Designation: D7742 – 17

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 practice 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[2] 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 test 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

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

[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.

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[2] Igepal is a trademark of Rhodia Operations, Aubervilliers, CA.

[3] 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)

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

2.2 *Other Standard:*[4]

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

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 *alkylphenol ethoxylates, n—in this practice*, nonylphenol polyethoxylates (NPnEO, $3 \leq n \leq 18$) and octylphenol polyethoxylates (OPnEO, $2 \leq n \leq 12$) collectively.

3.2.2 *screening limit, SL, n*—the estimated concentration of the lowest-level calibration standard used for quantification accounting for the sample dilution.

3.3 *Abbreviations:*

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

3.3.2 *ND*—non-detect

3.3.3 *ppt*—parts per trillion, ng/L

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 U.S. 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

[4] Available from National Technical Information Service (NTIS), 5301 Shawnee Rd., Alexandria, VA 22312, http://www.ntis.gov.

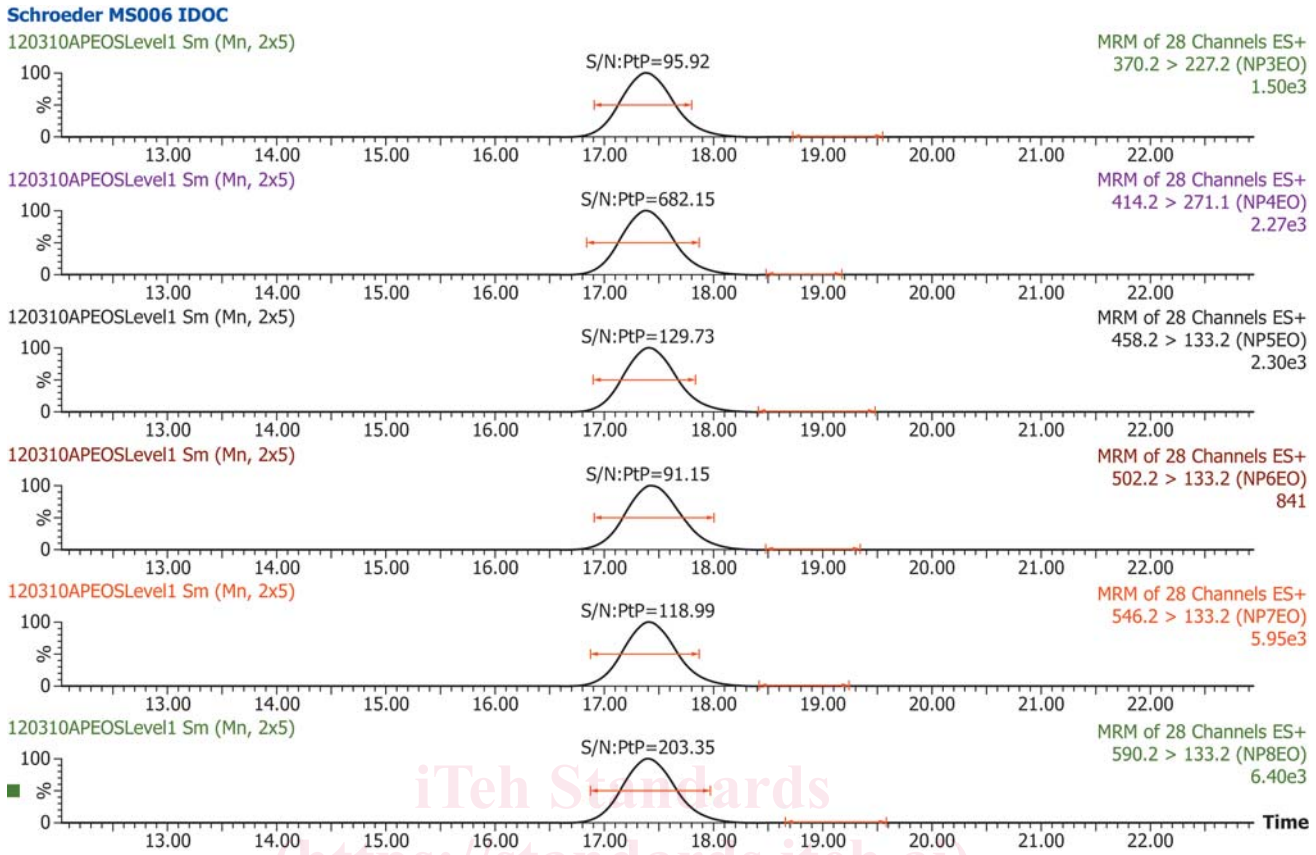

FIG. 1 SRM Chromatograms NP3EO-NP8EO

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 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, Det-o-Jet, Luminox, or Citrajet,[5] 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.[6] 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,[7] 2.1 × 150 mm, 3 µm particle size was used to develop this practice. Any

[5] Alconox, Det-o-Jet, Luminox, and Citrajet are trademarks of Alconox, Inc., White Plains, NY.

[6] Waters ACQUITY H-Class Ultra Performance Liquid Chromatography (UPLC) System, a trademark of the Waters Corporation, Milford, MA, was used to develop this practice. All parameters in this practice are based on this system and may vary depending on your instrument.

[7] Waters Atlantis dC18 is a trademark of the Waters Corporation, Milford, MA.

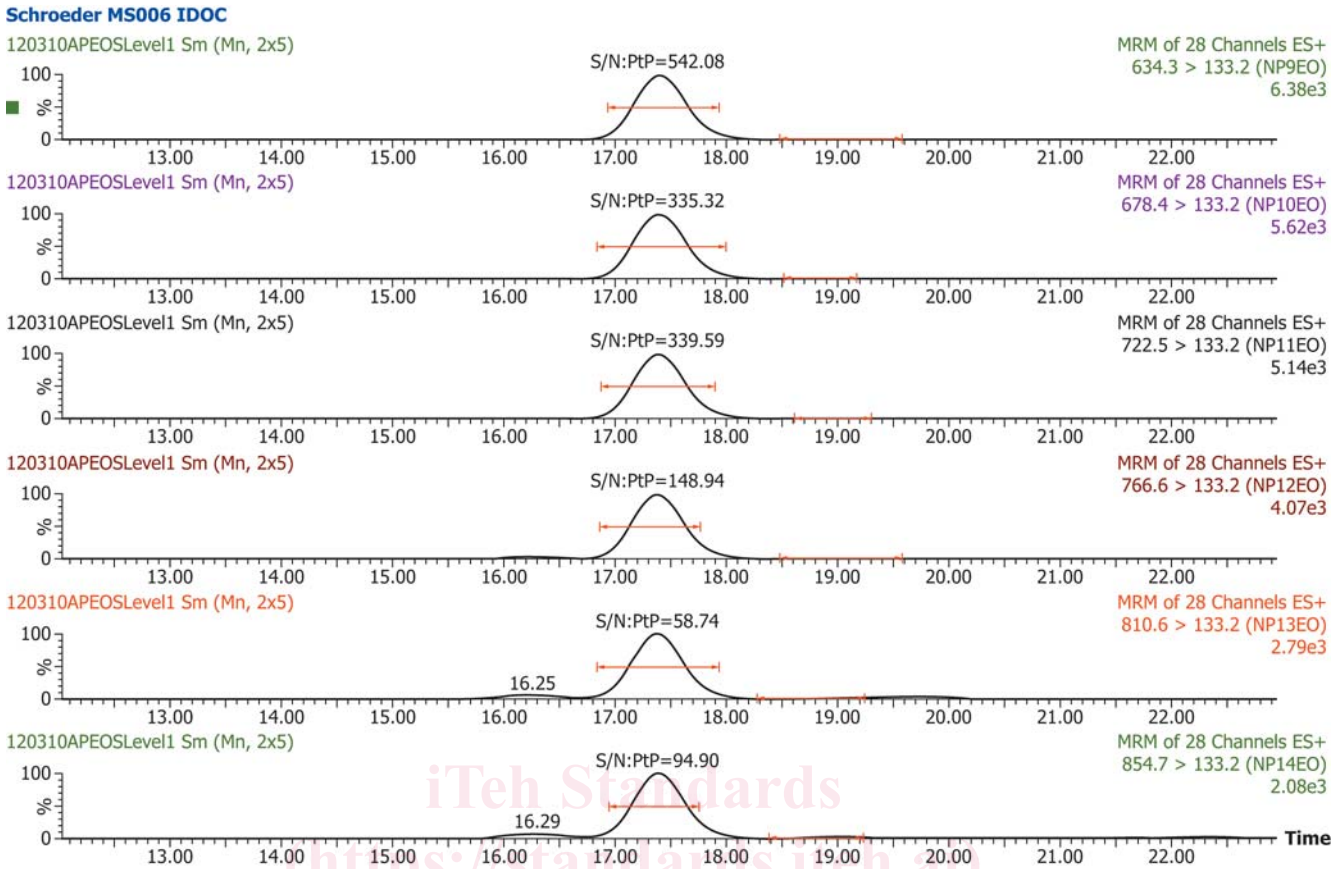

FIG. 2 SRM Chromatograms NP9EO-NP14EO

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.[8] 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,[9,10] or similar, may be used.

7.2.1.1 A 25–mL lock-tip glass syringe size is recommended for this practice.

7.2.2 *Filter*—Millex HV Syringe Driven Filter Unit PVDF 0.45 µm 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.[11] 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 D1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

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

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

[8] A Waters Quattro Micro tandem quadrupole mass spectrometer, a trademark of the Waters Corporation, Milford, MA., was used to develop this practice. All parameters in this practice are based on this system and may vary depending on your instrument.

[9] The sole source of supply of the Millex HV Syringe Driven Filter Unit PVDF 0.45 µm known to the committee at this time is Millipore Corporation, Catalog # SLHV033NS. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[10] Millex is a trademark of Merck KGAA, Darmstadt, Germany.

[11] *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 *Analar 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.

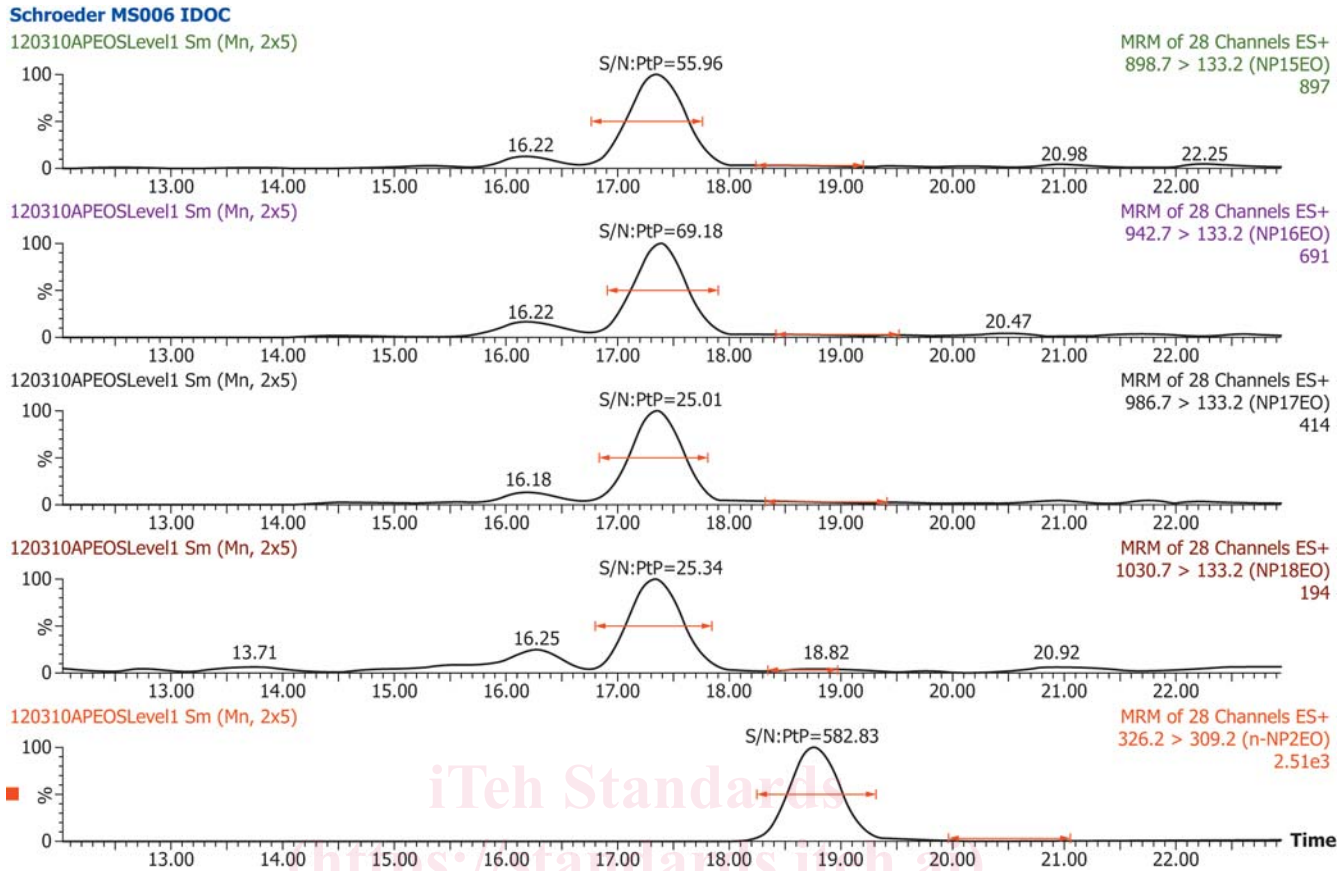

FIG. 3 SRM Chromatograms NP15EO-NP18EO and n-NP2EO

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.

10. Sampling

10.1 Grab samples may be collected in 40-mL pre-cleaned amber glass vials with TFE-fluorocarbon–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:*[6]

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.

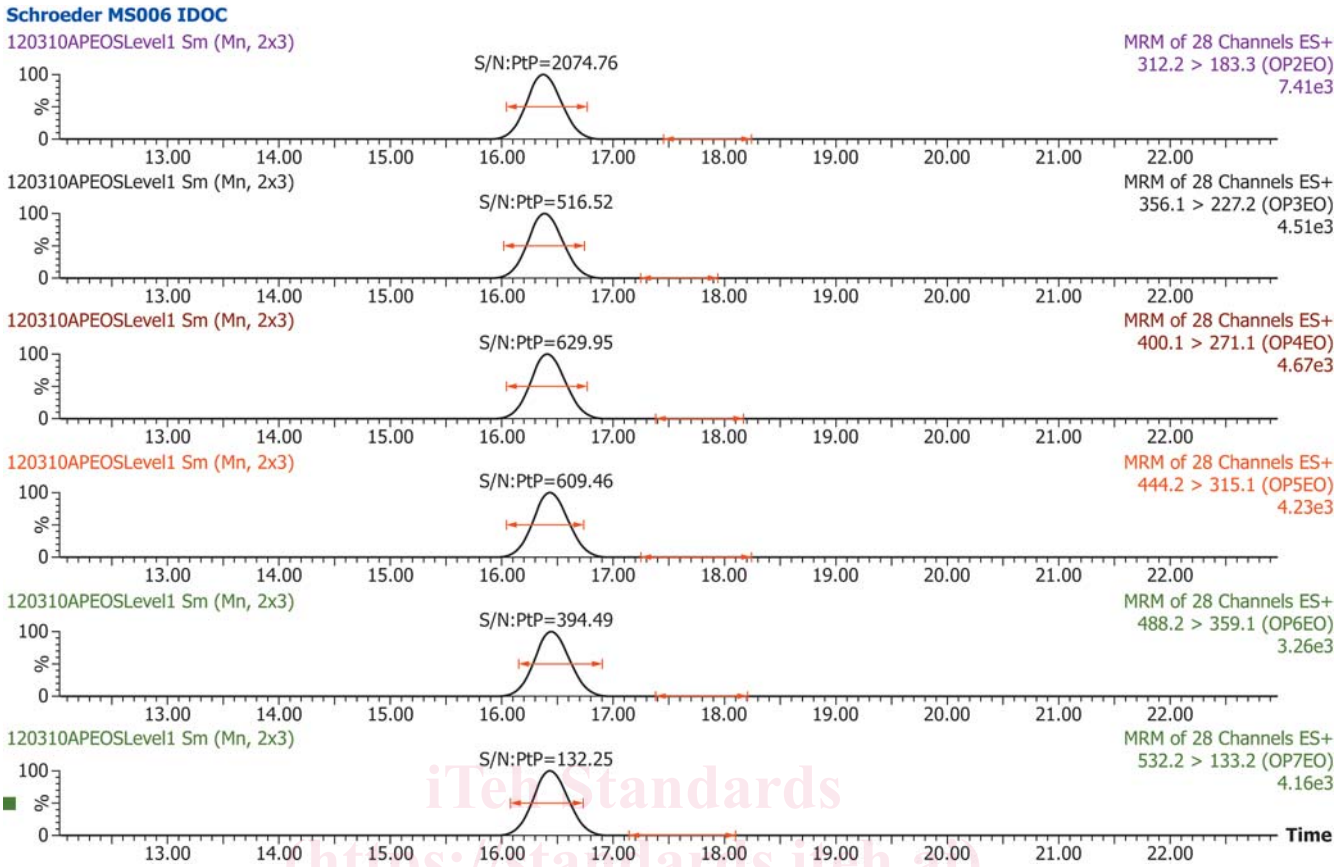

FIG. 4 SRM Chromatograms OP2EO-OP7EO

11.3 *Mass Spectrometer Parameters:*[8]

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[12] and Torrents[13] 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:

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:	7.0×10^{-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 in accordance with manufacturer specifications before analysis. In order to obtain accurate analytical values through using this practice, the following procedures must be followed when

[12] 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.

[13] 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.