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GAS CHROMATOGRAPHIC ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

The use of high-resolution capillary columns for the separation of polynuclear aromatic hydrocarbons (PAHs) by gas chromatography is described. With the exception of 1,2-benz[*a*]anthracene and chrysene, the other PAHs, with 2–7 rings, are at least 50% resolved.

The retention indices of 70 polycyclic aromatic hydrocarbons in temperature-programmed gas chromatography have been calculated by improved linear interpolation.

The efficiency of the gas chromatographic separation of PAHs was evaluated during these investigations. The accuracy and the reproducibility of the calculated retention indices and also the influence of several chromatographic parameters on the retention indices have been investigated.

The gas chromatographic detection of 5 ng of 10 PAHs indicated that the glass capillary column (16 m, 2.5% SE-52) allows a limit of detection of 0.5 ng.

INTRODUCTION

The carcinogenic properties of certain polycyclic aromatic hydrocarbons (PAHs), especially the benzpyrene fraction, have been recognized for more than 40 years[1]. Polynuclear arenes occur extensively in airborne particulate matter and their occurrence in smoked food products is of particular concern. The polynuclear arenes are formed during the incomplete combustion of all kinds of organic materials and dissipate via the atmosphere. It has been proved that this organic fraction, containing many PAHs, represents the main source of carcinogenic hydrocarbons ingested by humans[2–4], and it is therefore important to identify and determine these compounds.

The detection and identification of PAHs are generally carried out by UV spectroscopy[5], fluorescence[6–8], thin-layer chromatography[9–12], paper chromatography[13] and high-performance liquid chromatography[14–19]. These techniques have been used with varying success.

As the number of known carcinogenic polyaromatics has increased considerably, their detection has been carried out by more sophisticated techniques, in particular capillary column gas chromatography (GC) combined with mass spectrometry[20–24].

The objectives of this study were three-fold. Firstly, the retention indices of 70 polycyclic aromatic hydrocarbons in temperature-programmed gas chromatography were calculated by improved linear interpolation[25]. Secondly, the influence of several GC parameters (column length, concentration of the stationary phase, gas flow-rate, temperature programming and the injection system) on the separation and the reproducibility of the retention indices of the PAHs was studied. Thirdly, the GC detection limit of the PAHs was determined.

EXPERIMENTAL

A Varian 2700 gas chromatograph, equipped with a flame-ionization detector, was used for the analysis of the PAHs. The retention times of the PAHs and *n*-alkanes with even carbon number (C_{10}–C_{36}) were measured with a Hewlett-Packard 3352C data system. The capillary columns, statically coated with SE-52 as stationary phase, were conditioned at 300° for 16 h. The chromatographic operating conditions are summarized in Table I.

TABLE I

CHROMATOGRAPHIC OPERATING CONDITIONS FOR THE CAPILLARY COLUMNS

Detector	FID
Detector temperature	320°
Injector temperature	320°
Injection system	Glass insert of 2 mm I.D.; Chrompack glass solid injector
Liquid sample volume	1 μl with glass insert; 1–10 μl with solid injector
Column	Length 33.3 or 16.6 m; I.D. 0.5 or 0.25 mm; coated with 5 or 2.5 mg/ml of SE-52
Carrier gas (helium) flow-rate	6 or 3 ml/min
Column temperature	50° held for 5 min, then programmed at 6°/min to 320°; 70° held for 5 min, then programmed at 4°/min to 320°
Recorder attenuation	8, 32, 80 and 256

The restriction of the solid injector used was adapted in such a way that two thirds of the helium gas flowed through the restriction and one third through the capillary column. With temperature programming from 70° to 320°, the flow-rate of helium through the end of the capillary column was decreased from 6.3 to 3.7 ml/min. For capillary columns with a small internal diameter (0.25 mm), the back-pressure was so high that the use of a solid injector was excluded.

The PAH compounds were obtained from Aldrich (Milwaukee, Wisc., U.S.A.), Carlo Erba (Milan, Italy), Sigma (St. Louis, Mo., U.S.A.), Ferak (Berlin, G.F.R.), ICN (Plainview, N.J. U.S.A.) and Fluka (Buchs, Switzerland). The materials supplied by Carlo Erba were dissolved in benzene at a concentration of 10 ± 0.05 ppm. The other aromatic hydrocarbons were solids with purities of 95–99%.

The retention indices of the PAHs were calculated by improved linear interpolation. If $V(X)$ is the net retention volume of a PAH compound eluted between hydrocarbons with net retention volumes $V(1)$ and $V(2)$, the retention index of the unknown PAH X is defined by

$$I'(X) = I(1) + I(2) - I(1) \cdot \frac{\log V(X) - \log V(1)}{\log V(2) - \log V(1)} \quad (1)$$

where $I_t(X)$ represents the isothermal retention index. Replacing the logarithm of the retention volumes by the retention temperature, the equation becomes

$$I_p(X) = I(1) + I(2) - I(1) \cdot \frac{T(X) - T(1)}{T(2) - T(1)} \quad (2)$$

where $I_p(X)$ is the temperature-programmed GC retention index of the PAH X. After simplification of eqn. 2, the retention index of a PAH X, eluted between two hydrocarbons with even carbon number, is given by

$$I_p(X) = I(1) + I(2) - I(1) \cdot \frac{t(X) - t(1)}{t(2) - t(1)} \quad (3)$$

where $t(X)$, $t(1)$ and $t(2)$ represent the retention times of the PAH X and the two hydrocarbons.

The influence of gas chromatographic parameters on the resolution of the capillary column and the deviation of the retention indices of PAHs were carried out with a mixture containing 200 ng each of phenanthrene, anthracene, fluoranthene, 2,3-benzofluorene, pyrene, chrysene, perylene and benzo[*a*]pyrene standards.

RESULTS AND DISCUSSION

The retention indices of 70 polycyclic aromatic hydrocarbons are given in Table II.

TABLE II

RETENTION INDICES OF 70 PAHs IN TEMPERATURE PROGRAMMED GAS CHROMATOGRAPHY CALCULATED BY IMPROVED LINEAR INTERPOLATION

Peak No.	*PAH*	*Retention index, I*
1	Naphthalene	1172
2	Tetrahydroacenaphthene	1357
3	Biphenyl	1364
4	*o,o'*-Bitolyl	1387
5	Acenaphthylene	1426
6	1,8-Dimethylnaphthalene	1450
7	Acenaphthene	1461
8	Perhydrophenanthrene	1503
9	Fluorene	1555
10	*m,m'*-Bitolyl	1574
11	9-Methylfluorene	1579
12	*p,p'*-Bitolyl	1590
13	Octahydroanthracene	1667
14	2-Methylfluorene	1673
15	1-Methylfluorene	1679
16	Octahydrophenathrene	1693

(Continued on p. 112)

TABLE II *(continued)*

Peak No.	*PAH*	*Retention index, I*
17	Phenanthrene	1742
18	Anthracene	1752
19	7,8-Benzoquinoline	1755
20	Carbazole	1766
21	3,4-Benzoquinoline	1787
22	5,6-Benzoquinoline	1800
23	Acridine	1808
24	2-Methylphenanthrene	1860
25	2-Methylanthracene	1872
26	1-Methylphenanthrene	1883
27	9-Methylanthracene	1901
28	9-Butylanthracene	1934
29	Fluoranthene	2011
30	Pyrene	2057
31	9,10-Dimethylanthracene	2084
32	*p*-Terphenyl	2154
33	1,2-Benzofluorene	2161
34	Retene	2176
35	2,3-Benzofluorene	2178
36	Triptycene	2179
37	1-Methylpyrene	2212
38	Benzo[*c*]phenanthrene	2336
39	*o*-Quaterphenyl	2365
40	9-Phenylanthracene	2374
41	9,10-Benzophenanthrene	2392
42	1,2-Benzanthracene	2400
43	Chrysene	2400
44	2,3-Benzanthracene	2426
45	1,10-Dimethylbenz[*a*]acridine	2611
46	5,7-Dimethylbenz[*a*]acridine	2662
47	2,10-Dimethyl[*a*]acridine	2670
48	3,4-Benzofluoranthene	2694
49	11,12-Benzofluoranthene	2702
50	7,12-Dimethylbenz[*a*]anthracene	2711
51	Benzo[*a*]pyrene	2778
52	Perylene	2800
53	20(3)-Methylcholanthrene	2900
54	*m*-Quaterphenyl	2923
55	7-Methylbenz[*a*]anthracene	2939
56	9,10-Diphenylanthracene	3000
57	1,2,5,6-Dibenzacridine	3047
58	1,2,7,8-Dibenzacridine	3054
59	*o*-Phenylenepyrene	3081
60	1,2,5,6-Dibenzanthracene	3095
61	1,2,3,4-Dibenzanthracene	3103
62	1,2,7,8-Dibenzphenanthrene	3140
63	Benzo[*ghi*]perylene	3146
64	7*H*-Dibenzcarbazole	3157
65	Ananthrene	3183
66	1,2,3,4-Dibenzopyrene	3468
67	Coronene	3523
68	1,2,4,5-Dibenzopyrene	3540
69	3,4,9,10-Dibenzopyrene	3570
70	3,4,8,9-Dibenzopyrene	3588

TABLE III

THE RETENTION INDICES OF PAHs AS A FUNCTION OF THE CONCENTRATION OF THE STATIONARY PHASE

Column, 16.6 m × 0.5 mm I.D.; temperature programmed at 6°/min; gas flow-rate, 6 ml/min.

Injection	*Compound*	*Concentration of the SE-52 stationary phase*		*ΔI*
		2.5 mg/ml	*5 mg/ml*	
Glass insert	Phenanthrene	1753	1763	10
	Anthracene	1762	1773	11
	Fluoranthene	2023	2042	19
	Pyrene	2070	2086	16
	2,3-Benzofluorene	2190	2206	16
	Chrysene	2413	2431	18
	Benzo[*a*]pyrene	2790	2812	22
	Perylene	2815	2837	22
Solid injector	Phenanthrene	1739	1756	17
	Anthracene	1749	1765	16
	Fluoranthene	2009	2031	22
	Pyrene	2055	2080	25
	2,3-Benzofluorene	2176	2200	24
	Chrysene	2400	2424	24
	Benzo[*a*]pyrene	2769	2800	31
	Perylene	2795	2832	37

The retention data for phenanthrene, anthracene, fluoranthene, pyrene, 2,3-benzofluorene, chrysene, benzo[*a*]pyrene and perylene, calculated as functions of several GC parameters, are given in Tables III–VI.

TABLE IV

RETENTION INDICES OF PAHs AS A FUNCTION OF COLUNN LENGTH

Concentration of stationary phase (SE-52), 2.5 mg/,ml; temperature programme, 6°-min; gas flow-rate, 6 ml/min.

Injection	*Compound*	*Column length*		*ΔI*	*ΔI/m*
		16.6 m	*33.3 m*		
Glass insert	Phenanthrene	1753	1757	4	0.2
	Anthracene	1762	1766	4	0.2
	Fluoranthene	2023	2030	7	0.4
	Pyrene	2070	2079	9	0.5
	2,3-Benzofluorene	2190	2200	10	0.6
	Chrysene	2413	2422	9	0.5
	Benzo[*a*]pyrene	2790	2800	10	0.6
	Perylene	2815	2825	10	0.6
Solid injector	Phenanthrene	1739	1761	22	1.3
	Anthracene	1749	1770	21	1.3
	Fluoranthene	2009	2037	28	1.7
	Pyrene	2055	2086	31	1.9
	2,3-Benzofluorene	2176	2207	31	1.9
	Chrysene	2400	2434	34	2.0
	Benzo[*a*]pyrene	2769	2823	54	3.2
	Perylene	2795	2847	48	2.9

TABLE V

RETENTION INDICES OF PAHs AS A FUNCTION OF TEMPERATURE PROGRAMMING RATE

Column, 16.6 m × 0.5 mm I.D.; concentration of stationary phase (SE-52). 2.5 mg/ml; gas flow-rate, 6 ml/min.

Injection	*Compound*	*Temperature programming rate*		*ΔI*	*ΔI/°C · min*
		4°/min	*6°/min*		
Glass insert	Phenanthrene	1740	1753	13	7
	Anthracene	1750	1762	12	6
	Fluoranthene	2009	2023	14	7
	Pyrene	2054	2070	16	8
	2,3-Benzofluorene	2175	2190	15	8
	Chrysene	2394	2413	19	10
	Benzo[*a*]pyrene	2766	2790	24	12
	Perylene	2791	2815	24	12
Solid injector	Phenanthrene	1730	1739	9	5
	Anthracene	1740	1749	9	5
	Fluoranthene	1997	2009	12	6
	Pyrene	2041	2055	14	7
	2,3-Benzofluorene	2161	2176	15	7
	Chrysene	2378	2400	22	11
	Benzo[*a*]pyrene	2744	2769	25	13
	Perylene	2769	2795	26	13

The gas chromatographic resolution of 36 standard PAHs is illustrated in Fig. 1. Fig. 2 illustrates the gas chromatogram of these PAH standards and *n*-alkanes with even carbon number (C_{10}–C_{36}).

TABLE VI

RETENTION INDICES OF PAHs AS A FUNCTION OF CARRIER GAS FLOW-RATE

Column length, 16.6 m × 0.5 mm I.D.; concentration of stationary phase (SE-52), 2.5 mg/ml; temperature programming rate, 6°/min.

Injection	*Compound*	*Gas flow-rate*		*ΔI*	*ΔI/ml · min*
		3 ml/min	*6 ml/min*		
Glass insert	Phenanthrene	1768	1753	15	5.0
	Anthracene	1778	1762	16	5.5
	Fluoranthene	2044	2023	21	7.0
	Pyrene	2093	2070	23	7.5
	2,3-Benzofluorene	2212	2190	22	7.5
	Chrysene	2437	2413	24	8.0
	Benzo[*a*]pyrene	2824	2790	34	11.5
	Perylene	2848	2815	33	11.0
Solid injector	Phenanthrene	1754	1739	15	5.0
	Anthracene	1764	1749	15	5.0
	Fluoranthene	2030	2009	21	7.0
	Pyrene	2078	2055	23	7.5
	2,3-Benzofluorene	2200	2176	24	8.0
	Chrysene	2422	2400	22	7.5
	Benzo[*a*]pyrene	2800	2769	31	10.5
	Perylene	2828	2795	32	10.5

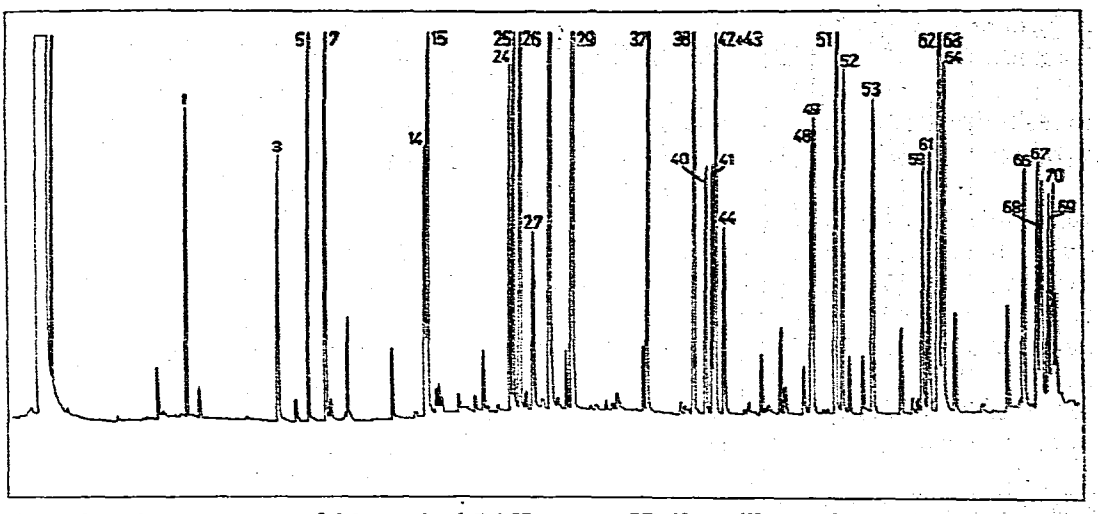
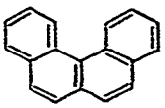


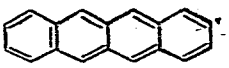
Fig. 1. Gas chromatogram of 36 standard PAHs on an SE-52 capillary column.

The signal-to-noise ratio for an injection of 5 ng each of biphenyl, phenanthrene, fluorene, fluoranthene, chrysene, benzo[*a*]pyrene, benzo[*ghi*]perylene, 1,2,3,4-dibenzanthracene, coronene and 3,4,9,10-dibenzopyrene standards is represented in Fig. 3.

The retention indices given in Table II show that the elution of PAHs on an SE-52 capillary column is a function of their molecular weights and their boiling or sublimation points. Another important factor is the stereochemical structure of the molecule. Considering the two isomers benzo[*c*]phenanthrene and 2,3-benzanthracene, there is a difference of 90 Kováts retention index units.



Benzo[*c*]phenanthrene
Mol. wt. 228
$I = 2336$



2,3-Benzanthracene
Mol. wt. = 228
$I = 2426$

The higher retention index for 2,3-benzanthracene may be mainly explained by the planar structure of the molecule. Therefore, the equilibrium between the two phases and also molecular diffusion will be faster for the benzo[*c*]phenanthrene molecule than for 2,3-benzanthracene.

An increase in the retention index is also observed when the PAHs are substituded by alkyl groups. On lengthening the alkyl group, the retention index tends to decrease; thus, the difference between 9-butylanthracene and 9-methylanthracene is only 33 Kováts retention index units. On the other hand, the attachment of a benzene ring to an aromatic hydrocarbon considerably increases the retention index (for 9-phenylanthracene $I = 2374$).

As we can observe from the results given in Table II, the adsorption of alkyl-aromatic hydrocarbons on the SE-52 stationary phase is dependent on the individual

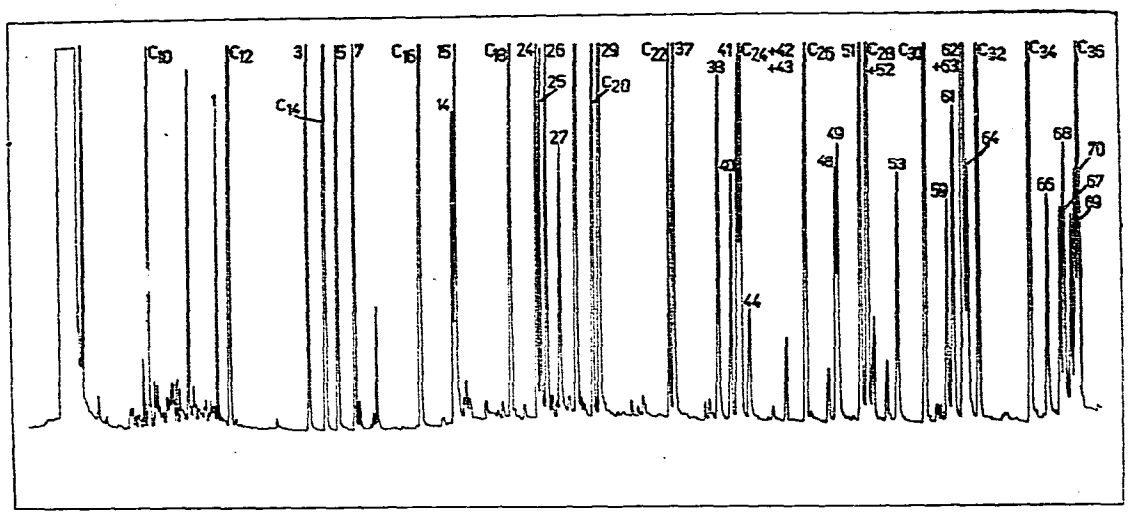


Fig. 2. Gas chromatogram of 36 standard PAHs and *n*-alkanes with even carbon number (C_{10}–C_{36}) on an SE-52 capillary column.

positions of the alkyl groups. Methylation of fluorene in the 2- or 1-position increases the retention index more than that in the 9-position. In fact, substitution by a methyl group to give branching at the α- or β-carbon atom was found to cause steric hindrance to adsorption, so that branching of an alkyl group has an effect on the retention indices of alkylaromatic hydrocarbons. These findings cannot be generalized to all alkylaromatic hydrocarbons, because 9-methylanthracene has a higher retention index than 2-methylanthracene. Finally, we can conclude that the retention index of the PAHs increases proportionately with the number of carbon atoms, with the

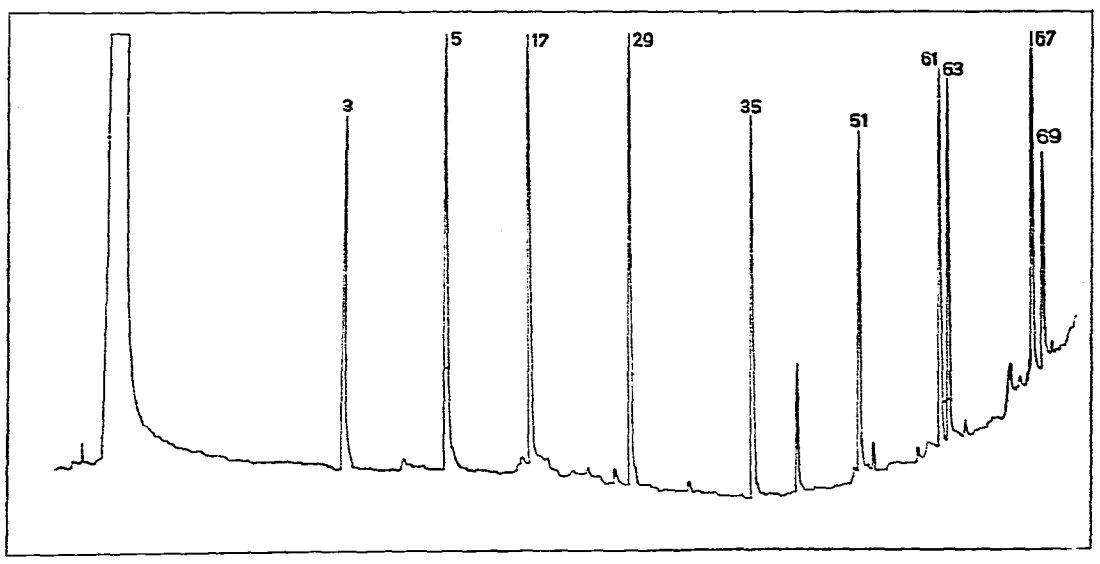


Fig. 3. Gas chromatogram of a 5-ng injection of 10 PAHs with 2–7 rings on an SE-52 capillary column.

molecular weight and with the boiling or sublimation point of the compound. In addition, the retention indices of alkyl-substituted polynuclear arenes are affected by steric interactions.

Many experiments have shown that the reproducibility of the retention indices of PAHs injected with a glass insert system is ± 1 Kováts retention index unit, whereas injection with a solid injector gives a reproducibility of ± 4 units.

The gas chromatographic analysis of the PAHs illustrated in Fig. 1 shows the resolution of several isomeric groups. In the chrysene group, consisting of six compounds, 1,2-benzanthracene and chrysene are not resolved. However, the other constituents are well separated. From the chromatogram it can also be seen that the important benzopyrene fraction, consisting of 3,4- and 11,12-benzfluoranthene, benzo[*a*]pyrene and perylene, are sufficiently well separated for identification. The significant improvements in resolution are illustrated by the separation of the constituents of the dibenzanthracene and the dibenzopyrene groups. The four isomers of dibenzopyrene and coronene are about 90% resolved.

In accordance with the gas chromatograms illustrated, we can conclude that a difference of 10 Kováts retention index units is sufficient to separate two PAHs.

From the results in Tables III–VI the following conclusions can be drawn.

(1) The retention indices of the PAHs are influenced by the concentration of the stationary phase, the length of the column, the temperature programming rate, the gas flow-rate and the injection system. Deviations of the retention indices as a function of the chromatographic parameters are given in ΔI units.

(2) Using a glass insert system, the ΔI value increases slightly with the column length, whereas it remains approximately constant with variation in the other chromatographic parameters. However, injection with a solid injector gives a minimal ΔI for an increase in the temperature programming rate and a maximal ΔI for an increase in the column length.

In addition to these results, as a function of the column length the ΔI value is especially dependent on the injection system. A similar effect, although less pronounced, is observed when the concentration of the stationary phase is increased. On changing the temperature programming rate or the gas flow-rate, this effect hardly occurs. This phenomenon may be attributed to the presence of solvent when a solution is introduced in an insert glass system. In fact, the influence of the solvent on the adsorption of the PAHs has been well established by the observation of the calculated retention indices. The results in Tables III–VI show that, with constant chromatographic parameters, the retention indices of the PAHs are higher for injection with a glass insert system than for an introduction with a solid injector.

(3) On varying the chromatographic parameters, it was found that ΔI increases according to higher molecular weight, higher boiling or sublimation point and increasing size of the molecule. Consequently, the adsorption of PAHs on SE-52 phase is retarded and the elution time increases.

Fig. 3 illustrates the gas chromatogram for a 5-ng injection of ten PAHs with 2–7 rings. The PAH constituents eluting between 70° and 200° have a minimal signal-to-noise ratio of 70:1, and those eluting between 200° and 320° have a minimal signal-to-noise ratio of 40:1. Considering the high resolving power of our capillary columns and the relatively short elution time, we can conclude that 0.5 ng of a PAH constituent can be detected and identified by capillary column gas chromatography.

CONCLUSION

The retention data given and the gas chromatograms illustrated show that two polycyclic aromatic hydrocarbons are resolved if their retention indices differ by at least 10 Kováts units.

The calculated ΔI values indicate that the most efficient gas chromatographic analysis of PAHs can be obtained under the following operating conditions: injection with a solid injector; concentration of the stationary phase (SE-52), 2.5 mg/ml; column length, 16–18 m; temperature programming rate, 4°/min; carrier gas (helium) flow-rate, 6 ml/min.

Considering the signal-to-noise ratio for the injection of 5 ng of a PAH with a solid injector and knowing that 1 μg of PAH per kilogram of sample corresponds to 1 ppb, it can be assumed that the detection limit for PAHs is about 0.5 ppb.

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