~~Designation:D7065–06~~ Designation: D7065 – 11

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

This standard is issued under the fixed designation D7065; 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 determination of nonylphenol (NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), octylphenol (OP), and bisphenol A (BPA) that are partitioned into organic solvent, separated using gas chromatography and detected with mass selective detection. These compounds or isomer mixtures are qualitatively and quantitatively determined by this method. This method adheres to selected ion monitoring mass spectrometry but full scan mass spectrometry has also been shown to work well under these conditions. Either analysis may be used.

1.2 The method detection limit (MDL) reporting limit (RL) for NP, NP1EO, NP2EO, OP, and BPA 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 to determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

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

D3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and 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

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 NP represents nonylphenol which is mixed isomers of branched p-nonylphenol. Commercial nonylphenol (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 BPA represents bisphenol A. Commercial bisphenol A is produced by the condensation reaction of phenol and acetone to produce predominantly the 4,4′-isopropylidenediphenol isomer.

3.4 NP1EO represents branched nonylphenol monoethoxylate.

3.5 NP2EO represents branched nonylphenol diethoxylate.

3.6 n-NP and n-NP1EO represent normal straight chain nonylphenol and nonylphenol ethoxylate. n-NP and n-NP1EO are used in this method as surrogates. They are not produced commercially and are not expected to be found in environmental waters.

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

[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.

~~Current edition approved Dec. 15, 2006. Published January 2007. DOI: 10.1520/D7065-06.~~

Current edition approved June 15, 2011. Published July 2011. Originally published 2006. Last previous version approved 2006 as D7065–06.DOI: 10.1520/D7065-11.

[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.

TABLE 1 MDL and Reporting Limits

Analyte	MDL, (µg/L)	Reporting Range (µg/L)
NP	0.9	5.0-80.0
NP1EO	1.2	10.0-160.0
NP2EO	1.8	20.0-320.0
OP	0.2	1.0-16.0
BPA	0.3	1.0-16.0

4. Summary of Test Method

4.1 For NP, NP1EO, NP2EO, BPA, and OP analysis, continuous liquid-liquid extraction technique is used for water samples.

4.2 *Continuous Liquid-Liquid Extraction Technique*—A 1-L volume of sample adjusted to pH 2 is extracted with methylene chloride. The methylene chloride extract is dried using sodium sulfate if needed, concentrated to a volume of 0.5 mL, and then analyzed by GC/MS operated in the selected ion monitoring (SIM) or full scan mode.

4.3 The target compounds are identified by retention time and confirmed by comparing the sample mass spectrum to that of a known standard. The target compounds are quantitated using the quantitation ions of the target compounds utilizing the internal standards acenaphthene-d_{10}, and phenanthrene-d_{10} . The final report issued for each sample lists total concentration of NP, NP1EO, NP2EO, BPA, and OP, if detected, or MDLs, if not detected, in µg/L for water samples.

5. Significance and Use

5.1 Nonylphenol, octylphenol, and bisphenol A have been shown to have toxic effects in aquatic organisms. The source of nonylphenol and octylphenol is prominently from the use of common commercial surfactants. The most widely used surfactant is NPEO which has an average ethoxylate chain of 9 mol of ethoxylate. The ethoxylate chain is readily biodegraded to form NP1EO and NP2EO, nonylphenol carboxylate (NPEC) and, under anaerobic conditions, nonylphenol. Nonylphenol will also biodegrade, but may be released into environmental waters directly at trace levels. This method has been investigated for use with surface water and waste treatment effluent samples and is applicable to these matrices. It has not been investigated for use with salt water or solid sample matrices.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus that lead to discrete artifacts or elevated baseline in the selected ion current profiles. 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 min. All glassware is subsequently cleaned with acetone and methylene chloride. 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 will vary considerably from sample source to sample source, depending on variations of the sample matrix.

7. Apparatus

7.1 *GC/MS System*:

7.1.1 *Gas Chromatograph (GC) System*—An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, autosamplers, and gases. The injection port must be designed for split/splitless when using the capillary columns.

7.1.2 *Analytical Column*—An analytical column (DB-5MS 30-m × 0.25 mm i.d; film thickness—0.25 µm or equivalent; (5 %-phenyl)-methylpolysiloxane) suitable for the analysis of target alkylphenols.[3]

7.1.3 *Mass Spectrometer (MS) System*—An MS system capable of scanning 45 to 500 amu every 2 s or less, using 70 eV in the electron impact mode, and producing a mass spectrum which meets all the criteria when 50 ng of decafluorotriphenylphosphine (DFTPP) is injected through the GC inlet.

7.2 *CLLE Apparatus*.

7.3 *Organic Solvent Evaporation Device*.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all

[3] The sole source of supply of the columns known to the committee at this time is J&W Columns, Agilent Technologies, Inc., 2850 Centerville Rd., Wilmington, DE 19808. 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, which you may attend.

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.

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 *Carrier Gases*—Research grade nitrogen or helium of highest purity are used as carrier gases.

8.4 *Methylene Chloride*, chromatography grade.

8.5 *Methanol*, purge and trap grade.

8.6 *Branched Nonylphenol Monoethoxylate (NP1EO)*, available as a high purity custom standard.

8.7 *Branched Nonylphenol Diethoxylate (NP2EO)*, available as a high purity custom standard.

8.8 *Branched Nonylphenol Ethoxylate Blend (NP1EO–NP3EO)*, where the composition is determined by gas chromatography.[5]

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

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

8.11 *Bisphenol A (BPA)*, 99+ % 4,4′-ispropylidenediphenol (CAS #80-05-7).

8.12 *Concentrated H_2SO_4 (CAS #7664-93-9).*

8.13 *Internal Standard Mix*, containing acenaphthene-d_{10} and phenanthrene-d_{10}.

8.14 *n-nonylphenol (CAS #104-40-5).*

8.15 *n-NP monoethoxylate (n-NP1EO, CAS #104-35-8).*

8.16 *Acetone, Reagent Grade (CAS # 67-64-1).*

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves and lab coats when working with acids. Methylene chloride is used as an extraction solvent for this method. Analysts should review the MSDS for all reagents used in this method.

10. Sample Collection, Preservation, and Storage

10.1 *Sampling*:

10.1.1 Grab samples must be collected in glass sample containers. 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. Samples must be iced or kept at 0 to 4ºC. Samples must be prevented from freezing.

10.2 *Preservation*:

10.2.1 Adjust to pH 2 with H_2SO_4. Store samples between 0 and 4ºC from the time of collection until extraction. Extract the sample within 28 days of collection and completely analyze within 40 days of extraction.

10.2.2 Sample extracts may be stored in sealed glass containers at < 0°C indefinitely.

11. Preparation of GC/MS

11.1 *Chromatograph Operating Conditions* (approximate values, your instrument may require different settings):

Carrier Gas:	Helium
Carrier Flow:	Variable (1.3 to 0.4 mL/min), Constant Pressure (11.16 psi approximately)
Average Velocity	42 cm/s
Injection Port Parameters:	Splitless Injection
Injection Pressure:	10 to 12 psi
Injection port temperature:	290°C
Purge flow to split vent:	30mL/min at 0.75 min, Total Flow 34.2 mL/min
Column oven temperature:	50°C initially and hold 2 min 50 to 320°C at 10°C/min 320°C – hold 5 min
Injection Volume:	1 µL
Injection Liner:	Single taper liner
MS Transfer Line Temperature:	290°C

11.2 *Mass Spectrometer Parameters* :

[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] The sole source of supply of the blend known to the committee at this time is Schenectady International, Inc., 2750 Balltown Road, Schenectady, NY 12309. 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, which you may attend.

Mode:	Electron Ionization
Electron Multiplier:	Set electron multiplier to achieve optimal performance.
Tune:	Autotune using PFTBA masses 69, 219, and 502.
Dwell Time:	30 ms
SIM Ions:	Refer to Table 2.

NOTE 1—For details regarding retention times and quantitation ions refer to Table 2.

TABLE 2 Retention Time (RT) and Electron Impact Ions.[A]

Parameter	RT (Minutes)	Quantitation Ions	Confirmation Ions
Octylphenol	16.50	135	107, 91
Nonylphenol (NP) Isomer Group 1	17.58	121	107, 163, 220
NP Isomer Group 2	17.71	135	107, 121, 220
NP Isomer Group 3	17.80	149	135, 107, 220
NP Isomer Group 4	17.87	149	121, 220
NP Isomer Group 5	17.90	135	121, 107, 220
NP Isomer Group 6	17.94	149	121, 107, 220
NP Isomer Group 7	18.05	135	107, 163, 220
NP Isomer Group 8	18.16	149	121, 107, 220
NP Isomer Group 9	18.18	163	121, 107, 220
NP Isomer Group 10	18.28	135	107, 149, 220
NP Isomer Group 11	18.37	149	107, 121, 220
NP Isomer Group 12	18.39	135	149, 107, 220
NP1EO Isomer Group 1	20.45	165	207, 221, 264
NP1EO Isomer Group 2	20.58	179	135, 107, 264
NP1EO Isomer Group 3	20.66	179	193, 107, 264
NP1EO Isomer Group 4	20.71	179	193, 165, 264
NP1EO Isomer Group 5	20.78	179	193, 165, 264
NP1EO Isomer Group 6	20.91	179	207, 135, 264
NP1EO Isomer Group 7	20.97	193	179, 221, 264
NP1EO Isomer Group 8	21.05	207	165, 107, 264
NP1EO Isomer Group 9	21.12	179	135, 193, 264
NP1EO Isomer Group 10	21.22	193	179, 107, 264
Bisphenol A	22.82	213	228, 119
NP2EO Isomer Group 1	23.29	251	265, 209, 308
NP2EO Isomer Group 2	23.44	223	135, 308
NP2EO Isomer Group 3	23.49	237	223, 279, 308
NP2EO Isomer Group 4	23.53	223	135, 237, 308
NP2EO Isomer Group 5	23.59	223	135, 237, 308
NP2EO Isomer Group 6	23.63	237	209, 279, 308
NP2EO Isomer Group 7	23.78	237	223, 265, 308
NP2EO Isomer Group 8	23.84	251	237, 223, 308
NP2EO Isomer Group 9	23.92	223	135, 308
NP2EO Isomer Group 10	24.03	237	223, 149, 308
n-NP (Surrogate)	19.63	107	135, 220
n-NP1EO (Surrogate)	22.47	107	151, 264, 91
Acenaphthene-d_{10}(Internal Std.)	15.04	164	140
Phenanthrene-d_{10}(Internal Std.)	18.86	188	94, 160

[A] Refer to Figs. 1-5, which will make the quantitation method more apparent.

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* :

12.2.1 To calibrate the instrument, analyze 5 calibration standards containing 5 increasing concentration levels of NP, NP1EO, NP2EO, BPA, OP, n-NP, and n-NP1EO prior to analysis of sample. The values in Table 3 are shown as approximate concentrations. A calibration stock standard solution is prepared from standard materials or purchased as certified solutions. Stock standard solution A (Level 5) containing NP, NP1EO, NP2EO, BPA, OP, n-NP, and n-NP1EO is prepared at Level 5 concentration and aliquots of that solution are diluted to prepare Levels 1 through 4. There are many ways to accomplish this; the following steps in this section will produce standards with the concentrations values shown in Table 3. The analyst is responsible for recording initial component weights carefully when working with the pure materials, and carrying the weights through the dilution calculations correctly.

12.2.2 Prepare stock standard Solution A (Level 5) by adding to a 10 mL volumetric flask solutions of the following: 20 µL of NP (80 000 µg/mL), 20 µL of NP1EO (160 000 µg/mL), 20 µL of NP2EO (320 000 µg/mL), 8 µL of octylphenol (40 000 µg/mL), 8 µL of bisphenol A (40 000 µg/mL), 32 µL of n-NP (10 000 µg/mL), 32 µL of n-NP1EO (10 000 µg/mL) then dilute to 10 mL with methylene chloride. The preparation of the Level 5 standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory.

12.2.3 Aliquots of Solution A are then diluted with methylene chloride to prepare the desired calibration level. A 0.50-mL aliquot of each diluted standard is transferred to a 2-mL crimp-top GC autosampler vial and 6.25 µL of a 2000 ng/µL Internal Standard solution (12.9) is added. The vials are stored in the freezer at 0ºC or less and protected from light. Calibration standards are routinely replaced every six months if not previously discarded for QC criteria failure.

12.2.4 Inject each standard and obtain a chromatogram for each one. The average response factors are calculated as described in 12.2.6. These values are used to calculate the amount of each individual target compound (OP, BPA) and surrogates n-NP, n-NP1EO, as well as isomer groups for NP, NP1EO, and NP2EO. The isomer groups that are present, as confirmed by matching mass spectra, are added to yield the total amount of the compound. NP, NP1EO, and NP2EO are reported as total NP, NP1EO, and NP2EO, and not as their individual isomers. Calculate the concentration in ppb for each analyte. NP, NP1EO, or NP2EO 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.

12.2.5 *Relative Response Factor (RRF) Calculations*—Calculate the relative response factor (RRF) for each target and surrogate compound using Eq 1. The primary characteristic ions used for quantitation are listed in Table 2. Assign the target compounds and surrogate compound to an internal standard according to Table 4. If an interference prevents the use of a primary ion for a given internal standard, use a secondary ion listed in Table 2.

NOTE 2—Unless stated otherwise, the area response of the primary characteristic ion is the quantitation ion.

12.2.6 If the RRF value over the working range is a constant (<35 % RSD), the RRF can be assumed to be invariant and the average response factor (ARF) can be used for calculations. Alternatively, the results can be used to plot a calibration curve of the response ratios A_x/A_{is} versus concentration ratios C_x/C_{is}.

12.2.6.1 *Relative Response Factor (RRF)*

$$RRF = \frac{A_x C_{is}}{A_{is} C_x} \quad (1)$$

where:

A_x = area of the characteristic ion (EICP) for the compound the be measured (see Table 2),
A_{is} = area of the characteristic ion (EICP) for the specific internal standard (see Table 2 and Table 4),
C_{is} = concentration of the internal standard, and
C_x = concentration of the compound to be measured.

12.2.6.2 *Average Response Factor (ARF)*—Average of the relative response factors (RRF) is shown in Eq 2.

TABLE 3 Concentrations of Calibration Standards (ng/µL)

MSP/Surrogate	LV 1 (ng/µL)	LV 2 (ng/µL)	LV 3 (ng/µL)	LV 4 (ng/µL)	LV 5 (ng/µL)
NP	10	20	40	80	160
NP1EO	20	40	80	160	320
NP2EO	40	80	160	320	640
Bisphenol A	2	4	8	16	32
Octylphenol	2	4	8	16	32
n-NP	2	4	8	16	32
n-NP1EO	2	4	8	16	32
Internal Standards	25	25	25	25	25

TABLE 4 Compounds Quantitated Against Selected Internal Standards

Internal Standards	Acenaphthene-d_{10}	Phenanthrene-d_{10}
Compounds Quantitated	Octylphenol NP	n-NP n-NP1EO Bisphenol A NP1EO NP2EO

$$Average\ response\ factor = \frac{\sum_{i=1}^{n} RRF_n}{n} \quad (2)$$

where:

RRF_n = relative response factor for each calibration standard,
n = number of calibration standards (5 recommended).

12.2.6.3 *Percent Relative Standard Deviation (RSD)*—Eq 3 is used to calculate the RSD of the RRF values over the calibration range.

$$RSD = \frac{\sigma}{\bar{x}} \times 100 \quad (3)$$

where:

Standard Deviation = $\sqrt{\frac{\sum_{i=1}^{n} (xi - \bar{x})^2}{n - 1}}$

D7065-11_4

x_i = each individual value used to calculate the mean,
x = the mean of n value,
n = the total number of values, and
σ = standard deviation.

12.3 *Initial Demonstration of Laboratory Capability*:

12.3.1 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., a precision and bias study must be performed to demonstrate laboratory capability.

12.3.2 Analyze at least four replicates of a sample solution containing NP, NP1EO, NP2EO, BPA, OP, n-NP, and n-NP1EO 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 the collaborative study. Each replicate must be taken through the complete analytical test method including any sample preservation and pretreatment steps. The replicates may be interspersed with samples.

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

12.3.4 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 Practice 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 an LCS prepared to contain NP, NP1EO, NP2EO, BPA, and OP at concentrations near the midpoint of the calibration curve. The LCS is taken through all of the steps of the analytical method including sample preservation and pretreatment and analyzed with each batch of 20 samples or less. The result obtained for the LCS shall fall within the limits in Table 5.

TABLE 5 QC Criteria Acceptance Criteria

Analyte	Test conc (ug/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 (%)
BisphenolA	8	53	119	13	52	120	52	120	22
NP1EO	80	58	119	13	57	121	57	121	22
NP2EO	160	56	126	17	54	128	54	128	28
Nonylphenol	40	57	110	13	56	112	56	112	22
Octylphenol	8	56	106	14	55	108	55	108	24
n-NP	8	58	115	14	56	116	56	116	23
n-NP1EO	8	54	139	13	53	140	53	140	21