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Unique selectivity of perfluorinated stationary phases with 2,2,2-trifluoroethanol as organic mobile phase modifier

K. Valkó[a,*], S. Espinosa[b], C.M. Du[c], E. Bosch[b], M. Rosés[b], C. Bevan[a], M.H. Abraham[d]

[a]Computational, Analytical and Structural Sciences, GlaxoSmithKline, Stevenage, UK
[b]Department of Analytical Chemistry, University of Barcelona, Barcelona, Spain
[c]pIon Ltd, Boston, MA, USA
[d]Department of Chemistry, University College London, London, UK

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Abstract

The selectivity of Luna C_{18}, Xterra™ C_{18} and Fluophase (perfluorinated C_6) stationary phases has been investigated with aqueous acetonitrile, methanol and 2,2,2-trifluoroethanol mobile phases using linear solvation equations. The gradient retention times of a set of 60 compounds with known molecular descriptors have been determined. Linear solvation equations have been set up to describe the relationship between the gradient retention times and the molecular properties. The selectivity of the stationary phase/mobile phase systems was characterised by the regression coefficients of the molecular descriptors. The perfluorinated stationary phase showed very different selectivity using 2,2,2-trifluoroethanol (TFE) as co-solvent. Compounds with H-bond donor functionality were retained much less than in the other investigated high-performance liquid chromatography (HPLC) systems. This unique selectivity can be explained by the stronger adsorption of trifluoroethanol on the perfluorinated stationary phase surface, than on the hydrocarbon surface. It suggests the importance of the adsorbed organic modifiers in the separation mechanism during reversed-phase HPLC. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Mobile phase composition; Selectivity; 2,2,2-Trifluoroethanol

1. Introduction

We have found that fast gradient reversed-phase retention times can be used for characterising compound lipophilicity for compound selection/optimisation purposes in drug discovery [1,2]. In order to compare the chromatographic lipophilicity with the traditionally used octanol/water lipophilicity we have used the linear solvation equation approach [3,4]. The general solvation Eq. (1) has been widely used to describe chromatographic retention selectivity:

$$SP = c + eE + sS + aA + bB + vV \quad (1)$$

where SP is a linear free energy related solute property, and E, S, A, B and V are the molecular descriptors (E = excess molar refraction, S = dipolarity/polarisability, A = H-bond acidity, B = H-bond basicity and V = McGowan volume). The regression coefficients and constants (c, e, s, a, b, v)

*Corresponding author. Tel.: +44-1438-763-309.
E-mail address: klv39154@gsk.com (K. Valkó).

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are obtained by linear regression analysis and are characteristic of the particular system (i. e. partitioning solvents, stationary phase and mobile phase). They reflect the difference of the complimentary parameters between the two-phase system. The solvation equation provides an excellent tool to describe the polar and non-polar selectivity contributions of the stationary/mobile phase system [5]. However, this approach is not capable of detecting any shape selectivity that might occur during reversed-phase chromatography.

In our earlier studies we have found that the chromatographic hydrophobicity index (CHI) derived from the gradient retention times measured on C_{18} stationary phase with acetonitrile gradient was different from the lipophilicity characterised by the octanol/water partition [6]. The major difference is in the sensitivity towards H-bond donor compounds. The partition of H-bond donor compounds to octanol is favoured by the strong H-bond acceptor property of the OH group in octanol. The coefficient of H-bond acidity is practically zero in the solvation equation for octanol/water partition, which suggests the equally strong H-bond acceptor property of water and octanol. However, in gradient reversed-phase retention with acetonitrile, the coefficient of H-bond acidity is negative, which now suggests that the C_{18} stationary phase (saturated with the mobile phase) is less basic than the mobile phase.

In the search for orthogonal reversed-phase high-performance liquid chromatography (HPLC) systems for the easy measurements of molecular descriptors we have characterised a great number of stationary phases and organic modifiers [7–9]. It was found that in all reversed-phase columns with acetonitrile or methanol gradient the coefficient of the size term (v) and the excess molar refraction term (e) were positive; this accounts for non-polar effects. The polar properties of the compounds (dipolarity/polarisability, H-bond acidity and basicity) always led to a decrease in retention (negative s, a, and b coefficients). In almost all cases the b coefficient is more negative than the a coefficient indicating the stronger H-bond donor character of the mobile phase in comparison to its H-bond acceptor character relative to the stationary phase. Table 1 shows the Kamlet–Taft solvatochromic parameters of water, acetonitrile, methanol and trifluoroethanol. At the beginning of our fast gradient chromatography only water molecules are present in the mobile phase, but later at higher organic modifier concentration we can assume that large amounts of the organic solvent are adsorbed on the stationary phase surface. Studying the coefficients of the solvation equations with methanol and acetonitrile organic modifier, a smaller H-bond acidity coefficient (a) is observed relative to the H-bond basicity coefficient (b) with methanol than with acetonitrile. As methanol has an H-bond donor group, and acetonitrile is only an H-bond acceptor, this suggests that adsorbed organic solvent molecules on the stationary phase surface play an important role in the separation selectivity.

Table 1
Kamlet–Taft solvatochromic parameters of the studied solvents [20]

Solvent	π^*_1	α_1	β_1
Water	1.09	1.17	0.47
Acetonitrile	0.75	0.19	0.31
Methanol	0.60	0.93	0.62
2,2,2-Trifluoroethanol	0.73	1.51	0.00

The importance of the sorbed water and organic modifier on the stationary phase that provides a ''near-surface region'' have already been described and discussed, in order to explain the retention mechanism in reversed-phase HPLC [10–14].

In our previous study to search for ''orthogonal'' HPLC systems, a very different selectivity was found in terms of the solvation equation using perfluorooctyl stationary phase with 2,2,2-trifluoroethanol (TFE) gradient [9]. The a coefficient was much more negative than the b coefficient. It means that the H-bond acid functionality on a solute molecule reduces its retention more strongly than the H-bond basicity. This could be explained by the sorption of TFE onto the stationary phase surface, as the OH group of the trifluoroethanol is a much stronger H-bond acid and a much weaker H-bond base in comparison to the OH groups in methanol, ethanol or isopropanol and water itself. Properties of trifluoroethanol and water mixtures have been studied by molecular dynamics simulations [15,16] as TFE is a commonly used co-solvent in studies of peptides and proteins. Concentration-dependent TFE effects were observed and explained by the aggregation of the TFE molecules. The selectivity of the fluorinated

Table 2
The linear gradient program used on a 50×4.6 mm column

Time (min)	% Organic solvent	Flow-rate (ml/min)
0.0–0.5	0	2
0.5–3.0	0–100	2
3.0–3.5	100	2
3.5–3.7	100–0	2
3.7–5.0	0	2

stationary phases was investigated by Yamamoto and Rokushika [17], who described the special selectivity with high concentration of methanol in the mobile phase on Fluofix bonded-fluoroalkyl column.

According to our hypothesis trifluoroethanol adsorbs more strongly to the fluorinated stationary phases than to C_{18} phases, therefore, its strong H-bond acidity and very weak H-bond basicity dominates the retention selectivity. In order to test this hypothesis we have designed a series of experiments using conventional C_{18} stationary phase (Luna C_{18}), the mixed polymer/silica base Xterra™ C_{18} phase and the perfluorinated FluoPhase with acetonitrile, methanol and 2,2,2-trifluoroethanol gradient. We thought that if the solvation equation was very different for the perfluorinated stationary phase with trifluoroethanol, it would demonstrate the importance of the adsorption of the mobile phase molecules onto the stationary phase surface in the separation selectivity.

2. Experimental

Gradient retention data were measured on a Hewlett-Packard 1100 or 1090 series HPLC. Data acquisition and processing were performed on a Viglen IBM-compatible PC with HP Chemstation software (Hewlett-Packard).

Gradient mixing was carried out by a low-pressure gradient mixer built into the HPLC and was controlled by the Chemstation program. The gradient program used can be found in Table 2.

The aqueous mobile phase was 50 m*M* ammonium acetate (Fisher Chemicals, for analysis) adjusted to pH 10.5 with concentrated ammonia solution for basic compounds. For acids or neutral compounds, the aqueous phase was prepared from 0.1% phosphoric acid, pH 2. The organic solvents were HPLC grade acetonitrile (AcN) (Rathburn Chemicals, Walkerburn, UK), HPLC grade methanol (MeOH) (Rathburn Chemicals) and 2,2,2-trifluoroethanol (TFE) (purity=>99.0%, Fluka Chemicals. Dorset, UK). pH measurements were taken with a Ross semi-micro combination electrode Orion 8103 (glass electrode and a reference electrode with a 3.0 *M* KCl solution in water as a salt bridge) in a Radiometer Copenhagen PHM93 reference pH meter with a precision of ±0.1 mV (±0.002 pH unit).

The reversed-phase columns used in this study are listed in Table 3 with their main properties and suppliers.

The 60 solutes used in this study are all commercially available and their molecular descriptors [18] are shown in Table 4. The solutions were prepared at 0.2 mg/ml in buffer/acetonitrile mixtures (1:1). Moreover, the gradient system was calibrated with a test mixture containing paracetamol, acetanilide, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. This test mixture was injected at the start and the end of the run in order to ensure that the physical conditions during the measurement were the same and to check the condition of the column.

In this study, for the sake of simplicity we used the gradient retention time values to set-up solvation equations, for easy comparison of the absolute retention times. The gradient retention times of the

Table 3
Description of the HPLC columns used in this work

Column	Dimension	Supplier	pH stability range
Luna C_{18} 5 μm (C_{18})	50×4.6 mm	Phenomenex	2–10
XTerra MS C_{18} 5 μm (Xter)	50×4.6 mm	Waters	1–12
Fluophase RP 5 μm (Fluo)	50×4.6 mm	Keystone Scientific	2–8

Table 4
Solute descriptors of the compounds studied

Name	E	S	A	B	V
n-Octanophenone	0.720	0.95	0	0.50	1.8593
n-Heptanophenone	0.720	0.95	0	0.50	1.7184
n-Hexanophenone	0.719	0.95	0	0.50	1.5775
n-Valerophenone	0.795	0.95	0	0.50	1.4366
n-Butyrophenone	0.797	0.95	0	0.51	1.2957
n-Propiophenone	0.804	0.95	0	0.51	1.1548
Acetophenone	0.818	1.01	0	0.48	1.0139
Dibenzothiophene	1.959	1.31	0	0.20	1.3791
Caffeine	1.500	1.60	0	1.33	1.3632
Indazole	1.180	1.22	0.53	0.35	0.9053
Benzonitrile	0.742	1.11	0	0.33	0.8711
Chlorobenzene	0.718	0.65	0	0.07	0.8388
1,4-Dinitrobenzene	1.130	1.63	0	0.46	1.0648
Hydrocortisone	2.030	3.49	0.71	1.90	2.7976
Cortisone-21-acetate	1.820	3.11	0.21	2.13	3.0521
Pyrene	2.808	1.71	0	0.28	1.5846
Progesterone	1.450	3.29	0	1.14	2.6215
Butalbarbital	1.030	1.14	0.47	1.18	1.6557
3,4-Di-Cl-phenol	1.020	1.14	0.85	0.03	1.0199
Phenol	0.805	0.89	0.60	0.30	0.7751
2-Cl-phenol	0.853	0.88	0.32	0.31	0.8975
4-I-phenol	1.380	1.22	0.68	0.20	1.0333
Resorcinol	0.980	1.00	1.10	0.58	0.8338
4-CN-phenol	0.940	1.63	0.80	0.29	0.9298
4-Nitrobenzoic acid	0.990	1.07	0.62	0.54	1.1059
4-OH-benzyl alcohol	0.998	1.15	0.88	0.85	0.9747
Salicylic acid	0.890	0.70	0.72	0.41	0.9904
Phenylacetic acid	0.730	0.97	0.60	0.61	1.0726
n-Hexylbenzene	0.591	0.50	0	0.15	1.5618
n-Nitropropane	0.242	0.95	0	0.31	0.7055
Testosterone	1.540	2.59	0.32	1.19	2.3827
Dexamethasone	2.040	3.51	0.71	1.92	2.9132
Cortexalone	1.910	3.45	0.36	1.60	2.7389
Corticosterone	1.860	3.43	0.40	1.63	2.7389
Aldosterone	2.010	3.47	0.40	1.90	2.6890
Hydroquinone	1.000	1.00	1.16	0.60	0.8338
Barbituric acid	1.090	1.19	0.46	1.16	0.8103
3-F phenol	0.667	0.98	0.68	0.17	0.7928
1,2-DiNObenzene	1.170	1.70	0	0.38	1.0648
Di-Et pthalate	0.729	1.40	0	0.88	1.7106
1,3,5-OH benzene	1.355	1.12	1.40	0.82	0.8925
Ibuprofen	0.700	0.92	0.60	0.60	1.7771
Anthracene	2.290	1.34	0	0.28	1.4544
Dimethylphthate	0.780	1.41	0	0.88	1.4288
3-OH benzoic acid	0.910	0.90	0.85	0.57	0.9904
3-OH benzyl alcohol	0.998	1.12	0.88	0.81	0.9747
4-F benzoic acid	0.600	0.91	0.61	0.29	0.9414
3-F benzoic acid	0.600	0.89	0.64	0.27	0.9414
3-Et barbituric acid	1.060	1.14	0.46	1.16	1.0921
3-NO_2 acetanilide	1.110	2.05	0.64	0.57	1.2875
Indomethacin	2.240	2.85	0.40	1.08	2.5299
Cortisone	1.960	3.50	0.36	1.87	2.7546
3-CN phenol	0.930	1.55	0.84	0.25	0.9298
4-F aniline	0.760	1.09	0.28	0.41	0.8339
2-Et aniline	0.962	0.85	0.23	0.65	1.0980
Lidocaine	1.010	1.49	0.11	1.27	2.0589
4-Nitroaniline	1.220	1.91	0.42	0.38	0.9904
p-Toluidine	0.923	0.95	0.23	0.52	0.9571
Aniline	0.955	0.96	0.26	0.50	0.8162
3-Nitroaniline	1.200	1.71	0.40	0.35	0.9904

60 compounds have been determined consecutively in one sequence.

3. Results and discussion

The gradient retention times of the solutes obtained on the three columns with the three different organic solvent gradients are listed in Table 5. By comparing the retention times we can see that the Xterra C_{18} phase selectivity is very similar to the selectivity of Luna C_{18} using acetonitrile, methanol or trifluoroethanol. Fig. 1 shows the plot of the gradient retention times of the 60 compounds obtained on Xterra C_{18} and Luna C_{18} column with acetonitrile gradient. The weakest correlation between the gradient retention times was obtained when the Luna C_{18} with methanol gradient was compared with the Fluophase with trifluoroethanol (TFE) gradient see Fig. 2. Fig. 3 shows the plot of gradient retention times obtained on Luna C_{18} and Fluophase with trifluoroethanol gradient. Slight curving of the data points can be seen that suggests a different wetting property of the TFE on Luna C_{18} and the perfluorinated fluophase. Fig. 4 shows the plot of the gradient retention times obtained on the Fluophase column with TFE and MeOH. As no curve can be noticed it suggests that the wetting mechanism of methanol is similar to the trifluoroethanol. However, the scatter shows selectivity differences that are probably due to the different H-bond acidity and basicity of the two OH groups in methanol and trifluoroethanol.

However, to properly describe the selectivity differences, the gradient retention time values of the solutes studied in the different HPLC systems were linearly regressed against the five descriptors (Table 4) in the solvation Eq. (1). The coefficients obtained are summarised in Table 6. The regression coefficients *e*, *s*, *a*, *b*, and *v* reflect how that particular descriptor influences the gradient retention. When a coefficient is negative it shows that that particular molecular property will cause a decrease in retention. So it is not surprising that the coefficients of the polar descriptors (S, A and B) are negative, while the non-polar terms have positive coefficients (increase in retention). When a coefficient is not significantly different from zero it means that that particular

Table 5
Measured gradient retention times of the 60 compounds under nine HPLC conditions

Name	Xter/ AcN	C_{18}/ AcN	Fluo/ AcN	Xter/ MeOH	C_{18}/ MeOH	Fluo/ MeOH	Xter/ TFE	C_{18}/ TFE	Fluo/ TFE
n-Octanophenone	3.03	3.10	2.50	3.15	3.22	2.89	3.41	3.41	2.64
n-Heptanophenone	2.90	2.97	2.42	3.06	3.13	2.82	3.24	3.23	2.56
n-Hexanophenone	2.75	2.83	2.33	2.96	3.03	2.73	3.06	3.06	2.48
n-Valerophenone	2.58	2.67	2.21	2.83	2.92	2.63	2.87	2.89	2.39
n-Butyrophenone	2.41	2.51	1.98	2.67	2.76	2.48	2.66	2.70	2.30
n-Propiophenone	2.21	2.32	1.84	2.48	2.58	2.29	2.44	2.51	2.20
Acetophenone	1.96	2.08	1.53	2.23	2.33	2.09	2.20	2.28	2.08
Dibenzothiophene	2.93	2.88	2.24	3.11	3.17	2.50	3.57	3.67	2.32
Caffeine	1.32	1.42	0.88	1.71	1.78	1.25	1.53	1.64	1.03
Indazole	1.72	1.83	1.15	2.15	2.27	1.57	1.89	2.01	1.74
Benzonitrile	1.98	2.10	1.37	2.14	2.25	2.19	2.09	2.17	2.11
Chlorobenzene	2.45	2.56	1.79	2.76	2.85	2.35	2.70	2.74	2.11
1,4-Dinitrobenzene	2.11	2.22	1.20	2.18	2.34	1.58	1.76	1.87	1.88
Hydrocortisone	1.78	1.85	1.24	2.47	2.56	1.97	1.90	1.99	1.74
Cortisone-21-acetate	2.13	2.20	1.57	2.59	2.68	2.47	2.18	2.23	2.03
Pyrene	3.14	3.03	2.26	3.23	3.30	2.55	3.97	3.87	2.38
Progesterone	2.62	2.67	2.22	2.94	3.01	2.92	2.84	2.82	2.82
Butalbarbital	1.78	1.88	1.15	2.27	2.40	1.64	1.83	1.95	1.70
3,4-Di-Cl-phenol	2.22	2.30	1.44	2.68	2.81	1.89	1.90	2.03	1.10
Phenol	1.66	1.81	0.50	1.87	2.07	0.81	1.35	1.50	0.42
2-Cl-phenol	1.94	2.05	0.97	2.25	2.40	1.34	1.69	1.83	0.91
4-I-phenol	2.15	2.24	1.21	2.56	2.70	1.59	1.89	2.04	0.94
Resorcinol	1.26	1.42	0.26	1.36	1.51	0.38	1.06	1.20	0.23
4-CN-phenol	1.67	1.78	0.89	1.94	2.09	1.30	1.31	1.45	0.68
4-Nitrobenzoic acid	1.83	1.96	1.29	2.27	2.44	1.50	1.49	1.65	1.11
4-OH-benzyl alcohol	1.20	1.33	0.39	1.39	1.50	0.43	1.08	1.21	0.21
Salicylic acid	1.83	1.99	1.27	2.31	2.49	1.85	1.61	1.80	1.14
Phenylacetic acid	1.74	1.87	0.98	2.11	2.27	1.37	1.69	1.85	0.93
n-Hexylbenzene	2.85	3.24	2.65	3.14	3.39	2.96	3.28	3.40	2.65
n-Nitropropane	1.67	1.90	1.19	1.63	1.81	1.66	1.63	1.80	1.72
Testosterone	2.19	2.25	1.58	2.78	2.85	2.81	2.54	2.56	2.48
Dexamethasone	1.92	1.99	1.38	2.57	2.65	2.32	2.07	2.15	1.87
Cortexolone	2.00	2.06	1.45	2.61	2.69	2.53	2.22	2.28	2.11
Corticosterone	1.96	2.03	1.39	2.60	2.69	2.40	2.18	2.25	2.06
Aldosterone	1.70	1.77	1.24	2.33	2.41	2.09	2.01	2.07	1.96
Hydroquinone	1.04	1.21	0.23	1.06	1.19	0.29	0.70	0.75	0.22
Barbituric acid	0.37	0.53	0.19	0.38	0.40	0.22	0.36	0.53	0.19
3-F phenol	1.82	1.96	0.69	2.08	2.29	1.08	1.40	1.55	0.53
1,2-DiNObenzene	2.11	2.22	1.45	2.22	2.34	2.16	1.80	1.89	2.02
Di-Et phthalate	2.31	2.42	2.03	2.57	2.66	2.44	2.59	2.64	2.32
1,3,5-OH benzene	1.00	1.14	0.21	1.03	1.13	0.26	0.64	0.91	0.20
Ibuprofen	2.48	2.59	2.21	2.92	3.02	2.76	2.85	2.94	2.25
Anthracene	2.98	2.98	2.27	3.14	3.19	2.55	3.55	3.52	2.35
Dimethylphthate	2.01	2.12	1.37	2.26	2.36	2.02	2.21	2.29	2.10
3-OH benzoic acid	1.46	1.58	0.53	1.83	1.96	0.90	1.18	1.30	0.32
3-OH benzyl alcohol	1.28	1.41	0.31	1.51	1.65	0.49	1.14	1.27	0.24
4-F benzoic acid	1.82	1.94	1.20	2.29	2.43	1.77	1.69	1.82	1.19
3-F benzoic acid	1.82	1.95	1.24	2.30	2.45	1.91	1.70	1.82	1.29
3-Et barbituric acid	1.12	1.26	0.21	1.25	1.38	0.26	1.07	1.22	0.21
3-NO_2 acetanilide	1.77	1.90	1.09	2.11	2.26	1.54	1.56	1.68	0.99
Indomethacin	2.43	2.53	2.09	2.90	2.99	2.52	2.69	2.76	2.03
Cortisone	1.81	1.90	1.30	2.39	2.49	2.21	1.95	2.02	1.85
3-CN phenol	1.73	1.88	0.89	2.00	2.15	1.29	1.42	1.55	0.84
4-F aniline	1.64	1.82	1.06	1.76	1.92	1.12	1.45	1.64	1.00
2-Et aniline	2.04	2.19	1.56	2.29	2.43	1.71	2.06	2.20	1.78
Lidocaine	2.34	2.48	2.23	2.74	2.87	2.71	3.00	3.00	2.63
4-Nitroaniline	1.74	1.91	1.15	1.83	1.96	1.22	1.37	1.52	1.10
p-Toluidine	1.81	1.97	1.34	2.05	2.20	1.53	1.89	2.06	1.69
Aniline	1.54	1.74	0.93	1.63	1.82	1.00	1.45	1.66	0.86
3-Nitroaniline	1.84	1.99	1.27	1.93	2.08	1.30	1.49	1.63	1.11

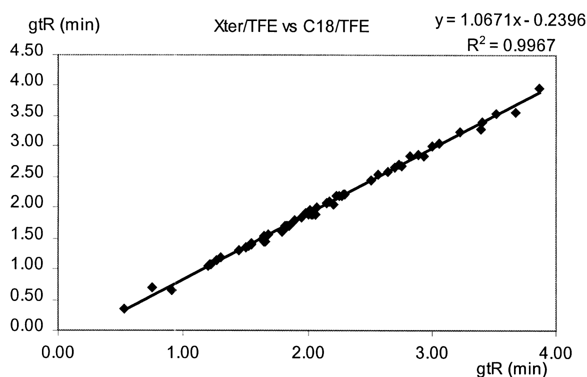

Fig. 1. The plot of the gradient retention times of the 60 compounds obtained on Luna C_{18} and Xterra C_{18} columns with trifluoroethanol gradient.

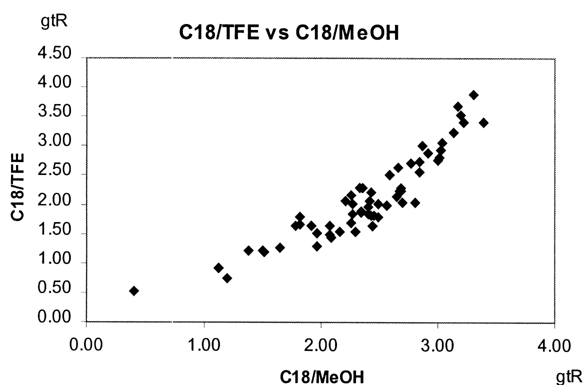

Fig. 3. The plot of the gradient retention times of the 60 compounds obtained on Luna C_{18} with methanol and trifluoroethanol gradient.

molecular property does not influence the retention. Comparing the coefficients of the various molecular descriptors we can reveal which property of the molecule causes an increase or decrease in its retention. Fig. 5 shows the non-linear mapping [19] of the stationary phase/mobile phase systems from a five dimensional space where the dimensions are the coefficients of the five molecular descriptors listed in Table 6. The closer the points are to each other, the more similar are the coefficients of the molecular descriptors in the solvation equation. It can be seen that X-terra C_{18} and Luna C_{18} columns have very similar selectivities whatever organic solvent is used in the gradient. It also can be seen that the three solvents show very different selectivities on each phase. It also can be seen that the Fluophase has distinct selectivity from the other two phases with all the three solvents. The special feature of the Fluophase is that the H-bond acidity coefficients have more negative values with each solvent in comparison to the other two C_{18} phases. The Fluophase with trifluoroethanol shows an even more negative H-bond acidity coefficient compared to the H-bond basicity coefficient. This property is very similar to TFE itself, which is a strong H-bond donor and a very weak H-bond acceptor. This supports the hypothesis that trifluoroethanol adsorbs on the stationary phase surface and ''repels'' all H-bond acid compounds, whereas H-bond basic compounds are

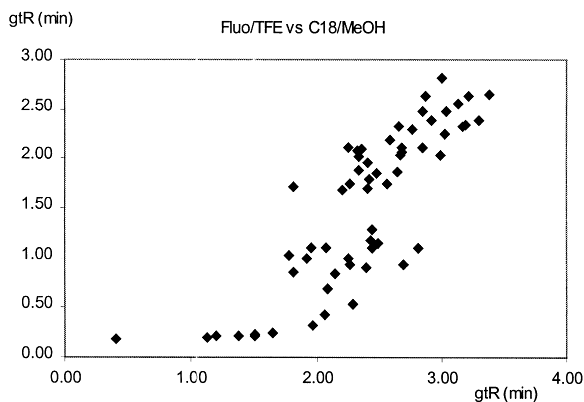

Fig. 2. The plot of the gradient retention times of the 60 compounds obtained on Luna C_{18} with methanol and Fluophase column with trifluoroethanol gradient.

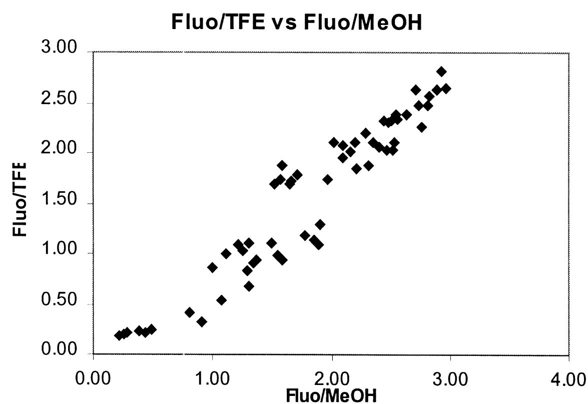

Fig. 4. The plot of the gradient retention times of the 60 compounds obtained Fluophase with trifluoroethanol and methanol gradient.

Table 6
Coefficients of the solvation equation for the different HPLC systems

Column/solvent	c	e	s	a	b	v	r	SD
C_{18}/TFE	1.43	0.43	−0.62	−0.75	−1.11	1.56	0.98	0.16
Xter/TFE	1.26	0.47	−0.65	−0.81	−1.17	1.66	0.98	0.17
Flu/TFE	1.11	−0.11*	−0.17*	−1.17	−0.83	1.32	0.95	0.27
C_{18}/MeOH	1.63	0.07*	−0.26	−0.24	−1.26	1.45	0.96	0.18
Xter/MeOH	1.47	0.11*	−0.28	−0.28	−1.23	1.46	0.96	0.17
Fluo/MeOH	1.04	−0.18*	−0.16*	−0.76	−1.23	1.68	0.96	0.22
C_{18}/AcN	1.70	0.08	−0.28	−0.42	−1.15	1.19	0.98	0.10
XterAcN	1.49	0.18	−0.29	−0.44	−1.18	1.22	0.98	0.11
Fluo/AcN	0.89	0.05*	−0.37	−0.69	−1.07	1.41	0.97	0.17

$gt_R = c + eE + sS + aA + bB + vV$where gt_R is the gradient retention time; c, e, s, a, b and v are regression coefficients of the corresponding molecular descriptors; r is the multiple regression coefficient; SD is the standard error of the gradient retention time by the model. The number of compounds was always 60.
* The coefficient is not significantly different from zero.

retained more. On the other hand, with a methanol gradient, the stationary phase is covered by the H-bond acid and base methanol molecules, and hence both H-bond donor and H-bond acceptor compounds are retained. Interestingly the biggest negative H-bond acidity coefficient using methanol was obtained on the Fluophase.

To show the differences in selectivity between these HPLC systems, a mixture of solutes was analysed: 3,4-dichlorophenol, caffeine, 4-nitrophenol, anisole and theophylline. The solutes were selected to cover a wide variety and range of the molecular descriptors, see Table 7.

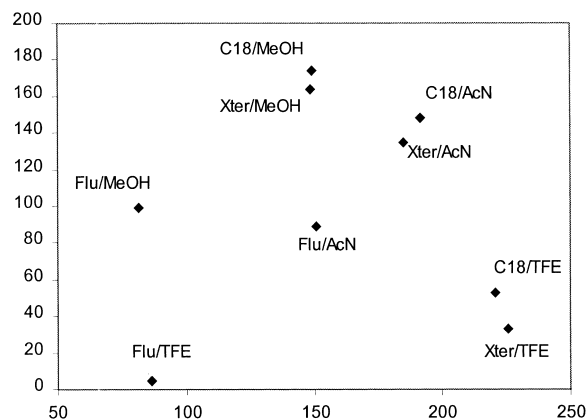

Fig. 5. The non-linear map showing the positions of the HPLC systems in a multidimensional space where the dimensions are the five coefficients of the molecular descriptors obtained from the linear solvation equation. The systems are represented by the same names assigned in Table 6.

Fig. 6 shows the obtained chromatograms for this mixture using trifluoroethanol gradient on the three investigated columns. The biggest selectivity difference between the stationary phases was observed when trifluoroethanol gradient was used. Even the retention order is changed. The H-bond donor compounds elute first leaving behind the H-bond acceptors (caffeine and anisole).

4. Conclusions

In conclusion, the separation selectivity of Xterra C_{18}, Luna C_{18} and Fluophase (perfluorohexyl-silica) using acetonitrile, methanol and trifluoroethanol mobile phases have been characterised by the linear solvation equation. By consideration of the molecular descriptors of the solvents and solutes, and the obtained solvation equations, we have been able to point out the importance of the adsorbed organic phase molecules in the separation selectivity. The solvation equations revealed the special selectivity of the fluorinated stationary phase when trifluoroethanol

Table 7
Solute descriptors of the model compounds

Name	E	S	A	B	V
Theophylline	1.500	1.60	0.54	1.34	1.2223
Caffeine	1.500	1.60	0.00	1.33	1.3632
Anisole	0.708	0.75	0.00	0.29	0.9160
3,4-Dichlorophenol	1.020	1.14	0.85	0.03	1.0199
4-Nitrophenol	1.070	1.72	0.82	0.26	0.9493

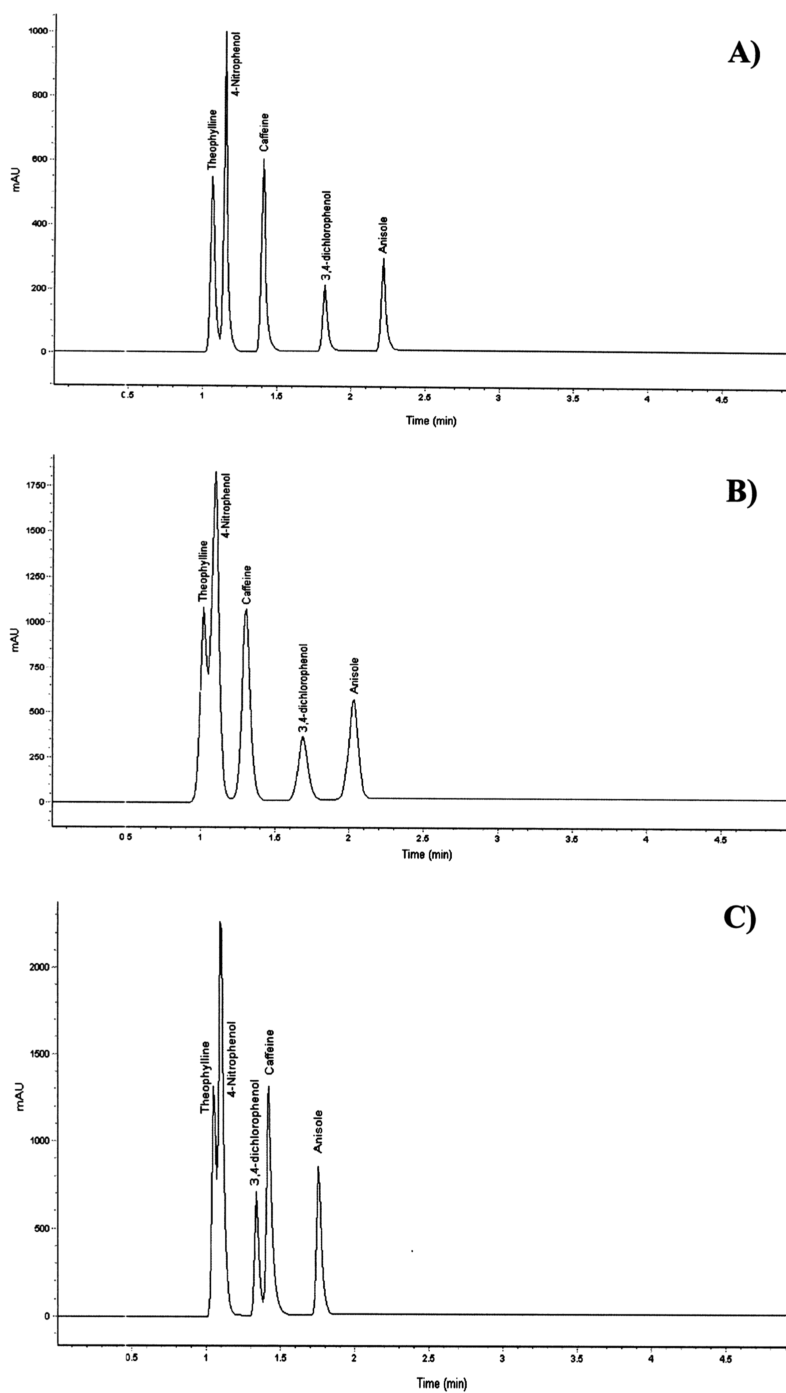

Fig. 6. The chromatograms of theophylline, caffeine, 4-nitrophenol, anisole and 3,4-dichlorophenol obtained with 0.1% H_3PO_4 (pH=2)/2,2,2-trifluoroethanol gradient in: (A) Luna C_{18}, (B) Xterra MS C_{18} and (C) Fluophase RP columns.

gradient was used; this selectivity can be explained by the properties of adsorbed solvent molecules on the stationary phase surface. Finally, we have demonstrated by sample chromatograms that the perfluorinated stationary phase (Fluophase) has a unique selectivity when trifluoroethanol gradient is used.

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