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ON-LINE ENRICHMENT OF THREE PHENOLIC COMPOUNDS USING A POLY(N-ISOPROPYLACRYLAMIDE-CO-VINYL ESTER RESIN) MONOLITHIC COLUMN

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Abstract

A poly (N-isopropylacrylamide-co-vinyl ester resin) monolithic column for HPLC was synthesized via a free-radical polymerization technique and was used as a high selective sorbent for phenolic compounds. The monolith showed excellent mechanical strength, permeability, and high selectivity. 2-Amino-4-methylphenol, 4-nitrophenol, and 2, 4-dichlorophenol could be enriched by the monolithic column and on-line analyzed by using RP-C18 column. Good linearity was obtained from 0.06 to 60μg mL^{-1}. Precision for inter-and intra-day assay showed acceptable results for quantitative assay with relative standard deviation (RSD) less than 3.36%. The recoveries were higher than 91.4% at three different concentrations and the limit of detection of the method was 1.0-4.5 ng-mL^{-1}. The results indicated that the monolith was feasible to be used as an on-line SPE sorbent material to enrich phenolic compounds from solution.

Keywords and phrases: poly (NIPAAm-co-vinyl ester resin), monolithic column, SPE, phenolic compounds.
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1. Introduction

Phenol and substituted phenols, mainly nitrophenols and chlorophenols, are determined in tap and surface water because they can be toxic to most aquatic organisms, even at concentrations lower than $1\mu gL^{-1}$ a nontoxic concentration, phenols affect the taste and odour of water and fish. Phenolic compounds of environmental interest come from a wide variety of industrial sources, such as the plastics and dye industries and particularly from pulp processing [1]. Due to their toxicity and persistence in the environment, a variety of phenols were included in different monitoring programs, such as those of the US Environmental Protection Agency (EPA) and of the European Union (EU) [2]. The Integrated Wastewater Discharge Standard GB 8978-1996 of the People's Republic of China requires that the maximum admissible concentration of phenols in wastewater should be $0.5\,mg\,L^{-1}$ for the total content of volatile phenols and $0.6\,mg\,L^{-1}$ for 2, 4-dichlorophenol. For human health protection and environmental control, it is important to develop a selective and sensitive method for the detection of phenolic compounds in water.

Analytical techniques used in determination of phenols are mainly high-performance liquid chromatography (HPLC), particularly reversed-phase liquid chromatography (RPLC). However, in order to achieve necessary levels of sensitivity, the substances interfered with the chromatographic analysis should be firstly removed before the determination of phenols. Thus, sample preparation is a key step in quantitative analysis and often be a bottleneck in the process in developing robust and efficient environment analytical methodology [3, 4]. Different techniques have been applied to recover antioxidant phenolic compounds from natural sources including solid-liquid extraction with organic solvents, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluids extraction, and high pressure processes [5 - 7]. In recent years, solid phase extraction (SPE) [8 - 12] has become an important sample preparation technique for either matrix simplification

or trace enrichment [13 - 17]. This extraction technique has been developed in the off-line and on-line mode. Both modes have advantages and limitations, but the on-line approach is preferred due to its advantages such as higher sensitivity, absence of organic solvent, and less manipulation of the samples, which leads to greater precision and much easier to be automated. An important consideration in SPE is the rational selection of the sorbent depending on the characteristics of the analytes to be extracted and also on the complexity of the sample matrix. A very important feature of polymeric sorbents is their porous character, which provides the surface area necessary for sorption of the compounds. Therefore, porous character is directly related to the efficacy of the sorbent in the SPE process [18].

In this work, a porous poly (N-isopropylacrylamide-co-vinyl ester resin) [poly (NIPAAm-co-vinyl ester resin)] monolithic column was prepared by in situ free-radical polymerization technique and applied as a high selective absorbent material. An on-line solid phase extraction combining with reversed-phase liquid chromatography was used to determine three phenolic compounds.

2. Materials and Methods

2.1. Chemicals and instruments

N-isopropylacrylamide (NIPAAm) was purchased from Kohjin (Tokyo, Japan). Vinyl ester resin was made by Hebei University laboratory. 2, 2-Azobisisobutyronitrile (AIBN) was produced by Shanghai Chemical Plant (Shanghai, China) and refined before use. Cetyl alcohol and methanol were purchased from Tianjin Kemiou Com (Tianjin, China). 2-Amino-4-methylphenol and 2, 4-dichlorophenol were purchased from Aladdin Chemistry Co. Ltd. 4-Nitrophenol was purchased from Beijing Chemical Ltd. All reagents were of analytical reagent (AR) grade. Triple distilled water was used throughout all experiments. All media were filtered through a 0.45μm membrane before injection for LC analysis.

HPLC analysis was performed by using a Shimadzu HPLC system equipped with two LC-20AD Solvent Delivery Units, a SUS-20A gradient controller, and an SPD-20A UV-VIS Detector (Shimadzu, Kyoto, Japan). An LC-solution work station (Shimadzu, Kyoto, Japan) was used as a data acquisition system. The synthetic monolithic column was used as a pre-column and a ZORBAX Eclipse XDB-C18 (150mm × 4.6mm I.D.; 5 μm, Aglilent, USA) was used as the analytical column. Infrared (IR) spectrometer (FT IR8400S, Shimadzu Co., Japan) and scanning electron microscopy (SEC) (KYKY 1000B, Chinese Academy of Sciences Scientific Instruments Co., China) were also used.

2.2. Standard solution

2-Amino-4-methylphenol, 4-nitrophenol, and 2, 4-dichlorophenol were dissolved with methanol to obtain concentration of 1mg mL^{-1}, respectively. The mixed stock solution was obtained by dissolving 2-amino-4-methylphenol, 4-nitrophenol, and 2, 4-dichlorophenol with methanol to obtain the concentration of 1mg mL^{-1}. Then, the stock solution was diluted with ultrapure water to prepare standards at concentration of 0.06μg mL^{-1}, 0.6μg mL^{-1}, 1.5μg mL^{-1}, 6μg mL^{-1}, 15μg mL^{-1}, 30μg mL^{-1}, 45μg mL^{-1}, and 60μg mL^{-1}. Wastewater was centrifuged at 5000r min^{-1} for 10 min. All the solutions mentioned above were stored in a refrigerator at 4°C until use. Quality control samples at three different concentration levels of 0.6μg mL^{-1}, 6.0μg mL^{-1}, and 30μg mL^{-1} were prepared for the evaluation of precision, accuracy, and recovery in analysis of samples.

2.3. Preparation and characterization of monolith

The monolithic column was prepared briefly as follows: 0.07g NIPAAm and 0.7g vinyl ester resin were dissolved in the mixture of 1.0g cetyl alcohol and 0.82mL methanol. The mixture was sonicated for 1 min. Then, 0.01g AIBN was added into the mixture. The mixture was sonicated again and degassed briefly for 30 min. Lastly, the stainless-steel columns (50mm × 4.6mm i.d.) sealed at the bottom were filled with the

polymerization mixture and then sealed at the top. After the polymerization was allowed to proceed at 60°C for 24h, the seals were removed from the tubes and the columns were provided with fittings, attached to the HPLC system and washed, respectively, with methanol and water at flow rate of $1\text{mL}\,\text{min}^{-1}$ for 60 min to remove unreacted NIPAAm, cetyl alcohol, and other soluble compounds present in the polymer rod. Scanning electron microscope (SEM) shows the morphological properties of the monolith. The pressure drop across the column was also investigated at different flow rates.

2.4. HPLC analysis

The mobile phase for enrichment of 2-amino-4-methylphenol, 4-nitrophenol, and 2, 4-dichlorophenol was ultrapure water, the mobile phase for separation and analysis was methanol-water (65:35, v/v). The detection wavelength was set at 280nm. The flow rate was set at $1\text{mL}\,\text{min}^{-1}$. The system was operated at room temperature.

2.5. Investigation of the sorption and desorption ability of the monolith

The sorption ability of the monolithic column was tested by directly injecting 1μL mixed solution of 2-amino-4-methylphenol, 4-nitrophenol, and 2, 4-dichlorophenol into the prepared column when using deionized water as the mobile phase at 280nm. Similarly, the desorption ability of monolithic column was studied by using pure methanol as the mobile phase, respectively.

2.6. SPE

The prepared monolith, which was used as SPE column for sample enrichment, was placed in the sample-loop position of the injection valve. Firstly, the SPE column was equilibrated with ultrapure water at a flow rate of $1\text{mL}\,\text{min}^{-1}$ for 10 min. Secondly, 30μL solution was directly injected into the SPE column in the “load” position of six-port injector valve and washed with ultrapure water for 10 min. Thirdly, C18 column

was balanced using methanol-water (65:35, v/v). Then, the six-port valve was switched to "inject" position to connect the SPE column and the analytical column in series. As a result, the retained analytes on the SPE column were eluted to the analytical C18 column. It is worth noting that the monolithic column should be washed with pure methanol and balanced with ultrapure water before re-using for subsequent SPE.

3. Results and Discussions

3.1. Characteristic features of the monolith

The prepared monolith was washed with pure methanol until a stable baseline was observed. Then, it was pushed out from the stainless-steel column and cut into little pieces. The pieces of monolith were dried in vacuo at 60°C for 24h for scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) characterization. Figure 1 shows the pore structure of the prepared monolith.

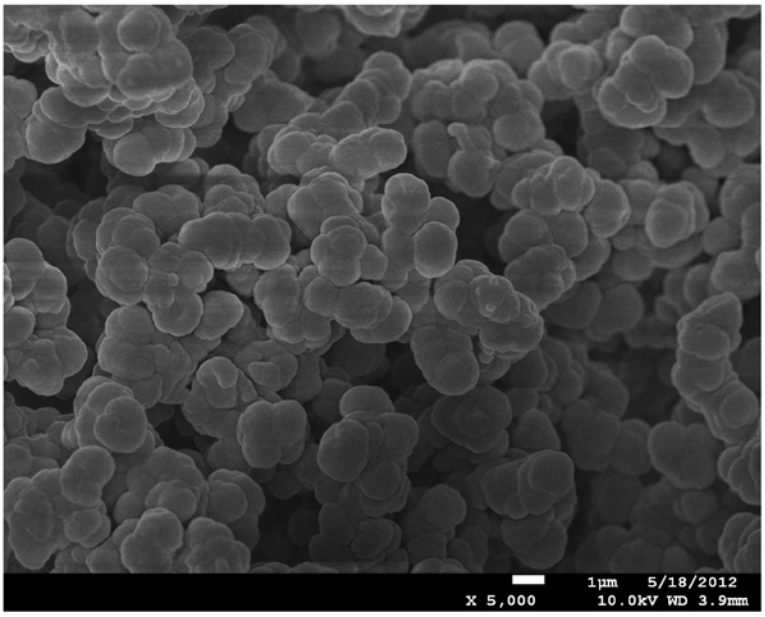

Figure 1. SEM image of poly (NIPAAm-co-vinyl ester resin) monolith.

Figure 2 shows FT-IR spectrum of the poly (NIPAAm-co-vinyl ester resin) monolith. The typical absorption bands were shown as following: $3050\text{-}2900\,cm^{-1}$ showed stretching vibration of the aromatic ring or alkene C-H; the strong absorption band at $1725\,cm^{-1}$ was the characteristic of the ester carbonyl. The spectrum at $1654\,cm^{-1}$ was due to the amide carbonyl vibration peak and contraction vibration peak of the C-N of the amide in the $1548\,cm^{-1}$; the strong band observed at $1500\,cm^{-1}$ was due to the benzene ring band. Pairs of methyl of isopropyl symmetric vibration coupling split peaks was shown at 1386 and $1368cm^{-1}$; the spectrum at $1173\,cm^{-1}$ was due to the contraction vibration peak of the C-C of isopropyl.

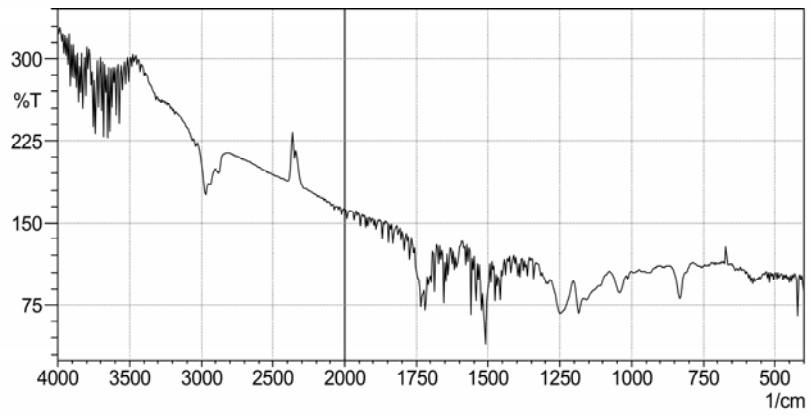

Figure 2. FT-IR spectrum of the poly (NIPAAm-co-vinyl ester resin) monolith.

Moreover, the effect of the flow rate on the back pressure was investigated when water and methanol was used as the mobile phase. An excellent linear relationship was shown in Figure 3. The low back pressure was owed to the macroporous structure of monolithic columns.

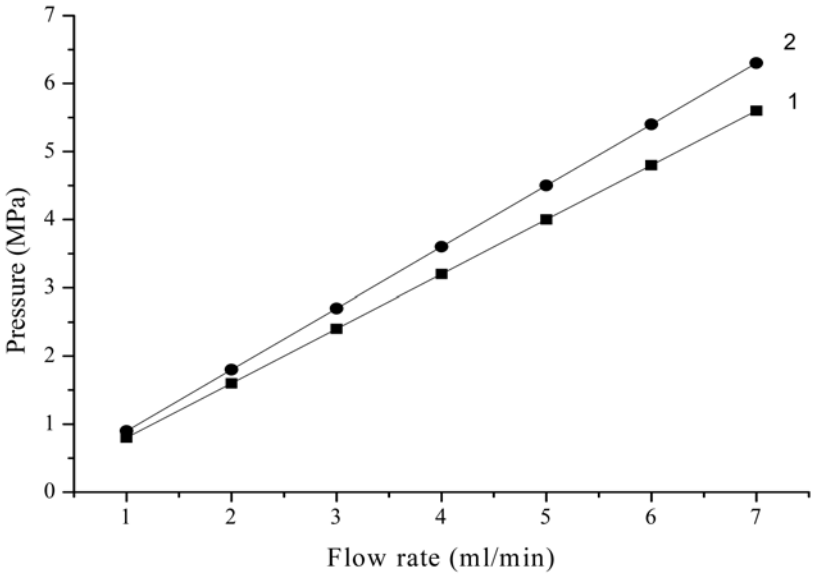

Figure 3. Effect of mobile phase flow rate on column back pressure. Mobile phase: (1) methanol and (2) water.

3.2. Investigation of the sorption and desorption ability of the monolith

Figure 4 showed that 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol could not be eluted when pure water (a) was used as the mobile phase. However, when pure methanol was used as mobile phase, 2, 4-dichlorophenol (b), 2-amino-4-methylphenol (c), and 4-nitrophenol (d) were eluted quickly from the monolithic column. Therefore, a conclusion could be drawn that the monolithic column could be used as SPE column to retain and elute the analytes using different mobile phases.

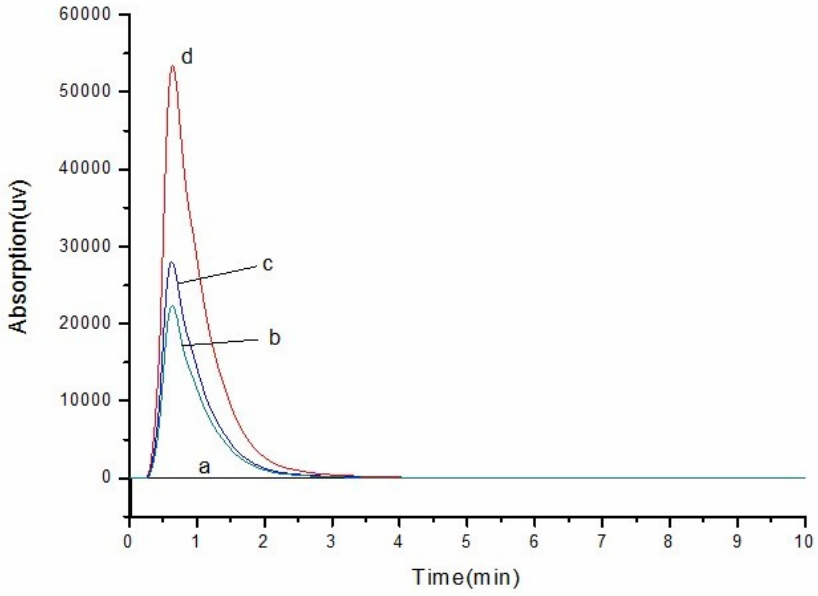

Figure 4. Chromatograms of three phenolic compounds on the monolithic column.

Column: 50mm × 4.6mm I.D.; Flow rate: 1.0 mL min^{-1}; Temperature: room temperature. 1μL solution of 2, 4-dichlorophenol (b), 2-amino-4-methylphenol (c), and 4-nitrophenol (d) were eluted with methanol and ultrapure water (a), respectively; wavelength: 280nm.

3.3. SPE-HPLC

30 μL of mixed solution was directly injected into the SPE column with ultrapure water as mobile phase and then analyzed on the RP-C18 column. The separation conditions on the RP-C18 column were optimized, the results demonstrated that 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol could be separated on C18 column when methanol-water (65:35, v/v) was used as the mobile phase at a flow rate of 1 mL min^{-1}. The chromatogram was shown in Figure 5, in which the blank was deducted.

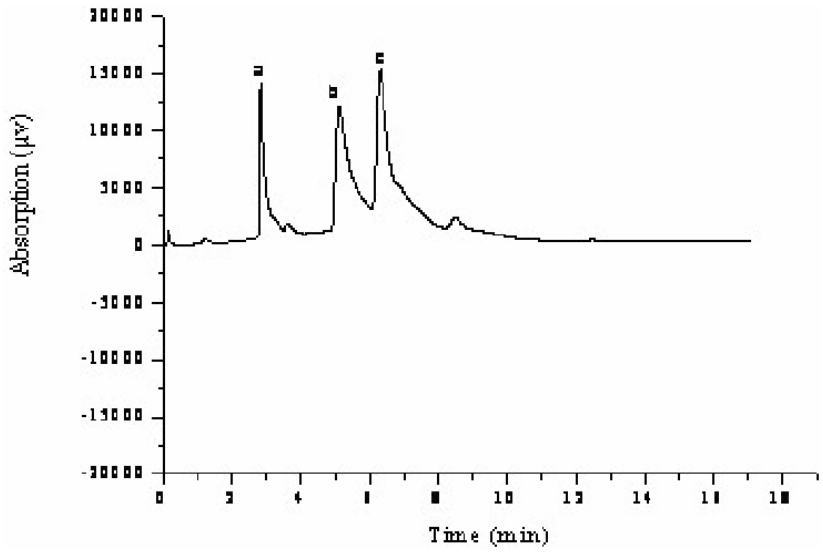

Figure 5. Chromatograms for the enrichment and separation of the mixture of (a) 2-amino-4-methylphenol, (b) 4-nitrophenol and (c) 2, 4-dichlorophenol.

Moblie phase: methanol-water (65:35, v/v); Flow rate: 1.0 mL min^{-1}; Column: a ZORBAX Eclipse XDB-C18 (150mm × 4.6mm I.D.; 5μm, Aglilent, USA); UV detection: at 280nm.

3.4. Method validation

Under the described extraction conditions, the parameters, such as selectivity, the linearity range, limits of detection (LODs), limits of quantification (LOQs), precision (intra-and inter-day), recoveries, and repeatability were measured. Results of the validation parameters were shown as follows.

3.4.1. Selectivity

The selectivity of the method was evaluated by the chromatograms obtained from the spiked samples containing 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol. As shown in Figure 5, it did

not be affected by other substances. These results showed that the developed method were selective.

3.4.2. Linearity studies

The linearity of the method was studied with a series of concentrations ($0.06\,\mu g\,mL^{-1}$, $0.6\,\mu g\,mL^{-1}$, $1.5\,\mu g\,mL^{-1}$, $6\,\mu g\,mL^{-1}$, $15\mu g\,mL^{-1}$, $30\mu g\,mL^{-1}$, $45\mu g\,mL^{-1}$, $60\mu g\,mL^{-1}$), and was obtained in the range of $0.06\text{-}60\,\mu g\,mL^{-1}$ for standard solutions. Each concentration was injected at least three times. Coefficients of correlation obtained were higher than 0.998 for three analytes. As can be seen from Table 1, the results showed a good linearity in the selected range.

3.4.3. Limits of detection (LODs) and limits of quantification (LOQs)

The LOD was calculated as the lowest concentration giving a response of three times the average of the baseline noise defined from three unfortified samples. The LOQ, defined at $S/N = 10$. The LODs indicated that the proposed method have satisfactory sensitivity and could be fully applied to the determination of 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol at trace level concentrations in wastewater.

Table 1. Calibration curve, LOD and LOQ of 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol after enrichment and separation

Analytes	Calibration equations	Correlation coefficient	LOQ ($ng\,mL^{-1}$)	LOD ($ng\,mL^{-1}$)
2-Amino-4-methylphenol	$Y = 68828x+64644$	$r^2 = 0.9992$	3.6	1.1
4-Nitrophenol	$Y = 228254x+318644$	$r^2 = 0.9986$	3.3	1.0
2, 4-Dichlorophenol	$Y = 89558x+25699$	$r^2 = 0.9994$	15	4.5

3.4.4. Precision

The precision of the experimental procedure was also evaluated by using quality control (QC) samples at low, medium, and high concentrations. The precision included an intra-day precision and inter-day precision was expressed as relative standard deviation (RSD) of analytes concentration. Method precision evaluated during the same day (intra-day) and 3 different days during a 3-week period (inter-day) using a $0.6\,\mu g\,mL^{-1}$ level standard are shown in Table 2, Intra-day method precision ranged from 1.8 to 2.49%, while for the inter-day assay, it varied from 3.1 to 3.36%. The satisfactory results showed that the reproducibility of method was excellent.

3.4.5. Recovery

Method recoveries were initially determined using a low $(0.6\,\mu g\,mL^{-1})$, medium $(6\,\mu g\,mL^{-1})$, and high-spiked $(30\,\mu g\,mL^{-1})$ concentrations to evaluate the proportion of analyte added to the matrix, which indicated that the method is able to accurately quantify. All analyzes were repeated five times. The absolute recoveries were calculated via comparing the peak areas measured after SPE-LC analysis of spiked ultrapure water samples to the peak area obtained by injection of samples dissolved in methanol without SPE pretreatment. Table 2 shows the results of recoveries.

Table 2. Recovery of 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol after enrichment and separation

Spiked analytes	$0.6\,\mu g\,mL^{-1}$		$6\,\mu g\,mL^{-1}$		$30\,\mu g\,mL^{-1}$	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2-Amino-4-methylphenol	98.6	2.9	106.7	3.5	101.0	3.6
4-Nitrophenol	95.9	2.3	104.9	3.2	108.2	1.9
2, 4-Dichlorophenol	95.8	3.9	108.2	1.6	91.5	1.6

3.4.6. Repeatability

A good monolith-to-monolith repeatability was important to the process. A good repeatability characterized by relative standard deviations (RSDs) for the retention times (in the range of 1.4-2.9%) was achieved on poly (NIPAAm-co-vinyl ester resin) monoliths by using the same process and conditions. The results proved that the preparation process had a good repeatability and the monoliths were stable.

3.5. Genuine water sample analysis

The proposed method was applied to analysis of actual water samples. Genuine water samples are taken from the sewage of Square of Baoding city. The fetched sewage was firstly centrifugated at 5000 r min^{-1} for 10 min, and then filtered through a 0.45 μm membrane. As described above, 20 μL water sample was directly injected, The result was shown in Figure 6. No interfering peaks were observed near the retention time of 2, 4-dichlorophenol, 2-amino-4-methylphenol, and 4-nitrophenol, which demonstrated the sample enrichment could be achieved by this approach.

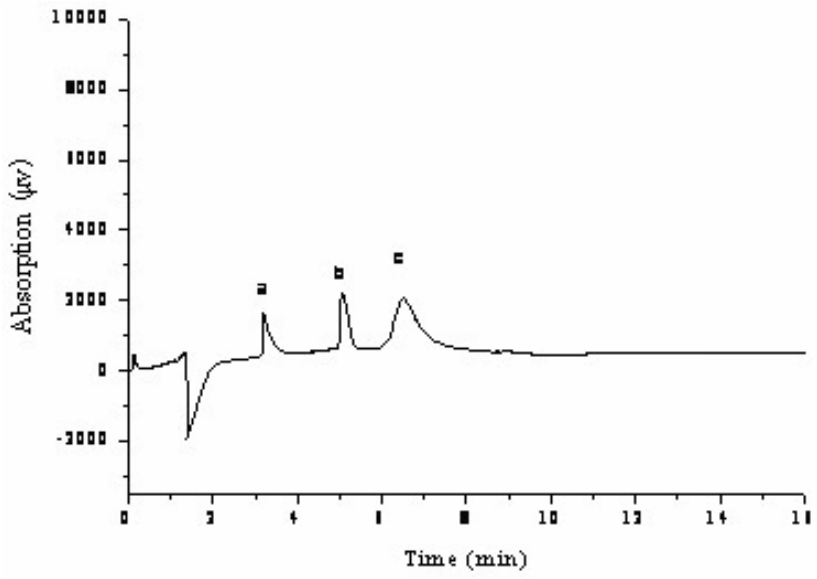

Figure 6. Chromatograms for the enrichment and separation of genuine water sample.

Mobile phase: methanol-water (65:35, v/v); Flow rate: 1.0mLmin^{-1}; Column: a ZORBAX Eclipse XDB-C18 (150mm × 4.6mm I.D.; 5μm, Aglilent, USA); UV detection: at 280nm.

4. Conclusion

A poly (NIPAAm-co-vinyl ester resin) monolithic column was prepared as SPE adsorption material to enrich three phenolic compounds. By using the monolithic column as on-line cartridge, the spiked samples were directly injected and the analytes could be selectively enriched and separated. Good linearity, precision, accuracy, and recovery have been achieved. This approach was appropriate for determination of trace phenolic compounds in water sample.

Acknowledgements

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