Alkylphenol and ethoxylates in soils and sediments by LC-MS/MS

Parameter

Nonylphenol(NP), nonylphenol monoethoxylate(NP1EO), nonylphenol diethoxylate(NP2EO), nonylphenol triethoxylate(NP3EO), nonylphenol tetraethoxylate(NP4EO), nonylphenol pentaethoxylate(NP5EO), nonylphenol hexaethoxylate(NP6EO), nonylphenol heptaethoxylate(NP7EO), nonylphenol octaethoxylate(NP8EO), octylphenol(OP), octylphenol monoethoxylate(OP1EO), octylphenol diethoxylate(OP2EO), octylphenol triethoxylate(OP3EO), octylphenol tetraethoxylate(OP4EO), octylphenol pentaethoxylate(OP5EO), octylphenol hexaethoxylate(OP6EO), octylphenol heptaethoxylate(OP7EO), and octylphenol octaethoxylate(OP8EO).

MDL(s) and EMS Analyte Codes

Analytes	CAS#	Approx. MDL µg/Kg	EMS Analyte codes
NP (Nonylphenol)	84852-15-3	0.5	N032
NP1EO(Nonylphenol Monoethoxylate)	104-35-8	0.5	
NP2EO(Nonylphenol Diethoxylate)	20427-84-3	0.5	
NP3EO(Nonylphenol Triethoxylate)		0.5	
NP4EO(Nonylphenol Tetraethoxylate)		0.5	
NP5EO(Nonylphenol Pentaethoxylate)		0.5	
NP6EO(Nonylphenol Hexaethoxylate)		0.5	
NP7EO(Nonylphenol Heptaethoxylate)		0.5	
NP8EONonylphenol Octaethoxylate)		0.5	
OP (Octylphenol)	140-66-9	0.5	
OP1EO(Octylphenol Monoethoxylate)	2315-67-5	0.5	
OP2EO(Octylphenol Diethoxylate)	2315-61-9	0.5	
OP3EO(Octylphenol Triethoxylate)		0.5	
OP4EO(Octylphenol Tetraethoxylate)		0.5	
OP5EO(Octylphenol Pentaethoxylate)		0.5	
OP6EO(Octylphenol Hexaethoxylate)		0.5	
OP7EO(Octylphenol Heptaethoxylate)		0.5	
OP8EO(Octylphenol Octaethoxylate)		0.5	

Analytical Method

SPE extraction – LC-MS/MS

Introduction

This method is for the quantitative determination of nonylphenol, octylphenol, and their ethoxylates in soils and sediments samples by LC-MS/MS.

Endocrine disruptors (EDs), such as nonylphenol, affect reproduction in humans, as well as wildlife. Thus, the need to monitor EDs is of importance for public safety. For more than forty years alkylphenols such as NP, NPE, OP, and OPE have been used as detergents, emulsifiers, wetting agents and more. Their presence in the environment is solely due to human activities. Soil contamination is mainly from pesticide applications, or sewage and pulp and paper sludge application to agricultural fields. Over 80% of alkylphenol produced are nonylphenol based compounds. The rest is mostly octylphenol based compounds.

NPEs are degraded to shorter chain nonylphenol ethoxylates as well as shorter nonylphenol ethoxycarboxylates under aerobic condition, before being biodegraded to nonylphenol. Shorter ethoxylate chain alkylphenols have greater toxicity than longer ones. CCME describes Toxic Equivalent Factors from Environment Canada for NPnEO ($1 \leq n \leq 8$) and OPnEO($1 \leq n \leq 8$) as half the relative toxicity of NP and OP.

Certified nonylethoxylate and octylethoxylate standards greater than NP3EO and OP2EO are almost nonexistent and therefore characterization of ethoxylate distribution in tech grade standards such as nonoxynol and triton-X are suitable alternatives.

Method Summary

Solid samples are extracted by successive sonication before SPE cleanup. Samples are weighed in a glass tube, spiked with a labelled surrogate solution, covered with a methanol/DCM solution, and placed in a sonic bath for 60 minutes. Extraction is repeated and extracts are recovered. After centrifugation, extracts are diluted in acidified water. SPE cleanup follows the same steps as the water extraction protocol.

Samples are cleaned up using polymeric sorbent SPE. Cartridges are cleaned with dichloromethane (DCM) to eliminate all alkylated phenols leftover from manufacturing, packaging or handling. SPE sorbents are conditioned with methanol and acidified water. Samples are loaded onto the SPE. After washing the sorbent beds with a methanol solution in water, they are vacuum dried. Elution is done by percolating a methanol/DCM solution through the SPE cartridges. A labelled internal standard is added before LC-MS/MS analysis.

Matrices

This method is applicable for determination of selected alkylphenols from soil and sediment samples.

Interferences and Precautions

a) Contaminants present in solvents, reagents, sample containers, or sample processing equipment may cause interferences or yield artefacts. Plastic containers should be avoided. High purity grade solvents should be used.
b) Matrix dependant interferences can cause signal suppression or signal enhancement in the Electrospray Ionisation source (ESI). Extract dilution may help to damper these effects.
c) A method blank helps to demonstrate a contamination free procedure. Background subtraction of method blank is not allowed.
d) Solvent blanks should be run before and after LC-MS/MS analysis to clean the system of alkylphenol contaminants.
e) Solvent blanks should also be run after highly contaminated samples to eliminate carryover.

Sample Handling and Preservation

Container: Amber glass 250mL jars.
Preservation: No preservatives.

Stability

Holding time.

Sample: Extract samples within 7 days after sampling.
Extract: Analyze extract up to 60 days after extraction.
Storage: Freeze samples below -10^{o}C. Keep extracts below -10^{o}C.

Procedure

Calibration stock:

Individual standards can be purchased as neat material or in solution but are only available for Nonyphenol, Octylphenol and mono- and di- ethoxylates. A technical grade mix must be used to calibrate for the remainder of the ethoxylates. This requires the technical grade mix to be characterized initially by HPLC to determine the ethoxylate distribution. Individual mono- and di- ethoxylate standards should be used as to provide a more accurate quantification than technical grade mix for the primary components.

Extraction:

Representative sub-samples are weighed in a glass tube for extraction. Sub-samples are spiked with labelled surrogate solution, covered with a methanol/DCM solution, and placed in a sonic bath for 60 minutes. Samples are centrifuged, and supernatants are collected in different flasks. Extraction is repeated by covering the sample with a methanol/DCM solution, and placed in a sonic bath for another 60 minutes. After centrifugation and supernatant collection, extracts are diluted in acidified water for SPE cleanup.

SPEs are mounted on a vacuum manifold and cleaned by percolating DCM through the cartridge. DCM is flushed away under full vacuum. SPE are conditioned with methanol and acidified water. Cartridges should not go dry during or after conditioning. Diluted extracts are loaded on to the cartridges at a rate of 2-5mL per minute.

Once all the extracts have completely passed through the SPE beds, flasks are washed with a methanol/water solution and applied to the cartridges and eluted. SPEs are dried under full vacuum for 15-30 minutes, until sorbent is visibly dry and free flowing.

Elution is done by percolating a methanol/DCM solution. Elution solution is drawn into the sorbent and left to soak for 1 minute before percolation. Extracts are diluted to volume with elution solution. A labelled internal standard solution is added to extracts before LC-MS/MS analysis.

UPLC Parameters

Column: ODS UPLC column

Mobile Phase A: Ammonium acetate in water

Mobile Phase B: Acetonitrile

Mode: Gradient elution

MS/MS Parameters

MS/MS transitions:

Analytes	Transitions (m/z)	ESI Polarity
NP	219->133;147	Neg.
NP1EO	282->127;265	Pos.
NP2EO	326->183;121	Pos.
NP3EO	370->227;353	Pos.
NP4EO	414->271;397	Pos.
NP5EO	458->315;440	Pos.
NP6EO	502->89;485	Pos.
NP7EO	546->89;529	Pos.
NP8EO	591->89;573	Pos.
OP	205->133;106	Neg.

OP1EO	268->113;250	Pos.
OP2EO	312->295;183	Pos.
OP3EO	356->339;295	Pos.
OP4EO	400->383;271	Pos.
OP5EO	444->427;315	Pos.
OP6EO	488->471;359	Pos.
OP7EO	532->515;403	Pos.
OP8EO	576->559;447	Pos.

Calculations

Total NP TEQ = ([NP] X 1) + ([NP 1EO X 0.5) + ([NP2EO] X 0.5) + ([NP3EO X 0.5) + ([NP3EO] X 0.5) + ([NP4EO] X 0.5) + ([NP5EO] X 0.5) + ([NP6EO] X 0.5) + ([NP7EO] X 0.5) + ([NP8EO] X 0.5)

Total OP TEQ = ([OP] X 1) + ([OP 1EO X 0.5) + ([OP2EO] X 0.5) + ([OP3EO X 0.5) + ([OP3EO] X 0.5) + ([OP4EO] X 0.5) + ([OP5EO] X 0.5) + ([OP6EO] X 0.5) + ([OP7EO] X 0.5) + ([OP8EO] X 0.5)

Toxic equivalency factors (TEFs) for NP, NPEs, NPECs, OP, OPEs, and OPECs (Servos et al. 2000; Environment Canada 2002). Chemical TEFs (relative to NP)

NP	1
NPnEO (1≤n≤8)	0.5
NPnEO(n≥9)	0.005
NP1EC	0.005
NP2EC	0.005
OP	1
OPnEO (1≤n≤8)	0.5
OPnEO(n≥9)	0.005
OP1EC	0.005
OP2EC	0.005

NPEC = Nonylphenol ethyl carboxylate

Performance Requirements

Any analytical method options selected for this analysis must meet or exceed the performance requirements specified below.

Accuracy and Precision requirements are distinct from daily QC requirements, and apply to measures of long-term method performance (averages and standard deviations). Achievement of these requirements is to be demonstrated during initial and ongoing method revalidation studies. For Initial Validations, averages of at least 8 Lab Control Samples or Reference Materials must be assessed. Ongoing Revalidations (performance reviews) should assess QC data encompassing longer periods (e.g. 6 months to 1 year). A minimum frequency of 2 years is recommended for Ongoing Revalidations.

Accuracy Requirement: Laboratories must demonstrate method accuracy (measured as average recovery) through repeat analysis of Laboratory Control Samples at concentrations above ten times the MDL. Average accuracy must be between 60-140% for all analytes.

Precision Requirement: Laboratories must demonstrate method precision through repeat analysis of Laboratory Control Samples at concentrations above ten times the MDL. Precision measured as percent relative standard deviation

(%RSD) must be <20% for all analytes.

Sensitivity Requirement: Where possible, the method should support Reporting Limits (and MDLs) that are less than 1/5 of applicable numerical standards. The method is not fit-for-purpose if an MDL exceeds a guideline, standard, or regulatory criteria against which it will be used for evaluation of compliance.

Quality Control

Summary of QC Requirements		
QC Component	**Minimum Frequency**	**Minimum Data Quality Objectives**
Method Blank (MB)	One per batch (max 20 samples)	Less than reported DL
Laboratory Control Sample (LCS)	One per batch (max 20 samples)	60-140%
Laboratory Duplicate (DUP)	One per batch (max 20 samples)	≤35% RPD [or within 3x reported DL for low level results]
Surrogate Compounds	All samples	40-140%
Internal Standards	All samples	Peak area counts for all internal standards in all injections must be within ± 50% of the average peak area calculated during the initial calibration.
Calibration Verification Standard (CVS)	Minimum 1 per initial calibration	70-130%
Continuing Calibration Verification (CCV)	One per batch (max 20 samples)	70-130%
If DQOs are not met, repeat testing or report qualified test results. DQOs do not apply to MS results where sample background exceeds spike amount.		

Method Blank: Required. An aliquot of reagent water that is treated exactly like a sample including exposure to equipment, solvents and reagents, sample preservatives, internal standards and surrogates that are used in the analysis batch .

Laboratory Duplicates: Required. Data quality objectives are listed above.

Laboratory Control Sample (Method Spike): Required. Prepare a Laboratory Control Sample by fortifying a field sample with known concentrations of analytes.

Surrogate Compounds: Required. Suggested: 4-n-NP-$^{13}C_6$, NP3EO-$^{13}C_6$ and/or OP1EO-$^{13}C_6$

Calibration Verification Standard (CVS): Required. A CVS from a source separate from the calibration standard must be analyzed with each initial calibration to monitor calibration accuracy.

Continuing Calibration Verification (CCV): Required. A mid-point calibration standard must be analyzed throughout the instrument run at least every 20 samples and at the end of the run to monitor calibration drift.

Prescribed Elements

The following elements are mandatory:

1. Analysis must be done by LC-MS/MS with MRM transition for each compound.
2. Confirmation should be done with second MRM transition.
3. Different surrogate standards must be used with ESI negative and ESI positive modes. Suggested: 4-n-NP-$^{13}C_6$, NP3EO-$^{13}C_6$ and/or OP1EO-$^{13}C_6$
4. Different internal standards must be used with ESI negative and ESI

positive modes. Suggested: BPA-d_{16}, NP2EO-$^{13}C_6$.

5. All performance requirements and QC requirements must be met.

References

ASMT. Determination of Nonylphenol, p-tert-octylphenol, Bisphenol A, Nonylphenol Monoethoxylate and Nonylphenol Diethoxylate in Environmental Waters by Liquid Chromatography/Tandem Mass Spectrometry. Designation: D7485-09. 2009.

Revision History

Jan 13, 2017	New method added to the BC Lab Manual to correspond with updates to the BC CSR. Effective date for this method is November 1, 2017.