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Correlation study of retention data and antimalarial activity of 1,2,4,5-mixed tetraoxanes with their molecular structure descriptors and LSER parameters

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ABSTRACT

The chromatographic behavior of mixed 1,2,4,5-tetraoxanes, cholic and deoxycholic acid derivatives with distinct biological activity, was examined by high-performance thin-layer chromatography in order to correlate their structure and retention. Chromatographic systems were consisted of RP-18 or CN-silica as stationary phase, and binary mixtures of water with methanol, dioxane or acetone as mobile phase. Based on the respective retentions, the lipophilicity of the investigated compounds was determined. Multiple linear regression and partial least squares have been used to select variables that best describe the behavior of the investigated compounds in chromatographic systems and to quantify influences of most important parameters. The validation and cross-validation of the QSRR model suggest its applicability for prediction and understanding of retention of congeners. The models indicate the importance of nonpolar properties of the solutes and their ability for hydrophobic interactions, as well as the importance of proton donating abilities, hydrophilic and π interactions pointing out on that way the possible separation mechanism in the studied chromatographic systems. Observed correlations between structure and biological activity of mixed 1,2,4,5-tetraoxanes, indicate that

the antimalarial activity against W2 and D6[1] *P. falciparum* strains, is governed by hydrophobic feature (measured with lipophilicity parameter), hydrophilic feature (measured with HLB, %HS, HB and HBA descriptors), and electronic feature (HOMO).

Keywords: Mixed 1,2,4,5-tetraoxanes, Lipophilicity, Quantitative structure-retention relationship (QSRR), Quantitative structure activity relationship (QSAR), Reversed-phase thin layer chromatography (RPTLC)

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1. Introduction

Retention, as a property of the analyte under defined chromatographic condition, has much in common with the pharmacokinetic/pharmacodynamic processes of drug behavior. It is considered that the major molecule structure features (*i.e*. lipophilicity, electrical charge, polarity and steric effects) which govern retention of compound also affect transport processes and drug–target interactions [1, 2]. Lipophilicity of compounds is one of the most important parameter that has strong influence on their biological activity [3, 4].

[1] Abbreviations: W2 chloroquine resistant and mefloquine susceptible *P. falciparum* strain (Indochina clone). *P. falciparum* D6 chloroquine and mefloquine susceptible *P. falciparum* strain (African clone).

Nowadays, traditional shake-flask procedure for lipophilicity determination is completely replaced with chromatographic methods like reversed-phase high-performance liquid chromatography (RP-HPLC) and reversed-phase high-performance thin-layer chromatography (RP-HPTLC). The real importance of the lipophilicity has been pointed out within the Quantitative Structure–Activity Relationship (QSAR), Quantitative Structure–Retention Relationship (QSRR) or Quantitative Structure–Property Relationship (QSPR) [5, 6]. Relationships obtained from such analysis describes physicochemical properties of analytes and identify the most important structural descriptors that govern the separation mechanism in a given chromatographic system, generate knowledge about the various interactions between the solute and the stationary and mobile phases and could be of importance for explanation of biological behavior of compounds.

As a continuation of our previous research on chromatographic behavior of steroidal tetraoxanes [7, 8] in this study the QSRR and QSAR studies of new class of mixed 1,2,4,5-steroidal tetraoxanes, cholic and deoxycholic acid derivatives with observed antimalarial activity [9-11], were performed. The first goal of this article was to characterize the lipophilic properties of 1,2,4,5-mixed tetraoxanes and to understand the separation mechanism in a given chromatographic system by applying different methods of multivariate analysis. In addition, QSAR was performed on antimalarial activity against W2 and D6 strains of 1,2,4,5-mixed tetraoxanes, in order to identify the interaction profile of the inhibitors, as well as key structural elements which mostly contribute biological properties.

2. Material and methods

2.1. Thin-Layer Chromatography

The chromatographic investigations were performed using the horizontal TLC on 10 × 10 cm plates covered with octadecyl and cyano modified silica. An HPTLC developing chamber (Camag, Muttenz, Switzerland) in the tank configuration was used for this purpose. The investigated substances were dissolved in dichloromethane, and the plates were spotted with 1.0 mL aliquots of freshly prepared solutions (C ~ 2 mg/mL). The following chromatographic systems were used: RP-18 W F254s (Art. 5559, Merck, Darmstadt, Germany) with mobile phase 92-100 Vol% methanol in water in (increment 2%), 60-100% Vol% acetone in water (increment 10%) and 70–90 Vol% dioxane in water in (increment 5%) and CN F_{254S} (Art. 16464, Merck) with mobile phase 50-90 Vol% acetone in water in (increment 10%). All solvents used were of analytical-grade purity. Dichloromethane, methanol and acetone were purchased from Sigma – Aldrich (Steinheim, Germany) and dioxane from Merck. Development distance was 4.5 cm. Detection of individual zones was performed by spraying the plates with 50% sulphuric acid and heating until the spots became visible. All experiments were performed at ambient temperature (22 ± 2 ºC).

2.2. Structural descriptors and molecular modeling

Optimized geometrical representations of the studied compounds were obtained by Hyperchem Professional software (version 7.0, Hybercube, Gainesville, FL, USA). Molecular Modeling Program Plus (MMP Plus) (http://norgwyn.com/mmpplus.html) software was used for the calculation of physicochemical properties and the values of connectivity indices. The calculated molecular descriptors are listed in Table S1 (Supplementary data).

The coefficients *A*, *B*, *E*, *S*, and *V* for LFER analysis (Table S2, Supplementary data) were calculated by ADMET software (http://.https://ilab.acdlabs.com/iLab2/).

2.3. Multivariate statistical analysis and modeling

Partial least square regression (PLS) was carried out by a PLS_Toolbox software package (v. 5.7 Eigenvectors Inc.) for MATLAB (v. 7.8.0 R2009) (MathWorks, Natick, MA, USA). The LSER methodology was done using the NCSS software (Hintze, J. (2001), NCSS and PASS Number Cruncher Statistical Systems, Kaysville, Utah; www.ncss.com).

The dimensions of the data set were $N \times K$ (40 compounds × 32 variables or descriptors). In addition to the cross-validation procedure, the entire data set of 40 compounds was divided into two subsets: calibration set consisting of the 26 randomly selected solutes (**6, 10, 12, 3, 15, 16, 19, 39, 33, 32, 37, 27, 7, 14, 25, 5, 35, 31, 28, 30, 18, 29, 40, 9, 17, 22**) and the testing set composed of the rest of the studied compounds (**1, 2, 4, 8, 11, 13, 20, 23, 24, 26, 34, 38, 21, 36**). The data were mean-centered and scaled to unit variance before statistical analyses.

3. Results and discussion

3.1. Chromatographic behavior of mixed 1,2,4,5- tetraoxanes

Mixed 1,2,4,5- tetraoxanes (Table 1) possess the steroid moiety at one side of the 1,2,4,5-tetraoxane ring and simply cycloalkane or isopropylidene chain at the opposite ones. Spirocycloalkane and isopropylidene moieties participate in hydrophobic interactions, polar tetraoxane ring act as H-bond acceptor, and steroidal moiety participate in both hydrophobic and polar interactions (hydrogen bonding).

One example graph of dependence of R_M on the Vol% of dioxane in the mobile phase is presented in Figure S1 (Supplementary data). Chromatographic behavior of mixed-tetraoxanes revealed stronger retention of acids in comparison to primary amides probably because of their dimerization by formation of intermolecular hydrogen bond. Such dimerization decreases the possibility of hydrogen bonds formation of carboxylic groups with mobile phase molecules, and making whole structure more lipophilic. Also, the retention of esters is stronger than the

corresponding acids. It was established a good selectivity in separation of mixed tetraoxanes differing from one another in $–CH_2$ groups within substituents on C(24) as well as in separation of 4″(*R*) and *4″*(*S*) epimers. However, there was no regularity in epimers retention. Increasing the number of $–CH_2$ groups within substituents on C(24) resulted in increasing retention.

The R_M^0 values obtained by extrapolation of R_M values to 0 Vol% of organic solvent are summarized in Tables S3-S6 (Supplementary data).

As is well known, different retention in a given chromatographic system, is a result of a different physicochemical properties of each solute. Knowing the properties of analytes, their chromatographic behavior could be explained using linear solvation energy relationship (LSER) analysis. [12]. All models obtained using LSER parameters as independent variable, and R_M^0 values as dependent ones are summarized in Table 2. The parameters *A*, *B*, *S*, *E* or *V* determined as non-significant for a specific model, were excluded. Exclusion of a certain parameter resulted in almost the same proportion of explained variance and a corresponding *RMSEC* value (Table 2, equation 2).

In systems RP-18 / water- dioxane the values of hydrogen bond donor (A), and dipolarity/polarizability (S) parameters of the investigated substances are negative suggesting that this type of interactions are stronger with mobile than with stationary phase. The higher the values of these coefficients are, the lower are the R_M^0. On the other hand, the coefficients of the excess of molar refraction, *E*, and McGowan's volume, *V*, have the positive sign. Descriptor *E* is a measure of the interaction of the stationary phase with the solute's *n*- or π-electrons, and *V* is a measure of the cohesiveness as well as dispersion interactions. The increasing of these interactions lead to increased R_M^0 values indicating a higher lipophilicity.

According to the model obtained for RP-18 stationary phase and mobile phase containing methanol, parameters *A* and *B* are structural characteristics responsible for the dominant type of interactions occurring between the investigated substances and mobile phase, since the most dominant interactions between investigated substances and stationary phase are cohesiveness as well as dispersion interactions. MLR model obtained for RP-18 /water-acetone system indicates the importance of dipolarity/polarizability (S) of the investigated substances in interactions with mobile phase while the parameters *E* and *V* are important in their interaction with stationary phase. In model obtained for CN-silica the most dominant interactions between the solutes and mobile phase are governed by parameters *A* and *S*, since the dominant in interactions with stationary phase are parameters *B* and *E*.

The R_M values obtained by Eq. (1) depend linearly on the concentration of the organic modifier in the mobile phase and, therefore, they might be used to assess lipophilicity.

Considering the obtained R_M^0 values it can be concluded that the deoxycholic acid derived tetraoxanes exhibited the highest lipophilicity and the cholic acid derived mixed tetraoxanes containing isopropylidene subunit exhibited the lowest lipophilicity values.

3.2 Modeling of retention and biological activity

QSRR analysis and QSAR on antimalarial activity against W2 and D6 strains of mixed 1,2,4,5- tetraoxanes, was performed in addition to previously described LSER model.

The models obtained for quantification of relationships between factors governing retention, *i.e.* lipophilicity, are summarized in Table 3. The assessment of descriptors that have the greatest influence on retention was done based on Variable importance in the projection (VIP) scores. The descriptors included in the final QSRR model are presented in Table 3 in descending order of regression coefficients, with notification of the sign of their contribution on

the dependent variable. All models are statistically significant, while the model obtained for chromatographic system with dioxane shows the best statistical performance. The descriptors included in the final models are of similar nature and significance.

All models confirm the importance of the lipophilicity parameter, log*P*. This parameter describes the nonpolar properties of the compounds and the ability to engage in hydrophobic (dispersive) interactions with the stationary phase, and thus positively contribute to retention. Descriptors such as the HLB, %HS, HBD, HB, and HBA (abbreviations of structural descriptors are presented in Table S1, Supplementary data), describe polarity of the investigated compounds, *i.e.* their abilities to engage in hydrophilic interactions. These molecular parameters negatively contribute to R_M^0 values. The model obtained for chromatographic system CN-silica/acetone-water contains dipole moment (DM) as an important variable. DM as a measure of the compound's ability to take part in the dipole-dipole and dipole-induced dipole interactions with components of the mobile and stationary phases influences negatively on retention. Topological descriptors encoding information about the degree of branching and size of the molecules and a positively sign of VI3 means that the third-order branching is a favorable parameter for getting higher R_M^0 values.

Some of the investigated substances exhibited impressive *in vitro* and *in vivo* antimalarial activity [9-11]. Recently, QSAR models based on correlation of biological activity against human cervix carcinoma (HeLa) and human malignant melanoma (Fem-X) cell lines and structures of *bis*-steroidal tetraoxanes, revealed the importance of descriptors relating to the size and shape of a molecule and those describing the ability of hydrogen bonding [7]. The similar conclusion was reported by Cvijetić et al. [13] in the case of 1,2,4,5-tetraoxane derivatives who claimed that hydrophobicity and H-bond donor properties were the main parameters influencing

potency of compounds towards HeLa and Fem-X strains. The in *vitro* antimalarial activity against two *P. falciparum* strains, W2 and D6 (Table S7, Supplementary data) towards structural descriptors was analyzed by PLS. The obtained QSAR models are not of satisfactory statistical quality to be used for accurate prediction of biological activity, but they may qualitatively indicate descriptors that play an important role in the activity exhibited by these compounds. The variables that have the greatest influence on antimalarial activity against W2 and D6 strains, together with the standardized regression coefficient that reveals the significance of an individual variable in the regression models, are shown in Figure S2 (Supplementary data).

The most relevant descriptors influencing activity against both strains are HLB, %HS, HB, Log*P*, HBA, CI 2, HOMO, MWd, SA(Ap). In addition, in model for W2 strain MW descriptor is included. From the graphic of VIP scores, for both strains, it is observed about two times higher influence of HLB, %HS and HB compared to other stated descriptors.

Log*P* and HOMO influence the biological activities in a positive manner, *i.e.* higher lipophilicity point to higher potency toward W2 and D6 strains. Also, the increasing of the HOMO energy indicates higher reactivity of the molecule, *i.e.* it is easier for those molecules to donate electrons from the accessible (near-zero) HOMO level toward appropriate acceptor (electrophile or reductant). That is in accordance to assumed mechanism of action which comprise transfer of single electron from 1,2,4,5-tetraoxane moiety toward Fe(II) [14]. Contrary to that, HLB, %HS, HB, HBA, CI 2, MWd, SA(Ap) and MW influence the biological activity in a negative manner, indicating that the higher these values are, the lower is their potency toward W2 and D6 strains.

A three-dimensional QSAR pharmacophore model for antimalarial activity of *bis*-steroidal and steroidal mixed 1,2,4,5-tetraoxanes was previously developed [15]. Model contains

two hydrogen bond acceptors (lipid) and one hydrophobic (aliphatic) feature, and maps well onto the potent analogues as well as many other active peroxide antimalarials. It appears that the presence of at least one hydrogen bond acceptor in the trioxane or the tetraoxane moiety is necessary for good activity of peroxide antimalarials. Consequently, the influence of the HB and HBA descriptors emphasizes the importance of the compound's nitrogen- and oxygen-containing components, and Log*P* parameter highlighting the influence of hydrophobic feature. Docking calculations with heme suggests that the proximity of the Fe(II) and oxygen atom of the trioxane or the tetraoxane moiety favors potent activity of the compounds and that electron transfer from peroxide oxygen is crucial for mechanism of action [15]. Hence, the contribution of the HOMO parameter appear to be of additional importance to the observed antimalarial activities of the mixed 1,2,4,5- tetraoxanes.

4. Conclusion

Proposed LSER and QSRR models correctly represent the relationship between the retention factors of mixed 1,2,4,5- tetraoxanes and five selected LSER descriptors, *i.e*. molecular descriptors calculated solely from molecular structures. Taking into account the statistical quality of the model, the best results are obtained with dioxane-water mobile phase on RP-18 sorbent. Also, the validation and cross-validation of the QSRR model suggest that the model can be used to make prediction of chromatographic behavior of congeners and understanding of their retention.

The proposed QSAR models suggest a great importance of lipophilicity parameter for antimalarial activity of mixed 1,2,4,5- tetraoxanes. These results are different to those obtained for antiproliferative activities of *bis*-steroidal tetraoxanes where the size of the molecule are critical for its transport through the membrane and for 1,2,4,5-tetraoxanes where the

hydrophobicity has negative influence. Such results indicate different mechanisms of action for antimalarial and antiproliferative activity of the investigated compounds.

Acknowledgements

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Highlights

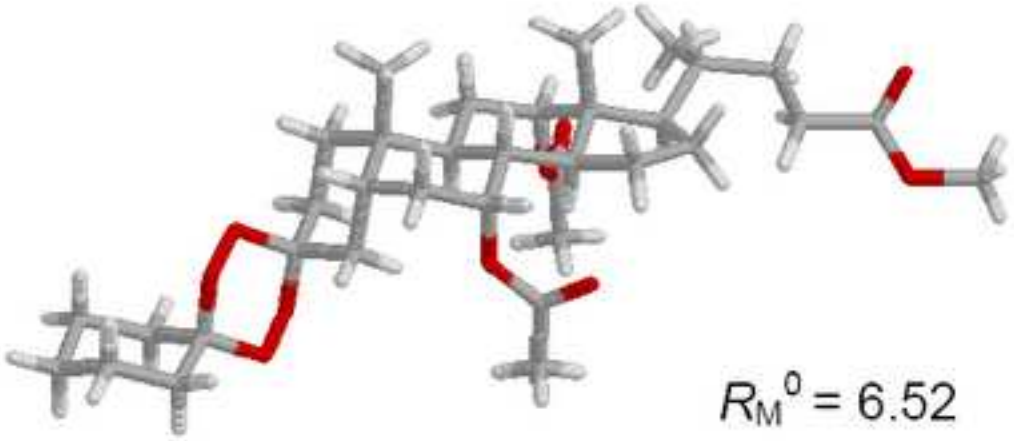
1,2,4,5-tetraoxanes derivatives which inhibit two unrelated pathogens were studied.

Lipophilicity was determined.

Correlations between structure, retention, and activity were performed.

Physically meaningful descriptors governing retention and activity were obtained.

***Graphical Abstract**



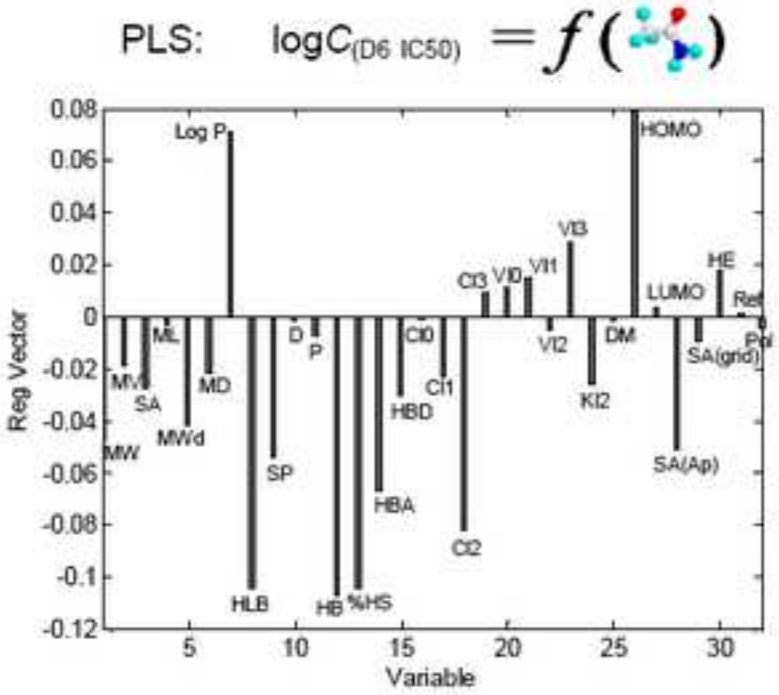
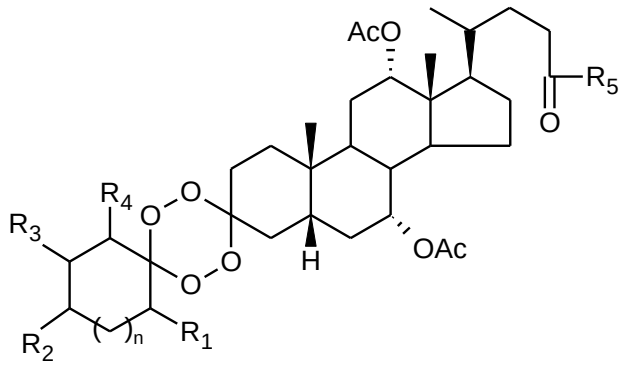
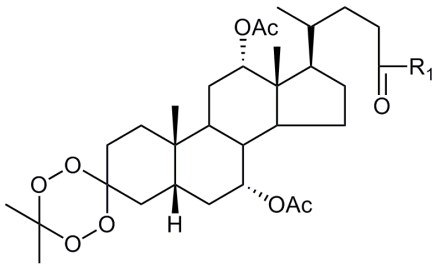


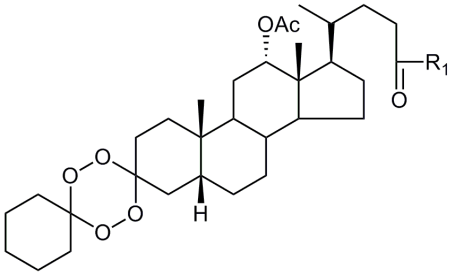
Table 1. The structures of the investigated substances



	n	R_1	R_2	R_3	R_4	R_5
1	1	H	H	H	H	OH
2	1	H	H	H	H	OCH_3
3	1	H	H	H	H	NH_2
4	1	H	H	H	H	$NHPr^n$
5	1	H	H	H	H	$NHCH_2CO_2CH_3$
6	1	CH_3	H	H	H	OCH_3
7	1	CH_3	H	H	H	NH_2
8	1	CH_3	H	H	H	NHMe
9	1	CH_3	H	H	H	$NHPr^n$
10	1	CH_3	H	H	CH_3	OCH_3
11	0	H	H	H	H	OCH_3
12	3	H	H	H	H	OCH_3
13	1	i-Pr	H	CH_3	H	OCH_3
14	1	H	(*R*)-CH_3	H	H	OH
15	1	H	(*R*)-CH_3	H	H	OCH_3
16	1	H	(*R*)-CH_3	H	H	NH_2
17	1	H	(*R*)-CH_3	H	H	NHMe
18	1	H	(*R*)-CH_3	H	H	$NHPr^n$
19	1	H	(*S*)-CH_3	H	H	OH
20	1	H	(*S*)-CH_3	H	H	OCH_3
21	1	H	(*S*)-CH_3	H	H	NH_2
22	1	H	(*S*)-CH_3	H	H	NHMe
23	1	H	(*S*)-CH_3	H	H	$NHPr^n$



	R_1
24	OH
25	OCH_3
26	NH_2
27	NHMe
28	$NHPr^n$
29	$N(Me)_2$
30	$NHCH_2COOCH_3$
31	NHPh
32	$NHNH_2$



	R_1
33	OH
34	OCH_3
35	NH_2
36	$NHPr^n$
37	$NHNH_2$
38	$NH(Pr^n)_2$
39	NHPh
40	

Table 2. LSER models with accompanying statistical performances

Chromatographic system	**Equation**	**Statistical parameters**[a]
RP-18 / Dioxane - water	R_M^0 = 4.7761 (± 1.2882) - 0.9474 (± 0.4071)*A* + 0.4546 (± 0.7793)*B* + 2.7447 (± 0.6355)*E* – 4.1664 (±0.7291)*S* + 1.9008 (±0.2918)*V*	R^2 = 0.8728, MSE = 0.1604, F = 46.656, P = 0.0000, N = 40, SSY = 42.8658, PRESS = 7.9191, R^2_{CV} = 0.8153
	R_M^0 = 4.9771 (± 1.2294) - 0.9264 (± 0.4016)*A* + 2.5066(± 0.6355)*E* – 3.7795(±0.3003)*S*+ 1.9025(±0.2890)*V*	R^2 = 0.8715, MSE = 0.3967, F = 59.3533, P = 0.0000, N = 40
RP-18 / Methanol – water	R_M^0 = 11.9407 (±2.5415) – 2.9636 (±0.8031)*A*– 3.6273 (±1.5374)*B* + 2.5562(±1.2538)*E* – 2.4118(±1.4385)*S* + 1,5171(±0.5757)*V*	R^2 = 0.8121, MSE = 0.6242, F = 29.393, P = 0.0000, N = 40, SSY = 2.8966, PRESS = 31.1547, R^2_{CV} = 0.7242
	R_M^0 = 12.9304(±2.5643) – 2.4424(±0.6941)*A* – 5.8745(±0.6533)*B* + 1.6856(±0.55352)*V*	R^2 = 0.7888, MSE = 0.8140, F = 44.8296, P = 0.003284, N = 40
RP-18 / Acetone - water	R_M^0 = 0.2844 (±2.3530) – 1.1969 (±0.7435)*A* – 0.1894 (±1.4234)*B* + 3.4881(±1.1608)*E* – 3.2544(±1.3318)*S*+ 2.1143(±0.5330)*V*	R^2 = 0.6610, MSE = 0.5351, F = 13.260, P = 0.0000, N = 40, SSY = 53.6697, PRESS = 24.8323, R^2_{CV} = 0.5373
	R_M^0 = 0.8363 (±2.1960) + 3.003(±0.8216)*E* – 3.5820(±0.5493)*S* + 2.6134(±0.4396)*V*	R^2 = 0.6344, MSE = 0.7382, F = 20.8246, P = 5.37E-08, N = 40
CN-silica / Acetone - water	R_M^0 = 4.7420 (±1.7823) – 1.7753 (±0.5637)*A* + 3.7007 (±1.0791)*B* + 4.6174 (±0.8807)*E* – 5.2208(±0.9691)*S* + 0.1794 (±0.4043)*V*	R^2 = 0.6068, MSE = 0.3074, F = 10.496, P = 0.0000, N = 40, SSY = 0.26.5844, PRESS = 14.9288, R^2_{CV} = 0.4387
	R_M^0 = 5.3109 (±1.2233) – 1.9191(± 0.4558)A + 3.7067(±1.0666)B + 4.6922(±0.8544)E – 5.1690(± 0.9915)S	R^2 = 0.6046, MSE = 0.3003, F = 13.378, , P = 5.37E-08, N = 40

[a] R^2, squared correlation coefficient, *MSE*, mean square error, *P*, significance level, *F*, Fischer's significance value, R^2_{cv}, cross-validation squared correlation coefficient, R^2_{cv} = (*SSY* – *PRESS*)/*SSY*, *SSY* , the sum of the squared deviations of the dependent variable values from their mean, *PRESS*, prediction residual sum of squares (reflects the overall predictive ability of the model).

Table 3. Results obtained via PLS analysis

Chromatographic system	Statistical performance of the model[a]						Molecular descriptors included in model
	R^2_{cal}	R^2_{CV}	R^2_{pred}	*RMSEC*	*RMSECV*	*RMSEP*	
RP-18 / Dioxane-water	0.9395	0.8176	0.8316	0.2414	0.4414	0.4544	Log *P*(+), HLB(-), %HS(-), P(-), HBA(-), VI3(+), HB(-), SA(Ap)(-)
RP-18 / Methanol-water	0.7461	0.6349	0.6944	0.7831	1.0125	1.0619	Log *P*(+), HLB(-), %HS(-), HBA(-), P(-), HBD(-), HB(-), VI3(+)
RP-18 /Acetone-water	0.7748	0.5820	0.5656	0.5078	0.7285	0.9750	Log *P*(+), HLB(-), %HS(-), HBA(-), P(-), HB(-), VI3(+), SA(Ap)(-)
CN-silica /Acetone-water	0.6957	0.5081	0.6747	0.4643	0.6005	0.5139	HLB(-), %HS(-), Log *P*(+), P(-), DM(-), MWd(-), HBA(-), HB(-)

[a] R^2_{cal}, R^2_{CV}, and R^2_{Pred}, squared values of Pearson's correlation coefficients for calibration, cross-validation, and prediction, respectively; *RMSEC*, *RMSECV*, and *RMSEP*, root mean-square errors of calibration, cross-validation, and prediction, respectively.