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PREPARATION OF ENVIRONMENTAL SAMPLES FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY THIN-LAYER CHROMATOGRAPHY

SALWA K. POOLE, THOMAS A. DEAN and COLIN F. POOLE*
Department of Chemistry, Wayne State University, Detroit, MI 48202 (U.S.A.)

SUMMARY

An evaluation of extraction procedures, liquid–liquid distribution systems, Sep-Pak cartridges, liquid–solid chromatography using silica, alumina and chemically modified silica packings (acid–base treated ethylammonium nitrate and picric acid impregnated), macroreticular resins and gel permeation columns for the analysis of polycyclic aromatic hydrocarbons (CPAHs) in environmental samples by thin-layer chromatography is discussed. For particulate samples solvent extraction using a Soxhlet apparatus or ultrasonication was found to be preferable to sublimation and liquid–liquid distribution between hexane and dimethyl sulfoxide followed by silica gel column chromatography was the preferred method for sample clean-up. Using this procedure enabled six PAHs (anthracene, fluoranthene, benz[*a*]anthracene, perylene, pyrene, and coronene) to be determined quantitatively in urban air particulate, diesel engine exhaust particulate, laboratory ventilator dust, household dust, river water, and tea samples. The PAHs were identified by coincidence of retention between the sample and standards in the same chromatographic system and by adequate agreement with standards for their normalized emission response ratios. The two-point calibration method was used for quantitation. Good agreement for the concentration of PAHs in the air particulate and diesel particulate extracts with published data using gas chromatography–mass spectrometry and high-performance liquid chromatography was found.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. They are formed by the incomplete combustion of organic matter and thus enter the environment from a wide variety of natural and man-made sources. As several PAHs are known or suspect mutagens and carcinogens a considerable amount of effort has been devoted to their analysis in environmental extracts[1–3]. As the PAHs themselves are a complex group of substances generally present at low concentrations in complex sample matrices the determination of individual PAHs or groups of regulated PAHs remains a challenge to the analytical community. Present methods of analysis usually employ high-resolution chromatographic techniques

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combined with relatively sophisticated or lengthy isolation, pre-concentration and group separation schemes to obtain fractions from environmental or biological samples suitable for chromatographic analysis. The most elegant separations of the PAHs have been obtained by gas chromatography on specially deactivated and stabilized thin-film open-tubular columns[4–6]. Even these high-efficiency columns can not completely resolve all PAH isomers found in environmental extracts, and because of volatility restrictions, information about the higher-molecular-weight PAHs is not generally accessible by this technique. The alternative method of analysis to gas chromatography is high-performance liquid chromatography with fluorescence detection[5,7–9]. The principal problem with this method remains the lack of reproducibility of individual reversed-phase columns, the need for a programmable detector to permit all PAHs to be determined with reasonable sensitivity in the same separation, and the rapid deterioration in column performance usually encountered when real environmental samples are analyzed.

Thin-layer chromatography (TLC) has some unique advantages for the separation of PAHs. The amount of sample clean-up required for analysis is reduced. TLC plates are discarded at the end of each analysis so are free from the problem of contamination and time consuming column rejuvination procedures. Multiple samples can be analyzed simultaneously by TLC thus providing a much higher sample throughput than the sequential separations possible with closed bed techniques. Up to 36 samples can be applied to a standard size modern high-performance thin-layer chromatography (HPTLC) plate. Also, TLC being an open bed technique offers certain advantages in detection simplicity. After the development process is complete the sample is essentially static on the plate which can be scanned several times under different detection conditions to provide complementary information about the sample composition or to optimize detection conditions for individual components.

Conventional TLC techniques have been evaluated for the detection of PAHs and the literature consisting of over 200 papers has been comprehensively reviewed[1,2,10,11]. In many of these studies PAHs were separated on alumina or silica gel, the spots or zones corresponding to expected PAHs excised, and the concentration of individual PAHs determined by elution and solution fluorometry. This process is time consuming and plagued by unstandardized operator errors. Thus, it is not a useful approach to the problem under consideration. The selectivity of silica gel for the separation of PAHs is poor and superior results were obtained using a mixture of alumina–acetylated cellulose sorbent or by using cellulose which had been partially acetylated. Partially acetylated cellulose plates became widely used for PAH separations using either normal[12,13] or reversed-phase[14,15] solvent systems. However, even under optimum conditions, many commonly encountered PAHs are not separated. In the last few years a considerable change in the practice of TLC generally known as high-performance thin-layer chromatography (HPTLC), has occurred. This change was spurred by the introduction of new sorbents, sample application devices, new development techniques, and the availability of scanning densitomers for accurate and precise in situ quantitation[16–21]. The result of these improvements in technique and materials has been to improve the separating power, reduce the analysis time, and to increase the sensitivity and reliability of quantitative measurements by HPTLC compared to conventional TLC.

Several studies have been made of the separation of PAH mixtures using dif-

ferent sorbents and mobile phases in HPTLC.[22–27] The selectivity of silica gel, cellulose, 3-aminopropylsilanized silica gel, and 3-cyanopropylsilanized silica gel was inadequate for resolving complex PAH mixtures. Furthermore, extensive quenching of the fluorescence signal of PAHs on silica gel layers was observed[26,27]. Among reversed-phase sorbents octadecylsilanized silica provided much better resolution of the PAHs than ethyl-, octyl-, and diphenylsilanized silica plates[22]. The superiority of multiple solvent development, taking advantage of the spot reconcentration mechanism, over manual and continuous development for the separation of PAHs by reversed-phase HPTLC has also been demonstrated[22–28]. Under optimized separation conditions poor resolution of perylene, benzo[*a*]pyrene, benzo[*e*]pyrene and the three benzofluoranthene isomers and the co-migration of chrysene and benz[*a*]anthracene remains a problem for the analysis of all environmentally important PAHs by reversed-phase HPTLC. Other PAHs of interest incompletely resolved by reversed-phase HPTLC can be determined selectively using sequential wavelength scanning with different combinations of excitation and emission wavelengths[28,29]. Complementary separation characteristics of acetylated cellulose HPTLC plates to those found for octadecylsilanized silica gel have been demonstrated, particularly for the separation of benzo[*a*]pyrene, benzo[*e*]pyrene, and perylene and chrysene and benz-[*a*]anthracene[22]. Unfortunately, these plates are not available commercially and are difficult to prepare reproducibly in the laboratory.

Certain features of scanning densitometry have been optimized for the determination of PAHs in complex mixtures. These include optimization of instrumental parameters to maximize sample detectability without compromising resolution[19,20,30,31], the standardization of a protocol for calculating normalized emission response ratios used for qualitative sample identification[28,29], and the development of a single standard method, the two-point calibration method, for sample quantitation[28,32]. The two-point calibration method enables multiple PAHs to be quantified in a single sample using one track for all calibration standards.

In this paper we will evaluate the relationship between identification and quantitation of PAHs in environmental samples and the need for sample clean-up. For sample screening a method requiring minimal sample preparation is desirable. In this respect TLC has the inherent advantage that the separation medium is used only once per analysis so can be loaded with crude sample without regard to the affects that strongly sorbed sample co-extractants might have on the analysis of subsequent samples. However, as we will demonstrate, for very complex mixtures some sample clean-up is always required if reliable quantitation is to be obtained, but in keeping with the desire of exploiting HPTLC for rapid sample screening, the extent of sample clean-up required is less rigorous than is needed to analyze the same samples by modern gas and liquid chromatography.

EXPERIMENTAL

Anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*g,h,i*]-perylene, chrysene, coronene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, perylene, phenanthrene, pyrene, triphenylene, dimethyl sulfoxide, dimethylformamide, and nitromethane were obtained from Aldrich (Milwaukee, WI, U.S.A.). Benzo-[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-

c,d]pyrene were obtained from the Community Bureau of Reference (Brussels, Belgium) and dibenzo[*a,i*]pyrene and Sephadex LH-20 from Sigma (St. Louis, MO, U.S.A.). Bio-Beads SX-2 were obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.), Amberlite XAD-2 from Rohm and Haas (Philadelphia, PA, U.S.A.), alumina (80–200) mesh) from Fisher Scientific (Fair Lawn, NJ, U.S.A.), and silica gel (60–200 mesh), Sep-Pak C_{18} cartridges and picric acid from J.T. Baker (Phillipsburg, NJ, U.S.A.). Ethylammonium nitrate was prepared as described previously.[33] Reversed-phase KC_{18} HPTLC plates were obtained from Whatman (Clifton, NJ, U.S.A.). Unless otherwise stated all other solvents and chemicals were analytical grade in the highest purity available.

The urban air particulate extracts, the diesel engine exhaust particulate extract, shale oil (NBS 1580) and river sediment sample (NBS 1645) were obtained from the National Bureau of Standards (Washington, DC, U.S.A.). The particulate extracts were supplied as solutions obtained by Soxhlet extraction of the respective samples for 48 h with dichloromethane. The laboratory dust sample was scrapped from the air vents providing conditioned air to the laboratory. The house dust sample was obtained by normal vacuum cleaning of several residences in urban Detroit. The dust samples were sieved through a 80-mesh sieve to eliminate debris, etc. Water samples were obtained in 2-gallon solvent bottles from the St. Claire River (Saint Clair Shores, MI, U.S.A.). Tea samples were obtained from a local supermarket (Detroit, MI, U.S.A).

Extraction of particulate samples

The dust samples were extracted in the dark using a Soxhlet extractor under the following conditions. A laboratory dust sample (27.23 g) was extracted with 100 ml of toluene, and 89.23 g of household dust with 300 ml of toluene. The extraction time was 28 h at a rate of approximately 5 cycles per h. The toluene soluble extract was reduced to a residue using initially room temperature rotary evaporation and subsequently a gentle stream of nitrogen. The organic soluble extracts weighed 2.25 g for the laboratory dust sample and 6.53 g for the household dust sample. These residues were redissolved in hexane to prepare stock solutions containing 1 mg/ml of organic extract.

For ultrasonic extraction 5 g of household dust sample and 1 g of sediment sample were extracted two times in an ultrasonic bath (Bransonic, Shelton, CT, U.S.A.) at room temperature for 15 min using 25 ml and 5 ml of acetonitrile for each extraction, respectively. After centrifugation the solvent was gently evaporated to a residue and redissolved in 1 ml of hexane.

Vacuum sublimation was performed in a standard sublimation apparatus (Ace Glass, Vineland, NJ, U.S.A.) by heating 3 g of household dust sample to 300°C at a reduced pressure of 0.1 Torr. After sublimation for 90 min. the sublimate was collected as a pale yellow slick that covered 30–40% of the bulb. The sample was transferred to a 5-ml sample vial with a conical tip using 3 ml of cyclohexane–acetone (2:1). The solvent was evaporated just to dryness by a gentle stream of nitrogen and redissolved in 1.0 ml of hexane.

Fractionation by liquid–liquid distribution

Crude organic extracts were fractionated by acid–base extraction[34] or by

liquid–liquid distribution between dimethyl sulfoxide and hexane[35], between dimethylformamide and cyclohexane[36], or between nitromethane and cyclohexane[37].

For acid–base extraction 1.0 ml of dust extract was evaporated to a residue with a gentle stream of nitrogen and redissolved in 1.0 ml of dichloromethane. The dichloromethane solution was extracted successively with 1.0 ml of 5% (w/v) aqueous sodium bicarbonate, 1.0 ml of 1.0 *M* sodium hydroxide, 1.0 ml of 1.0 *M* hydrochloric acid, and finally 1.0 ml of distilled water. After extraction the dichloromethane residue weighed 0.9 mg.

For extraction with dimethyl sulfoxide 1.0 ml of extract, in hexane, containing 1.0 mg of residue was partitioned three times with an equal volume of dimethyl sulfoxide. The dimethyl sulfoxide layers were combined and diluted with two volumes of distilled water. The PAHs were then back extracted three times with an equal volume of hexane. The final solution was washed once with an equal volume of water to remove traces of dimethyl sulfoxide and then concentrated at room temperature on the rotary evaporator to 1.0 ml, then just to dryness by a stream of nitrogen. The residue was redissolved in 1.0 ml of hexane.

For extraction with dimethylformamide 1.0 ml of extract containing 1.0 mg of residue was evaporated just to dryness and redissolved in 1.0 ml of cyclohexane. The cyclohexane solution was extracted with two volumes of dimethylformamide–water (9:1). The PAHs in the dimethylformamide–water phase were back extracted into cyclohexane after dilution with water in the system dimethylformamide–water–cyclohexane (1:1:2). The cyclohexane layer was evaporated just to a residue and redissolved in 1.0 ml of hexane.

For extraction with nitromethane 1 ml of extract containing 1 mg of residue was reduced just to dryness, redissolved in 1.0 ml of cyclohexane and extracted with an equal volume of methanol–water (2:1). The cyclohexane layer was then further extracted, five times, with an equal volume of nitromethane. The nitromethane extracts were combined, evaporated to a residue, and redissolved in 1.0 ml of hexane.

The river water sample, divided into 1 l aliquots, was extracted three times with 60 ml of dichloromethane. The dichloromethane layers were combined, reduced to 1.0 ml by a rotary evaporator at room temperature, and then just to dryness with a stream of nitrogen. The residue was dissolved in 1.0 ml of hexane and cleaned-up by dimethyl sulfoxide extraction followed by silica gel column chromatography.

The tea sample, 1.0 g, was saponified by refluxing for 6 h in 25 ml of 2 *M* aqueous methanol (methanol–water, 9:1) and, after cooling to room temperature, extracted two times with 5.0 ml of hexane. The hexane layers were combined, washed with distilled water, and finally evaporated just to dryness. The residue was redissolved in 1.0 ml of hexane and then further cleaned-up by dimethyl sulfoxide extraction followed by silica gel column chromatography.

Fractionation by column chromatography

Sep-Pak C_{18} cartridges, 5.0 ml, were flushed with two 5 ml volumes of acetonitrile–water (70:30) before application of the sample, 100 µl of extract in acetone, followed by elution with acetonitrile–water (70:30). The PAHs were eluted in the 2–5 ml fraction.

Columns containing Amberlite XAD-2 were slurry packed in toluene to give a bed height of 12 cm and a diameter of 1 cm. The column was washed with successive

volumes of 10 ml of toluene, 10 ml of ethanol, and 15 ml of methanol. The column was loaded with 100 μl of the hexane phase from the dimethyl sulfoxide extract and eluted successively with 20 ml of methanol; 10 ml ethanol, 10 ml hexane, and 10 ml ethanol; and finally 10 ml of toluene and 10 ml of ethanol.[38–40] The PAHs are concentrated in the third fraction eluted with toluene followed by ethanol.

For gel permeation chromatography Bio-beads SX-2 swollen overnight in tetrahydrofuran and Sephadex LH-20 swollen in 2-propanol were used. Sufficient slurry was used to provide a packed bed height of 12 cm in a column of 1 cm diameter. Each column was loaded with 100 μl of the hexane reconstituted extract after sample fractionation using dimethyl sulfoxide–hexane partition. The PAHs were eluted in the 1–4 ml fraction from the Bio-beads column and between 12 and 35 ml from the Sephadex column.

For adsorption chromatography columns packed with silica, alumina, or chemically modified silica were used. Silica gel was cleaned prior to use by Soxhlet extraction with dichloromethne for 4 h. Alumina was heated at 450°C overnight and allowed to cool to room temperature in an evacuated desiccator. Adsorbent columns were slurry packed in the first solvent used for elution to give a bed of 12 cm × 1 cm. Each column was loaded with 100 μl of the reconstituted hexane phase obtained after partitioning with dimethyl sulfoxide. Silica gel columns were eluted with 80 ml of cyclohexane. The PAHs were obtained in the second fraction, 6–80 ml. For the chemically modified silica gel columns the eluting solvent was hexane–dichloromethane (3:1). The PAHs eluted in different fractions depending on the column treatment as indicated in the results and discussion section. The alumina column was eluted with 50 ml of hexane, 75 ml of hexane–diethyl ether (95:5), and 100 ml of hexane–diethyl ether (72:28). The PAHs were eluted in the third fraction. For analysis by reversed-phase HPTLC the fraction containing PAHs was reduced to 1 ml using a rotary evaporator at room temperature, evaporated just to dryness with a stream of nitrogen, and redissolved in 1 ml of acetone.

Silica gel impregnated with ethylammonium nitrate (15%, w/w) was prepared by adding a methanol solution of ethylammonium nitrate to silica gel and removing excess solvent on the rotary evaporator. A similar technique was used to prepare silica gel impregnated with sodium hydroxide (10%, w/w), hydrochloric acid (10%, w/w), and picric acid (10%, w/w). The picric acid impregnated column was eluted several times with hexane–dichloromethane (3:1) until the eluent was colorless. For the picric acid impregnated column the bottom 3 cm portion of the bed was packed with unmodified silica gel. A mixed column bed was prepared from successive aliquots of 2 g of unmodified silica gel, 2 g of silica gel impregnated with sodium hydroxide, and 2 g of silica gel impregnated with hydrochloric acid.

Environmental extracts in acetone (200 μl) were applied to reversed-phase HPTLC plates using a contact spotting device (Transpot, Clarke Analytical Industries, Santa Clare, CA, U.S.A.). HPTLC plates were developed in position four of a short-bed continuous development chamber (Regis Chemical, Morton Gove, IL, U.S.A.). Samples were separated by multiple development using the sequence 9, 10, 11, 12 and 13-min developments with the mobile phase acetonitrile–methanol–water (1:5:1).

In situ sample detection was performed with a CS-910 scanning densitometer (Shimadzu, Columbia, MD, U.S.A.) fitted with a 100 W high-pressure mercury dis-

charge source for excitation at 254, 266, 313, and 365 nm. Interference filters were used to isolate the fluorescence emission signal from the excitation background. The peak maximum transmittance of the 400-, 450-, 500-, 550-, and 600-nm interference filters is 15, 33, 32, 42, and 43%, respectively. The auto feedback control for the photomultiplier tube (PMT) voltage was disabled and the PMT voltage set independently using the "H.V." control and a digital multimeter wired into a diagnostic socket[29,41]. The PMT was operated at − 650 V. All separations were scanned with a slit width of 1.0 mm, a slit height of 8.0 mm and a scan rate of 24 or 48 mm min^{-1}; conditions previously determined to maximize sensitivity without degrading resolution[20,30]. Peak profiles were recordedf on a U-135 strip chart recorder (Shimadzu) and peak areas with an SP-4100 computing integrator (Spectra-Physics).

PAHs in environmental extracts were identified by the coincidence of retention with standards run on the same plate and by adequate agreement between the normalized emission response ratios for the standards and extracts[28,29]. The emission response ratios were standardized from plate to plate using perylene as a reference compound according to eqn. 1.

$$\mathrm{ERR(ex,em)} = 100\,\frac{[\mathrm{PAH(ex,em)}]/[\mathrm{ng}]}{[\mathrm{Per(ex,em)}]/[\mathrm{ng}^*]} \tag{1}$$

Where ERR(ex,em) = emmission response ratio at a given excitation and emission wavelength; PAH(ex,em) = peak area of the test compound at the given excitation and emission wavelength; Per(ex,em) = peak area for perylene at the given excitation and reference emission wavelength; ng = nanograms of PAH and ng* = nanograms of perylene.

The two-point calibration method was used for sample quantitation[28,32]. Each sample track was scanned twice with a slit width setting of 0.8 and 0.4 mm. The slope of the sample response *vs.* slit width curve was then ratioed to a single standard of known amount. The amount of unknown PAH in the extract was calculated using eqn. 2.

$$C_u = \frac{C_s M_u}{M_s} \tag{2}$$

where C_u = amount of unknown PAH; C_s = amount of the standard; M_u = slope of the response *vs.* slit width curve for the unknown; M_s = slope of the response *vs.* slit width curve for the standard.

RESULTS AND DISCUSSION

To render possible the determination of low levels of biologically important PAHs in environmental samples some form of sample clean-up is invariably required. Normally the PAHs are present in low concentrations among materials spanning a wide polarity range that interfere in their chromatographic determination by causing detector saturation, loss of separation reproducibility, and contamination or fouling of columns. These processes represent a loss of analytical information with an increase in cost and time associated with re-establishing the optimum chromatographic

operating conditions. One perceived advantage of TLC for the analysis of environmental extracts is its ability to tolerate crude extracts that are unsuitable for analysis by column techniques. This arises because the separation medium, the plate, is used only once and then discarded. Thus, the separation medium is not conditioned by the problems encountered in analyzing the previous sample as in the case for closed-bed or column systems. TLC should thus be useful for the rapid screening of environmental extracts by minimizing the need for sample clean-up associated with an individual analysis as well as taking advantage of the high sample throughput that is possible in TLC due to its ability to separate several samples simultaneously. To establish these goals we have investigated the integration of several sample preparation procedures for environmental samples with a previously developed TLC method for the optimized separation of PAHs[22,28].

The bulk of most particulate environmental samples is comprised of inorganic and carbonaceous material that must be separated from the soluble organic portion prior to chromatographic analysis. This is generally achieved by solvent extraction using a Soxhlet apparatus[42–46], ultrasonic agitation[15,46–50], or alternatively, by using sublimation[13,51,52]. The extraction yield of PAHs by sublimation is dependent on the vapor pressure of the PAHs at the temperature and pressure used for the extraction and the binding forces between the PAHs and the matrix. Components of low vapor pressure require a substantially longer sublimation time to yield constant recoveries. However, with prolonged extraction times, a considerable loss of the more volatile PAHs occurs. These losses can be controlled using a two-step sublimation, but the considerable variation in the recovery of spiked samples with changes in sample size or sample type make the procedure less attractive. Sublimation was employed originally in the hope that it would provide a more selective extraction procedure than solvent extraction but a comparison of the particulate extracts by TLC showed similar profiles. Soxhlet and ultrasonic solvent extraction procedures were simpler to optimize and showed less variability than sublimation. For Soxhlet extraction the extractor should be shielded from light to avoid photodecomposition of the PAHs by wrapping with aliminum foil. Using an extraction time of 8 h with either dichloromethane, toluene, or toluene–ethanol (1:2) was sufficient to recover greater than 90% of PAHs from spiked particulate samples. Toluene was selected for final use as it tended to co-extract a smaller mass of the more polar organic and inorganic matrix components. Soxhlet extraction was also used to prepare standard extracts on a relatively large scale, grams of extract, from particulate samples for competitive testing of the effectiveness of additional sample fractionation schemes.

Ultrasonic extraction is suitable for the rapid preparation of small scale extracts. Different solvents were examined to optimize the extraction of PAHs from particulate samples. The recovery of spiked samples with hexane, toluene, and dimethyl sulfoxide was relatively low; dichloromethane gave good recoveries of spiked samples but frequently produced suspensions that were difficult to break by prolonged centrifugation; acetonitrile was the most efficient solvent for particulate and sediment samples giving greater than 95% recovery of spiked PAHs in a two-fold extraction requiring about 30 min. These results are in broad agreement with a recent detailed study of extraction techniques for PAHs in particulate samples by Junk and Richard[44]. We should also mention the need for care in reducing the size of solvent extracts by common laboratory procedures such as rotary evaporation and the gas

blowdown method. Spurious results can be otained if losses due to spluttering and vaporization of volatile PAHs are not avoided by using low temperatures and slow evaporation rates. A detailed account of reasonable precautions for solvent removal, followed in these studies, is given in reference[53].

The crude organic extracts obtained by solvent extraction are too complex to provide all but the most rudimentary qualitative information of the presence of a few PAHs when analyzed by TLC. Some actual examples wil be presented shortly. For quantitative analysis further sample fractionation is needed. The simplest and fastest approach to isolating a PAH enriched fraction for direct application to a TLC plate is liquid–liquid distribution. For this purpose the following distribution systems were investigated: cyclohexane–nitromethane, cyclohexane–dimethylformamide, hexane–dimethyl sulfoxide, and an acid–base fractionation scheme. Removal of water soluble acidic and basic substances from the organic soluble extract did little to reduce the interferences observed on analysis by TLC (Fig. 1). Superior results were obtained using solvents that can selectively isolate PAHs by formation of reversible charge-transfer complexes. Of these systems cyclohexane–nitromethane was considered the least useful because: (i) five distributions were necessary to completely extract the PAHs from cyclohexane, (ii) impurities in nitromethane interfered in the determination of PAHs, (iii) a substantial loss of PAHs through volatilization and/or thermal degradation occurred at the solvent removal step (recoveries varied between 10% for low-molecular-weight PAHs to 80% for the higher-molecular-weight homologues), and (iv) traces of nitromethane not eliminated prior to spotting the extract on the plate caused quenching of the fluorescence intensity of some PAHs. None of these inconveniences were observed for the dimethylformamide and dimethyl sulfoxide solvent systems. As preliminary studies using the household dust extract and the urban air particulate extract indicated similar effectiveness in sample clean-up the

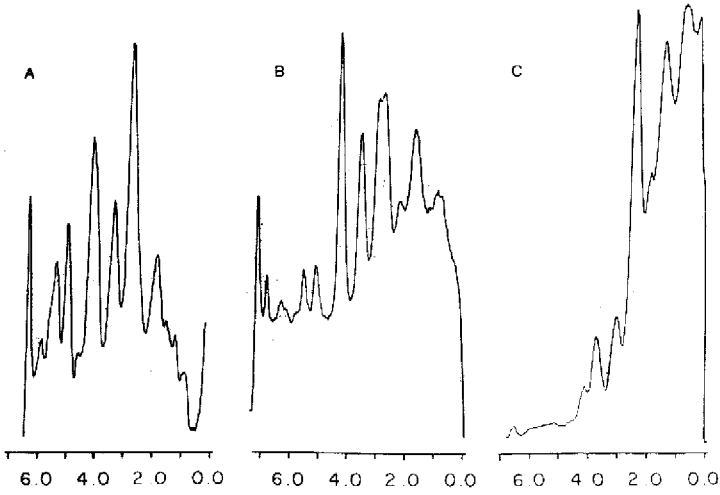

Fig. 1. Comparison of liquid–liquid distribution procedures for the clean-up of a household dust extract for TLC analysis. (A) Hexane–dimethyl sulfoxide; (B) cyclohexane–nitromethane; (C) acid–base extraction.

TABLE I

RECOVERY OF PAHs IN THE LIQUID–LIQUID DISTRIBUTION SYSTEM HEXANE–DIMETHYL SULFOXIDE

PAH	*Recovery (%) (5-μg level)*					
Recovery of standard substances (n = 10)						
Anthracene	95 ± 1.5					
Fluoranthene	95 ± 1.5					
Benz[*a*]anthracene	92 ± 1.2					
Perylene	91 ± 2.5					
Pyrene	94 ± 2.1					
Coronene	94 ± 0.6					
	Amount of standard added (μg)					
	1	*3*	*5*	*10*	*20*	*40*
Recovery of spiked household dust sample						
Anthracene	90			89	82	80
Fluoranthene	89	94		86	82	78
Benz[*a*]anthracene	96	91	91	86	85	
Coronene	95	92	93	85	83	

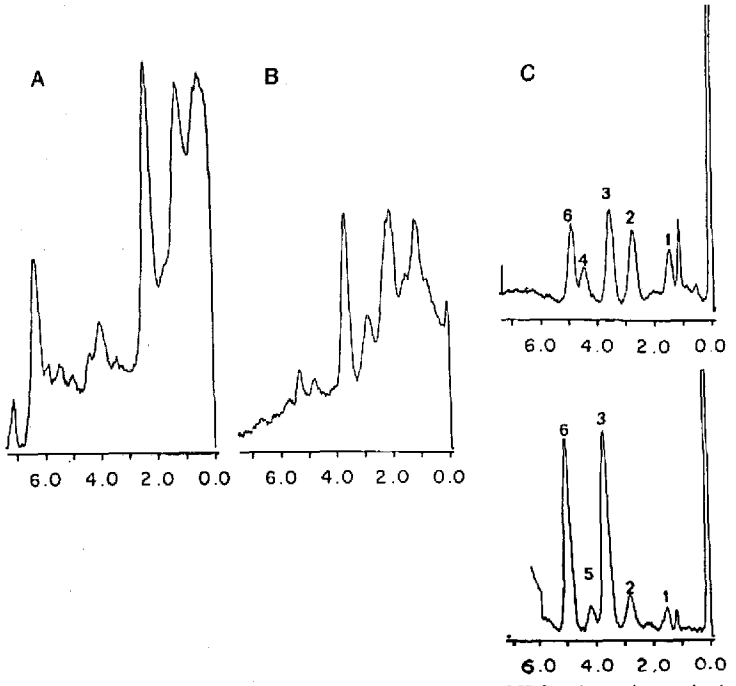

Fig. 2. Extraction and determination of PAHs in an NBS urban air particulate extract. (A) Sample obtained by Soxhlet extraction; (B) Soxhlet extract after clean-up by distribution between hexane and dimethyl sulfoxide; (C) extract B after further clean-up by silica gel column chromatography. Excitation wavelength = 313 nm and emission = 450 nm (top) and 400 nm (bottom).
Peak identification: 1 = coronene, 2 = benzofluoranthene isomers, 3 = benz[*a*]anthracene, 4 = pyrene, 5 = fluoranthene, 6 = anthracene.

hexane–dimethyl sulfoxide system was adopted in all subsequent studies. Following the protocol detailed in the experimental section better than 90% of PAH standards were recovered in the final hexane extract and generally better than 85% for spiked samples of household dust extract (Table I). It was found that low recoveries result unless the following precautions are considered: (i) at each extraction step shaking of the phases by a gentle rocking action should last for at least 3 min, (ii) after each extraction step the mixture should be allowed to stand until phase separation is complete, (iii) in difficult cases a small portion of sodium chloride can be added to help phase separation, and (iv) for solvent removal the volume of solvent should be reduced to 1 ml by a rotary evaporator at room temperature and then evaporated just to dryness with a gentle stream of nitrogen. Combining liquid–liquid distribution procedures, such as acid–base extraction and hexane–dimethyl sulfoxide distribution, hexane–nitromethane and hexane–dimethyl sulfoxide, etc., was not an effective means of further simplifying the extracts applied to the TLC plates. Representative examples of the sample clean-up achieved by liquid–liquid distribution using hexane–dimethyl sulfoxide for the urban air particulate extract, and the diesel exhaust extract are given in Fig. 2 and 3, respectively.

Micro columns packed with bonded-phase sorbents have been used for the rapid isolation of PAHs from environmental extracts[54,55]. The influence of loading a sample of the household dust extract onto a reversed-phase Sep-Pak C_{18} column

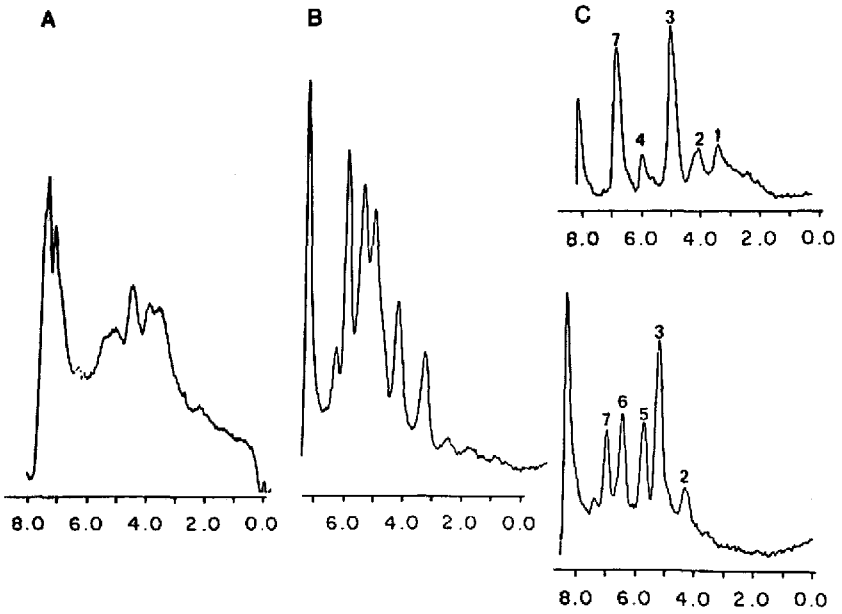

Fig. 3. Extraction and determination of PAHs in an NBS diesel exhaust particulate extract. (A) Sample obtained by Soxhlet extraction; (B) Soxhlet extract after clean-up by distribution between hexane and dimethyl sulfoxide; (C) extract B after further clean-up by silica gel column chromatography. Excitation wavelength = 313 nm and emission 450 nm (top) and 400 nm (bottom). Peak identification: 1 = coronene, 2 = benzo[*g*,*h*,*i*] perylene, 3 = benz[*a*]antracene, 4 = fluoranthene, 5 = benzofluoranthene isomers, 6 = pyrene, 7 = anthracene.

in various portions of acetonitrile–water was investigated. The extract was unretained when acetonitrile was used as solvent but adding water to the organic solvent reduced the concentration of co-eluted extractants with the PAHs. Different volumes of water to give 5, 10, 15, and 30% (v/v) solutions were applied. As the water concentration was increased the amount of co-eluted material decreased, however, when the water concentration exceeded 15% (v/v) removal of the aqueous solvent without loss of PAHs was difficult. With a mobile phase of acetonitrile–water (85:15) the PAHs were eluted in the fraction between 2 and 5 ml from the cartridges. Separation of the household dust sample on the micro column and by liquid–liquid distribution between hexane–dimethyl sulfoxide showed qualitatively similar results with one important difference. The capacity of the micro columns was limited to sample loadings of 50 μl of extract whereas much larger sample sizes were readily accommodated in the liquid–liquid distribution system. However, small samples could be processed in a shorter time and with a smaller volume of solvent than for liquid–liquid distribution. For large sample volumes liquid–liquid distribution is preferable. Using the two

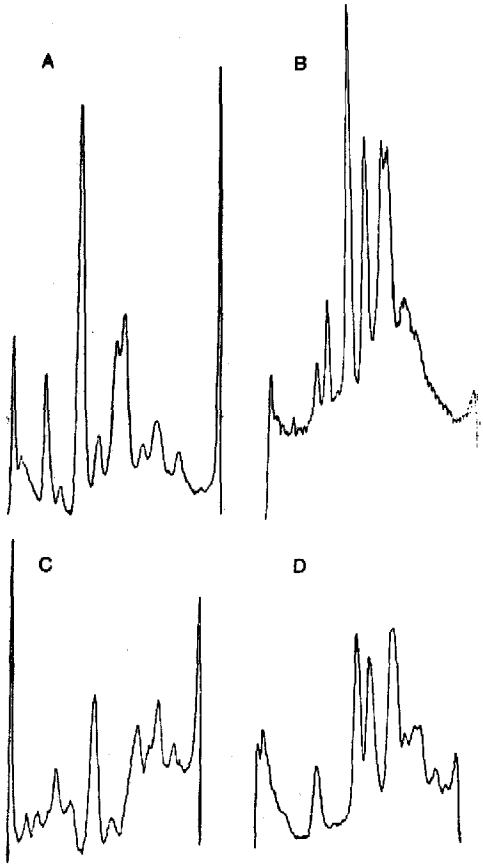

Fig. 4. Comparison of column chromatographic procedures for the clean-up of the household dust extract after fractionation by liquid–liquid distribution between hexane and dimethyl sulfoxide. (A) Silica gel; (B) Sephadex LH-20; (C) alumina; (D) Amberlite XAD-2.

systems, liquid–liquid distribution and Sep-Pak C_{18} cartridge extraction in tandem did not reduce the complexity of the extract as determined by TLC compared to the use of either method alone.

The mixtures obtained by liquid–liquid distribution were still quite complex and resisted further fractionation by similar methods. To improve further the prospects of obtaining accurate quantitative measurements different column chromatographic procedures were combined with liquid–liquid distribution for the analysis of environmental extracts. The results obtained using macroreticular resins, Sephadex LH-20, alumina, and silica gel for the household dust extract are summarized in Fig. 4. The macroreticular resin, Amberlite XD-2, was only partially successful in further reducing the complexity of the extract and was difficult to free of impurities that interfered in the quantitative measurement of some PAHs. Superior results were obtained using the gel permeation columns Bio-bead SX-2 and Sephadex LH-20. A separation is derived for these columns by a combination of size-exclusion and selective adsorption by formation of charge-transfer complexes between the PAHs and the polymer backbone of the column packing[56]. Little improvement in reducing the complexity of the household dust extract using either tetrahydrofuran, methanol, or 2-propanol as eluting solvent was observed for the Bio-beads column. Superior results were obtained using Sephadex LH-20 swollen in 2-propanol. However, the PAHs were still masked by substantial quantities of organic co-extractants and the time required to elute the PAHs from these columns was inconveniently long. The greatest discrimination between the PAHs and co-extractants was obtained using liquid–solid chromatography with either alumina or silica gel packings. The elution volume for PAHs on alumina seemed to be more sensitive to small changes in the degree of activation than for silica and the recovery of some PAHs from alumina was less than quantitative[57]. The recovery of PAH standards from silica gel columns was invariably high, 91–97% (Table II). Silica gel was found to be the more practical choice for our studies.

To improve upon the fractionation of the extractants obtained by liquid–liquid distribution followed by liquid–solid chromatography on silica gel different chemically modified silica gel packings were investigated. As chemical modifying agents sodium hydroxide, hydrochloric acid, ethylammonium nitrate, and picric acid were evaluated. Some representative examples of the results obtained are given in Fig. 5. Quantitative differences can be seen in the various chromatograms but the perform-

TABLE II

RECOVERY OF PAHs BY SILICA GEL CHROMATOGRAPHY USING CYCLOHEXANE AS ELUENT

PAH	*Recovery (%) (n = 5)*
Anthracene	91 ± 2.0
Fluoranthene	96 ± 2.3
Benz[*a*]anthracene	97 ± 2.9
Perylene	96 ± 1.1
Pyrene	93 ± 2.6
Coronene	94 ± 1.2

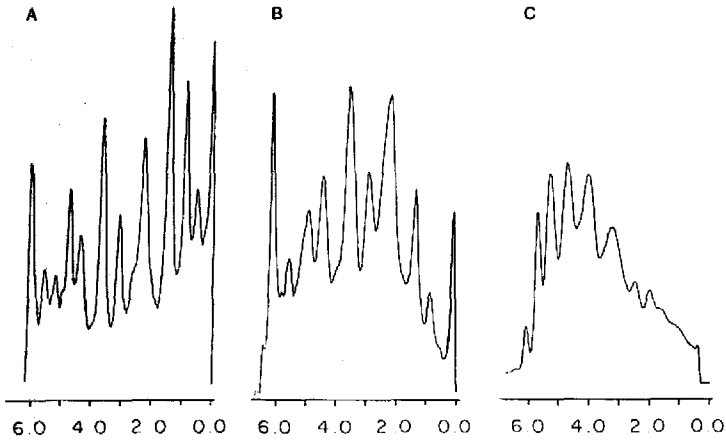

Fig. 5. Comparison of chemically modified silica gel sorbents for the clean-up of the household dust extract after fractionation by liquid–liquid distribution between hexane and dimethyl sulfoxide. (A) Silica gel impregnated with hydrochloric acid and sodium hydroxide (3 segment column, see Experimental); (B) ethylammonium nitrate; (C) picric acid.

ance of the chemically modified silica columns can not be considered superior to the use of silica gel itself. Unmodified silica gel was used in all subsequent studies. The use of silica gel alone or any of the above chemically modified silica gels without prior fractionation of the extracts by liquid–liquid distribution gave an inadequate clean-up of the extracts for quantitative analysis.

For quantitative analysis different environmental samples were extracted by

TABLE III

DETERMINATION OF PAHs IN AN NBS AIR PARTICULATE AND DIESEL ENGINE EXHAUST PARTICULATE EXTRACT

PAH	*Concentration in extract (μg/ml)*		
	TLC (n = 6)	*GC–MS* (n = 3)*	*HPLC* (n = 4)*
Urban air particulate			
Anthracene	1.90 ± 0.10		
Fluoranthene	1.89 ± 0.21	2.68 ± 0.23	3.34 ± 0.08
Benz[*a*]anthracene	1.02 ± 0.09	1.06 ± 0.10	1.22 ± 0.07
Pyrene	1.71 ± 0.08	2.17 ± 0.17	2.56 ± 0.09
Coronene	3.44 ± 0.35		
Diesel particulate			
Anthracene	4.06 ± 0.9		
Fluoranthene	4.84 ± 0.9	4.04 ± 0.39	4.02 ± 0.04
Benz[*a*]anthracene	3.50 ± 0.6	0.45 ± 0.07	0.58 ± 0.01
Pyrene	7.05 ± 1.5	3.69 ± 0.38	3.61 ± 0.07
Coronene	5.00 ± 0.8		

* Data from ref. 58.

TABLE IV

CONCENTRATION OF PAHs IN ENVIRONMENTAL SAMPLES DETERMINED BY TLC

PAH	*Fluorescence conditions (nm)*		*Concentration*
	Excitation	*Emission*	
Laboratory ventilator dust sample			
Anthracene	313	400	2.1 μg/g
Fluoranthene	266	550	3.2 μg/g
Benz[*a*]anthracene	313	500	2.4 μg/g
Perylene	266	450	4.2 μg/g
Pyrene	266	400	1.2 μg/g
Coronene	313	450	1.5 μg/g
Household dust sample			
Anthracene	313	450	8.1 μg/g
Fluoranthene	266	550	3.4 μg/g
Benz[*a*]anthracene	313	500	1.3 μg/g
Perylene	266	450	3.5 μg/g
Pyrene	266	400	2.1 μg/g
Coronene	313	450	1.2 μg/g
River water sample			
Anthracene	313	450	10.2 ng/l
Fluoranthene	266	550	6.3 ng/l
Benz[*a*]anthracene	313	500	11.5 ng/l
Pyrene	266	400	9.8 ng/l
Tea sample			
Anthracene	313	450	8 ng/g
Fluoranthene	266	550	12 ng/g
Benz[*a*]anthracene	313	500	2 ng/g
Pyrene	266	400	6 ng/g

either Soxhlet extraction or ultrasonic extraction and further fractionated by liquid–liquid distribution between hexane–dimethyl sulfoxide followed by liquid–solid chromatography using silica gel and cyclohexane as eluting solvent prior to final analysis by reversed-phase TLC with fluorescence scanning densitometry. Chromatograms for the National Bureau of Standards air particulate and diesel engine exhaust particulate extract are presented in Figs. 2 and 3, respectively. Quantitative measurements are given in Table III together with the values obtained by gas chromatography–mass spectrometry and high-performance liquid chromatography at the National Bureau of Standards[58]. Neither of these extracts have been certified but the results presented are considered the best available estimate of the concentration of the principal PAHs and are believed to be reliable. The agreement between the TLC data and the other methods for the air particulate extract is very good. Greater variation is seen for the diesel particulate extract with the assessment of PAH concentrations by TLC generally being higher. This is probably due to the presence of unresolved contaminants as the normalized emission response ratios are slightly

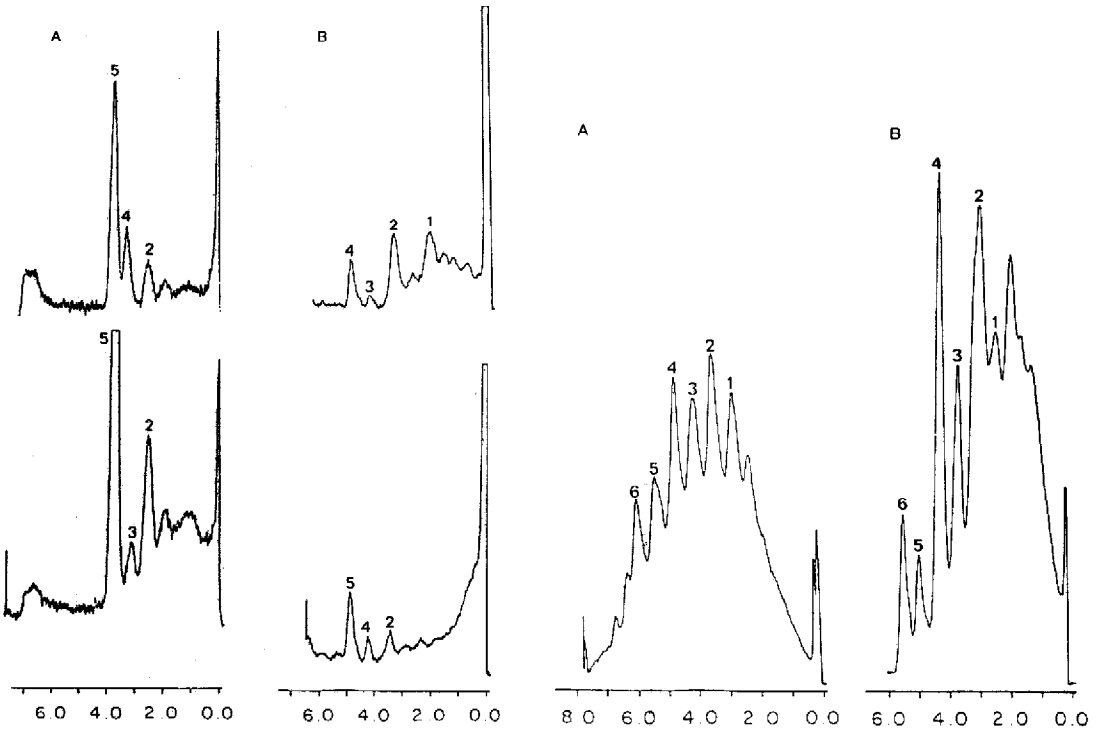

Fig. 6. Determination of PAHs in (A) a river water and (B) tea sample after sample clean-up using liquid–liquid distribution between hexane and dimethyl sulfoxide and silica gel column chromatography. Excitation wavelength = 313 nm and emission 450 nm (top) and 400 nm (bottom). Peak identification: 1 = benzo[*g,h,i*]perylene, 2 = benz[*a*]anthracene, 3 = fluoranthene, 4 = pyrene, 5 = anthracene.

Fig. 7. Qualitative identification of PAHs in (A) a shale oil and (B) river sediment sample after sample clean-up by liquid–liquid distribution between hexane–dimethyl sulfoxide and silica gel column chromatography. Peak identification: 1 = coronene, 2 = benzo[*g,h,i*]perylene, 3 = benzofluoranthene isomers, 4 = benzo[*a*]anthracene, 5 = fluoranthene, 6 = anthracene. Excitation = 313 nm and emission 450 nm.

at variance with expectations for fluoroanthene and pyrene in the diesel extract and show a substantial disagreement with calculated values for benz[*a*]anthracene and coronene. These latter two PAHs can not be considered reliably identified and quantified in the diesel particulate extract.

Six PAHs were quantified in the laboratory ventilator dust and household dust samples (Table IV). Good agreement for the normalized emission response ratios and a flat baseline were obtained. Thus, these PAHs can be considered as quantified with a reasonable degree of confidence.

Four PAHs were quantified in the river water extract and tea sample, (Table IV and Fig. 6). Benzo[*g,h,i*]perylene was also detected in the river water extract but was not quantified as its normalized emission response ratios were discordant with those of the standard analyzed simultaneously. For the other PAHs a reasonable baseline and good agreement between normalized emission response ratios was obtained. These PAHs can be considered to be reliably quantified.

Fig. 7 illustrates the results obtained when the sample preparation procedure was applied to a shale oil and river sediment sample. With the two-stage clean-up procedure the complexity of the two extracts was greatly reduced, however, many organic co-extractants still remained, and the background was too high for reliable sample quantitation. The PAHs in Fig. 7 are identified for qualitative purposes only as their assignment is based solely on the coincidence of retention with standards in the same chromatographic system.

CONCLUSIONS

It has been shown that TLC with fluorescence scanning densitometry can be used for the quantitative analysis of certain PAHs in complex environmental extracts. Although crude extracts can be applied directly to TLC plates only qualitative information can be obtained in this case unless adequate sample clean-up is used. For particulate extracts a combination of liquid–liquid distribution between hexane and dimethyl sulfoxide followed by silica gel chromatography gave extracts sufficiently clean for the quantitation of PAHs in air particulate, diesel exhaust particulate, household dust, laboratory ventilator dust, river water and tea samples. The extension of this clean-up procedure to a river sediment and shale oil sample was unsuccessful due to interference from the sample matrix that was not adequately reduced by the tandem sample clean-up procedure.

In general TLC is a rapid and reliable method of determining PAHs in environmental extracts. We see some strengths and weaknesses to its use that need to be explored in future studies. Compared to gas and liquid chromatographic methods cruder sample extracts can be processed routinely reducing the time required for sample preparation. Analysis of the household dust extract after clean-up resulted in an off-scale response with no useful information being obtained by high-performance liquid chromatography using fluorescence detection under standard conditions. However, the time required for sample preparation exceeds the time for TLC analysis, and for routine sample screening automation of the sample clean-up procedure possibly along the lines of the programmed flow preparation procedure employed by Oka *et al.*[59,60] for the analysis of drugs in biological fluids would be desirable. TLC systems do not provide sufficient resolving power to separate all PAHs of environmental interest so that a knowledge of the concentration of all target PAHs in an extract can not be obtained at present. Forced-flow TLC, also called overpressure TLC, may provide a breakthrough in this respect[15,61,62].

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