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# SEPARATION OF TRICYCLIC PSYCHOSEDATIVE DRUGS BY HIGH-SPEED ION-PAIR PARTITION AND LIQUID–SOLID ADSORPTION CHROMATO-GRAPHY

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SUMMARY

The application of high-speed ion-pair partition and liquid–solid adsorption chromatography to the separation of twenty common tricyclic tranquillizers and anti-depressant drugs is described.

In the ion-pair system, amine–perchlorate ion-pairs were extracted from an aqueous stationary phase supported on 10-$\mu$m silica gel by organic eluents containing a chloromethane and a higher aliphatic alcohol, and chromatographic parameters for elution by eight eluent mixtures are presented. Using 5 mm $\times$ 120 mm columns good separations, according to chemical class, were achieved.

For adsorption chromatography, the components were eluted from 20-$\mu$m spherical alumina using eluents containing methylene chloride, *n*-hexane or *n*-pentane, and acetic acid. Chromatographic parameters are given for eight eluent compositions. Components differing little in structure are well separated by liquid–solid adsorption chromatography. Compared with ion-pair partition chromatography, adsorption chromatography is much more selective for compounds of the same chemical type. The two methods are therefore complementary. Both methods gave plate heights in the range of 0.1 to 0.3 mm.

## INTRODUCTION

Since 1969 high-speed liquid chromatography (HPLC) has been applied to a great variety of pharmaceutical preparations, for example, narcotics such as the opium alkaloids, heroin[1–3] and methadone[3]; analgesics such as phenacetine[4], aspirin, caffeine and paracetamol[2,4–6]; barbiturates[7–9]; diphenylhydantoine[7,8]; sulphonamides[10]; actinomycin[11]; vitamins[12–14]; steroids[15–17]; and psychoreactive drugs such as lysergic acid[18,19], some phenethylamines[20], librium[21], and some benzodiazepines[22]. However, of the vast number of tricyclic antidepressants and tranquillizers present on the market only one of the phenothiazines, namely thioridazine, has been separated from its metabolites[23].

The present study describes the separation of some twenty common tricyclic

tranquillizers and antidepressant drugs by HPLC using two distinct separation methods, ion-pair partition and the liquid–solid adsorption chromatography.

The feasibility of chromatographic separation of these components in form of ion-pairs may be predicted from the work of Persson[24–27], who has shown that the psychosedative amines with condensed ring systems can be extracted from aqueous media by formation of ion-pairs with an appropriate counter-ion, and indeed the selectivity of the ion-pair distribution process has recently been applied in the separation by liquid chromatography of alkylammonium picrate ion-pairs[28] and of perchlorate ion-pairs of some biogenic amines[29].

The essential feature of ion-pair partition[24] of an amine, for example, is distribution according to the equilibrium

$$HA^+ + X^- \rightleftharpoons HAX$$
aqueous         organic
phase            phase
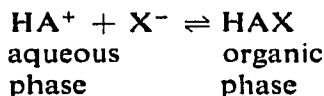
where A is an amine, $HA^+$ an ammonium ion, $X^-$ an anion and HAX the ion-pair. The extraction constant is then

$$E_{HAX} = \frac{[HAX]_{Org}}{[HA^+]_{aq} [X^-]_{aq}}$$

To a first approximation, if there are no complicating side-equilibria (e.g. hydrolysis of $HA^+$, dimerization of HAX, partition of A between the two phases), $E_{HAX}$ is equal to the conditional extraction constant

$$E_{HAX}^* = \frac{a_{Org}}{a_{aq} \, x_{aq}}$$

where $a$ and $x$ are the total concentrations of A and X in the appropriate phases irrespective of their actual molecular forms. Thus, for example,

$$a_{aq} = [HAX]_{aq} + [HA^+]_{aq} + [A]_{aq} + \cdots$$

The distribution coefficient between the two phases is then

$$D \equiv \frac{a_{aq}}{a_{Org}} = \frac{1}{E_{HAX}^* \, x_{aq}}$$

and the retention volume in a chromatographic system using the organic liquid as mobile phase is

$$V_R = V_m + DV_s$$

where $V_m$ and $V_s$ are the volumes of mobile and stationary phases. The column capacity ratio $k'$, which is obtained from the elution record as $k' = (t_R - t_0)/t_0 = (V_R - V_m)/V_m$ (where $t_R$ and $t_0$ are the retention times of retained and unretained solutes), is then given by

$$k' = DV_s/V_m$$

In the present work, ion-pairs of amine cations with perchlorate anions were extracted from an aqueous stationary phase by an organic mobile phase containing a higher aliphatic alcohol as a solvating agent. In order to assess the potential of high-speed ion-pair partition chromatography in the analysis of tranquillizers, a parallel study was carried out using liquid–solid adsorption chromatography.

## MATERIAL AND METHODS

### Solutes

We gratefully acknowledge gifts of tranquillizers whose names, formulae and sources are listed in Table I. The compounds belong to the dibenzocycloheptane, dibenzocycloheptene, dibenzazepine, dihydrodibenzazepine and phenothiazine groups. Samples to be analysed were made up by dissolving the appropriate compounds in mobile phase on the day of the experiment to give solutions containing approximately 5 mg/ml. Between 0.1 and 5 $\mu$l were injected using a high-pressure microsyringe.

### Equipment

The high-speed liquid chromatograph was constructed in the laboratory. It employed a gas-driven Haskel pump (Burbank, Calif., U.S.A.; Model No. 26920) and a UV monochromatic detector (254 nm) (DuPont). Operative pressures were between 80 and 200 p.s.i. Columns, 5 mm in bore and 125 mm long, of internally polished stainless steel were operated at ambient temperature. Column fittings were made according to designs described elsewhere[30]. Columns were terminated by 6-$\mu$m porosity metal frits. Porous PTFE frits blocked readily and were unsatisfactory.

### Column packings and eluents

For ion-pair partition chromatography, the column was packed with 10-$\mu$m Merckosorb S1100 using the balanced slurry techniques[31]. The packing, suspended in methyl iodide as a balanced density solvent, was pumped continuously into the column under a pressure of 3000 p.s.i. and compacted by several subsequent impulses. The column was dried by passage of air and loaded with stationary phase by drawing 5 ml of an aqueous solution of perchloric acid and sodium perchlorate (0.1 and 0.9 $M$, respectively) through the column using suction at the outlet. The organic mobile phase was then pumped into the column until the emerging liquid was clear. Thereafter no more bleeding of the stationary phase occurred. To saturate the eluent with stationary phase a stainless-steel pre-column, 5 mm in bore and 500 mm long, was placed before the main column. It was packed with 80–100 mesh Chromosorb W bearing 20% by weight of stationary phase. Eluents were mixtures of methylene chloride or chloroform and 1-butanol or isoamyl alcohol.

For adsorption chromatography, Spherisorb A20Y (20-$\mu$m-diameter spherical alumina manufactured by Material Preparations Unit, AERE, Harwell, Great Britain, and marketed by Phase Separations, Queensferry, Great Britain) was dry packed into standard columns using an automatic rotate-and-bounce device with additional sideways tapping[32]. The column was activated by passage of dry methanol (200 ml), sodium-dried diethyl ether (200 ml) and conditioned by passage of 200 ml of eluent. Eluents were mixtures of methylene chloride, acetic acid (1–10%), pentane or hexane (0–25%), and water (0–0.1%).

TABLE I

LIST OF TRANQUILLIZERS

Suppliers: AH = Allen and Hanbury, Bethnal Green, Great Britain; ERS = E.R. Squibb & Sons, Twickenham, Great Britain; EL = Eli Lilly & Co., Basingstoke, Great Britain; EU = Edinburgh University Laboratory Sample; G = Geigy Pharmaceuticals, Macclesfield, Great Britain; JW = John Wyeth Bros., Taplow, Maidenhead, Great Britain); M & B = May and Baker, Dagenham, Great Britain; MSD = Merck Sharpe and Dohme, Hoddesdon, Great Britain; SKF = Smith Kline and French Laboratories, Welwyn Garden City, Great Britain; SP = Sandos Products, Horsforth, Leeds, Great Britain; W = Wander, Horsforth, Leeds, Great Britain.
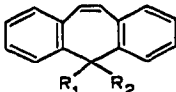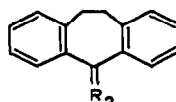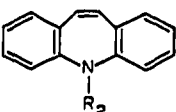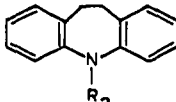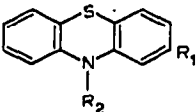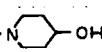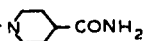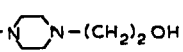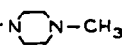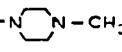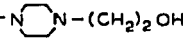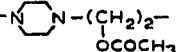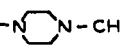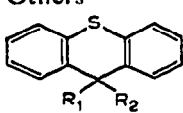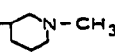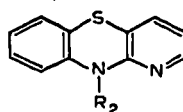
| Group | Name | $R_1$ | $R_2$ | Source |
|---|---|---|---|---|
| Dibenzocycloheptenes and heptanes | | | | |
| | Protriptyline · HCl | –H | –(CH$_2$)$_3$ N(H)(CH$_3$) | MSD |
| | Nortriptyline · HCl | | =CH(CH$_2$)$_2$ N(H)(CH$_3$) | EL |
| | Amitriptyline · HCl | | =CH(CH$_2$)$_2$ N(CH$_3$)$_2$ | EL |
| Dibenzazepine | | | | |
| | Opipramol · HCl | | –(CH$_2$)$_3$–N◯N–(CH$_2$)$_2$OH | G |
| Dihydrodibenzazepines | | | | |
| | Imipramine · HCl | | –(CH$_2$)$_3$ N(CH$_3$)$_2$ | G |
| | Trimipramine maleate | | –CH$_2$CH(CH$_3$)–CH$_2$N(CH$_3$)$_2$ | EU |
| Phenothiazines | | | | |
| | Promazine | –H | –(CH$_2$)$_3$ N(CH$_3$)$_2$ | EU |
| | Ethopromazine B.P. | –H | –CH$_2$CH(CH$_3$)CH$_2$ N(C$_2$H$_5$)$_2$ | M & B |
| | Methotrimeprazine maleate | –O–CH$_3$ | –CH$_2$CH(CH$_3$)CH$_2$ N(CH$_3$)$_2$ | M & B |
| | Propiomazine · HCl | –CO–C$_2$H$_5$ | –CH$_2$CH(CH$_3$)CH$_2$ N(CH$_3$)$_2$ | JW |

TABLE I *(continued)*

| Group | Name | $R_1$ | $R_2$ | Source |
|---|---|---|---|---|
| | Pericyazine | –CN | –(CH$_2$)$_3$–N◯–OH | M & B |
| | Pipamazine B.P. | –Cl | –(CH$_2$)$_3$–N◯–CONH$_2$ | M & B |
| | Perphenazine B.P. | –Cl | –(CH$_2$)$_3$–N◯N–(CH$_2$)$_2$OH | AH |
| | Prochloroperazine maleate* | –Cl | –(CH$_2$)$_3$–N◯N–CH$_3$ | M & B |
| | Trifluoroperazine | –CF$_3$ | –(CH$_2$)$_3$–N◯N–CH$_3$ | M & B |
| | Fluphenazine·HCl | –CF$_3$ | –(CH$_2$)$_3$–N◯N–(CH$_2$)$_2$OH | ERS |
| | Thiopropazate·HCl B.P. | –OCl | –(CH$_2$)$_3$–N◯N–(CH$_2$)$_2$– OCOCH$_3$ | M & B |
| | Thiethylperazine maleate* | –SCH$_2$CH$_3$ | –(CH$_2$)$_3$–N◯N–CH$_3$ | SP |
| Others | Methixen | –H | –CH$_2$◯N–CH$_3$ | W |
| | Prothipendyl·HCl hydrate | | –(CH$_2$)$_3$N(CH$_3$)$_2$ | SKF |

\* Insoluble in methylene chloride and studied only in ion-pair chromatography.

## RESULTS

To establish that columns were well packed their efficiencies were tested using a standard mixture of aromatic hydrocarbons (toluene, styrene, phenanthrene and biphenyl) with *n*-hexane (50% water-saturated) as eluent. Plots of reduced plate height, $h = H/d_p$, against reduced velocity, $v = ud_p/D_m$, where $u$ is the linear velocity, are shown in Fig. 1 for 10-$\mu$m Merckosorb SI100 and 20-$\mu$m Spherisorb A20Y packings. The performance of each adsorbent agrees with that reported previously[33]. The 5 × 125 mm columns gave a maximum efficiency corresponding to about 3500 theoretical plates ($h = 2$ to $4$) at linear velocities of 0.02–0.05 cm/sec ($v = 3$ to $10$). Column resistance parameters defined as $\varnothing = \Delta pL\eta/ud_p^2$ were 850 for Merckogel and 1300 for Spherisorb, where $\Delta p$ is the pressure drop, $L$ the column length and $\eta$ the viscosity of the eluent.

*Ion-pair chromatography*

Table II lists the column capacity ratios, $k'$, of various drugs when eluted by eight eluents from Merckosorb SI100 silica gel bearing 0.1 *M* HClO$_3$ + 0.9 *M* NaClO$_3$ as stationary phase.

Increase in the percentage of alcohol in the mobile phase caused $k'$ to decrease

TABLE II

k'VALUES FOR TRANQUILLIZERS IN ION-PAIR CHROMATOGRAPHY

Support: 10-μm Merckosorb SI100; stationary phase: 0.10 $M$ HClO$_4$ + 0.90 $M$ NaClO$_4$. Solvent systems: (1) chloroform–1-butanol (2:1); (2) chloroform–1-butanol (1:1); (3) chloroform–1-butanol (1:2); (4) chloroform–1-butanol (1:3); (5) chloroform–isoamyl alcohol (1:3); (6) methylene chloride–1-butanol (3:1); (7) methylene chloride–isoamyl alcohol (1:3); (8) methylene chloride–1-butanol–isoamyl alcohol (6:3:1).

| Major side chain | Class | Name | Solvent | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Aliphatic amino | Dibenzocycloheptene | Protriptyline | 0.7 | 0.6 | 0.5 | 0.4 | 0.6 | 0.6 | 0.2 | 0.2 |
| | Dibenzocycloheptane | Nortriptyline | 1.1 | 0.7 | 0.6 | 0.3 | 0.5 | 0.6 | 0.2 | 0.2 |
| | | Amitriptyline | 2.0 | 1.7 | 1.2 | 0.6 | 1.4 | 0.8 | 0.5 | 0.4 |
| | Dihydrodibenzazepine | Trimipramine | 2.1 | 1.5 | 1.3 | 0.7 | 1.1 | 0.9 | 0.5 | 0.5 |
| | | Imipramine | 2.1 | 1.7 | 1.4 | 0.9 | 1.3 | 0.8 | 0.5 | 0.5 |
| | Phenothiazine | Ethopromazine | – | 2.0 | 1.8 | 1.0 | 1.8 | 0.9 | 1.5 | 0.6 |
| | | Methotrimeprazine | 2.1 | 1.9 | 2.0 | 1.1 | 1.7 | 0.8 | 0.7 | 0.7 |
| | | Promazine | 2.6 | 2.4 | 2.3 | 1.3 | 1.8 | 0.6 | 0.9 | 0.6 |
| | | Propiomazine | – | 2.4 | 1.8 | 1.0 | 1.7 | 1.0 | 0.7 | 0.6 |
| | Other | Prothipendyl | 7.0 | 6.7 | 5.7 | 3.2 | 4.0 | 3.7 | 2.1 | 1.5 |
| Piperidine | Other | Methixen | 2.1 | 1.7 | 1.5 | 0.8 | 1.3 | 0.8 | – | 0.6 |
| | Phenothiazine | Pericyazine | 6.0 | 2.9 | 2.1 | 1.3 | 1.8 | 2.1 | 1.1 | 0.8 |
| | | Pipamazine | – | 3.2 | 2.5 | 1.3 | 2.1 | 4.0 | 1.2 | 1.0 |
| Piperazine | Phenothiazine | Thiopropazate | – | 4.7 | 3.9 | 1.8 | 2.4 | 3.8 | 1.7 | 0.8 |
| | | Thiethylperazine | – | 10.3 | 5.3 | 1.7 | 3.3 | 6.9 | 1.5 | 1.4 |
| | | Fluphenazine | 36.0 | 10.8 | 5.3 | 2.7 | 3.4 | 7.1 | 1.5 | 1.4 |
| | | Perphenazine | – | 10.7 | 5.9 | 2.9 | 3.5 | 7.6 | 1.7 | 1.3 |
| | | Prochlorperazine | – | 12.7 | 6.5 | 3.0 | 4.7 | 11.0 | 1.9 | 1.4 |
| | | Trifluoroperazine | 36.0 | 12.6 | 7.0 | 3.2 | 4.0 | 10.9 | 1.8 | 1.5 |
| Dibenzazepine | | Opipramol | 36.0 | 11.1 | 7.1 | 3.8 | 5.0 | 14.0 | 2.2 | 1.8 |
| | | Number of theoretical plates | 400–700 | 300–700 | 600–1500 | 500–800 | 400–600 | 700–1000 | 700–1200 | 1000–2500 |
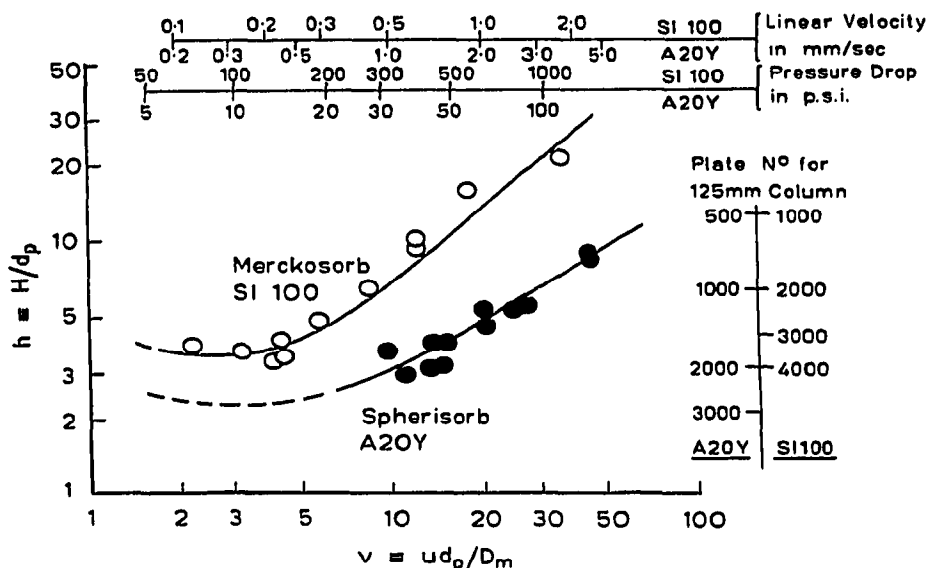
Fig. 1. Plots of reduced plate height, $h$, against reduced velocity, $v$, for elution of toluene from 10-$\mu$m Merckosorb SI100 (slurry packed) and 20-$\mu$m Spherisorb alumina AY (dry packed). Column dimensions: 125 × 5 mm.

(Solvents 1–4). Replacement of 1-butanol by isoamyl alcohol (Solvents 4 and 5) caused $k'$ to increase and replacement of chloroform by methylene chloride (Solvents 5 and 7) caused $k'$ to decrease. These changes are in the direction expected on the basis of the changes in Snyder's solvent-strength parameter, $\varepsilon_0$ (ref. 34), as shown in Table III; that is, the stronger the solvent (higher $\varepsilon_0$) the more weakly retained or the more strongly extracted is any ion-pair. Notwithstanding these general effects the response of individual components to changes in the percentage of alcohol in the mobile phase differed considerably. For example, the $k'$ values of the phenothiazines with a piperazine-containing side chain decreased about four times going from 1:1 to 1:3 methylene chloride–butanol (Solvents 2 and 4) while the $k'$ values of most other compounds were roughly halved, although the order of elution of components was more or less unaffected by the nature of the solvent.

In ion-pair partition chromatography the elution order is strongly influenced by the chemical type of the solute. Thus the dibenzocycloheptane derivatives were eluted first with the methylated compound, amitriptyline, the most retained. The dihydrodibenzazepines were retained to about the same extent.

All members of the phenothiazine group were retained longer than the members of the first two groups, but within the phenothiazine group those that had alkylaminoisobutyl side chains attached to the nitrogen in the central ring (ethopromazine, methotrimeprazine and propiomazine) were eluted first although they could not be well separated from each other in spite of different substituents for the hydrogen at carbon atoms 2 of the condensed ring system. However, using Solvent 3 they could be separated from promazine, which has an alkylaminopropyl side chain. The phenothiazines with a piperidine group in the side chain were significantly more strongly retained, while those with a piperazine ring were much more strongly re-

TABLE III

INFLUENCE OF SOLVENT CHANGE ON $\varepsilon_0$ AND $k'$

| Solvent change | $\varepsilon_0$ change | Effect on $k'$ |
|---|---|---|
| Butanol to isoamyl alcohol | 0.76 to 0.70* | 1.4 to 2 × increase |
| Methylene chloride to chloroform | 0.42 to 0.40 | 1.4 to 2.5 × increase |

* Extrapolated values[34] on the basis of first three numbers of series methanol (0.94), ethanol (0.88), 1-propanol (0.82).

tained. Among the latter fluphenazine and perphenazine (which differ only by the substituent at the carbon atom 2 in the condensed ring system) were not resolvable confirming that substituents in this position have a small influence on the $k'$.

Of the compounds with mixed structures, methixen was eluted before the phenothiazine group, while prothipendyl with a pyridine group in the condensed ring system was eluted towards the end of the phenothiazine group, its exact position being dependent upon the alcohol content of the mobile phase. Opipramol, a compound with an azepine ring and a long side chain containing the piperazine group, was generally retained longer than any of the other components. The effects of structure on retention may be summarized as follows.

(1) The central heterocylic group of the fused ring system has a major influence on retention. The groups in order of increasing effect on retention are:
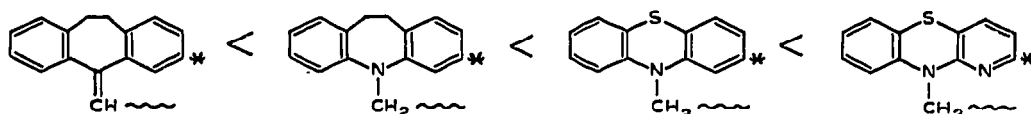


(2) Substituents in position 2 (marked *) have little effect on retention.

(3) In the phenothiazine and dibenzazepine series the order of elution is strongly influenced by the nature of the side chain attached to the central nitrogen atom. Groups in order of increasing effect on retention are:
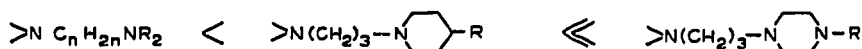


(4) The nature of R in the side chain has only a small effect on retention.

Ion-pair chromatography is most effective when a group separation is required, and the main discriminating factor is the number and basicity of the nitrogen atoms in the compounds. As expected the best separations were achieved with solvent combinations that produced the most theoretical plates. Although the effect of solvent composition on plate numbers or HETP was not fully studied, the results with chloroform and 1-butanol show that there is an optimal composition of chloroform–1-butanol (ca. 1:2), which gives a maximum number of theoretical plates (see last line of Table II). Fig. 2 illustrates the separation of seven different components obtained with such an eluent. Using methylene chloride in place of chloroform,

Fig. 2. Separation of tranquillizers by ion-pair chromatography. Support, 10-$\mu$m Merckosorb SI100; stationary phase, 0.1 $M$ HClO$_4$–0.9 $M$ NaClO$_4$ loaded *in situ*; eluent, chloroform–1-butanol (3:7); linear velocity, 0.5 mm/sec; sample, 1 $\mu$l containing 100–300 ng of each solute. Solutes: (1) nortriptyline; (2) trimipramine; (3) propiomazine; (4) pipamazine; (5) thiopropazate; (6) perphenazine; (7) opipramol.

Fig. 3. As Fig. 2 but eluent, methylene chloride–butanol–isoamyl alcohol (3:6:1). Solutes: (1) protriptyline; (2) amitriptyline; (3) promazine; (4) propiomazine; (5) perphenazine; (6) opipramol.

somewhat higher plate efficiencies were obtained. A separation obtained with a combination of methylene chloride with alcohols is shown in Fig. 3.

*Adsorption chromatography*

All samples were soluble in mobile phase except the salts of thiethylperazine and prochlorperazine, which were not studied further. The remaining 18 compounds could be eluted as well-shaped peaks from alumina with eluents composed of methylene chloride, acetic acid (1–10%) and *n*-pentane or *n*-hexane (0–25%). The presence of acetic acid was apparently essential in order to obtain stable elution conditions: water or methanol alone were insufficient. For example, an adsorbent that had been activated by passage successively of 200 ml each of dry tetrahydrofuran, sodium-dried ether and dry methylene chloride strongly retained all components when dry methylene chloride was used as eluent. When 0.01–0.02% water was added to the methylene chloride, the amines were at first readily eluted but their retention gradually increased while the peak shape deteriorated, and eventually complete retention took place.

A similar situation arose when 1–2% of methanol was added to dry methylene chloride: elution was at first accelerated but gradually slowed down over a period of hours until complete retention occurred. When acetic acid was used, however, stable elution conditions were rapidly obtained and could be guaranteed from day to day. When changing solvents, flushing with 200 ml of the new solvent was sufficient provided no water-containing eluents were used.

We believe that these rather unusual results can be explained in the following way. When eluent containing a small proportion of water or methanol is passed through the column, the adsorbent is deactivated in the normal way and accelerated elution occurs. When small samples of amine hydrochlorides are injected, the hydrochloric acid is removed by the adsorbent and unionized amine is eluted, but gradually with repeated injections the hydrochloric acid accumulates at the head of the column,

which now becomes acidic. The amines are then held very strongly as their hydro-chlorides, which are hydrophilic. This hypothesis was supported by the observation that the addition of 10 $\mu$l of water to a column from which the amines could readily be eluted caused immediate strong retention. The role of the acetic acid is thus both to deactivate the adsorbent and to provide a weak buffering acid in place of hydro-chloric acid. The amines may indeed be eluted as acetate ion-pairs, not as unassociated amines.

The results obtained with a number of eluents are presented in Table IV.

The broad effect of increasing the proportion of acetic acid in the range 1–10% is tc accelerate elution (Eluents 2, 4, 5 and 6) while substitution of n-pentane or n-hexane for methylene chloride retards elution (Eluents 1–5). The addition of small quantities of water (up to 0.1%) in the presence of acetic acid accelerates elution (Eluents 5, 7 and 8). These trends agree with what would be expected on grounds of polarity changes. More detailed examination reveals the following trends.

TABLE IV

$k'$ VALUES FOR TRANQUILLIZERS IN ADSORPTION CHROMATOGRAPHY

Support: Spherisorb 20-$\mu$m AY (Alumina). Eluents: (1) methylene chloride–acetic acid (99:1); (2) methylene chloride–acetic acid–n-pentane (89:1:10); (3) methylene chloride–acetic acid (97:3); (4) methylene chloride–acetic acid–n-heptane (87:3:10), (5) methylene chloride–acetic acid–n-heptane (77:3:20); (6) methylene chloride–acetic acid–n-heptane (70:10:20); (7) methylene chloride–acetic acid–n-heptane–water (77:3:20:0.01); (8) methylene chloride–acetic acid–n-heptane–water (77:3:20:0.1).

| End group in main chain | Class | Name | Eluent | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| –N(C$_2$H$_5$)$_2$ | Phenothiazine | Ethopromazine | 0.1 | 0.6 | 0.0 | 0.0 | 0.1 | 0.1 | 0.2 | 0.0 |
| N(CH$_2$)$_2$OCOCH$_3$ | Phenothiazine | Thiopropazate | 0.7 | 0.8 | 0.0 | 0.0 | 0.5 | 0.2 | 0.5 | 0.1 |
| –N(CH$_3$)$_2$ | Phenothiazine | Propiomazine | 1.6 | 3.1 | 0.4 | 0.7 | 1.1 | 0.3 | 0.4 | 0.2 |
| | Phenothiazine | Methotrimeprazine | 1.7 | 3.2 | 0.4 | 0.4 | 1.1 | 0.1 | 0.4 | 0.3 |
| N–CH$_3$ | Other | Prothipendyl | 1.3 | — | 1.0 | 1.3 | 1.1 | 0.2 | 0.4 | 0.5 |
| | Other | Methixen | — | 3.9* | 0.5 | 0.7 | 1.4 | 0.2 | 0.8 | 0.3 |
| | Phenothiazine | Trifluoroperazine | 2.7 | 3.9* | 0.9 | 1.5 | 1.7 | 0.2 | 0.9 | 0.5 |
| –N(CH$_3$)$_2$ | Dihydrodibenzazepine | Trimipramine | 4.7 | 5.5 | 0.5 | 0.8 | 1.3 | 0.1 | 0.6 | 0.1 |
| | Phenothiazine | Promazine | 5.2 | 6.3 | 0.7 | 1.2 | 2.1 | 0.2 | 1.2 | 1.0 |
| | Dihydrodibenzazepine | Imipramine | 8.0 | 8.8 | 0.7 | 1.2 | 1.9 | 0.2 | 0.9 | 0.3 |
| | Dibenzocycloheptane | Amitriptyline | 8.2 | 8.9 | 0.9 | 1.3 | 2.4 | 0.2 | 0.9 | 0.3 |
| –N CH–OH | Phenothiazine | Pericyazine | L** | L | 2.1 | 3.6 | 7.3 | 0.6 | 2.8 | 2.0 |
| –N N(CH$_2$)$_2$OH | Dibenzazepine | Opipramol | L | L | 2.6 | 3.2 | 6.7 | 0.6 | 2.4 | 1.5 |
| | Phenothiazine | Perphenazine | L | L | 2.6 | 4.7 | 8.1 | 0.8 | 2.6 | 2.5 |
| | Phenothiazine | Fluphenazine | L | L | 3.0 | 4.6 | 9.2 | 0.7 | 3.5 | 2.4 |
| –N CH–CONH$_2$ | Phenothiazine | Pipamazine | L | L | 4.3 | 7.1 | L | 0.8 | 4.4 | 2.5 |
| –NH–CH$_3$ | Dibenzocycloheptene | Protriptyline | L | L | 5.5 | t*** | t | 0.8 | 4.4 | 1.0 |
| | Dibenzocycloheptane | Nortriptyline | L | L | 5.7 | t | t | 0.7 | 4.7 | 1.1 |

\* 25% n-heptane.
\*\* L = Long retention, $k' > 10$.
\*\*\* t = Badly tailed peak.

*Order of elution*

The elution order differs markedly from that observed in ion-pair partition chromatography where the major influences on retention are the nature of the tricyclic condensed ring system and the presence of N-heterocyclic groups in the side chain. In adsorption chromatography on alumina the major influence appears to be the nature of terminal groups of the main side chain. Although the elution order depends to a considerable extent on the composition of the eluent, a general elution pattern can be discerned. Compounds may be grouped in order of increasing retention according to the nature of the terminal groups (see Table I) as follows.

(A)    $-N(C_2H_5)_2$    ethopromazine;⎱
(B)    $-O-CO-CH_3$    thiopropazate;  ⎰
(C)    $-N(CH_3)_2$    propiomazine, methotrimeprazine, prothipendyl;
(D)    $⎤N-CH_3$    methixen, trifluoroperazine;
(E)    $-N(CH_3)_2$    trimipramine, promazine, imipramine, amitriptyline;
(F)    $-N⎯CH-OH$    pericyazine;
(G)    $-CH_2OH$    opipramol, perphenazine, fluphenazine;
(H)    $-CONH_2$    pipamazine;
(J)    $-NH(CH_3)$    nortriptyline, protriptyline.

*Effect of acetic acid*

Increase of acetic acid content from 1 to 3% (Eluents 1–4) reduces $k'$ six to ten times for compounds of group E, about three times for group D, and about four times for group C with the exception that $k'$ for prothipendyl is unchanged. For the more strongly retained groups, F to J, increase of acetic acid content from 3 to 10% (Eluents 5 and 6) produces similar reductions of $k'$: ten to fourteen times for compounds of groups H and J, and six to ten times for compounds of groups F and G. In general the stronger the retention the greater the effect of changing the acetic acid content.

*Effects of added n-pentane and n-hexane*

Replacement of up to 20% of the methylene chloride by *n*-hexane increases retention by different amounts for different compounds (Eluents 3–5). For the majority of solutes addition of 20% *n*-hexane roughly doubled $k'$, but for a few solutes (*e.g.* prothipendyl) little change occurred. The order of elution can therefore be influenced by changing the alkane content. *n*-Pentane had an effect similar to *n*-hexane.

*Effect of water*

Addition of 0.01% water to eluents containing 3% acetic acid reduced the $k'$ values of most solutes two to three times (Eluents 5 and 7). The reductions were more or less independent of the proportion of hexane present. Further addition up to 0.1% of water had rather less effect on $k'$ and for a few solutes caused distortion of peak shapes; indeed, with pipamazine a double peak was formed. The reason for this is not clear and the effect was not studied further.

*Plate efficiencies*

Plate numbers of between 300 and 1200 were obtained in the adsorption system. These values were somewhat lower on average than observed for ion-pair chromatography and considerable peak tailing was often a problem. The plate number was independent of the eluent composition but was generally largest for components having $k'$ values in the range 0.5–1.5.

*Choice of conditions for particular separations by adsorption chromatography*

Adsorption chromatography contrasts with ion-pair chromatography, being much more selective to individual functional groups, and is preferable for separation of the members of a particular group, as shown by the following examples.

*Dihydrodibenzazepines.* Trimipramine with an isobutyl group in the side chain may be separated from imipramine with an *n*-propyl group using dry eluents containing 1–3% acetic acid. An example is shown in Fig. 4.



Fig. 4. Separation of trimipramine (1) and imipramine (2) by adsorption chromatography. Adsorbent, 20-*μ*m Spherisorb AY; eluent, methylene chloride–*n*-pentane–acetic acid (79:20:1); linear velocity, 1.0 mm/sec; sample, 2 *μ*l containing 0.3–3 *μ*g of each solute.

Fig. 5. Separation of amitriptyline (1) from nortriptyline (2) by adsorption chromatography. Adsorbent, 20-*μ*m Spherisorb AY; eluent for (A), methylene chloride–acetic acid (90:10); eluent for (B), methylene chloride–acetic acid–water (98:2:0.02); linear velocity, 5 mm/sec.

*Dibenzocycloheptanes.* Nortriptyline, a secondary amine, and amitriptyline, a tertiary amine, are widely separated using all eluents but the optimum eluents for their simultaneous analysis contain 7–10% acetic acid or 2% acetic acid and 0.02% water as shown in Fig. 5.

*Phenothiazines.* The phenothiazines differing in their aliphatic alkylamino side chains are best separated with low concentrations of acetic acid in the methylene chloride, with or without addition of *n*-pentane or *n*-hexane. However, the separation of methotrimeprazine from propiomazine required conditions illustrated in Fig. 6. These components differ only by the substitution of the carbon 2 in the condensed benzene ring and were not satisfactorily separated by ion-pair chromatography.
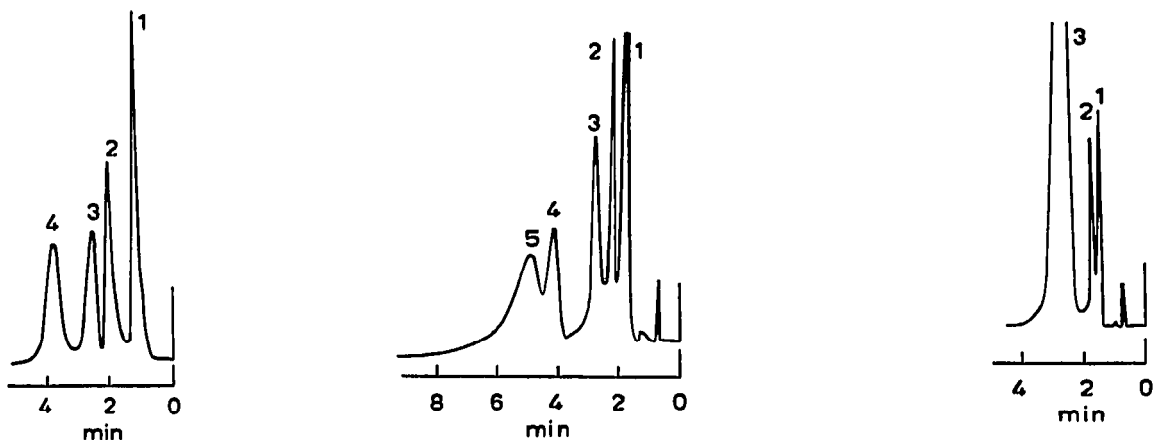
Fig. 6. Separation of phenothiazines: ethopropazine (1), methotrimeprazine (2) and propiomazine (3) from propazine (4) by adsorption chromatography. Adsorbent, 20-$\mu$m Spherisorb AY; eluent, methylene chloride–*n*-pentane–acetic acid (79:20:1); linear velocity, 3 mm/sec; sample, 2.5 $\mu$l containing 0.3–3 $\mu$g of each component.

Fig. 7. Separation of phenothiazines with cyclic side chains by adsorption chromatography. Adsorbent, 20-$\mu$m Spherisorb AY; eluent, methylene chloride–*n*-pentane–acetic acid (68:25:7); linear velocity, 1.5 mm/sec; sample, 300 ng–1 $\mu$g of each solute. Solutes: (1) thiopropazate; (2) methixen; (3) prothipendyl; (4) pericyazine; (5) pipamazine.

Fig. 8. Separation of fluorine-containing phenothiazines by adsorption chromatography. Support, 20-$\mu$m Spherisorb AY; eluent, methylene chloride–*n*-hexane–acetic acid–water (65:35:5:0.01); linear velocity, 1.7 mm/sec; sample, 1 $\mu$l containing 100 ng–1 $\mu$g of each solute. Solutes: (1) impurity in (3); (2) trifluorophenazine; (3) fluphenazine.

The separation of phenothiazines with cyclic groups in their side chains requires higher percentages of acetic acid (7–10%) and an example of such a separation is shown in Fig. 7; trifluorophenazine is easily separable from fluphenazine using the adsorption system (Fig. 8), but it is poorly separated by ion-pair chromatography.

## DISCUSSION

### Elution order and retention

In ion-pair chromatography the equilibrium between amine (Am) in the stationary and mobile phases may be written

$$ClO_4^- + HAm^+ \rightleftharpoons (HAm^+, ClO_4^-)$$
$$\text{aqueous phase} \qquad \text{organic phase}$$
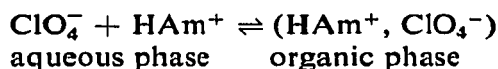
and it is clear that the more hydrophilic the organic phase the more strongly will the highly polar ion-pairs be solvated. Thus retention is reduced by making the eluent more polar. In our experiments (see Tables II and III) this was achieved (a) by increasing the alcohol content of the mobile phase, (b) by replacing a higher alcohol

(isoamyl) by a lower alcohol (butyl), and (c) by replacing a less polar solvent (chloroform) by a more polar solvent (methylene chloride).

Because the pH of the stationary phase was around 1, the basic nitrogen atoms within the solute molecules were almost entirely in their ammonium forms, the $pK_a$'s and ratios of [HAm$^+$] to [Am] that are relevant for comparison being: secondary amine, $\sim$11.1, $1.3 \cdot 10^{10}$; tertiary amine, $\sim$10.5, $3 \cdot 10^9$; piperidine, 11.3, $2 \cdot 10^{10}$; piperazine, 9.8, $6 \cdot 10^8$ and 5.6, $4 \cdot 10^4$; pyridine, 5.2, $1.6 \cdot 10^4$. Evidently, it would be more accurate to regard the amines as present in the organic phase as ion clusters in which several anions may be associated with a single amine molecule. It then becomes clearer why the number of basic nitrogen atoms in the solute molecule is the major factor governing retention and why group separation on the basis of the number of such atoms is the main feature of ion-pair chromatography.

In adsorption chromatography, the amines are probably present in the mobile phase as acetate ion pairs or at least in a form that is highly solvated by acetic acid. Such solvation could well mask or otherwise modify the normal adsorptive properties of the amino groups. Strength of adsorption and hence retention may then be expected to be largely dependent upon the nature of other functional groups in the solute molecules. Seen in this light the elution order is in tolerable agreement with that predicted by Snyder[34] and embodied in his group adsorption energy terms, $Q_i^0$, which are as follows for the important functional groups: –F, 1.64; –Cl, 1.82; –O–CO–CH$_3$, 5.0; >CHOH, 6.5; –CONH$_2$, 8.9. As explained above, the values for the amino groups –NR$_2$ (4.5) and –NHR (5.2) are unlikely to apply to the present systems because of the specific interaction between these groups and acetic acid. Evidently, the terminal amino groups are more strongly adsorbed than might otherwise be expected.

*Peak dispersion*

The plate numbers obtained in both ion-pair and adsorption chromatography are generally lower than those obtainable with the same columns run at the same elution speeds when using a standard mixture of aromatic hydrocarbons. There are a number of reasons for this. Fig. 1 shows the reduced plate height–reduced velocity curves for 10-$\mu$m Merckosorb SI100 and 20-$\mu$m Spherisorb A20Y. The scales to the right give the number of plates corresponding to the values of $h$ which will be obtained from a 125-mm-long column. Evidently, a maximum plate number of $\sim$3000 is expected with Spherisorb at $v \approx 4$ and $\sim$4000 with Merckosorb at $v \approx 3$, but in practice these reduced velocities correspond to very low linear velocities. Unfortunately, the solvents used for the ion-pair chromatography were much more viscous than those for adsorption chromatography. Typically the viscosity of a 1:1 mixture of a chloromethane and 1-butanol is about 2 cP compared with about 0.5 cP for an alkane–chloroalkane mixture, and the diffusion coefficients of any solute will be in roughly the same ratio. Thus, to achieve the same values of $v = ud_p/D_m$ the value of $u$ for Merckosorb must be about half that for Spherisorb as seen from the uppermost scales in Fig. 1. This coupled with the steepness of the $(h, v)$ curve means that analysis on 10-$\mu$m Merckosorb by ion-pair chromatography is expected to be markedly slower than by adsorption chromatography even when a higher pressure is used. This is evidenced by the 30- to 40-min analyses shown in Figs. 1 and 2 compared with the 2- to 8-min analyses shown in Figs. 3–8. In these analyses the reduced velocities were generally between 5 and 10 for Merckosorb and between 10 and 20 for

Spherisorb. The maximum plate efficiencies expected under ideal conditions would then be about 2500 for Merckosorb and about 1500 for Spherisorb. In practice, the maximum efficiencies obtained in the separation of the tranquillizers were only slightly below these figures, thus the fifth peak in Fig. 3 (ion-pair chromatography, $v = 8$) gives 1500 plates while the second peak in Fig. 5B (adsorption chromatography, $v = 15$) gives 1200 plates.

Where plate efficiencies are very much lower than expected, the peaks are significantly tailed and this is almost certainly an indication of non-linear isotherms. In ion-pair chromatography some residual adsorption might account for this. In adsorption chromatography tailing is a well-known phenomenon, which becomes more serious as solutes become more strongly polar and require very polar solvents for elution. Further work on the addition of suitable modifiers to the eluents for adsorption chromatography and experiments on deactivation of the support for ion-pair chromatography might improve matters. Under the best conditions, however, it has been possible to obtain almost as high plate efficiencies in the chromatography of complex amines as with simple test solutes with ideal distribution characteristics.

*Selectivity*

Structural differences between solutes have markedly different effects in ion-pair and adsorption chromatography and the general features of their effects have been noted. A number of more subtle effects are also detectable:

(a) Compounds differing by a methyl group attached to the terminal nitrogen atom of the side chain could be resolved by both techniques as illustrated by the separation of nor- and amitriptyline, which have $-NHCH_3$ and $-N(CH_3)_2$ terminal groups, respectively. In ion-pair chromatography the nortriptyline is eluted first, which is the same order as found by Persson[25] for the analogous pair desipramine and imipramine using chloride as anion. In adsorption chromatography (Fig. 5) the order was reversed and a very wide separation of nor- and amitriptyline was obtained.

(b) Compounds differing by a methyl group in the aliphatic part of the side chain (for example having *n*-propyl and isobutyl groups) were resolved only by adsorption chromatography. For example, trimipramine (isobutyl) is well separated from imipramine (*n*-propyl) in adsorption chromatography (Fig. 4), the isobutyl-containing compound eluting first. The failure to resolve the two by ion-pair chromatography is predictable from the small differences in their extraction coefficients measured by Persson[25].

(c) Compounds differing in substituents at position 2 of the condensed ring system, for example methotrimeprazine ($-OCH_3$) and propiomazine ($-CO-C_2H_5$) were again well resolved by adsorption chromatography (Fig. 6) but unresolved in ion-pair chromatography.

As expected, adsorption chromatography provides for greater selectivity in respect of small differences in substituent groups, while ion-pair chromatography selects on the basis of the molecular structure taken as a whole and in particular on the basis of the number of basic nitrogen atoms. Both techniques are important for the analysis of this general class of drugs. Adsorption chromatography is particularly suitable for testing purity where impurities are likely to be compounds of the same chemical class.

CONCLUSIONS

The study establishes that HPLC can be successfully applied to the analysis of tricyclic antidepressants and tranquillizers at the sub-microgram level. Analysis times are between 3 and 30 min. Analysis by adsorption chromatography is selective to the nature of individual functional groups in the molecules whereas ion-pair chromatography achieves group separation by chemical type. In each system peak sharpness can approach that obtained with simple aromatic test mixtures chromatographed under equivalent conditions.

The work presented has great potential as a basis for quality control methods in production and for the preparation of small pure samples. With some further development it can form the basis for methods of analysis of the relevant compounds in clinical toxicology.

REFERENCES

1 Cheng-Yi Wu, S. Siggia, T. Robinson and R. Waskiewicz, *Anal. Chim. Acta*, 63 (1973) 393.
2 J. H. Knox and J. Jurand, *J. Chromatogr.*, 82 (1973) 398.
3 J. H. Knox and J. Jurand, *J. Chromatogr.*, 87 (1973) 95.
4 M. W. Anders and J. P. Latorre, *J. Chromatogr.*, 55 (1971) 409.
5 R. L. Stevenson and C. A. Burtis, *J. Chromatogr.*, 61 (1971) 253.
6 E. Murgia, P. Richards and H. F. Walton, *J. Chromatogr.*, 87 (1973) 523.
7 M. W. Anders and J. P. Latorre, *Anal. Chem.*, 42 (1970) 1430.
8 R. W. Ross, *J. Pharm. Sci.*, 61 (1972) 1979.
9 J. E. Evans, *Anal. Chem.*, 45 (1973) 2428.
10 J. J. Kirkland and J. J. DeStefano, *J. Chromatogr. Sci.*, 8 (1970) 309.
11 W. J. Rzeszotarski and A. B. Mauger, *J. Chromatogr.*, 86 (1973) 246.
12 G. J. Krol, C. A. Mannan, F. Q. Gemmill, Jr., G. E. Hicks and B. T. Kho, *J. Chromatogr.*, 74 (1972) 43.
13 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
14 R. C. Williams, J. A. Schmit and R. A. Henry, *J. Chromatogr. Sci.*, 10 (1972) 494.
15 F. A. Fitzpatrick, S. Siggia and J. Dingman, *Anal. Chem.*, 44 (1972) 221.
16 J. C. Touchstone and W. Wortmann, *J. Chromatogr.*, 76 (1973) 244.
17 R. J. Dolphin, *J. Chromatogr.*, 83 (1973) 421.
18 I. Jane and B. B. Wheals, *J. Chromatogr.*, 84 (1973) 181.
19 J. D Wittwer and J. H. Kluckhohn, *J. Chromatogr. Sci.*, 11 (1973) 1.
20 P. J. Cashman, J. L. Thornton and D. L. Shelman, *J. Chromatogr. Sci.*, 11 (1973) 7.
21 *Waters Ass. Publication*, No. AN 138, Waters Ass., Framingham, Mass., December, 1973.
22 D. J. Weber, *J. Pharm. Sci.*, 61 (1972) 1797.
23 R. G. Muusze and J. F. K. Huber, *J. Chromatogr.*, 83 (1973) 405.
24 B.-A. Persson and G. Schill, *Acta Pharm. Suecica*, 3 (1966) 281.
25 B.-A. Persson, *Acta Pharm. Suecica*, 5 (1968) 335.
26 B.-A. Persson, *Acta Pharm. Suecica*, 5 (1968) 343.
27 B.-A. Persson, *Acta Pharm. Suecica*, 7 (1970) 343.
28 S. Eksborg and G. Schill, *Anal. Chem.*, 45 (1973) 2092.
29 B.-A. Persson and B. L. Karger, *J. Chromatogr. Sci.*, 12 (1974) 521.
30 J. H. Knox, *Chem. Ind.*, (1975) 29.
31 R. E. Majors, *J. Chromatogr. Sci.*, 1 (1973) 88.
32 J. N. Done, G. J. Kennedy and J. H. Knox, in S. G. Perry (Editor), *Gas Chromatography 1972*, Applied Sciences Publishers, London, 1973, pp. 145–155.
33 E. Grushka, J. H. Knox and L. R. Snyder, *J. Chromatogr. Sci.*, in press.
34 L. R. Snyder, *Principles of Adsorption Chromatography*, Edward Arnold (Publishers) Ltd., London, Marcel Dekker, New York, 1968.