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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES OF ENVIRONMENTAL CARCINOGENS IN CHINA

X. B. XU* and Z. L. JIN
Institute of Environmental Chemistry, Academia Sinica, Beijing (China)

SUMMARY

A review of high-performance liquid chromatographic (HPLC) studies of environmental carcinogens in China is presented, including the HPLC analysis of the ubiquitous polynuclear aromatic hydrocarbons (PAHs) and of nitro-substituted PAHs and aflatoxins. Some results of applying these methods to air particulates, emissions, smoke from coal combustion and water and to the inhibition of benzo[*a*]pyrene metabolism are reported.

INTRODUCTION

Since the mid-1970s, varies analytical studies of environmental pollutants have been carried out in China in research institutes of the Chinese Academy of Sciences, in research laboratories of universities and monitoring laboratories and stations of the Chinese Environmental Protection Office (now the Ministry of Urban and Rural Construction and Environmental Protection) as a result of the growing concern of the government about the impact of pollution on health and the environment. High-performance liquid chromatographic (HPLC) studies of environmental carcinogens have been an active, although small, part of this research. Different HPLC instruments are now made in China and also various types of stationary phase, such as YWG-CH bonded-phase microparticulate silica gel (ODS type). HPLC columns of high efficiency can be packed in the laboratory and fluorescence and UV detectors are also commercially available. Most of the research on environmental carcinogens has concentrated on various aspects of the ubiquitous polynuclear aromatic hydrocarbons (PAHs).

EXPERIMENTAL

HPLC methods[1–3], for the analysis of 10–18 PAHs have been developed and applied successfully to the study of different kinds of environmental samples, such as surface water, effluents, air particulates, fossil fuels and emissions or smoke produced by fuel combustion. Alternative pre-treatment methods and two different detectors [UV (254 nm) and multi-wavelength fluorescence detectors] have been used.

An SY-01 liquid chromatography with a UV detector[1] (Analytical Instruments

0021-9673/84/$03.00

Factory, Beijing, China) was used. Columns packed in our laboratories with the chemically bonded stationary phase YWG-CH (5 or 10 μm) have an efficiency of higher than 40,000 plates/m. The columns were 15 or 25 cm long and of 4 or 4.6 mm I.D. Depending on the purpose, 75:25, 85:15 or 95:5 methanol–water mixtures were used as mobile phases, with flow rates of 0.5 or 1–1.2 ml/min. Various solvent concentration programmes for gradient elution could be applied as necessary. The column temperature was 40 or 45°C. Other instruments and stationary phases have also been used[2,3].

Both vacuum sublimation (300°C, 10^{-2}–10^{-3} mmHg, 40 min) and ultrasonic extraction have been used in addition to conventional Soxhlet extraction for the pre-treatment of complex environmental particulates. Fig. 1 shows that the results obtained by three different methods are comparatively close. The vacuum sublimation method has been used mainly for analyses of PAHs in atmospheric particulates. The overall recovery may be higher than 84%. With ultrasonic extraction, the rate of recovery after three extractions can be higher than 90%.

For the rapid determination of benzo[*a*]pyrene [B(*a*)P], ultrasonic extracts after filtration could be injected directly into the column, equipped with a fluorescence detector, if necessary. Fluorescence spectra of chromatographic peaks corresponding to B(*a*)P, pyrene, and fluoranthene in the sample could be scanned during stopped-flow conditions and are consistent with those of authentic standards.

On the basis of the fluorescence spectra of individual standard PAHs, a multi-wavelength detection programme[3], in which different wavelengths of excitation and emission were selected in accordance with the respective retention time intervals, was specified. Under the above-mentioned conditions, PAHs can be well separated on the same column, except the chrysene–benz[*a*]anthracene pair and the benzo[*e*]-pyrene and benzo[*k*]fluoranthene isomers. The results obtained (see Table I) show that the detection limits for various PAHs are 2–700 pg. In this method, the error of the analytical results obtained can be less than ±5%.

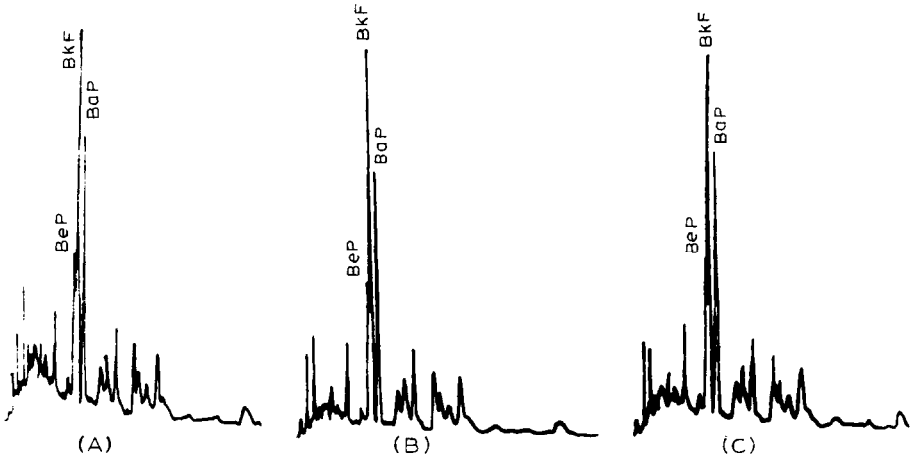

Fig. 1. HPLC traces of an air particulate sample with different methods of extraction: (A) supersonic extraction; (B) vacuum sublimation; (C) Soxhlet extraction. Columns, Zorbax ODS, 25 cm × 4.6 mm I.D., 6 μm; eluent methanol–water (85:15); flow-rate, 1 ml/min; column temperature, 40°C; λ_{ex} = 295 nm; λ_{em} = 427 nm. (From ref. 3.)

TABLE I

RETENTION INDICES, DETECTION LIMITS AND RECOVERIES OF 17 PAHs[3]

Naphthalene and biphenyl, λ_{ex} 265 nm, λ_{em} 330 nm; phenanthrene to benz[*a*]anthracene, λ_{ex} 285 nm, λ_{em} 400 nm; benzo(*e*)pyrene to coronene, λ_{ex} 300 nm, λ_{em} 425 nm.

PAH	*Retention index*	*Recovery (%)*	*Detection limit* $\times 10^{-12}$ *(g)*
Naphthalene	8.9	58.4	230
Biphenyl	9.8	65.4	110
Phenanthrene	11.9	71.8	150
Anthracene	12.3		
Fluoranthene	14.3	80.8	110
Pyrene	15.9	77.9	220
Triphenylene	17.3	72.2	240
Chrysene	17.6		
Benz[*a*]anthracene	18.3	90.5	6
Benzo[*e*]pyrene	24.0		
Benzo[*k*]fluoranthene	24.4	94.1	2
Perylene	24.5		
Benzo[*a*]pyrene	27.4	71.2	8
Dibenz[*a,h*]anthracene	29.9	84.5	10
Benzo[*ghi*]perylene	38.0	80.5	51
20-Methylcholanthrene	42.0	66.2	6
Coronene	60.5	85.2	700

Because the flow-rate is comparatively difficult to control with the SY-01, higher errors result, but with careful control of the flow-rate the analytical error may be about ±10%. However, the difficulty in smoke sampling also gave rise to higher errors.

Two methods for the quantitative analysis of inseparable peaks ($R_s < 1$) in PAH chromatograms were developed. One[4] was based on dual detectors (RI and UV) of the Knauer HPLC system for quantifying the anthracene–phenanthrene pair ($R_s < 0.4$) by solving simultaneous linear equations. The other[5] was based on selecting two different UV wavelengths to analyse two pairs of inseparable peaks with a variable-wavelength UV detector, *i.e.*, 290 and 270 nm for benzo[*a*]anthracene–chrysene and 290 nm and 254 nm for benzo[*e*]pyrene–perylene. The relative deviation of the method was less than ±8%, within the sample injection range of 6–300 ng.

RESULTS AND DISCUSSION

PAHs in air particulates

HPLC methods have been applied to the systematic study[1] of the distribution of 10–17 PAHs in the atmospheric suspended particulates from different areas of the city of Beijing. On the basis of the data collected, we now have a general picture of air pollution by the PAHs monitored in the districts selected and hope that this might serve as a reference for establishing air quality standards in China. Beijing is heavily contaminated by PAHs adsorbed on air particulates. In addition to well known PAH sources such as coke oven emissions and vehicle exhausts, smoke resulting from domestic cooking with coal is a serious pollution problem. Fig. 2 shows two examples

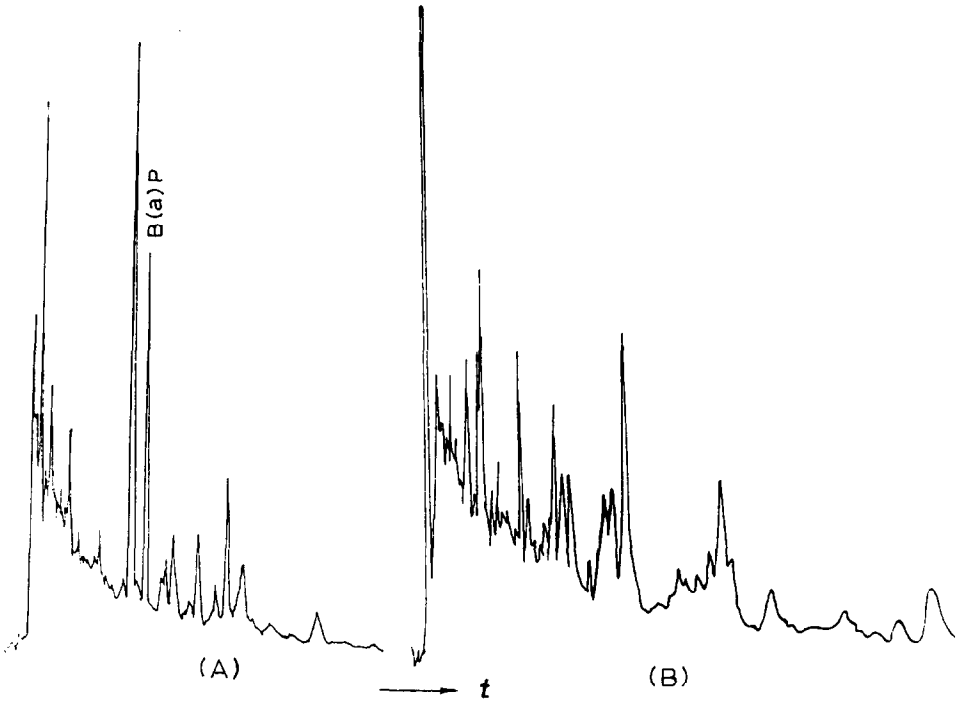

Fig. 2. HPLC traces of air particulate samples: (A) from an urban area; (B) from an electric power station. (From ref. 6.)

of PAH patterns in air particulates from different areas.

The distribution, seasonal variation and diurnal variation of PAHs have been systematically studied[6]. Fig. 3 shows the diurnal variation in B(*a*)P concentration of air particulates collected in Beijing. The maximum concentration occurred at 5–9 a.m., coincident with rush-hour traffic and morning cooking. Some degradation by sunshine and by higher wind velocities might be the causes of the minimum concentration, while the second maximum occurred in the evening, probably corresponding to traffic at the end of the working day and cooking.

In most places, samples collected at noon are richer in PAHs with less than four membered rings than those collected in the morning, while the reverse applies for four- and more-membered compounds.

Energy-related environmental studies

Table II[3] shows that PAHs in coal powders have different absolute and relative concentrations, depending on the source of the samples.

Work has also been carried out on the evaluation of alternative fuels for reducing pollution and saving energy sources. Fig. 4 shows the chromatograms of PAHs in smoke from ordinary honeycomb coal, used by people in Beijing. Fig. 4A shows the components collected on a glass-fibre filter and Fig. 4B shows the components collected by a GDX-101 (60–100 mesh) trap located after the glass-fibre filter.

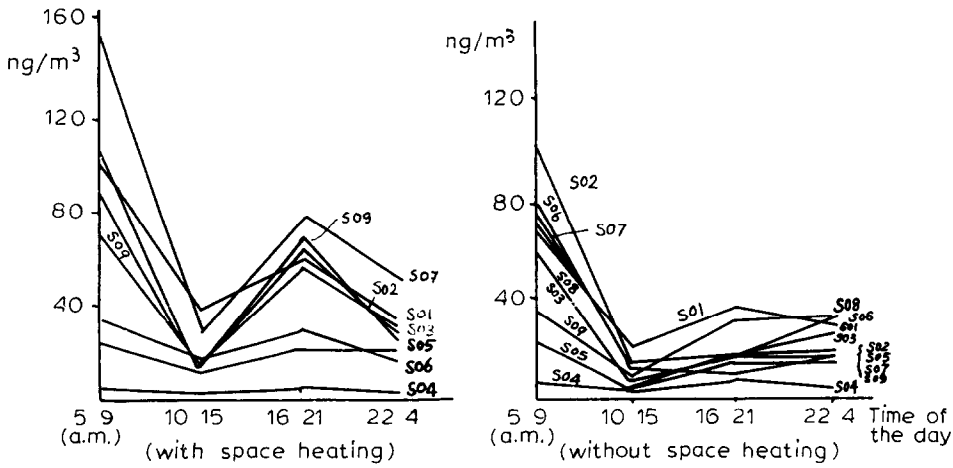

Fig. 3. Diurnal variation of B(*a*)P concentration of air particulates collected in Beijing (different locations). (From ref. 6.)

The variation of the B(*a*)P content in the exhausts from three different models of diesel engines using emulsified oils (diesel oil + water) as fuels under different working conditions was studied. It was found[7] that with the addition of water to diesel oil, one can reduce both the B(*a*)P emission and the consumption of fuel. The emission of B(*a*)P from diesel oil emulsified with 14.5% of water was lower than that from diesel fuel alone, and the stopped-flow spectra confirmed the B(*a*)P peaks.

PAHs in water

PAHs in various kinds of water have been either extracted with cyclohexane[2] or concentrated by Chinese GDX-type (styrene–vinylbenzene copolymer) adsor-

TABLE II

PAH CONCENTRATIONS IN COAL SAMPLES FROM DAITONG, JINGXI AND HUANGSHI

PAH	*Concentration (mg/kg)*		
	Daitong	*Jingxi*	*Huangshi*
Naphthalene	1.43	0.62	2.01
Biphenyl	1.62	0.56	0.66
Phenanthrene + anthracene	3.81	1.48	1.18
Fluoranthene	3.83	1.03	0.96
Pyrene	7.84	1.85	2.18
Triphenylene	5.57	ND*	ND
Benz[*a*]anthracene + chrysene	1.59	0.12	0.02
Benzo[*e*]pyrene + benzo[*k*]fluoranthene + perylene	0.54	0.03	0.01
Benzo[*a*]pyrene	1.23	0.05	0.02
Dibenz[*a,h*]anthracene	ND	ND	ND
Benzo[*ghi*]perylene	2.85	0.06	0.13
20-Methylcholanthrene	ND	ND	ND
Coronene	ND	ND	ND

* ND = not detected.

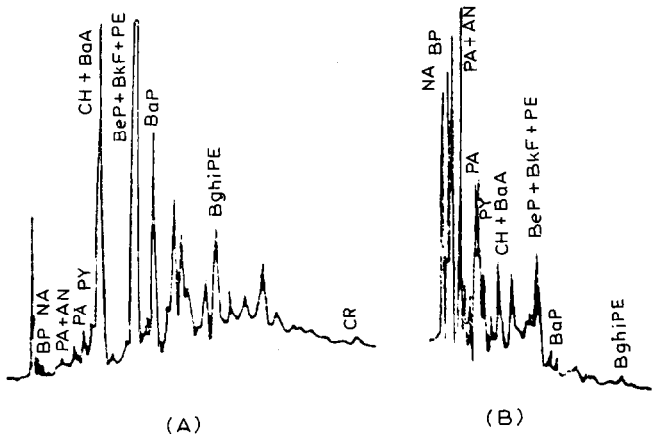

Fig. 4. HPLC traces of PAHs in smoke from ordinary domestic honeycomb coal. (A) Components collected on a glass-fibre filter; (B) components collected by a GDX-101 (60–100 mesh) trap located after the glass-fibre filter. NA = naphthalene; BP = biphenyl; PA = phenanthrene; AN = anthracene; FA = fluoranthene; PY = pyrene; CH = chrysene; BaA = benzo[*a*]anthracene; BeP = benzo[*e*]pyrene; BkP = benzo[*k*]fluoranthene; PE = perylene; BaP = benzo[*a*]pyrene; DBahA = dibenzo[*a,h*]anthracene; B(ghi) PE = benzo[*ghi*]perylene; CR = coronene. (From ref. 3.)

bents[8]. The PAH fractions obtained after cleanup and pre-separation were concentrated and evaporated under suction until just dry. The residues were dissolved in methanol and analysed by HPLC. The recoveries of various PAHs by different adsorbents were compared (Table III), and GDX-104, which gave the highest recovery, was chosen as the adsorbent for water analyses.

Fig. 5 shows examples of HPLC traces of PAHs from tap water, deep well water, surface water, water from a waterfall and from a clean pond.

TABLE III

RECOVERY (%) OF DIFFERENT PAHs WITH GDX ADSORBENTS

PAH	*Adsorbent*				
	GDX-101	*GDX-102*	*GDX-103*	*GDX-104*	*GDX-105*
Phenanthrene	80	61	73	85	78
Anthracene	86	85	79	82	71
Fluoranthene	90	84	71	83	98
Pyrene	91	77	81	88	73
9,10-Benzophenanthrene	74	81	78	99	99
Chrysene	66	73	61	85	71
Benz[*a*]anthracene					
Benzo[*c*]pyrene	77	73	56	80	74
2-Phenylanthracene					
Benzo[*a*]pyrene	68	77	73	94	94
9,10-dimethylbenz[*a*]anthracene	76	73	61	88	86
1,2,5,6-Dibenzanthracene	60	85	78	94	99
Benzo[*ghi*]perylene	76	63	53	79	74
20-Methylcholanthrene	64	47	58	70	85

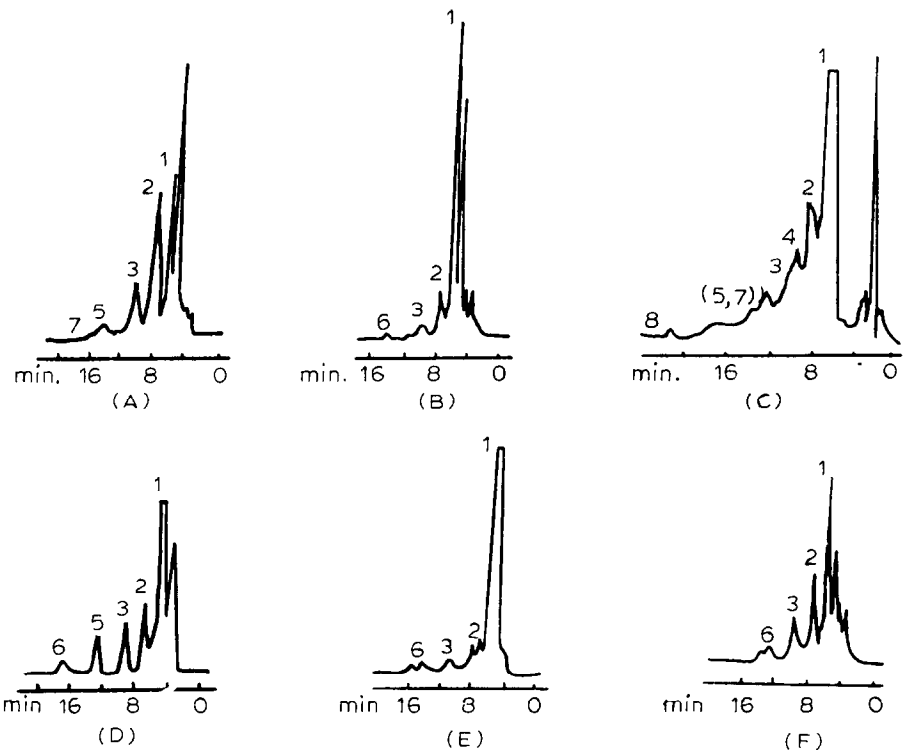

Fig. 5. HPLC traces of PAHs from different sources of water in Changchun City[8]. Column, YWG-CH ODS, 10 μm, 150 × 4 mm I.D.; eluent, methanol–water (77:23); flow-rate, 0.6 ml/min; column temperature, 47°C. (A) Tap water, λ_{ex} 284, λ_{em} 415 nm; (B) tap water, λ_{ex} 250, λ_{em} 365 nm; (C) deep well water, λ_{ex} 270, λ_{em} 392 nm; (D) water from South Lake, λ_{ex} 284, λ_{em} 415 nm; (E) water from Tianchi Pool, Changbai Mountain, λ_{em} 284, λ_{em} 415 nm; (F) water from waterfall, Changbai Mountain, λ_{ex} 250, λ_{em} 365 nm. (1) Phenanthrene + anthracene; (2) fluoranthene + pyrene; (3) benz[*a*]anthracene + chrysene; (4) 9,10-benzophenanthrene; (5) benzo[*e*]pyrene; (6) 2-phenylanthracene; (7) benzo[*a*]pyrene; (8) 20-methylcholanthrene. (From refs. 2 and 8.)

The sensitivity of the method and the detection limit for each PAH are dependent on the wavelengths of excitation and detection. The detection limits of eighteen PAHs using five sets of wavelengths pairs were determined. Under optimal conditions, PAHs down to parts per 10^{12} levels can be analysed.

Studies of inhibition of in vitro B(a)P metabolism

Several workers[9–14] have been actively involved in research on the inhibition of *in vitro* B(*a*)P metabolism (3-MC-induced Wistar rat liver microsomes) by various substances.

By metallic ions[9]. $FeCl_3$, $CoCl_2$, $MnCl_2$, $CuSO_4$ and $ZnSO_4$ (10 μg/ml) were introduced separately into the B(*a*)P metabolism system. It was found that these transition metal ions exert different effects on the metabolism. Zn^{2+} and Cu^{2+} significantly inhibit the formation of diol–B(*a*)P and phenol–B(*a*)P, and Se^{4+} inhibits it to a lesser extent. The results obtained show that the possible mechanism of meta-

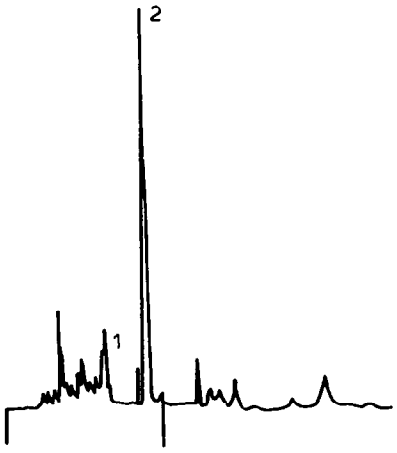

Fig. 6. HPLC trace of an airborne particulate sample from a tunnel. 1, 9-Nitroanthracene; 2, 1-nitropyrene. (From ref. 15).

bolic inhibition involves a significant inhibiting effect of these ions on the activities of microsomal aryl hydrocarbon hydroxylase (AHH) and epoxide hydrase (EH).

By other PAHs[10]. Anthracene (A), pyrene (P), benzanthracene (BA) and dibenz[*a*,*h*]anthracene (DBA) were found to have some inhibiting effect on B(*a*)P metabolism. The possible explanation of the inhibition is competition between B(*a*)P and other PAHs for the same amount of cytochrome P-448, which leads to a reduction in the formation of active metabolites. The results with ten PAHs, two or three as a group, have been presented and discussed[11].

By phenolic or hydroxy compounds[12]. Preliminary results showed that the natural products cucurbitacin B and paenol, the synthetic compound *p*-ethoxyphenol and the vitamin E intermediate 2,3,5-trimethylhydroquinone exert inhibitory effects on B(*a*)P metabolism similar to that of the reference hydroxycinnamic acid in the formation of 7,8-diol–B(*a*)P and 9,10-diol–B(*a*)P.

By naturally occurring quinones[10,13]. The natural quinones usually present in the Chinese traditional herbs used as the antipyretic, antidote, antiseptic and antitumour agents have been examined for their effects on B(*a*)P metabolism. It has been

TABLE IV

RESULTS OF NORMAL-PHASE HPLC ANALYSIS OF AFLATOXINS

Aflatoxin	*Detection limit (ng)*	*Retention time*			*Peak height*		
		Mean (sec)	*Standard deviation (sec)*	*C.V. (%)*	*Mean (mm)*	*Standard deviation (mm)*	*C.V. (%)*
B_1	3.7	340	1.08	0.32	60.1	1.80	3.0
B_2	4.2	372	1.2	0.32	48.4	1.34	2.7
G_1	6.5	439	1.33	0.31	31.8	1.29	4.0
G_2	7.7	491	1.53	0.31	29.6	1.08	3.6

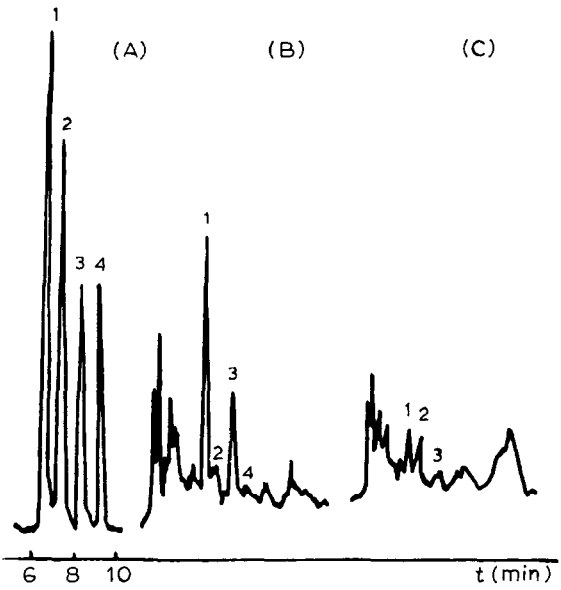

Fig. 7. HPLC traces of aflatoxins. (A) Mixture of standards; (B) peanut powder from Mali; (C) local peanut powder. Column, LiChrosorb Si 60, 25 cm × 4 mm I.D.; mobile phase, chloroform–methanol (100:0.8), 100% saturated with water; flow-rate, 1 ml/min; UV detection (355 nm). 1, Aflatoxin B_1; 2, aflatoxin B_2; 3, aflatoxin G_1; 4, aflatoxin G_2. (From ref. 16).

found that all of the quinones tested exerted inhibitory effects on B(*a*)P monooxygenation (with the addition of 100 μM of quinone the inhibition was in the range 34–70%). Among them, aloe-emodin is the strongest inhibitor; at a 50 μM concentration it reduces 7,8-diol–B(*a*)P and 3-OH–B(*a*)P by 62 and 41%, respectively.

HPLC analysis of nitro-substituted PAHs

As nitro-PAHs have been identified in diesel exhaust particulates as direct mutagens, an HPLC method[15,16] has been developed for the determination of nitro-PAHs in airborne particulate matter. After collection of airborne particulate samples on glass-fibre filters and Soxhlet extraction with dichloromethane, silica gel column chromatography is required for pre-separation. The nitro-PAHs in the moderately polar fraction, eluted with dichloromethane, were converted into amino-PAHs by reduction with hydrochloric acid and zinc powder. For sample clean-up, the reaction mixture was extracted twice with benzene and filtered. After the aqueous reaction solution had been made alkaline, the amino-PAHs were extracted with benzene and determined. The detection limit of 1-aminopyrene at wavelengths of 360 nm (excitation) and 430 nm (emission) was 20 pg. 1-Nitropyrene was detected in airborne particulate matter from a tunnel (1370 pg/m^3) and in an urban area (108 pg/m^3) with an overall recovery of 70 ± 2%. Fig. 6 shows the HPLC trace of an airborne particulate sample from a tunnel.

HPLC analysis of aflatoxins

Analyses of aflatoxins B_1, B_2, G_1 and G_2 have been performed by both nor-

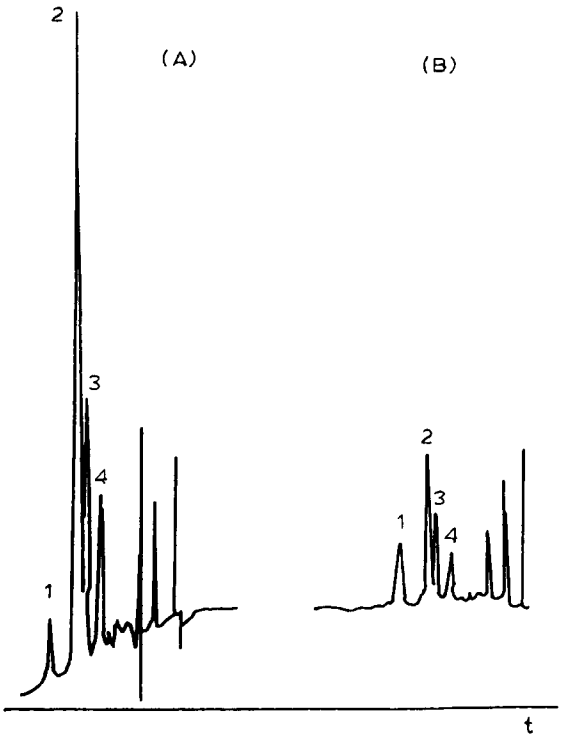

Fig. 8. HPLC traces of aflatoxins (reversed phase): (A) with 365 nm filter; (B) with 254 nm filter. 1, Aflatoxin B_1; 2, aflatoxin B_2; 3, aflatoxin G_1; 4, aflatoxin G_2. (From ref. 17).

mal-phase[17] and reversed-phase[18] HPLC, and different solvents and solvent mixtures have been compared. Chloroform, mixed with a small amount of methanol and then saturated with water, has been found to give the best separation on a LiChrosorb Si 60 column. The effects of changing the wavelength of the UV detector, flow-rate and column temperature have also been studied. Under the optimal conditions the results summarized in Table IV were obtained.

Fig. 7 shows the normal-phase HPLC traces of aflatoxins B_1, B_2, G_1 and G_2 in a mixture of standards and also in peanut powder samples.

Using a μBondpak C_{18} column (reversed-phase HPLC) with methanol–water (40:60) as the mobile phase, the detection limits can be reduced to 3.3, 3.9, 3.5 and 3.2 ng for aflatoxins B_1, B_2, G_1 and G_2, respectively. It has also been found that with a 365 nm filter the sensitivity is much higher than with a 254 nm filter (Fig. 8).

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