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EFFECT OF CHROMATOGRAPHIC CONDITIONS ON SEPARATION AND SYSTEM EFFICIENCY FOR HPLC OF SELECTED ALKALOIDS ON AMIDE C_{16} STATIONARY PHASES

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SUMMARY

Retention data for 29 alkaloids were determined on an amide embedded RP silica column with different aqueous mobile phases – mixtures of acetonitrile with water, mixtures of acetonitrile with aqueous buffers of pH 3 or pH 7.8, and mixtures of acetonitrile with aqueous buffers containing ion-pair reagents or silanol blockers. Improved peak symmetry and separation selectivity for basic solutes were observed when ion-pair reagents or silanol blockers were used as mobile phase additives. The best separation selectivity and most symmetric peaks for the alkaloids were obtained by use of mobile phases containing diethylamine. The effect of diethylamine concentration on retention, peak symmetry, and theoretical plate number was investigated.

INTRODUCTION

Basic compounds can interact with underivatized free silanol groups of silica-based chemically bonded phases. It has been observed that retention occurs by an ion-exchange process that involves protonated solutes and ionized silanols. This situation leads to peak tailing, increased retention, and poor column-to-column reproducibility. Interactions with the silanols can be reduced by use of mobile phases buffered at low pH, when silanol ionisation is suppressed, or at high pH, to suppress solute ionisation. In analysis of basic compounds anionic ion-pair reagents are used to form neutral associates [1–3]. Good peak symmetry and system efficiency for analysis of basic compounds is also achieved by use of systems containing organic amines as silanol blockers [4].

Use of alkylamide phases, which contain terminal alkyl chains at-

tached to the surface via an alkylamide group, also reduces interactions with free silanols, by an internal masking mechanism. These phases, with internal polar functional groups, are less hydrophobic and have selectivity somewhat different from that of C_{18} phases prepared from the same silica. This is because of possible repulsion or attraction of ionic analytes (bases or acids) owing to electrostatic interactions between the phase and the analytes.

Alkylamide phases have specific chromatographic properties. The alkylamide groups, located in the hydrophobic ligands (Fig. 1) have a significant effect on retention [5–10]. In addition, improved peak shape has been observed for polar solutes such as organic acids [11] and basic compounds [12–14]; this makes these phases attractive for separation of ionic analytes. One possible explanation of this enhanced performance is an internal masking mechanism. Free silanol groups present on the silica surface may interact by hydrogen bonding with the embedded amide groups. It is also feasible that some of these phases contain a positive charge – residual amino functionality – which results in repulsion of the protonated bases from the silica surface [15]. The basicity of the nitrogen atom in the amide group is very weak – when the amide group accepts a proton, it does so through the oxygen atom [5]. The alkylamide phase is reputed to have advantages such as stability under highly aqueous conditions, improved peak shape for basic solutes, and selectivity different from that of conventional alkyl-bonded phases. Polar-embedded phases such as the alkylamide phase are substantially less hydrophobic than other alkylamide phases as a result of incorporation of a polar amide group on the alkyl ligand [13,15].

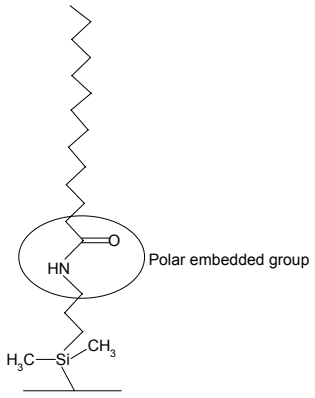

Fig. 1

The structure of the amide C_{16} stationary phase

The objective of the work reported in this paper was investigation of separation selectivity for selected alkaloids of an alkylamide stationary phase with different aqueous mobile phases, and identification of the most efficient and selective system.

EXPERIMENTAL

Liquid chromatography was performed with a Shimadzu LC-10 AT_{VP} equipped with a Shimadzu SPD-10AV_{VP} UV–visible detector and a Rheodyne 20-μL injector. Compounds were separated on a 150 mm × 4.6 mm, 5-μm particle, Discovery RP Amide C_{16} column (Supelco, Bellefonte, PA, USA). Detection was at 254 nm. All chromatography was performed at 22°C with a mobile phase flow rate of 1.0 mL min^{-1}.

Acetonitrile of chromatographic quality, octane-1-sulfonic acid sodium salt (OSA-Na), sodium dodecyl sulphate (SDS), tetrabutylammonium chloride (TBA-Cl), and diethylamine (DEA) were from Merck (Darmstadt, Germany). The pH of the 0.1 M phosphate buffers and the 0.2 M acetate buffer used in the experiments was measured for the aqueous solutions.

The alkaloids investigated are listed in Table I.

RESULTS AND DISCUSSION

Alkaloid standards, including fifteen isoquinoline alkaloids and fourteen alkaloids from other groups, were chromatographed on an alkylamide stationary phase by the use of a variety of aqueous mobile phases. The first experiment was performed with a mobile phase containing acetonitrile and water only. With this mobile phase most of the alkaloids were strongly retained by the amide stationary phase. In mobile phases containing only organic modifier and water, alkaloids – weak organic bases – are present in the ionized and neutral forms, which interact differently with the stationary phase. Alkaloids in the ionized form interact strongly with the underivatized free silanol groups of the silica-based alkylamide phase. This results in poor peak shape (for five alkaloids only was the asymmetry factor, A_S, acceptable), and poor system efficiency (Table I).

To obtain better peak shapes, higher efficiency, and improved separation selectivity the effects of conditions such as mobile phase pH, addition of an ion-pairing reagent, and concentration of silanol blockers were examined. The different selectivity of the chromatographic systems investigated are shown in the diagram presented in Figs 2A and 2B.

Table I

t_R, A_S, and N (m^{-1}) values for the alkaloids on the amide column with different mobile phases

Alkaloid	50% MeCN + H_2O			10% MeCN + 20% acetate buffer pH 3.5			40% MeCN + 20% buffer phosphate pH 7.8			40% MeCN + 20% acetate buffer pH 3.5 + 0.01 M OSA-Na			5% MeCN + 0.01 M TBA-Cl			15% MeCN + 20% acetate buffer pH 3.5 + 0.05 M DEA		
	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N
Berberine (Be)	FP[a]			28.86	7.01	690	FP			2.93	1.58	3280	FP			58.99	1.37	2250
Boldine (Bo)	10.30	1.95	3500	4.53	4.91	1550	8.50	2.58	12340	3.25	1.42	2660	5.18	1.35	3250	6.30	1.21	10800
Chelidonine (Chld)	FP			12.20	6.37	1960	61.58	0.97	90830	4.72	1.41	2300	5.32	1.55	2480	66.03	1.28	8580
Chelerithrine (Chlr)	1.78	0.66	4480	FP			64.73	1.29	3470	7.22	1.39	4560	25.14	2.60	2670	FP		
Dionine (D)	FP			3.18	1.17	2470	9.80	6.13	7530	3.33	1.14	3940	2.93	1.30	840	20.98	1.08	32150
Emetine (Em)	FP			3.29	1.69	3470	35.94	6.52	3080	5.05	3.00	840	FP			86.70	1.38	7800
Glaucine (G)	7.12	3.27	1150	17.99	5.33	640	22.30	3.15	24650	4.58	1.45	2830	4.73	1.26	2710	82.41	1.25	12350
Laudanosine (La)	1.79	0.66	2920	2.07	1.37	18080	4.38	2.17	4190	2.70	1.65	3000	FP			2.56	1.40	1420
Narceine (Nc)	FP			21.24	1.39	6210	3.43	1.25	21030	3.23	1.51	5250	12.18	1.29	2490	6.38	0.95	1310
Noscapine (No)	FP			10.67	4.73	740	37.83	1.10	41660	4.32	1.51	1790	6.54	0.96	2130	91.39	1.18	17320
Papaverine (P)	3.20	1.86	9210	20.73	6.49	3290	12.93	1.06	27560	4.54	1.46	4300	10.43	1.53	3340	FP		
Paracodine (Pa)	3.24	1.14	14740	FP			12.91	1.48	29800	4.61	1.23	5560	16.84	2.71	290	94.43	1.12	56890
Protopine (Pr)	19.76	3.15	1630	9.53	5.37	4050	14.64	1.21	11780	4.58	1.55	3450	4.99	1.48	3470	26.42	1.10	10810
Sanguinarine (S)	FP			FP			VSA			6.06	1.42	5080	15.32	1.98	3040	109.28	1.68	6540
Brucine (Br)	22.94	2.24	2510	5.07	3.49	3790	14.57	6.94	7110	4.53	1.91	2210	4.87	1.40	3330	14.08	1.07	3080
Quinine (Q)	FP			6.40	8.75	470	19.65	3.50	9930	6.10	1.82	390	5.32	1.55	2480	67.07	1.57	1990
Cinchonine (C)	FP			4.96	0.60	5440	14.59	2.98	2080	3.93	2.11	1200	FP			38.05	1.60	1170
Ephedrine (E)	FP			2.43	1.03	16520				2.95	1.47	1400	2.94	1.19	240	6.28	1.17	2610
Homatropine (Ho)	FP			FP			3.78	7.43	780	FP			2.45	1.05	2580	15.58	1.48	1440
Yohimbine (Y)	FP			FP			17.29	1.21	40110	4.48	1.39	2070	6.02	1.10	2010	16.77	1.55	1020
Caffeine (Caff)	2.22	0.92	1360	4.69	0.74	2910	2.38	1.02	10730	FP			2.94	1.31	950	2.81	1.04	1060
Colchicine (Co)	2.28	0.87	4820	78.51	1.45	6900	4.82	1.04	14230	2.77	1.66	5710	59.93	1.64	4850	12.31	1.05	1130
Lobeline (L)	FP			22.73	3.46	10500	36.31	1.82	2210	6.98	1.44	3450	FP			FP		
Pilocarpine (Pl)	FP			FP			FP			FP			16.84	2.71	290	13.85	1.00	2980
Santonine (Sa)	FP			53.47	1.11	28880	3.58	1.19	12860	3.70	0.78	3590	FP					
Scopolamine (Sc)	FP			2.68	0.93	5030	4.30	1.89	12820	3.03	1.18	2250	3.10	1.35	1030	6.44	1.22	1910
Strychnine (St)	20.31	1.70	6720	3.98	1.75	2520	17.00	6.13	7480	3.67	1.46	3290	4.12	1.43	3690	15.96	1.11	3370
Theophylline (T)	26.68	1.23	5090	5.23	1.31	1140	VSA			4.68	2.05	1030	FP		1237	8.02	0.98	1250

[a]Fuzzy peak
[b]Very strong adsorption

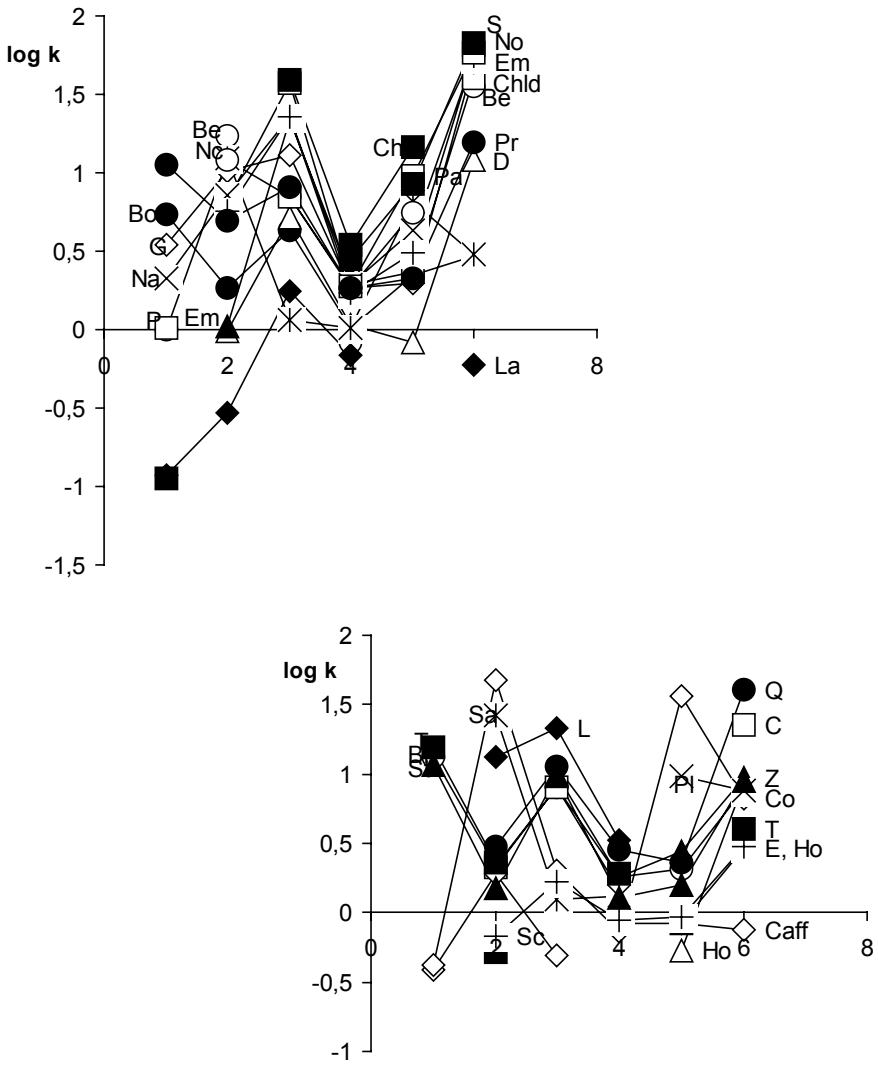

Fig. 2

Graphical comparison of log *k* values of the alkaloids on the amide C_{16} column with different mobile phases: 1, 50% acetonitrile in water; 2, 10% acetonitrile in aqueous acetate buffer at pH 3.5; 3, 25% acetonitrile in aqueous phosphate buffer at pH 7.8; 4, 40% acetonitrile in aqueous acetate buffer at pH 3.5 containing 0.01 M octane sulphonic acid sodium salt; 5, 5% acetonitrile in aqueous acetate buffer at pH 3.5 containing 0.01 M tetrabutylammonium chloride; 6, 15% acetonitrile in aqueous acetate buffer at pH 3.5 containing 0.01 M diethylamine

The diagram enables observation of the selectivity and sequence of elution of the alkaloids for use of different mobile phases on the alkylamide column. The different selectivity can be used in practice for rapid choice of the best system for separation of individual pairs or groups of compounds. For example, protopine and dionine, which are not separated by use of a mobile phase containing buffer at pH 7.8 or by addition of octane sulfonic acid sodium salt are well separated by use of mobile phases containing diethylamine or tetrabutylammonium chloride. Dionine and emetine are well separated by use of buffer at pH 7.8 or by addition of diethylamine but are not separated by use of buffer at pH 3.5. Good separation selectivity for most of the alkaloids, especially isoquinoline derivatives, was achieved by use of buffer at pH 7.8; somewhat worse separation was achieved by use of mobile phases containing silanol blockers. Asymmetry factors (A_S) and theoretical plate numbers (N) for chromatography of the alkaloids on the alkylamide column with different aqueous mobile phases are presented in Table I. In acetonitrile–water mobile phases the alkaloids are present in the ionised and un-ionised forms, which interact differently with stationary phase. For this reason, tailing peaks were obtained and efficiency was low. The asymmetry factor was acceptable for five alkaloids only, and only for narceine and papaverine was the number of theoretical plates greater than 10,000 m^{-1}. Buffer solutions were used to improve peak symmetry and system efficiency. Use of buffer solution at pH 3.5 suppressed ionisation of the free silanol groups which limited ion-exchange between free silanols and alkaloid cations. In this system symmetrical peaks were obtained for eight alkaloids but theoretical plate number was higher than 10,000 m^{-1} for four alkaloids only. Use of mobile phase containing buffer of pH 7.8 suppressed ionization of most of the alkaloids leading to reduced ion-exchange interaction between residual surface silanols and alkaloid ions. This resulted in improved peak shape (peak symmetry is good for twelve alkaloids) and, especially, improvement of system efficiency (for fourteen alkaloids $N > 10{,}000$ m^{-1}).

Figure 3 shows peak profiles obtained for papaverine with different mobile phases. With organic modifier–water mobile phases the peak was very asymmetrical and tailing. Better peak shape was obtained by use of mobile phases containing buffer at pH 3.5 and ion-pairing reagents, but peaks were still wide. The narrowest, most symmetric peaks were obtained by use of mobile phases containing buffer at pH 8 and, especially, by use of mobile phases containing diethylamine.

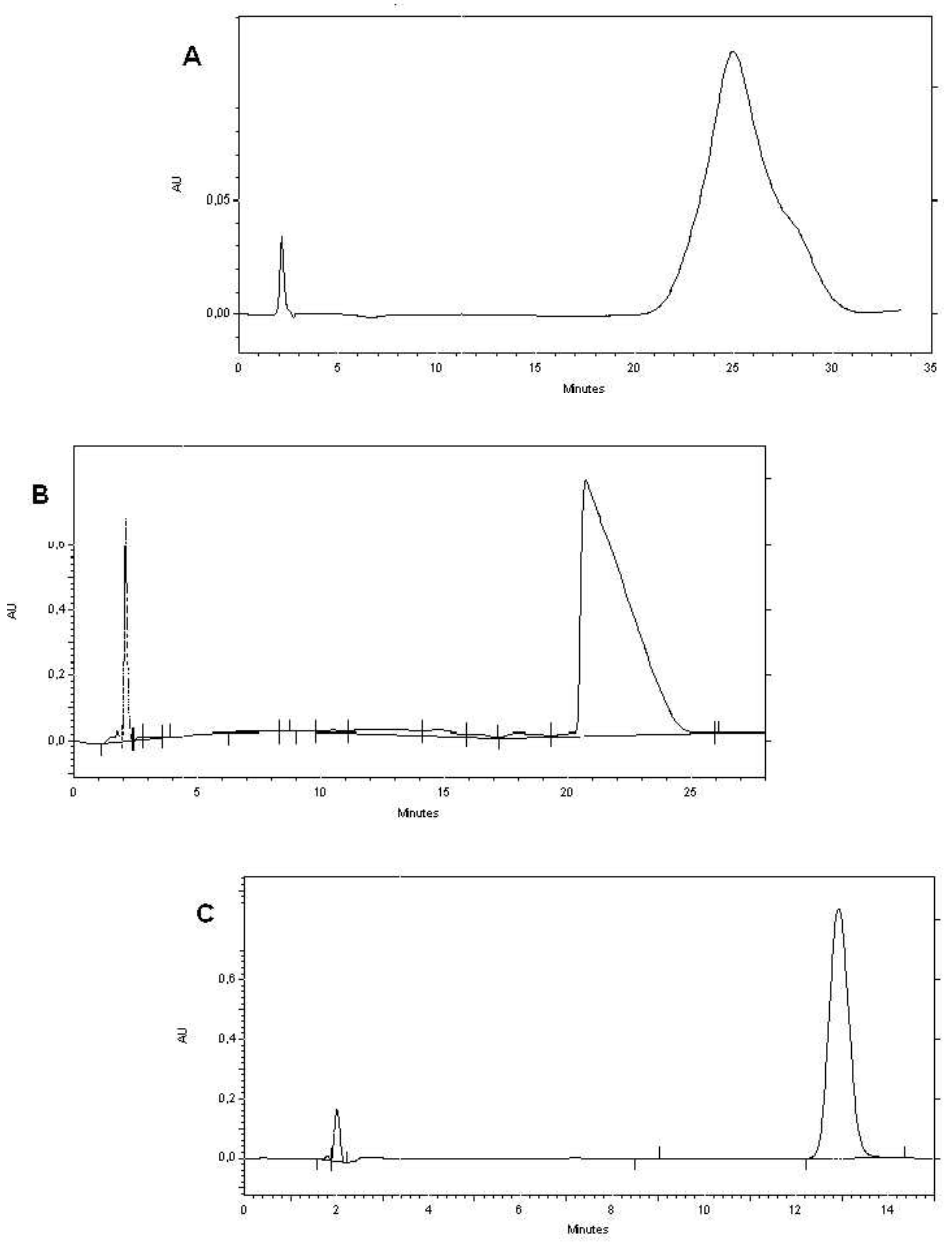

Fig. 3

Chromatograms obtained for papaverine with different mobile phases: A, 50% MeCN in water; B, 10% MeCN in aqueous acetate buffer at pH 3.5; C, 50% MeCN in aqueous phosphate buffer at pH 8

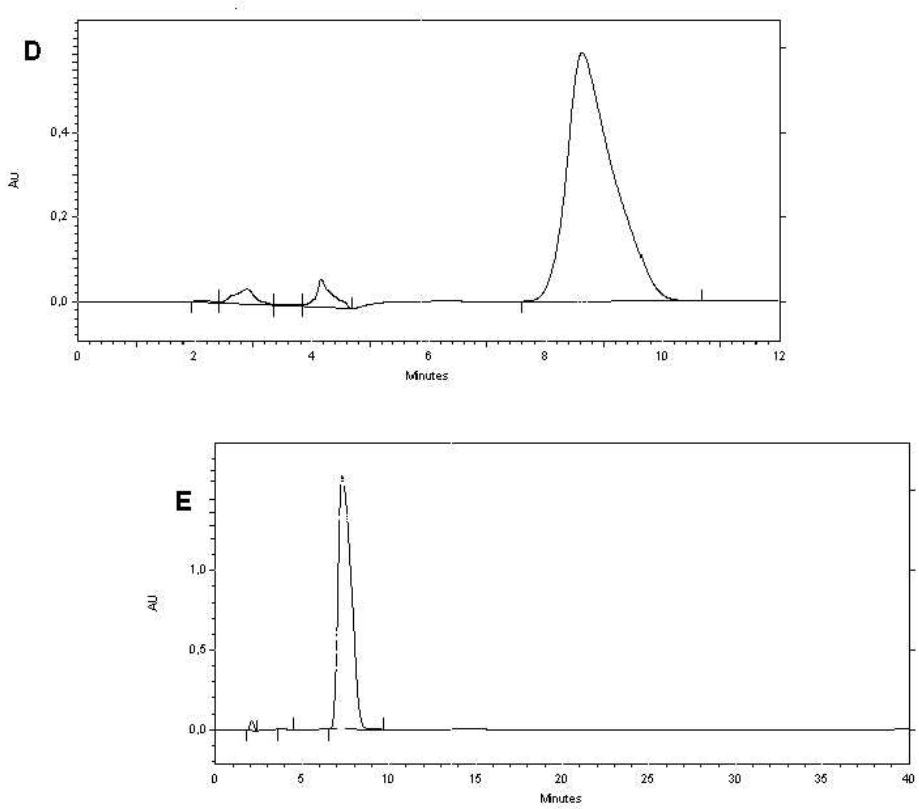

Fig. 3 (continued)

Chromatograms obtained for papaverine with different mobile phases: D, 40% MeCN in aqueous acetate buffer at pH 3.5 containing 0.01 M SDS; E, 15% MeCN in aqueous acetate buffer at pH 3.5 containing 0.01 M DEA

In subsequent experiments the effect of ion-pairing reagents on retention, peak symmetry, and system efficiency was examined. A schematic diagram of interactions in ion-pair RP systems is shown in Fig. 4. Symmetrical peaks were obtained for fourteen of the alkaloids, especially those in the isoquinoline group (nine alkaloids). The system with addition of octane sulfonic acid sodium salt as counter-ion proved poorly efficient, however. The number of theoretical plates per meter did not reach 10,000 for any of the compounds.

To improve peak shape and system efficiency, mobile phases containing the silanol blockers tetrabutylammonium chloride and diethylamine were used (a schematic diagram of the interactions occurring is presented in Fig. 5).

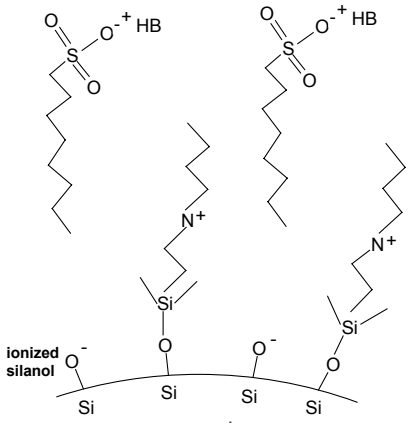

Fig. 4

Schematic diagram of interaction with the amide C_{16} stationary phase when ion-pairing reagent is present in the mobile phase

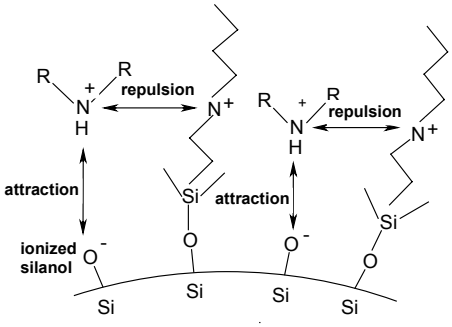

Fig. 5

Schematic diagram of interactions with the amide C_{16} stationary phase when silanol blockers are present in the mobile phase

In mobile phases containing TBA-Cl peak shape for some alkaloids is improved in comparison with mobile phases containing ion-pairing reagent. The best results were obtained by use of mobile phases containing diethylamine – symmetric peaks were obtained for twenty-one alkaloids. Addition of diethylamine to the mobile phase was especially useful for analysis of isoquinoline alkaloids. Only for sanguinarine was peak symmetry not acceptable. Figure 6 shows plots of log *k* as a function of the concentration of diethylamine in the mobile phase. Initially, with increasing DEA concentration, retention of the alkaloids decreases. When the concentration

of diethylamine was greater than 0.025 M, retention increases for most of the alkaloids; the exceptions are narceine, caffeine, boldine, and laudanosine, for which retention does not change with increasing DEA concentration. It is apparent that changing the concentration of DEA changes the separation selectivity and, occasionally, the sequence of elution. The best separation selectivity for most of the alkaloids (especially isoquinoline derivatives) was obtained by use of mobile phases containing 0.025 M diethylamine.

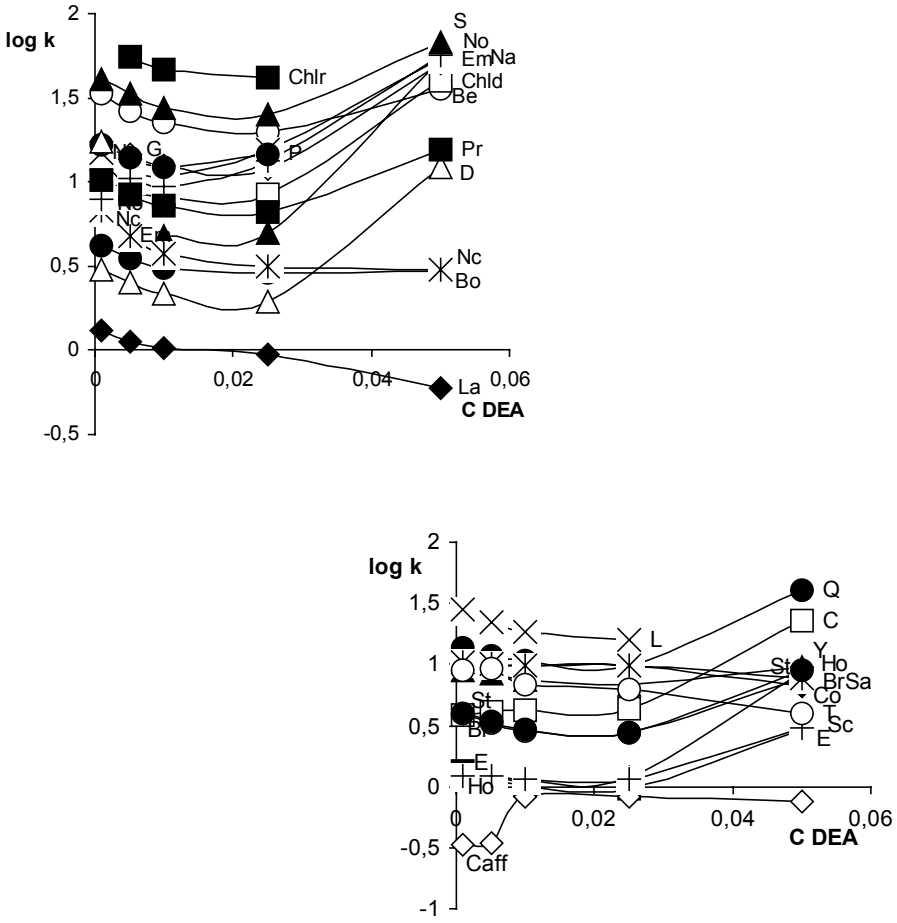

Fig. 6

Dependence on DEA concentration of alkaloid log *k* values on an amide C_{16} column eluted with 15% MeCN in aqueous acetate buffer at pH 3.5 containing DEA

Table II

t_R, A_S, and N (m^{-1}) values for the alkaloids obtained on the amide column with mobile phases containing 15% acetonitrile, 20% acetate buffer, and different concentrations of diethylamine.

Alka-loid	15% MeCN + pH 3.5 buffer + 0.001 M DEA			15% MeCN + pH 3.5 buffer + 0.005 M DEA			15% MeCN + pH 3.5 buffer + 0.01 M DEA			40% MeCN + pH 3.5 buffer + 0.025 M OSA-Na			15% MeCN + pH 3.5 buffer + 0.05 M DEA		
	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N
Be	54.73	2.03	240	43.90	1.99	570	37.61	1.94	1100	33.18	1.51	1630	58.99	1.37	2250
Bo	8.26	1.84	420	7.27	1.72	850	6.53	1.65	1240	6.17	1.55	1750	6.30	1.21	10800
Chld	23.27	1.71	1190	15.97	1.67	1240	14.68	1.49	2690	14.97	1.46	1630	66.03	1.28	8580
Chlr	113.54	2.19	1490	90.77	2.03	1570	76.22	2.15	2840	67.77	2.09	2400	VSA		
D	FP			5.67	1.34	3050	5.05	1.27	3160	4.71	1.19	3430	20.98	1.08	32150
Em	FP			FP			FP			9.56	2.10	980	86.70	1.38	7800
G	FP			FP			21.61	2.11	780	20.78	1.57	1040	82.41	1.25	12350
La	3.72	1.57	1020	3.41	1.47	1240	3.24	1.43	1360	3.12	1.41	1400	2.56	1.40	1420
Na	25.29	1.87	1180	20.48	1.70	1300	18.93	1.07	1580	26.26	1.07	1620	88.25	1.06	15610
Nc	12.33	1.43	1580	9.31	1.29	1640	7.63	1.25	2580	6.69	1.15	2710	6.38	0.95	1310
No	14.10	7.24	170	18.48	2.33	640	16.68	1.32	890	22.98	1.28	1100	91.39	1.18	17320
P	FP			23.78	1.76	1160	21.01	1.44	1630	24.98	1.24	1830	VSA		
Pa	29.20	1.45	4120	24.43	1.38	4750	21.61	1.92	7860	25.91	1.32	10980	94.43	1.12	56890
Pr	FP			15.23	1.98	410	13.05	1.85	810	12.29	1.55	1210	26.42	1.10	10810
S	66.91	1.81	2100	54.73	1.62	2270	45.99	2.28	1250	41.61	2.18	1330	109.28	1.68	6540
Br	7.73	1.72	960	6.91	1.65	1390	6.19	1.58	1780	6.04	1.46	2370	14.08	1.07	3080
Q	23.33	2.41	340	20.49	2.39	610	18.55	1.77	970	17.43	1.75	980	67.07	1.57	1990
C	7.81	4.09	310	8.18	2.85	460	8.28	2.45	500	8.60	1.91	650	38.05	1.60	1170
E	3.75	1.64	780	3.50	1.62	650	3.22	1.52	890	3.16	1.55	1930	6.28	1.17	2610
Ho	3.63	2.74	710	3.53	2.06	750	3.38	1.59	830	3.48	1.57	810	15.58	1.48	1440
Y	16.03	4.82	300	15.03	3.45	380	13.47	2.16	260	12.55	1.90	280	16.77	1.55	1020
Caff	2.14	1.35	4770	2.15	1.18	5950	2.94	1.03	720	2.93	1.06	880	2.81	1.04	1060
Co	18.23	1.23	3160	17.68	1.20	3250	17.20	0.92	1650	17.31	1.03	1690	12.31	1.05	1130
L	47.27	1.72	1670	37.08	1.82	1280	31.22	2.31	1100	27.18	2.30	1180	VSA		
Sa	18.18	2.80	540	17.55	2.42	610	17.28	1.40	830	17.29	1.10	930	13.85	1.00	2980
Sc	3.58	2.77	730	3.57	1.90	820	3.43	1.67	850	3.42	1.51	770	6.44	1.22	1910
St	7.93	1.66	750	7.07	1.63	1120	6.36	1.63	1190	6.07	1.68	1630	15.96	1.11	3370
T	15.78	2.00	1710	16.33	3.28	240	12.37	3.58	560	11.56	3.05	380	8.02	0.98	1250

[a]Fuzzy peak
[b]Very strong adsorption

Asymmetry factors and theoretical plate numbers for chromatography of the alkaloids on the alkylamide column with different aqueous mobile phases containing increasing concentrations of diethylamine are listed in Table II. Increasing the concentration of diethylamine results in improved peak symmetry and an increase in theoretical plate number for most of the alkaloids. When mobile phase containing 0.001 M DEA was used the asymmetry factor is acceptable for four alkaloids only and theoretical plate number was always <10,000 m^{-1}. When mobile phase containing 0.05 M DEA was used the asymmetry factor was excellent for 13 alkaloids and acceptable for another eight; for seven alkaloids N was >10,000 m^{-1}.

CONCLUSIONS

The alkaloids were strongly retained by the alkylamide phase when mobile phases containing acetonitrile and water were used. Peaks were highly asymmetric and system efficiency was poor. Use of buffered mobile phases, especially at pH 7.8, led to improved peak symmetry and system efficiency. Addition of ion-pairing reagent did not improve peak symmetry and system efficiency for most of the alkaloids compared with use of buffer at pH 7.8. The best efficiency and symmetric peaks were obtained by use of mobile phases containing diethylamine as silanol blocker.

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