Microchemical Journal 137 (2018) 148–154

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Cloud point extraction for simultaneous determination of 12 phenolic compounds by high performance liquid chromatography with fluorescence detection

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ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form 27 September 2017
Accepted 29 September 2017
Available online 03 October 2017

Keywords:
Cloud point extraction
Phenolic compounds
Tergitol 15-S-7
High liquid performance chromatography
Fluorescence

ABSTRACT

A sensitive method based on cloud point extraction was developed for the separation and preconcentration of 12 phenolic compounds (hydroquinone, resorcinol, catechol, phenol, β-naphthol, bisphenol A, α-naphthol, 4-*tert*-butylphenol, 4-*tert*-octylphenol, nonylphenol, octylphenol, and 4-*n*-nonylphenol) from environmental water samples for subsequent analysis by high performance liquid chromatography. The nonionic surfactant Tergitol 15-S-7 was chosen as the extractant. The analytes were detected using a fluorescence detector. Gradient elution was performed with a mobile phase mixture of acetonitrile and water at a flow rate of 1.0 mL min^{-1}. Various experimental parameters affecting the analytical performance were optimized in detail. Under the optimum conditions, the correlation coefficients were all above 0.997 and the limit of detection range for the analytes was 0.03 to 8.5 μg L^{-1}. The proposed approach was successfully applied to the determination of 12 analytes in environment water samples with good recoveries (88.7%–106%).

1. Introduction

Phenolic compounds are used extensively in the manufacture of various products (e.g. plastics, dyes, synthetic rubber, and household detergents) and can easily permeate into waterways [1,2]. This has resulted in severe contamination of the environment. Phenolic compounds are toxic, carcinogenic, and can interfere with the endocrine system, even at trace levels [3–6]. Furthermore, most of them are listed as priority pollutants by the European Union and the U.S. Environmental Protection Agency [7,8]. Different phenolic compounds usually coexist in water samples because they have similar structures and characteristics and are used in similar products [2,9]. For public health, it is highly desirable to develop a rapid, simple, and sensitive method for determination of different phenolic compounds in water samples. In this regard, 12 phenolic compounds, namely, hydroquinone (HQ), resorcinol (RS), catechol (CT), phenol (Ph), β-naphthol (2-NAP), bisphenol A (BPA), α-naphthol (1-NAP), 4-*tert*-butylphenol (4-*t*-BP), 4-*tert*-octylphenol (4-*t*-OP), nonylphenol (NP), octylphenol (OP), and 4-*n*-nonylphenol (4-*n*-NP) were selected as target analytes because of their widespread use and high toxicities (Table 1).

To date, various analytical methods have been applied to the analysis of phenolic compounds including gas chromatography (GC) [10], gas chromatography mass spectrometry (GC-MS) [11], high performance liquid chromatography (HPLC) [12–14], liquid chromatography mass spectrometry (LC-MS) [15], and capillary electrophoresis [2,16]. However, GC-MS and LC-MS are expensive analytical techniques. Additionally, derivatization of the selected phenolic compounds is required before GC analysis because they are semi- or non-volatile. Thus, HPLC is the preferred method for detection of phenolic compounds.

Although HPLC is a selective and sensitive analytical method, the complex matrices and low concentrations encountered in real samples dictate the need for separation and preconcentration steps before analysis. Various extraction methods have been developed for sample preparation, such as liquid-liquid extraction [17], liquid–liquid microextraction [18], pressurized liquid extraction [19], liquid-phase microextraction [20], solid-phase extraction [21], solid-phase microextraction [22], and cloud point extraction (CPE) [23]. All of these methods have their own disadvantages and advantages. In recent years, CPE has received increasing attention from researchers because it uses a nontoxic and environmentally friendly surfactant as an extractant to encapsulate analytes from aqueous solution. CPE is an inexpensive, green, simple, and effective preconcentration method. It has been successfully applied to the separation of trace metals [24], polycyclic aromatic hydrocarbons [25,26], and estrogens [27]. However, apart from the above-mentioned merits, conventional surfactants (PONPE series and Triton X series) containing benzene rings often introduce ultraviolet/fluorescent interference during HPLC analysis. After extensive

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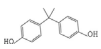
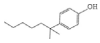
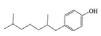
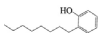
https://doi.org/10.1016/j.microc.2017.09.026
0026-265X/

Table 1
Properties of the 12 phenolic test compounds.

Compound	Structure	pKa	C_m (mg/L)[a]	Application	Toxicity	Ref.
Hydroquinone (HQ)		9.9	22.5	Plastic, fertilizer, paint, rubber, adhesive, pesticide, paper, petrochemical	Damage central nervous system, carcinogenic	[1]
Resorcinol (RS)		9.3	5.5	See HQ	Eczema, urticaria, carcinogenic, endocrine disruptor	[1]
Catechol (CT)		9.4	6.4	See HQ	Carcinogenic, abnormalities	[1]
Phenol (Ph)		9.9	3.6	See HQ	Irritating, corrosive effect, carcinogenic	[1]
β-naphthol (2-NAP)		9.5	0.1	Dyes, rubbers, pesticides, antioxidants, bactericides, pharmaceutical industries	Affect blood circulation, damage the kidneys and liver, carcinogenic,	[2]
Bisphenol A (BPA)		10.3	2.0	Industrial material, epoxy resins, polycarbonate, flame-retardants,	Endocrine disruptor	[2,14]
α-naphthol (1-NAP)		9.4	1.0	See 2-NAP	Decrease sperm mobility and concentration, carcinogen, cytotoxicity,	[2]
4-tert-butylphenol (4-*t*-BP)		10.2	2.9	Household detergents, textile, pesticides, plastics, tannery, oil industries	Endocrine disruptor	[3] [5]
4-tert-octylphenol (4-*t*-OP)		10.2	3.3	See 4-*t*-BP	Endocrine disruptor	[3]
Nonylphenol (NP)		10.7	7.0	See 4-*t*-BP	Endocrine disruptor	[5]
Octylphenol (OP)		9.5	3.5	See 4-*t*-BP	Endocrine disruptor	[6]
4-nonylphenol (4-*n*-NP)		10.5	2.4	See 4-*t*-BP	Endocrine disruptor	[6]

[a] The concentration of each analyte in the mixed standard.

research, two approaches were proposed to solve this problem. One approach is to use dual CPE whereby it is carried out twice for each sample. Then the targeted analytes are back-extracted into aqueous solution where the concentration of surfactant is approximately equal to the critical micelle concentration (CMC) [28]. Thus, the concentration of the surfactant is greatly reduced and the interference with the HPLC analysis can be overcome. However, to some extent, multiple extractions are time-consuming and can easily lead to sample loss [29]. The other approach is to select a novel surfactant that does not contain a benzene ring, although it is a challenge to select a new surfactant obtaining good extraction efficiency from numerous chemical reagents. Nevertheless, using an alternative surfactant appears more promising and practical than dual CPE because the method does not involve multiple extractions.

The nonionic surfactant Tergitol 15-S-7, which is a secondary ethoxylated alcohol without an aromatic ring, can be used directly for HPLC without interfering with the detection. It has some advantages including its low cloud-point temperature (37 °C) and CMC (38 mg/L). To the best of our knowledge, there are limited reports of Tergitol 15-S-7 being employed as an extractant in CPE [30,31]. Only one study has reported CPE with Tergitol 15-S-7 for analysis of phenolic compounds (CT, RS, HQ, Ph) in environmental water samples using HPLC with a ultraviolet detector (UVD) [32]. The density of Tergitol 15-S-7 is lower than that of water and in this earlier study, some of the upper micelle-rich phase was directly transferred and analyzed by HPLC. Unfortunately, because of the inhomogeneous distribution of analytes in the micelle-rich phase and the high viscosity of the micelles, this procedure affected the reproducibility and accuracy of the method. It is difficult to completely extract the upper micelle-rich phase or discard the lower aqueous phase. Therefore, we proposed a new way according to the principle of salting-out assisted liquid–liquid extraction (SALLE) to overcome this problem. In this method, a pump is used to remove excess water and acetonitrile (ACN) is added to dilute the micelle-rich phase. Then, in the presence of an appropriate salt solution, phase separation of the solution occurs after shaking and leaving the solution to settle for 10 min. This allows easy extraction of the diluted micelle-rich phase from the bottom of the conical centrifuge tube with good reproducibility and accuracy. Furthermore, a UVD or diode array detector should be used for CPE coupled with HPLC because most surfactants with an aromatic ring generate a fluorescence signal, and, in some cases, the analyte may not fluoresce. Consequently, the application of fluorescence detection (FLD) has been limited. However, FLD is more sensitive than UVD and a diode array detector. If FLD is applied, the sensitivity of the method will be further improved.

The novelty of this study is the development of a CPE-HPLC-FLD method to simultaneously concentrate, separate, and analyze 12 fluorescent phenolic compounds (HQ, RS, CT, Ph, 2-NAP, BPA, 1-NAP, 4-*t*-BP, 4-*t*-OP, NP, OP, and 4-*n*-NP) in water samples. To obtain lower detection limits, different wavelengths can be selected depending on the analyte of interest. Various experimental parameters affecting the analytical performance, such as the concentrations of Tergitol 15-S-7 and Na_2SO_4, pH, and equilibration temperature and time were studied in detail. The method was applied to environmental water samples to evaluate its applicability.

2. Experimental

2.1. Chemicals and materials

The target analytes HQ, RS, CT, Ph, 2-NAP, BPA, 1-NAP, 4-*t*-BP, 4-*t*-OP, NP, OP, and 4-*n*-NP were obtained from Merck (Darmstadt, Germany), and the relative physical properties and structures are listed in Table 1. A stock solution (1.0 mg mL^{-1}) of each analyte was prepared by dissolving the corresponding standard compound in acetonitrile (ACN). A working solution mixture was prepared fresh each week by diluting the stock solutions with ACN in a 10-mL glass volumetric flask. The concentrations of the 12 analytes in the working solution mixture are given in Table 1. To avoid degradation of the analytes, all the stock solutions were stored in a refrigerator at 4 °C. Tergitol 15-S-7 was purchased from Sigma-Aldrich (St. Louis, MO). A stock solution of Tergitol 15-S-7 (10% volume fraction) was prepared in ultrapure water and stored in a refrigerator at 4 °C. Ultrapure water (resistivity 18.2 MΩ cm^{-1}) was prepared using a Millipore water purification system (Milli-Q, Billerica, MA) and used in all experiments. Chromatographic grade ACN was obtained from TEDIA Co. Ltd. (Ohio, USA). Anhydrous sodium sulfate (analytical grade) was purchased from Jiangsu Qiangsheng Chemical Reagent Co. Ltd. (Jiangsu, China). Before HPLC analysis, all solvents and solutions were filtered through a nylon membrane (pore size 0.45 μm).

Table 2
Gradient elution program.

Time (min)	ACN (%)	H_2O (%)	Time (min)	ACN (%)	H_2O (%)
0–4.0	35	65	25.0–35.0	65	35
4.0–4.5	35–40	65–60	35.0–36.0	65–85	35–15
4.5–7.0	40	60	36.0–42.0	85	15
7.0–10.5	40–45	60–55	42.0–42.5	85–35	15–65
10.5–18.0	45	55	42.5–55.0	35	65
18.0–25.0	45–65	55–35			

2.2. Instrumental and analytical conditions

A Shimadzu LC-20A HPLC system (Kyoto, Japan) equipped with an automatic sampler and FLD was used for detecting the phenolic compounds. A Shim-pack VP-ODS C18 column (250 mm × 4.6 mm, 4.6 ± 0.3 μm, Shimadzu) was applied to separate the analytes. Separation was performed with a gradient elution (Table 2) of a mixture of ACN and H_2O at a flow rate of 1.0 mL min^{-1}. The column temperature was set at 30 °C and the injection volume was 10 μL. The excitation/emission wavelength program for the FLD was as follows: 270/315 nm from 0 to 13.0 min, 270/360 nm from 13.0 to 15.0 min, 230/360 nm from 15.0 to 16.3 min, 230/315 nm from 16.3 to 17.3 min, 230/440 nm from 17.2 to 18.5 min, and 230/315 nm from 18.5 to 55.0 min. A thermostatic bath (A DK-600, Shanghai Precision Experimental Equipment Co. Ltd., Shanghai, China) was used to maintain the temperature, and phase separation was achieved using a centrifuge (Anke KA-1000, Shanghai Anting Scientific Instrument Factory, Shanghai, China). The pH values were measured by a PHS-3C precision pH meter (Shanghai Hongyi Instrument Co. Ltd., Shanghai, China).

2.3. Cloud point extraction procedure

An aliquot (10 mL) of the working solution containing the target analytes or environmental water sample was mixed with 2.0 mL 10% of Tergitol 15-S-7, 0.78 g of Na_2SO_4 (the final concentration of Na_2SO_4 was 0.55 mol L^{-1}), and 2.0 mL of phosphate buffer (pH 6.0) in a conical centrifuge tube (step I and II). The tube was kept for 10 min in a thermostatic bath at 50 °C. Next, phase separation was accelerated by centrifugation at 3500 rpm for 10 min. After cooling in an ice-bath for 10 min, the aqueous phase was carefully removed from the bottom of the tube using a pump (step III). Then, to reduce surfactant-rich phase loss and the viscosity of the surfactant-rich phase, ACN was used to rinse the needle. After shaking the solution and leaving it to stand, the solution was allowed to separate again (step IV). The diluted surfactant-rich phase was easily transferred using a 1-mL syringe, and its volume was adjusted to 1.0 mL with ACN (step V). After filtering through a nylon membrane (pore size 0.45 μm) (step VI), 10.0 μL of this solution was injected into the HPLC-FLD system for analysis of the phenolic compounds (step VII). The steps for the proposed method are shown in Fig. 1.

2.4. Sample preparation

Three river water samples were collected from Zhujiang River (Guangdong, China), a river water sample was collected from a tributary near a plant, a tap water sample was collected from a tap in our laboratory (Guangdong, China), and two bottled water samples were purchased from a local supermarket (Guangdong, China). All of the water samples were filtered through 0.45-μm membranes and stored in the refrigerator at 4 °C.

3. Results and discussion

3.1. Optimization of the preconcentration step

3.1.1. Effect of the surfactant

In the CPE procedure, both the surfactant type and concentration greatly affected the extraction efficiencies (EEs) of the target compounds. In this study, the nonionic surfactants Triton X-114, PEG 6000, and Tergitol 15-S-7 (Fig. 2) were investigated as extractants. Triton X-114 contains a benzene ring, which has a strong fluorescence signal and gives very broad peaks in the chromatograms that interfere with the determination of 4-*t*-OP and NP. Additionally, the EEs, which were calculated as the ratio of the peak area of the analyte with CPE to that without CPE, obtained with Triton X-114 were lower than those obtained with the other extractants. The EEs for HQ, RS, CT, and Ph obtained with Triton X-114 were all below 30%. By contrast, PEG 6000 and Tergitol 15-S-7 do not have fluorescence signals in the selected wavelength region and gave higher EEs than Triton X-114. These results could be explained based on the structures of these surfactants. Tergitol 15-S-7 is amphiphilic with a hydrophobic head of an alkyl chain of 11–15 carbons, and its hydrophilic section is constructed using an average of 7.3 ethylene oxide units. The hydrophobic alkyl chains interact with the benzene rings of the phenolic compounds, and the hydroxyl group in the hydrophilic section interacts with the phenolic compounds through hydrogen bonding. Furthermore, because of its flexible long carbon chain, Tergitol 15-S-7

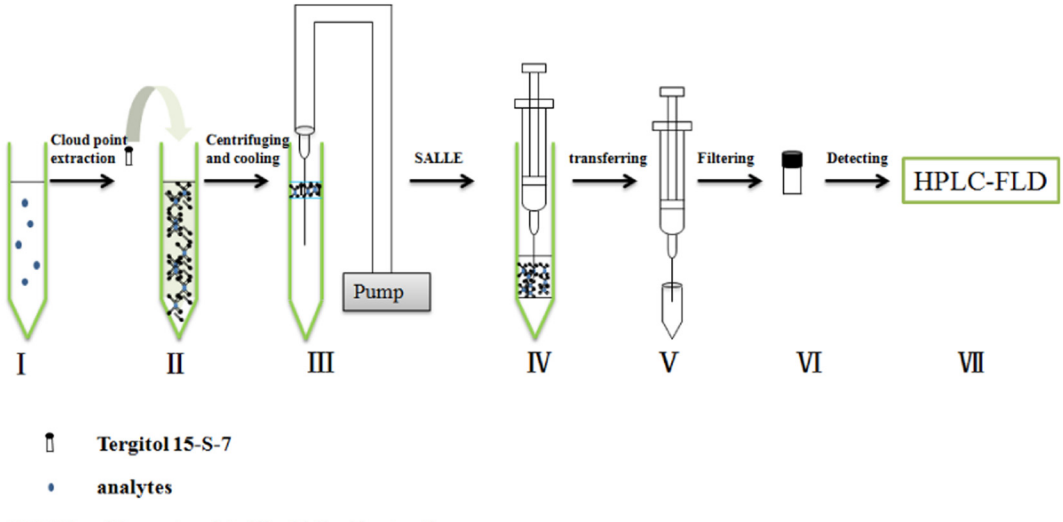


Fig. 1. Steps of the proposed method.

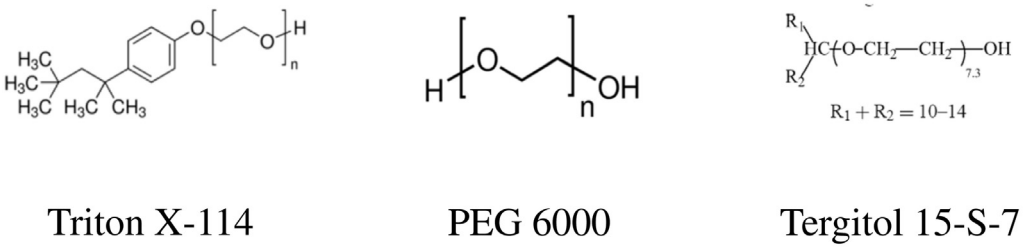


Fig. 2. The structure of three surfactants, triton X-114, PEG 6000, and Tergitol 15-S-7.

can be arranged compactly within or among the micelles, which can reduce the volume of the surfactant-rich phase and improve the enrichment factor. PEG 6000 has two hydroxyl groups that can form strong hydrogen bonds with the phenolic compounds. By contrast, the hydrophobic head of Triton X-114 contains a benzene ring that would sterically clash with the analytes. Therefore, PEG 6000 and Tergitol 15-S-7 gave higher EEs than Triton X-114. The CPTs of PEG 6000 and Tergitol 15-S-7 are approximately 100 and 37 °C, respectively [30,33], and a lower CPT is better than a higher CPT for this application. Hence, the nonionic surfactant Tergitol 15-S-7 was chosen as the extractant in this study.

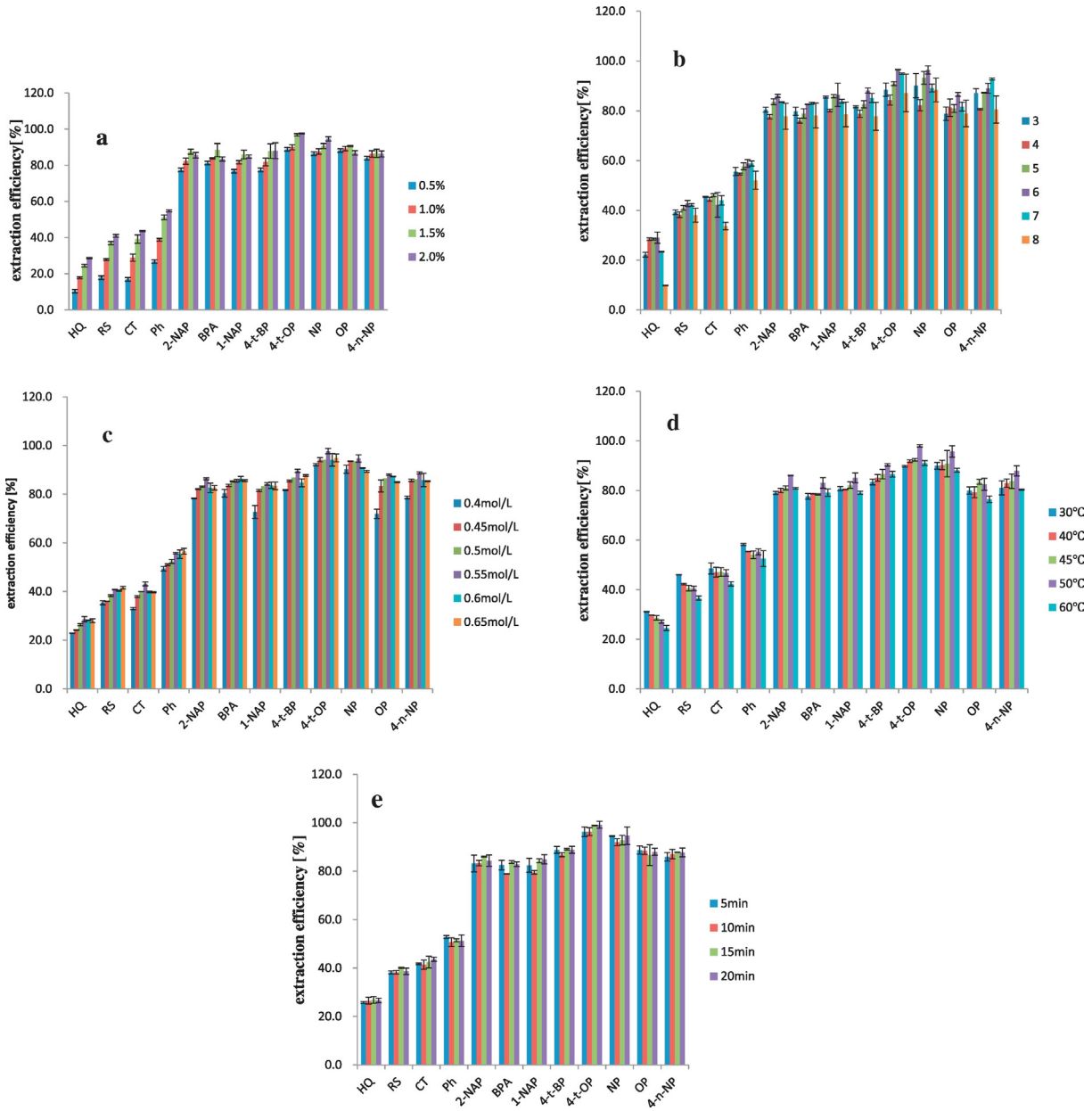


Fig. 3. Optimization of the following extraction conditions: (a) Tergitol 15-S-7 concentration, (b) pH, (c) Na_2SO_4 concentration, (d) equilibration temperature, and (e) equilibration time. Vertical segments correspond to error bars. Experimental conditions: Tergitol 15-S-7 volume fraction, 2%; Na_2SO_4 concentration, 0.55 mol L^{-1}; sample pH, 6.0; equilibrium temperature, 50 °C; equilibrium time, 10 min; centrifugal speed, 3500 rpm; centrifugation time, 10 min; ice-bath time, 10 min; volume of spiked standard mixture: 200 μL.

Table 3
Analytical performance and extraction efficiencies for phenolic compounds acquired by CPE-HPLC-FLD.

Analyses	range (μg L^{-1})	R^2	Regression equation Y = aX + b	C_{LOD} (μg L^{-1})	C_{LOQ} (μg L^{-1})	RSD (%)	EE (%)
HQ	23–1855	0.998	Y = 2978X + 7945	8.5	28.3	1.2	29
RS	6–446	0.998	Y = 9744X + 38,173	2.6	8.7	2.0	41
CT	7–530	0.997	Y = 9012X − 129,757	2.8	9.3	4.2	44
Ph	3–266	0.998	Y = 20,480X − 10,618	1.2	4.0	3.2	55
2-NAP	0.1–8.0	0.999	Y = 875,741X − 32,066	0.03	0.1	2.4	86
BPA	2–161	0.999	Y = 36,450X − 30,295	0.7	2.3	2.6	83
1-NAP	1–84	0.999	Y = 89,781X − 46,332	0.3	1.0	2.7	85
4-*t*-BP	3–253	0.999	Y = 28,374X + 103,260	0.9	3.0	3.0	88
4-*t*-OP	3–211	0.999	Y = 27,979X + 369,386	0.9	3.0	2.9	98
NP	5–432	0.999	Y = 13,447X − 10,022	1.9	6.3	1.9	95
OP	2–161	0.999	Y = 35,481X − 145,493	0.7	2.3	1.9	87
4-*n*-NP	2–192	0.999	Y = 33,502X + 68,199	0.7	2.3	3.3	86

Then, fractions of Tergitol 15-S-7 between 0.5% and 2% by volume were investigated (Fig. 3a). The EE increased monotonically with increasing surfactant concentration. The maximum EE was obtained with a Tergitol 15-S-7 fraction of 2%. Lower concentrations of surfactant would result in a micellar phase that is small, which would make it hard to extract more analytes from the bulk solution and this would affect the accuracy, repeatability, and EEs. The surfactant concentration and volume of the surfactant-rich phase were mutually dependent, which meant that the EE improved as the surfactant volume fraction increased. However, with a high-volume fraction of surfactant, the micelle will be too sticky, and this will affect the subsequent HPLC analysis. Consequently, 2% Tergitol 15-S-7 was selected for the following experiments.

3.1.2. Effect of pH

The sample pH also plays a crucial role in the CPE process because it affects the form of the target compound present in the solution. The ionic form of a molecule normally does not interact or bind as strongly as its neutral form with a nonionic surfactant [34]. Thus, the pH should be adjusted to ensure that the neutral species of the analytes are predominant during the CPE process. The target phenolic compounds are all weak acids with pKa values in the range from 9.0 to 10.7 (Table 1). In acidic solutions, the target compounds are mainly present as neutral compounds, and in alkaline solutions, they chiefly exist as phenolate ions. Therefore, the effect of pH on the CPE was investigated in the pH range of 3–8. The EEs were enhanced when the pH was increased from 3 to 6, and then decreased slightly when the pH was increased above 6 (Fig. 3b). This could be explained by the presence of neutral analyte species from pH 3–6, which are hydrophobic and bind strongly with Tergitol 15-S-7. However, when the pH increased, the analytes were gradually transformed to hydrophilic salt species, decreasing their hydrophobicity and weakening any hydrogen bonds between the analytes and the extractant. In summary, pH 6 was selected as the optimum pH for the following experiments.

3.1.3. Effect of the Na_2SO_4 concentration

Usually, addition of appropriate salts to a solution can change the cloud point temperature (CPT) and facilitate phase separation because of salting-in and salting-out effects [34]. In an earlier study, among a number of salts (NaCl, NaI, Na_3PO_4 and Na_2SO_4) [35], Na_2SO_4 had the best effect on decreasing the CPT to room temperature and thus, it was chosen for the present experiment. Na_2SO_4 concentrations between 0.4 and 0.65 mol L^{-1} were investigated (Fig. 3c). The highest EEs were obtained by CPE with a salt concentration of 0.55 mol L^{-1}, but above this concentration, the EEs remained stable and sometimes even decreased. The indicated that addition of a suitable electrolyte reduced the solubility of the phenolic compounds in the aqueous phase through a salting-out effect and decreased the "free water" concentration in the micelle phase. Consequently, the EEs improved as the salt concentration increased. However, if the Na_2SO_4 concentration is too high, the surfactant rich phase becomes milky-white and forms an unwanted viscous liquid crystalline phase, which makes it difficult to separate the

Table 4
Mean recoveries and relative standard deviations (±RSD, %) for spiked water samples.

Analyses	Spiked (μg L^{-1})	River water 1	River water 2	River water 3	River water 4	Tap water	Bottled drinking water 1	Bottled drinking water 2
HQ	112.5	101.1 ± 0.7	92.2 ± 2.2	99.3 ± 2.0	88.7 ± 3.9	91.0 ± 4.4	92. ± 2.3	90.3 ± 0.9
	450	100.5 ± 0.2	99.0 ± 1.0	100.3 ± 0.3	101.8 ± 5.1	95.1 ± 0.4	103.0 ± 0.5	103.6 ± 1.0
RS	27.5	101.3 ± 3.5	100.0 ± 2.2	105.0 ± 2.8	89.0 ± 6.9	95.2 ± 7.3	102.6 ± 2.7	102.2 ± 3.7
	110	101.3 ± 0.6	101.0 ± 2.8	102.9 ± 1.9	102.0 ± 5.9	103.5 ± 2.9	103.8 ± 1.0	105.7 ± 3.1
CT	32	105.7 ± 1.7	103.1 ± 1.7	103.0 ± 1.9	88.9 ± 6.8	92.0 ± 9.2	96.6 ± 1.9	100.2 ± 3.9
	128	100.6 ± 2.1	100.8 ± 1.3	98.9 ± 5.7	98.9 ± 4.1	95.7 ± 0.6	100.2 ± 0.5	96.1 ± 2.8
Ph	18	99.9 ± 1.2	104.1 ± 5.4	101.7 ± 1.9	91.6 ± 5.7	94.7 ± 6.2	100.1 ± 1.6	99.9 ± 4.2
	72	104.0 ± 2.9	103.8 ± 1.8	105.6 ± 2.3	90.0 ± 0.7	102.6 ± 6.9	104.4 ± 1.9	103.8 ± 4.1
2-NAP	0.5	100.7 ± 1.1	100.7 ± 4.1	104.0 ± 3.3	91.8 ± 5.7	100.0 ± 7.2	106.0 ± 1.9	100.7 ± 2.3
	2.0	100.0 ± 2.8	98.0 ± 0.9	99.8 ± 2.3	91.5 ± 2.5	101.8 ± 4.1	103.5 ± 2.1	103.2 ± 2.5
BPA	10	104.7 ± 0.8	101.5 ± 2.7	103.7 ± 0.4	89.5 ± 4.7	98.4 ± 10.1	104.2 ± 1.7	104.2 ± 5.8
	40	99.1 ± 2.6	96.1 ± 0.5	96.9 ± 2.2	101.8 ± 5.9	99.1 ± 4.3	97.8 ± 3.7	97.0 ± 2.4
1-NAP	5	96.3 ± 2.8	94.9 ± 1.2	95.6 ± 2.8	94.0 ± 2.7	94.1 ± 4.5	96.6 ± 4.0	97.2 ± 6.8
	20	98.7 ± 3.5	96.5 ± 2.1	97.9 ± 2.7	105.8 ± 4.8	97.7 ± 4.1	99.8 ± 1.3	97.9 ± 2.4
4-*t*-BP	14.5	103.9 ± 5.0	101.8 ± 4.2	102.7 ± 2.6	91.8 ± 6.2	98.9 ± 2.8	104.1 ± 2.3	101.0 ± 3.6
	58	97.6 ± 3.1	97.9 ± 2.2	98.0 ± 3.9	100.4 ± 2.8	99.5 ± 3.6	101.7 ± 1.8	101.4 ± 3.5
4-*t*-OP	16.5	101.9 ± 1.7	102.2 ± 2.1	100.3 ± 1.1	93.8 ± 7.2	101.1 ± 8.5	104.7 ± 8.7	99.8 ± 6.9
	66	96.4 ± 2.7	95.8 ± 2.2	96.8 ± 3.1	89.2 ± 1.4	98.4 ± 3.6	100.1 ± 1.2	99.2 ± 4.7
NP	35	95.9 ± 2.2	95.7 ± 1.4	95.3 ± 1.4	96.0 ± 5.7	94.4 ± 1.2	103.7 ± 4.1	97.4 ± 2.3
	140	100.1 ± 2.1	99.4 ± 1.1	101.5 ± 0.8	100.4 ± 4.8	100.8 ± 2.7	103.9 ± 1.8	102.4 ± 2.6
OP	17.5	103.8 ± 1.8	102.5 ± 1.4	104.5 ± 1.6	94.9 ± 6.7	99.5 ± 7.9	101.7 ± 1.8	101.7 ± 4.6
	70	100.3 ± 2.3	99.0 ± 1.1	99.9 ± 2.3	100.9 ± 6.3	97.9 ± 3.3	101.5 ± 0.9	100.4 ± 1.4
4-*n*-NP	12	88.7 ± 10.6	97.8 ± 4.6	98.7 ± 7.8	94.5 ± 4.0	93.8 ± 6.6	92.9 ± 3.9	92.4 ± 5.5
	48	94.1 ± 6.4	95.7 ± 2.5	98.7 ± 2.8	97.3 ± 2.7	97.7 ± 3.7	96.6 ± 1.8	95.4 ± 2.9

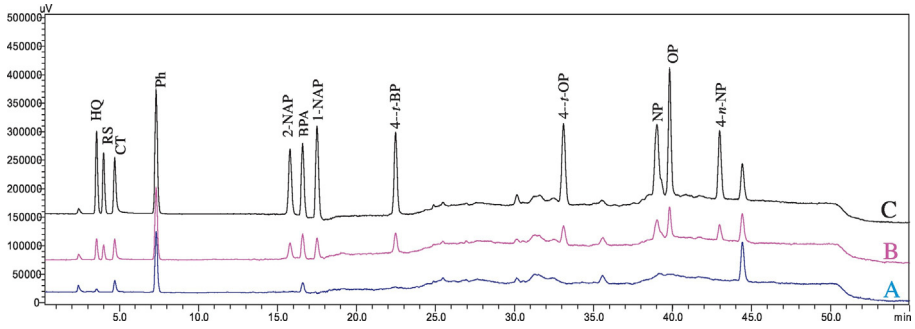


Fig. 4. Typical chromatograms of the water samples after CPE. (A) Positive water sample, (B) spiked water sample with 50 μL of the mixed standard, (C) spiked water sample with 200 μL of the mixed standard.

surfactant-rich phase. In conclusion, 0.55 mol L^{-1} Na_2SO_4 was chosen for the subsequent experiments.

3.1.4. Effect of the equilibration temperature and time

It is universally acknowledged that the CPT is a characteristic value of the surfactant and varies with the type of the surfactant [36]. According to the manufacturer, the CPT of a 1% (mass fraction) aqueous solution of Tergitol 15-S-7 is 37 °C. Theoretically, the most appropriate equilibration temperature for CPE is 15–20 °C above the CPT [37]. In this work, the equilibration temperature (30 to 60 °C, Fig. 3d) and time (5 to 20 min, Fig. 3e) were optimized. The EEs of four analytes (HQ, RS, CT, Ph) decreased as the incubation temperature increased, which could be attributed to the thermal instability of the phenolic hydroxyl group. The EEs of the other analytes increased as the temperature increased from 30 to 50 °C, and then decreased with further increases in the temperature above 50 °C. This could be explained by the processes of hydrogen bond breakage and dehydration occurring between 30 to 50 °C, and decomposition of the target analytes at temperatures above 50 °C. Therefore, an equilibration temperature of 50 °C was selected for the present experiments. When experiments were conducted to optimize the equilibration time, changes in the EEs as the extraction time increased were not remarkable. Therefore, an extraction time of 10 min was used for subsequent experiments.

3.2. Optimization of the high performance liquid chromatography conditions

Another crucial step in this study was optimization of the HPLC parameters for the separation and determination of the 12 analytes. The mobile phase will directly affect the separation of the compounds. A suitable mobile phase has good selectivity, low viscosity, and a UV cut-off wavelength shorter than the detection wavelength to avoid interference from background absorption peaks. In this study, we investigated mobile phases of ACN/H_2O, methanol/H_2O, and ACN/phosphate solution with pH levels from 3 to 7. Compared with methanol/H_2O, ACN/H_2O shortened the analysis time and did not give any background absorption peaks. These results were attributed to the lower viscosity and UV cut-off wavelength (λ = 190 nm) of ACN compared with those of methanol.

The other mobile phases tested did not greatly improve the performance compared with ACN/H_2O, and thus, ACN/H_2O was selected as the mobile phase because it was simple to prepare.

3.3. Analytical performance and extraction efficiency

Under the optimized conditions, the method performance was evaluated by determining the linearity, limits of detection (LODs), limits of quantification (LOQs), and precision (Table 3). Calibration curves were established using linear least squares regression and all the correlation coefficients (R^2) were higher than 0.997, which demonstrated excellent linearity. The LODs and LOQs were calculated as signal-to-noise ratios of 3 and 10, respectively. The range for the LODs was 0.03–8.5 μg L^{-1}. The precision of the method was evaluated using relative standard deviations (RSDs) obtained by spiking five replicates of ultrapure water with 200 μL of the mixed standard. All the RSDs were below 4.2%, which indicates the method has good precision. Overall, the proposed method for determination of these 12 phenolic compounds has good analytical characteristics. Furthermore, the EEs of most analytes were higher than 83%, and those for 4-*t*-OP and NP were higher than 95%.

3.4. Analysis of environmental water samples

Seven different environmental water samples were analyzed for the 12 target compounds to evaluate the applicability and reliability of the proposed method. Four analytes (HQ, CT, Ph, and BPA) were found in a river water sample at 26.7, 24.9, 52.7, and 6.9 μg L^{-1}, respectively. The other target analytes were not found in any of the water samples, possibly because they were present at trace levels below the LODs of the method. Next, recovery experiments were used to confirm the accuracy of the proposed method. Three replicates of a water sample were spiked with either 50 μL or 200 μL of the mixed standard. The range of recoveries of the 12 analytes was 88.7%–106% (Table 4). Example chromatograms of a positive sample before and spiked samples after CPE are shown in Fig. 4. Every target analyte was well separated and no large interfering peaks were detected around the retention times of them. This

Table 5
Comparison of limit of detections (μg L^{-1}) for the present work with other methods.

Method	HQ	RS	CT	Ph	2-NAP	BPA	1-NAP	4-*t*-BP	4-*t*-OP	NP	OP	4-*n*-NP	Ref.
SPE-GC-MS	–	–	–	–	–	0.14	–	–	–	–	–	0.03	[11]
CPE-CE	–	–	–	–	0.2	0.5	0.24	–	–	–	–	–	[2]
CPE-HPLC-UVD	–	–	–	–	–	0.18	–	0.98	0.3	0.28	0.96	–	[38]
CPE-HPLC-UVD	6.0	3.2	3.1	2.8	–	–	–	–	–	–	–	–	[32]
LPME-GC/MS						0.7		0.2			0.3		[7]
LLME-HPLC-FLD	–	–	–	–	–	0.9	–	–	1.6	1.6	1.4	–	[39]
DSPE[a]-HPLC-FLD	–	–	–	–	–	1.0	–	–	–	2.0	1.5	–	[5]
CPE-HPLC-FLD	8.5	2.6	2.8	1.2	0.03	0.7	0.3	0.9	0.9	1.9	0.7	0.7	This work

[a] DSPE = derivatization solid phase extraction.

indicates that the method could be applied to the determination of these 12 analytes in environment water samples.

3.5. Comparison of CPE-HPLC-FLD with other methods

A comparison of the LOD for the methods used to determine the target compounds are shown in Table 5. The developed method enabled synchronous quantitative extraction and analysis for twelve analytes. Table 5 shows the LODs for the developed method were better than or similar to those reported for other methods.

4. Conclusion

CPE is a robust method for extraction of HQ, RS, CT, Ph, 2-NAP, BPA, 1-NAP, 4-*t*-BP, 4-*t*-OP, NP, OP, and 4-*n*-NP from environmental water samples. This method has the following advantages over other techniques: it uses a nontoxic nonionic surfactant as the extractant and avoids the use of toxic organic solvents such as chloroform, it is simple, quick, and inexpensive, and it has high sensitivity (LODs between 0.03 and 8.5 μg L^{-1}), good recoveries (88.7%–106%), and high EE for most analytes. However, slightly hydrophilic compounds (e.g. HQ, RS, CT, and Ph) have low EEs, and future research will be focused on reducing their hydrophilicity in an effort to improve their EEs.

Acknowledgments

This work was supported by the Science and Technology Infrastructure Development Program of Guangdong, China (grant numbers 2014A040401085 and 2016A020226019), and the Science and Technology Program of Guangzhou, China (grant number 2014J4100085). We thank Renee Mosi, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

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