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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

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Abstract

The solvation equation [Eq. (1)] can be applied to reversed-phase HPLC capacity factors (i.e. $\log SP = \log k$).

$$\log SP = c + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_xS. \quad (1)$$

SP is a solute property (e.g., solubility or partition coefficient) and the explanatory variables are solute descriptors as follows: R_2 is an excess molar refraction, π_2^H is the solute dipolarity/dipolarisability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the solute overall or effective hydrogen-bond acidity and basicity, and V_x is the McGowan characteristic volume and c, r, s, a, b and v are constants that are characteristic of the particular mobile/stationary phase combination. Only a limited hydrophobicity range can be covered using $\log k$ values obtained by isocratic chromatography at a fixed mobile phase composition. Gradient elution is more versatile and 29 compounds were examined under 20 different reversed-phase HPLC conditions using automated fast gradient methods. Using Eq. (1) with gradient retention time (t_{R_g}) in place of $\log SP$ an excellent correlation was obtained with the solute descriptors. This provides an experimental demonstration that gradient retention times (t_{R_g}) can be treated as linear free-energy related parameters just like $\log k$ or $\log P$ values. Because gradient retention times cannot be used for inter-laboratory comparisons, they were scaled by conversion to chromatographic hydrophobicity index (CHI) values by correlation with data from a calibration set of compounds with known CHI values. By comparing the coefficients r, s, a, b and v of Eq. (1) as determined from different chromatographic systems, the relative effects of different solute properties can be revealed and thus the selectivity of HPLC columns can be predicted. It is also possible to compare chromatographic behaviour with that of other partition systems, such as octanol–water, cyclohexane–water and blood–brain barrier distributions. © 1998 Elsevier Science B.V.

Keywords: Hydrophobicity index; Immobilised artificial membranes; Stationary phases, LC; Solute descriptors; Principal component analysis; Chemometrics

1. Introduction

The importance of hydrophobicity/lipophilicity of

molecules, as a complex physico-chemical property, has long been recognised in drug action (absorption, blood–brain distribution, drug-receptor interaction, etc.) [1–4]. It is usually measured by determining the equilibrium concentration of the compound in two

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immiscible liquids and expressed as the logarithm of the partition coefficient. The octanol–water partition coefficients ($\log P$) are the most widely used measures of hydrophobicity [5], because it has been shown that this partition system is a good model for many biological partition processes [6]. Not only is a large data base available [5], but several calculation methods [7–10] provide a valuable help for $\log P$ prediction. Collander [11] first demonstrated linear relationships between the logarithmic values of partition coefficients measured in two different partitioning liquid systems and he showed the limitations as well. Later Leo [12] demonstrated by using data from 300 compounds in 12 partition systems that the correlations between different systems are very good only for structurally related compounds or when the partitioning solvents are similar in character. It is important to find a method for comparison of the various partition systems in order to find the best and most general model for particular types of biological partitioning.

Several years ago, Kamlet et al. [13,14] set out linear free-energy relationships between solute properties and solute descriptors based on solvatochromic measurements. The polarisability correction term, dipolarity/polarisability, hydrogen-bond acidity and basicity and a volume term [15] were used as solute descriptors. Abraham [16] modified these solute descriptors and derived them from solute properties as is shown by Eq. (1):

$$\log SP = c + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_x$$

where R_2 is an excess molar refraction, π_2^H is the solute dipolarity/polarisability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the solute overall or effective hydrogen-bond acidity and basicity, and V_x is the McGowan characteristic volume [17]. Eq. (1) has been applied to model partition using $\log P$ as dependent variable obtained in numerous water–organic solvent systems [18]. The solute factors affecting certain partitions can be revealed and the various partition systems can be compared [19,20] by comparing the regression coefficients.

In reversed-phase high-performance liquid chromatography (RP-HPLC) the retention factor of compounds ($\log k$) is related to the logarithm of the distribution coefficient of compound between the

mobile and the stationary phase and as such can be used as a measure of hydrophobicity [21–24]. Chromatographic experiments have a lot of practical advantages over the direct determination of partition coefficients, i.e. small amounts of material are required, impurities can be separated during the measurements, there is no need for concentration determination, and the process can be easily automated. However, there are certain limitations using $\log k$ instead of \log of the partition coefficients. Only a limited hydrophobicity range can be covered using $\log k$ values obtained from using isocratic chromatography at a fixed mobile phase composition. To cover a wider range of hydrophobicity it is necessary to determine $\log k$ values at several mobile phase compositions and extrapolate back to pure aqueous mobile phase ($\log k_0$) [25]. It has been shown that the correlation is weak between $\log P$ values and the $\log k_0$ values, when structurally unrelated compounds are investigated [26]. The correlation could be improved by using in addition the slope value (S) of the $\log k$ versus organic phase concentration plots [27]. Another approach was recently introduced [28] to overcome the low correlation between the chromatographic retention data and $\log P$ values when diverse sets of compounds are considered. The φ_0 values of a compound was defined as the percentage (by volume) of organic phase concentration required to achieve an equal distribution of compounds between the mobile and stationary phase, that is φ_0 is the percentage mobile phase composition when $\log k$ for a compound is zero. The φ_0 is an index (with physical meaning in the range 0 to 100) that characterises the compound hydrophobicity. Higher organic phase concentration is needed for the more hydrophobic compounds to achieve the equal distribution. A good correlation of φ_0 to the $\log P$ values was shown for almost 500 drug molecules [28]. A chromatographic hydrophobicity index (CHI) has been recently introduced [29] which showed excellent correlation to φ_0 . However, CHI can be obtained from a fast gradient chromatographic run which takes less than 10 min per sample. In this approach the retention time is directly related to an organic phase concentration when the compound is eluting from the column. In a recent report other authors [30] have independently demonstrated linear relationships between gradient elution parameters

and isocratic $\log k$ values for several compound series, and have shown that the observed relationship is derivable by theoretical considerations. The CHI values can be regarded as a high throughput alternative to the $\log k_0$ or φ_0 determination.

RP-HPLC $\log k$ values have previously been correlated with the solvation parameters [19,20]. Eq. (1) was successfully applied to $\log k$ values on various C_{18} columns with methanol–water, acetonitrile–water and tetrahydrofuran–water buffered mobile phases. Comparisons have been made between $\log k$ values and octanol–water partition coefficients.

In this paper we wanted to establish that the gradient retention times, and consequently the CHI parameters, would show a good correlation with the solute descriptors, in order to demonstrate that CHI values can be treated as linear free-energy related parameters just like the $\log P$, or $\log k$ values. Utilising a fast experimental procedure, the CHI

parameter has been measured in 20 different chromatographic systems for each of a diverse set of 29 compounds. This enables us to compare the various chromatographic columns and partitions, and by comparing the regression coefficients with those from other partition systems we can select the best for modelling biological partition processes.

2. Materials and methods

A Hewlett-Packard 1090 series high-performance liquid chromatograph was used. Data acquisition and processing were performed on a Viglen IBM compatible PC with HP Chemstation software (Hewlett-Packard, Amsterdam, Netherlands). The applied chromatographic columns are shown in Table 1.

The first seven columns were packed with octadecyl bonded silica stationary phases, each having

Table 1
The HPLC columns used in this study

Name	Abbreviated name	Particle size	Column dimension	Supplier
1. ODS2-IK5 Inertsil	IN	5 μm	150 \times 4.6 mm	A
2. Symmetry C_{18}	SY	3.5 μm	50 \times 4.6 mm	B
3. NovaPak RP	NRP	4 μm	75 \times 3.9 mm	B
4. Supelcosil ABZ	ABZ	3 μm	33 \times 4.6 mm	C
5. Selectosil RP	SRP	5 μm	150 \times 4.6 mm	D
6. Prodigy ODS2	PRO	5 μm	150 \times 4.6 mm	D
7. Spherisorb ODS1	OD1	5 μm	150 \times 4.6 mm	E
8. Unisphere- C_{18}	BRP	8 μm	100 \times 4.6 mm	F
9. Unisphere PBD	BPB	8 μm	100 \times 4.6 mm	F
10. Asahipak ODP	APO	5 μm	150 \times 4.6 mm	D
11. RPS40	POL	8 μm	50 \times 4.6 mm	G
12. Novapak Phenyl	NPH	4 μm	75 \times 3.9 mm	B
13. Novapak-CN	NCN	4 μm	75 \times 3.9 mm	B
14. Diol-YC5 Inertsil	Indiol	5 μm	150 \times 4.6 mm	A
15. Selectosil-diol	Sdiol	5 μm	150 \times 4.6 mm	D
16. RexChrom IAM PC2	IAM	12 μm	150 \times 4.6 mm	H
17. Nucleosil NH_2	NH2	5 μm	150 \times 4.6 mm	F
18. Nucleodex β PM	CD	5 μm	200 \times 4.6 mm	H

Suppliers:

A: Capital HPLC, Broxburn, UK.

B: Waters Chromatography, Watford, UK.

C: Supelco UK, UK.

D: Phenomenex UK, Macclesfield, UK.

E: Phase Separations, Deeside, UK.

F: Biotage UK, Division of Dyax, Hertford, UK.

G: Polymer Laboratories, Separation Science Division, Stratton, UK.

H: Fisher Scientific UK, Loughborough, UK.

different endcapping and carbon content. The Unisphere columns contain polymer coated alumina-based stationary phases, while the RPS40 is a polymer-based stationary phase. The IAM PC2 column represents a special stationary phase developed to mimic biological membrane partitioning [31] with chemically bonded phospholipid molecules on its surface. The permethylated β -cyclodextrin column was selected to represent a particular shape and size-specific interaction with the solutes.

The mobile phase was 50 mM aqueous ammonium acetate obtained from Fisons (Loughborough, UK). The pH was adjusted with concentrated ammonia solution to pH 7.4. The mobile phase flow rate was 1.5 ml/min. HPLC-grade acetonitrile (ACN) (Rathburn, Walkerburn, UK) was used as organic modifier. For the fast gradient retention time measurements the following gradient program was applied:

0–0.5 min	0% acetonitrile
0.5–4.0 min	0–100% acetonitrile
4.0–5.0 min	100% acetonitrile
5.0–5.2 min	100–0% acetonitrile
5.2–7.5 min	0% acetonitrile

On the Inertsil ODS2 stationary phase, 15 mM phosphate buffer (pH 7.4) was also used with acetonitrile as organic modifier. On the same column CHI values obtained by methanol gradient and the ammonium acetate buffer was also measured, in order to examine the effect of these mobile phase additives.

A shorter Inertsil ODS2 column (50×4.6 mm) with various gradient profiles has also been tested to check the reproducibility of the CHI determination using the same stationary phase. Table 2 shows the

various conditions under which the gradient retention time measurements were repeated.

The standard test mixture used for calibrating columns contained theophylline, paracetamol, acetanilide, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. The compounds were dissolved in acetonitrile–ammonium acetate buffer (50:50, v/v) in 1 mg/ml concentration and used for the quality control of the HPLC system. The φ_0 values of these standard compounds were determined by measuring their $\log k$ values at 3 to 5 different organic phase concentrations (φ). By plotting the $\log k$ as a function of φ , the slope and intercept values of the straight lines were calculated. The φ_0 values were obtained as $-\text{intercept}/\text{slope}$ [28]. The gradient retention times (t_R) were plotted against the isocratically determined φ_0 values (Table 3) of the standard compounds providing the constants [29] in the relation of $\varphi_0 = At_R + B$. The A and B constants (which are dependent on the flow rate, column length, gradient time, column, etc.) were used then to convert the measured gradient retention time values to the CHI values as $\text{CHI} = At_R + B$ for the compounds measured under the same conditions as the test mixture. In this manner we calibrate the actual gradient retention times and convert them to CHI values.

The 29 compounds investigated in this study (shown in Table 4) were all commercially available and were selected to represent a wide variety of solute properties. Table 4 shows the values of the molecular descriptors applied in Eq. (1) by Abraham [15]. They were dissolved in acetonitrile–50 mM ammonium acetate (pH 7.4) buffer (50:50, v/v) at 1 mg/ml concentration. The solutions (3 μ l) were

Table 2
The various conditions used with Inertsil ODS2 columns for investigating the reproducibility of CHI determination

Serial No	Column dimensions	Flow rate	Gradient time (min) 0–100%	Buffer A	Organic phase B
1	150×4.6 mm	1.5 ml/min	3.5	NH ₄ OAc	ACN
2	150×4.6 mm	1.5 ml/min	3.5	Na ₂ HPO ₄ pH 7.4	ACN
3	50×4.6 mm	1.5 ml/min	3.5	NH ₄ OAc	ACN
4	50×4.6 mm	1.5 ml/min	9.0	NH ₄ OAc	ACN
5	50×4.6 mm	1.0 ml/min	9.0	NH ₄ OAc	ACN
6	50×4.6 mm	1.0 ml/min	9.0	H ₃ PO ₄ pH 2	ACN

Table 3

The isocratically determined φ_0 values and regression constants from the relation $\varphi_0 = At_r + B$ that were used for the calibration of the HPLC gradient systems for the CHI determination

Column No.	1.	1.	6.	18.	16.	13.	14.	17.
Abbreviation	IN	IN	PRO	CD	IAM	NCN	Indiol	NH ₂
Eluent	MeCN	MeOH	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN
Constant <i>A</i>	35.80	34.165	29.60	34.60	30.14	23.31	47.56	37.55
Constant <i>B</i>	−86.51	−87.80	−91.60	−90.43	−80.14	−21.61	−122.56	−69.55
Compound	φ_0	φ_0	φ_0	φ_0	φ_0	φ_0	φ_0	φ_0
Octanophenone	99.28	96.11	99.70	70.53	49.4	46.11	40.2	48.50
Heptanophenone	94.67	93.95	95.70	67.54	45.7	44.12	36.6	37.61
Hexanophenone	89.86	91.32	91.20	64.22	41.8	42.16	32.3	29.61
Valerophenone	85.00	87.53	86.70	60.42	37.3	38.53	27.7	22.67
Butyrophenone	79.24	83.53	81.25	56.67	32.00	32.79	22.0	18.27
Propiophenone	72.31	78.29	74.40	53.40	25.9	25.48	15.5	12.56
Acetophenone	61.33	69.17	64.00	46.91	17.2	15.36	8.0	1.04
Acetanilide	37.94	54.50	42.45	32.75	11.5	4.72	−0.59	−0.41
Paracetamol	−1.93	40.80	6.26	11.84	2.9	−1.85	−	−

Table 4

The investigated compounds and their solvation parameters obtained from Ref. [16]

Name	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_s
Paracetamol	1.06	1.78	1.09	0.81	1.172
Acetophenone	0.82	1.01	0.00	0.48	1.014
Propiophenone	0.80	0.95	0.00	0.51	1.155
Butyrophenone	0.80	0.95	0.00	0.51	1.296
Valerophenone	0.80	0.95	0.00	0.50	1.437
Hexanophenone	0.72	0.95	0.00	0.50	1.578
Heptanophenone	0.72	0.95	0.00	0.50	1.718
Octanophenone	0.72	0.95	0.00	0.50	1.859
4-Iodophenol	1.38	1.22	0.68	0.20	1.033
Dibenzothiophene	1.96	1.31	0.00	0.20	1.379
4-Chlorophenol	0.92	1.08	0.67	0.20	0.898
4-CN-Phenol	0.94	1.63	0.80	0.29	0.930
Benzamide	0.99	1.5	0.49	0.67	0.973
Caffeine	1.50	1.6	0.00	1.33	1.363
Indazole	1.20	1.22	0.53	0.35	0.905
Anisole	0.71	0.75	0.00	0.29	0.916
Benzonitrile	0.74	1.11	0.00	0.33	0.871
Chlorobenzene	0.72	0.65	0.00	0.07	0.839
Naphthalene	1.34	0.92	0.00	0.20	1.085
Dinitrobenzene	1.13	1.63	0.00	0.46	1.065
Phenol	0.81	0.89	0.60	0.30	0.775
Trifluoromethyl-phenol	0.43	0.87	0.72	0.09	0.969
Toluene	0.60	0.52	0.00	0.14	0.857
Corticosterone	1.86	3.43	0.40	1.63	2.739
Aniline	0.96	0.96	0.26	0.41	0.816
Testosterone	1.54	2.59	0.32	1.19	2.383
Hydrocortisone-21-acetate	1.89	3.67	0.43	1.90	3.095
<i>p</i> -Toluidine	0.92	0.95	0.23	0.45	0.957
<i>m</i> -Nitroaniline	1.20	1.71	0.40	0.35	0.990

injected onto the HPLC system twice. The average gradient retention time was used for the CHI calculations.

The multiple regression analysis and the principal component analysis have been carried out using the Drugidea software package (Chemicro, Budapest, Hungary).

3. Results and discussion

3.1. Reproducibility of CHI measurements

Table 5 shows the measured retention times and the calculated CHI values obtained under the conditions listed in Table 2 on Inertsil ODS2 stationary phases. It can be seen from Table 5 that although gradient retention times for a single stationary phase are greatly dependent on the gradient speed, column dimensions and flow rate, CHI values are very similar. The average standard deviation of CHI values for the 29 compounds was less than 2 CHI units. Weakly basic compounds showed the highest standard deviations. The gradient retention times obtained on the other columns were converted to CHI values by using the corresponding data of the standard set of compounds shown in Table 3. The φ_0 values obtained on Inertsil ODS column were used to standardise the other reversed-phase systems. Tables

Table 5

Gradient retention times (t_R) and CHI values obtained on Inertsil ODS2 stationary phases under conditions listed in Table 2

Name	$t_R(1)$	CHI(1)	$t_R(2)$	CHI(2)	$t_R(3)$	CHI(3)	$t_R(4)$	CHI(4)	$t_R(5)$	CHI(5)	$t_R(6)$	CHI(6)	Mean CHI	S.D.
Paracetamol	2.70	10.1	2.84	10.1	1.82	12.0	3.16	15.0	3.71	13.6	3.69	12.0	12.13	1.92
Acetophenone	3.90	53.0	4.08	53.2	2.92	52.5	5.54	50.0	6.29	50.9	6.26	52.5	52.02	1.27
Propiophenone	4.24	65.4	4.42	65.0	3.28	65.5	6.49	64.0	7.24	64.7	7.20	65.5	65.00	0.59
Butyrophenone	4.51	74.8	4.68	74.0	3.54	75.0	7.18	74.3	7.92	74.6	7.90	75.0	74.59	0.39
Valerophenone	4.75	83.4	4.93	82.7	3.78	83.7	7.81	83.5	8.54	83.6	8.52	83.7	83.43	0.39
Hexanophenone	4.97	91.4	5.17	91.0	4.00	91.9	8.38	91.9	9.11	91.8	9.10	91.9	91.64	0.36
Heptanophenone	5.18	98.9	5.40	99.0	4.20	99.3	8.91	99.7	9.65	99.6	9.64	99.3	99.31	0.34
Octanophenone	5.40	107.0	5.66	108.0	4.39	106.0	9.41	107.1	10.14	106.7	10.13	106.0	106.82	0.75
4-Iodophenol	4.10	60.4	4.24	58.6	3.18	62.0	6.40	62.8	7.08	62.4	7.10	62.0	61.35	1.58
Dibenzothiophene	5.25	101.5	5.43	100.0	4.16	97.8	8.75	97.4	9.51	97.7	9.52	97.7	98.69	1.70
4-Chlorophenol	3.90	53.1	4.05	52.1	2.99	54.9	5.85	54.6	6.56	54.8	6.56	54.9	54.07	1.18
4-CN-Phenol	3.37	34.1	3.51	33.4	2.49	36.7	4.54	35.3	5.12	34.0	5.32	36.7	35.02	1.44
Benzamide	3.08	23.8	3.20	22.5	2.04	20.4	3.34	17.6	4.24	21.2	4.20	20.4	20.97	2.10
Caffeine	2.98	20.1	3.02	16.3	1.96	17.3	3.61	21.5	4.12	19.5	4.12	17.3	18.68	2.00
Indazole	3.63	43.3	3.70	40.1	2.58	40.0	4.91	40.7	5.57	40.5	5.51	40.0	40.75	1.30
Anisole	4.29	67.1	4.27	59.8	3.29	65.8	6.48	63.9	7.26	65.0	7.23	65.8	64.59	2.59
Benzonitrile	3.97	55.5	4.14	55.2	2.96	53.7	5.59	50.8	6.40	52.5	6.35	53.7	53.58	1.75
Chlorobenzene	4.64	79.5	4.83	79.2	3.69	80.7	7.33	76.4	8.10	77.2	8.10	77.6	78.43	1.62
Naphthalene	4.82	86.0	5.01	85.5	3.78	83.8	7.83	83.8	8.58	84.1	8.57	84.2	84.56	0.95
DiNitrobenzene	4.06	58.8	4.23	58.4	3.13	60.0	6.12	58.6	6.88	59.5	6.86	60.0	59.19	0.71
Phenol	3.48	38.2	3.71	40.3	2.52	37.9	4.51	34.9	5.29	36.4	5.24	37.9	37.60	1.83
Trifluoromethylphenol	4.05	58.6	4.23	58.4	3.18	62.0	6.45	63.5	7.13	63.1	7.14	62.0	61.27	2.22
Toluene	4.63	79.2	4.83	79.2	3.52	74.3	7.82	83.7	8.02	76.0	8.14	78.1	78.42	3.23
Corticosterone	3.91	53.5	3.96	49.0	2.86	50.2	5.81	54.0	6.41	52.7	6.41	50.2	51.59	2.07
Aniline	3.49	38.4	3.68	39.4	2.40	33.2	3.99	27.2	4.82	29.7	4.93	33.2	33.52	4.75
Testosterone	4.36	69.6	4.37	63.2	3.18	61.8	6.47	63.7	7.12	62.9	7.13	61.8	63.83	2.92
Hydrocortisone-21-acetate	3.97	55.6	4.09	53.6	3.03	56.4	6.18	59.5	6.80	58.4	6.79	56.4	56.63	2.09
<i>p</i> -Toluidine	3.79	49.2	3.95	48.6	2.76	46.5	5.11	43.7	5.88	45.0	5.66	46.5	46.59	2.08
<i>m</i> -Nitroaniline	3.71	46.2	3.94	48.2	2.78	47.3	5.12	43.9	5.95	45.9	5.24	47.3	46.46	1.51
	Mean standard deviation:													1.62

6 and 7 contains the CHI values of the 29 compounds on the investigated stationary phases. The correlations between CHI values on different reversed-phase type of columns were always high ($r \geq 0.991$ with a $0.917 < \text{slope} < 1.012$). The Supelcosil ABZ+ column represented slightly different selectivity. As an example, Fig. 1a shows the plot of the CHI values obtained on Prodigy ODS3 column as a function of CHI values on Inertsil ODS2. There is a good linear correlation demonstrating that there is little difference in selectivity for all the compounds examined. In Fig. 1b the CHI values for Supelcosil ABZ+ and Inertsil ODS2 columns are compared and it is apparent that in this case although there is still a good linear correlation, there are also selectivity differences for some compounds as manifested by deviations from the regression line. In

contrast when columns of different types are compared then good linear correlations are not observed. For example, in Fig. 2 the CHI values obtained on Inertsil ODS2 and IAM (immobilised artificial membrane) and on PC-CD (permethylated cyclodextrin) are compared. It can be seen that some of the compounds have much higher retention to these specific columns than would be expected from their retention on the ODS type of column. The reasons for this become clear when the data is analysed using the solvation equation [Eq. (1)] and an explanation will be presented later.

All the CHI values obtained on the ODS type columns are tabulated in Table 6 and on other columns in Table 7. The CHI values were obtained from the equation $\text{CHI} = A t_R + B$, where the constants A and B are derived from the calibration set

Table 6
CHI values obtained on the ODS type of columns^a

Column No.	CHI _{In}	CHI _{In MeOH}	CHI _{In Phosp}	CHI _{Sy}	CHI _{NRP}	CHI _{ABZ}	CHI _{SRP}	CHI _{PRO}	CHI _{OD1}	CHI _{BRP}
Abbreviation	IN	IN	IN	Sy	NRP	ABZ	SRP	PRO	OD1	BRP
Eluent	MeCN	MeOH	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN
Buffer	NH ₄ Ac	NH ₄ Ac	Na ₂ HPO ₄	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac
Paracetamol	10.1	40.8	10.1	25.7	23.5	26.6	24.0	20.8	23.2	22.7
Acetophenone	53.0	68.3	53.2	61.4	61.3	59.2	62.6	55.8	63.4	58.7
Propiophenone	65.4	75.7	65.0	72.9	72.5	72.6	73.7	67.1	73.8	71.8
Butyrophenone	74.8	80.7	74.0	81.4	81.2	82.2	81.7	75.9	81.6	80.9
Valerophenone	83.4	85.1	82.7	89.1	89.2	89.3	89.1	83.6	88.9	88.4
Hexanophenone	91.4	89.7	91.0	96.5	96.2	96.7	95.8	91.3	95.8	96.2
Heptanophenone	98.8	95.4	99.0	103.1	103.3	103.0	102.2	99.9	102.3	103.5
Octanophenone	107.0	100.5	108.0	109.0	109.4	108.9	109.9	109.4	108.5	110.7
4-Iodophenol	60.4	78.1	58.6	68.7	64.4	80.6	66.6	60.7	66.5	82.3
Dibenzothiophene	101.5	103.7	100.1	102.0	101.0	104.7	103.6	101.6	99.9	113.7
4-Chlorophenol	53.1	71.8	52.1	63.3	62.0	73.6	60.2	54.6	60.3	72.7
4-CN-Phenol	34.1	58.4	33.4	44.2	43.1	50.0	42.5	41.7	44.3	52.5
Benzamide	23.8	50.2	22.5	33.5	33.7	30.5	32.1	28.2	34.2	33.8
Caffeine	20.1	50.4	16.3	30.4	31.9	31.0	27.1	22.2	36.7	29.7
Indazole	43.3	65.3	40.1	50.5	50.4	54.4	50.0	42.4	53.9	58.3
Anisole	67.1	77.7	52.8	73.3	71.0	71.0	73.1	67.8	70.8	74.2
Benzonitrile	55.5	65.4	55.2	62.4	59.9	59.9	63.2	56.5	62.7	58.6
Chlorobenzene	79.5	84.0	79.2	84.1	81.1	83.4	84.4	79.6	80.8	90.7
Naphthalene	86.0	89.5	85.5	89.9	87.3	90.5	90.2	84.5	87.6	97.1
Dinitrobenzene	58.8	68.0	58.4	67.9	64.6	67.4	66.0	58.7	66.0	66.0
Phenol	38.2	60.0	40.3	48.6	44.4	50.0	48.2	43.5	45.0	50.0
Trifluoromethylphenol	58.6	74.3	58.4	69.4	67.4	78.8	65.1	59.0	65.1	73.2
Toluene	79.2	84.8	79.2	84.3	82.4	81.8	84.2	80.0	79.6	89.7
Corticosterone	53.5	75.6	49.0	59.9	60.4	66.0	59.4	50.2	75.2	68.5
Aniline	38.4	55.0	39.4	44.8	42.7	34.8	49.1	43.2	47.6	40.7
Testosterone	69.6	82.8	63.2	69.8	73.6	79.4	80.2	64.6	103.9	75.4
Hydrocortisone-21-acetate	55.6	75.5	53.6	64.7	65.8	72.0	63.3	54.0	69.5	67.6
<i>p</i> -Toluidine	49.2	64.6	48.6	55.9	55.1	53.5	58.4	50.9	60.5	54.9
<i>m</i> -Nitroaniline	46.2	61.1	48.2	56.6	54.3	58.0	56.9	49.8	57.2	58.1

^a Sometimes CHI values are higher than 100% which arises when compounds having longer retention than $\log k' = 0$ with 100% organic phase and sometimes they have negative values which arises when compounds have a shorter retention than $\log k' = 0$ with 0% organic phase.

(as described in the Section 2). The CHI is useful for two reasons: (1) CHI values provide a consistent approach to the scaling of gradient retention time so that, as long as the phase components are the same, direct comparison is possible even between different investigators using columns of different dimensions and different gradient rates, (2) CHI values approximate to the solvent strength φ (expressed as % by volume of organic solvent in the eluent) at which the isocratic $\log k = 0$ (i.e. for which retention time is double the dead time). If compounds are very hydrophilic (i.e. $\log k < 0$ when $\varphi = 0$) then CHI values can be negative and if compounds are very

lipophilic (i.e. $\log k > 0$ when $\varphi = 100\%$) then CHI values can exceed 100.

3.2. Principal components analysis

The CHI values obtained on the investigated column systems were subjected to principal component analysis of the correlation matrix. The explained variance is shown in Table 8. It can be seen that the first principal component explains 89% of the total variance. This indicates a high degree of similarity underlying the retention mechanisms which operate in all reversed-phase chromatography

Table 7
The CHI values of the 29 model compounds obtained on various stationary phases^a

Column No.	CHI _{PBD}	CHI _{APO}	CHI _{POL}	CHI _{NPH}	CHI _{NCN}	CHI _{Indiol}	CHI _{Sdiol}	CHI _{IAM}	CHI _{NH2}	CHI _{CD}
Abbreviation	PBD	APO	POL	NPH	NCN	Indiol	Sdiol	IAM	NH2	CD
Eluent	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN	MeCN
Buffer	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac	NH ₄ Ac
Paracetamol	23.0	23.1	17.4	23.9	-2.5	-24.9	-22.7	1.4	-9.3	15.7
Acetophenone	59.7	64.6	72.7	63.2	15.7	8.0	8.1	18.3	1.0	44.2
Propiophenone	73.9	75.2	74.9	75.0	23.6	15.5	15.4	26.6	12.6	51.8
Butyrophenone	82.8	82.2	82.5	83.4	31.3	22.0	20.9	32.2	18.3	55.7
Valerophenone	90.0	89.0	88.7	90.3	37.6	27.7	26.9	37.1	22.7	59.8
Hexanophenone	96.6	95.6	94.1	96.5	42.0	32.3	32.6	41.4	29.6	64.5
Heptanophenone	102.6	102.1	99.1	101.7	45.2	36.6	37.4	45.4	37.6	68.9
Octanophenone	108.5	108.4	105.7	106.4	47.4	40.2	41.4	49.2	48.5	73.2
4-Iodophenol	83.2	86.3	73.7	73.1	21.3	16.4	12.2	45.5	1.3	70.8
Dibenzothiophene	113.2	105.6	115.9	100.9	46.2	36.7	49.3	55.4	44.2	77.9
4-Chlorophenol	73.6	75.5	66.8	65.8	9.2	5.6	-4.3	38.9	-2.2	59.5
4-CN-Phenol	52.0	54.0	54.0	45.8	0.1	-31.0	-25.1	26.6	12.5	45.1
Benzamide	31.2	31.1	42.3	32.9	-1.5	-10.8	-9.4	1.9	-8.5	24.0
Caffeine	29.9	21.2	37.7	35.1	8.2	-3.7	-26.4	-4.5	-17.6	7.0
Indazole	60.0	58.8	58.3	53.5	13.5	12.7	13.5	24.1	2.9	49.4
Anisole	77.7	79.2	85.1	73.7	8.8	7.3	-2.8	27.2	-7.1	56.0
Benzonitrile	59.5	67.9	74.0	65.2	4.5	0.3	-5.0	19.6	-9.3	47.6
Chlorobenzene	92.8	93.0	94.7	83.3	15.6	20.6	4.7	37.0	-2.6	67.8
Naphthalene	98.6	102.9	103.2	90.4	36.2	29.0	31.9	42.8	25.9	68.0
Dinitrobenzene	66.3	76.9	78.6	76.9	4.6	-4.9	-27.1	23.8	-12.8	54.2
Phenol	47.0	59.5	56.06	45.4	-4.3	-21.6	-31.3	21.7	-9.9	47.6
Trifluoromethylphenol	75.0	72.5	67.4	72.3	18.4	8.5	-10.2	38.4	-3.7	56.1
Toluene	91.3	89.2	92.9	81.9	9.4	14.1	-7.6	34.1	-6.4	64.0
Corticosterone	71.3	53.8	53.8	69.9	32.7	16.6	10.1	30.9	30.1	39.6
Aniline	34.4	59.1	60.4	42.0	-4.7	-20.9	-26.7	7.6	-11.1	33.5
Testosterone	76.7	68.7	60.2	78.9	36.7	21.9	24.8	39.4	33.6	58.4
Hydrocortisone-21-acetate	69.3	58.8	56.6	73.3	34.6	18.6	9.5	28.7	30.4	43.8
<i>p</i> -Toluidine	53.4	64.5	66.5	56.1	1.8	-1.8	-6.9	19.5	-6.9	43.9
<i>m</i> -Nitroaniline	57.6	74.1	67.6	60.6	4.1	-5.2	-8.7	25.2	-9.9	48.6

^a Sometimes CHI values are higher than 100% which arises when compounds having longer retention than $\log k' = 0$ with 100% organic phase and sometimes they have negative values which arises when compounds have a shorter retention than $\log k' = 0$ with 0% organic phase.

columns. In effect the CHI value from each column provides a slightly different measure of hydrophobicity as a consequence of differing contributions from the same types of intermolecular interactions (e.g., dispersion forces, dipole–dipole and dipole–induced dipole, hydrogen bonding). Application of the solvation equation [Eq. (1)] provides a way of quantifying these differences and the first principal component score of a compound also provides a measure of hydrophobicity in the same way as an individual CHI value.

The non-linear map of the column principal

component loadings (the first 4 principal components were taken into account) is seen in Fig. 3a. The axes have arbitrary units. It can be seen that the points which represent non-polar phases (e.g., ODS) are very close to each other. The aminopropyl silica, diol and nitrile columns represent different selectivity and their position is above the ODS type of columns. On the opposite end the cyclodextrin and polymer based reversed-phase columns can be found. The immobilised artificial membrane column (IAM) represents another selectivity, different from the above mentioned columns. In order to differentiate further

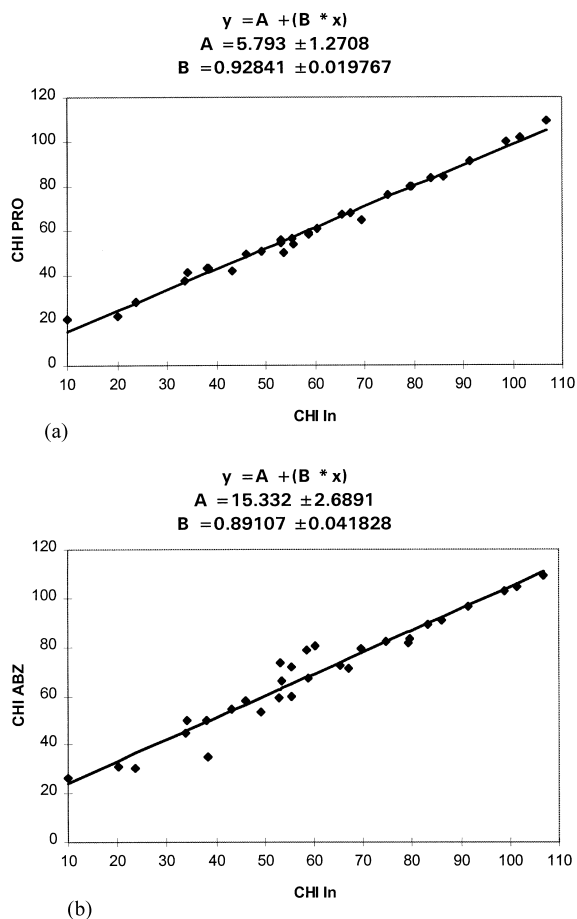


Fig. 1. (a) Plot of the CHI values on Prodigy ODS3 as a function of the CHI values on Inertsil ODS2 (correlation coefficient: 0.994). (b) Plot of the CHI values on Supelcosil ABZ+ as a function of the CHI values on Inertsil ODS2 (correlation coefficient: 0.971).

among the non-polar phases, the principal component analysis has been carried out on the CHI values obtained for only these columns. In this case the first principal component explained 97.58% of the variance, while the second explained 1.07% and the third 0.868%. Fig. 3b shows the non-linear map of the principal component loadings based on the first 4 principal components. It can be seen that Supelcosil ABZ+, the alumina based Biotage columns and the Spherisorb ODS1 column represented slightly different selectivity from the other third generation reversed-phase columns.

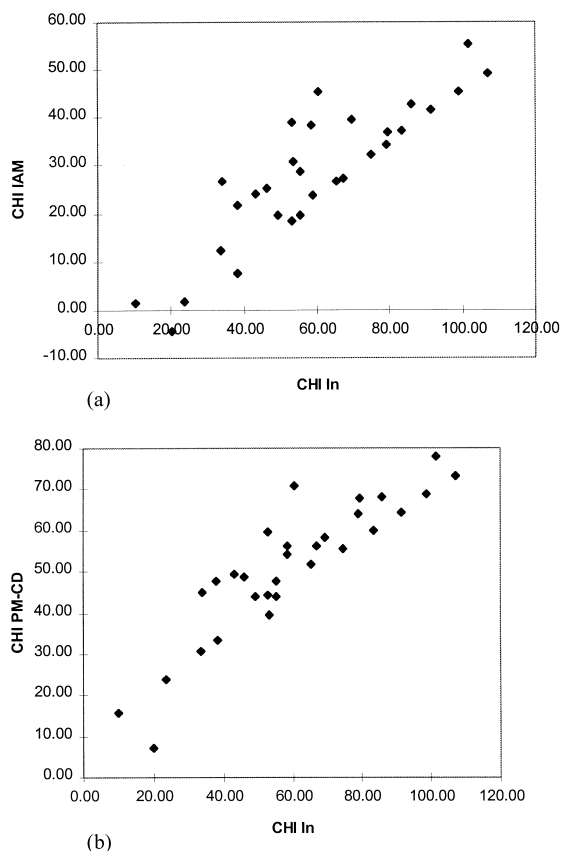


Fig. 2. (a) Plot of the CHI values on IAM (immobilised artificial membrane) column as a function of CHI values on Inertsil ODS2 column. (b) Plot of the CHI values on PC-CD (permethylated β -cyclodextrin) column as a function of CHI values on Inertsil ODS2 column.

3.3. Application of Eq. (1)

The principal component analysis and the non-

Table 8
Principal components and the percentage of the total explained variance based on the principal component analysis of CHI values for the 29 compounds in 20 chromatographic systems

Component	% of total explained variance
C-1	89.34
C-2	5.344
C-3	1.889
C-4	1.213
C-5	0.873
C-6	0.540

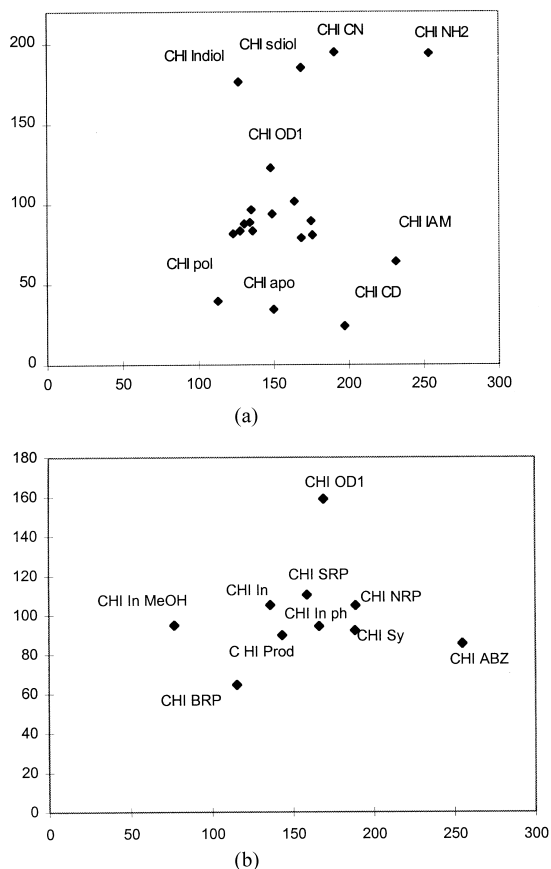


Fig. 3. The non-linear map of column principal component loadings (the first four principal components were taken into account). (a) Non-linear map of all columns. (b) Non-linear map of only the non-polar columns.

linear map technique provide an approximate tool for comparison of various HPLC partition systems. Using the solvation equation we can get a deeper insight into which molecular properties cause the retention differences on various columns. First we had to check whether the gradient retention times and the derived CHI values had a linear correlation with the molecular descriptors.

Eq. (2) shows the parameters of the solvation equation based on the gradient retention times on the Inertsil ODS column (t_{R_g}) from the first column of data in Table 5.

$$t_{R_g} = 3.349 + 0.1826R_2 - 0.351\pi_2^H - 0.759\Sigma\alpha_2^H - 1.8541\Sigma\beta_2^H + 1.737V_x$$

$$n = 29 \quad r = 0.996 \quad s = 0.064 \quad F = 646.5. \quad (2)$$

A series of such equations can be derived from the six different sets of retention times in Table 5. It is therefore better to set up the equations using the derived CHI values because they are all essentially the same.

$$\text{CHI}_{\text{In}} = 33.33 + 6.55R_2 - 12.57\pi_2^H - 27.15\Sigma\alpha_2^H - 66.38\Sigma\beta_2^H + 62.21V_x$$

$$n = 29 \quad r = 0.996 \quad s = 2.30 \quad F = 645.1. \quad (3)$$

To be able to compare the constants they can be normalised by dividing each constant by v , the coefficient of V_x . The normalised constants for R_2 , π_2^H , $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ are in both cases 0.105, -0.202 , -0.437 , -1.067 , respectively. Comparing the normalised constants and the statistics it can be seen that the two equations describe the same relationship. Gradient retention times are expected to be a complex function of the free energy of partition in two binary systems (i.e. stationary phase/aqueous buffer and aqueous buffer–organic phase). Their behaviour in the solvation equation demonstrates that in practice both the gradient retention times and the derived CHI values behave as linear free energy related terms. Table 9 summarises the constants and the mathematical statistical parameters obtained when the CHI values from 20 different column/eluent combinations were correlated with the five solute descriptors described by Eq. (1). In all cases good statistically significant correlations were found. Interestingly, the first principal component scores (PC-1) for the 29 compounds also showed excellent correlations with the solvation parameters as can be seen in Table 9.

In order to compare various partition systems, it is useful to set out the coefficient ratios (relative to $v=1$), as before [20,32]. These ratios are shown in Table 10 for a few selected columns. The most pertinent comparisons are $\log k$ values obtained from non-polar RP-HPLC systems operated with a fixed mobile phase. Abraham and Roses [19] listed numerous sets of coefficients for ODS columns with various water–methanol and water–acetonitrile mobile phases, but later work [32] showed that the coefficient ratios using six different ODS columns with water–methanol eluents that ranged from 30 to

Table 9

The constants, their confidence intervals, the multiple correlation coefficient (R), and the standard error of the estimate (S.D.) of Eq. (1) using the various CHI values from Tables 6 and 7 as dependent variables

CHI _x ^b	c	r	s	a	b	v	R	S.D.
CHI _{In} $n=32$	28.6	5.9±1.8	-15.3±2.0	-19.2±1.9	-63.7±2.4	65.0±1.6	0.987	4.5
CHI _{In MeOH} $n=33$	47.3	5.6±2.2	-13.5±3.3	-10.0±3.2	-45.1±4.2	52.0±2.1	0.965	5.5
CHI _{In Phos} $n=29$	32.9	5.6±2.9	-12.6±3.2	-25.9±3.0	-68.3±3.9	62.8±2.6	0.990	3.8
CHI _{Sy} $n=29$	44.6	3.4 ^a ±2.6	-10.8±2.9	-22.5±2.7	-60.3±3.5	56.2±2.4	0.990	3.4
CHI _{NRP} $n=29$	41.9	3.37 ^a ±2.3	-12.2±2.4	-22.6±2.3	-57.4±3.0	57.8±1.8	0.993	3.0
CHI _{ABZ} $n=29$	35.6	6.5 ^a ±4.3	-10.9±4.7	-13.4±4.4	-68.1±5.7	63.5±3.9	0.974	5.5
CHI _{SRP} $n=29$	43.2	4.0±1.8	-10.1±2.0	-25.1±1.9	-62.0±2.4	57.2±1.6	0.995	2.3
CHI _{PRO} $n=33$	39.8	3.4±2.3	-12.4±2.5	-23.2±2.4	-61.9±3.05	58.1±2.1	0.993	3.0
CHI _{OD1} $n=29$	37.5	4.0 ^a ±3.6	-7.5±3.9	-25.0±3.7	-56.4±4.7	57.7±3.2	0.982	4.6
CHI _{BRP} $n=29$	35.7	12.8±3.8	-12.1±4.2	-16.7±4.0	-72.6±5.1	63.0±3.5	0.981	4.9
CHI _{PBD} $n=29$	35.4	11.8±5.1	-10.4 ^a ±5.6	-18.4±5.3	-74.7±6.8	63.7±4.6	0.969	6.6
CHI _{APO} $n=29$	52.2	6.3 ^a ±3.9	-3.2 ^a ±4.2	-23.4±4.0	-78.5±5.1	51.2±3.5	0.980	5.0
CHI _{POL} $n=29$	60.6	10.0±2.7	-3.9±2.9	-36.1±2.7	-64.7±3.5	39.4±2.4	0.990	3.4
CHI _{NPH} $n=29$	43.1	1.8 ^a ±3.5	-3.1 ^a ±3.9	-26.9±3.6	-63.0±4.7	54.1±3.2	0.981	4.5
CHI _{NCN} $n=35$	-18.0	9.0±4.1	-13.1±4.5	-7.1±4.2	-30.0±5.4	48.8±3.7	0.957	5.3
CHI _{In diol} $n=29$	-22.8	16.5±6.0	-23.0±6.6	-12.1 ^a ±6.2	-28.9±8.0	50.0±5.4	0.930	7.7
CHI _{Sdiol} $n=29$	-41.3	27.0±8.3	-29.4±9.1	-4.3 ^a ±8.6	-39.3±11.1	61.6±7.5	0.903	10.7
CHI _{IAM} $n=33$	0.7	10.2±2.7	-11.0±2.9	6.5±2.8	-47.4±3.5	44.0±2.4	0.972	3.4
CHI _{NH2} $n=32$	-40.5	15.1±6.2	-17.4±6.7	3.7 ^a ±6.3	-35.4±8.2	57.1±5.5	0.939	7.9
CHI _{CD} $n=34$	36.5	7.5±3.8	-4.2 ^a ±4.2	-1.9 ^a ±4.0	-52.0±5.0	31.5±3.4	0.970	4.9
PC-1	1.46	-0.433±0.100	0.518±0.105	0.807±0.100	2.731±0.132	-2.576±0.079	0.993	0.128

^a Statistically not significant variable.

^b In those cases when the number of compounds exceeded 29, the data of some benzoic acid derivatives measured at pH 2 have been included in the calculations.

$$\text{CHI}_x = c + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_x \quad (n = \text{the number of compounds})$$

90% methanol were remarkably constant. Similarly, coefficient ratios were also constant for nine different ODS columns with water–acetonitrile eluents ranging from 20 to 90% acetonitrile. It is therefore not necessary to present coefficient ratios for all the ODS

columns and all the mobile phases, but just to give the two constant sets with one set obtained by the fast gradient method and the other obtained by the isocratic log k determinations. The coefficient ratios are remarkably similar to those for the ODS columns

Table 10

The normalised regression coefficients of selected equations from Table 6 and from the literature obtained for other HPLC systems

Partition	r/v	s/v	a/v	b/v
CHI _{In}	0.091	-0.235	-0.303	-0.979
CHI _{In MeOH}	0.108	-0.260	-0.193	-0.866
CHI _{In Phos}	0.090	-0.200	-0.413	-1.089
CHI _{Sy}	0.060*	-0.192	-0.400	-1.072
CHI _{ABZ}	0.102*	-0.172	-0.211	-1.091
CHI _{POL}	0.254*	-0.010 ^a	-0.915	-1.640
CHI _{NPH}	0.033*	-0.048 ^a	-0.497	-1.165
CHI _{NCN}	0.184	-0.268	-0.145	-0.614
CHI _{In diol}	0.330	-0.460	-0.243	-0.578
CHI _{IAM}	0.231	-0.249	0.147	-1.077
CHI _{NH2}	0.265	-0.304	0.065 ^a	-0.619
CHI _{CD}	0.236	0.134 ^a	-0.060 ^a	-1.648
PC-1	0.168	-0.201	-0.313	-1.060
ODS with aq. MeOH [31]	0.13	-0.32	-0.22	-0.90
ODS with aq. ACN [31]	0.18	-0.33	-0.26	-0.92
PRP-1 100% MeOH [20]	0.70	0.09	-1.84	-1.35
PRP-1 80% MeOH [20]	0.31	-0.03	-0.75	-1.17
PRP-1 100% ACN [20]	1.15	-0.66	-1.19	-1.75
PRP-1 67% ACN [20]	0.28	-0.10	-0.76	-1.45
IAM, 10% ACN [20]	0.43	-0.23	0.37	-1.07
DPC 10% ACN [20]	0.18	-0.16	0.01	-1.03

^a Statistically not significant variable.

in Table 10 (the first 5 entries). This strongly suggests that the factors that influence retention in ODS columns with a constant mobile phase are the same as those that influence retention in ODS columns using our fast gradient method. Furthermore, the factors must quantitatively be the same. We think this is a very important result, because it implies that the same information can be obtained, but more quickly, by the fast gradient method as by the usual constant eluent composition procedure.

Coefficient ratios for the polymer based column (CHI_{POL}) are very different to those for all the other columns, with large negative a/v and b/v ratios. Abraham and co-workers [20] have characterised a poly(styrene-divinylbenzene) column (RPR-1) using a number of eluents, and the coefficient ratios they obtained are also in Table 10. We would not expect two different polymer columns to behave in exactly the same way, but it is interesting that the PRP-1 column also gives rise to large negative a/v and b/v ratios.

The only other column for which we can make any similar comparison is the IAM column, No. 16. Coefficient ratios for this column in Table 10 can be

compared to those [20] for RP-HPLC studies with a fixed eluent for the IAM column of Nasal and co-workers [33]. There is but little agreement between the sets of coefficient ratios for these two different IAM columns. However, the phase obtained by Miyake and co-workers [34] by physically coating the silica gel with dipalmitoyl phosphatidylcholine has coefficient ratios slightly nearer those for IAM column, No. 16. Details are in Table 10 for an eluent of 10% acetonitrile; ratios for 20 and 30% aqueous acetonitrile are almost the same.

Finally, we can use the coefficient ratios in Table 10 to ascertain which, if any, of the columns we have studied by the fast gradient method are suitable for the determination of solute lipophilicity/hydrophobicity. Table 11 contains the coefficient ratios for other distribution systems, such as octanol–water, hexane–water, blood–brain barrier, etc.. There are very large variations in coefficient ratios obtained for these systems [18,35], and so it is important to specify the water–organic solvent system used for the log P determination when it is used as a measure of lipophilicity. If, as usual, we choose the water–octanol system as the standard then we have to

Table 11
The coefficient ratios in the solvation equations for several distribution systems

Distribution	r/v	s/v	a/v	b/v
Octanol–water [19]	0.15	–0.28	0.01	–0.91
Isobutanol–water [20]	0.17	–0.23	–0.02	–0.83
Pentanol–water [20]	0.18	–0.24	0.00	–0.87
Alkane–water [20]	0.15	–0.39	–0.82	–1.13
Cyclohexane–water [20]	0.18	–0.37	–0.81	–1.06
Hexadecane–water [20]	0.15	–0.36	–0.81	–1.10
Blood–brain barrier [32]	0.19	–0.69	–0.72	–1.28

match the coefficient ratios for HPLC systems. Of the fast gradient systems, the ODS systems No. 1–7 seem nearer to the octanol–water system than any other. However, the a/v ratio is not the same, as is also the case for ODS columns with fixed eluents, see Table 10. Thus, as pointed out previously, there will not be a good match as regards computed lipophilicity for solutes that are hydrogen-bond acids [36]. None of the other fast gradient systems, No. 8–18, yield coefficient ratios near to those for $\log P$ in the water–octanol or the water–alkane systems. This is not very surprising, because it has proved difficult to match RP-HPLC systems with the water–octanol or water–alkane measures of lipophilicity [20].

One other distribution, very important in the pharmaceutical industry, that has been investigated is that between blood and brain [37]. Coefficient ratios for $\log BB$, where BB is the blood–brain distribution, are in Table 11. Unfortunately, none of our fast gradient systems are in themselves good models, and neither are any water–solvent distributions [18]. One method of approach, applied with some success, is to obtain solvation descriptors for a given solute, and to estimate the blood–brain distributions from the known [37] blood–brain solvation equation.

To be able to find a good partition model system for biological partitions we believe that a combination of selected HPLC columns with different selectivity could be of valuable help. The solvation equations can be set up for a training set of compounds with known descriptors. Once the coefficients are known they can be used to derive the molecular descriptors for other compounds using CHI values obtained from high throughput determinations on several different systems. This will then

enable us to predict the behaviour of the compound in any other transport process for which the coefficients of the solvation equation have been determined.

3.4. Characterisation of HPLC columns

The methodology described above provides a high throughput approach for determination of the physicochemical properties of putative new drug molecules. Alternatively, in conjunction with a standard set of compounds, it provides an approach to characterise the selectivity of a large number of HPLC columns. By setting up the solvation equations, and determining the regression constants, we can conclude which of the molecular descriptors play the most important roles in determining the retention of the solute on that given column. Thus comparison of two rows of relative coefficients from Table 10 provides an explanation for the patterns seen when retention data from one column is plotted against that from another, e.g., Fig. 1 and Fig. 2.

As an example of how this data may be applied as an aid to the understanding of chromatographic retention mechanisms in molecular terms, the particular comparison between Inertsil ODS and the IAM column will be discussed in more detail (i.e. Fig. 2a and CHI_{In} and CHI_{IAM} coefficients in Table 9). The Inertsil phase consists of octadecylsilane moieties bonded to silica while the IAM column has phospholipid molecules bonded to silica [31]. In qualitative terms Inertsil is therefore a bonded non-polar phase while the IAM has mixed polar and non-polar character and one manifestation of this difference is the generally much lower reversed-phase retentivity of the IAM phase. Quantitatively in terms of Eq. (1) this is shown by the lower absolute value of the coefficient v (Table 9, $v=44$ for IAM and 65 for Inertsil). The molecular volume term V_x is very important and reflects the difference in free energy of cavity formation between the solvent and stationary phase and is the main determinant for hydrophobic interaction. In contrast the contributions (i.e. coefficients s , a and b) from the polar and hydrogen bonding terms (π_2^H , $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$) are all negative (i.e. reduce retention) for the Inertsil ODS phase while for IAM although coefficients s and b are still negative their magnitude is less while the

coefficient a is small (i.e. 6.5) but positive. The effect of the opposite contributions from $\Sigma\alpha_2^H$ (i.e. the hydrogen bond acidity) is striking (Fig. 2a and Tables 6 and 7) and results in compounds, such as the phenols, having relatively high retention on the IAM column. This effect can be explained by the presence of negatively charged phosphate groups on the phospholipids of the IAM phase which provide the H-bond acceptor partners for phenols and other H-bond donors.

Other investigators have used similar approaches for column characterisation. Jackson et al. [38] have used the solvation equation in conjunction with isocratic retention data to characterise novel carbon HPLC phases. Sándi et al. [39] used apparent gradient retention factors (k) for principal component analysis and then correlated the principal components with solvatochromic parameters [40]. Cruz et al. [41] also applied principal component analysis for the classification of commercially available stationary phases, based on the isocratic retention of test compounds, measuring hydrophobicity, H-bonding capacity, ion-exchange capacity, etc..

4. Conclusion

In conclusion, the recently developed high throughput chromatographic hydrophobicity index (CHI) values showed significant correlations with the linear free-energy related solvation descriptors of molecules. The CHI values obtained on various HPLC stationary phases showed differences due to the differing importance of the contributions from these solute descriptors as reflected in the relative sizes of the coefficients r , s , a , b and v . The method can be used for estimating the molecular descriptors of solutes, or for characterising the different selectivities of stationary phases according to the solute-stationary phase interaction properties of each particular phase.

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References

- [1] H. Meyer, Arch. Exp. Pathol. Pharmacol. 42 (1899) 109.
- [2] E. Overton, Z. Phys. Chem. 22 (1887) 189.
- [3] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616.
- [4] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 7 (1971) 525.
- [5] C. Hansch, A. Leo, Chem. Rev. 91 (1993) 1281.
- [6] R. Mannhold, H. Kubinyi, H. Timmerman, in: V. Pilska, B. Testa, H. van de Waterbeemd (Eds.), Methods and Principles in Medicinal Chemistry, Vol. 4, Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, New York, 1996.
- [7] R.F. Rekker, The Hydrophobic Fragmental Constant, Pharmacology Library, Vol. 1, Elsevier, Amsterdam, 1997.
- [8] C. Hansch, A. Leo, cLog P program, Pomona, CA, USA.
- [9] Pallas for Windows, ProLog P Module, CompuDrug Chemistry, Budapest, V. 2. 1994.
- [10] ACD/log P, Advanced Chemistry Development, Toronto, V. 1. 1996.
- [11] R. Collander, Acta Physiol. Scand. 13 (1947) 363.
- [12] A. Leo, in: R.G. Gould (Ed.), Biological Correlations — The Hansch Approach (Advances in Chemistry Ser. 114), American Chemical Society, Washington, DC, 1972, p. 51.
- [13] M.J. Kamlet, R.M. Doherty, J.-L.M. Abboud, M.H. Abraham, R.W. Taft, CHEMTECH 16 (1986) 566.
- [14] M.H. Abraham, R.M. Doherty, M.J. Kamlet, R.W. Taft, Chem. Brit. 22 (1986) 551.
- [15] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 660.
- [16] M.H. Abraham, University College London DataBase, 1997.
- [17] M.H. Abraham, J.C. McGowan, Chromatographia 23 (1987) 243.
- [18] M.H. Abraham, H.S. Chadha, in: V. Pilska, B. Testa, H. van de Waterbeemd (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996.
- [19] M.H. Abraham, M. Roses, J. Phys. Org. Chem. 7 (1994) 672.
- [20] M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kaliszan, A. Nasal, P. Haber, J. Chromatogr. A 766 (1997) 35.
- [21] M.S. Mirrlees, S.J. Moulton, C.T. Murphy, P.J. Taylor, J. Med. Chem. 19 (1976) 619.
- [22] S.H. Unger, J.R. Cook, J.S. Hollenberg, J. Pharm. Sci. 67 (1978) 1364.
- [23] Th. Braumann, G. Weber, L.H. Grimme, J. Chromatogr. 261 (1983) 329.
- [24] W.J. Lambert, J. Chromatogr. A 656 (1993) 469.
- [25] M. Harnisch, H.J. Mockel, G.J. Schulze, J. Chromatogr. 282 (1983) 315.
- [26] K. Valkó, in: H. Kalász, L. Etre (Eds.), Chromatography — The State of the Art, Akadémia, Budapest, 1995, p. 739.
- [27] K. Valkó, J. Liq. Chromatogr. 7 (1984) 1405.
- [28] K. Valkó, P. Slégel, J. Chromatogr. 631 (1993) 49.

- [29] K. Valkó, C. Bevan, D. Reynolds, *Anal. Chem.* 69 (1997) 2022.
- [30] J.D. Krass, B. Jastorff, H.-G. Genieser, *Anal. Chem.* 69 (1997) 2575.
- [31] C. Pidgeon, U.V. Venkatarum, *Anal. Biochem.* 176 (1989) 36.
- [32] M.H. Abraham, M. Roses, C.F. Poole, S. Poole, *J. Chromatogr. A*, in press.
- [33] A. Nasal, M. Sznitowska, A. Bucinski, R. Kaliszán, *J. Chromatogr. A* 692 (1995) 83.
- [34] K. Mikake, F. Kitaura, N. Mizuno, H. Terada, *J. Chromatogr.* 389 (1987) 47.
- [35] M.H. Abraham, H.S. Chadha, G.S. Whiting, R.C. Mitchell, *J. Pharm. Sci.* 83 (1994) 1085.
- [36] M.H. Abraham, H.S. Chadha, A.J. Leo, *J. Chromatogr. A* 685 (1994) 203.
- [37] M.H. Abraham, H.S. Chadha, R.C. Mitchell, *J. Pharm. Sci.* 83 (1994) 1257.
- [38] P.T. Jackson, M.R. Schure, T.P. Weber, P.W. Carr, *Anal. Chem.* 69 (1997) 416.
- [39] Á. Sándi, Á. Bede, L. Szepesy, G. Rippel, *Chromatographia* 45 (1997) 206.
- [40] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander, Cs. Horváth, *Anal. Chem.* 58 (1986) 2674.
- [41] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, *Chromatographia* 44 (1997) 151.