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Determination of Benzo(a)pyrene and Related Compounds in Cigarette Smoke*

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INTRODUCTION

In this paper a method using high temperature gas-liquid chromatography for the determination of benzo(a)pyrene in smoke is described. Some preliminary experiments are also discussed which demonstrate that the method can readily be extended to the examination of other polycyclic aromatic hydrocarbons.

Due to the low concentration of polycyclic hydrocarbons in smoke condensate, it is not practical to inject whole smoke onto a gas chromatogram, and determine the individual hydrocarbons. Consequently a simple solvent extraction scheme, essentially that of *Haenni* et al. (1), is used to isolate an enriched polycyclic hydrocarbon fraction. Sub-division of this fraction by chromatography on acetylated paper is also advantageous since the gas chromatogram will not resolve certain hydrocarbons from each other. For example, it has been shown by *Carugno* and *Waltz* (2) that even with the use of capillary columns, benzo(a)pyrene and benzo(e)pyrene could not be resolved.

An isotope dilution technique is incorporated in the determination of benzo(a)pyrene, so that the results can be corrected for the losses during the analytical procedure.

APPARATUS AND MATERIALS

Cigarette Smoking

Cigarettes are conditioned at 58% R. H. and 21° C and selected at an average weight ± 20 mg and average pressure drop ± 4% at a flow rate of 1050 ml/min. The cigarettes are smoked to a butt length of 23 mm at one puff a minute on a bellows type smoking machine as described by *Fordyce* et al. (3), using a puff of 35 ml volume and two seconds duration. The smoke is collected in spiral glass traps cooled in solid carbon dioxide.

Solvents

Dimethyl sulphoxide (laboratory reagent grade) is used without further purification. Other solvents are purified by distillation and chromatography on alumina (*Woelm* grade 1). It is not recommended that any solvents are stored over sodium since the sodium frequently contains paraffinic impurities which interfere with the gas-chromatographic analysis.

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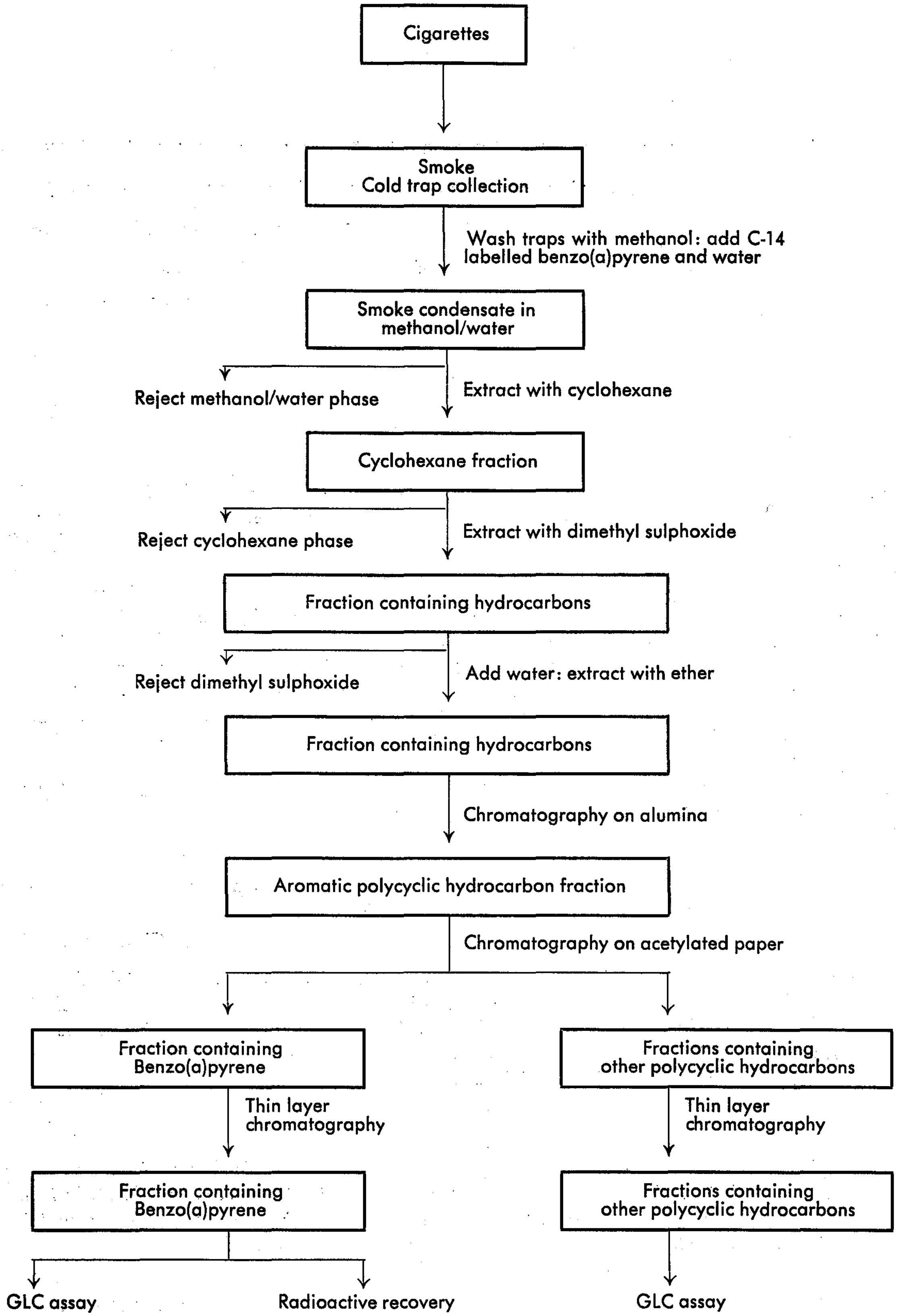
FIGURE 1

Isolation of polycyclic hydrocarbons from smoke



Paper Chromatography

Acetylated paper (23 % acetyl content) is prepared from *Whatman* 3MM paper by the method of *Spotswood* (4). The chromatogram is developed with methanol : ether : water (4 : 4 : 1 v/v).

Thin-Layer Chromatography

Plates (20 · 20 cm; 0.25 mm thick) are prepared from silica gel (Kieselgel G nach Stahl). The plates are activated at 110° C for 30 minutes and developed with cyclohexane : benzene (24 : 1 v/v).

Gas Chromatography

A *Perkin-Elmer* Model 800 instrument with dual columns and differential flame-ionisation detector is employed. Stainless steel columns (18 · $^{1}/_{8}$ inches) are packed with 5 % SE-30 methyl silicone polymer *(Fisher Scientific Co.)* carried on acid and alkali washed Celite (72–85 mesh). The nitrogen carrier gas flow rate is 25 ml/min.

Radioactive Counting Equipment

Determinations of radioactivity are made at "infinite thinness" by plating suitably sized aliquots (usually 0.2 ml) in quadruplicate onto dimple-type aluminium planchets and counting them in a thin end-window Mullard MX 123 geiger tube supplied by *Labgear Ltd.*

DETERMINATION OF BENZO(a)PYRENE

The method is shown in schematic form in Figure 1. Exposure to direct sunlight is avoided at all stages. The smoke condensate from 120 cigarettes is dissolved in methanol (120 ml), the solution evaporated (60 ml) and C-14 labelled benzo(a)pyrene (about 4 μg, accurately known; specific activity 6 μc/mg) in cyclohexane (2 ml) added, followed by water (15 ml). The solution is extracted with cyclohexane (5 · 25 ml) and the methanol/water layer rejected. The cyclohexane solution is extracted with dimethyl sulphoxide (3 · 25 ml) and the cyclohexane layer rejected. Water (150 ml) is added to the dimethyl sulphoxide solution, which is then extracted with ether (1 · 50 ml, 3 · 25 ml).

The ether solution is dried over anhydrous sodium sulphate, evaporated to dryness and the residue taken up as far as possible in hexane (5 ml) and chromatographed on alumina (100 g, *Woelm* neutral, grade 1) in a 25 · 3 cm column. The column is eluted with hexane (500 ml) and the eluate rejected. The small amount of residue which is insoluble in hexane is dissolved in benzene and added to the column which is then eluted with benzene (2000 ml).

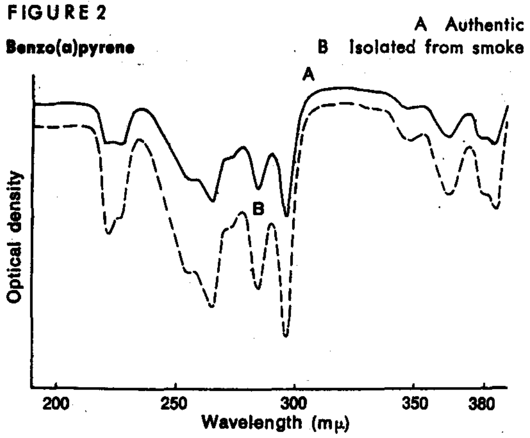
The benzene eluate is concentrated under nitrogen to a small volume (approximately 2 ml) and streaked in a narrow band across a strip (15 cm), of acetylated paper. The chromatogram is developed until the band containing the benzo(a)pyrene has moved 3–4 cm from the origin. The band is cut out and extracted with benzene (20 ml) in a soxhlet for 2 hours.

The benzene solution is evaporated under reduced pressure and in an atmosphere of nitrogen, the residue dissolved in ether (1 ml) and spotted on a thin layer plate. This stage is necessary to remove impurities extracted from the acetylated paper. The plate is developed for one hour, the benzo(a)pyrene spot dissolved in ether (5 ml) and an internal standard (n-triacontane, 10 μg) added for the gas-liquid chromatography stage. The solution is evaporated and the residue taken up in cyclohexane (2 ml). Aliquots of this solution are used to determine the percentage recovery from radioactivity measurements. The remainder of the solution is evaporated and the residue dissolved in ether or carbon disulphide and aliquots (10 μl) analysed by gas liquid chromatography. At a column temperature of 225° C, benzo(a)pyrene has a retention time of approximately ten minutes.

Examination of Other Polycyclic Hydrocarbons

Bands on the paper chromatogram, in addition to that due to benzo(a)pyrene, can be cut out, eluted and analysed in an analogous manner by gas-liquid chromatography. It may be necessary to change the internal standard from n-triacontane to another saturated hydrocarbon, e. g., eico-

FIGURE 2
Benzo(a)pyrene



sane, to prevent errors due to overlapping peaks, and with mixtures of polycyclic hydrocarbons it is advantageous to programme the column temperature from 150° C to 250° C at 5 degrees C per minute. The identity of a polycyclic hydrocarbon can be deduced from the R_f on the paper chromatogram, the retention time on the gas chromatogram and also by trapping out the peak and determining the ultra-violet absorption or fluorescence spectrum. For isothermally-operated gas chromatograms, the linear relationship between log retention time and carbon number, as used by *Ferrand* (5), has been found to be useful.

DISCUSSION AND RESULTS

Compared with an earlier method, developed by *Ayres, Horsewell* and *Hughes* (6), in which benzo(a)pyrene was determined by U.V. absorption spectroscopy, the present method has several advantages. It is more rapid, can be extended to other polycyclic hydrocarbons and, compared with U.V. absorption spectroscopy, the gas chromatogram offers increased sensitivity for measurement of very small amounts of benzo(a)pyrene. The gas-chromatographic peak given by less than 0.1 μg of benzo(a)pyrene can readily be measured. With more sensitive radioactive counting facilities, the number of cigarettes required for the determination of benzo(a)pyrene could be greatly reduced.

A comparison of the results obtained by both methods (Table 1) shows that good agreement was obtained, and the spectrum of benzo(a)pyrene isolated from smoke is given in Figure 2.

Approximate deliveries of other polycyclic hydrocarbons can be obtained if it is assumed that the percentage recovery of benzo(a)pyrene applies to allied compounds. The validity of this assumption is supported by an experiment in which a mixture of pyrene, benzo(a)fluorene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene (200 μg of each) was added to the smoke condensate from 100 cigarettes. At the end of the complete analytical procedure the individual

TABLE 1 **Delivery of benzo(a)pyrene**

Cigarette	GLC method. Recovery of benzo(a)pyrene (%)	Delivery (μg/100 cigs), corrected for recovery	
		GLC method	Previous method
A	76	5.0	5.2
A	64	4.6	
B	59	3.4	3.9
B	74	3.7	
C	65	3.0	2.9
C	57	3.2	
D	59	1.2	1.6
D	66	1.6	

TABLE 2 **Deliveries of polycyclic hydrocarbons**

Compound	Number of C-atoms from log. retention time	Delivery (μg/100 cigs)
11-H-Benzo(a)fluorene	17	4.1
Methyl-11-H-benzo(a)fluorene	18	3.5
Chrysene	18	9.6
Benzo(a)anthracene	18	7.6
Methylchrysene	19	10.3
Dimethylchrysene	20	2.6
Benzo(b)fluoranthene Benzo(j)fluoranthene	20	3.7
Benzo(a)pyrene	20	2.9
Indeno (1:2:3–c:d)pyrene	22	2.0

Qualitative identification of the following compounds has also been obtained: anthracene, phenanthrene, pyrene, fluoranthene, and a substituted chrysene (C_{21} compound).

FIGURE 3

11-H-Benzo(a)fluorene

A Authentic
B Isolated from smoke
C Methyl substituted 11-H-benzo(a)fluorene from smoke

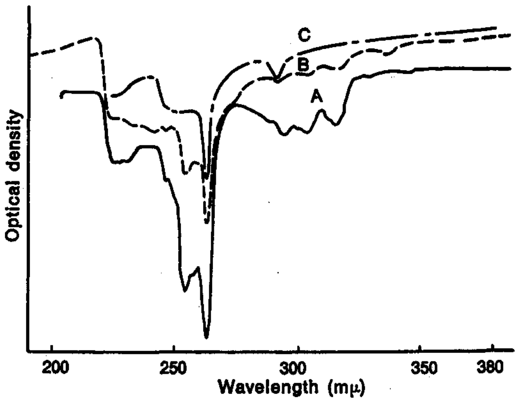


FIGURE 4

Chrysene

A Authentic
B Isolated from smoke
C Methyl substituted chrysene from smoke
D Dimethyl or ethyl substituted chrysene from smoke

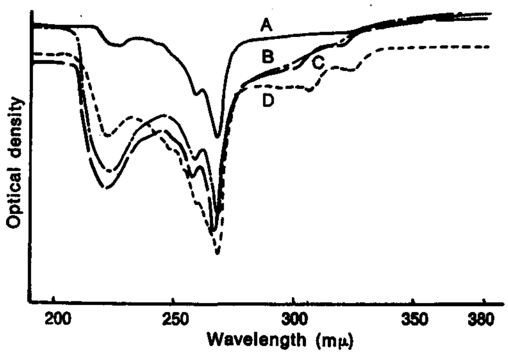


FIGURE 5

Benzo(a)anthracene

A Authentic
B Isolated from smoke

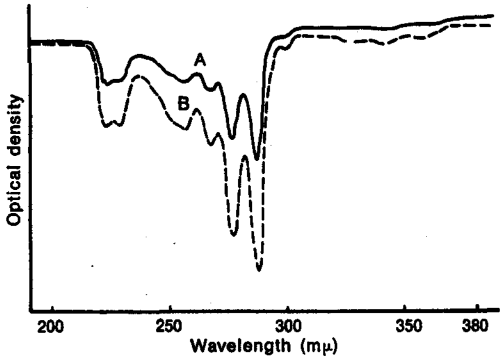


FIGURE 6

A Authentic
B Isolated from smoke

Benzo(b)fluoranthene

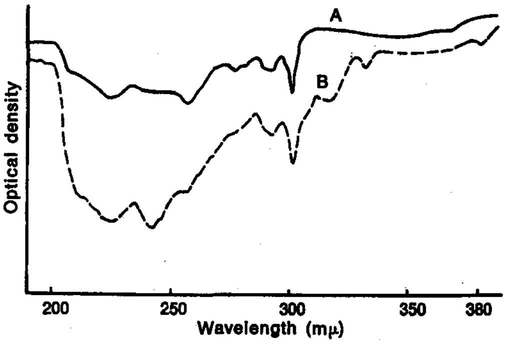


FIGURE 7

Indeno(1:2:3 – c:d)pyrene

A Authentic
B Isolated from smoke

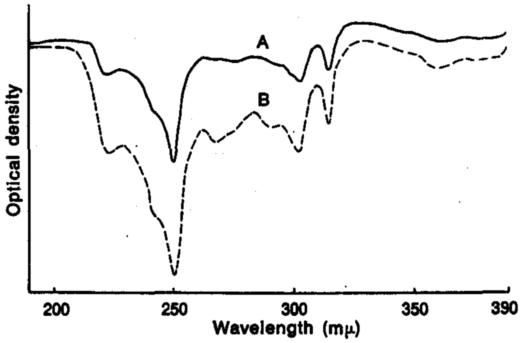
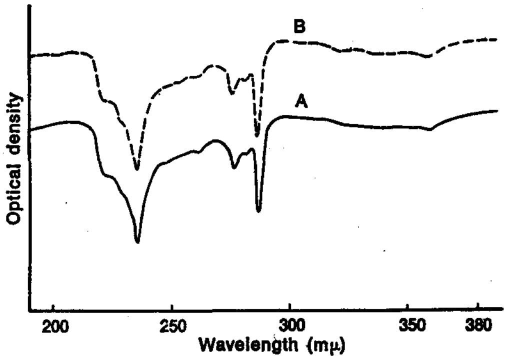


FIGURE 8

A Authentic
B Isolated from smoke

Fluoranthene



recoveries of these compounds, as determined by gas-liquid chromatography was in the range of 66 ± 3%.

The approximate deliveries of polycyclic hydrocarbons from a cigarette made from flue-cured tobacco are given in Table 2. In each case the column effluent has been trapped and satisfactory U.V. spectra obtained, e. g., Figures 3—8. Several of these compounds have almost identical retention times on the gas chromatogram and, without the prior separation of polycyclic hydrocarbon mixture on a paper chromatogram, complete resolution would not have been achieved.

SUMMARY

The determination of benzo(a)pyrene in cigarette smoke by a method based upon high-temperature gas-liquid chromatography is described. The extension of this method to the examination of other aromatic polycyclic hydrocarbons in smoke is briefly discussed.

ZUSAMMENFASSUNG

Die Bestimmung von in Cigarettenrauch enthaltenem Benzo(a)pyren mittels Gas-Flüssigkeits-Verteilungschromatographie bei hohen Temperaturen wird beschrieben. Die Anwendung dieser Methode auf die Untersuchung anderer in Rauch enthaltener aromatischer polycyclischer Kohlenwasserstoffe wird kurz erörtert.

RÉSUMÉ

La détermination du benzo-a-pyrène dans la fumée de cigarette au moyen d'une méthode de chromatographie gaz-liquide à hautes températures est décrite. L'extension de la méthode à l'examen d'autres hydrocarbures aromatiques polycycliques de la fumée est brièvement discutée.

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