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Simultaneous determination of degradation products of nonylphenol polyethoxylates and their halogenated derivatives by solid-phase extraction and gas chromatography–tandem mass spectrometry after trimethylsilylation

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Abstract

An efficient method for the simultaneous determination of the degradation products of nonylphenol polyethoxylates (NPnEOs, n = number of ethoxy units), i.e., nonylphenol (NP), NPnEOs (n = 1–3), nonylphenoxy carboxylic acids (NPnECs, n = 1–2, number of ethoxy units plus an acetate) and their halogenated derivatives (XNP, XNP1EO and XNP1EC; X = Br or Cl), in water samples were developed. After trimethylsilylation with *N,O*-bis(trimethysilyl)acetamide, all the analytes were determined by gas chromatography–tandem mass spectrometry (GC–MS–MS) with electron ionization (EI). The ion peaks of $[M - 85]^+$ of the derivatives were selected as precursor ions and their product ions showing the highest intensities were used for the quantitative analysis. The instrumental detection limits were in the range from 2.1 to 11 pg. The recoveries of the analytes from the water samples were optimized by using solid-phase extraction (SPE). The deuterated reagents of octylphenol, octylphenol monoethoxylate and octylphenoxyacetic acid were used as the surrogates. The method detection limits (500 ml water sample) using C_{18} SPE were from 2.5 to 18 ng/l. The recoveries from spiked pure water and the environmental water samples were greater than 78%. The method was successfully applied to environmental samples. Remarkably, the concentrations of the halogenated compounds (ClNP, ClNP1EO and BrNP1EO) were detected at the hundreds of ng/l levels in the Neya river.

Keywords: Water analysis; Environmental analysis; Solid-phase extraction; Derivatization, GC; Nonylphenol polyethoxylates; Nonylphenol; Nonylphenoxy carboxylic acids; Halogenated compounds

1. Introduction

The pollution by the degradation products of nonylphenol polyethoxylates (NPnEOs) such as

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nonylphenol (NP), short ethoxy chain NPnEOs and nonylphenoxy carboxylic acids (NPnECs) bearing a short ethoxy chain have received a significant amount of attention as they were recognized to exhibit ubiquitous, lipophilic, and refractory characteristics in the environment and, recently, potential estrogenicity although the evidence is still fragmentary [1–3].

In some researches concerned with the degradation products of the NPnEOs, some halogenated derivatives were detected. The formation of halogenated derivatives of the alkylphenols and acidic alkylphenols, mostly brominated compounds, was reported in effluent water and receiving river water after disinfection with chlorine in the presence of bromide ion in the wastewater treatment plant [4,5]. When evaluating the occurrence of NPnEOs and their related compounds in the effluents of 40 full scan sewage treatment plants in Japan, we found that halogenated nonylphenol ethoxylates (XNPnEOs, X = Cl or Br, n = 1–2) and halogenated nonylphenoxyacetic acid (XNPnEC, X = Cl or Br, n = 1) were in the range of hundreds of ng/l to μg/l on average [6]. In addition, the halogenated derivatives were also found in sediments from the New York Harbor Complex, USA [7], and in sludge from a Barcelona drinking water treatment plant, Spain, in concentrations of up to 220 μg/kg for bromononylphenol (BrNP), 430 μg/kg for BrNPnEOs (n = 1–2), 1600 μg/kg for BrNPnEOs (n = 3–15) and 660 μg/kg for ClNPnEOs [8]. Regarding the potential toxicity, Maki et al. [9] reported that both the BrNPnEOs and BrNPnECs showed a higher acute toxicity to *Daphnia magna* than their nonbrominated precursors, the NPnEOs and NPnECs. Because of the possible presence in the environment and the potential toxicities, the halogenated derivatives should be evaluated together with their precursors.

Gas chromatography–mass spectrometry (GC–MS) [10–16] and liquid chromatography–mass spectrometry (LC–MS) [7,8,17–20] have been shown to be efficient for the determination of alkylphenol polyethoxylates and their degradation products. The co-elution of the compounds and the lack of individual standards seem to be the reasons that halogenated derivatives were not determined in conjunction with their precursors, i.e., the NPnEOs and NPnECs. Until now, there is only one report on the simultaneous determination of NPnEOs, NPnECs and their halogenated derivatives by solid-phase extraction (SPE)–LC–MS [8]. On the other hand, MS–MS is a useful technique for their analysis in complex matrix such as environmental samples, however, such an application is still rare. Up to now, there is only one report in which Ding and Tzing confirmed the structure of the carboxyalkylphenol ethoxy carboxylates, the degradation products of alkylphenol polyethoxylates, in the environment by GC–MS–MS with chemical ionization (CI) [11].

In this study, we developed a sensitive and specific analytical method for the simultaneous determination of halogenated derivatives and their precursors in water by GC–MS–MS (ion-trap). The target analytes including the halogenated derivatives (XNP, XNP1EO and XNP1EC; X = Br or Cl), their precursors (NP, NPnEOs, n = 1–3; NPnECs, n = 1–2) and surrogates were synthesized in our laboratory. To derivatize all the analytes including the nonylphenols (NPs = NP, ClNP and BrNP), the alcohols (NPEOs = NP1EO, ClNP1EO, BrNP1EO, NP2EO and NP3EO) and the carboxylic acids (NPECs = NP1EC, ClNP1EC, BrNP1EC and NP2EC), we chose trimethylsilylation as their derivatization. The derivatization and SPE of the analytes were fully investigated.

2. Experimental

2.1. Materials

Unless otherwise stated, all chemicals and solvents for the analysis were of pesticide grade quality and the chemicals for the synthesis and methyl acetate were of reagent grade, which were purchased from Wako (Osaka, Japan). The silica gel [BW-127ZH (100–270 mesh)] was provided by Fuji Silysia (Aichi, Japan) and activated overnight at 120 °C. Acetone, methanol, methyl acetate and *n*-hexane were dehydrated by anhydrous sodium sulfate before use. Pure water (18 mΩ) produced by a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was passed through a methanol-rinsed 47 mm Empore C_{18} SPE disk (3M, USA) before use. Sodium sulfate was baked overnight at 200 °C under reduced pressure. All glassware was rinsed with purified water and pesticide grade solvents before use.

2.2. Standard reagents

Technical grade NP and NPnEO ($n_{ave} = 2$) were purchased from Kishida Chemical (Osaka, Japan) and TCI (Tokyo, Japan), respectively. The internal standards (phenanthrene-d_{10} and pyrene-d_{10}) were supplied by Kanto Chemical (Tokyo, Japan).

The nonylphenol mono-, di- and triethoxylates (NP1EO, NP2EO and NP3EO) were obtained by separating NPnEO ($n_{ave} = 2$) by silica gel column chromatography. Nonylphenoxyacetic acid (NP1EC) and nonylphenoxyethoxyacetic acid (NP2EC) were individually synthesized by Jones-oxidation of the ethoxy chain of the corresponding NP1EO and NP2EO [21]. The chlorinated derivatives (ClNP, ClNP1EO and ClNP1EC) were synthesized by reacting NP, NP1EO and NP1EC with sulfuryl chloride in chloroform, respectively [22]. The brominated derivatives (BrNP, BrNP1EO and BrNP1EC) were also obtained from NP, NP1EO and NP1EC, respectively, according to the previously reported method [23].

Deuterated *tert*-octylphenol (OP-d), deuterated *tert*-octylphenol monoethoxylate (OP1EO-d) and deuterated *tert*-octylphenoxyacetic acid (OP1EC-d) were synthesized and used as surrogates for the NPs, NPEOs and NPECs, respectively. OP-d was synthesized by Friedel–Craft reaction between phenol-d_6 and 2,4,4-trimethyl-1-pentene using $AlCl_3$ [24]. OP1EC-d was obtained by the reaction of OP-d with chloroacetic acid under alkaline conditions [21]. OP1EO-d was synthesized by reducing OP1EC-d with $LiAlH_4$. These surrogates were mixtures with wide deuterium contents. All the reactions were monitored by thin layer chromatography or GC with flame ionization detection. The products were purified by column chromatography on silica gel. Their structures and purities were confirmed by GC–MS and ^1H and ^{13}C NMR.

Except for the stock solutions of the surrogates and internal standards (50 mg/l), 100 mg/l stock standard solutions were individually prepared in acetone and stored at 4 °C in a refrigerator. The working standard solutions were prepared by diluting specific amounts of the analytes and the surrogates from the stock solutions in a 50 ml volumetric bottle with acetone. Two levels of working standard solutions, 100 μg/l (except for BrNP = 200 μg/l and NP2EC and NP3EO = 1000 μg/l) and 4000 μg/l (except for BrNP = 8000 μg/l and NP2EC and NP3EO = 40000 μg/l), were prepared. The internal standard solution containing phenanthrene-d_{10} and pyrene-d_{10} at 200 μg/l each in methyl acetate was also prepared from the stock solutions.

2.3. Sample preparation

For the recovery studies, two concentration ranges of the analytes were prepared in pure water as well as in an environmental sample matrix. The environmental samples were collected from the Ina river (Itami city, Hyogo prefecture) as a representative for a low polluted matrix and from the Neya river (Osaka city) as a representative for a highly polluted matrix (environmental data of those two rivers are not shown). The samples were stored at 4 °C and analyzed within 48 h after filtration using a 0.45 μm membrane filter (Millipore, USA) before use.

2.3.1. Extraction procedure

The SPE extraction procedure was modified from the previously described methods [8,15]. In the optimized procedure, a Bond Elut C18-HF (Varian, 500 mg, 3 ml) cartridge placed on a vacuum manifold (VAC Elute SPS 24) was successively conditioned with methyl acetate (5 ml), methanol (5 ml) and pure water (5 ml) at a flow rate of 1 ml/min. After acidification to pH 3 with concentrated HCl, a 500 ml water sample (200 ml for sample containing higher levels of the analytes) was loaded at a flow rate of 5–10 ml/min. The solid phase was then completely dried by drawing nitrogen gas for 20 min. The analytes were eluted from the solid phase by methyl acetate (7 ml) amended with 0.25 mM HCl under a positive pressure (flow rate = 1 ml/min). The extract was then dehydrated by passing it through 15 g of anhydrous sodium sulfate and collected in a vial. The solution was gently evaporated to dryness using nitrogen gas (flow rate = 500 ml/min). The residue of the extracts was then subjected to a derivatization reaction.

2.3.2. Derivatization

To a vial containing the residue of the extracts, 400 μl of methyl acetate containing 200 μg/l the internal standards and 100 μl of a derivatizing reagent were added. The vial was then closed and mixed

completely. The derivatization reaction was implemented at 25 °C for 1 h.

2.4. GC–MS analysis

The GC–MS analysis was done on a Varian 3800 gas chromatograph coupled with a Varian Saturn 2000 ion-trap mass spectrometer (Varian, Walnut Creek, CA, USA) and a 30 m (0.3 mm i.d. and film thickness = 0.25 μm) fused silica capillary column DB-5MS (J&W), which was directly connected to the mass spectrometer. A 2 μl of the derivatized sample was injected in a splitless mode from 0.2 to 2.2 min using programmed temperature vaporization injection. The oven temperature program was: 65 °C (2 min) at 14 °C/min, 160 °C at 5 °C/min, 240 °C at 10 °C/min, 290 °C (hold for 10 min). The injector temperature was set at 65 °C isothermal for 0.2 min and then increased to 280 °C (hold for 10 min) at a rate of 200 °C/min. Helium (99.999%) was used as carrier gas at the flow rate of 1.2 ml/min. The manifold and transfer line were set at 40 and 280 °C, respectively. The mass spectra were acquired using the EI–MS–MS technique with resonant collision-induced dissociation (CID) waveform amplitudes at a rate of 1 scan/s under the following conditions: ion-trap temperature, 220 °C; electron energy, 70 eV; emission current, 80 μA. Additional information is shown in Table 1.

3. Results and discussion

3.1. Mass and tandem mass spectra

A variety of derivatization reactions such as acylation [25–27], alkylation [16,28], silylation [12,14] and others [29] have been reported to enhance the GC performance of polar organic compounds. Very recently, Diaz et al. demonstrated that headspace solid-phase microextraction and GC–MS after in-sample methylation with dimethyl sulfate can be applicable to the analysis of NP and short ethoxy chain NPnEOs and NPnECs in water [16]. However, the detection limit increased with the increasing ethoxy chain due to lowering of the volatility. On the other hand, trimethylsilylation is the most well-known and the most convenient method for the analysis of polar organic pollutants as well as alkylphenols. To derivatize all analytes including the NPs, NPEOs and NPECs, we chose trimethylsilylation for their derivatization. The optimum EI–MS–MS conditions for the resulting trimethylsilyl ethers and esters were investigated (Table 1). The overall run time was split into 14 segments. For all the derivatives, the most significant ions in the EI–MS were $[M-85]^+$ corresponding to the α,α-dimethyl structures via benzylic cleavage of the nonyl chains [4,30]. To produce the product ions of higher intensity, the $[M-85]^+$ ions were selected

Table 1
EI–MS–MS conditions

Compound	Segment mass range (*m*/*z*)	Segment duration (min)	CID voltage (V)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)
OP-d	100–220	12.00–14.00	0.55	210	181
NP	100–210	14.00–15.34	0.55	207	179
Phenanthrene-d_{10}	100–205	15.34–16.61	0.60	188	160
ClNP	100–305	16.61–17.73	0.60	241	213
BrNP	100–295	17.73–18.78	0.70	285	191
OP1EO-d	100–295	17.73–18.78	0.60	254	210
OP1EC-d	100–285	18.78–19.80	0.60	268	210
NP1EO	100–261	19.80–20.87	0.55	251	207
Pyrene-d_{10}	100–275	20.87–21.88	0.90	212	210
NP1EC	100–275	20.87–21.88	0.60	265	207
ClNP1EO	100–295	21.88–23.23	0.65	285	241
BrNP1EO	100–340	23.23–24.50	0.65	329	285
ClNP1EC	100–340	23.23–24.50	0.65	299	241
BrNP1EC	100–355	24.50–25.80	0.75	343	285
NP2EO	100–355	24.50–25.80	0.60	295	207
NP2EC	100–320	25.80–27.50	0.55	309	207
NP3EO	100–350	27.50–32.00	0.60	339	161

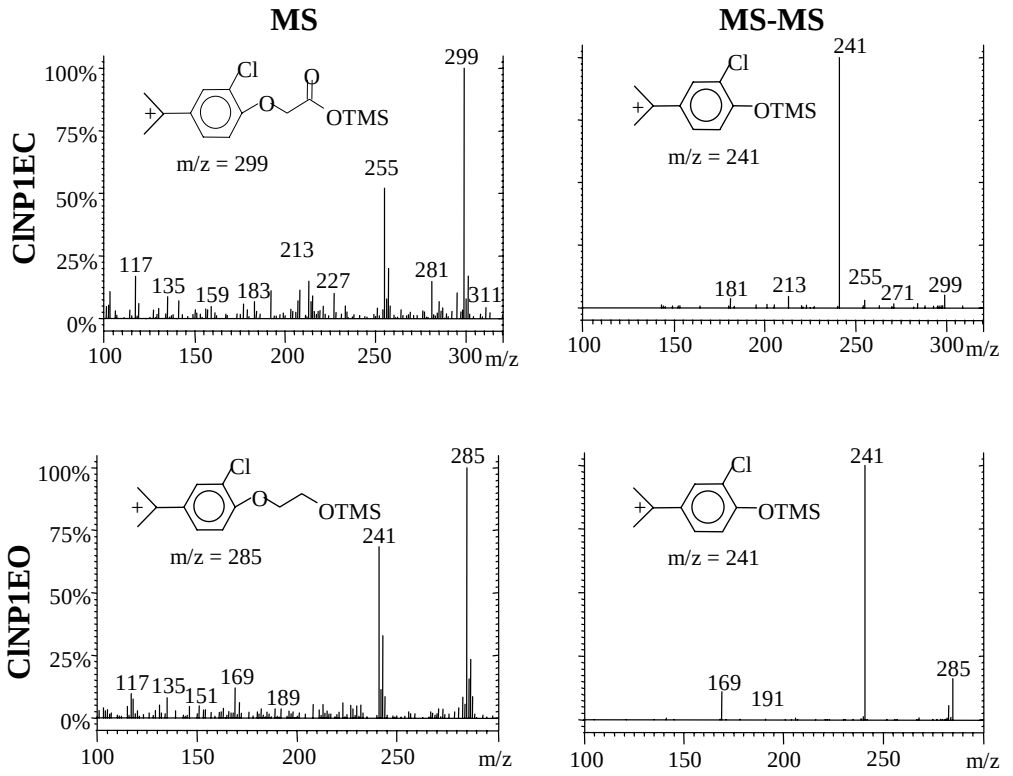

Fig. 1. EI–mass and EI–tandem mass spectra of ClNP1EO and ClNP1EC.

as the precursor ions. Fig. 1 shows the mass spectra of EI–MS and EI–MS–MS under the optimum CID conditions for ClNP1EO and ClNP1EC. For ClNP1EO, the CID of $m/z = 285$ produces the significant product ion $m/z = 241$, reflecting the loss of ethylene oxide ([precursor $- 44]^+$) via the rearrangement of the trimethylsilyl group. However, ClNP1EC also produced the same product ion as ClNP1EO, showing the loss of a three-membered lactone via the silyl rearrangement. The product ion of the highest intensity in the tandem mass spectra was selected for the quantitative analysis. Since the NP related compounds are isomeric mixtures of branched nonyl groups (C_9) that are separated by GC and the signals of these isomers are indicated in numerous peaks in the chromatogram, total concentration of a compound were determined by summing the concentrations of the two isomers having the highest intensity. These two isomers located at the start and the end of the isomer cluster of each compound.

3.2. Derivatization conditions

Many factors could affect the efficiency of the derivatization process. In this study, we investigated the effects of the reaction time, solvent, derivatizing reagent and water content using 5 ml of 20 μg/l standard solution (except for BrNP = 40 μg/l and NP2EC and NP3EO = 100 μg/l). At first, the solution was gently evaporated to dryness under a stream of nitrogen. Then 400 μl of the internal standard solution (200 μg/l) and 100 μl of the derivatizing reagent were added to the residue for the derivatization.

3.2.1. Effect of solvent, reaction time and derivatizing reagent

We evaluated the progress of the derivatization using three mediums (n-hexane, methyl acetate and acetone) and two derivatizing reagents (*N*,*O*-bis (trimethysilyl)trifluoroacetamide, BSTFA and *N*,*O*-bis (trimethysilyl)acetamide, BSA). The results shown in

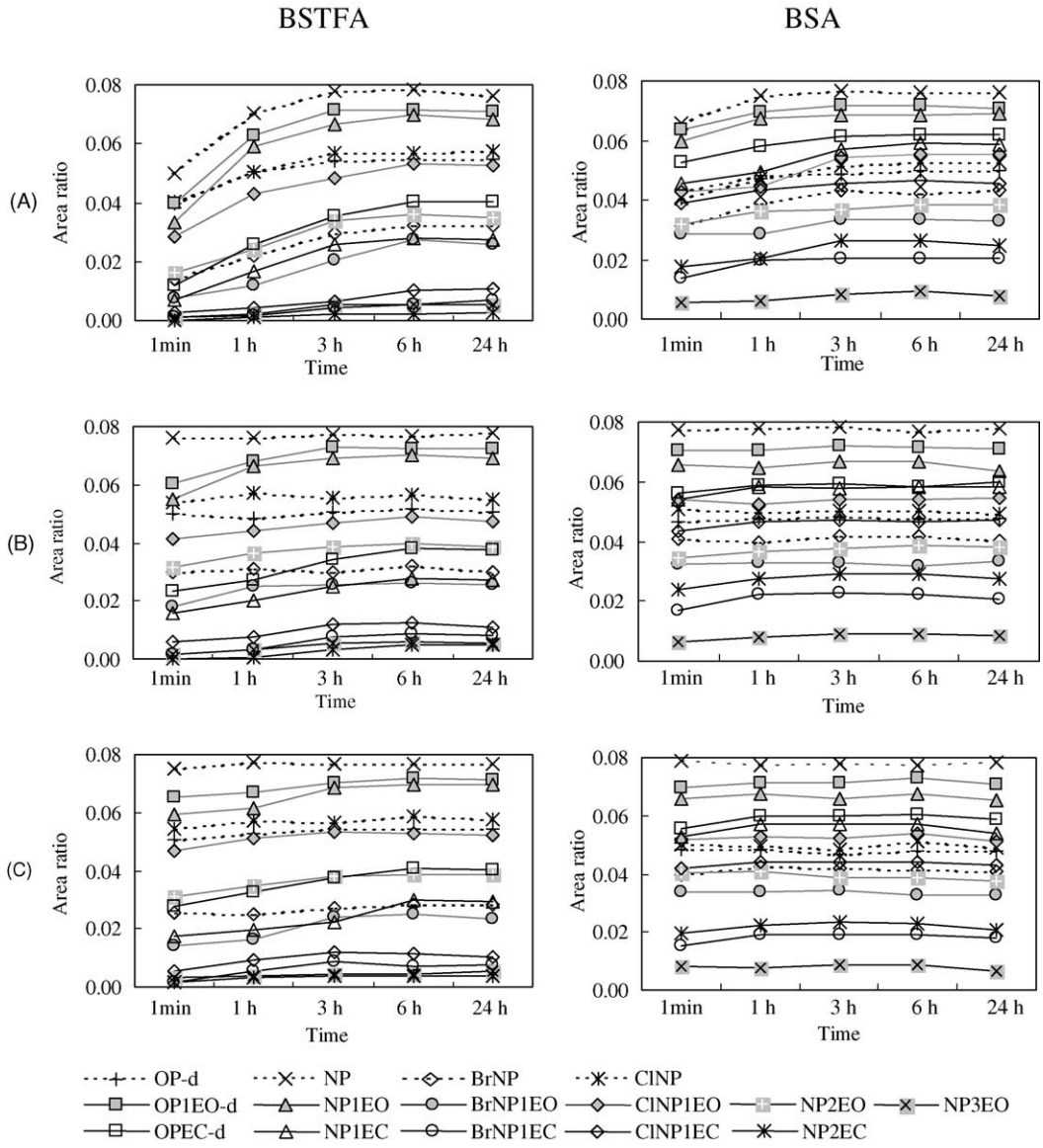

Fig. 2. Time dependence of trimethylsilylation with BSTFA and BSA in (A) *n*-hexane, (B) methyl acetate and (C) acetone.

Fig. 2 indicated that the derivatization yields depended on the analyte structure, solvent and reaction time. In general, the derivatization reactions for the phenolic hydroxyl group were completed faster than those for both the alcoholic hydroxyl and carboxyl groups.

The reaction rates in methyl acetate and acetone were similar and more favorable than those in n-hexane, confirming the results of Li et al. [14]. However, in these two mediums, BSA gave shorter reaction times (<1 h) as well as the higher yields than BSTFA (<6 h), especially for the carboxylic acids. The results made us choose BSA as the derivatizing reagent. Because methyl acetate was used for eluting the analytes from the SPE cartridge, this solvent was employed as the medium for the derivatization. A reaction time of 1 h was the minimal time needed for the

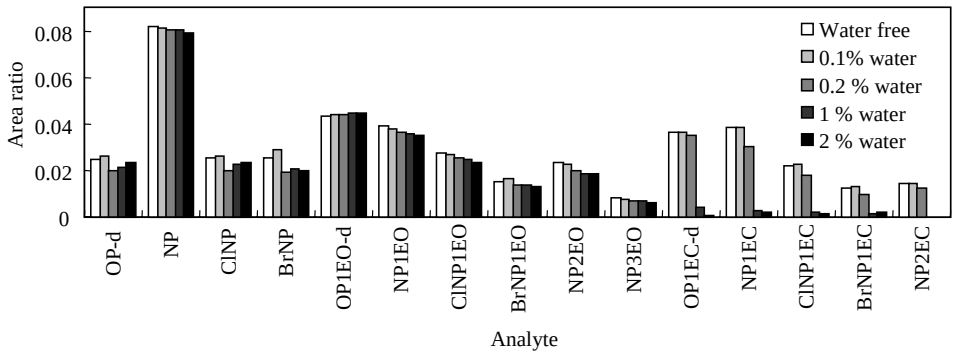

Fig. 3. Effect of water content on trimethylsilylation of the analytes.

quantitative derivatization. The yields of the derivatives in methyl acetate could be measured unchanged even after 15 days.

3.2.2. Effect of water content

The silylation reactions and the resultant derivatives are known to be adversely affected by the presence of water. In this study, the standard solutions containing 0.1, 0.2, 1 and 2% (v/v) of water were prepared in methyl acetate. To 400 μl of the standard solutions in a vial was added 100 μl of BSA. After closing the vial and mixing for 1 min, the vial was stored for 1 h in order to complete the reaction. Fig. 3 shows the results of the water-spiked standard solutions together with those of the water-free standard solution. The yields of the NPs revealed little affect by the presence of up to 2% water in the derivative solution. NPEOs showed a small decrease in yields with the increase of water content. However, the NPECs proved to be very sensitive to the presence of water. The yields became slightly lower for the standard solution containing 0.2% water, compared to those for a water-free standard solution, but drastically decreased in the solution containing 1% water.

3.3. Analytical performance

3.3.1. Quantitative analysis

The quantification of the analytes was carried out by the internal standard method using response factors of the analytes to the internal standards. While phenanthrene-d_{10} was employed for the early eluted compounds (OP-d and NPs), pyrene-d_{10} was used for the others more retained (OP1EO-d, OP1EC-d, NPEOs and NPECs). As shown in Table 2, the calibration curves had good linear relationships using the standard solutions at 10 different concentrations. The instrumental detection limits, that were calculated from the standard deviations estimate with $n - 1$ degrees of freedom and 97% confidence level of seven replicates of the working standard at a concentration five times the lowest working standard for quantitative calibration, ranged from 2.1 to 11 pg.

3.3.2. Solid-phase extraction from pure water

In order to find the optimum conditions for the SPE of the analytes, the solid phase for their adsorption, the eluent for their desorption and the pH of water samples were investigated.

The performance of five cartridges, Bond Elut C18-HF (500 mg, 3 ml) and Bond Elut ENV (500 mg, 3 ml) from Varian, Oasis HLB (500 mg, 5 ml) from Waters and ENVI-Carb (500 mg, 6 ml) and DPA-6S (500 mg, 6 ml) from Supelco were initially examined using 500 ml spiked pure water samples (data is not shown). Among the cartridges studied, C18-HF proved to provide the best performance for all the analytes. Our results confirmed the effectiveness of the C_{18} cartridge that was also applied for the simultaneous extraction of similar compounds by Petrovic et al. [8].

The optimum eluent was evaluated by desorbing the analytes loaded on the C_{18} cartridge by using 5 ml of a spiked pure water sample. The eluate was collected in a vial via a short column of sodium sulfate. The average recoveries ($n = 3$) of the analytes with methyl acetate are shown in Fig. 4. Methyl acetate

Table 2
Quantitative calibration and detection limits of analytes

Compound	Concentration range (μg/l)	Correlation coefficient (R)	IDL (pg)[a]	MDL (ng/l)[b]
NP	2–100	0.9961	2.1	2.9
ClNP	2–100	0.9956	3.5	5.1
BrNP	2–100	0.9976	4.2	6.4
NP1EO	2–100	0.9984	4.1	8.8
NP2EO	2–100	0.9991	5.0	2.5
NP3EO	10–1000	0.9987	11	18
ClNP1EO	2–100	0.9989	3.2	4.6
BrNP1EO	2–100	0.9988	4.8	3.8
NP1EC	2–100	0.9989	2.5	3.7
NP2EC	5–1000	0.9995	9.1	9.5
ClNP1EC	2–100	0.9983	2.8	3.9
BrNP1EC	2–100	0.9995	2.7	3.8
OP-d	2–100	0.9952	2.5	3.2
OP1EO-d	2–100	0.9987	3.1	3.9
OP1EC-d	4–100	0.9987	4.7	4.8

[a] Instrumental detection limit (97% confidence, $n = 7$); injecting level = 10 μg/l (except for BrNP = 20 μg/l, NP2EC and NP3EO = 100 μg/l).
[b] Method detection limit (97% confidence, $n = 5$); spiked level in 500 ml pure water = 20 ng/l (except for BrNP = 40 ng/l, NP2EC and NP3EO = 200 ng/l).

(7 ml) amended with HCl was sufficient to elute all the analytes (94–109% recoveries). However, the recoveries of the NPECs were less than 15% without HCl. In this case, all the NPECs were recovered from the short column of sodium sulfate by using methyl acetate amended with HCl. These results indicate that in the absence of HCl, methyl acetate cannot desorb NPECs from the surface of the sodium sulfate [12].

We examined the pH of the water sample in the range of 2–4, however, the pH had no significant effect on the recovery. In the recovery test using pure water samples (200 and 500 ml at pH 3) at two different spiked levels of 2000 ng/l (except for BrNP = 4000 ng/l, NP2EC and NP3EO = 20000 ng/l) and 20 ng/l (except for BrNP = 40 ng/l, NP2EC and NP3EO = 200 ng/l), satisfactory recoveries were obtained. The recoveries ($n = 5$) of the analytes and surrogates ranged from 86 to 114% with the R.S.D. values of 4.2–19% (at 20 ng/l) and 87–110% with the R.S.D. values of 1.0–5.7% (at 2000 ng/l). The method detection limits, that were calculated from the standard deviations estimate

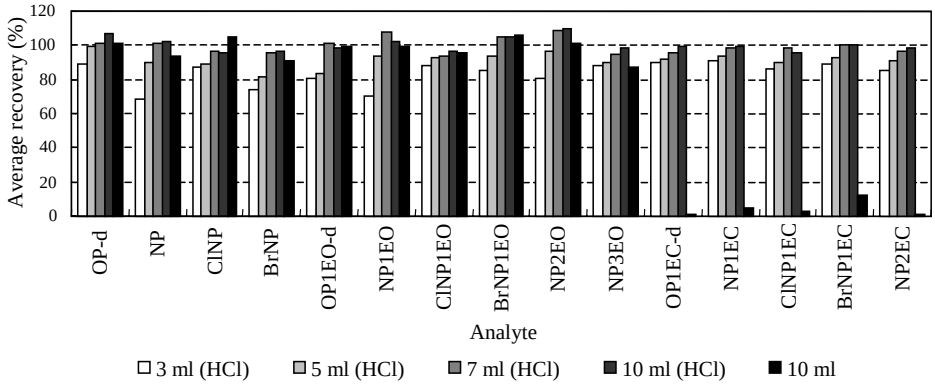

Fig. 4. Effect of eluent volume of methyl acetate on recovery of analytes.

Table 3
Recoveries from river waters and their concentrations

Compound	Ina river		Neya river	
	Recovery (20 ng/l)[a]	Concentration (ng/l)	Recovery (2000 ng/l)[b]	Concentration (ng/l)
NP	98 (7.5)	20 (9.4)	88 (1.7)	1600 (9.3)
ClNP	93 (2.7)	–	88 (1.7)	380 (18)
BrNP	93 (7.3)	–	100 (1.7)	–
NP1EO	98 (12)	–	88 (7.1)	480 (18)
NP2EO	106 (8.5)	3.8 (19)	80 (7.1)	560 (19)
NP3EO	84 (10)	–	96 (4.7)	–
ClNP1EO	80 (21)	–	86 (3.9)	330 (11)
BrNP1EO	78 (12)	4.2 (19)	101 (7.5)	110 (11)
NP1EC	89 (1.5)	12 (3.0)	100 (4.0)	2500 (5.7)
NP2EC	92 (11)	–	87 (4.6)	4900 (9.1)
ClNP1EC	108 (6.8)	–	95 (3.8)	–
BrNP1EC	111 (5.2)	–	97 (3.2)	–
OP-d	97 (4.4)	–	83 (3.7)	–
OP1EO-d	83 (4.6)	–	103 (3.6)	–
OP1EC-d	108 (5.0)	–	98 (4.6)	–

The relative standard deviation (R.S.D.) is given in parentheses ($n = 5$).
[a] Spiked level in 500 ml (except for BrNP = 40 ng/l, NP2EC and NP3EO = 200 ng/l).
[b] Spiked level in 200 ml (except for BrNP = 4000 ng/l, NP2EC and NP3EO = 20000 ng/l).

with $n - 1$ degrees of freedom and 97% confidence level of five replicates of the 500 ml spiked pure water samples at a concentration 10 times the lowest working standard for quantitative calibration, were relatively low (2.5–18 ng/l). Those values are about several times lower than the GC–MS method [12] and about one order of magnitude lower than the LC–MS method [8]. These results indicate the high performance of the developed analytical method.

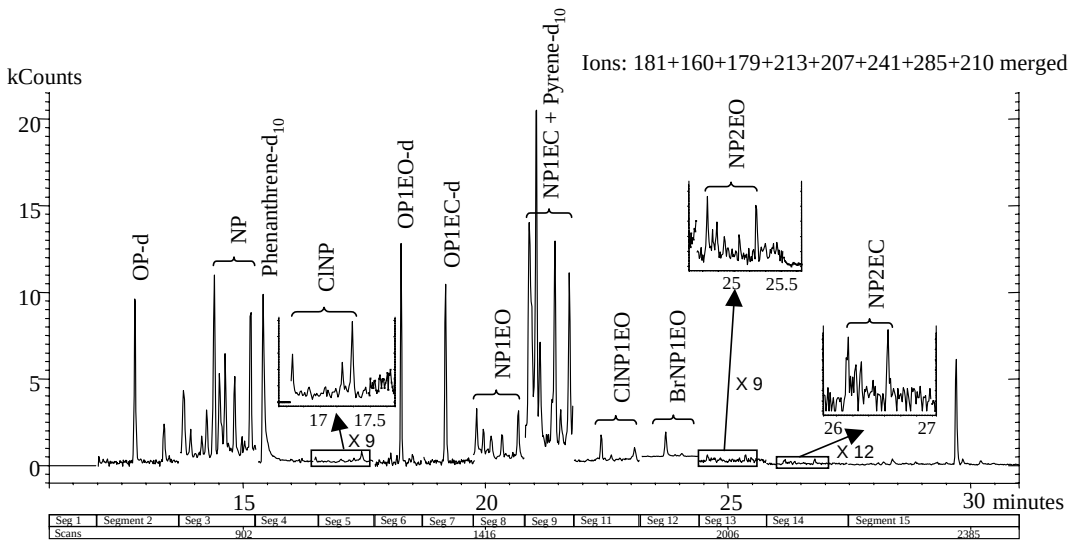

Fig. 5. Tandem mass chromatogram of NP related compounds detected in Neya river.

3.3.3. Application to environmental samples

The analytical performance of the developed method was tested through the recoveries of the analytes from two river samples. The analytes were spiked in the Ina river water (500 ml) and Neya river water (200 ml) in order to obtain their final concentrations at 20 and 2000 ng/l (except for BrNP, NP2EC and NP3EO), respectively. These results are summarized in Table 3. The high recovery values of 78–111 and 80–103% for the Ina and Neya river samples were obtained, respectively.

The concentrations of the analytes in the Neya river were measured about two orders of magnitude higher than those from the Ina river (Table 3). NP, BrNP1EO, NP1EC and NP2EO were detected at ng/l levels in the Ina river. The concentrations of NP1EC (2500 ng/l) and NP2EC (4900 ng/l) were much higher than those of NP1EO (480 ng/l) and NP2EO (560 ng/l) in the Neya river. Furthermore, the halogenated compounds (ClNP, ClNP1EO and BrNP1EO) were measured at hundreds of ng/l levels in the Neya river. Interestingly, BrNP1EC and ClNP1EC were not detected. The high recoveries (73–108 and 83–103%, data not shown) and good R.S.D. values (4.0–8.7 and 6.2–9.6%, data not shown) of the surrogates were obtained for the Ina and Neya rivers, respectively, indicating the high reliability of these data. A typical tandem mass chromatogram is shown in Fig. 5.

4. Conclusions

The simultaneous determination of the degradation products of NPnEOs and their halogenated derivatives was effectively demonstrated using trimethylsilylation and GC–EI–MS–MS. The C_{18} cartridge and methyl acetate amended with 0.25 mM HCl was found to be applicable for the extraction and elution of all the analytes. BSA was the effective derivatizing reagent, especially for the NPECs. Only 0.2% water present in the derivatizing medium reduced the derivatization yields of the NPECs. Further research on the toxicity as well as the occurrence of these compounds in receiving water and in sediments, particularly in the effluents of a wastewater treatment plant where chlorine is used for the disinfection process, is now in progress.

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References

[1] S.J. Jobling, J.P. Sumpter, Aquat. Toxicol. 27 (1993) 361.
[2] E.J. Routledge, J.P. Sumpter, Environ. Toxicol. Chem. 15 (1996) 241.
[3] T. Nishihara, J. Nishikawa, T. Kanayama, F. Dakeyama, K. Saito, M. Imagawa, S. Takatori, Y. Kitagawa, S. Hori, H. Utsumi, J. Health Sci. 46 (2000) 282.
[4] M. Reinhard, N. Goodman, K.E. Mortelmans, Environ. Sci. Technol. 16 (1982) 351.
[5] F. Ventura, A. Figueras, J. Caixach, I. Espadaler, J. Romero, J. Guardiola, J. Rivera, Water Res. 22 (1988) 1211.
[6] M. Fujita, M. Ike, K. Mori, H. Kaku, Y. Sakaguchi, M. Asano, H. Maki, T. Nishihara, Water Sci. Technol. 42 (2000) 23.
[7] P.L. Furguson, C.R. Iden, B.J. Brownawell, Anal. Chem. 72 (2000) 4322.
[8] M. Petrovic, A. Diaz, F. Ventura, D. Barceló, Anal. Chem. 73 (2001) 5886.
[9] H. Maki, H. Okamura, I. Aoyama, M. Fujita, Environ. Toxicol. Chem. 17 (1998) 650.
[10] J.A. Field, R.L. Reed, Environ. Sci. Technol. 30 (1996) 3544.
[11] W.H. Ding, S.H. Tzing, J. Chromatogr. A 824 (1998) 79.
[12] R.A. Rudel, S.J. Melly, P.W. Geno, G. Sun, J.G. Brody, Environ. Sci. Technol. 32 (1998) 861.
[13] J.A. Field, R.L. Reed, Environ. Sci. Technol. 33 (1999) 2782.
[14] D. Li, J. Park, J.R. Oh, Anal. Chem. 73 (2001) 3089.
[15] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[16] A. Diaz, F. Ventura, M.T. Galceran, Anal. Chem. 74 (2002) 3869.
[17] D.Y. Shang, R.W. Macdonald, M.G. Ikonomou, Environ. Sci. Technol. 33 (1999) 1366.
[18] A.D. Corcia, R. Cavallo, C. Crescenzi, M. Nazzari, Environ. Sci. Technol. 34 (2000) 3914.
[19] P.L. Furguson, C.R. Iden, B.J. Brownawell, J. Chromatogr. A 938 (2001) 79.
[20] N. Jonkers, T.P. Knepper, P.D. Voogt, Environ. Sci. Technol. 35 (2001) 335.
[21] A. Marcomini, A.D. Corcia, R. Samperi, S. Capri, J. Chromatogr. 644 (1993) 59.
[22] G.E. Stokker, A.A. Deana, S.J. deSolms, E.M. Schultz, R.L. Smith, E.J. Cragoe Jr., J.E. Baer, C.T. Ludden, H.F. Russo, A. Scriabine, C.S. Sweet, L.S. Watson, J. Med. Chem. 23 (1980) 1414.
[23] H. Kammerer, K. Eberle, V. Bohmer, M. Grossmann, Makromol. Chem. 176 (1975) 3295.

[24] S. Giovanni, B. Franca, J. Chem. Soc., Perkin Trans. 1 (1997) 257.
[25] H.B. Lee, T.E. Peart, Anal. Chem. 67 (1995) 1976.
[26] T.R. Croley, B.C. Lynn Jr., Rapid Commun. Mass Spectrom. 12 (1998) 171.
[27] R.J.W. Meesters, H.Fr. Sohröder, Anal. Chem. 74 (2002) 3566.
[28] U. Bolz, W. Körner, H. Hagenmaier, Chemosphere 40 (2000) 929.
[29] M. Kojima, S. Tsunoi, M. Tanaka, J. Chromatogr. A 984 (2003) 237.
[30] T.F. Wheeler, J.R. Heim, M.R. LaTorre, A.B. Janes, J. Chromatogr. Sci. 35 (1997) 19.