

CHROM. 19 228

ELUOTROPIC STRENGTH OF SOLVENTS

PREDICTION AND USE IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The importance of solvent strength in solvent optimization for reversed-phase high-performance liquid chromatography (RP-HPLC) is discussed. Several "strength" parameters are reviewed and their limitations and accuracy of predicting isoeluotropic solvent compositions are shown. Partition coefficient of the solvent is suggested as a solvent strength parameter. Its use resulted in relatively precise prediction of mobile phase compositions which are isoeluotropic in RP-HPLC. Comparison has been made between the compositions predicted by other strength parameters and empirical transfer rules. The unique advantages offered by the use of partition coefficient (P) for the calculation of strength parameter of mixed solvents are discussed. Experimental results support the view that $\log P$ of the solvent could be used for eluotropic strength calculations.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) is one of the most widely used analytical techniques. It is estimated¹ that 70–80% of all analytical separations are carried out using this technique. It is, therefore, not surprising that a considerable degree of interest exists in some fundamental aspects of this technique. One area which has attracted much attention is solvent optimization. The approach generally adopted for the prediction of optimum solvent composition for a specific separation problem is to chromatograph a given set of solutes using various combinations of solvents, and then to express the retentions by some quantitative parameter *e.g.* chromatographic optimization function (COF)². Optimization is sought by correlating COF with solvent composition. This is known as mixture-design statistical (MDS) technique². The first step, generally, is to find the composition of methanol–water which provides a chromatogram with capacity factors in the range of 1 to 10 or if required, 1 to 20. This is achieved by conventional "trial-and-error" methods or by predicting isocratic composition from gradient runs³. The latter method, unfortunately requires solvent-specific constants to be determined for

each column which requires considerable experimental work. The next step is to find compositions of acetonitrile–water and tetrahydrofuran THF–water giving similar chromatograms. Again the same method is generally used. However, it would be ideal if these compositions could be predicted beforehand. In order to achieve this goal various workers have proposed different solvent “strength” parameters such as P^4 , S^5 , δ_T^6 and also empirical transfer rules³ for RPLC and ϵ^0 for adsorption LC [liquid–solid chromatography (LSC)] for alumina as adsorbent⁷ or for carbon adsorbent⁸. Discussion of normal-phase LC (ϵ^0) is outside the scope of this paper. Research workers have made use of these parameters. However, these parameters have some limitations and do not provide adequate flexibility in their use as desired by chromatographers. In the present study we have explored the possibility of using the partition coefficient of a solvent as a strength parameter and comparison has been made with other parameters and an empirical approach.

THEORETICAL

In RPLC, retention of a solute is described mainly as a function of its solvophobic interaction^{9–13}. Hence retention is explained as a consequence of partitioning, or is due to the forces involved in the partitioning interaction of solute between stationary and mobile phase^{14–16}. Oscik¹⁷ was the first to derive an equation for LSC with mixed mobile phases which reflects a partition effect in the chromatographic process. Such effects are dominant in the typical reversed-phase system¹⁴. Whatever mechanism(s) may be responsible for the retention in RPLC, it is a fact that in this mode of chromatography retention parameters are correlated to octanol–water partition coefficients ($\log P$) of the solutes¹⁸. It is known that retention is a sensitive function of the quantitative (strength) and qualitative (selectivity) composition of the mobile-phase. However, it should be realised that whilst the strength of the mobile phase is a major factor in controlling the retention, minor changes (in the majority of cases) in retention are offered by selectivity changes employing isoeluotropic solvents. This can be shown by a multisolvent space diagram (Fig. 1). It can be seen that there are an infinite number of compositions which can be found in this space. However only those compositions which lie on the plane indicated are capable of producing chromatograms with retentions of all solutes within the desired limit of capacity factor. Therefore it is this triangular plane which is important for optimization. Ideally, knowing the composition of A by empirical means, or other, we should be able to predict the quantitative composition of B and C.

Snyder's polarity index (P')

Snyder¹⁹ proposed polarity index (P') as a chromatographic strength parameter. It was originally developed for gas chromatography (GC) and normal-phase LC solvents, but according to Snyder and Kirkland²⁰ and Glajch *et al.*² it can also be used for RPLC. Table I shows P' and other strength parameters for a few solvents widely used in RP-HPLC. The solvent strength is inversely proportional to its P' index. This relationship suggests that THF is the strongest solvent in this group. However it also shows that acetonitrile is a weaker solvent than methanol. In practice, generally, it is found that acetonitrile is stronger than methanol. This is shown more clearly by the comparison of experimental and predicted compositions using P' and

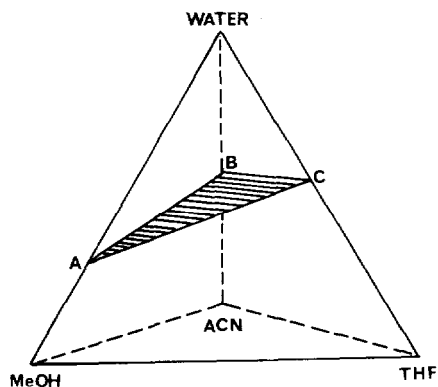


Fig. 1. Multisolvent space showing isoeluotropic plane. MeOH = Methanol; ACN = acetonitrile; THF = tetrahydrofuran. A = Methanol-water; B = acetonitrile-water; C = tetrahydrofuran-water.

other strength parameters in Table II. This discrepancy cannot be resolved simply. Hence it seems that we need a better parameter to measure the chromatographic strength of the solvents in RPLC.

The experimentally found equivalent compositions given in Table II were used by the authors, of the given reference, mostly for solvent optimization using the MDS technique. However, it should be noted that they are not strictly isoeluotropic, as the retentions of the last peak are not similar, in fact in some cases they are significantly different. Therefore judgement about the equivalence should be made with due consideration.

Solvent strength parameter (S)

According to Snyder *et al.*⁵ the retention ($\ln k$) of a solute in RPLC is best approximated, within experimental errors, by following expression:

$$\ln k = \ln k_0 - S \varphi \quad (1)$$

where, $\ln k_0$ is the extrapolated value of $\ln k$ when φ is zero, assuming that this relationship is true over the range $0 \leq \varphi \leq 1$. S is considered to be solvent-strength parameter and φ is the volume fraction of solvent in water.

TABLE I
STRENGTH PARAMETERS FOR THE SOLVENTS USED IN RPLC

Solvent	P' (ref. 4)	S (ref. 5)	δ_T (ref. 6)	$\log Ps^*$
Water	10.2	0.0	25.52	-1.38
Methanol	5.1	3.0	15.85	-0.82**
Acetonitrile	5.8	3.1	13.15	-0.34
THF	4.0	4.4	9.88	+0.46

* 1-Octanol-water $\log P$ of solvents (ref. 38).

** Although several values were available this value was chosen because it gave satisfactory predictions.

TABLE II
COMPARISON OF COMPOSITIONS PREDICTED BY DIFFERENT APPROACHES

Solvent	Experimental	Snyder		Schoenmakers <i>et al.</i>		This study log P_s	Last peak*** (retention)
		P' (ref. 4)	S (ref. 5)	Empirical (ref. 3)	δ_T (ref. 6)		
Methanol ²	63*	63.0	63.0	63.0	63.0	63.0	7.8 k'
Acetonitrile	52	73.0	61.0	48.6	49.2	46.7	7.9
THF	39	51.8	43.0	41.6	38.9	41.0	6.7
Methanol ²¹	50	50.0	50.0	50.0	50.0	50.0	35.8 k'
Acetonitrile	40	58.0	48.4	36.5	39.1	36.6	17.3
THF	37	41.1	34.1	33.0	30.9	32.2	10.3
Methanol ²²	60	60.0	60.0	60.0	60.0	60.0	15.0 t_R
Acetonitrile	40	69.6	58.1	45.7	46.9	44.3	23.0
THF	30	49.4	40.9	39.6	37.1	38.9	24.0
Methanol ²³	35	35.0	35.0	35.0	35.0	35.0	39.0 t_R
Acetonitrile	20	40.6	33.9	23.9	27.4	25.2	43.0
THF	12	28.8	23.9	23.1	21.6	22.3	57.0
Methanol ²⁴	41	41.0	41.0	41.0	41.0	41.0	6.15 cm
Acetonitrile	30	47.5	39.7	28.7	32.1	29.7	5.45
THF	28	33.7	27.9	27.0	25.3	26.2	6.20
Methanol ²⁵	65	65.0	65.0	65.0	65.0	65.0	5.9 cm
Acetonitrile	50	75.3	62.9	50.6	50.8	48.3	7.0
THF	45	53.5	44.3	42.9	40.2	42.3	6.4
Methanol ²⁶	50	50.0	50.0	50.0	50.0	50.0	11.6 t_R
Acetonitrile	32	58.0	48.4	36.5	39.1	36.6	12.6
THF	33	41.1	34.1	33.0	30.9	32.2	13.0
Methanol ²⁷	50	50.0	50.0	50.0	50.0	50.0	13.9 t_R
Acetonitrile	37	58.0	48.4	36.5	39.1	36.6	14.0
THF	32	41.1	34.1	33.0	30.9	32.2	14.0
Methanol**		100.0	100.0	100.0	100.0	100.0	
Acetonitrile		115.9	96.8	89.0	78.2	77.1	
THF		82.3	68.2	66.0	61.8	66.8	

* (% v/v) Solvent in water.

** Equivalent for 100% methanol (calculated).

*** Retention of the last peak in terms of k' , retention time (t_R) or distance from injection (cm).

They also pointed out that S is not a constant which is characteristic of a given solvent but varies, for unknown reasons, with other separation parameters and therefore suggested a definitive experimental study of Dolan *et al.*²⁸. This study found that for nine solutes the S value did not vary by more than ± 10 –20% for a given column and therefore argued that little variation is to be expected in S for other solutes as well. However S is not entirely independent of the nature of the solute as a relationship was observed between the number of carbon atoms in a homologous series and their retentions. Similar observations were also made by Poile²⁹, Englehardt³⁰ and Jandera^{31,32}. It was also shown²⁸ that S , for a given solute, is almost invariant with stationary phases, but does vary with the solvent type. Schoenmakers

*et al.*³ found that S is dependent on $\ln k_0$ by the following equation and on the solvent type:

$$S = p + q \ln k_0 \quad (2)$$

where p and q are constants for a given binary mobile phase system.

In our analysis of their data³ it was found that $\ln k_0$ is related with the $\log P$ (octanol-water) of the solutes ($n = 27$, $r = 0.943$, $s = 0.284$, $F = 200.3$) for methanol-water solvent systems. This result again confirms the view that S is dependent on the nature of the compounds under analysis. Therefore it seems that S cannot be used as an ideal strength parameter. This can be confirmed by the data shown in Table II, where predicted compositions, by S , are not in good agreement with experimental results. It is also apparent that according to the S values methanol and acetonitrile have little difference in strength, which again contradicts the experimental findings.

Schoenmakers' empirical transfer rules

Schoenmakers *et al.*³ obtained transfer rules empirically from experimental data on 32 solutes from three different classes, *viz.* acidic, basic and neutral compounds. The transfer equations are given below:

$$\varphi_a = 0.57\varphi_m + 0.32\varphi_m^2 \quad (3)$$

$$\varphi_t = 0.66\varphi_m \quad (4)$$

where m , a and t stand for methanol, acetonitrile and tetrahydrofuran, respectively.

These rules are found to be useful, although no theoretical basis was proposed to explain them and that it is not possible to calculate equivalents for systems containing more than two solvents. Furthermore similar rules should be established for solvents other than those given here. Compositions computed by eqns. 3 and 4 are found to be in good agreement with the experimental results (Table II). Once again it is shown that acetonitrile is stronger than methanol.

Solubility parameter (δ_T)

Schoenmakers *et al.*³³ were of the opinion that some of the problems that might occur when a solubility parameter is used as a solvent strength parameter, were as follows. (1) Water behaves so uniquely that it is difficult to describe it in terms of δ_T , (2) chemically bonded phases may not have the properties of bulk phases for which solubility theory has been derived, and (3) it is not possible to calculate the solubility parameter for mixed solvents especially when there are more than two solvents in the mixture. However, the following expression was used for two solvent mixtures:

$$\delta_{T_m} = \delta_{T_p} - \varphi(\delta_{T_p} - \delta_{T_q}) \quad (5)$$

where δ_T is total solubility parameter, and m stands for mixture, p for more polar solvent, q for less polar solvent.

This is a linear relationship, which does not agree with the experimental results^{3,4} where a non-linear relationship was shown. This means that the given expression should be considered only as an approximation and not an accurate way to calculate solvent strength. Similar difficulties with the use of δ_T were also reported earlier⁶, namely that accurate retentions in LC were not possible to predict using δ_T . Although the bulk partition behavior of solutes may be described by δ_T ($n = 7$, $r = 0.874$, $s = 0.615$), it is not as precise as the method of Leo *et al.*^{3,5} ($n = 7$, $r = 0.950$, $s = 0.287$). In spite of these reservations, δ_T is found to be a good predictor of the binary solvent compositions examined. However, the predictions are not in complete agreement with observations made by Schoenmakers *et al.*³ in their experimental study. For example, the acetonitrile equivalent for methanol showed a curvilinear relationship in the experimental results as compared to the linear relationship found in the predicted compositions by δ_T . Thus although δ_T is a good predictor of solvent strength it does have its limitations.

Partition coefficient (Ps)

The retention in RPLC, as mentioned earlier, is mainly hydrophobic in nature, and there are several reports* showing good correlation between $\ln k$ and $\log P$ of solutes. It shows that the competition between the solute and the mobile phase for the same retention site *i.e.* the stationary phase, controls the retention. Thus, it seems that the hydrophobic property of the solvent is also important for the solubility and/or elution of the solutes. Furthermore, the solubility of solutes-solvents is related to their partition coefficient and melting point as shown by Yalkowski *et al.*³⁶. Further support for the use of a hydrophobic parameter such as $\log P$ is provided by the findings of Tanaka and Thornton¹¹, who showed that pure water does not appear to be unique with respect to chromatographic property as compared to methanol-like solvents. The only difference found was that water is at the end of the continuum of hydrophobicity. Karger *et al.*¹⁰ reported that the hydrophobic selectivity of different solvents is approximately independent of their chemical nature, which again supports the idea that $\log P$ could be used as an absolute strength parameter, provided the chromatographic strength shows a good agreement with the experimental data. With these views in mind we thought of using partition coefficient as a solvent strength parameter (which shall now be referred to as Ps , where s indicates solvent). The experimental $\log Ps$ values for the four solvents widely used in RPLC were obtained from the literature³⁷. These values agreed well with theoretical $\log Ps$ values, calculated according to the method by Hansch and Leo³⁷, as shown in Table III. For pure solvents, partition coefficient can be correlated with solvent strength. However, for mixtures of solvents the partition coefficient of each solvent may be added according to their proportions *i.e.* their mol fractions times $\log P$. This can be expressed mathematically as follows:

$$\log P_{sm} = \sum_{i=1}^n x_i \log Ps_i \quad (6)$$

* For a comprehensive list see ref. 18.

TABLE III
THEORETICAL AND EXPERIMENTAL LOG P VALUES OF SOLVENTS

Solvent	$\log P$	
	Theoretical ³⁸	Experimental
Water	-1.41	-1.38
Methanol	-0.75	-0.82
Acetonitrile	-0.38	-0.34
THF	+0.46	+0.46

where m indicates mixture, x_i is the mole fraction of the i th solvent, $\log P_{S_i}$ is the octanol-water $\log P$ for the i th solvent, and n is the total number of solvents used in the mixture.

The proposed method (eqn. 6) was used to calculate $\log P_{S_m}$ of the solvent mixtures. The results are shown in Fig. 2. Theoretically, all compositions with same $\log P_{S_m}$ value are isoeluotropic. Based on this assumption we calculated the compositions of acetonitrile-water and THF-water which are isoeluotropic with respect to methanol-water. This involved calculation of the $\log P_{S_m}$ for the given mixture e.g. 50% methanol, and then to find the volume composition of the desired solvent mixture e.g. $x\%$ acetonitrile or $y\%$ THF in water, using a modified linear interpolation algorithm³⁸. This is an iterative interpolation process where the values of x or y are constantly changed until the $\log P_{S_m}$ value of these compositions matches (tolerance used for the match was $\pm 10^{-4}$) with the $\log P_{S_m}$ of the given composition,

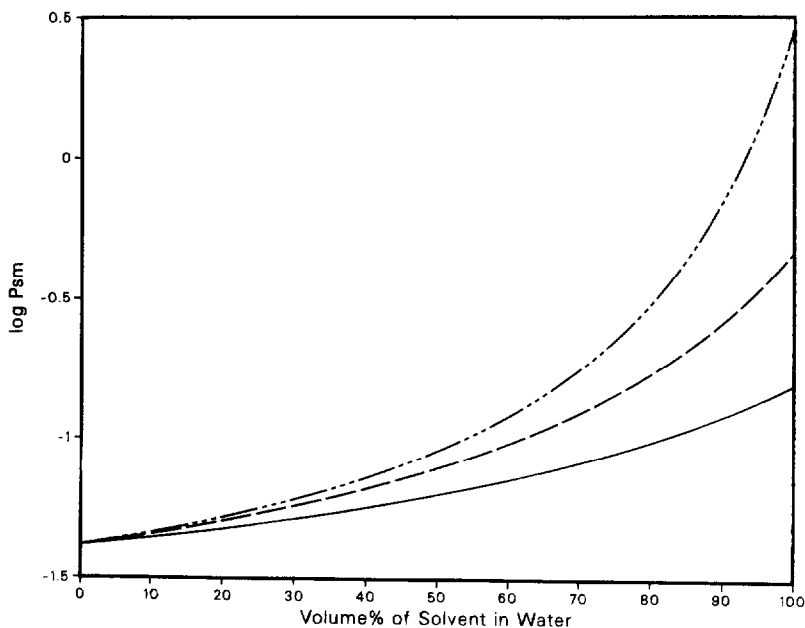


Fig. 2. Plot of solvent composition and its strength ($\log P_{S_m}$). Solvents: —, methanol; ---, acetonitrile; - · - ·, tetrahydrofuran.

in this case 50% methanol. This numerical method was found to be fast and provided sufficient accuracy required for the purpose. From these, data transfer equations similar to Schoenmakers' rules, were obtained. The equations are given below.

(1) This study:

$$V_a = 0.698V_m + 0.00081V_m^2 \quad (7)$$

$$V_t = 0.621V_m + 0.00046V_m^2 \quad (8)$$

(2) Schoenmakers':

$$V_a = 0.570V_m + 0.00320V_m^2 \quad (3a)$$

$$V_t = 0.660V_m \quad (4a)$$

where V stands for volume % and m, a and t for methanol, acetonitrile and tetrahydrofuran respectively.

The equations are compared graphically in Fig. 3. An examination of Fig. 3 suggests that the empirically obtained rules, of Schoenmakers, based on experimental data, compare very well with the rules derived on the theoretical basis in this study. It was possible to describe in linear form the relationship between V_t and V_m (eqn.

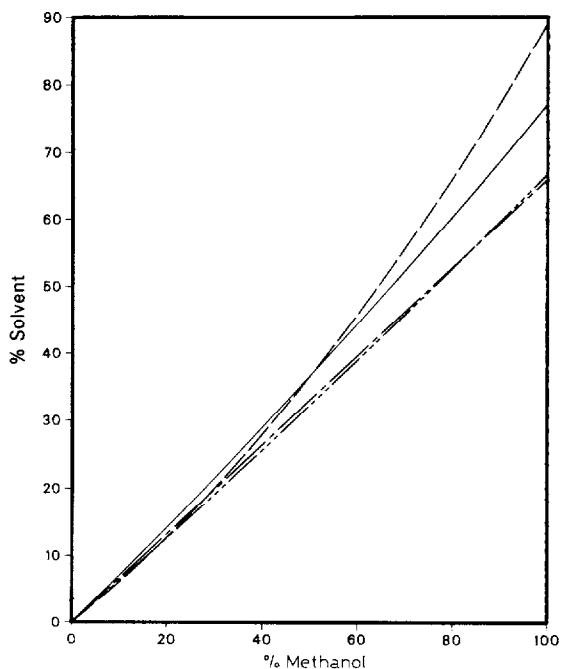


Fig. 3. Graphical comparison of transfer rules by Schoenmakers *et al.*³ (—, acetonitrile; ---, tetrahydrofuran) with those obtained in this study (—, acetonitrile; ---, tetrahydrofuran).

TABLE IV

COMPARISON OF CORRELATION BETWEEN OBSERVED AND PREDICTED COMPOSITIONS*

Predictor	Regression equation**	S.D.	r ²	r ² ***
P'	Exptl. = 2.90 + 0.622 Cal.	5.885	69.0	66.7
S	Exptl. = 3.32 + 0.738 Cal.	5.938	68.4	66.1
Empirical	Exptl. = -5.42 + 1.11 Cal.	4.572	81.3	79.9
δ _T	Exptl. = -3.99 + 1.07 Cal.	4.997	77.6	76.0
log P _s	Exptl. = -7.93 + 1.20 Cal.	4.517	81.7	80.4

* Data Table II.

** Exptl. is experimental and Cal. is calculated/predicted composition by the given strength parameter.

*** Correlation coefficient adjusted to degree of freedom.

8) without losing statistical significance but the quadratic relationship was retained for better accuracy. Although the curve for acetonitrile shows some departure from the empirical rule, compositions predicted seem to correlate very well as shown in Table II and IV.

Discussions so far have shown how log P_s compares with other parameters. In order to further check the validity of the predicted compositions by the log P_s approach, we conducted some experiments on simple un-ionizable molecules using RPLC.

TABLE V

MEAN RETENTIONS* (k') OF FIVE COMPOUNDS UNDER SIMILAR ELUOTROPIC CONDITIONS

log P _{s_m}	Solvent	Compounds**				
		C1	C2	C3	C4	C5
-1.0946	70.0% Methanol	1.87	3.44	5.35	7.58	12.13
	52.3% Acetonitrile	1.98	3.54	5.42	7.46	11.12
	45.8% THF	1.89	3.75	4.96	(4.96)***	(6.44)
-1.1557	60.6% Methanol	2.62	5.33	9.62	15.62	29.40
	44.3% Acetonitrile	3.21	5.94	10.02	15.21	25.58
	38.9% THF	2.83	6.19	8.94	(9.67)	(13.98)
-1.2074	50.0% Methanol	4.15	8.85	18.31	35.50	78.04
	36.6% Acetonitrile	4.83	9.40	17.54	30.15	57.60
	32.2% THF	4.15	10.15	16.62	(20.42)	(33.40)

* Average of three analysis.

** For C1-C5 see text.

*** k' values in parentheses are for those compounds for which THF probably shows different selectivity.

EXPERIMENTAL

Chromatographic studies were performed using a Spectra-Physics Model SP8100 liquid chromatograph with a UV-VIS detector, Model SP8440 and a computing integrator, Model SP4200, and a stainless-steel column, 150 × 4.6 mm I.D., packed with 6- μ m Zorbax ODS (DuPont). Methanol, acetonitrile and THF were HPLC grade (Fisons, Loughborough, U.K.). Glass-distilled deionized water was used to prepare phosphate buffer (0.0025 *M*) to maintain pH at 6.9 and is referred in this study as water. Samples were dissolved in a mixture of methanol-water. All injections were made by autoinjector. All the analyses were carried out at 35°C in a hot-air oven, and detection was monitored at 254 nm. Data analysis and computing was carried out on a Honeywell 68 DPS level 2 via RJE Honeywell Level 6/43 using MINITAB™ software or in FORTRAN77 and in BASIC using a SinclairQL micro-computer (Sinclair, Brentwood, UK) with 128K RAM.

A mixture was prepared containing benzonitrile (C1), benzene (C2), toluene (C3), naphthalene (C4) and biphenyl (C5). This mixture was analyzed in triplicate by isocratic mode using mobile-phase containing 70, 60 and 50% methanol in water and their predicted equivalents for acetonitrile and THF, which are shown in Table V, along with their log P_{s_m} values.

RESULTS AND DISCUSSION

Detailed examination of Table V reveals that, within the k' range of 1 to 10, isoelutropic compositions *i.e.* those compositions with identical log P_{s_m} values produced similar retention values, especially for compounds C1-C3. However C4 and C5 gave lower retentions than expected when in THF-water mixtures. This suggests that probably the selectivity of THF towards C4 and C5 is different from that of methanol and acetonitrile. It may be that the cyclic structure of THF permits better stacking with C4 and C5 during the solvation process, which would reduce retention. Nevertheless, as mentioned earlier, the compositions predicted using log P_s are well supported by Schoenmakers' transfer rules, which were obtained from data on 32 solutes. Hence we expect our predictions to be generally applicable.

CONCLUSIONS

The logarithm of partition coefficient (octanol-water) of a solvent log P_s could be used as a strength parameter, because its use has resulted in relatively precise predictions of isoelutropic compositions and it offers many advantages over the other parameters discussed.

(i) Isoelutropic compositions predicted by log P_s are in good agreement with those predicted by Schoenmakers' transfer rules, based upon experimental data from 32 solutes.

(ii) Experimentally, isoelutropic compositions predicted by log P_s gave good agreement between methanol and acetonitrile for five solutes. Tetrahydrofuran showed expected retentions of C1-C3 but the selectivity towards two solutes was different as compared to methanol and acetonitrile. A wider range of solutes is now under study.

(iii) The value of $\log P_s$ for any solvent is easily available from the literature or can be calculated theoretically, or can be determined experimentally, unlike other parameters which requires either extensive chromatographic analysis or rigorous calculations using basic molecular properties.

(iv) It is possible to calculate the strength of a mixture of any number of solvents, using $\log P_s$, and to find another isoeluotropic mixture of desired solvents which is of considerable value in optimization procedures.

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