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Reversed-phase ion-pair liquid chromatography of some quaternary ammonium drugs*

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Abstract: The resolving power of a reversed-phase liquid chromatographic method that makes use of a mobile phase system with two counter-ions of opposite charge (*N,N*-dimethyloctylamine and sodium octanesulphonate) for the separation of quaternary ammonium drugs, is compared with that obtained using more traditional ion-pairing systems. Efficient, selective and well-resolved separations could only be obtained by the combined effects of the eluent modifiers.

Keywords: *Reversed-phase ion-pair liquid chromatography; quaternary ammonium drugs; separation performance; eluent modifiers; simultaneous effect of amine and alkylsulphonate.*

Introduction

The resolving power of a reversed-phase high-performance liquid chromatographic (RP–HPLC) method on hydrophobic silica-based stationary phases for basic compounds [including quaternary ammonium drugs (QAD)] is dependent upon the type and concentration of modifiers incorporated into the organo-aqueous mobile phase. These modifiers are numerous; with organic amines, alkylsulphonates and inorganic salts being the most commonly used. They are added primarily to reduce interactions of the analytes with accessible residual silanol groups, which otherwise would result in tailing, poorly resolved and long retained peaks [1–3]. All of these eluent modifiers have been studied extensively and have been proved successful in solving numerous separation problems [4–6].

The separation of QAD using the above modifiers however is less well documented since these systems are often inadequate for the resolution of complex mixtures of QAD and because the liquid chromatographic separation of QAD is rather difficult due to their polarity and strong interactions with residual silanol groups at the surface of the hydrophobic stationary phase. A liquid chromatographic method, based on the combined effects of an amine [*N,N*-dimethyloctylamine (DMOA)] and an alkyl-sulphonate [sodium octanesulphonate (SOS)] has been developed to overcome such

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problems. This methodology has previously been applied to the separation and determination of 2-imidazoline drugs [7, 8] and quaternary ammonium drugs [9, 10]. Furthermore reproducibility in terms of retention dependence on the residual silanol groups in connection with column ageing also has been studied [11].

The present paper describes a comparison of the separations of QAD obtained with such combined modifiers (i.e. SOS and DMOA) with those obtained with conventional separation techniques.

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 Chimica (Belgium); 85% orthophosphoric acid and sodium perchlorate from Merck (FRG) and analytical grade methanol from U.C.B. (Belgium). Water was purified by ion-exchange chromatography and subsequent distillation. The quaternary ammonium and other basic drugs were kindly donated by the different manufacturers. These compounds were arylhydroxyacetic acid derived quaternary ammonium and other basic drugs (mepenzolate bromide, pentienate bromide, glycopyrolate, oxyphenonium bromide, oxyphencyclimine), phenylmethane derived QAD (isopropamide iodide, hexocyclium, tiemonium iodide), and xanthene-9-carboxylic acid derived QAD (propantheline bromide).

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 (Alltech-Europe, Eke, Belgium) was used throughout. The mobile phase was pumped at 1 ml min^{-1} 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 equipped with a 10-μl sample loop.

The mobile phases were prepared by dissolving the required amount of the modifier (or combination of modifiers) in about 990 ml of a mixture of methanol–water of the selected solvent strength. The mixture was adjusted to pH = 3.0 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.

Results and Discussion

Evaluation of established methods

The most successful approach to improve peak shape and efficiency of basic compounds in RP–HPLC is the addition of an organic amine to the eluent [12–15]. The main problem with such eluent systems, however, is a decrease in resolving power due to a strong capacity decrease, since the selectivity is usually not affected by varying amine

concentration. For most basic non-quaternary ammonium compounds, adequate selectivity (thus resolution) can be obtained by varying the proportion of organic modifier, the amine concentration and the pH of the eluent. In the case of QAD, chromatographically useful concentrations of the amine, i.e. $\leq$10 mM, still resulted in a parabolic dependence of retention upon increasing the water content in the eluent [11] and the gain in resolution at higher water concentrations ($\phi > 0.5$) was insufficient to resolve QAD mixtures. Further, since QAD are permanently positively charged compounds, their retention unlike other basic drugs, is virtually not affected by changing the pH of the eluent. Hence it was concluded that high-performance separations of QAD on octadecyl supports using an amine as the only modifier could not be achieved.

The addition of salts to aqueous–methanolic eluents has been shown to influence the retention, peak shape and selectivity of amino compounds and QAD in RP–HPLC [16, 17]. The choice of sodium perchlorate as an eluent modifier was prompted by the ability of the perchlorate anion to form a stable ion-pair with QAD [18]. The addition of sodium perchlorate (5–50 mM) to an eluent consisting of methanol–water (65:35), adjusted to pH = 3.0 enabled the elution of QAD but failed to give acceptable resolution. High salt concentrations (>20 mM) provided efficient, symmetrical and less retained peaks but showed poor resolution even in combination with high water concentrations in the eluent. Lower salt concentrations (<20 mM) resulted in less symmetrical peaks, increased retention and again poor resolution.

Another familiar approach to the separation of basic drugs is the incorporation of an alkylsulphonate into the eluent [19–20]. However, when applied to the separation of QAD, this technique also proved to fail. Increasing SOS concentrations up to 20 mM in methanol–water (65:35), pH = 3.0, resulted in the elution of QAD from the column but again resolution remained marginal owing to the occurrence of tailing peaks.

Representative chromatograms for the separation of QAD using DMOA, sodium perchlorate and SOS as the eluent modifiers are shown in Figs 1–3. It is apparent that all these separations lack selectivity. This is consistent with the observation that all these separation modes are basically related to the same retention mechanism, that is ion-exchange of the QAD with the residual silanols on the stationary phase surface. Evidence for this conclusion is obtained by a comparison of the separations obtained using the described eluent modifiers with those obtained using equimolar amounts of sodium chloride and ammonium chloride in the eluent [11]. Since QAD are extremely strongly retained on the support if no modifier is added, the concentration of the modifier must be kept high resulting in less flexible (selective) separation systems with inferior resolution, irrespective of the modifier used.

The combined use of amines and alkylsulphonates

Although for simple separations of QAD, the use of one modifier might be relatively straight forward, it is clear from the foregoing discussion that for complex mixtures, it is essential to have an additional means of optimisation of the mobile phase composition. The simultaneous use of two modifiers of opposite charge (DMOA and SOS) in acidic–aqueous–methanolic eluents was found to be well suited for the separation of complex mixtures of QAD. This eluent technique is essentially based on the capacity increasing effect of SOS with a concomitant enhancement of the overall resolution at relative high DMOA concentrations. Thus, the amine provides the required efficiency and the alkylsulphonate provides sufficient retention. The combined effect provides a flexible, efficient separation system with a high resolving power (Fig. 4).

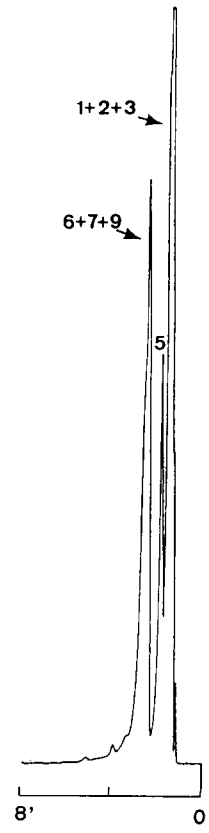

Figure 1
Separation of a mixture of quaternary ammonium drugs using DMOA as eluent modifier. Eluent: methanol–water (65:35, v/v), containing 10 mM DMOA, adjusted to pH = 3.0. Chromatographic conditions: flow rate 1 ml min^{-1}; temperature 25°C. Detection: 220 nm. Column: RSil C 18 5-μm (150 × 4.1 mm i.d.). Key: (1) isopropamide iodide; (2) tiemonium iodide; (3) mepenzolate bromide; (4) pentienate bromide; (5) glycopyrolate; (6) propantheline bromide; (7) oxyphenonium bromide; (8) oxyphencyclimine; (9) hexocyclium.

A systematic evaluation of the investigated eluent parameters led to the following conclusions. Increasing DMOA concentrations reduce retention, improve the peak shape, decrease resolution and affect selectivity but not the efficiency. DMOA concentrations higher than *ca.* 10–20 mM do not give rise to further increases of peak symmetry. The incorporation of SOS in the eluent increases retention, improves the peak shape and the efficiency, affects the selectivity and increases resolution. However, concentrations above *ca.* 20 mM do not further improve the peak shape and only slightly affect the selectivity, peak shape and resolution. The resolution of closely related QAD (for example compounds differing only by a methyl substituent) deteriorates with increasing SOS concentration through a selectivity decrease. Changing the volume fraction of water, ϕ, now showed a linear relationship of log k' versus ϕ, a relationship that is usually obtained in RP–HPLC. Increasing ϕ results in a continuous increase of resolution, although the peak symmetry and the efficiency only increase slightly above water concentrations of *ca.* 50% v/v.

The retention of the solutes appeared to be controlled by a combination of four processes: electrostatic interactions with the adsorbed modifiers, ion-exchange with residual silanols together with hydrophobic and polar non-ionic interactions with the stationary phase. Owing to this complex retention mechanism, unique retention and selectivity changes could be obtained not only towards charged compounds (including acids) but also towards neutral compounds. Such resolving power was demonstrated for

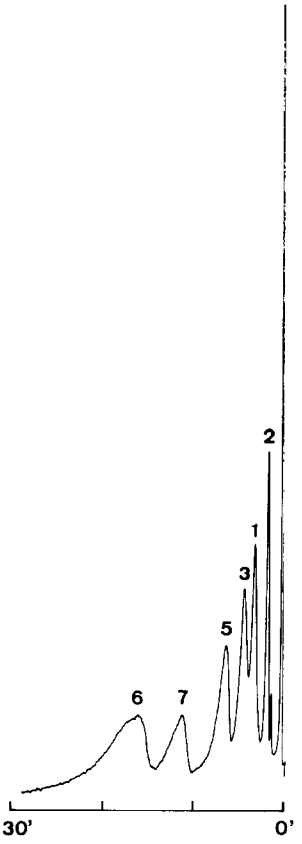

Figure 2
Separation of a mixture of quaternary ammonium drugs using sodium perchlorate as eluent modifier. Eluent: methanol–water (65:35, v/v), containing 20 mM $NaClO_4$, adjusted to pH = 3.0. Chromatographic conditions, Column and Key: see Fig. 1.

the separation of weak analgesic drugs, a miscellaneous class of pharmaceuticals frequently administrated as combined preparations [21].

Apart from a higher resolving power, the developed methodology proved to be far less dependent on the residual and generated silanol groups compared with eluents containing only one modifier, as was demonstrated by on-column silylation experiments with *N*-trimethylsilylimidazole [11].

A mobile phase containing both DMOA and SOS, despite its complexity, is well suited for quantitative analysis if injections are made in solvent of the same strength (methanol–water ratio) as that of the mobile phase. An optimisation strategy for quantitative analysis of QAD in pharmaceuticals was established as follows. Initially the solutes are eluted with moderate water concentrations (*ca.* 50% v/v) and relative high DMOA concentrations (5–20 mM) at an acidic pH. The resolution is subsequently optimised by adding SOS (5–20 mM) to the eluent. Finally the necessary (small) changes of ϕ and/or the DMOA concentration are made, depending on the lipophylic characteristics of the compounds investigated, in order to reduce analysis time without loss of resolution.

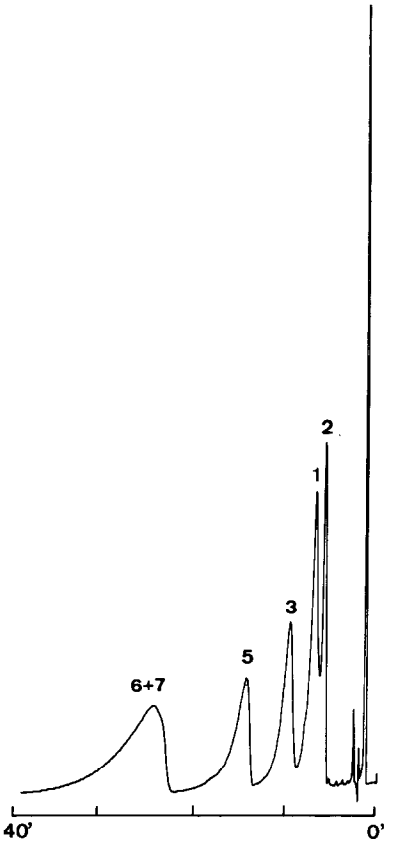

Figure 3
Separation of a mixture of quaternary ammonium drugs using SOS as eluent modifier. Eluent: methanol–water (65:35, v/v), containing 20 mM SOS, adjusted to pH = 3.0. Chromatographic conditions, Column and Key: see Fig. 1.

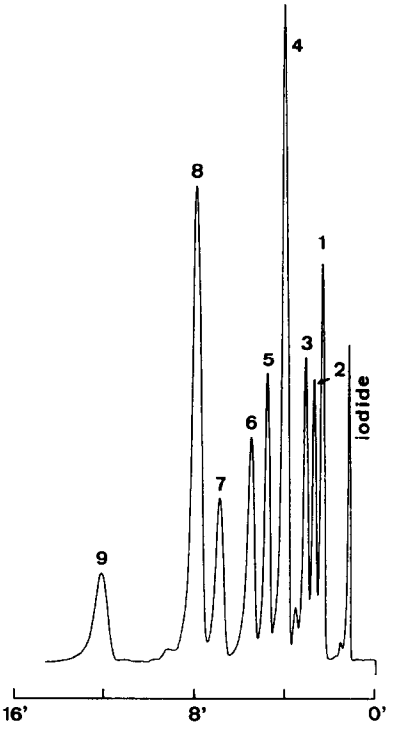

Figure 4
Separation of a mixture of quaternary ammonium drugs using SOS and DMOA as eluent modifiers. Eluent: methanol–water, (65:35, v/v), containing 10 mM DMOA and 20 mM SOS, adjusted to pH = 3.0. Chromatographic conditions, Column and Key: see Fig. 1.

Conclusions

It appears that aqueous–methanolic eluents containing two modifiers (counter-ions) of opposite charge are more suitable for the separation and determination of quaternary ammonium drugs than the commonly used mobile phases employed in reversed-phase ion-pair liquid chromatography.

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