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Direct extraction of alkylphenols, chlorophenols and bisphenol A from acid-digested sediment suspension for simultaneous gas chromatographic–mass spectrometric analysis

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Abstract

The direct extraction of alkylphenols, chlorophenols and bisphenol A from an acid-digested sediment suspension for GC–MS analysis was studied. The sediment was digested with acid while the hydrolyzed analytes were being extracted with dichloromethane. The conditions of the acid digestion and extraction were optimized in terms of time, acidity of digestion, and extracting solvent. It is possible to complete the extraction within 20 min with 5 ml of 0.1 *M* HCl digesting solution and three portions of 5 ml of dichloromethane. The recoveries of analytes were mostly around 90% with about 10% relative standard deviations. With this technique parallel treatment of large numbers of sediment samples is possible without any expensive special equipment or heating process. The analytical characteristics of this extraction technique were compared with Soxhlet extraction and the pressurized liquid extraction technique. The technique was examined and evaluated for real environmental sediment samples and certified reference material of natural matrix.

Keywords: Extraction methods; Sediments; Environmental analysis; Alkylphenols; Chlorophenols; Bisphenol A; Phenols

1. Introduction

Analysis of alkylphenols (APs), chlorophenols (CPs) and bisphenol A (BPA) from environmental samples has become a subject of great interested because of their estrogenic health effects in human and wildlife. These chemicals and their degradation products can be transferred to the environment at relatively high concentrations through aquatic or atmospheric systems because of their high solubility in water and high vapor pressure [1–4]. The environmental fate, toxicity and estrogenic health effects of the phenolic compounds to aquatic organisms, bird and mammals have been studied extensively [4–11].

Sediments or suspended solids are good adsorbents of the phenolic contaminants because of their high surface area and surface activities. The sediments can adsorb and accumulate the compounds in relatively high concentrations and affect the aquatic lives. So the analysis of the phenolic compounds in the sediment or suspended solid samples has been

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studied extensively because of their importance in the monitoring of contamination level of environments. Several techniques have been applied for the extraction of phenolic analytes from solid matrices such as soil or sediment samples. To get high extractability, polar solvents such as methanol, acetone, acetonitrile or their water mixtures were used in earlier works [12–15]. However, the use of polar solvents for the extraction is not desirable because of the co-extraction problems of other interfering polar compounds such as polar pigments or organic acids requiring complicated cleaning processes. So the use of nonpolar solvents such as dichloromethane or hexane is preferred. Ahel and Giger [16] successfully extracted the phenolic compounds from soil matrix by steam distillation, but this is time-consuming and laborious. Soxhlet extraction is one of the most popular techniques because of its simplicity and inexpensive extraction apparatus. Both polar and nonpolar solvents can be used [17–21], however, it requires a large amount of extracting solvent and a long extraction. Recently, pressurized liquid extraction (PLE; Dionex trade name for accelerated solvent extraction) [13,22,23], supercritical fluid extraction (SFE) [14,24,25] and microwave-assisted extraction (MAE) [26] techniques have been developed and applied to the extraction of the phenolic analytes from soil or sediment samples. It is possible to reduce the extraction time and the amount of extracting solvent, but expensive special equipment is still required. Moreover, parallel extraction for large numbers of environmental samples is not possible because of the limited availability of the expensive extraction equipment. So the extraction process is still the limiting step in the analysis of phenolic analytes. Furthermore, the sample should be dried prior to the extraction in most cases. Elevated extraction temperature in Soxhlet extraction, PLE or MAE technique may also cause the transformation or degradation of the analytes, resulting in lowered recoveries and reproducibility [12–14].

It is hard to find a systematic approach to the extraction of phenolic analytes from solid sample matrices. Extraction following acid digestion is an alternate method reported. Sediment samples were digested with strong hydrochloric acid for more than 12 h in the analysis of pentachlorophenol from sediment samples [6]. Acidic solutions containing the analytes were filtered, then extracted with dichloromethane. The recovery of pentachlorophenol ranged from 88.0 to 93.2%. A sufficient digestion time and strong acid condition seemed to be chosen empirically. However, lowered recoveries of some phenolic analytes were experienced when strong acid was used in our work. Furthermore, the recovery and reproducibility suffered due to prolonged digestion under such severe conditions.

Therefore we systematically explored the digestion and extraction conditions to develop a quick, simple and reliable extraction method for phenolic analytes from sediment samples. Here we report the optimized extracting conditions; the analysis can be completed within 20 min under mild conditions. It is a very simple and affordable method with reproducibly high recoveries for the phenolic analytes. Neither special expensive equipment nor a heating process is required for this procedure.

2. Experimental

2.1. Reagents and standards

APs, CPs and BPA, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), gas chromatographic internal standards {[$^2H_8$]-naphthalene, [$^2H_{10}$]-phenanthrene and [$^2H_{10}$]pyrene-d10} and surrogate standard ([$^2H_{14}$]bisphenol A) were purchased from Chem Service. Pesticide grade dichloromethane, hexane, acetone and methanol were purchased from Aldrich. Concentrated hydrochloric acid was obtained from Merck and used after washing with dichloromethane. Stock solutions of 100 mg/l were used for the preparation of spiking standards, working calibration standards, GC internal standards and surrogate standard.

2.2. Instrumental analysis.

Target compounds were separated using a Shimadzu GC-17A with a DB-1 (30 m×0.32 mm I.D., 25 μm film) capillary column and detected with a Shimadzu MS QP-5000 mass spectrometer. The analytical conditions for GC–MS can be found elsewhere [27]. The internal standards were added to the extract before the instrumental analysis. To

control the experimental quality the [$^{2}H_{14}$]bisphenol A was used as a surrogate standard in real sample analysis. It was spiked into the samples before acid digestion of the phenolic analytes. The analytes were silyl derivatized with BSTFA in dichloromethane–acetone and cleaned up using a silyl derivatization treatment kit developed in our laboratory [27].

2.3. Preparation of spiked sediment and real samples.

A 100-g amount of dry sediment (particle size ≤120 μm) was weighed exactly in an amber glass bottle with a PTFE-lined cap. It was saturated with 120 ml dichloromethane. The standards of the phenolic analytes of 10 mg/l were spiked to the sediment to the level of 100 ng/g. The spiked sediment was tumbled for 12 h using a tumbler and followed by evaporation of dichloromethane at room temperature. The dried sediment was tumbled again for 1 day, and then stored in a freezer at −20 °C until use. Real sediment samples were taken from the Han River passing through a metropolitan area, Lake Shihwa surrounded by an industrial area and a contaminated coastal area of Masan Bay in South Korea. Natural matrix certified reference material (CRM117-100) was obtained from Resource Technology (USA). The wet sediment was homogenized and frozen immediately. It was kept at −20 °C until use to avoid degradation of the target analytes.

2.4. Acid digestion and extraction

A 3-g amount of wet or dried sediment sample was weighed and transferred into a 50-ml PTFE or glass centrifuge tubes with PTFE-lined cap. A 100-μl volume of 1 mg/l surrogate standard was spiked for quality control. HCl solution (5 ml) was added for digestion and shaken vigorously using a vortex mixer for an appropriate period. A further, 5 ml of dichloromethane was added to the suspension. The mixture was shaken vigorously for several minutes using a vortex mixer or a horizontal orbit shaker followed by centrifugation for several minutes at >3000 rpm. The dichloromethane extract was transferred into a 20-ml glass tube. The extraction was repeated twice more and the extracts were added to the glass tube. The extract was concentrated to 1 ml under a gentle flow of dry nitrogen gas. Anhydrous sodium sulfate was added to remove water. The extract was collected and transferred to an 8-ml glass vial by rinsing twice with 1 ml of dichloromethane. Activated copper powder was added to the extracts to remove sulfur. It was concentrated to about 200 μl with a gentle flow of dry nitrogen gas, then derivatized by silyl and cleaned-up using the silylation treatment kit mentioned earlier [27].

2.5. Other extraction techniques

PLE was performed for comparison with the direct extraction technique using a Dionex 2000 PLE instrument (Sunnyvale, CA, USA). A 3-g sample was loaded into 33-ml stainless cell, which was then filled with glass beads. It was extracted with dichloromethane at 100 °C and 150 atm. (1 atm.= 101 325 Pa). Two cycles of extraction with 5 min static time were performed [28]. Soxhlet extraction was performed with dichloromethane for 18 h at a rate of 15–20 min/cycle as recommended by the US Environmental Protection Agency (EPA) SW846 Soxhlet extraction method 3540.

3. Results and discussion

Extraction of the phenolic analytes from sediment samples occurred in a two-step process. Hydrolysis of adsorbed phenolic analytes occurred by acid digestion, followed by extraction from the acidic digestion solution to an organic layer. Either step can be dependent on the acidity of the digesting medium. Phenolic compounds can be dissociated to phenolate ions or protonated to form phenoxonium ions, depending on the acidity of solution and their chemical structures. Extractability to the organic layer is very dependent on the acidity of the aqueous layer. A mixture of phenolic analytes was spiked into HCl solutions having different acidities of 10^{-4} to 12 *M* to the level of 100 μg/l followed by prolonged equilibration for 12 h. The analytes were extracted with dichloromethane and analyzed by GC–MS after silyl derivatization. High extraction efficiencies were observed under the conditions of acidity examined for the most analytes except bisphenol A. A slight decrease in the recoveries was noticed under very

strong acidic conditions above 6 *M* HCl. It was surprising that the recovery of bisphenol A decreased sharply at above 6 *M* HCl. It is assumed that the preferred formation of charged phenoxonium ion under such strong acidic conditions [29,30] may lower the extraction efficiency of phenolic analytes to the organic solvent. Degradation of analytes, especially bisphenol A also cannot be excluded under these strong acidic digesting conditions for prolonged period.

Since the extraction efficiency of the phenolic analytes is not highly dependent on the acidity of aqueous layer except above 6 *M* HCl, the digestion conditions seemed to be most critical in the extraction process. In order to optimize the hydrolysis conditions the recovery was investigated. The spiked sediment was treated with various HCl concetrations for 3 h. Then the digested phenolic analytes were extracted with dichloromethane. Under acidic conditions <0.01 *M* HCl, acceptable results were observed for some, but not all the target compounds. From 0.01 to 3 *M* HCl acceptable results (recovery> 85%) were obtained for the most of the analytes. As the acidity increased, the recoveries increased gradually. Maximum recoveries were observed at 0.1 *M* HCl (Fig. 1). The recoveries ranged from 86% for pentachlorophenol to 98% for *tert*.-octylphenol with <5% relative standard deviations (RSDs). Then the recoveries decreased again as the acidity increased further. The binding capacities and equilibrium behaviors of the phenolic analytes to the sediment are dependent on the pH [3,31]. At high pH most phenolic analytes should exist in dissociated form, so-called 'phenolates', which may interact strongly with the sediment matrix resulting in strong absorption. Their hydrophilic characteristics prevent their extraction to the organic solvent. As the acidity of the digesting solution gradually increases, neutral associated forms, so-called 'phenols' become prevalent, which interact less strongly with the sediment

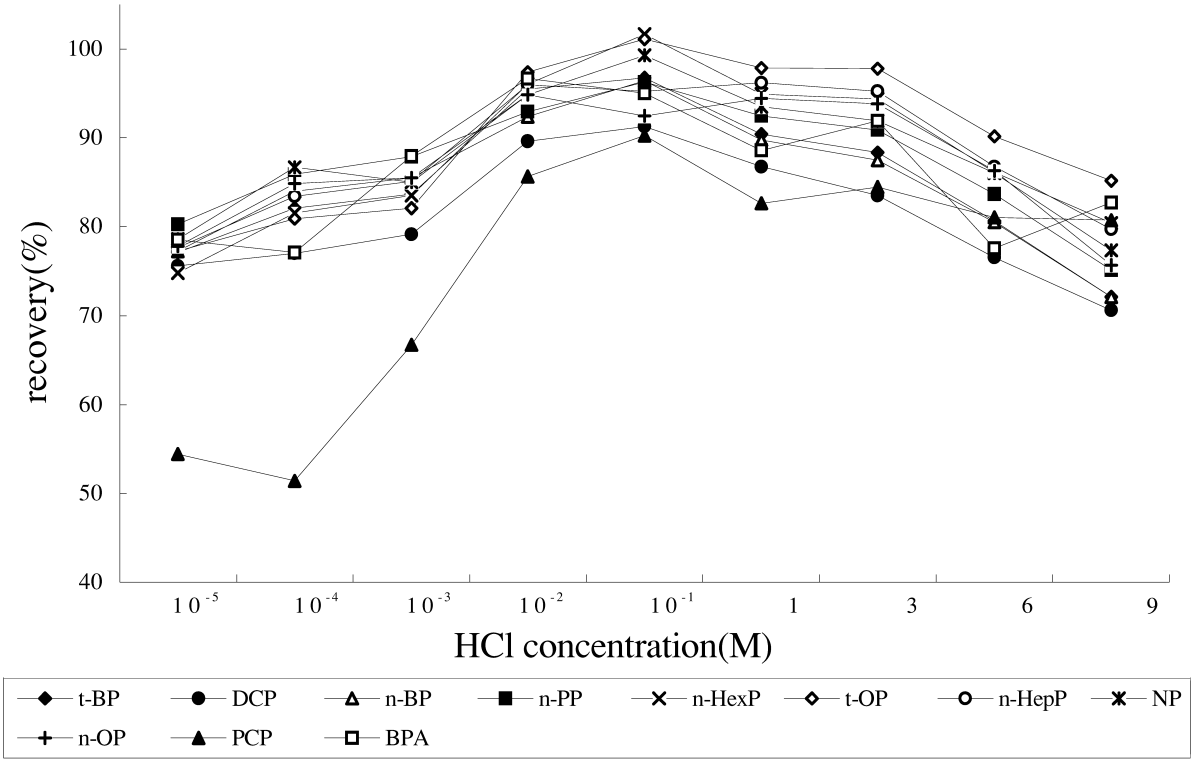

Fig. 1. Effect of acidity on the digestion efficiency represented as recoveries (n=6). 100 ppb spiked sediment sample was used. Refer the abbreviations for the analytes to Table 1).

matrix. At 0.1 *M* HCl, the phenolic analytes can be extracted easily to the extracting solvent resulting in high recoveries. However, under strong acidic conditions the phenolic analytes seem to form positively charged 'phenoxonium ions' which are strongly hydrophilic [30]. They may interact strongly with polar organic and inorganic matters in the sediment matrix resulting in lowered extractability.

Reduction of the acid digested extraction time is desirable because it is a time-consuming step. Moreover, the digestion time is an important factor affecting the accuracy of the analysis. Digestion kinetics was studied in terms of hydrolysis time (Fig. 2). Digesting acidity was controlled to 0.1 *M* HCl for the best results. Surprisingly the recoveries of the phenolic analytes completely leveled off within 5 min for the spiked samples. Longer hydrolysis—more than 3 h—resulted in slightly decreased recoveries. In the kinetic experiment for a real sediment sample contaminated with nonylphenol the recovery leveled off within 10 min (data not shown). Based on these results it can be concluded that the acid digestion can be completed within 10 min under optimized conditions.

The digestion and extraction can be performed simultaneously to shorten the procedure even more. The extracting solvent, dichloromethane can be added just after completion of wetting of the sediment by 0.1 *M* HCl in a centrifuge tube. The mixture should be shaken vigorously for 10 min followed by centrifugation. In this case the acid-digested extraction can be completed within 20 min. Wet sediment samples can be treated in the same way without the predrying step to save time, although the water content should be compensated for in the concentration calculation. The water content of the wet sample did not severely affect the acidity of the digesting solution and similar results to the dried sample were obtained.

The recoveries of the phenolic analytes were

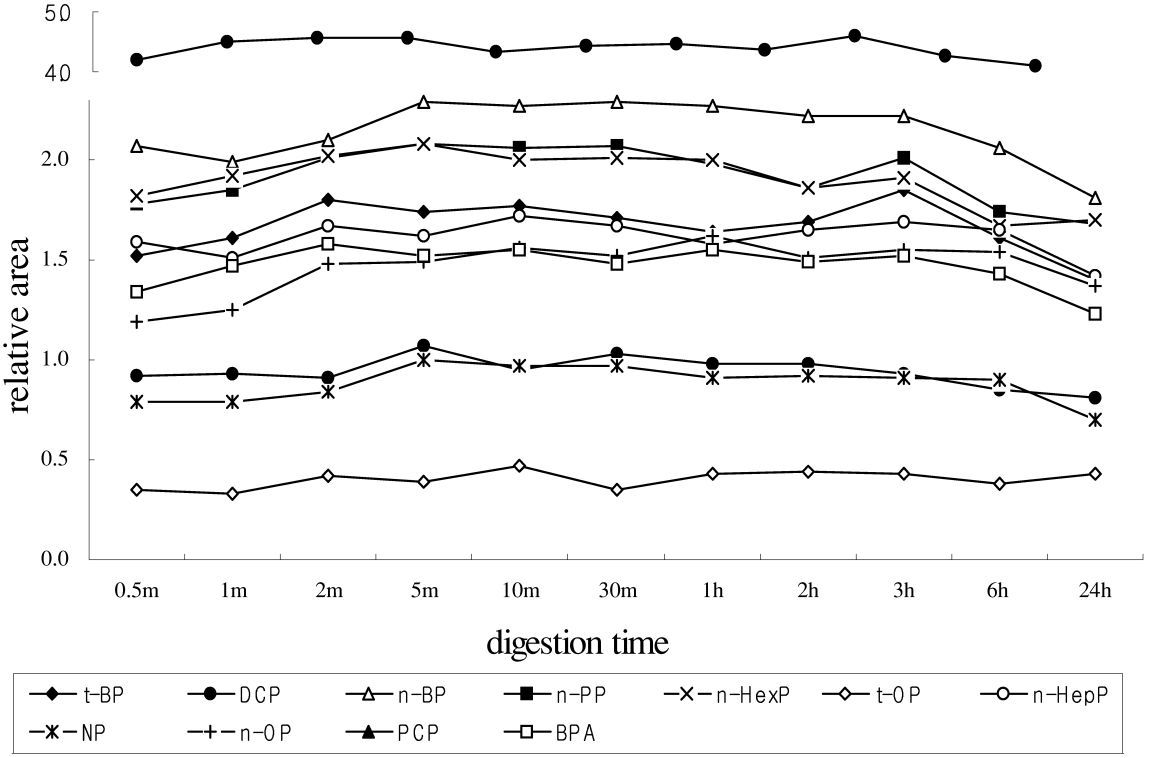

Fig. 2. Digestion kinetics of phenolic analytes for spiked sediment sample ($n=3$); 0.1 *M* HCl was used for the digestion of analytes; 100 ppb spiked sediment sample was used. Refer to Table 1 for abbreviations.

dependent on the polarity of extracting solvent. Excellent recoveries were obtained with the slightly polar dichloromethane but not with the nonpolar hexane. Since the 'phenol' forms of the phenolic analytes are slightly polar, the analytes should interact stronger with dichloromethane compared to hexane resulting higher recoveries. The deviations of the recoveries were also smaller when dichloromethane was used.

Fig. 3 compares the technique developed here to other extraction techniques. The recoveries in this technique were about 90% for the most analytes. The recoveries in the PLE and SE techniques ranged around 70–80% in most cases. The optimized acid-digested extraction technique is simpler, more time-saving and economical compared to other techniques currently applied in the analysis of the phenolic compounds from sediment samples.

The analytical characteristics of GC–MS analysis following the acid-digested extraction and silyl derivatization are given in Table 1 for wide ranges of phenolic analyte concentrations. The recoveries ranged from 81% for pentachlorophenol at 50 ng/g to 99% for *n*-octylphenol at 5 ng/g with <14% RSD. The method detection limits (MDL) varied from 0.6 ng/g for BPA to 4.1 ng/g for pentachlorophenol. This is similar or slightly lower compared to the results reported by others for most analytes [32].

In order to evaluate the reliability of the optimized acid digested extraction technique was applied to a certified reference material (CRM) of a natural matrix and real sediment samples. The real sediment samples were taken from three different environments, i.e. a river, lake and sea, where are known to be contaminated with phenolic pollutants. The analytical results were compared to those obtained by the SE technique. As listed in Table 2, the efficiency of the acid-digestion extraction in real sample experiments was better than SE in the case of the spiked

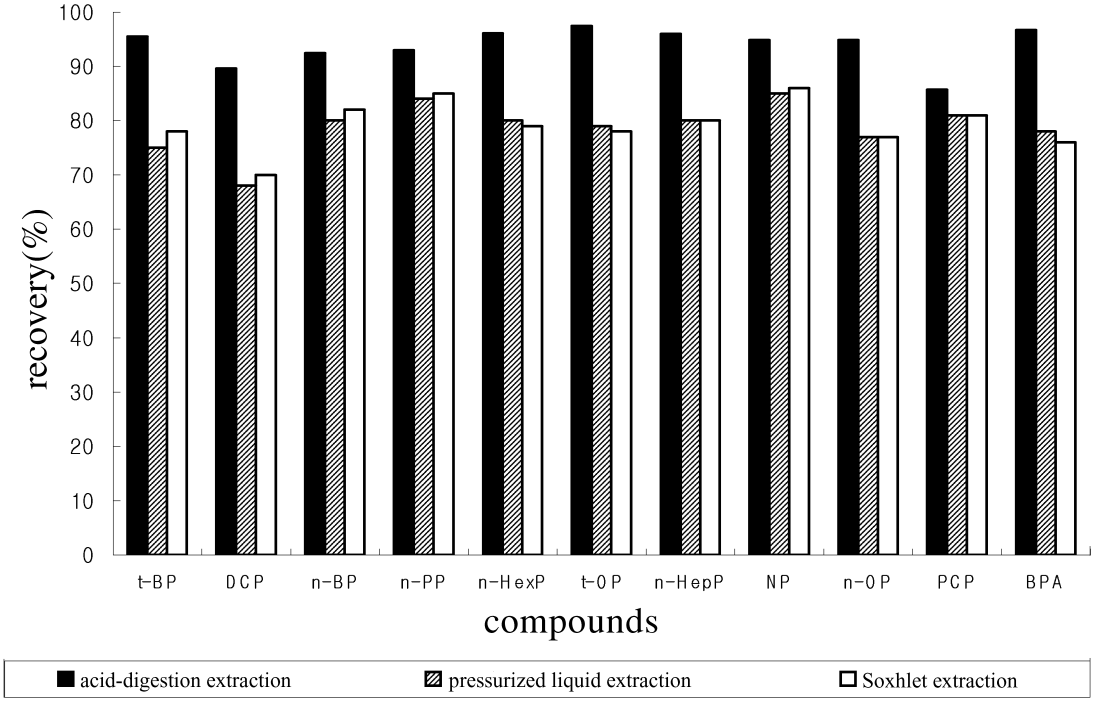

Fig. 3. Comparison of the extraction efficiency for the acid digested extraction, pressurized liquid extraction, and Soxhlet extraction techniques. 100 ppb spiked sediment sample was used for evaluation. Dichloromethane was used as extracting solvent. Refer to Table 1 for abbreviations.

Table 1
Analytical characteristics of the GC–MS analysis utilized the acid digestion extraction technique

Compounds (abbreviation)	MDL[a] (ng/g)	Recovery (%)±RSD(%)			
		5 ng/g (n=5)	20 ng/g (n=5)	100 ng/g (n=6)	1000 ng/g (n=3)
4-*t*-Butylphenol (*t*-BP)	1.1	96±13	87±7	94±4	86±5
2,4-Dichlorophenol (DCP)	1.3	92±14	81±10	89±3	91±10
4-*n*-Butylphenol (*n*-BP)	2.1	91±14	88±9	93±4	85±4
4-*n*-Pentylphenol (*n*-PP)	1.3	96±13	88±8	94±2	83±2
4-*n*-Hexylphenol (*n*-HexP)	1.6	89±5	96±3	98±7	87±6
4-*t*-Octylphenol (*t*-OP)	2.4	96±8	95±8	99±2	87±3
4-*n*-Heptylphenol (*n*-HepP)	1.2	93±10	92±2	96.8±0.5	86±1
Nonylphenol (NP)	2.8	91±12	97±16	96±3	92±6
4-*n*-Octylphenol (*n*-OP)	1.8	100±5	92±5	94±1	83±2
Pentachlorophenol (PCP)	4.1	81±10	85±8	86±4	84±11
Bisphenol A (BPA)	0.6	89±5	87±11	93±5	83.5±0.8

[a] MDL, method detection limit. A 1-g amount of sediment was used and the final volume was 0.5 ml. MDL=SD×t+blank, where t=3.143 for n=7 at 98% confidence level.

samples. In most cases it was >10% higher in comparison with SE. For the CRM, all the results by both techniques fell into the ranges of confidence interval but the results by the acid digestion technique appear to be higher than those of SE with better reproducibility. The accuracy was also better for chlorophenols in the CRM. There is no doubt that alkylphenols, chlorophenols and bisphenol A can be simultaneously extracted from environmental sediment samples using the acid-digestion extraction technique with improved reliability and convenience.

4. Conclusions

It was possible to develop a simple, time-saving and economical extraction technique for simultaneous GC–MS analysis of alkylphenols, chlorophenols and bisphenol A by optimizing digesting conditions. Digestion for 10 min under mild acidic conditions, i.e. 0.1 *M* HCl was sufficient for a high recovery. The technique is superior to currently applied techniques such as SE or PLE with regard to recovery, reproducibility, simplicity, extraction time

Table 2
Comparison of extraction efficiency between acid-digestion extraction technique and Soxhlet extraction from environmental sediment samples

	Han river ppb (RSD)[b]		Masan bay ppb (RSD)		Lake Shihwa ppb (RSD)		CRM[a] ppm (RSD)		Reference value
	Soxhlet	ADE[c]	Soxhlet	ADE	Soxhlet	ADE	Soxhlet	ADE	
t-OP	Nd[d]	Nd	6.2(9)[d]	8.6(8)	9.3(3)	9.9(2)	Nd	Nd	–
NP	127(5)	134(5)	768(8)	839(6)	1300(10)	1750(5)	Nd	Nd	–
BPA	5.0(4)	5.5(4)	29(10)	31(6)	10.5(5)	10.9(2)	Nd	Nd	–
DCP	Nd	Nd	Nd	Nd	Nd	Nd	7.2(8)	7.9(1.4)	8.02
PCP	Nd	Nd	Nd	Nd	Nd	Nd	6.1(10)	7.6(2.1)	6.99

[a] CRM, certified reference material.
[b] Relative standard deviation (%) for triplicate experiments.
[c] Acid-digestion extraction.
[d] Not detected.

and cost-effectiveness. The extraction can be completed in 20 min and a parallel extraction is possible for large numbers of environmental samples with simple equipment. Work is currently under way on environmental phenolic analyte monitoring utilizing this extraction technique.

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