time. In our experiments, carbazole had acceptable recovery criteria (Sect. 12.3) at 2 additional hours of drying, but had exceeded the acceptable range after 4 hours of additional time.

11.6 ANALYSIS OF SAMPLE EXTRACTS

11.6.1 Establish operating conditions as summarized in Table 1 of Section 17 for the HPLC system. Confirm that retention times, compound separation and resolution on the primary and secondary columns are similar to those listed in Tables 1 and 2 and Figures 1 and 2, respectively.

11.6.2 Determine the optimal injection volume. Establish a valid initial calibration following the procedures outlined in Section 10.2 using the

optimum injection size. Complete the IDC requirements described in Section 9.2.

11.6.3 Establish an appropriate retention time window for each target and surrogate to identify them in the QC and field samples. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the HPLC over the course of time. Plus or minus three times the standard deviation of the retention time for each compound while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

11.6.4 Check system calibration by analyzing a CCC (Sect. 10.3) and begin to inject aliquots of field and QC samples using the same injection volume and conditions used to analyze the initial calibration.

11.6.5 The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with methanol. Acceptable surrogate performance (Sect. 9.7) should be determined from the undiluted sample extract. If confirmation analyses require dilution, this must be done using reagent water/acetonitrile (Sect. 11.5) Incorporate the dilution factor into final concentration calculations. Any dilutions will also affect analyte MRL.

12. DATA ANALYSIS AND CALCULATION

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to retention time of an analyte peak in a calibration standard or the laboratory fortified blank. Surrogate retention times should be confirmed to be within acceptance limits (Sect. 11.6.3) even if no target compounds are detected.

12.2 Calculate the analyte concentrations using the initial calibration curve generated as described in Section 10.2. Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (Sect. 11.6.5).

12.3 Positive results should be confirmed on the confirmation column (Sect. 17, Table 2) that has been initially calibrated, and confirmed to still be in calibration by analyzing appropriate CCCs (Sect. 10.3) prior to the confirmation analysis. Quantitated values for the targets and surrogates on the confirmation column should be 50 - 150% of the primary column result. If so, report the more accurate primary column result. If not, report the lower of the 2 values and mark the results as

suspect/confirmation to inform the data user that the results are suspect due to lack of confirmation. If values are taken from the confirmation column, both surrogates must meet recovery acceptance criteria.
Note: The PDA spectra of these compounds do not have sufficient resolution to definitively identify each target compound in this method.

12.4 Adjust the calculated concentrations of the detected analytes to reflect the initial sample volume and any dilutions performed.

12.5 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

12.6 Analyte concentrations are reported in ug/L. Calculations should use all available digits of precision, but final concentrations should be rounded to an appropriate number of significant figures.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND MDLs – Method detection limits (MDLs) are presented in Table 3 and were calculated using the formula present in Section 9.2.4. Tables for these data are presented in Section 17. Single laboratory precision and accuracy data are presented for three water matrices: reagent water (Table 5); chlorinated, "finished" surface water (Table 6); and chlorinated, "finished" ground water (Table 7).

13.2 COMPOUND STABILITY – Thidiazuron and diflubenzuron are extremely sensitive to free chlorine. LFM recoveries were found to drop to 0% for thidiazuron and about 5% for diflubenzuron in the time required to extract samples in the presence of free chlorine. Other analytes are sensitive to free chlorine over time, even within the recommended sample holding time of 14 days. For example, LFM recoveries for diuron drop to about 12% on day 14 in a finished surface water with a residual free chlorine concentration of 0.8 mg/L. This illustrates the importance of proper sample preservation (Sect. 8). During method development, it was experimentally determined that the 2.5 g Trizma preset crystals (Sect. 7.1.6.2) was sufficient to dechlorinate a 500 mL sample with up to approximately 10 mg/L free chlorine as measured colorimetrically. This level is about 2.5 times the maximum allowable free chlorine residual in municipal tap waters promulgated in Stage I of the Disinfectant/Disinfection By-product Rule[6] and so should be sufficient to reduce free chlorine in samples from public water systems.

13.3 ANALYTE STABILITY STUDIES

13.3.1 FIELD SAMPLES – Chlorinated finished surface water samples, fortified with method analytes at 10 ug/L, were preserved and stored as required in

Section 8. The average of triplicate analyses, conducted on days 0, 2, 7, and 14, are presented in Section 17, Table 8. These data document the 14-day sample holding time.

13.3.2 EXTRACTS – Extracts from the day 0 extract holding time study described above were stored below 0 °C and analyzed on days 0, 8, 14, and 21. The data presented in Section 17, Table 9, document the 21-day extract holding time.

14. POLLUTION PREVENTION

14.1 This method utilizes solid phase extraction technology to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, D.C., 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16. REFERENCES

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5. ASTM Annual Book of Standards, Part II, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, 1986.

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE PRIMARY COLUMN

Peak Number (Figure 1)	Analyte	Retention Time (min.)
1	Tebuthiuron	2.03
2	Thidiazuron	2.48
3	Monuron (SUR)	2.80
4	Fluometuron	4.45
5	Diuron	5.17
6	Propanil	8.53
7	Siduron A	8.91
8	Siduron B	9.76
9	Linuron	11.0
10	Carbazole (SUR)	12.8
11	Diflubenzuron	13.9

Primary Column: Symmetry 4.6 × 150 mm packed with 3.5 um C_{18} stationary phase.

Conditions:

Solvent A	25 mM phosphate buffer
Solvent B	acetonitrile
40% B	0-9.5 minutes
linear gradient 40-50% B	9.5-10.0 minutes
linear gradient 50-60% B	10.0-14.0 minutes
linear gradient 60-40%B	14.0-15.0 minutes
Flow rate	1.5 mL/min
Wavelength	245 nm
Equilibration time prior to next injection 15 minutes.	

TABLE 2. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE CONFIRMATION COLUMN

Peak Number (Figure 1)	Analyte	Retention Time (min.)
1	Tebuthiuron	2.56
2	Monuron (SUR)	3.98
3	Thidiazuron	4.93
4	Fluometuron	5.94
5	Diuron	7.67
6	Siduron A	9.53
7	Propanil	10.1
8	Siduron B	10.8
9	Linuron	12.2
10	Carbazole (SUR)	14.3
11	Diflubenzuron	15.2

Confirmation Column: Supelcosil 4.6 × 150 mm packed with 5 um cyanopropyl stationary phase.

Conditions:

Solvent A	25 mM phosphate buffer
Solvent B	acetonitrile
linear gradient 20% B	0-11.0 minutes, 1.5 mL/min. flow rate
linear gradient 20-40% B	11.0-12.0 minutes, 1.5 mL/min flow rate
40% B	hold to 16 minutes at 1.5 mL/min. flow rate, step to 2.0 mL/min flow rate at 16 min. and hold to 20 min.
linear gradient 40-20% B	20.0-20.1 minutes, 2.0 mL/min. flow rate
Wavelength	240 nm
Equilibration time prior to next injection 15 minutes.	

TABLE 3. METHOD DETECTION LIMITS FOR 2 INJECTION VOLUMES IN REAGENT WATER WITH CARTRIDGE AND DISK EXTRACTION TECHNIQUES ON BOTH THE PRIMARY AND CONFIRMATION COLUMNS

Table 3A. Cartridge Extraction, Primary Column				
Analyte	Spiking Conc. (ug/L)		MDL[a] (ug/L)	
	20 uL inj.	10 uL inj.	20 uL inj.	10 uL inj.
Tebuthiuron	0.050	0.100	0.032	0.071
Thidiazuron	0.100	0.100	0.035	0.047
Fluometuron	0.050	0.100	0.013	0.027
Diuron	0.050	0.100	0.010	0.026
Propanil	0.050	0.300	0.023	0.084
Siduron A&B	0.100	0.600	0.024	0.091
Linuron	0.050	0.300	0.062	0.067
Diflubenzuron	0.050	0.100	0.014	0.033

Table 3B. Disk Extractions, Primary Column, 20 uL Injection		
Analyte	Spiking Conc. (ug/L)	MDL[a] (ug/L)
Tebuthiuron	0.050	0.046
Thidiazuron	0.100	0.047
Fluometuron	0.050	0.028
Diuron	0.050	0.018
Propanil	0.100	0.071
Siduron A&B	0.100	0.067
Linuron	0.100	0.032
Diflubenzuron	0.050	0.035

[a] Method detection limit samples extracted over 3 days for 7 replicates and analyzed using conditions described in Table 1.

Table 3C. Cartridge Extraction, Confirmation Column, 20 uL Injection		
Analyte	Spiking Conc. (ug/L)	MDL[b] (ug/L)
Tebuthiuron	0.300	0.145
Thidiazuron	0.300	0.143
Fluometuron	0.300	0.065[c]
Diuron	0.300	0.056
Propanil	0.300	0.066
Siduron A&B	0.300	0.136
Linuron	0.300	0.085
Diflubenzuron	0.300	0.126

[b] Method detection limit samples extracted over 3 days for 7 replicates and analyzed using conditions described in Table 2.
[c] MDLs for fluometuron were reinjected due to an interfering peak which tended to coelute with fluometuron.

TABLE 4. PRECISION, ACCURACY AND SIGNAL-TO-NOISE COMPARISON FOR TWO HPLC SYSTEMS FOR LOW LEVEL SPIKES IN REAGENT WATER EXTRACTED WITH CARTRIDGES AND ANALYZED USING THE PRIMARY COLUMN

Table 4. Precision, Accuracy and S/N Comparison, 10 uL Injection, Primary Column						
Analyte	HPLC System #1[a]			HPLC System #2[b]		
	Concentration = 1.0 ug/L(n=7)			Concentration = 1.0 ug/L(n=7)		
	Mean % Rec.[c]	RSD (%)	S/N Ratio[d]	Mean % Rec.	RSD (%)	S/N Ratio[c]
Tebuthiuron	100	1.8	84	105	2.7	17
Thidiazuron	103	2.4	33	102	1.9	36
Fluometuron	99	2.2	43	104	2.3	35
Diuron	102	1.8	44	104	3.8	34
Propanil	96	3.6	28	100	6.4	16
Siduron A&B[a]	100	3.0	16	109	11	40
Linuron	108	1.6	12	103	4.1	21
Diflubenzuron	100	2.0	22	98	3.3	11
Monuron (SUR)[a]	105	2.8	NC	94	2.3	NC
Carbazole (SUR)[a]	97	2.7	NC	104	2.7	NC

NC: Not Calculated

[a]HPLC System 1 was equipped with a photodiode array detector that employed a 1 cm path length.

[b]HPLC System 2 was equipped with a photodiode array detector that employed a 5 cm path length.

[c]Seven replicates of the low level reagent water spikes were processed through the cartridge extraction procedure and injected on both instruments.

[d]Signal-to-noise ratios were calculated for each peak by dividing the peak height for each compound by the peak-to-peak noise for each peak, which was determined for each component from the method blank over a period of time equal to the full peak width.

TABLE 5. PRECISION AND ACCURACY DATA IN REAGENT WATER

Table 5A. Cartridge Extraction, Primary Column, 20 uL Injection				
Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean Recovery (%)	Relative Standard Deviation (%)	Mean Recovery (%)	Relative Standard Deviation (%)
Tebuthiuron	107	2.4	97.9	1.4
Thidiazuron	106	1.1	96.7	1.6
Fluometuron	106	0.9	97.6	1.5
Diuron	107	1.0	97.5	0.0
Propanil	105	1.7	97.2	1.4
Siduron A&B	106	1.9	97.8	1.6
Linuron	104	1.6	97.4	1.5
Diflubenzuron	107	1.2	96.0	1.4
Monuron (SUR)[a]	100	1.8	100.	2.0
Carbazole (SUR)[a]	96.7	1.0	96.1	1.5

Table 5B. Disk Extractions, Primary Column, 20 uL Injection				
Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean Recovery (%)	Relative Standard Deviation (%)	Mean Recovery (%)	Relative Standard Deviation (%)
Tebuthiuron	104	2.2	101	2.3
Thidiazuron	99.8	4.8	101	2.4
Fluometuron	100	4.2	101	2.3
Diuron	104	5.9	101	3.2
Propanil	101	2.7	101	2.3
Siduron A&B	110	5.0	103	2.3
Linuron	99.2	4.8	99.0	2.5
Diflubenzuron	102	4.2	100	2.5
Monuron (SUR)[a]	102	2.6	99.0	2.8
Carbazole (SUR)[a]	96.9	3.3	95.1	3.0

[a]Surrogate concentration in all samples is 10 ug/L. Chromatographic conditions are described in Table 1.

Table 5C. Cartridge Extraction, Confirmation Column, 20 uL Injection				
Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Tebuthiuron	100	4.9	103	4.8
Thidiazuron	93.6	5.1	101	4.8
Fluometuron	87.6	12.4	97.0	5.7
Diuron	96.9	3.9	102	2.7
Propanil	88.0	5.9	103	4.9
Siduron A&B[a]	91.0	7.0	103	5.0
Linuron	79.8	13.3	99.8	5.6
Diflubenzuron	83.7	6.9	80.4	18.6
Monuron (SUR)[b]	105	4.3	102	4.5
Carbazole (SUR)[b]	93.8	6.0	83.6	7.6

[a]Total siduron concentration is twice the concentration of the other target analytes: 2.0 and 60.0 ug/L.

[b]Surrogate concentration in all samples is 10 ug/L. Conditions described in Table 2.

TABLE 6. PRECISION AND ACCURACY[a] OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED SURFACE WATER USING CARTRIDGES

Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%)	Mean % Recovery	Relative Standard Deviation (%)
Tebuthiuron	108	1.6	96	1.7
Thidiazuron	81	2.9	84	1.9
Fluometuron	109	1.0	96	1.5
Diuron	110	1.7	96	1.6
Propanil	104	1.8	96	1.5
Siduron A&B	102	1.8	96	1.6
Linuron	102	1.3	96	1.6
Diflubenzuron	109	1.5	95	1.7
Monuron (SUR)[b]	103	2.3	97	2.3
Carbazole (SUR)[b]	98	2.7	94	1.9

[a]All data collected using a 20 uL injection volume on the primary column and conditions described in Table 1.
[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 7. PRECISION AND ACCURACY[a] OF LOW AND HIGH LEVEL FORTIFIED CHLORINATED GROUND WATER USING CARTRIDGES

Analyte	Concentration = 1 ug/L (n=7)		Concentration = 30 ug/L (n=7)	
	Mean % Recovery	Relative Standard Deviation	Mean % Recovery	Relative Standard Deviation
Tebuthiuron	109	1.5	99	1.2
Thidiazuron	95	2.9	94	1.2
Fluometuron	103	1.9	97	1.9
Diuron	106	2.1	99	1.1
Propanil	106	1.9	99	1.1
Siduron A&B	106	4.2	99	1.2
Linuron	101	2.7	98	1.9
Diflubenzuron	104	2.6	98	1.0
Monuron (SUR)[b]	100	1.3	99	1.0
Carbazole (SUR)[b]	95	1.3	89	4.7

[a]All data collected using a 20 uL injection volume on the primary column and conditions described in Table 1.
[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 8. SAMPLE HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 10 ug/L, WITH CUPRIC SULFATE AND TRIZMA (Sect. 8.1.2)

Analyte	Day 0 % Recovery	Day 2 % Recovery	Day 7 % Recovery	Day 14 % Recovery
Tebuthiuron	93	95	98	96
Thidiazuron	72	76	84	77
Fluometuron	93	95	98	97
Diuron	94	97	99	97
Propanil	93	97	98	98
Siduron A&B	92	96	98	98
Linuron	93	97	98	99
Diflubenzuron	93	95	98	97
Monuron (SUR)[b]	96	101	103	102
Carbazole (SUR)[b]	88	96	92	95

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery] × 100) for replicate analyses were all less than 7.0%.
Samples analyzed using a 20 uL injection volume and conditions described in Table 1.
[b]Surrogate concentration in all samples is 10 ug/L.

TABLE 9. EXTRACT HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 10 ug/L, WITH CUPRIC SULFATE AND TRIZMA (Sect. 8.1.2)

Analyte	Initial Injection[b] % Recovery	Day 8 Reinjection % Recovery	Day 14 Reinjection % Recovery	Day 21 Reinjection % Recovery
Tebuthiuron	93	95	93	103
Thidiazuron	72	73	71	80
Fluometuron	93	95	93	107
Diuron	94	96	94	99
Propanil	93	96	93	105
Siduron A&B	92	94	93	106
Linuron	93	97	93	102
Diflubenzuron	93	95	93	107
Monuron (SUR)	96	98	96	94
Carbazole (SUR)	88	90	89	91

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery] × 100) for replicate analyses were all less than 5.0%.
[b]Same as day 0 sample hold time analysis.
Extract stability study consisted of the analysis of day 0 extracts stored at $< 0^{\circ}$ C and reinjected. Samples were analyzed using a 20 uL injection volume and conditions described in Table 1.

TABLE 10. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all target analytes are below ½ the intended MRL or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantitation of method analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at 10 ug/L (or mid cal).	RSD must be $\leq$ 20%.
Sect. 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery for replicates used in IDP.	Mean recovery $\pm$ 20% of true value.
Sect. 9.2.4	Method Detection Limit (MDL) Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the MDL. Analyze the replicates through all steps of the analysis. Calculate the MDL using the equation in Sect. 9.2.4.	Data from MDL replicates are <u>not required</u> to meet method precision and accuracy criteria. If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.
Sect. 10.2.3	Peak Gaussian Factor (PGF)	Calculated prior to establishing the initial calibration.	A PGF range of 0.90 to 1.10 is acceptable.

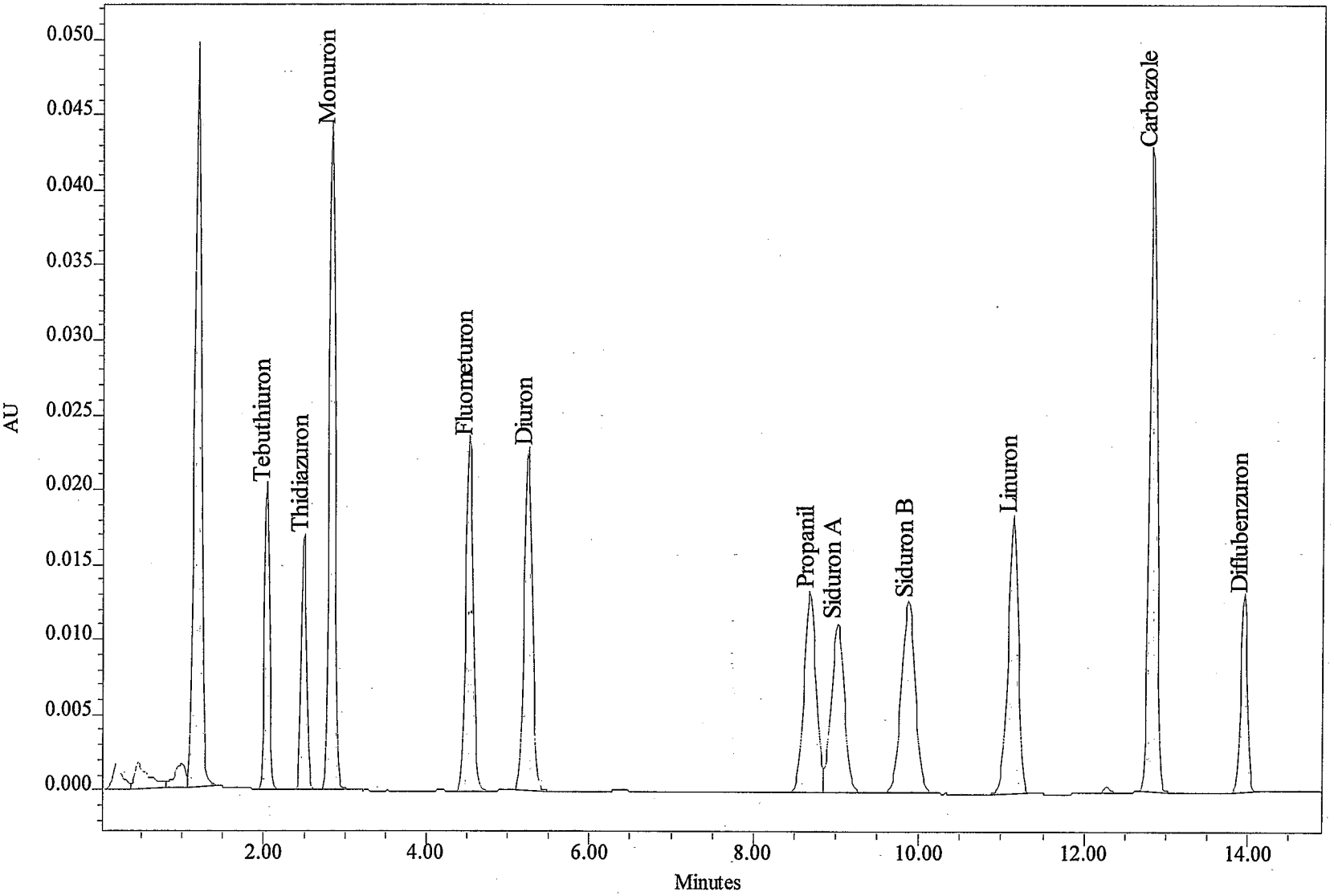
TABLE 11. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.4	Sample and Extract Holding Times	Properly preserved samples must be shipped at or below 10^0C and may be held in the lab at or below 6^0C for 14 days. Extracts in methanol may be stored at or below 0^0C for up to 21 days after extraction. Samples exchanged into 60/40 reagent water/acetonitrile may be held at or below 0^0C for 7 days, with the combined extract hold time not to exceed 21 days.	Do not report data for samples or extracts that have not been properly preserved or stored, or that have exceeded their holding time.
Sect. 9.4	Laboratory Reagent Blank (LRB)	Include LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free from interferences. Each analysis batch (Sect. 3.2) must include either a LRB or an instrument blank after the initial low level CCC injection.	Demonstrate that all target analytes are below 1/3 the intended MRL or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantitation of method analytes.
Sect. 9.7	Surrogate Standards	Surrogate standards are added to all calibration standards and samples, including QC samples.	Surrogate recovery must be 70-130% of the true value.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.8	Laboratory Fortified Sample Matrix (LFM)	With each extraction batch (Sect. 3.1), a minimum of one LFM is extracted and analyzed. A duplicate LFM, or LFMD, should be extracted when occurrence of target analytes is low. Laboratory duplicate analysis is not required for extraction batches containing a LFMD.	Recoveries not within 70-130% of the fortified amount may indicate a matrix effect, with the exception of thidiazuron which should have recoveries of 60-120%. If a LFMD is analyzed instead of a Laboratory Duplicate , target RPDs should be ± 30%.
Sect. 9.9	Field Duplicates (LD1 and LD2)	Extract and analyze at least one duplicate with each extraction batch (20 samples or less). A Laboratory Fortified Sample Matrix Duplicate may be substituted for a Field Duplicate when the occurrence of target analytes is low.	RPDs should be ± 30%.
Sect. 9.10	Quality Control Sample	Analyze either as a CCC or a LFB. Analyze at least quarterly, with each initial calibration, or when preparing new standards.	A QCS analyzed as a CCC will have the same acceptance criteria as a CCC, while a QCS analyzed as a LFB will have the same criteria as a LFB.

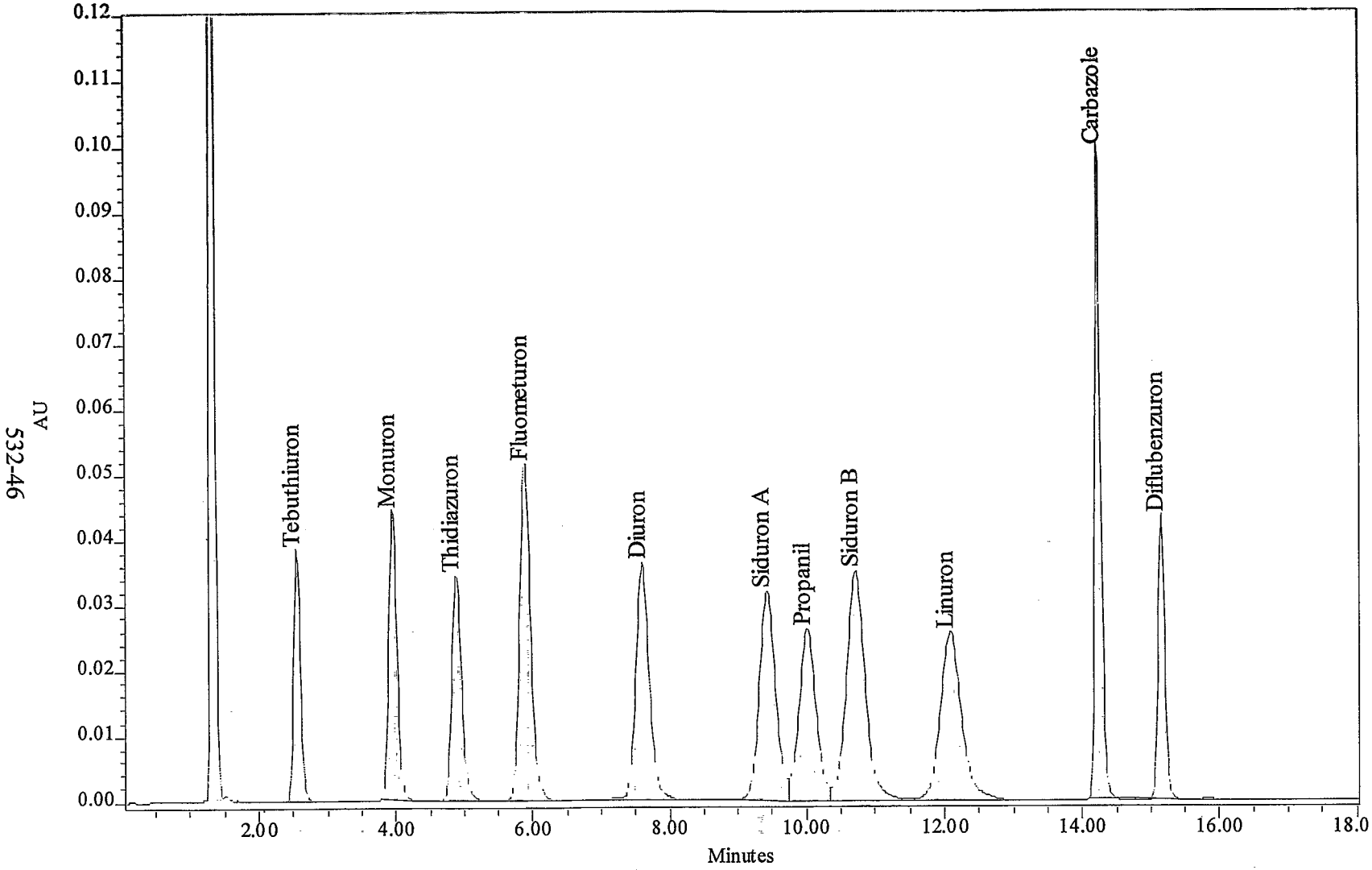
Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 10.2	Initial Calibration	Use external standard calibration technique to generate a calibration curve with five standards that span the approximate range of 1 to 30 ug/L sample concentration. Calibration curve fit options are discussed in Sect. 10.2.4. Analyze a QCS.	QCS must be ± 30% of true value. When each calibration standard is calculated as an unknown using the calibration curve, the results must be 70-130% of the true value for all but the lowest standard. The lowest standard must be 50-150% of the true value. The lowest CAL standard concentration must be as low or lower than the intended MRL.
Sect. 10.2.3	Peak Gaussian Factor (PGF)	Calculated prior to establishing the initial calibration.	A PGF range of 0.90 to 1.10 is acceptable.
Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte must be 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be 50-150% of the true value.
Sect. 12.3	Confirmation Column Results	Positive results should be confirmed using a chemically dissimilar column.	Quantitated target values should be ±50% of the primary result. Primary column results should be reported. If reporting confirmation results, both surrogates must meet ± 30% criteria.

Figure 1 Primary Column Chromatogram



532-45

Figure 2. Confirmation column chromatogram



METHOD 549.2 DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Revision 1.0

June 1997

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METHOD 549.2

DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

1. SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatography (HPLC) method for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride salt) in drinking water sources and finished drinking water (1).

Analytes	Chemistry Abstract Services Registry Number
Diquat	85-00-7
Paraquat	1910-42-5

1.2 When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. The use of a photodiode array detector provides ultraviolet spectra that can be used for the qualitative confirmation.

1.3 The method detection limits (MDL, defined in Sect. 13) (2) for diquat and paraquat are listed in Table 1.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sect. 9.3.

2. SUMMARY OF METHOD

2.1 A measured volume of liquid sample, approximately 250 mL, is extracted using a C_8 solid sorbent cartridge or a C_8 disk which has been specially prepared for the reversed-phase, ion-pair mode. The disk or cartridge is eluted with 4.5 mL of an acidic aqueous solvent. After the ion-pair reagent is added to the eluate, the final volume is adjusted to 5.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of diquat and paraquat in the extract by absorbance detection at 308 nm and 257 nm, respectively. A photodiode array detector is utilized to provide simultaneous detection and confirmation of the method analytes (1).

2.2 Analysis of diquat and paraquat is complicated by their ionic nature. Glassware should be deactivated to prevent loss of analytes. The substitution of polyvinylchloride (PVC) for glass is recommended.

3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate

analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentration which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 EXTERNAL STANDARD (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatogram. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Sect. 9.2.

4.1.1 Glassware must be scrupulously cleaned (3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and distilled water. It should then be drained dry and heated in a laboratory oven at 130°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 Before the initial use of all glassware, the procedure described in Sect. 4.1.1 should be followed. Silanization of all glassware which will come in contact with the method analytes is necessary to prevent adsorption of the diquat and paraquat cations onto glass surfaces (7.13).

4.1.3 Plasticware should be washed with detergent and rinsed in tap water and distilled water. It should be drained dry before use.

4.1.4 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.2 Interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source. Because of the selectivity of the detection system used here, no interferences have been observed in the matrices studied. If interferences occur, some additional cleanup may be necessary.

4.3 This method has been shown to be susceptible to interferences from Ca^{+2} and Mg^{+2} ions which may be present in hard water samples. These divalent cations can cause low recovery of method analytes, by interfering with the ion exchange process. Use of LFM samples can assist the analyst in evaluating the affect of these interferences in different matrices.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis.

6. EQUIPMENT AND SUPPLIES

6.1 SAMPLING EQUIPMENT, discrete or composite sampling.

6.1.1 Grab sample bottle -- Amber polyvinylchloride (PVC) high density, 1-L, fitted with screw caps. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with deionized water, and dried before use to minimize contamination.

6.2 GLASSWARE

6.2.1 Volumetric flask -- 5 mL, silanized

6.2.2 Autosampler vials -- 4 mL, silanized

6.3 BALANCE -- analytical, capable of accurately weighing 0.0001 g

6.4 pH METER -- capable of measuring pH to 0.1 units

6.5 HPLC APPARATUS

6.5.1 Isocratic pumping system, constant flow (Waters M6000A HPLC pump or equivalent).

6.5.2 Manual injector or automatic injector, capable of delivering 200 µL.

6.5.3 Analytical column -- any column which produces results equal to or better than those listed below may be used.

6.5.3.1 Phenomenex Spherisorb (3 µ, 100mm X 4.6mm), Hamilton PRP-1, (5 µ, 150 mm x 4.1 mm), or MICRA NPS RP-C18 (1.5 µ, 33 mm X 4.6mm) or equivalent.

6.5.3.2 Guard column, C_8 packing

6.5.4 Column Oven (Fiatron, Model CH-30 and controller, Model TC-50, or equivalent).

6.5.5 Photodiode array detector (LKB 2140 Rapid Spectral Detector or equivalent). Any detector which has the capability to switch between 257 nm and 308 nm may be used.

6.5.6 Data system -- Use of a data system to report retention times and peak areas is recommended but not required.

6.6 EXTRACTION APPARATUS

6.6.1 Liquid solid extraction cartridges, C_8, 500 mg or equivalent.

Note: EPA has observed significant variability between brands of C_8 LSE media, and also between lots of the same brand of C_8 LSE media. Verification of analyte recovery should be performed any time a new brand or lot of LSE media is used.

6.6.2 Liquid solid extraction system (Baker - 10 SPE, or equivalent).

6.6.3 Liquid solid extraction disks (C-8 Empore, 47 mm, or equivalent).

Note: EPA has observed significant variability between brands of C_8 LSE media, and also between lots of the same brand of C_8 LSE media. Verification of analyte recovery should be performed any time a new brand or lot of LSE media is used.

6.6.4 Liquid solid extraction system, Empore, 47 mm, 6 position manifold (Varian Associates or equivalent).

6.6.5 Vacuum pump, 100 VAC, or other source of vacuum, capable of maintaining a vacuum of 8-10 mm of Hg.

6.6.6 Membrane Filters, 0.45 µm pore-size, 47 mm diameter, Nylon.

7. REAGENTS AND STANDARDS

7.1 DEIONIZED WATER -- Water which has been processed through a series of commercially available filters including a particulate filter, carbon bed, ion exchange resin and finally a bacterial filter to produce deionized, reagent grade water. Any other source of reagent water may be used provided the requirements of Sect. 9 are met.

7.2 METHANOL -- HPLC grade or higher purity

7.3 ORTHOPHOSPHORIC ACID, 85% (w/v) -- Reagent grade

7.4 DIETHYLAMINE -- Reagent grade

7.5 CONCENTRATED SULFURIC ACID -- ACS reagent grade

7.6 SODIUM HYDROXIDE -- Reagent grade

7.7 CONCENTRATED HYDROCHLORIC ACID, 12 N -- Reagent grade

7.8 CETYL TRIMETHYL AMMONIUM BROMIDE, 95% -- Aldrich Chemical

7.9 SODIUM THIOSULFATE -- Reagent grade

7.10 1-HEXANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.11 1-HEPTANESULFONIC ACID, sodium salt, 98%, Aldrich Chemical

7.12 AMMONIUM HYDROXIDE, ACS, Concentrated

7.13 SYLON CT -- Silanization solution, Supelco

7.14 REAGENT SOLUTIONS

7.14.1 Conditioning solution A -- Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 mL of concentrated ammonium hydroxide in 500 mL of deionized water and dilute to 1000 mL in volumetric flask.

7.14.2 Conditioning solution B -- Dissolve 10.0 g of 1-hexanesulfonic acid, sodium salt and 10 mL of concentrated ammonium hydroxide in 250 mL of deionized water and dilute to 500 mL in volumetric flask.

7.14.3 Sodium hydroxide solution, 10% w/v -- Dissolve 50 g of sodium hydroxide into 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.4 Hydrochloric acid, 10% v/v -- Add 50 mL of concentrated hydrochloric acid to 400 mL of deionized water and dilute to 500 mL in a volumetric flask.

7.14.5 Disk or cartridge eluting solution -- Add 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine to 500 mL of deionized water and dilute to 1000 mL in a volumetric flask.

7.14.6 Ion-pair concentrate -- Dissolve 3.75 g of 1-hexanesulfonic acid in 15 mL of the disk or cartridge eluting solution and dilute to 25 mL in a volumetric flask with the disk eluting solution.

7.15 STOCK STANDARD SOLUTIONS

7.15.1 Diquat dibromide and Paraquat dichloride.

7.15.2 Stock diquat and paraquat solutions (1000 mg/L). Dry diquat and paraquat salts in an oven at 110°C for 3 hr. Cool in a desiccator. Repeat process to a constant weight. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt and place into a silanized glass or polypropylene 100-mL volumetric flask. Dissolve with approximately 50 mL of deionized water. Dilute to the mark with deionized water.

7.15.3 The salts used in preparing the stock standards (Sect. 7.15.2) were taken to be diquat dibromide monohydrate and paraquat dichloride tetrahydrate (4). The drying procedure described in Sect. 7.15.2 will provide these hydration levels.

7.16 MOBILE PHASE -- Make mobile phase by adding the following to 500 mL of deionized water: 13.5 mL of orthophosphoric acid; 10.3 mL of diethylamine; 3.0 g of 1-hexanesulfonic acid, sodium salt. Mix and dilute with deionized water to a final volume of 1 L.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Grab samples must be collected in either amber PVC high density bottles or silanized amber glass bottles. Conventional sampling procedures should be followed (5). Automatic sampling equipment must be free as possible of adsorption sites which might be extracted into the sample.

8.2 The samples must be iced or refrigerated at approximately 4°C from the time of collection until extraction. The analytes are light-sensitive, particularly diquat.

8.3 Samples which are known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L). Samples which are biologically active must be preserved by adding sulfuric acid to pH 2 to prevent adsorption of method analytes by the humectant material.

8.4 Analyte stability over time may depend on the matrix tested. All samples must be extracted within 7 days of collection. Extracts must be analyzed within 21 days of extraction (6).

9. QUALITY CONTROL

9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified matrix samples, and laboratory fortified blanks. The MDL (2) must also be determined for each analyte. The analyst should institute quality control practices to ensure that the brand and lot of LSE media being used show reliable recoveries of method analytes. The laboratory must maintain records to document the quality of the data generated. Additional quality control practices are recommended.

9.2 LABORATORY REAGENT BLANKS (LRB) -- Before processing any samples, the analyst must analyze a LRB to demonstrate that all deactivated glassware or plasticware, and reagent interferences are reasonably free of contamination. In addition, each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window (Sect. 11.4.2) of the analyte of interest, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples.

9.3 INITIAL DEMONSTRATION OF CAPABILITY

9.3.1 Prepare laboratory fortified blanks (LFBs) at analyte concentrations of 100 µg/L. With a syringe, add 25 µL of the stock standard (Sect. 7.15.2) to at least four 250 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Sect. 11.2.

9.3.2 Calculate the recoveries and relative standard deviation (RSD). The recovery (R) value for each sample, should be within ± 30% of the fortified amount. The RSD of the mean recovery should be less than 30%. For analytes that fail this critera, initial demonstration procedures should be repeated.

9.3.3 For each analyte, determine the MDL. Prepare a minimum of 4-7 LFBs (7 is recommended) at a low concentration. Fortification concentrations in Table 1

may be used as a guide, or use calibration data obtained in Section 10 to estimate a concentration for each analyte that will produce a peak with a 3-5 times signal to noise response. Extract and analyze each according to Sections 11 and 12. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day variations are reflected in precision measurements. Calculate mean recovery and standard deviation for each analyte. Use the standard deviation and the equation in Section 13 to calculate the MDL.

9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. As laboratory personnel gain experience with this method the quality of the data should improve beyond the requirements stated in Sect. 9.3.2.

9.4 The analyst is permitted to use other HPLC columns, HPLC conditions, or HPLC detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Sect. 9.3.

9.5 LABORATORY FORTIFIED BLANKS

9.5.1 The laboratory must analyze at least one laboratory fortified blank (LFB) sample per sample set (all samples extracted within a 24-hr period). The fortified concentration of each analyte in the LFB should be 10 times the MDL. If the recovery of either analyte falls outside the control limits (Sect. 9.5.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.5.2 Until sufficient data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess laboratory performance against the control limits in Sect. 9.3.2. When sufficient internal performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S_r) of the percent recovery. These data are used to establish upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = R + 3S_r$$
$$\text{LOWER CONTROL LIMIT} = R - 3S_r$$

After each five to ten new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should not exceed the limits established in Sect. 9.3.2.

9.6 LABORATORY FORTIFIED SAMPLE MATRIX

9.6.1 The laboratory must add a known fortified concentration to a minimum of 10% of the samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the original sample. Ideally, the fortified concentration should be the same as that used for the laboratory fortified blank (Sect. 9.5). Over time, samples from all routine samples sources should be fortified.

9.6.2 Calculate the accuracy as percent recovery (R) for each analyte, corrected for background concentrations measured in the original sample, and compare these values to the control limits established in Sect. 9.5.2 from the analyses of LFBs.

9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the original sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 QUALITY CONTROL SAMPLES (QCS) -- Each quarter the laboratory should analyze one or more QCS. If criteria provided with the QCS are not met, corrective action should be taken and documented.

9.8 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

10. CALIBRATION AND STANDARDIZATION

10.1 Establish HPLC operating conditions indicated in Table 1. The chromatographic system can be calibrated using the external standard technique.

10.2 In order to closely match calibration standards to samples, process standards by the following method: Using C_8 disks or C_8 cartridges conditioned according to Sect. 11.2.1, pass 250 mL of reagent water through the disk or cartridge and discard the water. Dry the disk or cartridge by passing 5 mL of methanol through it. Discard the methanol. Pass 4.0 mL of the eluting solution through the disk or cartridge and catch in a 5 mL silanized volumetric flask. Fortify the eluted solution with 100 µL of the ion-pair concentrate and with 500 µL of the stock standard and dilute to the mark with

eluting solution. This provides a 10:1 dilution of the stock. Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

10.3 Analyze a minimum of three calibration standards prepared by the procedure described in Sect. 10.2 utilizing the HPLC conditions given in Table 1. From full spectral data obtained, extract the 308 nm chromatographic trace for diquat and the 257 nm trace for paraquat. Integrate and record the analyte peak areas. Any mathematical manipulations performed to aid in data reduction must be recorded and performed on all sample chromatograms. Tabulate the peak area against quantity injected. The results may be used to prepare calibration curves for diquat and paraquat.

10.4 The working calibration curve must be verified on each working day by measurement of a calibration check standard, at the beginning of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 hr), it is strongly recommended that check standards be interspersed with samples at regular intervals. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

11. PROCEDURE

11.1 SAMPLE CLEANUP -- Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various sample types. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of the analytes is within the limits specified by the method.

11.1.1 If the sample contains particulates, or the complexity is unknown, the entire sample should be passed through a 0.45 µm Nylon membrane filter into a silanized glass or plastic container.

11.1.2 Store all samples at 4°C unless extraction is to be performed immediately.

11.2 CARTRIDGE EXTRACTION

11.2.1 Before sample extraction, the C_8 extraction cartridges must be conditioned by the following procedure.

11.2.1.1 Place a C_8 cartridge on the cartridge extraction system manifold.

11.2.1.2 Elute the following solutions through the cartridge in the stated order. Take special care not to let the column go dry. The flow rate through the cartridge should be approximately 10 mL/min.

11.2.1.2.1 Cartridge Conditioning Sequence

a. Deionized water, 5 mL
b. Methanol, 5 mL
c. Deionized water, 5 mL
d. Conditioning Solution A, 5 mL
e. Deionized water, 5 mL
f. Methanol, 10 mL
g. Deionized water, 5 mL
h. Conditioning Solution B, 20 mL

11.2.1.2.2 Retain conditioning solution B in the C_8 cartridge to keep it activated.

11.2.2 The C_8 cartridges should not be prepared more than 48 hr prior to use. After conditioning, the cartridge should be capped and stored at 4°C.

11.2.3 Measure a 250-mL aliquot of the sample processed through Sect.11.1 in a silanized, volumetric flask.

11.2.4 Measure the pH of the sample. If the pH is between 7.0 and 9.0, the sample can be analyzed without adjustment. If the pH is not in this range, or if the sample has been acidified for preservation purposes, adjust the pH of sample to between 7.0 and 9.0 with 10% w/v NaOH (aq) or 10% v/v HCl (aq) before extracting.

11.2.5 Place a conditioned C_8 cartridge on the solid phase extraction vacuum manifold. Attach a 60-mL reservoir to the C_8 cartridge with the appropriate adapter. Put a 250- mL beaker inside the extraction manifold to catch waste solutions and sample. Transfer the measured volume in aliquots to the reservoir. Turn on the vacuum pump or house vacuum and adjust the flow rate to 3 to 6 mL/min. Filter the sample through the C_8 cartridge, and wash the column with 5 mL of HPLC grade methanol. Continue to draw the vacuum through the cartridge for one additional minute to dry the cartridge. Release the vacuum and discard the sample waste and methanol.

11.2.6 Place a silanized 5-mL volumetric flask beneath the collection stem in the vacuum manifold. Add 4.5 mL of the eluting solution to the sample cartridge. Turn on the vacuum and adjust the flow rate to 1 to 2 mL/min.

11.2.7 Remove the 5-mL volumetric flask with the extract. Fortify the extract with 100 µL of the ion-pair concentrate. Adjust the volume to the mark with cartridge eluting solution, mix thoroughly, and seal tightly until analyzed.

11.2.8 Analyze sample by HPLC using conditions described in Table 1. Integration and data reduction must be consistent with that performed in Sect. 10.3.

11.3 DISK EXTRACTION -- The top surface of the disk matrix must remain covered with liquid at all times. If the disk is exposed to air at any step in the disk cleanup procedure, the elution procedure should be restarted. Eluants applied to the disk should be allowed to soak into the disk before drawing them through. Vacuum should then be applied to draw most of the eluant through the disk, leaving a thin layer of solution on the top of the disk. Flow rate through the disk is not critical.

11.3.1 Assemble the 47 mm disk in the disk holder or a filter apparatus. Be sure that the surfaces of the holder are either silanized glass or Teflon coated to avoid adsorption or decomposition of the analytes.

11.3.2 Measure the pH of the sample. If the pH is between 7.0 and 9.0, the sample can be analyzed without adjustment. If the pH is not in this range, or if the sample has been acidified for preservation purposes, adjust the pH of sample to between 7.0 and 9.0 with 10% w/v NaOH (aq) or 10% v/v HCl (aq) before extracting.

11.3.3 Apply 10 mL of methanol to the disk. Apply vacuum to begin elution, then immediately vent the vacuum when drops of liquid appear at the drip tip. Allow the methanol to soak into the disk for a minimum of 1 min, then reapply the vacuum to bring the methanol to just above the top surface of the disk.

11.3.4 Draw 2 10-mL aliquots of reagent water through to just above the top surface of the disk to remove the methanol.

11.3.5 Apply 10 mL of Conditioning Solution A to the disk. As with the methanol, draw a few drops through, then allow the disk to soak for at least 1 min. Draw the Conditioning Solution A through the disk to just above its top surface.

11.3.6 Draw 2 10-mL aliquots of reagent water through to just above the top surface of the disk.

11.3.7 Apply 20 mL of Conditioning Solution B to the disk. Draw a few drops through using vacuum and allow the disk to soak for at least 1 min. Draw the remaining Conditioning Solution B through to just above the top surface of the disk.

11.3.8 Measure 250 mL of the sample using a polypropylene graduated cylinder. Pour the sample aliquot into the filtration apparatus reservoir and apply

vacuum to draw the sample through the disk. Pass the entire sample through the disk, leaving no liquid on the top of the disk, then vent the vacuum.

11.3.9 Assemble a graduated collection tube under the drip tip with the tip descending into the tube slightly to prevent losses of eluants. Be sure the tube will hold at least 10 mL of eluate.

11.3.10 With the vacuum vented, drip enough methanol onto the disk to cover it completely (0.5-1.0 mL). Allow the methanol to soak into the disk for 1 min. Add more methanol as needed to keep the disk covered as it soaks.

11.3.11 Pipet 4 mL of Disk Eluting Solvent onto the disk. Apply vacuum until drops appear at the drip tip. Vent the vacuum and allow the disk to soak for 1 min.

11.3.12 Draw the Disk Eluting Solution through to just above the top surface of the disk. Add 4 mL of Disk Eluting Solution and draw it completely through the disk. Tap the disk holder assembly gently to loosen adhering drops into the collection tube.

11.3.13 Vent the vacuum, disassemble the disk extraction device, and remove the collection tube. Fortify the extract with 200 µL of the ion-pair concentrate. Add Disk Eluting Solution to the tube to a final volume of 10 mL.

11.3.14 Analyze samples by HPLC. Some suggested conditions, which were used in developing this method, are listed in Table 1. This table includes the retention times and MDLs that were obtained using the suggested conditions.

11.4 IDENTIFICATION OF ANALYTES

11.4.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits (Sect. 11.4.2), to the retention time of a standard compound, then identification is considered positive.

11.4.2 The width of the retention time window used to make identification should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.4.3 Identification requires expert judgment when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley

between two or more maxima), or any time doubt exists over the identification of a peak in a chromatogram, a confirmatory technique must be employed. Through the use of the photodiode array detector, full spectra of the analyte peaks are obtained (Figure 2). When a peak of an unknown sample falls within the retention time windows of method analytes, confirm the peak identification by spectral comparison with analyte standards.

If additional confirmation is required, replace the 1-hexanesulfonic acid salt with 1-heptanesulfonic acid, sodium salt in the mobile phase and reanalyze the samples. Comparison of the ratio of retention times in the samples by the two mobile phases with that of the standards will provide additional confirmation.

11.4.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with mobile phase and reanalyzed.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Determine the concentration of the analytes in the sample.

12.1.1 Calculate the concentration of each analyte injected from the peak area using the calibration curves in Sect. 10.3 and the following equation.

$$\text{Concentration, } \mu g/L = \frac{(A) \times (VF)}{(VS)}$$

where: A = Peak area of analyte in sample extract
VF = Final volume of sample extract, in mL
VS = Sample volume, in mL

12.2 Report results as micrograms per liter without correction for recovery data. When duplicate and fortified samples are analyzed, report all data obtained with sample results.

13. METHOD PERFORMANCE

13.1 METHOD DETECTION LIMITS -- The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level (2). The MDL data listed in Table 1 were obtained using disks with reagent water as the matrix.

$$MDL = S\, t_{(n-1,1\text{-alpha} = 0.99)}$$

where:

$t_{(n-1,1-alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses.

13.2 This method has been tested for linearity of recovery from fortified reagent water and has been demonstrated to be applicable over the range from approximately 4 x MDL to 100 x MDL.

13.3 Single-laboratory precision and accuracy results at several concentration levels in drinking water matrices using disks are presented in Table 2.

13.4 This methodology has been shown to be sensitive to brand differences and even lot differences in C_8 LSE media. This is presumed to be due to variations in manufacturing processes. If the method does not demonstrate performance data similar to those demonstrated in Sect. 17, C_8 LSE media should be obtained from a different source.

14. POLLUTION PREVENTION

14.1 Only an extremely small volume of an organic solvent is used in this method. A maximum of 15 mL of methanol is used per sample to condition each cartridge or disk. Methanol is not considered to be a toxic or hazardous solvent. All other chemicals used in this method are can be handled in a non-hazardous way when used in the prescribed manner and amounts.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1 There are generally no waste management problems involved with discarding spent or left over samples in this method since most often the sample matrix is drinking water. If a sample is analyzed which appears to be highly contaminated with chemicals, analyses should be carried out to assess the type and degree of contamination so that the samples may be discarded properly. The Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is

required with any sewage discharge restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Sect. 14.2.

16. REFERENCES

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5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water", American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

6. Hodgeson, J.W., Bashe, W.J. and J.W. Eichelberger, "Method 549.1 - Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection", Methods for the Determination of Organic Compounds in Drinking Water, Supplement II, EPA/600/R-92/129, U.S. Environmental Protection Agency, Envirinmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45268, 1992.

17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Method Detection Limits[a] (µg/L) (disks)
Diquat	0.72
Paraquat	0.68

HPLC Conditions:

Column:	Phenomenex Spherisorb, 3µ, 4.6 mm x 100 mm
Column Temperature	35.0° C
Flow Rate:	2.0 mL/min., Ion-Pair Mobile Phase (Sect. 7.16)
Injection Volume:	200 µL

Photodiode Array Detector Settings:

Wavelength Range:	210 - 370 nm
Sample Rate:	1 scan/sec.
Wavelength Step:	1 nm
Integration Time:	1 sec.
Run Time:	5.0 min.
Quantitation Wavelengths:	Diquat - 308 nm Paraquat - 257 nm

[a]MDL data were obtained from five samples fortified at 2.5 µg/L diquat and 2.5 µg/L paraquat.

TABLE 2. SINGLE OPERATOR ACCURACY AND PRECISION USING DISK (N = 5 FOR EACH TYPE OF WATER)

DIQUAT

Type of Water	Fortified 2.5 µg/L Mean % Rec.	% RSD	Fortified 10.5 µg/L Mean % Rec.	% RSD	Fortified 52.5 µg/L Mean % Rec.	% RSD
RW	90.9	8.4	94.1	5.2	92.1	2.9
TW	91.7	6.5	93.6	3.1	93.0	5.3
GW	91.4	6.4	93.7	3.0	90.2	3.3

PARAQUAT

Type of Water	Fortified 2.5 µg/L Mean % Rec.	% RSD	Fortified 10 µg/L Mean % Rec.	% RSD	Fortified 50 µg/L Mean % Rec.	% RSD
RW	94.7	7.7	92.3	5.5	88.8	4.2
DW	93.5	6.6	89.7	3.6	91.4	6.5
GW	92.0	8.1	89.9	2.5	90.4	2.5

RW = Reagent Water
TW = Tap Water (Dechlorinated with sodium thiosulfate)
GW = Ground Water
All samples adjusted to pH 7.

METHOD 556. DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY PENTAFLUOROBENZYLHYDROXYLAMINE DERIVATIZATION AND CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

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METHOD 556

DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY PENTAFLUORBENZYLHYROXYLAMINE DERIVATIZATION AND CAPILLARY GAS CHROMATOGRAPHY AND ELECTRON CAPTURE DETECTION

1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic method optimized for the determination of selected carbonyl compounds in finished drinking water and raw source water. The analytes applicable to this method are derivatized to their corresponding pentafluorobenzyl oximes. The oxime derivatives are then extracted from the water with hexane. The hexane extracts are analyzed by capillary gas chromatography with electron capture detection (GC-ECD) and quantitated using procedural standard calibration. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds:

Analyte	Chemical Abstract Services Registry Number
Formaldehyde	50-00-0
Acetaldehyde	75-07-0
Propanal	123-38-6
Butanal	123-72-8
Pentanal	110-62-3
Hexanal	66-25-1
Heptanal	111-71-7
Octanal	124-13-0
Nonanal	124-19-6
Decanal	112-31-2
Cyclohexanone	108-94-1
Crotonaldehyde	123-73-9
Benzaldehyde	100-52-7
Glyoxal (ethanedial)	107-22-2
Methyl glyoxal (2-oxopropanal or pyruvicaldehyde)	78-98-8

1.2 This method applies to the determination of target analytes over the concentration ranges typically found in drinking water. Analyte retention times are in Section 17, Table 1. Other method performance data are presented in Section17, Tables 2-6. Experimentally determined method detection limits (MDLs) for the above listed analytes are provided in Section 17, Table 3. The MDL is defined as the

statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero.[1] However, it should be noted that background levels of some method analytes (usually formaldehyde and acetaldehyde) are problematic. The minimum reporting level (MRL) for method analytes, for each analyst/laboratory that uses this method, will depend on their ability to control background levels (Sect. 4).

1.3 This method is restricted to use by or under the supervision of analysts skilled in liquid-liquid extractions, derivatization procedures and the use of GC and interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedures described in Section 9.

2.0 SUMMARY OF METHOD

2.1 A 20 mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 °C for 2 hr with 15 mg of O-(2,3,4,5,6-Pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL hexane. The extract is processed through an acidic wash step, and then analyzed by GC-ECD. The target analytes are identified and quantitated by comparison to a procedural standard (Sect. 3.9). Two chromatographic peaks will be observed for many of the target analytes. Both *(E)* and *(Z)* isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. However, the *(E)* and *(Z)* isomers may not be chromatographically resolved in a few cases. Compounds where two carbonyl groups are derivatized, such as glyoxal and methyl glyoxal, have even more possible isomers. See Section17, Table 1 and Figure 1 for the chromatographic peaks used for analyte identification.

NOTE: The absolute identity of the *(E)* and *(Z)* isomers was not determined during method development. Other researchers [2,3,4] have reported the first eluting peak as *(E)*, and the second peak as *(Z)*. For convenience, this method will follow this convention. Because more than 2 isomers are formed for glyoxal and methyl glyoxal, the peaks used for identification are referred to as "peak 1" and "peak 2."

2.2 All results should be confirmed on a second, dissimilar capillary GC column.

3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all

glassware, equipment, solvents and reagents, sample preservatives, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are introduced during sample shipping or storage. For this analysis the FRB should not be opened at the sampling site.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and

different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 PROCEDURAL STANDARD CALIBRATION -- A calibration method where aqueous calibration standards are prepared and processed (e.g. purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

3.10 INTERNAL STANDARD (IS) -- A pure analyte added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.11 SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.12 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.13 MATERIAL SAFETY DATA (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.14 CONTINUING CALIBRATION CHECK (CCC) -- A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.15 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration of an analyte that should be reported. This concentration is determined by the background level of the analyte in the LRBs and the sensitivity of the method to the analyte. Ideally, the MRL will be at or near the concentration of the lowest calibration standard.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in laboratory air, solvents, reagents (including reagent water), glassware, sample bottles and caps, and other

sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences (less than 1/2 the MRL) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3. **Subtracting blank values from sample results is not permitted.**

4.1.1 Before attempting analyses by this method, the analyst must obtain a source of reagent water free from carbonyl compounds and other interferences. The most likely interferences are the presence of formaldehyde and acetaldehyde in the reagent water. The most successful techniques for generating aldehyde free water are (1) exposure to UV light, or (2) distillation from permanganate.

4.1.2 Commercially available systems for generating reagent grade water have proved adequate, if a step involving exposure to UV light is included. For the data presented in this method, a Millipore Elix 3 reverse osmosis system followed by a Milli-Q TOC Plus polishing unit provided reagent water with background levels of 1 ug/L or less for each method analyte. Other researchers have reported typical blank values of 1-3 ug/L.[3,4]

4.1.3 Distillation of reagent water from acidified potassium permanganate has been reported as an effective method of eliminating background levels of aldehydes.[2] Distill 500 mL of reagent water to which 64 mg potassium permanganate and 1 mL conc. sulfuric acid have been added. In our laboratory, this procedure reduced formaldehyde levels to approximately 3 ug/L.

4.1.4 It may be necessary to purchase reagent grade water. If acceptably clean reagent grade water is purchased, care must also be taken to protect it from contamination caused by contact with laboratory air.

4.2 Formaldehyde is typically present in laboratory air and smaller amounts of other aldehydes may also be found. Care should be taken to minimize exposure of reagents and sample water with laboratory air. Because latex is a potential aldehyde contaminant source, protective gloves should not contain latex. Nitrile gloves, such as N-Dex Plus, are acceptable. Bottle caps should be made of polypropylene. Commonly used phenolic resin caps must be avoided because they can introduce formaldehyde contamination into samples.

4.3 Reagents must also be free from contamination. Many brands of solvents may contain trace amounts of carbonyl compounds.

4.4 Glassware must be scrupulously cleaned by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained, dried,

and heated in a laboratory oven at 130 °C for several hours before use. Solvent rinses with methanol or acetonitrile, followed by air drying, may be substituted for the oven heating. After cleaning, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.5 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled.

4.6 An interferant that elutes just prior to the acetaldehyde *(E)* isomer peak on the primary column is typically observed in chlorinated or chloraminated waters. If this peak interferes with the integration of the acetaldehyde *(E)* isomer peak, then acetaldehyde should be quantitated using only the acetaldehyde *(Z)* isomer, or from the confirmation column data.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[5-8]

5.2 Formaldehyde and acetaldehyde have been tentatively classified as known or suspected human or mammalian carcinogens. Glyoxal and methyl glyoxal have been shown to be mutagenic in in-vitro tests.[2]

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS -- Grab Sample Bottle (aqueous samples) -- 30 mL amber glass, screw cap bottles and caps equipped with Teflon-faced silicone septa. Screw caps should be polypropylene. **Typical phenolic resin caps should be avoided due to the possibility of sample contamination from formaldehyde.** Prior to use, wash bottles and septa according to Section 4.4.

6.2 VIALS -- 8 mL or 12 mL vials for the acid wash step (Sect. 11.1.10), and GC autosampler vials, both types must be glass with Teflon-lined polypropylene caps.

6.3 VOLUMETRIC FLASKS -- various sizes used for preparation of standards.

6.4 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.5 WATER BATH or HEATING BLOCK -- Capable of maintaining 35 ± 2 °C

6.6 GAS CHROMATOGRAPH -- Capillary Gas Chromatograph equipped with a split/splitless injector, or other injector suitable for trace analysis, and an electron capture detector.

6.6.1 Primary Column -- 30 m x 0.25mm J&W DB-5ms, 0.25 um film thickness (or equivalent). Note: The J&W DB-5 was not found to be equivalent for this application. The surrogate analyte is not resolved from octanal with the DB-5 column.

6.6.2 Confirmation Column -- 30 m x 0.25 mm Restek Rtx- 1701, 0.25 um film thickness (or equivalent)

7. REAGENTS AND STANDARDS

7.1 Reagent grade or better chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 REAGENT WATER --. **Reagent water as free as possible from interferences and contamination is critical to the success of this method. See Section 4.1.**

7.3 ACETONITRILE -- High purity, demonstrated to be free of analytes and interferences.

7.4 HEXANE -- High purity, demonstrated to be free of analytes and interferences: B&J Brand, GC^2 grade or equivalent.

7.5 POTASSIUM HYDROGEN PHTHALATE (KHP) -- ACS Grade or better.

7.6 0-(2,3,5,6-PENTAFLUOROBENZYL)-HYDROXYLAMINE HYDROCHLORIDE (PFBHA) -- 98+%, Aldrich cat# 19,448-4. (Store in a desiccator - Do not refrigerate).

7.7 SULFURIC ACID -- ACS Grade or better.

7.8 COPPER SULFATE PENTAHYDRATE -- ACS Grade or better.

7.9 AMMONIUM CHLORIDE, NH_4Cl or AMMONIUM SULFATE, $(NH_4)_2SO_4$.

7.10 SOLUTIONS

7.10.1 PFBHA REAGENT -- Prepare a fresh 15 mg/mL solution in reagent water daily. Prepare an amount appropriate to the number of samples to be derivatized. One mL of solution is added per sample. For example, if 14 sample vials are being extracted, prepare 15 mL of solution. For a 15 mL volume of solution, weigh 0.225 grams of PFBHA into a dry 40 mL vial, add 15 mL water and shake to dissolve.

7.10.2 0.2 N SULFURIC ACID -- Add 5 mL of concentrated sulfuric acid to 900 mL of reagent water.

7.11 STOCK STANDARD SOLUTIONS -- When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

7.11.1 INTERNAL STANDARD (IS) -- 1,2-DIBROMOPROPANE, 98+% purity. An alternate compound may be used as the IS at the discretion of the analyst. If an alternate is selected, an appropriate concentration will need to be determined.

7.11.1.1 INTERNAL STANDARD STOCK SOLUTION, (10,000 ug/mL) -- Accurately weigh approximately 0.1 gram to the nearest 0.0001g, into a tared 10 mL volumetric flask containing hexane up to the neck. After determining weight difference, fill to mark with hexane. Stock solutions can be used for up to 6 months when stored at -10 °C.

7.11.1.2 INTERNAL STANDARD FORTIFIED EXTRACTION SOLVENT, 400 ug/L in hexane -- This is the solvent used to extract the derivatized samples. The internal standard is added to the solvent prior to performing the extraction. The volume of this solvent to be prepared should be determined by the sample workload. The following example illustrates preparation of 1 L of fortified solvent. If fewer samples are to be analyzed each month, prepare smaller batches of working solvent. Add 40 uL of internal standard stock solution directly to 1 L of hexane in a volumetric flask. Cap flask and invert three times to ensure thorough mixing. Transfer to 1 L storage bottle with Teflon lined cap. This solution can be used up to 4 weeks. As a check, run a sample of this working solvent on the GC before the first extraction of aqueous samples. Have

enough working solvent available to extract all calibration and aqueous samples in each extraction set. Never use two different batches of working solvent for one set of extractions.

7.11.2 SURROGATE (SUR) -- 2',4',5' -TRIFLUOROACETOPHENONE
This compound was found to be an appropriate surrogate analyte for these analyses. However, the chromatograms for this analysis are very crowded, and all possible matrix interferences cannot be anticipated. An alternate carbonyl compound may be selected as the surrogate analyte if matrix interferences or chromatographic problems are encountered. Any surrogate analyte selected must form an oxime derivative, because its purpose is to monitor the derivatization process. If an alternate surrogate is selected, its concentration may also be adjusted to meet the needs of the laboratory.

7.11.2.1 SURROGATE STOCK SOLUTION, 10,000 ug/mL -- Accurately weigh approximately 0.1 gram SUR to the nearest 0.0001g, into a 10 mL tared volumetric flask containing acetonitrile up to the neck. After determining weight difference, fill to mark with acetronitrile. Stock solutions can be used for up to 6 months when stored at -10 °C or less.

7.11.2.2 SURROGATE ADDITIVE SOLUTION, 20 ug/mL -- Dilute the surrogate stock solution to 20 ug/mL in acetonitrile. This solution can be used up to 3 months when stored at 4 °C or less.

7.11.3 STOCK STANDARD SOLUTION (SSS)
Prepare stock standard solutions for each analyte of interest at a concentration of 1 to 10 mg/mL in acetonitrile, or purchase SSSs or primary dilution standards (PDSs) from a reputable supplier. Method analytes may be obtained as neat materials or as ampulized solutions from commercial suppliers. The stock standard solutions should be stored at -10 °C or less and protected from light. Standards prepared in this manner were stable for at least 60 days. Standards may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when their standards need to be replaced.

7.11.3.1 For analytes which are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.1 gram of pure material to the nearest 0.0001g in a 10 mL volumetric flask. Dilute to volume with acetonitrile.

7.11.3.2 Stock standard solutions for analytes which are liquid in their pure form at room temperature can be accurately prepared in the following manner.

7.11.3.2.1 Place about 9.8 mL of acetonitrile into a 10- mL volumetric flask. Allow the flask to stand, unstoppered, for about 10 min. to allow solvent film to evaporate from the inner walls of the volumetric, and weigh to the nearest 0.0001 gram.

7.11.3.2.2 Use a 100-uL syringe and immediately add 100 uL of standard material to the flask by keeping the syringe needle just above the surface of the acetonitrile. Be sure the standard material falls dropwise directly into the acetonitrile without contacting the inner wall of the volumetric.

7.11.3.2.3 Reweigh, dilute to volume, stopper, then mix by inverting several times. Calculate the concentration in milligrams per milliliter from the net gain in weight.

7.11.4 PRIMARY DILUTION STANDARD (PDS) -- The PDS for this method should include all method analytes of interest to the analyst. The PDS is prepared by combining and diluting stock standard solutions with acetonitrile to a concentration of 100 ug/mL. Store at -10 °C or less and protect from light. Standards prepared in this manner were stable for at least 60 days. Standards may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when their standards need to be replaced. This primary dilution standard is used to prepare calibration spiking solutions, which are prepared at 5 concentration levels for each analyte, and are used to spike reagent water to prepare the aqueous calibration standards.

7.11.5 CALIBRATION SPIKING SOLUTIONS -- Five calibration spiking solutions are prepared, each at a different concentration, and are used to spike reagent water to prepare the calibration standards. The calibration spiking solutions are prepared from the PDS. Store the calibration spiking solutions at -10 °C or less and protect from light. Solutions prepared in this manner were stable for at least 60 days. Solutions may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when solutions need to be replaced. An example of how the calibration spiking solutions are prepared is given in the following table. Modifications of this preparation scheme may be made to meet the needs of the laboratory.

PREPARATION OF CALIBRATION SPIKING SOLUTIONS

Cal. Level	PDS Conc., ug/mL	Vol. PDS Std., uL	Final Vol., Cal Spike Sol'n, mLs	Final Conc., Cal Spike Sol'n, ug/mL
1	100	250	5	5
2	100	500	5	10
3	100	1000	5	20
4	100	1500	5	30
5	100	2000	5	40

7.11.6 PROCEDURAL CALIBRATION STANDARDS -- A designated amount of each calibration spiking solution is spiked into five separate 20 mL aliquots of reagent water in a 30 mL sample container, to produce aqueous calibration standards. The reagent water used to make the calibration standards should contain the preservation reagents described in Section 8.1.2 (ammonium chloride or ammonium sulfate at 500 mg/L and copper sulfate pentahydrate at 500 mg/L). Aqueous calibration standards are processed and analyzed according to the procedures in Section 11. Resulting data are used to generate a calibration curve. An example of the preparation of aqueous calibration standards is given below. The lowest concentration calibration standard should be at or near (within 25% of) the MRL. Modifications of this preparation scheme may be made to meet the needs of the laboratory. Preparing aqueous calibration standards using varying volumes of one calibration spiking solution is an acceptable alternative to the example below.

PREPARATION OF PROCEDURAL (AQUEOUS) CALIBRATION STANDARDS

Cal. Level	Cal. Spike Sol'n Conc., ug/mL	Vol. Cal. Spike Sol'n., uL	Final Vol., Cal Std mL	Final Conc., Cal Std ug/L
1	5	20	20	5
2	10	20	20	10
3	20	20	20	20
4	30	20	20	30
5	40	20	20	40

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE VIAL PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [6] using amber glass 30 mL containers with PTFE-lined screw-caps, or caps with PTFE-faced silicon septa.

8.1.2 Prior to shipment to the field, 15 mg of copper sulfate pentahydrate must be added to each bottle. This material acts as a biocide to inhibit bacteriological decay of method analytes. If samples to be collected contain free chlorine, then 15mg of ammonium chloride or ammonium sulfate must also be added to the bottle prior to sample collection. The ammonium compound will react with the free chlorine to form monochloramine, and retard the formation of additional carbonyl compounds. Add these materials as dry solids to the sample bottle. The stability of these materials in concentrated aqueous solution has not been verified.

NOTE: Aldehydes have been demonstrated to be extremely susceptible to microbiological decay. The use of other chlorine reducing agents such as sodium thiosulfate or ascorbic acid, has also been shown to produce invalid data. Proper sample collection and preser-vation is important to obtaining valid data. The data in Section17, Table 6 illustrates the importance of proper sample preservation.

8.2 SAMPLE COLLECTION

8.2.1 Fill sample bottles to just overflowing but take care not to flush out the sample preservation reagents. The capped sample should be head-space free.

8.2.2 When sampling from a water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 3-5 min). Collect samples from the flowing system.

8.2.3 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample from a representative area, and carefully fill sample bottles from the container.

8.2.4 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 min.

8.3 SAMPLE STORAGE/HOLDING TIMES

8.3.1 Samples must be iced or refrigerated at 4 °C and maintained at these conditions away from light until extraction. Samples must be extracted within 7 days of sampling. However, since aldehydes are subject to decay in stored samples, all samples should be derivatized and extracted as soon as possible.

NOTE: A white or blue precipitate is likely to occur. This is normal and does not indicate any problem with sample collection or storage.

8.3.2 Extracts (Sect. 11.1.11) must be stored at 4 °C or less away from light in glass vials with Teflon-lined caps. Extracts must be analyzed within 14 days of extraction.

8.4 FIELD REAGENT BLANKS -- Processing of a field reagent blank (FRB) is required along with each sample set. A sample set is composed of the samples collected from the same general sampling site at approximately the same time. Field reagent blanks are prepared at the laboratory before sample vials are sent to the field. At the laboratory, fill a sample container with reagent water (Sect. 7.2), add sample preservatives as described in Section 8.1.2, seal and ship to the sampling site along with the empty sample containers. FRBs should be confirmed to be free (less than 1/2 the MRL) of all method analytes prior to shipping them to the field. Return the FRB to the laboratory with filled sample bottles. **DO NOT OPEN THE FRB AT THE SAMPLING SITE.** If any of the analytes are detected at concentrations equal to or greater than 1/2 the MRL , then all data for the problem analyte(s) should be considered invalid for all samples in the shipping batch.

9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. Minimum QC requirements are initial demonstration of laboratory capability (which includes calculation of the MDL), analysis of laboratory reagent blanks, laboratory fortified blanks, field reagent blanks, laboratory fortified sample matrices, and QC samples. Additional QC practices are encouraged.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) -- Requirements for the initial demonstration of laboratory capability are described in the following sections and summarized in Section 17, Table 7.

9.2.1 Initial demonstration of low system background. (See Sect. 9. 3)

9.2.2 Initial demonstration of precision. Prepare, derivatize, extract, and analyze 4-7 replicate LFBs fortified at 20 ug/L, or other mid-range concentration, over a period of at least 2 days. Generating the data over a longer period of time, e.g. 4 or 5 days may produce a more realistic indication of day to day laboratory performance. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 Initial demonstration of accuracy. Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.4 MDL[1] determination. Replicate analyses for this procedure should be done over at least 3 days (both the sample derivatization/extraction and the GC analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs at a concentration estimated to be near the MDL. This concentration may be estimated by selecting a concentration at 2-5X the noise level. Analyze the seven replicates through all steps of Section 11. Calculate the MDL

$$MDL = St_{(n-1,\ 1-\ alpha\ =\ 0.99)}$$

where:

$t_{(n-1,1-alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations.

9.2.5 Minimum Reporting Level (MRL) -- Although an MDL can be calculated for analytes that commonly occur as background contaminants, the calculated MDLs should not be used as the MRL for each analyte. Method analytes that are seen in the background (typically formaldehyde, acetaldehyde) should be reported as present in field samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentration + 3σ, or three times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as a minimum reporting level in order to avoid reporting false positive results.

9.3 LABORATORY REAGENTS BLANKS (LRB) -- Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Because background contamination is a significant problem for several method analytes, it is highly recommended that the analyst maintain a historical record of LRB data. If target analytes are detected in the LRB at concentrations equal to or greater than 1/2 the MRL (Sect. 9.2.5), then all data for the problem analyte(s) should be considered invalid for all samples in the extraction batch.

9.4 CONTINUING CALIBRATION CHECK/LABORATORY FORTIFIED BLANK -- Since this methodology is based on procedural standard calibration, a LFB and the calibration check sample (CCC) are prepared and analyzed in the same manner. Laboratory fortified blank QC requirements are therefore omitted. Calibration procedure options and the QC acceptance criteria associated with them are fully described in Sect 10.3. Please refer to that section for these criteria.

9.5 INTERNAL STANDARD--The analyst must monitor the IS response peak area of all injections during each analysis day. A mean IS response is determined from the five point calibration curve. The IS response for any chromatographic run should not deviate from this mean IS response by more than 30%. If a deviation greater than 30% occurs with an individual extract inject a second aliquot of that extract.

9.5.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.5.2 If a deviation of greater than 30% is obtained for the reinjected extract, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.5, recalibration is in order per Section 10. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.6 SURROGATE RECOVERY--The surrogate standard is fortified into the aqueous portion of all calibration standards, samples, FRBs and LRBs. The surrogate is a means of assessing method performance from derivatization to final chromatographic measurement.

9.6.1 When surrogate recovery from a sample, blank, or CCC is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument

performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.6.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.6.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.6.1, recalibration is in order per Section 10. If the calibration standard is acceptable, it may be necessary to extract another aliquot of sample if sample holding time has not been exceeded. If the sample reextract also fails the recovery criterion, report all data for that sample as suspect.

9.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM)

9.7.1 Within each analysis set, a minimum of one field sample is fortified as a LFM for every 20 samples analyzed. The LFM is prepared by spiking a sample with an appropriate amount of the calibration standard. The concentrations 5, 10, and 20 ug/L are suggested spiking concentrations. Select the spiking concentration that is closest to, and at least twice the matrix background concentration. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate vial of a sample that has already been analyzed, aids in the selection of appropriate spiking levels.

9.7.2 Calculate the percent recovery (R) for each analyte, after correcting the measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.,

$$R = \frac{(A-B)}{C} * 100$$

where C is the fortified concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion.

9.7.3 Recoveries may exhibit a matrix dependence. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the accuracy problem encountered with the fortified sample is judged to be

matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects. Repeated failure to meet the suggested recovery criteria indicates potential problems with the extraction procedure and should be investigated.

9.8 FIELD DUPLICATES -- Within each analysis batch, a minimum of one field sample should be analyzed in duplicate. Duplicate sample analyses serve as a check on sampling and laboratory precision.

9.8.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) as shown below.

$$RPD = \frac{FD1 - FD2}{(FD1 + FD2)/2} * (100)$$

9.8.2 Relative percent differences for laboratory duplicates should fall in the range of ± 30 %. Greater variability may be observed for target analytes with concentrations near their MRL.

9.9 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy (60-140% of the expected value), check the entire analytical procedure to locate and correct the problem source.

9.10 ASSESSING*(Z/E)* RATIOS -- In addition to monitoring analyte response from CCC/LFB, the ratio of the peak areas of each isomer pair should be monitored. When samples and standards are processed and analyzed by exactly the same procedure, the ratio of the *(Z/E)* isomers produced by each method analyte will be reproducible. This information can be used as a QC check to avoid biased results caused by an interferant with one isomer of the pair. Calculate and record the ratio of the peak area of the first eluting isomer (designated *(E)*) to the second eluting isomer (designated *(Z)*). This ratio will be used in data evaluation Section 12.4.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed, and is required intermittently throughout sample analysis. After initial calibration is successful, the analyst may choose one of two options for maintaining on-going calibration. The first option is to verify the initial calibration daily using a minimum of 2 calibration standards. The other option is daily calibration of the method with all 5 calibration standards. These options are further described in Section 10.3.

10.2 INITIAL CALIBRATION CURVE

10.2.1 Establish GC operating parameters equivalent to the suggested specifications in Section 17, Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other GC columns or GC conditions may be used if equivalent or better performance can be demonstrated.

10.2.2 Five calibration standards are recommended to calibrate over the range of approximately 2-40 ug/L. The lowest level standard will depend upon the level of blank contamination for each analyte (Sect. 7.11.6).

10.2.3 Prepare each calibration standard by the procedural standard calibration method. Method analytes are fortified into reagent water and carried through the entire extraction and derivatization procedure described in Section 11.

10.2.4 Inject 1 uL of each calibration standard extract into the GC and tabulate peak area response and concentration for each analyte and the internal standard. **NOTE:** The formaldehyde peak will be much larger (for the same concentration) than the other analyte peaks. The formaldehyde peak may need to be attenuated on some instruments/data systems to avoid signal saturation.

10.2.5 *(Z/E)* ISOMERS -- Two isomers, referred to as *(E)* and *(Z)*, are formed for most asymmetrical carbonyl compounds derivatized with PFBHA. Chromatographic resolution is usually obtained with the columns suggested in Section 6.6 for acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, and crotonaldehyde (see chromatograms in Fig. 1 and Fig 2). With dicarbonyl species such as glyoxal and methyl glyoxal, *(E)* and *(Z)* isomerism occurs from oxime formation with both carbonyl groups, increasing the number of isomers. The demonstration data included in this method has used two distinct isomer peaks each for glyoxal and methyl glyoxal. Use one of the following methods for both calibration and quantitation of each method analyte.

(a) Use the sum of the isomer peak areas for each constituent for both calibration and quantitation.

(b) Use the peak area of each individual isomer to independently calculate a concentration for each isomer. Then average the amount of the two isomers to report one value for the analyte.

10.2.6 Generate a calibration curve for each analyte by plotting the area ratios (A_a/A_{is}) against the concentration ratios (C_a/C_{is}) of the five calibration standards where:

A_a is the peak area of the analyte (or analyte isomer pair),
A_{is} is the peak area of the internal standard,
C_a is the concentration of the analyte, and
C_{is} is the concentration of the internal standard.

10.2.7 This curve must always be forced through zero and can be defined as either first or second order. Forcing zero allows for a better estimate of the background level of method analytes.

10.2.8 A data system is recommended to collect the chromatographic data, to calculate relative response factors, and calculate either linear or second order calibration curves.

10.2.9 VERIFICATION OF CALIBRATION STANDARD MATERIALS -- Analyze a LFB prepared from standard materials from a source other than those used to prepare the initial calibration curve (Sect. 3.8, QCS). Calculate the concentration of this QCS from the calibration curve. The calculated concentration of the QCS must agree within 60-140% of its true value. This step verifies the validity of calibration standard materials and the calibration curve prior to sample analyses.

10.3 OPTIONS FOR ON-GOING CALIBRATION

The time, temperature, pH, and PFBHA concentration will all affect the rate, efficiency and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters so that continuing calibration check standard criteria can be met. Some laboratories may find it more productive to prepare and analyze a calibration curve with each batch of samples. A batch of samples for this methodology should not exceed 20 samples, including field samples, FRBs, laboratory duplicates, and fortified sample matrices.

10.3.1 CONTINUING CALIBRATION CHECK (CCC) OPTION--The analyst must periodically verify calibration during the analysis of samples in order to ensure accuracy of analytical results. Prepare a minimum of one low-level (suggested concentration 2-5 ug/L) and one mid-level (suggested concentration 10-30 ug/L) calibration standard with each batch of samples. Verify calibration using these two standards, prior to analyzing any of the sample extracts from the batch. In addition, reanalyze one of these two standard extracts after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis

batch. Recovery must be within 70-130% of the true value for the mid-level standard, and within 50-150% of the true value for the low-level standard.

10.3.2 DAILY CALIBRATION OPTION -- The analyst may choose to create a new calibration curve for each batch of samples by preparing and analyzing a standard at all five calibration concentrations, with each batch of samples. If this option is selected, the calibration standard extracts should be analyzed prior to the analysis of sample extracts. To ensure that sensitivity and performance of the method has not changed significantly between sample batches , or changed since the IDC, the following performance check is required. The response (peak area) of the internal standard, surrogate and each method analyte in the mid-level standard (suggested concentration 10-30 ug/L), must be within 50-150% of the mean peak area for that analyte in the initial demonstration of precision replicates (Sect. 9.2.2). One of the calibration standard extracts must be reanalyzed after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis batch. Recovery must be within 70 to 130% of the true value for mid- and high-level calibration standards, and within 50-150% of the true value for the low-level standard (suggested concentration 2-5 ug/L).

11. PROCEDURE

11.1 SAMPLE EXTRACTION -- Once samples have been opened, process the samples straight through to step 11.1.11. There is no known "safe" stopping point once sample processing has begun. Samples are derivatized and extracted in the sample bottle in which they were collected. Transferring the sample to another container for derivatization and extraction has been shown to cause a loss of method analytes.

11.1.1 Remove the samples from storage and allow them to equilibrate to room temperature.

11.1.2 Remove 10 mL of sample and discard. Mark the level of the remaining sample volume on the outside of the bottle, for later sample volume determination.

11.1.3 Add 200 mg KHP to the sample for pH adjustment.

11.1.4 Add 20 uL surrogate solution (Sect 7.11.2.2).

11.1.5 Add 1 mL of freshly prepared PFBHA Reagent as per Section 7.10.1. Cap and swirl gently to mix.

11.1.6 Place all samples in a constant-temperature water bath set at 35 ± 2 °C for 2 hrs. Remove vials and cool to room temperature for 10 min.

11.1.7 To each vial add 0.05 mL (approximate 2 to 4 drops) of concentrated sulfuric acid. This prevents the extraction of excess reagent, which will cause chromatographic interferences.

11.1.8 Add 4 mL of hexane that contains the internal standard (as per Sect. 7.11.1.2).

11.1.9 Shake manually for 3 min. Let stand for approximately 5 min to permit phases to separate.

11.1.10 Draw off hexane layer (top layer) using a clean disposable Pasteur pipet for each sample into a smaller 8 mL vial containing 3 mL 0.2 N sulfuric acid. Shake for 30 sec and let stand for 5 min for phase separation. **NOTE:** This acid wash step further reduces the reagent and other interferants from the final extract. **Do not skip this step.**

11.1.11 Draw off top hexane layer using another clean disposable pipet for each sample and place in two 1.8 mL autosampler vials per sample. Store extra autosampler vials as a backup extract. Extracts may be stored for up to 14 days at 4 °C.

11.1.12 Sample Volume Determination -- Discard remaining water sample and hexane in each sample bottle. Fill with water to the level indicated by the mark made in Section 11.1.2. Pour the water into a 25 mL graduated cylinder and measure the volume to the nearest mL. Record the sample volume for each sample.

Alternately, if a laboratory has control over the brand and style of the sample bottles being used, the exact volume of a number of bottles from the same manufacturer and lot may be measured, and the average bottle volume minus 10 mL may be used as the sample volume for all samples using the same lot of sample bottles. A minimum of 10 % of the sample bottles obtained from the same manufacturer, from the same lot should be measured.

11.2 GAS CHROMATOGRAPHY

11.2.1 Analyze the extracts by GC/ECD. Table 1 (Sect. 17) summarizes recommended GC operating conditions and retention times observed using this method. Figure 1 illustrates the performance of the recommended primary column with the method analytes. Figure 2

illustrates the performance of the recommended confirmation column with the method analytes. Other GC columns or chromatographic conditions may be used if the requirements of Section 9 are met.

11.2.2 The width of the retention time window used to make identifications should be based on measurements of actual retention time variations of standards over the course of time. Plus or minus three times the standard deviation of the retention time for a compound can be used to calculate a suggested window size; however the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.2.3 If an analyte peak area exceeds the range of the calibration curve, the extract may be diluted with the hexane extraction solvent (that contains the internal standard) and reanalyzed. Incorporate the dilution factor into final concentration calculations. The analyst must not extrapolate beyond the calibration range established.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak (or isomer peaks) in a calibration standard or the laboratory fortified blank.

12.2 Calculate the analyte concentrations using the first or second order calibration curves generated as described in Section 10.

12.3 For any analytes that are found, adjust the calculated concentration to reflect the true sample volume determined in Section 11.1.12.

12.4 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration. If a confirmation column has been used, all identifications should be verified using the retention time data from that analysis. In addition, the *(Z/E)* isomer ratio should be within 50% of the ratio observed in standards. If the *(Z/E)* ratio does not meet these criteria, it is likely that an interferant occurred at the retention time of one of the isomer peaks. In this case, the amount indicated by the lower of the 2 isomer peaks should be reported. (This may require that the analyst recalculate the analyte amount using individual isomer peaks for quantitation.) If one peak of the isomeric pair is missing, the identification is not confirmed and should not be reported.

12.5 Analyte concentrations are reported in ug/L.

13. METHOD PERFORMANCE

13.1 Precision and accuracy data are presented in Section 17. Data are presented for three water matrices: reagent water (Table 2), chlorinated "finished" surface water (Table 4) , and untreated "raw" surface water (Table 5). These data, as well as the MDL data in Table 3, were generated in two laboratories. Data in Table 2 and column A of Table 3, were generated in one laboratory, while data in column B of Table 3 and in Tables 4 and 5 were generated in a second laboratory. Method performance in both laboratories was similar.

13.2 DERIVATIZATION PARAMETERS -- This method is a procedural standard method that will generate accurate and precise results when used as written. The time, temperature, pH, and PFBHA concentration will all affect the rate, efficiency and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters. Some laboratories may need to prepare and analyze a calibration curve with each batch of samples. Of all the method analytes, glyoxal, methyl glyoxal, benzaldehyde, crotonaldehyde and cyclohexanone are the most difficult to derivatize. Poor sensitivity for any of these compounds indicates that there may be a problem with the reaction conditions. Measurements of nonanal, decanal, glyoxal and methyl glyoxal appear to be less precise than the measurement of other analytes.

13.3 The importance of low background levels of formaldehyde and acetaldehyde cannot be overemphasized. Some laboratories or reagent waters may also contain background amounts of other method analytes. Care must be taken to avoid reporting false positive results that result from background contamination.

13.4 The importance of proper sample collection and preservation also cannot be overemphasized. Holding time studies in various matrices showed better than 70% recovery of all method analytes when samples were collected, preserved, and stored according to Section 8, and analyzed within 7 days. There were variations in the recovery of analytes from fortified samples from different matrices. Therefore, it is strongly recommended that samples be analyzed as soon as possible after collection. The data in Section 17, Table 6 illustrates the dramatic difference between a preserved and a non-preserved sample.

13.5 Data for crotonaldehyde is not listed in Section 17, Tables 4-6, because it was not included in the standard mixtures being used at the time that those data were collected. However, crotonaldehyde was included in many other studies not presented here, and its performance was similar to other method analytes.

14. POLLUTION PREVENTION

14.1 This method uses a micro-extraction procedure which requires very small quantities of organic solvents.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1 Due to the nature of this method tHere is little need for waste management. Only small volumes of solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND RETENTION DATA FOR ANALYTE DERIVATIVES

	Column A		Column B	
ANALYTE	RT (min)	Peak #, Fig 1	RT (min)	Peak #, Fig.2
Formaldehyde	10.69	2	13.35	2
Acetaldehyde (E)	14.23	3	16.71	3
Acetaldehyde (Z)	14.49	4	17.00	4
Propanal (E)	17.36	5	19.59	5
Propanal (Z)	17.62	6	19.86	6
Butanal (E)	20.58	7	22.62	7
Butanal (Z)	20.81	8	22.88	8
Crotonaldehyde (E)	22.67	9	24.79	not shown
Crotonaldehyde (Z)	23.00	10	25.31	not shown
Pentanal (E)	23.82	11	25.54	9
Pentanal (Z)	24.01	12	25.70	10
Hexanal (E)	26.99	13	28.74	11
Hexanal (Z)	27.16	14	28.93	12
Cyclohexanone	29.42	15	31.40	13
Heptanal (E)	30.05	16	31.64	14
Heptanal (Z)	30.16	17	31.78	15
Octanal (E)	33.01	19	34.45	16
Octanal (Z)	33.09	20	34.57	18
Benzaldehyde	33.88	21	36.74	19
Nonanal	35.89	22	37.18	20
Decanal	38.63	23	39.80	21

	Column A		Column B	
ANALYTE	RT (min)	Peak #, Fig 1	RT (min)	Peak #, Fig.2
Glyoxal (peak1)	40.87	24	43.74	22
Glyoxal (peak 2)	41.09	25	43.86	23
Methyl glyoxal (peak 1)	41.22	26	43.86	24
Methyl glyoxal (peak 2)	41.88	27	44.38	25
1.2 dibromopropane (Internal standard)	6.14	1	7.84	1
2',4',5' trifluoro-acetophenone (Surrogate)	32.84	18	34.45	17

Column A- DB-5ms, 30 m x 0.25mm i.d., 0.25 um film thickness, injector temp. 220 °C, head pressure 15 psi, detector temp. 300 °C, splitless injection, 1 min split delay. Temperature program: 50 °C for 1 min, program at 4 °C/min to 220 °C, program at 20 °C/min to 250 °C and hold at 250 °C for 10 min. (Figure 1)

Column B- Rtx-1701, 30 m x 0.25 mm i.d., 0.25 um film thickness, injector temp. 180 °C, head pressure 15 psi, detector temp. 300 °C,splitless injection, 1 min split delay. Temperature program: 50 °C for 1 min, program at 4 °C/min to 220 °C, program at 20 °C/min to 250 °C and hold at 250 °C for 10 min. (Figure 2)

Carrier gas- Helium

Detector gas- P5 Argon/Methane

TABLE 2. PRECISION AND ACCURACY IN REAGENT WATER (n=8) [a]

ANALYTE	Fortified Conc. (ug/L)	Mean Conc. Measured (ug/L)	Standard Deviation	Relative Std Dev (%)	Mean Accuracy (%)
Formaldehyde	20.0	19.5	0.792	4.0	98
Acetaldehyde	20.0	19.2	0.784	4.1	96
Propanal	20.0	19.5	0.873	4.5	97
Butanal	20.0	19.8	1.12	5.6	99
Crotonaldehyde	20.0	19.8	1.11	5.6	99
Pentanal	20.0	20.0	1.10	5.5	100
Hexanal	20.0	19.7	1.25	6.4	98
Cyclohexanone	20.0	19.2	0.729	3.8	96
Heptanal	20.0	19.2	1.37	7.1	98
Octanal	20.0	19.1	1.32	6.9	95
Benzaldehyde	20.0	19.1	0.362	1.9	95
Nonanal	20.0	18.8	1.09	5.8	94
Decanal	20.0	18.7	1.20	6.4	93
Glyoxal	20.0	18.4	0.571	3.1	92
Methyl glyoxal	20.0	17.9	1.36	7.6	90

a- Analyzed over 2 days on the primary chromatographic column (DB-5ms).

TABLE 3. METHOD DETECTION LIMITS IN REAGENT WATER (n=8)

ANALYTE	Fortified Conc. (ug/L)	MDL(ug/L) Column A	MDL(ug/L) Column B
Formaldehyde	2.0	0.36	0.11
Acetaldehyde	2.0	0.21	0.14
Propanal	2.0	0.41	0.06
Butanal	2.0	0.35	0.12
Crotonaldehyde	2.0	0.28	0.09
Pentanal	2.0	0.47	0.17
Hexanal	2.0	0.42	0.35
Cyclohexanone	2.0	0.29	0.10
Heptanal	2.0	0.43	0.71
Octanal	2.0	0.60	0.12
Benzaldehyde	2.0	0.31	0.06
Nonanal	2.0	0.74	0.40
Decanal	2.0	1.0	0.82
Glyoxal	2.0	0.59	0.21
Methyl glyoxal	2.0	0.81	0.22

Column A= DB-5ms

Column B= Rtx-1701

TABLE 4. PRECISION AND ACCURACY IN CHLORINATED TAP WATER(n=4) [a]

ANALYTE	Fortified Conc. (ug/L)	Mean Conc. Measured (ug/L)	Standard Deviation	Relative Std Dev (%)	Mean Accuracy (%)
Formaldehyde	20.0	21.6	0.197	0.9	108
Acetaldehyde*	20.0	19.6	0.263	1.3	98
Propanal	20.0	19.5	0.178	0.9	98
Butanal	20.0	20.2	0.254	1.3	101
Pentanal	20.0	20.3	0.318	1.6	101
Hexanal	20.0	20.2	0.382	1.9	101
Cyclohexanone	20.0	20.7	0.247	1.2	103
Heptanal	20.0	20.5	3.073	2.5	103
Octanal	20.0	20.3	0.979	4.8	102
Benzaldehyde	20.0	21.2	0.432	2.0	106
Nonanal	20.0	20.0	0.828	4.2	100
Decanal	20.0	19.8	1.203	6.1	99
Glyoxal	20.0	22.3	0.783	3.5	112
Methyl glyoxal	20.0	21.0	0.775	3.7	105

a- Analyzed on the primary chromatographic column (DB-5ms).
*- Values taken from the confirmation column.

TABLE 5. PRECISION AND ACCURACY IN UNTREATED SURFACE WATER (n=4) [a]

ANALYTE	Fortified Conc. (ug/L)	Mean Conc. Measured (ug/L)	Standard Deviation	Relative Std Dev (%)	Mean Accuracy (%)
Formaldehyde	20.0	19.4	0.941	4.8	97
Acetaldehyde*	20.0	20.0	0.891	4.4	100
Propanal	20.0	19.5	0.966	5.0	97
Butanal	20.0	18.9	0.965	5.1	94
Pentanal	20.0	18.7	1.00	5.4	93
Hexanal	20.0	18.2	1.080	6.0	91
Cyclohexanone	20.0	17.3	0.943	5.4	87
Heptanal	20.0	18.0	0.995	5.5	90
Octanal	20.0	18.3	1.017	5.6	92
Benzaldehyde	20.0	17.2	1.118	6.5	86
Nonanal	20.0	18.4	0.881	4.8	92
Decanal	20.0	18.1	0.908	5.0	90
Glyoxal	20.0	20.5	1.10	5.4	102
Methyl glyoxal	20.0	23.3	0.799	3.4	116

a- Analyzed on the primary chromatographic column (DB-5ms).
*- Values taken from the confirmation column.

TABLE 6. HOLDING TIME DATA FOR SAMPLES FROM AN UNTREATED SURFACE WATER SOURCE, FORTIFIED WITH METHOD ANALYTES AT 20 ug/L, WITH AND WITHOUT COPPER SULFATE BIOCIDE

	% Recovery without Copper Sulfate				% Recovery with Copper Sulfate			
Analyte	Day 0	Day 6	Day 14	Day 21	Day 0	Day 6	Day 14	Day 21
Formaldehyde	104	144	569	619	105	105	109	106
Acetaldehyde	96	23	21	24	98	99	103	98
Propanal	94	21	19	22	98	98	103	96
Butanal	92	20	18	21	99	98	102	91
Pentanal	87	19	16	21	96	98	100	94
Hexanal	83	21	17	22	93	97	100	92
Cyclohexanone	94	99	84	78	96	101	98	94
Heptanal	83	20	16	17	96	94	97	91
Octanal	82	18	<10	11	99	96	96	93
Benzaldehyde	94	83	74	79	98	100	104	92
Nonanal	72	15	<10	<10	104	98	92	84
Decanal	50	<10	<10	<10	107	101	93	82
Glyoxal	103	98	37	<10	106	108	106	90
Methyl glyoxal	108	68	<10	<10	111	105	94	73

- All samples were stored headspace free at 4 °C.
- Values at all time points are the mean of 5 replicate analyses. RSDs for replicate analyses of samples containing copper sulfate were <10%. RSDs for unpreserved samples were higher due to the degradation process.

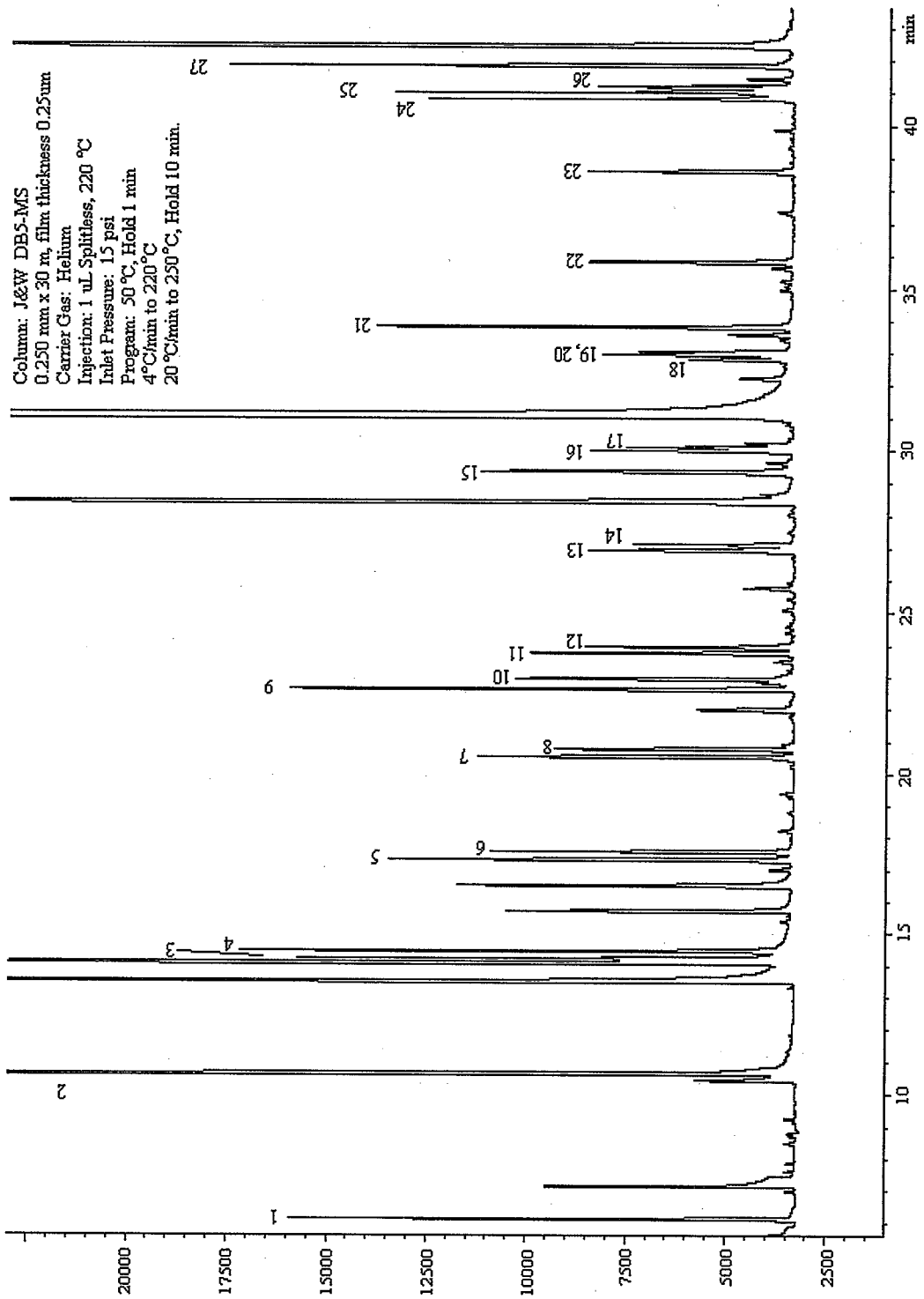
TABLE 7. INITIAL DEMONSTRATION OF CAPABILITY REQUIREMENTS

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3	Initial Demonstration of Low System Background	Analyze method blank and determine that all target analytes are below 1/2 the MRL prior to performing IDC	The LRB concentration must be ≤ 1/2 of the intended MRL
Sect 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LRBs fortified at 20.0 ug/L (or mid cal.) on at least 2 different days	RSD must be ≤ 20 %
Sect. 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery of IDP replicates	Mean recovery ± 20% of true value
Sect. 9.2.4	Method Detection Limit (MDL) Determination	a) select a fortifying level at 2 - 5 x the noise level b) analyze 7 replicates in reagent water taken thru all steps c) calculate MDL via equation - do not subtract blank d) replicate extractions and analyses must be conducted over at least 3 days	
Sect. 9.2.5	Minimum Reporting Levels (MRLs)	MRLs should be established for all analytes during IDC, and be updated as additional LRB data is available.	Establish the MRL for each analyte, as the LRB concentration + 3σ or 3 times the mean LRB concentration, whichever is greater.

TABLE 8. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 10.2	Initial Calibration	Use internal standard technique to generate curve with five standards that span the approximate range of 2-40 ug/L. First or second order curves must be forced through zero. Either sum E/Z isomer areas or average the amount of each isomer. Calculate E/Z ratios for analytes. Run QCS.	QCS must agree within 60 -140 %. Lowest concentration should be near MRL.
Sect. 9.3	Laboratory Reagent Blank (LRB)	Include LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free of interferences.	All analytes < 1/2 MRL
Sect. 10.3.1	Continuing Calibration Check (CCC) Option	Verify initial calibration by running CCCs prior to analyzing samples, after 10 samples, and after the last sample.	Recovery for mid-level CCC must be 70-130% of the true value, recovery for low level must be 50-150% of the true value.
Sect. 10.3.2	Daily Calibration Option	Calibrate daily, but verify that sensitivity and performance have not changed significantly since IDC.	Peak areas for IS, SUR, and method analytes for mid-level CAL std must be +/- 50% of the peak areas obtained for that CAL std during IDC.
Sect. 8.4	Field Reagent Blanks (FRB)	1 per shipping batch	All analytes < 1/2 MRL
Sect. 9.5	Internal Standard (IS)	1,2-Dibromopropane is added to all samples, blanks and standards	IS area counts must be 70 - 130% of the average initial calibration area counts

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.6	Surrogate Standard (SUR)	2',4',5' -Trifluoroacetophenone is added samples, blanks and standards	Surrogate recovery must be 70 - 130 % of the true value.
Sect. 9.7	Laboratory Fortified Sample Matrix (LFM)	Fortify at least one sample per analysis batch (20 samples or less) at a concentration close to that in the native sample.	Recoveries not within 70-130% may indicate matrix effect
Sect. 9.8	Field Duplicates	Extract and analyze at least one duplicate sample with every analysis batch (20 samples or less)	Suggested RPD ≤ 30 %
Sect. 9.9	Quality Control Sample (QCS)	Analyze a QCS at least quarterly from an external/second source.	QCS must agree within 60 -140 %.
Sect.9.10, Sect. 10.2.5 and Sect. 12.4	E/Z Isomer Ratio Agreement	Calculate the E/Z isomer ratio for target analytes and compare to E/Z ratio in initial calibration	E/Z ratio in standards, blanks, and samples must be within ± 50% of E/Z ratio in initial calibration. Do not report value if one isomer is missing.
Sect. 8.3.1	Sample Holding Time	Properly preserved samples may be stored in the dark at 4 °C for 7 days.	Do not report data for samples that have exceeded their holding time, or that have not been properly preserved or stored.
Sect. 8.3.2	Extract Holding Time	Extracts may be stored in the dark at 4 °C for 14 days.	Do not report data for extracts that have exceeded their holding time.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0.250 mm x 30 m, film thickness 0.25um
Carrier Gas: Helium
Injection: 1 uL Splitless, 220 °C
Inlet Pressure: 15 psi
Program: 50 °C, Hold 1 min
4°C/min to 220 °C
20 °C/min to 250 °C, Hold 10 min.
min
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17500
15000
12500
10000
7500
5000
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Figure 1

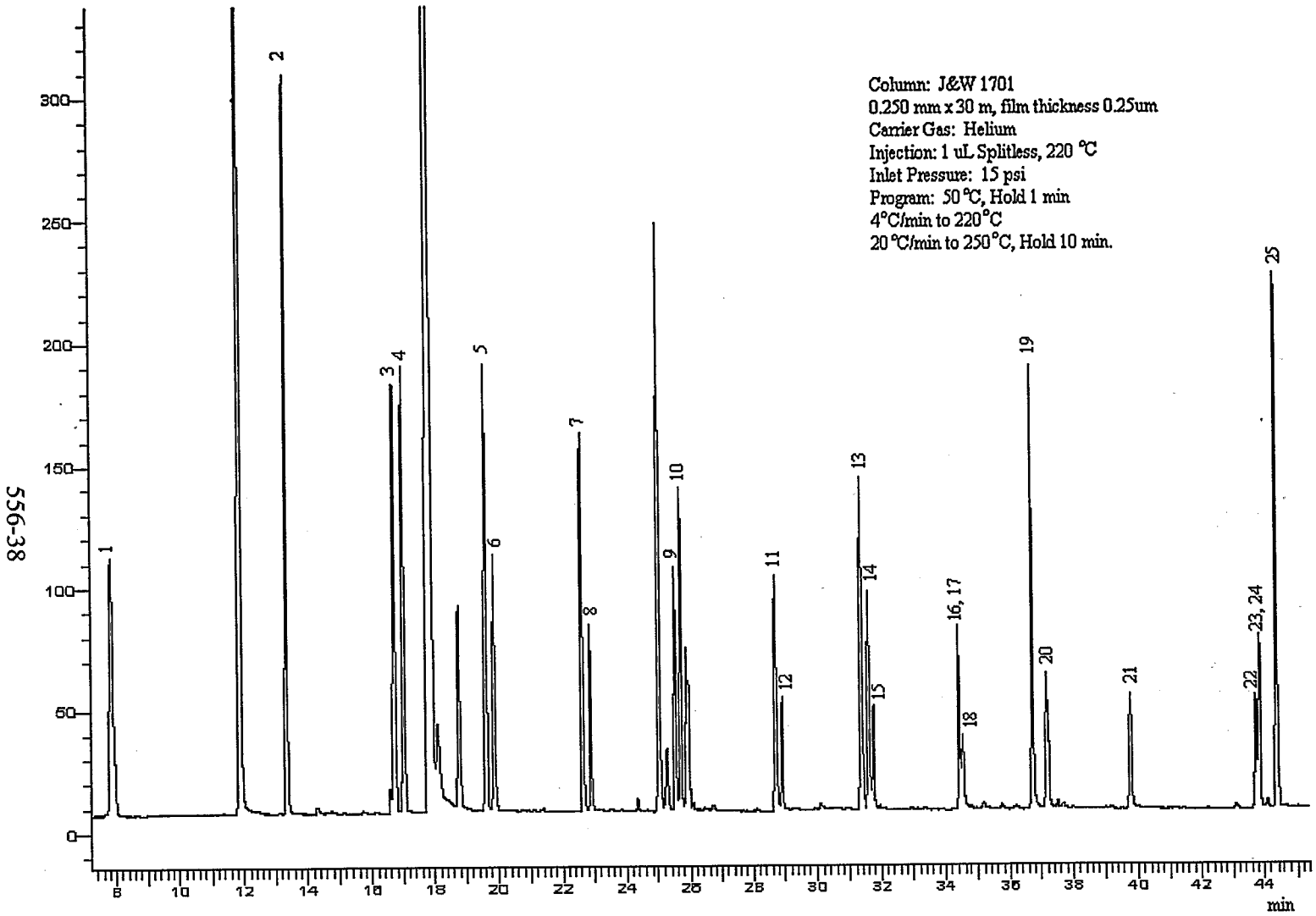
Column: J&W 1701
0.250 mm x 30 m, film thickness 0.25um
Carrier Gas: Helium
Injection: 1 uL Splitless, 220 °C
Inlet Pressure: 15 psi
Program: 50 °C, Hold 1 min
4°C/min to 220°C
20 °C/min to 250°C, Hold 10 min.
556-38
min

Figure 2

METHOD 556.1 DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY FAST GAS CHROMATOGRAPHY

Revision 1.0

September 1999

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D.J. Munch, USEPA, Office of Ground Water and Drinking Water and
S.D. Winslow, S.C. Wendelken, B.V. Pepich, ICF Kaiser Engineers, Inc. - Method 556, Revision 1.0 (1998)

S.C. Wendelken and B.V. Pepich (IT Corporation) and D.J. Munch, USEPA, Office of Ground Water and Drinking Water

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METHOD 556.1

DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY FAST GAS CHROMATOGRAPHY

1. SCOPE AND APPLICATION

1.1 This is a fast gas chromatographic method optimized for the determination of selected carbonyl compounds in finished drinking water and raw source water. The analytes applicable to this method are derivatized to their corresponding pentafluorobenzyl oximes. The oxime derivatives are then extracted from the water with hexane. The hexane extracts are analyzed by fast gas chromatography with electron capture detection (FGC-ECD). Fast GC uses a small diameter capillary column (≤ 100 μm i.d.), hydrogen carrier gas and a fast oven ramp rate to dramatically decrease analysis time. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds:

Analyte	Chemical Abstract Services Registry Number
Formaldehyde	50-00-0
Acetaldehyde	75-07-0
Propanal	123-38-6
Butanal	123-72-8
Pentanal	110-62-3
Hexanal	66-25-1
Heptanal	111-71-7
Octanal	124-13-0
Nonanal	124-19-6
Decanal	112-31-2
Cyclohexanone	108-94-1
Benzaldehyde	100-52-7
Glyoxal (ethanedial)	107-22-2
Methyl glyoxal (2-oxopropanal or pyruvic aldehyde)	78-98-8

1.2 This method applies to the determination of target analytes over the concentration ranges typically found in drinking water. Analyte retention times are in Section 17, Tables 1 and 2. Other method performance data are presented in Section 17, Tables 3-7. Experimentally determined method detection limits (MDLs) for the above listed analytes are provided in Section 17, Table 4. The MDL is defined as the statistically calculated minimum amount that can be measured with 99%

confidence that the reported value is greater than zero.[1,2] However, it should be noted that background levels of some method analytes (usually formaldehyde and acetaldehyde) are problematic. The minimum reporting level (MRL) for method analytes, for each analyst/laboratory that uses this method, will depend on their ability to control background levels (Section 4).

1.3 This method is restricted to use by or under the supervision of analysts skilled in liquid-liquid extractions, derivatization procedures and the use of GC and interpretation of gas chromatograms. The analyst should be thoroughly familiar with the practice of fast GC before significantly modifying the conditions in Table 1. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedures described in Section 9.

2. SUMMARY OF METHOD

2.1 A 20 mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 °C for 2 hr with 15 mg of O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL hexane. The extract is processed through an acidic wash step, and then analyzed by FGC-ECD. The target analytes are identified and quantified by comparison to a procedural standard (Section 3.9). Two chromatographic peaks will be observed for many of the target analytes. Both *(E)* and *(Z)* isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. The *(E)* and *(Z)* isomers may not be chromatographically resolved in a few cases. Compounds with two carbonyl groups, such as glyoxal and methyl glyoxal, can produce even more isomers. See Section 17, Table 1 and Figure 1 for the chromatographic peaks used for analyte identification.

NOTE: The absolute identity of the *(E)* and *(Z)* isomers was not determined during method development. Other researchers [3,4,5] have reported the first eluting peak as *(E)*, and the second peak as *(Z)*. For convenience, this method will follow this convention. Because more than 2 isomers are formed for glyoxal and methyl glyoxal, the peaks used for identification are referred to as "peak 1" and "peak 2."

2.2 All results should be confirmed on a second, dissimilar capillary GC column.

3. DEFINITIONS

3.1 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, internal standards, and surrogates that are used with other samples. The LRB is used to

determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are introduced during sample shipping or storage. For this analysis the FRB should not be opened at the sampling site.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.5 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.7 CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 PROCEDURAL STANDARD CALIBRATION -- A calibration method where aqueous calibration standards are prepared and processed (e.g. purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

3.10 INTERNAL STANDARD (IS) -- A pure analyte added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.11 SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

3.12 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.13 MATERIAL SAFETY DATA (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.14 CONTINUING CALIBRATION CHECK (CCC) -- A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.15 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration of an analyte that should be reported. This concentration is determined by the background level of the analyte in the LRBs and the sensitivity of the method to the analyte. The MRL will be at or near the concentration of the lowest calibration standard.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in laboratory air, solvents, reagents (including reagent water), glassware, sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than 1/2 the MRL) under the

conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3. **Subtracting blank values from sample results is not permitted.**

4.1.1 Before attempting analyses by this method, the analyst must obtain a source of sufficiently pure reagent water. This water must not contain target carbonyl compounds or interferences at a concentration greater than 1/2 of the MRL. The most likely interferents are formaldehyde and acetaldehyde in the reagent water. The most successful techniques for generating aldehyde free water are (1) exposure to UV light, or (2) distillation from permanganate.

4.1.2 Commercially available systems for generating reagent grade water have proved adequate, if a step involving exposure to UV light is included. For the data presented in this method, a Millipore Elix 3 reverse osmosis system followed by a Milli-Q TOC Plus polishing unit provided reagent water with background levels of 1 µg/L or less for each method analyte. Other researchers have reported typical blank values of 1-3 µg/L.[4,5]

4.1.3 Distillation of reagent water from acidified potassium permanganate has been reported as an effective method of eliminating background levels of aldehydes.[3] Distill 500 mL of reagent water to which 64 mg potassium permanganate and 1 mL concentrated sulfuric acid have been added. In our laboratory, this procedure reduced formaldehyde levels to approximately 3 µg/L.

4.1.4 It may be necessary to purchase reagent grade water. If acceptably clean reagent grade water is purchased, care must also be taken to protect it from contamination caused by contact with laboratory air.

4.2 Formaldehyde is typically present in laboratory air and smaller amounts of other aldehydes may also be found. Care should be taken to minimize exposure of reagents and sample water with laboratory air. Because latex is a potential aldehyde contaminant source, protective gloves should not contain latex. Nitrile gloves, such as N-Dex Plus, are acceptable. Bottle caps should be made of polypropylene. Commonly used phenolic resin caps must be avoided because they can introduce formaldehyde contamination into samples.

4.3 Reagents must also be free from contamination. Many brands of solvents may contain trace amounts of carbonyl compounds.

4.4 Glassware must be scrupulously cleaned by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained, dried, and heated in a laboratory oven at 130 °C for several hours before use. Solvent rinses with methanol or acetonitrile, followed by air drying, may be substituted

for the oven heating. After cleaning, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.5 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled.

4.6 An interferant that elutes just prior to the acetaldehyde *(E)* isomer peak on the primary column is typically observed in chlorinated or chloraminated waters. If this peak interferes with the integration of the acetaldehyde *(E)* isomer peak, then acetaldehyde should be quantified using only the acetaldehyde *(Z)* isomer, or from the confirmation column data.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[6-9]

5.2 Formaldehyde and acetaldehyde have been tentatively classified as known or suspected human or mammalian carcinogens. Glyoxal and methyl glyoxal have been shown to be mutagenic in in-vitro tests.[3]

5.3 Although hydrogen can be used as a carrier gas safely, the potential for fire or explosion does exist if the gas system is mishandled. If you are unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from your instrument manufacturer regarding its use.

6. EQUIPMENT AND SUPPLIES (All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS -- Grab Sample Bottle (aqueous samples) -- 30 mL amber glass, screw cap bottles and caps equipped with Teflon-faced silicone septa. Screw caps should be polypropylene. **Typical phenolic resin caps should be avoided due to the possibility of sample contamination from formaldehyde.** Prior to use, wash bottles and septa according to Section 4.4.

6.2 VIALS -- 8 mL or 12 mL vials for the acid wash step (Section 11.1.10), and GC autosampler vials, both types must be glass with Teflon-lined polypropylene caps.

6.3 VOLUMETRIC FLASKS -- various sizes used for preparation of standards.

6.4 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.5 WATER BATH or HEATING BLOCK -- Capable of maintaining 35 ± 2 °C

6.6 GAS CHROMATOGRAPH -- Capillary Gas Chromatograph (Hewlett Packard 6890 or equivalent), equipped for fast gas chromatography. Modifications will include a high pressure (≥50 psi) split/splitless injector, fast temperature ramp (30 °C/minute) oven and a low volume (150 µL) micro ECD detector. Additionally, a data system capable of fast sampling (20 points/peak) is required.

6.6.1 Primary Column -- 10 m x 0.10 mm J&W DB-5, 0.10 µm film thickness (or equivalent).

6.6.2 Confirmation Column -- 10 m x 0.10 mm Alltech AT- 1701, 0.10 µm film thickness (or equivalent)

6.6.3 Straight injection port liners (≤ 2 mm i.d.) packed with a central 2 cm plug of silanized glass wool. Hewlett Packard PN 5181-3317 or equivalent.

7. REAGENTS AND STANDARDS

7.1 Reagent grade or better chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 REAGENT WATER -- Reagent water as free as possible from interferences and contamination is critical to the success of this method. See Section 4.1.

7.3 ACETONITRILE -- High purity, demonstrated to be free of analytes and interferences.

7.4 HEXANE -- High purity, demonstrated to be free of analytes and interferences: B&J Brand, GC^2 grade or equivalent.

7.5 POTASSIUM HYDROGEN PHTHALATE (KHP) -- ACS Grade or better.

7.6 0-(2,3,5,6-PENTAFLUOROBENZYL)-HYDROXYLAMINE HYDROCHLORIDE (PFBHA) -- 98+%, Aldrich cat. # 19,448-4. (Store in a desiccator - Do not refrigerate).

7.7 SULFURIC ACID -- ACS Grade or better.

7.8 COPPER SULFATE PENTAHYDRATE -- ACS Grade or better.

7.9 AMMONIUM CHLORIDE, NH_4Cl or AMMONIUM SULFATE, $(NH_4)_2SO_4$ – ACS grade or better.

7.10 SOLUTIONS

7.10.1 PFBHA REAGENT -- Prepare a fresh 15 mg/mL solution in reagent water daily. Prepare an amount appropriate to the number of samples to be derivatized. One mL of solution is added per sample. For example, if 14 sample vials are being extracted, prepare 15 mL of solution. For a 15 mL volume of solution, weigh 0.225 grams of PFBHA into a dry 40 mL vial, add 15 mL water and shake to dissolve.

7.10.2 0.2 N SULFURIC ACID -- Add 5 mL of concentrated sulfuric acid to 900 mL of reagent water.

7.11 STOCK STANDARD SOLUTIONS -- When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

7.11.1 INTERNAL STANDARD (IS) -- 1,2-DIBROMOPROPANE, 98+% purity. An alternate compound may be used as the IS at the discretion of the analyst. If an alternate is selected, an appropriate concentration will need to be determined.

7.11.1.1 INTERNAL STANDARD STOCK SOLUTION, (10,000 µg/mL) -- Accurately weigh approximately 0.1 gram to the nearest 0.0001g, into a tared 10 mL volumetric flask containing hexane up to the neck. After determining weight difference, fill to mark with hexane. Stock solutions can be used for up to 6 months when stored at –10 °C.

7.11.1.2 INTERNAL STANDARD FORTIFIED EXTRACTION SOLVENT, 400 µg/L in hexane -- This is the solvent used to extract the derivatized samples. The internal standard is added to the solvent prior to performing the extraction. The volume of this solvent to be prepared should be determined by the sample workload. The following example illustrates

preparation of 1 L of fortified solvent. If fewer samples are to be analyzed each month, prepare smaller batches of working solvent. Add 40 µL of internal standard stock solution directly to 1 L of hexane in a volumetric flask. Cap flask and invert three times to ensure thorough mixing. Transfer to 1 L storage bottle with Teflon lined cap. This solution can be used up to 4 weeks. As a check, run a sample of this working solvent on the FGC before the first extraction of aqueous samples. Have enough working solvent available to extract all calibration and aqueous samples in each extraction set. Never use two different batches of working solvent for one set of extractions.

7.11.2 SURROGATE (SUR) -- 2',4',5' -TRIFLUOROACETOPHENONE
This compound was found to be an appropriate surrogate analyte for these analyses. However, the chromatograms for this analysis are very crowded, and all possible matrix interferences cannot be anticipated. An alternate carbonyl compound may be selected as the surrogate analyte if matrix interferences or chromatographic problems are encountered. Any surrogate analyte selected must form an oxime derivative, because one of its purposes is to monitor the derivatization process. If an alternate surrogate is selected, its concentration may also be adjusted to meet the needs of the laboratory.

7.11.2.1 SURROGATE STOCK SOLUTION, 10,000 µg/mL -- Accurately weigh approximately 0.1 gram SUR to the nearest 0.0001g, into a 10 mL tared volumetric flask containing room temperature (25 °C) acetonitrile up to the neck. After determining weight difference, fill to mark with acetonitrile. Stock solutions can be used for up to 6 months when stored at -10 °C or less.

7.11.2.2 SURROGATE ADDITIVE SOLUTION, 20 µg/mL -- Dilute the surrogate stock solution to 20 µg/mL in acetonitrile. This solution can be used up to 3 months when stored at 4 °C or less.

7.11.3 STOCK STANDARD SOLUTION (SSS)
Prepare stock standard solutions for each analyte of interest at a concentration of 1 to 10 mg/mL. Acetonitrile should be used as the solvent for all analytes except glyoxal. Glyoxal standards should be prepared using a volumetric 90:10 acetonitrile:water mixture due to the limited solubility of glyoxal in pure acetonitrile. Method analytes may be obtained as neat materials or as ampulized solutions from commercial suppliers. The stock standard solutions should be stored at –10 °C or less and protected from light. Standards prepared in this manner were stable

for at least 60 days. Laboratories should use standard QC practices to determine when their standards need to be replaced.

7.11.3.1 For analytes which are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.1 gram of pure material to the nearest 0.0001g in a 10 mL volumetric flask. Dilute to volume with solvent.

7.11.3.2 Stock standard solutions for analytes which are liquid in their pure form at room temperature can be accurately prepared in the following manner.

7.11.3.2.1 Place about 9.8 mL of solvent into a 10- mL volumetric flask. Allow the flask to stand, unstoppered, for about 10 min to allow solvent film to evaporate from the inner walls of the volumetric, and weigh to the nearest 0.0001 gram.

7.11.3.2.2 Use a 100-µL syringe and immediately add 100 µL of standard material to the flask by keeping the syringe needle just above the surface of the solvent. Be sure the standard material falls dropwise directly into the solvent without contacting the inner wall of the volumetric.

7.11.3.2.3 Reweigh, dilute to volume, stopper, then mix by inverting several times. Calculate the concentration in milligrams per milliliter from the net gain in weight.

7.11.4 PRIMARY DILUTION STANDARD (PDS) -- The PDS for this method should include all method analytes of interest. The PDS is prepared by combining and diluting stock standard solutions with acetonitrile to a concentration of 100 µg/mL. Store at –10 °C or less and protect from light. Standards prepared in this manner were stable for at least 60 days. Laboratories should use standard QC practices to determine when their standards need to be replaced. This primary dilution standard is used to prepare calibration spiking solutions, which are prepared at 5 concentration levels for each analyte, and are used to spike reagent water to prepare the aqueous calibration standards.

7.11.5 CALIBRATION SPIKING SOLUTIONS -- Five calibration spiking solutions are prepared, each at a different concentration, and are used to spike reagent water to prepare the calibration standards. The calibration spiking solutions are prepared from the PDS. Store the calibration spiking

solutions at –10 °C or less and protect from light. Solutions prepared in this manner were stable for at least 60 days. Laboratories should use standard QC practices to determine when solutions need to be replaced. An example of how the calibration spiking solutions are prepared is given in the following table. Modifications of this preparation scheme may be made to meet the needs of the laboratory.

PREPARATION OF CALIBRATION SPIKING SOLUTIONS

Calibration Level	PDS Concentration, (µg/mL)	Volume PDS Standard, (µL)	Final Volume Calibration Spike Solution, (mL)	Final Concentration Calibration Spike Solution, (µg/mL)
1	100	250	5	5
2	100	500	5	10
3	100	1000	5	20
4	100	1500	5	30
5	100	2000	5	40

7.11.6 PROCEDURAL CALIBRATION STANDARDS -- A designated amount of each calibration spiking solution is spiked into five separate 20 mL aliquots of reagent water in a 30 mL sample container, to produce aqueous calibration standards. The reagent water used to make the calibration standards should contain the preservation reagents described in Section 8.1.2 (ammonium chloride or ammonium sulfate at 500 mg/L and copper sulfate pentahydrate at 500 mg/L). Aqueous calibration standards are processed and analyzed according to the procedures in Section 11. Resulting data are used to generate a calibration curve. An example of the preparation of aqueous calibration standards is given below. The lowest concentration calibration standard must be at or below the MRL. Modifications of this preparation scheme may be made to meet the needs of the laboratory. Preparing aqueous calibration standards using varying volumes of one calibration spiking solution is an acceptable alternative to the example below.

PREPARATION OF PROCEDURAL (AQUEOUS) CALIBRATION STANDARDS

Calibration Level	Calibration Spike Solution Concentration, (µg/mL)	Volume Calibration Spike Solution, (µL)	Final Volume Calibration Standard (mL)	Final Concentration Calibration Standard (µg/L)
1	5	20	20	5
2	10	20	20	10
3	20	20	20	20
4	30	20	20	30
5	40	20	20	40

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE VIAL PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [7] using amber glass 30 mL containers with PTFE-lined screw-caps, or caps with PTFE-faced silicon septa.

8.1.2 Prior to shipment to the field, 15 mg of copper sulfate pentahydrate must be added to each bottle. This material acts as a biocide to inhibit bacteriological decay of method analytes. If samples to be collected contain free chlorine, then 15 mg of ammonium chloride or ammonium sulfate must also be added to the bottle prior to sample collection. The ammonium compound will react with the free chlorine to form monochloramine, and retard the formation of additional carbonyl compounds. Add these materials as dry solids to the sample bottle. The stability of these materials in concentrated aqueous solution has not been verified.

NOTE: Aldehydes have been demonstrated to be extremely susceptible to microbiological decay. The use of other chlorine reducing agents such as sodium thiosulfate or ascorbic acid, has also been shown to produce invalid data. Proper sample collection and preservation is important to obtaining valid data. The data in Section 17, Table 7 illustrates the importance of proper sample preservation.

8.2 SAMPLE COLLECTION

8.2.1 Fill sample bottles to just overflowing but take care not to flush out the sample preservation reagents. The capped sample should be head-space free.

8.2.2 When sampling from a water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap, and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.3 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample from a representative area, and carefully fill sample bottles from the container.

8.2.4 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 minute.

8.3 SAMPLE STORAGE/HOLDING TIMES

8.3.1 Samples must be iced or refrigerated at 4 ± 2 °C and maintained at these conditions away from light until extraction. Samples must be extracted within 7 days of sampling. However, since aldehydes are subject to decay in stored samples, all samples should be derivatized and extracted as soon as possible.

NOTE: A white or blue precipitate is likely to occur. This is normal and does not indicate any problem with sample collection or storage.

8.3.2 Extracts (Section 11.1.11) must be stored at 4 ± 2 °C away from light in glass vials with Teflon-lined caps. Extracts must be analyzed within 14 days of extraction.

8.4 FIELD REAGENT BLANKS -- Processing of a field reagent blank (FRB) is required along with each sample set. A sample set is composed of the samples collected from the same general sampling site at approximately the same time. Field reagent blanks are prepared at the laboratory before sample vials are sent to the field. At the laboratory, fill a sample container with reagent water (Section 7.2), add sample preservatives as described in Section 8.1.2, seal and ship to the sampling site along with the empty sample containers. FRBs should be confirmed to be free (less than 1/2 the MRL) of all method analytes prior to shipping them to the field. Return the FRB to the laboratory with filled sample bottles. **DO NOT OPEN THE FRB AT THE SAMPLING SITE.** If any of the analytes are detected at concentrations equal to or greater than 1/2 the MRL , then all data for

the problem analyte(s) should be considered invalid for all samples in the shipping batch.

9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. Minimum QC requirements are initial demonstration of laboratory capability (which includes calculation of the MDL), analysis of laboratory reagent blanks, laboratory fortified blanks, field reagent blanks, laboratory fortified sample matrices, and QC samples. Additional QC practices are encouraged.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) -- Requirements for the initial demonstration of laboratory capability are described in the following sections and summarized in Section 17, Table 8.

9.2.1 Initial demonstration of low system background. (Section 9. 3)

9.2.2 Initial demonstration of precision. Prepare, derivatize, extract, and analyze 4-7 replicate LFBs fortified at 20 μg/L, or other mid-range concentration, over a period of at least 2 days. Generating the data over a longer period of time, e.g., 4 or 5 days may produce a more realistic indication of day to day laboratory performance. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 Initial demonstration of accuracy. Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.4 MDL determination[1,2]. Replicate analyses for this procedure should be done over at least 3 days (both the sample derivatization/extraction and the GC analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs at a concentration estimated to be near the MDL. This concentration may be estimated by selecting a concentration at 2-5X the noise level. Analyze the seven replicates through all steps of Section 11. Calculate the MDL

$$MDL = St_{(n-1,\ 1-alpha\ =\ 0.99)}$$

where:
$t_{(n-1,1-alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations.

9.2.5 Minimum Reporting Level (MRL) -- Although an MDL can be calculated for analytes that commonly occur as background contaminants, the calculated MDLs should not be used as the MRL for each analyte. Method analytes that are seen in the background (typically formaldehyde, acetaldehyde) should be reported as present in field samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentration + 3σ, or three times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as a minimum reporting level in order to avoid reporting false positive results.

9.3 LABORATORY REAGENTS BLANKS (LRB) -- Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Because background contamination is a significant problem for several method analytes, it is highly recommended that the analyst maintain a historical record of LRB data. If target analytes are detected in the LRB at concentrations equal to or greater than 1/2 the MRL (Section 9.2.5), then all data for the problem analyte(s) should be considered invalid for all samples in the extraction batch.

9.4 CONTINUING CALIBRATION CHECK/LABORATORY FORTIFIED BLANK -- Since this methodology is based on procedural standard calibration, a LFB and the calibration check sample (CCC) are prepared and analyzed in the same manner. Laboratory fortified blank QC requirements are therefore omitted. Calibration procedure options and the QC acceptance criteria associated with them are fully described in Section 10.3. Please refer to that section for these criteria.

9.5 INTERNAL STANDARD--The analyst must monitor the IS response peak area of all injections during each analysis day. A mean IS response is determined from the five point calibration curve. The IS response for any chromatographic run should not deviate from this mean IS response by more than 30%. If a deviation greater than 30% occurs with an individual extract, inject a second aliquot of that extract.

9.5.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.5.2 If a deviation of greater than 30% is obtained for the reinjected extract, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria

of Section 10.3, recalibration is in order per Section 10. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.6 SURROGATE RECOVERY--The surrogate standard is fortified into the aqueous portion of all calibration standards, samples, FRBs and LRBs. The surrogate is a means of assessing method performance from derivatization to final chromatographic measurement.

9.6.1 When surrogate recovery from a sample, blank, or CCC is <70% or >130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

9.6.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.6.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.6.1, recalibration is in order per Section 10. If the calibration standard is acceptable, it may be necessary to extract another aliquot of sample if sample holding time has not been exceeded. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect.

9.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM)

9.7.1 Within each analysis set, a minimum of one field sample is fortified as a LFM for every 20 samples analyzed. The LFM is prepared by spiking a sample with an appropriate amount of the calibration standard. The concentrations 5, 10, and 20 µg/L are suggested spiking concentrations. Select the spiking concentration that is closest to, but greater than the concentration in the unfortified sample. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate vial of a sample that has already been analyzed, aids in the selection of appropriate spiking levels.

9.7.2 Calculate the percent recovery (R) for each analyte, after correcting the measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.,

$$R = \frac{(A-B)}{C} * 100$$

where C is the fortified concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion.

9.7.3 Recoveries may exhibit a matrix dependence. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects. Repeated failure to meet the suggested recovery criteria indicates potential problems with the extraction procedure and should be investigated.

9.8 FIELD DUPLICATES -- Within each analysis batch, a minimum of one field sample should be analyzed in duplicate. Duplicate sample analyses serve as a check on sampling and laboratory precision.

9.8.1 Calculate the relative percent difference (*RPD*) for duplicate measurements (L_d1 and L_d2) as shown below.

$$RPD = \frac{L_d1 - L_d2}{(L_d1 + L_d2)/2} * 100$$

9.8.2 Relative percent differences for laboratory duplicates should fall in the range of ± 30 %. Greater variability may be observed for target analytes with concentrations near their MRL.

9.9 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy (70-130% of the expected value), check the entire analytical procedure to locate and correct the problem source.

9.10 ASSESSING *(Z/E)* RATIOS -- In addition to monitoring analyte response from CCC/LFB, the ratio of the peak areas of each isomer pair should be monitored. When samples and standards are processed and analyzed by exactly the same procedure, the ratio of the *(Z/E)* isomers produced by each method analyte will be reproducible. This information can be used as a QC check to avoid biased results caused by an interferant with one isomer of the pair. Calculate and record the ratio of the peak area of the first eluting isomer (designated *(E)*) to the second eluting isomer (designated *(Z)*). This ratio will be used in data evaluation Section 12.4.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed, and is required intermittently throughout sample analysis. After initial calibration is successful, the analyst may choose one of two options for maintaining on-going calibration. The first option is to verify the initial calibration daily using a minimum of 2 calibration standards. The other option is daily calibration of the method with all 5 calibration standards. These options are further described in Section 10.3.

10.2 INITIAL CALIBRATION CURVE

10.2.1 Establish FGC operating parameters equivalent to the suggested specifications in Section 17, Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other GC columns or GC conditions may be used if equivalent or better performance can be demonstrated (Section 1.3).

10.2.2 Five calibration standards are recommended to calibrate over the range of approximately 5-40 µg/L. The lowest level standard will depend upon the level of blank contamination for each analyte (Section 7.11.6).

10.2.3 Prepare each calibration standard by the procedural standard calibration method. Method analytes are fortified into reagent water and carried through the entire extraction and derivatization procedure described in Section 11.

10.2.4 Inject 1 µL of each calibration standard extract into the FGC and tabulate peak area response and concentration for each analyte and the internal standard. **NOTE:** The formaldehyde peak will be much larger (for the same concentration) than the other analyte peaks. The formaldehyde peak may need to be attenuated on some instruments/data systems to avoid signal saturation.

10.2.5 *(Z/E)* ISOMERS -- Two isomers, referred to as *(E)* and *(Z)*, are formed for most asymmetrical carbonyl compounds derivatized with PFBHA. Chromatographic resolution is usually obtained with the columns suggested in Section 6.6 for acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, and octanal, (see chromatograms in Section 17, Figure 1 and Figure 2). With dicarbonyl species such as glyoxal and methyl glyoxal, *(E)* and *(Z)* isomerism occurs from oxime formation with both carbonyl groups, increasing the number of isomers. The demonstration data included in this method use two distinct isomer peaks each for

glyoxal and methyl glyoxal. Use one of the following methods for both calibration and quantitation of each method analyte.

(a) Use the sum of the isomer peak areas for each constituent for both calibration and quantitation.

(b) Use the peak area of each individual isomer to independently calculate a concentration for each isomer. Then average the amount of the two isomers to report one value for the analyte.

10.2.6 Generate a calibration curve for each analyte by plotting the area ratios (A_a/A_{is}) against the concentration ratios (C_a/C_{is}) of the five calibration standards where:

A_a is the peak area of the analyte (or analyte isomer pair),
A_{is} is the peak area of the internal standard,
C_a is the concentration of the analyte, and
C_{is} is the concentration of the internal standard.

10.2.7 This curve must always be forced through zero and can be defined as either first or second order. Forcing zero allows for a better estimate of the background level of method analytes.

10.2.8 A data system is required to collect the chromatographic data, to calculate relative response factors, and calculate either linear or second order calibration curves.

10.2.9 VERIFICATION OF CALIBRATION STANDARD MATERIALS -- Analyze a LFB prepared from standard materials from a source other than those used to prepare the initial calibration curve (Sections 3.8, and 9.9). Calculate the concentration of this QCS from the calibration curve. The calculated concentration of the QCS must agree within 70-130% of its true value. This step verifies the validity of calibration standard materials and the calibration curve prior to sample analyses.

10.3 OPTIONS FOR ON-GOING CALIBRATION--The time, temperature, pH, and PFBHA concentration will all affect the rate, efficiency and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters so that continuing calibration check standard criteria can be met. Some laboratories may find it more productive to prepare and analyze a calibration curve with each batch of samples. A batch of samples for this methodology should not exceed 20 samples, including field samples, FRBs, laboratory duplicates, and fortified sample matrices.

10.3.1 CONTINUING CALIBRATION CHECK (CCC) OPTION--The analyst must periodically verify calibration during the analysis of samples in order to ensure accuracy of analytical results. Prepare a minimum of one low-level (suggested concentration 2-5 µg/L) and one mid-level (suggested concentration 10-30µg/L) calibration standard with each batch of samples. Verify calibration using these two standards, prior to analyzing any of the sample extracts from the batch. In addition, reanalyze one of these two standard extracts after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis batch. Recovery must be within 70-130% of the true value for the mid-level standard, and within 50-150% of the true value for the low-level standard.

10.3.2 DAILY CALIBRATION OPTION -- The analyst may choose to create a new calibration curve for each batch of samples by preparing and analyzing a standard at all five calibration concentrations, with each batch of samples. If this option is selected, the calibration standard extracts should be analyzed prior to the analysis of sample extracts. To ensure that sensitivity and performance of the method has not changed significantly between sample batches, or changed since the IDC, the following performance check is required. The response (peak area) of the internal standard, surrogate and each method analyte in the mid-level standard (suggested concentration 10-30µg/L), must be within 50-150% of the mean peak area for that analyte in the initial demonstration of precision replicates (Section 9.2.2). One of the calibration standard extracts must be reanalyzed after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis batch. Recovery must be within 70 to 130% of the true value for mid- and high-level calibration standards, and within 50-150% of the true value for the low-level standard (suggested concentration 2-5µg/L).

11. PROCEDURE

11.1 SAMPLE EXTRACTION -- Once samples have been opened, process the samples straight through to step 11.1.11. There is no known "safe" stopping point once sample processing has begun. Samples are derivatized and extracted in the sample bottle in which they were collected. Transferring the sample to another container for derivatization and extraction has been shown to cause a loss of method analytes.

11.1.1 Remove the samples from storage and allow them to equilibrate to room temperature.

11.1.2 Remove 10 mL of sample and discard. Mark the level of the remaining sample volume on the outside of the bottle, for later sample volume determination.

11.1.3 Add 200 mg KHP to adjust the sample pH to approximately 4.

11.1.4 Add 20 µL surrogate solution (Sect 7.11.2.2).

11.1.5 Add 1 mL of freshly prepared PFBHA Reagent as per Section 7.10.1. Cap and swirl gently to mix.

11.1.6 Place all samples in a constant-temperature water bath set at 35 ± 2 °C for 2 hours. Remove vials and cool to room temperature for 10 minutes.

11.1.7 To each vial add approximately 0.05 mL (2 to 4 drops) of concentrated sulfuric acid. This prevents the extraction of excess reagent, which will cause chromatographic interferences.

11.1.8 Add 4 mL of hexane that contains the internal standard (Section 7.11.1.2).

11.1.9 Shake manually for 3 minutes. Let stand for approximately 5 minutes to permit phases to separate.

11.1.10 Draw off hexane layer (top layer) using a clean disposable Pasteur pipette for each sample into a smaller 8 mL vial containing 3 mL 0.2 N sulfuric acid. Shake for 30 seconds and let stand for 5 minutes for phase separation. **NOTE:** This acid wash step further reduces the reagent and other interferants from the final extract.

11.1.11 Draw off top hexane layer using another clean, disposable pipette for each sample and place in two 1.8 mL autosampler vials per sample. Store extra autosampler vials as a backup extract. Extracts may be stored for up to 14 days at 4 °C.

11.1.12 Sample Volume Determination -- Discard remaining water sample and hexane in each sample bottle. Fill with water to the level indicated by the mark made in Section 11.1.2. Pour the water into a 25 mL graduated cylinder and measure the volume to the nearest mL. Record the sample volume for each sample.

Alternately, if a laboratory has control over the brand and style of the 30 mL sample bottles being used, the exact volume of a number of bottles

from the same manufacturer and lot may be measured, and the average bottle volume minus 10 mL may be used as the sample volume for all samples using the same lot of sample bottles. A minimum of 10 % of the sample bottles obtained from the same manufacturer, from the same lot should be measured.

11.2 FAST GAS CHROMATOGRAPHY-- This method uses fast gas chromatography (FGC) for the analysis of the hexane extracts. Several important changes from "conventional GC" must be made to allow for the rapid analysis of the analytes. First the instrument must be capable of providing a fast temperature ramp (30 °C/minute) oven, a high pressure (≥50 psi) split/splitless injector, and a low volume (150 µL) micro ECD. Second, the column diameter, length and film thickness must all be decreased. Third, the carrier gas must be changed to a highly diffusive or "fast" gas such as hydrogen. **Although hydrogen can be used safely as a carrier gas, the potential for fire or explosion does exist if the gas system is mishandled. If you are unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from your instrument manufacturer regarding its use.** Finally, strict attention must be paid to established column installation guidelines with regard to the proper cutting and placement of the capillary columns within the instrument.

In addition to decreasing the column dimensions and changing the carrier gas, successful FGC depends on the minimization of extracolumn variance. Extracolumn variance refers to any source of bandbroadening other than those which occur within the chromatographic column itself. The major source of extracolumn variance in a properly designed FGC chromatographic system is the injector/injection process. The best chromatographic results for the oxime derivatives have been achieved using glass wool packed small i.d. liners (Section 6.6.3) combined with low split ratios. Although excellent precision and accuracy have been demonstrated using the listed chromatographic conditions (Section 17, Table 1.), it possible that the optimum conditions for a specific instrument will need to be empirically determined by the user.

11.2.1 Analyze the extracts by FGC/ECD. Tables 1 and 2 (Section 17) summarize recommended FGC operating conditions and retention times observed using this method. Figure 1 illustrates the performance of the recommended primary column with the method analytes. Figure 2 illustrates the performance of the recommended confirmation column with the method analytes. Other GC columns or chromatographic conditions may be used if the requirements of Section 9 are met.

11.2.2 The width of the retention time window used to make identifications should be based on measurements of actual retention time variations of standards over the course of time. Plus or minus three times the standard

deviation of the retention time for a compound can be used to calculate a suggested window size; however the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.2.3 If an analyte peak area exceeds the range of the calibration curve, the extract may be diluted with the hexane extraction solvent (that contains the internal standard) and reanalyzed. Incorporate the dilution factor into final concentration calculations. The analyst must not extrapolate beyond the calibration range established.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak (or isomer peaks) in a calibration standard or the laboratory fortified blank.

12.2 Calculate the analyte concentrations using the first or second order calibration curves generated as described in Section 10.

12.3 For any analytes that are found, adjust the calculated concentration to reflect the true sample volume determined in Section 11.1.12.

12.4 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration. If a confirmation column has been used, all identifications should be verified using the retention time data from that analysis. In addition, the *(Z/E)* isomer ratio should be within 50% of the ratio observed in standards. If the *(Z/E)* ratio does not meet these criteria, it is likely that an interferent occurred at the retention time of one of the isomer peaks. In this case, the amount indicated by the lower of the 2 isomer peaks should be reported. (This may require that the analyst recalculate the analyte amount using individual isomer peaks for quantitation.) If one peak of the isomeric pair is missing, the identification is not confirmed and should not be reported.

12.5 Analyte concentrations are reported in µg/L.

13. METHOD PERFORMANCE

13.1 Precision and accuracy data are presented in Section 17. Data are presented for three water matrices: reagent water (Table 3), chlorinated "finished" surface water (Table 5) , chlorinated "finished" ground water (Table 6).

13.2 DERIVATIZATION PARAMETERS -- This method is a procedural standard method that will generate accurate and precise results when used as written. The time, temperature, pH, and PFBHA concentration will all affect the rate,

efficiency and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters. Some laboratories may need to prepare and analyze a calibration curve with each batch of samples. Of all the method analytes, glyoxal, methyl glyoxal, benzaldehyde, and cyclohexanone are the most difficult to derivatize. Poor sensitivity for any of these compounds indicates that there may be a problem with the reaction conditions. Measurements of nonanal, decanal, glyoxal and methyl glyoxal appear to be less precise than the measurement of other analytes.

13.3 The importance of low background levels of formaldehyde and acetaldehyde cannot be overemphasized. Some laboratories or reagent waters may also contain background amounts of other method analytes. Care must be taken to avoid reporting false positive results that result from background contamination.

13.4 The importance of proper sample collection and preservation also cannot be overemphasized. Holding time studies in various matrices showed better than 70% recovery of all method analytes when samples were collected, preserved, and stored according to Section 8, and analyzed within 7 days. There were variations in the recovery of analytes from fortified samples from different matrices. Therefore, it is strongly recommended that samples be analyzed as soon as possible after collection. The data in Section 17, Table 7 illustrate the dramatic difference between a preserved and a non-preserved sample. Although this data was presented as Table 6 of Method 556, revision 1.0, it is included to illustrate the importance of proper sample preservation.

14. POLLUTION PREVENTION

14.1 This method uses a micro-extraction procedure which requires very small quantities of organic solvents.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect

the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE PRIMARY COLUMN (N=8)

Peak Number (Figure 1.)	Analyte	**Average Retention Time (min)**	**Standard Deviation**	**Relative Standard Deviation**
1	1,2 dibromopropane (IS)	0.673	3.26E-03	0.49%
2	Formaldehyde	1.08	1.67E-03	0.15%
3	E-Acetaldehyde	1.46	1.00E-04	0.01%
4	Z-Acetaldehyde	1.50	7.08E-04	0.05%
5	E-Propanal	1.85	4.51E-04	0.02%
6	Z-Propanal	1.88	4.63E-04	0.02%
7	E-Butanal	2.28	5.07E-04	0.02%
8	Z-Butanal	2.31	4.86E-04	0.02%
9	E-Pentanal	2.73	3.36E-04	0.01%
10	Z-Pentanal	2.76	3.27E-04	0.01%
11	E-Hexanal	3.19	3.18E-04	0.01%
12	Z-Hexanal	3.21	1.85E-04	0.01%
13	Cyclohexanone	3.54	4.33E-04	0.01%
14	E-Heptanal	3.64	3.47E-04	0.01%
15	Z-Heptanal	3.65	1.59E-04	0.00%
16	2,4,5, trifluoroacetophenone (S)	3.97	3.18E-04	0.01%
17	E-Octanal	4.07	3.25E-04	0.01%
18	Z-Octanal	4.08	4.88E-04	0.01%
19	Benzaldehyde	4.19	2.13E-04	0.01%
20	Nonanal	4.50	2.97E-04	0.01%
21	Decanal	4.91	2.76E-04	0.01%
22	Glyoxal (peak 1)	5.23	5.33E-04	0.01%
23	Glyoxal (peak 2)	5.27	4.91E-04	0.01%
24	Methyl Glyoxal (peak 1)	5.29	2.90E-04	0.01%
25	Methyl Glyoxal (peak 2)	5.41	2.85E-04	0.01%

Primary Column: DB-5, 10 m x 0.10 mm i.d., 0.10 μm film thickness, injector temp. 200 °C, liner 2 mm with a central 2 cm silanized glass wool plug, injection volume 1 μL, split ratio 30:1, constant head pressure @ 32 psi, detector temp. 300 °C, detector make up flow 20 mL/minute.

Temperature program: 70 °C initial, program at 27 °C/minute to 220 °C, ballistic heating to 280 °C for burnout and hold at 280 °C for 0.4 minutes. Data collection via HP GC Chemstation at a rate of 50 Hz.

Carrier gas: Hydrogen (UHP)

Detector gas: 95:5 Argon:Methane

TABLE 2. CHROMATOGRAPHIC CONDITIONS AND RETENTION TIME DATA FOR THE SECONDARY COLUMN (N=8)

Peak Number (Figure 2.)	Analyte	**Average Retention Time (min)**	**Standard Deviation**	**Relative Standard Deviation**
1	1,2 dibromopropane (IS)	0.808	1.48E-03	0.18%
2	Formaldehyde	1.29	1.75E-03	0.14%
3	E-Acetaldehyde	1.69	1.87E-03	0.11%
4	Z-Acetaldehyde	1.73	1.79E-03	0.10%
5	E-Propanal	2.07	1.79E-03	0.09%
6	Z-Propanal	2.10	1.87E-03	0.09%
7	E-Butanal	2.48	1.76E-03	0.07%
8	Z-Butanal	2.52	1.85E-03	0.07%
9	E-Pentanal	2.92	1.56E-03	0.05%
10	Z-Pentanal	2.96	1.58E-03	0.05%
11	E-Hexanal	3.37	1.59E-03	0.05%
12	Z-Hexanal	3.40	1.53E-03	0.05%
13	Cyclohexanone	3.76	1.43E-03	0.04%
14	E-Heptanal	3.80	1.46E-03	0.04%
15	Z-Heptanal	3.82	1.48E-03	0.04%
16	E-Octanal	4.22	1.14E-03	0.03%
17	Z-Octanal	4.23	7.25E-04	0.02%
18	2,4,5, trifluoroacetophenone (S)	4.36	1.09E-03	0.03%
19	Benzaldehyde	4.53	1.29E-03	0.03%
20	E-Nonanal	4.62	1.28E-03	0.03%
21	Z-Nonanal	4.63	1.31E-03	0.03%
22	Decanal	5.01	1.07E-03	0.02%
23	Glyoxal	5.57	9.91E-04	0.02%
24	Methyl Glyoxal	5.68	7.85E-04	0.01%

Secondary Column: AT-1701, 10 m x 0.10 mm i.d., 0.10 µm film thickness, injector temp. 200 °C, liner 2 mm with a central 2 cm silanized glass wool plug, injection volume 1 µL, split ratio 30:1, constant head pressure @ 32 psi, detector temp. 300 °C, detector make up flow 20 mL/minute.

Temperature program: 70 °C initial, program at 27 °C/minute to 220 °C, ballistic heating to 280 °C for burnout and hold at 280 °C for 0.4 minutes. Data collection via HP GC Chemstation at a rate of 50 Hz.

Carrier gas: Hydrogen (UHP)

Detector gas: 95:5 Argon:Methane

TABLE 3. PRECISION AND ACCURACY IN REAGENT WATER (N=8)

5 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Average Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	5.0	6.01	0.303	1.9%	114%
Acetaldehyde	5.0	4.46	ND	2.5%	89%
Propanal	5.0	6.61	1.46	2.0%	103%
Butanal	5.0	5.54	ND	2.4%	111%
Pentanal	5.0	5.54	ND	2.9%	111%
Hexanal	5.0	5.55	ND	4.0%	111%
Cyclohexanone	5.0	6.19	ND	3.2%	124%
Heptanal	5.0	6.77	1.68	5.0%	102%
Octanal	5.0	5.22	ND	5.4%	104%
Benzaldehyde	5.0	4.50	ND	4.1%	90%
Nonanal	5.0	5.42	0.403	4.6%	100%
Decanal	5.0	5.47	ND	5.1%	109%
Glyoxal	5.0	4.60	ND	6.3%	92%
Methyl Glyoxal	5.0	4.52	ND	5.7%	90%

20 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Average Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	20.0	20.8	0.303	2.3%	102%
Acetaldehyde	20.0	20.7	ND	6.3%	104%
Propanal	20.0	20.6	1.46	2.4%	96%
Butanal	20.0	20.0	ND	2.6%	100%
Pentanal	20.0	20.2	ND	3.0%	101%
Hexanal	20.0	20.3	ND	3.2%	101%
Cyclohexanone	20.0	20.7	ND	2.0%	104%
Heptanal	20.0	20.8	1.68	3.1%	95%
Octanal	20.0	20.2	ND	2.2%	101%
Benzaldehyde	20.0	19.6	ND	2.7%	98%
Nonanal	20.0	20.3	0.403	2.6%	100%
Decanal	20.0	20.3	ND	3.2%	101%
Glyoxal	20.0	19.5	ND	4.1%	98%
Methyl Glyoxal	20.0	19.3	ND	4.0%	97%

a = These recovery values were calculated using the equation in Section 9.7.2.

TABLE 4. METHOD DETECTION LIMITS IN REAGENT WATER (n = 7)

Analyte	Fortified Concentration (µg/L)	Primary Column MDL (µg/L)	Secondary Column MDL (µg/L)
Formaldehyde	1.0	0.09	0.08
Acetaldehyde	1.0	0.18	0.12
Propanal	1.0	0.11	0.06
Butanal	1.0	0.09	0.06
Pentanal	1.0	0.09	0.06
Hexanal	1.0	0.10	0.04
Cyclohexanone	1.0	0.19	0.09
Heptanal	1.0	0.40	0.24
Octanal	1.0	0.22	0.84
Benzaldehyde	1.0	0.19	0.04
Nonanal	1.0	0.62	0.64
Decanal	1.0	0.46	0.35
Glyoxal	1.0	0.39	0.13
Methyl Glyoxal	1.0	0.26	0.12

TABLE 5. PRECISION AND ACCURACY IN CHLORINATED SURFACE WATER (N=7)

5 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Average Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	5.0	8.45	3.40	3.3%	101%
Acetaldehyde*	5.0	6.53	1.76	2.7%	96%
Propanal	5.0	5.68	0.620	2.2%	101%
Butanal	5.0	5.73	0.390	2.4%	107%
Pentanal	5.0	5.43	ND	2.6%	109%
Hexanal	5.0	5.48	ND	2.8%	110%
Cyclohexanone	5.0	6.02	0.650	4.2%	107%
Heptanal	5.0	5.64	0.840	4.1%	96%
Octanal	5.0	4.84	ND	6.4%	97%
Benzaldehyde	5.0	4.92	ND	3.1%	98%
Nonanal	5.0	5.25	0.250	8.5%	100%
Decanal	5.0	5.78	ND	8.9%	116%
Glyoxal	5.0	7.92	1.40	9.2%	130%
Methyl Glyoxal	5.0	6.42	0.380	9.2%	121%

20 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Average Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	20.0	22.6	3.40	1.1%	96%
Acetaldehyde*	20.0	20.1	1.76	1.8%	91%
Propanal	20.0	20.4	0.620	1.3%	99%
Butanal	20.0	20.1	0.390	1.8%	99%
Pentanal	20.0	20.5	ND	1.9%	103%
Hexanal	20.0	20.7	ND	2.2%	103%
Cyclohexanone	20.0	20.5	0.650	2.1%	99%
Heptanal	20.0	19.1	0.840	4.1%	91%
Octanal	20.0	18.8	ND	7.7%	94%
Benzaldehyde	20.0	20.4	ND	2.2%	102%
Nonanal	20.0	19.9	0.250	11.2%	98%
Decanal	20.0	20.8	ND	10.5%	104%
Glyoxal	20.0	25.9	1.40	6.5%	122%
Methyl Glyoxal	20.0	23.0	0.380	10.3%	113%

* = Data for acetaldehyde were taken from the secondary column due to an interference with E-acetaldehyde.

a = These recovery values were calculated using the equation in Section 9.7.2.

TABLE 6 . PRECISION AND ACCURACY IN CHLORINATED GROUND WATER (N=7)

5 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Mean Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	5.0	7.10	2.21	1.3%	97.8%
Acetaldehyde*	5.0	6.13	1.13	3.8%	101%
Propanal	5.0	5.56	0.657	3.1%	98.0%
Butanal	5.0	5.58	0.437	3.5%	103%
Pentanal	5.0	5.36	ND	3.5%	107%
Hexanal	5.0	5.15	0.120	3.7%	101%
Cyclohexanone	5.0	6.02	0.534	4.7%	110%
Heptanal	5.0	5.40	0.883	7.0%	90.3%
Octanal	5.0	4.90	ND	7.9%	98.0%
Benzaldehyde	5.0	4.60	ND	5.3%	92.1%
Nonanal	5.0	5.02	ND	8.7%	100%
Decanal	5.0	5.29	ND	10.4%	106%
Glyoxal	5.0	5.82	0.471	10.9%	107%
Methyl Glyoxal	5.0	4.94	0.202	10.7%	94.7%

20 µg/L Fortification

Analyte	Fortified Concentration (µg/L)	Mean Concentration (µg/L)	Unfortified Sample (µg/L)	Relative Standard Deviation	Average Percent Recovery[a]
Formaldehyde	20.0	21.3	2.21	2.8%	95%
Acetaldehyde*	20.0	20.2	1.13	4.0%	96%
Propanal	20.0	19.9	0.657	3.3%	96%
Butanal	20.0	20.3	0.437	3.0%	99%
Pentanal	20.0	20.2	ND	3.7%	101%
Hexanal	20.0	19.7	0.120	5.8%	98%
Cyclohexanone	20.0	20.3	0.534	4.8%	99%
Heptanal	20.0	19.7	0.883	5.5%	94%
Octanal	20.0	20.5	ND	6.2%	102%
Benzaldehyde	20.0	19.6	ND	4.7%	98%
Nonanal	20.0	20.5	ND	6.6%	103%
Decanal	20.0	20.7	ND	8.9%	104%
Glyoxal	20.0	22.3	0.471	10.3%	109%
Methyl Glyoxal	20.0	19.9	0.202	8.9%	98%

* = Data for acetaldehyde were taken from the secondary column due to an interference with E-acetaldehyde.
a = These recovery values were calculated using the equation in Section 9.7.2.

TABLE 7. HOLDING TIME DATA FOR SAMPLES FROM AN UNTREATED SURFACE WATER SOURCE, FORTIFIED WITH METHOD ANALYTES AT 20 µg/L, WITH AND WITHOUT COPPER SULFATE BIOCIDE*

	% Recovery without Copper Sulfate				% Recovery with Copper Sulfate			
ANALYTE	Day 0	Day 6	Day 14	Day 21	Day 0	Day 6	Day 14	Day 21
Formaldehyde	104	144	569	619	105	105	109	106
Acetaldehyde	96	23	21	24	98	99	103	98
Propanal	94	21	19	22	98	98	103	96
Butanal	92	20	18	21	99	98	102	91
Pentanal	87	19	16	21	96	98	100	94
Hexanal	83	21	17	22	93	97	100	92
Cyclohexanone	94	99	84	78	96	101	98	94
Heptanal	83	20	16	17	96	94	97	91
Octanal	82	18	<10	11	99	96	96	93
Benzaldehyde	94	83	74	79	98	100	104	92
Nonanal	72	15	<10	<10	104	98	92	84
Decanal	50	<10	<10	<10	107	101	93	82
Glyoxal	103	98	37	<10	106	108	106	90
Methyl glyoxal	108	68	<10	<10	111	105	94	73

* = These data were collected and presented as Table 6 of Method 556, revision 1.0.

- All samples were stored headspace free at 4 °C.
- Values at all time points are the mean of 5 replicate analyses. RSDs for replicate analyses of samples containing copper sulfate were <10%. RSDs for unpreserved samples were higher due to the degradation process.

TABLE 8. INITIAL DEMONSTRATION OF CAPABILITY REQUIREMENTS

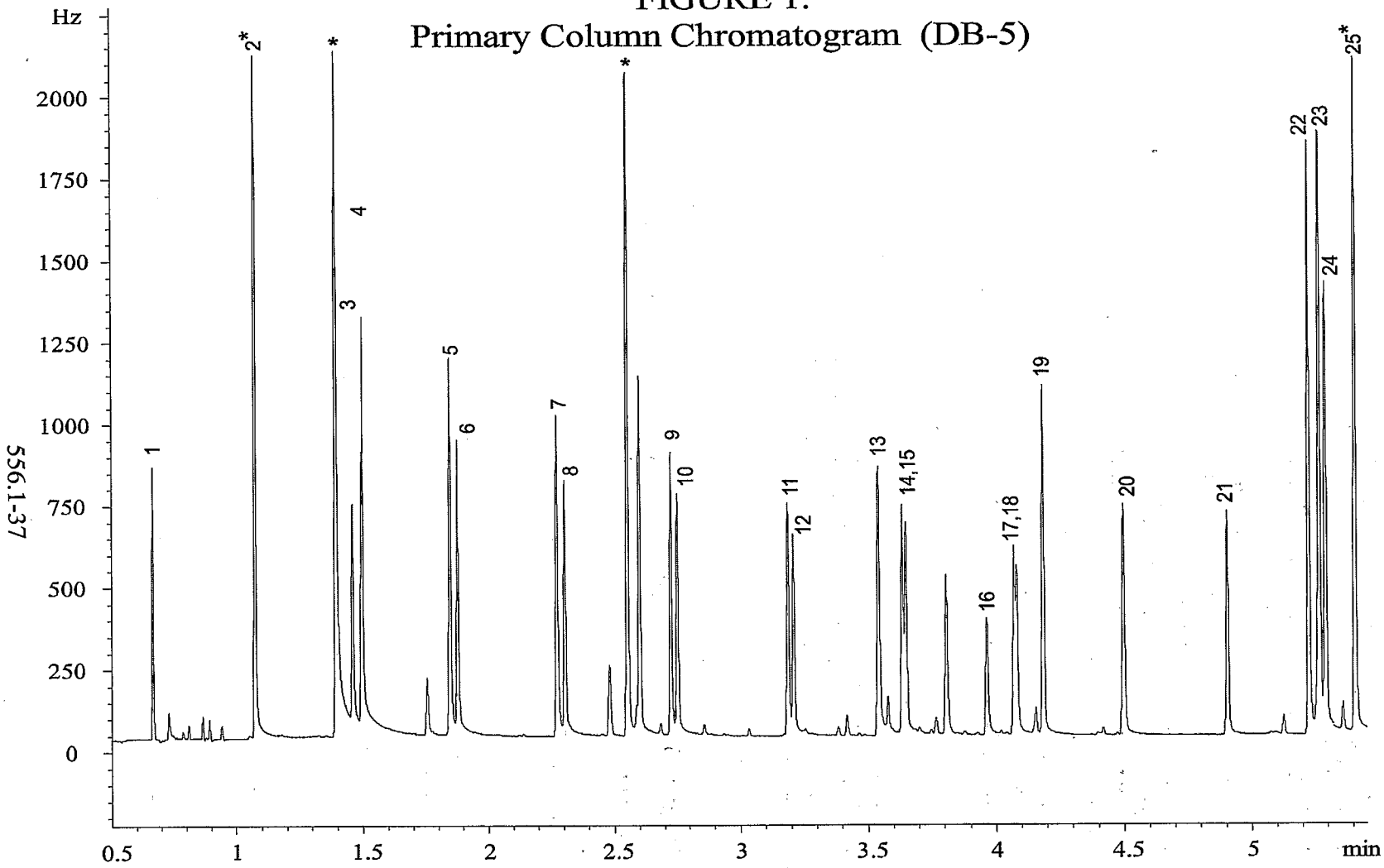
Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.3	Initial Demonstration of Low System Background	Analyze method blank and determine that all target analytes are below 1/2 the MRL prior to performing IDC	The LRB concentration must be ≤ 1/2 of the intended MRL
Section 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LRBs fortified at 20.0 μg/L (or mid cal.) on at least 2 different days	RSD must be ≤ 20 %
Section 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery of IDP replicates	Mean recovery ± 20% of true value
Section 9.2.4	Method Detection Limit (MDL) Determination	a) select a fortifying level at 2 - 5 x the noise level b) analyze 7 replicates in reagent water taken thru all steps c) calculate MDL via equation - do not subtract blank d) replicate extractions and analyses must be conducted over at least 3 days	
Section 9.2.5	Minimum Reporting Levels (MRLs)	MRLs should be established for all analytes during IDC, and be updated as additional LRB data is available.	Establish the MRL for each analyte, as the LRB concentration + 3σ or 3 times the mean LRB concentration, whichever is greater.

TABLE 9. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2	Initial Calibration	Use internal standard technique to generate curve with five standards that span the approximate range of 5-40 µg/L. First or second order curves must be forced through zero. Either sum E/Z isomer areas or average the amount of each isomer. Calculate E/Z ratios for analytes. Run QCS.	QCS must agree within 70-130 %. Lowest concentration should be near MRL.
Section 9.3	Laboratory Reagent Blank (LRB)	Include LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free of interferences.	All analytes < 1/2 MRL
Section 10.3.1	Continuing Calibration Check (CCC) Option	Verify initial calibration by running CCCs prior to analyzing samples, after 10 samples, and after the last sample.	Recovery for mid-level CCC must be 70-130% of the true value, recovery for low level must be 50-150% of the true value.
Section 10.3.2	Daily Calibration Option	Calibrate daily, but verify that sensitivity and performance have not changed significantly since IDC.	Peak areas for IS, SUR, and method analytes for mid-level CAL std must be +/- 50% of the peak areas obtained for that CAL std during IDC.
Section 8.4	Field Reagent Blanks (FRB)	1 per shipping batch	All analytes < 1/2 MRL
Section 9.5	Internal Standard (IS)	1,2-Dibromopropane is added to all samples, blanks and standards	IS area counts must be 70 - 130% of the average initial calibration area counts
Section 9.6	Surrogate Standard (SUR)	2',4',5' -Trifluoroacetophenone is added samples, blanks and standards	Surrogate recovery must be 70 - 130 % of the true value.

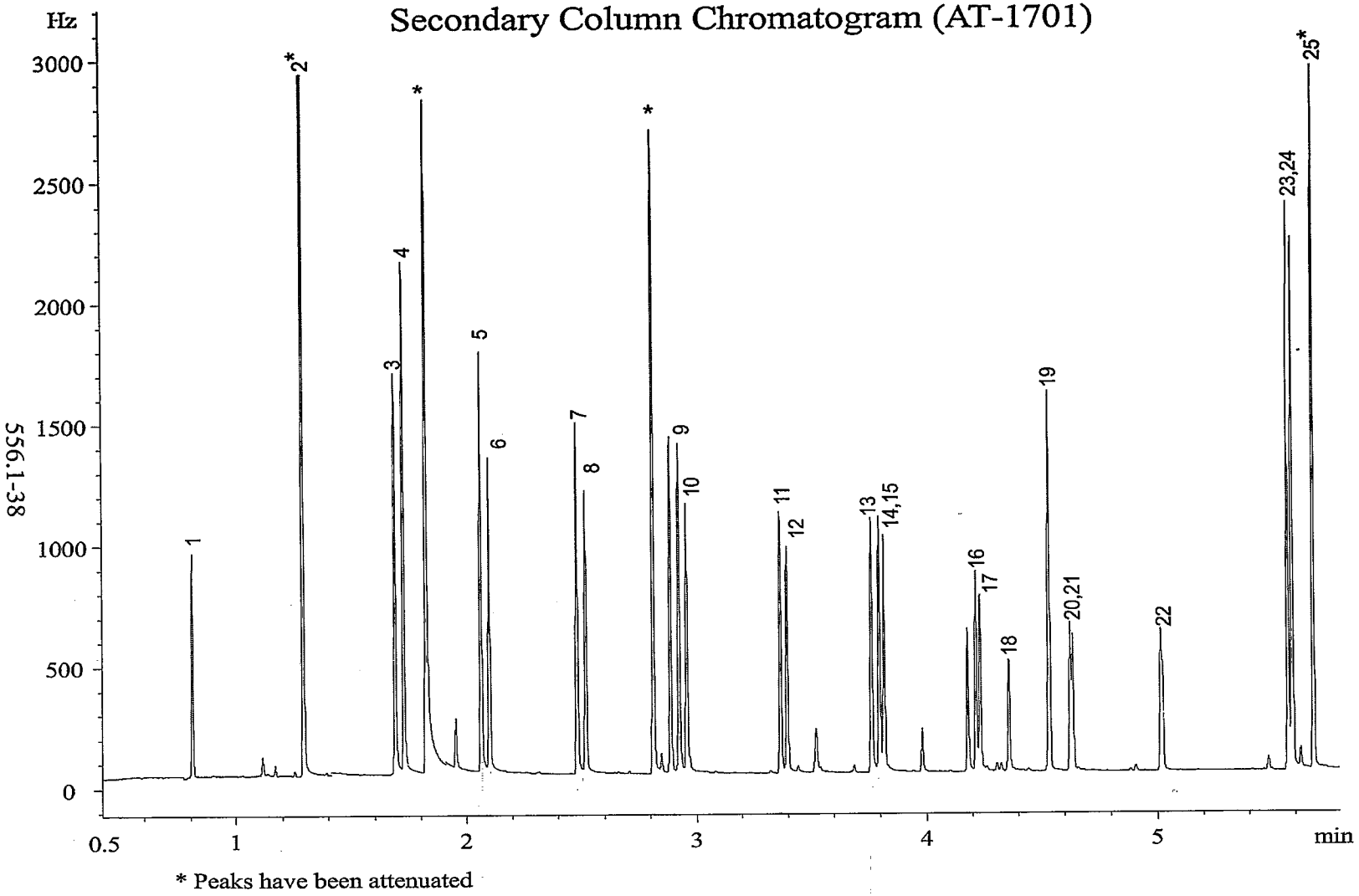
Section 9.7	Laboratory Fortified Sample Matrix (LFM)	Fortify at least one sample per analysis batch (20 samples or less) at a concentration close to that in the native sample.	Recoveries not within 70-130% may indicate matrix effect
Section 9.8	Field Duplicates	Extract and analyze at least one duplicate sample with every analysis batch (20 samples or less)	Suggested RPD ≤ 30 %
Section 9.9	Quality Control Sample (QCS)	Analyze a QCS at least quarterly from an external/second source.	QCS must agree within 70-130 %.
Section 9.10, Section 10.2.5 and Section 12.4	E/Z Isomer Ratio Agreement	Calculate the E/Z isomer ratio for target analytes and compare to E/Z ratio in initial calibration	E/Z ratio in standards, blanks, and samples must be within ± 50% of E/Z ratio in initial calibration. Do not report value if one isomer is missing.
Section 8.3.1	Sample Holding Time	Properly preserved samples may be stored in the dark at 4 °C for 7 days.	Do not report data for samples that have exceeded their holding time, or that have not been properly preserved or stored.
Section 8.3.2	Extract Holding Time	Extracts may be stored in the dark at 4 °C for 14 days.	Do not report data for extracts that have exceeded their holding time.

FIGURE 1.
Primary Column Chromatogram (DB-5)



* Peaks have been attenuated

FIGURE 2.
Secondary Column Chromatogram (AT-1701)



* Peaks have been attenuated

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