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Talanta 69 (2006) 1001–1006
Talanta
www.elsevier.com/locate/talanta

Determination of phenolic compounds in river water with on-line coupling bisphenol A imprinted monolithic precolumn with high performance liquid chromatography

Junjie Ou, Lianghai Hu, Ligang Hu, Xin Li, Hanfa Zou *

National Chromatographic Research & Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

Received 22 August 2005; received in revised form 29 November 2005; accepted 2 December 2005
Available online 6 January 2006

Abstract

The bisphenol A (BPA) imprinted monolithic precolumn has been prepared by in situ polymerization using 4-vinylpyridine (4-VP) and ethylene dimethacrylate (EDMA) as functional monomer and cross-linker, respectively. The column with good flow-through property was obtained by changing the molar ratio of the porogens (toluene and dodecanol). The selectivity and retention properties of the monolith for the BPA and other phenolic compounds were evaluated. The results show that the hydrophobic and hydrogen-bonding interaction plays important roles in the recognition process. The determination of BPA and other phenolic compounds with on-line solid-phase extraction (SPE) by monolithic precolumn coupled with conventional particulates packed and monolithic reversed-phase columns, respectively, was performed. The method was successfully applied to the analysis of phenolic compounds in river water.

Keywords: Molecularly imprinted polymer; Monolithic precolumn; Bisphenol A; Phenolic compound; On-line solid-phase extraction; Column switching

1. Introduction

Molecular imprinting polymer (MIP) is a class of tailor-made material with predetermined selectivity for analytical separation. The selectivity of MIP arises since the template molecule is present in the polymerization mixture during the synthesis of the MIP. Once the highly cross-linked polymer has formed, the template molecules are removed from the polymer revealing selective binding sites on the surface of the polymer matrix. The binding sites generated have suitable sizes and shapes complementary to a template molecule, resulting in the strong adsorption of the template [1–4]. It holds promise in the development of highly selective adsorbent for solid-phase extraction (SPE) of trace analytes, particularly in complex pharmaceutical and environmental samples. The application of SPE procedures involving molecularly imprinted polymers, called MISPE, has received increasing attention over the past decade as an attractive alternative for the analysis of complex samples. Successful applications of MIPs to SPE have been described in the literatures [5–11].

Phenolic compounds are environmental pollutants the sources of which can be as different as industrial effluents, the reaction products, and the degradation of some pesticides, etc. Bisphenol A (BPA) is an important intermediate in the industrial manufacture of polycarbonate plastics and phenolic resins, thus it is often contained in environmental water. In the past years a particular interest has focused on BPA since in vitro experiments have shown estrogenic activity even at concentration as low as 1 ng/l level. Different analytical methods have been developed for determination of BPA at the ng/l and μg/l concentration levels in various samples. Most of the methods are based on gas chromatography (GC) or high performance liquid chromatography (HPLC), and usually require a preliminary liquid–liquid or solid-phase extraction step [12,13].

Currently, a number of distinct approaches have been used to prepare BPA imprinted polymer. Ikegami et al. [14,15] have synthesized BPA imprinted polymer by a covalent imprinting technique using BPA-dimethacrylate or BPA-diacrylate as the template monomer. Binding sites in the polymers consisted of two hydroxyl groups are generated by reducing the ester bonds of the template monomer with $LiAlH_4$. Moreno-Bondi and co-workers [16] have applied multivariate analysis to the screening of MIP for BPA by the bulk polymerization method.

* Corresponding author. Tel.: +86 411 84379610; fax: +86 411 84379620.
E-mail address: hanfazou@dicp.ac.cn (H. Zou).

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doi:10.1016/j.talanta.2005.12.003

Haginaka and Sanbe [17] have prepared the MIP for BPA by a multi-step swelling and polymerization method using BPA as the template. Watabe et al. [18] have prepared uniformly sized polymer particles for BPA trapping using *p-tert*-butylphenol as a pseudo-template through the two-step swelling and polymerization method. In addition, they have analyzed BPA using these particles as an on-line pretreatment device.

In this study, BPA imprinted polymer has been prepared by in situ polymerization using 4-vinylpyridine (4-VP) as the functional monomer and ethylene dimethacrylate (EDMA) as the cross-linker. The MIP obtained shows the good selectivity for BPA. Further, an on-line procedure has been employed for quantitative determination of BPA and other phenolic compounds with preconcentration on the monolithic polymer precolumn coupling the ODS analytical column. The results indicated that the column based on 4-VP and EDMA copolymer (4-VP-*co*-EDMA) shows the hydrophobic property and could effectively extract the phenolic compounds.

2. Experimental

2.1. Materials

BPA, 4-*tert*-octylphenol (OP), 4-*n*-nonylphenol (NP) were purchased from Acros (Geel, Belgium). Phenol (Ph), hydroquinone (HQ), resorcin (Re), 4-methylphenol (MP), 2,3-dimethylphenol (DMP), and 4-*tert*-butylphenol (tBP) were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). 4-VP from Acros (Geel, Belgium) was distilled under vacuum. EDMA from Sigma (St. Louis, MO, USA) was extracted with 10% aqueous sodium hydroxide and water, and dried over anhydrous magnesium sulfate. 2,2′-Azobisisobutyronitrile (AIBN) was obtained from Shanghai Chemical Plant (Shanghai, China) and recrystallized in ethanol before used. Toluene and dodecanol were dried prior to use. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA). Acetonitrile (ACN) and other solvents were of HPLC or analytical grade.

2.2. Preparation of molecularly imprinted monolithic columns

The BPA imprinted monolithic column was directly prepared within a stainless-steel tube of 50 mm × 4 mm i.d. The template molecule, functional monomer (4-VP), cross-linker (EDMA) and initiator (AIBN) were dissolved in porogenic solvents (toluene and dodecanol) to form homogenous solution, in the compositions as indicated in Table 1, which was sonicated for 10 min and purged with dry nitrogen for 15 min to remove oxygen. The stainless-steel tube sealed at the bottom was full of the above polymerization solution, and then sealed at the top. The polymerization was carried out at 50 °C in a water-bath for 16 h. After that, the seals of column were replaced with fittings, and connected to an HPLC pump. The column was washed thoroughly with methanol/acetic acid (4/1 v/v) to remove the porogenic solvents and template molecule until no template was detected.

Table 1
Effect of porogen ratio on the retention of BPA and imprinting factors on the prepared monolithic imprinting column

Polymers	Percent of toluene in porogen (%)	k_{BP}	k_{MIP}	IF
P1	5	0.24	0.35	1.46
P2	10	0.32	0.48	1.50
P3	15	0.36	0.66	1.83
P4	20	0.45	0.85	1.89
P5	25	0.57	1.12	1.96

HPLC conditions: column size, 50 mm × 4.0 mm i.d.; column temperature, 20 °C; flow-rate, 0.3 ml/min; detection wavelength, 280 nm. The mobile phase was ACN.

Similarly, the non-imprinted blank monolithic column (BP) without the imprinted molecule was prepared according to the procedures mentioned above.

2.3. Instrumentation

For the evaluation of MIP, chromatography was performed on a Shimazdu LC-10A HPLC system (Shimazdu, Kyoto, Japan) consisting of two LC-10Advp pumps and an SPD-10Avp UV–vis detector. The data were acquired and processed with WDL-95 chromatographic workstation (National Chromatographic R&A Center, Dalian, China). For the on-line molecularly imprinted SPE (MISPE), a 6-port column-switching valve (Valco Instruments Co. Inc., USA), two Elite P230 pumps (Elite, Dalian, China) and a MIP monolithic column as the precolumn were installed. The schematic diagram of on-line SPE-HPLC system is illustrated in Fig. 1. The monolithic column was on-line coupled to a Shimazdu LC-10A liquid chromatographic system mentioned above. The analysis was performed on a column (200 mm × 4.6 mm i.d.) packed with Kromasil ODS (5 μm) purchased from Eka Chemicals (Bohus, Sweden) or a Chromonolith RP-18 column (50 mm × 4.6 mm i.d.) from Merck (Darmstadt, Germany).

2.4. Chromatographic conditions

The HPLC was carried out at room temperature. The detector was set at 280 nm. Acetone was injected as the void marker. Retention factor, k, was calculated by using the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention time of the analyte being investigated and the void marker, respectively. The molecular imprinting factor (IF) proposed for the evaluation of the recognition ability was calculated by the equation $IF = k_{MIP}/k_{BP}$, where k_{MIP} was the retention factor of the analyte on the MIP and k_{BP} was that on the BP.

2.5. On-line SPE procedures

Firstly, the precolumn was conditioned with pure ACN and acidified water (pH 3.0), respectively. Secondly, the water sample spiked phenolic compounds was applied to the conditioned precolumn, and then the precolumn was washed with different volume of 20% (v/v) ACN–water (pH 3.0). Flow-rate was

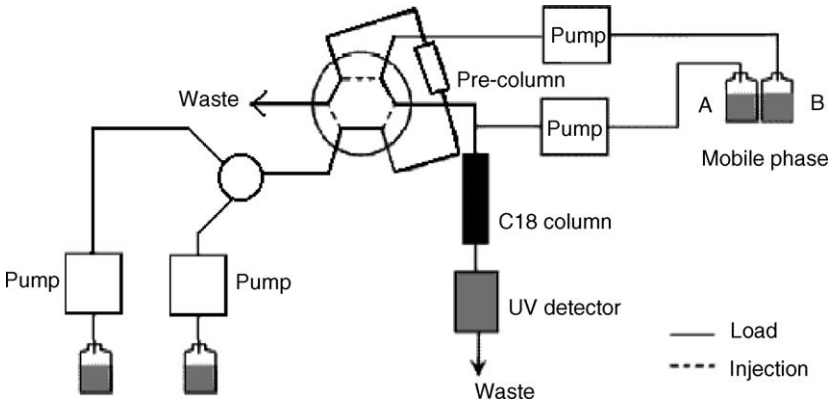

Fig. 1. Schematic diagram of an on-line SPE-HPLC system.

3.0 ml/min in all above steps. The analysis was performed using mixtures of two mobile phases: water/acetic acid (99/1 v/v, pH 3.0, mobile phase A) and ACN/acetic acid (99/1 v/v, mobile phase B) at the flow-rate of 1.0 ml/min. The compounds retained on the precolumn were eluted by the organic solvent (mobile phase B), and transferred into the analytical column in the back-flush mode. The organic solvent is mixed with the aqueous solvent (mobile phase A) prior to reaching the analytical column. The real samples were filtered through a 0.45 μm filter and adjusted to pH 3.0 before MISPE.

3. Results and discussion

3.1. Conditions for preparation of monolithic precolumn

Although the preparation of molecularly imprinted monolithic column is very simple, a large number of factors have effects on the molecular recognition properties of the synthesized polymer. These factors include the amount of the template molecule, the type and amount of functional monomer, cross-linking monomer and porogenic solvents and the polymerization initiation (thermal or UV). According to the results in our previous paper [19], the amounts of EDMA, 4-VP and BPA in polymerization solution at 4.0, 1.0 and 0.3 mmol, respectively, were the optimal composition for the preparation of MIPs. For the preparation of the monolithic stationary phase, the good flow-through property of the resultant polymer is needed, thus the choice of porogenic solvents is also important [20].

While toluene and dodecanol were used as porogen in our case, which show good solubility for all components in polymerization mixture, the effect of the molar ratio of them on the retention was investigated. As shown in Table 1, the retention of BPA on the both BP and MIP monolithic columns increased with increasing of the percent of the good solvent, toluene. If no toluene was added, the polymer had a soft gel-like appearance rather than being a stable monolith. As the volume percent of toluene was 20%, the MIP showed a remarkable imprinting effect, and the imprinting factor reached 1.89. However, when the toluene reached 25% in the porogenic mixture, the polymer became so dense that mobile phase could hardly flow through the monolith. These results may attribute to the toluene and dodecanol affect the pore structure of the monolithic polymer during the process of polymerization. Finally, 15% toluene was the optimal composition, while a balance had to be found between the requirements of good imprinting effect and low resistance for mobile phase. The backpressure on the monolith P3 was only 2.8 and 1.6 MPa at the flow-rate of 3.0 ml/min when the mobile phase was pure water and pure ACN, respectively.

3.2. Selectivity of the MIP monolithic precolumn for phenolic compounds

The imprinting factor (IF) proposed for the evaluation of the recognition ability was applied to examine the recognition ability of the prepared BPA imprinted polymer. Table 2 shows the retention factor and the IF of BPA and other phenolic compounds on the BPA imprinted and non-imprinted monolithic columns. The results indicated that the highest imprinting factor of 2.19 was obtained for BPA. The retention factor of other phenolic compounds on BPA-MIP is approximate to that on blank polymer, and the IF is slightly higher than 1.0, which could be attribute to the much more number of pyridyl groups covered on the surface of the MIP than that on the BP. As shown in Fig. 2, the separation of BPA and tBP was achieved on the BPA-MIP. These results indicated that there are two intermolecular hydrogen bonds between pyridyl groups in functional monomer, 4-VP, and two hydroxyl groups in BPA formed during the polymerization of MIP. It was deduced that BPA-MIP synthesized possesses recognition sites complementary to the spatial structure of the BPA, and the effective sites are mainly on the surface of the polymer. On the BPA-MIP, the retention of BPA became big-

Table 2
Retention and imprinting factors of BPA and its analogues on MIP and BP columns, respectively

Compound	k_{BP}	k_{MIP}	IF
Hydroquinone	0.48	0.57	1.18
Phenol	0.37	0.45	1.22
4-Methylphenol	0.32	0.37	1.16
4-*tert*-Butylphenol	0.31	0.34	1.10
4-*tert*-Octylphenol	0.24	0.25	1.04
Bisphenol A	0.42	0.92	2.19

HPLC conditions as Table 1.

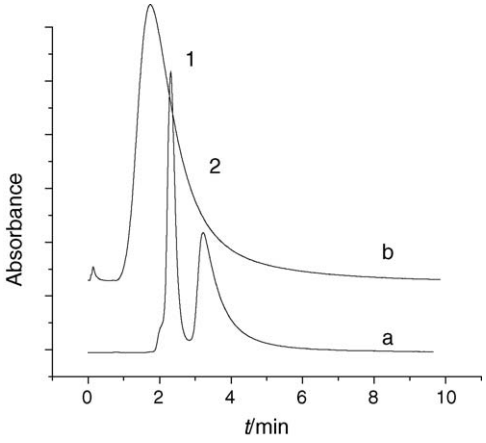

Fig. 2. Chromatograms for separation of BPA (peak 2) and tBP (peak 1) on the BPA imprinted (a) and non-imprinted (b) monolithic column, respectively. HPLC conditions as in Table 1.

ger than that on the BP because of the synergistic effect of two hydroxyl groups in BPA when binding to a MIP. Since there is no synergistic effect in the interaction of the BPA and the blank polymer, the retention of BPA and tBP on the blank polymer is almost similar, and only depends on the acidity of them.

3.3. Retention property of phenolic compounds in aqueous mobile phase

The effect of the concentration of water in ACN in the range of 0–80% (v/v) on the retention of BPA, phenol, hydroquinone and tBP on the MIP was investigated. As can be seen from Fig. 3, the retention of these four compounds decreased with the concentration of water increased. However, the amount of water was further increased, the retention of them began to increase, whereas that of BPA and tBP increased sharply. When water content in ACN was 65% (v/v) the retention factor of BPA and tBP reached 11.8 and 6.2, respectively. Although the phenol and hydroquinone could be eluted by water containing 20% (v/v) ACN, the retention of BPA and tBP was too strong to be eluted from the column. These results can be explained by considering that at lower content of water in ACN the contribution of hydrogen-bonding interaction to the retention and the resolution

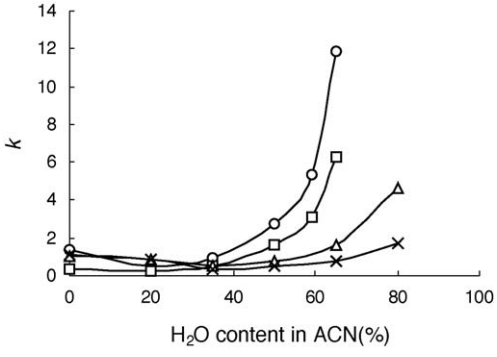

Fig. 3. Effects of the content of water in ACN on the retention of BPA and other compounds on the BPA imprinted column. (◯) BPA; (□) tBP; (△) Ph; (×) HQ. HPLC conditions as in Table 1.

disappeared almost completely. However, since the BPA and the polymer are relatively hydrophobic, the hydrophobic interaction came to play when the percentage of water was increased, the retention began to increase. It has been shown that intermolecular hydrogen-bonding between BPA and the polymer is weak in aqueous solution because of the competition from the high concentration of water molecules.

The retention of these compounds on the non-imprinted polymer was also investigated. The similar results obtained further revealed that hydrophobic interaction between BPA and monolithic polymer plays an important role in the retention of these phenolic compounds, since the hydrophobic interaction between BPA and 4-VP-*co*-EDMA material is so strong that both the intermolecular hydrogen-bonding and electrostatic interaction between the hydroxyl groups in BPA molecule and the pyridyl groups in MIP can only partly contribute to the retention of these phenolic compounds.

3.4. On-line SPE of phenolic compounds on the imprinted 4-VP-co-EDMA monolithic column

Before using monolithic precolumn for the on-line SPE of phenolic compounds, the separation condition of HPLC for BPA and other phenolic compounds was optimized. The linearity between 0.05 and 20 mg l^{-1} for all phenolic compounds was determined by injecting of 20 μl standard solutions of these compounds. The linear regression coefficients (R) obtained are higher than 0.998 for all tested analytes. To evaluate the monolithic precolumn for on-line SPE of phenolic compounds, 150 ml sample volume of water (pH 3.0) spiked nine phenolic compounds in concentration of 0.2–1.0 μg l^{-1} was on-line preconcentrated. The chromatogram for separation of these phenolic compounds was obtained, which was shown in Fig. 4(a). The peaks of the hydroquinone and resorcin were seriously tailing and broadening, and the peaks of the BPA and other strong retention compounds were narrowed. In the on-line SPE procedures, the precolumn should be as small as possible in order to prevent band broadening [21]. The peak broadening of weak retention compounds may be caused from the large void volume of the precolumn (50 mm × 4 mm i.d.) in our case. However, this effect can be minimized by using a suitable gradient mode which causes peak compression at the top of the analytical column. Furthermore, the equipment design has been modified to reduce this problem. In this design (Fig. 1), the compounds retained on the precolumn were eluted by the ACN containing a little acetic acid (mobile phase B) instead of the real mobile phase in the back-flush mode, which was mixed with aqueous solution (mobile phase A) before reaching the analytical column. Thus, the phenolic compounds can be eluted as fast as possible, and the peak broadening is effectively suppressed.

It can be seen from Table 3 that the recovery of these compounds except hydroquinone and resorcin were higher than 80%, and the recovery of BPA was reached to 95.2%. Fontanals et al. [22] has synthesized the 4-vinylpyridine-divinylbenzene (VP-DVB) beads as the sorbent for the on-line SPE of a group of polar compounds. Their results showed that the recovery of phenol with these VP-DVB beads reached to 83%, which was higher

Table 3
Recovery of phenolic compounds obtained by washing the imprinting monolithic precolumn with different volumes of ACN/H_2O (20/80 v/v)

Elution volume (ml)	Compound								
	Re	HQ	Ph	MP	DMP	BPA	tBP	OP	NP
0	45.2	61.8	84.3	86.3	94.9	95.2	91.6	94.2	93.7
2.0	0	37.3	81.0	84.8	92.5	97.3	94.4	95.9	94.0
4.0	0	0	54.2	72.0	95.4	95.8	92.2	97.8	95.8
6.0	0	0	32.9	65.5	91.6	96.0	93.1	94.5	96.2

Relative standard deviations (R.S.D.) were lower than 8% ($n = 5$).

than other highly cross-linked material, such as Envi-Chrom. In our case, the similar results suggest that the 4-VP-*co*-EDMA monolith for the SPE of phenolic compounds provided two types of interactions, not only hydrophobic interaction but also hydrogen-bonding interaction between the hydroxyl group in phenolic compounds and the pyridyl group in the polymer.

In order to reduce the peak interfering of the weak retention compounds, the volume of elution by water containing 20% (v/v) ACN was optimized (Table 3). After eluted by 4 ml 20% ACN/water, the hydroquinone and resorcin were completely eluted, and the recovery of phenol was reduced to 54.2%. However, the recovery of BPA and other strong retention compounds were not decreased even if eluted by much more volume of 20% (v/v) ACN/water. These results indicated that the determination of BPA and other phenolic compounds in real samples could be performed with the on-line SPE preconcentration by the 4-VP-*co*-EDMA precolumn.

3.5. Analysis of river water

To demonstrate the suitability of the method and its potential for sample pretreatment, the actual environmental sample was processed. Fig. 4(b) shows the chromatogram obtained

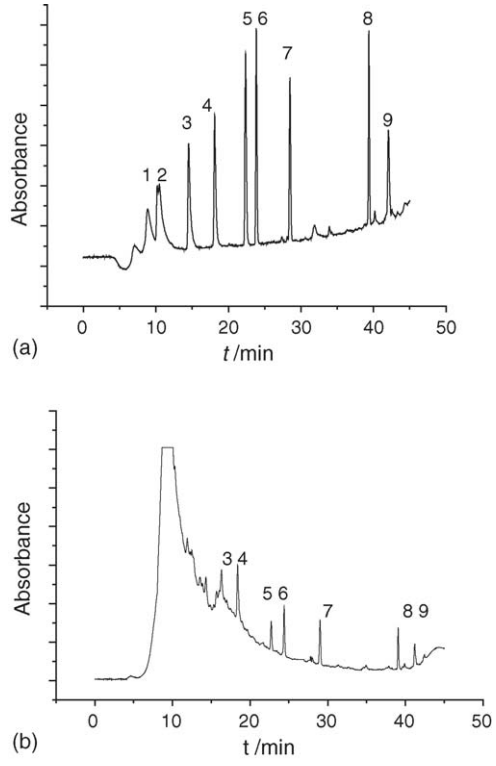

Fig. 4. Chromatograms obtained with on-line coupling a MIP monolithic precolumn to a conventional C18 column. Samples: 150 ml of purified water (a) and river water (b) spiked with 1.0 $\mu g\,l^{-1}$ Re and HQ, 0.2 $\mu g\,l^{-1}$ OP and NP, 0.5 $\mu g\,l^{-1}$ BPA and other compounds. Peak designation: (1) Re; (2) HQ; (3) Ph; (4) MP; (5) DMP; (6) BPA; (7) tBP; (8) OP; (9) NP. Analytical conditions: C18 column, 200 mm × 4.6 mm i.d.; mobile phase, (A) HOAc/H_2O (1.0/99.0 v/v, pH 3.0) and (B) HOAc/ACN (1.0/99.0 v/v); flow-rate, 1.0 ml/min; detection wavelength, 280 nm. Linear gradient elution from 15% B to 60% B in 30 min and from 60% B to 90% B in another 10 min, then stepwise gradient elution from 90% B to 100% B in 1 min and lasting for another 4 min.

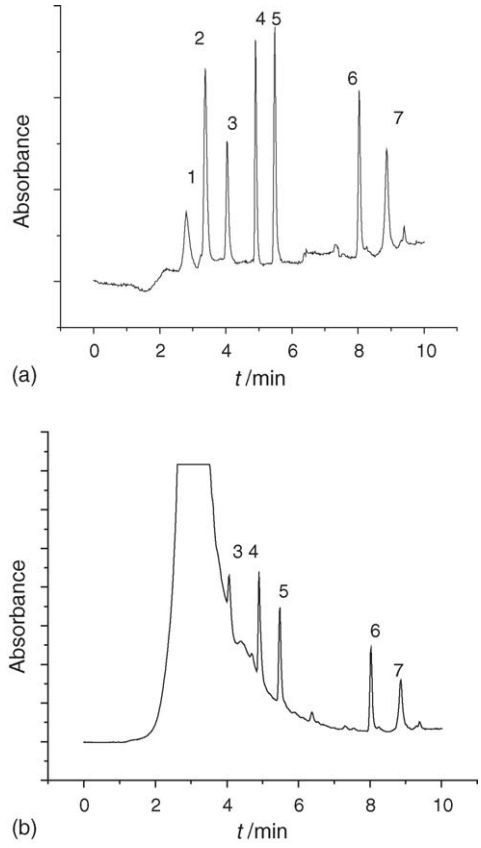

Fig. 5. Chromatograms obtained with on-line coupling a MIP monolithic precolumn to a Chromonolith C18 column. Samples: 150 ml of purified water (a) and river water (b) spiked with 0.2 $\mu g\,l^{-1}$ OP and NP, 0.5 $\mu g\,l^{-1}$ BPA and other compounds. Peak designation: (1) Ph; (2) MP; (3) DMP; (4) BPA; (5) tBP; (6) OP; (7) NP. Analytical conditions: a Chromonolith RP-18 column, 50 mm × 4.6 mm i.d.; flow-rate, 3.0 ml/min. Linear gradient elution from 10% B to 70% B in 8 min and from 70% B to 100% B in another 1 min, then lasting for 1 min. Other conditions as in Fig. 4.

by on-line enrichment of 150 ml of Malan river water spiked with BPA and other phenolic compounds in concentration of 0.2–1.0 $\mu g\,l^{-1}$. The peaks of some polar compounds eluted at the beginning of the chromatograms were masked by a broad-band corresponding to humic and fulvic acids [23]. However, the determination of BPA and other compounds, such as tBP, NP and OP, could not be influenced.

The recoveries of these compounds obtained with river water were similar to those obtained with distilled water. The detection limit (signal-to-noise, 3) and limit of quantification (signal-to-noise, 10) for BPA were 0.06 and 0.20 $\mu g\,l^{-1}$, respectively. The repeatability and reproducibility between days, expressed as the R.S.D. ($n=5$), were lower than 8% in all instances. The determination of tBP, OP and NP were also performed, and satisfied results were obtained. These results indicated that the 4-VP-*co*-EDMA based imprinting monolith could be successfully used as the precolumn for on-line solid-phase extraction of BPA and other phenolic compounds in river water.

Because of the good flow-through property of the prepared monolith, fast analysis could be achieved when a commercial silica monolithic reversed-phase column was used as the analytical column to couple the monolithic precolumn. As can be seen from Fig. 5, the analysis of a river sample could be completed within 10 min, which was remarkably faster than that using the conventional C18 column. The results further proved that the monolith is a good material used for on-line SPE preconcentration of analytes.

4. Conclusions

The BPA imprinted monolith was directly prepared in a stainless-steel column by in situ polymerization using 4-VP and EDMA as the monomer and cross-linker, respectively. Comparing with other methods, such as bulk polymerization, suspension polymerization and multi-step swelling polymerization, the method of in situ polymerization is very simple without many tedious procedures. The obtained monolith shows good flow-through property and selectivity. Thus, a method for determining of BPA in environmental water was successfully developed by using the 4-VP-*co*-EDMA monolith as the precolumn for on-line solid-phase extraction of BPA and other phenolic compounds, such as tBP, NP and OP. While a commercial C18 silica monolithic column was adopted as the analytical column to couple with precolumn, the fast-speed analysis of the samples can be achieved. These results indicate that the 4-VP-*co*-EDMA based imprinting monolithic column can be applied on the on-line SPE preconcentration of analytes.

Acknowledgements

Financial supports from the China State High-Tech Program Grants (2003AA233061), the Natural Science Foundation of China (200475054) and the Knowledge Innovation Program of DICP to Dr. Hanfa Zou are gratefully acknowledged.

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