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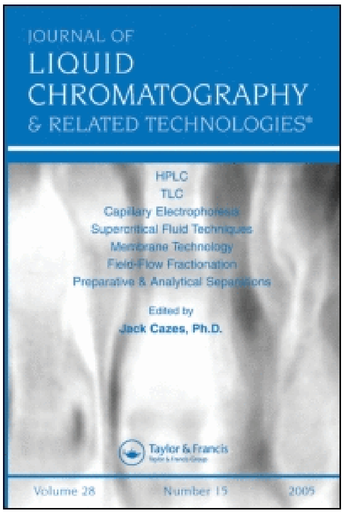

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Temperature—the Tool in Separation of Alkaloids by RP-HPLC

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Temperature—the Tool in Separation of Alkaloids by RP-HPLC

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Abstract: The retention behavior of 12 alkaloids on two different stationary phases, was based on silica matrix C_{18} and pentafluorophenyl by use of aqueous eluents consisting of acetonitrile, water, acetate buffer, and ion-pair reagent (OSA-Na) or silanol blocker (DEA). The retention coefficients and values of asymmetry factor and theoretical plate number were measured in the temperature range 10–50°C. Relationships between ln k vs. 1/T were created for alkaloids in all investigated chromatographic systems. In IP-RP systems typical van't Hoff plots – linear decrease of retention with the increase of temperature have been obtained. In the case of systems with DEA, relationships were untypical – the increase of retention with the increase of temperature in most cases. Temperature changes also caused changes in system efficiency and peak symmetry. In most cases, the increase of efficiency with the increase of temperature was observed to be the maximum, and than a slight decrease of theoretical plate number values were observed. Symmetry of peaks gets better with the increase of temperature in most cases.

Keywords: Alkaloids, RP-HPLC, Temperature, Retention, Efficiency, Peak symmetry

INTRODUCTION

The dependence of retention (log k) on absolute temperature (T) is usually given by the van't Hoff relationship:

$$\ln k = \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \log \Phi \qquad (1)$$

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where ΔH^0 and ΔS^0 are the retention enthalpy and entropy, R – gas constant and Φ - phase ratio.

ΔH^0 and ΔS^0 are usually independent of the temperature and, therefore, in the case of neutral compounds log k against $1/T$ is accurately described by a linear model.[1,2] When the temperature range was not very wide a classical linear relationship was applied,[2] especially when aqueous methanol was used as the mobile phase in reversed phase systems. Most often, the decrease of retention with the increase of temperature is observed.[1,2] When aqueous acetonitrile mobile phases were used, the relationships have been curvilinear also for simple solutes, similar to high molecular weight solutes,[3] but also with the decrease of retention at high temperatures. As pointed out by some authors,[2] this non-classical van't Hoff behavior can be explained by other modifications of stationary phases with solvent molecules by different temperatures or changes of retention mechanism.

The problem is more complex with ionizable compounds. This is because, with the change of temperature, not only are there changes in retention, but also changes in pKa values can be observed; this is especially distinct for basic solutes. When pKa values of solutes were compared with those measured chromatographically the largest differences were found at high temperatures for basic compounds, and the shift was usually positive.[1] This is because ΔpKa increases with the temperature increase. The basic solute may be under both neutral and ionized forms in aqueous solutions. Its retention enthalpy and entropy are expected to be different for both forms. As was earlier reported, the dissociation rate of a basic solute with a buffer prepared from a neutral or an anionic acid decreases when the temperature increases. This phenomenon will induce a competition between the decrease in retention due to a classical van't Hoff behavior and the increase in retention due to the decrease in the dissociation rate. As a result, a curvature of plots of log k versus $1/T$ occurs. In case of basic solutes, not only significant deviations to linearity are observed,[1] but also increases in the retention with the temperature.[1,4–9] It was reported that negative slopes in van't Hoff plots are expected when the buffer (component of aqueous mobile phase) has ionization enthalpy significantly different than the solute,[5,7] and the pH of the mobile phase is close to the pKa of analytes.

The situation can be more complex when not only the organic modifer and buffer, but also the mobile phases' additives such as ion-pair reagents[10–12] or silanol blockers[11,13] (short-chain amines), are aqueous mobile phase components. Such mobile phases are usually applied in the separation of basic analytes, for example, alkaloids.[14]

The aim of our paper was the examination of the temperature role as the factor influencing not only retention, but also peak symmetry, as well as system efficiency in the separation of selected alkaloids.

EXPERIMENTAL

Analysis was performed using an LC-10 AT_{VP} Shimadzu liquid chromatograph equipped with a SUPELCOSIL^{TM} LC-18 150×4.6 mm or SUPELCOSIL^{TM} PFP 250×4.6 mm column (Supelco, Bellefonte, PA, USA) $d_p = 5\ \mu m$, UV-VIS SPD-10AV_{VP} Shimadzu detector, and Rheodyne 20 μL injector. Detection was at wavelength 254 nm. All chromatographic measurements were carried out at 10–50°C controlled by a CTO-10AS_{VP} Shimadzu oven with the eluent flow rate of 1.0 mL/min. Acetonitrile of chromatographic quality, bidistilled water, octane-1-sulfonic acid sodium salt (OSA-Na), and diethylamine (DEA) were from Merck (Darmstadt, Germany). The pH of acetate buffer used in the experiments in $0.01\ M\ L^{-1}$ concentrations were measured in aqueous solutions. Alkaloid standards are listed in Table 1.

RESULTS AND DISSCUSSION

In our experiment, retention of 12 alkaloids was investigated in various chromatographic systems. Two columns based on silica matrix with different non-polar ligands were used: RP-18 and PFP (Pentafluorophenyl) with different aqueous mobile phases, containing acetonitrile, acetate buffer, and ion-pair reagent – octane sulphonic acid sodium salt (OSA-Na) or silanol blocker – diethylamine (DEA). The experiments were performed at the range of temperatures from 10°C–50°C. Obtained results are presented as lnk vs. 1/T relationships in Figures 1–4. Figures 1 and 2 concern IP-RP systems. It is seen that in all cases plots are linear and the retention decreases with the increase of temperature; it seems these are typical van't Hoff dependencies. However, alkaloids have basic character, their ions form with IP-reagents undissociated ion-pairs, which behave like molecules of non-ionizable compounds. On the basis of the data one must suppose that ion-pairs arise in mobile phase[15] and interact with stationary phase ligands as neutral molecules. Figures 3 and 4 show analogous plots created on the basis of retention data for investigated alkaloids obtained in systems with silanol blocking reagent – diethyl amine in mobile phase. In system RP-18 typical dependencies (decrease of retention with the increase of temperature) are obtained only for three alkaloids (Noscapine, Protopine, and Berberine). For all other compounds, the slight increase of retention (for Brucine, Codeine, Santonine, Cinchonine, Papaverine, Quinine, and Glaucine) or a distinct increase of retention (for Boldine and Emetine) can be observed. This phenomenon can be caused by the presence of diethylamine in mobile phase, which has ionization enthalpy significantly different than the investigated alkaloids. When one analyzes similar plots for retention data obtained on pentafluorophenyl stationary phase with the same mobile phases and analytes, results are similar to

Table 1. Values of asymmetry factor and theoretical plate number per meter obtained in system C 18 column/40% acetonitrile, 20% acetate buffer and 0.005 ML^{-1} OSA-Na at different temperature

Name of compounds	10°C			15°C		20°C		25°C		30°C		35°C		40°C		45°C		50°C
	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m
Berberine (Be)	11.23	5950	14.42	4590	12.94	3830	12.78	3580	11.74	2830	11.19	4630	10.44	3440	10.39	2470	10.43	1310
Boldine (Bo)	1.37	55460	2.29	49510	1.47	46790	1.51	50180	1.31	50940	1.82	62600	1.94	61390	1.57	58090	1.61	68680
Brucine (Br)	3.2	20150	5.19	19030	2.95	16660	2.92	18700	2.85	18360	4.08	23200	4.04	24450	3.55	22280	3.86	22910
Cinchonine (C)	8	6140	10.68	5930	5.39	5990	5.63	7150	5.16	8500	7.39	6590	8.93	5150	6.17	5740		
Emetine (E)	10.96	7380	11.01	7830	11.02	8780	7.74	9450	8.04	10600	9.53	8470	6.95	11380	6.68	11270		
Glaucine (G)	4.84	5950	10.07	4190	5.8	4360	6.39	7850	6.64	5260	7.54	4240	5.64	5780	3.74	5530		
Codeine (Cd)			2.05	24520	2.07	20940	2.38	20460	2.36	16870	2.9	31640	3.72	27970	2.63	26060	3.26	20490
Noscapine (No)	3.75	12720	9.27	19920	4.45	23020	2.88	27900	2.63	31560	5.41	24800	4.52	30290	3.22	27980	4.51	61860
Papaverine (P)	5.33	13660	9.88	13800	5.87	14140	5.33	14400	3.73	14480	6.88	15960	8.14	16220	6.13	16210	6.18	16660
Protopine (Pr)	5.66	13970	9.22	15100	5.1	16540	4.58	16460	3.46	18320	6.06	17580	6.01	17530	4.76	15930		
Strychnine (St)	8.41	5140	10.7	6100	6.81	5020	6.14	5380	5.3	5590	6.52	6580	5.51	5660	6.48	5270		

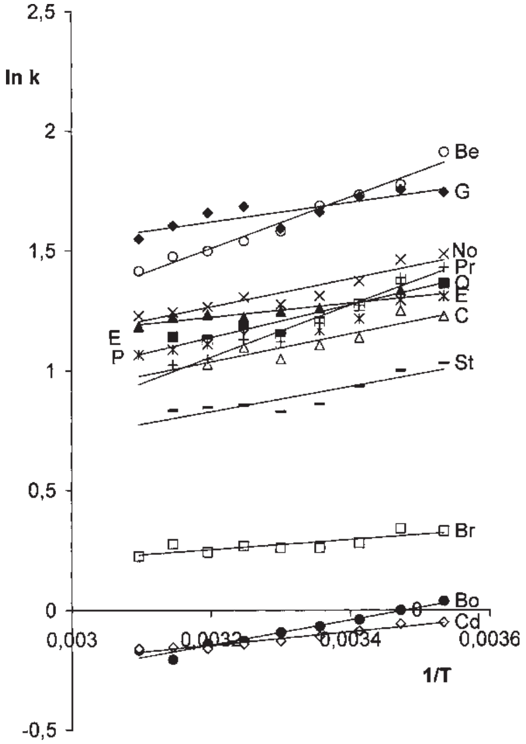

Figure 1. Plots of logk vs. 1/T for investigated alkaloids (symbols see Table 1) on C_{18} stationary phase. Eluent system: 40% MeCN + 20% acetate buffer at pH 3.5 + 40% water + 0.005 M OSA-Na.

those observed for C_{18} phases (compare Figures 3 and 4). In this system, the slight increase of retention with the increase of temperature of five of the alkaloids can be noticed, while seven other show typical van't Hoff behavior with the evident decrease of retention with the increase of temperature (see Figure 4).

It was ascertained that column efficiency and improvement of peak symmetry on various stationary phases increased with the increase of temperature in reversed-phase systems for neutral compounds.[2] In our experiment, values of theoretical plate number pro meter and asymmetry factor obtained in various systems for investigated alkaloids are presented in Tables 1–4. It can be seen from the presented values that theoretical plate numbers change with the increase of temperature. There are no rules when the changes in system C_{18}/IP are examined (see Table 1). In most cases, N/m increases with the increase of temperature when it reaches the maximum, and than slightly decreases. This maximum is reached at 35°C

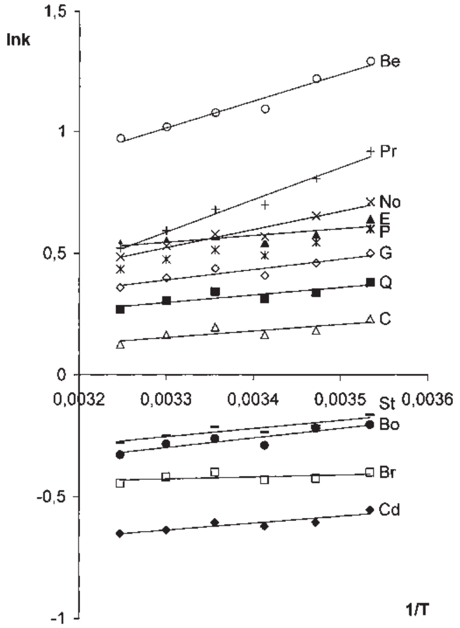

Figure 2. Plots of logk vs. 1/T for investigated alkaloids (symbols see Table 1) on PFP stationary phase. Eluent system: 40% MeCN + 20% acetate buffer at pH 3.5 + 40% water + 0.005 M OSA-Na.

in the IP-RP system on PFP stationary phase (Table 2). It should be noted, that peaks of alkaloids were wide and tailing on C_{18} columns in ion-pair systems. However, asymmetry factors decrease with the increase of temperature, in only a few cases were these values acceptable ($0.8 < As < 1.5$). When petafluorophenyl stationary phase was used in ion-pair systems, the symmetry of the peaks was much better. With the increase of temperature, usually asymmetry factors get better – at 10°C three compounds have correct values of asymmetry factor (Strychnine, Codeine, and Brucine), whereas at 50°C eight alkaloids (Strychnine, Protopine, Noscapine, Codeine, Glaucine, Cinchonine, Brucine, and Boldine) have acceptable A_S values. In the RP_{18} system with DEA as mobile phase additive, the maximum of efficiency in most cases is at 35–40°C, and in seven cases decrease at higher temperatures (see Table 3). In the system with the petafluorophenyl phase with aqueous acetonitrile + DEA, the maximum of efficiency visibly changes for various compounds, but in most cases it is in temperature range 40–50°C (Table 4). In systems with DEA, symmetry of peaks was in an acceptable range ($0.8 < As < 1.5$) and slightly changes with the change of temperature.

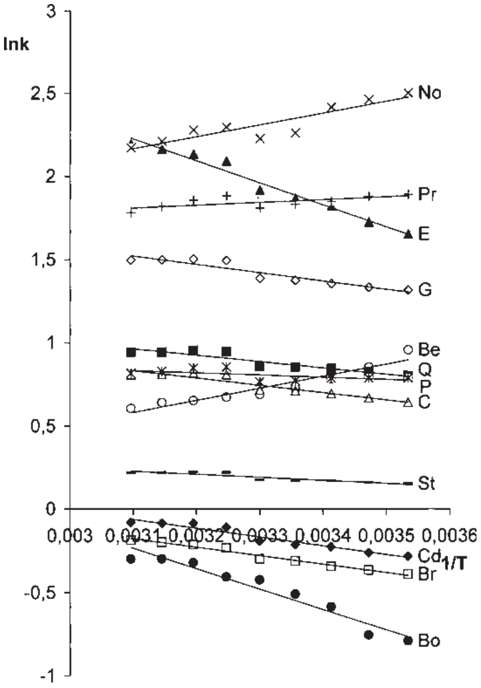

Figure 3. Plots of logk vs. 1/T for investigated alkaloids (symbols see Table 1) on C_{18} stationary phase. Eluent system: 40% MeCN + 20% acetate buffer at pH 3.5 + 40% water + 0.05 M DEA.

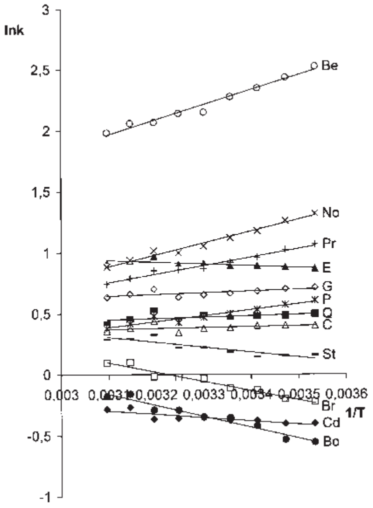

Figure 4. Plots of logk vs. 1/T for investigated alkaloids (symbols see Table 1) on PFP stationary phase. Eluent system: 40% MeCN + 20% acetate buffer at pH 3.5 + 40% water + 0.05 M DEA.

Table 2. Values of asymmetry factor and theoretical plate number per meter obtained in system PFP column/40% acetonitrile, 20% acetate buffer and 0.005 ML^{-1} OSA-Na at different temperature

Name of compounds	10°C		15°C		20°C		25°C		30°C		35°C		40°C		45°C		50°C	
	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m
Berberine																		
Boldine	1.61	139200	1.55	175750	1.46	189690	1.36	181880	1.36	170860	1.17	248970	1.56	205120	1.55	229780	1.49	216570
Brucine	1.14	184310	1.27	221460	1.13	221780	1.20	217270	1.11	237790	1.12	242720	1.29	197470	1.26	236550	1.25	263570
Quinine	1.96	167430	1.67	182960	1.92	197190	1.87	184280	1.75	205270	1.79	232390	1.94	195360	2.09	166100	1.83	211390
Cinchonine	1.60	234490	1.76	214400	1.48	233410	1.49	263050	1.42	289290	1.54	290470	1.70	240330	1.76	227810	1.58	249650
Emetine	4.25	28920	4.07	27600	4.26	36010	5.20	31970	4.80	36430	4.39	44120	6.04	28270	6.25	26200	6.28	25600
Glaucine	1.56	228130	1.54	263930	1.44	266090	1.51	304680	1.44	314750	1.49	337860	1.67	277080	1.58	283920	1.55	294550
Codeine	0.88	117680	1.08	138300	1.12	223280	1.26	254870	1.37	296840	1.24	342420	1.15	296160	1.28	271920	1.23	262140
Noscapine	1.88	177380	1.63	202210	1.88	213990	1.60	273960	1.56	289310	1.58	316750	1.05	263110	1.04	264100	0.93	273140
Papaverine	2.11	133110	2.01	146000	1.89	146490	1.59	182010	1.78	224070	1.69	232900	2.00	210490	1.92	193080	1.99	179870
Protopine	2.05	130610	2.15	117610	2.15	174000	2.00	228590	1.90	198000	1.85	225220	1.77	169040	1.74	184060	1.50	186590
Strychnine	1.46	242000	1.48	228660	1.32	257600	1.23	243570	1.18	256250	1.10	278450	1.46	252830	1.49	237890	1.48	231590

Table 3. Values of asymmetry factor and theoretical plate number per meter obtained in system C_{18} column/40% acetonitrile, 20% acetate buffer and 0.05 ML^{-1} DEA at different

Name of compounds	10°C		15°C		20°C		25°C		30°C		35°C		40°C		45°C		50°C	
	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m
Berberine																		
Boldine	1.19	8570	0.92	6690	0.95	2970	0.94	4740	0.96	5730	0.97	6950	1.14	9730	1.24	9830	1.23	11220
Brucine	0.91	4410	0.91	4410	1.11	5750	1.17	6380	1.23	7370	1.22	8120	1.20	9290	1.25	10700	1.22	12070
Quinnie	1.35	13370	1.30	14680	1.35	13280	1.57	14970	1.46	13480	1.49	16810	1.28	18810	1.38	18720	1.37	18630
Cinchonine	1.35	12810	1.28	13630	1.34	12540	1.24	14280	1.21	14910	1.35	17110	1.38	18110	1.35	14300	1.40	18760
Emetine	1.29	15830	1.36	13420	1.34	15010	1.39	15330	1.43	15540	1.36	16330	1.35	16630	1.35	15490	1.65	15800
Glaucine	1.15	17290	1.30	15700	1.19	19600	1.09	19660	1.05	19950	1.23	21640	1.22	23000	1.11	19950	1.13	19960
Codeine	0.91	6090	1.09	6040	1.14	7230	1.29	8860	1.28	9030	1.21	13650	1.17	14170	1.19	12820	0.90	10690
Noscapine	1.30	15950	1.31	17310	1.29	17910	1.34	20700	1.30	18280	1.18	25960	1.14	30060	1.00	19860	1.23	25410
Papaverine	1.16	15620	1.28	14920	1.26	18070	1.18	17280	1.16	17300	1.21	20700	1.20	19810	1.20	17110	1.15	18770
Protopine	1.16	18580	1.20	16290	1.39	21760	1.33	21380	1.37	21750	1.22	23550	1.19	25100	1.40	21750	1.33	22680
Strychnine	1.30	9740	1.33	9190	1.36	11820	1.38	10800	1.29	11790	1.38	13020	1.37	13690	1.34	13300	1.25	14420

Table 4. Values of asymmetry factor and theoretical plate number per meter obtained in system PFP column/40% acetonitrile, 20% acetate buffer and 0.05 ML

Name of compounds	10°C		15°C		20°C		25°C		30°C		35°C		40°C		45°C		50°C	
	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m	A_S	N/m
Berberine	4.46	2700	3.71	2980	3.45	3050	3.54	3120	3.02	3250	2.95	3480	2.83	3570	3.03	3610	2.52	3850
Boldine	2.34	5880	2.14	6160	2.14	7860	2.01	7700	1.94	9130	1.81	9990	1.78	11230	1.60	11230	1.47	11290
Brucine	3.57	8590	2.75	9370	2.93	15200	3.03	17220	2.53	27030	2.40	31130	2.07	36770	1.87	40140	1.00	36870
Quinnie	2.68	19560	2.66	21210	1.31	38810	1.36	50250	0.81	6130	1.48	5720	1.58	7100	1.74	8500	1.31	8650
Cinchonine	3.06	14920	2.82	17270	1.97	29220	2.16	32560	0.83	26620	0.87	21980	1.35	11690	1.55	4820	1.62	7390
Emetine	2.56	24660	2.39	26160	2.04	31620	1.42	37370	0.74	24970	0.69	19320	0.77	18930	0.77	10790	0.65	4290
Glaucine	1.69	35380	1.55	37860	1.13	45250	1.09	63670	1.17	10680	1.20	10010	1.68	11950	1.64	12900	1.89	10800
Codeine	1.98	13670	1.64	14580	1.62	19880	1.65	18860	1.48	17120	1.41	23090	1.46	22610	1.30	19117	1.21	13570
Noscapine	0.64	41220	0.95	45000	0.93	43020	0.94	42540	0.93	19990	1.03	22980	1.05	20460	1.07	22450	1.05	17310
Papaverine	0.93	41400	0.95	19720	1.01	27770	1.02	26470	1.00	22950	0.98	22780	0.99	21670	0.97	17820	0.96	17590
Protopine	0.86	46900	0.85	46310	0.92	44280	0.92	47510	0.86	4730	1.35	33740	0.96	16840	0.90	16580	0.84	13290
Strychnine	4.07	9530	3.80	9920	3.42	13950	3.48	15780	3.09	19310	2.92	21750	1.71	23340	1.54	44420	1.33	47930

CONCLUSIONS

In IP-RP systems, alkaloids behave like neutral compounds with retention decreasing as the temperature increases on C_{18}, as well as on the PFP stationary phase. It seems that ion-pairs form in mobile phases.

In RP systems with aqueous eluents containing diethylamine as silanol blockers, the increase of retention with the increase of temperature can be observed for most of the alkaloids.

In most cases, system efficiency increases with the increase of temperature reaching the maximum (at 35–45°C) and than slightly decreases.

Improvement of peak symmetry with the increase of temperature can be noticed only in few cases for alkaloids in the investigated RP systems.

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