

OPLC and HPTLC Methods in Physicochemical Studies of a New Group of Antimycotic Compounds

Jan K. Rózyło¹, Anna Zabinska¹, Joanna Matysiak², and Andrzej Niewiadomy

¹Faculty of Chemistry, M. Curie – Skłodowska University, 20-031 Lublin, Poland and ²Department of Chemistry, University of Agriculture, 20-950 Lublin, Poland

Abstract

In this study two high-performance methods [overpressured layer chromatography (OPLC) and high-performance thin-layer chromatography (HP-TLC) under reversed-phase conditions] are used to evaluate the hydrophobicity properties of newly synthesized bioactive compounds. The retention behavior of 24 2-(2,4-dihydroxyphenyl)benzothiazoles are examined for acquisition of log k data. With water–methanol mixtures as the mobile phases, the concentration range in which the correlation between log k and methanol content is linear is established for both methods and used to determine the hydrophobicity parameters of log k_W by linear extrapolation. The effect of substituents on retention constants is quantitated by using the group contribution parameters (t_W). The results suggest the use of OPLC methods for quick analysis of physicochemical properties of a large number of organic compounds.

Introduction

Overpressured layer chromatography (OPLC) is a hybrid between conventional TLC and HPLC and incorporates several of the attractive features of each technique (1).

In OPLC methods, in addition to capillary action, the solvent migration is carried out through the pump, which delivers the mobile phase. The vapor phase is completely eliminated, the chromatographic plate being covered with an elastic membrane under external pressure, and the analysis can thus be performed under controlled conditions. OPLC has a big role in the analysis of pharmaceuticals, drugs, foods, and toxicological samples, as well as in environmental analysis (2).

In this study, OPLC under reversed-phase conditions was used for the prediction of the physicochemical properties (especially hydrophobicity) of a new group of bioactive compounds. The results obtained for OPLC were compared with the results from conventional HPTLC. The examined substances were obtained by

a new synthesis method elaborated in our laboratory. These agents are a promising group of biologically active agents of a wide activity range tending towards yeast, depending on the kind of substitution.

Hydrophobic character often seems to be the most important physicochemical parameter in accounting or variations of biological activity within a series of chemical agents (3).

Reversed-phase chromatographic methods have been extensively applied to determine the hydrophobicity of many bioactive compounds (4). The chromatographic log k value is used in order to avoid the practical difficulties that often arise in the direct determination of the partition coefficient. The variation of log k of the sample with the organic modifier content of the mobile phase is the analytical tool to assess the selective effects of specific molecular structures (5,6).

In liquid chromatography the relationship between retention factors, log k, and the composition of the mobile phase can be described by the linear or quadratic equation (7,8):

$$\log k = \log k_W + S_o \quad \text{Eq. 1}$$

$$\log k = \log k_W + B_o + A_o^2 \quad \text{Eq. 2}$$

where log k_W represents the retention factor of a solute with pure water as the mobile phase, o is the volume fraction of organic solvent in the mobile phase, S is the slope of the regression curve, and A and B are coefficients of the quadratic equation.

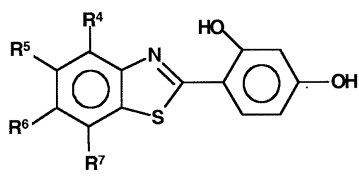
Experimental

The 2-(2,4-dihydroxyphenyl)benzothiazoles used (Table I) were synthesized in the laboratory at the Department of Chemistry in the University of Agriculture, Lublin, Poland (new synthesis method, patent pending).

TLC and OPLC was performed on precoated HPTLC plates of RP-8 F_{254S} (Merck, Darmstadt, Germany); 1- μ L samples of the

solutes (0.5 mg/mL in methanol) were spotted with the Desaga AS30 applicator.

Table I. The Chemical Structure of 2-(2,4-dihydroxyphenyl)benzothiazoles



Substituents

1	R4 - R7 = H	13	R6 = Br
2	R4 = F	14	R6 = I
3	R5 = F	15	R5 = Cl
4	R6 = F	16	R6 = Cl
5	R7 = F	17	R7 = Cl
6	R4 = R6 = F	18	R5 = R6 = Cl
7	R5 = R6 = F	19	R6 = R7 = Cl
8	R4 = R5 = R7 = F	20	R5 = CF3
9	R4 = R5 = R6 = R7 = F	21	R6 = CF3
10	R5 = CH3, R6 = F	22	R7 = OCF3
11	R5 = CH3	23	R5 = COOH
12	R4 = R5 = R7 = F, R6 = Br	24	R5 = C6H5CNS

The chromatograms were developed over a distance of 9.5 cm in horizontal "sandwich" chambers (Camag, Mittenz, Switzerland) for TLC and over a distance of 7.0 cm in an automatic OPLC BS-50 chamber (OPLC-NIT, Budapest, Hungary). The water-organic modifier (methanol) system was applied as the mobile phases in the concentration range of 0.5–1.0 v/v of organic solvent at 0.5 intervals. Two independent runs were carried out in all of the experiments.

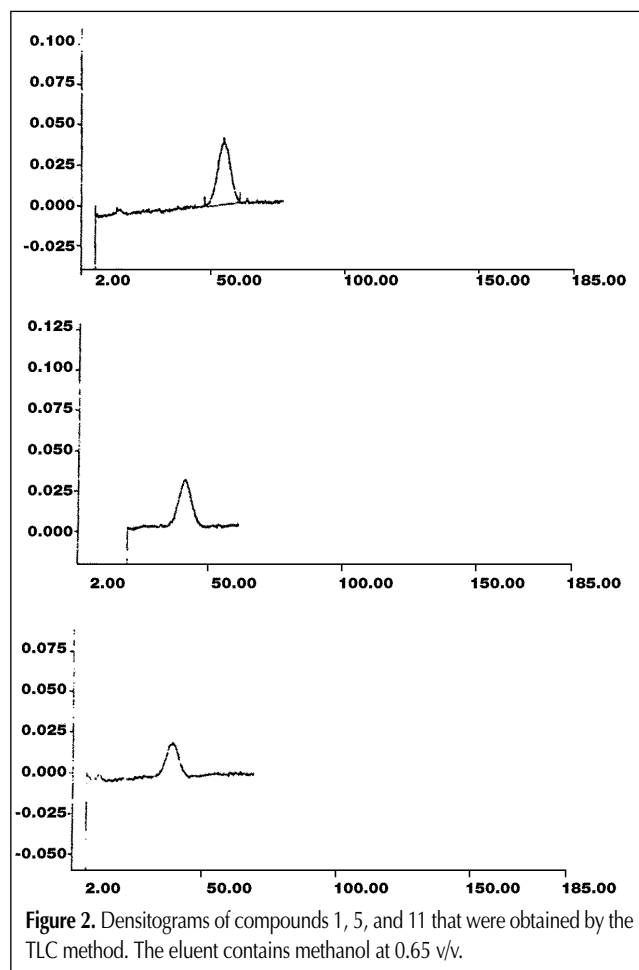
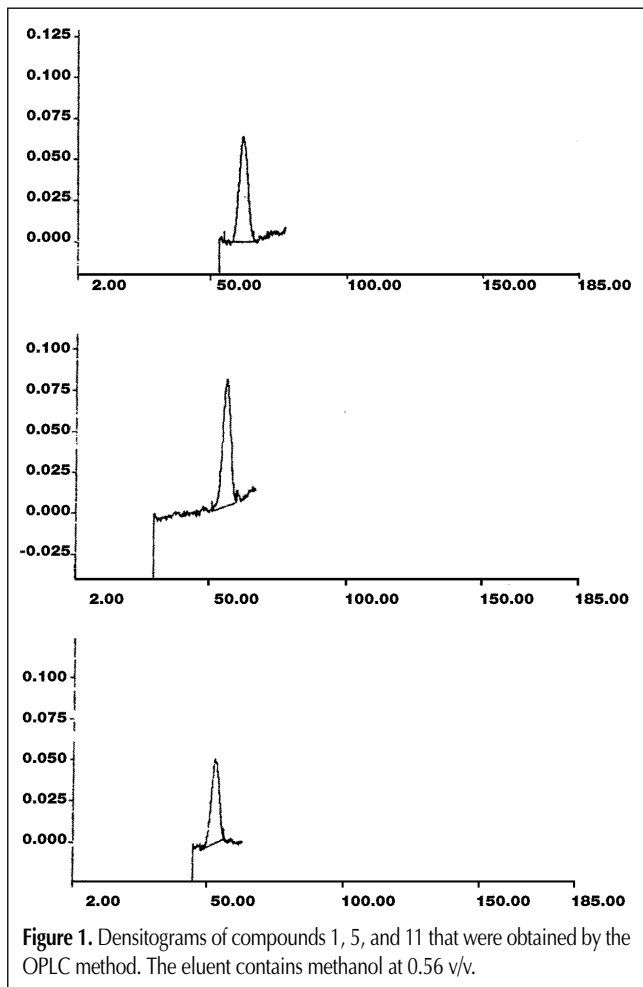
After development the plates were dried at room temperature and the spots were detected with a Shimadzu CS-9000 dual-wave-length TLC scanner at 325 nm.

Results and Discussion

The configurations of OPLC chambers enables the mobile phase to be pumped through the stationary layer, and the results could be compared with conventional TLC within a relatively shorter analysis time and with higher efficiency.

For TLC the analysis time was between 30 min for phases with a higher concentration of methanol and 70 min for water-rich mobile phases (distance migration of 9.5 cm). For the OPLC method the development time was 6 min for all mobile phases (distance migration of 7 cm).

Figures 1 and 2 illustrate the chromatograms obtained for sub-



stances 1, 5, and 11 with 0.65 v/v of methanol in the mobile phase. It is apparent that the use of the OPLC method resulted in sharper peaks. This enabled the development of chromatograms of all 24 compounds on one chromatoplate for OPLC and only 12 samples on one plate for conventional TLC.

Figure 3 depicts the different relationships between $\log k$ and organic modifier content for the TLC and OPLC methods. It is apparent that for OPLC the relationship is generally linear, in accordance with equation 1, over a wider range of concentration (0.5–0.8 v/v). For the TLC method, only part of the curve can be regarded as linear. Therefore, the mentioned correlation for TLC is described by the quadratic equation (equation 2).

The theoretical retention factors in pure water by linear extrapolation were obtained on the basis of these relationships because

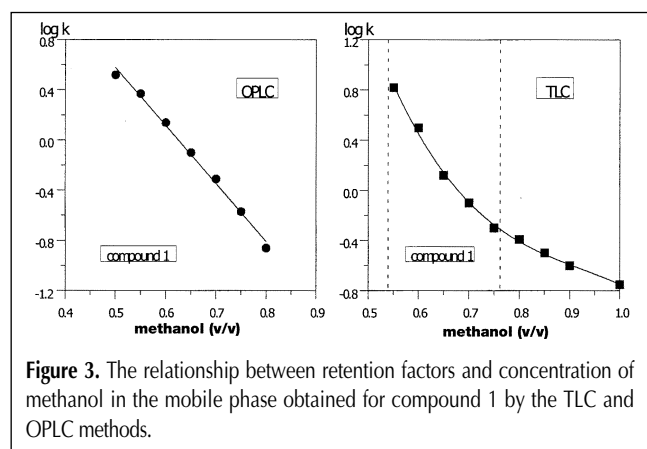


Figure 3. The relationship between retention factors and concentration of methanol in the mobile phase obtained for compound 1 by the TLC and OPLC methods.

Table II. Parameters S_1 and $\log k_{W1}$ Obtained From Linear Equation 1 for the OPLC Method

No.	S_1	$\log k_{W1}$	n_1	r_1
1	4.621	2.888	7	0.997
2	4.814	3.035	7	0.998
3	4.836	3.146	7	0.973
4	4.764	3.008	7	0.999
5	4.807	3.158	7	0.999
6	4.543	2.926	7	0.992
7	4.950	3.320	7	0.997
8	5.154	3.503	7	0.988
9	5.071	4.108	8	0.998
10	5.057	3.511	7	0.999
11	4.993	3.371	7	0.998
12	5.134	3.484	7	0.990
13	4.921	3.430	7	0.999
14	4.935	3.508	7	0.999
15	4.821	3.417	7	0.998
16	4.914	3.470	7	0.997
17	4.921	3.410	7	0.999
18	5.133	3.908	8	0.998
19	5.264	3.895	8	0.998
20	5.421	3.899	7	0.998
21	5.314	3.823	7	0.999
22	5.493	3.981	7	0.999
23	5.550	3.708	7	0.996
24	6.078	3.981	7	0.993

these compounds didn't migrate on the plates with pure water as the mobile phases (Tables II and III). For TLC to extrapolation, the retention data for mobile phases with the content of methanol in the range of 0.55–0.75 v/v were used.

The higher correlation coefficients were purchased for the OPLC method. The differences between calculated $\log k_{W}$ values for both methods were caused by different conditions in the development of chromatograms.

To show the contribution of a substituent to retention, functional group values (t_W) were calculated by:

$$t_W = \log k_{W(j)} - \log k_{W(i)} \quad \text{Eq. 3}$$

where k_W is the retention factors for pure water of solutes j and i , which differed by a substituent.

Table IV collects the t_W values for different compounds of Table I that were relative to the parent compound 1 and the lipophilicity parameters (π) that were used by Hansch (9).

These data show that the retention strongly depended on the molecular structure of the solute. The introduction of a small group into a large sample molecule significantly influenced the retention and hydrophobicity. The substituents that most increased hydrophobicity were $-\text{CF}_3$ (compounds 20 and 21), $-\text{OCF}_3$ (compound 22), two atoms of $-\text{Cl}$ (compounds 17 and 18), and four atoms of $-\text{F}$ (compound 9).

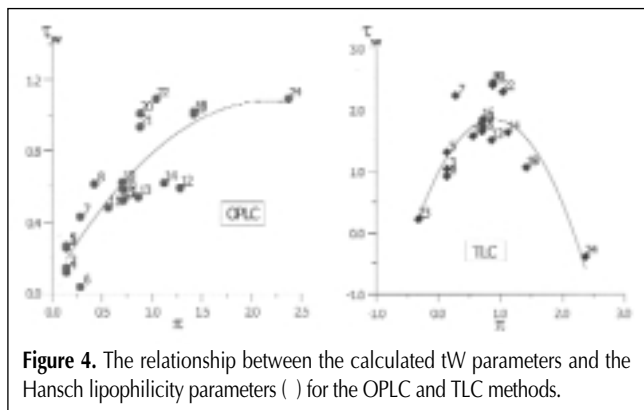
For both the OPLC and TLC methods, the correlation between calculated t_W values and the Hansch parameters (π) were examined. Different but good quadratic relationships (Figure 4) were obtained for both methods and described by the equations:

Table III. Parameters S_2 and $\log k_{W2}$ Obtained from Linear Equation 1 for the HPTLC Method

No.	S_2	$\log k_{W2}$	n_2	r_2
1	5.960	4.064	5	0.994
2	6.364	4.406	5	0.985
3	6.606	4.676	5	0.996
4	6.340	4.471	5	0.997
5	6.720	4.824	5	0.999
6	5.624	4.357	5	0.994
7	7.046	5.182	4	0.999
8	5.840	4.478	5	0.999
9	5.976	5.285	8	0.999
10	7.140	5.329	5	0.999
11	7.022	5.071	5	0.998
12	5.946	4.52	5	0.996
13	6.223	4.822	5	0.997
14	6.424	5.044	5	0.997
15	5.822	4.578	6	0.995
16	5.841	4.657	6	0.993
17	5.857	4.538	6	0.996
18	6.129	5.208	7	0.998
19	5.929	4.935	7	0.998
20	6.371	5.061	6	0.993
21	6.389	5.078	6	0.994
22	6.583	5.312	6	0.994
23	5.211	3.928	6	0.99
24	5.644	3.924	6	0.992

Table IV. Parameters and t_{W1} Calculated for the OPLC Method, and t_{W2} Obtained for TLC

Substituents		t_{W1}	t_{W2}
$R_4 - R_7 = H$			
$R_4 = F$	0.14	0.147	0.342
$R_5 = F$	0.14	0.258	0.612
$R_6 = F$	0.14	0.120	0.407
$R_7 = F$	0.14	0.270	0.760
$R_4 = R_6 = F$	0.28	0.038	0.293
$R_5 = R_6 = F$	0.28	0.432	1.118
$R_4 = R_5 = R_7 = F$	0.42	0.615	0.414
$R_4 = R_5 = R_6 = R_7 = F$	0.56	1.220	1.221
$R_5 = CH_3, R_6 = F$	0.70	0.623	1.265
$R_5 = CH_3$	0.56	0.483	1.007
$R_4 = R_5 = R_7 = F, R_6 = Br$	1.28	0.593	0.456
$R_6 = Br$	0.86	0.542	0.758
$R_6 = I$	1.12	0.620	0.980
$R_5 = Cl$	0.71	0.529	0.514
$R_6 = Cl$	0.71	0.582	0.593
$R_7 = Cl$	0.71	0.522	0.474
$R_5 = R_6 = Cl$	1.42	1.020	1.144
$R_6 = R_7 = Cl$	1.42	1.007	0.871
$R_5 = CF_3$	0.88	1.011	0.997
$R_6 = CF_3$	0.88	0.935	1.014
$R_7 = OCF_3$	1.04	1.093	1.248
$R_5 = COOH$	-0.32	0.820	-0.136
$R_5 = C_6H_5CNS$	2.37	1.093	-0.140

**Figure 4.** The relationship between the calculated t_W parameters and the Hansch lipophilicity parameters (π) for the OPLC and TLC methods.

$$t_{W1} = -0.213 \pi^2 + 0.930 \pi + 2.957 \quad (n = 21 \text{ and } r = 0.859) \quad \text{Eq. 4}$$

$$t_{W2} = -1.101 \pi^2 + 1.953 \pi + 7.987 \quad (n = 19 \text{ and } r = 0.869) \quad \text{Eq. 5}$$

The t_W values didn't fit the mentioned correlations for only a few compounds. For the OPLC method there were copunds 9 and 23, and for TLC there were compounds 6, 8, 9, and 12.

The differences between t_W and the π values suggested the presence of intramolecular interactions between various substituents in a given substance.

Conclusion

First, the present data confirmed the usefulness of the log k' values that were determined in the investigated reversed-phase methods as an expression of hydrophobic character of compounds. Second, the high efficiency, very short time of development of chromatograms, and increasing of the number of samples analyzed on one chromatoplate for the OPLC method allowed for the proposal of the use of OPLC for quick analysis of a large number of organic compounds.

References

1. E. Mincsovcics, K. Ferenczi-Fodor, and E. Tjihak. "Overpressure layer chromatography". In *Handbook of Thin-Layer Chromatography*. J. Sherma and B. Fried, Eds. Marcel Dekker, New York, NY, 1999, pp. 171–203.
2. Sz. Nyiredy. The bridge between TLC and HPLC: overpressured layer chromatography (OPLC). *Trends Anal. Chem.* **20**: 91–101 (2001).
3. G.L. Biagi, A.M. Barbaro, and A. Sapone. Determination of lipophilicity by means of reversed-phase thin-layer chromatography. *J. Chromatogr.* **662**: 341–61 (1994).
4. R. Kaliszan. *Quantitative Structure—Chromatographic Retention Relationship*. Wiley, New York, NY, 1987.
5. J.K. Rozylo, A. Zabinska, J. Matysiak, and A. Niewiadomy. Reversed-phase HPTLC and structure—activity relationship for fungicidal substances. *Chem. Environ. Res.* **7**: 65–75 (1998).
6. J. Matysiak, A. Niewiadomy, A. Zabinska, and J.K. Rozylo. Structure and retention of 2,4-dihydroxythiobenzanilides in a reversed-phase system. *J. Chromatogr.* **830**: 491–96 (1999).
7. L.R. Snyder, J.W. Dolan, and J.R. Grant. *J. Chromatogr.* **165**: 3 (1979).
8. J.K. Rozylo, J. Matysiak, A. Niewiadomy, and A. Zabinska. Reversed-phase HPLC and HPTLC in characterization of potential fungicides. *J. Planar Chromatogr.* **13**: 176–81 (2000).
9. C. Hansch and A. Leo. *Substituents Constants for Correlation Analysis in Chemistry and Biology*. Wiley, New York, NY, 1979.

Manuscript accepted May 20, 2002.