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SIMPLIFIED ANALYSIS OF BENZO[*a*]PYRENE IN AIRBORNE PARTICULATES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A simple high-performance liquid chromatographic (HPLC) method for the determination of benzo[*a*]pyrene (BaP) in airborne particulates is described. The method involves (i) ultrasonic extraction of hydrocarbons, (ii) clean-up of extracted polynuclear aromatic hydrocarbons (PAHs) by basic alumina and (iii) separation and determination of each PAH by HPLC with a fluorescence detector. The recovery of BaP was 99% and the detection limit (signal-to-noise ratio = 2) was 16 pg. The results were compared with those obtained by another method used for a nationwide survey in Japan, which consists in ultrasonic extraction of PAHs, separation of the PAHs by one-dimensional dual-band thin-layer chromatography and spectrofluorimetric determination. The results obtained by the two methods were in good agreement (r = 0.998) for 20 airborne particulate samples.

INTRODUCTION

Benzo[*a*]pyrene (BaP) is a widespread airborne contaminant with strong carcinogenic and mutagenic activities[1,2]. For this reason, there are many reports concerning BaP determination in airborne particulates[3–14] and liquid chromatographic methods have attracted much attention since the development of efficient detectors, pumps and column packings. Most of the published work on the routine analysis of BaP using high-performance liquid chromatography (HPLC)[15–21] is related to its isolation from interfering organic substances, detection limit and recovery. In this respect, more effective methods for clean-up of the extract solution, separation and detection are desirable for the determination of BaP in environmental samples. In this paper, we describe a simple HPLC method for the determination of BaP in airborne particulates, which includes a method for the clean-up of BaP from inter-

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fering polynuclear aromatic hydrocarbons (PAHs) by ultrasonic extraction using basic alumina. The results obtained by this method were compared with those obtained by the method generally used for the determination of BaP in a nationwide survey in Japan[22].

EXPERIMENTAL

Materials

Standard PAH samples were obtained from Aldrich (Milwaukee, WI, U.S.A.) and Wako (Osaka, Japan). A certain amount of each standard PAH dissolved in acetonitrile was used as a standard solution, and a mixed solution was also prepared. LiChrosorb RP-18 (Merck, Darmstadt, G.F.R.) beads of diameter 5 μm were used as the column packing material. Basic alumina was obtained from Wako. All other chemicals were of analytical-reagent grade.

Apparatus

All chromatographic experiments were carried out using a high-performance liquid chromatograph (Hitachi 638) equipped with a UV monitor (254 nm) and a Hitachi 650-10 LC fluorescence spectrophotometer.

Sample collection

The particulates were collected on a glass-fibre filter (20 × 25 cm, Gelman Type A/E) using a Hi-volume air sampler (Shibata) by continuous suction of air for 24 h. The amount of particulates was measured by weighing the filter before and after the collection of particulates.

Liquid chromatographic conditions

LiChrosorb RP-18 was packed into stainless-steel column (250 × 4.0 mm I.D.) by the slurry packing method. Acetonitrile–water (9:1) degassed by sonication for 10 min was used as the eluent. The separation of PAHs by HPLC was carried out at a flow-rate of 1.0 ml/min and a column temperature of 40°C. The measurement of PAHs was carried out by the fluorescence method at an excitation wavelength of 368 nm and an emission wavelength of 406 nm. Some chromatographic parameters such as retention time and peak area were printed out using a computing integrator with a Hitachi 638 liquid chromatograph.

Extraction and clean-up

A round piece of the glass-fibre filter bearing the collected particulates was cut out with a belt-punch 30 mm in diameter and used for BaP analysis. The PAHs, including BaP, in the sample were extracted with 4 ml of acetonitrile for 15 min at 15°C using the ultrasonic extraction method. Basic alumina (0.5 g) was added to each extract in a test-tube for clean-up of the sample by shaking. The acetonitrile solution was centrifuged for 5 min at 1300 *g*, and the supernatant was used for the determination of BaP by HPLC.

RESULTS AND DISCUSSIONS

Separation and determination by HPLC

It is well known that column temperature, proportions of water and acetonitrile in the eluent and flow-rate affect the retention time and separation of PAH by HPLC. Therefore, we tried to find the optimal conditions for the separation of PAHs in mixed standard samples, especially BaP. There was a linear relationship between the logarithm of the retention time and the reciprocal of the absolute temperature of the column. Further, linear relationships were observed between the carbon numbers of and/or numbers of double bonds in the PAHs and the logarithm of retention time. Fig. 1 shows the chromatographic profile of PAHs obtained (A) with the UV detector at 254 nm and (B) the fluorescence detector using acetonitrile–water (9:1) at a flow-rate of 1.0 ml/min as the mobile phase and a column temperature of 40°C. The nineteen PAHs in the mixed standard samples were detected as seventeen peaks by the UV detector and BaP was completely separated from benzo[*k*]fluoranthene, benzo[*e*]-

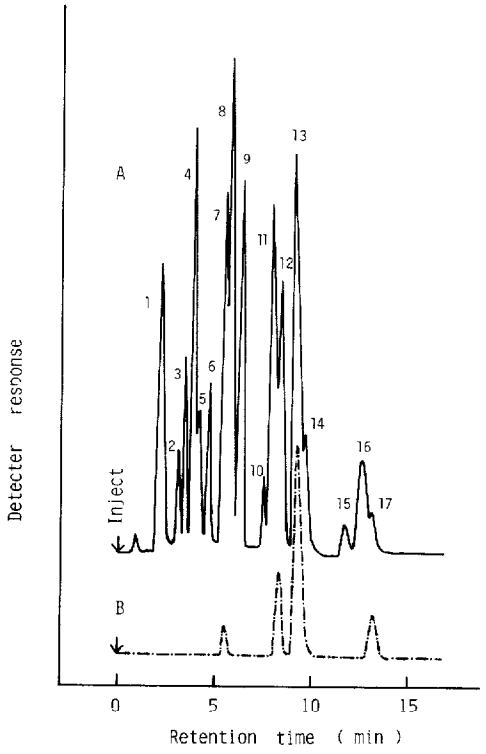

Fig. 1. High-performance liquid chromatograms of a standard aromatic hydrocarbons mixture. Column: LiChrosorb RP-18 (250 × 4.0 mm, I.D.). Mobile phase: acetonitrile–water (9:1). Column temperature: 40°C. Flow-rate: 1.0 ml/min. Detector: (A) UV, 254 nm; (B) fluorescence, excitation 368 nm, emission 406 nm. Peaks: (1) benzene; (2) naphthalene; (3) β-naphthoquinoline; (4) phenanthrene; (5) 2,3-benzofluorene; (6) fluoranthene; (7) pyrene; (8) naphthacene; (9) chrysene; (10) benzo[*b*]chrysene; (11) benzo[*b*]fluoranthene and perylene; (12) benzo[*k*]fluoranthene and benzo[*e*]pyrene; (13) benzo[*a*]pyrene; (14) dibenz-[*a,h*]anthracene; (15) picene; (16) indeno[1,2,3-*cd*]pyrene; (17) benzo[*ghi*]perylene.

pyrene, perylene and benzo[*b*]fluoranthene but not dibenzo[*a,h*]anthracene. As shown in Fig. 1B, nineteen PAHs in the same sample were detected as three peaks by the fluorescence detector. The overlapping peaks of BaP and dibenzo[*a,h*]anthracene obtained with the UV detector, shown in Fig. 1A, were separated to give a single peak of BaP by the fluorescence detector, as shown in Fig. 1B. Consequently, an accurate determination of BaP is easily obtainable by fluorescence measurement (excitation at 368 nm, emission at 406 nm), which is able to eliminate the interference from dibenzo[*a,h*]anthracene in the BaP[23].

Extraction and clean-up

As shown in Table I, BaP in airborne particulates was extracted quantitatively with ethanol–benzene[24] or acetonitrile by means of ultrasonic extraction. This treatment simplifies the extraction of PAHs and both of the above extracts can be used for the subsequent HPLC determination of BaP. The application of more than 10 μl of ethanol–benzene in HPLC has an adverse effect on the BaP determination. However, large amounts (up to 200 μl) of acetonitrile containing BaP can be applied because this solution is easily mixed with the mobile phase used.

TABLE I

COMPARISON OF EXTRACTED BaP USING DIFFERENT SOLVENTS (ETHANOL–BENZENE AND ACETONITRILE)

Sample No.	*Ethanol–benzene (1:3) (ng per 4.9 cm²)* (A)*	*Acetonitrile (ng per 4.9 cm²)* (B)*	*A/B × 100 (%)*
1	70.8	68.0	96.0
2	48.4	48.9	101.0
3	104.0	104.0	100.0
4	34.4	34.0	98.8
5	21.0	20.0	95.2
6	93.6	89.6	95.7
		Mean:	97.8

* 4.9 cm² equals the area of the glass-fibre filter used.

It is well known that HPLC columns are contaminated by the direct injection of ultrasonic extracts of airborne particulate and this causes interference with the BaP determination. Therefore, we tested several methods for eliminating substances that interfere in the determination of PAHs in ultrasonic extracts. Fig. 2 shows the HPLC fluorescence profiles obtained with (A) an ultrasonic extract of airborne particulates, (B) an ultrasonic extract treated with acidic alumina and (C) an ultrasonic extract treated with basic alumina. As shown in Fig. 2C, treatment of the extract with basic alumina eliminate some components that had shorter retention times than BaP. The removal of brownish components from the solution by basic alumina prevents contamination of the column and allows the repeated use of the column for the determination of BaP in many airborne particulate samples.

Identification

The peak of BaP (Fig. 2C, peak 1) was identified by comparing the retention time and fluorescence and/or excitation spectra with those of standard BaP; the re-

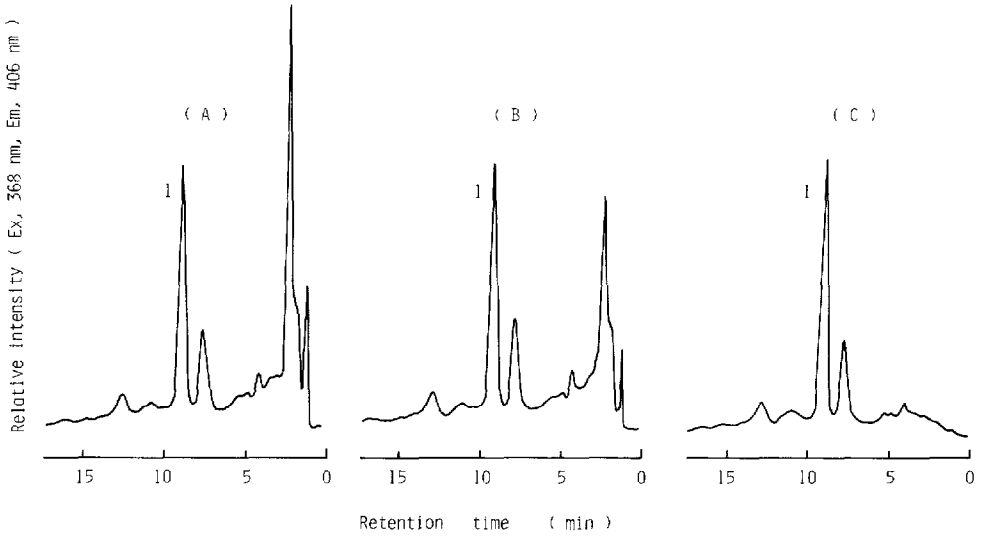

Fig. 2. High-performance liquid chromatograms of a solution obtained by ultrasonic extraction from airborne particulates and its clean-up solution. (A) Solution extracted by ultrasonic method with acetonitrile as solvent; (B) clean-up solution obtained from solution A by acidic alumina treatment; (C) clean-up solution obtained from solution A by basic alumina treatment.

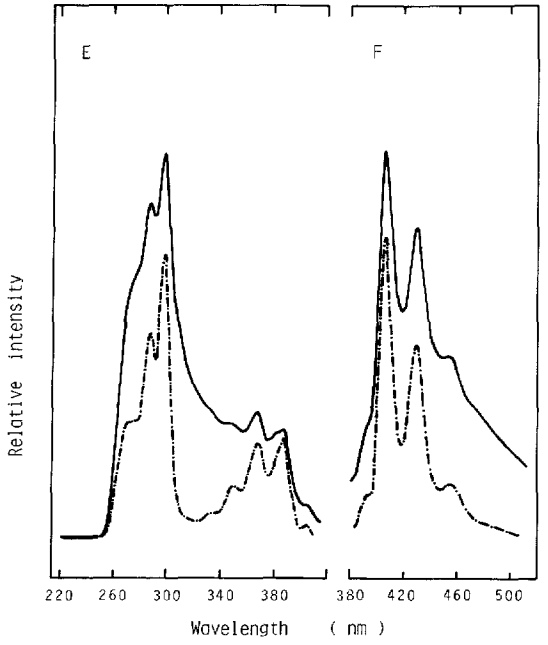

Fig. 3. Fluorescence and excitation spectra of BaP in sample and standard solution. E, Excitation (emission at 430 nm); F, fluorescence (excitation at 370 nm). ———, BaP HPLC fraction (Fig. 2C, peak 1); - · - · -, BaP.

TABLE II

RECOVERY OF BaP FROM AIRBORNE PARTICULATES BY THE PRESENT METHOD

Filter area: 4.90 cm^2.

Sample No.	*BaP added (ng) (A)*	*Sample (ng) (B)*	*Sample + A (ng) (C)*	*A + B*	*Recovery, C/(A + B) × 100 (%)*
1	50.7	48.0	96.8	99.8	97.2
2	203.0	394.0	620.0	597.0	103.8
3	30.4	24.8	52.8	55.3	95.7
4	101.0	112.0	223.6	213.0	104.9
5	152.0	150.0	289.2	302.0	95.8
6	304.0	260.0	549.6	564.0	97.4
				Mean:	99.1

TABLE III

COMPARISON OF BaP IN AIRBORNE PARTICULATES IN THE YAHATA DISTRICT (JAPAN) DETERMINED BY TWO METHODS

Present method: ultrasonic extraction and clean-up by basic alumina; HPLC using fluorescence detection. Previous method: ultrasonic extraction; one-dimensional dual-band thin-layer chromatography; solvent extraction of BaP spot; spectrofluorimetric determination.

Sample No.	*Concentration of BaP (μg per 1000 m^3 air)*		*A/B × 100 (%)*
	Present method (A)	*Previous method (B)*	
1	1.74	1.78	97.8
2	0.81	0.85	95.3
3	0.28	0.28	100.0
4	1.49	1.54	96.8
5	3.74	4.07	91.9
6	1.10	1.05	104.8
7	2.73	2.95	92.5
8	2.26	2.14	105.6
9	0.22	0.24	91.7
10	1.90	1.83	103.7
11	5.80	5.95	97.5
12	1.30	1.28	101.6
13	0.84	0.81	103.7
14	6.28	5.95	105.5
15	9.62	9.65	99.7
16	3.70	3.75	98.7
17	5.11	4.84	105.6
18	0.34	0.35	97.1
19	0.76	0.77	98.7
20	1.37	1.32	103.8
		Mean:	99.6
		C.V.*(%):	4.6

* Coefficient of variation.

tention time of peak 1 in Fig. 2C corresponded with that given by authenic BaP, and the fluorescence and excitation spectra of peak 1 in Fig. 3 are in agreement with those of standard BaP solution.

Recovery

In order to apply the present method to airborne particulate samples, a known amount of BaP was added to ultrasonic extracts of airborne particulate and the recovery of BaP was tested. As shown in Table II, 99% of BaP was recovered by this method.

Reliability

The reliability of the results was examined by comparison with those obtained by a method that consisted in ultrasonic extraction, one-dimensional dual-band thin-layer chromatography, solvent extraction of the BaP fraction, and spectrofluorometric determination[22]. As shown in Table III, the results from 20 airborne particulate samples were in good agreement, with a correlation coefficient of 0.998.

CONCLUSION

The proposed method for the routine determination of BaP in airborne particulates has the following advantages. (i) The procedure for the extraction of PAHs from airborne particulate was shortened and simplified by using acetonitrile as solvent. (ii) The incorporation of acetonitrile in the mobile phase makes possible the determination of BaP in dilute solution. (iii) The use of porous microspherical particles separates the BaP peak completely from that of benzo[*k*]fluoranthene, and BaP is separated within 10 min. (iv) The use of fluorescence detection increases the sensitivity to such an extent that down to 50 pg of BaP can be determined. An accurate determination of BaP is easily obtainable because of the elimination of interferences from other PAHs in the sample solution. (v) A clean-up using basic alumina prevents contamination of the column used for the HPLC analysis of sample solutions and can be used repeatedly for the determination of BaP in many airborne particulate samples.

We conclude that the present method has advantages over preivous methods for the determination of BaP in airborne particulates and should be useful for the routine determination of BaP in air.

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