Linear free energy relationship models for the retention of partially ionized acid-base compounds in reversed-phase liquid chromatography

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Abstract

The LFER model of Abraham is applied to the retention of the neutral and ionic forms of 94 solutes in a C18 column and 40% v/v acetonitrile/water mobile phase. The results show that polarizability and cavity formation interactions increase retention, whereas dipole and hydrogen bonding interactions favours partition to the mobile phase and thus, they decrease retention. The coefficients of the ionic descriptors measure the effect of the electrostatic interactions and their contribution to partition of the cation or anion between the two mobile and stationary chromatographic phases.

A new LFER model for application to the retention of partially dissociated acids and bases is derived averaging the descriptors of the neutral and ionic forms according to their degrees of ionization in the mobile phase. This new LFER model is satisfactorily compared to other literature modified Abraham models for a set of 498 retention data of partially dissociated acids and bases.

All tested models require the calculation of the ionization degrees of the compounds at the measuring pH. Calculation of the ionization degrees in the chromatographic mobile phase (i.e. from pH and pK_a in the eluent) give good correlations for all tested models. However, estimation of these ionization degrees from pH – pK_a data in pure water gives biased estimations of the retention of the partially ionized solutes.

Keywords: Chromatographic retention; Retention models; Acid-base ionization; Linear free energy relationships; Solvation parameter model.

1. Introduction

Retention in liquid chromatography is a complex process, which depends on different physical and chemical factors. Among the chemical factors, the most important are the nature of the solute, the composition of the mobile phase and the nature of the stationary phase [1]. Many different models have been developed to account for these factors and thus characterize different chromatographic systems [2,3]. Characterization with reliable and well designed models leads to significant advances in the knowledge of the fundamental chemical interactions that rule the complex chromatographic retention processes. From a practical point of view, characterization and parametrization of the different type of solute-solvent interactions in specific mobile-stationary phase systems allows one to predict the selectivity of these systems towards certain types of solutes and thus to select the most appropriate system for particular analytical separations.

A popular model is the one developed from the Linear Free Energy Relationships (LFER) of Abraham [4], also called Solvation Parameter Model (SPM) in its application to liquid chromatography. In LFERs, the variation of free energy of a process (ΔG^0) is assumed to be the sum of the free energies of the different molecular interactions [4]. Thus, ΔG^0 of a chromatographic partition process (or any linearly related parameter, such as log *k*) can be given as a linear combination of the product of solute and (mobile phase – stationary phase) difference descriptors [5]. These phase difference parameters, that characterize the chromatographic partition system, are obtained as the fitting coefficients of the linear correlation between the retentions of a series of solutes with varied and known descriptors and the descriptor values.

The model of Abraham for neutral solutes has been applied to many liquid chromatography separation systems, including reversed phase [6–37], normal phase [38–42] and hydrophobic interaction [43] liquid chromatographies (abbreviated RPLC, NPLC and HILIC, respectively), and even to micellar and microemulsion electrokinetic chromatography systems [31,44–52].

Initially, Abraham put forward the general LFER model for neutral solutes [4], but later he extended it to the partition of ions and ion-pairs into water and several organic solvents [53–57]. Additionally, some attempts were made to extend the Abraham model for neutral compounds to the chromatographic retention of ionized or partially ionized acid-base solutes [56–66]. The main handicap was the definition of appropriate descriptors for the ionized or partially ionized solutes and several ones were tested. Another big handicap is the measurement of the degree of ionization of the solute in the chromatographic system. It is well known that the degree of ionization of an acid-base solute depends on the pH of the medium and the pK_a of the solute. In most instances it can be well calculated in water where the pH of the buffer is easily measured and the pK_a of the compound is known or can be easily determined. However, when the organic modifier is added to the aqueous buffer, the pH of the buffer and the pK_a of the solute change in different degrees, and thus the degree of ionization is no longer the same as in water. Since pH measurement and pK_a determination in water-organic solvent mobile phases is more difficult than in water, some attempts were made to use the degree of ionization in water, with partially successful results [64,65].

The purpose of this work is to compare and set up chromatographic models based on the Abraham LFER equation to describe and interpret the RPLC retention of neutral, ionic, and partially ionized acids and bases. In a previous work [67], the retention of 66 acid-base compounds in an octadecylsilica Kinetex EVO column with a 40% (v/v) acetonitrile/water mobile phase was studied at different pH values accurately measured in this mobile phase. These data have been complemented with the measured data retention of 29 neutral (unionized) compounds in the same chromatographic system and used to test the different chromatographic LFER models. Since as far as we know, the overall Abraham model for neutral plus ionic solutes has not been applied to RPLC systems, it will be tested and extended to partially ionized solutes by an accurate calculation of the ionization degrees in the working mobile phase.

2. Theory

2.1. Abraham models

In liquid chromatography, retention is usually characterized and related to partition and interaction processes by means of the retention factor (*k*), which is directly related to the partition constant (*K*) by the phase ratio. Since the logarithm of *K* is proportional to the free energy of the partition process and the phase ratio is difficult to measure, the Abraham LFER model applied to retention of neutral compounds in liquid chromatography is usually written in terms of retention factor as described in Eq. (1) [5].

$$\log k = c + e E + s S + a A + b B + v V \quad (1)$$

In this model, the *v V* term accounts for the difference in free energy for cavity formation in the two solvents (mobile and stationary phases) together with residual solute-solvent dispersion interactions. The *e E* term models the difference in polarizability contributions from *n*- and π-electron pairs, *s S* the dipole-type interactions (orientation and induction) differences, *a A* the hydrogen bond donation from the solute to solvent phases, and *b B* the hydrogen bond donation from solvents to solute. *c* is the system constant which includes the phase ratio, normalization of descriptors and other factors independent of the probe solutes terms.

E, *S*, *A*, *B*, and *V* are solute descriptors, either experimentally determined or calculated. *V* is the McGowan molar volume. *E* is the solute excess molar refractivity. *S* is the solute dipolarity/polarizability, *A* and *B* are the overall or summation hydrogen bond acidity and basicity, respectively [4]. The descriptors are known for about 9000 compounds [68,69] and free [68] and commercial [69] software is available for the calculation, if necessary. Recently, Poole has developed an alternative database of descriptors from chromatographic data [70].

e, *s*, *a*, *b*, and *v* are the system coefficients, reflecting the difference in solute interaction between the stationary and mobile phases. The sign (positive or negative) and magnitude of these coefficients lead to the characterization of chromatographic systems,

finding the key features responsible for retention and allowing the comparison between different retention modes, columns, and mobile phases.

The Abraham LFER model for neutral solutes has been successfully applied to a large number of physicochemical and biological processes [4], including many liquid chromatography ones [5–52] , to obtain chemical and biological information about the intermolecular interactions governing the processes being studied [5].

However, there are many important biological and chemical processes that proceed at a fixed pH, where acid-base compounds may be partially or fully ionized. Thus, Abraham developed a new model with two additional terms to account for specific electrostatic interactions of ions [53–57], defined in Eq. (2).

$$\log k = c + e\,E + s\,S + a\,A + b\,B + v\,V + j^{+}\,J^{+} + j^{-}\,J^{-} \quad (2)$$

J^{+} is used for univalent cations with a positive value, specific for each cation, and it is zero for anions and neutral molecules, whereas J^{-} has a non-zero positive value for univalent anions and zero for cations and neutral compounds. Eq. (2) is of application to both neutral and ionic compounds. It must be noticed that not only J^{+} and J^{-} change with the acid-form of each compound. Ionization change the charge of the molecule but also its molar volume, polarizability, dipolarity and hydrogen bonding capabilities. Thus, any acid-base compound will have different *E*, *S*, *A*, *B*, and *V* descriptors in its cationic, neutral (non-charged) and anionic forms. A detailed description of the effect of the ionization in the change of theses descriptors can be found in [56,57].

2.2. Related Abraham models for chromatographic retention of partially ionized solutes

A number of different attempts have been made to extend the Abraham equation to the retention of partially dissociated acids and bases in liquid chromatography. Among them, the most promising seem to be those that use the degree of ionization in the model. It is well known that in RPLC, retention of ions (cations and anions) is much lower than retention of the corresponding neutral species [67]. Conversely, in HILIC retention of ions

is higher than that of the neutral forms. The degree of ionization is a measure of the extend of the ionization and thus, of the retention of the partially dissociated compound. First attempts to include ionization in the Abraham equation were made by Boilet, Poole and Rosés [59,60]. Several descriptors based on the pK_a of the solute [59] or more properly in the degree of ionization [60] were tested. A successful model was obtained by including the degree of ionization of acids (D) as an additional descriptor for ionization in the Abraham model, as described in Eq. (3).

$$\log k = c + e\,E + s\,S + a\,A + b\,B + v\,V + d\,D \quad (3)$$

A more rigorous approach was derived considering that the retention factor of an amphiprotic solute partially ionized at the pH of the mobile phase can be calculated through Eq. (4)

$$k = D^+ k^+ + D^0 k^0 + D^- k^- \quad (4)$$

In this equation, k^+, k^0, and k^- indicate the retention factor observed at mobile phase pH values where the analyte is fully in cationic, neutral, or anionic form, respectively. D^+, D^0, and D^- are the molar fractions of the acid-base compound in cationic, neutral, and anionic forms. Supplementary Information gives detailed calculation of these descriptors. We shall restrict the discussion to univalent ions, although we will consider the simultaneous presence of univalent cations and anions, such in zwitterionic or ampholytic compounds. Based in Eq. (4) and in the observed proportionality between the retention factor of ionic and neutral forms of the compounds, Rosés et al. [60,61] derived modified models of Eq. (1) applicable to the RPLC retention of partially ionized acids and bases. If f^+ is a proportionality factor between the retention factors of the cationic and neutral forms of the solutes, and f^- is a proportionality factor between the anionic and neutral forms, Eq. (4) can be written as Eq. (5). Evidence of the proportionality factors was observed in the original works [60,61] and in the more recent previous work where good linear correlations between retentions of the cations or anions and retentions of the neutral corresponding forms were obtained [67].

$$k = (D^+ f^+ + D^0 + D^- f^-)\, k^0 \quad (5)$$

And since k^0 is retention factor of the neutral form, Eq. (1) can be directly applicable to it, giving Eq. (6).

$$\log k = c + e\,E + s\,S + a\,A + b\,B + v\,V + d \log (D^+ f^+ + D^0 + D^- f^-) \quad (6)$$

The equation can be also written in terms of the cationic (D^+) and anionic (D^-) ionization degrees as in Eq. (7).

$$\log k = c + e\,E + s\,S + a\,A + b\,B + v\,V + d \log (1 - D^+(1 - f^+) - D^-(1 - f^-)) \quad (7)$$

Hence, log (1 - D^+(1 - f^+) - D^-(1 - f^-)) was taken as an additional descriptor accounting for the effect of the ionization in the retention of partially dissociated acids and bases. For a neutral compound, where $D^+ = 0$ and $D^- = 0$, the descriptor becomes 0 and the equation becomes Eq. (1). For neutral acids or bases fully ionized, $D^+ = 0$ and $D^- = 1$ or $D^+ = 1$ and $D^- = 0$, respectively, and the descriptor becomes log f^+ or log f^- and the form of the equation would be quite similar to the form of Eq. (2) for ionic compounds.

In fact, derivation of the model predicts the *d* coefficient to be 1.00 , but it was calculated in the correlation to check the validity of the derived model. Results were very successful and *d* coefficients very close to unity were obtained.

Later, West [62] and Stalcup [63] groups generalized Eq. (3) to acids, bases, and zwitterionic compounds and proposed a model of the type:

$$\log k = c + e\,E + s\,S + a\,A + b\,B + v\,V + d^+ D^+ + d^- D^- \quad (8)$$

Eq. (8) gave reasonable good results in RPLC [63,66] and HILIC [62,64,65].

In rigor for an accurate calculation of ionization degrees and *D* descriptors, the pH of the mobile phase must be measured in the same water/organic solvent mobile phase and the pK_a value of the solute must be also determined or estimated in the same mobile phase. This has been the procedure used by some research groups [60,61,63], but other groups just use pK_a values in water because of its simplicity and availability [62,64,65].

2.3. Derivation of Abraham models for chromatographic retention of partially ionized solutes

Combination of Eqs. (2) and (4) allows to predict retention of any acid or base partially dissociated. Although a similar method has been used to predict the skin permeation of partially dissociated drugs [71], as far as we know, it has not been applied to HPLC retention. Nor pure LFER Abraham models have been derived and tested from the retention data of partially ionized acid-base.

According to the general Abraham model Eq. (4), the retention factor of the different pure cationic, neutral, and anionic forms of the acid-base compound can be written as in Eqs. (9)-(11).

$$\log k^{+} = c + e\,E^{+} + s\,S^{+} + a\,A^{+} + b\,B^{+} + v\,V^{+} + j^{+}\,J^{+} \quad (9)$$

$$\log k^{0} = c + e\,E^{0} + s\,S^{0} + a\,A^{0} + b\,B^{0} + v\,V^{0} \quad (10)$$

$$\log k^{-} = c + e\,E^{-} + s\,S^{-} + a\,A^{-} + b\,B^{-} + v\,V^{-} + j^{-}\,J^{-} \quad (11)$$

In these equations, descriptors with superscripts +, 0, and – indicate the descriptors of the respective cationic, neutral, and anionic forms of the compound. Replacing the equations in Eq. (4), we obtain the general Abraham LFER model in Eq. (12).

$$k = D^{+}\,10^{(c + e\,E^{+} + s\,S^{+} + a\,A^{+} + b\,B^{+} + v\,V^{+} + j^{+}\,J^{+})} + D^{0}\,10^{(c + e\,E^{0} + s\,S^{0} + a\,A^{0} + b\,B^{0} + v\,V^{0})} + D^{-}\,10^{(c + e\,E^{-} + s\,S^{-} + a\,A^{-} + b\,B^{-} + v\,V^{-} + j^{-}\,J^{-})} \quad (12)$$

Alternatively, and since c is the unique common parameter in all exponential terms, Eq. (12) can be written as Eq. (13).

$$\log k = c + \log\left(D^{+}\,10^{(e\,E^{+} + s\,S^{+} + a\,A^{+} + b\,B^{+} + v\,V^{+} + j^{+}\,J^{+})} + D^{0}\,10^{(e\,E^{0} + s\,S^{0} + a\,A^{0} + b\,B^{0} + v\,V^{0})} + D^{-}\,10^{(e\,E^{-} + s\,S^{-} + a\,A^{-} + b\,B^{-} + v\,V^{-} + j^{-}\,J^{-})}\right) \quad (13)$$

Eqs. (12) and (13) are too complex to be directly used for linear correlations and thus, we propose to test a modified LFER model of the type of Eq. (2) assuming additivity of the descriptors of the ionic and neutral forms according to their molar fractions in the mixture, i.e. Eq (14).

$$\log k = c + e E + s S + a A + b B + v V + j^{+} D^{+} J^{+} + j^{-} D^{-} J^{-} \quad (14)$$

with

$$E = D^{+} E^{+} + D^{0} E^{0} + D^{-} E^{-} \quad (15)$$

$$S = D^{+} S^{+} + D^{0} S^{0} + D^{-} S^{-} \quad (16)$$

$$A = D^{+} A^{+} + D^{0} A^{0} + D^{-} A^{-} \quad (17)$$

$$B = D^{+} B^{+} + D^{0} B^{0} + D^{-} B^{-} \quad (18)$$

$$V = D^{+} V^{+} + D^{0} V^{0} + D^{-} V^{-} \quad (19)$$

Eq. (14) is identical to Eq. (8) except for two significant differences:

1. The solute descriptors *E, S, A, B,* and *V* are an average of the solute descriptors of the different ionic and non-ionic forms of the solute according to its preponderance (molar fractions) in the medium. The same solute descriptors in Eq. (8) are solely the solute descriptors of the neutral form, regardless of the preponderance of ionic or neutral forms.
2. The ionic descriptors of Eq. (8) (i.e. D^{+} and D^{-}) depend only on the degrees of ionization. When solutes are fully ionized, D^{+} and D^{-} descriptors are unity for all ions, i.e. all anions and cations have the same descriptor value ($D^{+} = 1$ and $D^{-} = 1$). The ionic descriptors of Eq. (14) are solute dependent. When acid-base solutes are fully ionized ($D^{+} = 1$ or $D^{-} = 1$), ionic descriptors become the particular J^{+} or J^{-} value for the corresponding ion.

Therefore, Eq. (14) is expected to give better correlations than Eq. (8) and it shall be also tested and compared to the other models in this work.

3. Experimental

3.1. Equipment

An Agilent (Santa Clara, CA, USA) 1200 Series instrument equipped with G1312B binary pump, a G1367D autoinjector and an UHD 6540 Accurate-Mass Q-TOF detector with

268 electrospray ionization (ESI) was used for chromatographic measurement, except in
269 phosphate buffers. Solutes in these low volatile buffers were detected by a G1315C DAD
270 set at 254 nm. Instrument was controlled and the data processed by Masshunter
271 software 4.0. The column was a 100 mm, 4.6 mm i.d, 2.6 µm octadecylsilica Kinetex
272 EVO C18 from Phenomenex (Torrance, CA, USA).
273 Mobile phase pH and buffer aqueous pH were measured by a combined Crison 5202
274 electrode in a Crison 2001 pH meter (Hach Lange Spain, L'Hospitalet de Llobregat,
275 Spain). The electrode system was calibrated with ordinary aqueous buffers of pH 4.01,
276 7.00 and 9.21 (25 ºC).
277
278 *3.2. Chemicals*
279 Acetonitrile LCMS grade was purchased from Fluka Analytical VWR (West Chester, PA,
280 USA) and water was purified by Milli-Q deionizing system from Millipore (Billerica, MA,
281 USA) with a resistivity of 18.2 MΩ. The chemicals used to prepare the buffer solutions
282 were sodium phosphate monobasic monohydrate (Sigma-Aldrich, ≥ 99.0%), formic acid
283 (Scharlau, eluent additive for LC-MS), acetic acid (Fluka Analytical, eluent additive for
284 LC-MS), ethylenediamine (Fluka Analytical, ≥ 99.5%) and 25% w/w ammonia solution
285 Sharlau, extrapur). The 66 studied acid-base and 29 neutral compounds were from
286 Sigma-Aldrich (Steinheim, Germany), Fluka Analytical VWR (West Chester, PA, USA),
287 Riedel-de Haën (Seelze, Germany), Merck (Darmstadt, Germany), Carlo Erba (Milano,
288 Italy), Baker (Center Valley, PA, USA) or synthesized in ESTEVE (Barcelona, Spain).
289
290 *3.3. Procedure*
291 The 94 solutes studied were injected in the HPLC system at 6 different pH values,
292 between 2 and 11, approximately. The mobile phase composition was 40 % acetonitrile
293 and 60 % aqueous buffer. The pH of the aqueous HPLC buffers was measured before
294 ($^{w}_{w}$pH) and after ($^{s}_{w}$pH) mixing it with the organic modifier. A more detailed explanation of

buffer preparation can be found in the previous work [67]. All experiments were done at 25 ºC.

Stock solutions of the compounds at 5 mg mL^{-1} were prepared by dissolving the appropriate weight or volume in methanol. A more diluted solution at 0.1 mg mL^{-1} was prepared by dissolving an aliquot of the previous stock solution in an ACN-H_2O mixture (40:60). Isocratic conditions were used at a flow rate of 1 mL min^{-1} and the injection volume was 10 µL. Extra-column time was measured as described earlier [67] and subtracted from all retention measurements.

3.4. Data analysis

Linear regressions of the different models were performed using Microsoft® Excel® for Office 365.

4. Results and discussion

4.1. Determination of retention factors of neutral and ionic species

In a previous work [67] the retention times of a series of 66 acid-base compounds were determined at several mobile phase pH values. Retention times *vs*. pH fitting provided the retention times of the neutral and ionic forms of these compounds. Retention factors for the ionized and non-ionized species can be calculated from these retention times, but calculation is not straightforward because different hold-up times for anions than for neutral species were observed.

A hold-up time of 0.83±0.01 min was obtained from retention of the neutral DMSO hold-up marker, regardless of the buffer employed. This hold-up time agreed with the pycnometrically measured hold-up times of 0.84±0.01 min and 0.86±0.01 min using the pairs of solvents water/methanol and water/acetonitrile, respectively. However, different hold-up times were obtained for ionic markers (KBr, KI) depending on the pH of the mobile phase. This fact was attributed to electronic repulsion between the anionic marker (Br^- or I^-) and the ionized silanols of the column at basic pH values. A value of 0.65 min

323 was set as the hold-up time of anions at basic pH (where the acids are mostly or fully
324 ionized). Additional evidence of this different hold-up times was found from the linear
325 correlation between the retention times of the studied anions ($t_{R_{A^-}}$) *vs.* the retention time
326 of the corresponding neutral species ($t_{R_{HA}}$) given in Eq. (20).
327
328 $$t_{R_{A^-}} = 0.0430\ t_{R_{HA}} + 0.607 \qquad (20)$$
329
330 If we replace the retention time of the neutral form by its hold-up time of 0.83 min in the
331 equations, we get hold-up time of 0.64 min, very close to the one of 0.65 min estimated
332 from KBr and KI retention.
333 No cationic hold-up marker was measured and the same hold-up time of the neutral
334 marker was attributed to cations. The correlation of the retention times of cations ($t_{R_{HA^+}}$)
335 *vs*. the retention times of the neutral species (t_{R_A}) supports this assumption because it
336 gives a hold-up time of 0.81 min for cations from the hold-up time of 0.83 min of the
337 neutral marker, as it can be calculated in Eq. (21).
338
339 $$t_{R_{HA^+}} = 0.0845\ t_{R_A} + 0.740 \qquad (21)$$
340
341 Notice that a wrong value of 0.77 min as hold-up time for cations was given in [67]
342 because of a mistake in the calculation.
343 Therefore, in order to obtain the LFER retention factors for the same phase ratio (or as
344 close as possible) for ions and neutrals, we shall use a hold-up time of 0.83 min to
345 calculate the adjusted retention times of neutral and cationic species and 0.65 min for
346 the adjusted retention times of anions. The retention factor will be calculated as usual by
347 division of the adjusted retention time (residence time of the solute in the stationary
348 phase) by the common hold-up time of 0.83 min (the same residence time in the mobile
349 phase for all solutes). Notice that a change in the hold-up time value used in the

denominator (residence time in the mobile phase) only affects the intercept (*c* value) of all Abraham LFER models, not to the LFER coefficients.

The retention factors of all studied solutes in the different forms (neutral, cationic and anionic) are presented in Table 1 together with the corresponding Abraham descriptors. A few ions showed retention times equal or even slightly smaller than the corresponding hold-up time and thus *k* value is given as 0. The log *k* of these ions could not be calculated and the ions were excluded from the correlations. We present the *k* values of all solutes and species studied previously [67] plus a new set of 34 neutral compounds of diverse nature. These new solutes were measured at the 6 pH values, and retention times averaged.

Calculation of the retention factors when the acids are partially dissociated is also somewhat complex. According to the previous point, we should use a value of hold-up time between 0.67 and 0.83 min for calculation of the adjusted retention times. It should be close to 0.67 min when the acid is highly ionized, but close to 0.83 min when it is mostly neutral. Hence, in coherence with the rest of the study we just use the weighted average of the two hold-up times according to the molar fractions (degree of ionization) of the species in the mixture, Eq. (22) in a general form.

$$t_M = D^+ t_M^+ + D^0 t_M^0 + D^- t_M^- \quad (22)$$

In this equation, t_M indicates the hold-up time and +, 0, and – superscripts the corresponding cationic, neutral and anionic species, as usual. As in the retention factor calculation of the pure species, a common t_M value of 0.83 min has been used in the denominator for *k* calculation.

The obtained retention factor of all compounds at all studied pH values are given in the Supplementary material.

4.2. Abraham pure LFER models for neutral, ionic and partially ionized compounds.

The Abraham LFER model was initially tested for the studied set of 94 solutes in neutral form according to Eq. (1). The descriptors of these solutes are presented in Table 1 together with the one of the ionic solutes studied and the log *k* value of the solutes. Descriptor values are experimental except for some ions for which we could not get experimental values and then the calculated values are given and indicated [56,57]. A very good correlation was obtained which is presented in Figure 1A and in Eq. (23) together with the statistics of the fit.

$$\log k = -0.393(\pm0.043) + 0.104(\pm0.050)\,E - 0.453(\pm0.028)\,S - 0.487(\pm0.044)\,A - 1.421(\pm0.048)\,B + 1.644(\pm0.046)\,V$$

$$N = 91 \quad R^2 = 0.950 \quad \text{SE} = 0.122 \quad F = 324 \qquad (23)$$

In this equation and all the following ones, the standard deviation of the fitting coefficients is given in parenthesis after the coefficient. *N* is the number of solutes (or fitting points), R^2 the coefficient of determination, SE the standard error in the estimate and *F* the Fischer's statistic.

Only 3 out of 94 solutes presented residuals higher than 2.5 times the standard error of the linear regression and they were marked as outliers and eliminated from the correlation. These solutes were 5-fluorouracil (with very low retention, $k = 0.02$), digitoxin and oxycodone.

The sign and magnitude of the fitting coefficients are similar to those obtained for many other RPLC systems [43]. *s, a,* and *b* coefficients are negative showing that an increase in the dipolarity (*S*) and hydrogen bonding capabilities (*A* and *B*) of the solute favours partition into the aqueous mobile phase decreasing retention in the organic stationary phase. Reversely, an increase in solute polarizability (*E*) and volume (*V*) favours retention in the non-polar stationary phase. The most important interactions ruling chromatographic retention are reflected in the large values of *b* and *v* coefficients. A high

406 positive *v* coefficient means that it is much easier for the solute to create a cavity in the
407 non-polar stationary phase than in the polar aqueous mobile phase and then, retention
408 in C18 and in other RPLC mobile phases increases with the size of the solute. On the
409 contrary, the large negative *b* coefficient indicates that there is a much stronger hydrogen
410 bond donation from the aqueous mobile phase to the hydrogen bond acceptor solute
411 than from the poor hydrogen bond donating C18 stationary phase and then retention
412 decreases when the hydrogen bond donor ability of the solute (*B*) increases.
413 Even that Eq. (1) models the main interactions that lead to retention of neutral solutes, it
414 does not take into account additional electrostatic interactions for ionic solutes. Acid-
415 base ionization process changes the polarity, polarizability, hydrogen bonding properties
416 of the solute and slightly its volume (in fact it only changes in the molecular or McGowan
417 volume of one hydrogen ion), but this change is not enough to account for the big
418 changes observed in retention of ions in reference to retention of neutrals. Change in
419 these properties can be easily observed by comparison of the descriptors of the different
420 neutral and ionic forms of the solutes of Table 1. Ionization specially changes dipolarity
421 and hydrogen bonding properties. Protonation of neutral species increases solute
422 hydrogen bond donor (*A*) and decreases hydrogen bond acceptor (*B*) abilities. There is
423 also a small decrease of *E* descriptor. Ionization by deprotonation has the contrary effect.
424 In both cases, solute dipolarity (*S*) increases quite a lot because the molecule becomes
425 charged.
426 Eq. (23) allows to estimate the contributions of these different interactions and compare
427 them for the neutral and ionic forms of the acid-base solutes. An example is given in
428 Table 2 for some selected solutes: two acids and two bases, one acid and one base
429 highly retained and one acid and one base poorly retained.
430 The effect of the ionization in the variation in the interactions of creation of the cavity
431 term (*v V*) and *n*- and π-electron pairs polarizability (*e E*) is very small. However, variation
432 in dipole-dipole (*s S*) and hydrogen-bonding (*a A* and *b B*) interactions is very big. The
433 increase in dipolarity results in a decrease of retention for both types of ions (*s S* much

lower than for the neutral form). For anions, ionization increases retention by a minor hydrogen bond donation from solute to solvents (*a A* higher than for the neutral form) and decreases retention by a much higher hydrogen bond donation from solvents (mostly aqueous mobile phase solvent) to solute (*b B* much more negative than for neutrals). For cations, the contrary effects are observed. Hydrogen bond donation from solute to solvents (*a A*) increases, decreasing retention, and hydrogen bond donation from mobile phase to solute (*b B*) decreases, increasing retention. Combination of these interactions results in predicting that cations should be slightly and anions much more less retained than the corresponding neutral forms. However, comparison with experimental log *k* values show that anions are much more retained (log k_{exp} >> log k_{cal} by Eq. (1)) than expected from these interactions, whereas cations are less retained than predicted (log k_{exp} < log k_{cal}) from Eq. (1).

The difference comes from the electrostatic interactions of ions not considered in the model of Eq. (1) that can be modelled by the J^+ and J^- descriptors through Eq. (2).

To quantify these interactions, Eq. (2) was applied to the joint set of descriptors of neutral and ionic solutes. Some ions presented retention times very close to or even slightly lower than the hold-up time, and thus its log *k* value cannot be precisely determined. Therefore, we excluded all solutes with $k < 0.10$ from the correlation. The correlation obtained is presented in Eq. (24).

$$\log k = -0.463(\pm0.054) + 0.116(\pm0.061)\,E - 0.363(\pm0.030)\,S - 0.359(\pm0.043)\,A - 1.241(\pm0.065)\,B + 1.459(\pm0.061)\,V - 0.352(\pm0.053)\,J^+ + 1.161(\pm0.077)\,J^- \quad (24)$$

$N = 123$ $R^2 = 0.904$ SE = 0.191 $F = 154$

The 123 solutes comprise 15 anions, 15 cations and 93 of the 94 neutral solutes (5-fluorouracil was excluded because $k < 0.10$). No outliers with deviations higher than 2.5 times SE were observed. We had no experimental descriptors for 4 anions and 3 cations

and we used estimated values [56,57]. A more precise correlation can be obtained if only experimental descriptors are used, which is given in Eq. (25).

$$\log k = -0.484(\pm0.049) + 0.175(\pm0.057)\, E - 0.413(\pm0.033)\, S - 0.477(\pm0.047)\, A - 1.321(\pm0.057)\, B + 1.546(\pm0.053)\, V - 0.275(\pm0.052)\, J^{+} + 1.266(\pm0.072)\, J^{-}$$

$$N = 116 \quad R^2 = 0.927 \quad \mathrm{SE} = 0.162 \quad F = 196 \qquad (25)$$

The regression obtained is also presented in Figure 1B, where cations, anions and neutral compounds are indicated. We also show the points for solutes with *k* values lower than 0.10 or with no experimental Abraham descriptors, not considered in the correlation of Eq. (25). It is evident that many of these points are away from the regression line because of the large uncertainty in the calculation of log *k* value or Abraham descriptors. Eqs. (24) and (25) are similar to Eq. (23) for the coefficients reflecting the neutral interactions of solutes with the chromatographic phases (*e*, *s,* *a*, *b*, and *v*), but they also include the electrostatic interactions of ions with the mobile phase ($j^{+}J^{+}$ and $j^{-}J^{-}$). As expected from the discussion of Table 2 above, j^{-} is large and positive (+1.266) since the $j^{-}J^{-}$ counteracts the too small retention expected for anions from their neutral interactions (*e* E^{-}, *s* S^{-}, *a* A^{-}, *b* B^{-}, and *v* V^{-} terms). j^{+} is smaller in size and negative (-0.275), accounting for the too large retention expected from the *e* E^{+}, *s* S^{+}, *a* A^{+}, *b* B^{+}, and *v* V^{+} interactions (see Table 2 for illustrative examples).

Eqs. (24) and (25) have been obtained by multilinear regression of the retention factors (log *k*) of the fully neutral and ionized forms of the studied compounds. Nevertheless, they allow prediction of the retention factors of these forms, but also of partially ionized compounds through Eqs. (12) or (13) if the molar fractions of the ionic and neutral species in the mobile phase are known (D^{+}, D^{0}, and D^{-} descriptors). We have tested this prediction by Eq. (13) and the parameters of Eq. (25) for the studied compounds in 6 different mobile phase pH values.

488 Three different sets of *D* descriptors have been tested. In the first set, the true molar
489 fractions in the mobile phase were tested, i.e. pH and pK_a values measured or
490 determined in the mobile phase (40/60 acetonitrile/aqueous buffer). For simplicity in
491 practical chromatography, it is quite common to calculate degrees of ionization from the
492 pH measured in the aqueous buffer before mixing it with the organic modifier, and the
493 pK_a values determined in water. We have also tested the model using *D* descriptors
494 calculated with this procedure. Several authors [62,64,65], calculate ionization degree
495 descriptors from pK_a values in water and pH measured in the mobile phase. We have
496 also tested the third set of *D* descriptors in this way. The pH values and pK_a values were
497 measured, determined and presented in a previous work [67]. They are also presented
498 in the Supplementary information and pK_a values in the mobile phase in Table 1. All pH
499 and pK_a have been measured with pH calibrated in water as reference state.
500 Supplementary information is an Excel file with pH, pK_a, *k*, and Abraham and *D*
501 descriptors (calculated by the three procedures) for all studied compounds and pH
502 points.
503 The log *k* calculated *vs*. log *k* experimental plot obtained with *D* descriptors calculated
504 from pH and pK_a values in the mobile phase is presented in Figure 2A. As in Eq. (24),
505 points with $k < 0.10$ where not considered for the uncertainty in *k* calculation. We also
506 discarded the two most acidic pH points of chlorpheniramine, nicotine, *o*-
507 phenylenediamine, and ranitidine and the most basic points of 4-hydroxyphenylacetic
508 acid because these diprotic bases or acid should be charged twice at these pH values
509 and our model has been developed only for monoprotic ions. In fact, these ions are
510 shortly retained, giving $k < 0.10$ too, as calculated with the hold-up time of monocations
511 or monoanions.
512 As expected points are scattered around the theoretical line of zero intercept and unity
513 slope. Scattering is larger for the lowest retentions because of the higher uncertainty.
514 It may be argued that Figure 2A includes many pH points where the compound is fully
515 or almost fully in neutral or ionized forms and that this fact may force the correlation to

516 the expected one. Hence, we have repeated the plot taking only pH points where
517 ionization is between 5 and 95 %, i.e. $0.95 \geq D^0 \geq 0.05$. Results are presented in Figure
518 2B, which shows the agreement between calculated and expected retentions when the
519 compound is partially ionized. In fact, a linear regression between the log *k* calculated
520 (log k_{cal}) and log *k* experimentally measured (log k_{exp}) values gives Eq. (26) with its
521 corresponding statistics.
522
523 $\log k_{cal} = 0.016(\pm 0.022) + 0.936(\pm 0.042) \log k_{exp}$
524 $N = 80 \quad R^2 = 0.865 \quad SE = 0.180 \quad F = 500$ (26)
525
526 The slope and intercept of this correlation are not significantly different from 1 and 0,
527 respectively, according to Student *t*-test for 95 % confidence level, demonstrating the
528 good accuracy of the model. The standard error of the model is very similar to the one
529 obtained from the model correlation with pure forms (Eq. 25).
530 We have also tested the accuracy in using *D* descriptors calculated from the pH - pK_a in
531 water and pH in mobile phase – pK_a in water. The corresponding plots for partially ionized
532 acids and bases (i.e. $0.95 \geq D^0 \geq 0.05$) are presented in Figure 2C (*D* in water) and Figure
533 2D (*D* calculated from pH in mobile phase and pK_a in water). It is evident that in both
534 cases the calculated log *k* values are more dispersed than in Figure 2B (using *D* values
535 calculated in the mobile phase) and also that they fall in a parallel line below the
536 expected experimental line of zero intercept and unity slope. In fact, linear regressions
537 of log k_{cal} *vs*. log k_{exp} give slopes close to 1, but intercepts significantly lower than 0 (-
538 0.269±0.030 for *D* in water and -0.274±0.029 for *D* calculated in mixed mode pH mobile
539 phase pK_a in water). Statistics were also worse than those of Eq. (26). Therefore, we
540 may conclude that for application of Abraham models to partially dissociated acids and
541 bases, the degrees of dissociation, or *D* descriptors, must be calculated from data (pH
542 and pK_a) in the mobile phase. Approaches using data in other solvents, i.e. water in

543 practice, give much worse results. We shall use only *D* descriptors in the mobile phase
544 for the following correlations.
545 Given the complexity of the true Abraham model for retention of partially ionized acid-
546 base compounds (Eq. (12) or (13)), we have tested the feasibility of using the
547 approximate simplified model described in Eqs. (14)-(19).
548 We have used the same data than for the previous calculations ($k \geq 0.10$ and
549 monocharged ions only), but we have also excluded oxycodone, which was an outlier in
550 Eq. (23), because it gives very high deviations for all its pH points. With these data, the
551 correlation obtained is presented in Eq. (27) and Figure 3A.
552
553 $\log k = -0.461(\pm0.027) + 0.128(\pm0.034)\ E - 0.437(\pm0.018)\ S - 0.400(\pm0.024)\ A -$
554 $1.339(\pm0.033)\ B + 1.586(\pm0.030)\ V - 0.301(\pm0.029)\ J^{+} + 1.458(\pm0.041)\ J^{-}$
555 $N = 498 \quad R^2 = 0.890 \quad SE = 0.185 \quad F = 567$ (27)
556
557 This correlation is very similar to Eq. (25) in coefficients and statistics. Standard
558 deviations of coefficients are lower and *F* larger because of the much higher number of
559 data points. Thus, the developed Abraham simplified model for RPLC retention of
560 partially ionized acids and bases from the average of descriptors according to their molar
561 fractions in the mobile phase, Eqs. (14)-(19), has a similar performance than the model
562 for fully neutral or ionized compounds and can be used as a general model to correlate
563 retention at any dissociation degree.
564
565 *4.3. Related Abraham models for neutral, ionic and partially ionized compounds.*
566 In the Theory Section, several approaches for application of the LFER equation of
567 Abraham for neutral compounds to neutral, partially and totally ionized acid-base
568 compounds have been presented. The most elaborated models seem to be the ones of
569 Rosés and Poole [60,61], Eq. (6) or (7) and Stalcup and West [62–66], Eq. (8). These

models will be tested here and compared with the Abraham model derived in Section *2.3*, Eqs. (14)-(19) , tested in section 4.2, Eq. (27).

The model of Rosés and Poole implies a proportionality between the retention factors of the ionized and neutral forms of the acid-base compounds (f^+ and f^- parameters). We have tested this assumption for our compounds and chromatographic system by regressing the retention factors of the cationic (k^+) and anionic (k^-) forms of the solutes studied against the retention factors of the corresponding neutral forms (k^0). Equations (28) and (29) present the correlations obtained.

$$k^+ = -0.024(\pm 0.026) + 0.084(\pm 0.005)\, k^0$$

$N = 28 \quad R^2 = 0.908 \quad SE = 0.112 \quad F = 258$ (28)

$$k^- = -0.009(\pm 0.010) + 0.043(\pm 0.003)\, k^0$$

$N = 42 \quad R^2 = 0.823 \quad SE = 0.046 \quad F = 186$ (29)

The statistics of the correlations are quite good and in both cases the intercept is not significantly different from zero according to Student *t*-test for 95% confidence level, demonstrating the proportionality between the retention of cations and anions and the retention of the corresponding neutral forms. It is noteworthy to point out that this proportionality for cations is twice the proportionality for anions. Cations are more retained than anions (about twice) as expected from the retention of the neutral species. Hence, we repeated the correlations for zero intercept in order to obtain the *f* parameters, with the results: $f^+ = 0.082 \pm 0.004$ ($N = 28$, $R^2 = 0.929$, SE = 0.112, $F = 355$) and $f^- = 0.041 \pm 0.002$ ($N = 45$, $R^2 = 0.862$, SE = 0.045, $F = 321$).

Figure 4 presents the plot of k^+ and k^- *vs*. k^0 and the correlation lines obtained. It can be argued that two possible outliers can be removed from the k^+ *vs*. k^0 correlation: 2-amino-4-nitrophenol (– 2.5 times SE) and *N,N*-dimethylaniline (+ 3.0 times SE), but since

597 they are similar in magnitude and opposite in sign, their removal practically gives the
598 same f^+ parameter.
599 To compare the performance of the Rosés-Poole model with the Abraham model for
600 partially dissociated acid-base compounds, we have used these *f* parameters to correlate
601 the same compound and pH data used in Eq. (27) according to Eq. (6). The correlation
602 obtained is presented in Eq. (30) and Figure 3B.
603
604 $\log k = -0.423(\pm0.023) + 0.028(\pm0.029)\ E - 0.406(\pm0.017)\ S - 0.395(\pm0.023)\ A -$
605 $1.346(\pm0.027)\ B + 1.607(\pm0.027)\ V + 0.855(\pm0.021) \log (0.082\ D^+ + D^0 + 0.041\ D^-)$
606 $N = 498 \quad R^2 = 0.917 \quad SE = 0.161 \quad F = 899$ (30)
607
608 The coefficients of this equation are very similar to the ones of Eq. (27) for neutral
609 interactions, except for the *e* coefficient which is lower. The *d* coefficient for ionic
610 interactions is slightly lower than the expected value of 1.00. The statistics of Eq. (30)
611 are even better than those of Eq. (27). This model has the advantage that does not need
612 the Abraham descriptors of the ionic compounds, which are much less available than
613 those of the neutral compounds. Instead, it requires the proportionality factors between
614 the retentions of the ionic and neutral forms of the compounds correlated. If there is not
615 enough data for calculating them from the correlation of the pure ionic and neutral forms
616 of the compounds, they can be obtained together with all other correlation coefficients
617 by non-linear regression of the available pH data according to Eq. (6) (or Eq. (7)).
618 The Stalcup-West model does not require these proportionality parameters. It simply
619 correlates the data to the Abraham descriptors for neutral compounds and the cationic
620 (D^+) and anionic (D^-) degrees of dissociation according to Eq. (8).
621 We have tested this correlation with the same data used in correlations (27) and (30)
622 and the results are presented in Eq. (31) and Figure 3C.
623

624 $\log k = -0.389(\pm0.027) + 0.033(\pm0.033)\ E - 0.398(\pm0.019)\ S - 0.398(\pm0.027)\ A -$

625 $1.316(\pm0.032)\ B + 1.564(\pm0.030)\ V - 0.837(\pm0.034)\ D^+ - 0.898(\pm0.034)\ D^-$

626 $N = 498 \quad R^2 = 0.896 \quad SE = 0.180 \quad F = 603$ (31)

627

628 Stalcup-West correlation (31) is very similar in coefficients for neutral interactions and
629 statistics to the Abraham correlation (27), except for *e* coefficient which is much lower,
630 as in the Rosés-Poole correlation (30), which is similar in coefficients and somewhat
631 better in statistics. The main differences are in the coefficients for ionic interactions. On
632 the one hand, notice that the derived Abraham model uses a different J^+ or J^- descriptor
633 for each cation or anion, respectively. The Stalcup-West model does not distinguish
634 between the descriptors of the different cations, nor between the descriptors of the
635 different anions. This is equivalent to using and averaged descriptor for cations and
636 another averaged descriptor for anions and incorporates them to the fitted d^+ and d^-
637 coefficients. On the other hand, the Abraham model uses different neutral interactions
638 descriptors (*E*, *S*, *A*, *B*, and *V*) for cations, anions and neutrals. As discussed in Section
639 4.2, and showed in Table 2, the descriptors of ions for neutral interactions predict that
640 cations are more retained and anions much less retained than expected from these
641 interactions. Consequently, j^+ coefficient is negative and j^- highly positive to counteract
642 the prediction from the other descriptors. However, the Stalcup-West model uses the
643 same descriptors of non-ionic compounds for cations, anions and neutrals. Since
644 retention of cations is smaller and retention of anions much smaller than retention of
645 neutrals, d^+ and specially d^- must be negative to counteract the higher retention predicted
646 by the *E*, *S*, *A*, *B*, and *V* descriptors of the neutral form.
647 As indicated in the theory section, application of the Stalcup-West model by West and
648 Lindberg groups [62,64,65] was by using D^+ and D^- calculated from pH measured in the
649 mobile phase, but pK_a in water data. Discussion in Section 4.2 and Figure 2D when
650 testing the Abraham model, demonstrates that this procedure for *D* determination

produced poorer correlations biased for the pH points for compounds partially dissociated. However we have tested this procedure of *D* descriptors calculation for the Stalcup-West model with the results presented in Eq. (32) and Figure 3D.

$$\log k = -0.354(\pm0.034) + 0.020(\pm0.042)\,E - 0.384(\pm0.024)\,S - 0.388(\pm0.035)\,A - 1.300(\pm0.041)\,B + 1.532(\pm0.038)\,V - 0.678(\pm0.039)\,D^{+} - 0.632(\pm0.035)\,D^{-}$$

$$N = 498 \quad R^2 = 0.829 \quad \mathrm{SE} = 0.228 \quad F = 346 \qquad (32)$$

Although coefficients are similar to those using *D* descriptors in the mobile phase, statistics are poorer. Moreover, in Figure 3D the presence of many data points with solutes almost not ionized or fully ionized forces a good fitting of the correlation line. Hoewever, pH points for solutes partially ionized ($0.95 > D^0 > 0.05$) are biased and lay below the correlation line in a similar way than in Figure 2D. Thus, we recommend calculation of ionization descriptors from pH and pK_a data in the own mobile phase. pH can be easily measured in many RPLC mobile phases after calibration with buffers in water [63,72–75]. If pK_a cannot be measured in the mobile phase, it may be calculated for some mobile phases [63,76–78] or the degree of ionization determined from absorbance measurements [66].

Concluding remarks

The general LFER model of Abraham for unionized or fully ionized acid-base solutes can be satisfactorily applied to RPLC retention. Regression coefficients of the model provide useful information on the different interactions between the solute and the mobile and stationary phases that contribute to retention. For neutral compounds these interactions are: *n*- and π-electron pairs polarizability interactions (*e*), dipole-type interactions (*s*), hydrogen bond donation from the solute to chromatographic phases (*a*), hydrogen bond donation from the chromatographic phases to solute (*b*), and interactions for cavity formation in mobile and stationary phases (*v*). Interactions for cavity formation and *n*-

679 and π-electron pairs polarizability favour RPLC retention (positive coefficients), whereas
680 the other interactions favour partition of the solute to the mobile phase (negative
681 coefficients). The most important interactions are those of cavity formation (increasing
682 retention when solute size increases) and hydrogen bond donation from
683 chromatographic phases to solutes (decreasing retention when hydrogen bond acceptor
684 ability of solute increases).
685 j^+ and j^- coefficients provide information on additional interactions for charged solutes. j^+
686 is negative decreasing retention of cations from that expected for the rest of interactions.
687 However, j^- is positive, accounting for an additional retention of anions from that
688 expected from the polarizability, dipole, hydrogen bonding and cavity formation
689 interactions.
690 Extension of the Abraham model to partially ionized solutes, results in a complex
691 equation which cannot be solved by linear regression. Instead, an approximate linear
692 model for partially ionized solutes has been derived from the general Abraham equation
693 and satisfactorily compared to other linear models semi-empirically related to Abraham
694 model for neutral solutes. The model uses Abraham descriptors for ions and neutrals
695 averaged according to the degrees of ionization.
696 The Rosés-Poole model gives correlations slightly better than the derived Abraham
697 model. However, it requires to use proportionality factors between the retention of
698 cationic and neutral, on one side, and the anionic and neutral, on the other side, forms
699 of the acid-base compounds, together with Abraham descriptors for neutral compounds.
700 The proportionality factors can be obtained from the retention of the compounds at pH
701 values where they are fully ionized or fully uncharged. Alternatively, it may be calculated
702 from non-linear regression.
703 The Stalcup-West model is simpler because it uses directly the degrees of cationic and
704 anionic ionization as descriptors, together with Abraham descriptors for neutrals.
705 However, the correlations obtained are slightly worse than those obtained from the other
706 models.

In all models, accurate fits for partially ionized compounds are obtained only if the ionization descriptors are calculated from the proper ionization degrees, *i.e.* the ionization degrees calculated from the pH and pK_a measured in the mobile phase, after mixing aqueous buffer and organic modifier. Ionization descriptors calculated from pH and/or pK_a values measured in water, *i.e.* before mixing with the organic modifier, result in biased calculation of retention for partially ionized acids and bases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix with supplementary data

An Excel file with pH, pK_a, *k*, and Abraham and *D* descriptors (calculated by the three procedures) for all studied compounds and pH points.

A pdf file with the derivation of the equations for the calculation of the different ionization degrees.

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997 **Table 1.** Dissociation constant, retention factor and Abraham descriptors of the solutes.

		Mobile phase										Descriptor
z	**Compound**	**p*K*$^+$**	**p*K*$^-$**	***k***	***E***	***S***	***A***	***B***	***V***	***J*$^+$**	***J*$^-$**	**type**
0	2,4-Dichlorophenol	-	9.19	2.80	0.960	0.82	0.54	0.17	1.0199	0.0000	0.0000	Experimental
0	2-Chlorophenol	-	10.01	1.31	0.853	0.88	0.32	0.31	0.8975	0.0000	0.0000	Experimental
0	2-Hydroxybenzoic acid (Salicylic acid)	-	3.85	0.94	0.900	0.85	0.73	0.37	0.9904	0.0000	0.0000	Experimental
0	2-Isopropyl-5-Methylphenol (Thymol)	-	11.71	5.52	0.822	0.80	0.43	0.44	1.3387	0.0000	0.0000	Experimental
0	2-Naphtol	-	10.70	2.00	1.520	1.08	0.61	0.40	1.1441	0.0000	0.0000	Experimental
0	2-Nitrophenol	-	8.41	1.70	1.015	1.05	0.05	0.37	0.9493	0.0000	0.0000	Experimental
0	3-Methylphenol (m-Cresol)	-	11.23	1.10	0.822	0.88	0.57	0.34	0.9160	0.0000	0.0000	Experimental
0	3-Nitrophenol	-	9.57	1.05	1.050	1.57	0.79	0.23	0.9493	0.0000	0.0000	Experimental
0	4-Bromophenol	-	10.27	1.76	1.080	1.17	0.67	0.19	0.9501	0.0000	0.0000	Experimental
0	4-Chloro-3-methylphenol	-	10.52	2.29	0.920	0.99	0.67	0.22	1.0384	0.0000	0.0000	Experimental
0	4-Chlorophenol	-	10.34	1.51	0.915	1.08	0.67	0.20	0.8975	0.0000	0.0000	Experimental
0	4-Ethylphenol	-	11.25	1.82	0.800	0.90	0.55	0.36	1.0569	0.0000	0.0000	Experimental
0	4-Hydroxybenzyl alcohol	-	10.98	0.14	0.998	1.30	0.86	0.79	0.9747	0.0000	0.0000	Experimental
0	4-Hydroxyphenylacetamide	-	10.77	0.13	1.180	2.08	0.84	0.94	1.1724	0.0000	0.0000	Experimental
0	4-Hydroxyphenylacetic acid	-	4.91	0.22	1.030	1.45	0.94	0.74	1.1313	0.0000	0.0000	Experimental
0	4-Methylphenol (p-Cresol)	-	11.33	1.08	0.820	0.87	0.57	0.31	0.9160	0.0000	0.0000	Experimental
0	4-Nitrophenol	-	8.52	0.93	1.070	1.72	0.82	0.26	0.9493	0.0000	0.0000	Experimental
0	5,5-Diethylbarbituric acid (Barbital)	-	9.40	0.27	1.030	1.00	0.58	1.12	1.3739	0.0000	0.0000	Experimental
0	5-Ethyl-5-phenylbarbituric acid (Phenobarbital)	-	8.85	0.63	1.630	1.72	0.71	1.18	1.6999	0.0000	0.0000	Experimental
0	5-Fluorouracil	-	9.18	0.02	0.720	0.84	0.57	1.02	0.7693	0.0000	0.0000	Experimental
0	Acetylsalicylic acid (Aspirin)	-	5.31	0.53	0.781	1.69	0.71	0.67	1.2879	0.0000	0.0000	Experimental
0	Benzoic acid	-	5.40	0.66	0.730	0.90	0.59	0.40	0.9317	0.0000	0.0000	Experimental
0	Capsaicin	-	10.90	5.76	1.250	2.19	0.57	1.45	2.5971	0.0000	0.0000	Experimental
0	Catechol	-	10.52	0.34	0.970	1.10	0.88	0.47	0.8338	0.0000	0.0000	Experimental
0	Diclofenac	-	5.34	7.87	1.810	1.85	0.55	0.77	2.0250	0.0000	0.0000	Experimental
0	Estradiol	-	11.35	2.64	1.800	1.77	0.86	1.10	2.1988	0.0000	0.0000	Experimental
0	Estriol	-	11.49	0.47	1.970	1.74	1.06	1.63	2.2575	0.0000	0.0000	Experimental
0	Estrone	-	11.26	3.87	1.730	2.05	0.50	1.08	2.1558	0.0000	0.0000	Experimental

0	Flurbiprofen	-	5.53	6.10	1.440	1.45	0.62	0.76	1.8389	0.0000	0.0000	Experimental
0	Ibuprofen	-	5.84	8.05	0.730	0.70	0.57	0.79	1.7771	0.0000	0.0000	Experimental
0	Indomethacin	-	5.46	7.98	2.240	1.47	0.58	1.43	2.5299	0.0000	0.0000	Experimental
0	Ketoprofen	-	5.57	2.71	1.650	2.26	0.55	0.89	1.9779	0.0000	0.0000	Experimental
0	Ketorolac	-	5.15	1.49	1.600	2.03	0.65	1.05	1.8712	0.0000	0.0000	Experimental
0	Methyl 4-hydroxybenzoate	-	9.65	0.73	0.930	1.46	0.71	0.46	1.1313	0.0000	0.0000	Experimental
0	Naproxen	-	5.77	2.84	1.510	2.02	0.60	0.67	1.7821	0.0000	0.0000	Experimental
0	Phenol	-	11.09	0.70	0.805	0.89	0.60	0.30	0.7751	0.0000	0.0000	Experimental
0	Resorcinol	-	10.69	0.23	0.980	1.11	1.09	0.52	0.8338	0.0000	0.0000	Experimental
0	Warfarin	-	5.91	4.30	1.980	1.88	0.29	1.57	2.3077	0.0000	0.0000	Experimental
0	2-Nitro-*p*-phenylenediamine	3.42	-	0.37	1.525	2.05	0.35	0.70	1.0902	0.0000	0.0000	Experimental
0	2-Toluidine	3.43	-	1.06	0.966	0.92	0.23	0.45	0.9571	0.0000	0.0000	Experimental
0	Aminopyrine	4.10	-	0.59	1.680	1.74	0.00	1.60	1.8662	0.0000	0.0000	Experimental
0	Aniline	3.54	-	0.69	0.955	0.96	0.26	0.41	0.8162	0.0000	0.0000	Experimental
0	Atropine	8.24	-	2.59	1.200	1.58	0.26	1.73	2.2820	0.0000	0.0000	Experimental
0	Benzyl nicotinate	2.21	-	2.80	1.262	1.38	0.00	0.85	1.6393	0.0000	0.0000	Experimental
0	Chloropheniramine	7.79	-	8.16	1.465	1.41	0.00	1.33	2.2098	0.0000	0.0000	Experimental
0	Codeine	7.19	-	0.86	2.160	2.14	0.14	1.80	2.2057	0.0000	0.0000	Experimental
0	Diethylcarbamazine	6.93	-	0.53	0.645	1.30	0.00	1.55	1.7241	0.0000	0.0000	Experimental
0	Ephedrine	7.68	-	1.47	0.916	0.74	0.21	1.21	1.4385	0.0000	0.0000	Experimental
0	Fentanyl	7.40	-	10.99	1.830	1.75	0.00	1.81	2.8399	0.0000	0.0000	Experimental
0	Isoquinoline	3.79	-	1.17	1.211	1.00	0.00	0.54	1.0443	0.0000	0.0000	Experimental
0	Lidocaine	7.15	-	4.46	1.110	1.51	0.07	1.24	2.0589	0.0000	0.0000	Experimental
0	*N*,*N*-dimethylaniline	4.04	-	3.87	0.957	0.81	0.00	0.41	1.0980	0.0000	0.0000	Experimental
0	Nicotine	7.58	-	0.75	0.865	0.88	0.00	1.09	1.3710	0.0000	0.0000	Experimental
0	*o*-Phenylenediamine	3.59	-	0.24	1.260	1.40	0.24	0.73	0.9160	0.0000	0.0000	Experimental
0	Oxycodone	7.56	-	2.02	2.320	2.50	0.29	1.91	2.2644	0.0000	0.0000	Experimental
0	*p*-Phenylenediamine	6.91	-	0.12	1.300	1.66	0.44	0.83	0.9160	0.0000	0.0000	Experimental
0	Propanolol	7.58	-	5.69	1.840	1.43	0.44	1.31	2.1480	0.0000	0.0000	Experimental
0	Pyridine	3.70	-	0.35	0.631	0.84	0.00	0.52	0.6753	0.0000	0.0000	Experimental
0	Ranitidine	7.52	-	0.41	1.600	1.63	0.25	2.33	2.3985	0.0000	0.0000	Experimental
0	Scopolamine	6.92	-	0.55	1.686	1.32	0.09	2.17	2.2321	0.0000	0.0000	Experimental

0	Sufentanyl	7.19	-	18.90	1.800	2.28	0.00	1.91	3.1051	0.0000	0.0000	Experimental
0	Tramadol	8.48	-	4.87	1.350	1.15	0.00	1.47	2.2340	0.0000	0.0000	Experimental
0	2-Amino-4-nitrophenol	3.07	8.44	0.59	1.415	1.95	1.01	0.43	1.0491	0.0000	0.0000	Experimental
0	4-Amino-2-nitrophenol	2.82	9.29	0.67	1.360	1.50	0.30	0.66	1.0491	0.0000	0.0000	Experimental
0	Morphine	7.53	10.29	0.52	2.230	1.30	0.39	2.01	2.0648	0.0000	0.0000	Experimental
0	Piroxicam	1.61	5.40	1.58	2.560	2.90	0.17	1.49	2.2500	0.0000	0.0000	Experimental
0	2-Phenylethanol	-	-	0.70	0.811	0.82	0.31	0.66	1.0569	0.0000	0.0000	Experimental
0	3-Xylene	-	-	9.23	0.623	0.52	0.00	0.16	0.9982	0.0000	0.0000	Experimental
0	8-Methoxypsoralen	-	-	1.78	1.611	1.70	0.00	0.80	1.4504	0.0000	0.0000	Experimental
0	Antipyrine	-	-	0.30	1.300	1.83	0.00	1.37	1.4846	0.0000	0.0000	Experimental
0	Atrazine	-	-	2.00	1.220	1.29	0.17	1.01	1.6196	0.0000	0.0000	Experimental
0	Benzaldehyde	-	-	1.22	0.820	1.00	0.00	0.39	0.8730	0.0000	0.0000	Experimental
0	Benzene	-	-	2.96	0.610	0.52	0.00	0.14	0.7164	0.0000	0.0000	Experimental
0	Benzyl alcohol	-	-	0.51	0.803	0.87	0.39	0.56	0.9160	0.0000	0.0000	Experimental
0	Cafeine	-	-	0.16	1.500	1.82	0.08	1.25	1.3632	0.0000	0.0000	Experimental
0	Cortexolone	-	-	1.40	1.910	3.45	0.36	1.60	2.7389	0.0000	0.0000	Experimental
0	Corticosterone	-	-	1.25	1.860	3.43	0.40	1.63	2.7389	0.0000	0.0000	Experimental
0	Cortisone	-	-	0.68	1.960	3.50	0.36	1.87	2.7546	0.0000	0.0000	Experimental
0	Cumene	-	-	14.80	0.602	0.49	0.00	0.16	1.1391	0.0000	0.0000	Experimental
0	Dexamethasone	-	-	1.08	2.040	3.51	0.71	1.92	2.9132	0.0000	0.0000	Experimental
0	Digitoxin	-	-	3.38	3.460	5.63	1.33	4.35	5.6938	0.0000	0.0000	Experimental
0	Ethylbenzene	-	-	9.10	0.613	0.51	0.00	0.15	0.9982	0.0000	0.0000	Experimental
0	Fluocinonide	-	-	6.25	1.950	2.48	0.31	2.51	3.4601	0.0000	0.0000	Experimental
0	Griseofulvin	-	-	2.39	1.750	2.64	0.00	1.44	2.3947	0.0000	0.0000	Experimental
0	Hydrocortisone	-	-	0.60	2.030	3.49	0.71	1.90	2.7976	0.0000	0.0000	Experimental
0	Hydroquinone	-	-	0.15	1.063	1.27	1.06	0.57	0.8338	0.0000	0.0000	Experimental
0	Hydroxyprogesterone	-	-	3.96	1.640	3.35	0.25	1.31	2.6802	0.0000	0.0000	Experimental
0	Methyl 4-hydroxyphenylacetate	-	-	0.43	0.908	1.46	0.59	0.68	1.2722	0.0000	0.0000	Experimental
0	Methyl phenyl ether	-	-	2.67	0.708	0.75	0.00	0.29	0.9160	0.0000	0.0000	Experimental
0	Prednisolone	-	-	0.55	2.210	3.10	0.71	1.92	2.7546	0.0000	0.0000	Experimental
0	Pregnenolone	-	-	10.86	1.360	3.29	0.32	1.18	2.6645	0.0000	0.0000	Experimental
0	Progesterone	-	-	10.58	1.450	3.29	0.00	1.14	2.6215	0.0000	0.0000	Experimental

0	Testosterone	-	-	2.75	1.540	2.56	0.32	1.17	2.3827	0.0000	0.0000	Experimental
0	Toluene	-	-	5.21	0.601	0.52	0.00	0.14	0.8573	0.0000	0.0000	Experimental
-1	2,4-Dichlorophenol	-	9.19	0.02	1.110	4.45	0.00	2.49	0.9984	0.0000	2.7500	Experimental
-1	2-Chlorophenol	-	10.01	-0.02	1.003	2.98	0.00	2.20	0.8760	0.0000	1.7600	Experimental
-1	2-Hydroxybenzoic acid (Salicylic acid)	-	3.85	0.10	1.050	3.51	0.14	2.18	0.9689	0.0000	1.6351	Experimental
-1	2-Isopropyl-5-Methylphenol (Thymol)	-	11.71	0.23	0.972	2.52	0.00	2.29	1.3172	0.0000	1.5161	Experimental
-1	2-Naphtol	-	10.70	0.07	1.670	6.55	0.00	3.00	1.1226	0.0000	3.5335	Experimental
-1	2-Nitrophenol	-	8.41	0.01	1.165	2.95	0.00	2.20	0.9278	0.0000	1.7200	Experimental
-1	3-Methylphenol (m-Cresol)	-	11.23	0.04	0.972	2.80	0.00	2.10	0.8945	0.0000	1.6100	Experimental
-1	3-Nitrophenol	-	9.57	-0.01	0.972	2.80	0.00	2.10	0.8945	0.0000	1.6100	Experimental
-1	4-Bromophenol	-	10.27	0.07	1.230	3.50	0.00	2.46	0.9286	0.0000	2.3000	Experimental
-1	4-Chloro-3-methylphenol	-	10.52	0.08	1.070	3.39	0.00	2.36	1.0169	0.0000	2.1306	Estimated
-1	4-Chlorophenol	-	10.34	0.06	1.065	2.95	0.00	2.38	0.8760	0.0000	2.0200	Experimental
-1	4-Ethylphenol	-	11.25	0.07	0.950	2.84	0.00	2.27	1.0354	0.0000	1.6602	Experimental
-1	4-Hydroxybenzyl alcohol	-	10.98	0.00	1.080	4.40	0.00	2.22	1.1098	0.0000	1.7674	Estimated
-1	4-Hydroxyphenylacetamide	-	10.77	0.00	1.330	6.14	0.00	2.38	1.1509	0.0000	1.4954	Estimated
-1	4-Hydroxyphenylacetic acid	-	4.91	0.14	1.180	3.87	0.13	3.11	1.1098	0.0000	2.1812	Estimated
-1	4-Methylphenol (p-Cresol)	-	11.33	0.04	0.970	2.75	0.00	2.10	0.8945	0.0000	1.6560	Experimental
-1	4-Nitrophenol	-	8.52	0.01	1.220	4.85	0.00	2.09	0.9278	0.0000	2.2000	Experimental
-1	5,5-Diethylbarbituric acid (Barbital)	-	9.40	-0.01	1.180	3.61	0.04	3.74	1.3524	0.0000	2.3539	Estimated
-1	5-Ethyl-5-phenylbarbituric acid (Phenobarbital)	-	8.85	-0.02	1.780	4.90	0.07	3.77	1.6784	0.0000	2.4878	Experimental
-1	5-Fluorouracil	-	9.18	-0.04	0.870	2.92	0.00	3.46	0.7478	0.0000	2.0907	Estimated
-1	Acetylsalicylic acid (Aspirin)	-	5.31	0.04	1.000	4.15	0.00	3.28	1.2664	0.0000	2.2560	Experimental
-1	Benzoic acid	-	5.40	0.05	0.880	3.64	0.00	2.88	0.9102	0.0000	2.3950	Experimental
-1	Capsaicin	-	10.90	0.24	1.400	7.04	0.00	2.46	2.5756	0.0000	0.9790	Estimated
-1	Catechol	-	10.52	0.00	1.120	5.81	0.00	2.63	0.8123	0.0000	2.4860	Estimated
-1	Diclofenac	-	5.34	0.40	1.960	5.31	0.03	3.35	2.0035	0.0000	2.6243	Experimental
-1	Estradiol	-	11.35	0.11	1.950	5.32	0.16	3.82	2.1773	0.0000	2.6980	Estimated
-1	Estriol	-	11.49	0.01	2.120	5.47	0.24	4.49	2.2360	0.0000	2.7698	Estimated
-1	Estrone	-	11.26	0.16	1.880	6.71	0.00	2.81	2.1343	0.0000	2.1090	Estimated
-1	Flurbiprofen	-	5.53	0.18	1.590	4.56	0.07	3.36	1.8174	0.0000	2.5383	Experimental
-1	Ibuprofen	-	5.84	0.29	0.880	3.50	0.08	3.31	1.7556	0.0000	2.4188	Experimental

-1	Indomethacin	-	5.46	0.41	2.390	5.62	0.10	4.38	2.5084	0.0000	2.9899	Experimental
-1	Ketoprofen	-	5.57	0.14	1.800	5.49	0.01	3.39	1.9564	0.0000	2.4851	Experimental
-1	Ketorolac	-	5.15	0.13	1.750	5.20	0.05	3.60	1.8497	0.0000	2.4776	Experimental
-1	Methyl 4-hydroxybenzoate	-	9.65	0.04	1.080	3.79	0.04	2.77	1.1098	0.0000	2.1526	Estimated
-1	Naproxen	-	5.77	0.12	1.660	5.07	0.02	3.11	1.7606	0.0000	2.4261	Experimental
-1	Phenol	-	11.09	0.02	0.955	2.80	0.00	2.12	0.7536	0.0000	1.6760	Experimental
-1	Resorcinol	-	10.69	0.00	1.130	7.31	0.00	2.82	0.8123	0.0000	2.8860	Estimated
-1	Warfarin	-	5.91	0.13	2.130	5.62	0.00	4.40	2.2862	0.0000	2.7620	Experimental
-1	2-Amino-4-nitrophenol	3.07	8.44	-0.01	1.565	7.33	0.00	2.62	1.0276	0.0000	2.9767	Estimated
-1	4-Amino-2-nitrophenol	2.82	9.29	-0.02	1.510	5.63	0.00	2.49	1.0276	0.0000	2.2638	Estimated
-1	Morphine	7.53	10.29	0.01	2.380	5.26	0.01	4.98	2.0433	0.0000	2.8576	Estimated
-1	Piroxicam	1.61	5.40	0.13	2.710	6.81	0.00	3.78	2.2285	0.0000	2.7356	Experimental
+1	2-Nitro-p-phenylenediamine	3.42	-	-0.04	1.375	3.13	3.43	0.00	1.1117	0.7450	0.0000	Estimated
+1	2-Toluidine	3.43	-	-0.04	0.816	1.99	1.95	0.00	0.9786	0.8001	0.0000	Estimated
+1	Aminopyrine	4.10	-	-0.02	1.530	5.16	2.92	0.00	1.8877	2.1616	0.0000	Estimated
+1	Aniline	3.54	-	-0.05	0.805	1.62	1.93	0.00	0.8377	0.6200	0.0000	Experimental
+1	Atropine	8.24	-	0.20	1.050	5.40	2.19	0.00	2.3035	2.3363	0.0000	Experimental
+1	Benzyl nicotinate	2.21	-	0.22	1.112	3.17	1.88	0.00	1.6608	1.5646	0.0000	Experimental
+1	Chloropheniramine	7.79	-	0.81	1.315	4.35	1.85	0.00	2.2313	2.2856	0.0000	Experimental
+1	Codeine	7.19	-	0.12	1.810	5.72	2.48	0.00	2.2272	2.8717	0.0000	Experimental
+1	Diethylcarbamazine	6.93	-	0.07	0.495	4.83	1.97	0.00	2.2702	1.7995	0.0000	Estimated
+1	Ephedrine	7.68	-	0.06	0.766	3.74	1.38	0.00	1.4600	1.9412	0.0000	Experimental
+1	Fentanyl	7.40	-	0.89	1.680	5.67	2.22	0.00	2.8615	2.8615	0.0000	Experimental
+1	Isoquinoline	3.79	-	-0.04	1.061	2.34	1.63	0.00	1.0658	1.3067	0.0000	Estimated
+1	Lidocaine	7.15	-	0.25	0.960	4.18	2.12	0.00	2.0804	1.7490	0.0000	Experimental
+1	*N*,*N*-dimethylaniline	4.04	-	-0.02	0.807	2.00	0.96	0.00	1.1195	1.0483	0.0000	Experimental
+1	Nicotine	7.58	-	0.17	0.715	3.52	1.27	0.00	1.3925	1.8345	0.0000	Experimental
+1	*o*-Phenylenediamine	3.59	-	-0.05	1.100	2.89	2.56	0.00	0.9375	1.0294	0.0000	Estimated
+1	Oxycodone	7.56	-	0.10	2.170	6.27	3.16	0.00	2.2859	2.9311	0.0000	Estimated
+1	*p*-Phenylenediamine	6.91	-	0.08	1.150	3.26	2.93	0.00	0.9375	0.9758	0.0000	Estimated
+1	Propanolol	7.58	-	0.53	1.690	4.31	2.07	0.00	2.1695	2.4319	0.0000	Experimental
+1	Pyridine	3.70	-	-0.07	0.481	2.25	1.21	0.00	0.6968	1.0450	0.0000	Experimental

+1	Ranitidine	7.52	-	0.10	1.450	6.87	2.13	0.00	2.4200	3.3742	0.0000	Experimental
+1	Scopolamine	6.92	-	0.10	1.536	6.34	1.64	0.00	2.2536	3.5261	0.0000	Experimental
+1	Sufentanyl	7.19	-	1.59	1.650	6.16	3.02	0.00	3.1266	2.5848	0.0000	Experimental
+1	Tramadol	8.48	-	0.39	1.200	4.56	1.50	0.00	2.2555	2.5348	0.0000	Experimental
+1	2-Amino-4-nitrophenol	3.07	8.44	0.33	1.265	2.43	3.32	0.00	1.0706	0.4096	0.0000	Estimated
+1	4-Amino-2-nitrophenol	2.82	9.29	0.00	1.210	2.77	2.67	0.00	1.0706	0.9712	0.0000	Estimated
+1	Morphine	7.53	10.29	0.04	1.970	5.95	1.25	0.00	2.0863	3.9413	0.0000	Experimental
+1	Piroxicam	1.61	5.40	0.11	2.410	5.44	3.67	0.00	2.2715	2.3802	0.0000	Estimated

z: charge of the species

Table 2. Contributions of non-electrostatic interactions to retention of neutral and ionic forms of acids and bases

Compound	***z***	***E***	***S***	***A***	***B***	***V***	***c***	***e E***	***s S***	***a A***	***b B***	***v V***	**log *k* cal**	**log *k* exp**
Benzoic acid	0	0.73	0.90	0.59	0.40	0.93	-0.39	0.08	-0.41	-0.29	-0.57	1.53	-0.05	-0.18
Benzoic acid	-1	0.88	3.64	0.00	2.88	0.91	-0.39	0.09	-1.65	0.00	-4.09	1.50	-4.55	-1.32
Diclofenac	0	1.81	1.85	0.55	0.77	2.03	-0.39	0.19	-0.84	-0.27	-1.09	3.33	0.92	0.90
Diclofenac	-1	1.96	5.31	0.03	3.35	2.00	-0.39	0.20	-2.41	-0.01	-4.76	3.29	-4.08	-0.40
Codeine	0	2.16	2.14	0.14	1.80	2.21	-0.39	0.22	-0.97	-0.07	-2.56	3.63	-0.14	-0.07
Codeine	+1	1.81	5.72	2.48	0.00	2.23	-0.39	0.19	-2.59	-1.20	0.00	3.66	-0.34	-0.92
Fentanyl	0	1.83	1.75	0.00	1.81	2.84	-0.39	0.19	-0.79	0.00	-2.57	4.67	1.10	1.04
Fentanyl	+1	1.68	5.67	2.22	0.00	2.86	-0.39	0.17	-2.57	-1.08	0.00	4.70	0.83	-0.05

FIGURE CAPTIONS

Figure 1. Fits of the retention of pure acid-base species to Abraham LFERs. A: Fits of neutral species to Eq. (1) model (correlation in Eq. (23)). B: Fits of neutral, anionic, and cationic species to Eq. (2) model (correlation in Eq. (25)). Symbols: (●) fitted neutral species, (■) fitted cations, (▲) fitted anions, (○) outlier neutral species, (+) ions with estimated descriptors, (x) ions with $k < 0.10$.

Figure 2. Prediction by Eq. (13) of the retention of acid-base compounds partially dissociated from the Abraham LFER of Eq. (2) for pure acid-base species. A: all pH data. B, C and D: pH values where compounds are only partially dissociated ($0.95 > D^0 > 0.05$). A and B: dissociation degrees (D descriptors) calculated from the pH and pK_a in the mobile phase. C: D descriptors calculated from the pH and pK_a in water. D: D descriptors calculated from the pH in the mobile phase and the pK_a in water. Symbols: (●) $D^0 > 0.95$ (compounds poorly dissociated), (■) $0.95 > D^0 > 0.05$ (compounds partially dissociated), (▲) $D^0 < 0.05$ (compounds highly dissociated).

Figure 3. Comparison of the fits of the experimental retention data at different pH values to the different models for partially dissociated acid-base compounds. A: Simplified Abraham model of Eq. (14) (correlation in Eq. (27)). B: Rosés-Poole model of Eq. (6) (correlation in Eq. (30)). C: West-Stalcup model of Eq. (8) with D descriptors calculated from the pH and pK_a in the mobile phase (correlation in Eq. (31)). D: West-Stalcup model of Eq. (8) with D descriptors calculated from the pH in the mobile phase and the pK_a in water (correlation in Eq. (32)). Symbols as in Figure 2.

Figure 4. Correlations between the retention of the ionic and neutral forms of acid-base compounds. Symbols: (■) cations (k^+ *vs*. k^0), (▲) anions (k^- *vs*. k^0).