Sources of Nonlinear van’t Hoff Temperature Dependence in High-Performance Liquid Chromatography

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ABSTRACT: In HPLC, the nonlinear behavior of the retention factor $k'$ with temperature (dependence of $ln k'$ on $1/T$) can be attributed to the multiple interactions of a unique analyte in the separation process and/or to the existence in solution of multiple forms of the analyte (also leading to different free enthalpies of interaction). In this study, several examples of nonlinear retention—temperature dependence are evaluated for both reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) separations. The potential explanation for nonlinear retention—temperature behavior is evaluated for each example, some caused by multiple interactions in the separation system of a unique analyte and others by multiple forms of the analyte. In cases where the analyte does not have more forms and the separation is based predominantly on one type of interaction (e.g., hydrophobic interaction in RP-HPLC), the dependence is linear, as expected. By studying the changes in the chemical structure of a compound as a function of pH it is possible to decide, in many cases, if a unique form or multiple forms of a compound are present in the solution. The use of this information allows us to determine when the lack of linearity (when present) is caused by multiple interactions in the separation system (for one form of the compound) and when more forms are causing the lack of linearity. The approximation with a quadratic form for the nonlinear dependence has been verified in most cases to be good, and only minor improvements were obtained when using higher polynomial dependencies.

INTRODUCTION

The dependence of a partition equilibrium constant $K$ on temperature (e.g., ref 1) can be expressed by the formula

$$ln K = -\frac{\Delta G^0}{RT}$$ (1)

where $\Delta G^0$ is the variation in the standard free enthalpy (Gibbs free energy) for the process and $R$ is the gas constant, 8.314 J/mol·K. This formula can be applied in HPLC to the equilibrium $A_m \leftrightarrow A_s$ where analyte $A$ is distributed between the stationary phase “s” and the mobile phase “m”. For a chromatographic column having the phase ratio $\Phi$, the retention factor $k'$ for the analyte $A$ is given by the formula

$$k'(A) = K(A) \Phi$$ (2)

In this case, the dependence of $k'(A)$ on temperature can be expressed as follows:

$$ln k'(A) = \frac{\Delta S^0}{R} + ln \Phi - \frac{\Delta H^0}{R} \left(\frac{1}{T}\right)$$ (3)

In eq 3, the expression $\Delta G^0 = \Delta H^0 - T \Delta S^0$ was utilized ($\Delta H^0$ is the change in standard enthalpy, and $\Delta S^0$ is the change in standard entropy). This formula is known as the van’t Hoff equation and indicates a linear dependence of $ln k'$ on $(1/T)$ when $\Delta S^0$, $\Delta H^0$, and $\Phi$ are assumed independent of temperature, although this assumption is only an approximation. Equation 3 can be written in the form

$$ln k'(A) = a + b \left(\frac{1}{T}\right)$$ (4)

Various studies were performed for HPLC to verify experimentally eq 4, indicating that, for a number of HPLC separations, the linearity of the van’t Hoff equation is in good agreement with experimental data (e.g., refs 4–7). In such cases, parameters $a$ and $b$ can be determined by fitting the line representing $ln k'(A)$ as a function of $(1/T)$, and when $ln \Phi$ is known, the values for $\Delta S^0$ and $\Delta H^0$ of the process can be estimated using the expressions

$$\Delta H^0 = -Rb \text{ and } \Delta S^0 = R(a - ln \Phi)$$ (5)

However, in practice, the linearity of the dependence of $ln k'(A)$ on $(1/T)$ is not obtained for all HPLC separations (e.g., refs 8–11). Such cases are known as nonlinear van’t Hoff dependencies. Both secondary equilibria9,10,12 and multiple retention mechanisms12 or interactions13,14 have been suggested to cause nonlinear van’t Hoff dependencies. The

Received: August 20, 2019
Accepted: November 5, 2019
Published: November 14, 2019

DOI: 10.1021/acsomega.9b02689
ACS Omega 2019, 4, 19808–19817

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present study shows that a major cause of nonlinear van’t Hoff dependencies is the existence of multiple mechanisms of interaction between the analyte and the phases in HPLC. Multiple interactions can appear for some compounds because they are present in more than one form in solution, but in other cases, because the HPLC process involves different interactions of the analyte during the chromatographic process. Temperature may influence similarly or differently each retention mechanism, leading to linear or nonlinear van’t Hoff dependencies. Other changes with temperature, such as the conformation of the stationary phase, may contribute to the differences in retention, but such change would be expected to affect similarly a whole group of analytes.

**THEORY**

Assuming a unique compound and a unique separation mechanism, only one thermodynamic potential \( \Delta H^0 \) and one \( \Delta S^0 \) should be assigned, and the variation with the temperature \( T \) for these is given by the expressions (e.g., ref 15)

\[
\Delta H^0_T = \Delta H^0_0 + \Delta C_p(T - T_0)
\]

and

\[
\Delta S^0_T = \Delta S^0_0 + \Delta C_p \ln\left(\frac{T}{T_0}\right)
\]

where \( \Delta C_p \) is the variation in heat capacity. Although \( C_p \) for solid and liquid solutes also varies with temperature to a certain extent,\(^{16}\) this variation is usually very small. With these corrections, for temperatures in a range relatively close to \( T_0 \) by taking with a good approximation \( \Delta C_p \ln(T/T_0) \approx 0 \), the dependence of \( \ln k' \) on temperature will be described by the formula

\[
\ln k' = \frac{\Delta S^0_0}{R} - \frac{\Delta H^0_0}{R} \left(\frac{1}{T} \right) - \Delta C_p \left(1 - \frac{T_0}{T}\right) + \ln \Phi
\]

For a value \( T = T_p \) or \( T \neq T_0 \) but \( \Delta C_p = 0 \), eq 8 is identical with eq 3. Both eqs 3 and 8 indicate that, with a good approximation, \( \ln k' \) should depend linearly on \( 1/T \), as indicated by eq 4. In reality, the linear dependence of \( k' \) on \( 1/T \) is verified only in some cases, and nonlinear behavior is relatively common.

The nonlinear dependence of \( k' \) on \( 1/T \) can be explained considering that, during the process \( A_m \leftrightarrow A_s \) where the molecular species \( A \) is distributed between the mobile phase (m) and stationary phase (s), more than one type of interaction takes place between the analyte and the stationary phase and mobile phase in the HPLC process and these interactions are influenced differently by the temperature.\(^{15}\) The different interactions may be caused by the fact that the analyte is present in more than one form in the solution (e.g., tautomers\(^{10,12}\)) with each form having its own interaction with the stationary phase and consequently a different equilibrium constant. Each constant may vary differently with temperature. However, even when the presence of different forms of an analyte is not plausible, nonlinear dependencies are possible. It is known (e.g., ref 17) that the HPLC process frequently involves more than one mechanism of separation. Even in the reversed phase (RP-HPLC) where the hydrophobic interactions play a dominant role, other interactions are present.\(^{18}\) The separations in HILIC-type HPLC or in the case of chiral chromatography are, in particular, known to have contributions from a combination of mechanisms (e.g., refs 13, 14, 19 and 20). It is only in some cases of RP-HPLC in the separation of analytes of low polarity in that it can be considered that one truly dominant type of interaction (hydrophobic) explains the whole process. Even when more than one separation mechanism is involved, the linearity between \( \ln k' \) on \( 1/T \) can be maintained if all processes are influenced equally by the temperature. In other cases, either because of different forms of the analyte or because different separation mechanisms are affected differently by temperature, the linearity is not fulfilled.

Considering, for example, only two different mechanisms and, for simplification, that a partition process takes place between the stationary phase and mobile phase, the expression for \( K(A) \) can be written in the form

\[
K(A) = \frac{[A_1]_m + [A_2]_m}{[A_1]_m + [A_2]_m}
\]

where indices “1” and “2” indicate either different molecular structures or that the unique type of molecules undergo two different separation mechanisms. Each mechanism can be assumed as described by a different equilibrium constant:

\[
K_1 = \frac{[A_1]_m}{[A_1]_m}
\]

\[
K_2 = \frac{[A_2]_m}{[A_2]_m}
\]

A formal equilibrium can be considered to exist between the molecules separated by the two different interactions, such that a constant \( K_{21} \) can be defined by the formula

\[
K_{21} = \frac{[A_2]_m}{[A_1]_m}
\]

From eqs 9–11, the following formula can be written for \( K(A) \):

\[
K(A) = \frac{K_1 + K_2 K_{21}}{1 + K_{21}}
\]

Each constant is related to a corresponding free-enthalpy value \( \Delta G \) (index of “0” omitted for simplicity of writing). For the constant \( K_{21} \), the free energy corresponds to the difference in the free energies of the two retention processes. As a result, the expression for \( K(A) \) can be written in the form

\[
K(A) = \exp\left(-\frac{\Delta G_1}{RT}\right) + \exp\left(-\frac{\Delta G_1 + \Delta G_{21}}{RT}\right)
\]

\[
1 + \exp\left(-\frac{\Delta G_{21}}{RT}\right)
\]

The expression for \( \ln K(A) \) can be written in this case in the form

\[
\ln K(A) = -\frac{\Delta G_1}{RT} + \ln\left[1 + \exp\left(-\frac{\Delta G_2 + \Delta G_{21} - \Delta G_1}{RT}\right)\right]
\]

\[-\ln\left[1 + \exp\left(-\frac{\Delta G_{21}}{RT}\right)\right]\]

Equation 14 can be represented in a Taylor series and will have the general form (e.g., see ref 9)

\[
\ln K(A) = a' + b' \frac{1}{T} + c' \frac{1}{T^2} + \sum_{n=3}^{n} d' \left(\frac{1}{T}\right)^n
\]
known, a value for the expression of \( k \) of \( \ln \) dependence. In such cases, the values for \( \Phi \) will generate the expression for the approximation (eq 16) for the dependence of \( \ln \) convergence of the Taylor series and therefore how good is the approximation of the dependence of \( \ln \) on \( 1/T \).



By replacing the value of \( k \) and \( \Phi \), the nonlinear dependence described by eq 16 can be mathematically characterized by an extreme value of \( \ln k' \) (having a maximum or minimum point in the given temperature interval). The temperature for this value can be obtained from the condition \( d(\ln k')/d(1/T) = 0 \). This condition shows that, for \( T = -2c/b \), the value of \( \ln k' \) is either a maximum or minimum and can be situated within the studied temperature interval for van’t Hoff dependence or outside this interval.

### EXPERIMENTAL SECTION

**Materials and Instrumentation.** Methanol (MeOH) and acetonitrile (AcCN) were of HPLC (gradient) grade and purchased from Sigma-Aldrich (Germany). The studied compounds (uracil, benzene, toluene, ethylbenzene, propylbenzene, propylparaben, butylparaben, sildenafil, and histidine) were of proanalysis grade and purchased from Merck (Germany). The additives (phosphoric acid and ammonium acetate) used for specific aqueous components from the mobile phase were of proanalysis grade purchased from Merck (Germany). Water of HPLC purity was obtained within the laboratory with a TKA Lab HP 6UV/UF instrument (Thermo Scientific). Solutions with a 100 μg/mL concentration of the studied compounds were made in acetonitrile.

The HPLC experiments were performed using an Agilent 1100 Series LC system (Agilent Technologies) consisting of the following modules: a degasser (G1379A), binary pump (G1312A), autosampler (G1313A), column thermostat (G1316A), and diode array detector (G1315A). Chromatographic data were acquired by means of Agilent Chemstation software rev. B.01.03.

Several columns were used in this study, and some of their characteristics are described in Table 1. All columns that have a C18 stationary phase are endcapped, and they were new and tested before usage according to the manufacturer’s specifications.

**Chromatographic Conditions.** Injections of 1 μL of mixture solutions containing 200 μg/mL concentrations of each analyte were used. Retention times longer than 60 min were not considered in this study, and the compounds eluting after this value (at a high water content in the mobile phase) were washed out with a pure organic modifier, and the column followed a re-equilibration step at the mobile phase.

### Table 1. Columns Used in this Study and Some of Their Characteristics

| no. | column type | phase | geometric characteristics [L (mm) × i.d. (mm) × carbon load (%) | surface area (m²/g) | pore size (Å) |
|-----|-------------|-------|---------------------------------------------------------------|---------------------|-------------|
| 1   | Gemini 5u C18 (Phenomenex) | porous | 100 × 4.6 × 5 × 14 | 375 | 110 |
| 2   | Ultrasil XB-C18 (Welch) | core–shell | 150 × 4.6 × 5 × 17 | 320 | 120 |
| 3   | BDS Hypersil C18 (Thermo Scientific) | porous | 100 × 4.6 × 5 × 11 | 170 | 150 |
| 4   | Chromolith Performance RP-18 (Phenomenex) | monolithic | 100 × 4.6 × 18 | 300 | 130 |
| 5   | Gold HILIC (Thermo Scientific) | porous | 150 × 4.6 × 5 × n.d.a. | 220 | 175 |

*Note: n.d.a. indicates no data available.
composition applied to the next run. All separations were performed at a flow rate of 1 mL/min unless specified for a particular situation. Detection was performed in UV at 254 nm. The temperature domain used in this study was 20−50 or 20−60 °C for different compounds evaluated.

Various mobile-phase compositions were used, as described in the following section, for several cases discussed in this study. The mobile-phase compositions were generated using an organic solvent (AcCN or MeOH) and aqueous component by appropriate settings for the pump (the solvents were not premixed).

The values of the retention factor ($k'$) were calculated according to the common expression (e.g., see ref 17)

$$k' = (t_R - t_0)/t_0$$  \hspace{1cm} (22)

where $t_R$ is the absolute retention time for the analyte and $t_0$ is the dead-time indicator, which was measured for each composition and temperature from the retention time of uracil (for the RP mechanism) and toluene (for the HILIC mechanism).

**RESULTS AND DISCUSSION**

**Examples of Linear van’t Hoff Dependence.** An example of a linear van’t Hoff dependence is shown in Figure 1 for the homologous series from benzene to propylbenzene with separation on a Gemini C18 100 × 4.6 mm column with a 5 μm particle size in the temperature range between 20 and 50 °C. The separation (in the isocratic mode) used a mobile phase of 45% water and 55% acetonitrile (v/v) at various indicated column temperatures.

![Figure 1](image1)

Figure 1. Overlaid chromatograms for the separation of benzene, toluene, ethylbenzene, and propylbenzene on the C18 column using a mobile phase with 45% water and 55% acetonitrile (v/v) at various indicated column temperatures.

![Figure 2](image2)

Figure 2. Example of linear van’t Hoff dependence in the range of 20 to 50 °C for benzene, toluene, ethylbenzene, and propylbenzene on a Gemini C18 column with water/AcCN 55/45 v/v as the mobile phase.
The $R^2$ values for the linear correlations shown in Figure 2 prove that the separation follows closely the theoretical prediction, as expected for a process dominated by a single type of interaction (hydrophobic) and with molecules present in a unique form.

The results from Figure 2 allow the evaluation of $\Delta H^0$ and $\Delta S^0$ assuming at a given temperature a phase ratio of $\Phi = 0.25$ for the Gemini C18 column. These results are given in Table 2, and they indicate as expected that the retention process is enthalpy-driven and that some loss of entropy takes place when the molecules are retained in the stationary phase. The entropic contribution to the variation in the standard free enthalpy ($-T\Delta S$), calculated for 298.15 K based on eq 5, is situated within the interval of 0.06 and 2.5 kJ/mol, having a contribution for the evaluated temperature lower (in the absolute value) than that of the enthalpy term (Table 2). Also, it can be noticed that this contribution increases with the increase of the hydrophobic character of the aromatic hydrocarbon, as expected. Overall, the free-enthalpy variation ($\Delta G^0$, calculated at 298.15 K), increases with an increment of $-1.0$ to $-1.1$ kJ/mol for the methylene group, which is in accordance with some reported values for the retention of these aromatic hydrocarbons.

Other columns and mobile-phase compositions were evaluated. These included the columns Ultisil XB-C18 (Welch) and BDS Hypersil C18 (Thermo Scientific). For these columns two compositions were used for the mobile phase: water/ACN 45/55 and water/ACN 50/50. For all experiments, linear van’t Hoff dependencies were obtained with $R^2$ values between 0.9970 and 0.9995. In these cases, the $R^2$ values for the linear correlations shown in Figure 2 prove that the separation follows closely the theoretical prediction, as expected for a process dominated by a single type of interaction (hydrophobic) and with molecules present in a unique form.
separations, it can be assumed that the dominant type of interactions is the hydrophobic ones (e.g., see ref 17) since the analytes are not polar and the stationary phase is a nonpolar C18.

Linear van’t Hoff dependencies in the range of 15 to 60 °C were also obtained, for example, for the separation of two alkylparaben analytes separated on a monolithic C18 column (Chromolith Performance RP-18) with the mobile phase of a 50/50 aqueous solution of 0.1% H$_3$PO$_4$/methanol. The graphs are shown in Figure 3. The $R^2$ values for this case were between 0.9988 and 0.9990. The values of $\Delta H^0$ and $\Delta S^0$ for the two parabens (Table 2) are much higher than the values obtained for aromatic hydrocarbons, but the values of $\Delta G^0$ for parabens are very close to those of the studied hydrocarbons.

The linear van’t Hoff dependence can also be interpreted as caused by the dominance of hydrophobic interactions between the analytes and the C18 column with a minor contribution from other potential types of interactions due to the presence of polar groups in propyl or butylparabens.

Examples of Nonlinear van’t Hoff Dependences on C18 Columns. Nonlinear dependences of ln $k'$ on $1/T$ have been previously reported in the literature, for example, for piroxicam, drotaverine, vincamine, and epivincamine at different pH values for the mobile phase. For drotaverine, for example, the van’t Hoff plots on a Zorbax XDB-C18, 150 × 4.6 mm, column with 3.5 μm particles for three different pH values are shown in Figure 3 (according to the data reported in ref 9). The utilized mobile phase was 62.5/37.5 aqueous buffer/acetonitrile at an apparent pH ($\text{pH}_{\text{app}}$) of 2.5 or 4.5, and of the mobile phase 50/50 aqueous buffer/acetonitrile at $\text{pH}_{\text{app}}$ 9.0 is shown in Figure 4. The variation with the pH of the drotaverine forms in solution is shown in Figure 5. The results from Figure 5 indicate that, with a good approximation, at the pH values from 2.5 to 4.5, the ionic form of the compound is dominant (and unique).

For the nonionic form of the analyte where the interactions with the C18 column are expected to be mainly hydrophobic and with little contributions from other types of interactions, the van’t Hoff dependence is linear. On the other hand, for the ionic form of the analyte, nonlinear van’t Hoff plots are obtained. This is an indication that, besides the hydrophobic interactions typical for an RP-HPLC separation, the ionic form dominant at $\text{pH}_{\text{app}}$ = 2.5 or $\text{pH}_{\text{app}}$ = 4.5 has other types of strong interactions, and their intensity depends on the temperature. The presence of other forms at these $\text{pH}_{\text{app}}$ values is not likely for drotaverine, and the nonlinear dependence is likely to be caused only by different types of interactions between the analyte and the separation system (mobile phase/stationary phase).

Figure 6. Overlaid chromatograms for the elution of sildenafil on a C18 column for a mobile phase consisting of 65/35 (v/v) water/acetonitrile at various indicated column temperatures.

Figure 7. Nonlinear van’t Hoff dependence in the range of 20 to 50 °C on a C18 column for sildenafil for (A) uncontrolled pH (water/acetonitrile) and (B) with pH = 2.5.
The quadratic curve given by eq 16 for the nonlinear dependence of ln \( k' \) on \( 1/T \) for drotaverine at pH 2.5 provides a value of \( R^2 = 0.9973 \), which indicates a good fit. According to eq 15, eq 16 is however only an approximation, and including higher terms (in \( 1/T \)) should provide an even better fit of the data. This is indeed the case, and for a cubic approximation, \( R^2 = 0.9976 \), for a quartic approximation, \( R^2 = 0.9979 \), and for a quintic approximation, \( R^2 = 0.9980 \). The relatively small increase in \( R^2 \) for the description of the dependence of ln \( k' \) on \( 1/T \) with higher polynomial expressions shows that these higher approximations do not add significant advantage compared to the quadratic expressions, which was typically reported in the literature for some systems (e.g., refs 9, 10, 12, and 15).

Similar behavior was observed for sildenafil, eluted under two different mobile-phase compositions (several examples of chromatograms obtained by its elution in a mobile phase with 65/35 v/v water/acetonitrile are shown in Figure 6). Thus, the nonlinear dependences between ln \( k' \) and \( 1/T \) have been noticed for sildenafil separated on a Gemini C18 column using a mobile phase of 65/35 v/v water/acetonitrile (Figure 7A) or an aqueous solution of H\(_3\)PO\(_4\) at pH 2.5 and acetonitrile 60/40 v/v (Figure 7B).

A thermodynamic analysis of the van’t Hoff plot from Figure 7B shows two different contributions of the enthalpy and entropy to the retention process. Up to a maximum retention at a column temperature calculated from the previously mentioned formula \( (T = -2c/b = 308.15 \text{ K or } 35 \text{ }^\circ\text{C}) \), the retention is entropy-driven. For a higher column temperature, the retention process becomes enthalpy-driven. Thus, the variation of enthalpy for the compound transfer from the mobile phase to the stationary phase at 293.15 K calculated with eq 20 and the regression parameters given in Figure 7B becomes \( \Delta H = +9.0 \text{ kJ/mol} \), while for the temperature of 323.15 K, the value of \( \Delta H \) is \(-8.1 \text{ kJ/mol}\). Similarly, with the aid of eq 21, the variation of entropy at 293.15 K is \( \Delta S = +37.2 \text{ J/mol} \cdot \text{K} \), while for 323.15 K, the value of \( \Delta S \) becomes \(-18.6 \text{ J/mol} \cdot \text{K} \) when we consider a constant value of the phase ratio of \( \Phi = 0.25 \) over the entire temperature interval, which is only an approximation.\(^{21}\) The free Gibbs enthalpy (\( \Delta G \)) can then be computed with these values of \( \Delta H \) and \( \Delta S \), resulting in the following values: \( \Delta G = -1.9 \text{ kJ/mol} \) at 293.15 K and \( \Delta G = -2.1 \text{ kJ/mol} \) at 323.15 K.

The van’t Hoff plot from Figure 7A has no maximum on the temperature interval, and the values of \( \Delta H \) are always positive on this temperature interval used for the retention study. For example, the values of \( \Delta H \) are \(+20.4 \text{ kJ/mol} \) at 293.15 K and \(+1.6 \text{ kJ/mol} \) at 323.15 K. In this case, the values of \( \Delta S \) are \(+95.6 \text{ J/mol} \cdot \text{K} \) at 293.15 K and \(+34.4 \text{ J/mol} \cdot \text{K} \) at 323.25 K.
Different molecular structures as a function of pH for sildenafil are shown in Figure 8. Two protonated forms of sildenafil (form 1 and form 2) are present at pH values below 2, and most likely, they exist in mutual equilibrium. However, for a pH = 2.5, Figure 8 shows a unique dominant molecular species with one positive charge. Similar to the case of drotaverine, at pH 2.5, multiple types of interactions are possible with the C18 stationary phase. For the mobile phase water/acetonitrile, the pH should be around neutral, and several molecular species are possibly present in the solution. Various species of the same analyte as well as the potential of multiple interactions in the case of forms that have a charge may explain the nonlinear vant’ Hoff behavior of the compound seen in Figure 7.

For drotaverine and sildenafil at pH = 2.5, the values of ln $k'$ have a maximum in the interval of evaluated temperatures as shown in Figures 4 and 7B, respectively. Similar to the case of drotaverine (e.g., at pH = 2.5), the curves describing the variation of ln $k'$ with $1/T$ are approximated better when using dependences including higher terms in $1/T$. However, the improvement is not necessarily significant. For the case of sildenafil separated without pH control, quadratic approximation gives $R^2 = 0.9532$, cubic approximation gives $R^2 = 0.9721$, quartic approximation gives $R^2 = 0.9904$, and quantic approximation gives $R^2 = 0.9990$. For the curve at pH = 2.5, quadratic approximation gives $R^2 = 0.9957$, cubic approximation gives $R^2 = 0.9977$, quartic approximation gives $R^2 = 0.9999$, and quantic approximation gives $R^2 = 1.0000$. These results indicate that, although the quadratic form provides a good approximation for the variation of ln $k'$ with temperature, the quadratic form still remains an approximation, and the consideration of higher terms in eq 15 still improves the agreement between the experiment and theory.

Examples of vant’ Hoff Dependences on HILIC Columns. On HILIC columns, deviations from linear vant’ Hoff dependences are more common. However, it is difficult to decide if the deviations from linearity are caused by different forms of the analyte (producing different inter-
actions\textsuperscript{26} or it is caused by one form of the analyte subject to different types of interactions. As an example, the variation of \( \ln k' \) with \( 1/T \) for histidine separated on a Gold HILIC 150 × 4.6 mm column with 5 μm particles with a mobile phase of 10 mM ammonium acetate in water (pH ≈ 7.0) at two different aqueous/acetonitrile compositions is shown in Figure 9.

The diagram showing the forms of histidine at different pH values is given in Figure 10. From Figure 10, it may be concluded that more than one form of the compound is present in a neutral mobile phase (pH ≈ 7). In such a case, different forms of the compound associated with different types of interactions would be the reason for nonlinear dependences between \( \ln k' \) and \( 1/T \) during the chromatographic separation of this compound. Similar to other nonlinear dependences, the approximations become slightly better when polynomial forms with higher powers of \( 1/T \) are included. For example, for glycyl-L-phenylalanine in the 25/75 Aq/AcCN phase, the \( R^2 \) values are \( R^2 = 0.9847 \) for quadratic, \( R^2 = 0.9956 \) for cubic, \( R^2 = 0.9987 \) for quartic, and \( R^2 = 0.9988 \) for quintic approximation. The same conclusion in that the addition of higher terms from eq 15 improves the agreement between the experiment and theory can be obtained here.

Nonlinear dependences between \( \ln k' \) and \( 1/T \) can also be seen for other compounds such as glycyl-L-phenylalanine (shown in Figure 11) and glycyl-L-tyrosine (shown in Figure 12). The separations were performed on a Gold HILIC 150 × 4.6 mm column with 5 μm particles with a mobile phase of 10 mM ammonium acetate in water (pH ≈ 7.0) at two different aqueous/acetonitrile compositions. For these compounds, the evaluated temperature interval does not cover a point where \( \ln k' \) reaches an extreme value within the used temperature interval.

These dependences could be explained by the role of the adsorbed water molecule from the mobile phase to the stationary phase\textsuperscript{27,28} when (i) the temperature increases, and thermal desorption of the adsorbed mobile phase leads to the formation of sites at which analyte adsorption is more exergonic owing to the reduced need to displace molecules from the stationary phase.

(ii) this process can increase the retention of the analyte, provided that the magnitude of the effect is sufficiently great.

(iii) the analyte retention can increase until the thermal desorption of solvent is near completion.

(iv) beyond this temperature a decrease in retention is expected with an increase in temperature.

■ CONCLUSIONS

Linear van’t Hoff dependences were obtained for compounds with unique structure, such as aromatic hydrocarbons and esters, which are involved only in hydrophobic interactions with C18 stationary phases with minor contributions from other potential types of interactions with the stationary phase (e.g., with residual silanols). On the contrary, compounds that can potentially participate in multiple interactions with the stationary phase generate deviations from van’t Hoff behavior. This was observed, for example, for drotaverine and sildenafil (as well as other compounds with nonlinear \( \ln k' \) vs \( 1/T \) dependencies reported in the literature) under a reversed-phase mechanism or for histidine and some dipeptides separated based on HILIC mechanisms. The quadratic form used to approximate nonlinear van’t Hoff dependence has been verified in most cases to be very good with only minor improvement when using higher polynomial dependencies.

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