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# Chromatographic retention of nine solutes by the using of linear solvation energy relationships in RP-HPLC

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**Abstract**−Linear solvation energy relationships (LSERs) are used to investigate the fundamental chemical interactions governing the retention of nine aromatic compounds on a $C_{18}$ column. The mobile phases studied involve ionic liquids 1-Methyl-3-octylimidazolium tetrafluoroborate ([OMIm][$BF_4$]) (0.003-0.009 M), with 5 to 20% acetonitrile in water as mobile phase modifiers. The ability of the LSERs to account for the chemical interactions underlying solute retention is shown. A comparison of predicted and experimental retention factors suggests that LSER formalism is able to reproduce adequately the experimental retention factors of the solutes studied in the different experimental conditions investigated.

Key words: Linear Solvation Energy Relationships, Chromatographic Retention, Ionic Liquid, [OMIm][$BF_4$], Modifier

## INTRODUCTION

Retention prediction and selectivity optimization are very important in rapid method development in reversed-phase liquid chromatography (RPLC) [1]. However, retention in RPLC is a very complicated process [2,3] that depends on many physical and chemical properties of the system, such as temperature [4,5], solute molecular properties [6], stationary phase characteristics [7], and mobile phase composition [8,9]. These years many practical retention models [10] for RP-HPLC, such as linear solvation energy relationships (LSER), have been developed and widely used.

To increase the chromatography effect, a spot of additives were added into the mobile phase. There are many additives that can be used to investigate the retention in the RP-HPLC, such as ionic liquid [11-13].

Ionic liquids are widely recognized as one of the key components of "green" chemistry. The solvent properties make them possible candidates in chromatography. And most applications of ionic liquids in RP-HPLC have been mainly as mobile phase additives [14]. The chemical nature of the ionic liquids makes it possible to conclude that when they are used as the mobile phase additives in HPLC, they exist in the mobile phase solution and they are also coated on $C_{18}$ column. Unfortunately, the influence of ionic liquids modifiers on chromatographic retention is still currently unclear.

In this study, nine solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol and pyridine) have been in terms of LSER. Several mobile phases using 1-Methyl-3-octylimidazolium tetrafluoroborate ([OMIm][$BF_4$]) as additives in acetonitrile/water mobile phases were characterized by using the previously mentioned solvation parameter LSER model.

## EXPERIMENTAL

### 1. Instruments

All experiments were performed on a Younglin M930 (Korea) equipped with a spectrophotometer (M 7200 Absorbance Detector, Young-In Scientific Co., Korea), and a Rheodyne injector (Hamilton Company, USA) valve with a 20 μL sample loop. The software Chromate (Ver. 3.0 Interface Eng., Korea) was used for system control and data handling. The detector was operated at 254 nm for LSER test solutes. Experiments were performed with a commercially available $C_{18}$ column (Optimapak, Korea, 4.6×150 mm, 5 μm). An injection volume of 2 μL was applied throughout the experiments. All procedures were performed at 30 °C.

### 2. Materials

All of the LSER test solutes and the ionic liquid [OMIm][$BF_4$] were purchased from C-TRI (Korea). The mobile phase modifier acetonitrile was purchased from Duksan (Korea). Deionized water was obtained via a water purification system from Millipore Corp. (Milford, MA).

### 3. Preparation of Mobile Phases and Standard Solutions

Ionic liquid was added into the acetonitrile/water solution directly. The molar concentrations were adjusted to 0.003 M, 0.006 M, and 0.009 M, respectively. The mixed mobile phase contained 5, 10, 15 and 20% (v/v) acetonitrile modifiers for the mixed solution. After thorough mixing in a sonicator for 30 minutes, the final running eluents were filtered through a syringe filter (HA-0.45, Division of Millipore, Waters, USA) and then sonicated for more 20 minutes prior to the experiments. All stock solute solutions were prepared at concentrations of 1 mg/mL each. All of the nine solute samples were dissolved in methanol. To avoid potential errors arising from decomposition, the working solutions were re-prepared every three days.

## THEORETICAL BACKGROUND AND CALCULATION

The general LSER equation used in this work is [15]:

$$\log k = \log k_0 + m(V_x/100) + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + rR_2 \quad (1)$$

where k is the experimental retention factor. The $V_x$, $\pi_2^H$, $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and $R_2$ terms are solute descriptors, where $V_x$ represents the solute's

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size/polarizability, $\pi_2^H$ is the dipolarity/polarizability, $\Sigma\alpha_2^H$ is the hydrogen bond (HB) donating ability, $\Sigma\beta_2^H$ is the HB accepting ability, and $R_2$ is the excess molar refraction. The subscript "2" simply signifies that these parameters are solute descriptors.

The coefficients of these descriptors m, s, a, b, and r reflect differences in the two bulk phases between which the solute is transferring [18] and are obtained through a multiparameter linear regression. The $\log k_0$ term is simply the intercept of the regression and is comprised of constant contributions from the solutes and the chromatographic system.

Since the parameters $V_x$ and $\pi_2^H$ are blends of two different interactions, the coefficients of these parameters are also blends of the corresponding properties. Specifically, m is the difference in the cohesivity/dispersive ability of the two bulk phases, and s is the difference in the ability of the two phases to interact through dipole-dipole and dipole-induced dipole interactions. Many reviews and examples of LSERs and their interpretations are available [4,9,15,16].

These coefficients provide quantitative information about solute-solute, solute-mobile phase, and solute-stationary phase interactions. The constant c represents the intercept and provides information about the separation phase ratio [17]. The m term is a measure of the relative proneness of cavity formation and general dispersion interactions for the solute with the stationary phase and the bulk aqueous phase, respectively. The difference in dipolarity/polarizability between the stationary phase and the bulk aqueous phase is represented by the coefficient s. The b and a terms represent the hydrogen bond donating ability and hydrogen bond accepting ability of the phase, respectively.

**1. Retention Factor Estimation**

The retention factor, k, of each solute was measured according to the following formula:

$$k=(t_R-t_M)/t_M \quad (2)$$

where $t_R$ and $t_M$ are the retention times of the retained analyte and the retention times of the unretained analyte (also known as dead time), respectively. Sodium nitrite was used as a $t_M$ marker and was measured from the time of injection to the first deviation from the baseline following a 5 μL injection of 1% sodium nitrite solution. The retention factors reported in this study are the averages of at least three determinations. The results of the chromatographic experiments were evaluated by using statistical techniques. The relative error of a single measurement did not exceed 5%.

**2. Linear Solvation Energy Relationship Estimations**

Retention factors were determined for the nine compounds used in this study, and the system constants were calculated by multiple linear regressions using Origin Pro 6.0 software (Microcal Software Inc., MA, USA) [19]. The statistical validity of the LSER models was evaluated through an F test, squared correlation coefficient ($r^2$), and root mean square error in the estimate (SD). The differences in LSER coefficients indicate the variations in the types of interactions between stationary phases and solutes. In the column, ionic liquid not only effected with solutes and additive, but also coated onto the silica in the column. Due to these different mechanisms, the LSER constants for different kinds of solutes are not identical.

## RESULTS AND DISCUSSION

The retention behaviors of the nine test solutes (acetophenone, aniline, caffeine, methylparaben, o-cresol, m-cresol, p-cresol, phenol and pyridine) in each mobile phase were examined and compared by using the solvation parameter LSER model, i.e., model described in Eq. (1). The test solutes and their descriptors used in this study are given in Table 1.

**Table 1. Test solutes and their descriptors for the solvation parameter model**

| Solute | Descriptors | | | | |
|---|---|---|---|---|---|
| | $V_X$ ($cm^3/mol^{-1}$) | $\pi^H$ | $\alpha_2^H$ | $\beta_2^0$ | $R_2$ ($cm^3/10$) |
| Caffeine | 1.5 | 1.6 | 0 | 1.35 | 1.363 |
| Phenol | 0.805 | 0.89 | 0.6 | 0.3 | 0.7751 |
| *p*-Cresol | 0.82 | 0.87 | 0.57 | 0.31 | 0.916 |
| Methylparaben | 0.9 | 1.37 | 0.69 | 0.45 | 1.131 |
| Acetopenone | 0.818 | 1.01 | 0 | 0.48 | 1.0139 |
| Aniline | 0.955 | 0.96 | 0.26 | 0.5 | 0.8162 |
| *o*-Cresol | 0.84 | 0.86 | 0.52 | 0.3 | 0.916 |
| Pyridine | 0.631 | 0.84 | 0 | 0.52 | 0.6753 |
| *m*-Cresol | 0.822 | 0.88 | 0.57 | 0.34 | 0.916 |

Some recommendations for selecting an appropriate set of solutes have been gathered from a survey of the literature: 1) mathematically, a minimum number of seven solutes is needed to solve a multiple linear regression equation for six unknowns (five system constants and the intercept); 2) there should be an absence of significant cross-correlation among the descriptors, and the clustering of individual descriptor values should be avoided; 3) since the used detection method in this work is UV absorption, the solutes should have a reasonable absorbance, between 200 and 250 nm, for convenient detection; and 4) solutes should be quite stable in the used solutions.

The coefficients for the LSER equations obtained for each mobile phase used in this work are grouped in Table 2. It shows the results obtained for [OMIm][$BF_4$] mobile phases, for which correlation coefficients ranged from 0.989 to 0.999 with standard errors ranging from 0.016 to 0.044.

In all the mobile phase investigated, the coefficient of $\Sigma\beta_2^H$ (b) and most of values for the coefficient of $\pi_2^H$ (s) were negative, that is, an increase in the HB basicity and solute dipolarity/polarizability decreases the overall retention of the molecule. Furthermore, most of the coefficients of $V_x$, $R_2$ and $\Sigma\alpha_2^H$ (m, r and a, respectively) were positive in all the mobile phases studied, indicating that increases in the solute volume, excess molar and HB acidity increases in the solute volume and excess molar. In terms of the magnitude of the coefficients, excess molar refractivity and solute dipolarity/polarizability generally play the largest role in determining the retention of solutes in all mobile phases' studied. HB basicity is also an important factor in the [OMIm][$BF_4$] mobile phases with coefficients comparable in magnitude to those of solute volume.

As shown in Fig. 1, the values of all the coefficients (m, s, a, b and r) changed very complicatedly as the concentration of [OMIm][$BF_4$] changed, but all series of the dates revealed a law that the relation curves between the concentrations of [OMIm][$BF_4$] and the coefficients mainfested as a hyperbola; they have a maximum or minimum. And during the three concentrations of the ionic liquid in the study (0.003, 0.006 and 0.009 M), the ones on 0.006 M were

**Table 2. Constants for the chromatographic mobile phases using solvation parameter model**

| IL concentration, M | | 0.003 | 0.006 | 0.009 |
|---|---|---|---|---|
| Type and concentration of modifier | | | 5% ACN | |
| Constants | $logk_0$ | 0.82(0.11) | 0.68(0.07) | 0.71(0.08) |
| | m | 0.35(0.25) | 0.21(0.17) | 0.32(0.19) |
| | s | −2.61(0.20) | −1.99(0.14) | −2.13(0.15) |
| | a | 0.28(0.12) | 0.17(0.08) | 0.24(0.10) |
| | b | −0.67(0.25) | −1.15(0.17) | −1.11(0.19) |
| | r | 3.42(0.22) | 3.33(0.15) | 3.27(0.17) |
| Statistics | $r^2$ | 0.989 | 0.995 | 0.995 |
| | SD | 0.044 | 0.030 | 0.034 |
| | F | 149.532 | 350.846 | 291.653 |
| Type and concentration of modifier | | | 10% ACN | |
| Constants | $logk_0$ | 0.40(0.06) | 0.40(0.08) | 0.41(0.09) |
| | m | 0.08(0.13) | 0.02(0.19) | 0.07(0.20) |
| | s | −1.07(0.11) | −1.14(0.15) | −1.07(0.16) |
| | a | 0.14(0.07) | 0.15(0.09) | 0.17(0.10) |
| | b | −1.47(0.14) | −1.51(0.19) | −1.56(0.21) |
| | r | 2.76(0.12) | 2.88(0.17) | 2.75(0.18) |
| Statistics | $r^2$ | 0.997 | 0.994 | 0.994 |
| | SD | 0.024 | 0.033 | 0.036 |
| | F | 542.408 | 297.047 | 266.995 |
| Type and concentration of modifier | | | 15% ACN | |
| Constants | $logk_0$ | 0.06(0.04) | 0.51(0.08) | 0.40(0.08) |
| | m | −0.88(0.09) | 0.40(0.20) | 0.18(0.19) |
| | s | −2.05(0.07) | −1.55(0.16) | −1.09(0.15) |
| | a | 0.37(0.04) | −0.00(0.10) | 0.08(0.10) |
| | b | −0.82(0.09) | −1.46(0.20) | −1.54(0.19) |
| | r | 4.35(0.08) | 2.64(0.17) | 2.51(0.17) |
| Statistics | $r^2$ | 0.999 | 0.993 | 0.994 |
| | SD | 0.016 | 0.035 | 0.034 |
| | F | 1581.476 | 244.351 | 263.741 |
| Type and concentration of modifier | | | 20% ACN | |
| Constants | $logk_0$ | 0.37(0.08) | 0.48(0.07) | 0.37(0.07) |
| | m | 0.26(0.19) | 0.41(0.16) | 0.27(0.17) |
| | s | −1.13(0.15) | −1.39(0.12) | −1.03(0.14) |
| | a | 0.02(0.09) | −0.01(0.08) | 0.01(0.08) |
| | b | −1.42(0.19) | −1.34(0.16) | −1.40(0.17) |
| | r | 2.28(0.17) | 2.26(0.14) | 2.13(0.15) |
| Statistics | $r^2$ | 0.993 | 0.995 | 0.994 |
| | SD | 0.033 | 0.028 | 0.030 |
| | F | 223.971 | 322.601 | 261.289 |

the maxinum or the minimum. It means that when the concentration of ionic liquid was smaller than 0.006 M, the mechanism was simple trace liquid just acting between the stationary phase and solution based on the polarity, but if the concentration was 0.009 M the mechanism was more complex; parts of the ionic liquid connected to the silica then effected the retention mechanism.

When the values of the LSER coefficients were based on the concentration of acetonitrile, it was also complex. Among the four concentrations of acetonitrile in water (5%, 10%, 15% and 20%), the largest changing range of the values was on 15%, even containing the biggest and smallest value. It means that when at this concentration the co-effect between acetonitrile and ionic liquid was most severe, and below 15%, the trend of the relation lines between concentration of acetonitrile and coefficients was: for m, a, b and r, 5%>10%, while for s, 5%<10%. With the increase of the acetonitrile the solute size increased but the polarizability decreased; also

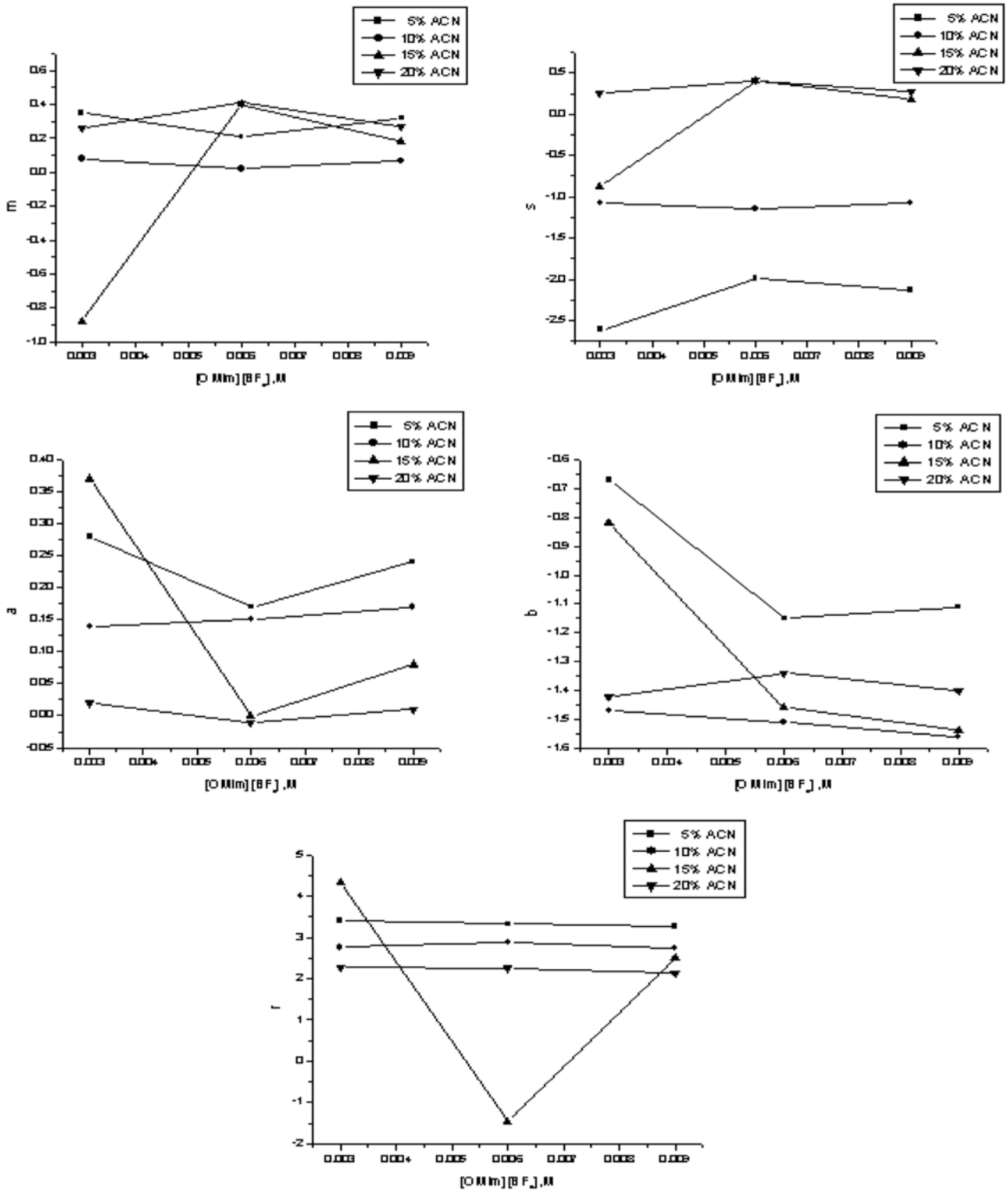


**Fig. 1. LSER coefficients as a function of [OMIm][$BF_4$] concentrations. Modifiers are: ■ 5% ACN, ● 10% ACN ▲ 15% ACN and ▼ 20% ACN Error bars have been omitted for clarity.**

the HB accepting or donating ability increased, but the dipolarity/polarizability decreased. But in acetonitrile/water (20%, v/v) solution, the situation cannot be compared to the 5% or 10% ones; the concentration of 15% should be used. Ignoring the concentration on 15%, the values of s increase, but the values of a and r decrease as the concentration of ACN in mobile phase increases in the main. In other words, as the concentration of ACN is increasing, the dipolarity/polarizability increases, but the hydrogen bond accepting ability and the excess molar refraction decrease. The polarity of water is larger than ACN, so when increasing the rate of ACN/water, the

**Table 3. The calculated (cal) and experimental (exp) log k with 5% v/v of ACN, using Eq. (2)**

| logk | Solute | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Caffeine | Phenol | p-Cresol | Methyl paraben | Aceto phenone | Aniline | o-Cresol | Pyridine | m-Cresol |
| | 0.003 M [OMIm][$BF_4$] | | | | | | | | |
| Exp | 0.92 | 1.44 | 1.92 | 1.30 | 1.64 | 1.15 | 1.90 | 0.79 | 1.88 |
| Cal | 0.93 | 1.40 | 1.92 | 1.32 | 1.62 | 1.18 | 1.95 | 0.81 | 1.88 |
| $\varepsilon$* | 1 | 4 | 0 | 2 | 2 | 3 | 5 | 2 | 0 |
| | 0.006 M [OMIm][$BF_4$] | | | | | | | | |
| Exp | 0.78 | 1.44 | 1.89 | 1.49 | 1.67 | 1.14 | 1.90 | 0.77 | 1.87 |
| Cal | 0.80 | 1.42 | 1.91 | 1.51 | 1.67 | 1.16 | 1.94 | 0.79 | 1.86 |
| $\varepsilon$ | 2 | 2 | 2 | 2 | 0 | 2 | 4 | 2 | 1 |
| | 0.009 M [OMIm][$BF_4$] | | | | | | | | |
| Exp | 0.72 | 1.44 | 1.90 | 1.41 | 1.61 | 1.12 | 1.88 | 0.73 | 1.84 |
| Cal | 0.74 | 1.42 | 1.91 | 1.44 | 1.60 | 1.15 | 1.93 | 1.75 | 1.85 |
| $\varepsilon$ | 2 | 2 | 1 | 3 | 1 | 3 | 5 | 2 | 1 |

* the relative error, %

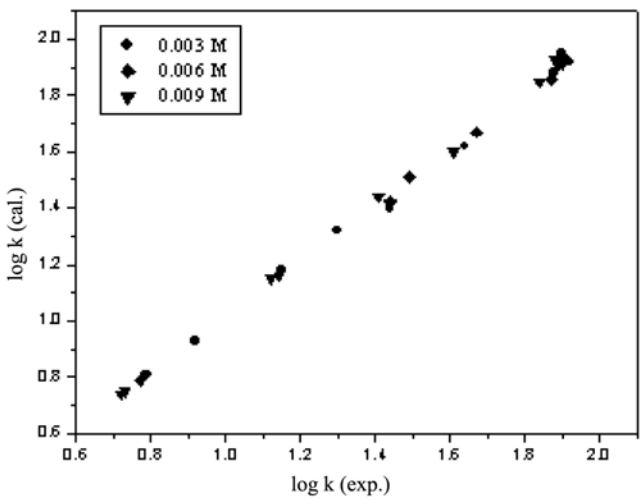


**Fig. 2. The correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from ACN 5% (v/v) with different concentrations of [OMIm][$BF_4$]).**

mobile phase becomes less polar. And there is no hydrogen bond in can, but in water when water is decreasing, the hydrogen bond accepting ability will decrease. Also, the excess molar fraction decreases as the polar water in the mobile phase.

Calculated log k values of the test solutes were computed for each mobile phase by using Eq. (2). The calculated (cal) and experimental (exp) log k and relative error ($\varepsilon$, %) for some mobile phases are given in Table 3.

The solvation parameter model is found to provide statistical and chemical results. This is evident when comparing the statistics (i.e., $r^2$, SD, and F values) of the solvation parameter model results in Table 2 with the results of prediction in Table 3. The correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from acetonitrile 5% (v/v) with different concentrations of [OMIm][$BF_4$] is demonstrated in Fig. 2. Also, good correlations were obtained for the experimental log k values versus predicted log k values for other mobile phases; that is, LSERs are able to approximately reproduce the experimental log k values for the solutes studied in the different mobile phases.

## CONCLUSION

Ionic liquid [OMIm][$BF_4$] and acetonitrile were applied as additives in the mobile phases. The LSER model, i.e., the solvation parameter model, was successfully applied to investigate the effect of the additive concentrations on retention of nine aromatic compounds in RP-HPLC. The results obtained from the solvation parameter model provide comparable information, for example, coefficient s and coefficient r play the most important role in retention behavior in all mobile phases. It is worth noting that, using the obtained LSER models, it is possible to predict retention factors with high correlation coefficients ($r^2$>0.99). It is evident from the results of the LSER model that the excess molar refraction and HB basicity have a dominant role on the solute ionic liquid interaction. This model is a helpful tool to understand the solute-ionic liquid interactions and evaluate the retention characteristic of liquid chromatography.

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