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Determination of bisphenol A, 4-*n*-nonylphenol, and 4-*tert*-octylphenol by temperature-controlled ionic liquid dispersive liquid-phase microextraction combined with high performance liquid chromatography-fluorescence detector

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ABSTRACT

Present study described a simple, sensitive, and viable method for the determination of bisphenol A, 4-*n*-nonylphenol and 4-*tert*-octylphenol in water samples using temperature-controlled ionic liquid dispersive liquid-phase microextraction coupled to high performance liquid chromatography-fluorescence detector. In this experiment, 1-octyl-3-methylimidazolium hexafluorophosphate ([C_8MIM][PF_6]) was used as the extraction solvent, and bisphenol A, 4-*n*-nonylphenol and 4-*tert*-octylphenol were selected as the model analytes. Parameters affecting the extraction efficiency such as the volume of [C_8MIM][PF_6], dissolving temperature, extraction time, sample pH, centrifuging time and salting-out effect have been investigated in detail. Under the optimized conditions, good linear relationship was found in the concentration range of 1.0–100 $\mu g\,L^{-1}$ for BPA, 1.5–150 $\mu g\,L^{-1}$ for 4-NP, and 3–300 $\mu g\,L^{-1}$ for 4-OP, respectively. Limits of detection (LOD, S/N = 3) were in the range of 0.23–0.48 $\mu g\,L^{-1}$. Intra day and inter day precisions (RSDs, $n = 6$) were in the range of 4.6–5.5% and 8.5–13.3%, respectively. This method has been also successfully applied to analyze the real water samples at two different spiked concentrations and excellent results were obtained.

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1. Introduction

During the past years endocrine disruptors (EDs) have been attracting much more attention because of their possible negative effects on human health [1]. They arise from many different sources including pesticides, industrial chemicals, pharmaceuticals, and phytochemicals [2]. Endocrine disruption is one of the highest priority research topics for the United States Environmental Protection Agency (US EPA), and a detailed research strategy has been developed to guide the placement of resources over the next several years [3]. The European Union and the US EPA have ordered further evaluation for their endocrine disruption role of a "priority" list [4].

Bisphenol A (BPA), 4-nonylphenol (4-NP), and 4-octylphenol (4-OP) have been reported to have endocrine disruption properties and become important pollutants. The effects of BPA are mediated by both genomic and non-genomic estrogen-response mechanisms with the disruption of the cell function occurring at doses as low as 1 pM (0.23 $ng\,L^{-1}$) [5]. US EPA has accepted the risks of nonylphenol and has prepared a guideline for ambient water quality that recommends nonylphenol concentrations in freshwater be below 6.6 $\mu g\,L^{-1}$ [6]. U.S. EPA drinking water relative source contribution yielded a 100 $\mu g\,L^{-1}$ BPA total allowable concentration (TAC) which could be used as a reference [7]. 4-NP and 4-OP are important precursors in the manufacture of non-ionic surfactants, household detergents, pesticide formulations and main degradation products of alkylphenol ethoxylates (APEOs) [8]. Human exposure to BPA, 4-OP and 4-NP is from food and drinking. Due to their endocrine disruptor potential, it is necessary to develop rapid, simple, and efficient methods for monitoring these compounds in the environment.

Several methods have been reported to determine these three analytes, such as solid phase extraction (SPE) [9–13], solid phase microextraction (SPME) [14–17] and liquid phase microextraction

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(LPME) [18,19]. Dispersive liquid–liquid microextraction (DLLME) was a new LPME mode, which was a successful extraction technique due to the large contact surface between extraction solvent and analytes, which speeded up the mass transferring processes of analytes from aquatic phase to the extractant phase [20]. DLLME method has some advantages such as simplicity, easy to operate, rapidity, low cost, high recovery and enrichment factor [21]. But DLLME often used a high-density solvent as the extraction solvent and a water miscible polar solvent as the disperser solvent. Usually, chlorobenzene, carbon tetrachloride, tetrachloroethylene and so on are used as the extraction solvent in the extraction procedure, and acetone, methanol and acetonitrile etc. are often utilized as the disperser solvent [22]. These solvents are of certain extent toxicity, and would generate secondary pollution.

Ionic liquids are nonvolatile and nonflammabe, have high thermal stability, tunable viscosity, miscibility with water and organic solvents. Moreover, the physical and chemical properties of ILs can be adjusted by selection of the suitable cation, anion, and substituent [23]. As result of these properties, ILs has been widely used in LLE [24,25], LPME [26,27] and SPME [28,29]. Recently, a new LPME mode termed as temperature controlled ionic liquid liquid phase microextraction has been developed [30,31] for the determination of organic pollutants. Temperature controlled ionic liquid liquid phase microextraction is a new extraction and enrichment mode based on the same principle of dispersive liquid liquid microextraction. However, it uses ionic liquid, known as "green solvent" with low volatility, as the extraction solvent, and need not to use toxic solvent as the dispersive solvent. The dispersing is performed by the change of temperature. Temperature controlled ionic liquid liquid phase microextraction avoided the large consumption of toxic solvents, and decreased the risk of exposure to toxic solvents. Due to the variety of ionic liquids, the developed method earned robust applicability. Meanwhile, the enrichment selectivity could be obtained by designing special ionic liquid. The enrichment performance could be improved by tuning the volume and the structure of ionic liquid. The results demonstrated that temperature controlled ionic liquid liquid phase microextraction was a simple, rapid, sensitive, and easy to operate method, and it would be very useful in the analysis of pollutants. However, no related reports on this new technique for the enrichment of BPA, 4-OP and 4-NP were found.

The goal of present study is to develop a simple, sensitive, method for the determination of BPA, 4-OP and 4-NP in water samples using temperature controlled ionic liquid liquid phase microextraction combined with HPLC-FLD method.

2. Experimental

2.1. Instrumentation

A high-performance liquid chromatography system, which consisted of two Waters 1525 Binary HPLC pumps and Waters 2475 Multiwavelength fluorescence detector were used for the analysis and separation. Analytical column temperature was controlled with a Model 1500 Column Heater (Waters, Singapore). The analytical column was a 150 mm × 4.6 mm Waters C18 column (particle size 5 μm). A Waters guard column (C18, 4.6 mm × 20 mm, particle size 5 μm) was used to protect the analytical column. The mobile phase was a mixture of methanol-pure water (75/25, v/v). The mobile phase flow-rate was 1.0 mL min^{-1}, the injection volume was 20 μL, and the detection wavelength was set as λ_{ex} = 230 nm, and λ_{em} = 305 nm. An Anke TDL80-2B (Shanghai, China) centrifuge was used to centrifuge the cloudy water solutions.

2.2. Reagents

BPA, 4-OP and 4-NP were obtained from AccuStandard, Inc. (New Haven, USA). The stock solutions were prepared by dissolving the suitable amount of them in methanol and then stored at 4 °C in the refrigerator. The stock solutions were further diluted to yield the appropriate working solutions with methanol.

1-octyl-3-methylimidazolium hexafluorophosphate ([C_8MIM][PF_6]) were synthesized by our own laboratory. HPLC grade methanol and acetonitrile were obtained from Jiangsu Guoda Chemical Reagent Co., Ltd. (Huaian, China). A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare ultrapure water. All the other solvents were analytical reagent grade unless stated. All glass wares used in the experiments were cleaned with pure water, then soaked in 6 mol L^{-1} nitric acid for 24 h and washed with ultrapure water before use.

2.3. Ionic liquid dispersive liquid-phase micro-extraction

In the extraction procedure, 10 mL ultra-pure water was added into a 10 mL conical tube and this solution was spiked with a concentration of 10 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 30 μg L^{-1} for 4-OP. Then 65 μL 1-oxyl-3-methylimidazolium hexafluorophosphate [C_8MIM][PF_6] was taken out with a HPLC syringe and added into the solution. The conical tubes were heated in the water bath with the temperature controlled at 80 °C. The IL was dissolved completely and mixed entirely. There-after the tube was cooled with ice-water for 20 min and led to a cloudy solution. Further the solution was centrifuged for 10 min at 4000 rpm. The upper aqueous phase was removed with a syringe, the sediment phase was dissolved in 200 μL methanol and 20 μL was injected into the HPLC system for analysis.

2.4. Water samples

River water was collected from Baihe, Nanyang city, Henan Province, China. Melted water was obtained from Henan Normal University in Xinxiang City, Henan Province. Drainage water was taken from Gongchanzhuyi drainage, Xinxiang City, Henan Province, China. Wastewater was collected from the outlet of Luotuowan sewage treatment plant, Xinxiang City, Henan Province, China. Before use, all the environmental water samples were filtered through 0.45 μm micro-pore membranes and stored in brown glass containers at the temperature of 4 °C.

3. Results and discussion

The extraction efficiency depends on some important experimental parameters. In order to obtain the maximal extraction efficiency, the amount of IL, temperature, sample pH, extraction time, centrifugation time, and ionic strength were investigated in detail.

3.1. Effect of the volume of ionic liquid

The volume of IL was a crucial parameter, which would have an important effect on the extraction performance. Small volume of IL resulted in lower extraction efficiency, but too much large volume of IL may led longer time to dissolved IL completely and mixed entirely. IL volume was examined within the range of 45–70 μL, and the results were shown in Fig. 1. From Fig. 1, it was found that the peak areas of them reached the largest at 65 μL, and decreased at 70 μL. Fine IL droplets increased along with the increase of the [C_8MIM][PF_6] volume and then more target analytes were transferred into the IL droplets, but larger volume led to the increase

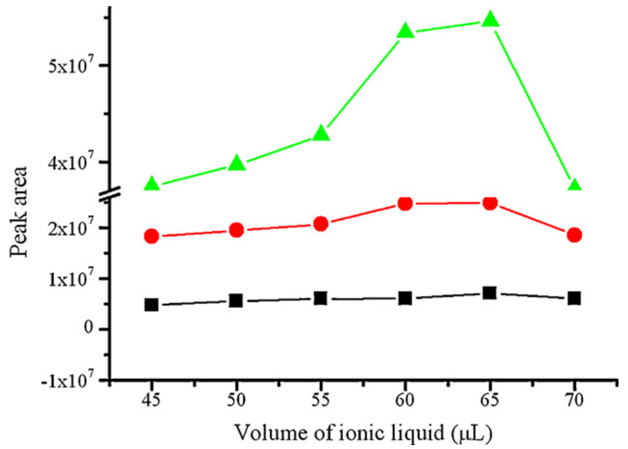

Fig. 1. Effect of ionic liquid volume. Conditions: kinds of ionic liquids, [C_8MIM][PF_6]; sample volume, 10 mL; spiked concentration, 10 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 30 μg L^{-1} for 4-OP; temperature, 80 °C; extraction time, 30 min; centrifugation time, 20 min; (■), BPA; (●), 4-NP; (▲), 4-OP.

of sedimented phase volume, which resulted in low enrichment factor. Therefore, 65 μL [C_8MIM][PF_6] was employed.

3.2. *Effect of dissolving temperature*

In this temperature-controlled ionic liquid dispersive liquid-phase microextraction, temperature is the key factor and the driving force to make [C_8MIM][PF_6] dispersed into the sample solution. Dissolving temperature was investigated in the range of 60–100 °C and the results were shown in Fig. 2. It was obviously that the largest peak areas were obtained at 80 °C. The reason was that at low temperature the performance of dispersing of IL [C_8MIM][PF_6] was poor and the diffused rate of the analytes was very low so the mass transfer coefficients were low. But at higher temperature, the diffused rate and mass transfer rates were increased. So the amounts of analytes increased with the rise of temperature. However, as all known, the rise of temperature also resulted in the increase of migrating out from the ionic liquid phase. That is to say, the rise of temperature has bifunctions, and the rise of temperature has enhanced the transferring into the ionic liquid phase, but at the same time, it also enhanced the migrating out from the ionic liquid. Hence a reason temperature is very important. From Fig. 2, we can see that the transferring rate into the ionic liquid was larger than the migrating out rate when the temperature was lower than 80 °C and a reverse result was obtained when the temperature was higher than 80 °C. So 80 °C was utilized in this new developed method.

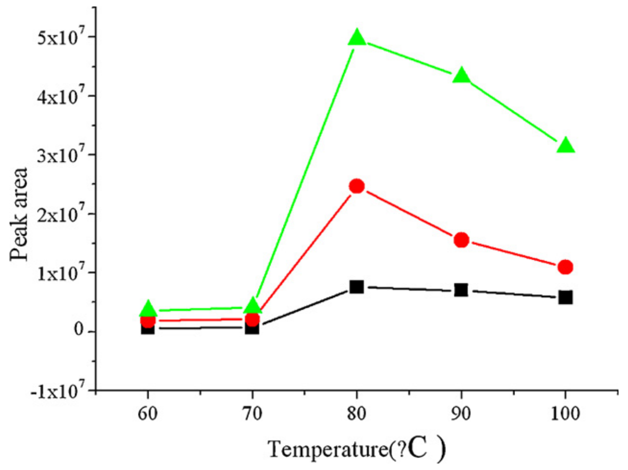

Fig. 2. Effect of temperature. Conditions: [C_8MIM][PF_6] volume, 6 5 μL; sample volume, 10 mL; spiked concentration, 10 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 30 μg L^{-1} for 4-OP; extraction time, 30 min; centrifugation time, 20 min; (■), BPA; (●), 4-NP; (▲), 4-OP.

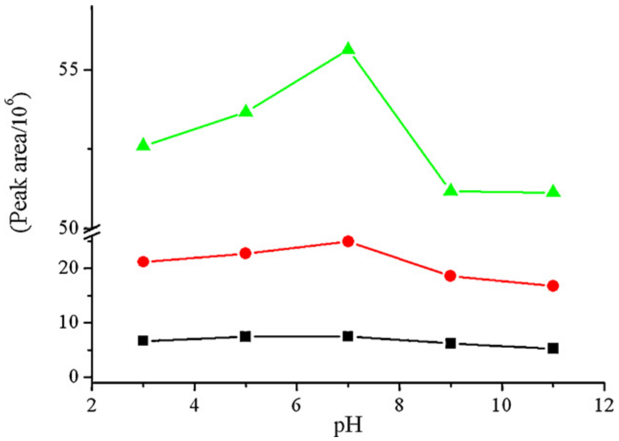

Fig. 3. Effect of sample pH. Conditions: [C_8MIM][PF_6] volume, 65 μL; sample volume, 10 mL; spiked concentration, 10 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 30 μg L^{-1} for 4-OP; temperature, 80 °C; extraction time, 20 min; centrifugation time, 20 min; (■), BPA; (●), 4-NP; (▲), 4-OP.

3.3. *Effect of sample pH*

Sample pH is another important parameter that might affect the extraction efficiency, because the analytes will be present at different forms at different pH environments. A series of experiments were performed to investigate the effect of pH. The sample pH was designed in the range of pH 3–11. The data were shown in Fig. 3. It was found that the peak areas of BPA and 4-NP had no significant effect in the range of pH 3–7, but slightly deceased in the range of pH 9–11. The peak area of 4-OP increased with the rise of sample pH in the range of pH 3–7, yet decreased with the continual rise of sample pH in the range of pH 7–11. The pK_a values of 4-NP, 4-OP, and BPA were 10.7, 10.3, and 9.5, respectively. Usually, the solution pH is three units lower than the pK_a value which could make the analyte existing as molecular form. From this point, the results would be in agreement to this experimental principle. Moreover these compounds are soluble in alkaline environment, so it is hard to be extracted. Based on these facts, the sample pH was adjusted to be neutral in the subsequent experiments.

3.4. *Effect of extraction time*

Extraction time is one of the most important factors in most of the extraction procedures, especially in microextraction methods such as SPME and LPME. In our study, it is defined as the time from the moment that the solution containing completely dissolved IL was put into ice water bath to the moment to start to centrifuge. The extraction time was tested in the range of 10–50 min, and the results were shown in Fig. 4. It was found that 20 min was the best time for extraction from the water samples. However, the peak areas would decrease when the extraction time was longer than 20 min. The reason maybe some of the analytes were adsorbed onto the wall of tube. Based on these results, 20 min was selected for further experiments.

3.5. *Effect of centrifuging time*

Centrifugation was a crucial step in the DLLME method, because it was required to break down the emulsion and accelerate the phase-separation process. The experiments indicated that the tur-

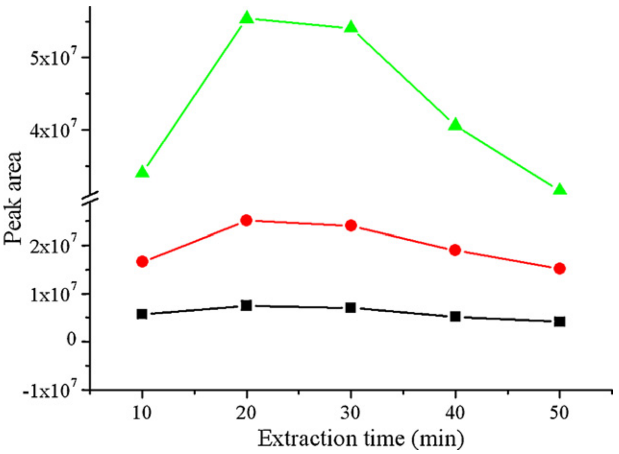

Fig. 4. Effect of extraction time. Conditions: [C_8MIM][PF_6] volume, 65 μL; sample volume, 10 mL; spiked concentration, 10 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 30 μg L^{-1} for 4-OP; temperature, 80 °C; centrifugation time, 20 min; (■), BPA; (●), 4-NP; (▲), 4-OP.

bidity phenomenon was easy to occur in the extraction procedure. Centrifugation time was examined in the range of 5–25 min at 4000 rpm. The results showed that the peak areas for the analytes reached largest at 10 min. We can see that the sedimented volume increased as the centrifugation time increased from 5 to 10 min, but decreased if the time exceeded 10 min. This can be explained that the [C_8MIM][PF_6] phase cannot be completely precipitated for a short time, and longer centrifuging time will result in parts of the [C_8MIM][PF_6] phase redissolved into water phase due to the heat generated from centrifugation. Hence, 10 min was chosen as the centrifuging time.

3.6. *Effect of salt addition*

Addition of salt to the aqueous sample can decrease the solubility of analytes in the aqueous phase and enhance the availability of analytes for extraction, but on the other hand, the obtained volume of organic phase is increased, resulting in a decrease of both the target analyte concentration and the enrichment factor [32]. For this proposes, experiments were carried out for investigating the effect of salt addition in the range of 0–30% (w/v) by adding NaCl. With the increase of the content of NaCl from 0 to 30%, no significant effect on peak areas was observed. Hence, no NaCl was added in follow experiments due to the fact that no significant positive changes were found in the extraction yields of all target analytes.

3.7. *Analytical performance of the proposed method*

Under the above optimum conditions, linear range, precisions and detection limits were investigated and the results were listed in Table 1. Good Linear relationships were found in the concentration range of 1.0–100 μg L^{-1} for BPA, 1.5–150 μg L^{-1} for 4-NP, and 3–300 μg L^{-1} for 4-OP, respectively. The correlation coefficients (R^2) were in the range 0.9981–0.9994. Reproducibility was investigated by series of 6 independent experiments carried out with spiked ultrapure water. Detection limits of proposed method based on a signal-to-noise ratio (S/N = 3) were in the range of 0.23–0.48 μg L^{-1}. The LODs were lower than the value of US EPA recommended level of 4-NP, and also lower than the total allowable concentration of BPA. They could be well used to monitor the residual concentration of such pollutants.

Meanwhile a comparison was made to compare the proposed method with SPE [11]. The SPE procedure was done as follows, the cartridge (HLB, 0.2 g) was washed with 5 mL of methanol and 5 mL of Milli-Q water at a flow rate of 5 mL min^{-1} before each extraction. The 10 mL sample percolated through the cartridge at a flow rate of 10 mL min^{-1}. A wash step was conducted using 5 mL of Milli-Q water to remove impurities retained in the cartridge. Subsequently, the cartridge was dried under vacuum for 10 min, and the retained analytes were eluted at a low flow rate (approximately 1 mL min^{-1}) with 2 mL of methanol. The eluent was evaporated to near dryness with mild nitrogen, then the residue was redissolved in 200 μL methanol and 20 μL of the solution was injected for HPLC analysis. The results were exhibited in Table 2. From Table 2, we can see that proposed method provided a relatively wide linear range than SPE. Although it uses much less solvent than LLE, the usage can still be considered. ILs was friendly to environment when it was used as the extraction solvent due to the merits such as negligible vapor pressure, good thermal stability, and tunable viscosity. Therefore,

Table 1
Linear ranges, precisions, detection limits for the enrichment of BPA, 4-NP and 4-OP by temperature controlled ionic liquid dispersive liquid phase micro-extraction.

Compound	Liner range (μg L^{-1})	R^2	Intra day precision (RSD%, n = 6)	Inter day precision (RSD%, n = 6)	Detection limits (μg L^{-1})
BPA	1–100	0.9994	4.8	13.3	0.23
4-NP	1.5–150	0.9981	4.6	8.5	0.35
4-OP	3–300	0.9983	5.5	11.5	0.48

Table 2
Linear ranges, precisions, detection limits for the enrichment of BPA, 4-NP and 4-OP by SPE (HLB).

Compound	Liner range (μg L^{-1})	R^2	Intra day precision (RSD%, n = 6)	Inter day precision (RSD%, n = 6)	Detection limits (μg L^{-1})
BPA	0.5–50	0.9991	3.4	10.1	0.15
4-NP	1.0–100	0.9967	4.1	9.8	0.19
4-OP	1.5–150	0.9992	4.0	6.4	0.22

Table 3
Spiked recoveries in samples by the proposed method.

Compound	Spiked level (μg L^{-1})	Gongchan zhuyi drainage	Wastewater	Melted snow water	Baihe river
BPA	7.5	96.5 ± 2.2	86.9 ± 3.6	95.2 ± 4.3	85.1 ± 4.1
	30	110 ± 1.5	116 ± 2.9	109 ± 1.8	116 ± 6.8
4-NP	15	97.2 ± 2.4	86.7 ± 4.2	93.7 ± 2.8	83.1 ± 3.1
	60	110 ± 1.2	117 ± 1.3	108 ± 1.8	117 ± 7.5
4-OP	22.5	96.4 ± 3.0	85.4 ± 4.1	94.9 ± 1.2	82.8 ± 3.4
	180	111 ± 1.5	114 ± 2.2	109 ± 2.7	114 ± 6.7

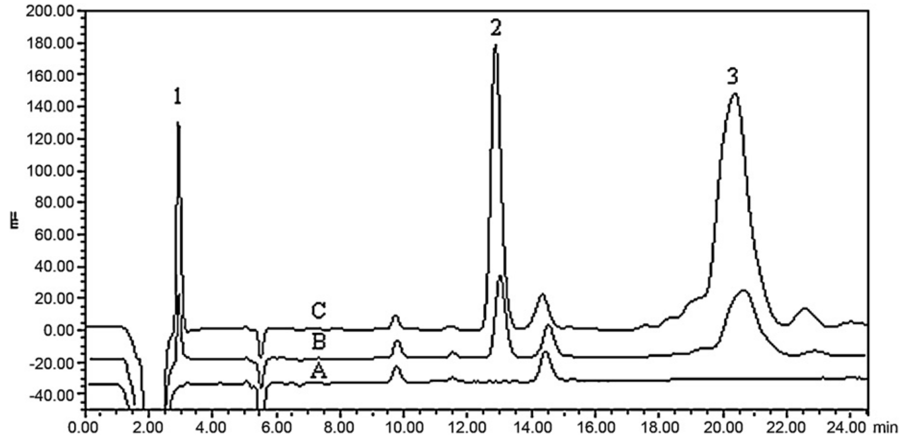

Fig. 5. Typical chromatogram of Baihe River. (A) Blank; (B) spiked at 7.5 μg L^{-1} for BPA, 15 μg L^{-1} for 4-NP, 22.5 μg L^{-1} for 4-OP; C, spiked at 30 μg L^{-1} for BPA, 60 μg L^{-1} for 4-NP, 180 μg L^{-1} for 4-OP. 1, BPA; 2, 4-NP; 3, 4-OP.

these merits indicated that it would be a competitive sample pretreatment technique and will play an important role in analytical and environmental fields.

3.8. Real water sample analysis

Four real water samples were analyzed with the established method based on three replicate extractions and the results were shown on Table 3. The typical chromatogram of water sample was shown in Fig. 5. The experimental results indicated that no BPA, 4-NP, and 4-OP were found in the blank samples. All these samples were spiked with two different concentrations. The spiked recoveries were good in the range of 82.8–117% with the precisions of 1.3–7.5% (RSD). All these results indicated the developed method was reliable alternative to existing methods and would have a great prospect in the future.

4. Conclusions

This paper described a temperature controlled ionic liquid dispersive liquid phase microextraction combined with HPLC-FLD for the determination of BPA, 4-OP and 4-NP in water samples. The experimental results demonstrated that the developed method met the requirement of concept and would be a viable and sensitive method for the monitoring of BPA, 4-NP and 4-OP. the proposed method provided good linear range and sensitivity. The experimental results indicated that the lower limits of detection, good linearity and repeatability of the proposed method will make it a competitive and viable alternative of the routine methods and have a good perspective in the future.

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