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METHOD 540. DETERMINATION OF SELECTED ORGANIC CHEMICALS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

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DETERMINATION OF SELECTED ORGANIC CHEMICALS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1 This is a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for determination of organic contaminants in drinking water. Accuracy and precision data have been generated in reagent water, and finished ground and surface waters for compounds listed in the table below.

Analyte	Chemical Abstract Services Registry Number (CASRN)
3-Hydroxycarbofuran	16655-82-6
Bensulide	741-58-2
Chlorpyrifos oxon	5598-15-2
Disulfoton sulfoxide	2497-07-6
Fenamiphos	22224-92-6
Fenamiphos sulfone	31972-44-8
Fenamiphos sulfoxide	31972-43-7
Methomyl	16752-77-5
Phorate sulfone	2588-04-7
Phorate sulfoxide	2588-03-6
Tebuconazole	107534-96-3
Tebufenozide	112410-23-8

1.2 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The single laboratory lowest concentration MRL (LCMRL) is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99%), between 50 and 150% recovery. Single laboratory LCMRLs for analytes in this method range from 0.30-2.7 ng/L, and are listed in Table 5. The procedure used to determine the LCMRL is described elsewhere.[1]

1.3 Laboratories using this method will not be required to determine the LCMRL for this method, but will need to demonstrate that their laboratory MRL meets the requirements described in Section 9.2.4.

1.4 Determining the Detection Limit (DL) for analytes in this method is optional (Sect. 9.2.6). Detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[2] The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance. DLs for analytes in this method range from 0.15-1.1 ng/L, and are listed in Table 5.

1.5 This method is intended for use by analysts skilled in solid phase extractions, operation of LC/MS/MS instruments, and the interpretation of associated data.

1.6 METHOD FLEXIBILITY – In recognition of technological advances in analytical systems and techniques, the laboratory is permitted to modify the evaporation technique, separation technique, LC column, mobile phase composition, LC conditions and MS and MS/MS conditions (Sect. 6.9, 9.1.1, 10.2, and 12.1). Changes may not be made to sample collection and preservation (Sect. 8), sample extraction steps (Sect. 11), or to quality control requirements (Sect. 9). Method modifications should be considered only to improve method performance. Modifications that are introduced in the interest of reducing cost or sample processing time, but result in poorer method performance, should not be used. Analytes must be adequately resolved chromatographically in order to permit the mass spectrometer to dwell on a minimum number of compounds eluting within a retention time window. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the initial demonstration of capability (IDC, Sect. 9.2), verify that all Quality Control (QC) acceptance criteria (Sect. 9) are met, and that acceptable method performance can be verified in a real sample matrix (Sect. 9.3.6).

NOTE: The above method flexibility section is intended as an abbreviated summation of method flexibility. Sections 4-12 provide detailed information of specific portions of the method that may be modified. If there is any perceived conflict between the general method flexibility statement in Section 1.6 and specific information in Sections 4-12, Sections 4-12 supersede Section 1.6.

2. SUMMARY OF METHOD

A 250-mL water sample is fortified with surrogates and passed through a solid phase extraction (SPE) cartridge to extract the method analytes and surrogates. Compounds are eluted from the solid phase with a small amount of methanol. The extract is concentrated by evaporation with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with methanol after adding the internal standards. A 10-µL injection is made into an LC equipped with a C_{18} column that is interfaced to an MS/MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique.

Surrogate analytes are added to all Field and QC Samples to monitor the extraction efficiency of method analytes.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period, including no more than 20 Field Samples, 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 and/or stock standard solution, internal standard(s), and the surrogate(s). The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 COLLISIONALLY ACTIVATED DISSOCIATION (CAD) – The process of converting the translational energy of the precursor ion into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

3.4 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes, internal standard(s) and surrogate(s). The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5 DETECTION LIMIT (DL) – 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. 9.2.6), and accurate quantitation is not expected at this level.[2]

3.6 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 SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

3.7 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 laboratory procedures.

3.8 INTERNAL STANDARD (IS) – A pure chemical added to an extract or standard solution in a known amount(s) and used to measure the relative response of other method analytes and surrogates that are components of the same solution. The internal

standard must be a chemical that is structurally similar to the method analytes, has no potential to be present in water samples, and is not a method analyte.

3.9 LABORATORY FORTIFIED BLANK (LFB) – A volume of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds 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.

3.10 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and 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 sample extraction and the measured values in the LFSM corrected for background concentrations.

3.11 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, extracted, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the occurrence of method analytes is low.

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 and reagents, sample preservatives, internal standards, and surrogates that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.13 LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50 and 150% recovery.[1]

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 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a method 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 QC criteria for this standard are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.4.

3.16 PRECURSOR ION – For the purpose of this method, the precursor ion is the protonated molecule ($[M+H]^+$) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by CAD to produce distinctive product ions of smaller *m/z*.

3.17 PRIMARY DILUTION STANDARD (PDS) SOLUTION – A solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.18 PRODUCT ION – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by CAD of the precursor ion.

3.19 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. The second source stock standard solution is used to fortify the QCS at a known concentration. The QCS is used to check calibration standard integrity.

3.20 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.

3.21 SURROGATE ANALYTE (SUR) – A pure chemical which chemically resembles method analytes and is extremely unlikely to be found in any sample. This chemical is added to a sample aliquot in known amount(s) before processing and is measured with the same procedures used to measure other method analytes. The purpose of the SUR is to monitor method performance with each sample.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by a reagent water rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for two hours or solvent rinsed. Volumetric glassware should be solvent rinsed and 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 chromatograms. All items must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3.1. **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. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent.[3-4] Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.4 Relatively large quantities of the preservatives (Sect. 8.1.2) are added to sample bottles. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks (Sect. 9.3.1), particularly when new lots of reagents are acquired.

4.5 SPE cartridges can be a source of interferences. Analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

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.[5-7]

5.2 Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES (Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

6.1 SAMPLE CONTAINERS – 250-mL amber glass bottles fitted with polytetrafluoroethylene (PTFE)-lined screw caps.

6.2 CENTRIFUGE TUBES – 15-mL conical glass centrifuge tubes (Corning #8082-15) or other glassware suitable for collection of the eluent from the solid phase after extraction.

6.3 AUTOSAMPLER VIALS – Amber glass 2.0-mL autosampler vials (National Scientific #C4000-2W or equivalent) with caps containing PTFE-faced septa (National Scientific #C4000-53 or equivalent).

6.4 MICRO SYRINGES – Suggested sizes include 5, 10, 25, 50, 100, 250, 500 and 1000-µL syringes.

6.5 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.6 SOLID PHASE EXTRACTION (SPE) APPARATUS FOR USING CARTRIDGES

6.6.1 SPE CARTRIDGES

6.6.1.1 Waters Oasis HLB, 150 mg, 6cc (Waters # 186003365) - divinylbenzene N-vinylpyrrolidone copolymer.

6.6.1.2 J.T. Baker Speedisk Column H_2O-Philic DVB, 200 mg, 6cc (Baker # 8108-09) – modified divinylbenzene polymer.

6.6.2 VACUUM EXTRACTION MANIFOLD

6.6.2.1 Manual Extraction - A manual vacuum manifold with Visiprep™ large volume sampler (Supelco #57030 and #57275 or equivalent) for cartridge extractions.

6.6.2.2 Automated Extraction – An automatic/robotic sample preparation system, designed for use with SPE cartridges, may be used if all QC requirements discussed in Section 9 are met. All sorbent washing, conditioning, sample loading, rinsing, drying and elution steps must be performed as closely as possible to the manual procedure. Solvents used for washing, conditioning, and sample elution must be the same as those used in the manual procedure; however, the amount used may be increased as necessary to achieve the required data quality. Solvent amounts may not be decreased. Sorbent drying times prior to elution may be modified to achieve the required data quality. Caution should be exercised when increasing solvent volumes. Increased extract volume will likely necessitate the need for extended evaporation times which may compromise data quality. Caution should also be exercised when modifying sorbent drying times. Excessive drying may cause losses due to analyte volatility, and excessive contact with room air may oxidize some method analytes.

6.6.3 SAMPLE DELIVERY SYSTEM – Use of a transfer tube system (Supelco "Visiprep," #57275 or equivalent), which transfers the sample directly from the sample container to the SPE cartridge is recommended.

6.7 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by evaporation with nitrogen using a water bath set no higher than 40 °C (Meyer N-Evap, Model 111, Organomation Associates, Inc. or equivalent).

6.8 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 10 to 15 inches of mercury for extracting cartridges.

6.9 LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER (MS/MS) WITH DATA SYSTEM

6.9.1 LC SYSTEM – Instrument capable of reproducibly injecting up to 10-µL aliquots, and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.3 mL/min). Usage of a cooled autosampler compartment and a column heater is optional. Method performance data were collected using an autosampler thermostated to 4 °C during analyses.

6.9.2 TANDEM MASS SPECTROMETER – The mass spectrometer must be capable of positive ion electrospray ionization (ESI) near the suggested LC flow rate of 0.3 mL/min. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.18) for method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision. Data demonstrated in Section 17 were collected using a triple quadrupole mass spectrometer.

6.9.3 DATA SYSTEM – 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 LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.9.4 ANALYTICAL COLUMN – An LC C_{18} column (2.1 x 100 mm) packed with 5 µm C_{18} solid phase particles (Restek Ultra Aqueous #9178512) was used. Any equivalent column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

7. REAGENTS AND STANDARDS

7.1 GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals should be used. 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 method analytes or interfering compounds greater than 1/3 the MRL for each method analyte of interest.

7.1.2 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher Optima LC/MS grade or equivalent).

7.1.3 ACETONITRILE (CH_3CN, CAS#: 75-05-8) – High purity, demonstrated to be free of analytes and interferences (Tedia Absolv grade or equivalent).

7.1.4 ACETONE [$(CH_3)_2CO$, CAS#: 67-64-1] – High purity, demonstrated to be free of analytes and interferences (Tedia Absolv grade or equivalent).

7.1.5 FORMIC ACID (CH_2O_2, CAS# 64-18-6) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade or equivalent).

7.1.6 AMMONIUM FORMATE (CH_5O_2N, CAS# 540-69-2) – High purity, demonstrated to be free of analytes and interferences (Fluka LC/MS grade or equivalent).

7.1.7 10 mM FORMATE BUFFER – To prepare 1 L, add 0.63 g ammonium formate and 0.5 mL formic acid to 1 L of reagent water. This solution is prone to volatility losses and should be replaced at least every 48 hours.

7.1.8 SAMPLE PRESERVATION REAGENTS – The following preservatives are solids at room temperature and may be added to the sample bottle before shipment to the field.

7.1.8.1 TRIZMA PRESET CRYSTALS, pH 7.0 (Sigma-Aldrich #T-7193 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxy-methyl)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 near 7.0 at 25 °C in reagent water. Trizma functions as a buffer (Sect. 8.1.2).

7.1.8.2 L-ASCORBIC ACID (CAS# 50-81-7) – Ascorbic acid reduces free chlorine at the time of sample collection (Sigma-Aldrich #255564 or equivalent).[8]

7.1.8.3 2-CHLOROACETAMIDE (CAS# 79-07-2) – Inhibits microbial growth and analyte degradation (Sigma-Aldrich #C0267 or equivalent).[8]

7.1.9 NITROGEN – Aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. Nitrogen used should meet or exceed instrument manufacturer's specifications.

7.1.10 ARGON – Used as collision gas during MS/MS experiments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen gas may be used as collision gas provided sufficient sensitivity (product ion formation) is achieved.

7.2 STANDARD SOLUTIONS – When the purity of a compound is assayed to be 96% or greater, the weight can be used without correction to calculate concentration of the stock standard. The suggested concentrations are a description of concentrations used

during method development, and may be modified to conform to instrument sensitivity. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize addition of excess organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should use standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL (IS) STOCK STANDARD SOLUTIONS – This method uses three IS compounds: carbofuran-$^{13}C_{6,}$ bensulide-d_{14} and phorate-d_{10}. These IS compounds were carefully chosen during method development because they encompass some of the functional groups of method analytes. Although alternate IS compounds may be used provided they are isotopically labeled compounds with similar functional groups as method analytes, the analyst must have documented reasons for using alternate IS compounds. Alternate IS compounds must meet the QC requirements in Section 9.3.4.

7.2.1.1 IS STOCK STANDARD SOLUTIONS – These IS stocks can be obtained as individual certified stock standard solutions or neat materials. During development of this method, commercially obtained 100 µg/mL stock standard solutions of carbofuran-$^{13}C_6$ in 1,4-dioxane (Cambridge Isotopes # CLM-1911-1.2) and phorate-d_{10} in acetone (Crescent Chemical # XA16080100AC) were used. Bensulide-d_{14} was prepared from neat material (Cambridge Isotopes # DLM-7152) at 1000 µg/mL in acetonitrile. IS stock standard solutions were stable for at least six months when stored at -5 °C or less in amber glass screw cap vials.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION STANDARD (IS PDS) (0.40 ng/µL) – Prepare, or purchase commercially, the IS PDS at a suggested concentration of 0.40 ng/µL using the three isotopically labeled chemicals in the table below. If prepared from individual stock standard solutions (Sect. 7.2.1.1), the table below can be used as a guideline for preparing the IS PDS although concentrations may need to be adjusted for instrument sensitivity. The IS PDS used in these studies was prepared in acetonitrile. The IS PDS has been shown to be stable for at least six months when stored at 6 °C or less in amber glass screw cap vials. Ten µL of this 0.40 ng/µL IS PDS was used to fortify the final 1-mL extracts (Sect. 11.4). This will yield a concentration of 4 pg/µL of each IS in 1-mL extracts.

IS	Conc. of IS Stock (µg/mL)	Vol. Of IS Stock (µL)	Final Vol. of IS PDS (mL)	Final Conc. of IS PDS (ng/µL)
carbofuran-$^{13}C_6$	100	20	5.0	0.40
bensulide-d_{14}	1000	2.0	5.0	0.40
phorate-d_{10}	100	20	5.0	0.40

7.2.2 SURROGATE (SUR) ANALYTE STANDARD SOLUTIONS – The two SUR(s) for this method are methomyl-$^{13}C_2$,^{15}N and tebuconazole-d_6. These isotopically labeled SUR standards were carefully chosen during method development because they encompass some of the functional groups, as well as the water solubility range of method analytes. Although alternate SUR standards may be used provided they are isotopically labeled compounds with similar functional groups as method analytes, the analyst must have documented reasons for using alternate SUR standards. Alternate SUR standards chosen must still span the water solubility range of method analytes. In addition, alternate SUR standards must meet the QC requirements in Section 9.3.5.

7.2.2.1 SUR STOCK STANDARD SOLUTIONS – These SUR stocks can be obtained as individual certified stock standard solutions. During development of this method, commercially obtained 100 µg/mL stock standard solutions of methomyl-$^{13}C_2$,^{15}N in methanol (Cambridge Isotopes #CNLM-7148-1.2) and tebuconazole-d_6 in acetone (Dr. Ehrenstorfer GmbH #XA17178710AC) were used. SUR stock standard solutions were stable for at least one year when stored at -5 °C or less in amber glass screw cap vials.

7.2.2.2 SURROGATE PRIMARY DILUTION STANDARD (SUR PDS) (0.40 ng/µL) – Prepare, or purchase commercially, the SUR PDS at a suggested concentration of 0.40 ng/µL. If prepared from individual stock standard solutions (Sect. 7.2.2.1), the table below can be used as a guideline for preparing the SUR PDS. The SUR PDS used in these studies was prepared in acetonitrile. This solution is used to fortify all QC and Field Samples. The PDS has been shown to be stable for at least six months when stored at 6 °C or less. Use 10 µL of this 0.40 ng/µL SUR PDS to fortify the 250 mL aqueous QC and Field Samples prior to extraction (Sect. 11.2.2). This will yield a concentration of 16 ng/L of each SUR in aqueous QC and Field Samples.

SUR	Conc. Of SUR Stock (µg/mL)	Vol. of SUR Stock (µL)	Final Vol. of SUR PDS (mL)	Final Conc. of SUR PDS (ng/µL)
methomyl-$^{13}C_2$, ^{15}N	100	20	5.0	0.40
tebuconazole-d_6	100	20	5.0	0.40

7.2.3 ANALYTE STANDARD SOLUTIONS – Analyte standards may be purchased commercially as ampulized solutions or prepared from neat materials (see Table 3 for analyte sources used during method development).

7.2.3.1 ANALYTE STOCK STANDARD SOLUTION (1000 µg/mL) – If preparing from neat material, accurately weigh approximately 5 mg of pure material to the nearest 0.1 mg and dilute to 5 mL with acetonitrile or

methanol for a final concentration of 1000 µg/mL. Repeat for each method analyte prepared from neat material. Alternatively, purchase commercially available individual stock standards of the analytes, preferably in methanol or acetonitrile, if available. For development of this method, commercially available stock standards of 1000 µg/mL were purchased except for phorate sulfoxide, tebufenozide, disulfoton sulfoxide and chlorpyrifos oxon which were purchased as neat materials. These stock standards were stable for at least six months when stored at -5 °C or less in amber glass screw cap vials.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION (0.16-0.40 ng/µL) – The analyte PDS contains all, or a portion, of method analytes at various concentrations in acetonitrile. ESI and MS/MS response varies by compound; therefore, a mix of concentrations may be needed in the analyte PDS. During method development, analyte PDS solutions were prepared such that approximately the same instrument response was obtained for all analytes. The analyte PDS was prepared in acetonitrile at concentrations of 0.4 ng/µL, except for fenamiphos, tebufenozide and tebuconazole at 0.16 ng/µL each. The analyte PDS is prepared by dilution of the combined Analyte Stock Standard Solutions (Sect.7.2.3.1) and is used to prepare CAL standards, and fortify LFBs, LFSMs, LFSMDs and FDs with the method analytes. The analyte PDS has been shown to be stable for six months when stored at 6 °C or less in amber glass screw cap vials.

7.2.4 CALIBRATION STANDARDS (CAL) – Prepare a series of at least five concentrations of calibration solutions in methanol (see note below), from dilutions of the analyte PDS (Sect 7.2.3.2). The suggested concentrations in this paragraph are a description of the concentrations used during method development, and may be modified to conform with instrument sensitivity. Concentrations ranging from 0.16-6.4 µg/L are suggested for fenamiphos, tebufenozide and tebuconazole, and 0.40-16 µg/L are suggested for the remaining analytes. Larger concentration ranges will require more calibration points. The IS and SUR are added to CAL standards at a constant concentration. During method development, the concentrations of the SUR(s) were 0.40 ng/µL in the standard (16 ng/L in the aqueous sample) and the IS(s) concentrations were 4 pg/µL. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. CAL standards may also be used as CCCs (Sect. 9.3.2). During method development, CAL standards were shown to be stable for two weeks when stored at 6 °C or less. Longer storage times are acceptable provided appropriate QC measures are documented demonstrating the CAL stability.

Note: Acetonitrile was not used as the solvent for calibration standards because injection of calibration standards prepared in acetonitrile distorted peak shapes for early eluting analytes, such as methomyl and 3-hydroxycarbofuran.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Samples must be collected in 250-mL amber glass bottles fitted with teflon-lined screw caps.

8.1.2 Preservation reagents, listed in the table below, are added to each sample bottle as a solid prior to shipment to the field (or prior to sample collection).

Compound	Amount	Purpose
Trizma	7.75 g/L	buffering reagent
2-Chloroacetamide	2 g/L	antimicrobial
Ascorbic acid	100 mg/L	dechlorinating agent

8.2 SAMPLE COLLECTION

8.2.1 Open the tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min). Collect samples from the flowing system.

8.2.2 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.3 After collecting the sample, cap the bottle and agitate by hand until preservative is dissolved. Note that 2-chloroacetamide is slow to dissolve especially in cold water. 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. Sample temperature must be confirmed to be at or below 10 °C when samples 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.

NOTE: Samples that are significantly above 10° C, at the time of collection, may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice to meet the above requirements.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Water samples should be extracted as soon as possible after collection but must be extracted within 28 days of collection, except for chlorpyrifos oxon which must be extracted within 7 days. Extracts must be stored at ≤ 6 °C and analyzed within 28 days after extraction.

9. QUALITY CONTROL

9.1 QC requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements that must be met when preparing and analyzing Field Samples. This section describes QC parameters, their required frequencies, and performance criteria that must be met in order to meet EPA quality objectives. QC criteria discussed in the following sections are summarized in Tables 14 and 15. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 METHOD MODIFICATIONS – The analyst is permitted to modify LC columns, LC conditions, evaporation techniques, internal standards or surrogate standards, and MS and MS/MS conditions. Each time such method modifications are made, the analyst must repeat the procedures of the IDC. **Modifications to LC conditions should still minimize co-elution of method analytes to reduce the probability of suppression/enhancement effects.**

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – The IDC must be successfully performed prior to analyzing any Field Samples. Prior to conducting the IDC, the analyst must first generate an acceptable Initial Calibration following the procedure outlined in Section 10.2.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of SPE cartridges, solvents, centrifuge tubes, disposable pipets, and autosampler vials are used, it must be demonstrated that an LRB is reasonably free of contamination and that criteria in Section 9.3.1 are met. If an automated extraction system is used, an LRB should be extracted on each port to ensure that all valves and tubing are free from potential contamination.

9.2.2 INITIAL DEMONSTRATION OF PRECISION (IDP) – Prepare, extract, and analyze four to seven 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 8.1.2 must be added to these samples. The relative standard deviation (RSD) of the results of replicate analyses must be less than 20%.

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 replicate values must be within ± 30% of the true value.

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The MRL may be established by a laboratory for their specific purpose or may be set by a regulatory agency. Establish an Initial Calibration following the procedure outlined in Section 10.2. The lowest CAL standard used to establish Initial Calibration (as well as the low-level CCC, Section 10.3) must be at or below the

concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm the MRL following the procedure outlined below.

9.2.4.1 Fortify, extract, and analyze seven replicate LFBs at the proposed MRL concentration. These LFBs must contain all method preservatives described in Section 8.1.2. Calculate the mean measured concentration (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963s$$

where

s = standard deviation
3.963 = a constant value for seven replicates.[1]

9.2.4.2 Confirm that the upper and lower limits for the Prediction Interval of Result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below

The Upper PIR Limit must be ≤ 150% recovery.

$$\frac{Mean + HR_{PIR}}{FortifiedConcentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{FortifiedConcentration} \times 100\% \geq 50\%$$

9.2.4.3 The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above (Sect. 9.2.4.2). If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

9.2.5 CALIBRATION CONFIRMATION – Analyze a QCS as described in Section 9.3.8 to confirm the accuracy of the standards/calibration curve.

9.2.6 DETECTION LIMIT DETERMINATION *(optional) – While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

Replicate analyses for this procedure should be done over at least three days (i.e., both the sample extraction and the LC/MS/MS analyses should be done over at least three days). Prepare at least seven replicate LFBs at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. DLs in Table 5 were calculated from LFBs fortified at various concentrations as indicated in the table. Appropriate fortification concentrations will be dependent upon the sensitivity of the LC/MS/MS system used. All preservation reagents listed in Section 8.1.2 must also be added to these samples. Analyze the seven replicates through all steps of Section 11.

NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the *DL* using the following equation

$$DL = s \times t_{(n-1,\,1-\alpha=0.99)}$$

where

s = standard deviation of replicate analyses

$t_{(n\text{-}1,\ 1\text{-}\alpha=0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates.

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

9.3 ONGOING QC REQUIREMENTS – This section summarizes ongoing QC criteria that must be followed when processing and analyzing Field Samples.

9.3.1 LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.6) to confirm that potential background contaminants are not interfering with identification or quantitation of method analytes. If more than 20 Field Samples are included in a batch, analyze an LRB for every 20 samples. If the LRB produces a peak within the retention time window of any analyte that would prevent 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 other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. Blank contamination is estimated by extrapolation, if the concentration is below the lowest CAL standard. This extrapolation procedure is not allowed for sample results as it may not meet data quality objectives. If method 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.

9.3.2 CONTINUING CALIBRATION CHECK (CCC) – CCC standards are analyzed at the beginning of each analysis batch, after every 10 Field Samples, and at the end of the analysis batch. See Section 10.3 for concentration requirements and acceptance criteria.

9.3.3 LABORATORY FORTIFIED BLANK (LFB) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. 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 low-level LFB analyses must be 50-150% of the true value. Results of medium and high-level LFB analyses must be 70-130% of the true value. If LFB results do not meet these criteria for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.3.4 INTERNAL STANDARDS (IS) – The analyst must monitor peak areas of the IS(s) in all injections during each analysis day. Internal standard responses (as indicated by peak areas) for any chromatographic run must not deviate by more than ± 50% from average areas measured during the initial calibration for the internal standards. If IS areas in a chromatographic run do not meet these criteria, inject a second aliquot of that standard or extract.

9.3.4.1 If the reinjected aliquot produces an acceptable IS response, report results for that aliquot.

9.3.4.2 If the reinjected extract fails again, the analyst should check the calibration by reanalyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL 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. Alternatively, collect a new sample and re-analyze.

9.3.5 SURROGATE RECOVERY – The SUR standard is fortified into all samples, CCCs, LRBs, LFBs, LFSMs, LFSMDs, and FD prior to extraction. It is also added to CAL standards. The SUR is a means of assessing method performance from extraction to final chromatographic measurement. Calculate the recovery (%R) for the SUR using the following equation

$$\%R = \left(\frac{A}{B}\right) \times 100$$

where

A = calculated SUR concentration for the QC or Field Sample

B = fortified concentration of the SUR.

9.3.5.1 SUR recovery must be in the range of 70-130%. When SUR 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. Correct the problem and reanalyze the extract.

9.3.5.2 If the extract reanalysis meets the SUR recovery criterion, report only data for the reanalyzed extract.

9.3.5.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by injecting the last CAL standard that passed. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. If the re-extracted sample also fails the recovery criterion, report all data for that sample as suspect/SUR recovery to inform the data user that the results are suspect due to SUR recovery. Alternatively, collect a new sample and re-analyze.

9.3.6 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (FD) (Sect. 9.3.7); however, infrequent occurrence of method analytes would hinder this assessment. If the occurrence of method analytes in samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFSMD, must be prepared, extracted, and analyzed from a duplicate of the Field Sample. Extraction batches that contain LFSMDs will not require extraction of a FD. If a variety of different sample matrices are analyzed regularly, for example, drinking water from ground water and surface water sources, method performance should be established for each. Over time, LFSM data should be documented by the laboratory for all routine sample sources.

9.3.6.1 Within each extraction batch (Sect. 3.6), a minimum of one Field Sample is fortified as an LFSM for every 20 Field Samples analyzed. The LFSM is prepared by spiking a sample with an appropriate amount of the Analyte PDS (Sect. 7.2.3.2). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Use historical data and rotate through low, mid and high concentrations when selecting a fortifying concentration.

9.3.6.2 Calculate percent recovery (%R) for each analyte using the equation

$$\%R = \frac{(A - B)}{C} \times 100$$

where

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.3.6.3 Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70-130%, except for low-level fortification near or at the MRL (within a factor of two-times the MRL concentration) where 50-150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and laboratory performance for that analyte is shown to be in control in CCCs, 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.

9.3.7 FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or LFSMD) – Within each extraction batch (not to exceed 20 Field Samples, Sect. 3.6), a minimum of one FD or LFSMD must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If method analytes are not routinely observed in Field Samples, an LFSMD should be analyzed rather than an FD.

9.3.7.1 Calculate relative percent difference (*RPD*) for duplicate measurements (*FD1* and *FD2*) using the equation

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \times 100$$

9.3.7.2 RPDs for FDs should be ≤ 30%. Greater variability may be observed when the matrix is fortified at analyte concentrations at or near the MRL (within a factor of two times the MRL concentration). At these concentrations, FDs should have RPDs that are ≤ 50%. If the RPD of any analyte falls outside the designated range, and laboratory performance for that analyte is shown to be in control in the CCC, 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.

9.3.7.3 If an LFSMD is analyzed instead of a FD, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

$$RPD = \frac{|LFSM - LFSMD|}{(LFSM + LFSMD)/2} \times 100$$

9.3.7.4 RPDs for duplicate LFSMs should be ≤ 30% for samples fortified at or above their native concentration. Greater variability may be observed when the matrix is fortified at analyte concentrations at or near the MRL (within a factor of two times the MRL concentration). LFSMs fortified at these concentrations should have RPDs that are ≤ 50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and laboratory performance for that analyte is shown to be in control in the CCC, 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.

9.3.8 QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new Analyte PDS (Sect. 7.2.3.2) is prepared, and at least quarterly, analyze a QCS sample from a source different from the source of the CAL standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared and analyzed just like a CCC. Fortify the QCS near the midpoint of the calibration range. Acceptance criteria for the QCS are identical to mid- and high-level CCCs; the calculated amount for each analyte must be ± 30% of the true value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem. If the discrepancy is not resolved, one of the standard materials may be degraded or otherwise compromised and a third standard must be obtained.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. The MS tune check and initial calibration must be repeated each time a major instrument modification is made, or maintenance is performed.

10.2 INITIAL CALIBRATION

10.2.1 ESI-MS/MS TUNE

10.2.1.1 Calibrate the mass scale of the MS with the calibration compounds and procedures prescribed by the manufacturer.

10.2.1.2 Optimize the $[M+H]^+$ for each method analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). This tune can be done on a mix of method analytes. MS parameters (voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. Method analytes may have different optima requiring some compromise between the optima. See Table 2 for ESI-MS conditions used in method development.

10.2.1.3 Optimize the product ion (Sect. 3.18) for each analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). This tune can be done on a mix of method analytes. MS/MS parameters (collision gas pressure, collision energy, etc.) are varied until optimal analyte responses are determined. See Table 4 for MS/MS conditions used in method development.

10.2.2 Establish LC operating parameters that optimize resolution and peak shape. Suggested LC conditions can be found in Table 1. LC conditions listed in Table 1 may not be optimum for all LC systems and may need to be optimized by the analyst.

10.2.3 Inject a mid-level CAL standard under LC/MS conditions to obtain retention times of each method analyte. Divide the chromatogram into retention time windows (segments) each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M+H]^+$; Sect. 3.16) for the analytes in each window and choose the most abundant product ion. Product ions (also quantitation ions) chosen during method development are in Table 4, although these will be instrument dependent. For maximum sensitivity in subsequent MS/MS analyses, minimize the number of transitions that are simultaneously monitored within each segment.

10.2.4 Inject a mid-level CAL standard under optimized LC/MS/MS conditions to ensure that each method analyte is observed in its MS/MS window and that there are at least 10 scans across the peak for optimum precision.

10.2.5 Prepare a set of at least five CAL standards as described in Section 7.2.4. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the CAL standards are at a concentration greater than or equal to the MRL.

10.2.6 The LC/MS/MS system is calibrated using the IS technique. Use the LC/MS/MS data system software to generate a linear regression or quadratic calibration curve for each of the analytes. Curves may be concentration weighted, if necessary.

10.2.7 CALIBRATION ACCEPTANCE CRITERIA – Validate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. For calibration levels that are ≤ MRL, the result for each analyte should be within ± 50% of the true value. All other calibration points must calculate to be within ± 30% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. It is recommended that corrective action is taken to reanalyze the CAL standards, restrict the range of calibration, or select an alternate method of calibration.

CAUTION: When acquiring MS/MS data, LC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at appropriate times. As a precautionary measure, chromatographic peaks in each window must not elute too close to the edge of the segment time window.

10.3 CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Analyze a CCC to verify the initial calibration at the beginning of each analysis batch, after every tenth Field Sample, and at the end of each analysis batch. LRBs, CCCs, LFBs, LFSMs, FDs and LFSMDs are not counted as samples. The beginning CCC of 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 standards to meet this requirement. Alternatively, analyte concentrations in the analyte PDS may be customized to meet this criteria. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

10.3.1 Inject an aliquot of the appropriate concentration CAL standard 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 IS(s) are within 50-150% of the average areas measured during initial calibration. If any of the IS areas 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 Section 10.3.4. Major instrument maintenance requires recalibration (Sect 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect 10.3). Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and SUR in the CCC. The calculated amount for the SUR must be within ± 30% of the true value. Each analyte fortified at a level ≤ MRL must calculate to be within ± 50% of the true value. The calculated concentration of method analytes in CCCs fortified at all other levels must be within ± 30%. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken (Sect. 10.3.4) which may require recalibration. Any Field or QC Samples 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 CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular method analyte, and Field Sample extracts show no detection for that method analyte, 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 the electrospray probe, atmospheric pressure ionization source, mass analyzer, replacing the LC column, etc., requires recalibration (Sect 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect 10.3).

11. PROCEDURE

11.1 This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. Data presented in Tables 5-13 demonstrate data collected by manual extraction. If an automated 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. If an automated system is used, LRBs should be rotated among the ports to ensure that all valves and tubing meet LRB requirements (Sect. 9.3.1).

NOTE: SPE cartridges described in this section are designed as single use items and should be discarded after use. They may not be refurbished for reuse in subsequent analyses.

11.2 SAMPLE PREPARATION

11.2.1 Samples are preserved, collected and stored as presented in Section 8. All Field and QC Samples, including the LRB, and LFB, must contain the preservatives listed in Section 8.1.2. Before extraction, verify that the sample pH is 7 ± 0.5. If the sample pH does not meet this requirement, discard the sample. If the sample pH is acceptable, proceed with the analysis. Before extraction, mark the level of the sample on the outside of the sample bottle for later sample volume determination (Sect. 11.5). If using weight to determine volume, weigh the bottle with collected sample before extraction.

11.2.2 Add an aliquot of the SUR PDS (Sect. 7.2.2.2) to each sample to be extracted, cap and invert to mix. During method development, a 10-µL aliquot of the 0.40 ng/µL SUR PDS (Sect. 7.2.2.2) was added to 250 mL for a final concentration of 16 ng/L in the aqueous sample.

11.2.3 In addition to SUR(s) and preservatives, if the sample is an LFB, FD, LFSM, or LFSMD, add the necessary amount of analyte PDS (Sect. 7.2.3.2). Cap and invert each sample to mix.

11.3 CARTRIDGE SPE PROCEDURE

11.3.1 CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 5 mL of methanol. Next, rinse each cartridge with 10 mL of

reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Add 4-5 mL of reagent water to each cartridge, attach sample transfer tubes (Sect. 6.6.3), turn on the vacuum, and begin adding sample to the cartridge.

11.3.2 SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is 10-15 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

11.3.3 CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the cartridge with 5 mL of reagent water. Draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

11.3.4 CARTRIDGE ELUTION – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Elute analytes from the cartridge by pulling 5 mL of methanol through the cartridge. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.

11.4 EXTRACT CONCENTRATION – Concentrate the extract to less than 1 mL (but not less than 0.5 mL) under a gentle stream of nitrogen in a heated water bath (40 °C). Add the appropriate amount of methanol and IS PDS (Sect. 7.2.1.2) to the collection vial to bring the volume to 1 mL and vortex. (10 µL of the 0.40 ng/µL IS PDS for extract concentrations of 4 pg/µL were used during method development). Transfer a small aliquot to an autosampler vial.

11.5 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 weight from the original sample weight (Sect. 11.2.1). Assume a sample density of 1.0 g/mL. In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 12.2).

11.6 EXTRACT ANALYSIS

11.6.1 Establish operating conditions equivalent to those summarized in Tables 1-4 of Section 17. Instrument conditions and columns should be optimized prior to initiation of the IDC.

11.6.2 Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in CAL standard solutions analyzed on the LC over the course of time. A value of plus or minus three times the standard deviation of the retention time obtained

for each method analyte 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.

11.6.3 Establish a valid initial calibration following the procedures outlined in Sect. 10.2 or confirm that the calibration is still valid by running a CCC as described in Sect. 10.3. If establishing an initial calibration, complete the IDC as described in Section 9.2.

11.6.4 Begin analyzing Field Samples, including QC samples, at their appropriate frequency by injecting the same size aliquots (10 µL was used in method development), under the same conditions used to analyze the CAL standards.

11.6.5 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard. Comparison of MS/MS mass spectra is not particularly useful given the limited ± 0.5 dalton mass range around a single product ion for each method analyte.

11.6.6 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 and the appropriate amount of IS added to match the original concentration. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable SUR performance (Sect. 9.3.5.1) should be determined from the undiluted sample extract. The resulting data should be documented as a dilution and MRLs should be adjusted accordingly.

12. DATA ANALYSIS AND CALCULATION

12.1. Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Table 4. Other ions may be selected at the discretion of the analyst.

12.2. Calculate analyte and SUR concentrations using the multipoint calibration established in Section 10.2. 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 11.5.

12.3 Prior to reporting data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

12.4 Calculations must utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

NOTE: Some data in Section 17 of this method are reported with more than two significant figures. This is done to better illustrate method performance.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND MINIMUM REPORTING LEVELS – Tables for these data are presented in Section 17. LCMRLs and DLs for each method analyte are presented in Table 5. Precision and accuracy are presented for three water matrices: reagent water (Tables 6 and 7); chlorinated (finished) ground water (Tables 8 and 9); chlorinated (finished) surface water (Tables 10 and 11).

13.2 SAMPLE STORAGE STABILITY STUDIES – An analyte storage stability study was conducted by fortifying the analytes into chlorinated surface water samples that were collected, preserved, and stored as described in Section 8. Precision and mean recovery (n=4) of analyses, conducted on Days 0, 7, 14, 23 and 28 are presented in Table 12.

13.3 EXTRACT STORAGE STABILITY STUDIES – Extract storage stability studies were conducted on extracts obtained from a chlorinated surface water fortified with method analytes. Precision and mean recovery (n=4) of injections conducted on Days 0, 7, 14, 23, and 28 are reported in Table 13.

13.4 SECOND LABORATORY DEMONSTRATION – Performance of this method was demonstrated by multiple laboratories, with results similar to those reported in Section 17. The authors wish to acknowledge the assistance of the analysts and laboratories for their participation in the multi-laboratory verification studies: a) Dr. Andrew Eaton and Mr. Ali Haghani of Eurofins Eaton Analytical (EEA), Monrovia, CA, b) Dr. Yongtao Li and Mr. Joshua S. Whitaker of UL LLC, South Bend, IN, and c) Dr. Damon Carl of Heritage Environmental Services, Indianapolis, IN.

14. POLLUTION PREVENTION

14.1 This method utilizes SPE 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 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: Guide to Minimizing Waste in Laboratories" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036 or on-line at http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf (accessed June 2012).

15. WASTE MANAGEMENT

15.1 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, laboratory waste management practices must 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.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. LC METHOD CONDITIONS

Time (min)	% Formate Buffer	% Methanol
Initial	90.0	10.0
8.0	60.0	40.0
9.0	50.0	50.0
28.0	17.7	82.3
28.1	10.0	90.0
30.0	10.0	90.0
30.1	90.0	10.0
40.0	90.0	10.0
Restek Ultra Aqueous 2.1 x 100 mm packed with 5 µm C_{18} stationary phase Flow rate of 0.3 mL/min 10 µL partial loop injection into a 20 µL loop		

TABLE 2. ESI-MS/MS METHOD CONDITIONS

ESI Conditions	
Polarity	Positive ion
Capillary needle voltage	4 kV
Cone gas flow	100 L/hr
Nitrogen desolvation gas	1100 L/hr
Desolvation gas temp.	350 °C

TABLE 3. METHOD ANALYTE SOURCE, RETENTION TIMES (RTs), AND SUGGESTED IS REFERENCES

Analyte	Method Analyte Source[a]	Peak # (Fig. 1)	RT (min)	IS# Ref
methomyl	AccuStandard	1	6.14	1
3-hydroxycarbofuran	Absolute Standards	3	8.09	1
fenamiphos sulfone	Absolute Standards	5	12.30	1
fenamiphos sulfoxide	Absolute Standards	6	12.56	1
phorate sulfone	Spex CertiPrep	7	13.12	3
phorate sulfoxide	Crescent Chemical	8	13.53	3
disulfoton sulfoxide	Chem Service	9	13.91	3
bensulide	Absolute Standards	11	19.14	2
tebufenozide	AccuStandard	12	19.17	2
chlorpyrifos oxon	Chem Service	13	19.62	3
fenamiphos	Crescent Chemical	14	19.64	2
tebuconazole	Crescent Chemical	17	20.93	2
methomyl-$^{13}C_{2,}$ ^{15}N(SUR)	Cambridge Isotopes	2	6.13	1
tebuconazole-d_6 (SUR)	Dr. Ehrenstorfer	16	20.88	2
carbofuran-$^{13}C_6$ (IS#1)	Cambridge Isotopes	4	11.45	-
bensulide-d_{14} (IS#2)	Cambridge Isotopes	10	18.96	-
phorate-d_{10} (IS#3)	Crescent Chemical	15	20.35	-

[a] Data presented in this method were obtained using analytes purchased from these vendors. Other vendors' materials can be used provided the QC requirements in Section 9 can be met.

TABLE 4. MS/MS METHOD CONDITIONS[a]

Segment[b]	Analyte	Precursor Ion[c] *(m/z)*	Product Ion[c,d] (*m/z*)	Cone Voltage (v)	Collision Energy[e] (v)
1	methomyl	163	88	15	10
2	3-hydroxycarbofuran	238	163	20	15
2	fenamiphos sulfone	336	266	30	20
2	fenamiphos sulfoxide	320	233	30	25
2	phorate sulfone	293	171	20	10
2	phorate sulfoxide	277	97	20	30
2	disulfoton sulfoxide	291	185	20	15
3	bensulide	398	314	20	10
3	tebufenozide	353	133	15	20
3	chlorpyrifos oxon	334	278	25	20
3	fenamiphos	304	217	30	25
3	tebuconazole	308	70	30	20
1	methomyl-$^{13}C_2$,^{15}N	166	91	15	10
3	tebuconazole-d_6	314	72	30	20
2	carbofuran-$^{13}C_6$	228	171	26	13
3	bensulide-d_{14}	412	316	20	10
3	phorate-d_{10}	271	75	15	10

[a] An LC/MS/MS chromatogram of the analytes is shown in Figure 1.

[b] Segments are time durations in which single or multiple scan events occur.

[c] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine the precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak place (e.g., *m/z* 398.1→313.9 for bensulide). These precursor and product ion masses (with one decimal place) should be used in the MS/MS method for all analyses.

[d] Ions used for quantitation purposes.

[e] Argon used as collision gas at a flow rate of 0.3 mL/min.

TABLE 5. DLs AND LCMRLs IN REAGENT WATER

Analyte	Fortified Conc. (ng/L)[a]	Oasis HLB		Speedisk H_2O-Philic DVB	
		DL[b] (ng/L)	LCMRL[c] (ng/L)	DL[b] (ng/L)	LCMRL[c] (ng/L)
methomyl	1.60	0.39	1.2	0.32	0.61
3-hydroxycarbofuran	1.60	0.45	1.3	0.25	1.2
fenamiphos sulfone	1.60	0.88	1.0	0.42	1.0
fenamiphos sulfoxide	1.60	0.39	0.86	0.32	1.6
phorate sulfone	1.60	0.57	0.99	0.32	1.0
phorate sulfoxide	1.60	0.70	2.0	0.46	1.1
disulfoton sulfoxide	1.60	0.68	2.0	0.37	1.1
bensulide	1.60	0.51	1.2	1.1	1.4
tebufenozide	0.64	0.26	0.81	0.15	0.73
chlorpyrifos oxon	1.60	1.0	2.0	0.77	2.7
fenamiphos	0.64	0.30	0.64	0.26	0.30
tebuconazole	0.64	0.25	2.0	0.30	0.33

[a] Spiking concentration used to determine DL.

[b] Detection limits were determined by analyzing seven replicates over three days according to Section 9.2.6.

[c] LCMRLs were calculated according to the procedure in reference 1.

TABLE 6. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN REAGENT WATER FORTIFIED AT A HIGH CONCENTRATION (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB		Speedisk H_2O-Philic DVB	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
methomyl	32.0	106	6.3	105	2.5
3-hydroxycarbofuran	32.0	104	2.6	102	3.5
fenamiphos sulfone	32.0	104	4.1	98.4	1.8
fenamiphos sulfoxide	32.0	106	4.5	102	3.4
phorate sulfone	32.0	104	5.1	111	1.5
phorate sulfoxide	32.0	111	2.6	116	3.1
disulfoton sulfoxide	32.0	115	4.4	117	3.8
bensulide	32.0	90.0	4.3	92.7	13
tebufenozide	12.8	103	5.6	95.5	11
chlorpyrifos oxon	32.0	97.1	5.6	104	4.3
fenamiphos	12.8	96.9	2.7	96.7	11
tebuconazole	12.8	100	5.2	97.6	10
methomyl-$^{13}C_2$,^{15}N	16.0	99.0	4.3	99.8	2.4
tebuconazole-d_6	16.0	98.1	8.6	92.6	12

TABLE 7. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN REAGENT WATER FORTIFIED AT A LOW CONCENTRATION (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB		Speedisk H_2O-Philic DVB	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
methomyl	4.00	102	1.9	103	2.6
3-hydroxycarbofuran	4.00	101	6.1	107	4.8
fenamiphos sulfone	4.00	101	3.8	99.6	3.1
fenamiphos sulfoxide	4.00	108	2.0	108	9.6
phorate sulfone	4.00	94.2	3.2	108	7.2
phorate sulfoxide	4.00	106	3.0	112	4.3
disulfoton sulfoxide	4.00	113	2.9	111	6.5
bensulide	4.00	93.6	5.5	93.0	3.0
tebufenozide	1.60	110	5.2	105	2.7
chlorpyrifos oxon	4.00	95.5	1.3	112	11
fenamiphos	1.60	92.6	6.8	104	6.6
tebuconazole	1.60	115	4.9	114	3.4
methomyl-$^{13}C_2$,^{15}N	16.0	101	3.4	98.1	2.1
tebuconazole-d_6	16.0	98.9	1.4	105	4.8

TABLE 8. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT A HIGH CONCENTRATION IN FINISHED DRINKING WATER FROM A GROUND WATER SOURCE[a] (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB		Speedisk H_2O-Philic DVB	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
methomyl	32.0	105	1.3	109	2.0
3-hydroxycarbofuran	32.0	101	2.3	107	1.5
fenamiphos sulfone	32.0	103	4.4	99.0	1.6
fenamiphos sulfoxide	32.0	106	3.2	105	1.3
phorate sulfone	32.0	97.8	4.9	103	1.5
phorate sulfoxide	32.0	102	4.2	104	2.5
disulfoton sulfoxide	32.0	103	4.3	108	2.1
bensulide	32.0	97.1	5.9	97.1	1.2
tebufenozide	12.8	100	4.2	102	4.3
chlorpyrifos oxon	32.0	91.8	3.5	95.1	2.2
fenamiphos	12.8	104	4.5	103	4.4
tebuconazole	12.8	100	5.2	101	4.0
methomyl-$^{13}C_2$,^{15}N	16.0	103	2.3	105	1.0
tebuconazole-d_6	16.0	93.5	7.5	98.6	3.9

[a] TOC = <0.50 mg/L and hardness = 342 mg/L as calcium carbonate.

TABLE 9. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT A LOW CONCENTRATION IN FINISHED DRINKING WATER FROM A GROUND WATER SOURCE[a] (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB		Speedisk H_2O-Philic DVB	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
methomyl	4.00	101	4.3	103	3.4
3-hydroxycarbofuran	4.00	103	5.4	107	4.4
fenamiphos sulfone	4.00	108	6.1	107	1.2
fenamiphos sulfoxide	4.00	99.4	3.2	100	2.0
phorate sulfone	4.00	98.1	2.4	100	2.9
phorate sulfoxide	4.00	109	4.0	108	7.6
disulfoton sulfoxide	4.00	115	1.8	111	6.2
bensulide	4.00	88.8	17	85.6	6.0
tebufenozide	1.60	100	5.1	90.6	8.9
chlorpyrifos oxon	4.00	124	14	102	5.1
fenamiphos	1.60	96.9	3.7	98.4	9.5
tebuconazole	1.60	98.4	3.2	85.9	7.0
methomyl-$^{13}C_2$,^{15}N	16.0	104	3.0	101	1.7
tebuconazole-d_6	16.0	96.3	5.5	91.7	4.4

[a] TOC = 0.61 mg/L and hardness = 377 mg/L as calcium carbonate.

TABLE 10. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT A HIGH CONCENTRATION IN FINISHED DRINKING WATER FROM A SURFACE WATER SOURCE[a] (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB		Speedisk H_2O-Philic DVB	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
Methomyl	32.0	106	2.2	111	2.5
3-hydroxycarbofuran	32.0	101	1.4	105	1.3
fenamiphos sulfone	32.0	106	0.6	104	2.5
fenamiphos sulfoxide	32.0	108	1.8	104	0.7
phorate sulfone	32.0	102	2.6	105	6.0
phorate sulfoxide	32.0	105	2.1	105	5.2
disulfoton sulfoxide	32.0	110	1.8	107	6.0
Bensulide	32.0	101	7.6	100	7.5
Tebufenozide	12.8	101	5.3	96.7	6.1
chlorpyrifos oxon	32.0	102	4.1	103	8.0
Fenamiphos	12.8	107	5.8	99.5	6.8
Tebuconazole	12.8	107	5.4	107	7.5
methomyl-$^{13}C_2$,^{15}N	16.0	100	3.5	107	1.8
tebuconazole-d_6	16.0	98.9	6.3	101	6.4

[a] TOC = 1.22 mg/L and hardness =154 mg/L as calcium carbonate.

TABLE 11. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT A LOW CONCENTRATION IN FINISHED DRINKING WATER FROM A SURFACE WATER SOURCE (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB[a]		Speedisk H_2O-Philic DVB[b]	
		Mean % Recovery	% RSD	Mean % Recovery	% RSD
methomyl	4.00	104	2.1	109	3.1
3-hydroxycarbofuran	4.00	96.6	4.3	106	3.4
fenamiphos sulfone	4.00	97.6	2.2	104	4.3
fenamiphos sulfoxide	4.00	106	3.1	105	7.4
phorate sulfone	4.00	90.3	3.3	98.1	6.6
phorate sulfoxide	4.00	102	5.5	105	5.7
disulfoton sulfoxide	4.00	111	4.8	107	4.4
bensulide	4.00	90.4	7.1	96.4	12
tebufenozide	1.60	98.9	9.5	105	11
chlorpyrifos oxon	4.00	94.0	4.6	104	7.6
fenamiphos	1.60	96.1	3.9	100	3.1
tebuconazole	1.60	111	6.6	120	5.2
methomyl-$^{13}C_2$,^{15}N	16.0	101	0.8	103	1.9
tebuconazole-d_6	16.0	100	2.8	101	3.7

[a] TOC = 1.22 mg/L and hardness =154 mg/L as calcium carbonate.

[b] TOC = 1.58 mg/L and hardness =137 mg/L as calcium carbonate.

TABLE 12. AQUEOUS SAMPLE HOLDING TIME DATA FOR SAMPLES OF FINISHED DRINKING WATER FROM A SURFACE WATER SOURCE[a], FORTIFIED WITH METHOD ANALYTES AND PRESERVED AND STORED ACCORDING TO SECTION 8 (n=4)[b]

Analyte	Fortified Conc. (ng/L)	Day 0		Day 7		Day 14		Day 23		Day 28	
		Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD
methomyl	32.0	106	2.2	104	2.5	109	1.3	109	3.8	113	1.6
3-hydroxycarbofuran	32.0	101	1.4	95.2	2.8	94.7	5.1	90.5	1.4	93.9	3.6
fenamiphos sulfone	32.0	106	0.6	103	1.5	100	3.2	99.4	2.1	104	4.3
fenamiphos sulfoxide	32.0	108	1.8	103	3.0	103	3.5	105	2.4	108	1.4
phorate sulfone	32.0	102	2.6	95.9	5.2	89.1	11	101	1.2	100	2.8
phorate sulfoxide	32.0	105	2.1	100	5.2	93.2	11	105	1.5	103	3.5
disulfoton sulfoxide	32.0	110	1.8	108	5.0	101	11	112	3.6	110	5.1
bensulide	32.0	101	7.6	99.2	5.5	100	4.1	106	6.0	100	4.6
tebufenozide	12.8	101	5.3	104	7.4	109	5.8	108	4.1	101	5.0
chlorpyrifos oxon	32.0	102	4.1	79.6	6.2	62.4	13	59.1	3.0	56.0	3.7
fenamiphos	12.8	107	5.8	98.4	4.1	104	4.4	107	5.5	98.7	5.9
tebuconazole	12.8	107	5.4	104	6.9	110	7.2	108	7.1	106	2.6
methomyl-$^{13}C_2$,^{15}N[c]	16.0	100	3.5	101	2.5	105	1.7	102	1.5	104	2.6
tebuconazole-d_6[c]	16.0	98.9	6.3	91.7	8.2	102	5.4	102	6.8	95.5	6.4

[a] TOC = 1.22 mg/L and hardness = 154 mg/L as calcium carbonate.

[b] Oasis HLB SPE cartridges used for holding time studies.

[c] Surrogates were not added to samples until the day of extraction.

TABLE 13. EXTRACT HOLDING TIME DATA FOR SAMPLES OF FINISHED DRINKING WATER FROM A SURFACE WATER SOURCE[a], FORTIFIED WITH METHOD ANALYTES AND PRESERVED AND STORED ACCORDING TO SECTION 8 (n=4)[a]

Analyte	Fortified Conc. (ng/L)	Day 0		Day 7		Day 14		Day 23		Day 28	
		Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD
methomyl	32.0	106	2.2	105	0.9	109	1.9	104	4.3	108	1.5
3-hydroxycarbofuran	32.0	101	1.4	99.0	1.5	106	2.1	99.8	2.0	105	2.3
fenamiphos sulfone	32.0	106	0.6	101	1.6	103	2.5	101	2.3	103	1.2
fenamiphos sulfoxide	32.0	108	1.8	103	1.8	106	1.2	106	2.8	109	0.5
phorate sulfone	32.0	102	2.6	98.8	6.7	90.1	2.5	95.5	3.5	101	2.8
phorate sulfoxide	32.0	105	2.1	103	7.6	94.1	4.1	101	2.3	102	3.0
disulfoton sulfoxide	32.0	110	1.8	108	6.5	98.9	5.5	104	3.1	106	3.8
bensulide	32.0	101	7.6	102	1.6	99.2	2.0	105	5.1	102	4.3
tebufenozide	12.8	101	5.3	107	2.0	107	2.1	109	4.6	105	3.8
chlorpyrifos oxon	32.0	102	4.1	97.7	8.0	86.2	4.1	92.1	3.4	97.7	5.9
fenamiphos	12.8	107	5.8	106	4.0	105	1.5	107	3.5	109	3.9
tebuconazole	12.8	107	5.4	104	2.2	106	2.3	111	4.9	115	3.6
methomyl-$^{13}C_2$,^{15}N	16.0	100	3.5	103	0.9	105	1.6	106	3.8	107	2.0
tebuconazole-d_6	16.0	98.9	6.3	94.4	4.0	94.4	2.1	99.2	4.1	96.8	2.7

[a] Oasis HLB SPE cartridges used for holding time studies.

TABLE 14. INITIAL DEMONSTRATION OF CAPABILITY QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1 and 9.3.1	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps.	Demonstrate that all method 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 four to seven replicate LFBs fortified near the midrange calibration concentration.	%RSD must be <20%
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP.	Mean recovery ± 30% of true value
Sect. 9.2.4	Minimum Reporting Limit (MRL) Confirmation	Fortify, extract and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the Mean and the Half Range (HR). Confirm that the upper and lower limits for the Prediction Interval of Result (Upper PIR, and Lower PIR, Sect. 9.2.4.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Sect. 9.2.5 and 9.3.8	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of IDC.	Results must be within 70-130% of true value.

NOTE: Table 14 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Section 9 supersedes any missing or conflicting information in this table.

TABLE 15. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.4	Sample Holding Time	28 days with appropriate preservation and storage as described in Sections 8.1-8.4 except chlorpyrifos oxon (7 days).	Sample results are valid only if samples are extracted within the sample holding time.
Sect. 8.4	Extract Holding Time	28 days when stored at ≤ 6 °C and protected from light.	Extract results are valid only if extracts are analyzed within the extract holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each extraction batch of up to 20 Field Samples.	Demonstrate that all method analytes are below 1/3 the MRL, and confirm that possible interferences do not prevent quantification of method analytes. If targets exceed 1/3 the MRL or if interferences are present, results for these subject analytes in the extraction batch are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	One LFB is required for each extraction batch of up to 20 Field Samples. Rotate the fortified concentrations between low, medium, and high amounts.	Results of LFB analyses must be 70-130% of the true value for each method analyte for all fortified concentrations except the lowest CAL point. Results of the LFBs corresponding to the lowest CAL point for each method analyte must be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standards, carbofuran-$^{13}C_6$, bensulide-d_{14} and phorate-d_{10}, are added to all standards and sample extracts, including QC samples. Compare IS areas to the average IS area in the initial calibration.	Peak area counts for all ISs in all injections must be within ± 50% of the average peak area calculated during the initial calibration. If ISs do not meet this criterion, corresponding target results are invalid.
Sect. 9.3.5	Surrogate Standards (SUR)	Surrogate standards, methomyl-$^{13}C_{2,}{}^{15}N$ and tebuconazole-d_6, are added to all CAL standards and samples, including QC samples. Calculate SUR recoveries.	SUR recoveries must be 70-130% of the true value. If a SUR fails this criterion, report all results for sample as suspect/SUR recovery.
Sect. 9.3.6	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration, if known. Calculate LFSM recoveries.	Recoveries at mid and high levels should be within 70-130% and within 50-150% at the low-level fortified amount (near the MRL). If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Extract and analyze at least one FD or LFSMD with each extraction batch (20 samples or less). A LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	See Sect. 9.3.6 and 9.3.7 for instructions on the interpretation of LFSM and FD results.
Sect. 9.3.8	Quality Control Sample (QCS)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Results must be within 70-130% of true value.

TABLE 15. (Continued)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 10.2	Initial Calibration	Use IS calibration technique to generate a linear or quadratic calibration curve for each analyte. Use at least five standard concentrations. Check the calibration curve as described in Sect. 10.2.7.	When each CAL standard is calculated as an unknown using the calibration curve, the analyte results must be 70-130% of the true value for all except CAL standards ≤ MRL, which must be 50-150% of the true value. If this criterion is not met reanalyze the CAL standards, restrict the range of calibration, or select an alternate method of calibration.
Sect. 9.3.2 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 Field Samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each SUR must be within 70-130% of the true value in all CCCs. Each analyte fortified at a level ≤ MRL must calculate to be within ± 50% of the true value. The calculated concentration of the method analytes in CCCs fortified at all other levels must be within ± 30%.

NOTE: Table 15 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Section 8-10 supersedes any missing or conflicting information in this table.

FIGURE 1. EXAMPLE CHROMATOGRAM (OVERLAID MS/MS SEGMENTS) OF A CALIBRATION STANDARD WITH METHOD 540 ANALYTES AT CONCENTRATION LEVELS OF 3.2-8.0 µg/L. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 3.