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Highlights

- The van't Hoff plot analysis was tested for several chromatographic circumstances.
- Two columns were serially connected to get a system containing two adsorption sites.
- Theoretical considerations were made to show the non-additivity of $\triangle$H and $\triangle$S values.
- $\triangle$H and $\triangle$S values were plotted against the average pressure drop and extrapolated.

The correctness of van 't Hoff plots in chiral and achiral chromatography

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Abstract

van 't Hoff plots (logarithm of the retention factor, ln *k*~~vs~~ , vs. the reciprocal of absolute temperature, $1/T$) are commonly used in chromatographic studies to characterize the retention mechanisms based on the determined enthalpy (~~ΔH~~$\Delta H°$) and entropy (~~ΔS~~$\Delta S°$) change of analyte adsorption. In reversed phase liquid chromatography~~these~~ , the thermodynamic parameters could help to understand the retention mechanism. In chiral chromatography, however, the conclusions drawn based on van 't Hoff plots can be deceptive because several different types of adsorption sites are present on the surface of stationary phase. The influence of heterogeneity, however, cannot be studied experimentally.

In this study, we employed two reversed phase columns with different retention mechanisms to show that by ~~serial connection of these~~ serially coupling the columns, the obtained thermodynamic parameters are not related to the results obtained on the ~~individual columns, respectively~~respective individual columns. Furthermore, our results show that the experimental conditions – such as flow-rate or choice of instrument – will strongly influence the calculated enthalpy and entropy values.

Keywords: van 't Hoff plot, entropy change, enthalpy change, adsorption sites, chiral separation mechanism, thermodynamics

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1. Introduction

Chiral separations have become a rather important chromatographic area in both analytical and preparative separations. ~~Retention~~ The retention behavior in chiral separations is often investigated via the estimation of enthalpy and entropy changes of enantiomer separation to unfold the mechanism of chiral recognition. In a typical case, several chemically related chiral analytes are involved and their retention is studied using a series of systematically changed experimental conditions. Nevertheless, it is nowadays clear that despite the simplicity of the van 't Hoff analysis, even the physical interpretation of its parameters is limited because the chromatographic column is an open system with constant pressure gradient, i.e. neither isobaric nor isochoric [1].

~~On the surface of chiral stationary phases, different types of adsorption sites (i.e. various enantioselective and nonselective sites) are present [2, 3], and the van 't Hoff procedure uses only the retention factor derived from the retention time of a retained and that of a non-retained compound to describe the bonding of an analyte to the stationary phase. Thus the individual bondings and their actual ratio in the chromatographic column are never determined and the conclusions drawn on the basis of the thermodynamic data derived can easily be deceptive.~~

The van 't Hoff analysis used in chromatographic practice is based on the equation

$$\ln k = -\frac{\Delta H}{RT}\frac{\Delta H^\circ}{RT} + \frac{\Delta S}{R}\frac{\Delta S^\circ}{R} + \ln \phi, \tag{1}$$

where k is the retention factor of the ~~observed peak, ΔH and ΔS are the~~ analyte, ΔH° and ΔS° are the standard molar enthalpy and entropy changes, respectively, R is the gas constant, T is the temperature and ϕ is the phase ratio (i.e. the ratio of the volume of the stationary phase and that of the mobile phase). This method assumes that by plotting $\ln k$ against $1/T$ a linear relationship is obtained. ~~ΔH~~ ΔH° is calculated from the slope~~while ΔS~~, whereas ΔS° is derived from the intercept of Eq. 1.

On the surface of chiral stationary phases, different types of adsorption sites (i.e. various enantioselective and nonselective sites) are present [2, 3], and the van 't Hoff procedure uses only the retention factor derived from the retention time of a retained and that of a non-retained compound to describe the interaction of an analyte with the stationary phase. Thus the individual interactions and their actual ratio in the chromatographic column are never determined and the conclusions drawn on the basis of the derived thermodynamic data can easily be deceptive.

Using a van 't Hoff plot in chiral chromatography such as the one illustrated in Fig. 2, several simplifications are made: the retention factor (k) does not refer to a single ~~bonding~~ type of interaction even in reversed phase chromatography [4, 5], and definitely not in chiral chromatography [3, 6–8] and the true phase ratio (ϕ) is actually never known [8–10].

Although Lämmerhofer [8] pointed out that the ~~the~~ information content of the thermodynamic studies and derived quantities is strongly limited and Asnin et al. [1] collected and demonstrated all the pitfalls possibly occurring when using van 't Hoff plots in chiral chromatography, there ~~are~~ is a great number of studies which favorably use it. It should be ~~mentioned,~~ noted

that when using the logarithm of the ~~selectivity, the errors made during the calculations cancel each other and the phase ratio also disappears~~separation factor (selectivity) and not the retention factor, the error introduced by the unknown phase ratio is eliminated. This argument, however, does not apply to ~~calculating~~ the calculation of molar enthalpies and entropies of the heterogeneous adsorption process where the errors are still present.

In this work~~we want to~~, we show the importance of distinguishing between the adsorption sites present in ~~the~~ a chromatographic column. To achieve this, we ~~used~~ use achiral conditions. The serial connection of two reversed phase columns ~~and caffeine as analyte. By serially connecting the columns,~~ results in a stationary phase with two ~~adsorption sites was obtained~~ different adsorption sites where both sites ~~were~~ are characterized individually using the van 't Hoff analysis. We also want to draw attention on instrumental and experimental circumstances that highly influence the results derived from van 't Hoff ~~plots~~analysis.

2. Theory

2.1. ~~Effect~~ The effect of two adsorption sites by column connection

For chiral stationary phases – where at least two types of adsorption sites are present – the retention factor is generally written [3] as the sum of the retention factor of the non-selective sites (ns) and that of the ~~selective sites such~~ enantioselective sites (es) as

$$k_{\mathrm{exp}} = k_{\mathrm{ns}} + k_{\mathrm{es}}. \tag{2}$$

~~By~~ Because the isolation of the selective and non-selective sites is not possible experimentally, we study a system containing two different reversed phase columns that are serially connected. This way, although, the results will not be based on chiral separations, we can get valuable information about a system where two different adsorption sites are present.

By the serial coupling of the columns, the expected retention time will be the sum of the individual retention times and also the void time is the sum of the respective void times. Thus, the retention factor of the ~~peak measured~~ analyte on the connected columns ~~must~~ will be

$$k = \frac{(t_{\mathrm{R},1} + t_{\mathrm{R},2}) - (t_{0,1} + t_{0,2})}{t_{0,1} + t_{0,2}}, \tag{3}$$

where $t_{R,1}$ and $t_{R,2}$ refer to the retention time of the same compound obtained on the first and the second column, while $t_{0,1}$ and $t_{0,2}$ are the void times of the first and second column, respectively. It follows that ~~by~~ using the individual retention factors to derive the retention times, $t_{\mathrm{R,i}} = t_{0,\mathrm{i}}(k_{\mathrm{i}} + 1)$, Eq. 3 will simplify to

$$k = \frac{t_{0,1}k_1 + t_{0,2}k_2}{t_{0,1} + t_{0,2}}, \tag{4}$$

thus the overall retention factor is calculated as the void-time weighted average of the respective k values.

By substituting Eq. 4 to Eq. 1 and using the individual enthalpy and entropy changes calculated for the corresponding columns (i.e. ~~ΔH_1 and ΔS_1~~ ΔH_1° and

ΔS_1° of the first column and ~~ΔH_2 and ΔS_2~~ ΔH_2° and ΔS_2° of the second column obtained using Eq. 1) one will get the following expression

$$k = \frac{V_{S,1} \exp\left(-\frac{\Delta H_1}{RT} + \frac{\Delta S_1}{R}\right) + V_{S,2} \exp\left(-\frac{\Delta H_2}{RT} + \frac{\Delta S_2}{R}\right)}{V_M} \frac{V_{S,1} \exp\left(-\frac{\Delta H_1^\circ}{RT} + \frac{\Delta S_1^\circ}{R}\right) + V_{S,2} \exp\left(-\frac{\Delta H_2^\circ}{RT} + \frac{\Delta S_2^\circ}{R}\right)}{V_M}, \tag{5}$$

where ~~we already used that~~ $\phi_1 V_{0,1} = V_{S,1}$ ~~and~~ , $\phi_2 V_{0,2} = V_{S,2}$ and $V_M = V_{0,1} + V_{0,2}$ is the mobile phase volume of the system. It is obvious from Eq. 5 that the enthalpy and entropy values of two adsorption sites ~~can not~~ cannot be summed as it is assumed in chiral chromatography ~~and~~ or whenever the adsorption is heterogeneous.

3. Experimental

The ~~mefloquin~~ mefloquine profiles were acquired using a Shimadzu HPLC system including a binary pump and diode-array detector using ACN/MeOH/H_2O 49/49/2 with 50 mM HCOOH and 30 mM $HCOONH_4$ as eluent in isocratic mode. Concentration of the sample was 0.1 mg/mL. 2 μL was injected, the flow-rate was set to 1.0 mL/min. The column was a Chiralpak ZWIX(+) with a particle diameter of 3 μm and dimensions of 4.6 × 150 mm. Temperature was set to 20, 25, 30 and 35 °C to obtain the profiles. Detection was done at 280 nm. The void time was obtained by injecting 1,3,5-tri-tert-butylbenzene (97+%, ThermoFisher GmbH). The mefloquine standard was purcased from Merck as hydrochloride salt with a purity of >98%. The molecular formula is $C_{17}H_{16}F_6N_2O$*HCl.

The column connection experiments were carried out on a Waters Acquity I Class instrument (Waters Corporation, Milford MA, USA). The system consists of a binary solvent manager, an autosampler with a flow-through-needle injector, a column manager, a diode-array detector and a computer data station running Empower 3 software.

Two reversed phase columns were used to perform the experiments: a Zorbax Eclipse Plus C18 (3.5 μm; 4.6 × 100 mm) and a Zorbax SB-CN (3.5 μm; 4.6 × 150 mm) column. The mobile phase was MeOH/H_2O 25:75 (V/V%). The sample contained caffeine (c_{inj} = 0.05 mg/mL, >99%, Fluka Analytical) and thiourea (c_{inj} = 0.06 mg/mL, >99%, Sigma-Aldrich) dissolved in mobile phase and the injected volume was 0.5 μL. The flow-rate was varied between 0.1 and 0.7 mL/min, at each temperature (25, 30, 35, 40, and 45 °C). ~~The sample contained caffeine (c_{inj} = 0.05 mg/mL) and thiourea (c_{inj} = 0.06 mg/mL) dissolved in mobile phase; V_{inj} = 0.5 μL.~~ Thus the separations were performed at 13 flow-rates and at 5 different temperatures to obtain the van 't Hoff plots for each column and when the columns were serially connected. Detection was done at 272 nm.

To obtain the retention factors, the retention times were obtained using PeakFit v4.12 software by fitting exponentially modified Gaussian functions to the peaks. Then the retention times were corrected. The correction contains instrumental values such as system volume and injection drift by increasing the flow-rate. The values used for the correction are summarized in Table 1.

4. Results and discussion

4.1. ~~Mefloquin~~ Mefloquine measurements

The retention behavior of mefloquine enantiomers on a ZWIX(+) column is rather unusual when carried out at several temperatures, as it is shown in Fig. 2. We can see that the retention of the first peak does not depend on the temperature and the second peak has significant tailing even using elevated temperatures. One would first assume overloading effects or slow adsorption–desorption kinetics [7], the situation is indeed rather complicated. From the peak shapes of the second peak shown on Fig. 2 we can conclude that several adsorption sites are present on this stationary phase.

The van 't Hoff analysis based on Eq. 1 shows that there is ~~one order of magnitude difference in ΔH between the two peaks (2.41~~ a big difference in ΔH° (-2.41 kJ/mol vs~~23.8~~ -23.80 kJ/mol~~, calculated via Eq. 1). The~~) and in ΔS° (-2.51 J/(K mol) vs. -62.40 J/(K mol)) values for the two enantiomers. The van 't Hoff plots are presented in Fig. 3~~.~~, while the chemical structure of the mefloquine enantiomers and the ZWIX(+) selector are shown in Fig. 1. These energies however could not refer to a single bond, even one H-bond ~~could~~ would be larger, and based on the molecule structure, two or more H-bonds can ~~formed~~ form between one molecule and one stationary phase ligand. There are several other possible interactions ~~like~~ such as ionic-bonding, π-stacking, enantiomer recognition, and it is also feasible that several molecules bond at the same time to one stationary phase ligand. ~~It is also interesting to observe that the peak~~ As mentioned, it is interesting that the retention time of the less retained enantiomer does not change ~~by~~ significantly when increasing the temperature. This phenomenon is still unclear. Maybe one or more inhibitory ~~bonds~~ interactions are also present and their ~~interaction~~ combination is balanced by temperature change.

The peak shape observed for the second eluted enantiomer can only occur when at least three different adsorption sites are present [11]. Using the stochastic theory of chromatography [12], the fitting of the characteristic function accounting for three adsorption sites is possible in the Fourier domain. This was carried out using an algorithm written in-house in Fortran programming language. It was assumed that the first eluted enantiomer is retained by only one type of adsorption site while the second eluted enantiomer is retained by all the three sites. The fitting procedure provides the number of adsorption-desorption steps and the average time that a molecule spends bound to the stationary phase for each adsorption site.

For a three-site retention process, the retention factor is written as

$$k = \frac{t'_R}{t_0} = \frac{n_1\tau_1 + n_2\tau_2 + n_3\tau_3}{t_0} \tag{6}$$

where n_i is number of adsorption–desorption steps and τ_i the average sojourn time in the stationary phase during a single adsorption step on site i. The contribution of site i to the overall retention factor is $k_i = n_i\tau_i/t_0$.

From those results, the van 't Hoff plots of the individual adsorption sites can be obtained respectively. Table 2 contains the ΔH° and ΔS° values obtained from the chromatograms of the mefloquine enantiomers using the data obtained by the fitting procedure. While looking at the data presented in Table 2 it is

clear that there is a huge difference between the various sites, thus the overall ΔH° and ΔS° values obtained using retention factors cannot describe well the separation mechanism.

4.2. *Theoretical van 't Hoff considerations*

The retention data of the mefloquine ~~measurement~~ measurements were utilized to show the effect of more than one ~~bonding sites~~ type of interactions on the calculated thermodynamic parameters (~~ΔH and ΔS). For the~~ ΔH° and ΔS°). Although three adsorption sites and interactions were used in the fitting procedure, for the sake of simplicity only two sites ~~were assumed . The partitioning of the retention factor was done based on the data obtained for the~~ are assumed in this theoretical part of the study.

We artificially partitioned the retention factor of the more retained mefloquine enantiomer ~~. These k values were artificially partitioned~~ into certain percentages to obtain k_1 and k_2. To preserve the experimental behavior of the retention factor data e.g. k decreases as temperature is increased, we needed to partition the original data in different degrees at different temperatures. This is illustrated in Fig. 4 where k_1 is 60% of the original k value at 293 K thus k_2 is 40% while at 308 K k_1 is 90% of the original k and k_2 is only 10% of it. At intermediate temperatures, the percentage of the partitioning yields that the logarithm of both k_1 and k_2 becomes linear as plotted against the reciprocal of temperature (shown in Fig. 5).

From the partitioned retention factors, a van 't Hoff plot was ~~made and ΔH and ΔS~~ created and ΔH° and ΔS° were calculated. We should note at this point that for all entropy calculations the same phase ratio was ~~used~~ assumed to show the differences that are present only because of the partitioning.

To change the proportion of k_1 related to the original k, the $\ln k_1$ vs. $1/T$ lines obtained from the partitioned data (indicated by squares in Fig. 4) were shifted while $\ln k_2$ was unchanged and both the ~~ΔH~~ ΔH° – ~~ΔS~~ ΔS° and the corresponding k_1 and k values were calculated. This shifting procedure was repeated for several cases. The ~~result are illustrated~~ calculated thermodynamic values are plotted against k_1/k shown in Figs. 6 and 7~~for the calculated enthalpy and entropy changes, respectively~~. The values obtained using Figs. 4 and 5 are at k_1/k=0.6 when the partitioning procedure was k_1 60% $\rightarrow$ 90%.

The colors in Figs. 6 and 7 represent different partitioning procedures and it is indicated from which ~~rate constant~~ retention factor the thermodynamic data was calculated. It is obvious from these graphs that the ~~ΔH and ΔS~~ ΔH° and ΔS° values calculated from the partitioned retention factors k_1 and k_2 only agree with the ones calculated from the original retention factor, k when the other type of adsorption site is not present, i.e. $k_1 = k$ or $k_2 = k$. When a heterogeneous interaction is assumed, the overall thermodynamic parameters calculated from the retention factor k show a nonlinear transition between k_1 and k_2.

4.3. *Experiments with two adsorption sites*

In chiral separations, one ~~can not~~ cannot eliminate the heterogeneity of the retention mechanism. Therefore when one wants to study how two ore more interactions contribute to the overall retention, a simpler model system should be constructed. We designed a series of experiments using two reversed phase

columns of different retention mechanisms (but of the same inner diameter) to draw attention on the phenomenon discussed in the previous sections of this study. Separations were performed on each column separately and on the serially connected columns ~~,~~as well. The conclusions drawn are general for other separation mechanisms, thus also for chiral chromatography~~as well~~.

The first thing to decide when determining the thermodynamic parameters via the van 't Hoff procedure is whether or not the chromatographic circumstances ~~–~~ such as flow-rate or back pressure ~~–~~ matter. To obtain a van 't Hoff plot, one has to measure the retention behavior of the compound under investigation, and that of a non-retained compound at various temperatures but ~~Figure~~ Fig. 8 illustrates that the retention factor depends on the flow-rate on Zorbax Eclipse C18 column. The same phenomenon was observed when using the Zorbax SB-CN column and also when we serially ~~connected~~ coupled the two columns.

The flow is generated via pressure across the chromatographic system, which results in higher pressure drops along the column as the flow-rate is increased. One should note that for two temperatures (25 and 30 °C), ~~the~~ for either individual and for the serially coupled columns the change of k shows a contradictory trend than expected ~~for both columns and also for the serially connected one~~ (see Fig. 10). It has to be noted that this effect ~~can not~~ cannot be attributed to frictional heat. Frictional heat would result in a steep decrease in k as the flow-rate is increased. Our results at 25°C, however, show a constant retention factor in the 0.5–0.7 mL/min flow-rate range. Furthermore, if we omit the results obtained at 25°C, the van 't Hoff plots do not change significantly and the same thermodynamic values are calculated. When measuring and plotting classical van 't Hoff ~~plots~~data, it is not common to use e.g. water bath to thermostat the column, so the problem of flow-rate presented here is a general warning.

In all three cases (with the two respective columns and ~~when serially connected~~ ~~)~~ for the serially connected one), the van 't Hoff plots were ~~drawn~~ created for each investigated flow-rate (Fig. 9). It is obvious from Fig. 9 that both the slope and the intercept depend on the particular flow-rates, thus the calculated enthalpy and the entropy changes will differ as well. The pressure dependence of the retention is already known: even for small molecules, the pressure affects retention due to the change of the molar volume of the analyte during adsorption [13, 14].

As it was mentioned above, the calculation of the true phase ratio, ϕ is not possible in liquid chromatography. Even for the simplest case when only one bonding site is assumed on the surface of the stationary phase particles to be present, the ratio of the stationary phase and the mobile phase volumes ~~can not~~ cannot represent the true phase ratio. Thus the ~~ΔS~~ ΔS° values calculated not only in this study but in all van 't Hoff analyses in chromatography must be treated with reservations. The calculated thermodynamic values and the phase ratios are summarized in Table 3. The errors of the values (95% confidence limits) were calculated from regression data.

By connecting two columns serially, we get a heterogeneous stationary phase with two bonding sites, where the phase ratio should be known for the respective sites. Thus, with a simple van 't Hoff plot, the true energy of a single ~~bonding can not~~ interaction cannot be determined. The data presented in Table 3 show that

- the calculated ~~ΔH and ΔS~~ $\Delta H°$ and $\Delta S°$ values vary with the flow-rate of the separation;
- although similar values are obtained on the single columns, the thermodynamic data calculated ~~when the columns were connected significantly~~ for the serially coupled columns differ from any of them;
- the values obtained for the connected columns are not between the results obtained for the single columns.

It is really interesting to investigate the correctness of Eqs. 4 and 5 and thus the actual meaning of the thermodynamic parameters derived from the van 't Hoff plots. Fig. 10 illustrates the correctness of Eq. 4. It is clear that these equations describe the system well, for ~~every~~ each investigated flow-rate the ~~percentage~~ relative difference between the calculated and measured values is below 2.3% for Eq.4 and below 3.2% for Eq. 5.

The average column pressure is calculated ~~by~~ as

$$p_{\mathrm{avg}} = \frac{p_{\mathrm{in}} + p_{\mathrm{out}}}{2}, \tag{7}$$

where p_{out} was obtained as $p_{\mathrm{system}} - p_{\mathrm{detector}}$ so the pressure drop along the detector cell was ~~substracted~~ subtracted from the whole pressure drop. For that purpose, the detector was disconnected from the system and the pressure was monitored for all flow-rates. To obtain p_{in}, both the column and the detector were disconnected from the system and the pressure was ~~followed up along the flow-rates used~~determined for each flow-rate. Table 4 summarizes the measured and calculated pressure data for the Zorbax SB-CN column at 25°C.

When one plots the ~~ΔH and ΔS~~ $\Delta H°$ and $\Delta S°$ values obtained at various flow-rates against the average column pressure, a rather surprising result occurs. The data shown in Tables 3 and 4 indicate that the thermodynamic parameters vary more or less linearly with the average pressure drop which is shown in Figs. 11 and 12. Here not the linearity is emphasized but the change with the flow-rate. The ~~ΔH and ΔS~~ $\Delta H°$ and $\Delta S°$ values for the serially connected columns ~~can not~~ cannot be the combination of the individual results.

When the plots are extrapolated to $p = 0$ bar (dashed lines in Figs. 11 and 12) to eliminate the pressure effect, one gets ~~a more realistic ΔH and ΔS~~ more realistic $\Delta H°$ and $\Delta S°$ values referring to the original van 't Hoff analysis. For ~~ΔH~~$\Delta H°$, the extrapolation to $p = 0$ results in ~~-16.8~~ −16.8 kJ/mol whereas -8.5 kJ/mol is obtained at a widely used flow-rate 0.5 mL/min. For ~~ΔS~~$\Delta S°$, the extrapolation to $p = 0$ results in ~~-43.2~~ −43.2 J/(K mol) whereas ~~-15.8~~ −15.8 J/(K mol) is obtained at $F_v = 0.5$ mL/min. Thus it is indeed a problem that van 't Hoff ~~plot usually is~~ plots are usually determined at a single flow-rate. Furthermore, results ~~are usually~~ obtained on different systems or at different flow-rates are usually compared. Pressure has an important effect on the retention behavior and thus on the calculated thermodynamic parameters. ~~Heterogeneity~~ Surface heterogeneity further complicates the determination of the thermodynamic parameters. ~~ΔH~~ $\Delta H°$ values obtained on the single columns are independent and rather different from the value obtained on the serially connected columns. All the ~~ΔH and ΔS values~~ $\Delta H°$ and $\Delta S°$ values determined in chromatography are only apparent, ~~it is not related to the~~

~~bonding mechanisms~~they do not express the true thermodynamic parameters of the interactions.

5. Conclusions

We have demonstrated that, however, in some cases interesting conclusions can be drawn based on van 't Hoff plots [15, 16], the numerical molar thermodynamic values determined from the slope and intercept of the van 't Hoff plot can be erroneous because of the assumptions made compared to the original van 't Hoff equation and because of the significant influence of the chromatographic circumstances.

Both experimental and theoretical studies show that a more complex thermodynamic study of retention on any type of chromatographic stationary phase is necessary than the one offered by van 't Hoff plots.

We have created a heterogeneous stationary phase containing two types of adsorption sites by the serial connection of two columns. van 't Hoff analysis has been used to calculate the thermodynamic parameters for the individual sites and for the heterogeneous surface. It can be concluded that the heterogeneity of the stationary phase made the determination of the accurate ~~ΔH and ΔS~~ $\Delta H°$ and $\Delta S°$ values impossible. That observation leads to a serious constraint for van 't Hoff analysis in chiral chromatography, where stationary phases are intrinsically heterogeneous.

Our results also show that pressure drop along the column will strongly influence the calculated enthalpy and entropy values. Therefore, the value of the calculated thermodynamic parameters strongly depend on the length of the column, the particle size, ~~or~~ the flow-rate, or the instrument itself.

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Tables

Table 1: Correction values at different flow-rates on the Waters Acquity I Class instrument

Flow-rate (mL/min)	~~Void~~ Extracolumn time (s)	~~Void~~ Extracolumn volume (μL)
0.10	5.60	9.33
0.15	4.15	10.37
0.20	3.43	11.42
0.25	3.02	12.58
0.30	2.72	13.59
0.35	2.48	14.46
0.40	2.33	15.54
0.45	2.19	16.41
0.50	2.09	17.45
0.55	1.99	18.24
0.60	1.92	19.20
0.65	1.85	20.08
0.70	1.82	21.19

Table 2: Thermodynamic values calculated from the data obtained during the fitting of the stochastic model for the three adsorption sites.

	$\Delta H°$ kJ/mol	$\Delta S°$ J/(K mol)
1st site	-2.41	-2.51
2nd site	-31.10	-89.70
3rd site	-32.75	-123.30

Table 3: Calculated phase ratios and thermodynamic data for the Zorbax Eclipse C18, Zorbax SB-CN, and the serially connected columns at different flow-rates.

Flow-rate	C18			SB-CN			Connected		
	~~ΔH~~ ϕ	~~ΔS~~ $\Delta H°$	~~ΔH~~ $\Delta S°$	~~ΔS~~ ϕ	~~ΔH~~ $\Delta H°$	~~ΔS~~ $\Delta S°$	ϕ	$\Delta H°$	$\Delta S°$
(mL/min)		(kJ/mol)	J/(K mol)		(kJ/mol)	J/(K mol)		(kJ/mol)	J/(K mol)
0.1	0.70	-14.27± 0.40	-35.66± 1.31	0.44	-14.65± 0.58	-36.11±1.85	0.52	-15.01±1.04	-37.39±3.40
0.15	0.70	-13.83± 0.68	-34.17± 2.22	0.44	-13.86±0.50	-33.42±1.63	0.53	-14.18±0.82	-34.68±2.66
0.2	0.70	-13.08± 1.08	-31.70± 3.49	0.44	-13.18±0.67	-31.21±2.17	0.53	-13.34±0.95	-31.96±3.08
0.25	0.70	-12.42± 1.56	-29.52± 5.08	0.44	-12.53±0.79	-29.08±2.57	0.53	-12.39±1.19	-28.88±3.88
0.3	0.71	-11.85± 1.85	-27.62± 6.02	0.44	-11.89±0.88	-27.01±2.85	0.53	-11.57±1.45	-26.22±4.71
0.35	0.71	-11.19± 1.56	-25.50± 5.08	0.45	-11.29±0.89	-25.09±2.90	0.53	-10.78±1.60	-23.64±5.21
0.4	0.71	-10.52± 1.02	-23.32± 3.32	0.45	-10.73±0.91	-23.26±2.97	0.53	-10.03±1.85	-21.19±6.01
0.45	0.71	-10.07± 0.98	-21.81± 3.18	0.45	-10.20±1.00	-21.55±3.26	0.53	-9.31±2.51	-18.87±8.16
0.5	0.71	-9.64± 0.85	-20.41± 2.77	0.45	-9.72±1.00	-20.02±3.25	0.53	-8.55±2.62	-16.38±8.50
0.55	0.71	-9.32± 0.56	-19.35± 1.83	0.45	-9.40±1.03	-19.01±3.36	0.54	-8.31±1.59	-15.59±5.16
0.6	0.71	-9.01± 0.33	-18.31± 1.06	0.45	-8.88±1.08	-17.30±3.50	0.54	-7.98±1.80	-14.50±5.85
0.65	0.71	-8.60± 0.22	-16.97± 0.71	0.45	-8.49±1.08	-16.06±3.51	0.54	-7.76±2.75	-13.77±8.94
0.7	0.71	-8.18± 0.47	-15.60± 1.53	0.45	-8.17±1.09	-15.07±3.55	n/a	n/a	n/a

Table 4: Monitored and calculated pressures for Zorbax-SB-CN at 25°C at the investigated flow-rates. Pressures are shown in bar, while flow-rate is in mL/min. The shortened indices are: det.disc.=detector disconnected, col.disc.=column dicsonnected.

Flow-rate	p_{system}	$p_{\text{det.disc.}}$	$p_{\text{col.disc.}}$	p_{out}	p_{in}	p_{avg}
0.10	51	42	26	9	25	17
0.15	78	65	40	13	38	26
0.20	106	87	55	19	51	35
0.25	133	110	68	23	65	44
0.30	158	132	83	26	75	51
0.35	184	155	97	29	87	58
0.40	208	178	112	30	96	63
0.45	232	200	126	32	106	69
0.50	256	223	141	33	115	74
0.55	281	245	156	36	125	81
0.60	305	267	170	38	135	86
0.65	329	289	184	40	145	92
0.70	351	311	199	40	152	96

Figure captions

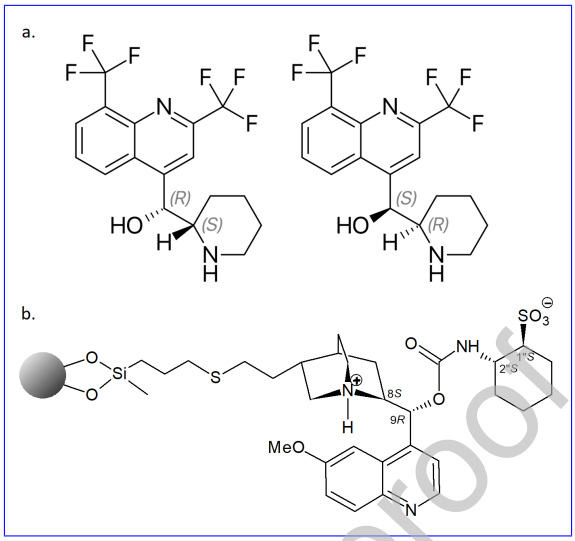

Figure 1: a. Molecular formula of mefloquine enantiomers. b. Molecular structure of ZWIX(+) selector.

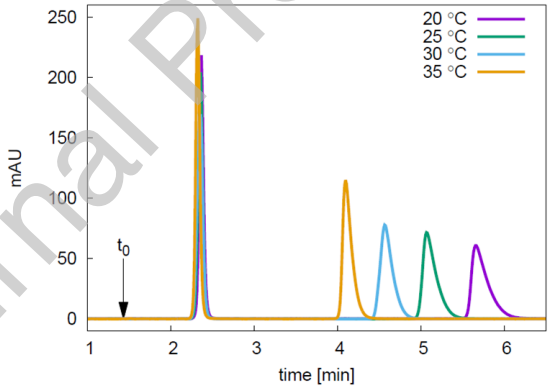

Figure 2: Chromatograms of mefloquine enantiomers recorded at different temperatures on a ZWIX(+) column.

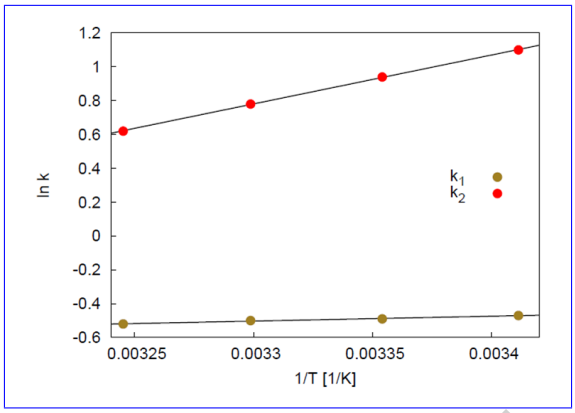

Figure 3: van 't Hoff ~~analysis~~ plots of mefloquine enantiomers recorded at different temperatures on a ZWIX(+) column.

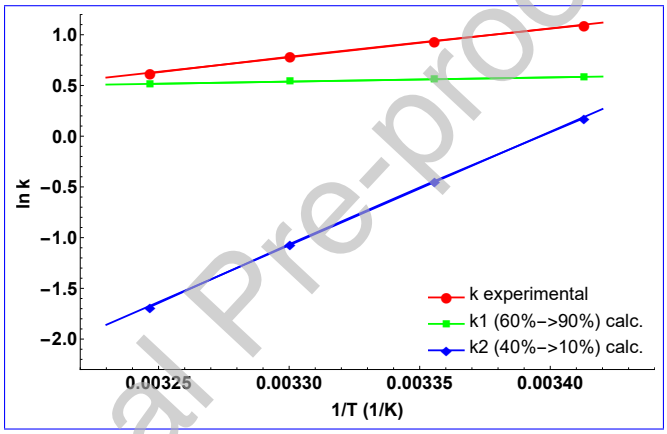

Figure 4: Example of theoretical partitioning the retention factor data. Red line shows the retention factors of the more retained ~~mefloquin~~ mefloquine enantiomer used for the calculations while the blue and green lines are the arbitrary fractions of the experimental data.

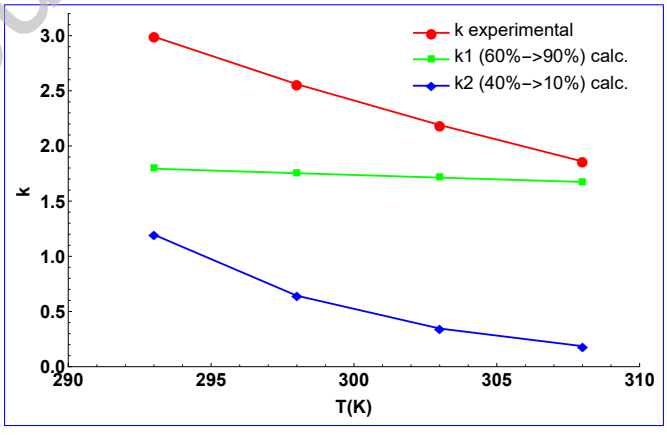

Figure 5: Plots of the partitioned and original retention factors shown in Fig. 4 used for the calculations.

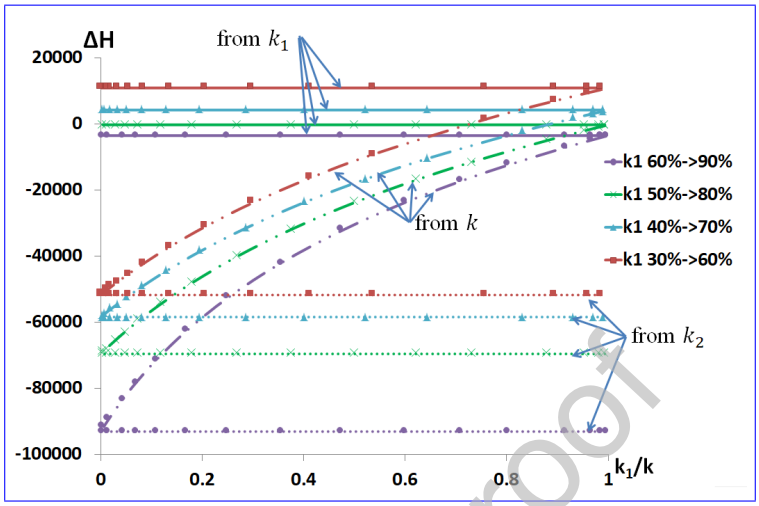

Figure 6: The calculated enthalpy changes when two adsorption sites are assumed and the retention factor is partitioned. Colors indicate different partitioning procedures shown in the legend. The retention factor from which the calculation was made (k_1, k_2 and k) is indicated with arrows.

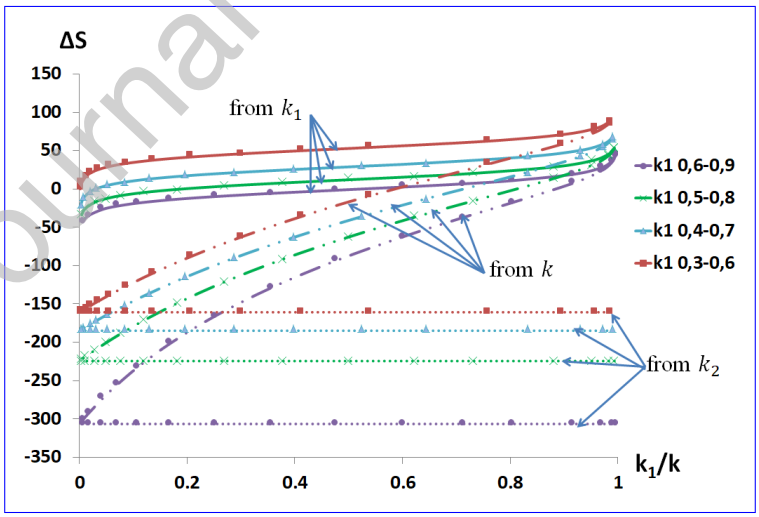

Figure 7: Calculated entropy changes when two adsorption sites are assumed and the retention factor is partitioned. Colors indicate different partitioning procedures shown in the legend. The retention factor from which the calculation was made (k_1, k_2 and k) is indicated with arrows.

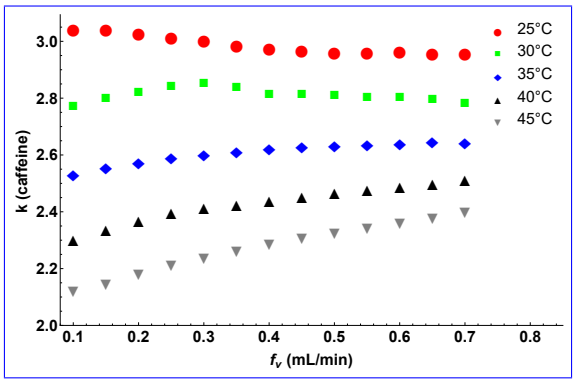

Figure 8: Dependence of the retention factor (k) on the flow-rate (F_V) on the Zorbax Eclipse Plus C18 column at 5 temperatures.

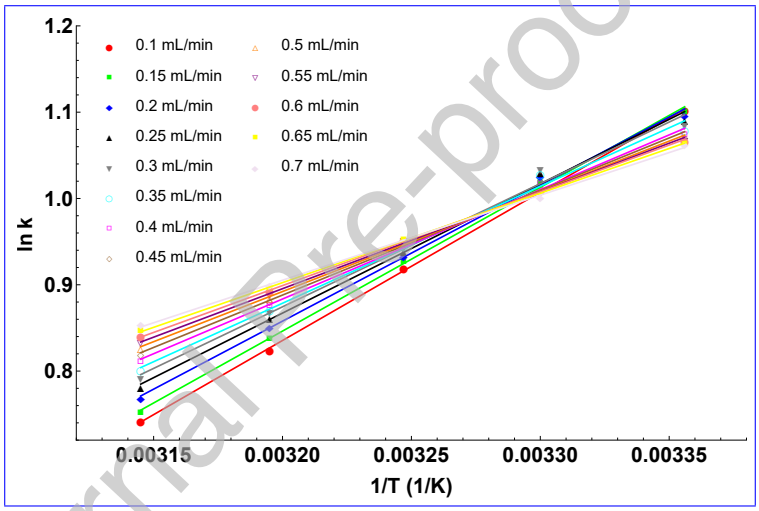

Figure 9: van 't Hoff plots measured at various flow-rates on the Zorbax Eclipse Plus C18 column.

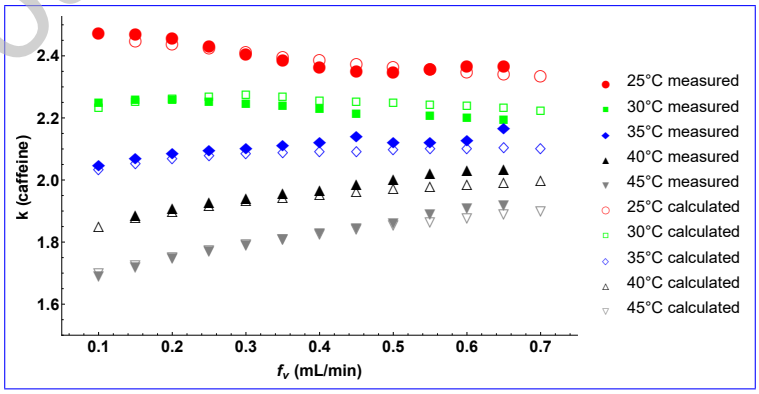

Figure 10: Dependence of the measured and calculated (Eq. 4) retention factors (k) at various flow-rates (F_V) on serially connected Zorbax C18 and Zorbax SB-CN columns at 5 temperatures.

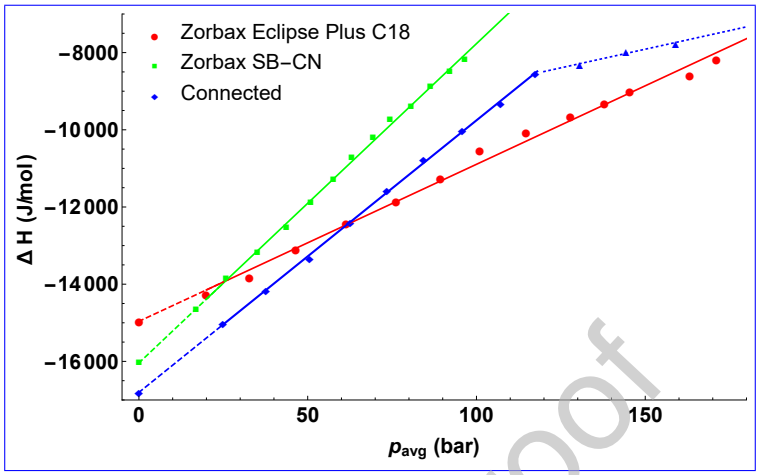

Figure 11: Dependence of the ~~ΔH~~ $\Delta H°$ values calculated via the van 't Hoff plot on the average pressure drop along the columns for the two single columns (Zorbax Eclipse Plus C18 and Zorbax SB-CN) and when the same columns are serially connected.

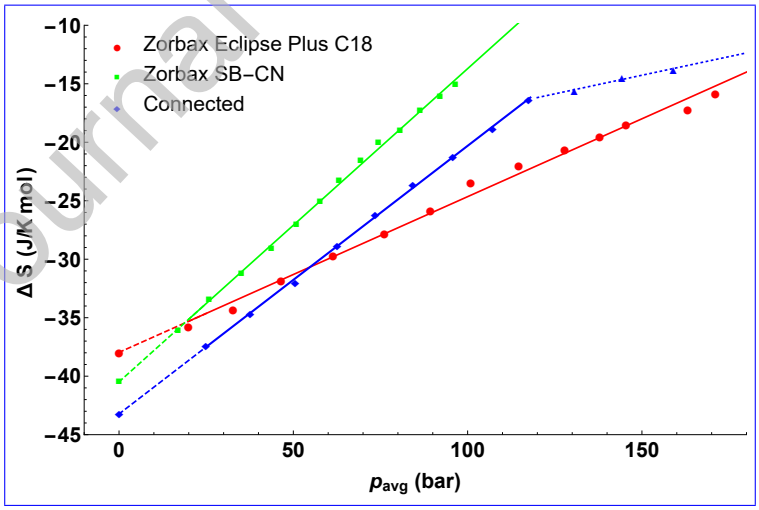

Figure 12: Dependence of the ~~ΔS~~ $\Delta S°$ values calculated via the van 't Hoff plot on the average pressure drop along the columns for the two single columns (Zorbax Eclipse Plus C18 and Zorbax SB-CN) and when the same columns are serially connected.