On-line enrichment and determination of polycyclic aromatic hydrocarbons in atmospheric particulates using high performance liquid chromatography with fluorescence as detector

HASHI Yuki[1,2], WANG Tian-ran[1], LI Yue-qi[2], LIN Jin-ming[1,*]

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, Beijing 100085, China. E-mail: jmlin@rcees.ac.cn
2. Shimadzu (Shanghai) Limited, Beijing Office, Analytical Application Center, Beijing 100020, China

Abstract

Seven polycyclic aromatic hydrocarbons (PAHs) in atmospheric particulates were determinated by high performance liquid chromatography (HPLC) with fluorescence detector using direction injection and an on-line enrichment trap column. The method simplified the sample pretreatment, saved time and increased the efficiency. With the on-line trap column, PAHs were separated availably even underground injecting 1.0 ml sample with relatively high column efficiency. The recoveries of the seven PAHs were from 85% to 120% for spiked atmospheric particulate sample. The limit of detection was 15.3–39.6 ng/L (S/N=3.3). There were good linear correlations between the peak areas and concentrations of the seven kinds of PAHs in the range of 1–50 ng/ml with the correlation coefficients over 0.9970. Furthermore, it also indicated that the method is available to determine PAHs in atmospheric particulates well.

Key words: on-line enrichment; on-line trap column; high performance liquid chromatography (HPLC); atmospheric particulate; polycyclic aromatic hydrocarbons (PAHs)

Introduction

Because atmospheric particulates can absorb many pollutants such as heavy metals, acidity oxide, organic pollutants and virus etc., they are of concern for both environmental and health reasons (Zhang *et al*., 2001; Wilson *et al*., 2001). Polycyclic aromatic hydrocarbons (PAHs) are well-known environmental pollutants owing to their toxicity, carcinogenicity and mutagenicity. And their derivatives with stronger mutagenicity and/or carcinogenicity, such as nitropolycyclic aromatic hydrocarbons (NPAHs), may be formed by reaction of PAHs with O_3 or NO_x in atmosphere (Tang *et al*., 2005). These years, the studies on PAHs in atmospheric particulates have been mainly concerned on their concentration, distribution, derivation and transformation or others. However, the sampling, the sample preparation and analysis method directly decide the results of determining PAHs in above studies. Because of the instability and volatilization of PAHs, the sample preparation of atmospheric particulates is of great importance and also difficult for many researchers (Li *et al*., 2006). The conventional pretreatment methods, such as SPE (solid phase extraction) or Soxhlet extraction,

need large amount of organic solvent, and the repeated sample transfer leading to sample loss and larger analysis deviation. On the other hand, it is difficult to keep off-line sample pretreatment identical, then resulting in bad repeatability. To overcome these limits, we developed a method by on-line high performance liquid chromatography (HPLC) with fluorescence detector using directly injecting and on-line enriching elution of atmospheric particulates to determine seven kinds of PAHs. Our main aim was to simplify the sample pretreatment, to save the time and increase the efficiency (Barnabe and Laurier, 2003; Gigliotti *et al*., 2003).

1 Experimental

1.1 Regents and chemicals

Seven PAH standards shown in Table 1 were purchased from Aldrich Chemical (Milwaukee, WI, USA). Acetonitrile was of HPLC grade from Fisher Company. The HPLC-grade water was obtained by purification of deionized water through a Milli-Q system (Bedford, USA) with a 0.22-μm fiber filter.

1.2 HPLC systems

A schematic flow diagram of the HPLC system used for the determination of PAHs is shown in Fig.1. The system

Table 1 Property of polycyclic aromatic hydrocarbons (PAHs)

PAHs	Molecular formula	Molecular weight	Boiling point (°C)
Fluoranthene	$C_{16}H_{10}$	202	384
Chrysene	$C_{18}H_{12}$	228	448
Benzo[b]fluoranthene	$C_{20}H_{12}$	252	481
Benzo[k]fluoranthene	$C_{20}H_{12}$	252	481
Benzo[a]pyrene	$C_{20}H_{12}$	252	500
Dibenzo[a,h]anthracene	$C_{22}H_{14}$	278	Sublimation
Benzo[g,h,i]perylene	$C_{22}H_{12}$	276	542

consisted of LC-20Avp sample analysis system, on-line enrichment system with trap column, and SIL-HTc auto sampler from Shimadzu Corporation (Kyoto, Japan). The pre-enrichment of samples was performed on Shim-Pack MAYI ODS trap column (10 mm × 4.6 mm) from Shimadzu Corporation. Subsequent separations were carried out on a Shimadzu VP-ODS (150 mm × 4.6 mm) analytical column. PAHs were detected using a fluorescence detector (SPD-10Avp, Shimadzu, Kyoto, Japan) operated at excitation wavelength 293 nm and emission wavelength 450 nm. LC-Solution software (Shimadzu, Kyoto, Japan) was used to acquire and process all chromatographic data.

1.3 Sample preparation

Standard stock solutions of PAHs (0.5 mg/ml) were prepared by dissolving the appropriate amount of crystal of each PAHs in acetonitrile. The stock solutions were further diluted and mixed to obtain working solutions (450, 90, 45, 18, 9, 1.8, 0.9 ng/ml and 1, 5, 10, 25, 50 ng/ml) of PAHs.

Atmospheric particulates were collected on the rooftop of about 30 m building in Research Center for Eco-Environmental Sciences of the Chinese Academy of Sciences by using a Kimmoto Denshi (Osaka, Japan) high-volume air sampler. Particulates were collected on pallflex products (GelmanScience, USA) 2500QAT-UP filter (20.3 cm × 25.4 cm) for 24 h at a flow rate of 700 L/min. Each filter was cut into small pieces and used in the following extraction. The particulates on filters were extracted into 30 ml of acetonitrile three times ultrasonically and centrifuge, then filtered with a 0.45-μm membrane filter. After that, 1.0 ml elution was directly injected into HPLC for detecting PAHs in sample.
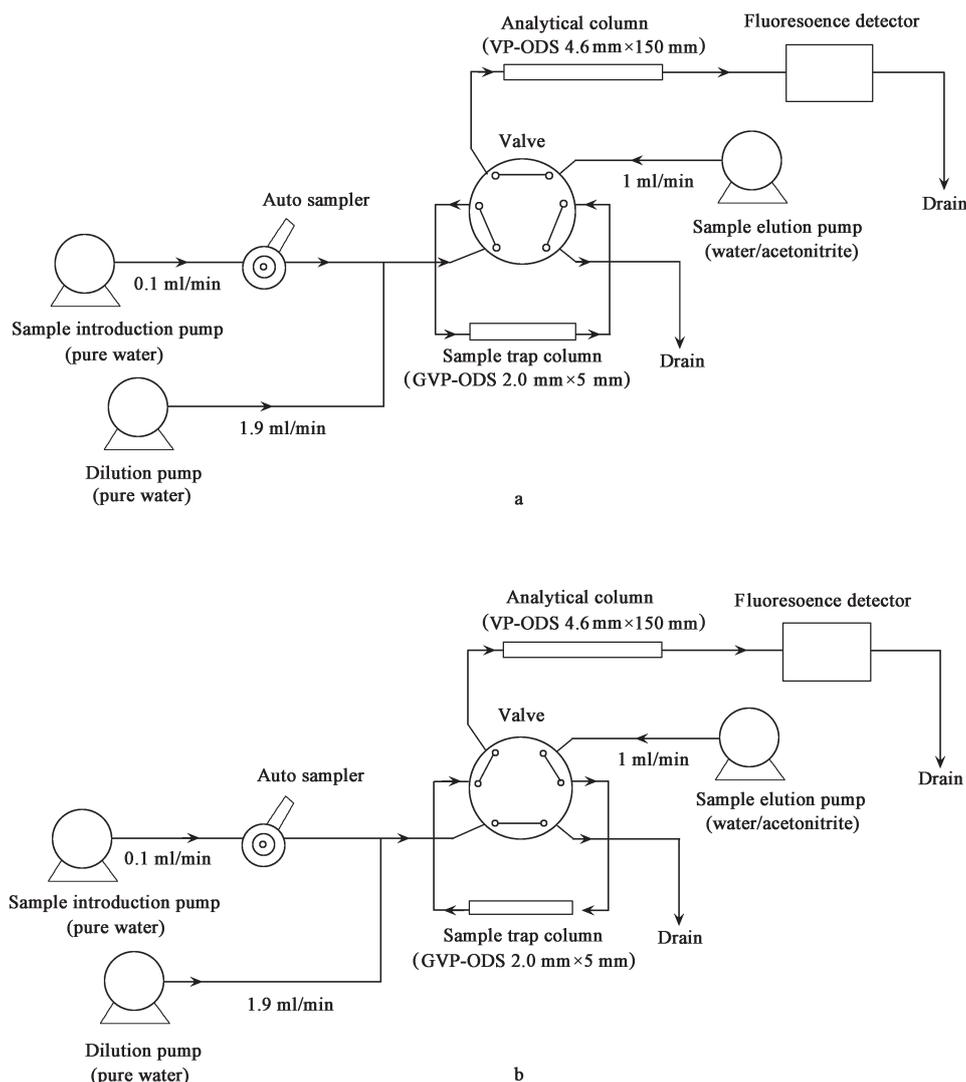

Fig. 1 Flow diagram of PAHs concentration trap (a) and analysis procedure (b).

1.4 Chromatographic conditions and operations

The analytical mobile phase consisting of water and acetonitrile in the ratio of 28:72 (v/v) was continuously passed through the analytical column (oven temperature at 35°C) with flow rate of 1.0 ml/min. At the same time, the sample carrier mobile phase premix of water and acetonitrile (90:10, v/v) at a total flow rate of 0.1 ml/min met the sample dilution mobile phase (water, 1.9 ml/min), and continuously passing through the trap column (oven temperature at 35°C) at a total flow rate of 2.0 ml/min.

After about 1.0 ml of samples was directly injected into the trap column, the sample concentration (Fig.1a) was carried out within about 15 min by pretreatment mobile phase at a total flow rate of 2.0 ml/min. Thereafter, the column switch valve was switched so that the analytical column was on line (Fig.1b) with the trap column and the detector (λ_{ex}=293 nm, λ_{em}=450 nm) underground of the analytical mobile phase flowing at the rate 1.0 ml/min. Later on, the column switch valve was switched again, the trap column was also being washed with pre-treatment mobile phase at a flow rate 2.0 ml/min, followed by equilibration for next injection.

2 Results and discussion

2.1 Determination of PAHs with HPLC using on-line enrichment system

Seven concentration PAHs mixtures with the same component absolute amount, different injection volume were respectively injected into HPLC with and without on-line enrichment system, and the results of theoretical plates were compared. Fig.2 shows the chromatogram of detecting seven kinds of PAHs mixtures by HPLC without on-line enrichment system with the same LC chromatographic conditions, injection volume rang from 2 to 1000 μl. While injection volume ranged from 2 to 50 μl, the peak shapes were fine. However, peak shapes became wider and column efficiency decreased when injection volume reached 100 μl, and then the sample components could not be separated when injection volume reached 1000 μl. Whereas, the same sample of even 1.0 ml injection volume were detected using HPLC with on-line enrichment system, the results obtained were fine and peak shape were symmetrical (Fig.3).
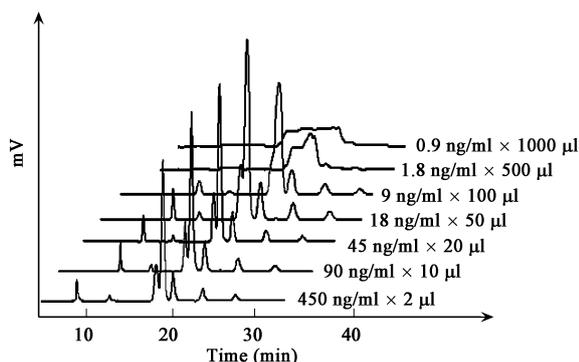
The theoretical plates of the two systems were respectively counted using flouranthene as model sample. As shown in Fig.4, the theoretical plates largely decreases using HPLC without on-line enrichment system while basically keep identical levels using HPLC with on-line enrichment system along with injection volumes increasing. Therefore, large volume injection can be realized by on-line enrichment system.

We thought the reason was as follows. Because PAHs were easy to solve into organic solvent but not water, and when large volume PAHs samples solved in organic solvent were injected into HPLC without on-line enrichment system, large volume organic solvent which eluting ability was strong made components easy to go out column, this led to a bad separation of samples. Therefore, pretreatment or concentration of large volume samples must be carried out before injecting into HPLC. But conventional sample off-line pretreatment procedure usually possessed so questions as complicated operation, large sample loss or needing large amount organic solvent.On-line enrichment system could realize on-line sample concentration by trap column. In Fig.2, we can see that, after large volume sample solvent were injected into carrying sample flow, the water from diluting flow could diluted organic solvent, then the sample were enriched by trap column, and after valve switching, the sample was carried into analytic column and separated. This method finished sample concentration in 15 min, and simplified sample pretreatment (Sonu *et al.*, 2004).

2.2 Analytical figures of merit

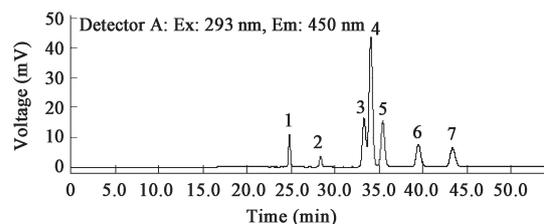The repeatability of method was assessed by five repeat injections of seven kinds PAHs mixed standard solution

Fig. 3 Chromatogram of PAHs using HPLC with enrichment system, injection 1000 μl. (1) fluoranthene; (2) chrysene; (3) benzo[b]fluoranthene; (4) benzo[k]fluoranthene; (5) benzo[a]pyrene; (6) dibenzo[a,h]anthracene; (7) benzo[g,h,i]perylene.

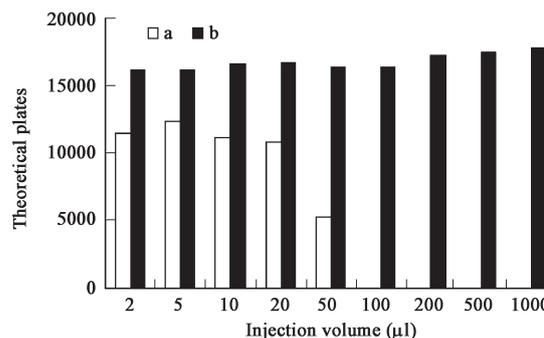Fig. 2 Chromatogram of PAHs using traditional HPLC system.

Fig. 4 Comparison of theoretical plates of PAHs using HPLC without enrichment system (a) and with enrichment system (b).

(10 ng/ml). As shown in Table 2, the relative standard deviation (RSD%) of retention time and area were less than 0.03% and 1.75%, respectively.

Table 2 Repeatability of PAHs (10 ng/ml) detected using enrichment system (*n*=5)

PAHs	RSD% (retention time)	Peak area (RSD%)
Fluoranthene	0.02	1.71
Chrysene	0.02	1.70
Benzo[b]fluoranthene	0.03	1.16
Benzo[k]fluoranthene	0.03	1.64
Benzo[a]pyrene	0.03	1.63
Dibenzo[a,h]anthracene	0.03	1.56
Benzo[g,h,i]perylene	0.03	1.28

Five concentration calibration standards (1, 5, 10, 25 and 50 ng/ml) were prepared for establishing the linearity. As shown in Table 3, the coefficient of correlation was better than 0.997, and there was a good linearity between the response to area and above concentrations. The method detection limit was determined as 15.3–39.6 ng/L in terms of *S/N*=3.3.

The experiment for evaluating recovery was carried out by repeating three times (*n*=3) for detecting three concentration (1, 10, 25 ng/ml) of PAHs added into atmospheric particulates. The recovery values were 85%–120% with RSD of 1.34%–1.71% as presented in Table 4.
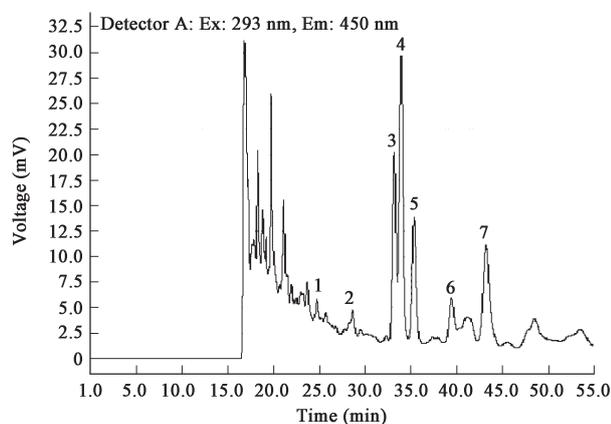
Fig. 5 Chromatogram of detecting PAHs in atmospheric particulates using HPLC with enrichment system. (1) fluoranthene; (2) chrysene; (3) benzo[b]fluoranthene; (4) benzo[k]fluoranthene; (5) benzo[a]pyrene; (6) dibenzo[a,h]anthracene; (7) benzo[g,h,i]perylene.

Table 4 The Recovery of PAHs

PAHs	Recovery (%)		
	1 ng/ml	10 ng/ml	25 ng/ml
Fluoranthene	119.3±1.6	100.4±1.6	97.3±1.6
Chrysene	120.5±1.3	118.7±1.4	102.4±1.7
Benzo[b]fluoranthene	118.3±1.6	93.4±1.5	86.7±1.6
Benzo[k]fluoranthene	114.3±1.7	100.3±1.6	93.2±1.6
Benzo[a]pyrene	112.3±1.7	95.6±1.7	92.1±1.6
Bibenzo[a,h]anthracene	119.8±1.7	107.3±1.7	101.2±1.7
Benzo[g,h,i]perylene	101.3±1.7	95.7±1.7	91.8±1.7

Table 5 Concentration of PAHs in atmospheric particulates and in atmosphere

PAHs	Conc. in atmospheric particulates (µg/g)	Conc. in atmosphere (ng/m^3)
Fluoranthene	0.66	0.17
Chrysene	1.26	0.32
Benzo[b]fluoranthene	2.69	0.68
Benzo[k]fluoranthene	1.34	0.34
Benzo[a]pyrene	2.00	0.50
Dibenzo[a,h]anthracene	1.71	0.43
Benzo[g,h,i]perylene	4.46	1.12

3 Determination of PAHs in atmospheric particulates

Atmospheric particulates sampling and eluting are shown as Section 1.3. The elution solution was subsequent injected into system, concentrated by on-line enrichment system, separated with HPLC and detected using a fluorescence detector. The chromatography of detecting PAHs in atmospheric particulates can be seen in Fig.5 and concentration in Table 5. The concentration of seven PAHs calculated in atmospheric particulates was 0.66–4.46 µg/g. Furthermore, results also indicated that this method is available to determine PAHs in atmospheric particulates.

4 Conclusions

A simple analytical method for the determination of seven kinds of PAHs by on-line HPLC with fluorescence detector using direct injection elution solution of atmospheric particulates has been developed and validated. Direction injection and on-line enrichment were realized by an trap column system named enrichment system. The method simplified pretreatment of sample, saved time and increased the efficiency. With enrichment system, PAHs were separated and analyzed availably even injection 1.0 ml sample with relatively high column efficiency. The recoveries of the seven kinds of PAHs are between 85%

Table 3 The regression equation and linear range

PAHs	Regression equation	Correlation coefficient (*r*^{2})	Detection limit (ng/L)
Fluoranthene	$Y = 1.44 \times 10^{-5} X - 0.571$	0.9974	18.4
Chrysene	$Y = 4.71 \times 10^{-5} X - 1.70$	0.9988	39.6
Benzo[b]fluoranthene	$Y = 6.14 \times 10^{-6} X - 0.566$	0.9976	16.1
Benzo[k]fluoranthene	$Y = 2.58 \times 10^{-6} X - 1.41$	0.9982	6.40
Benzo[a]pyrene	$Y = 6.49 \times 10^{-6} X - 1.17$	0.9983	15.3
Dibenzo[a,h]anthracene	$Y = 1.25 \times 10^{-5} X - 1.33$	0.9975	20.1
Benzo[g,h,i]perylene	$Y = 1.18 \times 10^{-5} X - 1.11$	0.9979	21.4

and 120% for spiked atmospheric particulates sample. The limit of detection is between 15.3–39.6 ng/L (S/N=3.3). There are good linear correlations between the peak areas and concentrations of the seven kinds of PAHs in the range of 1–50 ng/ml with the correlation coefficients over 0.9970. Furthermore, results also indicated that this method is available to determine PAHs in atmospheric particulates and providing a way for realizing on-line quick detecting PAHs in atmospheric particulates.

References

Barnabe N, Laurier P, 2003. Polycyclic aromatic hydrocarbons in the air in the St. Lawrence Basin[J]. Environ Sci Technol, 37: 2094–2099.

Gigliotti C L, Dachs J, Nelson E D *et al*., 2003. Influence of aeration on hydrophobic organic contaminant distribution and diffusive flux in estuarine sediments[J]. Environ Sci Technol, 37: 3547–3554.

Li X H, Ma L L, Liu X F *et al*., 2006. Polycyclic aromatic hydrocarbon in urban soil from Beijing, China[J]. Journal of Environmental Sciences, 18(5): 944–950.

Sonu S S, Manish J, Hiten S *et al*., 2004. Direct injection, column switching-liquid chromatographic technique for the estimation of rabeprazole in bioequivalence study[J]. Chromatogr, B813: 247–254.

Tang N, Hattori T, Taga R *et al*., 2005. Polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in urban air particulates and their relationship to emission sources in the Pan-Japan sea countries[J]. Atmospheric Environment, 39: 5817–5826.

Wilson W E, Chow J C, Claiborn C *et al*., 2001. Monitoring of particulate matter outdoor[J]. Chemosphere, 200(49): 1009–1043.

Zhang G S, Huang R H, Wang M X *et al*., 2001. Great progress in study on aerosol and its impact on the global environment[J]. Progress in Natural Science, 12(6): 407–415.