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Application of high-speed counter-current chromatography as a new pretreatment method for analysis of polycyclic aromatic hydrocarbons in environmental water samples

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Abstract

High-speed counter-current chromatography (HSCCC) was investigated as a new sample pretreatment method for the analysis of trace polycyclic aromatic hydrocarbons (PAHs) in water environmental samples. The experiment was performed with a nonaqueous binary two-phase solvent system composed of heptane-acetonitrile. The HSCCC column was first entirely filled with the upper stationary phase of the and a large volume of water sample was pumped into the column while the CCC column was rotated at 1600 rpm. Finally, the trace amount of PAHs extracted and enriched in the stationary phase were eluted out by the lower mobile phase. and analyzed by gas chromatography-flame ionization detector (GC-FID) or gas chromatography-mass spectrometry (GC-MS) after concentration. The enrichment and cleanup of PAHs can be fulfilled online by this method with high recoveries (84.1%–103.2%) and good reproducibility (RSDs 4.9–12.2%) for 16 EPA PAHs under the optimized HSCCC pretreatment conditions. This method has been successfully applied to determine PAHs in lake water where 8 PAHs were detected in the concentration of 40.9–89.9 ng/L. The present method is extremely suitable in the preparation of large volume of environmental water sample for the determination of a trace amount of organic pollutants including PAHs as studied in this paper.

Keywords

High-speed counter-current chromatography (HSCCC); Water sample; Polycyclic aromatic hydrocarbons (PAHs); Enrichment and cleanup; Pretreatment; Trace analysis of pollutants

1. Introduction

The analysis of contaminants in environmental samples is of a great challenge as they usually exist in a trace amount in complex matrix. Therefore, several enrichment and pretreatment methods have been developed to improve the sensitivity and selectivity of analysis, such as liquid-liquid extraction, solid phase extraction, solid phase microextraction, liquid phase microextraction, membrane extraction, supercritical fluid extraction, etc. Liquid-liquid extraction, a method with high recovery and without use of solid matrix, has

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been widely used in the concentration, pre-separation and cleanup of contaminants in environmental samples especially water samples. However, traditional liquid-liquid extraction is of low efficiency due to its multi-step operation and time-consuming procedure.

High-speed counter-current chromatography (HSCCC), being a support-free liquid-liquid partition chromatographic technique, eliminates irreversible adsorption of the sample onto the solid support [1]. It inherits the advantages of traditional liquid-liquid extraction with high recovery while its continuous elution system produces high partition efficiency in a short elution time. Although HSCCC has been widely used for the preparative separation of natural products during last thirty years [2], a few examples as presented below indicate that HSCCC also has a great potential in pretreatment of environmental and related samples.

Fedotov et al. first attempted to use counter-current chromatography for the extraction and separation of some polycyclic aromatic hydrocarbons (PAHs) from a sewage sludge medium [3]. Later he and his coworkers reported the dynamic recovery of polycyclic aromatic hydrocarbons from soils in rotating coiled columns used in CCC [4]. Hennebruder et al. also adopted CCC for the enrichment of rare-earth elements (REE) and Gd-DTPA in surface water samples prior to ICP-MS finding that CCC was more efficient than SPE from the recovery of the light and middle REE [5]. CCC has also been employed for the rapid sample preparation of N-methylcarbamate pesticides in vegetable oil and citrus fruit, where the carbamate pesticides can be easily separated from aliphatic sample matrix [6].

The present paper describes the establishment of a new enrichment and on-line cleanup method of 16 EPA PAHs in water samples by HSCCC using non-aqueous binary two-phase solvent systems. The method was applied in the pretreatment of an actual lake sample prior to Gas chromatography-mass spectrometry (GC-MS) analysis for the trace PAHs.

2. Experimental

2.1. Apparatus

The present study employed a Model GS 20 analytical HSCCC unit (Beijing Institute of New Technology Application, Beijing, China). The coil column was prepared by winding 0.8 mm I.D. polytetrafluoroethylene (PTFE) tubing in multilayer on a holder hub with a total capacity of 35 mL with the β values ranging from 0.4 to 0.72 ($\beta=r/R$, where r indicates the distance from the coil to the holder shaft and R , the distance from the holder shaft to the central axis of the apparatus).

The GC analyses were performed on an Agilent 6890 GC system coupled with split/splitless injector and flame ionization detector (FID).

The GC-MS equipment used was a Shimadzu QP2010 plus system equipped with AOC-20i auto sampler.

2.2. Chemicals and Reagents

All reagents were of HPLC-grade throughout the experiments. The 16 EPA PAHs standards (naphthalene, NA; acenaphthylene, ACN; acenaphthene, AC; fluorene, FL; phenanthrene, PHE; anthracene, AN; fluoranthene, FA; pyrene, PY; benzo[*a*]anthracene, B[*a*]A; chrysene, CHR; benzo[*b*]fluoranthene, B[*b*]F; benzo[*k*]fluoranthene, B[*k*]F; benzo[*a*]pyrene, B[*a*]P; indeno[1,2,3-*cd*]pyrene, IP; dibenz[*a,h*]anthracene, D[*ah*]A; benzo[*ghi*]perylene, B[*ghi*]P) each at 0.2 mg/mL in mixture were purchased from AccuStandard Inc. (CT, USA) and further diluted to obtain the desired concentration.

2.3. Sample preparation

The spiked water sample was prepared by dissolving 100 μL of diluted PAHs standards (each analyte at 20 $\mu\text{g/mL}$) in 500 mL ultrapure water (from Millipore ultrapure water system) to make a concentration of 4 $\mu\text{g/L}$ of each compound.

The actual environmental water sample was collected from a local lake, Beijing, China, and then filtered through a 0.45 μm membrane to remove the suspended particulates.

2.4. HSCCC pretreatment procedures

As PAHs are a group of hydrophobic compounds, a non-aqueous two-phase solvent system composed of n-heptane–acetonitrile (1:1, v/v) was used in this study. First, heptanes-rich upper phase was used as a stationary phase for enrichment of PAHs from the water sample. The multilayer coil column (35 mL) was entirely filled with n-heptane phase, and the column was rotated at 1600 rpm while the ultrapure water was introduced into the column at a flow-rate of 5 mL/min until the water emerged from the end of column indicating that dynamic equilibrium was established. (The retention factor S_f (%) was calculated as a ratio of the displaced stationary phase volume V_s to the total column volume V_c .) Then, the water sample (500 mL) spiked with PAH standards or real environmental water sample was pumped through the column at the same flow-rate of 5 mL/min, and the PAHs were extracted and enriched into heptane-rich upper stationary phase. Next, acetonitrile-rich lower phase was employed as the mobile phase at a flow-rate of 1 mL/min or 2 mL/min to elute the PAHs extracted in n-heptane-rich upper phase. The effluent was continuously monitored with a UV detector at 254 nm and the elution profile was recorded by online software. Finally, the peak fractions collected according to the chromatogram were subjected for further GC-FID or GC-MS analysis of PAHs.

2.5. Analysis of PAHs fractions

For the spiked water sample, an Agilent 6890 GC-FID system with HP-5 column (30 m $\times$ 0.32 mm I.D., and 0.25 μm film thickness) was employed. Nitrogen was the carrier gas with a flow-rate of 1.0 mL/min. The injector and detector temperature were 250°C and 280°C respectively. The GC oven was held at 50°C for 4 min and then increased to 280°C at a rate of 10°C/min, then maintained for 10 min. The injection volume was 1.0 μL in splitless mode. The recoveries of PAHs enriched through HSCCC method were analyzed quantitatively by external standard methods.

For the actual environmental water sample, GC-MS (Shimadzu QP2010 plus) with RxiTM-5ms column (30 m $\times$ 0.25 mm I.D., and 0.25 μm film thickness) was employed for the detection of the minor PAHs. The carrier gas was Helium with a constant flow of 1.2 mL/min. The GC oven temperature program was as follows: temperature held at 60°C for 3 min initially, then programmed to 160°C at the rate of 30°C/min, then increased to 275°C at the rate of 2°C/min, and maintained for 10 min. The injection volume was 1.0 μL in splitless mode. A solvent delay of 3 min was selected. The injector and interface temperature were 290°C and 250°C, respectively. In the scan mode, 70eV electron energy with a multiplier potential of 1.4 kv recorded the electron ionization mass spectra ranged from m/z 40 to 450.

3. Results and discussion

3.1. Optimization of HSCCC pretreatment condition

3.1.1. Selection of solvent system and rotation speed—Due to the low polarity of PAHs, two non-aqueous binary solvent systems composed of n-heptane–acetonitrile (1:1, v/v) and n-heptane–methanol (1:1, v/v) were selected and tested for the retention ability of stationary phase at various rotation speeds, as seen in Fig. 1A.

High stationary phase retention in HSCCC is critical for the recoveries of analytes. The results in Fig. 1 indicated that the stationary phase retention of n-heptane–acetonitrile (1:1, v/v) was generally higher than n-heptane–methanol (1:1, v/v) by 5–10%. Therefore, n-heptane–acetonitrile (1:1, v/v) was used in the following experiment. Meanwhile, it also indicated that the retention value of stationary phase increased with the rotation speed from 800 to 1800 rpm and reached a platform at 1600 rpm. Thus, 1600 rpm was finally chosen.

3.1.2. Impact of water loading flow-rate on the S_f —In the enrichment of PAHs from water sample, the flow-rate of water loading may influence the retention of stationary phase, so different flow-rates of 1.0, 3.0, 5.0 and 8.0 mL/min were tested as shown in Fig. 1B. It seems that higher flow-rate of water tends to produce lower retention of heptane, and meanwhile, higher back pressure was observed when the higher flow-rate was applied. Thus 5.0 mL/min was chosen as the water sample loading flow-rate when the retention of stationary phase and sample loading time were taken into account.

3.1.3. Impact of water sample volume on the S_f —To detect a trace amount of contaminants in environmental water samples, large volume of water sample was usually required for the enrichment. Long-term enrichment progress of a large volume of water samples may cause the bleeding of stationary phase. No obvious difference was observed for the stationary phase retention when 500–5000 mL of ultrapure water were loaded (seen in Fig. 1C). Thus it is feasible for the pretreatment of a large volume of water sample in the present system.

3.1.4. Impact of elution flow-rate on the S_f —After the loading of water sample, the enriched a trace amount of PAHs in heptane-rich stationary phase) was eluted out of the column by acetonitrile-rich mobile phase as mentioned above and semi-cleaned during the same time. Effect of flow-rates of mobile phase on retention of the stationary phase (S_f) was also checked as shown in Fig. 1D. The retention of stationary phase tends to decrease only slightly as the flow-rate was increased from 1.0 to 5.0 mL/min, and no significant difference is observed between 1.0 and 2.0 mL/min.

3.2. Method evaluation by spiked water sample

Under the optimized condition, the method described above was evaluated firstly by spiked water sample for the feasibility, recoveries and reproducibility.

Fig. 2 demonstrated the HSCCC extraction and elution profiles of PAHs from the spiked water sample. The GC analysis of each elution fractions (seen in Fig. 3) indicated that PAHs in the spiked water sample can be effectively extracted and enriched in the stationary phase, and eluted mainly in fraction III, while some interference materials are removed into fraction I and II. The elution time can be reduced to about one half as the flow-rate is increased from 1 mL/min to 2 mL/min.

The PAH fractions eluted at different flow-rates were collected, concentrated and subjected to GC-FID analysis to determine the recovery rate of each PAH. As shown in Fig. 4, the recoveries of PAHs ranged from 79.2% to 103.7% with the relative standard deviations (RSDs) 5.9–16% at a flow rate of 1 mL/min, and from 84.1% to 103.2% with RSDs 4.9–12.2% at the flow rate of 2 mL/min. Since, there is no significant difference between the results at two different flow-rates, the elution flow rate of 2 mL/min was used later. The recoveries of 11-B[b]F, 12-B[k]F, 13-B[a]P, 14-IP, 15-D[ah]A, 16-B[ghi]P are slightly higher than those of 1-NA, 2-CAN, 3-AC, 4-FL, 5-PHE, 6-AN, 7-FA, 8-PY, 9-B[a]A and 10-CHR apparently due to their different volatilities.

The PAH recoveries obtained through the above HSCCC pretreatment were acceptable compared with those from other conventional methods such as solid phase extraction (SPE) (33.5% to 122.4%) and solid phase microextraction (SPMS) (82.65% to 115.35%), as shown in the literatures[7,8].

The above results indicate that HSCCC can serve as an efficient method for the pretreatment of PAHs from water samples with high recovery and good reproducibility.

3.3. Application in lake water sample

An actual environmental water sample from a local lake was analyzed quantitatively for PAHs using our HSCCC pretreatment method described above followed by GC-MS. As shown in Fig. 5, HSCCC extraction and elution profile of PAHs of the lake water sample is similar to that of spiked sample, but no remarkable peak appeared in shadowed area where PAHs should be eluted due to their low concentration in the real water sample. However, GC-MS analysis of the shadowed elution fraction detected several PAHs in the concentration of 40.9–89.9 ng/L in lake water, the quantitative results of which are shown in Table 1.

4. Conclusion

The present study established a feasible and efficient pretreatment method for extraction and online cleanup of 16 EPA PAHs in environmental water samples by HSCCC. Using the selected two-phase solvent system composed of heptane-acetonitrile under the optimized HSCCC operation conditions, a trace amount of PAHs in a large volume of water sample was efficiently extracted and enriched in the stationary phase, and then eluted out online by the mobile phase to get rid of other interference materials. The method provided satisfied recovery of each PAH, ranging from 84.1% to 103.2% with good reproducibility at RSDs 4.9–12.2%. It was applied successfully to the pretreatment of actual lake samples for the analysis of PAHs by GC-MS. The overall results of our present studies suggest that HSCCC can be a potential pretreatment method for analysis trace organic pollutants in the environmental samples especially from water. Its further application in other pollutants and matrix are now under investigation in our laboratory.

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Fig. 1.

Impact of different conditions on the retention of stationary phase (S_f). A. solvent systems; B. flow-rate of loading water; C. volume of water sample; D. flow-rate of elution mobile phase. Other conditions: apparatus: GS20 analytical HSCCC; column: multilayer coil of 1.6 mm I.D. PTFE tubing with a total capacity of 35 mL; Stationary phase: upper phase; flow-rate: 1 mL/min (A and C).

Fig. 2. HSCCC extraction and elution profile of PAHs from spiked water sample. Conditions: elution flow-rate: 1 mL/min (A), 2 mL/min (B); detection: 254 nm; water sample: 500 mL ultrapure water spiked with 16 PAHs standards (4 $\mu\text{g/L}$ of each compound).

Fig. 3. GC-FID chromatograms of each fractions from HSCCC. A. standard PAH mixture; B-D. peak fractions I-III from HSCCC pretreatment (Fig. 2B); 1-NA; 2-ACN; 3-AC; 4-FL; 5-PHE; 6-AN; 7-FA; 8-PY; 9-B[a]A; 10-CHR; 11-B[b]F; 12-B[k]F; 13-B[a]P; 14-IP; 15-D[ah]A; 16-B[ghi]P

Fig. 4.

Recoveries of each PAH in the spiked water sample through HSCCC pretreatment.

Fig. 5. HSCCC extraction and elution profile of PAHs from the lake water sample. Conditions: elution flow-rate: 2 mL/min; detection: 254 nm; water sample: 500 mL local lake water

Table 1

Analysis of PAHs in lake water pretreated by HSCCC followed with GC-MS

No.	Analytes	Retention time (min)		Concentration (ng/L)	RSD (% n=3)
		Standards	Sample		
1	Nap	7.215		ND	
2	Acp	9.983		ND	
3	Ace	10.475		ND	
4	Flu	12.117		ND	
5	Phe	16.742	16.701	70.7	18.8
6	Ant	17.015	16.982	89.9	17.3
7	Fla	25.822	25.788	63.0	3.6
8	Pyr	27.726	27.694	67.2	7.1
9	BaA	40.453	40.454	87.3	5.7
10	Cry	40.862	40.857	78.6	8.7
11	BbF	51.983	51.957	79	2.4
12	BkF	52.263	52.215	40.9	11.4
13	BaP	54.999		ND	
14	InP	65.454		ND	
15	DBA	65.996		ND	
16	BPe	67.753		ND	

ND: Not detected.