



Standard Test Method for Determination of Nonylphenol, p-tert-Octylphenol, Nonylphenol Monoethoxylate and Nonylphenol Diethoxylate in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry¹

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](#)

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.

[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:

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, Luminex, 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*—Ultrapur 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 nonylphenol is suggested as an alternative surrogate

8.17 *Solid Phase Extraction Cartridges*¹⁰—An SPE extraction cartridge that will separate alkylphenols 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 0°C and 6°C 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 <0°C indefinitely.

11. Preparation of LC/MS/MS

11.1 LC Chromatograph Operating Conditions:

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

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.