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APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO WATER POLLUTION ANALYSIS

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SUMMARY

In different types of water (ground water, surface water and drinking water), the content of non-polar organic compounds, which are detectable by gas chromatography does not exceed 20–30% of total organic carbon. The remaining 70–80% represents mainly polar compounds, which can be analyzed by high-performance liquid chromatography (HPLC). Some applications of HPLC and analytical difficulties that arise from extremely low concentrations of trace organic compounds in water, are discussed and examples of investigations performed with the aid of this method are described.

In the G.F.R. Drinking Water Ordinance and a number of draft Directives issued by the Commission of the European Communities, reference is made to the analysis of six defined polycyclic aromatic hydrocarbons and maximum permissible concentrations are stated. An HPLC method has been developed that will provide comparable results within short time and opens up the possibility of automation.

For an evaluation of water quality in respect of naturally occurring water ingredients and reaction products from oxidative water treatment, the presence of amino acids and carboxylic acids is of special interest. The separation and determination of amino acids in surface and drinking waters after concentration have been achieved by using an amino acid analyser with fluoresence detection. Waters of different origin have been compared with respect to the type and amounts of its content of amino acids.

INTRODUCTION

Inorganic and organic constituents (micropollutants) are present in different types of water. They may reach ground water from the soil or surface water from the air via rain. Chemical substances resulting from household or industrial activities are discharged into waste waters in considerable amounts. In many instances these constituents will pass into rivers and ultimately even into drinking water, and the initial compounds may remain unchanged, although reactions due to chemical decomposition or conversion may occur. The presence of certain toxic compounds in

drinking water constitutes a potential risk for the population; dangerous effect may arise from an accumulation of pollutants in the food chain. However, water treatment cannot be fully effective unless there is adequate information on the amounts and toxicities of these pollutants.

Whereas the analysis of inorganic water ingredients, *e.g.*, toxic heavy metals, has been carried out for many years, the determination of organic constituents has been less well studied. In recent years, however, under a research project of the Commission of the European Communities, more than 1000 compounds in different types of water (waste water, ground water, surface water, rain water, drinking water) have been identified[1]. They are mostly non-polar organic substances, the determination of which has been possible only by the progress made in chemical analysis by means of gas chromatography (GC), specially if coupled with mass spectrometry (MS). However, these compounds constitute not more than 20–30% in relation to the total content of organic carbon in various water samples. On account of their high polarities or their high boiling-points, the remaining 70–80% organic substances in the water can be determined by GC only after the formation of suitable derivatives; however this requires an operational expenditure that is hardly justificable, considering the variety of samples to be evaluated. For an analysis of these polar organic substances, liquid chromatography, especially high-performance liquid chromatography (HPLC) means a suitable method in addition to thin-layer chromatography. The difficulties that previously prevented the wider application of HPLC in water analysis resulted principally from the inferior detection limits of HPLC in comparison with GC–MS, because part of the substances to be determined are often present in concentrations far below the micrograms per litre level in water. However, by using suitable procedures of concentration, water samples can be prepared that can be used for analysis by HPLC. In addition to the problems that originate from the sensitivity and specificity of detection when employing HPLC, some difficulties may also arise from separation procedures when it is necessary to determine individual compounds within a particular group.

EXPERIMENTAL

Polycyclic aromatic hydrocarbons

Reagents. Standard Ferapol solutions were obtained from Ferak (Berlin, G.F.R.). Other chemicals were Merck (Darmstadt, G.F.R.) analytical reagent.

Apparatus. A Knauer Model 5200 pulseless high-pressure pump and a Knauer Model 7200 filter fluorimeter were used. The flow-rate was 1 ml/min and the pressure 80 bar. Elution was carried out with methanol.

Column and packing material. A 250 × 4 mm I.D. column packed with Nucleosil reversed-phase C_{18} (particle size 5 μm) was used.

Sample preparation. Common procedures[6–8] were used. Extract a 1-l sample of water three times with 30 ml of cyclohexane. Concentrate the combined organic phase in a vacuum rotary evaporator to a volume of about 0.5 ml and filter through alumina, activity II. Elute the polycyclic aromatic hydrocarbons (PAHs) adsorbed to the alumina with 3 ml of cyclohexane–benzene (1:1, v/v) and evaporate to dryness. Take up the residue in 200 μl of methanol and inject 20 μl of the latter into the chromatograph.

Amino acids

Reagents and buffer. Amino acid standards, Fluescin and other chemicals were obtained from Merck. Citrate buffers of concentration (A) 0.2 *N*, pH 3.20, (B) 0.2 *N*, pH 3.50 and (C) 0.6 *N*, pH 4.00 were used.

Apparatus. A Biotronic amino acid analyser equipped with an Aminco Model SPF 125 fluorimeter with a 200-μl flow cell was used. The three temperature steps applied were T_A 45.0°, T_B 51.0° and T_C 64.5°. The flow-rate was 35 ml/h.

Column and packing material. A 250 × 6 mm I.D. column packed with Durrum DC 6 A resin was used. It was regenerated with 0.4 *N* sodium hydroxide solution and buffer A.

Sample preparation. For concentration of amino acids acidify 1 l water sample with 2 ml of 6 *N* hydrochloric acid (pH 2) and pump through a column (60 × 10 mm I.D.) filled with Lewatit S 100 cation exchanger (30–60 mesh) at a rate of *ca.* 3 ml/min. A peristaltic pump permits a simultaneous concentration of up to 25 samples. When the sample had been applied, wash the column with double-distilled water until the eluate is free from acid (*ca.* 20 ml). Elute the amino acids with 30 ml of 1 *N* ammonia solution at a flow-rate of 1 ml/min. Under these conditions, heavy metal ions, which might interfere with analysis later on, will be effectively retained by the ion-exchange resin. Evaporate under vacuum the eluate to dryness in a flask with tapering neck and dissolve the residue in 1.0 ml of buffer, pH 2.

RESULTS AND DISCUSSION

Polycyclic aromatic hydrocarbons

The references to literature include several publications describing a number of methods that can be used for the separation of PAH. However, these methods are not applicable to the Drinking Water Ordinance (Trinkwasserverordnung)[2] currently valid in G.F.R., or to several drafts of Directives of the Commission of the European Communities[3,4]. In these Directives, maximum admissible concentrations (MAC) which must not be surpassed have been established for PAHs. Control of adherence to these values is confined to the compounds listed in Table I; maximum limits are given in Table II.

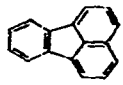
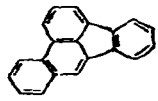
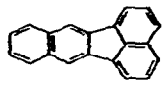
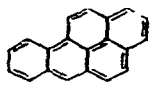
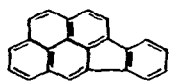
The Drinking Water Ordinance recommends the use of thin-layer chromatography, but this method involves considerable operation time[5,6]. As other procedures may be admitted by exemption, attempts have been made to have HPLC admitted for analysis of the PAHs[7,8].

The problem of separating these PAHs may be considered to have been principally solved. However, for routine application it is necessary to reduce the efficiency of the separation slightly in order to save time or to ensure the necessary reproducibility. In addition, the general expenditure on apparatus used in the analytic results is to be kept within certain limits. If thus a complete separation of all six PAH is not performed, since according to the Drinking Water Ordinance a statement of the cumulative content of these compounds is sufficient, it should be possible to establish good operating conditions for routine application. It was the main aim of the authors to optimize the essential operating conditions required for routine analysis of major series of samples, in accordance with the Drinking Water Ordinance.

Tests revealed that the use of reversed-phase columns for separation is gen-

TABLE I

POLYCYCLIC AROMATIC HYDROCARBONS REGULATED BY DIRECTIVES[2–4]

IUPAC name	*Other names*	*Formula*	*Remarks*	*Peak No.**
Fluoranthene	Benz[*a*]acenaphthene 1,2-Benzacenaphthene Idryl			1
Benz[*e*]acephenanthrene	Benzo[*b*]fluoranthene 2,3-Benzofluoranthene 3,4-Benzofluoranthene			2
Benzo[*k*]fluoranthene	8,9-Benzofluoranthene 11,12-Benzofluoranthene		Carcinogenic	3
Benzo[*d,e,f*]chrysene	Benzopyrene Benzo[*a*]pyrene 1,2-Benzopyrene 3,4-Benzopyrene		Carcinogenic	4
Indeno[1,2,3-*c,d*]pyrene	Indeno[*a,b*-3,4*a*,4]pyrene *o*-Phenylenepyrene 2,3-*o*-Phenylenepyrene 3,4-*o*-Phenylenepyrene		Carcinogenic	5
Benzo[*g,h,i*]perylene	1,12-Benzoperylene			6

* See Figs. 1–6.

erally advantageous. However, owing to the high viscosity of the methanol–water eluent usually employed, containing 10–20% of water, a pressure of up to 350 bar has to be applied and an analysis may take up to 2 h. When using reversed-phase columns it is preferable to use methanol alone as an eluent for PAH. By this means, and with consistent column quality, exept for the separation of benz[*e*]acephenanthrene and 11,12-benzofluoranthene, a good separation of the remaining four PAH can be achieved. Under these conditions, indeno[1,2,3-*c*, *d*]pyrene will be eluted before benzo[*g*, *h*, *i*]perylene (Fig. 1).

Satisfactory results were obtained when, before injecting the sample by means

TABLE II

MAXIMUM ADMISSIBLE CONCENTRATIONS OF PAH[2–4]

Total amount of all six PAHs.

Limit (carbon content)		
Drinking Water Ordinance[2]	*CEC Directive drinking water*[3]	*CEC Directive, surface water*[4]
0,02 mmole/m³ ≈250 ng/l	200 ng/l	200 ng/l

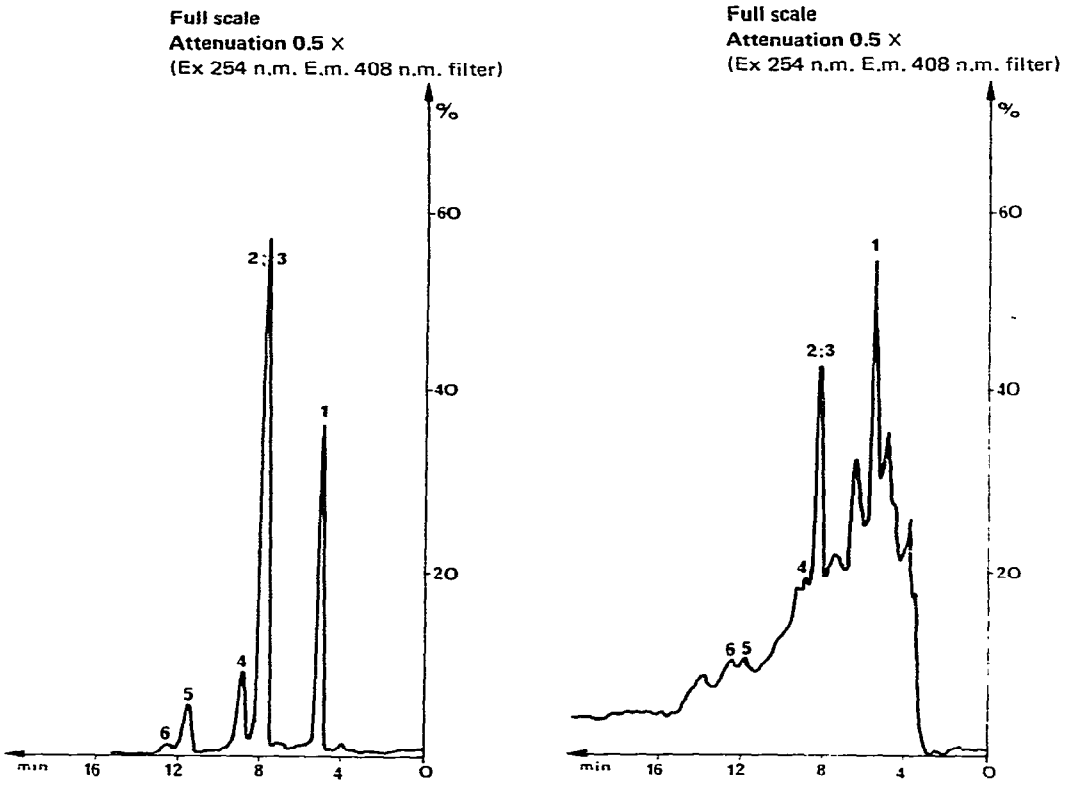


Fig. 1. Chromatogram of PAH standard (Ferapol). Sample, 1.0 μl. Total concentration, 20 ng/l. Peaks, see Table I.

Fig. 2. Chromatogram of PAHs in seepage from a waste dump. Extract, 10 μl from a 0.13-l water sample. Peaks, see Table I.

of a sample-loop valve, an equal volume of water was run through the column, thus counteracting the gradual broadening of the peak and improving the constancy of the retention times and retention volumes. However, the column can also be regenerated with water from time to time[8]. Difficulties arise on injection if the sample is dissolved in cyclohexane and the volume injected is greater than about 5 μl, because peak splitting occurs, possibly owing to the poor solubility of cyclohexane in methanol.

Fig. 2 shows an example of the determination of PAH in seepage from controlled refuse tip, Fig. 3 the chromatogram of a sample of river water and Fig. 4 that of a sample of drinking water.

For identification of peaks 1–6 in Figs. 1–4, the column eluate was collected in portions of 0.5 ml. Exitation- and emission-spectra of the fractions of interest were recorded and compared with those of reference compounds. For routine analysis, retention time was used for identification.

The detection limit is sufficient for the requirements of the Drinking Water Ordinance. If a filter fluorimeter is used, one tenth of the maximum permissible concentration of PAHs will then be easily detectable. This is, however, clearly to the debit of benzo[*k*]fluoranthene and indeno[1,2,3-*c*, *d*]pyrene, which show the lowest fluorescence.

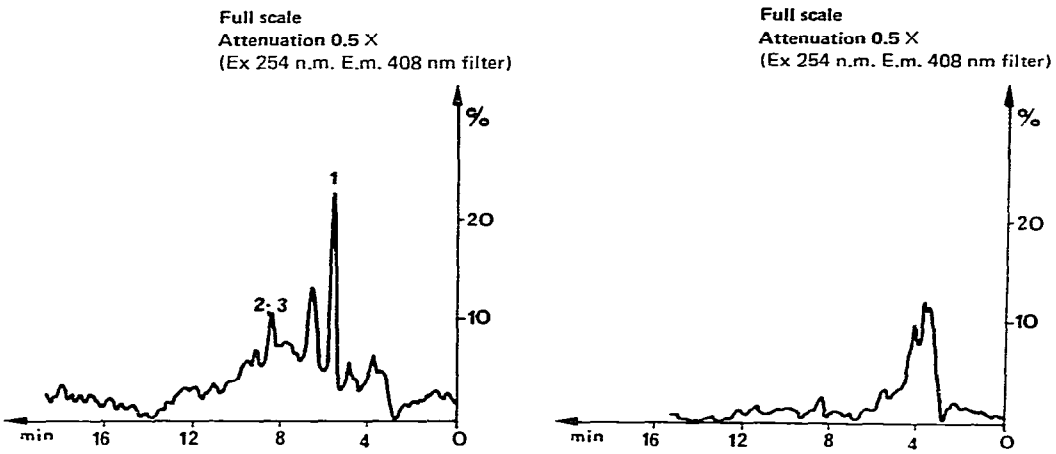


Fig. 3. Chromatogram of PAHs in river water. Conditions as in Fig. 2. Peaks, see Table I.

Fig. 4. Chromatogram of PAHs in drinking water. Conditions as in Fig. 2. Peaks, see Table I.

Amino acids

The main polar compounds in the different types of water are polysaccharides and proteins, which, by biological breakdown or oxidative processes in water treatment, are converted into organic carboxylic acids and amino acids, respectively. At present, the determination of carboxylic acids in concentrations below the parts per million level is difficult, because sufficiently sensitive detectors are not yet available. The actual separation of carboxylic acids, however, is not difficult, as has been shown by using isotope-labelled, biologically active carboxylic acids[9].

The development of the *o*-phthalaldehyde reagent Fluescin, means that now a sufficiently sensitive detector for the determination of amino acids has become available[10]. The concentration of amino acids in water samples is based on the following considerations[11,12]. Under acidic conditions, the amino groups are carrying a positive charge and therefore retained by cation-exchange resins, together with inorganic and other cations. Anions and non-ionic compounds pass through the columns, and they can be washed out. When injecting 1 *N* ammonia solution, the amino acids will lose their positive charge in the alkaline medium and are eluted as the pH front progresses. Since ammonia solution is a relatively weak base, inorganic cations will be eluted only slowly, so that there will be a good elimination of salts from the amino acids. The columns are regenerated by washing with hydrochloric acid, followed by water.

Fig. 5 shows the chromatogram of amino acids in a sample of river water and Fig. 6 that of a sample of drinking water. The different contents of acidic amino acids, which are considerably higher in the river water, can be seen. The content of the other amino acids is at almost the same low level in both samples. The longer the time after sampling, the greater will be the content of amino acids in the water samples, owing to the growth of algae; analyses should therefore be carried out as soon as possible after sampling.

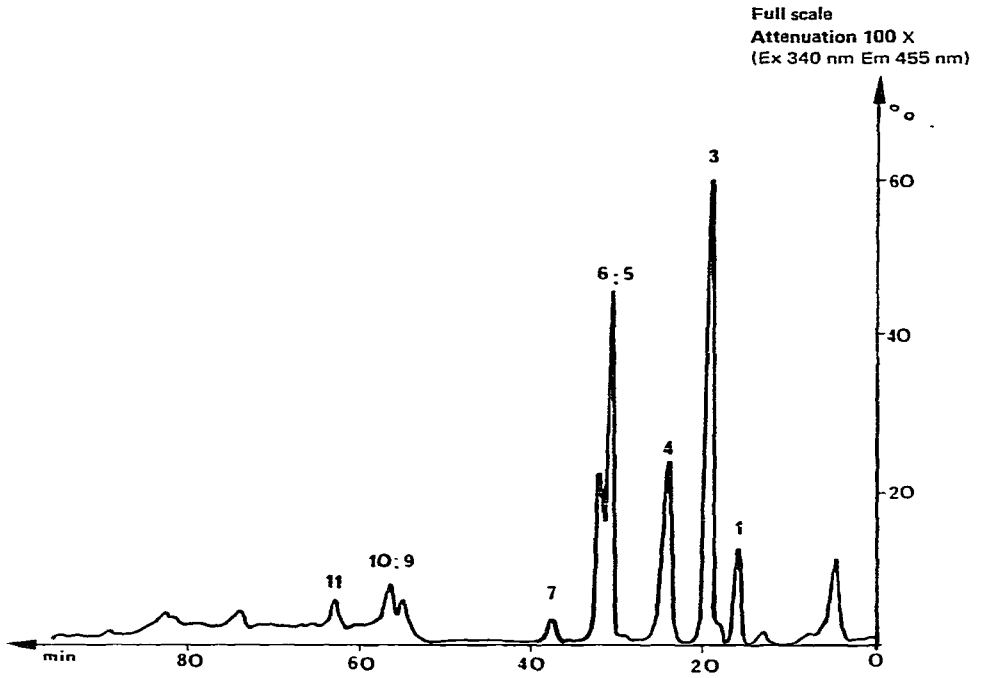


Fig. 5. Chromatogram of amino acids in river water. Peaks: 1 = Asp; 2 = Thr; 3 = Ser; 4 = Glu; 5 = Gly; 6 = Ala; 7 = Val; 8 = Met; 9 = Ile; 10 = Leu; 11 = Phe.

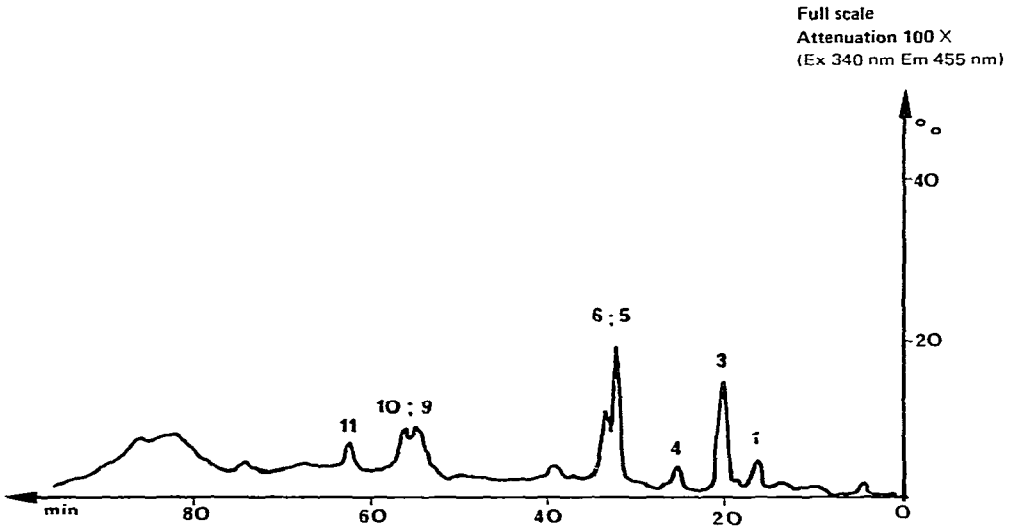


Fig. 6. Chromatogram of amino acids in drinking water. Peaks as in Fig. 5.

A comparison of the peak areas shows the concentrations given in Table III. The content of the remaining amino acids is below the detection limit. Analysis of swimming-pool water, showed a shift from the usual pattern of amino acids in other types of waters caused by human excreta (sweat, urine). Urea, being the chief component of organic nitrogen compounds in sweat and urine, could not be detected under the above conditions of analysis.

TABLE III

AMINO ACID CONTENTS (nmole/l) IN DIFFERENT WATER SAMPLES

Sample	*Asp*	*Ser*	*Glu*	*Gly*	*Ala*	*Leu*	*Phe*	*Lys*
Drinking water	5	10	5	10	5	2.5	5	n.d.*
River water	20	40	30	60	10	2.5	5	n.d.
Ground water	10	15	5	20	5	2.5	n.d.	n.d.
Waste water	20	40	10	40	5	2.5	5	10

* n.d. = Not detectable.

In the future, the distribution of organic acids, peroxides and other highly polar substances in waters will become more and more important. The extent to which HPLC as a method of identification may contribute to clarify the problems involved will depend mainly on the sensitivity of the detection systems used.

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