



## Simultaneous effect of pH, temperature and mobile phase composition in the chromatographic retention of ionizable compounds

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### ABSTRACT

The retention of 22 monoprotic acid–base solutes in 12 buffers (pH from 2 to 12) at 3 temperatures (25, 40 and 55 °C) and in 3 mobile phase compositions (20, 40 and 60% acetonitrile) was measured. The retention data for each solute, temperature and mobile phase compositions was fitted to pH by means of the common sigmoidal equation and the retention and acid–base parameters were obtained ( $\log k_{HA}$ ,  $\log k_A$  and  $pK_a$ ). The dependence of these parameters on temperature (van't Hoff plots), mobile phase composition ( $\phi$ , volume fraction of acetonitrile) and mobile phase polarity ( $P_m^N$  parameter) was investigated. Linear plots of the parameter values against the reverse of the absolute temperature, on one hand, and  $\phi$  or  $P_m^N$ , in the other hand, were generally obtained. From this analysis we propose 6-parameter equations to relate retention to pH and  $T$  at constant mobile phase composition, and to pH and  $\phi$  or  $P_m^N$  at constant temperature. A general 12 parameter equation is also proposed to relate retention simultaneously to pH,  $T$  and  $\phi$  or  $P_m^N$ . The general constancy of some terms of the equations allow to simplify the 12 parameter equation to a 8 parameter equation able to predict retention of the studied solutes. The accuracy of the proposed method provided excellent results with the advantage of modeling the effects of various optimization variables (modifier concentration, mobile phase pH and temperature) using a single equation, based on only eight fitting parameters.

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### 1. Introduction

Reversed-phase high performance liquid chromatography (RP-HPLC) is the most widely used separation technique in analytical chemistry. Despite of the wide range of applications, the separations are frequently developed by trial and error. The method development strategies for optimizing resolution consist in handling as many significant variables as possible. In many instances, the analytes include ionizable compounds, and mobile phase pH is a relevant variable. Thus, once the column characteristics have been chosen, the mobile phase pH, the modifier type and its concentration are then tested. Usually, the first preferred variable is the organic solvent concentration due to its large influence on retention for all type of solutes. However for weak protolytes, the choice of the mobile phase pH is critical due to its significant influence not only on retention factors but also on selectivity and sometimes on peak shapes [1]. Less frequent has been the use of temperatures higher than ambient for separation of low molecular weight solutes under typical RP-HPLC separations [2–13]. It is well known that an increase in temperature leads to an increase in solute diffusion coef-

ficient and an increase in the transfer kinetics, which decrease the mass transfer resistance at the common linear velocities, enhancing the peak width. Simultaneously, the reduced viscosity at higher temperatures allows to increase the flow-rates to speed-up the analysis [14,15]. Although the sequential trial and error approach is spread among chromatographers, it is a time consuming procedure and very often leads to resolution of the relevant peaks under non-optimum conditions.

Another way of facing the chromatographic behavior of compounds is based on predictions originated on theoretical models. Many efforts have been made in order to propose accurate and simple equations to describe the retention factor of solutes as function of the most important variables governing the separations. However, most of these equations describe the retention as a function of a single experimental variable or by considering simultaneously two factors, [16]. The concurrent influence of pH and solvent composition on retention of ionizable solutes have been largely studied [17–20]. Similarly, the changes in retention as a consequence of changes in solvent composition and temperature have been described [21–25] and, lately, the concurrent effect of pH and temperature on retention was theoretically studied [12,16,26,27]. Finally, Pous-Torres et al. [28] proposed a single equation to describe retention of eleven drugs as a function of the three variables.

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The aim of this study is to set up models to predict the retention data of ionizable compounds taking into account all the important chromatographic variables. Thus, a numerous group of acids and bases have been chromatographically studied. These weak protolytes have a wide range of  $pK_a$  and their retention factors have been experimentally measured in a typical octadecylsilica column. Different mobile phases buffered at twelve different pH, at three different acetonitrile/water compositions have been tested and each mobile phase condition was repeated at three temperatures within the range of 25–55 °C. Simple theoretical equations based on the sigmoidal dependence of retention with mobile phase pH, on the van't Hoff dependence of retention with the reciprocal of temperature and on the dependence of retention with either the solvent polarity or solvent composition are proposed. The models produced reasonably accurate predictions. Using such models, we are able to predict the retention data of these analytes under in the studied column, pH, temperature and mobile phase ranges, thus providing the best conditions for optimizing a given separation and, finally, saving time and costs.

## 2. Experimental

### 2.1. Instruments

Chromatographic measurements were conducted with a Shimadzu LC-10A instrument, equipped with LC10AD pump and Sil-10A autoinjector. A Shimadzu (Kyoto, Japan) Model SPD-10Avp UV–vis detector was used, set at 254 nm for the acids and the bases and 282 nm for the phenols. A 150 mm × 4.6 mm i.d. octadecylsilica X-Terra™ MS-C18 analytical column provided by Waters was used for all the determinations. It has been demonstrated that this material is stable within the pH range 2–12 [29,30]. Both, the column and the mobile phase were immersed into a temperature-controlled thermostatic bath. Temperature was measured by using a thermometer calibrated at  $\pm 0.1$  °C.

pH measurements of mobile phase were done with a Schott BlueLine combined glass electrode, connected to a 702 SM Titrimetric pH-meter (Metrohm) with a precision of  $\pm 0.01$  pH units.

### 2.2. Chemicals

All chemicals used here were of reagent grade or better. Acetonitrile (MeCN) HPLC-grade 99.9% was purchased from Mallinckrodt (Paris, KY). HPLC water was purified by a Milli-Q® deionizing system (Simplicity 185, Millipore). All other chemicals used to prepare the buffer solution were obtained from Fluka, Merck and Baker. Analytes were purchased from Fluka and Sigma-Aldrich. We used the following 22 analytes: naphthoic acid, 2-nitrobenzoic acid, 3-nitrobenzoic acid, 4-nitrobenzoic acid, benzoic acid, phenol, 2,4-dichlorophenol, 2,4-dinitrophenol,  $\beta$ -naphthol, 2-nitrophenol, 3,5-dichlorophenol, 3-bromophenol, 4-chlorophenol, *m*-cresol, 2,4,6-trimethylpyridine, 4-chloroaniline, aniline, *N*-ethylaniline, *N,N*-dimethylbenzylamine, *p*-toluidine, pyridine, and 2,6-dimethylpyridine.

### 2.3. Procedure

All the chromatographic experiments were done at three temperatures: 25, 40, 55 °C. The mobile phases were prepared at 25 °C by mixing the aqueous buffers described in Table 1 with acetonitrile, at 20%, 40% and 60% of organic solvent by volume. In order to measure the mobile phase pH, the electrode system was calibrated using the usual aqueous standard reference buffers of potassium hydrogenphthalate ( ${}^w\text{pH} = 4.00$  at 25 °C) and potassium dihydrogenphosphate–disodium hydrogen phosphate ( ${}^w\text{pH} = 7.02$  at 25 °C). 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.  ${}^s\text{pH}$  can be calculated subtracting the  $\delta$  value from the  ${}^w\text{pH}$  value [18,31]. Both  ${}^s\text{pH}$  and  ${}^w\text{pH}$  scales have been recommended by the IUPAC [32]. The mobile phase  ${}^s\text{pH}$  values at the three MeCN concentrations and at the three temperatures are presented in Table 1.

The solutes were dissolved in MeOH at a concentration of about 50 ppm. The chromatographic measurements were conducted after keeping the column and the mobile phase at the corresponding temperature for at least 1 h before injection. The eluent flow rate was 1 mL/min and the injection volume was 5  $\mu\text{L}$ . The hold-up time was measured by injections of water and it was found to be  $t_0 = 0.934$  min. All results are the average of triplicate injections.

### 2.4. Data treatment

The multiparametric non linear regressions of experimental retention factors with pH,  $T$  and solvent composition were performed using available commercial software (SigmaPlot v4.1) that applies Marquardt algorithm [33].

## 3. Theoretical part

### 3.1. Influence of pH

The theoretical sigmoidal function describing the retention factor,  $k$ , of ionizable compounds with pH in a typical RPLC column was first deduced by Horváth et al. [34] from the basic chromatographic equations. This sigmoidal dependence was lately verified experimentally by several authors [18,35–37]. Some of us have extensively reviewed retention models based in this relationship [19,20,38]. Thus, the retention factor of a monoprotic solute, HA, with an acid–base equilibrium ruled by an acidity constant,  $K_a$ , depends on the mobile phase pH according to Eq. (1).

$$k = \frac{k_{\text{HA}} + k_{\text{A}} 10^{(\text{pH} - \text{p}K_a)}}{1 + 10^{(\text{pH} - \text{p}K_a)}} \quad (1)$$

where  $k_{\text{HA}}$  and  $k_{\text{A}}$  represent the retention factors of the protonated and the dissociated forms of the solute at a given solvent concentration and temperature, respectively, and  $\text{p}K_a$  is the acid–base  $\text{p}K_a$  of the solute at the mobile phase composition and temperature at which the retention has been measured and in the same scale used to measure mobile phase pH.

This expression for retention, which has a strong dependence of pH in the region close to the solute  $\text{p}K_a$ , has been experimentally tested with several acidic and basic solutes. It has been extensively probed that the fitting capability of this equation is guaranteed only when pH and  $\text{p}K_a$  correspond to the true pH and  $\text{p}K_a$  values [17–20,36–38,46], i.e., the values measured in the solvent mixture regardless of the chosen standard state solvent for hydrogen ion (or solvent used for pH calibration: water ( ${}^s\text{pH}$  scale) or the same HPLC mobile phase ( ${}^w\text{pH}$  scale)), whereas the fitting becomes completely inappropriate when the  $\text{p}K_a$  and pH in pure water ( ${}^w\text{p}K_a$  and  ${}^w\text{pH}$ ) are used due to the different dependence with solvent composition for different ionizable compounds. For sake of simplicity, in this work the  ${}^s\text{p}K_a$  and  ${}^s\text{pH}$  values are used, i.e. pH was measured in the HPLC mobile phase with calibration with the usual aqueous pH standards, but at the working temperature.

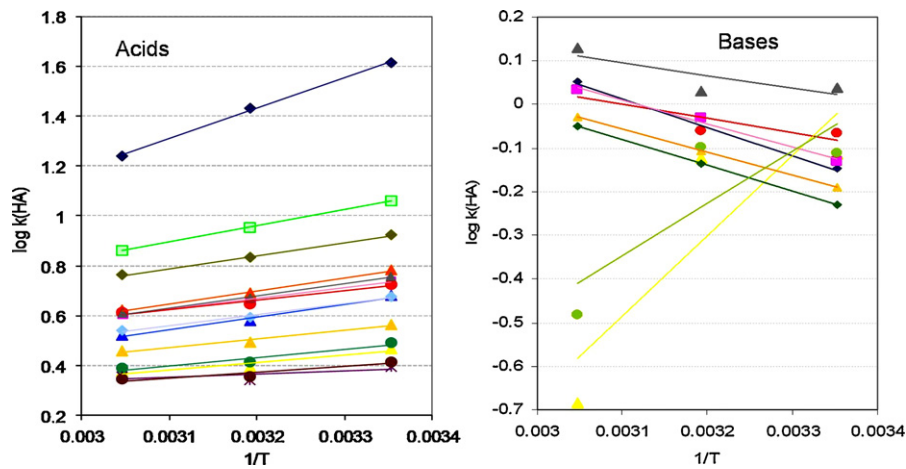
### 3.2. Influence of temperature

The influence of temperature on retention within a narrow temperature range is usually well described by the van't Hoff equation,

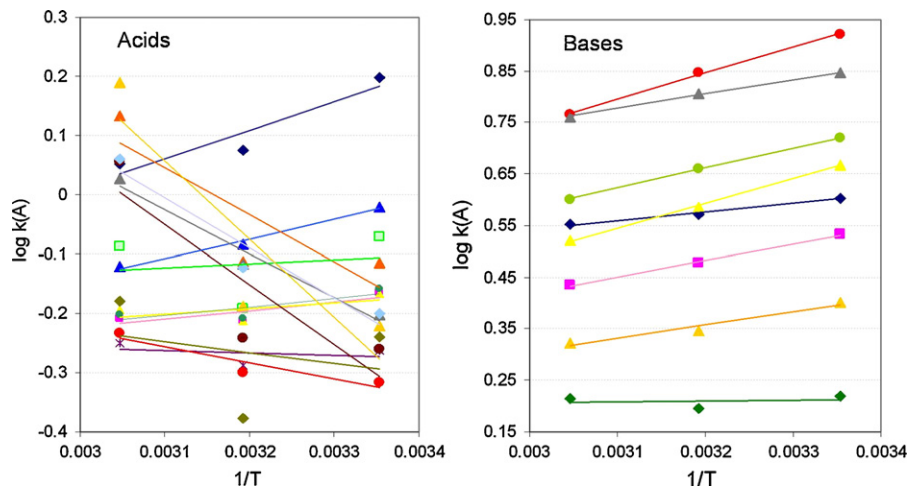
**Table 1** $pH_w^s$  values of the studied buffers measured at different temperatures and different modifier (MeCN) concentrations.

Aqueous buffer		25 °C			40 °C			55 °C		
		MeCN (%v/v)			MeCN (%v/v)			MeCN (%v/v)		
		20	40	60	20	40	60	20	40	60
A1	0.01 M HCl	1.96	1.96	1.84	1.98	1.95	1.82	2.00	1.94	1.81
A	0.01 M H <sub>3</sub> PO <sub>4</sub>	2.28	2.55	2.54	2.26	2.47	2.55	2.32	2.42	2.58
B	6.40 × 10 <sup>-3</sup> M H <sub>3</sub> Cit + 3.60 × 10 <sup>-3</sup> M KH <sub>2</sub> Cit	3.15	3.43	3.64	3.16	3.38	3.60	3.15	3.38	3.61
C	9.35 × 10 <sup>-3</sup> M KH <sub>2</sub> Cit + 6.52 × 10 <sup>-3</sup> M KNaHCit	4.32	4.65	5.01	4.29	4.63	5.04	4.33	4.63	5.04
D	3.46 × 10 <sup>-3</sup> M HAc + 6.54 × 10 <sup>-3</sup> M NaAc	5.39	5.80	6.47	5.41	5.80	6.45	5.46	5.86	6.47
E	5.81 × 10 <sup>-3</sup> M KNaHCit + 4.19 × 10 <sup>-3</sup> M Na <sub>3</sub> Cit	6.55	6.84	7.28	6.64	6.86	7.50	6.67	6.94	7.65
F	5.22 × 10 <sup>-3</sup> M KH <sub>2</sub> PO <sub>4</sub> + 4.78 × 10 <sup>-3</sup> M Na <sub>2</sub> HPO <sub>4</sub>	7.48	7.66	8.01	7.48	7.66	7.88	7.43	7.67	7.90
G	9.44 × 10 <sup>-3</sup> M KH <sub>2</sub> PO <sub>4</sub> + 9.06 × 10 <sup>-3</sup> M Na <sub>2</sub> HPO <sub>4</sub>	8.34	8.66	8.69	8.30	8.61	8.82	8.24	8.56	8.78
H	5.95 × 10 <sup>-3</sup> M H <sub>3</sub> BO <sub>3</sub> + 4.05 × 10 <sup>-3</sup> M NaH <sub>2</sub> BO <sub>3</sub>	9.01	10.11	10.28	8.78	9.69	10.09	8.67	9.55	9.93
I	7.84 × 10 <sup>-3</sup> M BuNH <sub>3</sub> <sup>+</sup> + 2.16 × 10 <sup>-3</sup> M BuNH <sub>2</sub>	9.79	9.39	9.25	9.31	8.94	8.76	8.91	8.65	8.37
J	1.64 × 10 <sup>-3</sup> M BuNH <sub>3</sub> <sup>+</sup> + 8.36 × 10 <sup>-3</sup> M BuNH <sub>2</sub>	10.78	10.63	10.26	10.30	10.13	9.80	9.99	9.72	9.41
K	0.01 M Na <sub>3</sub> PO <sub>4</sub>	11.68	11.99	12.02	11.30	11.55	11.75	11.02	11.25	11.50

Cit = citrate; Ac = acetate; Bu = butyl.



**Fig. 1.** van't Hoff plots. Graphs of  $\log k_{HA}$  of acids and bases at 40% (v/v) ACN as a function of the reciprocal of temperature. Symbols for acids (◆) naphthoic acid; (■) 2-nitrobenzoic acid; (▲) 3-nitrobenzoic acid; (●) 4-nitrobenzoic acid; (✕) benzoic acid; (●) phenol; (◆) 2,4-dichlorophenol; (▲) 2,4-dinitrophenol; (▲) β-naphthol; (●) 2-nitrophenol; (■) 3,5-dichlorophenol; (▲) 3-bromophenol; (◆) 4-chlorophenol; (▲) *m*-cresol. Symbols for bases: (◆) 2,4,6-trimethylpyridine; (▲) 4-chloroaniline; (●) *N*-ethylaniline; (▲) *N,N*-dimethylbenzylamine; (▲) aniline; (■) *p*-toluidine; (◆) pyridine; (●) 2,6-dimethylpyridine.



**Fig. 2.** van't Hoff plots. Graphs of  $\log k_A$  of acids and bases at 40% (v/v) ACN as a function of the reciprocal of temperature. Symbols as in Fig. 1.

**Table 2**  
Solute retention parameters and estimated  $s_w^0 pK_a$  obtained from fitting experimental retention factors to the mobile phase  $s_w^0 pH$  values. Eluent composition: 20% v/v MeCN. Column temperatures: 25, 40 and 55 °C.

Solute	Temperature (°C)												
	25				40				55				
	$k_{HA}$	$k_A$	$s_w^0 pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w^0 pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w^0 pK_a$	$r^2$	
1. Napthoic acid	41.4 ± 0.6	1.6 ± 0.3	4.38 ± 0.05	0.997	27.1 ± 0.4	1.2 ± 0.2	4.39 ± 0.05	0.9976	17.4 ± 0.2	1.1 ± 0.1	4.45 ± 0.04	0.9979	
2. 2-Nitrobenzoic acid	6.2 ± 0.4	0.68 ± 0.08	2.8 ± 0.1	0.984	4.5 ± 0.2	0.62 ± 0.07	3 ± 0.10	0.9777	3.5 ± 0.2	0.77 ± 0.07	3.2 ± 0.2	0.9613	
3. 3-Nitrobenzoic acid	10.4 ± 0.3	1.1 ± 0.1	3.85 ± 0.09	0.992	7.2 ± 0.2	1.0 ± 0.1	4.0 ± 0.1	0.9861	5.2 ± 0.2	0.9 ± 0.1	4.0 ± 0.1	0.9783	
4. 4-Nitrobenzoic acid	11.7 ± 0.4	1.3 ± 0.2	3.7 ± 0.1	0.986	7.8 ± 0.2	1.0 ± 0.1	3.9 ± 0.1	0.9878	5.6 ± 0.2	1.0 ± 0.1	3.9 ± 0.1	0.9806	
5. Benzoic acid	7.6 ± 0.2	0.9 ± 0.1	4.8 ± 0.1	0.988	5.8 ± 0.1	0.74 ± 0.09	4.8 ± 0.1	0.9912	4.4 ± 0.1	0.76 ± 0.08	4.9 ± 0.1	0.9861	
6. Phenol	5.65 ± 0.05	0.9 ± 0.2	10.48 ± 0.07	0.992	4.42 ± 0.06	0.9 ± 0.2	10.3 ± 0.1	0.9760	3.46 ± 0.02	1.1 ± 0.1	10.18 ± 0.07	0.9907	
7. 2,4-Dichlorophenol	56.7 ± 0.9	1.2 ± 1.3	8.35 ± 0.07	0.994	38.1 ± 1.0	0.9 ± 1.5	8.2 ± 0.1	0.9820	23.8 ± 0.5	0.9 ± 0.8	8.10 ± 0.08	0.9888	
8. 2,4-Dinitrophenol	16.8 ± 0.5	2.3 ± 0.3	4.1 ± 0.1	0.990	10.9 ± 0.4	1.7 ± 0.2	4.2 ± 0.1	0.9781	7.6 ± 0.3	1.4 ± 1.1	4.2 ± 0.1	0.9786	
9. β-Napthol	37.0 ± 0.5	1.6 ± 1.5	9.93 ± 0.09	0.988	24.1 ± 0.4	2.7 ± 1.2	9.8 ± 0.1	0.9795	15.2 ± 0.2	2.1 ± 0.7	9.87 ± 0.09	0.9846	
10. 2-Nitrophenol	16.2 ± 0.1	0.8 ± 0.1	7.66 ± 0.03	0.999	12.1 ± 0.2	0.6 ± 0.3	7.56 ± 0.07	0.9933	8.64 ± 0.09	0.7 ± 0.1	7.40 ± 0.04	0.9973	
11. 3,5-Dichlorophenol	92.2 ± 1.6	3.1 ± 2.4	8.51 ± 0.08	0.992	59.4 ± 2.0	1.9 ± 2.4	8.3 ± 0.1	0.9820	35.3 ± 0.7	1.8 ± 1.2	8.21 ± 0.08	0.9869	
12. 3-Bromophenol	27.5 ± 0.4	1.7 ± 0.9	9.41 ± 0.08	0.990	18.8 ± 0.3	1.3 ± 0.6	9.11 ± 0.07	0.9895	12.2 ± 0.2	1.8 ± 0.4	8.99 ± 0.07	0.9853	
13. 4-Chlorophenol	20.1 ± 0.3	1.1 ± 0.6	9.92 ± 0.07	0.991	14.2 ± 0.3	2.0 ± 0.6	9.92 ± 0.07	0.9782	9.7 ± 0.1	2.6 ± 0.3	9.5 ± 0.1	0.9846	
14. <i>m</i> -Cresol	11.3 ± 0.1	0.8 ± 0.5	10.71 ± 0.07	0.992	8.5 ± 0.2	0.9 ± 0.8	10.5 ± 0.2	0.9580	6.27 ± 0.06	1.6 ± 0.3	10.3 ± 0.1	0.9809	
15. 2,4,6-Trimethylpyridine	1.0 ± 0.4	17.0 ± 0.4	6.90 ± 0.09	0.990	1.1 ± 0.4	13.9 ± 0.3	6.7 ± 0.1	0.9870	1.1 ± 0.3	11.2 ± 0.3	6.4 ± 0.1	0.9877	
16. 4-Chloroaniline	1.6 ± 0.5	15.8 ± 0.2	3.6 ± 0.1	0.990	1.4 ± 0.3	11.6 ± 0.1	3.31 ± 0.07	0.9933	1.2 ± 0.6	8.0 ± 0.2	3.1 ± 0.2	0.9650	
17. Aniline	0.98 ± 0.08	4.63 ± 0.05	4.38 ± 0.07	0.994	0.9 ± 0.1	3.85 ± 0.06	4.1 ± 0.1	0.9849	0.9 ± 0.1	3.16 ± 0.05	3.8 ± 0.1	0.9825	
18. <i>N</i> -Ethylaniline	2.0 ± 0.7	28.0 ± 0.6	5.4 ± 0.1	0.990	1.0 ± 0.5	21.9 ± 0.3	4.67 ± 0.07	0.9948	1.0 ± 0.6	16.1 ± 0.4	4.5 ± 0.1	0.9836	
19. <i>N,N</i> -Dimethylbenzylamine	1.5 ± 0.2	10.4 ± 0.2	7.63 ± 0.09	0.990	1.31 ± 0.09	4.9 ± 0.1	7.0 ± 0.1	0.9881	1.23 ± 0.02	2.33 ± 0.02	5.73 ± 0.09	0.9931	
20. Toluidine	1.5 ± 0.2	9.0 ± 0.1	5.1 ± 0.1	0.991	1.2 ± 0.2	7.2 ± 0.1	4.5 ± 0.1	0.9885	1.2 ± 0.2	5.6 ± 0.1	4.3 ± 0.1	0.9802	
21. Pyridine	0.9 ± 0.1	2.9 ± 0.1	5.9 ± 0.2	0.949	0.78 ± 0.05	2.54 ± 0.03	4.6 ± 0.1	0.9900	0.80 ± 0.04	2.34 ± 0.02	4.49 ± 0.08	0.9932	
22. 2,6-Dimethylpyridine	1.6 ± 0.5	16.9 ± 0.2	3.60 ± 0.09	0.993	1.3 ± 0.6	13.2 ± 0.2	3.3 ± 0.1	0.9804	1.3 ± 0.6	9.9 ± 0.2	3.1 ± 0.2	0.9710	

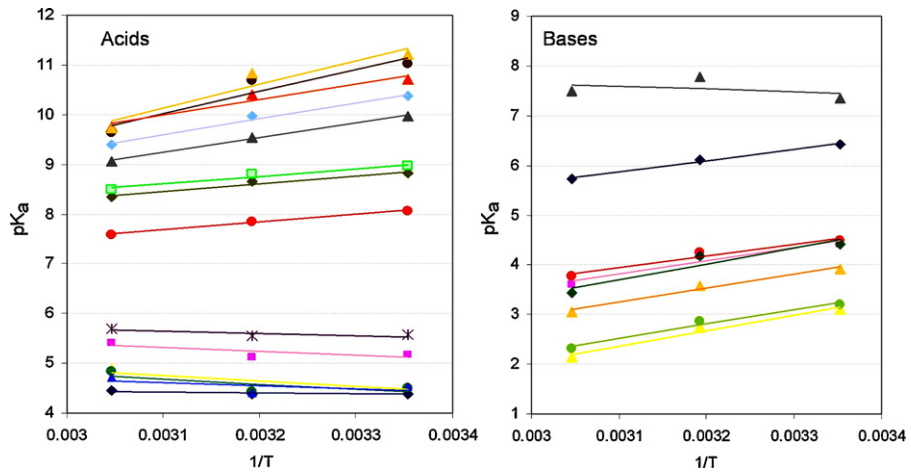


Fig. 3. Dependence of  $pK_a$  of acids and bases against the reciprocal of temperature. Symbols as in Fig. 1.

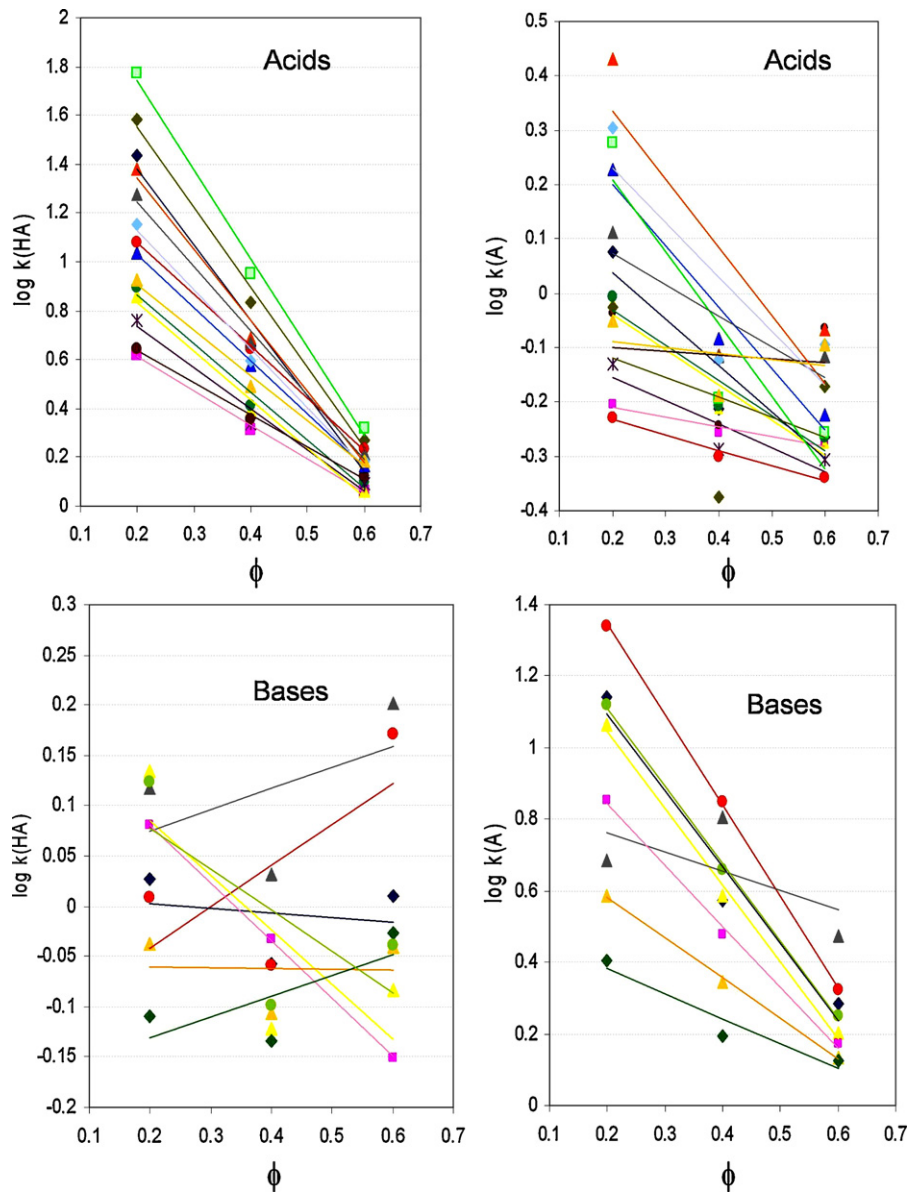


Fig. 4. Plots of logarithm of retention factors of protonated ( $k_{HA}$ ) and deprotonated ( $k_A$ ) species of neutral acids and bases as a function of  $\phi$ . Symbols as in Fig. 1.

**Table 3**  
Solute retention parameters and estimated  $s_w pK_a$  obtained from fitting experimental retention factors to the mobile phase  $s_w pH$  values. Eluent composition: 40% (v/v) MeCN. Column temperatures: 25, 40 and 55 °C.

Solute	Temperature (°C)											
	25				40				55			
	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$
1	5.5 ± 0.2	0.7 ± 0.1	5.2 ± 0.1	0.988	4.5 ± 0.1	0.61 ± 0.07	5.1 ± 0.1	0.990	4.0 ± 0.1	0.62 ± 0.07	5.4 ± 0.1	0.990
2	2.5 ± 0.1	0.62 ± 0.07	3.7 ± 0.2	0.934	2.0 ± 0.1	0.55 ± 0.05	3.8 ± 0.2	0.950	2.0 ± 0.1	0.62 ± 0.07	4.5 ± 0.3	0.915
3	2.9 ± 0.2	0.68 ± 0.09	4.6 ± 0.2	0.951	2.5 ± 0.1	0.61 ± 0.07	4.5 ± 0.2	0.950	2.4 ± 0.1	0.64 ± 0.07	4.9 ± 0.2	0.947
4	3.1 ± 0.1	0.69 ± 0.09	4.5 ± 0.2	0.958	2.6 ± 0.1	0.62 ± 0.08	4.4 ± 0.2	0.953	2.5 ± 0.1	0.63 ± 0.07	4.8 ± 0.2	0.951
5	2.47 ± 0.08	0.55 ± 0.06	5.5 ± 0.2	0.978	2.19 ± 0.07	0.52 ± 0.05	5.5 ± 0.1	0.976	2.27 ± 0.08	0.56 ± 0.06	5.7 ± 0.2	0.969
6	2.60 ± 0.01	0.55 ± 0.05	11.03 ± 0.04	0.997	2.26 ± 0.02	0.57 ± 0.08	10.7 ± 0.1	0.987	2.21 ± 0.06	1.1 ± 0.2	9.6 ± 0.3	0.816
7	8.37 ± 0.05	0.57 ± 0.08	8.83 ± 0.03	0.998	6.81 ± 0.08	0.4 ± 0.1	8.65 ± 0.05	0.995	5.8 ± 0.1	0.7 ± 0.2	8.36 ± 0.09	0.984
8	4.8 ± 0.2	0.9 ± 0.1	4.5 ± 0.2	0.962	3.8 ± 0.2	0.8 ± 0.1	4.4 ± 0.2	0.962	3.3 ± 0.2	0.7 ± 0.1	4.7 ± 0.2	0.949
9	6.05 ± 0.02	0.77 ± 0.07	10.48 ± 0.02	0.997	4.89 ± 0.05	0.8 ± 0.2	10.12 ± 0.08	0.995	4.2 ± 0.1	1.4 ± 0.3	9.5 ± 0.2	0.903
10	5.33 ± 0.05	0.48 ± 0.06	8.06 ± 0.05	0.998	4.43 ± 0.06	0.5 ± 0.08	7.86 ± 0.07	0.995	4.08 ± 0.09	0.6 ± 0.1	7.6 ± 0.1	0.988
11	11.47 ± 0.07	0.9 ± 0.1	8.96 ± 0.03	0.999	9.0 ± 0.1	0.6 ± 0.2	8.80 ± 0.05	0.995	7.3 ± 0.2	0.8 ± 0.3	8.5 ± 0.1	0.981
12	5.70 ± 0.03	0.63 ± 0.07	9.96 ± 0.03	0.999	4.67 ± 0.06	0.8 ± 0.2	9.55 ± 0.07	0.990	4.06 ± 0.09	1.0 ± 0.2	9.1 ± 0.1	0.964
13	4.73 ± 0.02	0.63 ± 0.06	10.37 ± 0.03	0.998	3.92 ± 0.05	0.8 ± 0.1	9.97 ± 0.07	0.985	3.48 ± 0.09	1.2 ± 0.3	9.4 ± 0.2	0.922
14	3.64 ± 0.03	0.5 ± 0.2	11.3 ± 0.1	0.986	3.11 ± 0.02	0.7 ± 0.2	10.8 ± 0.1	0.988	2.88 ± 0.08	1.6 ± 0.2	9.7 ± 0.3	0.799
15	0.71 ± 0.06	4.01 ± 0.05	6.42 ± 0.07	0.995	0.88 ± 0.03	3.73 ± 0.03	6.10 ± 0.04	0.998	1.13 ± 0.09	3.56 ± 0.06	5.7 ± 0.1	0.984
16	0.8 ± 0.1	4.66 ± 0.03	3.11 ± 0.05	0.996	0.8 ± 0.1	3.86 ± 0.03	2.74 ± 0.07	0.994	0.2 ± 1.5	3.32 ± 0.09	2.2 ± 0.5	0.857
17	0.65 ± 0.04	2.52 ± 0.02	3.92 ± 0.07	0.996	0.78 ± 0.04	2.22 ± 0.01	3.58 ± 0.06	0.996	0.9 ± 0.2	2.10 ± 0.06	3.1 ± 0.4	0.859
18	0.86 ± 0.08	8.34 ± 0.05	4.48 ± 0.03	0.998	0.9 ± 0.1	7.05 ± 0.06	4.25 ± 0.06	0.996	1.1 ± 0.3	5.8 ± 0.1	3.8 ± 0.2	0.973
19	1.1 ± 0.4	7.0 ± 0.4	7.4 ± 0.2	0.931	1.1 ± 0.1	6.4 ± 0.1	7.79 ± 0.08	0.992	1.3 ± 0.1	5.76 ± 0.1	7.48 ± 0.09	0.989
20	0.74 ± 0.05	3.41 ± 0.03	4.42 ± 0.06	0.996	0.93 ± 0.05	3.00 ± 0.03	4.17 ± 0.08	0.995	1.1 ± 0.2	2.72 ± 0.07	3.6 ± 0.3	0.930
21	0.59 ± 0.03	1.65 ± 0.02	4.42 ± 0.09	0.991	0.73 ± 0.03	1.57 ± 0.01	4.2 ± 0.1	0.986	0.9 ± 0.1	1.64 ± 0.05	3.4 ± 0.4	0.813
22	0.8 ± 0.1	5.25 ± 0.03	3.20 ± 0.05	0.997	0.8 ± 0.1	4.57 ± 0.03	2.87 ± 0.06	0.995	0.3 ± 1.1	4.0 ± 0.1	2.3 ± 0.3	0.903

which can be written as:

$$\log k = -\frac{\Delta H^\circ}{2.3RT} + \frac{\Delta S^\circ}{2.3R} + \log \Phi \quad (2)$$

where  $\Delta H^\circ$  and  $\Delta S^\circ$  represent the changes in enthalpy and entropy due to the transfer of solute from the mobile to the stationary phase,  $R$  is the gas constant and  $\Phi$  represent the phase ratio. In the situation in which the temperature range is narrow, a plot of  $\log k$  as a function of  $1/T$  usually shows a linear behavior, which means that all the parameters ( $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Phi$ ) are independent of temperature within that range. Thus, analogous expressions can be written for each form of a monoprotic substance, HA and A.

In the same way, a van't Hoff equation usually describes the dependence of the dissociation equilibrium constant  $K_a$  with temperature:

$$pK_a = \frac{\Delta H_a^\circ}{2.3RT} - \frac{\Delta S_a^\circ}{2.3R} \quad (3)$$

where  $\Delta H_a^\circ$  and  $\Delta S_a^\circ$  are the changes of enthalpy and of entropy due to solute dissociation.

**Table 4**  
Solute retention parameters and estimated  $s_w pK_a$  obtained from fitting experimental retention factors to the mobile phase  $s_w pH$  values. Eluent composition: 60% (v/v) MeCN. Column temperatures: 25, 40 and 55 °C.

Solute	Temperature (°C)											
	25				40				55			
	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$	$k_{HA}$	$k_A$	$s_w pK_a$	$r^2$
1	1.76 ± 0.09	0.53 ± 0.06	6.1 ± 0.3	0.938	1.55 ± 0.08	0.54 ± 0.06	6.3 ± 0.3	0.921	1.43 ± 0.08	0.55 ± 0.06	6.2 ± 0.4	0.894
2	1.3 ± 0.1	0.55 ± 0.06	5.0 ± 0.4	0.833	1.16 ± 0.08	0.53 ± 0.06	6.3 ± 0.05	0.810	1.2 ± 0.1	0.57 ± 0.06	5.2 ± 0.5	0.767
3	1.3 ± 0.1	0.55 ± 0.06	5.0 ± 0.4	0.833	1.16 ± 0.08	0.53 ± 0.06	6.3 ± 0.05	0.810	1.2 ± 0.1	0.57 ± 0.06	5.2 ± 0.5	0.767
4	1.41 ± 0.08	0.52 ± 0.06	6.1 ± 0.4	0.901	1.26 ± 0.08	0.54 ± 0.06	6.1 ± 0.4	0.853	1.2 ± 0.09	0.56 ± 0.06	6.0 ± 0.6	0.806
5	1.39 ± 0.08	0.52 ± 0.06	6.3 ± 0.3	0.896	1.23 ± 0.07	0.50 ± 0.07	7.2 ± 0.4	0.864	1.22 ± 0.09	0.56 ± 0.07	6.4 ± 0.5	0.808
6	1.41 ± 0.01	0.80 ± 0.01	11.12 ± 0.05	0.998	1.32 ± 0.01	0.86 ± 0.01	10.76 ± 0.04	0.998	1.25 ± 0.01	0.92 ± 0.01	10.38 ± 0.05	0.996
7	2.05 ± 0.05	0.47 ± 0.09	9.2 ± 0.1	0.969	1.87 ± 0.04	0.67 ± 0.08	9.0 ± 0.1	0.960	1.70 ± 0.08	0.7 ± 0.1	9.0 ± 0.3	0.829
8	1.7 ± 0.1	0.56 ± 0.07	5.8 ± 0.4	0.913	1.5 ± 0.1	0.60 ± 0.07	5.5 ± 0.5	0.847	1.4 ± 0.1	0.60 ± 0.07	5.3 ± 0.5	0.816
9	1.79 ± 0.01	0.77 ± 0.01	10.71 ± 0.02	0.968	1.63 ± 0.01	0.85 ± 0.02	10.34 ± 0.05	0.888	1.49 ± 0.01	0.90 ± 0.01	10.00 ± 0.03	0.952
10	1.87 ± 0.06	0.43 ± 0.08	8.6 ± 0.1	0.966	1.71 ± 0.06	0.46 ± 0.08	8.4 ± 0.2	0.951	1.57 ± 0.05	0.50 ± 0.07	8.3 ± 0.1	0.958
11	2.32 ± 0.05	0.49 ± 0.09	9.4 ± 0.1	0.978	2.08 ± 0.05	0.56 ± 0.09	9.1 ± 0.1	0.967	1.84 ± 0.05	0.60 ± 0.09	8.9 ± 0.2	0.955
12	1.79 ± 0.01	0.70 ± 0.03	10.13 ± 0.04	0.995	1.65 ± 0.02	0.76 ± 0.06	9.8 ± 0.1	0.969	1.50 ± 0.01	0.79 ± 0.04	9.62 ± 0.09	0.978
13	1.67 ± 0.01	0.76 ± 0.01	10.56 ± 0.02	0.999	1.54 ± 0.01	0.81 ± 0.02	10.25 ± 0.05	0.992	1.42 ± 0.01	0.84 ± 0.02	9.93 ± 0.05	0.994
14	1.67 ± 0.01	0.76 ± 0.01	10.56 ± 0.02	0.999	1.54 ± 0.01	0.81 ± 0.02	10.25 ± 0.05	0.992	1.42 ± 0.01	0.84 ± 0.02	9.93 ± 0.05	0.994
15	1.09 ± 0.08	2.11 ± 0.05	5.5 ± 0.3	0.939	1.02 ± 0.06	1.92 ± 0.04	5.5 ± 0.3	0.956	1.07 ± 0.04	1.76 ± 0.03	5.5 ± 0.3	0.959
16	0.9 ± 0.1	1.73 ± 0.01	2.2 ± 0.2	0.952	0.8 ± 0.23	1.59 ± 0.01	2.1 ± 0.3	0.940	0.4 ± 1.2	1.47 ± 0.01	1.5 ± 0.8	0.910
17	1.02 ± 0.04	1.45 ± 0.01	3.2 ± 0.2	0.954	0.91 ± 0.04	1.36 ± 0.01	2.9 ± 0.2	0.965	0.94 ± 0.04	1.29 ± 0.01	2.7 ± 0.2	0.944
18	1.06 ± 0.05	2.31 ± 0.02	3.7 ± 0.1	0.986	1.48 ± 0.05	2.11 ± 0.02	3.9 ± 0.2	0.941	1.01 ± 0.06	1.91 ± 0.02	3.3 ± 0.1	0.972
19	1.8 ± 0.2	3.3 ± 0.1	6.8 ± 0.4	0.864	1.59 ± 0.07	2.97 ± 0.07	7.1 ± 0.2	0.956	1.1 ± 0.1	2.37 ± 0.07	4.9 ± 0.3	0.917
20	1.08 ± 0.04	1.61 ± 0.02	3.9 ± 0.2	0.964	0.71 ± 0.09	1.49 ± 0.02	2.8 ± 0.2	0.948	1.03 ± 0.03	1.41 ± 0.01	3.5 ± 0.2	0.944
21	1.02 ± 0.03	1.41 ± 0.01	3.9 ± 0.2	0.946	0.94 ± 0.03	1.34 ± 0.01	3.4 ± 0.2	0.958	0.99 ± 0.03	1.29 ± 0.01	3.7 ± 0.3	0.927
22	0.9 ± 0.1	1.90 ± 0.02	2.4 ± 0.2	0.968	0.9 ± 0.1	1.77 ± 0.04	3.1 ± 0.3	0.888	0.8 ± 0.2	1.64 ± 0.01	2.0 ± 0.3	0.952

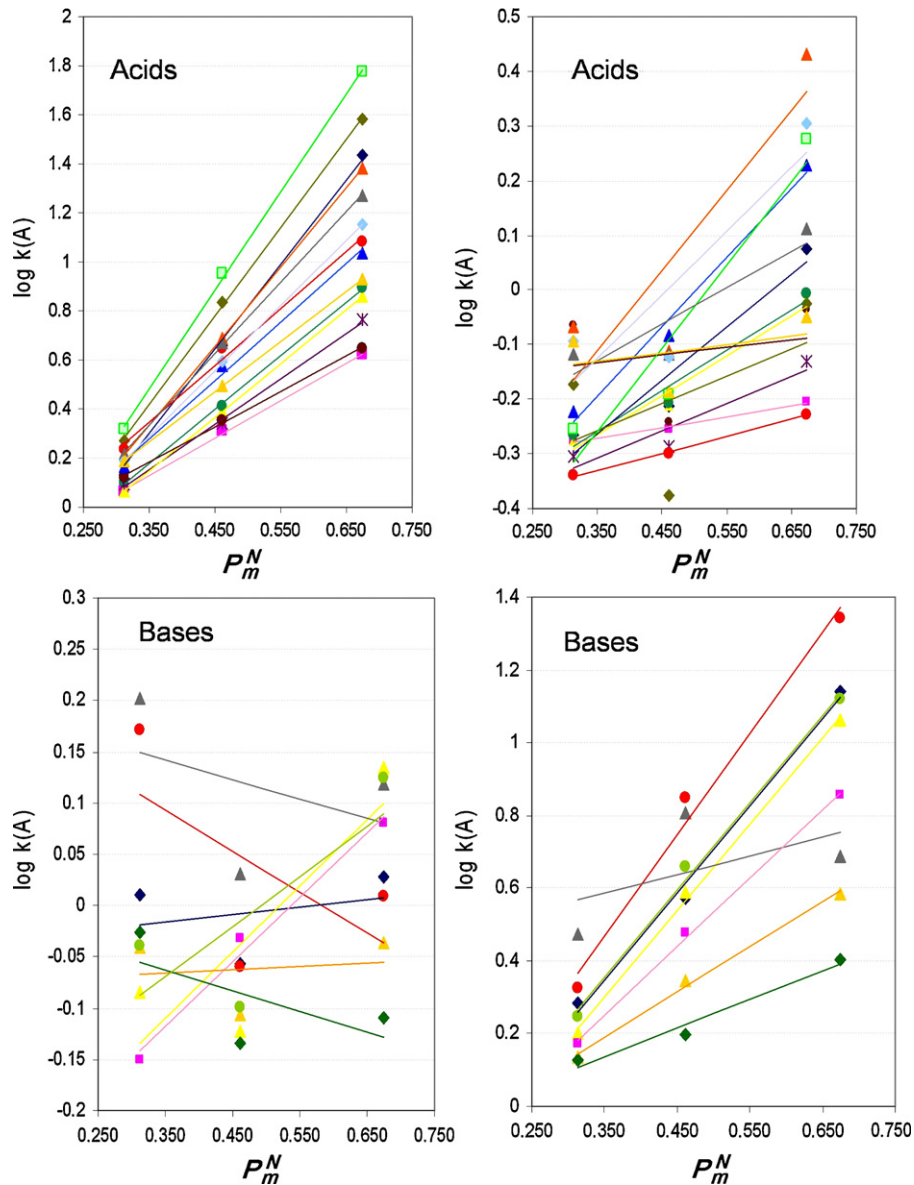


Fig. 5. Plots of logarithm of retention factors of protonated ( $k_{HA}$ ) and deprotonated ( $k_A$ ) species of neutral acids and bases as a function of  $P_m^N$ . Symbols as in Fig. 1.

### 3.3. Simultaneous influence of pH and temperature

By introducing Eqs. (2) and (3) into (1), the following expression can be written:

$$k = \frac{10^{a+b/T} + 10^{c+d/T} 10^{(pH-e-f/T)}}{1 + 10^{(pH-e-f/T)}} \quad (4)$$

where the fitting parameters contain the thermodynamic quantities associated to the dissociation and to the transfer of both forms of the analyte between phases, being these quantities functions of composition, i.e.,  $a = (\Delta S_{HA}^\circ / 2.3R + \log \Phi)$ ,  $b = -\Delta H_{HA}^\circ / 2.3R$ ,  $c = (\Delta S_A^\circ / 2.3R + \log \Phi)$ , and  $d = -\Delta H_A^\circ / 2.3R$ ,  $e = \Delta S_a^\circ / 2.3R$  and  $f = -\Delta H_a^\circ / 2.3R$ , where again the subscripts HA and A indicate the protonated and the deprotonated forms of the acid–base solute, respectively. The validity of Eq. (4) has also been experimentally tested with several acid–base systems [3,26,27,39,40].

### 3.4. Influence of solvent composition

Since the composition of the mobile phase has been the main variable used to optimize retention and selectivity in RPLC, several models describing the dependence of retention factors with solvent concentration have been proposed in the literature. Recently, a very interesting review of these models, in which the authors explained the fundamentals behind the differences between them, has been published [16].

The simpler equation suggested to describe this behavior correspond to the linear solvent strength model:

$$\log k = \log k_w - S\phi \quad (5)$$

where  $k_w$  and  $S$ , the intercept and the slope of the equation, respectively, represent the solute retention factor in pure water and the sensitivity of the solute molecule to the solvent strength [41].  $\phi$  is the volume fraction of organic modifier in the mobile phase.

Another approach to accurately describe the retention factor through a linear model that considers the polarity contributions of the solute, the stationary phase and the mobile phase has also

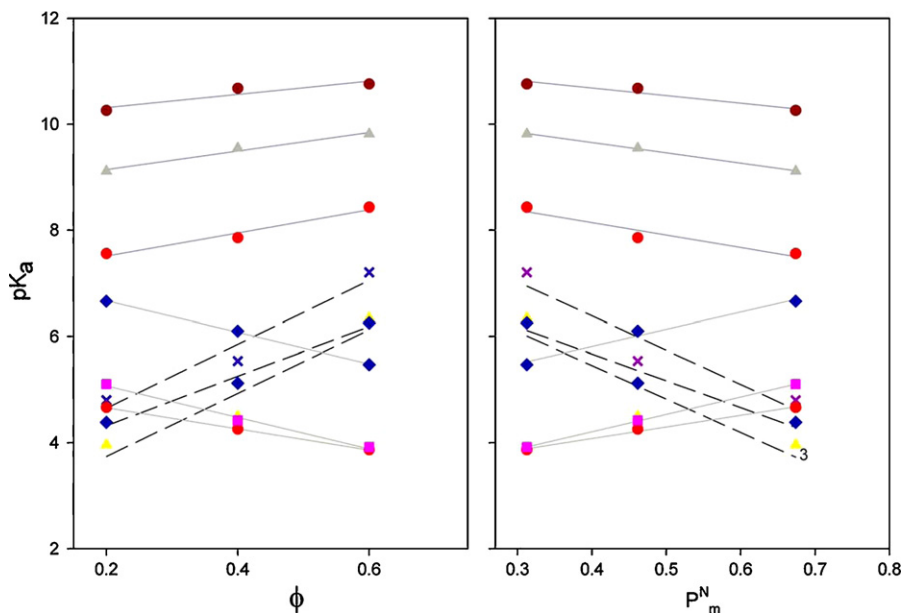


Fig. 6. Dependence between  $pK_a$  and solvent composition. Plots of  $pK_a$  of a group of representative several protogenic solutes against  $\phi$  and against  $P_m^N$ . Symbols as in Fig. 1.

been proposed [38,42–46]:

$$\log k = (\log k)_0 - p(P_m^N - P_s^N) \quad (6)$$

where  $p$  represents a descriptor for the solute polarity,  $P_m^N$  and  $P_s^N$  are normalized polarity parameters for the mobile and the stationary phases, respectively, whereas the term  $(\log k)_0$  represents the retention factor in a hypothetical mobile phase with the same polarity as the stationary phase. For acetonitrile–water mixtures, the dependence between  $P_m^N$  and  $\phi$  is as follows:

$$P_m^N = 1.00 - \frac{2.068\phi}{1 + 1.341\phi} \quad (7)$$

Eq. (6) yields a linear relationship between retention and the polarity of the eluent.

A variation of Eq. (6) has been proposed removing the restriction that all  $\log k$  vs.  $P_m^N$  lines must cross at the same point [38,43,45]. In this model, the solute is characterized by two descriptors ( $q$  and  $p$ ) according to the following equation:

$$\log k = q + pP_m^N \quad (8)$$

Since the number of solute fitting parameters is twice, the quality of the predictions with this model improved and the linearity range extended in reference to Eq. (6).

The change in the mobile phase composition affects not only to retention, but also to ionization of the acid–base solute. The addition of an organic solvent to a solution of an ionogenic compound in water changes its aqueous  $pK_a$  value. The variation of  $pK_a$  depends on different factors, such as the intrinsic acidities of the solute and solvents, solute size and charge, dielectric constant of the solvent and specific solute–solvent interactions [18]. For a particular solute, the solute parameters are constant and  $pK_a$  depends only on solvent properties (and temperature). When working in solvent mixtures, such as HPLC mobile phases, the solvent properties and thus  $pK_a$ , change monotonously with the solvent composition. Thus, several equations have been proposed to relate  $pK_a$  values to solvent composition expressed in solvent volume and also weight fraction. For acetonitrile–water mixtures at least up to 60% of acetonitrile this dependence can be described by a straight line between  $pK_a$  and the solvent volume fraction [40]:

$$pK_a = E + F\phi \quad (9)$$

Similarly, we can test a linear relationship of the same type between  $pK_a$  and the mobile phase polarity parameter:

$$pK_a = E + FP_m^N \quad (10)$$

### 3.5. Combined effect of pH and solvent composition

There are several models relating retention to simultaneous variation of pH and mobile phase composition, which can be obtained by combination of the pH and the different solvent composition models. Some of these models have been recently reviewed [38]. For simplicity and since we have data at only three mobile phase composition, we shall consider here only the models obtained by combination of the pH model (Eq. (1)) and the two-parameter solvent composition models (or linear models defined by Eqs. (5) or (8) for  $\log k$  and (9) or (10) for  $pK_a$ ).

With these premises the general equation obtained is

$$k = \frac{10^{A+Bx} + 10^{C+Dx} 10^{(pH-E-Fx)}}{1 + 10^{(pH-E-Fx)}} \quad (11)$$

where

$$\log k_{HA} = A + Bx \quad (12)$$

$$\log k_A = C + Dx \quad (13)$$

$$pK_a = E + Fx \quad (14)$$

and  $x$  is the appropriate variable used to describe mobile phase variation, i.e. it can be  $\phi$  or  $P_m^N$  depending on the solvent model chosen.

The  $A$ ,  $B$ ,  $C$ ,  $D$ ,  $E$ , and  $F$  parameters of this model have an easy chemical interpretation. If  $x$  is the volume fraction of organic modifier in the mobile phase,  $A$ ,  $C$ , and  $E$  are the extrapolations of the logarithms of retention factor of the acidic and basic forms and  $pK_a$  of the compound to pure water, respectively, and  $B$ ,  $D$  and  $F$  the variation of these parameters from pure water to pure organic modifier. In the same way, if the fits are done with the polarity parameter as  $x$  variable,  $A$ ,  $C$ , and  $E$  would be the retentions (in logarithmic scale) and  $pK_a$  in an hypothetical medium of zero polarity, and  $B$ ,  $D$ , and  $F$  the variation from this medium to pure water (which by definition has  $P_m^N = 1$ ).



### 3.6. Combined effect of pH, mobile phase composition and temperature

Eqs. (4) and (11) can be combined taking into account the simultaneous influence of variations in pH, mobile phase composition and temperature, to get the following single expression:

$$k = \frac{10^{(A_0 + (A_1/T) + B_0x + B_1(x/T))} + 10^{(C_0 + (C_1/T) + D_0x + D_1(x/T))} 10^{(pH - E_0 - (E_1/T) - F_0x - F_1(x/T))}}{1 + 10^{(pH - E_0 - (E_1/T) - F_0x - F_1(x/T))}} \quad (15)$$

This expression is obtained from Eq. (11) considering that the fitting parameters  $A$ ,  $B$ ,  $C$ ,  $D$ ,  $E$ , and  $F$  (which in fact are  $\log k$  or  $pK_a$  parameters) follow van't Hoff linear plots (Eqs. (2) and (3)). Again, the fits can be done at the different pH and temperatures in reference to mobile phase composition ( $\phi$ ) or polarity ( $P_m^N$ ).

## 4. Results and discussion

The results of retention factors of twenty-two ionizable compounds measured at twelve mobile phase pH, three acetonitrile compositions and three column temperatures (108 data points per solute) have been adjusted to Eq. (1). The solutes include several carboxylic acids, phenols and basic amines, with  $pK_a$  values between 4 and 11, appropriate for the wide mobile phase pH range studied (from 2 to 12 approximately, see Table 1). Tables 2–4 gather the parameters  $k_{HA}$ ,  $k_A$  and  $pK_a$  obtained from the fitting along with their standard deviations at the three temperatures and at 20, 40 and 60% (v/v) acetonitrile, respectively. The Tables show excellent fittings obtained for most of the experimental data, the only exceptions are those solutes poorly retained, i.e., the fitting of retention factors at the higher acetonitrile content and at 55 °C for a few poorly hydrophobic solutes.

The behavior exhibited by these parameters have been studied as follows. On one hand, the influence of temperature on these three parameters was studied. Figs. 1–3 show, as an example, the representation of  $\log k_{HA}$ ,  $\log k_A$ , and  $pK_a$ , respectively, at 40% (v/v) MeCN as a function of  $1/T$  for the studied compounds. Clearly, good linear trends can be observed in Figs. 1 and 2 (as it is expected from Eq. (2) when  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\log \phi$  are temperature independent) for the  $\log k$  vs.  $1/T$  plots of the neutral species of the studied compounds, i.e.  $\log k_{HA}$  for neutral acids (Fig. 1) and  $\log k_A$  for neutral bases (Fig. 2). Apparently, the linearity is not so good for the retention of the ionic species:  $\log k_A$  for neutral acids, which corresponds to retention of the anionic form (Fig. 2) and  $\log k_{HA}$  for neutral bases, which corresponds to retention of the cationic form (Fig. 1). However, it must be taken into account that the retention parameters of the ionic species are smaller than those of the neutral species

(see Tables 2–4) and thus the relative errors in these parameters are larger. Also, linear plots with very good correlation coefficients were exhibited by the representation of the  $pK_a$  vs.  $1/T$  (Fig. 3). From these results, we can conclude that the linear model can be adequate for the variation of retention of acid–base compounds with  $1/T$ . Even if the variation of the retention of ionic species may be not completely linear, these species are poorly retained and thus, the contribution of the error, in assuming linearity, to the overall retention of a partially ionized acid–base compounds is small.

On the other hand, the influence of solvent composition on the  $k_{HA}$ ,  $k_A$  and  $pK_a$  values (by keeping constant the temperature) was then evaluated. In Fig. 4, the retention of the acidic and basic solutes ( $\log k_{HA}$  and  $\log k_A$ ) as a function of mobile phase composition at a fixed temperature (40 °C) is depicted. In Fig. 5, the same retention parameters are presented but as a function of the polarity parameter  $P_m^N$ . In both figures, the points can be well represented by a straight line, as suggested by Eqs. (5) and (8), respectively. Again, the plots seem to be more linear for the  $\log k$  values of the neutral forms of the solute ( $\log k_{HA}$  for neutral acids and  $\log k_A$  for neutral bases) than for the ionic forms ( $\log k_A$  for neutral acids and  $\log k_{HA}$  for neutral bases), although the error introduced assuming linearity is small because of the low  $k$  values of these species.

The regression results indicate that, as a rule, slightly better fittings are obtained with the polarity parameter,  $P_m^N$  (Fig. 5), than with the solvent volume fraction as independent variable (Fig. 4). These observations are also extensive to the data obtained at the other two temperatures.

Fig. 6 shows the dependence of the  $pK_a$  of three carboxylic acids, three phenols and three amines at 40 °C with the eluent composition and properties. On the  $x$ -axis we included  $\phi$  and  $P_m^N$ . It is clear that the fitting quality depends on the solute. In some cases regression against  $P_m^N$  yields the best results, in other cases the regression against  $\phi$  yield the best ones. Similar results are obtained for 25 °C and 55 °C.

From this study we can conclude that there is a quite good linear dependence of  $\log k_{HA}$ ,  $\log k_A$ , and  $pK_a$  of the mobile phase composition or properties expressed in volume fraction of organic modifier ( $\phi$ ) or polarity parameter ( $P_m^N$ ), respectively. The linear dependence is slightly better for  $P_m^N$  than for  $\phi$ . However, there is not much difference in using any of these two parameters. This fact can be explained from the particular dependence of  $P_m^N$  on  $\phi$  at the three studied compositions, depicted in Fig. 7. Although, according to Eq. (7), the dependence is hyperbolic in the whole composition range (from 0 to 100% acetonitrile), a quite good straight line can be traced for the points of the three studied compositions (20, 40 and 60% acetonitrile) which lay in the middle part of the plot where curvature is small.

### 4.1. Modeling $k$ as a function of pH, temperature and solvent composition

After verifying the good linear behavior between the fitting parameters of Eq. (1) with both  $1/T$  and also solvent composition, we could combine two of these variables in a single equation, that is, by fitting  $k$ -values either to Eq. (4) or to Eq. (11).

On one hand, experimental  $k$ -values were fitted according to Eq. (4). The fitting parameters  $a$ ,  $b$ , ... and  $f$  for all the solutes at the three compositions were obtained and their dependence of eluent composition and properties was studied. Though clear trends of each parameter with solvent composition could be observed, the standard deviations associated to these fitting parameters were large, which prevented to get clear conclusions about linearity.

On the other hand, the fitting of the  $k$ -experimental data with pH and composition according to Eq. (11) yielded more clear results.

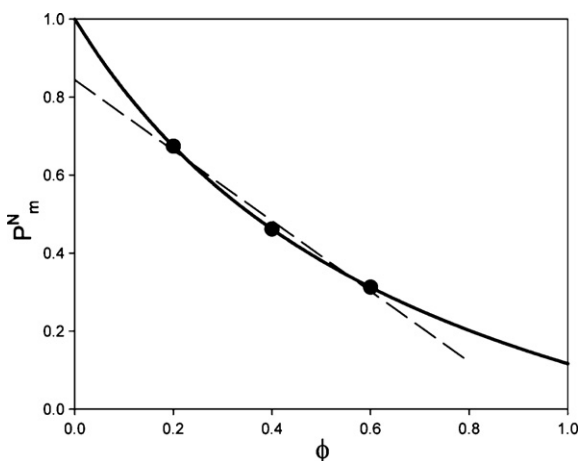


Fig. 7. Relationship between mobile phase polarity parameter  $P_m^N$  and volume fraction of acetonitrile ( $\phi$ ).

**Table 5**  
Results from the fitting of retention factors at different  $\xi_w$  pH, three temperatures and three mobile phase compositions ( $\phi$ ) to Eq. (15).

Solute	Fitting parameters													SE	R <sup>2</sup>
	A <sub>0</sub>	A <sub>1</sub> × 10 <sup>-3</sup>	B <sub>0</sub>	B <sub>1</sub> × 10 <sup>-3</sup>	C <sub>0</sub>	C <sub>1</sub> × 10 <sup>-2</sup>	D <sub>0</sub>	D <sub>1</sub> × 10 <sup>-3</sup>	E <sub>0</sub>	E <sub>1</sub> × 10 <sup>-3</sup>	F <sub>0</sub>	F <sub>1</sub> × 10 <sup>-3</sup>			
1	-4.1 ± 0.4	1.9 ± 0.1	8.5 ± 1.7	-3.8 ± 0.5	-2.3 ± 1.8	8.4 ± 5.6	4 ± 5	-1.6 ± 1.7	2.8 ± 3.8	2 ± 11	10 ± 18	-2.2 ± 5.6	0.48	0.997	
2	-3.3 ± 0.6	1.3 ± 0.2	5.5 ± 1.7	-2.2 ± 0.5	-0.3 ± 1	0.3 ± 3	-0.07 ± 2	-0.02 ± 0.8	0.8 ± 4.3	0.4 ± 1.3	28 ± 16	-7.3 ± 5.0	0.19	0.961	
3	-3.7 ± 0.4	1.6 ± 0.1	7.5 ± 1.6	-3.0 ± 0.5	-0.8 ± 1.1	3.1 ± 3.3	1.0 ± 3.0	-0.5 ± 0.9	3.5 ± 4.0	-0.4 ± 1.3	10 ± 17	-2.4 ± 5.3	0.26	0.984	
4	-4.1 ± 0.5	1.6 ± 0.1	7.8 ± 1.8	-3.1 ± 0.6	-1.9 ± 1.2	6.6 ± 3.8	3.1 ± 3.4	-1.2 ± 1.1	4.9 ± 4.5	-0.5 ± 1.4	6.4 ± 19	-0.99 ± 5.9	0.31	0.991	
5	-2.8 ± 0.4	1.2 ± 0.1	5.9 ± 1.3	-2.4 ± 0.4	-1.1 ± 1.2	3.3 ± 3.8	1.7 ± 3.2	-0.7 ± 1.0	3.2 ± 4.1	0.3 ± 1.2	12 ± 16	-2.6 ± 5.1	0.23	0.982	
6	-2.3 ± 0.1	1.01 ± 0.04	4.0 ± 0.4	-1.6 ± 0.1	2.5 ± 1.6	-8.0 ± 5.2	-2.1 ± 3.8	0.6 ± 1.1	11.8 ± 2.8	-0.5 ± 0.9	-29 ± 10	9.2 ± 3.3	0.12	0.993	
7	-3.5 ± 0.4	1.8 ± 0.1	6.9 ± 2.0	-3.3 ± 0.6	-4.0 ± 8.5	1.3 ± 2.6	10 ± 21	-3.4 ± 6.7	7.1 ± 4.3	0.2 ± 1.3	-8 ± 21	3 ± 6	1.20	0.995	
8	-3.9 ± 0.4	1.7 ± 0.1	6.6 ± 1.7	-2.7 ± 0.5	-3.4 ± 1.1	1.2 ± 0.3	5.8 ± 3.7	-2.2 ± 1.2	4.8 ± 3.9	-0.3 ± 1.2	1 ± 16	0.2 ± 5	0.40	0.986	
9	-4.1 ± 0.3	1.94 ± 0.09	8.0 ± 1.5	-3.5 ± 0.4	3.7 ± 4.4	-10 ± 14	-3 ± 13	0.8 ± 4	11.0 ± 4.8	-0.4 ± 1.4	-32 ± 22	10.2 ± 6.8	0.73	0.996	
10	-2.5 ± 0.2	1.27 ± 0.05	4.4 ± 0.6	-2.0 ± 0.2	-1.2 ± 1.9	3.6 ± 6.2	3.5 ± 5.2	-1.2 ± 1.6	5.8 ± 1.6	0.42 ± 0.49	-4.9 ± 6.7	2.1 ± 2.1	0.24	0.997	
11	-3.7 ± 0.5	1.96 ± 0.17	6.6 ± 2.6	-3.3 ± 0.8	-4.8 ± 9.0	1.7 ± 2.8	11 ± 33	-4 ± 10	5.7 ± 5.5	0.7 ± 1.7	-4.2 ± 26	1.9 ± 8.2	1.95	0.995	
12	-3.6 ± 0.3	1.72 ± 0.08	6.8 ± 1.2	-3.0 ± 0.3	-0.15 ± 3.2	1.5 ± 10	3.0 ± 9.5	-1.2 ± 3.0	7.8 ± 3.4	0.3 ± 1.0	-17.8 ± 16	5.97 ± 4.03	0.54	0.996	
13	-3.4 ± 0.3	1.58 ± 0.09	6.3 ± 1.2	-2.8 ± 0.4	7.8 ± 3.2	-2.3 ± 1.0	-13.8 ± 9.6	4.1 ± 3.0	0 ± 4	2.8 ± 1.3	-1.7 ± 19	1.1 ± 6.0	0.50	0.993	
14	-2.7 ± 0.2	1.25 ± 0.05	4.8 ± 0.7	-2.12 ± 0.2	5.9 ± 3.5	-1.8 ± 1.1	-7.1 ± 8.2	2.12 ± 2.6	12.8 ± 3.7	-0.7 ± 1.1	-35 ± 15	11.1 ± 4.9	0.26	0.994	
15	47 ± 7.9	-1.6 ± 2.4	-346 ± 469	11.7 ± 14.5	-8.76 ± 3.17	4.21 ± 0.99	575 ± 226	-260 ± 71	111 ± 34	-34 ± 11	-7960 ± 2492	-7.96 ± 2.49	0.70	0.979	
16	-0.08 ± 3.2	0.08 ± 0.9	-2.9 ± 10	0.8 ± 3.1	-2.87 ± 0.19	13.7 ± 0.6	4.66 ± 0.77	-2.16 ± 0.2	3.0 ± 3.9	0.2 ± 1.2	-23 ± 17	6.5 ± 5.4	0.34	0.997	
17	0.55 ± 0.97	-0.19 ± 0.30	-1.7 ± 2.7	0.5 ± 0.8	-1.54 ± 0.13	7.33 ± 0.42	2.21 ± 0.43	-1.04 ± 0.13	4.0 ± 3.3	0.1 ± 1.0	-27.7 ± 13	7.91 ± 4.02	0.12	0.989	
18	-3.2 ± 3.8	1.1 ± 1.2	3.9 ± 11.4	-1.3 ± 3.4	-1.25 ± 0.24	9.62 ± 0.74	1.0 ± 1.0	-1.1 ± 0.3	0 ± 4	1.7 ± 1.2	-0.02 ± 0.02	5.2 ± 5.3	0.70	0.993	
19	1.1 ± 2.8	-0.3 ± 0.9	-3.1 ± 6.4	1.0 ± 2.0	-6.70 ± 0.84	23.6 ± 2.6	11.5 ± 2.1	-3.76 ± 0.7	0 ± 9	2.3 ± 2.9	15.8 ± 24.3	-4.89 ± 7.5	0.88	0.870	
20	-0.9 ± 1.2	0.33 ± 0.40	2.0 ± 3.5	-0.7 ± 1.1	-1.89 ± 0.19	9.68 ± 0.57	3.05 ± 0.69	-1.51 ± 0.21	0.08 ± 4	1.5 ± 1.2	-14 ± 16	3.6 ± 5.1	0.23	0.991	
21	0.3 ± 1.3	-0.1 ± 0.4	-1.7 ± 3.6	0.6 ± 1.1	-0.53 ± 0.27	3.38 ± 0.85	0.77 ± 0.75	-0.47 ± 0.23	0 ± 6	1.8 ± 1.9	-36.0 ± 24	10.0 ± 7.4	0.16	0.935	
22	1.3 ± 4.1	-0.3 ± 1.2	-9.9 ± 15.7	2.8 ± 4.7	-1.84 ± 0.18	10.62 ± 0.6	2.95 ± 0.74	-1.62 ± 0.23	3.5 ± 3.4	0.09 ± 1.0	-25.2 ± 14	7.21 ± 4.47	0.37	0.995	

**Table 6**  
Results from the fitting of retention factors at different  $\xi_w$  pH, three temperatures and three mobile phase polarities ( $P_m^N$ ) to Eq. (15).

Solute	Fitting parameters													SE	R <sup>2</sup>
	A <sub>0</sub>	A <sub>1</sub> × 10 <sup>-3</sup>	B <sub>0</sub>	B <sub>1</sub> × 10 <sup>-3</sup>	C <sub>0</sub>	C <sub>1</sub> × 10 <sup>-2</sup>	D <sub>0</sub>	D <sub>1</sub> × 10 <sup>-3</sup>	E <sub>0</sub> × 10 <sup>-2</sup>	E <sub>1</sub> × 10 <sup>-3</sup>	F <sub>0</sub>	F <sub>1</sub> × 10 <sup>-3</sup>			
1	2.5 ± 0.9	-1.1 ± 0.3	-7.3 ± 1.4	3.4 ± 0.4	1.2 ± 3.1	-5.7 ± 9.6	-4.1 ± 5.1	1.7 ± 1.6	0.13 ± 0.10	-1.8 ± 3.1	-11 ± 15	2.4 ± 4.7	0.43	0.997	
2	1.7 ± 0.9	-0.65 ± 0.30	-5.8 ± 1.6	2.3 ± 0.5	-0.1 ± 1	-0.5 ± 4	-0.2 ± 3	0.1 ± 0.8	0.2 ± 0.1	-4.4 ± 3.1	-21 ± 15	5.0 ± 4.9	0.18	0.963	
3	2.3 ± 0.9	-0.91 ± 0.29	-6.8 ± 1.5	2.8 ± 0.4	0.2 ± 1.7	-2.1 ± 5.3	-1.2 ± 2.9	0.6 ± 0.9	11.7 ± 10.0	-1.7 ± 3.1	-9.2 ± 15	1.8 ± 4.7	0.24	0.986	
4	2.4 ± 1.0	-0.92 ± 0.32	-7.2 ± 1.6	2.9 ± 0.5	0.9 ± 1.9	-4.5 ± 6.12	-3.4 ± 3.4	1.31 ± 1.05	8.9 ± 10.8	-0.8 ± 3.3	-3.9 ± 16	-0.09 ± 5	0.28	0.984	
5	2.1 ± 0.7	-0.80 ± 0.23	-5.5 ± 1.2	2.3 ± 0.4	0.7 ± 1.7	-3.8 ± 5.4	-2.2 ± 3.0	0.85 ± 0.96	11.5 ± 8.9	-1.3 ± 2.7	-8.7 ± 13	1.52 ± 4.3	0.20	0.986	
6	1.2 ± 0.3	-0.45 ± 0.084	-4.0 ± 0.4	1.7 ± 0.1	1.9 ± 2.6	-6.6 ± 8.4	0.1 ± 4.8	0.06 ± 1.5	-0.14 ± 0.07	8.04 ± 2.4	31 ± 12	-9.9 ± 3.7	0.12	0.993	
7	1.8 ± 1.3	-0.80 ± 0.39	-5.9 ± 1.9	2.9 ± 0.6	5.4 ± 14	-1.8 ± 4.6	-10 ± 25	3.6 ± 7.9	0.007 ± 0.1	2.7 ± 4.0	7.0 ± 19	-2.7 ± 5.9	1.16	0.995	
8	1.4 ± 1.0	-0.59 ± 0.33	-6.0 ± 1.6	2.6 ± 0.5	1.6 ± 2.3	-7.2 ± 7.3	-5.7 ± 3.8	2.2 ± 1.2	0.05 ± 0.10	0.04 ± 3.2	-0.7 ± 15	-0.47 ± 4.8	0.39	0.986	
9	1.9 ± 0.8	-0.85 ± 0.25	-6.6 ± 1.2	-3.08 ± 0.4	-2.3 ± 10	5.3 ± 32	8.1 ± 16.4	-21 ± 5.2	0 ± 14	3.5 ± 4.3	6.5 ± 21	-2.7 ± 6.5	0.65	0.997	
10	1.1 ± 0.5	-0.44 ± 0.15	-4.1 ± 0.7	1.9 ± 0.2	2.1 ± 3.9	-8 ± 12	-4.1 ± 7.1	1.4 ± 2.2	0 ± 5.2	2.2 ± 1.6	4.9 ± 8.0	-2.1 ± 2.5	0.30	0.996	
11	1.4 ± 1.6	-0.71 ± 0.50	-5.7 ± 2.4	2.9 ± 0.7	3.7 ± 24	-1.4 ± 7.5	-9.2 ± 37	3.4 ± 11	2.9 ± 16	2.1 ± 5.1	2.8 ± 24	-1.5 ± 7.6	1.93	0.995	
12	1.6 ± 0.7	-0.69 ± 0.21	-5.7 ± 1.0	2.7 ± 0.3	1.3 ± 7.2	-0.6 ± 2.2	-1.3 ± 11	0.7 ± 3.6	0 ± 9	3.3 ± 2.9	6.1 ± 14	-2.4 ± 4.4	0.50	0.996	
13	1.5 ± 0.7	-0.66 ± 0.22	-5.4 ± 1.1	2.5 ± 0.3	-3.8 ± 6.8	1.0 ± 2.2	13 ± 11	-3.7 ± 3.5	0 ± 11	2.4 ± 3.4	-5.4 ± 17	0.9 ± 5.1	0.46	0.994	
14	1.0 ± 0.4	-0.46 ± 0.11	-4.0 ± 0.6	1.89 ± 0.18	-1.4 ± 4.8	0.3 ± 1.5	8.5 ± 8.6	-2.5 ± 2.7	0 ± 11	3.5 ± 3.5	8.2 ± 17	-2.9 ± 5.3	0.22	0.996	
15	0.4 ± 2.8	-0.1 ± 0.8	0.5 ± 5.5	-0.18 ± 1.7	1.1 ± 0.8	-5.2 ± 2.4	-2.7 ± 1.2	1.65 ± 0.37	0 ± 13	1.6 ± 3.9	2.1 ± 19	0.008 ± 5	0.46	0.991	
16	4.4 ± 6.1	-1.4 ± 1.9	-7.9 ± 9.9	2.6 ± 3.1	1.0 ± 0.5	-4.4 ± 1.5	-4.2 ± 0.7	2.0 ± 0.2	0 ± 12	0.5 ± 3.8	-3 ± 18	1.6 ± 5.7	0.33	0.995	
17	1.7 ± 1.6	-0.5 ± 0.5	-2.5 ± 2.9	0.8 ± 0.9	0.49 ± 0.31	-2.2 ± 0.95	-2.4 ± 0.5	1.11 ± 0.15	0 ± 9	0.7 ± 2.9	-3.5 ± 15	1.8 ± 4.5	0.14	0.986	
18	5.1 ± 7.1	1.1 ± 1.2	3 ± 11	-1.3 ± 3.4	-1.25 ± 0.24	9.62 ± 0.74	1.0 ± 1.0	-1.1 ± 0.3	0 ± 4	1.7 ± 1.2	-0.02 ± 0.02	5.2 ± 5.3	0.70	0.993	
19	0.4 ± 3.6	-0.04 ± 1.1	-0.7 ± 7.5	0.1 ± 2	5.47 ± 1.32	-1.5 ± 0.4	-15 ± 2	4.95 ± 0.7	0.4 ± 0.1	-1.1 ± 4.3	-77 ± 27	23.3 ± 8.1	0.91	0.862	
20	2.7 ± 2.0	-0.88 ± 0.64	-5.0 ± 3.6	1.7 ± 1.1	0.7 ± 0.4	-3.3 ± 1.2	-2.9 ± 0.6	1.49 ± 0.20	0 ± 10	0.8 ± 3.2	-5 ± 16	2.4 ± 4.9	0.22	0.992	
21	1.5 ± 1.3	-0.4 ± 0.4	-2.8 ± 2.5	0.8 ± 0.8	0.42 ± 0.37	-1.7 ± 1.1	-1.28 ± 0.66	0.66 ± 0.21	0 ± 12	2.2 ± 0.5	-19.2 ± 3.1	7.3 ± 0.9	0.14	0.953	
22	5.5 ± 5.5	-1.8 ± 1.7	-9.6 ± 9.5	3.2 ± 3.0	0.62 ± 0.50	-3.17 ± 1.55	-2.7 ± 0.8	1.56 ± 0.24	0 ± 11	1.92 ± 0.45	-5.7 ± 1.9	2.45 ± 0.55	0.41	0.994	

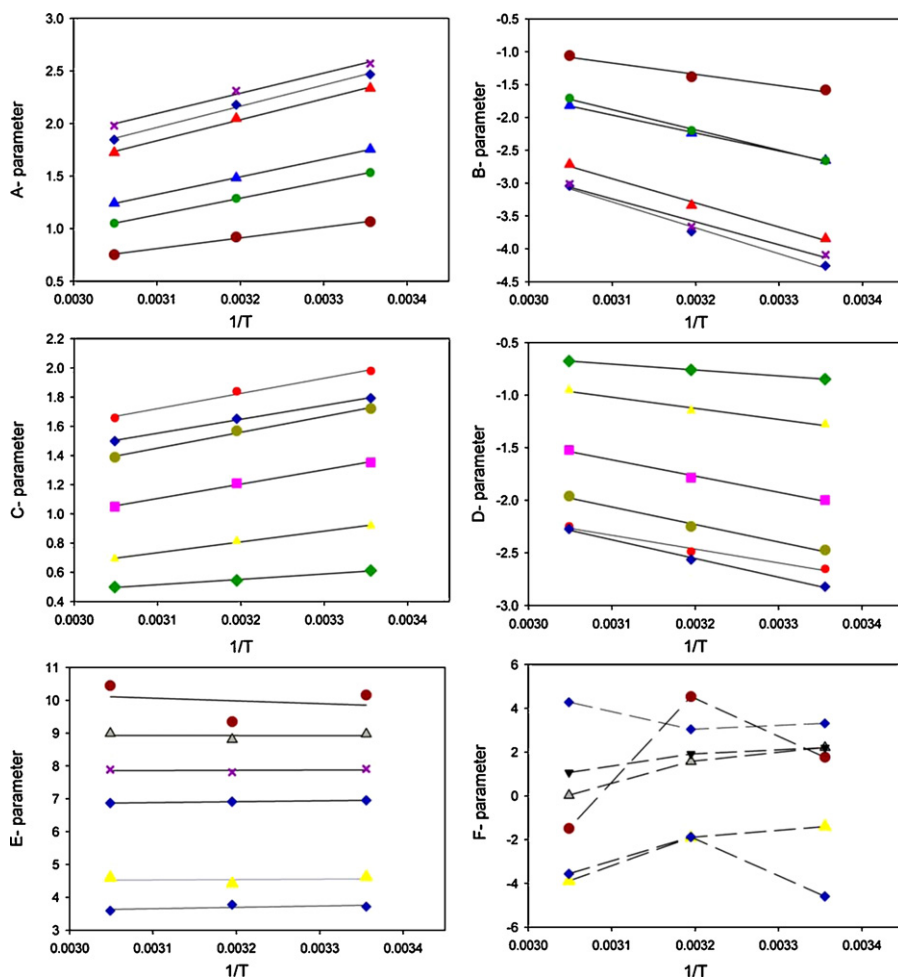


Fig. 8. Plots of the fitting parameters A, B, C, D, E and F of Eq. (11) against  $1/T$ . Symbols as in Fig. 1.

**Table 7**  
Results from the fitting of retention factors at twelve  $s_w$  pH, three temperatures and three mobile phase compositions ( $\phi$ ) to Eqs. (16) and (17) for acids and bases, respectively.

Solute	Fitting parameters									SE	$R^2$
	$A_0$	$A_1 \times 10^{-3}$	$B_0$	$B_1 \times 10^{-3}$	$C_0$	$E_0$	$F_0$	$F_1 \times 10^{-3}$			
1	$-4.14 \pm 0.39$	$1.97 \pm 0.14$	$8.6 \pm 1.8$	$-3.8 \pm 0.5$	$-0.07 \pm 0.03$	$3.83 \pm 0.15$	$5.4 \pm 3.0$	$-0.78 \pm 0.9$	0.55	0.996	
2	$-3.39 \pm 0.46$	$1.34 \pm 0.14$	$5.6 \pm 1.3$	$-2.22 \pm 0.42$	$-0.22 \pm 0.01$	$2.16 \pm 0.15$	$22.8 \pm 5.9$	$-5.7 \pm 1.8$	0.19	0.960	
3	$-3.75 \pm 0.43$	$1.58 \pm 0.13$	$7.3 \pm 1.7$	$-2.96 \pm 0.53$	$-0.133 \pm 0.02$	$3.53 \pm 0.17$	$10.6 \pm 5.6$	$-2.6 \pm 1.8$	0.29	0.978	
4	$-3.95 \pm 0.48$	$1.65 \pm 0.15$	$7.6 \pm 1.9$	$-3.04 \pm 0.59$	$-0.113 \pm 0.023$	$3.46 \pm 0.20$	$11.7 \pm 6.4$	$-2.9 \pm 2.0$	0.35	0.974	
5	$-2.85 \pm 0.35$	$1.24 \pm 0.11$	$6.0 \pm 1.3$	$-2.44 \pm 0.41$	$-0.20 \pm 0.02$	$4.31 \pm 0.17$	$7.0 \pm 5.8$	$-1.31 \pm 1.8$	0.24	0.979	
6	$-2.30 \pm 0.12$	$1.00 \pm 0.04$	$3.91 \pm 0.4$	$-1.64 \pm 0.13$	$-0.06 \pm 0.03$	$10.23 \pm 0.09$	$-15.2 \pm 3.3$	$4.9 \pm 1.0$	0.12	0.993	
7	$-3.62 \pm 0.41$	$1.84 \pm 0.13$	$7.1 \pm 1.9$	$-3.33 \pm 0.59$	$-0.10 \pm 0.13$	$7.89 \pm 0.15$	$-12.6 \pm 3.3$	$4.4 \pm 1.0$	1.18	0.995	
8	$-3.87 \pm 0.60$	$1.67 \pm 0.18$	$6.4 \pm 2.4$	$-2.68 \pm 0.76$	$0.03 \pm 0.03$	$4.10 \pm 0.23$	$2.5 \pm 7.5$	$-0.5 \pm 2.2$	0.60	0.967	
9	$-4.13 \pm 0.29$	$1.93 \pm 0.09$	$7.8 \pm 1.4$	$-3.47 \pm 0.42$	$0.13 \pm 0.08$	$9.85 \pm 0.16$	$-19.3 \pm 4.0$	$6.3 \pm 1.2$	0.73	0.996	
10	$-2.62 \pm 0.15$	$1.29 \pm 0.45$	$4.65 \pm 0.59$	$-2.11 \pm 0.18$	$-0.24 \pm 0.03$	$7.25 \pm 0.05$	$-10.0 \pm 1.9$	$3.61 \pm 0.61$	0.24	0.997	
11	$-3.79 \pm 0.51$	$1.98 \pm 0.16$	$6.9 \pm 2.5$	$-3.41 \pm 0.76$	$0.11 \pm 0.13$	$8.09 \pm 0.20$	$-16.3 \pm 3.6$	$5.5 \pm 1.1$	1.95	0.995	
12	$-3.71 \pm 0.25$	$1.73 \pm 0.07$	$6.99 \pm 1.13$	$-3.08 \pm 0.35$	$0.06 \pm 0.05$	$9.04 \pm 0.12$	$-22.2 \pm 3.1$	$7.21 \pm 0.94$	0.54	0.995	
13	$-3.55 \pm 0.29$	$1.63 \pm 0.09$	$6.8 \pm 1.3$	$-2.96 \pm 0.39$	$0.12 \pm 0.05$	$9.41 \pm 0.15$	$-34.0 \pm 4.4$	$10.8 \pm 1.3$	0.53	0.992	
14	$-2.62 \pm 0.17$	$1.23 \pm 0.05$	$4.53 \pm 0.67$	$-2.04 \pm 0.21$	$-0.04 \pm 0.06$	$10.49 \pm 0.13$	$-15.0 \pm 4.0$	$4.8 \pm 1.2$	0.26	0.994	
Solute	Fitting parameters									SE	$R^2$
$A_0$	$C_0$	$C_1 \times 10^{-2}$	$D_0$	$D_1 \times 10^{-3}$	$E_0$	$F_0$	$F_1 \times 10^{-3}$				
15	$0.02 \pm 0.04$	$-1.36 \pm 0.34$	$9.39 \pm 1.06$	$3.0 \pm 1.4$	$-1.74 \pm 0.45$	$6.99 \pm 0.19$	$-27.4 \pm 5.1$	$8.1 \pm 1.6$	0.56	0.986	
16	$0.08 \pm 0.04$	$-2.84 \pm 0.18$	$1.36 \pm 0.06$	$4.54 \pm 0.74$	$-2.12 \pm 0.23$	$3.66 \pm 0.12$	$-23.6 \pm 3.6$	$6.8 \pm 1.1$	0.34	0.995	
17	$-0.04 \pm 0.02$	$-1.52 \pm 0.13$	$7.28 \pm 0.40$	$2.16 \pm 0.41$	$-1.03 \pm 0.13$	$4.58 \pm 0.10$	$-29.1 \pm 3.7$	$8.3 \pm 1.1$	0.11	0.990	
18	$0.07 \pm 0.06$	$-1.13 \pm 0.24$	$9.27 \pm 0.74$	$0.6 \pm 1.0$	$-0.97 \pm 0.31$	$5.50 \pm 0.15$	$-38.4 \pm 4.6$	$10.9 \pm 1.4$	0.72	0.993	
19	$0.14 \pm 0.04$	$-6.38 \pm 0.75$	$2.27 \pm 0.23$	$10.6 \pm 1.9$	$-3.51 \pm 0.58$	$7.77 \pm 0.27$	$-3.4 \pm 10.5$	$0.7 \pm 3.2$	0.87	0.868	
20	$0.03 \pm 0.02$	$-1.83 \pm 0.18$	$9.49 \pm 0.56$	$2.87 \pm 0.68$	$-1.45 \pm 0.21$	$4.94 \pm 0.14$	$-30.4 \pm 4.7$	$8.9 \pm 1.4$	0.24	0.991	
21	$-0.07 \pm 0.02$	$-0.41 \pm 0.26$	$3.02 \pm 0.83$	$0.48 \pm 0.74$	$-0.39 \pm 0.23$	$5.95 \pm 0.23$	$-50.7 \pm 7.8$	$14.4 \pm 2.4$	0.16	0.930	
22	$0.06 \pm 0.05$	$-1.80 \pm 0.17$	$1.05 \pm 0.05$	$2.82 \pm 0.72$	$-1.58 \pm 0.22$	$3.64 \pm 0.11$	$-22.9 \pm 3.4$	$6.6 \pm 1.0$	0.37	0.994	

**Table 8**Results from the fitting of retention factors at several  $s_w$  pH, three temperatures and three mobile phase polarities ( $P_m^N$ ) to Eqs. (16) and (17) for acids and bases, respectively.

Solute	Fitting parameters									SE	$R^2$
	$A_0$	$A_1 \times 10^{-3}$	$B_0$	$B_1 \times 10^{-3}$	$C_0$	$E_0$	$F_0$	$F_1 \times 10^{-2}$			
1	2.7 ± 1.0	-1.1 ± 0.3	-7.4 ± 1.5	3.4 ± 0.5	-0.07 ± 0.03	6.55 ± 0.45	-2.4 ± 1.0	-2.3 ± 2.5	0.50	0.997	
2	2.3 ± 0.8	-0.82 ± 0.26	-6.7 ± 1.5	2.6 ± 0.5	-0.22 ± 0.02	6.4 ± 0.4	0.7 ± 2.6	-18 ± 7	0.19	0.961	
3	2.5 ± 1.0	-0.9 ± 0.3	-7.0 ± 1.6	2.8 ± 0.5	-0.14 ± 0.02	5.7 ± 0.5	0.1 ± 2	-8.3 ± 5.9	0.28	0.980	
4	2.3 ± 1.1	-0.92 ± 0.35	-7.1 ± 1.7	2.92 ± 0.55	-0.12 ± 0.02	5.8 ± 0.5	0.3 ± 2.3	-9.6 ± 6.6	0.33	0.977	
5	2.1 ± 0.7	-0.82 ± 0.23	-5.6 ± 1.2	2.34 ± 0.36	-0.20 ± 0.02	6.9 ± 0.4	-2.1 ± 2.1	-3.2 ± 6.2	0.22	0.983	
6	0.90 ± 0.26	-0.37 ± 0.08	-3.6 ± 0.4	1.56 ± 0.13	-0.09 ± 0.03	11.5 ± 0.3	-5.7 ± 1.4	13 ± 4	0.13	0.992	
7	1.5 ± 1.1	-0.71 ± 0.36	-5.5 ± 1.7	2.8 ± 0.5	-0.14 ± 0.14	9.45 ± 0.47	-6.2 ± 1.2	14 ± 3	1.15	0.995	
8	1.3 ± 1.5	-0.56 ± 0.47	-5.8 ± 2.4	2.5 ± 0.7	0.03 ± 0.03	4.9 ± 0.7	-0.4 ± 2.7	-2 ± 7	0.60	0.967	
9	1.67 ± 0.77	-0.7 ± 0.24	-6.3 ± 1.2	3.0 ± 0.4	0.09 ± 0.09	11.4 ± 0.5	-7.8 ± 1.3	17 ± 3	0.67	0.996	
10	0.82 ± 0.46	-0.36 ± 0.14	-3.7 ± 0.7	1.8 ± 0.2	-0.25 ± 0.04	8.65 ± 0.20	-5.8 ± 0.9	13 ± 3	0.31	0.995	
11	1.1 ± 1.5	-0.61 ± 0.47	-5.2 ± 2.3	2.8 ± 0.7	0.08 ± 0.14	9.5 ± 0.6	-7.0 ± 1.4	17 ± 3	1.94	0.995	
12	1.28 ± 0.67	-0.60 ± 0.21	-5.3 ± 1.0	2.5 ± 0.3	0.03 ± 0.06	10.22 ± 0.37	-8.8 ± 1.1	23 ± 3	0.52	0.996	
13	1.31 ± 0.72	-0.60 ± 0.22	-5.1 ± 1.1	-2.4 ± 0.3	0.08 ± 0.05	10.67 ± 0.45	-13.0 ± 1.5	35 ± 4	0.50	0.993	
14	0.94 ± 0.36	-0.4 ± 0.1	-3.94 ± 0.55	-1.87 ± 0.17	-0.09 ± 0.07	8.86 ± 0.85	-72 ± 88	614 ± 167	0.23	0.995	

Solute	Fitting parameters									SE	$R^2$
	$A_0$	$C_0$	$C_1 \times 10^{-2}$	$D_0$	$D_1 \times 10^{-3}$	$E_0$	$F_0$	$F_1 \times 10^{-3}$			
15	0.01 ± 0.03	1.2 ± 0.7	-5.5 ± 2.3	-2.9 ± 1.1	1.70 ± 0.35	5.22 ± 0.49	-5.9 ± 1.6	2.53 ± 0.42	0.45	0.991	
16	0.06 ± 0.05	1.0 ± 0.5	-4.5 ± 1.4	-4.36 ± 1.44	2.07 ± 0.22	2.15 ± 0.39	-5.6 ± 1.3	2.29 ± 0.38	0.33	0.995	
17	-0.04 ± 0.02	0.55 ± 0.29	-2.4 ± 0.9	-2.47 ± 0.50	1.14 ± 0.16	2.51 ± 0.31	-7.9 ± 1.9	3.2 ± 0.6	0.14	0.986	
18	0.07 ± 0.07	-0.2 ± 0.7	-0.3 ± 2	-1.2 ± 1.1	1.16 ± 0.34	2.73 ± 0.48	-11.2 ± 1.8	4.46 ± 0.54	0.82	0.991	
19	0.14 ± 0.04	3.8 ± 1.1	-1.06 ± 0.34	-12 ± 2	4.08 ± 0.65	7.37 ± 0.53	-7.9 ± 7.7	2.5 ± 2.3	0.91	0.858	
20	0.03 ± 0.02	0.7 ± 0.4	-34 ± 1.3	-2.99 ± 0.65	1.50 ± 0.20	3.35 ± 0.41	-8.7 ± 1.7	3.3 ± 0.5	0.23	0.991	
21	-0.078 ± 0.016	0.4 ± 0.3	-1.8 ± 1.1	-1.3 ± 0.6	6.7 ± 2.0	1.9 ± 0.4	-19 ± 3	7.23 ± 0.86	0.14	0.952	
22	0.05 ± 0.05	0.6 ± 0.5	-3.3 ± 1.6	-2.82 ± 0.77	1.58 ± 0.23	2.31 ± 0.39	-5.8 ± 1.3	2.30 ± 0.40	0.41	0.993	

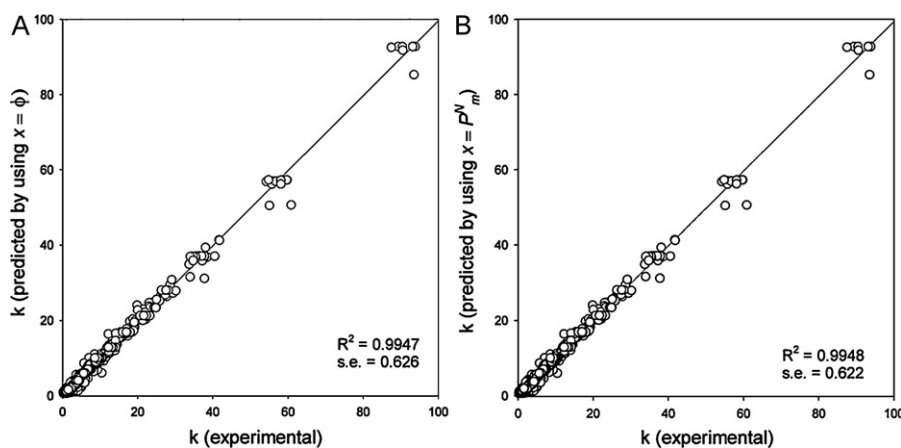
The fitting parameters  $A, B, \dots, F$  along with their standard deviations were estimated; their values were then plotted against  $1/T$ . The results for some solutes are depicted in Fig. 8. The main conclusions that could be made from these plots are as follows:

- Parameters  $A$  and  $B$  (related to  $\log k_{HA}$ ) as a function of  $1/T$  are linear for neutral acids. Large relative standard deviations for neutral bases, however, prevented of observing any clear trend for these parameters and solutes. This was expected since these two parameters reflect retentive properties of the protonated forms of the neutral bases, which are poorly retained.
- Parameters  $C$  and  $D$  (related to  $\log k_A$ ) corresponding to neutral bases plotted against  $1/T$  were linear. Similarly, the  $C$  and  $D$  values for neutral acids have associated large relative errors due to they represent the retentive features of ionized species (anionic carboxylates and phenolates) poorly retained.
- Parameter  $E$  (related to  $pK_a$ ) is slightly dependent on  $T$  for all the solutes and

- the plots of parameter  $F$  (related to  $pK_a$  variation with mobile phase composition) with  $1/T$  did not show a defined linear trend for most solutes.

Analogous conclusions could be obtained when the  $k$ -data were fitted to Eq. (11) but by using the polarity parameter  $P_m^N$  instead of acetonitrile volume fraction and, then, the corresponding fitting parameters are plotted against the reciprocal of temperature.

Two practical decisions can be obtained from these last results. First, they indicate that the retention of the ionized species studied in this work, i.e.  $A^-$  for acids and  $HA^+$  for bases, is almost insensitive to solvent strength or temperature. From a practical point of view, this means that we can consider their retention factors as constant at all the temperatures and mobile phase compositions studied. Second, since the  $E$  values are almost not dependent on  $T$ , we can consider they are constant and the same  $E$  fitting parameter value holds for all studied temperatures.



**Fig. 9.** Predicted  $k$ -values for 2376 data points corresponding to the 22 solutes run in 12 buffers at three temperatures and at three solvent compositions as a function of the corresponding experimental data. Plot A: predictions by using  $x = \phi$ ; plot B: predictions with  $x = P_m^N$  (see the text).

In order to test the validity of the general model, the whole set of experimental data were fitted simultaneously to the three relevant parameters: pH, temperature and composition, expressed as volume fraction and also by its polarity (Eq. (15)). According to this equation, twelve fitting parameters would be necessary to describe retention. The results obtained are presented in Table 5 (for  $\phi$ ) and Table 6 (for  $P_m^N$ ).

The parameters of Table 5 confirm the main conclusions obtained above, i.e., that for the solutes included in this study, some of these parameters are statistically equal to zero, and only eight fitting parameters are strictly necessary to describe the variation of retention with pH, temperature and mobile phase composition. Neutral acids can be fitted to a modified Eq. (15) which do not include  $C_1$ ,  $D_0$ ,  $D_1$  and also  $E_1$  parameters:

$$k = \frac{10^{(A_0 + (A_1/T) + B_0x + B_1(x/T))} + 10^{C_0} 10^{(pH - E_0 - F_0x - F_1(x/T))}}{1 + 10^{(pH - E_0 - F_0x - F_1(x/T))}} \quad (16)$$

where  $x$  may be either the volume fraction ( $\phi$ ) or the mobile phase polarity parameter ( $P_m^N$ ). On the other hand, neutral bases can be well fitted to a similar equation without  $A_1$ ,  $B_0$ ,  $B_1$  and  $E_1$  parameters, instead of  $C_1$ ,  $D_0$ ,  $D_1$  and  $E_1$  parameters:

$$k = \frac{10^{A_0} + 10^{(C_0 + (C_1/T) + D_0x + D_1(x/T))} 10^{(pH - E_0 - F_0x - F_1(x/T))}}{1 + 10^{(pH - E_0 - F_0x - F_1(x/T))}} \quad (17)$$

Two new fittings were performed for each solute, corresponding to Eqs. (16) and (17) in which the volume fraction ( $\phi$ ) or polarity parameter ( $P_m^N$ ) are the input  $x$ -values. The results of these calculations are presented in Table 7 ( $\phi$ ) and Table 8 ( $P_m^N$ ). The results of both Tables are quite similar in terms of precision, although the statistics are slightly better using  $P_m^N$  as the independent input for modeling the dependence of retention factor with mobile phase variation. As it can be noted, the fittings of  $k$ -data with eight parameters were quite good, similar to those of the general model with the 12 parameter equations.

Fig. 9 shows a representation of the correlations for 2396  $k$ -data points corresponding to the 22 solutes run in 12 buffers at three temperatures and at three solvent compositions. Plot A corresponds to predictions obtained by using  $\phi$  as mobile phase composition variable, and plot B correspond to values predicted by using  $P_m^N$ . The correlation coefficients and residual errors in each case are also indicated in the plots.

## 5. Conclusions

In this study, we focused in the development of simple models to predict RPLC retention factors of ionizable solutes over the chromatographic useful pH range (between pH 2 and 12) within a wide range of acetonitrile composition (20–60%) and in the range of temperatures between 25 and 55 °C. To achieve the aims, we explore different equations. First, the dependence of retention with the mobile phase pH was studied according to the common sigmoidal model, and three fitting parameters,  $k_{HA}$ ,  $k_A$  and the inflection point of the sigmoidal curve, which should be coincident with the  $pK_a$ , were obtained for all the solutes.

On one hand, the dependence of the three fitting parameters in logarithmic form, i.e.  $\log k_{HA}$ ,  $\log k_A$ , and  $pK_a$ , on temperature was studied according to van't Hoff model, concluding that van't Hoff plots close to linearity hold in most instances. Thus, a linear model can be employed to describe the variation of retention parameter and  $pK_a$  with the reverse of the absolute temperature. This model can be combined with the pH model to get a 6 parameter model describing variation of retention with simultaneous variation of pH and temperature in a fixed mobile phase composition.

On the other hand, good linear relationships of  $\log k_{HA}$ ,  $\log k_A$  and  $pK_a$  with mobile phase composition (volume fraction of ace-

tonitrile) and mobile phase polarity ( $P_m^N$ ) were also observed, allowing proposal of linear models for the variation of these parameters with eluent composition. Combination of these models with the pH model leads to simple 6 parameter models that relates retention to pH and mobile phase composition or polarity at constant temperature.

We also derived two general 12 parameter equations to relate retention of acid–base compounds to mobile phase pH, temperature and composition or polarity. The hardly retained ionic forms of the solutes included in this study showed that their retention factors were almost independent of temperature and also of solvent composition.  $pK_a$  variation with temperature is also negligible for most compounds. Thus, the 12 parameter equations can be simplified to 8 parameters equations able to predict retention at given pH,  $T$  and mobile phase composition between the studied ranges.

## List of symbols

$a$	intercept for the linear variation of $\log k_{HA}$ with $1/T$
$A$	intercept for the linear variation of $\log k_{HA}$ with $\phi$ or $P_m^N$
$A_0$	intercept for the linear variation of $A$ with $1/T$
$A_1$	slope for the linear variation of $A$ with $1/T$
$b$	slope for the linear variation of $\log k_{HA}$ with $1/T$
$B$	slope for the linear variation of $\log k_{HA}$ with $\phi$ or $P_m^N$
$B_0$	intercept for the linear variation of $B$ with $1/T$
$B_1$	slope for the linear variation of $B$ with $1/T$
$c$	intercept for the linear variation of $\log k_A$ with $1/T$
$C$	intercept for the linear variation of $\log k_A$ with $\phi$ or $P_m^N$
$C_0$	intercept for the linear variation of $C$ with $1/T$
$C_1$	slope for the linear variation of $C$ with $1/T$
$d$	slope for the linear variation of $\log k_A$ with $1/T$
$D$	slope for the linear variation of $\log k_A$ with $\phi$ or $P_m^N$
$D_0$	intercept for the linear variation of $D$ with $1/T$
$D_1$	slope for the linear variation of $D$ with $1/T$
$e$	intercept for the linear variation of $pK_a$ with $1/T$
$E$	intercept for the linear variation of $pK_a$ with $\phi$ or $P_m^N$
$E_0$	intercept for the linear variation of $E$ with $1/T$
$E_1$	slope for the linear variation of $E$ with $1/T$
$f$	slope for the linear variation of $pK_a$ with $1/T$
$F$	slope for the linear variation of $pK_a$ with $\phi$ or $P_m^N$
$F_0$	intercept for the linear variation of $F$ with $1/T$
$F_1$	slope for the linear variation of $F$ with $1/T$
$\phi$	volume fraction of organic modifier
$\Phi$	volumetric phase ratio
$\Delta H^\circ$	change in enthalpy for the transfer of one molecule of solute between the mobile and stationary phases
$\Delta H_a^\circ$	change in enthalpy due to solute dissociation
$k$	retention factor
$k_A$	retention factor of the basic form of an ionizable compound
$k_{HA}$	retention factor of the acid form of an ionizable compound
$(\log k)_0$	intercept of the correlation in the polarity parameter model
$\log k_w$	retention of the compound extrapolated to pure water
$P_m^N$	polarity parameter of the mobile phase in the polarity parameter model
$P_s^N$	polarity parameter of the stationary phase in the polarity parameter model
$p$	solute polarity parameter in the polarity parameter model
$p_w^H$	pH value in water
$p_s^H$	pH value in solvent $s$ in reference to water ( $w$ ) as standard state solvent In practice, pH measured in a mobile phase with an electrode system calibrated with aqueous standards

$\xi$ pH	pH value in solvent <i>s</i> in reference to the same solvent as standard state solvent In practice, pH measured in a mobile phase with an electrode system calibrated with standards prepared in the same mobile phase
${}^w_pK_a$	aqueous $pK_a$ value
${}^s_pK_a$	$pK_a$ value in solvent <i>s</i> in the ${}^s_pH$ scale
<i>q</i>	solute descriptor in the polarity parameter model
<i>R</i>	gas constant
<i>S</i>	sensitivity of the solute retention to the solvent strength
$\Delta S^\circ$	change in entropy for the transfer of one molecule of solute between mobile and stationary phases
$\Delta S_a^\circ$	change of entropy due to solute dissociation
<i>T</i>	absolute temperature

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