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COLUMN SWITCHING IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-pressure micro multiport switching valve, its performance and application are described. It is shown that the contribution of column switching to the peak width is negligible with this valve. Different modes of operation are demonstrated:

(1) Control of the total elution of the sample, by-passing the column.
(2) Stripping of a part of the sample, using a sectional column system.
(3) Separation of a complex mixture on a given phase system using columns of different phase ratio.

INTRODUCTION

Column switching is a chromatographic technique in which the sample is separated on a system of columns in which the same or different types of columns are used. The principle of operation is to transfer selectively fractions of the mobile phase from the outlet of one column to the inlet of another column. The transferred volume can correspond to a group of peaks, a single peak or a fraction of a peak. In principle, the method can be carried out either off-line or on-line, and with programmed operation. It is obvious that the on-line operation is preferable. Column switching chromatography is also called coupled column chromatography, multiple column chromatography and column programming chromatography. The method is widely used in process gas chromatography[1] and to a smaller extent also in laboratory gas chromatography. It has also been used in low-pressure liquid chromatography[2–4], but did not find wide application. Its use in high-pressure, high-efficiency liquid chromatography has not yet been reported. The main problem in this field of application is the switching device. A switching valve must be capable of operating many times at high pressure and must have a very low hold-up volume, so that the peaks passing the valve are not significantly broadened. Such a valve was constructed and its testing is described in this paper.

THEORETICAL

Mixing phenomena in tubes

In order to clarify the discussion of the experimental results, a short review of the theory of the longitudinal dispersion of a compound in a fluid flowing through a tube is given here. The discussion is restricted to laminar flow, as chromatography is concerned with this type of flow. It is assumed that the sample is initially present in a very short section of the tube and uniformly distributed over the cross-section. As a longitudinal concentration gradient exists, molecular diffusion will occur in this direction. Another dispersion phenomenon, convective dispersion, is caused by the flow profile in the cross-section. Owing to the flow profile, different fractions of the sample will move with different velocities, depending on their position in the cross-section. Because of this different displacement of the sample, a transverse concentration gradient is created which causes transverse diffusion. The combined effect of the different displacement and transverse diffusions is convective dispersion. If the tubes are not straight but are bent or twisted, secondary flow arises, which causes additional transverse mass transport. All of these phenomena can be included in the theoretical treatment[5,6] which results in an expression for the total longitudinal dispersion coefficient:

$$D_{ll} = \lim_{t \to \infty} \tfrac{1}{2} \frac{\mathrm{d}(\sigma_{zl}^2)}{\mathrm{d}t} = D_{lm} + k \frac{d^2 v^2}{192\, D_{lm}} \tag{1}$$

where

D_{ll} = longitudinal dispersion coefficient
σ_{zl}^2 = longitudinal variance of the sample distribution
z = length co-ordinate
t = time
D_{lm} = diffusion coefficient of the component l in the moving fluid m
v = average fluid velocity in the cross-section
k = characteristic of the eccentricity of the tube cross-section (circular, $k = 1$; elliptic, $^5/_{12} \leqslant k < 1$)
d = diameter of the tube cross-section.

The longitudinal dispersion coefficient is defined as half of the rate of increase of the longitudinal variance. Integration of eqn. 1 gives, for circular cross-section:

$$\sigma_{zl0}^2 = (2D_{lm} + \frac{r^2 v^2}{24 D_{lm}})\, t + \text{constant} \tag{2}$$

where $r = d/2$ = radius. The integration constant is the initial value, σ_{zl0}^2, of the longitudinal variance (constant $= \sigma_{zl0}^2$).

Substitution of t, v and σ_z by means of $t = L/v$, $v = w/r^2\pi$ and $\sigma_{zl} = \sigma_{Vl}/r^2\pi$ gives the expression

$$\Delta(\sigma_{Vl}^2) = \left(2\pi^3 \frac{D_{lm}\, r^6}{w} + \frac{\pi}{24} \cdot \frac{r^4 w}{D_{lm}}\right) L \tag{3}$$

where

$\Delta(\sigma_{vt}^2) = \sigma_{vt}^2 - \sigma_{v0}^2$
L = length of the tube
w = flow-rate

This expression[7,8] is correct only for long, straight tubes, as it is the limiting value for $t \to \infty$, neglecting secondary flow effects.

EXPERIMENTAL

Apparatus

A high-pressure liquid chromatograph (Siemens S200) with an ultraviolet (UV) spectrophotometric detector was used, modified by the installation of a high-pressure multiport micro valve (Siemens). A schematic diagram of the valve is shown in Fig. 1. It is a pneumatically operated piston valve, with one inlet which can be connected in turn to one of two or three outlets, depending on the type of valve. It has a smooth flow path. The specifications are given in Table I. Consideration was given also to a switching device in which the switching was performed without the sample having to pass a valve. Such an arrangement has been used successfully in gas chromatography[9], but would be too expensive in liquid chromatography as more than one pumping system has to be used.

All the flow lines downstream from the sampling device were made of stainless-steel tubing of 0.25 mm I.D. Branchings were carefully constructed so as to avoid introducing any additional volume. If necessary, the flow-rates in the different lines were matched by means of a high-pressure needle valve (Siemens) without a significant dead volume.

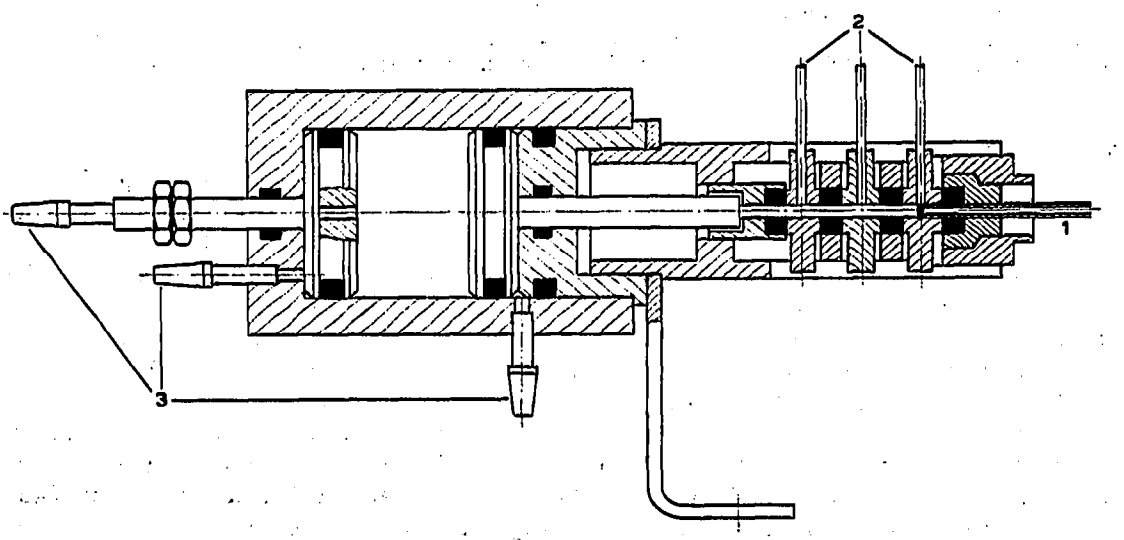


Fig. 1. Schematic diagram of the switching valve. 1 = Inlet, 2 = outlets, 3 = air supply.

TABLE I

SPECIFICATION OF THE HIGH-PRESSURE MULTIPORT SWITCHING MICRO VALVE

Property	*Specification*
Volume (including feed pipes)	20 μl
Tightness	Up to 300 bar
Temperature stability	Up to 180°
Lifetime (without change of parts)	10^6 actuations at 100°
Pressure for pneumatic operation	2.5–5 bar
Number of outlet ports	2 or 3
Connectors	Siemens high-pressure screwing
Construction materials	PTFE, stainless steel, tantalum (virtually no corrosion problems)

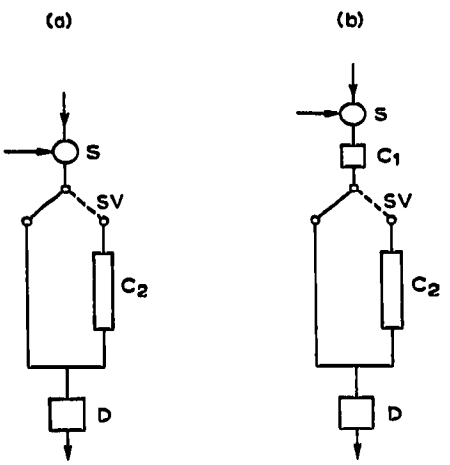


Fig. 2. Systems for column switching. S = sampling device, SV = switching valve, C_1, C_2 = chromatographic columns, D = detector. (a) Total elution control system; (b) single channel sequential column system (for column stripping or for adaption of the phase ratio).

Operating modes in column switching chromatography

The valve can be used in different arrangements with sampling device, columns and detector. Flow diagrams of the arrangements tested are shown in Fig. 2. In the first arrangement, the column is by-passed in order to control the total elution of the sample. In the second arrangement, two columns are connected in series by the switching valve. Columns of the same type varying only in length, or different types of columns having the same eluent, can be used. The sectional column system can be applied for stripping, in which only the first short column section is used for the later part of the chromatogram, the longer column section then being by-passed. The stripped part of the sample can be led to the detector or to a vent. The column combination consisting of different types of columns is used to handle samples with a large distribution coefficient range. The first column has a low phase ratio, *i.e.*, a low surface area in adsorption chromatography or a low stationary liquid volume in solution chromatography. The second column has a high phase ratio, *i.e.*, a high surface area

or a high stationary liquid volume. Only the first part of the sample eluted from the first column is led into the second column, while the remaining part is led immediately to the detector. The first part is stored in the second column during the elution of the second part from the first column, after which it is eluted from the second column and led to the detector.

Another arrangement which is still being tested is a two-channel system. It consists of two chromatographic systems, each with its own eluent delivery unit, sampling device, sectional column system, switching valve, and detector. Both systems are interconnected by the two switching valves which allow the transfer of any part of the sample from a column in one system to a column in the other system. Both systems can be operated independently.

The switching valve can also be used in a flexible high-pressure sampling system, as an allocating valve in a fraction collector or in a recycling chromatograph.

RESULTS AND DISCUSSION

Mixing in circular tubes

Theory is not able to describe the mixing phenomena in the short tubes that are used as connecting and by-pass lines in liquid chromatography, and the mixing characteristics therefore had to be determined experimentally. Stainless-steel capillary tubes with various lengths (90, 150, 350 and 600 mm) and diameters (0.25, 0.4 and 0.5 mm) were connected on one side to the injection block and on the other side to the detector cell, which had a volume of 2 or 8 μl depending on the volume standard deviation of the peaks to be measured. In order to record a peak without significant broadening, the detector cell must be smaller than one third of the volume standard deviation of the peak[10]. Other effects on the width of the elution peaks are caused by the sample volume, the injection technique, the time constants of the detector and the recorder, and we tried to minimize these effects. A sample volume of 0.9 μl was used and injected in less than 0.1 sec. The main error is probably caused by the time constant of the detector, which was 0.2 sec, and the pen speed of the recorder, which was 80 cm/sec. The mobile phase was 2,2,4-trimethylpentane and the flow-rate was varied between 0.03 and 6 ml/min. A solution of toluene in the mobile phase was used as the sample, and the sample concentration was adjusted to give a detector signal of less than 0.5 absorption units. Typical results are shown in Fig. 3. The peaks for short tubes and high flow-rates were significantly asymmetric, whereas for low flow-rates and long tubes, fairly symmetrical peaks were found. Eqn. 3 predicts a parabolic relationship for the plots in Fig. 3. This prediction is approached only at low flow-rates, small diameters and large lengths, where the assumptions for eqn. 3 are fulfilled to some extent. The results obtained with long tubes[11,12] cannot be used to draw conclusions about the results with short tubes.

Mixing characteristics of the switching valve

In order to test the switching valve, its inlet was connected directly to the injection system and its outlet, by means of stainless-steel capillary tubing (200 × 0.25 mm), to the detector cell. This arrangement was compared with an arrangement in which the capillary was connected directly to the injection block, leaving out the valve. The standard deviation of the recorded peak was calculated statistically and

converted into the volume standard deviation by using the recording velocity and the flow-rate. Peak asymmetry was characterized by the ratio of the standard deviations calculated from the width of the peak at 0.1 and 0.6 of the height, assuming a gaussian peak in which these widths are given by $W_{0.1} = 4.3\sigma$ and $W_{0.6} = 2\sigma$.

The results are shown in Table II, from which it can be concluded that the standard deviation caused by the valve is only small. Table III demonstrates that it is insignificant in chromatographic applications. A change in the resolution caused by a change in the standard deviation of the peaks of 10% can hardly be recognized. Peaks with a standard deviation of less than 25 μl cannot be measured without peak broadening with a detector cell of 8 μl.

TABLE II

DISPERSION CAUSED BY THE SWITCHING VALVE

Inclusive valve			*Exclusive valve*			$\sqrt{\Delta(\sigma_V^2)}$ (μl)*
w (ml/min)	σ_V (μl)	$\sigma_{0.1}/\sigma_{0.6}$	*w (ml/min)*	σ_V (μl)	$\sigma_{0.1}/\sigma_{0.6}$	
0.036	24.5	0.9	0.033	22.7	0.9	9
0.065	27.0	1.0	0.064	24.8	1.0	11
0.164	31.0	1.2	0.140	24.5	1.3	18
0.320	31.8	1.3	0.336	25.5	1.5	19
0.53	31.0	1.4	0.53	26.9	1.6	15
0.72	30.3	1.4	0.74	30.3	1.6	0
0.91	29.2	1.3	0.93	29.4	1.6	0
1.14	31.0	1.4	1.12	26.1	1.6	17
1.34	29.9	1.4	1.33	26.7	1.5	14
1.52	29.0	1.4	1.52	27.1	1.4	10
1.88	28.6	1.4	1.87	27.8	1.4	7

* The standard deviations of the inclusive valve were interpolated for the flow-rates of the exclusive valve.

TABLE III

CALCULATION OF THE EFFECT OF THE VALVE ON THE STANDARD DEVIATION OF THE ELUTION PEAK

σ_V *column* (μl)	*Max.* σ_V *valve* (μl)	*Max.* $(\Sigma\sigma_V^2)^{\frac{1}{2}}$	*Max.* $\Delta\sigma_V/\sigma_V$ (%)
25	20	32	21.9
50		53.9	7.7
100		102.0	2.0
150		151.3	0.9
200		201.0	0.5

Control of total sample elution

One of the problems in column chromatography is the fact that only those compounds which are eluted from the column in a reasonable time can be detected.

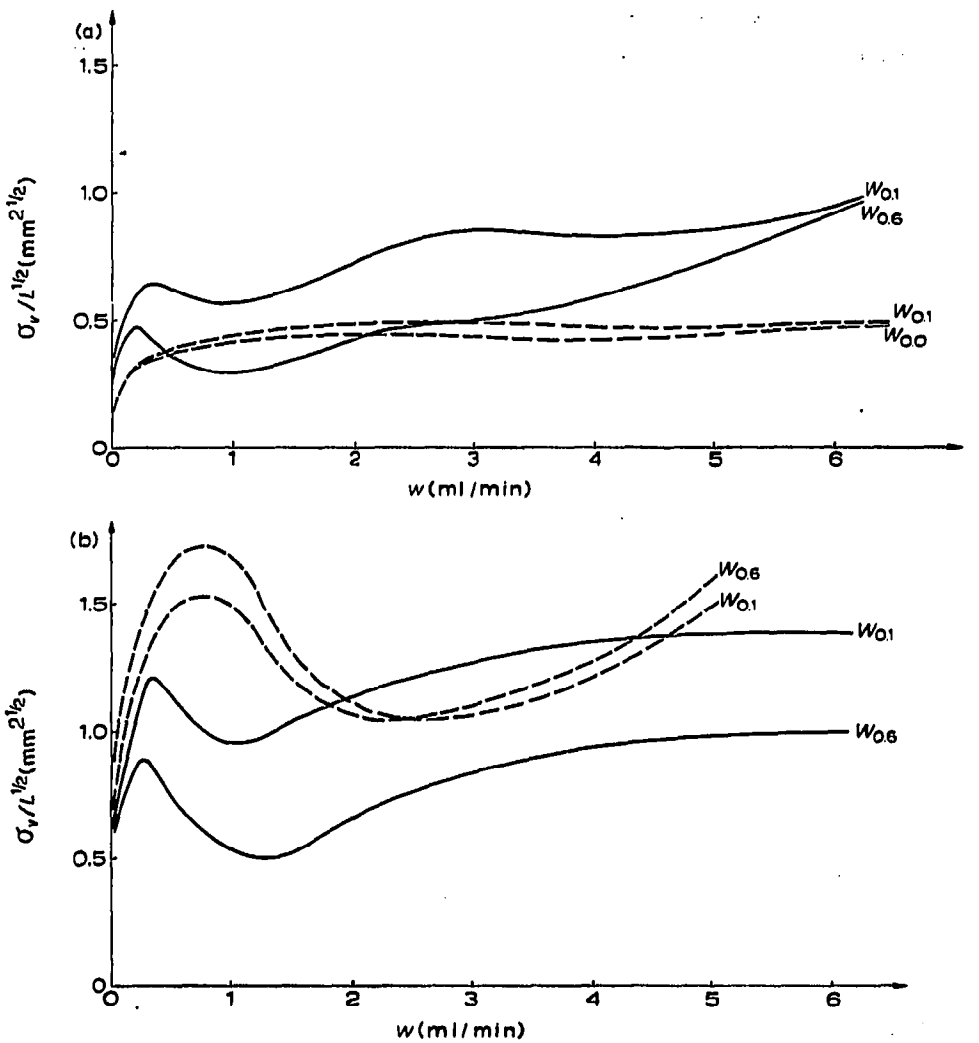


Fig. 3. Mixing characteristics of straight circular tubes (plot of the normalised volume standard deviation $\sigma_V/L^{\frac{1}{2}}$ versus the flow-rate w). Length 90 (———), 600 (— — —) mm; diameter 0.25 (a), 0.50 (b) mm; σ_V calculated from the width at 0.1 ($W_{0.1}$) and 0.6 ($W_{0.6}$) of the height of the peak.

It is, therefore, desirable to be able to control the total elution of the sample. Such a control can be performed in the system shown in Fig. 2a, by column switching. The flow-rates in the by-pass capillary tube and the column have to be exactly matched. The flow-rate was adjusted by means of a high-pressure micro needle valve and determined by collecting and weighing the effluents. Fig. 4 shows chromatograms obtained from the column and the by-pass line, respectively. Detector sensitivity and recording speed were chosen differently in both cases in order to make possible a better visual comparison of the peaks. The experiments were carried out four-fold. The precision of the peak area was found to be 2.5% for the unresolved peak and 0.5% for the sum of the single peaks. The mean values of both areas differed by 2.8%, the sum

of the peakshaving the smaller area. Although the difference is not really significant, two additional small peaks with a retention time of about 2 h were discovered after recording a longer time.

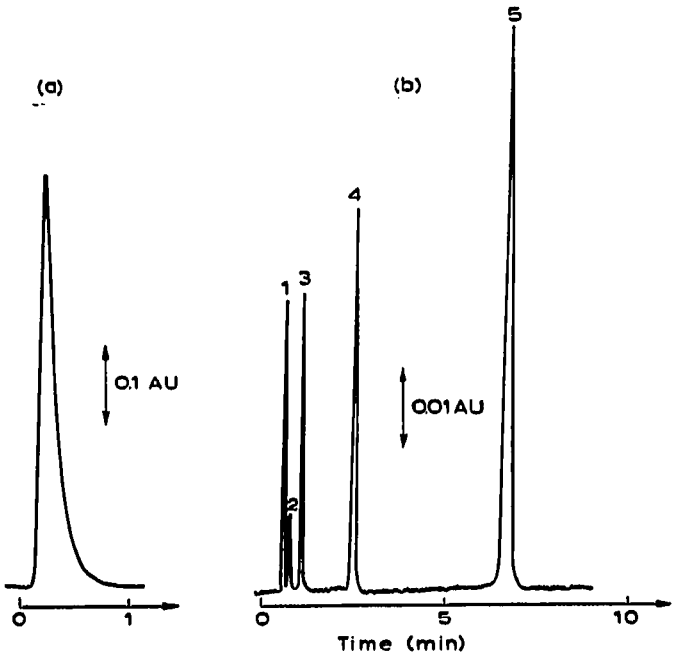


Fig. 4. Total elution control. Sample: 1 = toluene, 2 = azobenzene, 3 = 2-nitrobenzene, 4 = 2,6-dimethylphenol, 5 = 2,4-dimethylphenol. Column: 290 × 3 mm; packing, 1,2,3-tris(cyanoethoxy) propane on silica support, 4–8 μm, 10% (w/w); eluent, 2,2,4-trimethylpentane. Detector: UV, 270 ± 10 nm. Injection volume 10 μl. (a) Sampling device connected to by-pass line; (b) sampling device connected to column. Flow-rate. 0.4 μml/min.

Stripping the column

For the column stripping experiments, the set-up shown in Fig. 2b was applied using a short first and a longer second column of the same type. Fig. 5a shows a chromatogram obtained with both column sections together. Fig. 5b shows a chromatogram in which the peak-free space in the first chromatogram is cut out by column switching. The first group of components is separated on both column sections; the second group only on the short first section. The separation time is reduced in this way from about 45 min to 8 min. This type of column switching can be applied if only the first components of a sample are to be determined. In this case it is not necessary to resolve the rest of the sample which should be eluted as fast as possible. The length of the first column section has to be carefully chosen with regard to the problem in hand.

Phase ratio adaption

In general, a complex mixture containing very different types of com-

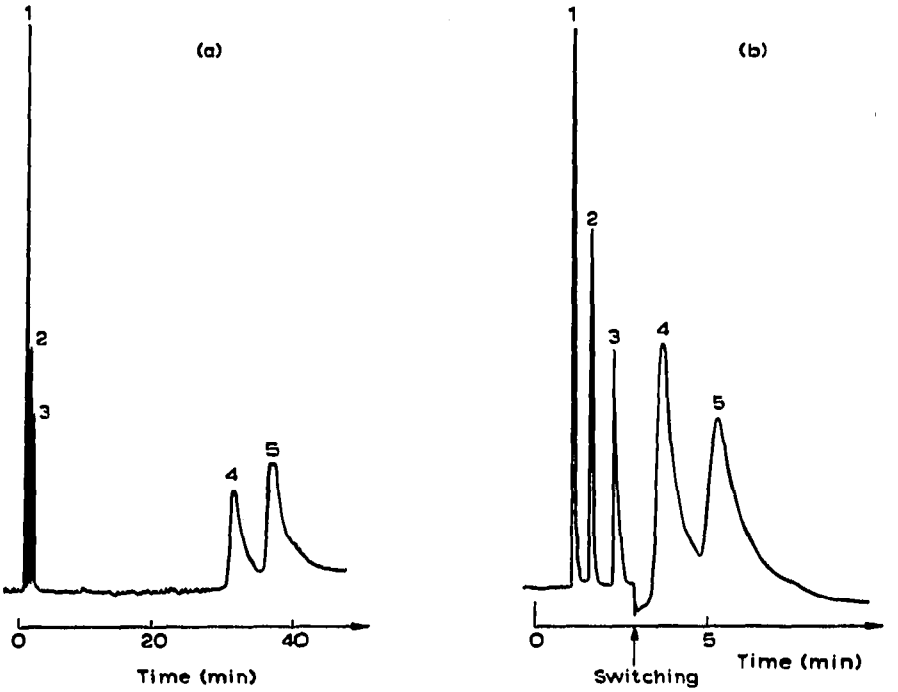
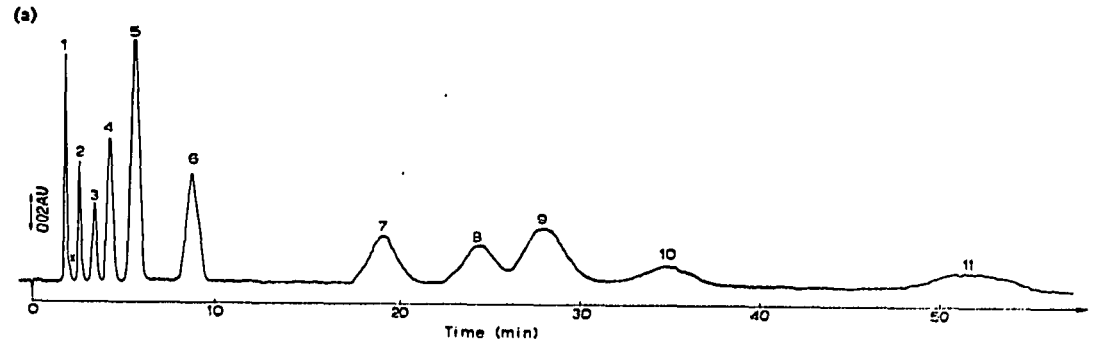
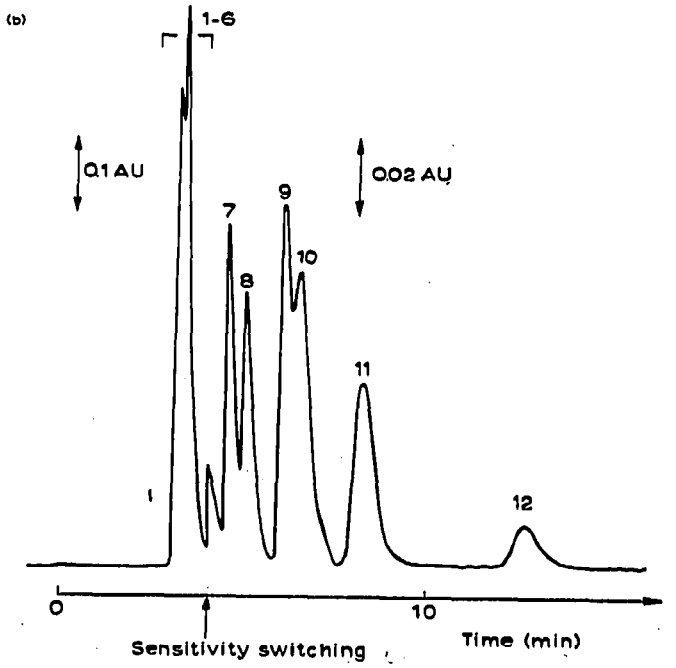


Fig. 5. Column stripping. Sample: 1 = toluene, 2 = azobenzene, 3 = 2-nitrobenzene, 4 = *p*-cresol, 5 = phenol; injection volume, 10 μl. Column: packing, 10% (w/w) 1,2,3-tris(cyanoethoxy)propane on silica support, 4–8 μm; eluent, 2,2,4-trimethylpentane. Detector: UV, 270 ± 10 nm. (a) Columns 1 + 2 for total sample; (b) columns 1 + 2 for components 1–3, column 1 alone for components 4 + 5. Column 1, 30 × 3 mm; column 2, 290 × 3 mm; flow-rate: column 1, 2.27 ml/min; columns 1 + 2, 1.33 ml/min.

pounds cannot be separated on a single column. In phase systems with high selectivity the range of distribution coefficients is too large, and in systems with a moderate range of distribution coefficients, the selectivity is insufficient. In the first case the separation time is too long and the detection of the later peaks becomes impossible. In the second case the resolution of the first peaks is greatly decreased. The problem can often be solved by using two columns with the same phase system and different phase ratios. The components with high distribution coefficients are separated on the column with a low phase ratio, and the components with low distribution coefficients on the column with a high phase ratio. The whole operation can be carried out with the set-up shown in Fig. 2b. The first column of the system has the low phase ratio, the second column the high phase ratio. Fig. 6 shows an application of this technique. Chromatogram (a) was obtained on both columns and shows the reduced detectability of the later peaks. Chromatogram (b) was obtained on the first column and it can be seen that the first six peaks are not resolved. Chromatogram (c) shows the result of column switching. The first peaks were stored in the second column whereas the later peaks were eluted from the first column. After the last peak was detected, column 1 was connected to column 2 and the separation of the first peaks was completed. It can be seen that all the peaks are resolved and can be detected. The separa-


(a)
0.02AU
1
2
3
4
5
6
7
8
9
10
11
0
10
20
30
40
50
Time (min)


(b)
1-6
0.1 AU
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7
8
9
10
11
12
1
0
10
Sensitivity switching
Time (min)

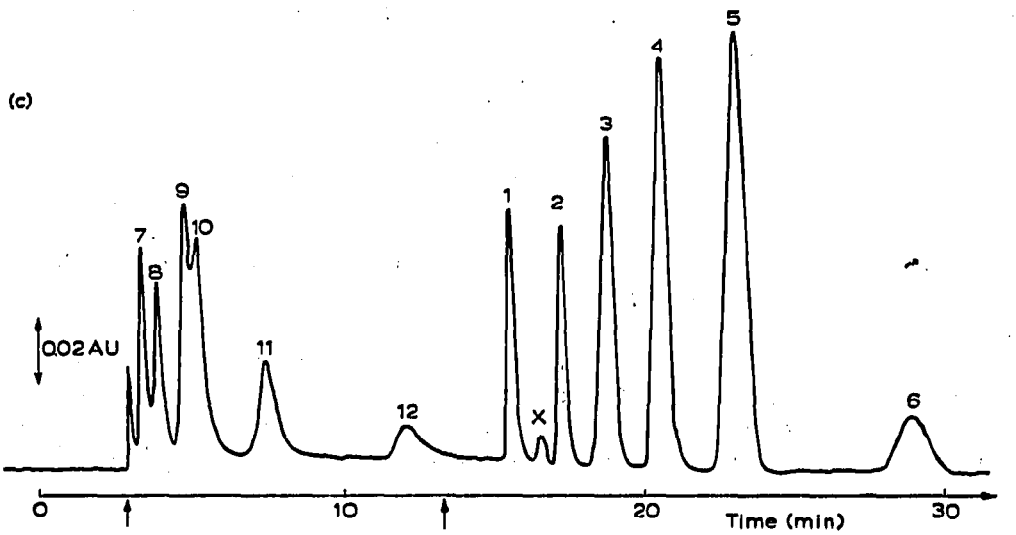
(c)
0.02AU
7
8
9
10
11
12
1
2
X
3
4
5
6
0
10
20
30
Time (min)

Fig. 6. Phase ratio adaption. Sample (distribution coefficient): 1 = decylbenzene (0), X = impurity, 2 = progesterone (9), 3 = androstenedione (26), 4 = methyltestosterone (36), 5 = testosterone (65), 6 = andrenosterone (122), 7 = 16α-hydroxy-pregn-4-ene-3,20-dione (300), 8 = 19 hydroxy-androst-4-ene-3,17-dione (380), 9 = corticosterone (560), 10 = 11-dehydrocorticosterone (700), 11 = cortisone (1300), 12 = cortisol (2900); injection volume, 30 μl. Columns: liquid–liquid system water–ethanol–2,2,4-trimethylpentane; % (w/w), water-rich phase (stationary) = 25.5:71.5:3.0, water-poor phase (mobile) = 0.1:3.0:96.9; column 1, 250 × 2.7 mm, diatomite support, 2 m^2/g, 5–10 μm; column 2, 250 × 2.7 mm, silica support, 15 m^2/g, 5–10 μm. Detector: UV, 236 nm. (a) Columns 1 + 2; (b) column 1; (c) first part column 1, second part columns 1 + 2.

tion time is significantly shorter than was needed for a complete resolution on columns 1 + 2. The distribution coefficients of the components vary by three orders of magnitude for this sample[13].

The problem could probably also be solved by gradient elution. This method has the disadvantage that the column has to be brought back to its initial state in order to start programming the solvent again.

CONCLUSION

The examples shown in this paper prove the extreme usefulness of column switching in high-pressure liquid chromatography. Another very important type of column switching is evaluated at present. A two-channel multicolumn system is used which allows an imitation of two-dimensional chromatography with columns. Such a set-up makes it possible to detect minor components in complex mixtures, the peaks of which are always covered by larger peaks on all types of column.

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