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# Relationship between the hydrophobicity and specific hydrophobic surface area of pesticides determined by high-performance liquid chromatography compared with reversed-phase thin-layer chromatography[1]

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**Abstract**

The hydrophobicity and specific hydrophobic surface area of 12 commercial pesticides have been determined by reversed-phase high-performance liquid chromatography (RP-HPLC) using octadecyl-bonded silica support and water–methanol mixtures as eluents. The pesticides showed regular retention behaviour, their log $k'$ values decreased linearly with increasing concentration of methanol in the eluent. The hydrophobicity and specific hydrophobic surface area of pesticides were significantly intercorrelated, although the chemical structures of the pesticides were different. Principal component analysis proved that the hydrophobicity parameters determined by RP-HPLC and reversed-phase thin-layer chromatography are slightly different and herbicides and fungicides could not be distinguished according to their hydrophobicity parameters. This indicates that differences in the biological activity of these pesticides cannot be attributed to either their hydrophobicity or specific hydrophobic surface area alone.

*Keywords:* Hydrophobicity; Surface area; Chemometrics; Pesticides

## 1. Introduction

In the last decades quantitative structure–activity relationship (QSAR) studies have found growing acceptance and application in up-to-date agrochemical research [1,2]. Hydrophobicity as one of the most important molecular parameters has been frequently used in various QSAR studies [3,4]. Besides other methods hydrophobicity can also be determined by reversed-phase thin-layer (RP-TLC) [5,6] and reversed-phase high-performance liquid chromatography (RP-HPLC) [7]. Many RP-HPLC supports have been used for the determination of the hydrophobicity parameters of bioactive compounds. Octadecylsilica columns have been used the most frequently [8,9], however, other RP supports such as $C_1$, $C_2$, $C_6$, $C_8$ and polyethylene-coated silica have also been applied [10]. The reliability of the determination of hydrophobicity can be considerably enhanced by calculating linear correlations between the logarithm of the capacity factor and the con-

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centration of organic modifier in the eluent [11]. The intercept value of the equation ($\log k'_0$) is related to the molecular hydrophobicity and the slope ($b$) is related to the specific hydrophobic surface area of the solutes. New hydrophobicity parameters determined by RP-HPLC [12] and a new method for the determination of hydrophobicity have been recently reported [13].

The objective of this work was to determine the hydrophobicity and specific hydrophobic surface of a non-homologous series of pesticides by RP-HPLC, to find a correlation between the hydrophobicity parameters, and to compare the results of RP-TLC and RP-HPLC methods. Only the pesticides frequently used in high amounts in the modern agrochemical practice were included in the experiments.

## 2. Experimental

The common and IUPAC names and the biological activity of the pesticides are compiled in Table 1. A 25 cm×4 mm I.D. column filled with octadecylsilica (particle diameter 5 μm, Macherey–Nagel, Düren, Germany) was used in each experiment. The HPLC equipment consisted of a Liquopump Model 312 (Labor MIM, Budapest, Hungary) pump, a Cecil CE-212 variable-wavelength UV detector (Cambridge, UK), a Valco injector (Houston, TX, USA) with a 20-μl sample loop and a Waters 740 integrator (Water-Millipore, Milford, MA, USA). The flow-rate was 1.0 ml $min^{-1}$ and the detection wavelength was set to 220 nm. Mixtures of methanol–water were used as eluents, the methanol concentration ranged from 0 to 70%, v/v (in steps of 5.0%, v/v). The use of the very large concentration range of methanol was motivated by the highly different hydrophobicity of the pesticides. The pesticides were separately dissolved in the eluents at the concentration of 0.05 mg $ml^{-1}$. As the solutions were always freshly prepared the decomposition of thiram during the separation process was not observed. Linear correlation was used to describe the dependence of the log $k'$ value on the concentration of methanol ($C$):

$$\log k' = \log k'_0 + bC \quad (1)$$

To test the validity of the hypothesis that in the case of homologous series of solutes the intercept ($\log k'_0$) and slope ($b$) values of Eq. (1) are intercorrelated linear correlation was calculated between the two hydrophobic chromatographic parameters [11]:

$$\log k'_0 = A + Bb \quad (2)$$

To compare the hydrophobic parameters determined by RP-TLC and RP-HPLC principal component analysis (PCA) was used [14]. The hydrophobicity parameters determined by RP-HPLC and RP-TLC were the variables. For the easier visualization of the

Table 1
Chemical names and biological activities of pesticides

| No. | Activity | Common name | IUPAC name |
|---|---|---|---|
| 1 | F | Thiram | Bis(dimethylthiocarbamoyl)disulfide |
| 2 | H | Isoproturon | 3-(4-Isopropylphenyl)-1,1-dimethylurea |
| 3 | H | Chlorotoluron | 3-(3-Chloro-*p*-tolyl)-1,1-dimethylurea |
| 4 | F | Thiophanate methyl | 4,4′-*o*-Phenylenebis(3-thioallophanic acid) dimethyl ester |
| 5 | H | Oxabetrinil | (*Z*)-1,3-Dioxolan-2-ylmethoxyimino(phenyl)acetonitrile |
| 6 | F | Flutriafol | (*RS*)-2,4′-Difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol |
| 7 | F | Carboxin | 5,6-Dihydro-2-methyl-1,4-oxathiine-3-carboxanilide |
| 8 | H | Terbacil | 3-*tert*.-Butyl-5-chloro-6-methyluracyl |
| 9 | H | Terbutryn | $N^2$-*tert*.-Butyl-$N^4$-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine |
| 10 | H | Aziprotryne | 4-Azido-*N*-isopropyl-6-methylthio-1,3,5-triazine-2-ylamine |
| 11 | H | Triasulfuron | 1-[2-(2-Chloroethoxy)phenylsulfonyl]-3-(4-furon methoxy-6-methyl-1,3,5-triazin-2-yl)urea |
| 12 | H | Fuberidazole | 2-(2-Furyl)benzimidazole |

F, Fungicide; H, herbicide.

results the two-dimensional nonlinear maps of PC loadings and variables were also calculated [15]. The hydrophobicity values and the specific hydrophobic surface areas of the pesticides determined by RP-TLC were taken from Ref. [16].

## 3. Results and discussion

The pesticides tested separate well on an octadecyl-bonded silica column and they give symmetrical peaks with each eluent system. Their retention times differ considerably, hence octadecyl-bonded silica column can be used successfully for the determination of the hydrophobicity parameters of the pesticides. The parameters of Eq. (1) and the hydrophobicity parameters of the pesticides determined by RP-TLC are given in Table 2. The relationship between log $k'$ and methanol concentration is linear and the correlation coefficient is in most instances is higher than 0.98, confirming the applicability of Eq. (1). The intercept and slope values differ considerably from each other, supporting our previous qualitative conclusions that an octadecyl-bonded silica column is suitable for the separation of the pesticides.

Significant linear correlation was found between the hydrophobicity and specific hydrophobic surface area of pesticides (Fig. 1), $r_{calc.}$ and $r_{99.9\%}$ values are the calculated and tabulated values of the coefficient

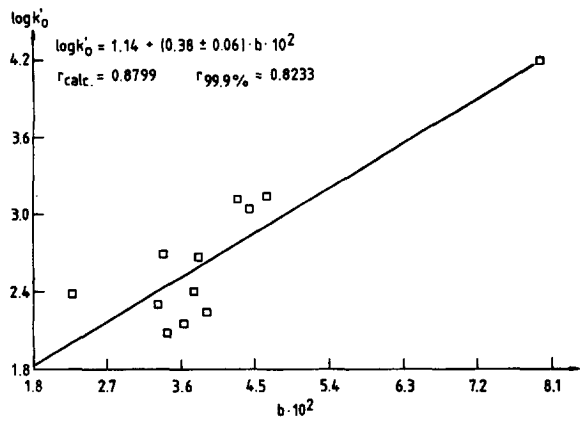


Fig. 1. Relationship between the hydrophobicity (log $k'_0$) and specific hydrophobic surface area ($b$) of the non-homologous series of pesticides, $r_{calc}$ and $r_{99.9\%}$ values are the calculated and tabulated values of the coefficient of correlation.

of correlation. This finding indicates that chromatographically these pesticides behave as a homologous series of solutes, despite their markedly different chemical structure.

The results of PCA are compiled in Table 3. The overwhelming majority of the information about the retention behaviour of pesticides in RP-HPLC and RP-TLC can be described by two background variables. In other words, two theoretical chromatographic parameters are sufficient to describe the retention behaviour of pesticides both in RP-HPLC and RP-TLC. Unfortunately, PCA does not define

Table 2
Parameters of linear correlations between log $k'$ and methanol concentration ($C$) in the eluent (log $k' = \log k'_0 + bC$) and the hydrophobicity parameters of the pesticides ($R_{M0}$ and $b$) taken from [16]

| No. | Pesticide | Log $k'_0$ | $-b\cdot10^2$ | $s_b\cdot10^3$ | $r_{calc.}$ | $R_{M0}$ | $b$ |
|---|---|---|---|---|---|---|---|
| 1 | Thiram | 2.07 | 3.44 | 2.69 | 0.9939 | 0.77 | 0.25 |
| 2 | Isoproturon | 2.66 | 3.82 | 3.22 | 0.9895 | 1.81 | 0.33 |
| 3 | Chlorotoluron | 2.30 | 3.31 | 1.74 | 0.9945 | 1.69 | 0.33 |
| 4 | Thiophanatemethyl | 2.15 | 3.64 | 3.45 | 0.9868 | 1.74 | 0.24 |
| 5 | Oxabetrinil | 3.11 | 4.31 | 3.95 | 0.9876 | 1.95 | 0.34 |
| 6 | Flutriafol | 3.14 | 4.67 | 3.48 | 0.9917 | 1.91 | 0.37 |
| 7 | Carboxin | 2.40 | 3.75 | 4.86 | 0.9758 | 1.61 | 0.37 |
| 8 | Terbacil | 4.18 | 7.99 | 22.42 | 0.9294 | 1.27 | 0.30 |
| 9 | Terbutryn | 2.39 | 2.28 | 3.15 | 0.9906 | 1.85 | 0.33 |
| 10 | Aziprotryn | 2.68 | 3.39 | 4.16 | 0.9998 | 1.77 | 0.31 |
| 11 | Triasulfuron | 2.23 | 3.92 | 2.68 | 0.9953 | −0.15 | 0.12 |
| 12 | Fuberidazol | 3.03 | 4.46 | 15.33 | 0.9456 | 1.70 | 0.34 |

$s_b$ = standard deviation of the slope ($b$) value.
$r_{calc.}$ = calculated coefficient of variation indicating the fitness of the equation to the experimental data.

Table 3
Similarities and dissimilarities between the hydrophobicity parameters of pesticides determined by RP-HPLC and RP-TLC

| No. of principal components | Explained variance (%) | Cumulative values (%) |
|---|---|---|
| 1 | 54.61 | 54.61 |
| 2 | 40.87 | 95.49 |
| 3 | 3.03 | 98.51 |
| Principal component | No. of principal components | |
| loadings | 1 | 2 |
| Log $k'_0$ | 0.83 | 0.53 |
| $b_{RP\text{-}HPLC}$ | 0.59 | 0.79 |
| $R_{M0}$ | 0.70 | −0.67 |
| $b_{RP\text{-}TLC}$ | 0.80 | −0.54 |

$R_{M0}$ = hydrophobicity value determined by RP-TLC.
$b_{RP\text{-}TLC}$ = specific hydrophobic surface area determined by RP-TLC.

these two parameters as concrete physical or physicochemical entities, only indicates their mathematical possibility. The data clearly indicate that both reversed-phase liquid chromatographic systems are similar and both can be used for the determination of the hydrophobicity parameters of pesticides.

The two dimensional non-linear map of PC loadings indicate that the information content of RP-HPLC and RP-TLC methods is similar but not identical (Fig. 2). The hydrophobicity parameters determined by RP-HPLC and RP-TLC form two distinct clusters suggesting that the application of the two methods can result in slightly different results. The cluster formation of hydrophobicity parameters on Fig. 2 further indicate that these molecular parameters contain markedly different information, therefore the separate application of these molecular parameters is justified in future QSAR studies. The distribution of the pesticides according to their hydrophobicity parameters (two dimensional non-linear map of principal component variables) is shown in Fig. 3. The pesticides do not form separate clusters either according to the chemical structure or to their biological activity. This result indicates that (taking both hydrophobicities and specific hydrophobic surface areas into consideration) herbicides and fungicides cannot be distinguished from each

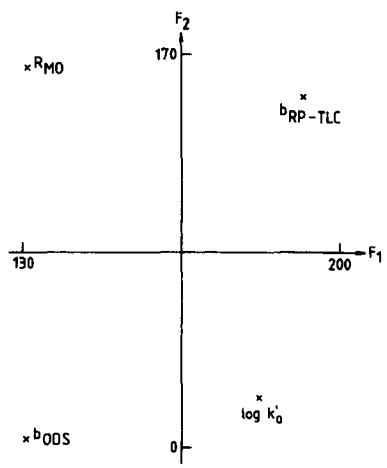


Fig. 2. Similarities and dissimilarities between the hydrophobicity parameters of pesticides determined by RP-HPLC and RP-TLC. Two-dimensional nonlinear map of principal component loadings. No of iterations: 24; Maximal error: $7.74 \cdot 10^{-4}$.

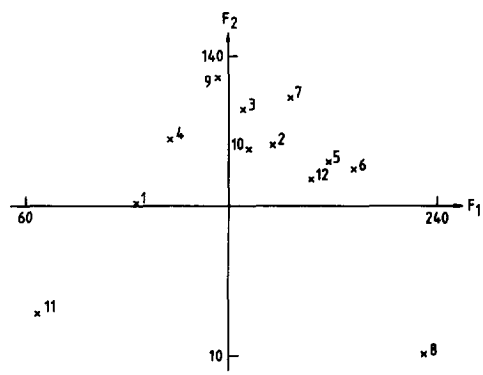


Fig. 3. Similarities and dissimilarities of pesticides according to their hydrophobicity parameters. Two-dimensional non-linear map of principal component variables. No. of iterations: 240; maximal error: $5.56 \cdot 10^{-3}$. Numbers refer to pesticides in Table 1.

other. This means that the hydrophobicity parameters alone do not determine the specificity of the biological activity of the pesticides.

## 4. Conclusions

The hydrophobicity and specific hydrophobic surface area of the pesticides can be determined by RP-HPLC using methanol–water eluent mixtures as eluents. The determination of hydrophobicity parameters by RP-HPLC is easy to carry out and the results are accurate and reliable. However, the hydrophobicity parameters determined by RP-HPLC and RP-TLC are slightly different probably due to the different coverage of silica support by the hydrophobic ligand.

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