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Calculation of chromatographic parameters by molecular topology: sulphamides

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Abstract

This investigation was undertaken to test the ability of the molecular connectivity model to predict R_F values in thin-layer chromatography (TLC) for a group of sulphamides using multi-variable regression equations with multiple correlation coefficients, standard error of estimate, *F*-Snedecor function values and Student's *t*-test as criteria of fit. Regression analyses showed that the molecular connectivity model predicts the values for this property in different silica gel stationary phases and different polar mobile phases. Corresponding stability and random studies were made on the selected prediction models which confirmed their goodness of fit. The results also demonstrated that different structural features determine the R_F values in TLC of sulphamides.

1. Introduction

Molecular topology has been shown to be a very important structural model for describing the chromatographic [1–4] and environmental [5,6] behaviour of chemicals. This method transcribes molecular structure into a topological graph from which a number is derived, the topological index. Topological parameters, such as molecular connectivity indices [7], can be used to quantify these properties.

In quantitative structure–activity relationship (QSAR) studies, the kind of descriptor parameters mentioned above are used to explain or predict the pharmacological behaviour of drug molecules. During the last 5 years, molecular connectivity indices have been used to predict several parameters related to the biological activities of drugs [8]. It was concluded that the direct correlation of molecular topology with biological activity is possible [9]. Therefore, it might be possible that the chromatographic behaviour of drugs in phases of different polarity contains information that is useful in describing their pharmacological behaviour, *e.g.*, for barbiturates [10] and neuroleptics [11].

In a previous paper [12] it was demonstrated that the molecular connectivity model [13,14] successfully predicts retention parameters of benzodiazepines in gas–liquid chromatography (GLC) and thin-layer chromatography (TLC) on polar and low-polarity eluents. In this study, we examined the relationship between R_F values in

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TLC and the connectivity indices of a group of sulphamides.

2. Method of calculation

Several extensive reviews have been published [8,15–18] which give detailed descriptions of the theory and method of calculation of all-valence and non-valence molecular connectivity indices used in this investigation.

Connectivity indices are calculated from a hydrogen-supressed formula or graph of the molecule, following the method of Kier and Hall [7]. Thus, for a graph of m edges and s subgraphs (binding between $m+1$ atoms), the general form of the indices, ${}^{m}\chi_t$, is calculated according to the equation

$$ {}^{m}\chi_t = \sum_{s=1}^{n_m} \prod_{i=1}^{m+1} (\delta_i)_s^{-1/2} \qquad (1) $$

where n_m is the number of paths. Connectivity indices describing non-linear arrangements of bonds, such as clusters of three bonds, ${}^{3}\chi_c$, and path-clusters of four bonds, ${}^{4}\chi_{pc}$, are calculated in the same way.

Each non-hydrogen atom is described by its valence delta value, δ^v, which is calculated by the expression $\delta^v = Z^v - N_H$, where Z^v is the number of valence electrons in the atom and N_H is the number of hydrogen atoms attached to it [8].

Single and multiple regression analyses were used to find the relationship between the TLC properties and the connectivity indices, and were calculated from the equation

$$ P = A_0 + \sum_{m,t} A_{m,t}\, {}^{m}\chi_t \qquad (2) $$

where P is a property, and A_0 and $A_{m,t}$ represent the regression coefficients of the equation obtained.

Eq. 2 was obtained by multilinear regression with program 9R of the biostatistic package BMD (Biomedical Computer Programs) [19]. To test the quality of the regression equations, the following statistical parameters were used: multiple correlation coefficient (r), standard error of estimate (s), F-Snedecor function values (F) and Student's t-test (statistical significance).

Random and stability studies were performed on the selected equations as follows. (a) Randomness was achieved by randomly modifying the value of the independent variables which intervene in the equation, subsequently modifying the value of the dependent (property), also done randomly; after each modification the BMDP 9R was executed, passing on to compare the calculated correlation coefficient with the one obtained for the selected equation [20]. (b) Stability: using the jack-knife method [21], the elimination of n observations was effected, by means of a random process, and a regression program was executed, repeating the process as many times as necessary until all the observations had been eliminated a minimum of once a maximum of 5 times. The correlation coefficients, standard deviations and the residuals with those obtained are subsequently compared with those of the selected equation.

The different experimental R_F values in TLC were obtained with precoated silica gel 60 F_{254} plates, 20 cm × 20 cm with a 0.25-mm layer thickness, activated for 1 h in a saturated chamber, as the ascending method with a length of run of 12 cm at 20°C with different stationary and mobile phases (Table 1). Development was achieved with a 0.1% solution of p-dimethylaminobenzaldehyde in 0.5% HCl. The sulphamide solutions were prepared at a 0.2% concentration in ethanol–water (70:30, v/v). Six chromatograms were obtained for each of the molecules studied in each of the systems employed, calculating the mean and error standard (see Table 2).

3. Results and discussion

The experimental R_F values and molecular connectivity indices of the eighteen sulphamides examined are given in Tables 2 and 3, respectively. Essentially, the R_F value represents the degree of affinity between the solute considered and the stationary and mobile phases. This

Table 1
Stationary and mobile phases used in the study of experimental R_F values

TLC	Stationary phase	Mobile phase
A	Toluene–castor oil (92:8, v/v)	Sörensen solution (pH 6.2)
B	Toluene–castor oil (92:8, v/v)	Sörensen solution–acetone (80:20, v/v) (pH 6.2)
C	Toluene–silicone DC-200 (95:5, v/v)	Sörensen solution (pH 6.2)
D	Toluene–silicone DC-200 (95:5, v/v)	1% sodium chloride Sörensen solution (pH 6.2)

affinity is quantified by the distribution coefficient for the solute in the two phases.

Multi-variable regression equations were screened to find the simplest equation that generated the experimental elution sequence. Both the order and number of connectivity indices were varied.

The selected equations for R_{F_A}, R_{F_B}, R_{F_C} and R_{F_D} of the compounds studied were as follows:

$$R_{F_A} = 0.299\ {}^1\chi^v - 1.110\ {}^3\chi_p - 1.647\ {}^3\chi_c^v + 1.698\ {}^4\chi_{pc} + 0.380 \quad (3)$$
$$n = 18;\quad r = 0.939;\quad s = 0.046;\quad F = 24.16$$

$$R_{F_B} = 0.303\ {}^0\chi^v - 1.380\ {}^3\chi_p - 1.275\ {}^3\chi_c + 1.331\ {}^4\chi_{pc} + 1.841 \quad (4)$$
$$n = 18;\quad r = 0.924;\quad s = 0.074;\quad F = 18.96$$

Table 2
Experimental R_F values (mean ± standard error) of sulphamides

Compound	R_{F_A}	R_{F_B}	R_{F_C}	R_{F_D}
Sulphasomidine	0.391 ± 0.003	0.699 ± 0.003	0.408 ± 0.010	0.522 ± 0.013
Sulphafurazole	0.503 ± 0.014	0.396 ± 0.013	0.316 ± 0.016	0.535 ± 0.018
Sulphadiazine	0.465 ± 0.011	0.612 ± 0.001	0.486 ± 0.017	0.497 ± 0.030
Sulphasimazine	0.323 ± 0.017	0.326 ± 0.012	0.169 ± 0.012	0.342 ± 0.013
Sulphamerazine	0.320 ± 0.014	0.552 ± 0.005	0.334 ± 0.013	0.402 ± 0.013
Sulphamethazine	0.241 ± 0.008	0.514 ± 0.005	0.239 ± 0.007	0.327 ± 0.008
Sulphadoxine	0.298 ± 0.016	0.398 ± 0.007	0.201 ± 0.009	0.362 ± 0.010
Sulphamethoxypryidazine	0.240 ± 0.006	0.499 ± 0.010	0.219 ± 0.005	0.331 ± 0.008
Sulphamethoxazole	0.308 ± 0.013	0.337 ± 0.008	0.307 ± 0.023	0.433 ± 0.012
Sulphalene	0.278 ± 0.016	0.410 ± 0.017	0.278 ± 0.014	0.416 ± 0.013
Sulphametomidina	0.232 ± 0.008	0.438 ± 0.008	0.203 ± 0.008	0.343 ± 0.011
Sulphamonomethoxine	0.260 ± 0.014	0.340 ± 0.007	0.231 ± 0.009	0.386 ± 0.014
Sulphaethoxypyridazine	0.160 ± 0.011	0.363 ± 0.010	0.111 ± 0.006	0.203 ± 0.007
Sulphenazole	0.158 ± 0.012	0.164 ± 0.006	0.086 ± 0.005	0.209 ± 0.017
Sulphadimethoxine	0.129 ± 0.006	0.178 ± 0.006	0.078 ± 0.005	0.185 ± 0.017
Sulphazamet	0.124 ± 0.005	0.137 ± 0.004	0.056 ± 0.004	0.153 ± 0.017
Sulphaquinoxaline	0.121 ± 0.005	0.118 ± 0.006	0.072 ± 0.006	0.172 ± 0.017
Sulphamoprine	0.087 ± 0.004	0.158 ± 0.008	0.076 ± 0.004	0.149 ± 0.017

Table 3
Connectivity indices used in the correlations of a group of sulphamides

Compound	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{2}\chi$	$^{3}\chi_{p}$	$^{3}\chi_{c}$	$^{3}\chi_{c}^{v}$	$^{4}\chi_{p}$	$^{4}\chi_{p}^{v}$	$^{4}\chi_{pc}$
Sulphasomidine	10.787	5.943	6.423	3.761	1.658	0.719	2.788	1.860	2.075
Sulphafurazole	10.171	5.582	6.247	4.265	1.601	0.713	2.451	1.486	2.579
Sulphadiazine	8.942	2.102	5.412	3.322	1.325	0.475	2.213	1.417	1.760
Sulphasimazine	12.071	6.934	6.792	4.408	1.561	0.617	2.967	1.892	2.213
Sulphamerazine	9.864	5.523	5.916	3.562	1.492	0.604	2.439	1.561	1.930
Sulphamethazine	10.787	5.943	6.423	3.761	1.658	0.733	2.788	1.833	2.075
Sulphadoxine	11.734	6.300	6.689	4.570	1.500	0.591	3.222	1.947	2.398
Sulphamethoxypyridazine	10.273	5.642	6.097	3.910	1.443	0.542	2.510	1.576	2.014
Sulphamethoxazole	9.248	5.159	5.901	3.597	1.529	0.608	2.172	1.372	1.986
Sulphalene	10.273	5.631	6.216	4.072	1.437	0.527	2.735	1.629	2.128
Sulphametomidine	11.195	6.056	6.607	4.084	1.609	0.642	2.871	1.788	2.144
Sulphamonomethoxine	10.325	5.682	6.264	4.012	1.469	0.550	2.748	1.643	2.037
Sulphaethoxypyridazine	10.980	6.230	6.493	4.041	1.443	0.542	2.730	1.692	1.979
Sulphenazole	11.751	6.910	6.942	4.651	1.505	0.614	3.011	2.093	2.285
Sulphadimethoxine	11.604	6.168	6.792	4.408	1.561	0.583	2.967	1.743	2.213
Sulphazamet	12.674	7.331	7.445	4.880	1.671	0.743	3.308	2.295	2.449
Sulphaquinoxaline	11.096	6.517	6.608	4.293	1.492	0.619	2.966	2.096	2.129
Sulphamoprine	11.604	3.168	6.792	4.408	1.561	0.581	2.967	1.720	2.213

$$R_{F_C} = -0.482\ ^{2}\chi + 0.838\ ^{3}\chi_{c} + 0.254\ ^{4}\chi_{p} + 1.347 \quad (5)$$

$$n = 18; \quad r = 0.927; \quad s = 0.053; \quad F = 28.52$$

and

$$R_{F_D} = -0.750\ ^{3}\chi_{p} - 0.884\ ^{3}\chi_{c}^{v} + 0.290\ ^{4}\chi_{p}^{v} + 1.168\ ^{4}\chi_{pc} + 0.943 \quad (6)$$

$$n = 18; \quad r = 0.915; \quad s = 0.059; \quad F = 16.79$$

Statistically, all the equations are significant above the 99.9% level. All the variables are statistically significant above the 99.9% level, except $^{1}\chi^{v}$ in Eq. 3 and $^{3}\chi_{c}$ in Eqs. 4 and 5, which are significant above the 99% level, $^{3}\chi_{c}^{v}$ in Eq. 6 above the 95% level and $^{4}\chi_{p}$ in Eq. 5 and $^{4}\chi_{p}^{v}$ in Eq. 6 above the 90% level.

The study of randomness of these equations (Table 4) demonstrates their non-randomness.

For R_{F_A}, four correlation coefficients >0.7 are obtained when the independent variable is studied and three correlation coefficients >0.7 when the dependent variable is studied; therefore, the probability (p) that a correlation coefficient >0.9 can be obtained is considerably less than 0.04 and 0.03, respectively. For R_{F_B}, two correlation coefficients >0.8 are obtained when the independent variable is studied and one correlation coefficient >0.8 when the dependent variable is studied; therefore, the probability that a correlation coefficient >0.9 can be obtained is less than 0.02 and 0.01, respectively. For R_{F_C}, two correlation coefficients >0.7 are obtained when the independent variable is studied and one correlation coefficient >0.7 when the dependent variable is studied; therefore, the probability that a correlation coefficient >0.9 can be obtained is considerably less than 0.02 and 0.01, respectively. For R_{F_D}, one correlation coefficient >0.8 is obtained when the independent variable is studied and eight correlation coefficients >0.7 when the dependent variable is studied; therefore, the probability that a correlation coefficient >0.9 can be obtained is less than 0.01 and 0.08, respectively.

The stability study of the equations was carried out by varying the number of eliminations done (between one and five) and the number of runs (eighteen runs in the case of one elimination or twenty runs with the rest), observing that by raising the number of eliminations the model was made more unstable, which was expected because the degrees of freedom were consider-

Table 4
Correlation coefficients computed from random number variables for a four-variable model of R_{F_A}, R_{F_B}, R_{F_C} and R_{F_D} value data for sulphamides

Modification variable								
Range of r	Independent (100 runs)				Dependent (100 runs)			
	Number of values				Number of values			
	R_{F_A}	R_{F_B}	R_{F_C}	R_{F_D}	R_{F_A}	R_{F_B}	R_{F_C}	R_{F_D}
<0.1	0	0	0	0	1	0	2	1
0.1–0.2	1	6	6	2	1	3	10	4
0.2–0.3	9	8	24	10	8	10	14	16
0.3–0.4	27	21	25	14	23	26	30	13
0.4–0.5	26	20	21	19	26	18	22	26
0.5–0.6	21	22	18	29	25	28	13	21
0.6–0.7	11	19	4	12	14	13	8	11
0.7–0.8	4	2	2	13	3	1	1	8
0.8–0.9	0	2	0	1	0	1	0	0
>0.9	0	0	0	0	0	0	0	0

ably diminished. In all instances the corresponding stability was chosen at two eliminations (twenty runs), which corresponds approximately to 10% of eliminated observations, the value recommended by some workers [8] (Tables 5–8). Comparison of the results between the obtained values for the selected model and the model of two eliminations shows that the selected equations are more stable, as is clear from the equality of the terms obtained and from the low standard deviations of each of these terms. The analysis of the residuals obtained for the selected model and for the model of two eliminations shows minimum discrepancies for the means and

Table 5
Statistical stability test information for the regression model of R_{F_A} values for sulphamides

Parameter	Original model (no deletions)		Two deletions per run (20 runs)	
	Regression value	Standard deviation	Regression value	Standard deviation
Correlation coefficient	0.939		0.939	0.009
Standard deviation	0.046		0.047	0.003
Coefficient of $^1\chi^v$	0.299	0.069	0.298	0.014
Coefficient of $^3\chi_p$	−1.110	0.161	−1.110	0.036
Coefficient of $^3\chi_c^v$	−1.647	0.339	−1.642	0.115
Coefficient of $^4\chi_{pc}$	1.698	0.239	1.693	0.062
Constant	0.380	0.144	0.392	0.038
Average residual	0.031		0.037	0.003
Residuals less than one standard deviation	77.8%		76.3%	
Residuals between one and two standard deviations	22.2%		23.7%	
Residuals greater than two standard deviations	0%		0%	

Table 6
Statistical stability test information for the regression model of R_{F_B} values for sulphamides

Parameter	Original model (no deletions)		Two deletions per run (20 runs)	
	Regression value	Standard deviation	Regression value	Standard deviation
Correlation coefficient	0.924		0.925	0.008
Standard deviation	0.074		0.075	0.005
Coefficient of $^{0}\chi^{v}$	0.303	0.073	0.297	0.033
Coefficient of $^{3}\chi_{p}$	−1.380	0.234	−1.364	0.110
Coefficient of $^{3}\chi_{c}$	−1.275	0.413	−1.244	0.205
Coefficient of $^{4}\chi_{pc}$	1.331	0.300	1.312	0.156
Constant	1.841	0.336	1.866	0.147
Average residual	0.057		0.058	0.005
Residuals less than one standard deviation	77.8%		77.5%	
Residuals between one and two standard deviations	22.2%		22.5%	
Residuals greater than two standard deviations	0%		0%	

for their standard deviations, an aspect of the study which strengthens the predictive quality of the model.

The regression analyses show that the most significant structural factor influencing the R_F values is the substitution pattern and typically the branching parameter, given by the $^{4}\chi_{pc}$ index (Eqs. 3, 4 and 6). In a previous paper [12] we suggested that this index is a measure of the eluent's polar character: more polar eluents (*e.g.*, Sörensen solution, pH 6.2) will make a higher contribution (higher regression coefficient for the $^{4}\chi_{pc}$ index) to the property studied than other less polar eluents [Sörensen solution–ace-

Table 7
Statistical stability test information for the regression model of R_{F_C} values for sulphamides

Parameter	Original model (no deletions)		Two deletions per run (20 runs)	
	Regression value	Standard deviation	Regression value	Standard deviation
Correlation coefficient	0.927		0.929	0.007
Standard deviation	0.053		0.052	0.004
Coefficient of $^{2}\chi$	−0.482	0.056	−0.485	0.045
Coefficient of $^{3}\chi_{c}$	0.838	0.188	0.849	0.091
Coefficient of $^{4}\chi_{p}$	0.254	0.339	0.252	0.047
Constant	1.347	0.241	1.349	0.136
Average residual	0.040		0.042	0.003
Residuals less than one standard deviation	61.1%		69.4%	
Residuals between one and two standard deviations	38.9%		30.6%	
Residuals greater than two standard deviations	0%		0%	

Table 8
Statistical stability test information for the regression model of R_{F_D} values for sulphamides

Parameter	Original model (no deletions)		Two deletions per run (20 runs)	
	Regression value	Standard deviation	Regression value	Standard deviation
Correlation coefficient	0.915		0.914	0.012
Standard deviation	0.059		0.061	0.004
Coefficient of $^3\chi_p$	−0.750	0.148	−0.750	0.050
Coefficient of $^3\chi_c^v$	−0.884	0.415	−0.894	0.119
Coefficient of $^4\chi_p^v$	0.290	0.155	0.289	0.064
Coefficient of $^4\chi_{pc}$	1.168	0.261	1.169	0.085
Constant	0.943	0.169	0.952	0.034
Average residual	0.037		0.048	0.004
Residuals less than one standard deviation	72.2%		76.3%	
Residuals between one and two standard deviations	27.8%		23.7%	
Residuals greater than two standard deviations	0%		0%	

tone (80:20, v/v), pH 6.2]. The size of the sulphamides, described and quantified by the $^1\chi^v$ and $^0\chi$ indices, whose numerical value is directly proportional to the number of ties, also contributes to the increase in the value of the property. The other factors that control the magnitude of the R_F values are $^3\chi_p$ (Eqs. 3, 4 and 6), $^3\chi_c$ (Eqs. 4 and 5) and $^3\chi_c^v$ (Eqs. 3 and 6). A measure of the molecular symmetry is given by the index $^3\chi_p$ [8]: sulphamides are non-symmetrical molecules, which explains why this index has a negative influence on the R_F values for this group of molecules. The indices $^3\chi_c$ and $^3\chi_c^v$ take into account the solvation effects, closely related to steric aspects. In Eqs. 5 and 6 other indices such as $^4\chi_p$, $^4\chi_p^v$ and $^4\chi_{pc}$ appear, characteristic of the presence of branchings. These results suggest that these indices, particularly $^4\chi_{pc}$, appear with more polar eluents [12].

Comparisons between experimental and theoretical R_F values following Eqs. 3, 4, 5 and 6 are given in Figs. 1, 2, 3 and 4, respectively.

This investigation has demonstrated that a relationship exists between molecular connectivity and R_F values for a group of sulphamides; with a three- or four-variable model a good degree of correlation can be obtained.

4. Conclusions

The molecular connectivity method has been used for the prediction of different R_F values in TLC using mobile phases of different polarity. The statistical studies of randomness show that the predictive models selected are not random, and the stability studies suggest that they are good statistical models because of their stability and predictive capacity. It is necessary to highlight the presence of the $^3\chi_p$ index, which in a

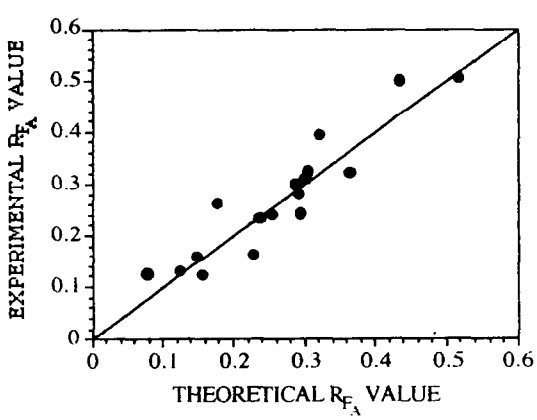

Fig. 1. Correlation between experimental and calculated (Eq. 3) R_{F_A} values of eighteen sulfamides.

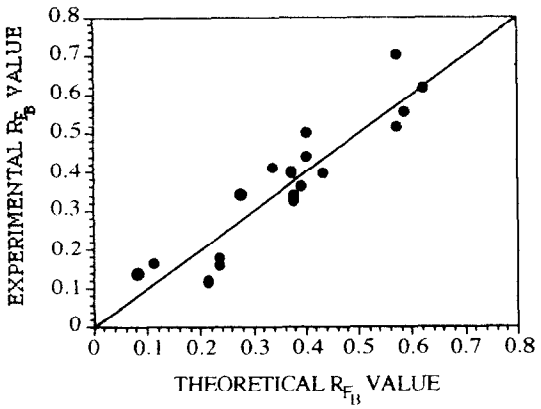

Fig. 2. Correlation between experimental and calculated (Eq. 4) R_{F_B} values of eighteen sulphamides.

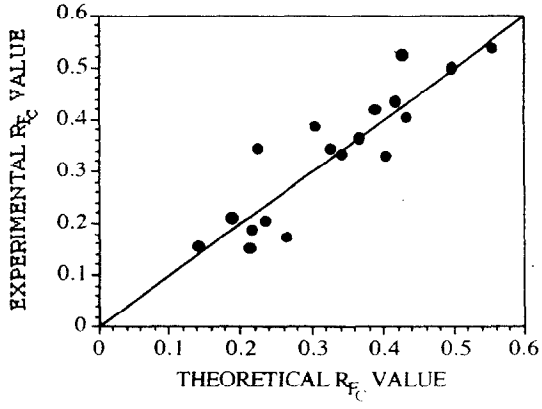

Fig. 3. Correlation between experimental and calculated (Eq. 5) R_{F_C} values of eighteen sulphamides.

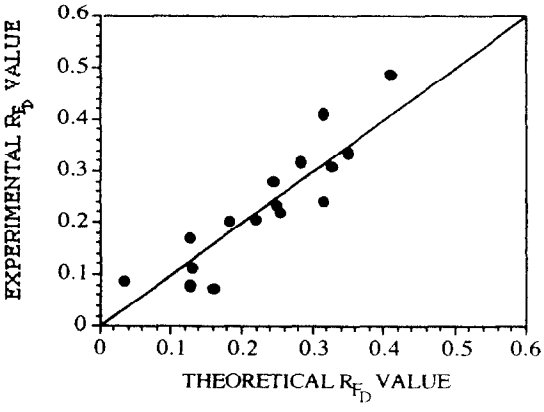

Fig. 4. Correlation between experimental and calculated (Eq. 6) R_{F_D} values of eighteen sulphamides.

certain way evaluates the molecular symmetry [12], because of which non-symmetrical molecules such as sulphamides will have a negative influence on this index. In all the equations the indices $^3\chi_c$ and $^3\chi_c^v$ are indicative of the solvent's solvation effects on the molecules and are also accompanied by $^4\chi_{pc}$, a parameter that gives information about the polar character of the eluents [12] and for $^2\chi$ index. All of this leads us to conclude that the selected equations predict the R_F values correctly, as was expected by the presence in the connectivity indices of information on factors that directly influence the studied property.

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