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# Enthalpy–entropy compensation of halogenated benzylamines in reversed–phase liquid chromatography

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Received for review February 10, 2006. Revised manuscript received March 24, 2006. Accepted April 6, 2006.

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## Abstract

Retention behavior of halogenated benzylamines on an ODS stationary phase was studied using phosphate buffer (20 mM, pH 2.0) / acetonitrile (95/5, v/v, %) as a mobile phase. The thermodynamic properties for the transfer of halogenated benzylamines from the mobile phase to the stationary phase were evaluated for understanding on the retention mechanism in the reversed–phase high–performance liquid chromatography. The data were analyzed to sufficiently confirm the possibility of enthalpy–entropy compensation (EEC). The EEC indicates that enthalpic gain for retention is canceled out to one half by the entropic loss, and that entropy–driven retention could take place without enthalpic contribution.

*Keywords:* desolvation, enthalpy–entropy compensation, halogenated benzylamine, retention behavior, van't Hoff plot.

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## 1. Introduction

Chromatography is a useful and reliable separation method that is widely used for both analytical and preparative purposes. Among actual implementations, reversed–phase liquid chromatography (RPLC) is the most suitable tool for fundamental studies on the retention behavior. Such information can be obtained by measuring retention data over a wide range of temperature. Retention data obtained at different temperatures allow an evaluation of the variations in enthalpy change and entropy change, which govern the transfer of the solute from the mobile phase to the stationary phase. Many investigations on the thermodynamics have been performed in the reversed–phase mode [1–7]. The most important technique for the investigation of retention mechanisms from the view point of thermodynamics is the enthalpy–entropy compensation (EEC). An EEC is experimentally demonstrated in several publications [8–13], and the possibility of EEC is supported on the theoretical bases in some reports [14, 15]. However, the linear correlation between enthalpy change and entropy change might be ob-

served with the random errors in the measurement of thermodynamic parameters. Krug *et al.* outlined the methods to avoid the observation of the false correlations [16–18]. Unfortunately, these methods were scarcely used in chromatographic studies of EEC.

In order to understand a mechanistic interpretation on the separation by RPLC, we have developed an extra–thermodynamic approach based on EEC by analyzing the chromatographic retention data obtained in the present study. The aim of this study is to provide the information concerning the relation between enthalpy change and entropy change involved in the retention using halogenated benzylamines as the model compounds on an octadecyl–silica stationary phase in the reversed–phase mode. Retention behavior of halogenated benzylamines was measured under different sets of experimental conditions to calculate enthalpy change and entropy change for the mass transfer from the mobile to the stationary phase. Correlation between enthalpy change and entropy change was verified by the methods proposed by Krug *et al.* in order to determine whether an EEC does exist for retention in RPLC

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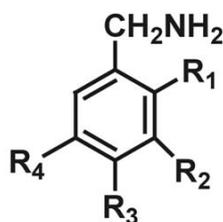
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or not. Then, the retention mechanism was discussed.

## 2. Experimental

### 2.1. Chemicals and reagents

Acetonitrile of HPLC grade, methanol, phosphoric acid, potassium dihydrogen phosphate, and potassium nitrate were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Water was deionized prior to usage. Potassium dihydrogen phosphate was dissolved in deionized water, and phosphoric acid was added to make 20 mM phosphate buffer at pH 2.0. The halogenated benzylamines (Figure 1) used in this study were 3-bromobenzylamine, 4-bromobenzylamine, 3-bromo-4-fluorobenzylamine, 5-bromo-2-fluorobenzylamine, 4-chloro-2-fluorobenzylamine from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Derived benzylamines were dissolved in methanol for HPLC analysis.



| Halogenated benzylamine      | R <sub>1</sub> | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub> |
|------------------------------|----------------|----------------|----------------|----------------|
| 3-Bromobenzylamine           | H              | Br             | H              | H              |
| 4-Bromobenzylamine           | H              | H              | Br             | H              |
| 3-Bromo-4-fluorobenzylamine  | H              | Br             | F              | H              |
| 5-Bromo-2-fluorobenzylamine  | F              | H              | H              | Br             |
| 4-Chloro-2-fluorobenzylamine | F              | H              | Cl             | H              |

Figure 1. Chemical structures of halogenated benzylamines.

### 2.2. Apparatus

HPLC measurements were performed on a Hitachi (Tokyo, Japan) D-7000 System equipped with two isocratic pumps (L-7100), variable wavelength detector (L-7420), variable volume injector (L-7200) and column oven (L-7300). Unison UK-C18 (4.6 mm in inside diameter and 15 cm in length, octadecylsilanized silica (ODS) gel for liquid chromatography, 3 μm in particle diameter, Imtakt Corp., Kyoto, Japan) was employed for the analytical separation. The mobile phase consisted of 20 mM phosphate buffer at pH 2.0 and acetonitrile (95/5, v/v, %) and was delivered at a flow-rate of 1.0 mL min<sup>-1</sup>. The column temperature was adjusted at 15.0–40.0 °C, and the precision of the column temperature adjustment was ± 0.1 °C. The injection volume was set at 10 μL. Duplicate injections were performed at each column temperature. The elution of halogenated benzylamines was monitored at 221 nm. The chromatographic system was equilibrated by passing the mobile phase until a stable baseline signal was obtained. Potassium ni-

trate was used as a void volume marker [19].

### 2.3. Thermodynamic properties of liquid chromatography

This study is based on enthalpy–entropy compensation (EEC). EEC is conveniently expressed by the following equation:

$$\Delta H = T_c \Delta S + \Delta G_{T_c} \quad (1)$$

where  $T_c$  is the compensation temperature,  $\Delta G_{T_c}$  denotes the Gibbs free energy change of a physico–chemical interaction at  $T_c$ , and  $\Delta H$  and  $\Delta S$  mean the corresponding enthalpy change and entropy change, respectively. These thermodynamic parameters ( $\Delta H$  and  $\Delta S$ ) are estimated by analyzing the temperature dependence of retention factor according to the van't Hoff equation. If the mechanism of the process is invariant over the temperature range studied and the enthalpy is constant, the van't Hoff plot yields a straight line. The retention factor,  $k$ , was expressed in terms of the following van't Hoff equation:

$$\ln k = -\Delta H/RT + \Delta S/R + \ln \phi \quad (2)$$

where  $R$  is the gas constant,  $T$  the absolute temperature of the column, and  $\phi$  the phase ratio of the column (the volume of the stationary phase divided by the volume of the mobile phase). The phase ratio of Unison UK-C18 column employed in this study is calculated to be 0.21.

There are experimental errors from the determination of  $\Delta H$  and  $\Delta S$  based on the van't Hoff plots, resulting that an apparent correlation between  $\Delta H$  and  $\Delta S$  may be observed when there is no real EEC effect. Krug *et al.* proposed the methods to clarify whether EEC results from actual physicochemical effects or arises from the statistical compensation due to experimental errors. In this study, we examined the possibility of EEC according to the four methods recommended by Krug *et al.* These four methods are described below.

#### 1) Correlation between enthalpy change and Gibbs free energy change at the harmonic mean of the experimental temperature.

If EEC is actually present, the plot generated from  $\Delta H$  and Gibbs free energy change at the harmonic mean of the experimental temperature ( $\Delta G_{T_{hm}}$ ) will be linear.

#### 2) Comparison of compensation temperature with the harmonic mean of the experimental temperature.

Krug *et al.* proposed the following equations to test the null hypothesis that  $T_c$  was equal to  $T_{hm}$ . Maximum and minimum values of  $T_c$  at 95% confidence level are calculated by the following equations.

$$T_c (\text{minimum}) = \frac{\sum (\Delta H - \langle \Delta H \rangle) (\Delta S - \langle \Delta S \rangle) / \sum (\Delta S - \langle \Delta S \rangle)^2}{-t (3,0.05) V(T_c)^{1/2}}$$

$$T_c (\text{maximum}) = \frac{\sum (\Delta H - \langle \Delta H \rangle) (\Delta S - \langle \Delta S \rangle) / \sum (\Delta S - \langle \Delta S \rangle)^2}{+t (3,0.05) V(T_c)^{1/2}}$$

where  $\langle \Delta H \rangle$  and  $\langle \Delta S \rangle$  are the average values of  $\Delta H$  and  $\Delta S$ , respectively. The  $t$ -value,  $t(3, 0.05)$ , is the value of the Student's  $t$  for 3 of freedom degree and a confidence level of 95%. Sample statistic,  $V(T_c)$ , is calculated by the following equation:

$$V(T_c) = \frac{\sum (\Delta H - \Delta G_{T_c} - T_c \Delta S)^2}{3 \sum (\Delta S - \langle \Delta S \rangle)^2}$$

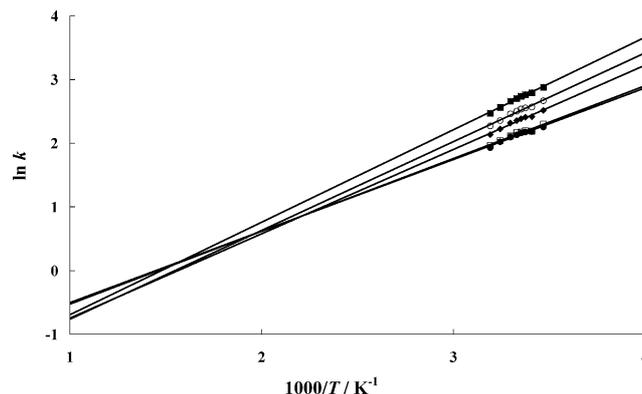
**3) Convergence of the van't Hoff plots at compensation temperature.** According to equation 1, when enthalpy-entropy compensation is observed for a group of compounds in chromatographic retention, all of the compounds have the same Gibbs free energy change at compensation temperature. When retention is influenced by EEC, the van't Hoff plot must intersect at a single point corresponding to the inverse of  $T_c$ .

**4) Analysis of variance (ANOVA).** Probability for the intersection of the linear regression lines was compared with that for the non-intersection on the basis of the statistical data derived by ANOVA. The probability for non-intersection was also compared with the precision of the experimental data in the same manner [7].

### 3. Results and discussion

Halogenated benzylamines were separated on an ODS column in the reversed-phase mode. Complete separation could not be achieved in this study, especially between 4-chloro-2-fluorobenzylamine and 5-bromo-2-fluorobenzylamine. The representative chromatogram obtained at a column temperature of 15.0 is shown in Figure 2.

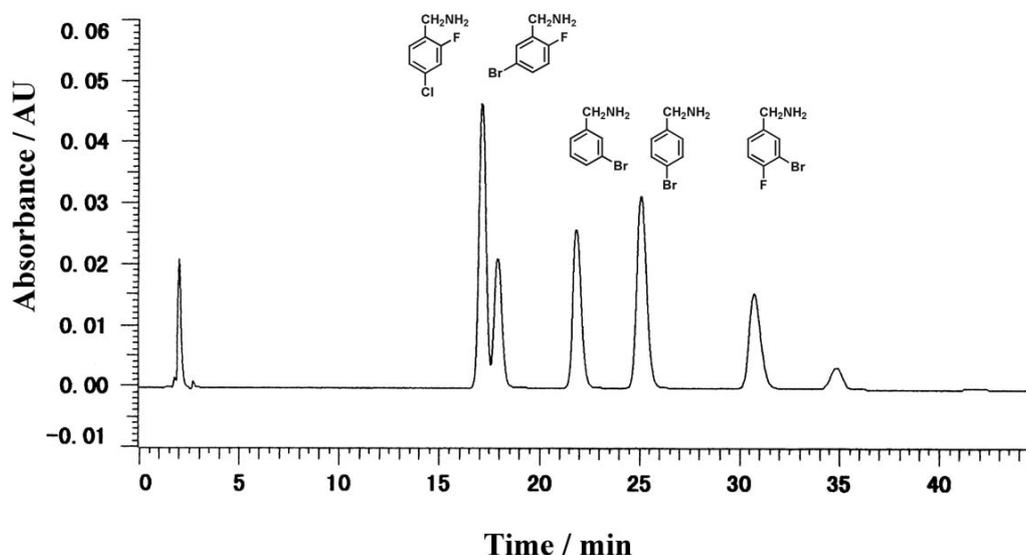
Figure 3 illustrates the van't Hoff plots with correlation coefficients of more than 0.975 for halogenated benzylamines. The thermodynamic parameters calculated according to equation 2 are listed in Table 1. It was found that values of Gibbs free energy



**Figure 3.** Plots of natural logarithms of retention factors of halogenated benzylamines as a function of the inverse of absolute temperature.  $\square$ , 3-Bromo-4-fluorobenzylamine;  $\circ$ , 4-bromobenzylamine;  $\triangle$ , 3-bromobenzylamine;  $\diamond$ , 5-bromo-2-fluorobenzylamine;  $\times$ , 4-chloro-2-fluorobenzylamine.

**Table 1.** Gibbs free energy change at 298.15 K, enthalpy change, entropy change times temperature (298.15 K) concerning mass transfer from the mobile to the stationary phase (unit:  $\text{kJ mol}^{-1}$ )

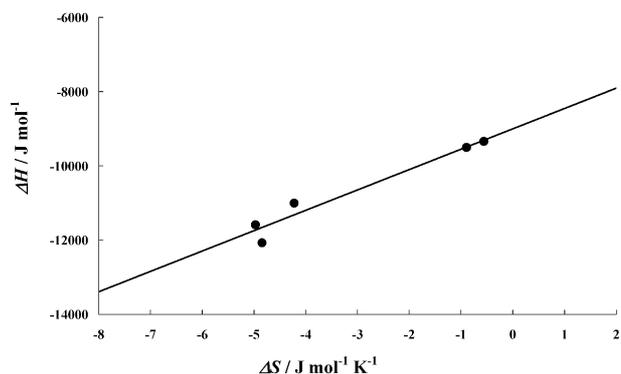
| Halogenated benzylamine      | Gibbs free energy change | Enthalpy change | Entropy change times temperature |
|------------------------------|--------------------------|-----------------|----------------------------------|
| 3-Bromobenzylamine           | -9.7                     | -11.0           | -1.3                             |
| 4-Bromobenzylamine           | -10.1                    | -11.6           | -1.5                             |
| 3-Bromo-4-fluorobenzylamine  | -10.7                    | -12.1           | -1.4                             |
| 5-Bromo-2-fluorobenzylamine  | -9.2                     | -9.5            | -0.3                             |
| 4-Chloro-2-fluorobenzylamine | -9.1                     | -9.3            | -0.2                             |



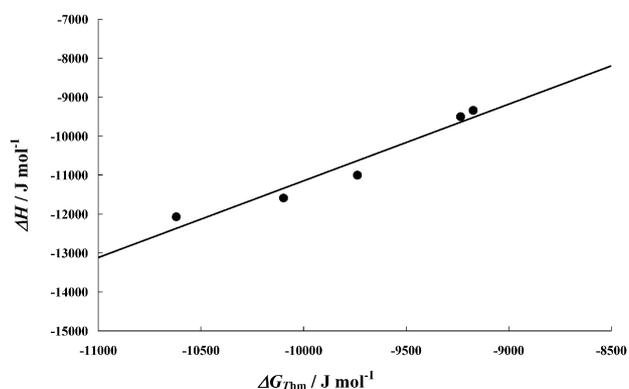
**Figure 2.** Chromatogram of halogenated benzylamines obtained in the reversed-phase mode. Column temperature, 15.0, other chromatographic conditions see in text.

change were all negative. This means all the solutes are favored to stay in the stationary phase rather than in the mobile phase. The solute transfer from the mobile to the stationary phase are enthalpically favorable (negative value) and entropically unfavorable (negative value), and the enthalpic contribution is predominate compared to the entropic contribution on C<sub>18</sub> column. It is obvious that the differences in Gibbs free energy change are smaller than those in enthalpy change owing to a canceling effect of entropy change. The linear enthalpy–entropy relationship observed experimentally is depicted in Figure 4, indicating that the value of  $T_c$  according to equation 1 is 560 K.

To evaluate whether EEC took place in data plotted in Figure 4, the procedures recommended by Krug *et al.* [16–18] were applied for the verification of the possibility of EEC. First, correlation between  $\Delta H$  and  $\Delta G_{T_{hm}}$  was examined. The graph with a correlation coefficient of 0.971 in Figure 5 might offer the proof of possibility of EEC. The value of  $T_c$  was calculated from the slope of the straight line in Figure 5 as 600 K, according to  $T_c = T_{hm} / \{1 - 1/(\text{slope})\}$ . The second method was the comparison of  $T_{hm}$  and  $T_c$  calculated by the statistical procedure. The calculated value of  $T_c$  (560 K) is approximately in agreement with those obtained from



**Figure 4.** Plots of enthalpy–entropy compensation for halogenated benzylamines.



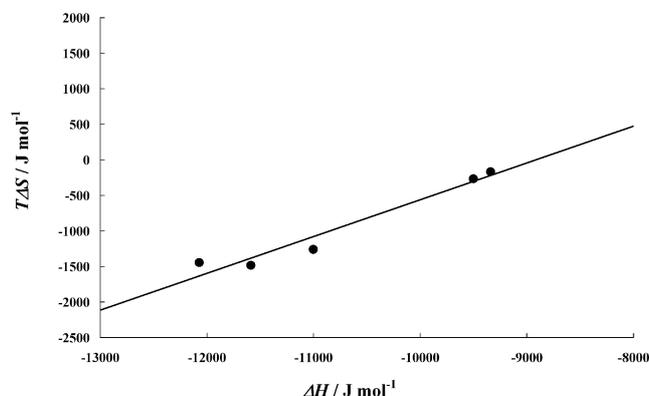
**Figure 5.** Linear correlations between enthalpy change and Gibbs free energy change at the harmonic mean of the experimental temperature for halogenated benzylamines.

Figures 4 and 5. The value of  $T_c$  was in the range of the 330–790 K at the 95% confidence level, and the value of  $T_{hm}$  (300 K) was outside this  $T_c$  range. The hypothesis of a coincidence of  $T_c$  with  $T_{hm}$  was rejected by the 95% confidence level by the  $t$ -test. The third method consisted in checking that the van't Hoff plots do intersect at  $T_c$ . As shown in Figure 3, the van't Hoff plots intersected at some points near  $T_c$  due to the influence of experimental errors. In this study, retention behavior was measured in a relatively narrow temperature range (15.0–40.0 °C) compared to the wide distance from the intersection points ( $T_c$ ) to the experimental temperatures. This may explain the distribution of the intersection points. Finally, the probability of intersection was verified by ANOVA (Table 2). The ratio, the mean sum of squares of the intersection ( $MS_{con}$ ) to that of the non–intersection ( $MS_{noncon}$ ), was much larger than the  $F$ -value,  $F(1, 3, 0.975) = 216$ , indicating that the probability of intersection was much larger than that of non–intersection. Similarly, the ratio of  $MS_{noncon}$  to the mean sum of squares of the residuals ( $MS_e$ ) was much smaller than the corresponding  $F$ -value,  $F(3, 24, 0.975) = 14.1$ . The probability of non–intersection was smaller than the precision of the experimental data. Intersection of van't Hoff plots was verified by the statistical procedure. Consequently, possibility of EEC was confirmed by the results obtained from the methods proposed by Krug *et al.*

Using these thermodynamic properties for the retention of

**Table 2.** ANOVA table for the retention of halogenated benzylamines

| Source of variation            | Mean sum of squares                       | Ratio   | $F$ -value |
|--------------------------------|---|---|------------|
| Intersection (concurrence)     | $1.5 \times 10^4$<br>( $MS_{con}$ )       | $1.1 \times 10^5$<br>( $MS_{con} / MS_{noncon}$ ) | 216        |
| Intersection (non–concurrence) | $1.4 \times 10^{-1}$<br>( $MS_{noncon}$ ) | $2.6 \times 10^{-3}$<br>( $MS_{noncon} / MS_e$ )  | 14.1       |
| Residuals                      | $53 \times 10$<br>( $MS_e$ )              |   |            |



**Figure 6.** Compensation plot of the entropy change against the enthalpy change for the transfer from the mobile to stationary phase.

halogenated benzylamines in RPLC, entropy change was plotted against enthalpy change to generate the straight line with a correlation coefficient of 0.975, the slope of 0.52 and the intercept of 4500 J mol<sup>-1</sup> at 25 °C, as shown in Figure 6. As the magnitude of the enthalpy change decreases, an offsetting increase in the entropy change occurs. For this straight line the resulting change in entropic term is proportional to the accompanying change in enthalpy change as described in the following equation:

$$T\Delta S = \alpha\Delta H \quad (3)$$

where  $\alpha$  means the slope of this straight line. Integration of equation 3 gives equation 4, where  $T\Delta S_0$  refers to the value at  $\Delta H = 0$  in Figure 6.

$$T\Delta S = \alpha\Delta H + T\Delta S_0 \quad (4)$$

Inserting equation 3 in differential form of Gibbs free energy change function, equation 5 is obtained.

$$\Delta\Delta G = (1 - \alpha)\Delta\Delta H \quad (5)$$

Thus, the slope ( $\alpha$ ) of the  $T\Delta S$  vs.  $\Delta H$  plot (equation 4) indicates how the enthalpic gain ( $\Delta\Delta H$ ) is canceled by the accompanying entropic loss ( $\Delta\Delta S$ ). In other words, only  $(1 - \alpha)$  proportion of the increment in enthalpy change ( $-\Delta H$ ) may contribute toward raising the enhancement of retention ( $-\Delta G$ ). As can be seen in Figure 6, linear plot with a slope of 0.52 demonstrates that the enthalpic gain is reduced to half by the entropic loss on the retention of halogenated benzylamines in this chromatographic system.

The intercept ( $T\Delta S_0$ ) is ascribed to the extent of desolvation upon interaction [20–26]. In the mixture of acidic phosphate buffer and acetonitrile (95/5, v/v, %), halogenated benzylamines are surrounded by the polar molecules due to the hydrogen-bonding network between protonated amino group and water molecules [19]. In the process of the transfer from the mobile to the stationary phase, the bulky aggregates are broken down, and halogenated benzylamines are immersed into the stationary phase. Consequently, when a solute is introduced into a stationary phase from the mobile phase, desolvation from the halogenated benzylamines will occur.

Inserting equation 4 in equation 2, equation 6 is obtained.

$$\ln k_T = -(1 - \alpha)\Delta H/RT + \Delta S_0/R + \ln \phi \quad (6)$$

where  $k_T$  denotes the retention factor at a column temperature  $T$ . The term,  $\Delta S_0/R + \ln \phi$ , provides the minimum time at which the halogenated benzylamines are eluted in the RPLC system studied. Equation 6 indicates that the retention time of halogenated benzylamines at a column temperature,  $T$ , is determined by the reduced enthalpic contribution,  $(1 - \alpha)\Delta H$ , and the entropic contribution,  $\Delta S_0$ , induced by desolvation and phase ratio. This means that the hypothetical halogenated benzylamine with no enthalpic

contribution will be retained for 3.6 min (retention factor: 1.30) by the effect of desolvation. Actual halogenated benzylamines will be retained on an ODS column more strongly than the hypothetical one by the enthalpic contribution reduced by the entropic loss.

#### Acknowledgement

The authors are grateful to Mr. I. Yazawa, Imtakt Corp, for useful discussion and insights into the column (Unison UK-C18) employed in this study.

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