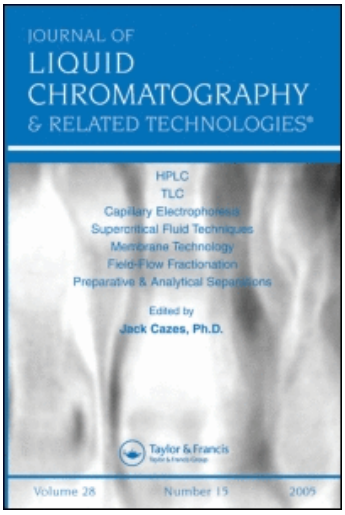

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COMPARISON OF VARIOUS COLUMNS FOR THE HIGH-SPEED HPLC ANALYSIS OF DRUGS OF FORENSIC INTEREST

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ABSTRACT

A comparison of the use of various commercially available columns for the high-speed reverse-phase ion-pair high performance liquid chromatographic separation of drugs of forensic interest is discussed. The columns include a Partisil 5 ODS-3 RAC, a Partisil 5 C8 RAC, a Radial Pak microBondapak C18 cartridge, a Perkin-Elmer HS/5 C18 and a Perkin-Elmer HS/3 C18. The mobile phases employed contain water, acetonitrile, phosphoric acid, and sodium hydroxide, with or without hexylamine. When a mobile phase without an amine modifier is employed, retention times were at least halved, except with a HS/3 C18 column, over those obtained with conventional columns. Basic drugs did not elute when the above mobile phase is used with a HS/3 C18 column. In addition, the selectivities of the other high speed columns were similar. Further reductions in retention times and different selectivities were obtained when an amine modifier is utilized. Column performance parameters such as n, V and v are presented for the columns examined. A new column performance parameter S which is $(n/V)^{\frac{1}{2}}$ is introduced and discussed.

INTRODUCTION

Reverse-phase ion-pair HPLC has been employed for the analysis of drugs of forensic interest (1, 2). The microBondapak C18 column (30cmx3.9mm) and Partisil 10-ODS-3 (25cmx4.6mm) column have been shown to exhibit similar selectivities (2). The goal of the present study was to investigate ways of obtaining faster separations using systems designed for high-speed analysis. There are three ways of obtaining faster separations with a given mobile phase assuming the column packing material to be employed is of a similar nature to the previously utilized column.

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1) An increased number of plates per second could be obtained by using 7-15cm length and 4-5mm id columns with 3 or 5 micron particle size packing material. The use of injectors, detectors and connecting tubing which minimize band spreading would be required. The Perkin-Elmer HS/5 C18 and Perkin-Elmer H8/3 C18 are examples of such columns.

2) An increased number of plates per second could be obtained by using 10cm length and 9.5mm id columns with 5micron particle size packing material. Unlike the columns in Category 1) conventional equipment would be employed. The Partisil 5 ODS-3 RAC and Partisil 5 C8 RAC are examples of such columns.

3) An increased number of plates per second could be obtained by using 10cm length and 5-8mm id cartridges with 5 or 10 micron particle size packings. A compression module is required for these cartridges of which the Radial Pak microBondapak C18 is an example.

Another way of obtaining faster separations is to modify the mobile phase. For the reverse-phase ion-pair chromatography of basic drugs, the addition of a competing amine to the mobile phase will reduce k' values (3, 4).

In this paper various ways of performing reverse-phase ion-pair high-speed HPLC analysis for drugs of forensic interest are compared. Columns were employed from each of the above categories utilizing mobile phases with or without the presence of a competing amine. Only one column from each commercial type was employed and therefore this study did not take into account variations between columns from the same manufacturer.

EXPERIMENTAL

Equipment

Two liquid chromatographs were employed in this study. One chromatograph consisted of the following components: Model 6000A pump (Waters Associates); Model U6K injector (Waters) or Model 7125 injector (Rheodyne); Model 440 fixed wavelength uv detector at 254 nm with a 12.5 μl flow cell (Waters); Systems 1VB data system (Spectra Physics) or Sigma 15 data system interfaced with a Model 3600 data station (Perkin-Elmer). Columns employed on the first chromatographic system consisted of two prepacked 8mmx10cm stainless steel columns each with 5 micron C18 and 5 micron C8 packing material (Partisil 5 ODS-3 RAC and Partisil 5 C8

RAC, Whatman); a prepacked 4.6mmx25cm stainless steel column with 10 micron C18 packing material (Partisil 10-ODS-3, Whatman); and a prepacked 8mmx10cm polyethylene cartridge with 10 micron C18 packing material (Radial Pak microBondapak C18 cartridge, Waters) mounted inside a radial compression chamber (Z-Module, Waters).

The other liquid chromatograph consisted of the following components: Model 8800 4-solvent gradient system (DuPont); Model LC85 variable UV detector, containing a 2.5 microliter flow cell or 1.5 microliter flow cell at 214 or 254nm (Perkin Elmer); Sigma 15 Data System interfaced with a Model 3600 Data Station (Perkin-Elmer). A prepacked 4.6mmx12.5cm stainless steel column with 5 micron C18 packing material (Perkin Elmer HS/5 C18) and a prepacked 4.6mmx10cm stainless steel column with 3 micron C18 packing material (Perkin-Elmer HS/3 C18) were used with this system.

Materials

The following chemicals were used: hexylamine (Eastman Chemicals, Rochester, N.Y.); methenamine (Merck, Rahway); acetonitrile (Burdick and Jackson). Other chemicals were reagent grade. Authentic drug standards of USP/NF quality were employed.

One mobile phase consisted of 20% acetonitrile, 79% water, and 1% phosphoric acid adjusted to pH 2.2 with 2N sodium hydroxide. This was prepared by filtering and degassing through a 0.45 micron filter a solution consisting of 1580ml water, 400ml acetonitrile, 20ml phosphoric acid and 60ml 2N sodium hydroxide.

The second mobile phase consisted of 12% acetonitrile, 87% water, and 1% phosphoric acid adjusted to pH 2.2 with 2N sodium hydroxide. This mobile phase was prepared as previously reported (2).

Hexylamine was added to mobile phases after they were filtered and degassed resulting in a final molarity for amine of 0.072M with the pH raised to approximately 3.

RESULTS AND DISCUSSION

Table 1 presents a comparison of retention times (RT's) and relative retention times (RRT's) for 4 of the high speed columns and a conventional column, the Partisil 10-ODS-3 using a mobile phase without an amine modifier. The high speed columns gave RT's at least one-half those obtained with the conventional column in addition to similar selec-

TABLE 1

Retention Data for Drugs of Forensic Interest Using Various Alkylsilica Stationary Phases With Mobile Phase Without Hexylamine

Column	HS/5 C18 flow 2.5 ml/min.		5 ODS-3 RAC flow 8.0 ml/min.		5 C8 RAC flow 8.0 ml/min.		RADIAL PAK flow 8.0 ml/min.		10 ODS-3 flow 2.0 ml/min.	
Mobile phase: see Figure 1.										
Drug	RT	RRT	RT	RRT	RT	RRT	RT	RRT	RT	RRT
Heroin	3.0	1.0	3.1	1.0	2.8	1.0	2.5	1.0	7.8	1.0
Caffeine	1.2	.42	1.2	.38	1.2	.44	.88	.35	3.2	.41
Phenobarbital	6.0	2.0	6.4	2.1	6.1	2.1	4.1	1.6	12.1	1.6
Methamphetamine	1.3	.42	1.2	.40	1.2	.43	1.1	.42	3.1	.40
Acetyl-codeine	2.9	.97	3.0	.95	2.8	.98	2.4	.94	7.4	.96
Noscapine	6.0	2.0	6.3	2.0	5.8	2.1	4.9	2.0	15.0	1.9
Papaverine	5.7	1.9	5.9	1.9	5.6	2.0	4.6	1.8	16.0	2.1
Cocaine	3.8	1.3	3.9	1.2	3.8	1.4	3.2	1.3	10.4	1.3
PCP	7.3	2.4	7.7	2.5	7.3	2.6	6.1	2.4	18.3	2.4
LSD	5.2	1.8	5.5	1.8	5.4	1.9	4.4	1.8	14.0	1.8

tivities. Therefore these high speed columns should be applicable to the analysis of drugs of forensic interest using mobile phases which had been previously utilized with the microBondapak C18 and Partisil ODS-3 columns.

Results from the HS/3 C18 column were omitted from Table 1 because basic drugs did not elute from the column under these conditions. The Perkin-Elmer HS/3 C18 column required a much more liphophilic mobile phase to elute heroin and the resulting peak is very assymetrical* with only 75 theoretical plates.

Although the addition of methenamine to the mobile phase resulted in reduced tailing and shortened retention time for heroin, the peak shape was still unsatisfactory and the plate count was only 300. There appears to be unbonded silanol sites on the HS/3 C18 column. It has been reported that using a similar mobile phase, with alkyl-bonded columns containing residual silanol groups, the addition of a competing amine to the eluent reduced peak tailing and decreased RT's for basic drugs (3, 4). The nature of the added amine affects both peak shape

* Satisfactory peak shape is taken to be an Assymetry Factor (A) of 1.8 or less, measured at 10% of peak maximum. (A) is computed by dividing the distance from peak center to trailing edge by the distance from peak center to leading edge.

and retention time for basic drugs. Since the addition of methenamine to the mobile phases did not result in a satisfactory peak shape for heroin, the effect of hexylamine was investigated since it has been shown to be one of the best blocking agents for silanol groups (4). Satisfactory peak shape was obtained with over 1000 theoretical plates for the heroin peak.

Table 2 shows results from measurements made under the same conditions as Table 1, except for the addition of hexylamine to the mobile phase. The addition of hexylamine further decreased RT's for the HS/5 column by a factor of 1.5-2. In addition, the amine reduced RT's with the HS/3 column to values less than those obtained with the HS/5 column.

Hexylamine in the mobile phase also affects column selectivity, as illustrated by the changes in RRT's for the HS/5 column for caffeine, phenobarbital, PCP, and LSD. RRT's for other substances changed little. Although satisfactory peak shape was obtained for heroin on the 3 micron column, some bases exhibited excessive tailing.

The parameter, v, described by Desty, et al. (5), was evaluated for the columns studied and is defined by

$$v = n/RT \quad (1)$$

where n = theoretical plates/column

RT = retention time (min)

The number of theoretical plates (n) was calculated as follows:

$$n = 25(RT/W_{4.4})^2 \quad (2)$$

where $W_{4.4}$ is the width of the peak at 4.4% of peak height.

The performance parameter, S, which is introduced in this paper is defined by

$$S = (n/V)^{1/2}$$

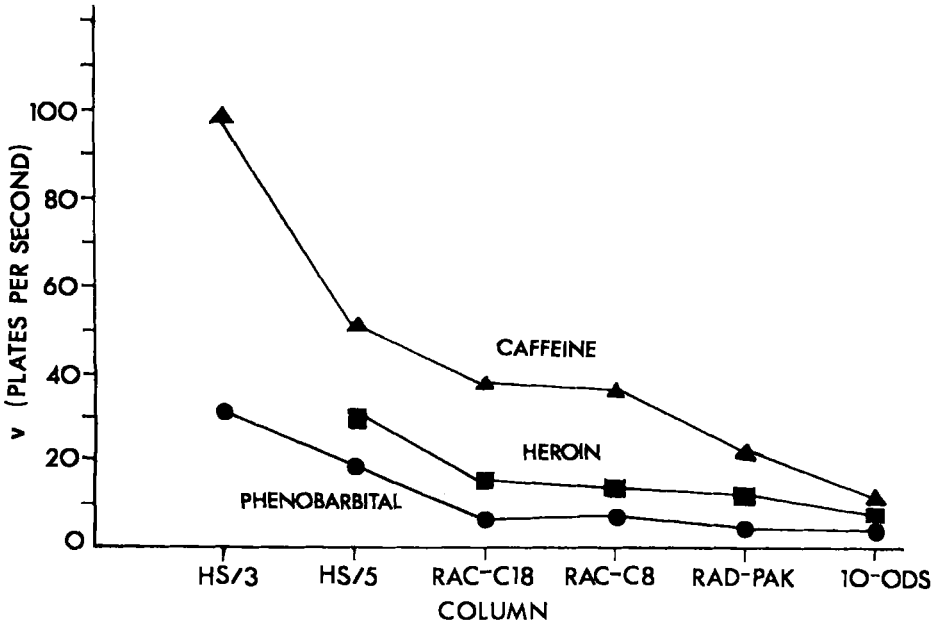
where V = retention volume (ml)

The parameter S is useful for the evaluation of column performance because it is a function of both the separating power of the column and of solvent consumption. The rate of production of theoretical plates, v, is of use where separating power is of primary interest. Graphical presentations of the v factor (Figure 1) and S factor (Figure 2) were obtained from experimental results for three compounds of forensic interest (caffeine, phenobarbital and heroin), using the five high speed columns and a conventional column. The column performance parameters

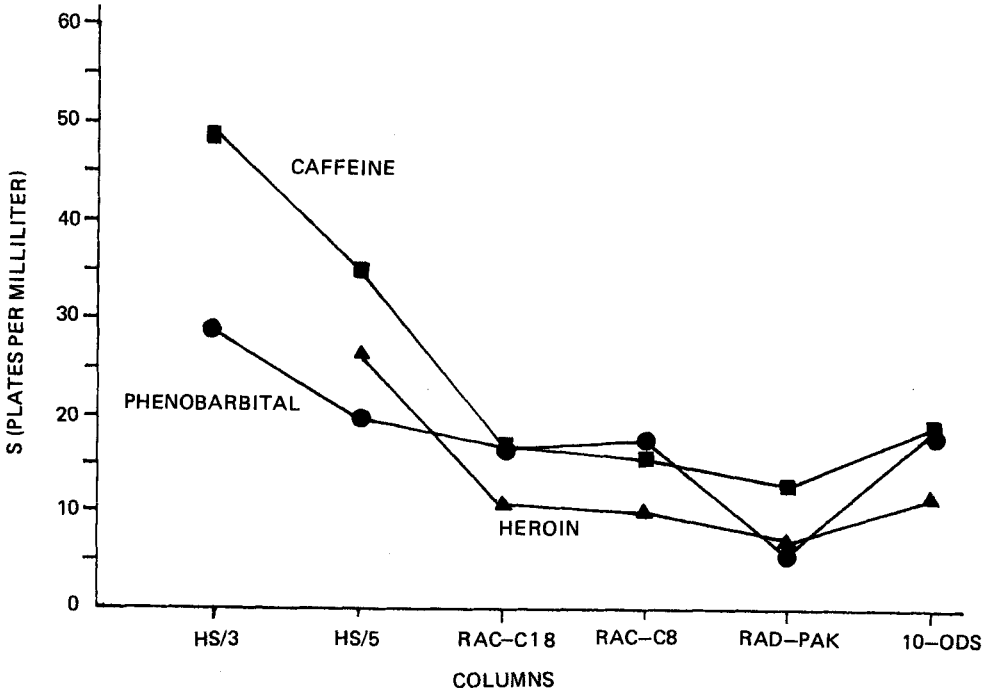
TABLE 2

Retention Data for Drugs of Forensic Interest Using the HS/5 C18 and HS/3 C18 Columns With Mobile Phase Containing Hexylamine

Column	HS/5 C18 flow 2.5 ml/min.		HS/3 C18 flow 2.5 ml/min.	
Mobile phase: same as Figure 1 except .072M hexylamine				
Drug	RT	RRT	RT	RRT
Heroin	1.6	1.0	1.1	1.0
Caffeine	1.2	.75	.70	.61
Phenobarbital	4.8	3.0	3.1	2.7
Methamphetamine	.83	.52	.62	.54
Acetyl-codeine	1.6	1.0	1.1	1.0
Noscapine	3.4	2.1	2.2	1.9
Papaverine	2.8	1.8	1.9	1.6
Cocaine	1.7	1.0	1.6	1.36
PCP	2.8	1.8	4.6	4.0
LSD	2.3	1.4	1.8	1.5



1. Plot of plates per second (v) versus column type for various solutes in mobile phase consisting of 20% acetonitrile, 79% water, 1% phosphoric acid adjusted to pH 2.2 with 2N sodium hydroxide.

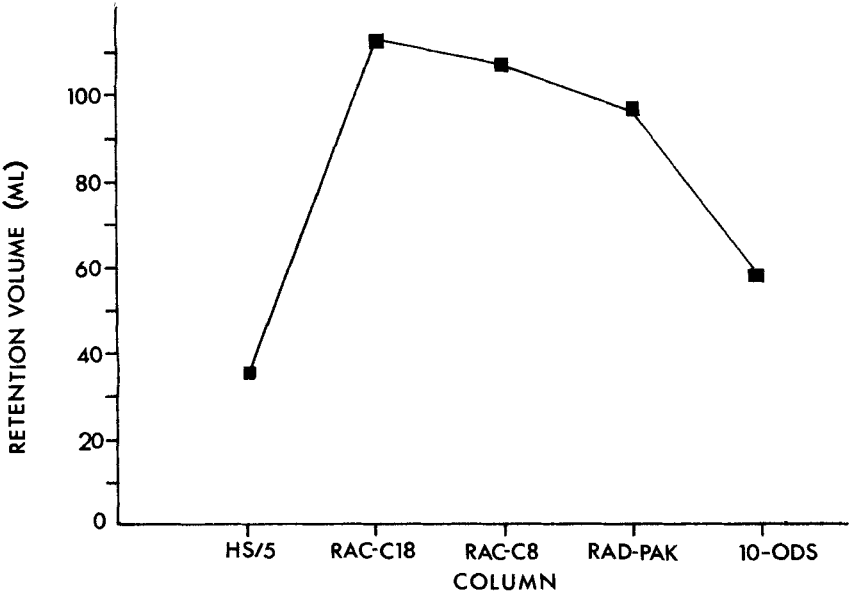


2. Plot of square root of plates per volume (S) versus column type for various solutes. See Figure 1 for mobile phase.

TABLE 3

Performance Parameters for Caffeine, Phenobarbital and Heroin On Various Alkyl-Silica Stationary Phases With Mobile Phase Without Hexylamine

Drug	Caffeine				Phenobarbital				Heroin			
Mobile phase and flow rates: see Tables 1 and 2												
Column	V	n	v	S	V	n	v	S	V	n	v	S
See text for description of performance parameter symbols												
HS/3 C18	2.1	4900	98	48	10.8	8425	32	28				
HS/5 C18	3.1	3802	51	35	14.9	6289	18	20	7.5	4960	28	26
5 ODS-3 RAC	9.6	2644	37	17	51.5	2633	7	17	24.9	2844	15	11
5 C8 RAC	9.9	2640	36	16	48.6	2900	8	18	22.7	2203	13	10
Radial Pak	7.0	1150	22	13	33.0	1080	4	6	20.0	1010	7	7
10 ODS-3	6.4	2258	12	19	24.3	2860	4	19	15.5	2243	5	12



3. Plot of retention volume (V) versus column type for heroin. See Figure 1 for mobile phase.

n, v, V and S for these compounds are presented in Table 3. A mobile phase without hexylamine was used at the same linear velocity. For caffeine and phenobarbital the highest values for v and S were obtained with the Perkin-Elmer HS/3 C18 column. The high value for v using the HS/3 column is consistent with the 3 micron particle size. This is a result of the lower value of the A and the C terms in the Van Deempter equation which are proportional to the particle diameter (6). Similar results have been reported by other authors using columns with 3 micron packing (7-9). The appreciably higher values for S obtained with the Perkin-Elmer HS/3 and HS/5 C18 columns as compared to the other high speed columns tested apparently resulted from their greater efficiencies and lower solvent consumption. The lower solvent consumption of the Perkin-Elmer high speed columns, illustrated in Figure 3, is due of course to their smaller internal diameters. As indicated in Figure 2, the S value of the conventional 10-ODS-3 column is higher than some of the high speed columns as a result of reduced solvent consumption. When

TABLE 4

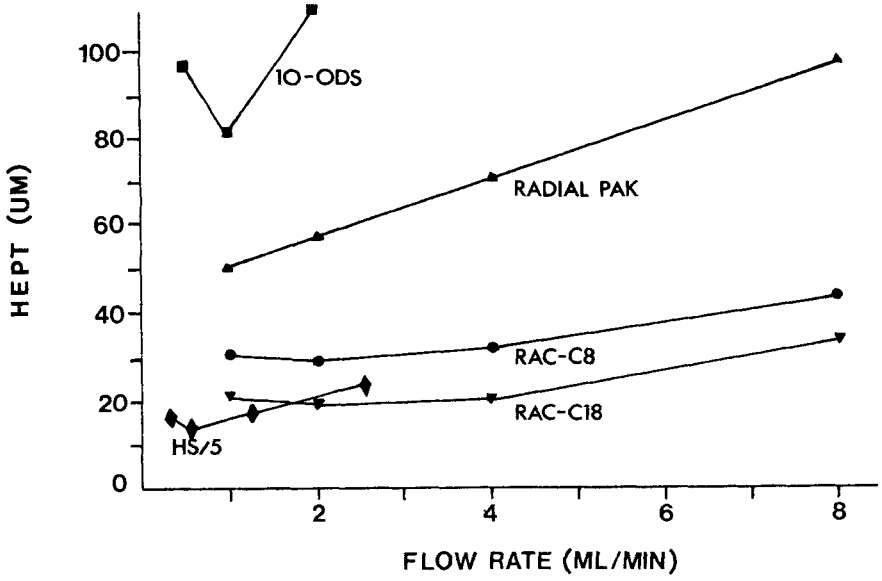
Performance Parameters for Caffeine, Phenobarbital and Heroin On the HS/5 C18 and HS/3 C18 Columns With Mobile Phase Containing Hexylamine

Drug	Caffeine				Phenobarbital				Heroin			
Mobile phase and flow rates: see Table 2												
Column	V	n	v	S	V	n	v	S	V	n	v	S
See text for description of performance parameter symbols												
HS/3 C18	1.7	2889	67	41	7.8	8742	47	34	2.8	1427	21	22
HS/5 C18	3.0	3540	49	34	12.0	6356	22	23	4.0	3053	32	28

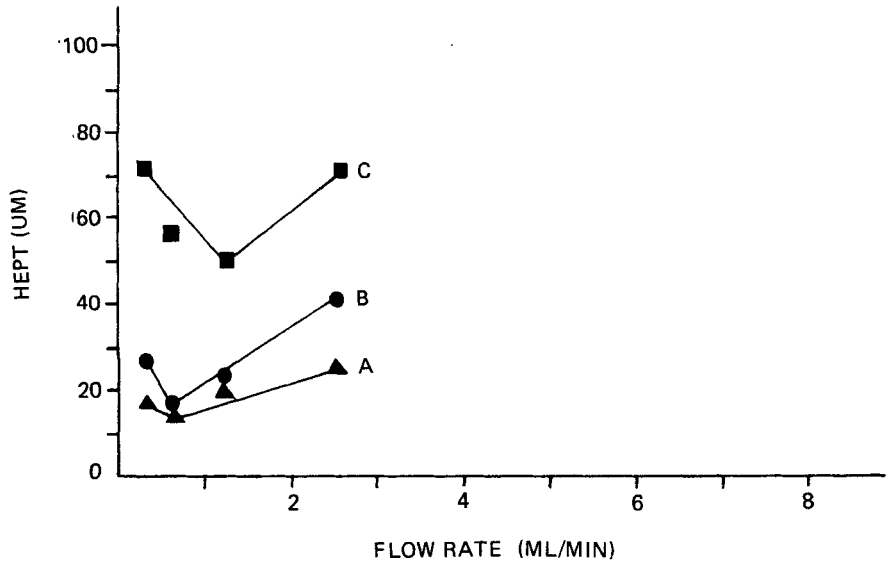
employing a mobile phase without hexylamine, the highest values for v and S are obtained for heroin with the Perkin-Elmer HS/5 C18 column.

A comparison of Table 3 and 4 indicates that the addition of hexylamine to the mobile phase has little effect on v or S for the compounds studied with the HS/5 column. The number of theoretical plates of the heroin peak is reduced on the HS/5 column when hexylamine is added to the mobile phase. The number of theoretical plates of heroin and caffeine on the HS/3 column is significantly lower than that of the HS/5 column using the latter mobile phase. For the HS/5 column using a mobile phase containing hexylamine, there is a slight increase in both v and S for heroin due to the column's significantly reduced retention volume.

As Figure 1 and Table 3 indicate, the v and n values are considerably higher for the HS/5 C18 column than the RAC-C18 and RAC-C8 columns. Neglecting extra-column effects (which is reasonable for phenobarbital due to its large k' value) one would expect as a result of the infinite diameter phenomenon that the larger diameter RAC columns would be closer in efficiency to the narrower HS/5 column. Indeed, it has been reported that for 10cm length columns packed with 5 micron packing material the 9.5 mm id column showed marked increases in efficiencies over a 4.5mm id column (10). Therefore it would appear that the RAC columns are not as well packed as the HS/5 column which results in a higher A term in the van Deemter equation.



4. Plot of HETP versus flow rate for heroin on various columns. See Figure 1 for mobile phase.



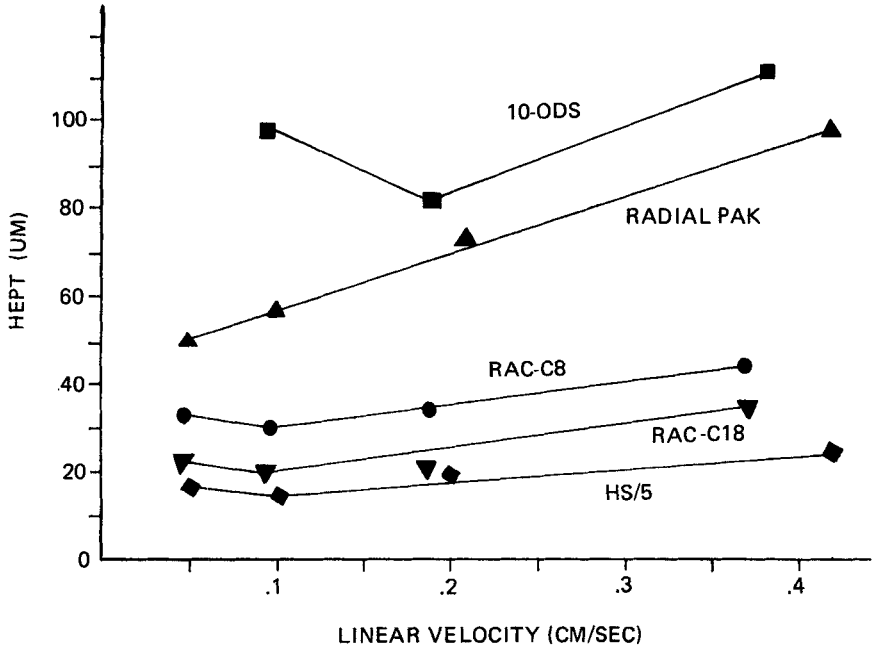
5. Plot of HETP versus flow rate for heroin; on a HS/5 C18 column with mobile phase without hexylamine (A) (see Figure 1); same column as (A) except mobile phase contains .072M hexylamine (B); on a HS/3 C18 column with mobile phase containing .072M hexylamine (C).

Figures 4 and 5 are plots for heroin of HETP versus flow rate for the various chromatographic systems. In order to obtain lower analysis times, higher than optimum flow rates were used and found to result in a small sacrifice in the number of theoretical plates.

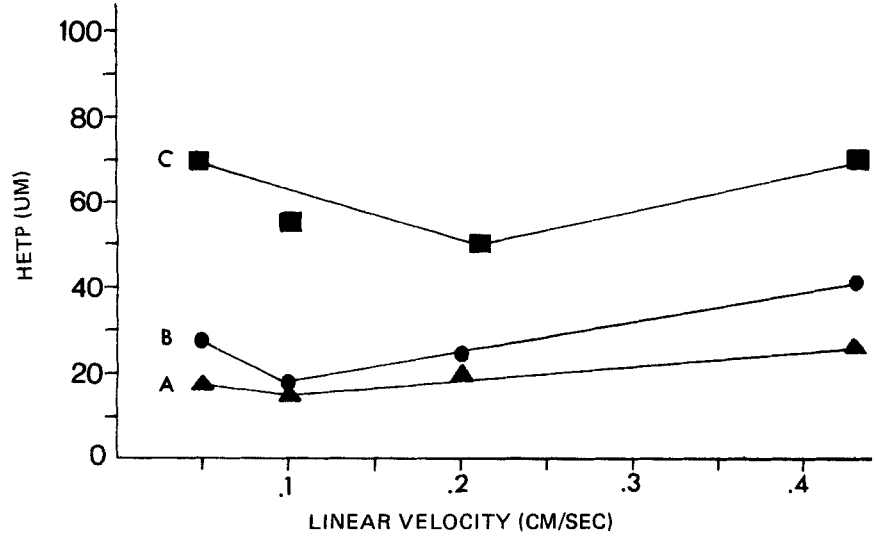
Figure 6 gives van Deemter plots for heroin using various columns without hexylamine. Van Deemter plots for the HS/5 C18 and ODS-3 RAC with other solutes have been reported and the curve shape is very similar to those illustrated in Figure 6 (7, 9). The smaller HETP values and smaller increase in HETP with linear velocity for the columns concontaining 5 micron particle sizesis a result of the effect of the smaller particle's size on the A and C term of the van Deemter equation. The shape of the curve for the Radial Pak microBondapak cartridge is very similar to that obtained for the microBondapak C18 column (11). The lower values for HETP on the Radial Pak microBondapak C18 column than on the 10-ODS-3 column are due to a smaller A term in the van Deemter equation as a result of the elimination of wall effects.

Figure 7 illustrates the effect of the addition of hexylamine to the mobile phase on the van Deemter plot for heroin on the HS/3 and HS/5 C18 columns. The higher HETP values and greater increase in HETP with linear velocity when an amine is added may be a result of the contribution of the D term in the van Deemter equation. This term relates to mass transfer effects in the stationary phase and could increase because of the presence of two differing absorption sites which result when a competing amine is added to the mobile phase (12). The two kinds of sites consist of bonded C18 groups and unbonded silanol groups (11). The higher HETP values for the HS/3 C18 column are consistent with a larger number of unbonded silanol sites and therefore a greater inhomogeneity of absorption sites. The HS/3 C18 column because it contains the smaller 3 micron particle size packing material would be expected to contain lower HETP values than the HS/5 C18 column (7).

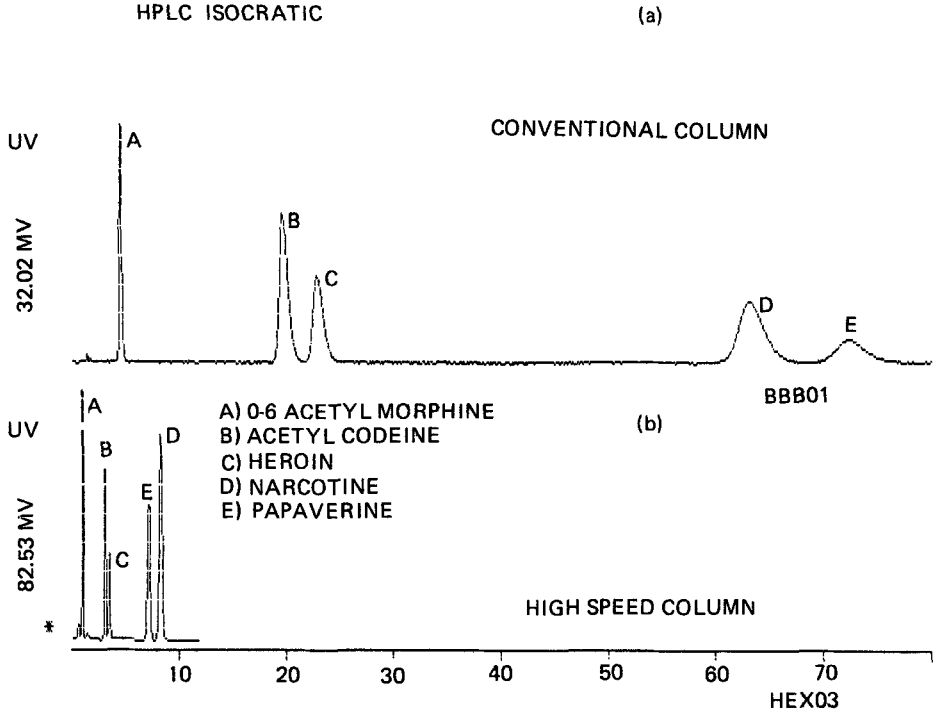
The HS/5 C18 column with a mobile phase containing hexylamine gave the best values of v and S for heroin. The use of this column with a mobile phase like one that has been previously reported for heroin analysis (2), except that methanesulfonic acid is replaced by hexylamine, gave a greatly improved separation for heroin and its major by-products. It was found that O^6-acetylmorphine, acetylcodeine, heroin,



6. Plot of HETP versus linear velocity for heroin on various columns. See Figure 1 for mobile phase.



7. Plot of HETP versus linear velocity for heroin. For description of (A), (B), and (C) see Figure 5.



8. Comparison of the separation of O^6-acetylmorphine (A), acetylcodeine (B), heroin (C), noscapine (D) and papaverine (E) on two chromatographic systems. System a consists of a Partisil 10-ODS-3 column with mobile phase which doesn't contain hexylamine. System b consists of a HS/5 C18 column with the same mobile phase as system a except that hexylamine is present. Flow rate on both systems is 3.0ml/min. The mobile phases are described in text.

noscapine and papaverine were resolved in almost one-tenth the previously reported (2) time, as illustrated in Figure 8. The addition of methanesulfonic acid or hexylamine to the mobile phase was necessary to produce a linear isotherm for heroin below a concentration of 2 mg/ml. The use of the above chromatographic system for the analysis of both uncut and adulterated samples is being studied.

ACKNOWLEDGEMENTS

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