BENZENE/WATER PARTITION CONSTANTS AND THERMODYNAMIC PARAMETERS ESTIMATED FROM LIQUID CHROMATOGRAPHY RETENTION OF SOME HERBICIDES USING PHENYL-SILICA STATIONARY PHASE

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Different elution conditions were used to study the retention behavior of some phenylurea and propanil herbicides in reversed-phase high performance liquid chromatography based on phenyl-silica stationary phase. In order to estimate the benzene/water partition constants of each analyte, the linear relationships between the logarithm of the retention factor and the acetonitrile or methanol content of the mobile phase obtained by isocratic runs were extrapolated for mobile phase containing only water. These values were compared to the octanol/water partition coefficients owing to the lack of data on benzene/water partition constants. The results of the study of column temperature influence have shown a high linear correlation between the logarithm of capacity factor and the reciprocal value of absolute temperature for each studied analyte. The thermodynamic parameters implicated in chromatographic retention were also determined using van't Hoff method.

INTRODUCTION

Herbicides derived from urea form a large group of chemical compounds widely used in agriculture in substantial amounts to control weeds in cereal, vegetable and fruit tree crops. As a consequence of their persistency and toxicological effects the monitoring of these micro-contaminants in natural ecosystems and foodstuff, consumption has become in the last decades an essential aspect of environmental protection and human health safeguard policy. The study of mixture of these compounds is of general interest because they appear generally together in well-known commercial formulation.[1-3] The most appropriate analytical technique for multi-residues monitoring of herbicides in all kinds of environmental samples is high-performance liquid chromatography (HPLC) due to its versatility and capability to separate between them or from matrix components, *e.g.*[4-7]

Unfortunately, ideal separations are often achieved only after optimization of chromatographic method parameters through costly and time consuming trial-and-error.[8] This problem has generated many researches with the aim to elucidate the mechanism of analyte retention and to develop some models that relate the retention to several chromatographic parameters and molecular properties that lead the analyte partition between a mobile phase and a stationary phase. In the literature, there are mentioned essentially three main factors determining distribution of an analyte between the two phases in liquid chromatography. At constant temperature of separation, these factors are: chemical structure of studied analyte, physico-chemical properties of the mobile phase and physico-chemical properties of the stationary phase.[9]

The lack of a general acceptable model for explaining the retention process in reversed-phase liquid chromatography (RP-LC) has not influenced the possibility of interpreting in various ways the chromatographic data and exploiting them for analytical and extra-analytical purposes.[10] Practically, the study of an analyte retention

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process in RP-LC, as function of the experimental parameters such as mobile phase composition, stationary phase nature, temperature, is the only possibility to observe the complex phenomena that take place during this process. The aim of this paper is to study the retention behavior of some phenylurea and propanil herbicides using phenyl silica stationary phase and to estimate some extra-analytical parameters characterizing the mentioned chromatographic process, such as benzene/water partition constants obtained from extrapolated capacity factors or thermodynamic parameters for the partition of analytes between mobile and stationary phases.

EXPERIMENTAL

1. Solvents and chemicals

All pesticides in the study (Diuron 99.5%, Isoproturon 99.8%, Linuron 99.7%, Monolinuron 99.9%, Propanil 99.7%) were supplied by Riedel-de-Haën (Seelze, Germany) and were used without further purification. Individual stock solutions (0.50 g/L) were prepared by dissolving 25 mg of each compound in 50 mL of methanol and were stored at 4°C. These were used to prepare by appropriate dilution the standard working samples. The HPLC-grade methanol (MeOH), acetonitrile (ACN) and water obtained from Merck (Darmstadt, Germany) were used for the preparation of the mobile phases. All the solvents were filtered through a 0.45 μm cellulose filter from Scharlau (Barcelona, Spain) before use. The lack of acidic-basic character of the studied compounds has allowed the use of the HPLC grade water, without a pH control. Uracil (2,4-dihydroxypyrimidine) used to estimate the dead time (t_0) of the column was purchased from Fluka Chemie (Buchs SG, Switzerland).

2. Instrumentation

Retention of the herbicides was investigated by using an Agilent 1100 liquid chromatograph equipped with a degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detector (MWD). The separations were performed on an Agilent Eclipse XDB-Phenyl column (150 mm length, 4.6 mm i.d.; 5 μm particle size) packed with a reversed phase featured by phenyl groups bonded to silica-organic support, double endcapped. The constructive data calculated for this chromatographic column are the following: volume of the mobile phase (V_{mp}, calculated from averaged dead time values) is 1.587 mL and the volume of the stationary phase (V_{sp} by the difference from geometric column volume and mobile phase volume) is 0.904 mL.

System control and data acquisition were achieved by means of a computer equipped with an Agilent ChemStation program. The chromatographic system was operationally qualified before performing this study.

3. Determination of retention parameters

The HPLC experiments for estimating dependences of capacity factor on mobile phase composition were carried out at 25°C with a flow-rate of 1 mL/min under isocratic conditions. The injection volume was 1 μL. Retention data were collected by injecting methanol solutions of single herbicides (50 μg/mL), in duplicate. Detection was achieved at 254 nm. The retention behavior of each analyte was investigated as a function of mobile phase composition ranging from 25 to 50% (v/v) acetonitrile and respectively from 45 to 67.5% (v/v) methanol, by steps of 2.5%. The dead time of the column was determined by injection of uracil-containing solution.

In order to estimate the main thermodynamic parameters (standard enthalpy and entropy) involved in the elution process of herbicides of interest, an isocratic elution was applied (acetonitrile/water = 30/70) for each individual solution at the same chromatographic conditions, but at different temperatures situated in the interval 20-45°C with an increment of 5°C.

RESULTS AND DISCUSSION

Benzene/water partition constants

The capacity factors (k') for mentioned herbicides (their commercial name, IUPAC name, CAS number, and their structure are shown in Table 1) were studied using two organic modifiers in the composition of mobile phase: acetonitrile (ACN) and methanol (MeOH). The retention time values of uracil were used to estimate t_0, for each chromatographic separation. The retention time values were measured for eight concentrations of acetonitrile or methanol in the mobile phase as organic modifier, within the following intervals: [25 - 50%] (v/v) for acetonitrile and [45 - 67.5%] (v/v) for methanol. The change in retention with solvent composition for a binary mobile phase in reversed-phase liquid chromatography is described, if only a limited range of mobile phase composition is considered, by the following relationship:[12-15]

$$\log k' = \alpha + \beta \, C_m \qquad (1)$$

where C_m is the volume concentration of organic solvent, α becomes the logarithm of the capacity factor for studied solutes extrapolated to a mobile phase containing only water ($C_m = 0$), β the slope of the experimental data after fitting to a linear regression model, sometimes taken as a general measure of the elution strength of the organic solvent.

The plots for the linear dependences (1) for both organic modifiers are given in Fig. 1. As can be seen, strong linear correlations between log k' and percentage of ACN or MeOH were found for all compounds ($r^2 > 0.9900$). The regression parameters obtained for dependences between log k' and organic modifier content in mobile phase for all the studied analytes are given in Table 2.

Table 1

Identification data on the studied herbicides[11]

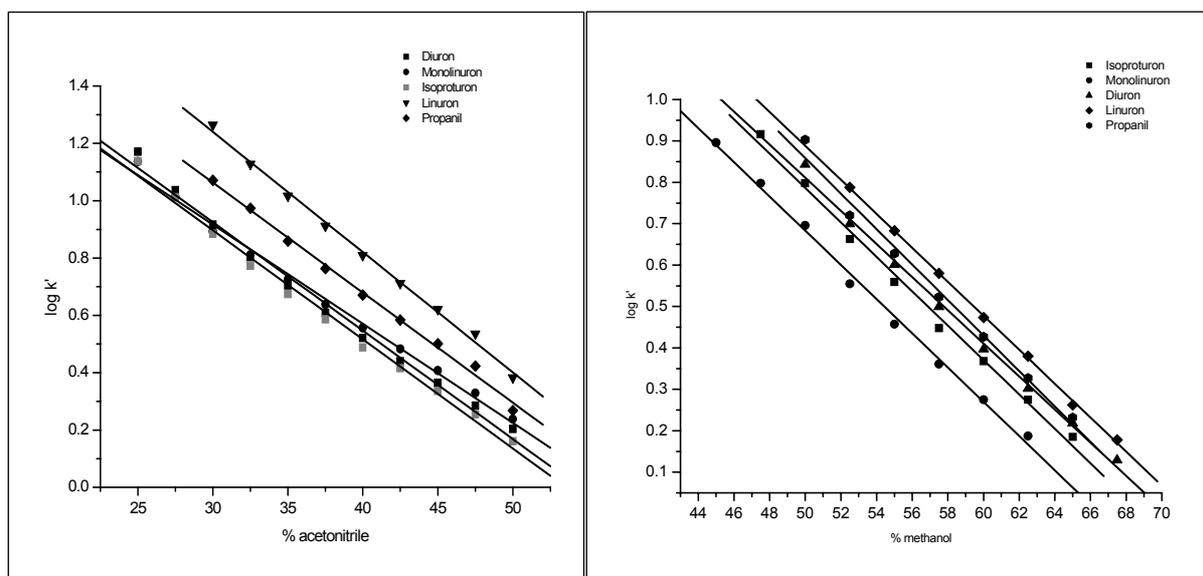| Compound	IUPAC name (CAS number)	Structure
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea (000330-54-1)	
Isoproturon	3-(4-isoprpylphenyl)-1,1-dimethylurea (034123-59-6)	
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (000330-55-2)	
Monolinuron	3-(4-chlorophenyl)-1-methoxy-1-methylurea (1746-81-2)	
Propanil	3'4'-dichloropropionanilide (000709-98-8)	

Fig. 1 – The relationship between retention (log k') and mobile
phase composition on phenyl silica stationary phase.

Table 2

Regression parameters from the retention dependences of studied herbicides on organic modifiers

Analyte	Regression parameters					
	α		β		r^2	
	ACN	MeOH	ACN	MeOH	ACN	MeOH
Diuron	2.060	2.811	-0.0378	-0.0400	0.9909	0.9951
Isoproturon	2.000	2.865	0.0373	-0.0416	0.9948	0.9939
Linuron	2.497	2.938	0.0419	-0.0410	0.9963	0.9992
Monolinuron	1.954	2.748	-0.0345	-0.0413	0.9940	0.9958
Propanil	2.216	3.002	-0.0384	-0.0426	0.9955	0.9887

The parameter α from eq. (1) that represents the logarithm of the capacity factor of the studied analyte for a mobile phase consisting of only water (log k' = α; C_m = 0) was used to estimate the extrapolated partition constant for analyte between the mobile phase containing only water and stationary phases. By similarity with extrapolated log K_{ow} value (octanol/water partition constants) obtained by using octyl-silica stationary phase, we may consider that for phenyl-silica stationary phase the extrapolated dependences given by eq. 1 fairly lead to estimation of benzene/water partition constants (log K_{bw}) for studied analytes. In the situation of using a stationary phase based on phenyl-silica the value of extrapolated log K_{bw} is given by the following relationship:

$$\log K_{bw} = \alpha - \log \frac{V_{sp}}{V_{mp}} \qquad (2)$$

Taking into consideration the data for V_{mp} and V_{sp} given in Experimental section, the second term from eq. (3) is: -0.245. Experimental values for log K_{bw} obtained by shake-flask methods for these compounds, as well as a theoretical methodology used for estimating log K_{bw} values are not still available. For this reason, in Table 3 we compared the experimental values for log K_{bw} with theoretical values of log K_{ow} obtained by means of the previous mentioned approaches,[16] although the organic solvent supposes different kinds of solvation forces with dissolved analytes.

Table 3

The octanol/water partition constants estimated from retention experiments, shake-flask methods and fragment methodology

Analyte	log K_{ow}		log K_{bw} from retention data	
	theoretic	shake-flask	ACN	MeOH
Diuron	2.67	2.68	2.30	3.05
Isoproturon	2.50	2.87	2.25	3.11
Linuron	2.91	3.20	2.74	3.18
Monolinuron	2.20	2.30	2.20	2.99
Propanil	2.88	3.07	2.46	3.25

In both situations, log K_{bw} obtained from retention experiments using acetonitrile or methanol as organic modifier in mobile phase are of the same magnitude order with the corresponding values from theoretic procedure (fragment methodology) and shake-flask methods.

However, some recent literature data[17] indicate only the values of water solubility (S_w, in mg/L at 25°C) of these studied compounds: S_w^{diuron} = 42; $S_w^{isoproturon}$ = 62; $S_w^{linuron}$ = 75; $S_w^{monolinuron}$ = 930; $S_w^{propanil}$ = 152. The only experimental value for benzene solubility (S_b) was found for diuron,[18] as a fraction solubility: S_b^{diuron} = 8.41·10⁻³. Taking into consideration the molecular weight of diuron (M_{diuron} = 233.1) and density of benzene ($\rho_{benzene}$ = 0.88 kg/L), one can estimate the ratio $S_b^{diuron} / S_w^{diuron}$ as a fair value for K_{bw}. The ten-base logarithm of K_{bw} for this herbicide leads to 1.72 value.

According to Table 3, correlations between log K_{ow} and log K_{bw} have been observed only between experimental shake-flask values and values obtained for retention study using methanol as organic modifier (r = 0.9099), respectively. This property can be explained by linear free-energy relationships, which allow linear correlations between different extraction systems (*e.g.* benzene/water and octanol/water).[19] However, the low correlations coefficient might be explained by small number of studied compounds. In case of acetonitril as organic modifier the correlation was poor (r = 0.8250), due to possible implications of acetonitrile molecules in π-π interactions with phenyl groups in stationary phase. According to the literature, comparison of these two non-miscible water solvents in extraction of carboxylic acids,[20] or their metallic complexes[21] revealed better linear relationships.

Thermodynamic parameters

The dependence of the capacity factor on the chromatographic column temperature, at which the retention process of investigated herbicides takes place, has been examined using mixture of acetonitrile-water (30 : 70, v/v) as mobile phase. Elution was performed in isocratic mode at the

following temperatures: 20; 25; 30; 35; 40; and 45°C. For a given mobile phase composition the effect of temperature on retention is usually described by the van't Hoff relationship:[22]

$$\ln k' = \ln \frac{V_{sp}}{V_{mp}} + \frac{\Delta S^0}{R} - \frac{\Delta H^0}{R} \cdot \frac{1}{T} \qquad (3)$$

where ΔS^0 is the standard partial molar entropy of transfer of solute from the mobile phase to the stationary phase, ΔH^0 is the standard partial molar enthalpy of transfer of solute from mobile phase to the stationary phase, R is the gas constant and T is the absolute temperature of the chromatographic

column. The linear representations of the dependence of ln k' on 1/T were checked for each analyte and given by the equation:

$$\ln k' = a + b \cdot \frac{1}{T} \qquad (4)$$

where *a* and *b* are regression parameters.

The plots depicted in Fig. 2 show good correlations between ln k' and 1/T characterized by very high coefficients ($r^2 > 0.9900$). This linear dependence has proved that the retention mechanism is based mainly on hydrophobic interactions.[23]
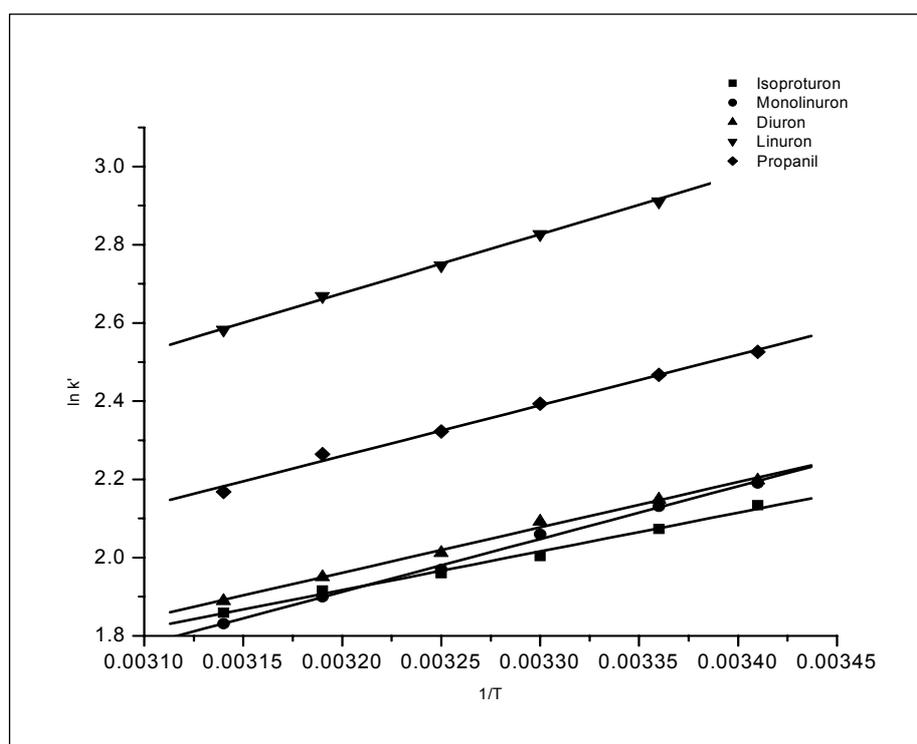

Fig. 2 – The relationship between retention (ln k') and 1/T.

The parameters implicated in the thermodynamic model of chromatographic retention, the enthalpies (ΔH^0) and entropies (ΔS^0) of the transfer of analytes from mobile phase to the stationary phase were calculated with the values of slope (b) and y-coordinate intersection (a). The value of R was taken as 8.314 J·K^{-1} mol^{-1} and ln (V_{sp}/V_{mp}) = -0.563 (this time as a natural logarithm). The calculated values of ΔH^0 and ΔS^0 from retention data are given in Table 4.

As can be seen, the values of enthalpy and entropy variation are high for these hydrophobic compounds. The enthalpy contribution to the reversed-phase retention process (between -0.94 and -11.41 kJ·mol^{-1}) is higher than the entropy

variation (between -6.21 and -15.96 J·mol^{-1}K^{-1}) at the partition of the investigated analytes between mobile and stationary phases. That signifies that the retention process is thermodynamically determined by the enthalpy, as has been proved in other applications for octyl- and octadecyl-silica based stationary phases, such as for aromatic hydrocarbons,[24] phenols,[25] or amino compounds.[26]

It can be concluded that this thermodynamic model is useful in the theoretic treatment of the retention process in reversed-phase liquid chromatography. A negative change in the entropy is related to the structure of the mobile phase and to the ordering of these analytes due to the hydrophobic and π-π interactions with phenyl groups in stationary phase.

Table 4

Regression parameters at the temperature variation and thermodynamic properties
estimated for the retention of some herbicides on phenyl-silicagel

Analyte	Regression parameters			Thermodynamic parameters	
	a	b	r^2	ΔH^0 (kJ·mol^{-1})	ΔS^0 (J·mol^{-1}K^{-1})
Diuron	-1.8108	1178.02	0.9936	-9.79	-10.37
Isoproturon	-1.3103	1007.79	0.9955	-8.38	-6.21
Linuron	-2.2426	1536.01	0.9995	-2.77	-13.97
Monolinuron	-2.4825	1372.51	0.9961	-11.41	-15.96
Propanil	-1.9545	1316.11	0.9927	-0.94	-11.57

CONCLUSIONS

The liquid chromatographic retention behavior of some herbicides using a phenyl silica stationary phase was studied. The experiences showed a high linear dependence between the capacity factor on mobile phase composition and temperature. That allowed obtaining the extrapolated retention factor values with pure water as eluent and then these values have been used in estimating the benzene/water partition constants and compared to their hydrophobicity as given by the octanol-water partition coefficient. The values of partition constants of the investigated herbicides estimated from retention were in a good agreement with the values of octanol/water partition coefficients estimated by means of fragment methodology, or from „shake-flask" experiments.

The values of the enthalpy and entropy variations determined for the retention of the herbicides derived from urea and propanil were also in good agreement with those obtained for some other organic compounds on octyl- or octadecyl-silica stationary phases. Taking into consideration the fact that the enthalpy contribution at the retention process is higher than the entropy variation of the partition of these solutes between mobile and stationary phases it can be concluded that the hydrophobic and π-π interactions are playing a major role in the retention mechanism of these herbicides in liquid chromatography based on phenyl-silica stationary phase.

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