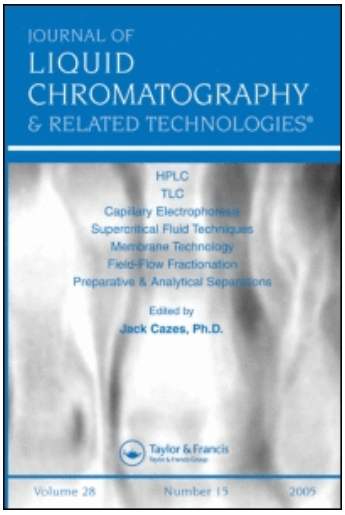

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Optimization of Chromatographic Systems for Determination of Lipophilicity for Selected Isoquinoline Alkaloids

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Abstract: Nine isoquinoline alkaloids were investigated on C_{18} stationary phase in different eluent systems containing acetonitrile, methanol, tetrahydrofuran, and dioxane as organic modifier with addition of buffers at different pH, ion pairing reagents, or silanol blockers. The dependencies between the concentration of organic modifier and retention parameters were plotted. Log k_w values were determined by the extrapolation method. The values, obtained in different eluent systems, were correlated with a predicted lipophilicity parameter (log P). The correlations between chromatographic values and predicted lipophilicity values are different, depending on the eluent system applied and the estimation method concerning partition coefficients: octanol/water. The best correlations were obtained for eluents containing the addition of ion pair reagents, especially for systems containing the sodium dodecyl sulfate.

Keywords: Alkaloids, Ion pairing reagents, Lipophilicity, Silanol blockers

INTRODUCTION

Liquid chromatography is a powerful technique for measurement of physicochemical properties, eg., parameters of lipophilicity such as a

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partition coeficient.[1] Lipophilic character often seems to be the most important physicochemical property accounting for variations of biological activity among series of chemical compounds.[2–5] The hydrophobic character of a solute is the main parameter responsible for its affinity for biological membranes.[6] These data are important to determine the transport inside an organism, as well as biopartitioning and bioconcentration of compounds. The lipophilic nature of chemical substances may be represented by the logarithm of the partition coefficient obtained from the determination of the substance distribution between an immiscible polar and nonpolar solvents.[7,8] The partition coefficient (log P) of a substance dividing into n-octanol–water in a two-phase system is used to predict or correlate its biological activity.[9,10] The log P has also been used in the estimation of toxicity and environmental parameters.[11] The measurement of partition coefficients by equilibration methods is often difficult, or even impossible, because of the impurity or instability of compounds in one of two phases of systems. Difficulties can also be caused by the formation of stable emulsions after shaking. The method cannot be applied for very hydrophilic or very hydrophobic compounds. Moreover, it is time and material consuming.[12] The chromatographic retention factor is, by definition, related to the solute distribution between the mobile and stationary phases.[13] The basic rules of the solute partitioning in aqueous two-phase systems were reported to be similar to those of water organic solvent systems. Partition behaviour of a solute in an aqueous two-phase system provides the information about relative affinity of the solute for aqueous media of different composition and different solvent properties. The logarithms of the retention factor of solutes are linearly correlated with the logarithm of their partition coefficients.

$$\log P = a \log k + b \tag{1}$$

where a and b are constants. Most often, retention factors extrapolated to 100% water (log k_w). Generally, the extrapolation to 100% water is based on a quadratic relationship between the isocratic capacity factor log k and the volume fraction of an organic solvent in the mobile phase (φ). When methanol is used as the organic modifier, a linear relationship is often obtained:[14,15]

$$\log k = -S\varphi + \log k_w \tag{2}$$

For other organic modifiers such as: acetonitrile, tetrahydrofuran, or dioxane, quadratic relationships are often obtained:[16]

$$\log k = \log k_w + B\varphi + A\varphi^2 \tag{3}$$

The retention factor, obtained by the extrapolation of retention data in binary solvent systems to pure water, is suitable for eliminating selective effects, and, thus, it can quantitatively describe the hydrophobic nature of solutes independently of the nature of an organic modifier, as well as it can show the correlation with biological activity in a better way.

For most neutral and ionizable compounds, e.g., drugs,[12,17–19] hormones,[20] bile acids,[21] ditrpenes,[11] eburnane alkaloids,[22] and potentially biologically active substances,[23–26] the obtained correlations between log P and log k_w are good. Worse correlations between retention data and lipophilicity parameters were often obtained for more ionizable compounds.[18] Alkaloids, weak bases, appear in aqueous solution in neutral and ionized forms. The distribution coefficient, in such cases, is the ratio between the concentration of a compound in the organic phase and the concentration of neutral and ionized forms of a compound in the aqueous phase. The organic phase is assumed to contain only a neutral form of a compound. When basic or acidic species are presented in the solution, the observed ratio of concentrations becomes pH-dependent and is called the distribution coefficient (D).[27] For the case of ionization in the aqueous phase, log D are related by equation (2) for a weak base that is partially protonated in the aqueous phase:[2,28,29]

$$\log D = \log P + \log[1/(1 + 10^{pK_a - pH})] \tag{4}$$

The decrease in the correlation between capacity factors and log P for basic solutes results from specific interactions of compounds with the residual silanol groups on the silica surface.[9] To reduce the effects of free silanols, various practical methods have been proposed: using a low pH mobile phase (2.0–3.5) because in these pH values the ionisation of silanol groups is suppressed;[30] using a high pH mobile phase (>7.0) when ionisation of some basic compounds is suppressed;[31] addition of ion pairing reagents to the mobile phase in order to form neutral associates between cations of basic solutes and an anion of an ion pairing reagent;[32–34] addition of a silanol blocker to the eluent, $pK_{a\ \text{silanol blocker}} > pK_{a\ \text{basic analyte}}$, when a more basic compound interacts stronger with residual silanols allowing a less basic compound to interact solely with the alkyl ligand of the stationary phase;[35,36] and selecting a stationary phase.[37–41] Silanol blockers also cause suppression of base dissociation.

Lombardo et al. have also investigated the influence of 1-octanol in the mobile phase on the correlation between log k_w and log P values for neutral and ionic drugs, and obtained the improvement of these correlations.[41]

Good correlations between lipophilicity parameters and retention parameters were obtained in systems containing reagents, forming

micelles.[1,42] Stationary phases modified by micellar reagents are structurally similar to biomembranes.

The aim of this paper was to investigate the influence of various parameters (pH of the eluent, addition of silanol blockers (amines, liquid ionic), addition of ion pairing reagents to the mobile phase) on the retention and correlation of the determined log k_w values with the lipophicity parameter (log P) of the selected isoquinoline alkaloids.

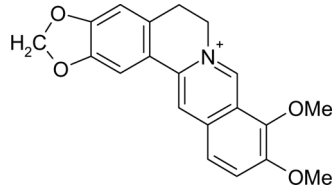
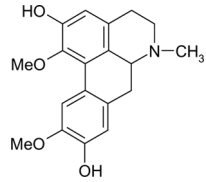
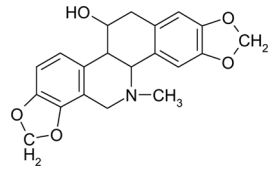
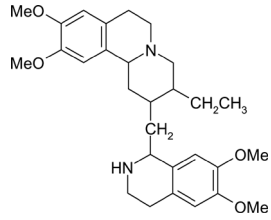
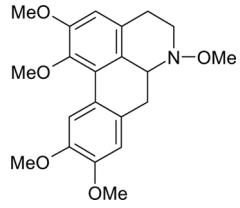
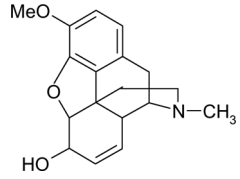
EXPERIMENTAL

Chromatographic analysis was performed using liquid chromatograph LC-10 AT_{VP} Shimadzu equipped with Xbridge^{TM} C_{18} 150×4.6 mm column (Waters, Ireland) $d_p = 5\,\mu m$, UV-VIS SPD-$10AV_{VP}$ Shimadzu detector, and Rheodyne 20 μL injector. Detection was at the wavelength of 254 nm. All chromatographic measurements were carried out at 22°C (controlled by termostat CTO $10AS_{VP}$ Shimadzu) with eluent flow rate of 1.0 mL/min. Acetonitrile, methanol, tetrahydrofuran, and dioxane of chromatographic quality, octane-1-sulfonic acid sodium salt (OSA-Na), diethylamine (DEA), were from Merck (Darmstadt, Germany). Bis-(2-ethylhexyl) phosphate (HDEHP), sodium dodecyl sulphate (SDS), isobutylamine (iBA), hexylamine (HA), were from Fluka (Buchs, Switzerland). Ionic liquids (1-methyl-3-octylxymethylimidazolium tetrafluoroborate) were from Polish Reagents (Gliwice, Poland). Each reagent was used at 0.01 M L^{-1} concentration in mobile phase. The pH of acetate buffer used in experiments at 0.2 M L^{-1} concentrations and ammonia buffer at 0.1 M L^{-1} were measured in aqueous solutions. Alkaloid standards purchased from Sigma-Aldrich are listed in Table 1.

RESULTS AND DISCUSSION

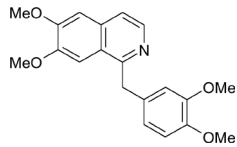
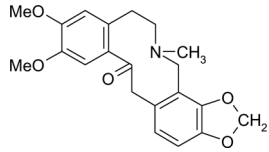
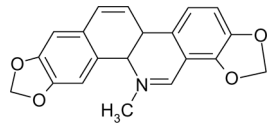
Nine isoquinoline alkaloid standards (Table 1) were chromatographed on a C_{18} column in various eluent systems containing different concentrations of modifiers: methanol, acetonitrile, tetrahydrofuran, and dioxane, buffers at different pH, different ion pairing reagents, or silanol blockers. In the first series of the experiment, the relationships between log k values and the concentration of an organic modifier in the mobile phase have been determined for all investigated alkaloids. The log k values of alkaloids decreased linearly with the increase of the volume fraction of methanol in all eluent systems. For eluent systems containing other organic modifiers these dependencies were not linear and had a polynomional shape in all cases. Afterwards, the retention factor for pure aqueous eluents (log k_w) was determined from the linear or polynominal retention eluent concentration relationships by extrapolation to $\varphi = 0$. The highest

Table 1. Structure and log P values of investigated alkaloids

Name of alkaloid	Structure	Log P
Berberine		2.08
Boldine		2.43
Chelidonine		2.91
Emetine		5.2
Glaucine		3.49
Codeine		1.19

(*Continued*)

Table 1. Continued

Name of alkaloid	Structure	Log P
Papaverine		2.95
Protopine		3.47
Sanguinarine		2.65

values of r ($r > 0.99$) proved the excellent fit between the obtained data and the applied equations in most cases (in 81 out of 102 cases). The values of the F statistic are always higher than the critical value of F for all investigated relationships. The best fit (the highest r values) of the experimental data to linear or quadratic equations were obtained in eluent systems containing ion pairing reagents: OSA-Na in the eluent containing dioxane, HDEHP in the eluent with tetrahydrofuran, and SDS in the eluent containing acetonitrile as a modifier. Different regression curves for each compound indicate selective effects upon the retention due to solute-solvent and solute stationary phase interactions.

The intercept values (log k_w) of plots – retention vs. organic modifier concentration obtained using the silanol blockers in the mobile phase are highly different from these obtained with ion pairing reagents as the component of eluents (r from 0.22 to 0.53) (Figure 1a). High correlation coefficients (r from 0.84 to 0.96) are obtained in eluent systems containing only ion pairing reagents or only silanol blockers. The log k_w values obtained in the same kind of eluent systems are similar (see correlations in Figures 1b and 1c). This good correlation could suggest that the retention behaviour is similar for the analyzed alkaloids in eluent systems containing the same kind of eluent additives. The log k_w values obtained in systems containing silanol blockers were lower in comparison to those obtained in systems with the addition of ion pairing reagents, where alkaloids appeared as associates with counter ions for all investigated isoquinoline alkaloids. The low correlation between log k_w values obtained in

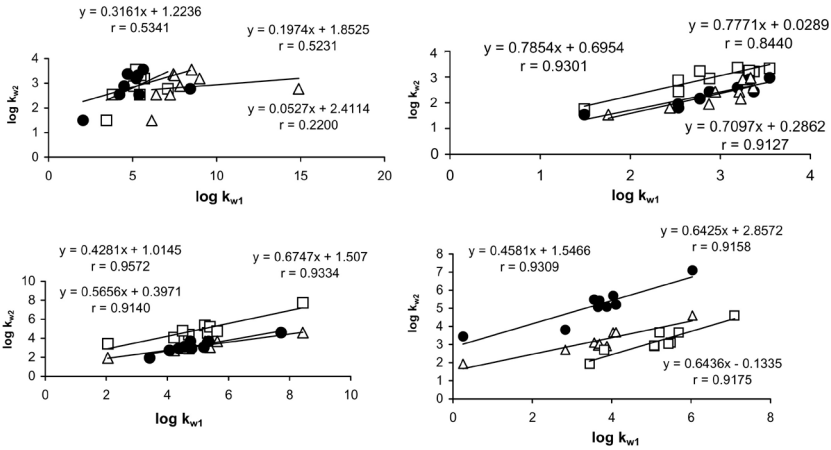


Figure 1. (a) Correlations between log k_w values obtained in the system containing acetonitrile +20% acetate buffer at pH 3.5 and DEA with log k_w values obtained in eluent systems containing acetonitrile +20% acetate buffer and HDEHP (•); SDS (Δ); OSA (□). (b) Correlations between log k_w values obtained in eluent systems containing acetonitrile +20% acetate buffer at pH 3.5 and additives of: DEA and HA (•); iBA and HA (Δ); DEA and iBA (□). (c) Correlations between log k_w values obtained in the eluent systems containing acetonitrile +20% acetate buffer at pH 3.5 and additives of: SDS and OSA-Na (•); HDEHP and OSA-Na (Δ); HDEHP and SDS (□). (d) Correlations between log k_w values obtained in eluent systems containing 20% acetate buffer at pH 3.5, OSA-Na and organic modifiers: MeOH and MeCN (•); MeOH and THF(Δ); MeCN and THF (□).

both eluent systems can be explained by the differences in the interactions of alkaloid molecules (neutral or ionised) and associates formed between alkaloid cations and ion pairing reagent anions with components of eluent and stationary phase. High correlation of the log k_w values were also obtained in eluent systems containing the same kind of additives (silanol blockers or ion pairing reagents) in the mobile phase which contained different organic modifiers (Figure 1d). The influence of the kind of addition to eluent (ion pairing reagent, silanol blockers) on log k_w values are higher then the influence of the organic modifier applied in the mobile phase. The values of log k_w indicate that emetine has the highest lipophilicity and codeine the lowest one in all eluent systems containing ion pairing reagents (Figures 1b–d). However, in other eluent systems the highest and the lowest values of log k_w were obtained for different alkaloids in various systems.

The log k_w values obtained for selected eluent systems and determined by extrapolation were linearly regressed against log P values

calculated using different computer programs. A number of different computer programs have been developed for the calculation of lipophilicity of chemical compounds based on their structure. In this work, six computer programs based on different calculation methods for computing log P were compared (Table 2). All calculated methods were based on the division of molecules into appropriate fragments for which log P values are known. Log P value for the whole molecule is the sum of log P values for all fragments to multiply by correction coefficients for intramolecular and intermolecular interactions. Table 3 presents the parameters of correlations of log P values calculated by different computer programs vs log k_w used in selected investigated eluent systems. The lowest correlation was obtained with Milog P values for seven out of ten compared systems. These log P values are calculated as a sum of group contributions and correction factors.[43] Group contributions were obtained by fitting the calculated log P values with experimental log P values for a training set of several thousands of drug like molecules. Somewhat higher values of correlation coefficients of dependencies log P vs log k_w were obtained for log $P_{CDI\text{-}REKKER}$.[44] The CDI-Rekker database is based on Rekker's collection of hydrophobic fragmental constants derived from an extended set of about a thousand log P values in the octanol-water system. Similar correlation coefficient values were obtained for correlations with ANN log P values, based on the atomic fragment collection of Ghose and Crippen.[45] Higher correlations were obtained with ACD log P values, which are calculated for the neutral form of a molecule based on a database containing one or more experimental log P values for over 3600 structures with 500 different functional groups.[46] Further improvement (especially for eluent systems containing ion pairing reagents) of the correlations with log k values extrapolated from pure water was obtained for X log P values, based on the summation of atomic contributions, but it included ten additional correction factors for some intramolecular interactions.[47] Atoms are classified by their hybridization states and neighboring atoms. The highest correlation between log k_w and log P values was obtained for frequently investigated systems (7 out of 10), where log K_{OW} values were calculated by ChemIDplus computer program.[48] The log P value is calculated by simply summing up all atom/fragment contribution values, correction factors, and the linear equation constant. For all calculation methods, higher correlation was obtained for eluent systems containing ion pairing reagents in comparison to the systems containing buffer or silanol blockers. For this reason, log K_{OW} values were applied in further consideration.

The selected group of isoquinoline alkaloids contains molecules which indicate great differences in lipophilicity (from 1.19 for codeine to 5.20 log P units for emetine). Table 3 and Figures 2a–d present the parameters of equations describing these dependencies, obtained for

Table 2. The values of slope (a), intercept (b) and correlation coefficient (r) for equations log k_w vs. log P values calculated by different computer programm

	Mi log P			Log P rekker			ANN log P			ACD log P			X log P			Log K_{ow}		
Eluent system	a	b	r	a	b	r	a	b	r	a	b	r	a	b	r	a	b	r
MeCN + B. pH 10.5	0.32	1.04	0.3407	0.64	1.69	0.5179	0.84	−0.62	0.6956	0.74	0.10	0.6556	0.49	0.90	0.7378	0.67	0.42	0.7854
MeOH + H_2O + iBA	0.16	1.69	0.1493	0.73	1.68	0.4999	0.70	0.23	0.4855	1.24	−0.99	0.7374	0.51	1.07	0.5868	0.52	1.23	0.5089
MeCN + H_2O + iBA	0.42	1.19	0.4009	0.53	2.76	0.3831	1.12	−0.24	0.8195	0.70	1.07	0.5644	0.29	2.01	0.3571	0.46	1.65	0.4760
MeOH + pH 3.5 + OSA-Na	0.28	1.24	0.3493	0.69	1.61	0.6573	0.60	0.37	0.5822	0.59	0.77	0.6118	0.53	0.86	0.8446	0.64	0.66	0.8695
MeCN + pH 3.5 + SDS	0.20	0.54	0.4418	0.35	1.18	0.5717	0.44	−1.18	0.7423	0.35	−0.11	0.6424	0.25	0.66	0.6948	0.37	−0.12	0.8669
MeCN + pH 3.5 + HDEHP	0.27	0.72	0.5346	0.34	2.20	0.5004	0.50	−0.23	0.7489	0.42	0.51	0.6772	0.27	1.25	0.6744	0.43	0.59	0.9003
THF + pH 3.5 + SDS	0.55	−0.49	0.5520	0.66	0.83	0.4970	1.04	−2.58	0.7947	0.90	−1.61	0.7488	0.53	0.13	0.6756	0.89	−1.45	0.9652
DX + pH 3.5 + OSA-Na	0.41	0.59	0.3103	1.28	−1.04	0.7335	1.07	−1.75	0.6233	1.01	−1.16	0.6287	0.90	0.84	0.8693	1.06	−1.28	0.8671
THF + pH 3.5 + OSA-Na	0.68	0.06	0.4214	1.46	−0.56	0.6798	1.39	−1.90	0.6598	1.50	−1.84	0.7495	1.08	0.71	0.8553	1.34	−1.32	0.8974
THF + pH 3.5 + HDEHP	0.35	0.48	0.4769	0.62	0.97	0.6420	0.63	−0.66	0.6669	0.64	−0.35	0.7299	0.49	0.29	0.8568	0.62	−0.21	0.9322

Table 3. Equations, correlation coefficient and F statistic test values obtained for correlations between extrapolated log k_w and predicted log P or log D values

Mobile phase	Equation of the dependence log P vs. log k_w	r	F
MeCN + b · PH 3.5*	$y = 1.4803x - 3.6997$	0.7459	1.19
MeCN + b · pH 10.5	$y = 0.6706x + 0.4184$	0.7854	1.86
MeOH + H_2O + iBA	$y = 0.5159x + 1.2293$	0.5100	0.96
MeOH + b · pH 3.5 + iBA*	$y = 1.3498x - 2.3134$	0.5642	0.70
MeCN + b · pH 3.5 + DEA*	$y = 1.2812x + 0.4184$	0.6387	0.93
MeCN + b · pH 3.5 + iBA*	$y = 1.6868x - 3.6038$	0.7428	1.35
MeCN + b · pH 3.5 + HA*	$y = 1.0276x - 1.0998$	0.4217	0.76
MeCN + b · pH 3.5 + IL*	$y = -2.8257x + 1.9022$	0.4461	0.52
THF + H_2O + iBA	$y = 0.4612x + 1.6498$	0.5906	0.97
THF + b · pH 3.5 + iBA*	$y = 1.63529x - 0.8789$	0.7011	1.24
MeOH + b · pH 3.5 + OSA-Na	$y = 0.6383x + 0.6573$	0.8695	2.91
MeCN + b · pH 3.5 + OSA-Na	$y = 0.9526x - 1.9709$	0.9103	4.17
MeCN + b · pH 3.5 + SDS	$y = 0.3671x - 0.1214$	0.9484	7.09
MeCN + b · pH 3.5 + HDEHP	$y = 0.4264x + 0.5863$	0.9003	3.75
THF + b · pH 3.5 + OSA-Na	$y = 1.3387x - 1.3238$	0.8974	3.65
THF + b · pH 3.5 + SDS	$y = 0.891x - 1.450$	0.9652	10.40
THF + b · pH 3.5 + HDEHP	$y = 0.622x - 0.2125$	0.9322	5.43
DX + b · pH 3.5 + OSA-Na	$y = 1.0560x - 1.2765$	0.8671	2.88

*Corerlation with log D.

twenty different eluent systems. The presented data show that eluent composition: kind of organic modifier, different additives to the mobile phase (buffers at acidic or basic pH, silanol blockers, ion-pairing reagents) significantly influences the correlation between log k_w and predicted lipophilicity values. Weak correlations were obtained in all chromatographic systems in which the examined alkaloids occurred in the ionic form. The improvement of these correlations – the increase of correlation coefficient values – was observed after the influence of dissociation constants (K_a) and hydrogen ion concentration on lipophilicity parameters and calculating log D (Equation 4), which had been taken into account. However, the correlations were often still poor ($r > 0.4 < 0.78$).

Initially, the influence of the pH of the mobile phase on log k_w vs log P correlations were examined. The correlation of these values was higher in the system containing strongly basic buffer (pH 10.5), when ionisation of alkaloids is suppressed, in comparison to the corerelation for the system containing buffer at pH 3.5. In the chromatographic system containing acidic buffer, alkaloids – weak bases occur in an ionised form.

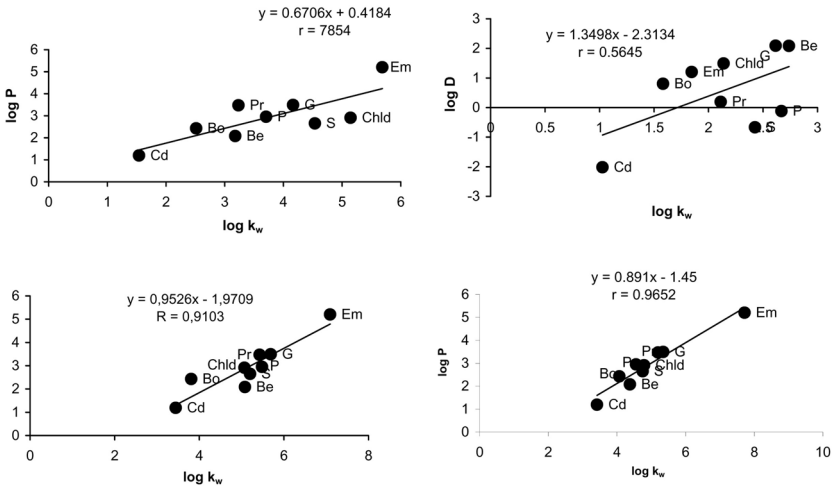


Figure 2. (a) Correlations between log k_w obtained in eluent systems containing acetonitrile + 20% ammonia buffer at pH 10.5 and log P values. (b) Correlations between log k_w obtained in eluent systems containing methanol + 20% acetate buffer at pH 3.5, 0.01 ML^{-1} iBA and log D values. (c) Correlations between log k_w obtained in eluent systems containing acetonitrile + 20% acetate buffer at pH 3.5, 0.01 ML^{-1} OSA-Na and log P values. (d) Correlations between log k_w obtained in eluent systems containing acetonitrile + 20% acetate buffer at pH 3.5, 0.01 ML^{-1} SDS and log P values.

Therefore, the correction for log P values should be taken into consideration. The correlation coefficient for log D values significantly increased from r = 0.2452 (for the correlation with log P) to r = 0.7459.

Afterwards, the usefulness of chromatographic systems containing different silanol blockers also playing the role of an ion suppressant in eluents for determination of lipophilicity was examined. Diethylamine, hexylamine, isobutylamine, and ionic liquid were applied. In all the systems containing silanol blockers, especially ionic liquid, very symmetric peaks and high efficiency were obtained, but the correlation of log k_w values with log P or log D values were lower than those obtained in the systems with buffer addition only. The lowest correlation coefficients were obtained for eluent systems consisting of acetonitrile, acetate buffer, and hexylamine or ionic liquid (r = 0.4217 and 0.4461, respectively). Better correlations were obtained in systems with isobuthylamine in the eluent containing acetonitrile or tetrahydrofurane and acetate buffer −0.7428 and 0.7011, respectively. The poor correlations can be explained that in systems containing silanol blockers and buffer at pH 3.5 non-hydrophobic interaction are suppressed, but alkaloids still exist in ionic form. The correlation between log P

and log k_w values obtained in eluent systems containing organic modifier, water, and amines are better. In these eluent systems, dissociation of alkaloids are suppressed and alkaloids strongly interact with hydrophobic stationary phase. For all the systems containing the mixtures of buffer, organic modifier, and different silanol blockers, the correlations for all log k_w and predicted lipophilicity parameters are not statistically significant. The F statistic test values are lower than critical values of the F test in these cases.

Eluent systems containing ion pairing reagents proved to be the most useful systems for the determination of log k_w values. In these systems, the peak shape and system efficiency were somewhat worse in comparison with the systems containing silanol blockers, but the correlations of log k_w obtained using the systems with log P values were significantly better. In all investigated chromatographic systems containing different organic modifiers and different ion pairing reagents, the r values for the correlation with log P were higher than 0.865, and the F statistic test values were higher then F critical values in most cases ($F_{critical} = 3.44$). In the partition process, only neutral forms of alkaloids, such as associates between alkaloid cations and ion pairing reagent anions, may penetrate into the organic phase. Good correlations of log k_w values obtained in the systems with log P values are the result of an easy transfer of neutral associates into the organic phase. The best correlation were obtained in the systems with the addition of HDEHP ($r = 0.9003$ for the eluent containing acetonitrile and 0.9322 for the mobile phase with tetrahydrofuran as a modifier), and for the SDS ($r = 0.9484$ for the eluent containing acetonitrile, and 0.9652 for the system with tetrahydrofuran).

CONCLUSIONS

Log k_w values determined by the use of various chromatographic systems are different but well correlated only in the same type of systems (IP, amines).

The highest correlations between log k_w and log P values for most investigated systems were obtained, where log P values were calculated by ChemIDplus computer program.

For the systems containing buffer at pH 3.5, log k_w values are better correlated with log D, taking into consideration the dissociation of analytes and the pH of the mobile phase. However, the determined lipophilicity values still poorly correlated with log P values. The correlation between log P and log k_w values obtained in eluent containing strongly basic buffer, when ionisation of alkaloids is suppressed was higher.

In the systems containing silanol blockers, very symmetric peaks and high efficiency were obtained, but the correlation of lipophilicity with log P or log D values were lower than those obtained in the systems with buffer addition only.

A significant improvement of log k_w vs log P correlation were used in all eluent systems containing different ion pairing reagents, especially for eluents with addition of SDS.

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