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ON-LINE PRE-CONCENTRATION AND LIQUID CHROMATOGRAPHY OF AZAARENES USING NITROAROMATIC SORBENTS

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SUMMARY

3-(2,4-Dinitrobenzenesulphonamido)propylsilica and 3-(2,4-dinitroanilino)-propylsilica were used for the liquid chromatographic separation of azaaromatics, anilines and alkylaromatic amines. As polycyclic aromatic hydrocarbons with up to three or four rings and weak nitrogen bases of the pyrrole and aniline classes are retained less than pyridines and other azaarenes, the use of the above sorbents for on-line pre-concentration makes it possible to determine trace amounts of azaarenes in gasoline, kerosene and diesel fuel. Azaarenes are retained reversibly in mobile phases of moderate polarity such as dichloromethane, and this enables their quantitative recovery to be achieved.

INTRODUCTION

On-line pre-concentration has become popular in the trace reversed-phase liquid chromatography (RPLC) of aqueous samples such as industrial waste water, drinking water or biological fluids[1–3]. The pre-concentration is achieved as a result of the strong sorption of non-polar organic compounds on silanized silica[4,5], carbonaceous sorbents[6] or styrene–divinylbenzene copolymers[3]. The sorbents used for this purpose are placed in a small pre-column connected with an injection valve[1–6]. The pre-concentrated analyte is transferred into an analytical columns after switching the valve from the "load" to the "inject" position.

Nevertheless, the on-line pre-concentration is not inevitably linked with RPLC. It might be possible to pre-concentrate polar impurities that are present in non-polar samples using alumina or silica. An interesting means of achieving more selective pre-concentration is to use specific sorbents that form complexes with analytes. This mode of adsorption has been used by Gowie *et al.*[7] and Werkhoven-Goewie[8] for the on-line pre-concentration of substituted phenylureas and catecholamines on metal-loaded sorbents.

As azaarenes and other nitrogen bases are efficient donors of π- and n-electrons, the use of chemically bound electron acceptors for their on-line pre-concentration and subsequent LC analysis seems to be promising. The separation of azaar-

enes by charge-transfer LC has been atempted by Ray and Frei[9]. They prepared the first chemically bonded nitroaromatic stationary phase for LC by the reaction of surface silanols with p-nitrophenyl isocyanate and reported the successful separation of five azaarenes. Tetrachlorophthalimidopropylsilica which has electron-accepting properties, and a commercial NO_2-silica[11] have also been used for the LC separation of various azaarenes[10]. Recently, a novel sorbent, 3-(2,4-dinitrobenzenesulphonam-ido)propylsilica (DNSP-silica), has been found to be an efficient electron donor[12].

In this work, the concept of on-line trace enrichment in high-performance liquid chromatography (HPLC) was extended to charge-transfer interactions between an electron-accepting stationary phase and electron-donating azaaromatics. In addition to DNSP-silica, another nitroaromatic sorbent, 3-(2,4-dinitroanilino)propyl-silica (DNAP-silica), which has recently been examined by several workers[13–15], was also tested. The trace analysis of azaarenes present in primary light gasoline, kerosene or diesel fuel serves as an example of the practical utilization of these nitroaromatic stationary phases.

EXPERIMENTAL

Chemicals and solvents

2,4-Dinitrobenzenesulphonyl chloride (purum grade) was supplied by Fluka (Buchs, Switzerland). n-Heptane (Loba Chemie, Vienna, Austria) and dichlorometh-ane (Lachema, Brno, Czechoslovakia), used for the preparation of mobile phase, and the other solvents used, were of analytical-reagent grade.

Primary light gasoline with a boiling range of 90–160°C and a low content of aromatics and kerosene with a boiling range of 150–240°C and 18.8% of aromatics were obtained from Chemical Works (Litvínov, Czechoslovakia). Diesel fuel of normal quality was obtained at a filling station.

Solutes obtained either as commercial samples (Koch-Light Labs., Colnbrook, U.K.; Fluka; Lachema) or generously supplied by Prof. Ferles and Dr. Černý were used as obtained.

Preparation of sorbents

A 5-g amount of Separon SI VSK silica gel for HPLC (10–12 μm) (Laboratorní Přístroje, Prague, Czechoslovakia) modified with 3-aminopropyltriethoxysilane was used for the preparation of DNSP-silica; 0.92 mmol/g of amino groups was found by titration. The sorbent was suspended together with 2 g of finely powdered sodium hydrogen carbonate in 50 ml of water–methanol (1:1); 0.5 mmol of 2,4-dinitroben-zenesulphonyl chloride (1.33 g) was dissolved in 10 ml of dry acetone and added dropwise to the suspension of silica gel. After 30 min the reaction was completed and the mixture was left to stand overnight. The silica gel modified with DNSP groups was filtered off, washed with warm water, methanol and acetone and dried at 60°C. The bright orange product was sieved through a 30-μm mesh and used for the prep-aration of columns. The amounts of DNSP-silica with particle diameter d_p = 5 and 20 μm were prepared in the same way. The preparation of DNAP-silica has been described elsewhere[13].

Chromatographic experiments

The sorbents were packed under a pressure of 6000 p.s.i.g. into stainless-steel columns (250 × 4 mm I.D.) using tetrachloromethane for the preparation of a slurry (0.2 g/ml). Chromatographic measurements were performed on an apparatus composed of an Altex 110A reciprocating pump (Altex, Berkeley, CA, U.S.A.), a Rheodyne 70-10 sampling valve (Rheodyne, Berkeley, CA, U.S.A.) and a UVM-4 detector (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) operated at 254 nm. The retention of polynuclear aromatic hydrocarbons (PAHs) was checked by means of a fluorimeter (Model FS 970; Kratos, Westwood, NJ, U.S.A.) operated at λ_{ex} = 254 nm and equipped with a KV 370 emission filter.

Dichloromethane was used as the mobile phase; the addition of 0.5% (v/v) of isopropanol suppressed tailing. The columns were equilibrated overnight with the mobile phase before measurement. The solutes dissolved in dichloromethane are stable for many days if kept in a cool and dark place; the use of chloroform or tetrachloromethane for sample preparation should be avoided owing to their reactivity towards amines (*e.g.*, ref. 16). Benzene was used as an unretained solute to determine t_0 (the retention time of an unretained solute). Each measurement, carried out at ambient temperature, was repeated three times.

A pre-column described elsewhere[4] was used for on-line pre-concentration. The sorbents used for pre-concentration were packed manually into the pre-column (3 × 4 mm I.D.) connected to the sampling valve instead of a capillary loop. During the pre-concentration step, samples were passed through the pre-column by means of a glass syringe.

Breakthrough curves

The pre-column was connected directly with the UV detector. The solutions of sorbates were pumped through the pre-column by means of the Altex 110A pump (valve in the "inject" position). Desorption was carried out in dichloromethane pumped through the pre-column by means of a second pump or a syringe (valve in the "load" position). The breakthrough curves were observed at 254 nm.

RESULTS AND DISCUSSION

Retention of nitrogen bases

The capacity factors (k') of several selected azaaromatics, anilines and alkylaromatic amines are summarized for both sorbents in Table I. Pure dichloromethane was been modified with 0.5% of isopropanol to suppress the tailing of strongly retained solutes. All solutes examined showed a higher retention greater on DNSP-silica than on DNAP-silica. The former sorbent seems to be the more efficient electron acceptor, as also found in the separation of PAHs[12].

The values of k' suggest that the retention of the above solutes is influenced by their basicity. Thus, relatively weak bases such as anilines or pyrroles are retained less than azaaromatics that contain a pyridine ring. Alkylaromatic amines such as benzylamine and phenylethylamine are very strongly retained, as also are azaaromatics with several pyridine rings. Thus, *o*-phenanthroline and 2,2'-bipyridyl are retained very strongly. Polycondensed aromatic hydrocarbons (PAHs) with up to three or four rings are eluted before weak nitrogen bases, which permits a group separation.

TABLE I

CAPACITY FACTORS OF AZAAROMATICS, ANILINES AND OTHER NITROGEN BASES ON DNAP- AND DNSP-SILICA

Column, 250 × 4 mm I.D.; mobile phase, dichloromethane containing 0 5% of isopropanol; k' values calculated assuming $k'_{benzene} = 0$.

Compound	Capacity factor	
	DNSP-silica	DNAP-silica
Aniline	0.7	0.3
2-Methylaniline	0.6	0.3
Diphenylamine	0.2	0.1
N,N-Dimethylaniline	0.2	0,1
1,2,3,4-Tetrahydroquinoline	0.4	0.2
Indole	0.5	0.3
Pyrrole	0.6	0.3
Carbazole	0.2	0.1
Pyridine	4 0	2.6
2,6-Dimethylpyridine	1.8	1.0
2,4,6-Trimethylpyridine	6.4	4.1
Quinoline	3.2	1 7
2-Methylquinoline	2.7	1.5
3-Methylquinoline	2.8	1.5
4-Methylquinoline	4.6	2.9
8-Methylquinoline	1.1	0 6
Isoquinoline	4 6	2 7
Acridine	2.9	1.1
2,2'-Bipyridyl	> 20	13 5
o-Phenanthroline	> 20	18.2
Benzylamine	> 20	> 20
2-Phenylethylamine	> 20	> 20
Anthracene*	0.2	0.1
Pyrene*	0.4	0.2
Picene*	0.7	0.2

* k' values of PAHs added for comparison

A methyl group attached to the parent azaaromatics such as pyridine or quinoline influences their retention by both polar and steric effects. Substitution of the *para* position enhances retention whereas *ortho* substitution leads to decreased retention (Table I). Thus, 2,6-dimethylpyridine is retained more weakly than pyridine, despite the greater basicity of the former compound. The results in Table I therefore indicate that n–π complexes are probably formed between azaaromatics and bonded nitroaromatic ligands.

Pre-concentration procedure
        The relatively strong but reversible complexation between the nitrogen bases and the nitroaromatic stationary phases makes it possible to pre-concentrate these compounds from relatively non-polar hydrocarbon mixtures such as gasoline or diesel fuel, in which they are often present as trace impurities. The on-line pre-concentration is carried out with a small pre-column packed with DNAP- or DNSP-silica and placed up-stream of the analytical column.

The optimal volume of the pre-column, V_p, depends on the volume, V_c, and the plate number, N_c, of the analytical column used and also the k'_A value of the analyte A that is pre-concentrated. Using the conditions derived by Van Deemter et al.[17], we found[4] that

$$V_p/V_c \leq (1 + k'_A)/2\sqrt{N_c} \tag{1}$$

In this work, N_c is $4 \cdot 10^3$ plates and the dimensions of analytical column are 250 × 4 mm I.D. This means that the use of a 3 × 4 mm I.D. pre-column should not cause additional band broadening for analytes with $k'_A > 1$.

We proposed that the enrichment factor, f, of an analyte A should be expressed on the basis of mass balance[4]. Thus, f is the ratio between the volume of sample passed through the pre-column, V_s, and the void volume of the pre-column, V_0. As V_s may not exceed the break through volume, V_b, as otherwise the pre-concentration does not proceed quantitatively, the following conditions holds:

$$f = V_s/V_0 \leq V_b/V_0 = (1 + k'_{A,sample}) (1 - 2\sqrt{hd_p/L_p}) \tag{2}$$

where L_p is the length of the pre-column and h is the reduced plate height, depending on the flow-rate of the sample through pre-column and other factors according to the Van Deemter equation.

The term $(1 + k'_{A,sample})$ is the relative sorption capacity of the pre-column for A and the term $(1 - 2\sqrt{hd_p/L_p})$, which takes into consideration axial dispersion, express how large a part of the sorbent bed can be used for the quantitative pre-concentration. Thus, the best way to achieve a suitable f is to use sorbents that ensure the largest $k'_{A,sample}$.

Werkhoven-Goewie[8] recently recommended the use of relatively long and narrow-bore pre-columns having the term $(1 - 2\sqrt{hd_p/L_p})$ close to unity. These pre-columns are packed with particles of $d_p = 5$ or 10 µm using the slurry packing technique. We have packed short pre-columns with particles of $d_p = 5$, 10 and 20 µm using the dry "tap and bounce" technique. Satisfactory reproducibility of the pre-column efficiency was found only if 20-µm particles were used; the term $(1 - 2\sqrt{hd_p/L_p})$, calculated from the breakthrough curves, is about 0.7, indicating that the values of the reduced plate height are about 3.5 when the flow-rate of the sample is about 1 ml/min. The bed formed by 20-µm particles has a good permeability, allowing easy manual sampling. The use of 5- and 10-µm particles often leads to pre-columns packed very poorly ($h > 20$).

It is an advantage if V_s is close to V_b and the maximum value of f is achieved. A knowledge of $k'_{A,sample}$ is therefore very important. It can be either determined experimentally or predicted from semi-empirical relationships as proposed by Brinkman and co-workers[6,7,18]. The authors used k'_A values measured in water–organic mobile phases of varying composition to extrapolate the value of $k'_{A,water}$ according to a relationship proposed by Schoenmakers et al.[19]. The second method used by the

above authors[6] is to calculate $k'_{A,water}$ from increments tabulated for pyrocarbon–water systems. In principle, it might also be possible to use a similiar approach in our work. Nevertheless, these semi-empirical procedures have several weak points if applied to samples with varying properties.

Breakthrough volumes for real samples

The polarity of industrially produced hydrocarbon mixtures such as gasoline, kerosene or diesel fuel depends on their composition, which is usually unknown. Also, the samples may contain varying amounts of PAHs with up to three or four rings and other strongly retained polar compounds.

If the adsorption of an analyte is described by the Langmuir isotherm, *e.g.*, the amount of analyte preconcentrated, m_A, is given by

$$m_A = V_P \rho S (1 - \varepsilon_T) \Theta_A C_{ads,A} \tag{3}$$

where the dimensionless surface coverage Θ_A is given by

$$\Theta_A = \frac{K_A c_A}{1 + K_A c_A + \sum K_i c_i} \tag{4}$$

In eqns. 3 and 4, K_A and K_i are the adsorption equilibrium constants, c_A and c_i are the concentrations of sorbates, S is the specific area of the sorbent, ε_T is the total porosity of bed, V_P is the volume of the pre-column, ρ is the density of the sorbent and $C_{ads,A}$ is the maximum adsorption capacity per unit area. The same equations hold for all other n components adsorbed.

The amount adsorbed, m_A, is proportional to c_A and independent of the varying concentration of the other n components only if

$$1 \gg \sum_{i=1}^{n} K_i c_i + K_A c_A \tag{5}$$

Also, V_b is constant only if the above condition is fulfilled.

We examined the adsorption of 2,6-dimethylpyridine as a model sorbate. The pre-concentrated amount of this compound depends on the volume of sample, V_s, according to Fig. 1. It is evident that the range of linearity depends on the nature of the sample examined. In some instances, the amount of 2,6-dimethylpyridine adsorbed is displaced by another sorbate, as observed during the experiments with diesel fuel (Fig. 1D).

The breakthrough curves of the individual compounds such as 2,6-dimethylpyridine, N,N-dimethylaniline and isoquinoline dissolved in *n*-heptane were measured. If the breakthrough curves of the individual compounds are compared with the breakthrough curve of their mixture, the latter is not a superposition of the individual curves (Fig. 2). Thus, the adsorption from the mixture does not obey linear adsorption conditions as required by eqn. 5.

Another possible explanation of the above discrepancy is that the weakly adsorbed N,N-dimethylaniline is partially displaced from the sorbent bed by the suc-
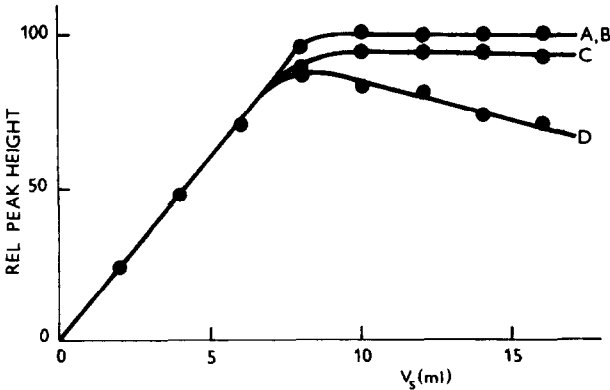
Fig. 1. Dependence of the amount of 2,6-dimethylpyridine adsorbed on 20-μm DNSP-silica (given as relative peak height) on V_s. A and B = pre-concentrated from n-heptane and light gasoline; C = kerosene; D = diesel fuel. UV detection at 254 nm; flow-rate of mobile phase, 1 ml/min; concentration of sorbate in samples, 10 ppm

ceeding zones of 2,6-dimethylpyridine and isoquinoline. It is very interesting that the breakthrough curves are different if measured on the fresh DNAP- or DNSP-silica used for the first time and on the same sorbent after the desorption of pre-concentrated sorbates into dichloromethane (Fig. 2).
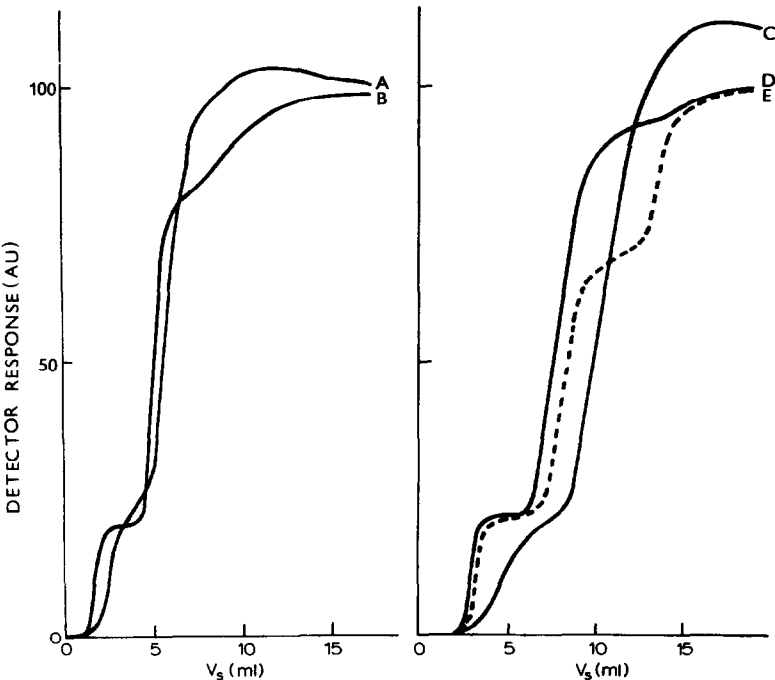

Fig. 2. Breakthrough curves of N,N-dimethylaniline, 2,6-dimethylpyridine and isoquinoline (10 ppm of each in n-heptane) in a pre-column (3 × 4 mm I.D.) packed with 20-μm DNAP-silica (A, B) or DNSP-silica (C, D). B and D = fresh sorbents; A and C = pre-concentration repeated after desorption in dichloromethane and purging with 5 ml of n-heptane. The dashed curve is a superposition of the individual breakthrough curves observed on fresh DNSP-silica.

The differences may be caused by various states of the layer of nitroaromatic ligands. In non-polar solvents, such as *n*-heptane, the layer is completely impermeable, as found by Hammers *et al.*[15]. In dichloromethane, the layer is swollen and the subsequent shrinking of the swollen layer in *n*-heptane is relatively slow. The layer swollen in dichloromethane probably has a higher adsorption capacity than in the non-swollen state. Also, penetration of sorbates into the swollen layer seems to be the reason for the increased axial dispersion, as is evident from Fig. 2. The distinct maxima in the breakthrough curves observed in several instances may be caused either by the displacement of weakly sorbed N,N-dimethylaniline or by the shrinking of the swollen layer, which in *n*-hexane loses its enhanced adsorption capacity.

The breakthrough curves of above solutes measured on bare silica and octadecylsilica were also observed in order to compare the adsorption capacities of these sorbents with those of nitroaromatic sorbents (Fig. 3). The interaction of nitrogen bases with the silanol groups of silica, which are known to be weakly acidic, seems to be stronger than the charge-transfer complexation. As a consequence, the desorption of basic sorbates from silica is incomplete if pure dichloromethane is used.
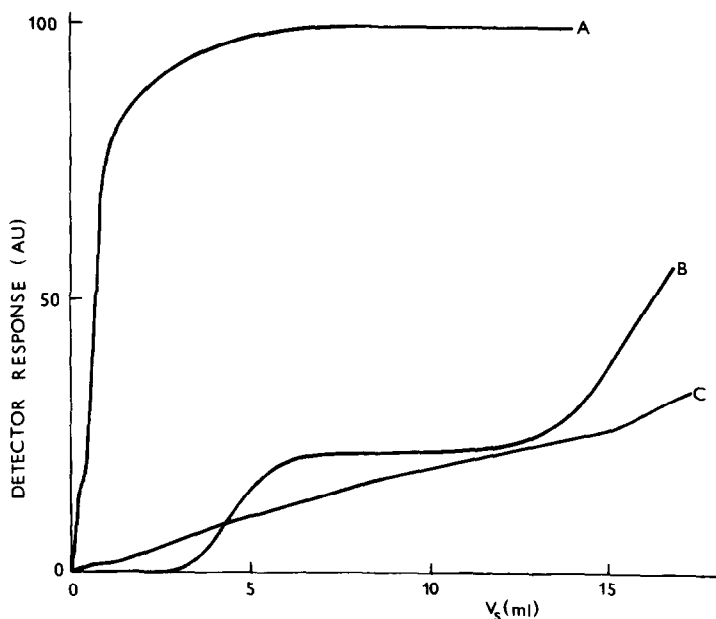

Fig. 3. Breakthrough curves of N,N-dimethylaniline, 2,6-dimethylpyridine and isoquinoline for (A) octadecylsilica, (B) fresh bare silica and (C) bare silica after desorption in dichloromethane and purging with *n*-heptane. For details, see Fig. 2.

Application

The diesel fuel, which we found to be virtually free of azaaromates, was spiked with 0.5, 1 and 5 ppm of pyridine and 2 ml of these samples were pre-concentrated on both sorbents. Reproducibility, studied by the repetitive pre-concentration of these samples, was achieved with a relative standard deviation of 3-4% ($n = 5$). The detection limit of pyridine calculated for a signal-to-noise ratio of 2:1 is about 50 and

100 ppb (10^9) using DNAP- and DNSP-silica, respectively. The recoveries of N,N-dimethylaniline and 2,6-dimethylpyridine, calculated from the peak areas obtained with loop injection and pre-concentration of the same amounts, were over 95% using DNSP-silica and over 90% using DNAP-silica. A recovery of about 80% was observed for *o*-phenanthroline and 2,2'-bipyridyl on DNAP-silica; the residual silanols are probably responsible for the slow desorption of a portion of the pre-concentrated solutes.

The repeatability of the adsorption–desorption cycle is very important for routine analysis. Therefore, we pre-concentrated trace amounts of nitrogen bases present in raw kerosene. After pre-concentration from 2.5 ml of kerosene, the compounds analysed were transferred into the analytical column and the pre-column was flushed with 5 ml of *n*-heptane. When this cycle was repeated five times, the reproducibility of the heights of the individual peaks was about 5% (relative standard deviation). Examples of the trace analysis of nitrogen bases present in petrochemical samples are shown in Figs. 4 and 5. Peaks 1–4 have been tentatively identified by comparing their capacity factors with the values given in Table I.

CONCLUSIONS

On-line pre-concentration can also be used in combination with charge-transfer LC for the trace analysis of nitrogen bases in non-polar hydrocarbon mixtures.
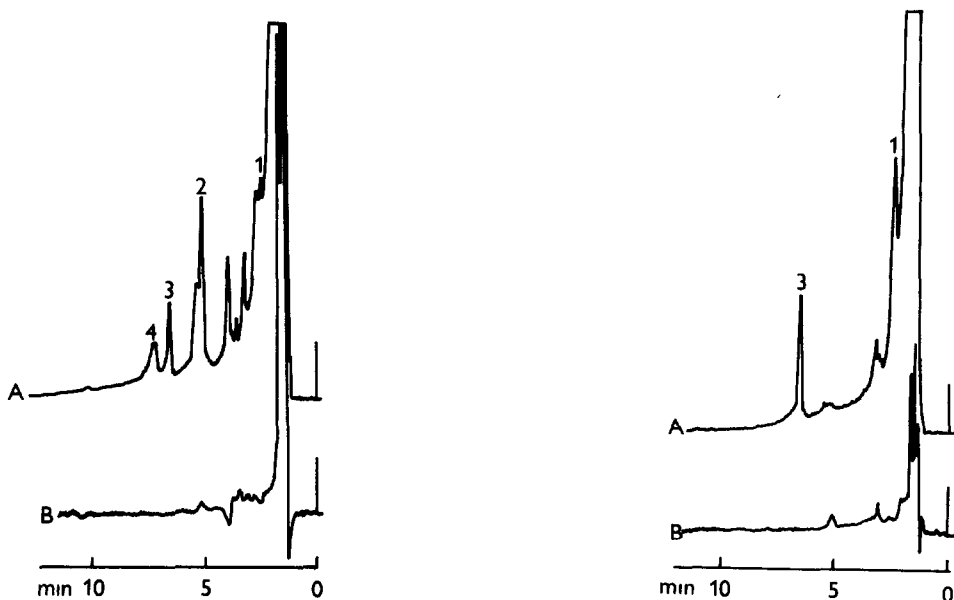

Fig. 4. On-line pre-concentration of various impurities present in kerosene: peak 1 is identical with aniline, 2 with quinoline, 3 with pyridine and 4 with isoquinoline. All the impurities are present in parts per million amounts. The retention of PAHs was checked by means of fluorimetric detection (B): λ_{ex} = 254 nm, emission filter 320 nm. Upper chromatogram (A), UV detector. Flow-rate of mobile phase, 1 ml/min; V_s = 2.5 ml.

Fig. 5. On-line pre-concentration of impurities present in light gasoline. Peak identities and experimental details as in Fig. 4.

The breakthrough volumes of individual compounds are dependent on the composition of the sample, especially if other strongly polar impurities are present.

The possibility of non-equilibrium conditions during pre-concentration must be taken into account. Hence the breakthrough volumes determined under equilibrium conditions may differ from those obtained with repeated of pre-concentration and desorption cycles.

ACKNOWLEDGEMENTS

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