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An approach to the evaluation and comparison of reversed-phase high-performance liquid chromatography stationary phases

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Abstract

An approach to the evaluation and comparison of reversed-phase high-performance liquid chromatography stationary phases with particular emphasis on data analysis and presentation is described. Assessment is based on the peak efficiency, asymmetry (USP tailing factor) and relative retention properties shown by 24 basic compounds having a wide range of structural and physico-chemical properties. A novel approach to data normalisation and presentation is described. This overcomes the problems associated with the quality of the column packing process, as well as differences in stationary phase selectivity which in conjunction with extra column band broadening effects can make comparisons meaningless. © 1998 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Sensitive and accurate analysis, whether in the pharmaceutical or bioanalytical field necessitates the use of high-performance liquid chromatography (HPLC) stationary phases which give symmetrical and efficient peaks. In an effort to meet the demands of current analytical problems, manufacturers are continuously improving and introducing new HPLC phases. In particular, the poor performance seen with basic compounds has been addressed through the development and introduction of so called base-deactivated materials. These are claimed to be superior to standard stationary phases for the analysis of basic compounds, due to reduced silanophilic interactions. From the analysts standpoint it is preferable to have only one or two columns which give good all-round performance with neutral, acidic and basic solutes. Consequently, procedures are required for the comparison of evaluation of phases which should give an unambiguous outcome.

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A variety of practical approaches for the evaluation and comparison of reversed-phase materials, with particular reference to basic solutes, have been reported in the literature. These have been based on the use of one or two [1–4], up to five [5–10] or 32 [11] basic solutes, and have included simple aromatics [3,7,8] or drug compounds [2,4–6,9–11]. These test compounds were usually selected on the basis of their pK_a and steric bulk around the basic centre. Occasionally compounds with high pK_a (>7) were selected [2,6,9] although it is only recently that clear evidence has been presented linking high pK_a and poor chromatographic performance [11]. The parameters assessed in these studies, include peak efficiency [3–5,8–10] and asymmetry [2,4,5,7,8,10,11] as well as the alpha value for a given pair of compounds, typically a neutral species and a basic compound [2,3,7,8]. A number of the earlier approaches have been reviewed [12]. Virtually all the reports have concentrated on the operating conditions (pH, buffers etc.) or the choice of compounds, with little or no consideration being given to the treatment or presentation of the data. Although a number of columns can meet given test criteria, they do not always give good performance with every compound [8,13].

We have assumed that the chromatography of basic solutes is the most challenging and to that end we have focused our approach on compounds of this type. Therefore, to allow identification of the best overall stationary phase we propose measuring the efficiency, symmetry and column retentivity with a diverse set of basic compounds. A similar approach employing the measurement of efficiency and symmetry has been recommended by Vervoort et al. [11]. The compounds we selected include both strong and weak bases, with hindered and unhindered nitrogens and compounds with multiple basic centres.

Column evaluation however, is not simply a matter of chromatographing a set of basic compounds. To overcome problems associated with the effects of hardware, the quality of the packing process, stationary phase selectivity differences as well as data presentation and interpretation, we have investigated data normalisation procedures and improved methods of graphical data presentation which we report here.

The aim of this report is not to make recommendations with regard to which stationary phase is the best, but to demonstrate an alternative approach to evaluation and data handling. The methods presented can be used with data generated under any chromatographic condition i.e. buffer types, pH, eluent modifier, etc. In developing the current approach we have used data generated on four HPLC columns under a predefined set of conditions. These columns all contained stationary phases which were claimed to be suitable for the analysis of bases.

2. Experimental

2.1. Equipment

The HPLC system consisted of an LDC/Milton Roy constametric 3000 pump, a Waters Associates 712 Wisp auto injector and a Perkin Elmer LC 135 diode array detector set at 255 nm. The system extra column band broadening was measured according to the literature [14] and was found to be around 40 μl which is considered typical of a modern HPLC system. Data acquisition and analysis were performed using the Beckman Peakpro data system, the data collection rate was 1.7 or 3.2 data points per second for the 15 and 10 cm columns respectively.

2.2. Materials

Methanol was HPLC grade from Fisons (Loughborough, UK), ammonium acetate was Analar grade from BDH (Liverpool, UK) and deionised water was produced in the laboratory using a multi stage ELGA system.

The test analytes were obtained from a variety of sources and were used as received. These compounds are listed in Table 1, along with their basic pK_a values where known, these values were taken from various literature sources. The compounds included simple test markers, e.g. phenol, naphthalene as well as basic compounds which have been used previously [2–6,8,9,15] and are known to be difficult to chromatograph on standard reversed-phase materials, e.g. *N*,*N*-diethy-

Table 1
Test solutes used in the study along with the basic pK_a where known

No.	Compound	pK_a	No	Compound	pK_a
1	Phenyl β-D-galactopyranoside	—[a]	**17**	*N*,*N*-Dimethylaminopyridine	9.6
2	Phenol	—	**18**	Pindolol	9.13
3	Benzyl alcohol	—	**19**	Amiloride	8.7
4	Phenetole[c]	—	**20**	Pyridine	5.25
5	Acetophenone	—	**21**	*d*,*l*-Homatropine	9.25
6	Naphthalene[c]	—	**22**	Caffeine	0.6
7	Anisole	—	**23**	Cycloguanil	NA[b]
8	*p*-Aminophenol	5.62	**24**	Levamisole	8.0
9	Thiamine	9.0	**25**	Scopolamine	7.55
10	*m*-Aminophenol	4.21	**26**	Codeine	8.2
11	Paraquat	—	**27**	Diphenhydramine[c]	9.1
12	Atenolol	9.6	**28**	Chlorpheniramine[c]	9.1
13	*o*-Aminophenol	NA[b]	**29**	*N*,*N*-Diethylaniline[c]	6.57
14	Procainamide	9.24	**30**	Chlorhexidine[c]	10.78
15	Practolol	9.45	**31**	ICI M207828[c]	NA[b]
16	Aniline	4.6			

[a] Not applicable.
[b] Not available.
[c] Chromatographed using eluent B.

laniline, diphenhydramine, procainamide. We also included a number of additional compounds from our own laboratory which had proved particularly troublesome, e.g. chlorhexidine and ZM207828.

The columns (I–IV) employed in the development of this methodology all contained reversed-phase materials, described by their manufacturers as base-deactivated or suitable for the analysis of basic compounds. The stationary phases in columns I–III were C8 materials and that in column IV mixed alkyl chain. They were all 5 μm particles, column III was 100×4.6 mm I.D., all the other columns were 150×4.6 mm I.D.

The test solutes were dissolved in methanol at concentrations of 1 mg ml^{-1} and diluted into eluent (≤ 50 μg ml^{-1}) for HPLC analysis. Duplicate 5 μl injections were made, except for some of the long retained compounds ($k' > 10$) where 20 μl was used to facilitate good integration. Each column required two eluents to allow the test compounds (which varied widely in lipophilicity) to be eluted within a reasonable k' range. The eluents consisted of mixtures of methanol and water, with the addition of ammonium acetate at a concentration of 0.1 M overall. Ammonium acetate was selected as the eluent buffer despite having limited buffering [16] compared with the more commonly used phosphate. This choice was based on its volatility which makes it eminently suited to mass-spectroscopic (MS) detection and its high solubility in common HPLC solvents [17]. The silanol masking activity of the ammonium ion is considered to be relatively weak such that it should have little effect on the outcome of the work. Eluent A, which was used for the more polar compounds (Table 1) varied between 25 and 30% methanol for the four columns. These eluents had an apparent pH of 7.06–7.16. Eluent B varied between 50 and 60% methanol and these eluents showed an apparent pH of 7.08–7.17. A flow rate of 1 ml min^{-1} was used throughout and experiments were carried out at room temperature.

Efficiency (N) was calculated using the following equation,

$$N = 16 \times (t_R/W)^2 \quad (1)$$

where t_R is retention time and W is width of peak at the base (both in min).

Capacity factor (k') was calculated as:

Table 2
Capacity factor (k'), efficiency (N, plates m^{-1}) and peak tailing (Tf) data for the 31 test compounds on columns I–IV

No.	I			II			III			IV		
	k'	N	Tf	k'	N	Tf	k'	N	Tf	k'	N	Tf
1	1.04	30 547	1.2	0.88	25 433	1.7	1.39	21 120	1.2	1.43	30 253	1.2
2	3.79	70 807	1.1	3.16	49 613	1.7	5.61	60 360	1.1	5.79	68 307	1.1
3	4.13	72 193	1.0	3.13	47 227	1.8	5.43	59 531	1.1	5.54	68 873	1.1
4	6.92	73 013	0.9	2.66	48 893	1.6	9.98	70 260	1.0	4.97	65 113	1.0
5	12.66	84 787	1.0	8.28	56 240	1.6	13.1	66 530	1.1	12.95	74 920	1.1
6	13.23	85 513	1.0	5.05	56 173	1.6	21.02	76 940	1.0	9.48	73 607	1.1
7	16.26	88 727	0.9	12.70	57 307	1.3	22.76	69 030	1.0	24.3	71 107	1.0
8	0.47	16 367	1.3	0.23	11 067	1.6	0.36	94 99	1.3	0.32	11 720	1.3
9	0.53	4813	2.5	0.13	7367	1.6	0.37	3510	1.7	0.16	6013	1.5
10	0.69	28 920	1.3	0.47	20 020	1.8	0.74	18 900	1.3	0.73	26 947	1.3
11	0.91	173	4.9	0.03	3227	5.6	0.88	370	5.2	0.30	900	2.9
12	0.97	8947	2.6	0.47	13 793	1.7	0.63	6610	1.3	0.49	12 160	1.2
13	1.28	30 980	1.5	0.92	21 027	1.9	1.56	24 570	1.5	1.57	34 147	1.4
14	1.35	4960	2.9	0.39	11 633	2.0	0.76	3390	1.5	0.38	10 467	1.2
15	1.59	17 707	1.6	0.73	16 660	1.8	0.98	7279	1.3	0.77	16 400	1.2
16	2.59	66 100	1.1	1.78	40 727	1.7	3.25	48 221	1.1	3.26	61 540	1.1
17	3.19	680	4.1	0.32	5113	3.3	1.24	840	3.6	0.43	1187	3.9
18	3.58	25 493	1.7	2.11	25 227	2.2	2.57	12 930	1.4	2.15	26 680	1.3
19	4.26	49 560	1.2	2.36	39 267	1.7	2.87	28 541	1.2	2.86	43 360	1.1
20	4.34	34 240	2.2	1.62	16 147	2.5	3.00	12 911	2.1	2.69	8540	2.7
21	4.37	6333	4.5	1.64	5267	1.4	2.77	2509	1.3	1.83	3347	1.1
22	5.37	30 060	2.6	1.77	32 967	1.8	2.22	16 031	1.3	1.93	33 880	1.3
23	7.33	25 727	2.2	5.10	40 953	1.9	6.64	21 420	1.4	6.38	46 920	1.2
24	7.58	36 420	2.5	3.63	9647	0.8	6.35	9030	1.1	5.50	4007	0.6
25	7.93	20 233	1.1	4.70	10 213	0.8	6.83	8929	0.7	6.39	7040	0.6
26	10.27	6027	6.5	4.36	22 427	1.0	6.31	7819	1.7	4.90	8147	0.6
27	10.50	11 213	4.0	2.06	20 087	2.8	8.91	11 861	2.3	2.84	36 380	1.6
28	11.70	2007	6.8	1.52	14 013	3.1	7.95	2730	3.1	2.01	25 227	1.6
29	18.77	78 813	1.0	6.31	58 233	1.6	26.86	73 250	1.0	12.14	70 933	1.1
30	24.55	20 207	2.2	5.61	29 913	2.0	35.55	13 370	1.5	5.86	25 780	1.4
31	42.20	2427	5.0	6.60	19 007	3.5	31.9	739	3.4	7.72	28 327	1.5

The lipophilic compounds **4**, **5** and **27**–**31** were chromatographed with eluent B (50–60% methanol) all other compounds were chromatographed with eluent A (25–30% methanol).

$$k' = (t_R/t_0) - 1 \quad (2)$$

where t_R is the retention of the solute in question and t_0 the retention time of the unretained solute. The latter was measured using a 5 μl injection of lithium nitrate solution (20 mg ml^{-1}).

The USP tailing factor (Tf) has been used to measure peak symmetry and this was calculated according to [18] using the equation.

$$Tf = 0.5(t_b - t_a/t_R - t_a) \quad (3)$$

where t_a and t_b are the retention times of points on the leading and tailing edges of the peak, measured at 5% of peak height.

3. Results and discussion

3.1. Efficiency

The k' and raw efficiency data (N, plates m^{-1}) for all the compounds on the four columns are shown in Table 2. The poor performance for some of the compounds may reflect the operating conditions, particularly the pH that was used. However the choice of an ammonium acetate buffer with a pH near neutral reflected our desire to have a system which was ultimately compatible with MS detection.

Although there is a high degree of variability in the data (Table 2), superficial examination would suggest that the stationary phase in column I is superior to the other three for the analysis of basic compounds, since this gives the highest efficiencies for the most bases (10/24). However, the data for the simple test markers (compounds **1**–**7**), which should all chromatograph with good efficiency irrespective of the nature of the stationary phase, also show column I to be superior to the others.

Column I was supplied by a manufacturer known for packing highly efficient HPLC columns. Therefore, the data in Table 2 is not just a reflection of the base deactivated properties of the phase, but it also reflects the column packing procedure and possibly the geometry of the column and end fittings. Consequently, the high efficiency seen for the basic compounds on column I may not be due to the base deactivated nature of the stationary phase but because the column was very well packed.

A second problem evident from the data for the simple compounds (Table 2) is the reduction in efficiency with decreasing k'. This phenomenon, which is mainly due to extra column band broadening (ECBB) [19] is present in all chromatographic work, but is particularly noticeable for compounds with low k', i.e. <4. In the present work this factor becomes problematical when one considers that there are very great selectivity differences between the four columns. Even though the eluent strength was selected to give a similar elution range for each column, the k' for a given compound often varied widely. For example, chlorpheniramine (**28**) eluted with k' of 11.7, 1.52, 7.95 and 2.01 on columns I–IV respectively. Even if all the columns were truly base deactivated with respect to chlorpheniramine, the efficiency on columns II and IV would always look worse than that of I or III, due to ECBB. Similar differences were evident for compounds 17, 21 and 22. The variation in efficiency with k' also makes the comparison of the efficiency shown by different compounds on a single column difficult.

Although this problem could be overcome by adjusting the eluent for each compound, so that they all chromatograph with a similar, fixed k', this would have involved considerable extra work. Furthermore, the variation in the eluent composition could have resulted in differing degrees of secondary interaction which may have confounded the results.

To overcome these problems in a simpler manner, and focus the comparison on the stationary phase per se, the efficiency data for the basic test solutes was normalised. This normalisation was carried out by referencing the efficiency for a basic test compound against the efficiency which would be shown by a simple neutral test compound running at the same k'. The efficiency for a simple test compound at a given k' (the ideal efficiency) was obtained from the neutral test solutes (compounds **1**–**7**) by interpolation, using simple transformations of chromatographic equations [14] as described below.

The dispersion in any chromatographic system can be described by Eq. (4):

$$\sigma^2_{\text{obs}} = \sigma^2_{\text{col}} + \sigma^2_{\text{extra}} \tag{4}$$

where σ^2 is the peak variance. The subscripts 'obs', 'col' and 'extra' in this (and other equations) refer to the observed or experimental peak dispersion, the dispersion due to the column and the dispersion due to extra column effects respectively. From basic theory:

$$\sigma^2_{\text{col}} = t^2_{\text{R}}/N_{\text{true}} \tag{5}$$

where N_{true} is the true or intrinsic column efficiency (i.e. the efficiency shown by a simple solute chromatographed under ideal conditions with no extraneous effects). Substituting into Eq. (4) gives

$$\sigma^2_{\text{obs}} = t^2_{\text{R}}/N_{\text{true}} + \sigma^2_{\text{extra}} \tag{6}$$

Eq. (2) can be rearranged in terms of t_{R}. which can then be substituted into Eq. (6) to give Eq. (7) which has been previously described by Freebairn and Knox [14].

$$\sigma^2_{\text{obs}} = [t^2_0(k'+1)^2]/N_{\text{true}} + \sigma^2_{\text{extra}} \tag{7}$$

By analogy with Eq. (5) one can make the substitution for σ^2_{obs} to give Eq. (8)

$$t^2_{\text{R}}/N_{\text{obs}} = [t^2_0(k'+1)^2]/N_{\text{true}} + \sigma^2_{\text{extra}} \tag{8}$$

Thus a plot of $t^2_{\text{R}}/N_{\text{obs}}$ against $(k'+1)^2$ for a series of simple neutral solutes should give a straight

line of the slope t_0^2/N_{true} and intercept σ^2_{extra}. This plot was generated using the data for the neutral markers on all four columns, all of which gave good straight lines with an $r^2 > 0.994$. If we make the not unreasonable assumption that N_{true} is independent of k' then (from a knowledge of the regression constants (i.e. t_0^2/N_{true} and σ^2_{extra}) and the retention time for a given basic solute) it is thus possible with Eq. (8) to calculate the expected efficiency for that solute (which we will call (N_{ideal}), assuming that it acts in a regular manner with no silanol interactions. This N_{ideal} should always be greater than or equal to the observed efficiency (N_{obs}). For practical convenience the N values (observed or ideal) can be converted into their equivalent H values.

From the observed or experimental efficiency (H_{obs}) for a basic solute and its predicted or ideal efficiency (H_{ideal}), it is thus possible to calculate the difference which can be attributed to secondary interactions which we consider to be due in the main, to residual silanols, this difference we have termed H_{sil}, where:

$$H_{sil} = H_{obs} - H_{ideal}$$

A stationary phase with a low H_{sil} value indicates reduced silanol interactions and would suggest that the stationary phase would be suitable for the analysis of basic solutes.

Thus through the use of the H_{sil} parameter, column packing and column geometry differences, as well as effects resulting from selectivity differences coupled with ECBB effects are eliminated, allowing a direct comparison of the stationary phase characteristics. Although it is a simple matter to apply this form of normalisation, the raw efficiency data should be first checked to ensure that the column is producing acceptable efficiency values, i.e. it is well packed and the maximum efficiency observed with a simple test solutes at

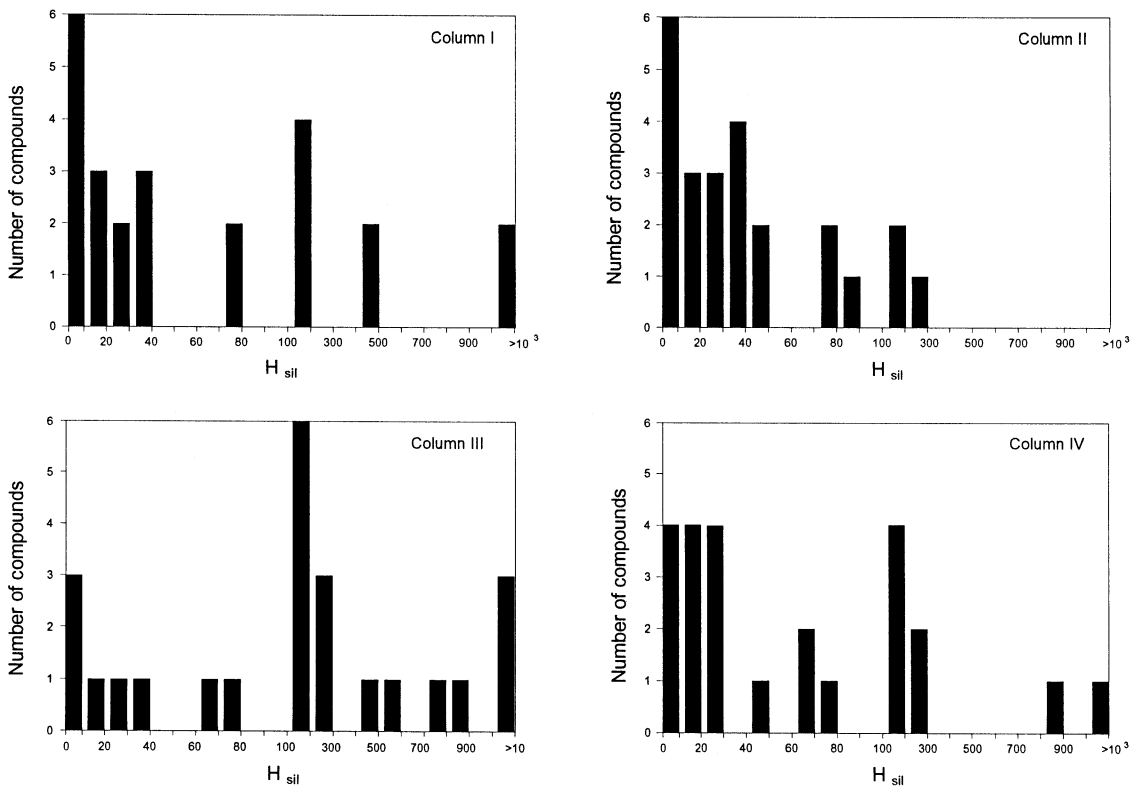

Fig. 1. Frequency histograms for stationary phases in columns I–IV showing the spread of H_{sil} values for the 24 basic compounds.

high k' (i.e. where extra column effects are minimal, typically $k' > 4$) is considered satisfactory. In this instance the lowest maximum was around 57000 plates m^{-1} (column II), which although substantially lower than the best (column I) would still be considered to be satisfactory.

Data for the four stationary phase (using results for the 24 basic compounds), normalised as described above, are presented as frequency histograms in Fig. 1. From this simple but commonly used form of data presentation [10,11,13], the stationary phase in column II would appear to be the best in terms of giving lower H_{sil} values for most of the basic solutes. This is in contrast to assessment of the raw data discussed above which indicated the stationary phase in column I to be the best. However, even with data normalisation, it is still difficult to assess the best overall stationary phase especially in terms of making a decision between what appears to be the best two columns; II and IV. The difficulty in making a quantitative assessment of the stationary phase would be further aggravated if the test data set involved more that four columns as in other studies [10,13].

A simple, but more explicit means of presenting the data was sought and the use of cumulative frequency plots was adopted. Such plots for the four stationary phases are shown in Fig. 2 where cumulative frequency (rather than frequency as in Fig. 1) is plotted against H_{sil}. This form of presentation has the advantage that data for many stationary phases can be presented concisely and simultaneously in a single plot. The best overall stationary phase is that with a steep profile, shifted farthest to the left. In this case it is readily discernible as II, followed by I and IV both of which have similar properties. The stationary phase in column III however, is clearly the worst.

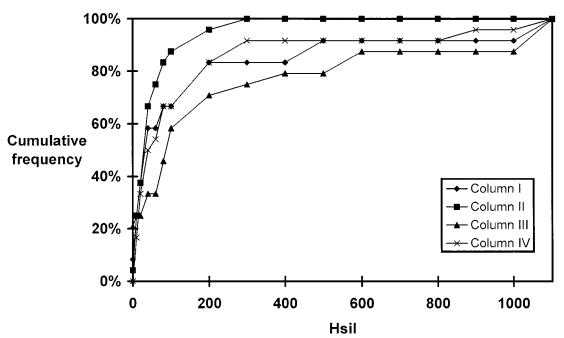

Fig. 2. Cumulative frequency profiles for H_{sil} calculated with the four columns.

Apart from the advantage of good visual presentation, the use of cumulative frequency plots also allows the calculation of a single quantifiable parameter for each stationary phase. For instance the number (or preferably the percentage) of compounds giving a predefined H_{sil} (e.g. $H_{sil} = 50$), or the H_{sil} shown by 90% of the compounds studied (H_{sil} 90%) can be easily obtained and compared. Using the data in Fig. 2 it can be shown that the stationary phase in column II has an H_{sil} 90% of 120 whereas that in IV (which was difficult to distinguish from II using the simple histograms (Fig. 1) has a value of 280. The worst H_{sil} (> 1000) was observed with III. Thus, unlike the frequency histograms the cumulative frequency plots allow clear visual and quantitative comparisons to be made between stationary phases as well as giving a single quantifiable parameter for each phase.

3.2. Peak tailing

A similar approach can be applied to the peak tailing factors (Tf) where one can consider the observed peak tailing to be made up of the following Tf contributions.

$$Tf_{obs} = Tf_{ideal} + Tf_{sil}$$

once again the subscripts 'obs', 'ideal' and 'sil' refer to: the observed tailing, the tailing shown by an ideal solute and the tailing contribution due to silanol interactions, respectively. We have defined Tf_{ideal} as the mean tailing factor of the seven neutral solutes (Table 2). It is worth noting that the mean tailing factor for the neutral solutes on column II was relatively high, approximately 1.6, which fits with the manufacturer's test data which reported an asymmetry (A_s) for anthracene of 1.38. These values are higher than most manufacturers and users would normally accept. However, we subjected the column to a full evaluation since it actually showed the best performance in terms of normalised efficiency (Fig. 2).

It has been reported that peak symmetry for basic solutes varies with k' [11], however we be-

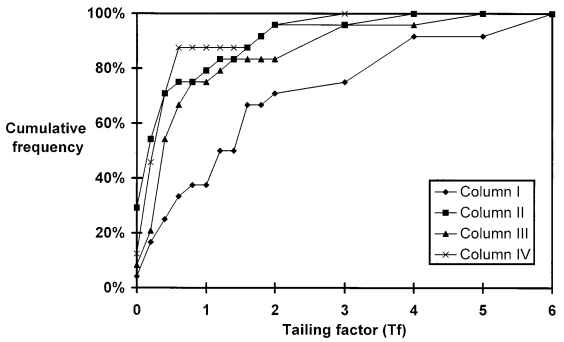

Fig. 3. Cumulative frequency profiles for Tf_{sil} calculated with the four columns.

lieve that these observations are related to the changing eluent composition in the reported work which resulted in variable secondary interactions. In the present experiments, where the eluent composition for a given set of compounds is relatively constant (within 10% methanol concentration), then the Tf values form a coherent data set which are amenable to comparison both within and between columns.

Fig. 3. shows a plot of cumulative frequency against Tf_{sil}. As measured by Tf_{sil} 90% values, the stationary phases in columns II and IV look to be the best closely followed by III. Although the Tf_{sil} measurements show II to have reduced silanol effects, examination of the raw Tf data for the neutral solutes (Table 2) would suggest that it is much worse than either III or IV. This shows therefore, that it is very important to extract from the data the contribution due to the silanols. Assessing the raw data alone, which contains contributions due to column packing etc., can result in invalid conclusions being drawn. The conclusion here therefore, is that in terms of minimal silanol interactions (as assessed through Tf_{sil} measurements) the stationary phases in columns II and IV are the best. The performance of II with the simple solutes suggests however that this column is less than optimally packed.

It is interesting to note that although the overall conclusion from the analysis of efficiency and asymmetry data are similar, at least in terms of the two best phases (II and IV), the ranking for I and III is reversed and the differences with either parameter are quite marked.

3.3. Capacity factors (k′)

The retention data as well as the efficiency and symmetry data can also contain information on silanol activity which is amenable to analysis, since it is a widely observed phenomena that silanol interactions can lead to over-retention for basic compounds. In an effort to quantify this effect, the retention data for the basic solutes was converted into relative retention (RRt) using a neutral marker as the reference compound. A stationary phase with lower silanol activity should show a narrower relative retention range for a given set of basic compounds compared with one which shows high silanol activity. Using the present data, the capacity factors of all the compounds chromatographed with eluent A were normalised to the capacity factor of acetophenone (compound **5**) and all the compounds chromatographed with eluent B were normalised to the capacity factor of compound **4** (phenetole). Cumulative frequency profiles were generated with this relative retention data and the relative retention time equivalent to the elution of 90% of the compounds was determined. This was found to be approximately 2.0, 1.01, 1.01, and 0.98 for columns I–IV respectively. Thus the phases in II, III and IV are relatively similar and they appear to show superior silanol deactivation to I, as determined by the influence of the silanols on retention.

3.4. Choice of test compounds

The results of this present work were very dependent on the test compounds used. For example, *N*,*N*-diethylaniline (DEA, compound **29**) which has been widely used as a test compound (albeit mainly with unbuffered eluents) ran well on all columns, (Table 2). The performance shown by a number of other compounds was very poor however. In particular the pyridines (**17** and **20**) appear highly problematic on columns II–IV. In terms of efficiency, some compounds (**18**, **22**) performed badly on one column but reasonably

well on the others, and then other compounds (**24**) only performed well on one column. It may be possible to use chemometric methods to help aid compound selection for this type of work and possibly reduce the number of compounds used [10]. However the variability seen here suggests that for a full and meaningful evaluation a wide range of compounds need to be studied.

In terms of the nature of the compounds used, there is some evidence to indicate that it is the most basic compounds which chromatograph badly [11] and hence these should be more discriminating for stationary phase evaluation purposes. Analysis of our data, using both normalised efficiency (H_{sil}) and tailing factor (Tf_{sil}) values showed there to be only a very weak correlation between solute pK_a and stationary phase performance. To a large extent this is not unexpected since the size of the molecule and in particular the steric bulk around the basic centre will influence its ability to penetrate the alkyl chains on the stationary phase and interact with the silanols. In support of this view are the present results for pyridine, which whilst being a relatively weak base (pK_a 5.25), shows significant tailing on all phases, presumably due its small size and hence greater ability to interact with the residual silanols. A plot of H_{sil} versus pK_a for column IV, which is typical of those seen, is shown in Fig. 4. This plot is similar to that shown previously [11], although the scatter in the data, especially at high pK_a, is much greater. This latter factor may be related to the test compounds used here in comparison to those employed in the previous work [11]. In the case of the work of Vervoort et al. [11] not all the 32 structures were given and of the 11 that were, seven were lipophilic, tri or tetra cyclic bases with similar pK_a.

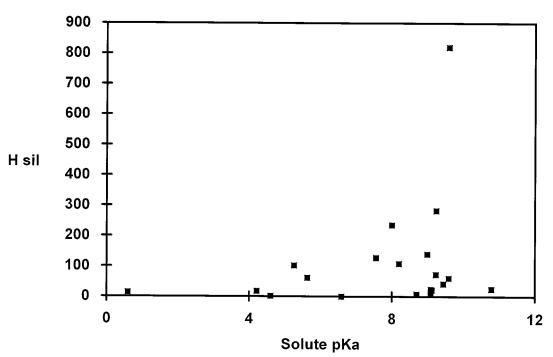

Fig. 4. Plot of H_{sil} versus solute pK_a determined with column IV.

Similar treatment of asymmetry data presented by Ascah and Feibush [20], who evaluated a different base deactivated material showed the same general lack of correlation. In general therefore, it would appear that pK_a alone does not control the chromatographic characteristics of a given solute and consideration should be given to the size of the molecule and the steric bulk around the basic centre(s). This view is well supported by work of McCalley [13,21].

3.5. Overall assessment

The selection of the best stationary phase will depend very much on the use to which it is to be put and whether a narrow retention range is to be preferred over symmetric peaks etc. The present work was aimed at devising methods to select the best all round stationary phase for drug analysis with special emphasis on suitability with basic compounds. In summarising our findings therefore we have given equal weight to each parameter: efficiency, tailing and relative retention. We have also taken into account the relatively good efficiency shown by column I and the poor tailing shown by column II, by including the minimum H and the mean tailing factor shown by the neutral markers. This raw data is presented in Table 3 along with the same data which has been scaled by reference to the median value for each of the five parameters. The table also includes the sum of these scaled parameters which acts as a 'score' and shows that with equal weighting, the stationary phase in column IV is the best overall, closely followed by II. It is clear from the data in Table 3 that II was let down by the poor peak symmetry for all the solutes, which is probably attributable to poor packing.

4. Conclusions

This work shows that for meaningful evaluation of HPLC stationary phases, careful thought

Table 3
The measured parameters and final 'scores' for the four columns studied

Column	Raw data					Scaled data					
	H min[a]	Mean Tf[b]	H_{sil} 90%	Tf_{sil} 90%	RRt 90%	*H* min[a]	Mean Tf[b]	H_{sil} 90%	Tf_{sil} 90%	RRt 90%	'Score'
I	11	1.0	490	4.0	1.95	0.7	0.9	1.3	1.9	1.95	6.8
II	17	1.6	120	1.7	1.01	1.1	1.5	0.4	0.8	1.0	4.7
III	19	1.1	2750	2.5	1.01	1.3	1.0	7.1	1.2	1.0	11.5
IV	13	1.1	280	1.7	0.98	0.9	1.0	0.7	0.8	1.0	4.3

[a] Taken from the most efficient neutral marker, compound **5**, **6** or **7** depending on the column.
[b] The mean of the neutral markers.

needs to be applied to the selection of the test compounds and test parameters, as well as the treatment and presentation of the results. Since efficiency, asymmetry and column retentivity appear to give different information about the phases under study, the use of all three parameters would appear to be necessary. What is clearly apparent is that interpretation of the raw data alone can lead to the wrong conclusions being made in terms of which is the best phase. Extraction of the contribution made by residual silanols to peak shape (e.g. the use of H_{sil} or Tf_{sil}), is seen as important for meaningful conclusions. Normalisation of the efficiency data in terms of H_{sil} is essential for a number of reasons. It overcomes both differences in the way the columns have been packed, as well as problems associated with extra column band broadening which can give misleading results, especially when selectivity varies, as in this present study.

Whilst basicity of an analyte, as defined by its pK_a, does have some influence on whether a given compound will show good symmetry or efficiency it is not a universal guide to good chromatographic performance. Solute size and the stereochemistry around the basic centre would appear to be equally important parameters. Furthermore, as no two stationary phases handle every compound in the same way, we therefore recommend the use of the widest range of test compounds possible. The test set used here were selected on the basis of previous work and personal experience, and with one exception are readily available.

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