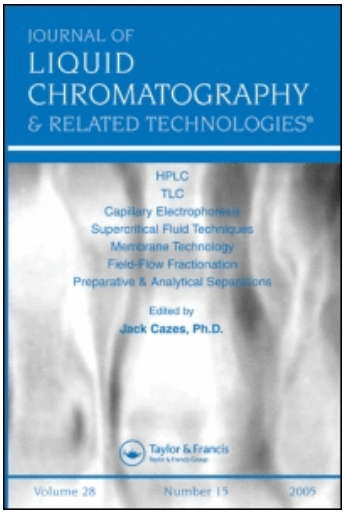

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Reversed Phase Thin Layer Chromatographic Behavior of Some Acylanilide Fungicides

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Abstract: The R_f values of some acylanilide derivatives was determined under reversed phase thin layer chromatographic (RP-TLC) conditions using acetone-water and methanol-water mixtures as mobile phases. The R_{M0} and b values related to the molecular lipophilicity and to the specific hydrophobic surface area of the analytes were calculated. The relationships between the chromatographic parameters and physicochemical characteristics were calculated by principal component analysis (PCA), cluster analysis (CA), and stepwise linear regression analysis. The results indicated that the type of organic modifier exerts a negligible effect on the measured RP-TLC parameters. It was further established that the results of PCA cannot be used for differentiation among the physicochemical parameters. CA calculations indicated that the highest similarities were between metalaxyl and furalaxyl, as well as between oxadixyl and RE26745. Stepwise regression analysis proved that computed parameters can be successfully used for the prediction of the retention behavior of acylanilide derivatives in RP-TLC.

Keywords: Acylanilide fungicides, Quantitative structure-retention relationship (QSRR), Reversed phase thin layer chromatography

*To Professor Janos Hollo at his 90th birthday.

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INTRODUCTION

Because of their marked antifungal activity acylanilide derivatives have been extensively used in up to date agricultural practice. In addition to their beneficial effect, they also show marked toxicity and can cause environmental pollution.[1] The considerable commercial importance of acylanilide derivatives promoted the development of various analytical techniques suitable for their separation and quantitative determination in various more or less complicated accompanying matrices. Thus, chemiluminescence and multicommutation continuous flow methodology have been successfully applied for the investigation of the photodegradation of furalaxyl and ofurace.[2] The high separation capacity, precision, and reliability of the chromatographic methods have been exploited frequently in the quantitative analysis of acylanilide derivatives. Gas chromatography (GC) coupled to mass spectrometry (MS) was employed for the control of the removal of fungicides and insecticides (among them metalaxyl and oxadixyl) from wines by using various adsorbents.[3] Another GC-MS study determined six oxazole fungicides, among them, oxadixyl in malt beverages.[4] GC-MS has found application in the analysis of pesticides (three triazines, their desethyl derivatives, metolachlor and metalaxyl) in surface and ground waters. The limit of quantitation was 0.01 μ/L.[5] Oxadixyl and other fungicides (hymexazol, drazoxolon, vinclozolin, chlozolinate, farmaxadone) were determined in wines and juices by using two new sample preparation methods, stir bar sorptive extraction and membrane assisted solvent extraction prior to ultra-performance liquid chromatographic analysis.[6] GC has been further used for the measurement of benalaxyl, chlorothalonil, and methomyl in tomatoes grown in open fields[7] and for the investigation of the photodegradation of furalaxyl.[8]

Various high performance liquid chromatographic techniques (normal and reversed phase separation modes) have also found application in the analysis of acylanilide derivatives. Pesticides, among them, benalaxyl and metalaxyl, were determined in tobacco using RP-HPLC combined with MS.[9]

Not only GC-MS and HPLC but also micellar electrokinetic chromatography have been used been applied for the analyses of pesticides (among them metalaxyl) in red wines. Preconcentration of analytes was performed by solid phase microextraction and the injection was carried out by reversed electrode polarity stacking mode. The recovery varied between 90–107%.[10]

As enantiomer pairs may exhibit markedly different biological activity, decomposition rate, and toxicity, many efforts were dedicated to the separation and quantitative determination of the enantimers of acylanilide derivatives. The investigation usually employed various HPLC technologies. Thus, the chiral separation of benalaxyl extracted from soil

and water has been reported.[11] The majority of methods used the chiral stationary phase cellulose-tris(3,5-dimethylphenylcarbamate) (CDMPC). This stationary phase has been successfully used for the enantioseparation of benalaxyl in soil and water,[12] in soil, water, and grapes,[14] in tomatoes, tobacco, sugar beets, capsicum and soil,[15] and in plasma.[16] The parameters of the RP-HPLC separation using CDMPC stationary phase were investigated in detail.[17]

Mathematical statistical methods have been frequently employed for the evaluation of the relationship between various TLC or RP-TLC systems,[18] between the thin layer chromatographic retention data and biological activity (quantitative structure activity relationship, QSAR),[19] and/or the relationship between retention data and physicochemical parameters, QSRR).[20]

The mathematical statistical methods frequently employed for the evaluation of TLC retention data are multilinear regression analysis,[21] principal component analysis,[22] spectral mapping technique,[23] factor analysis,[24] and cluster analysis.[25]

The objectives of the study were the determination of the lipophilicity and hydrophobic surface area of some acylanilide derivatives by reversed phase thin layer chromatography, the assessment of the effect of the type and concentration of the organic modifier in the mobile phase, and the elucidation of the predictive power of computed physicochemical parameters for the calculation of retention parameters.

EXPERIMENTAL

Materials

The chemical structures of the acylanilide derivatives are shown in Figure 1. Their common name, chemical name, commercial name, and their provenance are listed in Table 1. DC-Alufolien Kieselgel 60 F_{254} plates (20×20 cm, layer thickness, 0.2 mm) were purchased from Merck AG (Darmstadt, Germany) and were not pretreated, either by prewashing or by activation at elevated temperature. Acetone, *n*-hexane and methanol of HPLC grade were purchased from Sigma-Aldrich Kft (Budapest, Hungary). Paraffin oil of pharmaceutical quality was purchased in a local pharmacy.

Determination of the Lipophilicity in Reversed Phase TLC

Stock solutions (0.2 M) of the analytes were prepared in methanol and 2 μL was spotted separately on the plates. The application of this experimental design was influenced by the fact that the aim of the

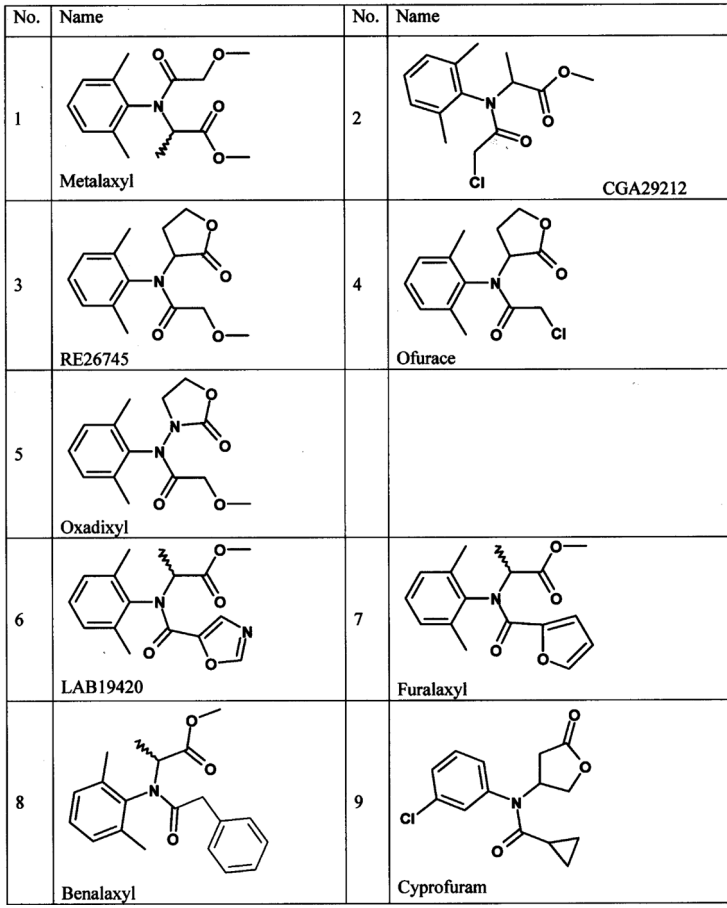

Figure 1. Chemical structures of acylanilide derivatives.

measurements was the determination of the retention behavior of acylanilide derivatives and not the study of the possibilities of their separation. Moreover, this arrangement excludes the competition of analytes for the active sites of the surface of the stationary phase.

Silica plates were impregnated by overnight development in *n*-hexane-paraffin oil (95:5, v/v). Mobile phases consisted of mixtures of acetone-water and methanol-water with the concentration of organic modifier varying in steps of 5 vol.%. Developments were carried out in sandwich chambers ($21 \times 20 \times 3$ cm). The chambers were not thermostatically controlled, developments were performed at ambient temperature ($22 \pm 2°C$). The running distance was, in each case, between 100–120 mm. After development, the plates were dried at room temperature and the

Table 1. The common name, chemical name, commercial name and provenance of acylanilide derivatives

No.	Common name	IUPAC name	Trade name	Manufacturer
1	Metalaxyl	methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate	Ridomil 25 wp	Ciba-Geigy, Switzerland
2	CGA29212	methyl *N*-(2,6-dimethyl-phenyl)-*N*-chloroacetyl-*DL*-alaninate	Experimental	Ciba-Geigy, Switzerland
3	RE-26745	*N*-(2,6-dimethylphenyl)-2-methoxy-*N*-(2-oxo-tetrahydrofuran-3-yl)-acetamide	Experimental	Chevron, USA
4	Ofurace	α-2-chloro-*N*-2,6-xylylacetamido-γ-butyrolactone	Milfuram 50 wp	Chevron, USA
5	Oxadixyl	methoxy-*N*-(2-oxo-1,3-oxazolidin-3-yl)acet-2′, 6′-xylidide	Sandofan 25 wp	Sandoz AG, Germany
6	LAB14202 F	2-[(2,6-dimethyl-phenyl)-(oxazole-5-carbonyl)-amino]-propionic acid methylester	Experimental	BASF AG, Germany
7	Furalaxyl	methyl-N-(2,6-xylyl)-N-(2-furanylcarbonyl)-DL-alaninate	Fongarid 25 wp	Ciba Geigy, Switzerland
8	Benalaxyl	methyl-*N*-phenylacetyl-*N*-2, 6-xylyl-*DL*-alaninate	Galben 25 wp	Montedison, Italy
9	Cyprofuram	(±)-a-[*N*-(3-chlorophenyl) cyclopropanecarboxa-mido]-ã-butyrolactone	Vinicur 50 wp	Schering AG, Germany

analytes were detected by their UV extinction. The spot center was determined visually. Each measurement was performed in triplicate, the data were omitted from the following calculations when the relative standard deviation was over 5%.

Calculation of the Relationship Between Retention Characteristics and Physicochemical Parameters of Acylanilides

The R_M value used for further computations was calculated for each analytes and each mobile phase composition by:

$$R_M = \log(1/R_f - 1) \quad (1)$$

In order to increase the reliability of the calculation of lipophilicity, the R_M values were extrapolated to zero concentration of the organic component of the mobile phase by:

$$R_M = R_{M0} + b \cdot C \quad (2)$$

Where R_M is the concrete R_M value of an acylanilide derivative determined at a given concentration of organic modifier in the mobile phase, R_{M0} is the hypothetic R_M value extrapolated to zero concentration of organic modifier (best estimation of molecular lipophilicity), and b is the change of R_M value caused by 1 vol.% change of organic modifier in the mobile phase (related to the hydrophobic surface area of the analyte[26,27]). Calculations were carried out separately for each acylanilide derivatives.

The capacity of a considerable number of computed physicochemical parameters was investigated for their ability to predict the retention behavior of these analytes. They are listed in Table 2. The similarities and dissimilarities among the acylanilide fungicides taking into consideration, simultaneously, the measured and computed parameters were elucidated by principal component analysis (PCA) and by cluster analysis (CA). The variance explained by the principal components was set to 95%. The linear regression between the measured and calculated physicochemical parameters was elucidated by stepwise regression analysis (SPA). The original data matrix of PCA and CA computations consisted of the acylanilide derivatives as variables and the computed and measured parameters as observations. SRA calculations were performed four times, the R_{M0} and b values measured in acetone-water and methanol water mobile phase systems were, separately, the dependent variables, and the computed molecular characteristic listed in Table 2 were, in each instance, the independent variables. The number of accepted independent variables was set to 1; the significance level of the acceptance was set to 95%. This decision was influenced by the high correlation between the computed molecular parameters.

RESULTS AND DISCUSSION

Each acylanilide derivatives displayed regular spot shapes in each RP-TLC system and deformed peak spots decreasing the reliability of the measurements were not observed. This finding indicated that these RP-TLC systems can be used for the analysis of this class of analytes. Acylanilide derivatives showed typical RP retention behavior: R_M values decreased with increasing concentration of the organic modifier in the mobile phase. The parameters of Equation 2 describing the dependence

Table 2. Physicochemical parameters included int he quantitative structure retention relationship (QSRR) study

Abbreviations	Parameter	Calculated by
miLog P	LogP value calculated as sum of molecular increments	Molinspiration property engine V2007.04
TPSA	Total Polar Surface Area	Molinspiration property engine V2007.04
Nrotb	Number of rotation barries	Molinspiration property engine V2007.04
miMolVol	Volume of molecule cubic angstrom calculated by	Molinspiration property engine V2007.04
CosmoQ	σ-profiles most likely also carry a large part of information required for the estimation of desolvation and binding processes, which are responsible for the inhibition of enzyme receptors by drug molecules. Thus, a high similarity with resoect to the ó-profiles appears to be necessary condition for drugs of similar physiological action.	
Molar refractivity	Molar refractivity is a measure of the volume occupied by an atom or group and is dependent on the temperature, the index of refraction, and the pressure. Therefore the molar refractivity is the volume of the substance (in cubic meters) taken up by each mole of that substance.	ACD/ChemSketch 11.01
Molecular volume	The van der Waals surface encloses a volume called molecular volume. The van der Waals surface area (abbreviated variously as vdWSA, VSA, WSA), also van der Waals surface or van der Waals envelope (after van der Waals) is the surface of the union of the union of the spherical atomic surfaces defined by the van der Waals radius of each componenet atom in the molecule. Both van der Waals surface and molecular volume are conventional abstractions, rather than ''real'' surface and volume of a molecule.	ACD/ChemSketch 11.01

(*Continued*)

Table 2. Continued

Abbreviations	Parameter	Calculated by
Parachor	Parachor is a scientific quantity defined according to the formula: $P = \gamma^{1/4} * M/d$ Where $-1^{/4}$ is the fourth root of surface tension, M is the molar mass, and d is the density.	ACD/ChemSketch 11.01
Polarizability	Polarizability is the relative tendency of a charge distribution, like the electron cloud of an atom or molecule, to be distorted from its normal shape by an external electric field,which may be caused by the presence of a nearby ion or dipole.	ACD/ChemSketch 11.01
LogPexp	Log P experimental In the fields of organic and medicinal chemistry, a partition (P) or distribution coefficient (D) is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium. Hence these coefficients are a measure of differential solubility of the compound these two solvents.	SYRRES Data Base, Syracusa Research Corporation http//www.syrres.com/
LogP	Computed	VCCl engine, Virtual Computational Chemistry Laboratory, http//www.vcclab.org
AC logP	Computed	Actelion Property Explorer, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Schwitzerland, http//www.actelion.com/
AB/LogP	Computed	Pharma Algorithms, BCE Place-TD Canada Trust Tower, 161 Bay Street, 27th Floor Toronto, Ontario, M5J 2S1, Canada http//www.pharma-algorithms.com/
ALOGP	Computed	DRAGON, TALETE srl, Via V.Pisani, 13-20124 Milano-Italy, http//www.talete.mi.it/
MLOGP	Computed	DRAGON, TALETE srl, Via V.Pisani, 13-20124

		Milano-Italy, http//www.talete.mi.it/
XLOGP2	Computed	Cheminfo, Cheminfo.pku.edu.cn
XLOGP3	Computed	XLOGP3 Online, wangrx@mail.sioc.ac.cn. Cheng,T., Zhao,Y., Li,X., Lin,F., Xu,Y., Zhang,X., Li,Y, Wang, R,.*, Lai,L. "Computation of Octanol-Water Partition Coefficients by Guiding an Additive Model with Knowledge",J.Chem.Inf. Model. 2007, 47, 2140–2148.
COSMOFrag	Computed	COSMOfrag Version 3.1, COSMOlogic GmbH & Co. KG, Burscheider Str. 515, D-51381 Leverkusen, Germany www.cosmologic.de
KOWWIN	Computed	SYRRES engine, Syracusa Research Corporation http//www.syrres.com/
ALOGPs	Computed	VCCL engine, Virtual Computational Chemistry Laboratory http//www.vcclab.org
ALOGpS	Computed	VCCL engine, Virtual Computational Chemistry Laboratory http//www.vcclab.org
AC logS	Solubility is a characteristic physical property referring to the ability of a given substance, the solute, to dissolve in a solvent. It is measured in terms of the maximum amount of solute dissolved in a solvent at equilibrium. The resulting solutions is called a saturated solution	Actelion
Dipole	Dipole Moment	SERENA Software, Inc., 1900 Seaport Bouleward, 2nd Floor, Redwood City, California 94063-5587 USA, http// www.serena.com

Table 3. Parameters of the linear relationships between the R_M values of acylanilide derivatives and the concentration of methanol (C) in the mobile phase (n = 10)

	$R_{Mmethanol} = R_{M0Methanol} + b \cdot C_{Methanol}$			
No.	Compound	R_{M0}	b	r
1	Metalaxyl	1.2087	−0.0249	0.9826
2	CGA29212	2.0500	−0.0366	0.9816
3	RE26745	1.1548	−0.0260	0.9730
4	Ofurace	1.9869	−0.0357	0.9802
5	Oxadixyl	1.2606	−0.0291	0.9648
6	LAB19420	1.9595	−0.0384	0.9750
7	Furalaxyl	2.2359	−0.0430	0.9867
8	Benalaxyl	3.8093	−0.0676	0.9767
9	Cyprofuram	1.6086	−0.0286	0.9624

of the R_M values on the concentration of the organic modifiers (methanol and acetone) in the mobile phase are listed in Tables 3 and 4. The correlation between the R_M value and the concentration of the organic modifier in the mobile phase was, in each case highly significant, confirming the regular retention behavior of the analytes. In the majority of cases both chromatographic parameters show marked differences, suggesting that these RP-TLC systems can be employed for the separation of acylanilide derivatives.

PCA calculations indicated that the first principal component explained more than 94% of the total variance. This result suggests that

Table 4. Parameters of the linear relationships between the R_M values of acylanilide derivatives and the concentration of acetone (C) in the mobile phase (n = 10)

	$R_{MAcetone} = R_{M0Acetone} + b \cdot C_{Acetone}$			
No.	Compound	R_{M0}	b	r
1	Metalaxyl	0.8815	−0.0276	0.9821
2	CGA29212	1.5490	−0.0375	0.9979
3	RE26745	0.5153	−0.0251	0.9995
4	Ofurace	1.3280	−0.0361	0.9979
5	Oxadixyl	0.5326	−0.0246	0.9985
6	LAB19420	1.4507	−0.0362	0.9891
7	Furalaxyl	1.4044	−0.0353	0.9974
8	Benalaxyl	2.2286	−0.0493	0.9896
9	Cyprofuram	1.2424	−0.0313	0.9976

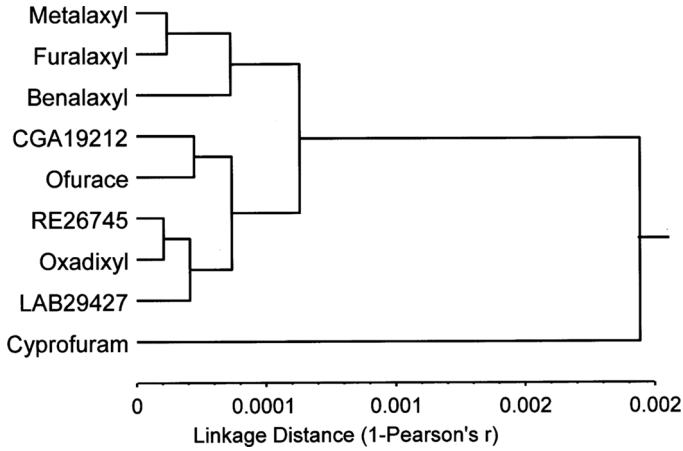

Figure 2. Similarities and dissimilarities between the acylanilide fungicides taking into consideration their measured and computed physicochemical parameters. Results of cluster analysis.

marked intercorrelations occur between the computed molecular parameters and between the measured lipophilicity characteristics of the acylanilide derivatives. The results of CA are depicted in Figure 2. The greatest similarities were observed between metalaxyl and furalxil, as well as between oxadixyl and the experimental preparation RE26745. Interestingly, cyprofuram differs considerably from the other acylanilide derivatives. This discrepancy can be tentatively explained by the unique presence of three ring structure.

The results of SRA are listed below:

$$R_{MOMethanol} = 0.108 + 0.914.XLOGP2 \quad r = 0.9312$$

$$b_{Methanol} = -3.6.10^{-3} - 1.53.10^{-2}.MLLOGP \quad r = 0.8156$$

$$R_{MOAcetone} = -0.426 - 0.593.ALOGPS \quad r = 0.9432$$

$$b_{Acetone} = -1.60.10^{-2} - 8.80.10^{-3}.XLOGP2 \quad r = 0.9332$$

$n = 9$ in each case.

Highly significant correlations were found between the measured and calcutated physicochemical parameters of acylanalide derivatives. As expected, only log P values calculated by various softwares were selected as an independent variable by SRA. This finding indicated that the retention of this class of analytes is governed by the molecular lipophilicity and by the dimensions of hydrophobic surface area. Moreover, the highly

significant correlations suggested that computed molecular characteristics can be successfully used for the prediction of the RP-TLC retention behavior of acylanilide fungicides.

CONCLUSIONS

In conclusion, from the measurements and calculations made herein, acylanilide derivatives can be easily separated by RP-TLC using both methanol and acetone as organic modifiers in the mobile phase. PCA and CA can be successfully used for the elucidation of the similarities and dissimilarities between acylanilide derivatives. It was further established that the computed physicochemical parameters included in the calculations can be used for the prediction of the retention behavior of this class of analytes by RP-TLC.

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