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Comparison of the performance of conventional C_{18} phases with others of alternative functionality for the analysis of basic compounds by reversed-phase high-performance liquid chromatography

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Abstract

The performance of ten reversed-phase columns which included conventional C_{18} phases, phases with embedded polar groups, short chain and cyano phases, and a high-pH stable phase, was evaluated with a variety of basic compounds of low and high pK_a. The aim of the work was to determine if these alternative phases offered any advantages over conventional C_{18} phases for the analysis of basic compounds. Mobile phases which were unbuffered, buffered with phosphate at a pH of 7.0 and 3.0, and modified with either methanol or acetonitrile, were investigated. Phases with embedded polar groups exhibited reduced hydrophobicity, somewhat different selectivity, and greater inertness towards basic compounds compared with C_{18} phases prepared on the same silica. Phases with shorter alkyl chains also produced improved peak shape for basic compounds; selectivities were similar for alkyl bonded phases but completely different for a cyanopropyl phase at pH 7.0. At high pH (pH 11.0) a novel bidendate phase gave improved peak shapes for some bases together with different selectivity compared with operation at pH 7.0. However, a contributing factor to this improvement may be the silanol masking ability of buffer components utilised in addition to reduced ion-exchange interactions with ionised silanols.

Keywords: Stationary phases, LC; Column performance; Basic compounds

1. Introduction

There is continued interest in the development and application of testing procedures which can be used to evaluate the ''activity'' of reversed-phase (RP) columns towards basic compounds. Considerable advances have been made by column manufacturers in the last 10 years in the production of columns which are more suitable for analysis of this group of compounds, which contain many important pharmaceuticals and compounds of biomedical and environmental significance. Much of the improvement in the peak shape of bases shown by modern packings can be attributed to the use of high purity silicas containing low levels of important metals such as iron and aluminium, which when incorporated into the silica structure may contribute to the acidity of residual silanol groups. These can give rise to serious peak tailing on RP columns, due to a variety of complex mechanisms [1]. There still appear to be considerable differences in the ''activity'' of these purer silica packings, the cause of which is not

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entirely clear [2,3]. The evaluation of columns is not straightforward, since it appears that performance can be influenced by many factors, including the nature of the basic substance, the organic modifier and the pH of the mobile phase. In some detailed studies using chemometric procedures to assist in data evaluation, we have concluded that a reasonable overall assessment of a column's activity towards bases can be carried out using at least five or six basic probes analysed with three different mobile phases: methanol–phosphate buffer pH 7.0, acetonitrile–phosphate buffer pH 7.0 and acetonitrile–phosphate buffer pH 3.0 [4,5].

Most analyses of bases are carried out on C_{18} phases, and best results are obtained on those prepared from pure ''new generation'' silicas. Recently, columns containing alternative ligands bonded to these pure silicas have become available. It is possible that these new phases may be useful for analysis of bases either due to further improvements in peak shape, or by offering a different selectivity to C_{18} phases. In some situations, it is useful to change the stationary phase rather than the mobile phase composition in order to achieve different selectivity effects. For example, the use of acetonitrile may be obligatory for separations at very low UV wavelengths; or solubility considerations for the sample may dictate a particular mobile phase [6]. It may just be that changes in mobile phase composition are inadequate to produce the desired separation [7]. Especially for the study of basic compounds, columns prepared from the same base silica are essential to investigate ligand effects, otherwise differences may be merely due to differences in silanophilic activity of the base silica between the different columns. Amongst these alternative columns are:

Phases containing embedded polar groups. Columns with embedded amide groups were first introduced by Supelco [8], who recently produced an improved version of the original phase based on a pure silica [9]. Similar phases with embedded carbamate groups were introduced by Waters [10]. These phases are claimed by manufacturers to give superior peak shape, and also to offer a different selectivity to conventional phases, although little comparative data has been published for columns using the same silica. The ability of such phases to trap a deactivating layer of water, or the competitive interaction of the embedded group with silanols, have been proposed as reasons for improved peak shape [11].

Columns with shorter alkyl ligands. Few systematic studies have been published which compare the peak shape or selectivity for basic solutes of columns made from the same silica but with shorter chain alkyl (C_8, C_4), or with polar bonded phases such as cyano groups. One study [12] claimed that peak shape for basic compounds generally improved as the chain length of the alkyl bonded phase decreased from C_{18} to C_1. However, this study was performed using only one type of basic probe (substituted pyridines) and a single mobile phase for the bases. Other bases or mobile phases might conceivably give different results. Furthermore, the pyridines gave extremely poor peak shape (asymmetry factors ranged from 6.2 for C_1 to about 20 for C_{18}) on the classical phase used (Hypersil ODS) which has a high metal content. Recently, these alternative ligands have become available on high purity silicas enabling a more detailed study of these effects to be made with high pK_a bases, which may show different behaviour, giving in general higher peak asymmetry. Other studies [6,7] have looked in some detail at selectivity differences between phases with different bonded ligands, although these studies included few basic compounds.

Columns with extended stability at high pH. Whereas better peak shape for a given compound is usually obtained by working at acid pH where the ionisation of silanol groups is suppressed and thus ion-exchange is reduced [2,3], an alternative approach is to work at a pH well above the pK_a where reduced ion-exchange is achieved by chromatography of bases as neutral species. Until recently, this approach has been limited by the pH stability of conventional RP columns, allowing analysis of only weak bases in the unprotonated state. However, recently, phases with enhanced pH stability have been prepared [13] allowing the investigation of the chromatography also of much stronger bases as neutral compounds. Another potential advantage of such phases might be a different selectivity offered by separation of bases as neutral rather than as partially ionised compounds.

The aim of the present study was to investigate the performance of these alternative types of phase, both in terms of peak shape and selectivity. By using a

variety of columns from different manufacturers and by comparing identical silicas from the same manufacturer bonded with different ligands, it was hoped that some conclusions could be reached on what generic types of phases might offer promise in the solution of the problem of the analysis of bases. The columns were evaluated using very similar equipment and procedures as used previously [2,3] allowing direct comparison with previously published data. This large body of data may also assist in the selection of both suitable columns and mobile phases for the analysis of the test compounds (or compounds closely related to them) of which many are important pharmaceuticals or significant chemicals in their own right.

2. Experimental

The HPLC system consisted of P200 pump, UV 100 detector (1μl flow cell) operated at 254 or 215 nm (Thermo Separation Products, San Jose, USA) and 7725 valve injector with 2μl loop (Rheodyne, Cotati, USA). Connections were made with minimum lengths of 0.0127 cm I.D. tubing. These precautions were taken to minimise extra-column effects. The columns were 1=Discovery Amide (surface area 200 $m^2\ g^{-1}$, %C=12%), 2=Discovery C_{18} (surface area 200 $m^2\ g^{-1}$, %C=13.5), 3=Discovery C_8 (surface area 200 $m^2\ g^{-1}$, %C=7.3), all from Supelco, Bellafonte, USA, 4=Inertsil 3-C_8 (surface area 450 $m^2\ g^{-1}$, %C=9.0, 5=Inertsil 3-C_4 (surface area 450 $m^2\ g^{-1}$, %C=6.4), 6=Inertsil 3-CN (surface area 450 $m^2\ g^{-1}$, %C=13.8), all from GL Sciences, Tokyo, Japan, 7=SymmetryShield RP-18 (surface area 340 $m^2\ g^{-1}$, %C=17.6), 8=SymmetryShield C_8 (surface area 340 $m^2\ g^{-1}$, %C=14.8), both from Waters, Milford, USA, 9=Luna C_{18}–2 (surface area 410 $m^2 g^{-1}$, %C=17.4), Phenomenex, Torrance, USA, 10=Zorbax bidentate C_{18}/C_{18} (surface area 185 $m^2\ g^{-1}$, %C=12.1). All columns used were 25 cm×0.46 cm and packed with (nominally) 5μm particles. N was determined from peak widths at half height ($w_{0.5}$) using the formula $N=5.54[t_r/w_{0.5}]^{2,}$, or using the Dorsey-Foley Eq., $N_{df}=41.7[t_r/w_{0.1}]^2/[A_s+1.25]$. A_s was calculated at 10% of the peak height from the ratio of the widths of the rear and front sides of the peak; all measurements were made using a model 2000 data station (Trivector, Bedford, UK). All results were the mean of at least duplicate injections. Preparation of buffers was as described previously with pH measured before addition of organic solvent [2,3]. All analyses were performed at 30°C with the column thermostatted in a block heater (Model 7980, Jones Chromatography, Hengoed, UK). Test solutes included codeine (pK_a= 8.0), quinine (8.5), procainamide (9.2), diphenhydramine (9.0), nortriptyline (10.0), nicotine (7.9), amphetamine (9.9), pyridine (5.2) and benzylamine (9.3) 0.2 μg of analyte was injected in each case to prevent significant overloading of the column, which can be especially problematic at low pH [14]. Solutes were generally injected singly, rather than in mixtures, due to possible mutual influence on peak shape of closely eluting substances [2]. Data for two further columns (Inertsil ODS-3 and Symmetry C_{18}) have been abstracted from previous studies [2,3], and are used again here for comparative purposes, although measurements for benzylamine at pH 7.0 were not available for these two columns. Full details of these columns were given previously [2]. Column void volume was estimated by injection of uracil using 55:45 methanol–water (v/v) or 40–60 acetonitrile–water (v/v) as appropriate. Some previously reported k values were re-calculated to make them compatible with the method for k calculation used for the present data.

3. Results and discussion

3.1. Overview of column performance

Table 1 gives some performance data for all ten columns using unbuffered methanol (55:45, v/v) and acetonitrile (40/60, v/v) which are roughly isoelutropic, giving broadly similar retention times for the probes. We have argued against testing columns with ionogenic solutes in unbuffered mobile phases which we believe give unrepresentative results and suffer from poorer reproducibility than tests in buffered mobile phases [2,5,15]. However, because pyridine and aniline are easily separated in a single run from uracil (used to measure void volume) and benzene (used to measure hydrophobicity), further

Table 1
Column performance data for low pK_a bases and benzene using (a) methanol–water (55:45, v/v) and (b) acetonitrile–water (40:60, v/v)

		Pyridine				Aniline				Benzene			
		k	N	N(d–f)	A_s	k	N	N(d–f)	A_s	k	N	N(d–f)	A_s
Supelco Dis. Am a	(a)	0.19	26 100	20 500	1.28	0.34	27 800	20 500	1.22	1.70	30 200	28 700	1.04
b	(b)	0.31	29 300	22 500	1.32	0.76	31 300	27 200	1.16	2.62	27 800	26 800	1.05
Supelco Dis.		0.29	15 300	7150	1.90	0.39	22 100	16 400	1.31	2.34	20 600	16 300	1.26
		0.39	11 400	4340	2.59	0.77	18 700	15 700	1.25	3.31	16 600	15 900	1.10
Supelco Dis. C_8		0.21	20 100	16 300	1.27	0.31	22 800	20 700	1.06	1.41	22 600	21 000	1.05
		0.27	19 400	15 500	1.18	0.62	24 900	22 800	1.08	2.31	23 000	21 900	1.05
Inertsil3-C_8		0.67	15 500	11 100	1.34	0.80	16 100	12 200	1.22	4.39	17 500	14 700	1.22
		0.80	15 100	6900	2.25	1.61	20 300	18 100	1.05	6.04	18 300	18 000	0.96
Inertsil3-C_4		0.49	13 600	9600	1.46	0.57	14 200	10 900	1.35	2.14	16 800	13 400	1.35
		0.71	18 600	15 000	1.27	1.47	21 000	19 500	1.10	4.25	19 000	18 600	1.03
Inertsil3-CN		0.58	13 200	8130	1.52	0.89	14 800	11 100	1.26	1.67	13 700	9220	1.52
		0.36	20 400	14 700	1.34	0.73	14 000	4600	2.57	1.50	24 200	22 600	1.00
Symm.Shield 18		0.45	5040	1290	3.04	0.83	20 100	16 900	1.18	4.30	23 000	19 800	1.18
		0.55	8450	2640	2.68	1.54	25 800	23 700	1.18	6.32	24 800	23 900	1.07
Symm.Shield 8		0.43	5200	1260	3.13	0.73	20 300	17 600	1.18	3.03	23 100	20 500	1.13
		0.59	10 100	3030	2.70	1.49	27 100	24 800	1.12	5.08	23 900	23 100	1.07
Luna C_{18} (2)		0.59	11 700	5060	2.08	0.86	25 800	24 200	1.07	5.50	25 300	24 900	1.00
		0.67	17 200	7270	2.02	1.71	26 400	24 700	1.03	8.14	20 200	19 000	1.06
Zorbax Bidentate		0.55	4420	1010	3.48	0.64	22 400	17 100	1.30	4.99	22 900	20 500	1.19
		0.62	4410	964	3.62	1.19	24 900	19 700	1.26	6.37	23 500	22 600	1.11

data for these low pK_a bases was obtained. It has been suggested recently that simple k data for neutral compounds represent column hydrophobicity better than so called ''hydrophobic selectivity'' or ''methylene group'' selectivity tests where the relative retention of compounds such as ethyl benzene and toluene is measured [16,17]. From this initial ''screen'' poorly packed or damaged columns can be rejected, by observation of benzene data. There is some evidence of slightly enhanced peak asymmetry for benzene on the Inertsil-3 columns only when using methanol as modifier; this was also noted previously for Inertsil-3 C_{18} [2]. However, in general all columns gave excellent performance for benzene: the extremely high efficiency shown by some (eg Discovery amide yields >30 000 plates using methanol) may indicate the use of particles somewhat smaller than the claimed 5 μm. However, the back pressure generated by this column was not excessive, even with the 25 cm lengths used.

Despite the simplicity of testing in unbuffered mobile phases, the data in Table 1 confirm some of our previous doubts about such methods [5]. Only low pK_a probes can be used for evaluation in unbuffered mobile phases; the popular test compound aniline does not distinguish well between modern, less active columns, with most giving very good results. Pyridine appears to be a much more demanding probe, giving differentiation between the columns. However, a range of probes is necessary to give a meaningful overall assessment, and few other challenging low pK_a probes of significantly different structure are available. Another difficulty is the supposed connection between the performance of low pK_a compounds in unbuffered mobile phases to that of high pK_a compounds in buffered mobile phases at low or intermediate pH, which appears unlikely.

Table 2 shows performance data for nine columns tested with approximately isoeluotropic methanol and acetonitrile modified pH 7.0 phosphate buffers, together with data for acetonitrile–phosphate buffer pH 3.0. We have previously suggested that these three mobile phases are suitable for giving a reasonable overall evaluation of column performance with basic compounds [4,5]. Ranking of columns according to peak shape for bases at pH 3.0 and 7.0 was shown to be clearly different even using the same modifier, and significant differences were noted between use of different modifiers at pH 7.0. For the

Table 2

(a) Column performance data for basic solutes using acetonitrile–0.0375 *M* phosphate buffer pH 7.0 (40:60, v/v)

	Procainamide				Pyridine				Nicotine				Amphetamine				Codeine			
	k	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s
Supelco Dis. Am	0.10	10 000	3810	2.47	0.31	30 500	23 900	1.30	0.54	4620	920	4.36	0.25	20 000	10 100	1.90	0.53	12 100	4830	2.57
Supelco Dis.C_{18}	0.03	15 200	9190	1.53	0.31	17 500	12 400	1.41	0.52	4750	876	4.22	0.13	12 800	3930	2.44	0.37	14 200	8330	1.64
Supelco Dis. C_8	0.05	19 330	13 960	1.30	0.29	22 700	17 800	1.24	0.39	13 900	5030	2.45	0.12	20 200	9610	1.71	0.33	19 200	14 300	1.30
Inertsil3-C_8	0.07	14 300	10 900	1.28	0.75	21 800	18 700	1.11	0.91	14 300	8760	1.66	0.19	16 300	11 200	1.41	0.72	14 600	12 600	1.14
Inertsil3-C_4	0.18	10 400	6540	1.61	0.72	18 200	14 900	1.26	0.89	13 000	8020	1.76	0.28	12 800	7300	1.68	0.85	12 100	9360	1.38
Inertsil3-CN	1.32	10 800	4870	1.83	0.34	21 000	16 000	1.28	0.59	12 500	6650	1.74	2.79	12 460	8250	1.42	1.01	12 700	10 100	1.23
Symm.Shield 18	0.11	19 100	15 200	1.27	0.57	13 100	4560	2.35	0.53	18 500	12 700	1.43	0.31	13 600	6070	2.01	0.48	18 400	15 400	1.19
Symm.Shield 8	0.21	17 000	11 700	1.44	0.57	12 900	4960	2.21	0.53	9570	2760	2.46	0.47	14 600	6820	2.01	0.50	17 300	13 400	1.30
Luna C_{18} (2)	0.02	20 200	15 400	1.28	0.6	18 700	9800	1.73	0.68	17 000	7490	2.10	0.15	21 600	7930	2.31	0.58	19 700	16 200	1.21
Mean	0.23	15 148	10174	1.56	0.50	19600	13669	1.54	0.62	12016	5912	2.46	0.52	16040	7912	1.88	0.60	15589	11613	1.44

	Quinine				Benzylamine				Diphenhydramine				Nortriptyline				Mean Column			
	k	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s
Supelco Dis. Am	1.36	5450	1080	4.85	0.11	15 000	9810	1.49	2.12	14 600	5230	3.11	3.01	9070	2690	3.87	0.93	13 482	6930	2.88
Supelco Dis. C_{18}	0.67	11 100	5060	2.06	0.05	14 100	5190	2.12	2.41	9130	2630	3.15	2.32	6790	1590	3.87	0.76	11 730	5466	2.49
Supelco Dis. C_8	0.61	17 000	10 100	1.66	0.06	20 200	11300	1.54	2.06	17 300	8040	2.10	1.88	17 300	8130	2.05	0.64	18 570	10 919	1.71
Inertsil3-C_8	2.20	5620	1090	4.34	0.08	15 500	8470	1.65	3.95	15 100	9560	1.76	2.96	9210	1910	4.53	1.31	14 081	9243	2.10
Inertsil3-C_4	1.77	9930	5560	1.94	0.12	12 200	5840	1.89	3.44	14 200	10 400	1.42	2.86	13 900	9240	1.56	1.23	12 970	8573	1.61
Inertsil3-CN	5.43	7940	3970	1.83	2.08	11 200	4850	1.87	4.90	14 200	9290	1.41	10.4	10 500	3670	2.18	3.21	12 589	7516	1.64
Symm. Shield 18	1.08	14 900	10 500	1.46	0.11	13 500	6660	1.86	3.19	13 100	7750	1.76	3.77	8160	3940	2.24	1.13	14 707	9198	1.73
Symm. Shield 8	1.25	14 900	9170	1.60	0.21	15 200	6410	2.07	3.44	16 300	9870	1.75	4.45	13 400	7050	2.13	1.29	14 574	8016	1.89
Luna C_{18} (2)	1.09	14 900	6460	2.40	0.04	18 700	6520	2.26	3.95	18 000	6730	2.70	3.09	12 300	2370	4.51	1.13	17 900	8767	2.28
Mean	1.72	11 304	5888	2.46	0.32	15 067	7228	1.861	3.27	14 659	7722	2.13	3.86	11 181	4510	2.99	1.29	14 511	8292	2.04

(b) Column performance data for basic solutes using methanol–0.0643 *M* phosphate buffer pH 7.0 (65:35, v/v)

	Procainamide				Pyridine				Nicotine				Amphetamine				Codeine			
	k	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s
Supelco Dis. Am	0.12	14 700	9500	1.67	0.12	28 400	22 900	1.28	0.27	16 800	10 100	1.65	0.21	13 900	10 900	1.53	0.38	15 420	10 910	1.44
Supelco Dis.C_{18}	0.11	14 100	10 200	1.28	0.19	16 200	9200	1.63	0.44	11 800	4300	2.25	0.24	12 400	5110	2.07	0.53	13 400	9460	1.37
Supelco Dis. C_8	0.07	15 200	14 300	1.00	0.13	21 100	17 200	1.19	0.32	17 300	14 300	1.18	0.17	17 800	14 100	1.19	0.35	16 400	14 700	1.10
Inertsil3-C_8	0.25	9400	8340	1.11	0.41	16 700	14 200	1.14	0.87	11 100	9100	1.23	0.41	11 000	8130	1.32	0.98	9313	8130	1.16
Inertsil3-C_4	0.29	7220	6320	1.09	0.27	16 400	13 300	1.22	0.55	11 600	9710	1.24	0.33	11 200	8880	1.24	0.74	8560	6960	1.30
Inertsil3-CN	1.49	8180	5570	1.49	0.45	16 000	10 400	1.44	0.46	12 000	8810	1.35	4.05	7900	4900	1.49	0.77	8170	6090	1.31
Symm.Shield 18	0.20	12 500	11 000	1.13	0.27	10 200	3700	2.29	0.52	14 800	11 800	1.26	0.44	12 700	7460	1.58	0.65	12 300	10 800	1.14
Symm.Shield 8	0.19	11 000	9350	1.22	0.24	9210	3950	2.13	0.43	11 600	8590	1.39	0.43	11 900	7902	1.50	0.50	11 500	10 100	1.21
Luna C_{18} (2)	0.26	14 400	13 800	0.93	0.36	14 900	7580	1.76	0.92	17 500	15 300	1.14	0.48	15 800	9880	1.50	1.15	14 500	13 500	1.05
Mean Solute	0.33	11 856	9820	1.21	0.27	16 568	11381	1.56	0.53	13833	10223	1.41	0.75	12733	8585	1.49	0.67	12174	10 072	1.23

	Quinine				Benzylamine				Diphenhydramine				Nortriptyline				Mean Column			
	k	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s	*k*	*N*	*N*(d−f)	A_s
Supelco Dis. Am	1.43	14 100	10 300	1.46	0.12	21 700	15 500	1.45	1.88	19 200	14400	1.53	2.00	15 900	11 800	1.45	0.73	17 791	12 923	1.50
Supelco Dis. C_{18}	2.16	11 300	7170	1.55	0.14	13 200	4380	2.33	3.46	11 040	4840	2.45	2.96	7714	1850	3.46	1.14	12 350	6279	2.04
Supelco Dis. C_8	1.42	13 900	10 900	1.31	0.09	18 200	13 200	1.27	2.12	15 600	9390	1.78	1.75	14 300	10 400	1.40	0.71	16 644	13 166	1.27
Inertsil3-C_8	6.09	3610	859	4.56	0.24	12 700	8420	1.39	6.77	9690	5540	2.13	4.65	6710	2900	2.41	2.30	10 025	7291	1.83
Inertsil3-C_4	2.63	5390	1920	3.11	0.20	8200	6680	1.31	2.87	9740	6570	1.71	2.30	8280	5910	1.45	1.13	9621	7361	1.52
Inertsil3-CN	2.72	3890	2370	1.64	3.19	8150	4990	1.50	3.17	5219	4683	1.18	12.2	5340	2960	1.69	3.17	8317	5641	1.45
Symm. Shield 18	2.73	10 900	10 000	1.14	0.23	12 400	5550	1.91	4.32	14 300	11 600	1.33	3.98	10 700	6890	1.60	1.48	12 311	8756	1.49
Symm. Shield 8	2.03	9620	7870	1.23	0.24	11 600	5620	1.85	3.10	12 000	10 100	1.30	3.12	9920	7320	1.42	1.14	10 928	7867	1.47
Luna C_{18} (2)	5.29	12 900	11 800	1.13	0.27	16 200	7790	1.77	8.48	17 000	14 200	1.31	6.07	14 100	7780	1.96	2.59	15 256	11 292	1.39
Mean Solute	2.94	9512	7021	1.90	0.52	13 594	8014	1.64	4.02	12 643	9036	1.64	4.34	10 329	6423	1.87	1.60	12 583	8953	1.55

Table 2 (continued)

(c). Column performance data for basic solutes using acetonitrile–0.0265 *M* phosphate buffer pH 3.0 (15:85,v/v). [for diphenhydramine and nortriptyline, 28:72 v/v]

	Pyridine				Nicotine				Amphetamine				Codeine				Quinine			
	k	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s
Supelco Dis. Am	0.00	29 300	21 400	1.43	0.00	24 700	16 900	1.45	0.34	27 600	23 000	1.25	0.19	23 000	19 100	1.23	0.82	19 100	16 500	1.15
Supelco Dis.C_{18}	0.02	20 100	15 600	1.40	0.04	19 000	13 400	1.40	1.14	16 700	14 000	1.33	0.65	14 900	12 600	1.26	2.43	13 800	11 500	1.29
Supelco Dis. C_8	0.04	21 100	19 100	1.09	0.06	20 600	17 300	1.14	1.08	20 800	17 400	1.22	0.59	19 100	17 400	1.07	2.65	16 800	14 600	1.15
Inertsil3-C_8	0.11	20 800	15 800	1.23	0.18	16 500	13 900	1.22	2.07	18 600	16 700	1.17	1.33	15 500	14 600	1.05	5.57	13 700	11 800	1.19
Inertsil3-C_4	0.16	17 200	12 200	1.28	0.24	13 100	9310	1.43	1.74	15 800	13 900	1.15	1.20	12 900	11 400	1.14	5.26	11 600	10 700	1.10
Inertsil3-CN	0.00	17 300	8760	1.85	0.00	16 200	10 400	1.48	0.09	16 900	12 800	1.27	0.02	12 900	10 500	1.21	0.21	8210	6230	1.30
Symm.Shield 18	0.02	25 200	19 300	1.28	0.02	21 000	18 200	1.18	1.13	19 500	17 100	1.23	0.65	17 300	15 900	1.12	2.18	13 500	12 300	1.15
Symm.Shield 8	0.06	19 300	11 600	1.77	0.08	18 800	14 000	1.51	1.23	18 900	16 500	1.27	0.75	15 900	14 300	1.15	3.32	14 600	12 600	1.22
Luna C_{18} (2)	0.07	25 000	20 000	1.00	0.10	22 700	18 900	1.19	2.08	22 500	18 400	1.37	1.19	18 500	16 900	1.12	4.86	18 500	13 900	1.49
Mean Solute	0.05	21 700	15 973	1.37	0.08	19 178	14 701	1.33	1.21	19 700	16 644	1.25	0.73	16 667	14 744	1.15	3.03	14 423	12 237	1.227

	Benzylamine				Diphenhydramine				Nortriptyline				Mean Column			
	k	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s	*k*	*N*	*N*(d–f)	A_s
Supelco Dis. Am	0.05	28 900	23 000	1.26	0.89	24 700	20 000	1.30	2.42	23 400	19 800	1.26	0.59	25 088	19 963	1.29
Supelco Dis. C_{18}	0.29	19 500	15 800	1.31	3.02	13 800	10 600	1.50	7.82	13 600	12 300	1.29	1.93	16 425	13 225	1.35
Supelco Dis. C_8	0.28	22 600	19 200	1.15	2.91	18 700	14 700	1.32	7.17	17 600	14 200	1.29	1.85	19 663	16 738	1.18
Inertsil3 – C_8	0.61	20 900	18 300	1.21	4.93	16 500	13 000	1.37	10.3	17 000	14 200	1.29	3.14	17 438	14 788	1.22
Inertsil3-C_4	0.59	14 700	13 100	1.16	4.53	16 000	14 200	1.14	8.46	16 000	14 800	1.10	2.77	14 663	12 451	1.19
Inertsil3-CN	0.00	18 300	13 200	1.28	0.33	14 000	10 800	1.26	0.81	12 900	10 500	1.21	0.18	14 589	10 399	1.36
Symm. Shield 18	0.3	22 300	19 800	1.18	2.30	16 900	13 500	1.40	5.79	18 400	14 800	1.39	1.55	19 263	16 363	1.24
Symm. Shiele 8	0.36	21 300	18 000	1.25	2.50	17 600	12 700	1.55	5.96	18 900	15 200	1.38	1.78	18 163	14 363	1.39
Luna C_{18} (2)	0.53	26 600	21 900	1.29	5.27	19 700	13 800	1.64	13.5	20 900	17 400	1.42	3.45	21 800	17 650	1.32
Mean Solute	0.33	21 678	18 033	1.23	2.96	17 544	13 700	1.39	6.91	17 633	14 800	1.29	1.92	18 565	15 104	1.28

present group of columns, the mean A_s for all probes on all columns using buffered methanol at pH 7.0 was 1.55 compared with 2.04 using buffered acetonitrile (see Table 2). This confirms our previous findings that peak symmetry is on the whole improved by use of methanol at pH 7.0 [2,5]. Increased silanophilic interactions in acetonitrile may lead to both increased peak asymmetry and greater than expected retention. However, it is clear that there is inconsistency between the behaviour of individual columns in these two mobile phases, confirming the necessity of separate testing. For example, Discovery Amide shows good overall peak shape for the bases (in terms of overall average *N* and average A_s calculated for all compounds) when using methanol–buffer pH 7.0. However, the worsening of peak shape in acetonitrile –buffer pH 7.0 appears greater for Discovery Amide than for most other columns; the average A_s is almost double in acetonitrile (2.88) compared with that in methanol (1.50). Conversely, the average A_s of the bases using SymmetryShield RP18 (which is of the same embedded polar group type as Discovery amide) is relatively little changed when using either methanol (A_s=1.49) or acetonitrile (1.73) as modifier in combination with pH 7.0 buffer. The Inertsil columns show behaviour more similar to the SymmetryShield column, with only slight increases in A_s when moving from methanol to acetonitrile: indeed, the column efficiency of the Inertsil columns shows some improvement in acetonitrile, even when the measurement is made by the more accurate Dorsey-Foley procedure which takes into account peak asymmetry in the calculation. This may be partially due to the improved peak shape shown by the Inertsil-3 columns even for benzene when using acetonitrile rather than methanol (see above). The behaviour of the Luna 2 column is more like that of Discovery Amide, giving considerably worse mean peak asymmetry and reduced column efficiency (Dorsey-Foley method) in acetonitrile. The most demanding probes using acetonitrile–buffer pH 7.0, as given by the mean solute A_s over the nine columns are nortriptyline (mean A_s=2.99), quinine and nicotine (both 2.46), and diphenhydra-

mine (2.13). three of the same four compounds are also the most demanding in methanol–pH 7.0 buffer, although it is interesting to note that nicotine becomes one of the easier probes to analyse in methanol (mean $A_s = 1.41$). These results all indicate that silanophilic effects are generally worse in acetonitrile than methanol at pH 7.0, and that for a comprehensive assessment, testing in both modifiers is advisable. It was previously shown that peak shapes in acetonitrile and methanol– modified pH 3.0 buffers were much more similar than in pH 7.0 buffers. Thus we have only tested the columns using acetonitrile to save time; it was found that acetonitrile generally gave higher column efficiency than methanol for bases at pH 3.0 [3] making it a reasonable ''first choice'' modifier at this pH.

Peak shapes for this column set are much better at pH 3.0 than at pH 7.0 (see mean peak asymmetries in Table 2) as shown previously for the same solutes on a different set of (mostly C_{18}) columns [2,3]. The overall mean asymmetry factor for all eight solutes on all nine columns was only 1.28 in acetonitrile–pH 3.0 buffer (Table 2) compared with 2.10 for acetonitrile–pH 7.0 buffer (omitting procainamide from the calculation, since data for this compound were not obtained at pH 3.0). Indeed, few of the basic probes gave problems on any of the columns at pH 3.0, the asymmetry factors are almost always below 1.5. However, pH 3.0 is not the inevitable choice for analysis of bases, due to the different selectivity at pH 7.0 and the rather low retention (and thus possibly poorer resolution) of many bases at low pH. Comparison of data for this column set (all of which have become commercially available only in the last year or so) both at pH 3.0 and pH 7.0, with similar data for a previous set of ''new generation'' RP columns (commercially available for about five years) tested previously under identical conditions [2,3], indicates promise in the use of these alternative ligands, since five of the columns in the present study (Inertsil and Waters columns) are prepared from the same silica as the columns used in the previous study. In other cases (Supelco columns) the improvement may be attributed to the use of a purer silica as column material. Figs. 1 and 2 show the order of elution of bases is quite similar from one C_{18} column to another in a given mobile phase (eg compare results for Inertsil-3 ODS, Symmetry C_{18}, Discovery C_{18} and even for the Discovery Amide and SymmetryShield RP 18 phases using acetonitrile–phosphate buffer pH 7.0). This finding agrees with our previous data [4], and with results from other workers [18,20].

3.2. Evaluation of phases containing embedded polar groups

Table 2 shows performance data for Discovery Amide, Symmetryshield C_{18} and Symmetryshield C_8, all of which contain embedded polar groups. Since Discovery Amide and Discovery C_{18} are made from the same silica, as are Symmetryshield RP18 and Symmetry C_{18} (evaluated previously [2,3]), direct comparison of the effect of incorporation of the embedded polar group is possible. Some retention data on SymmetryShield RP18 and C_8 has been published by other workers, although no comparative data on the equivalent conventional phases was given [19]. It has been claimed that the selectivity of these new phases is significantly different from classical RP packings, especially for polar analytes [18], also that such phases give improved inertness towards bases compared with conventional phases [11].

Table 1 and Fig. 1 show that the hydrophobicity of Discovery Amide is less than that of Discovery C_{18} as shown by reduced *k* for benzene using either methanol or acetonitrile as modifier. There are some differences in the selectivity of the column pair; for example, in acetonitrile–phosphate buffer pH 7.0, nortriptyline also is eluted just before diphenhydramine (peak 9) on Discovery C_{18}, but considerably after diphenhydramine on Discovery Amide (Fig. 1a). Furthermore, codeine (peak 4) moves to relatively higher retention on Discovery Amide. Differences in selectivity of these two columns are (peak 8) shown with methanol–phosphate buffer pH 7.0. (Fig. 1b). When using methanol–buffer pH 7.0, Discovery Amide shows reduced retention of bases compared with Discovery C_{18} (Fig. 1b and Table 2b), which is hardly apparent for these columns when acetonitrile is used (Fig. 1a and Table 2a). This may be a reflection both of reduced hydrophobicity of the Amide column (reduced *k* for benzene, Table 1) and its improved peak asymmetry (lower silanophilic effects), giving a mean solute asymmetry of

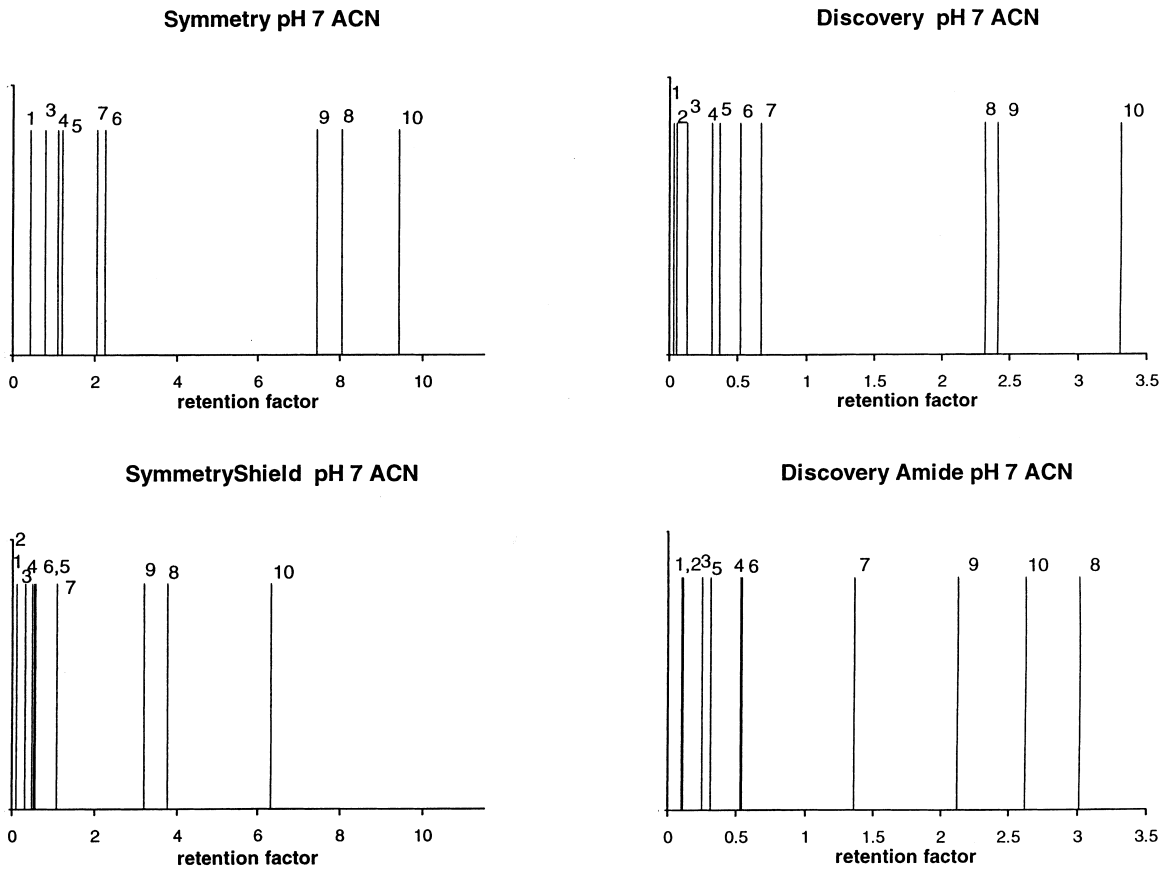

Fig. 1. Retention factor plots for Symmetry C_{18}, SymmetryShield RP-18, Discovery C_{18} and Discovery Amide. Mobile phase (a) acetonitrile–0.0375 *M* phosphate buffer pH 7.0 (40:60 v/v), (b) methanol–0.0643 *M* phosphate buffer pH 7.0 (65:35, v/v), (c) acetonitrile–0.0265 *M* phosphate buffer pH 3.0 (15:85, v/v) [for diphenydramine and nortriptyline, 28:72 v/v]. Peak identities: 1=procainamide, 2=benzylamine, 3=amphetamine, 4=codeine, 5=pyridine, 6=nicotine, 7=quinine, 8=nortiptyline, 9=diphenhydramine, 10=benzene. For other conditions, see Experimental Section.

1.50 in comparison with 2.04 on the C_{18} column. The higher than expected retention on the Amide column using acetonitrile may be attributable to increased silanophilic effects in this modifier, as evidenced by the much increased mean peak asymmetry of this column in in acetonitrile compared with methanol at pH 7.0. In acetonitrile–buffer pH 3.0, silanophilic effects are supressed, with both columns giving excellent peak symmetry. The difference in average plate counts seems merely a reflection of the increased column efficiency of the Amide column for benzene (Table 1). The reduced retention for bases of the Amide column (mean $k=0.59$ compared with 1.93 for the C_{18} column) using acetonitrile-pH 3.0 buffer may be merely a reflection of its reduced hydrophobicity, in the absence of gross silanophilic effects at pH 3.0.

A comparison of the Symmetry and SymmetryShield RP-18 columns gives similar results, although there are also some differences. The hydrophobicity of SymmetryShield RP18 (*k* for benzene in 55–45 methanol–water, 40–60 acetonitrile–water is 4.30 and 6.32 respectively) is considerably less than for Symmetry C_{18}(6.24 and 9.42 respectively). This decrease in hydrophobicity is greater than between the equivalent Supelco columns. Explanation of this is difficult since the exact nature of the ligand used in the RP-18 column has not been disclosed. The

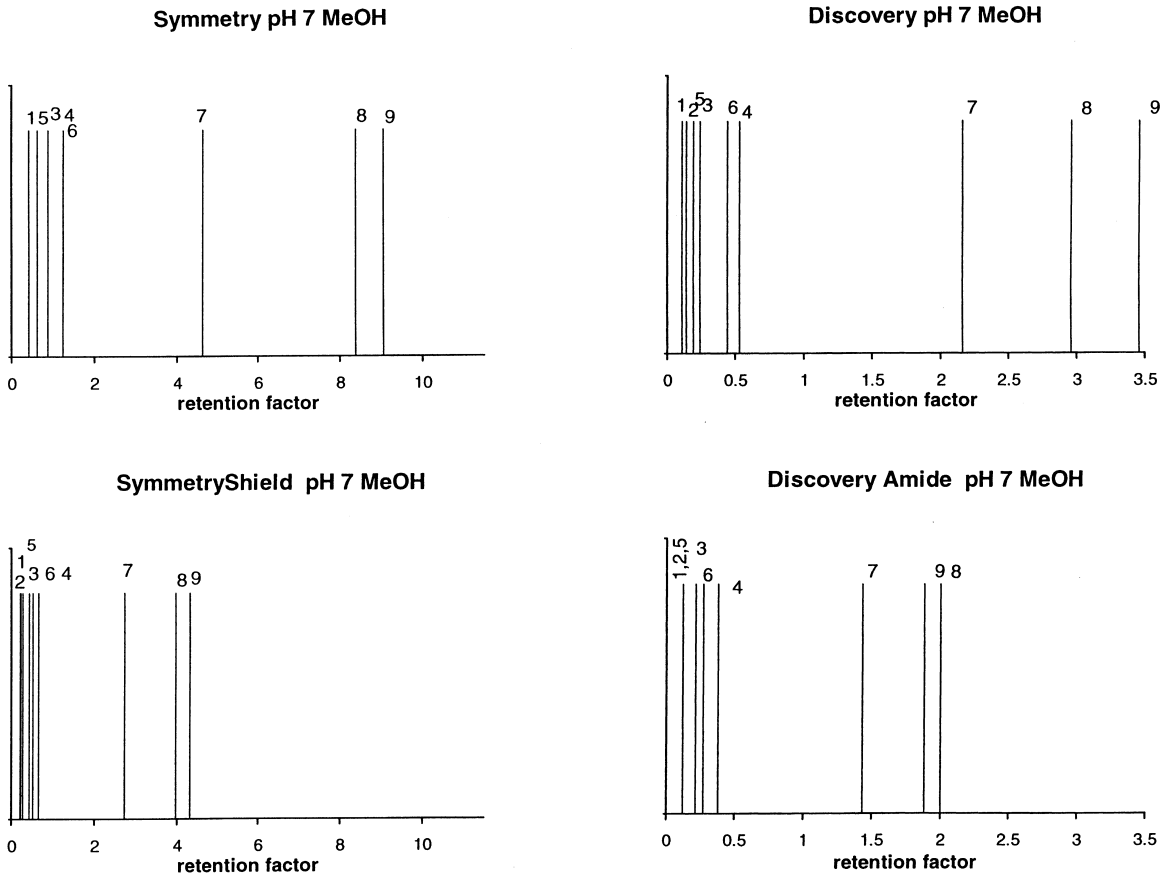

Fig. 1. (*continued*)

drop in retention of basic compounds (see Figs. 1a and 1b) of SymmetryShield RP-18 is evident in results for *both* acetonitrile and methanol–phosphate buffer pH 7.0 mobile phases. This may be due to the greater difference in the hydrophobicity of the two Waters columns. For the Waters columns, silanophilic effects are more similar in methanol and acetonitrile as evidenced by the more similar mean asymmetry factors (mean A_s=1.73 and 1.49 in acetonitrile–phosphate buffer pH 7.0 and methanol–phosphate buffer pH 7.0 respectively). The comparative figures for Symmetry C_{18} [2] are also similar, with acetonitrile giving only a small increase in mean peak asymmetry compared with methanol. Thus, lower silanophilic retention in acetonitrile of the Waters columns may mean a better correlation with hydrophobic retention. The mean A_s shown by Symmetry C_{18} in methanol-pH 7.0 buffer, acetonitrile–pH 7.0 buffer and acetonitrile–pH 3.0 buffer was 2.48, 3.06 and 2.21 respectively [2,3] in comparison with SymmetryShield RP-18 which gives mean A_s of 1.49, 1.73 and 1.24 respectively. These differences in peak asymmetry are greater than those between the Discovery and Discovery Amide phases. Again, there are relatively small differences in the selectivity toward bases of the shielded and conventional C_{18} Symmetry phases, in all three of the mobile phases studied, as can be seen by inspecting Fig. 1.

Overall, it would appear that columns with embedded polar groups have reduced hydrophobicity and silanophilic activity compared with conventional C_{18} columns made from the same silica. The reduced hydrophobic retention of bases may be masked

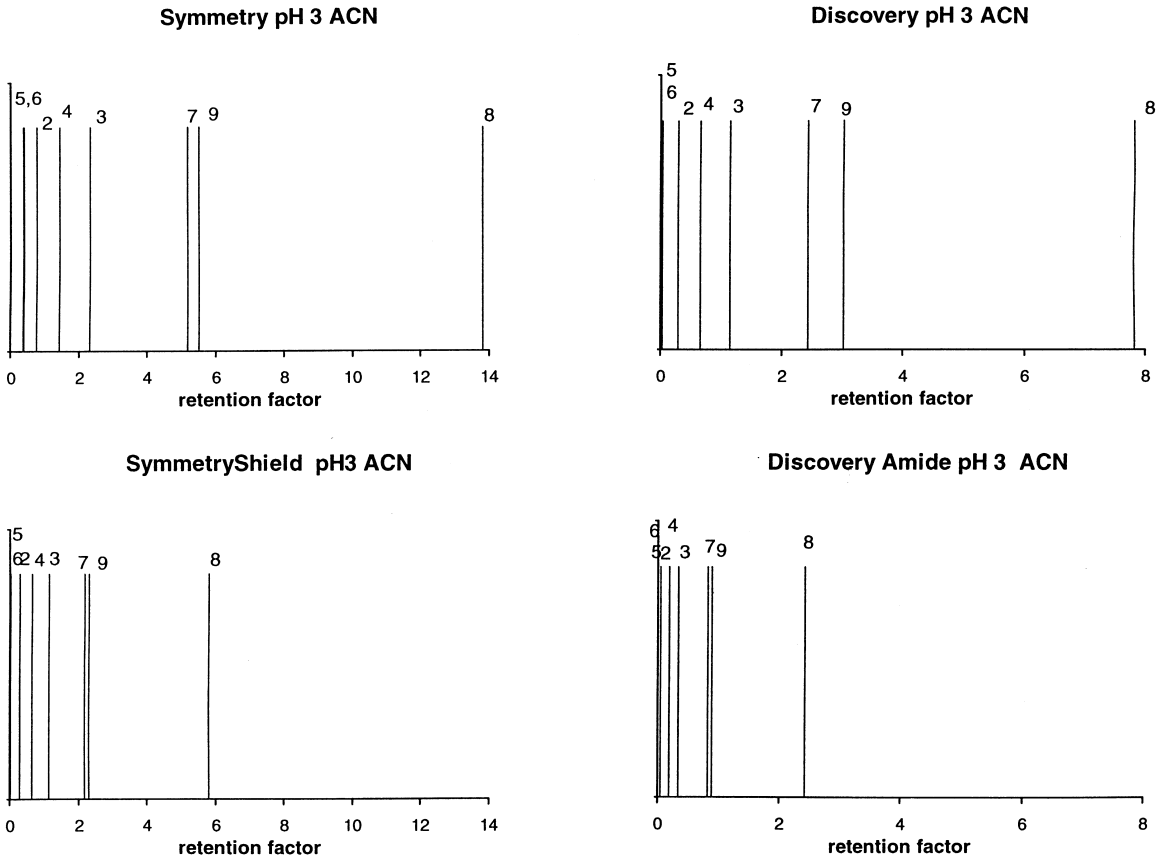

Fig. 1. (*continued*)

somewhat by increased silanophilic retention in acetonitrile compared with methanol. Differences in the selectivity towards exclusively basic compounds are rather small. It is interesting to note that this is true even for columns with quite large differences in silanophilic effects (for example Symmetry C_{18} and SymmetryShield RP18); thus more active columns elute a series of compounds in the same order but at higher *k* than inert columns on the same silica. Others have extended this principle to columns made from different silicas, and have used this as a way of monitoring column silanophilic activity [18].

3.3. Effect of chain length and nature of the bonded ligand on peak shape and retention.

Table 2 gives performance data for C_8, C_4 and cyanopropyl columns all made from Inertsil-3 silica, which is a pure silica known to give relatively low silanophilic activity. We previously obtained similar data for Inertsil-3 C_{18} [2,3], and the comparative retention data at pH 7.0 is plotted in Fig 2 for both acetonitrile and methanol. Previous studies [6,18] have indicated relatively small selectivity differences between phases with different length alkyl chains, although cyano phases may give much greater changes. In another study [7], a group of 22 probes was used to categorise the ''strength'' and polarity of a number of such phases; however, only one of the probes used (methylbenzylamine) was a basic compound. Column ''strength'' was measured by calculating the value of % acetonitrile (mixed with water) to give the same average *k* for the 22 compounds as on a C_8 column, which was used as a reference. It was found that column ''strength'' was similar for C_{18} and C_8 columns, but much less for a

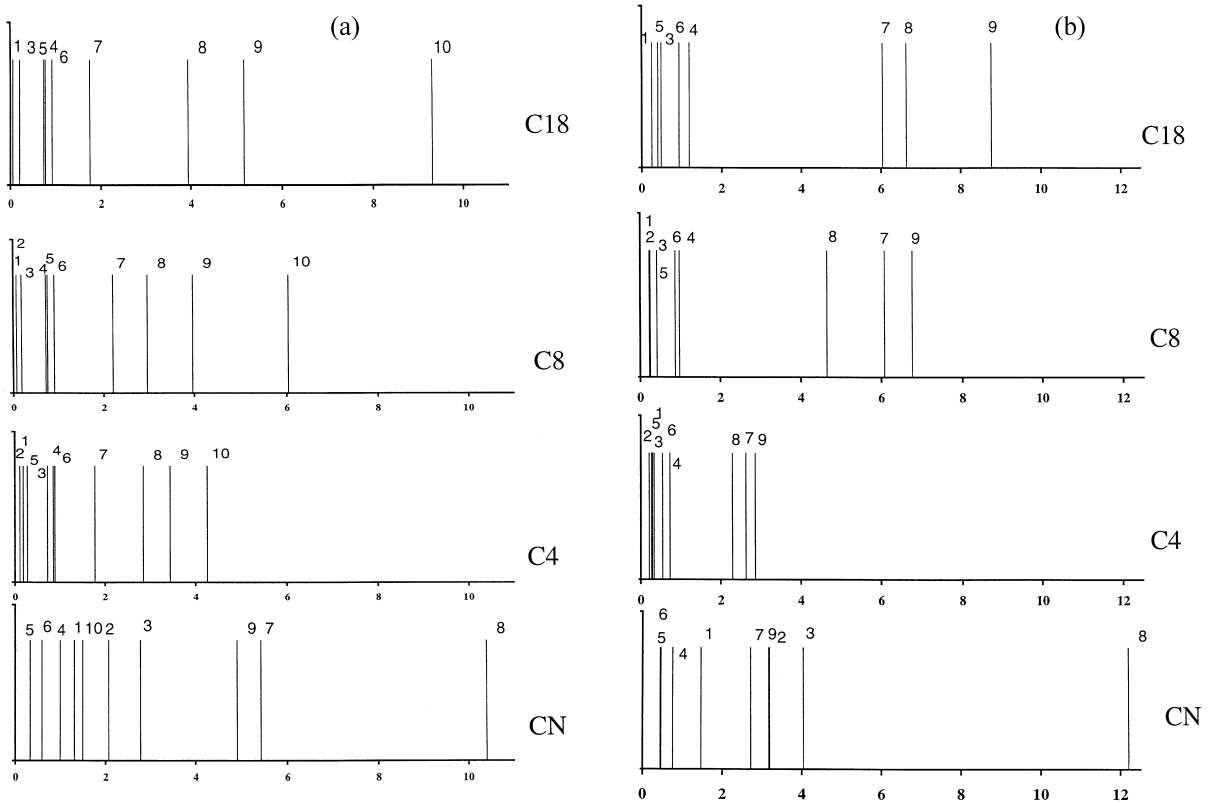

Fig. 2. Retention factor plots for Inertsil-3 C_{18}, Inertsil-3 C_8, Inertsil-3 C_4, Inertsil-3 cyanopropyl. Mobile phase (a) acetonitrile–0.0375 *M* phosphate buffer pH 7.0 (40:60 v/v), (b) methanol–0.0643 *M* phosphate buffer pH 7.0 (65:35, v/v). Peak identities as Fig. 1.

cyano column, which required 20% less acetonitrile to generate the same average *k* as the C_8 column. Furthermore, the polarity of the phases was found to increase with decreasing chain length, and incorporation of more polar functional groups like cyano groups obviously also leads to an increase in polarity.

To our knowledge however, the relative retention of exclusively basic compounds on these different types of phase has not been investigated in detail, and there are few indications of peak shape variations between these phases apart from one study [12]. A complication is the greater surface coverage often achieved with short chain phases due to the reduced steric hindrance involved in the bonding process. The coverage of the Inertsil-3 phases was 1.3 μmol m^{-2}, 1.7 μmol m^{-2}, 2.2 μmol m^{-2} and 2.8 μmol m^{-2} for the C_{18}, C_8, C_4 and cyano columns respectively (manufacturers' data).

Fig. 2 shows plots of *k* for bases and benzene for these four columns using acetonitrile–phosphate pH 7.0. *k* for benzene (peak 10) decreases in the order 9.31, 6.04, 4.25 and 1.50 for the series C_{18}, C_8, C_4 and cyanopropyl columns. The data for benzene are in accord with the expected hydrophobicity decrease of the columns and previously reported relative column ''strength'' [7]. This decrease is shown in spite of the increase in coverage of the phases reported above. However, for the C_{18}, C_8 and C_4 columns using acetonitrile–phosphate pH 7.0, there is only a minor decrease in retention factor of bases despite this reduced hydrophobicity. As before, it may be that silanophilic retention processes make a considerable contribution to retention in acetonitrile, even on this silica (Inertsil-3), which has previously been shown to give very good peak shape for bases [2,3]. The marked increase in the retention of bases on the cyano column is likely to be due to increased

dipolar interactions between the polar bases and the polar column group [7]. Whilst for the C_{18}, C_8 and C_4 columns there are likely to be selectivity differences between neutrals (considered as a group) and bases, Fig. 2 shows that there are few selectivity differences between individual bases, which are all eluted in more or less the same order on each of these three columns. However, in addition to a higher average retention for bases, the cyanopropyl column shows very marked selectivity differences, eluting the compounds in a completely different order from the alkyl bonded phases. These selectivity differences, using a set of exclusively basic compounds, seem larger than those reported by other workers for a set of more general test compounds [6,7]. In methanol–buffer pH 7.0 for the C_{18}, C_8 and C_4 phases, *k* for bases (Fig. 2) decreases much more in line with column hydrophobicity measured with benzene (Table 1). Again, the difference between the results for methanol and acetonitrile may be due to reduced silanophilic interactions in methanol, with hydrophobic retention of bases being a more significant retention process. Once again, there are no major selectivity differences in methanol–buffer pH 7.0 for the bases themselves between the C_{18}, C_8 and C_4 columns, although the order of elution of quinine (peak 7) and nortriptyline (peak 8) is switched on the C_8 and C_4 phases compared with the C_{18} phase. However, again there are major changes both in the increased average retention and in the selectivity of bases on the cyanopropyl column. Finally, it can be seen that there are as expected, some selectivity differences when interchanging methanol and acetonitrile as modifiers in admixture with phosphate buffer pH 7.0. For example, on the C_8 column the positions of nortriptyline (peak 8) and quinine (peak 7) are reversed in the different modifiers.

Considering now data for the Inertsil-3 columns at pH 3.0 using acetonitrile, the average *k* for the eight bases studied at pH 3.0 was 4.21, 3.14, 2.77 and 0.18 for C_{18}, C_8, C_4 and CN columns respectively. This reflects more nearly the decrease in hydrophobicity of the columns than does the data for the same modifier with pH 7.0 buffer, and may be due to the supression of silanophilic effects at pH 3.0. The elution order on the C_8 column in order of increasing *k* was:

pyridine<nicotine<benzylamine<codeine<amphetamine<diphenhydramine<quinine<nortriptyline.

This order is virtually identical on all the other columns, even on the cyano column despite very low retention factors. Thus, the enhanced retention and different selectivity of the cyanopropyl column shown at pH 7.0 is not mirrored at pH 3.0.

The average A_s for the set of compounds (see Table 2 and [2,3]) was for C_{18}, C_8, C_4 and CN 2.25, 2.10, 1.61 and 1.64 using acetonitrile–pH 7 buffer; 2.29, 1.83, 1.52 and 1.45 in methanol–pH 7.0 buffer and 1.60, 1.22, 1.19 and 1.36 in acetonitrile–pH 3.0 buffer. It can be seen that at pH 7.0 in either modifier, there are significant improvements in peak shape which result from the use of shorter alkyl chain columns. At pH 3.0, there is also some advantage of using shorter chain ligands, although the differences are less marked, presumably due to the general suppression of silanol effects at pH 3.0.

Although we have limited comparative data for other columns with shorter bonded ligands, comparisons can be made between Discovery C_{18} and C_8 (Table 2) Kromasil C_{18} and Kromasil C_8 [2,3]. In each case it can be seen that the same general conclusions can be drawn ie that there are only small selectivity differences for analysis of bases between the C_{18} and C_8 versions of a column based on the same silica, but there are quite marked improvements in peak shape for the shorter chain length column. For example, the mean A_s for Discovery C_{18} and Discovery C_8 was 2.49 and 1.71 using acetonitrile–pH 7.0 buffer; 2.04 and 1.27 in methanol–pH 7.0 buffer; and 1.35 and 1.18 in acetonitrile–pH 3.0 buffer (see Table 2). Indeed it can be seen that at least for the Discovery columns, reducing the chain length of the bonded ligand from C_{18} to C_8 is more efficaceous in reducing peak asymmetry than incorporating a polar group into the ligand, especially considering the results for acetonitrile–pH 7.0 buffer. For Kromasil C_{18} and C_8 the corresponding data [2,3] are 6.82 and 4.55 in acetonitrile–pH 7 buffer, 5.66 and 3.40 in methanol–pH 7 buffer, 2.52 and 1.96 in acetonitrile–pH 3 buffer. There may be several reasons for this improved performance including the increased coverage generally achieved with shorter ligands or less restricted access of buffer ions and solutes to silanol groups in these phases [21]. An exception to this rule appears to be the Symmetry Shield phases, where the C_8 column appears to give very similar mean peak asymmetry for bases to the RP-18 column, in any of the three

Table 3
Column performance data for Zorbax bidentate column

	Procainamide				Pyridine				Nicotine				Amphetamine				Codeine			
	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s
Methanol–0.0643 *M* phos pH 7, 65:35 v/v	0.16	12 500	6610	1.59	0.33	8240	2710	2.65	0.77	1610	177	5.22		n/e			0.79	10 400	5730	1.70
Methanol–0.0643 *M* phos pH 11, 65:35 v/v	0.40	10 600	4520	1.99	0.35	9120	3650	2.29	0.81	2900	481	4.00		n/e			0.97	10 400	6300	1.58
Methanol–0.05 *M* TEA pH 11, 65:35 v/v	0.32	14 900	12 400	1.23	0.30	10 800	4650	2.14	0.66	13 600	8620	1.55		n/e			0.84	13 500	11 600	1.20
ACN–0.0265 *M* phos pH 3 15:85 v/v (nb for nortrip. and diphen, 28:72 v/v)	N/d				0.09	18 600	8220	1.87	0.10	16 000	6080	2.48	1.4552	16 500	10 500	1.90	0.81	17 700	14 000	1.36

	Quinine				Benzylamine				Diphenhydramine				Nortriptyline				Mean Column			
	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s	k	N	N(d−f)	A_s
Methanol–0.0643 *M* phos pH 7, 65:35 v/v	3.76	7190	2670	2.34		n/e			7.39	1820	147	8.04		n/e				N/d		
Methanol–0.0643 *M* phos pH 11, 65:35 v/v	5.69	8940	4790	1.77		n/e			12.0	4900	588	6.10	33.8	23	5	10.0		N/d		
Methanol–0.05 *M* TEA pH 11, 65:35 v/v	4.81	11 500	9280	1.27		n/e			9.37	14 600	9800	1.69	18.7	3900	742	4.90		N/d		
ACN–0.0265 *M* phos pH 3 15:85 v/v (nb for nortrip. and diphen, 28:72 v/v)	4.80	4370	320	8.43	0.36	24 100	15 200	1.76	3.72	12 800	5790	2.75	9.76	12 600	5720	2.66	2.64	15 334	8229	2.90

N/d = not determined.
n/e = no peak eluted after 2 h.

mobile phases investigated. However, more results are needed for these types of phases on different silicas.

3.4. Columns with extended stability at high pH

The availability of columns stable at pH values where most organic bases are largely unprotonated offers an opportunity to study both selectivity and peak shape effects under these conditions. Table 3 shows comparative data for bases on a pH stable Zorbax bidentate phase [13] using methanol–phosphate buffer pH 7.0 and pH 11.0. For compounds of relatively low pK_a (pyridine 5.2, nicotine 7.9, codeine 8.0) there appears to be little change in k of compounds in the phosphate mobile phases at pH 7.0 and pH 11.0. This is somewhat unexpected for nicotine and codeine, since in aqueous solution there would be expected to be a considerable decrease in the degree of protonation of the compounds at the higher pH. These data are probably explicable due to the presence of the considerable amount of organic solvent present (65% methanol) which changes both the pH of the buffer components and the pK_a of the solute. Evidently, even nicotine and codeine are not appreciably protonated in methanol–phosphate buffer pH 7.0 (65:35, v/v). For the other compounds, which have higher pK_a, there are significant increases in retention at the higher pH. The magnitude of the increase is more or less in line with the aqueous pK_a value. In most cases, there is a slight improvement in the peak shape at pH11.0 compared with 7.0 when exclusively phosphate buffers are used. However, the peak shape of some compounds is still unsatisfactory, indicating deleterious effects of small amounts of protonated base, or influence of other factors [22]. Comparing k for the compounds when triethylamine is used as a buffering agent instead of phosphate at pH 11, it can be seen that there are fairly considerable reductions in k together

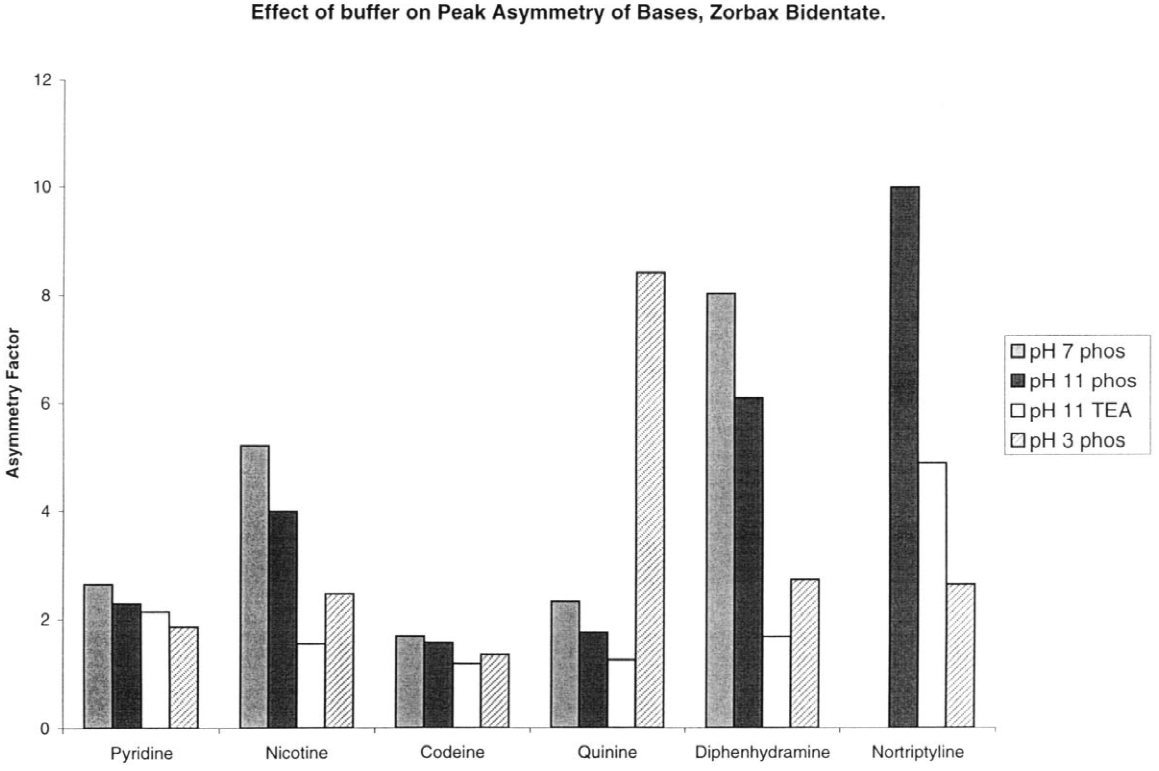

Fig. 3. Asymmetry factors for six basic solutes using Zorbax bidentate column with different mobile phases. For full description of mobile phases, see Table 3.

with large improvements in peak shape. For example, the retention factor of diphenhydramine decreases from 12.0 to 9.4 with a reduction in asymmetry factor from 6.1 to 1.7, indicating large reductions in silanophilic effects. The asymmetry factors for six bases in the four different mobile phases used are shown in Fig 3. Great care must be taken in comparing these results. It has been shown that the pH measured *after* the addition of organic solvent to an aqueous buffer depends on the nature of the buffer, even if the pH of the buffers measured *before* addition of solvent is the same [23]. Indeed the apparent pH of the methanol–TEA buffer (pHapp approximately 10.0) was considerably lower than that of the methanol–phosphate buffer (pHapp approximately 11.2) suggesting that pH effects were not responsible for the comparatively poorer performance of the phosphate buffered mobile phase. It is possible that TEA acts as a competing base or ''silanol masking agent'', giving rise to much improved peak shapes compared with phosphate buffer. Finally, it can be seen that whereas phosphate buffer at pH 3.0 almost always gives better results than phosphate buffer at pH 7.0 (apart for quinine, which is anomalous), in about half the cases, better results are obtained at pH 11 with TEA than at pH 3.0. However, a better comparison might have been achieved by adding TEA also to the acid mobile phase; it should also be noted that results at pH 3.0 were obtained with acetonitrile and are thus not directly comparable. The column was unaffected by use with only a few hundred column volumes of pH 11 phosphate buffer, which was shown by ''before and after'' testing. Phosphate buffers are not generally recommended for high pH work, because they lead to much more rapid column deterioration than the use of organic buffers [24]. It is even possible that these differences in deterioration may be due to the differences in pH of the mobile phases after organic solvent addition, as described above. Finally, it should be noted that better peak shapes have been reported using the bidentate column by other workers for some of the compounds investigated in the present study [13]. This might be attributed to a variety of differences between the two studies eg the use of acetonitrile rather than methanol used here, different buffering agents (eg piperidine), higher mobile phase strength [1] and higher temperatures than used in the present study. Despite the possibly non-optimised conditions used in the present study however, we believe our results are still consistent and valuable for comparative purposes.

4. Conclusions

1. In accord with our previous findings it has been confirmed that:
   1.1. Tests in unbuffered mobile phases are not particularly useful for evaluating RP columns.
   1.2. Silanophilic effects appear to be greater in buffered mobile phases at pH 7.0 modified with acetonitrile rather than methanol.
   1.3. At least for high pK_a bases, peak shapes are better at pH 3.0 than at pH 7.0 using the same modifier, due to suppression of silanol effects.
   1.4. Column evaluations are dependent on the nature of the probe compound utilised. Thus columns should be evaluated with a range of different bases [25].
2. Columns with embedded polar groups are less hydrophobic than conventional columns bonded with ligands of similar chain length, and they give somewhat different selectivity effects. Reduced retention of bases is not inevitably shown by these columns however, especially using acetonitrile as modifier at pH 7.0, where silanophilic retention may be a significant contributor to overall *k*. These columns can in some cases give marked improvement in peak shape for bases over equivalent conventional columns, although there may be variation in the magnitude of these effects in columns from different manufacturers.
3. Reduction in chain length for a family of RP columns based on the same silica (Inertsil-3) from C_{18} to C_4 shows the expected reduction in hydrophobicity as measured with neutral compounds. However, in acetonitrile modified mobile phases at pH 7.0, the retention of bases is not greatly reduced, possibly due again to the high contribution of silanophilic effects to retention. Thus these columns may show marked selectivity differences between neutrals and basic compounds, although selectivity effects between bases

themselves were relatively small. Using methanol modified pH 7.0 buffer, or acetonitrile–buffer at low pH, the reduction in retention of bases mirrors more the reduction in column hydrophobicity measured with a neutral compound. A cyanopropyl column based on the same high purity silica showed very large selectivity differences amongst basic compounds, using either methanol or acetonitrile modified pH 7.0 buffer as well as very good peak shape. Furthermore at pH 7.0 this column gave considerably reduced retention of neutral compounds relative to bases, giving additional useful selectivity effects. Significant improvements in peak shape were obtained by reducing the chain length from C_{18} to C_4 for the Inertsil-3 family of columns. For a variety of other columns from different manufacturers, reduction in chain length from C_{18} at least to C_8 confirmed this trend, as do results from other workers [12]. However, the long term stability of short chain and cyano phases needs further investigation, especially in acidic buffers, where increased ligand hydrolysis may be problematic.

4. Columns with high pH stability may be a useful additional aid in the separation of strongly basic compounds. Different selectivity is obtained amongst bases, since those of highest pK_a which are partially ionised at pH 7.0 (even considering the presence of organic solvents) move to higher k at higher pH where they are largely unionised. The retention of even moderately basic compounds (pK_a as high as 8) may in contrast be largely unaffected, because these substances are already largely unprotonated at pH 7.0 due to the presence of organic solvent which affects the buffer pH and the solute pK_a. However, it appears that even a small degree of residual protonation of bases at high pH (e.g. pH 11.0) or other factors, can lead to significant tailing. Improved peak shapes may be obtained by using organic bases as buffering components, which in any case are recommended to reduce dissolution of silica at high pH. However, some of the success in the use of these organic buffers may be due to competitive silanol masking effects in addition to suppression of the ionisation of the solute bases at high mobile phase pH.

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