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Characterisation of reversed-phase stationary phases for the liquid chromatographic analysis of basic pharmaceuticals by thermodynamic data

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Abstract

This paper describes the characterisation of reversed-phase liquid chromatography (RPLC) columns using thermodynamic measurements. Retention versus $1/T$ data were used to construct Van't Hoff plots. The slope of these plots indicates the standard enthalpy of transfer of the analyte from the mobile to the stationary phase. The standard entropy can be calculated from the intercept. Van't Hoff plots were linear for the investigated RPLC columns, meaning that for basic analytes over the temperature range studied no changes in the retention mechanism occurred. Enthalpies and entropies of transfer of basic analytes from the mobile to the stationary phase revealed information about the types of interaction of protonated and neutral compounds with the stationary phases. However, a clear view using the present set of basic compounds on how these thermodynamic data may explain the observed substantial differences in peak symmetry cannot be given. It is considered that addition of *N*,*N*-dimethyloctylamine (DMOA) to the eluent will results in a dynamically coating of the stationary phase. Addition of DMOA to the eluent resulted for protonated basic compounds in a reduction of both enthalpy and entropy. In practice, with DMOA in the eluent symmetrical peaks were obtained. It is assumed that this is due to blocking residual silanols and/or ion exclusion effects.

Keywords: Stationary phases, LC; Thermodynamic parameters; Basic drugs; *N*,*N*-Dimethyloctylamine

1. Introduction

Test methods for column characterisation can be divided into three groups: (i): empirical test methods, (ii): thermodynamically based test methods and (iii): test methods based on a retention model [1]. This paper describes the characterisation of reversed-phase liquid chromatography (RPLC) columns for the analysis of basic pharmaceuticals using a thermodynamically based method. For analytes enthalpies and entropies of transfer from the mobile to the

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stationary phase can be calculated from retention data by evaluation of the Van't Hoff plots [2].

As a theoretical basis for the Van't Hoff plots the retention factor is expressed in terms of standard enthalpies and entropies of transfer from mobile to stationary phase. The relation between the logarithm of the retention factor (ln k) and enthalpies and entropies equals:

$$\ln k = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \quad (1)$$

where k is the measured retention value, ΔH^0 the enthalpy, ΔS^0 the entropy, T the absolute temperature, R the gas constant and ϕ the phase ratio of the column. Enthalpy (ΔH^0) represents the measure of energy exchange in a system and entropy (ΔS^0) represents the chaos of a system. Van't Hoff plots are obtained by regressing ln k vs. $1/T$. Such plots are linear if ΔH^0 and ΔS^0 are independent of the temperature. The slope of the Van't Hoff plot indicates the standard enthalpies of transfer. The standard entropies of transfer of the analyte from the mobile to the stationary phase are calculated from the intercept. Non-linear Van't Hoff plots are indicative for a change of retention mechanism: e.g., ΔH^0 is not constant over the whole temperature range studied [2].

Stationary phases are compared by studying the changes in mechanism of retention of test analytes with changing column temperature. Examples are the studies of Dorsey and co-workers [3,4] who examined the influence of bonding density of stationary phases on the retention of nonpolar solutes. They found that partition, rather than adsorption, was found to be the relevant model of retention for non-polar compounds. The columns that were compared differed in bonding densities ranging from 1.60 to 4.07 $\mu mol/m^2$. The entropic contribution to retention becomes more significant with respect to the enthalpy contribution as the stationary bonding density is increased. Using benzene as a test compound for columns with a bonding density ≥ 2.84 $\mu mol/m^2$ non-linear Van't Hoff plots were obtained. Other examples are the studies of Purcell et al. [5] using Van't Hoff plots to study changes in the secondary structure of peptides and the mechanism of interaction with hydrophobic surfaces, and Philipsen et al. [6] using Van't Hoff plots to study the retention of polystyrene and polyester oligomers. For basic solutes, retention is considered to be a combination of hydrophobic and ionic interaction [7]. Non-linear Van't Hoff plots might be indicative of a change of retention mechanism [2,6,8]. For basic compounds this means that the ratio of the hydrophobic and ionic interaction could change.

This paper shows the comparison of RPLC stationary phases for the analysis of basic compounds using a thermodynamically based test method. Van't Hoff plots as well as standard enthalpies and entropies of transfer of neutral and basic analytes from the mobile to the stationary phase were determined for six RPLC columns.

2. Experimental

2.1. Apparatus

The high-performance liquid chromatography (HPLC) experiments were performed using a HP1100 liquid chromatograph consisting of a quaternary pump, a solvent degasser, an autosampler, a column oven and a diode array detector (Agilent Technologies, Amstelveen, The Netherlands). LC–UV chromatograms were collected using a HPLC 3^D Chemstation (Agilent Technologies).

The determinations of eluent pH were carried out using a Methrom 713 pH meter, Metrohm (Herisau, Switzerland) and a combined glass electrode Hamilton (Bonaduz, Switzerland). The pH meter was calibrated using buffers of pH 4.00, 7.00 and 9.00. The titrations of the eluent were performed using a Metrohm 670 Titriprocessor.

2.2. Chemicals

Org 2447, Org 2463 and Org 10490 were obtained from N.V. Organon (Oss, The Netherlands). In Fig. 1 the molecular structures and related pK_a values are shown. The other compounds were obtained from various manufacturers and are of analytical-reagent grade quality. As organic modifier methanol (MeOH), supplied by J.T. Baker (Deventer, The Netherlands) was used. For the preparation of the electrolyte solutions acetic acid (99–100%) and

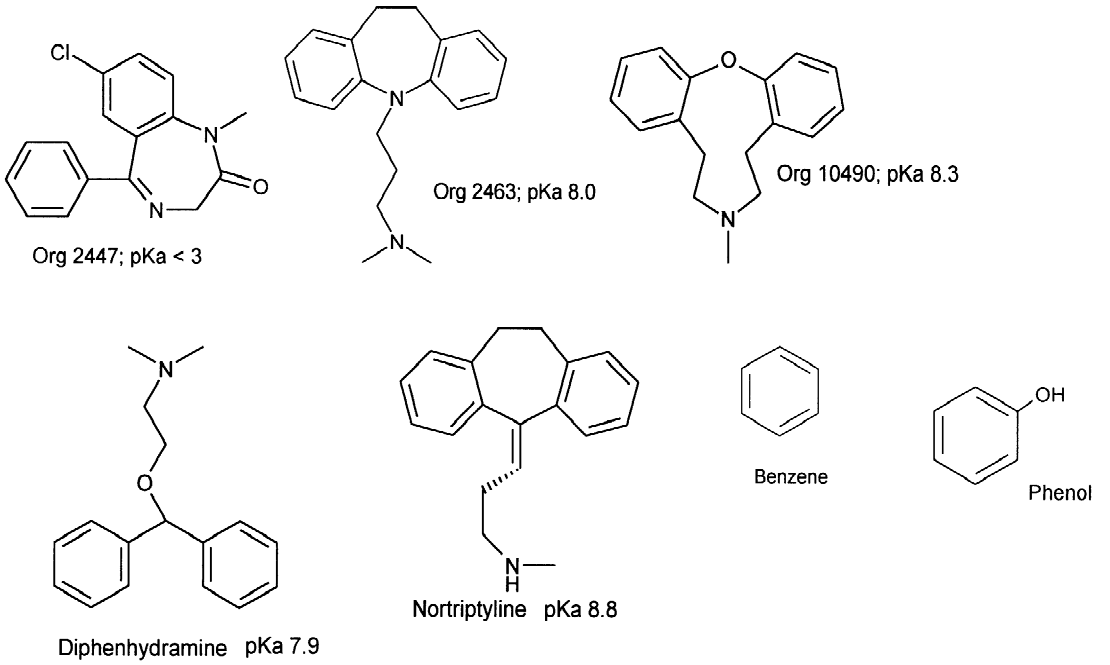

Fig. 1. Molecular structures of the test compounds used in this study. The pK_a values of Org 2447, Org 2463 and Org 10490 were measured in MeOH–water (60:40, v/v). The pK_a values of diphenhydramine and nortriptyline were measured in water.

ammonium hydroxide, supplied by J.T. Baker and ammonium acetate from Janssen (Geel, Belgium) were used. The mobile phase electrolyte solutions were prepared by titration of an electrolyte solution with an equimolar electrolyte solution with different pH, until the desired pH value was reached. E.g., 25 m*M* ammonium acetate, pH 7 was obtained by titration of 25 m*M* ammonium acetate with 25 m*M* ammonium hydroxide until pH 7.

To avoid overloading of the stationary phases by basic compounds, as recently discussed [9], amounts of 0.1 μg of each test compound were injected onto the column. As marker for the unretained peak the first deviation of the baseline after sample injection was taken. The flow-rate was set to 1.0 ml/min. The investigated stationary phases are shown in Table 1.

3. Results and discussion

Enthalpy and entropy of transfer of basic analytes from the mobile to the stationary phase using the columns in Table 1 were determined to study possible differences in the retention process between the RPLC columns. From Van't Hoff plots enthalpies and entropies were calculated [2,3]. Particularly at higher temperatures limited lifetimes of columns can be expected [10]. Therefore Van't Hoff plots for only

Table 1
Investigated stationary phases; dimensions are 150×4.6 mm I.D

Stationary phase	Manufacturer	Abbreviation	Bonding characteristics
Symmetry Shield C_{18}	Waters	SYSH	Embedded polar group
Symmetry C_{18}	Waters	SYMM	High-purity silica
Zorbax SB-C_{18}	Agilent Technologies	ZOSB	Sterically protected
Zorbax Extend-C_{18}	Agilent Technologies	ZOBI	Bidentate bonded alkyl chains
Alltima C_{18}	Alltech	ALLT	Polymeric bonded phase,
Luna C_{18}	Phenomenex	LUNA	High-purity silica

one eluent (MeOH–25 m*M* NH_4Ac, 50:50, v/v) at electrolyte solution, pH values 3 and 7) were determined in the temperature range 10 to 80 °C. Since the ammonium acetate solutions were not used at the optimal buffering pH first aspects related to the eluent properties will be discussed.

3.1. Eluent properties

Although it is known that the pH of the mobile phase changes after addition of methanol and acetonitrile, today it still common practice to measure the pH before mixing with the organic modifier. For organic modifier–buffer mixtures the pH can be calculated using measurements with conventional pH electrodes:

$$^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{pH} - \delta \quad (2)$$

where $^{s}_{s}$pH is the pH value in an aqueous–organic system, $^{s}_{w}$pH is the measured (apparent) value in an aqueous–organic system and δ is a correction factor for the liquid junction between the electrode and the eluent. For eluents containing 50% (v/v) methanol the correction factor (δ) is approx. 0.1 [15].

Fig. 2 shows the relation between the pH of the pure electrolyte solution and the pH of the methanol–25 m*M* ammonium acetate (50:50, v/v) solutions from pH 3 up to pH 7. As can be seen, addition of methanol to 25 m*M* ammonium acetate resulted in an increased eluent pH. In addition, in the pH range studied the relation between the pH of the electrolyte solution and the pH of the methanol–25 m*M* ammonium acetate (50:50, v/v) solutions is linear ($r = 0.98$). From these data it is obvious that for eluents containing 50% (v/v) methanol and prepared from 25 m*M* ammonium acetate solutions pH 3 and 7 the $^{s}_{w}$pH values are 3.8 and 7.5.

Fig. 3 shows the buffering range of a MeOH–25 m*M* ammonium acetate (50:50, v/v) solution. The solution was titrated with a 0.5 *M* HCl solution. As can be seen the $^{s}_{w}$pH values 3.8 and 7.5 are outside the buffering range of the eluent. However, since small amounts of analyte were injected onto the column (0.1 μg), $^{s}_{w}$pH will not change upon injection of analyte into the eluent. In addition, taking into account the effect of modifier concentration on the pK_a values of basic analytes [12] it is obvious that at $^{s}_{w}$pH 3.8 the basic compounds are protonated, whereas at $^{s}_{w}$pH 7.5 these compounds are not or partly

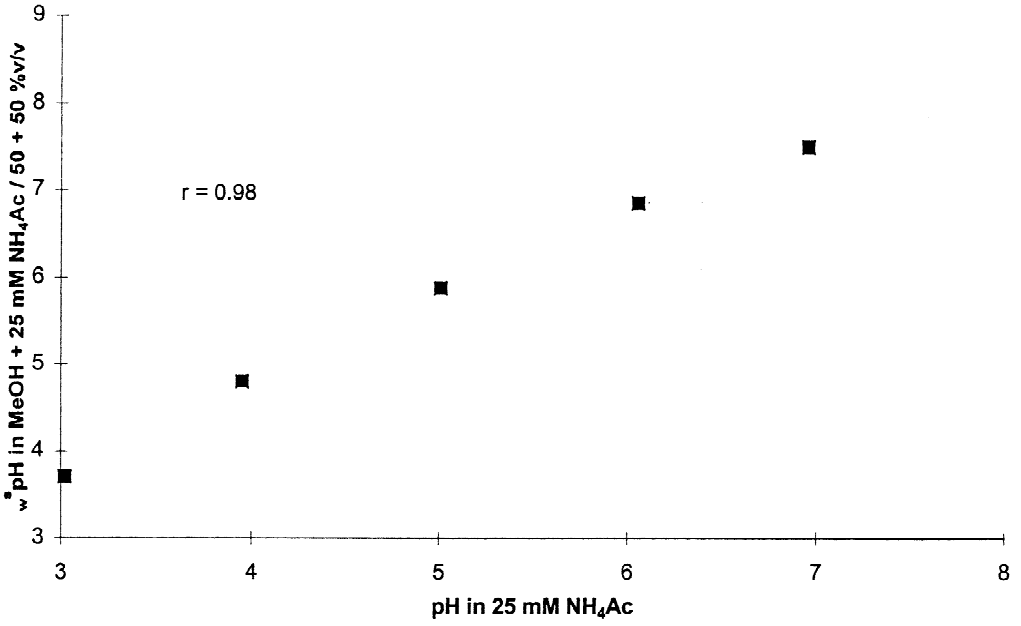

Fig. 2. Relation between pH of 25 m*M* NH_4Ac and $^{s}_{w}$pH of MeOH–25 m*M* NH_4Ac (50:50, v/v).

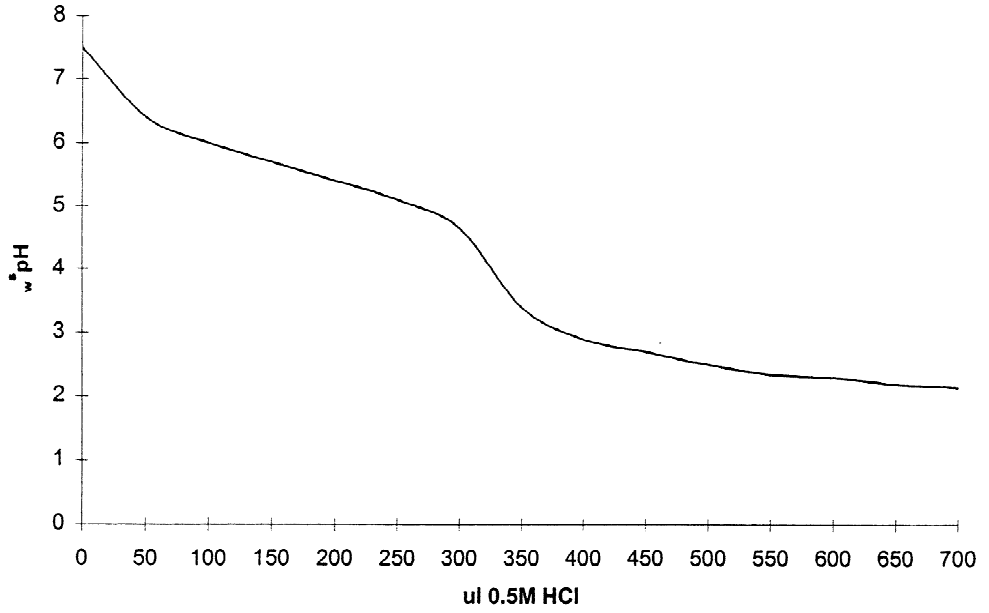

Fig. 3. Buffering range of an MeOH–25 m*M* NH_4Ac (50:50, v/v) solution.

protonated. This is also confirmed by the obtained retention data. Using the eluent at $^{s}_{w}$pH 7.5 the basic analytes are significantly more retained compared to using the eluent at $^{s}_{w}$pH 3.8.

3.2. Linearity of the Van't Hoff plots

The Van't Hoff plots showed no significant deviation from linearity ($r \geq 0.995$). Therefore it was concluded that over the studied temperature range no changes in the mixed retention mechanism for basic compounds occurred. As an example the Van't Hoff plots for Org 2463 and diphenhydramine obtained with $^{s}_{w}$pH 3.8 and $^{s}_{w}$pH 7.5 eluents are shown in Fig. 4. Comparable data were obtained for the other compounds.

Recently McCalley reported Van't Hoff plots of basic and neutral compounds obtained on an Inertsil ODS-3V column [11]. The eluent consisted of acetonitrile–phosphate buffers pH 3 and 7. The Van't Hoff plots for the neutral and basic compounds were linear, which is in agreement with the results observed in our study. For the neutral analyte benzene ΔH^0 values of -1.93 and -2.05 kcal/mol were found. This is comparable with our data where ΔH^0 values for benzene on the six RPLC columns were between -2.34 and -2.80 kcal/mol. Depending on the pH of the buffer and the nature of the analyte positive and negative slopes of the Van't Hoff plots were obtained by McCalley. For the six RPLC columns in our study however, only negative ΔH^0 values were obtained. We therefore assume that the nature of the buffered eluent substantially influences magnitude and sign of ΔH^0 values, and thus the mechanism of retention.

3.3. Enthalpy and entropy of transfer of the analyte from the mobile to the stationary phase

Table 2 shows the enthalpy data for the neutral and basic test compounds. The eluent conditions were the same for the whole set of columns, meaning the values for the various compounds reflect the differences between the stationary phases. In all cases ΔH^0 values were negative under the experimental conditions, demonstrating that retention of basic analytes is an exothermic process. It is energetically more favourable for the analytes to remain in the stationary phase than in the mobile phase. The value of ΔH^0 reflects the degree of interaction between analyte and stationary phase and a more negative ΔH^0 indicates a higher degree of

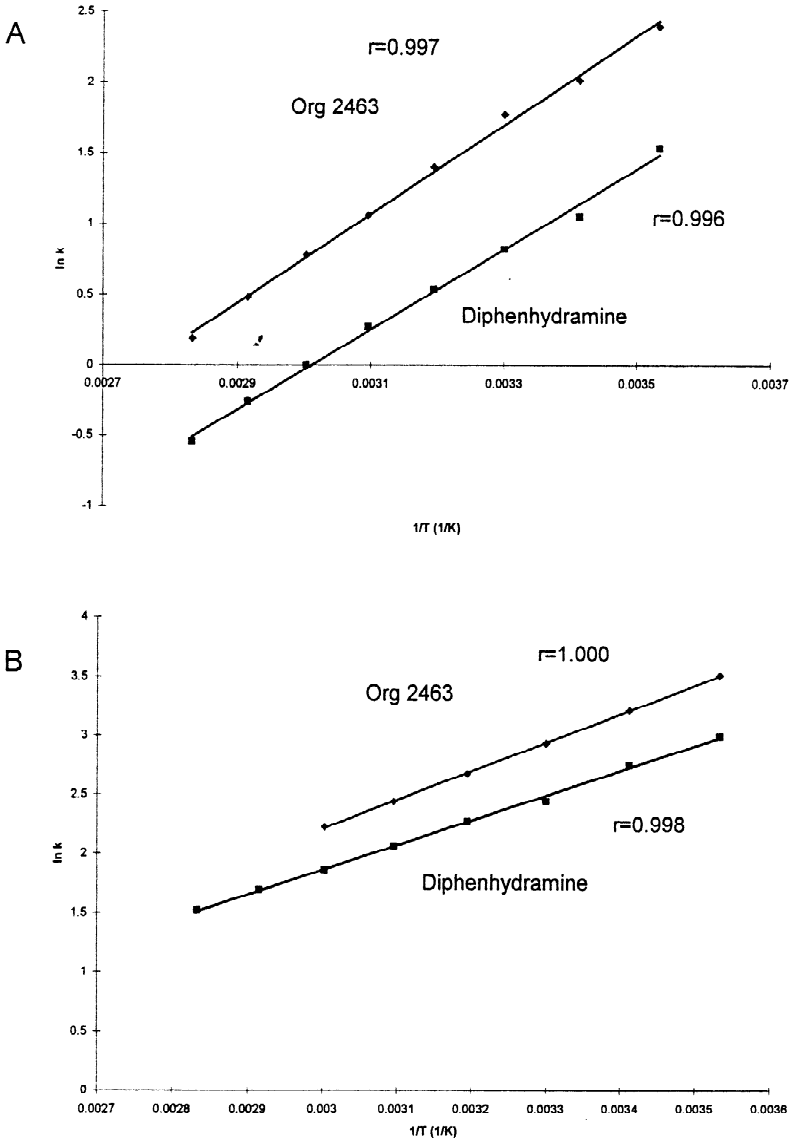

Fig. 4. Typical example of Van't Hoff plots for Org 2463 and diphenhydramine using MeOH–25 m*M* NH_4Ac (50:50, v/v) at electrolyte solution pH 3 (A) and 7 (B).

interaction. For the neutral compounds, i.e., Org 2447, benzene and phenol, the ΔH^0 values were comparable for both buffers pH 3 and pH 7. Typically for the latter compounds on all columns ΔH^0 values between -10 and -15 kJ/mol were obtained. For the strong basic Org 2463 and nortryptiline, however, significant differences in ΔH^0 values were obtained for the electrolyte solutions of pH 3 and pH 7 on the LUNA, SYSH and SYM*M* columns. For the latter columns the ΔH^0 values obtained using buffer, pH 3 (protonated compounds) were significantly more negative, compared to the ΔH^0 values obtained using buffer pH 7 (compounds are not or partly protonated). Moreover, the enthalpy

Table 2
ΔH^0 (kJ/mol) of selected test compounds using MeOH–25 m*M* NH_4Ac (50:50, v/v) as eluent

Compound	SYSH		SYMM		ZOSB		ZOBI		ALLT		LUNA	
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
Diphenhydramine	[a]	−10.1	[a]	−13.8	−23.7	−17.4	−26.2	−12.3	−21.2	−17.4	[a]	−9.1
Nortryptilline	−32.5	−19.2	−33.3	−22.2	−27.7	−24.2	−28.0	−23.3	−24.4	−24.5	−35.3	−21.0
Org 2447	−12.9	−13.8	−13.9	−14.3	−15.9	−15.9	−14.9	−15.6	−15.2	−15.2	−14.6	−15.7
Org 2463	[a]	−14.8	−35.9	−19.9	−26.6	−42.8	−27.3	−17.7	−25.0	−23.7	−41.9	−14.4
Org 10490	[a]	−8.3	[a]	−14.1	−20.9	−17.2	[a]	−10.1	−20.5	−19.1	[a]	−7.1
Benzene	−10.1	−9.8	−9.8	−11.7	−10.1	−9.8	−11.1	−11.6	−9.8	−10.1	−10.4	−10.4
Phenol	−11.1	−10.8	−10.3	−10.4	−10.4	−11.7	−13.3	−11.8	−11.7	−10.4	−10.6	−11.1

[a] Insufficient data to perform linear regression.

values at low pH were significantly more negative for protonated compounds than for neutral compounds like Org 2447.

For protonated compounds both ionic and hydrophobic interactions with the stationary phase will occur. For non-protonated compounds mainly hydrophobic interactions will take place. Combining the results from Table 2 and Ref. [12] obviously the differences in interaction between protonated compounds (pH 3) compared to non or partly protonated compounds (pH 7) with the stationary phase is larger for columns generally showing good peak shapes. This is particularly true for the SYSH, SYMM and LUNA columns. With the ALLT column, which with respect to peak asymmetry was the ''worst'' column, this effect was not observed. For this latter column comparable ΔH^0 values were obtained with the electrolyte solutions of pH 3 and 7. In addition the ΔH^0 values of the ALLT column are of the same order of magnitude as the data of pH 7 on the other columns. As can be seen in Ref. [12] the best peak shapes were obtained on the LUNA, SYSH and SYMM columns. Asymmetrical peaks were obtained using the ALLT column, for both electrolyte solutions of pH 3 and 7, which is taken as indicative for more ionic interactions between the basic analytes and this column. Therefore, one would expect lower ΔH^0 values (more negative) for the ALLT column compared to the LUNA, SYMM and SYSH columns. However, the present data suggest that peak symmetry improves at larger ionic interaction between basic solutes and the stationary phase.

In Table 3 the intercepts from the Van't Hoff plots, representing entropy plus the column phase ratio are presented. For RPLC columns phase ratios (ϕ) are different and difficult to obtain. Column manufacturers as a part of their quality control do not determine phase ratios. Therefore, in contrast to ΔH^0 values the absolute entropy data between the various columns cannot be directly compared. ΔS^0 is a measure of

Table 3
$\Delta S^0/R + \ln \phi$ values of selected test compounds using MeOH–25 m*M* NH_4Ac (50:50, v/v)

Compound	SYSH		STMM		ZOSB		ZOBI		ALLT		LUNA	
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
Diphenhydramine	[a]	−2.46	[a]	−3.33	−4.06	−4.42	−10.68	−2.94	−6.91	−4.17	[a]	−1.66
Nortryptilline	−14.11	−5.17	−13.60	−6.01	−8.83	−6.34	−9.95	−6.51	−7.05	−6.35	−16.76	−5.59
Org 2447	−2.63	−2.99	−2.67	−2.79	−3.48	−3.51	−3.22	−3.40	−2.75	−2.95	−2.78	−3.21
Org 2463	[a]	−3.33	−15.09	−4.72	−8.67	−5.59	−10.11	−4.13	−7.59	−5.80	−16.98	−2.80
Org 10490	[a]	−2.21	[a]	−3.88	−7.98	−4.68	[a]	−2.62	−7.16	−5.05	[a]	−1.44
Benzene	−2.29	−2.23	−1.95	−1.98	−2.37	−2.28	−2.52	−2.72	−1.89	−1.95	−2.18	−2.23
Phenol	−3.76	−3.71	−3.70	−3.81	−4.06	−3.91	−4.30	−4.48	−3.47	−3.57	−3.77	−3.98

[a] Insufficient data to perform linear regression.

ordering of the system, i.e., ordering of the stationary phase chains after bonding the analyte. For a single column R and ln ϕ are constants. Therefore the values in Table 3 for a single column can be considered as a measure for ΔS^0 and differences between ΔS^0 values obtained at buffer, pH 3 and pH 7 can be studied. Again, with all columns for the neutral compounds Org 2447, benzene and phenol minor differences between the ΔS^0 values, obtained with electrolyte solutions of pH 3 and 7, were observed. However, comparing the ΔS^0 values of benzene and phenol showed that for the more polar compound phenol ΔS^0 is slightly more negative in all cases.

For the basic compounds, comparison of the differences in ΔS^0 for each individual column at both pH values shows a certain similarity with the earlier discussed ΔH^0 values. Again columns producing better symmetrical peaks show a larger difference between their ΔS^0 values at pH 7 and 3, compared to columns producing severe tailing peaks. For example $\Delta\Delta S^0$ ($\Delta S^0_{\mathrm{pH\ 3}} - \Delta S^0_{\mathrm{pH\ 7}}$) values for nortryptiline follow the order: ALLT > ZOBI > ZOSB > SYMM + SYSH > LUNA. This is in fair agreement with column ordering according to the asymmetry values obtained at pH 7 for nortryptiline: ALLT > ZOBI > SYMM > ZOSB > SYSH > LUNA [12].

To investigate whether the discussed differences were due to ionic interactions, the silanol blocking compound *N*,*N*-dimethyloctylamine (DMOA) was added to the pH 3 eluent. 0.1% (v/v) DMOA [13] was added to the electrolyte solution of the eluent, which resulted in symmetrical peaks with both the LUNA and ALLT columns. Enthalpy and entropy values were determined for Org 2463, nortryptiline and Org 2447. Table 4 shows ΔH^0 and ΔS^0 values on the columns, with and without the addition of DMOA to the eluent. The addition of DMOA can be considered as a dynamically coating of RPLC columns. It is assumed that addition of this latter reagent result in a dynamically modified more densely bonded RPLC phase. This will result in a further ordering of the system. For the neutral compound (Org 2447) ΔS^0 values for both columns increase (more negative) slightly but significantly. This is taken as proof for better ordering of the system upon DMOA addition.

As for the neutral compounds, it was expected that addition of DMOA for protonated basic compounds would also result in a better ordering of the system. However, addition of DMOA to the eluent resulted for both the LUNA and the ALLT columns in a reduction (less negative) of both ΔH^0 and ΔS^0 for protonated basic compounds. This is interpreted that for protonated basic compounds it is energetically less favourable to remain in the stationary phase, after dynamically coating of the stationary phase with DMOA resulting in less negative ΔH^0 values. Without DMOA protonated basic compounds can interact with ionic sites and alkyl chains of the stationary phase. However, after addition of DMOA residual silanols can be blocked and/or ion exclusion effects can take place and only interaction with alkyl

Table 4
ΔH^0 and ΔS^0 values obtained using the LUNA and ALLT columns, with and without the addition of DMOA to the MeOH–25 m*M* NH_4Ac, pH 3 (50:50, v/v) eluent

Compound	ΔH^0(kJ/mol)			
	LUNA		ALLT	
	Without DMOA	With DMOA	Without DMOA	With DMOA
Org 2447	−14.6	−16.1	−15.2	−15.9
Org 2463	−41.9	−18.2	−25.0	−14.9
Nortryptiline	−35.3	−19.2	−24.5	−16.2
	$\Delta S^0/R + \ln \phi$			
Org 2447	−2.78	−3.40	−2.75	−3.21
Org 2463	−16.98	−6.55	−7.59	−5.03
Nortryptiline	−16.76	−6.57	−7.05	−5.18

chains occur. As a consequence, analytes may increase their degrees of freedom. In turn, this will increase the disorder of the system resulting in less negative ΔS^0 values of Org 2463 and nortryptiline.

Summarising all the data above, it is recalled that on the ALLT column asymmetrical peaks were obtained without DMOA. From the peak asymmetry data we expected that, compared to the LUNA column, the ionic activity with the ALLT column would be higher. From ΔH^0 values it is obvious that for protonated basic compounds the total amount of ionic interactions with the LUNA, SYSH and SYMM columns are larger compared to the ZOSB, ZOBI and ALLT columns. To some extend this can be explained by looking at silanol groups present on the stationary phase. The ionic interactions responsible for asymmetrical peaks do not necessarily originate from the whole silanol population. We assume that in the case of the ZOSB, ZOBI and ALLT columns a small part of ''bad silanols'' ($<1\%$ of the total population [14]) maybe responsible for the observed worser peak asymmetry for the latter three columns. Therefore, it is possible that asymmetrical peaks are obtained using columns with few ionic sites and that symmetrical peaks are obtained using columns with considerable amounts of ionic sites present.

From this study it is clear that determination of enthalpies and entropies of transfer from the mobile to the stationary phase for basic analytes reveals information about the types of interaction between protonated and neutral compounds with RPLC stationary phases. A clear view however on how these thermodynamic data may explain the observed substantial differences in peak symmetry cannot be given with the present set of basic compounds.

4. Conclusions

The obtained Van't Hoff plots were linear for the investigated set of RPLC columns ($r>0.99$). Therefore it was concluded that over the studied temperature range no changes in the (mixed) retention mechanism for basic compounds occurred. The negative ΔH^0 values obtained for the analytes showed that retention of basic compounds is an exothermic process under the actual eluent conditions. For strong basic compounds (nortryptiline and Org 2463) significant differences in ΔH^0 and ΔS^0 values were obtained for the electrolyte solutions of pH 3 and pH 7 on the LUNA, SYSH and SYMM columns. For the same compounds this effect was less observed on the ZOSB, ZOBI and ALLT columns. Using the latter columns generally asymmetrical peaks are obtained for these basic compounds. From the ΔH^0 data it is concluded that the total amount of ionic interactions with the LUNA, SYSH and SYMM columns are larger compared to the ZOSB, ZOBI and ALLT columns. It is proposed that in the case of the ZOSB, ZOBI and ALLT columns a small amount of ''bad silanols'' maybe responsible for the observed higher peak asymmetry for the columns.

Addition of DMOA to the eluent resulted for both the LUNA and the ALLT columns in a reduction (less negative) of both ΔH^0 and ΔS^0 for protonated basic compounds. For the neutral compound the differences in ΔS^0 upon addition of DMOA are relatively small but significant. This latter effect can be interpreted that addition of DMOA results in a dynamical modification of the column. In turn this contributes to a better ordering of the system. Furthermore, for protonated compounds it is energetically less favourable to remain in the stationary phase after dynamically coating of the stationary phase with DMOA. The latter effect is probably due to blocking silanols and/or ion exclusion effects. As a consequence, the degree of freedom for the protonated basic analytes nortryptiline and Org 2463 increases. This is reflected by an increase (less negative) of the corresponding ΔS^0 values.

To obtain more insight in the use of Van't Hoff plots for the characterisation of RPLC columns for the analysis of basic compounds further research is necessary. Probably this requires data obtained from many RPLC columns under various eluent conditions using a large set of diverse test compounds.

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