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Note

Separation of polycyclic aromatic hydrocarbons and determination of benzo-[*a*]pyrene in liquid smoke preparations

A. RADECKI, H. LAMPARCZYK, J. GRZYBOWSKI and J. HALKIEWICZ
Department of Physical Chemistry, Medical Academy, K. Marksa No. 107, 80-416 Gdańsk (Poland)
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The assay of polycyclic aromatic hydrocarbons (PAHs) in liquid smoke preparations is difficult owing to the nature of the material to be analysed. Liquid smoke preparations contain a variety of hydrocarbons, mostly at low levels, some of which exhibit carcinogenic activity. The carcinogenic components are accompanied by neutral substances. Safety standards established for these preparations require highly selective and sensitive methods that afford reproducible results to be developed.

Most work on the determination of PAHs in smoke preparations has been based on extraction with a non-polar solvent followed by purification of the extract by column or thin-layer chromatography and final spectrophotometric[1,2] or high-performance liquid chromatographic (HPLC) assay[3] of benzo[*a*]pyrene [B(*a*)P].

The use of gas–liquid chromatography (GLC) in the final step would simplify the extraction procedure. However, the main difficulty in introducing GLC or HPLC lies in the separation of some PAH isomers, especially of B(*a*)P, perylene (Per) and benzo[*e*]pyrene [B(*e*)P]. It is worth noting that procedures checked on artificial PAH mixtures do not always give reproducible results when applied to extracts of natural products owing to the presence of extraneous matter.

Various workers[4–12] have investigated the separation of the isomers, and the following measures have commonly been adopted: use of capillary columns of high resolving power[4,5]; use of selective detectors[6,7]; use of highly efficient classical columns[8,9]; and use of nematic phases in GLC[10–12].

The main aim of this work was to develop a precise method (GLC or HPLC) for assaying B(*a*)P in liquid smoke preparations.

EXPERIMENTAL

Reagents and materials

Benzene, ethanol, dimethyl sulphoxide (DMSO) and cyclohexane were purified by repeated distillation.

Florisil (Fluka, Buchs, Switzerland) was prepared for use by the method of Howard *et al.*[13].

Deactivated alumina was prepared in the following way, using Woelm neutral grade 1 alumina. The apparent water content of the alumina was established by

determination of the weight loss when 10 g of alumina were heated in a platinum dish at red heat for 15 min. An amount of water was then added to the dehydrated residue such that its content was 15%. The product was shaken for 15 min and stored in a dark bottle.

Gas chromatography

All determinations were carried out on a Pye Unicam gas chromatograph equipped with a flame-ionization detector (FID) and a nickel-63 electron-capture detector (ECD).

The separation of standard mixtures was carried out using nine chromatographic columns, the characteristics of which are given in Table I.

TABLE I
CHROMATOGRAPHIC COLUMNS USED

No.	*Stationary phase*	*Support*	*Degree of coating (%)*	*Column length (m) × I.D. (mm)*
1	GE SE 30	Celite 545	0.8	4 × 2
2	GE SE 30	Diatomite CQ (100–120 mesh)	3	4 × 2
3	OV-1	Diatomite CQ (100–120 mesh)	3	4 × 2
4	SE-52	Chromosorb W (60–80 mesh)	1	4 × 2
5	OV-7	Chromosorb W (80–100 mesh)	1	10 × 2
6	OV-101	Gas-Chrom Q (100–120 mesh)	5	10 × 2
7	BMBT*	Chromosorb W HP (100–120 mesh)	1.5	3 × 2
8	BBBT**	Chromosorb W HP (100–120 mesh)	1.5	1.5 × 2
9	BBBT**	Chromosorb W HP (100–120 mesh)	1.5	3 × 2

* N,N'-Bis[*p*-methoxybenzylidene]-α,α'-bi-*p*-toluidine.
** N,N'-Bis[*p*-butoxybenzylidene]-α,α'-bi-*p*-toluidine.

Column packing. Column No. 1 was packed as follows. Celite 545 was prepared as described by Aue and Younker[14] and coated with 6% of GE SE-30 mobile phase. The stationary phase was then conditioned for 18 h at 335° in the thermostating chamber of the chromatograph. After conditioning, the stationary phase was extracted with chloroform for 36 h in a Soxhlet apparatus. The final degree of coating of the phase was 0.8%.

The packings of columns 2–6, 8 and 9 were prepared by a common technique of solvent evaporation. Column No. 7 was packed with a ready-to-use packing manufactured by Analabs (North Haven, Conn., U.S.A.).

Chromatographic conditions. Columns 7–9, packed with nematic phases, were extremely susceptible to oxygen contamination of the carrier gas. For this reason, nitrogen was replaced with argon.

Both programmed and isothermal heating of the columns were employed. With columns 1–4, programmed heating gave better separations of PAHs. However, our efforts were aimed at elimination of two-column and two-detector system. Benzo[*b*]chrysene and benzo[*ghi*]perylene were used as internal standards.

High-performance liquid chromatography

An ALC/GPC 204 chromatograph was used with a Model 404 UV detector (Waters Assoc., Milford, Mass., U.S.A.). A column (30 cm × 4 mm I.D.) packed with μBondapak C_{18}/Corasil. The solvent used was methanol–water (7:3) at a flow-rate of 2 cm^3/min. The wavelength of the detector was 280 nm.

Procedure

The following procedure was developed for the analysis of an industrial liquid smoke preparation (PDW) manufactured by extraction of a tar from pyrolysis products of wood[15] with organic solvents.

To about a 150-g sample of PDW, internal standards (benzo[*b*]chrysene and benzo[*ghi*]perylene) were added, followed by 150 cm^3 of ethanolic potassium hydroxide solution (obtained by mixing 500 cm^3 of 20% aqueous potassium hydroxide solution with 1200 cm^3 of ethanol) and the mixture was maintained at 60° for about 30 min. Then the mixture was transferred into the first separation funnel to which 250 cm^3 of the ethanolic KOH solution and 200 cm^3 of cyclohexane had been added. The contents were shaken for 3 min, and the lower aqueous layer was transferred to a second separation funnel and again extracted with 200 cm^3 of cyclohexane. The same procedure was repeated in a third separation funnel. After the third extraction, the aqueous layer was rejected.

Each cyclohexane extract was washed three times with 100 cm^3 of 5% aqueous potassium hydroxide solution and 100 cm^3 of warm (50°) distilled water. The combined cyclohexane extracts (600 cm^3) were passed through a column (250 mm × 20 mm I.D.) packed with 20 g of alumina (activity grade V, 15% of water). According to McGinnis and Norris[16], alumina of activity grade IV should be used. However, with this type of support losses of benzo[*b*]chrysene occurred. The column was protected from light by wrapping it with aluminium foil.

The eluate from the column was extracted three times with 100 cm^3 of DMSO equilibrated with cyclohexane. The combined DMSO extracts (300 cm^3) were poured into a 1-dm^3 separation funnel containing 600 cm^3 of distilled water. After cooling, the mixture was extracted four times with 50-cm^3 portions of cyclohexane. The cyclohexane extracts were combined, washed five times with 100-cm^3 portions of distilled water, then applied on to top of a column (300 × 35 mm I.D.) packed with 60 g of Florisil and 50 g of sodium sulphate (at the top). The cyclohexane eluates were rejected and hydrocarbons were eluted of the column with 175 cm^3 of benzene and evaporated under reduced pressure to about 5 cm^3. This volume was further reduced to a few microlitres by evaporation under nitrogen. This amount was chromatographed on a GLC column.

RESULTS AND DISCUSSION

With columns 1–4 (Table I) the separation of B(*a*)P from B(*e*)P and perylene could not be achieved. The assay of B(*a*)P was carried out using the ECD, the response of which to this hydrocarbon has been found to be about 100 times greater than that to Per. The use of short columns in this instance markedly reduced the time required for the analysis. However, the high sensitivity of the detector to contaminants necessitated its frequent cleaning and breaks in the procedure occurred.

The main advantage of using GLC seems to be the possibility of using internal standards at the beginning of the procedure. On the other hand, the use of selective detectors requires the construction of calibration graphs, and this drawback outweighs the advantage of the short retention times of the hydrocarbons.

Fig. 1 shows the results of the HPLC separation of PAHs contained in the extract. By using this technique, an artificial mixture of B(*a*)P, B(*e*)P and Per could

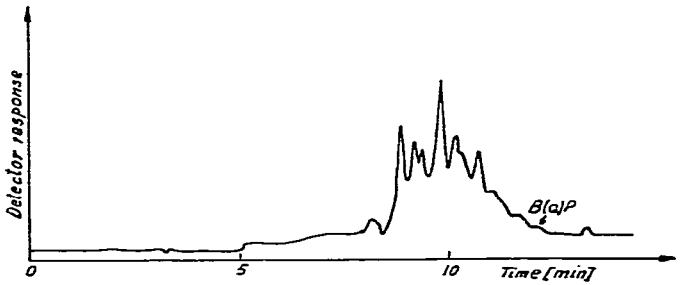

Fig. 1. HPLC separation of PAHs in liquid smoke condensate sample.

be separated. However, the separation of the components of extracts was unsatisfactory. In such instances an additional purification of the extract by thin-layer chromatography has usually been recommended[3,16]. The introduction of this step, however, complicates the procedure.

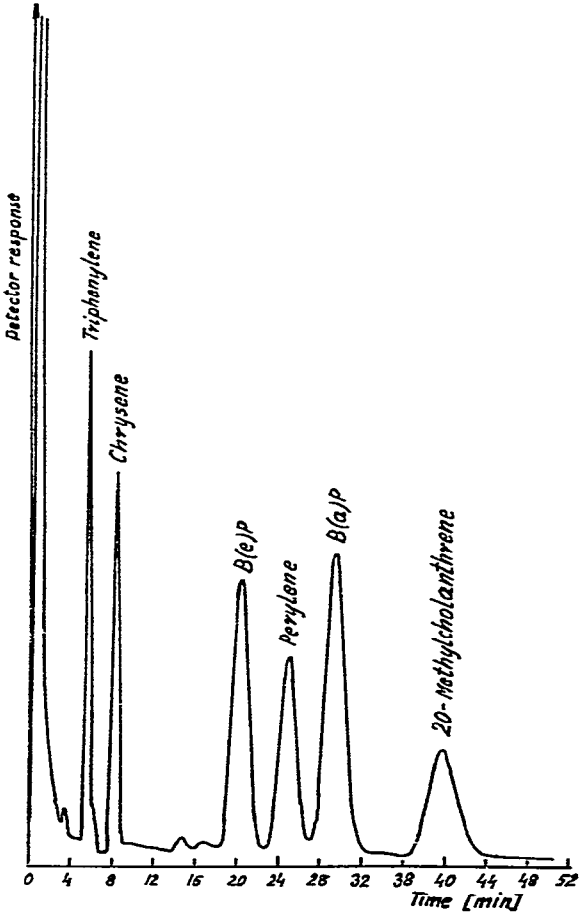

Fig. 2. Separation of a standard mixture of PAHs on column with BBBT stationary phase.

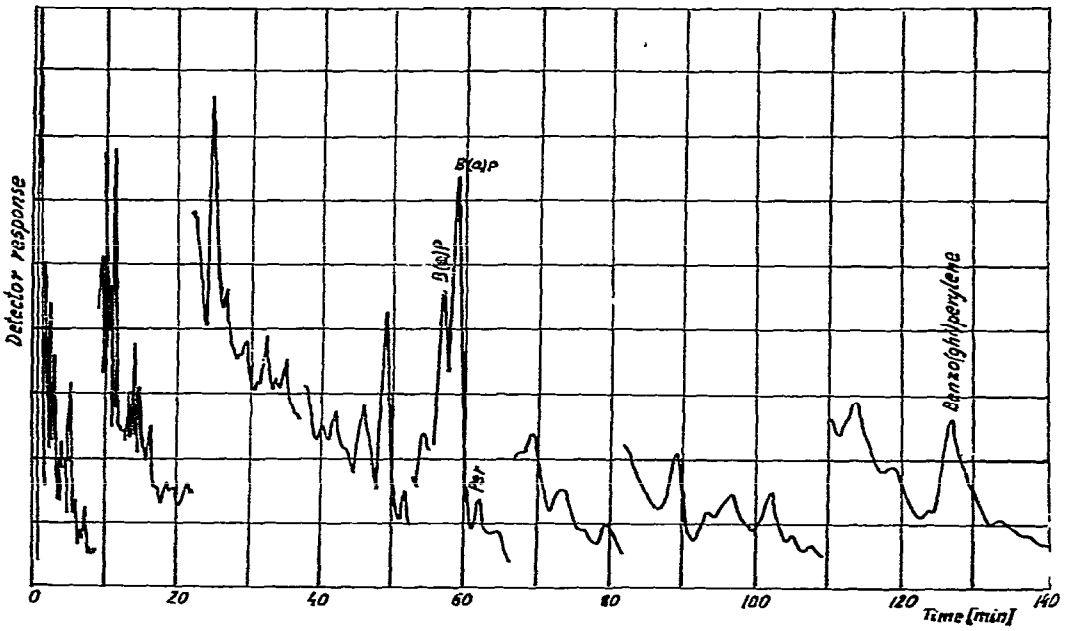

Fig. 3. Separation of PAH components in liquid smoke condensate by gas chromatography with an isotropic stationary phase (OV-101).

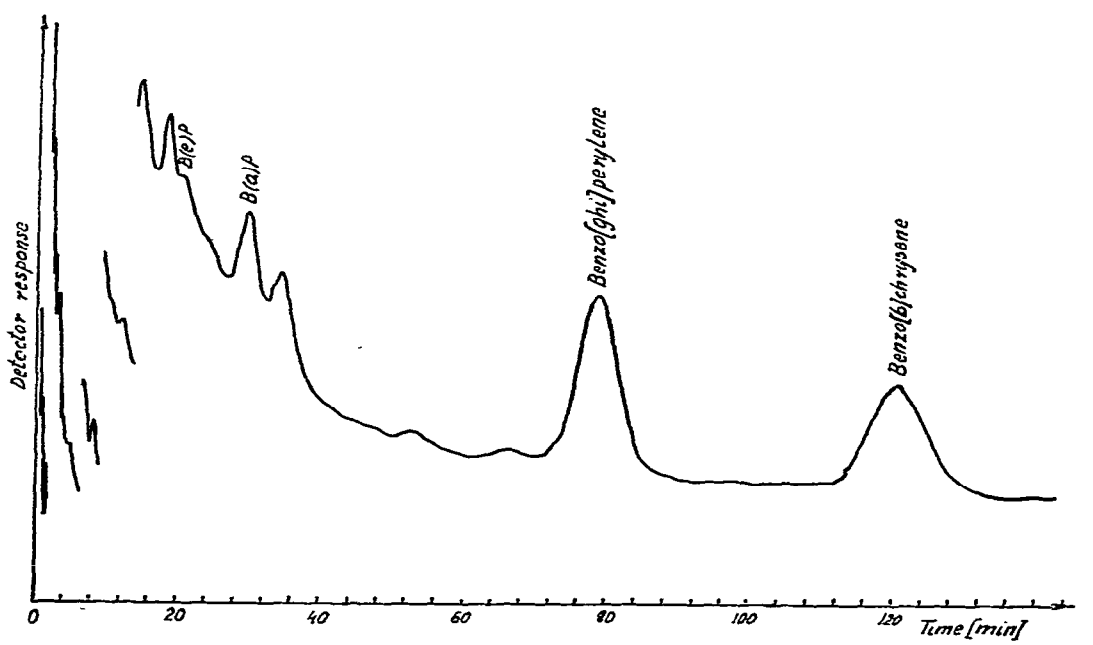

Fig. 4. Analysis of liquid smoke condensate PAH extract on the nematic liquid crystal column.

A recent report[7] recommended the use of a selective wavelength for B(*a*)P in the UV detector. This, however, requires calibration graphs to be constructed.

The nematic phase in column 7 gives a good separation of the B(*a*)P isomers, but is unsuitable for trace analysis owing to a poor thermal stability of the BMBT phase.

The separation of a standard mixture of hydrocarbons, and especially of the B(*a*)P isomers, down to the baseline was achieved with column 9 (Fig. 2).

In the group of columns with isotropic packings (columns 1–6, Table I), separation of the B(*a*)P isomers was achieved on column 6. In Fig. 3 the separation of the components of an extract in column 6 is shown.

The small number of peaks obtained when analyzing the extract on the nematic phases (Fig. 4) compared with the separation performed on the isotropic phase (Fig. 3) is noteworthy. This large difference arises because the main factor responsible for differentiation of the hydrocarbons on the isotropic phase is their size, whereas on the nematic phase their shape is a decisive factor. This feature is of importance to the analyst, as the simultaneous application of columns 6 and 9 in the analysis of B(*a*)P eliminates errors due to the interference of unidentified hydrocarbons.

The analysis is carried out isothermally and a linear response of the FID to all aromatic hydrocarbons allows internal standards to be used in place of a time-consuming calibration procedure. The recovery of B(*a*)P at the 2 ppb* level was 87–90%. The minimum detectable amount of B(*a*)P was 30 ng when using the FID.

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* The American billion (10^9) is meant.