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Research Article

Application of liquid–liquid–liquid microextraction and high-performance liquid chromatography for the determination of alkylphenols and bisphenol-A in water

A method termed liquid–liquid–liquid microextraction (LLLME) was utilized to extract 4-t-butylphenol, 4-t-octylphenol, 4-n-nonylphenol, and bisphenol-A from water. The extracted target analytes were separated and quantified by high-performance liquid chromatography using a fluorescence detector. In LLLME, the donor phase (i.e. water sample) was made weakly acidic by adding monobasic potassium phosphate (KH_2PO_4); the organic phase adopted was 4-chlorotoluene; the acceptor phase (i.e. enriched extract) was 0.2 M tetraethylammonium hydroxide dissolved in ethylene glycol. This study solves a problem associated with the surface activity of long-chain alkylphenolate ions, permitting LLLME to extract long-chain alkylphenols. Experimental conditions such as acceptor phase composition, organic phase identity, acceptor phase volume, sample agitation, extraction time, and salt addition were optimized. The relative standard deviation (RSD, 2.0–5.8%), coefficient of determination (r^2 0.9977–0.9999), and detection limit (0.017–0.0048 ng/mL) of the proposed method were achieved under the selected optimized conditions. The method was successfully applied to analyses of lake and tap water samples, and the relative recoveries of target analytes from the spiked lake and tap water samples were 92.8–106.3 and 93.6–105.6%, respectively. The results obtained with the proposed method confirm this microextraction technique to be reliable for the monitoring of alkylphenols and bisphenol-A in water samples.

Keywords: Alkylphenols / Bisphenol-A / Fluorescence detector / High-performance liquid chromatography / Liquid–liquid–liquid microextraction
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1 Introduction

Recently, great attention has been paid to endocrine disruptors (EDs) [1, 2]. EDs are exogenous substances that interfere with the endocrine system in the body and consequently disturb physiological functions of natural hormones [3]. The EDs in water can be concentrated by orders of magnitude by bioaccumulation [4]. It is therefore necessary to monitor trace levels of these compounds in water. In 2001, 4-*tert*-butylphenol (4-t-BP), 4-*tert*-octylphenol (4-t-OP), 4-n-nonylphenol (4-n-NP), and some polymeric derivatives of bisphenol-A (BPA) were listed as candidate EDs by the European Commission [5]. The analytical methods for these compounds in water were mainly solid-phase extraction (SPE), followed by either derivatization and detection using gas chromatography combined with mass spectrometry (GC-MS) or detection using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) without derivatization. These methods allowed the determination of the target analytes with limits of detection in the range from a few to several tens of ng/L [6–9]. Currently, microextraction is being increasingly used for aqueous sample analysis. The microextraction technique is environmentally friendly, less expensive, and simple to use. In 2004, Cai et al. [10] developed a solid-phase microextraction (SPME) technique with high-performance liquid chromatography-fluorescence detection (HPLC-FLD) to analyze BPA and alkylphenols in water. The performance of the method (the lowest concentration of linearity, 2 ng/mL; detection limit, 0.16–0.43 ng/mL) for determining the target analytes was established. Nevertheless, these target

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Abbreviations: AMADP, automated movement of acceptor and donor phases; **4-t-BP**, 4-t-butylphenol; **BPA**, bisphenol-A; **EDs**, endocrine disruptors; **FLD**, fluorescence detection; **LLLME**, liquid–liquid–liquid microextraction; **4-n-NP**, 4-n-nonylphenol; **4-t-OP**, 4-t-octylphenol; **SPME**, solid-phase microextraction

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analytes cannot be properly determined at low concentrations in practical water samples [10]. This may be due to matrix interference competing with the target analytes on the adsorptive sites of the SPME fiber.

In 1999, Pedersen-Bjergaard and Rasmussen [11] used a novel technique, liquid–liquid–liquid microextraction (LLLME). The target analytes were under the control of pH value and converted to either uncharged or charged species, then they can be extracted from the donor phase (i.e. water sample) into an organic phase and re-extracted into an aqueous acceptor phase inside the lumen of the hollow fiber. The enriched acceptor phase could be directly injected into either a HPLC or a CE. But there is a difficulty in extracting long-chain alkylphenols by the LLLME technique. The long-chain alkylphenol analytes cannot migrate to the acceptor phase from the organic phase in LLLME. This is due to a surfactant effect, long-chain alkylphenolate ion adsorbing on the interface between the organic phase and the aqueous acceptor phase.

In this study, the aim is to develop an LLLME technique combined with HPLC-FLD to allow the routine determination of ultratrace levels of alkylphenols and BPA in water samples. To diminish the surfactant effect, the acceptor solvent adopted is a polyhydric alcohol instead of water. Polyhydric alcohols can be directly injected into a reversed-phase liquid chromatograph. Otherwise, adding an ion-pair reagent to the acceptor phase causes the long-chain alkylphenolate ion to become an ion pair compound and thus eliminates the surfactant effect.

2 Materials and methods

2.1 Reagents and samples

BPA, 4-t-BP, and 4-t-OP) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 4-n-NP was purchased from Alfa Aesar Johnson Matthey (Ward Hill, MA, USA). Ethylene glycol, packed in glass bottles, was purchased from J.T. Baker (Phillipsburg, NJ, USA). Diethylene glycol and triethylene glycol were purchased from Sigma-Aldrich Chemie GmbH. Tetrabutylammonium hydroxide (40% m/m solution) was purchased from Sigma-Aldrich. Tetraethylammonium hydroxide (35% m/m solution), packed in a glass bottle, was purchased from Sigma-Aldrich Chemie GmbH. Sodium disulfite was purchased from Merck (Darmstadt, Germany). Methanol and acetonitrile (J.T. Baker) used for this study were of HPLC grade. Chlorobenzene (J.T. Baker), 1,2-dichlorobenzene (Riedel de Haën, Seelze, Germany), 1,2,4-trichlorobenzene (Janssen Chimica, Geel, Belgium), 2-nitrophenyl octyl ether (Sigma-Aldrich), and 4-chlorotoluene (T.C.I., Tokyo, Japan) were also used. Potassium phosphate monobasic (KH_2PO_4), added to the donor phase to make it weakly acidic, was purchased from Sigma-Aldrich Laborchemikalien GMBH (Seelze, Germany). $KH_2PO_{4(s)}$ may be contaminated with target analytes, so it must be purified before use. The purification procedure used was as follows: Acetone was slowly added to 10% (m/m) $KH_2PO_{4(aq)}$ with agitation; the then pure $KH_2PO_{4(s)}$ precipitated and was collected and dried. The stock solutions of the target analytes were prepared by dissolving each analyte in methanol to obtain 1 mg/mL solutions. Aliquots of these stock solutions were diluted with water to prepare standard working solutions of 10 ng/mL of each analyte. All other reagents and solvents used were of analytical reagent or LC grade unless otherwise mentioned. A Q3/2 Accurel polypropylene hollow fiber membrane (600 μm id, 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). The hollow fiber was cut into 4.0-cm segments and cleaned with acetone and dried before use. Deionized water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Samples of lake water and tap water were collected from Hsinchu county (Taiwan).

2.2 Instrumentation

An LC system assembled from modular components consisted of a Waters 2695 Separations Module and a Waters 2475 Multi λ Fluorescence Detector (Waters, Milford, MA, USA). Waters Empower Software was utilized to control the system and also to analyze data. A Waters XTerra phenyl column (3.0 × 250 mm, particle size 5 μm) was used for the separation of the target compounds. The mobile phase consisted of 90% (v/v) acetonitrile$_{(aq)}$ and pH 3 buffer solution (i.e. 0.05 M $(NH_4)H_2PO_{4(aq)}$ with added conc. H_3PO_4) at the gradient compositions shown in Table 1. Mixtures of water and 90% (v/v) acetonitrile$_{(aq)}$ with different ratios were used to clean the column for 15 min between successive injections. Detection was done with an excitation wavelength at 275 nm and an emission wavelength at

Table 1. Composition of mobile phase in HPLC

Time (min)	Flow rate (mL/min)	90% (v/v) acetonitrile$_{(aq)}$	pH 3 solution (0.05 M $(NH_4)H_2PO_{4(aq)}$ added H_3PO_4)
0	0.4	45%	55%
10	0.4	45%	55%
11	0.7	50%	50%
20	0.7	50%	50%
21	0.8	50%	50%
35	0.8	55%	45%

305 nm. A Model 200 series programmable syringe pump (KD Scientific, Holliston, MA, USA) and a 25-μL Hamilton 1702 syringe (Reno, NV, USA) were used in this study.

2.3 Extraction process and initial conditions

In the study, we adopted a dynamic LLLME technique, termed as LLLME with automated movement of acceptor and donor phases (LLLME/AMADP), to extract target analytes. As compared with conventional LLLME, LLLME/AMADP shows better performance and lower run-time cost [12]. The LLLME/AMADP apparatus is illustrated in Fig. 1. Initial extraction settings were as follows:

(i) A 14-mL aqueous solution (0.02 M KH_2PO_4) containing all analytes at concentrations of 10 ng/mL each and a 1-cm stirrer bar were placed in a 16-mL brown sample vial previously loaded with 1 g NaCl; the solution was stirred at 1200 rpm for 2 min to dissolve the NaCl. The sample vial was then gently patted to remove bubbles.

(ii) 12.5 μL of acceptor solution (0.2 M NaOH) was withdrawn into the microsyringe, followed by 3.5 μL of the solvent chlorobenzene and a little air in sequence. The needle tip of the microsyringe was then inserted into the hollow fiber, and the fiber was impregnated with the same organic solvent by capillary attraction through the fiber pores. After impregnation, the organic solvent and 7.5 μL of the acceptor phase in the syringe was injected into the hollow fiber to remove and retrieve excess organic solvent, leaving 5 μL of acceptor solution in the syringe. Next, the fiber was immersed in deionized water. Then, approximately 5 μL of deionized water was withdrawn back into the fiber, and the immersed fiber was moved to the aqueous sample solution from the de-ionized water.

(iii) The microsyringe was fixed on a syringe pump, which was programmed to run repetitively from 5 to 12.5 μL at a maximum speed with regular triphase, at 40 μL/min. The magnetic stirrer was set at a speed of 700 rpm. After extracting for 40 min the syringe pump and stirrer were stopped. All acceptor phase was withdrawn back into the microsyringe. Then, the fiber was discarded and the needle was cleaned with lens cleaning paper. Finally, the acceptor phase was injected into the HPLC system for separation and quantification.

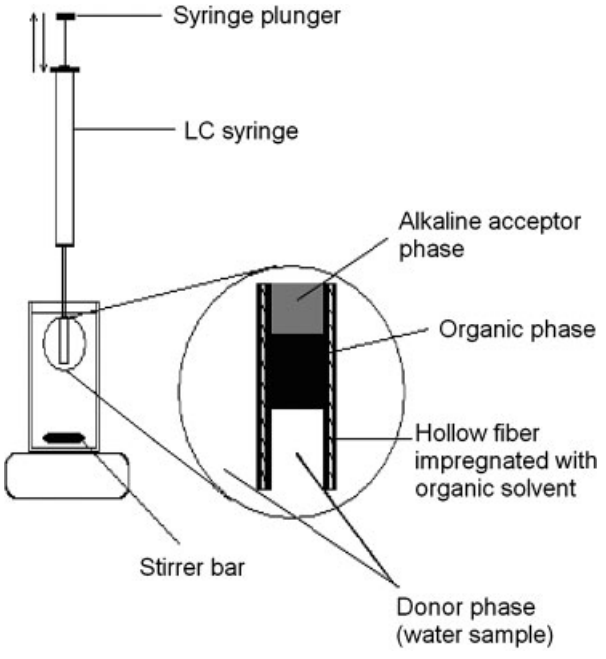

Figure 1. Design of LLLME/AMADP.

3 Results and discussion

The non-charged molecular forms of the analytes were extracted from an acidified donor phase into an organic phase. Then, the analytes in the organic phase were re-extracted into an alkaline acceptor phase, in which a non-charged molecular phenol was converted to a phenolate ion. The extracted analytes in the acceptor phase were separated and quantitated by HPLC-FLD. The pH of the acceptor phase should be maintained above pK_a+2, i.e. ≧99% of anion form in target analytes, and the pH of the donor phase should be maintained below pK_a 1, i.e. ≧90% of target analytes in non-charged molecular form. Seeing that non-target materials could also be extracted into the acceptor phase from the matrix of a practical water sample, these materials will neutralize the basicity of the acceptor phase. Therefore, 0.2 M hydroxide anion (OH^-) was adopted as the acceptor phase. Similarly, 0.02 M $KH_2PO_{4(aq)}$ was used for the donor phase to deal with potential matrix interference in a practical water sample.

3.1 Optimization of extraction settings

The univariant method was conducted to identify the optimal experimental conditions for LLME/AMADP. Analytical parameters, including acceptor phase composition, organic phase identity, acceptor phase volume, stirring rate, extraction time, and salt addition, were investigated and assigned to improve the extraction efficiency. The extraction settings for LLLME/AMADP were finally selected as follows: 14 mL of 0.02 M $KH_2PO_{4(aq)}$ without adding salt as donor phase, 15 μL of 0.2 M tetraethylammonium hydroxide dissolved in ethylene glycol as acceptor phase, 4-chlorotoluene as organic phase, 1100 rpm stirring speed, and 50 min extraction time.

3.1.1 Composition of acceptor phase

The elimination of the surfactant effect of long-chain alkylphenolate ions is exhibited in Fig. 2. The efficiencies

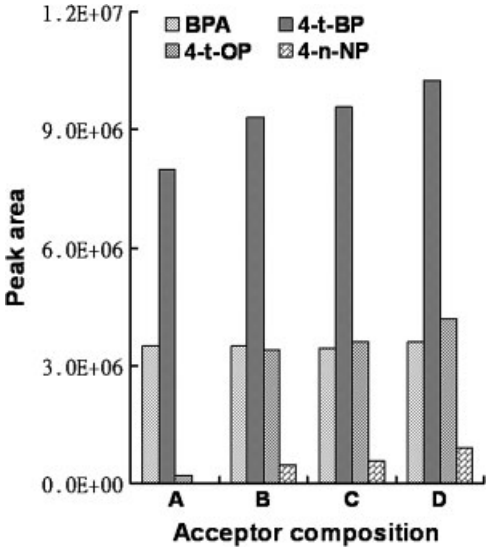

Figure 2. Effect of acceptor composition on extraction efficiency. (A) 0.2 M $NaOH_{(aq)}$; (B) 0.2 M NaOH/ethylene glycol solvent; (C) 0.2 M $(butyl)_4NOH$/ethylene glycol solvent; (D) 0.2 M $(ethyl)_4$-NOH/ethylene glycol solvent Experimental parameters: 0.02 M $KH_2PO_{4(aq)}$ as donor phase with 1 g added NaCl; 12.5 μL of acceptor phase; chlorobenzene as organic phase; 700 rpm stirring rate; 40 min extraction time.

of extracting 4-n-NP and 4-t-OP were elevated when the acceptor phase was the solvent ethylene glycol and an added ion-pair reagent. In addition, the efficiency of extracting 4-n-NP was improved more by adding tetraethylammonium hydroxide than by adding tetrabutylammonium hydroxide. This is due to the fact that the solubility of the ion-pair tetraethylammonium cation/4-n-nonyl phenolate anion is greater in ethylene glycol than is the solubility of the other ion pair. Therefore, the solvent ethylene glycol with added tetraethylammonium hydroxide was selected as the acceptor phase composition for the following trials.

3.1.2 Selection of organic solvent

The selection criteria for a suitable organic solvent are as follows: (i) The solvent should possess appropriate density and viscosity. (ii) The solvent must be of low volatility and immiscible with the polyhydric alcohol used. (iii) The solubilities of the analytes in the organic solvent must be much higher than they are in the aqueous donor phase. Given that the target analytes are phenyl derivatives, those aromatic organic solvents exhibiting both low miscibility with ethylene glycol and possessing a variety of functional groups were candidates for extracting analytes. Accordingly, chlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene, 4-chlorotoluene, and 2-nitrophenyl octyl ether were tested for their effectiveness in extraction; the results are shown in Table 2. The solvent 2-nitrophenyl octyl ether exhibits poor extraction effectiveness for 4-n-NP. This may be due to ion-pair chemicals of low polarity having high solubilities in nitrobenzene derivatives. The solvent 1,2,4-trichlorobenzene presents an unreasonably low extraction effectiveness for 4-t-OP. This is because 4-t-OP is co-eluted with 1,2,4-trichlorobenzene in HPLC. The solvent 1,2,4-trichlorobenzene, whose halogen groups cause a heavy atom effect, quenches the fluorescence of 4-t-OP [13]. These results indicated that acceptable extraction efficiencies were achieved with 1,2-dichlorobenzene. Consequently, the solvent 1,2-dichlorobenzene was used for the following extractions.

Table 2. Extraction efficiency in different organic solvent

Organic solvent	Enrichment factor[a)]			
	BPA	4-t-BP	4-t-OP	4-n-NP
Chlorobenzene	204	328	218	63
1,2-Dichlorobenzene	152	400	268	92
1,2,4-Trichlorobenzene	88	432	65	101
4-Chlorotoluene	182	411	268	70
2-Nitrophenyl octyl ether	173	299	169	3

Experimental parameters: 0.02 M $KH_2PO_{4(aq)}$ as donor phase with 1 g added NaCl; 12.5 μL of 0.2 M tetraethyl ammonium hydroxide dissolved in ethylene glycol as acceptor phase; 700 rpm stirring rate; 40 min extraction time.

a) Enrichment factor is the ratio of analyte concentration in final acceptor phase to that in initial donor phase.

3.1.3 Solvent effect of acceptor phase

The solvent 1,2-dichlorobenzene, of rather low polarity, was used as the organic phase. Instead of ethylene glycol, a polyhydric alcohol possessing higher solvent strength for 4-n-nonylphenolate ion-pair could be adopted as acceptor solvent to raise the efficiency of extracting 4-n-NP. As trial results indicated, diethylene glycol and triethylene glycol are more efficient than ethylene glycol for extracting analyte 4-n-NP, but triethylene glycol is deficient in extracting analyte BPA. Because of its capability to extract all analytes adequately, diethylene glycol was selected as the acceptor solvent for the following trials.

3.1.4 Effect of acceptor phase volume

In a three-phase LLLME system, a smaller volume of acceptor phase involves a higher analyte concentration (or enrichment) in the acceptor phase. Considering injecting all of the acceptor phase into HPLC, the important factor for LLLME is not only the concentration but also the volume in the acceptor phase. The acceptor phase should be of a sufficiently large volume to promote analyte transport into the acceptor phase. Given the limitations of syringe capacity (25 μL) and injector loop (20 μL), the acceptor phase volume was examined over a range of 7.5–20 μL. As trial results indicated, 15 μL of acceptor phase yields the maximum extraction efficiency and this volume was used for later extractions.

3.1.5 Effect of stirring rate

Agitation of the donor solution reduces the required extraction time and increases extraction efficiency. Stirring provides fresh donor solution for the organic phase to extract and reduces the effect of the stationary boundary layer close to the organic phase; this promotes analyte transport from the donor phase to the organic phase [14, 15]. However, agitation in excess of the optimal stirring rate also causes lower extraction efficiency because the hollow fiber is vibrated by the surrounding turbulent flow. To evaluate the effect of stirring, donor solution was extracted at varying stirring rates (500–1300 rpm). The extraction efficiency for these analytes increased at higher stirring rates and reached a maximum at 1100 rpm. Consequently, 1100 rpm was chosen for subsequent extractions.

3.1.6 Effect of extraction time

A dynamic LLLME is dependent on analyte migration amount rather than equilibrium extraction [16]. The trial results clearly indicate that the effect of analyte polarity on extraction efficiency varies with extraction time. The efficiency of extracting BPA was raised as the extraction proceeded to longer times. The extraction of 4-n-NP exhibited a maximum efficiency of 40–50 min. This may be due to re-extracting the 4-n-nonylphenolate ion-pair to the organic phase, perhaps even to the donor phase. The extraction time of 50 min was acceptable and it matched the duration of one HPLC analysis.

3.1.7 Effect of salt addition

Adding salt to the analytes may have a double-edged effect [17]. First, a positive effect results from salting out when the ionic strength of the donor phase is increased. This is due to the decrease in solubilities of the analytes in the aqueous phase and enhances their partitioning into the organic phase. Second, a negative effect is attributed to changes in the physical properties of the Nernst diffusion film, which reduces the rate of diffusion of the analytes from the donor phase into the organic phase. To examine the effect of salt addition, 0 to 2 g of $NaCl_{(S)}$ was added to 14 mL of the donor phase. Accordingly, only BPA, with its higher polarity, resulted in a positive effect. The efficiencies of extraction of 4-t-OP and 4-n-NP were obviously reduced as the amount of added $NaCl_{(S)}$ was increased. The addition of 1 g $NaCl_{(S)}$ was provisionally adopted.

3.1.8 Selection of triphase composition

Owing to the difference of analyte polarity, the optimum conditions for BPA extraction are sometimes different from those for 4-n-NP. Therefore, three groups of triphase combinations were tested to evaluate extraction effects. These include the following:

(i) Combination A consists of the following: diethylene glycol as acceptor solvent; 1,2-dichlorobenzene as organic phase; adding 1 g of NaCl to donor phase.
(ii) Combination B: ethylene glycol as acceptor solvent; 1,2-dichlorobenzene as organic phase; donor phase without added salt.
(iii) Combination C: ethylene glycol as acceptor solvent; 4-chlorotoluene as organic phase; donor phase without added salt.

As shown in Fig. 3, Combination A was somewhat more successful than the others. Additionally, 1,2-dichlorobenzene contaminating the acceptor phase interferes little with the HPLC/FLD chromatogram because two chlorine atoms of 1,2-dichlorobenzene cause a heavy-atom effect and quench the 1,2-dichlorobenzene fluorescence. Nevertheless, 1,2-dichlorobenzene is more toxic than 4-chlorotoluene. To protect workers from potential harm, Combination C was finally selected.

3.2 Quantitative aspects

The target analytes were determined under the selected optimized experimental conditions to assess repeatability, linearity, coefficient of determination, and detection limit. The results are given in Table 3. Linearity was established over

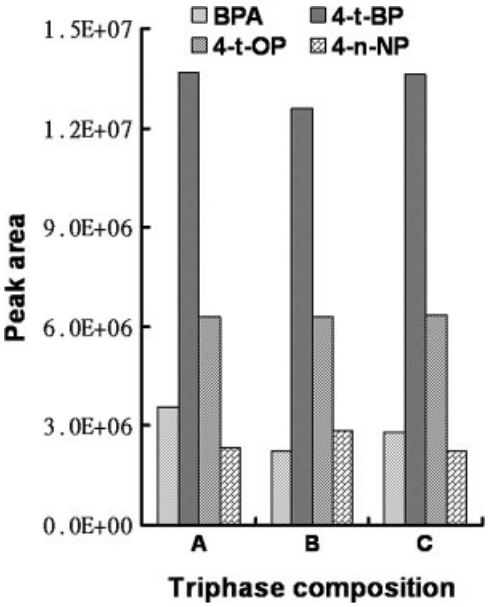

Figure 3. Effect of triphase composition on extraction efficiency. Combination A – acceptor solvent: diethylene glycol; organic phase: 1,2-dichlorobenzene; donor phase with 1 g added NaCl. Combination B – acceptor solvent: ethylene glycol; organic phase: 1,2-dichlorobenzene; donor phase without salt. Combination C – acceptor solvent: ethylene glycol; organic phase: 4-chlorotoluene; donor phase without salt. Experimental parameters: 0.02 M $KH_2PO_{4(aq)}$ as donor phase; 15 μL of 0.2 M tetraethyl ammonium hydroxide dissolved in acceptor solvent as acceptor phase; 1100 rpm stirring rate; 50 min extraction time.

the concentration range of 0.05–200 ng/mL for 4-t-BP and 4-t-OP as well as from 0.1 to 200 ng/mL for BPA and 4-n-NP. The coefficient of determination r^2 varied from 0.9977 to 0.9999. The limit of detection (LOD) values were calculated as three times the standard deviation of seven replicate runs of water spiked with each analyte at about the lowest concentration of the calibration curve [18]. The relative standard deviation (RSDs) values were calculated based on the peak areas for seven replicated runs, and these values were less than 5.8%. The low bias is due to the direct injection into HPLC after extraction, without derivatization and volume reduction. In addition, a benzene type solvent was used as the organic phase to impregnate the polypropylene hollow fiber, causing the fiber to be transparent. Such a fiber allows the interfaces of the LLLME triphase to be observed and its assembly is simple to organize and regulate. The achievement of acceptable LOD (i.e. 4.8–17 ng/L) resulted from that LLLME highly concentrates target compounds from the donor phase to the acceptor phase. By the virtue of this mechanism, the LLLME relative to SPME has a better extraction capacity.

Table 3. Performance of the method

Compound	Linearity rang (ng/mL)	r^2	LOD[a] (ng/mL)	RSD[b] (%)	RSD[c] (%)
BPA	0.1, 0.2, 1, 5, 10, 50, 200	0.9999	0.014	4.7	2.0
	0.1, 0.2, 1, 5, 10	0.9997			
4-t-BP	0.05, 0.2, 1, 5, 10, 50, 200	0.9999	0.0051	3.4	3.3
	0.05, 0.2, 1, 5, 10	0.9998			
4-t-OP	0.05, 0.2, 1, 5, 10, 50, 200	0.9995	0.0048	3.2	4.0
	0.05, 0.2, 1, 5, 10	0.9977			
4-n-NP	0.1, 0.2, 1, 5, 10, 50, 200	0.9995	0.017	5.8	3.4
	0.1, 0.2, 1, 5, 10	0.9985			

a) LOD are calculated as three times the standard deviation of seven replicated runs of standard solution. Concentrations: BPA and 4-n-NP 0.1 ng/mL; 4-t-BP and 4-t-OP 0.05 ng/mL.

b) Data obtained by extraction in seven replicates. Concentrations: BPA and 4-n-NP 0.1 ng/mL; 4-t-BP and 4-t-OP 0.05 ng/mL.

c) Data obtained by extraction in seven replicates. Concentrations: BPA and 4-n-NP 1 ng/mL; 4-t-BP and 4-t-OP 0.5 ng/mL.

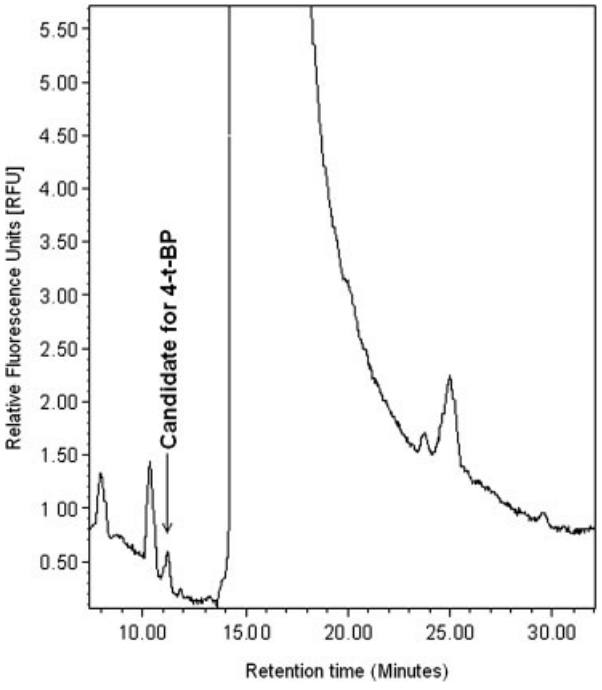

Figure 4. Chromatogram of lake water extraction. The peak of candidate for 4-t-butylphenol is less than LOD of 4-t-butylphenol. Experimental conditions under selected optimized conditions: 14 mL of 0.02 M KH2PO4(aq) without adding salt as donor phase; 15 μL of 0.2 M tetraethylammonium hydroxide dissolved in ethylene glycol as acceptor phase; 4-chlorotoluene as organic phase; 1100 rpm stirring rate; 50 min extraction time.

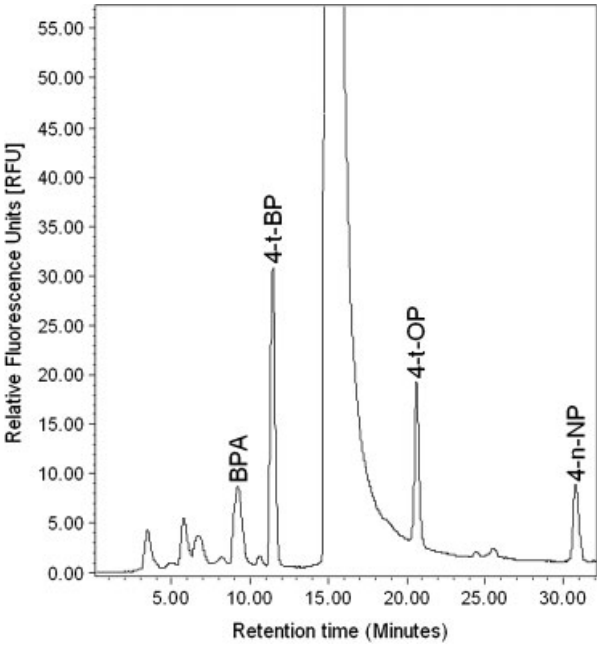

Figure 5. Chromatogram of lake water extraction. Sample was spiked with 0.5 ng/mL of 4-t-BP and 4-t-OP as well as 1 ng/mL of BPA and 4-n-NP. Experimental conditions under selected optimized conditions: 14 mL of 0.02 M KH2PO4(aq) without adding salt as donor phase; 15 μL of 0.2 M tetraethylammonium hydroxide dissolved in ethylene glycol as acceptor phase; 4-chlorotoluene as organic phase; 1100 rpm stirring rate; 5 min extraction time.

Table 4. Relative recovery of real field sample ($n = 3$)

Compound	Relative recovery (%)[a] (mean ± SD)		Relative recovery (%)[b] (mean ± SD)	
	Lake water	Tap water	Lake water	Tap water
BPA	100.4 ± 3.4	93.6 ± 3.5	104.9 ± 0.1	101.9 ± 3.4
4-t-BP	101.0 ± 4.2	96.7 ± 3.8	95.4 ± 1.1	95.8 ± 3.9
4-t-OP	106.3 ± 4.4	105.6 ± 4.6	98.4 ± 2.9	100.6 ± 3.7
4-n-NP	98.2 ± 6.6	98.6 ± 4.8	92.8 ± 4.7	94.1 ± 4.5

a) Performed on 0.05 ng/mL of 4-t-BP and 4-t-OP as well as 0.1 ng/mL of BPA and 4-n-NP spiked to practical samples, where SD is standard deviation.

b) Performed on 0.5 ng/mL of 4-t-BP and 4-t-OP as well as 1 ng/mL of BPA and 4-n-NP spiked to practical samples, where SD is standard deviation.

3.3 Real field aqueous sample analysis

Two water samples, lake water and tap water, were analyzed to demonstrate the practical applicability of this technique. These two samples were filtered with Advantec number 2 filter paper, and the tap water sample was dosed with sodium disulfite (5 μg/mL) to neutralize residual chlorine or hypochlorite. The analytical results of these samples indicated that the tap water sample is free from the target analytes, but a target analyte can be present in the lake water sample. The detected analyte, whose peak signal was close to the detection limit, coincides with standard 4-t-BP in retention time. The HPLC chromatogram is presented in Fig. 4. Figure 5 shows the results for the lake water sample, which was spiked with each target analyte. To verify the detected analyte to be 4-t-BP, the HPLC column XTerra RP8 (5 μm 3.0 × 250 mm) was substituted for original XTerra phenyl (5 μm 3.0 × 250 mm) and operated with the same mobile phase composition. The trial result, under LOD 5.1 ng/L, indicated that the analyte 4-t-BP was undetected in the lake water sample. As to recovery, this LLLME method depends on migration amount rather than exhaustive extraction. Instead of absolute recoveries, relative recoveries determined as the ratio of the real field sample outcome to that of the deionized water sample are summarized in Table 4. These results indicate that the relative recoveries of target analytes from the spiked lake and tap water samples were 92.8–106.3 and 93.6–105.6%, respectively. These relative recoveries of the practical water samples provide information about the matrix effect on the extraction efficiency. It is therefore suggested that the matrix effect, interfering in the transport of target compounds from the donor phase through the organic phase and finally into the acceptor phase, was limited.

4 Concluding remarks

This study solves the problem of the surfactant effect, in which long-chain alkylphenolate ions are adsorbed on the interface between the acceptor and the organic phases. Thus, it is feasible to use the LLLME technique to extract alkylphenols from water. From the studies of adequate repeatability, linearity, LOD, and recoveries shown above, the LLLME combined with HPLC-FLD is able to determine BPA and alkylphenols at ng/L levels. A feature of the sample pretreatment is that the acceptor phase, walled in by organic phase, is simply immersed in the water sample to extract target analytes before injecting into HPLC-FLD without derivatization, elution, or volume reduction. These processes are simple and cause the extraction to be of high precision. Additionally, the use of a widely available instrument and an environmentally friendly extraction is also an obvious advantage of the method.

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The authors have declared no conflict of interest.

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