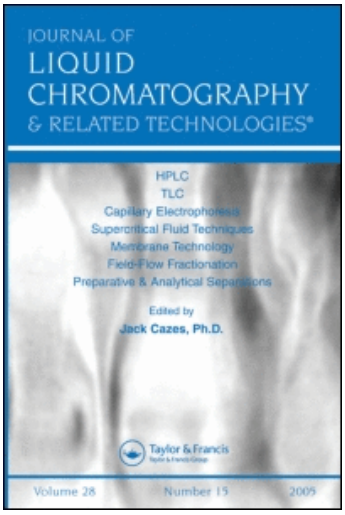

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Study of HPLC Retention behavior Of 1-Hydroxy-Imidazole-3-N-Oxides, Substituted Imidazoles, and Imidazole Derivatives Using Molecular and Adsorption Parameters

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STUDY OF HPLC RETENTION BEHAVIOR OF 1-HYDROXY-IMIDAZOLE-3-N-OXIDES, SUBSTITUTED IMIDAZOLES, AND IMIDAZOLE DERIVATIVES USING MOLECULAR AND ADSORPTION PARAMETERS

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ABSTRACT

High performance liquid chromatography (HPLC) was applied to study the behavior of a series of 1-hydroxy-imidazole-3-N-oxides (HIO) in solution. Some thermodynamic parameters influencing solute retention were determined from the temperature dependence of the capacity factor (k). A linear relation was derived between k and molecular parameters considering intermolecular association, which was predicted and confirmed. ΔG_{ads} values are also highly correlated with connectivity index, molar refractivity and Van der Waals volumes. Retention data for twenty closely related imidazoles were measured and analyzed in relation to hydrophilic parameters.

INTRODUCTION

Correlation between molecular structure and retention in RP-HPLC is a growing interest in different fields. Reports were published for correlations between retention of restricted groups of compounds containing similar functionalities[1-5] and molecular physicochemical properties such as Van der Waals volume (V_w),[6-8] molecular connectivity index (χ),[1,2] hydrophobicity,[3] molecular refractivity (R_M)[3] and shape parameters.[5] In this direction a high enough correlation coefficient is not always a reliable criterion predicting the correct elution sequence, since also adsorption enthalpy effects[9] can account for solute-phase interactions.

Although 1-hydroxyimidazoles have been known for some time, little is known about their 3-oxide derivatives. They have application in biochemistry, corrosion science,[10] and paint production. Thin layer and paper chromatography work on imidazoles is known,[11,12] however HPLC data are practically reported only for 5-nitroimidazoles,[13] benzimidazoles,[14] imidazoline surfactants,[15] and drugs.[16-21] The HPLC determination of the antihistomoniasis drugs Dimetridazole,[22] Benzoylmetronidazol,[23] and Metronidazol[23-29] is also found in the literature.

With one exception[18] there is no published work providing a study on retention of imidazole derivatives in HPLC as a function of their structure and not at all in relation to adsorption.

In this paper, two sets of compounds are studied,1-hydroxy-imidazole-3-N-oxide compounds and imidazole derivatives. The relationships between retention as well as adsorption, and the structural descriptors R_M, χ, V_W and correlation factor (F) of HIO are analized.

Based on the premise of intermolecular association, the establishment of a retention-molecular size relation was an interesting task for the limited group of HIO compounds. The experience gained in the chromatographic work with HIO, allowed the separation of their electrolysis products for further identification.

For a larger set of imidazole derivatives we were expecting to find a relation exhibiting linearity between retention and hydrophobicity in terms of molecular size and interaction ability with water. For this purpose the concept of hydrophilicity parameter is used and considers the calculated partial molar volume of solutes in water (MV_w) and the energy of their interaction with water (ΔG_w).

EXPERIMENTAL

Materials

The HIO (series A in Table 1) chosen as sample solutes were prepared from aldoximes according to the literature procedure.[30] The four HIO and their electrooxidation products were identified by IR, UV and RMN-H spectroscopy.[31] Their purity was checked by RP-HPLC at different wavelengths using methanol-water eluent in the composition interval 1:4 - 3:2. Imidazoles of the series B-D were purchased from different suppliers: A2, A6, A7, A9, A12, A13, C16, E20 (Aldrich Chemical, Milwaukee, WI, USA), A8, A11, E19 (Fluka, Fluka Chemie AG, Buchs, Switzerland), A3, A4 (Chemservice), A5, A10 (Merck, Darmstadt, Germany), and A1 (Kodak). B14, B15, C17 were obtained by crystallization from pharmaceutical forms (Globe Chemical, Rhone-Poulenc Rorer and Xian-Janssen, respectively). HPLC-grade methanol was obtained from J. T. Baker Chemical (Phillipsburg, NJ, USA).

Equipment

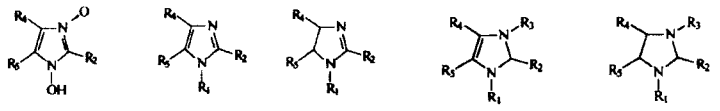
The chromatographic system consisted of a Model Constametric I pump (LDC Analytical, Riviera Beach, Fl, USA) and a Model 7125 valve injector (Rheodyne, Cotati, CA, USA) with a 20 μL loop. For the HIO, the study was carried out using a SpectroMonitor D (LDC Analytical) absorbance detector operated at the maximum absorbance wavelengths of each compound (λ_{max}(A1) = 244 nm, λ_{max}(A2) = 275 nm, λ_{max}(A3) = 270 nm, λ_{max}(A4) = 275 nm). The detector outputs were monitored by a Shimadzu C - R5A Chromatopac (Kyoto, Japan) integrator. For the substituted imidazoles, a Waters 994 programmable photodiode array detector (Waters Chromatogaphy Division, Milford, MA, USA) operated at 220 and 254 nm was used. The temperature control consisted of a Goldenfoil TM (Systec, Inc., Minneapolis, MI, USA) dual zone column temperature control system (± 0.5°C).

The molecular refractivity was achieved by refraction index and solution density measurements using a Reichert Abbe Mark II (Buffalo, NY, USA) refractometer at thermostated (25°C) temperature and 25 cm^3 pycnometers. For the determination of the molecular weight of the associates of A2 and A4, an Osmometer Osmomat 070-B (Gonotec GmbH, Berlin, Germany) was used. The equipment used in the electrochemical work is described in reference 31.

Table 1

Structural Formulae and Names of the Studied 1-Hydroxy-Imidazole-Oxides, Substituted Imidazoles, and Imidazole Derivatives

A B C D E



Series	Number	Compound Name	R_1	R_2	R_3	R_4	R_5
A	1	1-Hydroxy-imidazole-3-oxide		-H		-H	-H
	2	1-Hydroxy-2,4,5-trimethyl-imidazole-3-oxide		$-CH_3$		$-CH_3$	$-CH_3$
	3	1-Hydroxy-2-benzoyl-5-phenyl-imidazole-3-oxide		$-COC_6H_5$		-H	$-C_6H_5$
	4	1-Hydroxy-2,4,5-phenyl-imidazole-3-oxide		$-C_6H_5$		$-C_6H_5$	$-C_6H_5$
B	1	Imidazole	-H	-H		-H	-H
	2	1-Methylimidazole	$-CH_3$	-H		-H	-H
	3	2-Methylimidazole	-H	$-CH_3$		-H	-H
	4	4-Methylimidazole	-H	-H		$-CH_3$	-H
	5	1,2-Dimethylimidazole	$-CH_3$	$-CH_3$		-H	-H
	6	2-Ethylimidazole	-H	$-C_2H_5$		-H	-H
	7	4,5-Dicyanoimidazole	-H	-H		-CN	-CN
	8	1-Bencylimidazole	$-CH_2C_6H_5$	-H		-H	-H
	9	1-Phenylimidazole	$-C_6H_5$	-H		-H	-H
	10	2-Phenylimidazole	-H	$-C_6H_5$		-H	-H
	11	4-Phenylimidazole	-H	-H		$-C_6H_5$	-H
	12	4,5-Diphenylimidazole	-H	-H		$-C_6H_5$	$-C_6H_5$
	13	2,4,5-Triphenylimidazole	-H	$-C_6H_5$		$-C_6H_5$	$-C_6H_5$
	14	Metronidazole $C_6H_9N_3O_3$	$-C_2H_4OH$	$-CH_3$		-H	$-NO_2$
	15	Benzoylmetronidazol $C_{13}H_{43}N_3O_4$	$-C_2H_4OCOC_6H_5$	$-CH_3$		-H	$-NO_2$
C	16	2-Phenyl-2-Imidazoline	-H	$-C_6H_5$		$-H_2$	$-H_2$
	17	Astemizol $C_{28}H_{31}FN_4O$	$-CH_2C_6H_4F$	-NH-R_6*		Benzimidazol ring	
D	18	4,5-Diphenyl-2-imidazolone	H	=O	-H	$-C_6H_4$	$-C_6H_5$
E	19	2-Imidazolidone	-H	=O	-H	$-H_2$	$-H_2$
	20	1,3-Dimethyl-2-imidazolidinone	$-CH_3$	=O	$-CH_3$	$-H_2$	$-H_2$

* R_6: $-CHC_4H_8NC_2H_4PhOCH_3$

Sample Preparation

For dissolving imidazoles a few organic solvents are available. Here, we used pure ethanol and water-methanol mixture in the same proportion as in the mobile phase. For the chromatographic work and the refraction index measurements compounds (A1-A4) were dissolved in the mobile phase solution (water/methanol, 1:1), adjusting the pH at value 9 using 50% NH_4OH solution. For the RP-HPLC of substituted imidazoles and osmotic pressure measurements of A2 and A4, solutions were prepared in ethanol. The solutes were injected individually and the concentration of the solutions was in all cases 1 x 10^{-3} M.

Chromatographic Conditions

The following HPLC columns were used: C_{18} silica ISCO (Lincoln, Nebraska, USA) for the HIO and C_{18} Beckman (Fullerton, CA, USA) for the 20 imidazole derivatives. The column dimensions were 25 cm long with a 4.6 mm I.D. The packing specifications of the ISCO-RP column reported by the manufacturer were Spherisorb ODS-2 column material with a mean particle diameter of 5 μm, a mean pore diameter of 8 nm, a surface area of 220 m^2g^{-1} and 12% carbon content. The Beckman C_{18} column consisted of Ultrasphere column material with a mean particle diameter of 5 μm and a mean pore diameter of 8 nm, a surface area of 200 m^2g^{-1} and 12% carbon content. Mobile-phase was prepared from deionized water by measuring separately and then mixing known volumes of methanol and water. Every mixture was sonified prior to its use for at least 20 min. The column was equilibrated with at least 30 column volumes of the liquid solvent mixtures prior to any injection. A controlled flow rate of 0.5 mL/min was established by monitoring the time taken for the eluent to fill a 10.0 cm^3 graduated buret in a measured period of time. The retention time of the individual compounds (A1-A4) was measured isothermally in the temperature interval 25-50°C, in 5°C steps.

Determination of Adsorption and Structural Parameters

The Henry's adsorption constant (K_H) and the thermodynamic adsorption properties were calculated from the temperature dependence using the formulas:

$$K_H = V_{m,1} = \lim_{c \to 0} V_m = \lim_{c \to 0} V_R'/m \qquad (1)$$

where m is the adsorbent mass packed into the column.

$$\ln V_{m,1} = \overline{\Delta S_{ads}}/R + \overline{\Delta H_{ads}}/RT \quad (2)$$

$$\Delta G_{ads} = \overline{\Delta H} - T\overline{\Delta S} \quad (3)$$

Dead volume was measured by eluting a 50 ppm aqueous solution of sodium nitrite.[32] The values were 2.27 cm^3 for the C_{18} ISCO column and 1.93 cm^3 for the C_{18} Beckman column. These values were measured at 25°C, flow rate of 0.5 cm^3/min and mobile phase composition methanol-water (1:1). All measurements were made in at least triplicate. The average reproducibility was 1.0%. The hydrophobic parameter log k_w was calculated using the extrapolation technique of the relation between log k and the composition of the mobile phase.

Molecular refractivity (R_M) of HIO in solution was calculated after the Lorentz-Lorentz equation taking the solution density of the mobile phase (methanol-water 1:1). Molecular connectivity index, Van der Waals volume and correlation factor were calculated by the usual formulas.[33] The dipole moment (μ), the total energy (E_t), and the polarity parameter (Δ)[34] were achieved by the MMX force field method with the software PC Model version 1.0 from Serena Software (Bloomington, IN, USA). The partial molar volume of the compounds in water (MV_w) and the energy of their interaction with water (ΔG_w) as well as k_{calc} values were calculated by the Software Chromdream version 1.07 from Knauer (Herbert Knauer GmbH, Berlin, Germany). The regression equations were calculated on a Compaq 486 personal computer using the Quattro Pro program.

RESULTS AND DISCUSSION

The series of studied compound solutes consisted of four HIO, 15 substituted imidazoles, two imidazolines, one imidazolone, and two imidazolidone derivatives. In order to organize the compounds better, the entries in Table 1 are grouped according to the type of imidazole derivative and within a group are ranked in order of increasing number and bulkiness of the substituents.

In RP-HPLC, two types of forces dominate the interactions between solute molecules and stationary and mobile phases: the polar forces, arising from permanent or induced electric fields of solute, stationary and mobile phases, and the nonspecific forces resulting from dispersive interactions. The ability of a solute to undergo the last type of interactions are most readily parameterized

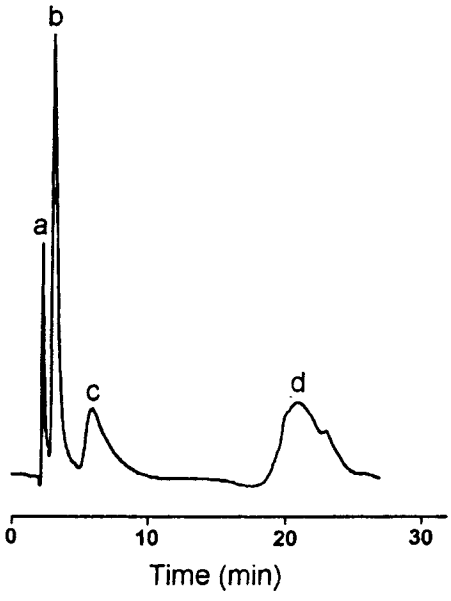


Figure 1. Chromatographic separation of a model mixture of 1-hydroxyimidazole-N-3-oxides. Stationary phase: Spherisorb ODS-2. Mobile phase: water-methanol (1:1). Flow rate 0.7 cm^3 min^{-1}. Temperature 45°C. UV detection at 220 nm. a = 1-hidroxyimidazole-3-N-oxide, b = 1-hydroxyimidazole-2-benzoyl-5-phenyl-3-N-oxide, c = 1-hydroxyimidazole-2,4,5-trimethyl-3-N-oxide, d = 1-hydroxyimidazole-2,4,5-triphenyl-3-N-oxide.

by common measures of solute "bulk" properties, like Van der Waals volumes, surface area, molar refractivity, or connectivity indices. By the studied HIO one can expect a large hydrogen bonding and electrostatic interaction ability, as well as a good capacity of the functional groups for dispersive interaction. Therefore the interaction of the solute molecules with the polar mobile phase in RP-HPLC and with themselves cannot be neglected. The role of the N-3-oxide group in the HIO in intermolecular hydrogen bonding has been demonstrated by different techniques.[35] From the RP-HPLC elution pattern for HIO, the role for intermolecular hydrogen bonding in polar aqueous-organic solution was expected to be established. Because spatial considerations prevent the 2-benzoyl-5-phenyl HIO (A3) from intermolecular interactions between hydroxyl and N-oxide groups of two A3 molecules, the retention properties of this HIO relative to the trimethyl and triphenyl-HIO should demonstrate the effect of intermolecular interactions. This structure-retention behaviour study was conducted on C_{18} chemically bonded silica stationary phases using a variety of

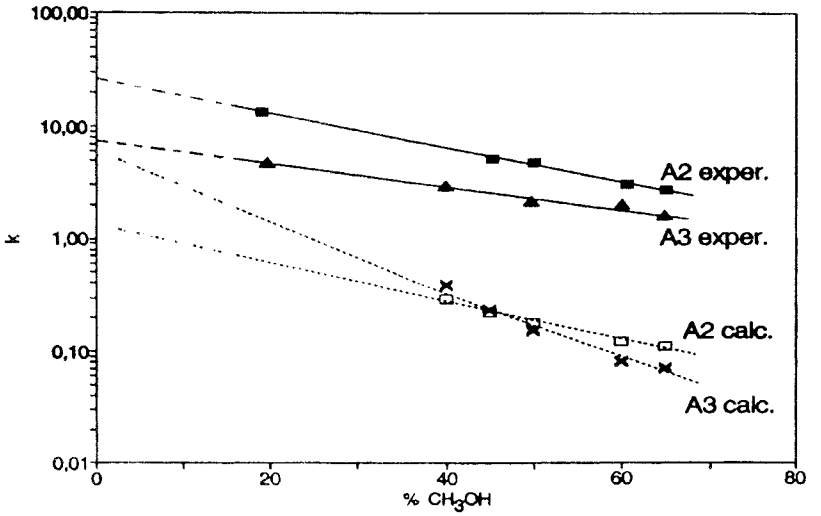


Figure 2. Relationships between log k and the volume fraction of methanol in mobile phase for 1-hydroxyimidazole-2,4,5-trimethyl-3-N-oxide (A2) and 1-hydroxyimidazole-2-benzoyl-5-phenyl-3-N-oxide (A3). Solid line, retention data determined experimentally on Spherisorb ODS-2; dotted line, calculated data on Spherisorb ODS-2.

methanol-water mobile phase compositions and temperatures. The initial results are illustrated in Figures 1 through 3. Figure 1 shows the reversed-phase C_{18} separation of a model mixture of HIO. The non substituted HIO has the lowest k, followed by the 2-benzoyl-5-phenyl and trimethyl-HIO. Here triphenyl-HIO (A4) displays the highest k value. The increased affinity of the A4 for the C_{18} stationary phase should be related to its increased hydrophobicity caused by the formation of a dimer A4-D. As the mobile phase composition becomes weaker, the retention of all four HIO increases with the trimethyl-HIO (A2) showing greatly enhanced retention over the 2-benzoyl-5-phenyl-HIO due to the formation of the trimer A2-T. Figure 2 illustrates the experimental and calculated log k values plotted against the solvent composition for the 2,4,5-trimethyl-HIO (A2) and 2-benzoyl-5-phenyl-HIO (A3).

The experimental plots for A2 and A3 are very similar over the entire solvent range, and the formation of an intermolecular hydrogen bond shifts the plot for the A2-HIO to higher log k values at each solvent composition. For these compounds the experimental capacity factors are greater than the calculated ones. It is worthseeing that experimental and calculated lines for A2 lie parallel, while for the A3-HIO they tend to almost the same k value at 0%

methanol content in the mobile phase. Since over limited ranges of binary composition, a linear relationship between ln k and mobile phase composition can often be used as a good approximation, we used a linear least-squares program to compare the experimental and calculated log k data for the A2 and A3-HIO. The Y-intercept, experimental log k_w (k_w=capacity factor at 100% H_2O mobile phase composition), for the A2 and A3-HIO was 1.44 and 0.82 (R=0.993 and 0.936) respectively and the calculated log k_w was 0.11 and 0.70 (R=0.986 and 0.971) respectively. It has been stated in other works that log k_w can be used directly as an hydrophobic parameter and it may be regarded as a useful substitute for log P. This gives as a result, that A2 presents an experimental hydrophobicity greater than A3, while the calculated hydrophobicity in terms of log k_w is greater for A3 than for A2. The same happened with the calculated log P [36] values, where A3 showed a value of 1.27 and A2 of 0.93. The reversed phase retention data for these HIO illustrated in Figures 1 through 2 certainly point out the unique hydrophobic nature of the 2,4,5-trimethyl-HIO relative to the 2-benzoyl-5-phenyl-HIO. Thus, these retention results demonstrate the existence of N-OH···O intermolecular hydrogen bonding which can form a n-member associate. The additional carbonyl group in the A3-HIO is suggested to stabilize the N-OH···O association between two molecules through the formation of an intramolecular hydrogen bond (O-H···O) between the carbonyl oxygen and the 1-hydroxy group. Another explanation for the lack of association between A3 molecules is the relative strengths of the hydrogen bonds, where the O-H···O association is known to be stronger than N-OH···O interactions. The curve for the 2-benzoyl-5-phenyl-HIO (Fig. 2) is shifted well below that of the 2,4,5-trimethyl-HIO suggesting not only the lack of enhanced hydrophobicity due to intermolecular association but a significant increase in polarity. Figure 1 has demonstrated the dramatic effect of intermolecular hydrogen bonding on the C_{18} retention of the 2,4,5-trimethyl-HIO. Here we can also observe that the 2,4,5-triphenyl-HIO (A4) displays marked retention relative to the A3 and A2-HIO. Our additional studies determining the molecular weight showed that A2-HIO exists in the three-member pseudoring form (A2-T) as reported in,[35] while A4 forms a two member associate (A4-D) in aqueous solution through N-OH···O association.

Much effort has been directed to describing retention in terms of classical thermodynamics. In an attempt to extend this study, we have investigated the effect of temperature on the retention times observed for HIO in RP-HPLC. As can be seen from Fig. 1 and Table 2 the Henry's adsorption constant and the enthalpy of adsorption in the HIO series on the reversed phase increases in the same way as the elution order. The plot of log k of HIO measured at 40°C against the corresponding enthalpy change is linear (R = 0.944) indicating a possible enthalpy-entropy compensation.

Table 2

Thermodynamic Characteristics of the Adsorption of HIO from Methanol-Water (1:1) Solution on Hydrophobic Silica Surface*

Compound	M	K_H (cm^3g^{-1})	$-\Delta H$ ($kJ\ mol^{-1}$)	ΔS ($J\ mol^{-1}\ K^{-1}$)
A1	100	0.9	1.9 ± 1.8	- 7.2 ± 0.2
A2-T**	426	7.8	12.6 ± 4.5	- 22.8 ± 0.7
A3	280	2.4	5.2 ± 1.1	- 8.2 ± 0.01
A4-D***	656	30.5	15.0 ± 4.4	- 20.1 ±0.9
B1	68	2.2	6.8 ± 2.0	- 15.2 ± 0.2

* Henry's constant measured at 40°C.

** A2-T is a trimer of 1-hydroxyimidazole-2,4,5-trimethyl-N-3-oxide.

*** A4-D is a dimer of 1-hydroxylimidazole-2,4,5-triphenyl-N-3-oxide.

When only the free energies of retention of the different HIO are compared, no accurate information on the hydrophobicity of the solutes can be obtained. Therefore the possibility of relating structural parameters of the studied compounds with retention offers an indirect approach to hydrophobicity.

Some authors suggested that retention is influenced by two terms: the bulk term, related to the solute polarizability, via its molar refractivity and thus to the ability for nonspecific interaction between the solute and the stationary phase, and the polar term, related to the solute polarity via its dipole moment. Various measures of the molecular ability of a solute to undergo dispersive interactions have been successfully applied to explain the retention of homologous series. Molar refractivity is found to be a good descriptor of retention of solutes chromatographed in GC. In our case it was seen that, as would be expected, the refractive index of A2 and A4 was higher than that of either A1 or A3 and log k resulted highly correlated with R_M (see Table 3) when A2 and A4 were taken in its associated form. Up to this moment, molecular descriptors V_W, χ and F were calculated taking A2 and A4 as the associated species A2-T and A4-D. It should be mentioned that log k of HIO gives a much poorer correlation with molar refractivity calculated from bond refractivities,[33] than with the experimental R_M values. In[4] the role of R_M in the RP-HPLC retention of benzodiazepines has been demonstrated. Dispersive, inductive, and orientative interactions taken together can be primarily related to molecular size, or van der Waals volume.

Table 3

Relationship Between Log k of HIO at 40°C and Various Structural Descriptors, Using Aqueous Methanol (1:1) Solution as Mobile Phase

Type of Parameter	Structural Descriptor	Standard Deviation*	Correlation Coefficient*
Bulk	R_M	0.002** 1.986*** (0.367)	0.999** 0.813*** (0.999)
Bulk	V_W	7.400 1.000	0.923 (0.998)
Bulk	M	3.228	0.996
Shape	F	0.663	0.972
Shape	X	0.179 (0.188)	01984 (0.975)

* In parenthesis, the relationship between ΔG_{ads} at 40°C and the structural descriptor is given.

** Correlation between log k and the experimental molar refractivity.

*** Correlation between log k and the calculated molar refractivity.

Alkanes, alkylbenzenes, and alkylalcohols each showed a good linear relationship between log k in HPLC and Van der Waals volumes.[6,8,37,38] The correlation factor obtained between log k of HIO in RP-HPLC and V_W is markedly lower (R=0.923) than the factor in log k vs. R_M, but is still significant. It has been reported that the retention in HPLC of pyrazine carbothioamide derivatives[39] and of alkylbenzenes[40] depends quantitatively on the shape of the molecule. We obtained significant correlations with R coefficients of 0.972 and 0.984 for the shape parameters F and χ respectively. A high correlation between log k and F has been found; for example, for PAHs[1] and in reference 37, connectivity has been used as structural parameter in the construction of a retention system for alkylbenzene derivatives. In the case of the studied HIO, the bulk parameter R_M predominates, and thus, the shape contribution to retention becomes less significant.

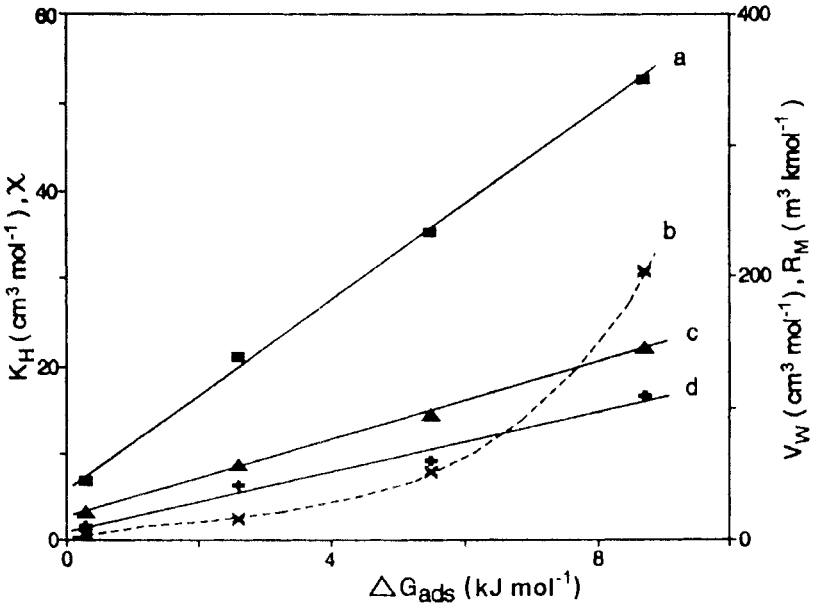


Figure 3. Correlation between ΔG_{ads} and Van der Waals volume (a), Henry's constant (b), molar refractivity (c) and connectivity (d) of 1-hydroxyimidazole-3-N-oxides. ΔGads on Spherisorb ODS-2 was calculated at 40°C using water-methanol (1:1) as mobile phase.

The structural descriptors used in this work are highly interrelated, therefore no attempt has been made to fit retention to a multiparameter equation. On the average, the coefficient of correlation varies from 0.923 to 0.999 as the bulk and shape structural descriptors were varied.

Finally, to investigate whether association of HIO is reflected on adsorption, the effect of calculated structural descriptors on the correlation between ΔG_{ads} and structure was tested. Figure 3 shows the correlation between the discussed structural parameters, Henry's adsorption constant, and ΔG_{ads}.

A linear relationship was achieved for R_M, V_W and χ. Table 3 shows again a better fit to linearity when log k is plotted against the bulk parameter R_M. With K_H a parabolic relationship was observed.

Table 4

Capacity Factor of Substituted Imidazoles and Imidazole Derivatives in 60% CH_3OH/H_2O

Compound	k	μ*, D	E_t*, kcal/mol^{-1}	Δ*
A1		4.28	6.638	1.03
A2	5.09	5.03	6.445	1.14
A3	1.44	1.87	232.358	0.98
A4		3.65	244.573	0.98
B1	0.89	3.03	10.594	0.76
B2	0.46	3.47	14.144	0.55
B3	0.68	3.71	12.434	0.69
B4	0.70	3.26	13.160	0.58
B5	0.82	3.60	14.555	0.69
B6	0.70	3.71	13.518	0.69
B7	0.03	6.96	21.404	0.97
B8	2.05	3.40	22.348	0.55
B9	1.71	4.10	133.475	0.55
B10	2.62	3.27	25.283	0.49
B11	1.21	3.61	99.511	0.53
B12	10.75	3.37	42.916	0.55
B13	36.35	3.15	56.288	0.48
B14	0.22	5.59	12.019	0.74
B15	2.15	4.53	21.673	0.73
C16	19.87	2.98	15.353	0.58
C17	14.62	3.68	55.542	0.83
D18	6.08	5.65	25.867	0.70
E19	0.12	4.23	-3.606	0.44
E20	0.25	4.32	3.227	0.41

* Calculated by the Software PC Model.

It appeared that, because of the close structural similarities of the compounds of the series B - E, they would all interact in a similar way with the column. Capacity factor k was measured in different RP-HPLC systems and some results are given in Table 4. An interesting and challenging task was to relate the electronic structural information to the chromatographic retention of solutes. The majority of reports published to date concerning structure-retention behaviour deal with homologous series of compounds. Less attention has been paid to cogeneric groups of compounds. It is known that when the

imidazole ring has polar substituents, the dipole moments of the molecule are of the order of 3.8 - 4.0 D. N-arylation lowers the dipole moment due to conjugation of the imidazole and aryl rings. Neither N-alkylation, nor carboxilic rings condensed with imidazole have much effect on μ. Its magnitude, however, indicates considerable polarization of the ring, although the extent of polarization is much less than the required to yield a ionic structure. The values obtained are indeed very similar among the compounds of the B series, except for the 4,5-dicyanoimidazole. In this part of the work we have considered that molecular shape is not as important for retention, as solute polarity and its dispersive properties, so we considered the total dipole moment. It is known however, that the total dipole moment of a solute differs from the effective dipole in a chromatographic system. Therefore, among the quantum chemical indices, the total energy (E_t) and the submolecular polarity parameter (Δ) were found to be meaningful for relating chromatographic capacity factors. The numerical data are listed in Table 4.

A retention decreasing effect has been found for μ^2 in the RPLC of benzodiazepines,[4] while on the other side, μ is involved in a retention equation.[38,41] Dipole moment was found however to be a worse descriptor of retention than E_t and Δ.[34] Although the calculated total energy is the parameter of deciding importance for retention on nonpolar stationary phase of a cogeneric group of solutes of nearly equal polarity, when log k of imidazoles was plotted against each of these molecular descriptors, a poor correlation was obtained. Dipole moments as well as substituent increments[42,43] have been occasionally used to correlate structure with chromatographic behaviour. Taking the twenty compounds of the series B - E, the linear relationship between retention and the substituent increment ($\Delta s_i = k_i - k_{imidazole}$), is given by the following equation: $k_{exp,i} = 0.97 + 1.00\ \Delta s_i$ ($R = 0.999$).

Although total energy has also been considered the most reliable descriptor of the ability of solutes to participate in dispersive chromatographic interactions, the parameters reflecting size of the solutes may be correctly interpreted as reflecting the ability of solutes to participate in nonspecific interactions. Molar volume is a molecular descriptor that can be used as a measure of solute dispersive properties. It describes the dependence of log k satisfactorily in RP-HPLC of cogeneric nonpolar solutes like PAHs[1] or alkylbenzenes[40] and gives a well known relationship for homologous series of organic and even organosilicon compounds in GC.[44] For this purpose we tested first the description of imidazoles retention by the molar volume in water MV_w, which together with the energy of interaction with water, constitutes "hydrophilic" interaction parameters.

Table 5

Correlation Between Retention in 60% CH_3OH / H_2O of Imidazoles of the Series B and the Calculated Energy of Their Interaction with Water ΔG_W

Stationary Phase	Equation	n*	R
C_{18} Ultrasphere	$\log k_{exprt} = -1.905 - 0.032\ \Delta G_W$	12	0.946
C_{18} Spherisorb**	$\log k_{cacl} = -1.935 - 0.035\ \Delta G_W$	12	0.882

* n is the number of points taken in the regression analysis.

** Log k was calculated by the Software Chromdream. Compound B7 was excluded.

However, neither electronic descriptors nor molar volumes were useful for describing the retention of series B - E in RP-HPLC (the retention-hydrophobicity study will be published later). The energy of interaction with water, proved more promising than any other parameter in the system investigated. Therefore, the use of ΔG_w as the parameter for constructing the system for the substituted imidazoles with C_{18} packing seems an appropriate compromise, though the system could be constructed with a combination of ΔG_w and any related parameter bulk parameter. This will be discussed at a later date. Table 5 shows that while there is reasonable agreement between the dependence of log k calculated from experimental data and that calculated from theoretical values, the correlation factor is much greater when calculated from experimental data. The capacity factor values calculated from theory are consistently smaller than the experimental measurements. They are linearly related by the expression $k_{exper} = 0.366 + 0.639\ k_{calc}$ with $R = 0.987$, where the two imidazolines were excluded from regression due to its significant deviation from the regression line. The correlation was especially high when the k values lay below 10. The results indicate that the Chromdream calculation software based on the parameters MV_w and ΔG_w is suitable for the calculation of log k, of imidazole compounds with and without polar groups.

As an example of the application of HPLC of HIO in electrochemical investigations, the chromatograms of a mixture after electrolysis of 1-hydroxyimidazole-2,4,5-trimethyl-3-N-oxide is shown in Figure 4. Collected fractions of each component were identified by independent spectroscopic methods as diacetylmonoxime, dimethylglioxime and 2-acetoxyimino-3-oximinobutanone. Here HPLC served in the establishment of the electrochemical oxidation mechanism of HIO[31,45] and in the influence of pH.

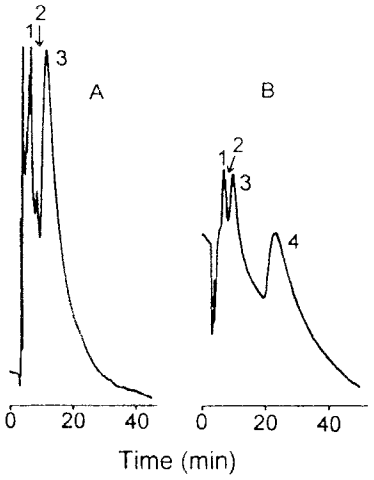


Figure 4. Chromatograms obtained after electrolysis of 1-hydroxyimidazole-2,4,5-trimethyl-3-N-oxide at 950 mV on Au electrode in Britton-Robinson buffer. (A) Electrooxidation at pH 11 and extraction with chloroform. (B) Electrooxidation at pH 4 and extraction with dietylether. Stationary phase: Spherisorb ODS-2. Mobile phase: water-methanol (1:1). Flow rate 0.5 cm^3 min^{-1}. Ambient temperature. UV detection at 230 nm. 1 = diacetylmonoxime, 2 = dimethylglyoxime, 3 = 2-acetoxyimino-3-oximinobutanone, 4 = 1-hydroxi-2,4,5-trimethylimidazole-3-oxide.

CONCLUSIONS

In the case of HIO, their chromatographic behaviour proved to be very informative since it served to confirm intermolecular association, determined a priori. The trimer formed by the association of the N-oxide and the hydrogen of the 1-hydroxy group in 1-hydroxyimidazole-2,4,5-trimethyl-3-N-oxide and the dimer of 1-hydroxyimidazole-2,4,5-triphenyl-3-N-oxide, produces enhanced reversed-phase retention relative to the 1-hydroxyimidazole-2-benzoyl-5-phenyl-3-N-oxide. Retention and ΔG_{ads} of HIO are highly correlated with bulk and shape parameters.

In our work, neither total energy or submolecular polarity descriptors gave high correlation with log k of substituted imidazoles. Hydrophilicity in terms of energy of interaction with water succeeded in the description of retention of substituted imidazoles with nonpolar moieties in RP-HPLC.

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