Designation: D 7485 – 09

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 D 7485; 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.*

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.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water
D 2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D 3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents
D 3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water
D 5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
D 5905 Practice for the Preparation of Substitute Wastewater

2.2 *Other Documents*

The Code of Federal Regulations 40 CFR Part 136, Appendix B[3]

3. Terminology

3.1 Nonylphenol (NP) 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 C_9H_{18} 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 OP represents octylphenol. Commercial octylphenol is produced by the reaction of phenol and diisobutylene to produce predominantly the 4-(1,1,3,3-tetramethylbutyl)phenol isomer.

3.3 NP1EO represents branched nonylphenol monoethoxylate.

3.4 NP2EO represents branched nonylphenol diethoxylate.

[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.

3.5 n-NP2EO 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.

3.6 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 n-nonylphenol is suggested as an alternative surrogate.

3.7 *Units*—parts per trillion (ng/L, ppt), parts per billion (µg/L, ppb), parts per million (mg/L, ppm)

3.8 Environmental water shall refer to water tested using this method. See Section 5.

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 scrupulously cleaned. 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 Liquid Chromatography system is needed in order to analyze samples. A Waters (registered trademark) ACQUITY Ultra Performance Liquid Chromatography (UPLC (trademark)) System was used to develop this test method, but a different system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes and requirements of the standard may be used.

7.1.2 *Analytical Column—Waters*—ACQUITY UPLC (trademark) HSS T3, 1.8 mm, 2.1 × 50 mm column or equivalent.

7.1.3 *Tandem Mass Spectrometer (MS/MS) System*—A MS/MS system capable of MRM analysis. A Waters Quattro Premier XE mass spectrometer was used to develop this test method, but another system that is capable of performing at the requirements in the standard may be used.

7.2 *SPE Vacuum Manifold System*—Supelco Visiprep 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.[4] 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.[5]

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification D 1193. 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).

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[4] *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.

[5] 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.

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).

8.17 *Solid Phase Extraction Cartridges*—Sep-Pak (registered trademark) Vac (500 mg) tC18 Cartridges 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 Material Safety Data Sheets (MSDS) 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 D 3856 and Practices D 3694. 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*:

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 Sample Manager 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.

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

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