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METHOD 538. DETERMINATION OF SELECTED ORGANIC CONTAMINANTS IN DRINKING WATER BY DIRECT AQUEOUS INJECTION-LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (DAI-LC/MS/MS)

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1. SCOPE AND APPLICATION

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

Analyte	Chemical Abstract Services Registry Number (CASRN)
Acephate	30560-19-1
Aldicarb	116-06-3
Aldicarb sulfoxide	1646-87-3
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Fenamiphos sulfone	31972-44-8
Fenamiphos sulfoxide	31972-43-7
Methamidophos	10265-92-6
Oxydemeton-methyl	301-12-2
Quinoline	91-22-5
Thiofanox	39196-18-4

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 0.011-1.5 µg/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 LCMRLs, 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 sample preparation, sample matrix, fortification concentration, and instrument performance.

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

1.6 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 and MS/MS conditions (Sect. 6.6, 9.4, 10.2, 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, 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 in this method (Sect. 9) are met, and that acceptable method performance can be verified in a real sample matrix (Sect. 9.3.5).

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

2.1 A 40-mL water sample is collected in a bottle containing sodium omadine and ammonium acetate. An aliquot of the sample is placed in an autosampler vial and the internal standards are added. A 50-µL or larger injection is made into an LC equipped with a C_{18} column that is interfaced to an MS/MS operated in the electrospray ionization (ESI) mode. The 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 internal standard calibration using procedural standards.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples analyzed on the same instrument, not exceeding a 24-hour period and including no more than 20 Field Samples, beginning and ending 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 and the internal standard(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 precursor ion's translational energy 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 and internal standard(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 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.

3.7 INTERNAL STANDARD (IS) – A pure chemical added to a standard solution in a known amount(s) and used to measure the relative response of other method analytes 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.

3.8 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 reagents 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.

3.9 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 and the measured values in the LFSM corrected for background concentrations.

3.10 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, 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.

3.11 LABORATORY REAGENT BLANK (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, and internal standards 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.

3.12 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]

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

3.14 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.

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

3.16 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.

3.17 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.

3.18 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 QCS is used to check calibration standard integrity.

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

4. INTERFERENCES

4.1 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 2 h or solvent rinsed. Volumetric glassware should be solvent rinsed and not be heated in an oven above 120 °C. Store clean glassware inverted, covered with foil, or capped.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other laboratory supplies or hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing LRBs 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 in 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 present in the sample at high levels can cause enhancement and/or suppression in the electrospray ionization source.[3-4] Total organic carbon (TOC) is a good indicator of humic content of the sample. Under the LC conditions used during method development, matrix effects due to total organic carbon (TOC), up to 8.7 mg/L, were not observed.

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 LRBs (Sect. 9.3.1), particularly when new lots of reagents are acquired.

5. SAFETY

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 analyses. Additional references to laboratory safety are available.[5-7]

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 – Amber glass bottles (40 mL or larger) fitted with teflon-lined screw caps (Fisher Cat. No.: 02-912-377 or equivalent).

6.2 STANDARD SOLUTION STORAGE CONTAINERS – Amber glass bottles (10 mL or larger) (Kimble Cat. No.: 60815-1965 or equivalent) fitted with teflon-lined screw caps (Kimble Cat No.: 73802-15425 or equivalent).

6.3 AUTOSAMPLER VIALS – Amber glass 2.0-mL autosampler vials (National Scientific Cat. No.: C4000-2W or equivalent) with caps (National Scientific Cat. No.: 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 LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER (MS/MS) WITH DATA SYSTEM

6.6.1 LC SYSTEM – Instrument capable of reproducibly injecting at least 50-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). The LC must be capable of pumping the ammonium formate/methanol mobile phase without the use of a degasser which pulls vacuum on the mobile phase **bottle** (other types of degassers are acceptable). Degassers which pull vacuum on the mobile phase **bottle** will volatilize the ammonium formate mobile phase causing the analyte peaks to shift to earlier retention times over the course of the analysis batch. The use of a column heater is optional.

6.6.2 TANDEM MASS SPECTROMETER – The MS/MS instrument (Waters Micromass Quattro Premier or equivalent) must be capable of positive ion 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. 3.17) for the method

analytes within retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision.

6.6.3 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 construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.6.4 ANALYTICAL COLUMN – A Waters Atlantis T_3 C_{18} column (2.1 x 150 mm) packed with 5 µm d_p C_{18} solid phase particles (Cat. No.: 36003736 or equivalent) was used. Any 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, Cat. No.: A-456 or equivalent).

7.1.3 AMMONIUM FORMATE (NH_4CHO_2, CAS#: 540-69-2) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich LC/MS grade, Cat. No.: 55674 or equivalent).

7.1.4 AMMONIUM ACETATE ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich ACS grade, Cat. No.: 238074 or equivalent).

7.1.5 SODIUM OMADINE® (Sodium 2-pyridinethio-1-oxide, CAS#: 3811-73-2) – High purity demonstrated to be free of analytes and interferences (Sigma-Aldrich Cat. No.: H3261 or equivalent; Omadine is a registered trademark of Arch Chemicals, Inc.).

7.1.6 20 mM AMMONIUM FORMATE/REAGENT WATER MOBILE PHASE – To prepare 1 L, add 1.26 g ammonium formate to 1 L of reagent water. This solution is prone to volatility losses and should be replaced at least every 48 hours.

7.1.7 2 M AMMONIUM ACETATE/REAGENT WATER – To prepare 100 mL, add 15.4 g ammonium acetate to a 100-mL volumentric flask. Dilute to volume with reagent water. This solution should be kept refrigerated at ≤6ºC and replaced at least every two weeks due to potential volatility losses.

7.1.8 32 g/L SODIUM OMADINE® IN REAGENT WATER – To prepare 25 mL, add 0.80 g of sodium omadine to a 25-mL volumetric flask. Dilute to volume with reagent water. This solution should be kept refrigerated at ≤6ºC.

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

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

7.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Alternate concentrations may be used as necessary depending on instrument sensitivity and the calibration range used. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should use standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL (IS) STOCK STANDARD SOLUTIONS – This method uses the five IS compounds listed in the table below. The internal standards were obtained from Cambridge Isotopes Laboratories, except for methamidophos-d_6 which was obtained from EQ Laboratories. The IS(s) may be purchased from alternate sources. Although alternate IS standards may be used provided they are isotopically labeled compounds with similar functional groups as the method

analytes, the analyst must have documented reasons for using alternate IS(s). Alternate IS(s) must meet the QC requirements in Section 9.3.4.

Internal Standards
Methamidophos-d_6
Acephate-d_6
Oxydemeton-methyl-d_6
Quinoline-d_7
Diisopropyl methylphosphonate-d_{14} (DIMP-d_{14})

7.2.1.1 IS STOCK STANDARD SOLUTIONS (100-1000 µg/mL) – These IS stock standards can be obtained as individual certified stock standard solutions. During the development of this method, commercially obtained 100 µg/mL or 1000 µg/mL stock standard solutions in acetonitrile or methanol were used. IS stock standard solutions were stable for at least six months when stored at -15 °C or less.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION (IS PDS) STANDARD (0.4-12.5 ng/µL) – Prepare, or purchase commercially, the IS PDS at a suggested concentration of 0.40-12.5 ng/µL in acetonitrile. If prepared from the individual stock standard solutions (Sect. 7.2.1.1), the table below can be used as a guideline for preparing the IS PDS. The IS PDS has been shown to be stable for at least six months when stored in amber glass bottles (Sect. 6.2) at -4 °C. Fortification of the final 1-mL samples with 10 µL of this 0.40-12.5 ng/µL solution (Sect. 11.2.1) will yield a concentration of 4-125 µg/L of each IS in the 1-mL samples. **The total volume of acetonitrile added to the 1-mL sample should not exceed 2% to avoid peak broadening of the early eluting analytes.** Acetonitrile volumes in excess of 2% may be used provided the laboratory can document that these volumes do not adversely affect the peak shape of any of the method analytes under the laboratories' operating conditions.

IS	Conc. of IS Stock (µg/mL)	Vol. of IS Stock (µL)	Final Conc. of IS PDS (ng/µL)[a]
Methamidophos-d_6	100	40	0.40
Acephate-d_6	100	40	0.40
Oxydemeton-methyl-d_6	100	40	0.40
Quinoline-d_7	1000	125	12.5
Diisopropyl methylphosphonate-d_{14}	1000	4.0	0.40

[a] Final concentrations based upon preparing the IS PDS in a 10-mL volumetric and diluting to the mark with acetonitrile.

7.2.2 ANALYTE STANDARD SOLUTIONS – Standard solutions may be prepared from certified, commercially available solutions or from neat compounds. If the neat compounds used to prepare solutions are of 96% or greater purity, the weight may be used without correction for purity to calculate the concentration of the stock standard. **Solution concentrations listed in this section were used to develop this method and are included as example concentrations**. With the exception of dicrotophos and quinoline, the method development work was done with commercially obtained stock standard solutions, which are readily available from most suppliers of environmental standards. Quinoline was obtained from Aldrich and dicrotophos from Chem Service as neat materials. At the time of method development, DIMP was only available from Cerilliant CIL, Inc. Prepare the Analyte Stock and Primary Dilution Standards as described below. **Analysts are permitted to use other PDS and calibration standard concentrations and volumes as necessary to achieve adequate sensitivity.**

7.2.2.1 ANALYTE STOCK STANDARD SOLUTION (SSS) (1 mg/mL, except as noted) – If preparing from neat material, accurately weigh approximately 10 mg of pure material to the nearest 0.1 mg into a tared, 10-mL volumetric flask. Dilute to the mark with methanol for a final concentration of 1 mg/mL. Repeat for each method analyte. In the case of quinoline, accurately weigh approximately 20 mg of pure material to the nearest 0.1 mg into a tared, 1-mL volumetric flask. Dilute to the mark with methanol for a final concentration of 20 mg/mL. These stock standards were stable for at least six months when stored at -15 °C or less. Alternatively, individual stock standards of the analytes in methanol or acetonitrile may be purchased commercially. For the development of this method, commercially purchased stock standards of 1 mg/mL were used to

make primary dilution standards for all analytes except quinoline and dicrotophos.

7.2.2.2 METHANOLIC ANALYTE PRIMARY DILUTION STANDARD (MEOH PDS) SOLUTION (40 -1600 ng/µL) – The analyte MEOH PDS contains all the method analytes of interest at various concentrations in methanol. The ESI and MS/MS response varies by compound; therefore, a mix of concentrations may be needed in the analyte MEOH PDS. During method development, the analyte MEOH PDS was prepared such that approximately the same instrument response was obtained for all the analytes. The analyte MEOH PDS is prepared (table below) by dilution of the combined analyte SSSs with methanol in a 1-mL volumetric flask and is used to prepare the analyte WATER PDS (Sect. 7.2.2.3). The analyte MEOH PDS has been shown to be stable for six months when stored at -15 °C. Longer storage times are acceptable provided appropriate QC measures are documented demonstrating the analyte MEOH PDS stability.

Analyte	Analyte SSS Conc. (mg/mL)	Analyte SSS Volume (µL)	Final Analyte MEOH PDS Conc. (ng/µL) [a]
Methamidophos	1.0	40	40
Acephate	1.0	40	40
Aldicarb sulfoxide	1.0	80	80
Oxydemeton-methyl	1.0	40	40
Dicrotophos	1.0	40	40
Aldicarb	1.0	80	80
Quinoline	21.6	80	1728
DIMP	1.0	40	40
Fenamiphos sulfoxide	1.0	40	40
Fenamiphos sulfone	1.0	40	40
Thiofanox	1.0	160	160

[a] Final concentration calculation based upon a 1-mL final volume.

7.2.2.3 AQUEOUS ANALYTE PRIMARY DILUTION STANDARD (WATER PDS) SOLUTION (0.25 -10.7 ng/µL) – The analyte WATER PDS contains all the method analytes of interest at various concentrations in reagent water containing 10% methanol. The analyte WATER PDS is prepared by placing 62 µL of the analyte MEOH PDS in a 10-mL volumetric flask and diluting with reagent water containing 10% methanol and is used to prepare the CAL standards, and fortify the LFBs, the LFSMs, the LFSMDs and FDs with the method analytes. The analyte WATER PDS has been shown to be stable for at least one month when stored at 4 °C. Longer storage times are acceptable

provided appropriate QC measures are documented demonstrating the analyte WATER PDS stability.

7.2.3 CALIBRATION STANDARDS (CAL) – Prepare a procedural calibration curve from dilutions of the analyte WATER PDS in preserved reagent water. At least five to seven calibration concentrations are required to prepare the initial calibration curve spanning a 50 to 100-fold concentration range (Sect. 10.2). Prepare the calibration standards, adding appropriate amounts of the ammonium acetate and sodium omadine concentrated stocks (Sect. 7.1.7 and 7.1.8) as shown in the table below. The target analyte concentrations found in Tables 5-11 can be used as a starting point for determining the calibration range. An example of the dilutions used to prepare the CALs, that were utilized to collect data in Section 17, is shown below. The lowest concentration CAL must be at or below the MRL, which will depend on system sensitivity. The CALs may also be used as CCCs. If stored in containers (Sect. 6.2), the aqueous standards must be refrigerated in the same manner as the samples. A constant amount of the IS PDS is added to each prepared CAL. This is accomplished for each CAL by taking 990 µL of the final CAL containing the 20 mM ammonium acetate and 64 mg/L sodium omadine, and placing it in a 2.0-mL autosampler vial and adding 10 µL of the IS PDS (Sect. 7.2.1.2). During method development, the CAL standards were shown to be stable for at least two weeks when stored at <6 ºC. Longer storage times are acceptable provided appropriate QC measures are documented demonstrating the CAL stability.

CAL Level	Analyte WATER PDS Conc. (ng/µL)[a]	Analyte WATER PDS Volume (µL)	2 M Ammonium Acetate Stock Volume (µL)	32 g/L Sodium Omadine Stock Volume (µL)	Final CAL Std. Conc. (µg/L)[b]	Final Quinoline Conc. (µg/L)[b]	Final Aldicarb Conc. (µg/L)[b]	Final Thiofanox Conc. (µg/L)[b]
1	0.25	2	100	20	0.050	2.1	0.10	0.20
2	0.25	5	100	20	0.12	5.4	0.25	0.50
3	0.25	10	100	20	0.25	11	0.50	0.99
4	0.25	20	100	20	0.50	21	0.99	2.0
5	0.25	40	100	20	0.99	43	2.0	4.0
6	0.25	100	100	20	2.5	107	5.0	9.9
7	0.25	200	100	20	5.0	214	10	20

[a] Quinoline = 10.7 ng/µL, Aldicarb and Aldicarb sulfoxide= 0.50 ng/µL, Thiofanox = 1.0 ng/µL

[b] Final concentrations based upon preparing the CALs in a 10-mL volumetric and diluting to the mark with reagent water.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Samples must be collected in amber glass bottles fitted with PTFE-lined screw caps (Sect. 6.1).

8.1.2 Prior to shipment to the field, ammonium acetate and sodium omadine must be added to each amber bottle. If using the suggested 40-mL bottles (Sect. 6.1) to collect a 40-mL aliquot, add 400 µL of the ammonium acetate concentrated stock (Sect. 7.1.7) and 80 µL of the concentrated sodium omadine stock (Sect. 7.1.8). 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.

Compound	Amount	Purpose
Sodium omadine	64 mg/L	Antimicrobial
Ammonium acetate	1.5 g/L	Binds free chlorine

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 a representative sample from the flowing stream using a beaker of appropriate size. Use this bulk sample to generate individual samples as needed. Transfer a volume of approximately 40 mL into each collection container. Alternatively, collect the sample directly in the sample bottle containing the preservatives.

8.2.2 When filling sample bottles, take 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 to mix the sample with the preservation reagents. Keep the sample sealed from time of collection until analysis.

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 the samples are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until analysis, 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.

NOTE: Samples that arrive at the laboratory on the same day of sample collection (exclusively due to the close proximity of the sampling site to the laboratory), may not yet have stabilized to 10ºC or less when they arrive at the lab. These samples are acceptable ONLY if packed on ice or with frozen gel packs immediately after sample collection and hence, delivered while samples are in the process of reaching an equilibrium temperature less than 10ºC. These samples must contain the preservatives as described in Section 8.1.2.

8.4 SAMPLE HOLDING TIMES – Results of the sample storage stability study (Table 12) indicated that all compounds listed in this method have adequate stability for 14 days when collected, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.3. Therefore, aqueous samples must be analyzed within 14 days of collection.

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 the QC parameters, their required frequencies, 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 Tables 13 and 14. 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 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 solvents, reagents, and autosampler vials are used, it must be demonstrated that an LRB is reasonably free of contamination and that the criteria in Section 9.3.1 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION (IDP) – Prepare and analyze four to seven replicate LFBs (same as a CCC – Section 9.3.3) fortified near the midrange of the initial calibration curve according to the procedure described in Section 11. The sample preservative, as described in Section 8.1.2, must be added

to these samples. The relative standard deviation (RSD) of the concentrations of the 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 mean recovery. The mean recovery of the 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 the 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 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 the method analytes in these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) for each analyte using the equation below

$$HR_{PIR} = 3.963s$$

where

s = the 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 confirmed again at a higher concentration.

9.2.5 CALIBRATION CONFIRMATION – Analyze a QCS as described in Section 9.3.7 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 preparation 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 (e.g., three LFBs individually fortified on day one, two LFBs individually fortified on day two, and two LFBs individually fortified on day three). This concentration may be estimated by selecting a concentration at two to five times the noise level. The DLs in Table 5 were calculated from LFBs fortified at various concentrations as indicated in the table. The 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. The DL is a statistical determination of precision only.[2] If the DL replicates are

fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs, and may result in a calculated DL that is higher than the fortified concentration. Therefore, no precision and accuracy criteria are specified.

9.3 ONGOING QC REQUIREMENTS – This section summarizes the 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 analysis batch (Sect. 3.1) to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. If the LRB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or 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 the method analytes are detected in the LRB at concentrations equal to or greater than 1/3 the MRL, then all data for the problem analyte(s) must be considered invalid for all samples in the analysis batch.

9.3.2 CONTINUING CALIBRATION CHECK (CCC) – CCC standards, containing the preservatives, 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) – Since this method utilizes procedural calibration standards, which are fortified reagent waters, there is no difference between the LFB and the CCC. 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 IS(s) in all injections during each analysis day. The IS peak areas in any chromatographic run must be within 50-150% of the average IS areas in the most recent calibration curve. If the IS areas in a chromatographic run do not meet these criteria, inject a second aliquot from the same autosampler vial.

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 aliquot fails the IS criterion, 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, report results obtained from the reinjected aliquot, but annotate as "suspect/IS recovery." Alternatively, prepare another aliquot of the sample as specified in Section 11.2 or collect a new sample and re-analyze.

9.3.5 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – Analysis of an LFSM is required in each analysis 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. 9.3.6); however, infrequent occurrence of method analytes would hinder this assessment. If the occurrence of method analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM, or LFSMD, must be prepared and analyzed from a duplicate of the Field Sample. Analysis batches that contain LFSMDs will not require the analysis of a FD. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be established for each. Over time, LFSM data should be documented by the laboratory for all routine sample sources.

9.3.5.1 Within each analysis batch (Sect. 3.1), 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 WATER PDS (Sect. 7.2.2.3). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Use historical data and rotate through the low, mid and high concentrations when selecting a fortifying concentration.

9.3.5.2 Calculate the 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.

9.3.5.3 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.

9.3.5.4 If the accuracy of any analyte falls outside the designated range in the LFSM, and the laboratory performance for that analyte is shown to be in control in the CCCs, and the CCCs for the batch were not freshly prepared on the day of analysis, the Analyte WATER PDS used to fortify the matrix sample must be checked for analyte losses. Check the accuracy of the Analyte WATER PDS by using it to prepare a fresh CCC. The fresh CCC must meet the criteria in Section 10.3. If the fresh CCC does not meet the criteria in Section 10.3, a new Analyte WATER PDS must be prepared and the LFMS analysis repeated.

9.3.5.5 If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, as well as in the Analyte WATER PDS, 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.

9.3.6 FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or LFSMD) – Within each analysis batch (not to exceed 20 Field Samples, Sect. 3.1), 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.

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

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

9.3.6.2 RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of two of the MRL. At these concentrations, FDs should have RPDs that are ≤50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the 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.

9.3.6.3 If an LFSMD is analyzed instead of a FD, calculate the 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% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are fortified at analyte concentrations that are within a factor of two of the MRL. LFSMs fortified at these concentrations should have RPDs that are ≤50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the 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 QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new Analyte MEOH PDS (Sect. 7.2.2.2) 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 near the midpoint of the calibration range and analyzed as a CCC. Acceptance criteria for the QCS are identical to the CCCs; the calculated amount for each analyte must be ± 30% of the expected value. 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 LC columns, LC conditions, internal standards, and MS and MS/MS conditions. Each time such method modifications are made, the laboratory must confirm that the modifications provide acceptable method performance as defined in the Sections 9.4.1-9.4.3. **Modifications to LC conditions should still produce conditions such that co-elution of the method analytes is minimized to reduce the probability of ESI suppression/enhancement effects.**

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. 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

(TOC) (e.g., 2 mg/L or greater) and a hard groundwater (e.g., 250 mg/L or greater as calcium carbonate).

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 Quality Assurance (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 LFSMs (Sect. 9.3.5), FDs or LFSMDs (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 initial calibration is required before any samples are analyzed. After the initial calibration is successful, a CCC is required at the beginning and end of each period in which analyses are performed, and after every tenth Field Sample.

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 1.0-5.0 µg/mL of each analyte (prepared in water containing 10% methanol) 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 the method analytes. The MS parameters (voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. The 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. 3.17) for each analyte by infusing approximately 1.0-5.0 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 the method analytes. The 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. The LC conditions listed in Table 1 may not be optimum for all LC systems and may need to be optimized by the analyst. If possible, optimize chromatographic conditions such that a unique quantitation ion is available for each analyte that is free from interference due to an identical product ion in any co-eluting (or overlapping) peak(s).

NOTE: During method development, the LC flow was diverted to waste for the first three minutes of the analysis. The flow was diverted to prevent potential fouling of the ESI source from sample components including preservatives. Laboratories are not required to divert the LC flow, but more frequent maintenance may be necessary if the flow is not diverted prior to elution of the first analyte.

10.2.3 Inject a mid-level CAL standard under LC/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into retention time windows so that each window contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M+H]^+$; Sect. 3.15) for the analytes in each window and choose the most abundant product ion. The product ions (also the quantitation ions) chosen during method development are in Table 4, although these will be instrument dependent. For maximum sensitivity during method development, small mass windows of ±0.5 daltons around the product ion mass were used for quantitation.

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.

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 the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the segment time window.

10.2.5 Prepare a set of at least five CAL standards as described in Section 7.2.3. The lowest concentration CAL standard must be at or below the MRL, which will 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. The curves may be concentration weighted, if necessary.

10.2.7 CALIBRATION ACCEPTANCE CRITERIA – When quantitated using the initial calibration curve, each calibration point, except the lowest point, for each analyte should calculate to be within 70-130% of its true value. The lowest CAL point should calculate to be within 50-150% of its 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.

10.3 CONTINUING CALIBRATION CHECK (CCC) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. In this context, a "sample" is considered to be a Field Sample. LRBs, CCCs, 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, the 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 in the most recent 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 in the CCC. The calculated amount for each analyte for medium and high level CCCs must be within ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50% of the true value. If these conditions cannot be met, then all data for the problem analyte must be considered invalid, and remedial action should be taken (Sect. 10.3.4). This 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 at the end of the batch fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular method analyte, and Field Samples 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, cleaning the 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. SAMPLE PREPARATION

11.1.1. Samples are preserved, collected and stored as presented in Section 8. All Field and QC Samples must contain the preservatives listed in Section 8.1.2, including the LRB.

11.1.2. In addition to the preservatives, if the sample is an LFSM or LFSMD, add the necessary amount of analyte WATER PDS (Sect. 7.2.2.3). Cap and invert each sample to mix.

NOTE: If the laboratory is concerned that a particular matrix may contain high particulate levels that could cause clogging of the LC system, sample filtration may be incorporated into the procedure. 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 Section 9.2 to ensure that they do not introduce interferences or retain any of the method analytes. Verification of subsequent lots of filters can be accomplished by examining a filtered LRB and duplicate samples of filtered LFBs fortified at the MRL. The filtered LFBs should calculate to be within ±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. During method development, Pall Gelman GHP Acrodisc, 25 mm syringe filters with 0.45 µm GHP membranes (Cat. No.: 4560T) were evaluated and found to pass all QC criteria. Other filter materials may be used provided the QC criteria in Section 9 are met.

11.2. SAMPLE ANALYSIS

11.2.1. Transfer a 990 µL aliquot of the sample to an autosampler vial. Add 10 µL of the IS PDS (Sect. 7.2.1.2) to the autosampler vial. Cap and invert each vial to mix.

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

11.2.3. 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.

11.2.4. Calibrate the system by either the analysis of a set of CAL standards (Sect. 10.2) or by confirming the existing calibration is still valid by analyzing a CCC as described in Section 10.3. If establishing an initial calibration, complete the IDC as described in Section 9.2.

11.2.5. Begin analyzing Field Samples, including QC samples, at their appropriate frequency by injecting the same size aliquots (50 µL was used in method development) under the same conditions used to analyze the CAL standards.

11.2.6. 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 the 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.

11.2.7. The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the calibration curve, the new aliquot of sample may be diluted with reagent water and the appropriate amount of IS added. Re-inject the diluted sample. Incorporate the dilution factor into the final concentration calculations. The resulting data should be documented as a dilution, with an increased MRL.

12. DATA ANALYSIS AND CALCULATION

12.1. In validating this method, concentrations were calculated by measuring the product ions listed in Table 4. Other product ions may be selected at the discretion of the analyst.

12.2. Calculate analyte concentrations using the multipoint calibration established in Section 10.2. Do not use daily calibration verification data to quantitate analytes in samples.

12.3 Prior to reporting the 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.

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 four tap water matrices: reagent water (Tables 6 and 7); chlorinated surface water (Tables 8 and 9); chlorinated (finished) ground water (Table 10); chlorinated surface water fortified with natural organic matter (Table 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. The chlorinated surface water was adjusted to pH=9.0 before adding the preservatives and analytes to simulate the worst case scenario for compounds with potential to degrade under basic pH conditions. The precision and mean recovery of analyses (n=7), conducted on Days 0, 7, and 14, are presented in Table 12.

13.3 MULTIPLE LABORATORY VERIFICATION – The performance of this method was demonstrated by multiple laboratories, with accuracy and precision results similar to those reported in Section 17. The authors wish to acknowledge the assistance of the analysts and laboratories listed below for their participation in the multi-lab demonstration.

13.3.1 Dr. Andrew Eaton and Mr. Ali Haghani of MWH Laboratories, Monrovia, CA.

13.3.2 Dr. Yongtao Li of Underwriters Laboratories, Inc., South Bend, IN.

13.3.3 Ms. Tiffany Payne and Mr. Ed George of Varian, Inc., Walnut Creek, CA.

14. POLLUTION PREVENTION

14.1 This method utilizes DAI-LC/MS/MS for the analysis of method analytes in water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste

Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036 or on-line at http://membership.acs.org/c/ccs/pub_9.htm (accessed November 2009).

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, 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.

15.2 Local regulations may prohibit sink disposal of water containing sodium omadine. Therefore, the laboratory should determine with local officials how to safely dispose of Field and QC samples containing sodium omadine.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. LC METHOD CONDITIONS

Time (min)	% 20 mM Ammonium Formate	% Methanol
Initial	90.0	10.0
3.0	90.0	10.0
5.0	70.0	30.0
8.0	70.0	30.0
20	30.0	70.0
20.1	10.0	90.0
25	10.0	90.0
25.1	90.0	10.0
30	90.0	10.0
Waters Atlantis® T_3 2.1 x 150 mm packed with 5.0 µm C_{18} stationary phase Flow rate of 0.3 mL/min 50 µL injection		

TABLE 2. ESI-MS METHOD CONDITIONS

ESI Conditions	
Polarity	Positive ion
Capillary needle voltage	+4 kV
Cone gas flow	100 L/hr
Nitrogen desolvation gas flow	1000 L/hr
Desolvation gas temp.	350ºC

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

Analyte	Peak # (Fig. 1)	RT (min)	IS# Ref
Methamidophos	2	3.66	1
Acephate	4	5.24	2
Aldicarb sulfoxide	5	6.51	2
Oxydemeton-methyl	7	7.50	3
Dicrotophos	8	9.59	3
Aldicarb	9	14.74	3
Quinoline	11	15.77	4
DIMP	13	16.65	5
Fenamiphos sulfoxide	14	17.63	3
Fenamiphos sulfone	15	18.07	3
Thiofanox	16	18.79	5
Methamidophos-d_6	1	3.57	IS#1
Acephate-d_6	3	5.16	IS#2
Oxydemeton-methyl-d_6	6	7.44	IS#3
Quinoline-d_7	10	15.57	IS#4
DIMP-d_{14}	12	16.44	IS#5

TABLE 4. MS/MS METHOD CONDITIONS[a,b]

Segment[c]	Analyte	Precursor Ion[d] *(m/z)*	Product Ion[d,e] (*m/z*)	Cone Voltage (v)	Collision Energy[f] (v)
1	Methamidophos	142	94	20	15
1	Acephate	184	143	20	15
2	Aldicarb sulfoxide	207	132	20	10
2	Oxydemeton-methyl	247	169	20	15
3	Dicrotophos	238	112	25	15
3	Aldicarb	208	89	10	15
4	Quinoline	130	77	35	30
4	DIMP	181	97	15	10
4	Fenamiphos sulfoxide	320	233	30	25
4	Fenamiphos sulfone	336	266	30	20
5	Thiofanox	219	57	20	20
1	Methamidophos-d_6	148	97	20	15
1	Acephate-d_6	190	149	20	15
2	Oxydemeton-methyl-d_6	253	175	20	15
4	Quinoline-d_7	137	81	35	30
4	DIMP-d_{14}	195	99	15	15

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

[b] These conditions were optimized during method development and used to collect the data in Section 17. Optimum conditions may vary on different LC/MS/MS instruments.

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

[d] 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 (e.g., *m/z* 141.9→93.9 for methamidophos). These precursor and product ion masses (with one decimal place) should be used in the MS/MS method for all analyses.

[e] Ions used for quantitation purposes.

[f] 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. (µg/L)[a]	DL[b] (µg/L)	LCMRL[c] (µg/L)
Methamidophos	0.050	0.017	0.032
Acephate	0.050	0.019	0.044
Aldicarb sulfoxide	0.10	0.060	0.088
Oxydemeton-methyl	0.050	0.010	0.019
Dicrotophos	0.050	0.025	0.039
Aldicarb	0.10	0.030	0.030
Quinoline	2.1	1.2	1.5
DIMP	0.050	0.014	0.022
Fenamiphos sulfoxide	0.050	0.034	0.042
Fenamiphos sulfone	0.050	0.0087	0.011
Thiofanox	0.20	0.090	0.18

[a] Spiking concentration used to determine DL.

[b] Detection limits were determined by analyzing eight 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 IN REAGENT WATER FORTIFIED AT 0.05-2.1 µg/L (n=8)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.050	107	11
Acephate	0.050	96.0	13
Aldicarb sulfoxide	0.10	96.9	21
Oxydemeton-methyl	0.050	105	6.5
Dicrotophos	0.050	114	15
Aldicarb	0.10	106	9.5
Quinoline	2.1	94.0	20
DIMP	0.050	103	9.2
Fenamiphos sulfoxide	0.050	118	19
Fenamiphos sulfone	0.050	108	5.4
Thiofanox	0.20	112	13

TABLE 7. PRECISION AND ACCURACY IN REAGENT WATER FORTIFIED AT 0.99-43 µg/L (n=7)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.99	105	1.3
Acephate	0.99	107	4.3
Aldicarb sulfoxide	2.0	105	1.9
Oxydemeton-methyl	0.99	96.0	3.6
Dicrotophos	0.99	95.9	5.2
Aldicarb	2.0	97.1	4.9
Quinoline	43	101	5.3
DIMP	0.99	101	3.3
Fenamiphos sulfoxide	0.99	94.5	6.5
Fenamiphos sulfone	0.99	92.3	4.7
Thiofanox	4.0	93.2	4.2

TABLE 8. PRECISION AND ACCURACY IN CHLORINATED SURFACE WATER FORTIFIED AT 0.05-2.1 µg/L (n=7)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.050	102	20
Acephate	0.050	113	18
Aldicarb sulfoxide	0.10	106	18
Oxydemeton-methyl	0.050	99.8	14
Dicrotophos	0.050	93.9	16
Aldicarb	0.10	116	15
Quinoline	2.1	97.4	19
DIMP	0.050	98.9	15
Fenamiphos sulfoxide	0.050	111	16
Fenamiphos sulfone	0.050	111	18
Thiofanox	0.20	119	14

TABLE 9. PRECISION AND ACCURACY IN CHLORINATED SURFACE WATER FORTIFIED AT 0.99-43 µg/L[a] (n=7)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.99	102	2.4
Acephate	0.99	103	3.2
Aldicarb sulfoxide	2.0	98.4	2.5
Oxydemeton-methyl	0.99	101	2.7
Dicrotophos	0.99	98.4	3.5
Aldicarb	2.0	112	2.1
Quinoline	43	98.5	3.7
DIMP	0.99	102	2.7
Fenamiphos sulfoxide	0.99	110	3.5
Fenamiphos sulfone	0.99	113	3.9
Thiofanox	4.0	95.2	3.3

[a] TOC = 1.49 mg/L and hardness = 120 mg/L as calcium carbonate.

TABLE 10. PRECISION AND ACCURACY IN FORTIFIED CHLORINATED GROUND WATER FORTIFIED AT 0.99-43 µg/L[a] (n=7)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.99	98.1	7.0
Acephate	0.99	106	4.4
Aldicarb sulfoxide	2.0	102	5.8
Oxydemeton-methyl	0.99	97.0	5.2
Dicrotophos	0.99	95.6	5.3
Aldicarb	2.0	103	5.9
Quinoline	43	94.3	7.6
DIMP	0.99	99.2	4.5
Fenamiphos sulfoxide	0.99	101	7.9
Fenamiphos sulfone	0.99	102	7.3
Thiofanox	4.0	89.3	2.4

[a] TOC = 0.726 mg/L and hardness = 342 mg/L as calcium carbonate.

TABLE 11. PRECISION AND ACCURACY IN CHLORINATED SURFACE WATER CONTAINING NATURAL ORGANIC MATERIAL[a] AND FORTIFIED WITH ANALYTES AT 0.99-43 µg/L (n=5)

Analyte	Fortified Conc. (µg/L)	Mean % Recovery	% RSD
Methamidophos	0.99	102	2.5
Acephate	0.99	106	3.8
Aldicarb sulfoxide	2.0	98.6	4.0
Oxydemeton-methyl	0.99	103	3.5
Dicrotophos	0.99	102	3.4
Aldicarb	2.0	109	3.1
Quinoline	43	98.8	3.0
DIMP	0.99	103	1.8
Fenamiphos sulfoxide	0.99	114	3.1
Fenamiphos sulfone	0.99	116	3.2
Thiofanox	4.0	104	4.9

[a] Prepared using commercially available (International Humic Substances Society) aquatic natural organic matter (NOM) isolated from the Suwannee River. The chlorinated surface water was fortified with NOM to obtain a TOC measurement of 8.7 mg/L.

TABLE 12. AQUEOUS SAMPLE HOLDING TIME DATA FOR SAMPLES FROM CHLORINATED SURFACE WATER[a], FORTIFIED WITH METHOD ANALYTES AND PRESERVED AND STORED ACCORDING TO SECTION 8 (n=7)

Analyte	Fortified Conc. (µg/L)	Day 0		Day 7		Day 14	
		Mean %Rec	% RSD	Mean %Rec	% RSD	Mean %Rec	% RSD
Methamidophos	0.99	94.4	2.8	97.5	1.9	91.1	2.5
Acephate	0.99	105	3.3	106	4.4	94.7	4.1
Aldicarb sulfoxide	2.0	97.7	2.9	100	6.2	84.5	3.2
Oxydemeton-methyl	0.99	92.9	3.4	85.9	2.5	80.0	2.3
Dicrotophos	0.99	91.4	5.7	94.5	2.9	93.1	4.6
Aldicarb	2.0	104	4.4	110	3.7	97.6	2.6
Quinoline	43	97.8	2.6	98.0	4.0	99.9	2.4
DIMP	0.99	96.8	2.7	98.5	3.6	98.3	1.2
Fenamiphos sulfoxide	0.99	102	4.4	112	3.3	99.0	3.0
Fenamiphos sulfone	0.99	101	4.3	117	3.0	99.6	2.6
Thiofanox	4.0	87.7	2.9	88.1	3.0	101	2.9

[a] TOC = 1.49 mg/L, pH=9.0 and hardness =120 mg/L as calcium carbonate.

TABLE 13. INITIAL DEMONSTRATION OF CAPABILITY QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low System Background	Analyze LRB prior to any other IDC steps and any time a new lot of solvents, reagents, and autosampler vials are used.	Demonstrate that all method analytes are below 1/3 the MRL and that possible interferences 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 mid-range calibration concentration.	%RSD must be <20%
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate mean recovery for replicates used in IDP.	Mean recovery ± 30% of true value
Sect. 9.2.4	Minimum Reporting Limit (MRL) Confirmation	Fortify 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 9.3.7	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of the IDC, each time a new Analyte MEOH PDS (Sect. 7.2.2.2) is prepared, and at least quarterly.	Results must be within 70-130% of expected value.
Sect. 9.2.6	Detection Limit (DL) Determination (optional)	Over a period of three days, prepare a minimum of seven replicate LFBs fortified at a concentration estimated to be near the DL. Analyze the replicates through all steps of the analysis. Calculate the DL using the equation in Sect. 9.2.6.	Data from DL replicates are not required to meet method precision and accuracy criteria. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria for CCCs.

NOTE: Table 13 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 14. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.4	Sample Holding Time	14 days with appropriate preservation and storage as described in Sections 8.1-8.4.	Sample results are valid only if samples are analyzed within the sample holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each analysis 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 analysis batch are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	LFB not required unless LFSM fails QC criteria (Sect. 9.3.5.4).	If an LFB must be analyzed due to failure of the LFSM, results of LFB analyses must be 70-130% of the true value for each method analyte for all except the lowest standard, which should be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standards are added to all standards and samples including QC samples. Compare IS areas to the average IS areas in the most recent calibration.	Peak areas (or calculated concentrations) for all ISs in all injections must be within ±50% of the average peak areas calculated during the most recent calibration. If this criterion is not met, results are labeled "suspect/IS recovery."
Sect. 9.3.5	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per analysis 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.	See Sect. 9.3.5 for instructions on the interpretation of LFSM results.
Sect. 9.3.6	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Analyze at least one FD or LFSMD with each analysis batch (20 samples or less). An LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	See Sect. 9.3.6 for instructions on the interpretation of LFSMD or FD results.
Sect. 9.3.7	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 expected value.
Sect. 10.2	Initial Calibration	Use IS calibration technique to generate linear or quadratic calibration curves. 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 should be 70-130% of the true value for all except the lowest standard, which should be 50-150% of the true value.
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 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte must be within 70-130% of the true value for all but the lowest level of calibration. Recovery for each analyte in the lowest CAL level CCC must be within 50-150% of the 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 8-10 supersedes any missing or conflicting information in this table.

FIGURE 1. EXAMPLE CHROMATOGRAM FOR REAGENT WATER FORTIFIED WITH METHOD 538 ANALYTES AT CONCENTRATION LEVELS INDICATED IN TABLE 7. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 3.