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Article

Effect of Ionic Liquid Additives to Mobile Phase on Separation and System Efficiency for HPLC of Selected Alkaloids on Different Stationary Phases

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The silica-based stationary phases with favorable physical characteristics are the most popular in liquid chromatography. However, there are several problems with silica-based materials: severe peak tailing in the chromatography of basic compounds, non-reproducibility for the same chemistry columns, and limited pH stability. Ionic liquids (ILs) as mobile phase components can reduce peak tailing by masking residual free silanol groups. The chromatographic behavior of some alkaloids from different classes was studied on C18, phenyl, and pentafluorophenyl columns with different kinds and concentrations of ionic liquids as additives to aqueous mobile phases. Ionic liquids with different alkyl substituents on different cations or with different counterions as eluent additives were investigated. The addition of ionic liquids has great effects on the separation of alkaloids: decrease in band tailing, increase in system efficiency, and improved resolution. The retention, separation selectivity, and sequence of alkaloid elution were different when using eluents containing various ILs. The increase of IL concentration caused an increase in silanol blocking, thus conducted to decrease the interaction between alkaloid cations and free silanol groups, and caused a decrease of alkaloids retention, improvement of peak symmetry, and increase of theoretical plate number in most cases. The effect of ILs on stationary phases with different properties was also examined. The different properties of stationary phases resulted in differences in analyte retention, separation selectivity, peak shape, and system efficiency. The best shape of peaks and the highest theoretical plate number for most investigated alkaloids in mobile phases containing IL was obtained on pentafluorophenyl (PFP) phase.

Introduction

The separation of basic compounds in liquid chromatography still remains problematic due to their interactions with free silanol groups. Many of these problems are associated with the complex structure of the surface in silica-based reversed-phase (RP)-packings. Silica gel is rigid and exhibits better performance and higher efficiency than other solid supports. C18 ligands are too bulky to react completely with all silanols, and after reaction with smaller silylating agents a maximum coverage of 50% can be achieved (1, 2).

Basic compounds are retained on silica-based RPs by a combination of electrical (charge-charge) and hydrophobic interactions with the stationary phase and with the ions of the mobile phase (3). The mixed mechanism involves ion-pairing, ion-exchange and hydrophobic partitioning. The basic compound peak shape depends on the kinetics of the interactions. In aqueous mobile phases, charge-charge interactions are usually stronger and slower than hydrophobic interactions.

The silanol ion-exchange interactions can be reduced by different methods, e.g., by using mobile phases with a low pH when the silanol ionization is suppressed, or mobile phases with a high pH to suppress solute ionization. In the analysis of basic compounds, anionic ion-pairing reagents are also employed (4–6). Good peak symmetry and system efficiency in analysis of basic compounds can also be obtained in systems containing organic amines as silanol blockers (7). These basic additives play also the role of ion-suppressant for basic analytes. To suppress free silanol groups, an addition of ionic liquids (ILs) to mobile phases can be applied. IL salts that are liquid at ambient temperature are normally composed of relatively large organic cations and inorganic or organic anions. ILs have many applications in organic synthesis, catalysis, sample pre-treatment techniques and analytical chemistry. Their usefulness for analytical chemistry can be attributed to their favorable physicochemical properties, such as the lack of vapor pressure, good thermal and chemical stability and very good dissolution properties regarding both organic and inorganic compounds (8). ILs can be used as organic modifiers in high-performance liquid chromatography (HPLC) (9), stationary phases in gas chromatography (GC) (10) and background electrolytes in capillary electrophoresis (11) or electrokinetic chromatography (12).

The retention mechanism using IL additives is very complex, because both the cation and the anion contribute to the retention of analytes. On one hand, cations of ILs coat the surface of the stationary phase to suppress free silanols and thus improve peak shape; on the other hand, part of the ionic liquids move with mobile phase and interact with the analytes: the anions of ILs paired with the cations of analytes to make the analytes more retentive, whereas the cations of ILs repulse the positively charged analytes to reduce their retention (13).

The IL additives were applied for RP-LC separation of different compounds. He et al. separated ephedrines by using only water containing 1-butyl-methylimidazolium tetrafluoroborate ionic liquids at pH 3 as the eluent (14). Different ILs as additives to mobile phase were used to analyze some amines (15, 16). Basic analytes were separated by thin-layer chromatography (TLC) and HPLC methods with mobile phases containing different kinds and concentrations of ILs (17–20). The addition of ILs to the eluent was used to separate and identify peptides by a TLC method (21). Addition of 1-butyl-3-methylimidazolium hexafluorophosphate or 1-octyl-3-methylimidazolium tetrafluoroborate to mobile phase was applied for the separation of β-blockers (22). Addition of 1-butyl-3-methylimidazolium

chloride, 1-octyl-3-methylimidazolium chloride, 1-decyl-3-methylimidazolium chloride was applied to separate phenoxy acid herbicides and phenols (13).

The aim of this paper was a study of the retention behavior of selected alkaloids by RP-HPLC in systems containing ionic liquids as eluent additives and different stationary phases. The paper covers systematic investigations of the ILs' effect on separation selectivity, peak symmetry and system efficiency of groups of alkaloids.

The use of ILs on pentafluorophenyl (PFP) stationary phase caused a significant improvement of peak shape. In addition to dispersive interactions available on traditional alkyl phases, the PFP also allows for dipole-dipole, π- π, charge transfer and ion-exchange interactions. Solutes with π-electrons will display a different retention behavior on such columns than on ordinary RP columns. The π-π interactions between aromatic moieties of solutes and pentafluorophenyl ligands on stationary phase are significant for retention on the PFP column and are partially blocking the interactions between basic analytes and free silanol groups. The use of mobile phases containing addition of ILs caused further improvement of peak shapes. The relatively small cations of ILs strongly interact with silanol groups, and they are blocking. The use of the double protection against interaction between aromatic basic solutes and free silanol groups allows symmetrical peaks and good system efficiency to be obtained.

Systems with the best parameters and highest selectivity were used for the separation of alkaloid standards mixture.

Experimental

Analysis was performed using an LC-10 AT_{VP} Shimadzu equipped with a Shimadzu detector SPD−10 AV_{VP} and a Rheodyne 20 μL injector. Detection was carried out at 254 nm. Five analytical columns of 4.6 × 150 mm were used: XBridge C18, Xbridge Phenyl (Waters, Milford, MA), Supelcosil C18, Supelcosil C18-DB and Supelcosil LC-F (Supelco, Bellefonte, PA). All chromatographic measurements were carried out at 22°C controlled by CTO-$10AS_{VP}$ thermostat with eluent flow rate of 1.0 mL/min. Acetonitrile of chromatographic quality was from Merck (Darmstadt, Germany). Water was double distilled. The pH of the 0.2 M acetate buffer used in experiments was measured for the aqueous solutions. ILs 3-methyl-1-octylimidazolium tetrafluoroborate (IL1), 1-butyl-2,3-dimethylimidazolium tetrafluoroborate (IL2), *N*-butyl-3-methylpyridinium tetrafluoroborate (IL3), methyltrioctylammonium trifluoroacetate (IL4) and 1-butyl-3-methylimidazolium tetrafluoroborate (IL5) were from Merck (Darmstadt, Germany); 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate (IL6) was from Polish Reagents (Gliwice, Poland).

Results and Discussion

Our previous research concerned the optimization of chromatographic systems for analysis of alkaloids with applying different adsorbents and eluents containing buffers at different pHs, eluents with the addition of a ion-pairing reagents or an amines used as free silanol blockers (23–25). The best shape of peaks, system efficiency and selectivity were obtained on different stationary phases by using mobile phases containing amines as silanol blockers. In the present work, the addition of different ionic liquids to eluents was tested on five stationary phases.

Thirteen alkaloid standards (Table I) were chromatographed on C18 or phenyl columns with various eluents. With basic compounds with polar functional groups, severe band tailing, band broadening and low theoretical plate number in the chromatogram often occur in eluent systems containing only organic modifier and water because of the residual silanols on the surface of stationary phases. IL cations can interact with silanol groups and compete for the groups on the stationary phase surface with the polar groups of the analytes. Therefore, they can effectively mask the residual silanols and improve the peak shapes while also decreasing the retention of basic analytes.

In the first series of experiments, alkaloids were chromatographed on an Xbridge C18 column in eluents containing a mixture of acetonitrile, acetate buffer at pH 3.5 and 2% of different ionic liquids. The buffer was added to the mobile phase to ionize alkaloids and to obtain better peak shapes. Great differences in retention, peak shape and system efficiency were obtained in eluents containing different kinds of ILs. Figure 1

Table I

Retention time (t_r), assymetry factor (A_S) and theoretical plate number (N/m) values for investigated alkaloids obtained on Xbridge C18 column in eluent system containing MeCN + acetate buffer at pH 3.5 and 2% IL

Alkaloid	Abbreviation	5%MeCN + 20%b.pH3.5 + 2% IL1			5%MeCN + 20%b.pH3.5 + 2% IL2			15%MeCN + 20%b.pH3.5 + 2% IL3			5%MeCN + 20%b.pH3.5 + 2% IL4			5%MeCN + 20%b.pH3.5 + 2% IL5		
		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
Allocryptopine	A	2.73	1.78	1880	13.87	1.49	1260	11.47	2.84	640	4.52	0.79	1210	2.67	1.48	1310
Berberine	Be	•			•			29.85	2.08	790	19.93	7.35	260	•		
Boldine	Bo	2.73	1.34	400	9.82	1.46	8590	4.16	2.31	300	5.63	2.20	610	1.83	1.92	50
Brucine	Br	1.87	1.07	5620	9.34	1.18	5520	4.76	2.08	630	12.07	8.42	240	•		
Chelidonine	Chld	4.02	1.99	3440	15.79	1.68	1780	21.10	3.14	330	5.08	0.99	2370	5.98	3.46	190
Quinine	Q	1.71	2.16	300	6.71	0.66	3600	8.45	0.60	2210	5.07	1.02	1410	1.66	1.80	700
Cinchonine	C	•			4.63	0.82	6770	5.53	0.58	5650	3.63	1.89	1490	1.53	1.94	1160
Emetine	Em	2.00	1.32	3000	25.93	1.74	7350	12.98	1.86	8240	5.09	3.24	810	1.83	1.49	520
Glaucine	G	6.17	1.50	6040	33.58	1.37	12050	16.32	1.15	10040	18.15	2.77	2420	3.53	4.61	170
Codeine	Cd	1.97	1.98	680	5.03	1.12	16250	•			2.59	0.85	5470	1.81	2.01	510
Laudanosine	L	1.96	1.61	1230	3.08	1.34	8100	3.58	1.47	7080	3.13	1.59	6050	1.55	2.02	480
Noscapine	No	3.05	1.58	2900	17.60	1.03	12610	26.13	1.40	12030	7.20	1.48	5600	3.29	2.48	960
Papaverine	P				31.88	2.15	1750	15.43	0.89	4210	17.58	3.32	540	3.49	1.84	1020

• Fuzzy peak.

presents a comparison of log k values obtained on the C18 column with mobile phases containing five different ILs. The diagram allows the selectivity and sequence of elution for alkaloids to be observed with the different mobile phases. The investigated alkaloids were more retained when eluents contained IL2, IL3 and IL4 and less retained when they contained IL1 and IL5 with octyl substituent on imidazolium cation. The ILs containing one long alkyl chain best coated the stationary phase surface. The higher covering of the surface caused blocking of most free silanol groups and increased hydrophobicity of the stationary phase. Polar alkaloid cations were weakly retained on stationary phase modified by ILs with octyl substituent. The retention, separation selectivity and sequence of alkaloids elution were different in eluents containing various ILs. For example, emetine and chelidonine are not separated in systems containing IL4, partly separated in systems with IL2 and IL3 and very well separated in eluents containing IL1 or IL5, whereas emetine and codeine are practically not separated in eluents containing IL1 or IL5 and well separated in systems with addition of IL2 or IL4. The selectivity diagram can be used in practice for the rapid choice of the best system for separation of individual pairs or groups of compounds.

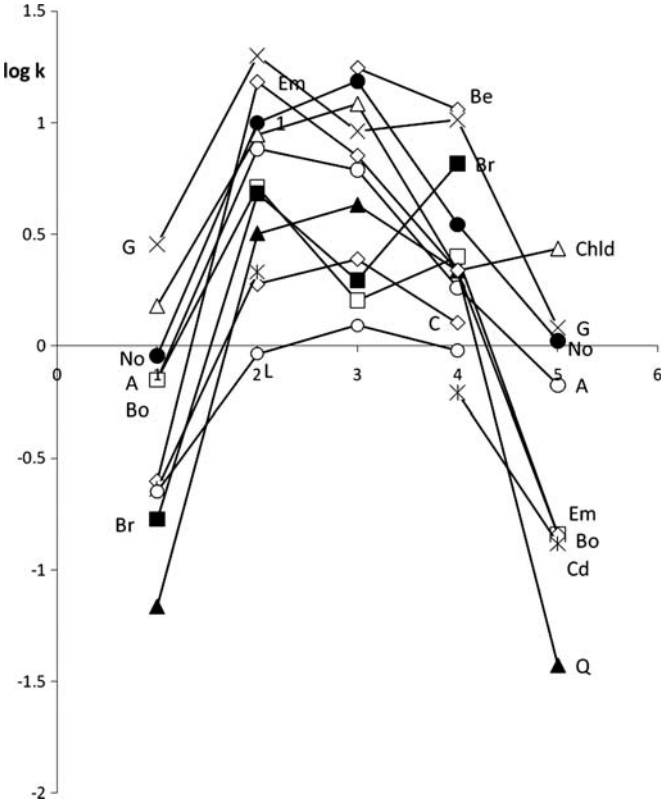

Figure 1. Graphical comparison of log k values of the alkaloids on Xbridge C18 column with mobile phases containing as eluent: acetonitrile (for 1 and 3– 15% MeCN, for 2, 4 and 5 – 5% MeCN), acetate buffer at pH 3.5; and 2% ILs: IL1, IL2, IL3, IL4 IL5.

The use of different ILs in the mobile phase caused differences in peaks symmetry and system efficiency (Table I). In the eluent system with IL5, a good peak shape was obtained only for allocryptopine and emetine, and a good symmetry was obtained with addition of IL1, IL3 and IL4 for four alkaloids. The best symmetrical peaks were obtained in systems containing IL2: for eight alkaloids, A_s was in acceptable range (0.8–1.5). In most cases, the highest efficiency was obtained with the system using IL2. Thus, this system appears to be the most suitable in terms of separation selectivity, system efficiency and peak shape.

The next set of experiments was performed on the PFP column. The effect of different IL additives to eluents on retention, peak symmetry and system efficiency was examined (Table II). Asymmetry factor for codeine obtained in mobile phase containing IL6 was higher than 5: the peak was very fuzzy, while in system with IL2 the peak was symmetric $A_s = 1.23$; for emetine, asymmetrical peaks were obtained with addition of IL1, IL3, IL4 and IL6, but with the addition of IL2, an ideal peak shape was obtained: $A_s = 1.0$. The change in system efficiency was observed for eluents containing different ILs, for example, for glaucine $N/m = 700$ when IL1 was added, but $N/m = 8140$ for system with IL6, for quinine N/m was only 780 in system with addition of IL4, but in eluent containing IL3 N/m was > 10 000. In comparison to the C18 column, the PFP column gave more symmetrical peaks and highest theoretical plate numbers in all examined eluent systems. The

Table II

Retention time (t_r), assymetry factor (A_S) and theoretical plate number (N/m) values for investigated alkaloids obtained on SUPELCOSIL LC-F column in eluent system containing MeCN + acetate buffer at pH 3.5 and 2% IL

Alkaloid	10%MeCN + 20%b.pH3.5 + 2%IL1			10%MeCN +20%b.pH3.5 + 2%IL2			10%MeCN +20%b.pH3.5 + 2%IL3			10%MeCN +20%b.pH3.5 + 2%IL4			10%MeCN +20%b.pH3.5 + 2%IL6		
	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
Allocryptopine	6.68	1.18	6160	9.51	1.23	3870	9.67	1.44	110	4.21	1.53	1470	12.24	1.40	3980
Berberine	24.18	2.20	7070	•			•			9.53	1.51	8420	•		
Boldine	•			6.31	1.16	1580	6.39	1.32	1640	4.04	1.43	870	7.96	1.26	5950
Brucine	5.55	0.86	6520	6.09	1.17	7030	6.18	1.34	4450	3.90	1.94	2480	7.58	1.25	7530
Chelidonine	6.40	1.27	14750	9.63	1.05	11410	9.88	1.01	890	4.57	1.04	4310	12.40	1.04	14470
Quinine	5.68	0.86	7140	5.95	0.95	7810	5.54	1.13	10330	3.30	1.05	780	7.38	0.81	5760
Cinchonine	4.58	2.11	3190	4.78	2.04	3200	4.58	0.79	8460	•			5.38	0.94	5630
Emetine	7.14	2.13	1550	14.41	1.00	5083	•			•			19.78	1.62	4000
Glaucine	12.63	1.48	700	16.83	1.12	6520	17.38	1.14	8640	•			21.42	1.48	8140
Codeine	4.22	1.40	8090	3.91	1.23	8510	•			4.23	1.41	9880	4.33	5.05	7580
Laudanosine	2.76	1.95	6770	3.30	1.49	7480	3.21	0.95	1400	•			3.63	1.37	8270
Noscapine	7.65	1.46	10530	10.87	1.38	9590	10.97	1.44	9080	5.95	1.79	8630	13.46	1.62	10620
Papaverine	11.48	1.30	9610	15.48	1.21	10980	15.88	1.35	730	6.29	2.52	2980	19.90	1.28	9650

least number of symmetrical peaks was obtained in system containing IL4: A_s were in acceptable range for four compounds only, in eluent containing IL1, peaks were symmetrical for eight alkaloids, in mobile phases containing IL3 and IL4, A_s values were in acceptable range for nine alkaloids. The highest number of symmetrical peaks was obtained in system containing IL2: A_s values were in acceptable range for 11 investigated alkaloids (peaks were asymmetrical only for berberine and cinchonine).

The influence of IL2 concentration on retention, peak shape and efficiency was examined on a C18-DB column. The increase of IL concentration in mobile phase caused a decrease of retention and a considerable improvement of peak shape and system efficiency. Mean log k values from six independent experiments served to construct the relationships given in Figure 2. The increase of IL concentration caused an increase in silanol blocking, and hence a reduced interaction between alkaloid cations and free silanol groups, and caused a decrease in alakloid retention. In addition, the repulsion between IL cations and alkaloid cations also played an important role and contributed to the decrease of retention.

Increasing the IL concentration caused improved in peak symmetry and increased the theoretical plate number in almost all cases (Table III). In eluent systems containing a mixture of acetonitrile and acetate buffer at pH 3.5 for most investigated alkaloids, peaks were highly asymmetrical; the system was only appropriate for the analysis of codeine (A_s was in acceptable

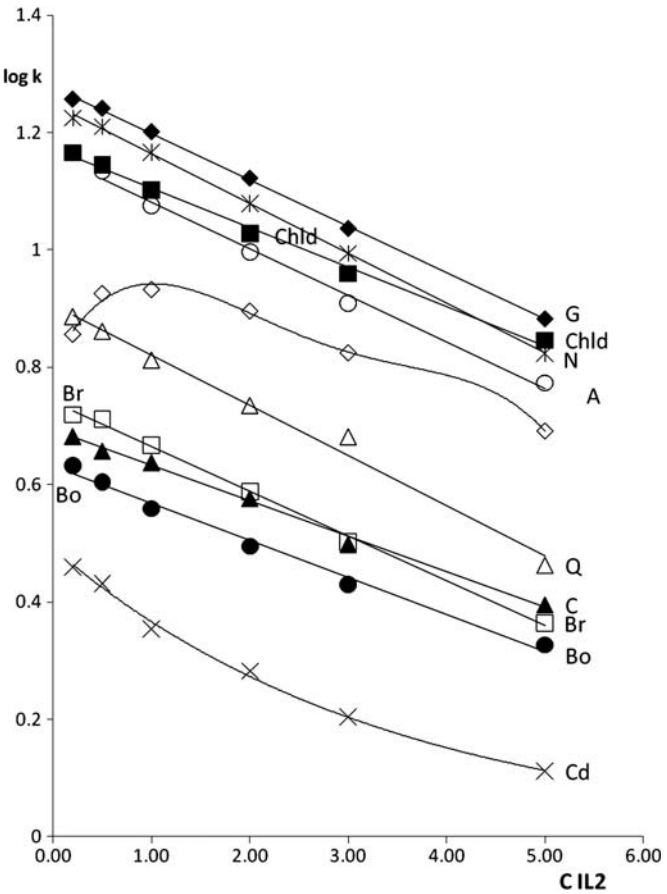

Figure 2. Dependence of alkaloid log k values versus IL2 concentration on Supelcosil LC-18-DB column eluted with 10% MeCN and acetate buffer at pH 3.5 containing IL2.

Table IV

Retention time (t_r), assymetry factor (A_S) and theoretical plate number (N/m) values for investigated alkaloids obtained on different columns in eluent system containing MeCN + acetate buffer at pH 3.5 and 2% IL

Alkaloid	15%MeCN +20%b.pH3.5 + 2%IL2 SUPELCOSIL C18			10%MeCN +20%b.pH3.5 + 2%IL2 SUPELCOSIL C18-DB			10%MeCN +20%b.pH3.5 + 2%IL2 XBridge Phenyl		
	t_R	A_S	N	t_R	A_S	N	t_R	A_S	N
Allocryptopine	42.19	1.49	5670	40.83	1.38	8100	21.69	1.19	12050
Berberine	14.93	1.00	1320	•			51.23	4.43	890
Boldine	7.16	0.98	8990	7.79	0.93	8530	4.73	0.90	6690
Brucine	9.23	0.89	13000	10.33	0.99	9750	6.02	1.05	8010
Chelidonine	•			45.68	1.46	7540	26.29	1.14	18430
Quinine	17.06	0.85	17400	14.19	0.84	4320	8.31	0.91	1420
Cinchonine	8.53	0.68	10100	7.97	0.73	7590	6.02	0.80	7880
Emetine	16.83	1.02	36860	28.72	1.45	8140	13.50	0.95	9930
Glaucine	65.34	1.24	11650	64.03	1.30	10050	22.63	1.14	14930
Codeine	3.84	1.02	13090	4.43	1.20	14870	3.29	0.87	15680
Laudanosine	2.93	0.66	4360	3.25	1.81	2800	2.63	0.73	7070
Noscapine	24.04	1.75	8120	54.93	1.25	15010	24.93	1.64	7120
Papaverine	53.23	1.69	14270	53.00	1.29	12480	18.17	1.00	18520

Table III

Retention time (t_r), assymetry factor (A_S) and theoretical plate number (N/m) values for investigated alkaloids obtained on SUPELCOSIL C18-DB column in eluent system containing 10%MeCN + acetate buffer at pH 3.5 and IL2

Alkaloid	10%MeCN + 20%b.pH3.5			10%MeCN + 20%b.pH3.5 +0.2% IL2			10%MeCN + 20%b.pH3.5 +0.5% IL2			10%MeCN + 20%b.pH3.5 +1% IL2			10%MeCN + 20%b.pH3.5 + 3%IL2			10%MeCN + 20%b.pH3.5 + 5%IL2		
	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
Allocryptopine				54.18	4.41	6200	66.87	2.40	6150	54.18	1.41	6200	30.03	1.48	10770	18.86	1.21	8480
Berberine	•						•						47.93	-	-	•		
Boldine	10.13	1.64	1020	9.45	1.06	6460	10.86	1.38	-	9.45	1.06	6460	6.47	1.17	6830	4.93	1.06	7220
Brucine	14.75	1.85	-	13.27	1.18	10340	15.37	1.32	10400	13.27	1.08	10340	7.95	1.02	11220	5.42	0.93	11510
Chelidonine	•			59.48	2.09	13150	69.38	1.39	13500	59.48	1.29	13650	35.87	1.37	17470	24.15	1.11	17630
Quinine	19.51	1.83	4240	23.27	1.30	5620	27.48	1.14	7710	23.27	1.00	7110	13.89	0.93	8650	7.09	0.84	5680
Cinchonine	12.48	2.41	-	12.09	1.84	13730	13.88	1.05	13470	12.09	0.84	13730	7.88	0.85	16750	5.89	0.86	15760
Emetine	9.53	-	-	32.63	-	-	31.86	2.22	3100	32.63	1.89	4890	22.47	1.68	8800	14.38	1.50	9020
Glaucine	•			85.08	1.45	10080	98.29	1.26	10160	85.08	1.05	10080	47.12	1.15	11020	27.44	1.10	11850
Codeine	8.26	0.88	10130	5.28	1.13	13160	6.51	1.28	11850	5.28	1.13	13160	3.72	0.79	12620	3.11	0.92	10500
Laudanosine	•			3.28	0.62	2640	3.30	0.63	2600	3.28	0.72	2640	3.05	0.81	3010	2.85	0.86	3100
Noscapine	•			74.99	2.45	8470	87.78	2.10	8420	74.99	1.35	8470	40.53	1.30	15540	23.93	1.36	14500
Papaverine	•			73.18	-	-	86.76	-	-	73.18	1.83	4520	38.60	1.54	4610	22.34	1.48	4910

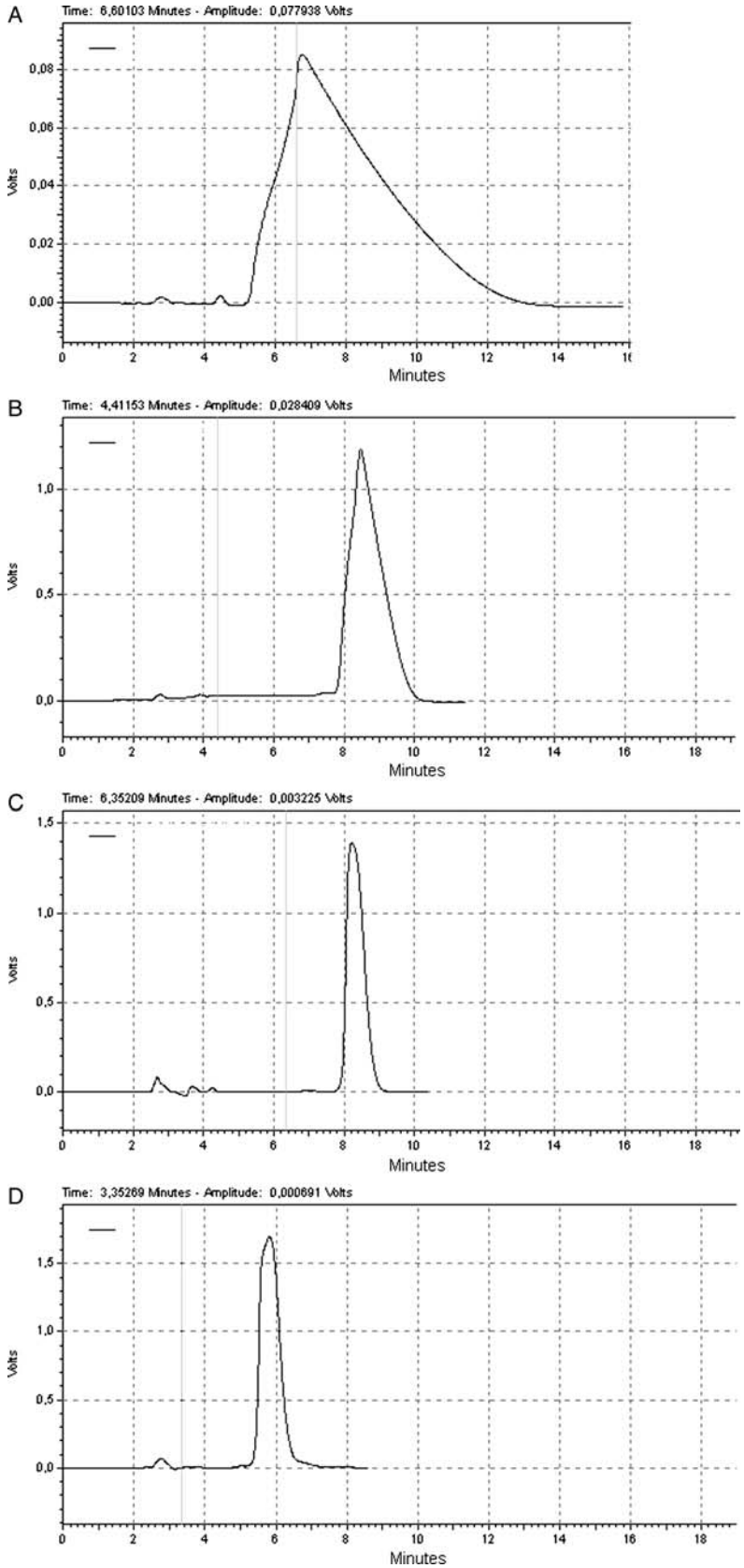

Figure 3. Chromatograms obtained for boldine on PFP column with different mobile phases: (A) 15% MeCN and 20% acetate buffer at pH 3.5; (B) 15% MeCN, 20% acetate buffer at pH 3.5 and 0.5% IL2; (C) 15% MeCN, 20% acetate buffer at pH 3.5 and 2% IL2; (D) 15% MeCN, 20% acetate buffer at pH 3.5 and 5% IL2.

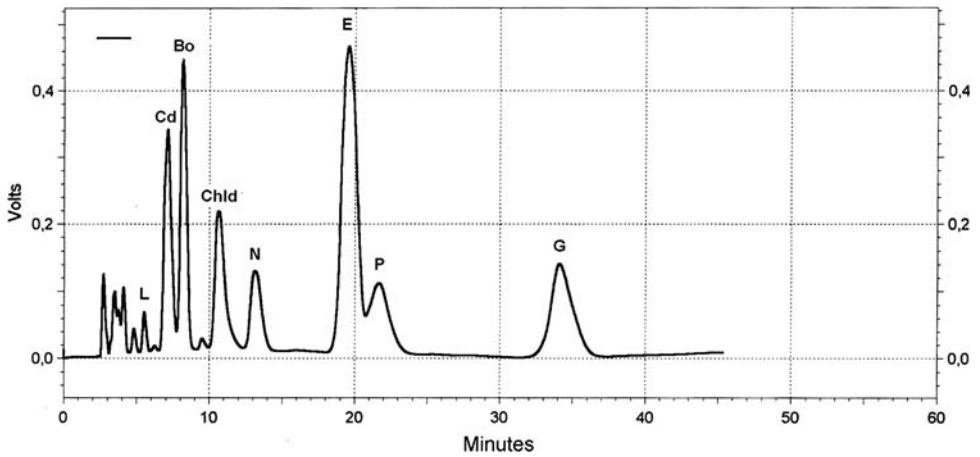

Figure 4. Chromatogram obtained for isoquinoline alkaloid standards obtained on PFP column with mobile phase containing 10% MeCN, 20% acetate buffer at pH 3.5 and 2% IL2.

range and N/m > 10 000). The addition of only 0.2% IL2 to the eluent caused an improvement in peak shape: for 5 alkaloids, A_s values were in the range of 0.8–1.5 and N/m was higher than 10 000. Further increase in IL concentration caused an improvement of peak symmetry and theoretical plate number. With a system containing 5% IL, only the berberine peak was asymmetrical and N/m was > 10 000 for 6 alkaloids. A spectacular improvement in peak shape was observed after addition of 0.2% IL2 compared to a system without IL; e.g., for boldine in a system containing only acetonitrile and buffer $A_s = 1.64$, while in eluents with the addition of 0.2% IL2 $A_s = 1.06$; for brucine in the first system $A_s = 1.85$; in the second $A_s = 1.18$. Further increase of IL2 concentration from 0.2 to 5% slightly changed the A_s value for some investigated compounds; e.g., for brucine from $A_s = 1.18$ to 0.93; for some alkaloids, peak shape was importantly improved; e.g., for chelidonine from $A_s = 2.09$ to 1.11. When the concentration of IL2 was over 1%, the band tailing of most investigated alkaloids was suppressed into an acceptable range.

The differences in retention, peak shape and system efficiency were obtained on different types of stationary phases. Optimization of the stationary phase for analysis of basic compounds was achieved by minimizing the interaction between analytes and residual silanols. The introduction of hydrophobic π-π active aromatic moieties (phenyl, pentafluorophenyl) to the common *n*-alkyl chain RP-sites generates a concerted π-π RP retention mechanism, which, as a consequence of the new functionality, diversifies the common RP-interaction properties without severely altering the latter. The consequence of different properties of stationary phases is the differences in analyte retention, separation selectivity, peak shape and system efficiency (Table IV). For example, allocryptopine on the C18 Supelcosil column in eluent system containing 15% MeCN was eluted after 42 min, its A_s value was 1.49, N/m = 5670, while on the PFP Supelcosil column its $t_R = 9.51$ min, $A_s = 1.23$ and N/m = 3870. On the C18 XBridge and Supelcosil columns, less symmetrical peaks were obtained: A_s was in acceptable range for seven or eight alkaloids, respectively, but on the C18 Supelcosil column for seven alkaloids, N/m was > 10 000. More symmetrical peaks were obtained on basic compounds with deactivated octadecyl silica (C18-DB Supelcosil), the most peaks with good shape were obtained on phases with π- π active aromatic moieties (LC-F Supelcosil and Phenyl Xbridge columns): for 10 or 11 alkaloids, A_s values were ranged from 0.8 to 1.5.

The use of eluent systems containing ILs as silanol blockers allowed more symmetrical peaks to be obtained than by using systems with addition of amines on the same stationary phases (25). The theoretical plate number obtained for most investigated alkaloids in both systems was similar.

Figure 3 shows peak profiles obtained for boldine with mobile phases containing different concentrations of IL2. With the organic modifier–buffer phase, the peak was very asymmetrical and tailing. Significantly better peak shape was obtained by using a mobile phase containing only 0.5% of IL2. Further improvement of peak shape was obtained in eluent with the addition of 2% IL2. The narrowest, most symmetrical peak was obtained by using a mobile phase containing 5% of IL2.

The most selective chromatographic system containing acetonitrile, acetate buffer at pH 3.5 and 2% IL2 was used on a PFP stationary phase for the separation of alkaloid standards (Figure 4).

Conclusions

These results confirmed that the application of ILs to HPLC was a simple and effective way to analyze and separate basic compounds. The addition of ILs to the mobile phase resulted in decreased band tailing, increased retention, and improved system efficiency and resolution.

The use of different ILs in the mobile phase caused differences in retention, peak symmetry, and system efficiency. The system containing 1-butyl-2,3-dimethylimidazolium tetrafluoroborate proved to be most suitable in terms of selectivity, efficiency, and peak symmetry.

An increase of IL concentration caused a decrease in alkaloid retention, an improvement in peak symmetry, and increase of theoretical plate number in most cases.

The best shape of peaks and the highest theoretical plate number for most investigated alkaloids were obtained in eluent systems containing IL on PFP phase, when the double

protection against disadvantageous interaction between cations of basic analytes and free silanol was used.

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