ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 95–103

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Use of ANN modelling in structure–retention relationships of diuretics in RP-HPLC

S. Agatonovic-Kustrin [a,*], M. Zecevic [b], Lj. Zivanovic [b]

[a] *School of Pharmacy, University of Otago, PO BOX 913 Dunedin, New Zealand*
[b] *Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia, Yugoslavia*

Received 17 October 1998; received in revised form 30 January 1999; accepted 15 February 1999

Abstract

Structure–retention relationship study, conducted by RP–HPLC, was used to investigate physical chemical parameters related to the RP retention times of amiloride, hydrochlorothiazide and methyldopa in order to predict the separation of amiloride and methylclothiazide from Lometazid® tablets. Retention data were obtained with an ODS column using a mobile phase methanol–water (pH adjusted with phosphoric acid). Physical chemical properties were calculated directly from the molecular structure. Artificial neural networks (ANNs) were used to correlate chromatograms retention times with mobile phase composition and pH, and with physical chemical properties of amiloride, hydrochlorothiazide and methyldopa and to predict separation of amiloride and methylclothiazide from Lometazid® tablets. Sensitivity analysis was performed to interpret the meaning of the descriptors included in the models. Results confirmed the dominant role of the polar modifier in such chromatographic systems. Within a series of solutes chromatographed under identical conditions, the retention parameters could be approximated by a non-linear combination of log P, log D, pK_a, surface tension, parachor, molar volume and to minor extend by polarisability, rexractivity index and density. This study has demonstrated that the use ANNs techniques can result in much more efficient use of experimental information. As HPLC is the most popular analytical technique, improvements in HPLC methods development can yield significant gains in the overall analytical effort. The ANNs extension presented could be the method of choice in some advanced research settings and serves as an indication of the broad potential of neural networks in chromatography analysis. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Amiloride; Hydrochlorothiazide; Methylclothiazide; Methyldopa; RP–HPLC; SRR; ANNs

1. Introduction

Predicting chromatographic behaviour from molecular structure of solutes is one of the main goals of the structure–retention relationships (SRR) methodology. The usefulness of Artificial neural Networks (ANNs) for modelling retention

* Corresponding author. Tel.: +64-3-479-5410; fax: +64-3-479-7034.
E-mail address: nena.kustrin@stonebow.otago.ac.nz (S. Agatonovic-Kustrin)

0731-7085/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(99)00133-8

times in HPLC optimisation to correlate the chromatographic behaviour of solutes (capacity factors) with mobile phase composition and pH has been previously investigated [1,2]. Over the last few years HPLC retention data have been used as a pseudo-molecular descriptor to estimate the aqueous solubility of aromatic hydrocarbons [3] and organic non-electrolytes [4], to estimate octanol/water partition coefficient [5,6], for determinations of log P_{oct} values of chlorosubstituted aromatic compounds [7] and for accurate estimations of pK_a [8,9]. Computer simulation methods has been used to predict separation as a function of simultaneous change in pH and solvent strength for reversed-phase high-performance liquid chromatography [10,11] and hydrophobicity coefficients for the prediction of peptide elution profiles [12].

The aim of this work was to find molecular parameters related to the RP retention times and to predict the retention as a function of changes in mobile phase pH and composition, in addition to molecular structure descriptors of separated solutes. ANN model was used to correlate the liquid chromatographic behaviour of a group of structurally diverse diuretics with their physical chemical and molecular descriptors and to create a model for the prediction of retention values of unanalysed molecules.

1.1. Artificial neural networks

An artificial neural network is an information-processing model inspired by the way biological nervous systems, process information. It tries to simulate its learning process. It learns by example from experience during training phase. ANN is composed of a large number of highly interconnected processing units, the artificial neurons organised in layers. Connecting lines have associated connection 'weights', which can be modified during the training or 'learning' process. Under suitable conditions, a neural network can be trained to learn the relationship between a set of input/output pairs, called the training set.

The behaviour of neural network architecture is generally determined by the transfer functions of its neurons, by the learning rule, and by the architecture, itself.

We have used a supervised network with back-propagation learning rule. In this type of model, informations from various sets of inputs are fed forward through the ANN to optimise the weights between neurones, or to train it. The output of the neurone is related to the summed input by a sigmoid shaped transfer function. The optimisation is therefore non-linear. The network produces a response that is compared with the desired response. The difference is called the error. The error in the prediction is propagated back through the system to adjust the weights in the network so that the next time the same example is presented, the network will come closer to producing the desired response. This type of learning or training is called supervised.

If a network has a larger capacity for learning with respect to the training data set, over-training can result when the network continues to learn very specific features of the training data and looses the ability to generalise. Over-training can be measured by checking the results of test data set. If at some point during learning test data begins to produce worse results, even though the training data continues to produce improved results, over-training is occurring.

Thus, three types of data sets are used:

1. Training data: used to train network,
2. Test data: used to monitor the neural network performance during training,
3. Validation data: used to measure the performance of a trained application,

each with corresponding error.

1.2. Physical chemical properties

Since the work of Louis Hammett [13] (1894–1987) who correlated electronic properties of organic acids and bases with their equilibrium constants and reactivity, the development of mathematical models that correlate structure with reactivity is in increasing rate. The basic philosophy is that the structural changes that affect the biological activities of a set of compounds are of three major types: electronic, steric, and hydrophobic. Electronic variations have been largely treated by using Hammet and Taft [14] equations for pK_a values. Hydrophobic changes are mod-

elled with partition coefficient or distribution coefficient from the octanol–water system. In addition, molecular-orbital parameters are gaining in importance.

A number of commercial software products for physical property prediction exist. Prediction algorithms are developed on correlation between molecular structure and physical chemical properties. Such programs can demonstrate graphically and intuitively the effect of structural changes on these individual properties. Experimental determination of such properties can be time consuming and tedious as well as, in some cases, being subject to large experimental variation and errors.

The major differences between behaviour profiles of organic chemical are attributable to physical chemical properties. A large number of descriptors have been proposed and tested [15]. Among the most commonly used molecular descriptors are molecular weight and volume, the number of specific atoms, surface areas, refractivity, parachor, steric parameters and various topological parameters. It is likely that existing and new descriptors will be tested and eventually a generally preferred set of parameters will be adopted of routine use for correlating purpose.

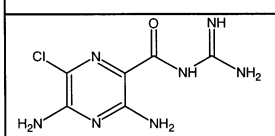
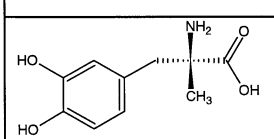
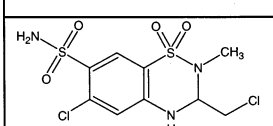
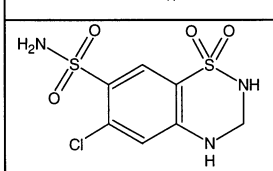
1.2.1. Data determined

t_R, retention time measured by reversed phase liquid chromatography (RPLC); time elapsed between sample introduction and maximum of response.

1.2.2. Chemical descriptors (Table 1)

M, the concentration of the organic modifier (methanol) (%) in the mobile phase; pH, pH of the mobile phase.

Table 1
Physical chemical descriptors calculated from the molecular structure

Molecular structure	logP	pKa	pKa^0	logD	V	nd_{20}	γ	d_{20}	polarizability	P
	-1.03	5.26	10	-3.57	108.5	1.884	112.5	2.11	19.78	353.6
	0.11	2.28	2.57	-2.67	150.4	1.635	72.6	1.403	21.36	439.3
	1.3	9.42	10.02	0.79	223.2	1.606	55.1	1.613	30.53	608.4
	-0.36	8.7	10.02	-0.84	175.8	1.632	62	1.693	24.86	493.3

1.2.3. Data calculated from molecular structure

log *P*, logarithm of the partition coefficient in octanol/water; measure of the hydrophobicity or lipophilicity properties [16]. *V*, (cm^3/mol) the solute molar volume. log *D*, distribution coefficient at pH 7.0 in octanol/water, (depends on partition coefficient (logP) and the dissociation constant(s) (pK_a) at different pH); measure of compound hydrophobicity that correlates well with chemical and biological properties such as solubility, the bioconcentration factor, and the adsorption coefficient. pK_a, acid–base ionisation constants under 25°C and zero ionic strength in aqueous solutions; related to the ionisation capabilities of chemical species; at a constant temperature the pK_a of a molecule is linearly related to the free energy change for the reaction of ionisation of a proton. pK_a^0, is the ionisation constant of the unsubstituted parent compound; pK_a value of the unsubstituted parent compound, and expresses the sensitivity of the parent compound to the effects of substituents. *P* (cm^3), parachor [17] is an additive physical property of a substance related to its molar volume; determined by the kind and the number of atoms in a molecule as well as their manner of arrangement and binding. $nd_{20,}$ index of refraction [18] models the dispersion forces of molecular substituents; additive constitutive property of molecules, fragment values have been calculated for groups of atoms. γ (dyne/cm), surface tension is correlated with the binding forces of polar, charged molecules within hydrogen bonding and Van der Waals forces are involved; solution and dispersion properties increases rapidly with the reduction of the surface tension. d_{20} (g/cm^3), density is one of physical characteristics of a substance that help identify the substance. Mass per unit volume; refers to the compactness of the matter (g/cm^3). Polarisability [19] (cm^3), is numerical derivative of the dipole moment and geometry charge of molecules; related to dielectric constants.

2. Experimental

2.1. Equipment

Separations were made on a Waters 5-μm μBondapak C-18 column (300 × 3.9 mm i.d. Waters Milford, MA, USA). The injection volume was 10 μl, elution was performed at a flow rate of 1.5 ml/min and the column was maintained at ambient temperature. The absorbance was monitored at 286 nm. The mobile phase was methanol–water (pH adjusted with phosphoric acid).

2.2. Solvents and chemicals

Standards of methyldopa (MD), hydrochlorothiazide (H) and amiloride (A) and Alatan® tablets (250 mg MD, 25 mg A, 2.5 mg H) were supplied by Lek (Ljubljana, Slovenia). Moduretic® tbl. (5 mg A, 50 mg M) were obtained from Merck Sharp (Dokme International Div. of Merck and Co., Inc, USA). Standard of methylclothiazide (M) and Lometazid® tablets (10 mg A, 5 mg M) were supplied by ICN Galenika (Belgrade, Serbia). The chromatographic internal standard was coffein. All solvents used for the preparations of the mobile phase were HPLC grade and the mixtures were filtered and degassed before use.

2.3. Solutions

2.3.1. Internal standard solution

An 8 μg/ml solution of coffein in methanol was prepared.

2.3.2. Solutions for Alatan® tablets

2.3.2.1. Stock solution. About 1000 mg of methyldopa reference material, 500 mg of hydrochlorothiazide reference material and 10 mg of amiloride reference material was precisely weighed, dissolved in internal standard solution and diluted to 100.0 ml with the same solvent. A 2.5-ml volume of this solution was diluted with internal standard solution to 10 ml to form a stock solution.

2.3.2.2. Sample preparation. A finely powdered Alatan® tablet was accurately transferred to a 100.0 ml calibrated flask and diluted to volume with internal standard solution. The mixture was sonicated for 5 min at room temperature and than centrifuged at 2500 × *g* for 5 min. The supernatant liquid was filtered through a 1.5-μm membrane filter.

2.3.2.3. Standard solutions. Working standard solutions were prepared by dilution of a 2.0 ml volume of these solutions to 100.0 ml with the internal standard solution.

2.3.3. Solution for Lometazid® tablets

2.3.3.1. Stock solution. About 10 mg of amiloride reference material and 5 mg of methyclothiazide reference material was precisely weighed, dissolved in internal standard solution and diluted to 100 ml with the same solvent to form a stock solution.

2.3.3.2. Sample preparation. A finely powdered Lometazid® tablet was accurately transferred to a 100 ml calibrated flask and diluted to volume with internal standard solution. The mixture was sonicated for 5 min at room temperature and than centrifuged at $2500 \times g$ for 5 min. The supernatant liquid was filtered through a 1.5-μm membrane filter.

2.3.3.3. Standard solutions. Working standard solutions were prepared by dilution of a 4-ml volume of these solution to 10 ml with the internal standard solution.

2.3.4. Solutions for Moduretic® tablet

2.3.4.1. Stock solution. About 5 mg of amiloride reference material and 50 mg of hydrochlorothiazide reference material was precisely weighted, dissolved in internal standard solution and diluted to 50 ml with the same solvent to form a stock solution.

2.3.4.2. Sample preparation. A finely powdered Moduretic® tablet was accurately transferred to a 50.0-ml calibrated flask and diluted to volume with internal standard solution. The mixture was sonicated for 5 min at room temperature and than centrifuged at $2500 \times g$ for 5 min. The supernatant liquid was filtered through a 1.5-μm membrane filter.

2.3.4.3. Standard solutions. Working standard solutions were prepared by dilution of a 0.7-ml volume of these solution to 100 ml with the internal standard solution.

3. Data analysis

3.1. ANN simulator software

MS-WINDOWS based artificial neural network simulator software, NNMODEL Version 1.404 (Neural Fusion) was used. For calculating drug properties from molecular structure PALLAS 2.1 (Compu Drug Int.) and CHEMSKETCH 3.5 freeware (ACD Inc.) were used. Calculations were performed on a Pentium personal computer.

The starting point in this study is a set of retention times for the active ingredients of Alatan®, Lometazid® and Moduretic® tablets obtained under different chromatographic conditions. Retention values for amiloride, methyl dopa and hydrochlorthiazide (Alatan® tbl.) and amiloride and hydrochlorothiazide (Moduretic® tbl.), together with their physical chemical properties were used to train the network and to establish the relationship.

3.2. Optimal network architecture

A standard feed-forward network, with back-propagation rule and with single hidden layer architecture was chosen. A single hidden layer was used for simplicity, and because there is little evidence to suggest that a larger number of hidden layers improves performance [20]. Problem was to determine the appropriate complexity of the network. This problem is similar to the problem of choosing the degree of smoothing in non-parametric estimation. Model selection for an ANN requires choosing the number of hidden units and connections thereof.

The multilayer perceptron [21] (MLP) model architecture was chosen. MLPs are general-purpose, flexible, non-linear models that, given enough hidden neurons and enough data can approximate virtually any function to any desired degree of accuracy when little knowledge about the form of investigated relationship is known. In this model, the inputs are fully connected to the

hidden layer and hidden layer neurons are fully connected to the outputs. Direct connections from the input layer to the output layer did not improve the network performance. Direct connection, which could be called main effect in statistical terminology, would speed the convergence if the relationship were simple.

In order to select the best ANN architecture pruning method [22] was applied, similarly to backward elimination in stepwise regression. Connections or units were eliminated during training based on sensitivity report, highest coefficient of multiple determination and minimal generalisation error.

The ANN used in this investigation consisted of 12 inputs (methanol percentage and pH in the mobile phase, pK_a, pK_a^0 $\log P$, $\log D$, molar volume, refractivity index, surface tension, density, polarisability and parachor of separated drugs), one hidden layer, and one output neuron for the corresponding retention time. The number of hidden neurons and number of training cycles as adjustable parameters were optimised.

During training and testing the number of hidden neurons has been varied from two to 12 and training cycles from 0 to 3000 and ANN performance was tested after each addition.

3.3. Training

At the start of the training run, both weights and biases were initialised with random values. During training, 25% of the data was used as the test set and was back propagated through the network to evaluate the trained network. The training set is used to configure the ANN and testing set to monitor network performance during training.

The error in mapping the training values decreased as the number of hidden neurons increased. By increasing the number of hidden neurons, the ANN more closely followed the topology of the training set that resulted in tracing the training pattern too closely above an optimum level. The system was over-trained and exhibited poor generalisation to unseen data.

3.4. Importance of the inputs

The system was trained with retention times obtained with Alatan® (containing amiloride, hydrochlorthiazide and methyl dopa) and Moduretic® tablets (amiloride and hydrochlorthiazide). For a sample composed of a mixture of two compounds, the chromatogram is a non-linear superposition of the retention times of each individual compound. Each drug presented to the chromatograph produces a characteristic chromatogram under specific separation conditions. By changing experimental conditions of separations, a database of chromatograms was constructed. From this database, training and testing sets were generated.

MLP model computes the output as a sum of non-linear transformations of linear combinations of the inputs. The number of weights and hidden units increases linearly with the number of inputs. The higher the dimensionality of the input space, the more training data sets is required. If the dimension of the input space is high the network uses almost all its resources to represent irrelevant portions of the input space. Careful feature selection and scaling of the input affects the complexity of the problem, as well as the selection of the best neural network model. Scaling the components according to their importance and selecting the optimal input subset with the best predictive ability is necessary. In order to choose the most significant model variables and to avoid a large number of inputs network was pruned until good generalisation was reached based on the sensitivity report (Table 2). Sensitivity reports show the sensitivity of the output variables, as a percentage, to the changes in the input variables. The sensitivity is calculated by summing the changes in the output variables caused by moving the input variables by a small amount over the entire training set.

The network was trained using 12, 11, ten, nine and eight input data points. The performance of the ANN was evaluated with testing data. Using only 75% of the sample data for training and performing the cross validation with the other 25% of the data carried out the training. The set used for testing was rotated and the results of the four runs were averaged.

Table 2
Sensitivity of the output variables to the changes in the input variables[a]

Inputs	Sensitivity				
M	0.32	0.33	0.37	0.32	0.34
pH	0.25	0.24	0.20	0.23	0.24
log P	0.07	0.08	0.09	0.09	0.08
V	0.06	0.06	0.08	0.08	0.09
pKA^{o}	0.05	0.07	0.05	0.08	0.09
γ	0.05	0.04	0.05	0.05	0.06
log D	0.04	0.04	0.04	0.06	0.05
P	0.04	0.05	0.04	0.06	0.04
pK_a	0.03	0.04	0.05	0.04	
d_{20}	0.04	0.03	0.02		
Polarisability	0.03	0.03			
nd_{20}	0.02				
NU	12	11	10	9	8
HU	9	8	7	6	5
Test SSE	0.0003	0.0002	0.0001	0.0002	0.0002
R^2	0.958	0.967	0.981	0.974	0.973

[a] NU, number of inputs; HU, number of hidden units; SSE, sum of squared error; R^2, coefficient of multiple determination.

3.5. Method validation

The testing error is not a good estimate of the generalisation error. One method for getting an unbiased estimate of the generalisation error is to present the ANN with a new, third set of data, that were not used at all during the training process. Separation of amiloride and methylclothiazide (Lometazid® tablets) was performed under different experimental conditions and experimental data were compared with predicted data. The relative error (ERR) [2] was used to compare generalisation ability of the models (Table 3).

4. Results and discussion

The general assumption in SRR modelling is that molecular structure causes the chromatographic behaviour. After the chromatographic behaviour of amiloride, methylchlorthiazide and methyl dopa was determined SRR model was constructed in a several steps. The first step was to calculate physical chemical parameters as mathematical representations of investigated diuretics. These parameters provided a description of the similarities and differences of investigated diuretics. Next step was to correlate molecular descriptors with the observed chromatographic behaviour using non-linear neural networks model. Lack of correlation between input variables and retention times made the selection of the important inputs. Sensitivity report was used as a statistical tool to reduce the number of inputs and to select the optimal combination of input variables (Table 2). Retention properties of the solutes were highly related to the concentration of the organic modifier (methanol) (%) and pH in the mobile phase as expected. These results proved the dominant role of the polar modifier in such chromatographic systems. Furthermore, within investigated solutes chromatographed under identical conditions the retention parameters could be approximated by a non-linear combination of the molar volume, of log P, log D, pK_a, surface tension, density, parachor and molar volume. The sensitivity report showed that descriptor contributions to a model varied from 2 to 32%.

The criteria for judging the best model were multiple correlation coefficient and sum of squared error. The resulting models gave an excellent correlation to HPLC retention data yielding

r^2 values from 0.958 to 0.981. The mean sum of squared error was less than 0.03%. Network structure was optimised by heuristic search. Seven to nine hidden neurons respectively were enough to achieve good convergence on the training data.

The best models were selected and used to predict separation of amiloride and methylclothiazide from Lometazid® tablets (Table 3). Estimated models showed a high degree of correlation between observed and calculated values of retention times. This study showed that the models have a good predictive ability for application.

5. Conclusion

ANN regression analysis to find the best relationship between the retention data and the structural descriptors gave highly significant correlation with reasonable good prediction power.

The results of this research have shown the benefits of the neural network application in predicting chromatographic behaviour from mobile phase composition and physical chemical properties and molecular descriptors of solutes.

Table 3
Experimentally measured and predicted by ANNs values of retention time

Measured t_R	[5]Predicted t_R with different number of inputs				
	12	11	10	9	8
10.97[a]	10.51	10.59	11.31	6.24	6.15
9.48[a]	8.81	9.30	8.48	5.47	6.12
8.42[a]	7.50	8.44	6.41	5.20	6.15
7.78[a]	8.78	7.79	8.25	5.81	3.52
6.94[a]	7.48	7.07	6.06	4.98	3.49
7.50[a]	6.47	6.58	4.98	4.46	3.50
8.05[a]	7.69	7.41	5.97	6.13	2.35
8.57[a]	6.60	6.77	4.86	5.10	2.23
8.90[a]	5.83	6.34	4.43	4.31	2.16
7.17[a]	7.48	7.07	6.06	4.98	3.49
10.81[a]	9.49	9.79	9.68	5.73	6.13
8.58[a]	9.37	9.09	9.67	5.67	5.27
7.18[a]	7.00	6.83	5.48	4.72	3.49
8.37[a]	7.35	7.21	5.56	5.82	2.31
21.92	21.20	22.86	19.74	23.20	20.93
22.17	23.28	23.58	23.42	23.20	21.77
31.18	33.54	34.78	35.23	35.49	35.38
8.52	8.25	8.09	9.33	9.45	9.81
10.45	12.01	11.55	11.90	11.75	11.20
12.21	17.32	16.43	15.80	15.89	17.43
7.22	5.61	5.92	6.21	6.87	6.81
8.42	7.54	8.01	7.82	8.18	7.48
8.61	11.12	11.64	10.66	10.81	10.90
10.32	12.01	11.55	11.90	11.75	11.20
20.43	21.96	22.55	21.41	22.33	20.64
13.72	14.72	15.33	14.99	15.59	14.12
11.64	14.17	13.52	13.42	13.27	12.90
6.78	6.03	6.38	6.56	7.14	6.90
Relative error	0.13	0.12	0.17	0.24	0.32

[a] Methylclothiazide retention time.

The retention mechanism of chromatographic systems depends mainly on interactions between the solutes, stationary phase and mobile phase. These interactions are explained by molecular descriptors using SRRs and by correlating molecular structure to solute retention. SRR studies are proven to be useful in retention prediction, finding the relevant structural descriptors for analytes and estimating the relative biological activities of a series of analytes. This study has demonstrated that the use of ANNs techniques can result in much more efficient use of experimental information. As HPLC is the most popular analytical technique, improvements in HPLC methods development can yield significant gains in the overall analytical effort.

References

[1] S. Agatonovic-Kustrin, M. Zecevic, Lj. Zivanovic, I.G. Tucker, Anal. Chim. Acta 364 (1998) 265–273.
[2] S. Agatonovic-Kustrin, M. Zecevic, Lj. Zivanovic, I.G. Tucker, J. Pharm. Biomed. Anal. 17 (1998) 69–76.
[3] B.G. Whitehouse, R.C. Cooke, Chemosphere 11 (1982) 689–699.
[4] T.L. Hafkenscheid, E. Tomlinson, J. Chromatogr. 218 (1981) 409–425.
[5] R.L. Swann, D.A. Laskowski, P.J. McCall, K. Vander Kuy, H.J. Dishburger, Residue Rev. 85 (1983) 17–28.
[6] W.A. Bruggeman, J. van der Steen, O. Hutzinger, J. Chromatogr. 238 (1982) 335–346.
[7] H. Konemann, R. Zelle, F. Busser, J. Chromatogr. 178 (1979) 559–565.
[8] H.Y. Ando, T. Heimbach, J. Pharm. Biomed. Anal. 16 (1997) 31–37.
[9] K. Valko, J. Liquid Chromatogr. 10 (1987) 1663–1686.
[10] J.A. Lewis, J.W. Dolan, L.R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 197–208.
[11] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, L.R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 183–195.
[12] M.T. Hearn, M.I. Aguilar, C.T. Mant, R.S. Hodges, J Chromatogr. 438 (1988) 197–210.
[13] L.P. Hammett, Physical Organic Chemistry; Reaction Rates, Equilibria, and Mechanisms, McGraw-Hill, New York, 1940.
[14] R.W. Taft, Steric Effects in Organic Chemistry, John Wiley, New York, 1956, p. 556.
[15] W. Lyman, W. Reehl, D. Rosenblatt, Handbook of Chemical Property Estimation, American Chemical Society, Washington, DC, 1990.
[16] C. Hansch, J.E. Quinlan, G.L. Lawrence, J. Org. Chem. 33 (1968) 347–350.
[17] A. Leo, C. Hansch, C. Church, J. Med. Chem. 12 (1969) 766.
[18] K. Yoshida, T. Shigeoka, F. Yamauchi, Ecotox. Environ. Saf. 7 (1983) 558–565.
[19] D. Ayma1, J.P. Campillo, M. Rérat, M. Causà, J. Comput. Chem. 18 (1997) 1253–1263.
[20] B.D. Ripley, Pattern Recognition and Neural Networks, Cambridge University Press, Cambridge, 1996.
[21] H. White, Artificial Neural Networks: Approximation and Learning Theory, Blackwell, Oxford, UK, 1992.
[22] R. Reed, IEEE Trans. Neural Networks 4 (1993) 740–747.