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## MOISTURE CONTROL SYSTEM FOR NON-POLAR ELUENTS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

### I. REPRODUCIBLE RETENTIONS ON ALUMINA\*

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#### SUMMARY

In order to achieve reproducible absolute and relative retentions on alumina with non-polar eluents, a simple and inexpensive glass moisture control system has been developed for stabilizing the water content of *n*-heptane or dichloromethane. This system can be inserted into any chromatographic equipment, between the detector outlet and the inlet of the pump. The system is filled with silica or alumina impregnated with different amounts of water, and the water content and temperature of the moisture control system determine the water concentration in the eluent and consequently the absolute and relative retentions in the alumina column.

Over 500 samples can be injected into this closed-loop system because all the contaminants of the eluent are removed by the moisture control system. The equilibration time is 8-50 h, depending on the analytical parameters. The water content of the eluent can be characterized by the capacity ratios of standard samples. The effect of the water content of the eluent is demonstrated with some practical separations.

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#### INTRODUCTION

In high-performance liquid chromatography (HPLC), retention on active stationary phases such as silica and alumina with non-polar and moderately polar eluents (*e.g.*, *n*-heptane and dichloromethane, respectively) is strongly influenced by the water content of the eluent. At room temperature, the solubility of water in *n*-heptane is about 100 ppm<sup>1,2</sup>. Small changes in the moisture content of the eluent alter the separation significantly, as demonstrated in Figs. 1 and 2. On a column packed with alumina and with "dry" *n*-heptane (water content  $\leq 20$  ppm) as eluent, five polynuclear aromatic hydrocarbons (naphthalene to chrysene, capacity ratios  $k' =$

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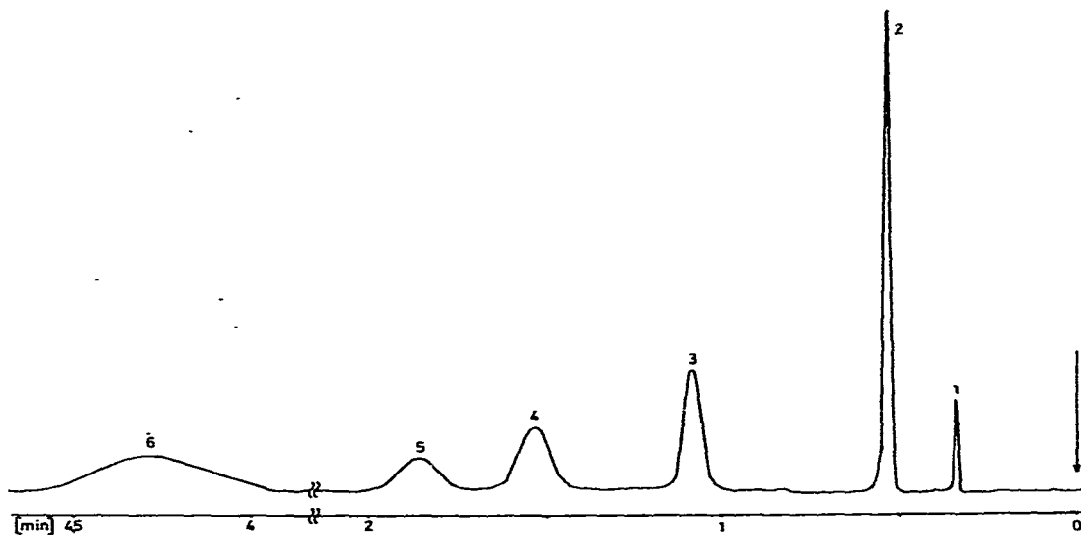


Fig. 1. Separation of aromatic hydrocarbons with "dry" *n*-heptane (water content *ca.* 20 ppm). Column: 12.5 cm  $\times$  4.1 mm I.D. Stationary phase: alumina (Woelm), 5  $\mu$ m. Eluent: *n*-heptane (*ca.* 20 ppm of water, not controlled).  $\Delta p = 130$  atm;  $u = 6.4$  mm/sec;  $F = 4.5$  ml/min. Samples: 1 = tetrachloroethylene ( $k' = 0$ ); 2 = naphthalene ( $k' = 0.59$ ); 3 = anthracene ( $k' = 2.25$ ); 4 = pyrene ( $k' = 3.59$ ); 5 = fluoranthene ( $k' = 4.59$ ); 6 = chrysene ( $k' = 11.9$ ).

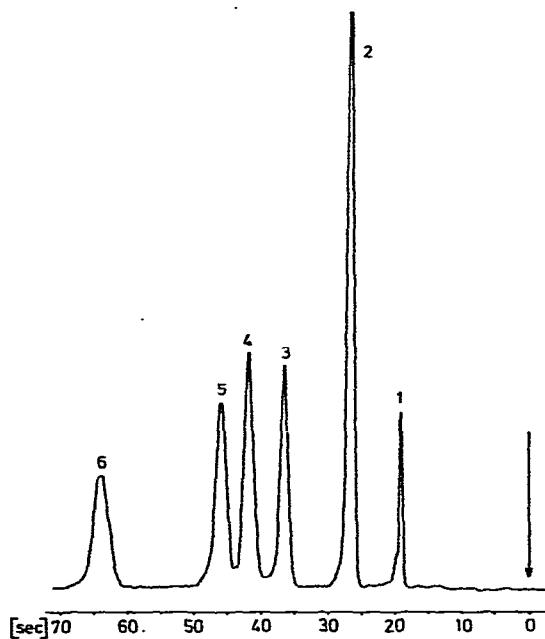


Fig. 2. Separation of aromatic hydrocarbons with "moist" *n*-heptane (water content *ca.* 40 ppm). Conditions as in Fig. 1, except eluent was *n*-heptane (*ca.* 40 ppm of water, not controlled). Samples: 1 = tetrachloroethylene ( $k' = 0$ ); 2 = naphthalene ( $k' = 0.38$ ); 3 = anthracene ( $k' = 0.97$ ); 4 = pyrene ( $k' = 1.28$ ); 5 = fluoranthene ( $k' = 1.54$ ); 6 = chrysene ( $k' = 2.14$ ).

0.6–11.9) can be separated within 5 min, as shown in Fig. 1. With “moist” *n*-heptane (water content *ca.* 40 ppm) the separation is completed in about 1 min, although all of the other parameters remain unchanged (Fig. 2). The increase of about 20 ppm in the water content results in a decrease in the  $k'$  value of chrysene from 11.9 to 2.2.

Not only the absolute but also the relative retentions are sensitive functions of the water content of the eluent, which accounts for the poor reproducibility of the above separations and for the contradictions that exist in the literature. The effects demonstrated here are well known in both classical column chromatography and HPLC<sup>3–5</sup>. In column chromatography, attempts have been made to standardize the water content of the adsorbents<sup>6</sup> and eluents<sup>7</sup>. In HPLC, the use of eluents with a defined water content in a recycling system has been recommended<sup>4</sup>, but it is extremely difficult to prepare eluents with defined and easily determinable low water contents and also to achieve and maintain water contents below 20 ppm by the drying methods commonly used, such as percolation over active alumina or molecular sieves. Similar problems arise with moderately high water concentrations in non-polar eluents. As soon as the reservoir is opened or the liquid decanted into another container, a change in the water content of the eluent is virtually unavoidable, which is one of the reasons why it would be impracticable to produce and sell eluents for chromatography with standardized water contents.

Recycling of the eluent results in more or less reproducible retentions, but their magnitude varies with the original water content and temperature of the eluent. Moreover, the addition of further eluent to the reservoir is likely to change the capacity ratios of the samples, because it is difficult to prepare two batches of eluent with identical water contents. In fact, our experience has shown that the use of two batches of the same eluent with identical water contents as determined by the Karl Fischer method<sup>8</sup> frequently leads to inconstant retentions for a given compound on a given stationary phase.

In this paper, a closed-loop moisture control system for producing and maintaining the desired water content of an eluent is described. The effect of the water content of the eluent on sample retentions is also discussed.

## EXPERIMENTAL AND RESULTS

### *Karl Fischer titration*

According to the standard method, as described, for example, in DIN 51777 (G.F.R.), the water content of gasoline can be determined to below 30 ppm (w/w). The accuracy of the Karl Fischer titration is 5 ppm and its reproducibility is 3 ppm if a 200-ml sample is used. The sample size required increases if the water concentration is below 10 ppm.

For practical reasons, the volume of our eluent reservoir was either 500 or 1000 ml. Ten or more titrations were required in order to determine the water content of the eluent in the course of the equilibration and consequently the maximal sample size was 10 ml of eluent. The accuracy and reproducibility stated above could be achieved if the water concentration was higher than 15 ppm, but water concentrations below 5 ppm could not be determined with a 10-ml sample with acceptable accuracy.

The water concentration was determined with an automatic dead-stop Karl Fischer titrator (Metrohm, Herisau, Switzerland).

### Chromatography

**Eluents.** Before use, the eluents (dichloromethane and *n*-heptane) were distilled in order to increase their UV sensitivity, and then contained 160–220 and about 30–40 ppm of water, respectively.

**Stationary phases.** The chromatographic columns were always packed with alumina: (1) neutral alumina (Woelm, Eschwege, G.F.R.) with particle size  $d_p = 5 \mu\text{m}$  and (2) Spherisorb A5Y (an alumina distributed by Phase Separations, Queensferry, Great Britain) with  $d_p = 5 \mu\text{m}$ .

**Columns and column packing.** All columns (I.D. 4.1 mm) were drilled before use<sup>9</sup>. Their lengths varied from 12.5 to 30 cm, depending on the particle size of the stationary phase. The columns were packed by a slurry method<sup>9</sup>, using a mixture of tetrabromoethane and dioxan (9:1, v/v).

**Chromatographic system.** Self-built equipment was used. The pumping system consisted of either a three-headed membrane pump (Model M3-S4, Orlita KG, Giessen, G.F.R.) or a piston pump (Model 6000A, Waters Assoc., Milford, Mass., U.S.A.). Samples were always injected with a syringe on to the top of the column. A UV detector (254 nm) was used exclusively. The eluent was thermostated by passing it through a heat exchanger (a 100 cm  $\times$  0.25 mm I.D. capillary) directly before the injection port. The column was additionally thermostated with a water-jacket and both the heat exchanger and the water-jacket were kept at the same temperature.

### Moisture control system (MCS)

The MCS was constructed from standard laboratory glassware, and is shown schematically in Fig. 3. The eluent reservoir (A) was a three-necked Woulff flask (500 or 1000 ml) with ground-glass joints. A thermostated dropping funnel (B), of volume either 100 or 250 ml, was mounted in the central port. A perforated glass plate fused into the bottom of the funnel was covered with filter-paper in order to retain the contents. This arrangement is superior to the use of a fritted glass disk, which tends to become clogged by very fine particles. The flow-rate through the funnel was regulated by means of a stopcock (or a syphon system) so that the alumina contents were always covered with the eluent. The other ports served to connect a suction line (C) to the pump and to provide an opening to the atmosphere via the drying tube (D), which was filled with blue silica gel. The eluent in the reservoir was mixed with a magnetic stirrer.

The MCS system was connected to the pump and the column effluent was recycled on to the top of the funnel. Although the eluent was gradually contaminated by the injected samples, most of these impurities were removed by the contents of the funnel. No difficulties with eluent purity were encountered even with 500 (or more) injections of sample.

Prior to a run, the dropping funnel was filled with silica or alumina of "large" particle diameter (63–200  $\mu\text{m}$ ). This silica (SI 40 or SI 200, Merck, Darmstadt, G.F.R.) and neutral alumina (Woelm) were used either dry or impregnated with water. Before impregnation with water, the silica and alumina were activated for 4 h at 150° and 200°, respectively, then, after cooling in a tightly closed flask, the desired amount of water was added and the mixture equilibrated for 1 h with occasional shaking. Typically, the funnel of the MCS (100 ml) was loaded with 100 g of alumina or 40 g of silica. The alumina was coated with 3, 6, 10, or 15% (w/w) and the silica with 5 or

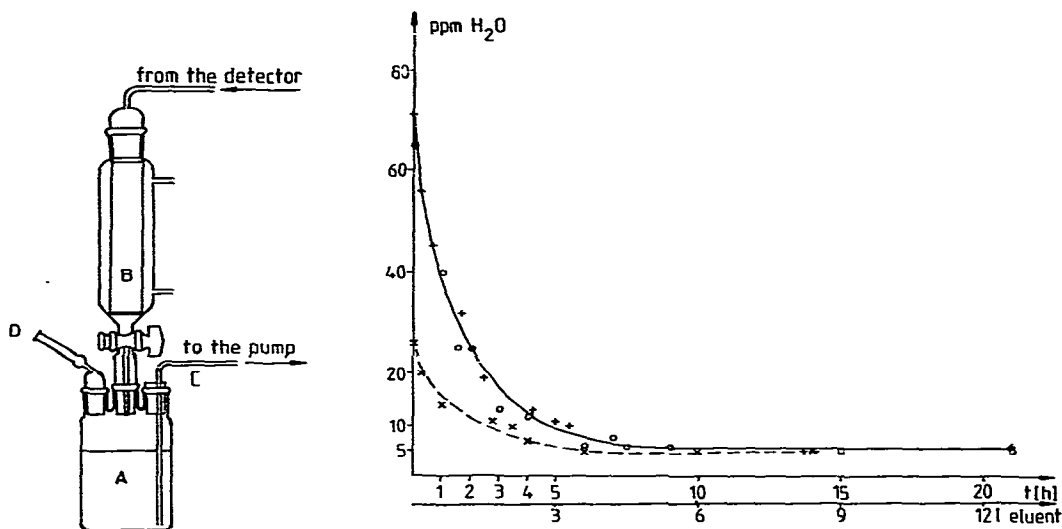


Fig. 3. Moisture control system (MCS). A = Eluent reservoir (Woufff flask); B = thermostated funnel for alumina or silica coated with defined amounts of water; C = suction line to the pump; D = drying tube filled with blue silica gel.

Fig. 4. Drying rate of *n*-heptane in the MCS. O, MCS filled with activated silica (150°, 4 h); water-saturated *n*-heptane (initial water concentration 70 ppm). +, MCS filled with activated alumina (200°, 4 h); water-saturated *n*-heptane. x, MCS filled with activated silica; distilled *n*-heptane (initial water concentration 25 ppm).

20% (w/w) of water. The eluent was then introduced through this filled funnel and/or recycled through it and was consequently dried or moistened to an equilibrium water content. The water content of the silica or alumina in the MCS and also its temperature determined the absolute and relative water content of the eluent.

In order to establish the properties of the MCS and its ability to regulate the water content of the eluent, the water concentration of *n*-heptane was measured as a function of time. The funnel was filled with freshly activated silica and "wet" *n*-heptane, with an initial water concentration of 65–75 ppm, was recycled through the MCS at a flow-rate of 10 ml/min. The temperature of the funnel was maintained at  $25 \pm 0.25^\circ$ . Fig. 4 shows the decrease in the water concentration as measured by Karl Fischer titration using 10-ml samples. After about 10 h, a final (minimum determinable) water concentration of 5 ppm was reached. The content of the MCS reservoir was recycled at least six times before a constant water concentration was attained.

If *n*-heptane with a lower initial water concentration (25 ppm) was used, the final concentration was reached, of course, in a shorter time (broken line in Fig. 4).

When alumina was used in the MCS, the rate of equilibration of the *n*-heptane with water was the same as with silica (crosses in Fig. 4).

The time of equilibration is a function of the flow-rate, and the equilibration time is inversely proportional to the flow-rate although owing to the limitations of the system, there is a certain upper limit.

### Moisture control system connected to a chromatographic column

The MCS was loaded with freshly activated silica and the *n*-heptane in the reservoir was equilibrated for about 18 h, by which time the water-concentration as measured by Karl Fischer titration had been constant at about 5 ppm for more than 5 h. The chromatographic system, including the column packed with alumina, was then connected to the MCS and the  $k'$  values of different aromatic hydrocarbons were determined as a function of time at a constant flow-rate (5 ml/min) and at a constant temperature of the MCS and the column (25°). As shown in Fig. 5, the capacity ratios of the samples increase with increasing equilibration time of the chromatographic column (the MCS system was in "equilibrium" at  $t = 0$ ). As the "dry" eluent removes sorbed water from the chromatographic column, its activity increases. In the experiment shown in Fig. 5, the initial water content of the alumina in the chromatographic column was greater than the value obtained after equilibration of the system. By coincidence, the eluent and column may already be in equilibrium at the beginning of a run. The opposite situation to that presented in Fig. 5 is also possible: the column is initially "dry" and water is transported with the eluent into the column and deposited on the stationary phase. This is possible, as will be demonstrated later, only if the weight of water in the MCS is much greater than that required in the column for equilibration. To summarize, the MCS is a buffer system that can donate water to or remove water from the chromatographic column, depending on the water content of the adsorbent in the MCS funnel and column at a given temperature.

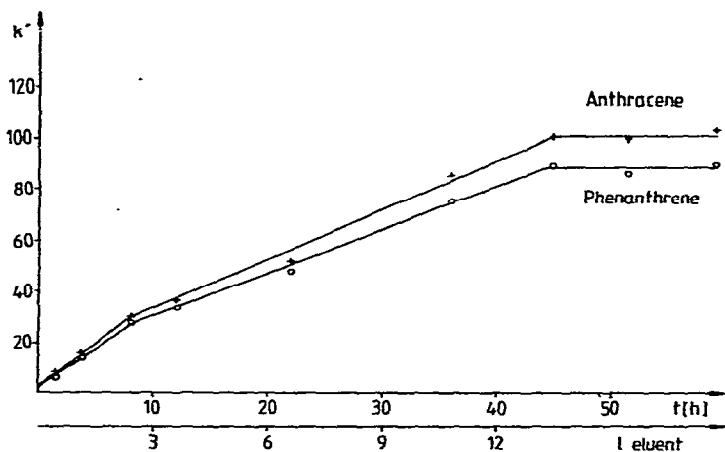


Fig. 5. Removal of water from an analytical column (MCS in equilibrium). MCS: silica SI 40 (activated at 150° for 4 h); temperature = 25°. Column: alumina (Woelm), 5  $\mu$ m; 15 cm  $\times$  4 mm I.D.; temperature = 25°. Eluent: *n*-heptane, equilibrium water concentration <5 ppm.  $\Delta p = 160$  atm;  $F \approx 5$  ml/min;  $u = 7$  mm/sec. Samples: anthracene ( $k' = 103$ ) and phenanthrene ( $k' = 88$ ).

Fig. 5 shows the increase of the  $k'$  values of anthracene and phenanthrene as the chromatographic column becomes increasingly drier, the value for anthracene increasing from 2.5 to about 100. The time required to obtain constant  $k'$  values was more than 50 h.

The equilibration time of the MCS as determined by Karl Fischer titration,

with the restrictions mentioned above (*i.e.*, 10-ml samples), appears to be much shorter than that determined by the chromatographic method, *i.e.*, measuring the  $k'$  values of standard samples.

From Figs. 4 and 5, it follows that after the MCS system is equilibrated (on the basis of the Karl Fischer titration), a further 50 h are required to achieve constant retentions of the samples. It is possible, but unlikely, that the equilibration time between water and stationary phase in the analytical column requires much more time than the same equilibration (however, with larger particles) in the MCS. It is more likely that the chromatographic method (*i.e.*, the determination of  $k'$  values of selected standards) is a more sensitive way of determining the "water content" of the eluent, especially if it is low and the amount of eluent available for the titration is limited. Regardless of which of these methods is the more sensitive, constant retentions are required for routine chromatography. From the point of view of chromatography, the water content of a given eluent can be characterized by the capacity ratios of selected standard samples. It will be demonstrated that these capacity ratios (*i.e.*, the "water concentrations" in the eluent) can be adjusted by changing the temperature of the MCS (*i.e.*, that of funnel B and/or the temperature of the chromatographic column). Of course, the same effect can be achieved by changing the water content of the material in the MCS funnel.

Because the time required to achieve constant  $k'$  values with the chromatographic column is longer than that required to attain equilibrium in the MCS (as measured by Karl Fischer titration), in subsequent experiments the whole system, *i.e.*, the MCS and the chromatographic column, was always equilibrated together. Karl Fischer titration was used only to determine the water concentration in the eluent after equilibrium conditions had been approached.

#### *Separations with eluents containing reproducible concentrations of water*

The two eluents studied, *n*-heptane and dichloromethane, are representative non-polar and moderately polar eluents, respectively. For analytical applications, a completely "dry" stationary phase is undesirable because even non-polar aromatic hydrocarbons such as anthracene would have excessively large  $k'$  values. Instead, "moderate" water concentrations are desirable, which can be obtained by loading the MCS with silica or alumina containing a definite amount of water.

*I. Silica in the MCS with n-heptane as eluent.* Table I summarizes the results obtained when the MCS contained silica impregnated with different amounts of water (5 and 20%, w/w). Approximately 50 h were required to reach equilibrium between the silica in the MCS and the water in the entire system, regardless of whether the silica added or removed water. In addition, the  $k'$  values from of Fig. 5 (0% water coating) are included in Table I.

When the MCS contained silica impregnated with 5% of water, the  $k'$  value of anthracene was only 2.6 compared with 103 for the "dry" system, while the corresponding values for naphthalene were 0.6 and 4.1. The concentration of water in *n*-heptane as determined by Karl Fischer titration was 18 ppm.

If more water (20%, w/w) was added to the silica in the MCS, a further decrease in the  $k'$  values was noted. At equilibrium, about 55 ppm of water could be measured in the eluent by Karl Fischer titration.

In the experiments described above, the MCS was filled with a silica with an

TABLE I  
EQUILIBRIUM CAPACITY RATIOS OF AROMATIC HYDROCARBONS

MCS filling: silica (40 Å) with different water contents. Eluent: *n*-heptane. Column: packed with neutral alumina (5 μm). Temperature of MCS and column: 25 ± 0.25°.

Water coating on the silica (%, w/w)	Water concentration in the eluent (ppm)	Capacity ratio ( $k'$ )			
		Benzene	Naphthalene	Anthracene	Chrysene
0	<5	0.35	4.1	103	>120
5	18	0.14	0.59	2.6	15.5
20	55	0.06	0.19	0.45	1.3

average pore diameter of 40 Å, and the equilibration time remained unchanged if silica with an average pore diameter of 200 Å was used.

*Temperature dependence.* Increasing the temperature of the contents of the MCS funnel increases the water concentration in the recycled eluent. Consequently, if the temperature of the chromatographic column was kept constant (25°), the  $k'$  values of the samples decreased. Fig. 6 demonstrates the decrease in the  $k'$  values for chrysene, anthracene and phenanthrene as the temperature of the MCS was increased

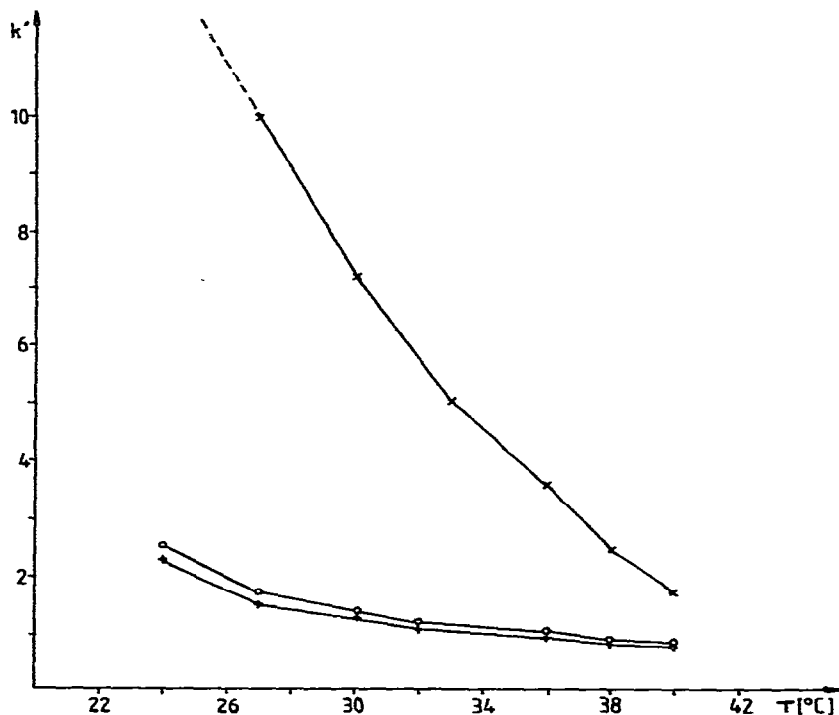


Fig. 6. Capacity ratios ( $k'$ ) as a function of the temperature of the MCS. MCS: silica SI 40 (activated at 150° for 4 h, 6% of water added). Column: Spherisorb alumina, 5 μm; 20 cm × 4.1 mm I.D.; temperature = 25°. Eluent: *n*-heptane. Samples: +, phenanthrene; O, anthracene; ×, chrysene.



from 24° to 40°; these are, of course, equilibrium values. As was predictable from the heat of sorption, the larger the  $k'$  values the more rapidly they decreased as the temperature of the MCS was increased. The  $k'$  value of chrysenes for example, decreased from 15.5 at 22° to about 2 at 40°. Equilibrium conditions were reached more rapidly at higher than lower temperatures of the MCS; it took about 30 h at an MCS temperature of 40° to obtain constant  $k'$  values.

These results demonstrate the necessity for exact control of the temperatures of the MCS and the analytical column, especially if non-polar eluents with low water concentrations are to be used.

*II. Alumina filling in the MCS with n-heptane as eluent.* Constant water concentrations as measured by Karl Fischer titration were achieved at identical rates when either silica or alumina was placed in the MCS funnel. The time required to achieve equilibrium conditions defined by chromatographic parameters ( $k'$  values) is too long for practical analyses if the MCS contains silica, and alumina was therefore also tried as a water regulator in the MCS.

It was pointed out earlier that the time of equilibration (*ca.* 10–15 h) was independent of whether the MCS contained silica or alumina, when the water concentration was determined by Karl Fischer titration (Fig. 4). With silica in the MCS, constant capacity ratios were achieved after *ca.* 50 h (Fig. 5).

In subsequent experiments, the MCS was loaded with alumina ( $d_p = 63\text{--}200 \mu\text{m}$ ) that had been dried at 200° for 4 h. The chromatographic column was packed with alumina, as usual. The temperature of the MCS and the column was 25°. Fig. 7 demonstrates that the equilibration time is about 40 h, based on the  $k'$  values of naphthalene. In this instance, both the eluent and the chromatographic column were "wet" initially (*i.e.*, the capacity ratios of the naphthalene increased with time). In Figs. 5 and 7, the water in the eluent was removed by the MCS. It should be emphasized that the time required for the equilibration becomes shorter at higher flow-rates of the recycled eluent. An increase in the flow-rate increases the heat of friction<sup>10</sup>. At high flow-rates, the equilibrium may be governed by the thermal equilibrium of the system.

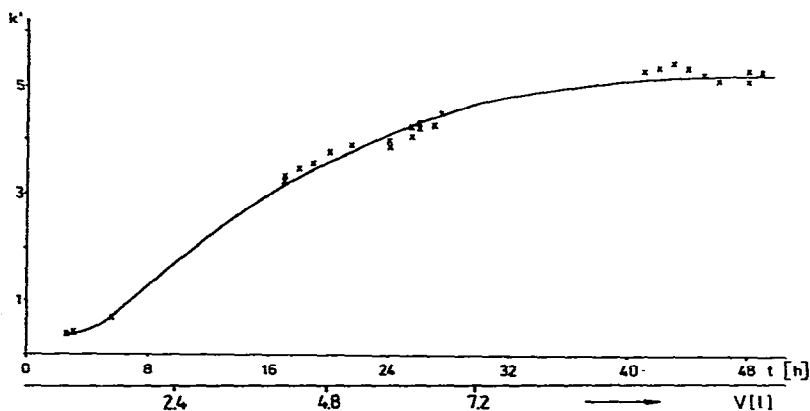


Fig. 7. "Drying" a chromatographic column with MCS. MCS: alumina with 0% of water; temperature = 25°. Column: alumina (Woelm), 5  $\mu\text{m}$ ; 15 cm  $\times$  4.1 mm I.D.; temperature = 25°. Eluent: *n*-heptane, final water concentration < 5 ppm.  $\Delta p = 140$  atm;  $F = 4$  ml/min. Sample: naphthalene.

Fig. 7 represents a system in which the chromatographic column was "wet" initially and was dried by means of a "dry" eluent; the capacity ratio of naphthalene increased with increasing equilibration time. Fig. 8 demonstrates the reverse procedure: the chromatographic column was "dry" initially and was then flushed with "wet" eluent. The MCS system was filled with alumina coated with 15% (w/w) of water. The capacity ratio of naphthalene decreased with time. During this procedure, water was transported from the MCS into the chromatographic column. For all of the systems described thus far, about 50 h were required for equilibration. Under the experimental conditions given in Fig. 8, constant  $k'$  values were achieved after about 10 h.

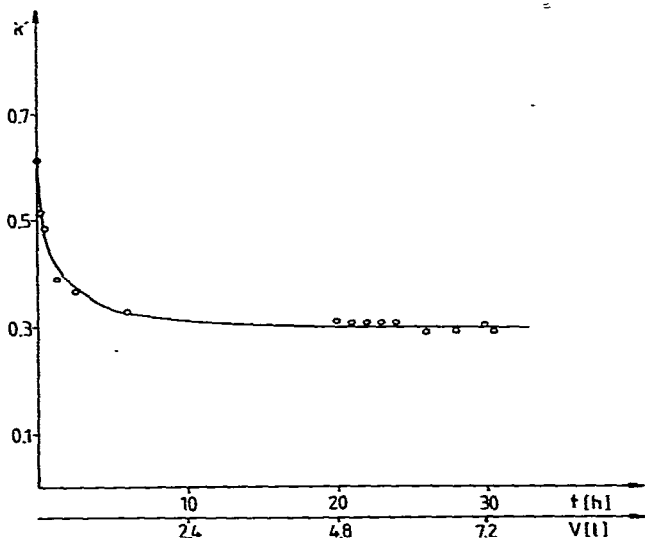


Fig. 8. "Wetting" a chromatographic column with MCS. MCS: alumina with 15% (w/w) of water; temperature = 25°; Column: alumina (Woelm), 5  $\mu$ m; 12.5 cm  $\times$  4.1 mm I.D.; temperature = 25°. Eluent: *n*-heptane, final water concentration 65 ppm.  $\Delta p = \pm 115$  atm;  $F = 4$  ml/min. Sample: naphthalene.

Table II demonstrates the effect of the water alumina ratios in the MCS filling on the  $k'$  values of three aromatic hydrocarbons. The highest water concentration used in the MCS was 15% (w/w) because at higher water concentrations the alumina tends to become sticky. It can be seen that the  $k'$  values of these hydrocarbons are a very sensitive function of the water content of the eluent. The  $k'$  value of anthracene decreased from 120 to 26 on changing from dry alumina in the MCS to one impregnated with 3% (w/w) of water; the corresponding water concentration increased from 5 to 12 ppm. Again, it was demonstrated that the  $k'$  values are a much more sensitive way of establishing when equilibrium conditions have been attained.

*III. Alumina in the MCS with dichloromethane as eluent.* An important difference between the use of dichloromethane and *n*-heptane as eluent was the higher rate of equilibration. The slowest process, the removal of water from the chromatographic column when the MCS contained freshly activated alumina, took about 10–12

h (overnight). The reverse process, deactivation of the analytical column by increasing the water content of the eluent, was much faster and the  $k'$  values approached constancy within 5–7 h. During the increase in the water concentration of the eluent and consequently in the chromatographic column, variations in the elution order are likely<sup>3</sup>. When relatively dry dichloromethane (water content *ca.* 20 ppm) was used, *m*-nitroaniline was eluted after vitamin D<sub>2</sub> and the relative retention of *m*-nitroaniline to vitamin D<sub>2</sub> in this system was 1.30. At higher water concentrations (*ca.* 100 ppm), *m*-nitroaniline was eluted before vitamin D<sub>2</sub> and the relative retention of the former changed to 0.79. There exists, of course, a water concentration at which both components are eluted unresolved.

TABLE II

## CAPACITY RATIOS OF AROMATIC HYDROCARBONS

MCS filling: neutral alumina (Woelm). Eluent: *n*-heptane. Column: packed with neutral alumina (5  $\mu$ m). Temperature of MCS and column: 25°.

Water concentration in MCS (% w/w)	Water concentration in eluent (ppm)	Capacity ratio ( $k'$ )		
		Naphthalene	Anthracene	Chrysene
0	<5	5.3	117	≥100
3	12	2.1	26	≥100
6	40	0.33	0.90	2.4
10	56	0.32	0.71	1.62
15	60	0.30	0.67	1.50

In Table III, the capacity ratios of standard samples are tabulated as a function of the water content of the eluent. The results given in the bottom row of Table III could be achieved either with 15% (w/w) of water coating in the MCS or with a water-saturated eluent: the MCS system contained a thin layer of water floating on top of the eluent. At equilibrium, the pores of the alumina in the column were partially filled with water<sup>11</sup>. The total porosity is only  $\epsilon_T = 0.65$  compared with  $\epsilon_T = 0.8$  for all the other columns.

TABLE III

## CAPACITY RATIOS OF SOME ORGANIC SAMPLES

MCS filling: neutral alumina (Woelm). Eluent: dichloromethane. Column: packed with Spherisorb A5Y (alumina). Temperature of MCS and column: 25°.

Water concentration in MCS (% w/w)	Water concentration in eluent (ppm)	Capacity ratio ( $k'$ )			
		Cholestenone	<i>m</i> -Nitroaniline	1,4,5-Xylenol	Phenol
0	<10	3.58	13.5	—	—
3	700	0.52	0.57	1.15	3.9
6	1150	0.34	0.35	0.77	2.05
10	1800	0.28	0.31	0.68	1.72
15	2100	0.18	0.24	0.62	1.53

### *Reproducibility of $k'$ values using the MCS*

It can be seen from the results presented above that the  $k'$  values depend on the amount of water impregnated on the material in the MCS, on its temperature and on the temperature of the analytical column. If all of these parameters were kept constant, the  $k'$  values could be measured with a repeatability of better than 5% over a period of several weeks, although for  $k'$  values below 4 the repeatability decreased below 2.5%. Only if the MCS was thermostated while the chromatographic column remained at ambient temperature could the  $k'$  values be determined to within 10%; with no thermostating the repeatability was not better than  $\pm 20\%$ .

The above reproducibilities were achieved without changing the temperature of the MCS or that of the chromatographic column. Further, the water content of the MCS was also kept constant. If the adsorbent in the MCS funnel and the temperature of the entire system were changed, the reproducibility of the  $k'$  values of the standards was always within 10% ( $k' > 20$ ). For smaller  $k'$  values, the reproducibility was better than 10%.

### *Loadability*

The loadability (maximum sample size) is also a function of the water content of the eluent: the lower the water content, the smaller is the loadability. For a water content of 45 ppm in dichloromethane the loadability was about  $1 \cdot 10^{-5}$  g of sample per gram of stationary phase, and an increase to 180 ppm increased the loadability to  $5 \cdot 10^{-5}$  g/g. This change could be attributed to greater surface homogeneity with increasing water content in the eluent and consequently on the surface of the stationary phase<sup>3</sup>. At water concentrations below 45 ppm, the loadability was barely measurable because it approached the detection limit. For high water concentrations in dichloromethane (1000 ppm), the loadability of the system was about  $3 \cdot 10^{-4}$  g/g, leading to values obtained with heavily loaded columns of about  $10^{-3}$  g/g (ref. 12).

### *Applications*

From experience, it appears that for routine separations typical water concentrations in the MCS (packed with alumina) are of 0, 3, 6, 10 and 15% (w/w). By equilibrating *n*-heptane or dichloromethane with the above water concentrations, eluents with reproducible water contents are obtained, as presented in Tables II and III. These water concentrations are additionally defined in terms of the capacity ratios of the standards. In Figs. 9–11, some typical separations illustrating the effect of the water content of the eluent are shown. It should be noted that, in order to optimize the time of analysis, it is sometimes advantageous to use water concentrations different to those proposed in the tables.

Fig. 9 shows separations of some aromatic hydrocarbons with water contents of the *n*-heptane of 31, 25 and 15 ppm.

The separation of carcinogenic polynuclear aromatic hydrocarbons is demonstrated in Fig. 10. The compounds in this mixture are typical of those present in automobile exhaust gases<sup>13</sup>.

The separations of some steroids with dichloromethane (with different water contents) are shown in Fig. 11. Whether 1150 or 1800 ppm of water in the eluent is preferable depends on the importance of the analytical problem to be solved.

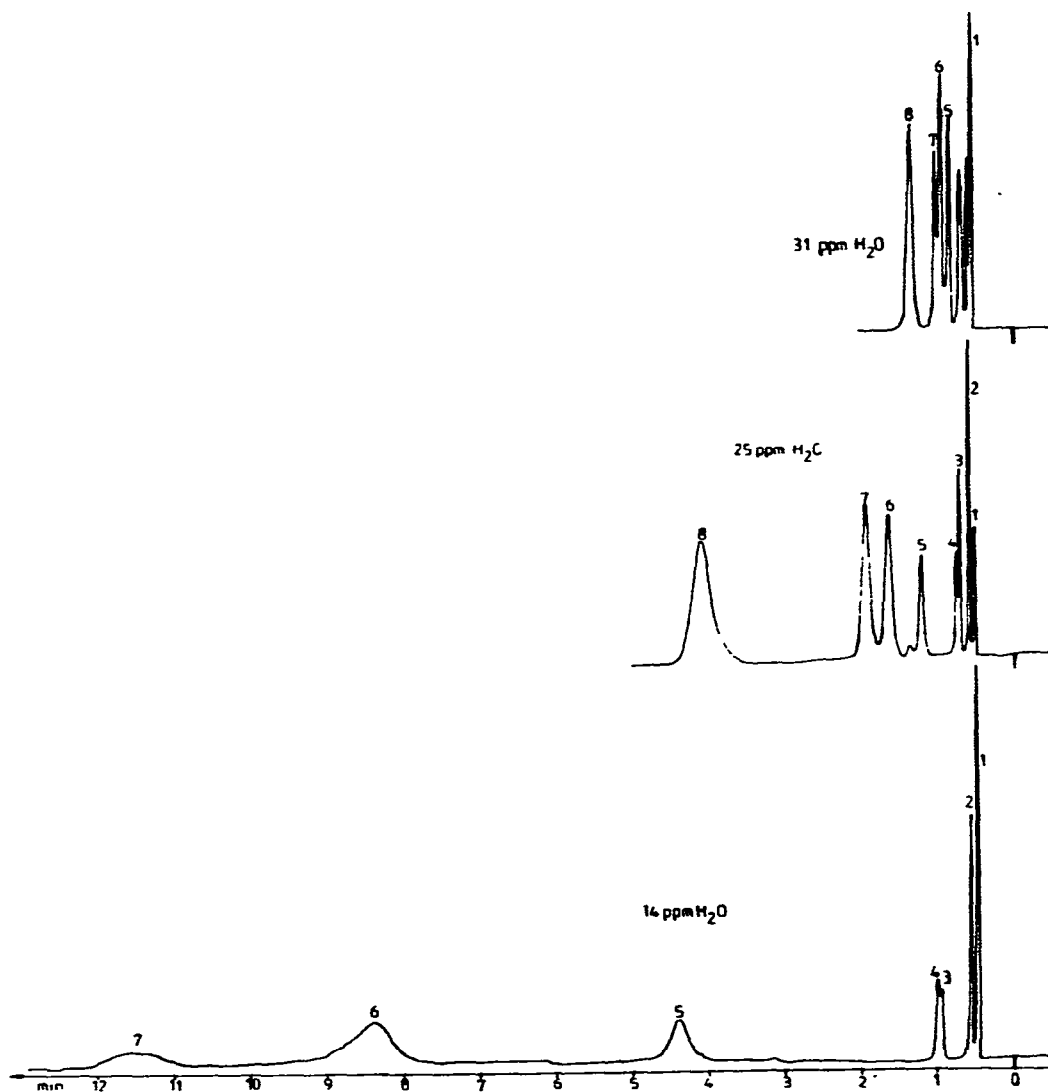


Fig. 9. Influence of the water concentration of the eluent on the separation of aromatic hydrocarbons (equilibrium conditions). MCS: alumina with 4.5, 6 or 9% (w/w) of water; temperature = 25°. Column: alumina (Woelm), 5  $\mu$ m; 15 cm  $\times$  4.1 mm I.D.; temperature = 25°. Eluent: *n*-heptane with 15, 25 or 31 ppm of water.  $\Delta p$  = 100 atm;  $F$  = 2.8 ml/min;  $u$  = 4.2 mm/sec. Samples: 1 = tetrachloroethylene; 2 = benzene; 3 = naphthalene; 4 = biphenyl; 5 = anthracene; 6 = pyrene; 7 = fluoranthene; 8 = 1,2-benzanthracene.

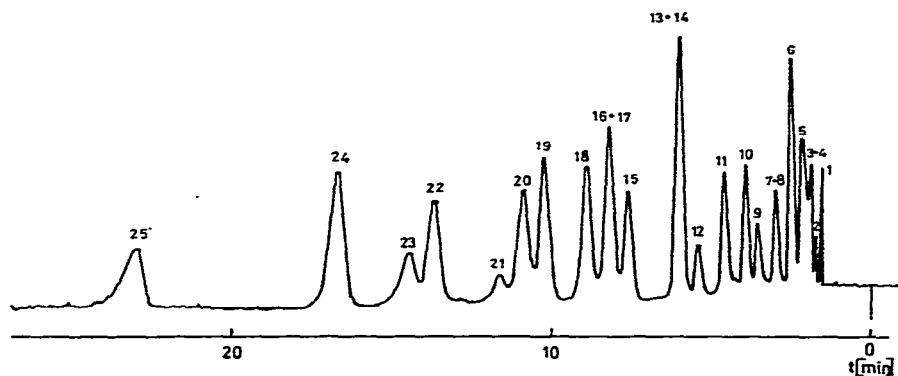


Fig. 10. Separation of polynuclear aromatic hydrocarbons. MCS: alumina with 10% (w/w) of water; temperature = 25°. Column: Spherisorb alumina, 5  $\mu$ m; 20 cm  $\times$  4.1 mm I.D.; temperature = 25°. Eluent: *n*-heptane with 37 ppm of water.  $\Delta p$  = 30 atm;  $F$  = 1.6 ml/min;  $u$  = 2.4 mm/sec. Samples: 1 = tetrachloroethylene; 2 = benzene; 3 = naphthalene; 4 = biphenyl; 5 = dichloromethane (sample solvent); 6 = acenaphthene; 7 = anthracene; 8 = phenanthrene; 9 = pyrene; 10 = fluoranthene; 11 = 1,10-benzfluoranthene; 12 = triphenylene; 13 = 1,2-benzanthracene; 14 = chrysene; 15 = 1,2-benzpyrene; 16 = 3,4-benzpyrene; 17 = perylene; 18 = 3,4-benzfluoranthene; 19 = 11,12-benzfluoranthene; 20 = 1,12-benzperylene; 21 = anthanthrene; 22 = 2,3-phenylenepyrene; 23 = coronene; 24 = 1,2,7,8-dibenzanthracene; 25 = 1,2,5,6-dibenzanthracene.

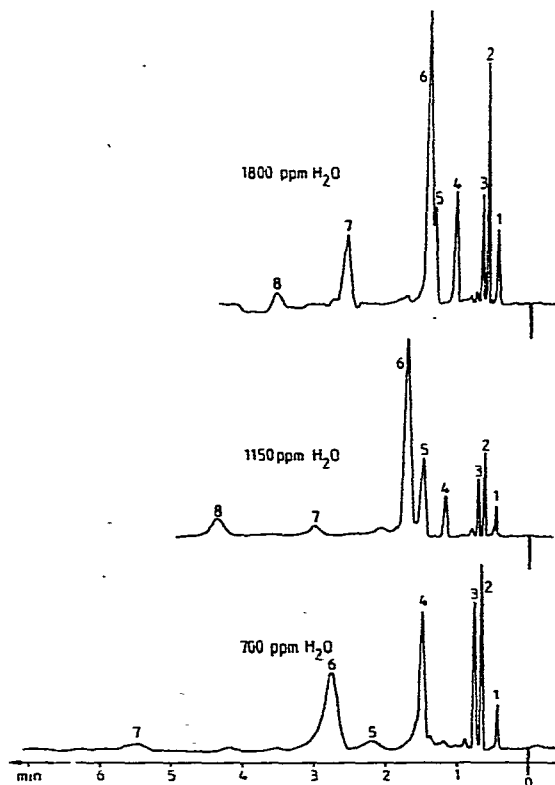


Fig. 11. Influence of the water concentration of the eluent on the separation of steroids (equilibrium conditions). MCS: alumina with 3, 6 or 10% (w/w) of water; temperature = 25°. Column: Spherisorb alumina, 5  $\mu$ m; 20 cm  $\times$  4.1 mm I.D.; temperature = 25°. Eluent: dichloromethane with 700, 1150 or 1800 ppm of water.  $\Delta p$  = 120 atm;  $F$  = 5.4 ml/min;  $u$  = 8.1 mm/sec. Samples: 1 = tetrachloroethylene; 2 = cholestenone; 3 = progesterone; 4 = 17-methyltestosterone; 5 = estrone; 6 = 17-hydroxyprogesterone; 7 = estradiol; 8 = prednisolone.

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