

CHROMSYMP. 238

OPTIMIZATION OF REVERSED-PHASE ION-PAIR CHROMATOGRAPHY BY OVER-PRESSURIZED THIN-LAYER CHROMATOGRAPHY

II. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION ON SILICA BY UTILIZING PRE-INVESTIGATION DATA FROM OVER-PRESSURIZED THIN-LAYER CHROMATOGRAPHIC EXPERIMENTS

M. GAZDAG, G. SZEPESI*, M. HERNYES and Z. VÉGH
Chemical Works Gedeon Richter Ltd., 1475 Budapest (Hungary)

SUMMARY

Ion-pair formation and the separation of fifteen organic acids by high-performance liquid chromatography (HPLC) have been studied on silica columns, using the optimal conditions found in prior thin-layer chromatographic (TLC) experiments. It was found that the data obtained over-pressurized TLC (OPTLC) could be advantageously applied in the HPLC experiments, a good correlation between the OPTLC relative retention values and the logarithm of the capacity ratios obtained by HPLC being achieved.

INTRODUCTION

In Part I¹ we demonstrated that over-pressurized thin-layer chromatography (OPTLC) is suitable for carrying out reversed-phase ion-pair separations of organic acids on both silica and reversed-phase layers using cetrinide as an ion-pair reagent. Because the most promising results were obtained on silica layers, we tried to use the same experimental conditions in high-performance liquid chromatographic (HPLC) experiments as in the previous OPTLC study. The use of bare (unbonded) silica was successfully applied by Bidlingmeyer *et al.*² for the separation of organic amine compounds using reversed-phase eluents. Their work contrasts with the popular belief that silica should not be used in aqueous eluents. The main aims of this work were to study the applicability of OPTLC for modelling reversed-phase ion-pair systems in HPLC and to investigate the use of silica columns for ion-pair separations using a cationic ion exchanger.

EXPERIMENTAL

All solvents and reagents were of analytical-reagent grade and were obtained from Reanal (Budapest, Hungary).

A Liquochrom 2010 high-pressure liquid chromatograph (Labor MIM, Bu-

TABLE I
COMPOUNDS INVESTIGATED

No.	Name	No.	Name
0	2-Chloronicotinic acid	8	Cresol (mixture of <i>ortho</i> and <i>para</i> isomers)
1	Nicotinic acid	9	Resorcinol
2	Isonicotinic acid	10	Pentachlorophenol
3	Salicylic acid	11	5-Sulphosalicylic acid
4	Acetylsalicylic acid	12	<i>p</i> -Toluenesulphonic acid
5	Niflumic acid	13	Benzenesulphonic acid
6	Veratric acid	14	1,4-Naphthoquinone-4-sulphonic acid
7	Phenol		

dapest, Hungary) equipped with a variable-wavelength UV detector (Labor MIM), a loop-type injector and a recorder (Type 185, Kutesz, Budapest, Hungary) was used for the HPLC experiments. The separations were performed on a LiChrosorb Si 60 (10 μm) column (250 \times 4.6 mm I.D.) (Chrompack, Middleburg, The Netherlands). The eluent was methanol (HPLC grade, E. Merck, Darmstadt, F.R.G.)–0.01 *M* aqueous phosphate buffer (pH 5.0) (1:1) containing different amounts of cetrimide. The eluent was filtered and degassed prior to chromatography. The flow-rate was 1.0 cm^3/min and the compounds were detected at 260 nm.

The OPTLC instrument and conditions were the same as in Part I¹. To investigate the adsorption and desorption characteristics of cetrimide on a silica layer, a Polygram Sil G/UV₂₅₄ (Macherey, Nagel & Co., Düren, F.R.G.) plate was used. To determine the amount of cetrimide on the silica surface, the plate was sprayed with a 0.25 *M* solution of $\text{K}_2[\text{Co}(\text{SCN})_4]$. The plate was covered with an empty glass plate and densitometry was carried out at 630 nm in the remission mode. The compounds investigated are listed in Table I.

RESULTS AND DISCUSSION

To investigate the applicability of OPTLC for modelling reversed-phase ion-pair systems in HPLC, the adsorption and desorption characteristics of cetrimide bound to the silica surface during the TLC experiments was first studied. The TLC data can be transferred to HPLC systems only if the separation conditions are similar, *i.e.*, if the cetrimide concentration is constant during the development. The adsorption isotherm of cetrimide on a silica layer was determined by *in situ* densitometry after spraying (see Experimental) when the cetrimide concentration of the eluent was constant and the reagent concentration of the solution used for impregnation was varied, as shown in Fig. 1.

As can be seen from Fig. 1, if the cetrimide concentration in the eluent is lower than 0.02 mole/ dm^3 , independent of the reagent concentration used in impregnation of the layer, the amount of cetrimide adsorbed varies during the run. When the cetrimide concentrations in the eluent and in pre-treatment are 0.1 and 0.05 mole/ dm^3 , respectively, the amount of cetrimide adsorbed does not change with the running distance, which provides similar conditions to those in the HPLC experiments. A similar results was obtained by the investigating the desorption character-

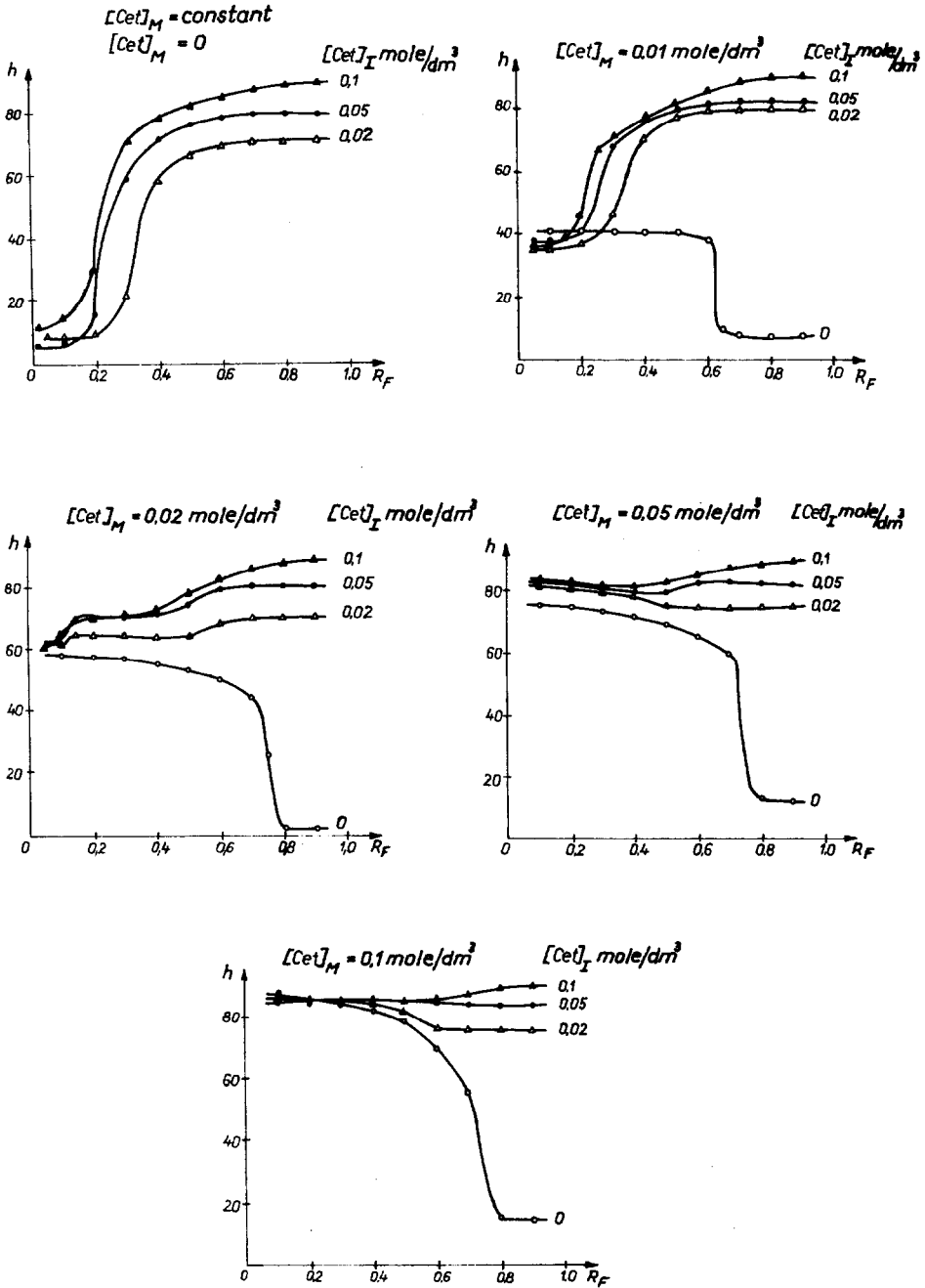


Fig. 1. Adsorption characteristics of cetrимide on a silica layer. Plate, Polygram Sil G/UV₂₅₄; eluent, methanol-0.01 mole/dm³ phosphate buffer (pH 5.0) (1:1) containing different amount of cetrимide. For other conditions, see Experimental. $[Cet]_M$ = cetrимide concentration in the mobile phase; $[Cet]_I$ = cetrимide concentration on immersion. $h = 100 - \text{remission \%}$; after visualisation of the cetrимide with colour reaction on the layer.

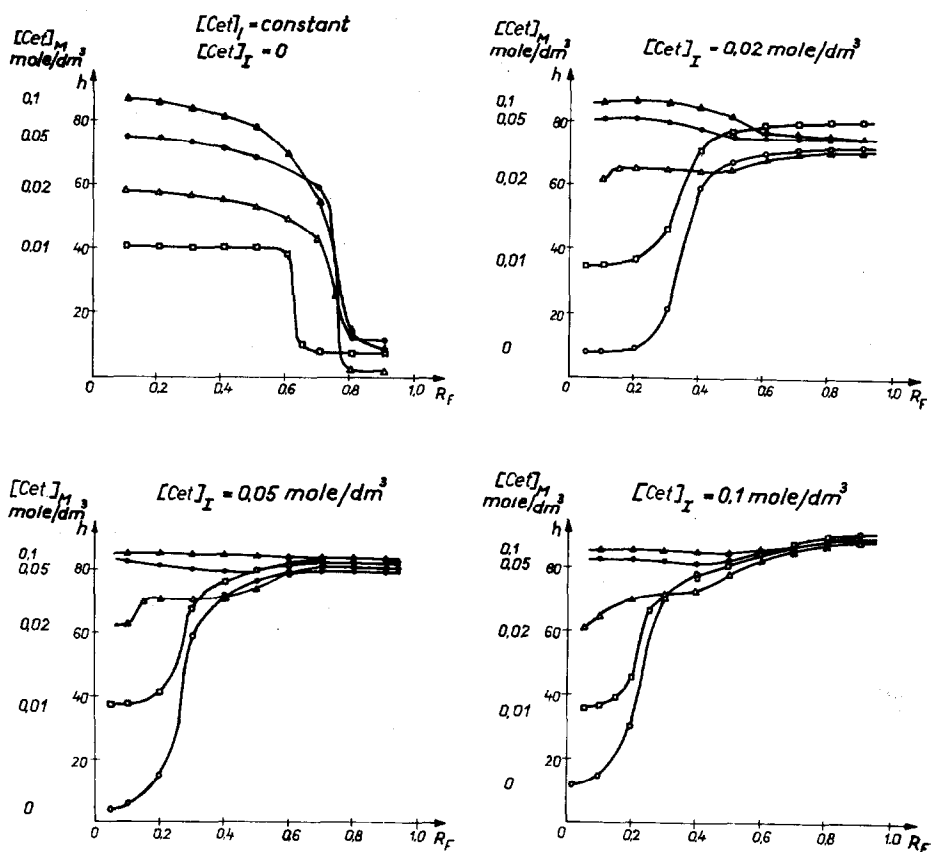


Fig. 2. Desorption characteristics of cetrime on a silica layer. Conditions as in Fig. 1.

istics of cetrime from the layer. In this instance the cetrime concentration in impregnation was constant and the reagent concentration of the eluent was varied, as shown in Fig. 2.

From these results, it can be concluded that the optimal separation system for OPTLC investigations is when the plate is immersed in 0.05 mole/dm³ cetrime solution and the eluent contains 0.1 mole/dm³ of cetrime. The results support our observations¹ that the best separation was achieved by using these conditions.

Based on the results obtained by OPTLC, for the HPLC experiments a silica column, a 1:1 mixture of methanol and buffered water containing different amounts of ion-pair reagent (pH \approx 5.0) were selected.

Fig. 3 shows the dependence of the capacity ratios of the compounds investigated on the cetrime concentration of the eluent.

Considering the influence of the reagent concentration in the eluent on the retention of the compounds illustrated in Fig. 3, the significant changes in the retentions of sulphosalicylic acid, niflumic acid and pentachlorophenol can be explained by micellar formation of cetrime in the mobile phase^{3,4}. It can also be seen from Fig. 3 that the use of cetrime at a concentration of 0.05 or 0.1 mole/dm³ (the latter

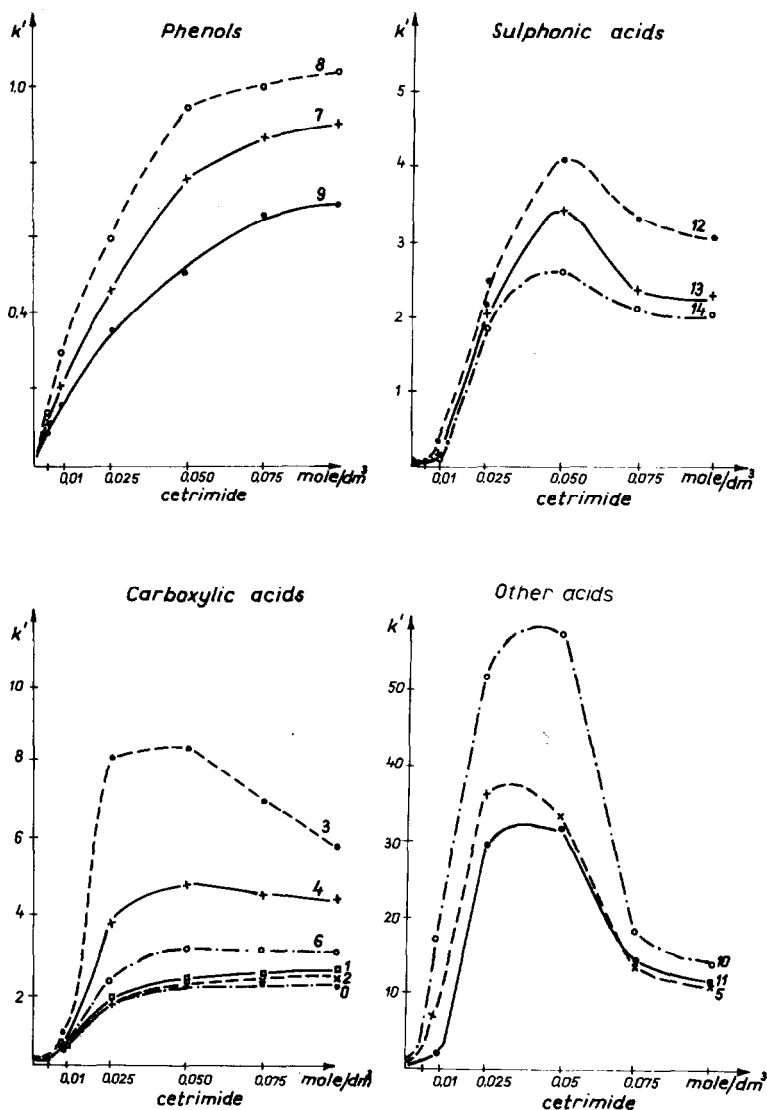


Fig. 3. Dependence of the capacity ratios of the compounds investigated on the cetrimide concentration of the eluent. Column, LiChrosorb Si 60 (10 μ m) (250 \times 4.6 mm I.D.); eluent, methanol-0.01 mole/dm³ phosphate buffer (pH 5.0) (1:1) containing different amounts of cetrimide. For compounds, see Table I.

is used with strongly retarded compounds) provides the best conditions for the separation.

This is demonstrated in Figs. 4 and 5, where the chromatograms of a model mixture using 0.05 and 0.1 mole/dm³ of cetrimide in the eluent are shown. The peak symmetry in Figs. 4 and 5 is good, with a similar elution order to that obtained by OPTLC [see Fig. 5 (A) in Part I].

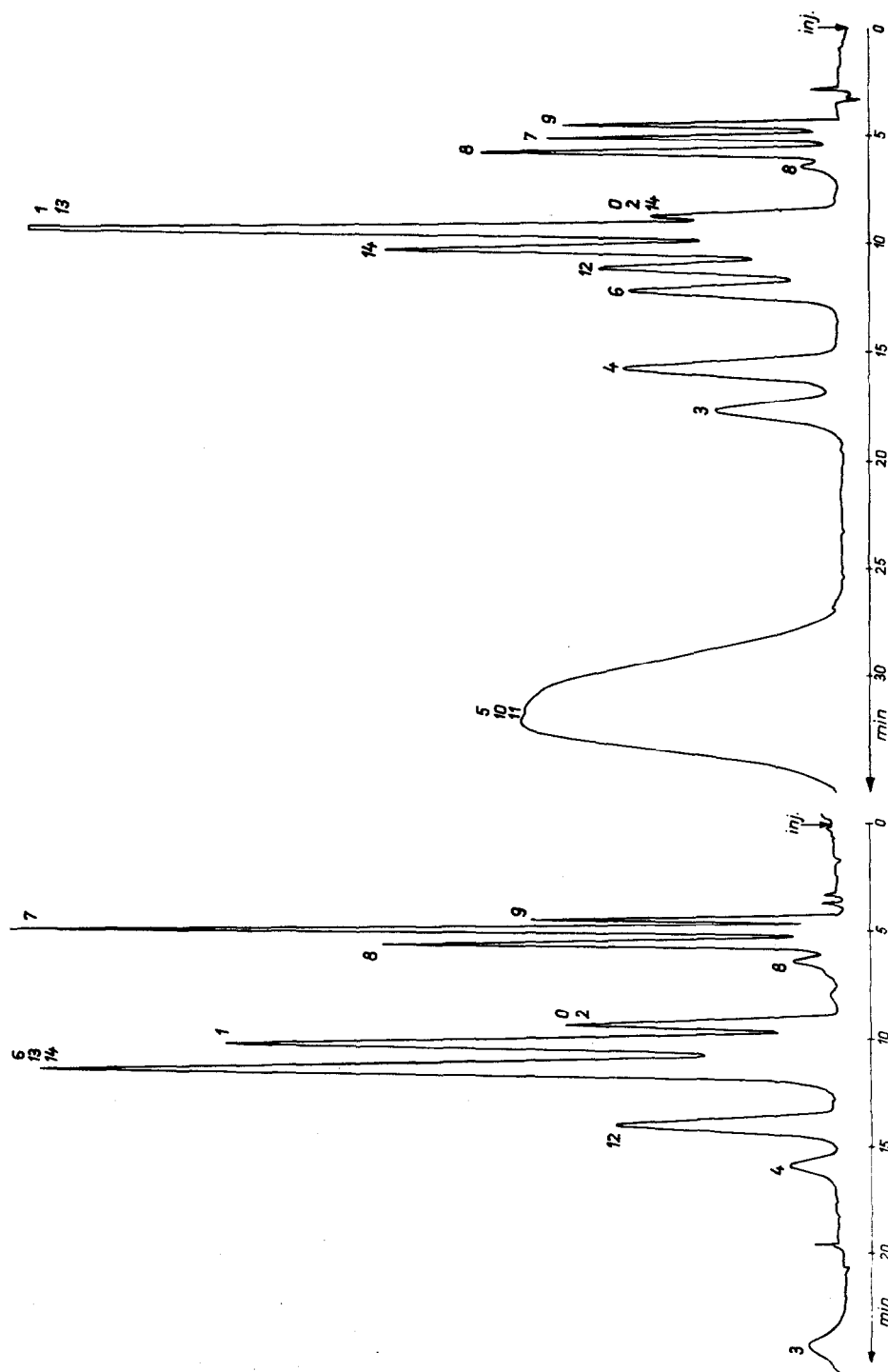


Fig. 4. Separation of a model mixture using cetrimide at a concentration of 0.05 mole/dm³. Conditions as in Fig. 3.

Fig. 5. Separation of a model mixture using cetrimide at a concentration of 0.1 mole/dm³. Conditions as in Fig. 3.

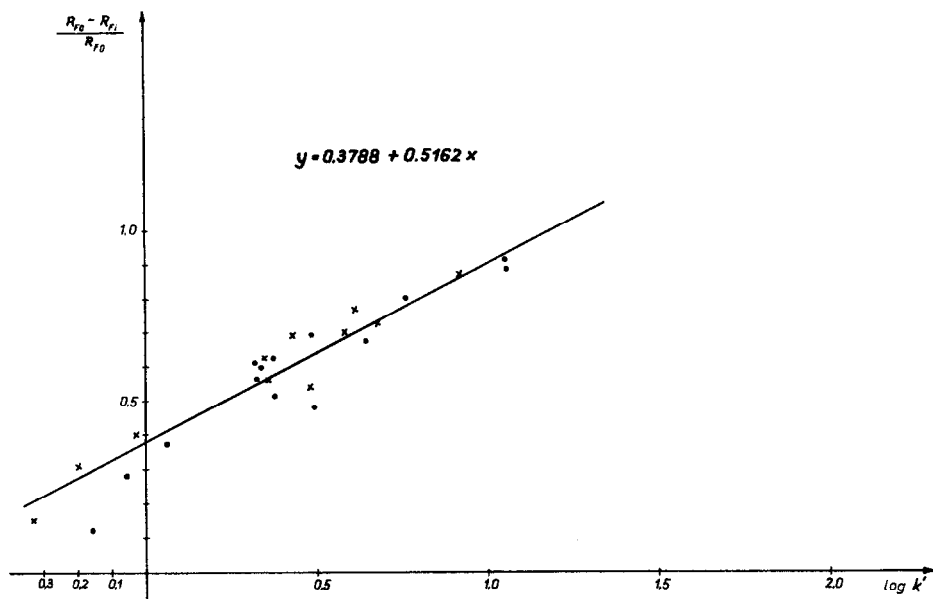


Fig. 6. Correlation between the relative retention obtained by OPTLC and capacity ratios in HPLC. Cetrimide concentration in the mobile phase: ●, 0.05 mole/dm³; ×, 0.1 mole/dm³.

CONCLUSIONS

The good agreement in selectivity between HPLC and OPTLC ion-pair systems using the same eluent composition offers an excellent possibility that reversed-phase ion-pair chromatographic methods could be modelled by OPTLC (or *vice versa*), so the retentions of compounds in HPLC could be predicted in advance.

Fig. 6 shows the correlation between the relative retentions in OPTLC ($(R_{F0} - R_{Fi})/R_{F0}$, where R_{F0} and R_{Fi} are the R_F values of the same component in the absence and presence of an ion-pair reagent) and the capacity ratios in HPLC.

The good efficiency obtained on the silica column supports our conclusion that reversed-phase ion-pair chromatography of ionizable compounds can be carried out on silica using aqueous eluents, so many ion-pair separations that are very difficult on bonded reversed phases should be easily accomplished with good peak symmetry on silica. This work is in progress and will be published later.

REFERENCES

- 1 G. Szepesi, Z. Vég, Zs. Gyulay and M. Gazdag, *J. Chromatogr.*, 290 (1984) 127.
- 2 B. A. Bidlingmeyer, J. K. Del Rios and J. Korpi, *Anal. Chem.*, 54 (1982) 442.
- 3 C. P. Terwey-Groen, S. Heemstra and J. C. Kraak, *J. Chromatogr.*, 161 (1978) 69.
- 4 J. G. Dorsay, M. T. DeEchegaray and J. B. Landy, *Anal. Chem.*, 55 (1983) 924.