

Analysis of Polycyclic Hydrocarbons*

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Because of the emphasis which the polycyclic aromatic hydrocarbons in cigarette smoke have received, a means of monitoring their presence in smoke is desirable. We wish to present the preliminary results of a gas-chromatographic investigation of the polycyclic hydrocarbon fraction of cigarette smoke as well as an improved spectrofluorometric procedure for the determination of benzo(a)pyrene in smoke.

There has been little application of the technique of gas chromatography to the polycyclic compounds of cigarette smoke. The use of conventional detectors, such as thermal conductivity or flame ionization devices, has not been fruitful, since the polycyclic aromatic compounds occur in the hydrocarbon fraction of smoke in minute concentrations accompanied by larger amounts of substances of closely similar chromatographic properties.

In this respect, the use of the electron capture detector appeared promising. In this device, the electrons emitted from a tritium source are collected and the resulting current is amplified. When a substance capable of capturing electrons flows through the device, the signal is diminished and the peak is recorded. This detector is extremely sensitive to the higher polycyclic aromatics and relatively insensitive to more saturated substances which might be present in the polycyclic fraction of smoke (1).

The gas-chromatographic conditions used are presented in Table 1. Good separation of known polycyclics was obtained with a silicone rubber phase. As little as .01 microgram of benzo(a)pyrene and corresponding amounts of other polycyclics could be detected. The detector response was a linear function of concentration for amounts less than one microgram, making quantitative measurements possible.

No information was obtained from chromatograms of unfractionated cigarette smoke due to "flooding" of the detector by excessive quantities of interfering substances. However, a simple fractionation yielded samples which gave well resolved gas chromatograms. The particulate phase of smoke

TABLE 1

Instrument:	Wilkens Aerograph Hy-Fi 600-B
Detector:	Electron capture
Column:	15-foot, 15 % SE-30 silicone on ABS anakrom
Column temperature:	Isothermal operation at 180°, 200°, 250°, 280° C
Detector voltage:	40 V. D. C.
Carrier gas:	Nitrogen

Gas-chromatographic
procedure for polycyclics
in smoke

* Presented to the 5th General Assembly of the CORESTA, Vienna, October 1964

FIGURE 1

Fraction 4

Aliquot = .08 cigt.

Column temperature = 200° C

EC detector

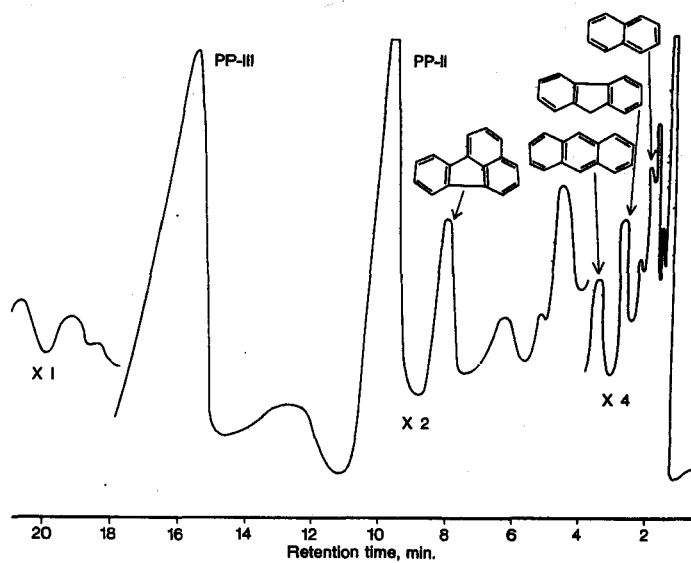


FIGURE 2

Fraction 5

Aliquot = .16 cigt.

Column temperature = 200° C

EC detector

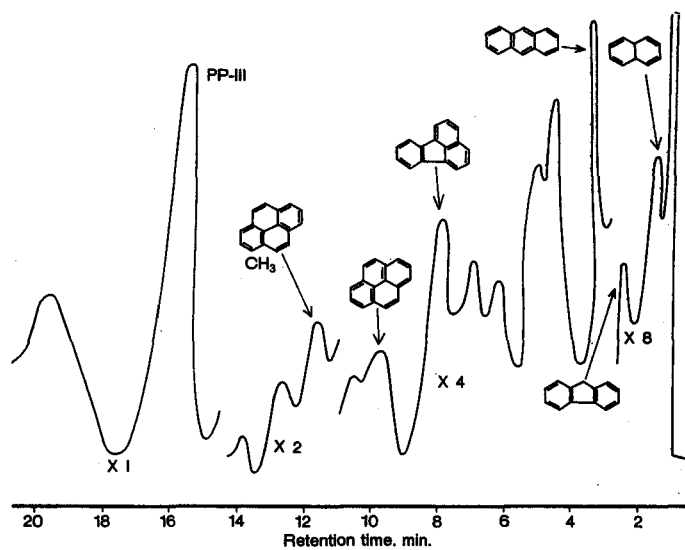


FIGURE 3

Fraction 6

Aliquot = .16 cigt.

Column temperature = 200° C

EC detector

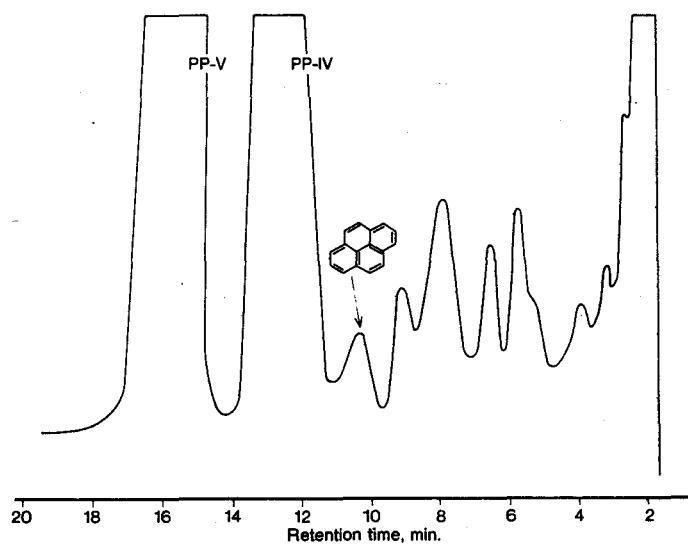


FIGURE 4

Fraction 7

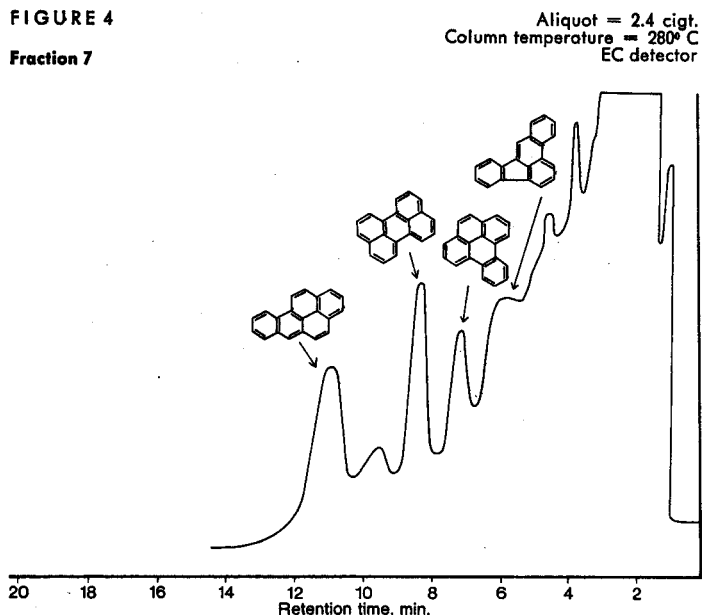


FIGURE 5

Fraction 8

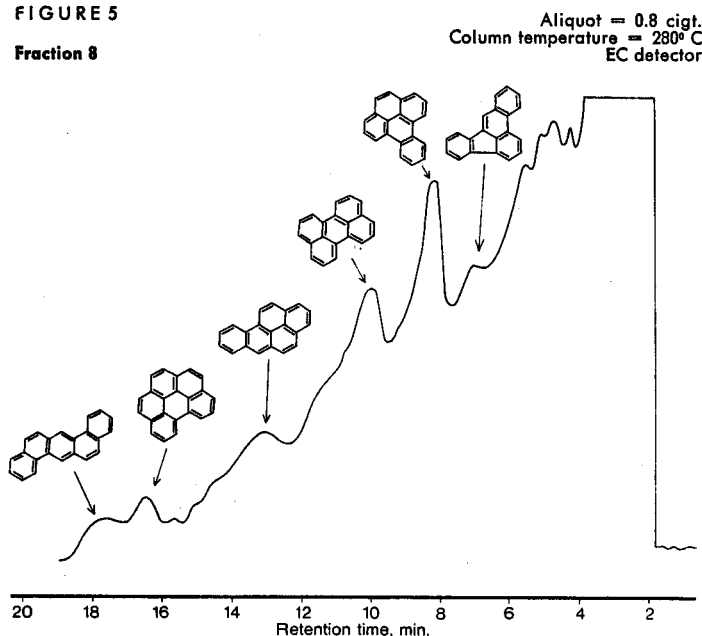


TABLE 2 Compounds identified in fractions 4-9

Compound	Fraction	Compound	Fraction
Naphthalene	3, 4	Carbazole	7
9-Methylantracene	3	Benzo(e)pyrene	7, 8
Fluorene	4, 5	Benzo(a)pyrene	7, 8
Biphenyl	4, 5, 6	Perylene	7, 8
Phenanthrene	4	Benzo(b)fluoranthene	7, 8
Anthracene	4, 5	Benzo(ghi)perylene	8, 9
Pyrene	5, 6	Dibenz(ah)anthracene	8, 9
1-Methylpyrene	5	Fluoranthene	4, 5

from 80 cigarettes was adsorbed on 80 g. of silica gel, and, after removal of the waxes by elution with 800 ml. of hexane, a series of 125 ml. fractions were eluted with 1:9 benzene:hexane. Preliminary experiments in which C¹⁴ - labeled anthracene and benzo(a)pyrene were added to smoke established that the former was removed in fractions 4 and 5 and the latter in fractions 7 and 8.

In Figure 1 is shown a chromatogram of fraction 4. The peaks marked in this and subsequent figures were identified by comparing retention times and fluorescence spectra with those of known samples*. In this fraction are found mainly polycyclic compounds with two and three rings; naphthalene, fluorene, anthracene, and fluoranthene are prominent**. In fraction 5 (Figure 2), the same compounds occur, along with pyrene and methyl-pyrene. In the following fraction (Figure 3), only pyrene was identified. Other 4-ring polycyclics anticipated in this fraction, such as chrysene or benz(a)-anthracene are obscured by two large peaks which will be discussed below.

In fractions 4, 5, and 6 the aliquots chromatographed corresponded to .08 to .16 cigarette. In subsequent fractions, the much smaller quantities of the higher polycyclics made necessary a considerably larger sample size. In fraction 7 (Figure 4), benzo(b)fluoranthene, benzo(e)pyrene, perylene, and benzo(a)pyrene are prominent. In fraction 8 (Figure 5) these are also seen, along with benzo(ghi)perylene and dibenz(ah)anthracene.

* This was accomplished by repetitive temperature programmed gas-chromatographic separations followed by isothermal chromatography until satisfactory fluorescence spectra were obtained. Purification stages were monitored with electron capture detection.

** Biphenyl, phenanthrene, and 9-methylantracene were detected in this fraction by isothermal gas chromatography at 180° C.

A summary of the compounds identified is given in Table 2. Of these, 9-methyl anthracene and biphenyl have not been previously reported in cigarette smoke (2). No attempt was made to establish the quantities present of these compounds. It is evident from the chromatograms that the amounts present generally decrease with increasing number of rings.

The substances designated PP in fractions 4, 5, and 6 are the large peaks mentioned above. These are not polycyclic aromatic compounds. Several of these were isolated. Their infrared spectra revealed that they were unsaturated aliphatic hydrocarbons having both di- and tri-substituted double bonds and having a CH_3 to CH_2 ratio of about 1. They were fluorescent, having emission maxima in the region 360–420 millimicrons. Their ultraviolet spectra had only smoothly increasing background adsorption from 400 to 250 millimicrons with no distinct maxima.

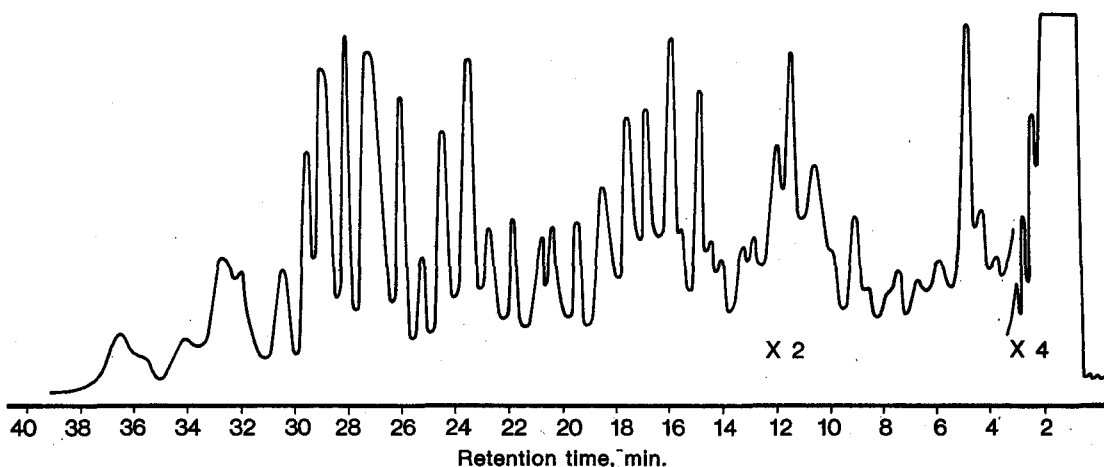
We believe these compounds to be linear polyisoprenoid polyenes of the type represented by phytadiene or solanesol, both of which occur in cigarette smoke. Their fluorescence implies the presence of at least 4 conjugated double bonds, although the fluorescence may be due to a minor aromatic impurity. These compounds have also been observed in the hydrocarbon fraction of smoke by other workers. We have found that these materials are separable from benzo(a)pyrene only by repeated chromatography on acetylated cellulose, and we conclude that they are the source of the background absorption in the ultraviolet which obscures the ultraviolet spectrum of benzo(a)pyrene isolated from cigarette smoke.

The chromatograms presented here give an overly simple picture of these fractions of cigarette smoke, because of the greater sensitivity of the electron capture detector to polycyclic aromatic compounds. In Figure 6 is shown a chromatogram of fractions 3 through 9 combined, using a thermal conductivity detector and temperature programming. The sample size was such that no polycyclic compounds were present in amounts great enough to give a visible peak with this detector. It is evident that a great many compounds other than polycyclics occur in these fractions.

Aliquot = 24 cigt.
Column temperature programmed 100/min. from 50° to 300° C
TC detector

FIGURE 6

Fractions 3–9 combined



The procedure outlined here was readily adapted for quantitative measurements. This was demonstrated for benzo(a)pyrene. The particulate phase of smoke* from 80 cigarettes was chromatographed on silica gel as described above. Fractions 7 and 8 were gas chromatographed, using the electron capture detector. Benzo(a)pyrene was estimated by comparing its peak height with a

TABLE 3

Comparison of gas-chromatographic B(a)P analyses with reported values

Cigarette	B(a)P, μg per cigarette	
	This work	Reported (3)
Bright tobacco	.026	.053
Burley tobacco	.011	.024

calibration curve of known benzo(a)pyrene. The results for Bright and Burley cigarettes are shown in Table 3, along with reported analyses of the same cigarette types (3). The gas-chromatographic results are too low, since no correction was made for any losses of benzo(a)pyrene in the procedure. There is,

* Collected by the procedure of W. B. Wartman, Jr., E. C. Cogbill, and E. S. Harlow, Anal. Chem. 31 (1959) 1705.

Particulate phase from 20 cigarettes

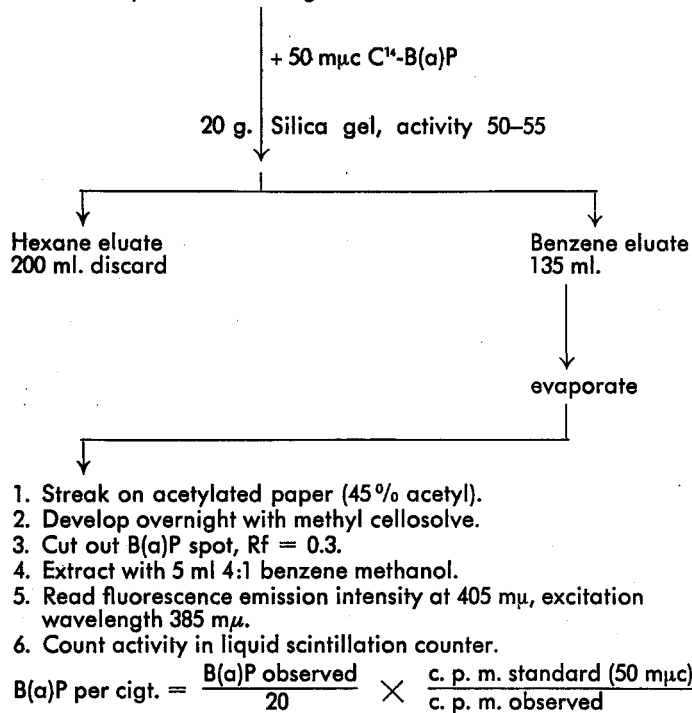


FIGURE 8

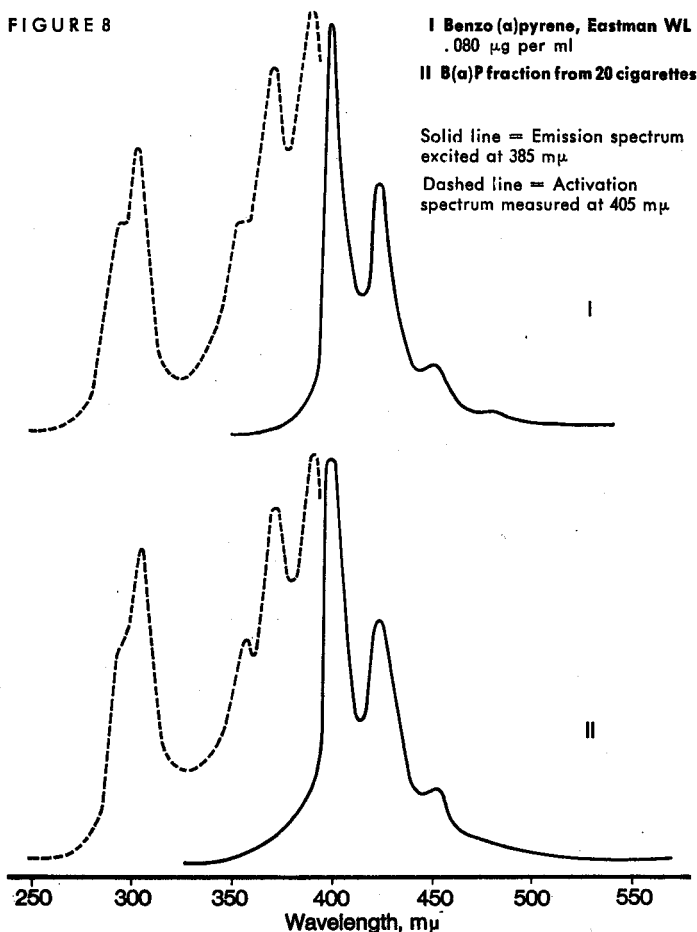


FIGURE 7

Analysis of B(a)P in cigarette smoke

however, good relative agreement with the published results.

The chief advantage of this method of analysis is that it can easily be extended to the determination of any of the compounds identified in the gas chromatograms of these fractions. In practice, however, benzo(a)pyrene is often taken as representative of the higher polycyclic aromatic compounds. Because of this, we have developed a procedure for benzo(a)pyrene alone which is suitable for routine multiple analyses.

This procedure, shown in Figure 7, is a modification of a previously published procedure (4). The modifications we have made represent an improvement of existing procedures in three respects. First, the use of methyl cellosolve as the developing solvent for the paper chromatogram gives better separation of benzo(a)pyrene from other polycyclic compounds than do previously reported solvent systems. This permits us to apply the total benzene eluate to the paper chromatogram, eliminating the need for careful fractionation in the silica gel chromatography with a concomitant saving in analysis time.

Second, the higher specificity of fluorescence excitation and emission as compared with ultraviolet absorption eliminates the necessity for multiple paper chromatograms. In particular, the materials responsible for the "background" absorption in the ultraviolet do not fluoresce when activated at 385 millimicrons, and a single paper chromatogram of the total benzene eluate gives a benzo(a)pyrene fraction whose fluorescence spectrum shows no evidence of any interfering fluorescent substances (Figure 8).

Finally, the greater sensitivity of fluorescence measurements permits the use of smaller sample sizes which in turn allows us to eliminate most of the previously reported initial separation steps. We have found, using the particulate phase from 20 cigarettes, that extractions to remove acidic and basic components and partitions between solvent pairs such as nitromethane: cyclohexane are entirely unnecessary.

In order to correct for losses during the procedure, C^{14} - labeled benzo(a)pyrene is added to the initial smoke sample and the final benzo(a)pyrene fraction, after the fluorescence measurement, is counted. Recoveries range between 80% and 90%, and the final figures are corrected for this loss. The recovery of known benzo(a)pyrene, added to smoke was 96% (Table 4). Multiple determinations on a commercial filter-tip cigarette indicated a standard deviation of about 10% of the mean value.

TABLE 4

Recovery of added benzo(a)pyrene

Sample	B(a)P
85 mm filter cigarette	.0179 μg per cigt.
Same + .0185 mg B(a)P per cigt.	.0357 μg per cigt.
Difference	.0178 μg per cigt.
Recovery of added B(a)P	96 %

It is difficult to compare the results of this analysis with published figures for benzo(a)pyrene in cigarette smoke, since the tobacco blend, cigarette construction, and smoking procedure all could affect the delivery of benzo(a)pyrene. The consensus of more recent reported values is approximately one part per million of benzo(a)pyrene in the particulate phase from commercially made American cigarettes (5). Our results are in good agreement with this value.

SUMMARY

A gas-chromatographic procedure using the electron-capture detector is presented. By fractionation of cigarette smoke on silica gel, followed by gas-chromatographic analysis of the fractions, fifteen polycyclic aromatic hydrocarbons were identified. Application of this procedure to quantitative analysis was demonstrated in the case of benzo(a)pyrene.

An analysis for benzo(a)pyrene in cigarette smoke which utilizes paper-chromatographic separation and fluorescence measurements is also presented. This procedure, which gives good precision with a 20-cigarette sample, represents a great simplification of existing methods.

ZUSAMMENFASSUNG

Es wird ein gaschromatographisches Verfahren beschrieben, das sich des Elektroneneinfang-Detektors bedient. 15 polycyclische aromatische Kohlenwasserstoffe wurden durch Fraktionierung des Zigarettenrauches auf Kieselgel und nachfolgende gaschromatographische Analyse der Fraktionen identifiziert. Die Anwendung der Methode wurde am Beispiel der quantitativen Analyse des Benzo(a)pyrens dargestellt.

Eine Bestimmung des Benzo(a)pyrens im Zigarettenrauch mittels papierchromatographischer Auftrennung und Fluoreszenzmessungen wird ebenfalls beschrieben. Diese Methode liefert bei Proben von 20 Zigaretten genaue Ergebnisse und stellt eine bedeutende Vereinfachung der bisher angewandten Verfahren dar.

RÉSUMÉ

Un procédé se servant d'un chromatographe à gaz muni d'un détecteur à capture d'électrons est décrit. 15 hydrocarbures aromatiques polycycliques ont été identifiés par le fractionnement par passage sur colonne de silice de la fumée de cigarette et, subséquemment, par l'analyse des fractions par chromatographie gazeuse. L'application de la méthode était démontrée par l'exemple de l'analyse quantitative du benzo-a-pyrène.

Une détermination du benzo-a-pyrène dans la fumée de cigarette utilisant la séparation par chromatographie sur papier suivie par une mesure de spectrofluorométrie est également décrite. Ce procédé donne des résultats bien précis lors de l'emploi d'un échantillon de 20 cigarettes et représente une simplification remarquable des méthodes précédentes.

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