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Simultaneous effects of amines and alkylsulphonates in reversed-phase ion-pair liquid chromatography — application to the separation of 2-imidazoline drugs

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Abstract: The reversed-phase liquid chromatographic separation of several pharmaceutically important 2-imidazoline derivatives using eluents containing both amines and alkylsulphonates, is described. The addition of *N,N*-dimethyloctylamine and sodium octanesulphonate to an acidic aqueous methanolic eluent, resulted in an eluent with much higher separation power than mobile phases containing only one modifier. The combined effect of the amine and of the alkylsulphonate enabled baseline (or near-baseline) separations of all the 2-imidazolines studied.

The use of such eluents is described in terms of efficiency, selectivity, peak symmetry and separation time and the mechanism of retention is discussed. Some separation examples are given to demonstrate the applicability of the developed technique in the quality control of these drugs in pharmaceuticals.

Keywords: *Reversed-phase ion-pair liquid chromatography; combined effect of amines and alkylsulphonates; selectivity; peak symmetry; efficiency; resolution; 2-imidazolines; pharmaceutical preparations.*

Introduction

2-Imidazolines are in wide use as therapeutic agents and many of them are listed in official pharmacopoeias. They all act as vasoconstrictors and reduce congestion and oedema of the nasal mucosa. Most analytical methods described are either colourimetric or ultraviolet determinations, and non-aqueous titrations are still used to assay the bulk compounds. These methods are rather time-consuming (extraction steps, colour development times) and lack specificity both with regard to degradation compounds and to related 2-imidazolines.

Despite their widespread use, very few column liquid chromatographic studies have been reported for the separation and determination of structurally closely-related 2-imidazolines such as naphazoline, tolazoline, tetrahydrozoline, tramazoline, coumazoline, antazoline, oxymetazoline and xylometazoline. The structures of these drugs are

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Table 1
Chemical structure of the investigated decongestants*

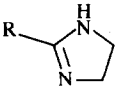
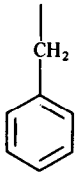
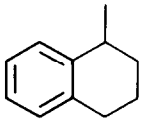
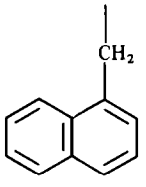
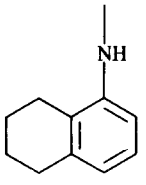
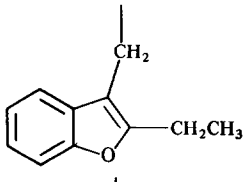
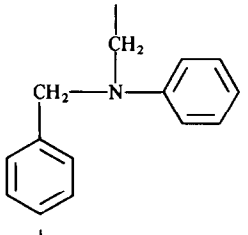
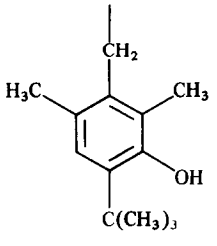
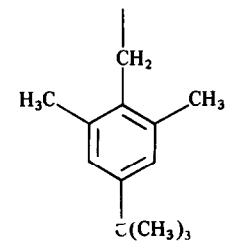
Decongestant	Abbreviation	N	R
			
Tolazoline	TOL	**1**	
Tetrahydrozoline	TET	**2**	
Naphazoline	NAP	**3**	
Tramazoline	TRA	**4**	
Coumazoline	COU	**5**	
Antazoline	ANT	**6**	
Oxymetazoline	OXY	**7**	

Table 1
(continued)

Decongestant	Abbreviation	N	R
Xylometazoline	XYL	8	

*Structure shown is for the free base.

presented in Table 1. Two papers have described HPLC stability-indicating assays for tetryzoline hydrochloride [1] and for tetryzoline hydrochloride and naphazoline hydrochloride [2], both in ophthalmic preparations. Neither of them gave additional information concerning the separation of related pharmaceutically important 2-imidazoline derivatives. Both methods described mobile phases adjusted to rather unsuitable pH values. The mobile phase adjusted to pH 7 [1] caused severe tailing of the eluting peaks through an increased interaction of the amine functions of the solutes with the residual silanol groups of the packing material. The mobile phase of pH 2.2 [2] would shorten the column life-time and consequently the reproducibility of the separation, by cleavage of the Si–C bonds at the surface of the stationary phase.

As it proved to be difficult to obtain adequate chromatographic separations of the above compounds using current ion-pairing methods, an acidic aqueous methanolic eluent containing an amine (*N,N*-dimethyloctylamine) and an ion-pairing agent (sodium octanesulphonate) was developed.

This approach permitted the separation of the 2-imidazolines studied as well as their respective degradation products on a common C-18 reversed-phase support. A systematic investigation of the eluent parameters (water content, *N,N*-dimethyloctylamine concentration, pH, nature and concentration of salt added, sodium octanesulphonate concentration) and of the column temperature was made to evaluate the contribution of each individual eluent parameter to the retention and to the chromatographic performance of the separation.

Experimental

Chemicals and solvents

N,N-Dimethyloctylamine (DMOA) was obtained from Aldrich (Milwaukee, USA) and was used as received; anhydrous sodium 1-octanesulphonate (SOS) from Janssen (Belgium), 85% orthophosphoric acid from Merck (FRG) and analytical grade methanol from UCB (Belgium). Water was purified by ion-exchange chromatography and subsequent distillation. Naphazoline hydrochloride and tolazoline hydrochloride were purchased by Janssen. Tetrahydrozoline hydrochloride was a kind gift from Pfizer,

Tramazoline hydrochloride from Boehringer and coumazoline from Labaz. Antazoline, oxymetazoline and xylometazoline were kindly supplied by Essex.

HPLC equipment

Chromatography was performed on a SP 8000 liquid chromatograph (Spectra Physics, Darmstadt, FRG) equipped with a Model 770 variable wavelength detector (Spectra Physics, Darmstadt, FRG) and with a BD 8 single channel recorder (Kipp and Zonen, Delft, The Netherlands).

Chromatographic conditions

A 5-μm particle size, 150 × 4.1 mm RSIL C-18 column (RSL, Eke, Belgium) was used throughout. The mobile phase was pumped at 1 ml/min and the column effluent was monitored at 220 nm. All separations were performed at 25°C (heated air oven). Injections were made with a Valco six-port injection valve and a 10-μl sample loop. The mobile phases were prepared by dissolving the required amount of SOS and the required volume of DMOA in *ca* 990 ml of a mixture of methanol–water. The mixture was adjusted to pH with orthophosphoric acid and subsequently diluted to exactly 1000 ml. Before chromatography, the mobile phase was filtered through a 5-μm filter and degassed with helium.

Chromatographic performance

The chromatographic performance parameters [selectivity (α), plate count (N) and resolution (R_S)] were calculated according to the well-known basic formulae of liquid chromatography [3]. The peak asymmetry (Asf) was measured by drawing a perpendicular from the peak maximum to the baseline and by dividing the rear portion of the peak by the front portion at 10% of the peak height.

Results and Discussion

The 2-imidazoline derivatives either failed to elute from an octadecyl column using acidic aqueous methanolic eluents or were excessively retained resulting in badly tailing peaks (Fig. 1). In reversed-phase high-performance liquid chromatography (RP-HPLC), it is known that the elution of cationic compounds can be significantly affected by the addition of an amine to the eluent [4–7]. The chromatographic performance in terms of excessive retention times, asymmetric peak shape and poor efficiency greatly improve

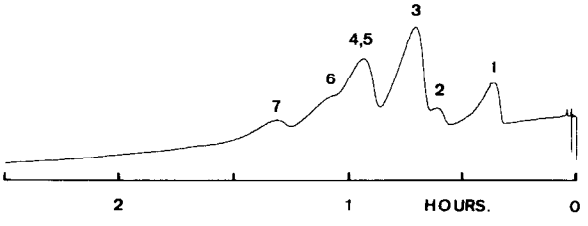


Figure 1
Chromatogram showing the elution of the 2-imidazolines with a mobile phase containing no modifier. Eluent: methanol–water (55:45, v/v), adjusted to pH = 3.0; chromatographic conditions: flow rate 1 ml/min; temperature 25°C; detection 220 nm UV; key: see Table 1.

and DMOA was selected as it was previously shown that this amine is very efficient in avoiding such problems [8, 9]. However, preliminary experiments revealed that upon the addition of DMOA to the eluent, the resolution deteriorated rapidly (Fig. 2). Therefore, SOS was also added to the eluent to introduce sufficient resolution to separate all of the 2-imidazolines studied.

As the eluent composition became quite complex, careful optimization of the chromatographic (eluent) conditions was necessary, in order to control the separation for efficiency, peak shape, selectivity, separation time and quantitative analysis of any of the investigated drugs.

DMOA concentration

Increasing the DMOA concentration in the eluent methanol–water (55:45, v/v), containing 20 mM SOS adjusted to pH 3.0, strongly reduced the retention of all compounds (Fig. 3), but selectivities (α values) were found not to change except for the peak pair XYL/OXY (Table 2). Although the efficiency of the separation remained virtually unaffected by rising DMOA concentration, resolution decreased due to falling k' values. According to the ion-interaction model developed by B. A. Bidlingmeyer [10, 11] the following explanation could be given. The positively charged DMOA molecules compete with the solutes for electrostatic interaction with the adsorbed negatively charged SOS molecules resulting in less retention (interaction) of the solutes with increasing DMOA concentration. Yet, silanophylic interactions [12] cannot be excluded as DMOA also strongly reduces the retention, in the absence of SOS in the eluent (Fig. 2).

The α change can be explained by assuming a decreased hydrophobicity of the stationary phase through adsorption of DMOA molecules (octyl chain versus octadecyl

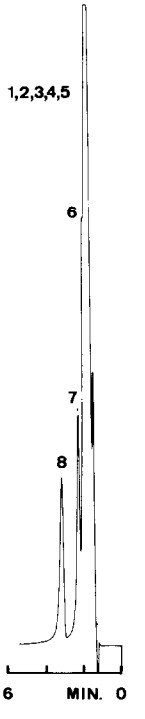


Figure 2
Chromatogram showing the elution of the 2-imidazolines with a mobile phase methanol–water (55:45, v/v) containing 5 mM DMOA, adjusted to pH = 3.0. Chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

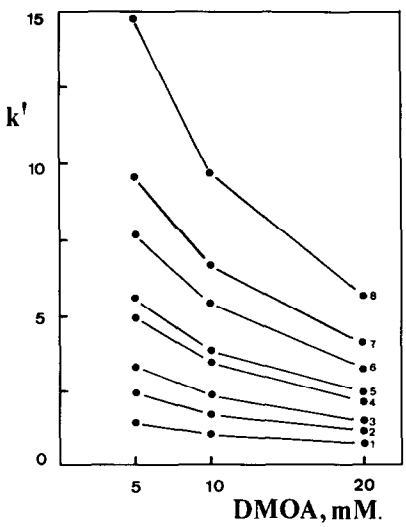


Figure 3
Change in retention as a function of the DMOA concentration in the eluent. Eluent: methanol–water (55:45, v/v) containing 20 mM SOS, adjusted to pH = 3.0; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

Table 2
Change of selectivities as a function of some eluent parameters

Eluent parameter	(A) DMOA (mM)			(B) ϕ (v/v)			(C) pH*			(D) Na^+ (mM)		
α	5	10	20	0.25	0.35	0.45	4.5	5.9	7.4	0	30	80
TET/TOL	1.85	1.80	1.90	1.29	1.53	1.85	1.75	1.68	1.48	1.61	1.66	1.59
NAP/TET	1.37	1.43	1.32	1.15	1.21	1.37	1.38	1.40	1.40	1.22	1.23	1.32
TRA/NAP	1.52	1.53	1.49	1.12	1.36	1.51	1.46	1.44	1.31	1.41	1.40	1.43
COU/TRA	1.13	1.11	1.12	1.14	1.09	1.13	1.13	1.16	1.21	1.09	1.10	1.10
ANT/COU	1.39	1.41	1.41	1.06	1.30	1.39	1.38	1.35	1.25	1.26	1.25	1.26
OXY/ANT	1.25	1.25	1.25	1.21	1.23	1.25	1.24	1.24	1.26	1.26	1.29	1.28
XYL/OXY	1.55	1.47	1.40	1.55	1.52	1.55	1.54	1.55	1.57	1.50	1.51	1.52

(A) Influence of the DMOA concentration (methanol–water (55:45, v/v), 20 mM SOS, pH = 3.0).
(B) Influence of the volume fraction of water (methanol–water, 20 mM SOS, 5 mM DMOA, pH = 3.0).
(C) Influence of the apparent pH (methanol–water (55:45, v/v), 20 mM SOS, 5 mM DMOA).
(D) Influence of the Na^+ concentration (methanol–water (65:35, v/v), 20 mM SOS, 10 mM DMOA, pH = 3.0).

chain). The solutes showing highest retention (i.e. the peak pair XYL/OXY) would be most sensitive to such a hydrophobicity decrease resulting in a slightly decreased separation factor.

The peak symmetry, measured as indicated in the Experimental section, improved at higher DMOA concentrations. However this improvement was less pronounced than is usually the case in the absence of an alkylsulphonate in the eluent. This is due to the ion-pairing mechanism involved in these separations that also eliminates tailing of eluting peaks to a great extent.

Water content

A plot of the logarithm of the capacity ratio versus the volume fraction of water (ϕ) yielded a linear relationship in all cases ($r > 0.9970$) (Fig. 4). The slopes of the lines

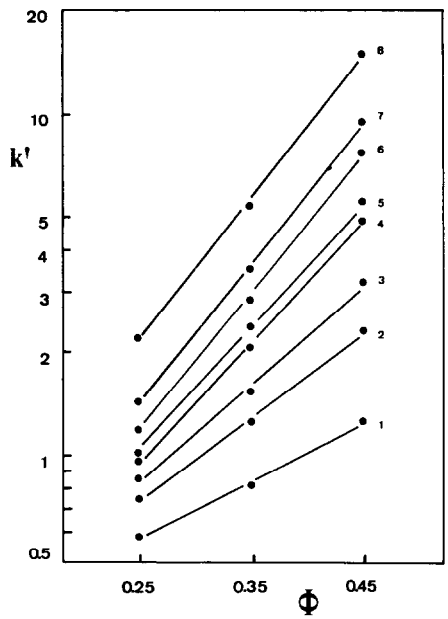


Figure 4
Change in retention as a function of the volume fraction of water in the eluent. Eluent: methanol–water, containing 20 mM SOS and 5 mM DMOA, adjusted to pH = 3.0; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

calculated for TRA and COU were 0.124 and for ANT, OXY and XYL were 0.105, respectively. The slopes of the lines calculated for TOL (0.2561), TET (0.1757) and NAP (0.1512) differ significantly from each other and from those calculated above. A selectivity change will only be obtained if the slope of the lines differs for a given pair of compounds. Thus, no selectivity change was observed for the peak pairs COU/TRA, OXY/ANT and XYL/OXY, whereas the selectivity increased for the peak pairs TET/TOL, NAP/TET, TRA/NAP and ANT/COU (Table 2).

The gain in resolution observed at higher water concentrations was due to the k' increase and to a selectivity increase for some pairs of compounds, as the efficiency of the separations (expressed by the number of theoretical plates, N) only slightly improved (about 10% in the concentration range tested). The peak symmetry, remained virtually unaffected by the water content of the eluent.

pH

The elution behaviour as a function of the apparent pH of the eluent is shown in Fig. 5. In the lower pH range retention was not influenced by pH changes but at pH values greater than 5, the retention of all compounds increased. This observed relationship has been attributed to a competition of the residual silanol functions with the ion-pairing agent SOS for the charged solutes, resulting in stronger interactions at higher pH values [13]. The selectivity of the separation depended on the pH of the eluent (Table 2) and the chemical nature of the compounds.

Resolution as a function of pH in general decreased (except for the peak pair COU/TRA) with rising pH due to a peak broadening effect and a selectivity decrease, whereas the peak symmetry was found not to change with varying pH. An illustration of these observations is given in Fig. 6. Throughout the experiments a pH = 3.0 was maintained because small variations in the pH of the eluent would not result in significant retention differences.

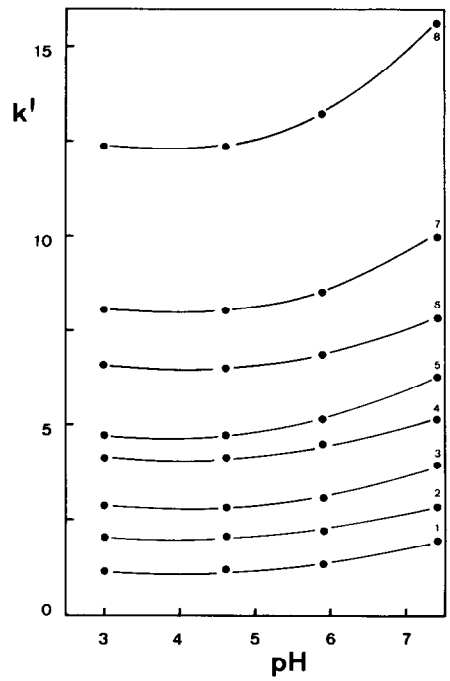


Figure 5
Change in retention as a function of the apparent pH of the eluent. Eluent: methanol–water (55:45, v/v), containing 20 mM SOS and 5 mM DMOA; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

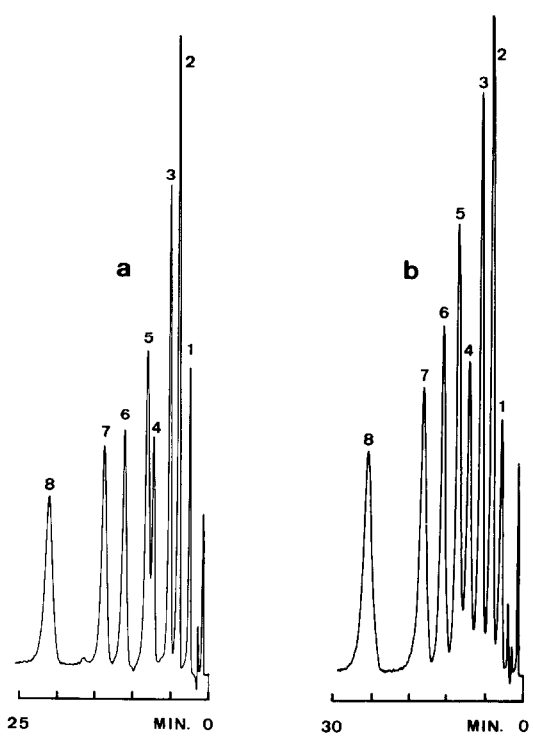


Figure 6
Influence of the apparent pH of the eluent on the chromatographic performance of the separation. (a) Eluent: methanol–water (55:45, v/v), containing 20 mM SOS and 5 mM DMOA (pH = 3.0). (b) Eluent: as in (a), but pH adjusted to 7.35. Chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

The concentration and nature of the cation

To examine the influence of salts, i.e. the ionic strength, on the retention of cationic solutes, the 2-imidazolines were chromatographed with a mobile phase (methanol–water (65:35, v/v) containing 10 mM DMOA and 20 mM SOS, adjusted to pH 3.0), containing different amounts of sodium chloride. It was found that retention decreased with increasing salt concentration (Fig. 7). Replacing sodium chloride by equimolar amounts of lithium chloride or ammonium chloride yielded similar results and it was observed that the decrease in retention depended on the nature of the cation. The retention-decreasing effect increased in the order $NH_4^+ > Na^+ > Li^+$. A similar order has been reported for the elution strength of these cations in ion-exchange chromatography [14]. These observations are clearly illustrated in Fig. 8. The addition of 60 mM ammonium chloride to the eluent (Fig. 8c) reduced retention to the same extent as 80 mM sodium chloride (Fig. 8b). The best separation was obtained without salt added (Fig. 8a).

Two possible explanations for these results can be formulated. As the nature of the cation influences the retention, the occurrence of an underlying ion-exchange mechanism cannot be excluded as described by the "dynamic ion-exchange model" [15]. However, the capacity factor was not linearly dependent on the reciprocal of the concentration of the $[Na^+]$ in the eluent, a relationship usually obtained in ion-exchange chromatography. More likely is the explanation given by Bidlinmeyer *et al.* in their developed "ion-interaction model" [10, 11, 16]. They stated that retention results from both electrostatic and lipophylic forces. At high salt concentrations, the electrostatic interactions decrease, resulting in less retention. Thus, the effects of inorganic salts and DMOA are similar in nature and are ascribed to a competition (of any added positively charged ion) with the solutes for the adsorbed negatively charged ion-pairing agent. Depending on the type of positive ion added (nature, dimension, charge, functional groups, alkyl chain length) the complex ion-pairing agent–ion formed near the surface of the stationary phase is more or less stabilized resulting in a different elution power for the various ions. These assumptions might explain the difference in retention-decreasing power observed between the inorganic ions and between these ions and DMOA, the latter being far more effective (Fig. 3) through additional non-polar forces, i.e. interaction of the alkyl chain with the stationary phase.

Selectivity changes as a function of the Na^+ concentration were not observed (Table 2). Changing the type of inorganic cation again did not alter the selectivity of the separation. These observations are rationalized by assuming that the ions only affect the association between solute and pairing-ion and do not affect the physicochemical behaviour of the uncharged moieties of the solutes [17]. The same explanation might hold true for DMOA as there again no selectivity changes were observed (Table 2).

The resolution decreased with increasing salt concentration through a capacity factor decrease as the efficiency of the separation was not affected in the investigated concentration range of salt added.

Column temperature

The column temperature is an additional parameter to control chromatographic separations. Plots of the logarithm of the capacity values versus the reciprocal of the absolute temperature yield straight lines ($r > 0.9980$) (Fig. 9). This linear relationship indicates a regular retention behaviour [18], i.e. one single retention mechanism dominates the retention process. This conclusion is confirmed by the linear relationship obtained upon plotting the log k' values versus ϕ (Fig. 4). This generally observed

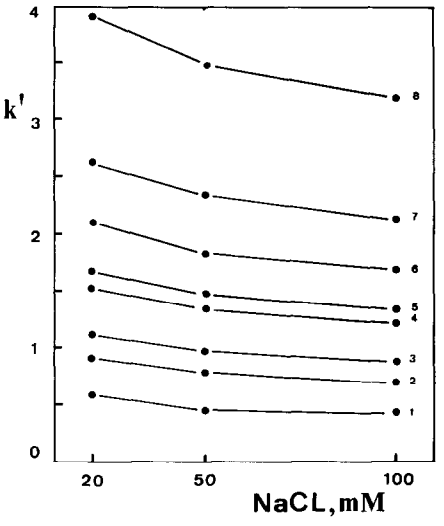


Figure 7
Change in retention as a function of the salt concentration. Eluent: methanol–water (65:35, v/v), containing 20 mM SOS and 10 mM DMOA (pH = 3.0); chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

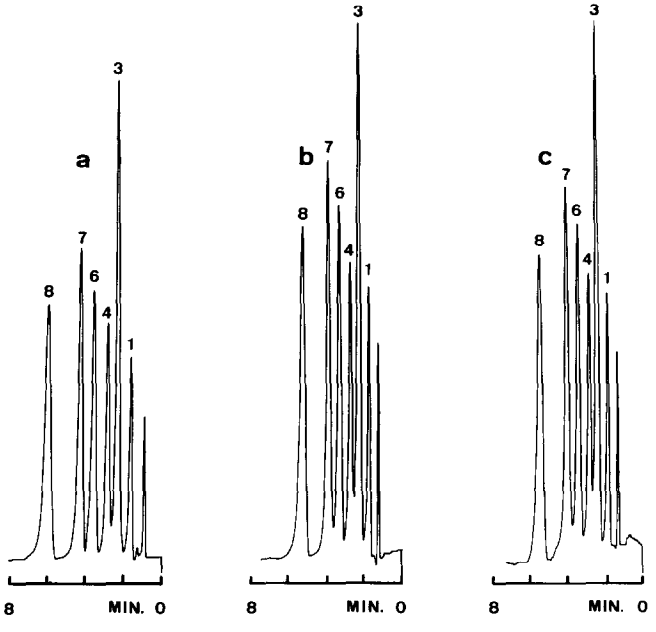


Figure 8
Influence of the type and concentration of salt added on the chromatographic performance of the separation. Eluent: (a) as in Fig. 7; (b) as (a) with 80 mM sodium chloride added; (c) as (a) with 60 mM ammonium chloride added; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

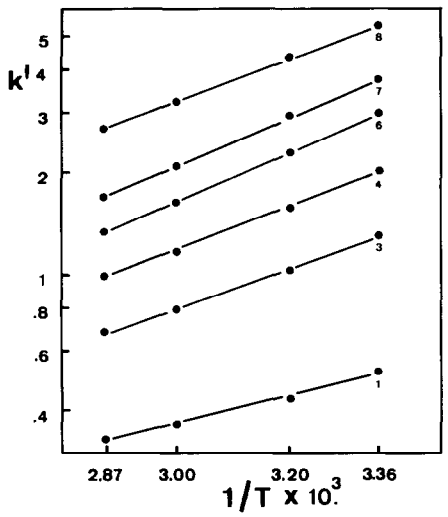


Figure 9
Change in retention as a function of the column temperature. Eluent: methanol–water (55:45, v/v), containing 20 mM SOS and 20 mM DMOA, adjusted to pH = 3.0; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

relationship is also an indication of regular retention behaviour. As both experiments were performed with different amounts of DMOA in the eluent, the conclusion may be drawn that the retention mechanism is not altered by changing the DMOA concentration from 5 to 20 mM. The slopes of the lines calculated for XYL, TRA and NAP were the same (0.6933) whereas a similar slope was obtained for OXY and ANT (0.5622) and a different slope was calculated for TOL (1.13).

Minor selectivity changes are thus possible by a variation of the column temperature. An increase in column temperature improved the chromatographic efficiency due to a rise in diffusion coefficients caused by a reduced viscosity of the mobile and stationary phase [19], but decreased resolution mainly through reduced capacity factors. Thus, all separations were performed at 25°C throughout this study.

Sodium octanesulphonate concentration

The experiments so far performed suggested that the eluent should contain high water concentrations (45% v/v) to achieve sufficient resolution as well as relatively high DMOA concentrations for peak shape improvement and efficiency gain. The combined effect of both eluent parameters however resulted in unsatisfactory separations. 2-Imidazolines are strong bases (pKa > 10) and quite polar compounds [20]. Such compounds usually exhibit poor chromatographic performance and excessive retention on octadecyl silica based columns by an interaction with the unreacted (residual) silanol groups at the surface of the stationary phase layer. Upon addition of DMOA to the eluent, these active sites are blocked resulting in low retention times and poor resolution even at high water concentrations (75% v/v) and low DMOA concentrations (2.5 mM) (Fig. 10a). The early eluting 2-imidazoline derivatives were poorly resolved and the analysis time increased. Note also the considerable tailing due to the low DMOA concentration and the absence of SOS in the eluent (peak 8 in Fig. 10a).

Incorporating SOS in the eluent, without adding DMOA, greatly improved the separation but resulted in excessive elution times (>1 h) (Fig. 10b). Several mobile phases were tried, including the use of other alkylsulphonates in order to reduce the separation time. However, with such attempts, faster analysis was associated with lower

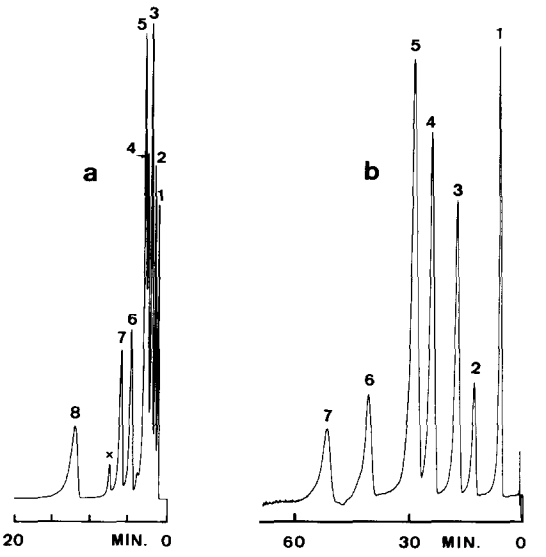


Figure 10
Chromatograms showing the (optimized) separation of the 2-imidazoline drugs using eluents containing either DMOA or SOS. (a) Eluent: methanol–water (25:75, v/v), containing 2.5 mM DMOA (pH = 3.0). (b) Eluent: methanol–water (55:45, v/v), containing 20 mM SOS (pH = 3.0). Chromatographic conditions and key: as in Fig. 1.

resolution in all cases. Therefore, a combination of both modifiers was used to separate the 2-imidazolines studied.

By adding SOS to the eluent, methanol–water (50:50, v/v), containing 5 mM DMOA (pH = 3.0), the retention of all 2-imidazoline drugs could be increased (Fig. 11). Yet, the plot of k'-values versus SOS concentration showed no pronounced levelling-off effect in the concentration range tested (0–35 mM). This can be explained by the rather low methanol content of the eluent (50% v/v). Indeed, in ion-pair chromatography, retention depends mainly on the amount of ion-pairing agent (SOS) adsorbed onto the stationary phase surface, which in turn is related to the mobile phase concentration of SOS and to the organic modifier concentration [21]. Since low methanol concentrations allow the adsorption of more ion-pair agent at the stationary phase surface, a curvature as shown in Fig. 11 is obtained. The expected levelling-off effects [16] will be observed only at SOS concentrations greater than 35 mM SOS.

In order to rationally select the conditions for separating all the 2-imidazolines studied, some basic performance parameters were calculated. A pronounced effect on the selectivity (α-values) was observed upon changing the SOS content of the eluent (Fig. 12). In the absence of SOS, α is highest for the stronger retained compounds (OXY/ANT, XYL/OXY) and the peak pairs TET/TOL and COU/TRA and lowest for the less retained compounds (NAP/TET, TRA/NAP) and the peak pair ANT/COU. Upon increasing the SOS concentration (0–10 mM), α rapidly decreased or increased, whereas at higher SOS concentrations α became almost independent in all instances, except for the peak pair NAP/TET where a continuous increase of α was observed.

The addition of SOS to the eluent greatly improved the efficiency of the separation (Fig. 13). In particular at low SOS concentrations (<10 mM) a dramatic increase of the plate count was observed. However, little change was seen above this concentration.

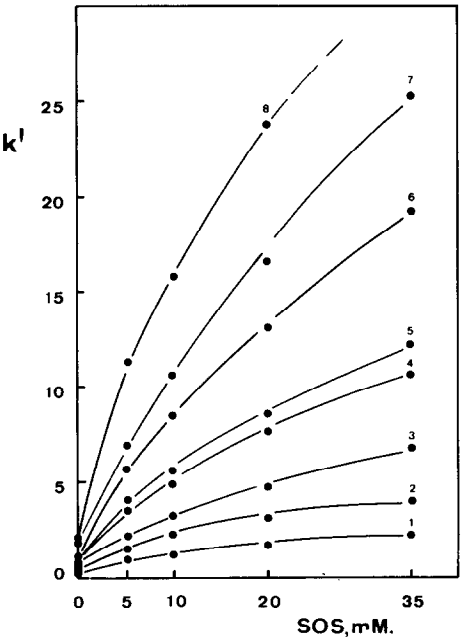


Figure 11
Change in retention as a function of the SOS concentration in the eluent. Eluent: methanol–water (50:50, v/v), containing 5 mM DMOA, adjusted to pH = 3.0; chromatographic conditions: as in Fig. 1; key: as in Fig. 1.

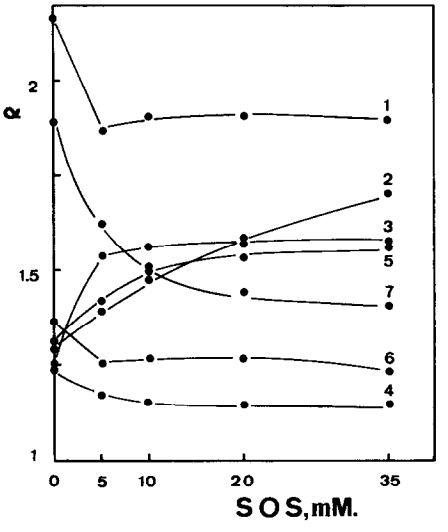


Figure 12
Influence of the SOS concentration on the selectivity (α values) of the separation. Eluent and chromatographic conditions: as in Fig. 11. Key: (1) TET/TOL; (2) NAP/TET; (3) TRA/NAP; (4) COU/TRA; (5) ANT/COU; (6) OXY/ANT; (7) XYL/OXY.

The gain in efficiency was accompanied by reduced peak tailing and again this peak shape improvement was particularly pronounced at low SOS concentration (<10 mM). Higher concentrations only slightly improved peak symmetry (Fig. 14). Nevertheless, the solutes could be eluted as very symmetrical peaks (Asf <1.5) with 35 mM SOS in the eluent. In one case (OXY), peak fronting occurred. The resolution calculated from the data shown in Figs 11, 12 and 13 is depicted in Fig. 15. Initially, the resolution was strongly enhanced, followed by a smaller increase at higher SOS concentrations. The

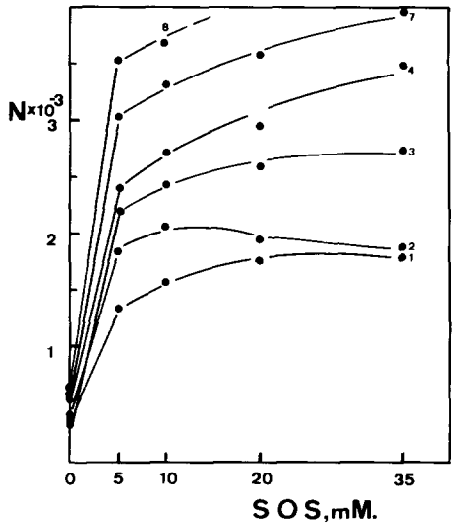


Figure 13
Influence of the SOS concentration on the efficiency (plate count) of the separation. Eluent, chromatographic conditions and key: as in Fig. 11.

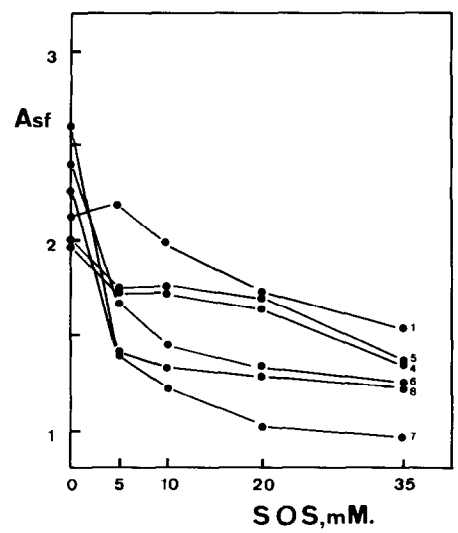


Figure 14
Dependence of the peak symmetry on the SOS concentration in the eluent. Eluent, chromatographic conditions and key: as in Fig. 11.

strongest retained compounds (XYL/OXY and OXY/ANT) showed a decrease in resolution at SOS concentrations greater than 20 mM.

The described chromatographic optimization allowed the selection of optimum chromatographic conditions resulting in excellent separations of all of the 2-imidazolines studied. Figure 16 shows the great separation power of the described eluent approach providing baseline separations (or a near-baseline separation for the peak pair COU/TRA) in a reasonable elution time.

Analysis of pharmaceutical preparations

To illustrate the applicability of RP-IPC using eluents containing both positively and negatively charged modifiers, some pharmaceutical preparations containing 2-imidazoline drugs were analysed. The percentage of organic modifier, the DMOA

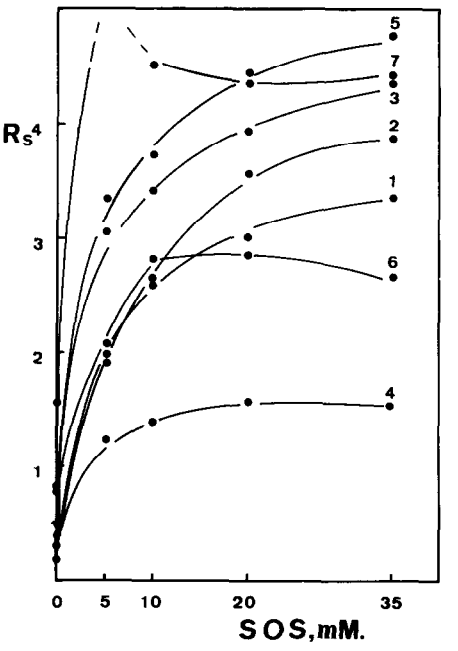


Figure 15
Resolution as a function of the SOS content of the eluent. Eluent, chromatographic conditions and key: as in Fig. 12.

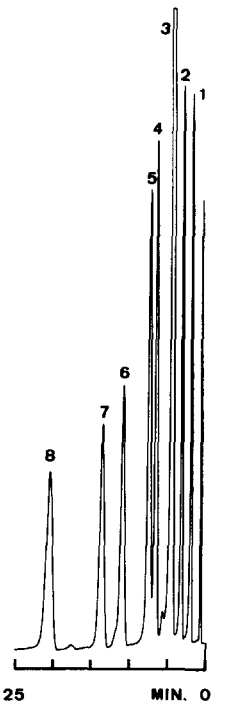


Figure 16
Representative chromatogram of the optimized separation of the 2-imidazolines investigated. Eluent: methanol–water (50:50, v/v), containing 10 mM SOS and 5 mM DMOA. Chromatographic conditions and key: as in Fig. 11.

concentration and the SOS concentration were adjusted to optimize the separation in terms of analysis time and baseline separation of the active drug (and the internal standard) from possible degradation products and from other ingredients present in the pharmaceutical formulation. Figure 17 shows a chromatogram for the analysis of coumazoline in Galenyl® (Labaz), a commercially available nasal solution, after the addition of 0.05 ml 1 N NaOH and subsequent sample preparation. Another example is given in Fig. 18, in which the commercially available nasal solution Neocor-Tyzine®

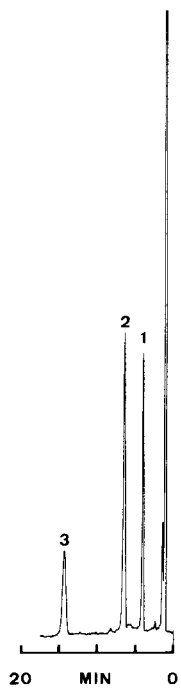


Figure 17
Representative chromatogram for the stability-indicating determination of coumazoline in Galenyl® nasal solution. Eluent: methanol–water (40:60, v/v), containing 20 mM SOS and 20 mM DMOA (pH = 3.0); chromatographic conditions: as in Fig. 1; key: (1) naphazoline hydrochloride (internal standard); (2) coumazoline; (3) degradation product of coumazoline.

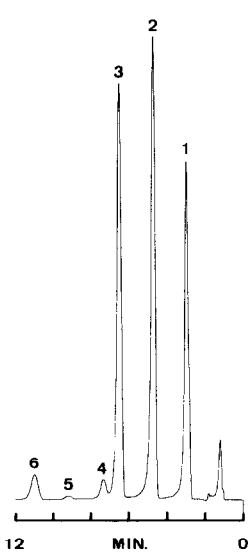


Figure 18
Representative chromatogram for the stability-indicating determination of tetryzoline hydrochloride in Neocor-Tyzine® nasal solution. Eluent: methanol–water (40:60, v/v), containing 20 mM SOS and 5 mM DMOA (pH = 3.0); chromatographic conditions: as in Fig. 1; key: (1) *p*-hydroxybenzoic acid; (2) tetrahydrozoline hydrochloride; (3) nipagine; (4) prednisolone; (5) and (6) unidentified degradation products.

(Roerig) was treated with 1 N NaOH and the resulting degradation mixture chromatographed.

In both cases, the system precision, determined on 10 consecutive standard responses and expressed as the relative standard deviation, was excellent (RSD $< \pm 1\%$) demonstrating that a convenient, specific stability-indicating determination could be employed. More details concerning the quantitative analysis of this class of compounds will be presented in a forthcoming paper.

Conclusions

The 2-imidazoline drugs can be completely separated as symmetrical bands (Asf about 1.5) with sufficient efficiency (N around 2500) using acidic aqueous methanolic eluents containing both DMOA and SOS on a common reversed-phase support. The combination of both modifiers is essential for satisfactory chromatography in terms of analysis time and resolution.

The control of separation is based upon three major eluent parameters, i.e. the DMOA concentration, the SOS concentration and the volume fraction of water in the eluent. The column temperature, the apparent pH of the eluent and the addition of salts to the eluent, only slightly affected the performance of the separation. Addition of DMOA to the eluent reduced retention, improved the peak shape, sometimes changed the selectivities and decreased the resolution whereas the efficiency was not affected. Incorporating SOS in the eluent increased retention, improved the peak shape and the efficiency, affected the selectivity and increased resolution. Increasing ϕ resulted in stronger retention, different selectivities and enhanced resolution. The efficiency was only slightly increased whereas the peak symmetry was not affected.

The observed elution characteristics are, at least partially, in agreement with the hypothesis of the ion-interaction model.

The reproducibility and high separation power of the presented chromatographic method allow adequate quantitative analysis of the 2-imidazoline drugs in pharmaceuticals.

References

[1] G. Andermans and A. Richard, *J. Chromatogr.* **298**, 189–192 (1984).
[2] J. Bauer and S. Krogh, *J. Pharm. Sci.* **72** (11), 1347–1349 (1983).
[3] L. R. Snyder and J. J. Kirkland, in *Introduction to Modern Liquid Chromatography*, 2nd edn, pp. 12–37. Interscience, New York (1979).
[4] J. S. Kiel, S. L. Morgan and R. K. Abramson, *J. Chromatogr.* **320**, 313–323 (1985).
[5] N. J. Eggers and C. M. Saint-Joly, *J. Liq. Chromatogr.* **6** (11), 1955–1967 (1983).
[6] R. Gill, S. P. Alexander and A. C. Moffat, *J. Chromatogr.* **247**, 39–45 (1982).
[7] B.-A. Persson, S.-O. Jansson, M.-L. Johansson and P.-O. Lagerström, *J. Chromatogr.* **316**, 291–300 (1984).
[8] A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.* **189**, 299–316 (1980).
[9] K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.* **151**, 299–310 (1978).
[10] B. A. Bidlingmeyer, *Liq. Chromatogr. Magazine* **1** (6), 344–349 (1984).
[11] B. A. Bidlingmeyer, S. N. Deming, W. P. Price, Jr, B. Sachok and M. Petrusek, *J. Chromatogr.* **186**, 419–434 (1979).
[12] K. E. By, C. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.* **203**, 65–84 (1981).
[13] B. L. Karger, J. N. LePage and N. Tanaka, in *High-Performance Liquid Chromatography — Advances and Perspectives* (C. Horváth, Ed.), Vol. I, pp. 149–161. Academic Press, New York (1980).
[14] J. C. Kraak, in *Techniques in Liquid Chromatography* (C. F. Simpson, Ed.), pp. 310–315. Wiley, New York (1982).

[15] W. R. Melander and C. Horváth, in *Ion-Pair Chromatography* (M. T. W. Hearn, Ed.), pp. 27–77. Marcel Dekker, New York (1985).
[16] B. A. Bidlingmeyer, *J. Chromatogr. Sci.* **18**, 525–539 (1980).
[17] E. Tomlinson and C. M. Riley, in *Ion-Pair Chromatography* (M. T. W. Hearn, Ed.), pp. 91–92. Marcel Dekker, New York (1985).
[18] A. Nahum and C. Horváth, *J. Chromatogr.* **203**, 53–63 (1981).
[19] G. B. Cox, in *Techniques in Liquid Chromatography* (C. F. Simpson, Ed.), pp. 65–67. Wiley, New York (1982).
[20] J. A. Molica and H. C. Stober, in *GLC and HPLC determination of Therapeutic Agents* (K. Tsuji, Ed.), Part 2, pp. 723–728. Marcel Dekker, New York (1978).
[21] R. P. W. Scott and P. Kucera, *J. Chromatogr* **175**, 51–63 (1979).

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