

Characterization of drug purity by liquid chromatography*

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Abstract: Applications of liquid chromatography (LC) in the characterization of drug purity are reviewed. Examples are given of the development of methods for the separation of impurities in acids, amines and neutral drug substances. A variety of methods and techniques are discussed including normal-phase and reversed-phase LC, gradient elution, and multidimensional separation systems.

Keywords: *Drug purity; ion-pair chromatography; ionic modifiers; gradient elution; dynamically modified silica.*

Introduction

During the last ten years column liquid chromatography (LC) has been increasingly used for the characterization of drug purity. In recent times new versions of other chromatographic methods have been developed commercially, such as instrumental thin-layer chromatography and gas chromatography with bonded-phase capillary columns. These techniques may be reasonable alternatives to LC for the determination of impurities in drugs.

However, in most instances LC is still preferred because of its separation power, based on the combination of a wide range of mobile-phase solvents and column packings and its applicability to different sample types, often without pretreatment of the sample. The high accuracy and precision, even using simple single-point calibration, makes LC in many instances superior to other chromatographic techniques. For a detailed discussion, including practical directions for the use of LC in purity determinations of drugs, reference can be made to recent textbooks on LC [1-3].

In the present review different aspects of the characterization of drug purity by liquid chromatography are discussed. Examples are given from work in the author's laboratories on separation systems for acids, amines and neutral drug substances in which normal- and reversed-phase LC, gradient elution and column switching were used.

Column capacity

One limiting factor in liquid chromatographic tests for impurities is the limited capacity of the system. The peak capacity, defined as the number of peaks within a

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certain time period, is infinite in theory but limited in practice. With increasing retention the peak height decreases and as a consequence it is more difficult to detect small amounts of separated impurities. The sample cannot exceed a certain size due to overloading of the stationary phase and there is a linear increase of the plate height with the sample load, an effect that is more pronounced at higher retention times [4].

Normal-phase LC

Most analyses are performed today in the reversed-phase mode since this technique better enables retention to be regulated. However, in the purity determination of a new drug the most useful information is often obtained by a comparison with a normal-phase separation. Normal-phase chromatography may be the only alternative in the determination of lipophilic drugs with low aqueous solubility. Another reason to use an organic solvent as the mobile phase may be improved stability of the sample solution.

Normal-phase LC has been used for the isolation of impurities in aspirin in order to avoid hydrolysis of the drug in the mobile phase [5-7]. An example from such a separation is shown in Fig. 1, where aspirin anhydride and two acids, salicylic acid and acetylsalicylsalicylic acid, are separated from aspirin [5]. The separation system comprised a stationary phase of perchloric acid applied on silica *in situ* and a mobile phase of dioxane and hexane. Acceptable stability was obtained by dissolving aspirin in a mixture of hexane and dioxane from which perchloric acid was excluded. However, when the method was applied to a conventional tablet rapid hydrolysis of aspirin to salicylic acid was observed; magnesium oxide in the formulation had a catalytic effect on

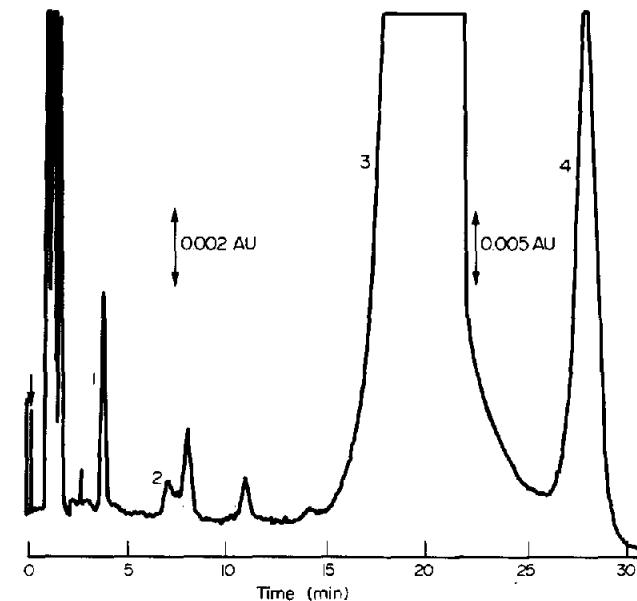


Figure 1

Separation of salicylic acid (1), acetylsalicylic anhydride (2) and acetylsalicylsalicylic acid (4) from aspirin (3). Solid phase: 10- μ m LiChrosorb SI 100. Mobile phase: Dioxane-hexane (8:92, v/v) saturated with 0.1 M HClO_4 . Stationary phase: 0.1 M HClO_4 , 0.2 ml g $^{-1}$ support. Flow rate 1.9 ml min $^{-1}$. Detection at 243 nm. Sample: 0.5 mg of aspirin in dioxane-hexane (3:7, v/v).

the degradation of aspirin. Later, reversed-phase methods were applied for this separation; the same problem of degradation was encountered [8, 9].

Reversed-phase LC

Reversed-phase methods with surface-modified silica as a nonpolar adsorbent are most used today. The manufacturers of bonded silica have tried to improve their products by endcapping in order to decrease the amount of free silanol groups. However, there seem to be different types of sites on the surface of the bonded phase with different binding ability [10, 11]. This influences chromatographic behaviour and may cause asymmetric peaks with low chromatographic efficiencies for certain solutes such as moderately to strongly hydrophobic amines and ammonium compounds, some commonly used drug substances possess these characteristics.

Studies by Wahlund and Sokolowski [12, 13] showed that peak shapes could be improved by addition of amine modifiers to the mobile phase. Later several studies have confirmed their observations [14–17]. Figure 2 shows the effect of addition of such an amine modifier, *N,N*-dimethyloctylamine (DMOA) to the mobile phase. DMOA causes a considerable decrease in the retention of the phenoxypropanolamines owing to competition with these solutes for the retaining phase [15]. The modifier also has a drastic effect on peak tailing by blocking highly active binding sites and, at a concentration of about 1 mM, better peak symmetry is attained.

Chromatographic Studies

After several years' experience with phenoxypropanolamines as beta-adrenoreceptor antagonists, these substances have been used as model compounds in the author's studies on reversed-phase chromatography. The possibility of regulating the retention of

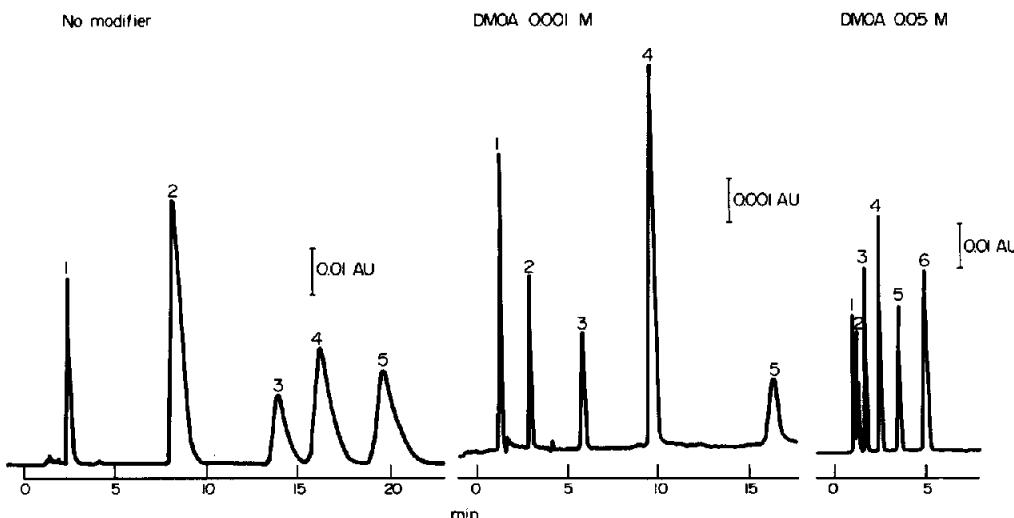


Figure 2
Influence of an ionic modifier (dimethyloctylamine, DMOA) on the separation of phenoxypropanolamines. Solid phase: LiChrosorb RP 8. Mobile phase: DMOA (various concentrations) and 0.11 M 1-pentanol in phosphate buffer (pH 2.2). Sample: 1-phenoxy-3-isopropylamino-2-propanol derivatives.

potential impurities by an ion-pair system is discussed below with examples from studies on pafenolol [18].

Like most other beta-blocking drugs, pafenolol is a secondary amine, which is manufactured from tyramine in three steps [18]. The mobile phase used in the study on pafenolol was based on phosphate buffer (pH = 2.2) with addition of a neutral modifier, pentanol, a cationic modifier DMOA or *N,N,N',N'*-tetramethylenediamine (TMEA), and an ion-pair forming counter-ion, 3,5-dimethylcyclohexylsulphate (DMCHS). Four of the compounds studied are amines and distributed as ion-pairs to the adsorbing phase, LiChrosorb RP 8. The two other compounds, a phenol and a diol, are uncharged at pH 2.2 and are retained as such. A mobile phase with DMCHS (0.001 M) gave a moderate retention of the most hydrophilic amine, tyramine, while pafenolol was too strongly retained. Addition of pentanol and DMOA or TMEA was used to decrease and optimize retention of the hydrophobic solutes.

Effect of neutral modifiers

Increasing the concentration of pentanol in the mobile phase decreases the retention of pafenolol and the related amine to an extent larger than that of the uncharged phenol and diol as illustrated in Fig. 3. This is probably due to effects on adsorption sites with high and selective affinity for amines. However, with higher concentrations of a more strongly adsorbed amine modifier such as DMOA, the competing effect of pentanol is of the same magnitude on all solutes studied since the retention of amine solutes on the strong sites is then prevented by the amine modifier.

The same effect is obtained with more polar neutral solvents such as methanol and acetonitrile. A concentration of pentanol of about 1% gave a retention of the same magnitude as a mobile phase containing 20% v/v of acetonitrile. No significant difference in selectivity between pentanol and acetonitrile or methanol was observed. Acetonitrile is nowadays more commonly used as a neutral modifier in mobile phases for beta-blockers.

Effect of ionic modifiers

The addition of increasing concentrations of the cationic modifier, DMOA, (Fig. 4) decreases the capacity ratios for all compounds. The effect is much stronger on the

Figure 3
Influence of neutral modifier (1-pentanol) on the separation of various compounds. Solid phase: LiChrosorb RP 8; Mobile phase: 1-pentanol (various concentrations) with 0.025 M tetramethylenediamine and 0.01 M dimethylcyclohexylsulphate in phosphate buffer (pH 2.2).

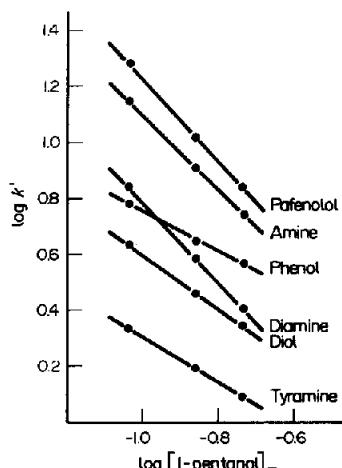
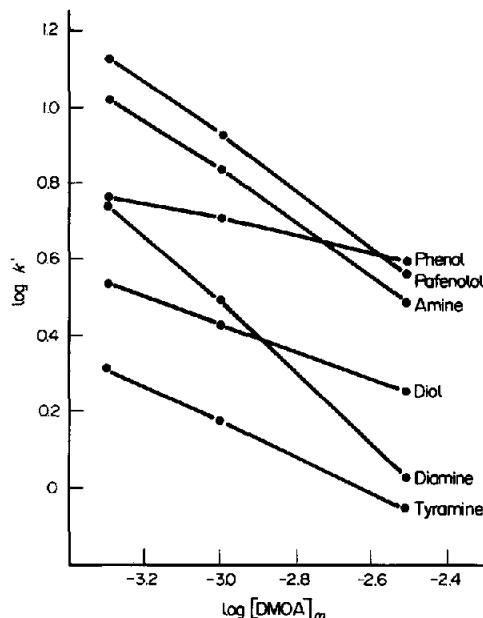


Figure 4

Influence of an ionic modifier (dimethyloctylamine, DMOA) on the separation of various compounds. Solid phase: LiChrosorb RP 8. Mobile phase: DMOA (in various concentrations) with 0.01 M dimethylcyclohexylsulphate and 0.092 M 1-pentanol in phosphate buffer (pH 2.2).



amines than on the neutral compounds probably owing to the selective competition for adsorption sites with high affinity for amines. The influence of DMOA seems to be stronger on the divalent than on the monovalent amines. A selective effect is thus obtained not only between different types of amines but also between amines and other compounds.

The retention of the amines increases with increasing concentration of the counter-ion, DMCHS (Fig. 5), an ion-pairing effect. However, this effect is counteracted by a simultaneous increased adsorption of amine modifier and approaches zero since the capacity of the adsorbing phase is limited. When the solid phase becomes completely covered with the ion-pair between DMOA and DMCHS, retention is unaffected by the counter-ion concentration and could well be described as an ion-exchange between amine solutes and DMOA. By changing to counter-ions more hydrophilic than DMCHS, such as perchlorate or phosphate, the capability of regulating retention by the counter-ion may be regained [19].

The decrease in the capacity factors of the neutral compounds, the phenol and the diol, may be explained according to the retention model by displacement from an increased amount of DMOA adsorbed as an ion-pair with the counter ion (Fig. 5).

Optimization of separation conditions

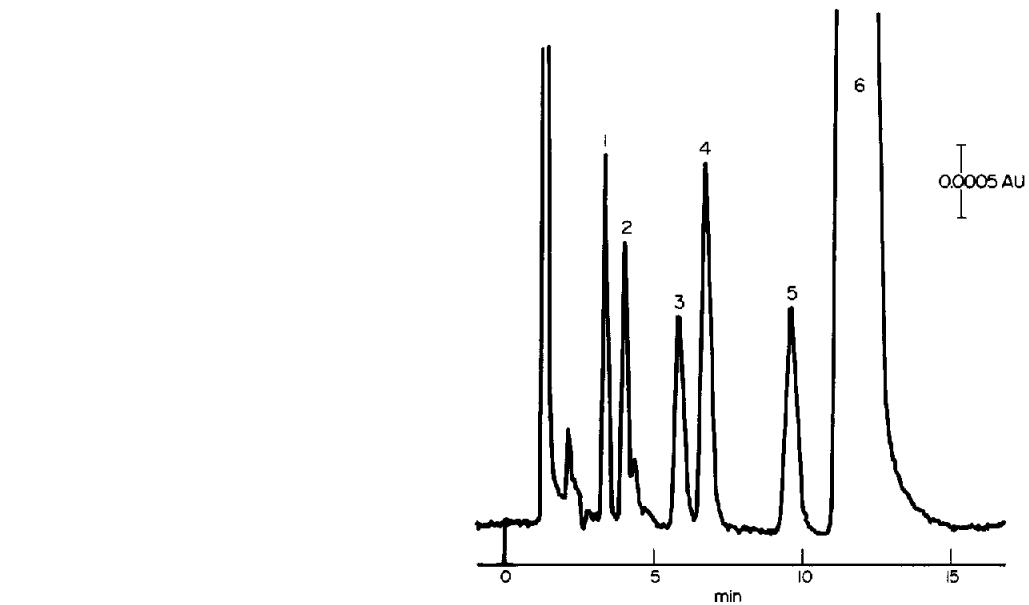
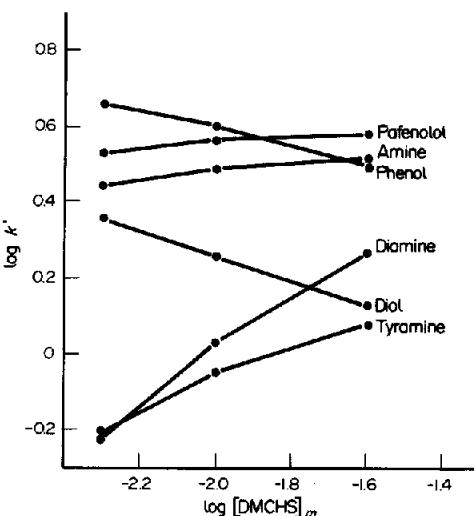
By use of the plots demonstrated in Figs 3–5 it was possible to select a suitable separation system for impurities in pafenolol (Fig. 6). Small amounts of unidentified impurities in the sample or even lot-to-lot variations in the properties of the solid phase may necessitate final adjustment of the separation conditions in daily use. This is preferably done by a small change in the amount of added counter-ion.

Gradient elution

In determinations of purity profiles the character of the impurities can vary from much more polar to much less polar than the drug itself. Such problems may be solved most

Figure 5

Influence of counter ion (dimethylcyclohexylsulphate, DMCHS) on the separation of various compounds. Solid phase: LiChrosorb RP 8. Mobile phase: DMCHS (in various concentrations) with 0.003 M dimethyloctylamine and 0.092 M 1-pentanol in phosphate buffer (pH 2.2).

**Figure 6**

Separation of about 0.3% of each of the studied impurities in pafenolol. Mobile phase: 0.003 M DMOA, 0.015 M DMCHS and 0.092 M 1-pentanol in phosphate buffer (pH 2.2). Detection at 270 nm. Samples: 1, tyramine; 2, diol; 3, diamine; 4, phenol; 5, amine; 6, pafenolol.

easily by use of a chromatographic system with gradient elution as demonstrated for metoprolol (Fig. 7) (S. Larsson, personal communication, 1978). As with pafenolol, some of the impurities are isomers or close homologues originating from impurities in the starting material. Other compounds result from possible side-reactions in the synthesis, with structures which deviate considerably from that of metoprolol. To achieve this separation of a sample containing metoprolol and spiked with seven potential impurities the gradient profile adopted was: $t = 0$ min, % (v/v) acetonitrile = 7.5%; 7.5 min, 7.5%; 18 min, 50%; 20 min, 50%.

Detection of impurities

The chromatogram in Fig. 7 was evaluated by measuring the peak area by use of an integrator. Most of the impurities have the same chromophore and the area percentage

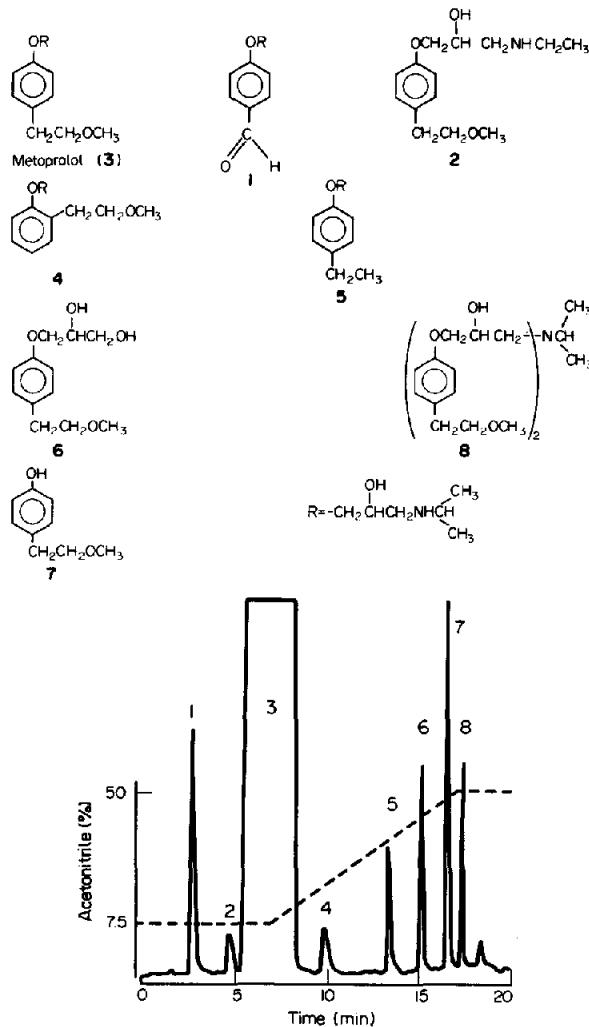


Figure 7

Separation of potential impurities in metoprolol tartrate. Solid phase: LiChrosorb RP 8. Mobile phase: 4 mM Tetrabutylammonium and acetonitrile (gradient elution) in phosphate buffer (pH 2.2). Detection at 280 nm.

will be representative for their respective concentrations in the sample. The aldehyde compound, eluted in the first peak, is an exception as it contains a conjugated system, which gives a change in the UV-spectrum and a considerable increase in absorptivity.

When the wavelength of the UV-detector was changed from 280 to 295 nm and the same sample injected, a different chromatogram was obtained showing detectable amounts of the main compound, metoprolol, and only one of the impurities, the aldehyde. This example demonstrates that it is always necessary to check the absorbance spectrum of an unknown impurity to verify that it contains the same chromophore as the main compound. Monitoring at two or more different wavelengths and measurement of absorbance ratios for the peaks may contribute to establishing the nature of the impurities. For such studies it is convenient to use a photodiode array detector that enables a UV spectrum of each separated compound to be obtained in combination with the chromatogram [20, 21].

Figure 8 shows a three-dimensional diode array chromatogram and UV spectra from the separation of potential impurities in alprenolol (S. Larsson and B. Persson, personal communication, 1984). A salt of alprenolol, the benzoate, was dissolved in a mobile phase of pH 3 and the first peak in the chromatogram is derived from benzoic acid. The second peak represents added diol, the main peak is alprenolol and the last peak is the phenol used as starting material.

Dynamically modified silica

An alternative or complement to separations on chemically bonded phases is to use dynamically modified silica. Several studies have shown that it is possible to alter the

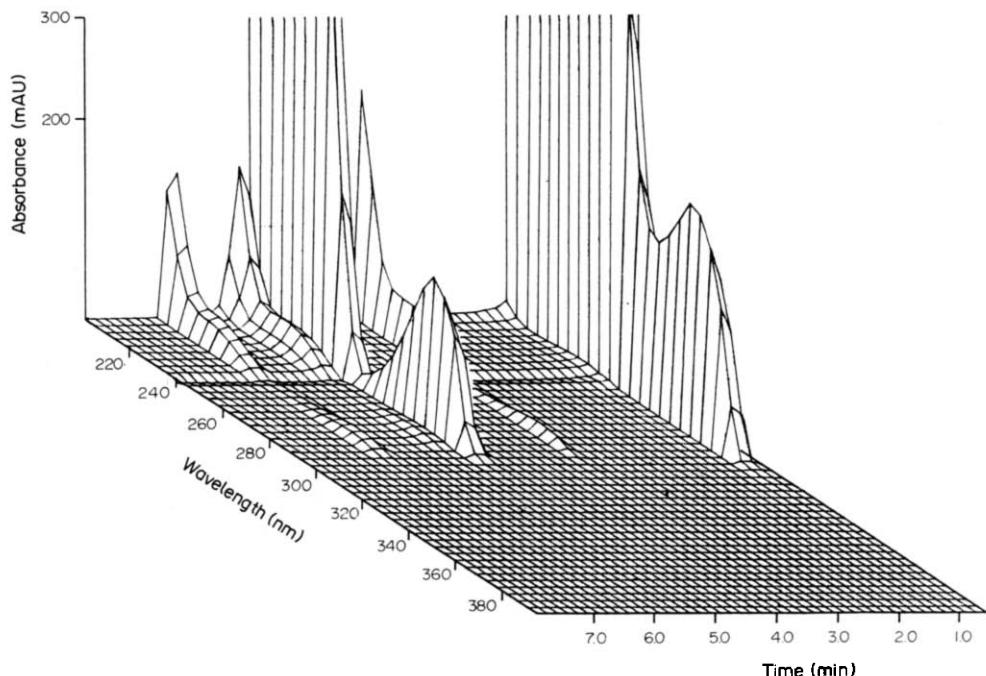


Figure 8

Separation of potential impurities in alprenolol benzoate. Solid phase: LiChrosorb RP 8. Mobile phase: 6 mM Dimethylcyclohexylsulphate and acetonitrile (35%, v/v) in phosphate buffer (pH 3).

silica surface by the addition of surfactants to a mobile aqueous phase. Non-ionic or quaternary ammonium cationic surfactants have been adsorbed substantially on to silica at pH 5–9 [22–24]. Retention of the solutes seems to be due to hydrophobic interaction with the adsorbed compound in a way very similar to that for alkylbonded silica.

It is even possible to adsorb a neutral ion-pair between hydrophobic cationic and anionic compounds present in the mobile phase [25]. When the hydrophobic cation, *N,N,N*-trimethyloctylammonium (TMOA) was combined with a hydrophobic anion, such as DMCHS, a change in retention pattern was found (Fig. 9) as the content of modifiers in the mobile phase was increased [26]. The capacity factors of the beta-blockers decrease initially with increasing concentration of TMOA and reach an almost constant level in a region below 0.03 M. This is followed by an increase in capacity ratios and a change in retention order of the solutes. At high concentration of modifiers the retention follows almost entirely the same order as in a reversed-phase system.

Multidimensional separation systems

The different retention characteristics on unmodified silica and bonded-phase silica enable these systems to be used in combination for the separation of complex mixtures of impurities. Such a system was used by Weidolf [27] for the separation of metabolites of a drug substance, felodipine. This drug is a dihydropyridine derivative with both a methyl ester and an ethyl ester as functional groups. Felodipine is metabolized by oxidation of the dihydropyridine moiety to its pyridine analogue followed by hydrolysis of one or both ester functions. These three metabolites, the ethyl mono-acid, the methyl mono-acid and the di-acid, as well as their corresponding hydroxymethyl-acids have been identified in human urine [28].

The system used was composed of bare silica in the first column and alkylbonded silica in the second column connected by column-switching equipment. Complete separation of the six metabolites was attained by the different selectivity of the two supports. One

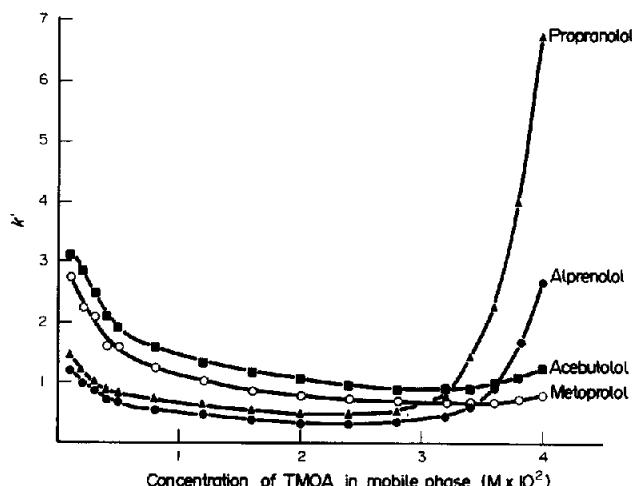


Figure 9
Influence of trimethyloctylammonium (TMOA), on the retention of amines. Solid phase: LiChrosorb SI 60 (silica). Mobile phase: TMOA and potassium bromide (total concentration 0.04 M) with 0.01 M dimethylcyclohexylsulphate in phosphate buffer (pH 2.2).

group of four metabolites was eluted from the silica column and separated by a linear methanol gradient on the bonded-phase column. After restoring the mobile phase composition, the remaining second group of metabolites was eluted from the silica column and separated on the bonded-phase column by use of a new gradient with methanol.

This multidimensional separation method for drugs illustrates its capability for separating complex mixtures of related substances such as impurities and degradation products. It may even enable the main compound to be switched out before the last separation column to avoid disturbances from overloading, thus increasing the sensitivity of the method [29, 30].

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