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EFFECT OF PHASE SYSTEM SELECTIVITY IN PREPARATIVE COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

In the application of chromatography in preparative work, the sample load and the resolution are the most important characteristics. An equation describing both the sample load and the chromatographic resolution as a function of the other process variables is derived. The influence of the selectivity coefficient is discussed on the basis of this equation.

The exploitation of the selectivity of the phase system in preparative work was investigated experimentally. The influence of the feed volume on the maximum and the width of the elution function as well as on the resolution of two components is described. For constant resolution, the maximum allowable sample load increases with increasing selectivity coefficient.

It is demonstrated that the maximum sample load for simple mixtures can be very large if a highly selective column is chosen, and can be further increased by two-column operation.

INTRODUCTION

At present, the primary application of chromatography is in analytical chemistry and preparative chromatography is used to only a minor extent. Among chromatographic methods, column liquid chromatography is the most suitable for preparative application.

In preparative chromatography, two limiting cases can be distinguished. On the one hand, the aim may be to separate very similar compounds, requiring high column efficiencies, and only a small sample load on the column is allowed. On the other hand, the problem may be to separate relatively dissimilar compounds, requiring only low column efficiencies, and a large sample load on the column can be used. The latter application is often suitable in synthetic work where relatively simple mixtures are formed.

Previously, column liquid chromatography has been applied in preparative work without a profound systematic investigation of the basic methodology. In this

paper, an attempt is made to discuss the main methodological aspects in the preparative separation of simple mixtures of dissimilar compounds.

THEORETICAL

The outline of a general theory for preparative chromatography is given in the following. The effectiveness of chromatographic separations in analytical and preparative applications is determined by the chromatographic resolution and the quantitative proportions of the substances to be separated:

$$R_{ji} = \frac{V_{Rj} - V_{Ri}}{\sigma_{Vi}} \tag{1}$$

($V_{Rj} > V_{Ri}$), where R_{ji} = resolution of components j and i; V_{Rj}, V_{Ri} = retention volumes of components j and i, respectively; σ_{Vi} = volume standard deviation of the output function of the chromatographic column for component i; and

$$\alpha_{ji} = \frac{Q_j}{Q_i} \tag{2}$$

where α_{ji} = quantitative proportion of components j and i; Q_j, Q_i = amounts of components j and i, respectively.

For example, the resolution necessary to achieve a certain purity and yield of a compound is lower if that compound has to be separated from another compound present in about the same amount than if it has to be separated from another compound which is present in a much larger amount.

The main factors characterizing a preparative separation are illustrated in Fig. 1. The characteristics with respect to the product are output, purity and yield, while the process characteristics determining the product characteristics are resolution, quantitative proportion and peak shapes of the components.

The sample input to a chromatographic column is determined by the feed volume and the feed concentration, both of which influence the resolution. An increase

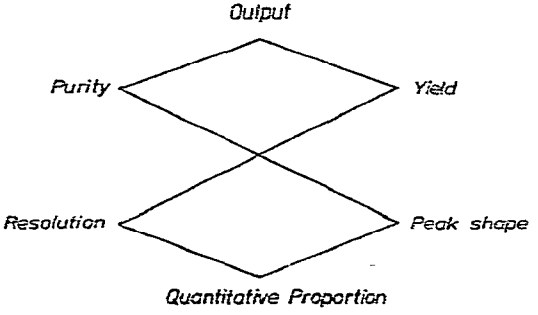

Fig. 1. Illustration of the dependence of the main product characteristics (purity, output, yield) on the main process characteristics (resolution, quantitative proportion, peak shape) in preparative chromatography.

in the feed volume increases the peak width and changes the peak shape, resulting in a reduction in the resolution. An increase in the feed concentration increases the peak width and changes the peak shape, resulting in asymmetric concentration profiles and a reduced resolution. Below certain limits, however, changes in the feed volume and feed concentration have only negligible influence on the resolution. In this paper, the influence of the feed volume on the preparative results is discussed.

The volume variance of the output function is determined by the volume variances of the input function and the chromatographic process[1,2]:

$$\sigma_{VL}^2 = \sigma_{V0}^2 + \sigma_{VC}^2 \tag{3}$$

where σ_{VL}^2 = volume variance of the distribution function of the component at the end of the column (output function); σ_{V0}^2 = volume variance of the distribution function of the component at the beginning of the column (input function); σ_{VC}^2 = volume variance generated by the chromatographic process. This equation describes the influence of the feed volume on the peak width, as σ_{V0} depends on the feed volume.

Feed volume and resolution

The effect of the feed volume in preparative column liquid chromatography has already been discussed less rigorously in several papers on, *e.g.*, ion-exchange chromatography[3] and more recently adsorption chromatography[4].

An expression[2,5] for the dependence of the resolution on the feed volume can be derived by combining eqns. 1 and 3, assuming proportionality between σ_{V0} and V_0 and inserting $\sigma_{VC}^2 = V_{Ri}^2/N_i$:

$$R_{ji} = \frac{V_{Rj} - V_{Ri}}{[\psi^2 V_0^2 + (V_{Ri}/N_i)]^{\frac{1}{2}}} = \frac{R_{ji}^{max}}{\left(\frac{\psi^2 V_0^2}{V_{Ri}^2} \cdot N_i + 1\right)^{\frac{1}{2}}} \tag{4}$$

where V_0 = feed volume; $\psi = \sigma_{V0}/V_0$ = proportionality factor depending on the mixing at the sampling; if one assumes that no mixing due to the sampling occurs, then a rectangular input function results for which $\psi = 1/\sqrt{12}$; N_i = theoretical plate height of the column for component i; $R_{ji}^{max} = (V_{Rj} - V_{Ri})/\sigma_{VCi}$ = maximum value of the resolution caused by the chromatographic process only.

The maximum resolution is achieved at a negligibly small feed volume and can be expressed by the chromatographic process variables[1,2]:

$$\lim_{V_0 \to 0} R_{ji} = R_{ji}^{max} = (r_{ji} - 1) \frac{\kappa_i}{\kappa_i + 1} \cdot N_i^{\frac{1}{2}} \tag{5}$$

where $r_{ji} = \kappa_j/\kappa_i$ = selectivity coefficient of the phase system with respect to components j and i; κ_j, κ_i = capacity ratios of components j and i, respectively.

In order to optimize a preparative chromatographic separation, the product of the resolution and the sample input has to be made a maximum. Considering

$Q_i = c_i^0 V_0$ and $V_{Ri} = V_m(1 + \kappa_i)$, from eqns. 4 and 5 an expression for the optimization can be derived:

$$R_{ji}\,Q_i = (r_{ji} - 1)\cdot\frac{c_i^0}{\left[\frac{\psi^2}{(V_m\,\kappa_i)^2} + \frac{(1+\kappa_i)^2}{\kappa_i^2\,N_i\,V_0^2}\right]^{\pm}} \tag{6}$$

where Q_i = sample input for component i; c_i^0 = initial concentration of component i in the sample; V_m = volume of mobile phase in the column.

Two limiting cases are possible for eqn. 6. Firstly, for $V_0^2 \ll (1 + \kappa_i)^2/\kappa_i^2 N_i$

$$\lim R_{ji}\,Q_i = (r_{ji} - 1)\,\frac{\kappa_i}{1+\kappa_i}\,N_i^{\frac{1}{2}}\,c_i^0\,V_0 \tag{7}$$

and secondly, for $V_0^2 \gg (1 + \kappa_i)^2/\kappa_i^2 N_i$

$$\lim R_{ji}\,Q_i = (r_{ji} - 1)\,\kappa_i\,V_m\frac{c_i^0}{\psi} = (\kappa_j - \kappa_i)\,V_m\frac{c_i^0}{\psi} \tag{8}$$

Eqn. 7 applies if the resolution is so small that a feed volume which does not reduce the resolution has to be used; in this instance, the feed volume should be increased to the limit where the resolution starts to decrease. Eqn. 8 applies if the resolution is so large that the feed volume can be increased up to the point were the dispersion due the chromatographic process becomes negligible compared with the width of the input peak. The peak shape in this instance corresponds to a rectangular function for which $\psi = 1/\sqrt{12}$, and the feed volume should be increased as far as the peaks meet.

The product output obtained with a given sample input depends on the purity of the product required, the resolution achieved in the chromatographic separation and the shapes of the output peaks. The yield for a given component at a given product purity is defined by the ratio of the product output and the sample input for this component. If the resolution is so large that the yield is near to 100%, then the sample input and the product output are about equal. The maximum sample input corresponding to certain requirements with respect to the resolution and the peak shapes is called the load capacity.

Feed volume and output concentration

In general, dilution of the sample occurs in the elution chromatographic process under isocratic conditions. The maximum of the output function of a component depends on the load of the component and the width and shape of the output concentration peak:

$$(c_{iL}^m)_{max} = \varphi_i\cdot\frac{c_i^0\,V_0}{\sigma_{VLi}} \tag{9}$$

where $(c_{iL}^m)_{max}$ = maximum concentration of the component i in the mobile phase, m, at the end (L) of the column; σ_{VLi} = volume standard deviation of the output peak

of component i; φ_i = factor depending on the shape of the output peak, being between $1/\sqrt{2\pi} \approx 0.40$ (gaussian shape) and $1/\sqrt{12} \approx 0.29$ (rectangular shape).

Combining eqns. 9 and 3 and taking into account $\sigma^2_{V0} = (\psi V_0)^2$ and $\sigma^2_{VC} = V^2_{Ri}/N_i$, an expression[2,5] for the dilution of the sample in the column and its dependence on the feed volume is obtained:

$$\frac{(c^m_{iL})_{max}}{c^0_i} = \frac{\varphi_i}{\left(\psi^2 + \frac{V^2_{Ri}}{V^2_0 N_i}\right)^{\frac{1}{2}}} \tag{10}$$

This equation indicates that two limiting cases exist for the dependence of the dilution on the feed volume; for $V^2_0 \gg V^2_{Ri}/N_i$

$$\lim (c^m_{iL})_{max}/c^0_i = \frac{\varphi_i}{\psi} = 1 \tag{11}$$

and for $V^2_0 \ll V^2_{Ri}/N_i$

$$\lim (c^m_{iL})_{max}/c^0_i = \varphi_i \cdot \frac{N_i^{\frac{1}{2}}}{V_{Ri}} \cdot V_0 \tag{12}$$

In the first limiting case, the dilution is independent of the chromatographic process and approaches a value of unity, as φ_i becomes equal to ψ as the shape of the output peak approaches that of the input peak. In the second limiting case, the dilution is proportional to the feed volume, the proportionality factor depending on chromatographic parameters.

EXPERIMENTAL

Chemicals

The reaction extract of 3-nitrophenacetin as well as the testsubstance 3-nitro-4-ethoxyanaline was obtained from Sandoz Ltd. (Basle, Switzerland). The test substances (phenol, anisole, benzophenone, benzyl alcohol, 5-phenyl-1-pentanol, 4-ethoxyanaline and phenacetin) were of analytical-reagent grade (Fluka, Buchs, Switzerland and Merck, Darmstadt, G.F.R.). The solvents used for the preparation of the mobile phases were of analytical-reagent grade (Fluka) and the stationary phases were LiChrosorb SI-100 and LiChrosorb RP-8 of particle size 10μm (Merck).

All of the samples were dissolved in the respective mobile phase. The concentrations of the test solutions were 0.5 mg/ml of each compound for peak-broadening measurements and 2 mg/ml of reaction mixture for preparative separation experiments.

Apparatus

A chromatograph of our own construction[6] was used. The pressure was generated by an air-driven fluid pump (Haskel, Burbank, Calif., U.S.A.; Type 26 980-40,

nominal ratio 46:1), and was measured with a manometer (Wika, Klingenberg, G.F.R.; Type 111.63.600). The feeding of the sample solutions to the columns was effected with a high-pressure syringe-loading sample injector (Rheodyne, Model 7105) fitted to a 40-ml sample loop constructed from stainless-steel tubing of length 12.7 m, O.D. 3.2 mm and I.D. 2.0 mm.

The column tubing was made of stainless steel, length 250 mm and I.D. 4.6 mm (6.4 mm O.D.). It was packed for hydrophilic adsorption chromatography with LiChrosorb SI-100 and for hydrophobic adsorption chromatography with LiChrosorb RP-8.

The columns were filled by the slurry technique at a pressure of 250 bar. A 15-cm stainless-steel tube of I.D. 35 mm and O.D. 50 mm was used as a container for the slurry. The slurry solvent for both LiChrosorb SI-100 and RP-8 was tetrahydrofuran–water (1:1, v/v). The slurry (5%, w/w), was degassed in an ultrasonic generator and was pumped into the column at constant flow-rates of 8 and 16 ml/min for LiChrosorb RP-8 and SI-100, respectively. A pressure-regulated Haskel pump was used for this purpose.

A UV–visible photometer–detector with a cell volume of 8 μl was used (Perkin-Elmer, Norwalk, Conn., U.S.A.; Model LC 55) and operated at wavelengths at which no saturation of the linear absorbance range occurred.

RESULTS AND DISCUSSION

It was our intention to study the potential use of highly selective columns in preparative liquid chromatography, as such columns are expected to have a high load capacity. It was decided to study primarily the maximum allowable feed volume; the influence of the feed concentration will be considered in a subsequent paper. In order to exclude concentration effects on the resolution, the linear capacities of the columns were determined and found to be about 1 mg/ml. Therefore, the initial sample concentration was chosen to be at this level so as to achieve maximum sample input within the linear range of the distribution isotherm. Under these conditions, the column contribution, σ_{VC}^2 in eqn. 3, is independent of the sample load.

If large amounts of sample or large numbers of samples have to be treated, the sample throughput, being the product output per unit time, becomes an important characteristic of the process.

Influence of feed volume

The influence of the feed volume on the width and maximum value of the output peak and on the resolution was studied for a number of columns and compounds, and the results are shown in Figs. 2–4.

The increase in the peak width with increasing feed volume is shown in a normalized form in Fig. 2. This plot can be theoretically explained by eqn. 3, taking into account that the input standard deviation increases with the feed volume. We recognize that the peak width remains constant up to a feed volume about equal to the volume standard deviation caused by the column. With increasing feed volumes, the shape of the output peak changes from gaussian to rectangular, making the determination of the peak variance more complicated. In order to simplify the experimental work, an approximate method was chosen for this determination. The inter-

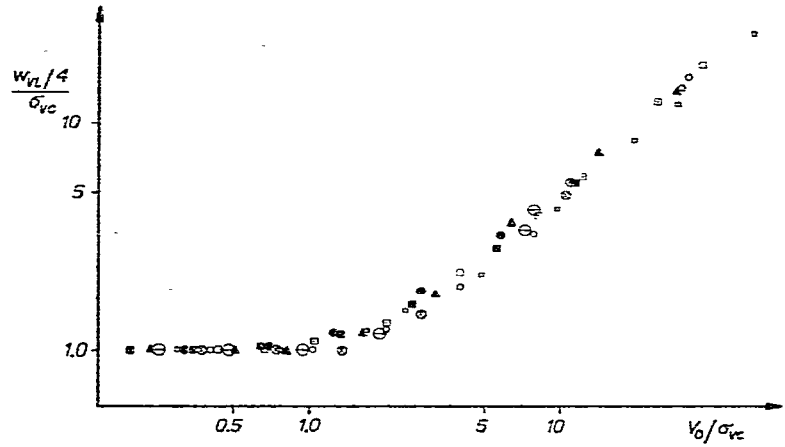

Fig. 2. Influence of the feed volume on peak broadening. ○, Phenol; ⊗, anisole; ⊖, benzophenone (stationary phase, LiChrosorb RP-8; mobile phase, acetonitrile–water–triethylamine, 447:547:6); ▲, benzyl alcohol; ■, 5-phenyl-1-pentanol; ●, benzophenone (stationary phase, LiChrosorb RP-8; mobile phase, acetonitrile–water–triethylamine, 348:646:6); □, 3-nitroaniline; ⊡, 3-nitrophenacetin (stationary phase, LiChrosorb SI-100; mobile phase, dichloromethane–methanol–water, 989:10:1).

section on the baseline of the chromatogram defined by the two tangents to each peak was assumed to be four times the standard deviation of the peak. This assumption is correct only for a true gaussian peak, but the inaccuracy is small for the peaks involved in this work. The volume base width, w_{VL}, of the output peak varies from the value valid for a gaussian shape towards the value valid for a rectangular shape, depending on the feed volume. These two values are nearly identical: gaussian peak, $w_{VL} = 4\sigma_{VL}$; rectangular peak, $w_{VL} = -(\sqrt{12})\sigma_{VL} = 3.5\,\sigma_{VL}$.

The increase in the maximum value of the output peak with the feed volume is shown in Fig. 3; the experimental results correspond to eqn. 10. It can be seen that the height of the output peak reaches a plateau, the concentration of which is equal

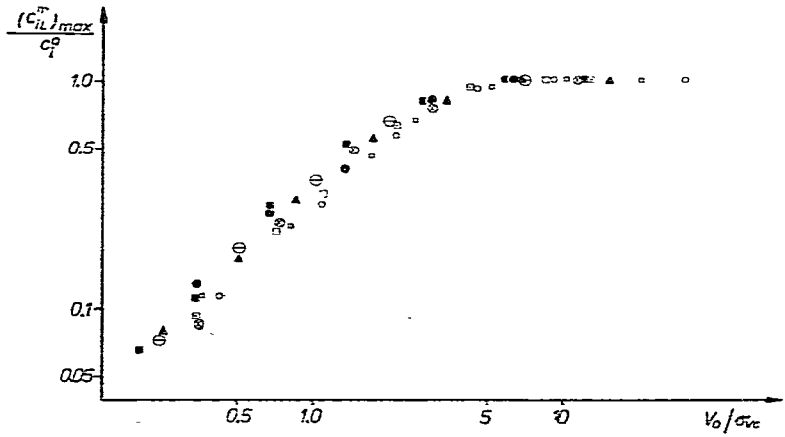

Fig. 3. Influence of the feed volume on the maximum concentration of the peak. Symbols as in Fig. 2.

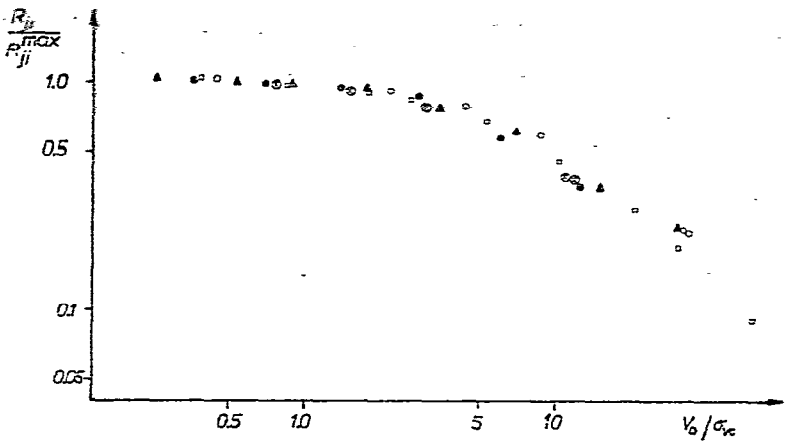

Fig. 4. Influence of the feed volume on the resolution. ○, Phenol–anisole; ⊗ = anisole–benzophenone; ▲, benzyl alcohol–5-phenyl-1-pentanol; ●, 5-phenyl-1-pentanol–benzophenone; □, 3-nitroaniline–3-nitrophenacetin. Columns as in Fig. 2.

TABLE I

MAXIMUM VALUES OF RESOLUTION IN FIG. 4

Component pair		R_{ji}^{max}
j	*i*	
Anisole	Phenol	63
Benzophenone	Anisole	17
5-Phenyl-1-pentanol	Benzyl alcohol	43
Benzophenone	5-Phenyl-1-pentanol	17
3-Nitrophenacetin	3-Nitroaniline	55

to the initial concentration of the sample. This plateau is approached at a feed volume of about five times the volume standard deviation of the column.

Fig. 4 shows the decrease in the resolution with increasing feed volume. Corresponding to Fig. 2, it can be seen that the resolution starts to decrease at a feed volume about equal to the volume standard deviation generated by the chromatographic column. This observation is in agreement with eqn. 4. Table I gives the maximum values of the resolution that are achieved at low feed volumes.

Preparative exploitation of phase system selectivity

In the preparative chromatographic separation of a mixture, it is the aim to find a column for which, according to eqn. 6, the smallest selectivity coefficient that occurs is as large as possible. If the selectivity coefficients and the capacity ratios are large enough, a high sample volume can be used in a single run and eqn. 8 applies. At a given resolution, by which the required purity is achieved, the load capacity then depends on the difference of the capacity ratios.

In order to increase the throughput, one can attempt to carry out the separation in two steps. In the first step, the sample is fractionated only partially, with the intention of achieving a very high load capacity. Those groups of components which

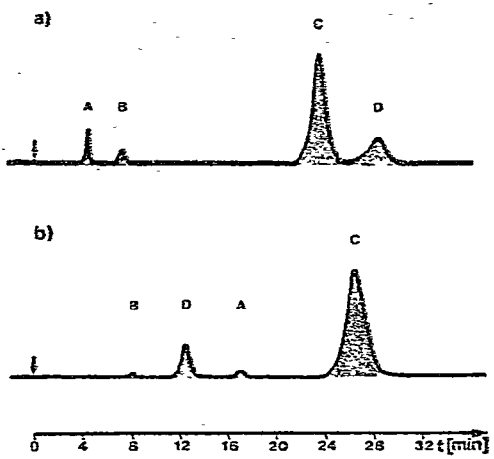

Fig. 5. Chromatograms of 3-nitrophenacetin reaction mixture. (a) Column, 250 × 4.6 mm I.D.; stationary phase, LiChrosorb SI-100, $d_p = 10\,\mu m$; mobile phase, *n*-hexane–dichloromethane–acetonitrile–water (195:780:24:0.1); flow-rate, 2.5 ml/min. (b) Column, 250 × 4.6 mm I.D.; stationary phase, LiChrosorb RP-8, $d_p = 10\,\mu m$; mobile phase, water–acetonitrile–triethylamine (870:124:6); flow-rate, 2.5 ml/min. Temperature 22°, feed volume 150 μl and total sample concentration 2 mg/ml in both chromatograms.

TABLE II

CAPACITY RATIOS, SELECTIVITY FACTORS AND MAXIMUM VALUES OF RESOLUTION CORRESPONDING TO FIG. 5 FOR RELEVANT COMPONENTS

Column	*Component*	κ_i	r_{ji}	R_{ji}^{max}
LiChrosorb SI-100	B (*i*)	4.5	3.8	65
(Fig. 5a)	C (*j*)	17.1	—	—
LiChrosorb RP-8	B (*i*)	5.3	2.3	33
(Fig. 5b)	A (*j*)	12.2	—	—
	D (*i*)	8.6	2.3	49
	C (*j*)	19.4	—	—

could not be separated sufficiently on the first column are collected in fractions and separated on a second column that must have a high selectivity towards these components.

The potential for exploiting the phase system selectivity to increase the load capacity and throughput in preparative work was tested with a reaction mixture encountered in synthetic experiments. The result of the search for phase systems with high selectivity is shown in Fig. 5 and values of the parameters are given in Table II.

From Fig. 5, it can be seen that the LiChrosorb RP-8 column is more suitable than the LiChrosorb SI-100 column for the preparative separation of the test mixture. Its maximum feed volume is 9 ml and the separation with this feed volume takes 32 min. The LiChrosorb SI-100 column, on the other hand, is suitable for the separation of the test mixture into two groups of compounds. The maximum feed volume for the separation into groups of components is determined by the distance between the last peak of one group and the first peak of the next group. In Fig. 5, the latter can be seen with peaks B and C, for the separation of which the maximum feed volume

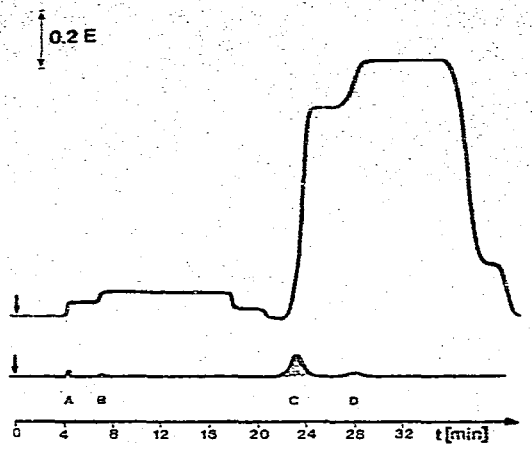

Fig. 6. Chromatograms of 3-nitrophenacetin reaction mixture. Column conditions as in Fig. 5a. Feed volume: in analytical chromatogram, 150 μl; in preparative chromatogram, 37 ml. Total sample concentration in both chromatograms, 2 mg/ml.

is 37 ml, as can be seen from Fig. 6. It is recognizable that the sample is separated into two groups of compounds. The chromatogram shows the superposition of two nearly rectangular peaks in each group. Fractions of the column effluent corresponding to the two groups of compounds were collected manually and transferred as samples to the LiChrosorb RP-8 column after evaporating the first eluent under nitrogen and redissolving the residue in the second eluent. A complete separation of all four compounds is achieved in this manner with the initial feed volume of 37 ml, as can be

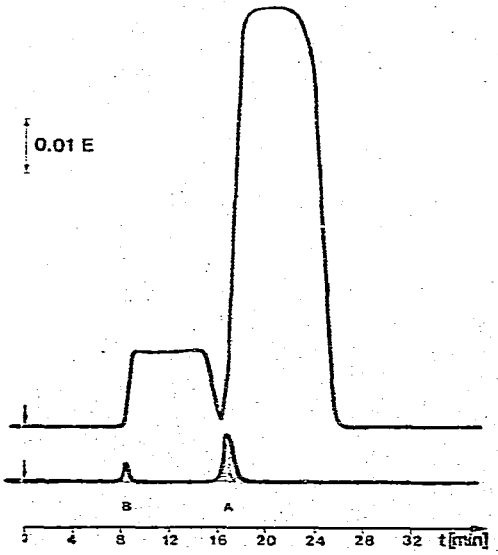

Fig. 7. Chromatograms of fraction 1 (compounds A and B). Column conditions as in Fig. 5b. Feed volume: in analytical chromatogram, 150 μl; in preparative chromatogram, 20 ml.

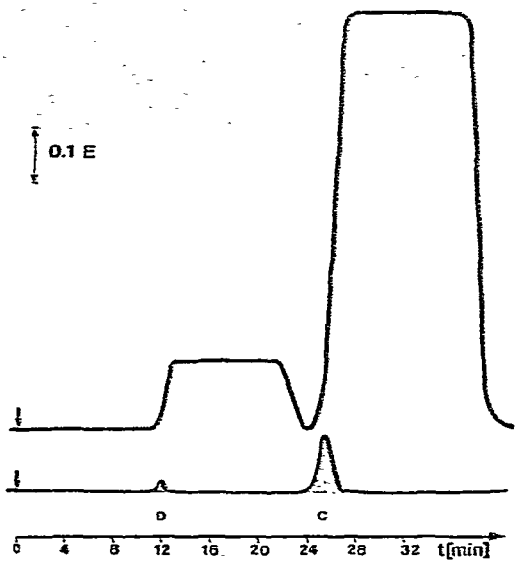

Fig. 8. Chromatograms of fraction 2 (compounds C and D). Column conditions as in Fig. 5b. Feed volume: in analytical chromatogram, 150 μl; in preparative chromatogram, 30 ml.

seen in Figs. 7 and 8. The total separation time for all three chromatographic separations involved is 108 min.

With regard to throughput, the successive separation of the test mixture on two columns of different selectivity is only slightly better than repetitive separation of the same sample volume on the LiChrosorb RP-8 column alone. For other types of samples, there may be more advantage in preparative separation on more than one type of column, especially if the mobile phase is the same and only the stationary phase differs.

The fractions containing the separated components were extracted into chloroform, which was then evaporated partially under nitrogen. The concentrated fractions were analyzed for the structure of the test compounds by NMR, IR and mass spectrometry. The final step in the identification was a comparison of the spectral and retention data with reference data for the presumed compounds. This procedure led to the following identities of the compounds: A = 3-nitro-4-ethoxyaniline; B = 4-ethoxyaniline; C = 3-nitrophenacetin; D = phenacetin.

CONCLUSIONS

The use of highly selective columns has been shown to be of great value in preparative chromatography. In the separation of simple mixtures, a very high load capacity and throughput can be achieved. Even with columns of small diameter, the product output is sufficient to permit the use of a number of methods for structure determination. With columns of large diameter, a maximum feed volume of the order of one l can be expected, which requires, however, the design of new sampling devices.

REFERENCES

1 J. F. K. Huber and J. A. R. J. Hulsman, *Anal. Chim. Acta*, 38 (1967) 305.
2 J. F. K. Huber, J. A. R. J. Hulsman and C. A. M. Meijers, *J. Chromatogr.*, 62 (1971) 79.
3 F. H. Spedding and J. E. Powell, in F. C. Nachod and J. Schubert (Editors), *Ion-Exchange Technology*, Academic Press, New York, 1956, p. 359.
4 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 119 (1976) 467.
5 J. F. K. Huber, *Z. Anal. Chem.*, 277 (1975) 341.
6 A. Wehrli, *Z. Anal. Chem.*, 277 (1975) 289.