INVESTIGATION OF AROMATIC HYDROCARBONS BY REVERSED PHASE HPLC

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Introduction

Liquid chromatography has been widely used for the separation of aromatic hydrocarbon compounds both on analytical and preparative scale.

On the one hand the amount and composition of aromatics present in the petroleum fractions furnish important information for evaluating feedstocks and products. On the other hand the analysis of polycyclic aromatic hydrocarbons (PAH) is one of the most important tasks in environmental analysis. Polycyclic aromatic hydrocarbons have long been recognized as hazardous environmental chemicals. Several members of this class of compounds show considerable carcinogenic activity and the number of potentially dangerous compounds is increasing by the advance of systematic research carried out in this field.

They can occur as very complex mixtures and are wide-spread throughout the environment being detected in trace quantities in a wide variety of matrices varying from oils to sediments, soils, foodstuffs and airborn particulates.

In early publications open-column liquid chromatography has been used for the separation of aromatic hydrocarbon compounds.

As column packing materials mostly silica [1—3] and alumina [4—6] have been used by application of gradient elution techniques with different solvents. More recently gel packings, mainly Sephadex LH 20 have also been successfully used for separation of aromatics [7—10].

The main object of these fractionation steps was to provide a class separation of complex mixtures to reduce its complexity for further analysis. Aromatics can be separated from aliphatics and other compounds on each of the above adsorbents. The separation of aromatics according to ring numbers can advantageously be carried out on alumina. It is considered that the selectivity of alumina for aromatics is practically independent of substituent, whereas that of silica and Sephadex LH 20 gel is dependent [6, 11] of it.

A number of prefractionation procedures have been developed and utilized including the application of multistage techniques by using different adsorbents connected in series [9, 10, 12—13]. These combined prefractionation techniques are used to furnish simplified samples for further separation and determination of the individual aromatic compounds. For this application, in general, gas chromatography, recently mainly capillary gas chromatography and gas chromatography-mass spectrometry (GC-MS) have been used.

Because of the inherent low efficiency and time requirement of open-column liquid chromatography the resolution is restricted and only more or less overlapping fractions can be produced. With the introduction of high pressure, high performance liquid chromatography (HPLC) the separating power of the technique has considerably been increased. On the one hand, HPLC technique can be used for the prefractionation of complex samples furnishing well separated, more simplified fractions for further analysis [8, 11, 14—16]. On the other hand, analytical HPLC by using different column packings is suitable for the analysis of different samples furnishing additional or in some cases sufficient information for the identification and determination of aromatic compounds.

In addition to the adsorbents used for the separation of aromatic compounds the reversed phase packings developed for HPLC give new possibilities and specific selectivities for the above separation [17—18].

In this publication some results are given about the possible applications of reversed phase packings in HPLC for the separation and determination of aromatic compounds.

Experimental

Apparatus

The apparatus used in this work was a Waters high-pressure liquid chromatograph ALC Model 244 equipped with Model U6K universal injector, Model 6000 A pump and Model 440 UV detector set at 254 szm.

A 3.9 mm i.d. × 30 cm column packed with Waters - Bondapak C_{18} was employed. This packing has a monomolecular layer of octadecyltrichlorosilane chemically bonded to Waters 10 μ Porasil (silica).

Reagents

As eluent methanol-water and acetonitrile-water mixtures were used with different compositions at a flow rate of 1 ml/min.

Analytical grade methanol and acetonitrile (99% min) was obtained from Reanal Co. and distilled in glass before use. Distilled water was bidistilled and percolated through a column containing Porapak Q (100—120 mesh) packing.

All solvents were filtered through a Millipore type 0.5 μm filter and degassed in an ultrasonic bath before application.

Alkylbenzenes and polyaromatic hydrocarbon compounds were obtained from various sources and used as received.

Results and discussion

1. Separation of aromatic compounds

The retention of a great number of alkylbenzenes of different structures were measured using three solvent systems of different strength (methanol-water 6 : 4, 7 : 3 and 8 : 2).

By decreasing the methanol content of the mobile phase retention is increased.

To compare the retention of the compounds studied k' values (chromatographic capacity factors) are quoted as follows:

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

where t_R is the retention time of the compound investigated

t_0 is the retention time of an unretained compound.

For the determination of t_0, i.e. the dead time of the system, the retention time of methylene chloride or potassium iodide was taken.

The k' values of the alkylbenzenes investigated are given in *Table I.*

It has been shown in literature that the log k' values of alkylbenzenes increase linearly with increasing aliphatic carbon number on reversed phase

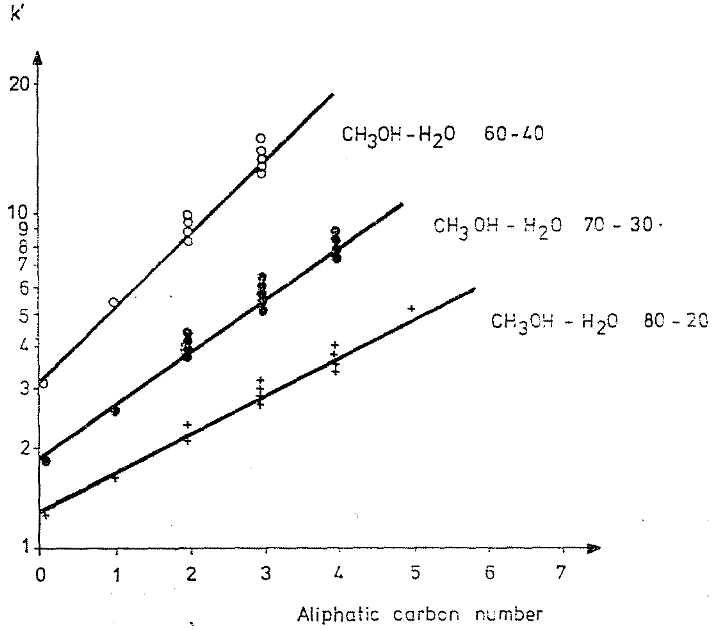

Fig. 1. Log k' values for alkylbenzenes as a function of aliphatic carbon number

Table 1

k' values for some alkylbenzenes on μ-Bondapack C_{18} reversed phase packing

Compound \ Solvent	$CH_3OH—H_2O$ 80 : 20	$CH_3OH—H_2O$ 70 : 30	$CH_3OH—H_2O$ 60 : 40	F Correlation factor
Benzene	1.30	1.88	3.20	3
Toluene	1.67	2.68	5.35	4
Ethylbenzene	2.09	3.79	8.68	5
o-Xylene	2.09	3.75	8.33	5
m-Xylene	2.18	4.10	9.29	5
p-Xylene	2.27	4.15	9.55	5
Isopropylbenzene	2.54	5.00	—	5
n-Propylbenzene	2.90	5.67	—	6
2-Ethyltoluene	2.73	—	13.33	6
3-Ethyltoluene	2.91	5.90	14.38	6
4-Ethyltoluene	2.82	—	—	6
1,2,3-Trimethyl-benzene	2.82	5.39	13.28	6
1,2,4-Trimethyl-benzene	2.91	5.77	14.86	6
1,3,5-Trimethyl-benzene	3.09	6.27	16.36	6
1,2-Diethylbenzene	3.36	7.27	—	7
Izobutylbenzene	3.54	8.18	—	6
n-Butylbenzene	3.82	8.77	—	7
1,2,3,4-Tetramethylbenzene	3.91	7.86	—	7
1,2,4,5-Tetramethylbenzene	3.91	—	—	7
Pentamethyl-benzene	5.10	—	—	6

packing [17]. First, we wanted to check the validity of this statement for alkylbenzenes of different structures.

The log k' values given in Table I are plotted as a function of aliphatic carbon number *in Fig. 1.* Although a certain amount of scatter is evident in the graph the experimental values can be fitted with a reasonably good straight line for each solvent system.
The equation of the straight line is

$$\log k' = a + bC \tag{2}$$

where C is the number of carbon atoms in the substituent alkyl chains;

a and b are experimental constants for the given solvent system.

On the basis of the above equation a rough prediction of the *k*' value of an alkylbenzene seems possible. However, as it is seen in the figure, alkylbenzenes having the same number of aliphatic carbons as substituents but in different chain lengths and positions, show considerably different retentions.

In order to study the influence of the length and position of the alkyl substituents an enlarged version of Fig. 1 was prepared, shown in *Fig. 2.*

It can be seen in the figure that there is no overlapping among the experimental values obtained for derivatives containing different numbers of aliphatic carbon. On the other hand, it can also be observed that there is a definitive trend in the retention values depending on the position of the alkyl-substituents. In this study no attempt was made to elucidate the mechanism of the observed retention behaviour, as this is beyond the scope of the work. Recent studies have shown that the retention mechanism on alkyl-bonded stationary phases is a complex phenomenon depending on several factors.

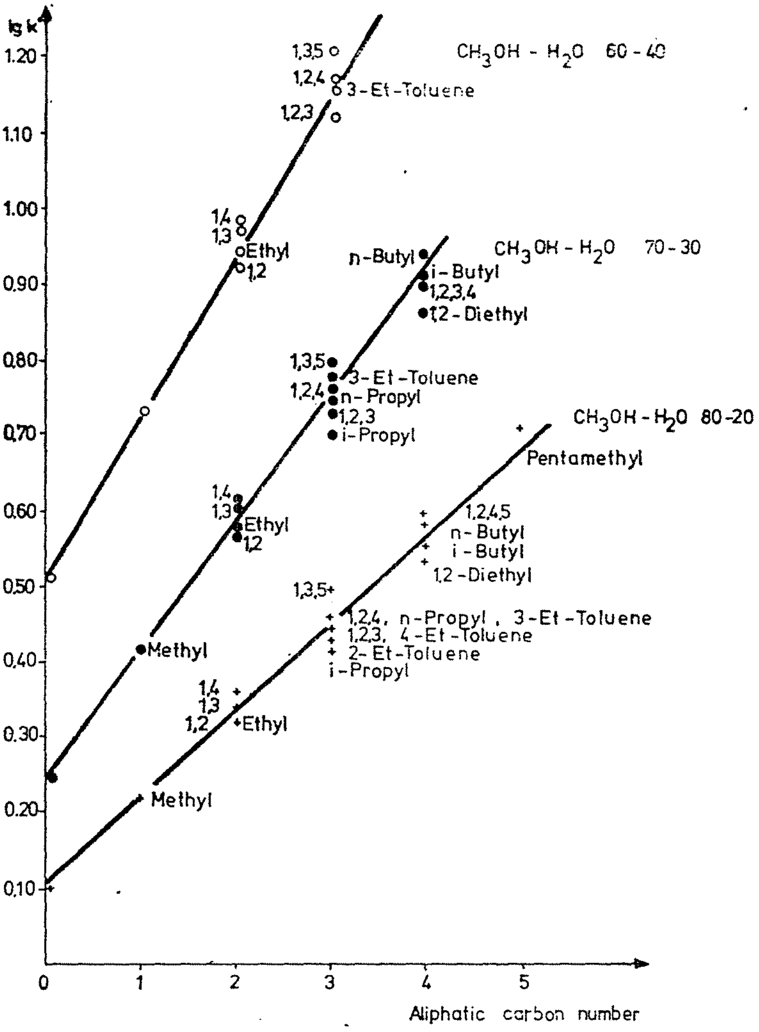

Fig. 2. Log k' values for alkylbenzenes as a function of aliphatic carbon number

Most recent studies apply the solvophobic theory for describing retention in reversed phase chromatography (19—20) where the solvatation of the solute molecule and its interaction with the solvent will determine the extent of retention.

From the values shown in Fig. 2 it can be seen that the planarity of the solute considerably influences its retention. On the other hand, tertiary and quaternary carbons, as well as, steric hidrance of solvatation may also have some effect on retention. From the practical point of view it can be established that positional isomers of benzene derivatives can also be separated and determined on reversed phase packings.

2. *Determination of polycyclic aromatic hydrocarbons (PAH)*

The determination of PAH compounds as potential carcinogenics is one of the most important tasks in enviromental analysis concerning a great number of different enviromental samples (water, sediments, airborn particulates, engine exhaust gases etc.).

In order to investigate the application of reversed phase packings for the determination of PAH retention values of available standards were measured in different solvent systems. *k*' values for some polycyclic aromatic compounds are given in *Table 2.*

Table 2

k' values for some polycyclic aromatic compounds on μ-Bondapack C_{18} reversed phase packing

Compound \ Solvent	$CH_3OH—H_2O$ 80 : 20	$CH_3OH—H_2O$ 70 : 30	$CH_3CN—H_2O$ 80 : 20	$CH_3CN—H_2O$ 60 : 40	F Correlation factor
Benzene	1.30	1.88	—	—	3
Naphtalene	1.97	3.42	—	—	5
Biphenyl	2.59	5.08	—	—	6
Acenaphtylene	2.32	4.18	—	—	5.5
Fluorene	3.14	6.46	—	—	7
Phenantrene	3.18	6.95	—	—	7
Anthracene	3.42	7.75	—	—	7
Fluoranthene	4.00	9.00	2.08	9.27	8
Pyrene	4.62	10.47	2.17	9.91	8
Chrysene	6.07	16.16	—	—	8
1,2-Benzoanthracene	5.73	14.54	2.58	13.73	9
Perylene	7.82	19.54	3.25	17.00	10
3,4-Benzofluoranthene	7.91	21.27	3.25	18.48	10
11,12-Benzofluoranthene	8.51	22.82	3.33	18.55	10
3,4-Benzopyrene	8.27	22.27	3.50	19.55	10
1,12-Benzoperylene	11.63	32.63	4.58	25.64	11

As it is seen in the table the retention increases by increasing ring number. First a correlation was attempted by plotting log *k*' values as a function of the ring number of compounds investigated. *Fig. 3* shows that the experimental values can roughly be represented by straight line, but there is a considerable scattering even overlappings among compounds having adjacent ring numbers. Again it can be seen in the figure that the structure, the planarity of the polycyclic aromatics has a considerable influence on their retention.

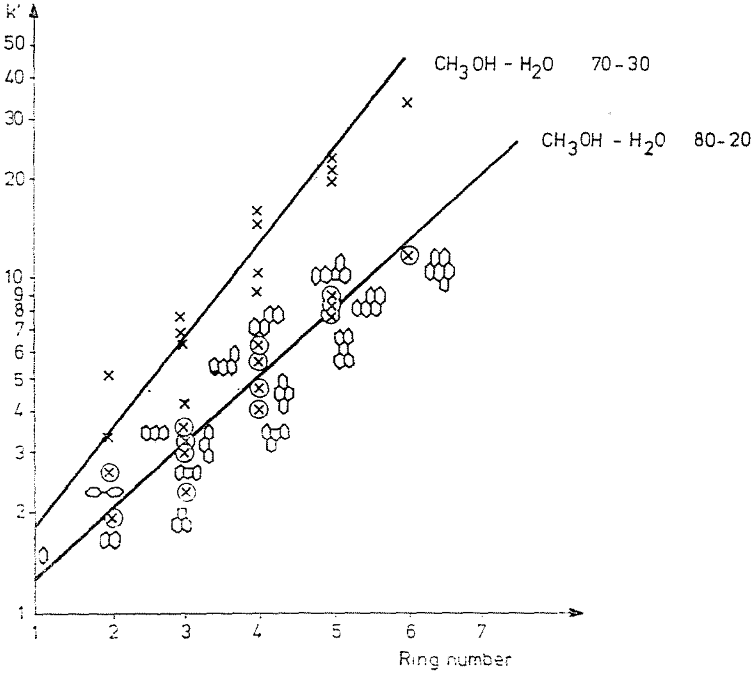

Fig. 3. Log k' values for polycyclic aromatic hydrocarbons as a function of ring number

Next we tried the correlation suggested by SCHABRON et al. [21] using an empirical correlation factor, denoted by F. The F correlation factor applies to alkyl-substituted and insubstituted aromatic hydrocarbons and relating log *k*' values to certain structural features of the compounds. The correlation factor F was defined as:

F = number of double bonds + the number of primary and secondary carbons—0.5 for a nonaromatic ring.

F values for alkyl-substituted benzenes and polycyclic aromatic compounds were calculated and are also given in Table 1 and 2, respectively.

Figure 4 shows the log *k*' values measured for all aromatic compounds in two solvent systems as a function of the correlation factor F. It can be seen that this type of correlation is really better than the former one and the experimental values can be fitted with a straight line. However, a certain amount of scattering can also be observed which indicates that all the structural features influencing retention are not included in the empirical correlation factor *F*.

There is a second point of practical importance, the overlapping of retentions among alkylbenzenes and polycyclic aromatic compounds. It can be seen in the figure that alkylbenzenes with increasing number of aliphatic carbons overlap with polycyclics of higher ring number.

This is demonstrated in *Fig. 5* which shows the chromatogram of a model mixture containing some alkylbenzenes and polycyclic aromatic compounds. It can be seen in the chromatogram that n-butylbenzene elutes after the three-ring compounds, pentamethylbenzene even among the four-ring compounds. From the practical point of view it means that samples containing alkylbenzenes and PAH compounds cannot be analysed on reversed phase packing because of overlapping retentions, only after an initial aromatic class separation according to ring number. As the alkyl-substituted derivatives of polycyclic compounds will also have higher retentions than the unsubstituted ones, the ring-type separation is required also for the higher compounds. This aromatic class

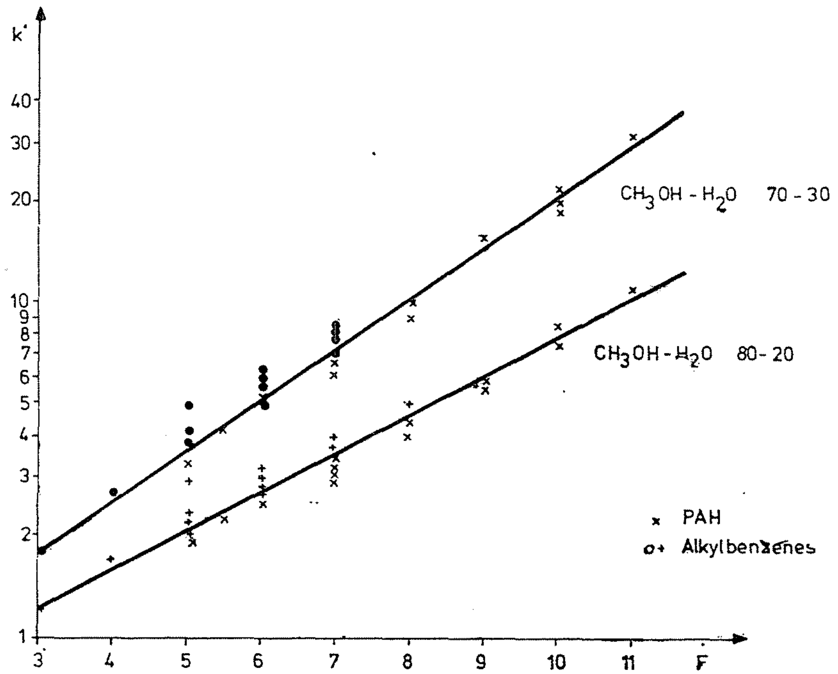

Fig. 4. Log k' values for aromatic compounds as a function of correlation factor F

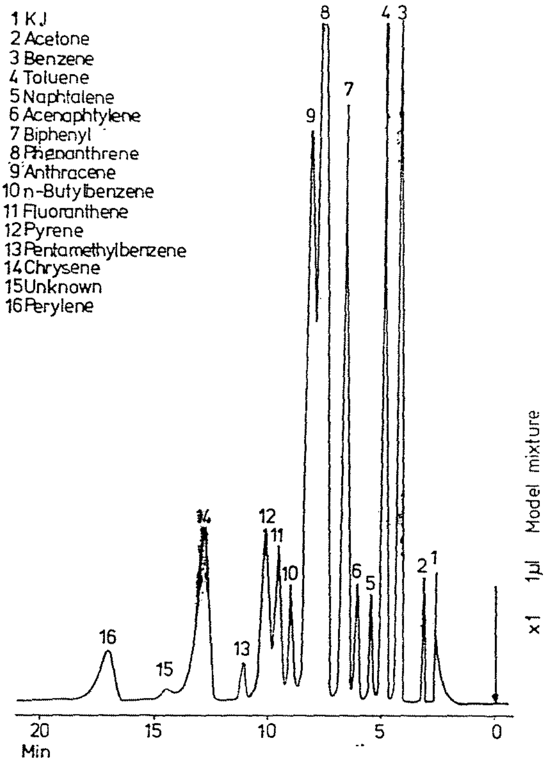

Fig. 5. Separation of a model mixture containing alkylbenzenes and polycyclic aromatic compounds

separation according to ring number can most efficiently be carried out on alumina column where the separation is less affected by the presence of alkyl substituents [6, 22]. Rechromatographing the fractions of ring type separation on reversed phase packing will reveal the extent of alkylation within these classes.

By inspecting the chromatogram of a model mixture shown in Fig. 5 it can be stated however, that reversed phase HPLC has an appropriate efficiency, selectivity and speed for the separation of aromatic hydrocarbons after an initial ring type separation.

3. *Direct analysis of aqueous samples*

The spectacular development of reversed phase chromatography can be explained by the fact that it emerges as a most general type of liquid chromatography which can replace various conventional techniques.

In enviromental analysis one of the most important advantages of reversed phase chromatography is that aqueous samples can directly be injected and analysed.

In order to investigate the application of the technique for direct analysis of water pollutants, first the limit of detection was studied. The waters U6K injector system renders possible the introduction of sample volumes up to 2 ml. In this experiment water samples each of 1 ml were introduced. Of course column efficiency and peak symmetry for such a large sample volume are inferior to those obtained for μl samples.

Figure 6 shows the chromatogram of 1 ml distilled water injected as a blank. For the purification of the water some preliminary investigations were carried out (bidistillation, ion exchange, open-column chromatography on Porapak Q) but we failed to obtain high-purity water. The contaminants remained were mostly chlorinated hydrocarbons revealed by other chromatographic techniques (HPLC, GC). As we wanted to investigate first of all the presence of PAH compounds no effort was made to produce ultra high purity water.

Figure 7 shows the chromatogram of 1 ml distilled water with measured amount (40 ng) of three PAH compounds.

Figure 8 shows the chromatogram obtained with the addition of 4 ng of each compound. Under the given conditions this seems to be the limit of detection, i.e. ng/ml or μg/l PAH contaminants can be detected from direct sample injec-

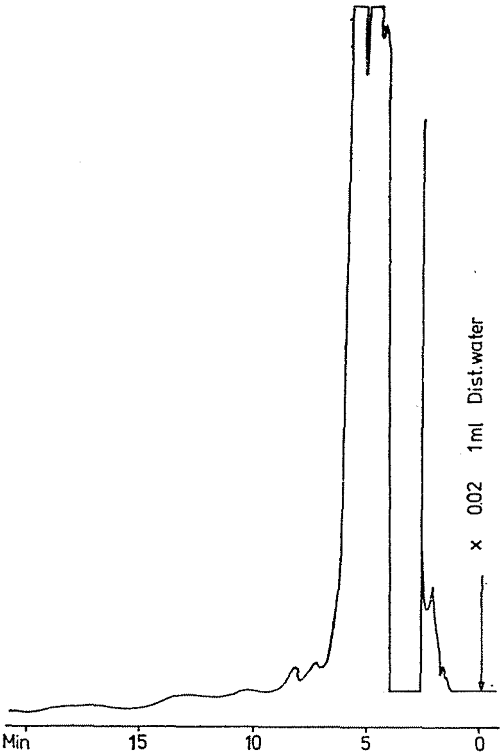

Fig. 6. Chromatogram of distilled water

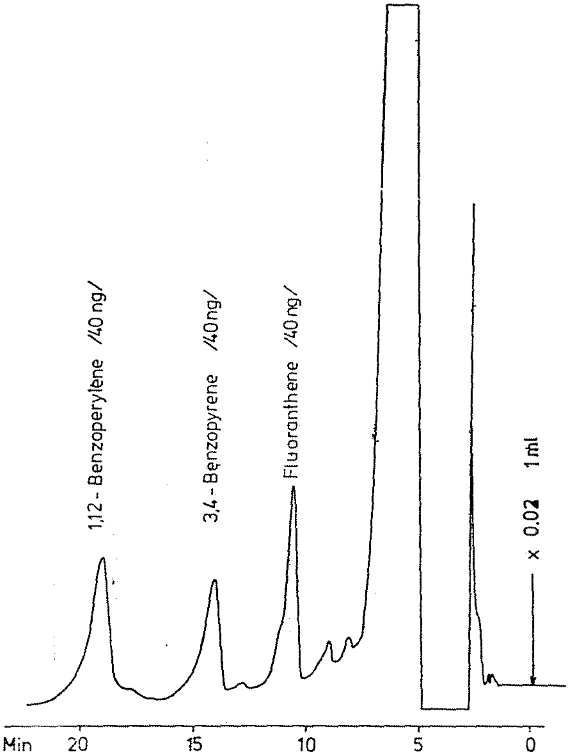

Fig. 7. Chromatogram of distilled water with measured amount (40 ng) of three PAH compounds

tion. This limit refers, of course, only to the conditions investigated, i.e. UV absorption at 254 nm. With variable wave length UV detector maximum absorbance can be found for the individual compounds at other than 254 nm and different wavelengths. Moreover, with the application of a spectrofluorometric detector sensitivity can be increased at least by an order of magnitude rendering possible the detection of PAH contaminants in the ng/l region.

Other possibility for trace analysis with reversed phase chromatography is the application of a short pre-column for enrichement of minute amount of contaminants. *Figure 9* shows a simple set-up for pre-column enrichement. A pre-column of 5 cm length, 3.9 mm i.d. was packed with 10 μ C_{18} reversed phase packing and connected between the injector and the analytical column. Parallel with the eluent reservoir a water reservoir was also connected. First water sample (100—500 ml) was pumped through the pre-column and directed by a three-way walve to the first collection flask. Then the water reservoir was closed, the three-way valve turned to connect pre-column to the analytical column and the eluent flow started. The eluent stream eluted the contaminants adsorbed on the pre-column directly to the analytical column and the chromatogram was recorded.

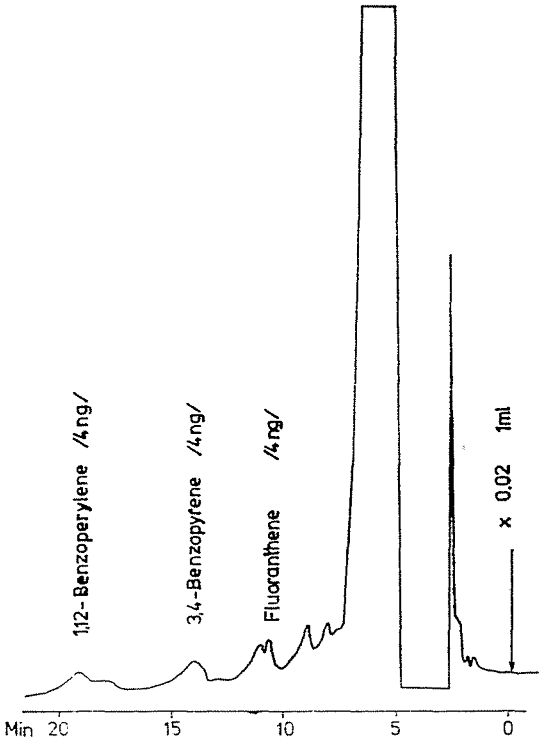

Fig. 8. Chromatogram of distilled water with measured amount (4 ng) of three PAH compounds

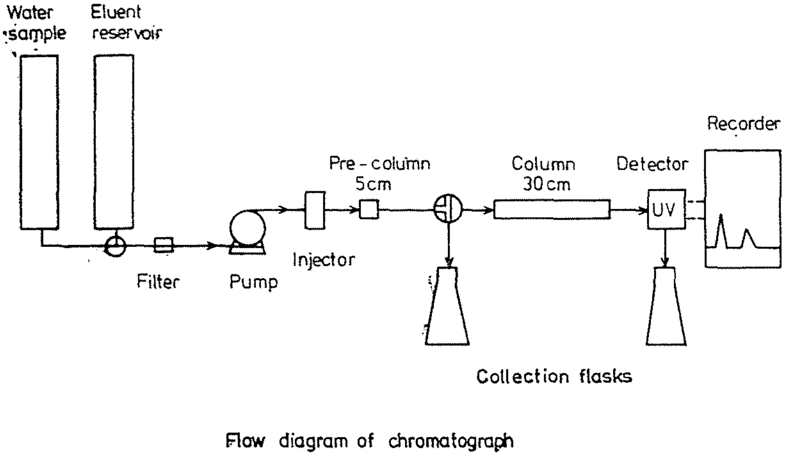

Fig. 9. Flow chart for pre-column enrichement technique

Figure 10 shows the chromatogram obtained with pre-column for a water sample of 100 ml containing added amounts of PAH compounds. By comparing Fig. 10 to Fig. 8 it can be seen that the limit of detection can be decreased by about two order of magnitude by using pre-column enrichement. By appropriate filling technique, using shorter pre-column and eliminating dead volumes in

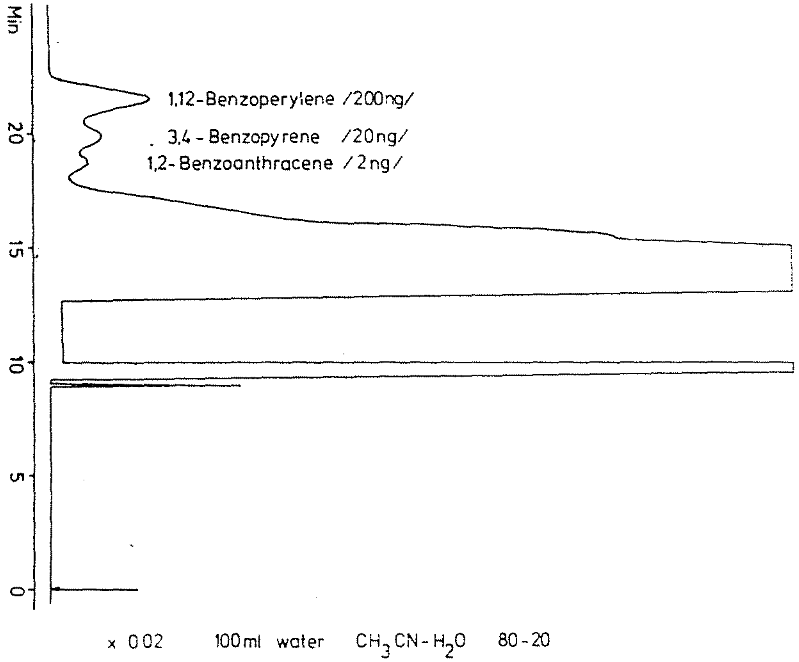

Fig. 10. Chromatogram of PAH compounds in water enriched by pre-column technique

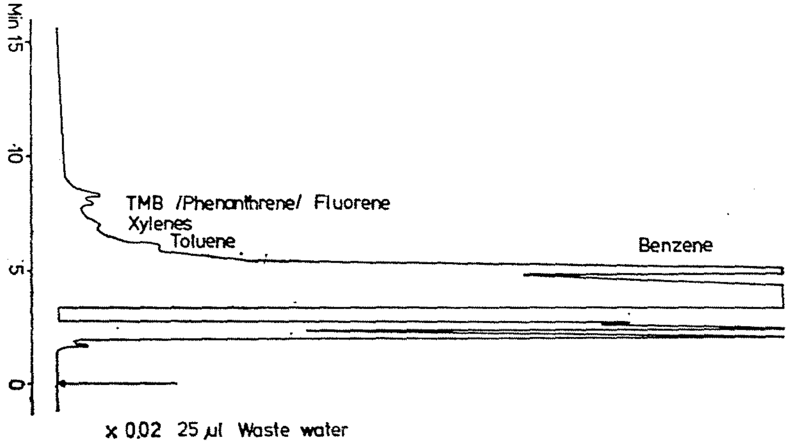

Fig. 11. Chromatogram of waste water from a petrochemical plant

the connections better efficiency and resolution can be achieved. By using a more sensitive detection technique as indicated before PAH compounds can be determined in water samples, i.e. in drinking water in the sub-nanogram/liter region by the direct enrichement technique on a pre-column.

Reversed phase chromatography can directly be applied also for the analysis of waste water of refineries, petrochemical and other chemical plants. *Figure 11* shows the chromatogram of 25 μl of waste water obtained from a petrochemical plant after the separation of the oil layer. The last peaks representing alkylbenzenes and polycyclic aromatic compounds correspond to ng amounts which means that these compounds are present in 0.1 mg/l i.e. in 0.1 ppm concentration. In this way the water quality after waste treatment or on the other hand, the efficiency of water treatment operations can be rapidly checked and evaluated.

Conclusions

Reversed phase chromatography is an efficient and simple technique for separation of aromatic compounds. Retention of alkyl-derivatives increases by increasing number of aliphatic carbons and a linear relationship can be given between log k' values and aliphatic carbon numbers. Retention is also influenced by the structure of the molecule and some of the isomeric alkyl benzenes can also be separated. Polycyclic aromatic hydrocarbons can be separated according to ring number on reversed phase column. The retention of alkyl-derivatives increases by increasing the number of aliphatic carbons and these will overlap with higher ring number compounds. The behaviour of PAHs with dissimilar ring structures but the same carbon content remains obscure. Alkylbenzenes of higher aliphatic carbon number overlap with the PAH compounds. For determination of PAH compounds in petroleum or enviromental samples a preliminary ring-type aromatic class separation is required on other adsorbents, expediently on alumina.

Aqueous samples can directly be injected and PAH compounds determined in the μg/l or under optimum condition in the ng/l region. The limit of detection can be improved by using pre-column technique for enrichement of the water pollutants.

Reversed phase chromatography furnishes a simple method for the determination of hydrocarbon pollutants in industrial waste water and for the evaluation of water treatment operations.

Acknowledgement

We would like to express our sincere gratitude to the Management of Waters GmbH, Kőnigstein for the half year loan of the high pressure liquid chromatograph used in this experiment.

Summary

Application possibilities of reversed phase HPLC were investigated for separation and assay of aromatic compounds. Retentions were measured and correlated for a number of alkylbenzenes and polycyclic aromatic compounds (PAH). The advantages of direct aqueous sample injection were investigated and detection limits under the given conditions determined. A simple arrangement for pre-column enrichement of water pollutants was shown and possibilities of this technique discussed.

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