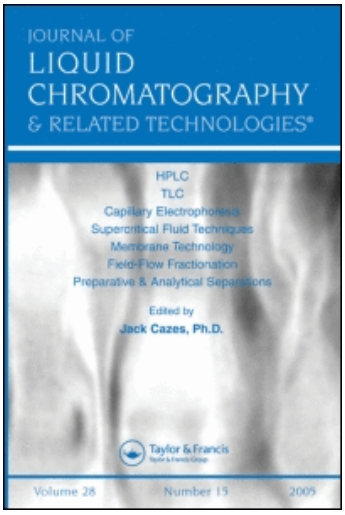


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# Column Switching Technique for Group-Type Separation of Different Pah Classes by use of $C_{18}$-Modified Silica and Polystyrene Packings

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# COLUMN SWITCHING TECHNIQUE FOR GROUP-TYPE SEPARATION OF DIFFERENT PAH CLASSES BY USE OF $C_{18}$-MODIFIED SILICA AND POLYSTYRENE PACKINGS

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## ABSTRACT

A column switching technique was developed to realize a group-type separation of PAHs and nitrogen containing PAHs (N-PAHs) applying a $C_{18}$-immobilized polystyrene packing as well as a $C_{18}$-modified silica stationary phase. On the first column the group-type separation and also the separation of the N-PAH fraction in single compounds was performed. After backflush and transfer to a second column, the separation of the PAH fraction could be achieved.

## INTRODUCTION

Reversed-phase liquid chromatography (RP-HPLC) generally is performed with chemically modified silica

stationary phases, for example ODS. Polymer based packings formed from crosslinked styrene-divinylbenzene have recently become of great interest in reversed-phase separation. Many papers have been devoted to their characterization (1-7) as well as to the description of a retention mechanism as a function of the mobile phase composition (8-12).

The great advantage of polystrene-divinylbenzene (PS-DVB) is its stability covering a wide range of pH-values, involving no limitations in the choice of mobile phases. This is an important fact for the liquid chromatographic investigation of more polar samples including chlorophenols (13-15), penicillins (16), amino acids and peptides (17), proteins (18), nucleosides (19), triazines (20), gibberellins (21), thiamine derivatives (22), alkaloids (23), and acidic compounds (24). Their separation has been described by the use of organic polymers like PRP-1 or PLRP-S.

As already mentioned by McBlane (25) and Benson (26) polystyrene-divinylbenzene, like PRP-1, shows peak asymmetry, taling and long retention times for unpolar aromatic compounds, which can be attributed to the high density of π-electrons of the unmodified aromatic matrix. To decrease the effect of π-electron interactions during the chromatographic separation process, it seems advantageous to use chemically modified polystyrene packings where the π-electron density is reduced or screened by appropriate functional groups. Examples for modified polystyrenes are given by Benson (26), Verzele (27) and Howang (28).

A wide variety of stationary phases have been applied for the group-type separation of PAHs and nitrogen containing PAHs (N-PAHs) (29-32). At normal-phase stationary phases like silica, cyanopropylsilica, nitroaromatic silica or aminopropylsilica the PAHs elute first whereas the polar N-PAHs are stronger retained due to possible polar, ionic or acid-base

interactions. The disadvantage of silica includes poor recovery for some polar species and sensitivity to water in the mobile phase, involving reduced adsorbent activity such that overlap of chemical classes occurs (33).

Although bonded stationary phases like nitroaromatic silica or aminopropylsilica show improved reproducibility and recovery rates for polar compounds, the obtained group-type separation is not always complete. Blümer and Zander compared normal-phase Nuclosil $NO_2$ and reversed-phase $C_{18}$ for their ability to separate a large number of PAHs from nitrogen heterocycles. Both stationary phases investigated yielded overlap of large PAHs into the nitrogen heterocycle fraction.

The limitations of existing stationary phases encourage the development and application of new materials for the group-type separation of complex mixtures. Most of these new developed stationary phases are working under normal-phase conditions, for example (34). Separation occurs by exploiting polar, ionic or acid-base interactions, involving long retention times for some polar species. In contrast to this, marcophase MP-1 may enable a compound class separation of PAHs and N-PAHs by utilization of the expected high affinity of PAHs to the polystyrene based packing.

HPLC is a powerful separation technique, but frequently it approaches to its limits when applied to complex mixtures. To overcome this difficulty, selectivity enhancing steps such as the use of more efficient columns, gradients or derivatization techniques may be successful. Another important method involving better resolution, group-type separation (35-37), sample cleanup (38,39) or sample enrichment (14,15,40) is the application of a column switching technique. A fraction

of the effluent from a primary column is selectively transfered to a second column for a further chromatographic investigation. Highly selective separations are achieved by using different transfer techniques and/or switching functions or by changing the chromatographic modes of separation during the overall process (41-44).

We recently described a group-type separation of four polycyclic aromatic hydrocarbons and the corresponding aminosubstituted arenes on a $C_{18}$-modified polystyrene packing (45). We continued our work dealing with additional investigations of more complex standard samples containing PAHs, aminosubstituted arenes and nitrogen heterocycles. To realize a separation of PAHs and N-PAHs a column switching technique was developed, applying a $C_{18}$- modified silica stationary phase as well as a $C_{18}$-immobilized polystyrene packing.



## MATERIALS AND METHODS

### Apparatus

The chromatographic equipment consisted of two Merck Hitachi 655A-12 liquid chromatographs (Darmstadt, FRG), two Merck Hitachi L-5000 LC controllers, a Merck Hitachi L-3000 photodiode array detector, a Merck Hitachi D-2000 integrator and a Merck Hitachi 655A-40 autosampler. The column switching technique was performed with two Krannich (Göttingen, FRG) switching valves type ELV 7000 and ELV 7040 respectively and one Krannich switching valve operating with an Valco 10-port valve type C10-U. The pH of the hydrochlorid acidic solution was measured with a pH-meter modell 522 (Wissenschaftlich-Technische Werkstätte, FRG)

## Chemicals and Reagents

1-Aminoanthracene, 2-aminoanthracene, 1-aminopyrene and acridine were supplied from Janssen (Beerse, Belgium), all others were purchased from Merck (Darmstadt, FRG). The standard compounds were used without further purification. Acetonitrile was of HPLC grade and supplied by Baker (Gross-Gerau, FRG)

## Columns

Macrophase MP-1, is a $C_{18}$-derivatized polystyrene-divinylbenzene and was obtained from Interaction Chemicals Co. (Frankfurt, FRG) as loose material with a particle size of 10 µm. The column (125 x 4.1 mm) was packed with methanol as mobile phase using the slurry packing technique. The silica based $C_{18}$-modified stationary phase was a Bakerbond Wide Pore column (250 x 4.1 mm) and purchased from Baker (Gross-Gerau, FRG).



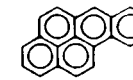
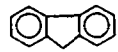
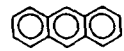
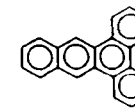
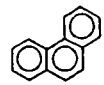
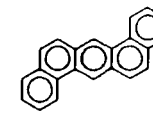
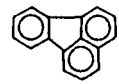
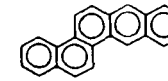
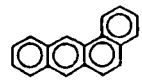
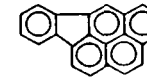
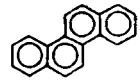
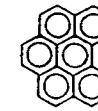
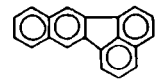
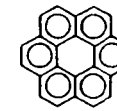
# RESULTS AND DISCUSSION

## Chromatography on Macrophase MP-1

Macrophase MP-1 is a high performance polymer, covalently derivatized with $C_{18}$ functional groups. Only carbon-carbon and carbon-hydrogen bonds are used throughout the polymer matrix. Therefore the material behaves chromatographically in a manner similar to an ODS silica packing, but exhibits extraordinary chemical stability (25).

TABLE 1

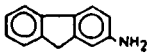
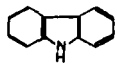
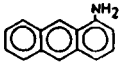
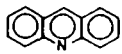
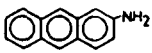
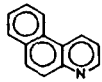
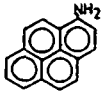
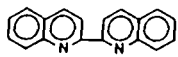
Investigated polycyclic aromatic Hydrocarbons (PAHs)

| COMPOUND | STRUCTURE | COMPOUND | STRUCTURE |
|---|---|---|---|
| NAPHTHALENE |  | BENZO(A)PYRENE |  |
| FLUORENE |  | | |
| ANTHRACENE |  | DIBENZ(AC) ANTHRACENE |  |
| PHENANTHRENE |  | DIBENZ(AH) ANTHRACENE |  |
| FLUORANTHENE |  | | |
| PYRENE |  | BENZO(B)CHRYSENE |  |
| BENZ(A)ANTHRACENE |  | INDENO(1,2,3,CD) PYRENE |  |
| CHRYSENE |  | BENZO(GHI)PERYLENE |  |
| BENZO(K) FLUORANTHENE |  | CORONENE |  |

As already mentioned in a previous paper a group-type separation of four polycyclic aromatic hydrocarbons and the corresponding aminosubstituted arenes was realized on MP-1 (45). In order to decide whether macrophase MP-1 is a suitable stationary phase

TABLE 2

Investigated Amino-PAHs and Nitrogen Heterocycles (N-PAHs)

| COMPOUND | STRUCTURE | COMPOUND | STRUCTURE |
|---|---|---|---|
| | | INDOLE |  |
| 1-AMINONAPHTHALINE |  | CHINOLINE |  |
| 2-AMINOFLUORENE |  | CARBAZOLE |  |
| 1-AMINOANTHRACENE |  | ACRIDINE |  |
| 2-AMINOANTHRACENE |  | 5,6-BENZOCHINOLINE |  |
| 1-AMINOPYRENE |  | 1-AZAPYRENE |  |
| | | 2,2'-BICHINOLINE |  |

for the group-type separation of more complex samples, the retention behaviour of 16 PAHs (see Table 1) and 11 N-PAHs (see Table 2) was investigated.

Figure 1 shows the k'-values of some PAHs as a function of the mobile phase composition. It is well documented that this compound class is strongly retained on macrophase MP-1. For PAHs with more than three rings, tailing and peak asymmetry was observed

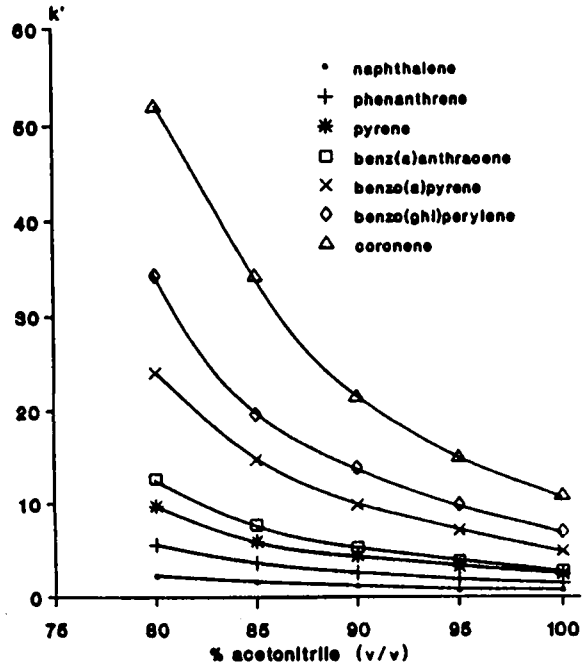



Figure 1: k'-values of some PAHs on macrophase MP-1 as a function of the mobile phase composition (acetonitrile/water, v/v).



even at a low water content of the mobile phase. This phenomen can be attributed to the strong interactions of PAHs and the polystyrene based packing. Therefore a complete separation of the PAHs presented in Table 1 could not be realized.

Aminosubstituted PAHs and nitrogen heterocycles were investigated with acetonitrile/water pH7 and acetonitrile/water pH2 eluent respectively. As can be seen in Figure 2a, 2b, 3a and 3b the k'-values are strongly reduced, when the pH changes from seven to two. Occu-

ring protonation at a pH of two involves a considerable decrease of the k'-values for aminosubstituted PAHs. For pyridine based nitrogen heterocycles the k'-values are now lower than those obtained for 100 % acetonitrile. There are only two exceptions in the group of nitrogen heterocycles. Indole and carbazole are not protonated at a pH value of two, due to their low pka values.

A further important retention behaviour can be shown by comparing the capacity factors of PAHs and N-PAHs. Due to the unpolar character of PAHs this compound class is much stronger retained on MP-1. As a consequence it can be assumed that MP-1 is an appropriate stationary phase for the group-type separation of PAHs and N-PAHs.



Figure 4 shows the separation of 11 N-PAHs (see Table 2) at MP-1, applying an acetonitrile/water pH2 gradient, whereas Figure 5 illustrates the expected group separation of PAHs and N-PAHs, accompanied by a simultaneous, nearly complete, separation of the N-PAH fraction into the induvidual substances. The distance between the last eluting N-PAH (2,2'-bichinoline, (8)) and the first eluting PAH (naphthalene, (9)) could be enlarged or reduced as a function of the applied gradient.

To realize a complete separation of the compounds listed in Table 1 and Table 2 a column switching technique was developed, applying a $C_{18}$-modified polystyrene packing in combination with a $C_{18}$-immobilized silica stationary phase.

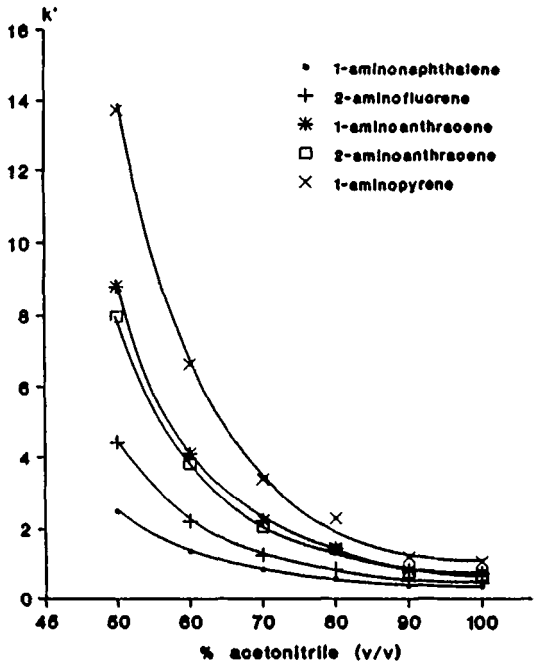
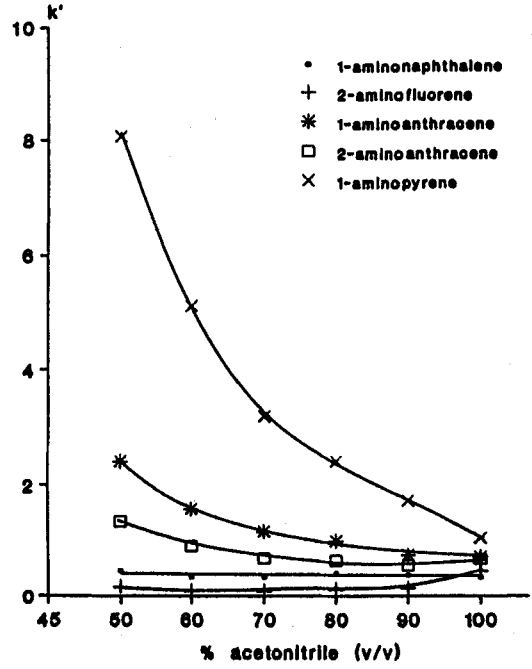



Figure 2a:
k'-values of amino-PAHs on macrophase MP-1 as a function of the mobile phase compo-sition (acetonitrile/water, v/v) at pH7.



## Column Switching Technique Part I

As pointed out before MP-1 is an excellent stationary phase for the group-type separation of PAHs and N-PAHs. Furthermore the separation of the N-PAH fraction in single compounds could be accomplished. Figure 6a shows the developed column switching technique where one ten-port valve and two six-port




2b:
k'-values of amino-PAHs on macrophase MP-1 as a function of the mobile phase composi-tion (acetonitrile/water, v/v) at pH2.



valves are used. Valve number one controlls the flow direction on MP-1 (PRP-$C_{18}$ phase), whereas valve number two enables a separate purge and operating of the ODS silica stationary phase as well as the direct combination of macrophase MP-1 (column 1) and the bakerbond phase (column 2). By the use of valve number three the detector can be positionated behind column 1 or column 2 respectively.

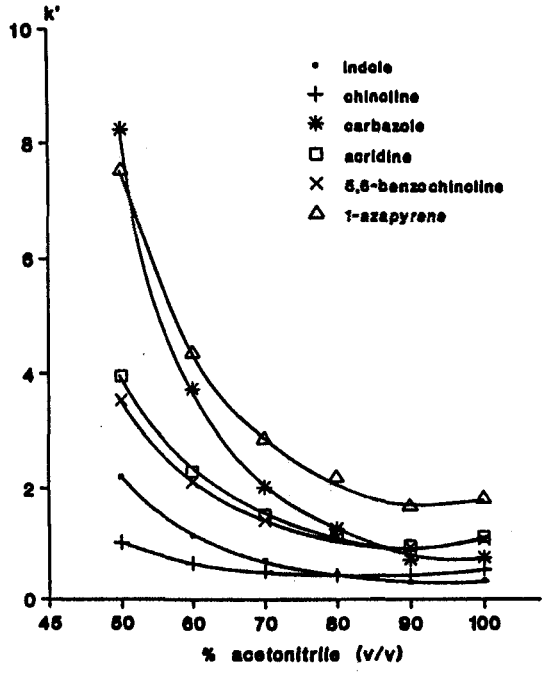
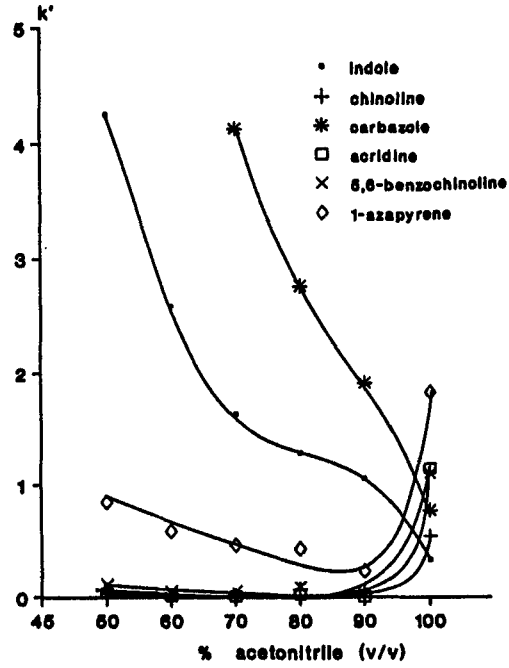



Figure 3a:
k'-values of nitrogen heterocycles on macrophase MP-1 as a function of the mobile phase composition (acetonitrile/water, v/v) at pH7.



When starting the column switching technique all valves are in position one. The route of the mobile phase is indicated in Figure 6a, whereas the resulting chromatogram is illustrated in Figure 6b. During the course of part I of the regarded column switching technique N-PAHs and PAHs are separated in compound classes accompanied by a simulaneous nearly complete separation




3b:
k'-values of nitrogen heterocycles on macrophase MP-1 as a function of the mobile phase composition (acetonitrile/water, v/v) at pH2.

of the N-PAH fraction into the individual substances. As soon as the last eluting N-PAH (2,2'-bichinoline) has been detected, the first part of the column switching technique is finished. Now valve number one changes from position one (p1) to position two (p2) initiating part II of the column switching technique.

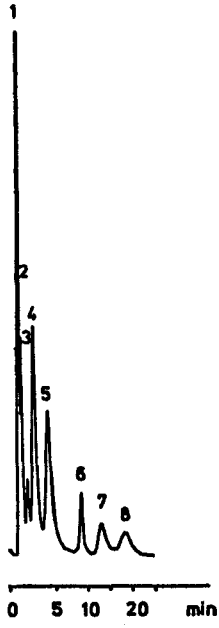



Figure 4: Separation of 11 N-PAHs on macrophase MP-1 applying an acetonitrile/water (v/v) pH2 gradient: 0.0 min 40%$CH_3CN$/60%$H_2O$; 20.0 min 55%$CH_3CN$/45%$H_2O$; 22.0 min 100%$CH_3CN$; wave length: 260 nm; flow rate: 1.0 ml/min; (1) acridine, chinoline, 5,6-benzochinoline, 2-aminofluorene; (2) 1-aminonaphthalene; (3) 1-azapyrene; (4) 2-aminoanthracene; (5) 1-aminoanthracene; (6) indole; (7) 1-amino-pyrene; (8) 2,2'-bichinoline.



## Column Switching Technique Part II

The second part of the column switching technique consists of the backflush of the PAH fraction on macrophase MP-1 as well as its transfer to the ODS silica phase (see Figure 7a). A successfully performed backflush is indicated by a narrow and symmetric backflush

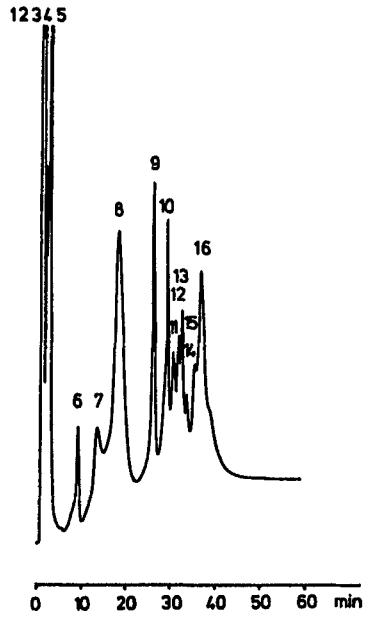



Figure 5: Separation of 10 N-PAHs and 16 PAHs on macrophase MP-1 applying an acetonitrile/water (v/v) pH2 gradient; conditions as presented in Figure 4; (1)-(8) N-PAHs, see Table 2; (9)-(16) PAHs, see Table 1.



peak (see Figure 7b). Tests applied at distinct acetonitrile/water compositions and at divers flow rates have demonstrated that the best results are obtained with an increase of the flow rate from 1.0 ml/min to 2.5 ml/min and an acetonitrile/water composition of 50/50 (v/v) during the backflush process. In contrast to silica based material, MP-1 can not be used with pressures more than 2000 psi. Therefore the flow rate was reduced to 1.0 ml/min during the transfer periode. The high water content of

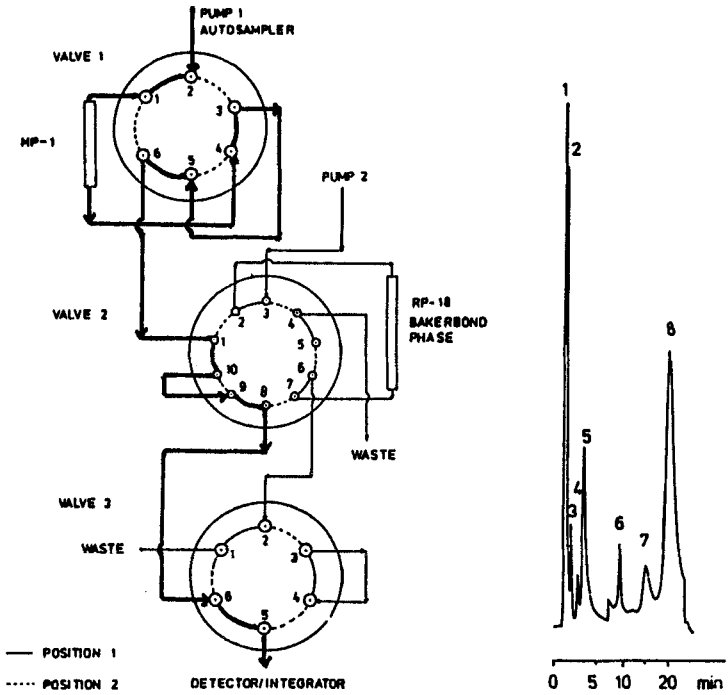



Figure 6a: Column switching technique part I; group-type separation of N-PAHs and PAHs as well as the separation of the N-PAHs fraction into the individual compounds.

6b: Separation of 10 N-PAHs applying column switching technique part I; identification see Figure 4; conditions pump 1:

| time [min] | $CH_3CN$ [%] | $H_2O$(pH2) [%] | flow rate [ml/min] | valve position |
|---|---|---|---|---|
| 0.0 | 40 | 60 | 1.0 | all p1 |
| 20.0 | 55 | 45 | 1.0 | |
| 22.0 | 100 | 0 | 1.o | |

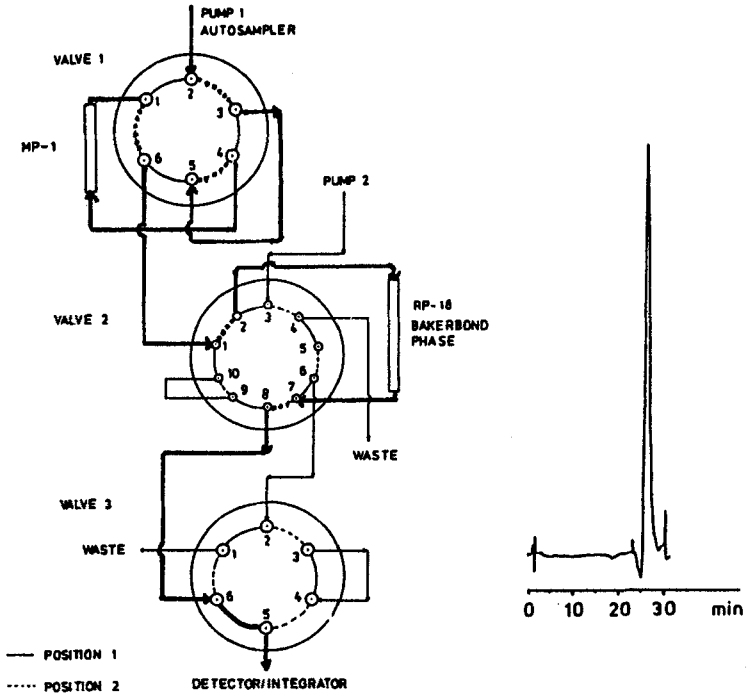



Figure 7a: Column switching technique part II; back-flush of the PAH fraction on macrophase MP-1 and its transfer to the ODS silica packing.

7b: Backflush peak applying column switching technique part II; conditions pump 1:

| time [min] | $CH_3CN$ [%] | $H_2O$ [%] | flow rate [ml/min] | valve position |
|---|---|---|---|---|
| 23.0 | 100 | 0 | 1.0 | v1 p2 |
| 23.5 | 50 | 50 | 1.0 | |
| 25.5 | 50 | 50 | 2.5 | |
| 25.2 | 50 | 50 | 2.5 | |
| 25.3 | 50 | 50 | 1.0 | v2 p2 |
| 28.5 | 50 | 50 | 1.0 | v2 p1 |

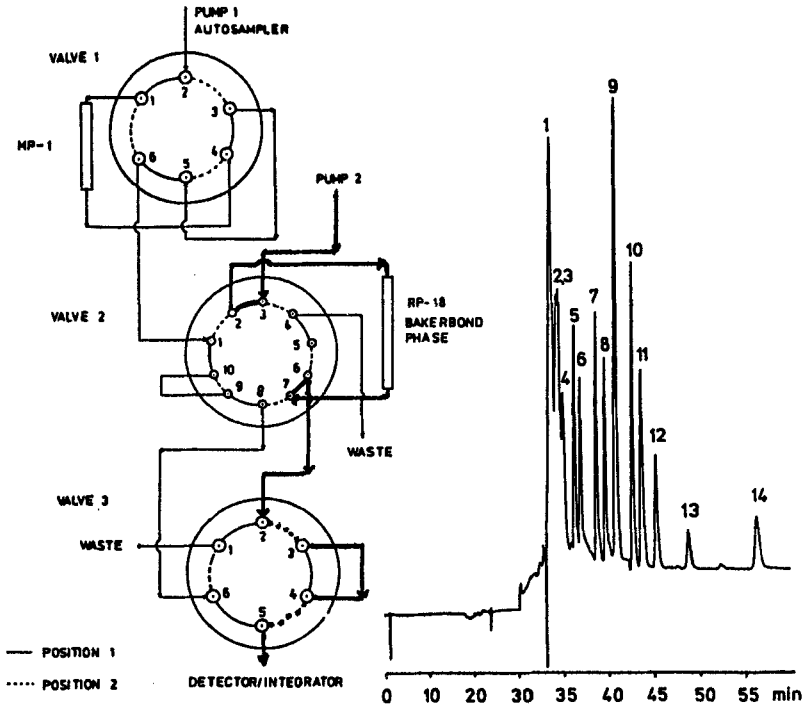



Figure 8a: Column switching technique part III, sepa-ration of the PAH fraction on a $C_{18}$-modi-fied silica packing.

8b: Separation of the PAH fraction on the Bakerbond Wide Pore column; conditions:

| time [min] | $CH_3CN$ [%] | $H_2O$ [%] | flow rate [ml/min] | valve pos. | pump |
|---|---|---|---|---|---|
| 25.3 | 50 | 50 | 1.0 | v2 p2 | 1 |
| 28.5 | 50 | 50 | 1.0 | v2 p1 | 1 |
| 28.5 | 100 | 0 | 1.0 | v3 p2 | 2 |
| 60.0 | 100 | 0 | 1.0 | | 2 |

(1) naphthalene, fluorene, phenanthrene; (2) anthracene; (3) fluoranthene; (4) pyrene; (5) benz(a)anthracene; (6) chrysene; (7) dibenz(ac)anthracene; (8) benzo(k)-fluoranthene; (9) benzo(a)pyrene; (10) di-benz(ah)anthracene; (11) benzo(ghi)pery-lene; (12) indeno(1,2,3,cd)pyrene;(13) ben-zo(b)chrysene; (14) coronene.

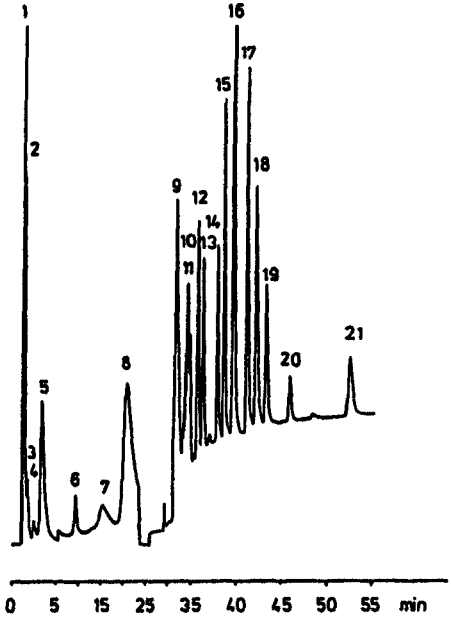



Figure 9: Complete separation of 10 N-PAHs and 16 PAHs by use of the developed column swit-ching technique; conditions and identi-fication as illustrated in Figure 6b-8b



the mobile phase (acetonitrile/water 50/50) during the transfer periode promotes on column concentration of the PAH fraction at the Bakerbond stationary phase which was equilibrated with acetonitrile/water 70/30 (v/v) before use.

## Cloumn Switching Technique Part III

The last step of the column switching technique is illustrated in Figure 8a and Figure 8b. After backflush

on MP-1 and transfer to the bakerbond stationary phase, the separation of the PAH fraction could be realized. The chromatogram in Figure 8b shows low resolution for the first eluting PAHs. This can be attributed to the high transfer volume of the PAH fraction. Although the backflushpeak is narrow and symmetric detailed investigation has shown, that coronene was detected in the front, whereas naphthalene composed the back side. So the first eluting PAHs have to surpass all the others on the ODS silica surface involving a further contribution to low resolution.

While the PAHs are separated on the RP-18 stationary phase, macrophase MP-1 is equilibrated for the next analysis. The Bakerbond stationary phase can be reequilibrated during the first part of the column switching technique.



## Complete Analysis of 16 PAHs and 10 N-PAHs

Figure 9 shows the separation of 16 PAHs and 10 N-PAHs applying the developed column switching technique. A comparision with Figure 5 documents the great improvement, which has been achieved by the use of a $C_{18}$ modified organic polymer in combination with a $C_{18}$ immobilized silica packing. The presented RP-HPLC method may be applied for complex samples, where a group-type separation is necessary before the analysis of single compounds can be accomplished.

## CONCLUSION

A column switching technique was developed to realize a group-type separation of PAHs and N-PAHs. On a $C_{18}$-immobilized polystyrene-divinylbenzene packing a

group-type separation of N-PAHs and PAHs, accompanied by a nearly complete separation of the N-PAH fraction into the individual compounds was observed. After backflush and transfer to a $C_{18}$-modified silica stationary phase, the separation of the PAH fraction could be achieved.

Organic polymers should not be regarded as an universal alternative but , rather, as an useful complement to ODS silica gel packings.

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