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New aspects of quantitative structure–retention relationships in chromatography

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ABSTRACT

The usual task of quantitative structure–retention relationships (QSRR) in chromatography is to predict retentions, whereas in spectroscopy the resulting spectra are used for structure elucidation. It is shown that if an equation with a good correlation is found, QSRR can also be used for a similar purpose, with consideration of exceptions from the rule. Equations for the dependence of the retention in gas, high-performance liquid and thin-layer chromatography on molecular mass and selected structural fragments of eighteen benzodiazepines are proposed. Any deviation from the rule is connected with a given influence of a neighbour on the corresponding structural fragment atoms. A new quantity given to fragment evaluation is used for considering the intramolecular group influences.

INTRODUCTION

One of the challenges in quantitative structure–property relationship (QSPR) investigations is to predict the properties of a substance when the structure is known. There is also another possibility, namely to predict the structure from the properties. To use system and solute properties for precalculation of retention is the usual task of quantitative structure–retention relationships (QSRR) in chromatography, whereas in the spectroscopy the resulting spectra are used for structure elucidation. In this paper we show that QSRR can be also used for a similar purpose. We assume that the chromatographic retention on a given stationary phase is influenced both by extensive (molecular mass, number of atoms, etc.) and intensive (structural features) solute properties. Such a differentiation is similar to that in spectroscopy. The absorption band in IR spectroscopy, for example, of a ketone C=O group is at about 1720 cm^{-1}. This value is due to the vibration of the bond between two atoms with corresponding mass (extensive property). This band moves down to 1670 cm^{-1} or up to 1820 cm^{-1} depending on the neighbouring atoms and/or functional groups. Hence a band anywhere between 1670 to 1820 cm^{-1} could be connected with the presence of a C=O group. Its exact location serves for structural elucidation. If a valid rule (equation) about the dependence of chromatographic retention on some general properties of

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a series of compounds can be defined, any deviation from this rule can also be connected with a given influence of corresponding functional groups and, as a next step, the influence of neighbouring atoms on the functional group in question. Therefore, if QSRR is exact enough it can serve for reliable considerations on the structure of compounds the retention of which differs from the rule.

The present status of QSRR studies shows the following problems in modelling: (i) lack of reproducibility of the experimental retention data for substances of interest, (ii) correct representation of the corresponding substance structure and (iii) adequate mathematical data handling.

Analytical results in biological, pharmacologial, clinical studies, etc., show poor reproducibility. This situation, does not apply, however, with chromatographic determinations. Modern experimental methods based on high-resolution gas (GC), high-performance liquid (HPLC) and thin-layer chromatography (TLC) give very repeatable results for retention, which can be used in QSRR investigations. There are also a great variety of topological, geometric, quantum chemical, etc., indices [1,2], which make the second problem also less difficult. Mathematical data handling on a purely theoretical base is not yet possible. There are however, many empirical and semi-empirical equations [3–30] connecting the retention with the solute properties. The accuracy achieved can be considered satisfactory for identification purposes in limited cases only, *e.g.*, with one chromatographic technique used for similar compounds. No general mathematical approach for the adequate solution of the third problem in all chromatographic modes is available.

We advocate the use of a linear model, given elsewhere [30], for retention data handling. This model has been used for the derivation of equations describing chromatographic retention in different modes. It has been already applied with success for hydrocarbons and halogenated hydrocarbons [31–33]. These compounds however, cannot be analysed by TLC or HPLC and therefore it is not possible to compare the significant parameters and their estimates in different chromatographic modes. This study deals with the retention data of eighteen benzodiazepines, obtained in adsorption (thin-layer and liquid) and partition (gas and liquid) chromatographic modes.

THEORY

It is well known that the general physico-chemical equation connecting the absolute retention with the enthalpy of solution, ΔH_s, and the entropy of solution, ΔS_s, is

$$RT \ln V_g = \Delta H_s - T\Delta S_s \quad (1)$$

where V_g is the specific retention volume, T is the absolute temperature and R is a constant. Rearranging eqn. 1 to

$$RT \ln r_{1,2} = \Delta(\Delta H_s)_{1,2} - T\Delta(\Delta S_s)_{1,2} \quad (2)$$

where $r_{1,2}$ is the relative retention of solutes 1 and 2, it can be seen that the relative retention depends on the relative changes in ΔH_s and ΔS_s. If the solute molecules differ

significantly in their ΔH_s values (*e.g.*, homologous neighbours separated by GC on a non-polar stationary phase), their retention is governed mostly by $\Delta(\Delta H_s)_{1,2}$. When the difference in $\Delta(\Delta H_s)_{1,2}$ is negligible (*e.g.*, closely related structures and equal molecular masses), then the retention still depends on ΔH_s, but separation can be achieved if the stationary phase provides a greater difference in the solution entropy (*e.g.*, use of liquid crystals). The experimentally obtained numerical values show that $\Delta(\Delta S_s)_{1,2}$ usually plays a modifying role for retention [34,35].

Different solution theories based on quantum-chemical calculations lead to a general equation analogous in its form and meaning [36]:

$$\Delta E = \varepsilon_1 + \Sigma\varepsilon_r \tag{3}$$

where ΔE is the change in the system energy after non-destructive interactions (*e.g.*, solutions), ε_1 is the magnitude of dispersive forces and ε_r are different other forces. Again there is a basic contributor ε_1 and modifying contributors $\Sigma\varepsilon_r$.

We have tested the validity of analogous equations reported in a series of papers [30–33,37–42] and we have proposed a biparametric model based on the additivity principle as a general model for QSRR studies in GC [30]:

$$R = b_0 + \sum_{i=1}^{n} b_i B_i + \sum_{j=n+1}^{n+k} b_j T_j \tag{4}$$

where R represents the corresponding retention (usually the retention index in GC and R_F in TLC or the capacity factor (k') in HPLC in this work. B_i are basic and T_j are tuning contributors to retention. The constants $b_0 - b_j$ are regressor (parameter) estimates. It was accepted [30] that the B term in eqn. 4 includes solute properties, allowing the calculation of a value for $R_{calc.}$, which does not differ from $R_{exp.}$ by more than $\pm$10–15%. Every solute property answering the above demands and even linear or non-linear [28,29,43] equations including the corresponding property can be used as the B term.

The T term includes also solute properties, which can correlate insignificantly with retention and do not correlate with the properties included in the B term. They have to possess a high discrimination power and be able to approximate the roughly calculated $R_{calc.}$ to the value of $R_{exp.}$.

RESULTS AND DISCUSSION

The retention data used in this investigation were taken from the literature [44,45]. The molecular mass, *Mm*, is a general solute property that can be used in all of the studied chromatographic modes and it was tested as a B contributor. The molecular fragments (see Fig. 1) C=O, –OH, –F, $-NO_2$, N–R_2 and flat rings (phenyl and cyclopropane) were tested as T contributors. We assumed that the presence of a given fragment is counted as 1 and its absence as 0. The parameter –F for halazepam was taken to be 3, because there are three –F atoms (see Fig. 1). The parameter C=O for camazepam was taken as 2 for a similar reason, assuming no difference in their retention contributions. Correspondingly, prazepam has three flat rings, whereas tetrazepam has only one.

TABLE I

RETENTION DATA (R_F FOR TLC, k' FOR HPLC AND RETENTION INDEX I FOR GC) FOR 15 BENZODIAZEPINES, THEIR MOLECULAR MASS (*Mm*) AND PARAMETRIC VALUES

No.	Compound	R_F[a]	R_F[b]	k'[c]	k' (RP)[d]	I[e]	*Mm*[f]	C=R$_3$ (N–CO)	R$_4$ (–OH)	R$_{2,5}$ (–F)	R$_1$ (–NO$_2$)	Flat ring	N–R$_2$
1	Medazepam	40	74	3.66	–	2272	270	0	0	0	0	2	1
2	Prazepam	36	74	1.49	–	2652	324	1	0	0	0	3	1
3	Tetrazepam	34	76	2.03	21.44	2437	288	1	0	0	0	1	1
4	Pinazepam	31	79	1.32	10.96	2529	308	1	0	0	0	2	1
5	Flurazepam	30	48	6.10	–	2771	387	1	0	1	0	2	1
6	Halazepam	18	76	1.05	16.46	2292	352	1	0	3	0	2	1
7	Camazepam	13	79	0.08	13.61	2930	371	2	0	0	0	2	1
8	Nimetazepam	12	77	1.12	3.62	2662	295	1	0	0	1	2	1
9	Flunitrazepam	10	72	0.34	3.1	2600	313	1	0	1	1	2	1
10	Temazepam	8	59	0.42	5.76	2589	300	1	1	0	0	2	1
11	Lormetazepam	6	67	0.10	6.39	2674	334	1	1	0	0	2	1
12	Nordazepam	4	55	1.36	8	2493	270	1	0	0	0	2	0
13	Lorazepam	1	36	0.11	4.6	2423	320	1	1	0	0	2	0
14	Nitrazepam	0	36	0.99	3	2720	281	1	0	0	1	2	0
15	Oxazepam	0	40	0.47	4.62	2350	286	1	1	0	0	2	0
Intercorrelation coefficients													
							Mm	0.5	0.1	0.5	0.2	0.2	0.4
							N–CO		0	0	0	0	0
							–OH			0.2	0.3	0	0.3
							–F				0	0	0.2
							–NO$_2$					0	0.1
							Flat ring						0

[a] R_F × 100 on silica gel 60 F_{254} with cyclohexane–toluene–diethylamine (75:15:10) [44,45] (basic mobile phase).
[b] R_F × 100 on silica gel 60 F_{254} with chloroform–methanol (90:10) [44,45] (neutral mobile phase).
[c] k' on Spherisorb S5W with 100 μl of perchloric acid in 1000 cm^3 of methanol [44] (adsorption HPLC).
[d] RP: ODS-Hypersil, methanol–water–0.1 *M* phosphate buffer (55:25:20) [44] (reversed-phase HPLC).
[e] *I* on DB1 (SE-30) wide-bore capillary column (modern GC) at 250°C [44].
[f] *Mm* is taken in all calculations as *Mm* × 10^{-2}.

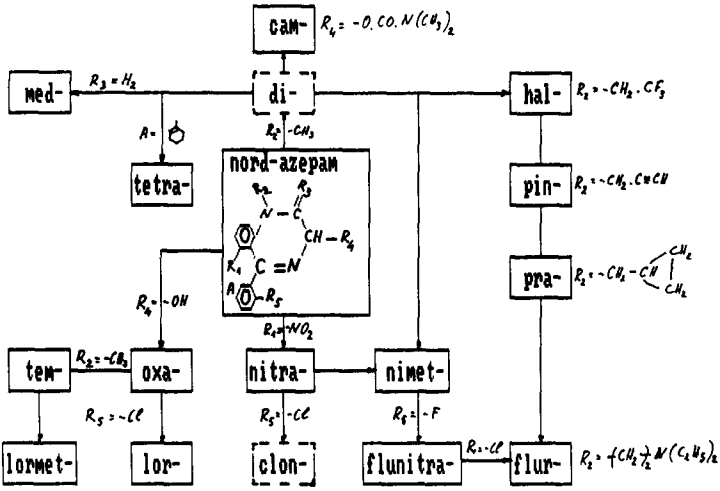

Fig. 1. Molecular fragments tested.

The corresponding retention data, the values of the molecular mass (*Mm*) and the values of selected parameters (fragments) are summarized in Table I.

The intercorrelation between the selected parameters was calculated and was found to be negligible (Table I). Therefore, we correlated first the retention with all seven selected factors in all chromatographic modes. Because of the high probability of chance correlations [46] we checked the validity of the parametric estimates in two ways: applying the leave-one test and reducing the number of factors with the requirement that the variances remain statistically equal. Surprisingly, the parametric estimates remain with a constant sign and within a limited range of values. The maximum and minimum quantities obtained in the leave-one test are given in Table II. For comparison of both full and reduced equations, see eqns. 5–9a.

The studies show that in GC just the parameter molecular mass satisfies the requirements of the *B* term in eqn. 4. In TLC such a parameter is the N–R_2 group. In the other chromatographic modes neither parameter as a basic contributor covers the experimental retention value by more than 85%. Nevertheless, there is always a leading contributor. Taking into account the negligible intercorrelation between the parameters (see Table I) and both the leave-one test and reducing parameters, we consider the sign and quantity of the estimate of a given parameter to represent a real quantitative evaluation of its influence on retention under the given chromatographic conditions.

The two types of equations can be given as follows for the various chromatographic modes:

for GC:

$$I = 1219 + 353.6Mm + 150.4(\text{N–CO}) - 50(\text{–OH}) - 143(\text{–F}) + 183(\text{–NO}_2) + 29(\text{flat}) + 43(\text{N–R}_2) \quad (5)$$

$$I = 992 + 502.7Mm - 161.2(\text{–F}) + 232.7(\text{–NO}_2) \quad (5a)$$

TABLE II

MAXIMUM AND MINIMUM VALUES (ACCORDING TO THE LEAVE-ONE TEST) OF PARAMETER ESTIMATES b_1–b_7 IN ALL CHROMATOGRAPHIC MODES

Mode	b_1 (*Mm*)	b_2 (N–CO)	b_3 (–OH)	b_4 (–F)	b_5 ($-NO_2$)	b_6 (flat ring)	b_7 ($N-R_2$)
GC	324	176	−54	−117	171	32	13
	360	119	−67	−154	226	81	38
RP-HPLC	1.9	2.9	−4	−1.3	−6.1	−10	0.6
	3.1	1.6	−6	1.3	−8	−11.8	1.2
HPLC (SiO_2)	4	−4	−1.3	−0.5	−0.9	1	−1.0
	5	−5	−2	0.7	0.4	1	−1.6
TLC (neutral)	−29	18	−8	−4	−14	−0.2	10
	−33	20	−11	4.5	−9	4	35
TLC (basic)	6	−16	−16	−4	−14	−0.2	10
	9	−19	−19	−5	−15	0.3	13

with statistically equal variances;
for reversed-phase HPLC:

$$k' = 1.25 + 2.26Mm + 1.54(\text{N–CO}) - 4.90(\text{–OH}) + 1.41(\text{–F}) - 7.32(\text{–NO}_2) - 11(\text{flat}) + 0.88(\text{N–R}_2) \quad (6)$$

$$k' = -6.53 + 5.777Mm - 6.03(\text{–OH}) - 7.35(\text{–NO}_2) - 11.33(\text{flat}) \quad (6a)$$

with statistically equal variances;
for adsorption-mode HPLC:

$$k' = -7.6 + 4.74Mm - 4.22(\text{N–CO}) - 2.12(\text{–OH}) + 0.57(\text{–F}) - 0.82(\text{–NO}_2) + 1.15(\text{flat}) - 1.06(\text{N–R}_2) \quad (7)$$

$$k' = -3.43 + 2.65Mm - 3.13(\text{N–CO}) - 1.38(\text{–OH}) \quad (7a)$$

with statistically equal variances;
for TLC with chloroform–methanol (neutral phase):

$$R_F = 110.3 - 31Mm + 18.1(\text{N–CO}) - 8.4(\text{–OH}) + 3.59(\text{–F}) - 8.4(\text{–NO}_2) + 4.57(\text{flat}) + 34.5(\text{N–R}_2) \quad (8)$$

$$R_F = 46.21 - 8.91(\text{–OH}) + 26.41(\text{N–R}_2) \quad (8a)$$

with statistically equal variances.
for TLC with diethylamine as modifier (basic phase):

$$R_F = 10.4 - 7.53Mm - 17.3(\text{N–CO}) - 18.6(\text{–OH}) - 4.7(\text{–F}) - 14.6(\text{–NO}_2) - 0.3(\text{flat}) + 13.1(\text{N–R}_2) \quad (9)$$

$$R_F = 27 - 13.5(\text{N–CO}) - 16.7(\text{–OH}) - 15.5(\text{–NO}_2) + 14(\text{N–R}_2) \quad (9a)$$

with statistically equal variances.

If the parametric estimations from eqns. 5–9a are compared with the corresponding values in Table II, several conclusions can be drawn:

(1) The values of regressor (parametric) estimates coincide with quantity and sign in most instances, which confirms the lack of chance correlation.

(2) The greater difference between the estimates of *Mm* in Table II or eqn. 5 and in eqn. 5a for GC can be explained if the assumption regarding the additivity of the contributions of the different parameters is correct: the sum of estimates of *Mm* and N–CO– from Table II (353.6 + 150.4) is equal to the estimate of *Mm* in eqn. 5 (502.7). Hence the N–CO– group does not make a specific individual contribution to the retention and its estimation is incorporated in the *Mm* parameter estimate of eqn. 5.

The role of *Mm* in TLC with a neutral mobile phase (eqn. 8) is also clear: the greater the molecular mass, the greater is the retention and the lower the R_F value. This well known qualitative observation is now quantified.

(3) According to eqn. 8a (neutral mobile phase), the retention depends mostly on the N–R_2 fragment, whereas Table II and eqn. 8 give two significant parameters: N–CO and *Mm*. If the additivity of the parameter contibutions is again valid, then the sum of the estimates b_1, b_2 and b_7 (Table II or eqn. 8) should be equal to the estimate of the N–R_2 parameter in eqn. 8a, which is indeed the case.

(4) The case described by eqns. 8, 8a and 13 shows that if the azepine N atom is substituted (the parameter $\text{N–R}_2 = 1$), the solute is retained more weakly than the pattern nordazepam (Tables I and III). The only drastic exception when the reduced equation 8a is used is flurazepam (Table III). However, its structure (Fig. 1) contains an additional N atom is observed in its –R_2 substituent, and it can be assumed that this N atom compensates for the decrease in retention due to the substitution of the azepine N atom. It is known that the interaction between a basic N atom and a silica surface with neutral mobile phases is of the greatest importance for the retention, and this was quantified in this study.

(5) Considering the case described by eqn. 9 (basic mobile phase), the influence of N–R_2, as can be expected, decreases, the influence of the other fragments also becomes significant and the discrimination is better.

(6) The hydroxyl group is an important parameter in all chromatographic modes. An –OH group in the solute molecule increases the retention in chromatographic modes with liquid mobile phases, because of silanophilic interactions. Again, a behaviour known in chromatographic practice was quantified. The insignificant role of the –OH group in the GC of benzodiazepines is probably due to the high temperature of analysis.

(7) Considering the influence of the –NO_2 group, we observed that its presence increases the molecular polarity and its GC and TLC retentions increase. The retention in reversed-phase HPLC decreases, probably because of a decrease in the solubility of the solute in the non-polar C_{18} stationary phase.

TABLE III

COMPARISON OF EXPERIMENTAL AND CALCULATED R_F VALUES ACCORDING TO EQNS. 8a AND 13

No.	Compound	R_F		
		Exp.	Calc. (eqn. 8a)	Calc. (eqn. 13)
1	Medazepam	74	73	76
2	Prazepam	74	73	76
3	Tetrazepam	76	73	76
4	Pinazepam	79	73	76
5	Flurazepam	48	73	48
6	Halazepam	76	73	76
7	Camazepam	79	73	76
8	Nimetazepam	77	73	76
9	Flunitrazepam	72	73	76
10	Temazepam	59	64	60
11	Lormetazepam	67	64	65
12	Nordazepam	55	46	54
13	Lorazepam	36	37	36
14	Nitrazepam	36	46	35
15	Oxazepam	40	37	41

Taking into account that in GC and reversed-phase HPLC the retention is mostly due to solubilization, whereas in adsorption-mode HPLC and TLC both solubilization and adsorption take place, some more general conclusions may be drawn. Solubilization is a bulk process, in which a solute molecule interacts with the whole surface, whereas in the adsorption mode only a few atoms can interact with the stationary phase surface. The results show that probably the influence of solubilization in the mobile phase predominates over adsorption in adsorption HPLC. This can explain why the molecular mass and the $-NO_2$ and -OH groups act in the observed manner. In TLC probably adsorption on the surface predominates and the retention is mostly due to the presence of an active fragment, in this instance $N-R_2$. The decrease in k' in HPLC modes could be explained on a similar basis: the solubility in the mobile phase predominates over adsorption on the silica surface.

Eqns. 5a–9a can serve for considering the influence of different parameters (fragments) on the retention, and also for the selection of compounds for which the calculated retention differs from the rule. The differences obtained between $R_{exp.}$ and $R_{calc.}$ may be connected with unequal contributions of equal chromatophores, owing to specific structural features (*e.g.*, intramolecular interactions such as $H \cdots O$ bonding or shielding). In other words, the contribution of the fragments, called chromatophores, might be tuned by the influence of specific neighbouring atoms, groups or other structural peculiarities. This tuning effect cannot be evaluated in advance but if, in the cases of greater discrepances between $R_{calc.}$ and $R_{exp.}$, we exchange the arbitrary value of 1.0 given to the fragments in question, we can decrease this discrepancy. The new fragment evaluation is accepted as a quantification of this

influence. In other words, we expect to evaluate the difference between equal functional groups due to different adjacent atoms. A new series of equations can now be formulated, allowing a better description of the retention of the studied compounds in the interpolation region.

The evaluations of N–CO– and –OH fragments in GC have to be corrected:

$$I = 1116.7 + 429.67Mm + 123.4(\text{CO–N})^{\text{cor.}} - 77.9\ (\text{–OH})^{\text{cor.}} - 151.2(\text{–F}) + 164.8(\text{–NO}_2) \quad (10)$$

with almost the same parametric estimates as in Table II, eqn. 10 has a higher correlation coefficient, $r = 0.993$, maximum discrepancy, $\Delta_{\max} = 40$ i.u., and only two incorrect arrangements, those of the peaks of temazepam and lormetazepam.

The new equation for reversed phase HPLC is

$$k' = -8.98 + 6.544Mm - 5.45(\text{–OH})^{\text{cor.}} - 8.28(\text{–NO}_2)^{\text{cor.}} - 11.58(\text{flat}) \quad (11)$$

again with the same values and signs as in Table II, but with higher $r = 0.987$, $\Delta_{\max} = 2.4$ and only two incorrect arrangements, halazepam and lorazepam.

For adsorption-mode HPLC, the new equation is

$$k' = -0.927 + 1.949Mm - 2.77(\text{CO–N})^{\text{cor.}} - 1.34(\text{–OH})^{\text{cor.}} + 0.62(\text{flat}) - 0.65(\text{N–R}_2)^{\text{cor.}} \quad (12)$$

with $r = 0.999$, $\Delta_{\max} = 0.3$ and only one incorrect arranged compound (nordazepam).

For TLC with a neutral mobile phase, we have

$$R_F = 35.66 - 10.4(\text{–OH})^{cor.} + 40.2(\text{N–R}_2)^{cor.} \quad (13)$$

with $r = 0.99$, $\Delta_{\max} = 3.8$ and a 100% correct arrangement of the R_F values of the spots.

For TLC with a basic mobile phase, the equation becomes

$$R_F = 28.28 - 13.49(\text{CO–N})^{\text{cor.}} - 14.85(\text{–OH}) - 14.49(\text{–NO}_2) + 11.73(\text{N–R}_2)^{\text{cor.}} \quad (14)$$

with $r = 0.998$, $\Delta_{\max} = 1.6$ and a 100% correct arrangement of the R_F values of the spots.

The superscript cor. means that the value for the presence of a given fragment differs from 1. For example, in reversed-phase HPLC the presence of an –OH group in the compounds lormetazepam and lorazepam is evaluated as 1.2 units instead of 1.0, in order to minimize the discrepancy between $k'_{\text{exp.}}$ and $k'_{\text{calc.}}$. We assume that this change in fragment evaluation is necessary in order to compensate for the influence of the –Cl atom in the adjacent phenyl ring. In adsorption-mode HPLC the necessary change is even greater, 1.7 units instead of 1. Similar corrections are necessary in TLC with a

neutral mobile phase; the presence of an –OH group in temazepam and lormetazepam has a greater influence on the retention than in lorazepam and oxazepam.

The fragment evaluations are found on a chemical logic basis. They are not constants; their values depend rather on the kind of chromatographic conditions used. They are meaningful only for the interpolation region. They could be used, however, for considering the intramolecular group influences on intermolecular interactions, and we see in this a new aspect of QSRR application.

The kind of substituent $-R_2$ in the $N-R_2$ group is also very important, and this has already been shown in TLC with a neutral mobile phase.

The results presented illustrate that comparable equations for all the studied chromatographic modes describing quantitatively the corresponding chromatographic retentions can be created on a single model. There are fragments selected from the solute molecule which are responsible for the retention in chromatography and these can be called chromatophores. Their contributions are additive, but in some instances the fragment evaluations differ from 1.0, depending on the neighbouring atoms. Therefore, after obtaining preliminary results, the fragment evaluation can be tuned so that more accurate interpolation equations can be obtained (eqns. 10–14). The evaluations from both the first and second groups of equations allow quantitative considerations of the contributions of different solute fragments, while the difference of a given fragment evaluation from 1.0 could be used for considering intramolecular interactions. The equations could also be used for formulating preliminary assumptions about the retention mechanism in particular chromatographic techniques.

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