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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF DRUGS

AN EVALUATION OF AN OCTADECYLSILANE STATIONARY PHASE

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SUMMARY

The performance of a commonly used high-pressure liquid chromatographic stationary phase, octadecylsilane, has been evaluated for 30 compounds selected as representative of a wide variety of drug substances. Chromatographic behaviour is found to be highly predictable on the basis of pK_a and partition coefficient, and the stationary phase should be especially valuable for the separation of acidic and neutral drugs. For basic drugs, however, the column efficiency is poor, detracting from the overall usefulness.

INTRODUCTION

Thin-layer and paper partition chromatography are well-established methods for the separation of drug substances. Apart from normal-phase operation, using a polar stationary phase with non-polar eluents, chromatographic layers may be impregnated with a liquid of low polarity (*e.g.*, tributyrin, silicone oil) and used in a reversed-phase fashion. In a recent survey of thin-layer and paper chromatographic systems, such a reversed-phase method has been found to be amongst the most successful for the discrimination of basic drugs[1]. Gas–liquid chromatography is also extensively used in drug analysis, but is not always suitable for highly polar substances which may be non-volatile or thermally labile.

High-pressure liquid chromatography (HPLC) may be expected to have many of the advantages of previously used liquid-phase separation systems (high selectivity, application to non-volatile samples, preparative capability) together with those advantages hitherto enjoyed only by gas chromatography (speed, good precision, high efficiency, and ease of quantitation). For such reasons HPLC is finding increasing use in drug analysis.

By their very nature, many drug substances comprise both ionic and non-polar functional groups, and it is not surprising that similar separations have been achieved by several modes of liquid chromatography. Mixtures containing diamorphine, for

TABLE I

HPLC RETENTION VOLUMES* (ml) OF SOME DRUGS ON A MICROPARTICULATE OCTADECYLSILANE COLUMN WITH AQUEOUS METHANOL ELUENTS OF DIFFERENT pH VALUES

Drug	*pH of eluent*									
	3.0					*5.0*				
	Methanol concentration (%)					*Methanol concentration (%)*				
	0	*20*	*40*	*60*	*80*	*0*	*20*	*40*	*60*	*80*
Salicylic acid	>46	24.1	10.7	5.5	3.8	17.3	6.6	4.2	3.4	3.1
Acetylsalicylic acid	—	24.7	11.0	5.5	3.8	18.4	6.6	4.2	3.4	3.2
Ibuprofen	—	—	>40	22.0	5.5	—	—	>44	17.9	5.4
Paracetamol	34.3	7.1	4.0	3.4	3.2	32.8	6.9	4.1	3.4	3.1
Phenylbutazone	—	—	>74	13.7	4.6	—	—	>36	9.0	4.1
Phenytoin	—	—	21.0	5.4	3.5	—	—	18.4	5.3	3.6
Glutethimide	—	—	20.2	5.8	3.7	—	—	17.5	5.6	3.7
Barbitone	>60	12.1	5.4	3.7	3.3	69.2	11.8	5.3	3.7	3.3
Phenobarbitone	—	36.8	9.5	4.4	3.5	—	34.0	8.8	4.3	2.9
Quinalbarbitone	—	—	30.2	6.9	3.9	—	—	26.2	6.6	3.8
Thiopentone**	—	—	34.5	7.8	4.0	—	—	30.0	7.2	4.0
Nitrazepam	—	—	24.7	6.2	3.7	—	—	25.0	6.3	3.9
Chlordiazepoxide	—	—	19.1	5.9	4.1	—	—	46.8	9.0	4.4
Phenacetin	—	44.5	11.0	4.9	3.6	—	41.8	10.0	4.7	3.6
Sulphacetamide	25.8	6.4	3.9	3.2	3.1	18.1	5.5	3.7	3.2	3.1
Sulphanilamide	8.8	4.2	3.4	3.1	3.1	9.2	4.3	3.4	3.1	3.0
Amphetamine	61.6	13.5	6.1	4.0	3.6	>110	17.1	7.0	4.4	4.0
Methylamphetamine	—	15.1	6.4	4.2	3.7	—	20.3	7.5	4.6	4.2
Ephedrine	31.3	7.8	4.4	3.5	3.3	19.8	8.9	4.9	3.8	3.7
Nicotine***	25.3	7.7	4.7	4.2	5.4	>100	8.8	5.5	4.5	5.0
Caffeine	—	17.6	5.7	3.8	3.5	—	17.3	5.5	3.9	3.6
Morphine	46.0	7.1	4.0	3.4	3.5	>46	8.8	4.5	3.8	4.0
Pethidine	—	—	—	6.5	4.6	—	—	—	7.8	5.1
Cocaine	—	—	21.1	6.4	4.7	—	—	24.5	7.9	5.5
Quinine	—	—	20.5	5.5	4.4	—	—	37.0	7.2	4.7
Diphenhydramine	—	—	48.0	8.7	4.9	—	—	60.0	10.4	6.0
Chlorpromazine	—	—	—	>54	11.4	—	—	—	>80	13.8
Amitriptyline	—	—	—	22.8	8.2	—	—	—	37.5	10.0
Tubocurarine	—	>80	14.2	4.9	4.6	—	—	26.0	7.3	9.0
Paraquat***	8.4	6.4	5.4	5.2	8.6	39.0	11.1	8.4	15.2	>54

* In general retention volumes in excess of 40 ml were not measured.
** Detection wavelength 290 nm.
*** Detection wavelength 260 nm.

example, have been separated with varying degrees of success by high speed anion[2,3] and cation exchange[3–5], liquid–solid adsorption[6] and normal[7] and reversed-phase[4] liquid–liquid partition chromatography.

The development and commercial availability of hydrolytically stable stationary phases chemically bonded onto microparticulate substrates has given the chromatographer an efficient and versatile partition medium for HPLC. One of the most widely used HPLC bonded phase functional groups is octadecylsilane, which is employed in a reversed-phase mode with aqueous methanol eluents. Amongst the advantages of aqueous solvent systems is the compatibility with biological samples. This may lead

7.0					9.0				
Methanol concentration (%)					*Methanol concentration (%)*				
0	*20*	*40*	*60*	*80*	*0*	*20*	*40*	*60*	*80*
12.6	5.4	3.4	2.9	2.5	7.9	4.0	3.0	2.6	2.4
9.4	4.2	3.2	2.7	2.4	6.0	3.4	2.9	2.6	2.4
—	>48	28.3	6.7	2.9	—	>75	16.6	4.5	2.8
32.1	7.1	4.1	3.4	3.1	26.0	6.1	3.9	3.4	3.1
—	>50	13.9	4.3	2.7	—	43.6	8.5	3.4	2.5
—	>62	17.4	5.2	3.5	—	35.4	9.5	4.5	3.45
—	>32	17.3	5.6	3.8	—	>50	16.4	5.5	3.8
56.8	11.0	5.2	3.7	4.0	10.8	4.2	3.4	3.1	3.0
>50	28.2	8.0	4.2	2.6	17.6	5.2	3.5	3.0	2.5
—	>44	24.3	6.5	3.8	—	32.9	10.4	4.8	3.75
—	—	26.9	7.1	4.0	>48	22.8	8.1	4.3	3.65
—	—	23.8	6.2	3.8	—	>50	21.1	6.0	3.6
—	—	50.2	9.2	4.4	—	—	44.7	8.7	4.5
—	42.2	9.6	4.7	3.6	—	34.3	9.3	4.6	3.6
4.4	3.1	2.9	2.8	2.9	3.3	2.6	2.6	2.5	2.5
9.2	4.2	3.4	3.2	3.0	8.5	3.9	3.4	3.1	3.0
—	27.6	11.1	6.2	6.0	—	>40	28.1	11.4	7.7
—	36.6	13.4	7.7	6.7	—	—	39.6	18.0	10.8
>54	14.0	7.3	5.0	5.1	—	52.0	17.5	9.0	7.0
—	29.7	12.0	7.1	6.0	—	>46	16.0	6.4	4.5
21.0	17.0	5.5	3.9	3.6	>48	16.0	5.4	3.8	3.5
—	29.7	9.7	6.1	6.7	—	>50	20.0	7.7	5.2
—	—	>42	14.0	10.0	—	—	>40	18.2	7.8
—	—	43.7	12.1	7.9	—	—	>43	12.6	5.9
—	—	>48	16.0	8.8	—	—	—	34.8	9.9
—	—	>42	21.9	11.3	—	—	—	45.5	11.0
—	—	—	>41	30.2	—	—	—	>47	34.0
—	—	—	>46	22.6	—	—	—	>45	30.0
—	>45	>35	20.0	21.6	—	—	—	38.6	22.4
—	>41	>42	>56	>43	—	—	—	>54	>42

to the possibility of the direct analysis of body fluids without the necessity for prior solvent extraction.

In order to evaluate this octadecylsilane microparticulate column for the analysis of drugs, 30 compounds have been chosen as representative of a wide variety of polar drug substances, *viz.*, strongly acidic, weakly acidic, neutral and basic drugs, and of varying chemical structure, molecular weight, lipid solubility and pharmacological action. A knowledge of the chromatographic characteristics of these thirty compounds should allow the prediction of the chromatographic behaviour of many other drugs on this column.

EXPERIMENTAL

A constant-flow pump M-6000 (Waters Ass., Stockport, Great Britain) was used to deliver eluent at a rate of 2 ml/min to a column (30 cm × 4 mm I.D.) packed with a microparticulate (10 μm) octadecylsilane bonded phase (μ-Bondapak-C_{18}, Waters Ass.). Aqueous methanolic solutions of the drugs (2 μl) were introduced under full flow using a microlitre syringe and septum injection port. The eluted compounds were detected by their ultraviolet (UV) absorbance using a variable wavelength UV monitor fitted with an 8-μl flowcell (CE-212, Cecil, Cambridge, Great Britain). Except where otherwise stated the wavelength of detection was 220 nm.

Eluent solutions (pH 3.0, 5.0, 7.0 and 9.0) were made up from 0.025 *M* NaH_2PO_4 and/or 0.025 *M* Na_2HPO_4 solutions and various amounts of methanol (0–80%). The solutions were adjusted to the specified pH (± 0.04 pH units) by the addition of 5% sodium hydroxide or phosphoric acid solution. The phosphate eluent was chosen because of the high UV transparency and the wide pH range. However, because of the low buffering capacity at the extremes of pH, only small quantities of solute were injected (1–10 μg of drug). Retention volumes were calculated from the point of injection.

RESULTS AND DISCUSSION

Table I gives the retention volumes of the 30 drugs studied using aqueous

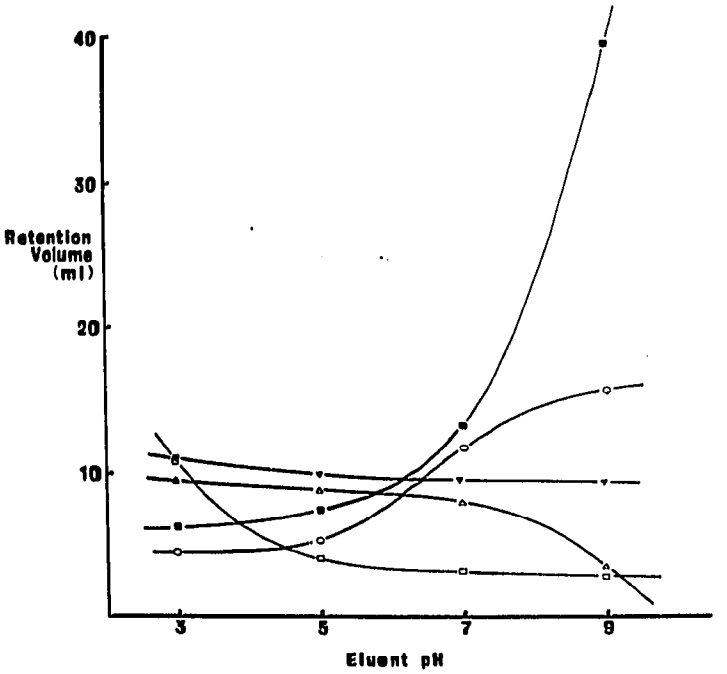

Fig. 1. Effect of variation of the eluent pH upon elution volumes (methanol concentration 40%). □—□, Salicylic acid; △—△, phenobarbitone; ▼—▼, phenacetin; ○—○, nicotine; ■—■, methylamphetamine.

TABLE II

PHYSICO-CHEMICAL CONSTANTS AND CHROMATOGRAPHIC PROPERTIES OF SOME DRUGS ON AN OCTADECYLSILANE COLUMN

Drug	*pK_a**	*log P***	*Retention volume (ml)*
Barbitone	7.8	0.7	3.7***
Phenobarbitone	7.4	1.4	4.4***
Quinalbarbitone	7.9	2.3	6.9***
Thiopentone	7.6	3.0	7.8***
Ephedrine	9.6	0.9	9.0§
Nicotine	8.0	1.2	6.4§
Quinine	8.5	1.8	35§
Diphenhydramine	9.0	3.3	45§
Amitriptyline	9.4	4.9§§	>45§
Chlorpromazine	9.3	5.3	>45§

* Data given in ref. 9.

** log P = logarithm of the partition coefficient between water and n-octanol; data as given in ref. 8.

*** Measured at pH = 3.0; 60% methanol.

§ Measured at pH = 9.0; 60% methanol.

§§ pH 7.4.

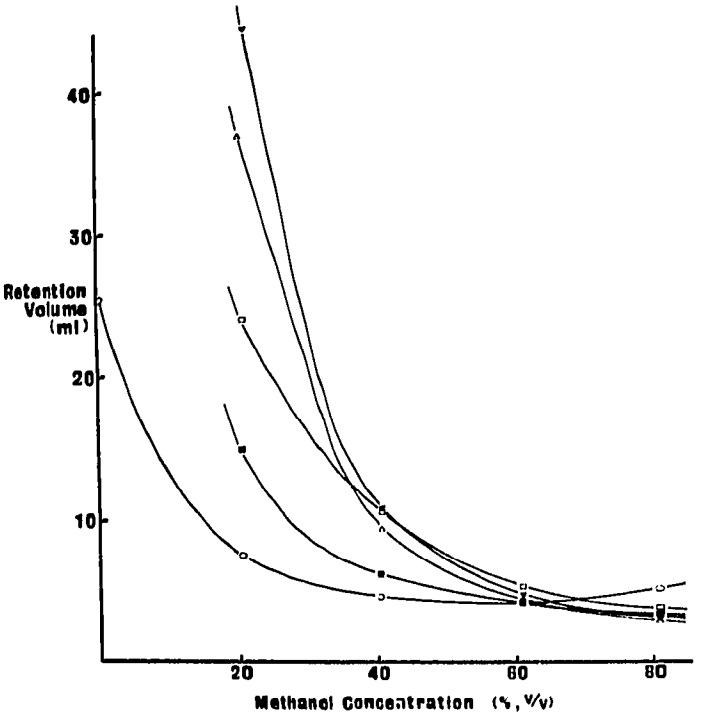

Fig. 2. Effect of variation of the eluent methanol content upon elution volumes at pH 3.0. □—□, Salicylic acid; △—△, phenobarbitone; ▼—▼, phenacetin; ○—○, nicotine; ■—■, methylamphetamine.

methanol mixtures of differing pH values. The order of the drugs listed is: strong acids, weak acids, neutral compounds, bases and quaternary ammonium compounds. It can be seen from these results that in the octadecylsilane reversed-phase system, the partition is primarily dependent on three physico-chemical properties:

(a) the proportion of the drug present in the unionised form (a function of the pH of the eluent and the pK_a of the drug);

(b) the relative lipid solubility of the unionised form of the drug in the stationary phase and in the eluent;

(c) the methanol content of the eluent.

The profound effect of the eluent pH and pK_a of the drug upon retention characteristics is clearly shown in Fig. 1. A strong acid such as salicylic acid (pK_a 3.0) shows a marked decrease in retention volume when the pH rises above 3 whilst a weak acid, *e.g.*, phenobarbitone (pK_a 7.4) has a relatively constant elution volume with increase in pH until the pH exceeds the pK_a of the drug when the elution volume decreases. This is not unexpected, as only the unionised form of the drug would partition into the stationary phase. In contrast to the acids, the retention volumes of basic drugs such as nicotine and methylamphetamine (pK_a values 8.0 and 10.1, respectively) increase as pH increases. Neutral drugs such as phenacetin show virtually no change in retention characteristics with pH of the eluent since they do not contain ionisable groupings.

The lipid solubility of a drug is usually quantified in terms of the partition

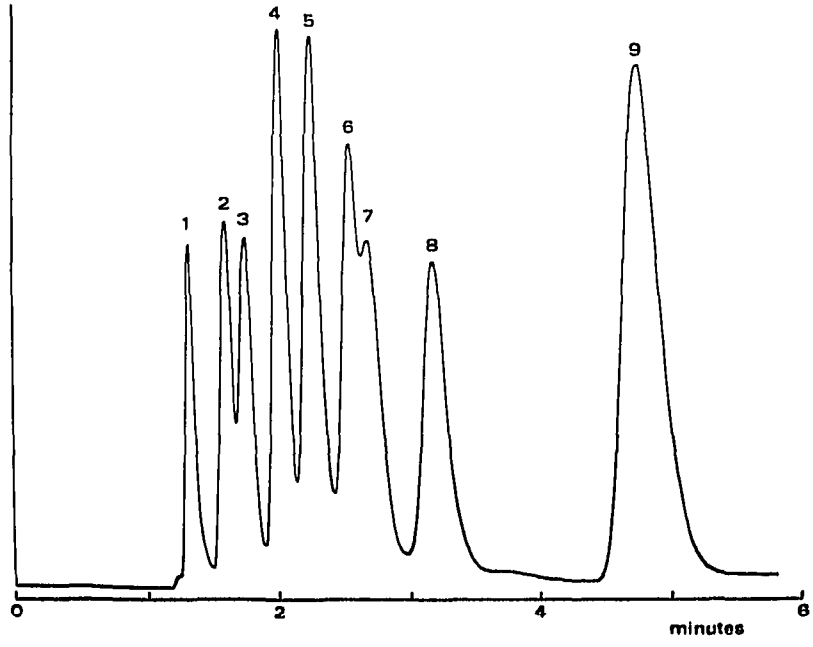

Fig. 3. High pressure partition chromatography of some acidic and neutral drugs. Eluent: 55% methanol, pH 7.0; flow-rate, 2 ml/min. 1 = Acetylsalicylic acid; 2 = paracetamol; 3 = barbitone; 4 = phenobarbitone; 5 = phenacetin; 6 = phenytoin; 7 = glutethimide; 8 = quinalbarbitone; 9 = chlordiazepoxide.

coefficient between water and an organic solvent, *n*-octanol being must frequently used for this purpose. In Table II, the *n*-octanol partition coefficients for some acidic and basic drugs are compared with the retention volumes measured at a pH appropriate to the type of drug, and a good quantitative correlation is observed within each series of drugs. It was not expected that *n*-octanol–water partition coefficients would mirror exactly the partitioning of partially ionised drug molecules between an aqueous methanol buffer and an octadecylsilane stationary phase, but since a high correlation was obtained, the prediction of the chromatographic behaviour of other drug substances should be possible if the appropriate pK_a and partition coefficients are known.

The effect of the increase in organic content of the mobile phase on the elution volume is illustrated in Fig. 2. At this pH the more ionised basic drugs are more rapidly eluted than the acids and for nearly all the drugs studied, the elution volumes are reduced by increasing the proportion of methanol in the eluent. In some cases, *e.g.*, quaternary ammonium compounds, morphine and nicotine, the elution volume at pH 3.0 first decreased with increase in methanol content of the eluent and then increased (Table I); a satisfactory explanation for this was not apparent.

It would appear that with few exceptions the behaviour of drugs on the octadecylsilane stationary phase is quite predictable on the basis of pK_a and lipid solu-

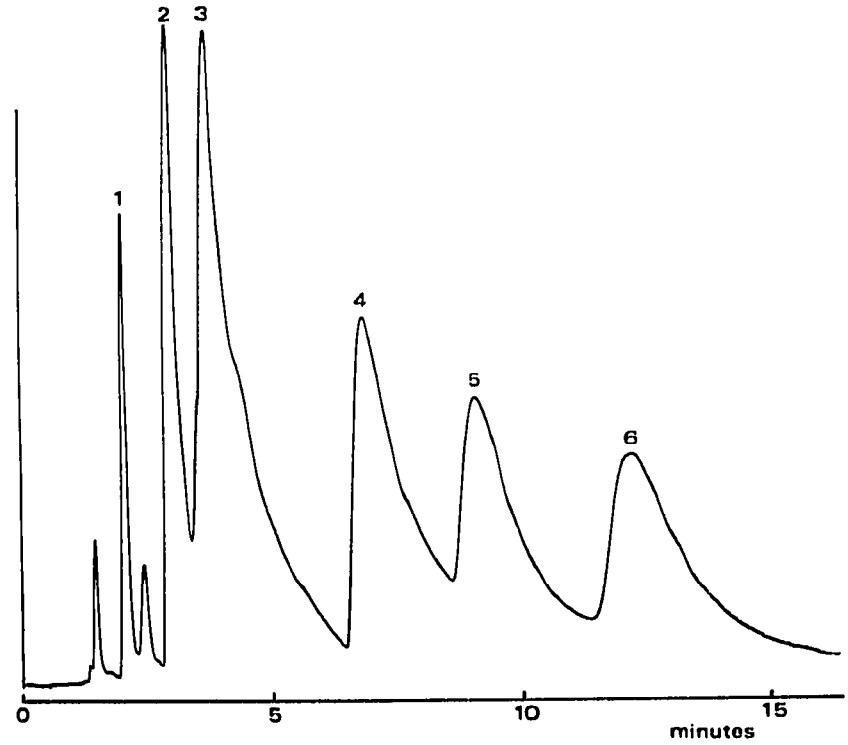

Fig. 4. High-pressure partition chromatography of some basic drugs. Eluent: 55% methanol, pH 7.0; flow-rate, 2 ml/min. 1 = Caffeine; 2 = ephedrine; 3 = methylamphetamine; 4 = cocaine; 5 = quinine; 6 = tubocurarine.

TABLE III

COLUMN EFFICIENCY FOR SOME DRUGS ON A MICROPARTICULATE OCTADECYLSILANE COLUMN

Column efficiency, $N = 5.54 (R/W_{\frac{1}{2}})^2$, where R = retention time (min) and $W_{\frac{1}{2}}$ = peak width at half height (min). Column capacity factor, $k' = [(V_r - V_0)/V_0]$, where V_r = elution volume of solute and V_0 = column void volume.

Drug	*pH 9; 60% methanol*		*pH 3; 60% methanol*	
	N	*k'*	*N*	*k'*
Acetylsalicylic acid	1900	0.08	1300	1.3
Phenobarbitone	1300	0.25	1200	0.8
Nitrazepam	1550	1.50	1450	1.6
Sulphanilamide	2150	0.3	1250	0.3
Amphetamine	450	3.75	500	0.7
Methylamphetamine	350	6.50	300	0.75
Nicotine	1400	1.7	250	0.75
Amitriptyline	—	—	60	8.5
Tubocuraine	60	15.1	70	1.0

bility, and drugs of any character may be chromatographed if an appropriate eluent is chosen. For a useful chromatographic system, however, high resolution is essential and this is governed by column efficiency as well as selectivity. The excellent efficiency and resolution afforded by this column for the acidic and neutral drugs (Fig. 3) allows their separation, and quantitation, but in contrast, the basic drugs have much poorer chromatographic peak shapes (Fig. 4). Table III gives the chromatograph efficiencies (in theoretical plates) for a representative selection of compounds. For most basic

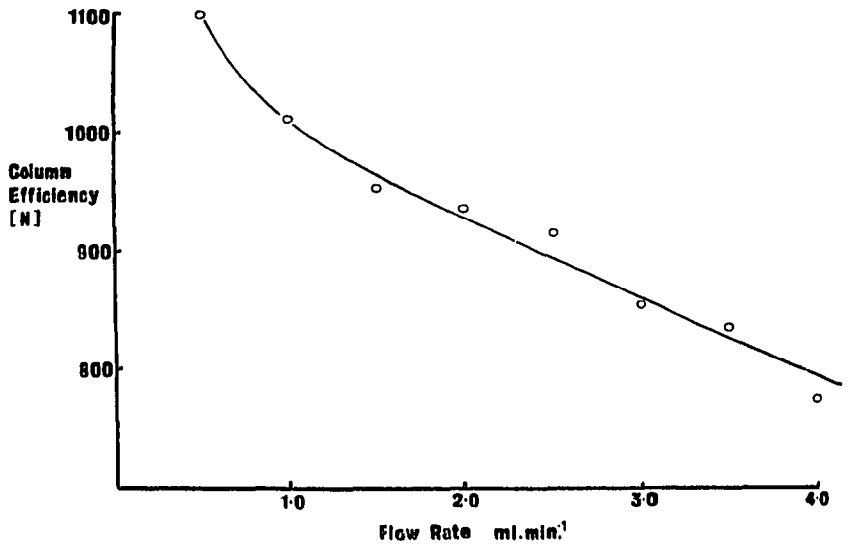

Fig. 5. Effect of eluent flow-rate on the chromatographic efficiency (in theoretical plates) for amphetamine. Eluent: 80% methanol, pH 9.0.

drugs, the efficiency is seen to be very poor indeed and even a reduction in eluent flow rate does not lead to any substantial enhancement in efficiency for basic drugs such as amphetamine (Fig. 5). It must be concluded that although an octadecylsilane stationary phase may be used for the chromatography of a wide variety of substances, it is only with acidic and neutral solutes that satisfactory results are to be obtained.

NOTE ADDED IN PROOF

Since the completion of this work, Carlson *et al.*[10] have reported the correlation of *n*-octanol–water partition coefficients for some substituted phenols and anilines with retention in an octadecylsilane–acetone–water chromatographic system.

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