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Comparative Analysis of Anisotropic Lipophilicity of a Series of 6-Chloro-1,3,5-Triazines Determined in Reversed Phase Ultra High Performance Liquid Chromatography System

Strahinja Kovačević [1], Milica Karadžić Banjac [1,*], Jasmina Anojčić [2], Vojislav Banjac [3], Petar Ilić [3], Benjamin Salaković [1], Sanja Podunavac-Kuzmanović [1] and Lidija Jevrić [1]

[1] Department of Applied and Engineering Chemistry, Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; strahko@uns.ac.rs (S.K.); benjamin.salakovic@uns.ac.rs (B.S.); sanya.podunavac@uns.ac.rs (S.P.-K.); lydija@uns.ac.rs (L.J.)

[2] Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia; jasmina.anojcic@dh.uns.ac.rs

[3] Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; vojislav.banjac@fins.uns.ac.rs (V.B.); petar.ilic@fins.uns.ac.rs (P.I.)

* Correspondence: mkaradza@uns.ac.rs; Tel.: +381-21-4853666

Abstract: Triazine derivatives are well-known commercially available compounds used for selective weed control in different crops, such as corn and sugarcane. Some of them are considered persistent organic compounds in the environment and it is important to improve the features of herbicide formulae, to estimate their physicochemical properties and to determine their retention behavior in modern analytical techniques that can be used in the determination of pesticides in environmental samples. The present study deals with a comprehensive analysis of the chromatographic behavior of a series of 6-chloro-1,3,5-triazines with alkyl and cycloalkyl substituents, among which some compounds possess herbicidal and fungicidal activity. The anisotropic lipophilicity of triazine derivatives was determined using reversed-phase ultra high performance liquid chromatography with octadecyl and phenyl columns and applying binary (methanol/water and acetonitrile/water) and ternary (methanol/acetonitrile/water) mobile phases under isocratic conditions. The retention data were analyzed using chemometric pattern recognition methods (hierarchical cluster analysis and principal component analysis) and sum of ranking differences method. The obtained results are excellent indicators of the retention behavior and the lipophilicity of the analyzed series of triazines and can serve as an outstanding basis for the development of new chromatographic methods for the determination of triazines in environmental samples.

Keywords: chemometrics; chromatography; lipophilicity; pesticides; triazines

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1. Introduction

In the modern world of ever-increasing population, the food production industries play a crucial role in providing substantial food quantities [1]. The completion of this task is particularly difficult considering all the factors that influence the quality of raw materials, the yield of crops, and agricultural production, such as weed, insects, bacteria, fungi, etc. [1–3]. Modern agriculture is widely based on the application of various synthesized chemicals (pesticides) that provide high yield of crops, yet, at the same time contribute to the environmental pollution [4]. For example, the residues of some less expensive and older pesticides (dichlorodiphenyltrichloroethane (DDT) and lindane) can persist for years in soil and water [5]. Today's pesticide chemistry has to meet the criteria of modern society that take into account economic and ecological requirements [4]. New pesticide compounds must be environmentally friendly and must have favorable toxicological properties in order to be safe for non-target organisms [4,6]. The population that is exposed to pesticides via

residues of pesticides in food and water is usually in the risk of low-concentration exposure, whilst the agricultural workers who directly manipulate with pesticides are in much higher risk of heaving health issues caused by pesticide toxicity [5].

Triazine derivatives are chemical compounds with six-membered heterocyclic nitrogen-containing rings. Triazines that have symmetrically distributed nitrogen in the ring are known as symmetrical triazines or *s*-triazines. *s*-Triazines are effective pesticides especially for weed control in crops, including corn, fruit crop, sorghum, and sugarcane [7]. Some *s*-triazines express antifungal [8] and antibacterial [9] activity as well, and their effects in agriculture can be multiple. On the other hand, due to their long half-life in the environment, triazines possess high bioconcentration factor and soil adsorption coefficient [10]. Considering the fact that *s*-triazines and their metabolites can be toxic to humans and animals [11–13], particularly for aquatic organisms, the analysis of the physicochemical properties (especially lipophilicity) and environmental behavior of new *s*-triazine derivatives, as pesticide candidates, should be performed. The development of new analytical methods for the analysis of triazine residues and their metabolites is of great importance for risk assessment.

The chromatographic techniques are most commonly used for analysis of *s*-triazines. The chromatographic analysis of *s*-triazines can provide significant data regarding their physicochemical properties, such as lipophilicity profile [14,15]. Additionally, the chromatographic techniques are used for triazine separation and determination in environmental and food samples [16,17]. The retention behavior of several series of *s*-triazine derivatives has been studied applying normal-phase (NP) and reversed-phase (RP) high-performance thin-layer chromatography (HPTLC) [18–20] and reversed-phase high-performance liquid chromatography (RP-HPLC) on octadecyl column [21]. Gas chromatography coupled with mass spectrometry (GC-MS) technique has been applied for triazine analysis as well [22].

The retention mechanism in a particular chromatographic system can be often explained by lipophilic properties of the compounds [23–25]. Chromatographic lipophilicity of biologically active compounds, including pesticides, is considered to be an excellent predictor of their biological activity and environmental behavior. Chromatographic lipophilicity is usually expressed as capacity factor (log$k$) in HPLC and R_M factor in TLC. However, there are some alternative parameters that are used for this purpose as well, such as C_0 or ϕ_0 parameters [26]. The estimation of the chromatographic lipophilicity can be performed in different chromatographic systems with various types of mobile phases (binary or ternary mixtures of methanol, acetonitrile and water) and different stationary phases (octyl, octadecyl, cyano, etc.). As columns with a non-polar stationary phase, octadecyl (C18) columns are widely used in the estimation of chromatographic lipophilicity of numerous biologically active compounds and more non-polar compounds have higher retention on C18 columns than the compounds with moderate or high polarity. The application of phenyl columns for this purpose is particularly interesting due to their ability to form π–π interactions with analytes containing unsaturated functional groups [27].

The present study is focused on the estimation of the retention parameters (chromatographic lipophilicity) of a series of *s*-triazine derivatives with alkyl and cycloalkyl substituents, which includes one commercially available herbicide (propazine), and their comparative analysis. Also, in order to compare the influence of structural isomerism, there are two pairs of structural isomers in the investigated series. The group of the compounds with cycloalkyl substituents contains the substituents from homologous series of cycloalkanes including cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl group. In some previous studies, the retention of some cycloalkyl *s*-triazines was examined in RP(C18)-TLC [28] and RP(C18)-HPLC systems [29] with binary mobile phases. The present study provides a new approach to the analysis of retention mechanism and lipophilicity of the studied herbicide candidates based on *s*-triazines with RP-UHPLC system with binary and ternary mobile phases and C18 and phenyl columns. The comparative analysis of the retention parameters was carried out applying pattern recognition chemometric tools that provided

useful information and revealed (dis)similarities between the studied compounds and applied chromatographic systems.

2. Materials and Methods

2.1. The Series of 6-Chloro-1,3,5-Triazine Derivatives

The series of the analyzed compounds included eight 6-chloro-1,3,5-triazine derivatives with acyclic substituents (propyl, isopropyl, 2-methylpropyl and isobutyl) and cyclic substituents (cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl) in the positions 2 and 4 of the triazine ring. The molecular structures and IUPAC names of the compounds are given in Figure 1. The compounds were synthesized at the Faculty of Technology and Metallurgy, University of Belgrade, based on the procedure given in the literature [30]. The purity of the substances was checked by gas chromatography, infrared spectroscopy, and nuclear magnetic resonance spectroscopy [30].

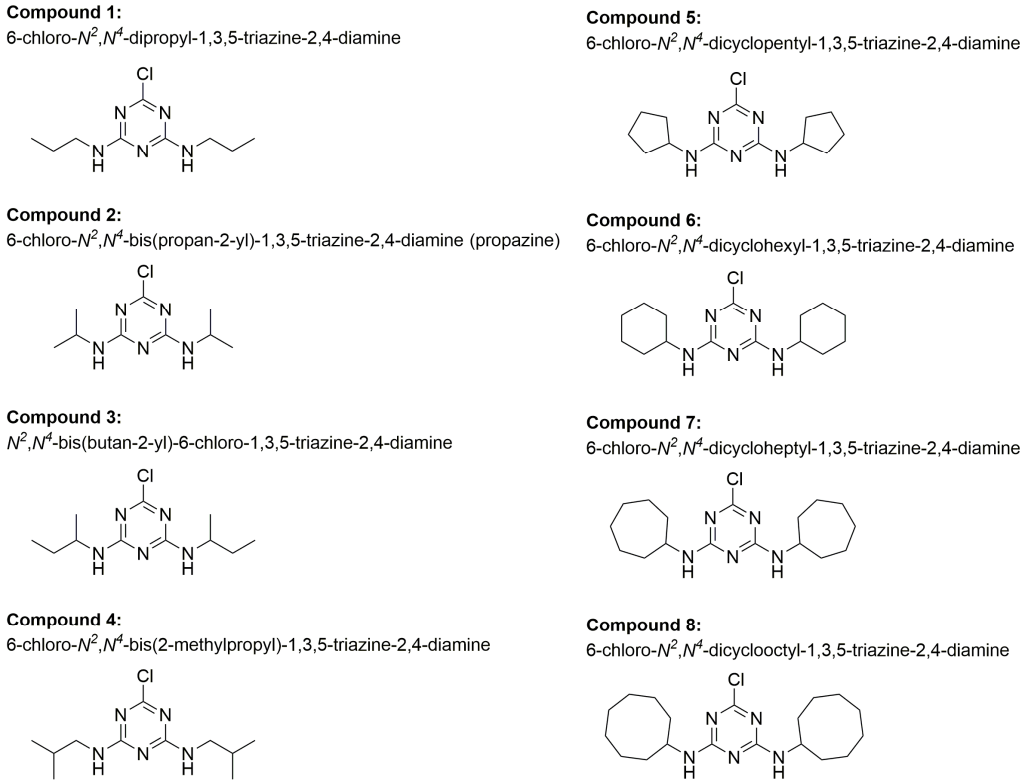

Figure 1. Molecular structures and IUPAC names of the analyzed *s*-triazine derivatives (**1**–**8**).

Compounds **1** and **2**, as well as **3** and **4**, are structural isomers, while compounds **5**–**8** contain the substituents that belong to the homologous series of cycloalkanes. Compound **2** is a commercial herbicide known as propazine, which is considered to be an environmental contaminant [31].

2.2. Chromatographic Analysis

The chromatographic analysis was performed on the UHPLC Agilent 1290 Infinity LC system with Diode Array Detector. Two types of columns were used:

(1) ZOBRAX Eclipse C18, 2.1 × 50 mm, 1.8 µm (1200 bar pressure limit, LC platform, Low Dispersion UHPLC);
(2) ZORBAX Eclipse XDB-Phenyl, 95 Å, 2.1 × 150 mm, 5 µm.

Prior to analysis, the compounds were dissolved in acetone (for HPLC analysis, Carlo Erba, Emmendingen, Germany) in concentration of 1 mg/mL and filtered using Captiva Econofilter (nylon membrane, 25 mm diameter, 0.45 µm pore size, 1000/pk). The injection

volume of the sample was 10 µL. The analysis was carried out, so the column temperature was maintained at 25 °C with the flow of 0.3 mL/min (C18 column) and 0.5 mL/min (phenyl column) under isocratic conditions. The mobile phases used were binary or ternary mixtures of methanol (HPLC gradient grade, J.T. Baker, Phillipsburg, NJ, USA), acetonitrile (for HPLC analysis, Acros Organics, Geel, Belgium), and water (HPLC grade distilled water):

(1) Mobile phase A: Methanol/Water (protic modifier).
(2) Mobile phase B: Methanol/Acetonitrile/Water (mixture of protic and aprotic modifiers).
(3) Mobile phase C: Acetonitrile/Water (aprotic modifier).

The volume fraction of modifiers in mixtures ranged from 0.5 to 0.85 v/v. In the mobile phase B, the volumes of the modifiers were equal. The peaks on chromatograms were recorded at the wavelength of 254 nm.

The chromatographic parameters (the capacity factor, k) were calculated based on the retention time recorded for the peak of a compound (t_r) and the retention time of a peak of the solvent (dead time, usually the first disturbance on the chromatogram, t_0) [26]:

$$k = (t_r - t_0)/t_0 \quad (1)$$

The capacity factor was used in further analysis in its logarithmic form (log$k$). Besides the log$k$ values, which were determined using the certain amount of modifier(s) in the mobile phase, there are logk_0 values that were predicted by extrapolation of the dependence between volume fraction of modifier in the mobile phase (ϕ) and log$k$ values [26]. The parameters logk_0 practically represents the retention of a compound when ϕ = 0 (the retention in a pure water as a mobile phase). The slope of ϕ–log$k$ values (S) also represents the significant retention parameter [26].

Also, the alternative retention parameter (C_0) was calculated based on the following equation [26]:

$$C_0 = -\log k_0/S \quad (2)$$

2.3. Calculation of the Lipophilicity Descriptor

The *in silico* lipophilicity descriptors were calculated based on the software and procedure described in the literature [32]. The programs used for calculation of lipophilicity parameters are the following: ALOGPS 2.1, $SWISS_{ADME}$, MarvinSketch 14.09.15.0 and ChemBioDraw 13 [32]. The consensus logP values were selected as the representative values of the *in silico* lipophilicity parameters and afterwards correlated with the retention data.

2.4. Chemometric Analysis

Chemometric analysis included the application of pattern recognition and ranking techniques such as hierarchical cluster analysis (HCA), principal component analysis (PCA), and sum of ranking differences (SRD). HCA was carried out using NCSS 2023 software [33]. PCA was performed in Statistica v.14 software [34]. SRD analysis was conducted in the program created in Microsoft Excel 2013 [35].

HCA was based on Ward's minimum variance algorithm and the Euclidean distance method. The results are presented in the form of double dendrogram (clustered heat maps). PCA was based on correlations and the number of the significant principal components was selected based on Eigenvalues higher than 1. SRD analysis was performed based on row average as the reference ranking. The HCA and PCA analyses were carried out on non-scaled log$k$ data since all the data were on the same scale. However, the SRD analysis was carried out on the data that included other retention parameters (S and C_0), hence the data were scaled between 0.01 and 0.99 applying *min–max* normalization method. The SRD procedure was validated by comparison of ranks by random numbers (CRRN) approach and seven-fold cross-validation [35].

3. Results and Discussion

The methods for chromatographic lipophilicity estimation of *s*-triazine derivatives in the RP-UHPLC system with C18 and phenyl columns and binary and ternary mobile phases with acetonitrile or/and methanol as modifiers, were developed. A ternary mobile phase was applied due to its unique contribution to the chromatographic selectivity [36] which can be reflected in better differentiation of lipophilicity of structurally similar compounds. The retention factors, $\log k_0$, for all the analyzed compounds were obtained by extrapolation of ϕ–log$k$ linear relationship. Those factors present the retention of the compounds in pure water (which is not possible to determine experimentally for the studied triazines but only by extrapolation). Also, the log$k$ values determined using specific volume fractions of modifiers (ϕ = 0.75 and 0.80) were taken into account in comparative analysis ($\log k_{0.8}$ and $\log k_{0.75}$). It should be emphasized that the ϕ–log$k$ linear relationships were not defined for all the compounds based on the same volume fractions of modifiers due to the practical reasons: for more lipophilic derivatives (compounds **5**–**8**), the range of ϕ values was narrower than in the case of compounds **1**–**4** because higher fractions of water in the mobile phase caused the peak broadening and difficulties with determination of the retention time.

3.1. The Results of RP-UHPLC Analysis on C18-Column

The results of the RP-UHPLC analysis on C18-column are summarized in Table 1. The $\log k_0$ values were defined applying extrapolation based on the graphs presented in Figure 2. Taking into account the presented retention parameters, it can be seen that all the compounds with acyclic substituents (compounds **1**–**4**) have lower retention than the compounds with cycloalkyl substituents (**5**–**8**). In mobile phases A and B, there is a reverse order considering the $\log k_0$ of compounds **4** and **5**, so the experiment showed that compound **4** has higher retention in pure water than compound **5**. Also, the increase of the volume fraction of modifiers led to the decrease in retention of the analyzed compounds. The change between the modifiers used in the analysis in the following order methanol < acetonitrile/methanol < acetonitrile influenced the decrease of the retention. Therefore, the lowest retention was in the system with acetonitrile, whilst the highest retention was determined with methanol as a modifier. Acetonitrile is generally a stronger modifier than methanol due to the presence of carbon-nitrogen triple bond in acetonitrile causing the reduction of hydrogen bonding between the solvent molecules; furthermore, the reduction of enthalpic contribution to the retention when acetonitrile is used is another reason for decrease of the retention [36]. Generally speaking, methanol, as a protic solvent and more polar than acetonitrile, influenced higher retention of the compounds on C18 column. The slope of the ϕ–log$k$ linear relationship is generally higher for more lipophilic compounds (**5**–**8**) than more hydrophilic compounds (**1**–**4**).

Considering the structural isomerism of compounds **1** and **2**, it can be noted that their retentions in all three mobile phases are quite similar; however, some differences can be observed. Namely, compound **1**, which possesses *n*-propyl substituents, has a higher $\log k_0$ parameter than compound **2** with isopropyl substituents. With some exceptions, compound **1** generally has higher retention parameters than compound **2**, particularly in the systems with mobile phases with high volume fractions of modifiers. This could be the consequence of the fact that the *n*-propyl group, as a linear substituent, expresses stronger interactions with C18-stationary phase than isopropyl group as a branched substituent.

In the case of the second pair of structural isomers (compounds **3** and **4**), the differences between the retention parameters determined with mobile phases A and B (Table 1) are more significant than in the case of compounds **1** and **2**. The highest difference is noticeable with methanol as a modifier (mobile phase A) and it decreases when acetonitrile is introduced in the mobile phase (mobile phase B); the lowest difference is recorded in mobile phase C (with acetonitrile as a modifier). Compound **4** has higher retention parameters than compound **3**, except in the mobile phase C, in which their retentions are very close. This could imply that 2-methylpropyl substituents contribute to stronger interactions

with C18 stationary phase than butan-2-yl substituents in the studied molecules when the mobile phase contains methanol or methanol mixed with acetonitrile.

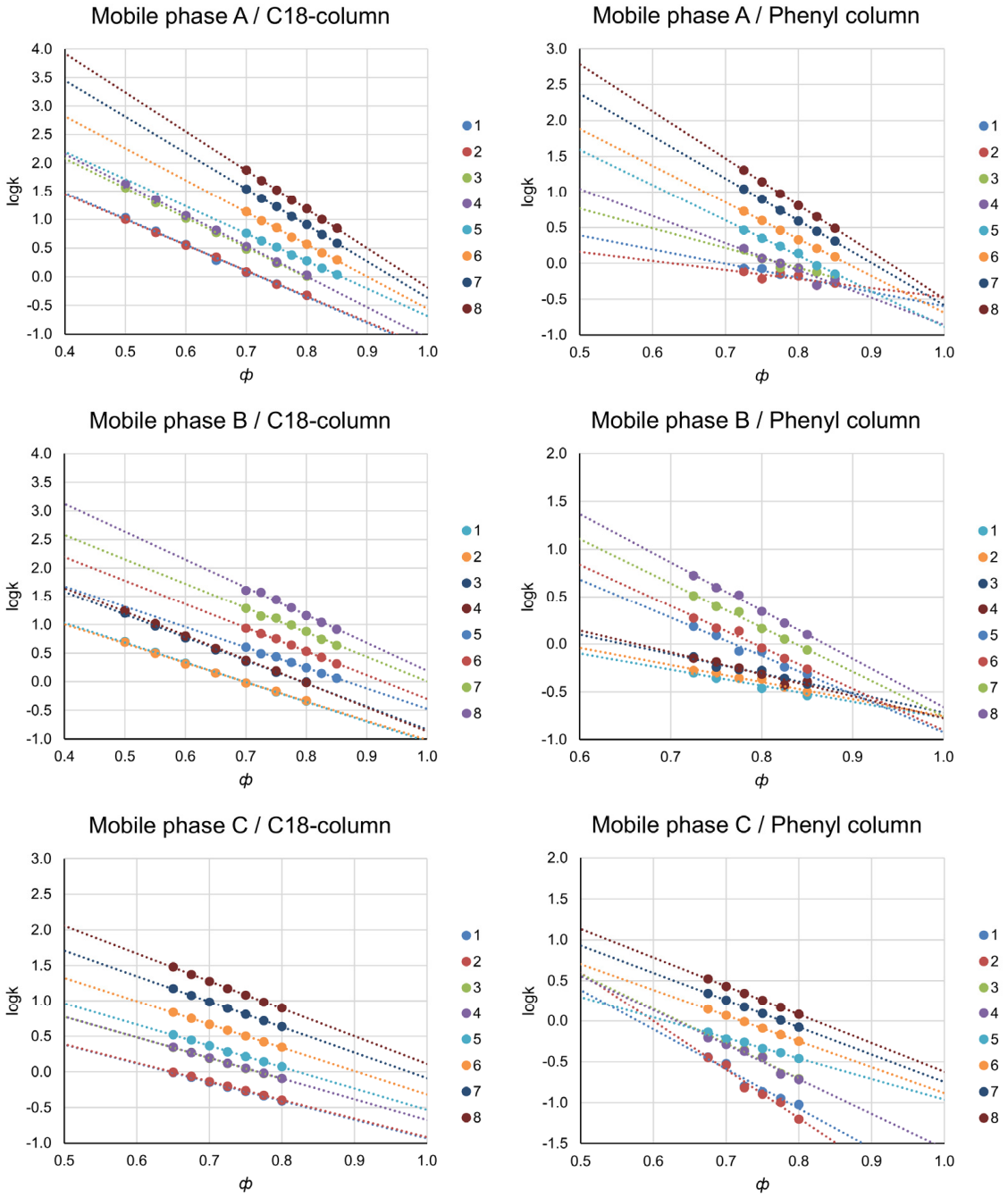

Figure 2. The comparison of the ϕ–log$k$ dependences determined using C18 and phenyl columns.

Considering compounds **5**–**8** as derivatives that contain cycloalkyl substituents which belong to the homologous series (cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl), the increase in retention in terms of ring size is expected. Hence, compound **8** has the highest retention in all applied chromatographic systems with C18 stationary phase. Also, quite interesting observation is the fact that compound **5**, which has two cyclopentyl rings and higher lipophilicity, has lower retention in the systems with mobile phases A and B, than compound **4**, which has two 2-methylpropyl groups. Only in the mobile phase C, does the opposite phenomenon occurs.

Table 1. The retention data obtained using C18-column.

Compound	Mobile Phase	$\log k_{0m}$ [1]	$\log k_{0.8m}$	$\log k_{0.75m}$	S_m	C_{0m}
1	A	3.290	−0.321	−0.124	−4.555	0.722
2		3.224	−0.320	−0.131	−4.454	0.724
3		4.165	0.013	0.237	−5.223	0.797
4		4.307	0.031	0.259	−5.377	0.801
5		4.115	0.279	0.517	−4.802	0.857
6		5.071	0.568	0.860	−5.633	0.900
7		5.990	0.912	1.227	−6.360	0.942
8		6.657	1.189	1.525	−6.845	0.973
Compound	**Mobile Phase**	$\log k_{0ma}$	$\log k_{0.8ma}$	$\log k_{0.75ma}$	S_{ma}	C_{0ma}
1	B	2.411	−0.335	−0.179	−3.456	0.698
2		2.348	−0.331	−0.167	−3.366	0.698
3		3.191	−0.004	0.165	−4.028	0.792
4		3.321	−0.014	0.188	−4.189	0.793
5		3.107	0.244	0.437	−3.583	0.867
6		3.853	0.530	0.750	−4.155	0.927
7		4.293	0.879	1.113	−4.288	1.001
8		5.081	1.156	1.440	−4.892	1.039
Compound	**Mobile Phase**	$\log k_{0a}$	$\log k_{0.8a}$	$\log k_{0.75a}$	S_a	C_{0a}
1	C	1.693	−0.408	−0.277	−2.628	0.644
2		1.688	−0.397	−0.265	−2.605	0.648
3		2.228	−0.095	0.044	−2.909	0.766
4		2.225	−0.093	0.048	−2.901	0.767
5		2.462	0.070	0.212	−2.997	0.822
6		2.967	0.344	0.502	−3.286	0.903
7		3.507	0.634	0.809	−3.597	0.975
8		4.005	0.899	1.084	−3.895	1.028

[1] The meaning of the letters in the subscript of retention parameters: a—determined using acetonitrile as modifier; m—determined using methanol as modifier; ma—determined using methanol and acetonitrile as modifiers in the same volume ratio. A—methanol/water mobile phase, B—methanol/acetonitrile/water mobile phase, C—acetonitrile/water mobile phase.

In silico lipophilicity parameters of the analyzed compounds, based on consensus value of various logP values calculated using different approaches [32], are presented in Table A1. All the retention parameters determined on C18 stationary phase are very well correlated with ConsensusLogP descriptor, as it can be seen from the data presented in Table A2. The determination coefficients for almost all linear equations are very close to 1. Therefore, the retention parameters determined applying RP(C18)-UHPLC system with methanol/water, methanol/acetonitrile/water and acetonitrile/water mobile phases can be considered chromatographic lipophilicity measures of the studied *s*-triazine derivatives.

3.2. The Results of RP-UHPLC on Phenyl Column

The retention data obtained when applying the RP-UHPLC analysis on phenyl column are presented in Table 2. As in the case of C18 column, the $\log k_0$ values were defined based on extrapolation on the graphs given in Figure 2. The retention behavior of the studied compounds in the system with phenyl column is influenced not only by cavity formation, dispersive forces, dipole–dipole interactions, hydrogen bonding, and lipophilicity, but also by π–π interactions between π-electrons of double bond(s) present in analyte molecules (*s*-triazine ring) and π-electrons of the phenyl stationary phase. These interactions are sometimes crucial for separation of structurally similar compounds, although hydrophobic interaction has stronger influence on the retention [37].

Table 2. The retention data obtained using phenyl column.

Compound	Mobile Phase	$\log k_{0m}$ [1]	$\log k_{0.8m}$	$\log k_{0.75m}$	S_m	C_{0m}
1	A	1.379	−0.389	−0.072	−1.978	0.697
2		0.790	−0.176	−0.216	−1.261	0.626
3		2.147	−0.065	0.066	−2.763	0.777
4		2.947	−0.068	0.070	−3.808	0.774
5		4.068	0.139	0.349	−4.952	0.821
6		4.455	0.329	0.601	−5.145	0.866
7		5.331	0.592	0.899	−5.916	0.901
8		6.061	0.809	1.145	−6.557	0.924
Compound	**Mobile Phase**	$\log k_{0ma}$	$\log k_{0.8ma}$	$\log k_{0.75ma}$	S_{ma}	C_{0ma}
1	B	0.913	−0.464	−0.357	−1.686	0.542
2		0.229	−0.366	−0.302	1.035	−1.791
3		0.406	−0.277	−0.242	1.325	−2.043
4		1.521	−0.317	−0.186	−2.296	0.663
5		3.090	−0.079	0.092	−4.015	0.770
6		3.448	−0.040	0.169	−4.351	0.792
7		3.898	0.164	0.396	−4.652	0.838
8		4.411	0.344	0.597	−5.071	0.870
Compound	**Mobile Phase**	$\log k_{0a}$	$\log k_{0.8a}$	$\log k_{0.75a}$	S_a	C_{0a}
1	C	2.808	−1.025	−0.869	−4.850	0.579
2		3.569	−1.202	−0.899	−5.950	0.600
3		2.720	−0.708	−0.437	−4.281	0.635
4		2.678	−0.718	−0.451	−4.239	0.632
5		1.555	−0.462	−0.338	−2.518	0.618
6		2.287	−0.251	−0.090	−3.171	0.721
7		2.606	−0.078	0.091	−3.354	0.777
8		2.890	0.081	0.255	−3.512	0.823

[1] The meaning of the letters in the subscript of retention parameters: a—determined using acetonitrile as modifier; m—determined using methanol as modifier; ma—determined using methanol and acetonitrile as modifiers in the same volume ratio. A—methanol/water mobile phase, B—methanol/acetonitrile/water mobile phase, C—acetonitrile/water mobile phase.

Based on the data given in Table 2, it can be concluded that, considering the majority of the retention parameters, the retention of compounds **1**–**4** is lower than the retention of compounds **5**–**8** in the mobile phases A and B. The compounds from the two groups (**1**–**4** and **5**–**8**) cannot be discriminated based on the $\log k_0$ values determined using the mobile phase C, but only based on $\log k_{0.8a}$ and $\log k_{0.75a}$ parameters.

Considering the parameters S_{ma} and C_{0ma}, some significant deviations from expected results are observable for compounds **2** and **3**. Even after repeating the analysis, similar values were obtained. These deviations are the reasons for some poor correlations between ConsensusLogP values and S_{ma}, C_{0ma}, $\log k_{0a}$, and S_a retention parameters (bold R^2 values in Table A2). The rest of the retention parameters determined using phenyl column are very well correlated with ConsensusLogP values ($R^2 > 0.8$) and, therefore, can be considered to be lipophilicity measured of the analyzed compounds. These parameters mostly reflect the hydrophobic interactions in the applied chromatographic system. However, considering the fact that the *s*-triazine ring is an aromatic ring, π–π interactions contribute to the retention as well, so slightly worse correlations between the retention parameters determined by phenyl column and *in silico* lipophilicity were obtained than in the case of C18 column.

The phenyl column provided better selectivity regarding the retention of the pairs of structural isomers (compounds **1** and **2** and compounds **3** and **4**). The differences between their retention parameters are generally higher than on C18 column. Introducing acetonitrile in the mobile phase, the parameter *S* decreased for all compounds with cyclic substituents. Acetonitrile reduces π-π interactions because the triple bond between carbon and nitrogen in acetonitrile interferes with them leading to the reduced retention of a solute.

3.3. Comparative Analysis of Chromatographic Behavior

To compare the retention behavior of the analyzed compounds in different chromatographic systems, several chemometric methods were applied. The logk_0 and S parameters were determined based on the graphs presented in Figure 2. The relationships defined using C18 column are consistent for all three mobile phases and the retention behavior of the compounds is comparable and predictable. On the other hand, the graphs defined for the phenyl column show certain deviations for compounds with acyclic substituents (**1**–**4**). For the same mobile phase composition, the retention increased from compound **1** to compound **4**, with several exceptions on phenyl column, particularly when higher volume fraction of modifier was used (the overlapping of the points or reversed retention order occurred).

How retention changes when the phenyl column is used instead of C18 column can be seen in Figure A1 (Appendix A), in which the chromatograms of the representative compound (**7**) are presented. The retention time of compound **7** in the system with phenyl column is around two times shorter than the retention time achieved in the system with C18 column.

The first step of the chemometric analysis was a hierarchical cluster analysis of the retention parameters in the form of clustered heat maps (double dendrograms). The obtained results are presented in Figure 3.

Taking into account the log$k$ values (Figure 3a), it can be noted that the highest retention (logk_0) was recorded in the C18 phase, followed by logk_0 determined on the phenyl column. The clustering of the compounds is very clear: the compounds with alkyl substituents (**1**–**4**) belong to a separate cluster (vertical pale green cluster), while the compounds with cycloalkyl substituents (**5**–**8**) are separated into two sub-clusters, so compounds **5** and **6** belong to the first (vertical pale blue sub-cluster) and compounds **7** and **8** to the second sub-cluster (vertical pale red sub-cluster). The HCA indicates that there is a significant difference in terms of retention behavior, expressed as log$k$ values, of *s*-triazine derivatives with acyclic and cyclic substituents.

The HCA based on C_0 parameters indicates a different clustering (Figure 3b). Namely, compounds **5**–**8** are placed in a separate cluster (pale green vertical cluster) and their alternative parameters of chromatographic lipophilicity are different from the rest of the compounds. The grouping of compounds **1**–**4** has a different pattern than the grouping based on log$k$ values. Here, compounds **1** and **4** belong to the same sub-cluster (vertical pale red sub-cluster), whilst compounds **2** and **3** are placed in a separate sub-cluster (vertical pale blue sub-cluster) due to the significant differences between their C_{0ma} parameters defined on phenyl column.

Taking into account the S parameters, the HCA resulted in one separate cluster (vertical pale green cluster) with compounds **5**–**8** and two sub-clusters, among which one contains compounds **1** and **4** (vertical pale red sub-cluster) and the second contains compounds **2** and **3** (vertical pale blue sub-cluster) (Figure 3c). The main reason for the clustering of compounds **1**–**4** is the high similarity between S_{ma} values determined on the phenyl column. This parameter is actually marked as an outlier (it does not belong to any horizontal cluster) in Figure 3c.

Considering all the HCA results, it can be concluded that the retention of *s*-triazine derivatives with cycloalkyl substituents is statistically different from the retention of derivatives with alkyl substituents. The behavior of structural isomers are similar with regard to one aspect (log$k$), but different when considering the other (C_0 and S).

In order to gain an overview of the similarities and dissimilarities of the analyzed derivatives in the space of all retention parameters, the PCA was applied. The first PCA model was based on log$k$ parameters and covered 98.60% of total variance (PC1 covers 92.58% and PC2 6.02%). The second PCA model took into account alternative chromatographic lipophilicity measures (S and C_0). This model covers 94.27% of total variability so the PC1 has 83.63% and PC2 10.64% of total variability.

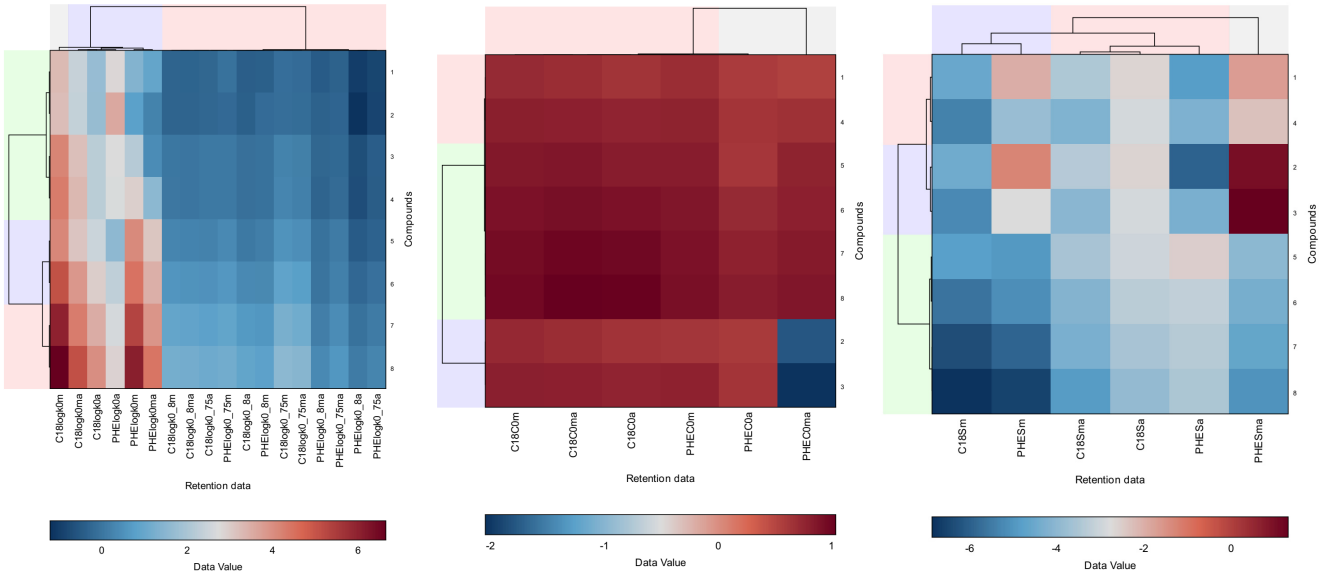

Figure 3. The clustering of (**a**) log*k* parameters, (**b**) C_0 parameters, and (**c**) *S* parameters, based on Ward's minimum variance algorithm.

The results of the first PCA model are presented in Figure 4 in the form of loadings (Figure 4a) and score plot (Figure 4b). Going along the PC1 axis on the score plot, the clear separation of the compounds based on the presence of alkyl and cycloalkyl substituents in their structure exists. The compounds are distributed along the PC1 axis so compounds **6**, **7,** and **8** are placed towards the negative end, compound **5** is on the 0 vertical axis, and compounds **1**–**4** are located on the positive PC1 end. The projections of compounds **1** and **2**, as well as **3** and **4**, overlap on the PC1 since they have similar values of parameters that are located on the negative end of the PC1 axis on the loadings plot (the majority of the log*k* values). The only exception is the PHElogk_{0a} parameter which has the strongest influence on the distribution of the compounds along the PC2 axis (Figure 4a). Based on the obtained results, it can be said that compounds **5**–**8** are arranged in the same way as the homologous series of cycloalkane substituents, and that compounds from group **1**–**4** are arranged so that the structural isomers coincide on the PC1 axis.

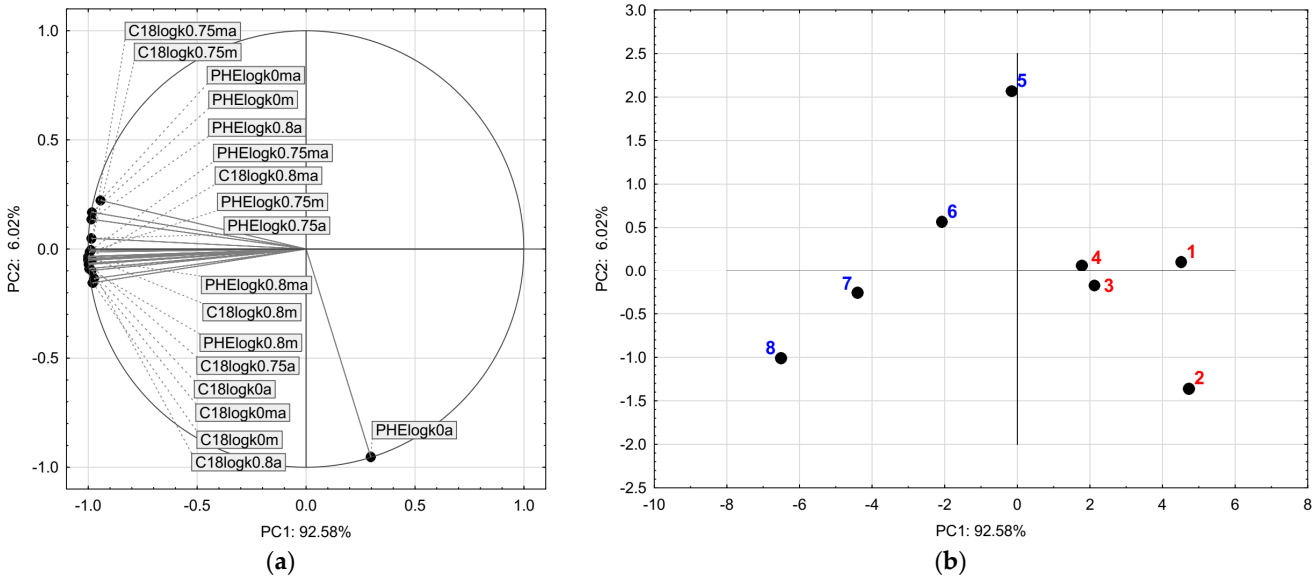

Figure 4. The results of the PCA based on log*k* parameters: (**a**) loadings and (**b**) score plot.

The PCA model based on alternative chromatographic lipophilicity parameters (S and C_0) is presented in Figure 5. The loadings plot (Figure 5a) indicates the strong influence of the analyzed parameters on the PC1 axis. The C_0 parameters have a negative influence, whilst the S parameters express positive influences on the distribution of the compounds on the PC1 axis. PHES$_{ma}$ and PHEC$_{0ma}$ parameters have the highest influence on the PC2 axis.

The distribution of the compounds along the PC1 axis is similar to their distribution presented in Figure 4b. There is a clear separation between the *s*-triazines with alkyl and cycloalkyl substituents. The close projection of the structural isomers (**1** and **2**, **3** and **4**) is also noticeable on the PC1 axis.

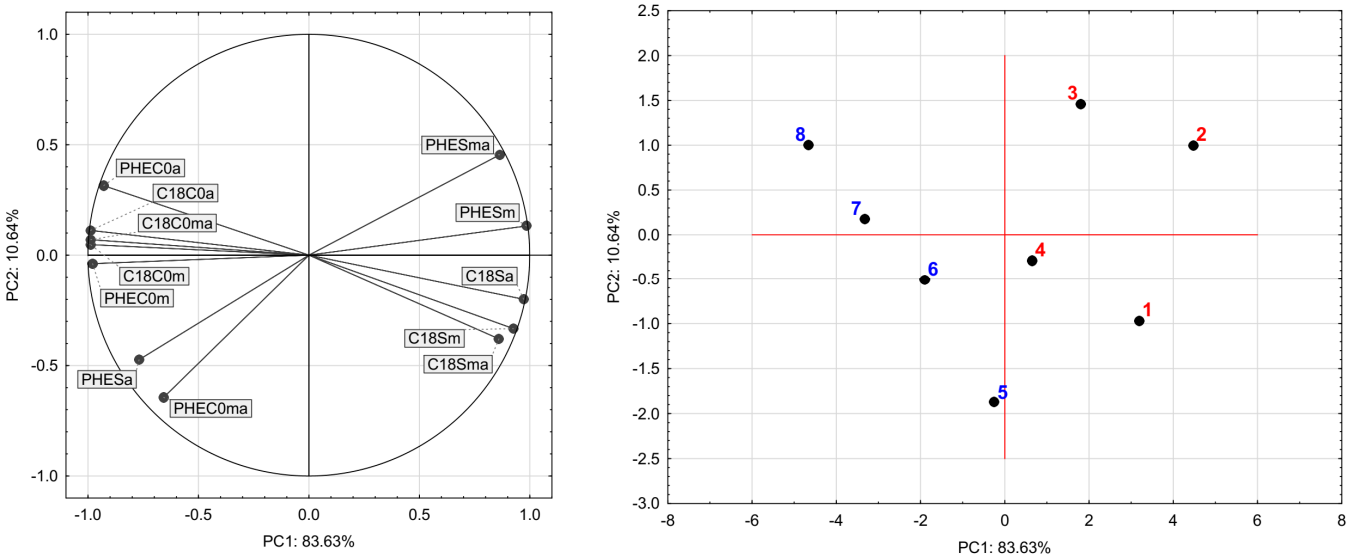

Figure 5. The results of the PCA based on alternative chromatographic lipophilicity parameters (S and C_0): (**a**) loadings and (**b**) score plot.

Considering that the lipophilicity is closely related to the retention behavior of the analyzed *s*-triazine derivatives, it can be concluded that the PCA showed a clear separation between the compounds so that more lipophilic compounds are located towards the negative end whilst the less lipophilic compounds are placed towards the positive end of the PC1 axis in Figures 4b and 5b.

There are certain differences between the retention behavior of the analyzed compounds on C18 and phenyl columns; however, the retention parameters determined with these columns are highly correlated, as can be seen from the heat map of the squared values of the Pearson correlation matrix (Figure A2). The lipophilicity determined on the phenyl column can be more differentiated for structurally similar samples (particularly for *s*-triazines with alkyl substituents) in accordance with the theory of chromatographic mechanisms.

Despite the fact that HCA and PCA can provide very useful information regarding similarities and dissimilarities among the retention parameters of the analyzed *s*-triazine derivatives, these methods do not reveal whether those (dis)similarities are statistically significant or not. In order to overcome this, the SRD analysis was applied. As a non-parametric comparison, the SRD analysis was applied to rank determined chromatographic lipophilicity. The ranking was based on consensus since the reference ranking (so-called "golden standard") was the arithmetic mean average. This consensus-based approach has two main advantages: it cancels out the systematic and random errors, and the arithmetic mean estimates the most probable observation [38]. On the SRD graph, the retention parameters are arranged in ascending order in terms of SRD values so the variables that

have lower SRD values are placed closer to the reference ranking. The ranking procedure was validated by the CRRN approach and sevenfold cross-validation.

The results of the ranking analysis are presented in Figure 6 and Table 3. Figure 6 shows the comparison of ranks with random numbers so the *x*-axis and left *y*-axis present the interval scaled SRD numbers (%), whilst the right *y*-axis shows the relative frequencies of random numbers. The SRD score number is scaled between 0 and 100 when applying the following equation:

$$\text{SRD (\%)} = 100 \times \text{SRD}/\text{SRD}_{\text{max}} \quad (3)$$

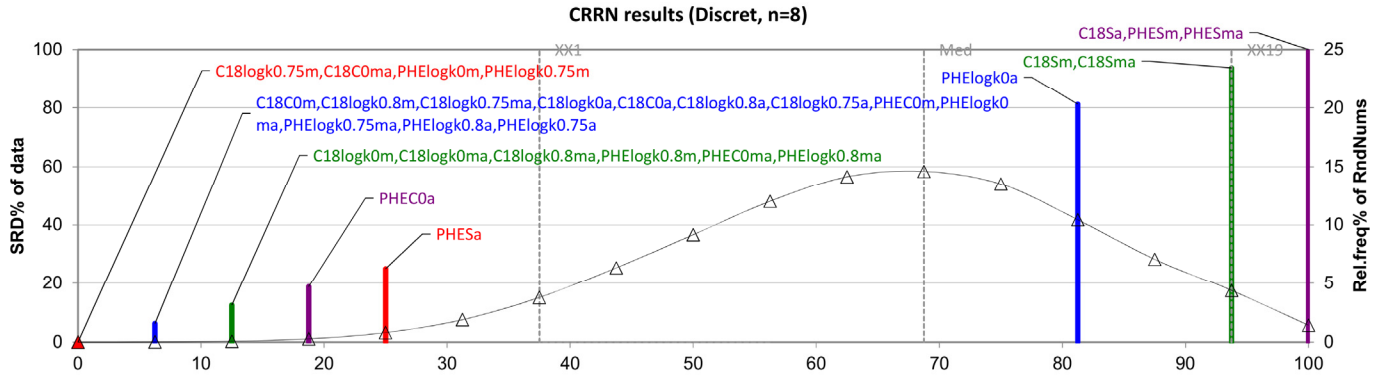

Figure 6. The ranking of the standardized chromatographic lipophilicity parameters determined using C18 and phenyl (PHE) columns and different mobile phases (a—determined using acetonitrile as modifier; m—determined using methanol as modifier; ma—determined using methanol and acetonitrile as modifiers in the same volume ratio); the statistical characteristics of the function of theoretical distribution are first icosaile (5%), XX1 = 12; first quartile, Q1 = 18; median, Med = 22; last quartile, Q3 = 24; last icosaile (95%), XX19 = 30.

The results reveal that the majority of parameters are separated into three main groups relatively close to the reference ranking. Also, there are some parameters that are placed alone on the SRD graph, as well as a group of parameters which is placed at the end of *x*-axis (furthest from the ideal rank).

The parameters that have the same ranking as the reference ranking (SRD = 0) are placed in the first group; that includes: C18log$k_{0.75m}$, C18C_{0ma}, PHElogk_{0m}, and PHElog$k_{0.75m}$. Therefore, these experimental parameters can be considered the best choice for lipophilicity measures among all determined parameters. All of these parameters were determined using the mobile phase with methanol as a modifier (C18log$k_{0.75m}$, PHElogk_{0m}, and PHElog$k_{0.75m}$) or methanol together with acetonitrile in ternary mixtures (C18C_{0ma}).

On the other hand, the parameters C18S_a, PHES_m, and PHES_{ma} are placed in the same group, which is furthest from the ideal rank, meaning that these parameters are not the ideal choice for chromatographic lipophilicity estimation. The parameters PHElogk_{0a}, C18S_{ma}, and C18S_m are also significantly distant from the reference ranking, meaning that their application as lipophilicity measures is questionable.

The rest of the retention parameters are placed into three groups, among which the group marked as blue on the graph in Figure 6 contains the highest number of the parameters. All of these parameters are close to the reference ranking and can therefore be considered good chromatographic lipophilicity measures. The green group on the graph contains the parameters that are ranked further from the reference ranking than the parameters from the red and blue group; nevertheless, these parameters can also be considered acceptable lipophilicity measures of the analyzed series of *s*-triazine derivatives. No strict separation of the chromatographic parameters determined using C18 column and phenyl column was detected.

The SRD results also indicate that among the alternative lipophilicity parameters (*S* and C_0), *S* parameters are not a good choice for the lipophilicity depiction of the studied

series of *s*-triazine derivatives. All *S* parameters are placed at a significant distance from the reference ranking, as can be noticed in Figure 6. Better solutions from alternative chromatographic lipophilicity measures are C_0 parameters which are located much closer to the reference ranking than *S* parameters.

The probabilities of random ranking (Table 3) are very low for the parameters from the first three groups on the SRD graphs, meaning that their rankings are not of a random nature. However, those probabilities are quite high for the rest of the parameters. Besides the CRRN procedure, the seven-fold cross-validation method confirmed the validity of conducted SRD analysis.

Table 3. The SRD values and probabilities of random ranking of the standardized chromatographic lipophilicity measures.

Retention Parameter	SRD	*p*%:	$x <$ SRD $\leq y$
C18log$k_{0.75m}$	0	0	2.48×10^{-3}
C18C_{0ma}	0	0	2.48×10^{-3}
PHElogk_{0m}	0	0	2.48×10^{-3}
PHElog$k_{0.75m}$	0	0	2.48×10^{-3}
C18C_{0m}	2	2.48×10^{-3}	1.98×10^{-2}
C18log$k_{0.8m}$	2	2.48×10^{-3}	1.98×10^{-2}
C18log$k_{0.75ma}$	2	2.48×10^{-3}	1.98×10^{-2}
C18logk_{0a}	2	2.48×10^{-3}	1.98×10^{-2}
C18C_{0a}	2	2.48×10^{-3}	1.98×10^{-2}
C18log$k_{0.8a}$	2	2.48×10^{-3}	1.98×10^{-2}
C18log$k_{0.75a}$	2	2.48×10^{-3}	1.98×10^{-2}
PHEC_{0m}	2	2.48×10^{-3}	1.98×10^{-2}
PHElogk_{0ma}	2	2.48×10^{-3}	1.98×10^{-2}
PHElog$k_{0.75ma}$	2	2.48×10^{-3}	1.98×10^{-2}
PHElog$k_{0.8a}$	2	2.48×10^{-3}	1.98×10^{-2}
PHElog$k_{0.75a}$	2	2.48×10^{-3}	1.98×10^{-2}
C18logk_{0m}	4	1.98×10^{-2}	0.10
C18logk_{0ma}	4	1.98×10^{-2}	0.10
C18log$k_{0.8ma}$	4	1.98×10^{-2}	0.10
PHElog$k_{0.8m}$	4	1.98×10^{-2}	0.10
PHEC_{0ma}	4	1.98×10^{-2}	0.10
PHElog$k_{0.8ma}$	4	1.98×10^{-2}	0.10
PHEC_{0a}	6	0.10	0.39
PHES_a	8	0.39	1.20
XX1	12	3.10	6.87
Q1	18	22.37	34.40
Med	22	48.56	63.17
Q3	24	63.17	76.70
PHElogk_{0a}	26	76.70	87.14
C18S_m	30	94.20	98.57
C18S_{ma}	30	94.20	98.57
XX19	30	94.20	98.57
C18S_a	32	98.57	100.00
PHES_m	32	98.57	100.00
PHES_{ma}	32	98.57	100.00

3.4. Further Recommendations

Considering the fact that the obtained results define the experimental lipophilicity of the studied derivatives, these results can be considered significant guidelines for the development of new chromatographic methods for the separation and isolation of the studied compounds from environmental samples—they predict their behavior in the applied RP-UHPLC chromatographic system based on their lipophilicity.

It is worth investigating the chromatographic behavior of the series of studied triazine derivatives in the chromatographic system with gradient elution which could probably

provide better selectivity than isocratic elution. Additionally, it would be interesting to compare the experimental lipophilicity determined under isocratic vs. gradient conditions.

4. Conclusions

In the present study, a comprehensive analysis of the chromatographic behavior of a series of 6-chloro-1,3,5-triazines, with alkyl and cycloalkyl substituents, was carried out using reversed phase ultra high performance liquid chromatography system with C18 and phenyl columns and binary and ternary mobile phases with methanol and acetonitrile as modifiers. The retention behavior of the studied compounds was expressed by capacity factors (log$k$ and logk_0) and alternative chromatographic lipophilicity measures (S and C_0). The comparative analysis of chromatographic behavior was conducted through the application of chemometric methods: hierarchical cluster analysis, principal component analysis, and sum of ranking differences.

Based on the results, it can be concluded that the compounds behave in the C18 system in accordance with hydrophobic interaction (which are dominant), whilst in the system with phenyl column, besides hydrophobic interactions, π–π interactions also contribute to the retention. The correlations of the retention parameters, determined on the C18 column, with *in silico* lipophilicity measures are better than in the case of the retention parameters estimated on phenyl column. As a protic solvent that is more polar than acetonitrile, methanol influenced higher retention of the compounds on the C18 column than acetonitrile. The same conclusion is true for the retention recorded on the phenyl column.

Hierarchical cluster analysis and principal component analysis indicated a clear separation of the compounds based on the presence of alkyl and cycloalkyl substituents according to all retention parameters. Sum of ranking differences analysis pointed out that C18log$k_{0.75\mathrm{m}}$, C18$C_{0\mathrm{ma}}$, PHElog$k_{0\mathrm{m}}$, and PHElog$k_{0.75\mathrm{m}}$ parameters can be considered to be the best chromatographic lipophilicity measures, while the application of the parameters PHElog$k_{0\mathrm{a}}$, C18S_{m}, C18S_{ma}, C18S_{a}, PHES_{m}, and PHES_{ma} for the lipophilicity estimation of the studied compounds is questionable due to their distance from the reference ranking. Considering the alternative chromatographic lipophilicity measures, C_0 parameters are a better solution for lipophilicity estimation than S parameters.

Further research can be related to the development of methods that will be suitable for the separation of the studied triazine derivatives from the environmental samples and their quantitative chromatographic analysis. Also, the chromatographic analysis based on the gradient elution of the analyzed series of triazine derivatives would be worth further investigating.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CRRN—comparison of ranks by random numbers, **HCA**—hierarchical cluster analysis, **HPTLC**—high performance thin layer chromatography, **HPLC**—high performance liquid chromatography, **NP**—normal phase, **PCA**—principal component analysis, **RP**—reversed phase, **SRD**—sum of ranking difference, **UHPLC**—ultra high performance liquid chromatography.

Appendix A

Table A1. Consensus *in silico* lipophilicity measures of the studied *s*-triazine derivatives [32].

Compounds	ConsensusLogP
1	2.83
2	2.63
3	3.57
4	3.49
5	3.70
6	4.53
7	5.36
8	6.28

Table A2. The correlations between consensus *in silico* lipophilicity measures and retention parameters of the analyzed *s*-triazine derivatives.

$y = k \times x + n$				
x	y	k	n	R^2
C18logk_{0m}	ConsensusLogP	0.9682	0.6831	0.9883
C18S_m	ConsensusLogP	−0.6562	−2.7497	0.9427
C18C_{0m}	ConsensusLogP	0.0725	0.5461	0.9294
C18log$k_{0.8m}$	ConsensusLogP	0.4372	−1.4761	0.9797
C18log$k_{0.75m}$	ConsensusLogP	0.4802	−1.3976	0.9803
C18logk_{0ma}	ConsensusLogP	0.7297	0.4965	0.9781
C18S_{ma}	ConsensusLogP	−0.3643	−2.5198	0.8113
C18C_{0ma}	ConsensusLogP	0.1003	0.4457	0.9468
C18log$k_{0.8ma}$	ConsensusLogP	0.4321	−1.4834	0.9816
C18log$k_{0.75ma}$	ConsensusLogP	0.4649	−1.4140	0.9834
C18logk_{0a}	ConsensusLogP	0.6618	−0.0822	0.9936
C18S_a	ConsensusLogP	−0.3636	−1.6301	0.9980
C18C_{0a}	ConsensusLogP	0.1102	0.3731	0.9572
C18log$k_{0.8a}$	ConsensusLogP	0.3732	−1.3918	0.9879
C18log$k_{0.75a}$	ConsensusLogP	0.3889	−1.3048	0.9887
PHElogk_{0m}	ConsensusLogP	1.4221	−2.3600	0.8947
PHES_m	ConsensusLogP	−1.3973	1.6090	0.8459
PHEC_{0m}	ConsensusLogP	0.0751	0.4945	0.8609
PHElog$k_{0.8m}$	ConsensusLogP	0.3141	−1.1252	0.9500
PHElog$k_{0.75m}$	ConsensusLogP	0.3811	1.1875	0.9725
PHElogk_{0ma}	ConsensusLogP	1.1770	−2.5252	0.7940
PHES_{ma}	ConsensusLogP	−1.5211	3.6937	**0.5723**
PHEC_{0ma}	ConsensusLogP	0.5082	−1.9772	**0.2664**
PHElog$k_{0.8ma}$	ConsensusLogP	0.2151	−1.0000	0.9399
PHElog$k_{0.75ma}$	ConsensusLogP	0.2692	−1.0688	0.9347
PHElogk_{0a}	ConsensusLogP	−0.0078	2.9538	**0.0296**
PHES_a	ConsensusLogP	0.5313	−6.1353	**0.3815**
PHEC_{0a}	ConsensusLogP	0.0697	0.3909	0.9616
PHElog$k_{0.8a}$	ConsensusLogP	0.3441	−1.9384	0.9224
PHElog$k_{0.75a}$	ConsensusLogP	0.3210	−1.6419	0.9393

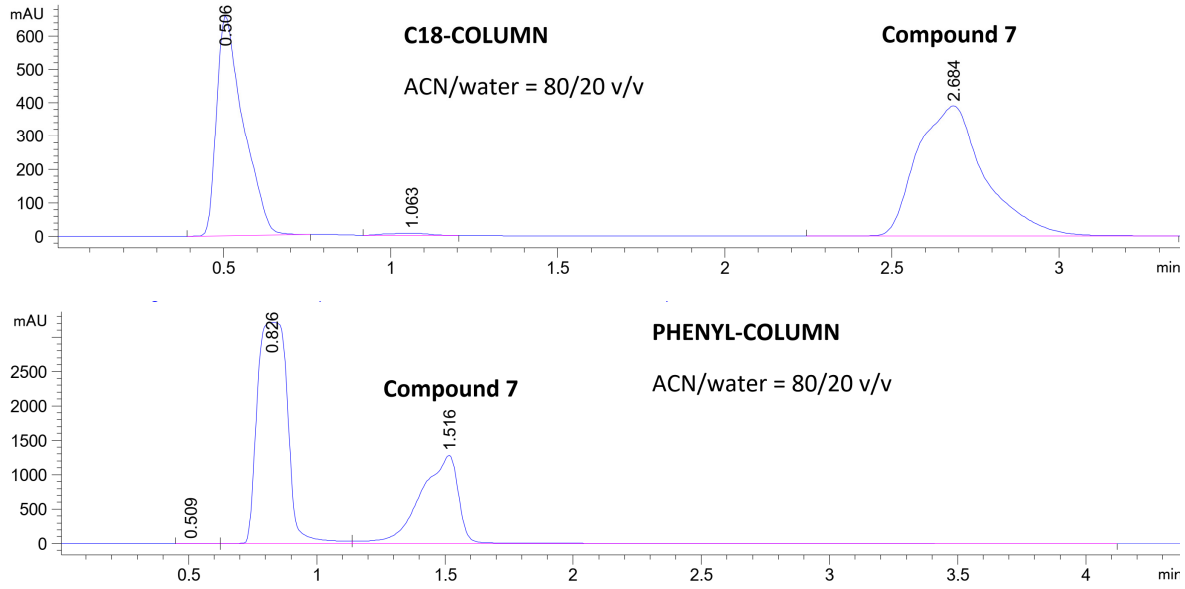

Figure A1. Representative chromatograms of compound **7** in two main applied chromatographic systems.

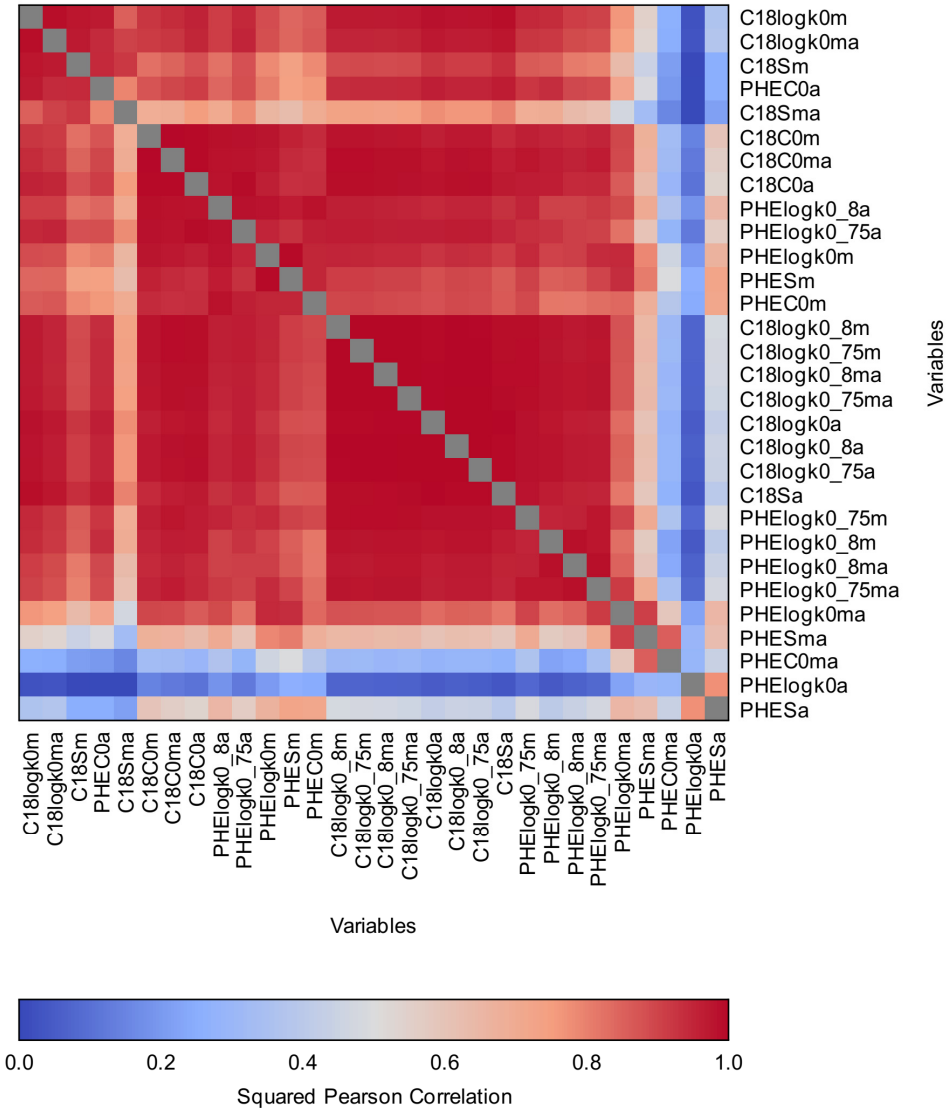

Figure A2. Heat map of the squared values of the Pearson correlation matrix.

References

1. Leoci, R.; Ruberti, M. Pesticides: An Overview of the Current Health Problems of Their Use. *J. Geosci. Environ. Prot.* **2021**, *9*, 1–20. [CrossRef]
2. Akash, S.; Sivaprakash, B.; Rajamohan, N.; Muruga Pandiyan, C.; Vo, D.V.N. Pesticide pollutants in the environment—A critical review on remediation techniques, mechanism and toxicological impact. *Chemosphere* **2022**, *301*, 134754. [CrossRef] [PubMed]
3. Rajmohan, K.S.; Chandrasekaran, R.; Varjani, S. A Review on Occurrence of Pesticides in Environment and Current Technologies for Their Remediation and Management. *Indian J. Microbiol.* **2020**, *60*, 125–138. [CrossRef]
4. Klausener, A.; Raming, K.; Stenzel, K. Modern Tools for Drug Discovery in Agricultural Research. In *Pesticide Chemistry, Crop Protection, Public Health, Environmental Safety*; Ohkawa, H., Miyagawa, H., Lee, P.W., Eds.; Wiley-VCH: Weinheim, Germany, 2007; pp. 55–63.
5. World Health Organization (WHO). Available online: https://www.who.int/news-room/fact-sheets/detail/pesticide-residues-in-food (accessed on 27 September 2023).
6. Pathak, V.M.; Verma, V.K.; Rawat, B.S.; Kaur, B.; Babu, N.; Sharma, A.; Dewali, S.; Yadav, M.; Kumari, R.; Singh, S.; et al. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. *Front. Microbiol.* **2022**, *13*, 962619. [CrossRef] [PubMed]
7. Gianessi, L.P. Benefits of Triazine Herbicides. In *Triazine Herbicides: Risk Assessment*; Ballantine, L.G., McFarland, J.E., Hackett, D.S., Eds.; American Chemical Society: Washington, DC, USA, 1998; pp. 1–8.
8. Salaković, B.; Kovačević, S.; Karadžić Banjac, M.; Podunavac-Kuzmanović, S.; Jevrić, L.; Pajčin, I.; Grahovac, J. New Perspective on Comparative Chemometric and Molecular Modeling of Antifungal Activity and Herbicidal Potential of Alkyl and Cycloalkyl *s*-Triazine Derivatives. *Processes* **2023**, *11*, 358. [CrossRef]
9. Riyahi, Z.; Asadi, P.; Hassanzadeh, F.; Khodamoradi, E.; Gonzalez, A.; Karimi Abdolmaleki, M. Synthesis of novel conjugated benzofuran-triazine derivatives: Antimicrobial and in-silico molecular docking studies. *Heliyon* **2023**, *9*, e18759. [CrossRef] [PubMed]
10. Wang, R.; Zhang, S.; Cai, M.; Zhu, J.; Xu, Z.; Zheng, H.; Xiao, K.; Wang, F. Screening triazine herbicides in drinking water in the Yangtze River Delta, China: Occurrence and health risk. *J. Hazard. Mater. Adv.* **2023**, *10*, 100277. [CrossRef]
11. Tchounwou, P.B.; Wilson, B.; Ishaque, A.; Ransome, R.; Huang, M.-J.; Leszczynski, J. Toxicity Assessment of Atrazine and Related Triazine Compounds in the Microtox Assay, and Computational Modeling for Their Structure-Activity Relationship. *Int. J. Mol. Sci.* **2000**, *1*, 63–74. [CrossRef]
12. Abass, K.; Pelkonen, O.; Rautio, A. Chloro-s-triazines-toxicokinetic, Toxicodynamic, Human Exposure, and Regulatory Considerations. *Curr. Drug Metab.* **2021**, *22*, 645–656. [CrossRef]
13. Schuler, L.J.; Trimble, A.J.; Belden, J.B.; Lydy, M.J. Joint toxicity of triazine herbicides and organophosphate insecticides to the midge *Chironomus tentans*. *Arch. Environ. Contam. Toxicol.* **2005**, *49*, 173–177. [CrossRef]
14. Perišić-Janjić, N.U.; Djaković-Sekulić, T.L.J.; Jevrić, L.R.; Jovanović, B.Ž. Study of Quantitative Structure-Retention Relationships for *s*-Triazine Derivatives in Different RP HPTLC System. *J. Planar. Chromatogr.* **2005**, *18*, 212–216. [CrossRef]
15. Djaković-Sekulić, T.; Smolinski, A.; Perisić-Janjić, N.; Janicka, M. Chemometric characterization of (chromatographic) lipophilicity parameters of newly synthesized s-triazine derivatives. *J. Chemom.* **2008**, *22*, 195–202. [CrossRef]
16. Okihashi, M.; Akutsu, K.; Obana, H.; Hori, S. Determination of triazine herbicides in foods with liquid chromatography mass spectrometry. *Analyst* **2000**, *125*, 1966–1969. [CrossRef] [PubMed]
17. Sun, X.; Gao, J.; Xing, J.; Xing, L.; Guo, M.; Peng, J.; Li, Z.; Tan, Z. Simultaneous determination of triazine herbicides and their metabolites in shellfish by HPLC-MS/MS combined with Q/E-Orbitrap HRMS. *Anal. Bioanal. Chem.* **2021**, *413*, 6239–6252. [CrossRef] [PubMed]
18. Perišić-Janjić, N.; Jovanović, B.Ž.; Janjić, N.J.; Rajković, O.S.; Antonović, D.G. Study of the Retention Behavior of Newly Synthesized s-Triazine Derivatives in RP TLC Systems, and the Lipophilicity of the Compounds. *JPC-J. Planar. Chromat.* **2003**, *16*, 425–432. [CrossRef]
19. Janicka, M.; Perišić-Janjić, N.U.; Różyło, J.K. Thin-Layer and Overpressured-Layer Chromatography for Evaluation of the Hydrophobicity of s-Triazine Derivatives. *JPC-J. Planar. Chromat.* **2004**, *17*, 468–475. [CrossRef]
20. Perišić-Janjić, N.U.; Jovanović, B.Ž. Reversed-Phase Thin-Layer Chromatographic Behavior of Some *s*-Triazine Derivatives. *JPC-J. Planar. Chromat.* **2003**, *16*, 71–75. [CrossRef]
21. Wu, B.; Niu, Y.; Bi, X.; Wang, X.; Jia, L.; Jing, X. Rapid analysis of triazine herbicides in fruit juices using evaporation-assisted dispersive liquid–liquid microextraction with solidification of floating organic droplets and HPLC-DAD. *Anal. Methods* **2022**, *14*, 1329–1334. [CrossRef]
22. Ma, W.T.; Fu, K.K.; Cai, Z.; Jiang, G.B. Gas chromatography/mass spectrometry applied for the analysis of triazine herbicides in environmental waters. *Chemosphere* **2003**, *52*, 1627–1632. [CrossRef]
23. Vastag, G.; Apostolov, S.; Perišić-Janjić, N.; Matijević, B. Multivariate analysis of chromatographic retention data and lipophilicity of phenylacetamide derivatives. *Anal. Chim. Acta* **2013**, *12*, 44–49. [CrossRef]
24. Pallicer, J.M.; Sales, J.; Rosés, M.; Ràfols, C.; Bosch, E. Lipophilicity assessment of basic drugs (log P(o/w) determination) by a chromatographic method. *J. Chromatogr. A* **2011**, *1218*, 6356–6368. [CrossRef] [PubMed]

25. Ciura, K.; Fedorowicz, J.; Andrić, F.; Greber, K.E.; Gurgielewicz, A.; Sawicki, W.; Sączewski, J. Lipophilicity Determination of Quaternary (Fluoro)Quinolones by Chromatographic and Theoretical Approaches. *Int. J. Mol. Sci.* **2019**, *20*, 5288. [CrossRef] [PubMed]
26. Nasal, A.; Siluk, D.; Kaliszan, R. Chromatographic retention parameters in medicinal chemistry and molecular pharmacology. *Curr. Med. Chem.* **2003**, *10*, 381–426. [CrossRef] [PubMed]
27. Yang, M.; Fazio, S.; Munch, D.; Drumm, P. Impact of methanol and acetonitrile on separations based on pi-pi interactions with a reversed-phase phenyl column. *J. Chromatogr. A* **2005**, *1097*, 124–129. [CrossRef] [PubMed]
28. Milošević, N.; Janjić, N.; Milić, N.; Milanović, M.; Popović, J.; Antonović, D. Pharmacokinetics and toxicity predictors of new s-triazines, herbicide candidates, in correlation with chromatogrpahic retention constants. *J. Agric. Food Chem.* **2014**, *62*, 8579–8585. [CrossRef] [PubMed]
29. Janicka, M.; Kwietniewski, L.; Perišić-Janjić, N.U. Determination of Retention Factors of s-Triazines Homologous Series in Water Using a Numerical Method Basing on Ościk's Equation. *Chroma* **2006**, *63*, S87–S93. [CrossRef]
30. Antonović, D.; Bončić-Caričić, G.A. Gas chromatographic retention indices for *N*-substituted amino s-triazines on DB-1 and DB-5 capillary columns. *J. Serb. Chem. Soc.* **1994**, *59*, 993–996.
31. Thomas, M.C.; Flores, F.; Kaserzon, S.; Reeks, T.A.; Negri, A.P. Toxicity of the herbicides diuron, propazine, tebuthiuron, and haloxyfop to the diatom *Chaetoceros muelleri*. *Sci. Rep.* **2020**, *10*, 19592. [CrossRef]
32. Salaković, B.; Kovačević, S.; Karadžić Banjac, M.; Jevrić, L.; Podunavac-Kuzmanović, S.; Antonović, D. Comparative chemometric analysis, ranking and selection of lipophilicity parameters of 6-chloro-1,3,5-triazine derivatives with acyclic and cyclic substituents. *Acta Period. Technol.* **2022**, *53*, 88–99. [CrossRef]
33. *NCSS 2023 Statistical Software*; NCSS, LLC.: Kaysville, UT, USA, 2023. Available online: https://www.ncss.com/software/ncss/ (accessed on 27 September 2023).
34. TIBCO Software Inc. *Data Science Workbench*; Version 14; TIBCO Software Inc.: Palo Alto, CA, USA, 2020; Available online: http://tibco.com (accessed on 27 September 2023).
35. Héberger, K.; Kollár-Hunek, K. Sum of ranking differences for method discrimination and its validation: Comparison of ranks with random numbers. *J. Chemom.* **2011**, *25*, 151–158. [CrossRef]
36. Coym, J.W. Evaluation of ternary mobile phases for reversed-phase liquid chromatography: Effect of composition on retention mechanism. *J. Chromatogr. A* **2010**, *1217*, 5957–5964. [CrossRef] [PubMed]
37. Reubsaet, J.L.; Vieskar, R. Characterisation of π–π interactions which determine retention of aromatic compounds in reversed-phase liquid chromatography. *J. Chromatogr. A* **1999**, *841*, 147–154. [CrossRef]
38. Šegan, S.; Božinović, N.; Opsenica, I.; Andrić, F. Consensus-based comparison of chromatographic and computationally estimated lipophilicity of benzothiepino[3,2-c]pyridine derivatives as potential antifungal drugs. *J. Sep. Sci.* **2017**, *40*, 2089–2096. [CrossRef] [PubMed]

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