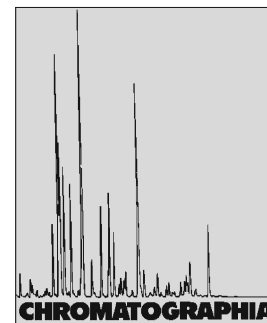


Analysis of PAHs in Water and Fruit Juice Samples by DLLME Combined with LC-Fluorescence Detection



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Abstract

A simple, rapid and efficient method termed dispersive liquid-liquid microextraction combined with liquid chromatography-fluorescence detection, has been developed for the extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in water and fruit juice samples. Parameters such as the kind and volume of extraction solvent and dispersive solvent, extraction time and salt effect were optimized. Under optimum conditions, the enrichment factors ranged from 296 to 462. The linear range was 0.01–100 $\mu\text{g L}^{-1}$ and limits of detection were 0.001–0.01 $\mu\text{g L}^{-1}$. The relative standard deviations (RSDs, for 5 $\mu\text{g L}^{-1}$ of PAHs) varied from 1.0 to 11.5% ($n = 3$). The relative recoveries of PAHs from tap, river, well and sea water samples at spiking level of 5 $\mu\text{g L}^{-1}$ were 82.6–117.1, 74.9–113.9, 77.0–122.4 and 86.1–119.3%, respectively. The relative recoveries of PAHs from grape and apple juice samples at spiking levels of 2.5 and 5 $\mu\text{g L}^{-1}$ were 80.8–114.7 and 88.9–123.0%, respectively. It is concluded that the proposed method can be successfully applied for determination of PAHs in water and fruit juice samples.

Keywords

Column liquid chromatography-fluorescence detection
Dispersive liquid-liquid microextraction
Polycyclic aromatic hydrocarbons
Water and fruit juice samples

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contami-

nants arising from a variety of sources including fossil fuel combustion, oil spills and some industrial processes [1]. Increased attention has been paid to these compounds

in environmental chemistry due to the mutagenic, teratogenic and carcinogenic character of some of these molecules [2]. Therefore, they have been included in the European Union (EU) and the Environmental Protection Agency (EPA) priority pollutant lists. Due to hazardous characteristics, identification and determination of PAHs is an important analytical issue. The main problem with PAHs monitoring is their very low concentration and complexity of environmental matrices [3, 4]. As a result, preconcentration and separation are needed to achieve the required sensitivity and selectivity.

Liquid-liquid extraction (LLE) [5] and solid-phase extraction (SPE) [6, 7] are still the most common methods to extract PAHs from environmental samples. LLE is a very useful technique, but it is tedious, time-consuming and requires large amounts of toxic and flammable solvents. SPE is a commonly used technique, but it also has some disadvantages, such as particle blockage and slow sample processing rate [8]. Additionally, solid-phase microextraction (SPME) has been developed to extract PAHs from aqueous samples [9]. Compared to LLE, SPME is a solvent-free process that includes simultaneous extraction and preconcentration of analytes from aqueous samples. However, SPME is expensive, its fiber is fragile

and has limited lifetime, and sample carry-over could be a problem [10].

In the last few years, headspace solvent microextraction (HSME), belonging to one kind of liquid-phase microextraction (LPME), was developed to determine PAHs in water samples [11]. Compared with conventional methods, HSME is an extremely simple, low cost and virtually solvent-free sample-preparation technique. However, the disadvantages of this method are as follows: fast stirring would tend to format air bubble, extraction is time-consuming and equilibrium could not be attained after a long time in most cases [12, 13]. Cloud point extraction (CPE) is another technique that has been developed for extraction of PAHs from aqueous samples [14, 15]. Despite many benefits of using CPE, the main disadvantage is not compatibility of extraction phase with some instrumental analysis such as GC and LC [16].

Recently, a novel microextraction technique termed as dispersive liquid-liquid microextraction (DLLME) has been developed by Assadi [17]. The advantages of the DLLME method are simplicity of operation, rapidity, low cost, high recovery and enrichment factor. Now, DLLME has been successfully utilized for extraction of antioxidants, triazine herbicides, volatile phenols, chlorophenols, chlorobenzenes, organophosphorus flame retardants, phthalate esters and lead in liquid samples [18–25].

So far, PAHs has been determined by using various chromatographic techniques including GC [26], LC [27] and supercritical fluid chromatography (SFC) [28]. LC-FLD is the most widely used method for its sensitivity. The aim of the present study was to investigate the feasibility of using DLLME combined with LC-FLD to extract and determine PAHs in water and fruit juice samples. The effects of various experimental parameters, such as the kind and volume of extraction solvent and dispersive solvent, extraction time and salt effect were optimized.

Experimental

Reagents and Standards

All PAHs stock standard solutions: 100 mg L⁻¹ of anthracene, pyrene,

benzo[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*a*]pyrene; 200 mg L⁻¹ of fluoranthene and benzo[*b*]fluoranthene were obtained in 1:1 methanol:methylene chloride from Supelco, Bellefonte, PA, USA. Appropriate dilutions of the standard solution with methanol were made to the working solutions. Acetone, carbon tetrachloride, chloroform and 1,1,2,2-tetrachloroethane were of analytical grade and redistilled twice before use. Acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Sodium chloride was of the highest purity available from Merck. Deionized water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Tap, river, well and sea water samples were collected from Wenzhou, China, and filtered through a 0.45 μm Nylon filter. Grape juice and apple juice were bought in a local supermarket. All samples were kept in darkness at 4 °C and analyzed within 48 h. The fresh juice was centrifuged at 3,000 rpm for 15 min, and then the supernatant was filtered through a 0.45-μm nylon filter. 25 mL of filtrate was diluted at 1:1 ratio with deionized water in a 50 mL volumetric flask.

Instrumentation

The chromatographic analysis was performed on an Agilent 1200 LC system equipped with a manual injector and a fluorescence detector. A Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5-μm particle size) was used and all injections were performed manually with 20.0-μL sample loop. The mobile phase was a mixture of methanol–water (75:25, v/v) and the flow rate was 0.8 mL min⁻¹. The column temperature was set at 40 °C. Fluorescence detection was carried out as follows: 0–20 min λ_{ex} at 256 nm and λ_{em} at 441 nm, 20–35 min λ_{ex} at 270 nm and λ_{em} at 390 nm, 35–55 min λ_{ex} at 290 nm and λ_{em} at 410 nm.

Extraction Procedure

For the DLLME, an aliquot (5.00 mL) of an aqueous solution containing the analytes was placed in a 10 mL glass test tube

with conical bottom. 1.00 mL of acetonitrile (as dispersive solvent) containing 16.0 μL C₂H₂Cl₄ (as extraction solvent) was injected into sample solution by syringe, rapidly and then the mixture was gently shaken. A cloudy solution was formed. Then the mixture was centrifuged for 3.0 min at 3,000 rpm. After centrifugation, the fine droplets of the extraction phase were separated at the bottom of tube. 5.0 μL of the separated phase were withdrawn into a 10.0 μL microsyringe for further analysis.

Results and Discussion

Selection of Extraction Solvent

In the selection of extraction solvent, several factors should be considered: (1) higher density than water; (2) low solubility in water, (3) good chromatographic behavior, and (4) high extraction capability of interested compounds. Thereby, the following solvents, carbon tetrachloride, chloroform and 1,1,2,2-tetrachloroethane were selected. A series of sample solution were studied by using 1.00 mL acetonitrile containing different volumes of extraction solvent (26.0 CCl₄, 36.0 μL CHCl₃ and 16.0 μL C₂H₂Cl₄) in order to achieve 10.0 μL of settled phase. The results show that C₂H₂Cl₄ has the highest extraction recovery (64.8–101.1%) in comparison with the CHCl₃ (40.8–69.4%) and CCl₄ (26.6–45.2%). Also, standard deviations by using C₂H₂Cl₄ are lower than other solvents. Therefore, C₂H₂Cl₄ was selected as the extraction solvent in subsequent experiments.

Selection of Dispersive Solvent

The dispersive solvent is selected on the basis of its miscibility in the organic phase and aqueous phase. Accordingly, acetone, acetonitrile, and methanol were selected. A series of sample solution were studied by using 1.00 mL of each dispersive solvent containing 16.0 μL C₂H₂Cl₄. The recoveries by using acetonitrile, acetone and methanol were in the range 64.8–101.1%, 57.5–85.0%, and 38.7–74.8%, respectively. According to these results, acetonitrile exhibits the

highest extraction efficiency among three solvents. Thus, acetonitrile was chosen as the dispersive solvent for subsequent experiments.

Effect of Extraction Solvent Volume

In order to examine the effect of extraction solvent volume on the extraction efficiency of analytes, a constant volume of dispersive solvent (acetonitrile, 1.0 mL) containing different volumes of $C_2H_2Cl_4$ (16.0–31.0 μL , at 5 μL interval) was subjected to the same DLLME procedure. The results show that the volume of the separated phase increases from 10.0 to 40.3 μL by increasing the volume of $C_2H_2Cl_4$ from 16.0 to 31.0 μL . The extraction efficiency for most of the analytes is almost constant, indicating the quantitative extraction and high distribution coefficients of PAHs in the conditions. However, enrichment factor decreases because the volume of the separated phase increases. As a result, 16.0 μL of $C_2H_2Cl_4$ were selected as the volume of extraction solvent in order to obtain good recovery and high enrichment factor.

Effect of Dispersive Solvent Volume

To investigate the effect of dispersive solvent volume, various experiments were performed by using different volumes of acetonitrile (0.5, 1.0, 1.5 and 2.0 mL) containing 15.5, 16.0, 17.0 and 21.5 μL tetrachloroethane, respectively. Under the conditions, the volume of separated phase was constant. The result showed that the extraction efficiency at first increased by increasing the acetonitrile volume up to 1.0 mL, and then decreased when a higher volume of acetonitrile (>1.0 mL) was used. At a low volume, acetonitrile cannot disperse extraction solvent properly and the cloudy solution is not formed completely, which resulted in low extraction efficiency. Additionally, the solubility of analytes in water increased at a high volume of acetonitrile, which also

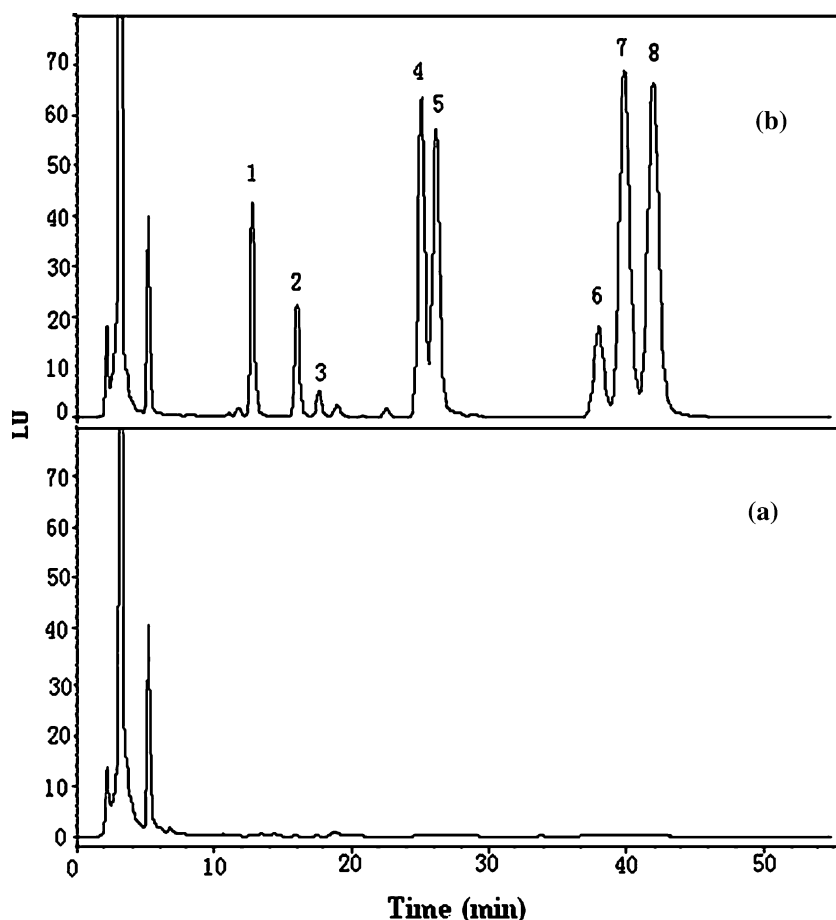


Fig. 1. Chromatograms of unspiked apple juice (a) and spiked apple juice (b) at concentration level of $5 \mu g L^{-1}$ for most of analytes obtained using DLLME combined with HPLC-FLD. Extraction conditions: water sample volume, 5.00 mL; concentration of most PAHs, $5 \mu g L^{-1}$; dispersive solvent (acetonitrile) volume, 1.00 mL; volume of tetrachloroethane, 16.0 μL ; sedimented phase volume, $10.0 \pm 0.5 \mu L$. Peak identification: 1 = Anthracene; 2 = Fluoranthene; 3 = Pyrene; 4 = Benzo[a]anthracene; 5 = Chrysene; 6 = Benzo[b]fluoranthene; 7 = Benzo[k]fluoranthene; 8 = Benzo[a]pyrene

decreased the extraction efficiency. Thus, 1.00 mL of acetonitrile was chosen as optimum volume.

Effect of Extraction Time

For the DLLME process, the extraction time is defined as the interval between injection of the mixture of disperser and extraction solvents, and starting to centrifuge. The effect of extraction time (0–60 min) was tested under constant experimental conditions. The result showed that DLLME is a time-independent method. It is revealed that there is no interface between the water phase and the water-immiscible organic solvent phase after formation of the cloudy solution. The transition of the analytes

from the aqueous phase to the extraction solvent is very fast and equilibrium state is achieved quickly, which is the obvious advantage of DLLME technique.

Salt Addition

It is necessary to examine the influence of salt addition on the efficiency of DLLME. For this purpose, various experiments were performed by adding different amounts of NaCl (0–5%, w/v) under constant experimental conditions, the results showed that the volume of the separated phase increased from 9.7 to 21.8 μL by increasing the amount of NaCl from 0 to 5%, because the solubility of extraction solvent in aqueous phase decreased in the presence of salt.

Table 1. Relative recoveries and relative standard deviations of PAHs from real water samples^a

PAHs	Tap water		River water		Well water		Sea water	
	Relative recovery ^b (%)	RSD (%) <i>n</i> = 3	Relative recovery ^b (%)	RSD (%) <i>n</i> = 3	Relative recovery ^b (%)	RSD (%) <i>n</i> = 3	Relative recovery ^b (%)	RSD (%) <i>n</i> = 3
Anthracene	99.7	9.6	79.5	5.1	77.0	9.2	94.3	9.0
Fluoranthene	105	9.2	110.5	1.3	108.1	6.5	119.3	1.0
Pyrene	117.1	8.0	113.9	2.0	110.2	8.1	103.74	2.5
Benzo[<i>a</i>]anthracene	82.6	7.6	86.7	8.1	122.4	10.3	101.2	4.4
Chrysene	84.5	7.9	74.9	4.3	105.7	11.5	86.1	8.4
Benzo[<i>b</i>]fluoranthene	85.7	6.9	82.9	5.3	115.7	10.1	97.7	8.7
Benzo[<i>k</i>]fluoranthene	98.9	11.8	81.9	7.6	122	7.5	95.3	10
Benzo[<i>a</i>]pyrene	85.1	8.0	87.1	6.9	80.8	9.6	110.5	8.2

^a Extraction conditions: as in Fig. 1^b Spiked with 5 µg L⁻¹ for most of analytes**Table 2.** Relative recoveries and standard deviations of PAHs from spiked fruit juice samples^a

Grape juice	Apple juice							
	Relative recovery ^b (%)	RSD ^b (%) <i>n</i> = 3	Relative recovery ^c (%)	RSD ^c (%) <i>n</i> = 3	Relative recovery ^b (%)	RSD ^b (%) <i>n</i> = 3	Relative recovery ^c (%)	RSD ^c (%) <i>n</i> = 3
Anthracene	113.3	4.1	80.8	1.0	99.8	9.4	102.3	3.0
Fluoranthene	114.7	11.3	86.7	9.7	113.9	9.5	123	9.4
Pyrene	106.7	11.6	87.4	10.8	98.6	9.8	122	4.9
Benzo[<i>a</i>]anthracene	108.5	9.8	87.8	10.2	107.3	5.0	115.4	9.7
Chrysene	102.4	11.0	89.9	1.2	88.9	9.6	115.8	9.7
Benzo[<i>b</i>]fluoranthene	99.0	5.7	87.1	5.9	99.6	4.6	99.8	8.2
Benzo[<i>k</i>]fluoranthene	102.7	10.1	86.9	6.9	98.0	3.9	104.1	8.6
Benzo[<i>a</i>]pyrene	104.5	8.5	88.7	3.2	92.8	7.6	90.2	1.7

^a Extraction conditions: as in Fig. 1^b Spiked with 2.5 µg L⁻¹ for most of analytes^c Spiked with 5 µg L⁻¹ for most of analytes**Table 3.** Comparison of DLLME with HSME and SPME for the determination of PAHs in water samples

Methods	LR ^a (µg L ⁻¹)	LOD ^b (µg L ⁻¹)	Extraction time (min)	Sample volume (mL)	References
HSME-GC-FID	10–240	4–41	12	6	[11]
SPME-GC-FID	0.5–100	0.05–0.16	10–120	10	[29]
DLLME-HPLC-FLD	0.01–100	0.001–0.01	A few seconds	5	Represented method

LR linear range

LOD limit of detection

Salt addition had no effect on the extraction recovery but decreased the enrichment factor. Therefore, all extraction experiments were carried out without the addition of salt.

Quantitative Analysis

The characteristic of calibration curves was obtained under optimized conditions. Linearity was observed in the range of 0.01–100 µg L⁻¹, with the correlation

coefficients (*r*) ranging from 0.9995 to 0.9998. The enrichment factors ranged from 296 to 462. The LODs, based on signal-to-noise ratio (S/N) of 3, ranged from 0.001 to 0.01 µg L⁻¹. The RSDs for 5 µg L⁻¹ of PAHs in water samples were in the range of 1.0–11.5% (*n* = 3).

Real Samples Analysis

The efficiency of the proposed method was evaluated by concentration and

determination of PAHs in four water and two fruit juice samples. The proposed samples were extracted using DLLME and analyzed by LC-FLD. The results showed that they were all free of PAH contamination. These water and fruit juice samples were spiked with PAHs at different concentration levels to assess matrix effects. Figure 1 shows the chromatograms of unspiked and spiked apple juices at a concentration level of 5 µg L⁻¹ for most of the analytes. As can be seen from Table 1, the relative

recoveries for PAHs in tap, river, well and sea water samples were 82.6–117.1, 74.9–113.9, 77.0–122.4 and 86.1–119.3%, respectively. According to Table 2, the relative recoveries for PAHs in grape juice and apple juice samples were 80.8–114.7 and 88.9–123.0%, respectively. The previous results demonstrated that the different matrices of real water and fruit juice samples, in our present experiments, had little effect on DLLME performance.

Comparison of DLLME to HSME and SPME

The performance of DLLME-LC-FLD was compared to that of other methods such as HSME [11] and SPME [29] from the viewpoints of linear range (LR), LODs, extraction time and sample volume required. According to Table 3, DLLME had low LODs (0.001–0.01) and wide linear range (0.01–100 $\mu\text{g L}^{-1}$). In addition, the extraction time in DLLME was very short. However, extraction time for other methods ranged from 10 to 120 min. The volume of the sample solution required for DLLME was small. The previous analysis suggested that it is very simple, rapid, inexpensive, easy to use and benign to the environment.

Conclusion

In the present study, DLLME-LC-FLD was presented for concentration and determination of PAHs in water and fruit juice samples. The technique provided better repeatability, higher enrichment factor and good recovery within a shorter time compared to other techniques. In addition, consumption of toxic organic solvents was minimized without affecting the sensitivity of the

method. In conclusion, the proposed method is very simple, fast and of low cost, and can be successfully applied for preconcentration and determination of the PAHs in water and fruit juice samples.

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