Designation: D7485 – 16

Standard Test Method for Determination of Nonylphenol, p-*tert*-Octylphenol, Nonylphenol Monoethoxylate and Nonylphenol Diethoxylate in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry[1]

This standard is issued under the fixed designation D7485; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of nonylphenol (NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), and octylphenol (OP), extracted from water utilizing solid phase extraction (SPE), separated using liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS). These compounds are qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 The method detection limit (MDL) and reporting limit (RL) for NP, NP1EO, NP2EO, and OP are listed in Table 1.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water

D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis

D5905 Practice for the Preparation of Substitute Wastewater

2.2 *Other Documents:*[3]

The Code of Federal Regulations 40 CFR Part 136, Appendix B

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *environmental water, n*—shall refer to water tested using this method. See Section 5.

3.2.2 *nonylphenol, NP, n*—is a mixture of branched p-nonylphenol isomers. Commercial NP is produced by the reaction of phenol with commercial nonene. Commercial nonene is not simply a linear C9H18 alpha olefin; it is a complex mixture of predominantly nine-carbon olefins, called propylene trimer, containing no linear isomers. This synthesis results in a mixture of various branched nonylphenol isomers rather than a discrete chemical structure. The branched nonyl group is positioned predominantly in the *para* position on the phenol ring.

3.2.3 *octylphenol, OP, n*—commercial octylphenol is produced by the reaction of phenol and diisobutylene to produce predominantly the 4-(1,1,3,3-tetramethylbutyl)phenol isomer.

3.2.4 *independent reference material, IRM, n*—a material of known purity and concentration obtained either from the National Institute of Standards and Technology (NIST) or other reputable supplier. The IRM shall be obtained from a different lot of material than is used for calibration.

3.3 *Acronyms:*

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

TABLE 1 MDL and Reporting Limits

Analyte	MDL[A] (ng/L)	Reporting Range[B] (ng/L)
NP	33	100–2000
NP1EO	9	100–2000
NP2EO	9	100–2000
OP	24	100–2000

[A] MDL Determined Following The Code of Federal Regulations, 40 CFR Part 136, Appendix B.
[B] Lowest Point of the Reporting Range is Calculated from the LV 1 Concentration Calibration Standard in Table 4.

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *IC, n*—Initial Calibration

3.3.3 *LC, n*—Liquid Chromatography

3.3.4 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.5 *MDL, n*—Method Detection Limit

3.3.6 *MeOH, n*—Methanol

3.3.7 *mM, n*—millimolar, 1×10^{-3} moles/L

3.3.8 *MRM, n*—Multiple Reaction Monitoring

3.3.9 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.10 *NA, adj*—Not Available

3.3.11 *ND, n*—non-detect

3.3.12 *NP1EO, n*—represents branched nonylphenol monoethoxylate.

3.3.13 *NP2EO, n*—represents branched nonylphenol diethoxylate.

3.3.14 *n-NP2EO, n*—represents normal straight chain nonylphenol diethoxylate. n-NP2EO is used in this method as a surrogate. It is not produced commercially and is not expected to be found in environmental waters.

3.3.15 *P&A, n*—Precision and Accuracy

3.3.16 *PPB, n*—parts per billion

3.3.17 *PPT, n*—parts per trillion

3.3.18 *QA, adj*—Quality Assurance

3.3.19 *QC, adj*—Quality Control

3.3.20 *RL, n*—Reporting Limit

3.3.21 *RSD, n*—Relative Standard Deviation

3.3.22 *RT, n*—Retention Time

3.3.23 *SDS, n*—Safety Data Sheets

3.3.24 *SRM, n*—Single Reaction Monitoring

3.3.25 *SS, n*—Surrogate Standard

3.3.26 *TC, n*—Target Compound

3.3.27 *μM, n*—micromolar, 1×10^{-6} moles/L

3.3.28 *VOA, n*—Volatile Organic Analysis

4. Summary of Test Method

4.1 This is a performance-based method and modifications are allowed to improve performance.

4.2 For NP, NP1EO, NP2EO, and OP analysis, solid phase extraction is used to extract water samples.

4.2.1 *Solid Phase Extraction*—250 milliliter volume of sample adjusted to pH 2 is extracted using a solid phase extraction cartridge. The acetonitrile/water extract is concentrated to a volume of 1.0 mL, and then analyzed by LC/MS/MS operated in the multiple reaction monitoring (MRM) mode.

4.3 The target compounds are identified by retention time and SRM transition and are quantitated using the SRM transition of the target compounds utilizing external calibration. The final report issued for each sample lists the concentration of NP, NP1EO, NP2EO, and OP.

5. Significance and Use

5.1 NP and OP have been shown to have toxic effects in aquatic organisms. The source of NP and OP is prominently from the use of common commercial surfactants. The most widely used surfactant is nonylphenol ethoxylate (NPEO) which has an average ethoxylate chain length of nine. The ethoxylate chain is readily biodegraded to form NP1EO, NP2EO, nonylphenol carboxylate (NPEC) and, under anaerobic conditions, NP. NP will also biodegrade, but may be released into environmental waters directly at trace levels. This method has been investigated and is applicable for environmental waters, including seawater.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus producing discrete artifacts or elevated baselines. All of these materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples.

6.2 All glassware is washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with acetone and methanol. Detergents containing alkylphenolic compounds must not be used.

6.3 All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences can vary considerably from sample source to sample source, depending on variations of the sample matrix.

7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography System*—A complete LC system is needed in order to analyze samples.[4] This should include a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature controlled column compartment. A system that is capable of performing at

[4] A Waters ACQUITY UltraPerformance LC (a trademark of the Waters Corporation, Milford, MA) system, or equivalent, was found suitable for use.

the flows, pressures, controlled temperatures, sample volumes and requirements of the standard may be used.

7.1.2 *Analytical Column*[5]—A LC analytical column with the ability to separate alkylphenols or equivalent.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis.[6] A system that is capable of performing at the requirements in this standard may be used.

7.2 *SPE Vacuum Manifold System*[7]—A solid phase extraction vacuum manifold or similar may be utilized.

7.3 Organic solvent evaporation device.

8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[8] Other reagent grades may be used provided it is first ascertained that they are of sufficiently high purity to permit their use without affecting the accuracy of the measurement.[9]

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification D1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Isopropanol (CAS # 67-63-0).

8.7 Acetone (CAS # 67-64-1).

8.8 Branched nonylphenol monoethoxylate (NP1EO) available as a high purity custom standard.

8.9 Branched nonylphenol diethoxylate (NP2EO) available as a high purity custom standard.

8.10 Nonylphenol, NP, >95 % para isomer (CAS # 84852-15-3).

8.11 Octylphenol, OP, 99 + % 4-(1,1,3,3-tetramethylbutyl)phenol (CAS # 140-66-9).

8.12 Concentrated HCl (CAS # 7647-01-0).

8.13 Ammonium Acetate (CAS # 631-61-8) (ACS Reagent Grade or Better).

8.14 n-Nonylphenol diethoxylate (n-NP2EO).

8.15 n-Nonylphenol (suggested alternate surrogate, if needed).

8.16 2-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol (Br-OP).

8.16.1 2-Bromo-4-(1,1,3,3-tetramethylbutyl)phenol (Br-OP) is used in this method as a surrogate. It is not produced commercially and is not expected to be found in environmental waters. It was reported that compounds in highly chlorinated bromide rich wastewaters could potentially interfere with the Br-OP surrogate. If this interference is encountered nnonylphenol is suggested as an alternative surrogate

8.17 *Solid Phase Extraction Cartridges*[10]—An SPE extraction cartridge that will separate alkypphenols from the matrix or equivalent.

NOTE 1—Alkylphenols have been found in SPE cartridges therefore it is advisable that the cartridges be lot certified alkylphenol free. Glass cartridges should have a much lower risk of alkylphenol contamination.

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves and lab coats when working with acids. Analysts should review the Safety Data Sheets (SDS) for all reagents used in this method.

10. Sample Collection, Preservation, and Storage

10.1 *Sampling*—Grab samples must be collected in 250 mL amber glass bottles. This must be done in order to allow for the rinsing of the bottle with acidified water and acidified 10 % methanol/water in order to get complete transfer of the sample into the SPE cartridge and extraction process. Alkylphenols tend to adsorb to glassware and rinsing will allow optimum recoveries. Conventional sampling practices should be followed. Refer to Guide D3856 and Practices D3694. Automatic sampling equipment should be as free as possible of Tygon tubing and other potential sources of contamination.

NOTE 2—Pre-cleaned bottles demonstrated to be free of interferences may be used.

10.2 *Preservation*—Adjust sample to pH 2 with concentrated HCl at time of collection. Store samples between 0ºC and 6ºC from the time of collection until extraction. Extract the sample within 14 days of collection and completely analyze within 14 days of extraction.

10.3 Sample extracts may be stored in sealed glass containers at <0ºC indefinitely.

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*

[5] A Waters ACQUITY UPLC (a trademark of the Waters Corporation, Milford, MA) HSS T3, 1.8 µm, 2.1 × 50 mm column, or equivalent, was found suitable for use.

[6] A Waters Quattro Premier (a trademark of the Waters Corporation, Milford, MA) mass spectrometer, or equivalent, was found suitable for use.

[7] A Supelco Visiprep (a trademark of Sigma-Aldrich Co., LLC, St. Louis, MO) was found suitable to use, any SPE extraction manifold may be used.

[8] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[9] Two sources of the alkylphenol standards are: Cambridge Isotope Laboratories, 50 Frontage Road, Andover, MA 01810-5413 and Accustandard, Inc., 125 Market Street, New Haven, CT 06513. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[10] Sep-Pak (a trademark of the Waters Corporation, Milford, MA) Vac (500 mg) tC18 Cartridges or equivalent were found suitable for use.

11.1.1 Injections of all calibration standards and samples are made at a 50 µL volume using a full loop injection. If a 50 µL volume loop is installed in your LC, a "full loop" mode is the preferred technique when performing fast, qualitative analyses. This mode should be used whenever accuracy and precision are the primary concerns. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in Table 2.

11.2 *LC Auto Sampler Conditions:*

11.2.1 *Wash Solvents*—Weak wash is 1.2 mL of 95 % water/5 % acetonitrile, Strong wash is 1 mL of 30 % acetonitrile, 30 % methanol, 30 % isopropyl alcohol, 10 % water. The strong wash solvent is needed to eliminate carry-over between injections of alkylphenol samples. The weak wash is used to remove the strong wash solvent. Specific instrument manufacturer specifications should be followed in order to eliminate sample carry-over in the analysis of alkylphenols.

11.2.2 *Temperatures*—Column, 30°C; Sample compartment, 15°C.

11.2.3 *Seal Wash*—5 minutes.

11.3 *Mass Spectrometer Parameters:*

11.3.1 Your instrument may require different settings.

11.3.2 Variable parameters depending on analyte are shown in Table 3.

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent 95 % CH_3CN/5 % Water 2 mmolar NH_4OAc	Percent 95 % Water/5 % CH_3CN 2 mmolar NH_4OAc
0	300	0	100
1	300	0	100
3	300	50	50
4	300	60	40
6	300	70	30
7	300	70	30
9	300	100	0
13	300	100	0
14	300	0	100
16	300	0	100

The instrument is set in the Electrospray source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.1 Volts
Source Temperature: 120°C
Desolvation Temperature: 300°C
Desolvation Gas Flow: 900 L/hr
Cone Gas Flow: 300 L/hr
Low Mass Resolution 1: 14
High Mass Resolution 1: 14
Ion Energy 1: 0.5
Entrance Energy: –1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 2
Low Mass Resolution 2: 14
High Mass resolution 2: 14
Ion Energy 2: 0.5
Multiplier: 650
Collision Cell Pirani Gauge: 7×10^{-3} Torr
Analyser Penning Gauge : 3×10^{-5} Torr
Inter-Channel Delay : 0.02 seconds
Inter-Scan Delay: 0.1 seconds
Repeats: 1
Span: 0 Daltons
Dwell: 0.1 Seconds

11.3.3 In order to acquire the maximum number of data points per MRM channel, the above scan, delay and dwell times may be changed and optimized according to your instrument. Fig. 1 displays a SRM chromatogram of each analyte and the number of scans per peak which data was generated. Each peak requires at least 10 scans per peak for adequate quantitation. This standard contains only 4 target compounds and 2 surrogates which can be broken up into MRM experiment windows in order to optimize the number of scans and sensitivity of your instrument. For details regarding retention times and SRM transitions cone and collision energies refer to Table 3. If the instrument can only acquire in one mode effectively, two analyses will need to be performed, one in electrospray ionization (ESI) negative and one in ESI positive mode in order to optimize speed and sensitivity.

12. Calibration and Standardization

12.1 In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following procedures must be followed when performing the test method.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze eight calibration standards containing the eight concentration levels of NP, NP1EO, NP2EO, OP,

TABLE 3 Retention Times, MRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Analyte	ESI Mode	Retention Time (min)	Cone Voltage (Volts)	Collision Energy (eV)	SRM Mass Transition (Parent > Product)
Octylphenol	neg	6.77	40	25	205.2 > 133
NP2EO	pos	7.71	20	12	326.3 > 183.2
NP1EO	pos	7.78	15	10	282.3 > 127.1
Nonylphenol	neg	7.82	40	30	219.2 > 133
Br-OP (surrogate)	neg	7.73	35	25	283.1 > 78.8
n-NP2EO (surrogate)	pos	8.62	20	17	326.3 > 88.9

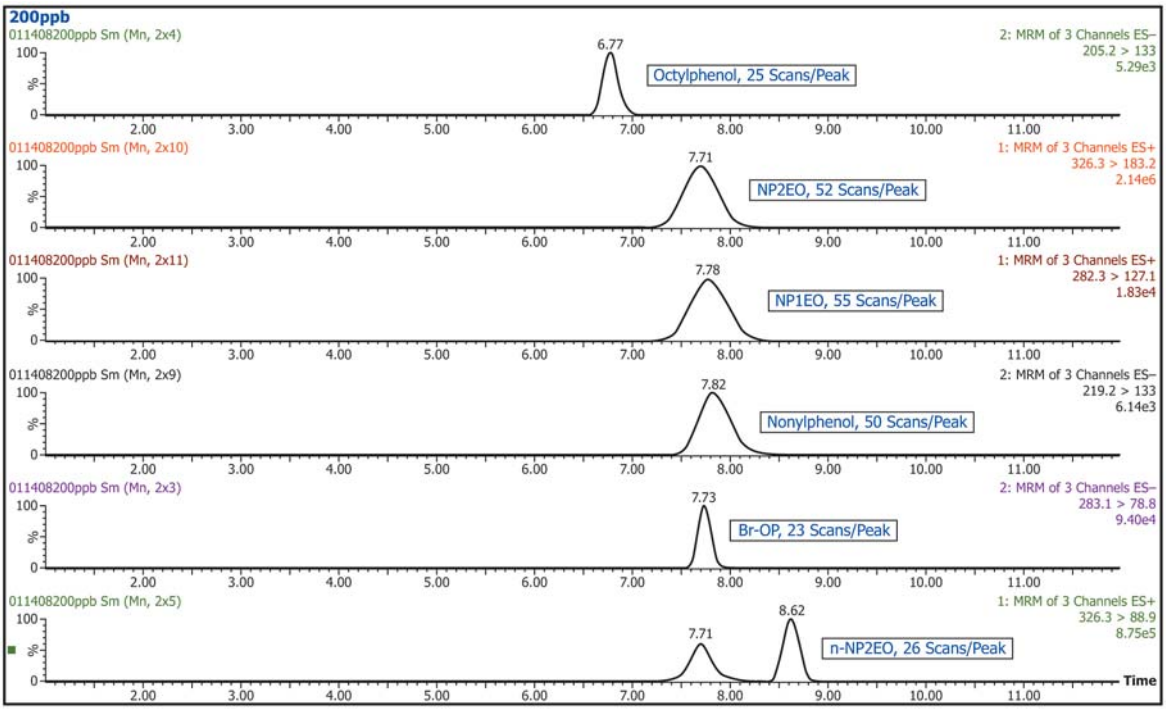

FIG. 1 Example SRM Chromatograms and Number of Scans per Peak

n-NP2EO, and Br-OP prior to analysis as shown in Table 4. A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock standard solution A (Level 8) containing NP, NP1EO, NP2EO, OP, n-NP2EO and Br-OP is prepared at Level 8 concentration and aliquots of that solution are diluted to prepare Levels 1 through 7. The following steps will produce standards with the concentrations values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with the pure materials, and correctly carrying the weights through the dilution calculations.

12.2.1 Prepare stock standard Solution A (Level 8) by adding to a 100 mL volumetric flask individual acetonitrile/methanol solutions of the following: 20 µL of NP, OP, NP1EO, NP2EO, Br-OP and n-NP2EO each at 2500 ppm, dilute to 100 mL with 75 % water/25 % acetonitrile. The 2500 ppm individual stock solutions are made in predominantly acetonitrile with methanol added to ensure solubility. Nonylphenol 2500 ppm stock may require up to a 50 % concentration of methanol, the OP, NP1EO, NP2EO, Br-OP and n-NP2EO are predominantly in acetonitrile. The preparation of the Level 8 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the stock concentrations prepared, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Solution A are then diluted with 75 % water/25 % acetonitrile to prepare the desired calibration levels in 2 mL amber LC vials. The calibration vials must be used

TABLE 4 Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6	LV 7	LV 8
NP	25	50	75	125	200	250	350	500
NP1EO	25	50	75	125	200	250	350	500
NP2EO	25	50	75	125	200	250	350	500
Octylphenol	25	50	75	125	200	250	350	500
Br-OP	25	50	75	125	200	250	350	500
n-NP2EO	25	50	75	125	200	250	350	500

within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every six months if not previously discarded for QC criteria failure.

12.2.3 Inject each standard and obtain a chromatogram for each one. An external calibration is used monitoring the SRM transition of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates. The SRM transition of each analyte is used for quantitation and confirmation. This gives confirmation by isolating the parent ion, fragmenting it to the product ion fragment, and also relating it to the retention time in the calibration standard.

12.2.4 The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt or ppb units as long as the analyst is consistent.

12.2.5 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98 this point must be re-injected or a new calibration curve must be regenerated. If the low or high (or both) point is excluded, minimally a six point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.6 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards, other than the high or low, causes the curve to be <0.99 this point must be re-injected or a new calibration curve must be regenerated. If the low or high point is excluded, a seven point curve is acceptable using a quadratic fit. An initial 8 point curve over the calibration range is suggested in the event that the low or high point must be excluded to obtain a coefficient of determination >0.99. In this event, the reporting range must be modified to reflect this change. Each calibration point used to generate the curve must have calculated percent deviation less than 25 % from the generated curve.

12.2.7 The retention time window of the MRM transitions must be within 10 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., perform a precision and bias study to demonstrate laboratory capability.

12.3.1 Analyze at least four replicates of a sample solution containing NP, NP1EO, NP2EO, OP, Br-OP, and n-NP2EO at a concentration near the midpoint of the calibration curve. The matrix and chemistry of the solution should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of quality control (QC) acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5. If a concentration other than the recommended concentration is used, refer to Test Method D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

12.4 *Laboratory Control Sample (LCS):*

12.4.1 To ensure that the test method is in control, analyze a LCS prepared with NP, NP1EO, NP2EO, and OP at concentrations near the midpoint of the calibration curve. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing NP, NP1EO, NP2EO, and OP at 1.25 ppm. Spike 150 µL of this stock matrix solution into 250 mL of water to yield a concentration of 750 ppt of each analyte in the sample. The result obtained for the LCS shall fall within the limits in Table 5.

12.4.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.5 *Method Blank:*

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of NP, NP1EO, NP2EO,

TABLE 5 QC Acceptance Criteria

Analyte	Test Conc. (ng/L)	Initial Demonstration of Performance			Lab Control Sample		MS/MSD		
		Recovery (%)		Precision	Recovery (%)		Recovery (%)		Precision
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit	Lower Limit	Upper Limit	Maximum RPD (%)
NP1EO	750	48	100	20	42	100	52	100	37
NP2EO	750	49	100	18	43	100	53	102	37
Nonylphenol	750	40	100	34	29	100	48	106	46
Octylphenol	750	46	110	40	29	126	66	119	24
n-NP2EO	750	26	100	32	19	100	30	100	47
Br-OP	750	53	107	34	39	122	35	103	28

and OP found in the blank must be below the detection limit or significantly below the confidence limits of the known concentration of the analyte in the associated test sample. If the concentrations of NP, NP1EO, NP2EO, and OP are found above this level, analysis of samples is halted until the contamination is eliminated and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.6 *Matrix Spike (MS):*

12.6.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 20 or fewer samples by spiking the sample with a known concentration of NP, NP1EO, NP2EO, and OP and following the analytical method. Prepare a stock matrix spiking solution in methanol containing NP, NP1EO, NP2EO, and OP at 1.25 ppm. Spike 150 µL of this stock matrix spiking solution into 250 mL of water to yield a concentration of 750 ppt for each analyte in the sample.

12.6.2 If the spiked concentration plus the background concentration exceeds that of the Level 8 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.6.3 Calculate the percent recovery of the spike (P) using Eq 1:

$$P = 100\,\frac{|A(V_s+V) - BV_s|}{CV} \quad (1)$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = Percent Recovery.

12.6.4 The percent recovery of the spike shall fall within the limits in Table 5. If the percent recovery is not within these limits, a matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 5 were generated using a secondary sewage treatment plant (STP) effluent, sea water, river water and a modified Practice D5905 artificial wastewater by a single laboratory.

12.7 *Duplicate:*

12.7.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the concentration of the analyte is less than five times the detection limit for the analyte, a MSD should be tested.

12.7.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. Compare to the RPD limit in Table 5.

$$RPD = \frac{|MSR - MSDR|}{(MSR+MSDR)/2} \times 100 \quad (2)$$

where:

RPD = relative percent difference,
MSR = matrix spike recovery, and
$MSDR$ = matrix spike duplicate recovery.

NOTE 3—The vertical bars in Eq 2 indicate the absolute value of the difference, hence RPD is always expressed as a positive value.

12.7.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.8 *Surrogate Spiking Solution:*

12.8.1 A surrogate standard solution containing Br-OP and n-NP2EO is added to all samples. A stock surrogate spiking solution is prepared in methanol containing Br-OP and n-NP2EO at 3.75 ppm. Spiking 50 µL of this spiking solution into 250 mL of water results in a concentration of 750 ppt of each surrogate in the sample.

12.9 The mass spectrometer must be calibrated per manufacturer specifications before analysis.

13. Sample Collection and Solid Phase Extraction Procedure

13.1 The water sample is acidified in the field to pH 2 with concentrated hydrochloric acid and shipped chilled between 0°C and 6°C in 250 mL amber glass bottles.

13.2 If the samples are received by the laboratory at greater than 6°C or greater than pH 2 (or both), the data is qualified estimated and noted in a case narrative that accompanies the data.

13.3 Additional acid is added if necessary in the laboratory to bring sample to a pH of 2. The samples are then appropriately spiked as required in Section 12.

13.4 *Solid Phase Extraction Procedure:*

13.4.1 *Step 1*—The solid phase extraction cartridge is placed on the vacuum manifold system under negative pressure according to the manufacturer specifications. Once the extraction process begins, the cartridge is not allowed to dry until the drying step. Alkylphenols tend to adhere to surfaces of glassware, plastics and tubing. The use of reservoirs and automatic SPE systems involving tubing should be avoided unless proven not to affect the performance of the method.

13.4.2 *Step 2*—The cartridge is washed with 8 mL of acetonitrile followed by 8 mL of methanol and then with 8 mL of pH 2 hydrochloric acid acidified water at a flow rate of 5 mL/minute. These solvents can either be poured or transferred by glass disposable pipet directly to the SPE cartridge. It is best to add the solvents in small portions to the cartridge in order to minimize mixing with the previous wash solvent.

13.4.3 *Step 3*—Once the cartridge is conditioned, the acidified water sample is poured directly from the 250 mL bottle to the SPE cartridge in order to minimize loss of alkylphenols to other surfaces. The water sample is added to the cartridge at a rate of 10 mL/minute. High sediment content waters may reduce the flow rate.

13.4.4 *Step 4*—Once the sample bottle is emptied, it is washed with 10 mL of pH 2 HCl acidified water and the wash

is added to the cartridge, followed by a second wash of 10 mL pH 2 HCl acidified 10 % methanol/90 % water solution which is also added.

13.4.5 *Step 5*—The cartridge is then dried with vacuum for 2 minutes.

13.4.6 *Step 6*—After drying, 4 mL of acetonitrile is added to the SPE cartridge and it is soaked for 5 minutes. After soaking, the cartridge is eluted into a 10 mL Kuderna-Danish graduated concentrator.

13.4.7 *Step 7*—The cartridge is then eluted into the same 10 mL Kuderna-Danish graduated concentrator tube with an additional 6 mL of acetonitrile at a 4 mL/minute flow rate. Ensure that the volume of the Kuderna-Danish is not exceeded if more solvent is added. It is important to fully wash the sides of the SPE cartridge with the acetonitrile elution solvent to remove the alkylphenols that may have adhered to the sides. The sides can be washed down by taking 1 mL portions of acetonitrile using a glass disposable pipet and rinsing the sides of the SPE cartridge while eluting into the Kuderna-Danish.

13.4.8 *Step 8*—The acetonitrile extract is reduced to 0.25 mL under nitrogen blow-down at 50 °C while washing the sides of the Kuderna-Danish concentrator tube using a glass pipet with approximately 2 mL of acetonitrile.

13.4.9 *Step 9*—The 0.25 mL acetonitrile extract is then diluted to 1 mL with water. The 25 % acetonitrile/75 % water extract is then transferred to a LC vial for LC/MS/MS analysis. The extract contains acetonitrile to ensure solubility of the analytes.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the analytes and surrogates, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. External calibration curves are used to calculate the amount of each individual target compound and surrogate. Calculate the concentration in ppt for each analyte. NP, NP1EO, NP2EO, or OP can be reported if present at or above their method detection limit as long as their values are accompanied by appropriate qualification codes. No qualification codes are needed if the values are at or above their respective reporting limits. If the concentration of an analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the mid-point of the calibration range and re-analyzed.

15. Report

15.1 Determine the results in units of ng/L (ppt) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Single Laboratory Precision and Bias

16.1 Standard Methods under the jurisdiction of the ASTM committee D19 may be published for a maximum of five years to the completion of a full collaborative study validation. Such standards are deemed to have met all other D19 qualifying requirements but have not completed the required validation studies to fully characterize the performance of the Methods across multiple laboratories and matrices. Publication of standards that have not been fully validated is done to make current technology accessible to users of standards, and to solicit additional input from the user community.

16.2 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on reagent water to determine precision and bias. The samples were spiked with target compounds and surrogates to obtain a 750 ppt concentration of each as described in Section 12. Table 6 contains the recoveries and standard deviation (SD) for all surrogates and target compounds.

16.3 This test method was tested by US EPA Region 5 Chicago Regional Laboratory (CRL) on various environmental waters to determine QC acceptance criteria. The samples were spiked with target compounds and surrogates to obtain a 750 ppt concentration of each as described in Section 12. Table 7 contains the recoveries for all surrogates in the various environmental waters. Tables 8-11 contain the recoveries for all target compounds in the various environmental waters.

16.4 This test method was tested by CRL on Practice D5905 substitute wastewater. Substitute wastewater was prepared in accordance with Practice D5905. Triton X-100 was not used in the formulation because it contains alkylphenol ethoxylates. The resulting wastewater was diluted by 25 times to better represent a sewage treatment plant (STP) effluent. Table 8 contains the Youden Pair spiking levels and recoveries for all target compounds.

TABLE 6 Single-Laboratory Recovery Data in Reagent Water

Precision and Accuracy Samples	Measured ppt from 750 ppt Spikes					
	NP	OP	NP1EO	NP2EO	Br-OP	n-NP2EO
1	344	464	400	408	616	256
2	448	512	448	472	768	360
3	408	536	408	408	584	248
4	536	768	504	504	504	288
5	472	640	464	464	544	264
Average Recovery	442	584	445	451	603	283
Average Percent Recovery	59 %	78 %	59 %	60 %	80 %	38 %
Standard Deviation	72	121	43	42	101	45
% Relative SD	16 %	21 %	10 %	9 %	17 %	16 %

TABLE 7 Single-Laboratory Surrogate Recovery Data in Various Environmental Waters

Samples	Measured ppt from 750 ppt Surrogate Spikes							
	ASTM Substitute		STP Effluent		Sequim Bay, WA		Chicago River	
	Br-OP	n-NP2EO	Br-OP	n-NP2EO	Br-OP	n-NP2EO	Br-OP	n-NP2EO
1	357	298	446	381	461	502	419	420
2	379	485	514	440	397	614	416	394
3	460	481	575	486	520	534	419	421
4	449	523	507	447	523	610	425	457
5	315	245	641	486	576	514	413	314
6	436	278	611	438	515	496	447	372
7	398	231	646	403	841	586	434	308
8			709	490	738	585	473	321
Average Recovery	399	363	581	447	571	555	431	376
Average Recovery %	53 %	48 %	77 %	60 %	76 %	74 %	57 %	50 %
Standard Deviation	53	127	87	40	147	49	20	57
% Relative SD	13 %	35 %	15 %	9 %	26 %	9 %	5 %	15 %

TABLE 8 Single-Laboratory Recovery Data for Diluted Practice D5905 Substitute Wastewater

Sample	Youden Pair	Target Compound Spike (ppt)	NP Measured (ppt)	NP Percent Recovery	OP Measured (ppt)	OP Percent Recovery
Blank						
Sample 1	1	150	154	102	121	81
Sample 2		180	159	88	150	84
Sample 3	2	750	608	81	578	77
Sample 4		900	483	54	704	78
Sample 5	3	1500	1008	67	1258	84
Sample 6		1800	1094	61	1590	88
Sample	Youden Pair	Target Compound Spike (ppt)	NP1EO Measured (ppt)	NP1EO Percent Recovery	NP2EO Measured (ppt)	NP2EO Percent Recovery
Blank						
Sample 1	1	150	112	75	127	85
Sample 2		180	133	74	157	87
Sample 3	2	750	600	80	630	84
Sample 4		900	438	49	455	51
Sample 5	3	1500	882	59	958	64
Sample 6		1800	987	55	1025	57

TABLE 9 Single-Laboratory Recovery Data for Secondary STP Effluent

Sample	Youden Pair	Target Compound Spike (ppt)	NP[A] Measured (ppt)	NP Percent Recovery	OP Measured (ppt)	OP Percent Recovery
Unspiked Effluent 1			126			
Unspiked Effluent 2			179			
Sample 1	1	150	139	93	192	128
Sample 2		180	114	63	209	116
Sample 3	2	750	737	98	782	104
Sample 4		900	826	92	1046	116
Sample 5	3	1500	1231	82	1556	104
Sample 6		1800	1573	87	1880	104
Sample	Youden Pair	Target Compound Spike (ppt)	NP1EO Measured (ppt)	NP1EO Percent Recovery	NP2EO Measured (ppt)	NP2EO Percent Recovery
Unspiked Effluent 1						
Unspiked Effluent 2						
Sample 1	1	150	126	84	126	84
Sample 2		180	126	70	153	85
Sample 3	2	750	563	75	594	79
Sample 4		900	680	76	722	80
Sample 5	3	1500	1066	71	1113	74
Sample 6		1800	1343	75	1378	77

[A] Subtracted 153 ppt from NP sample results to account for average NP concentration in Unspiked Effluent.

16.5 This test method was tested by CRL on secondary STP effluent. Table 9 contains the Youden Pair spiking levels and recoveries for all target compounds.

16.6 This test method was tested by CRL on Sequim Bay, WA seawater. Table 10 contains the Youden Pair spiking levels and recoveries for all target compounds.

TABLE 10 Single-Laboratory Recovery Data for Sequim Bay, WA Seawater

Sample	Youden Pair	Target Compound Spike (ppt)	NP[A] Measured (ppt)	NP Percent Recovery	OP Measured (ppt)	OP Percent Recovery
Unspiked Seawater 1			30			
Unspiked Seawater 2			39			
Sample 1	1	150	108	72	146	93
Sample 2		180	135	75	173	92
Sample 3	2	750	547	73	663	88
Sample 4		900	595	66	678	75
Sample 5	3	1500	1566	104	1718	114
Sample 6		1800	1358	75	1508	83
Sample	Youden Pair	Target Compound Spike (ppt)	NP1EO Measured (ppt)	NP1EO Percent Recovery	NP2EO Measured (ppt)	NP2EO Percent Recovery
Unspiked Seawater 1						
Unspiked Seawater 2						
Sample 1	1	150	135	90	134	90
Sample 2		180	154	85	172	96
Sample 3	2	750	588	78	624	83
Sample 4		900	672	75	702	78
Sample 5	3	1500	1534	102	1650	110
Sample 6		1800	1276	71	1335	74

[A] Subtracted 35 ppt from NP sample results to account for average NP concentration in Unspiked Seawater.

TABLE 11 Single-Laboratory Recovery Data for Chicago River Water

Sample	Youden Pair	Target Compound Spike (ppt)	NP[A] Measured (ppt)	NP Percent Recovery	OP Measured (ppt)	OP Percent Recovery
Unspiked River Water 1			65			
Unspiked River Water 2			57			
Sample 1	1	150	87	58	133	89
Sample 2		180	156	87	174	96
Sample 3	2	750	456	61	682	91
Sample 4		900	591	66	742	82
Sample 5	3	1500	1051	70	1206	80
Sample 6		1800	1239	69	1493	83
Sample	Youden Pair	Target Compound Spike (ppt)	NP1EO Measured (ppt)	NP1EO Percent Recovery	NP2EO Measured (ppt)	NP2EO Percent Recovery
Unspiked River Water 1						
Unspiked River Water 2						
Sample 1	1	150	120	80	118	79
Sample 2		180	151	84	147	82
Sample 3	2	750	540	72	512	68
Sample 4		900	681	76	642	71
Sample 5	3	1500	1009	67	1006	67
Sample 6		1800	1200	67	1206	67

[A] Subtracted 61 ppt from NP sample results to account for average NP concentration in Unspiked River Water.

16.7 This test method was tested by CRL on Chicago River water. Table 11 contains the Youden Pair spiking levels and recoveries for all target compounds.

17. Quality Control

17.1 A crucial part of a test method is quality control. A laboratory should follow their in-house QA/QC procedures and should meet or exceed the criteria given in this test method. The quality-control criteria are given in the various test method sections. Section 10 contains the sampling and preservation requirements and Section 12 contains the majority of quality control requirements when following this test method. Section 12 includes requirements for calibration, precision and bias study to demonstrate laboratory capability, initial demonstration of performance, surrogate, method blank, reporting limit check, laboratory control, matrix spike and duplicate sample requirements. An IRM should be incorporated into the analysis periodically to verify that standard concentrations are comparable between sources. The IRM criteria should be based upon the laboratories QA/QC policies and the individual data quality objectives.

18. Keywords

18.1 diethoxylate; liquid chromatography; monoethoxylate; nonylphenol; octylphenol; solid phase extraction; tandem mass spectrometry; water