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## Retention of ionizable compounds in high-performance liquid chromatography

### IX. Modelling retention in reversed-phase liquid chromatography as a function of pH and solvent composition with acetonitrile–water mobile phases<sup>☆</sup>

Sonia Espinosa, Elisabeth Bosch, Martí Rosés\*

*Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain*

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#### Abstract

The influence of pH and solvent composition of acetonitrile–water mobile phases on the retention of acids and bases on a polymeric stationary phase is studied. Very good relationships between retention and mobile phase pH are obtained if the pH is measured in the proper pH scale. The fit of retention to pH for a particular solvent composition provides the  $pK_a$  values of the equilibria between the different acid–base species and the retention parameters of these species at this solvent composition. Several models are tested that relate these parameters to solvent composition and properties in order to propose a general model to predict retention for any mobile phase pH and composition. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Retention models; Mobile phase composition; pH effects

#### 1. Introduction

Development and optimization of analytical separation methods by liquid chromatography require the

manipulation of retention and selectivity through variation of suitable parameters [1–7]. In reversed-phase liquid chromatography, manipulation is usually performed through modification of the composition of the mobile phase. This can be achieved by change of the type and percentage of the organic modifier, and for ionizable solutes, variation of the pH of the mobile phase. This is not straightforward because variation of the organic modifier percentage also causes variation of the pH of the mobile phase.

In previous publications [8–14], we have studied the relationships between retention of acids and

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\*Corresponding author. Tel.: +34-93-4021-796; fax: +34-93-402-1233.

E-mail address: marti@apolo.qui.ub.es (M. Rosés).

bases on reversed-phase stationary phases and the pH of the mobile phase, measured on different pH scales. We have also noted the importance of proper pH measurement of the mobile phase and studied the relationships between the measured pH and the different pH scales, defined according to IUPAC recommendations [15]. Moreover, we have demonstrated that good relationships between retention and mobile phase pH of general validity for any solute and buffering solution can be only achieved when the pH is measured in the actual mobile phase used, i.e., after mixing the aqueous buffer with the organic modifier [11–13].

In this paper, we test the relationships between retention and the different pH scales for a variety of acids and bases on a polymeric stationary phase with acetonitrile–water mobile phases (20%, 40% and 60%, v/v, of acetonitrile) at different pH values. Moreover, we extend the relationships to variation of the composition of the mobile phase, since change of the acetonitrile percentage implies variation of retention of the neutral and ionic forms of each solute and variation of its  $pK_a$  value. We test several equations and parameters to fit the variations in order to optimize the model.

## 2. Theory

Retention models of ionizable solutes in liquid chromatography as a function of pH and solvent composition have been reviewed by Schoenmakers and co-workers [1,2]. For a particular mobile phase composition (percentage of organic modifier), the relationship between retention and mobile phase pH is based on the acid–base equilibria between the different species of the solute and the distribution equilibria of these species between mobile and stationary phases.

The overall retention factor ( $k$ ) of a solute with an unique acid–base equilibrium can be given as an average of the retention factors of the two species ( $k_{HA}$  and  $k_A$ ) according to the mole fraction of each species ( $\alpha_{HA}$  and  $\alpha_A$ ) at the mobile phase pH [1–6,9,11–13,16–19]:

$$k = \alpha_{HA}k_{HA} + \alpha_Ak_A \quad (1)$$

which leads to the following relationship between retention and pH:

$$k = (k_{HA} + k_A 10^{pH - pK'_a}) / (1 + 10^{pH - pK'_a}) \quad (2)$$

where  $pK'_a$  is the  $pK_a$  value in terms of the concentration of the two species, instead of activities, i.e.:

$$\begin{aligned} pK'_a &= -\log K'_a = -\log a_H[A]/[HA] \\ &= -\log K_a \gamma_{HA} / \gamma_A \end{aligned} \quad (3)$$

where  $\gamma_{HA}$  and  $\gamma_A$  are the activity coefficients of the acidic (HA) and basic (A) forms of the solute, which are usually estimated by the Debye–Hückel equation.

In fact, retention factors should be replaced by distribution constants in Eqs. (1) and (2) because the latter are the true thermodynamic constants of the distribution equilibria. However, if the mobile phase volume remains constant with pH variation, the retention factor is proportional to the distribution constant and since the former is measured more easily, Eq. (2) is usually written in terms of retention factor. The assumption that the mobile phase volume is constant for all pH values implies that Eqs. (1) and (2) can be also written in terms of other parameters linearly related to  $k$ , such as retention time or volume.

For a solute involved in multiple acid–base equilibria, Eq. (1) should be written as:

$$k = \sum \alpha_i k_i \quad (4)$$

and an equation similar to Eq. (2), with as many terms in the numerator and the denominator as there are species, is derived to relate retention to pH.

In order to obtain good relationships between retention and pH, a crucial point is the practical measurement of pH. Three different pH scales can be used to relate retention to mobile phase pH: the pH scale in water ( $^w\text{pH}$ ), the pH scale in the mobile phase with water as standard state solvent ( $^s\text{pH}$ ) and the pH scale in the mobile phase with the actual mobile phase as standard state solvent ( $^s\text{pH}$ ) [11,12]. In practice, the three scales agree with the measurement of pH in the aqueous buffer before mixing with the organic modifier ( $^w\text{pH}$ ) and measurement of pH in the mobile phase with calibration of the electrode system with aqueous buffers ( $^s\text{pH}$ ) or with buffers

in the same mobile phase ( $^s\text{pH}$ ), provided that an electrode system with a negligible residual liquid junction potential is used [11,12].

Although the unique rigorous pH scales recommended by IUPAC [15] for pH measurements in non-aqueous and mixed solvents are  $^s\text{pH}$  and  $^w\text{pH}$ , the most used scale in liquid chromatography has been  $^w\text{pH}$ . However, in this instance the  $\text{p}K'_a$  parameter of Eq. (2) has no physical meaning [1]. We have demonstrated that good relationships between retention and pH are obtained only when buffers of the same type are used over the pH range studied [11–13].

In contrast, Eq. (2) correlates retention and pH well for all kinds of buffers if the pH is measured in the mobile phase ( $^w\text{pH}$  or  $^s\text{pH}$  scales), instead of measuring it in the aqueous buffers. In addition, the  $\text{p}K'_a$  values obtained in these fits are the  $\text{p}K$  values valid in the solvent medium (mobile phase), either in the  $^w\text{pH}$  scale ( $^w\text{p}K'_a$ ) or the  $^s\text{pH}$  scale ( $^s\text{p}K'_a$ ) [11,12].

For a given compound, the fitting parameters of Eq. (2) ( $k_{\text{HA}}$ ,  $k_A$  and  $\text{p}K'_a$ ) change with the composition of the mobile phase. There is not yet a general model established for these variations. For moderately large solvent composition ranges the model:

$$\log k = A + Bv \quad (5)$$

fits the data very well [1,2], where  $v$  is the volume fraction of the organic modifier in the mobile phase. Over an extended mobile phase composition range, a quadratic exponential model is more satisfactory [1,2].

Eq. (5) is applied to the retention factors of both acid–base species (i.e., to  $k_{\text{HA}}$  and  $k_A$ ). Therefore, two sets of  $A$  and  $B$  parameters are required for each acid–base solute. We have found that for a wide set of solutes the ratio ( $f$ ) of the retention factor of the ionized form of the solute ( $k_{\pm}$ ) and the retention factor of the neutral ( $k_0$ ) form is rather constant [16], i.e.:

$$\log f = \log k_{\pm} - \log k_0 \quad (6)$$

If this ratio remains constant for the solutes and conditions tested here, the model would become simplified, because the retention of the ionic form

could be easily calculated from that of the neutral form of the solute.

The model defined by Eq. (5) and others with higher order polynomials are not the unique models used to fit retention to mobile phase composition. In particular, we have proposed a new solvent parameter,  $P_m^N$ , to measure the polarity of the mobile phase and good relationships were obtained between  $\log k$  and this parameter [20]:

$$\log k = q + pP_m^N \quad (7)$$

where  $q$  and  $p$  are constants depending on the solute. However, it was found that the constants were interrelated and a general equation which depends only on one solute parameter,  $p$ , one mobile phase parameter,  $P_m^N$ , and two stationary phase constant parameters,  $(\log k)_0$  and  $P_s^N$ , could be established [20]:

$$\log k = (\log k)_0 + p(P_m^N - P_s^N) \quad (8)$$

where  $P_s^N$  is a fitting parameter that measures the polarity of the stationary phase.

Equations were also established to relate  $P_m^N$  to the mobile phase composition for methanol–water and acetonitrile–water. For the latter the relationship is:

$$P_m^N = 1.00 - 2.13v/(1 + 1.42v) \quad (9)$$

The  $P_m^N$  parameter was derived from relationships between  $\log k$  and the Dimroth–Reichardt  $E_T(30)$  parameter of the mobile phase. It has been pointed out that plots of  $\log k$  vs. the  $E_T(30)$  parameter are, in general, more linear than plots of  $\log k$  vs.  $v$  [6,21–24].

The variation of  $\text{p}K'_a$  with solvent composition is more complex because of the preferential solvation of the solute by one of the two solvent components of the mobile phase. We have proposed several models to account for this preferential solvation. The models were derived for polarity and hydrogen bond parameters of solvent mixtures [25] and were later extended to  $\text{p}K_a$  values [9,26,27]. The general model has five fitting parameters and would require measurements at a minimum of five different mobile phase compositions, which is quite time consuming. However, simplified models can be applied in many instances. We shall test here linear relationships of

the type of Eqs. (5) and (7) between  $pK_a$  and solvent composition or polarity  $P_m^N$  of the mobile phase, i.e.:

$$pK_a = A_{pK} + B_{pK}v \quad (10)$$

$$pK_a = q_{pK} + p_{pK}P_m^N \quad (11)$$

where  $A_{pK}$  and  $B_{pK}$  are the intercept and slope, respectively, of the  $pK_a$  vs.  $v$  plot and  $q_{pK}$  and  $p_{pK}$  the intercept and slope, respectively, of the  $pK_a$  vs.  $P_m^N$  plot. The two equations are tested in order to decide which one gives the best straight line.

### 3. Experimental

#### 3.1. Apparatus

pH measurements were taken with a Ross combination electrode Orion 8102 (glass electrode and a reference electrode with a 3.0 M KCl solution in water as a salt bridge) in a Crison micropH 2002 potentiometer with a precision of  $\pm 0.1$  mV ( $\pm 0.002$  pH units). The retention data were measured in a 15 cm  $\times$  4.6 mm I.D. Polymer Labs. PLRP-S 100 Å column (15–20  $\mu$ m) with a flow-rate of 1 ml/min in an Isco (Lincoln, NE, USA) Model 2350 dual-pump system with a 20  $\mu$ l loop valve. A Shimadzu (Kyoto, Japan) Model SPD-10Avp UV–Vis detector was used set at 254 nm for the acids and the bases, 282 nm for the phenols and 200 nm for potassium

bromide (0.01%), which was used as the hold-up time marker. All data were taken by triplicate at 25 °C with the potentiometric cell and the column thermostated with water jackets. Flow rate was 1 ml/min for the 40% and 60% acetonitrile and 3 ml/min for the 20% acetonitrile mobile phases.

#### 3.2. Chemicals

Acetonitrile was HPLC grade from Merck and water purified by the Milli-Q plus system from Millipore. Other chemicals were reagent grade or better and obtained from Fluka, Aldrich, Merck or Carlo Erba.

#### 3.3. Procedure

The mobile phases were prepared by mixing the aqueous buffers described in Table 1 with acetonitrile, at 20%, 40% and 60% (v/v) of organic solvent. The buffers were the same used in previous work [12]. In order to measure the mobile phase pH, the electrode system was calibrated using the usual aqueous standard reference buffers of potassium hydrogenphthalate ( $^w\text{pH}=4.00$ ) and potassium dihydrogenphosphate–disodium hydrogenphosphate ( $^w\text{pH}=7.02$ ). Then, the pH of the aqueous HPLC buffer was measured before and after mixing it with the organic modifier, obtaining the  $^w\text{pH}$  and the  $^s\text{pH}$  values, respectively.  $^s\text{pH}$  was calculated by subtracting the  $\delta$  term from the  $^w\text{pH}$  value. The  $\delta$  term is the

Table 1  
pH values of the studied buffers measured on different scales

Aqueous buffer	$^w\text{pH}$	Acetonitrile (% , v/v)					
		20		40		60	
		$^s\text{pH}$	$^s\text{pH}$	$^s\text{pH}$	$^s\text{pH}$	$^s\text{pH}$	$^s\text{pH}$
(A) 0.01 M $\text{H}_3\text{PO}_4$	2.00	2.07	2.10	2.20	2.34	2.24	2.70
(B) $6.40 \cdot 10^{-3}$ M $\text{H}_3\text{Cit}$ – $3.60 \cdot 10^{-3}$ M $\text{KH}_2\text{Cit}$	3.00	3.24	3.27	3.53	3.67	3.77	4.23
(C) $9.35 \cdot 10^{-3}$ M $\text{KH}_2\text{Cit}$ – $6.52 \cdot 10^{-3}$ M $\text{KNaHCit}$	3.99	4.31	4.34	4.70	4.84	5.13	5.59
(D) $3.46 \cdot 10^{-3}$ M $\text{HAc}$ – $6.54 \cdot 10^{-3}$ M $\text{NaAc}$	5.00	5.38	5.41	5.99	6.13	6.35	6.81
(E) $5.81 \cdot 10^{-3}$ M $\text{KNaHCit}$ – $4.19 \cdot 10^{-3}$ M $\text{Na}_3\text{Cit}$	6.01	6.49	6.52	6.89	7.03	7.11	7.57
(F) $5.22 \cdot 10^{-3}$ M $\text{KH}_2\text{PO}_4$ – $4.78 \cdot 10^{-3}$ M $\text{Na}_2\text{HPO}_4$	7.01	7.43	7.46	7.80	7.94	8.02	8.48
(G) $9.44 \cdot 10^{-4}$ M $\text{KH}_2\text{PO}_4$ – $9.06 \cdot 10^{-3}$ M $\text{Na}_2\text{HPO}_4$	8.02	8.41	8.44	8.62	8.76	8.99	9.45
(H) $5.95 \cdot 10^{-3}$ M $\text{H}_3\text{BO}_3$ – $4.05 \cdot 10^{-3}$ M $\text{NaH}_2\text{BO}_3$	9.01	9.65	9.68	10.25	10.39	10.74	11.20
(I) $7.84 \cdot 10^{-3}$ M $\text{BuNH}_3^+$ – $2.16 \cdot 10^{-3}$ M $\text{BuNH}_2$	10.03	9.78	9.81	9.52	9.66	9.36	9.82
(J) $1.64 \cdot 10^{-3}$ M $\text{BuNH}_3^+$ – $8.36 \cdot 10^{-3}$ M $\text{BuNH}_2$	11.01	10.84	10.87	10.73	10.87	10.42	10.88
(K) 0.01 M $\text{Na}_3\text{PO}_4$	12.04	12.38	12.41	12.70	12.84	13.19	13.65

operational difference between the two pH scales ( $^s_w\text{pH}$  and  $^s\text{pH}$ ). It includes the primary medium effect for hydrogen ion ( $-\log {}^s_w\gamma_{\text{H}}^0$ ), which is directly related to the Gibbs energy of transfer of  $\text{H}^+$  from water to the acetonitrile–water mixture, and also the difference of the liquid-junction potentials ( $\bar{E}_j$ ) between the two solvents expressed in pH units. Previous studies [11–13] have shown that the use of appropriate salt bridges containing a solution of an equitransferent binary salt (e.g., KCl) at a much higher concentration than the sample and standard solutions, as recommended by the IUPAC [15], minimizes the residual liquid-junction potential  $\bar{E}_j$ . In this instance,  $\delta$  agrees with the hydrogen ion medium effect and it is a general parameter that depends only on the solvent composition. The  $\delta$  (or  $-\log {}^s_w\gamma_{\text{H}}^0$ ) values for acetonitrile–water mixtures up to 60% in volume were determined in a previous work [12]. Table 1 presents the different pH values for each mobile phase.

## 4. Results and discussion

### 4.1. Variation of retention with mobile phase pH

The retention volumes of several compounds with acid–base properties in the studied polymeric column were measured at the pH values of Table 1 with 20, 40 and 60% of acetonitrile as mobile phase. The studied set included benzoic acid, phenol, pyridine and amine derivatives. The retention volumes were fitted to the pH of the mobile phase through an equation analogous to Eq. (2) in terms of retention volumes, i.e.:

$$V_{\text{R}} = [V_{\text{R(HA)}} + V_{\text{R(A)}} 10^{\text{pH} - \text{pK}'_{\text{a}}}] / (1 + 10^{\text{pH} - \text{pK}'_{\text{a}}}) \quad (12)$$

The pH was measured on the three scales already explained:  $^w\text{pH}$ ,  $^s_w\text{pH}$  and  $^s\text{pH}$ . Figs. 1 and 2 present the plots obtained for some representative solutes on the  $^s_w\text{pH}$  and  $^w\text{pH}$  scales. When the pH is measured in the mobile phase after mixing the aqueous buffer with acetonitrile, good fits are obtained for all compounds and pH points, as it can be observed in Fig. 1 for the  $^s_w\text{pH}$  scale. The same good fits are obtained for the  $^s\text{pH}$  scale, because there is a

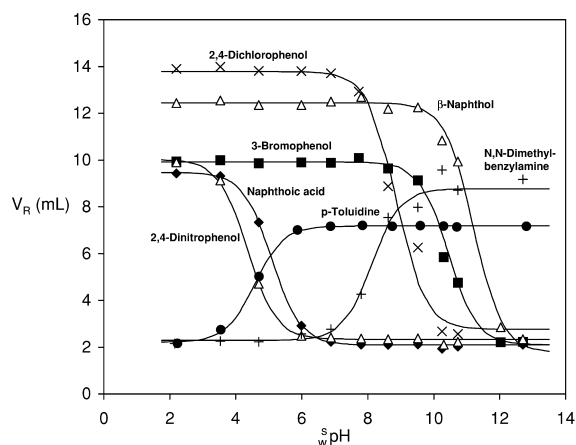


Fig. 1. Fit of the retention volumes of several acid–base solutes in 40% acetonitrile to the pH of the mobile phase measured after mixing the aqueous buffer and the organic modifier and calibrating the glass electrode with aqueous buffers (Eq. (12)).

constant difference between both scales for each acetonitrile percentage. This differences ( $^s_w\text{pH} - ^s\text{pH}$ ) are  $-0.03$ ,  $-0.14$  and  $-0.46$  for 20, 40 and 60% of acetonitrile, respectively [12].

With the common procedure of measuring the pH of the aqueous buffers before mixing it with acetonitrile ( $^w\text{pH}$  scale) good fits are obtained if buffers of similar type are used in the pH range studied (pH values close to  $\text{pK}'_{\text{a}}$ ), because the differences between the  $^w\text{pH}$  and  $^s_w\text{pH}$  scales are quite similar for all buffers [11,12]. This can be observed in Fig. 2 for naphthoic acid, 2,4-dinitrophenol and *p*-toluidine, which have  $\text{pK}'_{\text{a}}$  values below 5.00. The buffers covering this pH range have been prepared from neutral or anionic acids [phosphoric acid, citric acid ( $\text{H}_3\text{Cit}$ ), potassium dihydrogen citrate ( $\text{KH}_2\text{Cit}$ ) and acetic acid (Hac)] and their conjugate bases (Table 1), and the  $^s_w\text{pH} - ^w\text{pH}$  differences are positive for all these buffers.

However, the pH-dependent variation of retention for  $\beta$ -naphthol, 2,4-dichlorophenol, 3-bromophenol and *N,N*-dimethylbenzylamine (from 7 to 12 approximately) is covered by buffers prepared from neutral (boric acid), anionic (dihydrogenphosphate) and cationic (butylammonium) acids. The  $^s_w\text{pH} - ^w\text{pH}$  differences for these buffers are quite different, as can be observed in Table 1. The difference is positive for borate and phosphate buffers, but negative for butylamine buffers. Consequently, the fits in

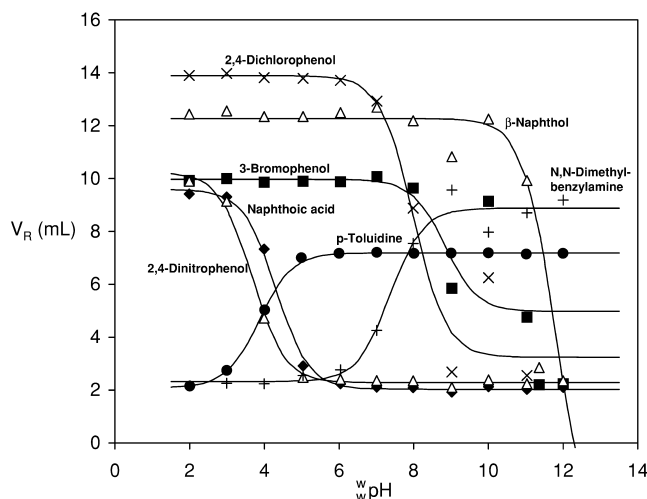


Fig. 2. Fit of the retention volumes of several acid–base solutes in 40% acetonitrile to the pH of the mobile phase measured in the aqueous buffer before mixing it with the organic modifier (Eq. (12)).

Fig. 2 for  $\beta$ -naphthol, 2,4-dichlorophenol, 3-bromophenol and *N,N*-dimethylbenzylamine cannot be as good as those in Fig. 1. These results confirm the recommendation of measuring the pH of the HPLC mobile phase after mixing of the aqueous buffer and the organic modifier [11,12], instead of measuring only the pH of the aqueous buffer ( $^w\text{pH}$  scale). The electrode system may be calibrated with the usual aqueous buffers ( $^s\text{pH}$  scale) or with buffers prepared in the same mobile phase ( $^s\text{pH}$  scale). Because of its convenience and simplicity, we choose to calibrate with the common aqueous buffers, and therefore the  $^s\text{pH}$  scale will be mainly used through all this work.

Table 2 presents the fits obtained in the application of Eq. (12), using the  $^w\text{pH}$  scale, to all acid–base compounds studied. The  $\text{p}K'_a$  values obtained should agree with the thermodynamic  $^s\text{p}K_a$  values of the compounds in each particular mobile phase.

3-Aminophenol has two acid–base equilibria. Since the two  $\text{p}K_a$  values are quite different, the retention of this compound can be fitted to two different Eqs. (12), one for the pH range covered by buffers A–F, and another for the pH range covered by buffers G–K. Alternatively, the whole retention data can be fitted to an unique equation derived from Eq. (4):

$$V_R = [V_{R(\text{H}_2\text{A})}10^{\text{p}K_{a1}' - \text{pH}} + V_{R(\text{HA})} + V_{R(\text{A})}10^{\text{pH} - \text{p}K_{a2}'}] / (10^{\text{p}K_{a1}' - \text{pH}} + 1 + 10^{\text{pH} - \text{p}K_{a2}'} \quad (13)$$

The results presented in Table 2 for 3-aminophenol are based on this equation.

#### 4.2. Variation of retention with mobile phase composition

The retention volumes presented in Table 2 follow the trends expected in reversed-phase liquid chromatography. The neutral species are much more retained than the ionic species and retention decreases with increasing acetonitrile content of the mobile phase. In a previous work [16], we observed a constant ratio ( $f$ ) between the retention factors of the neutral and ionic forms of different compounds studied in the same methanol–water mobile phase (Eq. (6)). We have tested this relationship for the compounds studied in the three acetonitrile–water mobile phases. The retention factors have been calculated from the retention volumes presented in Table 2 with 1.80 ml as the volume of the mobile

Table 2

Retention parameters for the studied solutes obtained using Eqs. (12) and (13) with the  $s_w\text{pH}$  values of the mobile phase (Eq. (13) for 3-aminophenol)

Substance	Acetonitrile (% v/v)														
	20					40					60				
	$V_{R(HA)}$	$V_{R(A)}$	$s_w\text{p}K_a$	SD	$F$	$V_{R(HA)}$	$V_{R(A)}$	$s_w\text{p}K_a$	SD	$F$	$V_{R(HA)}$	$V_{R(A)}$	$s_w\text{p}K_a$	SD	$F$
Naphthoic acid	118.83	2.40	4.41	2.49	1664	9.46	2.07	5.10	0.06	12 178	3.85	1.81	5.80	0.04	2731
2-Nitrobenzoic acid	14.61	2.25	2.92	0.22	1152	3.97	2.02	3.60	0.05	665	2.57	1.80	4.34	0.04	221
3-Nitrobenzoic acid	21.87	2.55	3.91	0.25	3787	4.50	2.05	4.40	0.06	1306	2.72	1.81	5.00	0.04	339
4-Nitrobenzoic acid	25.71	2.64	3.79	0.26	4982	4.70	2.07	4.31	0.07	1108	2.75	1.81	4.93	0.04	317
Benzoic acid	14.76	2.19	4.74	0.23	2428	3.95	2.01	5.30	0.05	1437	2.71	1.80	5.79	0.04	427
Resorcinol	3.93	1.83	10.48	0.05	1111	2.64	1.94	10.99	0.03	683	2.20	1.72	11.46	0.00	7665
Phenol	12.75	2.16	10.77	0.27	773	4.54	1.96	11.55	0.05	1105	2.93	1.80	11.92	0.01	4154
2,4-Dichlorophenol	159.00	5.82	8.15	4.41	1183	13.78	2.61	8.88	0.68	281	4.81	1.82	9.68	0.13	332
2,4-Dinitrophenol	82.11	4.23	4.04	1.38	2095	10.02	2.30	4.37	0.10	4210	4.03	1.92	4.79	0.05	1242
$\beta$ -Naphthol	134.25	7.86	10.24	5.22	374	12.41	2.04	11.18	0.24	809	4.58	1.83	11.62	0.05	1369
2-Nitrophenol	92.04	3.90	7.37	5.79	238	13.49	2.24	7.92	0.23	2605	5.34	1.89	8.74	0.11	871
3,5-Dichlorophenol	237.30	6.48	8.68	2.70	6819	16.85	2.40	9.33	0.95	222	5.30	1.84	9.82	0.12	498
3-Bromophenol	79.50	3.57	9.6	1.26	2903	9.94	2.28	10.32	0.21	837	4.13	1.86	10.79	0.02	4205
4-Chlorophenol	49.95	3.21	10.08	0.96	1615	7.77	2.16	10.76	0.18	493	3.63	1.84	11.20	0.03	1935
<i>m</i> -Cresol	25.89	1.89	11.03	0.72	506	6.02	1.92	11.59	0.07	1898	3.28	1.78	12.19	0.02	1965
3-Aminophenol (phenol)	3.74	1.92	10.84			2.65	1.96	11.43			2.24	1.74	12.35		
3-Aminophenol (amino)	2.34	3.73	4.28	0.09	119	2.00	2.65	3.68	0.04	103	1.87	2.24	3.40	0.00	3948
2,4,6-Trimethylpyridine	2.36	20.31	7.03	0.19	9661	2.13	4.94	6.58	0.04	5037	2.04	3.29	6.11	0.02	2264
4-Chloroaniline	2.61	65.42	3.55	0.98	2175	2.14	10.82	3.11	0.07	5041	2.63	4.78	2.93	0.03	1436
Aniline	2.35	14.60	4.35	0.14	5650	2.10	5.43	3.96	0.05	2271	2.00	3.52	3.57	0.02	2599
<i>N</i> -Ethylaniline	4.89	159.66	4.95	4.08	1252	2.33	23.40	4.57	0.24	5717	1.91	7.79	3.87	0.08	2555
<i>N,N</i> -Dimethylbenzylamine	2.70	44.97	8.51	0.36	13 000	2.30	8.98	8.15	0.39	329	1.96	4.49	7.68	0.06	1242
<i>p</i> -Toluidine	2.52	28.66	4.83	0.29	6686	2.21	7.18	4.58	0.06	5448	2.00	3.99	4.08	0.02	3237
Pyridine	2.14	4.52	4.91	0.03	4175	2.08	2.97	4.61	0.02	1340	1.99	2.61	4.03	0.01	1486
2,6-Dimethylaniline	3.48	70.86	3.57	1.92	682	2.18	12.70	3.22	0.20	1069	1.95	5.41	2.78	0.04	2124

phase (calculated from the hold-up time of potassium bromide with 60% acetonitrile). The results are presented in Table 3: the log  $f$  value is rather constant for each mobile phase. In fact, the differences between the log  $f$  values of the three mobile phase are small compared with the standard deviations and an average value of log  $f = -1.4$  can be given for all compounds and mobile phases.

The constancy of the log  $f$  values simplifies the study of the variation of retention with mobile phase composition, because the study is now limited to the variation of one unique species. The most convenient to study is the variation of the retention of the neutral species. In Table 4, we compare the fits obtained with Eqs. (5) and (7). The fits of log  $k$  to the volume fraction of acetonitrile in the mobile phase (Eq. (5))

are quite good, but the fits to the polarity parameter  $P^N$  (Eq. (7)) are much better.

Since the compounds studied present good correlations with  $P^N$ , we have also tested the application of Eq. (8). The results are presented in Table 4. The best fitting parameters were  $-0.02$  for the polarity of the stationary phase ( $P_s^N$ ) and  $-1.22$  for  $(\log k)_0$ . The polarity of the polymeric stationary phase studied is similar to that of the silica- $C_{18}$  previously studied [17] for which  $P_s^N = 0.00$ .  $(\log k)_0$  would be the retention of any solute in a hypothetical mobile phase with the same polarity as that of the stationary phase ( $P_m^N = P_s^N$ ). In this instance, retention would be only related to the phase ratio  $V_s/V_M$  where  $V_s$  and  $V_M$  are the volumes of the stationary and mobile phases, respectively. Comparison of the statistical

Table 3  
Retention factors of the acid–base forms of the studied solutes

Substance	Acetonitrile (% v/v)								
	20			40			60		
	$k_{HA}$	$k_A$	Log $f$	$k_{HA}$	$k_A$	Log $f$	$k_{HA}$	$k_A$	Log $f$
Naphthoic acid	65.02	0.33	−2.29	4.26	0.15	−1.45	1.14	0.01	−2.31
2-Nitrobenzoic acid	7.12	0.25	−1.45	1.21	0.12	−0.99	0.43	0.00	−
3-Nitrobenzoic acid	11.15	0.42	−1.43	1.50	0.14	−1.03	0.51	0.01	−1.96
4-Nitrobenzoic acid	13.28	0.47	−1.45	1.61	0.15	−1.03	0.53	0.01	−1.98
Benzoic acid	7.20	0.22	−1.52	1.19	0.12	−1.01	0.51	0.00	−
Resorcinol	1.18	0.02	−1.85	0.47	0.08	−0.78	0.22	−0.04	−
Phenol	6.08	0.20	−1.48	1.52	0.09	−1.23	0.63	0.00	−
2,4-Dichlorophenol	87.33	2.23	−1.59	6.66	0.45	−1.17	1.67	0.01	−2.18
2,4-Dinitrophenol	44.62	1.35	−1.52	4.57	0.28	−1.22	1.24	0.07	−1.27
$\beta$ -Naphthol	73.58	3.37	−1.34	5.89	0.13	−1.65	1.54	0.02	−1.97
2-Nitrophenol	50.13	1.17	−1.63	6.49	0.24	−1.42	1.97	0.05	−1.59
3,5-Dichlorophenol	130.83	2.60	−1.70	8.36	0.33	−1.40	1.94	0.02	−1.94
3-Bromophenol	43.17	0.98	−1.64	4.52	0.27	−1.23	1.29	0.03	−1.59
4-Chlorophenol	26.75	0.78	−1.53	3.32	0.20	−1.22	1.02	0.02	−1.66
<i>m</i> -Cresol	13.38	0.05	−2.43	2.34	0.07	−1.55	0.82	−0.01	−
3-Aminophenol (phenol)	1.08	0.07	−1.21	0.47	0.09	−0.73	0.24	−0.03	−
3-Aminophenol (amino)	0.30	1.08	−0.56	0.11	0.47	−0.63	0.04	0.24	−0.80
2,4,6-Trimethylpyridine	0.31	10.28	−1.52	0.18	1.74	−0.98	0.13	0.83	−0.79
4-Chloroaniline	0.45	35.34	−1.90	0.19	5.01	−1.42	0.46	1.66	−0.56
Aniline	0.31	7.11	−1.37	0.17	2.02	−1.08	0.11	0.96	−0.93
<i>N</i> -Ethylaniline	1.72	87.70	−1.71	0.29	12.00	−1.61	0.06	3.33	−1.74
<i>N,N</i> -Dimethylbenzylamine	0.50	23.98	−1.68	0.28	3.99	−1.16	0.09	1.49	−1.23
<i>p</i> -Toluidine	0.40	14.92	−1.57	0.23	2.99	−1.12	0.11	1.22	−1.04
Pyridine	0.19	1.51	−0.90	0.16	0.65	−0.62	0.11	0.45	−0.63
2,6-Dimethylaniline	0.93	38.37	−1.61	0.21	6.06	−1.46	0.08	2.01	−1.38
Average			−1.56				−1.17		−1.45
SD			0.37				0.29		0.54

parameters presented in Table 4 (correlation coefficient  $R^2$ , standard deviation of residuals SDs, and  $F$  value for the analysis of variance of the residuals) clearly demonstrates that the fits obtained with this model are not as good as those obtained from Eq. (7), but better than those obtained from Eq. (5). The advantage of the model over the models based on Eqs. (5) and (7) is that it requires only one unique solute parameter ( $p$ ) instead of two. The  $p$  value is a measure of the ability of the solute to interact with the stationary and mobile phases. The larger the  $p$  value, the larger solute retention.

For the variation of the  ${}^s_w pK_a$  values with the mobile phase composition, where  ${}^s_w pK_a$  is equal to the  $pK'_a$  fitting parameter of Eq. (12), Eqs. (10) and (11) have been used and the results obtained are presented in Table 5. For this parameter, the best

relationships have been obtained with the mobile phase composition in volume fraction. Relationships of  ${}^s_w pK_a$  with the  $P_m^N$  value of the mobile phase are worse for most compounds. Also, these relationships cannot be converted to the one solute parameter model similar to that of Eq. (8). Therefore, the linear model of  ${}^s_w pK_a$  vs. mobile phase composition (volume) has been selected. The plot obtained is similar to that presented by Sarmini and Kenndler [28]. These authors found that the plot of  ${}^s_w pK_a$  (apparent  $pK_a$  in their terminology) of substituted benzoic acids vs. the volume percentage of acetonitrile is close to linearity in the 20–80% acetonitrile range, although it curves for lower acetonitrile percentages. The slope ( $B$ ) of the correlations is positive for neutral acids, negative for neutral bases. This agrees with previous studies [8–12,26–33] that demonstrate



Table 4

Correlations of  $\log k$  values of the uncharged forms of the studied solutes with the mobile phase composition ( $v$ ) and polarity ( $P_m^N$ ) according to Eqs. (5), (7), and (8)

Substance	Log $k = A + Bv$ (5)					Log $k = q + pP_m^N$ (7)					Log $k = -1.22 + p(P_m^N + 0.02)$ (8)			
	A	B	R <sup>2</sup>	SD	F	q	p	R <sup>2</sup>	SD	F	p	R <sup>2</sup>	SD	F
Naphthoic acid	2.59	-4.39	0.961	0.25	25	-1.54	4.96	0.991	0.12	110	4.21	0.966	0.16	57
2-Nitrobenzoic acid	1.41	-3.05	0.977	0.13	43	-1.45	3.43	0.998	0.04	436	2.88	0.970	0.11	66
3-Nitrobenzoic acid	1.65	-3.35	0.971	0.16	33	-1.49	3.77	0.995	0.07	206	3.14	0.965	0.13	55
4-Nitrobenzoic acid	1.75	-3.50	0.969	0.18	32	-1.54	3.94	0.995	0.07	186	3.23	0.959	0.14	47
Benzoic acid	1.37	-2.88	0.960	0.17	24	-1.34	3.26	0.990	0.08	103	2.92	0.979	0.09	92
Resorcinol	0.42	-1.82	0.996	0.03	236	-1.27	2.02	0.999	0.02	697	1.86	0.991	0.03	233
Phenol	1.24	-2.47	0.985	0.09	66	-1.06	2.76	1.000	0.01	2537	2.96	0.994	0.04	340
2,4-Dichlorophenol	2.71	-4.29	0.971	0.21	33	-1.32	4.83	0.995	0.09	206	4.50	0.990	0.09	197
2,4-Dinitrophenol	2.36	-3.89	0.976	0.17	41	-1.29	4.37	0.997	0.06	357	4.10	0.993	0.07	282
$\beta$ -Naphthol	2.62	-4.20	0.970	0.21	32	-1.32	4.72	0.995	0.09	196	4.39	0.990	0.09	190
2-Nitrophenol	2.34	-3.52	0.978	0.15	44	-0.95	3.95	0.998	0.05	450	4.31	0.988	0.08	171
3,5-Dichlorophenol	2.94	-4.57	0.969	0.23	32	-1.35	5.14	0.995	0.10	189	4.73	0.988	0.10	162
3-Bromophenol	2.32	-3.81	0.973	0.18	36	-1.25	4.28	0.996	0.07	260	4.09	0.994	0.06	332
4-Chlorophenol	2.07	-3.55	0.975	0.16	39	-1.26	3.99	0.997	0.06	329	3.79	0.994	0.05	348
<i>m</i> -Cresol	1.68	-3.03	0.980	0.12	48	-1.15	3.40	0.998	0.03	630	3.41	0.998	0.02	1254
3-Aminophenol	0.34	-1.61	0.996	0.03	238	-1.16	1.79	0.999	0.02	690	1.84	0.998	0.02	868
2,4,6-Trimethylpyridine	1.48	-2.74	0.947	0.18	18	-1.09	3.10	0.984	0.10	60	3.23	0.981	0.08	106
4-Chloroaniline	2.15	-3.32	0.975	0.15	39	-0.96	3.73	0.997	0.05	325	4.09	0.987	0.08	155
Aniline	1.25	-2.18	0.978	0.09	45	-0.79	2.45	0.998	0.03	487	3.16	0.906	0.14	19
<i>N</i> -Ethylaniline	2.60	-3.55	0.985	0.13	65	-0.72	3.98	1.000	0.02	2264	4.77	0.956	0.15	43
<i>N,N</i> -Dimethylbenzylamine	1.92	-3.01	0.968	0.15	30	-0.91	3.39	0.994	0.07	170	3.86	0.974	0.10	75
<i>p</i> -Toluidine	1.67	-2.72	0.974	0.13	37	-0.89	3.06	0.997	0.05	286	3.58	0.965	0.10	56
Pyridine	0.41	-1.32	0.951	0.08	19	-0.83	1.49	0.986	0.05	69	2.16	0.768	0.13	7
2,6-Dimethylaniline	2.17	-3.20	0.979	0.13	48	-0.83	3.60	0.998	0.04	590	4.20	0.968	0.12	60
Average				0.15					0.06				0.09	

than in water–methanol and water–acetonitrile solvent mixtures, the  $pK_a$  of neutral and anionic acids increases with the organic solvent concentration, whereas the  $pK_a$  of cationic acids (protonated bases) remains rather constant.

Electrostatic and specific interactions contribute to the variation of  ${}^s_w pK_a$  values [26,27,29–31,33]. For a protonated base, the dissociation equilibria do not alter the number and charge of ions and therefore they are not affected by electrostatic interactions. Specific interactions between the solvent and the different solute species determine that in acetonitrile–water and methanol–water mixtures, the  ${}^s_w pK_a$  of protonated bases show a minimum for solvent compositions around 60–80% of organic solvent. However, neutral and anionic acids create more charged species when they dissociate, and the electrostatic interactions, which can be estimated by Born's approach, determine an increase in the  ${}^s_w pK_a$

value when the acid is transferred from water to a lower dielectric constant solvent, such as a methanol–water or acetonitrile–water mixture [26,27,29–31,33].

Finally, we have tested the accuracy of the overall method by calculation of the  $\log k$  values of the compounds at all pH and mobile phase compositions studied and compared them with the experimental values. The  ${}^s_w pK_a$  of each compound and acid–base equilibrium at each mobile phase composition has been calculated through Eq. (10) and the  $A$  and  $B$  parameters of Table 5. The  $\log k$  values of the neutral species at each mobile phase composition have been calculated by Eq. (8) and the constants of Table 4. The  $\log k$  values of the ionic forms have been estimated through Eq. (6). From the  ${}^s_w pK_a$  values and the  $\log k$  values of the species involved, the retention ( $\log k$ ) of the compound at each mobile phase composition and  ${}^s_w pH$  has been calculated by

Table 5

Correlations of the  ${}^s_pK_a$  values of Table 2 with the mobile phase composition ( $v$ ) and polarity ( $P_m^N$ ) according to Eqs. (10) and (11)

Substance	${}^s_pK_a = A_{pK} + B_{pK}v$ (10)					${}^s_pK_a = q_{pK} + P_{pK}P_m^N$ (11)				
	$A_{pK}$	$B_{pK}$	$R^2$	SD	$F$	$q_{pK}$	$P_{pK}$	$R^2$	SD	$F$
Naphthoic acid	3.71	3.47	0.9998	0.01	6440	6.94	-3.84	0.9868	0.11	75
2-Nitrobenzoic acid	2.20	3.55	0.9989	0.03	945	5.49	-3.91	0.9818	0.14	54
3-Nitrobenzoic acid	3.34	2.72	0.9953	0.05	211	5.86	-2.99	0.9708	0.13	33
4-Nitrobenzoic acid	3.20	2.85	0.9963	0.05	271	5.84	-3.13	0.9735	0.13	37
Benzoic acid	4.22	2.62	0.9997	0.01	3675	6.66	-2.91	0.9926	0.06	133
Resorcinol	10.03	2.45	0.9804	0.10	50	12.32	-2.75	0.9986	0.03	706
Phenol	10.23	2.87	0.9890	0.09	90	12.92	-3.21	1.0000	0.00	$2 \cdot 10^5$
2,4-Dichlorophenol	7.37	3.82	0.9988	0.04	867	10.92	-4.21	0.9814	0.15	53
2,4-Dinitrophenol	3.65	1.87	0.9929	0.04	139	5.38	-2.05	0.9653	0.10	28
$\beta$ -Naphthol	9.64	3.45	0.9480	0.23	18	12.89	-3.91	0.9839	0.13	61
2-Nitrophenol	6.64	3.42	0.9853	0.12	67	9.80	-3.74	0.9505	0.22	19
3,5-Dichlorophenol	8.14	2.85	0.9918	0.07	120	10.80	-3.18	0.9999	0.01	7014
3-Bromophenol	9.09	2.97	0.9465	0.20	18	11.89	-3.37	0.9831	0.11	58
4-Chlorophenol	9.59	2.80	0.9511	0.18	19	12.23	-3.17	0.9856	0.10	69
<i>m</i> -Cresol	10.44	2.90	0.9996	0.02	2523	13.13	-3.20	0.9850	0.10	66
3-Aminophenol (phenol)	10.02	3.80	0.9836	0.14	60	13.53	-4.15	0.9475	0.25	18
3-Aminophenol (amino)	4.64	-2.15	0.9610	0.12	25	2.62	2.43	0.9908	0.06	108
2,4,6-Trimethylpyridine	7.49	-2.30	0.9998	0.01	6348	5.36	2.54	0.9867	0.07	74
4-Chloroaniline	3.82	-1.55	0.9446	0.11	17	2.36	1.76	0.9820	0.06	54
Aniline	4.74	-1.95	1.0000	0.00	$5 \cdot 10^{28}$	2.93	2.16	0.9895	0.06	94
<i>N</i> -Ethylaniline	5.54	-2.70	0.9716	0.13	34	3.06	2.93	0.9277	0.21	13
<i>N,N</i> -Dimethylbenzylamine	8.93	-2.08	0.9996	0.01	2296	7.01	2.29	0.9848	0.07	65
<i>p</i> -Toluidine	5.25	-1.88	0.9643	0.10	27	3.52	2.03	0.9166	0.16	11
Pyridine	5.40	-2.20	0.9674	0.11	30	3.38	2.39	0.9212	0.18	12
2,6-Dimethylaniline	3.98	-1.98	0.9957	0.04	231	2.15	2.17	0.9719	0.97	35
Average				0.08					0.14	

means of Eq. (2) (Eq. (4) for 3-aminophenol)). The results obtained are presented in Figs. 3 and 4. Fig. 3 presents the results obtained taking an average value of  $\log f = -1.4$  for the three mobile phases; Fig. 4 shows a different  $\log f$  value for each mobile phase ( $-1.6$ ,  $-1.2$ , and  $-1.4$  for 20, 40 and 60% acetonitrile, respectively). In both plots, the points are more scattered for low retention because there is a larger error in the  $\log k$  calculation and measurement. The approximation of taking an average value of  $\log f$  to calculate the  $\log k$  value of the ionized form contributes also to this scattering, as can be observed by comparison of Fig. 3 with Fig. 4.

## 5. Conclusions

The model proposed to fit retention to mobile

phase pH and composition consists of relating the retention in any isocratic mobile phase to pH through equations of the type of Eqs. (2) and (12) for an unique acid–base equilibrium, or the more general expressions Eqs. (4) or (13) if more than two acid–base species are present. The pH must be measured in the mobile phase, calibrating the electrodes with aqueous standards ( ${}^s_pH$  scale) or with standards prepared in the same mobile phase ( ${}^m_pH$  scale). The  $\log k$  values of the uncharged forms of the solutes are related to mobile phase composition through Eq. (8) which requires an unique parameter for each solute. The  $\log k$  values of the ionized forms can be easily related to the  $\log k$  values of the uncharged forms by the  $\log f$  parameter (Eq. (6)). The  $pK$  values of the solutes are related to solvent composition through Eq. (10). With this model, retention can be related to pH and mobile phase composition

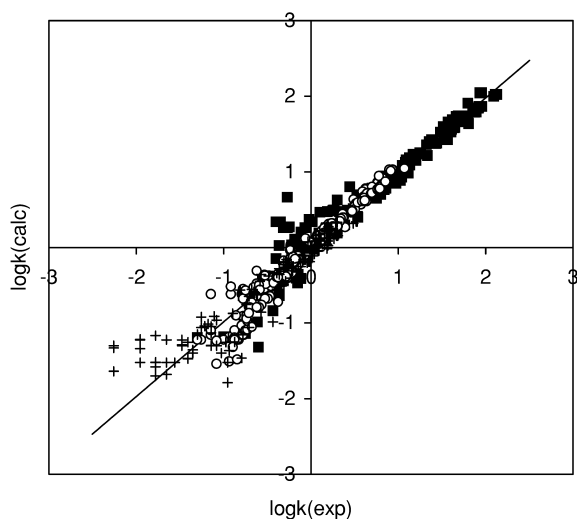


Fig. 3. Plot of  $\log k$  calculated with the proposed model vs.  $\log k$  experimental for the solutes, pH buffers and mobile phase compositions studied. An average value of  $\log f = -1.4$  has been taken for all mobile phases: (■) 20% acetonitrile, (○) 40% acetonitrile, and (+) 60% acetonitrile.

by only three solute parameters ( $A_{pK}$  and  $B_{pK}$  of Eq. (10) and  $p$  of Eq. (8)). Additional parameters which are equal for all solutes studied on the same column with the same organic modifier in the mobile phase

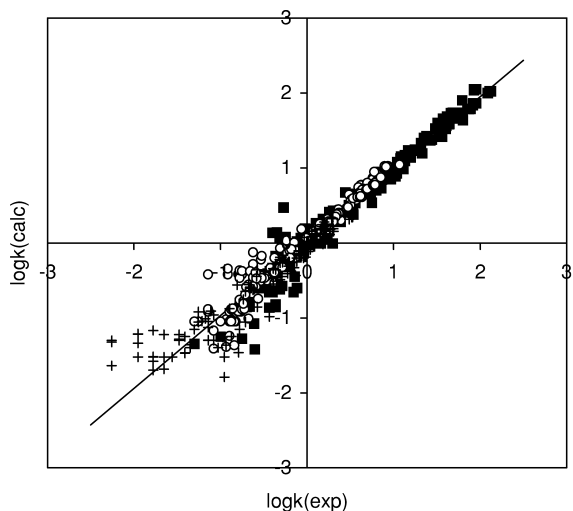


Fig. 4. Plot of  $\log k$  calculated with the proposed model vs.  $\log k$  experimental for the solutes, pH buffers and mobile phase compositions studied. Different  $\log f$  values have been taken for each mobile phase (see text). Mobile phases as in Fig. 3.

are  $(\log k)_0$  and  $P_s^N$  in Eq. (8), the two coefficients of Eq. (9) ( $-2.13$  and  $1.42$  for acetonitrile) and the  $\log f$  parameter of Eq. (6).

This method can be very useful in the development and optimization of liquid chromatographic separations. Retention data of the solutes to be separated should be obtained at several mobile phase pH values, at different mobile phase compositions. Given the linearity of the model, the number of mobile phase compositions studied can be limited to 2–3. Determination of the  $k_{HA}$ ,  $k_A$ , and  $pK_a$  parameters by Eqs. (2) or (12) at each mobile phase composition requires measurements at a minimum of three different mobile phase pH values, although to get parameters accurate enough we recommend to measure retention at 2–3 mobile phase pH values close to the solute  $pK_a$  and at two more pH values, one at least 1 pH unit higher than  $pK_a$  and another at 1 pH unit lower than  $pK_a$ . From these data, the model should provide an accurate estimation of the retention of the solute at any mobile phase pH and composition between the range studied.

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