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Thin-layer reversed-phase ion-pair chromatography of some sulphonamides*

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Abstract: The retention behaviour of 15 sulphonamides 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.

Keywords: *Reversed-phase ion-pair chromatography; isocratic elution; gradient elution; cationic counter-ions; anionic counter-ions; sulphonamides.*

Introduction

Sulphonamides have been analysed by various classical liquid chromatographic techniques such as column [1–5], thin-layer [6–9] and paper chromatography [10] but there have been relatively few reports on the use of ion-pair thin-layer chromatography [11–16] for the determination of these drugs.

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

Experimental

Chemicals

Tricaprylylmethylammonium chloride, tetra-*n*-butylammonium chloride, tetra-*n*-butylammonium bromide and di(2-ethylhexyl)-orthophosphoric acid were from Fluka (Buchs, Switzerland), camphoric acid was obtained from Sigma (St Louis, MO, USA). Cetyltrimethylammonium bromide and tetra-*n*-butylammonium iodide and all other reagents and solvents were of analytical grade from E. Merck (Darmstadt, Germany); HPTLC RP-18 $F_{254}S$, precoated plates (Fertigplatten) were also obtained from E. Merck.

Chromatographic method

Experiments were carried out in a horizontal sandwich chamber [17] with a glass distributor of the mobile phase using: 10 × 10 cm precoated HPTLC RP-18 plates; and plates covered with a 0.25-mm layer of the slurry of the sorbent obtained by mixing 25.0 g of silanized silica gel Si 60 HF_{254} (E. Merck) with either 60 ml of methanol–water mixture (1:2, v/v) or 60 ml of methanol containing an appropriate concentration of ion-pairing reagent.

Ion-pairing coated HPTLC RP-18 plates were prepared by dipping the plate for 5 min in a 3% w/v ethanolic solution of the counter-ion. The dipped plates were then allowed to dry in air and 2-μl samples of a 0.2% w/v solution of each solute in methanol were applied 1 cm from the edge of the plate and eluted over a distance of 8 cm. Buffer solutions used as the mobile phase were prepared by dissolving 0.5 ml of 85% w/w orthophosphoric acid in 80 ml of water and adjusting the pH to the appropriate value with a saturated sodium hydroxide solution. The pH of the buffer was measured in the aqueous solutions and not in the final eluent.

Stepwise gradient elution was carried out by introducing consecutively under the distributor a series of 0.4-ml portions of the eluent which contained decreasing concentrations of the ion-pairing reagent. The spots of the compounds were localized under UV light at 254 nm. The results (the mean values of three measure-

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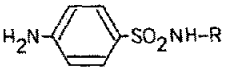
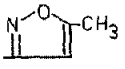
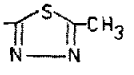
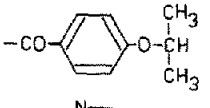
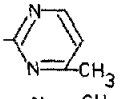
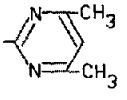
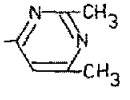
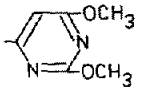
ments differing by no more than 0.05 R_F units) and chromatographic conditions are given in the figures.

Results and Discussion

Results for the sulphonamides listed in (Table 1) are illustrated graphically as plots of R_M against pH of the phosphate buffer solution in methanol as the mobile phase (Fig. 1). As expected, the retention is a sigmoidal function of the hydronium ion concentration and is very typical for sulphonamides. The pH dependence of retention provides preliminary information on the acidic–basic properties of the solutes. The inflection points are at the pH

Table 1
Investigated compounds



No.	Generic name	R	Commercial name in Poland	Manufacturer
1	Sulphanilamide	-H	Pabiamid	Polfa P.
2	Sulphacarbamide	$-CO-NH_2$	Urenil	Polfa P.
3	Sulphaguanidine	$-C(=NH)-NH_2$	Sulphaguanidinum	Polfa P.
4	Sulphacetamide	$-CO-CH_3$	Sulphacetamidum	Polfa P.
5	Sulphamethoxazole		Gantanol	Roche CH.
6	Sulphadicramide	$-CO-CH=C(CH_3)-CH_3$	Irgamid	Dispersa D.
7	Sulphafurazole		Amidoxal	Polfa P.
8	Sulphathiazole		Sulphathiazol	Spofa CS.
9	Sulphamethizole		Rufol	Debat F.
10	Sulphaproxyline		Merafin	Polfa P.
11	Sulphadiazine		Adiazine	Theraplix F.
12	Sulphamerazine		Sulphamerazine	Polfa P.
13	Sulphadimidine		Sulphadimidin	Spofa CS.
14	Sulphisomidine		Elkosin	Ciba CH.
15	Sulphadimethoxine		Madroxin	Polfa P.

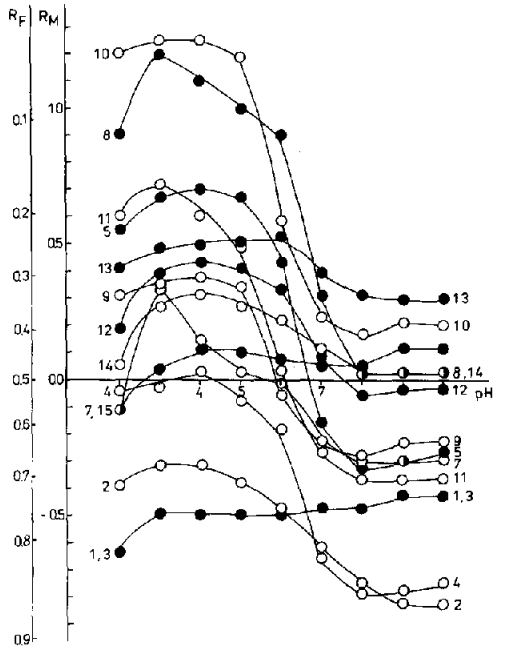


Figure 1
Plots of R_M values versus pH of the mobile phase of methanol–0.09 M phosphate buffer (1:3, v/v). Adsorbent: silanized silica gel. For the identification of the solutes see Table 1.

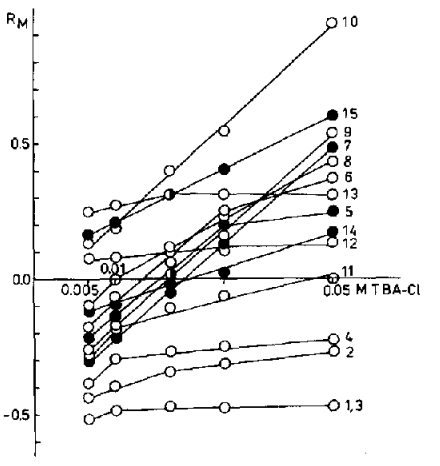


Figure 2
Plots of R_M values versus molar concentration of tetrabutylammonium chloride (TBA-Cl) in the mobile phase of methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v). Adsorbent: silanized silica gel.

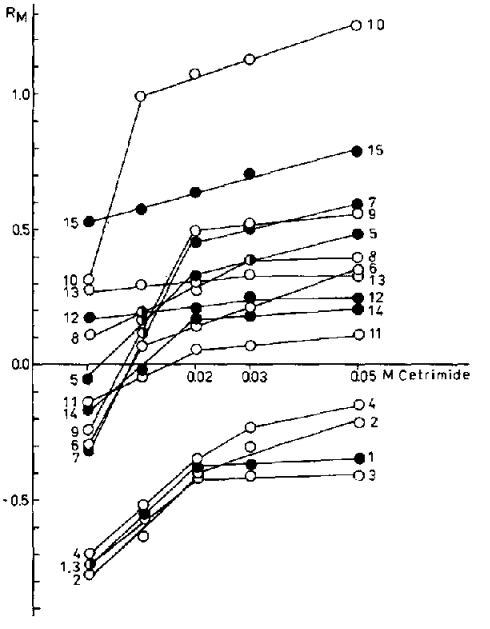


Figure 3
Plots of R_M values versus molar concentration of cetyltrimethylammonium bromide (cetrimide) in the silanized silica gel. Eluent: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v).

equal to the pK_a of the compounds, where solute retention is very sensitive to the mobile phase pH. Therefore a small change in pH results in a large change in the R_M value of the solute. At low pH the retention is low whereas at pH 3–5 the R_M is at a maximum value; at pH 6–7 retention decreases sharply and at higher pH values (pH > 7) the retention of the anionic form of sulphonamides is low and independent of pH. The R_M values of some sulphonamides (sulphanilamide, sulphaguanidine and sulphadimethoxine) did not depend on the pH of the mobile phase; similar R_M values were obtained in the pH range studied. Good separation was obtained at pH 4–5 in all cases.

Separation by RP-IPC was carried out in the buffer solutions of pH 3.5 and 7.85 for the anionic and cationic ion-pairing reagents, respectively. Plots showing the effect of the molar concentration of the tetra-*n*-butylammonium chloride (TBA-Cl) in the mobile phase (Fig. 2) or cetyltrimethylammonium bromide (cetrimide) in the stationary phase (Fig. 3) on retention of the compounds are presented.

In both cases in the appropriate ranges of the concentration of the ion-pairing reagent, linear relationships were mostly obtained in accordance with the equation: $R_M = m\ C_s + k$, where C_s is the concentration of the interacting component ion-pairing reagent of the developing solvent and k is a constant. 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. More polar compounds such as sulphanilamide, sulphacarbamide, sulphaguanidine and sulphacetamide had higher R_F values.

In Figs 4–8 the specific differences in adsorption affinity are graphically presented as chromatographic 'spectra' by plotting the R_M value of the sulphonamides against the mobile or stationary phase used, which enables the selectivity of the systems to be compared.

The systems in which the mobile phase is the same and the ion-pairing reagents in the stationary phase are different are presented in Fig. 4. The different chromatographic behaviour of sulphonamides is due to the nature of the counter-ions used. For almost all the sulphonamides investigated, the R_f values are in the optimal range (0.2–0.8) and only slight differences in selectivity were obtained. Sulphaproxyline and sulphadimethoxine were not separated in the system containing TBA-I as a counter-ion; sulphacetamide and sulphacarbamide were best separated in the system containing TBA-Cl as a counter-ion.

The R_M values obtained for various systems containing TBA-Cl as an ion-pair forming counterion are shown in Fig. 5. In stepwise gradient elution (system IV) the separation

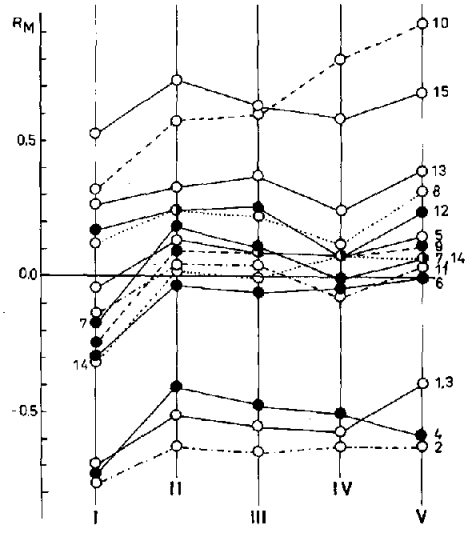


Figure 4
R_M values of the sulphonamides for the systems. I, Eluent: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v) for all systems; adsorbent: silanized silica gel; II, adsorbent: silanized silica gel with 0.01 mM g^{-1} TBA-Cl; III, adsorbent: silanized silica gel with 0.01 mM g^{-1} TBA-IC (tetrabutylammonium iodide); IV, adsorbent: silanized silica gel with 0.01 mM g^{-1} cetrimide; V, adsorbent: silanized silica gel with 0.01 mM g^{-1} TCMA-Cl (tricaprylylmethylammonium chloride).

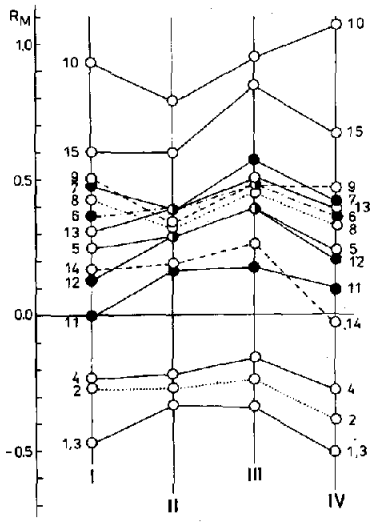


Figure 5
R_M values of the sulphonamides for the systems. I, Eluent: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v) containing 0.5 mM TBA-Cl, adsorbent: silanized silica gel; II, eluent: methanol–water–0.9 M phosphate buffer (pH 7.85) (3:3:4, v/v/v), adsorbent: silanized silica gel with 0.05 mM g^{-1} TBA-Cl; III, eluent: as in system I. Adsorbent as in system II; IV, four-step gradient elution: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v) containing 0.03, 0.02, 0.01 and 0.005 M TBA-Cl, respectively, adsorbent: silanized silica gel.

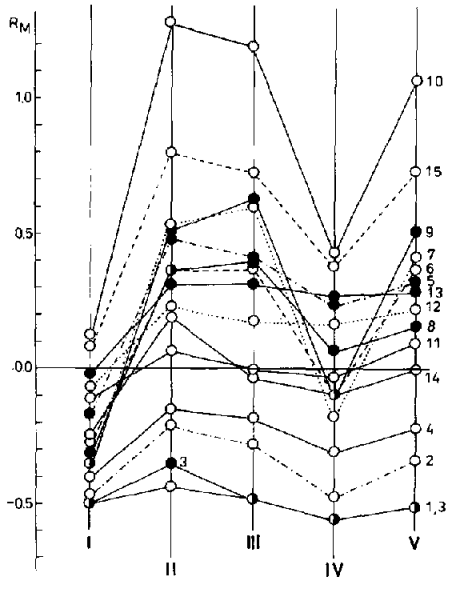


Figure 6
R_M values of the sulphonamides for the systems. I, Eluent: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v) with 0.5 M g^{-1} cetrimide, adsorbent: silanized silica gel; II, eluent: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v), adsorbent: silanized silica gel with 0.05 mM g^{-1} cetrimide; III, eluent as in system I, adsorbent as in system II; IV, four-step gradient elution: methanol–water–0.09 M phosphate buffer (pH 7.85) (3:3:4, v/v/v) containing 0.03, 0.02, 0.01 and 0.005 M cetrimide, respectively, adsorbent: silanized silica gel; V, as in system IV, adsorbent: silanized silica gel with 0.05 mM g^{-1} cetrimide.

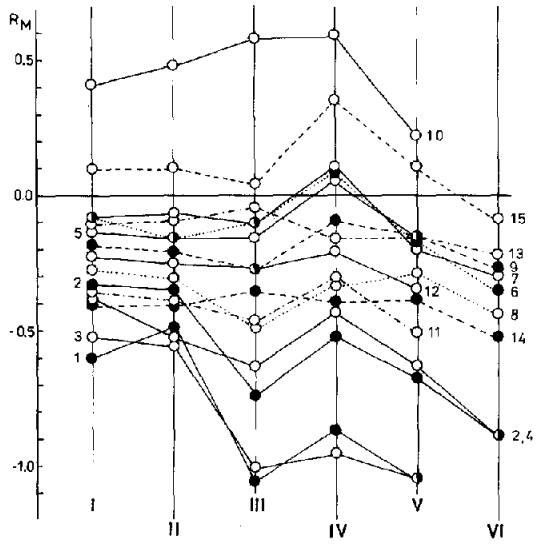


Figure 7
R_M values of the sulphonamides for the systems: I, Eluent: methanol–0.09 M phosphate buffer (pH 7.85) (6:4, v/v), adsorbent: HPTLC RP-18 plates dipped in 3% w/v TBA-Cl in ethanol; II, eluent: as in system I, adsorbent: HPTLC RP-18 plates dipped in 3% w/v TBA-Br in ethanol; III, eluent as in system I, adsorbent HPTLC RP-18 plates dipped in 3% w/v cetrimide in ethanol; IV, four-step gradient elution: methanol–0.09 M phosphate buffer (pH 7.85) (6:4, v/v) with 0.03, 0.02, 0.01 and 0.005 M cetrimide, respectively, adsorbent as in system III; V, eluent: methanol–0.09 M phosphate buffer (pH 7.85) (6:4, v/v), adsorbent: HPTLC RP-18 plates dipped in 3% w/v HDEHP (di-2-(ethylhexyl)orthophosphoric acid) in the eluent; VI, eluent: methanol–water (6:4, v/v), adsorbent as in system V.

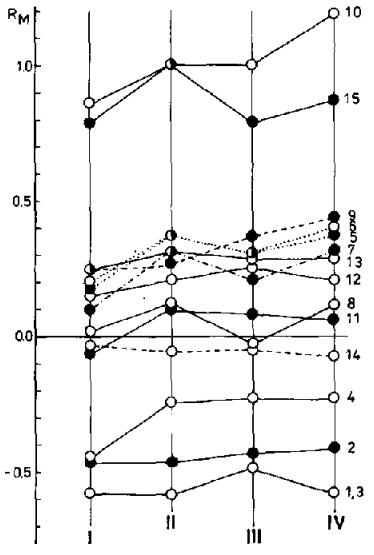


Figure 8
R_M values of the sulphonamides in the system. I, Eluent: methanol–water–0.09 M phosphate buffer (pH 3.5) (3:3:4, v/v/v) containing 0.5 mM camphoric acid, adsorbent is silanized silica gel; II, eluent: methanol–water–0.09 M phosphate buffer (pH 3.5) (3:3:4, v/v/v); adsorbent: silanized silica gel with 0.05 mM g^{-1} camphoric acid; III, eluent as in system I, adsorbent as in system II; IV, four-step gradient elution: methanol–water–0.09 M phosphate buffer (pH 3.5) (3:3:4, v/v/v) with 0.03, 0.02, 0.01 and 0.005 M camphoric acid, respectively, adsorbent: silanized silica gel.

efficiency is better than in isocratic development owing to the enhanced mutual displacement of the solutes [18]. In this system almost all compounds were separated.

Figure 6 shows the R_M values of sulphonamides obtained for various systems containing cetrimide as an ion-pairing reagent; the best results were obtained when the counter-ion was present in both phases simultaneously (system III) and in gradient elution (system V). The elution sequence of the compounds changed with the addition of the counter-ion in the stationary phase. These changes are advantageous for optimization of the separation of individual pairs of compounds.

The R_M values obtained on precoated HPTLC RP-18 plates dipped in the counter-ion reagent solution showed that the best results were obtained for gradient elution with cetrimide as a counter-ion (Fig. 7, system IV).

Good results were also obtained for anionic ion-pair reagents, especially for camphoric acid (Fig. 8). The selectivity parameter, ΔR_M, for each pair of investigated compounds, can be easily estimated directly from the plots (Figs 4–8). The ΔR_M ($R_{M2} - R_{M1}$) values are different for the various systems owing to the differences in the polarity of the substituents in the structure of the sulphonamides and to their interaction with mobile and stationary phases containing ion-pairing reagents of different properties.

The largest effect on retention and selectivity was obtained when the ion-pairing reagent was incorporated in the stationary phase.

The possible application of the ion-pair reversed-phase thin-layer data in the study of quantitative structure–activity relationships (QSAR) is demonstrated in Fig. 9 [19, 20].

The following regression equation for correlation between the logarithm of the partition coefficient, log P from the n-octanol–water system [15] and R_M was obtained:

$$\log P = 2.03\, R_M - 10$$
($r = 0.952$, SD of the slope = 0.837).

This indicates indirectly that there may be a correlation between R_M and biological activity.

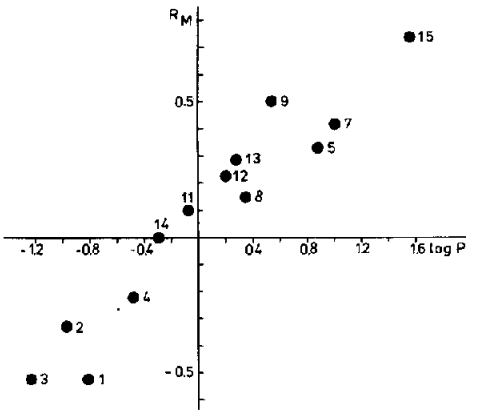


Figure 9
Relationship between the R_M value of each sulphonamide (obtained by gradient elution Fig. 6, system V, with cetrimide as the ion-pairing reagent) and the logarithm of the partition coefficient (log *P* value) [15].

The relatively high correlation coefficient (*r*) indicates that the major factor (among other factors [21–24]) that influences the retention of solutes in the reversed-phase ion-pair chromatographic systems is solvophobic interaction and that these systems can be used as an approach for predicting the activities of congeneric series of compounds [25–27].

Conclusions

Thin-layer reversed-phase ion-pair chromatography with cationic and anionic counterions and isocratic or gradient elution has been shown to be an efficient, straightforward and cheap method for the analysis of mixtures of sulphonamides. Stepwise gradient elution (not widely used in planar chromatography) increased the efficiency of separation and improved the shape of the spots.

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