Quantitative Structure-Retention Relationships with Model Analytes as a Means of an Objective Evaluation of Chromatographic Columns

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Abstract

The performance of several previously designed model series of test analytes has been tested to characterize in an objective, quantitative manner modern stationary phases for reversed-phase high-performance liquid chromatography (RP-HPLC) using quantitative structure-retention relationships (QSRRs). Three QSRR approaches and three respective series of test analytes recommended for studies of the molecular mechanism of chromatographic retention are employed: the reduced linear solvation energy relationship (LSER)-based model of Abraham, a model employing structural descriptors from molecular modeling, and a model relating retention to the *n*-octanol–water partition coefficient log P. All of the models and test analytes proposed provide reliable QSRR equations. Those equations discriminate in quantitative terms individual columns and chromatographic systems and can be interpreted in straightforward rational chemical categories. In view of QSRRs, the differences in the intermolecular interactions between a given stationary phase and a structurally defined analyte rationalize the observed differences in retention. The QSRR models (previously derived retrospectively) are demonstrated to work well on new sets of RP-HPLC data. At the same time, it has been confirmed that the three test series of analytes have properly been designed and can be recommended for comparative studies of analytical columns. QSRRs once derived on a given column for model analytes can be used to predict the retention of other analytes of a defined structure. That in turn can facilitate the procedure of the rational optimization of chromatographic separations.

Introduction

Quantitative structure-retention relationships (QSRRs) are one of the most extensively studied manifestations of linear free-energy relationships (LFERs). QSRRs are the statistically derived relationships between the chromatographic parameters determined for a representative series of analytes in a given separation system and the quantities (descriptors) accounting for structural differences among the analytes tested (1).

Among the several areas of application of QSRRs (2), a wide interest from analytical chemists has recently developed in the studies on the molecular mechanism of separation operating in individual chromatographic systems both in high-performance liquid chromatography (HPLC) (3,4) and gas chromatography (GC) (5). The QSRR approach has allowed for the rationalization of differences in analyte retention on various stationary phases in terms of intermolecular interactions of a particular class involving the analyte, the stationary phase (zone), and the eluent.

The wide variety of the presently available reversed phase (RP)-HPLC phases differ in the ligand type of support material and the way in which the ligands are immobilized on the matrix. However, the polar and ionic properties of such support materials (such as silica or alumina) are responsible for secondary intermolecular interactions that often determine the unique character of an RP-HPLC phase (6). Numerous stationary phases for HPLC have nominally been identical, suggesting that they show similar chromatographic properties. However, as pointed out more recently by Sandi et al. (7), Barrett et al. (8), Cruz et al. (9), and Carr et al. (10), despite the widespread application of both analytical and preparative RP-HPLC, the underlying principles and molecular mechanism of retention are still subjects of a long-standing study and debate.

The active role in the retention of the stationary phase has long been acknowledged (11–15). The bonded phase is a complicated heterogeneous medium in which chemical composition and configuration vary with the mobile phase composition, the nature of the support, the bonding density of the ligand, and the alkyl ligand chain length (12,16–18).

Quantitative comparisons of stationary phases are difficult because there are no unequivocal quantitative tests (6). Best suited for that purpose might be the analysis of QSRRs. Tan et al. (19) and Abraham et al. (20) found in their QSRR studies that the relative importance of analyte structural descriptors in QSRR equations describing retention does not differ signifi-

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cantly among various hydrocarbon silica stationary phases. However, the QSRRs reported in literature have been derived for different sets of test analytes. In such a situation it appeared advisable to design a model series of test analytes that could next be recommended for individual types of QSRR analysis aimed at an explanation of the mechanism of separation operating in a given chromatographic system. Recently, we succeeded in identifying such a model series of test analytes for QSRR studies of stationary phases (21).

Three QSRR models have recently been recommended for studies of the molecular mechanism of HPLC retention: the reduced linear solvation energy relationship (LSER)-based model of Abraham, a model employing structural descriptors of analytes from molecular modeling, and a model correlating retention to an *n*-octanol–water partition coefficient (21). All of these models have been demonstrated to provide QSRR equations that are directly interpretable in rational chemical terms and discriminate quantitatively individual chromatographic systems. In view of QSRR analysis, the retention processes clearly emerged as the net effects of fundamental intermolecular interactions involving the analyte and components of the chromatographic system.

The LSER model of QSRRs originated from the solvatochromic comparison method introduced in 1976 by M.J. Kamlet, R.W. Taft, and their co-workers (22–25). Abraham (26) has proposed the following LSER-based approach to chromatographic data:

$$\log k = \log k_0 + rR_2 + vV_x + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \qquad \text{Eq. 1}$$

where R_2 denotes an excess molar refraction of the analyte, V_x denotes its characteristic molecular volume according to McGowan (27), π_2^H is a dipolarity–polarisability parameter, $\Sigma\alpha_2^H$ denotes the sum of the hydrogen-bond acidity, and $\Sigma\beta_2^H$ denotes the sum of the hydrogen-bond basicity of the analyte. The coefficients log k_0, r, v, s, a, and b denote respective bulk stationary and mobile phase properties.

Abraham and co-workers (28,29) applied equation 1 extensively. The approach was introduced to chromatography in order to study the molecular mechanism of retention and characterize RP-HPLC columns. A series of papers and review articles by other authors followed. However, most of the researchers report equations that comprised of only the V_x (or R_2), $\Sigma\beta_2^H$, and (less frequently) $\Sigma\alpha_2^H$ terms of equation 1 as statistically significant. Also, the π_2^H descriptor has been reported occasionally as being significant for specific analytes and chromatographic systems (7,30–33). In single cases in which the researchers select the test analytes very carefully in order to avoid noncorrelation between V_x and R_2, the two descriptors may be present in the same regression equation. Otherwise, only one of the two descriptors is statistically significant.

The objective of this study was to test and further develop a method to characterize in an objective quantitative manner stationary phases for RP-HPLC. It has been assumed that such a method is provided by QSRR analysis with a carefully designed reference series of test analytes. In this study, we used V_x, $\Sigma\alpha_2^H$, and $\Sigma\beta_2^H$ descriptors that appeared significant in the reduced LSER equation accounting for retention differences within series I of the test analytes (Table I). The previously proposed (21) reduced model of the LSER type is summarized by the following equation:

$$\log k_w = k_1 + k_2\Sigma\alpha_2^H + k_3\Sigma\beta_2^H + k_4V_x \qquad \text{Eq. 2}$$

where log k_w denotes the retention parameter corresponding to pure water as a hypothetical eluent and is obtained by linear extrapolation from several isocratic data and k_1–k_4 denote regression coefficients in which physical meaning is similar to that of the corresponding coefficients of equation 1.

The following general QSRR equation employing structural parameters from the molecular modeling of a previously designed set of test analytes (series II, Table II) was applied in this study:

$$\log k_w = k_1' + k_2'\mu + k_3'\delta_{min} + k_4'A_{WAS} \qquad \text{Eq. 3}$$

where μ denotes the total dipole moment of an energy-optimized analyte molecule, δ_{min} is the electron excess charge on the most negatively charged atom, and A_{WAS} is the water-accessible van der Waals surface area of the molecule.

The relationship between the chromatographic retention

Table I. Series I of 18 Analytes Proposed for QSRR-Based Testing of HPLC Columns Employing the LSER-Based Structural Descriptors of Analytes According to Abraham*

No.	Analyte	No.	Analyte
1'	Anisole	10'	Indole
2'	Benzamide	11'	Isopropylbenzene
3'	Benzene	12'	1-Methyl-2-pyrrolidinone
4'	Benzonitrile	13'	Naphthalene
5'	Biphenyl	14'	Nitrobenzene
6'	2-Chloroaniline	15'	4-Nitrophenol
7'	4-Cyanophenol	16'	Phenanthrene
8'	Hexachlorobutadiene	17'	Pyrene
9'	Indazole	18'	1,3,5-Triisopropylbenzene

* Obtained from references 26 and 33.

Table II. Series II of 18 Analytes Proposed for QSRR-Based Testing of HPLC Columns Employing Structural Descriptors of Analytes Generated by Molecular Modeling

No.	Analyte	No.	Analyte
1''	Aniline	10''	2,2-Dinaphthyl ether
2''	Anisole	11''	Indazole
3''	Benzamide	12''	Indole
4''	Benzene	13''	Naphthalene
5''	Benzonitrile	14''	2-Naphthol
6''	Benzyl chloride	15''	1-Naphthylacetonitrile
7''	Biphenyl	16''	Phenanthrene
8''	2-Chloropyridine	17''	Phenol
9''	4-Cyanophenol	18''	Pyrene

data and the logarithm of the *n*-octanol–water partition coefficients (log P) is the third model of QSRRs proposed for the comparative studies of RP-HPLC stationary phases (21):

$$\log k_w = k_1'' + k_2'' \log P \qquad \text{Eq. 4}$$

A model series of ten test analytes (series III, Table III) previously proposed was used to derive QSRR equations of the type of Eq. 4.

QSRR equations concerning the three model series of test analytes were used to test the previously (21) formulated hypothesis that the approach allows for an objective evaluation of any new stationary phase as well as a rational interpretation of the molecular mechanism of chromatographic separation operating in a given mobile or stationary-phase HPLC system.

Experimental

Materials

The HPLC columns used in the study were as follows: an Aluspher RP-select B (119- × 4.00-mm i.d., particle diameter 5 µm) from E. Merck (Darmstadt, Germany), a Supelcosil LC-Hisep (150- × 4.6-mm i.d., particle diameter 5 µm) from Supelco (Bellefonte, PA), a Nova-Pak C18 (150- × 3.9-mm i.d., particle diameter 4 µm, pore diameter 60 Å) from Waters Corporation (Milford, MA), a Luna C18 (250- × 4.6-mm i.d., particle diameter 5 µm) from Phenomenex (Torrance, CA), a Discovery RP-Amide C16 (150- × 4.6-mm i.d., particle diameter 5 µm) from Supelco (Bellefonte, PA), a Discovery Cyano (150- × 4.6-mm i.d., particle diameter 5 µm) from Supelco (Bellefonte, PA), and a Mix-Cholesterol-AP (125- × 4.6-mm i.d., particle diameter 5 µm) from Nicolaus Copernicus University (Torun, Poland).

Methanol (MeOH) of analytical reagent grade was obtained from Odczynniki Sp. z o.o. (Lublin, Poland), acetonitrile (ACN) (super gradient) was from Lab-Scan Ltd. (Dublin, Ireland), and water was prepared with a Milli-RQ 5 Plus water purification system (Millipore, Milford, MA).

Test analytes were purchased from recognized reagent suppliers.

Chromatographic parameters

Analytes were chromatographed using a Merck-Hitachi (Wien, Austria) apparatus equipped with a thermostat, an integrator, and a variable-length UV detector. The temperature 38°C was chosen for the tests in order to provide the best reproducibility of the HPLC analyses (34). The eluent flow rate was 1.0 mL/min.

Table III. Series III of Ten Analytes Proposed for QSRR-Based Testing of HPLC Columns Employing Logarithms of the *n*-Octanol–Water Partition Coefficient of Analytes

No.	Analyte	No.	Analyte
1'''	Aniline	6'''	*n*-Hexylbenzene
2'''	2-Chloropyridine	7'''	Indazole
3'''	4-Cyanophenol	8'''	Isopropylbenzene
4'''	3-Cyanopyridine	9'''	Naphthalene
5'''	Hexachlorobutadiene	10'''	Phenol

Retention coefficients were determined for five to eight compositions of the binary organic solvent-water mobile phase ranging from 95:5 to 5:95 (v/v). A signal of sodium nitrite was the dead-time marker. MeOH and ACN were the organic solvents employed, and both were diluted with water.

Linear relationships were found between log k and the volume percentage of the organic solvent in the eluent. Based on these linear relationships (in each case the correlation coefficient was > 0.99), the values of log k_w corresponding to 100% water eluent were obtained by extrapolation. The data (the mean of three independent determination runs) are summarized in Tables IV and V.

Structural descriptors of analytes

The analytes and their corresponding structural descriptors are given in Table VI. The LSER parameters of Abraham for test analytes were taken from references 26 and 33 when available (Table VI).

The test analytes (Table I) of series I that appeared significant in QSRR equations were V_x, $\Sigma\alpha^H$, and $\Sigma\beta$.

The test analytes (Table II) of series II that appeared significant in QSRR equations were A_{WAS}, μ, and δ_{min}.

In Table VI, log P was given as recommended in specialist literature (35) including the test analytes of series III (Table III).

Chemometric calculations

Multiple regression equations were derived employing a Statgraphics Plus-6.0 program (Manugistics, Rockville, MD) run on a personal computer. The results are collected in Tables VII, VIII, and IX. In these tables, the regression coefficients (± standard deviations), multiple correlation coefficients, standard errors of estimate, and values of the F-test of significance are given. In order to exclude chance correlations, cross validation of the procedure was performed (36).

Results and Discussion

Parameters log k_w in Tables IV and V represent the retention of the total number of 27 analytes forming the test series I, II, and III on Aluspher, Hisep, Nova-Pak, Luna, Discovery Amide, Discovery Cyano, and Mix-Cholesterol-AP columns in MeOH–water (Table IV) and ACN–water (Table V) eluent systems. The analytes for the QSRR studies were as previously designed (21) not only to cover a wide range of values of individual structural descriptors, but also to provide well-shaped peaks and a good linearity ($R \geq 0.99$) of the log k versus the percentage of organic modifier relationships. It has been demonstrated (21) on the basis of retrospective analysis that the test series I, II, and III of analytes yield three respective QSRR models that account quantitatively for the differences in the retention mechanism of three distinctive stationary phase materials: Inertsil ODS-3 (GL Sciences, Tokyo, Japan), Sym-

metry C8 (Waters, Milford, MA), and IAM.PC.C10/C3 (Regis, Morton Grove, IL).

An important question was whether the models derived previously were of general validity and would work on retention data determined a posteriori on diverse RP-HPLC columns of present analytical interest.

In QSRR studies, the log k_w data are preferred instead of individual isocratic log k data. Log k_w is a standardized retention parameter that is believed to be more reliable than any arbitrarily selected isocratic log k (1). Log k_w is an intercept of the linear Snyder–Soczewinski relationship (37) between the isocratic log k values and the corresponding contents of the organic modifier in the eluent. Log k_w appears to depend on the nature of the organic modifier. Thus, this parameter for a given analyte derived on a given column when using MeOH–water systems differs normally from the parameter derived on the same column when using ACN–water systems (38). However, both log k_w values of analytes are characteristic for a given column with respect to another one.

The multiple regression equations given in Table VII with three significant LSER descriptors of analytes from series I (V_x, $\Sigma\beta_2^H$, and $\Sigma\alpha_2^H$ from Table VI) make good physical sense. Coefficient k_4 at the McGowan volume term was positive. This meant that attractive dispersion interactions of London-type between an analyte and the bulky ligand of the stationary phase were stronger than the same nonspecific attractive interactions between the analyte and the small molecules (water, MeOH, and ACN) of the eluent. If one compares the magnitude of k_4 in equations 5–11 with that in equations 12–18, the stronger dispersivity of ACN (MW 41) than MeOH (MW 32) may account for the differences.

The rationalization is that the net positive input of V_x to log k_w is a result of a stronger attraction of an analyte by the ligand (and the adsorbed eluent components) than between the analyte and the bulk eluent. However, the dispersive attraction of the analyte by ACN as the eluent was stronger than by MeOH. Therefore, the retention-increasing effects of V_x on the same column were more evident (larger k_4) in the MeOH–water systems than in the ACN–water systems (smaller k_4) (Table VII, equations 5–18).

According to the k_3 coefficient at $\Sigma\beta_2^H$ in equations 5–18, the net effect on the retention of attractive interactions of a hydrogen-bond acceptor analyte with the nonpolar reversed-phase ligand and the polar components of the eluent (which is an efficient hydrogen-bond donor) was naturally negative. This is documented by the negative sign at k_3 (Table VII).

When k_3 values for different stationary phases and the same eluent system are compared, the phases can be ordered as follows. In MeOH–water systems: Luna ≥ Aluspher ≥ Nova-Pak >

Table IV. Retention Parameters of Test Analytes in MeOH–Water Systems

No.	Analyte	log k_w (Aluspher)*	log k_w (Hisep)*	log k_w (Nova-Pak)*	log k_w (Luna)*	log k_w (Discovery Amide)*	log k_w (Discovery Cyano)*	log k_w (Mix-Cholesterol-AP)*
1	Aniline	–0.0875	0.164	0.8293	1.0811	0.5798	0.0383	0.2114
2	Anisole	0.7214	0.617	1.8914	2.1731	1.4927	0.4285	1.0274
3	Benzamide	–0.2797	0.2745	0.7526	1.0217	0.673	0.0179	0.3117
4	Benzene	0.7126	0.2963	1.7552	2.1013	1.3878	0.2775	0.8134
5	Benzonitrile	0.4184	0.511	1.4712	1.7838	1.1639	0.3219	0.7569
6	Benzyl chloride	1.1172	1.2544	2.4026	2.6444	2.0058	0.8365	1.684
7	Biphenyl	2.8614	2.0781	3.6533	3.7298	3.0943	1.5445	3.1237
8	2-Chloroaniline	0.5758	0.7269	1.5801	1.9245	1.2842	0.4055	1.1818
9	2-Chloropyridine	–0.0218	0.3938	1.1427	1.3951	0.8584	0.0977	0.5502
10	4-Cyanophenol	0.2372	0.9598	1.1483	1.5635	1.2555	0.4536	0.9758
11	3-Cyanopyridine	–0.6397	–0.073	0.4118	0.7376	0.4262	–0.1571	0.0239
12	2,2-Dinaphthyl ether	4.8581	3.8919	5.7091	5.617	4.9729	3.1429	4.9087
13	Hexachlorobutadiene	3.5561	2.3079	4.4461	4.5299	3.736	1.9416	3.8675
14	*n*-Hexylbenzene	4.3201	2.7044	5.4613	5.5533	4.3766	2.314	4.471
15	Indazole	0.4312	0.8832	1.5969	1.91	1.3546	0.4412	1.0726
16	Indole	0.9034	1.0883	1.6723	1.9863	1.5813	0.651	1.3219
17	Isopropylbenzene	2.2269	1.5704	3.4407	3.4774	2.8411	1.1267	2.5633
18	1-Methyl-2-pyrrolidinone	–0.6912	–0.4638	0.2049	0.2802	0.059	–0.402	0.0034
19	Naphthalene	2.1057	1.5751	3.1188	3.1728	2.6138	1.1041	2.3129
20	2-Naphthol	1.4008	1.6415	2.3461	2.509	2.2735	0.9692	2.1813
21	1-Naphthylacetonitrile	1.574	1.8289	2.7531	2.8525	2.3602	1.2266	2.3498
22	Nitrobenzene	0.6602	0.5724	1.6074	1.9578	1.3331	0.4445	1.1732
23	4-Nitrophenol	0.5221	1.5759	1.2573	1.719	1.4867	0.4852	1.1915
24	Phenanthrene	3.0912	2.4908	4.0858	3.8264	3.38	1.9187	3.5308
25	Phenol	0.1127	0.4929	1.0048	1.3839	0.9521	0.1741	0.8898
26	Pyrene	3.6974	2.7579	4.5733	4.2843	3.7737	2.0296	3.9243
27	1,3,5-Triisopropylbenzene	4.7233	3.343	6.1109	6.3161	5.086	2.925	5.0661

* Extrapolated to a hypothetical 100% water eluent as determined on given column employing a series of water–MeOH compositions of mobile phase.

Discovery Amide ≥ Mix-Cholesterol-AP > Hisep > Discovery Cyano (Table VII, equations 5–11). In ACN–water systems: Nova-Pak ≥ Aluspher ≥ Luna ≥ Discovery Amide > Mix-Cholesterol-AP ≥ Hisep > Discovery Cyano (Table VII, equations 12–18).

The magnitude of k_3 can reflect differences in hydrogen-bond donor properties between MeOH and ACN. For a given stationary phase, k_3 tended to be less in the case of ACN-modified eluents as compared with the MeOH-modified ones (with the exception of Luna columns). However, the differences in k_3 were insignificant statistically.

The coefficient k_2 in QSRR equations 5–18 (Table VII) stayed within the hydrogen-bond acidity parameter. It was positive in the case of Hisep columns, and for other phases it was negative. Thus, on all columns except Hisep, the net effect on the retention of attractive interactions of a hydrogen-bond donor analyte with the stationary phase ligand and with the components of the eluent that were efficient hydrogen-bond acceptors was negative. A reverse situation was with the Hisep column. This observation can probably be explained by the unique properties of that column (39). According to the producer, the Hisep column is "silica-based material covered with a thin polymer consisting of hydrophobic regions in a hydrophilic network" (40). This hydrophilic network was evidently very polar and accessible to the small analytes tested.

The Hisep column had a relatively high specific polarity (as quantitated by k_3 and k_2) and a low nonspecific attractivity (as quantitated by k_4). The next most polar and least dispersive of the seven phases studied appeared to be the Discovery Cyano column. The Discovery Amide and Mix-Cholesterol-AP (aminopropyl-silica-bound) columns were of similar polarity, but dispersivity of the latter column was significantly larger. There were no big differences between the k_2, k_3, and k_4 parameters in the case of the Aluspher, Luna, and Nova-Pak columns. This would suggest a generally similar retention mechanism on these modern reversed-phases.

This discussion illustrates the actual potency of equations 5–18 in regards to the effects of stationary and mobile phases on the retention in reversed-phase HPLC. The series of test analytes (series I, Table I) provided physically interpretable QSRR equations employing the most important LSER-based structural descriptors. The series of 18 test analytes of Table I had been designed observing the requirements of a statistically correct and physically meaningful QSRR analysis.

Apart from routine statistical requirements, additional quality measures had also been taken into consideration. One was that intercorrelations between the pairs of individual descriptors employed in equations 5–18 were less than R = 0.33. Also, the range and distribution of log k_w values for test analytes were appropriate as evident in a representative figure

Table V. Retention Parameters of Test Analytes in ACN–Water Systems

No.	Analyte	log k_w (Aluspher)*	log k_w (Hisep)*	log k_w (Nova-Pak)*	log k_w (Luna)*	log k_w (Discovery Amide)*	log k_w (Discovery Cyano)*	log k_w (Mix-Cholesterol-AP)*
1	Aniline	–0.0148	0.2851	0.4576	0.7859	0.4602	0.0627	0.1763
2	Anisole	0.6366	0.7599	1.6821	1.7552	1.2715	0.4929	0.9892
3	Benzamide	–0.4505	0.1971	–0.0472	0.2726	0.1196	–0.0539	0.1496
4	Benzene	0.7111	0.6631	1.6484	1.7552	1.2632	0.4455	0.7476
5	Benzonitrile	0.3194	0.6132	1.3147	1.387	0.9938	0.3372	0.6206
6	Benzyl chloride	1.3073	1.2111	1.9311	1.9399	1.7393	0.895	1.4801
7	Biphenyl	2.4846	1.9589	2.7364	2.5999	2.2635	1.5603	2.4467
8	2-Chloroaniline	0.532	0.8814	1.2686	1.5183	1.189	0.4575	0.9082
9	2-Chloropyridine	–0.1159	0.2564	0.6386	0.8882	0.6445	0.0375	0.379
10	4-Cyanophenol	0.252	0.9004	0.7762	0.918	0.8671	0.3514	0.7389
11	3-Cyanopyridine	–0.6989	–0.0388	0.0666	0.3867	0.0843	–0.1522	–0.0849
12	2,2-Dinaphthyl ether	3.3904	3.0773	3.7407	3.5113	3.2588	2.6665	3.4806
13	Hexachlorobutadiene	2.9224	2.209	3.4138	3.0041	3.1427	1.941	2.9241
14	*n*-Hexylbenzene	3.0136	2.4838	3.6068	3.5327	3.3719	2.3218	3.158
15	Indazole	0.4206	0.754	1.1878	1.3541	1.1395	0.3343	0.8475
16	Indole	0.8363	1.1051	1.4736	1.5382	1.3783	0.6876	1.1723
17	Isopropylbenzene	2.2289	1.4710	2.5808	2.5608	2.1202	1.1784	2.2218
18	1-Methyl-2-pyrrolidinone	–0.8658	–0.5887	–0.1944	0.034	–0.2831	–0.4004	–0.3077
19	Naphthalene	1.9572	1.4882	2.3963	2.4021	2.2137	1.1602	2.0244
20	2-Naphthol	1.2599	1.4859	1.5729	1.7731	1.3598	0.9558	1.656
21	1-Naphthylacetonitrile	1.525	1.6037	2.137	2.0329	1.9164	1.2207	1.8617
22	Nitrobenzene	0.6723	0.7868	1.4054	1.6182	1.188	0.5233	0.9067
23	4-Nitrophenol	0.5496	1.5468	1.1164	1.1784	0.7817	0.5631	1.0077
24	Phenanthrene	2.5358	2.184	2.9263	2.7261	2.5415	1.689	2.7212
25	Phenol	–0.0089	0.5878	0.7656	0.9352	0.7386	0.1498	0.6992
26	Pyrene	2.9546	2.4436	2.8537	2.8139	2.5647	1.9346	2.6987
27	1,3,5-Triisopropylbenzene	3.7126	3.1997	3.6689	3.5113	3.4166	2.669	3.492

* Extrapolated to a hypothetical 100% water eluent as determined on given column employing a series of water–ACN compositions of mobile phase.

presented for the sake of illustration (Figure 1). In this figure, log k_w data determined experimentally on the Discovery Cyano column with a series of ACN–water mobile phases were plotted against the corresponding quantities calculated by equation 17 from Table VII.

A former QSRR analysis describing retention on three selected columns and employing calculation chemistry descriptors (21) resulted in series II of the test analytes (Table II). The following structural parameters were found as the most significant for retention: A_{WAS}, μ, and δ_{min} (21,41) (Table VI). The QSRR equations (equations 19–32) for the columns studied here are collected in Table VIII.

The physical meaning of equations 19–32 was similar to that of equations 5–18. As expected, the net positive input to retention was because of the A_{WAS} parameter (see coefficient k'_4 in Table VIII). This parameter was evidently related to the ability of analytes to take part in London-type interactions. Obviously, these attractive dispersion interactions were stronger between an analyte and the bulky ligand of the stationary phase than between the same analyte and the small molecules of the eluent. Therefore, there was a positive sign at the k'_4 regression coefficient in equations 19–32.

The changes in the magnitude of the regression coefficient k'_4 at A_{WAS} in equations 19–32 paralleled the changes in the coefficient k_4 at V_x in the LSER-based equations 5–18. There was a high intercorrelation between the two coefficients (R = 0.99) for all the analytes listed in Table VI. Therefore, V_x was practically interchangeable with the easily calculated A_{WAS}.

In the case of MeOH–water systems, when considering coefficient k'_4 (Table VIII), the phases studied are ordered as follows: Aluspher > Mix-Cholesterol-AP ≥ Nova-Pak > Discovery Amide ≥ Luna > Hisep > Discovery Cyano. This would indicate a ranking of the dispersion attractivity of the phases in general accordance with that emerging from the LSER-based QSRR equations of Table VII.

The inputs to retention by the specific polar intermolecular interactions are reflected by the coefficients k'_2 and k'_3 in equations 19–32 in Table VIII. Coefficient k'_2 proved that the net effect to retention provided by μ is negative. It appeared reasonable because the dipole–dipole and the dipole–induced dipole attractions were obviously stronger between an analyte and the polar molecules of the eluent than between the same analyte and the nonpolar ligands (mainly hydrocarbons) of the stationary phase.

An analogous explanation is valid in regards to the coefficient k'_3 at δ_{min} in equations 19–32. The positive sign at k'_3 is because the δ_{min} values in Table VI are negative (they represent electron excess in the most-charged atom of the analyte molecule). The

Table VI. Structural Descriptors of Test Analytes

No.	Analyte	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	Vx (cm^3/mol)*	μ (D)	δ_{min} (electrons)	A_{WAS} ($Å^2$)	log P†
1	Aniline	0.955	0.96	0.26	0.41	0.816	1.583	–0.412256	264.96	0.90
2	Anisole	0.708	0.75	0.00	0.29	0.916	1.249	–0.211650	288.21	2.11
3	Benzamide	0.990	1.50	0.49	0.67	0.973	3.583	–0.433323	292.72	0.64
4	Benzene	0.610	0.52	0.00	0.14	0.716	0.000	–0.130121	244.95	2.13
5	Benzonitrile	0.742	1.11	0.00	0.33	0.871	3.336	–0.134902	277.62	1.56
6	Benzyl chloride	0.821	0.82	0.00	0.33	0.980	1.494	–0.127880	295.59	–
7	Biphenyl	1.360	0.99	0.00	0.22	1.324	0.000	–0.131476	358.38	4.01
8	2-Chloroaniline	1.033	0.92	0.25	0.31	0.939	1.676	–0.401070	285.38	1.90
9	2-Chloropyridine	0.738	1.03	0.00	0.37	0.798	2.823	–0.182290	262.35	1.22
10	4-Cyanophenol	0.940	1.63	0.79	0.29	0.930	3.311	–0.244030	289.20	1.60
11	3-Cyanopyridine	0.750	1.26	0.00	0.62	0.829	2.892	–0.185682	269.24	0.23
12	2,2-Dinaphthyl ether	–	–	–	–	–	1.464	–0.160379	508.89	–
13	Hexachlorobutadiene	1.019	0.85	0.00	0.00	1.321	0.000	–0.073001	342.76	4.78
14	*n*-Hexylbenzene	0.591	0.50	0.00	0.15	1.562	0.349	–0.210585	423.24	5.52
15	Indazole	1.180	1.25	0.54	0.34	0.905	1.547	–0.203378	285.49	1.77
16	Indole	1.200	1.12	0.44	0.22	0.948	1.883	–0.219424	292.55	2.14
17	Isopropylbenzene	0.602	0.49	0.00	0.16	1.139	0.247	–0.205658	321.85	3.66
18	1-Methyl-2-pyrrolidinone	0.491	1.50	0.00	0.95	0.820	3.593	–0.352950	271.74	–0.54
19	Naphthalene	1.340	0.92	0.00	0.20	1.085	0.000	–0.127744	313.25	3.30
20	2-Naphthol	1.520	1.08	0.61	0.40	1.144	1.460	–0.251779	325.25	2.70
21	1-Naphthylacetonitrile	–	–	–	–	–	3.031	–0.138098	365.76	–
22	Nitrobenzene	0.871	1.11	0.00	0.28	0.891	5.239	–0.358573	278.37	1.85
23	4-Nitrophenol	1.070	1.72	0.82	0.26	0.949	5.264	–0.363381	289.73	1.91
24	Phenanthrene	2.065	1.29	0.00	0.26	1.454	0.020	–0.127882	376.31	4.46
25	Phenol	0.805	0.89	0.60	0.30	0.775	1.233	–0.252623	256.22	1.25
26	Pyrene	2.808	1.71	0.00	0.29	1.585	0.000	–0.127331	393.84	4.88
27	1,3,5-Triisopropylbenzene	0.627	0.40	0.00	0.22	1.985	0.080	–0.205552	477.78	–

* Obtained from references 26 and 33.
† Obtained from reference 35.

more charged the atom is, then the higher is the absolute value of the $k'_3\ \delta_{min}$ term and thus the less retained is the analyte.

The coefficient k'_2 at μ in Table VIII differentiated the phases from each other well. It also clearly differentiated the MeOH–water from the ACN–water system. According to k'_2, the most polar of the phases studied would be Hisep and Discovery Cyano. The Discovery Amide, Luna, and Mix-Cholesterol-AP stationary phases would be of lesser dipolarity. The least polar would be the Aluspher column followed by Nova-Pak. These observations agree with the conclusions drawn from QSRR equations based on the LSER model.

Equations 19–32 of Table VIII quantitatively differentiate stationary and mobile phases in regards to their effects on

Table VII. Coefficients of Equation 2 for Test Series I of Analytes Listed in Table I Relating log k_w to the LSER-Based Structural Descriptors of Abraham*

log k_w	k_1	k_2	k_3	k_4	R†	S§	F‖	Eq. No.
log k_w (Aluspher, MeOH)	–1.6398 (± 0.2853)	–0.9680 (± 0.2853)	–2.4572 (± 0.3042)	3.6731 (± 0.2061)	0.9893	0.2482	215	5
log k_w (Hisep, MeOH)	–1.2497 (± 0.2411)	0.4393 (± 0.1832)	–1.6278 (± 0.2571)	2.6852 (± 0.1742)	0.9817	0.2098	124	6
log k_w (Nova-Pak, MeOH)	–0.7414 (± 0.2419)	–1.1084 (± 0.1838)	–2.4341 (± 0.2580)	3.7781 (± 0.1748)	0.9928	0.2105	319	7
log k_w (Luna, MeOH)	–0.1763 (± 0.2260)	–0.8021 (± 0.1717)	–2.6290 (± 0.2410)	3.4366 (± 0.1633)	0.9926	0.1966	312	8
log k_w (Discovery Amide, MeOH)	–0.6725 (± 0.1997)	–0.4876 (± 0.1518)	–2.2000 (± 0.2130)	3.2035 (± 0.1443)	0.9927	0.1738	314	9
log k_w (Discovery Cyano, MeOH)	–1.1730 (± 0.1045)	–0.1887 (± 0.0794)	–1.2363 (± 0.1114)	2.2527 (± 0.0755)	0.9954	0.0909	502	10
log k_w (Mix-Cholesterol-AP, MeOH)	–1.4489 (± 0.2857)	–0.5372 (± 0.2171)	–2.0095 (± 0.3048)	3.6959 (± 0.2065)	0.9878	0.2486	187	11
log k_w (Aluspher, ACN)	–0.8657 (± 0.2851)	–0.8171 (± 0.2166)	–2.6475 (± 0.3041)	2.7892 (± 0.2060)	0.9848	0.2481	149	12
log k_w (Hisep, ACN)	–0.6640 (± 0.1668)	0.2864 (± 0.1268)	–1.9273 (± 0.1779)	2.2296 (± 0.1205)	0.9894	0.1452	215	13
log k_w (Nova-Pak, ACN)	0.8312 (± 0.2026)	–1.0367 (± 0.1540)	–2.8923 (± 0.2161)	1.8433 (± 0.1464)	0.9893	0.1763	214	14
log k_w (Luna, ACN)	1.2222 (± 0.1776)	–0.9412 (± 0.1349)	–2.5297 (± 0.1894)	1.3927 (± 0.1283)	0.9880	0.1545	191	15
log k_w (Discovery Amide, ACN)	0.3975 (± 0.2029)	–0.6964 (± 0.1542)	–2.4896 (± 0.2165)	1.8659 (± 0.1466)	0.9868	0.1766	173	16
log k_w (Discovery Cyano, ACN)	–0.7514 (± 0.1069)	–0.2966 (± 0.0812)	–1.4363 (± 0.1140)	1.9333 (± 0.0772)	0.9944	0.0930	415	17
log k_w (Mix-Cholesterol-AP, ACN)	–0.4631 (± 0.2596)	–0.4468 (± 0.1972)	–2.1250 (± 0.2768)	2.4136 (± 0.1875)	0.9814	0.2258	121	18

* Obtained from references 26 and 33. The numerical values of the retention parameters and structural descriptors of the analytes were taken from Tables IV, V, and VI.
† R, Multiple correlation coefficient.
§ S, Standard error of estimate.
‖ F, Value of the F-test of significance.

Table VIII. Coefficients of Equation 3 for Test Series II of Analytes Listed in Table II Relating log k_w to Structural Descriptors of Analytes from Molecular Modeling*

log k_w	k'_1	k'_2	k'_3	k'_4	R†	S§	F‖	Eq. No.
log k_w (Aluspher, MeOH)	–3.3477 (± 0.2798)	–0.3490 (± 0.0385)	2.3799 (± 0.5331)	0.0180 (± 0.0007)	0.9938	0.1775	375	19
log k_w (Hisep, MeOH)	–2.6759 (± 0.3111)	–0.1100 (± 0.0429)	1.4997 (± 0.5929)	0.0140 (± 0.0008)	0.9847	0.1974	148	20
log k_w (Nova-Pak, MeOH)	–1.9956 (± 0.2318)	–0.3054 (± 0.0319)	3.0880 (± 0.4417)	0.0171 (± 0.0006)	0.9955	0.1471	513	21
log k_w (Luna, MeOH)	–1.2007 (± 0.1909)	–0.2477 (± 0.0263)	3.0623 (± 0.3638)	0.0149 (± 0.0005)	0.9960	0.1211	579	22
log k_w (Discovery Amide, MeOH)	–1.9653 (± 0.2150)	–0.2284 (± 0.0296)	2.3735 (± 0.4097)	0.0151 (± 0.0005)	0.9946	0.1364	431	23
log k_w (Discovery Cyano, MeOH)	–2.3211 (± 0.1397)	–0.1128 (± 0.0192)	1.2506 (± 0.2661)	0.0114 (± 0.0004)	0.9952	0.0886	505	24
log k_w (Mix-Cholesterol-AP, MeOH)	–2.7928 (± 0.3394)	–0.2563 (± 0.0468)	2.3630 (± 0.6468)	0.0172 (± 0.0009)	0.9894	0.2153	217	25
log k_w (Aluspher, ACN)	–1.8284 (± 0.3359)	–0.3111 (± 0.0463)	2.8433 (± 0.6402)	0.0126 (± 0.0009)	0.9856	0.2131	158	26
log k_w (Hisep, ACN)	–1.5144 (± 0.2671)	–0.1398 (± 0.0368)	1.5771 (± 0.5090)	0.0103 (± 0.0007)	0.9819	0.1694	125	27
log k_w (Nova-Pak, ACN)	–0.1658 (± 0.2653)	–0.2328 (± 0.0365)	4.1331 (± 0.5055)	0.0096 (± 0.0007)	0.9881	0.1683	192	28
log k_w (Luna, ACN)	0.1658 (± 0.2245)	–0.2223 (± 0.0309)	3.2134 (± 0.4277)	0.0081 (± 0.0006)	0.9881	0.1424	192	29
log k_w (Discovery Amide, ACN)	–0.3196 (± 0.2164)	–0.1828 (± 0.0298)	3.1903 (± 0.4124)	0.0086 (± 0.0006)	0.9888	0.1373	205	30
log k_w (Discovery Cyano, ACN)	–1.6697 (± 0.1627)	–0.1346 (± 0.0224)	1.5310 (± 0.3101)	0.0095 (± 0.0041)	0.9922	0.1032	292	31
log k_w (Mix-Cholesterol-AP, ACN)	–14724 (± 0.2868)	–0.2112 (± 0.0395)	2.31 (± 0.5465)	0.0115 (± 0.0007)	0.9855	0.1819	157	32

* Numerical values of the retention parameters and structural descriptors of the analytes were taken from Tables IV, V, and VI.
† R, Multiple correlation coefficient.
§ S, Standard error of estimate.
‖ F, Value of the F-test of significance.

retention. Series II of the test analytes (Table II) provided physically interpretable QSRR equations employing simple structural descriptors generated by computational chemistry.

In the third kind of QSRR analysis, the log k_w data (Tables IV and V) were linearly regressed against log P (Table VI) for series III of the test analytes (Table III). Respective QSRR equations (equations 33–46) are presented in Table IX.

Table IX shows that slopes (k''_2) in equations relating log k_w to log P were markedly larger in the case of log k_w data determined with MeOH than with ACN as an eluent modifier, excepting the Discovery Cyano column (equations 38 and 45). This means that there was a stronger dependence of retention on analyte hydrophobicity (log P) in the case of MeOH-type eluents as compared with the ACN-modified eluents. For the Discovery Cyano column, slopes (k''_2) determined in the two eluent systems did not differ significantly.

The k''_2 values in the case of ACN-modified eluents (equations 40–46) were much less than unity. For the MeOH-modified eluents, the k''_2 values were close to unity—closer in the case of the Aluspher column (k''_2 = 0.9873), the Nova-Pak (k''_2 = 0.9774), the Luna (k''_2 = 0.9138), and less close in the case of Mix-Cholesterol-AP (k''_2 = 0.8793) and Discovery Amide (k''_2 = 0.7873). Far from unity were the k''_2 values for Hisep (k''_2 = 0.5171) and Discovery Cyano columns (k''_2 = 0.4737).

This observation is rational in view of the report by Knox and Ross (42). According to these authors, k''_2 or the gradient—d(logk)/d(log P)—reflects the degree to which the analyte is surrounded by the stationary phase. For a bonded stationary phase, this should be somewhat less than for liquid octanol so that the partitioning into the bonded phase is likely to be less with a bonded phase than with octanol, and the gradient is also less. Knox and Ross (42) have concluded that even with pure water eluent, the gradient for C18 silica phases is less than unity. According to this way of thinking, the more similar to octanol the stationary phase (solvated) is, the closer k''_2 should be to 1. The MeOH-solvated C18 silica-phase Nova-Pak and the polybutadiene-encapsulated alumina-phase Aluspher were more similar to octanol than similarly solvated Luna, Mix-

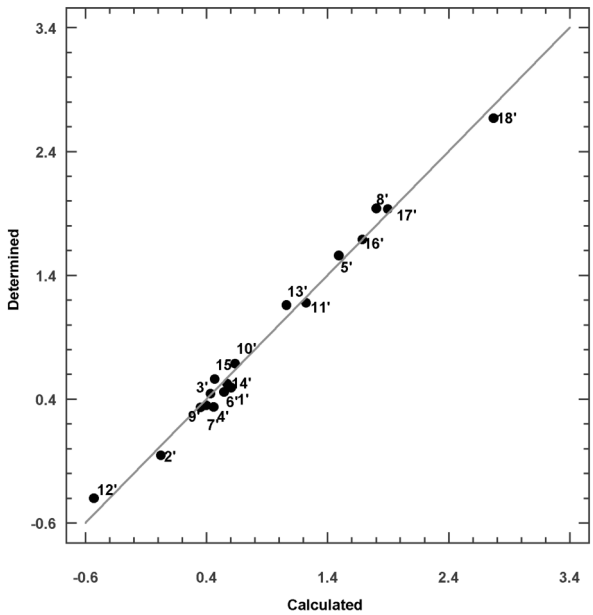

Figure 1. Plot of log k_w determined experimentally (Discovery Cyano column, ACN as organic modifier) for series I of the test analytes from Table I versus those calculated theoretically by equation 17 from Table VII.

Table IX. Coefficients of Equation 4 for Test Series III of Analytes Listed in Table III Relating log k_w to log P*

log k_w	k''_1	k''_2	R†	S§	F‖	Eq. No.
log k_w (Aluspher, MeOH)	–1.1357 (± 0.0971)	0.9655 (± 0.0328)	0.9954	0.1736	867	33
log k_w (Hisep, MeOH)	–1.1659 (± 0.0806)	0.5171 (± 0.0272)	0.9891	0.1442	360	34
log k_w (Nova-Pak, MeOH)	–0.1286 (± 0.1133)	0.9774 (± 0.0383)	0.9939	0.2026	652	35
log k_w (Luna, MeOH)	0.2473 (± 0.0967)	0.9138 (± 0.0326)	0.9949	0.1729	783	36
log k_w (Discovery Amide, MeOH)	–0.0248 (± 0.0699)	0.7873 (± 0.0236)	0.9964	0.1250	1112	37
log k_w (Discovery Cyano, MeOH)	–0.4043 (± 0.0654)	0.4737 (± 0.0221)	0.9914	0.1170	459	38
log k_w (Mix-Cholesterol-AP, MeOH)	–0.4330 (± 0.0832)	0.8702 (± 0.0281)	0.9959	0.1487	960	39
log k_w (Aluspher, ACN)	–0.8958 (± 0.1330)	0.7739 (± 0.0449)	0.9868	0.2377	297	40
log k_w (Hisep, ACN)	–0.1234 (± 0.0737)	0.4759 (± 0.0249)	0.9893	0.1317	366	41
log k_w (Nova-Pak, ACN)	–0.1897 (± 0.0839)	0.7278 (± 0.0283)	0.9940	0.1501	659	42
log k_w (Luna, ACN)	0.1703 (± 0.0804)	0.6164 (± 0.0272)	0.9923	0.1439	514	43
log k_w (Discovery Amide, ACN)	–0.1140 (± 0.0668)	0.6515 (± 0.0225)	0.9952	0.1194	835	44
log k_w (Discovery Cyano, ACN	–0.4489 (± 0.0641)	0.4858 (± 0.0216)	0.9922	0.1145	504	45
log k_w (Mix-Cholesterol-AP, ACN)	–0.3077 (± 0.0648)	0.6613 (± 0.0219)	0.9957	0.1158	914	46

* Numerical values of the retention parameters and log P of the analytes were taken from Tables IV, V, and VI.
† R, Multiple correlation coefficient.
§ S, Standard error of estimate.
‖ F, Value of the F-test of significance.

Cholesterol-AP, and Discovery Amide phases. Hisep and Discovery Cyano phases were evidently dissimilar to *n*-octanol. This is proved by equations 33–39 in Table IX. Clearly, the effect on the partition by polar functional groups of ligands was very strong in the case of Discovery Cyano and Hisep stationary phases. The ACN-solvated stationary phases were less similar to octanol than the MeOH–solvated ones, thus k''_2 values in equations 40–46 were significantly less than 1.

The differences in magnitude of the k''_2 coefficients in equations 33–46 may be explained in view of the observations that organic modifiers differently solvate hydrocarbon-like stationary phases used in RP-HPLC (43,44). It is known that ACN adsorbs more strongly on such phases than low alcohols (45). Because of this, the increase of eluting power of the eluent from the increasing content of ACN (at a fairly constant attraction by the solvated stationary phase) will be less pronounced than the analogous increase of the eluent strength accompanying the increasing MeOH concentrations. This is also why the log k_w data in Table IV that were extrapolated from MeOH–water systems were larger than the respective data from the ACN–water systems collected in Table V.

Equations 33–46 (Table IX) discriminated well the stationary and mobile phases in regards to their effects on analyte retention. The proposed series III of test analytes provided precise QSRR equations with very small standard deviations of regression coefficients. Therefore, it proves to be a proper design of the model series of analytes.

Conclusion

It has been demonstrated that the test series of analytes previously proposed by us (21) are proper for QSRR comparisons of RP-HPLC stationary phases and shed light on the molecular mechanism of retention operating on individual phases. The statistical quality of fit was excellent in the correlating retention data on reversed-phase materials of diverse chemical structure. The present study proves that the QSRR analysis was able to distinguish individual modern analytical columns for RP-HPLC in regards to the prevailing molecular mechanism of retention. Observations on different retention properties of individual stationary phases were confirmed by the statistical significance of the specific structural descriptors present in the QSRR equations of three distinctive types. The described method applies to diverse stationary phases and can be recommended as an objective column testing method. At the same time, the QSRR equations once derived on a given column can be used to predict the retention of any analyte of the known structural descriptors and optimize the separations. The model employing structural parameters from molecular modeling (which may nowadays be readily obtained for any defined analyte) appears especially feasible.

References

1. R. Kaliszan. *Quantitative Structure–Chromatographic Retention Relationships*. John Wiley & Sons, New York, NY, 1987, pp 49–65.
2. R. Kaliszan. Quantitative structure retention relationships. *Anal. Chem.* **64:** 619A–31A (1992).
3. E. Forgács and T. Cserháti. *Molecular Basis of Chromatographic Separation*. CRC Press, Boca Raton, FL, 1997, pp 116–18.
4. R. Kaliszan. *Structure and Retention in Chromatography. A Chemometric Approach*. Harwood, Amsterdam, The Netherlands, 1997, pp 127–47.
5. L. Rohrschneider. Prototypical test substances for a reversed phase in a retention parameter model. *J. High Resol. Chromatogr.* **22:** 1–5 (1999).
6. H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, and B. Buszewski. Comparative study of test methods for reversed-phase columns for HPLC. *J. Chromatogr. A* **826:** 135–56 (1998).
7. A. Sandi, A. Bede, L. Szepesy, and G. Rippel. Characterization of different RP-HPLC columns by a gradient elution technique. *Chromatographia* **45:** 206–214 (1997).
8. D.A. Barret, V.A. Brown, P.N. Shaw, M.C. Davies, H. Ritchie, and P. Ross. Characterization of a range of alkyl-bonded silica HPLC stationary phases: chromatographic behavior of neutral, acidic, and basic test solutes. *J. Chromatogr. Sci.* **34:** 146–56 (1996).
9. E. Cruz, M.R. Euerby, C.A. Johnson, and C.A. Hackett. Chromatographic classification of commercially available reverse-phase HPLC columns. *Chromatographia* **44:** 151–61(1997).
10. P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, and L.C. Tan. Revisionist look at solvophobic driving forces in reversed-phased-phase liquid chromatography. *J. Chromatogr. A* **656:** 113–33 (1993).
11. D.E. Martire and R.E. Boehm. Unified theory of retention and selectivity in liquid chromatography. 2. Reversed-phase liquid chromatography with chemically bonded phases. *J. Phys. Chem.* **87:** 1045–62 (1983).
12. K.A. Dill. The mechanism of solute retention in reversed-phase liquid chromatography. *J. Phys. Chem.* **91:** 1980–88 (1987).
13. M.R. Bohmer, L.K. Koopal, and R. Tijssen. Partition and adsorption of chain molecules at chemically modified surfaces in reversed phase liquid chromatography. *J. Phys. Chem.* **95:** 6285–97 (1991).
14. W.J. Cheong and P.W. Carr. Study of partition models in reversed-phase liquid chromatography based on measured mobile phase solute activity coefficients *J. Chromatogr.* **499:** 373–93 (1990).
15. C.R. Yonker, T.A. Zwier, and M.F. Burke. Comparison of stationary phase formation in RP-18 for methanol-water systems. *J. Chromatogr.* **241:** 257–68 (1982).
16. C.R. Yonker, T.A. Zwier, and M.F. Burke. Investigation of stationary phase formation in RP-18 using various organic modifiers. *J. Chromatogr.* **241:** 269–80 (1982).
17. E.H. Slaats, W. Markowski, J. Fekete, and H. Poppe. Distribution equilibria of solvent components in reversed-phase liquid chromatographic columns and relationship with the mobile phase volume. *J. Chromatogr.* **207:** 299–323 (1981).
18. B. Buszewski, J. Schmid, K. Albert, and E. Bayer. Chemically bonded phases for the reversed-phase high-performance liquid chromatographic separation of basic substances. *J. Chromatogr.* **552:** 415–27 (1991).
19. L.C. Tan, P.W. Car, and M.H. Abraham. Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships. 1. The stationary phase. *J. Chromatogr. A* **752:** 1–18 (1996).
20. M.H. Abraham, M. Ross, C.F. Poole, and S.K. Poole. Hydrogen bonding. 42. Characterization of reversed-phase high performance liquid chromatography C18 stationary phases. *J. Phys. Org. Chem.* **10:** 358–68 (1997).
21. M.A. Al-Haj, R. Kaliszan, and A. Nasal. Test analytes for studies of the molecular mechanism of chromatographic separations by

quantitative structure retention relationships. *Anal. Chem.* **71:** 2976–85 (1999).
22. M.J. Kamlet and R.W. Taft. The solvatochromic comparison method. 1. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *J. Am. Soc. Chem.* **98:** 377–83 (1976).
23. R.W. Taft and M.J. Kamlet. The solvatochromic comparison method. 2. The α-scale of solvent hydrogen-bond donor (HBD) acidities. *J. Am. Chem. Soc.* **98:** 2886–94 (1976).
24. P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, and M.H. Abraham. Study of retention processes in reversed-phase high-performance liquid chromatography. *Anal. Chem.* **57:** 2971–76 (1985).
25. P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander, and C. Horvath. Study of temperature and mobile phase effects in reversed-phase high performance liquid chromatography by use of the solvatochromic comparison method. *Anal. Chem.* **58:** 2674–83 (1986).
26. M.H. Abraham. Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* **22:** 73–83 (1993).
27. M.H. Abraham and J.C. McGowan. The use of characteristic volumes to measure cavity terms in reversed phase liquid chromatography. *Chromatographia* **23:** 243–46 (1987).
28. K. Valko, M. Plass, C. Bevan, D. Reynolds, and M.H. Abraham. Relationships between the chromatographic hydrophobicity indices and solute descriptors obtained by using several reversed-phase, diol nitrile, cyclodextrin and immobilised artificial membrane-bonded high-performance liquid chromatography columns. *J. Chromatogr. A* **797:** 41–55 (1998).
29. M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kaliszan, A. Nasal, and P. Haber. Determination of solute lipophilicity, as log P (octanol) and log P (alkane), using poly(styrene-divinylbenzene) and immobilized artificial membrane stationary phases in reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **766:** 35–47 (1997).
30. P.T. Jackson, M.R. Schure, T.P. Weber, and P.W. Carr. Intermolecular interactions involved in solute retention on carbon media in reversed-phase high-performance liquid chromatography. *Anal. Chem.* **69:** 416–25 (1997).
31. J.H. Park, M.H. Yoon, J.W. Ryu, and M.D. Jang. Characterization of some normal-phase liquid chromatographic stationary phases based on linear solvation energy relationships. *J. Chromatogr. A* **796:** 249–58 (1998).
32. L.C. Tan and P.W. Carr. Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships. II. The mobile phase. *J. Chromatogr. A* **799:** 1–19 (1998).
33. M.H. Abraham. University College London DataBase, 1997.
34. H. Engelhardt, M. Arangio, and T. Lobert. A chromatographic test procedure for reversed-phase HPLC column evaluation. *LC-GC Int.* **15:** 856–65 (1997).
35. C. Hansch, A. Leo, and D. Hoekman. *Exploring QSAR. Hydrophobic, Electronic, and Steric Constants.* ACS, Washington, DC, 1995, pp 3–193.
36. T.F. Woloszyn and P.C. Jurs. Prediction of gas chromatographic retention data for hydrocarbons from naphthas. *Anal. Chem.* **65:** 582–87 (1993).
37. L.R. Snyder, J.J. Kirkland, and J.L. Glajch. *Practical HPLC Method Development,* 2nd ed. John Wiley & Sons, New York, NY, 1997, pp 236–42.
38. T. Dzido and H. Engelhardt. Retention parameters of aromatic hydrocarbons with mono-substiuted polar groups in binary reversed phase high-performance liquid chromatography systems. *Chromatographia* **39:** 51–61 (1994).
39. B. Feibush and C. Santasania. Hydrophillic shielding of hydrophobic, cation and anion-exchange phases for separation of small analytes: direct injection of biological fluids onto high-performance liquid chromatographic columns. *J. Chromatogr.* **544:** 41–49 (1991).
40. *Information About the Unique Hisep Qualities and Capabilities.* Supelco, Bellefonte, PA. Publication 609005.
41. R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, and H.A. Claessens. Molecular mechanism of retention in reversed-phase high-performance liquid chromatography and classification of modern stationary phase by using quantitative structure-retention relationships. *J. Chromatogr. A* **855:** 455–86 (1999).
42. J.H. Knox and P. Ross. Carbon-based packing material for liquid chromatography: application. *Adv. Chromatogr.* **37:** 73–119 (1997).
43. R.S. Helburn, S.C. Rutan, J. Pompano, D. Mitchem, and W.T. Patterson. Solvatochromic studies of solvated chromatographic stationary phases. *Anal. Chem.* **66:** 610–18 (1994).
44. R.M. McCormick and B.L. Karger. Distribution phenomena of mobile phase components and determination of dead volume in reversed-phase liquid chromatography. *Anal. Chem.* **52:** 2249–57 (1980).
45. J.H. Knox and R. Kaliszan. Theory of solvent disturbance peaks and experimental determination of thermodynamic dead-volume in column liquid chromatography. *J. Chromatogr.* **349:** 211–34 (1985).

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