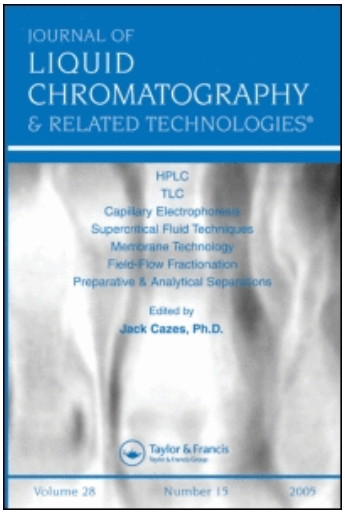

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Investigations into the HPLC Retention Behavior of Simvastatin and Structurally Related Compounds

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Abstract: The HPLC retention behavior of simvastatin and its β,δ-dihydroxy acid and methylester forms were investigated. The retention of the lactone and the methylester was found to be highly dependent on the eluent composition and mildly dependent on temperature at low temperatures. The retention of the β,δ-dihydroxy acid was found to be highly dependent on eluent pH and temperature as well as the composition. Atypical dependence of the retention of the β,δ-dihydroxy acid on temperature prompted further investigation. It was determined that the observed temperature–retention relationship for the β,δ-dihydroxy acid was caused by a shift in the ${}^{s}_{w}$pK of the acid under the elution conditions. As the elution temperature increases, the ${}^{s}_{w}$pK of the acid changes resulting in an increase in retention at low temperatures. Thermodynamic analysis shows that retention of simvastatin and the methyl ester is predominantly enthalpy driven. Similar analysis for the β,δ-dihydroxy acid shows that retention is entropy driven at low temperatures at all pH's, but it is enthalpy driven at higher temperatures.

Keywords: Retention behavior, Simvastatin, HPLC, Related compounds

INTRODUCTION

Simvastatin[1] is an effective 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitor for the treatment of hypercholesterolemia.[2] More recent reports have suggested its efficacy in the prevention of coronary disease[3,4] and bone loss.[5]

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Administered as an inactive lactone, simvastatin (I, Figure 1) is hydrolyzed to the active β,δ-dihydroxy acid (II, Figure 1) in vivo. The presence of both forms in vivo has led to several papers on the HPLC-UV,[6–8] HPLC-Fluorescence,[9] and HPLC-MS[10–13] analysis of simvastatin in biological matrixes. Additional chromatographic work has been presented regarding the HPLC purification of simvastatin and other reductase inhibitors.[14] In all of these reports, reverse phase chromatographic conditions using various C_{18} and C_8 columns are discussed. Water/acetonitrile mixtures are predominantly used as the mobile phase with the elution temperature ($T = 20^{\circ}C$ to $50^{\circ}C$) and pH (from pH 2 to pH 7), being adjusted according to the individual goals of the analysts. Adequate resolution was reported under all of the described conditions.

Shen et al. found that the retention time of simvastatin was linear in both water/methanol (70 to 100% methanol) and water/acetonitrile (50 to 100% acetonitrile) elution conditions.[8] However, a systematic evaluation of mobile phase composition, temperature, pH effects on the retention of both I and II has not been reported. This work expands on Shen, et al., and investigates the retention dependence of both simvastatin and the β,δ-dihydroxy acid on the various elution conditions. Interesting chromatography was observed for the β,δ-dihydroxy acid prompting further investigation. The retention behavior of the β,δ-dihydroxy acid was observed to be highly

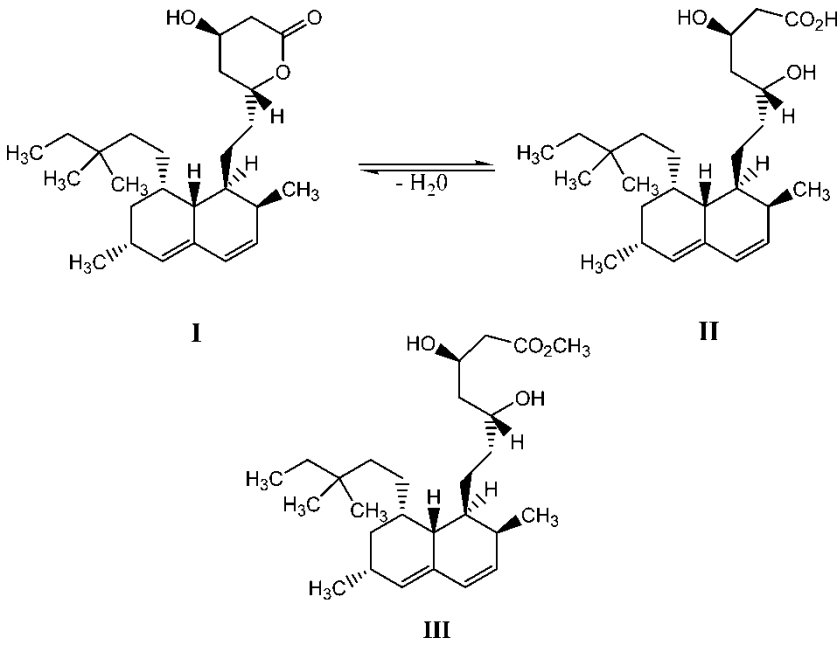

Figure 1. Chemical structures of the lactone (**I**), β,δ-dihydroxy acid (**II**), and methyl-ester (**III**) forms of simvastatin.

dependent on mobile phase composition, mobile phase pH, and anomalous behavior was observed when changing the separation temperature. The retention behavior of simvastatin was found to be much less dependent on mobile phase pH and temperature, but sensitive to mobile phase composition. Van't Hoff plots were used to investigate the thermodynamic properties of retention for both compounds.

EXPERIMENTAL

Chemicals and Reagents

The HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Citric acid (monohydrate) and potassium citrate (monohydrate) were purchased from Aldrich (Milwaukee, WI, USA). Potassium phosphate (monobasic) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). All were used without further purification. The water was purified using a Hydro water filtration system. Simvastatin samples were obtained from Merck Manufacturing Division, and the ammonium salt of the β,δ-dihydroxy acid form of simvastatin was provided by the Process Research Department of Merck Research Laboratories (Rahway, NJ, USA).

High Performance Liquid Chromatography

An Agilent 1100 high performance liquid chromatography (HPLC) instrument comprised of a vacuum degasser, quaternary pump, autosampler, thermostated column compartment, and variable wavelength detector was used for all analyses (Wilmington, DE, USA). Chromatographic data was collected and processed using Turbochrom 6.1 (Perkin Elmer, Cupertino, CA, USA). Three different HPLC columns were used in the study; YMC Basic (250×4.6 mm; 5 μm dp proprietary pore size; 7% carbon load; 300 m^2/g surface area) distributed by Waters Corp. (Milford, MA, USA); ACE-C8 (250×4.6 mm; 5 μm dp; proprietary pore size; 7% carbon load; surface area not available) distributed by MAC_MOD Analytical (Chadds Ford, PA, USA); and Luna Phenyl-Hexyl (100×4.6 mm; 5 μm dp; 100 Å; 17.5% Carbon load; 400 m^2/g surface area) distributed by Phenomenex (Torrance, CA, USA). The weak mobile phase (A) was 10 mM citrate buffer at varying pH values controlled through the relative ratio of citric acid and potassium citrate unless otherwise noted. The strong mobile phase (B) was pure acetonitrile (ACN). Chromatograms were obtained under isocratic elution conditions. The injection volume was 10 μL, the detection wavelength was 238 nm, and the column flow rate was 1.2 mL/min unless otherwise noted. All data points generated from HPLC analyses are the average of three injections.

pH and pK Determinations

All pH measurements were made on an Accumet AR15 pH meter (Fisher Scientific, Fair Lawn, NJ, USA). The pH meter was calibrated using aqueous standards buffered at pH = 4, 7, and 10 daily. Apparent pH ($^{s}_{w}$pH) values of aqueous/organic mixtures were measured based on the same purely aqueous standards. A Brinkman model 716 DMS Titrino automatic titrator was used for dissociation constant ($^{s}_{w}$pK) determinations in various solvent mixtures. Approximately 450 mg of II was dissolved in water/acetonitrile with 40% ACN and titrated with 0.1 N NaOH. Progression of the titration was followed potentiometrically after each addition of titrant.

Sample Preparation

Samples were prepared by dissolving approximately 0.2 g/L of simvastatin in diluent. The diluent composition was predominately 50/50 (v/v) citrate buffer/ACN. However, for comparison purposes, 50/50 (v/v) H_2O/ACN, and 20/80 (v/v) mixtures were also investigated.

RESULTS AND DISCUSSION

Chromatographic Parameter Investigation

Evaluations of both simvastatin (I) and the β,δ-dihydroxy acid (II) retention were performed on a YMC Basic column (250 × 4.6 mm; 5 μm). Previous reports have shown that adequate separation between both forms of simvastatin can be achieved with a number of different reversed phase HPLC columns,[6–13] so the YMC Basic column was arbitrarily selected to investigate the general retention behavior of I and II. The influence on the mobile phase composition, pH, and temperature on retention is shown in Figures 2 and 3. Typical retention behavior was observed for simvastatin with respect to the amount of strong solvent and pH. Figure 2 demonstrates this behavior with the mobile phase buffered at pH = 3.9 and 7.2. The natural logarithm of the retention factor of I decreased linearly ($R^2 > 0.98$) with increasing acetonitrile eluent. As expected, no pH dependency was observed in the retention of the lactone, as the experimental retention factor using pH = 7.2 buffered mobile phase is nearly identical to that obtained with the pH = 3.9 buffered mobile phase. It is also interesting to note that temperature did not have a large influence on the retention of I between 0°C and 30°C as might be expected.

Using the same conditions, the retention behavior of the β,δ-dihydroxy acid form of simvastatin was observed to be highly dependent on mobile phase composition and separation temperature (Figure 3). A non-linear depen-

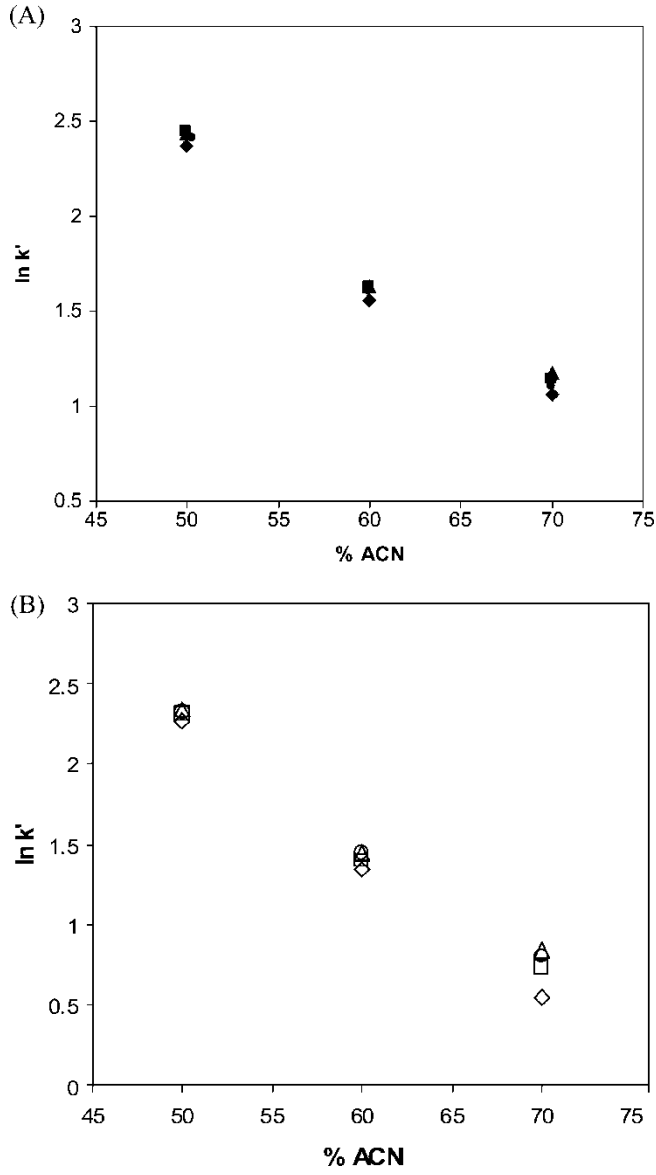

Figure 2. The effect of mobile phase composition on the retention behavior of **I** for isocratic elution in various mobile phase conditions using a YMC Basic column. Retention factors are for isocratic elutions with citrate buffer (pH = 3.9)/acetonitrile mixtures at elution temperatures of 0°C (filled triangles), 10°C (filled squares), 20°C (filled circles), and 30°C (filled diamonds) and citrate buffer (pH = 7.2)/acetonitrile mixtures at elution temperatures of 0°C (open triangles), 10°C (open squares), 20°C (open circles), and 30°C (open diamonds). **I** was prepared as a 0.2 g/L solution in 80/20 (v/v) acetonitrile/water.

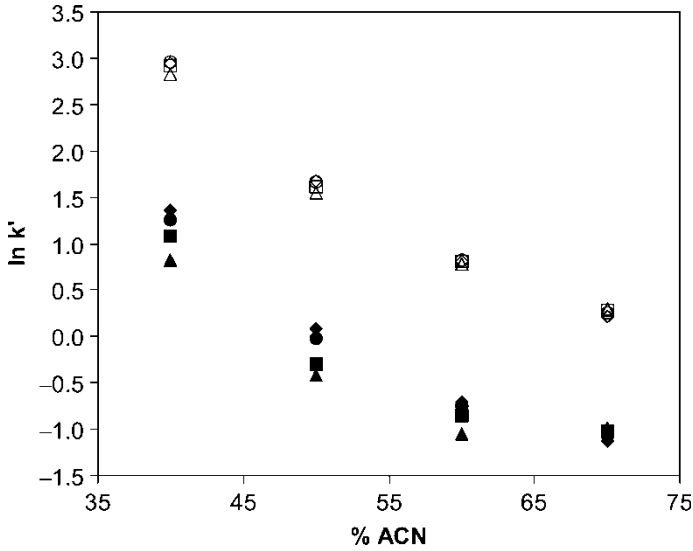

Figure 3. The effect of mobile phase composition on the retention behavior of **II** for isocratic elution in various mobile phase conditions using a YMC Basic column. Retention factors are for isocratic elutions with citrate buffer (pH = 7.2)/acetonitrile mixtures at elution temperatures of 0°C (filled triangles), 10°C (filled squares), 20°C (filled circles), and 30°C (filled diamonds) and citrate buffer (pH = 3.9)/acetonitrile mixtures at elution temperatures of 0°C (open triangles), 10°C (open squares), 20°C (open circles), and 30°C (open diamonds). **II** was prepared as a 0.2 g/L solution in 80/20 (v/v) acetonitrile/water.

dence on the amount of acetonitrile was observed, and as expected, its retention is also pH dependent. It has been shown that the pK_a of the buffer component,[15–17] and therefore, the buffer pH[18,19] changes with changes in the amount of organic modifier in the mobile phase. In the conditions in Figure 3, the apparent pH (^s_wpH) of the mobile phase buffered at pH = 3.9 (prior to ACN addition) increases by 0.2 ^s_wpH units per 10% increase of ACN from 40% ACN (^s_wpH = 4.6) to 70% ACN (^s_wpH = 5.2). The ^s_wpH of the mobile phase buffered at pH = 7.2 (prior to ACN addition) shows a similar dependence on the mobile phase composition between 40% ACN (^s_wpH = 8.1) to 70% ACN (^s_wpH = 8.5). Furthermore, the pK_a shifts observed with changes in mobile phase composition are not limited to the buffer components. Ionogenic analytes are also susceptible to these mobile phase composition induced pK_a shifts.[20,21] The β,δ-dihydroxy acid exhibits this behavior. The apparent pK_a (^s_wpK) of the acid increases by 0.2 ^s_wpK units per 10% increase of ACN in the mobile phase from 40% ACN (^s_wpK = 5.3) to 70% ACN (^s_wpK = 5.9). A combination of these mobile phase influences is likely the cause of the non-linear retention dependence on mobile phase composition observed in Figure 3.

The influence mobile phase pH has on the retention of I and II was also investigated further. Mobile phases consisting of 10 mM citrate buffers ranging in pH from 2.5 to 8.5 (measured prior to mixing with acetonitrile) with 40% acetonitrile were investigated to determine the β,δ-dihydroxy acid's retention dependence over a wide and continuous pH range. A characteristic acid/base sigmoidal curve was observed when the retention factor was plotted against pH (Figure 4). The amount of acetonitrile was decreased from 50% to 40% for this figure in order to achieve greater retention of the acid. However, it is worth noting that a similar curve is observed when 50% acetonitrile is used. Solving the second derivative of these sigmoidal plots reveals inflection points of pH = 4.7 and pH = 4.6 (pH of aqueous phase measured prior to ACN addition) at 0°C and 30°C, respectively. In a separate experiment, the apparent pH of this mobile phase mixture was measured to be ${}^{s}_{w}\text{pH} = 5.3$ at 0°C and ${}^{s}_{w}\text{pH} = 5.2$ at 30°C. Therefore, the chromatographically determined ${}^{s}_{w}\text{pK}$ of II is ${}^{s}_{w}\text{pK} = 5.3$ at 0° and ${}^{s}_{w}\text{pK} = 5.2$ at 30°C. These values are in reasonable agreement with the titrimetrically determined ${}^{s}_{w}\text{pK}$ of the β,δ-dihydroxy acid in a mixture of water and 40% acetonitrile at 0° (${}^{s}_{w}\text{pK} = 5.5$) and 30°C (${}^{s}_{w}\text{pK} = 5.3$). Therefore, similar to other ionogenic species, HPLC can be used to estimate the pK of the β,δ-dihydroxy acid form of simvastatin in various aqueous/organic mixtures. Both the chromato-

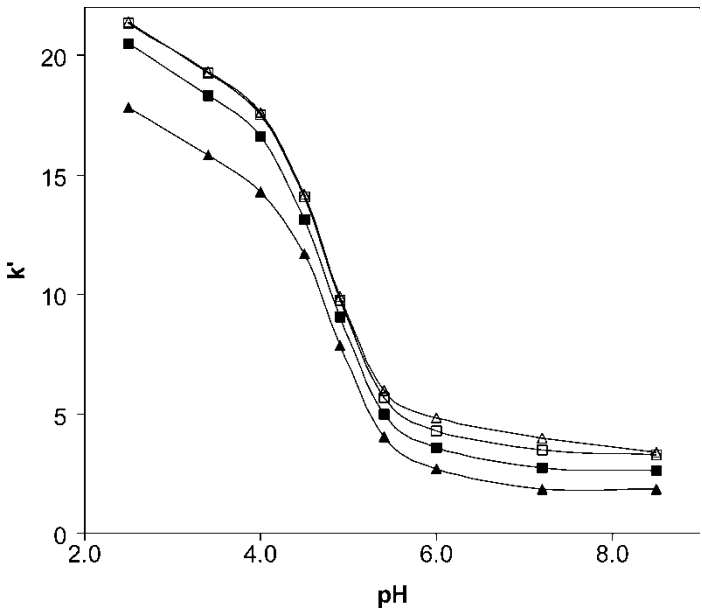

Figure 4. The effect of mobile phase pH on the retention of the β,δ-dihydroxy acid form of simvastatin using a YMC Basic column with 40% acetonitrile. Temperatures at 0°C (filled triangles), 10°C (filled squares), 20°C (open squares), and 30°C (open triangles) are plotted.

graphic and titrimetric experiments also show the temperature dependence of the ${}^{s}_{w}$pK for II.

It is also interesting to note the effect temperature has on retention for both compounds. A significant direct relationship between the retention of the β,δ-dihydroxy acid and the elution temperature is observed in Figures 3 and 4. There is little change in retention time for simvastatin between 0°C and 30°C (Figure 2). The retention/temperature relationship is typically inversely proportional for most organic compounds. These results, in addition to the pH and mobile phase composition dependencies of retention, encouraged further investigation into the retention behavior of simvastatin and the β,δ-dihydroxy acid.

Van't Hoff Analysis

The van't Hoff relationship has been used to relate the chromatographic retention factor (k′) of an analyte to the thermodynamic properties associated with its transfer between the stationary phase and mobile phase.[22] This is based on the relationship between the Gibbs free energy of transfer, ΔG°, and chromatographic retention factor, k′.

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} = -RT \ln k' \tag{1}$$

In Equation (1), T is the column temperature and $R = 8.314\,J/(K * mol)$. This relationship can be rearranged to allow the determination of the enthalpy, ΔH° (J/mol), and entropy, ΔS° (J/mol * K), of analyte transfer from the mobile phase to the stationary phase using,

$$\ln(k') = \frac{\Delta H^{\circ}}{RT} + \frac{\Delta S^{\circ}}{R} + \ln \varphi \tag{2}$$

where φ is the phase ratio. For comparison purposes, the phase ratio is considered to be constant over the temperature range studied. Provided that the enthalpy and entropy are not dependent on temperature (e.g., the separation mechanism is constant), plots of ln k′ vs. 1/T (van't Hoff plots) are linear allowing for the determination of ΔH° and ΔS° for the retention process.

Representative van't Hoff plots for simvastatin and the β,δ-dihydroxy acid form of simvastatin between 0°C and 60°C are presented in (Figure 5). van't Hoff plots of II were determined across a range of mobile phase pH. For simplicity, only the plots for pH = 3.9 and 7.2 are shown. The curvature of the plots increases with increasing pH, but all exhibit two linear regions on either side of a maximum temperature. These plots also show the significant retention dependence of II on pH. Simvastatin also has a non-linear van't Hoff plot, but the curvature is much less pronounced. In addition, the retention and curvature do not exhibit the same pH dependence as II.

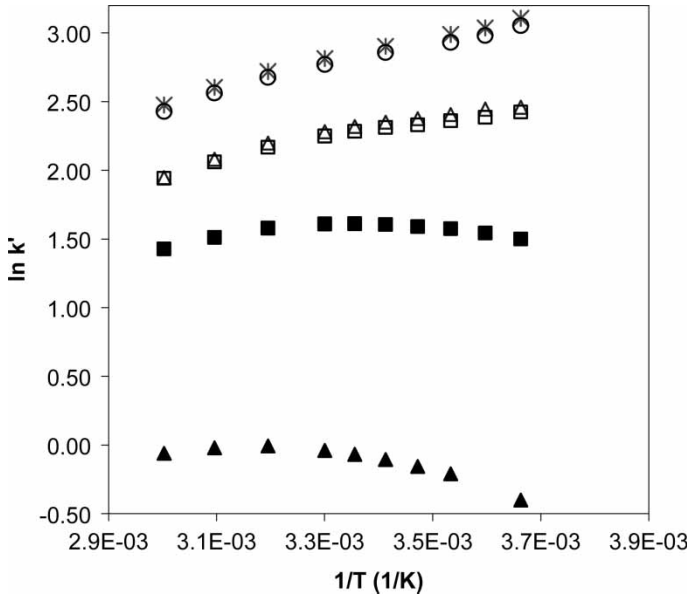

Figure 5. Van't Hoff plots for the lactone, β,δ-dihydroxy acid, and methylester forms of simvastatin. Plots were produced using 50% ACN with 10 mM citrate buffer. The lactone is plotted using pH = 7.2 (open triangles) and pH = 3.9 (open square). The data points for the β,δ-dihydroxy acid represent pH = 7.2 (filled triangle) and pH = 3.9 (filled squares), and those for the methylester also represent pH = 7.2 (open circles) and pH = 3.9 (asterisks).

Non-Linear Van't Hoff Analysis

Non-linear Van't Hoff plots are observed when temperature dependent changes in the analyte or stationary phase conformation(s), or their interaction are present. To elucidate the source of the non-linearity observed for the β,δ-dihydroxy acid, additional studies were performed. To determine if the nonlinearity is the result of thermal changes in the YMC Basic stationary phase, temperature studies using an ACE C8 column and a Luna Phenylhexyl HPLC column were performed. Both columns were investigated with pH = 7.2 citrate buffer/acetonitrile mobile phases (Figure 6). The experiments using the YMC Basic column were performed with 40% ACN to increase the retention of the acid. The experiments using the ACE C8 column were performed with 42% ACN, and those using the Luna Phenylhexyl column were performed with 35% ACN. The percentage of acetonitrile used was adjusted slightly for these columns to achieve comparable retention of II. Despite having different stationary phase chemistries, particle size, and column dimensions, all three columns still produced van't Hoff plots with the same curvature. These studies suggest that the non-

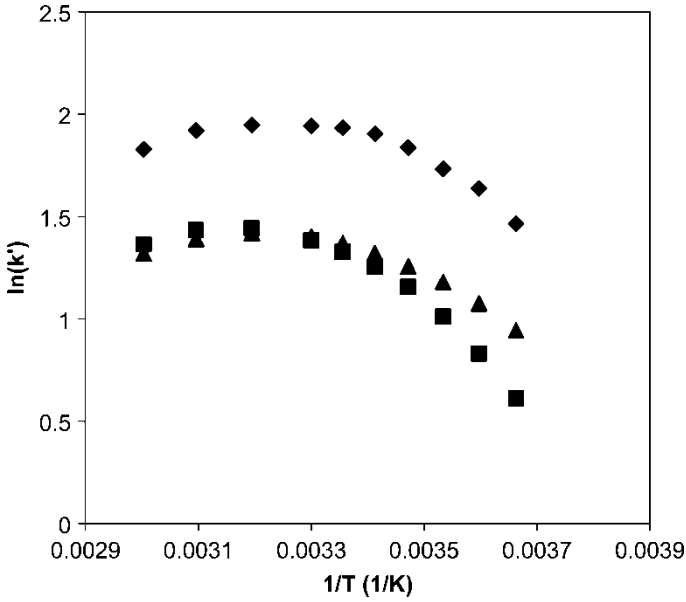

Figure 6. Van't Hoff plots of II using the a YMC Basic (filled squares), an ACE C8 (filled triangles), and a Phenomenex Luna Phenyl Hexyl (filled diamonds) column. See text for detailed discussion of experimental conditions.

linearity is not caused by thermally induced changes to the stationary phase. It does, therefore, suggest that the observed retention behavior for II on all three columns is caused by a temperature induced change in II.

The influence of organic modifier concentration and pH of the diluent were also investigated. An organic modifier concentration range of 50% to 80% ACN and a diluent pH range of pH 2.5 to pH 8.5 were investigated with both simvastatin and the β,δ-dihydroxy acid. These changes had no effect on the retention time of either compound.

The temperature effect on the eluent apparent pH ($^{s}_{w}$pH) was also considered as a potential source of the observed nonlinearity. The $^{s}_{w}$pH of a citrate buffer (pH = 4.0 prior to addition of ACN) with 40% ACN decreased from $^{s}_{w}\text{pH} = 4.9$ at 0°C to $^{s}_{w}\text{pH} = 4.5$ at 50°C. Conversely, the $^{s}_{w}$pH of a citrate buffer (pH = 7.0 prior to addition of ACN) with 40% ACN increased from $^{s}_{w}\text{pH} = 7.5$ at 0°C and $^{s}_{w}\text{pH} = 8.0$ at 50°C. A decrease in $^{s}_{w}$pH of the acidic eluent with an increase in temperature could cause the curvature for an acidic compound by increasing protonation of the conjugate base. However, one might, therefore, expect to see a linear plot using the pH = 7.2 eluent conditions where the $^{s}_{w}$pH increases with temperature. As seen in Figure 6, this is not observed. Furthermore, the curvature for the β,δ-dihydroxy acid using a phosphate buffer with 40% ACN at pH = 3.2 and pH = 7.2 is nearly identical to the curvature observed when the citrate

buffer is used. Phosphate buffers are not sensitive to changes in temperature, therefore, these results show that changes in eluent pH with temperature do not appear to play a significant role in the observed retention behavior of the β,δ-dihydroxy acid. It is also worth noting that buffer concentration does not effect the retention behavior of these compounds. In separate studies, HPLC citrate concentrations from 5 to 15 mM were studied using 40% and 50% ACN, and no retention differences were observed.

In addition, a closer look at the inflection points in the van't Hoff plots at different temperatures reveals that this transition is also pH dependent. The inflection temperatures, or the temperatures at which $\Delta(\ln k')/\Delta(T)$ equals zero, are shown in Figure 7. A sudden jump in inflection temperature occurs at pH ~5.3, which again, is close to the ${}^{s}_{w}$pK for the β,δ-dihydroxy acid in water with 40% ACN. As mentioned previously, titrating the acid showed a decrease in the ${}^{s}_{w}$pK from 0°C (${}^{s}_{w}\text{pK} = 5.5$) to 55°C (${}^{s}_{w}\text{pK} = 5.1$).

As a result, all of these findings support the conclusion that the observed curvature is caused by changes in ${}^{s}_{w}$pK of the β,δ-dihydroxy acid with temperature. To confirm this conclusion, the methyl ester analog of simvastatin (III, Figure 1) was prepared and analyzed. Blocking the sight of protonation of II, one would expect the methyl ester to have similar retention behavior to that of simvastatin. A slight excess of sulfuric acid was added to a 3 g/L solution of simvastatin in MeOH. After heating to 58°C for one hour, the methyl ester analog was observed by HPLC analysis. The solution was appropriately diluted in MeOH for HPLC analysis and analyzed using 50/50 citrate buffer (pH = 3.9 and pH = 7.2)/acetonitrile mobile phase mixtures. As expected, the methyl ester does not display any pH dependence and the van't Hoff plots are only slightly curved (Figure 5) similar to the lactone. This provides further evidence that

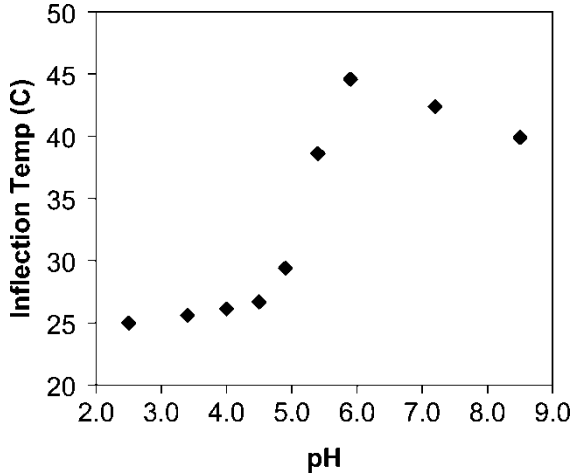

Figure 7. Van't Hoff inflection temperature as a function of pH.

changes in the ${}^{s}_{w}$pK of II with temperature is the likely source of the observed retention behavior.

The source of the curvature for the lactone and methyl ester is less clear. However, a small degree of curvature previously observed in highly bonded stationary phases has been attributed to a phase transition in the stationary phase at low temperatures.[23] As the temperature increases from very low temperatures, the stationary phase becomes more accessible to the analyte, therefore increasing solute retention, until the enthalpic contribution dominates the retention process. However, the only information available on the YMC Basic is the 7% carbon load. Both the pore size and information about the "neutral" silanol groups are considered proprietary, and the phase is only described as a monomeric phase consisting of alkyl silanes with chain lengths $\leq$C8 to maximize surface coverage. Therefore, it is not possible to test this hypothesis and perform suitable comparison studies with this column.

Thermodynamic Analysis

Similar curvature was observed for three different reverse phase columns, suggesting the observed retention is driven by thermally induced changes to the ${}^{s}_{w}$pK of the β,δ-dihydroxy acid. Therefore, it can be useful to evaluate the thermodynamics of retention of the analytes on one column. Although the absolute values of this thermodynamic data on the YMC Basic column may not be identical to that on other reverse phase columns, using it can still provide insight into the general retention of simvastatin and structurally related compounds.

The non-linear van't Hoff relationships in chromatography that possess maxima or minima can be analyzed in different ways. The thermodynamic properties of these non-linear systems can be solved by breaking the van't Hoff plots into two separate linear regions, one on either side of the inflection zone.[24] Alternatively, a quadratic curvilinear fit can be applied Equation (3).[22,25–27]

$$\ln k' = a + \frac{b}{T} + \frac{c}{T^2} + \ln\varphi \tag{3}$$

The use of this expression to solve non-linear van't Hoff plots is possible when the heat capacity (Cp) of the retention process is dependent on elution temperature.[24] Therefore, according to Kirchoff's relationships, the temperature dependence of ΔH° and ΔS° can be determined by

$$\Delta H^\circ = \Delta Cp(T - T_H) \tag{4}$$

and

$$\Delta S^\circ = \Delta Cp \ln(T/T_S) \tag{5}$$

where ΔH° and ΔS° are as defined previously, ΔCp is the change in heat capacity, and T_H and T_S are the temperatures at which ΔH° and ΔS° are zero, respectively. The following relationships can then be derived for ΔH°, ΔS°, and ΔCp using the quadratic fit of the data and Kirchoff's relationships.

$$\Delta H^\circ = -R\left(b + \frac{2c}{T}\right) \tag{6}$$

$$\Delta S^\circ = R\left(a - \frac{c}{T^2}\right) \tag{7}$$

$$\Delta Cp = \frac{2Rc}{T^2} \tag{8}$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{9}$$

It is also worth noting at this time, that previous studies have shown that it is adequate to assume the phase ratio, φ, is constant over the temperature range used in this HPLC study,[22–25,27,28] which simplifies the relationship for comparison studies.

As mentioned previously, the second order temperature dependence observed in concave van't Hoff plots is valid only if Kirchoff's relationships hold. That is, the heat capacity of analyte association with the stationary phase changes with temperature. In this study, all compounds and all eluent conditions produced linear plots of ΔCp vs. temperature with $R^2 > 0.99$. The slope and intercept of representative systems for simvastatin, the β,δ-dihydroxy acid, and the methylester are presented in Table 1. A general trend is that the heat capacity change of retention for the β,δ-dihydroxy

Table 1. Slope and intercept of the linear plots of ΔCp vs. temperature

Analyte	Slope	Intercept
β,δ-Dihydroxy acid		
pH = 2.5; 40% ACN; YMC Basic	2.352	−424.9
pH = 3.9; 40% ACN; YMC Basic	2.416	−436.3
pH = 5.5; 40% ACN; YMC Basic	2.408	−434.9
pH = 7.2; 40% ACN; YMC Basic	4.141	−747.9
pH = 8.5; 40% ACN; YMC Basic	3.582	−647.0
pH = 7.2; 35% ACN; Luna Phenylhexyl	3.291	−594.5
pH = 7.2; 42% ACN; ACE C8	2.812	−507.9
pH = 3.9; 50% ACN; YMC Basic	1.634	−295.1
pH = 7.2; 50% ACN; YMC Basic	1.965	−354.9
Lactone		
pH = 3.9; 50% ACN; YMC Basic	0.948	−171.3
Methylester		
pH = 7.2; 50% ACN; YMC Basic	0.579	−104.6

acid is more sensitive to temperature at higher pH's, but the heat capacity change of retention for the lactone and the methylester is not pH dependent. In addition, when the plots of ΔCp vs Temperature are extrapolated, all the systems studied for all three compounds converge to zero at the same temperature of 180.6°C.

The thermodynamic properties of the retention of simvastatin after fitting the van't Hoff plots with the quadratic expression in Equation (3) are presented in Table 2. The quadratic yielded a good fit with $R^2 > 0.99$ for all systems investigated. The values of ΔH° (J/mol), ΔS° (J/mol-K), and ΔG° (kJ/mol) for the β,δ-dihydroxy acid, simvastatin, and the methylester at pH = 3.9 using the YMC Basic column and 50% acetonitrile were calculated using Equations (6), (7), and (1), respectively. From the data, it can be seen that the retention of the lactone and the methylester is driven by both enthalpic and entropic processes between 0 and 20°C. Above 20°C, retention is predominantly enthalpy driven. The β,δ-dihydroxy acid retention, however, is entropy driven between 0 and approximately 25°C. Above this temperature its retention is enthalpy dominated.

Table 3 explores the pH dependence on the thermodynamic properties of the β,δ-dihydroxy acid retention. For all pH conditions, the enthalpic contribution to retention is endothermic at lower temperatures, showing that retention at these temperatures is entropically driven. Enthalpy starts to drive the retention at increasingly higher temperatures as the eluent pH is increased. This trend corresponds well with the observed change in the maximum temperature observed in the van't Hoff plots depending on eluent pH. Although the absolute values of the thermodynamic variables were slightly different for the Luna Phenyl-Hexyl column and the ACE C8 column, the same trend was observed.

Table 2. Thermodynamic properties for various forms of simvastatin pH = 3.9 with 50% ACN. Units and chromatographic conditions defined in text

T	β,δ-dihydroxy acid			Lactone			Methylester		
(°C)	ΔH°	ΔS°	ΔG°	ΔH°	ΔS°	ΔG°	ΔH°	ΔS°	ΔG°
0	6520	36.3	−3.39	−1480	14.6	−5.47	−4580	8.5	−6.90
5	5049	31.0	−3.56	−2333	11.5	−5.53	−5150	6.4	−6.93
10	3230	25.9	−3.70	−3157	8.6	−5.58	−5699	4.5	−6.96
20	937	16.6	−3.91	−4719	3.1	−5.64	−6741	0.8	−6.98
30	−1578	8.1	−4.03	−6178	−1.8	−5.65	−7714	−2.4	−6.97
40	−3932	0.5	−4.08	−7544	−6.2	−5.61	−8625	−5.4	−6.93
50	−6141	−6.5	−4.05	−8826	−10.2	−5.52	−9480	−8.1	−6.87
60	−8217	−12.8	−3.95	−10,031	−13.9	−5.40	−10,284	−10.5	−6.77

Table 3. Thermodynamic properties for β,δ-dihydroxy acid simvastatin as a function of pH using 40% ACN. Units defined in text

T	pH = 2.5			pH = 3.9			pH = 5.5			pH = 7.2		
(°C)	ΔH°	ΔS°	ΔG°	ΔH°	ΔS°	ΔG°	ΔH°	ΔS°	ΔG°	ΔH°	ΔS°	ΔG°
0	9879	60.2	−6.56	10,535	61.0	−6.04	14,927	66.1	−3.13	27,842	107.1	−1.58
5	7761	52.5	−6.84	8361	53.1	−6.37	12,759	58.2	−3.44	24,114	93.6	−1.92
10	5718	45.2	−7.08	6263	45.6	−6.62	10,668	50.8	−3.71	20,518	80.8	−2.36
15	3747	38.3	−7.29	4238	38.5	−6.83	8650	43.7	−3.95	17,047	68.6	−2.73
20	1842	31.7	−7.46	2282	31.8	−6.98	6700	37.0	−4.15	13,694	57.1	−3.05
25	1	25.5	−7.61	392	25.4	−7.10	4816	60.6	−4.32	10,454	46.1	−3.31
30	−1779	19.6	−7.72	−1436	19.3	−7.23	2994	24.6	−4.46	7321	35.7	−3.51
40	−5168	8.6	−7.86	−4917	8.0	−7.38	−476	13.3	−4.64	1354	16.4	−3.77
50	−8348	−1.4	−7.90	−8183	−2.2	−7.40	−3731	3.1	−4.72	−4243	−1.2	−3.84
60	−11,337	−10.5	−7.83	−11,252	−11.6	−7.34	−6791	−6.3	−4.71	−9505	−17.3	−3.75

CONCLUSIONS

As discussed in previous reports,[6–13] simvastatin and its β,δ-dihydroxy acid were separated under a variety of chromatographic conditions without noting how the retention of both was influenced by mobile phase composition, pH, and elution temperature. As might be expected, retention of both compounds decreased with an increase in organic modifier. Also not surprisingly, the acid retention was highly dependent on mobile phase pH, but simvastatin shows no pH dependence. However, neither had linear dependence on elution temperature. The non-linearity of the β,δ-dihydroxy acid was determined to be caused by a temperature induced shift in the acid's ${}^{s}_{w}$pK. The non-linearity of simvastatin is believed to be driven by small stationary phase changes at low temperatures. As the temperature increases from 0°C to 20°C, the stationary phase becomes more accessible to the analyte.

These studies suggest that analysis of simvastatin and/or its β,δ-dihydroxy acid is best performed at pH <3.0 or pH >6.0 and ambient temperature regardless of the column or amount of acetonitrile used for the separation. As with all other ionogenic analytes, mobile phase ${}^{s}_{w}$pH near the solutes ${}^{s}_{w}$pK should be avoided, because small fluctuations in ${}^{s}_{w}$pH can cause large shifts in retention time. Additionally, the temperature dependence of retention for both is flat near 25°C, making for a more robust method. The column and acetonitrile content can then be selected to optimize the selectivity and run time for the individual needs of the analysis.

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