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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF DRUGS

II. AN EVALUATION OF A MICROPARTICULATE CATION-EXCHANGE COLUMN

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SUMMARY

A microparticulate cation-exchange column has been evaluated for the chromatography of thirty compounds selected as representative of a wide variety of drug substances. Although the column exhibited a strong partition effect besides the expected ion-exchange mechanism, the retention of drugs could be predictably influenced by variation of the eluent ionic strength and organic solvent content.

For acidic drugs the column showed little selectivity (although a salting-out effect increased retention at high eluent ionic strengths), but for basic substances Partisil SCX may afford a useful separative medium offering reasonable chromatographic efficiency (HETP $\approx$ 0.1 mm). The column longevity, however, is at present questionable.

INTRODUCTION

During the past three years, high-pressure liquid chromatography (HPLC) has evolved to a state of sophistication and usefulness on a par with the more established separative methods of gas and thin-layer chromatography. Although the number of HPLC column packings is not as large as for gas chromatography, a wider range of separation modes is available. However, while many separation problems involving drugs may be approached by partition, adsorption, ion-exchange or even steric exclusion chromatography, there are few data to enable the selection of the most effective of these modes for the separation and quantitation of members of a particular class of drugs. The present series of communications is designed to evaluate the usefulness of various HPLC systems for the chromatography of a wide variety of drug substances.

In a previous paper[1] we described the evaluation of a microparticulate octadecylsilane stationary phase. Although such a column may be used for many different types of drug, it was found that column efficiency varied considerably according to the solute, and only with acidic and neutral drugs was the efficiency acceptable.

For the chromatography of a predominantly ionic group of compounds, ion-exchange would appear to be an obvious method, and, indeed, the pellicular anion and cation exchangers such as Zipax® SAX and SCX have found extensive use in separations involving analgesics[2,3], barbiturates[4], sulphonamides[5], vitamins[6], morphine[7,8] and other alkaloids. For the separation of complex mixtures, however, the low efficiency of such supports frequently necessitated recourse to gradient elution. This paper describes the evaluation of a cation-exchange column based on microparticulate silica, which was expected to combine the unique selectivity of the ion-exchange mechanism with the superlative efficiency of the microparticulate substrate.

The thirty compounds chosen for this study were the same as those used previously in the evaluation of an octadecylsilane reversed-phase system[1]. The substances were selected as representative of a wide range of drug classes of varying polarity, lipid solubility and base strength. Acidic and neutral drugs were included as preliminary experiments indicated that interactions other than ion-exchange would lead to some retention for non-basic compounds.

EXPERIMENTAL

A constant-flow pump (M-6000; Waters Assoc., Stockport, Great Britain) was used to deliver eluent to a column (25 cm × 4.6 mm I.D.) packed with a sulphonic acid cation exchanger based on irregular-shaped microparticulate (10 μm) silica (Partisil SCX®; Whatman–Reeve-Angel, Maidstone, Great Britain). The eluents used were made from aqueous solutions of ammonium dihydrogen phosphate (AnalaR grade; BDH, Poole, Great Britain) and methanol (AnalaR; BDH) or acetonitrile (puriss. grade; Koch-Light, Colnbrook, Great Britain). Eluents were adjusted to the specified apparent pH ($\pm$ 0.02 units) by the addition of ammonium hydroxide or phosphoric acid solution.

Aqueous methanolic solutions of the drugs (2 μl) were injected under full flow using a microlitre syringe and a septum injection port. Eluted substances were detected by their ultraviolet (UV) absorbance using a fixed-wavelength (254 nm) UV monitor (Waters Assoc.). The column was operated at ambient temperature (*ca.* 22°) and, except where stated, the eluent flow-rate was 2.0 ml/min, generating a back-pressure of 450–1000 p.s.i. according to the eluent.

The chromatographic behaviour of the Partisil SCX column was investigated with respect to three variables of the eluent: ionic strength, pH and the effect of added organic solvent (methanol or acetonitrile). Eluents of pH 3, 5 and 7 with ionic strengths, of 0.5, 0.1, 0.05 and 0.01 *M* ammonium dihydrogen phosphate and containing 0, 20, 40, or 60% (v/v) methanol were employed. Some eluents were also made up using acetonitrile in place of methanol.

RESULTS AND DISCUSSION

Effect of eluent ionic strength, methanol concentration and pH upon retention

In Table I are given the retention volumes (V_R) for the thirty drugs studied using eluents of pH 3, 5 and 7 containing methanol (40%) with aqueous phosphate buffers of varying molarity. The drugs are listed in the order: strong acids, weak acids, neutral compounds, bases and quaternary ammonium compounds.

TABLE I

RETENTION VOLUMES (ml) OF SOME DRUGS ON A MICROPARTICULATE CATION-EXCHANGE COLUMN USING ELUENTS CONTAINING 40% METHANOL AND OF VARYING pH AND IONIC STRENGTH

Drug	*Eluent pH = 3*				*Eluent pH = 5*				*Eluent pH = 7*			
	Ionic strength (M)				*Ionic strength (M)*				*Ionic strength (M)*			
	0.5	*0.1*	*0.05*	*0.01*	*0.5*	*0.1*	*0.05*	*0.01*	*0.5*	*0.1*	*0.05*	*0.01*
Salicylic acid	3.2	3.2	3.0	3.4	3.4	3.0	3.2	2.6	3.5	3.1	2.9	2.6
Acetylsalicylic acid	3.2	3.5	3.1	3.4	3.6	3.2	3.1	2.6	3.3	3.5	3.0	2.4
Ibuprofen	3.4	3.2	3.2	3.4	3.5	3.2	3.3	3.0	3.4	3.1	2.9	2.5
Paracetamol	3.4	3.3	3.3	3.4	3.5	3.5	3.5	3.4	3.6	3.6	3.5	3.6
Phenylbutazone	3.8	3.4	3.6	3.5	3.3	3.2	3.3	2.9	3.3	3.1	3.0	2.5
Phenytoin	3.3	3.4	3.3	3.2	3.3	3.3	3.4	3.4	3.5	3.5	3.5	3.6
Glutethimide	3.6	3.5	3.6	3.4	3.4	3.6	3.6	3.5	3.4	3.6	3.5	3.7
Barbitone	3.2	3.5	3.3	3.1	3.6	3.6	3.4	3.4	3.4	3.5	3.4	3.4
Phenobarbitone	3.4	3.2	3.4	3.2	3.2	3.4	3.3	3.4	3.2	3.5	3.3	3.4
Quinalbarbitone	3.2	3.5	3.2	3.2	3.2	3.5	3.6	3.4	3.2	3.4	3.3	3.4
Thiopentone	3.2	3.3	3.2	3.1	3.5	3.4	3.4	3.3	3.4	3.4	3.4	3.5
Nitrazepam	3.8	4.0	4.1	5.8	3.5	3.6	3.7	3.7	3.5	3.7	3.7	3.8
Chlordiazepoxide	4.5	6.0	7.2	17.0	4.0	4.5	5.0	5.6	3.8	4.0	4.0	4.0
Phenacetin	3.6	3.8	3.6	3.5	4.0	3.6	3.8	3.6	3.5	3.7	3.6	3.6
Sulphacetamide	3.4	3.2	3.4	3.4	4.0	3.6	3.5	3.4	3.3	3.4	3.2	2.8
Sulphanilamide	3.2	3.4	3.5	3.8	3.8	3.4	3.5	3.6	3.3	3.7	3.8	3.6
Amphetamine	3.2	4.8	5.4	12.0	4.1	4.7	5.8	13.0	4.6	5.8	6.1	12.8
Methylamphetamine	3.2	5.3	6.2	13.4	4.6	5.5	6.6	15.6	5.4	7.1	7.6	15.4
Ephedrine	3.9	4.8	5.5	12.0	4.0	5.0	5.9	13.6	4.8	6.4	6.8	13.2
Nicotine	7.0	16.3	26.9	—	8.1	12.6	17.0	—	14.5	22.3	24.2	—
Caffeine	4.2	4.0	4.0	4.0	4.3	4.2	4.3	4.2	4.0	4.3	4.4	4.1
Morphine	4.3	5.8	7.0	16.8	4.9	6.2	7.7	20.0	7.8	10.7	15.2	23.2
Pethidine	4.8	6.3	7.5	17.4	4.9	6.4	7.9	20.6	6.8	9.6	10.2	20.7
Cocaine	6.4	9.2	11.1	24.5	6.5	9.1	11.5	30.0	8.0	10.8	11.7	25.6
Quinine	5.9	18.4	36.0	>60	5.5	9.3	12.0	52.0	7.3	10.5	10.6	23.4
Diphenhydramine	4.8	6.5	7.6	18.1	4.8	6.6	7.8	22.8	6.1	8.0	8.4	18.4
Chlorpromazine	4.9	7.0	8.5	20.0	4.9	7.0	8.6	23.6	6.7	9.0	9.6	20.8
Amitriptyline	4.8	6.7	7.9	18.4	4.8	6.9	8.0	20.8	6.3	8.1	11.7	19.2
Tubocurarine	8.3	22.4	—	—	8.9	23.2	47.0	—	19.6	46.2	—	—
Paraquat	20.3	>50	—	—	32.0	>80	—	—	>60	—	—	—

Retention by a cation-exchange mechanism is expected to be dependent upon:

(a) the ionic strength of the eluent;

(b) the proportion of drug present in the ionised form (which is controlled by the pH of the eluent and the pK_a of the drug.).

Retention due to non-ionic interactions is dependent upon:

(c) the proportion of drug present in the unionised form;

(d) the lipid solubility of the unionised form of the drug;

(e) the organic solvent content of the eluent.

From Table I it is seen that acidic drugs are rapidly eluted while more basic compounds are strongly retained on the column. Although retention is roughly in order of base strength, it is only for closely related compounds, *e.g.* amphetamine (pK_a 9.8) and methylamphetamine (pK_a 10.1), that a precise order of basicity is

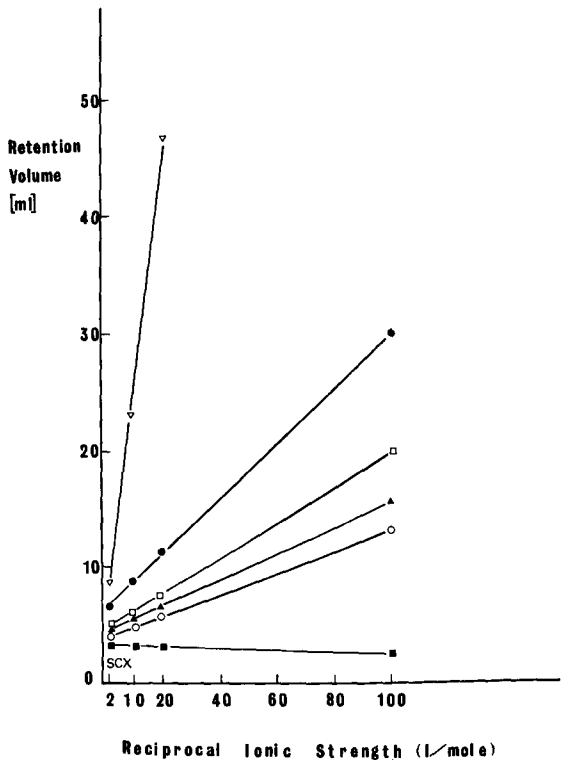

Fig. 1. Effect of variation of the eluent ionic strength upon retention volume. Methanol concentration 40%; pH 5. ■, Acetylsalicylic acid; ○, amphetamine; ▲, methylamphetamine; □, morphine; ●, amitriptyline; ▽, tubocurarine.

followed. Not surprisingly[7] for basic solutes, retention was also found to be inversely proportional to the eluent ionic strength (Fig. 1). For acidic drugs, the increase in retention with increasing ionic strength was attributed to a salting-out effect.

The observation that some of the plots in Fig. 1, although linear, do not pass through V_0 (the column void volume $\approx$ 1.8 ml) indicates that some mechanism other than ion-exchange is also operative. Indeed, the fact that acidic and neutral compounds are retained at all by the column supports this, as does the reduction in retention volumes when eluents containing organic solvent are used (Fig. 2). The ability of ion-exchange materials based on styrene–divinylbenzene materials to act as solid solvents for organic compounds is well known. It has been proposed[7] that for Zipax SCX (a pellicular cation-exchange material) the preferential sorption of organic solvents by the resin phase may influence retention by reducing the affinity for ions and by increasing the sorption of unionised molecules. In theory, the addition of methanol to the eluent could therefore either increase or decrease retention on the column. Alternatively, the column packing may act to some extent as a reversed-phase partition system (possibly in part owing to the presence of unsulphonated aromatic or aliphatic groups) and, indeed, the effect of added methanol (Fig. 2) is very similar to that observed for an octadecylsilane column[1].

The effect of the eluent pH on retention is shown in Table I and illustrated in

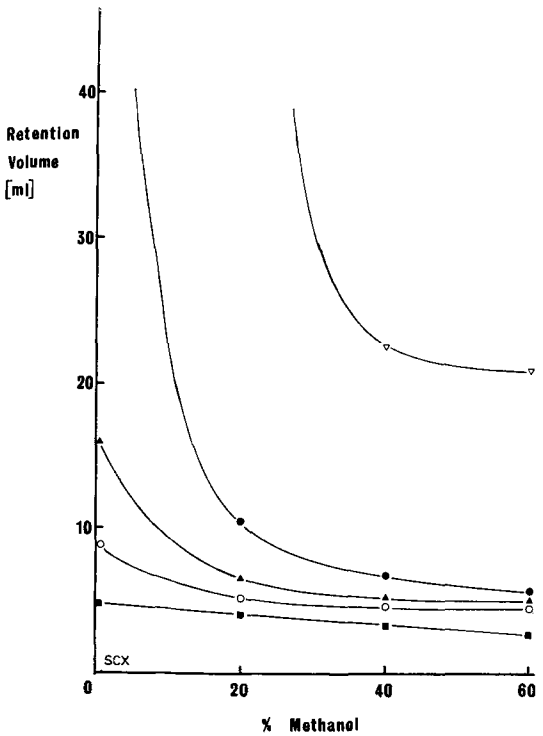

Fig. 2. Effect of variation of the eluent methanol concentration upon retention volumes. Ionic strength, 0.1 *M*; pH 3. ■, Acetylsalicylic acid; ○, amphetamine; ▲, methylamphetamine; ●, amitriptyline; ▽, tubocurarine.

Fig. 3. In general, for basic drugs, retention increases with increasing eluent pH from pH 3 to pH 7, while for acidic and neutral compounds there is a decrease in V_R. Variation of the eluent pH may be expected to influence the chromatographic system in several ways. As the pH is increased basic drugs will become less ionic in nature leading to:

(a) less retention by an ion-exchange mechanism, and

(b) more retention by partition chromatography.

Although the method used in this work to adjust the eluent pH will lead to changes in the eluent ionic strength, this is not considered significant in accounting for the effects shown in Fig. 3. Similarly, variation of the pH between 3 and 7 is not expected to have a significant effect on the ionisation of the strongly acidic sulphonic acid groups of the column itself. The observed pH effect is therefore at variance with that expected for cation-exchange chromatography, but is comparable with that expected for a reversed-phase partition system where only the unionised form of the drug would partition into the stationary phase. As the eluent pH is increased, phenobarbitone, for example, becomes more ionised and less lipid-soluble, while basic drugs such as methylamphetamine become more lipid-soluble and more strongly retained by a partition mechanism. It is possible that only over a pH range comparable to the pK_a of basic drugs would the effect of pH on the ion-exchange mechanism be predominant.

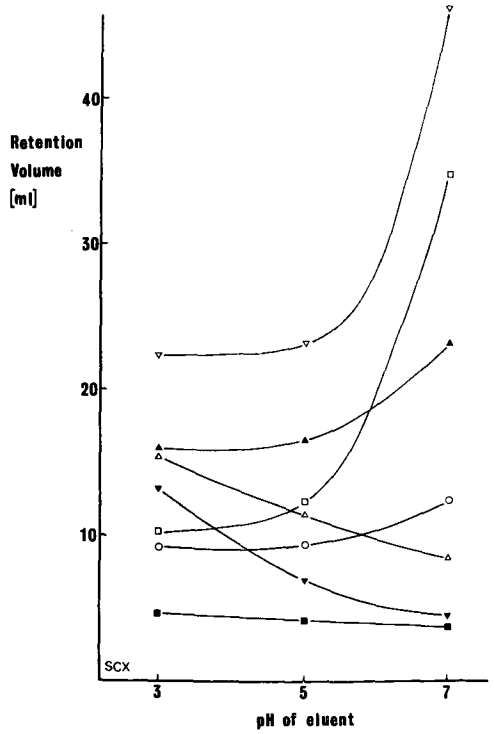

Fig. 3. Effect of variation of the eluent pH upon retention volume. Ionic strength, 0.1 *M*; methanol concentration 40% for tubocurarine; 0% for other drugs. ■, Acetylsalicylic acid; ▼, phenobarbitone; △, nitrazepam; ○, amphetamine; ▲, methylamphetamine; □, morphine; ▽, tubocurarine.

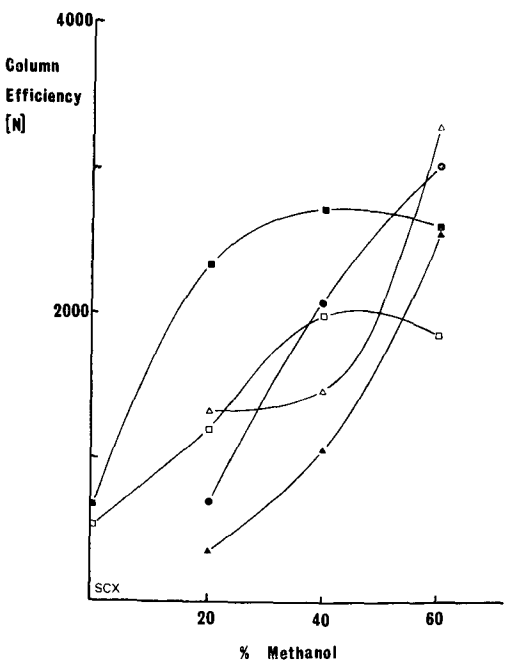

Fig. 4. Effect of the eluent methanol concentration upon column efficiency. Ionic strength, 0.01 *M*; pH 3. ■, Acetylsalicylic acid; △, nitrazepam; ▲, methylamphetamine; □, morphine; ●, amitriptyline.

Column efficiency

As well as the effect of added organic eluent on retention times, the chromatographic efficiency was considerably enhanced for all types of drug when a methanolic eluent was used (Fig. 4). This may be due to a reduction in partition effects, as reversed-phase partition chromatography may be very inefficient for certain drug types. The column efficiency for some drugs was also influenced by the eluent pH (Fig. 5), but in an unpredictable manner.

Acetonitrile-containing eluents were found to have an effect on retention volumes similar to those containing methanol, but, owing to the lower viscosities of acetonitrile–water mixtures, operating pressures were much lower and column efficiencies significantly higher than for methanolic eluents.

Column efficiency was also strongly dependent upon the eluent flow-rate. For amphetamine, for example, an efficiency of 2500 theoretical plates at a flow-rate of 0.5 ml/min ($v = 0.12$ cm/sec) was reduced to 1500 plates at 5.0 ml/min ($v = 1.2$ cm/sec).

Column longevity

It had been envisaged that a useful variation in selectivity could be achieved by changing the eluent pH around the pK_a of the drug to be chromatographed and

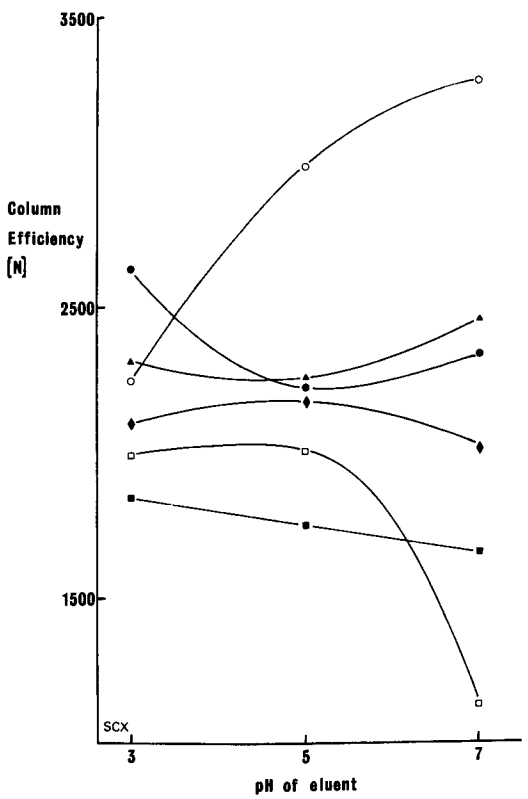

Fig. 5. Effect of eluent pH upon column efficiency. Ionic strength, 0.01 M; methanol concentration, 60%. ■, Acetylsalicylic acid; ◆, phenylbutazone; ○, amphetamine; ▲, methylamphetamine; □, morphine; ●, amitriptyline.

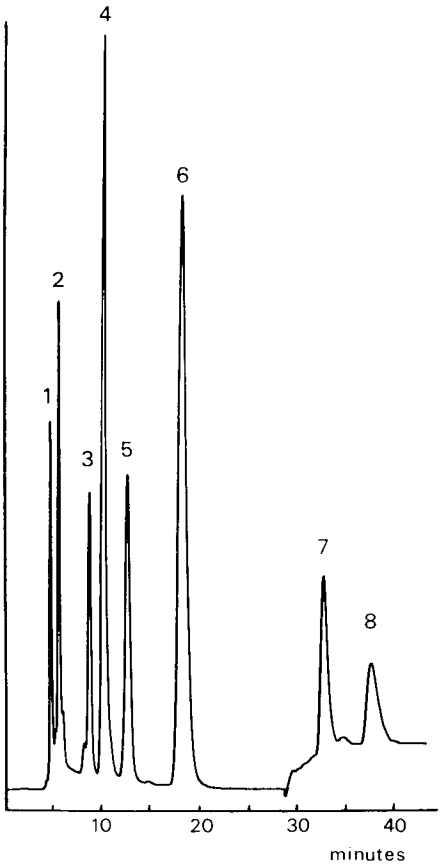

Fig. 6. High-pressure cation-exchange chromatography of a wide variety of drugs. Eluent 0.1 *M*, pH 3, 60% methanol. Flow-rate, 0.5 ml/min for 30 min, then 4.0 ml/min. 1 = Salicylic acid; 2 = nitrazepam; 3 = amphetamine; 4 = methylamphetamine; 5 = morphine; 6 = cocaine; 7 = quinine; 8 = tubocurarine.

initial recommendations from the suppliers of the column stated that eluents with a pH range of 1.5–10 could be used. However, use of the column at pH 9 gave a rapid and progressive deterioration in performance accompanied by a shrinking of the column bed. The manufacturers recommended pH limits were subsequently revised (pH 3–7) and the work described in this paper was performed using fresh columns and eluents of pH 3–7. Despite this, the life expectancy of Partisil SCX columns appears to be shorter than that found previously for Zipax SCX, and we have no experience of the columns surviving more than a few months use, after which the ion-exchange capacity was reduced markedly.

CONCLUSIONS

In view of the deliberately heterogeneous composition of the thirty drugs studied, and the multiplicity of factors affecting column retention, it is not possible to make detailed comment on the effect of drug structure and pK_a upon retention.

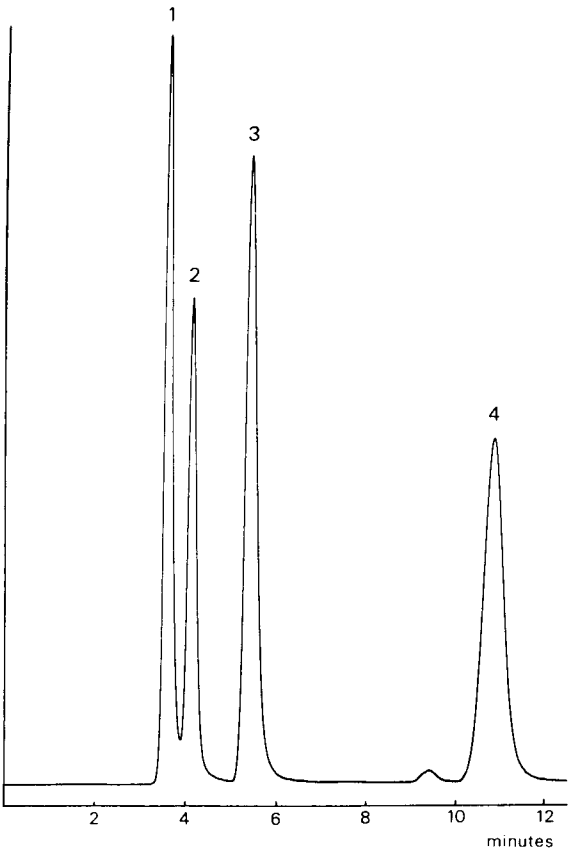

Fig. 7. High-pressure cation-exchange chromatography of some benzodiazepins. Eluent 0.05 *M*, pH 3, 60% methanol. Flow-rate, 1.0 ml/min. 1 = Oxazepam; 2 = nitrazepam; 3 = diazepam; 4 = chlordiazepoxide.

It is clear, however, that for closely related compounds such as amphetamine (pK_a 9.8) and methylamphetamine (pK_a 10.1) the elution order is as expected from the base strengths, and for strongly basic quaternary ammonium compounds retention is very great indeed. The utility of the column is enhanced by the manner in which retention varies predictably in inverse proportion to the eluent ionic strength, but it is unfortunate that changes in selectivity cannot be similarly engineered by variation of the eluent pH to take advantage of a useful feature in ion-exchange chromatography.

In contrast to an octadecylsilane HPLC system[1], the cation-exchange column displays tolerable efficiency for most drugs if eluents containing methanol or, better, acetonitrile are used. The optimum conditions for the chromatography of basic drugs may therefore be selected by:

(1) using eluents comprising at least 40% acetonitrile or methanol to obtain good efficiencies;

(2) varying the eluent ionic strength to obtain the desired retention;

(3) changing the eluent pH which may affect retention (apparently by partition effects only) and influence column efficiency in an unpredictable fashion.

Fig. 6 illustrates the wide range of drugs that may be separated on the column and Fig. 7 exemplifies the use of the column to separate a group of closely related benzodiazepines.

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