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DETERMINATION OF ENDOGENIC AND EXOGENIC HYDROCARBONS BY THE COMBINED APPLICATION OF LIQUID, THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

A method is described for the combined application of high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and capillary gas chromatography (GC) to the rapid determination of hydrocarbons in hydrobionts. Specific procedures have been developed for preliminary and final separations. The analytical scheme consists of saponification, extraction, TLC, GC and HPLC determinations. Alkanes, alkenes, mono-, di-, tri- and polynuclear arenes were investigated. The method is suitable for routine determinations.

INTRODUCTION

The analysis of environmental contamination products in food, particularly hydrocarbons, has received much attention. Even the presence of *n*-alkanes in nutriments probably results in pathological changes in living organisms[1–4]. Moreover, polynuclear aromatic hydrocarbons (PAHs), such as 3,4-benzo[*a*]pyrene are known to be carcinogens. The concentration limits for such compounds in several food products have been established[5,6]. The detrimental action of mono-, bi- and trinuclear aromatic hydrocarbons on mammals has been investigated in less detail, but their toxic and in several cases carcinogenic action is not in doubt[7–9].

When determining the content of aromatic hydrocarbons in foodstuffs it is necessary to take into account both natural and introduced hydrocarbons. The danger of contamination is larger for hydrobionts because of constant deterioration in their habitat. The principal sources of contamination of such ecological systems are oil-well sinking, oil spillage, creosote-impregnated wooden piles, untreated sewage and airborne particles from the combustion of fossil fuels[10]. Some work has been carried out on the biosynthetic mechanisms and metabolism of hydrocarbons and particularly of oil components in the lipids of hydrobionts[11,12].

In order to determine hydrocarbons in foodstuffs we have developed methods generally based on the combination of thin-layer (TLC) and gas chromatography (GC) and UV spectrophotometry with subsequent interpretation of the compounds isolated by chromatography–mass spectrometry[13–15]. Previously, quasi-linear

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Spollskiy spectra[16] were generally used for the determination of PAHs. With the rapid development of high-performance liquid chromatography (HPLC), several publications concerning the analysis of aromatic hydrocarbons by this method and applying photometric and fluorescence detection have appeared[17–20].

The present work concerns the application of multidimensional TLC[15] to the separation of aromatic hydrocarbons into fractions of mono-, bi-, tri- and polynuclear arenes, with subsequent further separation of each fraction by HPLC on a reversed-phase column. The advantage of this method is the clear quantitation of a number of components within each fraction and also the determination of changes in concentration and in the quantity of components in the course of the analysis of samples, indicating the occurrence of contamination.

EXPERIMENTAL

Chemicals

The solvents used were reagent grade, purified by distillation, and free from aromatic hydrocarbons[15,25].

All adsorbents used (sodium sulphate, alumina) were reagent grade, purified by washing with chloroform–methanol (1:1), at an adsorbent/solvent ratio of 1:5[13], followed by annealing.

Plates for HPTLC (Merck, Darmstadt) were washed in chloroform–methanol (1:1) and then activated.

Only glass vessels were used, washed with chrome mixture.

Hydrocarbon standards for GC were supplied by Wako (Gazukuro, Japan) and Koch-Light (F.R.G.). Standard solutions of hydrocarbons were prepared using absolute water-free methanol.

Hydrobiont samples

Hydrobionts for testing were collected in different regions: midies (*Mytilus galloprvicialis*), the Black Sea coast; sardines (*Sardinops-sagax melanosticta*), the Sea of Japan. Before analysis, samples were stored at −30°C. After defrostation, the sardine samples were cut and the viscera were taken for analysis. Midies were separated from their shells and homogenized.

Instrumentation

The following instrumentation was used: Nichonseiki Kaisha homogenizer (Japan); Yamato RE-51 rotational evaporator (Japan); Camag UV cabinet (Switzerland); Shimadzu UV-VIS 260 spectrophotometer (Japan); Shimadzu RF-540 spectrofluorimeter (Japan); Yanako G-180U gas chromatograph (Japan) equipped with a flame ionization detector, silica capillary wall-coated open tubular column OV-101, 50 m × 0.3 mm; temperature program, 60–280°C at 7°C/min; carrier gas, nitrogen. Shimadzu LC-5A liquid chromatograph (Japan), equipped with a Zorbax ODS column (300 × 4.6 mm); mobile phase, methanol, flow-rate 1.0 ml/min; column temperature, ambient. Detection conditions: (a) UV detector, SPD-2AS, wavelength 260 nm, sensitivity 0.005 a.u.f.s.; (b) fluorescence detector RF-530, excitation wavelength 252 nm, emission wavelength 360 nm; sensitivity ×4; sample volume injected 1 μl; thin-layer chromatograph with flame ionization detector, TN-10, Iatron (Japan).

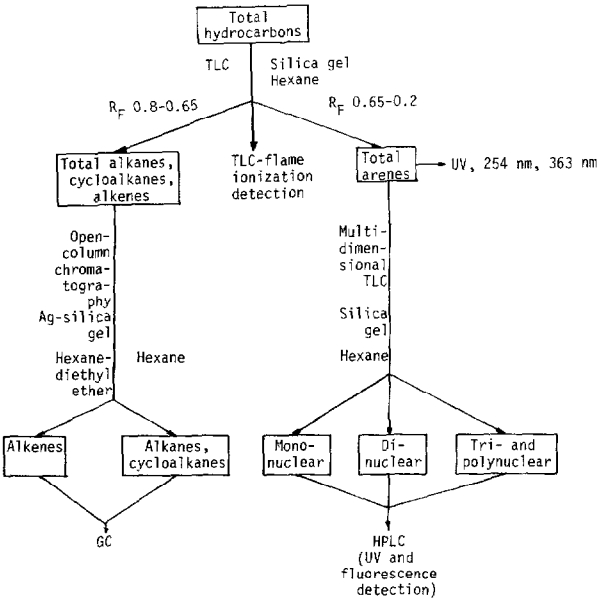

Fig. 1. Scheme for hydrocarbon determination.

Method

The procedure is shown in Fig. 1. The liquid fraction left after saponification was placed on an unsupported alumina layer and eluted with hexane. The collected mixture of hydrocarbons was subjected to TLC on silica gel with hexane as eluent and fractionated into aliphatic (R_F 0.8–0.65) and aromatic (R_F 0.65–0.2) hydrocarbons. The mixture of aliphatic hydrocarbons was then separated into alkene and alkane fractions on the argentated silica gel column by applying eluents with different polarities, *i.e.*, hexane–diethyl ether (1:1) and pure hexane, respectively. The fractions obtained were analyzed by GC. The extent of separation was monitored by micro-TLC[15]. The chromatograms obtained were interpreted by using appropriate standards and by the internal normalization method[26]. The mixture of aromatic hydrocarbons was fractionated into mono-, bi-, tri- and polynuclear arenes by multidimensional TLC (the eluent was passed four times in three directions, Fig. 2). Further analysis was performed as shown in Fig. 1.

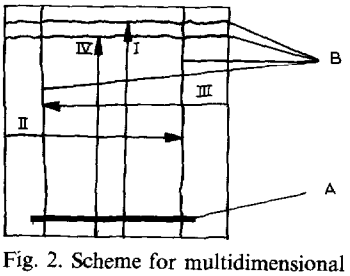

Fig. 2. Scheme for multidimensional HPTLC developed specially for the determination of PAHs: A = start; B = front of solvent. Development directions are shown by arrows, order of development by Roman numerals.

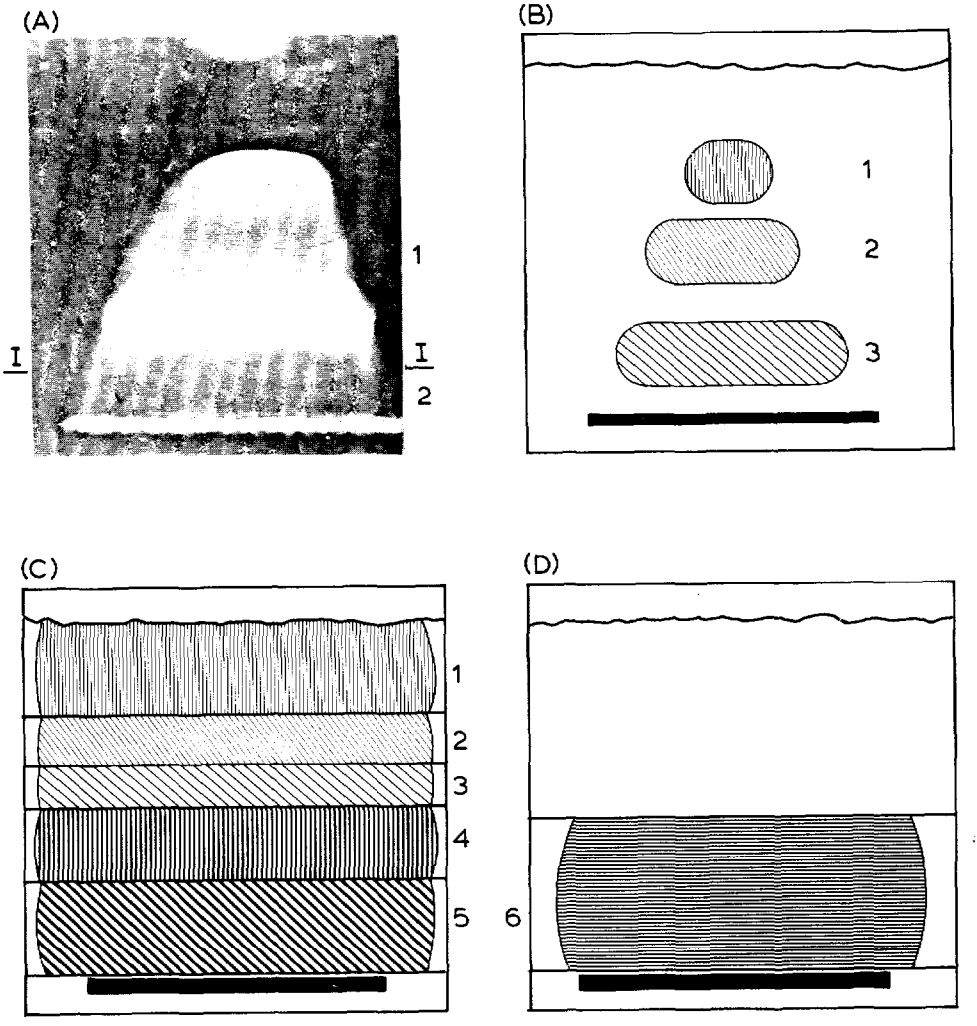

Fig. 3. TLC plates of: (A) sample with high PAH content; (B) sample with low PAH content; (C) spot 1 from plate A after rechromatography along line I-I; taken for HPLC are indicated by numerals; (D) spot 2 from plate A after rechromatography; fraction 6 was taken for HPLC.

RESULTS AND DISCUSSION

The studies performed indicate that the hydrocarbon contents in the samples were different, ranging from 0.26 to 0.05 g/kg, being largest in sardine viscera. The content of aromatic hydrocarbons did not exceed 0.15 μg/kg.

Fig. 3 shows the thin-layer chromatograms of samples containing different amounts of aromatic hydrocarbons. As is seen from the photograph, overlapping of zones takes place at 254 and 363 nm. By rechromatographing of plate A, zones for HPLC separation were selected (plates C and D). Fractions from the plates were

eluted and analyzed spectrophotometrically. The spectra obtained confirmed that the fractions correspond to both aromatic hydrocarbons and sulphur-containing compounds: 1 = mononuclear arenes; 2 and 3 = trinuclear arenes; 4 = polynuclear arenes; 5 and 6 = sulphur-containing compounds.

The fractions were subjected to HPLC (Figs. 4–9). Fig. 4 shows that fraction 1 indeed comprises mononuclear arenes, since the fluorometric response did not reveal luminescence. There were more than ten components and it was confirmed by use of appropriate standards that none of them was pure benzene or benzene having short chain substituents.

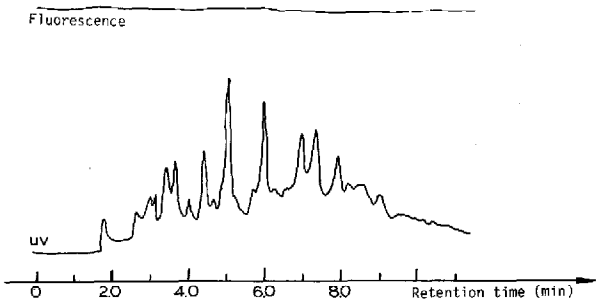

Fig. 4. HPLC separation of fraction 1 (Fig. 3C).

It was also confirmed by use of standards that in some cases when multidimensional TLC was applied for fractionating the mixture of aromatic hydrocarbons, the position on the plate, occupied by fraction 2, generally corresponded to binuclear aromatic compounds (Fig. 3, plate B). However, in the present case, analysis of the

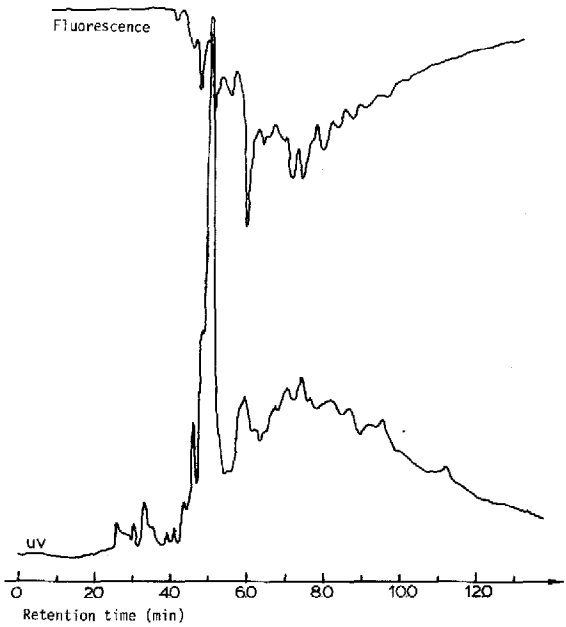

Fig. 5. HPLC separation of fraction 2 (Fig. 3C).

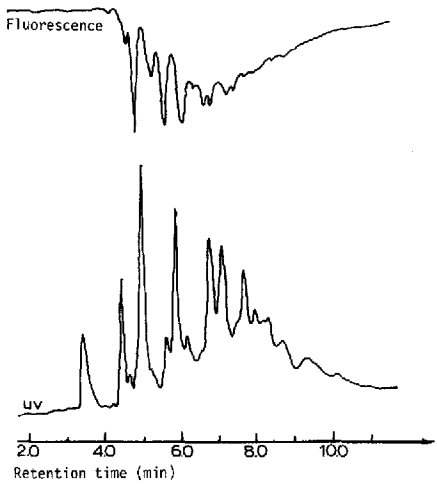

Fig. 6. HPLC separation of fraction 3 (Fig. 3C).

spectral parameters showed that fraction 2 contained trinuclear arenes, which fact can be explained by the high content of this class of hydrocarbons in the sample studied. Fraction 3 consisted of trinuclear arenes. The difference in hydrocarbon composition revealed by chromatograms (Figs. 5 and 6) suggests that due to the high content of trinuclear aromatic hydrocarbons the latter separated on the plate into two zones apparently as a function of the nature of the substituents in the aromatic ring. As is shown in Figs. 5 and 6, the trinuclear arene-containing fractions were comprised of more than 30 components, with phenanthrene being predominant (13% of the total amount). The lack of naphthalene-type hydrocarbons and the high content of phenanthrene-type ones in contaminated hydrobionts is in agreement with

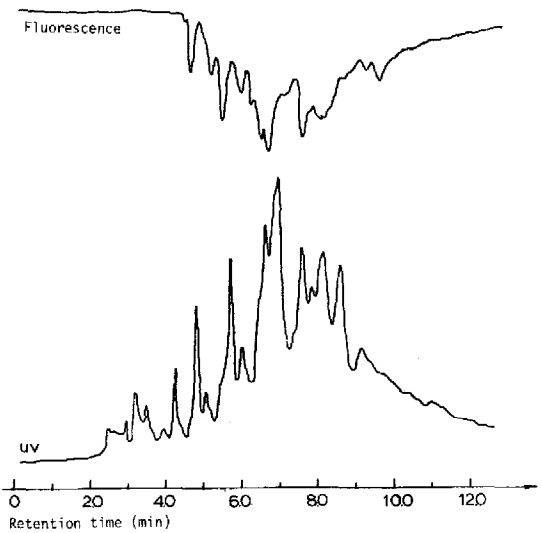

Fig. 7. HPLC separation of fraction 4 (Fig. 3C).

previously published work concerning the analysis of hydrocarbons in a number of hydrobionts from New York Bight[20].

Fig. 7 shows the HPLC separation of PAHs (fraction 4) which, according to their luminescence spectra (right chromatogram), are mainly represented by 3,4-benzo[α]pyrene derivatives. However, it is difficult to identify the exact content since the selection of appropriate standard —aromatic hydrocarbons probably present in the sample (taking into ccount that hydrocarbons entering the hydrobiont organism can either simply accumulate in tissues and organs or can undergo transformations by biosynthesis and metabolism— is impossible[20–23]. The small arene content in the samples analyzed makes it impracticable to perform chromatographic–mass spectrometric identification of this class of hydrocarbons.

Fractions 5 and 6 did not show luminescence. This is attributable to the presence of sulphur-containing compounds, which conclusion was supported by the separation of the appropriate standards and samples with the Iatroscan TN-10 instrument and by HPLC. Chromatograms are presented in Figs. 8 and 9.

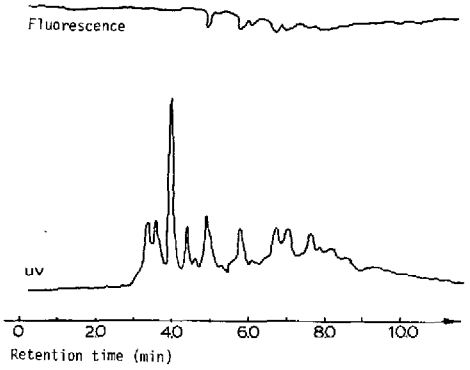

Fig. 8. HPLC separation of fraction 5 (Fig. 3C).

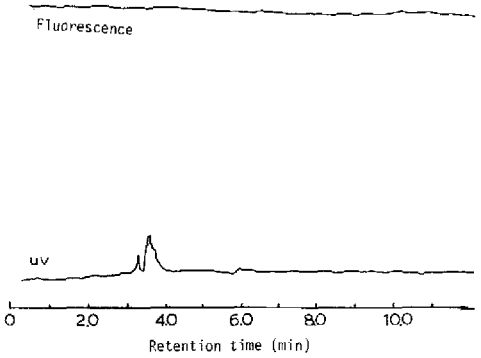

Fig. 9. HPLC separation of fraction 6 (Fig. 3D).

Thus, the described analysis scheme, based on the combination of multidimensional TLC with further HPLC separation of each fraction into mono-, bi- and trinuclear arenes, enabled us to determine not only the overall content of the compounds but also their content in each fraction separated.

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