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High-performance liquid chromatography with non-aqueous solvents
Relative elution strength on a polystyrene–divinylbenzene column

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Abstract

A polymeric resin-based stationary phase was used to study the retention of alkylphenols, alkylbenzenes, substituted benzenes and fused-ring compounds with different eluents. Various 100% organic solvents were used as eluents in order to observe the relative ability to solvents to elute compounds of given chemical structure. Plots of log k' vs. number of carbons in a chain attached to the aromatic ring showed linear correlations with slopes that are dependent on solvent type and solute functionality. Of the solvents studied, acetonitrile exhibited the highest solvating ability for the benzene ring and for several functional groups. Ethanol was shown to have the strongest eluting power for the methylene group. The relative ability of several solvents to elute polynuclear aromatic hydrocarbons in solid-phase extraction was determined. The functional group contribution (τ) was calculated for selected compounds and acceptable agreement was found in the correlation between experimental and predicted values for solute capacity factor.

Keywords: Mobile-phase composition; Retention behaviour; Alkylbenzenes; Alkylphenols; Benzenes; Polynuclear aromatic hydrocarbons

1. Introduction

Retention of analytes in liquid chromatography is determined both by the properties of the stationary phase and by the mobile phase. The composition of the mobile phase plays a critical role in determining the success of any HPLC separation. Most frequently, the mobile phase is a mixture of an organic solvent and water. Relatively complex theoretical treatments of mobile phase selectivity have tended to give way to simple models using easily measured parameters [1]. These include eluotropic series [2], solvatochromic comparison [3] and the solubility parameter scale [4].

In the present work, the ability of solvents to solvate organic analytes was studied by measuring the capacity factors of substituted benzenes (C_6H_5X) in various media. These experiments were performed with a pure organic solvent as the mobile phase in order to eliminate any effects from different interactions of the organic

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solvent with water. Such interactions in mixed organic–water phases are a very real possibility. Methanol–water complexes in binary mixtures commonly used as the mobile phase in reversed-phase HPLC have already been shown to occur [5]. The authors suggested that the capacity factor (k') is a function of the free (uncomplexed) methanol and not the total methanol concentration. Similar interactions between water and other organic solvents are definitely possible.

Although bonded-phase silica materials are generally used in reversed-phase HPLC, we selected polystyrene–divinylbenzene (PS–DVB) resins for several reasons. These provide larger surface areas of up to 800 m^2/g as opposed to about 250 m^2/g for bonded-phase silica. The larger surface area of PS–DVB gives larger capacity factors and permits the measurement of solutes in pure solvents where the k' might be too small to measure accurately using silica particles. PS–DVB resins are also more robust under extreme pH conditions [1,6] and have a more homogeneous surface. Bonded-phase silicas have hydrocarbon chains extending from the surface and are apt to contain free silanol groups.

Another goal of this research was to provide better data regarding the elution of sample analytes in solid-phase extraction (SPE). Polymeric resins in mini-columns or membranes have been shown to be superior to cartridges containing silica-based sorbents for SPE [7,8]. However, only limited information has been available concerning the relative ability of pure organic solvents to elute analytes of different chemical structure from polymeric stationary phases.

2. Experimental

2.1. Reagents and chemicals

Solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA), except 200 proof ethanol (Quantum Chemical, Newark, NJ, USA) and ethylene glycol monomethyl ether (2-methoxyethanol), (Sigma–Aldrich, Milwaukee, WI, USA). All solvents were of HPLC grade or analytical-reagent grade. Analyte compounds were purchased from Aldrich, Fisher, Mallinckrodt (St. Louis, MO, USA) or Eastman (Rochester, NY, USA) and were of analytical-reagent grade, except phenylacetaldehyde (90%).

2.2. Apparatus

The chromatograph consisted of a Model 302 HPLC pump (Gilson, Middleton, WI, USA), a Model 783A UV absorbance detector (Applied Biosystems, Stone Mountain, GA, USA), a Model 7000 switcher (Rheodyne, Cotati, CA, USA, used as an injector), a Hitachi D-2000 Chromato-integrator (EM Science, Cherry Hill, NJ, USA) or model C-R3A Chromatopac integrator (Shimadzu, Kyoto, Japan) and a Model LP-21 Lo-Pulse pulse damper (Scientific Systems, State College, PA, USA). The stainless-steel column (10 cm × 4.6 mm I.D.) was slurry-packed in our laboratory with polystyrene–divinylbenzene beads of average particle size 5 μm and average pore diameter 80–100 Å (Sarasep, Santa Clara, CA, USA).

2.3. Procedure

Sample compounds were dissolved in methanol or acetonitrile. Chromatographic eluents were sparged with helium (Air Products, Des Moines, IA, USA) for 0.5 h before transfer to the eluent reservoir. Pure organic solvents were used as mobile phases at a flow-rate of 1 ml/min. Analyte concentrations ranged from 1 to 500 μg/ml and were injected using a 5-μl injection loop (Rheodyne, Cotati, CA, USA) individually or in sets, depending on resolution. Chromatographic peaks were detected by measuring the UV absorbance at 254 nm. Retention times were recorded with an integrator and the capacity factor, k', was calculated for each analyte using the relationship $k' = (t_R - t_0)/t_0$. The column hold-up time was determined by measuring the time of the refractive index disturbance in the baseline following injection, as described by Dolan [9]. The values of the hold-up time were checked by estimating the hold-up volume as roughly one tenth of the column length and

dividing this value by the flow-rate to obtain the column hold-up time, as discussed in Ref. [9]. The column temperature was held at 25°C with an Eldex Model III temperature control unit (Eldex Laboratories, San Carlos, CA, USA). Isocratic elution was used throughout.

3. Results and discussion

3.1. Retention as a function of carbon number

The linear relationship between the logarithm of the capacity factor and the carbon number within a homologous series is well known [10,11]. We studied the retention of several alkylbenzenes and several *p*-alkylphenols using each of three pure organic solvents as the mobile phase. The results in Figs. 1 and 2 show satisfactory linearity for plots of log k' against the carbon number of the alkyl group. In both figures log k' is lowest in acetonitrile (ACN) and highest in methanol (MeOH). This indicates that solvation of a benzene ring follows the order ACN > EtOH > MeOH.

The slope of the plot of log k' vs. carbon number for alkylbenzenes is the greatest in methanol and the smallest in ethanol (EtOH). This indicates that solvation of methylene and methyl groups follows the order EtOH > ACN > MeOH. For *p*-alkylphenols (Fig. 2), the slopes in methanol and acetonitrile are almost the same whereas the slope in ethanol is significantly lower. This again shows that ethanol provides the strongest solvation for methylene and methyl groups.

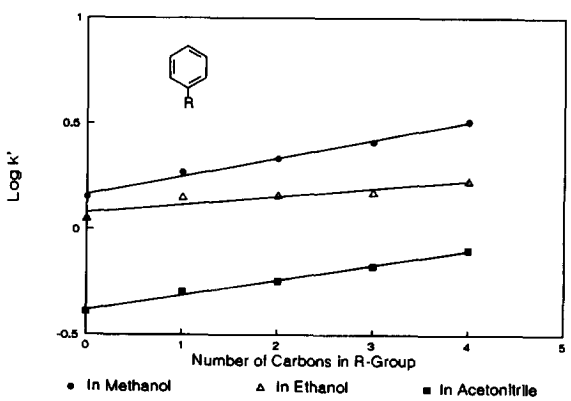

Fig. 1. Logarithmic capacity factor vs. carbon number for alkylbenzenes. Methanol, slope = 0.085, S.D = 0.005, r^2 = 0.991; ethanol, slope = 0.038, S.D. = 0.009, r^2 = 0.858; acetonitrile, slope = 0.071, S.D. = 0.003, r^2 = 0.993.

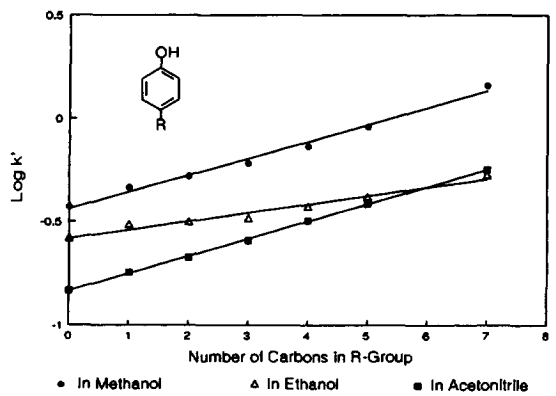

Fig. 2. Logarithmic capacity factor vs. carbon number for alkylphenols. Methanol, slope = 0.081, S.D. = 0.004, r^2 = 0.990; ethanol, slope = 0.041, S.D. = 0.003, r^2 = 0.968; acetonitrile, slope = 0.083, S.D. = 0.001, r^2 = 0.999.

Comparison of log k' of an alkylphenol with that of the corresponding alkylbenzene gives a measure of the relative solvation of the phenolic OH by each of the solvents studied. Phenolic solvation follows the order EtOH > MeOH > ACN. The stronger solvation by the two alcoholic solvents could be due to hydrogen bond formation between the alcohol and the phenol.

3.2. Elution and separation of polynuclear aromatic hydrocarbons

In solid-phase extraction (SPE), it is customary to extract small amounts of organic solutes from predominantly aqueous samples on to a small column filled with a porous solid sorbent. The extracted substances are then eluted from the SPE column by a small volume of organic solvent. The literature contains many recipes for elution with single or mixed solvents, but numerical comparisons of the eluting ability of solvents are generally lacking.

We studied the ability of seven different solvents to elute polynuclear aromatic hydrocar-

bons (PAHs) from porous polystyrene resin columns by measuring their capacity factors. Several of the solvents eluted benzene so quickly that comparison of their relative eluting abilities was difficult. However, fused-ring aromatic compounds eluted more slowly and it was possible to compare the capacity factors obtained with the various solvents. The values obtained are given in Table 1.

These results show that tetrahydrofuran (THF) and methylene chloride (CH_2Cl_2) provide the lowest capacity factors. However, for practical SPE methylene chloride has the disadvantage of not being miscible with water and thereby possibly being less efficient for elution after having passed an aqueous sample through the column. Ethyl acetate also gives low capacity factors and has the property of being volatile when gas chromatography is used to separate and measure the eluted sample components.

The data in Table 1 show that methanol is a very poor solvent for eluting fused-ring aromatic hydrocarbons and that ethanol and acetonitrile are also weak eluents. The chromatogram in Fig. 3a shows that benzene and naphthalene are readily eluted by methanol but anthracene elutes much later in a very broad peak. Chrysene failed to elute in any reasonable time. These results suggest that PAH compounds with more than two rings can be isolated selectively by SPE. Smaller, more polar compounds in methanol will pass through a short, polymeric SPE column whereas the larger PAH compounds will be strongly retained. These, however, are quickly eluted by THF, ethyl acetate (EtOAc) or methylene chloride. We tried a simple experiment using about 50 mg of underivatized 5-μm PS–DVB in a 1-ml cartridge. A mixture of 75 μg/ml toluene, 1 μg/ml anthracene and 5 μg/ml chrysene dissolved in ethanol and diluted with water was forced over the PS–DVB using positive air pressure. The packing was washed with 0.5 ml of methanol to remove toluene, then 1 ml of tetrahydrofuran was used to elute the PAH compounds from the SPE packing. The effluent was collected in a volumetric flask, diluted to 10 ml with methanol and analyzed by HPLC. Perylene was added as an internal standard and recoveries were calculated by comparison with a separate standard solution. The toluene peak was satisfactorily removed while anthracene and chrysene were recovered at 88% and 90%, respectively.

A good chromatographic separation of naphthalene, anthracene and chrysene was obtained with acetonitrile (Fig. 3b). Incomplete separation of the same mixture was obtained with ethylene glycol monomethyl ether (EGME, Fig. 3c). With ethyl acetate all three compounds were eluted together as a single early peak (Fig. 3d).

3.3. Functional group contributions to capacity factor

Several workers have studied the effect of a substituent on the retention of a given solute [12,13]. In many studies the functional group contribution is given by τ_X, defined by

$$\tau_X = \log k'_{R-X} - \log k'_{R-H} \quad (1)$$

Table 1
Chromatographic capacity factors for selected solutes in pure solvents

Solute	Solvent						
	MeOH	EtOH	ACN	THF	EGME	EtOAc	CH2CI2
Benzene	1.44	1.13	0.40	–[a]	–[a]	–[a]	–[a]
Naphthalene	4.65	3.60	1.02	0.53	1.78	0.90	0.59
Anthracene	30.07	20.65	5.61	0.52	2.65	1.08	0.58
Chrysene	–[b]	–[b]	10.85	0.49	3.12	1.20	0.78

[a] Very low values.
[b] Very high values.

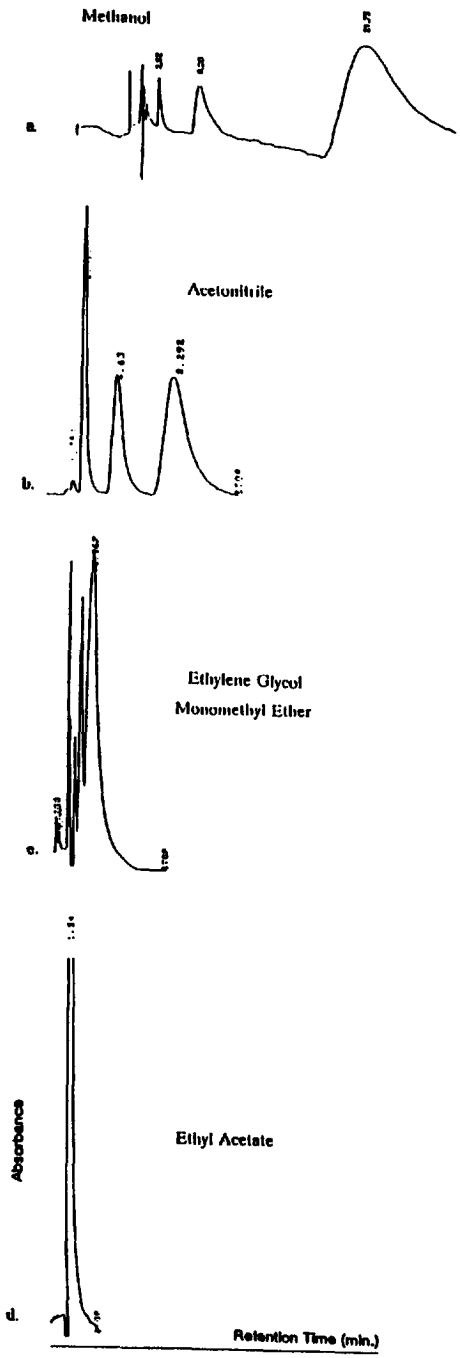

Fig. 3. Chromatograms of (a) 150 μg/ml benzene, 25 μg/ml naphthalene and 20 μg/ml anthracene with 100% methanol eluent, (b) 10 μg/ml naphthalene, 1 μg/ml anthracene and 10 μg/ml chrysene with 100% acetonitrile eluent, (c) 10 μg/ml naphthalene, 1 μg/ml anthracene and 10 μg/ml chrysene with 100% ethylene glycol monomethyl ether eluent and (d) 5 μg/ml naphthalene, 5 μg/ml anthracene and 5 μg/ml chrysene with 100% ethyl acetate eluent. Similar chart speeds.

We measured the capacity factors of a number of substituted benzene and naphthalene compounds in methanol, ethanol and acetonitrile. The capacity factors are given in Table 2 and τ values are listed in Table 3.

The capacity factors of several substituted compounds were predicted by adding the τ values for each substituent to log k' for benzene or naphthalene. Table 4 compares the predicted

Table 2
Capacity factors for various compounds in organic solvents on polystyrene–divinylbenzene

Compound	MeOH	EtOH	ACN
Bromobenzene	2.57	1.85	0.77
Chlorobenzene	1.99	1.24	0.60
Benzonitrile	1.16	–[a]	0.23
Nitrobenzene	1.80	1.63	0.30
Benzaldehyde	1.60	1.35	0.37
Benzoic acid	0.22	0.62	0.01
Aniline	0.60	0.66	0.22
Naphthalene	4.65	3.60	1.02
1-Chloronaphthalene	6.40	4.52	1.62
1-Naphthol	1.09	0.71	0.47
Quinoline	1.71	1.42	1.19
Phenethyl alcohol	0.46	0.29	0.16
3-Phenyl-1-propanol	0.54	0.31	0.21
Cinnamaldehyde	2.91	2.22	0.43
Phenylacetaldehyde	1.40	1.31	0.28
Cinnamyl alcohol	0.62	0.40	0.24
Benzylamine	4.20	3.25	1.01
Benzyl chloride	1.65	–[a]	0.39
Benzyl bromide	2.12	1.65	0.38
Benzyl cyanide	1.13	1.17	0.14
Toluenethiol	2.65	2.08	0.48
Benzyl acetate	1.69	1.50	0.23
Benzylacetone	1.59	1.35	0.23
Phenylacetic acid	0.34	–[a]	0.01
Benzene	1.42	1.13	0.40
Toluene	1.86	1.42	0.50
Ethylbenzene	2.15	1.45	0.57
Propylbenzene	2.57	1.49	0.66
Butylbenzene	3.23	1.69	0.80
Phenol	0.37	0.26	0.15
p-Cresol	0.46	0.31	0.18
4-Ethylphenol	0.53	0.32	0.21
4-Propylphenol	0.61	0.33	0.26
4-*n*-Butylphenol	0.73	0.37	0.32
4-*n*-Amylphenol	0.91	0.42	0.39
4-*n*-Heptylphenol	1.44	0.53	0.57
p-Chlorotoluene	2.67	1.82	0.75
2-Nitrotoluene	2.04	1.87	0.33

[a]Data not collected for these solute–solvent systems.

Table 3
Functional group contributions τ_X (Eq. 1) for the benzene ring

Functional group	Contribution, τ_X		
	Methanol	Ethanol	Acetonitrile
Br	0.26	0.21	0.28
Cl	0.15	0.04	0.17
Cn	−0.09	–[a]	−0.25
NO_2	0.10	0.16	−0.14
CHO	0.05	0.08	−0.03
COOH	−0.81	−0.26	−1.69
NH_2	−0.37	−0.23	−0.26
CH_2NH_2	0.47	0.46	0.40
CH_2Cl	0.07	–[a]	−0.01
CH_2Br	0.17	0.17	−0.03
CH_2CN	−0.10	0.01	−0.46
CH_2SH	0.27	0.27	0.08
OH	−0.58	−0.63	−0.44
CH_2COCH_3	0.05	0.08	−0.25
CH_2COOCH_3	0.08	0.12	−0.24
CH_2COOH	−0.62	–[a]	−1.69
Carbons in alkyl chain			
Alkylbenzenes:			
C_1	0.12	0.10	0.09
C_2	0.18	0.11	0.15
C_3	0.26	0.12	0.22
C_4	0.36	0.18	0.30
Alkylphenols[b]:			
C_1	−0.49	−0.57	−0.35
C_2	−0.43	−0.55	−0.28
C_3	−0.37	−0.54	−0.20
C_4	−0.29	−0.48	−0.10
C_5	−0.19	−0.43	−0.02
C_7	0.01	−0.33	0.15

[a] Not measured.
[b] (E.g., $\log k'_{p\text{-cresol}} - \log k'_{\text{benzene}}$).

and actual values of log k' for several compounds. For example, the difference in log k' values for chlorobenzene and benzene was calculated, giving $\tau = 0.15$ for the chloro group in methanol. Log k' for chloronaphthalene was predicted by adding this same τ value to log k' for naphthalene. Similar treatment was used for other compounds and reasonable agreement was obtained between predicted and actual values, as shown in Fig. 4. The slope of this plot was very close to 1 and the intercept was 0.006. However,

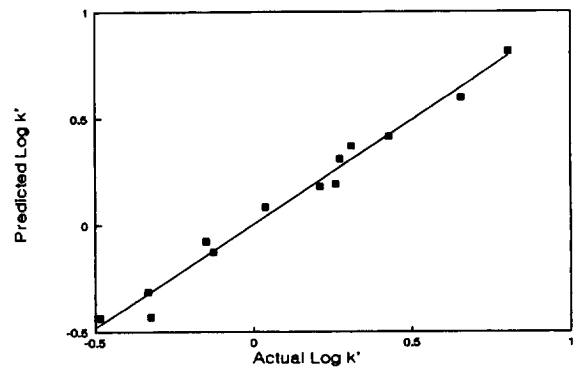

Fig. 4. Correlation of predicted and actual log k' based on functional group contribution to capacity factor. Slope = 0.98, S.D. = 0.03, intercept = 0.006, S.D. = 0.05, $r^2 = 0.987$.

there are some obvious limitations to this approach. For example, the actual value for 2-nitrotoluene is lower than the predicted value in each solvent. This could be due to weak interaction between the methyl hydrogen and the NO_2 group.

Most of the chromatographic peaks were sharp in the solvents studied. A number of separations are feasible, as illustrated by the separation of nitrobenzene and bromobenzene using pure methanol as the mobile phase (Fig. 5).

Table 5 lists the relative solvation of the various substituent groups by the solvents studied. The groups having the smallest (or most negative) τ values are considered to be the most strongly solvated. In general, polar groups are the most strongly solvated and more non-polar groups are less solvated, although the CH_2NH_2 group seems to be an exception. The order of solvation varies among the solvents studied.

It is perhaps more meaningful to note for each analyte which solvent gives the greatest reduction in τ and therefore the greatest degree of solvation. Such a comparison can be made from the values in Tables 2 and 3. Here we see that methanol gives the strongest solvation for NH_2; ethanol gives the strongest solvation for OH, Cl, Br and $-CH_2-$; and acetonitrile most strongly solvates COOH, CHO, CN, NO_2, CH_2Cl, CH_2Br, CH_2CN, CH_2SH, benzene and naphthalene. Further comparison of these substituted benzenes in the three solvents shows that the

Table 4
Predicted and actual log k' values using functional group contributions

Solvent	Compound	Functional group(s)	R–H	Log k'	
				Predicted	Actual
Methanol	*p*-Cresol	OH, CH_3	Benzene	−0.31	−0.33
	p-Chlorotoluene	Cl, CH_3	Benzene	0.42	0.43
	2-Nitrotoluene	NO_2, CH_3	Benzene	0.37	0.31
	1-Chloronaphthalene	Cl	Naphthalene	0.81	0.81
	1-Naphthol	OH	Naphthalene	0.09	0.04
Ethanol	*p*-Cresol	OH, CH_3	Benzene	−0.48	−0.51
	p-Chlorotoluene	Cl, CH_3	Benzene	0.19	0.26
	2-Nitrotoluene	NO_2, CH_3	Benzene	0.31	0.27
	1-Chloronaphthalene	Cl	Naphthalene	0.60	0.65
	1-Naphthol	OH	Naphthalene	−0.07	−0.15
Acetonitrile	*p*-Cresol	OH, CH_3	Benzene	−0.74	−0.74
	p-Chlorotoluene	Cl, CH_3	Benzene	−0.12	−0.13
	2-Nitrotoluene	NO_2, CH_3	Benzene	−0.44	−0.48
	1-Chloronaphthalene	Cl	Naphthalene	0.18	0.21
	1-Naphthol	OH	Naphthalene	−0.43	−0.32

Based on functional group contribution (τ) from Table 3. Log $k'_{R-X(predicted)} = \log k'_{(R-H)} + \tau_{X_1} + \tau_{X_2} + \cdots$.

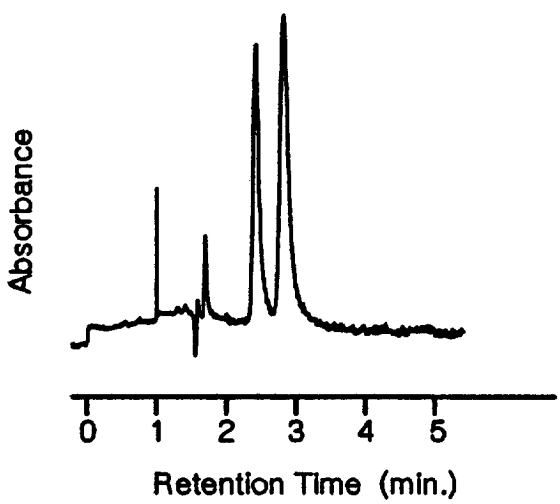

Fig. 5. Separation of 1 μg/ml nitrobenzene and 100 μg/ml bromobenzene using 100% methanol on 5-μm PS–DVB.

Table 5
Order of solvation from top to bottom: (best solvated to least solvated) of selected functional groups in three 100% organic eluents

Methanol	Ethanol	Acetonitrile
COOH	OH	COOH
CH_2COOH	COOH	CH_2COOH
OH	NH_2	CH_2CN
NH_2	Benzene	OH
CH_2CN	CH_2CN	NH_2
CN	Cl	CH_2COCH_3
Benzene	CH_2COCH_3	CH_2OOCH_3
CH_2COCH_3	CHO	CN
CHO	CH_3	NO_2
CH_2Cl	CH_2OOCH_3	CHO
CH_2OOCCH_3	NO_2	CH_2Br
NO_2	CH_2Br	CH_2Cl
CH_3	Br	Benzene
Cl	CH_2SH	CH_2SH
CH_2Br	CH_2NH_2	CH_3
Br	Naphthalene	Cl
CH_2SH		Br
CH_2NH_2		CH_2NH_2
Naphthalene		Naphthalene

following groups are the most poorly solvated by each solvent: for methanol, CH_2–, CH_3, CN; for ethanol, NH_2, COOH, CHO, NO_2, CH_2CN; and for acetonitrile, OH, Cl, Br.

4. Conclusions

By comparing the capacity factors of substituted benzenes and naphthalenes with that of the parent hydrocarbon, it has been possible to measure the relative degrees of solvation of the substituent groups by various organic solvents. The use of pure solvents as the mobile phase in HPLC avoids uncertainties related to solvent–water interactions that may occur when organic–water mixtures are used [5]. Measurement of the capacity factors of PAH compounds in each of seven pure solvents has given a numerical comparison of the relative elution efficiencies of these solvents in SPE. Methanol and ethanol have been shown to be very poor for eluting PAHs from SPE columns. However, advantage can be taken of this factor to isolate selectively PAHs from other organics by SPE in methanol or ethanol with subsequent rapid elution by a more effective solvent such as ethyl acetate or methylene chloride.

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