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Improved cleanup technique for gas chromatographic–mass spectrometric determination of alkylphenols from biota extract

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Abstract

A simple and economical cleanup technique was developed to determine alkylphenols by GC–MS from biological extracts containing relatively high lipids. The lipids were successfully removed from bivalve extracts through a two-step cleanup. The new method is a combination of Florisil adsorption chromatography and silyl derivatization technique. Low and high (non-polar and highly polar) molecular weight lipids were removed from the biota extract with deactivated Florisil column in the first step. And in the second step, middle molecular weight (middle polar) lipids were removed in an activated Florisil column after the alkylphenols were converted to corresponding silyl derivatives with bis(trimethylsilyl)trifluoroacetamide (BSTFA). On the basis of the above results, a simple cleanup kit was developed for convenience. The technique was optimized with reference to the activity of packing materials and polarity of eluting solvents. Only 3 g of Florisil, 25 mL of hexane and 10 mL of dichloromethane were required for one sample. The recoveries of alkylphenols from spiked samples varied from 88 to 103% with a low relative standard deviation (mean value: 5.3%) and the recovery was similar or even higher than other methods currently in use. The technique was successfully applied to mussel samples from Masan Bay, South Korea. Simultaneous measurement of these compounds in water, sediment and biota; the resulting bio-concentration factor and their relationships confirm previously published works, validating the method applied.

Keywords: Alkylphenols; Nonylphenols; Biota; Cleanup; Lipids; GC–MS

1. Introduction

Alkylphenols (APs) are the degradation products of alkylphenol polyethoxylates (APnEOs) during wastewater treatment [1]. APnEOs have been widely used in the preparation of detergents, wood preservatives in households and industries. Approximately 500,000 t of APnEOs are produced globally each year [2]. The main APs used are nonylphenols (NPs) and octylphenols (OPs), with nonylphenol polyethoxylates (NPnEOs) taking approximately 80% of the world market, and octylphenol polyethoxylates (OPnEOs) taking the remaining 20% [3].

There are several reports relating to their distribution characteristics, persistency, toxicity and estrogenic properties. During the last several decades, APs such as NPs and OPs were detected in various matrices such as river water, seawater, suspended particles and sediment samples [4–7]. APs are stable in the environment with a half-life of about 2 months in water and even years in sediments [2]. APs are toxic to organisms with LC_{50} values between 50 and 2000 μg/L in different species [8]. Toxicity may occur when they partition to lipid membranes in mitochondria, leading to uncoupling of energy production [9]. The toxicity of APs usually increases as the length of the hydrophobic chain increases [10]. In particular, endocrine disrupting properties of APs are important. APs structure is similar to that of the natural estrogen 17β-estradiol. APs affect the estrogen receptors in the same way as 17β-estradiol. They influence the males to have some female characters under long-term exposure due to endocrine disruption. Ishibashi et al. reported that 4-NP adversely affect the reproduction (fecundity and fertility) of adult *medaka* and accumulated in eggs with adverse effects [11]. Human exposure to APs, in particular, can result from different routes, like seafood contamination and water supply. It is estimated from the seafood intake of Italians that their total NPs intake was 12.2 μg day^{-1} $person^{-1}$ and OPs intake was 0.05 μg day^{-1} $person^{-1}$ [12]. NPs are found to be ubiquitous [13], present in human blood and urine [14,15]. Thus, a

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potential impact on organisms and human health is expected from these chemicals. Therefore, a detailed study on the effect of APs is needed. However, our current knowledge on the fate and transport of APs is insufficient to adequately assess the risk of incorporated concentrations of these chemicals in organisms and their effect on the ecosystem. Hence the analysis of the phenolic compounds in organisms is essential.

Most approaches towards the analysis of APs, utilize HPLC [16,17], LC–MS [18,19], and GC–MS [20,21]. GC–MS is widely used for its high sensitivity. However, biological samples such as mussel, oyster, and fish are unsuitable for GC–MS analysis due to the presence of large amount of lipids and other interferences. These co-extracts will interfere in the final instrumental determination if not removed. Obviously, lipids overload in GC columns deteriorate the chromatographic performance rapidly. Selective cleanup techniques need to be developed to overcome this. But separation of trace amount of APs from a large portion of lipids is very difficult. There are several methods in use for the removal of co-extracted lipids. Gel permeation chromatography (GPC) [22] is used but the main disadvantage of the GPC system is the difficulty to completely remove lipids, more over it requires large volume of solvent which is expensive and time consuming [23]. On the other hand, classical column chromatography using absorbents such as silica gel, alumina, and Florisil are also applied for the removal of lipids [24,25]. These treatments are tedious, solvent and time consuming. Matrix solid-phase dispersion extraction (MSPD) is efficient, time and solvent saving, but the difference in rubbing granularity sizes and priming would change the elution curve, so standardization of the method is difficult [26]. Solid-phase extraction (SPE) is simple and convenient [27], but it is advantage to liquid samples and its enrichment ability is restricted. Thus it is necessary to develop a convenient, economical, highly efficient and reliable cleanup technique for GC–MS determination of APs for biota samples.

Phenols are amenable to GC analysis without derivatization, but at lower concentration, peak tailing might occur and give low sensitivity and reproducibility, especially when environmental samples are analyzed [28,29]. In our previous works, silyl derivatization techniques using bis(trimethylsilyl)trifluoroacetamide (BSTFA) was successfully applied to the cleanup and determination of phenolic analytes in various environmental water and sediment samples [30]. On this basis, we developed an improved cleanup technique for GC–MS determination of APs for biota samples.

2. Experimental

2.1. Chemicals and reagents

Alkylphenol standards [4-*tert*-butylphenol, 4-*n*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*tert*-octylphenol, 4-*n*-octylphenol, 4-octylphenols (technical mixture) and 4-nonylphenols (technical mixture)] were purchased from Chem Service (Hatfield, PA, USA) and the purities were higher than 99%. GC internal standards (I.S.s) [$^2H_{10}$]phenanthrene (phenanthrene-d_{10}), surrogate standard (*n*-heptylphenol) and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA: 99%) were purchased from Chem Service (USA). Dichloromethane, hexane, acetone and methanol were purchased from Aldrich (St. Louis MO, USA). All solvents were pesticide grade. For sample cleanup, Florisil (LC grade) obtained from Supelco (Bellefonte, PA, USA), after activation at 400 °C for 12 h, was used for column cleanup. Anhydrous sodium sulfate and all glassware were burned at 400 °C for 12 h and washed with solvent before using.

2.2. Instrumental analysis

Target compounds and low polar lipids were separated using a Shimadzu GC2010 (Tokyo, Japan) with DB-5MS capillary column (30 m × 0.32 mm i.d.; coated film thickness: 25 μm). One microliter of sample was introduced by splitless mode using an autoinjector AOC-5000 from Shimadzu. The temperature of the injection port was 280 °C. The oven temperature was held at 80 °C for 2 min, then elevated to 100 °C at 20 °C/min, from 100 to 200 °C at 10 °C/min, and from 200 to 280 °C at 20 °C/min, and finally held at 280 °C for 2 min. A Shimadzu QPMS 2010 mass spectrometer was interfaced with the chromatographic system. The interface temperature was 280 °C. The selected ion-monitoring (SIM) mode was used with a sampling rate of 0.2 s. Quantification was done by an internal standard method. The GC internal standards were added to the extract before the instrumental analysis. To control the experimental quality, *n*-heptylphenol was used as a surrogate standard in real sample analysis. Samples were spiked with surrogate before extraction.

The middle polar lipids were analyzed with a Shimadzu HPLC 6A system equipped with gradient pump, 10 μL injection loops, and a C_{18} analytical column (250 mm × 4.6 mm). The constant flow was kept at 1 mL/min. The solvent composition was CH_3OH:H_2O = 85:15.

2.3. Preparation of spiked biota extract and real samples

Twenty grams of non-contaminated dry biota samples (mussel and oyster) were weighed and transferred to a Soxhlet thimble. The Soxhlet extraction was performed with 200 mL of dichloromethane for 16 h, then concentrated and solvent was exchanged to 10 mL of hexane. The phenolic analyte standards were spiked to the biota extracts according to the level of experimental demand. The spiked biota extracts were vigorously shaken and then stored in a freezer at −20 °C until use. To determine alkylphenols from real environment, the mussel samples were taken from Masan Bay, South Korea. The samples were homogenized and frozen immediately. It was kept at −20 °C until use.

2.4. Extraction

Three grams of wet or 0.5 g of homogenized dry sample was weighed exactly. Fifty microliters of 5 mg/L surrogate standard was spiked for quality control. Then it was transferred into a Soxhlet extractor and extracted with 200 mL of dichloromethane for 16 h. The extract was collected and rotary-vacuum

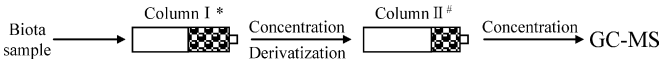

Fig. 1. Schematic diagram of cleanup unit. The Column I* contains 5% deactivated Florisil absorbent (2 g). The non-polar and slightly polar solvents were used as mobile phase. As a result, APs (in slightly polar fraction) were separated from non-polar lipids (in non-polar fraction) and high polar lipids (retain in the column). Column II# contains fully activated Florisil absorbent (1 g). Only non-polar solvent was used as mobile phase. So, only silyl derivatized APs (non-polar) were eluted from the column and middle polar lipids were still retained in the column.

evaporated to 1 mL at 40 °C. To remove water from the extract, 0.5–2 g of anhydrous sodium sulfate was added to every extract. Then the extract was transferred into an 8 mL glass vial by rinsing twice with 1 mL of dichloromethane. It was concentrated to below 200 μL under a gentle stream of nitrogen at room temperature. Then the solvent was exchanged to about 200 μL of hexane. It was subjected to cleanup column or kit, for further analysis.

2.5. Cleanup and derivatization

The cleanup course contained two steps (Fig. 1). The first step was performed in column I. A part of non-polar and high polar lipids were removed through column I. Column I contains 2 g of hexane-rinsed Florisil (deactivated with 5% H_2O) between two 1.5 g anhydrous sodium sulfate layers in a 10 mL glass syringe. A standard solution or an extract of biota samples was added on to the column. Hexane (18 mL) was eluted first and discarded, and then phenolic analytes were eluted with 10 mL of dichloromethane. The first 2 mL was discarded, and the next 8 mL was collected, it was concentrated with a gentle stream of nitrogen, and then the solvent was exchanged with about 200 μL of hexane for column II [20]. The column II contains 1 g of activated Florisil between two anhydrous sodium sulfate layers in a 10 mL glass syringe. A 0.7 mL aliquot of acetone was added to the sodium sulfate layer as solvent medium for derivatization and then followed by addition of 100 μL of BSTFA (99% in 1% TMCS). The sample was added. The derivatization reaction can be completed for 15 s. The analytes (alkylphenol silyl derivatives) were eluted with 3 mL of hexane twice. The elute was concentrated to below 0.2 mL with a gentle stream of nitrogen followed by addition of I.S.s (50 μL × 5 mg/L) and then it was subjected to GC–MS analysis after the final volume was set to 0.5 mL.

To improve the convenience of a cleanup course, a cleanup kit was designed as shown in Fig. 2. An adapter with three-way valves was used for collection and use of elution, concentration of solvent, derivatization of APs and connection of the two columns. On the basis of this kit, a semi-automatic cleanup step was possible.

3. Results and discussion

Extracts of biota samples such as oysters, mussels and fish contain large amount of lipids such as fats, wax, fatty acids, steroids, phospholipids, *etc*. These lipids affect GC–MS determination of APs. Therefore, it is necessary to separate APs from

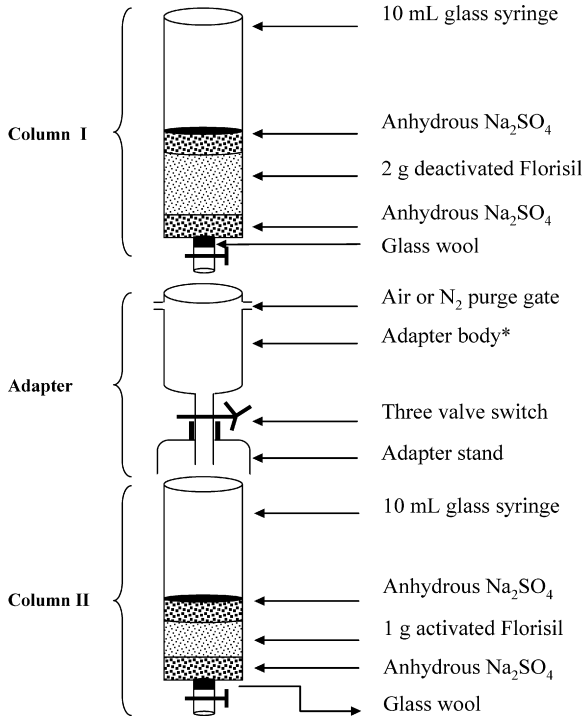

Fig. 2. Semi-automatic cleanup kit assembly for the separation of APs from biota extracts. *The adapter body was used in collection and concentration of eluting solvents, derivatization of APs and connecting Columns I and II.

lipids before GC–MS analysis. Physical and chemical properties of the lipids are much similar to those of APs due to similar alkyl and hydroxyl groups. As a result, separation of the lipids and APs is much more difficult with traditional chromatography and chemical reaction techniques. Li et al. [20,31] reported simple and fast APs cleanup techniques and it was successfully applied to sediment samples. But the technique could not be directly applied to biota samples due to lipid overload. Thus an improved cleanup technique combining the Florisil adsorption chromatography and silyl derivatization was taken up.

OPs were selected as target analytes for optimization of various cleanup conditions. It is reported that 4-octylphenols (4-OPs) was one of the key APnEOs metabolites [32,33]. In this work after acceptable results were obtained from a series of experiments using OPs, the same analytical procedure was applied to other phenolic analytes and also to real environmental samples.

3.1. Remove low and high polar lipids from biota extract

In the liquid absorbent chromatographic separation techniques, absorbent and eluting solvents are most important amongst the various parameters. Lipids were roughly classified as three groups: low (fatty acid, *etc*.), middle (steroids, *etc*.) and high polar lipids (phospholipids, *etc*.). Based on polarity of lipids and APs, Florisil was selected as an absorbent, and then the effects of Florisil activity to the recoveries of OPs were studied.

The OPs standards (1 mL × 1 mg/L) dissolved in hexane were introduced into the surface of the column containing 2 g of

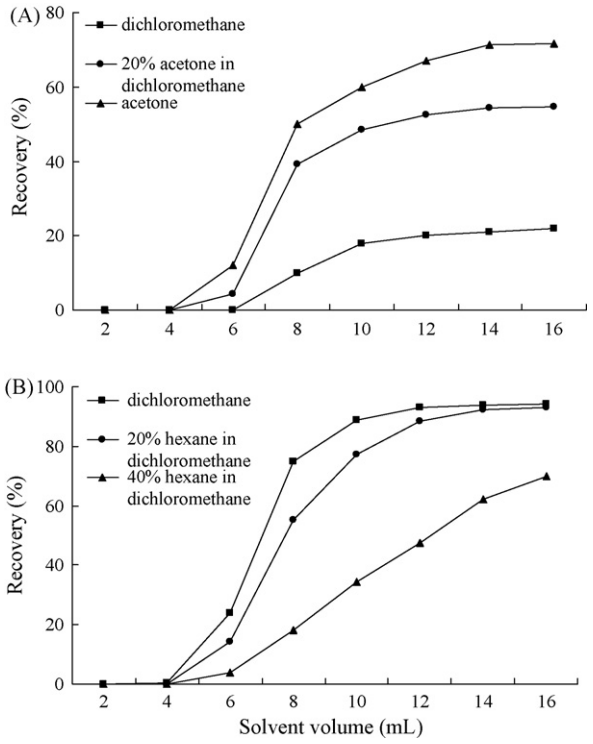

Fig. 3. Comparison of the elution efficiency in Column I with reference to solvent polarity and activity of florisil ($n=7$). (A) Elution efficiency of 0, 20 and 100% acetone in dichloromethane in Column I packed with fully activated florisil; (B) elution efficiency of 0, 20 and 40% hexane in dichloromethane in Column I packed with deactivated (5%) Florisil. OPs standards (1 mL × 1 mg/L) were used. *Note*: The first 18 mL hexane elution was discarded.

activated or deactivated (5%) Florisil (Column I), and then it was eluted with various solutions. For activated Florisil absorbent columns, target OPs were eluted with 0, 20 and 100% acetone in dichloromethane after elution of 18 mL of hexane which contained low polar lipids such as fatty acids and wax. As shown in Fig. 3A, low recoveries were obtained even while using polar eluting solvent such as acetone. This may be due to strong interaction between Florisil and alkylphenols. In this case, more polar solvent is also not suitable for elution due to co-elution of high polar lipids which affect silyl derivatization and GC–MS determination.

In the case of deactivated Florisil absorbent columns, the OPs were eluted with 0, 20 and 40% hexane in dichloromethane after elution with 18 mL of hexane. As shown in Fig. 3B, the elution recovery increased with an increase in the polarity of eluting solvent. Just 10 mL of slightly polar dichloromethane solvent was needed for eluting OPs. As a result, excellent recovery (≥94%) was obtained with 5% relative standard deviation.

In order to characterize the lipids eluted from column I, the biological extract spiked with OPs was treated in the same way. The hexane fraction was analyzed in GC–MS after the silyl derivatization and dichloromethane fraction was analyzed with HPLC. As shown in Fig. 4A, various fatty acids (hexadecenoic acid, octadecanoic acid, eicosatetraenoic acid, *etc*.) and wax (pentadecane, hexadecane, octadecane tetradecadienic acetate, *etc*.) were eluted in the hexane fraction. The typical liquid chromatogram of dichloromethane fraction was shown in Fig. 4B. Many kinds of lipids still remained in the dichloromethane fraction. However, the target analyte, OPs from the biological extract were completely separated with low polar and high polar lipids due to strong interactions with Florisil.

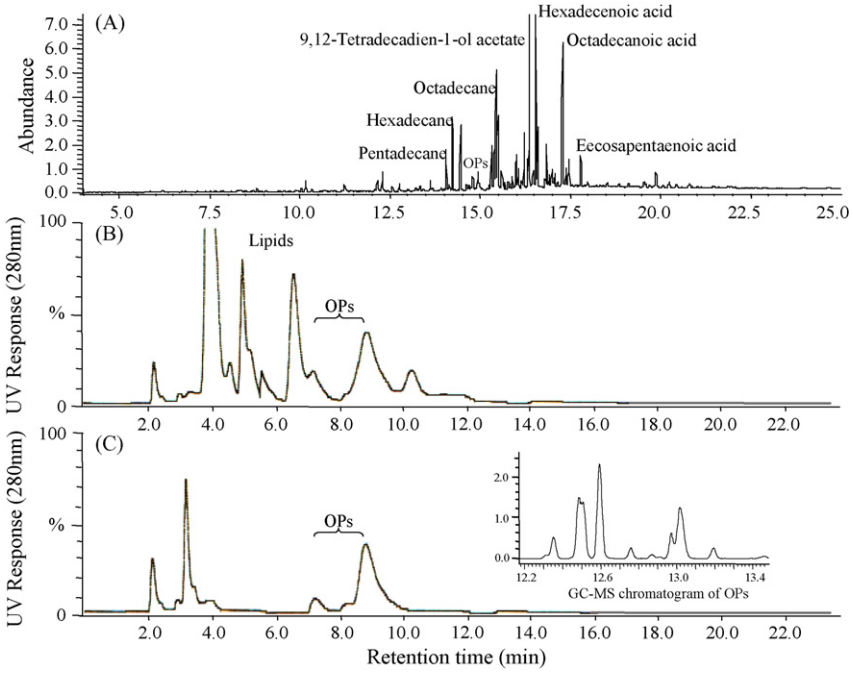

Fig. 4. Removal of lipids in spiked biota extracts. (**A**) GC–MS chromatogram of first 18 mL hexane elution in Column I. This fraction contained many fatty acids and waxes. (B) HPLC-UV chromatogram of 8 mL dichloromethane elution in Column I. This fraction contained middle polar lipids. (**C**) HPLC-UV chromatograms of 8 mL hexane elution through Column II. The OPs were fully separated from the lipids containing biota extracts. The picture in the insertion was GC–MS chromatogram of this fraction.

In summary, although the fraction containing OPs carry lipids (middle polar lipids), it is possible to separate them from the low polar and high polar lipids using the traditional Florisil absorbent liquid chromatographic technique.

3.2. Removal of middle polar lipids from biota extract

As discussed in the previous section, some lipids (middle polar lipids) were retained in the dichloromethane fraction which contained OP analytes. The polarities of the lipids are similar to those of OPs, so they showed similar elution behavior in the liquid absorption chromatography. In order to separate OPs analytes from the lipids, it is necessary to make a difference in their polarity.

It is reported that APs can be easily converted to low polar silyl derivatives in a medium such as acetone [20]. Also alkylphenolic silyl derivatives could improve various gas chromatographic parameters of APs, such as accuracy, reproducibility, sensitivity, and resolution by suppressing tailing and enhancing thermal stability. The mass spectrometric properties of the derivatives could also be improved by producing favorable diagnostic fragmentation patterns for structure investigation and characteristic ions for selected ion monitoring in trace analyses.

The dichloromethane fraction containing middle polar lipids and OPs were concentrated and derivatized with BSTFA in acetone medium. It was concentrated again, and then subjected to a second column separation which contained 1 g of activated Florisil. The derivatized APs were easily eluted with a nonpolar hexane solvent due to their weak polarity. Only 6 mL of hexane was used as an eluting solvent and it was subjected to HPLC/UV (Fig. 4C) analysis after concentrating it to an appropriate volume. As shown in Fig. 4C, the lipids found in the dichloromethane fraction from column I disappeared completely after a second cleanup. Because 18 mL of hexane was eluted in the first cleanup step (column I), the hexane fraction in the second cleanup step (column II) did not contain that part of lipids, and the recoveries of OPs in the second cleanup step ranged from 92 to 103% with an average of 97%.

In summary, it is possible that alkylphenolic analytes could be separated from low, middle and high polar lipids using combined liquid absorbent chromatography and derivatization techniques.

3.3. Preparation of alkylphenols cleanup kit

By using the above experimental results, APs cleanup kit was designed as shown in Fig. 2. The kit consisted of three parts: column I, adapter and column II. The sample collection, concentration, transfer and derivatization were performed in an adapter system including connection to columns I and II. Fabrication and operation becomes much simpler by using this kit. The experimental procedures were as following. The second fractions from the column I were collected with the adapter and it was simultaneously evaporated to just dryness using stream of nitrogen. A 0.7 mL of acetone and 100 μL BSTFA was added sequent to the adapter for derivatization. After waiting for several seconds, the reactant was dropped into column II. And then it was eluted with 6 mL hexane. The cleanup procedure for biota extract could be finished within 10–15 min with a high recovery (≥90%). In addition, it gives minimum contamination because the works were conducted in one continuous system.

3.4. Analytical features

A series of experiments were carried out systematically to evaluate the applicability of the kit to environmental samples. Initially, recovery and reproducibility of the cleanup treatment kit, was evaluated using different concentrations of spiked biota extract (mussels and oysters), and then the results were compared with that of pure standards. The results are given in Table 1. The recoveries and relative standard deviations (R.S.D.) ranged from 88 to 103% with an average of 95% and from 0.4 to 7.6% with an average of 5.3% for spiked biota extract, respectively. The results were very similar to those obtained in the standards. The similarities (recovery from standards/recovery from spiked biota extract) ranged from 0.93 for 4-*n*-butylphenol to 1.1 for 4-*n*-OP. The recovery was similar or even higher than those obtained from other methods currently in use [22,26,29]. The typical mass chromatogram for spiked biota extract is given in

Table 1
Recovery of pure chemicals and spiked chemicals from the cleanup kit applied in the separation of lipids and alkylphenolic analytes in biota extracts

Compounds	MDL[a] (ng/g)	5 ng/g[a]		250 ng/g[a]		1000 ng/g[a]	
		Standard sample ($n=7$)	Spiked sample ($n=7$)	Standard sample ($n=7$)	Spiked sample ($n=7$)	Standard sample ($n=7$)	Spiked sample ($n=7$)
4-*tert*-Butylphenol	1	94.4 ± 5.3	88.0 ± 7.6	93.5 ± 2.5	90.3 ± 6.8	94.6 ± 1.2	91.4 ± 4.6
4-*n*-Butylphenol	1	92.6 ± 2.4	90.1 ± 5.9	93.4 ± 2.0	92.4 ± 5.8	91.5 ± 1.0	90.2 ± 5.3
4-*n*-Pentylphenol	1	94.2 ± 4.5	92.3 ± 6.3	94.6 ± 2.5	94.5 ± 4.9	96.8 ± 1.2	92.2 ± 5.1
4-*n*-Hexylphenol	1	95.8 ± 2.0	94.7 ± 4.5	96.5 ± 1.4	96.0 ± 4.9	97.1 ± 0.8	93.5 ± 0.4
4-*n*-Heptylphenol	1	94.3 ± 1.8	91.2 ± 7.4	95.8 ± 0.8	101.1 ± 5.7	95.5 ± 0.8	98.2 ± 5.0
4-*tert*-Octylphenol	3	93.2 ± 4.6	93.7 ± 5.2	96.8 ± 1.2	100.3 ± 5.9	98.2 ± 0.6	96.8 ± 4.5
4-*n*-Octylphenol	1	94.4 ± 4.3	92.5 ± 4.9	96.2 ± 1.5	97.6 ± 5.3	95.3 ± 1.0	102.1 ± 4.4
Nonylphenol	3	98.6 ± 1.2	103.2 ± 5.6	99.4 ± 0.4	101.5 ± 7.1	98.4 ± 0.7	100.4 ± 3.8
Average	1.5	94.7 ± 3.3	93.2 ± 5.9	95.7 ± 1.5	96.7 ± 5.8	95.9 ± 0.9	95.5 ± 4.1

MDL: method detection limit.
[a] Recovery (%) ± RSD (%).

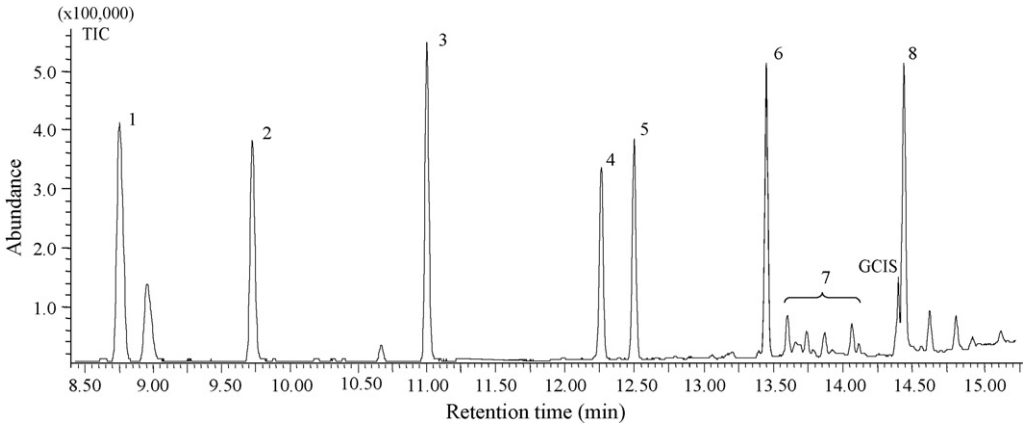

Fig. 5. Chromatogram of phenolic analytes in biota extracts after two steps cleanup kit. (1) 4-*tert*-Butylphenol (*t*-BP), (2) 4-*n*-butylphenol (*n*-BP), (3) 4-*n*-pentylphenol (*n*-PP), (4) 4-*n*-hexylphenol (*n*-HexP), (5) 4-*tert*-octylphenol (*t*-OP), (6) 4-*n*-heptylphenol (*n*-HepP), (7) nonylphenol (NP), and (8) 4-*n*-octylphenol (4-*n*-OP).

Fig. 5. In this chromatogram, few interfering peaks are observed. Thus, a simple, fast and economical cleanup for APs in biota extract is possible. The advantage of the method is that it is highly sensitive and reproducible.

The method detection limits (MDLs) were also calculated from seven replicate samples at 2 ng/g levels using a 0.5 g of dry biota sample and the final volume was set at 0.5 mL. MDL was derived by using the following formula: MDL = SD × t + blank, where $t = 3.143$ for $n = 7$ at 98% confidence level and SD is the standard deviation. As shown in Table 1, MDLs of NPs and *t*-OPs were 3 ng/g and about 1 ng/g for other six APs.

3.5. Application to biota extracts

The method was applied for the analysis of NPs in mussels from the Masan Bay of Korea. The analytical results are shown in Table 2. NPs were detected in every sample and the concentrations determined ranged 50.5–289.2 ng/g dry weight. The recoveries of heptylphenol (surrogate) are between 85.8 and 97.5% and the RSD values were lower than 11.3%. The concentrations of NPs in water and sediment sampled at the same site and time are also shown in Table 2. The applicability of this technique, including the cleanup kit can be established from measuring the concentration of NPs in water, sediment and biota, their relationships and by calculating the bio-concentration factor (BCF). As shown in Fig. 6, the concentrations of NPs in mussels showed high correlation with that of water ($R^2 = 0.98$) and sediments ($R^2 = 0.90$). For sediment, because the sediment was containing large amount of sand, NPs was not detected at St5 (shown in Table 2). The BCF was obtained using the following equation:

$$\text{BCF} = \frac{\text{NPs concentration in mussel}}{\text{NPs concentration in water}}.$$

On the basis of the above equation, the average BCF was 2990. Ekelund et al. reported BCF of 4-NP in mussels to be 3400 in laboratory experiments [34]. Recently, Cheng et al. reported BCF of 4-NP in oysters to be 2900 from the coast of Taiwan [33]. These results are in excellent agreement with our results. This strongly suggests that the cleanup treatment kit could be successfully applied to the analysis of APs in real environmental samples and the method is applicable to samples with high lipid content.

Table 2
Concentrations of NPs in water, sediments and mussels from the Masan Bay, Korea

Sites	Concentration of nonylphenols		
	Water[a] (ng/L)	Sediment (ng/g dw)	Mussel (ng/g dw)
St1	94 (6.2)[b]	447 (3.6)	289.2 (8.3)
St2	54 (7.4)	364 (5.5)	169.5 (12.3)
St3	26 (4.5)	92 (10.2)	76.8 (5.4)
St4	22 (3.2)	110 (7.7)	50.5 (6.8)
St5	32 (7.6)	NA	111.0 (6.5)

[a] In order to obtain a representative value, concentrations of nonylphenol in seawater, for example St1, St2 and St5 were derived from five, three and five sampling stations, respectively.
[b] Relative standard deviation ($n = 3$). NA: not analyzed.

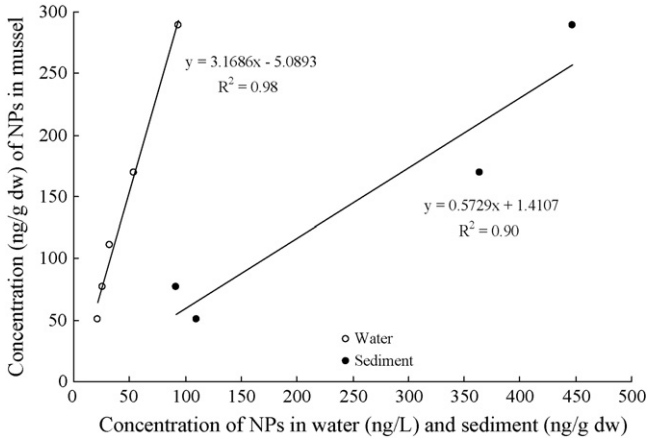

Fig. 6. Relationships of NPs in mussels against their concentrations in water and sediments.

4. Conclusions

By using an advanced, two-step cleanup combining adsorb chromatography and derivatization technique, we have demonstrated a substantial improvement in the separation of

alkylphenols from biota extracts containing large amount of lipids, and the alkylphenols may be detected reliably using GC–MS. The proposed method is a time saving and economic technique with high precision and sensitivity. Understanding distributions and behaviors of alkylphenols in environmental biota are now possible.

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