Designation: D7742 – 11

Standard Practice for Determination of Nonylphenol Polyethoxylates (NPnEO, 3 ≤ n ≤ 18) and Octylphenol Polyethoxylates (OPnEO, 2 ≤ n ≤ 12) in Water by Single Reaction Monitoring (SRM) Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D7742; 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 procedure covers the determination of nonylphenol polyethoxylates (NPnEO, 3 ≤ n ≤ 18) and octylphenol polyethoxylates (OPnEO, 2 ≤ n ≤ 12) in water by Single Reaction Monitoring (SRM) Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS) using direct injection liquid chromatography (LC) and detected with tandem mass spectrometry (MS/MS) detection. This is a screening Practice with qualified quantitative data to check for the presence of longer chain ethoxylates in a water sample.

1.1.1 All data are qualified because neat standards of each alkylphenol ethoxylate (APEO) are not available and the synthesis and characterization of these neat standards would be very expensive. The Igepal® Brand standards, which contain a mixture of various chain lengths of the alkylphenol ethoxylates (APEOs), were used. The mixture was characterized in-house assuming the instrument response at an optimum electrospray ionization cone and collision voltage for each APEO was the same. This assumption, which may not be accurate, is used to determine qualified amounts of each ethoxylate in the standards. The n-Nonylphenol diethoxylate (n- NP2EO) surrogate was available as a neat characterized standard, therefore, this concentration and recovery data was not estimated. APEOs are not regulated by the EPA, but nonylphenol, a breakdown product of NPnEOs, is regulated for fresh and saltwater dischargers. A request by a sewage treatment plant (STP) was made to make this Practice available through ASTM in order to screen for the influent or effluent from sources of APEOs coming into the STP. The interest lies in stopping the source of the longer chain APEOs from entering the STP in order to meet effluent guidelines. Based upon the above, this is a Practice rather than a Standard Method. A comparison between samples is possible using this Practice to determine which has a higher concentration of APEOs.

1.2 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this Practice.

1.3 The estimated screening range shown in Table 1 was calculated from the concentration of the Level 1 and 7 calibration standards shown in Table 4. These numbers are qualified, as explained in Section 1, and must be reported as such. Figs. 1-5 show the SRM chromatograms of each analyte at the Level 1 concentration with the signal to noise (S/N) ratio. This is a screening Practice and method detection limits are not given. The S/N ratio for each analyte at the Level 1 concentration must be at least 5:1 for adequate sensitivity. If the instrument can not meet the criteria, the screening limit must be raised to an acceptable level.

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]

D1193 Specification for Reagent Water

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

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

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

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

E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques

[1] This Practice 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 June 15, 2011. Published July 2011. DOI: 10.1520/D7742-11.

[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 Estimated Screening Range

Analyte	Estimated Screening Range (µg/L)
Nonylphenol triethoxylate (NP3EO)	0.73-11.6
Nonylphenol tetraethoxylate (NP4EO)	1.1-18.3
Nonylphenol pentaethoxylate (NP5EO)	1.4-22.1
Nonylphenol hexaethoxylate (NP6EO)	1.8-28.2
Nonylphenol heptaethoxylate (NP7EO)	1.9-30.1
Nonylphenol octaethoxylate (NP8EO)	1.8-29.2
Nonylphenol nonaethoxylate (NP9EO)	1.6-26.3
Nonylphenol decaethoxylate (NP10EO)	1.5-24.1
Nonylphenol undecaethoxylate (NP11EO)	1.3-21.3
Nonylphenol dodecaethoxylate (NP12EO)	1.0-15.7
Nonylphenol tridecaethoxylate (NP13EO)	0.64-10.3
Nonylphenol tetradecaethoxylate (NP14EO)	0.41-6.5
Nonylphenol pendecaethoxylate (NP15EO)	0.21-3.4
Nonylphenol hexadecaethoxylate (NP16EO)	0.11-1.7
Nonylphenol heptadecaethoxylate (NP17EO)	0.05-0.80
Nonylphenol octodecaethoxylate (NP18EO)	0.023-0.4
Total NPnEO	16-250
Octylphenol diethoxylate (OP2EO)	0.14-2.3
Octylphenol triethoxylate (OP3EO)	1.4-22.2
Octylphenol tetraethoxylate (OP4EO)	2.2-35.2
Octylphenol pentaethoxylate (OP5EO)	2.9-45.8
Octylphenol hexaethoxylate (OP6EO)	2.6-41.9
Octylphenol heptaethoxylate (OP7EO)	2.5-40.4
Octylphenol octaethoxylate (OP8EO)	1.8-28.8
Octylphenol nonaethoxylate (OP9EO)	1.1-17.6
Octylphenol decaethoxylate (OP10EO)	0.62-9.9
Octylphenol undecaethoxylate (OP11EO)	0.26-4.2
Octylphenol dodecaethoxylate (OP12EO)	0.11-1.8
Total OPnEO	16-250
n-Nonylphenol diethoxylate (n-NP2EO)	15.6-250 (Not Estimated)

2.2 *Other Standard:*[3]

EPA SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods

3. Terminology

3.1 *Definitions:*

3.1.1 *Screening Limit, SL, n*—the estimated concentration of the lowest-level calibration standard used for quantification accounting for the sample dilution.

3.1.2 *Alkylphenol Ethoxylates, n*—in this Practice, nonylphenol polyethoxylates (NPnEO, $3 \leq n \leq 18$) and octylphenol polyethoxylates (OPnEO, $2 \leq n \leq 12$) collectively.

3.2 *Abbreviations:*

3.2.1 ppt– parts per trillion, ng/L

3.2.2 mM– millimolar, 1 x 10^{-3} moles/L

3.2.3 ND– non-detect

4. Summary of Practice

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

4.2 For APEOs analysis, samples are shipped to the lab between 0°C and 6°C containing 1% formaldehyde and analyzed within 7 days of collection. In the lab, an aliquot of the sample is filtered, spiked with surrogate, and analyzed directly by LC/MS/MS.

4.2.1 Field samples from sewage systems propose a challenging analysis. Since this is a screening technique to determine if APEOs are present, a 10-25 mL aliquot of the sample is filtered through a PVDF syringe driven filter unit before spiking with surrogate. It was demonstrated that similar recoveries of the APEOs are achieved filtered and unfiltered using PVDF filters. Filtering using PTFE filters produced much lower recoveries. This Practice does not account for the APEOs adhered to particulates or the sample bottle.

4.3 Nonylphenol polyethoxylates (NPnEO, $3 \leq n \leq 18$), octylphenol polyethoxylates (OPnEO, $2 \leq n \leq 12$), and n-nonylphenol diethoxylate (n-NP2EO, surrogate) are identified by retention time and one SRM transition. The target analytes and surrogates are quantitated using the SRM transition by external calibration. The final report issued for each sample lists their qualified concentration and the surrogate recovery.

5. Significance and Use

5.1 This Practice has been developed in support of the US EPA Office of Water, Office of Science and Technology by the Chicago Regional Laboratory (CRL).

5.2 Nonylphenol (NP) and Octylphenol (OP) have been shown to have toxic effects in aquatic organisms. The prominent source of NP and OP is from common commercial surfactants which are longer chain APEOs. The most widely used surfactant is nonylphenol polyethoxylate (NPnEO) which has an average ethoxylate chain length of nine. The APEOs are readily biodegraded to form NP1EO, NP2EO, nonylphenol carboxylate (NPEC) and NP. NP will also biodegrade, but may be released into environmental waters directly at trace levels. This Practice screens for the longer chain APEOs which may enter the STP at elevated levels and may cause a STP to violate its permitted discharge concentration of nonylphenol.

6. Interferences

6.1 Practice interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus producing

[3] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

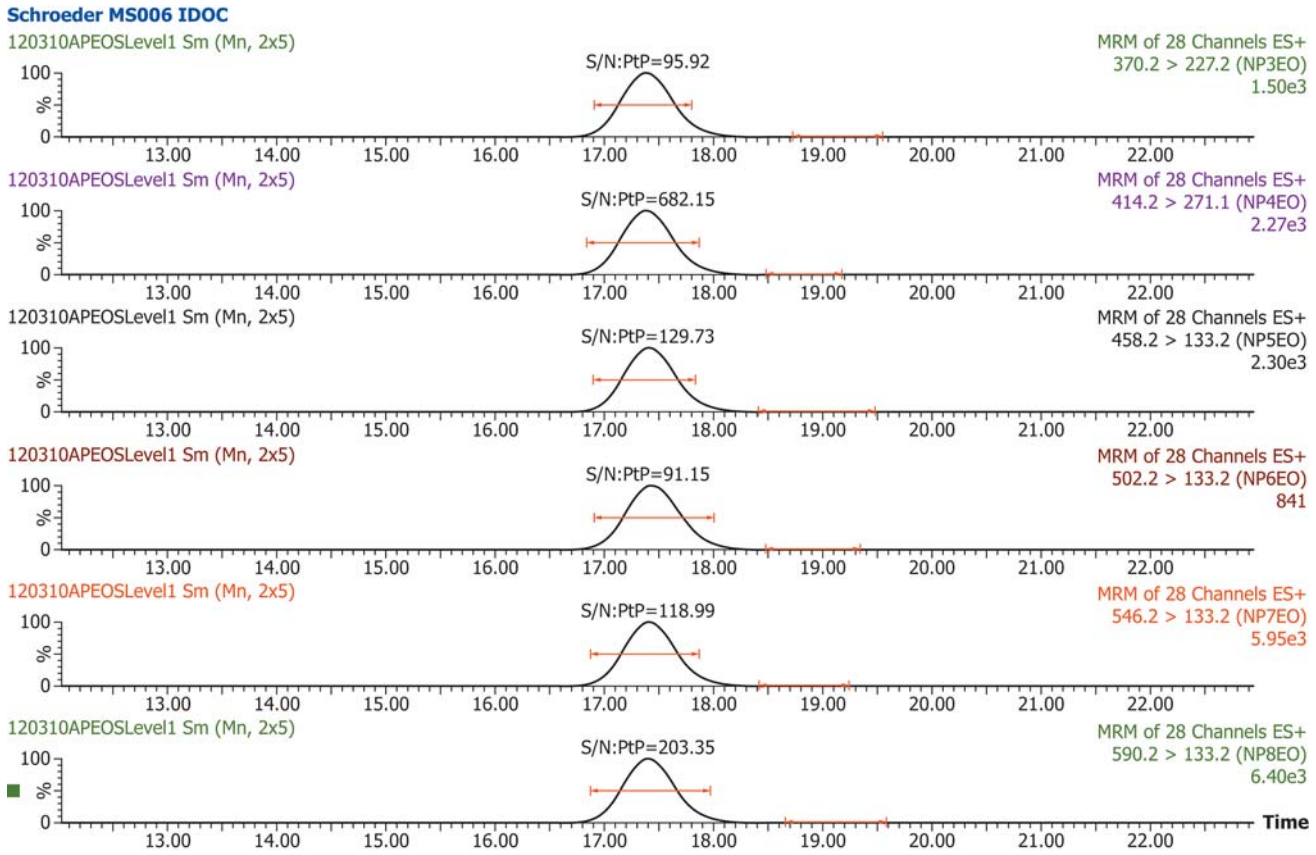

FIG. 1 SRM Chromatograms NP3EO-NP8EO

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 250ºC 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] Any 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*—Atlantis™ dC18, 2.1 x 150 mm, 3 µm particle size was used to develop this Practice. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 *Tandem Mass Spectrometer System*—A MS/MS system capable of MRM analysis.[5] Any system that is capable of performing at the requirements in this Practice may be used.

7.2 Filtration Device

7.2.1 *Hypodermic syringe*—A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.45 µm or similar may be used.

7.2.1.1 A 25 mL Lock Tip Glass Syringe size is recommended for this Practice.

[4] Waters ACQUITY H-Class Ultra Performance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[5] A Waters Quattro Micro™ tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

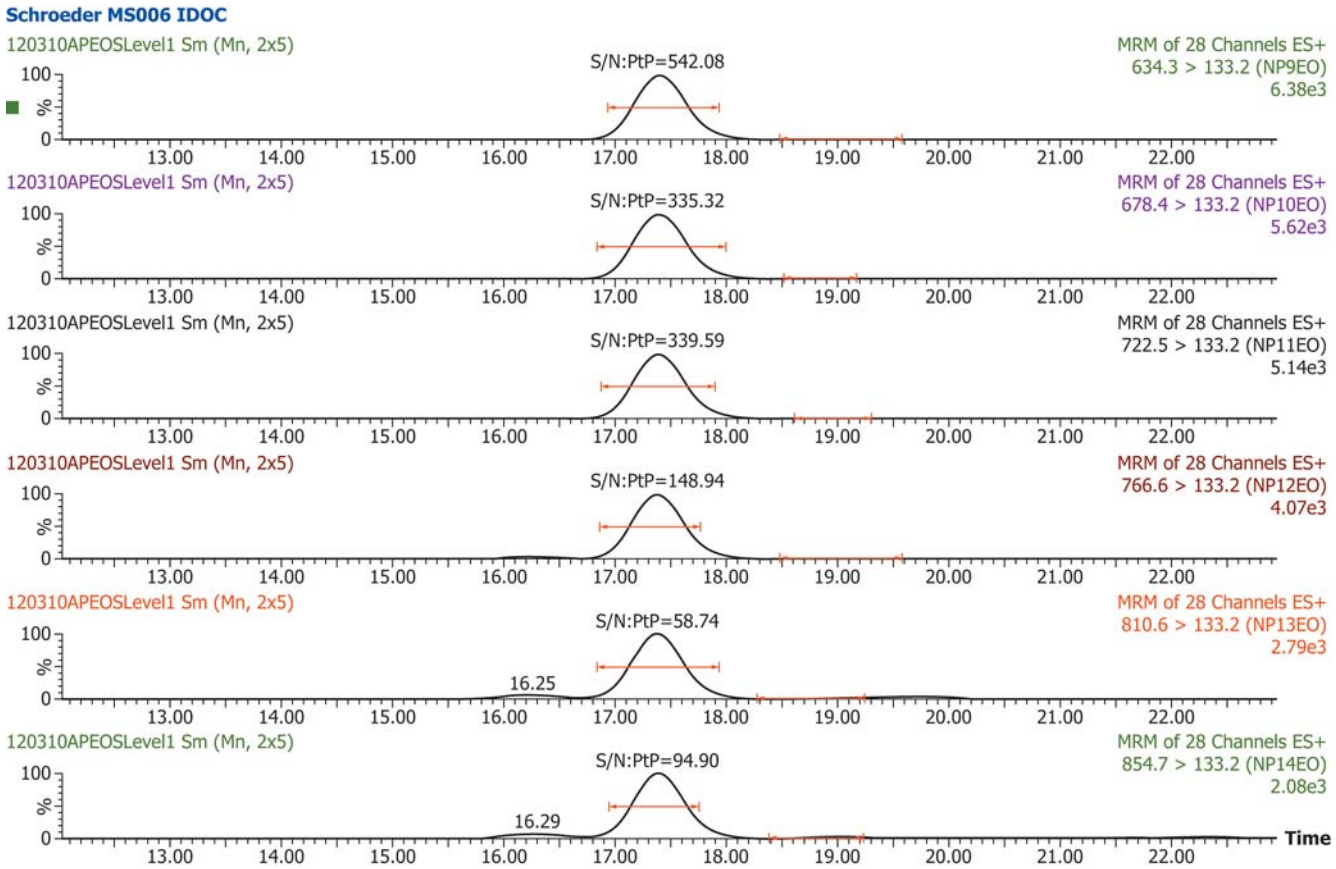

FIG. 2 SRM Chromatograms NP9EO-NP14EO

7.2.2 *Filter*—Millex® HV Syringe Driven Filter Unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV033NS) was used to develop this Practice, any similar filter may be used.

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.[6] 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 indicated, references to water shall be understood to mean reagent water conforming to Type I of Specification . 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 2-Propanol (CAS # 67-63-0)

8.7 Acetone (CAS # 67-64-1)

8.8 Nonylphenol pentaethoxylate mixture (several NPnEO isomer groups with an average of NP5EO, Igepal® CO-520)

8.9 Nonylphenol nonaethoxylate mixture (several NPnEO isomer groups with an average of NP9EO, Igepal® CO-630)

8.10 Octylphenol diethoxylate mixture (several OPnEO isomer groups with an average of OP2EO, Igepal® CA-210)

8.11 Octylphenol pentaethoxylate mixture (several OPnEO isomer groups with an average of OP5EO, Igepal® CA-520)

8.12 Formaldehyde (CAS # 50-00-0, 37 wt. % solution in water)

8.13 Ammonium Acetate (CAS # 631-61-8)

8.14 n-Nonylphenol diethoxylate (n-NP2EO)

9. Hazards

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

[6] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. 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 Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

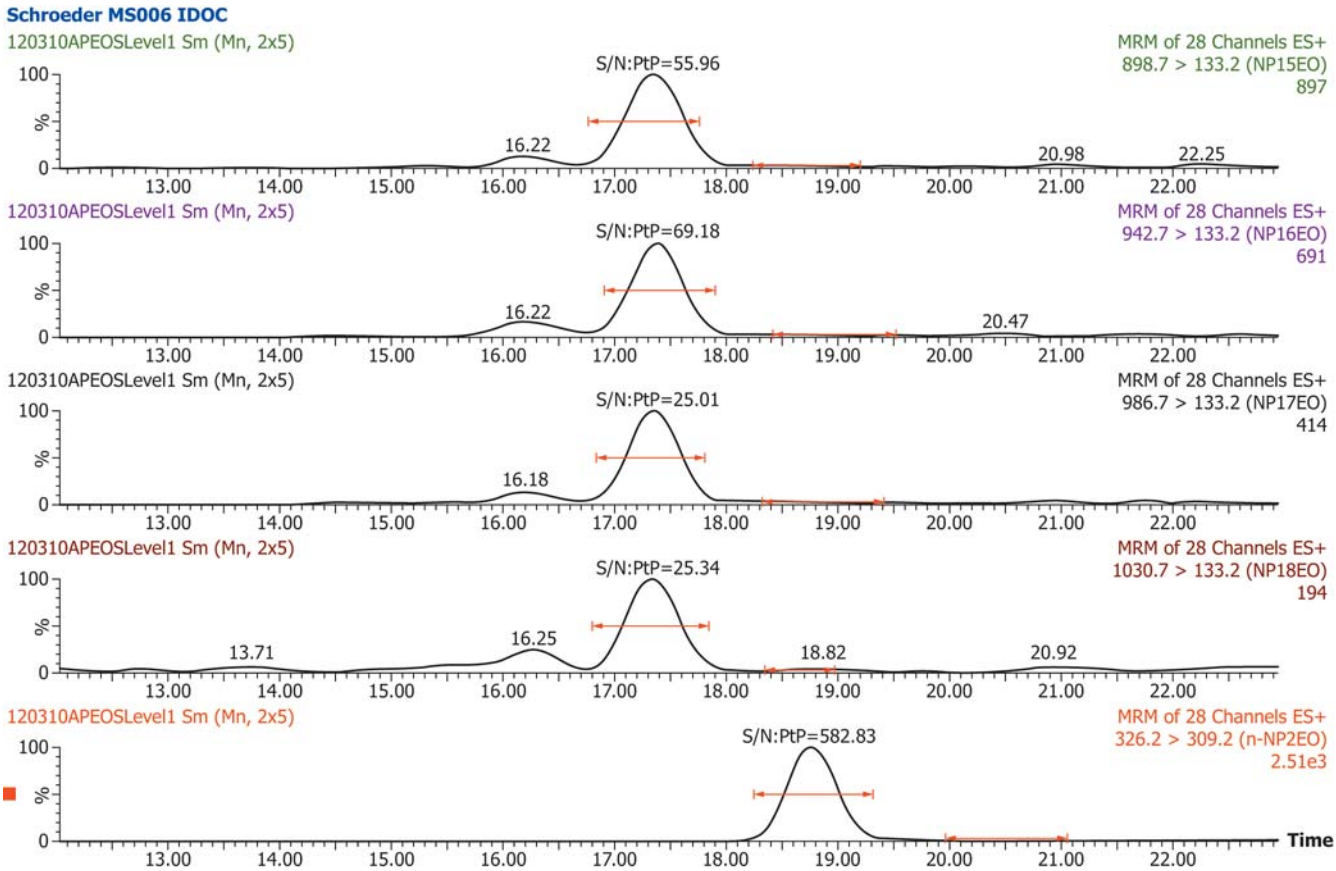

FIG. 3 SRM Chromatograms NP15EO-NP18EO and n-NP2EO

10. Sampling

10.1 Grab samples may be collected in 40 mL pre-cleaned amber glass vials with Teflon® lined caps demonstrated to be free of interferences, larger sample sizes may be used since a subsample aliquot is only required. All samples are preserved with 1% concentration of formaldehyde, shipped between 0°C and 6°C, and stored in the laboratory between 0°C and 6°C. 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 or cause adhesion of APEOs. Analyze the sample within 7 days of collection.

11. Preparation of LC/MS/MS

11.1 LC Chromatograph Operating Conditions[4]

11.1.1 Injection volumes of all calibration standards and samples are made at 100 µL volume. 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 Solvent*—Pre-inject and post-inject wash are both 8 seconds of 60% CH_3CN/40% 2-propanol.

11.2.2 *Purge Solvent*—50% Water/50% CH_3CN

11.2.3 *Temperatures*—Column, 35°C; Sample compartment, 15°C.

11.2.4 *Seal Wash*—Solvent: 50% CH_3CN /50% Water; Time: 5 minutes

11.3 Mass Spectrometer Parameters[5]:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters must be optimized according to the instrument. Each peak requires at least 10 scans per peak for adequate quantitation. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Previous studies by Houde[7] and Torrents[8] demonstrated that the $[M+NH_4]^+$ adducts for the APEOs are suitable for tandem mass spectrometry analysis and the fragmentation was very reproducible for these target analytes. It is best to use $[M+H]^+$ or $[M-H]^-$ as the precursor ion, but in this case, the ammonium adduct allowed for the best sensitivity and ease of analysis in one electrospray ionization mode. Mass spectrometer parameters used in the development of this Practice are listed below, others may vary and require optimization:

[7] Houde, F., DeBlois, C., and Berryman, D., "Liquid Chromatographic-Tandem Mass Spectrometric Determination of Nonlylphenol Polyethoxylates and Nonylphenol Carboxylic Acids in Surface Water," Journal of Chromatography A, Vol 961, 2002, pp. 245-256.

[8] Loyo-Rosales, J.E., Schmitz-Alfonso, I., Rice, C.P., Torrents, A., "Analysis of Octyl- and Nonylphenol and Their Ethoxylates in Water and Sediments by Liquid Chromatography/Tandem Mass Spectrometry," Analytical Chemistry, Vol. 75, No. 18, September 15, 2003, pp. 4811-4817.

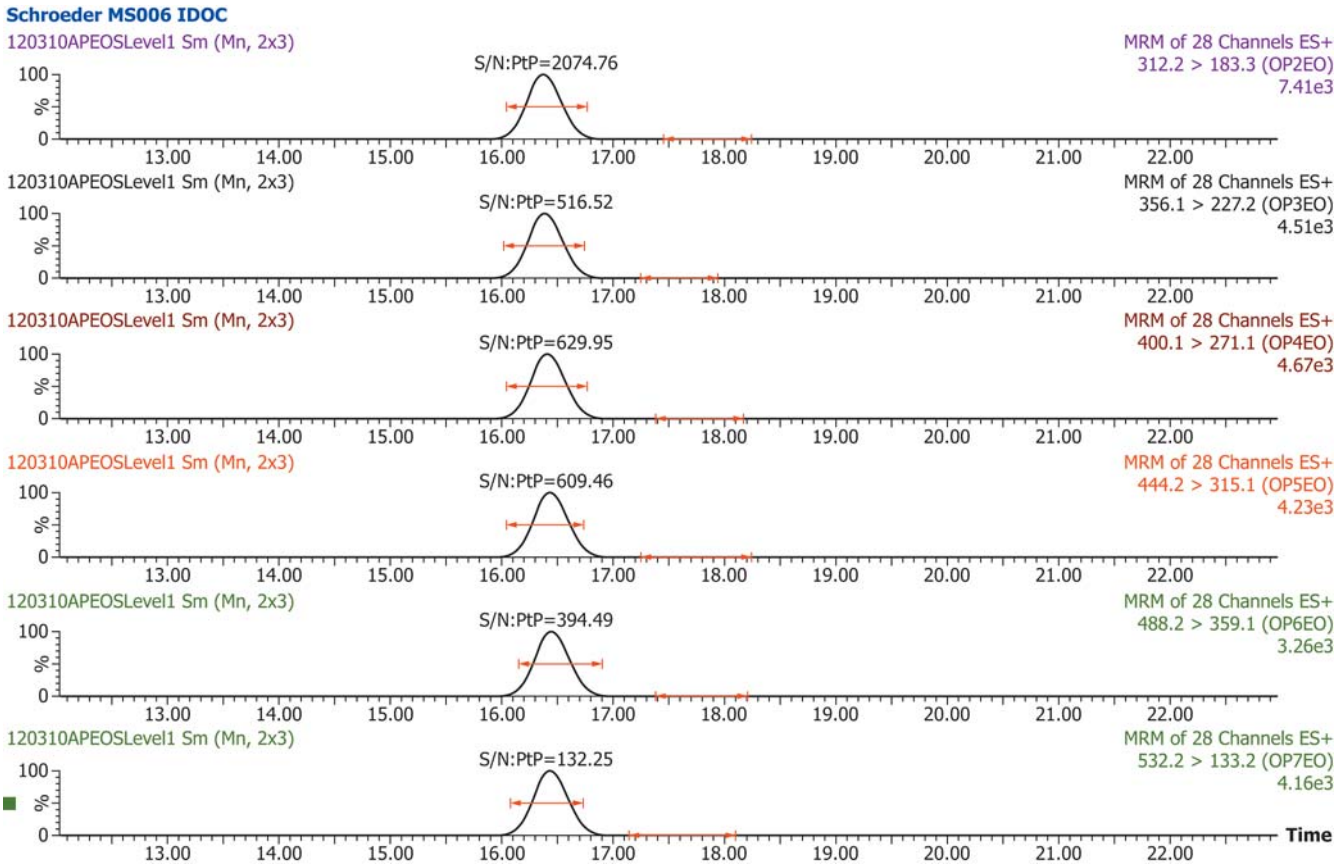

FIG. 4 SRM Chromatograms OP2EO-OP7EO

The instrument is set in the electrospray positive 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: 325°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.0
High Mass Resolution 1: 14.0
Ion Energy 1: 0.8
Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 2
Low Mass Resolution 2: 14.0
High Mass resolution 2: 14.0
Ion Energy 2: 1.5
Multiplier: 650
Gas Cell Pirani Gauge: 7.0 x 10^{-3} Torr
Inter-Channel Delay: 0.02 seconds
Inter-Scan Delay: 0.1 seconds
Dwell: 0.05 seconds

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this Practice, the following procedures must be followed when performing the Practice. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze seven calibration standards containing the seven concentration levels of the APEOs 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 containing the APEOs and n-NP2EO surrogate is prepared, and aliquots of that solution are diluted to prepare Levels 1 through 7. The following steps produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations.

12.2.1 Prepare stock standard solution A by adding to a 100 mL volumetric flask individual 50% CH_3CN/50% water with 1% formaldehyde solutions of the following: 100 µL of 250 ppm Igepal® CO-520, 100 µL of 250 ppm Igepal® CO-630, 100 µL of 250 ppm Igepal® CA-210, 100 µL of 250 ppm Igepal® CA-520, and 200 µL of 250 ppm n-NP2EO and dilute to 100 mL with 90% water/10% CH_3CN with 1% formaldehyde. Preparation of the stock standard solution A can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory.

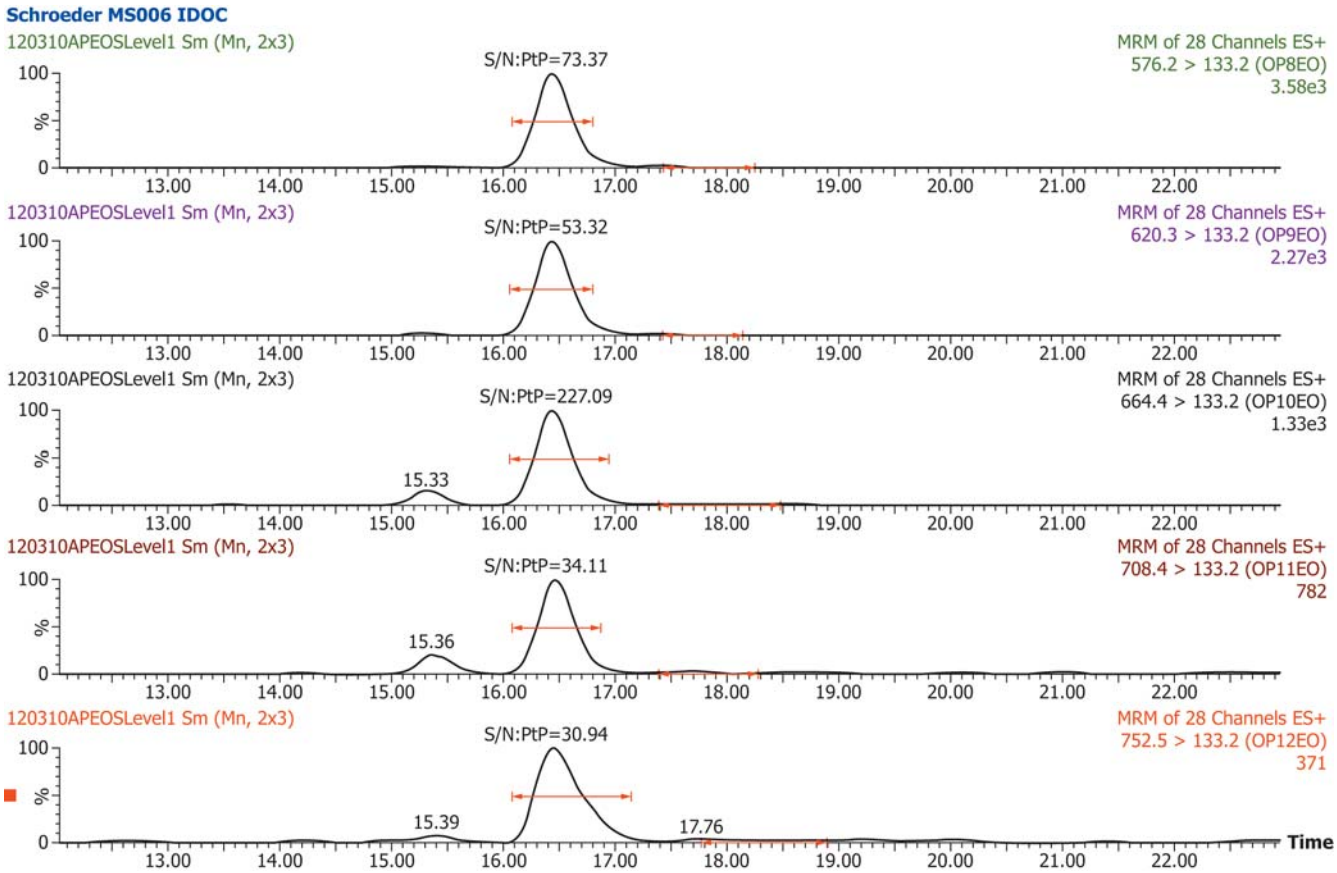

FIG. 5 SRM Chromatograms OP8EO-OP12EO

TABLE 2 Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	Percent 95% Water/ 5% CH_3CN, 5 mM NH_4OAc	Percent 95% CH_3CN/ 5% Water, 5 mM NH_4OAc
0	300	100	0
2	300	100	0
5	300	70	30
10	200	60	40
15	200	25	75
20	200	20	80
25	300	15	90
30	300	0	100
35	300	0	100
40	300	100	0

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

	SRM Mass Transition				
Analyte	Retention Time (Minutes)	Precursor (m/z)	Product (m/z)	Cone Voltage (V)	Collision Energy (eV)
NP3EO	17.4	370.2	227.2	21	14
NP4EO	17.4	414.2	271.1	19	17
NP5EO	17.4	458.2	133.2	24	22
NP6EO	17.4	502.2	133.2	29	33
NP7EO	17.4	546.2	133.2	31	25
NP8EO	17.4	590.2	133.2	32	26
NP9EO	17.4	634.3	133.2	34	28
NP10EO	17.4	678.4	133.2	36	28
NP11EO	17.4	722.5	133.2	38	30
NP12EO	17.4	766.6	133.2	39	30
NP13EO	17.4	810.6	133.2	42	31
NP14EO	17.4	854.7	133.2	43	32
NP15EO	17.4	898.7	133.2	45	32
NP16EO	17.4	942.7	133.2	47	35
NP17EO	17.4	986.7	133.2	47	40
NP18EO	17.4	1030.7	133.2	49	37
OP2EO	16.4	312.2	183.3	19	12
OP3EO	16.4	356.1	227.2	20	13
OP4EO	16.4	400.1	271.1	19	16
OP5EO	16.4	444.2	315.1	18	18
OP6EO	16.4	488.2	359.1	28	18
OP7EO	16.4	532.2	133.2	29	23
OP8EO	16.4	576.2	133.2	32	26
OP9EO	16.4	620.3	133.2	33	27
OP10EO	16.4	664.4	133.2	35	28
OP11EO	16.4	708.4	133.2	38	30
OP12EO	16.4	752.5	133.2	40	30
n-NP2EO	18.8	326.2	309.2	20	9

Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of solution A are then diluted with water containing 1% formaldehyde to prepare the desired calibration levels in 2 mL amber glass LC vials as shown in Table 5. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 14 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transition of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogate using the SRM transition. The calibration software manual

TABLE 4 Estimated Concentrations of Calibration Standards (PPB)

Analyte	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
NP3EO	0.73	1.46	2.91	4.66	5.82	8.78	11.64
NP4EO	1.14	2.29	4.58	7.33	9.16	13.74	18.32
NP5EO	1.38	2.76	5.53	8.84	11.06	16.59	22.11
NP6EO	1.76	3.52	7.04	11.26	14.08	21.12	28.16
NP7EO	1.88	3.77	7.53	12.06	15.07	22.61	30.14
NP8EO	1.82	3.65	7.29	11.67	14.59	21.89	29.17
NP9EO	1.64	3.29	6.57	10.51	13.14	19.71	26.28
NP10EO	1.50	3.01	6.02	9.63	12.04	18.06	24.07
NP11EO	1.33	2.67	5.33	8.53	10.67	16.01	21.33
NP12EO	0.98	1.96	3.91	6.26	7.83	11.75	15.65
NP13EO	0.64	1.29	2.58	4.12	5.16	7.74	10.31
NP14EO	0.41	0.82	1.63	2.61	3.26	4.89	6.52
NP15EO	0.21	0.43	0.85	1.36	1.70	2.55	3.41
NP16EO	0.11	0.21	0.43	0.68	0.86	1.29	1.71
NP17EO	0.050	0.10	0.20	0.32	0.40	0.60	0.80
NP18EO	0.023	0.045	0.090	0.14	0.18	0.27	0.36
OP2EO	0.14	0.28	0.56	0.90	1.13	1.70	2.26
OP3EO	1.39	2.77	5.55	8.88	11.10	16.65	22.20
OP4EO	2.20	4.40	8.80	14.08	17.61	26.42	35.21
OP5EO	2.86	5.72	11.45	18.32	22.90	34.35	45.80
OP6EO	2.62	5.24	10.48	16.77	20.96	31.44	41.92
OP7EO	2.52	5.05	10.09	16.15	20.19	30.29	40.38
OP8EO	1.80	3.59	7.19	11.50	14.38	21.57	28.76
OP9EO	1.10	2.20	4.40	7.04	8.79	13.19	17.59
OP10EO	0.62	1.23	2.47	3.94	4.93	7.40	9.86
OP11EO	0.26	0.53	1.05	1.68	2.10	3.15	4.21
OP12EO	0.11	0.23	0.45	0.73	0.91	1.37	1.82
n-NP2EO	15.6	31.3	62.5	100	125	187	250

TABLE 5 Calibration Level Preparation

Solution	LV1	LV2	LV3	LV4	LV5	LV6	LV7
A[A]	31.3 µL	62.5 µL	125 µL	200 µL	250 µL	375 µL	500 µL
B[B]	968.7 µL	937.5 µL	875 µL	800 µL	750 µL	625 µL	500 µL

[A]Solution A: Stock solution prepared in Section 12.2.1
[B]Solution B: Water with 1% by weight formaldehyde

should be consulted for proper use. The quantitation method is set as an external calibration using the peak areas in ppt or ppb units, but consistency is warranted. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 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 to give 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 and/or high point is excluded, minimally a five point curve is acceptable, but the screening range must be modified to reflect this change.

12.2.5 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 to give emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. Minimally a six point curve is acceptable using a quadratic fit. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% from the generated curve.

12.2.5.1 An initial eight point curve over the calibration range is an option in the event that the low and/or high point must be excluded to obtain a coefficient of determination >0.99. In this event, the screening range must be modified to reflect this change.

12.2.6 The retention time window of the SRM transitions must be within 5% 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.

12.2.7 A midpoint calibration check standard must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated. This end calibration check should be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard must have a percent deviation less than 30% from the calculated concentration for the target analytes and surrogate. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they do not fall within the performance criteria of the Practice. If the analyst inspects the vial containing the end calibration check standard and notices that the sample evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation greater than 30% from the calculated concentration for the target analytes and surrogate, the results must be further qualified detailing how the end quality control check failed or the sample set including quality control must be re-analyzed.

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

12.3.1 Analyze at least four replicates of a sample solution containing the APEOs and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 4 concentration of the 7 point calibration curve was used to set the QC acceptance criteria in this Practice. The matrix and chemistry should be similar to the solution used in this Practice. Each replicate must be taken through the complete analytical Practice 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 the QC acceptance criteria for the Initial Demonstration of Performance in Table 6. This data for each NPEO, OPEO, and n- NP2EO must fall in this range.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 6. 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.

TABLE 6 QC Acceptance Criteria

Analyte	Test Conc. (µg/L)	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
(Each NPnEO, $3 \leq n \leq 18$)	100[A]	30	130	30	30	130
Each OPnEO, $2 \leq n \leq 12$)	100[A]	30	130	30	30	130
n-NP2EO (Surrogate)	100	30	130	30	30	130

[A]Total NPnEO, $3 \leq n \leq 18$ and OPnEO, $2 \leq n \leq 12$. Individual concentrations are listed in Table 4 Level 4.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in are preliminary until a collaborative study is completed, acceptance criteria in your laboratory should be much tighter. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this Practice. References on how to generate QC acceptance criteria are ASTM Standards D2777, D5847, E2554 or Method 8000B in EPA publication EPA SW-846EPA SW-846.

12.4 *Surrogate Spiking Solution :*

12.4.1 A surrogate spiking solution of 75% water/25% CH_3CN with 1% formaldehyde containing n-NP2EO is added to all samples. A stock surrogate spiking solution is prepared at 4 ppm. Spiking 25 µL of this spiking solution into a 975 µL water sample results in a concentration of 100 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 6. If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the Practice. Surrogate spiking solutions are routinely replaced every 14 days if not previously discarded for quality control failure.

12.5 *Method Blank:*

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of the APEOs found in the blank must be below the screening limit. If the concentrations of the APEOs 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 Practice.

12.6 *Laboratory Control Sample (LCS):*

12.6.1 To ensure that the Practice is in control, analyze a LCS prepared with the APEOs at a concentration of Level 4. The LCS is prepared following the analytical Practice and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in 75% water/25% CH_3CN with 1% formaldehyde containing the total NPnEO, $3 \leq n \leq 18$ at 4 ppm and total OPnEO, $2 \leq n \leq 12$ at 4 ppm. Spike 25 µL of this stock solution into 950 µL of water plus the 25 µL surrogate spike to yield a concentration of 100 ppb for the total NPnEO, $3 \leq n \leq 18$ and 100 ppb for the total OPnEO, $2 \leq n \leq 12$ in the sample. The result obtained for the LCS must fall within the limits in Table 6. Matrix spiking solutions are routinely replaced every 14 days if not previously discarded for quality control failure.

12.6.2 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 Practice.

12.7 *Matrix Spike (MS)/Matrix Spike Duplicate(MSD):*

12.7.1 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 APEOs and following the analytical Practice. Prepare a stock matrix spiking solution as shown in 12.6.

12.7.2 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.

12.7.3 Calculate the percent recovery of the spike (P) using:

$$P = 100\frac{(A(V_S+V) - BV_S)}{CV} \quad (1)$$

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
P = percent recovery

12.7.4 The percent recovery for the matrix spikes may be variable depending on the matrix interferences. The percent recovery for matrix spikes should meet the preliminary limits in Table 6 for laboratory control samples. 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 Practice or 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 Practice.

12.7.5 The matrix variation between the different waters may tend to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate an individual in-house QC acceptance criteria. No matrix spike limits are given in this Practice since it is a screening Practice and due to the variations in matrix. Recovery data is given in the Precision and Bias Section 16 for real world samples in this Practice.

12.7.5.1 The laboratory should generate an in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria are ASTM Standards D2777, D5847, E2554 or Method 8000B in EPA publication EPA SW-846EPA SW-846.

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If

the sample contains the analyte at a level greater than 5 times the detection limit of the Practice, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. The RPD limit is 30% for this Practice.

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

RPD=relative percent difference
MSR=matrix spike recovery
MSDR=matrix spike duplicate recovery

12.8.3 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 Practice.

13. Procedure

13.1 This Practice is based upon a 975 µL sample size per analysis. The samples must be analyzed within 7 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 7 days of collection, the data is qualified estimated and noted in the case narrative that accompanies the data.

13.1.1 In the laboratory, a 25 mL aliquot from the sample container is filtered through the syringe driven filter unit in 7.2 into a clean glass 40 mL vial. A 975 µL portion of that filtered aliquot is measured into a 2 mL glass LC vial. Every sample is then spiked with the surrogate as described in Section 12. The laboratory control and matrix spike samples are then spiked with the target compounds as described in Section 12. In the case of matrix spike samples and laboratory control samples a 950 µL sample aliquot is measured into the 2 mL LC vial in order to maintain a 1 mL final volume. The samples are then shaken in order to mix the spike solutions throughout the water sample.

13.1.2 The syringe must be cleaned between each filtration. It is the responsibility of the analyst to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of acetone, then 3 volumes of methanol, and a final rinse with water.

13.2 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be method blank(s), laboratory control sample(s), sample(s), duplicate(s), matrix spike sample(s) followed by an end calibration check standard.

14. Calculation or Interpretation of Results

14.1 For analysis of the APEOs and surrogate, 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 estimated amounts of APEOs and surrogate. Calculate the concentration in ng/L (ppt) for each analyte. The individual APEOs may be reported if present at or above the screening limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the midpoint of the calibration range and re-analyzed.

14.2 The analysis of APEOs includes a sample dilution step with the addition of surrogate, laboratory control and/or matrix spikes. This dilution factor must be accounted for in the reported concentration. For example, the concentrations in Table 4 are used to set up the quantitation method. The curves generated would be based upon an undiluted sample. The diluted 1 mL sample (V_f) results in an uncorrected concentration of 100 ppb (C_u) from the generated calibration curve. The corrected concentration is 103 ppb (C_f) in the initial 975 µL sample (V_i). (See Eq 3)

$$\frac{V_f}{V_I}(C_u) = C_f \quad (3)$$

Where:

V_f = Final Volume
V_I = Initial Volume
C_u = Uncorrected Concentration
C_f = Final Concentration (Corrected for Dilution)

15. Report

15.1 Determine the results in units of ng/L (ppt) or µg/L (ppb) 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 Practice must be appropriately qualified. All data generated from this Practice must be clearly identified as estimated, as all concentrations reported are qualified based on the unavailability of neat characterized standards.

16. Precision and Bias

16.1 This is an analytical Practice for qualitatively identifying and semi-quantitatively determining a concentration based upon an assumption that the individual APEOs have an equal response at their optimum electrospray cone and collision voltages, which may not be correct, to determine their individual concentrations in the target mixes. Therefore, without the availability of neat characterized individual standards, all data is qualified. However, Precision and Bias information should be made available demonstrating the performance and reproducibility of the Practice.

16.2 The determination of Precision and Bias was conducted through EPA and generated applicable data to determine the precision and bias as described in D2777.

16.3 This Practice was tested by CRL on reagent water. The samples were spiked with nonylphenol polyethoxylates (NPnEO, $3 \leq n \leq 18$) and octylphenol polyethoxylates (OPnEO, $2 \leq n \leq 12$) to obtain a 100 ppb concentration of total NPnEO, total OPnEO and surrogate as described in Section 12. Tables 7-11 contain the recoveries and standard deviation (SD) for the surrogate and target compounds.

16.4 This Practice was used by CRL on influent and effluent from sewage treatment plants. The samples were prepared as described in this Practice. Figs. 6-8 show the recoveries for the surrogate, total nonylphenol polyethoxylates (NPnEO, $3 \leq n \leq$

TABLE 7 Recovery Data for NP3EO-NP8EO

	Theoretical Spike Concentration (ppb)					
Precision and Accuracy Samples	4.7 NP3EO	7.3 NP4EO	8.8 NP5EO	11.3 NP6EO	12.1 NP7EO	11.7 NP8EO
1	4.8	7.7	8.9	11.0	12.2	11.9
2	5.1	7.9	9.2	11.5	12.4	12.1
3	4.9	7.9	9.5	11.0	12.6	12.1
4	5.1	7.9	9.4	11.5	12.7	12.2
5	5.2	8.0	9.4	11.7	12.6	12.3
Average Recovery:	5.0	7.9	9.3	11.3	12.5	12.1
Average % Recovery:	108	107	105	100	104	104
Standard Deviation:	0.16	0.11	0.23	0.34	0.21	0.17
% Relative SD	3.2	1.4	2.4	3.0	1.7	1.4

TABLE 8 Recovery Data for NP9EO-NP14EO

	Theoretical Spike Concentration (ppb)					
Precision and Accuracy Samples	10.5 NP9EO	9.6 NP10EO	8.5 NP11EO	6.3 NP12EO	4.1 NP13EO	2.6 NP14EO
1	10.6	9.4	8.4	6.3	4.0	2.6
2	10.8	9.7	8.7	6.3	4.2	2.7
3	11.0	10.1	9.0	6.3	4.1	2.6
4	10.9	10.2	8.8	6.3	4.2	2.7
5	10.9	10.3	8.7	6.4	4.1	2.6
Average Recovery:	10.8	9.9	8.7	6.3	4.1	2.6
Average % Recovery:	103	103	102	101	100	100
Standard Deviation:	0.16	0.35	0.23	0.043	0.10	0.048
% Relative SD	1.5	3.6	2.6	0.69	2.5	1.9

TABLE 9 Recovery Data for NP15EO-NP18EO and n-NP2EO

	Theoretical Spike Concentration (ppb)				
Precision and Accuracy Samples	1.4 NP15EO	0.68 NP16EO	0.32 NP17EO	0.14 NP18EO	100 n-NP2EO
1	1.3	0.65	0.28	0.12	106
2	1.4	0.66	0.30	0.14	99
3	1.4	0.65	0.30	0.15	104
4	1.3	0.65	0.30	0.14	95
5	1.4	0.68	0.31	0.14	105
Average Recovery:	1.4	0.66	0.30	0.14	102
Average % Recovery:	100	97	93	99	102
Standard Deviation:	0.058	0.013	0.011	0.011	4.51
% Relative SD	4.2	2.0	3.7	7.9	4.4

18) and total octylphenol polyethoxylates (OPnEO, $2 \leq n \leq 12$) from those sample analyses.

TABLE 10 Recovery Data for OP2EO-OP7EO

	Theoretical Spike Concentration (ppb)					
Precision and Accuracy Samples	0.90 OP2EO	8.9 OP3EO	14.1 OP4EO	18.3 OP5EO	16.8 OP6EO	16.2 OP7EO
1	0.91	9.2	14.0	18.2	17.3	16.4
2	1.0	10.0	15.1	19.3	17.7	17.1
3	1.0	9.8	15.2	19.5	18.1	17.1
4	1.0	10.0	15.1	19.4	18.3	17.6
5	1.1	9.8	14.9	19.6	17.9	17.5
Average Recovery:	1.0	9.8	14.9	19.2	17.8	17.2
Average % Recovery:	112	110	106	105	106	106
Standard Deviation:	0.061	0.35	0.52	0.56	0.38	0.45
% Relative SD	6.0	3.6	3.5	2.9	2.1	2.6

TABLE 11 Recovery Data for OP8EO-OP12EO

	Theoretical Spike Concentration (ppb)				
Precision and Accuracy Samples	11.5 OP8EO	7.0 OP9EO	3.9 OP10EO	1.7 OP11EO	0.73 OP12EO
1	11.4	7.0	4.0	1.7	0.7
2	11.9	6.9	4.1	1.7	0.6
3	12.0	7.1	4.3	1.7	0.7
4	11.9	7.0	4.2	1.8	0.7
5	12.0	7.2	4.4	1.7	0.7
Average Recovery:	11.8	7.1	4.2	1.7	0.66
Average % Recovery:	103	100	106	101	90
Standard Deviation:	0.26	0.10	0.17	0.060	0.025
% Relative SD	2.2	1.4	4.1	3.5	3.8

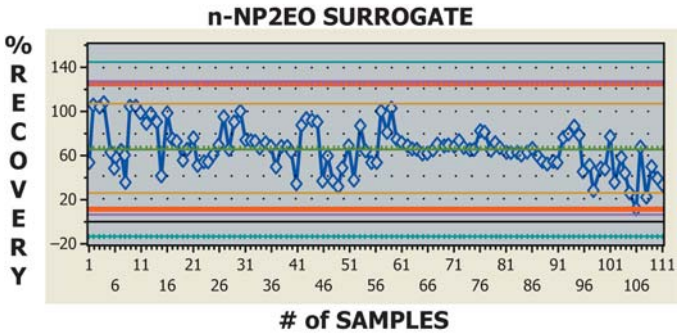

FIG. 6 N-NP2EO Surrogate Recovery

17. Keywords

17.1 Alkylphenols; Ethoxylates; Liquid Chromatography; Mass Spectrometry

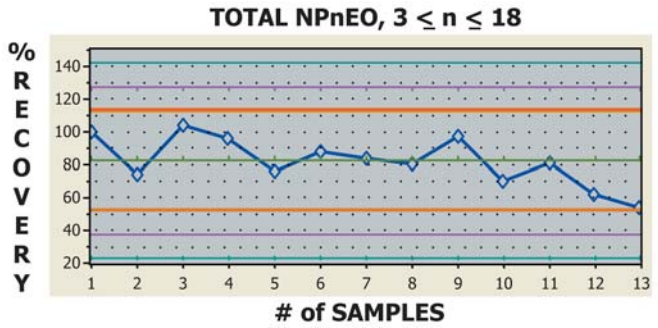

FIG. 7 Total NPnEO Recovery

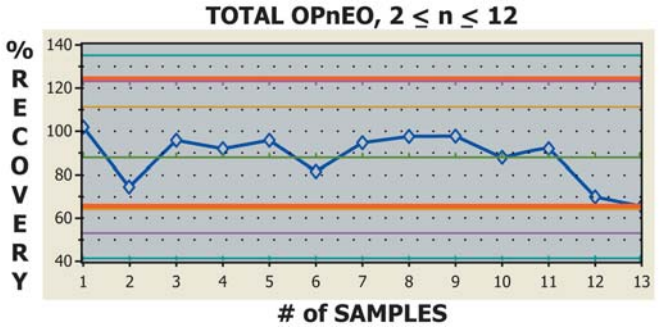

FIG. 8 Total OPnEO Recovery