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Influence of variation in mobile phase pH and solute pK_a with the change of organic modifier fraction on QSRRs of hydrophobicity and RP-HPLC retention of weakly acidic compounds

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ABSTRACT

The variation in mobile phase pH and ionizable solute dissociation constant (pK_a) with the change of organic modifier fraction in hydroorganic mobile phase has seemingly been a troublesome problem in studies and applications of reversed phase high performance liquid chromatography (RP-HPLC). Most of the early studies regarding the RP-HPLC of acid–base compounds have to measure the actual pH of the mixed mobile phase rigorously, sometimes bringing difficulties in the practices of liquid chromatographic separation. In this paper, the effect of this variation on the apparent *n*-octanol/water partition coefficient (K_{ow}'') and the related quantitative structure–retention relationship (QSRR) of log K_{ow}'' vs. log k_w, the logarithm of retention factor of analytes in neat aqueous mobile phases, was investigated for weakly acidic compounds. This QSRR is commonly used as a classical method for K_{ow} measurement by RP-HPLC. The theoretical and experimental derivation revealed that the variation in mobile phase pH and solute pK_a will not affect the QSRRs of acidic compounds. This conclusion is proved to be suitable for various types of ion-suppressors, i.e., strong acid (perchloric acid), weak acid (acetic acid) and buffer salt (potassium dihydrogen phosphate/phosphoric acid, PBS). The QSRRs of log K_{ow}'' vs. log k_w were modeled by 11 substituted benzoic acids using different types of ion-suppressors in a binary methanol-water mobile phase to confirm our deduction. Although different types of ion-suppressor all can be used as mobile phase pH modifiers, the QSRR model obtained by using perchloric acid as the ion-suppressor was found to have the best result, and the slightly inferior QSRRs were obtained by using acetic acid or PBS as the ion-suppressor.

1. Introduction

Hydrophobicity, generally expressed by *n*-octanol/water partition coefficient (K_{ow}), constitutes an important physicochemical parameter conventionally used in quantitative structure–retention relationship (QSRR) studies for various bioactive compounds including pharmaceuticals and natural products [1–5]. The relationship between log *Kow* and log k_w, the logarithm of retention factor (k) of analyte obtained by extrapolating to neat aqueous mobile phase in reversed-phase high performance liquid chromatography (RP-HPLC), is usually adopted for *Kow* determination, which is also known as Collander equation [6–9]. In general, Collander equation is limited to neutral solutes. In fact, most biomedical molecules are more or less dissociated, therefore, buffers including acids and bases are added into the mobile phase to suppress the dissociation of compounds with acid–base properties, which results in improved chromatographic retention and peak shape in RP-HPLC [10–12]. However, the dissociation is completely suppressed only when the pH of mobile phase was adjusted to at least 2 pH units lower than dissociation constant (pK_a) of the solute, which means that very strong acidity of mobile phase is necessary for those compounds with small pK_a value (e.g., p$K_a \leq 4$), decreasing the life of chromatographic columns as well as apparatus. The apparent n-octanol/water partition coefficient (K_{ow}'') has been proposed to correct K_{ow} so as to describe the hydrophobicity of ionizable solutes more precisely. Moreover, we have reported the RP-HPLC retention behavior of carboxylic acids and phenols by using acetic or perchloric acid as the ion-suppressor in binary hydroorganic mobile phase, as well as the QSRRs of hydrophobicity and retention for these weak acids. A better linear

Abbreviations: *Kow*, *n*-Octanol/water partition coefficient; QSRR, Quantitative structure–retention relationship; k, Retention factor; RP-HPLC, Reversed-phase high performance liquid chromatography; K_a, Dissociation constant; K_{ow}'', Apparent n-octanol/water partition coefficient; SFM, Shake-flask method; SSM, Slow-stirring method; t_R, Retention time; DP-RTC, Dual-point retention time correction

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relationship relating $\log K_{ow}''$ with $\log k_w$ than that relating $\log K_{ow}$ with $\log k_w$ has been established and applied successfully to K_{ow} measurement and retention prediction of weakly acidic compounds in our previous studies [13–16].

In RP-HPLC procedure, the retention of an ionizable compound depends on its hydrophobicity and ionization degree, which in turn depends on mobile phase pH and analyte pK_a. It is generally acknowledged that both mobile phase pH and solute pK_a vary with the change of organic modifier fraction in the mobile phase [17]. Therefore, precise measurement and control of pH and pK_a are essential to correct analysis results. The IUPAC has endorsed rules and procedures for pH standardization of electrode systems in water and in binary aqueous-organic solvent mixtures commonly used as mobile phases in RP-HPLC [18–20]. On the basis of IUPAC recommendations, three different pH scales are usually employed in pH measurement of mobile phase in RP-HPLC. The most common is the aqueous pH scale (${}^{w}_{w}$pH), which is obtained by calibrating the electrode system with aqueous buffers (w), and mobile phase pH is measured in aqueous fraction (w) before mixing it with the organic modifier [21]. If the electrode system is calibrated with aqueous buffers, but mobile phase pH is measured after mixing the aqueous fraction with the organic modifier, the pH scale in the mobile phase solvent (s) relative to water (w) as standard-state solvent is obtained as ${}^{s}_{w}$pH. If the electrode system is calibrated with buffers prepared by mobile phase solvent (s), and pH is measured in the same mobile phase solvent (s), the pH scale is obtained as ${}^{s}_{s}$pH. ${}^{s}_{w}$pH or ${}^{s}_{s}$pH scale is recommended to express true mobile phase acidity, because they clearly represent variation of the mobile phase pH with the change of the organic modifier content. However, working in the ${}^{s}_{s}$pH scale requires preparation and maintenance of the standard mixed solvents, most of which are not commercially available, thus ${}^{s}_{w}$pH is usually chosen to describe mobile phase pH in practice. Similarly, the symbols s and w used for pH scales can also be extended to solutes pK_a scales, and accordingly there are three different pK_a scales, i.e., ${}^{w}_{w}$pK_a, ${}^{s}_{w}$pK_a or ${}^{s}_{s}$pK_a, in dissociation characterization of analytes in RP-HPLC mobile phase [22].

Subirats et al. [17] pointed out that the variation of mobile phase pH and solute pK_a resulting from the change of organic modifier fraction in the mobile phase affect the retention of acid–base compounds on RP-HPLC. They established the precise relationships between true mobile phase pH and organic modifier fraction, which are very useful to estimate the actual pH variation of mobile phase with the change of the organic modifier content when the measurement of mobile phase pH is not easy to operate, i.e., in the case of highly automated liquid chromatographic experiments where different mobile phase components from independent reservoirs are pumped into and mixed within the apparatus. They also derived a number of models that relate the retention of an acid–base compound with its pK_a and the organic modifier fraction in the mobile phase. These models are greatly helpful in predicting the proper mobile phase composition in which the differences on ionization degree between analytes with similar aqueous pK_a values are significant, thereupon improving the selectivity of RP-HPLC for acid–base compounds and explaining the relative separation mechanism, as well as avoiding fruitless experimental time and reagent consuming.

Since the retention times of acid–base compounds are affected by mobile phase pH and solute pK_a, the influence of the variation in pH and pK_a seems involuntarily necessary to be considered when studying the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for weakly ionizable compounds. However, in our previous studies on the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for acidic compounds, direct adoption of ${}^{w}_{w}$pH and ${}^{w}_{w}$pK_a always gave satisfactory results. This phenomenon has attracted our attention on the reason behind.

In definition, the k_w value of a weak acid at a certain mobile phase pH is the weighted average of retention factors of all the neutral and ionic species formed in the neat aqueous fraction of mobile phase [23]. Accurate pH value of the neat aqueous fraction of mobile phase refers to ${}^{w}_{w}$pH. At a specific ${}^{w}_{w}$pH, the ratios of all the neutral and ionic species of the weak acid and their contributions to k_w are invariable. Therefore, k_w value is definitely independent of the organic modifier fraction in the mobile phase. The main purpose of this paper is to find out whether K_{ow}'', as well as the related QSRRs of $\log K_{ow}''$ vs. $\log k_w$ for weakly acidic compounds are influenced by the variation in ${}^{w}_{w}$pH or ${}^{s}_{s}$pH of mobile phase and ${}^{s}_{w}$pK_a or ${}^{s}_{s}$pK_a of acidic solute arising from the change of organic modifier fraction. In addition, the QSRRs of $\log K_{ow}''$ vs. $\log k_w$ modeling by 11 substituted benzoic acids were compared by using three types of ion-suppressors, i.e., strong acid, weak acid and buffer salt, and the role of different ion-suppressors on the retention mechanism was preliminarily investigated.

2. Theoretical basis

An acid–base equilibrium for a monoprotic weak acid in its diluted aqueous solution ruled by ${}^{w}_{w}$pK_a is described as

$$HA = H^+ + A^- \tag{1}$$

with ${}^{w}_{w}K_a = a_{(H^+)} \times a_{(A^-)}/a_{(HA)}$. Due to the diluted solution of the studied system, the activities of species can be replaced by concentrations

$${}^{w}_{w}K_a = \frac{c_{(H^+)} \times c_{(A^-)}}{c_{(HA)}} \tag{2}$$

When the aqueous solution is diluted with an organic modifier, the concentrations of species existing in the solution are changed. Given the addition of the organic modifier affects all the species to the same degree [24], the concentrations of species change from $c_{(H^+)}$, $c_{(A^-)}$ and $c_{(HA)}$ to $c_{(H^+)}\varphi_{H_2O}$, $c_{(A^-)}\varphi_{H_2O}$ and $c_{(HA)}\varphi_{H_2O}$, respectively, where φ_{H_2O} is the volume fraction of water in the mobile phase, thereby ${}^{s}_{s}$pK_a is represented as

$${}^{s}_{s}K_a = \frac{c_{(H^+)} \times \varphi_{H_2O} \times c_{(A^-)} \times \varphi_{H_2O}}{c_{(HA)} \times \varphi_{H_2O}} = \frac{c_{(H^+)} \times c_{(A^-)} \times \varphi_{H_2O}}{c_{(HA)}} = {}^{w}_{w}K_a \times \varphi_{H_2O} \tag{3}$$

The difference between ${}^{s}_{s}$pK_a and ${}^{w}_{w}$pK_a, ΔpK_a, is obtained

$$\Delta pK_a = {}^{s}_{s}pK_a - {}^{w}_{w}pK_a = -\log\varphi_{H_2O} \tag{4}$$

In general, buffers used as ion-suppressors may be strong or weak acids or bases. If an aqueous buffering solution is prepared from a strong monoprotic acid, e.g., perchloric acid, as Espinosa et al. discussed [25], the difference between ${}^{s}_{s}$pH and ${}^{w}_{w}$pH of the solution, i.e., ΔpH is

$$\Delta pH = {}^{s}_{s}pH - {}^{w}_{w}pH = -\log\varphi_{H_2O} \tag{5}$$

The equalities $\Delta pH = {}^{s}_{s}pH - {}^{w}_{w}pH = {}^{s}_{s}pK_a - {}^{w}_{w}pK_a = \Delta pK_a$ are obtained by comparing Eq. (4) with Eq. (5), which can be alternated to

$${}^{s}_{s}pH - {}^{s}_{s}pK_a = {}^{w}_{w}pH - {}^{w}_{w}pK_a \tag{6}$$

As Subirats et al. proposed [17], ${}^{s}_{w}pH - {}^{s}_{s}pH = {}^{s}_{w}pK_a - {}^{s}_{s}pK_a = \delta$, where δ is a constant that depends only on the organic modifier used. These equalities can be transformed to

$${}^{s}_{s}pH - {}^{s}_{s}pK_a = {}^{s}_{w}pH - {}^{s}_{w}pK_a \tag{7}$$

By comparing Eq. (6) with Eq. (7), the following equalities are obtained

$${}^{s}_{s}pH - {}^{s}_{s}pK_a = {}^{s}_{w}pH - {}^{s}_{w}pK_a = {}^{w}_{w}pH - {}^{w}_{w}pK_a \tag{8}$$

${}^{w}_{w}\mathrm{pH}$ and ${}^{w}_{w}\mathrm{p}K_a$ are acidity of the neat aqueous fraction of mobile phase modified by a strong acid and dissociation constant of a monoprotic acid in the aqueous solution, respectively. They are both independent of the fraction of organic modifier in the mobile phase. Therefore, for a given ${}^{w}_{w}\mathrm{pH}$, the value of Eq. (8) is a constant, indicating that although the true pH value (${}^{s}_{s}\mathrm{pH}$ or ${}^{s}_{w}\mathrm{pH}$) of the mobile phase and the true pK_a (${}^{s}_{s}\mathrm{p}K_a$ or ${}^{s}_{w}\mathrm{p}K_a$) of the eluted acidic solute continuously vary as the content of organic modifier in the mobile phase changes, the difference between them is a constant.

Another common case in RP-HPLC is the use of a weak acid, e.g., acetic acid as the ion-suppressor in mobile phase. Espinosa et al. [25] also gave ΔpH between different mobile phase pH scales (the symbols of ΔpH and ΔpK_a are the same as above)

$$\Delta\mathrm{pH} = \frac{\Delta pK_a - \log\varphi_{\mathrm{H_2O}}}{2} \tag{9}$$

Eq. (9) can be transformed into

$${}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_a = ({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_a) + \boxed{({}^{w}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH}) - \log\varphi_{\mathrm{H_2O}}} \tag{10}$$

By substituting Eq. (9) into the polynomial in the above frame on the right side of Eq. (10), the following expression can be obtained

$$({}^{w}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH}) - \log\varphi_{\mathrm{H_2O}} = \frac{1}{2}\log\frac{{}^{s}_{s}\mathrm{p}K_a}{{}^{w}_{w}\mathrm{p}K_a \times \varphi_{\mathrm{H_2O}}} \tag{11}$$

${}^{w}_{w}\mathrm{p}K_a$ and ${}^{s}_{s}\mathrm{p}K_a$ can be individually expressed by Eqs. (2) and (3), therefore, Eq. (11) is described as

$$({}^{w}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH}) - \log\varphi_{\mathrm{H_2O}} = \frac{\log 1}{2} = 0 \tag{12}$$

Hence, Eq. (10) is simplified to Eq. (6).

Equalities ${}^{s}_{w}\mathrm{pH} - {}^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{p}K_a - {}^{s}_{s}\mathrm{p}K_a = \delta$ have been previously established when a weak acid was used as the ion-suppressor [17], thus Eq. (8) can also be available. In this case, ${}^{w}_{w}\mathrm{pH}$ represents the acidity of neat aqueous fraction of mobile phase, and ${}^{w}_{w}\mathrm{p}K_a$ represents the dissociation constant of the eluted monoprotic acid in the aqueous solution. Therefore, (${}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_a$) in RP-HPLC using a weak acid as the ion-suppressor is also independent of organic modifier fraction in the mobile phase, and ${}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_a = {}^{s}_{w}\mathrm{pH} - {}^{s}_{w}\mathrm{p}K_a = {}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_a$ is a constant at a fixed ${}^{w}_{w}\mathrm{pH}$ for a monoprotic acid.

The pK_a values corresponding to the dissociation of high levels of polyprotic weak acids are several orders of magnitude larger than pH values of mobile phase, thus these pK_a values can be neglected in practice. Consequently, the value of Eq. (8) approximating to a constant at fixed ${}^{w}_{w}\mathrm{pH}$ is reasonable for a polyprotic weak acid.

In early studies about retention behaviors of weak acids, the most common RP-HPLC buffers are prepared from an acid at concentration ca and its conjugated base at concentration cb, e.g., acetic acid/acetate. Espinosa et al. [25] has already demonstrated that ${}^{s}_{s}\mathrm{pH} - {}^{w}_{w}\mathrm{p}H = {}^{s}_{s}\mathrm{p}K_a - {}^{w}_{w}\mathrm{p}K_a$ in RP-HPLC mobile phase using acid/base buffer salts as ion-suppressors. Therefore, the value of Eq. (8) for a given ${}^{w}_{w}\mathrm{pH}$ is also a constant for buffer salts.

The above discussions indicate that the difference between true pH of the mobile phase and true pK_a of the eluted acidic solute in the mobile phase equals to the difference between ${}^{w}_{w}\mathrm{pH}$ of neat aqueous fraction of the mobile phase and ${}^{w}_{w}\mathrm{p}K_a$ of the solute in the aqueous fraction. Moreover, at a fixed mobile phase ${}^{w}_{w}\mathrm{pH}$, this difference keeps invariable, regardless of the change of organic modifier content in the mobile phase during RP-HPLC procedure

$${}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_a = {}^{s}_{w}\mathrm{pH} - {}^{s}_{w}\mathrm{p}K_a = {}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_a = \text{constant} \tag{13}$$

Therefore, it is reasonable to calculate K_{ow}'' by using ${}^{w}_{w}\mathrm{pH}$ and ${}^{w}_{w}\mathrm{p}K_a$ through Eq. (14) in our previous works [14–16]

$$K_{ow}'' = \frac{K_{ow}}{1 + ({}^{w}_{w}K_{a1}/[H^+]_w) + ({}^{w}_{w}K_{a1}\,{}^{w}_{w}K_{a2}/[H^+]^2_w) + \cdots + ({}^{w}_{w}K_{a1}\,{}^{w}_{w}K_{a2}\cdots{}^{w}_{w}K_{an}/[H^+]^n_w)} \tag{14}$$

As $\log {}^{w}_{w}K_{a1}/[H^+]_w = ({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a1})$, $\log {}^{w}_{w}\mathrm{p}K_{a1}\,{}^{w}_{w}K_{a2}/[H^+]^2_w = 2\,{}^{w}_{w}\mathrm{pH} - ({}^{w}_{w}\mathrm{p}K_{a1} + {}^{w}_{w}\mathrm{p}K_{a2}) = ({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a1}) + ({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a2})$, and $\log {}^{w}_{w}K_{a1}\,{}^{w}_{w}K_{a2}\cdots{}^{w}_{w}K_{a\mathbf{n}}/[H^+]^n_w = n\,{}^{s}_{s}\mathrm{pH} - ({}^{s}_{s}\mathrm{p}K_{a1} + {}^{s}_{s}\mathrm{p}K_{a2} + \cdots + {}^{s}_{s}\mathrm{p}K_{a\mathbf{n}}) = ({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a1}) + ({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a2}) + \ldots + ({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a\mathbf{n}})$, the required data in Eq. (15) is only the difference value between pH of the mobile phase and pK_a of the acidic solute, but not the individual value of them in calculating procedure for K_{ow}''. That is, the calculation of K_{ow}'' is independent of the organic modifier content in the mobile phase.

$$K_{ow}'' = \frac{K_{ow}}{1 + 10^{({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a1})} + 10^{({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a1}) + ({}^{w}_{w}\mathrm{pH} - {}^{w}_{w}\mathrm{p}K_{a2})} + \cdots + 10^{({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a1}) + ({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a2}) + \cdots + ({}^{s}_{s}\mathrm{pH} - {}^{s}_{s}\mathrm{p}K_{a\mathbf{n}})}} \tag{15}$$

This deduction result is of very significant importance for studying the QSRRs of hydrophobicity and retention behavior of ionizable compounds by RP-HPLC, because it directs us to avoid the rigorously defined pH and pK_a measurements.

3. Experimental section

3.1. Chemicals

Water for mobile phase was Wahaha purified water (Wahaha Group, Hangzhou, China). The mobile phases were prepared from methanol (HPLC grade, Merck, Darmstadt, Germany) and aqueous acidic solution. The employed ion-suppressors are acetic acid (analytical-reagent grade, Sinopharm Group Chemical Reagent, Shanghai, China), perchloric acid (70–72%, analytical-reagent grade, Nanjing Chemical Reagent, Nanjing, China), phosphoric acid ($\geq$85%, guaranteed-reagent grade, Sinopharm Group Chemical Reagent), and potassium dihydrogen phosphate ($\geq$99.5%, analytical-reagent grade, Nanjing Chemical Reagent). Table 1 lists all substances investigated in this experiment with their reliable literature log K_{ow} and pK_a data. They were all with the purity of 98% or higher checked by RP-HPLC, and then used without further purification. Stock solutions of these compounds were respectively prepared in methanol (about 1.0 mg mL^{-1}) and stored in refrigerator before use.

3.2. Apparatus

A Waters 2695 Alliance separation module (Milford, MA, USA) was employed consisting of a vacuum degasser, a quaternary pump and an auto-sampler, and a Waters 996 photodiode-array (PDA) detector set at the respective optimum absorption wavelength for each eluted compound. The chromatographic column used was an Agela Venusil XBP C18, 5 μm, 150 mm × 2.1 mm i.d. (Bonna-Agela Technologies, Tianjin, China) maintained at 30 °C. Data acquisition and processing were performed on a Waters Empower chromatography manager system. All experimental retention times (t_R) were obtained by averaging the results of at least three independent injections at 0.2 mL min^{-1} mobile phase flow rate.

The pH values of mobile phase were measured with a Seven-Multi electrochemical analytical meter (Metter-Toledo, Schwerzenbach, Switzerland). The electrode system was standardized with ordinary aqueous buffers of pH 2.00 and 4.01 at 25 °C (Mettler-Toledo). All pH readings were carried out in ${}^{w}_{w}\mathrm{pH}$ scale,

Table 1
Substituted benzoic acids studied.

Compounds	Log K_{ow} [a]	pK_a [b]	Log K_{ow}''		
			Mobile phase ${}^{w}_{w}$pH		
			2.80	3.20	3.60
Benzoic acid	1.87[a1]	4.20	1.85	1.83	1.77
2-Methylbenzoic acid	2.18[a2]	3.90	2.15	2.10	2.00
3-Methylbenzoic acid	2.37[a3]	4.27	2.36	2.33	2.28
4-Methylbenzoic acid	2.27[a4]	4.36	2.26	2.24	2.20
4-Ethylbenzoic acid	2.89[a5]	4.35	2.88	2.86	2.82
4-(1-Methylethyl)benzoic acid	3.40[a6]	4.35	3.27	3.25	3.21
2-Chlorobenzoic acid	2.05[a7]	2.88	1.79	1.56	1.25
3-Chlorobenzoic acid	2.68[a8]	3.83	2.64	2.59	2.48
4-Chlorobenzoic acid	2.65[a9]	3.99	2.62	2.58	2.50
2-Bromobenzoic acid	2.20[a10]	2.85	1.92	1.69	1.38
3-Bromobenzoic acid	2.87[a11]	3.81	2.83	2.77	2.66

[a] Only reliable Shake-flask method/Slow-stirring method (SFM/SSM) data (presented in BioByte Star List) were adopted: [a1,a3,a4,a8,a9,a11] from [26]; [a2,a7,a10] from [27]; [a5,a6] from [28].
[b] From [29].

i.e., the pH of aqueous fraction before mixing it with organic modifiers.

3.3. Procedure

All compounds studied were eluted isocratically by the mobile phase consisting of methanol and water at pH 2.80, 3.20 and 3.60. Each mobile phase pH was adjusted by acetic acid, perchloric acids, and 20 mM potassium dihydrogen phosphate/phosphoric acid (PBS), respectively. At each pH adjusted by every ion-suppressor, at least four different methanol contents were required to elute each solute according to its lipophilicity. The t_R value was recorded at each methanol-aqueous solution ratio, then corrected by dual-point retention time correction (DP-RTC) using 2-chlorobenzoic acid and 3-bromobenzoic acid as "anchor compounds". The k value was calculated according to the equation $k=(t_R-t_0)/t_0$, where t_0 was determined by using sodium nitrate eluted on the "standard column". The detailed process of DP-RTC refers to our previous work [30]. For each solute, the logarithm of k was plotted against the volume fraction of methanol (φ_{CH_3OH}),

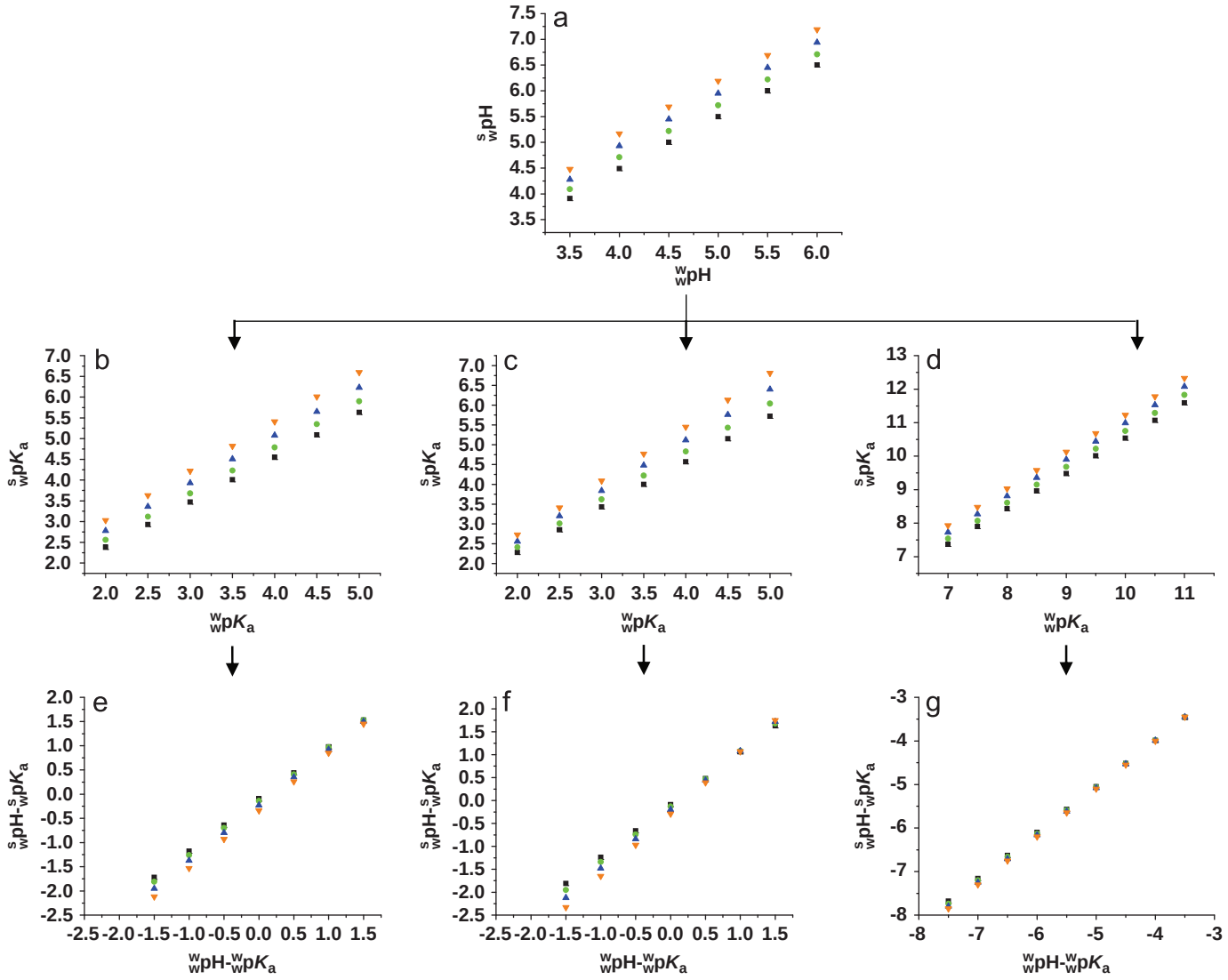

Fig. 1. Deviations between ${}^{w}_{w}$pH and ${}^{s}_{w}$pH, ${}^{w}_{w}pK_a$ and ${}^{s}_{w}pK_a$, as well as (${}^{w}_{w}$pH$-{}^{w}_{w}pK_a$) and (${}^{s}_{w}$pH$-{}^{s}_{w}pK_a$) for three different compound families at ${}^{w}_{w}$pH 3.20 adjusted by acetic (a–g) and phosphoric (h–n) acid, respectively [17]. Three compound families are aromaticcarboxylic acid-with orthosubstitution (b), (e), (i) and (l); aromaticcarboxylic acid-without orthosubstitution (c), (f), (j) and (m); and phenols (d), (g), (k) and (n). Symbols: ■ 30% methanol; ● 40% methanol; ▲ 50% methanol; ▼ 60% methanol.

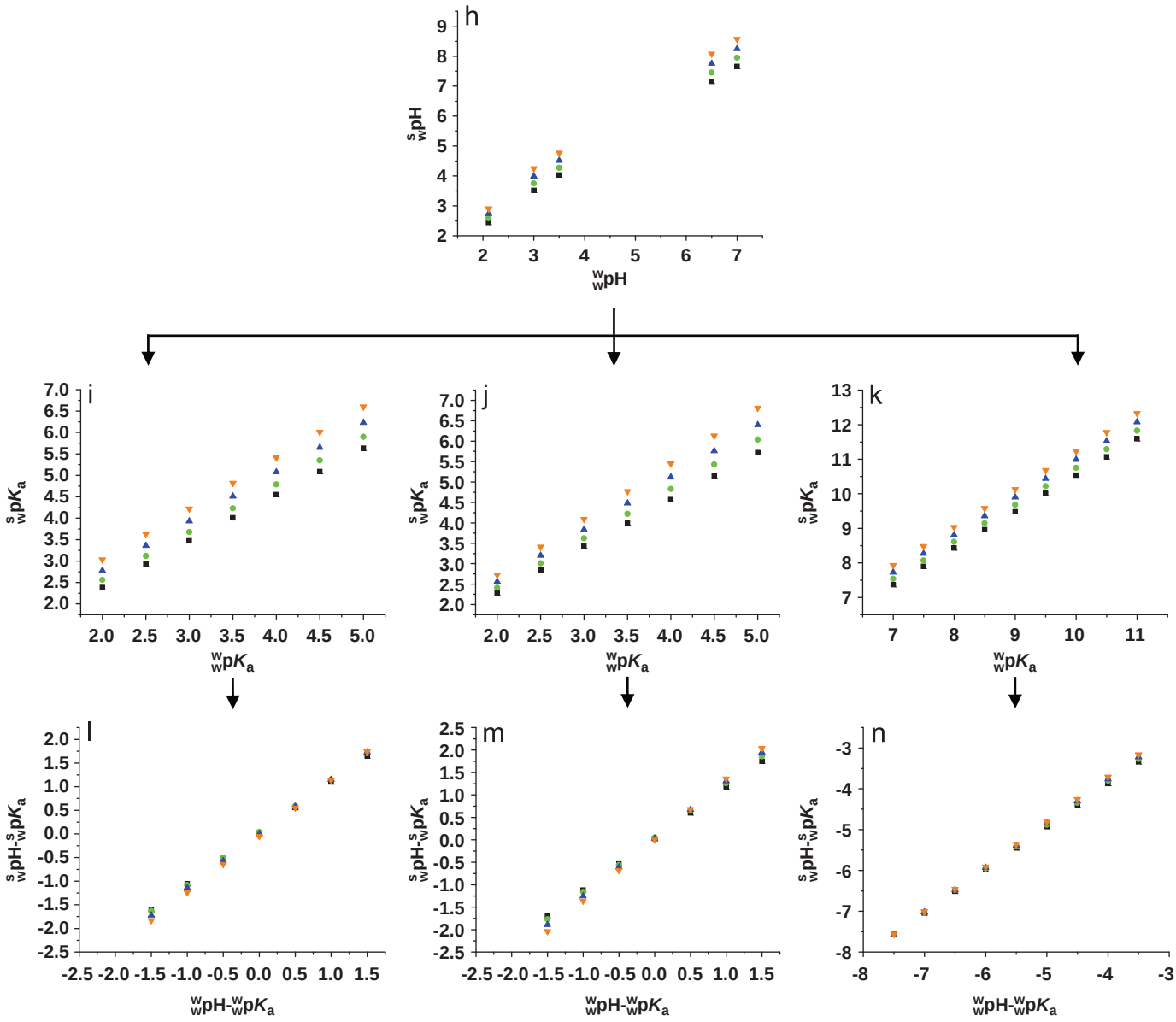

Fig 1. *Continued.*

and log k_w of the solute was subsequently obtained by extrapolation of retention factor to neat aqueous mobile phase via Snyder–Soczewinski equation [8]. The literature K_{ow} value of each compound was calibrated to the corresponding K_{ow}'' through Eq. (14). Then the correlations relating log K_{ow}'' and log k_w of acidic compounds at various elution conditions were derived with different ion-suppressors at different mobile phase pH.

The statistical analysis for regression model was accomplished by SPSS V16.0.0 (SPSS, Chicago, Illinois, USA) and MATLAB Software V7.10.0 (R2010.a) (The MathWorks, Natick, MA, USA).

4. Results and discussion

4.1. Influence of organic modifier fraction on K_{ow}'' of acidic compounds

Subirats et al. [17] revealed the change of mobile phase pH, as well as that of pK_a for different families of compounds identified by acidic functional groups, with the addition of methanol into neat aqueous mobile phase. These data were further analyzed in this present paper. It can be seen from Table S-1 (see Supporting Information) that the change values of pK_a with the addition of methanol are different for various families, but are constant for the compounds in the same family. At a specific $^{w}_{w}$pH, although the true pH value ($^{s}_{s}$pH or $^{s}_{w}$pH) of mobile phase and the true pK_a ($^{s}_{s}pK_a$ or $^{s}_{w}pK_a$) of every eluted acidic solute continuously varied as methanol content changed in the mobile phase, the difference value between pH and the corresponding pK_a was a constant. Moreover, this difference value is numerically equal to that between $^{w}_{w}$pH of the mobile phase and $^{w}_{w}pK_a$ of the solute. Table S-1 also lists the precision of the difference between $^{w}_{w}$pH and $^{s}_{w}$pH at various organic modifier fractions by using acetic or phosphoric acid as the ion-suppressor. The standard deviations (SDs) were 0.25–0.30 for acetic acid and 0.20–0.40 for phosphoric acid, indicating the significant deviation of ($^{w}_{w}$pH$-^{s}_{w}$pH) at different methanol contents for both ion-suppressors. Analogously, the deviations of ($^{w}_{w}pK_a-^{s}_{w}pK_a$) were also large for all the compound families. However, the SDs of ($^{s}_{w}$pH$-^{s}_{w}pK_a$) for each compound family with different acidity were very small. As collected in Table S-1, these

SDs presented the values lower than or close to 0.10 with the high proportion of 73.9%. In contrast, only 3.8% of the SDs was larger than 0.20. Fig. 1 illustrates deviations between ${}^{w}_{w}$pH and ${}^{s}_{w}$pH, ${}^{w}_{w}pK_a$ and ${}^{s}_{w}pK_a$, as well as $({}^{w}_{w}\mathrm{pH}-{}^{w}_{w}pK_a)$ and $({}^{s}_{w}\mathrm{pH}-{}^{s}_{w}pK_a)$ for three different compound families in different methanol contents at ${}^{w}_{w}$pH 3.20. It can be observed that for each compound family, plots of $({}^{s}_{w}\mathrm{pH}-{}^{s}_{w}pK_a)$ and corresponding $({}^{w}_{w}\mathrm{pH}-{}^{w}_{w}pK_a)$ nearly overlapped at different methanol contents, indicating that the value of $({}^{s}_{w}\mathrm{pH}-{}^{s}_{w}pK_a)$ equals to that of $({}^{w}_{w}\mathrm{pH}-{}^{w}_{w}pK_a)$. Moreover, all the zero-crossing linear fittings had slopes approximating to 1.0, although plots of ${}^{w}_{w}$pH vs. ${}^{s}_{w}$pH and ${}^{w}_{w}pK_a$ vs. ${}^{s}_{w}pK_a$ had distinct difference at each methanol fraction.

The results of data analysis verified the conclusion derived in "Theoretical basis" section, that is, the direct use of ${}^{w}_{w}$pH and ${}^{w}_{w}pK_a$ has no influence on the accuracy of K_{ow}'' calculation, as well as the QSRRs of log K_{ow}'' vs. log k_w for weakly acidic compounds. Therefore, it is not necessary to use the complex pH (${}^{s}_{s}$pH or ${}^{s}_{w}$pH) and pK_a (${}^{s}_{s}pK_a$ or ${}^{s}_{w}pK_a$) scales in similar works, which means that the experimental procedure can be greatly simplified, and furthermore, that the choice of organic modifier content in mobile phase only depends on solute hydrophobicity, but not the corresponding variation of mobile phase pH and solute pK_a.

4.2. QSRRs of log K_{ow}'' (log K_{ow}) vs. log k_w at different elution conditions

Table 2 lists relationships between log K_{ow}'' (log K_{ow}) and log k_w for 11 substituted benzoic acids eluted by methanol-aqueous solutions at different ${}^{w}_{w}$pH. The better linearity between log K_{ow}'' and log k_w was obtained than that between log K_{ow} and log k_w at all the mobile phase pH. Moreover, the high consistency of log K_{ow}''—log k_w linear fittings was observed at different ${}^{w}_{w}$pH adjusted by the same ion-suppressor in most cases. On the contrary, the corresponding log K_{ow}—log k_w fitting equations varied at different mobile phase pH. When perchloric acid was used as the ion-suppressor, slopes of log K_{ow}''—log k_w linear relationships were all very close to one, implying that the apparent n-octanol/water partitioning and chromatographic retention were homo-energetic processes [31–33], and that log k_w can simulate log K_{ow}'' well. Furthermore, the intercepts of log K_{ow}''—log k_w equations established at different ${}^{w}_{w}$pH were almost the same. The possible reason for the prominent homo-energetic processes is explained as below: perchloric acid can also be considered as an ion-pair agent, prevailing over the ionized acidic solutes in combining with the residual silanols on reversed-phase C18 stationary phase, thereby the secondary interaction is eliminated within the mobile phase pH range investigated. When acetic acid was used as the ion-suppressor, slopes of log K_{ow}''—log k_w linear relationships were approximate to one, but the slope values slightly increased as mobile phase pH increased. A little deviation of the slope from 1.0 at ${}^{w}_{w}$pH 2.80 (0.96) was due to the role of organic modifier played by acetic acid especially at low mobile phase pH [15,16,34]. The change in "total" organic modifier content (higher than original methanol content arising from the additional contribution of acetic acid) had an impact on the acquirement of accurate log k_w by extrapolation, which resulted in a little influence on the simulation accuracy of the chromatographic procedure for *n*-octanol/water partitioning. The departure of the slope from 1.0 at ${}^{w}_{w}$pH 3.60 (1.04) was probably caused by the interaction between ionized solutes and the residual silanols on C18 stationary phase. The proportion of dissociated acidic solutes increases with mobile phase pH increase, thus the attraction of silanols to anions cannot be neglected at high ${}^{w}_{w}$pH. This interaction inevitably exerts an influence on the retention mechanism. The continuous decrease of the intercepts as mobile phase pH increases could also be attributed to this interaction. As the adsorption strengthened the retention of acidic solutes on reversed-phase C18 stationary phase, especially for more hydrophilic ones (log $k_w < 2.0$), the trend of retention decrease of these solutes became weaker at higher ${}^{w}_{w}$pH. In consequence, the intercepts of log K_{ow}''—log k_w linear relationships decreased. The same phenomenon was observed by using PBS as the ion-suppressor. The slopes of QSRRs of log K_{ow}'' vs. log k_w also increased with the increase of mobile phase pH, however, the closest value to one was obtained at ${}^{w}_{w}$pH 3.60. This is probably because in comparison to the ionized acidic solute, potassium dihydrogen phosphate was competitive in interacting with the residual silanols on C18 stationary phase. The content of potassium dihydrogen phosphate increased as ${}^{w}_{w}$pH increased, which reduced the interaction between ionized acidic solutes and the residual silanols. Therefore, the slopes of the QSRRs more and more approached to one with ${}^{w}_{w}$pH increase. The statistical results summarized in Table 2 indicated that it is most suitable by using strong acid as the ion-suppressor for modeling the QSRRs of log K_{ow}'' vs. log k_w for weakly acidic compounds. Whereas, the linearity of log K_{ow}''—log k_w correlations is inevitably affected by the secondary interaction between acidic solutes and residual silanols by using weak acid or buffer salt as the ion-suppressor.

5. Conclusions

In general, true pH of mobile phase and true pK_a of acidic solute vary with the change of organic modifier content in RP-HPLC, which has been demonstrated in many works. It seems that these variations of pH and pK_a inevitably have effect on the QSRRs of hydrophobicity and retention of acidic compounds. Thus, most of the early studies on these QSRRs insistently tried to fix mobile

Table 2
The relationships between log K_{ow}'' (log K_{ow}) and log k_w for 11 substituted benzoic acids eluted by various methanol-aqueous buffer solutions at different ${}^{w}_{w}$pH (95% confidence limits are in parentheses, R^2 the squared correlation coefficient, R^2_{cv} the cross-validated correlation coefficient, SD the standard deviation, and *F* the Fisher's test value).

Ion-suppressor	${}^{w}_{w}$pH	Log K_{ow} − log k_w						Log K_{ow}'' − log k_w					
		Slope	Intercept	R^2	R^2_{cv}	SD	*F*	Slope	Intercept	R^2	R^2_{cv}	SD	*F*
Perchloric acid	2.80	0.88 (0.07)	0.54 (0.15)	0.946	0.931	0.10	176.75	0.99 (0.04)	0.22 (0.09)	0.985	0.982	0.06	676.32
	3.20	0.77 (0.08)	0.83 (0.18)	0.897	0.855	0.14	87.81	0.99 (0.04)	0.21 (0.09)	0.984	0.978	0.07	609.09
	3.60	0.68 (0.10)	1.10 (0.21)	0.820	0.736	0.19	46.68	1.02 (0.04)	0.14 (0.08)	0.984	0.978	0.08	622.64
Acetic acid	2.80	0.84 (0.07)	0.73 (0.15)	0.937	0.918	0.11	150.95	0.96 (0.04)	0.42 (0.08)	0.986	0.983	0.06	681.27
	3.20	0.79 (0.08)	0.87 (0.17)	0.905	0.860	0.14	95.76	1.01 (0.04)	0.29 (0.08)	0.985	0.979	0.07	655.43
	3.60	0.70 (0.10)	1.06 (0.22)	0.822	0.739	0.19	47.10	1.04 (0.04)	0.10 (0.09)	0.985	0.977	0.08	664.29
PBS	2.80	0.80 (0.07)	0.80 (0.16)	0.921	0.890	0.13	118.05	0.91 (0.04)	0.48 (0.08)	0.985	0.983	0.06	672.76
	3.20	0.74 (0.09)	0.92 (0.21)	0.857	0.792	0.17	60.77	0.97 (0.04)	0.29 (0.08)	0.986	0.983	0.07	705.83
	3.60	0.65 (0.10)	1.16 (0.22)	0.791	0.690	0.21	38.84	0.99 (0.04)	0.21 (0.08)	0.985	0.981	0.08	668.15

phase pH using acid–base buffer solutions, as well as to keep acidic solutes in the neutral form. In this work, it is manifested that although ${}^{s}_{w}\mathrm{pH}$ or ${}^{s}_{s}\mathrm{pH}$ of the mobile phase and ${}^{s}_{w}\mathrm{p}K_a$ or ${}^{s}_{s}\mathrm{p}K_a$ of the acidic solute continuously change at various organic modifier fraction, the difference between them, i.e., $({}^{s}_{w}\mathrm{pH}-{}^{s}_{w}\mathrm{p}K_a)$ and $({}^{s}_{s}\mathrm{pH}-{}^{s}_{s}\mathrm{p}K_a)$ are a constant. This result reinforced that the calculation of $\log K_{ow''}$ from $\log K_{ow}$ and $\mathrm{p}K_a$ of solute, and pH of mobile phase is independent of the organic modifier content in hydro-organic mobile phase, as only the different between pH and $\mathrm{p}K_a$ is required in the calculation procedure. In addition, the type of available ion-suppressors is significantly expanded, as the mobile phase pH is not necessary keep invariable, i.e., strong and weak acids, and buffer salts all can be employed as the ion-suppressors. The QSRRs of $\log K_{ow''}$ vs. $\log k_w$ obtained by using different types of ion-suppressors were also compared in this work. It is suggested that for studying the QSRRs between the hydrophobicity and retention of acidic compounds in RP-HPLC, the use of strong acids, e.g., perchloric acid as the ion-suppressor is recommended with priority.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.08.051.

References

[1] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616–1626.
[2] H. van de Waterbeemd, B. Testa., in: B. Testa (Ed.), Advances in Drug Research, Academic Press, New York, 1987, pp. 85–227.
[3] C. Hansch, A.J. Leo, D. Hoekman, Fundamentals and Applications in Chemistry and Biology in Exploring QSAR, American Chemical Society, Washington DC, 1995.
[4] D.A. Smith, B.C. Jones, D.K. Walker, Med. Res. Rev. 16 (1996) 243–266.
[5] K. Ballschmiter, D. Klingler, S. Ellinger, R. Hackenberg, Anal. Bioanal. Chem. 382 (2005) 1859–1870.
[6] J.E. Garst, W.C. Wilson, J. Pharm. Sci. 73 (1984) 16l6–1629.
[7] OECD, Guideline for Testing of Chemicals, No.117: Partition Coefficient (n-octanol/water)-High Performance Liquid Chromatography Method, 1989.
[8] L.R. Snyder, J.W. Dolan, J.R. Gant, J. Chromatogr. 165 (1979) 3–30.
[9] T. Braumann, J. Chromatogr. 373 (1986) 191–225.
[10] R.E.A. Escott, P.G. McDowell, N.P. Porter, J. Chromatogr. 554 (1991) 281–292.
[11] C. Crescenzi, A. Di Corcia, S. Marchese, R. Samperi, Anal. Chem. 67 (1995) 1968–1975.
[12] R.B. Cole, Electrospray Ionization Mass Spectrometry, John Wiley and Sons, New York, 1997.
[13] H.Z. Lian, W.H. Wang, D.N. Li, J. Sep. Sci. 28 (2005) 1179–1187.
[14] X. Ming, S.Y. Han, Z.C. Qi, D. Sheng, H.Z. Lian, Talanta 79 (2009) 752–761.
[15] S.Y. Han, X. Ming, Z.C. Qi, D. Sheng, H.Z. Lian, Anal. Bioanal. Chem. 398 (2010) 2731–2743.
[16] S.Y. Han, J.Q. Qiao, Y.Y. Zhang, H.Z. Lian, X. Ge, Talanta 97 (2012) 355–361.
[17] X. Subirats, M. Rosés, E. Bosch, Sep. Purif. Rev. 36 (2007) 231–255.
[18] IUPAC, Compendium for Analytical Nomenclature, Definitive Rules, Blackwell, Oxford, 1998.
[19] T. Mussini, A.K. Covington, P. Longhi, S. Rondinini, Pure Appl. Chem. 57 (1985) 865–876.
[20] S. Rondinini, P.R. Mussini, T. Mussini, Pure Appl. Chem. 59 (1987) 1549–1560.
[21] J. Flieger, J. Chromatogr. A 1175 (2007) 207–216.
[22] R. Ruiz, C. Ràfols, M. Rosés, E. Bosch, J. Pharm. Sci. 92 (2003) 1473–1481.
[23] C. Horváth, W. Melander, I. Molnar, Anal. Chem. 49 (1977) 142–154.
[24] I. Canals, J.A. Portal, E. Bosch, M. Rosés, Anal. Chem. 72 (2000) 1802–1809.
[25] S. Espinosa, E. Bosch, M. Rosés, Anal. Chem. 74 (2002) 3809–3818.
[26] T. Fujita, J. Iwasa, C. Hansch, J. Am. Chem. Soc. 86 (1964) 5175–5180.
[27] H. Tomida, T. Yotsuyanagi, K. Ikeda, Chem. Pharm. Bull. Japan 26 (1978) 2824–2831.
[28] K. Gerogi, K.S. Boos, Chromatographia 63 (2006) 523–531.
[29] J.G. Speight (Ed.), 16th ed.,McGraw-Hill, New York, 2005.
[30] S.Y. Han, J.Q. Qiao, Y.Y. Zhang, L.L. Yang, H.Z. Lian, X. Ge, H.Y. Chen, Chemosphere 83 (2011) 131–136.
[31] D.J. Minick, D.A. Brent, J. Frenz, J. Chromatogr. 461 (1989) 177–191.
[32] X.L. Liu, H. Tanaka, A. Yamauchi, B. Testa, H. Chumana, J. Chromatogr. A 1091 (2005) 51–59.
[33] C. Giaginis, S. Theocharis, A. Tsantili-Kakoulidou, Anal. Chim. Acta 573–574 (2006) 311–318.
[34] Z.C. Qi, S.B. Mei, H.Z. Lian, Sci. China (Series B) 39 (2009) 806–818.