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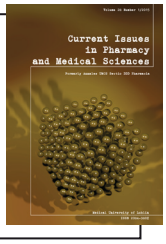

Retention behavior of selected alkaloids in Reversed Phase micellar chromatographic systems

Anna Petruczynik, Grzegorz Noster, Monika Waksmundzka-Hajnos

Department of Inorganic Chemistry Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland

ARTICLE INFO

Received 31 March 2015
Accepted 08 May 2015

Keywords:
alkaloids,
HPLC,
micellar chromatography,
SDS,
system efficiency.

ABSTRACT

In this work, the effects of sodium dodecyl sulfate (SDS) concentrations on retention, separation selectivity, peak shapes and systems efficiency were investigated. Herein, the retention data for 11 alkaloids were determined on an RP18 silica column with mobile phases containing methanol as organic modifier, with acetate buffer at pH 3.5, and, subsequently, with the addition of sodium dodecyl sulfate (SDS). The results of this study indicate that the retention of alkaloids decreases with the increase of SDS concentration in the mobile phase. The increase of SDS concentration, however, leads to the significantly improvement of peak symmetry and the increase of theoretical plate number in all cases. The best system efficiency for most of the investigated alkaloids was obtained in a mobile phase containing 0.1 M SDS, while most symmetrical peaks were obtained through the addition of 0.3 M of SDS to the mobile phase.

INTRODUCTION

Analysis of basic compounds is very often performed by reversed-phase (RP) liquid chromatography (LC), using octadecylsilica (C18) or octyl (C8) stationary phases. However, several problems are found in such practice, among them being severely low efficiencies, tailed peaks and poor separation selectivity. Such behavior is caused mostly by the ion-exchange interaction of the positively charged analytes with free silanol groups of the silica matrix. The silanol ion-exchange interactions can be reduced by using a mobile phase with a buffer at low pH to suppress silanol ionization, or by employing a mobile phase with a buffer at high pH to suppress solute ionization, as well as by the application of a mobile phase incorporating the addition of anionic ion-pairing (IP) reagents (which form neutral associates), or the addition of amines as silanol blockers. However, it is possible to undertake RP-LC with conventional C18 columns and mobile phases containing a micellar surfactant e.g. sodium dodecyl sulfate (SDS). This approach has been shown to yield good performance in the analysis of basic compounds. In these conditions, the stationary phase is modified with an approximately constant amount of surfactant monomers, and the solubilising capability of the mobile phase is altered by the presence of micelles, giving rise to diverse interactions (hydrophobic, ionic and steric). The anionic surfactant layer adsorbed on the stationary phase interacts strongly with the positively charged basic compounds, increasing the retention and masks the silanol groups that are the origin of the poor efficiencies and tailing peaks in the RPLC system.

Micellar liquid chromatography (MLC) is a mode of reversed-phase liquid chromatography which employs surfactants in the mobile phases at concentrations above the critical micellar concentration [10]. In MLC, the principal parameters are concentration of organic modifiers, pH and concentration of surfactant. The use of these modified mobile phases increases the hydrophobicity of the stationary phase and reduces the interaction between basic analytes and free silanols. The micelles in the mobile phases provide hydrophobic and electrostatic interactions, and they contribute towards enhancing the separations by HPLC. Micellar liquid chromatography offers a number of advantages to the researcher e.g. low toxicity, low cost, low volatility of mobile phase constituents, the possibility of simultaneous separation of ionic and non-ionic compounds. Moreover, it offers different separation selectivity, when compared to other chromatographic methods, owing to the involvement of a large number of parameters [7].

SDS is a most adequate surfactant for use in the analysis of compounds with basic functional groups, such as quinolines [7], Fluconazole and Tinidazole, in pharmaceuticals

* **Corresponding author**
e-mail: anna.petrucznik@umlub.pl

 DOI: 10.1515/cipms-2015-0058

and biological fluids [2], as well as with Timolol maleate in the presence of its degradation products [11].

Ruiz-Angel *et al.* investigated the influence of an SDS addition to mobile phase on peak shape and separation selectivity of β-blockers [12]. In this work, the micellar mobile phase containing 0.1M sodium dodecyl sulfate and 3% (v/v) butanol on ODS-2 column was used for determination of clorazepate, diazepam and diltiazem in examined pharmaceuticals [6]. Moreover, analysis of urine samples containing cardiovascular drugs was performed by micellar liquid chromatography by way of a C18 column with a mixture of 0.11M SDS, 8% propanol, and 0.01M NaH_2PO_4 at pH 3 or 0.15M SDS, and 15% propanol at pH 3 as mobile phases [3]. Furthermore, the determination of Enalapril and Hydrochlorothiazide in pharmaceutical preparations was then performed at the cyano bonded stationary phase, with the mobile phase containing 0.2M sodium dodecyl sulfate, 1% octanol, 10% n-propanol and 0.3% triethylamine in 0.02M phosphoric acid [8]. In addition, quantification of Paroxetine was carried out using a C18 column and a mobile phase of 0.15M sodium dodecyl sulfate, 6% 1-pentanol, 0.01M NaH_2PO_4 at pH 3 [1].

In such work, SDS can be applied in lower concentration in the mobile phase as an ion-pairing reagent. The retention of analytes in ion-pair chromatography system can be controlled by changing the type and concentration of the ion-pair reagent, type and concentration of organic modifier, as well as the pH of the mobile phase.

Chromatographic systems with ion pairing reagents being added to their buffered aqueous mobile phases have often been employed for the analysis of different basic compounds. For example, the alkaloids Nitidine and Chelerythrine, were determined by way of a C18 column with an eluent containing acetonitrile, sodium dodecyl sulphate (17.8 mM), 20 mM citric acid, pH 2.98, 57:43, v/v [9]. What is more, caffeine and alkaloids from *Citrus aurantium* contained in dietary weight loss products were determined by way of a C18 column with a mobile phase containing acetonitrile and sodium lauryl sulfate adjusted to pH 2.5 with concentrated sulfuric acid [5]. Sodium dodecyl sulfate was also employed as an ion pair reagent for the determination of Metformin in combination with Rosiglitazone, in tablets [20]. Plus, the addition of SDS as an ion-pairing reagent to the mobile phase was successfully utilized for the analysis of Gatifloxacin in bulk and formulations [13]. Finally, for the determination of the alkaloid, ephedrine, in medicinal plants, an addition of 5 mM of SDS was applied in one research work [4].

The aim of our study was to bring about an understanding of the retention behavior of selected alkaloids by RP-HPLC in systems containing different concentrations of SDS as an eluent additive. The resulting paper covers systematic investigations of the SDS effect on separation selectivity, peak symmetry and system efficiency of selected alkaloids. In our work, the most symmetrical peaks were obtained in an eluent system with the addition of 0.3M SDS, and the best efficiency of system for most investigated alkaloids was observed in a mobile phase containing 0.1M SDS.

EXPERIMENTAL

In our study, the analysis was performed using a liquid chromatograph LC-10 ATVP Shimadzu, equipped with column: Xbridge C18 150 mm × 4.6 mm, 5 μm particle (Waters). Detection was performed by a Shimadzu detector SPD – 10 AVP at 254 nm wavelength. All chromatographic measurements were carried out at 22ºC, controlled by way of a CTO-10ASVP thermostat with eluent flow rate of 1.0 ml/min. Methanol of chromatographic quality was from Merck (Darmstadt, Germany), while sodium dodecyl sulfate (SDS) was obtained from Sigma-Aldrich (Steinheim, Germany). Other reagents were of analytical grade and obtained from Merck. The employed water was double distilled. The pH of 0.2 M acetate buffer used in experiments was measured for the aqueous solutions. Alkaloid standards listed in Table 1 were introduced to the system by use of a Reodyne 20 μL injector.

RESULTS

Alkaloid standards were chromatographed through the use of a C18 column, in eluent systems containing MeOH as organic modifier, acetate buffer at pH 3.5 and SDS in various concentrations. The first experiment was performed with a mobile phase containing 15% MeOH, acetate buffer and different concentrations of SDS. Figure 1 presents the obtained dependencies of log k values vs SDS concentration (0.025-0.3M) for the investigated alkaloids. In this part of the study, we found that the retention of alkaloids decreases with the increase of SDS concentration in the mobile phase, dramatically in the range 0.025-0.1M. This effect probably came about because, in mobile phases containing near 0.1M and higher concentrations of SDS, the formation of micelles is predominant. In a system with 0.025M SDS, with regard to the six alkaloids, retention was found to be very strong (t_R >120 min). Changes in SDS concentration, hence, lead to changes in separation selectivity of the alkaloids. Of note, the better separation selectivity was in systems containing higher concentrations of SDS.

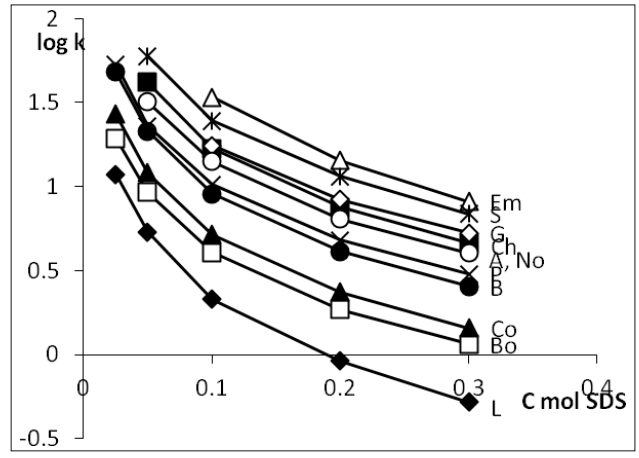

Figure 1. Relationships between log k and SDS concentration in a mobile phase obtained by way of a C18 column, and a mobile phase containing 15% MeOH and 20% acetate buffer at pH 3.5, for the investigated alkaloids. (See Table 1 for abbreviations)

The change of SDS concentrations also brought about differences in system efficiency and peak symmetry (Table 1). The best system efficiency for most investigated compounds was observed when 0.1M SDS was added to the mobile phase. In this system, for 6 alkaloids, N/m > 10 000, while in a system with a higher (0.3M) SDS concentration, chelidonine alone showed N/m > 10 000. Furthermore, in eluent with lower concentration (0.025M) SDS, an N/m >10 000 was evidenced for 3 of the alkaloids, and a lot of peaks were wide and strongly retained. Symmetry of peaks for most alkaloids improved with the increase of SDS concentration, e.g. for berberine, As = 2.72 in a system containing 0.025M SDS, but in the mobile phase, with the addition of 0.3M SDS, As = 1.18; for snaquinarine, As = 2.34 when the concentration of SDS was 0.05M, while As = 1.22 was obtained in a system with 0.3M SDS. It should be pointed out that all alkaloids have peaks with acceptable symmetry in systems with 0.2 or 0.3M SDS.

In the next step of our experiments, we examined the relationships between retention, peak symmetry, as well as system efficiency versus concentrations of organic modifier in a mobile phase containing various levels of SDS. The results of this work showed that for eluent systems with the addition of an 0.01M SDS agent, in most cases, the highest separation selectivity was obtained when eluent containing 55-60% MeOH was applied (Table 2). What is more, the most symmetrical peaks for 10 alkaloids were obtained in a mobile phase containing 75% MeOH. Herein, values were in the optimal range (0.8<As<1.5). In addition, higher efficiencies were seen for most alkaloids in a mobile phase containing 60 or 65% of MeOH.

The influence of organic modifier on retention, peak symmetry and system efficiency was also investigated by way of chromatographic systems supplemented with 0.2M SDS in the mobile phase (Table 3). In most systems investigated, the peaks for the majority of tested alkaloids were symmetrical. Furthermore, in systems with 20 and 30% of MeOH, As values for all investigated compounds were in the optimal range. Finally, the highest theoretical plate number was obtained when eluent systems containing 50% of organic modifier were applied.

Beyond the aforementioned work, the retention behavior of the investigated alkaloids was investigated by way of a mobile phase containing different concentrations of buthanol (BuOH) and acetate buffer at pH 3.5 and 0.15M SDS (Table 4). It should be noted that higher alcohols are usually applied in micellar chromatography systems. Our work demonstrated that the retention of alkaloids in systems with BuOH, compared to systems with MeOH, was significantly weaker. However, for almost all the investigated alkaloids, a good symmetry of peaks was obtained in all systems containing BuOH. Moreover, for most compounds, the highest system efficiency was obtained in systems containing 7 or 9% of BuOH.

In order to compare the efficiency of different chromatographic systems, chromatograms were obtained for chelidonine in eluents incorporating various concentrations of SDS. These are presented in Figure 2. In examining these, it is evident that, in the system containing 0.05M SDS within the mobile phase, the peak is asymmetric and tailing, while a more symmetric peak was obtained when the mobile phase was supplemented with 0.1M SDS. Furthermore, the most

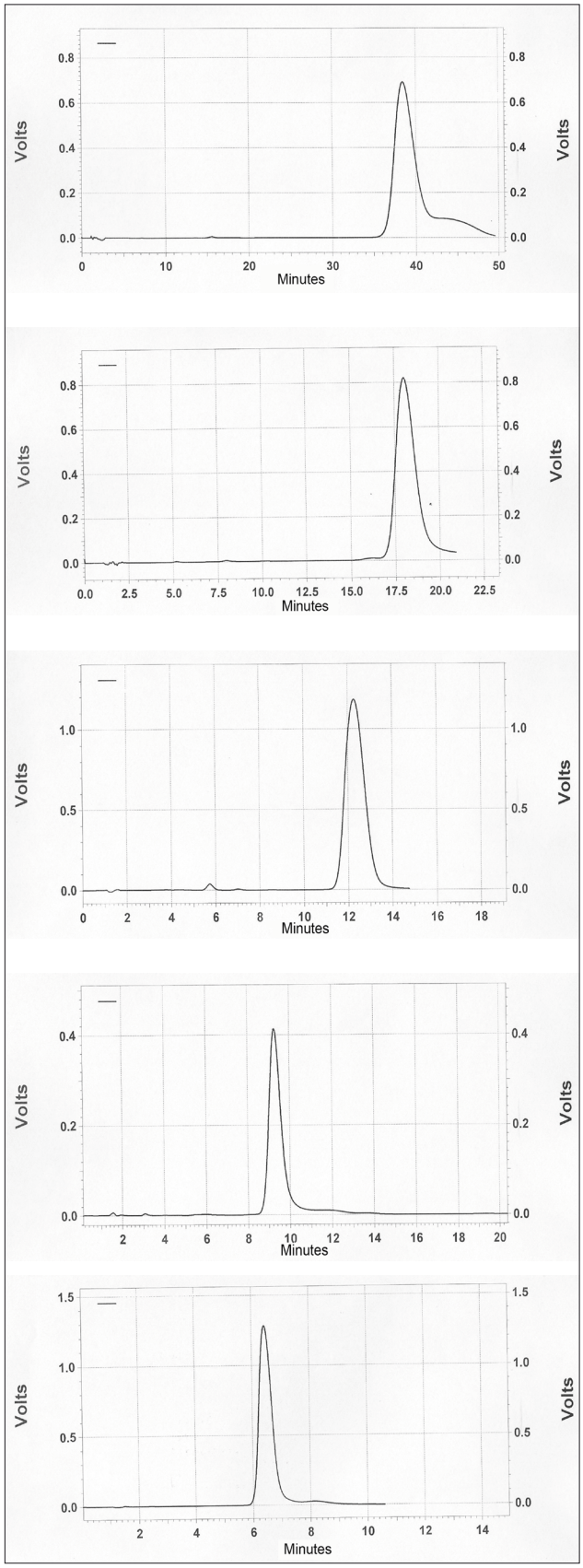

Figure 2. Chromatograms of papaverine obtained by way of a C18 column with a mobile phase containing 15% MeOH, 20% acetate buffer at pH 3.5 and SDS in the concentrations of: A, 0.025M; B, 0.05M; C, 0.1M; D, 0.2M; and E, 0.3M

Table 1. Values of retention time (t_R), asymmetry factor (A_S), and theoretical plate number per meter (N/m) for investigated alkaloids obtained on C18 column in eluent systems containing different concentration of SDS

Name of compounds	Abbreviations	15% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.025M L^{-1} SDS			15% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.05M L^{-1} SDS			15%(v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.1M L^{-1} SDS			15%(v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			15%(v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.3M L^{-1} SDS		
		t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m
Allocryptopine	A				52.08	1.31	2890	22.90	1.61	3120	11.75	1.24	4720	7.917	1.18	6880
Berberine	B	78.40	2.72	4520	35.78	1.53	7560	16.04	1.31	9030	8.18	1.23	7890	5.67	1.18	7820
Boldine	Bo	32.55	1.13	24860	16.43	1.08	14970	8.07	1.18	12260	4.56	1.30	8442	3.45	1.19	6710
Chelidonine	Ch				68.37	2.14	12590	28.44	1.40	15400	13.63	1.22	11200	8.99	1.15	10600
Emetine	E							56.09	0.98	25170	24.43	1.20	10480	14.61	1.24	3220
Glaucine	G							29.74	1.47	9240	14.98	1.16	8810	10.08	1.12	5740
Codeine	Co	44.87	1.24	14850	20.96	1.25	21780	9.88	0.93	14500	5.36	1.46	11970	3.91	1.18	9410
Laudanozine	L	20.54	0.94	21880	10.21	1.05	15260	5.03	1.62	10170	3.07	1.43	7050	2.43	1.20	6290
Noscapine	N				52.74	1.31	10150	24.19	1.18	12360	11.85	1.17	11110	8.03	1.06	9750
Papaverine	P	87.02	2.23	5180	38.43	1.97	8940	18.04	1.18	8880	9.28	1.17	8350	6.44	1.14	7760
Sanquinarine	S				97.32	2.34	7140	41.08	1.66	8060	19.21	1.42	7150	12.58	1.22	6630

Table 2. Values of retention time (t_R), asymmetry factor (A_S), and theoretical plate number per meter (N/m) for investigated alkaloids obtained on C18 column in eluent systems containing different concentration of MeOH and 0.01M SDS

Name of compounds	55% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0,01M L^{-1} SDS			60% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0,01M L^{-1} SDS			65% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0,01M L^{-1} SDS			70% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0,01M L^{-1} SDS			75% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0,01M L^{-1} SDS		
	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m
Allocryptopine	45.14	1.18	3230	21.81	1.11	3940	11.35	1.10	13350	6.30	1.09	16450	4.19	1.01	15100
Berberine	68.93	0.89	30180	29.66	0.79	33260	13.93	0.78	35000	9.46	0.77	28210	4.51	1.03	22040
Boldine	16.63	0.71	23850	9.93	0.74	26660	6.77	0.85	25950	4.98	0.74	24950	3.23	1.15	31450
Chelidonine	55.23	0.78	39810	25.61	0.85	40320	13.12	0.90	37870	9.08	1.00	30940	4.33	0.98	16510
Emetine										113.42	3.92	11430	36.98	3.07	9260
Glaucine	64.93	1.14	49820	29.38	1.04	49850	14.19	1.07	41950	7.42	0.90	26680	4.68	0.91	14630
Codeine	16.75	1.40	34510	10.69	0.98	33940	7.49	0.94	24500	6.03	1.05	19450	3.56	1.29	8840
Laudanozine	10.42	1.03	30330	7.02	1.15	12630	5.33	1.11	41380	4.18	1.06	5690	2.98	1.37	18360
Noscapine	47.89	1.17	51860	22.83	1.15	47710	12.03	1.09	42110	8.33	1.02	29770	4.17	1.03	15800
Papaverine	40.37	1.19	27840	19.44	1.04	34800	11.88	1.05	30510	7.47	1.08	20240	3.81	1.21	14130
Sanquinarine	68.93	0.89	25410	29.66	0.79	28750	14.19	0.82	29300	9.93	0.79	14510	4.69	1.24	22570

Table 3. Values of retention time (tR), asymmetry factor (AS), and theoretical plate number per meter (N/m) for investigated alkaloids obtained on C18 column in eluent systems containing different concentration of MeOH and 0.2M SDS

Name of compounds	5% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			10% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			20% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			30% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			40% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS			50% (v/v) MeOH+20% (v/v) acetate buffer pH 3.5+0.2M L^{-1} SDS		
	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m
Allocryptopine	12.66	1.48	4730	11.54	1.34	3720	10.50	1.27	5720	9.10	1.24	2590	6.32	1.49	2950	4.98	2.32	2380
Berberine	9.01	1.28	5170	8.24	1.20	7720	7.45	1.08	8050	6.65	1.03	6050	5.33	0.96	8850	4.71	0.93	10240
Boldine	4.78	1.04	8940	4.51	1.03	8480	4.23	0.96	8430	3.53	1.05	8990	3.03	0.98	8940	2.66	0.93	9550
Chelidonine	14.48	1.37	7970	13.42	1.12	10240	12.49	0.95	10140	9.81	1.00	12209	8.15	0.92	9640	6,68	0.88	11530
Emetine	26.27	2.44	1080	23.93	1.78	6140	21.22	1.19	12080	19.94	1.18	12410	15.73	1.34	12970	14.89	1.41	14120
Glaucine	16.00	1.07	6640	15.02	1.09	8850	13.38	1.10	10140	10.35	0.95	10890	8.44	0.63	9120	6.83	0.77	10340
Codeine	5.56	1.23	11470	5.14	1.16	11980	5.03	0.97	12190	4.26	1.01	12360	3.73	0.93	11140	3.30	0.96	12160
Laudanozine	3.07	1.04	8510	3.01	1.03	8470	2.96	0.89	8530	2.73	1.03	9440	2.55	0.97	10010	2.38	1.01	11490
Noscapine	13.00	1.23	9510	12.04	1.17	10010	10.71	0.97	10140	8.15	1.00	11130	6.37	0.91	9540	4.94	0.91	11060
Papaverine	10.17	1.26	8140	9.37	1.19	8350	8.38	1.05	5860	6.57	1.03	7060	5.38	0.96	6780	4.38	0.98	6490
Sanquinarine	22.56	1,23	2080	19.36	1.17	4680	16.78	1.11	7790	14.25	1.10	7820	9.31	0.94	7860	7.43	0.94	8920

Table 4. Values of retention time (t_R), asymmetry factor (A_S), and theoretical plate number per meter (N/m) for investigated alkaloids obtained on C18 column in eluent systems containing different concentration of BuOH and 0.15M SDS

Name of compounds	3% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS			5% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS			7% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS			9% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS			11% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS			15% (v/v) BuOH+20% (v/v) acetate buffer pH 3.5+0.15M L^{-1} SDS		
	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m	t_R	A_s	N/m
Allocryptopine	7.58	0.86	11200	6.23	1.00	13470	5.40	1,07	13840	4.77	1.06	13120	4.25	0.90	13850	3.16	0.89	10620
Berberine	4.89	1.06	8120	4.30	1.04	10330	4.02	0,99	10970	3.78	0.92	10930	3.53	0.97	10470	2.83	1.38	9810
Boldine	4.33	0.93	11020	4.12	0.91	11850	3.96	0,99	8220	3.66	0.87	13090	3.42	0.88	11640	2.71	0.99	10600
Chelidonine	8.49	1.03	12570	6.74	0.99	15030	5.73	0,99	15470	4.98	0.95	16360	4.43	0.93	15570	3.28	0.91	14330
Emetine	15.87	1.45	870	14.26	1.37	3250	13.35	0,73	8750	11.97	0.70	9320	10.27	0.70	10470	6.03	0.80	12910
Glaucine	11,64	0.87	8720	9.11	0.95	13110	7.26	0,98	14300	5.84	0.94	15050	4.85	0.99	14360	3.37	0.99	11900
Codeine	1.75	0.95	5090	1.72	0.87	8310	1.71	0,80	8390	1.69	0.86	5410	1.72	0.73	5030	1.70	0.74	3530
Laudanozine	2.97	0.74	6330	2.97	0.78	7560	2.95	0,81	8470	2.88	0.89	9080	2.77	0.83	7880	2.34	0.82	2790
Noscapine	7.08	0.95	11540	5.73	0.97	13560	4.90	0,96	14340	4.28	0,95	14570	3.79	0.97	14390	2.87	1.03	12040
Papaverine	5.65	1.07	5840	6.62	1.06	6840	4.00	1,07	6960	3.56	1.03	6820	3.23	1.08	6030	2.59	1.16	5040
Sanquinarine	10.66	1.02	6070	7.48	1.09	11200	5.83	1,13	12770	4.81	1.09	12530	4.16	1.14	11890	3.07	1.26	10480

symmetric peak was obtained in an eluent system containing 0.3M SDS. Of note, the highest theoretical plate number for chelidonine was obtained in an eluent system containing 0.1M SDS.

CONCLUSIONS

The results presented herein indicate that the retention, separation selectivity, symmetry of peaks and system efficiency for investigated alkaloids depends significantly on the concentration of SDS in the mobile phases. What is more, the noticed decrease of alkaloid retention indicates that the formation of micelles in all applied mobile phases is predominant.

Our work also demonstrated that in eluent systems containing lower concentrations of SDS, alkaloid retention was strongest and the shape of peaks for most investigated compounds were poor, in comparison to systems with higher concentrations of SDS. Moreover, systems efficiency was highest in mobile phases containing 0.1M SDS.

Finally, more symmetrical peaks for most investigated alkaloids were obtained in a mobile phase containing MeOH and BuOH as organic modifiers, when higher concentrations of SDS (0.2-0.3M) were applied.

REFERENCES

1. Agrawal N. *et al*.: Determination of Paroxetine in Blood and Urine Using Micellar Liquid Chromatography with Electrochemical Detection. *J. Chromatogr. Sci.,* 52, 1217, 2014.
2. Belal F. *et al*.: Micellar HPLC and Derivative Spectrophotometric Methods for the Simultaneous Determination of Fluconazole and Tinidazole in Pharmaceuticals and Biological Fluids. *J. Chromatogr. Sci.,* 52, 298, 2014.
3. Carda-Broch S. *et al*.: Analysis of Urine Samples Containing Cardiovascular Drugs by Micellar Liquid Chromatography with Fluorimetric Detection. *J. Chromatogr. Sci.,* 37, 93, 1999.
4. Enga A.T.W., Henga M.Y., Ong E.S.: Evaluation of surfactant assisted pressurized liquid extraction for the determination of glycyrrhizin and ephedrine in medicinal plants. *Anal. Chim. Acta,* 583, 289, 2007.
5. Evans R.L., Siitonen P.H.: Determination of Caffeine and Sympathomimetic Alkaloids in Weight Loss Supplements by High-Performance Liquid Chromatography. *J. Chromatogr. Sci.,* 46, 61, 2008.
6. Gil-Agustí M. *et al*.: Use of Micellar Mobile Phases for the Chromatographic Determination of Clorazepate, Diazepam, and Diltiazem in Pharmaceuticals, *J. Chromatogr. Sci.,* 38, 521, 2000.
7. Hadjmohammadi M.R., Kamel K.: Optimization of the Separation of Quinolines in Micellar Liquid Chromatography by Experimental Design and Regression Models. *Chin. J. Chem.,* 26 2197, 2008.
8. Hammouda M.E.A. *et al*.: Simultaneous Determination of Enalapril and Hydrochlorothiazide in Pharmaceutical Preparations Using Microemulsion Liquid Chromatography. *J. Chromatogr. Sci*., 53, 90, 2015.
9. Jia C.-P., Feng F.: Optimization of the Separation and Determination of Nitidine and Chelerythrine in Zanthoxylum nitidum by High-Performance Liquid Chromatography with Fluorescence Detection. *J. Chromatogr. Sci.,* 52, 164, 2014.
10. Martín L. *et al*.: Fluorescence quenching of β –carboline alkaloids in micellar media. A study to select the adequate surfactant to use in analytical techniques. *Luminescence,* 20, 152, 2005.
11. Rizk M.S. *et al*.: Development and Validation of a Stability-Indicating Micellar Liquid Chromatographic Method for the Determination of Timolol Maleate in the Presence of Its Degradation Products. doi:10.1093/chromsci/bmu075
12. Ruiz-Angel M.J. *et al*.: Improvement of Peak Shape and Separation Performance of b-Blockers in Conventional Reversed-Phase Columns Using Solvent Modifiers. *J. Chromatogr. Sci.,* 41, 350, 2003.
13. Venugopal K. *et al*.: Development and Validation of an Ion-Pairing RP-HPLC Method for the Estimation of Gatifloxacin in Bulk and Formulations. *J. Chromatogr. Sci.,* 45, 220, 2007.