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Note

Gas–liquid chromatographic analysis of benzo[*a*]pyrene in cigarette smoke on a nematic liquid crystal

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Polycyclic aromatic hydrocarbons (PAHs) represent the class of compounds credited with the highest carcinogenic activity in tobacco smoke condensate[1]. Accurate and reliable methods for the separation and quantification of these compounds in environmental, occupational, and biological samples have long been a target of many scientists[2,3]. Synthetic PAH mixtures have been separated by high-pressure liquid chromatography (HPLC) using reversed-phase[4], adsorption[5], partition[6], and complexation[7] methods. No HPLC method has as yet shown complete separation of the benzpyrene fraction (PAH containing 20 carbons), which includes benzo[*a*]fluoranthrene, benzo[*j*]fluoranthrene, benzo[*b*]fluoranthrene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene and pentacene. Although the application of HPLC methods for analysis of PAH environmental air pollutants has been unsatisfactory[8]. HPLC methods have recently been attempted for the quantitation of PAH in suspended particulate matter[8] and cigarette smoke[9].

In contrast, gas–liquid chromatography (GLC) has played a more important role in the analysis of PAH[2,3]. Some PAH isomers can be separated by GLC using packed[10] and capillary columns[11], although PAH isomers such as benzo[*a*]pyrene and benzo[*e*]pyrene as well as the other members of the benzpyrene fraction are normally incompletely separated[8,10]. It was recently demonstrated that novel GLC base-line separations of isomeric 3–5 ring PAH components were achieved on the nematic liquid crystal, N,N′-bis(*p*-methoxybenzylidene)α,α′-bi-*p*-toluidine (BMBT)[12]. The separations achieved were superior to those obtained with GLC using conventional stationary phases. More recently, higher molecular weight liquid crystals were synthesized and shown to provide the same unique separations with significantly diminished column bleed[13,14]. The low bleed levels and high efficiency characteristics observed for the liquid crystal N,N′-bis(*p*-phenylbenzylidene)α,α′-bi-*p*-toluidine (BPhBT) suggested its application as a liquid phase in a gas chromatographic–mass spectrometric system[14], and made it the preferred liquid crystal stationary phase for the present study.

In this communication, we report a quantitative method for the determination of benzo[*a*]pyrene in cigarette smoke. GLC in the liquid crystalline region of BPhBT has shown baseline separation of components of the benzpyrene fraction in cigarette

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smoke. The polycyclic aromatic hydrocarbon fraction was isolated from cigarette smoke condensate prior to the GLC analysis by conventional solvent extraction and column chromatography. The separations noted are dramatically superior to those obtained with GLC using conventional liquid phases. Utilizing a flame-ionization detector, 25 ng of benzo[*a*]pyrene in a 5-μl injection volume were detected.

EXPERIMENTAL

Materials

The preparation of BPhBT was outlined previously[14]. The elemental analysis results were within 0.3% of calculated values and the phase transition temperatures were 257° (solid–nematic) and 403° (nematic–isotropic)[14]. All solvents used were glass-distilled (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.). PAH standards were obtained from sources identified previously[12].

Sampling and extraction

Unsmoked portions of various brands of filter cigarettes were collected and the filters were carefully separated from the tobacco. 165 filters in two batches were collectively Soxhlet-extracted for 10 h using 250 ml of methylene chloride for each

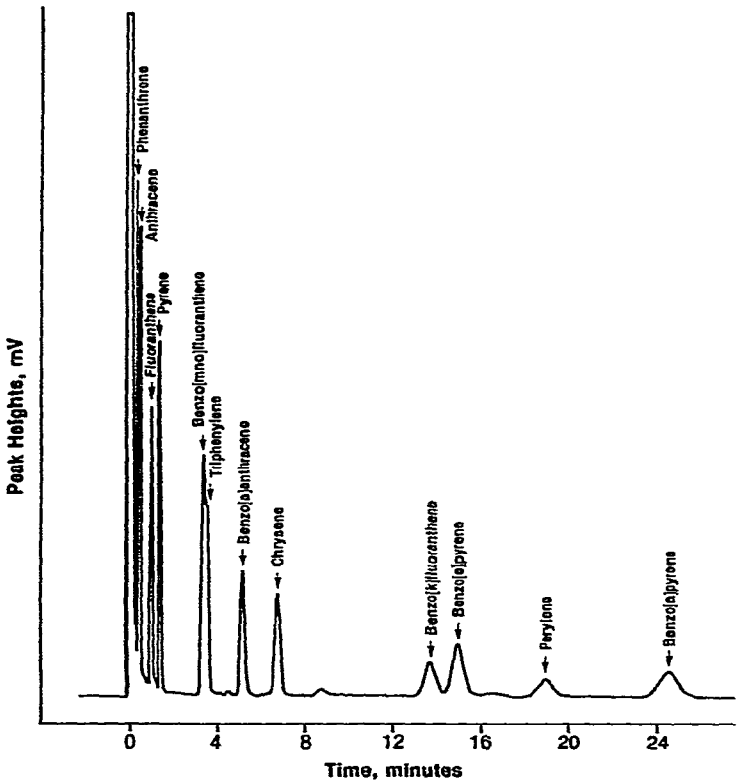

Fig. 1. Gas–liquid chromatogram of a synthetic mixture of polycyclic aromatic hydrocarbons. Column: 6 ft × 2 mm I.D.., glass. Packing: 2.5% (w/w) BPhBT on 100–120 mesh HP Chromosorb W. Conditions: flow-rate, 20 ml/min; oven, 270°; injector and detector, 275°. Electrometer setting: 4×10^{-12} A.f.s. Sample size: 0.5 μl. Concentration: about 50–100 ng of each component.

extraction. The extractions were combined and evaporated to dryness in a rotary evaporator at 35°. The dry weight of the extract was 3.9 g. The condensate was fractionated according to a partition scheme outlined by Novotny *et al.*[15]. The nitromethane extract (dry weight 125 mg) was transferred with *n*-hexane to a silicic acid column (50 × 1 cm I.D., containing 2 g of silicic acid). *n*-Hexane was passed through the column and four 100-ml fractions were collected. This step was found necessary for the removal of interfering compounds that were carried through the extraction scheme into the nitromethane extract[16]. The four *n*-hexane fractions were recombined and concentrated to 200 μl in a 2-ml screw-cap septum vial. Further concentration of sample resulted in excessive solvent peak tailing when 1–5-μl injection volumes were delivered.

Gas–liquid chromatography

A Hewlett-Packard Model 7610 gas chromatograph equipped with a dual flame ionization detector was employed. The packing material (2.5% (w/w) on 100–120 mesh HP Chromosorb W) was prepared by the solvent slurry method. BPhBT was only slightly soluble in chloroform, but formed a fine dispersion which was amenable to normal coating procedures. Helium carrier gas flow-rate was regulated by a cali-

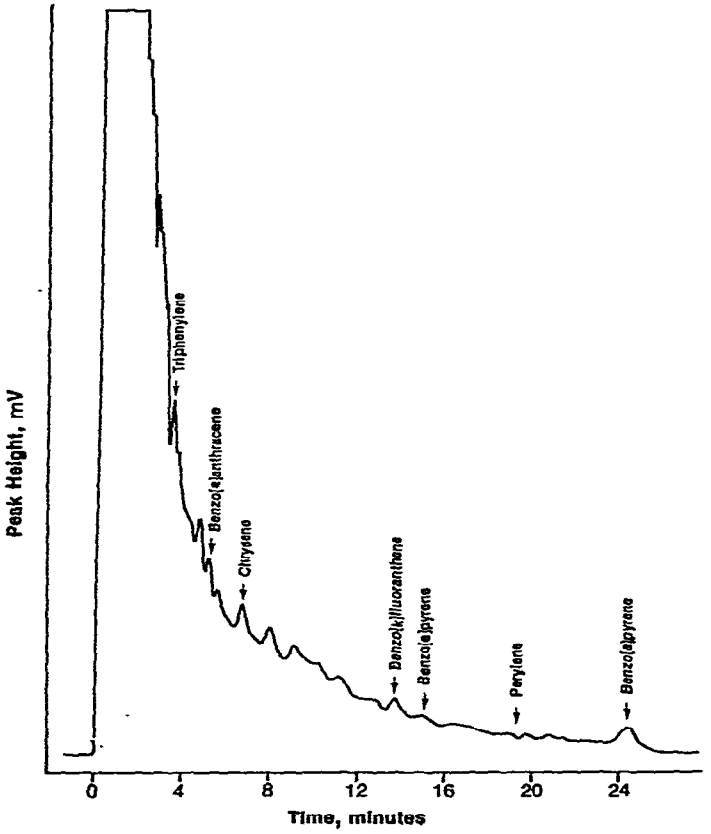

Fig. 2. Gas–liquid chromatogram of the polycyclic aromatic hydrocarbon fraction from cigarette smoke condensate. Chromatographic conditions same as Fig. 1. Sample size: 5 μl.

brated Brooks 5840 dual mass flow controller. Air flow-rate was maintained at 300 ml/min, and hydrogen flow-rate was kept at 20 ml/min.

RESULTS AND DISCUSSION

The principal objective of this study was to evaluate the utility of BPhBT for the analysis of the benzpyrene fraction from environmental samples. This liquid crystal was especially suited for this application since it had previously been shown to exhibit low bleed levels and high efficiency[14]. The shorter analysis time required to effect PAH separations on BPhBT columns resulted in narrower peak widths and greater solute concentration per unit time, allowing smaller samples to be analyzed. To illustrate, Fig. 1 shows the separation of a synthetic mixture of 3–5 ring PAH. The baseline separation of benzo[*a*]pyrene from benzo[*e*]pyrene (as well as from the other PAH) in a relatively short analysis time is as yet unmatched by other separation techniques[8,10] including GLC using other liquid crystalline phases[12,13]. Fig. 2 shows a chromatogram resulting from a 5-μl injection volume of the PAH fraction isolated from cigarette smoke condensate. Several PAH components were readily identified by matching retention times with the standard mixture (Figs. 1 and 2). Other peaks were not identified because of their low concentrations and/or lack of standards. The amount of benzo[*a*]pyrene present in the 5-μl injection volume (25 ng) was obtained by comparing its peak area with that resulting from a known volume injection of standard benzo[*a*]pyrene solution. The minimum detection limit for benzo[*a*]-pyrene from cigarette smoke under the same experimental conditions is estimated to be less than 10 ng.

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