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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF BASIC COMPOUNDS ON NON-MODIFIED SILICA GEL AND ALUMINIUM OXIDE WITH AQUEOUS SOLVENT MIXTURES

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## SUMMARY

A comparison is made between the use of aluminium oxide and non-modified silica gel as cation-exchange materials for the separation of basic drugs (amines) with aqueous solvent mixtures. The retention behaviour of the amines is studied and appears to be controlled predominantly by the pH and the concentration and nature of the modifier; the nature and concentration of the competing ions and the buffer components of the mobile phase also exert some influence on the retention. Preparations with imidazoline and tetracycline derivatives have been analysed as examples of the application of these ion-exchange systems on non-modified silica gel and aluminium oxide in the analysis of pharmaceutical formulations.

## INTRODUCTION

The analysis of basic compounds and quaternary ammonium compounds by reversed-phase high-performance liquid chromatography (HPLC), with aqueous mobile phases on chemically modified silica gel, is often hindered by the occurrence of badly tailing peaks. Poorly reversible interaction of the solute with residual free silanol groups and/or complexation with metal ions in the stationary phase may be the cause of this undesirable behaviour.

Paired-ion chromatography[1,2] of basic solutes increases the retention, but it frequently does not yield improved peak-shapes. The use of tailing-suppressing agents (*e.g.* N,N-dimethyloctylamine)[3] often results in improved peak-shapes but shortens the column life. Other possibilities are ion-suppression techniques[4], adsorption paired-ion chromatography with non-aqueous solvents[5,6], ion-exchange chromatography on special materials[7], normal-phase chromatography[8,9], isolation of the solute by paired-ion extraction, followed by HPLC analysis of the ion-pair[10], and the use of hydrophobic materials other than silica gel-based materials, such as PRP-1[11].

However, these systems are sometimes still inadequate. An interesting development is the application of non-modified polar stationary phases, such as aluminium oxide and silica gel, in combination with aqueous solvent mixtures for the sep-

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aration of amines. Bare silica gel can be used in adsorption paired-ion systems with aqueous buffers (pH *ca.* 2), containing competing cations and counter-anions[12,13]. To achieve acceptable $k'$ values, small concentrations (1%) of a polar organic solvent are added to the mobile phase. However, not only does this cause a reduction in retention of the solute, but the separation power of the system is reduced as well.

Hansen and Helboe[14,15] used long-chain quaternary ammonium compounds (*e.g.* cetyltrimethylammonium bromide) to modify the silica gel surface. They concluded that under these circumstances non-ionic compounds are separated mainly by hydrophobic interactions, anionic solutes are separated by a combination of hydrophobic and paired-ion effects, and, except for the separation of some quaternary ammonium compounds, the ion-exchange mechanism plays only a minor role in the retention of protonated amines[16]. The disadvantage of this type of system is the long equilibration time[17]. The retention mechanism is rather complex, but can be simplified by using symmetrical quaternary ammonium compounds. These cationic species are adsorbed to only a minor extent on the silica gel surface[18]. The retention of cations is then predominantly governed by ion exchange.

After its introduction by Jane[19], Wheals[20] and Law *et al.*[21] have described the analysis of amines on non-modified silica gel with aqueous ammonium nitrate–methanol systems as the eluent. Despite the high pH values (*ca.* 10) used in these studies, the majority of the amines are at least partially protonated and are separated predominantly by ion-exchange interactions. To avoid the dissolution of the silica gel material, Bidlingmeyer and co-workers[22,23] used bare silica gel with aqueous ammonium phosphate (pH 7.8)–acetonitrile mixtures as the mobile phase; they pointed out that hydrophobic interactions also must be effective. The disadvantages of these systems are in some cases the extreme pH values of the mobile phase[12,13,20,21], the poor peak symmetry[12,13] and the limited discriminating power (selectivity) of the systems[20–23].

To achieve a more flexible system with good discriminating power, Laurent *et al.*[24–26] used aluminium oxide instead of silica gel; aluminium oxide is stable over a wider pH range (2–12), and it can be used as a cation-exchange material by the addition of competing ions to a mobile phase consisting of an aqueous buffer and an organic modifier (acetonitrile or methanol). Excellent chromatographic behaviour of protonated amines has been achieved with this type of ion-exchange system. Although the discriminating power of the aluminium oxide system is comparable with that of silica gel-based systems, the retention behaviour of organic cations is more complex and, therefore, not easily predictable. This can be explained by the substantial number of parameters that influence the retention and by the pronounced amphoteric character of aluminium oxide[24]. An advantage of the available silica gel over aluminium oxide is the much higher specific surface area: *ca.* 500 $m^2/g$ for neutral silica gel and 70 $m^2/g$ for basic aluminium oxide resulting in a higher capacity of silica gel[27]. However, this amphoteric character and consequently the zero point of charge of aluminium oxide which depends on the type of buffer anions introduces an additional possibility, in comparison with silica gel; the chromatography of anions as well as cations[24,25].

Recently, Flanagan and Jane[28] reported the successful analysis of several amines and quaternary ammonium compounds in a comparable silica gel-based system with semi-aqueous, buffered eluents. It would seem that aluminium oxide and

silica gel, in combination with semi-aqueous, buffered mobile phases containing competing ions, offer good possibilities for the analysis of basic compounds and quaternary ammonium compounds.

In the present investigation, the retention behaviour of amines and quaternary ammonium ions was studied as a function of the type and concentration of organic modifier, the pH, and the type and concentration of the competing ion and buffer components in the mobile phase, with both silica gel and aluminium oxide as the stationary phase.

The usefulness of these ion-exchange systems with silica gel as stationary phase is demonstrated by the analysis of imidazoline and tetracycline derivatives, which are troublesome compounds to chromatograph in reversed-phase systems on modified silica gel. The analysis of pharmaceutical formulations is discussed, with xylometazoline-containing nasal drops and formulations containing tetracyclines as examples.

## EXPERIMENTAL

### *Apparatus*

The chromatographic system consisted of a Model 6000 A solvent delivery system and a U6K injector (Waters Assoc., Milford, MA, U.S.A.), equipped with three on-line detection systems, a Model 440 differential UV absorbance detector, operating at 254 nm, and a Model R 401 refractive index detector (both from Waters Assoc.). The third detection system, installed for measuring pH shifts in the eluent, was a Radiometer G 299A capillary glass pH electrode (Radiometer, Copenhagen, Denmark), which was connected to the outlet of the refractive index detector. The capillary glass electrode and the reference electrode were connected to a Radiometer PHM 64 pH meter[29].

Stainless-steel columns of standard dimensions (30 cm × 3.9 mm I.D.), packed with LiChrosorb SI-60, particle size 10 $\mu$m, or with LiChrosorb Alox T, particle size 5 $\mu$m (both from Merck, Darmstadt, F.R.G.), were used. The eluent solutions were ultrasonically deaerated; a flow-rate of 1 ml/min was maintained. Chromatography was performed at ambient temperature. The retention times were measured with a SP-4000 central processor (Spectra Physics, Santa Clara, CA, U.S.A.).

### *Chemicals*

2-Propanol, methanol (pro analysis) and tetramethylammonium bromide (TMABr) were obtained from Merck, tetraethylammonium bromide (TEABr) and tetrabutylammonium bromide (TBABr) were supplied by Fluka (Buchs, Switzerland). Disodium hydrogen citrate, disodium hydrogen phosphate, and sodium acetate were purchased from Brocacef (Maarssen, The Netherlands). Vibramycin capsules, terramycin capsules and terramycin ointment from Pfizer (New York, U.S.A.) and doxycycline hydrochloride from Sigma (St. Louis, MO, U.S.A.) were used in the analysis of tetracycline derivatives. Otrivin from Ciba (Arnhem, The Netherlands), naphazoline nitrate and antazoline hydrochloride from Nogepha (Alkmaar, The Netherlands) and xylometazoline hydrochloride from Sigma were used in the analysis of imidazoline derivatives. Adriamycin was supplied by Farmitalia (Milan, Italy). Water was purified by deionization. The other compounds used in this study came from various commercial sources or were obtained as gifts; they were used without

further purification. The test compounds used in the chromatographic studies are numbered and are mentioned in the legends of the figures.

*Column packing and equilibration*

The analytical columns were slurry-packed with a Shandon HPLC packing pump (Shandon, Zeist, The Netherlands) at a pressure of *ca.* 50.000 kPa. Before use the silica gel columns were heated and washed with eluent 1 [60% (w/w) aqueous methanol containing 0.004 *m* TMABr and 0.004 *m* disodium citrate (pH 5.9)] for 12 h at a flow-rate of 0.5 ml/min at 50°C. The aluminium oxide material was decanted several times with the slurry liquid (methanol) before packing the column, and the silica gel columns were packed with a mixture of equal volumes of 2-propanol and methanol as the slurry liquid.

To equilibrate the silica gel system, 25–50 ml of the mobile phase were passed through the system until the signals of the three on-line detectors were stable and a constant capacity factor was obtained[30]. The equilibration volume for the aluminium oxide system was *ca.* 1000 ml when equilibration was performed with the mobile phase. Instead of this rather time-consuming procedure, equilibration at a certain pH was achieved by eluting 100 ml of a concentrated solution of the competing- and counter-ions (10-fold excess), followed by 20 ml of the appropriate eluent[26]. The retention time of an unretained compound was determined by injection of a solution of 10 mg/ml toluene in the mobile phase.

*Determination of pH and dissociation constants*

The pH measurements were performed with a standard pH glass electrode. All p$K_a$ values were determined by titration at 20 ± 1°C of 0.01 *m* solutions, using the equipment described. If the measurements were performed in aqueous organic solvent mixtures, the pH and p$K_a$ were denoted as apparent pH (pH′) and apparent p$K_a$ (p$K_a$′), respectively.

## RESULTS AND DISCUSSION

Silica gel can be used as a cation-exchange material at medium to high pH values[19–22]. The protonated amines (analytes) and the ions in the mobile phase (*e.g.* $H^+$, $K^+$, $Na^+$, $TMA^+$, $TEA^+$, $TBA^+$) compete for the ion-exchange sites of the silica gel. Apart from the type and concentration of the competing ions, the degree of dissociation of the silanol functions, the degree of protonation of the amines, the organic co-solvent, and the ionic strength of the solvent will influence the retention of the solutes. Ion exchange is not necessarily the only mechanism contributing to the retention of the amines; hydrophobic and hydrogen-bonding mechanisms can also take part[30,31].

Aluminium oxide shows anion-exchange properties at pH values below the zero point of charge (ZPC) and cation-exchange behaviour above this pH value[24,25]. This means that for the analysis of positively charged compounds the pH of the mobile phase must be higher than the ZPC (*ca.* 3.5 for a citrate buffer).

For silica gel the situation is different[27,32]. This material shows (weak) cation-exchange properties, even at pH values as low as 2[33]. Because this cation-exchange capacity falls sharply with decreasing pH, two quaternary ammonium com-

pounds were chromatographed with a series of eluents differing only in pH, in order to establish the ion-exchange behaviour of the silica gel material in this system (Fig. 1). The pH of the eluents was regulated with 70% perchloric acid, and the sodium concentration was kept constant. TBABr, a symmetrical quaternary ammonium compound, was chosen as the competing ion. A citrate buffer was chosen to allow a large pH range. Obviously (Fig. 1), below a pH′ value of *ca.* 4 the cation-exchange capacity of the silica gel column is very limited. The difference in $k'$ values between methylatropine sulphate[7] and benzalkonium chloride[22] at pH 2 can possibly be explained by hydrogen-bonding interactions of the solute ions with the silica gel surface[31]; these interactions are possibly of greater importance than the hydrophobic interactions with the siloxane functions on the silica surface[16,22,34]. The variable ratio for compounds 7 and 22 in Fig. 1 suggests that the charge of the stationary phase is not the only retention-determining mechanism. The dissociated as well as the undissociated silanol groups can take part in the binding of the cations[30].

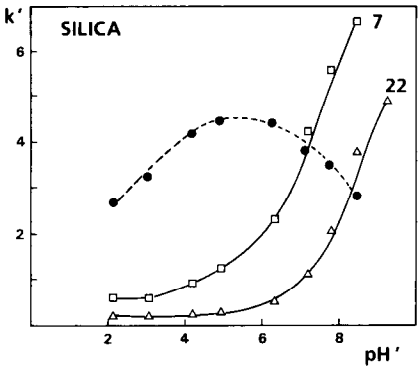


Fig. 1. Effect of pH′ on the retention of quaternary amines methylatropine sulphate (7) and benzalkonium chloride (22). Stationary phase, LiChrosorb Si-60; mobile phase, methanol–water (60:40, w/w) containing 0.004 *m* TBABr and 0.004 *m* trisodium citrate. The dotted line represents the $k'_7/k'_{22}$ ratio.

In the silica gel systems the influence of the temperature was investigated in the range 25–55°C and was found to be negligible (results not shown), although the ion-exchange model predicts a slight decrease in $k'$ at higher temperatures[35].

Chromatograms of some test compounds, obtained with the aluminium oxide and the silica gel systems, are shown in Fig. 2. Sharp peaks with good peak symmetry could be achieved with both systems.

*Effect of pH*

Fig. 3 shows the retention behaviour of six test compounds with different $pK_a$ values on silica gel, with three eluents that differ only in pH′ from eluent 1 (see Experimental). The ionic strength of the solvents was kept constant. When a phosphate buffer, with a different buffer capacity in comparable pH regions, was used instead of a citrate buffer in the silica gel system, the same effects were measured. For compound 5 (a quaternary ammonium cation) and compound 8 ($pK'_a$ 10.3), which are completely protonated over the entire pH range studied, the $k'$ values

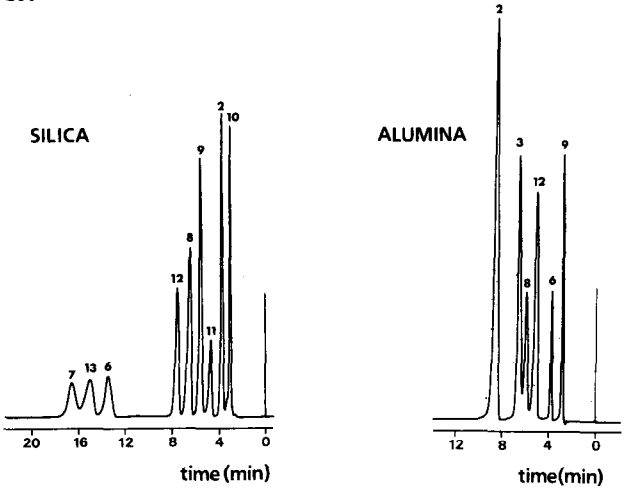


Fig. 2. Chromatograms of amines on LiChrosorb Si-60 or LiChrosorb Alox T. Mobile phase, methanol–water (60:40, w/w) containing 0.004 *m* TMABr and 0.004 *m* disodium citrate (pH 5.9). Peaks: 2 = narcotine, 3 = scopolamine, 6 = atropine, 7 = methylatropine sulphate, 8 = xylometazoline, 9 = dansylcadaverine, 10 = dansylamide, 11 = adriamycin, 12 = naphazoline and 13 = codeine.

appear to increase linearly with pH′. The enhanced retention at higher pH′ values is caused by a higher degree of ionization of the silanol functions, resulting in an increased negative charge of the column. For compounds 4 ($pK'_a$ 7.2), 6 ($pK'_a$ 8.9) and 13 ($pK'_a$ 7.5) the increase of the capacity factor is less than linear with pH′ because the degree of protonation of these compounds decreases at the higher pH′. The retention of compound 1 ($pK'_a$ 5.6) declines with increasing pH′ due to the low $pK'_a$ value of this drug. The peak-shape of the analysed amines depends on the pH′ of the eluent. At pH′ values above 8 the peak symmetry is slightly impaired.

With aluminium oxide the situation is different (Fig. 3). For compounds 1, 4,

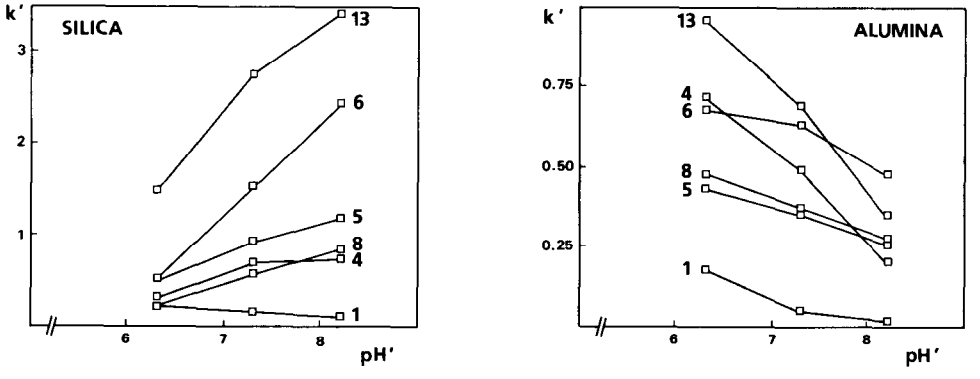


Fig. 3. Effect of mobile phase pH′ on *k*′. Stationary phase, LiChrosorb Si-60 or LiChrosorb Alox T; mobile phase, methanol–water (50:50, w/w) containing 0.002 *m* disodium citrate, 0.016 *m* sodium perchlorate and 0.01 *m* TMABr. Keys: 1 = papaverine, 4 = apomorphine, 5 = butylscopolamine bromide, 6 = atropine, 8 = xylometazoline and 13 = codeine.

6, and 13 the decrease in retention with increasing pH can be at least partly attributed to the reduced degree of protonation of these compounds at higher pH'. However, the retention of compound 8 (completely protonated over the entire pH' range) and compound 5 (quaternary ammonium compound) also decreases with increasing pH. A possible explanation is the occurrence of an increase of the ZPC value of the column, owing to the change in charge of the anionic constituent of the buffer, resulting in a decrease of the negative charge of the column.

Characteristic of both systems is that neutral molecules and anions are hardly retained, which is in agreement with the assumption that the most important interactions in these types of aluminium oxide and silica gel systems are electrostatic in nature[28].

*Effect of competing ions*

The influence of different competing ions has been determined, with the silica gel system as well as with the aluminium oxide system, by chromatography of butylscopolamine bromide and some protonated amines. Fig. 4 shows the $k'$ values of the test compounds, obtained with an eluent containing a 0.01 *m* concentration of TMABr, TEABr or TBABr. The general conclusion for both systems is that with increasing chain length of the competing ion the capacity factor of the solute becomes larger. Larger competing ions are more easily displaced by the solute ions than smaller competing ions, including the hydrated sodium and potassium ions[36,37]. This can be explained by the greater distance between the charges of the larger cations and the ion-exchange sites and the enhanced solvation of larger organic competing ions, resulting in weaker interactions with the stationary phase.

When the ionic strength of the solution is enhanced by addition of different concentrations of the salt of a competing ion (sodium chloride, sodium perchlorate or sodium sulphate) to the mobile phase, the retentions of the test solutes were found to decrease, in the silica gel system as well as in the aluminium oxide system, owing to increased competition of the competing ions for the ion-exchange sites of the stationary phase (Fig. 5). The effects due to the competing ions confirm that the retention is largely controlled by electrostatic interactions[37,38]. Except for a change

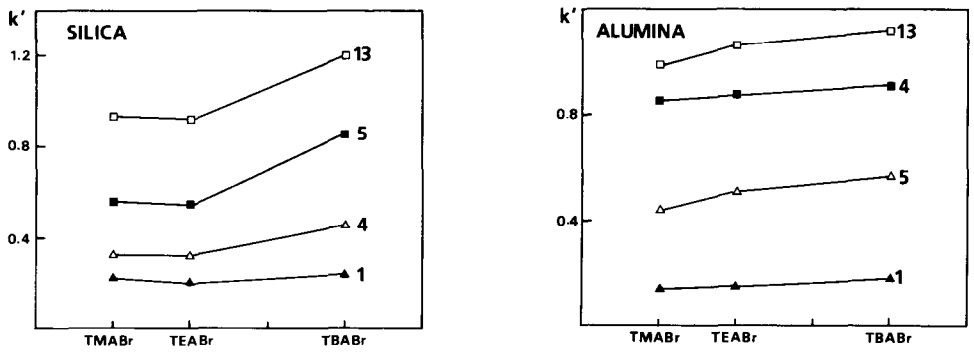


Fig. 4. Effect of competing ions on $k'$. Stationary phase, LiChrosorb Si-60 or LiChrosorb Alox T; mobile phase, methanol–water (50:50, w/w) containing 0.01 *m* TMABr, TEABr or TBABr and 0.01 *m* disodium citrate (pH 5.1). Keys: 1 = papaverine, 4 = apomorphine, 5 = butylscopolamine bromide and 13 = codeine.

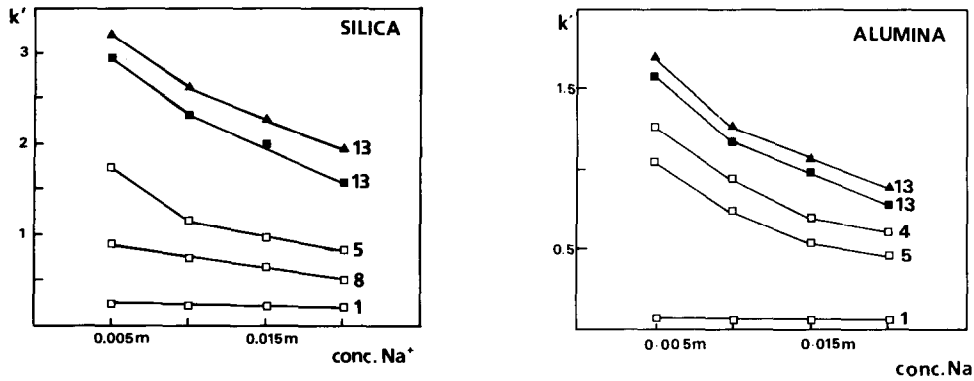


Fig. 5. Effect of ionic strength and counter ions on $k'$. Stationary phase, LiChrosorb Si-60 or LiChrosorb Alox T; mobile phase, methanol–water (50:50, w/w) containing 0.005 *m* TMABr, 0.001 *m* disodium citrate and 0.005–0.02 *m* sodium salt (pH 5.5). Keys: 1 = papaverine, 4 = apomorphine, 5 = butylscopolamine bromide, 8 = xylometazoline and 13 = codeine; (□), sodium chloride, sodium perchlorate and sodium sulphate; (▲), sodium chloride and sodium perchlorate; (■), sodium sulphate.

in retention, the addition of electrolytes strongly influences the peak-shape of the analytes. At very low ionic strengths the peak-shape deteriorates.

*Effect of counter ions*

The effect of different concentrations of counter ions is also shown in Fig. 5. The curves obtained with eluents containing sodium chloride or sodium perchlorate were identical for all solutes. For most compounds (codeine excluded) the sodium sulphate curve, with the same sodium concentration as in the sodium chloride and sodium perchlorate solutions, coincides with the chloride and perchlorate curves. The deviating behaviour of the sodium sulphate curve of codeine must be a specific effect of codeine. In the series of twelve test amines, codeine was the only exception.

It can be concluded that the nature of the counter ions hardly influences $k'$ in either system, and that the influence of the added salt can be attributed to a competing effect of the positive ion[17,37–39]. Because a half molal solution of sodium sulphate has an ionic strength different from a one molal solution of sodium chloride or sodium perchlorate, and the curves of $k'$ *versus* the salt concentration are nevertheless identical, the nature and concentration of the competing ions, not the ionic strength, must be responsible for the observed effect on $k'$.

If salts of weak acids are used the effect on $k'$ in the silica gel and aluminium oxide systems is different. After correction for differences in pH and ionic strength, no difference in $k'$ was observed in silica gel systems. With aluminium oxide, however, a shift in $k'$ was observed caused by a change in the ZPC value, different buffer anions, of the stationary phase as discussed by Laurent *et al.*[40].

*Effect of co-solvent*

The selectivity of ion-exchange chromatography on aluminium oxide or silica gel can be improved by the addition of organic solvents to the aqueous mobile phase. In Fig. 6 the effect of co-solvent addition is plotted against $k'$ with silica gel as the

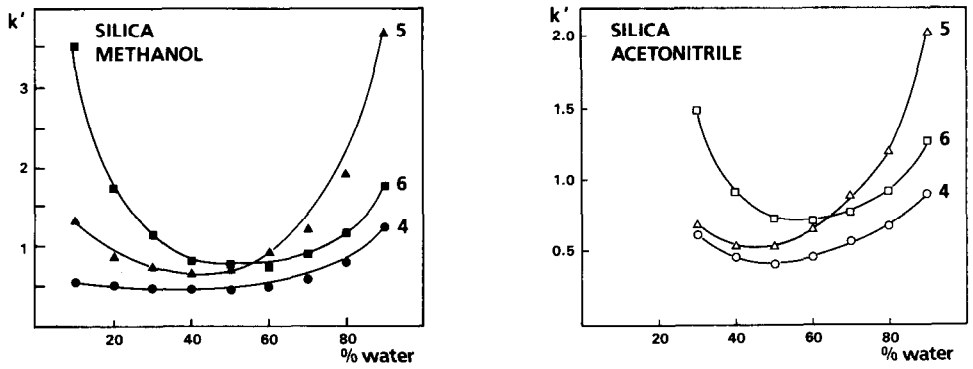


Fig. 6. Effect of co-solvent on $k'$. Stationary phase, LiChrosorb Si-60; mobile phase: 10–90% (w/w) methanol or 10–70% (w/w) acetonitrile in water containing 0.0065 *m* disodium citrate and 0.0065 *m* TMABr (pH 4.9). Keys: 4 = apomorphine, 5 = butylscopolamine bromide and 6 = atropine.

stationary phase. For all of the chromatographed test amines a minimum in the curve was observed at *ca.* 50% water, if methanol or acetonitrile was used as the co-solvent. The exact position of the minimum depends on the degree of protonation and on the polarity of the solute[41].

In reversed-phase chromatography, capacity factors are sensitive to changes in the surface tension of the eluent. If there is more water in the eluent, then the dielectric constant is higher and the surface tension greater. This facilitates hydrophobic interaction with the stationary phase, resulting in higher retention times[42]. The log $k'$ values of all the test amines were found to increase linearly with the water content at volume fractions of 60% and higher. This behaviour, the same as in reversed-phase chromatography, may be explained by the presence of siloxane groups on the silica gel surface[22]. Thus, in the water-rich part of the curves, hydrophobic expulsion possibly overrules the ion-exchange effects.

In the other part of the curve, at high co-solvent concentrations, the increase of the capacity factors can be explained by the increased solvation of the organic competing ions compared with the solute ions. A counteracting effect is the increase of pH′ at higher co-solvent fractions, which causes a decrease in the degree of ionization of the solute. This latter effect explains the behaviour of compound 4, apomorphine ($pK'_a$ 7.2), as shown in Fig. 6. Concentrations of acetonitrile above 70% were not investigated, because of solubility problems with the inorganic salts in these eluents.

Although the first additions of water to the ionic–methanolic eluents in this study were found to cause changes in the elution order of the analytes and a remarkable decrease in the retention of some amines, these effects were not observed in the study carried out by Flanagan and Jane[28]. In agreement with these authors was the observed decrease in peak symmetry with a substantial increase of the water fraction in the eluent.

When aluminium oxide was used as the stationary phase and methanol as the co-solvent, the observed effects were different (Fig. 7). For most compounds, $k'$ was found to decrease at higher water concentrations in the eluent. Laurent *et al.*[25] ex-

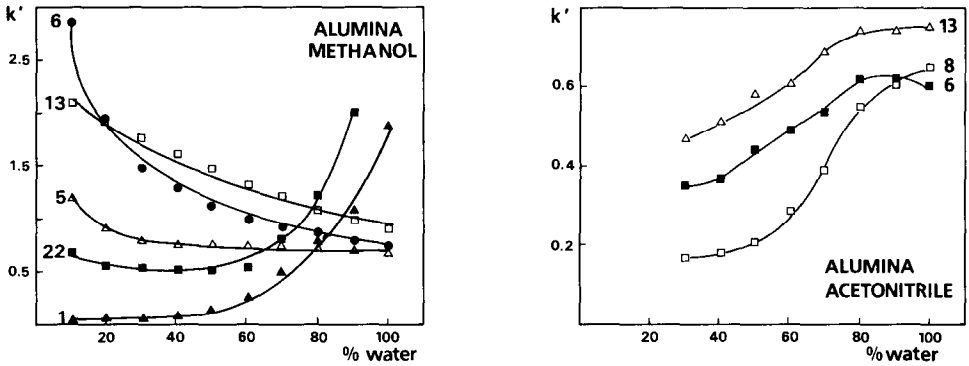


Fig. 7. Effect of co-solvent on $k'$. Stationary phase, LiChrosorb Alox T; mobile phase: 0–90% (w/w) methanol or 0–70% (w/w) acetonitrile in water containing 0.0065 *m* disodium citrate and 0.0065 *m* TMABr (pH 4.9). Keys: 1 = papaverine, 5 = butylscopolamine bromide, 6 = atropine, 8 = xylometazoline, 13 = codeine and 22 = benzalkonium chloride.

plain this by assuming that the solvation of the competing ion decreases with increasing water content. The behaviour of papaverine (1) is the result of the incomplete protonation of this compound in mobile phases with a water content of 60% or more.

In the case of benzalkonium chloride (22) an increase in $k'$ was observed at higher water concentrations, which is the opposite of the behaviour of another quaternary ammonium compound (5) and the results of amines on aluminium oxide, obtained by Laurent *et al.*[25]. This might mean that the solvation of benzalkonium chloride is reduced in water-rich solutions sooner than that of the organic competing ion.

For the silica gel system the methanol and acetonitrile curves were similar, but for the aluminium oxide system the acetonitrile curve showed a slight increase in retention for most compounds with increasing water content, whereas the opposite was observed with methanol. A more general treatment of the effect of co-solvent in the aluminium oxide system has been provided by Laurent *et al.*[26].

Figs. 6 and 7 clearly show that in the aluminium oxide system the addition of a co-solvent is necessary to achieve resolution of the different solutes, whereas in the silica gel system resolution can be achieved even with water-rich eluents. However, the addition of a co-solvent can improve the separation between certain solutes. In general, the shape of the chromatographic peaks was better in the case of eluents rich in co-solvent.

*Applications in pharmaceutical preparations*

The ion-exchange silica gel system was used for the analysis of tetracycline and imidazoline derivatives in some pharmaceutical formulations. Tetracycline derivatives form strong complexes with several metal ions and are unstable in solution. It is therefore desirable to analyse the parent compounds simultaneously with the degradation products. The retention times of seven tetracycline derivatives and three of the degradation products are summarized in Table I. Evidently, tetracycline drugs

TABLE I

CAPACITY FACTORS OF TETRACYCLINE DERIVATIVES WITH THE ION-EXCHANGE–SILICA SYSTEM

Stationary phase: LiChrosorb Si-60. Mobile phase: methanol–water 5:95 (w/w) with 0.0013 *m* disodium citrate, 0.001 *m* TMABr, 0.0011 *m* citric acid and 0.008 *m* EDTA.

| | $k'$* |
|---|---|
| Chlortetracycline | 1.43 |
| Demethylchlortetracycline | 1.12 |
| Doxycycline | 0.56 |
| Oxytetracycline | 0.39 |
| Rolitetracycline | 3.23 (0.90, 0.63, 1.58) |
| Rondomycin | 0.49 |
| Tetracycline | 1.56 (0.88, 0.64) |
| Anhydrotetracycline | 1.91 (1.23) |
| Epi-anhydrotetracycline | 1.22 (1.89) |
| Epi-tetracycline | 0.88 (1.57) |

* The numbers between brackets are the $k'$ values of accompanying (degradation) products.

can be chromatographed with this system, and the parent compounds can be separated from the degradation products. The addition of 0.008 *m* EDTA to the eluent was found to improve the peak symmetry.

To investigate the usefulness of the system for the determination of tetracyclines in pharmaceutical formulations, an oxytetracycline dihydrate containing ointment (terramycin) was analysed (Table II). An aliquot of the ointment was dissolved in petroleum ether and extracted with the same volume of 70% ethanol. After dilution with methanol to a concentration of 0.1 mg/ml, 10 $\mu$l of this solution were injected into the chromatographic system. The results of the analysis of capsules containing

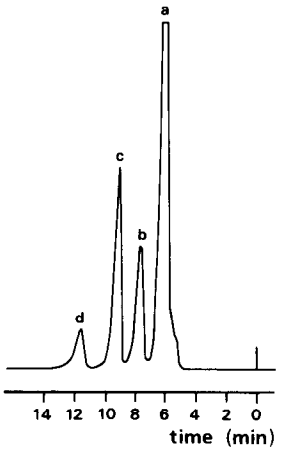


Fig. 8. Chromatogram of the analysis of xylometazoline hydrochloride in nasal drops. Stationary phase, LiChrosorb Si-60; mobile phase, methanol–water (60:40, w/w) containing 0.004 *m* TBABr and 0.004 *m* disodium citrate (pH′ 6.0). Peaks: a = Thiomersal and inorganic salts, b = benzalkonium chloride, c = xylometazoline and d = domiphen bromide.

TABLE II

IMIDAZOLINE AND TETRACYCLINE DERIVATIVES ANALYZED WITH THE ION EXCHANGE–SILICA SYSTEM

| *Drug* | *Pharmaceutical preparation* | *Calibration range (mg/ml)* | *Calibration line (± S.D.)* | *r (n)** | *Recovery (n)* ± S.D. (%)* |
|---|---|---|---|---|---|
| Doxycycline | Capsules | 0.05 – 0.5 | $y = 5 (\pm 1) + 383 (\pm 7)x$ | 0.997 (11) | 104.0 ± 1.7 (4) |
| Oxytetracycline | Ointment | 0.05 – 0.5 | $y = 12 (\pm 2) + 529 (\pm 13)x$ | 0.994 (12) | 99.8 ± 1.5 (4) |
| Demethylchlor-tetracycline | Capsules | 0.05 – 0.5 | $y = 9 (\pm 3) + 189 (\pm 5)x$ | 0.994 (11) | |
| Xylometazoline | Nasal drops | 0.1 – 1.0 | $y = 3 (\pm 2) + 202 (\pm 4)x$ | 0.998 ( 7) | 99.4 ± 1.3 (7) |
| Antazoline | Nasal drops | 0.1 – 1.0 | $y = 13 (\pm 1) + 222 (\pm 2)x$ | 0.999 ( 7) | 98.9 ± 0.5 (4) |
| Naphazoline | | 0.02 – 0.1 | $y = 6 (\pm 2) + 1910 (\pm 36)x$ | 0.999 ( 7) | 103.2 ± 2.2 (4) |

* *n* is number of determinations.

doxycycline hydrochloride or demethylchlortetracycline hydrochloride are also shown in Table II. Before chromatography the contents of the capsules were extracted with methanol and diluted with the eluent. The chromatographic system for the analysis of the capsules is shown in Table I.

A second application is the analysis of imidazoline derivatives for which the reversed-phase HPLC analysis is complicated[43]. Table II summarizes the results of the analysis of nasal drops containing xylometazoline hydrochloride or antazoline hydrochloride and naphazoline nitrate. The chromatogram obtained from the xylometazoline hydrochloride-containing nasal drops (Otrivin) is shown in Fig. 8; the other components of the nasal drops are: thiomersal, inorganic salts, benzalkonium chloride, and domiphen bromide, which were all separated from xylometazoline. In the drops containing antazoline and naphazoline the concentration of antazoline is twenty times higher than that of naphazoline. Nevertheless, both drugs could be quantified in one HPLC run [60% (w/w) aqueous methanol containing 0.004 *m* TBABr and 0.002 *m* disodium citrate (pH 5.7)].

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