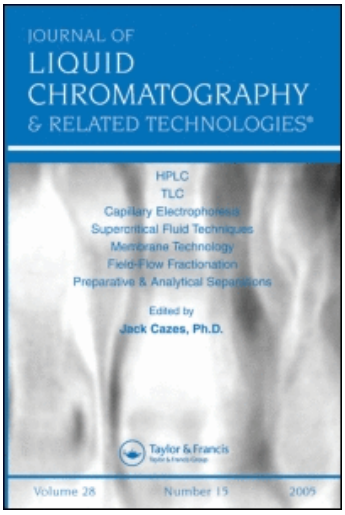

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A NEW METHOD OF STUDYING TEMPERATURE DEPENDENCE AND THE EFFECT OF MOBILE PHASE COMPOSITION ON THE RETENTION MECHANISM IN REVERSED PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

In this work, a rapid procedure to examinate the effect of temperature and eluent composition on thermodynamic properties in high performance liquid chromatography is presented. The use of an experimental design is proposed to study thermodynamic solution property trends for ten Benzodiazepines. Enthalpies and entropies of transfert (mobile to stationary phase) are calculated by evaluation of Van't Hoff plots. For all cases examined enthapies of transfert are negative. These data showed that the entropic contribution to retention becomes more significant as the solvant polarity decreases. Enthalpy-enthropy compensation behavior is tested for varying mobile phase composition.

INTRODUCTION

The mechanism of retention in reversed phase liquid chromatography has been the subject of much debate. The many interactions that a solute may undergo in both the stationary and mobile phases explain the difficulty in elucidating the mechanism of retention. Explanation of solute retention in "reversed phase" separation is described qualitatively through partioning models (1,2,3,4) or by the use the hydrophobic effect (5,6,7). One of the first thermodynamic

investigations was made by Knox and Vasvari (8). Knox plotted lnk' vs $\frac{1}{T}$ for a group of compounds separated on two different columns. The resultant Van't Hoff plots gave absolute enthalpies and relative entropies of transfert for the solute. This method necessitated a great number of experiments. More recently linear Van't Hoff plots have been observed for eight retinoates (9). In this study, the effect of temperature and the nature of the organic modifier were studied. Enthalpies of transfert were studied using the slopes of the Van't Hoff plots, but $\Delta S°$ values were not provided due to an ambiguity in the calculation of the phase ratio of the commercial columns. Our aim in carrying out this research was to show the advantage of using an experimental design which can reduce the number of experiments. With only 13 experiments, thermodynamic solution property trends are examined as a function of eluent composition. The relative effect of entropy and enthalpy on k' is discussed and enthalpy-enthropy compensation behavior is tested as a function of mobile phase composition.

MATERIALS AND METHODS

CHROMATOGRAPHIC CONDITIONS

APPARATUS : The HPLC system consisted of a HPLC Waters pump 501 (Saint Quentin en Yvelines, France), an Interchim rheodyne injection valve Model 7125 (Montluçon, France) fitted with a 20µl sample loop, a Merck L 4000 variable wavelength UV spectrophotometer detector and a Merck D 2500 chromato integrator (Nogent-sur-Marne, France). A Waters 150 mm × 3.9 mm ID. RP 18 column (Nova pak, 5 µm particle size) was used with a controlled temperature in an Interchim crococil oven TM N° 701 (Montluçon, France). Overall temperature control was maintained within ± 1° C with a variation from 26° C to 50° C. The detection wavelength was 254 nm. The flow rate used varied from 0.6 to 1.6 mL/min. The mobile phase was a methanol-water mixture with varied percentages of methanol from 50 % to 80 %. Weaker percentages were not used because of the excessively high column pressure obtained with 50 % of methanol with a flow rate of 1.6 mL/min.

REAGENTS AND SAMPLES : Methanol was HPLC grade determine analytical. (1) Bromazepam (2) Nitrazepam (3) Flunitrazepam (4) Clobazam (5) Lorazepam (6) Oxazepam

(7) Tofisopam (8) Chlordiazepoxide (9) Chlorazepate dipotassic and (10) Diazepam were obtained from HOFFMANN LA ROCHE (Basel, Switzerland). These were diluted in methanol in a concentration range of 10-80 mg/mL.

METHODS

CHEMOMETRIC METHODOLOGY : The traditional approach studies each factor separately to find the influence of mobile phase composition and temperature on the retention mechanism. A chemometric approach is based on the use of matrix experiments which study the simultaneous variation of all factors. This way the number of experiments can be reduced compared with the traditional methods. A mathematical model is used which linked the observed response (Y) and the influencing factor (X). Variables were coded to have a variation from -1 to +1. The experimental quantitative factors included the mobile phase composition, its flow rate and column temperature.

THERMODYNAMIC RELATIONSHIPS : Valuable information concerning the retention mechanism in HPLC may be gained by examining the temperature dependence of retention which is given by the equation :

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \Phi$$

where k' is the capacity factor of the solute

$$k' = \frac{t_R - t_0}{t_0}$$

t_R is the retention time of the compound and t_0 the retention time of an unretained peak such as sodium nitrate. ΔS° is the enthalpy of transfert of the solute from the mobile phase to the stationary phase, ΔH° is the entropy of transfert from the mobile phase to the stationary phase, T is the temperature and Φ the phase ratio (volume of the stationary phase divided by the volume of the mobile phase). Numerical values for the phase ratio may be estimated from the physical constant of the packing material. To estimate the phase ratio, a physical model was used which links the percent carbon loading with other physical properties of the stationary phase (10). It must be noted that ΔH° values are independent of the phase ratio. In addition any uncertainty in the phase ratio affects the ΔS° values equally and thus the ΔS° trends, as a function of the eluent compositions, are unaffected.

RESULTS AND DISCUSSION

The principle of the statistical method is based on a second order polynomial between the capacity factor k' of each compound and the factors studied [mobile phase composition-flow rate-column temperature]. In order to investigate the effect of these factors, a modified Box and Benhken experimental design was used (11). Thirteen experiments were carried out and the capacity factor of each compound was determined. The k' values were then fitted into a second order polynomial.

$$\ln k' = a_0 + a_1 \ln x_1 + a_2 \ln x_2 + a_3 \ln x_3 + a_{12}(\ln x_1)(\ln x_2) + a_{13}(\ln x_1)(\ln x_3) + a_{23}(\ln x_2)(\ln x_3) + a_{11}(\ln x_1)^2 + a_{22}(\ln x_2)^2 + a_{33}(\ln x_2)^2$$

where x_1 is the percentage of methanol in the methanol/water mixture, x_2 the flow rate, x_3 the column temperature and a_0, a_1, a_2, a_3, a_{12}, a_{13}, a_{23}, a_{11}, a_{22}, a_{33} the model coefficients (11). Using this model, seven mobile phase compositions and seven column temperatures lnk' versus these two factors for Flunitrazepam were plotted (Fig. 1). k' decreased when x_1 and x_3 increased. Linear Van't Hoff behavior was obtained for all solutes. The Van't Hoff plot for the solute Flunitrazepam with a mobile phase composition of 50 % of methanol is given in (Fig. 2). The correlation coefficient (r) for the linear fit of this plot was 0.999. Since these data were linear, it was possible to calculate $\Delta H°$ and $\Delta S°$ for this system. $\Delta H°$ was found to be -2.36 kcalmol^{-1} and $\Delta S°$ was calculated to be -2.64 calmol^{-1} K^{-1}. Table 1 contains a complete list of the $\Delta H°$ and $\Delta S°$ values obtained for all the solutes with the different mobile phase compositions. The r values listed for each solute demonstrate the good linearity of these data over the temperature range 25 to 50° C. Three groups of compounds were distinguished according to the variation of $\Delta H°$ versus solvent polarity. In each group, most entropies transfer were negative and values increased with an increased solvent polarity (decreased methanol concentration) (Fig. 3). This phenomenon has been attributed to an ordering of water molecules adjacent to the surface of the solute molecule which is relatively large and non polar. This hydrophobic effect can be described as the tendency of a large and relatively non polar solute to reduce its surface area exposed to water either through association with other relatively large and non polar molecules or through removal from the solution by adsorption. Thereby, an increase in the mutual association of solute molecules in the mobile phase and this

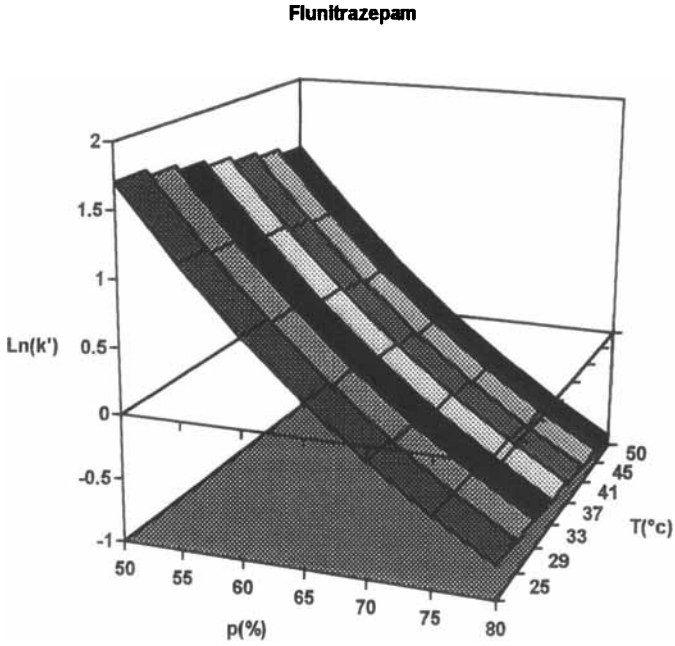

Figure 1 : plot of the logarithme of the capacity factor k' versus percentage of methanol P (%) in the mixture methanol/water and column temperature T(°C) for flunitrazepam.

process of inserting the solute molecule into water would thus explain the observed trend of Δ S° values to positively increase.

In each instance, enthalpies of transfer were negative. It is estimated that maximum variation uncertainty in the calculation ΔH° was inferior or equal to 7 %. For the first group of compounds, when the percentage of methanol in the mixture increased enthalpies of transfer values increased (Fig. 4). When the percentage of methanol increased from 50 % to 80 % ΔH° increased for Bromazepam, Nitrazepam and Flunitrazepam by respectively 28 %, 44 % and 42 % :

Bromazepam < Flunitrazepam < Nitrazepam.

This behavior can be explained since interactions between the solute molecule and a relative *non polar mobile* phase can be expected to be stronger than for solute-mobile phase combinations of dissimilar polarity. For the second group of solutes when the percentage of

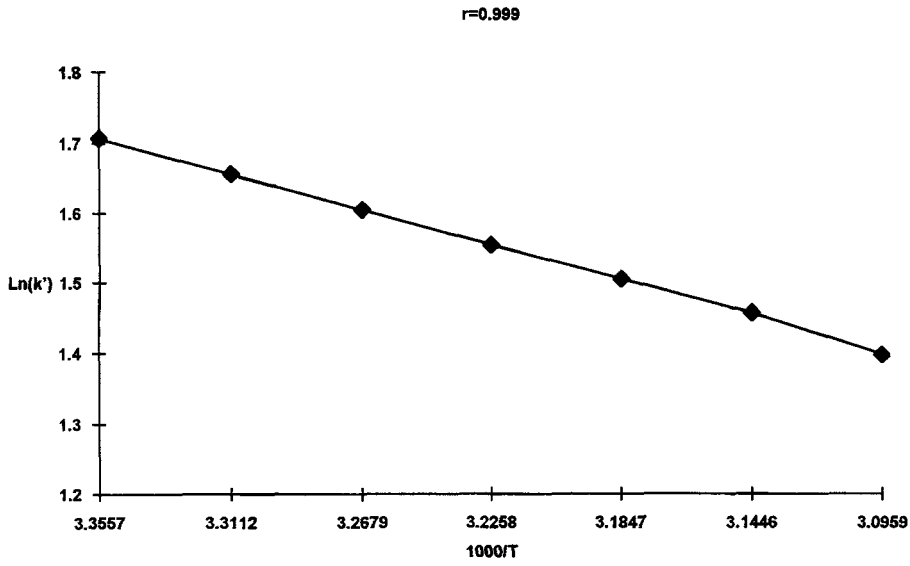

Figure 2 : Van't Hoff plot for the solute flunitrazepam with a mobile phase 50/50 methanol/water.

methanol increased $\Delta H°$ decreased . If this percentage increased from 50 % to 80 %, $\Delta H°$ decreased for Oxazepam and Diazepam by respectively 16 % and 24 % : Oxazepam < Diazepam.

It became energetically more favorable for the solute to be in the stationary phase than in the mobile phase. The small decrease of $\Delta H°$ values with a decrease of solvant polarity was attributed to decreased solute-mobile phase interaction. The third group of compounds was made up of Chlorazepate dipotassic, Chlordiazepoxide, Clobazam, Tofisopam and Lorazepam. For these solute molecules the enthalpy of transfert was constant or its decrease was not significant (≤ 4 %) as the mobile phase composition of methanol was increased. Energetically it is not better for these solutes to be either in the mobile phase or in the stationary phase. For every solute when $\Delta H°$ is compared to $\Delta S°$ over the temperature range studied, the magnitude of $\Delta H°$ was always greater than that of $T\Delta S°$ (Table 2). This indicates, that enthalpy plays a greater role in the transfer of a solute from the mobile phase to the stationary phase and

Table 1 : $\Delta H°$, $\Delta S°$ values for all solutes and for seven percentages of methanol in the methanol/water mixture

	Percentage of methanol											
	50 %			55 %			60 %			65 %		
Compound [a] N°	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/mol/K)	r [b]	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/mol/K)	r	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/mol/K)	r	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (cal/mol/K)	r
1	1.76	1.39	0.997	1.71	2.17	0.999	1.61	2.64	0.999	1.51	3.03	0.998
2	2.63	3.72	0.999	2.39	3.94	0.999	2.18	4.11	0.998	1.98	4.24	0.998
3	2.36	2.65	0.999	2.16	3.06	0.999	1.98	3.39	0.999	1.81	3.65	0.999
4	1.68	0.03	0.998	1.67	1.04	0.998	1.66	1.92	0.998	1.65	2.68	0.998
5	1.97	0.61	0.999	1.97	1.85	0.999	1.98	2.94	0.999	1.97	3.90	0.999
6	1.74	-0.19	0.999	1.82	1.15	0.999	1.88	2.33	0.999	1.94	3.38	0.999
7	1.85	-0.45	0.999	1.84	0.75	0.999	1.83	1.78	0.999	1.83	2.65	0.999
8	2.01	-0.10	0.999	2.01	0.98	0.999	2.01	1.97	0.999	2.01	2.87	0.999
9	2.15	0.11	0.999	2.16	1.30	0.999	2.15	2.37	0.999	2.15	3.32	0.999
10	1.35	-3.08	0.987	1.47	-1.50	0.990	1.56	-0.12	0.992	1.64	1.10	0.993

(continued)

Continuation of Table 1

Compound N°	Percentage of methanol								
	70 %			75 %			80 %		
	- ΔH° (kcal/mol)	- ΔS° (cal/mol/K)	r	- ΔH° (kcal/mol)	- ΔS° (cal/mol/K)	r	- ΔH° (kcal/mol)	- ΔS° (cal/mol/K)	r
1	1.42	3.36	0.998	1.34	3.63	0.998	1.26	3.86	0.997
2	1.80	4.34	0.997	1.62	4.41	0.997	1.46	4.45	0.994
3	1.65	3.86	0.999	1.51	4.03	0.999	1.37	4.16	0.997
4	1.63	3.37	0.998	1.63	3.98	0.998	1.62	4.53	0.997
5	1.97	4.76	0.999	1.97	5.52	0.999	1.97	6.21	0.999
6	1.99	4.32	0.999	2.03	5.17	0.999	2.07	5.93	0.999
7	1.81	3.42	0.999	1.80	4.07	0.999	1.79	4.63	0.999
8	2.01	3.69	0.999	2.00	4.43	0.999	2.00	5.11	0.999
9	2.15	4.18	0.999	2.15	4.95	0.999	2.16	5.65	0.999
10	1.70	2.20	0.994	1.75	3.18	0.994	1.78	4.06	0.995

a : (1) Bromazepam (2) Nitrazepam (3) Flunitrazepam (4) Clobazam (5) Lorazepam (6) Oxazepam

(7) Tofisopam (8) Chlordiazepoxide (9) Chlorazepate dipotassic (10) Diazepam

b : r value for the linear fit of the Van't Hoff plot

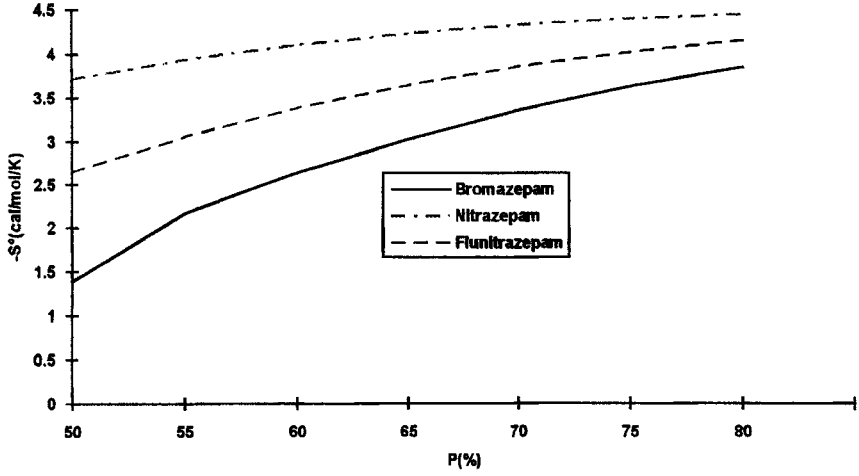

Figure 3 : dependence of the standard entropy change, - ΔS° on the percentage of methanol, P (%) in the mixture methanol/water for the first group of compounds.

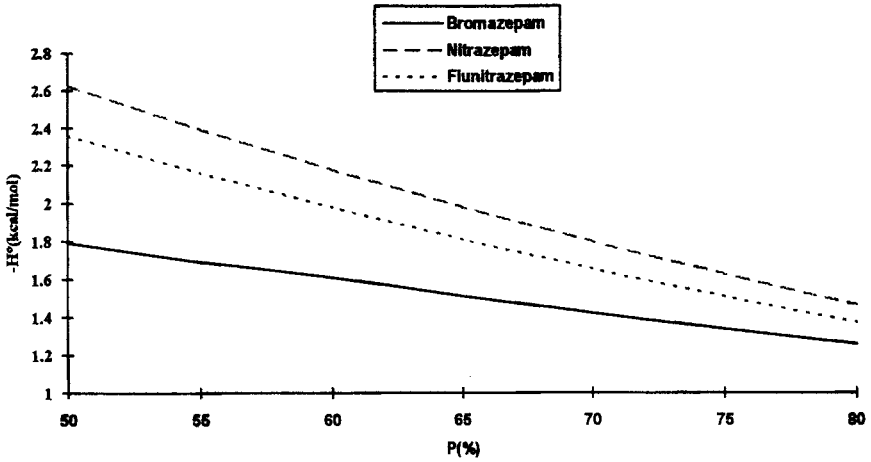

Figure 4 : dependence of the standard enthalpy change, - ΔH° on the percentage of methanol, P (%) in the mixture methanol/water for the first group of compounds.

Table 2 : $\Delta H°$, $\Delta S°$ and $T\Delta S°$ values for all solutes for two different compositions of the mobile phase :

[1] : 50 % of methanol in the methanol/water mixture

[2] : 80 % of methanol in the methanol/water mixture

Compound [a] N°	Percentage of methanol					
	[1]			[2]		
	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol/K)	$T\Delta S°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol/K)	$T\Delta S°$ [b] (kcal/mol)
1	-1.76	-1.39	-0.43	-1.26	-3.86	-1.19
2	-2.63	-3.72	-1.15	-1.46	-4.45	-1.38
3	-2.36	-2.65	-0.82	-1.37	-4.16	-1.29
4	-1.68	-0.03	-0.00	-1.62	-4.53	-1.40
5	-1.97	-0.61	-0.19	-1.97	-6.21	-1.93
6	-1.74	+0.19	+0.06	-2.07	-5.93	-1.84
7	-1.86	+0.45	+0.14	-1.79	-4.63	-1.44
8	-2.01	+0.10	+0.31	-2.00	-5.11	-1.58
9	-2.15	-0.11	-0.34	-2.16	-5.05	-1.75
10	-1.35	+3.08	+0.95	-1.78	-4.06	-1.26

[a] See Table 1

[b] For T = 310 K

therefore in the retention process than does entropy. Nevertheless, it appears that entropy plays an increasing role in retention as the percentage of methanol in the eluent increased (Table 3). It is of interest to study the similarity in the retention mechanism for this family of compounds. Enthaly-entropy compensation (12) is a term used to describe a compensation temperature which is a system that is independant for a class of experimental systems. Enthalpy-entropy

Table 3 : Correlation coefficients of regressions between $\ln k'_T$ and $\Delta H°$ for each compound of the first two groups

Group N°	Compounds	Correlation coefficient r
I	Bromazepam	0.991
	Nitrazepam	0.999
	Flunitrazepam	0.999
II	Oxazepam	0.999
	Diazepam	0.993
III	Tofisopam	For these solutes $\Delta H°$ is constant or its variation with percentage of methanol is not significant
	Clobazam	
	Chlordiazepoxide	
	Chorazepate dipotassic	
	Lorazepam	

compensation has been applied to chromatographic systems to evaluate the retention mechanism (13, 14, 15, 16, 17). The following equation relates the compensation temperature (β) to the capacity factor at temperature T (k'_T)

$$\ln k'_T = \ln k'_0 - \frac{\Delta H°}{R}\left(\frac{1}{T} - \frac{1}{\beta}\right)$$

A plot of lnk' versus (- $\Delta H°/R$) should yield a slope line of $\frac{1}{T} - \frac{1}{\beta}$. The compensation temperature (β) is the temperature at which the studied compound had the same value of k' whatever the mobile phase composition. Compensation "enthalpy-entropy" was obviously tested for the first two groups of solutes when the percentage of methanol varied from 50 % to 80 % at a temperature of 310 K. Table 3 contains a complete list of correlation coefficients r. The high degree of correlation indicates that the retention mechanism for a solute is the same whatever the mobile phase composition.

CONCLUSION

This new approach has enabled us to study the effect of temperature and eluent composition on the retention mechanism of ten benzodiazepines with a limited number of experiments. This procedure meant that 13 experiments instead of 49 were carried out (7 different mobile phase compositions x 7 different temperatures). The Van't Hoff plot curve shape was used to evaluate similitaries and differences between the compound retention mechanism when different mobile phases were used. Results of this work show the strong influence of solvant polarity on thermodynamic properties and this must be considered in order to accurately described the retention process.

REFERENCES

1) Martire D.E., Boehm R.E., J. Phys. Chem., 87, 1045, 1983.

2) Dill K.A., J. Phys. Chem., 91, 1980, 1987.

3) Dill K.A., Naghizadeh J., Marqusee J.A., Annu. Rev. Phys. Chem., 39, 425, 1988.

4) Dorsey J.G., Dill K. A., Chem. Rev., 89, 331, 1989.

5) Horvath C.S., Melander W., Molnar I., J.Chromatogr., 125, 129, 1976.

6) Melander W.R., Horvath C.S., In high performance liquid chromatography, Advances and perspectives, Horvath C.S., Ed., Academic press : New York, 2, 113, 1980.

7) Horvath C.S., Melander W.R., Am Lab., 17, 1978.

8) Knox John H., Vasvari Gabor., J. Chromatogr., 83, 181, 1973.

9) Marja S., Heikki V., Jaoko H., J. Chromatogr., 592, 127, 1992.

10) Sentell K.B., Dorsey J.G., J. Liq. Chromatogr., 11, 1875, 1988.

11) Guillaume Y., Guinchard C., J. Liq. Chromatogr., 16, 3457, 1993.

12) Boots H.M.J., De Bokx P.K., J. Phys. Chem., 93, 8240, 1989.

13) Tchapla A., Heron S., Colin H., Guiochon G., Anal. Chem., 60, 1443, 1988.

14) Yamamoto F.M., Rokushika S., Hatono H., J. Chromatogr. Sci., 27, 704, 1989.

15) Sander L.C., Field L.R., Anal. Chem., 52, 2009, 1980.

16) Kuchar M., Kraus E., Rejholec V., Miller V., J. Chromatogr., 449, 391, 1988.

17) Melander W.R., Campbell D.E., Horvath C.S., J. Chromatogr., 158, 215, 1978.

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