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Linear solvation energy relationships in the study of the solvatochromic properties and liquid chromatographic retention behaviour of benzodiazepines

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ABSTRACT

The solvatochromic behaviour of a series of benzodiazepines was studied in order to characterize their molecular properties [polarity/polarizability and hydrogen bonding (HB) acidity and basicity]. Such experimental parameters, compared with those calculated according to Kamlet and co-workers, were employed in the study of the chromatographic retention behaviour by means of linear solvation energy relationships. Various high-performance liquid and thin-layer chromatographic systems were analysed for testing the confidence of this methodology in the study of different retentions in reversed-phase (RP) and normal-phase (NP) chromatographic systems. The most important parameters influencing benzodiazepine retention were established as size and HB basicity for RP retention and polarity and HB acidity or basicity for NP retention. For this series of solutes the present method also appears suitable for predicting retention in the studied systems and for selecting the optimum chromatographic conditions for analytical purposes.

INTRODUCTION

Solvent effects are often small and not easily measured accurately, as they are commonly the resultant of several individual effects which sometime reinforce one another and sometimes cancel each other out. Over the past decade, Kamlet and co-workers have developed a methodology for quantifying such interactions and the influence of bulk solvents on a wide variety of solution-phase processes [1,2]. They made use of the phenomenon of solvatochromism [3,4] (*i.e.*, the effect of a solvent on a spectroscopic property) to establish three carefully constructed scales representing solvent dipolarity (π^*) and hydrogen bond (HB) acidity (α) and basicity (β). Based on these parameters, linear solvation energy relationships (LSERs) have been widely

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used to deconvolve, evaluate and rationalize the multiple interaction effects that influence various solubility properties (SPs) [5,6]. The general LSER form is

$$SP = SP_0 + mV/100 + s\pi^* + a\alpha + b\beta \quad (1)$$

and includes (i) a cavity term ($mV/100$) to measure the endoergic process of separating the solvent molecules to provide a suitably sized enclosure for the solute (V being the molecular volume), (ii) a dipolarity/polarizability term ($s\pi^*$) to measure the exoergic effects of solute–solvent dipole–dipole and dipole–induced dipole dielectric interactions and (iii) hydrogen bonding terms to measure the exoergic effects of HBs involving the solute as an HB donor (HBD) acid ($a\alpha$) and as an HB acceptor (HBA) base ($b\beta$). V, π^*, α and β are parameters characteristic of a solute and are measures of differences in ground- and excited-state properties. The regression coefficients, m, s, a and b, represent how sensitive the solubility properties are to the characteristics of each solute. A large number of physico-chemical processes in condensed phases can be discussed in terms of LSERs, particularly liquid chromatographic capacity factors [7–10].

In this work, the solvatochromic behaviour of selected benzodiazepines (BDZs) was studied in different solvents to test if a reliable indication of their properties (*i.e.*, dipolarity, HBD acidity and HBA basicity) as a function of their molecular structure can be obtained. Through the use of LSERs these solvatochromic parameters were used to study the effects of BDZ molecular structure on retention in different chromatographic systems and to establish the solute properties that determine retention. For pharmacologically active compounds, such as BDZs, a comparison with the molecular properties determining their binding to the receptor, and therefore essential for their pharmacological activity, may be useful. Moreover, taking into account all the complex interactions that affect the retention, this method makes it possible to select the best solvent conditions for BDZ separation and analysis.

EXPERIMENTAL

High-performance liquid chromatographic (HPLC) measurements were performed with a Waters Assoc. Model 600 multi-solvent delivery system with a Waters Assoc. Model 990 photodiode-array detector. The column was μBondapak C_{18} (30 cm × 3.9 mm I.D.) from Waters Assoc. Different mixtures of methanol–water, acetonitrile–water and tetrahydrofuran–water were used as mobile phases. For the solvatochromic measurements the following solvents were used: *n*-hexane, cyclohexane, *n*-heptane, ethyl acetate, dioxane, tetrahydrofuran (THF), acetonitrile (ACN), dichloromethane, chloroform, 2-propanol and methanol (CH_3OH), all of HPLC grade from Carlo Erba. The standard BDZ compounds (whose molecular structures are reported in Table I) were analysed as 1 mM methanol solutions. The previously reported thin-layer chromatographic (TLC) retention data set was completed with measurements carried out under the same experimental conditions [11].

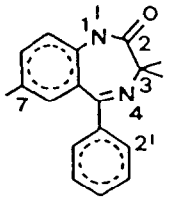
RESULTS AND DISCUSSION

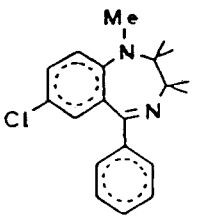
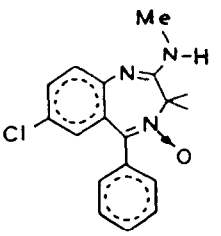
Solvatochromic measurements

The solvent effects on the electronic spectra of selected BDZs were studied.

TABLE I

MOLECULAR STRUCTURES OF THE STUDIED BENZODIAZEPINES



No.	Compound	Substituents			
		1[a]	3	2'	7
1	Diazepam	Me	H	H	Cl
2	Demethyldiazepam	H	H	H	Cl
3	Nitrazepam	H	H	H	NO_2
4	Flunitrazepam	Me	H	F	NO_2
5	Medazepam				
6	Chlordiazepoxide				
7	Oxazepam	H	OH	H	Cl
8	Lorazepam	H	OH	Cl	Cl

[a] Me = methyl.

Solvent-induced frequency shifts have been interpreted in terms of specific solute–solvent interactions by means of the solvatochromic equation

$$\nu_{max} = \nu_0 + s\pi^* + a\alpha + b\beta \quad (2)$$

which correlates solvatochromic effects on electronic spectral transitions, $\nu_{max} - \nu_0$, with solvent properties, defined as the parameters π^*, α and β. The BDZ solvatochromic behaviour was studied in twelve selected solvents with different π^*, α and β values, as reported in Table II. As both solvents and solutes are HB donors and acceptors, a stepwise version of the solvatochromic comparison method was chosen in order to reveal and quantify the multiple effects of solvent properties on the UV–VIS spectra of BDZs [15]. Table III reports all the coefficients s_{ex}, a_{ex} and b_{ex} for the three variable regressions (eqn. 2) of each studied BDZ.

TABLE II

π^*, α AND β PARAMETER VALUES OF THE SOLVENTS USED IN THE SOLVATOCHROMIC MEASUREMENTS

Solvent	V_x	π^*	α	β
n-Hexane	220.24	−0.08	0	0
n-Heptane	253.81	−0.08	0	0
Cyclohexane	202.62	0	0	0
Dioxane	159.94	0.55	0.37	0
Ethyl acetate	143.59	0.55	0.45	0
Tetrahydrofuran	147.5	0.58	0.55	0
Chloroform	87.91	0.58	0	0.44
Dichloromethane	66.96	0.82	0	0.30
2-Propanol	131.16	0.48	0.95	0.76
Methanol	63.62	0.60	0.62	0.93
Acetonitrile	73.22	0.75	0.31	0.19
Water	29.85	1.09	0.18	1.17

The interpretation of the solvatochromic frequency shift is difficult for HB donor-acceptor solutes, such as BDZs, because the polarity–polarizability effect is obscured by interference from hydrogen bonding. A multiple regression must be calculated to fit the data for the twelve solvents and some doubts may exist owing to the small data set used for evaluating each parameter. The present results show a very high correlation ($P < 0.005$) between ν_{max} and π^*, indicating the predominant role that polarity/polarizability forces play in determining solvent–solute interactions. The statistical procedure based on Student's *t*-test demonstrates that inclusion of the α variable is statistically justified in most instances ($P < 0.05$), except for diazepam and demethyldiazepam. Only for oxazepam and lorazepam is the statistical goodness of fit increased by introduction of the variable β. The negative value of s_{ex} shows a bathochromic shift with increased solvent polarity. The maximum in the absorption spectrum of the BDZ molecule is to be ascribed to a $\pi^* \leftarrow \pi$ transition involving the conjugated system. The red shift is consistent with an increase in the dipole moment and/or in the HB ability of the excited form compared with the ground state: the more polar solvent increases the solvation energy of the excited state [1,2,12].

It should be noted that the *s*, *a* and *b* coefficients are not directly affected by overall electronic molecular properties, but more properly they are thermodynamic measures of differences in ground- and excited-state properties of the solute [2,13–15]. Moreover, they may be regarded as good indicators of the chemical properties of the solute, mainly when the molecules considered belong to the same family, as was recently demonstrated [16]. The coefficients of the LSERs have been shown to be a linear combination of solute and solvent properties [17]. With the above-mentioned hypotheses, s_{ex}, a_{ex} and b_{ex} estimated from the multiple regression may be considered to be reliable indicators of the BDZ polarity/polarizability, basicity and acidity, respectively [16,18]. The s_{ex} terms in Table III show a reasonable trend with systematic variations in the BDZ structure: the higher the number of substituents able to donate or accept HBs with the solvent, the larger is *s*. Replacement of a Cl atom with an NO_2 group in position 7 [compounds 3 and 4 *vs.* 1 and 2 (Table I)] markedly increases the

TABLE III

COEFFICIENTS AND STATISTICAL PARAMETERS OF THE EQUATION $\nu_{max} = \nu_0 + s\pi^*_{ex} + a_{ex}\alpha + b_{ex}\beta$ CALCULATED FOR THE STUDIED BDZs

No.	Compound	s_{ex}	a_{ex}	b_{ex}	r	S.D.[a]
1	Diazepam	−1.91	0.56	0.13	0.972	0.283
		±0.19	±0.48	±0.16		
2	Demethyldiazepam	−1.88	0.74	−0.08	0.974	0.284
		±0.19	±0.58	±0.29		
3	Nitrazepam	−3.05	−0.08	0.5	0.989	0.152
		±0.41	±0.04	±0.29		
4	Flunitrazepam	−2.54	−0.08	0.07	0.990	0.140
		±0.25	±0.04	±0.05		
5	Medazepam	−0.91	−0.05	0.10	0.989	0.126
		±0.12	±0.03	±0.10		
6	Chlordiazepoxide	−3.06	−0.77	0.43	0.989	0.161
		±0.38	±0.38	±0.34		
7	Oxazepam	−3.13	−0.61	0.34	0.995	0.085
		±0.19	±0.19	±0.17		
8	Lorazepam	−3.07	−0.42	0.17	0.996	0.071
		±0.18	±0.18	±0.11		

[a] $n = 12$.

values of s. In fact, a quantum-mechanical approach to BDZs shows that the more electron-withdrawing the substituent in this position is, thus lowering the frontier orbital (HOMO and LUMO) energies, the stronger the electrostatic interactions of the negatively charged atoms of the molecule will be [19,20]. The negative values of a_{ex} (Table III) mean that an increase in HBD solvent acidity further increases the solvation energy: the BDZ molecule interacts with solvent mainly as an HBA base, probably with negatively charged O-2 and N-4 atoms via HBs accepted from the solvent. In the medazepam molecule, only the N-4 group may interact with the solvent, as confirmed by the low value of s_{ex}, whereas in chlordiazepoxide the O atom in position 4 is the HBA centre. The positive values of a_{ex} for diazepam and demethyldiazepam are contrary to the above. On the other hand, for these compounds, the α term was not statistically significant in eqn. 2. The improvement in the statistical significance of eqn. 2 obtained when the β term is added for oxazepam and lorazepam demonstrates that the presence of an OH group in the BDZ molecule increases its ability to interact as an HBD acid.

Calculated solvatochromic parameters

In order to check the reliability of s_{ex}, a_{ex} and b_{ex} coefficients as indicators of solute properties, their values were correlated with the calculated BDZ π^*, α and β values. Parameter estimation rules were used by Kamlet *et al.* [6,8,16] to arrive at π^*, α and β values for solid compounds when these were not otherwise available. These estimated parameters were validated by correlations of octanol–water partition coefficients, aqueous solubilities, solvent–water partition coefficients and HPLC capacity factors involving large numbers of solutes and stationary and mobile phases [6,21]. For complex solutes, containing more dipolar substituents, the assumption was made

TABLE IV

CALCULATED SOLVATOCHROMIC PARAMETERS OF THE STUDIED BDZs

No.	Compound	$V_x/100$	π^*	β	α
1	Diazepam	2.09	0.96	0.74	0
2	Demethyldiazepam	1.95	1.00	0.73	0
3	Nitrazepam	2.01	1.30	0.96	0
4	Flunitrazepam	2.23	1.29	0.94	0
5	Medazepam	2.06	0.38	0.93	0
6	Chlordiazepoxide	2.17	1.42	1.52	0
7	Oxazepam	2.01	1.40	1.18	0.33
8	Lorazepam	2.14	1.52	1.15	0.33

that polar and HB effects at the multiple sites are additive (*i.e.*, $\Sigma\pi_i$, $\Sigma\alpha_i$ and $\Sigma\beta_i$ for each substituent i were calculated). The values obtained are reported in Table IV. For the BDZs studied a good correlation exists between experimental s_{ex} and a_{ex} valves and calculated π^* and β values, respectively ($r = 0.971$ and $r = 0.945$, excluding compounds 1 and 2). This relationship is reliable evidence that this method is valid for obtaining good indicators of the solute polarity and basicity. The relationship between b_{ex} and α is not informative ($r = 0.148$), as only two solutes (7 and 8) have appreciable HB acidity.

The cavity term, $V/100$, was also calculated for the studied BDZs (Table IV). McGowan's characteristic volume V_x was used [22]. This value, which can easily be calculated, has proved to be entirely equivalent to the Leahry computer-calculated intrinsic volume and therefore appropriate as a cavity term in LSERs [23].

LSER in RP-HPLC systems

The solvatochromic parameters and LSERs were used to test the ability of this methodology to evaluate the multiple interaction effects that influence RP-HPLC capacity factors. Retention values in various RP chromatographic systems were studied by means of solvatochromic LSERs. The experimental s_{ex}, a_{ex}, b_{ex} values were used, together with the characteristic volume V_x in a stepwise LSER method involving subsequent single-parameter correlations. Table V reports the coefficients and the statistical parameters of the final calculated multiple linear regressions. Different mobile phases were used on a C_{18} column to analyse the effects of the organic modifier (CH_3OH, ACN and THF) and the mobile phase composition on BDZ retention. Some measurements on C_{18} TLC plates were also studied to compare corresponding HPLC and TLC systems. When methanol is the strong solvent, in both HPLC and TLC systems, the leading term in the correlation equations is the measure of the cavity formation ($V_x/100$): this term alone can explain more than 99% of the total variance of retention times, the other terms being, in most instances, nearly zero and not statistically significant at the 95% confidence level. Also, the term relating to solute HB basicity is statistically significant at the 99% confidence level in those equations where ACN and THF are the organic modifiers. The statistical goodness of the calculated equations also demonstrates that experimental solvatochromic parameters are highly suitable for prediction purposes.

TABLE V

COEFFICIENTS AND STATISTICAL PARAMETERS OF THE LSER EQUATION LOG $k' = SP_0 + mV_x/100 + ss_{ex} + ba_{ex} + ab_{ex}$ CALCULATED FOR LOG k' VALUES IN DIFFERENT RP CHROMATOGRAPHIC SYSTEMS

SP_0	m	s	b	a	r	S.D.[d]	Modifier
1.04	1.22	0.01	−0.01	−0.01	0.997	0.009	40% CH_3OH[b]
±0.14	±0.07[a]	±0.01	±0.01	±0.01			
−1.60	1.13	−0.01	−0.01	−0.01	1	0.002	50% CH_3OH[b]
±0.02	±0.01[a]	±0.01	±0.01	±0.01			
−1.82	1.12	0.01	0.02	0.01	0.996	0.010	60% CH_3OH[b]
±0.09	±0.04[a]	±0.01	±0.04	±0.01			
−2.08	1.10	−0.01	0.03	0.01	0.997	0.009	70% CH_3OH[b]
±0.08	±0.04[a]	±0.01	±0.04	±0.01			
−1.34	1.26	0.01	−0.03	−0.01	1	0.004	30% ACN[b]
±0.04	±0.02[a]	±0.01	±0.01[a]	±0.01			
−1.37	1.08	−0.01	0.04	0.01	1	0.003	40% ACN[b]
±0.03	±0.01[a]	±0.01	±0.01[a]	±0.01			
−1.55	0.97	−0.01	−0.03	0.01	0.999	0.005	50% ACN[b]
±0.04	±0.02[a]	±0.01	±0.01[a]	±0.01			
−1.37	0.77	−0.01	−0.10	−0.03	0.997	0.009	60% ACN[b]
±0.25	±0.12[a]	±0.01	±0.03[a]	±0.07			
−0.70	0.77	−0.01	−0.02	0.01	0.999	0.002	20% THF[b]
±0.02	±0.01[a]	±0.01	±0.01[a]	±0.01			
−0.83	0.71	−0.02	−0.02	−0.01	0.998	0.005	30% THF[b]
±0.02	±0.01[a]	±0.01	±0.01[a]	±0.01			
−0.90	0.66	−0.01	−0.02	0.01	0.998	0.004	40% THF[b]
±0.03	±0.02[a]	±0.02	±0.01[a]	±0.01			
−1.13	0.62	0.01	−0.02	−0.01	0.996	0.005	50% THF[b]
±0.05	±0.02[a]	±0.01	±0.01[a]	±0.02			
−1.69	1.03	+0.01	−0.01	0.01	0.993	0.012	60% CH_3OH[c]
±0.12	±0.06[a]	±0.01	±0.04	±0.01			
−1.75	0.92	+0.01	0.05	0.01	0.998	0.005	70% CH_3OH[c]
±0.05	±0.02[a]	±0.01	±0.02	±0.01			

[a] $p < 0.01$.
[b] HPLC.
[c] TLC.
[d] $n = 8$.

Table V also shows the following:

(i) In all instances m values increase in the order THF < ACN < CH_3OH. This reflects the order of solvent cohesiveness, as measured by the cohesive energy δ_H^2: in fact, the Hildebrand solubility parameter (δ_H) is 14.4, 11.8 and 9.1 for CH_3OH, ACN and THF, respectively [9]. The dependence of solute retention on the solvent solubility parameter is well established in LC [24].

(ii) For each solvent, the m term increases regularly with increasing water content in the mobile phase. This is an expected result, as δ_H, the solvent parameter influencing the cavity term, would also be expected to increase regularly with increase in water content [7].

(iii) When the retention in the corresponding TLC and HPLC systems was considered, the resulting equations were very similar. This result is further evidence

TABLE VI

COEFFICIENTS AND STATISTICAL PARAMETERS OF THE EQUATION LOG $k'_w = SP_0 + mV_x/100 + ss_{ex} + ab_{ex} + ba_{ex}$ FOR LOG k' VALUES EXTRAPOLATED TO PURE WATER

SP_0	m	s	a	b	r	S.D.[b]	Column
2.93	1.42	−1.02	0.18	−0.61	0.997	0.058	C_{18}
±0.59	±0.28[a]	±0.04[a]	±0.11	±0.07[a]			
1.40	1.54	−0.71	−0.04	−0.32	0.997	0.037	Phenyl
±0.38	±0.18[a]	±0.02[a]	±0.11	±0.04[a]			
2.44	0.18	−0.42	0.06	−0.22	0.973	0.099	Cyano
±0.47	±0.08[a]	±0.03[a]	±0.14	±0.05[a]			

[a] $P < 0.01$.
[b] $n = 8$.

that the chromatographic behaviour of corresponding HPLC and TLC systems is nearly the same [25].

In order to study the behaviour of different stationary phases, the retention values, extrapolated to pure water, were employed in LSERs. Table VI reports the coefficients and the statistical parameters of the final multiple linear regressions. The major factors influencing HPLC properties are the cavity ($mV_x/100$), dipolar ($s\pi^*$) and hydrogen bonding ($b\beta$) terms. The HBA basicity of BDZs contributes significantly to changes in retention, while the dependence on HBD acidity is not statistically significant at the $P < 0.01$ level. This result confirms, as reported above, that the BDZ molecules mainly act as HBA bases. The signs of the terms in the equation were as expected: (i) increasing solute size (V) causes an increase in retention, *i.e.*, free energy concepts favour solute transfer from the more cohesive mobile phase to the less cohesive stationary phase; (ii) increases in solute dipolarity (π^*) and HB basicity (β) lead to lower log k' values because the solutes have increased affinities for the more dipolar and HB-donating aqueous mobile phase.

The value of the m term reflects the endoergity of cavity formation in the elution process. The very similar values for C_{18}- and phenyl-bonded stationary phases (m = 1.42 and 1.54, respectively) may be interpreted in terms of the similar hydrophobicities of the two phases, whereas the lower m value for the cyano-bonded phase (m = 0.18) may be ascribed to the higher polarity of this column.

LSER in NP-HPLC systems

The solvatochromic parameters were also used to study the retention behaviour of BDZs in NP systems, reported previously [26]. LSERs were built up in a stepwise fashion in order to relate log k' values (as intercepts of the Soczewinski–Snyder equations) to solute molecular properties, as described by the parameters V_x, s_{ex}, a_{ex} and b_{ex} (Table VII). The statistical parameters of the calculated equations show that for the present compounds the solvatochromic values are sufficiently unbiased to describe the retention behaviour in NP-LC systems.

For each stationary phase, a close similarity is noted in the equations describing retention with ethyl acetate and 2-propanol as polar solvents. Unlike RP-LC, here the dominant solute properties are dipole–dipole interactions and HB formation when

TABLE VII

COEFFICIENTS AND STATISTICAL PARAMETERS OF THE LSER EQUATION LOG $k' = SP_0 + mV_x/100 + ss_{ex} + ab_{ex} + ba_{ex}$ CALCULATED FOR THE INTERCEPT (LOG k') OF THE SOK-ZEWINSKI–SNYDER EQUATIONS (LOG k' *VS.* LOG X_s)

Strong solvent	SP_0	m	s	a	b	r	S.D.[b]	Column
Ethyl acetate	−0.61	−0.40	0.73	3.31	−0.65	0.999	0.026	Amino
	±0.19	±0.09[a]	±0.01[a]	±0.09[a]	±0.03[a]			
2-Propanol	−2.55	−0.32	0.73	2.64	−0.44	0.916	0.238	Amino
	±0.23	±0.11[a]	±0.16[a]	±1.07[a]	±0.26[a]			
Ethyl acetate	−1.44	−0.13	0.37	0.47	0.16	0.988	0.037	Cyano
	±0.34	±0.16	±0.02[a]	±0.16[a]	±0.06[a]			
2-Propanol	−0.89	−0.37	0.35	0.75	0.05	0.993	0.027	Cyano
	±0.25	±0.11[a]	±0.02[a]	±0.12[a]	±0.04			
Ethyl acetate	1.04	−1.51	0.20	0.22	0.10	0.960	0.058	Silica
	±0.55	±0.26[a]	±0.04[a]	±0.27	±0.09			
2-Propanol	1.73	−1.35	0.17	0.53	0.19	0.921	0.080	Silica
	±0.75	±0.36[a]	±0.05[a]	±0.36	±0.12			

[a] $P < 0.01$.
[b] $n = 8$.

the solute acts as an HB acid. The signs of these coefficients are all positive: an increase in dipole–dipole and dipole–induced dipole interactions increases retention, whereas more HBD acidic and HBA basic solutes give stronger HBs with the stationary phase, thus increasing retention. The only exception is the amino-bonded phase, where the negative sign of the solute basicity term indicates the basic properties of this stationary phase. The study of solute acidity and basicity coefficients in the equation given in Table VII makes it possible to examine the relative HB acidities and basicities of the different stationary phases. The amino-bonded phase is markedly more basic than the silica and cyano-bonded phases (higher dependence on α values), whereas the cyano-bonded and silica phases are definitely more acidic than the amino-bonded phase (positive dependence on β values), exhibiting a similar behaviour [27–31]. In

TABLE VIII

COEFFICIENTS AND STATISTICAL PARAMETERS OF THE EQUATION LOG $k' = SP_0 + mV_x/100 + ss_{ex} + ab_{ex} + ba_{ex}$ CALCULATED FOR ISOCRATIC LOG k' VALUES IN DIFFERENT NP CHROMATOGRAPHIC SYSTEMS

Column	SP_0	m	s	a	b	r	S.D.[b]	X_s (ethyl acetate)
Amino	−0.90	−0.79	0.43	0.31	−0.17	0.961	0.226	−0.1
	±0.46	±0.13[a]	±0.16[a]	±0.20[a]	±0.13			
Cyano	−1.08	−1.07	0.45	0.32	−0.03	0.995	0.096	−0.3
	±0.26	±0.09[a]	±0.07[a]	±0.18[a]	±0.10			
Silica	0.34	−0.50	0.25	0.74	0.37	0.945	0.124	−0.2
	±0.16	±0.12[a]	±0.09[a]	±0.38[a]	±0.12[a]			

[a] $P < 0.01$.
[b] $n = 8$.

comparison with RP retention studies, the influence of the V_x term is much less important and not always statistically significant at the 99% confidence level. Even if the displacement model of NP chromatography neglects mobile phase solute–solvent interactions at the first level of analysis [27–29], the V_x term accounts for the cavity formation energy in the solvent.

The ability of the present methodology to predict NP retention of BDZs was investigated with an LSER study of the log k' values at a fixed composition of ethyl acetate in the mobile phase on amino, cyano and silica columns. Table VIII reports the coefficients and the statistical parameters of LSERs calculated with V_x, s_{ex}, a_{ex} and b_{ex} values. The chief factors controlling NP retention are solute size, polarity and HBD acidity, solute HBA basicity being, in this instance, a less important factor. The predictive power is good for these systems, particularly for the cyano phase.

Analytical application

When similar solutes belonging to the same family are studied, the shift of the UV–VIS adsorption spectrum maxima with various solvents as a function of solvent–solute interactions and of the differences in solute ground- and excited-state properties may give information regarding solute molecular structure. In this case, with a photodiode-array detector, it is possible to measure both the chromatographic and the spectroscopic data fundamental to solute characterization.

When the solvatochromic parameters of the solutes are known (both calculated or experimentally determined), the correct solvent for optimizing elution can be chosen on the basis of its solvatochromic properties. Methanol is a strong HBA base and an intermediate acid and polar solvent; ACN is mostly a polar solvent, as it is only a weak HB base and acid; THF has, above all, a high molecular volume (see Table II). Therefore, CH_3OH would exhibit a specific selectivity towards polar, HBD acid solutes, and THF towards large apolar molecules.

The effects that different solvents have on the group increment to retention were

TABLE IX

EFFECT OF DIFFERENT SOLVENTS (CH_3OH, ACN AND THF) ON THE GROUP INCREMENT TO THE RETENTION OF SOME SUBSTITUENT GROUPS OF THE BDZ MOLECULE

Group	BDZs	V_x	π^*	β	α	ΔLog k'[a]		
						CH_3OH	ACN	THF
OH	7 – 2	0.06	0.44	0.41	0.33	−0.17 (±0.03)	−0.07 (±0.01)	−0.05 (±0.01)
NO_2	4 – 2	0.06	0.30	0.23	0	−0.14 (±0.02)	−0.06 (±0.01)	−0.04 (±0.01)
CH_3	1 – 2	0.14	−0.04	−0.03	0	0.08 (±0.02)	0.08 (±0.02)	0.15 (±0.03)
Cl	8 – 7	0.13	0.12	0.03	0	0.09 (±0.02)	0.10 (±0.02)	0.16 (±0.03)
F	4 – 8	0.22	−0.01	0	0	0.05 (±0.01)	0.06 (±0.01)	0.18 (±0.03)

[a] Mean data over four solvent compositions with their standard deviations. CH_3OH concentration range, 40–70%; ACN concentration range, 30–60%; THF concentration range, 20–50%.

studied for some substituents of the BDZ molecule (Table IX). The $\Delta \log k'$ values, a function of free energy changes involved in the retention process, are related to the solvatochromic parameters of the solvent. The reported $\Delta \log k'$ values are mean data over four solvent compositions (Table IX). For each solvent studied the group contributions are constant and independent of the organic modifier concentration. In the BDZ skeleton, the OH and NO_2 groups, mainly affecting solute polarity and HB acidity and basicity, are best separated by CH_3OH, whereas the effects of ACN and THF are low. The selectivity of THF for CH_3, Cl and F substituents is high. In fact, these groups mainly give rise to variations in molecular volume. The selectivity of ACN and CH_3OH is low owing to the low variation in the molecular polarity.

To confirm these effects and to analyse how these results are affected by the molecular environment, an attempt was made to compare the present results with the literature. However, as only a few, heterogeneous data concerning the simpler benzene nucleus were available [9,32], it was impossible to obtain unequivocal, reliable information. If the solvatochromic comparison method were applied to a wider homogeneous series of compounds, this might help in identifying and evaluating the individual solute–solvent interactions contributing to retention selectivity.

CONCLUSION

The results showed that solvatochromic studies are suitable for examining the chemical and physical solute characteristics when a set of similar compounds, such as BDZs, are analysed. Moreover, for these solutes LSERs may be applied to recognize molecular properties governing retention and to predict retention behaviour by means of solute solvatochromic parameters. For the studied BDZs the solvatochromic method has identified the same solute properties (polarity and HB acidity and basicity) as essential to their pharmacological activity [19,20]. More complex mathematical methods, *e.g.*, partial least squares in latent variables (PLS) or principal component regression (PCR) may also be appropriate models of multi-component statistical analysis to be applied to these studies.

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