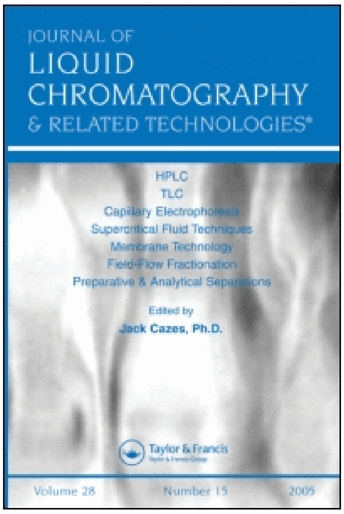

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF NEW GENERATION PESTICIDES IN COMPLEX PLANT MATRICES BY HPLC USING VARIOUS STATIONARY PHASES

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To cite this Article Michel, Monika(2010) 'DETERMINATION OF NEW GENERATION PESTICIDES IN COMPLEX PLANT MATRICES BY HPLC USING VARIOUS STATIONARY PHASES', Journal of Liquid Chromatography & Related Technologies, 33: 2, 239 — 249

To link to this Article: DOI: 10.1080/10826070903442246

URL: http://dx.doi.org/10.1080/10826070903442246

Journal of Liquid Chromatography & Related Technologies, 33:239–249, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903442246

DETERMINATION OF NEW GENERATION PESTICIDES IN COMPLEX PLANT MATRICES BY HPLC USING VARIOUS STATIONARY PHASES

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☐ *Author interest is focused on method optimization to determine the new compounds using stationary and mobile phases with different physicochemical properties. This work deals with 5 substances composed of nitrogen containing heterocycles, 1,2,4-triazoles. The relationships between the chromatographic retention factor (k) and those physicochemical properties which are relevant in quantitative structure retention relationship (QSRR) studies were investigated. The accuracy of the simple linear regressions between the chromatographic retention and the descriptors for all of the compounds was satisfactory (correlation coefficient, $0.8 \leq r^2 \leq 1.0$). The QSRR models of compounds could be predicted with a multiple linear regression equation having the statistical index, $r^2 = 1.000$. Evaluation of chromatographic properties of the new stationary phases and description of the molecular separation mechanism using the QSRR method including molecular modeling will be suggested.*

Keywords HPLC columns, QSRR, retention factor, triazole fungicides

INTRODUCTION

The investigation of the quantitative structure retention relationship (QSRR) of substances is an important issue in chromatographic science and bioanalytical chemistry, as well as in new compounds discovery. One of the most central areas of chemistry, QSRR provides information that is useful for organic chemistry, physical chemistry, molecular design, and bioanalytical chemistry.[1,2] This information is composed of mathematical equations relating the chemical structure of compounds to a wide variety of their physical, chemical, biological, and technological properties. The derived relationships between molecular descriptors and retention in chromatographic systems are used to estimate the properties of other molecules and/or to find the parameters affecting their biological activity.[3,4]

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Over the past few years, different methods have been used to correlate chromatographic retention behavior with various molecular structure and physicochemical parameters, such as the Van der Waals and molecular volume, the molecular connectivity index, the number of carbon atoms, the *n*-octanol/water partition coefficient (log$P$), the hydrophobic substituent constant (log$S$), and the solubility parameters.[5,6] Correlations between chromatographic retention and molecular parameters provide significant information on the effect of the molecular structure on the retention time and on the possible mechanism of adsorption and elution. These correlations can be investigated using QSRR, which provides statistical equations that relate the molecular structure to the retention phenomena.

The incorporation of the heterocyclic triazole nuclei into various compounds therapeutic agents is the main synthetic strategy in agrochemistry. The high fungitoxic properties have encouraged chemists to synthesize large numbers of novel chemotherapeutic agents. Triazole agents have a broad scope in remedying various disorders in plant protection. Chemoterapeutic properties include a wide spectrum of biological activities such as antifungal, antimycotic, and antiulcer. At present, there is a real perceived need for the discovery of new compounds endowed with biological activity.

In the present paper, a quantitative structure retention relationship analysis was conducted to investigate the quantitative effect of the physicochemical properties of various 1,2,4-triazole molecules on their chromatographic retention. The investigation of multiple linear regression equations between descriptors and retention factors was the purpose of this work.

EXPERIMENTAL

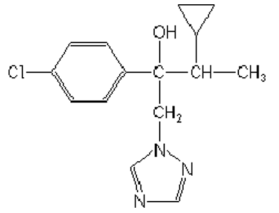
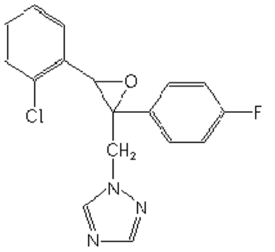
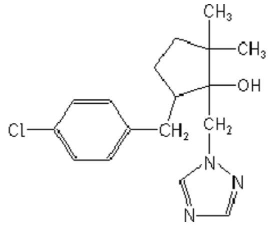
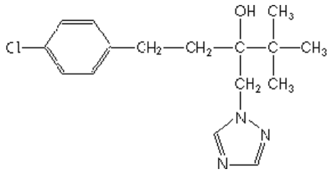
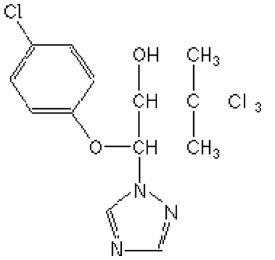
Reagents, Materials, and Apparatus

Five triazole fungicides were purchased from Sigma-Aldrich (St. Louis, MO, USA). The names and structures are presented in Table 1. HPLC grade acetonitrile was obtained from J.T. Baker (Deventer, The Netherlands). All water used in the experiments was filtered by a Millipore ultra pure water system (Millipore, Belford, MA, USA).

The HPLC system consisted of a CM3500 and a CM3200 pump, UV-DAD detector type SM 5000 set at $\lambda = 250\,\mathrm{nm}$ (TSP, Riviera Beach, FL, USA); 20 µL injection loop (Supelco, Bellefonte, PA, USA); Rheodyne Sample Injector Model 7125 (Rheodyne, Cotati, CA, USA). The data were collected and analyzed with an LCtalk computing system (TSP LCtalk HPLC software, version 2.03.02).

The liquid chromatographic separations were performed using HPLC columns listed in Table 2. The column temperature was kept ambient.

TABLE 1 Chemical Structure of Triazoles

No.	Structure	Name
1		**cyproconazole** (2*RS*,3*RS*;2*RS*,3*SR*)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1*H*-1,2,4-triazol-1-yl) butan-2-ol
2		**epoxiconazole** (2*RS*,3*SR*)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl) propyl]-1*H*-1,2,4-triazole
3		**metconazole** (1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol
4		**tebuconazole** (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
5		**triadimenol** (1*RS*,2*RS*;1*RS*,2*SR*)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl) butan-2-ol

The mobile phase consisted of a methanol/water mixture (10:90, v/v) (A) and methanol (B) and was delivered at a flow rate of 1 mL/min. The gradient program consisted of increasing the concentration of B from 5% to 90% over a period of 20 min and then holding it at this concentration

TABLE 2 HPLC Column Characteristics

Column Name	Stationary Phase Type	Producer	Geometrical Parameters					
			I.D. (mm)	d_p (μm)	P_c (%)	S_{BET} (g/m^2)	D (Å)	EC
Alltima C18	Octadecyl-bonded silica	Alltech Ass., Inc., Deerfield, IL, USA	250 × 4.6	5	16	350	100	yes
PGC Hypercarb	Porous graphitized carbon	Thermo Electron Corp., Waltham, MA, USA	100 × 3	5	100	–	250	–
Kromasil NH_2	Amino ligands bonded to silica	Nicolaus Copernicus University, Toruń, Poland	250 × 4.6	5	4.47	310	100	–
Nucleosil CN	Cyanopropyl ligands bonded to silica	BGB, Anvil, Switzerland	250 × 4.6	5	5	350	100	–
HILIC column (ZIC-HILIC)	Sulfobetaine covalently attached to silica	SeQuant, Umea, Sweden	250 × 4.6	5	–	–	100	–

I.D. – column ID.
d_p – particle size.
P_c – carbon load.
S_{BET} – specific surface area.
D – pore size.
EC – end-capping.

for another 2 min. The run time, from injection to injection, was 25 min. In the case of the HILIC column, the gradient program was 0% A to 80% A due to different properties of stationary phase.

Theoretical Background

Retention Factor

The retention factor (k) can be calculated according to Equation (1).

$$k = (t_R - t_M)/t_M \tag{1}$$

where t_M is the holdup time and t_R is the retention time.

Descriptors

The descriptors of the molecules were drawn using HyperChem® software (Hypercube Inc., FL, USA, version 7) on a personal computer, using semi empirical AM1 calculations for the determination of the molecular dynamics (293 K) of the molecules. Extending the empirical approach can produce methods with good accuracy and excellent computational performance. In this extended method, the calculation is generally sufficiently reliable and the results in good agreement with those obtained experimentally. To obtain the quantitative effects of the structural parameters of the 1,2,4-triazole fungicides on their chromatographic retention, a QSRR analysis with the molecular descriptors, including their electronic and physicochemical adjectives, was operated. The polarizability (α), molar refractivity (MR), lipophilicity (log P), dipole moment (μ), total energy (E_{tot}), heat of formation (ΔH_f), molecular surface area (S_M), and binding energy (E_b) are the properties that can be calculated for each molecule. Therefore, these data were used to determine the QSRR models. The values of the calculated descriptors and retention factors of the substances are summarized in Table 3.

TABLE 3 The Values of the Descriptors of the Triazoles

Comp	MW	log P	MR	α	S_M	E_{tot} [kcal/mol]	μ [D]	E_b [kcal/mol]	ΔH_f [kcal/mol]
Triad	295.8	3.08	56.16	19.73	368	−2249	3.51	−2471	43.97
Cypr	291.8	2.91	85.06	29.39	475	−3634	4.48	−3665	84.14
Tebuc	307.8	3.70	75.48	25.91	428	−3211	1.98	−3236	66.75
Metc	319.8	3.85	56.16	19.37	362	−2449	3.88	−2468	46.85
Epox	329.8	3.44	85.06	29.39	489	−3630	3.35	−3658	90.53

Multiple Linear Regression

The mathematical foundation of the quantitative structure retention relationship is based on the principle of polylinearity. The QSRR equations were obtained by forward stepwise multiple regression techniques using the multilinear forms:

$$P = f(D) = a_0 + a_1D_1 + a_2D_2 + \cdots + a_nD_n \tag{2}$$

where P is the property; in our case, P is the retention factor k, D_1, D_2, and D_n are the descriptors and n is the number of descriptors. The intercept a_0 and regression coefficients of the descriptors (a_1, $a_2 \ldots a_n$) were determined using the least squares method. The statistical evaluation of the data was performed using the Origin program package. To test the quality of the regression equation, the correlation coefficient r was utilized as the statistical parameter.

RESULTS AND DISCUSSION

In this paper, the retention factors of the triazoles listed in Table 1 were estimated using the mathematical relationships expressed as a linear relationship in terms of their physicochemical descriptors. The absence of strict homology in a sequence set of investigated substances poses a serious problem in the creation of a "structure retention" model.[7,8] In such cases, the linear correlation equation is not always applicable to the description of the chromatographic retention within the limit of a full non-homologous set.

Using QSRR methodology, the relationships between the experimental parameter k and theoretical descriptors were revealed. Usually, QSRR studies involve three components; these are a relevant description of molecular properties, informative data on properties, and correct and meaningful correlations. To obtain the optimal QSRR model using the subset of the molecular structural descriptors, the next followed procedures were realized in this work. First, a linear mathematical analysis with all eight theoretical physicochemical molecular descriptors as predictor variables were performed (Table 3). These data served as a basis for further statistical analysis.

The linear regression equations were obtained for each of the columns and a summary of these is given in Table 4. Some of the results had a high value of r, which indicate that these equations represent a satisfactory model for some of the calculated descriptors. As can be observed in this experiment, the linear models were adequate ($0.8 \leq r^2 \leq 1.0$). Most of the regression equations showed that the retention was primarily influenced by the size, steric factors, and polar effects. The theoretical descriptors

TABLE 4 The Multiple Linear Models $k=f(D)=a_0+a_1 log_P+a_2 MR+a_3\alpha+a_4 S_M+a_5 E_{tot}+a_6\mu+a_7 E_b+a_8\Delta H_f$

Column	a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	r^2
C18	−6.607	−1.500	−0.066	−0.375	−0.004	−0.003	0.147	−0.006	−0.009	0.997
PGC	8.525	−6.006	0.084	−0.292	0.003	0.003	−3.461	−0.005	−0.005	0.979
NH_2	−2.654	3.293	−0.043	0.130	−0.001	−0.002	0.924	−0.001	−0.037	0.832
CN	−1.837	7.338	−0.099	0.862	0.003	−0.001	0.143	0.001	−0.147	0.871
HILIC	−2.874	3.041	−3.768	0.997	0.0022	−0.151	0.560	0.016	0.122	0.963

calculated in this study include a wide spectrum of molecular properties, which all have high values, because each of them influences the chromatographic retention to some extent.

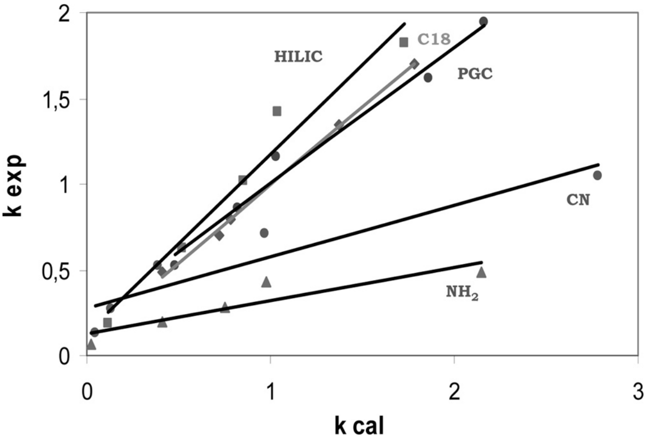
In this paper for QSRR studies involving descriptors, the physicochemical parameters are of utmost importance, as a basic model. Electronic effects, in contrast to global molecular properties such as lipophilicity (log*P*), normally refer to a certain atom or group. Steric parameters describe and parameterize the molecules in terms of, for example, molecular surface area (S_M). They significantly influence physicochemical interactions in chromatography that involve weak bonds, in particular, electrostatic and dispersion interactions.[9–11] These descriptors provide information about the fundamental properties of molecules that is not available by other means. Undoubtedly, these effects have been of interest to chromatographers and other chemists.

Chromatographic retention results from the solvation and partition of individual compounds in a stationary phase. The retention is affected by various intermolecular forces, which include hydrogen bonding, ion-dipole, dipole-dipole, specific, and dispersion (non-specific) interactions, etc.

A common practice employed in the development of QSRR methodology has been to identify relationships employing self chosen single or more physicochemical descriptors. However, regardless of which type of variable is chosen, it is usually difficult to predict in advance the descriptor variables of value in describing the properties variation. Understandably, it is very difficult, or even impossible, to handle a large number of descriptors while aiming to produce an unambiguous and understandable selection. The latter fact is one of the disadvantages of QSRR. As a result, in this study, it is attempted to consider the entire spectrum of the calculated descriptors. To overcome the difficulty, however, a software program like HyperChem® has been used that can calculate the standard descriptor values. However, some of the simple linear correlations shown in Table 4 gave a low (inadequate) regression coefficient r^2 of less than 0.80.

Therefore, the multiple linear statistical analysis technique described by Equation (2) was used, in order to obtain a reliable expression, which considers all of the descriptors calculated in this work. Equation (2) was examined and the numeric coefficients were determined by linear regression. The multiple linear regressions of the retention factors for all columns used in the study and their regression coefficients are listed in Table 4. The result of chromatographic retention was estimated with calculated values of regression coefficients, and the result showed clearly that each obtained descriptor with different molecular properties has different a effect. It is not difficult to see that coefficients a_4, a_5, and a_7 are small and close to zero. This testifies that the physicochemical parameters such as surface area, total energy, and binding energy weakly influence the retention of the investigated sorbates. However, even such low informative descriptors should be considered also since their use unambiguously increases the predictive ability of the equations. The obtained multiple linear equations showed that they possess the best predictive ability, in comparison with the linear equations.

The calculated retention times are compared with the corresponding experimental values in Figure 1 for different stationary phases. These plots clearly show the relationships between the observed and calculated retention times. The multi-linear correlations obtained for the observed and



C18	$y = 0.9071x + 0.088$	$r^2 = 0.997$
PGC	$y = 0.7934x + 0.212$	$r^2 = 0.979$
NH_2	$y = 0.1929x + 0.128$	$r^2 = 0.832$
CN	$y = 0.3008x + 0.276$	$r^2 = 0.871$
HILIC	$y = 1.0429x + 0.127$	$r^2 = 0.963$

FIGURE 1 Comparision between the calculated retention factors and the corresponding experimental values.

calculated retention factors are of excellent quality. PGC and HILIC phases display significantly higher retention factors than NH2 and CN phases. The C_{18} phase appears to be a potential alternative for the separation owing to the retention of some compounds.

The advantages of the approach used in this paper, are that they are not restricted to closely related compounds, they can be easily obtained, and describe clearly defined molecular properties. Because of their advantages, they are widely used in the QSRR studies. The small errors of prediction make it possible for these models to be applied to the problem of peak identification in a chromatogram, particularly in the case where multiple retention times are observed. It is undoubtedly possible to further improve the ability of this technique to identify unknown triazole's compounds. Unfortunately, in the present study, the small data sets limited our ability to predict the retention data, due to the inevitable statistical irregularities. Another approach to identifying unknown triazoles peaks in a chromatogram is to use a similar QSRR approach to correlate the structural properties of various triazoles compounds. The use of the retention times and structural correlations should offer the possibility of making further significant progress in this regard.

Basically, two types of interaction between solute and solvated stationary phase surface can be considered. First, the solute can interact with the adsorbed solvent layer and then remain on the layer. This interaction will take place when the interactions between the solute and the stationary phase are relatively weak compared with interaction between the solvent and the stationary phase.[12,13] This has been known as sorption interaction. Second, the solute can displace the solvent molecules from the stationary phase surface and interact directly with the surface. This displacement interaction occurs when the interaction between the solute and the stationary phase is much stronger than interaction between the solvent and the stationary phase.

If there are multi-layers of solvent on the stationary phase surface and the solute interacts with the lower layer of solvent by displacing solvent from the upper layer, the cavity formation energy in the solvent layer increases with the surface area of the solute and, hence, retention of the solute decreases as the surface area increases.

It has been considered that there are two interactions which play a role in solute retention in RPLC, dispersive interaction and electrostatic interaction. Dispersive interaction might be considered between nonpolar groups of solute and nonpolar stationary phase. On the other hand, electrostatic interaction would be expected between polar groups of solute and mobile phase. The regression models for triazoles included both dispersive descriptors, such as solvent accessible surface area and log$P$, and electrostatic descriptors such as dipole moment. Of course, their contribution to the retention would be different. Therefore, the driving force of the retention of triazoles would be the combination of dispersive and electrostatic interactions.

CONCLUSION

The retention times of 5 triazoles were modeled using multi-linear models based on the calculated physicochemical descriptors. The obtained models showed perfect correlation and predictive ability. The resulting eight parameter multiple linear regressions for the retention times of the triazoles can be used to predict the retention times and indices of unknown but structurally similar compounds with a considerable degree of confidence. Also, this study clearly demonstrates that QSRR models can be used to predict the retention times and indices without the need for chemical standards.

ACKNOWLEDGMENTS

I thank Prof. Bogusław Buszewski (Nicolaus Copernicus University, Toruń, Poland) for valuable discussions and suggestions.

This work was supported by Ministry of Science and Higher Education during 2005–2008, grant No. 2P06R 075 29 (Warsaw, Poland).

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