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ISOCRATIC MULTI-COLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AS A TECHNIQUE FOR QUALITATIVE ANALYSIS AND ITS APPLICATION TO THE CHARACTERISATION OF BASIC DRUGS USING AN AQUEOUS METHANOL SOLVENT

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SUMMARY

A variety of high-performance liquid chromatography packing materials were prepared and their chromatographic properties compared for separating several basic drugs using a single solvent system. The three most promising packing materials (silica, a mercapto propyl modified silica and a *n*-propyl sulphonic acid modification) were subsequently used to provide retention volume data for a large number of drugs.

INTRODUCTION

In modern analytical chemistry the unequivocal identification of a compound is usually achieved by a combination of spectroscopic, chromatographic and chemical tests. In some circumstances it may not be possible or appropriate to carry out such an exhaustive series of examinations and if this is the case chromatographic methods alone can often be used to make an identification possible. When using separative methods to characterise the identity of the components in a mixture it is necessary to obtain retention volume data (or R_F data in the case of thin-layer chromatography) under two or more conditions for comparison with values obtained from known compounds. For effective qualitative analysis it is essential that the separations made should differ substantially in their selectivity and hence produce sets of data which are not highly correlated. In addition the detection method used is also important and as its selectivity increases so too does the specificity of the analysis.

In high-performance liquid chromatography (HPLC) there are three practical methods for carrying out sample identification using chromatographic parameters alone. These methods involve separations on

(a) A single column eluted with different solvents *e.g.* using a C_{18} bonded-phase support eluted in reversed-phase and reversed-phase ion-pair partition modes.

(b) Two or more columns eluted with different solvents *e.g.* a reversed-phase and a normal-phase separation.

(c) Two or more columns eluted with the same solvent.

The third approach has a number of practical advantages, for example, if a single chromatograph is being used it is possible to obtain data on more than one column without having to change the eluent in the pump or detector. This avoids a troublesome feature of HPLC for most operational problems are encountered during such changes. In addition it is possible to mount several different columns in parallel to provide an even more convenient configuration. Despite these obvious advantages multi-column isocratic HPLC does not seem to have attracted any attention. In part this can be attributed to the fact that HPLC has mainly been used for quality control purposes where mixtures of relatively simple composition are examined, but in addition the versatility of reversed-phase separations on chemically bonded C_{18} packing materials has also had a marked influence on developments. The selectivity of separations on such supports can be varied by adjustments to the solvent composition and hence there has been relatively little stimulus to develop bonded-phase packings for HPLC which display different selectivity with a common solvent, in marked contrast to the situation in gas–liquid chromatography.

In fields such as forensic drug analysis samples of unknown origin and composition are frequently examined and the emphasis of the analysis is on the identification of compounds rather than on their quantitation. Chromatographic methods, including HPLC, are mainly used for screening purposes as a preliminary to a more definitive characterisation by spectroscopic methods, and a series of chromatographic separations, provided they yield non-correlated data, can impart much useful information. For some years analysts in this laboratory have separated mixtures of basic drugs by HPLC on columns packed with silica using a methanol–2 *M* ammonium hydroxide–1 *M* ammonium nitrate (27:2:1) mobile phase[1,2]. Very similar solvent systems have also been applied by others to achieve thin-layer[3] and HPLC[4,5] analyses. It is worth noting that despite the high pH of the eluent (*ca.* pH 10.3) we have not run into problems arising fom solvent attack on the silica, although it seems to be a popular misconception amongst chromatographers that high pH should be avoided at all costs in order to prevent siliceous materials from dissolving. In fact if the methanol content of the solvent is high and ammonia is the source of hydroxyl ions it appears that attack of the support is unlikely[6,7].

The aim of the study reported here was to develop and assess alternative packing materials capable of giving different separations of basic drugs under the same solvent conditions that we have used with silica.

EXPERIMENTAL

Preparation of packing materials

Packing materials for the primary assessment of different bonded-phases were all prepared from the same batch of silica that was size graded by aqueous sedimentation. This material had the following characteristics: particle size 3–7 μm, pore diameter 130 Å (mean value), pore volume 1.25 ml/g, surface area (BET) 320 m^2/g. Several different procedures were used to prepare bonded-phase packings from this material.

Method 1. The silica was dried overnight at 175° and 50 g were weighed into a conical flask and 250 ml of hexane (sodium dried) and 25 ml of the chlorosilane

reagent were added (see Table I for details of the silylating agents used). The mixture was heated under reflux for 1 h and the product was filtered through a sintered glass filter funnel and washed several times with hexane and finally acetone. The product was vacuum dried at 70°.

TABLE I

CHEMICALLY-BONDED PACKING MATERIALS USED FOR STUDYING THE SEPARATION OF BASIC DRUGS

Packing designation	*No.*	*Reagent*	*Preparation method**	*Loading (%)***
C_3	16	*n*-Propyltrichlorosilane	1	5.1
C_8	17	*n*-Octyltrichlorosilane	1	8.2
C_8/TMCS	62	*n*-Octyltrichlorosilane + TMCS		8.2
C_{18}	8	Octadecyltrichlorosilane	1	10.6
C_{18}/TMCS	59	Octadecyltrichlorosilane + TMCS		—
Gly	13	Glycidoxypropyltrimethoxysilane		17.8
Vinyl	4	Vinyltrichlorosilane	1	3.2
CN (1)	22	3-Cyanopropyltrimethoxysilane	2	11.8
CN (2)	23	3-Cyanopropyltrichlorosilane	1	10.0
CN (3)	11	2-Cyanoethyltriethoxysilane	2	8.9
NH_2 (1)	10	3-Aminopropyltriethoxysilane	2	10.6
NH_2 (2)	12	3,2-Aminoethylaminopropyltrimethoxysilane	2	18.1
SH	60	3-Mercaptopropyltrimethoxysilane	2	10.4
SCX (1)	49	Prepared from SH		9.2
SCX (2)	—	Prepared from 2-phenylethyl		—
Phenyl (1)	21	Phenyltriethoxysilane	2	7.9
Phenyl (2)	14	Phenyltrichlorosilane	1	8.1
Phenyl/TMCS	63	Phenyltrichlorosilane + TMCS		—
Phenylethyl	—	2-Phenylethyltrichlorosilane	1	—
Phenylmethyl	18	Phenylmethyldichlorosilane	1	8.9
Diphenyl	15	Diphenyldichlorosilane	1	6.3
Dichlorophenyl	19	Dichlorophenyltrichlorosilane	1	10.7

* The preparation method indicated is that given in the text. Where no method is indicated a detailed preparation is given in the text.

** Defined as follows: weight loss at 600° × 100/residue weight.

Method 2. A 50-g amount of dried silica was weighed into a conical flask and was shaken for 3 h with 250 ml of hexane and 2 ml of water. The equilibrated mixture was then refluxed for 1 h with 25 ml of a trialkoxysilane reagent. The product was filtered, washed and dried as before.

Specific methods. A glycidoxypropyl bonded-phase material was prepared as follows: 50 g of dried silica, 250 ml of water and 25 ml of glycidoxypropyltrimethoxysilane were acidified to pH 3.5 with dilute hydrochloric acid and heated at 90° with continuous stirring for 1 h. The product was filtered, washed several times with water and was then vacuum dried.

An aliphatic strong cation exchanger based on *n*-propylsulphonic acid modified silica was prepared by oxidising a portion of the mercaptopropyl modified material that had been prepared by method 2. The procedure was essentially the same as one described previously[8] and involved stirring 20 g of the mercapto material with

100 ml of 2 *M* sulphuric acid in a conical flask whilst adding a saturated, filtered solution of potassium permanganate in 2 *M* sulphuric acid. When a permanent permanganate colour was attained the excess oxidising agent was removed by adding a solution of oxalic acid. The product was filtered, washed and dried as before.

The method used to prepare the aromatic strong cation exchanger was that described by Cox *et al.*[9].

Trimethylsilyl modifications of some of the materials produced by method 1 were prepared as follows: 25 g of the bonded-phase packing were stood for several hours in methanol to ensure the alcoholysis of any unreacted chloro groups. The sample was then dried under vacuum and refluxed with 250 ml of hexane and 25 ml of trimethylchlorosilane for 1 h. The product was washed with dry hexane several times and finally acetone before vacuum drying.

The silylating reagents used and the method of preparation for each bonded-phase product are shown in Table I.

Chromatographic comparison of the packing materials

The packings were slurry packed into 25 cm × 5 mm I.D. (1/4 in. O.D.) stainless-steel columns terminated with 1/4–1/16 in. zero dead volume end fittings. The top of the packing material bed was smoothed off about 0.5 cm from the end of the column and was covered with a stainless-steel mesh over-laid with glass beads (*ca.* 200 μm diameter). Stop-flow syringe injections were made via a modified tee[10]. The eluent used throughout this study was methanol–2 *M* ammonium hydroxide–1 *M* ammonium nitrate (27:2:1) pumped at 1 ml/min using a Waters Assoc. Model 6000 pump. Detection at various wavelengths was carried out using a UV detector (Cecil Instruments, Cambridge, Great Britain). Eleven test compounds were run on each column and the retention time data directly compared with that obtained on silica.

Collection of retention data on three different columns under isocratic conditions

Reference data on various drugs were built up by injecting suitable aliquots of their aqueous (or water–methanol) solutions. The three columns studied in depth were those packed with silica, the mercaptopropyl bonded-phase and the aliphatic strong cation exchanger (in all subsequent text these three packings are designated as Si, SH, and SCX, respectively). The eluent used was slightly different to that applied in the screening experiments in that it contained 50 mg of sodium sulphite per litre of solvent. This was to minimise *in situ* oxidation of the SH packing.

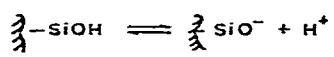
In addition to the above experiments the effect of ionic strength and pH on the elution of drugs on silica was also studied in order to provide more information on the separation mechanism.

RESULTS AND DISCUSSION

The retention volumes of the 11 test compounds on 25-cm columns packed with the different supports are shown in Table II, together with the correlation coefficient obtained when each set of data is compared with that obtained on silica. It is apparent that many of the bonded-phase packings give basic drug separations

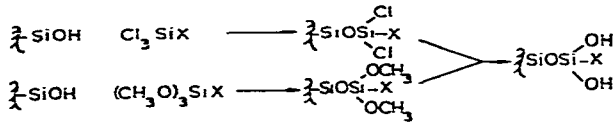
which closely resemble that found on silica. To understand this it is important to appreciate the separation mechanism on silica itself.

Although it seems likely that a variety of complex mechanisms apply, the dominant process occuring when basic drugs are separated on silica at high pH is probably ion exchange[11]. The cation-exchange properties of silica can be attributed to the ionisation of silanol groups at the silica surface.

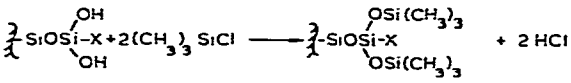


In common with all ion-exchange separations it is to be expected that ionic strength and pH should exert an effect on the chromatographic process on silica and that this is indeed the case was shown by two series of experiments. For example it is apparent from Table III that increasing the ionic strength of the eluent results in reduced retention of five basic drugs and Table IV illustrates the effect of pH. The latter effect is somewhat complex because the degree of ionisation of both the test drugs and the silica is being changed simultaneously.

If we assume that surface silanol groups on the silica are the major means of retaining and separating basic drugs why should so many bonded-phase packings display separating properties that so closely resemble that of silica? This probably arises because of the presence of silanol groups on the surface of bonded-phase packings, either from lack of effective coverage or because the silylating reagents themselves are introducing silanol groups as a result of hydrolysis.



This type of effect would explain why many of the *n*-alkyl and phenyl modifications give rise to greater retention than that displayed on the unmodified silica, for in some cases as many as two silanol groups may be introduced where only one existed before. Removal of such silanol groups can be achieved by reaction with trimethylchlorosilane



and one would anticipate that where this is carried out a decrease in retention is to be expected. In the three instances where this type of reaction was done the packing material after trimethylchlorosilylation gave separations which were less correlated with silica, *e.g.* the values changed from 0.93 to 0.70, 0.89 to 0.29 and 0.93 to 0.60 in the case of the C_8, C_{18} and phenyl modifications, respectively. In each case the reaction also led to a marked reduction in the retention volumes of all test drugs. There thus seems to be considerable evidence to indicate that under the solvent conditions used in this study basic drug separations are dominated by the influence of silanol groups even on bonded-phase packing materials.

The surface modifications producing separations that showed the least correla-

TABLE II

RETENTION DATA OF ELEVEN BASIC DRUGS ON A VARIETY OF DIFFERENT BONDED-PHASE PACKING MATERIALS

The data recorded in this table were obtained using 25 cm × 0.5 mm I.D. (1/4 in. O.D.) columns eluted with methanol–2 *M* ammonium hydroxide–1 *M* ammonium nitrate (27:2:1) at 1 ml/min. The correlation coefficient shown was obtained by comparing each set of results against that found on silica. The range indicated is the difference between the elution volume of the most strongly bound compound and the least strongly bound compound.

Test compound	*Retention volume (ml) on the bonded-phase specified*											
	Si	C_3	C_8	C_8/*TMCS*	C_{18}	C_{18}/*TMCS*	*Gly.*	*Vinyl*	*CN (1)*	*CN (2)*	*CN (3)*	NH_2 *(1)*
Quinine	4.9	5.3	8.2	6.8	8.7	5.7	3.9	5.3	4.3	4.6	4.9	3.9
Diacetyl morphine	5.5	5.5	6.4	5.2	6.3	4.3	3.8	5.6	4.7	5.2	5.1	3.9
Morphine	7.2	7.0	7.6	5.1	7.7	4.5	4.0	7.1	4.5	5.4	5.2	5.8
Codeine	6.9	6.7	7.7	5.3	8.0	4.8	4.0	7.0	4.8	5.7	5.6	4.3
Methadone	5.9	6.3	8.3	6.7	10.6	6.9	3.8	6.4	5.5	6.0	6.3	3.7
Dipipanone	4.5	4.9	6.6	6.2	7.6	6.8	3.6	4.9	5.5	5.3	5.8	3.6
Norephedrine	5.5	5.6	6.2	5.0	6.3	4.6	3.9	5.5	4.8	5.3	5.4	3.9
Ephedrine	8.6	8.9	10.5	6.1	11.3	5.6	4.1	8.4	6.1	7.6	7.6	3.8
Amphetamine	6.2	6.4	7.8	5.6	8.4	5.2	4.1	6.3	5.7	6.5	6.5	3.8
Methyl amphetamine	9.2	9.7	12.9	7.4	14.7	6.8	4.1	9.4	6.2	8.4	8.2	3.8
Strychnine	11.6	12.6	16.8	9.7	17.8	6.6	4.6	12.6	5.2	8.1	7.4	4.0
Correlation coefficient	–	0.99	0.93	0.70	0.89	0.29	0.91	0.99	0.39	0.88	0.76	0.12
Range (ml)	7.1	7.7	10.6	4.7	11.5	2.6	1.0	7.7	1.9	3.8	3.3	2.2

TABLE II *(continued)*

Test compound	*Retention volume (ml) on the bonded-phase specified*										
	NH_2 (2)	*SH*	*SCX (1)*	*SCX (2)*	*Phenyl (1)*	*Phenyl (2)*	*Phenyl/ TMCS*	*Phenyl-ethyl*	*Phenyl-methyl*	*Diphenyl*	*Dichloro-phenyl*
Quinine	3.8	6.4	4.6	5.9	6.6	6.6	5.0	6.8	6.5	6.0	8.2
Diacetyl morphine	3.7	6.6	4.2	5.5	8.1	7.5	4.9	6.7	7.2	7.1	8.2
Morphine	5.3	6.5	5.5	7.5	9.9	9.5	4.8	7.8	8.3	9.1	10.3
Codeine	3.8	7.2	5.3	7.5	9.9	9.6	5.0	8.2	8.4	9.1	10.3
Methadone	3.7	8.6	6.5	7.4	8.6	8.3	6.3	8.1	8.0	7.7	9.3
Dipipanone	3.7	8.1	6.1	7.0	6.4	6.0	5.5	6.0	6.0	5.5	6.8
Norephedrine	3.7	5.3	7.5	6.8	5.6	5.7	4.7	5.6	5.4	5.8	6.0
Ephedrine	3.6	7.2	10.1	11.2	9.7	10.7	5.0	9.0	8.4	9.9	10.7
Amphetamine	3.6	6.3	9.3	8.9	7.1	7.0	4.7	6.7	6.4	6.9	7.8
Methyl amphetamine	3.8	8.7	10.7	12.4	12.8	12.5	5.7	10.5	10.2	11.5	14.1
Strychnine	3.8	13.4	6.5	13.7	31.2	24.3	7.2	22.7	23.1	19.7	27.3
Correlation coefficient	0.07	0.75	0.42	0.93	0.87	0.93	0.6	0.88	0.86	0.95	0.90
Range (ml)	1.6	8.1	6.5	8.2	25.6	18.6	2.5	17.1	17.7	14.2	21.3

TABLE III

EFFECT OF IONIC STRENGTH ON THE SEPARATION OF BASIC DRUGS ON SILICA

An aqueous buffer solution was prepared by mixing 2 parts of 4 *M* ammonium hydroxide and 1 part of 2 *M* ammonium nitrate. Eluents A, B, C and D were prepared by mixing 450 ml of methanol with 50, 25, 12.5 and 6.25 ml of the buffer solution and adjusting the final volume to 500 ml with water. 25-cm columns of 5 mm I.D. packed with silica were used in the study and were eluted at a solvent flow-rate of 1 ml/min.

Test compound	*Elution volume (ml) with the solvent specified*			
	Solvent			
	A	*B*	*C*	*D*
Amphetamine	5.4	6.3	8.2	10.7
Methyl amphetamine	7.0	9.1	13.0	17.7
Morphine	6.4	7.3	8.7	10.4
Diacetyl morphine	5.2	5.6	6.5	7.6
Codeine	6.0	7.0	8.7	10.3

tion with that of silica were those involving the trimethylsilylated packings mentioned above, together with bonded-phases formed by introducing amino, cyano, mercapto and sulphonic acid groups. In making a decision with regard to which packings to use in an isocratic multi-column system it is not possible to rely solely on the correlation coefficient, for this number makes no allowance for the retention volume range over which the test components elute. Thus several of the packing materials displaying the lowest correlation with silica have little chromatographic value because most of the test compounds are insufficiently retained. In practice it is the three materials with acidic surface character that prove to be most useful, *i.e.* Si, SH and SCX for they exhibit a moderately low correlation value with a fairly wide retention volume range. Despite these three materials having similarity by virtue of their ability to ionise and produce a proton they undoubtedly differ in their degree of ionisation, and the presence of a *n*-propyl chain between the siloxane bond

TABLE IV

EFFECT OF pH ON THE SEPARATION OF BASIC DRUGS ON SILICA

Test compound	*Elution volume*			
	pH			
	10.3	*9.0*	*8.0*	*7.0*
Quinine	4.9	5.1	5.8	5.1
Diamorphine	5.5	—	3.7	3.7
Morphine	7.2	8.4	12.8	9.9
Codeine	6.9	8.2	13.1	10.7
Methadone	5.9	—	5.1	4.6
Dipipanone	4.5	4.3	4.5	4.3
Norephedrine	5.5	4.9	4.2	4.0
Ephedrine	8.6	6.3	4.8	4.2
Amphetamine	6.2	4.9	4.2	4.2
Methyl amphetamine	9.2	6.7	5.3	4.7
Strychnine	11.6	14.2	17.0	17.0

and the ionisable group presumably imparts some lipophilic properties to the SH and SCX materials not found with Si. In the second part of the investigation it was the Si, SH and SCX packings that were compared in depth.

The retention data of the large sample of basic drugs are shown in Table V and are given in the form of k' (*i.e.* the column capacity ratio) values on the three column systems arranged in order of increasing retention. The acid dissociation constants in aqueous solution are also shown, and these values were collected from a variety of literature sources. It can be seen that all the drugs examined elute in the k' region of 0–3.5 and that many of the compounds display marked differences in k' value on the different columns. Of the 161 drugs studied 93 have unique retention characteristics, but the remaining 68 compounds cannot be identified without ambiguity. Substances in the latter category are mainly found in the area of low retention, *i.e.* $k' \leqslant 0.2$. There is no apparent direct relationship between pK_a and k' although compounds with high pK_a values tend to be longer retained. On balance, therefore, the system provides an acceptable level of discrimination for basic drugs that are retained to an extent of $k' > 0.2$ and is of value in characterising many basic drugs by direct comparison of measured values with those of reference materials. However, a list of retention data such as that shown gives no indication of the potential of the three systems used for classifying unknown compounds. To bring out this feature of the method it is convenient to assign the various classes of drugs into groups based on the retention sequence on the three columns as shown in Fig. 1.

It can be seen that groupings of closely related compounds are occurring and this suggests that the three column systems must be retaining basic drugs by somewhat different mechanisms. At the high pH of the eluent (pH 10.3) most basic drugs are likely to be only partially ionised and hence four types of sample/packing material interaction can be visualised:

(1) Ion exchange of ionised molecules;

(2) liquid–liquid partition of the free base;

(3) liquid–liquid partition of the ion pairs formed by reaction of the ionised molecules and the eluent counter ion (*i.e.* NO_3^-);

(4) ill defined retention mechanisms, *e.g.* dipole interactions, binding by Van der Waals forces, hydrogen bonding etc.

In practice it is probable that the retention of any particular drug is attributable to a variety of such mechanisms, particularly as the packing material is itself complex (*e.g.* the bonded-phase packings studied will almost certainly contain residual silanol groups in addition to the acidic bonded groups). The observed separation patterns brought out in Fig. 1 may be explained if we assume the following simplistic patterns to apply

Column packing	*Dominant separation mechanism*
Si	Ion exchange and type 4 interactions
SH	Partition processes
SCX	Ion exchange

In broad terms the compounds which are not strongly retained are those with nitrogen atoms bound into an alicyclic ring system which is substituted with other

TABLE V

RETENTION VOLUME DATA OF BASIC DRUGS

The eluent used to achieve the retention shown below was methanol–2 *M* ammonium hydroxide–1 *M* ammonium nitrate (27:2:1) with 50 mg of sodium sulphite added to each litre of solvent. The class of compound is designated as follows: 1 = antihistamines and antinauseants, 2 = stimulants and anorexics, 3 = antidepressants and tranquilisers (not tricyclic compounds), 4 = local anaesthetics, 5 = narcotic analgesics (not morphine-like in structure), 6 = phenothiazines, 7 = narcotic analgesics (morphine-like compounds), 8 = tricyclic anti-depressants and other related compounds, 9 = antimalarial agents. The pK_a values shown in the table were taken from *The Extra Pharmacopoeia*, Martindale, 27th ed., The Pharmaceutical Press, London.

Compound	pK_a	*Class*	*k' on*			*Compound*	pK_a	*Class*	*k' on*		
			SI	*SH*	*SCX*				*SI*	*SH*	*SCX*
Buclizine	–	1	0	0	0	Amodiaquin	–	9	0.2	0.4	0.1
Meclizine	–	1	0	0	0	Chlorcyclizine	8.1	1	0.2	0.4	0.1
Benzphetamine	6.6	2	0	0	0	Piperacetazine	–	6	0.2	0.4	0.2
Chlormezanone	–	3	0	0	0	Isomethadone	–	5	0.2	0.4	0.2
Isocarboxazid	10.4	3	0	0	0	Ethopropazine	–	6	0.2	0.4	0.3
Benzocaine	2.5	4	0	0	0.1	Pentazocine	8.5	5	0.2	0.4	0.4
Phenadoxone	6.9	5	0	0.1	0	Noxiptiline	–	8	0.2	0.5	0.1
Amfepramone	–	2	0	0.1	0	Norpipanone	–	5	0.2	0.5	0.2
Paragyline	–	3	0	0.1	0	Methotrimeprazine	9.2	6	0.2	0.5	0.2
Haloperidol	8.3	3	0	0.1	0	Trimeprazine	–	6	0.2	0.5	0.2
Pimozide	7.3	3	0	0.1	0	Butriptyline	–	8	0.2	0.5	0.2
Trifluperidol	–	3	0	0.1	0	Chlorprothixene	–	8	0.2	0.5	0.2
Anileridine	–	5	0	0.2	0	Diethazine	9.1	6	0.2	0.5	0.3
Phenazocine	–	5	0	0.2	0	Lobeline	–	–	0.2	0.5	0.5
Diphenoxylate	–	5	0	0.2	0	Dipipanone	8.5	5	0.2	0.8	0.6
Phenbutrazate	–	2	0	0.2	0	Meperidine	8.7	5	0.3	0.3	0.2
Oxypertine	–	3	0	0.2	0	Metaproterenol	–	2	0.3	0.3	0.8
Papaverine	8.1	5	0	0.3	0	Triflupromazine	–	6	0.3	0.4	0.2
Pipamazine	8.6	6	0	0.3	0.1	Orphenadrine	–	1	0.3	0.4	0.2
Thiopropazate	7.3	6	0	0.4	0	Thonzylamine	8.9	1	0.3	0.4	0.2
Hydroxybuclizine	–	1	0.1	0.1	0	Chlorothen	8.4	1	0.3	0.4	0.2
Nialamide	–	3	0.1	0.1	0	Normethadone	–	5	0.3	0.5	0.2

Phenoxypropazine	–	3	0.1	0.1	0	Tripelennamine	9.0	1	0.3	0.5	0.3
Nalorphine	7.8	7	0.1	0.1	0.1	Trifluoperazine	8.1	6	0.3	0.6	0.1
Tranylcypromine	8.2	3	0.1	0.1	0.2	Cyproheptadine	–	1, 8	0.3	0.6	0.2
Caffeine	–	2	0.1	0.2	0	Promethazine	9.1	1, 6	0.3	0.6	0.2
Trifluomeprazine	–	6	0.1	0.2	0	Isothipendyl	–	8	0.3	0.7	0.2
Flupentixol	–	8	0.1	0.2	0	Clomipramine	–	8	0.3	0.7	0.3
Benoxinate	–	4	0.1	0.2	0.1	Thiethylperazine	–	6	0.3	0.9	0.2
Fluphenazine	8.1	6	0.1	0.2	0.1	Butaperazine	–	6	0.3	0.9	0.3
Isoproterenol	–	2	0.1	0.2	0.8	Viloxazine	–	3	0.4	0.4	0.2
Noscapine	6.2	5	0.1	0.3	0	Diphenhydramine	9.0	1	0.4	0.4	0.3
Pericyazine	–	6	0.1	0.3	0.1	Alphaprodine	8.7	5	0.4	0.4	0.2
Trimethoprim	7.2	9	0.1	0.3	0.2	Norpseudophedrine	–	2	0.4	0.4	1.1
Methylphenidate	–	2	0.1	0.3	0.2	Mepyramine	8.9	1	0.4	0.5	0.3
Phendimetrazine	7.6	2	0.1	0.3	0.2	Diacetylmorphine	7.6	7	0.4	0.5	0.2
Benzoctamine	7.6	8	0.1	0.3	0.5	Piperocaine	–	4	0.4	0.5	0.5
Acetophenazine	–	6	0.1	0.4	0.1	Propranolol	9.5	–	0.4	0.5	0.9
Carphenazine	–	6	0.1	0.4	0.1	Mepazine	9.7	6	0.4	0.6	0.3
Metopimazine	–	6	0.1	0.4	0.1	Amitriptyline	9.4	8	0.4	0.6	0.2
Perphenazine	7.8	6	0.1	0.4	0.1	Quinine	8.5	9	0.4	0.7	0.3
Clopenthixol	–	8	0.1	0.4	0.1	Chlorpromazine	9.3	6	0.4	0.7	0.3
Opipramol	–	8	0.1	0.5	0.1	Dothiepin	–	8	0.4	0.7	0.3
Fonazine	–	6	0.1	0.5	0.1	Pipradrol	–	2	0.4	0.7	0.9
Propiomazine	–	6	0.1	0.5	0.2	Prochlorperazine	8.1	6	0.4	0.8	0.2
Emetine	7.4, 8.3	–	0.2	0.1	0.2	Thiothixene	–	8	0.4	0.9	0.1
Scopolamine	7.6	–	0.2	0.1	0.2	Thioproperazine	–	6	0.4	0.9	0.2
Tetracaine	8.5	4	0.2	0.2	0.2	Fenfluramine	9.1	2	0.5	0.5	0.8
Cocaine	8.6	4	0.2	0.2	0.2	Chlorphentermine	9.6	2	0.5	0.6	1.2
Procaine	9.0	4	0.2	0.2	0.2	Phentermine	10.1	2	0.5	0.6	1.4
Cyclizine	8.2	1	0.2	0.3	0.2	Iprindole	–	8	0.5	0.7	0.3
Phenmetrazine	8.4	2	0.2	0.3	0.2	Perazine	–	6	0.5	0.8	0.2
Dibenzepin	–	8	0.2	0.4	0	Thioridazine	9.5	6	0.5	1.1	0.5
Trimipramine	–	8	0.2	0.4	0.2	Monoacetyl morphine	–	7	0.6	0.5	0.3

(Continued on p. 76)

TABLE V *(continued)*

Compound	*pK*$_a$	*Class*	*k' on*			*Compound*	*pK*$_a$	*Class*	*k' on*		
			SI	*SH*	*SCX*				*SI*	*SH*	*SCX*
Triprolidine	–	1	0.6	0.7	0.4	Methyl ephedrine	9.3	2	1.1	0.9	1.0
Amphetamine	9.9	2	0.6	0.7	1.5	Pentaquine	–	9	1.1	2.0	2.2
Amopyroquine	–	9	0.6	0.8	0.1	Pholcodine	9.3	7	1.1	1.1	0.6
Thebaine	8.2	7	0.6	0.8	0.4	Ephedrine	9.6	2	1.2	1.0	1.9
Methoxypromazine	–	6	0.6	0.8	0.4	Mesoridazine	–	6	1.2	1.4	0.7
Imipramine	9.5	8	0.6	0.8	0.4	Antazoline	10.0	1	1.3	1.6	2.6
Aminopromazine	–	6	0.6	0.9	0.8	Primaquine	–	9	1.3	1.9	2.7
Methadone	8.3	5	0.6	1.0	0.8	Methyl amphetamine	10.1	2	1.4	1.2	1.9
Narceine	9.3	5	0.7	0.5	0.4	Methdilazine	–	6	1.4	1.8	1.6
Amydricaine	–	4	0.7	0.6	0.9	Quinacrine	10.3	9	1.4	1.8	1.6
Hydroxyamphetamine	9.3	2	0.7	0.6	1.8	Desipramine	10.2	8	1.5	1.8	2.1
Codeine	8.2	7	0.7	0.8	0.5	Mephentermine	10.4	2	1.6	1.4	2.3
Promazine	9.4	6	0.7	0.9	0.5	Protriptyline	–	8	1.6	2.2	2.6
Prothipendyl	–	1, 8	0.7	0.9	0.5	Dihydrocodeine	8.8	7	1.8	1.1	0.9
Tofenacin	–	3	0.7	0.9	1.0	Hydromorphone	8.2	7	1.8	1.0	0.9
Diphenylpyraline	–	1	0.8	0.8	0.4	Strychnine	8.0	–	1.9	2.1	0.8
Benzylmorphine	–	7	0.8	0.8	0.3	Maprotiline	–	8	1.9	2.4	2.8
Procyclidine	–	1	0.8	0.8	0.8	Dihydromorphine	8.6	7	2.0	1.1	1.1
Nicomorphine	–	7	0.8	1.3	0.3	Levorphanol	8.2	7	2.0	1.4	1.3
Dimethoxanate	–	6	0.9	0.9	0.5	Levomethorphan	–	7	2.0	1.9	1.3
Morphine	9.9	7	1.0	0.5	0.6	Racemorphan	–	7	2.1	1.6	1.4
Pheniramine	9.3	1	1.0	0.7	0.5	Norcodeine	–	7	2.4	1.7	2.5
Chlorpheniramine	9.2	1	1.0	0.8	0.5	Normorphine	–	7	2.7	1.6	3.2
Pipazethate	–	8	1.0	1.2	0.8	Chloroquine	10.8	9	2.7	2.2	2.5
Ethoheptazine	–	5	1.0	1.4	0.8	Norlevorphanol	–	7	3.0	2.7	4.6
Nortriptyline	10.0	8	1.0	1.5	1.6	Atropine	9.9	–	3.4	1.8	1.9
Phenylephrine	10.1	2	1.1	0.7	2.9						

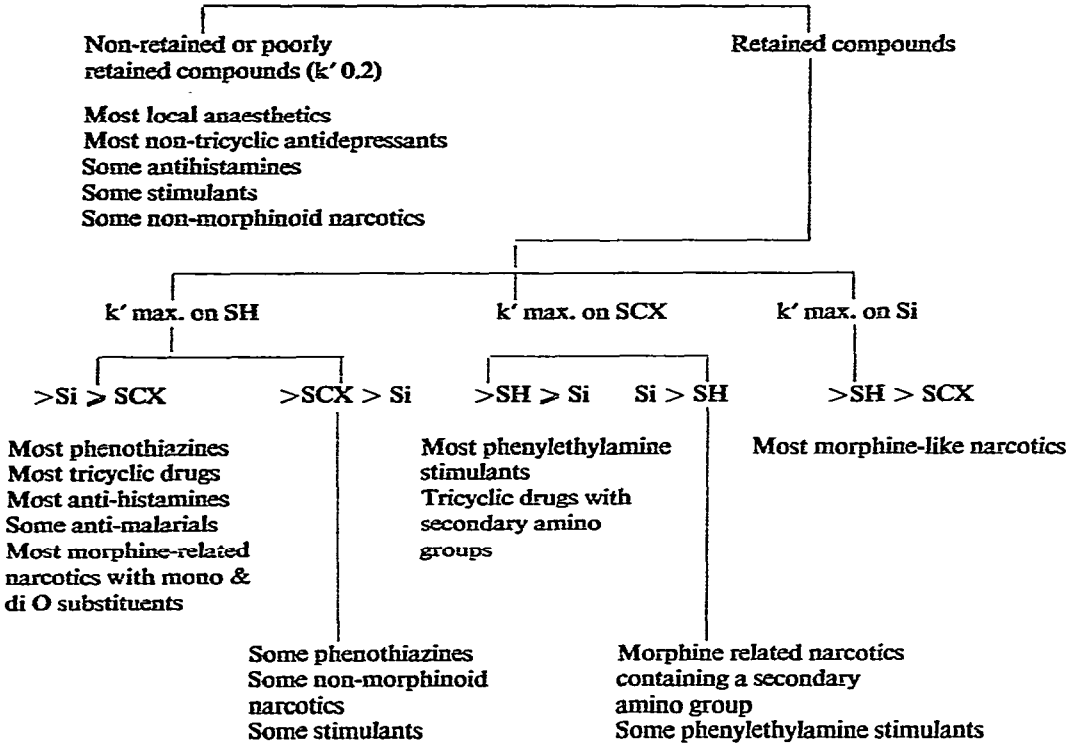
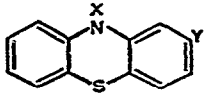


Fig. 1. Diagramatic representation of the interrelationship between drug type and the chromatographic properties on silica, a mercapto-modified silica and a strong cation-exchanger.

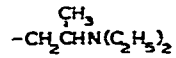
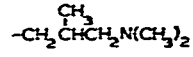
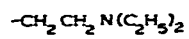
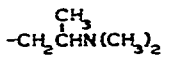
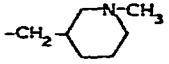
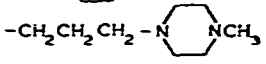
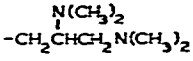
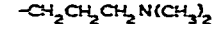
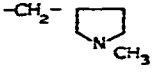
groups, particularly aromatic species. Drugs which are appreciably retained usually contain an alkylamino group substituted into an aromatic ring system. Such a classification is not particularly revealing and it is more useful to consider groups of compounds with similar properties or structures.

Phenothiazines

This group of compounds all have the general formula shown below



where X is an alkylamino group containing a tertiary nitrogen moiety or some other alicyclic nitrogen-containing ring system. Y can be either H or some other substituent such as Cl, CF_3, CN etc. As a class these compounds display a wide range of retention values but with few exceptions the SH packing material retains such drugs to a greater extent than either of the other two packings. The degree of retention is markedly dependent upon the character of the group X.

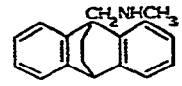
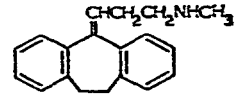
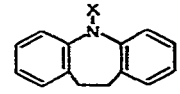
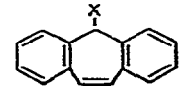
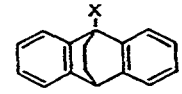
Compound (Y = H)	*X*	*k' (Si, SH, SCX)*
Ethopropazine		0.2, 0.4, 0.3
Trimeprazine		0.2, 0.5, 0.2
Diethazine		0.2, 0.5, 0.3
Promethazine		0.3, 0.6, 0.2
Mepazine		0.4, 0.6, 0.3
Perazine		0.5, 0.8, 0.2
Aminopromazine		0.6, 0.9, 0.8
Promazine		0.7, 0.9, 0.5
Dimethoxanate		0.9, 0.9, 0.5
Methdilazine		1.4, 1.8, 1.6

Substitution at the Y position in the ring with group X remaining constant results in far less variation in retention characteristics, although in general reduced retention is observed when H is replaced by electron attracting groups. Consider for example

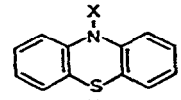
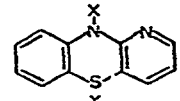
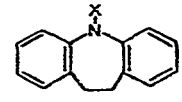
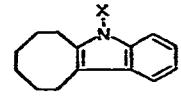
Compound X = $CH_2CH_2CH_2N(CH_3)_2$	*Y*	*k' (Si, SH, SCX)*
Triflupromazine	CF_3	0.3, 0.4, 0.2
Chlorpromazine	Cl	0.4, 0.7, 0.3
Methoxypromazine	OCH_3	0.6, 0.8, 0.4
Promazine	H	0.7, 0.9, 0.5

Tricyclic compounds other than phenothiazines

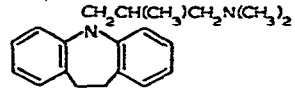
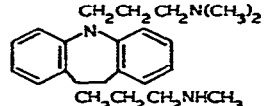
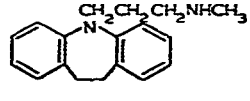
In general drugs falling into this category show a rather similar pattern of retention behaviour to that displayed by phenothiazines. One notable difference is that a number of the compounds have an alkylamino group in which the amino group is in a secondary form. This results in a more basic character and gives rise to longer retention on the SCX column.

Compound X = $CH_2CH_2CH_2NHCH_3$		*k′ (Si, SH, SCX)*
Benzoctamine		0.1, 0.3, 0.5
Nortriptyline		1.0, 1.5, 1.6
Desipramine		1.5, 1.8, 2.1
Protriptyline		1.6, 2.2, 2.6
Maprotiline		1.9, 2.4, 2.8

Those drugs with an alkylamino group with tertiary functionality or a heterocyclic nitrogen-containing substituent are less basic in character and maximum retention occurs with the SH column as is the case with phenothiazines. The substituent group again seems to be the major factor in determining the degree of retention for the variation in the fused ring system does not exert a marked influence.

Compound X = $CH_2CH_2CH_2N(CH_3)_2$		*k′ (Si, SH, SCX)*
Promazine		0.7, 0.9, 0.5
Prothipendyl		0.7, 0.9, 0.5
Imipramine		0.6, 0.8, 0.4
Iprindole		0.5, 0.7, 0.3

Whereas changing the substituent group has a pronounced effect.

Compound		*k′ (Si, SH, SCX)*
Trimipramine		0.2, 0.4, 0.2
Imipramine		0.6, 0.8, 0.4
Desipramine		1.5, 1.8, 2.1

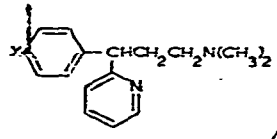
As with phenothiazines the introduction of an electron attracting group in the 2 position of the tricyclic ring gives rise to some reduction in retention.

Anti-depressants (non-tricyclic)

The compounds falling into this class display a variety of chemical structures almost all with the nitrogen atom(s) present in a heterocyclic ring system or in the form of a hydrazide. With the exception of Vilaxazine (a substituted morpholine) and Tofenacin (a compound with an alkylamino functional group) the drugs falling into this category were not appreciably retained.

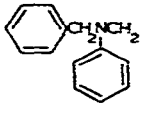
Antihistamines

Antihistamine drugs have diverse chemical structures but most of the compounds are retained to the maximum extent on the SH column with slightly lower retention on the Si and SCX systems. The SH solubility is apparently far less marked than for phenothiazines. The exceptions to the previous generalisation are the related compounds pheniramine and chlorpheniramine which have maximum retention on the Si column and antazoline which is strongly bound to the SCX material.



PHENIRAMINE X = H

CHLORPHENIRAMINE X = Cl



ANTAZOLINE

Cyproheptadine is a tricyclic antihistamine and displays chromatographic characteristics far more reminiscent of other tricyclic compounds.

Local anaesthetics

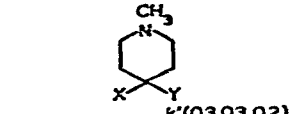
This class of materials are almost all ester derivatives of benzoic acid (or a substituted benzoic acid) with the alcoholic moeity of the molecule containing a basic nitrogen group. With the exception of amydricaine and piperocaine the compounds are not appreciably retained and display rather similar k' values on all three columns. The two exceptions are more basic in character and are retained to the greatest extent on the SCX column.

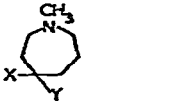
Antimalarials

Several of the drugs in this category are derivatives of isoquinoline but the substitution pattern is so diversified that it is not possible to discern meaningful interrelationships between their structure and their chromatographic characteristics. As a group, however, these compounds are retained to an appreciable extent on all three columns and this contrasts with the drugs trimethoprim and pyrimethamine which also have anti-malarial properties but are derivatives of 2,4-pyrimidine diamine. Quinacrine is a tricyclic compound and displays the characteristic maximum retention on the SH column.

Narcotic analgesics (non-morphinoid in structure)

This group of narcotic analgesics provide some interesting chromatographic phenomena. For example one structurally related group of compounds are derivatives of piperidine (*e.g.* diphenoxylate, anileridine, meperidine and α-prodine) and all are fairly weakly retained. A substantial increase in retention occurs, however, when the heterocyclic ring is increased by one carbon atom.



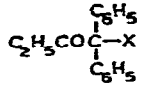


X = C_6H_5

Y = $COOC_2H_5$

k' (1.0, 1.4, 0.8)

Another structurally related group of drugs within this class are the compounds with the general formula:



Compound	*X*	*k' (Si, SH, SCX)*
Phenadoxone	$CH_2CH(CH_3)$ N O	0, 0.1, 0
Isomethadone	$CH(CH_3)CH_2N(CH_3)_2$	0.2, 0.4, 0.2
Normethadone	$CH_2CH_2N(CH_3)_2$	0.3, 0.5, 0.2
Methadone	$CH_2CH(CH_3)N(CH_3)_2$	0.6, 1.0, 0.8
Norpipanone	CH_2CH_2 N	0.2, 0.5, 0.2
Dipipanone	$CH_2CH(CH_3)$ N	0.2, 0.8, 0.6

can be seen that substituting a methyl group for a hydrogen atom on the carbon jacent to the nitrogen atom has a pronounced influence upon the basicity of the ter. Thus both methadone and dipipanone are retained quite appreciably on the X column, and the last compound displays a fourfold increase in retention on the column when compared to that on Si. As will be brought out later this type of aviour can be used to good effect. Methyl substitution on the β-carbon atom has less influence on the basicity (see isomethadone and methadone).

mulants

Many of the drugs in this class are derivatives of β-phenylethylamine. They olay a tendency to be strongly retained on the SCX column with lower retention Si and least on SH. The retention sequence closely parallels the order of inasing basicity and indicated by the pK_a values.

The morphine-like narcotics

These drugs are unusual in that they are distributed in all three retention subgroups. Thus the least basic members of the class, *i.e.* the narcotics with substituents on both of the phenolic OH groups are retained to the greatest extent on SH. Mono substituted analogues display a roughly equivalent retention on Si and SH, whereas morphine itself is retained most strongly on the Si column as are reduced derivatives such as hydromorphone, dihydromorphine, dihydrocodeine and levorphanol. Conversion of the tertiary nitrogen atom to a secondary amino form gives rise to compounds which are most strongly bound on the SCX column, whereas in the case of nalorphine substituting a CH_2-CH=CH_2 group in place of a CH_3 on the nitrogen atom reduces retention almost completely.

Practical applications

It is apparent from the foregoing text that isocratic multi-column HPLC can be a useful technique for characterising basic drugs and as the accompanying chromatograms show all three columns are able to provide efficient separations of this class of compound. In Fig. 2 for example, a test mixture of phenothiazines has been separated. It can be seen that substantial differences in retention occur on the three columns and there is even some change in the elution sequence on the SH column which retains this class of drug to the greatest extent. A changed elution sequence usually arises when a mixture of different classes of drug are chromatographed and in Fig. 3 a mixture of various phenylethylamine stimulants and tricyclic antidepressants have been separated on the different packings. Some very substantial changes occur in the elution sequence for the test components. A practical application

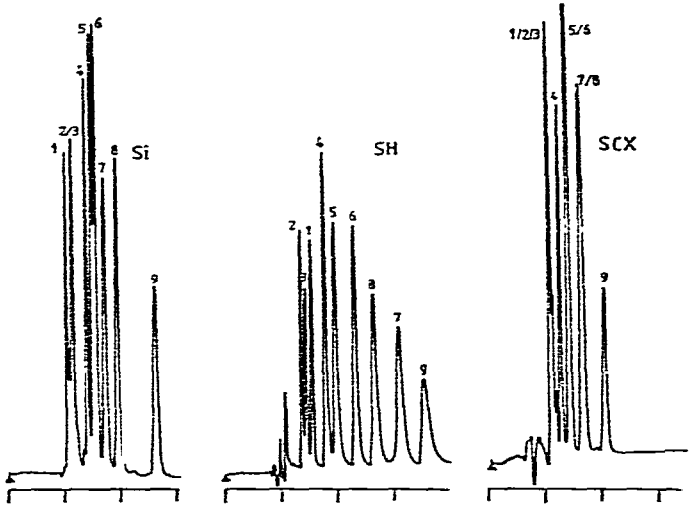


Fig. 2. Separation of pipamazine (1), fluphenazine (2), trifluomeprazine (3), trifluopromazine (4), mepazine (5), chlorpromazine (6), thioridazine (7), promazine (8) and methdilazine (9). Eluent: methanol–2 *M* ammonium hydroxide–1 *M* ammonium nitrate (27:2:1) + 50 mg of sodium sulphite per litre of solvent. Columns: 25 cm × 5 mm I.D. (1/4 in. O.D.) packed with Si, SH and SCX (1). Flow-rate: 2 ml/min. Detector: UV at 254 nm. Sensitivity: 0.1. Time intervals on the chromatogram: 4 min.

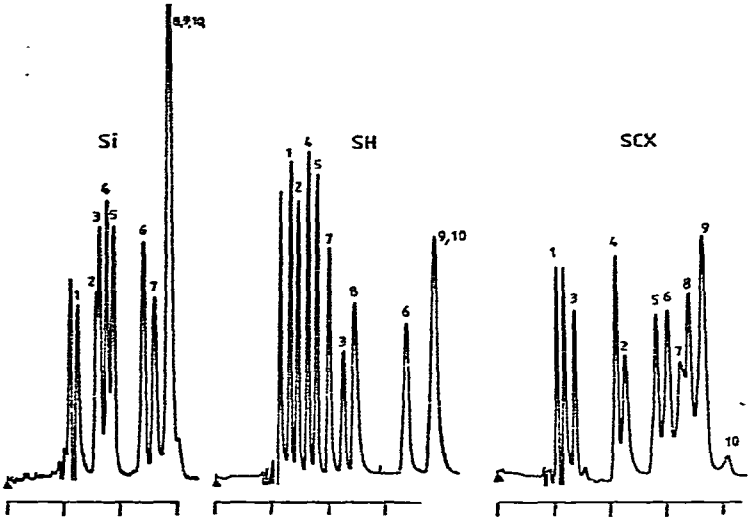


Fig. 3. Separation of benzphetamine (1), norephedrine (2), amitriptyline (3), fenfluramine (4), amphetamine (5), nortriptyline (6), ephedrine (7), methyl ephedrine (8), desipramine (9) and protriptyline (10). Chromatographic conditions as in Fig. 2.

of the method that has been of value in this laboratory concerns the analysis of cyclizine/dipipanone preparations (marketed under the proprietary name of Diconal). This particular drug combination is subject to some abuse and as such the detection of the drugs in admixture is of forensic importance. On the standard Si column the two drugs are inadequately separated (see Fig. 4) but excellent resolution occurs on the SH and SCX columns. Another drug separation of forensic importance is that of mixtures containing morphine and its monoacetyl and diacetyl derivatives. Fig. 5

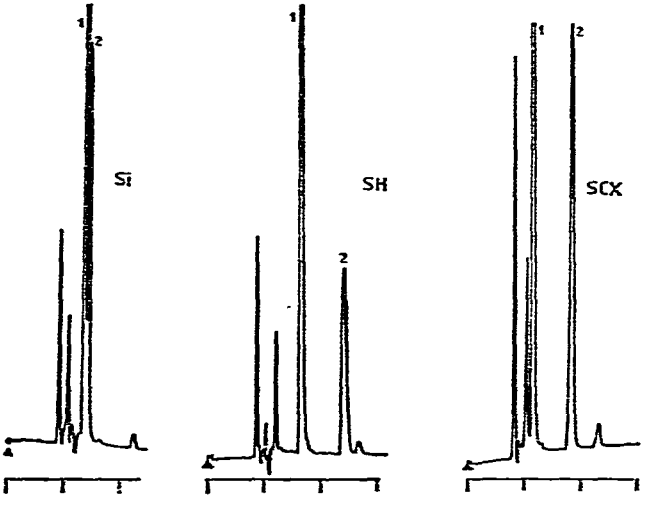


Fig. 4. Methanol extract of a Diconal tablet. 1 = Cyclizine, 2 = dipipanone. Chromatographic conditions as in Fig. 2.

shows that the three compounds are resolved on Si and SCX, but not on SH. Strychnine, an additive found in some illicit diamorphine preparations, can be eluted much more rapidly from the SCX column than from the Si.

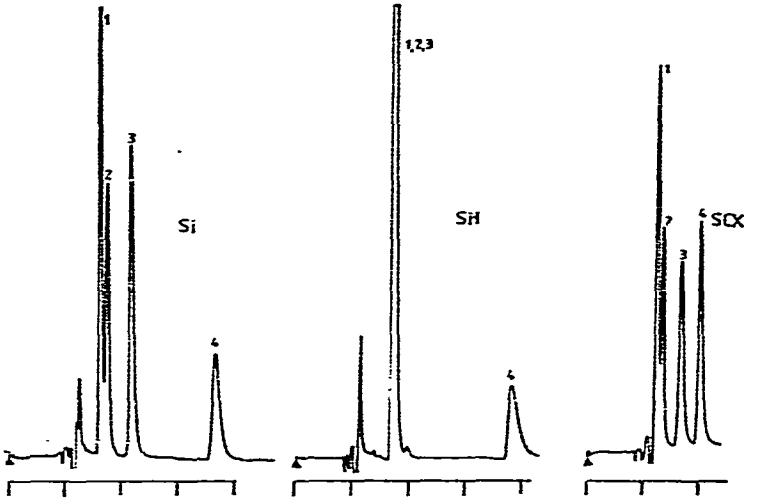


Fig. 5. Separation of diacetylmorphine (1), monoacetylmorphine (2), morphine (3) and strychnine (4). Chromatographic conditions as in Fig. 2.

It was mentioned in the introduction that one of the major advantages of isocratic multi-column HPLC is the capability that the method offers for achieving separations without having to change the solvent in either the pump or the detector. An even more convenient operational advantage can be gained by mounting in parallel two columns containing different packing materials. This is shown diagrammatically in Fig. 6 and it can be seen that the solvent emerging from a single pump can be split to pass down each column and then recombined before entering the detector. Provided the tee contains minimal dead volume we have found a negligible loss in chromatographic efficiency as a result of the post-column recombination. The main use of such a configuration is in those laboratories, such as our own, undertaking a variety of different analyses but with a sample input rate that does not justify assigning a particular HPLC system for one particular analysis. In this situation submission of a sample that could not be directly analysed on one of the separation systems already operating would require the introduction of a new column and/or solvent. This not only takes time but can lead to the introduction of air into both pump and detector, a process to be avoided if possible. A disadvantage of the type of column assembly shown is that to maintain a volumetric flow-rate down each column comparable to that which would normally be used with a single column one must accept a doubling of solvent usage (assuming comparable pressure drops down both columns). In addition sample dilution after the separation results in analyses having about half the sensitivity. These problems could be avoided by using a switching valve system at the solvent inlet side of the equipment, and no doubt if a low dead-volume multiple inlet/single outlet post-column adaptor could be made it

would be possible to mount several columns in parallel. At the present time in this laboratory it is only the configuration shown in the diagram that has been exploited.

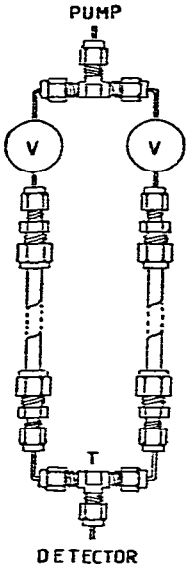


Fig. 6. Diagram of an isocratic dual column assembly. V = injection valve. T = a stainless-steel 1/16 in. union tee drilled out for zero dead volume and joined to each column with a length of 1/16 in. O.D., 0.010 in. I.D. stainless-steel tubing. The tubes are virtually butted together in the tee. The outlet line to the detector was PTFE 1/16 in. O.D., 0.006 in. I.D., this was also butted on to the two steel tubes leaving minimal space between.

Isocratic multi-column HPLC, particularly if used in conjunction with variable wavelength UV detection, can provide a very powerful method of characterising compounds. There seems no reason why it should not also be applied to separating acidic compounds, and possibly neutral molecules, provided a suitable solvent and set of columns can be developed.

REFERENCES

1 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
2 B. B. Wheals, *J. Chromatogr.*, 122 (1976) 85.
3 R. Verpoorte and A. Baerheim Svendson, *J. Chromatogr.*, 124 (1976) 152.
4 R. G. Achari and E. E. Theimer, *J. Chromatogr. Sci.*, 15 (1977) 320.
5 R. G. Achari, J. L. Baldridge, T. R. Koziol and L. Yu, *J. Chromatogr. Sci.*, 16 (1978) 271.
6 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109.
7 A. Wehrli, J. C. Hildenbrand, H. P. Keller, R. Stampfli and R. W. Frei, *J. Chromatogr.*, 149 (1978) 199.
8 B. B. Wheals, *J. Chromatogr.*, 177 (1979) 263.
9 G. B. Cox, C. R. Loscombe, M. J. Slucutt, K. Sugden and J. A. Upfield, *J. Chromatogr.*, 117 (1976) 269.
10 B. B. Wheals, *J. Liquid Chromatogr.*, 2 (1979) 91.
11 K. Sugden, G. B. Cox and C. R. Loscombe, *J. Chromatogr.*, 149 (1978) 377.