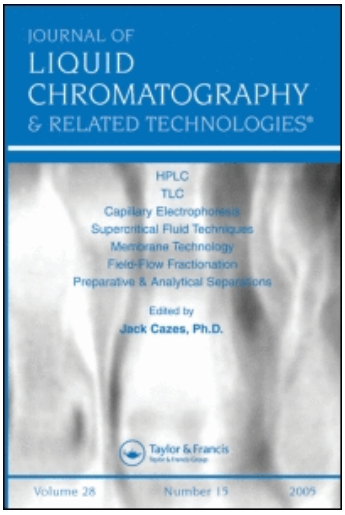


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# THIN-LAYER REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF SOME CEPHALOSPORINS

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# THIN-LAYER REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF SOME CEPHALOSPORINS

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## ABSTRACT

The retention behavior of five cephalosporins was investigated by thin-layer reversed-phase ion-pair chromatography (RP-IPC). Optimization of the retention and selectivity of these compounds was carried out by changing the pH, the concentration of the ion-pairing counter-ion, and the concentration of the organic solvent in the aqueous mobile phase. The effects of various cationic and anionic pairing reagents in the mobile phase and the stationary phase were investigated. The use of stepwise gradient elution improved the spot shape and the selectivity of the separation.

## INTRODUCTION

Cephalosporins are the group of β-lactam antibiotics. Chemically, they are derivatives of 7-aminocephalosporanic acid. Cephalosporins have been analysed by various classical liquid chromatographic techniques such as column[1,2] and thin-layer chromatography. [3-12]

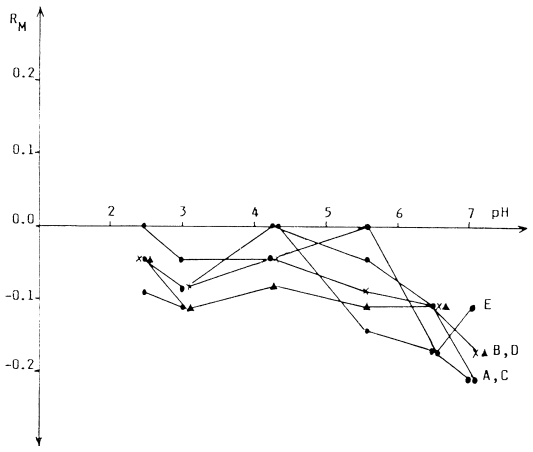



**Figure 1**. Plots of $R_M$ values versus pH of the mobile phase acetonitrile-0.09 M phosphate buffer (3:6). For the identification of the solutes, see Table 1.

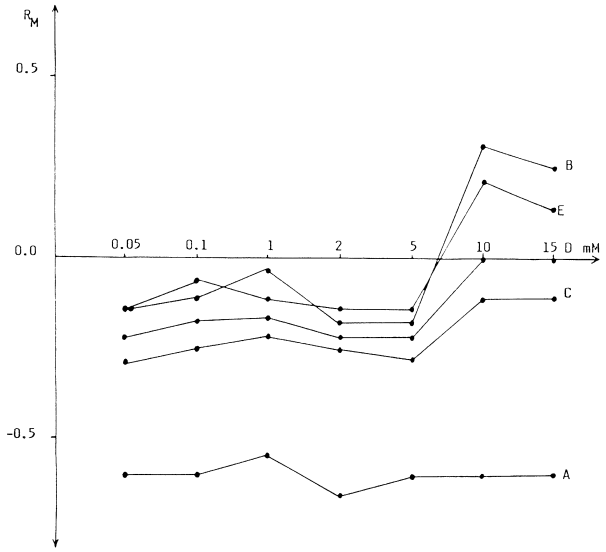



**Figure 2**. Plots of $R_M$ values versus amount mM of (TMA-Cl) in the mobile phase acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6, v/v).

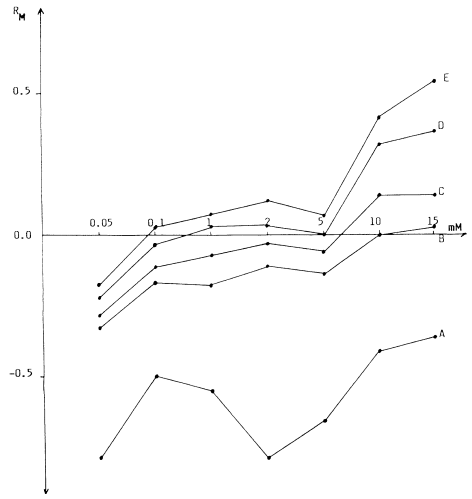
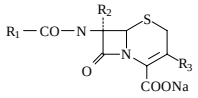
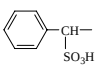
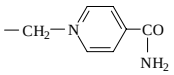
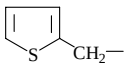
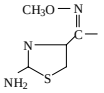
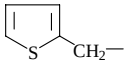
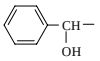
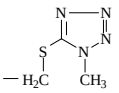



**Figure 3**. Plots of $R_M$ values amount mM of (TBA-OH) in the mobile phase acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6).

## Table 1

## Structures of Investigated Compounds




| No | Generic Name | | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|---|---|
| 1 | Cefsulodin | A |  | -H |  |
| 2 | Cefalothin | B |  | -H | $-CH_2OCOCH_3$ |
| 3 | Cefotaxime | C |  | -H | $-CH_2OCOCH_3$ |
| 4 | Cefoxitin | D |  | $-OCH_3$ | $-CH_2OCONH_2$ |
| 5 | Cefaman-dole | E |  | -H |  |

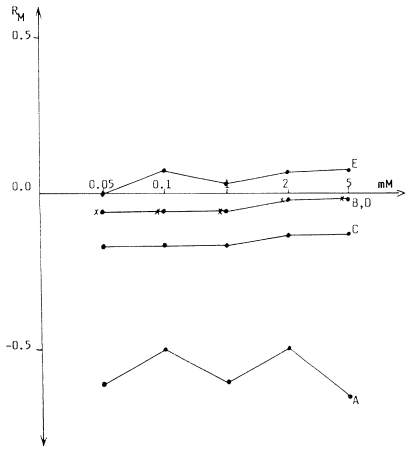



**Figure 4**. Plots of $R_M$ values versus amount mM of CA in the mobile phase tetrahydrofuran-0.09 M phosphate buffer (pH 2.47) (5:4).

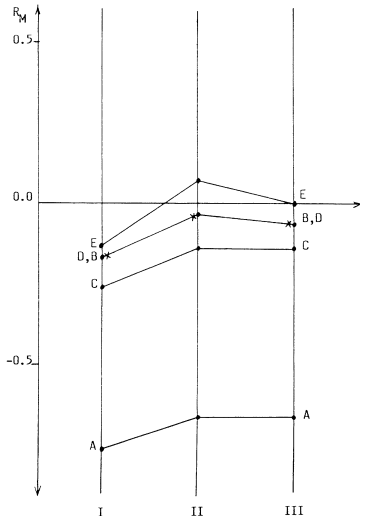



**Figure 5**. $R_M$ values of the cephalosporins for the systems: I. Mobile phase: tetrahydrofuran-0.09 M phosphate buffer (pH 2.47) (5:4); adsorbent: TLC RP-18 plates dipped in 3% w/v CA in ethanol. II. Mobile phase: tetrahydrofuran-0.09 phosphate buffer (pH 2.47) (5:4) containing 5 mM of camphoric acid; adsorbent: TLC RP-18. III. Mobile phase as in system II, adsorbent as in system I.

The aim of the present work was to compare the retention of cephalosporins in systems containing various cationic and anionic ion-pairing reagents in the mobile phase and/or in the stationary phase. Differences in the selectivity (as measured by $\Delta R_M$ values) of these systems in either isocratic or gradient elution have been determined.

## EXPERIMENTAL

### Reagents and Apparatus

Tetramethylammonium chloride (TMA-Cl) was obtained from Fluka (Buchs, Switzerland).Tetrabutylammonium hydroxide (TBA-OH), N-cetyl-pyridinium chloride monohydrate (NCP-Cl), benzyldimethyltetra-decylammonium chloride monohydrate (BDTDA-Cl), hexane-1-sulfonic acid sodium salt (HSA_Na), octane-1-sulfonic acid sodium salt (OSA-Na) and camphoric acid (CA) were purchased from (E. Merck Darmstadt, Germany). Cefsulodin, cefalothin, cefotaxime, cefoxitin, and cefamandole nafate were obtained from Sigma (St. Louis, MO,USA). All the other reagents and solvents were of analytical grade from E. Merck. TLC RP-18 $F_{254}S$ pre-coated plates (Fertigplatten) were also obtained from E. Merck.

The horizontal DS-type chamber was from Chromdes (Lublin, Poland) and a 25 μl micro-syringe (accuracy 0.5 μL) from Hamilton (Bonaduz, Switzerland).

### Procedure

Experiments were carried out in a horizontal sandwich chamber using 10x20 cm pre-coated TLC RP-18 plates. Ion-pair coated TLC RP-18 plates were prepared by dipping the plate in a 3% (w/v) ethanolic solution of the counter-ion for 5 min. The dipped plates were then allowed to dry in air, and 5 μL samples of a 0.1 % solute in phosphate buffer at pH 7.09 were applied 1 cm from the bottom edge of the plate and developed over a distance of 8 cm. Buffer solutions, used as the mobile phase, were prepared by dissolving 0.5 mL of 85% (w/v) orthophosphoric acid in 80 mL of water and adjusting the pH to the appropriate value with a saturated sodium hydroxide solution. Stepwise gradient elution was carried out by introducing consecutively a series of 0.5 mL portions of the mobile phase which contained decreasing concentrations of the ion-pairing reagent under the distributor. The spots of the compounds were detected under 254 nm UV light. The results (the mean values of three measurements differing by no more than 0.05 $R_f$ units) and chromatographic conditions are given in the figures.

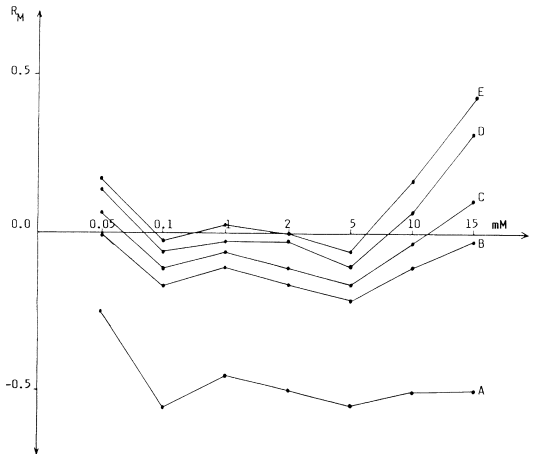



**Figure 6**. Plots of $R_M$ values versus amount mM of (BDTDA-Cl) in the mobile phase acetonitrile-0.09 phosphate buffer (pH 7.09) (3:6).



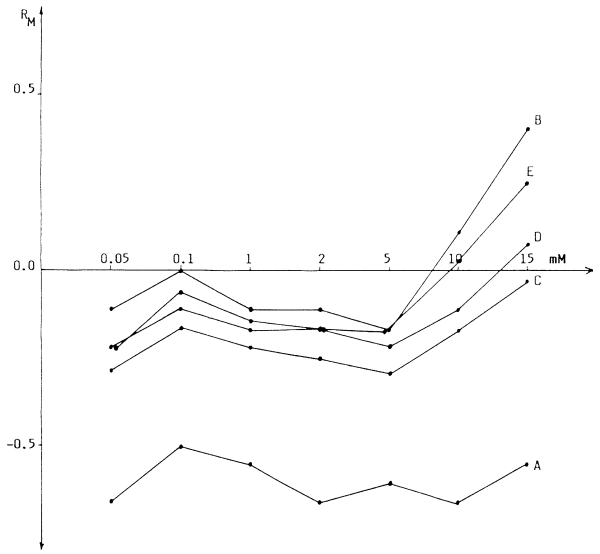



**Figure 7**. Plots of $R_M$ values versus amount mM of (NCP-Cl) of in the mobile phase acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6).

## RESULTS AND DISCUSSION

Results for the studied cephalosporins (listed in Table 1) are illustrated graphically as plots of $R_M$ against pH of the phosphate buffer solution in acetonitrile as the mobile phase (Fig. 1). The pH dependence of retention provides preliminary information on the acidic-basic properties of the solutes. The inflection points are at the pH equal to the pKa of the compounds, where solute retention is very sensitive to the mobile phase pH. At low pH the retention is low, whereas at pH 4-6 the $R_M$ is a maximum value; at pH 6-7 retention decreases sharply and at higher pH values (pH > 7) the retention of the anionic form of cephalosporins is low. The $R_M$ values of cefoxithim did not depend on the pH of the mobile phase. Good separation was obtained at pH 5.59 in all cases.

Separation by RP-IPC was carried out in the buffer solutions pH 2.47 and 7.09 for the anionic and cationic ion-pairing reagents, respectively. Plots showing the effect of the amount (mM) of tetramethylammonium chloride, tetrabutylammonium hydroxide, and camphoric acid in the mobile phase (Figs. 2, 3, 4) or 3% camphoric acid in the stationary phase (Fig. 5) on retention of the compounds are presented. The greatest reduction of $R_f$ values occurs at low concentrations of the ion-pairing reagent, but the best separation and strongest retention were observed at higher concentrations for cationic reagents.

The highest values were obtained for cefamandole in tetramethyl-ammonium chloride (Fig 2) and benzyldimethyltetradecylammonium chloride (Fig. 6) and for cefalothin in tetrabutylammonium hydroxide (Fig. 3) and in N-cetylpyridinium chloride (Fig. 7). Cefalothin and cefoxitin were not separated in the system containing hexane-1-sulfonic acid, octane-1-sulfonic acid, and camphoric acid. All of the studied substances were best separated in the system containing tetramethylammonium chloride as a counter-ion.

The separation efficiency is better with stepwise gradient elution than with isocratic development, owing to the enhanced mutual displacement of the solutes. The effect of the organic modifier content of the mobile phase was also investigated. The methanol, acetonitrile, and tetrahydrofuran concentrations were varied between 0 and 80% and the change in $R_f$ values for a range of test compounds was evaluated. In the case of the formation of ion-pairs with cationic reagents, acetonitrile turned out to be the best modifier and tetrahydrofuran was best in formation of ion-pairs with anionic reagents.

The $R_M$ values obtained on pre-coated TLC-RP-18 plates dipped in the counter-ion reagent solution showed that the best results were obtained for gradient elution with tetramethylammonium chloride, tetrabutylammonium hydroxide, and camphoric acid as a counter-ion (Figs. 5, 8, 9).

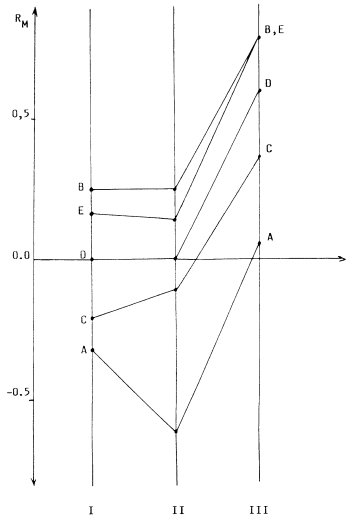



**Figure 8**. $R_M$ values of the cephalosporins for the systems: I. Mobile phase: acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6 ); adsorbent: TLC RP-18 plates dipped in 3% w/v TMA-Cl. II. Mobile phase: acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6) containing 15 mM of TMA-Cl; adsorbent: TLC RP-18. III. Mobile phase as in system II, adsorbent as in system I.

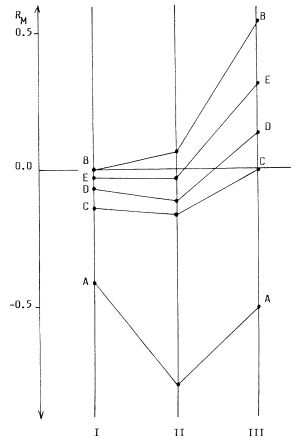



**Figure 9**. $R_M$ values of the cephalosporins for the systems: I. Mobile phase: acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6); adsorbent: TLC RP-18 plates dipped in 3% w/v TBA-OH in ethanol. II. Mobile phase: acetonitrile-0.09 M phosphate buffer (pH 7.09) (3:6) containing 5 mM of TBA-OH - adsorbent: TLC RP-18. III. Mobile phase as in system II, adsorbent as in system I.

The $\Delta R_M$ ($R_{MA}$ - $R_{MB}$) values are different for the various systems owing to the differences in the polarity of the substituents in the structures of the cephalosporins and to their interaction with mobile and stationary phases containing ion-pairing reagents of different properties. In Figs. 5, 8, 9, the specific difference in adsorption affinity is graphically presented as chromatographic "spectra" by plotting the $R_M$ value of the cephalosporins against the mobile or stationary phase used, which enables the selectivity of the systems to be compared.

Thin-layer reversed-phase ion-pair chromatography with cationic and anionic counter-ions and isocratic or gradient elution has been shown to be an efficient, straightforward, and cheap method for the analysis of mixtures of cephalosporins.

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