INTERPRETATION OF RETENTION BEHAVIOR OF *s*-TRIAZINE DERIVATIVES IN RP-UHPLC SYSTEM FROM THE ASPECT OF REACTIVITY DESCRIPTORS

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Abstract

The present study deals with the interpretation of the retention data of a series of *s*-triazine derivatives from the aspect of reactivity descriptors, including highest occupied molecular orbitals (HOMO), lowest unoccupied molecular orbitals (LUMO), the difference between HOMO and LUMO energies (HOMO-LUMO gap – Egap), chemical potential (μ) and electrophilicity index (ω). The retention behavior of the studied compounds was determined by using RP-UHPLC system with phenyl column and methanol/water mobile phase and it was expressed as capacity factor ($logk_0$) obtained based on extrapolation of the linear dependence between the volume fraction of modifier in the mobile phase (φ) and capacity factor ($logk$). The relationships between the retention parameters and reactivity descriptors were examined by univariate linear approach. Statistical characteristics of the resulting relationships provided an insight into the influence of the molecular characteristics on the retention behavior of the studied compounds in the applied chromatographic system. The established linear quantitative structure-retention relationships (QSRRs) can be considered to be preliminary ones, taking into account the limited number of compounds, however they could be significant guidelines for further detailed examination of the retention mechanisms.

Introduction

The application of *s*-triazine derivatives in agriculture is well-known. Some of the triazine derivatives are used as effective herbicides and fungicides [1]. Considering the fact that some of the triazine derivatives are persistent in environment, their efficient analysis is quite important task.

Chromatographic analysis provides an efficient, fast and precise determination of many compounds. One of the aspects of application of chromatographic approaches is examination of certain molecular properties that can be estimated based on their retention behavior in particular chromatographic system. Quantitative structure-retention relationship (QSRR) approach is a chemometric approach that integrates the molecular properties and retention behavior in the form of mathematical models [2]. QSRR approach is based on linear or non-linear approaches. These linear or non-linear relationships can correlate the retention behavior with various types of molecular descriptors, including physicochemical, topological, biological, etc [3].

The present study is based on univariate linear QSRR approach for examination of the influence of some reactivity descriptors, including highest occupied molecular orbitals (HOMO), lowest unoccupied molecular orbitals (LUMO), the difference between HOMO and LUMO energies (HOMO-LUMO gap – Egap), chemical potential (μ) and electrophilicity index (ω), on the retention behavior of a series of *s*-triazine derivatives in reversed-phase ultra high performance liquid chromatography system (RP-UHPLC) with phenyl column and methanol/water mobile

phase. The aim of the present study is contribution to the understanding of retention mechanisms of *s*-triazines in RP-UHPLC system from the aspect of the reactivity descriptors.

Experimental

The molecular structures of the series of the studied *s*-triazines are presented in Figure 1. The studied molecules are based on 6-chloro-1,3,5-triazine structure with acyclic and cyclic substituents. The compounds were synthesized at the Faculty of Technology and Metallurgy, University of Belgrade, following the procedures described in literature [4].

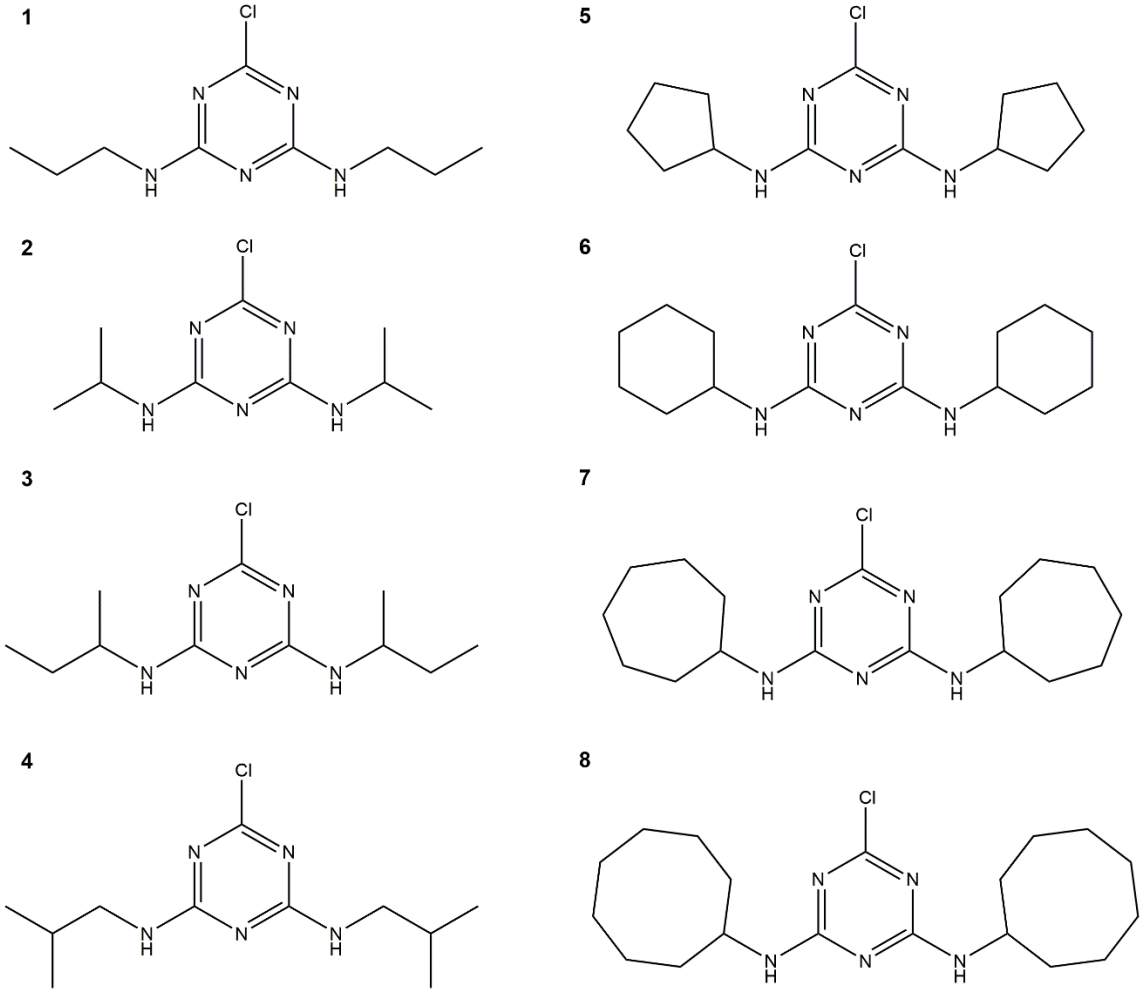

Figure 1. Molecular structures of the studied *s*-triazine derivatives

The chromatographic analysis was done by using UHPLC Agilent 1290 Infinity LC system with Diode Array Detector. The applied column was ZORBAX Eclipse XDB-Phenyl, 95 Å, 2.1 × 150 mm, 5 µm. The mobile phase was methanol/water mixture. The chromatographic analysis and determination of retention parameters ($logk_0$) are described in detail in a previous study [5]. The reactivity molecular descriptors were calculated by Gaussian 16 program. HOMO and LUMO descriptors represent the ability to donate and ability to receive an electron, respectively, whilst Egap is the energy difference between the HOMO and LUMO energies. Chemical potential (µ) represents the entropy change after addition of a particle to a system. Electrophilicity index (ω) measures the energy stabilization when the system gains an additional electronic charge in the environment.

Results and discussion

The molecular descriptors were obtained based on 2D molecular structures and their values are given in Table 1. Also, the retention data in the form of capacity factor obtained by extrapolation are provided in Table 1. Observing the values of the calculated molecular descriptors, it can be seen that the compounds with acyclic substituents (compounds **1**-**4**) possess generally lower HOMO energies and μ values, and higher Egap and ω values, than compounds with cyclic substituents (compounds **5**-**8**). There is no strict separation between these two groups of compounds regarding their LUMO energies. In order to examine their influence on the retention behavior of the studied compounds in RP-UHPLC system, the correlation analysis was performed. Electron excitations become easier as the Egap narrows. Increased mobility of π electrons in extensive conjugated π orbital systems enhances energy distribution throughout the molecule, thereby stabilizing it. Consequently, smaller Egap values are associated with greater stability, which is the case with *s*-triazine derivatives with cyclic substituents (**5**-**8**).

Table 1. Retention data and molecular descriptors of the analyzed *s*-triazine derivatives

Comp.	$logk_0$ [5]	HOMO	LUMO	Egap	μ (eV)	ω (eV)
1	1.379	-9.657	3.214	12.871	-3.22147	0.806
2	0.790	-9.628	3.199	12.826	-3.21457	0.806
3	2.147	-9.598	3.222	12.820	-3.18805	0.793
4	2.947	-9.614	3.178	12.792	-3.21807	0.810
5	4.068	-9.538	3.220	12.758	-3.15931	0.782
6	4.455	-9.552	3.199	12.751	-3.17644	0.791
7	5.331	-9.535	3.225	12.761	-3.15498	0.780
8	6.061	-9.504	3.260	12.764	-3.12172	0.763

The univariate linear relationships between HOMO, LUMO and Egap energies are presented in Figure 2. Here, it can be seen that there is a strong influence of HOMO and Egap energies on the retention parameters of the studied *s*-triazine derivatives considering significantly high correlation coefficients of the relationships. On the other hand, there is a weak correlation between LUMO energy and retention parameters.

The obtained results indicate that higher retention in the applied chromatographic system is associated with high HOMO energies and low Egap energies. Having in mind that the applied chromatographic system has a phenyl column as a stationary phase, it can be assumed that π-π interactions occur during the chromatographic separation.

LUMO energies are associated with higher retention, however there are some exceptions such as the compound **3** that has higher LUMO energy but lower retention parameter than compounds **4**, **5** and **6** which possess lower LUMO energies but higher retention parameters.

Since the chromatographic separation is a quite complex process, the retention behavior can rarely be explained by only one variable. Usually, it is explained by more complex mathematical equations (QSRR models) based on multivariate regression (multiple linear regression, partial least squares regression, principal component regression, etc.). However, there is the limitation in the present study since it was impossible to apply multivariate regression due to limited number of compounds. Nevertheless, the obtained results can be considered a preliminary ones and further investigation is needed to confirm the possibility of precise prediction of the retention parameters based on reactivity descriptors by establishing the QSRR models with strictly defined applicability domain and evaluated predictive ability based on internal and external validation procedures.

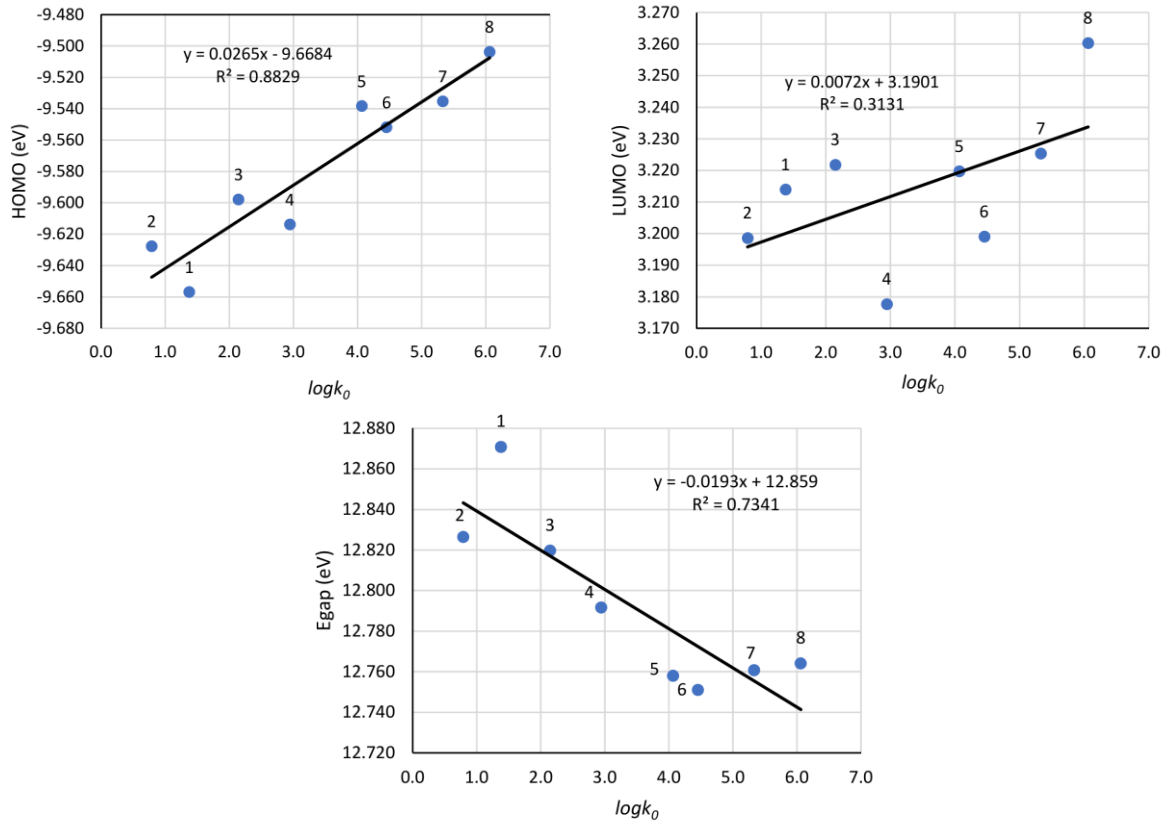

Figure 2. Linear relationships between retention data ($logk_0$) and HOMO, LUMO and Egap molecular descriptors

Linear relationships between the retention parameters and chemical potential and electrophilicity index are presented in Figure 3. There is a significant influence of these two chemical properties on the retention behavior of the studied derivatives, considering high regression coefficient.

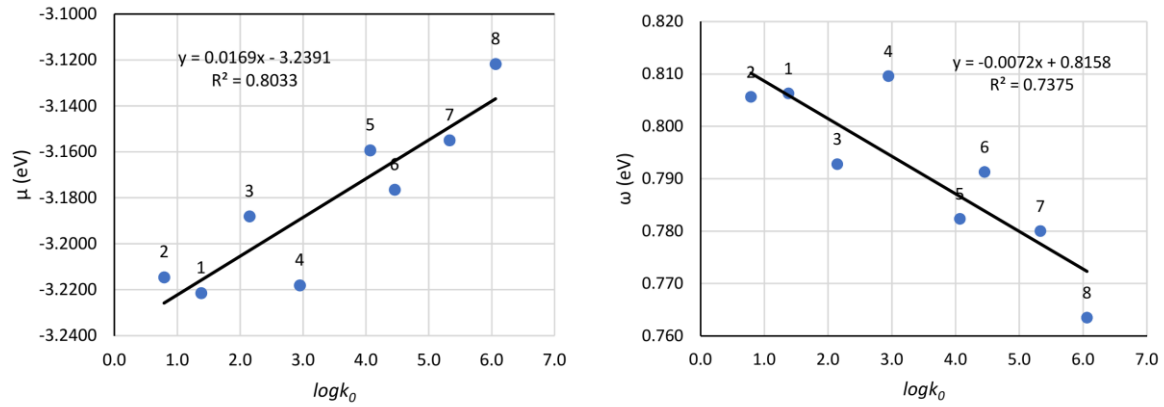

Figure 3. Linear relationships between retention data ($logk_0$) and μ and ω molecular descriptors

Statistical parameters of the established preliminary QSRR models are presented in Table 2. Based on the presented statistical data, it can be concluded that only the influence of the LUMO energies on the retention is questionable considering small determination coefficient (R^2),

adjusted determination coefficient (R^2adj), cross-validation determination coefficient (R^2_{CV}) and *F-test*. Those parameters indicate poor fitting of the data between retention parameter and LUMO energies. The other models have acceptable statistical parameters and can be considered statically significant, but with limited predictive ability.

Table 2. Statistical parameters of the established QSRR models

Parameter	Model 1	Model 2	Model 3	Model 4	Model 5
x	$logk_0$	$logk_0$	$logk_0$	$logk_0$	$logk_0$
y	HOMO	LUMO	Egap	μ	ω
R^2	0.8829	0.3131	0.7341	0.8033	0.7375
R^2adj	0.8635	0.1922	0.6882	0.7706	0.6939
R^2_{CV}	0.7919	-0.2455	0.4665	0.6670	0.5556
F-test	45.3	2.7	16.4	24.5	16.9

Conclusion

The presented results indicate the importance of reactivity descriptors in retention mechanism of the studied series of *s*-triazine derivatives in the applied RP-UHPLC system with phenyl column and methanol/water mobile phase. Particular influence of HOMO, Egap, μ and ω descriptors on the retention behavior of the studied compounds was confirmed by correlation analysis. Considering the aforementioned descriptors, there is a clear separation between the derivatives with acyclic and cyclic substituents. The compounds with cyclic substituents possess higher HOMO and μ descriptors than the derivatives with acyclic substituents. There is no clear separation among the derivative with acyclic and cyclic substituents regarding LUMO energies. HOMO and μ descriptors have the highest influence on the determined retention parameters ($logk_0$) of the studied compounds determined in the applied chromatographic system.

Acknowledgements

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