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Influence of modifier and molecular structure of some dihydroxythiobenzanilides on retention in reversed-phase high-performance thin-layer chromatography

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Abstract

The retention behaviour of ten dihydroxythiobenzanilides in a reversed-phase high-performance thin-layer chromatographic system has been examined. Using water–organic modifier (acetone, acetonitrile or methanol) as the mobile phase, a linear relationship between the volume fraction of the organic solvent and the R_M values over a limited range was established for each solute. The physico-chemical properties of these compounds were characterized by their hydrophobicity parameters ($R_{M,w}$), determined by extrapolation of the linear relationships for retention data in binary solvent systems to pure water. The effect of individual substituents on retention was quantified by using the group contribution parameter ΔR_M . © 1997 Elsevier Science B.V.

Keywords: Mobile phase composition; Organic modifiers; Dihydroxythiobenzanilides

1. Introduction

Lipophilicity is one of the inherent properties of chemical compounds, affecting their biological activity (the activity usually increases with increasing lipophilicity) [1]. It plays a determinant role in the transport of compounds through a biological system and it may also influence the formation of a complex between a compound and a receptor or a biomacromolecule at the site of action.

The use of partition coefficients P obtained from an *n*-octanol–water partitioning system has become a standard method of evaluating the hydrophobicity of

a given compound [2]. Log P is either determined experimentally or calculated [2,3].

In recent years attempts have been made to introduce chromatographic techniques for the determination of the lipophilicity of different chemicals, especially reversed-phase thin-layer chromatography (RP-TLC) [4–6], and reversed-phase high-performance liquid chromatography (RP-HPLC).

Application of partition chromatography for such a purpose follows from the relationship between suitable retention indices and the partition coefficient P , determined in the chromatographic system. A suitable index in TLC is R_M , which is related to the experimental R_F value and depends linearly on log P [7]. Log k' is equivalent to R_M in HPLC. R_M or log k' values can be used directly as hydrophobicity

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parameters. On the basis of R_M values determined experimentally or calculated for pure water substances can be ranked according to their hydrophobicity. The compounds with higher R_M values have a more hydrophobic character [5].

In RP-TLC the stationary phase is usually non-polar (e.g., alkyl-bonded phases), and the mobile phase is polar, e.g., water–acetone or methanol mixtures. In this technique there is a linear relationship between the R_M values and the organic solvent concentration in the mobile phase. The TLC equation [5]:

$$R_M = a_w + b (\% \text{ organic modifier}) \quad (1)$$

describing this relationship yields extrapolated R_{Mw} (a_w) values at 0% of organic modifier in the mobile phase, even for compounds which do not migrate with pure water alone.

These R_{Mw} values should be related to the partitioning of the compounds between water and the hydrocarbonaceous surface.

Biagi et al. [5] observed that R_{Mw} values are independent of the nature of the organic solvent in the mobile phase when the solvent is acetone, acetonitrile or methanol. However, it is questionable whether this aspect has general relevance for any chromatographic process. Some HPLC data seem to suggest a different influence of methanol and ace-

tonitrile on the retention of simple organic compounds [8,9].

This work will show how the type of organic modifier used and the structure of the solutes influence the retention and selectivity of the group of dihydroxythiobenzanilides.

The investigated dihydroxythiobenzanilides are new compounds with biological activity. They have fungicidal properties. The retention data for these substances have not yet been published. Dihydroxythiobenzanilides have a complicated structure with respect to hydrophilic and hydrophobic substituents (Table 1), so a study of the retention and partition behaviour should provide good information about pharmacologically important physico-chemical parameters.

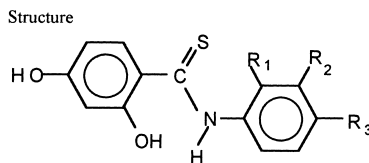
2. Experimental

2.1. Materials

The dihydroxythiobenzanilides used (Table 1) were synthesized in-laboratory in the Department of Chemistry, University of Agriculture (Lublin, Poland)¹. Analytical-reagent grade organic solvents

¹New synthesis method; patent pending.

Table 1
Chemical structure of dihydroxythiobenzanilides



Compound No.	Substituents
1	$R_1 = -H, R_2 = -H, R_3 = -H$
2	$R_1 = -OH, R_2 = -H, R_3 = -H$
3	$R_1 = -H, R_2 = -OH, R_3 = -H$
4	$R_1 = -H, R_2 = -H, R_3 = -OH$
5	$R_1 = -OH, R_2 = -H, R_3 = -CH_3$
6	$R_1 = -H, R_2 = -H, R_3 = -COCH_3$
7	$R_1 = -H, R_2 = -H, R_3 = -CONHCH_2COOH$
8	$R_1 = -H, R_2 = -H, R_3 = -N(CH_2CH_3)CH_2CH_2OH$
9	$R_1 = -H, R_2 = -NHCSC_6H_4(OH)_2, R_3 = -OH$
10	$R_1 = -H, R_2 = -H, R_3 = -NHCOCH_3$

were purchased from Baker (Phillipsburg, NJ, USA) and from POCH (Lublin, Poland).

2.2. Procedure

TLC was performed on 10×10 cm pre-coated HPTLC plates of RP-8F₂₅₄ (E. Merck, Darmstadt, Germany); 1- μ l samples of the solutes (1.0 mg cm⁻³ in methanol) were spotted with a Desaga AS30 Applicator.

The chromatograms were developed over a distance of 9.5 cm in horizontal “sandwich” chambers (Chromdes, Lublin, Poland) for TLC. The chambers were saturated with appropriate organic solvents vapour for 20 min.

For the studied series of dihydroxythiobenzanilides the mobile phases were: water–acetone, water–acetonitrile and water–methanol. The concentration of organic modifier in the mobile phase ranged from 50% to 85%. All TLC determinations were carried out at a temperature of 20°C. The spots were localized under UV light at 254 nm.

3. Results and discussion

The HPTLC results are shown as plots of R_M versus volume percentage of methanol (Figs. 1 and 2), as intercepts of the TLC equations represented by

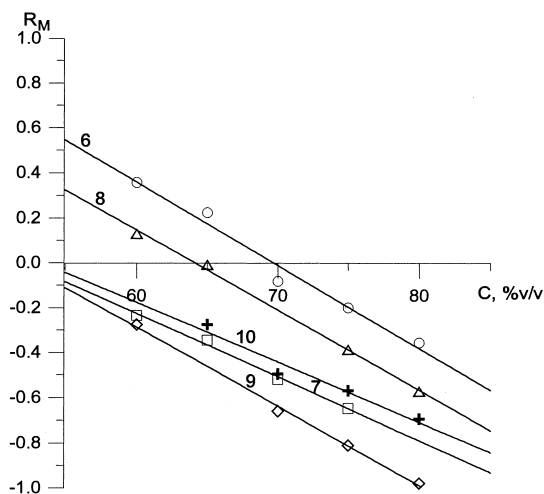


Fig. 1. Relationship between R_M values and the methanol concentration in the mobile phase. Notation of solutes as in Table 1.

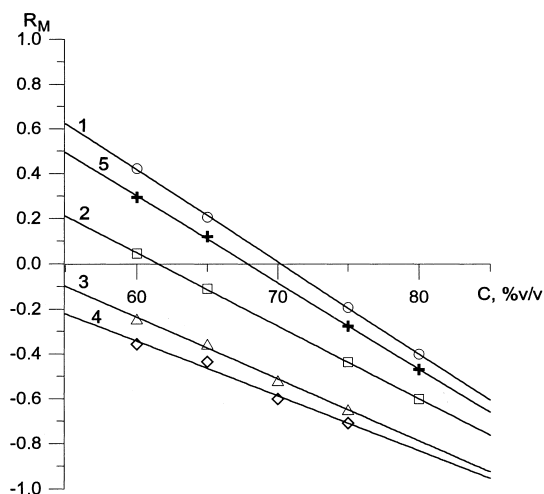


Fig. 2. As Fig. 1 for further solutes.

the extrapolated R_{Mw} values and slopes of these equations (Table 2), and also as a plot of $R_{M,extr.}$ versus $R_{M,extr.}$ relationships obtained using different systems (Fig. 3).

The results in Figs. 1 and 2 indicate that linear relationships were obtained over all of the investigated concentration ranges. The examined range was only from 50% to 85% because for mobile phases with concentration of organic solvent less than 50% the compounds were eluted near the start.

In reversed-phase systems, in which solvation effects are very important, the retention depends on the molecular structure of the solute; the introduction of small polar or non-polar groups into a large sample molecule significantly influences the retention (Figs. 1 and 2).

Compounds with polar hydroxy groups in the molecule, such as substances 2, 3 and 4, have the weakest retention if an aqueous–organic phase is used. The introduction methyl or other less polar groups increased the retention of e.g., substances 5, 6 and 8 (the spots were nearer the start).

All investigated compounds cannot be separated at low concentration of the modifier. In this instance the range of measurable data of dihydroxythiobenzanilides which retention (R_M) shows a linear dependence on the concentration of the modifier can be extended by extrapolation of experimental $R_M = f(\% \text{ organic modifier})$ relationship to a pure water medium.

Table 2

Parameters a_w (intercept), b (slope) and r (correlation coefficient) of the linear relationships in Eq. (1)

Compound	$R_M = a_w + b$ (% organic modifier)								
	Acetone			Acetonitrile			Methanol		
	a_w	b	r	a_w	b	r	a_w	b	r
1	3.08	-0.0428	0.989	2.93	-0.0462	0.999	2.89	-0.0411	0.999
2	2.20	-0.0332	0.999	2.30	-0.0405	0.999	2.00	-0.0325	0.999
3	2.07	-0.0326	0.998	1.98	-0.0372	0.997	1.42	-0.0276	0.995
4	2.01	-0.0323	0.999	1.88	-0.0376	0.997	1.12	-0.0244	0.980
5	2.64	-0.0377	0.999	2.51	-0.0424	0.999	2.61	-0.0385	0.999
6	2.55	-0.0370	0.999	2.47	-0.0380	0.999	2.59	-0.0372	0.976
7	1.68	-0.0288	0.996	1.63	-0.0305	0.997	1.49	-0.0285	0.991
8	2.41	-0.0368	0.986	2.36	-0.0400	0.990	2.29	-0.0357	0.998
9	1.85	-0.0316	0.978	1.85	-0.0391	0.994	1.83	-0.0352	0.998
10	1.50	-0.0266	0.999	1.56	-0.0306	1.000	1.44	-0.0268	0.976

The intercepts (a_w) in Eq. (1) represent the extrapolated R_{Mw} values, i.e., the theoretical R_M values at 0% organic solvent. The intercepts ($a_w = R_{Mw}$) and slopes of TLC equations for different organic solvent in the mobile phase are reported in Table 2.

The data in Table 2 show that the extrapolated R_{Mw} values from the TLC system with the acetone–water and acetonitrile–water mobile phases are very similar (Fig. 3). The correlation between the extrapo-

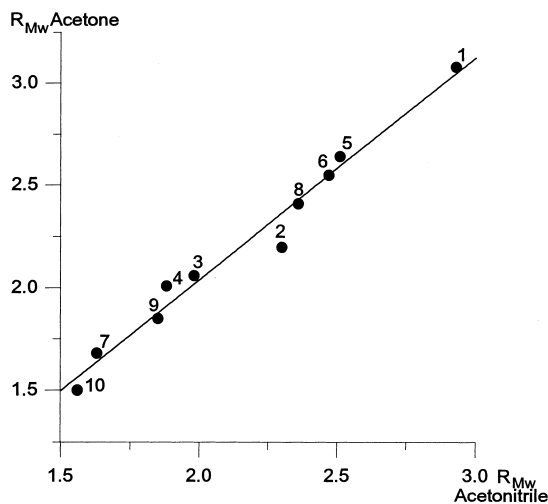


Fig. 3. Relationship between R_{Mw} values from TLC systems with acetone and acetonitrile.

lated R_{Mw} values is described by the following equation [5]:

$$R_{Mw,acetonitrile} = 1.086(R_{Mw,acetone}) - 0.132$$

$$(n = 10, r = 0.988, s = 0.077) \quad (2)$$

The slope and intercept, very close to 1 and 0, respectively, showed that in this case the nature of the organic modifier does not significantly affect parameters $a_w = R_{Mw}$ in the TLC equations. However, the extrapolated R_{Mw} values obtained using the methanol–water system are different from these obtained with the acetone or acetonitrile–water mobile phases and correlated with them in the following way [5]:

$$R_{Mw,methanol} = 1.079(R_{Mw,acetone}) - 0.381$$

$$(n = 10, r = 0.853, s = 0.336) \quad (3)$$

$$R_{Mw,methanol} = 1.227(R_{Mw,acetonitrile}) - 0.644$$

$$(n = 10, r = 0.882, s = 0.302) \quad (4)$$

The moderate correlation coefficient obtained for these systems showed that the molecular properties of the solute contribute differently to the retention for the methanol system.

The R_{Mw} values for the methanol–water phase are less than those for the acetone or acetonitrile–water

mixtures, especially for substances 2, 3 and 4 which have R_1 , R_2 or $R_3 = -OH$. It means that examined compounds migrate on the plate further than for acetone or acetonitrile. It can be assumed that because of the existence of hydroxy groups in the molecule there are strong solute–eluent interactions, i.e., formation of hydrogen bonds which increase solubility of substances in the mobile phase.

The negative slope of the TLC equation indicates the increase in migration per unit increase in organic solvent concentration, i.e., the rate at which the solubility of the compound increases in the mobile phase. The slope is also related to eluting power of the organic modifier, as expressed by its solvent strength parameter $1/E_0$ in a reversed-phase chromatographic system [6].

The absolute values of slopes of dihydroxythiobenzanilides (Table 2) are ranked for each compound (with the exception of the substance 9) as follows: acetonitrile > acetone \geq methanol.

The above ranking of slopes indicates that the migration of a given compound is more sensitive to the increasing concentration of acetonitrile in the mobile phase than to those of acetone or methanol in this order. The smallest absolute values of slopes for the most substances in the mobile phase with methanol compared with the other systems (Table 2) are related to the lowest eluting power of methanol. However, the most negative slopes in the acetonitrile system which has a lower eluotropic strength than acetone indicate that the slope of the TLC equation depends not only on the solvent strength but also on specific interactions between solutes, stationary phase and mobile phase.

From the data in Table 2 it can be shown that there is a linear relationship between the slopes and intercepts of TLC equations. The equations describing such a linear relationship for the present series of dihydroxythiobenzanilides are reported in Table 3.

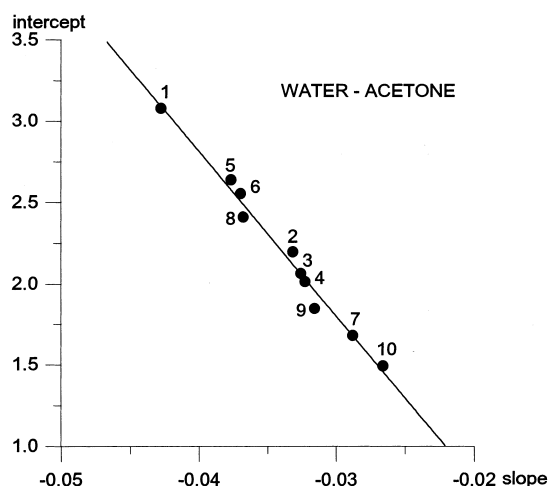


Fig. 4. Relationship between slopes and intercepts of the TLC equation as described by Eq. (5) in Table 3.

In the case of acetone no deviation was observed from the linear relationship between the intercepts and slopes of the TLC equation (Fig. 4).

In the instance of acetonitrile and methanol, substance 9 does not fit the straight line (Figs. 5 and 6).

In the methanol system compound 9 is an outlier for statistical reasons and it was omitted from the calculation of Eq. (7) (Table 3). The larger deviation from linearity of substance 9 seems to be related to the presence of the complicated substituent in position R_2 (the aromatic C ring with two polar OH groups). This might be the cause of a different frame of intra- and inter-molecular interactions.

The linear relationship between the slopes and intercepts of TLC equations is the important feature of the chromatographic determination of the lipophilicity [5]. There is such a linear relationship for our examined substances, so the intercepts and slopes ($a_w = R_{Mw}$ and b values in Table 2) can be

Table 3
Relationship between intercepts (R_{Mw}) and slopes of TLC equations

TLC mobile phase	$R_{Mw} = a + b$ (slope)					Eq.
	a	b	n	r	s	
Acetone	-1.227	-100.96	10	0.992	0.063	5
Acetonitrile	-1.037	-83.04	10	0.891	0.207	6
Methanol	-1.434	-104.41	9	0.992	0.0847	7

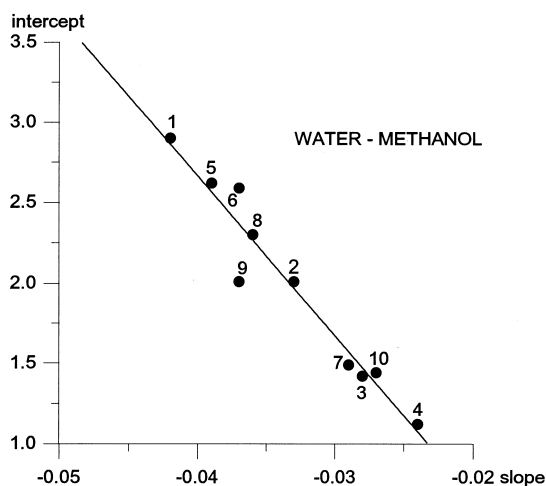


Fig. 5. Relationship between slopes and intercepts of the TLC equation as described by Eq. (6) in Table 3.

used as the hydrophobicity parameters for this group of dihydroxythiobenzanilides. The R_{Mw} values can be used as an expression of the partitioning between an aqueous mobile phase and the non polar stationary phase. The slopes (b) indicate the rate at which the solubility of the compounds increases in the mobile phase [5]. The increased migration can be related to a decrease in the surface tension of the

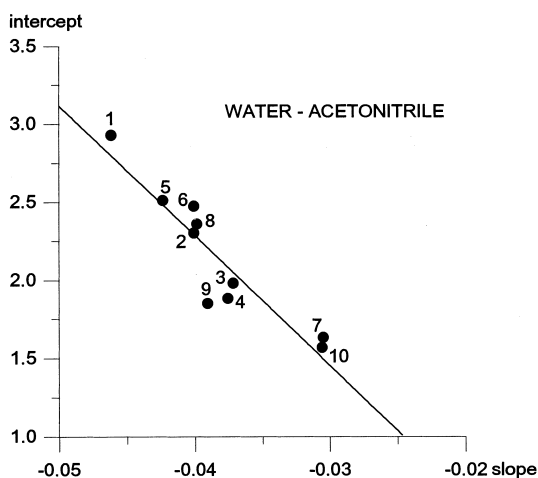


Fig. 6. Relationship between slopes and intercepts of the TLC equation as described by Eq. (7) in Table 3.

mobile phase, thus resulting in a better solubility [10].

The TLC selectivity parameter ΔR_M ($R_{M2} - R_{M1}$) shows the effect of individual substituents on the retention and the selectivity of the developing systems.

Table 4 summarizes ΔR_M values obtained for 65% organic modifier in the eluent.

The ΔR_M values are differentiated for the various investigated modifiers owing to the differences in the polarity of the substituents.

With 65% of methanol as modifier (Table 4), ΔR_M ($R_3 = -OH$) = -0.641 (compound 1 and 4) a methyl group in the molecule increases the hydrophobic properties, ΔR_M ($R_3 = -CH_3$) = -0.086 (compound 1 and 5). Also the position of the same substituent (substances 2, 3 and 4) significantly effect the retention. Compound 2 ($R_1 = -OH$) $\Delta R_M = -0.315$ is more hydrophobic than the substance 4 ($R_3 = -OH$) $\Delta R_M = -0.641$.

4. Conclusions

(1) The present data show that acetone and acetonitrile does not significantly affect the extrapolated R_{Mw} values, i.e., the theoretical R_M values in pure water for the investigated series of dihydroxythiobenzanilides. So the extrapolated R_{Mw} values could be used as an expression of the partitioning between water and the non-polar stationary phase.

Methanol has a different influence on the retention of examined substances, especially on substances which have $-OH$ groups as R_1 , R_2 or R_3 substituent. Because of strong molecular interactions with the solutes methanol is not good modifier to determine hydrophobicity parameters for these compounds.

(2) There is the linear relationship between slopes and intercepts of TLC equations for all examined modifier systems (it is the best for acetone without any deviation), which is the important feature of the chromatographic determination of lipophilicity. The intercept and slope should be interrelated because both seem to be related to the same physico-chemical factors.

(3) Methanol–water is the most selectivity eluent for examined series of dihydroxythiobenzanilides with respect to substituent effect on retention.

Table 4
 ΔR_M values of dihydroxythiobenzanilides

Compound	HPTLC RP-8, 65% of modifier in the eluent					
	Acetone		Acetonitrile		Methanol	
	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M
1	0.235	0.000	-0.064	0.000	0.205	0.000
2	0.037	-0.198	-0.335	-0.271	-0.110	-0.315
3	-0.064	-0.299	-0.447	-0.383	-0.358	-0.563
4	-0.092	-0.327	-0.547	-0.483	-0.436	-0.641
5	0.185	-0.050	-0.244	-0.180	0.119	-0.086
6	0.156	-0.079	-0.116	-0.052	0.224	-0.019
7	-0.176	-0.411	-0.506	-0.442	-0.345	-0.550
8	0.009	-0.226	-0.185	-0.121	-0.009	-0.214
9	-0.167	-0.402	-0.573	-0.509	-0.335	-0.540
10	-0.235	-0.470	-0.423	-0.359	-0.275	-0.480

Selectivities relative to compound 1 for solutes 2–10.

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