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ANALYSIS OF QUATERNARY AMMONIUM COMPOUNDS AND BASIC DRUGS BASED ON ION-PAIR ADSORPTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A general approach to the ion-pair adsorption high-performance liquid chromatography of basic drugs and quaternary ammonium compounds is described in which suitable counter ions such as Br^- and ClO_4^- are dissolved in the eluent. The columns are packed with silica (*e.g.*, LiChrosorb and LiChrosfer). The systems thus obtained showed high efficiencies and stability in which the capacity ratios were found to be exponentially dependent on the concentration of the counter ion. This indicates that the separation mechanism is dominated by adsorption processes. The degree of retention and the separation order can be varied by the nature and the concentration of the counter ion, the sorbent and the composition of the eluent.

INTRODUCTION

In recent years, ion-pair partition liquid chromatography has been developed into a useful technique for the analysis of drugs and metabolites, in particular by Schill and co-workers[1]. In the beginning, it was customary to coat the support with a hydrophilic liquid containing the counter ion, but more recently reversed-phase chromatography has also been carried out, in which the stationary phase is organic and the eluent is an aqueous solution containing the counter ion[2–4]. The term "soap chromatography" was suggested for systems in which quaternary ammonium detergents were used as the counter ion, in either the reversed-phase or the straight-phase mode[5]. The ion-pair partition systems show good stability and reproducibility if they are carefully thermostated and if the mobile and stationary phases are in equilibrium. Unfortunately, these conditions are not always easy to meet in practice, especially in situations in which system changes are often required (*e.g.*, in systematic toxicological analysis).

We recently introduced straight-phase thin-layer chromatographic(TLC) systems for the separation of quaternary ammonium compounds[6], in which the quaternary cations were converted into uncharged ion pairs based on the reaction

$$Q^+ + X^- \rightleftarrows QX \qquad (1)$$

Inorganic anions, such as Cl^-, Br^-, I^- and ClO_4^-, proved to be suitable counter ions and could be introduced by dissolving the sodium salts in the developing solvent and/or by impregnation of the sorbent prior to development. This approach also proved to be applicable to ionizable cationic substances such as basic drugs[7], according to the equation

$$BH^+ + X^- \rightleftarrows BHX \quad (2)$$

In the present work we have investigated the feasibility of ion-pair adsorption chromatography under high-performance liquid chromatographic (HPLC) conditions by adding an inorganic counter ion to the mobile phase. As HPLC, in contrast to TLC, is carried out under conditions that approach equilibrium situations much better, we also investigated the fundamental character of the ion-pair adsorption processes.

EXPERIMENTAL

Chemicals

Clomipramine (Clo), desmethylclomipramine (Dclo), imipramine (Im) and desipramine (Desi) were used as their hydrochlorides and were gifts from Ciba-Geigy (Basle, Switzerland). Amitriptyline (Ami), nortriptyline (Nor), protriptyline (Prot) and trimipramine (Trim) were purchased as hydrochlorides from Nogepha (Alkmaar, The Netherlands). Doxepine (Dox) was isolated as the base by extraction from Sinequan capsules (Pfizer, Rotterdam, The Netherlands). Thiazinamium methylsulphate (Th), thiazinamium sulphoxide methylsulphate (ThSO) and promethazine (Prom) hydrochloride were gifts from Specia (Paris, France). The substances were used as received and dissolved in the mobile phase in concentrations of 5–15 $\mu g \cdot ml^{-1}$. It could be ascertained that there was no effect on the chromatographic behaviour whether the free bases or the hydrochlorides were applied to the columns. For stability reasons, solutions of the hydrochlorides were preferred.

All other substances and solvents were of analytical-reagent grade, obtained from E. Merck (Darmstadt, G.F.R.) and used without purification.

Liquid chromatography

A Spectra-Physics (Berkeley, Calif., U.S.A.) liquid chromatograph was used with a Model SF 770 variable-wavelength UV detector, which was operated at a wavelength of 250 nm. Injections were made with a Valco high-pressure valve fitted with a 90-μl sample loop.

Stainless-steel columns with a length of 30 or 15 cm and an internal diameter of 0.46 cm were used, packed with silica (LiChrosorb SI-60 and SI-100 and LiChrosfer SI-100, particle size 5 μm, obtained from Merck) by a balanced-density slurry method[8]. Developments were carried out at room temperature at a flow-rate of approximately 1.2 $ml \cdot min^{-1}$.

Mobile phases were solutions of sodium bromide or sodium perchlorate in methanol. These solutions were prepared by sonication, filtered through a Millipore filter to remove any undissolved particles or impurities and finally degassed by sonication immediately before use.

RESULTS AND DISCUSSION

The separation of relatively polar substances such as quaternary ammonium compounds and protonated bases by means of adsorption chromatography is difficult because of the strong interactions of these cationic species with the adsorption sites of the stationary phase. When pure methanol was used as the mobile phase the quaternary compounds could not be eluted at all, whereas the tertiary and secondary amines showed broad, tailing peaks with very long retention times.

The addition of a suitable counter ion at a sufficiently high concentration directly to the mobile phase had a distinct effect. All substances could now be eluted in sharp, symmetrical peaks and in a relatively short period of time. Fig. 1a shows

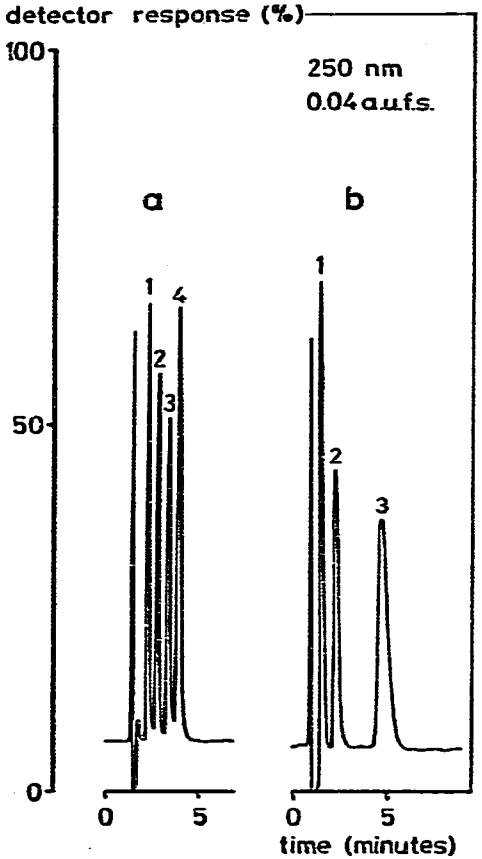


Fig. 1. Ion-pair adsorption HPLC of some basic drugs and quaternary ammonium compounds. (a) Separation of some tricyclic antidepressant drugs. Column, 15 × 0.46 cm, packed with LiChrosorb SI-60, particle size 5 μm. Eluent, 0.06 *M* NaBr in methanol. 1 = Desmethylclomipramine; 2 = trimipramine; 3 = clomipramine; 4 = imipramine. (b) Separation of some quaternary and tertiary phenothiazines. Column, 15 × 0.46 cm, packed with LiChrosfer SI-100, particle size 5 μm. Eluent, 0.1 *M* NaBr in methanol. 1 = Promethazine; 2 = thiazinamium; 3 = thiazinamium sulphoxide.

the separation of a selection of tricyclic antidepressants on LiChrosorb SI-60 with 0.06 *M* sodium bromide in methanol as the mobile phase, the elution order being Dclo, Tri, Clo, Im. It should be observed that under normal adsorption chromatographic conditions using methanol–ammonia (100:1.5) as mobile phase the separation order is Tri, Clo, Im, Dclo[9], whereas a reversed-phase system, LiChrosorb RP-8 with acetonitrile–water (7:3), yields the separation order Dclo, Im + Clo, Tri[10]. Fig. 1b shows the separation of the quaternary ammonium compound Th, its tertiary homologue Prom and its major metabolite ThSO, separated on LiChrosfer SI-100 using 0.1 *M* sodium bromide in methanol as mobile phase. It is remarkable that the highly polar sulphoxide is eluted relatively rapidly and as a symmetrical peak. On LiChrosorb similar results can be obtained except for some tailing of the ThSO peak.

We decided to choose relatively small inorganic halide ions as counter ions because they could be dissolved directly in the mobile phase and because they were known to give stable ion pairs with a large variety of cationic compounds. We also tested some larger organic counter ions but found that the latter are more likely to dominate the chromatographic behaviour of the resulting ion pair rather than the cationic moiety, which is undesirable, of course. Moreover, larger counter ions were found to give interferences due to interactions with the stationary phase. Of the halide ions tested, chloride in methanol gave corrosion of some of the metal parts of the chromatograph, whereas iodide in methanol gave a UV background in the detector and, moreover, occasionally resulted in iodine formation. We therefore preferred bromide or perchlorate as counter ions.

In order to determine the nature of the chromatographic mechanism, we investigated the effects of the counter ion concentration in the mobile phase on the capacity ratio, k'. In straight-phase ion-pair partition chromatography, the capacity ratio of a substance Q can be defined as[1]

$$k'_Q = \frac{1}{K_{ex(Q)}[X^-]} \cdot \frac{V_s}{V_m} \tag{3}$$

where $K_{ex(Q)}$ is the extraction constant of the ion pair QX in the partition system, $[X^-]$ is the equilibrium concentration of the counter ion and V_s and V_m are the volumes of the stationary and the mobile phases, respectively. From this equation it can be seen that in ion-pair partition, k' would be inversely proportional to the concentration of the counter ion.

We assume that in straight-phase ion-pair adsorption chromatography the bases and the quaternary compounds are predominantly adsorbed to the stationary phase in their positively charged form and present in the eluent phase predominantly as neutral ion pairs. Thereby, the free concentration of BH^+ or Q^+ in the eluent phase is determined by the stability constant of eqn. 1 or 2 and by the concentration of X^-. It has been derived for other HPLC adsorption systems that the chromatographic retention behaviour obeys a simple Freundlich-type equation[5,11]. Our straight-phase systems would then have to obey the equation

$$k'_Q = a[X^-]^b \tag{4}$$

where $[X^-]$ is the equilibrium concentration of the counter ion and a and b are

constants, with $a > 0$ and $-1 < b < 0$. A double-logarithmic plot of k' *versus* $[X^-]$ would thus give a straight line.

The occurrence of ion-exchange chromatography as a major mechanism could be excluded as it was found that the nature of the other cation(s) present in the system (Li^+, Na^+, K^+) had no influence on the retention behaviour. Moreover, it could be shown in comparable thin-layer systems that no migration at all occurred of basic substances on sodium phosphate-buffered acidic TLC-plates with methanol as eluent[6].

Table I shows the results of changing the counter ion concentration in a bromide system and Fig. 2 shows the double-logarithmic plots of k' *versus* $[X^-]$. Table II and Fig. 3 show similar results for a perchlorate system, although in Fig. 3 the data for only three cationic substances have been plotted for simplicity reasons. These results indicate that the underlying mechanism of the chromatographic behaviour is indeed adsorption chromatography. We also checked the possible existence of partition chromatography by means of eqn. 3 but the correlations for those data were considerably lower. In the straight-phase ion-pair chromatography of negatively charged species using quaternary detergents as counter ion, Knox and Laird[5], observing in increase in k' with increasing concentration of the counter ion, were unable to determine the underlying principles of chromatography. Mellström and Braithwaite[4], working with perchlorate in a mixed mobile phase containing water, methanol, dichloromethane and diisopropyl ether, found that their results did not obey the partition equation (eqn. 3). However, when we applied eqn. 4 to their data we found that straight lines could be obtained in double-logarithmic plots of k' *versus* $[ClO_4^-]$, indicating the occurrence of adsorption chromatography.

TABLE I

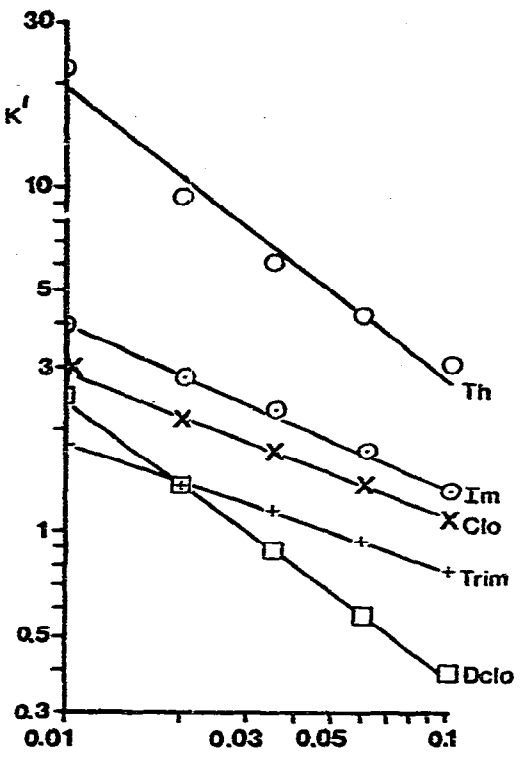
ION-PAIR ADSORPTION HPLC RETENTION DATA (k' VALUES) OF ION PAIRS OF SOME BASIC DRUGS USING Br^- AS COUNTER ION

Column: 15 cm × 0.46 cm I.D., packed with LiChrosorb SI-60, particle size 5 μm. Mobile phase: Sodium bromide in methanol; flow-rate 1.2 ml·min^{-1}.

Cationic drug	*Bromide concentration (M)*					*Curve fit parameters**		
	0.1	*0.06*	*0.035*	*0.02*	*0.01*	*a*	*b*	*r*
Clomipramine	1.03	1.36	1.70	2.08	2.87	0.39	−0.43	0.998
Desmethylclomipramine	0.39	0.57	0.87	1.31	2.47	0.06	−0.80	0.999
Imipramine	1.30	1.69	2.23	2.77	3.92	0.44	−0.47	0.999
Trimipramine	0.73	0.93	1.15	1.31	1.82	0.31	−0.38	0.995
Thiazinamium	3.03	4.19	6.00	9.31	22.2	0.39	−0.85	0.987

* Equation, $k' = a[X^-]^b$; r = correlation coefficient.

Changing the counter ion from bromide to perchlorate resulted in lower k' values for most of the compounds, as can be seen in Tables I and II. However, the reverse was found for the secondary amines Dclo, Desi, and Prot. As lower k' values would be an indication of stronger ion pairs, these observations seem to indicate that perchlorate forms stronger ion pairs with most of the compounds, which is in agreement with observations in solvent extraction studies. So far, we have no



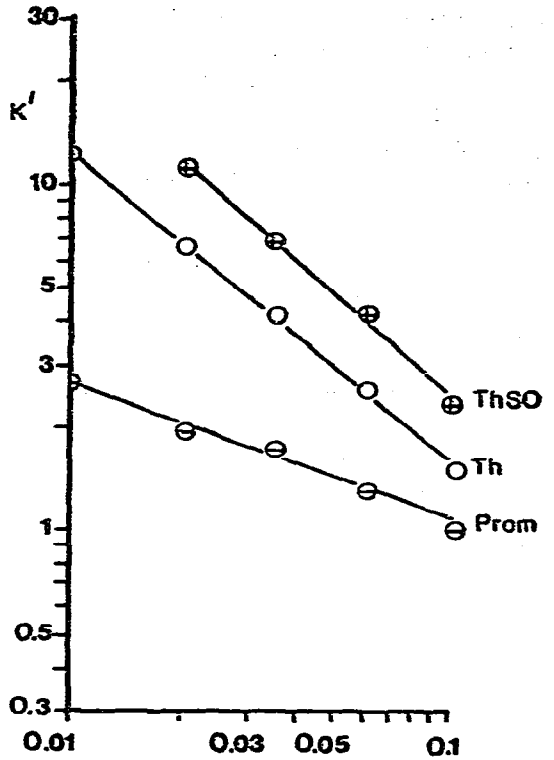


Fig. 2. Dependence of k' on the counter ion concentration in bromide systems (double-logarithmic plot). Column, 15 × 0.46 cm, packed with LiChrosorb SI-60, particle size 5 μm. Eluent, methanol containing different amounts of sodium bromide. Th = thiazinamium; Im = imipramine; Clo = clomipramine; Trim = trimipramine; Dclo = desmethylclomipramine.

Fig. 3. Dependence of k' on the counter ion concentration in perchlorate systems (double-logarithmic plot). Column as in Fig. 2. Eluent, methanol containing different amounts of sodium perchlorate. ThSO = thiazinamium sulphoxide; Th = thiazinamium; Prom = promethazine.

TABLE II

ION-PAIR ADSORPTION HPLC RETENTION DATA (k' VALUES) OF ION PAIRS OF SOME BASIC DRUGS USING ClO_4^- AS COUNTER ION

Column as in Table I. Mobile phase: Sodium perchlorate in methanol; flow-rate 1.2 ml·min^{-1}.

Cationic drug	*Perchlorate concentration (M)*					*Curve fit parameters**		
	0.1	*0.06*	*0.035*	*0.02*	*0.01*	*a*	*b*	*r*
Clomipramine	0.77	1.04	1.54	1.90	2.75	0.22	−0.55	0.995
Desmethylclomipramine	0.38	0.62	0.92	1.44	2.33	0.07	−0.78	0.998
Imipramine	0.85	1.36	1.85	2.39	3.67	0.23	−0.61	0.993
Trimipramine	0.55	0.71	1.08	1.26	2.00	0.15	−0.55	0.993
Thiazinamium	1.48	2.54	4.08	6.42	12.2	0.19	−0.90	0.999
Thiazinamium sulphoxide	2.31	4.15	6.85	11.2	n.d.**	0.26	−0.97	0.998
Promethazine	1.00	1.38	1.72	1.94	2.67	0.42	−0.40	0.988

* Equation $k' = a[X^-]^b$; r = correlation coefficient.

** n.d. = not determined.

explanation for the fact that the opposite would seem to hold for the secondary amines used in this study.

In order to check the possible effects of the molecular structure of a cationic compound on its chromatographic behaviour, we examined some tertiary tricyclics and some corresponding secondary tricyclics. From the results in Table III, it can be seen that there is a major difference between the two groups with regard to the slopes of the straight lines: the a values for the secondary amines are a factor of 4–5 lower than those of their corresponding tertiary analogues. The same trend is observed on other columns (Table I, Fig. 2) and with other counter ions (Table II).

TABLE III

ION-PAIR ADSORPTION HPLC RETENTION DATA (k' VALUES) OF SOME TERTIARY AND SECONDARY TRICYCLIC ANTIDEPRESSANTS USING Br^- AS COUNTER ION

Column: 15 cm × 0.46 cm I.D., packed with LiChrosorb SI-60, particle size 5 μm. Mobile phase: sodium bromide in methanol; flow-rate 1–4 ml·min^{-1}.

Cationic drug	*Bromide concentration (M)*			*Curve fit parameters*[*]		
	0.1	*0.05*	*0.01*	*a*	*b*	*r*
Imipramine	0.98	1.50	3.70	0.26	−0.57	1.000
Clomipramine	0.79	1.10	2.83	0.21	−0.56	0.999
Desmethylimipramine	0.36	0.55	2.11	0.06	−0.78	0.998
Desmethylclomipramine	0.28	0.48	1.75	0.04	−0.80	1.000
Amitriptyline	0.66	1.10	2.24	0.21	−0.52	0.991
Nortriptyline	0.28	0.54	2.02	0.04	−0.85	0.999
Protriptyline	0.28	0.54	2.21	0.04	−0.89	1.000
Doxepine	0.85	1.40	2.75	0.29	−0.49	0.990
Trimipramine	0.56	0.79	1.58	0.20	−0.45	0.999

[*] Equation: $k' = a[X^-]^b$; r = correlation coefficient.

The ion-pair adsorption systems presented here proved to be simple, rapid, versatile and reproducible. The chromatographic efficiency is equivalent to that usually obtainable in normal high-performance adsorption chromatography. Changing from one system to another, regardless of whether they contain a normal mobile phase or a mobile phase with a counter ion added, required an equilibration time of 20 min, after which stable baselines were obtained. Thermostating of the column was not found necessary.

Detrimental effects to either the column packing or the chromatograph and the detector itself were not observed with the bromide and perchlorate systems mentioned. However, it should be observed that when not required for chromatographic work (overnight, weekend) we flushed the system with methanol and left it in methanol to avoid crystallization in the pump, injection valves and other crucial parts. Reequilibration with an ion-pair system did not take longer than 20 min. Under these conditions the system worked properly for more than six months.

Ion-pair adsorption HPLC will be a valuable alternative for those mixtures which are difficult to resolve by other high-performance techniques, for cases in which a different separation mechanism is desired in order to facilitate identification, and for structural studies. Parameters that can be varied to attain optimal conditions are the nature and concentration of the counter ion, the composition of the mobile phase

(nature and ratio of the organic solvents) and the stationary phase. For ionized substances such as quaternary ammonium compounds this technique provides unique possibilites, both qualitatively and quantitatively, as these substances are usually very difficult to handle by other chromatographic techniques.

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