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Analysis of endocrine disrupting alkylphenols, chlorophenols and bisphenol-A using hollow fiber-protected liquid-phase microextraction coupled with injection port-derivatization gas chromatography–mass spectrometry

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Abstract

Liquid-phase microextraction (LPME) coupled with gas chromatography–mass spectrometry were used to determine alkylphenols (APs), chlorophenols (CPs) and bisphenol-A (BPA) in aqueous samples. APs, CPs and BPA are highly polar compounds and need to be derivatized before analysis by GC–MS. In this work, they were derivatized in the GC injection port with bis(trimethylsilyl)trifluoroacetamide (BSTFA). The analytes were extracted directly from 5 ml of sample solution using 5 μl of organic solvent though a porous polypropylene hollow fiber. The hollow fiber, filled with an immiscible organic solvent (ca. 5 μl), was immersed in the sample solution which was stirred during the 30-min extraction. An aliquot (2 μl) of the extract and 2 μl of BSTFA were then consecutively injected into the GC injection port. Extraction parameters such as extraction time, pH of sample, concentration of salt added, and stirring rate were optimised. The proposed LPME provided a good average enrichment factor of up to 162-fold, reproducibility ranging from 5.9 to 13.9% ($n = 4$), and good linearity ($r^2 = 0.995$) for spiked water samples. The limits of detection (LODs) ranged between 0.005 and 0.015 $\mu g\,l^{-1}$ (S/N = 3) using GC–MS with selective ion monitoring and limits of quantification were in the range of 0.012–0.026 $\mu g\,l^{-1}$. A comparative study was performed between LPME, headspace solid-phase microextraction (HS-SPME) and liquid–liquid extraction (LLE). The results obtained suggested that hollow fiber LPME was a rapid, simple and efficient technique for APs, CPs and BPA, and provided a good alternative to SPME and LLE. Finally, the proposed method was applied to monitor Singapore coastal water samples.

Keywords: Alkylphenols; Bisphenol-A; Derivatization; Seawater analysis; Hollow fiber membrane; Liquid-phase microextraction

1. Introduction

Many man-made chemicals have been found in the environment, in increasing amounts in recent years, generating awareness regarding their potential impact. The alkylphenols (APs) including, 4-*t*-octylphenol and 4-nonylphenol and chlorophenols (CPs) including 2,4-dichlorophenol and pentachlorophenol, and bisphenol-A (BPA) have been shown to exhibit endocrine disrupting properties in wildlife and laboratory animals [1,2]. Trace levels of these compounds can potentially cause adverse health effects in humans, and there is an increasing demand to quantify these ultratrace contaminants. Many countries classify these APs, CPs and BPA as priority hazardous compounds [3,4].

APs, CPs and BPA are a class of polar semi-volatile compounds. Their presence in environmental matrices present significant analytical challenges as several problems are usually encountered such as peak tailing (in gas chromatography (GC)) due to interaction of analytes with active sites in the analytical column. Trace enrichment can be performed by conventional techniques such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) and equilibrium extraction techniques such as solid-phase microextraction

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(SPME) and liquid-phase microextraction (LPME). Equilibrium methods are simpler, and generally result in lower losses of analyte [5]. Recently, LPME supported by hollow fiber has been found to overcome the drawback of microdrop LPME. The polypropylene fiber used for LPME is less expensive compared to commercial SPME fibers, and a fresh piece is used for each extraction to avoid contamination. In addition, hollow fiber-supported-LPME provides a high enrichment factor, and can also be used as a good clean up device for complex matrices [6,7]. LPME is generally compatible with GC [8,9], capillary electrophoresis [10,11], high-performance liquid chromatography (HPLC) [12] and analytical results with low detection limits and good reproducibility can be obtained. APs, CPs and BPA are polar compounds; hence their derivatives have to be generated prior to GC analysis. Derivatization has the advantage of converting polar analytes into their less polar forms, thus increasing their volatility. Methylation [13], acetylation [14] silylation [15,16] are the common derivatization procedures. Silyl derivatization can be accomplished by using bis(trimethylsilyl)trifluoroacetamide (BSTFA) and is the most common method [17].

Derivatization of APs, CPs and BPA using BSTFA by conventional procedures are more time consuming and expensive (requiring heat and 100 μl of BSTFA) [17,18]. Although the reported conventional procedures can be successfully carried out, large amounts of sample and BSTFA are required. Moisture content can affect the accuracy and reproducibility of analysis. In microanalytical techniques involving LPME only limited volume (5 μl) of extract is available for derivatization. Further dilution will decrease the sensitivity of the analysis. Therefore, injection-port derivatization was selected to enhance the efficiency of AP, CP and BPA analysis. Furthermore, as it shortens derivatization time, degradation of analytes due to exposure to moisture is much reduced, if not eliminated.

In this paper, a method for the analysis of APs, CPs and BPA from aqueous samples is proposed. The analytes were extracted by hollow-fiber protected LPME. The extract was injected into the GC, followed immediately by an injection of BSTFA to directly derivatize the phenols before analysis. Conditions essential to the extraction and derivatization were optimized. The optimised parameters were applied to the analysis of real environmental samples.

2. Experimental

2.1. Standard and reagents

The following chemical standards (purity ≥97%) were obtained from Wako (Tokyo, Japan): 4-*n*-butylphenol, 4-*tert*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol, 4-*n*-octylphenol, 4-*tert*-octylphenol, 4-*n*-heptylphenol, 4-nonylphenol, 2,4-dichlorophenol, pentachlorophenol and bisphenol-A. [$^2H_8$]Naphthalene (*n*-naphthalene-d_8) (98%), [$^2H_{10}$]pyrene (pyrene-d_{10}) (98%) and [$^2H_{10}$]phenanthrene (phenanthrene-d_{10}) (99%) were used as internal standards. Bisphenol-A-d_{14} (98%) was used as surrogate standard. The derivatization agent BSTFA (purity >98%) and all HPLC-grade organic solvents were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared on a Milli-Q (Milford, MA, USA) system. A standard stock solution of 50 μg ml^{-1} of each analyte was prepared in acetone. A working standard solution (10 μg ml^{-1} of each analyte) was prepared by dilution. Q3/2 Accurel polypropylene hollow fiber was bought from Membrana GmbH (Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm and the pore size was 0.2 μm. A SPME fiber holder and 85 μm polyacrylate fiber were purchased from Supelco (Bellefonte, PA, USA). SPME fibers were conditioned in the GC injector port according to the instructions provided by the manufacturer. Oasis-HLB SPE cartridges were purchased from Waters (Milford, MA, USA). APs, CPs and BPA are estrogenic compounds and safety measures were observed. All the experiments were performed in the fume cupboard and non-permeable gloves were used to handle them.

2.2. Liquid-phase microextraction

A 10 μl microsyringe (needle of 0.47 mm o.d.) (SGE, Sydney, Australia) was used for LPME. The experimental setup is shown in Fig. 1. Before extraction, the syringe was rinsed with acetone followed by toluene 10–15 times to avoid carryover and air bubble formation. Five microlitres of toluene was withdrawn into the syringe. The disposable hollow fiber (1.2 cm length) was fixed onto the conical tip of the syringe needle. The fiber was immersed in toluene for three seconds to dilate the pores prior to extraction of analytes from the sample solution. For extraction, the hollow fiber was held 5 mm below the surface of a 5 ml sample solution. The syringe plunger was depressed to fill the hollow fiber with toluene. The fiber was exposed to the sample for 30 min (under the optimum conditions). After extraction, the hollow fiber assembly was removed and 2 μl of extract was carefully withdrawn into the syringe and then the fiber was discarded. The extract was then injected into the GC–MS system. Two μl of BSTFA was injected immediately into the GC injection port using

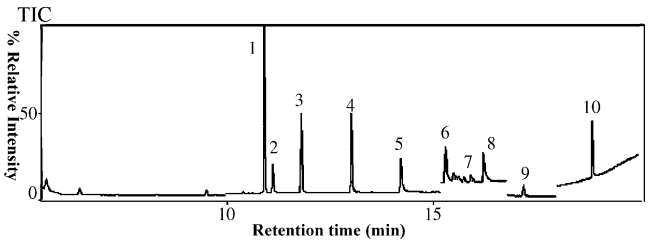

Fig. 1. Chromatogram of injection port-derivatized APs, CPs and BPA in spiked (40 μg l^{-1}) artificial seawater sample after LPME. Peak identification: (1) 4-*n*-butylphenol (2) 2,4-dichlorophenol (3) 4-*tert*-butylphenol (4) 4-*n*-pentylphenol (5) 4-*n*-hexylphenol (6) 4-*n*-heptylphenol (7) 4-nonylphenol (8) 4-*n*-octyl phenol (9) pentachlorophenol (10) bisphenol-A.

a separate syringe. Analytes were held in the GC injector port for 2 min to ensure complete derivatization before being channelled into the GC column.

A strict quality control procedure was followed that included the analysis of spiked and real seawater samples with each set of field samples analysed.

2.3. Solid-phase microextraction

An optimized HS-SPME method [19] was utilized for the extraction of APs, CPs and BPA. Briefly, 10 ml of ultrapure water containing 100 ng ml^{-1} of each analyte was placed in a 30 ml screw-cap glass vial containing a 12 mm PTFE-coated stir bar, 30% of sodium chloride, and 100 μl of acetone. The sample pH was adjusted to 2 with 0.1 M HCl. The vial was clamped in a thermostated water bath at 80 °C, which was placed on a magnetic hotplate. The SPME fiber was exposed to the headspace for 60 min. The sample solution was stirred at 105 rad s^{-1} (1000 rpm; 1 rpm = 0.1047 rad s^{-1}). The fiber was then exposed to the headspace of a derivatizing solution of 200 μl of BSTFA in 2 ml of acetone in a 10 ml vial, for 10 min at 60 °C. Finally, the fiber was desorbed in the injection port of the GC–MS for 3 min.

2.4. Liquid–liquid extraction

LLE was evaluated with 200 ml artificial seawater sample (i.e. Coral Red Sea salt (Red Sea Fish Pharm (P), Eilat, Israel)) dissolved in water to a salinity of 3.3%, pH 8.5 and conductivity 48.5 mS) spiked with a stock solution containing APs, CPs and BPA (at concentrations of 100 μg l^{-1} per analyte). Extraction was performed with 50 ml of dichloromethane. Prior to extraction, the sample pH was adjusted to 2 using dilute 1 M HCl. Anhydrous sodium sulphate was then added to the organic layer to remove trace amounts of water. The extract was then preconcentrated in a rotary evaporator to a total solvent volume of ∼2 ml, followed by SPE with an Oasis-HLB cartridge. The eluate was reduced to less than 1 ml with a flow of nitrogen gas. Finally, the extract was derivatized with 100 μl of BSTFA [17,18], and made up to 2 ml with acetone. Two microlitres of the derivatized extract was injected to the GC–MS for analysis. An internal standard calibration was established with three internal standards (i.e. naphthalene-d_8, pyrene-d_{10}, phenanthrene-d_{10}) and bisphenol-A-d_{14} as surrogate standard.

2.5. GC–MS analysis

Analysis was carried out using a Shimadzu (Tokyo, Japan) QP5050 GC–MS system equipped with a Shimadzu AOC-20i autosampler and a DB-5 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 2.1 ml min^{-1}. Two microlitres of sample was injected into the GC–MS system with a splitless injector under splitless mode after a sampling time (holding time) of 2 min (i.e. sample and derivatization agent were retained in the injector port for 2 min). The injection temperature was set at 300 °C, and the interface temperature at 270 °C. The GC temperature programme was as follows: initial temperature 50 °C, held for 2 min, then increased by 20 °C min^{-1} to 100 °C, a second increase at 10 °C min^{-1} to 200 °C, and a third increase at 20 °C min^{-1} to 300 °C, held for 7 min. The pressure programme was as follows: carrier gas pressure 40 kPa (for 5 min), then increased by 2 kPa min^{-1} to 70 kPa, held for 7 min. All standards and samples were analysed in selected ion monitoring (SIM) mode with a detector voltage of 1.5 kV and a mass scan range of *m/z* 50–500. The most abundant ion present was selected as the quantitative ion, while a further two ions were used for confirmation of individual compounds, as listed in Table 1.

2.6. Derivatization of alkylphenols, chlorophenols and bisphenol-A

Derivatization of APs, CPs and BPA is necessary to achieve good recoveries and precision. BSTFA is a suitable silylation agent for phenolic compounds by direct derivatization in solution. In this procedure, incomplete derivatization has often been reported due to excessive reagent and moisture content [20,21]. Removal of excess, unreacted BSTFA in the solution is necessary, otherwise poor resolution of chromatographic peaks results. Various approaches have been reported to remove excess BSTFA [8,22,23]. In our study, to overcome

Table 1
Elution order, retention times and characteristic ions used for GC–MS-SIM analysis

Analyte	Retention time (min)	Target ion (*m/z*) (relative intensity)	Confirmation ion (*m/z*) (relative intensity)
4-*tert*-Butylphenol	11.2	207.10 (100)	280.10 (16.50), 222.10 (13.79)
2,4-Dichlorophenol	11.4	218.95 (100)	220.95 (72.40), 234 (33.65)
4-*n*-Butylphenol	12.1	179.10 (100)	222.10 (18.59), 207.10 (33.65)
4-*n*-Pentylphenol	13.4	179.10 (100)	236.15 (14.19), 221.10 (9.50)
4-*n*-Hexylphenol	14.7	179.10 (100)	250.15 (12.03), 207.10 (4.17)
4-*n*-Heptylphenol	15.7	179.10 (100)	264.20 (13.00), 249.15 (3.60)
4-Nonylphenol	16.1	207.10 (100)	221.10 (8.90), 208.10 (10.57)
4-*n*-Octylphenol	16.5	179.10 (100)	278.20 (10.21), 263.10 (1.70)
Pentachlorophenol	16.7	322.80 (100)	324.80 (66.32), 337.80 (19.52)
Bisphenol-A	19.4	357.10 (100)	358.10 (36.32), 372.15 (14.24)

Table 2
Linearity, enrichment factors, precision (R.S.D.s, n = 3), LODs (S/N = 3) and LOQs of LPME

Analyte	Linear range ($\mu g\,l^{-1}$)	Linearity r^2	Enrichment factor	R.S.D. (%)	LODs ($\mu g\,l^{-1}$)	LOQs ($\mu g\,l^{-1}$)
4-*tert*-Butylphenol	2.5–250	0.9937	84	9.2	0.007	0.021
2,4-Dichlorophenol	2.5–250	0.9929	123	9.2	0.014	0.022
4-*n*-Butylphenol	2.5–250	0.9934	122	12.5	0.005	0.012
4-*n*-Pentylphenol	2.5–250	0.9942	162	13.8	0.007	0.016
4-*n*-Hexylphenol	2.5–250	0.9941	144	13.9	0.005	0.014
4-*n*-Heptylphenol	2.5–250	0.9947	156	9.9	0.011	0.019
4-Nonylphenol	2.5–250	0.9922	148	13.4	0.006	0.016
4-*n*-Octylphenol	2.5–250	0.9944	156	9.9	0.010	0.024
Pentachlorophenol	2.5–250	0.9839	136	5.9	0.015	0.026
Bisphenol-A	2.5–250	0.9891	105	10.9	0.014	0.024

these problems the analytes after LPME were directly derivatized in the GC injection port [24]. Derivatization of APs, CPs and BPA using BSTFA is a very quick procedure (15 s is enough to complete derivatization) [21]. Therefore, within 2 min (sample holding time in the injector port) analytes were completely derivatized. At a high GC injection port temperature, BSTFA and analytes are easily volatilised, ensuring complete derivatization of the phenols. Fig. 1 shows a chromatogram with sharp peaks with injection port-derivatization after LPME of spiked artificial seawater sample.

2.7. Enrichment factor and recovery

In LPME, the analytes are extracted from the aqueous sample into the organic solvent present in the pores and inside the lumen of the hollow fiber.

$$A_{(\text{aqueous sample})} \leftrightarrow A_{(\text{organic solvent})}$$

The enrichment factor E_f may be calculated (for diluted i.e. 5 ml sample and 5 μl of extraction solvent) based on the equation [7]

$$E_f = \frac{1}{(V_o/V_a + 1/K)}$$

where K is the distribution coefficient, V_o is the volume of organic solvent and V_a, the volume of aqueous sample.

The distribution coefficient K is calculated for the two-phase equilibrium condition

$$K = \frac{C_{o,eq}}{C_{a,eq}}$$

where $C_{o,eq}$ is the concentration of the analyte in the organic phase and the $C_{a,eq}$ is concentration of the analyte in the aqueous phase. The optimum conditions were applied to investigate enrichment factors. The latter are listed in Table 2; they ranged from 84 to 162. Relative recoveries were calculated (defined as the GC peak area ratios of extract in pure water and the respective water samples spiked at the same concentrations of analytes) and shown in Table 3.

3. Results and discussion

3.1. Method optimization

The selectivity of the current procedure was examined by studying extraction solvent, time, the influence of the extraction pH, amount of sodium chloride added to the sample solution, stirring speed and concentration of the derivatizing agent.

Since the extraction of analytes in LPME is based on an equilibrium distribution process, the amount of analyte extracted at a given time depends on the mass transfer of the analyte from the aqueous phase to the hollow fiber containing organic solvent. The extraction time taken to attain equilibrium was investigated. The aqueous solution was spiked with 40 $ng\,l^{-1}$ of each phenol and extracted for periods ranging from 10 to 50 min. The sample was continuously stirred with a 12 mm magnetic bar. Fig. 2 shows that for most of the analytes, equilibrium was attained after 30 min of extraction.

In conventional LLE, polarizable solvents such as chloroform [25] and dichloromethane [26] have been used for extracting from aqueous samples due to the relatively polar nature of phenols. Suitable extraction solvents used in LPME

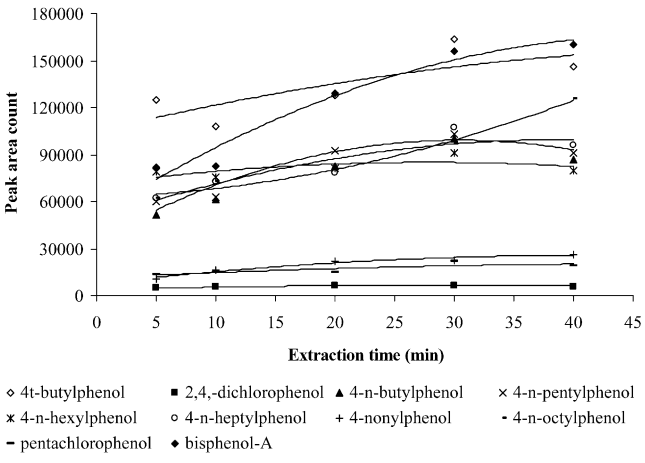

Fig. 2. Effect of extraction time on LPME. Toluene was used as extraction solvent, stirring speed 63 rad s^{-1}. Sample pH and ionic strength were not adjusted. One microlitre of BSTFA was used for injection port-derivatization.

Table 3
Extraction of alkylphenols, chlorophenols and bisphenol-A from tap water, reservoir water and artificial seawater by LPME combined with injection port-derivatization ($n = 3$) and LLE ($n = 5$), HS-SPME ($n = 4$)

Analyte	LPME			LLE[a]	HS-SPME
	Relative recovery (R.S.D., %)			Artificial seawater	
	Tap water	Reservoir water	Artificial seawater	Recovery (R.S.D., %)	Relative recovery (R.S.D., %)
4-*tert*-Butylphenol	103.4 (6.7)	94.0 (7.6)	97.1 (12.8)	83.1 (6.8)	91.9 (9.2)
2,4-Dichlorophenol	116.2 (13.5)	97.9 (10.7)	95.5 (8.1)	87.3 (15.5)	92.2 (9.7)
4-*n*-Butylphenol	91.0 (5.2)	87.1 (11.6)	89.0 (12.9)	77.2 (11.3)	92.3 (12.5)
4-*n*-Pentylphenol	83.1 (7.4)	87.0 (11.2)	84.6 (11.1)	96.5 (16.7)	94.0 (13.7)
4-*n*-Hexylphenol	87.3 (5.3)	86.8 (9.8)	90.2 (11.7)	92.8 (15.7)	94.1 (13.9)
4-*n*-Heptylphenol	91.8 (10.8)	nc	98.7 (12.6)	95.8 (18.4)	93.2 (9.9)
4-Nonylphenol	88.2 (5.6)	nc	88.5 (12.3)	86.8 (16.9)	92.0 (8.3)
4-*n*-Octylphenol	93.1 (10.0)	92.5 (10.7)	98.7 (6.8)	95.4 (16.5)	101.6 (13.4)
Pentachlorophenol	75.4 (13.5)	98.6 (13.6)	87.3 (11.4)	90.3 (7.7)	91.7 (9.9)
Bisphenol-A	106.3 (13.3)	103.5 (14.5)	119.7 (9.8)	73.9 (4.8)	81.0 (5.9)

[a] 100 $\mu g\,l^{-1}$ of each analyte spiked into artificial seawater. Recoveries were calculated using internal standard calibration; nc: not calculated.

are limited since they should be immiscible and preferably insoluble in water, and are of low volatility. Based on these conditions, toluene, hexane, *n*-nonane and isooctane were evaluated. These solvents have different chemical characteristics such as polarity, volatility and solubility in water. For each solvent, GC–MS peak area counts (for sampling volume of 2 μl) are shown in Fig. 3. The data obtained suggested that using toluene as the extraction solvent could achieve better results.

The influence of stirring speeds between 21 and 73 rad s^{-1} was evaluated. Higher stirring speeds increase the transfer rate of analytes to the organic solvent and thus the enrichment factor. However, due to the small volume of sample solution considered, higher stirring speeds (above 73 rad s^{-1}) caused air bubble formation and some solvent evaporation. This resulted in poor precision. Therefore, 73 rad s^{-1} was selected as the optimum stirring speed. Fig. 4 shows the effect of pH on extraction. A higher extraction yield was observed at pH 2. The optimum value of pH 2 was chosen for subsequent analysis.

In LLE it is common practice to add salt to aqueous samples in order to enhance the partition of polar analytes into the organic phase. The effect of decreasing solubility of organic compounds by the addition of salt is known as salting out [27]. Indeed, salting out effects have been commonly observed [28,29]. The addition of sodium chloride decreases the solubility of phenols and increases the partition of the analytes into the organic solvent held by the hollow fiber. Thirty percent sodium chloride appeared to be optimum, and was therefore used for subsequent extractions.

We investigated the influence on LPME of different volumes of BSTFA in the range of 1–3 μl. The results, when compared with those obtained with underivatized extracts (Table 4), indicated that the derivatization efficiencies were better when 2 μl of BSTFA was used. Excessive amount (>2 μl) of BSTFA caused poor GC resolution of the analytes and low precision of the analysis. After every 10 analyses a blank run was performed to assess BSTFA contamination caused by the "soiled" septum. Compared with LLE and

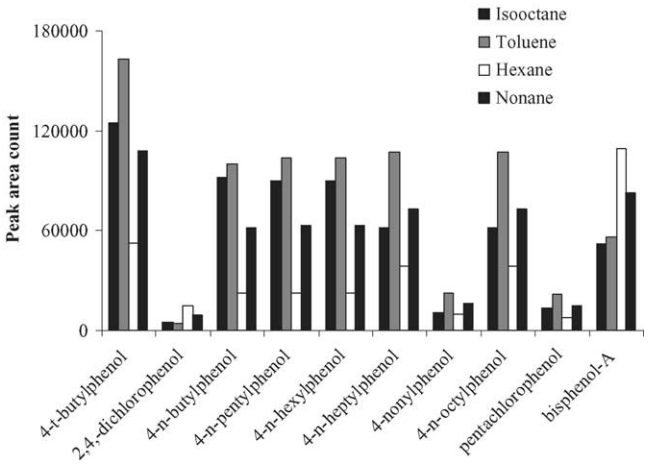

Fig. 3. Influence of organic solvent on LPME. Extraction time 30 min, extraction was performed at 63 rad s^{-1}. Sample stirring speed and sample pH and ionic content were not adjusted. One microlitre of BSTFA was used for injection port-derivatization.

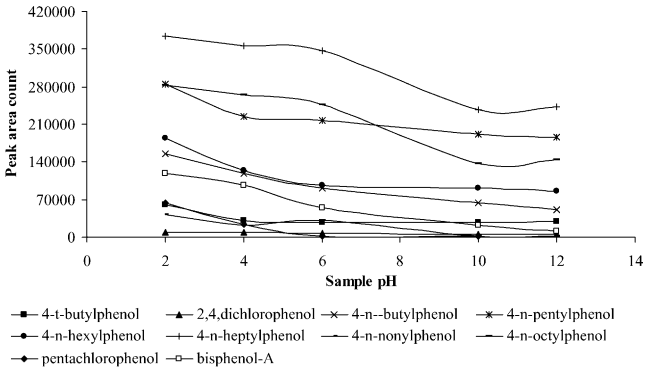

Fig. 4. Effect of pH of sample solution on LPME. Extraction time 30 min, toluene as extraction solvent, sample stirring speed 73 rad s^{-1}. Sample ionic strength was not adjusted. One microlitre of BSTFA was used for injection port-derivatization.

Table 4
Influence of BSTFA volume (on injection port-derivatization after LPME)

Analyte	R.S.D. (%) for the amount of BSTFA ($n = 3$)			
	Underivatized	1 μl	2 μl	3 μl
4-*tert*-Butylphenol	12.9	6.7	9.8	12.8
2,4-Dichlorophenol	19.8	13.5	12.1	8.1
4-*n*-Butylphenol	20.1	15.2	13.5	12.9
4-*n*-Pentylphenol	11.2	7.4	9.8	11.1
4-*n*-Hexylphenol	12.2	12.3	9.9	11.7
4-*n*-Heptylphenol	10.8	10.8	7.7	12.6
4-Nonylphenol	12.4	5.6	11.1	12.3
4-*n*-Octylphenol	13.8	10.0	7.8	6.8
Pentachlorophenol	13.7	13.5	7.8	11.4
Bisphenol-A	17.8	13.3	12.4	9.8

SPME conducted in this work, LPME coupled with injection port-derivatization GC showed good reproducibility for all target analytes.

3.2. Linearity, precision and detection limits

The linearity range, precision, reproducibility and limits of quantification for LPME were evaluated. The linearity of the method was tested at five different concentration levels, ranging from 2.5 to 250 $\mu g\,l^{-1}$. The extraction procedure was used for an external calibration and linearity between 0.995 and 0.984 was obtained. The reproducibility of the extraction procedure was determined by performing five consecutive extractions at the optimum extraction conditions. R.S.D. values ranged from 5.9% (pentachlorophenol) to 13.9% (4-*n*-hexylphenol), comparable with those obtained with HS-SPME. Limits of detection (LODs), at a signal to noise ratio of 3, under MS-SIM conditions, were in the range of 0.005–0.016 $\mu g\,l^{-1}$ (see Table 2). When determining LODs, a sample blank was carried out every time to confirm that no carryover occurred. Limits of quantification (LOQs) (i.e. the standard deviation of the replicate samples multiplied by 10) [30] were in the range of 0.012–0.026 $\mu g\,l^{-1}$ (Table 2).

Artificial seawater samples were extracted by LPME, LLE and HS-SPME. The recoveries and R.S.D.s of all three methods are shown in Table 3. In LLE the extracts were derivatized using the conventional procedure (i.e. 100 μl of BSTFA in 1 ml sample extract and kept it in a water bath for 30 min at 60 °C). In HS-SPME, on-fiber derivatization was performed (i.e. the extracted fiber was exposed to the headspace for 30 min at 60 °C) and in LPME, the extracts were derivatized in the GC injector port. LPME and HS-SPME offer comparable recoveries and R.S.D.s for artificial seawater whereas LLE recoveries are lower than both. HS-SPME gave precision of between 5.9 and 13.9% (with 60 min extraction time). LLE achieved precision of between 4.8 and 18.4% (with 70 min extraction time). These values are comparable with those obtained by the proposed LPME method (R.S.D.s between 5.2 and 17.7%) but the extraction time was only 30 min. Therefore, analysis of APs, CPs and BPA using LPME with GC injection port-derivatization was suitable for routine and complex environmental analysis.

3.3. Water samples

The present method was also used to analyse reservoir and tap water. Tap water showed no trace of APs, CPs and BPA. In reservoir water, nonylphenol and heptylphenol were detected at concentrations of 0.02 and 0.15 $\mu g\,l^{-1}$, respectively. The relative recovery, defined as the peak area ratio between ultrapure water and other water samples spiked at the same concentration (40 $\mu g\,l^{-1}$) was calculated and shown in Table 3. The mean recoveries obtained for APs, CPs and BPA spiked in the two types of water ranged between 75 and 116%. The results shown in Table 3 clearly indicate no matrix interferences.

To complete the study, the LPME procedure was applied to genuine seawater samples. Samples were collected from coastal environments (Straits of Singapore) in the vicinity of six major wastewater treatment plants. The determined concentrations are given in the Table 5 and a typical chromatogram generated from one such seawater extract is shown in Fig. 5. The detected concentrations of APs, and CPs ranged between "non-detected" (below limit of quantification (LOQ)) and 2.76 $\mu g\,l^{-1}$, and of BPA between 0.04 and 0.19 $\mu g\,l^{-1}$. The detected total concentration of APs and CPs (8.3 $\mu g\,l^{-1}$) was lower than the permitted value for total

Table 5
Alkylphenols, chlorophenols and bisphenol-A in seawater samples in Singapore coastal environment

Analytes	Concentration ($\mu g\,l^{-1}$)					
	Sembawang Park	Punggol	Pasir Ris	Changi	Jurong Pier	Tuas Jetty
4-*tert*-Butylphenol	0.03	[a]	0.03	0.03	1.06	0.95
2,4-Dichlorophenol	0.05	0.24	0.08	[a]	0.53	1.55
4-*n*-Butylphenol	[a]	0.11	0.05	0.02	0.44	0.59
4-*n*-Pentylphenol	[a]	0.02	[a]	[a]	0.2	0.06
4-*n*-Hexylphenol	0.02	0.03	0.04	[a]	0.55	1.86
4-*n*-Heptylphenol	0.03	[a]	0.02	0.02	0.54	0.32
4-Nonylphenol	1.03	1.63	0.37	0.32	2.4	2.76
4-*n*-Octylphenol	0.05	0.03	[a]	0.1	0.19	0.13
Pentachlorophenol	0.03	0.14	0.1	[a]	1.65	0.09
Bisphenol-A	0.04	0.19	0.04	0.05	0.17	0.04

[a] Below LOQ.

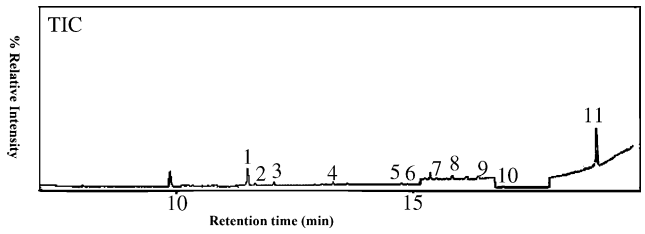

Fig. 5. Chromatogram of APs, CPs and BPA generated from LPME under optimized conditions, with injection port-derivatization (2 μl of BSTFA was used for derivatization) of real seawater collected from the Straits of Singapore. GC–MS conditions are described in the text.

phenols given by the Australian Environmental Protection Agency (i.e. 270 $\mu g\,l^{-1}$ for 99% protection) [31].

4. Conclusions

In the present work, a simple LPME procedure combined with GC injection port-derivatization has been developed for APs, CPs and BPA in aqueous samples. The results showed that LPME–GC injection port-derivatization is a promising method for APs, CPs and BPA and has good agreement with the performance of SPME and LLE. The newly developed microextraction procedure can achieve LOQs ranging from 0.012 to 0.026 $\mu g\,l^{-1}$ which exceed the requirement for environmental analysis. The method is fast, simple to use, and the hollow fiber can be discarded after each extraction to avoid carryover and cross-contamination.

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