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METHOD 559. DETERMINATION OF NONYLPHENOL AND 4-TERT-OCTYLPHENOL IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

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METHOD 559

DETERMINATION OF NONYLPHENOL AND 4-TERT-OCTYLPHENOL IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1. This is a solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of nonylphenol and 4-tert-octylphenol in drinking water. Accuracy and precision data have been generated in reagent water and drinking water for the compounds listed in the table below.

Analyte	Acronym	Chemical Abstract Services Registry Number (CASRN)
Nonylphenol	NP	84852-15-3[a]
4-tert-Octylphenol	4-t-OP	140-66-9

[a] This CASRN describes and this method reports technical nonylphenol, comprised mostly of branched C9-alkyl phenols, and not linear nonylphenol (CASRN 104-40-5) which is a laboratory generated chemical not typically found in the environment.

1.2. 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 are 4.9 ng/L for 4-tert-octylphenol and 24 ng/L for nonylphenol and are listed in Table 5. The procedure used to determine the LCMRL is described elsewhere.[1]

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

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

1.5. This method is intended for use by analysts skilled in solid phase extractions, 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. 6.13, 9.1.1, 10.2, and 12.1). **Changes may not be made to sample collection and preservation (Sect. 8), the sample extraction steps (Sect. 11.4), 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, must not be used. 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. 9.3.6).

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

2. SUMMARY OF METHOD

A 100-250 mL water sample is fortified with surrogate and passed through a copolymeric SPE cartridge to extract the method analytes and surrogate. The compounds are eluted from the solid phase sorbent with a small amount of acetone. The extract is adjusted to a 5 mL volume with acetone after addition of the internal standard. A 10 µL injection is made into an LC equipped with a C_{18} column that is interfaced to an MS/MS. The analytes are separated and identified by comparing retention times and signals produced by unique mass transitions to retention times and reference signals for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the internal standard technique. A surrogate analyte is added to all Field and QC Samples to monitor the extraction efficiency of the method analytes.

3. DEFINITIONS

3.1. ANALYSIS BATCH – A set of samples that is analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

3.2. CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution, internal standard, and the surrogate. 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, internal standard and surrogate. The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5. DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination of precision (Sect. 9.2.6), and accurate quantitation is not expected at this level.[2]

3.6. EXTRACTION BATCH – A set of up to 20 Field Samples (not including QC samples) extracted together by the same person(s) during a work-day using the same lot of SPE devices, solvents, surrogate, internal standard and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

3.7. FIELD DUPLICATES (FD1 and FD2) – Two separate samples collected at the same time and placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

3.8. FIELD REAGENT BLANK (FRB) – An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.9. INTERFERENCE CHECK STANDARD (ICS) – A standard injected to ensure nonylphenoxy carboxylic acids do not overlap retention time and interfere with nonylphenol quantitation.

3.10. INTERFERENCE PRIMARY DILUTION STANDARD (IPDS) SOLUTION – A solution containing the nonylphenoxy carboxylic acid interferents prepared in the laboratory from stock standard solutions and diluted as needed to prepare the interference check standard.

3.11. INTERFERENCE STOCK STANDARD SOLUTION (ISSS) – A concentrated solution containing one or more nonylphenoxy carboxylic acids for checking retention

time interference. The standard is prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

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

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

3.14. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved field sample to which known quantities of the method analytes are added in the laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate sample extraction and the measured values in the LFSM corrected for background concentrations.

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

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

3.17. 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]

3.18. 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.

3.19. PRECURSOR ION – For the purpose of this method, the precursor ion is the deprotonated molecule ($[M-H]^-$) 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*.

3.20. 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.

3.21. 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.

3.22. QUALITY CONTROL SAMPLE (QCS) – A solution containing the method analytes at a known concentration that is obtained from a source external to the laboratory and different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibration standards.

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

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

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

4. INTERFERENCES

4.1. All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by a reagent water rinse. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 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 or capped.

NOTE: Detergents could be a source of high NP or OP background. Laboratories should examine their detergent(s) if having difficulty meeting LRB requirements.

Alternate detergents may need to be evaluated to determine if background contamination levels can be lowered.

4.2. Method interferences may be caused by contaminants in detergents, solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment that include plastics. 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 laboratory reagent blanks as described in Section 9.3.1. Subtracting blank values from sample results is not permitted.

4.2.1 There are many potential sources of nonylphenol contamination in the laboratory, especially from plastics and chemicals that may have been stored in plastic containers. Care must be taken to minimize sources of contamination, and the QC criteria for LRBs must be met (Sect. 9.3.1). Special precautions must also be taken when creating calibration curves for analytes consistently found in LRBs (Sect. 10.2.6).

4.3. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water.

4.3.1 Two common nonylphenoxy carboxylic acids (nonylphenoxyethoxy acetic acid and nonylphenoxy acetic acid) are known to interfere[3] with nonylphenol because they share the same transition (219 > 133). These two carboxylic acids were tested during method development and found to be separated from nonylphenol by five minutes. Severely compressing the gradient program and increasing flow may allow these substances to interfere wth nonylphenol.

4.3.2 Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent.[4,5] 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) were not observed.

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

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

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of SDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[6-8]

5.2. Nonylphenol and 4-t-octylphenol are harmful if ingested, can cause skin and serious eye burns, and can cause reproductive harm. Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

6. EQUIPMENT AND SUPPLIES

Brand names and/or catalog numbers are included for illustration only, and do not imply endorsement of the product. Plastic materials must be avoided as they have the potential to contaminate standards and/or samples with alkylphenols. The usage of Teflon™ is acceptable.

6.1. SAMPLE CONTAINERS – Amber glass bottles (125-250 mL) fitted with Teflon™-faced screw caps.

6.2. CONICAL CENTRIFUGE TUBES – 15 mL conical glass tubes (Corning Cat. No.: 8082-15 or equivalent) or other glassware suitable for collection of the extracts.

6.3. LOW VOLUME AUTOSAMPLER VIALS – Amber glass 2 mL autosampler vials with 0.4 mL low volume insert (Thermo Fisher Cat. No.: C4000-LV2W or equivalent).

6.4. AUTOSAMPLER VIALS – Amber glass 2.0 mL autosamplers vials for calibration standard preparation and storage (Thermo Fisher Cat. No.: C4000-2W or equivalent).

6.5. AUTOSAMPLER VIAL CAPS – Vial caps with PTFE/Silicone septum (Thermo Fisher Cat. No.: C5000-54B or equivalent).

NOTE: Vial cap septa have the potential to introduce nonylphenol contaminants once punctured, therefore vials can only be used once for an injection. Autosampler injection needles cored vial cap septa with three layers (PTFE/Silicone/PTFE) more easily than two layer septa (PTFE/Silicone). The three layer septa introduced NP contamination into the vial as the sample was injected and are not recommended. Pre-slit septa are not permitted because they cause excess extract evaporation and have the potential to allow nonylphenol from laboratory air to intrude into standards and extracts.

6.6. GRADUATED CYLINDERS – Glass, suggested sizes include 25, 50, 100, 250 and 1000 mL cylinders.

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

6.8. VOLUMETRIC FLASKS – Suggested sizes include 5, 10, 25, 500, and 1000 mL.

6.9. GLASS PIPETS – Borosilicate glass disposable pipets (Fisher Cat. No.: 13-678-20D or equivalent).

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

6.11. SOLID PHASE EXTRACTION (SPE) APPARATUS FOR USING CARTRIDGES – Cartridges specified here were found to contain acceptable background levels of nonylphenol. Alternate cartridges with equivalent sorbents need to be evaluated to ensure absence of nonylphenol contamination prior to use with field samples.

6.11.1 SPE CARTRIDGES

6.11.1.1 Waters Oasis HLB, 150 mg, 6 cc (Waters Cat No.: 186003365 or equivalent) – divinylbenzene-N-vinylpyrrolidone copolymer.

6.11.1.2 Phenomenex Strata-X, 100 mg, 6 cc (Phenomenex Cat No.: 8B-S100-ECH or equivalent) – styrene divinylbenzene-N-vinylpyrrolidone copolymer.

6.11.2 VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold with Visiprep™ large volume sampler (Supelco Cat. No. 57030 and 57275 or equivalent) for cartridge extractions, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements discussed in Section 9 are met.

6.11.3 SAMPLE DELIVERY SYSTEM – Use of a transfer tube system (Supelco "Visiprep," Cat No.: 57275) which transfers the sample directly from the sample

container to the SPE cartridge, is recommended, but not mandatory. Standard extraction manifolds come equipped with PTFE transfer tube systems.

NOTE: Transfer tubes are preferred to limit potential nonylphenol contamination, but plastic reservoirs may be used provided LRBs meet QC criteria.

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

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

6.13.1 LC SYSTEM – Instrument capable of reproducibly injecting up to 10 µL aliquots and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.3 mL/min). The usage of a column heater and thermostated autosampler compartment are optional. Data in Section 17 were collected with autosampler vials thermostated at 10 °C.

NOTE: During the course of method development, nonylphenol was observed as a background peak in solvent blank injections. The source of this background peak is likely components of the LC systems and solvents. Analysts must evaluate their instrument background and determine if additional action is required to lower contamination to an acceptable level. A second LC column (Sect. 6.13.4.2) used to delay background nonylphenol may be installed after the pump mixer but prior to the autosampler injector. The delay column causes the system background contamination to elute later in the chromatogram and separates it from the injected nonylphenol peak.

6.13.2 LC/TANDEM MASS SPECTROMETER – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.3 mL/min. The system must be capable of performing MS/MS to produce unique product ions (Sect. 3.21) for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision. Data are presented in Tables 5-13 using a triple quadrupole mass spectrometer (Thermo Scientific TSQ Endura).

6.13.3 DATA SYSTEM – An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must

be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

6.13.4 LC COLUMNS

6.13.4.1 ANALYTICAL COLUMN – An LC C_{18} column (2.1 x 50 mm) packed with 3 µm C_{18} solid phase particles (Thermo Fisher Cat No.: 25003-052130 or equivalent) was used during method development. Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

NOTE: It is recommended the LC column be dedicated for use with only ammonium fluoride due to potential deterioration of LC column performance[9] when switching between ammonium fluoride and other mobile phase additives.

6.13.4.2 DELAY COLUMN (optional) – An LC C_{18} column (2.1 x 100 mm) packed with 4 µm C_{18} solid phase particles (Thermo Fisher Cat No.: 74104-102130 or equivalent) used to retain nonylphenol contamination present in the LC system and separate it from the injected nonylphenol. The delay column should be installed after the binary pump mixer but prior to the autosampler injector and be of sufficient length and particle size to move the delayed nonylphenol peak so it is separated by a minimum of one minute from the injected nonylphenol peak without significantly increasing the system back pressure. The one minute peak separation is required due to multiple isomers in technical nonylphenol causing significant tailing beyond the peak elution time.

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. Prior to daily use, reagent water should be flushed from the purification system to rinse out any build-up of analytes in the system's tubing.

7.1.2 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher LC/MS grade or equivalent). Methanol used as the mobile phase must be replaced every 48 hours if not using a delay column to avoid increases in background contamination due to potential nonylphenol in the laboratory air.

7.1.3 ACETONE [$(CH3)_2CO$, CAS#: 67-64-1] – High purity, demonstrated to be free of analytes and interferences (Fisher Optima grade or equivalent).

7.1.4 AMMONIUM FLUORIDE (NH_4F, CAS#: 12125-01-8) – High purity, demonstrated to be free of analytes and interferences (Sigma-Aldrich Cat. No.: 216011 or equivalent).

7.1.4.1 10 mM AMMONIUM FLUORIDE STOCK SOLUTION – To prepare 100 mL, add 0.037 g ammonium fluoride to a 100 mL volumetric flask and bring to volume with reagent water. This solution should be stored in the refrigerator at 4 ºC to minimize bacterial growth and replaced after one month.

7.1.4.2 0.2 mM AMMONIUM FLUORIDE – To prepare 500 mL, add 10 mL of the 10 mM ammonium fluoride stock solution to a 500 mL volumetric flask and bring to volume with reagent water. This solution is used as the LC aqueous mobile phase. The mobile phase must be replaced every 48 hours if not using a delay column to avoid increases in background contamination due to potential nonylphenol in the laboratory air, otherwise replace weekly to avoid microbial growth.

7.1.5 SODIUM BISULFATE (CAS# 7681-38-1) – Preserves the sample to a pH of 2 to inhibit microbial growth and prevent analyte degradation (Fluka #71656 or equivalent) (Sect. 8.1.2).

7.1.6 L-ASCORBIC ACID (CAS# 50-81-7) – Reduces free chlorine at the time of sample collection (Sigma-Aldrich Cat. No.: 255564 or equivalent). (Sect. 8.1.2).

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

7.1.8 ARGON – Used as 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 so the smallest volume that can be accurately measured is used to minimize the addition of excess organic solvent to aqueous samples. PDS and calibration standards were found to be stable for at least one month during method development. Laboratories should use standard QC practices to determine when standards need to be replaced. The target analyte manufacturer's guidelines may be helpful when making the determination.

NOTE: Stock standards (Sect. 7.2.1.1, 7.2.2.1 and 7.2.3.1) and primary dilution standards (Sect.7.2.1.2, 7.2.2.2 and 7.2.3.2) were stored at ≤6 °C. Standards should be allowed to come to room temperature and mixed prior to use.

7.2.1 INTERNAL (IS) STOCK STANDARD SOLUTION – This method uses 4-(1,3-dimethyl-1-ethylpentyl) phenol-$^{13}C_6$ as the IS compound. The IS compound was carefully chosen during method development because it mimics the structure of technical nonylphenol (mostly branched nonylphenols). Alternate IS compounds may be used provided it is an isotopically labeled branched nonylphenol, however the analyst must have documented reasons for using alternate IS compounds. Alternate IS compounds must meet the QC requirements in Section 9.3.4.

NOTE: Isotopically labeled linear alkylphenol compounds do not elute at the same retention time as the branched IS and would not sufficiently correct for matrix suppression or enhancement of nonylphenol.

7.2.1.1 IS STOCK STANDARD SOLUTION (IS SSS) – The IS stock can be obtained as an individual certified stock standard solution (Cambridge Isotopes Cat. No.: CLM-8356-1.2) or neat material (Cambridge Isotopes Cat. No.: CLM-8356-0; custom order). During development of this method, the IS was prepared from neat material at 1000 µg/mL in methanol. The IS stock standard solution was stable for at least six months when stored at 6 °C or less in amber glass screw cap vials.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION (IS PDS) STANDARD (1 µg/mL) – Prepare, or purchase commercially, the IS PDS at a suggested concentration of 1.00 µg/mL. If prepared from individual stock standard solutions (Sect. 7.2.1.1), the table below can be used as a guideline for preparing the IS PDS although concentrations may need to be adjusted for instrument sensitivity. The IS PDS used in these studies was prepared in methanol. The IS PDS has been shown to be stable for at least six months when stored at 6 °C or less in amber glass screw cap vials. Fifty µL of this 1.00 µg/mL IS PDS was used to fortify the final 5 mL extracts (Sect. 11.5). This will yield a concentration of 10.0 µg/L in 5 mL extracts.

Conc. of IS Stock (µg/mL)	Vol. of IS Stock (µL)	Final Vol. of IS PDS (mL)	Final Conc. of IS PDS (µg/mL)
1000	10	10	1.00

7.2.2 SURROGATE (SUR) STANDARD SOLUTION – The SUR for this method is 4-(1,1,3,3-tetramethylbutyl) phenol-$^{13}C_6$ (4-tert-octylphenol-$^{13}C_6$). This isotopically labeled SUR standard was carefully chosen during method development because it is an isotopically labeled analogue of a target analyte and behaves similarly to method analytes during SPE extraction. Although alternate SUR standards may be used provided they are isotopically labeled branched compounds of a C8 or C9 branched alkylphenol, the analyst must have documented reasons for using alternate SUR standards. In addition, any alternate SUR standard must meet the QC requirements in Section 9.3.5.

NOTE: During method development it was discovered that linear alkyl phenols do not elute efficiently from the sorbent during SPE extraction under the same conditions as the branched alkyl phenols, resulting in lower recoveries. The branched SUR used in this method was chosen to produce the best SUR recovery and mimic the branched alkyl phenol target analytes of the method.

7.2.2.1 SUR STOCK STANDARD SOLUTION (SUR SSS) –During development of this method, the SUR was prepared from neat material (Toronto Research Chemicals Cat. No.: O293782) at 1000 µg/mL in methanol. The SUR stock standard solution was stable for at least six months when stored at 6 °C or less in amber glass screw cap vials.

7.2.2.2 SURROGATE PRIMARY DILUTION STANDARD (SUR PDS) (1.25 µg/mL) – Prepare the SUR PDS at a suggested concentration of 1.25 µg/mL as shown in the table below. Use 50 µL of this 1.25 µg/mL solution to fortify all QC and Field Samples. (Sect. 11.5). This will yield SUR concentrations of 250 ng/L in 250 mL aqueous samples.

Conc. of SUR Stock (µg/mL)	Vol. of SUR Stock (µL)	Final Vol. of SUR PDS (mL)	Final Conc. of SUR PDS (µg/mL)
1000	12.5	10	1.25

7.2.3 ANALYTE STANDARD SOLUTIONS – Analyte standards may be purchased commercially as ampulized solutions or prepared from neat materials.

7.2.3.1 ANALYTE STOCK STANDARD SOLUTION (SSS) – If preparing from neat material, accurately weigh approximately 5 mg of pure material to the nearest 0.1 mg and dilute to 5 mL with methanol for a final concentration of

1000 µg/mL. Repeat for each method analyte prepared from neat material. Alternatively, purchase commercially available individual stock standards of the analytes, preferably in methanol or acetonitrile, if available. For development of this method, a commercially available stock standard of 1000 µg/mL in methanol was purchased for 4-tert-octylphenol (Spex Cat. No: S-4379). Technical grade nonylphenol (Sigma Cat. No: 290858) was purchased as a neat material and prepared at 1000 µg/mL in methanol. These stock standards were stable for at least six months when stored at 6 °C or less in amber glass screw cap vials.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARD (PDS) SOLUTION (1.0 µg/mL) – The analyte PDS contains all method analytes in methanol. The analyte PDS was prepared in methanol at a concentration of 1.0 µg/mL. The analyte PDS is prepared by diluting (10 µL of each SSS into 10 mL of methanol) the combined Analyte Stock Standard Solutions (Sect.7.2.3.1) and is used to prepare CAL standards, and fortify LFBs, LFSMs, and LFSMDs with the method analytes. The analyte PDS has been shown to be stable for at least six months when stored at 6 °C or less in amber glass screw cap vials.

7.2.4 NONYLPHENOXY CABOXYLIC ACID INTERFERENCE SOLUTION – The interference solution is prepared and analyzed only if the LC column and LC conditions in Table 1 are changed from method suggested parameters (See Section 9.1.1.1).

7.2.4.1 INTERFERENCE STOCK STANDARD SOLUTIONS (ISSS) – For development of this method, commercially available stock standards of 100 µg/mL in methanol were purchased for nonylphenoxy ethoxy acetic acid (CAS# 106807-78-7, Accustandard Cat. No: PEO-012S) and nonylphenoxy acetic acid, (CAS# 3115-49-9, Accustandard Cat. No: PEO-009S). These stock standards were stable for at least three months when stored at 6 °C or less in amber glass screw cap vials.

7.2.4.2 INTERFERENCE PRIMARY DILUTION STANDARD (IPDS) SOLUTION (5.00 µg/mL) – The IPDS contains all method analytes in methanol. The IPDS was prepared in methanol at a concentration of 5.00 µg/mL. The IPDS is prepared by diluting (50 µL of the ISSS into 1 mL of methanol) the combined Interference Stock Standard Solutions (Sect. 7.2.4.1). The IPDS has been shown to be stable for at least three months when stored at 6 °C or less in amber glass screw cap vials.

7.2.4.3 INTERFERENCE CHECK STANDARD (ICS) – The ICS is prepared at 100 µg/L and analyzed according to Section 9.1.1.1. The ICS is prepared by

diluting (20 µL of IPDS into 1 mL of acetone) the Interference Primary Dilution Standard (Sect. 7.2.4.2).

7.2.5 CALIBRATION STANDARDS (CAL) – At least five calibration concentrations are required to prepare the initial calibration curve spanning a 20-fold concentration range (Sect. 10.2). Larger concentration ranges will require more calibration points. Prepare the CAL standards over the concentration range of interest from dilutions of the analyte PDS in acetone. The suggested analyte concentrations found in Tables 5-11 can be used as a starting point for determining the calibration range. The IS and SUR are added to the CAL standards at a constant concentration. During method development, the concentration of the SUR was 12.5 µg/L in the standard (250 ng/L in the aqueous sample) and the IS concentration was 10.0 µg/L. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. The CAL standards may also be used as CCCs (Sect. 9.3.2). CAL standards should be stored at 6 °C or less. Calibration standards are valid for up to thirty days if properly stored. CAL standards must be aliquoted in separate vials for each injection so that no more than one injection occurs per vial. Example CAL standard preparations are found below.

CAL STD Prepared at 1 mL in 2 mL vial	Vol. of 1.00 µg/mL analyte PDS (µL)	Vol. of 1.25 µg/mL SUR PDS (µL)	Vol. of 1.00 µg/mL IS PDS (µL)	Final Conc. of Target Analytes[a] in CAL standard (µg/L)
CAL1	2.5	10	10	2.5
CAL2	5.0	10	10	5.0
CAL3	10.0	10	10	10.0
CAL4	15.0	10	10	15.0
CAL5	20.0	10	10	20.0
CAL6	40.0	10	10	40.0
CAL7	60.0	10	10	60.0

[a] Final standard concentration of SUR is 12.5 µg/L and IS is 10.0 µg/L.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1. SAMPLE BOTTLE PREPARATION

8.1.1 Collect samples in 250 mL (or smaller) amber glass bottles fitted with a Teflon™ lined screw-cap.

NOTE: Smaller sample volumes (100 mL minimum) can be collected if the laboratory demonstrates acceptable performance in meeting the required MRLs (Sect. 9.2.4) using the smaller sample volume. However, the entire sample volume collected must be processed (e.g., a 100 mL sample cannot be aliquoted from a 250 mL

sample). The amount of added preservatives and surrogate/analyte fortification levels must be adjusted accordingly.

8.1.2 The preservation reagents, listed in the table below, are added to each sample bottle as solids prior to shipment to the field (or prior to sample collection).

Compound	Amount	Purpose
Sodium bisulfate (Sect. 7.1.5)	1.0 g/L	Inhibits microbial growth
L-ascorbic acid (Sect. 7.1.6)	100 mg/L	Removes 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 samples from the flowing system.

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

8.2.3 After collecting the sample, cap the bottle and agitate by hand until preservatives are dissolved. Keep the sample sealed from time of collection until extraction.

8.3. FIELD REAGENT BLANKS (FRB)

8.3.1 An FRB must be handled along with each sample set. The sample set is composed of samples collected from the same sample site and at the same time. At the laboratory, fill the field blank sample bottle with reagent water, then seal, and ship to the sampling site along with the sample bottles. For each FRB shipped, a second FRB bottle containing only the preservative must also be shipped. At the sampling site, the sampler must open the shipped FRB and pour the preserved reagent water into the empty shipped sample bottle, seal and label this bottle as the FRB. The FRB is shipped back to the laboratory along with the samples and analyzed to ensure that nonylphenols were not introduced into the sample during sample collection/handling.

8.3.2 The same batch of preservatives must be used for the FRBs as for the field samples.

8.3.3 The reagent water used for the FRBs must be initially analyzed for method analytes as an LRB (using the same lot of sample bottles as the field samples) and must meet the LRB criteria in Section 9.3.1 prior to use. This requirement will ensure samples are not being discarded due to contaminated reagent water or sample bottles rather than contamination during sampling.

8.4. 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 extraction but must not be frozen.

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

8.5. SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Table 12) indicated that all compounds listed in this method have adequate stability for 28 days when collected, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.4. Therefore, water samples should be extracted as soon as possible but must be extracted within 28 days. Extracts must be stored at or below 6 ºC and analyzed within 28 days after extraction. The extract storage stability study data are presented in Table 13.

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 14 and 15. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 METHOD MODIFICATIONS – The analyst is permitted to modify LC columns, LC conditions, evaporation techniques, internal standards or surrogate standards (refer to cautions about substituting SUR and IS standards, Sections 7.2.1 and 7.2.2), and MS and MS/MS conditions. Each time such method modifications are made, the analyst must repeat the procedures of the IDC. **LC modifications to shorten the analytical run will move method analyte peaks closer to matrix interferences from humics/fulvics and nonylphenoxy carboxylic acids, potentially increasing the probability of suppression/ enhancement effects.**

9.1.1.1 DEMONSTRATION OF NON-INTERFERENCE FROM SELECT NONYLPHENOXY CARBOXYLIC ACIDS – If the LC column and conditions are modified from suggested parameters in Table 1, it must be demonstrated that nonylphenoxy carboxylic acids do not co-elute with nonylphenol. Prepare the interference check standard as specified in Section 7.2.4.3. Inject the ICS and search the chromatogram for nonylphenoxy

carboxylic acid peaks at the same transition (*m/z* 219→133) as nonylphenol. Both nonylphenoxy carboxylic acids must be separated from the nonylphenol peak by at least one minute to eliminate the possibility of interference. Nonylphenoxy carboxylic acids peaks may tail under method conditions and could interfere with nonylphenol since they share the same MS/MS transition.

9.2. INITIAL DEMONSTRATION OF CAPABILITY – 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. The IDC must be repeated if the laboratory changes the brand of SPE cartridge.

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

9.2.2 INITIAL DEMONSTRATION OF PRECISION (IDP) – Prepare, extract, and analyze four to seven replicate LFBs fortified near the midrange of the initial calibration curve according to the procedure described in Sections 11.3 and 11.4. Sample preservatives as described in Section 8.1.2 must be added to these samples. The percent relative standard deviation (%RSD) of the results of the replicate analyses must be less than or equal to 20%.

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

9.2.3 INITIAL DEMONSTRATION OF ACCURACY (IDA) – Using the same set of replicate data generated for Section 9.2.2, calculate average percent recovery (%R). The average recovery of the replicate values must be within ± 30% of the true value.

$$\%\text{R} = \frac{\text{Average Measured Concentration}}{\text{Fortified Concentration}} \text{ X } 100$$

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. The MRL may be established by a laboratory for their specific purpose or may be set by a regulatory agency. If there is a programmatic MRL requirement, the laboratory MRL must be set at or below this level. 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.

NOTE: Setting an MRL for method analytes that are consistently present in the background (e.g., nonylphenol) is particularly important so that false positive data are not reported for Field Samples. See Sect. 9.3.1 for guidance in setting an MRL for these analytes.

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

$$HR_{PIR} = 3.963s$$

where

s = 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.

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

The Lower PIR Limit must be ≥ 50% 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 (Sect. 9.2.4.2). If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration. Because background contamination can be a significant problem, some MRLs may be background limited.

9.2.5 CALIBRATION CONFIRMATION – Analyze a QCS as described in Section 9.3.9 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.

9.2.6.1 Replicate analyses for this procedure should be done over at least three days (i.e., both the sample extraction and the LC/MS/MS analyses should be done over at least three days). Prepare at least seven replicate LFBs at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. 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. Therefore, no precision and accuracy criteria are specified.

9.2.6.2 If a laboratory is establishing their own MRL, the calculated DLs should not be used as the MRL for analytes that commonly occur as background contaminants. Method analytes that are seen in the background should be

reported as present in Field Samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentrations + 3σ or 3 times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as an MRL in order to avoid reporting false positive results.

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 extraction batch (Sect. 3.6) to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. If more than 20 Field Samples are collected, analyze an LRB for every 20 samples. 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 or reduce as much as possible the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. 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. Background concentrations of method analytes must be less than or equal to one-third the MRL. If method analytes are detected in the LRB at concentrations greater than this level, then all positive field sample results (i.e., results at or above the MRL) for those analytes are invalid for all samples in the extraction batch. Because background contamination may be a problem for nonylphenol, maintaining a historical record of LRB data is highly recommended.

NOTE: It is extremely important to evaluate background values of analytes that commonly occur in LRBs. The MRL must be set at a value greater than three times the mean concentration observed in replicate LRBs. If LRB values are highly variable, setting the MRL to a value greater than the mean LRB concentration plus three times the standard deviation may provide a more realistic MRL.

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

9.3.3 LABORATORY FORTIFIED BLANK (LFB) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The low concentration LFB must be as near as practical to, but no more than two

times, the MRL. Similarly, the high concentration LFB should be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the low-level LFB analyses (within a factor of 2-times the MRL concentration) must be 50-150% of the true value. Results of the medium and high-level LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.3.4 INTERNAL STANDARDS (IS) – The analyst must monitor the peak areas of the IS in all injections during each analysis day. The IS responses (peak areas) in any chromatographic run must be within 70-130% of the response in the most recent CCC and must not deviate by more than 50% from the average area measured during initial analyte calibration. If the IS areas in a chromatographic run do not meet these criteria, inject a second aliquot of that extract placed into a new capped 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 extract fails again, the analyst should check the calibration by reanalyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the CAL standard is acceptable, extraction of the sample may need to be repeated provided the sample is still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect. Alternatively, collect a new sample and re-analyze.

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

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

where

A = calculated SUR concentration for the QC or Field Sample
B = fortified concentration of the SUR.

9.3.5.1 SUR recovery must be in the range of 70-130%. When SUR recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check 1) calculations to locate possible errors, 2) standard solutions for

degradation, 3) contamination, and 4) instrument performance. Correct the problem and reanalyze the extract.

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

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

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

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

9.3.6.2 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.

9.3.6.3 Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70-130%, except for low-level fortification near or at the MRL (within a factor of 2-times the MRL concentration) where 50-150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

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

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

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

9.3.7.2 RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of 2 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 influenced. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

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

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

NOTE: LFSMs and LFSMDs fortified at concentrations near the MRL, where the associated Field Sample contains native analyte concentrations

above the DL but below the MRL, should be corrected for the native levels in order to obtain meaningful %R values. This example, and the LRB extrapolation (Sect. 9.3.1), are the only permitted uses of analyte results below the MRL.

9.3.7.4 RPDs for duplicate LFSMs must 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 2 of the MRL. LFSMs fortified at these concentrations must have RPDs that are ≤50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and 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.

9.3.8 FIELD REAGENT BLANK (FRB) – The purpose of the FRB is to ensure that method analytes measured in the Field Samples were not inadvertently introduced into the sample during sample collection/handling. Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample. If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.

9.3.9 QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new Analyte PDS (Sect. 7.2.3.2) is prepared, and at least quarterly, analyze a QCS sample from a source different from the source of the CAL standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared at a mid-level concentration and analyzed just like 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.

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 µg/mL of each analyte directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). The mobile phase conditions may be varied to optimize sensitivity of the infused analytes(s). 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. 3.21) for each analyte by infusing approximately 1.0 µg/mL of each analyte directly into the MS at the chosen LC mobile phase flow rate (approximately 0.3 mL/min). The mobile phase conditions may be varied to optimize sensitivity of the infused analytes(s). 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. Ammonium fluoride was added to the aqueous phase at 0.2 mM to enhance sensitivity and to increase stability from potential matrix interference competing for ionization in the source. 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.

Cautions: LC system components, as well as the mobile phase constituents, may contain trace amounts of nonylphenol. Thus, nonylphenol will build up on the head of the LC column during mobile phase equilibration. To minimize the background nonylphenol peaks and to keep background levels constant, the time the LC column is held at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, prior to daily use, flush the column with 100% methanol for at least 15 min before initiating a sequence. It may be necessary on some systems to flush other LC components such as wash syringes, sample needles or any other system components before daily use. Acetone was found to rinse nonylphenol from syringes and valves better than methanol.

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 each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ($[M-H]^-$; Sect. 3.19) 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.

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

10.2.5 Prepare a set of at least five CAL standards as described in Section 7.2.5. The lowest concentration CAL standard must be at or below the MRL, which may depend on system sensitivity. It is recommended that at least four of the CAL standards are at a concentration greater than or equal to the MRL.

10.2.6 The LC/MS/MS system is calibrated using the IS technique. Use the LC/MS/MS data system software to generate a linear regression or quadratic calibration curve for each of the analytes. This curve **must always** be forced through zero and may be concentration weighted, if necessary. Forcing zero allows for a better estimate of the background levels of method analytes.

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

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.

10.3. 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, LFBs, LFSMs, FDs, FRBs

and LFSMDs are not counted as samples. The beginning CCC of each analysis batch must be at or below the MRL 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 these 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 70-130% of the areas measured in the most recent continuing calibration check, and within 50-150% from the average areas measured during initial calibration. If any of the IS areas has changed by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may include cleaning of the MS ion source, or other maintenance as indicated in Section 10.3.4. Major instrument maintenance requires recalibration (Sect. 10.2) and verification of sensitivity by analyzing a CCC at or below the MRL (Sect. 10.3). Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and SUR in the CCC. The calculated amount for each analyte and SUR 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% and the SUR must be within ± 30% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken (Sect. 10.3.4) which may require recalibration. Any Field or QC Samples that have been analyzed since the last acceptable calibration verification that are still within holding time must be reanalyzed after adequate calibration has been restored, with the following exception. **If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular method analyte, and Field Sample extracts show no detection for that method analyte, non-detects may be reported without re-analysis.**

10.3.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance, such as cleaning the electrospray probe, atmospheric pressure ionization source, 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. This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. The data presented in Tables 5-13 demonstrate data collected by manual extraction. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the LRBs should be rotated among the ports to ensure that all the valves and tubing meet the LRB requirements (Sect. 9.3.1).

11.2. Nonylphenol may adsorb to surfaces. Therefore, the aqueous sample bottles must be rinsed with the elution solvent whether extractions are performed manually or by automation. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected (Sect. 11.4.4). Due to this adsorption, the entire sample volume collected, excluding sample used for pH verification and residual chlorine testing must be extracted.

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

11.3. SAMPLE PREPARATION

11.3.1 Samples are preserved, collected and stored as presented in Section 8. All Field and QC Samples, including the LRB, LFB and FRB, must contain the preservatives listed in Section 8.1.2. Before extraction, verify that the sample pH is ≤3. If pH is outside this range, the sample must be rejected. Determine sample volume. An indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 1 g. After extraction, proceed to Section 11.6 for final volume determination. Nonylphenol may adsorb to surfaces, thus the sample volume may **NOT** be transferred to a graduated cylinder for volume measurement. The LRB, LFB and FRB may be prepared by measuring 250 mL of reagent water with a glass graduated cylinder or filling a 250 mL sample bottle to near the top.

11.3.2 Add an aliquot of the SUR PDS (Sect. 7.2.2.2) to each sample, cap and invert to mix. During method development, a 50 µL aliquot of the 1.25 µg/mL SUR PDS was added to each 250 mL sample for a final aqueous sample concentration of 250 ng/L for 4-tert-octylphenol-$^{13}C_6$.

11.3.2.1 In addition to the SUR, dechlorination agent and antimicrobial, if the sample is an LFB, LFSM, or LFSMD, add the necessary amount of analyte PDS

(Sect. 7.2.3.2), taking into account the final extract volume is 5 mL. Cap and invert each sample to mix.

11.4. CARTRIDGE SPE PROCEDURE

11.4.1 CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 15 mL of acetone. Next, rinse each cartridge with 15 mL of reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Add 2-3 mL of reagent water to each cartridge, attach the sample transfer tubes (Sect. 6.11.3), turn on the vacuum, and begin adding sample to the cartridge.

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

11.4.3 SAMPLE BOTTLE AND CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the sample bottles with one 5 mL aliquot of reagent water and draw each aliquot through the sample transfer tubes and the cartridges. Draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

11.4.4 SAMPLE BOTTLE RINSE AND CARTRIDGE ELUTION – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Rinse the sample bottles with 2 mL of acetone and elute the analytes from the cartridges by pulling the 2 mL of acetone through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 2 mL aliquot of acetone.

11.5. EXTRACT VOLUME ADJUSTMENT – Add IS PDS (50 µL of the 1.00 µg/mL IS PDS for extract concentrations of 10.0 µg/L were used for method development; Sect. 7.2.1.2) to the collection vial and bring the volume to 5 mL with acetone and vortex. Transfer a small aliquot with a glass pipet (Sect. 6.9) to a low volume autosampler vial.

NOTE: It is recommended that a small aliquot be transferred to a single autosampler vial because each vial can only be used for one injection due to the potential of extract contamination from nonylphenol after septa puncture. Extract aliquots can be stored in autosampler vials prior to puncture of the vial cap. Alternatively, extracts can be stored in 15 mL centrifuge tubes (Sect. 6.2)

with caps. Pre-slit septa are not permitted because they cause excess extract evaporation and have the potential to allow nonylphenol from laboratory air to intrude into the sample extract.

11.6. SAMPLE VOLUME DETERMINATION – If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 2 mL. If using weight to determine volume, weigh the empty bottle to the nearest 1 g and determine the sample weight by subtraction of the empty bottle weight from the original sample weight (Sect. 11.3.1). Assume a sample density of 1.0 g/mL. In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 12.2).

11.7. EXTRACT ANALYSIS

11.7.1 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.

11.7.2 Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in CAL standard solutions analyzed on the LC/MS/MS 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.

11.7.3 Calibrate the system by either the analysis of calibration standards (Sect. 10.2) or by confirming the initial 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.

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

11.7.5 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard. Comparison of 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.

11.7.6 The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract must be diluted with acetone and the appropriate amount of IS added to match the original concentration. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable SUR performance (Sect. 9.3.5.1) should be determined from the undiluted sample extract. The resulting data for the analyte peak that exceeded the initial calibration range must be documented as a dilution and MRL adjusted accordingly.

12. DATA ANALYSIS AND CALCULATION

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

12.2. Calculate analyte and SUR concentrations using the multipoint calibration as described in Section 10.2. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.6.

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.

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

13. METHOD PERFORMANCE

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

13.2. SAMPLE STORAGE STABILITY STUDIES – An analyte storage stability study was conducted by fortifying the analytes into chlorinated surface water samples that were

collected, preserved, and stored as described in Section 8. The precision and mean recovery (n=4) of analyses, conducted on Days 0, 7, 14, 21, and 28 are presented in Table 12.

13.3. EXTRACT STORAGE STABILITY STUDIES – Extract storage stability studies were conducted on extracts obtained from a chlorinated surface water fortified with the method analytes. The precision and mean recovery (n=4) of injections conducted on Days 0, 7, 14, 21, and 28 are reported in Table 13.

13.4. MULTI-LABORATORY DEMONSTRATION – The performance of this method was demonstrated by multiple laboratories, with results reported in Section 17. The authors wish to acknowledge the work of Bill Deckelmann and Katie Kohoutek at American Water Central Laboratory (Belleville, IL), David Schiessel and Susann Thomas at Babcock Laboratories, Inc. (Riverside, CA), William Lipps and Ali Haghani at Eurofins Eaton Analytical, LLC (Monrovia, CA), Yongtao Li and Joshua Whitaker at Eurofins Eaton Analytical, LLC (South Bend, IN).

14. POLLUTION PREVENTION

14.1. This method utilizes SPE to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2. For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Guide to Minimizing Waste in Laboratories" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C., 20036 or on-line at https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf (accessed November 2019).

15. WASTE MANAGEMENT

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. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. LC METHOD CONDITIONS

Time (min)	% 0.2 mM ammonium fluoride	% Methanol
Initial	90	10
1.0	90	10
15.1	5	95
19.0	5	95
19.1	90	10
23.0	90	10
10 µL injection into a 20 µL loop Flow rate of 0.3 mL/min Autosampler compartment thermostated at 10 °C	Analytical Column: Thermo Hypersil Gold C_{18} 2.1 x 50 mm packed with 3.0 µm C_{18} stationary phase thermostated to 30 ºC	Delay Column: Thermo Accucore C_{18} 2.1 x 100 mm packed with 4 µm C_{18} stationary phase

TABLE 2. ESI-MS METHOD CONDITIONS

ESI Conditions	Setting
Polarity	Negative ion
Capillary needle voltage	-2.8 kV
Sheath Gas	25 L/hr
Aux Gas	5 L/hr
Sweep Gas	1 L/hr
Ion Transfer Tube Temperature	325 °C
Vaporizer Temperature	300 °C

TABLE 3. METHOD ANALYTES AND RETENTION TIMES (RT)[a]

Analyte	Peak # (Fig. 1)	RT (min)
4-t-OP	1	13.44
NP	2	14.08
4-tert-octylphenol-$^{13}C_6$ (SUR)	3	13.43
4-(1,3-dimethyl-1-ethylpentyl) phenol-$^{13}C_6$ (IS)	4	14.08

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

TABLE 4. MS/MS METHOD CONDITIONS

Analyte	Precursor Ion [a] *(m/z)*	Product Ion[a,b] *(m/z)*	Collision Energy[c] (v)	RF Lens (v)
4-t-OP	205	133	23	99
NP[d]	219	133	29	93
4-t-OP-$^{13}C_6$ (SUR)	211	139	24	95
Branched-NP-$^{13}C_6$ (IS)	225	139	29	101

[a] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak place (e.g., *m/z* 219→133 for NP). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

[b] Ions used for quantitation purposes.

[c] Argon used as collision gas at 2 mTorr.

[d] Analyte has multiple unresolved chromatographic peaks due to multiple branched isomers. All peaks summed as one peak for quantitation purposes.

TABLE 5. DLs AND LCMRLs IN REAGENT WATER

Analyte	Fortified Conc. (ng/L)[a]	DL[b] (ng/L)	LCMRL[c] (ng/L)
4-tert-OP	6.0	3.4	4.9
NP	24	6.2	24

[a] Spiking concentration used to determine DL.

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

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

TABLE 6. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN REAGENT WATER FORTIFIED AT 600 ng/L (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	600	99.2	2.1	98.5	1.5
NP	600	96.4	1.6	97.4	0.76
4-t-OP-$^{13}C_6$	250	99.0	2.2	99.5	1.6

TABLE 7. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN REAGENT WATER FORTIFIED AT 100 ng/L (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	100	95.1	2.4	95.1	2.4
NP	100	100	3.8	97.4	4.5
4-t-OP-$^{13}C_6$	250	98.4	3.0	98.8	1.1

TABLE 8. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN DRINKING WATER FROM A GROUND WATER SOURCE FORTIFIED AT 600 ng/L (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	600	97.1	3.5	97.0	0.89
NP	600	96.0	3.4	93.9	0.59
4-t-OP-$^{13}C_6$	250	98.3	3.2	99.9	1.8

[a] TOC^{10} = 0.71 mg/L and hardness = 360 mg/L measured as calcium carbonate.

TABLE 9. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN DRINKING WATER FROM A GROUND WATER SOURCE FORTIFIED AT 100 ng/L (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	100	101	3.8	101	2.0
NP	100	100	3.3	103	3.6
4-t-OP-$^{13}C_6$	250	99.0	1.0	99.1	1.6

[a] TOC^{10} = 0.71 mg/L and hardness = 360 mg/L measured as calcium carbonate.

TABLE 10. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN DRINKING WATER FROM A SURFACE WATER SOURCE FORTIFIED AT 600 ng/ (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	600	96.4	3.4	98.2	2.1
NP	600	97.2	1.5	96.6	2.1
4-t-OP-$^{13}C_6$	250	99.4	3.4	99.8	3.0

[a] TOC^{10} = 1.5 mg/L and hardness = 86 mg/L measured as calcium carbonate.

TABLE 11. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES IN DRINKING WATER FROM A SURFACE WATER SOURCE FORTIFIED AT 100 ng/L (n=4)

Analyte	Fortified Conc. (ng/L)	Oasis HLB Mean % Recovery	Oasis HLB % RSD	Strata-X Mean % Recovery	Strata-X % RSD
4-t-OP	100	103	4.8	102	4.0
NP	100	102	1.2	101	3.7
4-t-OP-$^{13}C_6$	250	98.4	1.9	100	1.0

[a] TOC^{10} = 1.5 mg/L and hardness = 86 mg/L measured as calcium carbonate.

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

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Rec	Day 0 % RSD	Day 7 Mean % Rec	Day 7 % RSD	Day 14 Mean % Rec	Day 14 % RSD	Day 21 Mean % Rec	Day 21 % RSD	Day 28 Mean % Rec	Day 28 % RSD
4-t-OP	600	96.1	1.0	96.8	1.1	95.6	2.8	92.7	2.1	96.7	1.9
NP	600	98.2	0.85	93.9	1.5	94.0	2.4	92.9	1.4	95.4	0.80
4-t-OP-$^{13}C_6$	250	100	1.9	99.5	0.78	99.2	2.0	95.3	1.5	100	2.0

[a] TOC^{10} = 1.0 mg/L and hardness = 86 mg/L measured as calcium carbonate.

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

Analyte	Fortified Conc. (ng/L)	Day 0 Mean % Rec	Day 0 % RSD	Day 7 Mean % Rec	Day 7 % RSD	Day 14 Mean % Rec	Day 14 % RSD	Day 21 Mean % Rec	Day 21 % RSD	Day 28 Mean % Rec	Day 28 % RSD
4-t-OP	600	96.1	1.0	99.5	1.6	98.9	1.3	97.7	1.4	100	2.5
NP	600	98.2	0.85	96.6	1.2	97.4	1.0	100	1.2	99.7	1.6
4-t-OP-$^{13}C_6$	250	100	1.9	99.3	1.4	101	1.2	97.3	1.5	99.2	1.5

TABLE 14. 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.	Demonstrate that all method analytes are less than or equal to 1/3 the MRL and that possible interferences from extraction media do not prevent the identification and quantification of method analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze four to seven replicate LFBs fortified near the midrange calibration concentration.	%RSD must be ≤20%
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP.	Mean recovery ± 30% of true value
Sect. 9.2.4	Minimum Reporting Limit (MRL) Confirmation	Fortify, extract and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the Mean and the Half Range (HR). Confirm that the upper and lower limits for the Prediction Interval of Result (Upper PIR, and Lower PIR, Sect. 9.2.4.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Sect. 9.2.5 and 9.3.9	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of IDC.	Results must be within 70-130% of true 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. 9.2.6.1.	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.

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

TABLE 15. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.5	Sample Holding Time	28 days with appropriate preservation and storage as described in Sections 8.1-8.5.	Sample results are valid only if samples are extracted within the sample holding time.
Sect. 8.5	Extract Holding Time	28 days when stored at ≤ 6 °C in glass centrifuge tubes or vials with un-punctured septa.	Extract results are valid only if extracts are analyzed within the extract holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each extraction batch of up to 20 samples.	Demonstrate that all method analytes are less than or equal to 1/3 the MRL and confirm that possible interferences do not prevent quantification of method analytes. If analytes are >1/3 the MRL or if interferences are present, any samples from the extraction batch for the problem analyte(s) that yielded a positive result are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	One LFB is required for each extraction batch of up to 20 Field Samples. Rotate the fortified concentrations between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each method analyte for all fortified concentrations except the lowest CAL point. Results of the LFBs corresponding to the lowest CAL point for each method analyte must be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standard, 4-(1,3-dimethyl-1-ethylpentyl phenol-$^{13}C_6$, is added to all standards and sample extracts, including QC samples. Compare IS areas to the average IS area in the initial calibration and to the most recent CCC.	Peak area counts for IS in all injections must be within ± 50% of the average peak area calculated during the initial calibration and 70-130% from the most recent CCC. If ISs do not meet this criterion, corresponding target results are invalid.
Sect. 9.3.5	Surrogate Standards (SUR)	Surrogate standard, 4-tert-octylphenol-$^{13}C_6$, is added to all CAL standards and samples, including QC samples. Calculate SUR recovery.	SUR recoveries must be 70-130% of the true value. If a SUR fails this criterion, report all results for sample as suspect/SUR recovery.

TABLE 15. (Continued)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.6	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration, if known. Calculate LFSM recoveries.	Recoveries at mid and high levels must be within 70-130% and within 50-150% at the low-level fortified amount (near the MRL). If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Extract and analyze at least one FD or LFSMD with each extraction batch (20 samples or less). A LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	Method analyte RPDs for the LFMD or FD must be ≤30% at mid and high levels of fortification and ≤50% near the MRL. If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.8	Field Reagent Blank (FRB)	Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample.	If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.
Sect. 9.3.9	Quality Control Sample (QCS)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Results must be within 70-130% of true value.
Sect. 10.2 and Sect. 9.3.2	Initial Calibration	Use IS calibration technique to generate a first or second order calibration curve forced through zero. 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 and SUR results must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value. Recalibration is recommended if these criteria are not met.
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 and SUR 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 and the SUR must be within 70-130% of the true value.

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

FIGURE 1. EXAMPLE CHROMATOGRAM OF A CALIBRATION STANDARD WITH METHOD 559 ANALYTES AT CONCENTRATION LEVELS OF 5-12 µg/L. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 3.