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Determination of polycyclic aromatic hydrocarbons in water samples using high-performance liquid chromatography with amperometric detection

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Abstract

The determination of polycyclic aromatic hydrocarbons (PAHs) using high-performance liquid chromatography (HPLC) with UV and fluorescence detection has been well established. Although most of the PAHs can be detected by these methods, some environmentally important polyaromatic compounds, such as acenaphthylene, do not show fluorescence and can only be determined by UV detection at higher concentrations. A sensitive and selective determination of acenaphthylene, acenaphthene and the six PAHs listed in the TVO, the German drinking water standard, is also possible by amperometric detection following HPLC separation. The method was applied to the determination of PAHs in different water samples after solid-phase extraction (SPE). The efficiency of the amperometric determination was found to be superior to UV detection ($\lambda=300$ nm).

Keywords: Environmental analysis; Water analysis; Solid-phase extraction; Detectors, LC; Polynuclear aromatic hydrocarbons; Trichloroacetic acid

1. Introduction

Polycyclic aromatic hydrocarbons are often formed during pyrolysis processes by incomplete combustion of organic materials. As a result of vehicle and industrial emissions, PAHs accumulate in various environmental matrices. The determination of polyaromatic hydrocarbons, their substituted derivatives and analogous heterocyclic compounds is of great importance because some of them are known mutagens and carcinogens [1]. The ubiquity of PAHs in the environment requires ambient monitoring especially of air, water and soils by certified national

institutions using validated methods for their sensitive and selective identification and quantitation.

Several analytical methods are suitable for PAH studies, such as capillary gas chromatography with mass spectrometric detection [2], thin-layer chromatography in combination with direct fluorescence scan techniques [3], supercritical fluid chromatography with UV detection [4] and capillary electrophoresis with fluorescence detection [5].

The ability to handle high-molecular-mass, thermally unstable compounds and the possibility of using several selective detection systems makes high-performance liquid chromatography (HPLC) ideal for PAH analysis [6,7]. According to the USEPA Method 610 [8] and the TVO [9], 16 and 6

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PAHs, respectively, must be determined using HPLC in combination with UV and fluorescence detection. With these methods many of the polycyclic compounds can be detected in lower ppb concentrations because of their strong native fluorescence.

The electrochemical activity of polyaromatic hydrocarbons also allows a sensitive and selective determination by amperometric methods using normal-sized electrodes or microelectrodes as working electrodes [10–12]. The merits of amperometric detection, such as sensitivity, limits of detection and linear range are comparable with those of UV detectors; this was verified in our previous studies [13,14].

Although fluorescence detection is very sensitive for several PAHs, it is not universally applicable. Some environmentally important PAHs, such as acenaphthylene, do not fluoresce and can be determined only by UV detection with limited sensitivity. Amperometric detection should be universally applicable, since all PAHs can be determined by electrooxidation giving radical cations [15].

Our work shows that a selective and sensitive determination of acenaphthylene, acenaphthene and the six PAHs fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene which are listed in the German drinking water standard is possible by using HPLC with amperometric detection. The limits of detection of the eight PAHs investigated were reported and compared with ultraviolet detection ($\lambda=300$ nm).

Varying the applied potential allows for selective detection. The method which uses trichloroacetic acid (TCA) as supporting electrolyte in a methanol–water eluent, was applied to the determination of the eight PAHs in different water samples after enrichment by solid-phase extraction.

2. Experimental

2.1. Chromatography

The eight PAHs investigated were separated on a C₁₈-RP column, 3 μ m, 100×3.2 mm I.D. (Bioanalytical Systems). Methanol–water (85:15, v/

v) containing 2 g l⁻¹ trichloroacetic acid as supporting electrolyte was used as mobile phase. The eluent was delivered by a Knauer HPLC pump at a flow-rate of 0.5 ml min⁻¹ and degassed on line by a CMA 260 degaser (Axel Semrau).

The samples were injected via a Rheodyne 8125 injection valve fitted with a 20- μ l sample loop.

For UV detection of the substances, a Knauer variable-wavelength monitor ($\lambda=300$ nm) was used. Amperometric detection was performed with a Metrohm EC 656 cell in combination with a VA-detector 641 at a detector potential of +1350 mV.

All measurements were done in the 3-electrode mode using a glassy carbon electrode as working electrode, a Ag/AgCl electrode as reference electrode and a Au electrode as auxiliary electrode. The resulting signals were recorded on a Shimadzu C-R3A and on a Perkin-Elmer LCI 100 integrator.

2.2. Chemicals, reference standards and water samples

Methanol (Merck), trichloroacetic acid (Merck), 2-propanol (Merck), dichloromethane (Merck) and propylene carbonate (Fluka) used for HPLC and SPE were of analytical grade. Water was of HPLC grade and was delivered by a Milli-Q water purification system.

The mixture of the eight PAHs investigated was obtained by adding 1.7 μ g acenaphthylene and 1.5 μ g acenaphthene to 1 ml of a certified reference standard (Promochem; EC 6-component PAH mix, code PAH 7A) containing the polycyclic compounds fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene dissolved in acetonitrile. The PAH mixture was diluted with eluent to obtain standard solutions of different concentrations.

For the determination of the PAHs in ground water, surface water, rain water, drinking water and river water (the first two were kindly provided by the Institut für Wasserforschung in Schwerte, Germany), the water samples were treated with 2-propanol to avoid adsorption of the PAHs on the glass walls of the sample bottles. The reference values for the PAH concentrations in the ground water and the surface water sample were obtained by using HPLC with

fluorescence detection following enrichment by SPE at the Institut für Wasserforschung in Schwerte.

2.3. Solid-phase extraction

The solid-phase extraction system consisted of a Baker SPE-10 unit combined with a home-packed SPE glass column containing 1 g of C_{18} -sorbent (particle size, 40 μm ; Baker). The column was pretreated by washing with 5 ml of methanol followed by 5 ml of dichloromethane, 5 ml of 2-propanol and 5 ml of water–2-propanol (20:3, v/v). A 500-ml volume of the water sample was treated with 75 ml of 2-propanol. After passing through a 0.45- μm cellulose acetate filter, the sample was introduced onto the column at 2.5 ml min^{-1} . The column was washed with 2×10 ml of water–2-propanol (20:3, v/v) and then dried under the vacuum for 30 min. The extracted compounds were eluted with 1×1 ml and 2×500 μl of dichloromethane into a flask containing 50 μl of propylene carbonate. The dichloromethane was evaporated by a gentle nitrogen stream before the PAH residue was diluted with 350 μl of methanol–water (85:15, v/v) for HPLC analysis.

Recoveries were determined by addition of known amounts of the PAHs to water samples.

3. Results and discussion

3.1. Hydrodynamic voltammograms

The optimum detector potential for amperometric detection could be obtained by recording hydrodynamic voltammograms. All PAHs investigated were detectable at potentials between +1350 and +1450 mV (Fig. 1). At these potentials, the corresponding current reached the limiting current plateau for most of the polycyclic compounds. Although the responses for acenaphthylene, acenaphthene, fluoranthene and benzo[*b*]fluoranthene are higher at +1450 mV, peak evaluations were very difficult because the signal-to-noise ratio was bad. By comparison of reproducibility, calculated limits of detection, limits of determination and standard deviations at different potentials, the best results were obtained at a detector potential of +1350 mV.

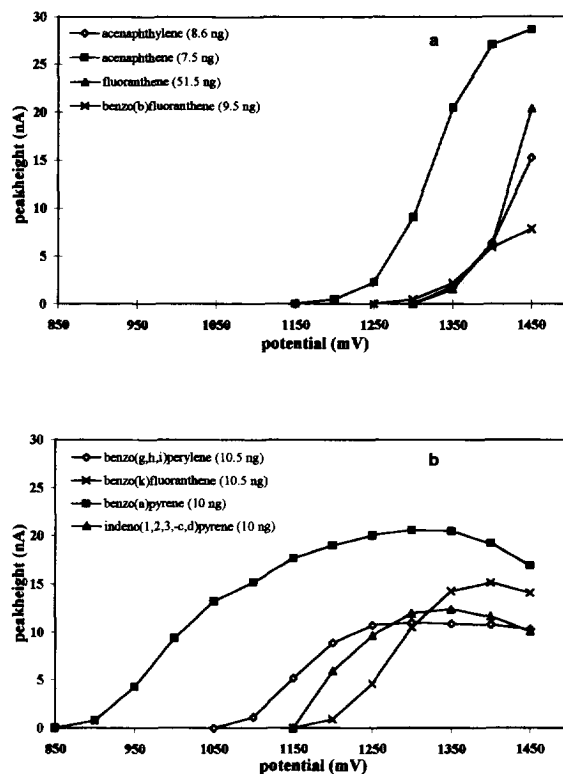


Fig. 1. Hydrodynamic voltammograms of the eight PAHs investigated. For chromatographic conditions, see Section 2.

3.2. Selectivity of amperometric detection

The amperometric response of the polyaromatic compounds mainly depends on the working potential. In fact, by variation of the potential, a selective determination of certain PAHs is possible (Fig. 2). At +1000 mV, benzo[*a*]pyrene could be determined amperometrically whereas all other PAHs show no chromatographic signal. For acenaphthylene, fluoranthene and benzo[*b*]fluoranthene a signal could not be obtained at a potential less than +1350 mV. Similar to fluorescence and UV detection, the selectivity of amperometry gives the possibility of measuring overlapping and unresolved peaks.

3.3. Comparison with UV detection

The chromatograms in Fig. 3 recorded by amperometric and UV detection ($\lambda=300$ nm) show that the amperometric response is much more sensitive

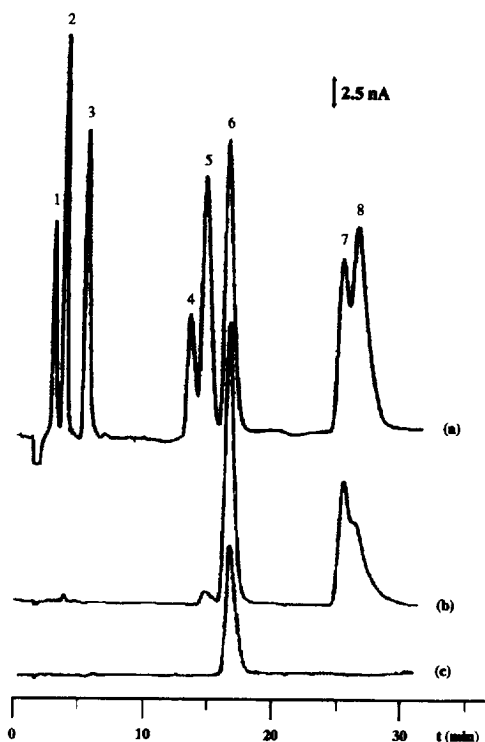


Fig. 2. Chromatogram of the PAHs using a detector potential of (a) +1450 mV, (b) +1200 mV, (c) +1000 mV vs. Ag/AgCl. Peaks: 1=acenaphthylene (8.6 ng); 2=acenaphthene (7.5 ng); 3=fluoranthene (51.5 ng); 4=benzo[*b*]fluoranthene (9.5 ng); 5=benzo[*k*]fluoranthene (10.5 ng); 6=benzo[*a*]pyrene (10 ng); 7=benzo[*g,h,i*]perylene (10.5 ng); 8=indeno[1,2,3-*c,d*]pyrene (10 ng). For chromatographic conditions, see Section 2.

for all PAHs investigated. At concentrations of 0.75 ng to 5.15 ng per injection (depending on the PAH), the limit of the UV detection was reached, whereas smaller amounts of the analytes could be measured with amperometric detection. This was verified by calculation of the limit of detection for each PAH (Table 1). The limits have been found to be between 0.09 and 0.25 ng per injection for the PAHs investigated. The limit for fluoranthene was found to be 1.8 ng per injection. In comparison to the limits obtained by UV detection, the sensitivity of the amperometric technique is about 5–10 times better. The calculation of the limits and standard deviations in Table 1 were performed by a special statistic evaluation program [16].

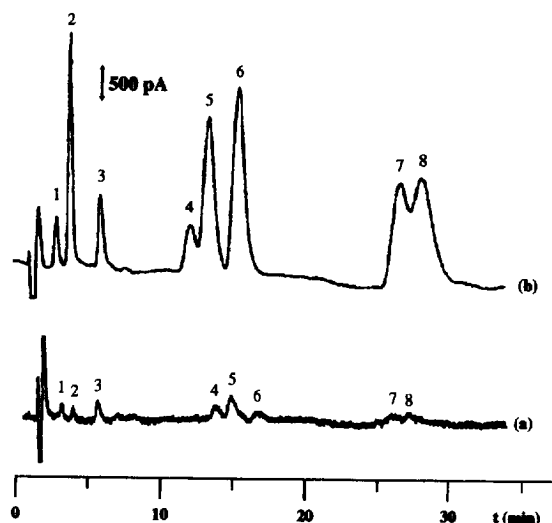


Fig. 3. Chromatogram of the PAHs recorded by (a) UV detection, attenuation=3, $\lambda=300$ nm, (b) amperometric detection, potential=+1350 mV vs. Ag/AgCl. Peaks: 1=acenaphthylene (0.86 ng); 2=acenaphthene (0.8 ng); 3=fluoranthene (5.15 ng); 4=benzo[*b*]fluoranthene (0.95 ng); 5=benzo[*k*]fluoranthene (1.05 ng); 6=benzo[*a*]pyrene (1.0 ng); 7=benzo[*g,h,i*]perylene (1.05 ng); 8=indeno[1,2,3-*c,d*]pyrene (1.0 ng). For chromatographic conditions, see Section 2.

3.4. Application to the determination of PAHs in environmental samples

According to the German drinking water standard, the limit value for the sum of six polycyclic aromatic hydrocarbons is 200 ng l^{-1} . For the PAH determination in water samples by the technique described above, it is necessary to concentrate the analytes using solid-phase extraction. In order to choose the most efficient SPE technique, the recovery rates of the eight PAHs were determined. Different amounts of the analytes were added to drinking water samples which were then extracted using different conditions (column conditioning, sample addition, washing and elution). The best results were obtained by using the SPE procedure described in Section 2. All compounds could be recovered with approximately 87% to 110% efficiency over the concentration range of $2\text{--}80 \text{ ng l}^{-1}$, except for benzo[*a*]pyrene (77%) and acenaphthene (130%). The results are summarized in Table 2. It is necessary to add the propylene carbon-

Table 1
Detection limits by UV and amperometric detection

PAH	Detection limits [ng/injection (S.D. in %, n=6)]	
	UV Detection	Amperometric detection
Acenaphthylene	2.42 (6.8)	0.25 (2.1)
Acenaphthene	1.81 (5.5)	0.09 (1.2)
Fluoranthene	12.45 (8.4)	1.84 (2.6)
Benzo[<i>b</i>]fluoranthene	1.72 (5.3)	0.17 (1.6)
Benzo[<i>k</i>]fluoranthene	1.61 (5.5)	0.19 (1.7)
Benzo[<i>a</i>]pyrene	1.15 (3.9)	0.17 (1.6)
Benzo[<i>g,h,i</i>]perylene	1.04 (3.6)	0.22 (1.5)
Indeno[1,2,3- <i>c,d</i>]pyrene	1.1 (3.8)	0.18 (1.3)

$\lambda=300$ nm, potential=+1350 mV vs. Ag/AgCl reference electrode (for HPLC conditions, see Section 2)

Table 2
Recovery rates and standard deviations, respectively, of the eight PAHs investigated from spiked drinking water with amperometric detection and UV detection

PAH	Method	Concentration (ng l ⁻¹)									
		2		4		20		40		80	
		Rec. (%)	S.D. ^b (%)	Rec. (%)	S.D. (%)	Rec. (%)	S.D. (%)	Rec. (%)	S.D. (%)	Rec. (%)	S.D. (%)
Acenaphthylene	Amp. ^c	-	-	-	-	132	7.9	127	5.7	134	5.9
	UV ^d	-	-	-	-	-	-	87	4.6	90	3.3
Acenaphthene	Amp.	-	-	-	-	91	7.8	84	1.9	91	6.2
	UV	-	-	-	-	-	-	89	4.7	96	3.3
Fluoranthene ^a	Amp.	120	10.4	107	6.7	117	8.4	107	3.1	107	5.2
	UV	-	-	-	-	-	-	93	4.3	96	3.3
Benzo[<i>b</i>]fluoranthene	Amp.	93	12.1	96	9.8	89	9.7	87	2.7	91	4.6
	UV	-	-	-	-	-	-	86	2.2	92	1.9
Benzo[<i>k</i>]fluoranthene	Amp.	92	4.7	83	6.6	89	6.9	89	1.9	92	3.6
	UV	-	-	-	-	-	-	88	2.3	92	4.0
Benzo[<i>a</i>]pyrene	Amp.	77	5.7	79	6.5	73	1.2	76	1.6	79	2.6
	UV	-	-	-	-	-	-	83	2.6	78	3.1
Benzo[<i>g,h,i</i>]perylene	Amp.	85	9.0	87	7.9	85	4.7	91	1.6	91	5.6
	UV	-	-	-	-	-	-	93	2.6	91	5.4
Indeno[1,2,3- <i>c,d</i>]pyrene	Amp.	87	8.0	85	6.7	87	6.4	87	4.6	90	4.5
	UV	-	-	-	-	-	-	90	4.6	93	4.5

^a For fluoranthene the spiking levels are five times higher.

^b Standard deviation, $n=6$.

^c Amperometric detection at +1.35 V.

^d UV detection at $\lambda=300$ nm.

For HPLC and extraction conditions, see Section 2.

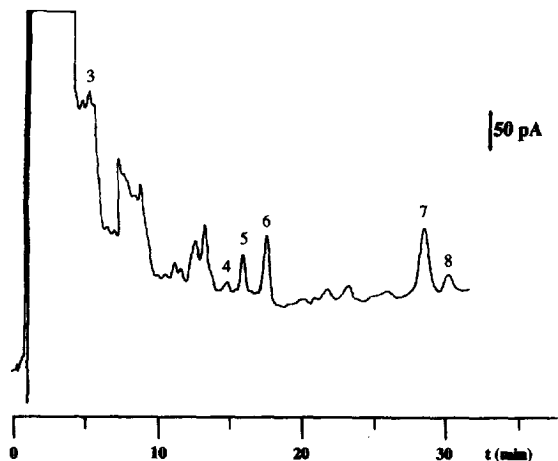


Fig. 4. Chromatogram of the eight PAHs in surface water by HPLC with amperometric detection. Potential = +1350 mV vs. Ag/AgCl. Same peak identification as Fig. 3; concentration values are listed in Table 3. For chromatographic conditions and the procedure for the sample extraction see Section 2.

ate before elution into a flask to improve the recoveries of acenaphthylene and acenaphthene. The enrichment obtained of 1:1250 using SPE with a

C_{18} -phase makes it possible to determine the eight PAHs investigated down to 1 ng l^{-1} for benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene, 2 ng l^{-1} for benzo[*b*]fluoranthene, 5 ng l^{-1} for fluoranthene and 10 ng l^{-1} for acenaphthylene and acenaphthene in drinking water samples. The use of this method also allowed a quantitation of PAHs in ground water, surface water (Fig. 4), rain water and river water samples (Table 3). The results obtained by HPLC with amperometric detection after SPE enrichment and the reference values determined by HPLC in combination with fluorescence detection following SPE at the Institut für Wasserforschung in Schwerte are in good agreement.

4. Conclusion

A procedure for the determination of eight environmentally important PAHs using HPLC in combination with amperometric detection was de-

Table 3

Results for the eight PAHs investigated in different water samples, obtained with solid-phase extraction and amperometric detection after HPLC-separation

PAH	Concentration found (ng l^{-1})			
	Ground water ^a	Surface water ^a	Rain water	River water (Elbe)
Acenaphthylene	<10 (n.n.)	<10 (n.n.)	<10	<10
Acenaphthene	<10 (<7.5)	<10 (<7.5)	10.5	<10
Fluoranthene	<5 (<5)	7.8 (7.3)	75	20
Benzo[<i>b</i>]fluoranthene	<2 (<1.5)	5.4 (5.8)	3.2	4
Benzo[<i>k</i>]fluoranthene	<1 (<0.5)	1.9 (1.8)	1.5	1.8
Benzo[<i>a</i>]pyrene	<1 (<1.5)	4.4 (4.1)	3.0	1.5
Benzo[<i>g,h,i</i>]perylene	<1 (<2)	9.7 (11.0)	2.5	1.3
Indeno[1,2,3- <i>c,d</i>]pyrene	<1 (<8)	2.5 (<8)	2.1	1.5

^a Water samples from the Institut für Wasserforschung in Schwerte.

Reference values of the water samples in parentheses; for HPLC and extraction conditions, see Section 2.

scribed. The limits of detection at +1350 mV in a methanol–water eluent (85:15) containing 2 g l⁻¹ trichloroacetic acid as supporting electrolyte were found to be between 0.09 and 1.84 ng per injection (depending on the PAH).

In comparison with the more sensitive fluorescence detection the amperometric method is suitable for the determination of all PAHs investigated. Further, it could be shown that the amperometric technique is about 5–10 times more sensitive than UV detection at $\lambda=300$ nm.

On the basis of these results, the method was applied to the PAH analysis in drinking water, surface water, ground water and river water samples. A concentration and clean-up step with octadecyl cartridges was necessary. PAH levels of 1–10 ng in 1 l of water could be detected amperometrically.

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