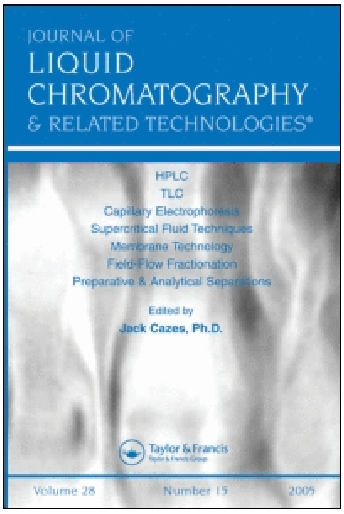

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RELATIONSHIP BETWEEN THE CALCULATED PHYSICOCHEMICAL PARAMETERS AND REVERSED PHASE THIN-LAYER CHROMATOGRAPHIC RETENTION BEHAVIOR OF CARBOXAMIDE FUNGICIDES AND RELATED COMPOUNDS

Gy. Oros[a]; T. Cserháti[b]
[a] Hungarian Academy of Sciences, Plant Protection Institute, Budapest, Hungary [b] Research Institute of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary

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RELATIONSHIP BETWEEN THE CALCULATED PHYSICOCHEMICAL PARAMETERS AND REVERSED PHASE THIN-LAYER CHROMATOGRAPHIC RETENTION BEHAVIOR OF CARBOXAMIDE FUNGICIDES AND RELATED COMPOUNDS

Gy. Oros[1] **and T. Cserháti**[2]
[1]*Hungarian Academy of Sciences, Plant Protection Institute, Budapest, Hungary*
[2]*Research Institute of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary*

☐ *The lipophilicity of 6 carboxamide fungicides and 11 phenylbenzamide derivatives were determined by reversed phase layer chromatography (RP-TLC) employing silica and alumina as stationary phases impregnated with paraffin oil. Mixtures of methanol-water, acetonitrile-water, tetrahydrofurane-water, and acetone-water were used as mobile phases. The R_{M0} and b values related to the molecular lipophilicity and to the specific hydrophobic surface area (b) of the solutes were calculated separately for each RP-TLC system and for each analyte. The correlations between the physicochemical parameters measured were calculated by linear regression analysis. The impact of the site and character of the substituent on the retention of analytes was assessed by Free-Wilson analysis.*

The measured lipophilicity parameters were compared with 24 molecular descriptors computed applying free engines of the web. Calculations proved that the character of the stationary phase and the type of organic modifier exerted a negligible effect on the measured lipophilicity parameters. In the majority of cases the R_{M0} and b values and computed molecular descriptors were intercorrelated, but the fitness of the points to the linear regression equation was not strong enough to replace the measured values with the computed ones. This finding indicates the importance of using not only computed but also measured parameters for the future studies of both quantitative structure retention relationship (QSRR) and quantitative structure activity relationship (QSAR) calculations.

Keywords carboxamide fungicides, phenylbenzamide derivatives, lipophilicity, reversed phase thin-layer chromatography, specific hydrophobic surface area

Address correspondence to T. Cserháti, Research Institute of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary. E-mail: tevi@chemres.hu

INTRODUCTION

Because of their simplicity, low cost, and rapidity various thin-layer chromatographic (TLC) methodologies have been frequently applied for the separation of a wide variety of organic and inorganic compounds. The theory and practice of TLC analytical tehniques have been previously discussed in detail.[1] TLC has been recently employed for the analysis of pesticides in lake water,[2] study of the behavior of some water soluble Co(III) complexes,[3] measurement of bicalutamide in bulk drug and liposomes,[4] determination of chlorogenic acid in fruits,[5] caffein in black and green teas,[6] rabeprazol and domperidone in formulations,[7] separation of aminoglycoside antibiotics (amikacin, gentamicin, kanamycin, neomycin, and tobramycin),[8] the active agents alprazolam and fluoxetine in tablets.[9] The combination of TLC with other techniques such as matrix-assisted laser desorption/ionization mass spectrometry,[10] and desorption electrospray ionisation ion mobility mass spectrometry[11] has also been reported.

Various TLC methodologies can be applied not only for the separation of analytes but also for the determination of physicochemical parameters of solutes such as lipophilicity,[12–14] free energy of adsorption, and surface area of adsorption.[15]

Discoveries in quantum chemistry and development of computer technologies made possible the calculation of the shape and other properties of molecules. Nowadays several program sets are available to compute a wide variety of molecular descriptors. Some programs, either installed on personal compouter or disposed by engines via web are widely used and in numerous cases such kind of in silico results are substituting physicochemical parameters determined by various physicochemical and biophysical methods.

Carboxamide derivatives have been extensively used in the up to date agrochemical practice with special regards to soil borne basidiomycetaceous infections. Thus, their impact on the dry root rot of Mungbean (*Vigna radiata*),[16] their influence on bradyrhizobial inoculants,[17] and their impact on the quality of sunflower seed was studied in detail.[18] Their effect on the grape quality,[19] and on postharvest gray mold of fruits as well as blue mold in apples[20] have also been investigated.

The aim of the study was the measurement of the lipophilicity parameters of carboxamide fungicides and related compounds on both silica and alumina supports using mixtures of methanol-water, acetone-water, acetonitirile-water, and tetrahydrofurane-water as mobile phases, and the assessment of the influence of the type of stationary phase and organic modifier of the mobile phase on the lipophilicity parameters of analytes. The relationship between measured and computed lipophilicity parameters has also been studied by using linear regression analysis.

EXPERIMENTAL

Materials

The chemical structures of carboxamide fungicides and related compounds are depicted in Fig. 1. The active ingredients of commercialized fungicides were purchased from Sigma Riedel-de Haën (Seelze, BRD). Phenylbenzamide derivatives 1–11 were synthetised in our laboratory of the Plant Protection Institute (Hungarian Academy of Sciences, Budapest, Hungary).

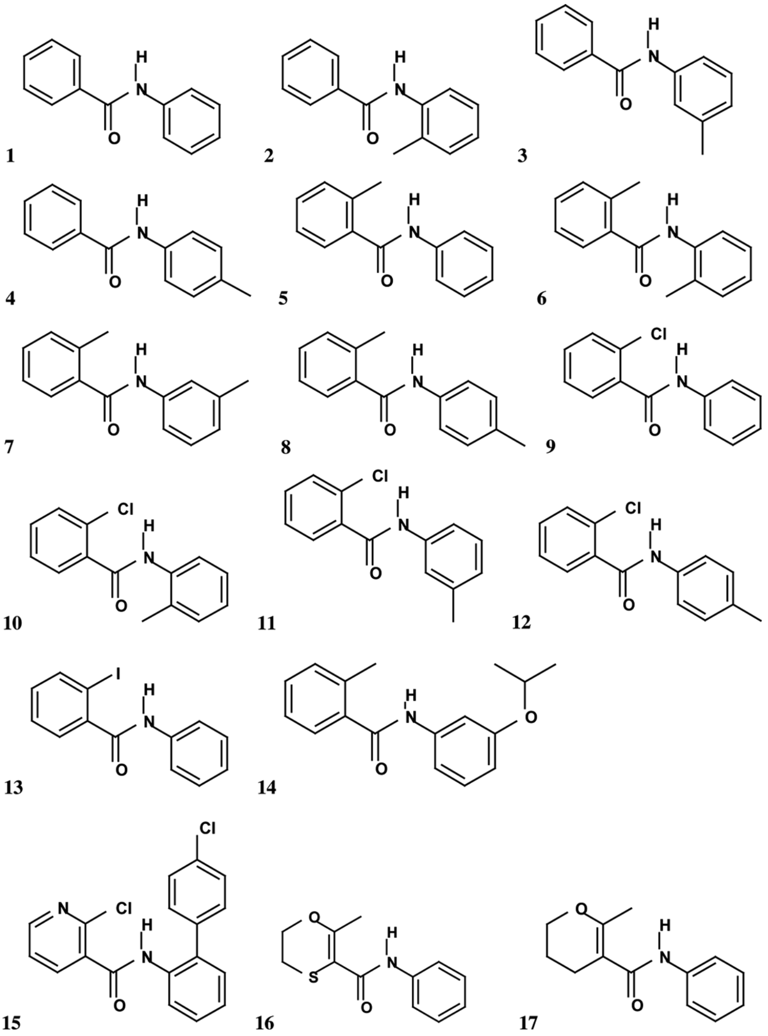


FIGURE 1 Chemical structure of carboxamide derivatives.

DC-Alufolien silica gel 60 F_{254} and DC-Alufolien aluminium oxide 60 F_{254} (both 20 × 20 cm; layer thickness, 0.2 mm) were purchased from Merck AG (Darmstadt, Germany) and were not pretreated, neither by prewashing nor by activation at elevated temperature. The solvents (*n*-hexane, benzene, acetone, acetonitrile, and tetrahydrofuran) were purchased from Sigma Aldrich Kft (Budapest, Hungary). Paraffin oil of pharmaceutical quality was purchased in a local pharmacy.

RP-TLC Determination of the Lipophilicity and Specific Hydrophobic Surface Area of Carboxamide Derivatives and Related Compounds

Silica and alumina plates were impregnated by overnight predevelopment in *n*-hexane-paraffin oil (95:5, v/v). Mobile phases were mixtures of methanol-water, acetone-water, acetonitrile-water, and tetrahydrofurane-water with the concentration of organic modifier varying in steps of 5 vol.%. Analytes were separately dissolved in methanol at a concentration of 0.02 M (w/v), and 2 μL of the solution was spotted onto the plates with a micropipette (Camag, Switzerland). Developments were carried out in separation chambers of 22 × 22 × 3 cm. The developing chambers were not thermostated, developments were performed at ambient temperature (22 ± 1)°C. After development, the mobile phase was evaporated at room temperature and the center of the spots was determined visually under an UV lamp. Measurements were performed in triplicate; the data were omitted from the following calculations when the relative standard deviation between the parallel determinations was higher than 5%. The R_M value used for further calculations was computed for each analyte in each RP-TLC system:

$$R_M = \log(1/R_F - 1) \tag{1}$$

In order to enhance the reliability of the measurements the R_M values were extrapolated to zero concentration of the organic modifier by

$$R_M = R_{MO} + b \cdot C \tag{2}$$

where R_M is the R_M value of a solute measured at a given concentration of organic modifier in the mobile phase, R_{M0} is the R_M value extrapolated to water (best estimation of molecular lipophilicity) and b is the change of R_M values caused by a 1% change of organic modifier (related to the specific hydrophobic surface are of the solute). Calculation was carried out separately for each analyte in each TLC system. The reliability of the measured parameters was assessed by the Fisher's test using Microsoft Office Excel

2003 statistical functions (Microsoft, Redmondton, USA). The relationships between the R_{M0} and *b* values determined under different RP-TLC conditions was examined by using linear regression analysis.

Calculation of the Relationship Between Molecular Parameters Measured by Reversed-Phase TLC and Between the "In Silico" Molecular Descriptors

The impact of the site and character of the individual substituents on the retention of phenylbenzamide derivatives was calculated by Free Wilson analysis. Each substituent at each position was regarded as separate independent variable.

The computed descriptors listed in Table 11 were employed for the assessment of the linear correlation between measured and calculated molecular characteristics.

RESULTS AND DISCUSSION

Analytes showed regular retention behavior, the reproducibility of R_f values was good ($F_{comp} = 0.18 < F_{repl} = 1.78 < F_{0.1} = 2.84$). R_M values decreased linearly with increasing solvent strength of the mobile phases.

TABLE 1 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationary Phase: Silica Impregnated with Paraffin Oil; Mobile Phase: Methanol-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit ($P = 5\%$)

No.	R_{M0}		Slope		R^2
1	1.3230	(1.2806–1.3654)	−0.0261	(−0.0267–−0.0254)	0.9862
2	1.3197	(1.2615–1.3779)	−0.0254	(−0.0262–−0.0245)	0.9728
3	1.9283	(1.8546–2.0020)	−0.0327	(−0.0338–−0.0316)	0.9737
4	1.8870	(1.8178–1.9563)	−0.0323	(−0.0334–−0.0313)	0.9762
5	1.4969	(1.4480–1.5458)	−0.0275	(−0.0283–−0.0268)	0.9835
6	1.2908	(1.2465–1.3351)	−0.0242	(−0.0248–−0.0235)	0.9824
7	1.7947	(1.7506–1.8389)	−0.0304	(−0.0311–−0.0297)	0.9889
8	1.6887	(1.6472–1.7301)	−0.0287	(−0.0294–−0.0281)	0.9891
9	1.4700	(1.4283–1.5117)	−0.0268	(−0.0274–−0.0262)	0.9924
10	1.5862	(1.5191–1.6533)	−0.0284	(−0.0295–−0.0274)	0.9821
11	1.7939	(1.7452–1.8427)	−0.0300	(−0.0307–−0.0293)	0.9862
12	1.9491	(1.8894–2.0088)	−0.0327	(−0.0336–−0.0318)	0.9826
13	1.8377	(1.7831–1.8922)	−0.0321	(−0.0329–−0.0313)	0.9878
14	1.7059	(1.6606–1.7512)	−0.0271	(−0.0278–−0.0265)	0.9917
15	2.5796	(2.4660–2.6933)	−0.0397	(−0.0391–−0.0371)	0.9583
16	1.5551	(1.4929–1.6174)	−0.0284	(−0.0242–−0.0234)	0.9751
17	1.1939	(1.1447–1.2432)	−0.0228	(−0.0207–−0.0199)	0.9813

TABLE 2 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationary Phase: Silica Impregnated with Paraffin Oil; Mobile Phase: Acetone-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit (P = 5%)

No.	R_{M0}		Slope		R^2
1	1.0482	(1.0176–1.0789)	−0.0246	(−0.0251–−0.0241)	0.9876
2	0.9539	(0.9290–0.9788)	−0.0234	(−0.0238–−0.0230)	0.9909
3	1.3400	(1.3131–1.3668)	−0.0258	(−0.0262–−0.0253)	0.9913
4	1.4203	(1.3920–1.4486)	−0.0291	(−0.0296–−0.0286)	0.9924
5	1.2050	(1.1829–1.2272)	−0.0261	(−0.0265–−0.0258)	0.9942
6	1.1117	(1.0822–1.1412)	−0.0255	(−0.0260–−0.0251)	0.9893
7	1.7047	(1.6561–1.7534)	−0.0336	(−0.0344–−0.0329)	0.9860
8	1.5660	(1.5265–1.6055)	−0.0305	(−0.0311–−0.0298)	0.9866
9	1.1929	(1.1767–1.2091)	−0.0254	(−0.0257–−0.0252)	0.9967
10	1.1488	(1.1282–1.1694)	−0.0255	(−0.0258–−0.0251)	0.9947
11	1.5556	(1.5235–1.5877)	−0.0302	(−0.0307–−0.0296)	0.9909
12	1.4714	(1.4499–1.4930)	−0.0288	(−0.0292–−0.0284)	0.9955
13	1.0685	(1.0445–1.0926)	−0.0224	(−0.0228–−0.0220)	0.9907
14	2.2331	(2.1694–2.2969)	−0.0401	(−0.0411–−0.0391)	0.9885
15	2.1271	(2.0657–2.1886)	−0.0381	(−0.0391–−0.0371)	0.9882
16	0.9911	(0.9670–1.0152)	−0.0238	(−0.0242–−0.0234)	0.9918
17	0.8158	(0.7929–0.8387)	−0.0203	(−0.0207–−0.0199)	0.9898

The R^2 values varied between 0.942–0.997 indicating that the linearity of the dependence of R_M values on the concentration of the organic modifier in the mobile phase was not influenced by the type of the RP-TLC system

TABLE 3 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationary Phase: Silica Impregnated with Paraffin Oil; Mobile Phase: Acetonitrile-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit (P = 5%)

No.	R_{M0}		Slope		R^2
1	2.1436	(1.9870–2.3003)	−0.0531	(−0.0558–−0.0504)	0.9533
2	3.0796	(2.8702–3.2891)	−0.0751	(−0.0788–−0.0715)	0.9581
3	3.2149	(2.9812–3.4486)	−0.0747	(−0.0787–−0.0706)	0.9688
4	3.6060	(3.3003–3.9117)	−0.0845	(−0.0897–−0.0794)	0.9488
5	3.1930	(3.0077–3.3783)	−0.0766	(−0.0798–−0.0734)	0.9716
6	2.8446	(2.6868–3.0024)	−0.0687	(−0.0714–−0.0660)	0.9743
7	3.6397	(3.4018–3.8775)	−0.0841	(−0.0881–−0.0801)	0.9719
8	3.3734	(3.2004–3.5465)	−0.0777	(−0.0807–−0.0747)	0.9839
9	3.3332	(3.1417–3.5247)	−0.0801	(−0.0834–−0.0768)	0.9689
10	2.6560	(2.6019–2.7102)	−0.0684	(−0.0694–−0.0674)	0.9973
11	3.6147	(3.4202–3.8091)	−0.0834	(−0.0867–−0.0801)	0.9781
12	3.6992	(3.5007–3.8977)	−0.0821	(−0.0854–−0.0787)	0.9832
13	3.3625	(3.2050–3.5199)	−0.0786	(−0.0813–−0.0759)	0.9780
14	3.7382	(3.5815–3.8948)	−0.0830	(−0.0857–−0.0803)	0.9884
15	4.2965	(4.1026–4.4905)	−0.0947	(−0.0979–−0.0915)	0.9878
16	2.6652	(2.5137–2.8167)	−0.0661	(−0.0688–−0.0633)	0.9760
17	2.4218	(2.2884–2.5552)	−0.0611	(−0.0635–−0.0587)	0.9782

TABLE 4 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationary Phase: Silica Impregnated with Paraffin Oil; Mobile Phase: Tetrahydrofuran-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit ($P = 5\%$)

No.	R_{M0}		Slope		R^2
1	1.0195	(0.9993–1.0397)	−0.0177	(−0.0180–−0.0174)	0.9958
2	0.8504	(0.8176–0.8832)	−0.0153	(−0.0158–−0.0148)	0.9854
3	1.5763	(1.5269–1.6257)	−0.0258	(−0.0266–−0.0251)	0.9883
4	1.5799	(1.5133–1.6465)	−0.0264	(−0.0274–−0.0254)	0.9799
5	1.5071	(1.4532–1.5610)	−0.0244	(−0.0252–−0.0236)	0.9846
6	1.1020	(1.0698–1.1341)	−0.0182	(−0.0187–−0.0178)	0.9901
7	1.5914	(1.5216–1.6612)	−0.0244	(−0.0254–−0.0234)	0.9798
8	1.7301	(1.6615–1.7986)	−0.0269	(−0.0279–−0.0259)	0.9859
9	1.2743	(1.2262–1.3225)	−0.0213	(−0.0220–−0.0205)	0.9837
10	1.0882	(1.0585–1.1180)	−0.0179	(−0.0184–−0.0175)	0.9912
11	1.6966	(1.6480–1.7453)	−0.0269	(−0.0276–−0.0262)	0.9947
12	1.5331	(1.4948–1.5715)	−0.0244	(−0.0250–−0.0239)	0.9946
13	1.6627	(1.5778–1.7476)	−0.0332	(−0.0344–−0.0320)	0.9781
14	1.9336	(1.8661–2.0011)	−0.0294	(−0.0304–−0.0284)	0.9885
15	1.8021	(1.7350–1.8692)	−0.0290	(−0.0299–−0.0280)	0.9883
16	0.6898	(0.6654–0.7143)	−0.0134	(−0.0137–−0.0130)	0.9924
17	0.6157	(0.5816–0.6499)	−0.0133	(−0.0139–−0.0128)	0.9878

($F_{supp} = 3.02 < F_{0.1} = 3.69$; $F_{solv} = 0.34$). Students t values varied between 7.2–57.3 and 9.0–55.3 for the lipophilicity and specific hydrophobic surface area ($t_{0,001} = 5.4$), respectively. This finding indicates the reliability of the measurements.

TABLE 5 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationary Phase: Alumina Impregnated with Paraffin Oil; Mobile Phase: Methanol-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit ($P = 5\%$)

No.	R_{M0}		Slope		R^2
1	1.3144	(1.2744–1.3545)	−0.0260	(−0.0266–−0.0253)	0.9835
2	1.2037	(1.1669–1.2405)	−0.0256	(−0.0262–−0.0251)	0.9891
3	1.8794	(1.7845–1.9743)	−0.0331	(−0.0345–−0.0317)	0.9681
4	1.8991	(1.8078–1.9904)	−0.0341	(−0.0354–−0.0328)	0.9722
5	1.5252	(1.4630–1.5873)	−0.0292	(−0.0301–−0.0283)	0.9846
6	1.4577	(1.4028–1.5125)	−0.0300	(−0.0308–−0.0291)	0.9810
7	1.8672	(1.7803–1.9541)	−0.0323	(−0.0336–−0.0311)	0.9764
8	2.0604	(1.9859–2.1349)	−0.0359	(−0.0370–−0.0349)	0.9831
9	1.6347	(1.5801–1.6894)	−0.0308	(−0.0316–−0.0300)	0.9931
10	1.7029	(1.6402–1.7657)	−0.0340	(−0.0350–−0.0331)	0.9821
11	2.1530	(2.0707–2.2352)	−0.0368	(−0.0380–−0.0356)	0.9804
12	1.8226	(1.7529–1.8922)	−0.0369	(−0.0379–−0.0359)	0.9818
13	1.8398	(1.7909–1.8887)	−0.0323	(−0.0330–−0.0316)	0.9930
14	2.1790	(2.1216–2.2363)	−0.0400	(−0.0407–−0.0392)	0.9965
15	2.5140	(2.3321–2.6960)	−0.0363	(−0.0371–−0.0355)	0.9963
16	1.3475	(1.3032–1.3917)	−0.0273	(−0.0280–−0.0267)	0.9896
17	1.2707	(1.2285–1.3129)	−0.0275	(−0.0281–−0.0268)	0.9872

TABLE 6 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationay Phase: Alumina Impregnated with Paraffin Oil; Mobile Phase: Acetone-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit (P = 5%)

No.	R_{M0}		Slope		R^2
1	1.3641	(1.2848–1.4433)	−0.0393	(−0.0408–−0.0377)	0.9835
2	1.2020	(1.1163–1.2877)	−0.0379	(−0.0396–−0.0363)	0.9854
3	1.9009	(1.8243–1.9775)	−0.0478	(−0.0492–−0.0464)	0.9879
4	1.8466	(1.7686–1.9246)	−0.0471	(−0.0486–−0.0456)	0.9888
5	1.5961	(1.5401–1.6521)	−0.0421	(−0.0432–−0.0410)	0.9940
6	1.4724	(1.3881–1.5567)	−0.0430	(−0.0446–−0.0413)	0.9869
7	1.9922	(1.9286–2.0557)	−0.0467	(−0.0479–−0.0456)	0.9912
8	2.0087	(1.9467–2.0707)	−0.0479	(−0.0490–−0.0468)	0.9921
9	1.6759	(1.5915–1.7603)	−0.0437	(−0.0453–−0.0421)	0.9849
10	1.5711	(1.4771–1.6651)	−0.0436	(−0.0454–−0.0417)	0.9868
11	2.1243	(2.0535–2.1951)	−0.0506	(−0.0518–−0.0493)	0.9907
12	2.0575	(1.9829–2.1321)	−0.0497	(−0.0512–−0.0483)	0.9935
13	1.6821	(1.5910–1.7733)	−0.0414	(−0.0430–−0.0397)	0.9773
14	2.1866	(2.0935–2.2798)	−0.0466	(−0.0483–−0.0449)	0.9813
15	2.3992	(2.3082–2.4901)	−0.0538	(−0.0555–−0.0522)	0.9865
16	1.2128	(1.1443–1.2814)	−0.0360	(−0.0373–−0.0347)	0.9853
17	1.1229	(0.9594–1.2864)	−0.0385	(−0.0419–−0.0351)	0.9421

The lipophilicity, specific hydrophobic surface area, and the confidency limit (5%) of analytes determined under different experimental conditions are compiled in Tables 1–8. The high R^2 values indicate that the

TABLE 7 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationay Phase: Alumina Impregnated with Paraffin Oil; Mobile Phase: Acetonitrile-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit (P = 5%)

No.	R_{M0}		Slope		R^2
1	1.3689	(1.2640–1.4738)	−0.0398	(−0.0418–−0.0377)	0.9801
2	1.3083	(1.2110–1.4056)	−0.0466	(−0.0486–−0.0445)	0.9865
3	1.8732	(1.7999–1.9464)	−0.0476	(−0.0490–−0.0462)	0.9931
4	1.8051	(1.7422–1.8679)	−0.0464	(−0.0476–−0.0452)	0.9947
5	1.6360	(1.5561–1.7159)	−0.0442	(−0.0458–−0.0427)	0.9906
6	1.4725	(1.3684–1.5766)	−0.0412	(−0.0434–−0.0391)	0.9790
7	2.0573	(1.9701–2.1445)	−0.0491	(−0.0507–−0.0474)	0.9891
8	2.0181	(1.9611–2.0751)	−0.0488	(−0.0499–−0.0477)	0.9945
9	1.6187	(1.5168–1.7205)	−0.0429	(−0.0449–−0.0409)	0.9885
10	1.5669	(1.4750–1.6588)	−0.0464	(−0.0483–−0.0445)	0.9849
11	2.1284	(2.0589–2.1979)	−0.0507	(−0.0521–−0.0494)	0.9924
12	2.0683	(2.0020–2.1347)	−0.0503	(−0.0516–−0.0490)	0.9930
13	1.8602	(1.7842–1.9362)	−0.0462	(−0.0477–−0.0448)	0.9922
14	2.6027	(2.4386–2.7669)	−0.0570	(−0.0601–−0.0538)	0.9763
15	2.7075	(2.5412–2.8737)	−0.0608	(−0.0640–−0.0576)	0.9786
16	1.4221	(1.2756–1.5686)	−0.0427	(−0.0458–−0.0396)	0.9735
17	1.0381	(0.9453–1.1310)	−0.0365	(−0.0384–−0.0345)	0.9852

TABLE 8 Lipophilicity (R_{M0}) and Specific Hydrophobic Surface Area (b) of Carboxamide Derivatives. Stationay Phase: Alumina Impregnated with Paraffin Oil; Mobile Phase: Tetrahydrofuran-Water Mixtures. Numbers Refer to Analytes in Fig. 1. Values in Brackets are Confidency Limit ($P = 5\%$)

No.	R_{M0}		Slope		R^2
1	1.0382	(1.0069–1.0694)	−0.0211	(−0.0216–−0.0206)	0.9872
2	0.7360	(0.7099–0.7620)	−0.0167	(−0.0171–−0.0163)	0.9858
3	1.4549	(1.4271–1.4827)	−0.0298	(−0.0302–−0.0294)	0.9968
4	1.4856	(1.4488–1.5224)	−0.0282	(−0.0287–−0.0276)	0.9926
5	1.4893	(1.4625–1.5162)	−0.0293	(−0.0297–−0.0289)	0.9967
6	1.0836	(1.0653–1.1019)	−0.0210	(−0.0213–−0.0207)	0.9959
7	1.7687	(1.7304–1.8070)	−0.0379	(−0.0384–−0.0373)	0.9951
8	1.7232	(1.6733–1.7731)	−0.0305	(−0.0313–−0.0298)	0.9917
9	1.5604	(1.5165–1.6043)	−0.0300	(−0.0306–−0.0293)	0.9907
10	1.2209	(1.1880–1.2538)	−0.0265	(−0.0270–−0.0260)	0.9920
11	1.6212	(1.5652–1.6771)	−0.0317	(−0.0325–−0.0309)	0.9887
12	1.5319	(1.4899–1.5739)	−0.0310	(−0.0316–−0.0304)	0.9927
13	1.6201	(1.5812–1.6590)	−0.0330	(−0.0336–−0.0324)	0.9944
14	1.9625	(1.9181–2.0068)	−0.0332	(−0.0339–−0.0326)	0.9944
15	1.7743	(1.6596–1.8889)	−0.0283	(−0.0297–−0.0270)	0.9693
16	0.8516	(0.8308–0.8724)	−0.0188	(−0.0191–−0.0185)	0.9928
17	0.4828	(0.4657–0.4998)	−0.0136	(−0.0139–−0.0134)	0.9909

dependence of both the lipophilicity and specific hydrophobic surface area of analytes on the concentration of organic modifyer in the mobile phase can be adequately described by a linear relationship. It can be further established from the data in Tables 1– 8 that both the R_{M0} and b values show

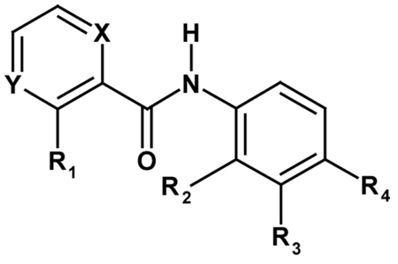
TABLE 9 Similarity Between the Lipophilicity (R_{M0}) Values and Specific Hydrophobic Surface Area (b Values) of Analytes Determined in Various RPTLC Systems (Pearson's Correlation Coefficients)

	Matrix A							
	Aluminiumoxide				Silicagel			
Matrix B	ACO	MEOH	ACN	THF	ACO	MEOH	ACN	THF
Aluminiumoxide								
ACO	1	0.964	0.868	0.951	0.961	0.786	0.892	0.870
MEOH	0.780	1	0.886	0.987	0.940	0.848	0.900	0.884
ACN	0.479	0.704	1	0.884	0.839	0.703	0.958	0.801
THF	0.682	0.929	0.600	1	0.933	0.838	0.894	0.876
Silicagel								
ACO	0.718	0.835	0.605	0.876	1	0.790	0.851	0.866
MEOH	0.571	0.752	0.544	0.723	0.505	1	0.649	0.702
ACN	0.545	0.801	0.838	0.685	0.588	0.663	1	0.827
THF	0.678	0.832	0.690	0.819	0.702	0.707	0.735	1

Matrix A and Matrix B=Pearson's correlation coefficients of R_{M0} values and specific hydrophobic areas, respectively ($r_{0.01} = 0.57$, $r_{0.05} = 0.43$, $n = 17$).

ACO = aceton, MEOH = methanol, ACN = acetonitrile, THF = tetrahyrofuran.

TABLE 10 The Impact of Substituents on the Retention Behavior of Phenylbenzamide Derivatives



General formula

	Substituents								
	R_1			R_2		R_3		R_4	
No.	H	CH_3	Cl	H	CH_3	H	CH_3	H	CH_3
Lipophilicity (R_{M0})									
Aceton-Al	*−0.55*	0.12	0.44	0.00	*-0.63*	0.39	0.39	−0.41	0.41
Methanol-Al	−0.45	−0.05	*0.50*	*0.65*	0.00	0.36	0.36	*−0.46*	*0.46*
THF-Al	*−0.76*	0.44	0.32	*0.74*	0.00	0.29	0.29	−0.19	0.19
Acetonitrile-Al	*−0.50*	0.17	0.33	*0.62*	0.00	*0.50*	*0.50*	−0.35	0.35
Aceton-Si	*−0.59*	0.30	0.30	0.41	−0.41	*0.72*	*0.72*	*-0.55*	0.00
Methanol-Si	−0.03	−0.44	*0.47*	*0.49*	0.00	0.30	0.30	−0.37	0.37
THF-Si	*−0.46*	0.38	0.07	0.74	0.00	0.39	0.39	−0.36	0.36
Acetonitrile-Si	−0.34	0.11	0.23	0.34	−0.34	*0.64*	*0.64*	*−0.47*	0.00
Specific Hydrophobic Area									
Aceton-Al	*0.52*	0.01	*−0.53*	−0.41	0.41	*−0.75*	*−0.75*	*0.57*	0.00
Methanol-Al	*0.49*	0.13	*−0.62*	*−0.46*	*0.46*	*−0.68*	*−0.68*	*0.56*	0.00
THF-Al	*0.67*	−0.33	−0.35	*−0.67*	0.00	−0.48	−0.48	−0.01	0.01
Acetonitrile-Al	0.26	0.08	−0.34	−0.09	0.09	*−0.51*	0.00	*0.70*	*−0.70*
Aceton-Si	*0.47*	−0.45	−0.03	0.00	*0.51*	−0.40	−0.40	0.30	−0.30
Methanol-Si	−0.11	0.33	−0.22	*−0.58*	0.00	−0.36	−0.36	0.42	−0.42
THF-Si	.32	−0.28	−0.04	*−0.77*	0.00	−0.35	−0.35	0.40	−0.40
Acetonitrile-Si	0.34	−0.10	−0.25	−0.24	0.24	*−0.54*	*−0.54*	0.38	0.00

Al = aluminiumoxide, Si = silicagel. The parcial regression coefficients were calculated by Stepwise regression analysis involving experimental compounds **1–12** ($r_{0.05} = 0.46$, FG = 11).

considerable variation between the analytes. This finding suggests that RP-TLC systems using impregnated silica and/or alumina stationary phases and any organic modifiers can be successfully employed for the separation of this class of analytes in reversed phase thin-layer chromatography. The separation capacity of organic modifiers showed slight dissimilarities (F = 0.34), however, the specific hydrophobic surface area of phenylbenzamide derivatives indicated that the interaction between stationary phase and organic modifier did not influence considerably their retention ($F_{supp,solv} = 0.41$).

TABLE 11 Relationships Between the Measured and Computed Physicochemical Parameters of Carboxamide Fungicides and Phenylbenzamide Derivatives (Pearson's Correlation Coefficients)

		Organic modifiers[a]							
		Aceton		Methanol		Acetonitrile		Tetrahydrofurane	
Calculated Parameters (descriptors)	WE[b]	R_{M0}	SHA	R_{M0}	SHA	R_{M0}	SHA	R_{M0}	SHA
Lipophilicity related									
LogP	A	0.75	−0.58	0.78	−0.79	0.85	−0.87	0.66	−0.49
LogP	M	0.81	−0.72	0.82	−0.85	0.82	−0.83	0.72	−0.59
AC_logP	T	0.77	−0.64	0.76	−0.79	0.76	−0.77	0.74	−0.64
AB/LogP	T	0.78	−0.79	0.70	−0.71	0.74	−0.76	0.58	−0.41
ALOGP	T	0.78	−0.68	0.74	−0.76	0.75	−0.75	0.73	−0.64
MLOGP	T	0.61	−0.45	0.59	−0.61	0.58	−0.56	0.71	−0.69
KOWWIN	S	0.85	−0.67	0.82	−0.81	0.82	−0.73	0.89	−0.83
XLOGP2	T	0.82	−0.63	0.84	−0.85	0.85	−0.83	0.83	−0.73
XLOGP3	T	0.82	−0.77	0.80	−0.84	0.78	−0.79	0.68	−0.56
ALOGPs	T	0.75	−0.68	0.77	−0.81	0.75	−0.75	0.67	−0.56
LogP	C	0.86	−0.70	0.84	−0.83	0.83	−0.77	0.87	−0.80
LogP	O	0.77	−0.64	0.76	−0.79	0.76	−0.77	0.75	−0.64
Water solubility related									
ALOGpS	T	−0.79	0.69	−0.79	0.83	−0.78	0.80	−0.72	0.63
AC_logS	T	−0.77	0.67	−0.80	0.84	−0.80	0.83	−0.65	0.51
AB/logS	T	−0.75	0.59	−0.76	0.79	−0.75	0.75	−0.76	0.70
OESol	O	−0.77	0.67	−0.80	0.84	−0.80	0.83	−0.65	0.51
Other parameters									
Mw	M	0.53	−0.40	0.63	−0.67	0.64	−0.64	0.46	−0.34
Mol volume	M	0.72	−0.67	0.75	−0.77	0.79	−0.83	0.51	−0.30
Mol volume	A	0.75	−0.68	0.76	−0.78	0.82	−0.84	0.55	−0.35
Parachor	A	0.72	−0.65	0.76	−0.79	0.80	−0.84	0.53	−0.32
Mol. refr.	A	0.74	−0.63	0.80	−0.82	0.83	−0.86	0.59	−0.40
Polarizability	A	0.74	−0.63	0.80	−0.82	0.83	−0.86	0.59	−0.40
pKa	C	−0.61	0.43	−0.69	0.74	−0.68	0.71	−0.66	0.53
pKb	C	0.16	−0.06	0.09	−0.04	0.17	−0.14	0.29	−0.23

[a] The Pearson's correlation coefficients between "in silico" molecural descriptors and lipophylicity parameters R_{M0} as well as specific hydrophopbic area (SHA) were calculated applying Excel2003 statistical functions ($r_{0.05} = 0.43$; $r_{0.01} = 0.57$).

[b] The following free programs, sets, and web engines were used for calculation of "in silico" descriptors: A=ACD, M=Molinspiration, T=VCCLab, S=Syrres, AC=Actelion, O=Osiris, C=Chemaxon.

It is interesting to note that the R_{M0} values show marked variation according to the type of stationary phase and the character of the organic modifier. Theoretically, the R_{M0} values extrapolated to zero concentration of organic modifier have to be identical independently of the type of organic modifier. The deviation of this rule can be tentatively explained by the supposition that the linearity is not strictly valid at very low concentration of organic modifier in RP-TLC.

The Pearson's correlation coefficients descripting the relationship between the lipophilicity and b values measured in different RP-TLC systems are compiled in Table 9. The data in Table 9 entirely support our previous qualitative conclusions illustrating that the measured molecular parameters are similar but not identical. This results draws the attention to the fact that the reliability of the determination of hydrophobicity parameters of this class of analytes can be considerably increased by using more then one RP-TLC systems for the determination of lipophilicity and specific hydrophobic surface area.

The impact of the type and situation of the individual substituents of phenylbenzamide derivatives on their retention is illustrated in Table 10. The results clearly show that both the position of the substituent and its chemical structure influences considerably its impact on the retention behavior of the molecule.

The correlation coefficients of the linear regression analyses computed between the measured and computed physicochemical parameters are listed in Table 11. The data in Table 11 show that in the majority of cases significant correlations can be found between the two sets of physicochemical parameters. Although the relationships are significantly linear their predictive power is relatively low. This finding suggests that the measured and computed physisochemical parameters of carboxamide fundicides and phenylbenzamide derivatives are similar but not identical, consequently they cannot be used for the reliable prediction of the RP-TLC retention behavior of these analytes.

CONCLUSIONS

It can be concluded from the measurements and computations that carboxamide fungicides and phenylbenzmaide drievatives can be easily separated by RPTLC using both alumina and silica stationary phases impregnated with paraffin oil and employing methanol, acetone, acetonitrile, and tetrahydrofurane as organic modifier of the mobile phase. It was found that the method is precise, reliable, and can be successfully employed for the detemination of molecular lipophilicity and specific hydrophobic surfa area. It was further established that the predictive power of computed parameters for the description of the retention behavior of this class of analytes is fairly low, although the relationships between these two sets of data are significant.

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