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Chromatographic quantitation of the hydrophobicity of ionic compounds by the use of micellar mobile phases

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Abstract

Many biologically active compounds of interest in structure–activity relationships are ionic at physiological pH. However, ionic organic compounds are only weakly or not retained in conventional RPLC which impedes the chromatographic estimation of their hydrophobicity and the development of quantitative retention–activity relationship studies. The use of micellar mobile phases allows the retention of ionic compounds. Hydrophobic and electrostatic forces govern the retention of ionic compounds in micellar liquid chromatography. In this paper three different retention models log k–log P for ionic compounds are tested (P=partition coefficient). The retention model (log $k=a$ log $P+b\alpha+c$) which includes the hydrophobicity and the molar total charge of compound at a given pH value has proven to be valid for all types of compounds tested, catecholamines, local anesthetics, diuretics and *o*-phthalaldehyde–N-acetyl-L-cysteine amino acid derivatives.

Keywords: Hydrophobicity; Retention models; Micellar liquid chromatography; Catecholamines; Anaesthetics; Diuretics; Amino acids

1. Introduction

The hydrophobicity quantification of solutes is of great importance in quantitative structure–activity relationship (QSAR) studies, drug design and toxicology [1–4]. Initially, in order to determine the hydrophobicity of compounds, the partition coefficients in the biphasic octanol–water solvent system, log P, were used. The determination of log P using the traditional shake-flask method has several drawbacks and different approaches for estimating it have been proposed [5]. Since the retention of a compound in reversed-phase liquid chromatography (log k, RPLC) is governed by hydrophobic interactions, linear relationships log k–log P could be expected (P=partition coefficient). This approach has received much attention, but until now there is no universally accepted method of performing these estimations [6–8].

Many biologically active compounds of interest in QSAR studies are ionic at physiological pH. Ionic organic compounds are only weakly or not retained in RPLC even with pure buffer or water as the eluent, because of the limited pH operating range of silica bonded phases. In addition, organic bases can interact with the unreacted silanol groups of the silica particles. The influence of solute ionization on hydrophobicity estimation by RPLC has been extensively studied [6–9], and different approaches have been proposed: (i) the estimation of the retention factor of the unionized form using a retention model [10,11]; (ii) the use of ion-pair RPLC methodology

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[12,13]; (iii) the use of polymeric resin stationary phases [14,15] and alumina bonded phases [16] as an attractive alternative to silica bonded phases because of their wider pH operating range and lack of residual silanol groups. However, over the physiological range of pH, the silica supports are stable and allow the determination of the exact partitioning behaviour of compounds, which is of major interest in pharmaceutical research.

Retention of a compound in micellar LC (MLC) depends on the type of interactions (electrostatic and/or hydrophobic) with the micelles and the surfactant-modified stationary phase [17–19]. Nonionic solutes should only be affected by hydrophobic interactions and adequate linear relationships log k–log P were obtained at low micellar concentrations [20–23]. However, for highly hydrophobic compounds and high micellar concentrations deviation on the linearity could be obtained [24,25].

For charged solutes, in addition to hydrophobic, electrostatic interactions exist and two distinct situations can be considered: (i) the charge on the solute and surfactant has the same sign or (ii) it has the opposite sign. In the first situation, the electrostatic repulsion between the solute and the surfactant-modified stationary phase decreases the retention or can even impede the retention. In this case the MLC would only be useful for hydrophobicity measurements of highly hydrophobic compounds. In the second case, electrostatic interactions of solutes with the modified stationary phase increase the retention of charged compounds, even for low hydrophobic ones. In our opinion, in this case, the use of micellar mobile phases should make it possible to quantify the hydrophobicity of charged compounds.

In a previous paper [26] we proposed a novel retention model which includes the hydrophobicity of compounds and the molar fraction of the charged form of compounds. The model was assayed for local anesthetics. In this paper, the model is tested using different groups of compounds with different degrees of ionization and net charge and the results were compared with those obtained using other previously reported models.

2. Experimental

Experimental data were collected from our laboratory in order to check the models. The retention factors corresponding to six catecholamines eluted with 0.1 M sodium dodecyl sulfate (SDS) at different pH values [27], eight local anesthetics measured using Brij 35 as micellar mobile phase at three different concentrations [26], twelve diuretics eluted using 0.15 M SDS mobile phases at different pH values [28] and sixteen o-phthalaldehyde–N-acetyl-L-cysteine (OPA–NAC) amino acid derivatives eluted with 0.05 M SDS mobile phases at different mobile phases pH [29] were studied.

Tables 1–4 show the structure, the logarithm of protonation constants (log K) and the log P values for the nonionic forms of the catecholamines, local anesthetics, diuretics, and amino acids studied respectively. The log P values for the nonionic forms of the compounds, and the protonation constants of the compounds were taken from the literature [30,31].

Excel 7.0 from Microsoft Office software was used to perform the statistical analysis of the multiple linear regression.

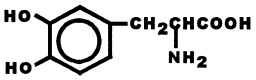
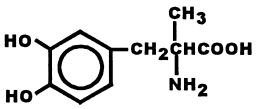
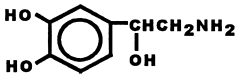
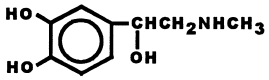
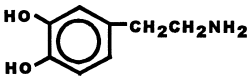
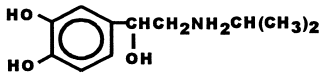
3. Results and discussion

3.1. Retention–log P relationships

When the retention of ionic compounds, log k values, obtained for a certain mobile phase are correlated with the corresponding log P values for the nonionic forms of compounds, poor correlations are generally obtained. This behavior is due to the fact that the retention of ionic compounds not only depends on the hydrophobic interactions but also, on the degree of ionization of the compounds. Thus, when the degree of ionization is the same for structurally related compounds, the difference in retention is due to the differences in hydrophobicity. However, for ionic compounds with different degrees of ionization, linear log k–log P relationships should not be obtained.

In order to obtain adequate log k–log P relationships, different approaches were assayed. In the first model, Eq. (1) was used. This equation was previously proposed to correlate the retention of ionic compounds with their octanol–water partition coefficients [32,33]:

Table 1
Structure, log K and log P values for the nonionic forms of the catecholamines studied

Compound	Abbreviation	Structure	Log K_1 [a]	Log K_2 [a]	Log K_3 [a]	Log K_4 [b]	Log P
L-DOPA	D		13.4	9.7	8.7	3.8	−0.22
2-Methyl-L-DOPA	MD		12	10.6	9.2	4.2	0.12
Norepinephrine	NE		12	9.8	8.6		−0.88
Epinephrine	E		12	10.2	8.7		−0.63
Dopamine	DA		13	9.9	8.7		0.12
Isoproternol	ISA		12	10.1	8.6		0.25

[a] Protonation constant of compounds in aqueous medium.
[b] Protonation constant of carboxylic group in SDS solution.

$$\log k = a \log P_{app} + b \quad (1)$$

where P_{app} is the apparent octanol–water partition coefficient [34,35]. It can be calculated as:

$$\log P_{app} = \log P + \log \delta_i \quad (2)$$

$$\log \delta_i = \log [\beta_i h^i / (1 + \beta_1 h + \beta_2 h^2 + \ \ldots \ + \beta_n h^n)] \quad (3)$$

In these equations, δ_i is the molar fraction of the neutral form of the compound, h is the proton concentration and β_i is the protonation cumulative constant (for the polyprotic system, $\beta_n = K_1 K_2 K_3 \ldots K_n$).

A limitation of this approach is that it considers the contributions of the hydrophobicity and the charge of the compounds to the retention to be the same. We developed a new model (model II) based on the independence of these contributions to the retention [26], according to Eq. (4):

$$\log k = a \log P + b \log \delta_i + c \quad (4)$$

Eqs. (1) and (4) take into account the ionization of the compounds, but both of them present an important limitation, in that their application is limited to pH values close to the log K values of compounds. For instance in Eq. (1), when pH $\ll$ log K, nonsense values of log k could be predicted since log P_{app} decreases systematically as the pH decreases, while in this case the retention is independent of the mobile phase pH.

In order to overcome these limitations, a novel retention model (model III, Eq. (5)) was assayed, which uses the α variable. This variable measures the molar total charge of compound at a given pH value.

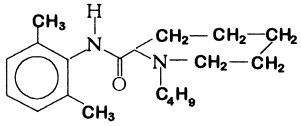
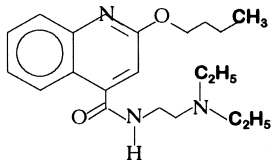
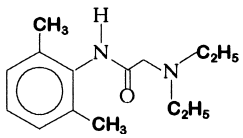
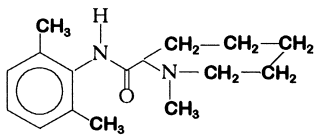
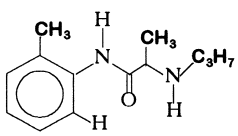
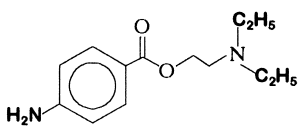
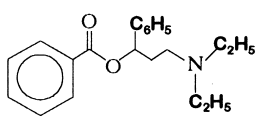
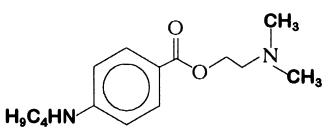
$$\log k = a \log P + b\alpha + c \quad (5)$$

For polyprotic compounds, the α values can be calculated as:

$$\alpha = \sum_{j=0}^{n} a_j \delta_j \quad (6)$$

where a_j is the value with its sign of the net charge of the considered specie (i.e. $+1, -1, 0, +2, \ldots$) and δ_j the molar fraction of the considered specie at the considered pH, i.e., for monoprotic acid or basic compounds, the α values can be calculated using Eqs. (7) and (8), respectively:

Table 2
Structure, log K and log P values for the nonionic forms of the local anesthetics studied

Compound	Abbreviation	Structure	Log K	Log P
Bupivacaine	BU		8.10	3.38
Dibucaine	DI		8.85	4.40
Lidocaine	LI		7.9	2.26
Mepivacaine	ME		7.7	1.75
Prilocaine	PRI		7.89	1.65
Procaine	PRO		8.80	2.24
Propanocaine	PROP		7.53	4.20
Tetracaine	TE		8.6	3.73

$$\alpha = a_0\delta_0 = (-1)1/(1 + K[H^+]) \quad (7)$$

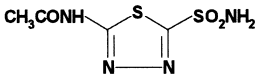
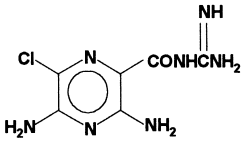
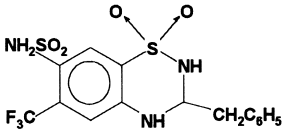
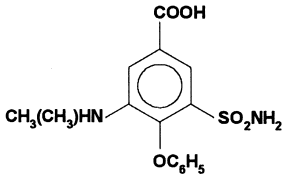
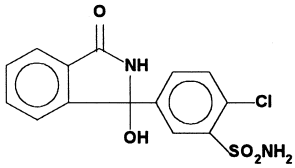
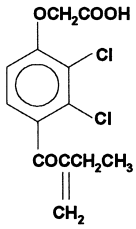
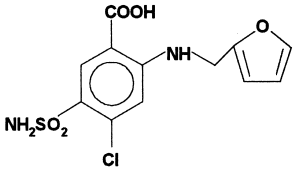
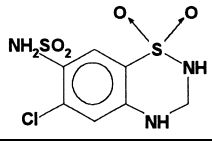
$$\alpha = a_1\delta_1 = (1)K[H^+]/(1 + K[H^+]) \quad (8)$$

According to model III (Eq. (5)), for pH values far from log K, the predicted retention remains constant.

3.2. Log k–log P relationships for catecholamines

In a previous paper [27], the retention factors for catecholamines eluted with 0.1 M SDS mobile phases at different pH values (2–7) were measured. In this case, an anionic surfactant, SDS, was used to increase the retention of the catecholamines.

Table 3
Structure, log K and log P values for the nonionic forms of the diuretics studied

Compound	Abbreviation	Structure	Log K	Log P
Acetazolamide	A		7.2	−0.26
Amiloride	AM		8.7	1.9
Bendoflumetazide	BEN		8.5	2.02
Bumetanide	BU		5.2	2.78
Chlortalidone	CHLOR		9.4	0.24
Etacrinic acid	EA		4.19	3.88
Furosemide	FURO		4.42	2.29
Hydrochloratiazide	HYDRO		7	−0.07

(*Continued overleaf*)

Table 3. Continued

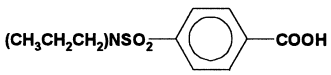
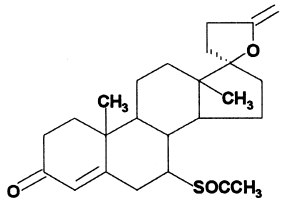
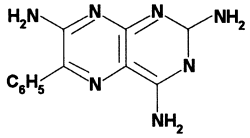
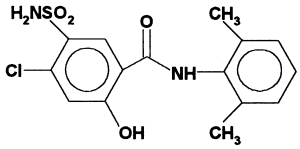
Compound	Abbreviation	Structure	Log *K*	Log *P*
Probenecid	PRO		4.65	3.03
Sipironolactone	SPIRO		–	5.053
Trianterene	TRI		6.2	1.3
Xipamide	XIPA		5.47	4.01

Table 1 shows the structure, protonation constants and log *P* values of the nonionic forms of catecholamines. As can be observed, catecholamines are compounds of very low hydrophobicity and present a positive or neutral charge as a function of the pH. In the 2–7 pH range norepinephrine (NE), epinephrine (E), dopamine (DA) and isoprotenerol (ISO) are positively charged, therefore α values were +1 in all the studied pH range, whereas L-DOPA (D) and 2-methyl-L-DOPA (MD) are in the zwitterionic form and have a net charge of zero at pH 7 and next to +1 at pH 2 (i.e. for D the α values were 0, 0.4 and 0.98 at pH 7, 4 and 2, respectively). For calculations the protonation constant of the carboxylate group of D and MD in SDS micellar medium (log $K_4 = 3.8$ and 4.2, respectively) was considered.

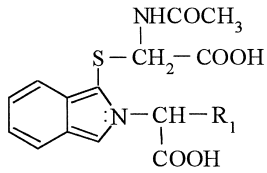
The retention factors (log *k*) for catecholamines obtained with 0.1 *M* SDS mobile phases at different pH values, the log *P* values, the logarithm of the molar fraction of the neutral form of compounds (log δ_2 for NE, E, DA and ISO and log δ_3 for D and MD) and the molar total charge of the compounds at these pH values (α values) were adjusted to models I, II and III (Eqs. (1), (4), (5)). Table 5 shows the results obtained from regression analysis of the data.

As can be observed, in all cases the best correlations were obtained using model III. Model I only provided adequate results for pH values higher than the log *K* values of D and MD. For pH values lower than 5 the linear relationships were considerably worse.

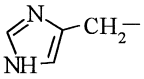
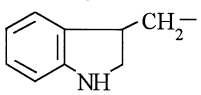
In all cases model II provides better results than model I, but in some cases the fitting coefficients *a* and *b* were not statistically significant. In addition, the fitting parameters *a* and *b* were different, which indicates that the contribution of hydrophobicity and the charge of compound to the retention is different. This fact could explain the results obtained using model I.

Using model III, the fitting parameters related with hydrophobicity, *a* coefficients, were statistically significant and remained practically constant when the mobile phase pH was modified. This fact indicates that the model isolates correctly the hydrophobicity contribution of compounds to the retention. On the other hand, the *b* coefficients were positive, which

Table 4
Structure, log K and log P values for the nonionic forms of the amino acids studied



GENERAL STRUCTURE

Compound	Abbreviation	R_1	Log K^a α-CO_2H	Log K α-NH_3^+	Log K (side chain)	Log P^b
Alanine	Ala	$-CH_2-CONH_2$	3.3	9.69	–	0.28
Arginine	Arg	$-(CH_2)_3-NH-C(NH_2)=NH$	4.02	9.04	13.2[c]	0.38
Aspartic acid	Asp	$-CH_2-COOH$	3.1	9.60	3.9[d]	0.78
Cysteine	Cys	$-CH_2-SH$	3.39	8.18	8.35[e]	0.12
Glutamic acid	Gly	$-CH_2CH_2-COOH$	3.1	9.67	4.32[d]	0.89
Glycine	Gly	–H	3.1	9.60	–	1.37
Histidine	His		2.9	9.17	6.05[c]	1.02
Isoleucine	Ile	$-CH(CH_3)-CH_2-CH_3$	3.72	9.68	–	2.89
Leucine	Leu	$-CH_2-CH(CH_3)-CH_3$	3.72	9.60	–	3.06
Lysine	Lys	$-(CH_2)_4-NH_2$	3.29	9.12	10.8[c]	1.53
Methionine	MET	$-CH_2-CH_2-sCH_3$	2.8	9.21	–	2.71
Phenylalanine	Phe	$-CH_2-Ph$	3.5	9.13	–	3.06
Threonine	Thr	$-CH(CH_3)-OH$	3.2	9.62	–	1.64
Tryptophan	Trp		3.2	9.39	–	3.52
Tyrosine	Tyr	$-CH_2-Ph-OH$	3.5	9.11	9.11[f]	2.32
Valine	Val	$-CH(CH_3)-CH_3$	3.4	9.62	–	2.32

[a] Protonation constants of carboxylic group in SDS solution.
[b] log P values of OPA–NAC amino acid derivatives, obtained using the ACD-log P software.
[c] Amine group.
[d] Carboxylic group.
[e] Thiol group.
[f] Phenolic group.

indicates that the electrostatic attractions between compounds and surfactant-modified stationary phase increase the retention of compounds.

3.3. Log k–log P relationships for local anesthetics

Local anesthetics are basic compounds with large hydrophobicity (see Table 2). At pH 7.4, the compounds are positively charged but the degree of ionization is different between compounds ranging from 0.57 for propanocaine to 0.97 for dibucaine. Previously [26], the retention of local anesthetics was measured using a nonionic surfactant, Brij 35, as micellar mobile phase at pH 7.4.

The retention of local anesthetics obtained for different concentrations of Brij 35 in the mobile phase, log k, the log δ_0 and α values at pH 7.4, and the log P values were adjusted to models I, II and III, by applying simple and multiple linear regression. Table 6 shows the regression statistics obtained.

Table 5
Statistical analysis of the linear regressions for catecholamines

pH	a (ts_a)	b (ts_b)	c (ts_c)	r^2	SE
$\log k = a \log P_{app} + b$ (model I, Eq. (1))					
2	0.02 (0.07)	1.2 (0.5)		0.18	0.14
3	0.01 (0.07)	1.1 (0.6)		0.06	0.15
3.5	0.00 (0.09)	1.0 (0.7)		0.002	0.17
4	−0.05 (0.10)	0.5 (0.8)		0.30	0.20
5	−0.18 (0.17)	0 (1)		0.70	0.30
5.4	−0.2 (0.2)	−1 (2)		0.75	0.30
5.8	−0.3 (0.2)	−2 (2)		0.80	0.30
6.2	−0.4 (0.3)	−3 (2)		0.80	0.30
6.6	−0.5 (0.4)	−3 (3)		0.70	0.40
7	−0.5 (0.5)	−4 (4)		0.70	0.40
$\log k = a \log P + b \log \delta_i + c$ (model II, Eq. (4))					
2	0.30 (0.12)	0.00 (0.02)	1.1 (0.2)	0.96	0.04
3	0.31 (0.17)	−0.01 (0.03)	1.0 (0.2)	0.91	0.05
3.5	0.3 (0.2)	−0.03 (0.04)	0.9 (0.3)	0.90	0.07
4	0.3 (0.3)	−0.09 (0.05)	0.3 (0.4)	0.92	0.08
5	0.3 (0.3)	−0.23 (0.08)	−0.9 (0.6)	0.96	0.10
5.4	0.3 (0.3)	−0.32 (0.07)	−1.6 (0.5)	0.98	0.08
5.8	0.29 (0.06)	−0.43 (0.02)	−2.62 (0.15)	0.9994	0.02
6.2	0.26 (0.08)	−0.54 (0.03)	−3.6 (0.2)	0.9991	0.02
6.6	0.30 (0.08)	−0.66 (0.03)	−4.5 (0.3)	0.9992	0.02
7	0.26 (0.07)	−0.80 (0.04)	−5.8 (0.3)	0.993	0.02
$\log k = a \log P + b\alpha + c$ (model III, Eq. (5))					
2	0.30 (0.11)	1 (8)	0 (8)	0.96	0.03
3	0.30 (0.13)	0.6 (1.0)	0.5 (1.0)	0.95	0.04
3.5	0.31 (0.12)	0.6 (0.4)	0.5 (0.3)	0.97	0.04
4	0.30 (0.08)	0.77 (0.14)	0.34 (0.12)	0.992	0.03
5	0.3 (0.2)	0.9 (0.2)	0.2 (0.2)	0.98	0.08
5.4	0.3 (0.2)	1.1 (0.2)	0.04 (0.16)	0.990	0.07
5.8	0.30 (0.05)	1.25 (0.05)	−0.12 (0.04)	0.9995	0.017
6.2	0.26 (0.03)	1.34 (0.02)	−0.20 (0.02)	0.99990	0.009
6.6	0.29 (0.05)	1.35 (0.04)	−0.23 (0.03)	0.9997	0.015
7	0.27 (0.08)	1.32 (0.07)	−0.21 (0.06)	0.9991	0.02

ts = 95% confidence intervals for the coefficients; r^2 = squared product-moment correlation coefficient; SE = standard error of regression.

As can be observed, model III in general provides better results than models I and II. However models I and II gave adequate results probably due to the proximity between the log K values of compounds and the pH of the mobile phase. On the other hand, the similarity between the fitting parameters values, a and b, obtained from model II indicates that for these compounds and in these chromatographic conditions, the contribution of the hydrophobicity and the charge of the compounds to the retention is similar, which justifies the agreement between the statistics obtained from models I and II. In model III, the fitting parameters related with the α values are negatives, indicating that the presence of charged compounds systematically decrease the retention.

The retention of local anesthetics was also measured at pH 3.5 using 0.04 M Brij 35 as mobile phase. At this pH, all compounds are essentially in cationic form and their retention was very low. At pH 3.5, model III also provided the best results (correlation coefficient values were 0.84, 0.91 and 0.93 for Eqs. (1), (4), (5), respectively).

Table 6
Statistical analysis of the linear regressions for local anesthetics

	a (ts_a)	b (ts_b)	c (ts_c)	r^2	SE
$\log k = a \log P_{app} + b$ (model I, Eq. (1))					
Brij 35, 0.02 *M*	0.53 (0.03)	0.65 (0.06)		0.98	0.05
Brij 35, 0.04 *M*	0.48 (0.10)	0.6 (0.2)		0.97	0.08
Brij 35, 0.06 *M*	0.44 (0.03)	0.54 (0.08)		0.97	0.08
$\log k = a \log P + b \log \delta_i + c$ (model II, Eq. (4))					
Brij 35, 0.02 *M*	0.53 (0.03)	0.51 (0.06)	0.62 (0.03)	0.98	0.05
Brij 35, 0.04 *M*	0.46 (0.13)	0.4 (0.3)	0.5 (0.3)	0.97	0.09
Brij 35, 0.06 *M*	0.44 (0.03)	0.40 (0.08)	0.51 (0.10)	0.97	0.08
$\log k = a \log P + b\alpha + c$ (model III, Eq. (5))					
Brij 35, 0.02 *M*	0.59 (0.05)	−1.4 (0.2)	1.13 (0.14)	0.97	0.07
Brij 35, 0.04 *M*	0.47 (0.07)	−1.0 (0.4)	1.0 (0.2)	0.990	0.05
Brij 35, 0.06 *M*	0.44 (0.02)	−1.03 (0.14)	0.97 (0.11)	0.990	0.05

3.4. Log k–log P relationships for diuretics

Diuretics are compounds with a wide variety of chemical structures, very different physico–chemical properties and protonation constants (see Table 3). Acetazolamide, bendroflumetazide, chlortalidone, hydrochlorothiazide, and xipamide are weak acids; bumetanide (BU), ethacrinic acid (EA), furosemide (FURO) and probenecid are diuretics with a strong acidic character, and they are negative charged at pH 7; amiloride and trianterene are basic compounds, and spironolactone is nonionic (neutral). For calculations the protonation constants of the carboxylate groups of FURO, EA and BU in SDS micellar medium was considered. The retention factors of these diuretics eluted using 0.1 *M* SDS at different pH values (pH 3.3–6.8) [28], together with the log *P* values and the corresponding log δ_i and α values were adjusted to models I, II and III. Table 7 shows the regression statistics obtained.

As can be observed, model I provided the worst results ($r^2 < 0.6$). The use of model II improved the correlations but the results were not adequate yet ($r^2 < 0.8$). As can be observed the a and b coefficients obtained from model II were very different, which suggests that the contribution of log *P* and log δ_i values to the retention are different. This fact could explain the poor results obtained using model I.

Model III provided adequate results ($r^2 = 0.9$) at all pH values studied, and the a coefficients were constants at all pH values. This behavior suggests that for compounds with different net charge only model III seems to be valid. Models I and II only considers that retention is due to hydrophobic forces and the ionization only decreases retention. However, model III considers that the retention depends

Table 7
Statistical analysis of the linear regressions for the diuretics

pH	a (ts_a)	b (ts_b)	c (ts_c)	r^2	SE
$\log k = a \log P_{app} + b$ (model I, Eq. (1))					
3.31	0.08 (0.11)	0.6 (0.3)		0.20	0.40
4.8	0.12 (0.13)	0.6 (0.3)		0.30	0.40
5.36	0.13 (0.14)	0.5 (0.3)		0.30	0.40
6.02	0.22 (0.16)	0.2 (0.3)		0.50	0.35
6.81	0.30 (0.17)	0.2 (0.6)		0.60	0.40
$\log k = a \log P + b \log \delta_i + c$ (model II, Eq. (4))					
3.31	0.24 (0.08)	−0.07 (0.08)	0.3 (0.2)	0.80	0.20
4.8	0.21 (0.11)	−0.09 (0.17)	0.3 (0.3)	0.70	0.30
5.36	0.18 (0.14)	0 (0.2)	0.3 (0.4)	0.50	0.35
6.02	0.23 (0.15)	0.5 (0.4)	0.4 (0.4)	0.65	0.30
6.81	0.25 (0.13)	0.5 (0.20)	0.3 (0.3)	0.80	0.30
$\log k = a \log P + b\alpha + c$ (model III, Eq. (5))					
3.31	0.25 (0.07)	0.4 (0.3)	0.3 (0.2)	0.90	0.17
4.8	0.25 (0.08)	0.4 (0.3)	0.2 (0.2)	0.90	0.18
5.36	0.26 (0.07)	0.7 (0.3)	0.3 (0.2)	0.90	0.17
6.02	0.25 (0.08)	0.8 (0.3)	0.3 (0.2)	0.90	0.19
6.81	0.23 (0.09)	1.1 (0.3)	0.5 (0.2)	0.90	0.20

on the hydrophobicity and electrostatic forces that can be attractive or repulsive and can increase or decrease retention, this information are included in the α values.

3.5. Log k–log P relationships for OPA–NAC amino acids derivatives

OPA reacts with the primary amino group of amino acids in the presence of NAC to form 1-alkylthio-2-alkyl substituted isoindole derivatives (OPA–NAC derivatives). The retention data, in the pH range 3.1–3.6, used in this study were taken from a previous paper [29], where the OPA–NAC derivatives of the proteic amino acids (Table 4) were separated in a C_{18} column with SDS and different mobile phase pH. The OPA–NAC derivatives of amino acids only differ in the nature of the R_1 substituents. Electrostatic interactions between the derivatives and charged surfactant only should be possible for the α-carboxylate group of the amino acid and for those amino acids with R_1 containing an ionizable group (i.e. arginine, histidine and lysine which present an amine group, aspartic acid and glutamic acid which present a carboxylic group, tyrosine which contains a phenol group and cysteine with a thiol group).

Table 8 shows the regression statistics obtained for models I, II and III. As can be observed, model I provided the worst results at all pH values studied ($r^2<0.3$). When model II was applied, the correlations were improved, but the correlation coefficients were still very low ($r^2<0.6$) and the fitting parameters were statistically nonsignificant. In contrast, model III provided adequate results in all cases ($0.90<r^2<0.94$). This behavior suggests, as in the case of diuretics, that for compounds with different net charge only model III seems to be valid.

In addition, the fitting parameters were statistically significant, and the fitting parameter '*a*' related with the hydrophobicity remained practically constant, indicating that, as expected, the contribution of hydrophobicity to the retention did not vary when the mobile phase pH was varied.

4. Conclusions

Some conclusions can be obtained form the results shown above. In all cases model III provided adequate results ($r^2>0.9$) better than those obtained

Table 8
Statistical analysis of the linear regressions for the amino acids

pH	a (ts_a)	b (ts_b)	c (ts_c)	r^2	SE
$\log k=a \log P_{app}+b$ (model I, Eq. (1))					
3.1	0.2 (0.2)	0.7 (0.4)	0.30		0.40
3.2	0.2 (0.2)	0.7 (0.5)	0.20		0.40
3.45	0.2 (0.3)	0.4 (0.8)	0.30		0.50
3.5	0.2 (0.2)	0.3 (0.5)	0.20		0.50
3.6	0.2 (0.3)	0.2 (0.7)	0.11		0.55
$\log k=a \log P+b \log \delta_i+c$ (model II, Eq. (4))					
3.1	0.30 (0.17)	−1 (1)	0.6 (0.3)	0.60	0.30
3.2	0.3 (0.2)	−1 (1)	0.6 (0.4)	0.50	0.30
3.45	0.4 (0.5)	−2 (6)	0.3 (0.9)	0.40	0.50
3.5	0.3 (0.3)	−1 (2)	0.2 (0.5)	0.30	0.50
3.6	0.3 (0.4)	−1 (2)	−0.1 (0.8)	0.30	0.50
$\log k=a \log P+b\alpha+c$ (model III, Eq. (5))					
3.1	0.32 (0.09)	0.7 (0.2)	0.58 (0.17)	0.90	0.16
3.2	0.34 (0.08)	0.73 (0.17)	0.62 (0.16)	0.94	0.12
3.45	0.39 (0.11)	0.9 (0.2)	0.5 (0.2)	0.94	0.14
3.5	0.29 (0.11)	0.9 (0.3)	0.5 (0.2)	0.90	0.20
3.6	0.33 (0.15)	1.0 (0.3)	0.3 (0.3)	0.90	0.20

with models I and II and, the results obtained with model II were better than those corresponding to model I.

A limitation of model I is that it considers the contributions of the hydrophobicity and the charge of the compounds to the retention to be the same. When these contributions are different, i.e., for catecholamines, diuretics and OPA–NAC amino acids derivatives, poor correlations with model I are obtained. In addition, for pH values far from log K values of compounds nonsense values of log K could be predicted with model I, i.e., when pH $\ll$ log K, log P_{app} decreases systematically as the pH decreases, while in this case the retention is independent of the mobile phase pH.

For compounds that show the same sign of charge, i.e, catecholamines and local anesthetics, the results obtained with models II and III are equivalent. However, for compounds with different net charge (diuretics and amino acids) only model III provided adequate correlations. In addition the fitting parameter 'a' in model III always remains constant for a series of compounds. This behaviour indicates that the contribution of hydrophobicity to the retention does not vary when mobile phase pH is varied. That is, model III isolates correctly the hydrophobicity contribution of compounds to the retention.

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