METHOD 536   **DETERMINATION OF TRIAZINE PESTICIDES AND THEIR DEGRADATES IN DRINKING WATER BY LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (LC/ESI-MS/MS)**

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METHOD 536

DETERMINATION OF TRIAZINE PESTICIDES AND THEIR DEGRADATES IN DRINKING WATER BY LIQUID CHROMATOGRAPHY ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (LC/ESI-MS/MS)

1.   SCOPE AND APPLICATION

1.1   This is a liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) method for the determination of triazine pesticides and their degradation products in finished drinking waters. This method requires the use of MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance selectivity. Precision and accuracy data have been generated for the detection of the triazine pesticides and their degradates in reagent water and finished drinking water from both groundwater and surface water sources. The single laboratory Lowest Concentration Minimum Reporting Level (LCMRL) has also been determined in reagent water.[1] The following compounds can be determined using this method:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Atrazine	1912-24-9
Atrazine-desethyl	6190-65-4
Atrazine-desethyl-desisopropyl	3397-62-4
Atrazine-desisopropyl	1007-28-9
Cyanazine	21725-46-2
Propazine	139-40-2
Simazine	122-34-9

1.2   The MRM mass spectrometry conditions described in this method were developed using a conventional LC/MS/MS system.

1.3   The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets the Data Quality Objectives (DQOs), which are based on the intended use of this method. The single laboratory LCMRL is the lowest true concentration for which the future recovery is predicted to fall between 50-150% recovery with 99% confidence. Single laboratory LCMRLs for the analytes in this method ranged from 0.020 – 0.14 µg/L, and are listed in Table 4. The procedure used to determine the LCMRL is described elsewhere.[1]

1.4   Laboratories using this method are not required to determine an LCMRL, but they will need to demonstrate that their laboratory Minimum Reporting Level (MRL) for this method meets the requirements described in Section 9.2.4.

1.5   Detection limit (DL) 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 dependent on sample matrix, fortification concentration, and instrument performance.

Determining the DL for analytes in this method is optional (Sect. 9.2.6). DLs for method analytes fortified into reagent water ranged from 0.010 – 0.043 μg/L. These values are also provided in Table 4.

1.6   This method is intended for use by analysts skilled in the operation of LC/MS/MS instrumentation and the interpretation of the associated data.

1.7   METHOD FLEXIBILITY – In recognition of technological advances in analytical systems and techniques, the laboratory is permitted to modify the separation technique, LC column, mobile phase composition, LC conditions and MS conditions (Sect. 6.10, 9.4, 10.2.2, 10.2.3, 10.2.4, and 12.1). **Changes may not be made to sample collection and preservation (Sect. 8) or to the 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, may not be used. **The analytes must be resolved chromatographically in order to avoid the effects of matrix suppression or enhancement and to provide analyte specificity**. 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 QC acceptance criteria in this method (Tables 9 and 10) are met, and verify method performance on a real sample matrix (Sect. 9.4).

2.   SUMMARY OF METHOD

2.1   Samples are pH adjusted and dechlorinated with ammonium acetate and protected from microbial degradation using sodium omadine during sample collection. Prior to analysis, isotopically enriched analytes [Atrazine-d5(ethyl-d5), Atrazine-desethyl-d7, Atrazine-desethyl-desisopropyl(^{13}C3), Atrazine-desisopropyl-d5(ethyl-d5), Cyanazine-d5(N-ethyl-d5), Propazine-d14(di-isopropyl-d14), and Simazine-d10(diethyl-d10)] are added to the samples as internal standards. An aliquot of the sample is injected without cleanup or concentration onto a chromatographic column (C18). The analytes are separated using an ammonium acetate-buffered binary mobile phase and subsequently detected by positive electrospray ionization tandem mass spectrometry. The concentration of each identified component is determined using the internal standard technique.

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. Each 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),
Continuing Calibration Check Standards (CCCs),
Laboratory Fortified Sample Matrix (LFSM), and
Laboratory Fortified Sample Matrix Duplicate or Laboratory Duplicate (LFSMD or LD).

3.2   CALIBRATION STANDARD (CAL) – A solution of the target analytes prepared from the primary dilution standard solution(s) or stock standard solution(s) and the internal standards. 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 the method analytes and internal standards which is analyzed periodically to verify the accuracy of the existing calibration.

3.4   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 (Sect. 9.2.6), and accurate quantitation is not expected at this level.[2]

3.5   INTERNAL STANDARD (IS) – A pure compound added to all standard solutions and field samples in a known amount. It is used to measure the relative response of the method analyte. The internal standard must be a compound that is not a sample component.

3.6   ION SUPRESSION/ENHANCEMENT – An observable loss or increase in target analyte response in complex (field) samples as compared to the response obtained in standard solutions.

3.7   LABORATORY DUPLICATES (LDs) – Two sample aliquots (LD_1 and LD_2), taken in the laboratory from a single sample bottle, and analyzed separately with identical procedures. Analyses of LD_1 and LD_2 indicate precision associated specifically with the laboratory procedures by removing variation contributed from sample collection, preservation and storage procedures.

3.8   LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water or other blank matrix to which a known quantity of the method analytes is added. The LFB is analyzed exactly like a sample including the preservation procedures in Section 8. Its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements.

3.9   LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – An aliquot of a field sample to which a known quantity of the method analytes is added. 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 concentration of the analytes in the sample matrix must be determined in a separate aliquot and the measured value in the LFSM corrected for background concentrations.

3.10   LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the field sample used to prepare the LFSM which is fortified and analyzed identically to the LFSM. The LFSMD is used instead of the Laboratory Duplicate to assess method precision and accuracy when the occurrence of the target analytes is infrequent.

3.11   LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all storage containers, buffers, preservatives and internal standards. The LRB is used to determine if the method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.12   LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which the future recovery is predicted to fall between 50-150% recovery with 99% confidence.[1]

3.13   MATERIAL SAFETY DATA SHEETS (MSDS) – These sheets contain 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   MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value for the target analyte in a sample following analysis. This concentration must meet the criteria defined in Section 9.2.4 and must be no lower than the concentration of the lowest calibration standard for the target analyte.

3.15   MULTIPLE REACTION MONITORING (MRM) – A mass spectrometric technique in which a precursor ion is first isolated then subsequently fragmented into product ion(s). Quantitation is accomplished by monitoring a specific product ion. As described in Section 10.2.2, MS parameters must be optimized for each precursor ion (Sect. 3.16) and product ion (Sect. 3.18).

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

3.17   PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing the method analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration standards and other 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 collisionally activated dissociation of the precursor ion.

3.19   QUALITY CONTROL SAMPLE (QCS) – A solution containing the method analytes at a known concentration which 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.

3.20   REAGENT WATER (RW) – Purified water which does not contain any measurable quantity of the target analytes or interfering compounds at or above 1/3 the MRL for the target analytes.

3.21   STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more of the method analytes that is prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source, so that the concentration and purity of analytes are traceable to certificates of analysis.

4.   **INTERFERENCES**

4.1   All glassware must be meticulously cleaned. Wash glassware thoroughly and rinse with reagent water.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware. These interferences may lead to discrete artifacts and/or elevated baselines in the chromatograms. All laboratory reagents and equipment must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for the target analytes) under the conditions of the analysis. This may be accomplished by analyzing LRBs as described in Section 3.11. **Subtracting blank values from sample results is not permitted.**

4.3 Equipment used for sample collection and storage has the potential to introduce interferences. The potential for interferences from these devices must be investigated during the Initial Demonstration of Capability (Sect. 9.2) by preparing and analyzing a Laboratory Reagent Blank (LRB). This procedure should be repeated each time a new brand or lot of equipment is used to ensure that background contamination does not interfere with the identification and quantitation of the analytes.

4.4 Matrix interferences may be caused by contaminants that are present in the sample. The extent of matrix interferences will vary considerably from source to source depending upon the nature of the water.

    4.4.1 ION SUPPRESSION/ENHANCEMENT – Field samples containing moderate-to-high levels of organic carbon or dissolved solids may cause suppression or enhancement of instrumental response for one or more of the analytes. Suppression and enhancement effects are introduced during the ionization process in the electrospray source when a co-eluting contaminant influences the ionization of the analyte of interest. MS/MS methods are not immune to suppression or enhancement effects because the fragmentation process occurs after ionization. Isotopically enriched internal standards can help mitigate the effect of matrix-induced suppression or enhancement, but good performance is best achieved by optimizing the chromatographic separation.

    4.4.2 INTERFERING COMMON IONS – While MS/MS detection offers increased selectivity relative to MS mode detection, interference can still occur in complex samples if common ions are present in co-eluting components. Due to a lack of LC/MS/MS reference spectral libraries, optimized chromatographic separation is required for mass resolution of the analytes of interest in the method.

5. <u>SAFETY</u>

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.

5.2 Pure standard materials and stock standard solutions of the method compounds should be handled with suitable protection for skin, eyes, etc. [3-5]

6. **EQUIPMENT AND SUPPLIES** (References to specific brands or catalog numbers are included as examples only and do not imply endorsement of the product. This does not preclude the use of other vendors or suppliers.)

6.1 SAMPLE CONTAINERS – Pre-cleaned, amber vials (40 mL or larger) fitted with PTFE/silicone septa and polypropylene screw caps (Fisher Cat. No. 02-912-377 or equivalent).

6.2 VIALS – Amber 2-mL glass autosampler vials with PTFE/silicone septa for storing standards (Fisher Cat. No. 03-375-20B or equivalent).

6.3 MICRO SYRINGES – Suggested sizes include 10, 25, 50, 100, 250, and 500 µL.

6.4 VOLUMETRIC FLASKS – Class A, suggested sizes include 5, 10, 50, 100, 250, and 500 mL for preparation of reagents and standards.

6.5 VOLUMETRIC PIPETTES – Class A, suggested sizes include 1, 4, 5, 10, 20, and 25 mL.

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

6.7 DESOLVATION GAS – High purity compressed gas (e.g., nitrogen or zero-air) used for desolvation in the mass spectrometer. The specific type of gas, purity and pressure requirements will depend on the instrument manufacturers' specifications.

6.8 COLLISION GAS – High purity compressed gas (e.g., nitrogen or argon) used in the collision cell of the mass spectrometer. The specific type of gas, purity and pressure requirements will depend on the instrument manufacturer's specifications.

6.9 DISPOSABLE PASTEUR PIPETS – 9" borosilicate glass, used to transfer samples to autosampler vials (Fisher Cat. No. 13-678-20C or equivalent).

6.10 LIQUID CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROMETRY SYSTEM (LC/ESI-MS/MS)

6.10.1 LC COLUMN – LC column, Waters Xterra® RP18, 2.1 x 150 mm, 3.5 µm d_p (Waters Part No. 186000410 or equivalent). Any column that provides adequate resolution, peak shape, capacity (Sect. 10.2.3 and 10.2.4), accuracy and precision (Sect. 9), and does not result in suppression or enhancement of analyte response (Sect. 13.4.2) may be used.

6.10.2 LC SYSTEM –The LC system (Waters 2695 or equivalent) must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate.

6.10.3 MASS SPECTROMETER –The MS (Micromass QuattroMicro or equivalent) must be capable of providing electrospray ionization with positive ion detection. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.18) for the target analytes within specified retention time windows. Data presented in Section 17, Tables 4 - 8 were collected using an ESI-MS/MS system.

6.10.4 DATA SYSTEM – An interfaced data system is required to acquire, store, and output MS data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the ion abundance of any specific ion between specified times or scan number limits. The software must be able to construct a linear regression or quadratic calibration curve, and calculate analyte concentrations using the internal standard technique.

7. <u>REAGENTS AND STANDARDS</u>

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used. 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, as long as the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 METHANOL – (CH_3OH, CAS# 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher GC Resolv grade or equivalent).

7.1.2 AMMONIUM ACETATE – ($NH_4C_2H_3O_2$, CAS# 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Fisher Brand HPLC grade or equivalent).

7.1.3 5 mM AMMONIUM ACETATE MOBILE PHASE – To prepare 1 L of mobile phase, add 0.3854 g ammonium acetate to 1 L of reagent water (final pH ~ 6.5). This solution is prone to volatility losses and should be replaced every 48 hours.

7.1.4 2.0 M AMMONIUM ACETATE/REAGENT WATER CONCENTRATED STOCK – Add 38.5 g of ammonium acetate to a 250-mL volumetric flask. Dilute to volume with reagent water. An aliquot of this concentrated stock solution is diluted 1:100 in all calibration standards and samples to yield a final concentration of 20 mM (1.5 g/L).

7.1.5 SODIUM OMADINE® (Sodium 2-pyridinethiol-1-oxide, CAS# 3811-73-2) – (C_5H_4NOSNa) – 40 wt.% in water (Omadine is a registered trademark of Arch Chemicals, Inc.).

7.1.6 SODIUM OMADINE CONCENTRATED STOCK SOLUTION (32 g/L) – Add 20 mL of the 40 wt.% sodium omadine in water to a 250-mL volumetric flask. Dilute to volume with reagent water. The concentrated stock solution is diluted 1:500 in all calibration standards and samples to yield a final concentration of 64 mg/L.

7.2 STANDARD SOLUTIONS – When a compound's purity is assayed to be 96 percent 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 only as an example**. Stock standard solutions are estimated to be stable for six months. Any fortified or dilute solutions made from the stock standards are stable for at least 60 days provided they are stored at a temperature $\leq 6\ ^{\circ}C$ and the stock standard solutions have not exceeded their six month stability period. **Although estimated stability times for standard solutions are given, laboratories should use standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTIONS – This method uses a separate isotopically enriched internal standard for each target analyte, specifically Atrazine-d5(ethyl-d5) (CAS# 163165-75-1), Atrazine-desethyl-d7, Atrazine-desethyl-desisopropyl(^{13}C3), Atrazine-desisopropyl-d5(ethyl-d5), Cyanazine-d5(N-ethyl-d5), Propazine-d14(di-isopropyl-d14), and Simazine-d10(diethyl-d10) (CAS# 220621-39-6). It should be noted that all of the internal standards except atrazine-desethyl-desisopropyl (^{13}C3) are commercially available; the atrazine-desethyl-desisopropyl (^{13}C3) internal standard used during method development was custom synthesized by Cambridge Laboratories.

7.2.1.1 INTERNAL STANDARD STOCK STANDARDS (ISSS) (500 μg/mL, except as noted) – Prepare the stock standards individually by weighing 2.5 mg of the solid standards into tared 5-mL volumetric flasks and diluting to volume with methanol. Atrazine-desethyl-desisopropyl (^{13}C3) and Simazine-d10 (diethyl-d10) exhibit limited solubility in methanol and must be prepared as ≤ 100 μg/mL stock solutions. To prepare the 100 μg/mL stock solutions, weigh 2.5 mg of the solid standards into tared 25-mL volumetric flasks and dilute to volume with methanol. Sonication may be required to achieve full dissolution of the standards.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION STANDARD (PDS) (1.0 μg/mL) – Prepare the internal standard PDS by adding enough of each ISSS to a volumetric flask partially filled with methanol to make the final concentrations near 1.0 μg/mL when filled to the mark with methanol. During method development, addition of 5 μL of the internal standard PDS to each 1.0-mL sample produced a final concentration of 5 μg/L. Analysts are permitted to use other PDS concentrations and volumes provided all field samples and CAL standards contain the same amount of internal standard, the concentration of the internal standard added provides adequate signal to maintain precision, and the volume added has a negligible effect on the final concentration.

7.2.2 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat standards. Prepare the Analyte Stock and Primary Dilution Standards as described below.

7.2.2.1 ANALYTE STOCK STANDARD SOLUTION (500 μg/mL, except as noted) – Prepare the stock standards individually by weighing 2.5 mg of the solid standards into tared 5-mL volumetric flasks and diluting to volume with methanol. Atrazine-desethyl-desisopropyl and Simazine exhibit limited solubility in methanol and must be prepared as ≤ 100 μg/mL stock solutions. To prepare the 100 μg/mL stock solutions, weigh 2.5 mg of the solid standards into tared 25-mL volumetric flasks and dilute to volume with methanol. Sonication may be required to achieve full dissolution of the standards.

7.2.2.2 ANALYTE PRIMARY DILUTION SOLUTION (PDS) (2.5 – 5 μg/mL) – The analyte PDS is prepared by dilution of the Analyte Stock Standard solutions and is used to prepare the calibration standards and to fortify the LFBs, LFSMs and LFSMDs with the target analytes.

7.2.3   CALIBRATION (CAL) STANDARDS – Prepare a procedural calibration curve from dilutions of the analyte PDS in reagent water. Transfer the standard to a proper storage container and add appropriate amounts of the ammonium acetate and sodium omadine concentrated stocks (Sect. 7.1.4 and 7.1.6) as shown in the table below. The target analyte concentrations found in Tables 5-7 can be used as a starting point for determining the calibration range. An example of the dilutions used to prepare the CALs that were used to collect data in Section 17 is shown below. The lowest concentration calibration standard must be at or below the MRL, which may depend on system sensitivity. The CAL standards may also be used as CCCs. If stored, the aqueous standards must be refrigerated in the same manner as the samples. A constant amount of the internal standard is added to each prepared Calibration Standard This is accomplished for each standard by taking 1.0 mL of the final calibration standard containing the 20 mM ammonium acetate and 64 mg/L sodium omadine, and placing it in a 2.0-mL autosampler vial and adding the appropriate volume of Internal Standard PDS (Sect. 7.2.1.2).

CAL Level	Analyte PDS Conc. (µg/mL)	Analyte PDS Volume (µL)	Ammonium Acetate Stock Volume [*] (mL)	Sodium Omadine Stock Volume [*] (mL)	Final CAL Std. Volume (mL)	Final CAL Std. Conc. (µg/L)
1	5	5	1.0	0.2	100	0.25
2	5	10	1.0	0.2	100	0.50
3	5	20	1.0	0.2	100	1.00
4	5	20	0.5	0.1	50	2.00
5	5	25	0.5	0.1	50	2.50
6	5	10	0.1	0.02	10	5.00

[*]Ammonium acetate stock (2.0 M) and sodium omadine stock (32 g/L) are added after the standards have been diluted to volume and transferred to storage containers.

7.2.3.1   This is a procedural calibration technique. If an alternate set of dilutions are used to prepare the calibration standards, the analyst must ensure that the dilution factor used to prepare the calibration standards remains consistent with the dilution factor during sample collection (Sect. 8.1.1). The dilution factor is currently 101.2/100 in the table above and in Section 8.1. The analyst must also ensure that all CAL standards, QC and field samples contain the preservation reagents at their proper concentrations (Sect. 8.1.1).

8.   SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1   SAMPLE COLLECTION

8.1.1.   Prior to shipment to the field, ammonium acetate and sodium omadine must be added to each amber bottle fitted with a PTFE-lined screw cap. If using the suggested 40-mL vials to collect a 40-mL aliquot, add 400 µL of the ammonium acetate concentrated stock (Sect. 7.1.4) and 80 µL of the concentrated sodium omadine stock (Sect. 7.1.6). If other collection volumes are used, adjust the amount of preservation reagent so that the final concentrations of ammonium acetate and sodium omadine in the sample containers are 1.5 g/L and 64 mg/L, respectively. Cap the vials to avoid evaporation of the preservation reagents.

8.1.2   When sampling from a cold water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually approximately 3 to 5 minutes). Collect a representative sample from the flowing system using a beaker of appropriate size. Use this bulk sample to generate individual samples as needed. Transfer a volume of at least 40 mLs into each collection container, cap the container, and invert it several times to mix the sample with the preservation reagents. Care should be taken not to overfill the vial and flush out the preservation reagents. Samples do not need to be headspace free.

8.1.3   When sampling from an open body of water, fill a beaker with water sampled from a representative area. Use this bulk sample to generate individual samples as needed.

8.2   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 stored at or below 6 °C until analysis. Samples should not be frozen.

8.3   SAMPLE HOLDING TIMES – Samples should be analyzed as soon as possible. Samples that are collected and stored as described in Sections 8.1 and 8.2 may be stored prior to analysis for a maximum of 28 days.

9.   **QUALITY CONTROL**

9.1   Quality Control (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 each QC parameter, its required frequency, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Section 17, Tables 9 and 10. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

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 meet the calibration requirements outlined in Sections 10.2 and 10.3. Requirements for the initial demonstration of laboratory capability are described in the following sections and are summarized in Table 9.

9.2.1   DEMONSTRATION OF LOW SYSTEM BACKGROUND – Analyze a LRB and confirm that the blank is reasonably free of contamination and that the criteria in Section 9.3.1 are met.

NOTE: It is good laboratory practice to include a blank in the calibration of any instrument. The method should also be checked for carry-over by analyzing a RW blank immediately following the highest CAL standard. If this RW sample does not meet the criteria outline in Section 9.3.1, then carry-over is present and should be identified and eliminated.

9.2.2   DEMONSTRATION OF PRECISION – Prepare and analyze 7 replicate LFBs. These samples should be fortified near the midrange of the initial calibration curve.

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Ammonium acetate and sodium omadine must be added to the samples as described in Section 8.1. The percent relative standard deviation (%RSD) of the concentrations of the replicate analyses must be ≤ 20% for all target analytes.

$$\% \, RSD = \frac{\text{Standard Deviation of Measured Concentrations}}{\text{Average Concentration}} \times 100$$

9.2.3 DEMONSTRATION OF ACCURACY – Using the same sets of replicate data generated for Section 9.2.2, calculate the average percent recovery. The average percent recovery of the replicate analyses must be within ± 30% of the true value.

$$\% \, Recovery = \frac{\text{Average Measured Concentration}}{\text{Fortified Concentration}} \times 100$$

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The lowest calibration standard used to establish the initial calibration (as well as the low-level Continuing Calibration Check) 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 or validate the MRL following the procedure outlined below.

9.2.4.1 Fortify and analyze seven replicate LFBs at or below the proposed MRL concentration. All samples must be fortified and processed using the sample collection protocols described in Section 8.1. Calculate the mean (*Mean*) and standard deviation (*S*) 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* is the standard deviation, and 3.963 is a constant value for seven replicates.[1]

9.2.4.2 Confirm that the upper and lower limits for the Prediction Interval of Results (*PIR = Mean ± HR*$_{PIR}$) meet the upper and lower recovery limits as shown below.

The Upper PIR Limit must be ≤ 150 percent recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\,Concentration} \times 100 \leq 150\%$$

The Lower PIR Limit must be ≥ 50 percent recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\,Concentration} \times 100 \geq 50\%$$

9.2.4.3 The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above. 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 QUALITY CONTROL SAMPLE (QCS) – Analyze a mid-level Quality Control Sample (Sect. 9.3.7) to confirm the accuracy of the calibration curve fit.

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.*

Analyses for this procedure should be done over at least 3 days. Prepare at least 7 replicate LFBs. Ammonium acetate and sodium omadine must be added to the samples as described in Section 8.1. Use the solutions described in Section 7.2 to fortify 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. 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:

$$DL = St_{(n-1, 1-\alpha = 0.99)}$$

where

$t_{(n-1, 1-\alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom (for seven replicate determinations, the Student's t value is 3.143 at a 99% confidence level),
n = number of replicates, and
S = standard deviation of replicate analyses.

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

9.3 ONGOING QC REQUIREMENTS – This section describes the ongoing QC criteria that must be followed when processing and analyzing field samples. Table 10 summarizes these requirements.

9.3.1 LABORATORY REAGENT BLANK (LRB) – A LRB is analyzed during the IDC and is required with each Analysis Batch (Sect. 3.1) to confirm that background contaminants are not interfering with the identification or quantitation of the target analytes. 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. The LRB must contain ammonium acetate, sodium omadine, and the internal standard at the same concentrations used to fortify all field samples and CAL standards. Background from target analytes or contaminants that interfere with the measurement of target analytes must be ≤ 1/3 the MRL. If the target analytes are detected in the LRB at concentrations equal to or greater than this level, then

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all data for the problem analyte(s) must be considered invalid for all samples which yielded a positive result.

NOTE: Although quantitative data below the MRL may not be accurate enough for data reporting, such data are useful in determining the magnitude of background interference. Therefore, blank contamination levels may be estimated by extrapolation when the concentration is below the MRL.

9.3.2   CONTINUING CALIBRATION CHECK (CCC) – CCC standards are analyzed at the beginning of each Analysis Batch, after every ten field samples and at the end of the Analysis Batch. See Section 10.4 for concentration requirements and acceptance criteria.

9.3.3   LABORATORY FORTIFIED BLANK (LFB) – Since this method utilizes procedural calibration standards, which are fortified reagent waters, there is no difference between the LFB and the continuing calibration check standard. Consequently, the analysis of a separate LFB is not required as part of the ongoing QC; however, the acronym LFB is used for clarity in the IDC.

9.3.4   INTERNAL STANDARDS (IS) – The analyst must monitor the peak areas of the internal standards in all injections of the Analysis Batch. The IS responses (as indicated by peak areas) for any chromatographic run must not deviate by more than $\pm$ 50% from the average areas measured during the initial calibration for the internal standards. If the IS area counts for a sample do not meet this criterion, inject a second aliquot of the suspect sample as part of the same or a new Analysis Batch.

   9.3.4.1   If the re-injected aliquot produces an acceptable internal standard response, report results for that aliquot.

   9.3.4.2   If the IS area counts of the re-injected aliquot still do not meet the IS criterion, check the IS area of the most recent CCC. If the IS criterion is met in the CCC but not the sample, report the sample results as suspect/matrix.

   9.3.4.3   If the IS area criterion is not met in both the sample and the CCC, instrument maintenance such as sample cone cleaning may be necessary. Perform the appropriate instrument maintenance and then re-inject the sample in a subsequent analytical batch.

9.3.5   LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of a LFSM (Sect. 3.9) is required in each Analysis Batch. 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 aliquot and the measured values in the LFSM corrected for background concentrations. If a variety of different sample matrices are analyzed regularly, for example drinking water from groundwater and surface water sources, performance data should be collected for each source.

   9.3.5.1   Within each Analysis Batch, a minimum of one field sample is fortified as an LFSM for every 20 samples analyzed. The LFSM is prepared by fortifying a sample with an appropriate amount of the analyte PDS (Sect. 7.2.2.2). Select a spiking

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concentration that is greater than or equal to the native background concentration, if known. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data and rotate through low, medium and high calibration concentrations when selecting a fortifying concentration.

9.3.5.2 Calculate the percent recovery (%REC) using the equation:

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

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

9.3.5.3 Recoveries for samples fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) should be within $\pm$ 50% of the true value. Recoveries for samples fortified at all other concentrations should be within $\pm$ 30% of the true value. If the accuracy for any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the 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.

9.3.5.3.1 Field samples that have native analyte concentrations above the DL but below the MRL and are fortified at concentrations at or near the MRL should be corrected for the native levels in order to obtain meaningful %REC values. This example and the LRB (Sect. 9.3.1) are the only permitted use of analyte results below the MRL.

9.3.6 LABORATORY DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LD or LFSMD) – Within each Analysis Batch, a minimum of one Laboratory Duplicate (LD) or Laboratory Fortified Sample Matrix Duplicate (LFSMD) must be analyzed. Laboratory Duplicates check the precision associated with laboratory procedures. If target analytes are not routinely observed in field samples, a LFSMD should be analyzed rather than a LD.

9.3.6.1 Calculate the relative percent difference (RPD) for duplicate measurements (LD_1 and LD_2) using the equation:

$$RPD = \frac{|LD_1 - LD_2|}{(LD_1 + LD_2)/2} \times 100$$

9.3.6.2 RPDs for Laboratory Duplicates should be $\leq$ 30%. Greater variability may be observed when Laboratory Duplicates have analyte concentrations that are within a factor of 2 x MRL. At these concentrations, Laboratory Duplicates should have

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RPDs that are ≤ 50%. If the RPD of the analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC, the precision is judged to be matrix influenced. The result from the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.6.3   If a LFSMD is analyzed instead of a Laboratory Duplicate, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation:

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

9.3.6.4   RPDs for duplicate LFSMs should be ≤ 30%. Greater variability may be observed when fortified LFSMs have analyte concentrations that are within 2 x MRL. LFSMs at these concentrations should have RPDs that are ≤ 50%. If the RPD of the analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC, the precision is judged to be matrix influenced. The result from the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.3.7   QUALITY CONTROL SAMPLE (QCS) – A QCS is required if an alternate commercial source is available for the target analytes. A QCS should be evaluated as part of the IDC (Sect. 9.2.5) and each time new PDS solutions are prepared. If standards are prepared infrequently, analyze a QCS at least quarterly. The QCS should be fortified near the midpoint of the calibration range and analyzed as a CCC. The acceptance criteria for the QCS are the same as the mid- and high-level CCCs (Sect. 10.4). If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

9.4   METHOD MODIFICATION QC REQUIREMENTS - The analyst is permitted to modify the separation technique, LC column, mobile phase composition, LC conditions and MS conditions.

9.4.1   Each time method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2) and verify that all QC criteria can be met in ongoing QC samples (Sect. 9.3).

9.4.2   The analyst is also required to evaluate and document method performance for the proposed method modifications in real matrices that span the range of waters that the laboratory analyzes. This additional step is required because modifications that perform acceptably in the IDC, which is conducted in reagent water, can fail ongoing method QC requirements in real matrices. This is particularly important for methods subject to matrix effects, such as LC/MS-based methods. If, for example, the laboratory analyzes finished waters from both surface and groundwater municipalities, this requirement can be accomplished by assessing precision and accuracy (Sects. 9.2.2 and 9.2.3) in a surface water with moderate to high total organic carbon (e.g., 2 mg/L or greater) and a hard groundwater (e.g., 250 mg/L as $CaCO_3$ equivalent, or greater).

9.4.3   The results of Sections 9.4.1 and 9.4.2 must be appropriately documented by the analyst and should be independently assessed by the laboratory's QA officer prior to analyzing field samples.

9.4.3.1   When implementing method modifications, it is the responsibility of the laboratory to closely review the results of ongoing QC, and in particular, the results associated with the LFSM (Sect. 9.3.5), LFSMD (Sect. 9.3.6), CCCs (Sect. 9.3.2), and the IS area counts (Sect. 9.3.4). If repeated failures are noted, the modification must be abandoned.

10   CALIBRATION AND STANDARDIZATION

10.1   Demonstration and documentation of acceptable MS calibration and initial analyte calibration are required before performing the IDC (Sect. 9.2) and prior to analyzing field samples. After initial calibration is successful, Continuing Calibration Check (CCC) standards are required at the beginning and the end of each analysis batch, and after every tenth sample (Sect. 10.4). Verification of the MS calibration and the initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2   LC/MS/MS CALIBRATION AND OPTIMIZATION

10.2.1   MASS CALIBRATION – Calibrate the mass spectrometer with the calibration compounds and procedures specified by the manufacturer.

10.2.2   OPTIMIZING MS PARAMETERS – This method requires the utilization of ESI and Multiple Reaction Monitoring as defined above (Sect. 3.15). LC/MS instruments have several instrumental parameters that must be optimized. Each LC/MS system will have different optimal conditions which are influenced by the source geometry and system design. Due to the differences in design, the recommendations of the instrument manufacturer should be followed when tuning the instrument. In the development of this method, instrumental parameters were optimized for the precursor and product ions listed in Section 17, Table 3. Literature resources to date describe similar precursor and product ions.[6,7] Product ions other than those listed may be selected; however, the analyst is cautioned to avoid using ions with lower mass and/or common ions that may not provide sufficient discrimination between the analytes of interest and co-eluting interferences.

10.2.2.1   Prepare 0.05 – 1.0 µg/mL solutions of the target analytes in the approximate mobile phase compositions that correspond to the elution mobile phase compositions. This step will need to be repeated and the parameters re-optimized if an analyte is prepared in a mobile phase composition that is significantly different from the mobile phase composition at the time the analyte elutes from the column.

10.2.2.2   Optimize the response of the precursor ion (Sect. 3.16) for each target analyte by infusing the prepared solutions (Sect. 10.2.2.1) directly into the mass spectrometer as recommended by the instrument manufacturer. The LC should be operating with a mobile phase composition and flow rate comparable to the elution conditions. Vary the MS parameters (source voltages, source and desolvation temperatures, gas

flows, etc.) until optimal analyte responses are determined. The target analytes may have different optima, thus requiring some compromise. LC/MS systems are subject to drift, which can affect run-to-run precision. Accordingly, the optimal conditions may be those that yield good response while minimizing the potential for drift. See Section 17, Table 2 for the ESI+ MS conditions used during method development.

10.2.2.3 In all cases, a precursor ion must be isolated (e.g., in a quadrupole or an ion trap) prior to fragmentation. Optimize the response of the product ion (Section 3.18) for each target by infusing the prepared solutions (Sect. 10.2.2.1) directly into the mass spectrometer as recommended by the instrument manufacturer. The LC should be operating with a mobile phase composition and flow rate comparable to the elution conditions. The MS/MS parameters (collision gas pressure, collision energy, etc.) are varied until optimal analyte responses are determined. The triazine compounds listed in this method exhibited very similar optimal MS/MS conditions. See Section 17, Table 3 for the MS/MS conditions used in method development.

10.2.3 LC INSTRUMENT CONDITIONS – LC operating conditions are described in Section 17, Table 1. Conditions different from those described (e.g., LC columns and mobile phases) may be used if the QC criteria in Sections 9.2 and 9.3 are met and chromatographic separation of the target analytes is achieved (resolution $\geq$ 50%).

NOTE: Chromatographic separation as defined does not include the isotopically enriched internal standards, which are mass separated. Co-elution of the internal standards with their analogous target analytes helps mitigate matrix suppression and/or enhancement effects.[8]

10.2.4 ESTABLISH LC/MS RETENTION TIMES AND MRM SEGMENTS – Inject a mid- to high-level CAL standard under optimized LC/MS conditions to obtain the retention times of each target analyte. Divide the chromatogram into segments that contain one or more chromatographic peaks. For maximum sensitivity in subsequent MS/MS analyses, minimize the number of MRM (Sect. 3.15) transitions that are simultaneously monitored within each segment.

10.3 INITIAL CALIBRATION – During method development, daily calibrations were performed; however, it is permissible to verify the calibration with daily CCCs. Calibration must be performed using peak areas and the internal standard technique. Calibration using peak heights or external calibration is not permitted.

10.3.1 CALIBRATION STANDARDS – Prepare a set of at least five CAL standards as described in Section 7.2.3. The lowest concentration of the calibration standards must be at or below the MRL. The MRL must be confirmed using the procedure outlined in Section 9.2.4 after establishing the initial calibration. Additionally, field samples must be quantified using a calibration curve that spans the same concentration range used to collect the IDC data (Sect. 9.2).

10.3.2 CALIBRATION – The LC/MS system is calibrated using peak areas and the internal standard technique. Concentrations may be calculated using the data system software to generate a linear regression or quadratic calibration curve. Forcing the calibration curve

through the origin is not recommended. The MS/MS instrument used during method development was calibrated using weighted (1/X) quadratic curves. Data may be fit with either a linear regression (response vs. concentration) or quadratic fit (response vs. concentration) and weighting may be used, if necessary.

10.3.3 CALIBRATION ACCEPTANCE CRITERIA – The validation of the calibration is determined by calculating the concentration of the analyte from each of the analyses used to generate the calibration curve. Calibration points that are ≤ MRL should calculate to be within ± 50% of their true value. All other calibration points should calculate to be within ± 30% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. In this case, corrective action should be taken to reanalyze the calibration standards, restrict the range of calibration, or select an alternate method of calibration.

10.4 CONTINUING CALIBRATION CHECKS (CCCs) – The CCC verifies the calibration at the beginning, after every tenth field sample and at the end of each Analysis Batch. CCCs are not counted as samples. **The beginning CCC for each Analysis Batch must be at or below the MRL**. This CCC verifies instrument sensitivity prior to the analysis of samples. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

10.4.1 Inject an aliquot of the appropriate concentration CAL standard and analyze with the same conditions used during the initial calibration.

10.4.2 Determine that the absolute areas of the quantitation ions of each of the internal standards have not changed by more than ± 50% from the average areas measured during the initial calibration. If IS areas have changed by more than ± 50%, remedial action is necessary (Sect. 10.4.4). Recalibrate according to Section 10.3 and verify sensitivity by analyzing a CCC at or below the MRL.

10.4.3 Calculate the concentration of each analyte in the CCC. The CCC fortified at ≤ MRL must calculate to be within ± 50% of its true value. CCCs fortified at all other levels must calculate to be within ± 30%. If these conditions do not exist, then all data for the analyte must be considered invalid, and remedial action (Sect. 10.4.4) must be taken. The remedial action may require recalibration. Any field samples that have been analyzed since the last acceptable CCC that are still within holding time should be reanalyzed after adequate calibration has been restored.

10.4.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria requires remedial action. Maintenance such as cleaning an ion source probe, sample cone, ion lenses and/or replacing LC columns may be required. Following major maintenance, the analyst must return to the initial calibration step (Sect. 10.3).

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper sample collection and storage (Section 8), ensuring that the instrument is properly calibrated (Section 10) and that all required QC are met (Section 9). This section describes the procedures for sample preparation and analysis.

11.2   SAMPLE PREPARATION

11.2.1 Samples are dechlorinated, preserved, collected and stored as described in Section 8.1. All field and QC samples must contain the dechlorinating and preservation agents listed in Section 8.1.1, including the LRB. Allow field samples to reach room temperature prior to analysis.

11.2.2 Transfer a 1.0 mL aliquot of each field or QC sample to an autosampler vial; as appropriate, samples that will be chromatographed using small particle diameter LC columns (< 2 μm) may be filtered prior to analysis (see note below). Add the IS PDS (Sect. 7.2.1.2), cap and mix well. Larger amounts of sample may be used, but the amount of internal standard must be increased to give a final concentration that is equal to the CAL IS concentration.

NOTE: If filtering is incorporated as part of the sample preparation, the first lot of filters must be subjected to the procedures outlined in the IDC (Sect. 9.2) and meet the acceptance criteria defined in Table 9 to ensure that they do not introduce interferences or retain any of the target analytes. Verification of subsequent lots of filters can be accomplished by examining a LRB and duplicate samples of LFBs fortified at the MRL. The LFBs should calculate to be within $\pm$ 50% of the true value. If the LRB or the LFBs fail this evaluation, the full IDC will need to be repeated with the new lot of filters. CAL standards and CCCs should not be filtered in order to identify potential losses associated with the sample filtration devices.

11.3   SAMPLE ANALYSIS

11.3.1 Establish MS operating conditions equivalent to those summarized in Tables 1–3 of Section 17 as per the guidance in Section 10.2. Column choice and instrument parameters should be optimized prior to initiation of the IDC.

11.3.2 Establish an appropriate retention time window (Sect. 10.2.4) for each analyte to identify them in QC and field sample chromatograms. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the LC 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 IDC (Sect. 9.2) 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.

11.3.3 Establish a valid initial calibration following the procedures outlined in Section 10.3 or confirm that the calibration is still valid by running a CCC as described in Section 10.4. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2 prior to analyzing field samples.

11.3.4 Analyze aliquots of field and QC samples at appropriate frequencies (Section 9) with the LC/MS/MS conditions used to acquire the initial calibration.

11.3.5 At the conclusion of data acquisition, use the same software settings that were used in the calibration procedure to identify peaks of interest in the predetermined retention time windows. Identify an analyte by comparison of its retention time with that of the corresponding analyte peak in a CAL standard or CCC.

11.3.6 EXCEEDING CALIBRATION RANGE – The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, the sample may be diluted using reagent water containing 20 mM ammonium acetate and 64 µg/L sodium omadine and with the appropriate amount of internal standard added to match the original level. Re-inject the diluted sample. Incorporate the dilution factor into final concentration calculations. The resulting data should be documented as a dilution and the dilution will affect analyte MRLs.

12. DATA ANALYSIS AND CALCULATIONS

12.1 While complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS in reagent water, it is important in real matrices. The analyst must avoid chromatographic modifications that result in suppression or enhancement of analyte responses due to co-eluting matrix components. This is especially a problem with samples that contain higher TOC levels (Sect. 13.4.2). In validating this method, concentrations were calculated using the product ions listed in Table 3 of Section 17. Other ions may be selected at the discretion of the analyst as discussed in Section 10.2.2.

12.2 Calculate analyte concentrations using the multipoint calibration established in Section 10.3. Report only those values that fall between the MRL and the highest CAL standard. Samples with target analyte responses that exceed the highest CAL standard require dilution and reanalysis (Sect. 11.3.6).

12.3 Calculations must use 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.

12.4 Prior to reporting the data, the chromatograms should be reviewed for any incorrect peak identifications or poor integrations.

12.5 Prior to reporting data, the laboratory is responsible for assuring that QC requirements have been met or that any appropriate qualifier is documented.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND DETECTION LIMITS – Tables for these data are presented in Section 17. Instrumental conditions are presented in Tables 1 through 3. DLs and LCMRLs are presented in Table 4. LCMRLs were determined and calculated using a procedure described elsewhere.[1] 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) groundwater (Table 7). Figure 1 shows an overlaid chromatogram of the normalized MS/MS responses for the analytes obtained under the conditions employed during method development.

13.2   SECOND LABORATORY EVALUATION – The performance of this method was demonstrated by a second laboratory, with results similar to those reported in Section 17. The authors wish to acknowledge the work of Dr. Jody Shoemaker of the U.S. EPA National Exposure Research Laboratory, Cincinnati, OH for conducting the second laboratory evaluation.

13.3   ANALYTE STABILITY STUDIES – Chlorinated surface water samples, fortified with target analytes at 5 μg/L, were preserved as required in Section 8 and stored over a 28-day period. The accuracy and precision of three replicate analyses, conducted on day 0, 7, 14, 21 and 28, are presented in Section 17, Table 8. These data were used to determine the 28-day holding time.

13.4   PROBLEM COMPOUNDS – During method development and the second laboratory evaluation, the following problems were observed for Atrazine-desethyl-desisopropyl (DACT):

   13.4.1 DACT is subject to hydrolysis at both high (> 9) and low (< 5) pH but has acceptable stability in neutral aqueous solutions. DACT is also degraded in the presence of free available chlorine, but stable in the presence of chloramines. Ammonium acetate mitigates both of these modes of loss and has sufficient buffer capacity at 20 mM to buffer finished groundwaters with relatively high pH that also have buffer capacity.

   13.4.2 DACT and its internal standard (DACT-^{13}C) exhibited ESI matrix suppression in the second laboratory evaluation after being fortified into a chlorinated surface water containing high total organic carbon (TOC) levels (9.5 mg/L). The DACT-^{13}C response was suppressed 26% relative to the calibration; however, since DACT was affected by the suppression in an equivalent manner, the DACT recovery was 99%. The matrix suppression occurred due to co-eluting TOC in the early part of the chromatographic run and DACT is the analyte most susceptible to matrix effects. As discussed in Section 4.4.1, good performance is achieved by optimizing chromatographic separation, and in particular, the separation of DACT from early eluting contaminants. Modifications that seek to shorten the chromatographic separation without accounting for the need to separate the analytes from early eluting interferences may result in failure to meet QC criteria.

14.   POLLUTION PREVENTION

14.1   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, or on-line at http://www.ups.edu/community/storeroom/Chemical_Wastes/wastearticles.htm.

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, see the publications of the American Chemical Society's Laboratory Environment, Health & Safety Task Force on the Internet at http://membership.acs.org/c/ccs/publications.htm. Additional waste management information can be found in "Laboratory Waste Minimization and Pollution Prevention," Copyright © 1996 Battelle Seattle Research Center, which can be located at http://www.p2pays.org/ref/01/text/00779/index2.htm.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1.   LC CONDITIONS [a]

Time (min.)	%5 mM NH$_4$OAc	% Methanol
Initial	90	10
5.0	90	10
25.0	70	30
25.1	60	40
35.0	60	40
35.1	50	50
45.0	50	50
45.1	90	10
65.0	90	10

[a] **Chromatographic conditions:** Column: Waters Xterra® RP18, 2.1 x 150 mm, 3.5 μm particle diameter; Injection Volume: 100 μL; Flow Rate: 0.250 mL/min.

TABLE 2.   ESI-MS/MS METHOD CONDITIONS

MS Parameter	Setting
Polarity	Positive Ion Mode
Capillary Voltage	0.5 kV
Source Temperature	100 $^{\circ}$C
Desolvation Temperature	260 $^{\circ}$C
Desolvation Gas Flow	616 L/hr
Cone Gas Flow	26 L/hr
Collision Cell Pressure	2.1 e-3 mbar
Collision Gas	Argon

TABLE 3.   LC/ESI-MS/MS RETENTION TIMES, PRECURSOR AND PRODUCT IONS, AND COLLISION ENERGIES

MRM Segment #	Analyte	Ret. Time (min.)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
1	Atrazine-desethyl-desisopropyl-[13]C (IS)	3.78	149	105.5	22
1	Atrazine-desethyl-desisopropyl	3.78	146	103.5	22
2	Atrazine-desisopropyl-d5 (IS)	11.26	179	137	22
2	Atrazine-desisopropyl	11.57	174	132	22
3	Atrazine-desethyl-d7 (IS)	18.66	195	147	22
3	Atrazine-desethyl	19.09	188	146	22
4	Simazine-d10 (IS)	30.48	212	137	20
4	Simazine	30.96	202	132	20
4	Cyanazine-d5 (IS)	32.03	246	219	20
4	Cyanazine	32.20	241	214	20
5	Atrazine-d5 (IS)	37.03	221	179	23
5	Atrazine	37.37	216	174	23
6	Propazine-d14 (IS)	42.87	244	196	20
6	Propazine	43.51	230	188	20

TABLE 4.   LC/ESI-MS/MS LOWEST CONCENTRATION MRLs AND DLs [a]

Analyte	LCMRL Concentration Levels (µg/L)	Calculated LCMRL (µg/L)	Calculated DL (µg/L)
Atrazine-desethyl-desisopropyl	0.104, 0.208, 0.260, 0.520	0.14	0.028
Atrazine-desisopropyl	0.102, 0.204, 0.255, 0.510	0.11	0.043
Atrazine-desethyl	0.021, 0.052, 0.104, 0.130	0.026	0.017
Simazine	0.020, 0.050, 0.100, 0.125	0.020	0.010
Cyanazine	0.019, 0.048, 0.096, 0.120	0.027	0.010
Atrazine	0.021, 0.052, 0.104, 0.130	0.037	0.010
Propazine	0.020, 0.050, 0.100, 0.125	0.028	0.010

[a] DLs calculated using data acquired on a single day at the lowest LCMRL concentration level (n=7 sample replicates).

TABLE 5. LC/ESI-MS/MS PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 0.5 µg/L AND 5.0 µg/L IN REAGENT WATER (n=7 SAMPLES)

Analyte	Fortified Conc. = 0.5 µg/L (n=7)		Fortified Conc. = 5.0 µg/L (n=7)	
	Mean % Recovery	Relative Standard Deviation (%RSD)	Mean % Recovery	Relative Standard Deviation (%RSD)
Atrazine-desethyl-desisopropyl	98.9	4.9	89.4	4.2
Atrazine-desisopropyl	100	3.4	96.1	3.7
Atrazine-desethyl	99.4	2.7	97.6	1.3
Simazine	101	3.0	101	1.9
Cyanazine	97.7	1.3	99.3	1.4
Atrazine	99.4	2.5	99.7	1.6
Propazine	103	2.9	99.0	2.0

TABLE 6. LC/ESI-MS/MS PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 0.5 µg/L AND 5.0 µg/L IN A CHLORINATED SURFACE WATER [a] (n=7 SAMPLES)

Analyte	Fortified Conc. = 0.5 µg/L (n=7)		Fortified Conc. = 5.0 µg/L (n=7)	
	Mean % Recovery [b]	Relative Standard Deviation (%RSD)	Mean % Recovery [b]	Relative Standard Deviation (%RSD)
Atrazine-desethyl-desisopropyl	102	8.4	103	2.7
Atrazine-desisopropyl	100	6.6	101	3.7
Atrazine-desethyl	95.5	1.5	97.9	0.9
Simazine	94.5	3.7	99.4	1.7
Cyanazine	95.3	4.2	99.4	1.7
Atrazine	97.4	6.1	101	0.9
Propazine	94.9	6.0	105	1.5

[a] Surface water physical parameters: pH=7.1; Hardness = 154 mg/L; Free chlorine = 1.5 mg/L (Hach).
[b] Recoveries corrected for native levels in the unfortified matrix: Atrazine-desethyl-desisopropyl (0.043 µg/L), Atrazine-desisopropyl (0.001 µg/L), Atrazine-desethyl (0.031 µg/L), Simazine (0.054 µg/L), Cyanazine (0.018 µg/L), Atrazine (0.316 µg/L) and Propazine (0.038 µg/L).

TABLE 7. **LC/ESI-MS/MS PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 0.5 µg/L AND 5.0 µg/L IN A CHLORINATED GROUNDWATER [a] (n=7 SAMPLES)**

Analyte	Conc. = 0.5 µg/L (n=7)		Conc. = 5.0 µg/L (n=7)	
	Mean % Recovery [b]	Relative Standard Deviation (%RSD)	Mean % Recovery[b]	Relative Standard Deviation (%RSD)
Atrazine-desethyl-desisopropyl	106	3.5	95.7	2.5
Atrazine-desisopropyl	99.6	5.2	103	3.0
Atrazine-desethyl	95.7	1.7	98.6	1.5
Simazine	94.6	3.1	98.0	1.5
Cyanazine	93.5	1.8	101	1.3
Atrazine	96.7	0.9	100	1.5
Propazine	91.7	3.7	99.7	1.4

[a] Groundwater physical parameters: pH = 7.5; Hardness = 360 mg/L; Free chlorine = 0.49 mg/L.
[b] Recoveries corrected for native levels in the unfortified matrix: Atrazine-desethyl-desisopropyl (0.032µg/L), Atrazine-desisopropyl (not detected), Atrazine-desethyl (0.036 µg/L), Simazine (0.030 µg/L), Cyanazine (0.018 µg/L), Atrazine (0.057 µg/L), and Propazine (0.036 µg/L).

TABLE 8. LC/ESI-MS/MS SAMPLE HOLDING TIME DATA FOR METHOD ANALYTES FORTIFIED AT 5.0 µg/L IN A CHLORINATED SURFACE WATER [a] (n=3 SAMPLES)

Analyte	Day 0		Day 7		Day 14		Day 21		Day 28	
	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD
Atrazine	98.0	4.1	97.4	2.9	99.4	1.1	94.3	7.0	99.3	0.8
Atrazine-desethyl	107	2.5	104	4.1	111	1.7	103	4.7	108	1.5
Atrazine-desethyl-desisopropyl	95.9	7.7	93.9	6.2	102	1.2	90.8	6.6	103	3.6
Atrazine-desisopropyl	105	3.5	89.8	7.6	95.4	1.1	93.5	7.9	102	1.8
Cyanazine	99.3	4.7	94.0	1.2	94.8	1.6	87.8	4.7	89.5	0.5
Propazine	112	2.9	102	4.8	109	2.5	103	6.1	109	1.6
Simazine	94.2	6.0	99.6	4.8	93.6	6.8	89.1	11.8	100	2.5

[a] Surface water physical parameters: pH = 7.0; Hardness=154 mg/L; Free chlorine = 1.2 mg/L (Hach).

TABLE 9.   INITIAL DEMONSTRATION OF CAPABILITY (IDC) QUALITY CONTROL REQUIREMENTS

| Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.1	Demonstration of Low System Background	Analyze a LRB prior to any other IDC steps.	Demonstrate that all target analytes are < 1/3 of the MRL and that possible interferences from reagents and glassware do not prevent the identification and quantitation of target analytes.
Section 9.2.1	Test For System Carryover	Analyze a reagent water blank after the high CAL standard during the IDC calibration.	Demonstrate that the target analytes are < 1/3 of the MRL and that carry-over from previous samples does not prevent the identification and quantitation of target analytes.
Section 9.2.2	Demonstration of Precision	Analyze 7 replicate CCCs fortified near the midrange concentration.	%RSD must be $\leq$ 20%
Section 9.2.3	Demonstration of Accuracy	Calculate average recovery for replicates used in Section 9.2.2.	Mean recovery within $\pm$ 30% of the true value.
Section 9.2.4	Minimum Reporting Limit (MRL) Confirmation	Fortify and analyze 7 replicate CCCs at the proposed MRL concentration. Calculate the mean and the Half Range (HR). Confirm that the Upper PIR and Lower PIR (Sect. 9.2.4.2) meet the recovery criteria.	Upper PIR $\leq$ 150% Lower PIR $\geq$ 50%
Section 9.2.5	Quality Control Sample (QCS)	Analyze mid-level QCS sample.	Results must be within $\pm$ 30% of the true value.

TABLE 10. ONGOING QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.3	Sample Holding Time	28 days when processed and stored according to Sections 8.1 and 8.2.	Sample results are valid only if samples are analyzed within the sample holding time.
Section 10.3	Initial Calibration	Use the internal standard calibration technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Validate the calibration curve as described in Section 10.3.3.	When each calibration standard is calculated as an unknown using the calibration curve, the lowest level standard should be within $\pm$ 50% of the true value. All other points should be within $\pm$ 30% of the true value.
Section 9.3.1	Laboratory Reagent Blank (LRB)	Daily, or with each Analysis Batch of up to 20 field samples, whichever is more frequent.	Demonstrate that all target analytes are below 1/3 the MRL, and that possible interference from reagents and glassware do not prevent identification and quantitation of target analytes.
Section 10.4	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low-level CCC at the beginning of each Analysis Batch. Subsequent CCCs are required after every 10 field samples, and after the last field sample in a batch.	The lowest level CCC must be within $\pm$ 50% of the true value. All other points must be within $\pm$ 30% of the true value.

Results for field samples that are not bracketed by acceptable CCCs are invalid. |

TABLE 10. ONGOING QUALITY CONTROL REQUIREMENTS (CONTINUED)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.3.4	Internal Standard (IS)	Isotopically labeled internal standards are added to all standards and samples.	Peak area counts for each IS must be within ± 50% of the average peak areas in the initial calibration.
Section 9.3.5	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per Analysis Batch. Fortify the LFSM with target analytes at a concentration close to but greater than the native concentrations (if known). Calculate LFSM recoveries.	For LFSMs fortified at concentrations ≤ MRL, the result should be within ± 50% of the true value. All other LFSMs should be within ± 30% of the true value.
Section 9.3.6	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Laboratory Duplicate(LD)	Analyze at least one LFSMD or LD with each Analysis Batch of up to 20 field samples.	For LFSMDs or LDs, RPDs should be ≤ 30%.
Sections 9.3.7	Quality Control Sample (QCS)	Analyze mid-level QCS sample when new PDS solutions are prepared, or at least quarterly if PDS solutions are prepared less frequently.	Results must be ± 30% of the true value.

FIGURE 1. **NORMALIZED, OVERLAID CHROMATOGRAMS OF LC/ESI-MS/MS TRANSITIONS FOR METHOD 536 ANALYTES FORTIFIED AT 0.5 µg/L IN REAGENT WATER [a]**
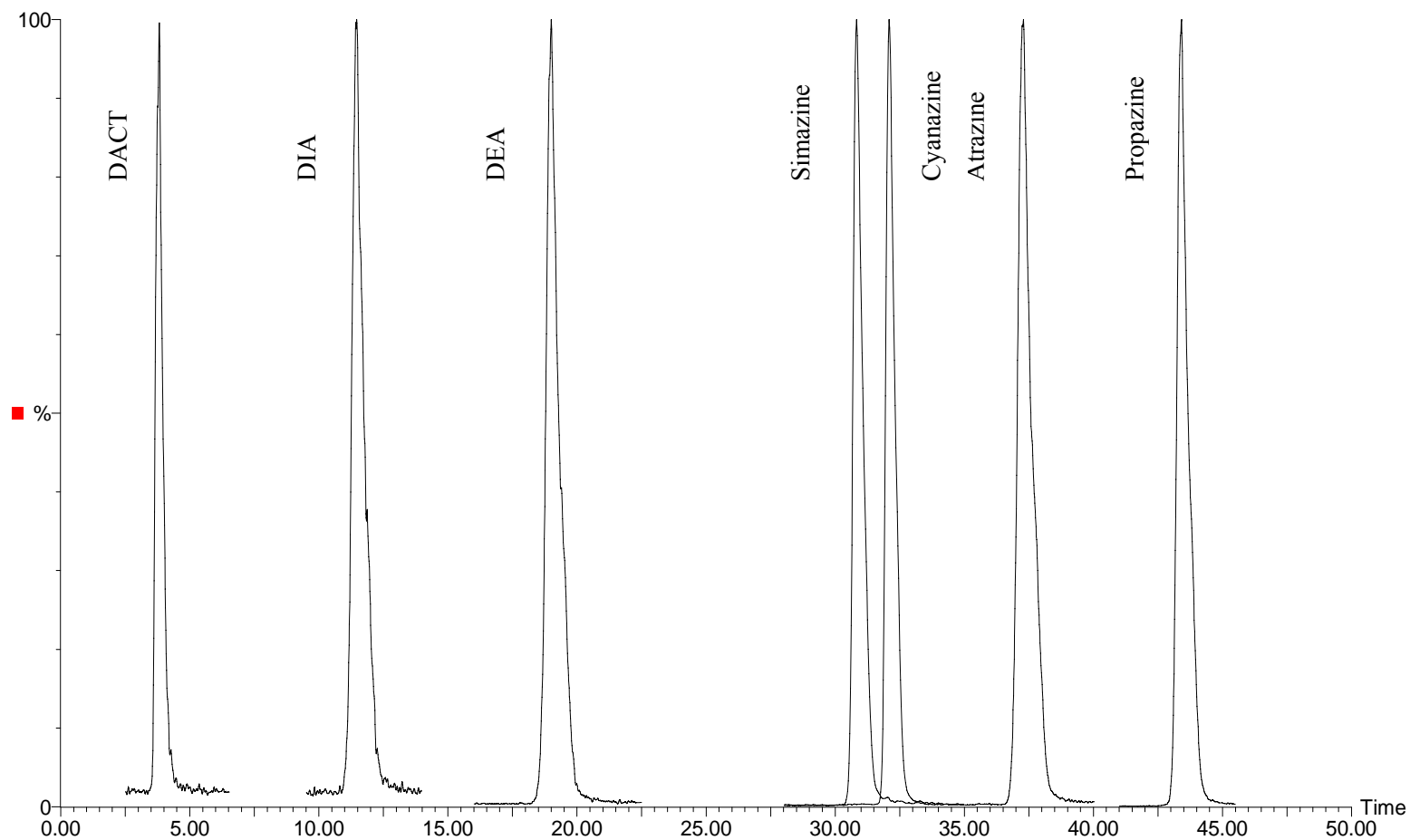
[a] The chromatograms for the isotopically-enriched internal standards are not included because they co-elute with their analogous target analytes.