



*Dedicated to Professor Eugen Segal  
on the occasion of his 80<sup>th</sup> anniversary*

## THERMODYNAMIC STUDY OF THE LIQUID CHROMATOGRAPHY RETENTION PROCESS FOR SOME PESTICIDES ON C8 AND C18 SILICA-BASED STATIONARY PHASES

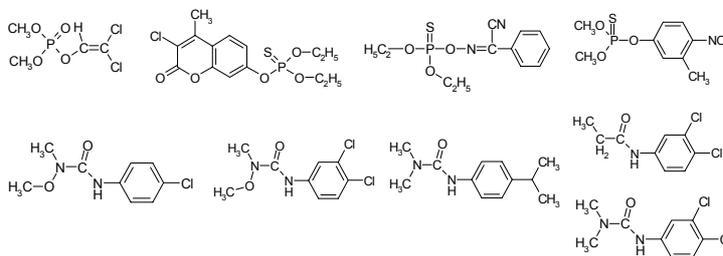
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Thermodynamic standard enthalpy ( $\Delta H^0$ ) and entropy ( $\Delta S^0$ ) of the liquid chromatographic retention process for several pesticide-type compounds by reversed-phase (RP-LC) mechanism were estimated using an octyl- (C8) and octadecyl-silica (C18) based stationary phase. The temperature range used to plot van't Hoff dependences was 20–45°C, which is the usual range for separation in RP-LC. Regression parameters of the linear dependences obtained between retention factor ( $\ln k'$ ) and absolute temperature ( $1/T$ ) together with the phase ratio were used to calculate the above thermodynamic parameters of the chosen compounds. Chromatographic conditions were following: acetonitrile/water as mobile phase composition in isocratic elution; and 1 mL/min as flow-rate. Chromatographic columns were Zorbax Eclipse XDB-C8 or C18 with characteristics: 4.6 mm inner diameter; 150 mm length, and 5 $\mu$ m particle size. The values of standard enthalpy and entropy were situated within the intervals (-12; -3 kJ/mol) and (-1; -26 J/mol K), respectively, which are in good agreement with data reported for other compounds in the literature.



### INTRODUCTION

Most of the liquid-chromatographic processes based on the partition of analyte between mobile and stationary phases<sup>1</sup> are influenced by temperature according to the relationship that describes the dependence of the retention factor  $k'$

the on absolute column temperature ( $T$ ), known as van't Hoff equation:

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

where,  $\Delta H^0$  and  $\Delta S^0$  are the standard enthalpy and entropy changes, respectively, for the transfer of

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the analyte from the mobile to the stationary phase;  $\phi$  is the phase ratio (*i.e.* the ratio of the volume of stationary phase to the volume of mobile phase from the chromatographic column);  $R$  is the universal gas constant ( $8.314 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ ). Since the retention is an exothermic process,  $\Delta H^0$  has negative values. Eq. 1 indicates that for typical separations, the value of  $k$  decreases as the temperature increases. On the other hand, a negative change of entropy is related to the structure of the mobile phase and to the ordering of these compounds due to the hydrophobic interactions with alkyl chains in stationary phase. Their ordering is more regular than interacting with surrounding molecules in the mobile phase. When the stationary phase, the analyte, and the mobile phase properties do not change with a temperature change,  $\Delta H^0$  and  $\Delta S^0$  can be considered temperature invariants.<sup>2,3</sup>

The most encountered situations in reversed-phase liquid chromatography (RP-LC) for temperature dependence of retention are those obeying the linear van't Hoff equation.<sup>4,7</sup> When studying compounds that are involved in structural modifications it is likely that the temperature dependence of their retention factors not to follow this rule.<sup>8-10</sup> For some chiral separations of enantiomers of a conformationally rigid spirolactam or  $\beta$ -blockers on a commercial column packed with a stationary phase derived from (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine the van't Hoff plots were non-linear.<sup>11,12</sup> Deviation from linearity has also been observed for the chiral separation mechanism of difunctional polymer imprinted with dansyl-L-phenylalanine as a stationary phase.<sup>13</sup> Keto-enolic tautomerism resulting in its keto- and

enol-forms of chlortetracycline posed problems in developing an LC-MS method for determining this antibiotic in biological samples. In this case, temperature was used as a major parameter in order to force the equilibrium in favour of the chlortetracycline keto-form.<sup>14</sup> Other deviations from linearity of  $\ln k$  versus  $1/T$  were observed for diphenhydramine, chlorprocaine, quinine, and codeine, but over the normal temperature range used in RP-LC.<sup>8</sup> Retention study of these compounds revealed an increase of  $k$  with the increase of  $T$  when column temperature was varied within 30-130°C. Chromatographic studies in RP-LC showed that compounds involved in tautomeric inter-conversion may have a different retention behavior with temperature: a deviation from the linearity of  $\ln k$  on  $1/T$ , or, on certain temperature ranges, the temperature increase may lead to a retention increase.<sup>15</sup>

The present study is focused on temperature dependences of the retention for nine pesticides, whose structure and hydrophobicity data (represented by the octanol/water partition constant,  $\log K_{ow}$ )<sup>16,17</sup> are given in Table 1. The aim of this study is the estimation of the thermodynamic parameters  $\Delta H^0$  and  $\Delta S^0$  from the van't Hoff plots resulted from chromatographic measurement of the retention factor for several column temperatures. These parameters were estimated for several mobile phase compositions in order to find out the influence of mobile phase composition on the thermodynamic of the partition of the chosen compounds in reversed-phase liquid chromatography.

Table 1

Structure and the identification data on the studied pesticides

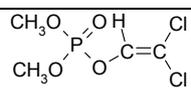
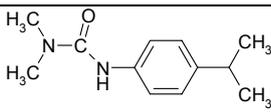
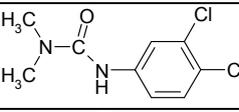
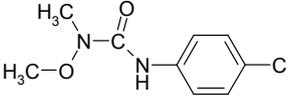
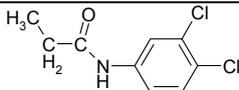
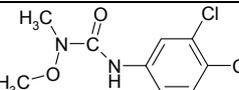
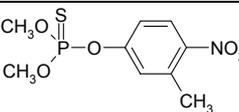
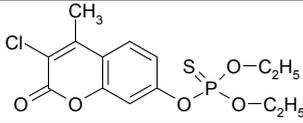
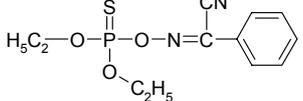
Commercial name	CAS number	Structure	$\log K_{ow}$ (theoretic)*	$\log K_{ow}$ (experimental)**
Dichlorvos	000062-73-7		0.60	1.43
Isoproturon	034123-59-6		2.84	2.87
Diuron	000330-54-1		2.67	2.68
Monolinuron	1746-81-2		2.26	2.30

Table 1 (continued)

Propanil	000709-98-8		2.88	3.07
Linuron	000330-55-2		2.91	3.20
Phenitrothion	000122-14-5		3.29	3.30
Coumaphos	000056-72-4		4.47	4.13
Phoxim	014816-18-3		4.39	4.39

\* By means of fragment methodology.<sup>18</sup>

\*\* By shake-flask method.

## EXPERIMENTAL

All experiments were performed with an Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) consisting of the following modules: degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detector (MWD). System control and data acquisition were made with the Agilent ChemStation software (Rev. B.03.02-SR1). The retention study was carried out with two analytical columns: double end-capped Zorbax Eclipse XDB-C18 (4.6 x 150 mm, 5 µm particle size); double end-capped Zorbax Eclipse XDB-C8 (4.6 x 150 mm, 5 µm particle size).

The applied chromatographic method was based on isocratic elution at a flow-rate set up at 1 mL/min, although this parameter may have a less significant influence on the thermodynamic parameters,<sup>19</sup> using as mobile phase constituents water and acetonitrile in different ratio ratios, as described in the following section. The chromatographic column was thermostated at several temperatures in the range 20 – 45°C following a 5°C step increase. The detection was achieved at 254 ± 2 nm with a response time of the detector of 2 seconds.

All retention time determinations were performed in triplicate to ensure data accuracy, and the calculated results (retention factor) were based on the average of these determinations (retention time). Column dead-time was determined from the retention time of uracil, injected into the column together with the studied compounds.

Retention data for all nine model compounds obtained for different temperatures were used to calculate the retention factor ( $k' = (t_r - t_0)/t_0$ ), which was further used to plot van't Hoff dependences.

All the compounds were obtained from commercial sources (Merck, Fluka, Sigma) in analytical grade. Acetonitrile and methanol were HPLC gradient-grade also from Merck. Water for chromatography (minimum resistivity

18 MΩ and maximum TOC 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments. The injection volume was by 1 µL from standard solutions prepared in methanol at a concentration level of 50 µg/mL for each studied compound.

## RESULTS AND DISCUSSION

According to the hydrophobicity data given in Table 1, these compounds have sufficient retention on hydrophobic stationary phases (C8 and C18) in order to measure retention factor for several temperatures and mobile phase compositions.<sup>17</sup> As mentioned in Experimental section, solutions of the nine compounds were injected into the column (C8 or C18) and the retention parameters were measured ( $t_r$  and  $t_0$ ) at 6 different temperatures within the interval 20 - 45°C. The retention study at each temperature was repeated three times and the mean values of  $t_r$  for the analyte and  $t_0$  for uracil were used in calculating the mean value of  $k'$ . The peak shapes were acceptable, with symmetry being close to 1. This can be seen from the example of six overlaid chromatograms shown in Fig. 1, where phenitrothion and phoxim elute at retention time value that decreases with the increase of the column temperature. Uracil has a retention time almost constant for all six temperatures: 1.308 min for C18 stationary phase, and 1.294 min for C8 stationary phase.

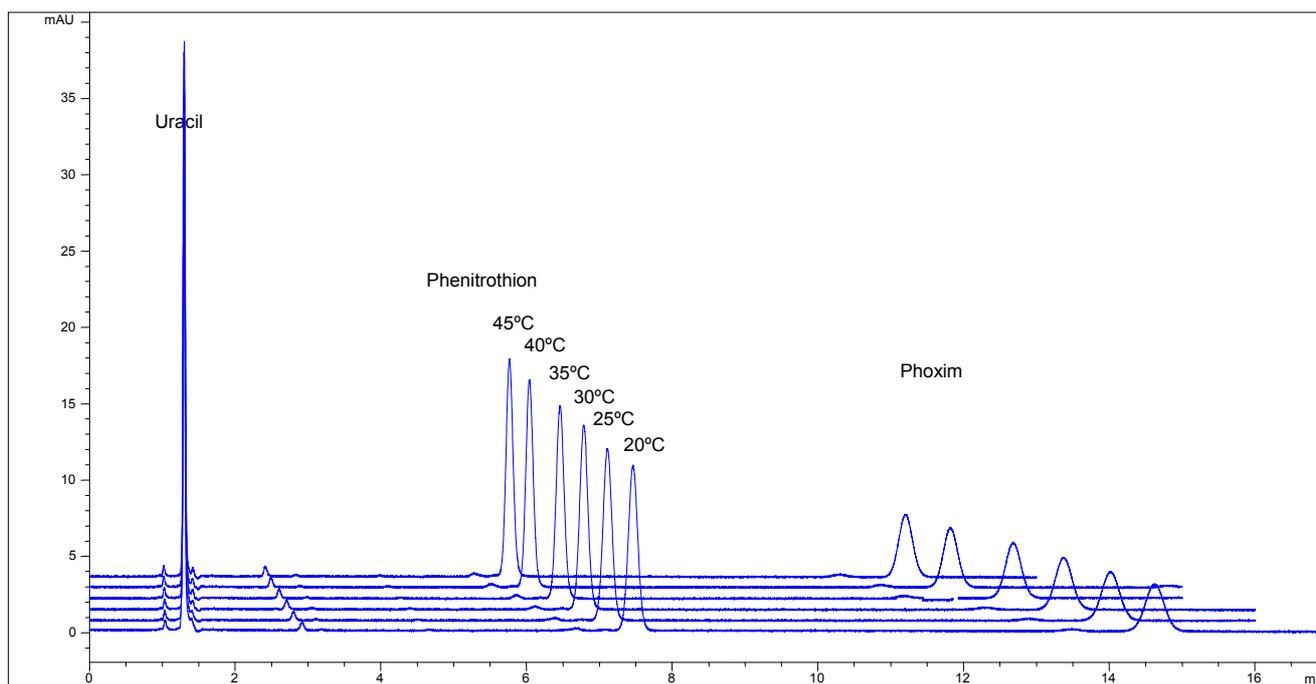


Fig. 1 – Overlaid chromatograms for obtained for fenitrothion and phoxim for several temperature and a mobile phase composed of 55% acetonitrile and 45% water (v/v) and octyl silica based stationary phase (C8).

The graphs representing  $\ln k$  as a function of  $1/T$  are known as van't Hoff plots ( $\ln k = a + b/T$ ). Using the retention data obtained for the chosen compounds as a dependence of column temperature, the corresponding van't Hoff plots were represented for each compound and mobile phase composition. Four examples of these plots are illustrated in Figs. 2 and 3, where both stationary phases (C8 and C18) were used for the retention study. The regression parameters  $a$ ,  $b$  and the correlation coefficient ( $r^2$ ) of the dependences  $\ln k = a + b/T$  for all studied compounds, the three mobile phase compositions and the two stationary phases used in this study are given in Table 2 and 3. As can be seen, all linear regressions were highly correlated ( $r^2 > 0.9900$ , in many cases  $r^2$  being even higher than 0.9990), which can be a proof of the accurate of the chromatographic data measured from the LC retention process. The thermodynamic parameters of the chromatographic partition process for the nine model compounds were calculated from the regression parameters  $a$  and  $b$  of the van't Hoff plot, as follows:

$$\Delta H^0 = -R \cdot b \quad (2)$$

$$\Delta S^0 = R(a - \ln \phi) \quad (3)$$

The calculated values of  $\Delta H^0$  and  $\Delta S^0$  from the regression parameters  $a$  and  $b$  are also given in Tables 2 and 3. The most debatable parameter in these calculations is however  $\phi$ .<sup>20</sup> For this reason many authors do not calculate the value of  $\Delta S^0$  which is dependent on this parameter. However, the phase ratio  $\phi = V_{sp}/V_{mp}$  can be approximately calculated for these columns as following: the volume of mobile phase ( $V_{mp}$ ) is calculated from the averaged dead time ( $t_0$  in min) multiplied by the flow-rate of the mobile phase  $F_{mp}$  (in mL/min), while the value of stationary phase ( $V_{sp}$ ) can be considered as the difference between geometric column volume ( $V_{column}$ ) and  $V_{mp}$ . Using the values of the  $t_0$  and the constructive data of the chromatographic column mentioned in Experimental one can be obtained the following values for C8 ( $\phi = 0.925$ ) and C18 ( $\phi = 0.904$ ) columns.

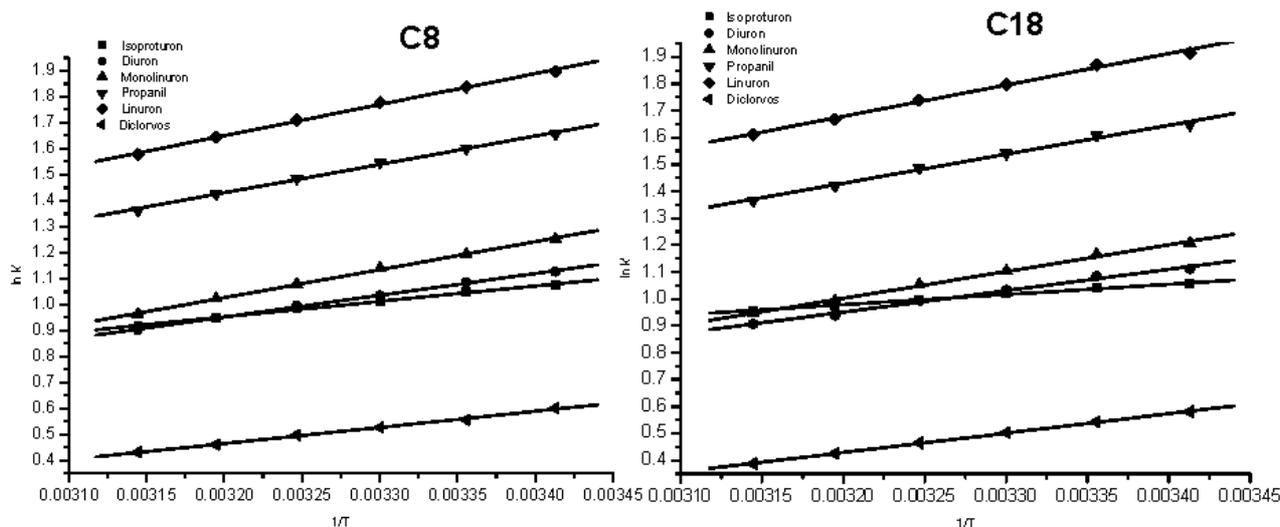


Fig. 2 – Van't Hoff plots for six of the studied pesticides obtained on octyl- and octadecyl silica based stationary phase for a mobile phase composed of 45% acetonitrile and 55% water (v/v).

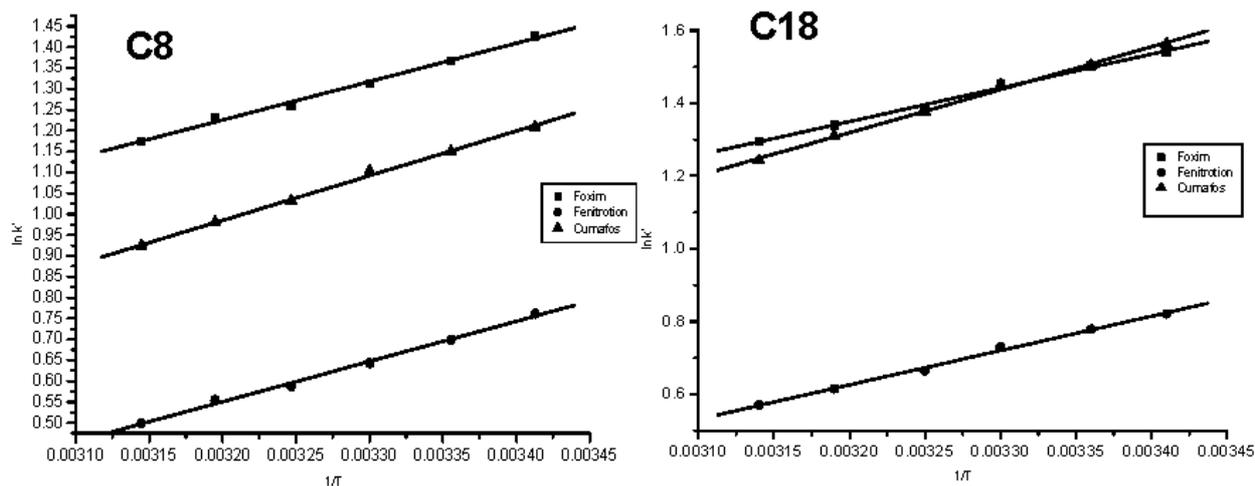


Fig. 3 – Van't Hoff plots for three of the studied pesticides obtained on octyl- and octadecyl silica based stationary phase for a mobile phase composed of 65% acetonitrile and 35% water (v/v).

These results for both  $\Delta H^0$  and  $\Delta S^0$  are in a fair agreement with thermodynamic data reported by previous cited works,<sup>21-25</sup> and generally they correspond to the hydrophobic-based interactions. For instance, the thermodynamic parameters describing the interactions between two hydrocarbon side chains of a protein are usually ranging in absolute value between 1.2–7.5 kJ/mol for  $\Delta H^0$  and 7–45 J/K mol for entropic term at 298 K.<sup>26</sup> Generally, the more hydrophobic character has the compound participating to the retention process, the more value of the standard enthalpy is assigned to the process. The entropic contribution to the partition process is of three orders of magnitude less than the enthalpy of the same process.

On the other hand, from this study no correlation has been observed between the mobile

phase composition and the value of the standard enthalpy or entropy calculated from the van't Hoff plots. For only one compound (isoproturon), the value of  $\Delta H^0$  and  $\Delta S^0$  decreased with the increase of the acetonitrile content in mobile phase (for C18 stationary phase), for the others these parameters reached a maximum or a minimum value for 40%, or 60% acetonitrile in mobile phase, according to the Tables 2 and 3. The nature of the stationary phase did not reveal significant differences for the standard enthalpy and entropy measured for the same compound and for the same mobile phase on the two studied stationary phases. Because all of the chosen compounds contain polar moieties in their molecules it is likely that some significant contribution of the polar-polar interactions between these compounds and residual silanols

from the surface of the two stationary phases to occur. However, according to the literature, the linear dependence of  $\ln k'$  on  $1/T$  is considered as

the real proof that the retention mechanism governed by hydrophobic interactions does not change with temperature change.<sup>1,27-29</sup>

Table 2

Regression parameters of the van't Hoff plots for C18 stationary phase and the corresponding values of standard enthalpy and entropy calculated from slope and intercept

Compound	%ACN	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	$\Delta H^0$ (kJ·mol <sup>-1</sup> )	$\Delta S^0$ (J·mol <sup>-1</sup> K <sup>-1</sup> )
Isoproturon	35	-0.4785	688.19	0.9976	-5.72	-3.18
	40	-0.2422	503.83	0.9953	-4.19	-1.17
	45	-0.2333	378.62	0.9956	-3.15	-1.10
Diuron	35	-0.6780	773.26	0.9979	-6.43	-4.79
	40	-1.1994	811.59	0.9970	-6.75	-9.13
	45	-1.5980	796.44	0.9916	-6.22	-12.4
Monolinuron	35	-1.2780	964.37	0.9945	-8.02	-9.78
	40	-1.4054	847.63	0.9904	-7.05	-10.8
	45	-2.1848	995.75	0.9965	-8.28	-17.3
Propanil	35	-1.5011	1215.10	0.9983	-10.10	-11.6
	40	-1.2160	933.23	0.9952	-7.76	-9.27
	45	-2.0180	1077.42	0.9948	-8.96	-15.9
Linuron	35	-1.5935	1339.24	0.9989	-11.13	-12.4
	40	-1.1886	1009.01	0.9965	-8.39	-9.04
	45	-2.0584	1167.66	0.9955	-9.71	-16.2
Diclorvos	35	-1.7539	880.77	0.9944	-7.32	-13.7
	40	-2.5500	961.39	0.9965	-7.99	-20.3
	45	-1.8709	718.58	0.9992	-5.97	-14.7
Fenitrothion	55	-1.1024	798.79	0.9935	-6.64	-8.32
	60	-3.3376	1382.13	0.9973	-11.49	-26.9
	65	-2.4628	964.47	0.9958	-8.02	-19.6
Coumaphos	55	-0.5882	864.11	0.9978	-7.18	-4.05
	60	-0.9480	831.42	0.9988	-6.91	-7.04
	65	-2.5245	1200.28	0.9951	-9.98	-20.1
Phoxim	55	0.0312	714.23	0.9965	-5.94	1.09
	60	-2.0875	1241.21	0.9907	-10.32	-16.5
	65	-1.6119	924.79	0.9924	-7.69	-12.5

Table 3

Regression parameters of the van't Hoff plots for C8 stationary phase and the corresponding values of standard enthalpy and entropy calculated from slope and intercept

Compound	%ACN	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	$\Delta H^0$ (kJ·mol <sup>-1</sup> )	$\Delta S^0$ (J·mol <sup>-1</sup> K <sup>-1</sup> )
Isoproturon	35	-0.2069	484.67	0.9953	-4.03	-1.07
	40	-0.7008	633.52	0.9971	-5.27	-5.17
	45	-0.9515	594.99	0.9924	-4.95	-7.26
Diuron	35	-0.7980	794.34	0.9986	-6.60	-5.98
	40	-1.5926	910.15	0.9983	-7.57	-12.5
	45	-1.7358	839.75	0.9978	-6.98	-13.7
Monolinuron	35	-1.6375	1064.33	0.9993	-8.85	-12.9
	40	-2.3492	1164.07	0.9995	-9.68	-18.8
	45	-2.4235	1078.06	0.9989	-8.96	-19.5
Propanil	35	-1.3453	1136.34	0.9998	-9.45	-10.5
	40	-1.9818	1192.05	0.9993	-9.91	-15.8
	45	-2.0700	1093.72	0.9970	-9.09	-16.5
Linuron	35	-1.5486	1283.96	0.9999	-10.67	-12.2
	40	-2.1486	1320.24	0.9994	-10.98	-17.2
	45	-2.1813	1197.19	0.9977	-9.95	-17.4
Diclorvos	35	-0.3174	455.42	0.9980	-3.78	-1.99
	40	-1.5676	741.53	0.9971	-6.16	-12.3
	45	-1.5215	620.75	0.9967	-5.16	-12.0

Table 3 (continued)

Fenitrothion	55	-2.6430	1234.84	0.9958	-10.27	-21.3
	60	-3.2296	1293.57	0.9948	-10.75	-26.2
	65	-2.5288	962.40	0.9955	-8.00	-20.3
Coumaphos	55	-1.0964	925.88	0.9903	-7.70	-8.46
	60	-2.0134	1064.21	0.9970	-8.85	-16.1
	65	-2.4379	1069.72	0.9974	-8.89	-19.6
Phoxim	55	-1.5515	1142.18	0.9920	-9.49	-12.2
	60	-2.1360	1179.08	0.9922	-9.80	-17.1
	65	-1.7173	919.65	0.9948	-7.65	-13.6

Graphical van't Hoff plots can also be used to check the separation selectivity. For example, the dependences  $\ln k'$  on  $1/T$  from Fig. 2 (C18) for three of the studied compounds indicate that within the temperature interval of 25 - 45°C the elution order of the monolinuron, diuron and isoproturon is tremendously changed. Their separation is critical within this interval, under elution conditions used in this study, and therefore temperature can not be used to improve the chromatographic separation. However, this can be obtained by modifying the organic modifier added to the mobile phase,<sup>1</sup> or the nature of the stationary phase.

### CONCLUSIONS

A high linear correlation was observed for the dependence of the logarithm of retention factor on the reciprocal value of the absolute temperature for the pesticides studied in this work. Using van't Hoff plots for the thermodynamic treatment of the chromatographic process, the values of the enthalpy and entropy variations have been determined for the retention of chosen pesticides. These values were in a good agreement with values obtained for other compounds reported by literature and generally they are of the same magnitude with values corresponding to the hydrophobic interactions between two protein chains. From this study one results that the hydrophobic interactions depend on the composition of the mobile phase and there are not significant differences of enthalpy and entropy for the retention of these compounds on octyl and octadecyl silica based stationary phases. The higher contribution of the enthalpy than entropy to the partition process of studied pesticides can be a proof of the main role played the hydrophobic interactions in the chromatographic retention mechanism, although significant contribution of the polar-polar interaction between pesticide

molecule and residual silanols from stationary phase is likely to occur.

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