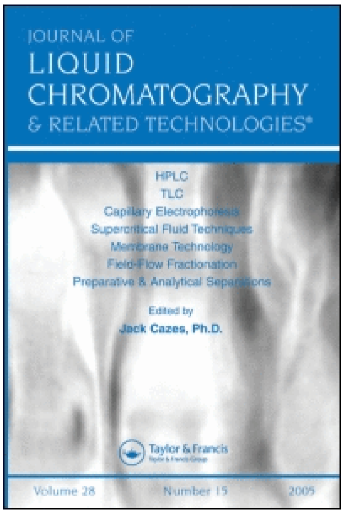

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High-Performance Liquid Chromatography of 1, 2, 6-Thiadiazinone 1, 1-Dioxides and Related Pyrazolones: A Comparative Study of Hydrophobicity

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF 1,2,6-THIADIAZINONE 1,1-DIOXIDES AND RELATED PYRAZOLONES: A COMPARATIVE STUDY OF HYDROPHOBICITY

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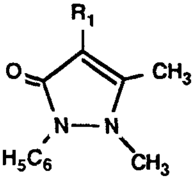
ABSTRACT

A comparative study of hydrophobicity in biological active pyrazolone derivatives **1** and **3** and related 1,2,6-thiadiazinone 1,1-dioxides **2** and **4** using HPLC (RPC) technique has been carried out. The capacity factors (K') and retention index (I) of the compounds studied have been measured in five different elution conditions (mobile phase in which the percentage of methanol varies). A correlation between hydrophobic parameters of series **1** and **2** has been established.

INTRODUCTION

The influence of hydrophobicity of drugs on their pharmacological *in vivo* activities is well known (1). For this reason, we have carried out a comparative study of the hydrophobic character in two related series of biological active compounds, pyrazolones **1** and **3** and 1,2,6-thiadiazinone 1,1-dioxides **2** and **4**. The compounds chosen for this study were the antipyretic and analgesic Antipyrine **1a**, their 4-substituted derivatives **1b-1e**, the antiinflammatory Phenylbutazone **3** and their analogues in 1,2,6-thiadiazine 1,1-dioxide series **2a-2e** and **4**. In previous works, the physico-chemical, chemical and biological parallelism between pyrazoles and related 1,2,6-thiadiazine derivatives was demonstrated (2,3,4).

Octanol-water partitions coefficients (log P) is an accepted model for hydrophobicity in quantitative structure-activity relationships (QSAR) (5,6). Nevertheless, measurement of log P by the "shake flask" method is subject to many inconveniences. Reversed-phase HPLC has been investigated previously as a means for determining hydrophobicity by attempting to correlate logK', a retention time parameter, with logP (7,8). Retention in HPLC has also been correlated directly with biological activity (7,9,10). Another approach, proposed by Baker, uses retention index (I) as a universal scale for hydrophobicity (11). The purpose was to make possible a direct comparison of retention data between different columns and mobile phases by using 2-ketoalkanes as retention reference compounds.



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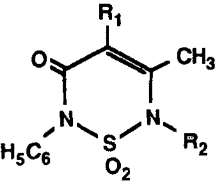

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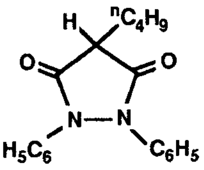

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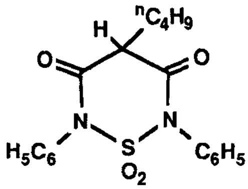

4

a $R_1 = H, R_2 = CH_3$
b $R_1 = R_2 = CH_3$
c $R_1 = Cl, R_2 = CH_3$
d $R_1 = Br, R_2 = CH_3$
e $R_1 = I, R_2 = CH_3$
f $R_1 = R_2 = H$

In this work, we have measured the capacity factors (K') and calculated the retention index (I) of related series **1**,**2** and **3**,**4** using reverse-phase HPLC. Correlations among K' or I of pyrazolone and thiadiazinone series have been found.

MATERIAL AND METHODS

Materials

The **C3-C9** 2-ketoalkanes were obtained from Alltech Europe (Belgium). High performance liquid chromatography grade methanol was obtained from E.M. Merck Co. and reagent grade water was generated by a Millipore Milli-Q water purification system. Antipyrine (**1a**) and Phenylbutazone (**3**) were obtained from Sigma Chemical Company. The other compounds studied were synthesized by reported methods: **1b** (12), **1c**, **1d** (13), **1e** (14), **2a-2f** (4) and **4** (3).

Chromatography

HPLC was performed on a Waters HPLC system consisting of a 6000A solvent delivery system and a U6K injector. The compounds studied on this system were detected by a Waters 440 absorbance spectrophotometric detector at 254 nm. Retention data were collected by a Waters 730 Data module at a rate of 0.5 cm./min.

For analysis a Hibare µ-Bondapack C-18 column at 25ºC was used. Mobile phase consisted of mixtures of MeOH/H_2O buffer solutions at 20%, 40%, 60%, 70% and 80% (v/v), at a flow rate of 0.9 ml/min.

Phosphate buffer has been reported to cause the least deviation from octanol-water values (15). The mobile phase solutions were used with a 0.025 NaH_2PO_4 buffer that had been adjusted to an apparent pH of 7.0 after the MeOH/H_2O had been added.

Methods

Compounds and 2-ketoalkanes were dissolved in methanol at a concentration of 250 µg/ml. Then they were injected separate from each other and in mixtures of five or six compounds. The experiments were carried out four times and the mean value of the retention time was obtained for each compound.

Calculations

The capacity factors (K') of compounds.studied and 2-ketoalkanes were determined from the observed retention time (Tr) and the retention time of the solvent front (T_0) using eq. 1

$$K' = \frac{T_r - T_0}{T_0} \quad [1] \qquad I = 100\,\frac{\log K'_D - \log K'_N}{\log K'_{N+1} - \log K'_N} + 100\,N \quad [2]$$

The retention index (I) of a given 2-ketoalkane standard was by definition (11) equal to 100 times the number of carbons in the compound. The retention index of a given test compound was calculated from the observed capacity factor for the compound (K'_D), the capacity factor for a 2-ketoalkane eluting just before the test compound (K'_N) and the capacity factorof the next higher homologous (K'_{N+1}) using eq. 2.

Table 1. HPLC parameters of studied products and 2-ketoalkane standards (C3 - C9).

Comp	20%MeOH			40%MeOH			60%MeOH			70%MeOH			80%MeOH		
	Tr	logK'	I	Tr	logK'	I	Tr	logK'	I	Tr	logK'	I	Tr	logK'	I
1a	31.99	1.028	559	7.34	0.252	497	3.68	-0.376	431	3.20	-0.638	408	2.99	-0.853	371
1b	41.50	1.146	595	8.55	0.326	526	3.98	-0.275	468	3.35	-0.568	440	3.04	-0.795	374
1c	47.12	1.206	610	9.60	0.418	541	4.16	-0.221	487	3.42	-0.522	450	3.10	-0.744	426
1d	64.32	1.361	647	19.42	0.801	636	5.42	0.033	576	3.92	-0.309	553	3.31	-0.585	510
1e	73.62	1.414	660	12.40	0.568	579	4.50	-0.124	518	3.55	-0.443	490	3.16	-0.677	458
2f	4.02	-0.327	291	2.65	-1.301	108	-	-	-	-	-	-	-	-	-
2a	85.60	1.485	678	13.44	0.620	592	4.37	-0.154	514	3.44	-0.494	460	3.05	-0.823	386
2b	-	-	-	22.29	0.877	656	5.45	0.041	579	3.85	-0.327	533	3.22	-0.638	479
2c	-	-	-	28.40	1.000	685	6.15	0.136	614	4.10	-0.244	582	3.31	-0.585	510
2d	-	-	-	30.87	1.036	695	6.29	0.161	621	4.16	-0.244	579	3.34	-0.568	515
2e	-	-	-	35.22	1.095	709	6.50	0.184	628	4.21	-0.221	591	3.35	-0.552	525
3	-	-	-	23.96	0.894	658	5.00	-0.031	552	3.37	-0.552	472	-	-	-
4	-	-	-	15.84	0.706	614	3.88	-0.309	455	2.94	-0.886	304	-	-	-
C3	4.12	-0.292	300	3.29	-0.552	300	3.00	-0.795	300	2.91	-0.958	300	-	-	-
C4	7.45	0.235	400	4.55	-0.124	400	3.45	-0.481	400	3.10	-0.744	400	-	-	-
C5	16.62	0.697	500	7.37	0.262	500	4.28	-0.180	500	3.64	-0.408	500	-	-	-
C6	43.50	1.166	600	14.31	0.655	600	5.84	0.103	600	4.30	-0.163	600	3.64	-0.397	600
C7	-	-	700	32.05	1.062	700	8.88	0.394	700	5.69	0.068	700	4.21	-0.214	700
C8	-	-	800	-	-	800	15.05	0.680	800	8.00	0.311	800	5.09	-0.022	800
C9	-	-	900	-	-	900	27.06	0.980	900	11.89	0.549	900	6.45	0.167	900

RESULTS AND DISCUSSION

Test compounds were chromatographed under a wide variety of conditions in which the percentage of methanol varies. The retention times (Tr), capacity factors (K') and retention index (I) of test compounds and 2-ketoalkane standards are gathered in table 1.

In general, it was found that the plots of logK' *vs* I were linear over a very wide range of conditions (11). In our case, this was performed taking into account the data of 2-ketoalkanes and compounds **1**, **2**, **3** and **4** as can be seen in figure1.

The calculated retention index of test compounds are not constant in all conditions studied. However, the variation of I is smaller in the case of pyrazolones (fig. 2) than in thiadiazinones (fig. 3).

The I variations found are due to the fact that 2-ketoalkane standards cannot account for all interactions that pyrazolones and thiadiazinones undergo in different elution conditions. Thus, the most acid compounds studied **2f**, **4** and-**3** (3) are those which present maxima deviations (figs. 2 and 3).

The retention times of investigated compounds increase with increasing water content in the mobile phase (fig. 4).

Phenylbutazone **3** is more hydrophobic (longer retention time) than its thiadiazinic analogue **4** (table 1). This fact is related to their pKa values, since, **4** is more acid than **3** (3). However, between series **1** and **2**, thiadiazinone derivatives **2** are more hydrophobic than pyrazolones **1**,

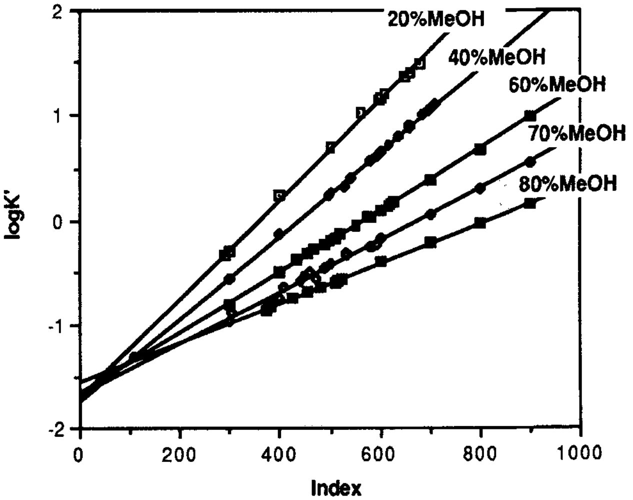

Figure 1. Correlation between logK' and I for studied compounds and 2-ketoalkanes.

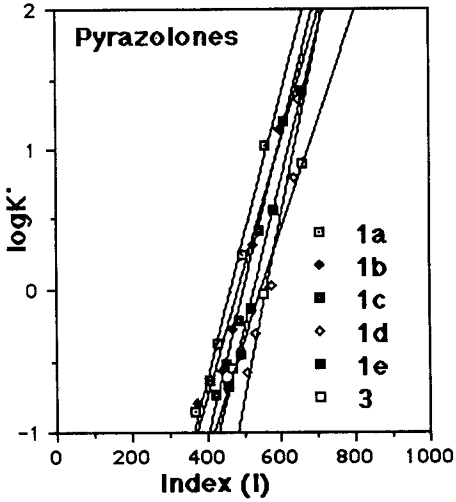

Figure 2. Plots of logK' *vs* **I** for each pyrazolone in the five elution conditions

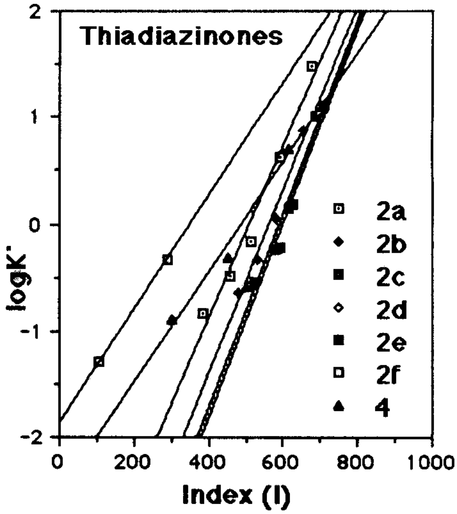

Figure 3. Plots of logK' *vs* **I** for each thiadiazinone in the five elution conditions

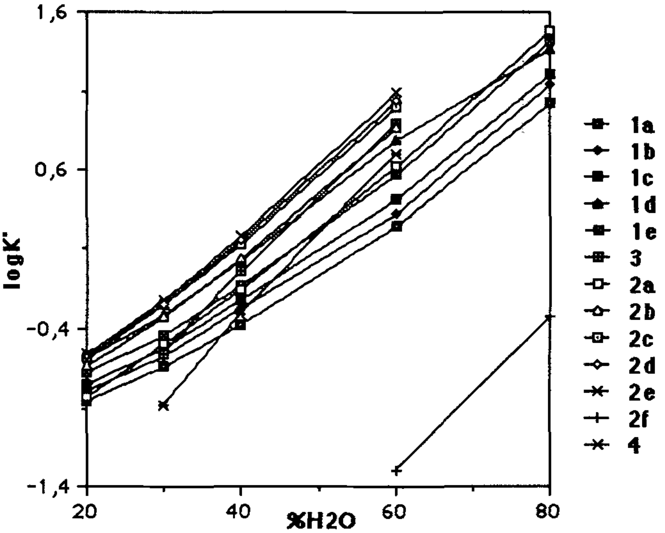

Figure 4. Semi-log plot of K' *vs* percentage of H_2O in the mobile phase for pyrazolones and thiadiazines.

Table 2. Correlation of hydrophobic parameters in series **1** and **2**.

run	eluent %MeOH	correlation equations [a]	statistical terms n^b	r^c	s^d	F^e
1	40	$\log K'_P = 0.61 \log K'_T - 0.16$	4	0.92	0.064	11.68
2	60	$\log K'_P = 0.68 \log K'_T - 0.28$	4	0.96	0.036	23.52
3	60	$I_P = 0.69\, I_T + 72.2$	4	0.97	11.422	28.44
4	70	$\log K'_P = 0.61 \log K'_T - 0.34$	4	0.93	0.037	12.56
5	20 / 40	$\log K'_P = 0.77 \log K'_T - 0.52$	5	0.92	0.084	17.07
6	miscellaneus	$I_P = -1.04\, I_T + 1083$	4	0.81	59.330	3.91

[a] K'_P and I_P : capacity factors and retention index of series **1** , K'_T and I_T: capacity factors and retention index of series **2.** [b] Number of points. [c] Correlation coefficient. [d] Standard error. [e] F test value.

except for compound **2f** which has the shorter retention time of all compounds studied due to its acidic NH proton (16).

This study has allowed us to establish the similar chromatographic behaviour of series **1** and **2.** The elution order of related pyrazolonic and thiadiazinonic derivatives is the same: **Ia<Ib<Ic<Id<Ie**, except for 4-bromopyrazolone **1d**, the elution order of which changes from 20% to 40% of methanol as eluent. Thus, 4-bromopyrazolone **1d** elutes before 4-iodo derivative **1e** on using 20% MeOH in the mobile phase, however, when the percentage of methanol increases (40%, 60%, 70%, 80%), **1d** has longer retention time than **1e.**

Correlations have been found between retention time parameters (logK', I) of series **1** and **2** (table 2).

Only fours pairs of compounds (**a**, **b**, **c** and **e**) have been taken into account (runs 1, 2, 3 and 4), since, 4-bromo derivatives **1d** and **2d** have been eliminated due to the anomalous behaviour of **1d**. Since the chromatographic behaviour of **1d** at 20% MeOH is similar to that of **2d** at 40% MeOH a correlation between logK'T (40% MeOH) and logK'P (20% MeOH) has been established for five pairs ofcompounds (run 5). The correlation coefficient found is the same as that found in run 1.

Correlation coefficients found for logK' or I have similar values when retention parameters are taken in the same elution conditions (runs 2 and 3). However, if the parameters are chosen at random among all elution conditions used, retention index give better results. Thus, in run 6 the parameters have been chosen as follows: **2a** (60% MeOH), **1a** (20% MeOH), **2b** (40% MeOH), **1b** (80% MeOH), **2c** (70% MeOH), **1c** (40% MeOH), **2e** (80% MeOH) and **1e** (70% MeOH), the correlation coefficient found for I_P *vs* I_T are |0.81|; whilst the correlation found for logK' in the same conditions give a coefficient of |0.35|.

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