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INFLUENCE OF MOBILE PHASE ADDITIVES IN LIQUID CHROMATOGRAPHY OF PHENOXYPROPANOLAMINES

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SUMMARY

Liquid chromatographic systems for the separation of phenoxypropanolamines were investigated. Bonded phases from different manufacturers were tested for column efficiency and peak symmetry. Phosphate buffer solutions containing acetonitrile were used as mobile phases. The effect of amine modifiers on the chromatographic performance was examined, and it was found that even for columns adapted for the separation of amines there was a significant improvement. Silica as the solid phase was modified by the presence of both hydrophobic ammonium ions and counter-ions in aqueous mobile phases of pH 2. The alkylammonium modifier is enriched as an ion pair on the solid phase, and the retention of hydrophobic compounds is strongly affected by the stationary phase formed. Selectivity changes were observed as the content of modifier was altered. Peak symmetry and efficiency were quite different for some pairs of amines with the same capacity factor, indicating a complex retention mechanism.

INTRODUCTION

Phenoxypropanolamine is a structural feature common to most β-adrenergic blocking drugs, but within this group of substances there is a wide range of hydrophobic properties. Liquid chromatography (LC) has become the method of choice for the separation and determination of these compounds in pharmaceutical analysis[1,2], and it is also an important complement to gas chromatography (GC) for the determination of phenoxypropanolamines in biological samples by use of highly sensitive fluorescence detection[3,4].

In bonded-phase LC, moderately to strongly hydrophobic compounds containing amino functions may create problems in terms of asymmetric peaks and low efficiency. In a series of papers[2,5,6], the retention behaviour of phenoxypropanolamines and the influence of amine modifiers in the mobile phase were thoroughly investigated using a retention model based on two different binding sites. Experiments were also performed where pure silica was used as the solid phase, the properties of which were modified by adsorption of lipophilic ion-pairing components[7].

This paper reports further studies of the influence of modifiers in the mobile

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aqueous phase on the selectivity and chromatographic performance of phenoxypropanolamines in order to optimize the separation. The investigation comprises both bonded phases of different origin and pure silica.

EXPERIMENTAL

Chemicals and reagents

N,N-Dimethyloctylamine (DMOA) was from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and octylsulphate from E. Merck (Darmstadt, F.R.G.). Organic solvents were from E. Merck or Rathburn (Walkerburn, U.K.). Buffer substances were of analytical grade. N,N,N-Trimethyloctylammonium (TMOA) bromide, 3,5-dimethylcyclohexylsulphate (DMCHS) and all drug substances were from the Department of Organic Chemistry, Hässle. The basic structure of phenoxypropanolamines is shown in Fig. 1.

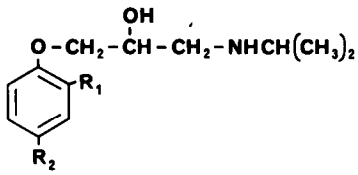

Fig. 1. Basic structure of phenoxypropanolamines (β-adrenergic blocking drugs).

Liquid chromatographic system

Pumps used in the liquid chromatographs were either Altex 110 A, Spectra-Physics 8700 or Waters M-45. The UV detector was either Spectromonitor III or Waters Model 440, operated at 270 or 254 nm. The injection valve was Rheodyne 7010 with a 20-μl loop. Chromatographic columns were packed either by the manufacturer or in the laboratory. The mobile phases were buffered by phosphate to pH 2 (I = 0.1) or pH 3 (I = 0.05). The flow-rate was usually 1 ml/min.

Determination of chromatographic parameters

Measurement of t_0 was performed by injection of a waterrich solvent. The amount of dynamically adsorbed modifier was determined by elution of the modifier and GC analysis. In some instances the results obtained were confirmed by breakthrough analysis with a refractive index detector.

The theoretical plate number was calculated by drawing the perpendicular from the peak maximum and measuring the peak width at 13.5% of the height[8], which also was used for estimating peak symmetry.

RESULTS AND DISCUSSION

Bonded phases

LC separation of amines and quaternary ammonium compounds on bonded phases often requires certain precautions as regards the composition of the mobile phase. Wahlund and Sokolowski showed that there were differences in chromato-

graphic performance between packing materials as well as between the ammonium solutes[9,10]. They also found that the peak shape could be improved significantly by the addition of ammonium modifiers to the mobile phase, although not all of these were equally efficient. A great number of later studies have confirmed their observations[11,12]. One of the objectives of this part of the study was to develop efficient chromatographic systems for the determination of phenoxypropanolamines in low concentrations in biological samples by means of fluorometric detection.

Manufactured columns with 3- and 5-μm particles were tested with different mobile phases in order to determine which systems were preferable. Some of the columns chosen are, according to the manufacturer, adapted for separation of amino compounds. As could be anticipated, most of the columns gave low efficiency and poor peak symmetry with acetonitrile as the only modifier in a mobile phase of phosphate buffer pH 3 (Fig. 2, Table I). Dimethyloctylamine was used in our previous studies[2,5,6] and, as can be seen in Fig. 3, the chromatographic performance was greatly improved by the presence of this amine modifier.

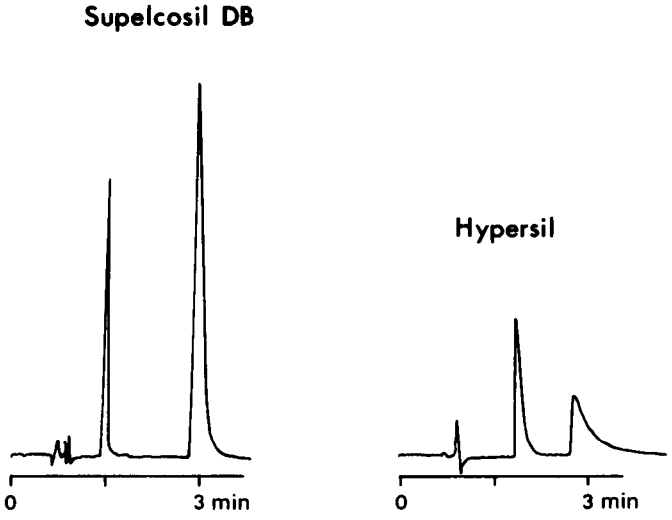

Fig. 2. Separation of phenoxypropanolamines in the absence of amine modifier. Mobile phase, 25% acetonitrile in phosphate buffer (pH 3).

In our study, no significant difference in efficiency was seen between the amine modifiers examined. There was a wide range of capacity factors between the different columns, which may influence the plate count and asymmetry factors. Table I lists the capacity factors, plate numbers and asymmetry factors for one phenoxypropanolamine on the different bonded-phase systems studied. It is obvious that it is possible to generate efficiencies in the range of 40–60,000 plates per metre with these manufacturer-packed columns.

Different chromatographic phenomena may occur in amine-modified separation systems. Impurities in the modifiers may lead to negative peaks or false positive ones by displacement effects, which, however, can also be utilized to increase the plate number drastically, as shown by Westerlund[13]. In certain systems we have

TABLE I

CHROMATOGRAPHIC PERFORMANCE FOR METOPROLOL ON DIFFERENT C_{18} BONDED PHASES

Sample, 20 μl of metoprolol 100 μmol/l in mobile phase.

Column	*Modifier**	*k'*	*N/m* × 10^{-3}	*Asf*
Perkin-Elmer	Ac	15.9	1	4.0
3 μm, 75 × 4.6 mm I.D.	DMOA	1.20	39	1.5
Rainin Microsorb	Ac	5.8	3	5.4
3 μm, 75 × 4.6 mm I.D.	DMOA	0.92	41	1.9
Hypersil**	Ac	1.93	3	8.5
3 μm, 75 × 4.6 mm I.D.	DMOA	0.89	44	1.6
Spherisorb ODS 2	Ac	1.30	21	2.7
5 μm, 150 × 4.6 mm I.D.	DMOA	0.76	42	1.4
LiChrosorb Hibar	Ac	1.15	9	2.0
5 μm, 125 × 4 mm I.D.	DMOA	0.77	34	1.4
Chrompack Microspher	Ac	1.08	15	4.2
3 μm, 100 × 4.6 mm I.D.	DMOA	0.77	54	1.5
Supelcosil DB	Ac	0.85	47	2.8
3 μm, 75 × 4.6 mm I.D.	DMOA	0.73	61	2.0
Waters NOVA-PAK	Ac	0.85	24	2.2
5 μm, 150 × 3.9 mm I.D.	DMOA	0.59	26	1.6
Polygosil	Ac	0.76	34	2.4
5 μm, 150 × 4.6 mm I.D.	DMOA	0.68	45	1.6
Nucleosil**	Ac	0.73	32	1.7
5 μm, 150 × 4.6 mm I.D.	DMOA	0.81	37	1.5

* Ac, phosphate buffer containing 25% acetonitrile; DMOA, phosphate buffer containing 21% acetonitrile and 0.001 *M* dimethyloctylamine.

** Laboratory packed.

observed a narrow linear range in the sample concentration, as well as distortion of sample peaks. Many disturbances observed can probably be attributed to effects in the starting zone, which are conveyed along the column (*cf.* ref. 14). This is a field where further studies are definitely needed. It is evident, however, that the addition of organic ammonium modifiers to the aqueous mobile phase is an appropriate way to improve the chromatographic performance of moderately to highly hydrophobic amines on bonded phases. It is also a means of standardizing the separation system and overcoming the problems of variation in properties of different brands and batches of packing material. In our experience, di- and trimethyl-substituted 1-octylamines are among the most efficient modifiers.

Silica

Pure silica has in the past few years found use as a solid phase in LC of amino compounds with aqueous mobile phases containing ammonium modifiers. One objective has been to develop more reproducible systems than is possible with bonded phases, another to use them in column switching systems.

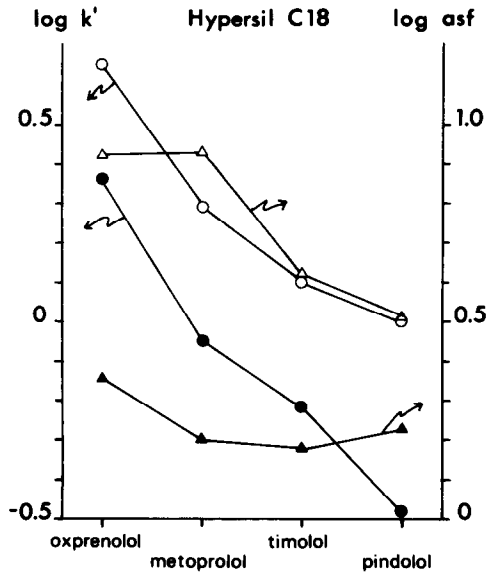

Fig. 3. Chromatographic performance of phenoxypropanolamines on Hypersil C_{18}. Mobile phases, phosphate buffer (pH 3) with 25% acetonitrile (○, △) or 0.001*M* dimethyloctylamine and 21% acetonitrile (●, ▲).

Crommen used mobile phases of pH 2 and 3[15,16], but Ghaemi and Wall[17] and Hansen[18] preferred pH 5–9, where the long-chain ammonium ion (CTMA) is adsorbed on silica substantially by ion exchange. The retention of solutes is then due to hydrophobic interaction with adsorbed CTMA[19]. In a previous paper[7] we showed that by combining a hydrophobic ammonium ion, N,N-dimethyloctylammonium (DMOA) or N,N,N-trimethyloctylammonium (TMOA), and a hydrophobic anion, 3,5-dimethylcyclohexylsulphate (DMCHS), we could change the retention pattern, going from low to high content of modifier. This indicated a change in the retention mechanism, hydrogen bonding being important at low concentrations of modifier and the lipophilic properties being important at high concentrations.

TABLE II

ADSORPTION OF DIMETHYLOCTYLAMINE ON LICHROSORB Si 60 AND LICHROSORB RP-8

Mobile phase: phosphate buffer (pH 2.2), DMOA and 0.05 *M* bromide or 0.01 *M* dimethylcyclohexylsulfate (DMCHS) as counter-ion.

Conc. of DMOA (M)	*Counter-ion*	*Amount of DMOA adsorbed (mmol/g of solid phase)*	
		LiChrosorb Si 60	*LiChrosorb RP-8*
0.001	Bromide	0.0037	0.11
0.05	Bromide	0.024	0.44
0.006	DMCHS	0.024	0.15
0.04	DMCHS	0.99	0.54

It was seen that by combining two hydrophobic ion-pairing ions some kind of stationary phase was built up on the silica with a steep increase at high concentration[7]. The amount of DMOA adsorbed on LiChrosorb Si 60 and LiChrosorb RP-8 is compared in Table II for a low and a high content of DMOA in the mobile phase. The influence of the lipophilic properties of the counter-ion is also demonstrated. With bromide and phosphate as counter-ions the adsorption is much higher on the bonded phase that on silica, as could be expected. By addition of lipophilic DMCHS, the adsorption on silica is greatly increased, and the amount is even higher than for the bonded phase. The effect of the anion on the capacity factor is illustrated in Fig. 4, where data with and without DMCHS for metoprolol and alprenolol at different concentrations of DMOA are given. When no DMCHS is present, the capacity factor decreases with the DMOA content. When DMCHS is present in the mobile phase there is at first a slow decrease in the capacity factor but, at 0.03 *M*, a pronounced increase occurs for the more lipophilic alprenolol. In separate batch extraction studies, no association processes in the aqueous phase, *e.g.* formation of micelles or ion-pairs, were discovered that may explain those drastic effects at high modifier content.

As a follow-up to the promising results with DMOA and TMOA combined with DMCHS, other lipophilic ammonium compounds were examined as modifiers, both symmetrical quaternary ones and homologues of DMOA. Either low solubility

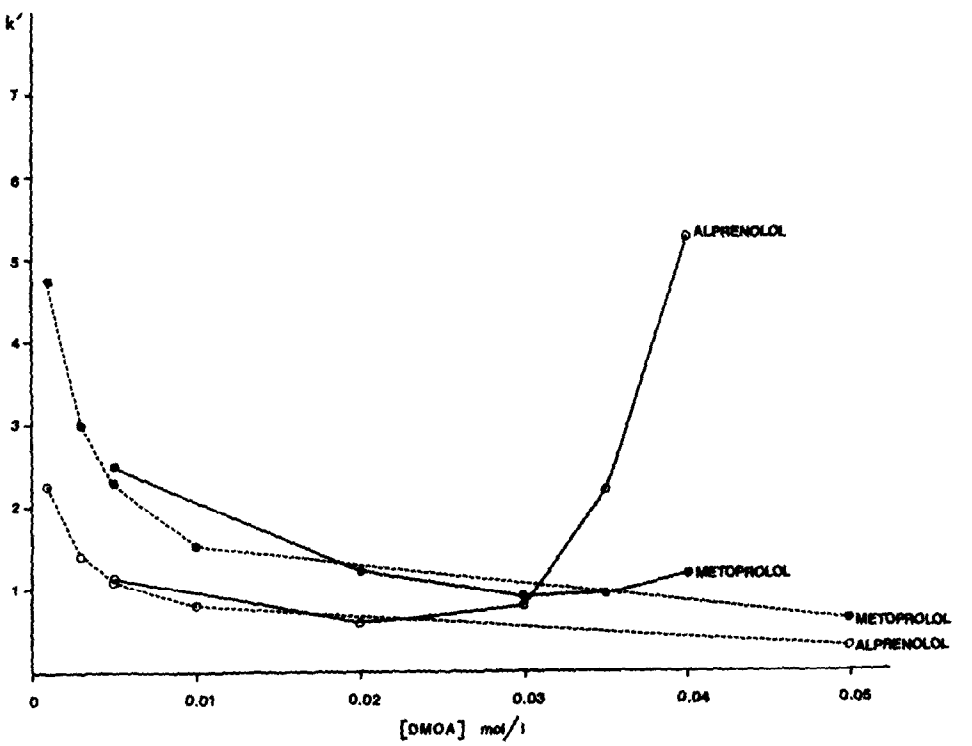

Fig. 4. Influence of dimethyloctylamine (DMOA) on the retention of alprenolol and metoprolol on silica. Solid phases, LiChrosorb Si 60. Mobile phase: ·····, phosphate buffer (pH 2.2); ———, 0.01*M* dimethylcyclohexylsulphate (DMCHS) in phosphate buffer (pH 2.2).

or absence of effect at high concentrations made these less suitable. Exchange of DMCHS for octylsulphate gave, with TMOA, a system with a lower solubility limit but interesting effects, as can be seen in Fig. 5, where the two anions are compared. Both at low concentrations of TMOA and even more so at high concentrations, the capacity factors for propanolol are of greater magnitude. The drastic change in retention properties occurs at 0.003 *M* octylsulphate, as compared with the ten times higher concentration of DMCHS.

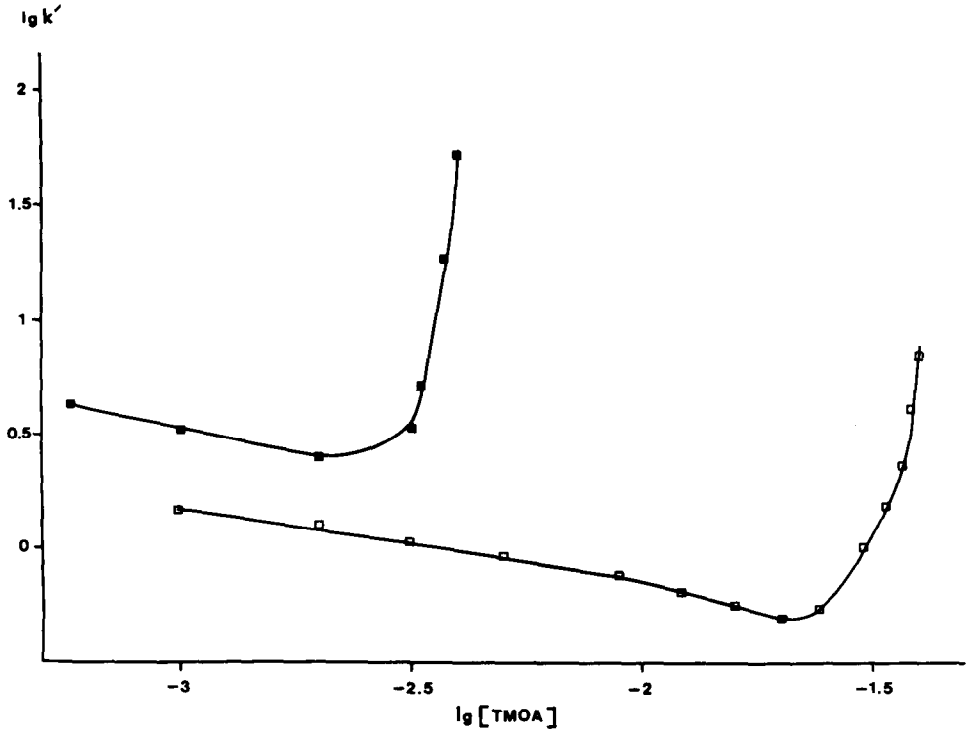

Fig. 5. Influence of trimethyloctylammonium (TMOA) on the retention of propranolol on silica. Solid phases, LiChrosorb Si 60; mobile phase, phosphate buffer (pH 2.2) with 0.008 *M* octylsulphate (■) or 0.01*M* dimethylcyclohexylsulphate (DMCHS) (□).

The increase in retention at high modifier content is less rapid for the less hydrophobic compounds, such as metoprolol and acebutolol. Propranolol is most retained both at low and high TMOA, which was not the case in the previous study[7]. The t_0 value, which is *ca.* 1.5 min and independent of the TMOA concentration below 0.004 *M*, is markedly decreased to 1.16 at 0.008 *M* TMOA with octylsulphate as counter-ion. This significant change coincides probably with the formation of a stationary phase of the ion-pair components.

In the systems with TMOA or DMOA and DMCHS as counter-ions, reasonable column efficiency and good peak symmetry were obtained both at low and high concentrations of the ammonium modifier[7]. In the system with TMOA and octylsulphate, there was a general tendency for peak shapes to be improved by increasing

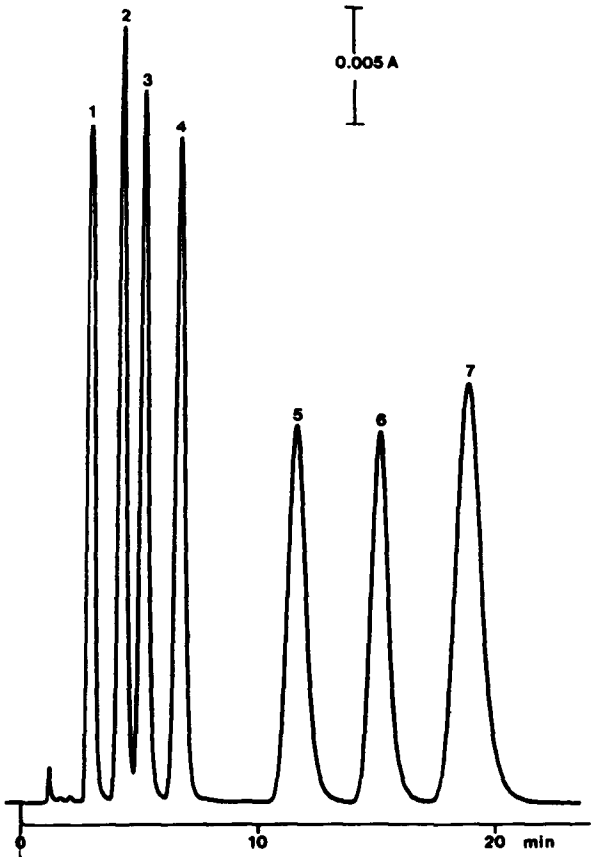

Fig. 6. Separation of phenoxypropanolamines on silica. Solid phase, LiChrosorb Si 60; mobile phase, 0.008 *M* trimethyloctylammonium (TMOA) and 0.008 *M* octylsulphate in phosphate buffer (pH 2.2). Samples: 1 = atenolol; 2 = practolol; 3 = O-desmethylmetoprolol; 4 = prenalterol; 5 = pafenolol; 6 = metoprolol; 7 = acebutolol.

the concentration of TMOA at constant octylsulphate concentration. At high concentrations of TMOA good peak symmetry was achieved (Fig. 6).

It was even more interesting to observe the differences in column efficiency between amines eluted with about the same capacity factors. This is illustrated in Figs. 7 and 8, where chromatograms from two pairs of phenoxypropanolamines are shown. Oxprenolol and alprenolol (Fig. 7) are structurally identical, except for an ether oxygen in the former. They are equally retained in the system, but the number of theoretical plates is four to five times higher for oxprenolol. Acebutolol and propanolol (Fig. 8) are also eluted with about the same retention time but with quite different peak efficiency, *ca.* 15-fold. At present it is hard to imagine the reason for this discrepancy in chromatographic performance. One would expect that the same retention and ability to bind to the stationary phase should give plate numbers of the same magnitude.

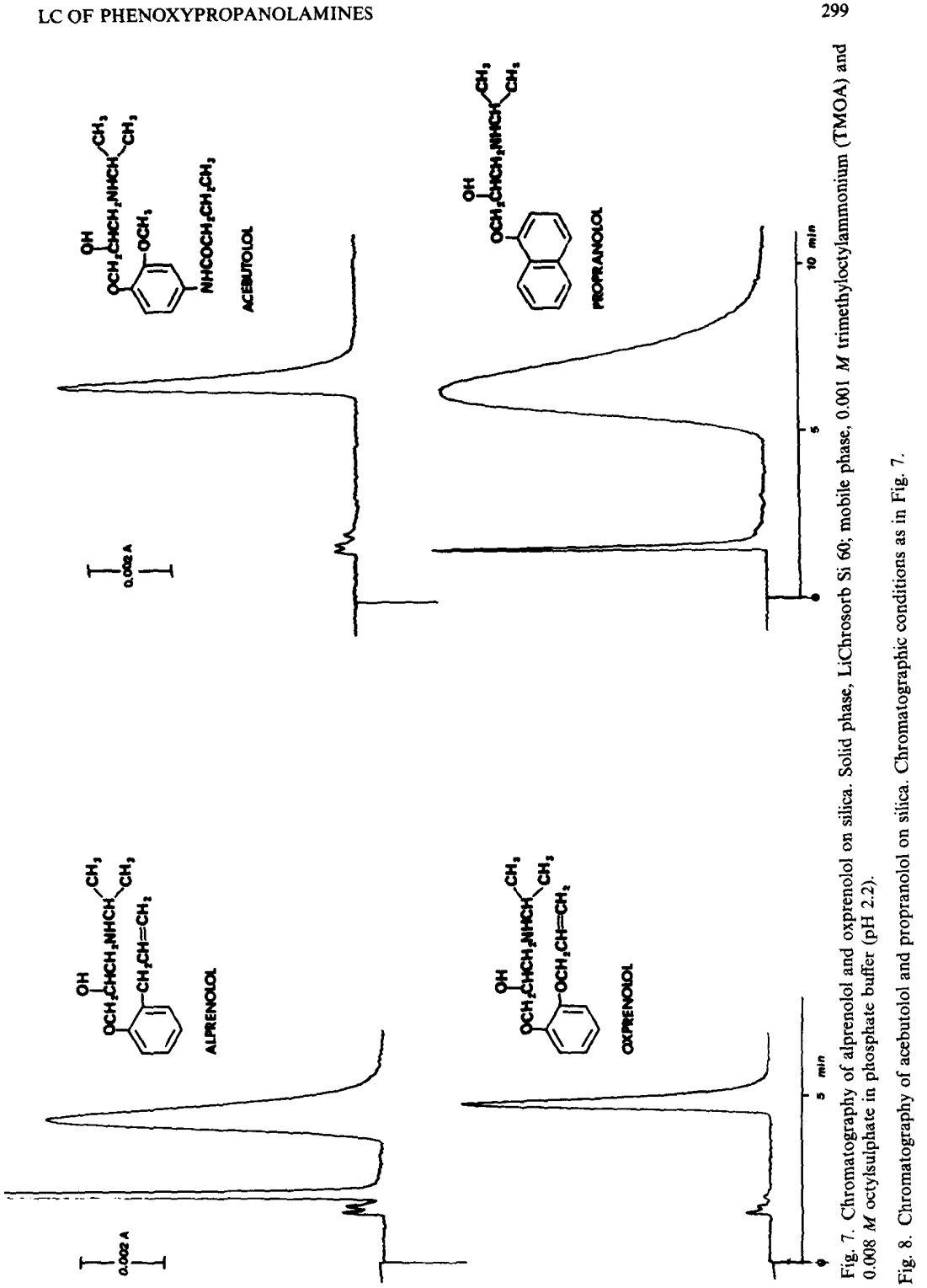

Fig. 7. Chromatography of alprenolol and oxprenolol on silica. Solid phase, LiChrosorb Si 60; mobile phase, 0.001 *M* trimethyloctylammonium (TMOA) and 0.008 *M* octylsulphate in phosphate buffer (pH 2.2).

Fig. 8. Chromatography of acebutolol and propranolol on silica. Chromatographic conditions as in Fig. 7.

As was proposed in the previous paper, hydrogen bonding seems to be involved at low concentrations of TMOA or DMOA, whereas lipophilic interaction as in bonded phases is more important at higher concentrations of modifier. The results here indicate that more than one retention mechanism or kind of binding site is involved, and these then influence the amine solutes to different degrees. These effects will have to be the subjects of further studies.

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