Determination of polycyclic aromatic hydrocarbons in precipitation using solid-phase extraction and column liquid chromatography

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Abstract

Solid-phase extraction (SPE) of polycyclic aromatic hydrocarbons (PAHs) on Sep-Pak C_{18} cartridges was studied. Eight 3–6-ring compounds from the 16 EPA priority pollutants were selected for investigation. Factors affecting the recovery of PAHs such as conditioning of the SPE cartridge, addition of organic modifier to the sample, flow-rate of the sample loaded on the cartridge, concentration of PAHs in the sample, drying after sample loading, elution of the analytes from the SPE cartridge with different organic solvents and recovery from spiked rainwater were included. Conditioning time was shortened by omitting the application of a 2-propanol–water mixture. The presence of 25% (v/v) 2-propanol was most appropriate for the enrichment of 3–6-ring aromatic compounds on Sep-Pak C_{18}. The flow-rate of the sample influenced the efficiency of sample preparation of compounds with a retention volume close to the sample volume. Recoveries did not change significantly in the 20–2200 ng/l concentration range. Drying of the SPE cartridge after sample loading improved recoveries of all PAHs. Elution with dichloromethane and tetrahydrofuran gave the highest recoveries. The detection limit was found to be less than 1 ng/l for several PAHs. The concentrations of PAHs in rainwater were between 3 and 1000 ng/l.

Keywords: Solid-phase extraction; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in our environment. They can be found in the atmosphere, aqueous media, soil, etc. PAHs are emitted during combustion processes. The heavier compounds are rapidly condensed or adsorbed onto particles, predominantly to sub-micrometer soot particles. The more volatile compounds remain longer in the gas phase, but their distribution is very sensitive to temperature, and therefore the ratio of the concentrations in the gas phase and the particulate fraction shows a large decrease in winter [1]. The small particles have long atmospheric residence times (of the order of weeks), and then they are deposited by rain or fallout processes, consequently facilitating the global distribution of these compounds. When they are deposited directly onto surface waters, the dissolved fractions pollute the water, while the hydrophobic contaminants are embedded in bottom sediments [2]. Although the water solubility of these compounds is very low [3], determination of their concentration in the natural environment is of great importance

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because of the carcinogenic and/or mutagenic nature of many PAHs [4].

The complexity of environmental samples and the low concentration of PAHs require thorough sample preparation. In the case of aqueous samples, solid-phase extraction (SPE) [5–12] is one of the most frequently used techniques. Since PAHs have low polarity SPE of pollutants from aqueous media is usually carried out on a bonded octadecyl-silica stationary phase.

There are a number of factors that can influence the recovery of PAHs during SPE. Several of those have partly been examined already. However, systematic investigation and interpretation can only be found for a small group of these compounds [5–12]. Recovery of 2- and 3-ring PAHs from different chemically bonded phases has been compared [7,8,12], and the application of a bonded octadecyl phase yielded recoveries of 64–100%. Brouwer et al. [12] compared the extraction efficiency of C_{18} stationary phase with copper phthalocyanine trisulphonate type phases and found similar recoveries for 3–6-ring PAHs. Kicinski et al. [10] published recoveries of 16–70% for 2–3-ring PAHs and 71–90% for 4–6-ring PAHs on a C_{18} SPE cartridge. In another study decreasing recoveries were reported with increasing ring number. For naphthalene, anthracene and pyrene, recoveries of 85%, 55% and 37% were found, respectively, and 5-ring compounds could not be enriched using SPE [6]. Recovery of some PAHs has been studied at different concentration levels on cyclohexyl [8] and octadecyl phases [11]. On the cyclohexyl phase no significant tendency was observed for the 2–3-ring compounds in the 20–100 μg/l concentration range, and recoveries ranged from 63% to 110%. Recoveries over 94% were achieved for fluoranthene, perylene, benzo[a]pyrene and benzo[g,h,i]perylene in the 4–140 ng/l concentration range on the octadecyl phase [11]. Loss of volatile compounds due to drying of the SPE cartridge after sample loading was reported [9]. However, there was no significant difference in the recovery of naphthalene and acenaphthene when drying was applied only for several minutes. The effect of elution solvents used for the removal of PAHs from the SPE cartridge has been investigated in some papers [5,7,9]. Benzene was the most efficient (83–89%) for the removal of naphthalene,

acenaphthylene and acenaphthene [9]. Dichloromethane, tetrahydrofuran and ethyl acetate yielded recoveries over or near 100% for naphthalene, while acetonitrile and hexane gave a lower efficiency. Dichloromethane proved to be more efficient than methanol to remove benzo[a]pyrene from both a C_{18} phase and a hydrophobic porous polymer [5].

Results of the investigation of several factors, e.g. addition of organic modifier to the sample, flow-rate of the sample through the SPE cartridge, concentration of PAHs in the sample, drying of the cartridge after sample loading and elution of PAHs from the SPE cartridge, are discussed in this paper. Application of the method to rainwater samples is also shown.

2. Experimental

2.1. Materials

Acetonitrile, methanol and 2-propanol (super purity solvents) were obtained from Romil (Loughborough, UK). Dichloromethane (HPLC grade), acenaphthylene (85%), fluoranthene (98%), pyrene (99%), benzo[a]anthracene (99%), benzo[a]pyrene (98%), dibenz[a,h]anthracene (97%) and benzo[g,h,i]perylene (98%) were purchased from Aldrich (Milwaukee, USA) and anthracene (99%) from Serva (Heidelberg, Germany). A standard solution (PAH mixture 610-M) containing the 16 EPA priority pollutant PAHs [naphthalene (NAPH), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHRY, 93%), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DIBahA), benzo[g,h,i]perylene (BghiP) and indeno[1,2,3-c,d]pyrene (INPY)] was obtained from Supelco (Gland, Switzerland). HPLC-grade water produced by a Milli-Q (Waters, Milford, MA, USA) purifier was used throughout the experiments. Sep-Pak Plus C_{18} SPE cartridges filled with 360 mg of packing were purchased from Waters (Milford).

Glassfibre filters (GF/F, 0.7 μm) were obtained from Whatman (Maidstone, UK).

2.2. Procedures

For simplicity reasons eight PAHs (ACY, ANTH, FLT, PYR, BaA, BaP, DlBahA, and BghiP) were selected for the first experiments. These compounds span the 3–6-ring size range but can easily be separated by reversed-phase HPLC. A stock solution was prepared with the concentration of the eight selected PAHs between 2 and 50 mg/l. The appropriate amount of organic modifier was added to 1 ml of stock solution, and then it was diluted to 50 ml to prepare the synthetic sample. When studying the effect of concentration and matrix, the recovery tests were extended to 16 PAHs. In these experiments the PAH mixture 610-M solution was diluted to the appropriate extent, and the 2-propanol content was adjusted to 25% (v/v). Recovery of PAHs was determined at five concentration levels (c1–c5). Sample solution c1 contained the aromatic hydrocarbons in the following concentrations: ACY, 44.4 μg/l; NAPH and ACE, 22.2 μg/l; FLU, FLT, BbF, DlBahA and BghiP, 4.44 μg/l; PHEN, ANTH, PYR, BaA, CHRY, BkF, BaP and INPY, 2.22 μg/l. Solutions c2, c3, c4 and c5 are 4, 10, 40 and 100 times diluted c1, respectively.

For conditioning of the C_{18} cartridges 5 ml of dichloromethane, then 4 ml of methanol or 2-propanol were used, depending on the organic modifier of the sample, and finally 4 ml of methanol–water or 2-propanol–water mixture, with the same composition as that of the sample [e.g., the SPE cartridge was conditioned with 5 ml of dichloromethane, then 4 ml of 2-propanol and finally with 4 ml of 2-propanol–water (25:75, v/v) when the organic modifier content of the sample was adjusted to 25% (v/v) 2-propanol]. Then the sample was sucked through the cartridge. Sample loading was followed by flushing with 3 ml of HPLC-grade water, then drying for 15 min by nitrogen flow. PAHs were eluted with different organic solvents, and the volume of the eluate was reduced to 100 μl under a gentle nitrogen flow. Finally, the concentrated eluate was diluted to 1 ml with methanol before HPLC analysis.

Effect of drying on recovery was studied as follows: first, 50 ml of sample were loaded onto the cartridge, and then it was dried for 60 s by a water aspirator, then for 15 min by nitrogen flow. Finally, PAHs were eluted with 2 ml of dichloromethane. In a further experiment, SPE was performed without 15 min drying, then these procedures were repeated, but instead of dichloromethane, tetrahydrofuran was used as the eluting solvent. When studying the elution efficiency of different organic solvents, sample loading and drying were performed as described above. Elution was performed with 1-ml portions of organic solvents. Each 1-ml fraction was analysed by HPLC to determine the amount of PAHs in the aliquot.

The matrix effect was examined as follows: 180 ml of rainwater was spiked with a solution containing the 16 EPA priority pollutant PAHs. Their concentrations ranged between 2 and 4 μg/l. The 25% (v/v) organic modifier content was adjusted by adding 60 ml of 2-propanol to the rainwater sample, and SPE was performed. After the sample loading, the cartridge was flushed with 3 ml of HPLC-grade water and then dried with a water aspirator for 1 min and with nitrogen for 15 min. PAHs were removed from the cartridge with 2 ml of dichloromethane, then the volume of eluate was reduced to about 100 μl by a gentle flow of nitrogen. The evaporated eluate was diluted to 1 ml with methanol before HPLC analysis.

Sampling of rainwater was performed by an automatic wet-only sampler with a cross-sectional area of 452 cm^2. The precipitation was collected through a funnel into a glass beaker. Samples were analysed within hours after rainfall.

2.3. Analysis

PAHs were analysed with a Waters HPLC consisting of a Waters 600E gradient pump, a Rheodyne 7125 injector (sample volume: 20 μl), a Waters 490E programmable multiwavelength UV detector (λ=254 nm) and a Waters 470 scanning fluorescence detector. Separation of the eight selected PAHs was performed on a monomeric type octadecyl silica column (Novapak C_{18}, 150×3.9 mm I.D., 4 μm packing) using gradient elution [0–5.5 min, methanol–water (83:17, v/v); then concave gradient to 100% methanol between 5.5 and 9 min; 100% methanol from 9 to 13 min) at a flow-rate of 1 ml/min. PAHs were detected by UV at 254 nm. Since the 16 EPA priority pollutant PAHs cannot be separated on monomeric type octadecyl silica col-

umns, a polymeric type C_{18} column (LiChrospher PAH, 250×4 mm I.D., 5 μm packing) was used. Gradient elution (linear gradient from 40% acetonitrile to 100% acetonitrile in 20 min, then 100% acetonitrile from 20 to 35 min) at 1 ml/min was applied again. Fluorescence detection was used at programmed wavelengths, based on settings suggested by Hansen et al. [13]. ACY was detected by UV at 225 nm due to the lack of fluorescence. Data acquisition and processing were accomplished by Maxima 820 software.

3. Results and discussion

3.1. Conditioning of the SPE cartridge

The first step of reversed-phase SPE is the cleaning of the cartridge with an apolar solvent, e.g. dichloromethane [8]. This is necessary since the housing, frit and packing of the cartridge may contain impurities that can interfere with the analysis, especially at low concentrations [14]. Then the SPE cartridge is usually prewetted with a water-miscible, more polar solvent, e.g. methanol, acetonitrile or 2-propanol, to activate the octadecyl chains. Finally, the cartridge is conditioned with water or an aqueous mixture [10]. For a shorter conditioning, the application of the 2-propanol–water mixture was omitted. Comparison of the recoveries with or without aqueous mixture is shown in Table 1.

It can be seen that recoveries of analytes were not significantly improved using the 2-propanol–water

mixture. Therefore, it can be neglected, and thus the conditioning time can be shortened.

3.2. Addition of organic modifier to the sample

If PAHs have to be concentrated on a C_{18} SPE cartridge, addition of organic modifier to the sample is necessary to keep the octadecyl chains in activated form [7,10,11]. Otherwise, a large amount of water will remove the activating organic solvent from the hydrocarboneous layer of the packing, and the alkyl chains will collapse to a tight film due to their hydrophobic character [15]. As a consequence, penetration of the solute molecules between the bonded octadecyl chains is hampered. Hence, a weak retention and therefore low recoveries of PAHs may be obtained [6,16].

The efficiency of sample preparation can be improved by the addition of organic modifier. Higher recoveries of the analytes may be expected with a higher organic modifier content as the film-like hydrocarboneous layer changes to a brush-like one. On the other hand, however, the eluotropic strength of the sample increases with increasing organic modifier content. This results in a smaller breakthrough volume of PAHs which is conducive to lower recoveries, especially for the 2- and 3-ring compounds. These two contradictory effects must be considered when choosing the type and amount of organic modifier.

In our experiments 50 ml of an aqueous solution containing methanol or 2-propanol in various ratios was used to examine the effect of organic modifiers.

Table 1
Effect of conditioning on recovery of PAHs

Compound	Conditioning with 2-propanol–water mixture		Conditioning without 2-propanol–water mixture	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Acenaphthylene	76	8	78	4
Anthracene	90	5	89	2
Fluoranthene	95	2	94	3
Pyrene	93	4	93	3
Benzo[a]anthracene	92	3	91	4
Benzo[a]pyrene	91	6	90	4
Dibenz[a,h]anthracene	88	6	91	4
Benzo[g,h,i]perylene	91	3	91	4

Conditions: $n=4$; 50-ml sample; 25% (v/v) 2-propanol as organic modifier; elution with 2 ml of dichloromethane.

First, the methanol content of the sample was increased from 2 to 40% (v/v), and recoveries of PAHs were determined (2% (v/v) methanol content originates from dilution of the stock solution). Results are plotted in Fig. 1.

The recoveries of ANTH, FLT and PYR were between 80% and 90% at a methanol content of 10 to 40% (v/v). The recovery of ACY ranged between 76% and 80% at a methanol content of 10 to 30% (v/v), but decreased to 70% with a methanol content of 40% (v/v). This can be explained by the breakthrough of the smallest PAH because of the increased eluotropic strength of the sample. It can also be observed that the lowest recoveries were experienced at a methanol content of 2% (v/v). A considerable improvement of the recovery of BaA and BaP was observed with increasing methanol content. However, the recoveries of DlBahA and BghiP varied between 6% and 20% with high R.S.D. at a methanol content of 2 to 30% (v/v). The irregular behaviour of these compounds needs further investigation. However, the low efficiency shows that this amount of methanol is not enough to fully activate the octadecyl chains, and therefore penetration of the largest PAHs between the alkyl chains is still hindered. At 40% (v/v) methanol the recoveries of the largest PAHs examined reached only about 35%. In all cases the non-retained amounts of PAHs were found in the solution passed through the cartridge. By adding more methanol to the sample, recoveries of the higher molecular mass PAH compounds could be increased, but this would result in three disadvan-
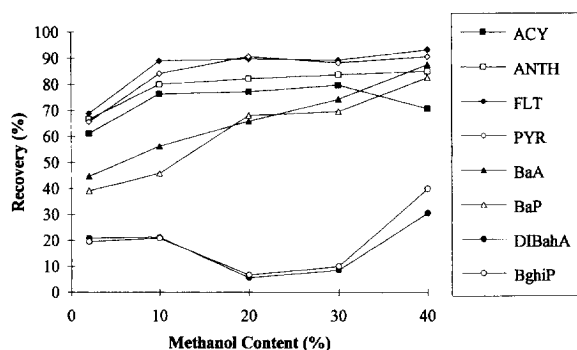
tages: (1) recoveries of the smaller PAHs decrease due to their earlier breakthrough; (2) sample will be diluted to a great extent; (3) considerable amount of organic modifier increases the cost of analysis.

Thus, instead of increasing the methanol content, 2-propanol was used as an organic modifier in the following experiments. Recoveries of the eight selected PAHs are presented in Fig. 2a and b.

As can be seen, 20 and 25% (v/v) of 2-propanol yielded high recoveries (81–94%) for the smaller PAHs, but at 30% (v/v) 2-propanol, recoveries of ANTH and ACY decreased considerably (Fig. 2a). Increasing the 2-propanol content resulted in improved recoveries for BaA, BaP and particularly DlBahA and BghiP (Fig. 2b). Based upon these results, 25% (v/v) 2-propanol is suggested as an organic modifier when all the 3–6-ring PAHs have to be concentrated on a Sep-Pak C_{18} SPE cartridge.

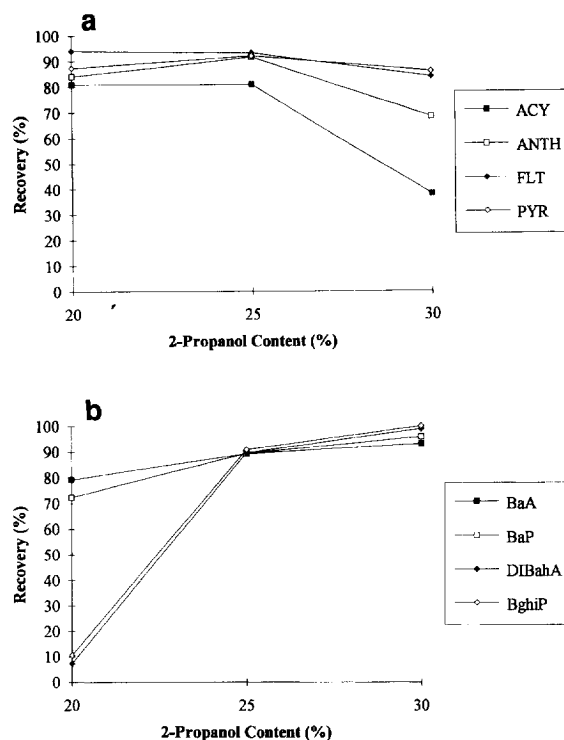Fig. 2. (a) Effect of 2-propanol as organic modifier on the recovery of ACY, ANTH, FLT and PYR: 50-ml sample, elution with 2 ml of dichloromethane. (b) Effect of 2-propanol as organic modifier on the recovery of BaA, BaP, DlBahA and BghiP: 50-ml sample, elution with 2 ml of dichloromethane.

Fig. 1. Effect of methanol as organic modifier on the recovery of PAHs, using a 50-ml sample and elution with 2 ml of dichloromethane.

This composition was used in the further experiments.

3.3. Effect of flow-rate of sample loaded on the SPE cartridge on the recovery of PAHs

Different values of flow-rate of samples can be found in the literature. Some researchers used 2–5 ml/min [5,10], manufacturers recommend 5–15 ml/min [17], while others apply 10–30 ml/min [7,18] or even 100 ml/min [9,19]. With the highest possible flow-rate a reduced sample preparation time should be maintained. However, at higher flow-rates, non-equilibrium processes can lead to lower retention volumes [20,21]. This may result in a loss of recovery, particularly for the compounds that have a retention volume close to the sample volume. Nevertheless, recoveries of the compounds having a retention volume much higher than the sample volume remains unaffected. This can be a reason why the flow-rate is found not to influence recovery in some experiments [9].

Effect of flow-rate on recovery with 25% (v/v) 2-propanol and sample volume of 50 ml was studied. Results are summarised in Fig. 3. The recovery of ACY decreased rapidly with increasing flow-rate since the retention volume of ACY on the SPE cartridge is about 50 ml under these conditions and decreases with increasing flow-rate. This was verified by breakthrough curves recorded at different flow-rates. Retention volumes as a function of flow-rate is shown in Fig. 4. Recoveries of the other PAHs were not influenced due to their much higher re-

Fig. 4. Effect of flow-rate on the retention volume of ACY in a solution containing 25% of 2-propanol as organic modifier.

tention volume ($V_R = 230$ ml for ANTH, $V_R = 280$ ml for FLT, $V_R = 290$ ml for PYR and $V_R > 400$ ml for the other PAHs under these conditions). So, it can be concluded that recoveries of PAHs can be significantly influenced by the flow-rate if the retention volume of the compounds is close to the sample volume.

3.4. Effect of concentration of PAHs in the sample on the recovery

Furthermore, the effect of concentration of PAHs in the sample on recovery was studied. Since in precipitation PAHs are usually in the ng/l concentration range [22,23], the method to be developed needs a series of experiments on trace levels. Results of this series of experiments are summarised in Table 2.

No significant changes in recovery were observed in the 20–2200 ng/l concentration range. This means that neither irreversible adsorption nor contamination during sample preparation hampers the determination of these compounds in rainwater samples. Ogan et al. [11] obtained similar results for BaP and BghiP using a C_{18} extraction column. They published a somewhat higher recovery for FLT. Our lower values can be explained with the applied higher organic modifier content [25% (v/v) 2-propanol vs. 20% (v/v) methanol] and the higher sample volume (240 ml vs. 100 ml). The R.S.D. of the recovery values usually increased with decreasing concentrations because of the increasing uncertainty of quantitative analysis in the ng/l range.
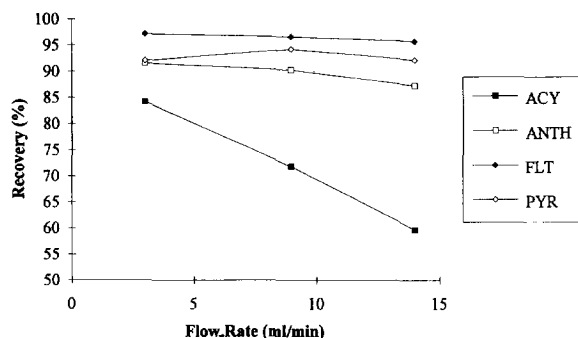

Fig. 3. Effect of flow-rate on the recovery of ACY, ANTH, FLT and PYR, using 50-ml sample, 25% of 2-propanol as organic modifier, elution with 2 ml of dichloromethane.
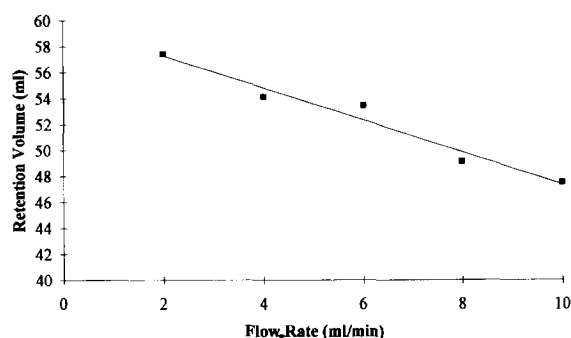
Table 2
Recovery of PAHs at different concentration levels

Compound	Concentration level									
	c1		c2		c3		c4		c5	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
NAPH	11	9	12	12	13	19	13	11	13	12
ACY	24	11	25	10	22	13	24	12	23	12
ACE	38	12	39	9	33	15	37	14	37	11
FLU	50	3	49	11	49	3	52	5	52	8
PHEN	64	6	65	5	64	6	65	7	66	11
ANTH	70	3	75	3	74	6	77	5	74	11
FLT	88	3	84	6	84	8	91	8	90	12
PYR	87	4	90	5	83	6	84	10	82	8
BaA	91	3	95	4	91	3	96	3	93	5
CHRY	93	3	93	5	91	3	95	3	90	5
BbF	91	3	94	3	91	4	93	3	89	5
BkF	89	3	93	4	91	3	90	3	88	3
BaP	87	3	94	3	94	6	96	7	92	5
DIBahA	88	3	91	4	91	6	91	5	90	5
BghiP	92	3	91	4	89	6	90	6	90	5
INPY	89	3	93	3	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.

n.q.=not quantitated due to poor fluorescence; c1–c5=concentration levels (for details see Section 2).
Conditions: $n=4$; 240-ml sample; 25% (v/v) 2-propanol as organic modifier; elution with 2 ml of dichloromethane.

3.5. Drying of the SPE cartridge after sample loading

After sample loading the concentrated analytes have to be removed from the cartridge with an apolar solvent. Water should be removed from the cartridge before elution, otherwise lower recoveries and higher standard deviations can be obtained. The loaded Sep-Pak C_{18} SPE cartridge contains 600–650 mg of water that can be eliminated by a water aspirator or a flow of nitrogen or air. In our experiments, the suction was applied for 60 s, and 250–300 mg of water were removed. Then drying was continued with a nitrogen flow of about 60 ml/s because suction may lead to the loss of more volatile compounds. The amount of water on the cartridge decreased rapidly in the first 10 min. After 15 min the original dry weight of the cartridge was usually achieved. The effect of drying on the recovery of PAHs was studied with tetrahydrofuran and dichloromethane as eluting solvents. Results of the four experiments are summarised in Table 3.

Lower recoveries and higher standard deviations were obtained without drying regardless of the

solvent. The difference in recoveries can be explained by the process of elution. If the cartridge is dried, the organic solvent is forced through by gravity, thus enabling complete elution of PAHs from all the pores of the stationary phase. However, if drying is neglected, the pores are filled with water, and the eluting solvent cannot or can only slowly penetrate into the pores because of miscibility and/or viscosity reasons. To accelerate the removal of the analytes positive or negative pressure (vacuum) must be applied. This results in faster elution but yields lower recoveries with higher standard deviations. Based on these results drying with a water aspirator for 60 s then with nitrogen for 15 min can be suggested after sample loading.

3.6. Elution of PAHs from the SPE cartridge

All the concentrated PAHs should be eluted in as small a volume as possible. A small volume of eluate is necessary to achieve a high enrichment factor. For the elution, 1–2 ml of organic solvents such as methanol [19] or acetonitrile [7,8] are usually applied. In this case the eluate can be directly injected

Table 3
Effect of drying on recovery (R) of PAHs

Compound	Elution with tetrahydrofuran				Elution with dichloromethane			
	With drying		Without drying		With drying		Without drying	
	R (%)	R.S.D. (%)	R (%)	R.S.D. (%)	R (%)	R.S.D. (%)	R (%)	R.S.D. (%)
ACY	78	5	67	4	81	7	46	25
ANTH	92	6	79	6	92	3	75	15
FLT	93	4	92	4	93	5	87	9
PYR	92	4	87	5	92	4	87	9
BaA	91	5	84	4	89	3	83	14
BaP	91	5	77	5	90	3	82	16
DIBahA	90	5	74	5	90	4	63	34
BghiP	92	5	84	4	91	6	71	31

Conditions: $n=4$; 50-ml sample; 25% (v/v) 2-propanol as organic modifier.

for HPLC separation. Nevertheless, in the case of 5- and 6-ring PAHs more apolar solvents such as 2-propanol, dichloromethane or tetrahydrofuran can be more efficient [8,10].

In our experiments methanol, acetonitrile, 2-propanol, dichloromethane and tetrahydrofuran were used as eluting solvents. Elution of PAHs from a previously loaded cartridge was performed with 1-ml portions of the selected organic solvent. Table 4 shows the attainable recovery of ACY, BaA, BaP and BghiP as a function of the 1-ml fractions of methanol, acetonitrile and 2-propanol.

It can be seen that 3×1 ml of methanol yields 73% recovery for the 3-ring ACY and 60% recovery for the 4-ring BaA. This can be increased only to 71% by an additional 2×1 ml of this solvent. The recoveries of the 5-ring BaP and the 6-ring BghiP reached only about 40% using 5 ml of methanol. Since no PAHs were usually detected in the 5th fraction, the cartridges were not washed with additional methanol. Using acetonitrile as the eluting

solvent, a more efficient removal of PAHs was achieved, but 5 ml of acetonitrile were still needed to yield 86% recovery for BaA, 59% for BaP and 61% for BghiP. Finally, the elution of PAHs was carried out by 2-propanol. The elution efficiency of this solvent was found to be similar to that of acetonitrile. Thus, methanol was the least powerful eluting solvent for 4–6-ring aromatic hydrocarbons, while acetonitrile and 2-propanol resulted in slightly higher recoveries. The 5- and 6-ring aromatic compounds could not be removed completely with any of the three solvents.

Using 2 ml of dichloromethane and tetrahydrofuran for elution, 90% recovery was achieved even for the 6-ring BghiP (see Table 1). Since the application of halogenated organic solvents is being reduced or even prohibited in many laboratories, tetrahydrofuran can be suggested for the removal of PAHs from the C_{18} SPE cartridge. On the other hand, solvents with low polarity are disadvantageous for reversed-phase HPLC separation. If PAHs are

Table 4
Attainable recovery of PAHs with different volumes of methanol, acetonitrile and 2-propanol

Compound	Methanol					Acetonitrile					2-Propanol				
	Number of 1-ml fractions of solvent					Number of 1-ml fractions of solvent					Number of 1-ml fractions of solvent				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
ACY	62	71	73	73	73	67	75	77	77	77	66	73	75	75	75
BaA	30	52	60	67	71	30	59	74	82	86	25	52	65	73	80
BaP	13	27	33	36	36	14	36	48	54	59	13	35	46	54	60
BghiP	7	30	38	42	42	10	36	49	56	61	14	42	53	60	66

Conditions: 50-ml sample; 25% (v/v) 2-propanol as organic modifier.

determined directly from the eluate (dissolved in dichloromethane or tetrahydrofuran), considerable band broadening will hinder the separation and reliable quantitative analysis. Therefore, polarity of the eluate must be increased before the liquid chromatographic separation. This is usually done by partial or complete evaporation of the eluate and dilution back of the compounds in a more polar solvent (e.g. methanol). Evaporation down to a volume not less than 50–100 μl can be suggested when the more volatile 2- and 3-ring PAHs are also to be determined because complete evaporation may lead to a remarkable loss of these compounds.

3.7. Recovery of PAHs from environmental sample matrix

With optimal conditions concerning adsorption and desorption of PAHs using SPE on a C_{18} cartridge, recoveries of these compounds were determined from spiked rainwater. The sample was collected on a day following several rainfalls. Therefore, anthropogenic organic pollutants were assumed to be washed out entirely. In addition, rainwater was stored in the laboratory for 24 h exposed to ambient light, then 180 ml of rainwater was spiked with a solution containing the 16 EPA priority pollutant PAHs, and SPE was performed. Recoveries of PAHs

from rainwater obtained in four parallel experiments are shown in Table 5. These data agree with those obtained with model solutions. The average recovery values are slightly higher with rainwater, but the difference is not significant. This indicates that no special matrix effect should be expected when analysing rainwater samples. Recoveries of the 4–6-ring compounds reach or even exceed 90% with small uncertainties. This enables reliable determination even of traces of PAHs in rainwater. Considering the results shown in Table 5 and the calibration lines obtained with HPLC, the limit of quantitation and limit of detection were determined. Results are summarised in Table 5 as well.

A chromatogram of a rainwater sample obtained by HPLC with programmed fluorescence detection after solid-phase extraction using the optimised method is shown in Fig. 5.

3.8. Determination of PAHs in rainwater

Rainwater was collected after a long dry period on 8 August 1994, at the centre of Veszprém, located in the west part of Hungary. The amount of the rainwater collected (655 ml) made only three analyses possible, so one extraction with a filtered (0.7-μm glassfibre filter) and two extractions with an unfiltered sample were performed. SPE was per-

Table 5
Recovery of PAHs from rain water ($n=4$), limits of quantitation and detection of the method

Compound	Recovery (%)	R.S.D. (%)	Limit of quantitation (ng/l)	Limit of detection (ng/l)
Naphthalene	10	32	140	36
Acenaphthylene	25	17	58	17
Acenaphthene	39	16	37	7
Fluorene	52	14	6	0.4
Phenanthrene	69	4	2	0.6
Anthracene	77	3	2	0.1
Fluoranthene	93	5	12	5
Pyrene	88	6	7	3
Benzo[a]anthracene	98	3	2	0.4
Chrysene	97	3	2	0.4
Benzo[b]fluoranthene	98	3	3	0.4
Benzo[k]fluoranthene	96	3	2	0.03
Benzo[a]pyrene	97	3	2	0.1
Dibenz[a,h]anthracene	95	3	3	0.2
Benzo[g,h,i]perylene	97	3	3	0.7
Indeno[1,2,3-cd]pyrene	95	4	60	30

Conditions: 240-ml sample; 25% (v/v) 2-propanol as organic modifier; elution with 2 ml of dichloromethane.
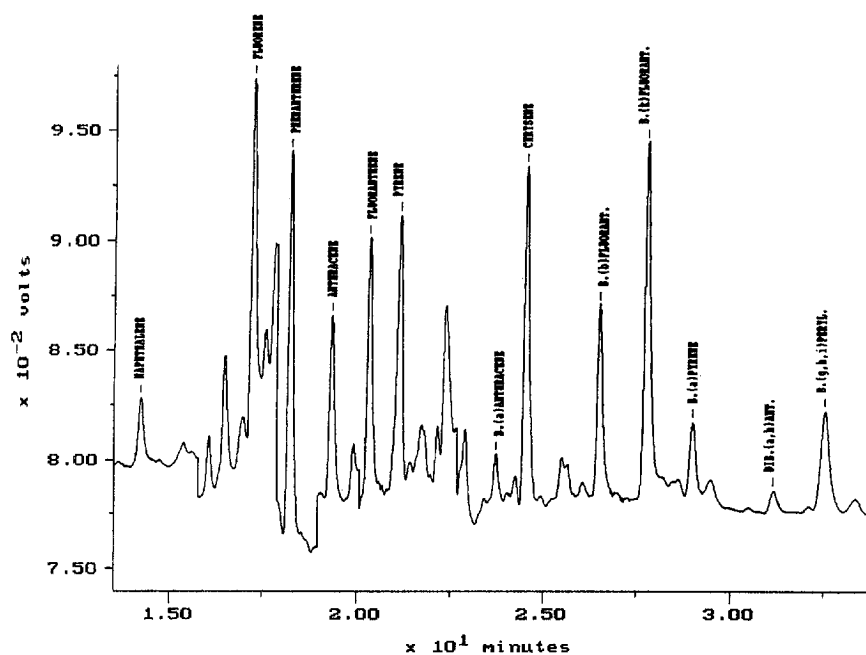
Fig. 5. HPLC chromatogram of a rainwater sample concentrated by the optimized method; 240-ml sample, 25% 2-propanol as organic modifier, drying for 60 s by water aspirator followed by 15 min with nitrogen, elution with 2 ml of dichloromethane.

formed with 180 ml rainwater as described in the previous section. Only the last step of the sample preparation was modified: the volume of the eluate was reduced to about 50 μl, and 150 μl methanol were added before HPLC analysis. Thus, an enrichment factor of about 900 was achieved, and this enabled us to carry out reliable quantitative analysis in the ng/l range. Another analysis was carried out with an unfiltered precipitation from 11 August 1994. Results of the analyses are compared in Table 6.

The concentration of PAHs was in the 3 ng/l to 1 μg/l range. The most prevalent compound was NAPH, which can be explained by its relatively high volatility and water solubility. A 30% deviation of the parallel analyses reflects the uncertainty experienced during sample preparation (Table 5). Concentration of the 4–6-ring compounds varied between 3 ng/l and 180 ng/l. These values show good agreement with those measured in Hannover [22] and in Dübendorf [23]. Somewhat higher results were obtained for 4–6-ring PAHs in the unfiltered

sample which can be explained by their partial removal during filtration. Due to their low vapour pressure, low polarity and low water solubility, PAHs exhibit a strong adsorption affinity with airborne particles. In summer NAPH, FLU and PHEN are present in the vapour phase, ANTH and the 4-ring compounds can be found both in the vapour phase and adsorbed onto particles, while the higher-molecular-mass ones occur primarily in the particle phase [24]. This tendency can be observed if the results of analyses of filtered and unfiltered rainwater are compared. Almost identical concentrations were obtained for FLU and PHEN. However, since particles larger than 0.7 μm were filtered out from one of the samples, the 4–6-ring PAHs adsorbed on those particles were also removed, and therefore lower concentrations were measured. Occurrence of 5–6-ring compounds in the filtered rainwater can be explained by the fact that the PAHs are mainly adsorbed on soot particles having a diameter of <0.3 μm [24]. The concentration of PAHs measured in the precipitation from 11 August 1994 was usually lower

Table 6
Concentration of PAHs in filtered and unfiltered rain water

Compound	8 August 1994			11 August 1994
	Filtered (ng/l, single analysis)	Unfiltered (ng/l, parallel analyses)		Unfiltered (ng/l, single analysis)
	1	2	3	4
Naphthalene	540	770	600	1000
Fluorene	18	17	15	23
Phenanthrene	84	81	77	120
Anthracene	3.2	5.8	3.9	6.0
Fluoranthene	36	94	87	66
Pyrene	120	170	180	52
Benzo[a]anthracene	12	13	25	16
Chrysene	4.7	7.5	11	8.1
Benzo[b]fluoranthene	5.5	12	14	14
Benzo[k]fluoranthene	2.5	5.7	6.1	6.1
Benzo[a]pyrene	14	23	25	10
Benzo[g,h,i]perylene	76	93	98	25

Conditions: 240-ml sample; 25% (v/v) 2-propanol as organic modifier; elution with 2 ml of dichloromethane.

than or equal to that found in rainwater 3 days earlier. This can be explained by the washing out of atmospheric pollutants during the earlier sampling.

4. Conclusion

Reliable sample preparation conditions were investigated for the determination of 3–6-ring PAHs in precipitation using SPE. Conditioning could be simplified by omitting the application of 2-propanol–water without any disadvantages. Eight compositions of organic modifiers were compared, and 10 to 30% (v/v) methanol and 20% (v/v) 2-propanol were found to be appropriate for ACY, ANTH, FLT and PYR but to yield low recoveries for the larger aromatic compounds. For BaA, BaP, DIBahA and BghiP, 30% (v/v) 2-propanol resulted in recoveries of over 90%. However, the increased eluotropic strength of the sample led to breakthrough of the 3-ring compounds. As a compromise, 25% (v/v) 2-propanol was found to be the most appropriate for the enrichment of 3–6-ring aromatic compounds on Sep-Pak C_{18} SPE cartridges. The recovery of PAHs from the 50-ml sample solution varied around 90% using this composition. Similarly high (90–100%)

recoveries were obtained by Brouwer et al. [12] from a 10-ml sample using Brij-35 surfactant instead of organic solvent. It was also shown that the sample flow-rate could considerably influence the efficiency of sample preparation for the compounds with a retention volume close to the sample volume. No significant changes of recoveries were observed in the 22–2200 ng/l range, showing that reliable quantitative analysis can be performed at this low concentration level. However, the R.S.D. of the measurements increased with decreasing concentration. Drying of the SPE cartridge after sample loading by 1-min suction followed by 15-min nitrogen flow was appropriate to obtain high recoveries with low R.S.D. A quantity of 2 ml of either dichloromethane or tetrahydrofuran was enough to elute aromatic compounds from the extraction cartridge completely. Recovery tests with spiked rainwater showed that the matrix effect did not hamper the determination of PAHs in precipitation. The limit of detection of the method ranged from 0.03 ng/l for BkF to 36 ng/l for NAPH. These values are similar to those obtained with on-line micelle-mediated sample preparation [12]. Results of the analysis of real rainwater samples showed that PAHs can partly be removed by filtering the sample through a 0.7-μm filter before SPE.

Acknowledgments

References

[1] J.L. Jaffrezo, P. Masclet, M.P. Clain, H. Wortham, S. Beyne and H. Cachier, Atmos. Environ., 27A (1993) 2781.

[2] G. Sanders, K.C. Jones and J. Hamilton-Taylor, Atmos. Environ., 27A (1993) 139.

[3] R.M. Harrison, R. Perry and R.A. Wellings, Water Res., 9 (1975) 331.

[4] B.K. Afghan and A.S.Y. Chau (eds), Analysis of Trace Organics in the Aquatic Environment, CRC Press, Boca Raton, FL, 1987, p. 206.

[5] J.L. Robinson, J. John, A.I. Safa, K.A. Kirkes and P.E. Griffith, J. Chromatogr., 402 (1987) 201.

[6] P. Loconto, LC·GC Int., 4, 11 (1991) 10.

[7] E. Chladek and R.S. Marano, J. Chromatogr. Sci., 22 (1984) 313.

[8] C.E. Rostad, W.E. Pereira and S.M. Ratcliff, Anal. Chem., 56 (1984) 2856.

[9] G.A. Junk and J.J. Richard, Anal. Chem., 60 (1988) 451.

[10] H.G. Kicinski, S. Adamek and A. Kettrup, Chromatographia, 28 (1989) 203.

[11] K. Ogan, E. Katz and W. Slavin, J. Chromatogr. Sci., 16 (1978) 517.

[12] E.R. Brouwer, A.N.J. Hermans, H. Lingeman and U.A.T. Brinkman, J. Chromatogr. A, 669 (1994) 45.

[13] A.M. Hansen, I.L.B. Olsen, E. Holst and O.M. Poulsen, Ind. Occup. Hyg., 35 (1991) 603.

[14] G.A. Junk, M.J. Avery and J.J. Richard, Anal. Chem., 60 (1988) 1349.

[15] H. Engelhardt, B. Dreyer and H. Schmidt, Chromatographia, 16 (1982) 11.

[16] G.E. Berendsen and L. de Galan, J. Chromatogr., 196 (1980) 21.

[17] Waters Sep-Pak Cartridge Care and Use Manual, Millipore Corporation, Milford, MA.

[18] A.R. Oyler, D.L. Bodenner, K.J. Welch, R.J. Llukkonen, R.M. Carlson, H.L. Kopperman and R. Caple, Anal. Chem., 50 (1978) 837.

[19] W.A. Saner, J.R. Jadamec and R.W. Sager, Anal. Chem., 51 (1979) 2180.

[20] I. Liska, J. Krupcik and P.A. Leclercq, J. High Resolut. Chromatogr., 12 (1989) 577.

[21] A. Lagana, G. Goretti, B.M. Petronio and M. Rotatori, J. Chromatogr., 219 (1981) 263.

[22] K. Levsen, S. Behnert and H.D. Winkeler, Fresenius' J. Anal. Chem., 340 (1991) 665.

[23] C. Leuenberger, J.Czuczwa, E. Heyerdahl and W. Giger, Atmos. Environ., 22 (1988) 695.

[24] T. Vo-Dinh (Editor), Chemical Analysis of Polycyclic Aromatic Compounds, Wiley and Sons, New York, NY, 1989, p. 34.