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Comparative characteristics of HPLC columns based on quantitative structure–retention relationships (QSRR) and hydrophobic-subtraction model

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Abstract

The study was aimed at quantitative comparison of retention properties of modern stationary phases for reversed-phase HPLC. Three approaches, the calculated logarithm of octanol/water partition coefficient (*clogP*)-based model, the molecular modeling descriptors-based model and the hydrophobic-subtraction model, were compared and discussed. Gradient retention time, t_R, of a series of test analytes was a dependent variable in the quantitative structure–retention relationship (QSRR) equations describing retention in terms of analytes' structure descriptors. The QSRRs derived were used to characterize in quantitative manner the specific retention properties of nine representative reversed-phase HPLC. Either the theoretically calculated logarithm of octanol/water partition coefficient, or the structural descriptors from molecular modeling were employed to quantitatively characterize the structure of the analytes. The three molecular modeling-derived structural descriptors considered were: the total dipole moment, the electron excess charge of the most negatively charged atom and the water-accessible molecular surface area. In addition to the above standard QSRR approaches, a recently developed parameterization of reversed-phase column selectivity based on the hydrophobic-subtraction model of Snyder et al. [L.R. Snyder, J.W. Dolan, J.W. Carr, The hydrophobic-subtraction model of reversed-phase column selectivity, J. Chromatogr. A 1060 (2004) 77] was considered. According to the hydrophobic-subtraction model, reversed-phase columns are characterized by five selectivity parameters derived from the linear solvation energy relationships (LSER) theory. Values of these parameters are available for more than 300 different columns. It has been demonstrated that the *clogP*-based model, the molecular modeling descriptors-based model and the hydrophobic-subtraction model provide generally similar classification of the HPLC columns studied. Some differences in column classification by the three approaches considered are discussed in terms of specific properties of individual stationary phases. All the approaches allow a quantitative, although multidimensional, characteristic of HPLC columns, however, the nonempirical QSRR-based approach is simpler and require less labor.

Keywords: Column selectivity; Mechanism of retention; Principal component analysis (PCA); Quantitative structure–retention relationships (QSRR); RP-HPLC columns

1. Introduction

There are three main factors determining distribution of an analyte between a mobile and a stationary phase in liquid chromatography. At constant temperature of separation, these factors are: chemical structure of the analyte, physicochemical properties of the mobile phase and physicochemical properties of the stationary phase. If one gets numerical measures of properties of the analyte, of the mobile phase and of the stationary phase, one can derive a general relationship linking these properties to retention. Evaluation of retention in terms of chemical structure of analytes and of physicochemical properties of both the mobile and the stationary phase is known under acronym QSRR: quantitative structure–retention relationships [1].

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QSRR are statistically derived relationships between chromatographic parameters and the quantities (descriptors) characterizing molecular structure of the analytes. QSRR found application to: (i) get insight into the molecular mechanism of separation operating in a given chromatographic system; (ii) identify the most informative structural descriptors of analytes; (iii) evaluate complex physicochemical properties of analytes; (iv) evaluate properties of stationary phases; (v) predict retention for a new analyte.

A number of reports appeared recently on retention properties of stationary phase materials for reversed-phase high-performance liquid chromatography (RP-HPLC). In several studies the QSRR analysis was applied [2–9].

The simplest QSRR approach consists in regressing retention against the theoretically calculated logarithms of octanol/water partition coefficients (*clogP*) [2,5–8]. Values of *clogP* are calculated from structural formula of analytes, usually by means of commercially available computer programs. A simple regression equation holds:

$$t_R = k_1 + k_2\, clog\, P \tag{1}$$

where k_1 and k_2 are regression coefficients.

The second types of QSRR equations relate retention to quantum chemical indices and/or other analyte structural descriptors from calculation chemistry [2–8]. Such obtained QSRR equations distinguish the RP-HPLC stationary phases of different chemical nature of the organic ligand and/or the material of support. The QSRR equations also indicate the kind of the analyte-stationary phase interactions which are decisive for retention on individual columns. Previous studies [2–8] demonstrated a good performance in quantitative comparison of retention properties of diverse HPLC columns of a QSRR model employing the following analyte descriptors: (i) total dipole moment, μ, (ii) electron excess charge of the most negatively charged atom, δ_{Min}, (iii) water-accessible molecular surface area, A_{WAS}. The following physical meaning of individual descriptors was assumed: μ as accounting for the dipole–dipole and dipole-induced dipole attractive interactions of the analyte with the components of the competing mobile and stationary phase; δ_{min} as reflecting the local, fragmental analyte polarity and hence its ability to participate in polar interactions with the phases like submolecular dipole–dipole, charge-transfer and hydrogen-bonding interactions; A_{WAS} as describing the strength of dispersive interactions (London-type interactions) of the analyte with the molecules forming the chromatographic phases.

A general QSRR equation based on the molecular modeling-derived descriptors has the form:

$$t_R = k'_1 + k'_2\mu + k'_3\delta_{Min} + k'_4 A_{WAS} \tag{2}$$

where k'_1–k'_4 are regression coefficients.

The third type of the most studied QSRR is based on the solvatochromic comparison method and the so-called linear solvation energy relationships (LSER). The approach was introduced to chromatography and extensively developed by Abraham and co-workers [10]. The general LSER equation is of the form:

$$\log k = \log k_0 + rR_2 + vV_x + s\pi_2^H + a\sum \alpha_2^H + b\sum \beta_2^H \tag{3}$$

where R_2 is the excess molar refraction of the analyte, V_x is its molecular volume from the McGowan algorithm, π_2^H is dipolarity/polarizability descriptor, $\sum \alpha_2^H$ is a measure of the ability of the analyte to donate a hydrogen, $\sum \beta_2^H$ is an analogous parameter corresponding to the hydrogen accepting potency, $\log k_0$ is a constant and r, v, s, a and b are regression coefficients accounting for the net complementary properties of the chromatographic system formed by the given stationary and mobile phases.

The model described by Eq. (3) served to develop an original approach to comparison of stationary phase selectivity [11–16]. On that approach is also based the hydrophobic-subtraction model of retention [11,17].

In the hydrophobic-subtraction approach a subtraction of the hydrophobicity contribution to the to RP-HPLC retention is done to better see the remaining contributions to retention, due to other than hydrophobicity analyte–stationary/mobile phase interactions. The resulting general equation describing column selectivity, α, is:

$$\log \alpha \equiv \log\left(\frac{k}{k_{EB}}\right) = H_\eta - S^*\sigma + A\beta + B\alpha + C\kappa \tag{4}$$

where k is the retention factor of a given analyte, k_{EB} the value of k for a non-polar reference analyte (for example, ethylbenzene), determined on the same column under the same conditions, and the remaining selectivity-related symbols represent either eluent- and temperature-dependent properties of the analyte ($\eta, \sigma, \beta, \alpha, \kappa$) or eluent- and temperature-independent properties of the column (H, S^*, A, B, C). The column parameters denote the following column properties: H, hydrophobicity; S, steric resistance to insertion of bulky analyte molecules into the stationary phase; A, column hydrogen-bond acidity, mainly attributable to non-ionized silanols; B, column hydrogen-bond basicity, hypothesized to result from sorbed water in the stationary phase; C, column cation-exchange activity due to ionized silanols. The parameters $\eta, \sigma, \beta, \alpha, \kappa$ denote complementary properties of the analyte: η, hydrophobicity; σ, molecular "bulkiness" or resistance of the analyte to its insertion into the stationary phase; β, hydrogen-bond basicity; α, hydrogen-bond acidity; κ, approximate charge (either positive or negative) on the analyte molecule. It must be emphasized here that the values of each analyte parameter, $\eta, \sigma, \beta, \alpha, \kappa$, are relative to the values for ethylbenzene, the reference analyte for which all the analyte parameters are zero.

The three types of the above-mentioned QSRR, the *clogP*-based model, the molecular modeling descriptors-based model and the hydrophobic-subtraction model, were compared for nine representative RP-HPLC stationary phases. The QSRR equations were derived using the retention data

for a predesigned series if test analytes [18], determined in this work on three columns, Aqua C18, Nova-Pak C18 and LiChrospher 60RP-select B, and the retention data reported previously [3,4] for a set of six other modern analytical reversed-phase HPLC columns.

QSRR analysis by multiple regression was supported with a principal component analysis (PCA) of columns similarity.

2. Experimental

2.1. Equipment

The following chromatographic columns served to generate retention factors for QSRR analysis: (i) LiChrospher 60RP-select B column, 12.5 cm × 0.4 cm, particle size 5 μm (Merck, Darmstadt, Germany), (ii) Nova-Pak C18 column, 15.0 mm × 0.39 mm, particle size 4 μm (Waters, Milford, MA, USA), (iii) Aqua C18 125 Å column, 15.0 mm × 0.46 mm, particle size 5 μm (Phenomenex, Torrance, CA, USA), (iv) Symmetry C18, 15.0 cm × 0.46 cm I.D., particle size 5 μm (Waters, Milford, MA, USA), (v) Supelcosil LC-18 column, 15.0 cm × 0.46 cm I.D., particle size 5 μm (Supelco, Bellefonte, PA, USA), (vi) XTerra MS C18, 15.0 cm × 0.46 cm I.D., particle size 5 μm (Waters, Millford, MA, USA), (vii) Inertsil ODS-3, 15.0 cm × 0.46 cm I.D., particle size 5 μm (GL Sciences Inc., Shinjuku-ku, Tokyo, Japan), (viii) Chromolith, 10.0 cm × 0.46 cm I.D. (Merck, Darmstadt, Germany), (ix) Discovery HS F5-3, 15 cm × 0.46 cm I.D., (Supelco, Bellefonte, PA, USA). All the columns were based on regular hydrocarbonaceous silica, except Chromolith, which was made of a highly porous monolithic rod of silica and Discovery HS F5-3, which was packed with a pentafluorophenylpropyl-terminated reversed phase material.

Retention measurements on LiChrospher 60RP-select B, Nova-Pak C18 and Aqua C18 columns were done on a Merck-Hitachi LaChrom HPLC system (Merck-Hitachi, Frankfurt-Tokyo, Germany-Japan), equipped with UV–vis detector (L-7400), autosampler (L-7200) and thermostat (L-7360). Chromatographic data were collected using D-7000 HPLC System Manager, version 4.1 (Merck-Hitachi). The mobile phase contained methanol and 100 mM Tris buffer of pH 7.2, necessary to suppress the dissociation of individual analytes. The buffer was prepared by dissolving tris(hydroxymethyl)aminomethane in water and adjusting the pH with 1 M HCl (Fluka, Buchs, Switzerland). The pH of the buffer was measured at 21 °C before mixing with the organic modifier. The pH measurements were done with an HI 9017 pH meter (Hanna Instruments, Bedfordshire, UK). All the chromatographic measurements were done at 40 °C at the eluent flow rate 1 ml/min. The injected sample volume was 20 μL.

Table 1
Molecular descriptors of test series of analytes used in QSRR analysis

Test analyte	*clogP*	μ (D)	δ_{Min} (electron)	A_{WAS} (Å^2)
Benzamide	0.74	3.583	−0.4333	293.46
4-Cyanophenol	1.60	3.311	−0.2440	290.90
Indazole	1.82	1.547	−0.2034	284.44
Benzonitrile	1.65	3.336	−0.1349	279.14
Indole	2.14	1.883	−0.2194	292.38
2-Naphthol	2.71	1.460	−0.2518	323.16
Anisole	2.13	1.249	−0.2116	288.94
Benzene	2.22	0.000	−0.1301	245.21
1-Naphthylacetonitrile	2.68	3.031	−0.1381	364.26
Benzyl chloride	2.49	1.494	−0.1279	296.17
Naphthalene	3.45	0.000	−0.1277	311.58
Biphenyl	3.98	0.000	−0.1315	358.08
Phenanthrene	4.68	0.020	−0.1279	374.73
Pyrene	5.17	0.000	−0.1273	392.41
2,2′-Dinaphthyl ether	6.67	1.463	−0.1606	510.36

Meaning of symbols is explained in the text.

The equipment and the experimental conditions used to determine retention data for the QSRR analysis in the case of the six other columns studied have been described previously [3,4]. Information on the equipment and the experimental conditions used to obtain the parameters *H*, S^*, *A*, *B*, and *C* (at pH 7.0) of the hydrophobic-subtraction model for all the nine columns studied is given in Ref. [17].

2.2. Chemicals

Methanol was from P.C. Odczynniki (Gliwice, Poland). Water was prepared with a Milli-Q Water Purification System (Millipore Corporation, Bedford, MA, USA).

The following series of test analytes was used as recommended [3,18] for comparative QSRR analysis: ben-

Table 2
Coefficients k_1 and k_2 (±standard deviations) with their significance levels, *p* (underneath in parenthesis), and statistical parameters, *R*, *s*, *F* and *p* (see text for explanation), of the regression equations of the forms: $t_R = k_1 + k_2\ clogP$, for a series of test analytes

Column	k_1	k_2	*R*	*s*	*F*	*p*
LiChrospher 60RP-select B	5.461 (±1.125)	4.066 (±0.339) (*p* = 2E−8)	0.958	2.01	144	2E−08
Nova-Pak C18	4.210 (±1.454)	4.912 (±0.439) (*p* = 5E−8)	0.952	2.59	125	5E−08
Aqua C18	4.375 (±1.460)	5.017 (±0.440) (*p* = 4E−8)	0.953	2.60	130	4E−08
Symmetry C18	9.428 (±1.656)	4.991 (±0.500) (*p* = 2E−7)	0.941	2.95	100	2E−07
Supelcosil LC-18	9.367 (±1.244)	3.906 (±0.375) (*p* = 1E−7)	0.945	2.22	108	1E−07
XTerra MS C18	11.227 (±1.353)	3.983 (±0.408) (*p* = 2E−7)	0.938	2.41	95	2E−07
Inertsil ODS-3	12.211 (±1.287)	3.666 (±0.388) (*p* = 3E−7)	0.934	2.29	89	3E−07
Chromolith	3.489 (±1.345)	4.882 (±0.406) (*p* = 2E−8)	0.958	2.40	145	2E−08
Discovery HS F5	14.100 (±1.071)	3.516 (±0.323) (*p* = 7E−8)	0.949	1.91	118	7E−08

zamide, indazole, benzonitrile, 2-naphthol, anisole, 1-naphthylacetonitrile, benzyl chloride, naphthalene, biphenyl, pyrene, 2,2′-dinaphthyl ether, all from Lancaster (Newgate, England); indole and benzene, both from P.C. Odczynniki; 4-cyanophenol from Aldrich Chemical (Gillingham, England) and phenanthrene from Koch-Light Laboratories (Koinbrook Bucks, England).

The molecular structure descriptors of test analytes (Table 1) which were employed in QSRR analysis, i.e., total dipole moment, μ, electron excess charge of the most negatively charged atom, δ_{Min}, and water-accessible molecular surface area, A_{WAS}, were calculated by standard molecular modeling, using the HyperChem program for personal computers with the extension ChemPlus (Hypercube, Waterloo, Canada). The software performed the geometry optimization by the molecular mechanics MM+ force field method, followed by quantum chemical calculations by the semiempirical AM1 method.

The *clogP* values of the test series of analytes were calculated using ACD software (Advanced Chemistry Development, Toronto, Canada). Numerical data obtained are collected in Table 1.

2.3. Methods

The gradient retention times, $t_{R(exp)}$, of the test analytes were measured with a linear gradient of 10–90% of methanol at gradient time, t_G, of 30 min [19]. The data from the gradient experiment were used to derive QSRR models for the individual column/eluent system. To derive QSRR equations multiple regression analysis was performed using Microsoft Excel software (Microsoft, Redmond, WA, USA). The regression coefficients (±standard deviations), the multiple correlation coefficients, R, the standard errors of the estimate of the equation, s, the significance levels of each term and of whole equations, p, and the values of the F-test of significance, F, are given in Tables 2 and 3.

The values of H, S^*, A, B and C of the hydrophobic-subtraction model were found in Ref. [17] for all the nine columns considered and are given in Table 4. The *clogP*-based model is described in Table 2. The molecular modeling descriptors-based model is characterized in Table 3. The regression coefficients of the hydrophobic-subtraction model are collected in Table 4.

Correlations between the regression coefficients of the three models considered are presented in Table 5.

Principal component analysis was carried out with a Statistica package (StatSoft, Tulsa, USA).

3. Results and discussion

Using the experimental gradient retention times of model analytes, determined in present work on LiChrospher 60RP-select B, Nova-Pak C18 and Aqua C18 columns and previously [3,4] on the remaining six columns, the coefficients of

Table 3
Coefficients k'_1–k'_4 (±standard deviations) with their significance levels, p (underneath in parenthesis), and statistical parameters, R, s, F and p (see text for explanation), of the regression equations of the form: $t_R = k'_1 + k'_2\mu + k'_3\delta_{Min} + k'_4 A_{WAS}$, for a series of test analytes

Column	k'_1	k'_2	k'_3	k'_4	R	s	F	p
LiChrospher 60RP-select B	2.008 (±2.138)	−1.852 (±0.316) (p = 1E−4)	22.942 (±5.123) (p = 9E−4)	0.069 (±0.005) (p = 7E−8)	0.986	1.28	124	9E−09
Nova-Pak C18	1.505 (±2.577)	−2.753 (±0.381) (p = 2E−5)	23.955 (±6.175) (p = 3E−3)	0.079 (±0.007) (p = 1E−7)	0.986	1.55	126	9E−09
Aqua C18	1.563 (±2.542)	−2.918 (±0.376) (p = 9E−6)	22.794 (±6.092) (p = 3E−3)	0.080 (±0.007) (p = 9E−8)	0.987	1.53	135	6E−09
Symmetry C18	9.475 (±2.511)	−2.992 (±0.371) (p = 6E−06)	27.911 (±6.017) (p = 7E−4)	0.074 (±0.006) (p = 2E−7)	0.987	1.51	141	5E−09
Supelcosil LC-18	8.624 (±1.981)	−2.242 (±0.293) (p = 1E−05)	21.517 (±44.746) (p = 9E−4)	0.060 (±0.005) (p = 1E−07)	0.987	1.19	137	5E−09
XTerra MS C18	11.713 (±1.977)	−2.314 (±0.292) (p = 7E−06)	24.345 (±4.737) (p = 3E−4)	0.059 (±0.005) (p = 2E−07)	0.988	1.19	145	4E−09
Inertsil ODS-3	13.425 (±1.893)	−2.116 (±0.280) (p = 1E−05)	24.124 (±4.535) (p = 2E−4)	0.052 (±0.005) (p = 3E−07)	0.987	1.14	135	6E−09
Chromolith	−0.261 (±2.5)	−2.678 (±0.374) (p = 2E−05)	22.044 (±6.060) (p = 4E−4)	0.080 (±0.006) (p = 8E−08)	0.986	1.52	128	8E−09
Discovery HS F5	11.807 (±2.720)	−1.351 (±0.402) (p = 6E−3)	23.870 (±6.518) (p = 4E−3)	0.058 (±0.007) (p = 4E−06)	0.969	1.63	56	6E−07

Table 4
Coefficients H, S^*, A, B and C, of the regression equations of the form: $\log\alpha \equiv \log(k/k_{EB}) = H\eta' - S^*\sigma' + A\beta' + B\alpha' + C\kappa'$

Column	H	S^*	A	B	C
LiChrospher 60RP-select B	0.747	−0.060	−0.042	0.006	1.773
Nova-Pak C18	1.048	0.005	0.096	−0.029	0.562
Aqua C18	0.979	0.024	0.004	0.005	0.236
Symmetry C18	1.053	0.062	0.020	−0.020	0.124
Supelcosil LC-18	1.019	−0.046	0.185	0.158	1.756
XTerra MS C18	0.985	0.012	−0.141	−0.014	0.051
Inertsil ODS-3	0.991	0.021	−0.142	−0.021	−0.333
Chromolith	1.003	0.028	0.009	−0.014	0.187
Discovery HS F5	0.631	−0.166	−0.325	0.023	0.940

Data extracted from Ref. [17].

multiple regression QSRR equations were derived, characterizing the columns tested (Tables 2 and 3). The parameters of the hydrophobic-subtraction model for all the columns studied were taken from Ref. [17].

The parameters of Eq. (1) for each column studied are collected in Table 1. The high t_R versus *clogP* correlations confirm the similarity of the slow-equilibrium octanol/water partition system and the fast-equilibrium partition chromatographic process. Hydrophobicity order of the stationary phases according to k_2 coefficient is as follows: Discovery HS F5 < Inertsil ODS-3 < Supelcosil LC-18 < XTerra MS C18 ≤ LiChrospher 60RP-select B < Chromolith ≤ Nova-Pak C18 < Symmetry C18 ≤ Aqua C18.

The coefficients at the three parameters of Eq. (2), k'_2, k'_3 and k'_4, relating the t_R of test analytes to their total dipole moment, electron excess charge of the most negatively charged atom and water-accessible molecular surface area, respectively, are statistically significant for all the columns tested (Table 3). The multiple regression equations based on these parameters make a good physical sense. The values of the coefficient k'_4 are positive, in accordance with a positive contribution to retention of the non-specific analyte-stationary phase interactions, characterized by the A_{WAS} parameter. These interactions require a close contact of the interacting molecules of molecular fragments. Larger values of A_{WAS} indicate a larger surface area of the stationary phase hydrocarbon moiety which is accessible to the analyte. Based on the numerical data from Table 3, the stationary phases Aqua C18, Chromolith, Nova-Pak C18 and Symmetry C18 have most developed surface area of external hydrocarbon ligands and hence, the highest non-specific London retentivity due to dispersion interactions (London interactions). At the other end, the stationary phases LiChrospher 60RP-select B, Supelcosil LC-18, XTerra MS C18, Discovery HS F5 and Inertsil ODS-3 are characterized by the lowest input to retention of London-type interactions.

The specific, polar intramolecular interactions are characterized by the coefficients k'_2 and k'_3. Negative values of the coefficients k'_2 suggest that the net effect to the retention of dipole–dipole (and dipole-induced dipole) attractions between the analytes and the stationary phase, on one hand, and the components of the eluent, on the other hand, is negative. This can be explained by a stronger attraction between the total dipole of the analyte and the total and/or fragmental dipoles (both permanent and induced) of the polar molecules of the eluent, as compared to the respective interactions between the analytes and the non-polar alkyl chains of the stationary phases. The values of the coefficient k'_2 and hence, the polarity of the stationary phase, increase in the order: Symmetry C18 ≤ Aqua C18 ≤ Nova-Pak C18 ≤ Chromolith < XTerra MS C18 ≤ Supelcosil LC-18 < Inertsil ODS-3 < LiChrospher 60RP-select B < Discovery HS F5.

A similar rationalization applies to the coefficient k'_3 in Eq. (2). Coefficient k'_3 has a positive sign because the δ_{Min} values (electron deficiencies), given in Table 1 are negative. Thus, the more charged an atom is, the higher is the absolute value of the $k'_3\delta_{Min}$ term, and the less retained the analyte is. Lower values of k'_3 can be interpreted as indicating stronger local (fragmental) dipole–dipole interactions and/or the formation of the electron-pair-donor/electron-pair-acceptor complexes between the analyte and the stationary phase, with regards to analogous interactions with the eluent. Therefore, low k'_3 values suggest a higher polarity of the stationary phase (Table 3). According to k'_3, the stationary phases can be ordered as follows with decreasing polarity: Symmetry C18 > XTerra MS C18 ≥ Inertsil ODS-3 ≥ Nova-Pak C18 > Discovery HS F5 > LiChrospher 60RP-select B ≥ Aqua C18 > Chromolith > Supelcosil LC-18. The k'_3 coefficient can also be interpreted as reflecting the ability of analytes to take part in hydrogen-bonding interac-

Table 5
Correlation between regression coefficients obtained with the use of the studied QSRR models of retention: *clogP* model, the molecular descriptors from calculation chemistry-based model and the hydrophobic-subtraction model

	k'_2	k'_3	k'_4	H	S^*	A	B	C
k_2	**−0.90**	0.21	**0.94**	0.58	**0.68**	0.57	−0.36	−0.29
k'_2		−0.26	**−0.71**	**−0.85**	**−0.88**	−0.66	0.26	0.45
k'_3			−0.00	0.15	0.33	−0.22	−0.51	−0.46
k'_4				0.32	0.43	0.50	−0.31	−0.06
H					0.88	**0.70**	−0.05	−0.46
S^*						0.50	−0.39	−0.63
A							0.39	0.27
B								**0.69**

Significant correlations ($p < 0.05$) are marked in bold.

Table 6
Summation of results of principal component analysis of regression coefficients of the both the *clogP*-based and the molecular modeling descriptors-based QSRR models in relation to data obtained for analogous analysis of regression coefficients of the hydrophobic-subtraction model and all the three models taken together

No. of principal component	Eigenvalue	Variance explained (%)
Coefficients k_2, k'_2, k'_3 and k'_4		
1	2.75	68.72
2	1.01	25.20
3	0.24	6.07
Sum		99.98
Coefficients H, S^*, A, B and C		
1	2.69	53.77
2	1.90	37.97
3	0.34	6.70
Sum		98.45
Coefficients k_2, k'_2, k'_3 and k'_4, H, S^*, A, B and C		
1	4.73	52.55
2	2.29	25.42
3	1.12	12.44
Sum		90.42

tions with free silanols of the stationary phases support material.

Classifications of the stationary phases based on QSRR employing either *clogP* or the molecular modeling descriptors, on the one hand, and the hydrophobic-subtraction model, on the other hand, were compared with the help of correlation analysis (Table 5) and principal component analysis (Table 6). Similarities and dissimilarities among the columns were quantitatively evaluated.

As evident from Table 6, most information on individual column properties (above 95% of total data variance) can be explained by the first three principal components. The distribution of the coefficients of Eqs. (1)–(3) indicates the similarities of k_2 (at *clogP*), k'_4 (at A_{WAS}), H (at η), and C (at κ). Similarity of k_2 and H is as expected for two types of hydrophobicity parameters.

Also, a close neighborhood of k'_4 is not surprising as the molecular surface of the stationary phase ligands might directly be related to column hydrophobicity. That observation is confirmed by a high correlation ($R = 0.94$) between k_2 and k'_4 in Table 5. However, it is difficult to explain, why the columns cation-exchange activity due to ionized silanols (coefficient C) would be related to their hydrophobicity (coefficients k_2 and H). Correlation analysis (Table 5) shows a high intercorrelation ($R = 0.88$) between H and S^*, which is not surprising, but shows a limited specificity of the two column parameters employed by the hydrophobic-subtraction model. Also, significant negative correlation between column dipolarity, k'_2, and H ($R = -0.85$), S^* ($R = -0.88$) and k_2 ($R = -0.90$) appear to be reasonable.

Comparison of stationary phases can be done on the basis of principal component scores (objects) plots, presented in Figs. 1 and 2. Projection of nine columns onto the space of two first principal components, PC1 and PC2, resulted from

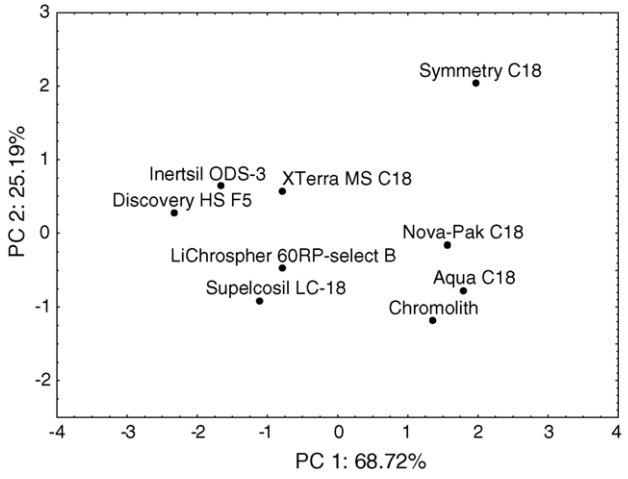

Fig. 1. Projection of nine RP HPLC columns onto the plane of first two principal components, PC1 and PC2, from principal component analysis of regression coefficients of the QSRR models of retention based on *clogP* and on the structural descriptors from molecular modeling.

PCA of the regression coefficients at the QSRR variables *clogP*, μ, δ_{Min} and A_{WAS} (k_2, k'_2, k'_3 and k'_4, respectively) indicates a close similarity of the Chromolith, Aqua C18 and Nova-Pak C18 columns. Similar conclusion can be drawn from Fig. 2. Here, the projection of nine columns onto the space of two first principal components from PCA of the H, S^*, A, B and C coefficients also localizes Chromolith, Aqua C18 and Nova-Pak C18 columns together. However, contrary to Fig. 1, in Fig. 2 the Symmetry C18 column is also located in the cluster.

In both Figs. 1 and 2, XTerra MS C18 and Inertsil ODS-3 columns are close to one another. In Fig. 2 these two columns are not far from the cluster of the Chromolith, Aqua C18 and Nova-Pak C18 columns. Fig. 1 would indicate that these five columns are not that similar. On the other hand, in Fig. 2,

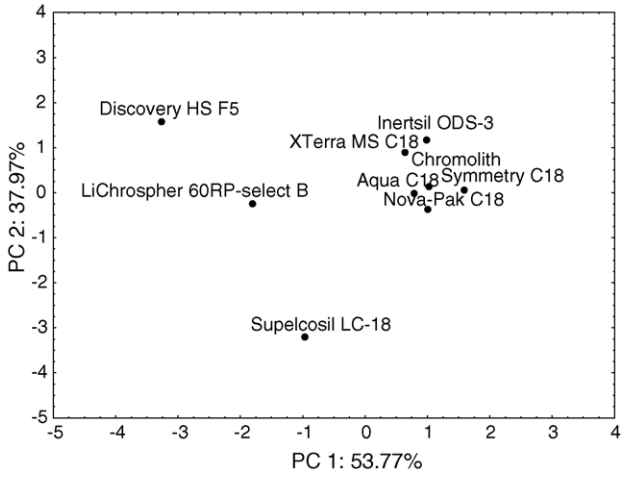

Fig. 2. Projection of nine RP HPLC columns onto the plane of first two principal components, PC1 and PC2, from principal component analysis of regression coefficients of the hydrophobic-subtraction model of retention.

based on the hydrophobic-subtraction model, similarity of LiChrospher 60RP-select B to Supelcosil LC appear to be less apparent than in Fig. 1, based on the QSRR models employing analytes' descriptors from calculation chemistry.

Summarizing discussion of columns' similarity/dissimilarity, illustrated in Figs. 1 and 2, one will note that the most striking difference in classification concerns the Symmetry C18 column. According to the coefficients of the hydrophobic-subtraction model, the Symmetry C18 stationary phase is like Nova-Pak C18, Aqua C18 and Chromolith. On the other hand, according to systematic information extracted by PCA from the set of coefficients of QSRR equations based on the structural descriptors from calculation chemistry, the Symmetry C18 column is no more similar to Nova-Pak C18, Aqua C18 and Chromolith than to XTerra MS C18 and Inertsil ODS-3. It is difficult to judge which classification of the Symmetry C18 column is more reliable. Anyway, that single difference does not disqualifies neither the empirical approach to column classification [17] nor the QSRR approach based on calculation chemistry. Certainly, both the approaches give means for quantitative, although multidimensional, comparison of RP HPLC columns' properties. In our opinion the QSRR-based approach is simpler and requires less labor. It is also more straightforward as regards physical meaning of individual descriptors of column properties.

4. Conclusions

QSRR analysis of retention times for a predesigned series of 15 structurally diverse test analytes allows a rationalization of the molecular mechanism of separation operating in individual RP-HPLC systems. QSRR equations employing either the empirical or the calculation chemistry-derived structural descriptors allow classification of modern RP HPLC materials according to the type and magnitude of intermolecular interactions affecting analyte retention. The *clogP*-based QSRR model, the model based on the descriptors of test analytes from molecular modeling and the hydrophobic-subtraction model provide quantitative, physically interpretable column characteristics. The approaches studied give comparable classification of modern RP HPLC columns, indicating similarities and differences in retention properties, which might be of use in rational column selection.

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