Journal of Chromatography A, 1218 (2011) 6454–6463

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Modeling the effects of type and concentration of organic modifiers, column type and chemical structure of analytes on the retention in reversed phase liquid chromatography using a single model

Abolghasem Jouyban[a,*], Somaieh Soltani[b], Ali Shayanfar[c], Adriani Pappa-Louisi[d]

[a] Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[b] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[c] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz 51664, Iran
[d] Laboratory of Physical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 11 November 2010
Received in revised form 19 June 2011
Accepted 11 July 2011
Available online 20 July 2011

Keywords:
RP-HPLC
Modeling
Retention time

ABSTRACT

A previously proposed model for representing the retention factor (k) of an analyte in mixed solvent mobile phases was extended to calculate the k of different analytes with respect to the nature of analyte, organic modifier, its concentration and type of the stationary phase. The accuracy of the proposed method was evaluated by calculating mean percentage deviation (MPD) as accuracy criterion. The predicted vs. observed plots were also provided as goodness of fit criteria. The developed model prediction capability compared with a number of previous models (i.e. LSER, general LSER and Oscik equation) through MPD and fitting plots. The proposed method provided acceptable predictions with the advantage of modeling the effects of organic modifiers, mobile phase compositions, columns and analytes using a single equation. The accuracy of developed model was checked using the one column and one analyte out cross validation analyses and the results showed that the developed model was able to predict the unknown analyte retention and the analytes retentions on unknown column accurately.

1. Introduction

Many efforts were made to provide mathematical models for prediction of the retention factor of analytes in high performance liquid chromatography (HPLC). These models were reviewed and their accuracies were compared [1,2]. Most of the proposed models treat the retention factor data in specific conditions, such as specified analyte, various analytes on a specified column or specified organic modifier, or in a given organic modifier concentration. Although these models provided acceptable predictions, however they could be employed as mathematical tools for a single variable optimization and models for simultaneous representation of various affecting parameters are in demand to facilitate the multivariate optimization in HPLC.

Organic modifier volume fraction based models (partition theory) have been used to predict the retention data for a given analyte in a given column with respect to mobile phase composition and known as linear model:

$$\log k_m = \log k_w + J\varphi \quad (1)$$

or quadratic model:

$$\log k_m = \log k_w + M_1\varphi + M_2\varphi^2 \quad (2)$$

where φ is the volume fraction of organic modifier, J, M_1 and M_2 are the model constants. These models have been widely checked in an applicable range of organic modifier fractions. Although the required number of experiments for obtaining the model parameters of Eq. (1) is low, the organic modifier fraction applicable range is narrow and Eq. (2) has the advantage of being useful for higher range of φ [2].

Due to the complex nature of retention, the partition and absorption theory have been combined in order to modeling the retention of analytes in RPLC [3], and the developed model (Oscik equation) have been used for the prediction of $\log k_w$ recently [4]. The general form of Oscik equation is:

$$\log k_m = x_1^l \log k_1 + x_2^l \log k_2 + (x_1^s - x_1^l)\left(\log \frac{k_1}{k_2} + A_m\right) \quad (3)$$

$\log k_1$ and $\log k_2$ ($=\log k_w$) could be estimated from a linear plot of $\log k_m$ against x_1^l (mole fraction of organic modifier in mobile phase). More details of these computations could be found in the original Ref. [4].

Linear solvation energy relationships (LSER) have been developed to modeling various possible interactions of the different

* Corresponding author. Tel.: +98 411 3379323; fax: +98 411 3363231.
E-mail address: ajouyban@hotmail.com (A. Jouyban).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.07.034

compounds by the stationary and mobile phases. The general form of the Abraham model is:

$$SP = c + e \cdot E + s \cdot S + a \cdot A + b \cdot B + v \cdot V \quad (4)$$

where SP is analyte property including the retention factor ($\log k$), E is the excess molar refraction, S is dipolarity/polarizability of analyte, A denotes the analyte's hydrogen-bond acidity, B stands for the analyte's hydrogen-bond basicity and V is the McGowan volume of the analyte. In Eq. (4) the coefficients c, e, s, a, b and v are the model constants representing solvent's properties. Eq. (4) was used for representing the retention factor of analytes in RP-HPLC with a given solvent composition (mono-solvents or mixed solvents) as:

$$\log k = c' + e' \cdot E + s' \cdot S + a' \cdot A + b' \cdot B + v' \cdot V \quad (5)$$

in which the regressed parameters (i.e. c', e', s', a', b' and v') refer to the differences of stationary and mobile phases; e' refer to the capability of interacting with analyte π- and n-electron pairs, s' dipolarity/polarizability, a' hydrogen-bond basicity (an acidic analyte interacts with basic phase), b' hydrogen-bond acidity and v' hydrophobicity [5]. In another compilation by Sandi and Szepesy [6] the regressed parameters of Eq. (5) were obtained using retention factors of 34 analytes using acetonitrile–water (30:70) mobile phase collected on 15 widely used stationary phases. In this work, we have adopted these parameters to represent the effect of column type on retention factor of analytes.

Sandi and Szepesy in a series of publications [6–10] studied the characteristics of different stationary phases using the linear free energy relationships. They introduced the ability of LSER approach to measure independently the contribution of individual molecular interactions to the retention process. In the first study [6] they used a set of 34 diverse polarity compounds retention factors which obtained in 15 different columns using a single mobile phase of acetonitrile–water (30:70 v/v) by isocratic method. As they used water instead of buffer the obtained phase system coefficients of LSER equations were due to the packing materials without any surface modifications. They compared the proposed LSER method with a PCA and concluded that the LSER approach provides more detailed and reliable description on the role and extent of the different molecular interactions. In the next paper [8] they used LSER to study the selectivity factors for various analyte pairs using previous data sets. They used these factors to characterize the hydrophobic properties and different types of molecular interactions of the columns. One year later, the selectivity in RPLC evaluated using retention factor of 31 diverse polarity analytes on 5 different columns using acetonitrile–water and methanol–water in a composition range of 20–70% (v/v). The results showed that the selectivity is a complex characteristics affected by stationary phase characteristics, mobile phase composition, organic modifier and analyte characteristics [9]. In another attempt they used these data to characterize the studied columns through the effect of the organic modifier and the mobile phase composition [10]. The calculated regression coefficients of the LSER equations for the different mobile phase compositions have been used to study the extent and relative importance of the individual molecular interactions due to mobile phase compositions. They concluded that the LSER is able to provide an estimate of selectivity under different operating conditions (mobile and stationary phases). Later, Szepesy evaluated the effect of molecular interaction on retention and selectivity in RPLC in order to establish that the retention and selectivity depend on all participants (structure and properties of the stationary phase, the type and composition of the mobile phase and the molecular properties of the analytes) of the system. He explained how the specific molecular interactions influence chromatographic behavior of analytes using the LSER approach [11]. However they did not develop any model to predict the retention factor according to the all studied effective parameters.

Wang et al. [12] and Wang and Carr [13], developed a global linear solvation energy relationship (GLSER) by combination of the linear solvent strength theory and local LSER models which simultaneously models retention in reversed-phase liquid chromatography as a function of both analyte LSER descriptors and mobile phase composition. The general form of their model is:

$$\begin{aligned}\log k_m = {} & (\log k_{0,w} - \log k_{0,J}\varphi) + (v_w - v_J\varphi)V + (s_w - s_J\varphi)S \\ & + (a_w - a_J\varphi)A + (b_w - b_J\varphi)B + (e_w - e_J\varphi)E\end{aligned} \quad (6)$$

considering the local LSER model for a number of analytes in a given mobile phase composition and column as:

$$\log k_m = \log k_w + vV + sS + aA + bB + eE \quad (7)$$

The J and $\log k_w$ of Eq. (1) as linear free energy parameters can be calculated using Eq. (7):

$$\log k_w = \log k_{0,w} + v_wV + s_wS + a_wA + b_wB + e_wE \quad (8)$$

$$J = \log k_{0,J} + v_JV + s_JS + a_JA + b_JB + e_JE \quad (9)$$

From these two equations we have:

$$\begin{aligned}\log k_m = {} & \log k_{0,w} + v_wV + s_wS + a_wA + b_wB + e_wE \\ & + (\log k_{0,J} + v_JV + s_JS + a_JA + b_JB + e_JE)\varphi\end{aligned} \quad (10)$$

and by rewriting this equation we have Eq. (6).

Combining these two methods, Wang et al. [12] investigated the prediction capability of the combined model and developed a global version of the LSER model which is able to predict the retention of a test analyte in the desired mobile phase composition in a given column. They concluded that the developed model needed fewer measurements comparing with LSER model and is more practical.

Abraham et al. [14] developed a general LSER by obtaining the system coefficients for different mobile phase-column systems and combined the obtained equations by calculating the ratio of the coefficients to the v values of each system (which is constant for all systems). The developed model is:

$$\log k_m = c + v\left(V + \frac{s}{v}S - \frac{a}{v}A - \frac{b}{v}B - \frac{e}{v}E\right) \quad (11)$$

where s/v, a/v, b/v and e/v coefficients are constant for a given column-solvent system and the trained version of this equation can be used to predict the retention factor of an analyte in different mobile phase compositions.

Another general LSER model has been provided based on quadratic model (Eq. (2)) [1]:

$$\begin{aligned}\log k_m = {} & (c_{0,w} + e_{0,w}E + s_{0,w}S + a_{0,w}A + b_{0,w}B + v_{0,w}V) \\ & + (c_{M1} + e_{M1}E + s_{M1}S + a_{M1}A + b_{M1}B + v_{M1}V)\varphi \\ & + (c_{M2} + e_{M2}E + s_{M2}S + a_{M2}A + b_{M2}B + v_{M2}V)\varphi^2\end{aligned} \quad (12)$$

which can be simplified to:

$$\begin{aligned}\log k_m = {} & (c_{0,w} + e_{0,w}E + s_{0,w}S + a_{0,w}A + b_{0,w}B + v_{0,w}V) \\ & + M_1\varphi + M_2\varphi^2\end{aligned} \quad (13)$$

The last model provided the possibility to extend the prediction capability of quadratic model to different analytes.

Recently, an ANN approach was established to predict the retention factors of 26 pesticides in 6 different stationary phases and several composition of acetonitrile–water (30–70% v/v) mobile phase. They used 5 analytes, one mobile phase and one stationary phase descriptors as inputs and the mean percentage deviation (MPD) for back calculated retention factors was about 45% [15]. These proposals are steps forward, however, the models should be trained for other organic modifiers, stationary phases, analytes, and they are only applicable for the trained dataset and there is no possibility to extend their application for other organic modifiers.

The aim of this work is to propose quantitative structure-property relationship (QSPR) models based on the Jouyban-Acree model for modeling the retention factor of analytes with respect to the nature of analyte, column, organic modifier as well as concentration of organic modifier using a single model.

2. Experimental

2.1. Experimental data

The details of the experimental retention factors collected from the literature [9] are listed in Table 1 of supplementary materials. The Abraham solvent coefficients of water, acetonitrile and methanol are listed in Table 1 [16]. The collected Abraham solvation parameters for representing the column interactions are reported in Table 2 [6]. Table 3 lists the numerical values of Abraham solute parameters investigated in this work taken from Refs. [6,17]. In addition to the experimentally measured solvation parameters, these descriptors could be computed using Pharma-Algorithms software [18] and this makes the prediction procedures more feasible.

2.2. Computational methods

The Jouyban-Acree model for representation of the retention factor of an analyte in a binary solvent mobile phase is [19]:

Table 1
The Abraham solvent coefficients used in this work taken from a Ref. [16].

Solvent	c	e	s	a	b	v
Acetonitrile	0.413	0.077	0.326	−1.566	−4.391	3.364
Methanol	0.329	0.299	−0.671	0.080	−3.389	3.512
Water	−0.994	0.577	2.549	3.813	4.841	−0.869

Table 2
The characteristics of the columns[a] and the mean percentage deviation (MPD) of fitting and leave one column out analyses using the proposed equations.

Abbreviation	Column	c'	e'	s'	a'	b'	v'	MPD Cross validated		MPD Fitted	
								Eq. (19)	Eq. (20)	Eq. (23)	Eq. (24)
M-C18e	LiChrospher 100 RP-18e	0.24	0.30	−0.48	−0.59	−1.95	1.95	11.9	28.0	11.3	27.2
M-C8	LiChrospher 100 RP-8	0.32	0.21	−0.35	−0.49	−1.53	1.58	11.1	28.8	10.3	22.9
M-PURe	Purospher RP-18e	0.28	0.31	−0.50	−0.62	−1.97	1.95	16.0	27.3	13.4	27.5
Sym-C18	SymmetryShield RP-C_{18}	0.02	0.39	−0.45	−0.43	−2.10	2.01	14.5	20.1	13.1	19.5
Sym-C8	SymmetryShield RP-C_8	0.05	0.33	−0.32	−0.34	−1.99	1.89	16.6	28.3	13.9	21.6
							Overall	14.0	26.5	12.4	23.7

[a] Further details of the columns could be found in the original Ref. [6].

Table 3
The Abraham solute parameters of the analytes investigated in this work taken from Refs. [6,17].

No.	Analyte	E	S	A	B	V
1	2,6-Dimethylphenol	0.86	0.79	0.39	0.39	1.06
2	3,5-Dimethylphenol	0.82	0.84	0.57	0.36	1.06
3	Acetophenone	0.82	1.01	0.00	0.48	1.01
4	alpha-Naphthol	1.52	1.05	0.60	0.37	1.14
5	alpha-Naphthylamine	1.67	1.26	2.00	0.57	1.19
6	Aniline	0.96	0.96	0.26	0.50	0.82
7	Anisole	0.71	0.75	0.00	0.29	0.92
8	Benzyl alcohol	0.80	0.87	0.39	0.56	0.92
9	Benzyl cyanide	0.75	1.15	0.00	0.45	1.01
10	beta-Naphthol	1.52	1.08	0.61	0.40	1.14
11	Bromobenzene	0.88	0.73	0.00	0.09	0.89
12	Butylparaben	0.86	1.35	0.69	0.45	1.55
13	Caffeine	1.50	1.60	0.00	1.33	1.36
14	Chlorobenzene	0.72	0.65	0.00	0.07	0.84
15	Dimethyl phalate	0.78	1.40	0.00	0.84	1.43
16	Ethylbenzene	0.61	0.51	0.00	0.15	1.00
17	Ethylbenzoate	0.69	0.85	0.00	0.46	1.21
18	Ethylparaben	0.86	1.35	0.69	0.45	1.27
19	Hydroquinone	1.00	1.00	1.16	0.60	0.83
20	Methylbenzoate	0.73	0.85	0.00	0.46	1.07
21	Methylparaben	0.90	1.37	0.69	0.45	1.13
22	N,N-dimethylaniline	0.96	0.84	0.00	0.42	1.10
23	o-Cresol	0.84	0.86	0.52	0.31	0.92
24	o-Nitrotoluene	0.87	1.11	0.00	0.28	1.03
25	o-Toluidine	0.97	0.92	0.23	0.59	0.96
26	p-Cresol	0.82	0.87	0.57	0.32	0.92
27	p-Ethylphenol	0.80	0.90	0.55	0.36	1.06
28	Phenol	0.81	0.89	0.60	0.30	0.78
29	Propylparaben	0.86	1.35	0.69	0.45	1.41
30	Pyridine	0.63	0.84	0.00	0.52	0.68
31	Toluene	0.60	0.52	0.00	0.14	0.86

$$\log k_m = \varphi_1 \log k_1 + \varphi_2 \log k_2 + \varphi_1\varphi_2 \sum_{j=0}^{2} B_j (\varphi_1 - \varphi_2)^j \quad (14)$$

where k is the retention factor of the analyte, φ denotes the volume fraction of the solvent in the binary solvent mobile phase, subscripts m, 1 and 2 are the mixed solvent mobile phase, components 1 and 2, respectively, B_j is the model constant which represents various solvent–solvent and analyte–solvent interactions and is calculated by using a no intercept least square analysis. The numerical values of k_1 and/or k_2 are very high/low, and the data could not be accurately measured, therefore, two extreme k values (in the water rich area and organic modifier rich area) were considered as alternative values. As reported in an earlier work [19], it is possible to employ two curve-fit parameters instead of $\log k_1$ and $\log k_2$ (see Eq. (3) of previous work [19]) and consider them as adjustable parameters. Eq. (14) should be considered as a mathematical representation rather than an equation derived from rigorous thermodynamic model [19]. However, the model produced reasonable accurate predictions after training by a minimum number of experimental data points (i.e. retention data in mono solvents and three data points in mixed solvent). The required retention data in mixed solvent mobile phases to train the Jouyban-Acree model is a limitation for the model and any attempt to overcome this limitation could improve its practical applicability. The B_j constants are functions of analyte's chemical nature and the separation system under investigation.

The constants of the Jouyban-Acree model could be correlated with the Abraham solvation parameters (of analytes and solvents) for building a generally trained version of the model for predicting the retention factor of analytes in mixed solvent mobile phases. Our main hypothesis is that the model constants are functions of the nature of the analytes, mobile and stationary phases and other parameters of the analytical systems under investigation.

Eq. (14) provided accurate results for retention factor of an analyte in a specified organic modifier and a given column. For various analytes in given analytical conditions with different solvent compositions, the model constants of Eq. (14) could be correlated with Abraham solute parameters (i.e. $B_j = W_1 + W_2E + W_3S + W_4A + W_5B + W_6V$). The Abraham solute parameters could represent all possible interactions in the column among separation process. This is a similar approach which was used to derive a general LSER model based on quadratic equation, i.e. Eq. (12). The model could be extended as:

$$\begin{aligned}\log k_m = {} & \varphi_1 \log k_1 + \varphi_2 \log k_2 \\ & + \varphi_1\varphi_2\{W_1 + W_2E + W_3S + W_4A + W_5B + W_6V\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)\{W'_1 + W'_2E + W'_3S + W'_4A + W'_5B + W'_6V\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2\{W''_1 + W''_2E + W''_3S + W''_4A + W''_5B + W''_6V\}\end{aligned} \quad (15)$$

and by considering $\log k_1 = \alpha_0 + \alpha_1E + \alpha_2S + \alpha_3A + \alpha_4B + \alpha_5V$ and $\log k_2 = \beta_0 + \beta_1E + \beta_2S + \beta_3A + \beta_4B + \beta_5V$ it is possible to convert Eq. (15) as:

$$\begin{aligned}\log k_m = {} & \varphi_1\{\alpha_0 + \alpha_1E + \alpha_2S + \alpha_3A + \alpha_4B + \alpha_5V\} \\ & + \varphi_2\{\beta_0 + \beta_1E + \beta_2S + \beta_3A + \beta_4B + \beta_5V\} \\ & + \varphi_1\varphi_2\{W_1 + W_2E + W_3S + W_4A + W_5B + W_6V\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)\{W'_1 + W'_2E + W'_3S + W'_4A + W'_5B + W'_6V\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2\{W''_1 + W''_2E + W''_3S + W''_4A + W''_5B + W''_6V\}\end{aligned} \quad (16)$$

for representing the retention factors of various analytes using two experimental retention data and the computational predictions, respectively [20]. When various organic modifiers were considered appropriate descriptors (in this work Abraham solvent parameters are employed) should be added to the model for representing the effects of solvents. The results of statistical analyses revealed that the most accurate form of the models are:

$$\begin{aligned}\log k_m = {} & \varphi_1 \log k_1 + \varphi_2 \log k_2 \\ & + \varphi_1\varphi_2 \left\{ \begin{matrix} L_1 + L_2[(c_1 - c_2)^2] + L_3[E(e_1 - e_2)^2] + L_4[S(s_1 - s_2)^2] \\ + L_5[A(a_1 - a_2)^2] + L_6[B(b_1 - b_2)^2] + L_7[V(v_1 - v_2)^2] \end{matrix} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2) \left\{ \begin{matrix} L'_1 + L'_2[(c_1 - c_2)^2] + L'_3[E(e_1 - e_2)^2] + L'_4[S(s_1 - s_2)^2] \\ + L'_5[A(a_1 - a_2)^2] + L'_6[B(b_1 - b_2)^2] + L'_7[V(v_1 - v_2)^2] \end{matrix} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2 \left\{ \begin{matrix} L''_1 + L''_2[(c_1 - c_2)^2] + L''_3[E(e_1 - e_2)^2] + L''_4[S(s_1 - s_2)^2] \\ + L''_5[A(a_1 - a_2)^2] + L''_6[B(b_1 - b_2)^2] + L''_7[V(v_1 - v_2)^2] \end{matrix} \right\}\end{aligned} \quad (17)$$

and

$$\begin{aligned}\log k_m = {} & \varphi_1\{\alpha_0c_1 + \alpha_1e_1E + \alpha_2s_1S + \alpha_3a_1A + \alpha_4b_1B + \alpha_5v_1V\} \\ & + \varphi_2\{\beta_0c_2 + \beta_1e_2E + \beta_2s_2S + \beta_3a_2A + \beta_4b_2B + \beta_5v_2V\} \\ & + \varphi_1\varphi_2 \left\{ \begin{matrix} L_1 + L_2[(c_1 - c_2)^2] + L_3[E(e_1 - e_2)^2] + L_4[S(s_1 - s_2)^2] \\ + L_5[A(a_1 - a_2)^2] + L_6[B(b_1 - b_2)^2] + L_7[V(v_1 - v_2)^2] \end{matrix} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2) \left\{ \begin{matrix} L'_1 + L'_2[(c_1 - c_2)^2] + L'_3[E(e_1 - e_2)^2] + L'_4[S(s_1 - s_2)^2] \\ + L'_5[A(a_1 - a_2)^2] + L'_6[B(b_1 - b_2)^2] + L'_7[V(v_1 - v_2)^2] \end{matrix} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2 \left\{ \begin{matrix} L''_1 + L''_2[(c_1 - c_2)^2] + L''_3[E(e_1 - e_2)^2] + L''_4[S(s_1 - s_2)^2] \\ + L''_5[A(a_1 - a_2)^2] + L''_6[B(b_1 - b_2)^2] + L''_7[V(v_1 - v_2)^2] \end{matrix} \right\}\end{aligned} \quad (18)$$

in which α, β, W and L terms are the model constants [20]. The $(c_1 - c_2)^2$, $(e_1 - e_2)^2$, $(s_1 - s_2)^2$, $(a_1 - a_2)^2$, $(b_1 - b_2)^2$ and $(v_1 - v_2)^2$ terms could represent the differences between mobile phase components (i.e. organic modifier and water), where multiplying these terms to analyte parameters (i.e. E, S, A, B and V) represents the differences between the interactions of analyte with each component of mobile phase lonely and the mobile phase as a single solvent. Since the number of curve-fitting parameters (the model constants) is relatively high, it is a difficult task to provide more theoretical justifications for the proposed model and from this point of view, it could be considered as an empirical expression. It is obvious

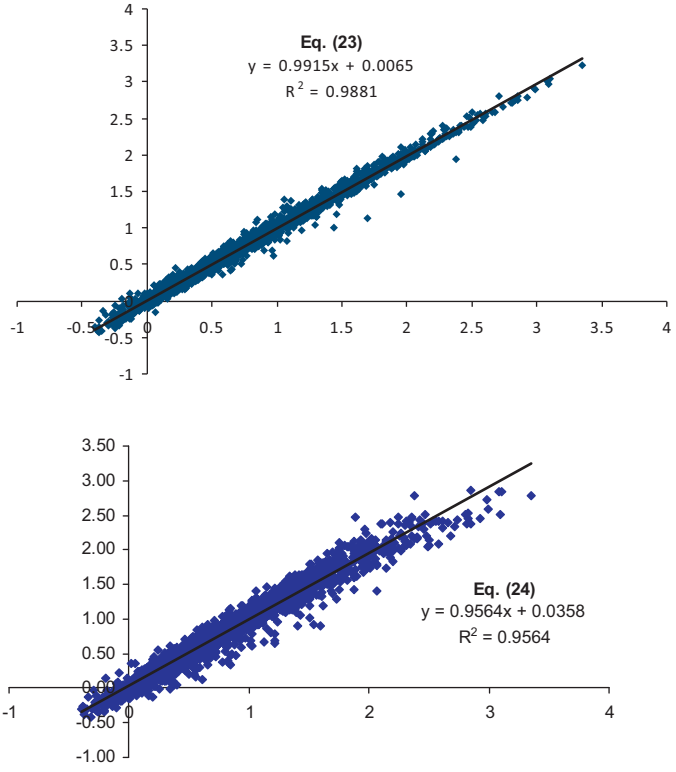

Fig. 1. Predicted vs. experimental logarithmic values of retention data.

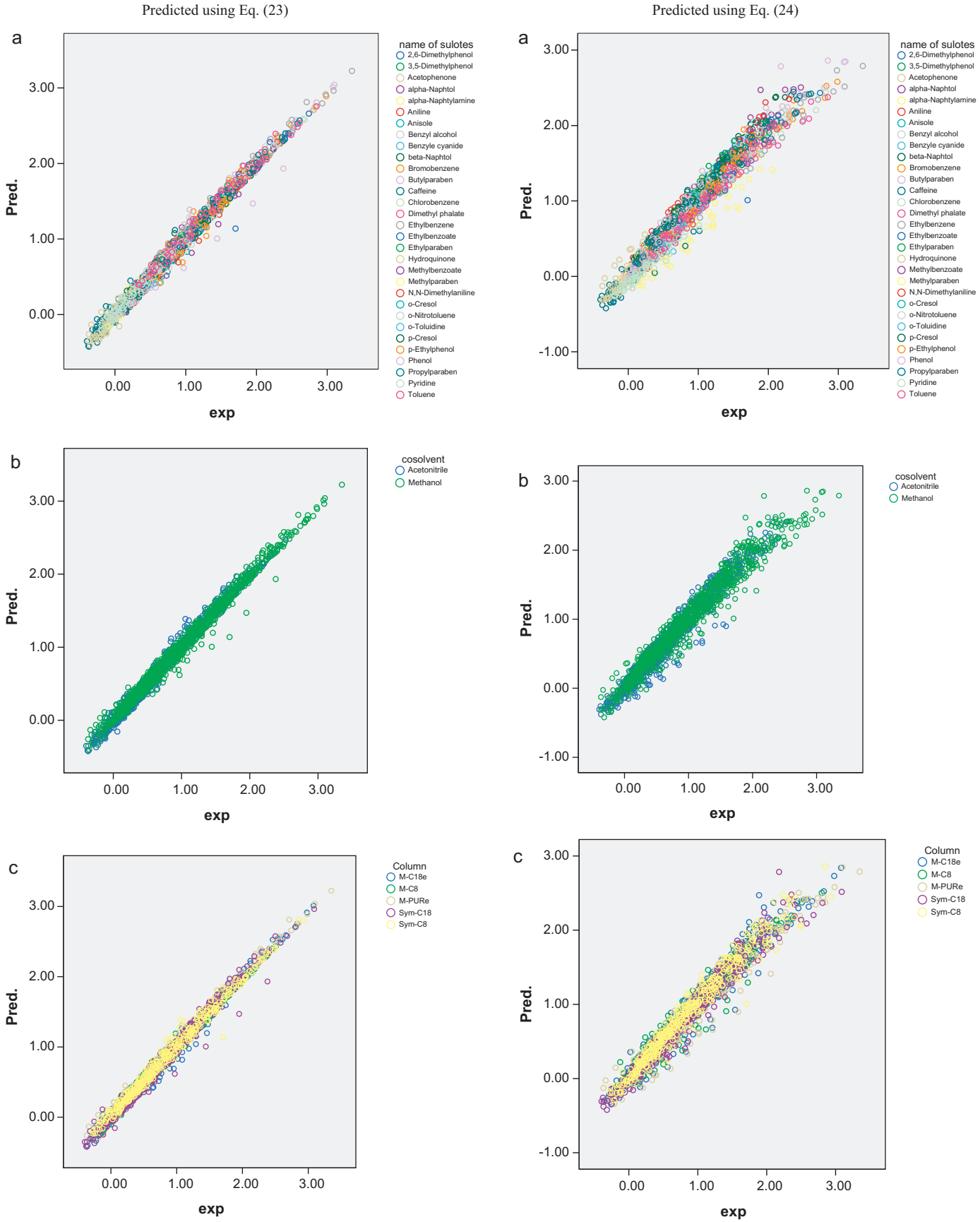

Fig. 2. Predicted vs. experimental data using Eqs. (23) and (24) regarding to analytes (a) mobile phases (b) and stationary phases (c).

Table 4
Variations of independent variables.

Variable	Range of variation	Variable	Range of variation	Variable	Range of variation
$\varphi_1\varphi_2c'(c_1-c_2)^2$	0.01–0.16	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[c'(c_1-c_2)^2]$	0.05 to −0.06	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[c'(c_1-c_2)^2]$	0.00–0.04
$\varphi_1\varphi_2e'E(e_1-e_2)^2$	0.00–0.04	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[e'E(e_1-e_2)^2]$	0.01 to −0.02	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[e'E(e_1-e_2)^2]$	0.00–0.01
$\varphi_1\varphi_2s'S(s_1-s_2)^2$	−0.13 to −2.08	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[s'S(s_1-s_2)^2]$	0.80 to −0.7	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[s'S(s_1-s_2)^2]$	0.00 to −0.48
$\varphi_1\varphi_2a'A(a_1-a_2)^2$	0.00 to −8.94	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[a'A(a_1-a_2)^2]$	3.43 to −3.00	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[a'A(a_1-a_2)^2]$	0.00 to −2.08
$\varphi_1\varphi_2b'B(b_1-b_2)^2$	−1.16 to −59.37	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[b'B(b_1-b_2)^2]$	22.8 to −19.95	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[b'B(b_1-b_2)^2]$	0.00 to −13.68
$\varphi_1\varphi_2\nu'V(\nu_1-\nu_2)^2$	3.05–14.98	$\varphi_1\varphi_2(\varphi_1-\varphi_2)\,[\nu'V(\nu_1-\nu_2)^2]$	5.03 to −5.57	$\varphi_1\varphi_2(\varphi_1-\varphi_2)^2\,[\nu'V(\nu_1-\nu_2)^2]$	0.00–3.45

that any difference in the retention of analytes is due to the interactions between analyte, column and mobile phases, and it is believed that the Abraham solvation parameters are the best descriptors to represent the extent of various possible interactions ranging from London to hydrogen bonding forces. The applicability of Eq. (18) was checked on 292 retention data sets of various analytes using mobile phases of water–methanol and water–acetonitrile mixtures and a certain stationary phase [20]. In these models, we used c, e, s, a, b and ν coefficients of the solvents collected from the literature [16].

The Abraham solvent coefficients of Eqs. (17) and (18) derived from solubility data and represent only solvent effects on the retention. In these equations no variable representing the effects of the stationary phases on the retention were employed. Considering the results of statistical analyses, the relevant independent variables could be considered in the model as:

$$\begin{aligned}\log k_m &= \varphi_1 \log k_1 + \varphi_2 \log k_2 \\ &+ \varphi_1\varphi_2 \left\{ \begin{matrix} Z_1 + Z_2[c'(c_1-c_2)^2] + Z_3[e'E(e_1-e_2)^2] + Z_4[s'S(s_1-s_2)^2] \\ +Z_5[a'A(a_1-a_2)^2] + Z_6[b'B(b_1-b_2)^2] + Z_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\} \\ &+ \varphi_1\varphi_2(\varphi_1-\varphi_2) \left\{ \begin{matrix} Z'_1 + Z'_2[c'(c_1-c_2)^2] + Z'_3[e'E(e_1-e_2)^2] + Z'_4[s'S(s_1-s_2)^2] \\ +Z'_5[a'A(a_1-a_2)^2] + Z'_6[b'B(b_1-b_2)^2] + Z'_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\} \\ &+ \varphi_1\varphi_2(\varphi_1-\varphi_2)^2 \left\{ \begin{matrix} Z''_1 + Z''_2[c'(c_1-c_2)^2] + Z''_3[e'E(e_1-e_2)^2] + Z''_4[s'S(s_1-s_2)^2] \\ +Z''_5[a'A(a_1-a_2)^2] + Z''_6[b'B(b_1-b_2)^2] + Z''_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\}\end{aligned} \tag{19}$$

where Z terms are the model constants.

As discussed in Eqs. (4), (5), (15) and (17), the Abraham solvation parameters of the analytes, solvents and columns, e.g. e, E, e', could represent the extent of various interactions in the separation system between analyte, mobile and stationary phases. It is obvious that there is no rigorous theoretical justification behind this combination and we prefer to consider Eq. (19) as an empirical correlation. However, Eq. (19) is able to represent the experimental data more accurate than other models reported in the literature. To the best of our knowledge, there is no such a single model to calculate the effects of these variables all together and the available models consider one, two or three independent variables in their model building process.

By employing computational method (without employing new experimental k_1 and k_2 values, and experimental analyte parameters), the general equation could be written as:

$$\begin{aligned}\log k_m &= \varphi_1\{\alpha_0c' + \alpha_1e'E + \alpha_2s'S + \alpha_3a'A + \alpha_4b'B + \alpha_5\nu'V\} \\ &+ \varphi_2\{\beta_0c' + \beta_1e'E + \beta_2s'S + \beta_3a'A + \beta_4b'B + \beta_5\nu'V\} \\ &+ \varphi_1\varphi_2 \left\{ \begin{matrix} Z_1 + Z_2[c'(c_1-c_2)^2] + Z_3[e'E(e_1-e_2)^2] + Z_4[s'S(s_1-s_2)^2] \\ +Z_5[a'A(a_1-a_2)^2] + Z_6[b'B(b_1-b_2)^2] + Z_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\} \\ &+ \varphi_1\varphi_2(\varphi_1-\varphi_2) \left\{ \begin{matrix} Z'_1 + Z'_2[c'(c_1-c_2)^2] + Z'_3[e'E(e_1-e_2)^2] + Z'_4[s'S(s_1-s_2)^2] \\ +Z'_5[a'A(a_1-a_2)^2] + Z'_6[b'B(b_1-b_2)^2] + Z'_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\} \\ &+ \varphi_1\varphi_2(\varphi_1-\varphi_2)^2 \left\{ \begin{matrix} Z''_1 + Z''_2[c'(c_1-c_2)^2] + Z''_3[e'E(e_1-e_2)^2] + Z''_4[s'S(s_1-s_2)^2] \\ +Z''_5[a'A(a_1-a_2)^2] + Z''_6[b'B(b_1-b_2)^2] + Z''_7[\nu'V(\nu_1-\nu_2)^2] \end{matrix} \right\}\end{aligned} \tag{20}$$

The numerical values of these terms could be computed by regressing $\log k_m$ against φ_1c', $\varphi_1e'E$, $\varphi_1s'S$, $\varphi_1a'A$, $\varphi_1b'B$, $\varphi_1\nu'V$, φ_2c', $\varphi_2e'E$, $\varphi_2s'S$, $\varphi_2a'A$, $\varphi_2b'B$, $\varphi_2\nu'V$, $\varphi_1\varphi_2$, $\varphi_1\varphi_2c'(c_1-c_2)$, $\varphi_1\varphi_2e'E(e_1-e_2)^2$, $\varphi_1\varphi_2s'S(s_1-s_2)^2$, $\varphi_1\varphi_2a'A(a_1-a_2)^2$, $\varphi_1\varphi_2b'B(b_1-b_2)^2$, $\varphi_1\varphi_2\nu'V(\nu_1-\nu_2)^2$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[c'(c_1-c_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[e'E(e_1-e_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[s'S(s_1-s_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[a'A(a_1-a_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[b'B(b_1-b_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)[\nu'V(\nu_1-\nu_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[c'(c_1-c_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[e'E(e_1-e_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[s'S(s_1-s_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[a'A(a_1-a_2)^2]$, $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[b'B(b_1-b_2)^2]$ and $\varphi_1\varphi_2(\varphi_1-\varphi_2)^2[\nu'V(\nu_1-\nu_2)^2]$ using a no intercept least square analysis. It should be noted that α, β and W terms in the above mentioned models possess various numerical values and the predictions should be made using the trained version of these models.

The predictive ability of the models was assessed in terms of the mean percentage deviation (MPD) of observed ($k_{obs.}$) and calculated ($k_{cal.}$) retention factors, defined by:

$$\mathrm{MPD} = \frac{100}{\mathrm{NDP}} \sum \frac{\left|k_{cal.} - k_{obs.}\right|}{k_{obs.}} \tag{21}$$

where NDP is the number of data points. In addition, we also calculated the individual percentage deviation (IPD):

$$\mathrm{IPD} = 100 \left\{ \frac{\left|k_{cal.} - k_{obs.}\right|}{k_{obs.}} \right\} \tag{22}$$

for each data point.

3. Results and discussion

3.1. General model

The available experimental k_m values collected from the literature were fitted to the proposed model and the model constants with probability of < 0.05 were included in the model. Non-significant contribution of some solvation parameters is very common in practical applications of the LSER models [5]. The obtained model after excluding the model constants with the p values of >0.05 is:

$$\begin{aligned}\log k_m = {} & \varphi_1 \log k_1 + \varphi_2 \log k_2 \\ & + \varphi_1\varphi_2 \left\{ \begin{array}{l} 0.085 - 0.537[c'(c_1 - c_2)^2] - 5.530[e'E(e_1 - e_2)^2] - 0.017[s'S(s_1 - s_2)^2] \\ -0.017\left[a'A(a_1 - a_2)^2\right] - 0.007\left[b'B(b_1 - b_2)^2\right] - 0.034\left[v'V(v_1 - v_2)^2\right] \end{array} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2) \left\{ \begin{array}{l} -0.547 + 2.113[e'E(e_1 - e_2)^2] - 0.023[a'A(a_1 - a_2)^2] \\ -0.025[b'B(b_1 - b_2)^2] - 0.113[v'V(v_1 - v_2)^2] \end{array} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2 \left\{ \begin{array}{l} 1.771[c'(c_1 - c_2)^2] + 22.330[e'E(e_1 - e_2)^2] + 0.045[a'A(a_1 - a_2)^2] \\ +0.013[b'B(b_1 - b_2)^2] + 0.046[v'V(v_1 - v_2)^2] \end{array} \right\} \end{aligned} \quad (23)$$

This correlation was significant at $p < 0.0005$ and the F value of 1647. The details of the computed MPD values were listed in Table 1 of supplementary materials (see column 5). The overall MPD (±SD) was 12.4 (±7.4)% and the number of data sets (NDS) were 310. When these MPD values were analyzed considering the organic modifier, the values were 11.6 (±6.4) and 13.2 (±8.2), respectively for acetonitrile (NDS = 155) and methanol (NDS = 155). The predicted values using this equation were fitted to the experimental data (Fig. 1 (for whole data set) and Fig. 2 (for subgroups of data sets (i.e. analytes, mobile phases and columns)) and the slope and regression coefficients were 0.996 and 0.988, respectively for all data.

Using a set of 32 analytes of different chemical properties, 19 different columns and a mobile phase of acetonitrile–water (30:70 v/v) it has been shown [22] that e' and v' coefficients favors the column interactions, where s', a' and b' act favorably in the mobile phase. In this study, we found that the analyte positive or negative effects on solvation coefficients of columns vanished. It seems that the column effect on retention depends on the differences between the column and mobile phase properties rather than their properties lonely. It is obvious from the developed model that the positive or negative effect of each single property depends on the organic modifier fraction in hydro-organic mobile phases. Table 4 shows the numerical ranges of independent variables investigated in this work in which all solvation coefficients and solvation parameters possess both positive and negative effects on retention due to the organic modifier fractions employed, i.e. $\varphi_1\varphi_2$, $\varphi_1\varphi_2(\varphi_1 - \varphi_2)$, or $\varphi_1\varphi_2(\varphi_1 - \varphi_2)^2$.

Table 5
Mean percentage deviation (MPD) of fitted and predicted retention factors using one analyte out trained models and the number of data points (N).

Analyte	Cross validated data, Eq. (19)	Cross validated data, Eq. (20)	Fitted data, Eq. (23)	Fitted data, Eq. (24)	N
2,6-Dimethylphenol	7.7	25.0	7.6	23.2	60
3,5-Dimethylphenol	9.2	29.6	9.0	27.5	60
Acetophenone	8.6	19.1	8.5	18.1	60
alpha-Naphthol	16.3	61.3	15.9	42.8	60
alpha-Naphthylamine	23.7	74.1	14.9	46.5	48
Aniline	12.1	30.3	11.9	28.6	12
Anisole	11.0	17.7	10.7	17.1	60
Benzyl alcohol	8.1	21.4	7.9	19.5	60
Benzyl cyanide	8.7	23.4	8.7	21.7	60
beta-Naphthol	13.7	65.8	13.5	45.4	60
Bromobenzene	17.6	25.6	16.3	22.5	24
Butylparaben	22.4	41.0	21.9	36.1	36
Caffeine	29.5	25.3	20.1	18.3	60
Chlorobenzene	15.6	23.2	14.7	21.2	60
Dimethyl phethalate	13.4	18.2	12.6	16.0	60
Ethylbenzene	22.5	30.2	20.9	26.5	60
Ethylbenzoate	12.2	17.2	12.2	16.3	60
Ethylparaben	10.2	25.7	10.0	22.7	60
Hydroquinone	14.9	34.3	14.1	26.4	60
Methylbenzoate	10.6	12.2	10.4	12.0	60
Methylparaben	10.1	19.4	9.7	17.0	60
N,N-dimethylaniline	10.2	23.4	9.9	22.0	60
o-Cresol	7.6	23.6	7.5	22.3	60
o-Nitrotoluene	11.3	19.1	10.9	17.8	60
o-Toluidine	9.6	16.7	9.5	16.0	60
p-Cresol	7.7	24.3	7.7	22.6	60
p-Ethylphenol	13.9	13.5	13.8	13.2	60
Phenol	10.5	22.7	10.2	20.5	60
Propylparaben	14.3	30.0	13.9	26.3	60
Pyridine	13.7	32.6	12.8	25.9	60
Toluene	17.8	26.6	16.9	23.3	60
Overall	13.4	25.6	12.4	23.7	

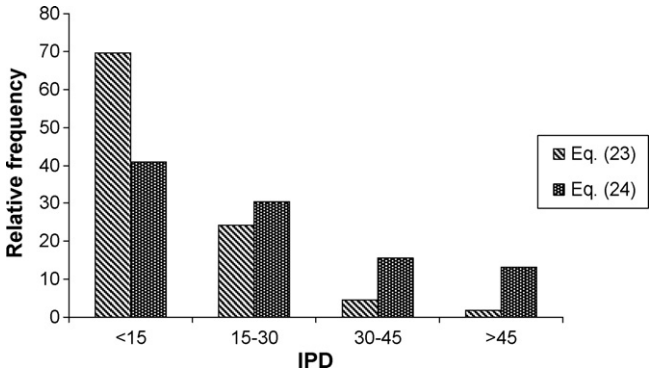

Fig. 3. Relative frequency of IPD values produced using Eqs. (23) and (24).

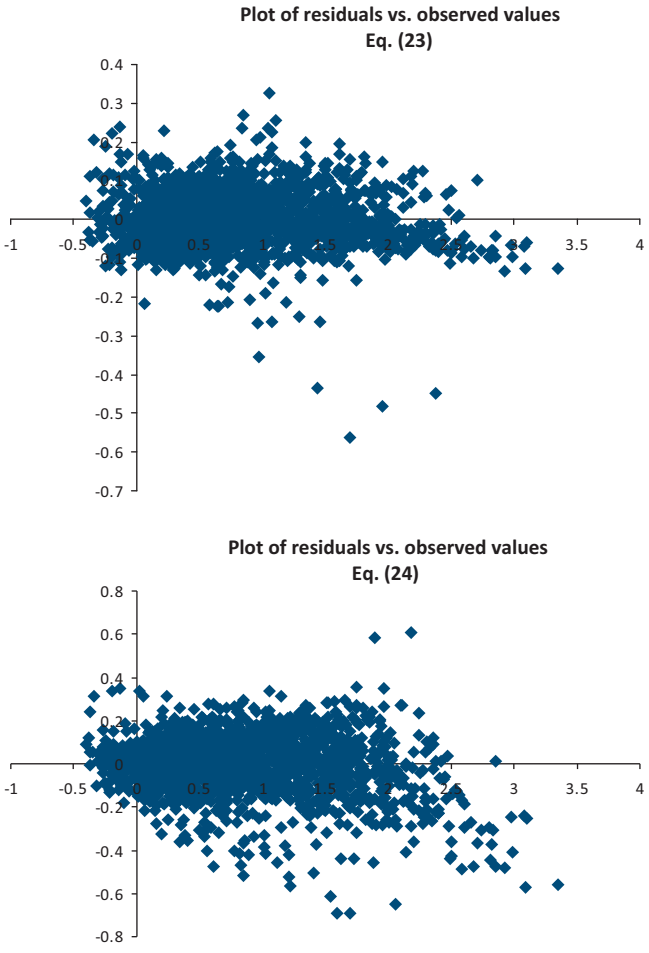

Fig. 4. The residuals vs. observed values plots for developed models.

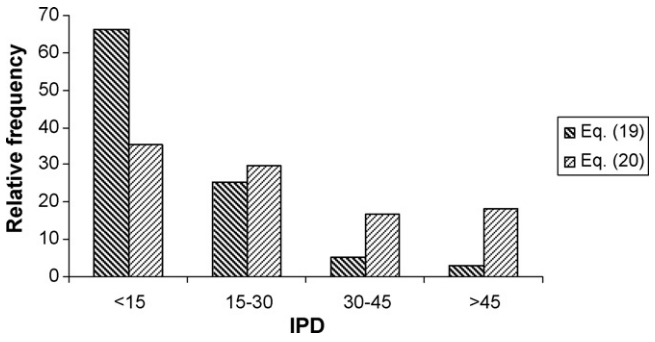

Fig. 5. Relative frequencies of IPD values for leave one analyte out cross-validation analysis.

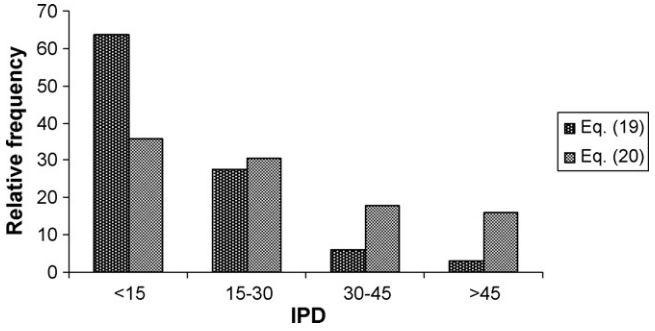

Fig. 6. Relative frequencies of IPD values for leave one column out cross-validation analysis.

This correlation was significant at $p < 0.0005$ and the F value of 4111 and the number of data points (NDP) fitted to the model was 1860. The back-calculated k_m values were used to compute the MPD and standard deviation values for the data sets studied in this work. The details of the values were listed in Table 1 of supplementary materials (see column 6). The overall MPD (±SD) was 23.7 (±14.4)% and the number of data sets (NDS) was 310. When these MPD values were analyzed considering the organic modifier, the values were 21.6 (±13.2) and 25.8 (±15.2), respectively for acetonitrile and methanol. A number of data sets produced very large MPD values using Eq. (24), where predicted accurately by Eq. (23) (e.g. data set numbers of 1, 12, 19, 38, . . .) which can be due to the calculated analyte parameters using software. Beyond this some datasets produced large MPD values both for Eqs. (23) and (24), which can be regarded as outlier characteristics of them.

Considering the proposed computational method, the accuracy of the predictions could be considered acceptable.

The optimum range of retention factors from practical point of view are $1 < k_m < 10$. When, these constrains were applied to Eqs. (23) and (24), the produced MPDs were reduced to 11.0 and 19.3%, respectively.

The relative frequencies of IPD values using Eqs. (23) and (24) showed in Fig. 3. About 70% of data predicted by the IPD values of less than 15% which is acceptable for validated HPLC methods.

When Eq. (20) was trained using the available data, the obtained model is:

$$\begin{aligned}\log k_m = {} & \varphi_1\{2.376c' + 0.782e'E - 0.555s'S + 0.358a'A + 1.202b'B - 2.520\nu'V\} \\ & + \varphi_2\{3.733c' + 2.446e'E + 3.495s'S + 0.929a'A + 1.775b'B + 1.031\nu'V\} \\ & + \varphi_1\varphi_2 \left\{ \begin{array}{l} -0.988 - 4.551[c'(c_1 - c_2)^2] - 11.480[e'E(e_1 - e_2)^2] - 0.143[s'S(s_1 - s_2)^2] \\ -0.035[a'A(a_1 - a_2)^2] - 0.040[b'B(b_1 - b_2)^2] + 0.330[\nu'V(\nu_1 - \nu_2)^2] \end{array} \right\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)\{0.738[s'S(s_1 - s_2)^2] - 0.029[b'B(b_1 - b_2)^2] + 0.197[\nu'V(\nu_1 - \nu_2)^2]\} \\ & + \varphi_1\varphi_2(\varphi_1 - \varphi_2)^2\{-2.863 - 3.593[c'(c_1 - c_2)^2] - 0.041[b'B(b_1 - b_2)^2] + 0.526[\nu'V(\nu_1 - \nu_2)^2]\}\end{aligned} \quad (24)$$

Table 6
Comparison of the proposed models with a number of basic and combined models.

Equation numbers	φ	Organic modifier	Analyte	Column	MPD	SD	R^2
(3)	Yes	No	No	No	571.54	431.11	0.7304
(7)	No	No	Yes	No	19.38	8.26	0.9679
(13)	Yes	No	Yes	No	32.44	19.86	0.9249
(23)	Yes	Yes	Yes	Yes	12.46	4.97	0.9885
(24)	Yes	Yes	Yes	Yes	23.71	10.77	0.9564

The plot of the residuals vs. the observed data (Fig. 4) showed the normal distribution of residuals and no overestimations or under estimations for a special set of analytes or organic modifier or columns have been determined.

3.2. Cross-validation of the general model

Eqs. (19) and (20) were trained using all data points except data of one analyte which was excluded from the training set (leave one out cross-validation). The trained models were used to predict the retention factor of the excluded analyte and the computed MPDs are listed in Table 5 along with the MPDs of Eqs. (23) and (24). The differences between the MPDs of the fitted and predicted data using Eq. (20) was small revealing that the model was robust and could be used for predicting retention factors of un-measured analytes.

In the next cross-validation procedure, the retention data of various analytes collected using a specific column were excluded from the training process of the models and then the retention factors of the excluded set were predicted. The obtained MPDs for the predicted data using Eqs. (19) and (20) are listed in Table 2.

Figs. 5 and 6 show the relative frequencies of described cross-validation procedures respectively for leave one analyte out and one column out anlaysis. The error trends are similar to the fitted IPD values and about 70% of data predicted by the errors less than 15%.

4. Comparison with basic models

The developed models' advantage and disadvantage in comparison with previously developed basic models (i.e. LSER, general LSER model, and Oscik's equation) are investigated using published data sets. The results are summarized in Table 6 and Fig. 7. The summary of calculation methods for each model is provided in introduction and the details could be found in the literature. Its clear from results that the newly developed general method is able to predict the retention data more accurately (even better than local models for each specific data set) while its high number of fitting parameters is a disadvantage.

About the applicable organic modifier range of the developed method, it should be noted that the predicted $\log k_2$ values are dependent to the organic modifier (such as all other models) and although applicable range were improved (10–90%), but it is not applicable for whole range (0–100%) of organic modifier.

The only model which provided an organic modifier independent column parameter is the proposed models (Table 6) and there is not a similar model which is able to model all retention affecting parameters (i.e. column, organic modifier, mobile phase composition and analyte) simultaneously. This model is also capable of modeling the 5th parameter (i.e. temperature) as shown in a previous work [21].

In comparison with other solvation parameter based models, this method is so simpler with regard to the nature of analyte solvation parameters and organic modifier solvation coefficients have been determined using solubility experiments, where for other models the model parameters should be computed using chromatographic data which are more difficult to obtain than solubility experiments. The only parameters which should be obtained using chromatographic data are column solvation parameters which have been determined for most of the common columns and could be found from the literature [6,14,23,24].

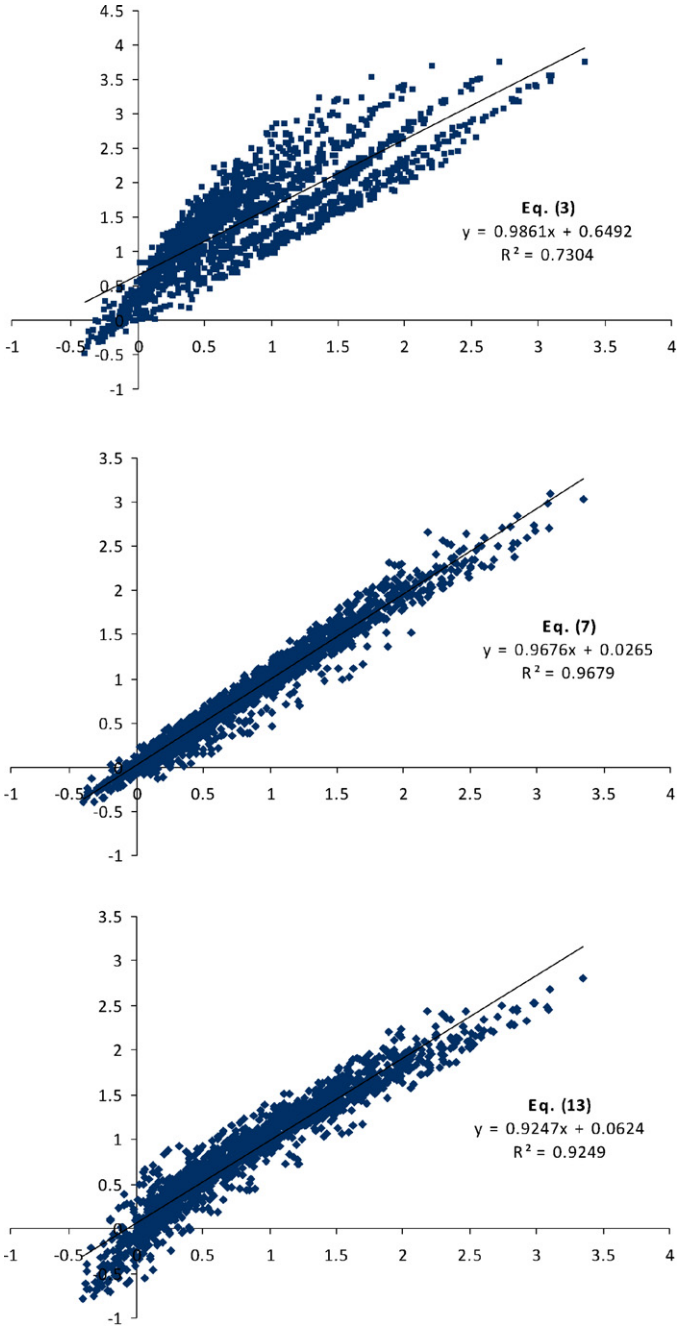

Fig. 7. Predicted vs. observed values for the studied models.

5. Conclusion

A generally trained model was proposed for predicting the retention factor of analytes in RP-HPLC by combining the Jouyban-Acree and Abraham models. The proposed model has the advantage of modeling four variables, i.e. the analyte structure, the column type, the nature and concentration of the organic modifier in the mobile phase using a single model. Eq. (19) employs two experimental k_m values, i.e. k_1 and k_2 values, for each analyte and provides better calculations. Whereas, Eq. (20) is a computational model and predicts the k_m values without any experimental input data. Considering the combined effect of column, mobile phase (type and concentration of organic modifier) and analytes' properties on the retention time, the developed general model can represent the variability of retention data well, while its prediction capability and applicability (for other columns and other organic modifiers) can be improved using more diverse set according to the organic modifier and column types in training procedure.

6. Precautionary note

The proposed general models were trained using a limited number of analytes, stationary phases, organic modifiers and to provide better predictions, more experimental data should be employed. In this work, we have shown the capability of the models to represent the effect of four discussed variables on the retention of analytes in HPLC.

Acknowledgment

The partial financial support under Grant No. 87-411 from the Drug Applied Research Center, Tabriz University of Medical Sciences was gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.07.034.

References

[1] J.R. Torres-Lapasio, M.J. Ruiz-Angel, M.C. Garcja-Alvarez-Coque, J. Chromatogr. A 1166 (2007) 85.
[2] P. Nikitas, A. Pappa-Louisi, J. Chromatogr. A 1216 (2009) 1737.
[3] D.E. Martire, M. Jaroniec, J. Liq. Chromatogr. 8 (1985) 1363.
[4] M. Janicka, J. Liq. Chromatogr. Relat. Technol. 32 (2009) 2779.
[5] M. Vitha, P.W. Carr, J. Chromatogr. A 1126 (2006) 143.
[6] A. Sandi, L. Szepesy, J. Chromatogr. A 818 (1998) 1.
[7] Á. Sándi, L. Bede, L. Szepesy, G. Rippet, Chromatographia 45 (1997) 206.
[8] Á. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 19.
[9] A. Sándi, L. Szepesy, J. Chromatogr. A 845 (1999) 113.
[10] Á. Sándi, M. Nagy, L. Szepesy, J. Chromatogr. A 893 (2000) 215.
[11] L. Szepesy, J. Chromatogr. A 960 (2002) 69.
[12] A. Wang, L.C. Tan, P.W. Carr, J. Chromatogr. A 848 (1999) 21.
[13] A. Wang, P.W. Carr, J. Chromatogr. A 965 (2002) 3.
[14] M.H. Abraham, M. Roses, C.F. Poole, S.K. Poole, J. Phys. Org. Chem. 10 (1997) 358.
[15] A.A. D'Archivio, M.A. Maggi, P.M. Mazzeo, F. Ruggieri, Anal. Chim. Acta 646 (2009) 47.
[16] D.M. Stovall, W.E. Acree Jr., M.H. Abraham, Fluid Phase Equilib. 232 (2005) 113.
[17] J.R. Torres-Lapasio, M.C. Garcja-Alvarez-Coque, M. Ross, E. Bosch, A.M. Zissimos, M.H. Abraham, Anal. Chim. Acta 515 (2004) 209.
[18] ADME Boxes, version 4.0; Pharma Algorithms: Toronto, 2008.
[19] A. Jouyban, M.R. Rashidi, Z. Vaez-Gharamaleki, A.A. Matin, D.J. Djozan, Pharmazie 60 (2005) 827.
[20] A. Jouyban, S. Soltani, A. Fathi-Azarbayjani, W.E. Acree Jr., Anal. Methods 2 (2010) 1286.
[21] A. Jouyban, Sh. Soltanpour, W.E. Acree Jr., D. Thomas, P. Agrafiotou, A. Pappa-Louisi, J. Sep. Sci. 32 (2009) 3898.
[22] L. Szepesy, J. Sep. Sci. 26 (2003) 201.
[23] W. Kiridena, C.F. Poole, A.A. Koziol, J. Chromatogr. A 1060 (2004) 177.
[24] L. Szepesy, V. Hada, Chromatographia 54 (2001) 99.