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Estimation of the lipophilic character of flavonoids from the retention behavior in reversed phase liquid chromatography on different stationary phases: A comparative study

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ABSTRACT

The retention behavior of some flavonoids in reversed phase liquid chromatography (RPLC) was investigated using different chemistries of the modified silicagel based stationary phases. Highly end-capped octadecyl silicagel (ODS), polar embedded linker octadecyl silicagel (SB-18 Aqua), phenyl silicagel and pentafluorophenyl modified silicagel (PFP) were used as stationary phases. The mobile phase consisted in acetonitrile/acidified water mixtures, at different fractions of volume. The lipophilicity was estimated through different chromatographic descriptors, as it follows: $\log k_w$, $m \log k$, S, φ_0 and PC1/$\log k$. The chromatographic behavior observed on the mentioned stationary phases was evaluated by means of various graphical profiles and correlation matrices. Additionally, new information about the characteristics of the stationary phases and their (dis)-similarities were provided through lipophilicity charts and by scatterplots of loadings obtained by applying principal component analysis (PCA) to retention data. Furthermore, the experimental lipophilicity indices estimated from retention data were correlated with the computed descriptors, at a high level of statistical significance.

1. Introduction

The increasing occurrence of cancer diseases during the last century lead to a constant interest in evaluation and characterization of compounds with antioxidant activity, to offer detailed insights on the most recommended alternatives to healthy and equilibrated diets. Polyphenols are amidst the natural compounds exhibiting proved antioxidant activity, abundantly found in plants. Over years, the biological behavior (toxicity, antioxidant or oxidant activity, etc.) was directly correlated to the chemical structure of the compounds and their ability to interact with the chemical entities that defines the biological environment. The recognized property that significantly describes the biological behavior of chemical compounds is their lipophilic character, defined by IUPAC as the affinity of a molecule or a moiety for a lipophilic environment. The lipophilicity is commonly measured by evaluating the distribution behavior of compounds in biphasic systems, either liquid–liquid or solid–liquid [1]. The lipophilicity is associated with an increased biologically activity, poorer aqueous solubility, faster metabolization and elimination, increased plasma protein binding, sometimes shorter duration of action. In the same time it plays an important role in the pharmacodynamic and toxicological profile of drugs [2,3].

A large variety of lipophilicity estimation methods are known, but only few of them are frequently used. According to Kaliszan [4] and Sangster [5] techniques designed for lipophilicity determination are classified in direct and indirect methods. The shake-flask procedure was the most used direct method, but because of the multiple drawbacks it was almost totally replaced by the indirect techniques like chromatographic ones, which are more versatile, flexible and from some points of view they are better simulating the biological conditions mainly because it involves a dynamic process of compound transfer between the used immiscible phases (stationary vs. mobile phase). In addition, these methods require only the determination of some retention parameters [6,7].

The lipophilic character is described by means of partition coefficients, denoted in few different ways ($\log K_{ow}$, $\log P$, $\log k_w$, $\log V$, R_{M0}, etc.), according to the determination method. These parameters are extensively used in the biological, biochemical, and environmental sciences as descriptors of the lipophilic character [8]. Their importance has been attested in

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the quantitative structure–activity relationships (QSARs), quantitative structure–property relationships (QSPRs), and quantitative structure–retention relationships (QSRRs) experiments [9–12]. The liquid chromatographic approaches for lipophilicity estimation are very popular in QSAR/QSPR/QSRR especially because the large variety of stationary phases may offer some insights concerning the in vivo behavior, and also well evidencing some specific interactions. Additionally, some real information about the capacity of a compound to produce a particular impact over the biological system may also be provided.

The chromatographic approach involved in the estimation of the lipophilic character is usually based on the reversed-phase partition mechanism, the stationary phase being totally non-polar (octadecyl or octyl chemically modified silicagels). However, since the biological environment is characterized by a large complexity described by various chemical entities, a pertinent analysis should be performed on stationary phases exhibiting different characteristics, even including the HILIC ones [13]. In the spirit of the above considerations, the purpose of this work is to evaluate different stationary phases (usually exploited under the reversed phase separation mechanism) in the estimation of the lipophilic character of the solutes. One of the major goals is to illustrate the differences and similarities existing between the investigated stationary phases through using advanced chemometric methods, such as principal component analysis (PCA). The stationary phase's lipophilicity charts and the PCA loadings scatterplots assume a new vision about the chromatographic separation mechanism. All experiments have been evaluated from the perspective offered by a significant group of flavonoids. Flavonoids are a class of phenolic compounds largely spread in the edible plants. The flavonoids are responsible with plant protection against a large variety of environmental stress factors, also being considered as potent phytoalexins. In human body, flavonoids are functioning as complex biological response modifiers [12], their wide spectrum of action involving antitumoral, antiviral, antibacterial, cardio-protective, anti-mutagenic, antioxidant, anti-inflammatory, anti-allergenic, anti-aging, and anti-carcinogenic characteristics being already highlighted [14–19].

2. Experimental

2.1. Reagents

All solvents (methanol, acetonitrile) were HPLC gradient grade from Merck (Darmstadt, Germany). Water for chromatography (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments. Formic acid was extra pure grade from Merck. Flavonoids, obtained from different commercial sources (Merck, Fluka, Sigma–Aldrich) were analytical grade. The following compounds have been considered: **1**. flavone; **2**. 2′-methoxyflavone; **3**. 3-methoxyflavone; **4**. 5-methoxyflavone; **5**. 6-methoxyflavone; **6**. 7-methoxyflavone; **7**. 7,8-dimethoxyflavone; **8**. 3-hydroxy-7-methoxyflavone; **9**. 5-hydroxyflavone; **10**. 6-hydroxyflavone; **11**. 7-hydroxyflavone; **12**. 3,6-dihydroxyflavone; **13**. 3,7-dihydroxyflavone; **14**. apigenin (4′,5,7-trihydroxyflavone); **15**. baicalein (5,6,7-trihydroxyflavone); **16**. galangin (3,5,7-trihydroxyflavone); **17**. kaempferol (4′, 3,5,7-tetrahydroxyflavone); **18**. luteolin (3′,4′,5,7-tetrahydroxyflavone); **19**. quercetin (3′,4′,3,5,7-pentahydroxyflavone); **20**. geraldol (3′-methoxy-4′,3,7-trihydroxyflavone); **21**. daidzein (4′,7-dihydroxyisoflavone); **22**. 6-methylflavone; **23**. 6-chloro-7-methylflavone. Their chemical structures are indicated in Fig. 1. Stock solutions having concentrations of 1 mg/mL were obtained from each of the tested compounds through direct dissolution in acetonitrile or acetonitrile/water mixture (1:1, v/v).

2.2. Equipments

Experiments were performed on a system built up from Agilent series 1100 modules (Agilent Technology, Waldbronn, Germany) as following: degasser (G1379 A); quaternary pump (G1311 A); thermostated autosampler (G1329 A and G1330 B); column thermostat (G1316 A). Detection was made through the DAD module (G1315 B). System control, data acquisition and interpretation were made with the Agilent Chemstation software version B 01.03.

2.3. Chromatographic conditions

Retention studies were made on the following chromatographic columns: **A**. Kinetex C18 100 Å (100 mm L × 2.1 mm i.d. × 2.6 μm d.p.) from Phenomenex (Cat. no. 00D-4462-AN) consisting in fused core particles of octadecyl chemically modified silicagel as a stationary phase; **B**. Zorbax SB-Aq (150 mm × 4.6 mm i.d. × 5 μm d.p.) from Agilent Technologies (Cat. no. 883975-914) consisting in polar embedded octadecyl modified silicagel as a stationary phase; **C**. Betasil Phenyl (150 mm L × 4.6 mm i.d. × 5 μm d.p.) from Thermo Electron Corporation, consisting in phenyl chemically modified silicagel as stationary phase; **D**. Luna PFP (2) 100 Å (100 mm L × 2.0 mm i.d. × 3 μm d.p.) from Phenomenex (Cat. no. 00D-4447-80) consisting in pentafluorophenyl chemically modified silicagel as stationary phase. Flow rate on columns **A** and **D** was 0.4 mL/min. Flow rate on columns **B** and **C** was 1.2 mL/min. Elution was made under isocratic conditions. Mobile phase components are acetonitrile and aqueous 0.1% formic acid, mixed in different volumetric ratios. All columns were operated at 25 °C. A volume of 0.1 μL from stock solutions was injected in columns **A** and **D**, while on **B** and **C** columns, 0.2 μL injections were made.

Isocratic compositions of the mobile phase used for experiments on columns **B**–**D** ranged from 35% to 55% acetonitrile, in steps of 5%. For column **A**, the volumetric ratio of acetonitrile ranged from 20% to 60% with increments of 5%, in order to obtain a minimum of 5 data points couples for each of the investigated analytes.

The injection valve switching signal was considered to indicate the column hold-up time (t_0 – dead time) used for determination of the retention factor (k). The following values were considered during the computation of the raw experimental data: for column **A**, t_0 was 0.571 ± 0.010 min ($t \pm s_t$); for column **B**, t_0 was 1.371 ± 0.043 min; for column **C**, t_0 was 1.545 ± 0.068 min; for column **D**, t_0 was 0.714 ± 0.024 min.

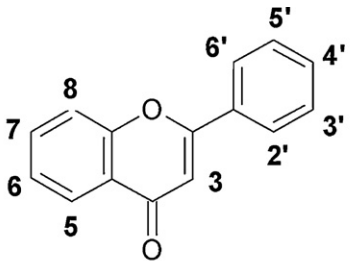
DAD was operated in the full acquisition mode over the 200–800 nm interval, chromatograms being simultaneously recorded at 220, 254, 270 and 300 nm analytical wavelengths (±2 nm spectral width).

2.4. Theory, methods

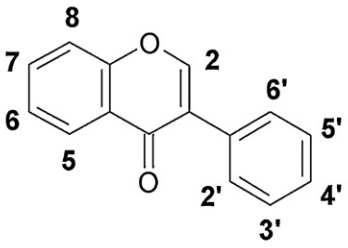
Many of the parameters resulting from a liquid chromatographic separation may be associated to the lipophilic character of the analytes, but most of them are based on the retention factor (k). The Soczewiński–Snyder [20] parameter of lipophilicity has gain in time the status of the most influent descriptor. It may be computed through equation (Eq. (1)):

$$\log k = \log k_w + S\varphi \quad (1)$$

where $\log k_w$ represents the intercept and it is associated with the k value of the analyte for a hypothetical mobile phase containing 100% water and φ is the volume fraction of the organic modifier in the mobile phase. $\log k_w$ is widely accepted as the most powerful lipophilicity descriptor derived from chromatographic retention



A



B

Compound id. #	Base Structure	Substitution in position									
		3	5	6	7	8	2'	3'	4'	5'	6'
1	A	H	H	H	H	H	H	H	H	H	H
2	A	H	H	H	H	H	OCH_3	H	H	H	H
3	A	OCH_3	H	H	H	H	H	H	H	H	H
4	A	H	OCH_3	H	H	H	H	H	H	H	H
5	A	H	H	OCH_3	H	H	H	H	H	H	H
6	A	H	H	H	OCH_3	H	H	H	H	H	H
7	A	H	H	H	OCH_3	OCH_3	H	H	H	H	H
8	A	OH	H	H	OCH_3	H	H	H	H	H	H
9	A	H	OH	H	H	H	H	H	H	H	H
10	A	H	H	OH	H	H	H	H	H	H	H
11	A	H	H	H	OH	H	H	H	H	H	H
12	A	OH	H	OH	H	H	H	H	H	H	H
13	A	OH	H	H	OH	H	H	H	H	H	H
14	A	OH	H	H	OH	H	H	H	OH	H	H
15	A	H	OH	OH	OH	H	H	H	H	H	H
16	A	OH	OH	H	OH	H	H	H	H	H	H
17	A	OH	OH	H	OH	H	H	H	OH	H	H
18	A	H	OH	H	OH	H	H	OH	OH	H	H
19	A	OH	OH	H	OH	H	H	OH	OH	H	H
20	A	OH	H	H	OH	H	H	OCH_3	OH	H	H
21	B	H	H	H	OH	H	H	H	OH	H	H
22	A	H	H	CH_3	H	H	H	H	H	H	H
23	A	H	H	Cl	CH_3	H	H	H	H	H	H

Fig. 1. Chemical structures of the considered solutes.

data, although its value is more often different from those experimentally determined through direct methods, when applicable. Even more, it is strongly dependent by the organic modifier being used [21]. The slope (S) and even $\log k$ are also alternatives for the estimation of lipophilicity. Moreover, S is associated with the solvent strength of the organic modifier and with sorbent surface area. S also considerably varies with solute structure, especially with solute size.

In the HPLC, the k value is directly determined from the retention time, as depicted in Eq. (2):

$$\log k = \log \frac{t_R - t_0}{t_0} \tag{2}$$

where t_R is the retention time and t_0 is the hold-up time. The t_0 value is considered to be the retention time of a non-retained marker.

An alternative to the Soczewiński–Snyder lipophilicity indices has been offered by Valkó [22,23], through a new lipophilicity descriptor, namely index of hydrophobicity (φ_0). It represents the volume of organic solvent in the mobile phase for which the amounts of solute distributed in the mobile and stationary phases are equal, so the k value will be 1 ($\log k = 0$). The index of hydrophobicity is computed as follows (Eq. (3)):

$$\varphi_0 = \frac{\log k_w}{S} \tag{3}$$

This descriptor has been recommended as being very efficient in case of non-homologous classes of compounds.

During the last decade new insights were offered by PCA, having an unraveled ability to provide highly descriptive lipophilicity indices. PCA is extracting the meaningful and interpretable features from the underlying information of the multivariate raw data. It has the ability to separate the pertinent information from the noise. Usually, the first few components account for the maximum information existing in the initial (raw) data [24]. Various studies have revealed that the score plot of the first two principal components (PCs) offer sustainable patterns that are highly related to the chemical reality [25–28]. In the same time by applying the PCA algorithm on the lipophilicity indices obtained on different stationary phases, a suggestive classification of stationary phases may be obtained. In addition, a careful investigation of eigenvalues and eigenvectors (loadings) can offer useful information concerning the chromatographic behavior of the compounds and the mobile phase influence over the obtained lipophilicity indices [29–31]. All the

experimental lipophilicity descriptors and graphs are computed through the Statistica 8.0 program (www.statsoft.com).

2.5. Theory, log P

The log *P* values are usually estimated by means of various computer softwares or Internet available modules that are applying different algorithms based on structural, atomistic, topological, electrotopological, or other considerations on a drawn chemical structure [32]. Some of the most common software for log *P* estimation are ChemOffice 8.0 (www.cambridgesoft.com), Alchemy 2000 (www.tripos.com) and Dragon Plus 5.4 (www.talete.mi.it). In addition, ALOGPS 2.1 (www.vcclab.org) Internet module is also contributing by various log *P* values. In this experiment, prior to the computation, the chemical structures were pre-optimized with the Molecular Mechanics Force Field procedure included in Hyperchem version 8.0 (www.hyper.com) and the resulting geometries were further refined by means of the semi-empirical method Parametric Method-3 using the Fletcher-Reeves algorithm and a gradient norm limit of 0.009 kcal $Å^{-1}$. The optimized geometries were loaded by the above presented software in order to calculate the lipophilicity descriptors. For the involved flavonoids, the Chem Office 8.0 had offered four indices (CLOGP, $\log(p)^C$-Crippen's method, $\log(p)^V$-Viswanadhan's method, $\log(p)^B$-Broto's method), Alchemy 2000 provided two values ($\log P_C$, log *P*), Dragon Plus gave three values (Hy-Hydrophobicity index, MLOGP-Moriguchi's method, ALOGP-Ghose-Crippen's method) and finally ALOGPS 2.1. allowed computation of eight values (ALOGPs, AC log *P*, AB/log *P*, mi log *P*, CosmoFRAQ, KOWWIN, XLOGP2, XLOGP3). As long as experiments were carried out with an acidic mobile phase (0.1% formic acid was used as aqueous component of the mobile phase) in order to produce accurate peak shape and symmetry, the correlation of the chromatographic lipophilicity indices with a computed log *D* (pH = 2–3) seems appropriate. Computation of log *D* values has been made by means of the Marvin Sketch 5.5.01 Internet module (http://intro.bio.umb.edu/111-112/OLLM/111F98/newclogp.html). All the descriptors are listed in Table 1.

3. Results and discussion

The Soczewiński–Snyder parameter of lipophilicity results from the linear regression according to Eq. (1), considering as experimental data couples the percent of the organic modifier in the mobile phase (φ) and the logarithm of the retention factor ($\log k$). However, a better correlation between the two experimental variables is frequently observed through using a binomial regression:

$$\log k = \log k_w + B\varphi + A\varphi^2 \quad (4)$$

The quadratic term is more often explained through the perturbation of the retention behavior of the analytes on a morphologically modified hydrophobic stationary phase when using water rich mobile phases. The collapse of the C8/C18 hydrocarbonate chains is readily invoked when using totally aqueous mobile phases, and leads to introduction in the experimental practice of the polar embedded hydrophobic C8/C18 phases. On analysis of the experimental data resulting from the retention behavior study of target compounds on the considered stationary phases, it clearly results that correlation coefficients (r_{xy}) for the binomial regressions are better than for the linear ones (exceptions are made only for compounds **2**, **5**, **9** on the stationary phase **A**). Fig. 2 illustrates the correlation between $\log k_w$ values obtained through applying linear and binomial regressions models on the retention data sets of the target analytes on the studied columns.

$\log k_w$ values computed through linear and binomial regression models are well correlated on columns **B**–**D**, while on column **A**,

Table 1
The computed lipophilicity indices for the investigated compounds.

Compound #	log D^a	$\log(p)^C$	$\log(p)^V$	$\log(p)^B$	CLOGP	$\log P_C$	log *P*	Hy	MLOGP	ALOGP	ALOGPs	AC log *P*	AB/log *P*	COSMO Fraq	mi log *P*	K_{ow} W_N	XLOGP2	XLOGP3
1	2.97	3.07	3.17	3.04	3.48	2.75	2.21	−0.86	3.15	3.14	3.10	3.59	3.42	3.11	3.74	3.51	3.21	3.56
2	2.81	2.95	2.92	3.17	2.89	0.96	1.87	−0.83	2.83	3.12	3.14	3.49	3.38	3.81	3.75	3.59	3.12	3.53
3	2.83	2.27	2.20	3.39	3.09	1.69	1.93	−0.83	2.83	2.64	2.83	3.45	3.70	3.80	3.72	2.80	2.96	3.73
4	2.81	2.95	2.92	3.17	3.59	1.03	1.90	−0.83	2.83	3.12	3.11	3.49	3.41	2.90	3.75	3.59	3.12	3.47
5	2.81	2.95	2.92	3.17	3.59	1.27	2.01	−0.83	2.83	3.12	3.18	3.49	3.25	3.08	3.77	3.59	3.12	3.95
6	2.81	2.95	2.92	3.17	3.59	1.07	2.36	−0.83	2.83	3.12	3.18	3.49	3.43	3.43	3.77	3.59	3.12	3.95
7	2.65	2.82	2.66	3.30	3.27	0.43	2.80	−0.80	2.53	3.10	3.09	3.38	3.06	3.67	3.56	3.67	2.77	3.12
8	2.56	1.78	1.67	3.05	2.87	0.36	1.90	−0.32	2.29	2.56	2.47	2.89	3.80	4.18	3.48	2.70	2.79	3.37
9	3.31	2.68	2.88	2.65	4.11	2.52	1.67	−0.33	3.09	2.87	3.37	3.29	3.46	3.56	3.47	3.80	2.80	2.45
10	2.66	2.68	2.88	2.65	3.21	2.55	1.68	−0.33	2.58	2.87	3.31	3.29	2.89	1.97	3.23	3.03	2.80	3.62
11	2.66	2.68	2.88	2.65	3.21	2.47	1.69	−0.33	2.58	2.87	3.27	3.29	3.14	2.52	3.23	3.03	2.80	3.62
12	2.42	1.52	1.64	2.53	2.30	0.37	1.19	0.34	2.04	2.31	2.29	2.70	3.27	2.87	2.94	2.14	2.47	3.05
13	2.42	1.52	1.64	2.53	2.30	0.35	1.17	0.34	2.04	2.31	2.24	2.70	3.51	3.29	2.94	2.14	2.47	3.05
14	2.71	1.90	2.32	1.87	2.91	0.31	0.75	1.10	1.76	2.33	2.47	2.69	2.43	2.44	2.46	2.84	1.15	1.74
15	2.71	1.90	2.32	1.87	3.00	0.31	1.19	1.10	1.76	2.33	2.66	2.69	2.84	3.02	2.68	3.27	2.64	1.74
16	2.76	1.13	1.35	2.14	2.56	0.31	1.05	1.10	1.76	2.04	1.46	2.40	3.42	2.73	2.65	2.44	2.06	2.25
17	2.46	0.74	1.07	1.75	1.90	0.31	0.94	1.92	0.99	1.77	1.23	2.10	2.80	2.03	2.17	1.96	0.81	1.90
18	2.40	1.51	2.03	1.48	2.31	0.31	0.36	1.92	0.99	2.07	2.15	2.40	1.96	2.36	1.97	2.36	0.75	1.38
19	2.16	0.35	0.78	1.36	1.30	0.57	0.30	2.78	0.23	1.50	1.07	1.80	2.34	1.94	1.68	1.48	0.41	1.54
20	1.96	1.00	1.10	2.27	1.49	0.39	0.50	1.07	0.73	2.02	1.73	2.30	2.74	3.37	2.28	1.49	1.56	2.30
21	2.73	2.13	2.25	2.20	2.08	0.33	1.87	0.34	1.77	2.33	2.77	2.24	2.52	1.90	2.56	2.55	2.08	2.47
22	3.48	3.56	3.64	3.45	3.98	2.80	2.38	−0.87	3.40	3.62	3.53	3.91	3.83	3.41	4.16	4.06	3.65	3.92
23	4.08	4.12	4.15	4.07	4.69	3.15	2.66	−0.83	3.91	4.29	3.92	4.52	4.54	3.99	4.77	4.70	4.06	4.55

[a] log *D* was computed at pH = 2–3.

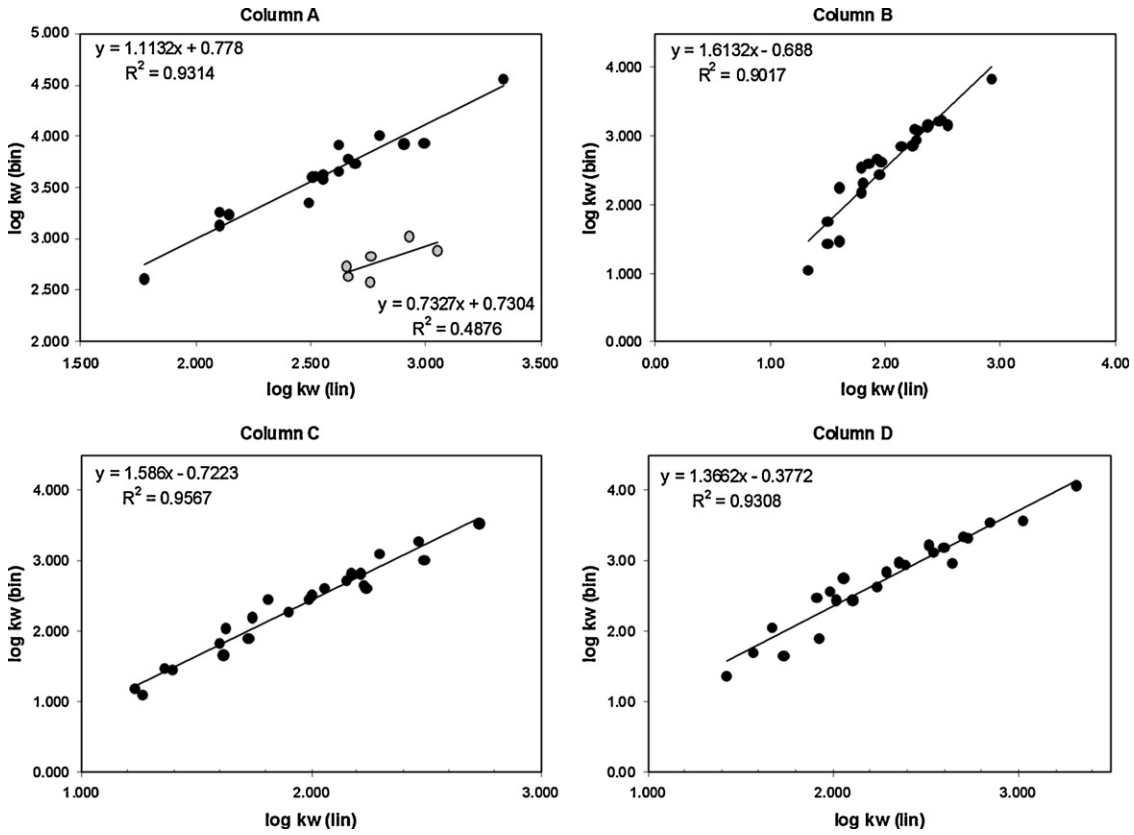


Fig. 2. Graphic correlations obtained between $\log k_w$ values determined from linear (lin) and quadratic (bin) fitting regressions for the studied compounds on the considered stationary phases (a detailed description of the stationary phases is given in Section 2).

two different behaviors may be observed. Values of $\log k_w$ determined through the two different regression models correlates differently for compounds **2**, **3**, **5**, **6**, **8** and **22** compared to the other compounds. This may be explained by the morphological modification of the stationary phase induced through the collapse of the alkyl chains when the chromatographic column is exploited with mobile phases having increased content of water. Indeed, stationary phases in columns **B**–**D** contains embedded polar moieties and their use with mobile phases containing large volumes of aqueous solutions does not induce essential morphological changes, making their behavior more uniform. The stationary phase in column **A** has a higher hydrophobic character and morphologically changes under the use of water rich mobile phase compositions, interacting differently with the target compounds, according to their structural characteristics. This may be also sustained by a broader interval of mobile phase compositions used during experiments to avoid extensive retention or no retention at all of the studied analytes.

The chromatographic lipophilicity indices resulting from the experimental protocol ($m \log k$ – the arithmetic mean of $\log k$, $\log k_w$ (lin), $\log k_w$ (bin), S, φ_0, PC1/$\log k$ – scores corresponding to the first principal component (PCs) of $\log k$) are enlisted in Table 2.

Fig. 3 illustrates the variation of the lipophilic character of the solutes according to the variation of each the chromatographic index taken into consideration. The ordinate in the graphics from Fig. 3 represents the occupied rank of each solute in the increasing order of the considered chromatographic index. When two or more compounds are exhibiting the same value of a chromatographic index, they have been ranked on the same level, the immediately following corresponding level(s) remaining unaddressed. The most uniform variation behaviors were obtained for $\log k_w$ (lin), φ_0, and PC1/$\log k$. The most non-uniform behavior is provided by the S lipophilicity index. $\log k_w$ (bin) and $m \log k$ behave in an uniform way for columns **B**–**D**, but evidently, data on column **A** vary differently. According to all chromatographic lipophilicity indices, the most lipophilic flavonoids are compounds **22** and **23** (6-methyl and 6-chloro-7-methyl substituted flavones, respectively). At the opposite limit of the interval, compound **21** (daidzein) behaves as the least lipophilic one, followed by **18** and **19** (luteolin and quercetin). If comparing the methoxy and hydroxy substituted flavonoids, it is obvious that the first ones are more lipophilic. In case of hydroxy flavones, the lipophilic character decrease with the increasing of the substitution with the hydroxyl groups. As already expected, the lipophilic character depends upon the nature and number of the functional groups introduced on the basic flavonic structure. The classical lipophilicity indices are also sustained by the PC1/$\log k$, which are confirming the same aspects. The PCA lipophilicity index is highly descriptive and according to the obtained eigenvalues this is a consequence of the fact that the first principal component (PC1/$\log k$) retains over 99.58% from the initial variation (information), while the first two PCs retain over 99.94%.

All determined chromatographic indices are in fact the expression of the lipophilic character of the analytes in the systems being studied. Accordingly, these indices should be correlated (directly or indirectly) one to another, as they are illustrating the same basic property (the preference for a lipophilic environment). Table 3 contains the correlation matrix for all lipophilicity indices obtained on the considered stationary phases.

It can be observed from Table 3 that $\log k_w$ (bin) on the stationary phase in column **A** is not statistically correlated with the other indices (obtained on the same stationary phase or on other stationary phases). The reasons for such a behavior have been already discussed at the beginning of the present section. Another lipophilicity index poorly correlating to other indices is the S parameter. Only one correlation of the S index obtained on the

Table 2
The chromatographic lipophilicity indices of the investigated compounds.

Compound	Column **A**						Column **B**					
	$\log k_w$ (lin)	$\log k_w$ (bin)	$m \log k$	S	φ_0	PC1/$\log k$	$\log k_w$ (lin)	$\log k_w$ (bin)	$m \log k$	S	φ_0	PC1/$\log k$
1	2.62	3.65	0.857	−0.044	−59.5	−0.53	2.14	2.85	0.575	−0.035	−61.5	−0.44
2	2.67	2.64	0.688	−0.044	−60.7	−0.66	2.28	3.08	0.628	−0.037	−62.1	−0.56
3	2.76	2.58	0.778	−0.044	−62.7	−0.84	2.24	2.86	0.594	−0.036	−61.3	−0.48
4	2.62	3.92	0.758	−0.050	−52.8	−0.04	1.93	2.65	0.445	−0.033	−58.5	−0.15
5	2.76	2.84	0.742	−0.045	−61.5	−0.77	2.36	3.13	0.670	−0.038	−62.8	−0.65
6	2.66	2.74	0.669	−0.044	−60.1	−0.62	2.37	3.15	0.691	−0.037	−63.5	−0.70
7	2.67	3.78	0.802	−0.047	−57.2	−0.41	2.26	3.10	0.627	−0.036	−62.3	−0.56
8	3.05	2.89	0.869	−0.048	−62.9	−1.04	2.49	3.24	0.676	−0.040	−61.8	−0.67
9	2.99	3.93	0.798	−0.044	−68.2		2.54	3.16	0.758	−0.040	−64.1	−0.85
10	2.49	3.35	0.625	−0.050	−50.0	0.21	1.80	2.32	0.248	−0.035	−52.2	0.29
11	2.55	3.63	0.658	−0.054	−47.2	0.46	1.86	2.61	0.321	−0.034	−54.4	0.13
12	2.80	4.00	0.801	−0.053	−52.6	−0.1	1.96	2.62	0.289	−0.037	−52.8	0.20
13	2.69	3.74	0.735	−0.052	−51.6	0.02	1.95	2.45	0.288	−0.037	−52.8	0.20
14	2.51	3.60	0.552	−0.056	−44.9	0.7	1.79	2.54	0.107	−0.037	−47.9	0.60
15	2.52	3.62	0.634	−0.054	−46.8	0.5	1.61	2.25	0.095	−0.034	−47.8	0.63
16	2.90	3.92	0.863	−0.051	−56.9	−0.51	2.27	2.95	0.462	−0.040	−56.5	−0.19
17	2.55	3.59	0.603	−0.056	−45.8	0.59	1.80	2.18	0.106	−0.038	−47.8	0.61
18	2.14	3.23	0.248	−0.054	−39.6	1.28	1.50	1.75	−0.147	−0.037	−41.0	1.17
19	2.10	3.13	0.267	−0.052	−40.1	1.21	1.50	1.43	−0.161	−0.037	−40.6	1.21
20	2.10	3.27	0.321	−0.051	−41.3	1.09	1.60	1.47	−0.063	−0.037	−43.3	0.98
21	1.78	2.61	0.140	−0.047	−38.0	1.38	1.34	1.05	−0.213	−0.034	−38.8	1.32
22	2.93	3.02	0.866	−0.046	−63.9	−1.02	2.46	3.23	0.736	−0.038	−64.2	−0.80
23	3.33	4.55	0.972	−0.047	−70.6		2.92	3.84	0.955	−0.044	−66.9	−1.29

Compound	Column **C**						Column **D**					
	$\log k_w$ (lin)	$\log k_w$ (bin)	$m \log k$	S	φ_0	PC1/$\log k$	$\log k_w$ (lin)	$\log k_w$ (bin)	$m \log k$	S	φ_0	PC1/$\log k$
1	2.00	2.51	0.505	−0.033	−60.2	−0.33	2.35	2.98	0.624	−0.038	−61.3	−0.37
2	2.17	2.82	0.577	−0.035	−61.3	−0.49	2.52	3.22	0.689	−0.041	−62.0	−0.52
3	2.23	2.65	0.647	−0.035	−63.4	−0.64	2.64	2.97	0.773	−0.041	−63.6	−0.71
4	1.81	2.46	0.340	−0.033	−55.4	0.04	2.06	2.76	0.372	−0.037	−54.9	0.19
5	2.22	2.82	0.587	−0.036	−61.2	−0.51	2.60	3.20	0.721	−0.042	−62.3	−0.59
6	2.15	2.73	0.540	−0.036	−60.1	−0.41	2.54	3.12	0.683	−0.041	−61.5	−0.51
7	2.06	2.62	0.514	−0.034	−60.0	−0.35	2.39	2.95	0.564	−0.041	−58.9	−0.24
8	2.47	3.27	0.698	−0.039	−62.8	−0.76	2.85	3.55	0.907	−0.043	−66.0	−1.01
9	2.49	3.01	0.783	−0.038	−65.6	−0.95	3.03	3.58	1.035	−0.044	−68.4	−1.29
10	1.75	2.19	0.229	−0.034	−51.8	0.29	1.98	2.56	0.246	−0.039	−51.4	0.47
11	1.63	2.04	0.156	−0.033	−49.8	0.45	1.91	2.47	0.188	−0.038	−49.9	0.60
12	1.99	2.45	0.370	−0.036	−55.3	−0.03	2.29	2.85	0.445	−0.041	−55.9	0.03
13	1.90	2.28	0.320	−0.035	−54.1	0.09	2.23	2.63	0.433	−0.040	−55.8	0.05
14	1.62	1.67	0.091	−0.034	−47.7	0.60	2.02	2.45	0.169	−0.041	−49.1	0.64
15	1.60	1.84	0.147	−0.032	−49.6	0.48	1.93	1.90	0.177	−0.039	−49.6	0.62
16	2.24	2.61	0.522	−0.038	−58.7	−0.37	2.71	3.35	0.684	−0.045	−60.2	−0.51
17	1.72	1.90	0.143	−0.035	−49.1	0.48	2.10	2.44	0.224	−0.042	−50.3	0.52
18	1.36	1.46	−0.129	−0.033	−41.1	1.09	1.67	2.06	−0.097	−0.039	−42.5	1.24
19	1.40	1.45	−0.084	−0.033	−42.5	0.99	1.73	1.65	−0.059	−0.040	−43.5	1.15
20	1.27	1.09	−0.050	−0.029	−43.3	0.92	1.57	1.69	−0.017	−0.035	−44.5	1.06
21	1.23	1.19	−0.135	−0.030	−40.6	1.11	1.43	1.37	−0.214	−0.036	−39.1	1.50
22	2.30	3.10	0.641	−0.037	−62.4	−0.63	2.73	3.33	0.817	−0.043	−64.2	−0.81
23	2.73	3.54	0.845	−0.042	−65.2	−1.09	3.31	4.08	1.134	−0.048	−68.4	−1.52

Table 3
The correlation matrix of the determined chromatographic lipophilicity indices corresponding to the investigated compounds (bold black characters indicates correlation coefficients higher than 0.8 as an absolute value while bold grey characters indicates correlation coefficients between 0.7 and 0.8).

Column	Column	A						B						C						D					
	Lipophilicity Indices	log k_w (lin)	log k_w (bin)	m log k	S	φ_0	PC1 / log k	log k_w (lin)	log k_w (bin)	m log k	S	φ_0	PC1 / log k	log k_w (lin)	log k_w (bin)	m log k	S	φ_0	PC1 / log k	log k_w (lin)	log k_w (bin)	m log k	S	φ_0	PC1 / log k
A	log k_w (lin)	1.00	0.40	**0.94**	0.32	**-0.89**	**-0.90**	**0.92**	**0.94**	**0.89**	-0.64	**-0.86**	**-0.89**	**0.94**	**0.93**	**0.91**	**-0.90**	**-0.89**	**-0.91**	**0.94**	**0.94**	**0.93**	**-0.82**	**-0.92**	**-0.93**
	log k_w (bin)		1.00	0.40	-0.36	-0.12	0.14	0.19	0.26	0.14	-0.24	-0.12	-0.14	0.19	0.17	0.14	-0.28	-0.12	-0.14	0.22	0.26	0.17	-0.33	-0.14	-0.17
	m log k			1.00	0.36	**-0.87**	**-0.90**	**0.85**	**0.92**	**0.88**	-0.40	**-0.89**	**-0.88**	**0.88**	**0.90**	**0.89**	-0.74	**-0.90**	**-0.89**	**0.86**	**0.89**	**0.88**	-0.64	**-0.90**	**-0.88**
	S				1.00	-0.71	-0.65	0.61	0.49	0.68	-0.17	-0.69	-0.68	0.58	0.59	0.68	-0.30	-0.69	-0.67	0.55	0.52	0.63	-0.22	-0.65	-0.63
	φ_0					1.00	**1.00**	**-0.97**	**-0.93**	**-0.98**	0.56	**0.96**	**0.98**	**-0.98**	**-0.97**	**-0.99**	**0.81**	**0.99**	**0.99**	**-0.96**	**-0.95**	**-0.99**	0.72	**0.99**	**0.99**
	PC1 / log k						1.00	**-0.97**	**-0.93**	**-0.98**	0.38	**0.96**	**0.98**	**-0.98**	**-0.97**	-1.00	0.77	**0.99**	**1.00**	**-0.96**	**-0.95**	**-0.99**	0.63	**0.99**	**0.99**
B	log k_w (lin)							1.00	**0.95**	**0.97**	-0.68	**-0.94**	**-0.97**	**0.98**	**0.96**	**0.97**	**-0.87**	**-0.95**	**-0.97**	**0.98**	**0.97**	**0.98**	**-0.80**	**-0.97**	**-0.98**
	log k_w (bin)								1.00	**0.96**	-0.52	**-0.95**	**-0.96**	**0.94**	**0.95**	**0.94**	**-0.82**	**-0.94**	**-0.94**	**0.92**	**0.96**	**0.94**	-0.74	**-0.94**	**-0.94**
	m log k									1.00	-0.49	**-0.99**	**-1.00**	**0.95**	**0.96**	**0.98**	-0.76	**-0.98**	**-0.98**	**0.93**	**0.95**	**0.96**	-0.67	**-0.97**	**-0.96**
	S										1.00	0.40	0.49	-0.67	-0.55	-0.56	**0.83**	0.48	0.56	-0.73	-0.65	-0.63	**0.87**	0.56	0.63
	φ_0											1.00	**0.99**	**-0.92**	**-0.95**	**-0.96**	0.71	**0.97**	**0.96**	**-0.89**	**-0.93**	**-0.94**	0.60	**0.96**	**0.94**
	PC1 / log k												1.00	**-0.95**	**-0.96**	**-0.98**	0.76	**0.98**	**0.98**	**-0.93**	**-0.95**	**-0.96**	0.67	**0.97**	**0.96**
C	log k_w (lin)													1.00	**0.98**	**0.98**	**-0.91**	**-0.96**	**-0.98**	**0.99**	**0.97**	**0.99**	**-0.83**	**-0.98**	**-0.99**
	log k_w (bin)														1.00	**0.97**	**-0.87**	**-0.96**	**-0.97**	**0.95**	**0.97**	**0.97**	-0.75	**-0.97**	**-0.97**
	m log k															1.00	**-0.82**	**-0.99**	**-1.00**	**0.97**	**0.95**	**0.99**	-0.73	**-0.99**	**-0.99**
	S																1.00	0.77	**0.82**	**-0.92**	**-0.89**	**-0.86**	**0.96**	**0.82**	**0.86**
	φ_0																	1.00	**0.99**	**-0.94**	**-0.94**	**-0.98**	0.67	**0.99**	**0.98**
	PC1 / log k																		1.00	**-0.97**	**-0.96**	**-0.99**	0.73	**0.99**	**0.99**
D	log k_w (lin)																			1.00	**0.96**	**0.99**	**-0.87**	**-0.97**	**-0.99**
	log k_w (bin)																				1.00	**0.96**	**-0.80**	**-0.96**	**-0.96**
	m log k																					1.00	-0.78	**-0.99**	**-1.00**
	S																						1.00	0.72	0.78
	φ_0																							1.00	**0.99**
	PC1 / log k																								1.00

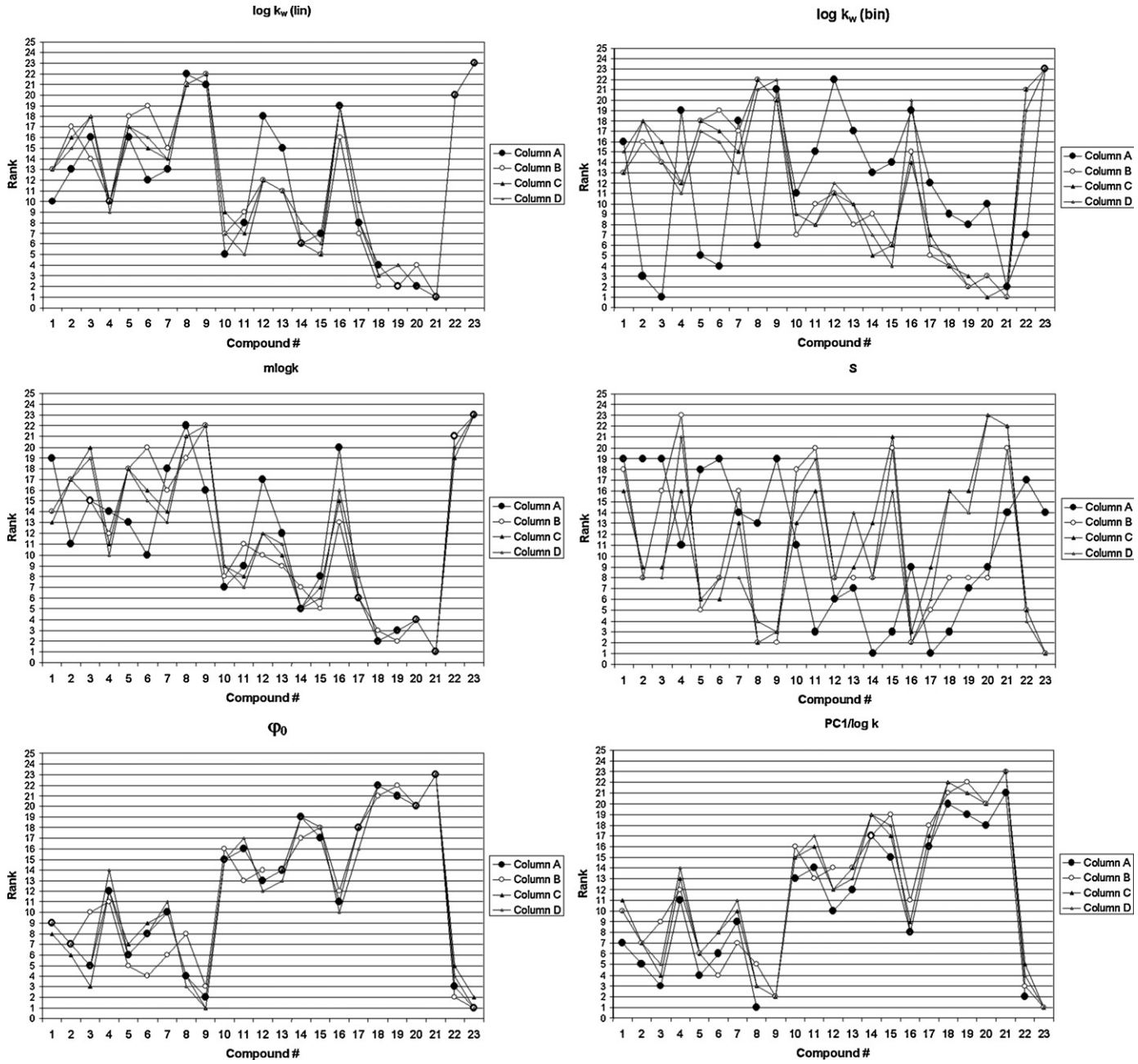


Fig. 3. Rank profiles according to the increase of the chromatographic lipophilicity indices determined for the considered compounds. The chromatographic lipophilicity indices are: $\log k_w$ (lin); $\log k_w$ (bin); $m \log k$; S; φ_0; PC1/$\log k$.

C18 stationary phase with the other 23 indices (6 indices per stationary phase, 4 stationary phases considered) is placed above the 0.8 threshold (taken as module). The same S index determined on the polar embedded C18 phase correlates well (correlation factor above 0.8) only with 2 other indices from the total of 23. The S indices obtained on phenyl and PFP stationary phases correlates better with the other determined indices (20 correlations above 0.7 for the value obtained on the phenyl column and 15 correlations above the same threshold for the value obtained on the PFP stationary phase).

Correlations coefficients higher than 0.8 (as a module) of the $\log k_w$ (lin), $m \log k$, φ_0 and PC1/$\log k$ indices to other indices on the studied stationary phases globally represents between 79.3 and 83.7% for the total number of possible correlations. Log k_w (bin) is following (64.1%), together with S (26.1%).

When considering correlation coefficients above 0.8 for lipophilicity indices obtained on a given stationary phase with respect to the total number of possible correlations, the phenyl stationary phase is placed on the first place (79%), followed by the PFP one (75.6%), the polar embedded C18 phase (67.4%) and finally the C18 phase (53.6%).

The investigated compounds are highly structurally related, so it was expected that the correlation between $\log k_w$ (lin) and S to be characterized by high correlation coefficients, which, according to some authors [33], reveals a congener series of compounds. However, the obtained results are contradictory because on stationary phases **A** and **B** the correlations are rather weak (−0.32 on stationary phase **A** and −0.68 on stationary phase **B**), while on stationary phases **C** and **D** the correlations may be considered as significant ones (−0.91 on stationary phase **C** and −0.87 on stationary phase

Table 4
The correlation matrix of the chromatographic and computed lipophilicity indices corresponding to the investigated compounds (**1**–**23**). Bold black characters indicates correlation coefficients higher than 0.8 (as absolute value) while italic bolded characters indicates correlation coefficients between 0.7 and 0.8.

Column	Chromatographic lipophilicity indices	Correlation to computed lipophilicity indices																	
		log *D*	log$(p)^C$	log$(p)^V$	log$(p)^B$	CLOGP	log PC	log *P*	Hy	MLOGP	ALOGP	ALOGPs	AC log *P*	AB/log *P*	COSMO Fraq	mi log P	K_{ow} W_{in}	XLOGP2	XLOGP3
A	log k_w (lin)	0.66	0.50	0.45	0.67	***0.71***	0.45	0.53	−0.55	***0.71***	0.61	0.44	0.67	**0.86**	0.68	***0.73***	0.60	0.66	0.58
B		***0.73***	0.64	0.57	**0.81**	***0.77***	0.53	***0.70***	−0.69	***0.79***	***0.74***	0.56	***0.78***	**0.88**	***0.77***	**0.84**	***0.71***	***0.73***	***0.70***
C		***0.71***	0.57	0.50	***0.76***	***0.73***	0.48	0.65	−0.65	***0.77***	0.66	0.51	***0.71***	**0.88**	***0.74***	**0.80**	0.66	***0.71***	0.65
D		***0.73***	0.54	0.48	***0.71***	***0.72***	0.47	0.60	−0.58	***0.73***	0.63	0.46	0.68	**0.86**	***0.73***	**0.76**	0.64	0.66	0.59
A	log k_w (bin)	0.29	0.07	0.12	0.02	0.25	0.18	−0.02	0.07	0.13	0.15	0.04	0.14	0.23	0.01	0.08	0.17	0.08	−0.05
B		***0.70***	0.68	0.62	***0.78***	**0.82**	0.50	0.69	***−0.71***	**0.82**	***0.75***	0.60	**0.81**	**0.83**	***0.72***	**0.84**	***0.76***	***0.75***	0.69
C		***0.71***	0.65	0.58	**0.81**	***0.78***	0.51	***0.73***	***−0.72***	**0.82**	***0.74***	0.59	***0.78***	**0.89**	***0.73***	**0.86**	***0.72***	***0.78***	***0.73***
D		***0.70***	0.61	0.55	***0.75***	***0.76***	0.50	0.63	−0.66	***0.78***	***0.70***	0.52	***0.74***	**0.84**	0.69	**0.80**	0.68	0.69	0.66
A	*m* log *k*	0.60	0.54	0.48	***0.72***	***0.71***	0.46	0.62	−0.65	***0.75***	0.63	0.47	***0.70***	**0.86**	0.65	**0.77**	0.61	***0.73***	0.65
B		***0.72***	***0.74***	0.66	**0.87**	**0.83**	0.58	***0.79***	**−0.81**	**0.87**	**0.81**	0.67	**0.85**	**0.88**	***0.77***	**0.91**	***0.78***	**0.83**	***0.78***
C		***0.70***	0.63	0.55	**0.82**	***0.76***	0.51	***0.72***	***−0.74***	**0.82**	***0.71***	0.57	***0.76***	**0.89**	***0.79***	**0.85**	***0.70***	***0.78***	***0.71***
D		***0.71***	0.59	0.52	***0.78***	***0.75***	0.51	0.66	−0.68	***0.78***	0.68	0.53	***0.74***	**0.89**	***0.79***	**0.82**	0.67	***0.73***	0.66
A	*S*	0.51	0.65	0.56	***0.73***	0.55	0.48	***0.74***	***−0.76***	0.68	0.63	0.61	0.63	0.55	0.58	**0.71**	0.60	0.68	0.65
B		−0.47	−0.09	−0.07	−0.28	−0.26	−0.16	−0.12	0.03	−0.21	−0.24	0.00	−0.22	−0.51	−0.46	−0.27	−0.19	−0.13	−0.17
C		−0.65	−0.36	−0.33	−0.51	−0.57	−0.33	−0.42	0.34	−0.55	−0.48	−0.29	−0.50	−0.74	−0.54	−0.57	−0.49	−0.46	−0.44
D		−0.67	−0.28	−0.27	−0.38	−0.52	−0.29	−0.31	0.19	−0.44	−0.39	−0.19	−0.41	−0.63	−0.44	−0.45	−0.44	−0.32	−0.28
A	φ_0	***−0.74***	−0.67	−0.60	**−0.83**	***−0.79***	−0.57	***−0.73***	***0.75***	**−0.84**	***−0.75***	−0.61	**−0.80**	**−0.89**	***−0.78***	**−0.87**	***−0.74***	***−0.79***	***−0.72***
B		−0.68	***−0.74***	−0.66	**−0.88**	**−0.83**	−0.57	**−0.80**	**0.83**	**−0.88**	**−0.80**	−0.67	**−0.86**	**−0.86**	***−0.76***	**−0.91**	***−0.78***	**−0.84**	***−0.79***
C		−0.66	−0.64	−0.55	**−0.82**	***−0.76***	−0.51	***−0.73***	***0.76***	**−0.83**	***−0.70***	−0.58	***−0.77***	**−0.87**	***−0.78***	**−0.85**	***−0.70***	***−0.79***	***−0.71***
D		−0.67	−0.60	−0.52	−0.79	***−0.74***	−0.50	−0.67	***0.71***	***−0.79***	−0.68	−0.53	***−0.75***	**−0.88**	***−0.79***	**−0.82**	−0.67	***−0.75***	−0.68
A	PC1/log *k*	−0.63	−0.58	−0.47	**−0.83**	***−0.70***	−0.38	***−0.71***	***0.75***	***−0.79***	−0.68	−0.49	***−0.75***	**−0.90**	***−0.74***	**−0.86**	−0.63	***−0.78***	***−0.77***
B		***−0.72***	***−0.74***	−0.66	**−0.87**	**−0.83**	−0.57	***−0.79***	**0.81**	**−0.87**	**−0.81**	−0.67	**−0.85**	**−0.88**	***−0.78***	**−0.91**	***−0.78***	**−0.83**	***−0.78***
C		***−0.70***	−0.63	−0.55	**−0.82**	***−0.76***	−0.51	***−0.72***	***0.74***	**−0.82**	***−0.71***	−0.57	***−0.76***	**−0.89**	***−0.79***	**−0.85**	***−0.70***	***−0.78***	***−0.71***
D		***−0.71***	−0.59	−0.52	***−0.78***	***−0.75***	−0.51	−0.66	0.68	***−0.78***	−0.68	−0.53	***−0.74***	**−0.89**	***−0.79***	**−0.82**	−0.67	***−0.74***	−0.66

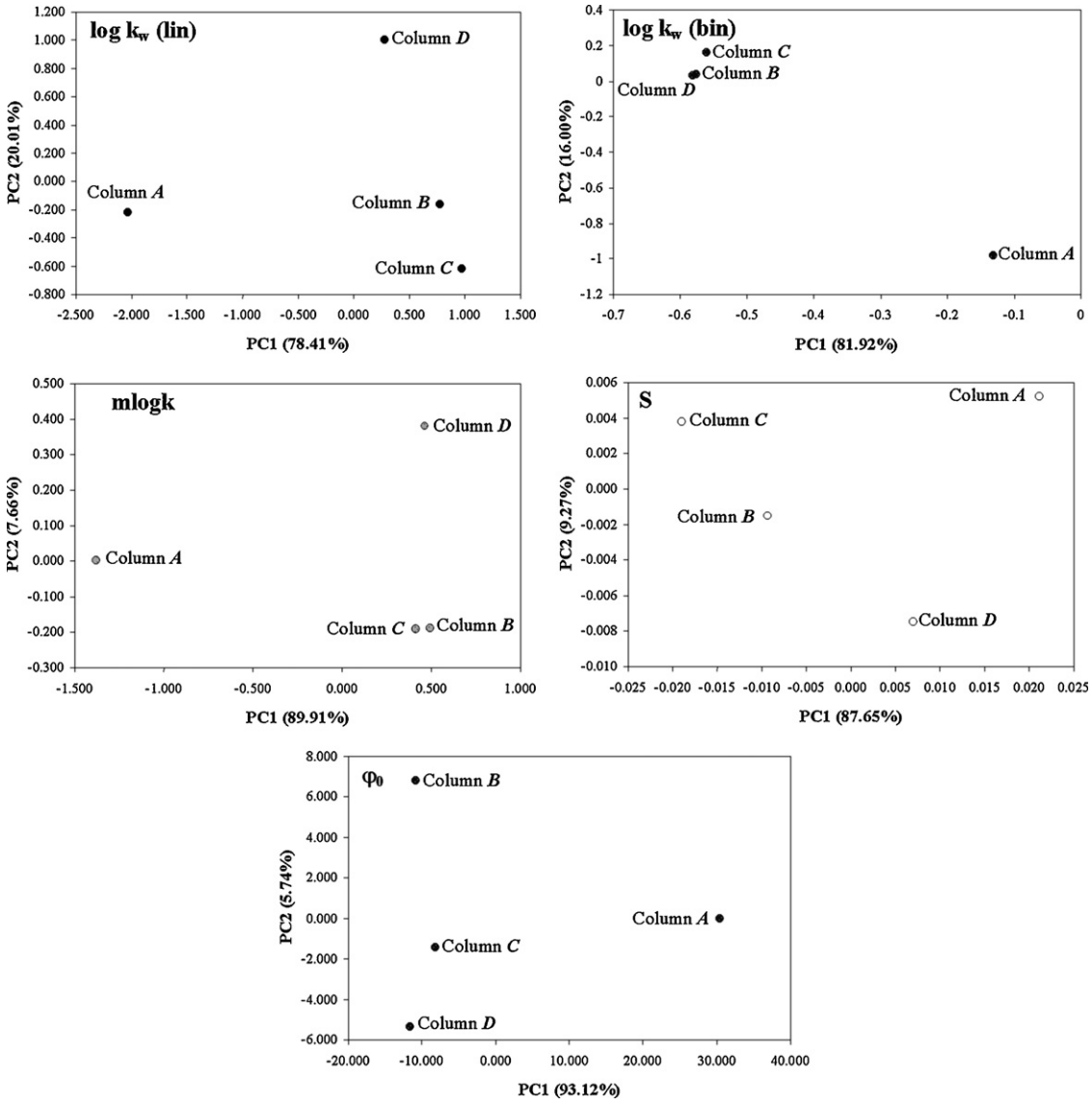


Fig. 4. Lipophilicity charts obtained for the studied stationary phases in columns **A**–**D** obtained through application of PCA to the matrix of the chromatographic determined indices $\log k_w$ (lin); $\log k_w$ (bin); $m \log k$; S; φ_0; PC1/$\log k$ (a detailed description of the stationary phases is given in Section 2).

D). Various studies [25–31] have led to similar results unconfirmed by the chemical reality, so it may be concluded that such correlation between $\log k_w$ (lin) and S is not highly significant in the lipophilicity studies, and may be taken into consideration only from a statistical point of view. However, S value retains some information about the specific interactions of the analytes with the stationary phases, allowing insights about their behavioral similarities/dissimilarities.

The considered stationary phases are different by taking into account their polar characteristics. Anyway, the per-fluorinated aromatic stationary phases (like stationary phase **D**) are difficult to classify, their polarity being intermediary placed between octadecyl modified and net silica gel materials. Moreover, they are characterized by both reversed (π–π) and normal phase (dipolar and H-bonding) interactions [34]. The comparison of the elution behavior of flavonoids on different stationary phases may be evidenced by means of the 2D correlation profiles (in Fig. 3) of the lipophilicity indices. It can be observed that the differences induced by the nature of the stationary phase are quite minor. Moreover, one can appreciate that stationary phase **D** induces mostly lipophilic interactions. However, the correlation matrix (in Table 3) of the chromatographic descriptors indicates significant difference between stationary phase **A** and the other stationary phases. The values obtained on stationary phases **B**–**D** are well correlated, evidencing the positive contribution to the chromatographic retention of some additional hydrophilic interactions between analytes and the stationary phases. Additionally, a classification of the stationary phases may be obtained if PCA is applied on the matrix of the chromatographic lipophilicity indices obtained on different stationary phases. In the lipophilicity charts pictured in Fig. 4 it may be observed that stationary phases **B**–**D** are always forming linear clusters, while stationary phase **A** induces a different chromatographic elution behavior. Log k_w (bin) is however the chromatographic lipophilicity index which is grouping the stationary phases **B**–**D** as a cluster. All these aspects are reflecting the capacity of the PCA approach to discriminate between large sets of data, allowing illustrative classifications of products or behaviors according to the interpretation of their characteristics.

Even more, the PCA loadings (eigenvectors) allow the investigation of the retention mechanism involved in the separation process by comparing their profiles depicted against the organic modifier

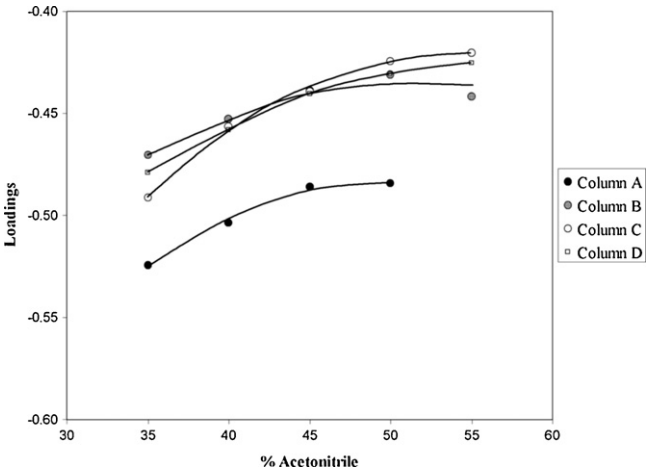


Fig. 5. The log k loadings quadratic scatterplot.

fraction used in the mobile phase (see Fig. 5). The log k loadings quadratic profiles illustrate once more that, even if the interactions are mainly lipophilic, stationary phases **B**–**D** allow some additional weak polar interactions. Considering all above mentioned aspects, it may be concluded that the lipophilicity of flavonoids on stationary phases **B**–**D** is characterized by similar values of the chromatographic indices, while the highly non-polar character of stationary phase **A** leads to a different lipophilicity scale.

The computed lipophilicity indices (see Table 1) are usually considered to be highly descriptive, but the question regarding their possibility to describe the chromatographic or the biological behavior is still debatable. The correlation matrix (in Table 4) between chromatographic lipophilicity and computed indices offers new insights about their adaptability to describe the chromatographic behavior.

From Table 4, it clearly results that $\log(p)^C$, $\log P_C$ and ALOGPs are practically not correlated with all chromatographic lipophilicity indices determined over the studied stationary phases. The higher number of correlations are provided by AB/log P, mi log P, CLOGP and $\log(p)^B$ computed descriptors. On the stationary phase **A**, $\log k_w$ (bin) and PC1/log P exhibit a lack of correlation with all the computed lipophilicity descriptors. The S index is evidently the chromatographic lipophilicity index the least correlated to all computed descriptors. Indices φ_0 and $m \log k$ are the chromatographic descriptors closest correlated to all computed values. On the stationary phase **A**, 29 correlations (correlation coefficient higher than 0.7, as a module) have been achieved between the chromatographic lipophilicity indices and the computed ones. The greatest number of correlations between chromatographic and computed descriptors was obtained on the stationary phase **B** (69 from a total possible of 108), followed by the stationary phase **C** (65) and finally by **D** (42).

4. Conclusions

The chromatographic lipophilicity indices of some flavonoids were estimated using different ACN-aqueous 0.1% formic acid mixtures as mobile phases on octadecyl modified silicagel, polar embedded octadecyl modified silicagel, phenyl modified silicagel, and pentafluorophenyl modified silicagel. The octadecyl modified stationary phase is prone to some morphological modifications when used with water rich mobile phase compositions, resulting in specific interactions with some of the studied analytes. The correlation matrices, lipophilicity charts and graphical profiles of loadings sustain that the separation process on octadecyl silicagel is totally based on lipophilic interactions, as expected, while the other stationary phases add some weak polar interactions too. Chromatographic lipophilicity indices are highly correlated one to another, especially on stationary phases inducing also some polar interactions. From far, the S index is the least descriptive when considering the lipophilicity character. However, S usefully describes how sensitive is the chromatographic retention of a target compound to the change of the organic modifier concentration in the mobile phase. Moreover, it may be concluded that the highest correlation between the chromatographic determined indices and the computed ones is achieved on the polar embedded octadecyl stationary phase. For this structurally related series of compounds, the φ_0 descriptor produces the best correlation to the computed indices. Amidst the computed lipophilicity indices, AB/log P, mi log P, CLOGP and $\log(p)^B$ are highly descriptive for the chromatographic behavior of flavonoids

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