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Molecular topology and chromatographic retention parameters for benzodiazepines

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ABSTRACT

The relationship between gas–liquid chromatographic (GLC) retention properties and R_F values in thin-layer chromatography (TLC) with molecular connectivity indices, $^m\chi_t$, was investigated for a series of benzodiazepines using multiple correlation coefficients, standard errors of estimate, *F*-Snedecor function values and Student's *t*-test as the criteria for best equation selection. Regression analyses show that the molecular connectivity model predicts the retention properties in GLC with the polar stationary phase OV-17 at 280°C and the R_F values in TLC with the stationary phase silica gel. However, zero- or second-order connectivity indices alone are not sufficient; higher-order indices are shown to be necessary. The effect of the polarity of the mobile phases in TLC was also investigated.

INTRODUCTION

Quantitative structure–activity relationship (QSAR) studies are used to explain or predict the physicochemical [1,2] or pharmacological [3–6] behaviour of drug molecules. Attempts have been made to develop a numerical description of a molecule derived not from experimental measurements of a property but from knowledge of the molecular structure itself [7]. Molecular topology transcribes molecular structure into a topological graph from which a number is derived, the topological index. Topological parameters, such as the molecular connectivity indices [8], can be used to quantify these properties.

The degree of retention in a chromatographic experiment depends on the structure and properties of the stationary phase and the molecular characteristics of the solute (volume, temperature, partition coefficient of each molecule, etc.). Experimental retention data of several groups of molecules on a given stationary phase can be correlated with parameters describing the molecular structure [9]. Unfortunately, the only criterion used to test the relationship between the observed and calculated retention properties in these experiments is the statistical correlation coefficient. This criterion is insufficient for predicting retention properties since a high correlation coefficient does not necessarily imply a correct elution sequence [10]. Other results indicate that the empirical additive scheme will not be able to reproduce adequately the retention indices of chlorinated benzenes unless a large number of parameters are employed [11,12].

Kier and Hall [13] have established that chromatographic behaviour depends on both topological and non-topological molecular structural characteristics. It seems that, for polar columns, the topological characteristics are more important. Other, later studies [14–16] have established that chromatographic parameters in a polar stationary phase system correlate better with the valence connectivity indices, whilst Kovat's parameters, obtained from

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the apolar phase interaction, show best correlation with the non-valence connectivity terms.

In this study, the connectivity indices of nineteen benzodiazepines with different chromatographic properties were compared: retention times (t_R) in seconds, retention indices (RI) and R_F values are those reported in ref. 17.

Some reports correlating the chromatographic behaviour of drugs with molecular connectivity, for example the barbiturates [18] and the neuroleptics [19], have been published. However, the benzodiazepines have not been investigated.

METHOD OF CALCULATION

Connectivity indices are calculated from a hydrogen-suppressed formula or graph of the molecule, following the method of Kier and Hall [20]. The general form of the indices, ${}^m\chi_t$, is found by assigning to each vertex (non-hydrogen atom) in the molecular graph a value (δ) which is the number of edges (bonds) to that atom, bonds to hydrogen being ignored. Thus, for a graph of m edges and s subgraphs (binding between $m+1$ atoms), ${}^m\chi_t$ is calculated according to eqn. 1.

$$ {}^m\chi_t = \sum_{s=1}^{n_m} \prod_{i=1}^{m+1} (\delta_i)_s^{-1/2} \quad (1) $$

where n_m is the number of paths. Connectivity indices describing non-linear arrangements of bonds, such as clusters of three bonds, ${}^3\chi_c$, and path clusters of four bonds, ${}^4\chi_{pc}$, are calculated in the same way.

The vertex valences, δ^v, of the unsaturated carbon atoms and the heteroatoms (N or O) can be calculated using eqn. 2.

$$ \delta^v = Z^v - N_H \quad (2) $$

where Z^v is the number of valence electrons of the atom and N_H is the number of hydrogen atoms attached to it. The empirically derived values for the halogens were also used [21].

Single and multiple regression analyses were used to find the relationship between the gas chromatographic properties and the connectivity indices, and are calculated from eqn. 3.

$$ P = A_0 + \sum_{m,t} A_{m,t}{}^m\chi_t \quad (3) $$

where P is a property, and A_0 and $A_{m,t}$ represent the regression coefficients of the obtained equation.

Eqn. 3 was obtained by multilinear regression with 9R and 5R programs of the biostatistic package BMDP (Biomedical Computer Programs) [22]. To test the quality of the regression equations, the following statistical parameters were used: multiple correlation coefficient (r), standard error of estimate (s), F-Snedecor function values (F) and Student's t-test (statistical significance).

The retention time (t_R) in seconds and the retention index (RI) values in gas–liquid chromatography (GLC) used in this study, reported by Schütz [17], were obtained at 280°C with a 1.5 m × 2 mm I.D. glass column packed with 3% OV-17 on Chromosorb G AW DMCS (80–100 mesh) as the polar stationary column and nitrogen as the carrier gas at a flow-rate of *ca.* 15 m/min. The R_F values in thin-layer chromatography (TLC) were obtained with precoated TLC plates, silica gel 60 F_{254}, 20 cm × 20 cm, layer thickness 0.25 mm, activated for 1 h (110°C), saturated chamber, ascending method, length of run 10 cm, 20°C, and two solvent systems: chloroform–acetone (90:10, v/v) and benzene–isopropanol–25% ammonia solution (85:15:1, v/v/v).

RESULTS AND DISCUSSION

The connectivity indices and experimental chromatographic properties of nineteen benzodiazepines examined in this study are shown in Tables I and II, respectively.

Essentially, all these parameters represent the degree of affinity between the solute considered and the two phases, namely stationary and mobile. This affinity is closely related to the molecular solubility in both phases, and it is quantified by the distribution coefficient value for the solute in the two phases. This solubility, in turn, basically depends on two factors: first, the polar character of the solute (evaluated by its dipolar moment value) and, second, the solvent's capacity for solute solvation.

The selected equations for the retention times and retention indices in GLC of the compounds studied were, respectively:

$$ t_R = 1047.2\,{}^2\chi - 797.0\,{}^2\chi^v - 556.7\,{}^4\chi_{pc} + 461.7\,{}^4\chi^v_{pc} - 1402.7 \quad (4) $$

$$ n = 19 \quad r = 0.946 \quad s = 87.54 \quad F = 29.66 $$

TABLE I

CONNECTIVITY INDICES USED IN THE CORRELATIONS OF A GROUP OF BENZODIAZEPINES

Compound	$^0\chi$	$^2\chi$	$^2\chi^v$	$^3\chi_p$	$^3\chi_c^v$	$^4\chi_p$	$^4\chi_p^v$	$^4\chi_{pc}$	$^4\chi_{pc}^v$
Chlordiazepoxide	13.110	5.945	5.198	4.273	0.556	3.176	2.641	1.530	1.050
Demoxepam	12.240	5.786	4.966	3.980	0.556	3.109	2.535	1.468	0.985
3-Desmethylchlordiazepoxide	12.403	5.780	5.082	3.913	0.647	3.072	2.513	1.446	1.126
Diazepam	12.188	5.659	5.122	4.231	0.541	2.912	2.739	1.697	1.063
Nordiazepam	12.163	5.285	4.568	3.554	0.485	2.497	2.147	1.278	0.746
3-Hydroxydiazepam	13.058	6.122	5.201	4.608	0.577	3.191	2.724	2.029	1.106
Oxazepam	12.188	5.731	5.139	4.118	0.655	2.828	2.480	1.614	1.175
Nitrazepam	12.179	5.496	4.566	3.981	0.430	2.884	2.262	1.201	0.887
7-Aminonitrazepam	11.317	5.275	4.384	3.676	0.452	2.684	2.135	1.195	0.849
7-Acetamidonitrazepam	13.232	6.240	5.021	4.090	0.541	3.076	2.399	1.326	0.928
Medazepam	11.688	5.553	5.039	4.063	0.443	2.967	2.815	1.419	0.914
Lorazepam	13.110	6.180	5.697	4.469	0.818	3.068	2.768	1.894	1.522
Prazepam	13.886	6.303	5.751	4.589	0.745	3.431	3.093	1.702	1.117
3-Hydroxyprazepam	14.757	6.766	5.882	4.977	0.791	3.652	3.108	2.047	1.192
Clonazepam	13.102	5.945	5.125	4.331	0.593	3.123	2.549	1.481	1.233
7-Aminoclonazepam	12.240	5.724	4.943	4.026	0.615	2.923	2.422	1.475	1.196
7-Acetamidoclonazepam	14.154	6.689	5.247	4.441	0.602	3.315	2.612	1.606	1.230
Clobazam	12.895	6.191	5.304	4.627	0.533	3.386	2.029	2.005	0.966
Norclobazam	12.025	5.808	4.928	4.071	0.549	3.180	2.570	1.503	0.864

TABLE II

EXPERIMENTAL VALUES FOR SEVERAL CHROMATOGRAPHIC PROPERTIES OF BENZODIAZEPINES USING THE MOLECULAR CONNECTIVITY METHOD

Compound	GLC		TLC	
	t_R (s)	RI	R_{F_A}	R_{F_B}
Chlordiazepoxide	180	3160	0.07	0.47
Demoxepam	169	3142	0.10	0.30
3-Desmethylchlordiazepoxide	474	3590	0.02	0.30
Diazepam	127	3020	0.52	0.74
Nordiazepam	162	3124	0.26	0.52
3-Hydroxydiazepam	194	3200	0.42	0.51
Oxazepam	93	2888	0.16	0.26
Nitrazepam	335	3455	0.24	0.48
7-Aminonitrazepam	369	3475	0.08	0.30
7-Acetamidonitrazepam	823	3815	0.04	0.18
Medazepam	71	2775	0.56	0.85
Lorazepam	115	2979	0.16	0.30
Prazepam	230	3178	0.63	0.79
3-Hydroxyprazepam	358	3375	0.56	0.61
Clonazepam	435	3518	0.27	0.48
7-Aminoclonazepam	470	3560	0.08	0.30
7-Acetamidoclonazepam	1200	3970	0.04	0.19
Clobazam	232	3170	0.52	0.59
Norclobazam	316	3297	0.22	0.45

and

$$RI = 1129.3\,{}^{2}\chi - 1210.0\,{}^{2}\chi^{v} + 1447.7\,{}^{3}\chi_{c}^{v} - 477.5\,{}^{4}\chi_{pc} + 2705.9 \quad (5)$$

$n = 19 \quad r = 0.911 \quad s = 124.12 \quad F = 17.05$

Statistically, eqns. 4 and 5 are significant above the 99.9% level, while the ${}^{2}\chi$, ${}^{2}\chi^{v}$ and ${}^{4}\chi_{pc}$ indices are significant above the 99.9% level and ${}^{3}\chi_{c}^{v}$ and ${}^{4}\chi_{pc}^{v}$ indices are significant above the 95% level. In both cases, there is dependence on the ${}^{2}\chi$ and ${}^{2}\chi^{v}$ indices and typically branching parameters such as ${}^{3}\chi_{c}^{v}$, ${}^{4}\chi_{pc}$ and ${}^{4}\chi_{pc}^{v}$ occur. The size of benzodiazepines is described and quantified by the ${}^{2}\chi$ indices, the numerical values of which are directly proportional to the number of bonds in a molecule, and by the substitution pattern given by structural parameters. The difference ${}^{2}\chi - {}^{2}\chi^{v}$ somehow measures the polar character of the molecule, whilst the branching indices, *i.e.* cluster and path cluster, take into account the solvation effects, closely related to steric aspects.

Graphical representations of the experimental and theoretical values for these properties following equns. 4 and 5 are given in Figs. 1 and 2, respectively.

For the R_F values (with high polar mobile phase, R_{F_A}, or lower polar mobile phase, R_{F_B}) in TLC, the best regression equations and their statistical parameters are as follows:

$$R_{F_A} = 0.47\,{}^{3}\chi_{p} - 0.78\,{}^{4}\chi_{p} + 0.80\,{}^{4}\chi_{p}^{v} - 0.66\,{}^{4}\chi_{pc}^{v} - 0.74 \quad (6)$$

$n = 19 \quad r = 0.927 \quad s = 0.07 \quad F = 21.31$

and

$$R_{F_B} = 0.23\,{}^{0}\chi - 0.87\,{}^{2}\chi + 0.85\,{}^{4}\chi_{p}^{v} + 0.54 \quad (7)$$

$n = 19 \quad r = 0.906 \quad s = 0.08 \quad F = 22.94$

Eqns. 6 and 7 are statistically significant above the 99.9% level and 99% level, respectively. ${}^{2}\chi$, ${}^{4}\chi_{p}$, ${}^{4}\chi_{p}^{v}$ and ${}^{4}\chi_{pc}^{v}$ are significant above the 99.9% level and ${}^{0}\chi$ and ${}^{3}\chi_{p}$ are significant at the 99% level. The dependence on the ${}^{4}\chi_{p}$, ${}^{4}\chi_{p}^{v}$ and ${}^{4}\chi_{pc}^{v}$ indices should be emphasized; it occurs when polar eluents [chloroform–acetone mixture (90:10, v/v)] are used. However, when mixtures with a lower polar character are

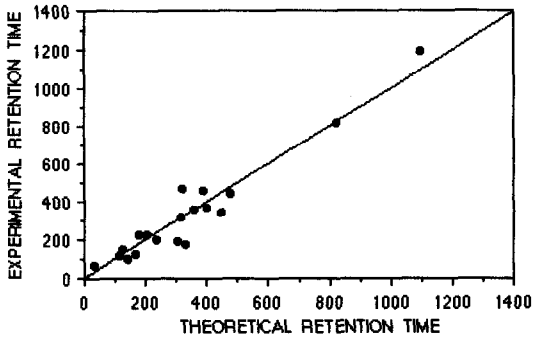

Fig. 1. Correlation between experimental (GLC with polar stationary phase, OV-17) and calculated (eqn. 4) retention times of nineteen benzodiazepines.

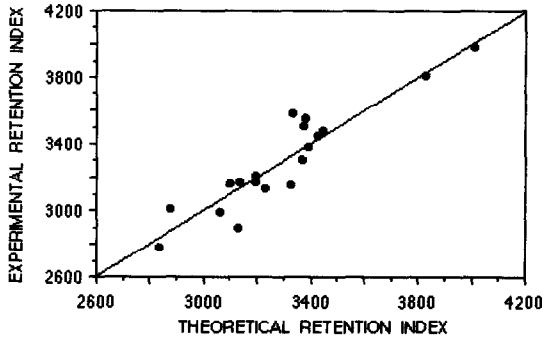

Fig. 2. Correlation between experimental (GLC with polar stationary phase, OV-17) and calculated (eqn. 5) retention indices of nineteen benzodiazepines.

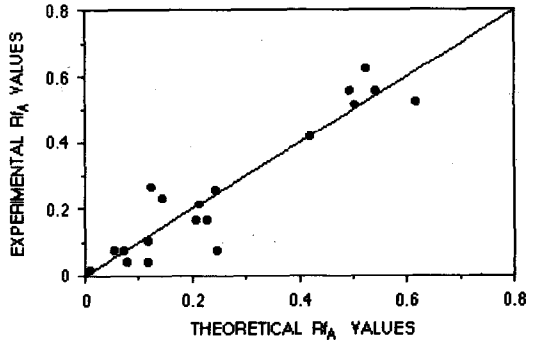

Fig. 3. Correlation between experimental (TLC with stationary phase silica gel and high polar mobile phase) and calculated (eqn. 6) R_{F_A} values of nineteen benzodiazepines.

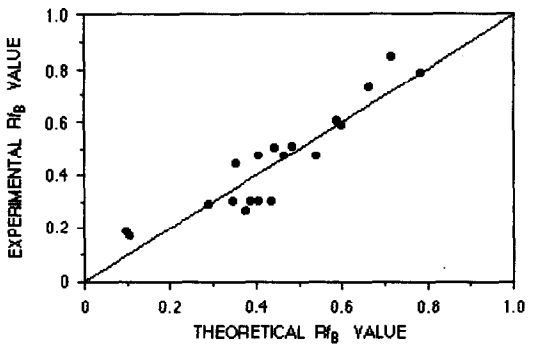

Fig. 4. Correlation between experimental (TLC with stationary phase silica gel and lower polar mobile phase) and calculated (eqn. 7) R_{F_B} values of nineteen benzodiazepines.

used, such as benzene–isopropanol–25% ammonia solution (85:15:1, v/v/v), these indices do not appear.

These results suggest that these indices, particularly $^4\chi^v_{pc}$, are a measure of the eluent's polar character. The comparison between experimental and theoretical R_F values is illustrated in Figs. 3 and 4.

This report demonstrates that a relationship exists between molecular connectivity and chromatographic retention parameters for a group of benzodiazepines. Generally a three or four-variable model is necessary to obtain a good degree of correlation.

CONCLUSION

The molecular connectivity model has been shown to be a useful tool for predicting and interpreting the different chromatographic retention parameters of benzodiazepines on different polarity phases. Statistical analyses show that the size of molecules and the structural terms control the drugs' chromatographic behaviour. In those cases concerning a system containing one polar phase, the dependence on the $^2\chi$, $^2\chi^v$ or $^4\chi_p$, $^4\chi^v_p$, together with a cluster or path cluster index, indicates that the three somehow evaluate the molecular dipolar moment, while the last estimates the solvent's solvation effects on the solute molecules.

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