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Application of artificial neural networks for prediction of retention factors of triazine herbicides in reversed-phase liquid chromatography

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Abstract

In this paper a quantitative structure-retention relationship (QSRR) method is used to model reversed-phase high-performance liquid chromatography (HPLC) behaviour of a series of triazine herbicides and their metabolites. Accurate description of the retention factors in terms of four descriptors related to the analytes and to the mobile phase is achieved by means of an artificial neural network (ANN). For comparison, a QSRR model is derived by multilinear regression (MLR). Validation of the two models shows a better ability in prediction of the ANN as compared with the MLR method. A solid-phase extraction (SPE) procedure allowing the simultaneous determination of the five triazinic compounds in groundwater analysis is also presented. The observed recoveries from water samples range between 85 and 100% for ng/ml concentration levels of all analytes.

Keywords: Quantitative structure-retention relationships; Artificial neural networks; HPLC optimisation; Triazine herbicides

1. Introduction

Triazines, owing to their extensive use as herbicides in modern agriculture, can be dispersed in surface and spring water at trace levels [1–3]. As a consequence of proven carcinogenic and endocrine disrupting action of these and other potentially hazardous compounds resulting from human activity, monitoring of groundwater has become an important aspect of environmental and health safeguard. Triazines are subjected to various abiotic and biotic degradation processes [4], and consequently, quantification of the metabolic products provides an additional analytical index to check water contamination.

High-performance liquid chromatography (HPLC) based on reversed stationary phase, coupled with a suitable preliminary sample preparation step able to concentrate the analytes and remove possible interferences, is one of the most powerful techniques for detection and quantification of triazine herbicides and their metabolites in water environment [2,5,6].

In the framework of the progress of chromatography, much effort has been concentrated in the last years to develop expert systems able to predict with good accuracy the retention behaviour of the analytes, providing an automatic means for the optimisation of chromatographic performance. In this perspective, quantitative structure-retention relationships (QSRR) methods [7,8] have been proposed, with the major aim of finding a mathematical model relating to the retention of a given analyte to physicochemical and structural parameters (descriptors). Besides practical application in optimisation strategies, QSRR studies can significantly contribute to get some insight into the molecular mechanism of separation [9–11].

Statistical treatment of QSRR multivariate data, consisting of a set of observed retention values and descriptors for a number of test molecules, is generally based on multilinear regression (MLR) [7,10–14]. In recent years, artificial neural networks (ANN) [15,16] have become a very popular and powerful chemometric tool to solve chemical problems,

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including optimisation of chromatographic analysis [17–23]. As compared with multivariate regression, ANN does not require knowledge of a mathematical model before fitting of the data. Thus, it is particularly useful in the case of hidden nonlinearity inside the data variables.

In the present paper, ANN was used to develop a QSRR model for the prediction of the retention factor *k* of triazinic herbicides. In addition to the effect of the molecular structure of the analytes on the retention behaviour, as expressed by suitable descriptors, our attention was focused on the influence of pH and composition of the mobile phase, that are some of the operative parameters optimised in HPLC in order to achieve adequate separation and analysis time. The ability in prediction of the best ANN model was compared with that given by MLR.

A solid-phase extraction (SPE) procedure allowing simultaneous preconcentration of the five analytes in groundwater samples was also proposed. As alternative to common sorbents, i.e. porous silica particles surface-bonded with C_{18} or other hydrophobic groups, we used a macroporous copolymer formed by [poly(divinylbenzene-co-*N*-vinylpyrrolidone)], exhibiting both hydrophilic and lipophilic retention characteristics.

2. Method

2.1. Involved parameters

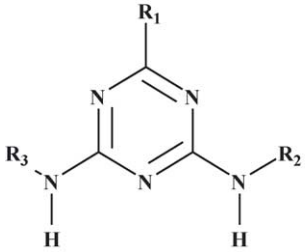
The triazinic herbicides used as test analytes in the present study are summarised in Fig. 1. The QSRR model was built by using descriptors related to the analyte and descriptors related to the eluent as inputs. The analyte descriptors were: the logarithm of the *n*-octanol–water partition coefficient ($\log K_{ow}$, taken from literature [2,24]), which is the standard hydrophobicity index widely used in QSRR research, and the total dipole moment (μ), related to the charge distribution within the molecule, obtained by ab-initio calculations. The descriptors related to the eluent were the eluent composition expressed by the percentage of methanol (%MeOH) and pH. In addition to $\log K_{ow}$ and μ, some other physico-chemical properties calculated from the molecular structure (molecular weight, refractive index, molar volume and polarisability), were considered in a preliminary step. However, information carried by these descriptors appeared to largely overlap with that provided by $\log K_{ow}$ and μ, as indicated by absolute values of coefficients of mutual correlation of these parameters with $\log K_{ow}$ (between 0.86 and 0.97) and μ (between 0.75 and 0.85). The influence of the above molecular properties on the retention behaviour was also evaluated by applying MLR based on a stepwise procedure, in which the number of descriptors to be selected and the order of entry are based on statistical criteria (see below). The regression model with the best statistics was that including only $\log K_{ow}$ and μ as analyte descriptors. These were finally chosen as the optimal parameters to describe the molecular properties.

2.2. Artificial neural networks analysis

Details on principles, functioning and applications of artificial neural networks can be found in references [15] and [16].

ANNs are computational models designed to simulate the way in which the human brain processes information. They consist of simple processing units (or neurons) linked with weighted modifiable interconnections. The neurons are generally organised into a layered structure, formed by one input layer, one output layer and at least one hidden layer. In a feed-forward network the signals are propagated from the input layer through the hidden layer(s) to the output layer. The feed-forward ANN architecture adopted in the present study consists of four inputs (the descriptors defined above) and one output (*k* values) connected to each other by one hidden layer with six neurons.

In addition to the network topology, an important component of most neural networks is a learning rule. A learning rule allows the network to adjust its connection weights in order to associate given inputs with corresponding outputs. The training of the network has been carried out by using a back-propagation algorithm, in which the network reads inputs and outputs from a proper data set (training set) and iteratively computes weights and biases in order to minimise the sum of squared differences between predicted and target values. The training is stopped when the error in prediction reaches a desired level of accuracy. However, if the network is left to train too long, it will overtrain and lose the ability



analyte	R_1	R_2	R_3
desisopropylatrazine	Cl	H	CH_2CH_3
desethylatrazine	Cl	H	$CH(CH_3)_2$
simazine	Cl	CH_2CH_3	CH_2CH_3
atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$
prometon	OCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$

Fig. 1. Structure of the triazine herbicides used in this work.

to generalise. In order to avoid over-training, the predictive performance of the trained ANN is checked by running the back-propagation algorithm on a data set not used in training (validation set).

The training set and validation set used in the present work are reported in Tables 1 and 2, respectively. The whole data set consists of $\log K_{ow}$ and μ values of four triazine herbicides (atrazine, desisopropylatrazine, desethylatrazine and prometon), pH and mobile phase composition and observed k values (the target of the model). The total number of data points is 58, 14 of them (about 24%) being used for validation. The data were distributed over training and validation sets in order to have for each set a quite uniform distribution of variables over the related range of variability. Before fitting the data, input and output variables were normalised to have 0 mean and unity standard deviation (SD). At the start of a

Table 1
Data set used in training of ANN and derivation of MLR model

Analyte	%MeOH	pH	$\log K_{ow}$	μ (D)	Observed k	Predicted k	
						ANN	MLR
Desisopropylatrazine	60	3.0			0.590	0.573	0.584
	50	3.0			0.983	0.963	1.128
	40	3.0			1.766	1.767	2.180
	70	3.4			0.365	0.364	0.302
	50	3.4			1.014	0.952	1.128
	40	3.4	1.2	3.70	1.778	1.790	2.180
	70	3.8			0.374	0.379	0.302
	60	3.8			0.641	0.562	0.584
	50	3.8			0.840	0.916	1.128
	70	4.2			0.439	0.403	0.302
	60	4.2			0.525	0.563	0.584
	40	4.2			1.641	1.562	2.180
Atrazine	70	3.0			1.716	1.587	2.031
	60	3.0			3.268	3.218	3.924
	50	3.0			7.473	7.427	7.580
	70	3.4			1.498	1.572	2.031
	60	3.4			3.041	3.163	3.924
	40	3.4	2.7	3.43	20.924	20.109	14.645
	60	3.8			3.637	3.225	3.924
	50	3.8			6.855	7.302	7.580
	40	3.8			19.850	19.663	14.645
	70	4.2			1.863	1.801	2.031
	50	4.2			7.442	7.265	7.580
	40	4.2			18.902	18.867	14.645
Prometon	70	3.2			1.650	1.672	2.158
	65	3.4			2.643	2.535	3.000
	65	3.8			2.977	2.719	3.000
	70	4.0	2.55	2.50	1.743	1.970	2.158
	65	4.0			3.232	2.838	3.000
	55	4.0			6.492	6.736	5.795
	45	4.2			19.382	19.083	11.196
Desethylatrazine	70	3.0			0.596	0.570	0.478
	60	3.0			0.946	0.966	0.923
	40	3.0			3.472	3.507	3.444
	70	3.4			0.530	0.560	0.478
	50	3.4			1.789	1.723	1.783
	40	3.4			3.543	3.526	3.444
	70	3.8	1.6	3.82	0.558	0.579	0.478
	60	3.8			1.041	0.928	0.923
	50	3.8			1.498	1.650	1.783
	40	3.8			3.471	3.340	3.444
	70	4.2			0.651	0.612	0.478
	60	4.2			0.833	0.928	0.923
	50	4.2			1.670	1.567	1.783
					ERR%	4.9	15.8
					R	0.999	0.977

Predicted k values from ANN and MLR models. Related correlation coefficients (R) and average percent error (ERR%) are given at the bottom of the table (see text for details).

Table 2
Data set used in validation of ANN and MLR models

Analyte	%MeOH	pH	log K_{ow}	μ (D)	Observed k	Predicted k	
						ANN	MLR
Desisopropylatrazine	70	3.0	1.2	3.70	0.404	0.365	0.302
	60	3.4			0.556	0.564	0.584
	50	4.2			0.948	0.872	1.128
	40	3.8			1.762	1.701	2.180
Atrazine	40	3.0	2.7	3.43	20.667	19.767	14.645
	50	3.4			7.772	7.363	7.580
	70	3.8			1.568	1.658	2.031
	60	4.2			2.904	3.349	3.924
Prometon	60	3.2	2.55	2.50	3.495	3.856	4.169
	55	3.8			6.147	6.584	5.795
	70	4.2			1.953	2.078	2.158
Desethylatrazine	50	3.0	1.6	3.82	1.744	1.761	1.783
	60	3.4			0.891	0.939	0.923
	40	4.2			3.293	3.064	3.444
					ERR%	6.5	15.7
					R	0.999	0.984

Predicted k values from ANN and MLR models. Related correlation coefficients (R) and average percent error (ERR%) are given at the bottom of the table (see text for details).

training run, the biases and weights were initialised at random values in the range between +1 and −1. At the end of each training cycle, the learned network was tested on the validation set. Typically, the training error decreases, whereas the validation error first decreases and subsequently begins to rise again, revealing that overtraining of the network is occurring. In addition to the network architecture, the performance of ANN can also depend on two important parameters, the learning rate and the momentum, that control the size and the speed of weight changes made by the back-propagation algorithm, respectively. The values of these parameters and the number of neurons in the hidden layer were tested to find the best performance of the network. The optimal architecture (six hidden neurons), training cycle number (3000), learning rate (0.15) and momentum (0.30) were defined as those providing the lowest validation error. The transfer function $\xi(\mathrm{Net}_k)$ used in all layers is the hyperbolic tangent function defined as:

$$\xi(\mathrm{Net}_k) = \frac{1 - \mathrm{e}^{-\alpha \mathrm{Net}_k}}{1 + \mathrm{e}^{-\alpha \mathrm{Net}_k}}$$

where the parameter α (the slope of the transfer function) is fixed to 1, that has provided a lower validation error than the more common sigmoid transform function.

2.3. Multilinear regression

MLR is a common method used in QSRR study. Equations relating the retention behaviour to the descriptors are developed with the following form:

$$\log k = a_0 + \sum a_i X_i$$

where a_0 is the intercept and a_i are the regression coefficients of the descriptors X_i. In the present work a forward stepwise MLR procedure [25] is applied. The optimal number of descriptors and the best regression equation are defined on the basis of the following statistical parameters: multiple correlation coefficient, F ratio, standard error (SE) of the estimate and statistical significance of individual descriptors.

3. Experimental

3.1. Solvents and chemicals

All used pesticides are certified materials and were provided by Labor Dr. Ehrenstorfer-Schäfers (Augsburg, Germany). Acetonitrile, dichloromethane and methanol were HPLC grade and provided by Carlo Erba Reagenti (Milan, Italy). The mobile phase was prepared with distilled water obtained from a milli-Q water filtration/purification system (Millipore, Bedford, MA, USA). Stock solutions were prepared by dissolving 10 mg of respective triazines in 10 ml of methanol. The stock solutions (1000 mg/l) were used to prepare the standard methanol solutions with concentration 0.2, 0.4, 0.6, 0.8, 1.0, mg/l, respectively. All solutions were stored at 4 °C.

3.2. Sampling and sample preparation

Spring water samples were collected in the agricultural–industrial settlement of Fucino plain, (L'Aquila, Italy) by a 1 l glass dark bottle and stored at 4 °C. Prior to analysis the water samples were filtered by filter paper Whatman 5, diameter 5 cm, pore size 0.45 μm (Whatman International Ltd. Maidstone, England). In order to evaluate the recovery of the extraction procedure, 1 l of sample was spiked with an equimolar mixture of triazine compounds in the concentration range 0.2–1.0 μg/l.

3.3. Solid phase extraction

Sample preconcentration was carried out by using OASIS HLB 6cc 200 mg cartridges, constituted by a copolymer of *N*-vinylpyrrolidone and divinylbenzene (Waters, Milford, MA, USA). The cartridges were cleaned with 10 ml of dichloromethane, and conditioned with 10 ml of methanol and successively with 10 ml of milli-Q water, forced through the cartridge by means of a positive pressure. The sample (1 l) was drawn through the cartridge at a flow of 10 ml/min by applying a moderate vacuum, after connecting the sample flask directly to the cartridge. The cartridge was then washed with a mixture water/methanol (95:5, v:v) and successively dried for 5 min by fluxing air. The adsorbed compounds were eluted by 5 ml of acetonitrile and successively by 5 ml of methanol. The collected eluate was evaporated to dryness in a Supelco drying attachment working under vacuum in nitrogen atmosphere. The sample was successively reconstituted by 500 μl of the same mobile phase used in HPLC analysis. Aliquots (20 μl) were used in chromatographic analysis.

3.4. Equipment

Separation was performed using an HPLC system equipped with a column Spherisorb ODS2 (5 μm, 250 mm × 4.6 mm, Waters), a precolumn LC 8 (Supelco), a 515 pump and a 996 Photodiode Array Detector (Waters). The chromatographic apparatus was controlled by a Millennium software (Waters). The pH of the mobile phase was measured by an Orion 420 A (Beverly, MA, USA) pH-meter equipped with an Orion 9107 electrode.

3.5. Determination of retention parameters for QSSR studies

The HPLC analyses were carried out at room temperature with a flow-rate of 1 ml/min at isocratic conditions. The absorbance of the analytes was measured in the spectral range 210–400 nm. The chromatographic peaks were monitored at 225 nm. pH of the aqueous phase, before mixing with methanol, was adjusted by addition of NaOH to partially neutralise H_3PO_4 (1%) previously added, and measured by a pH-meter. The retention behaviour of the analytes was investigated by varying the methanol content of the mobile phase between 35 and 70% and its pH between 3.0 and 4.8. This range of chromatographic conditions was able to guarantee good resolution and acceptable retention time of all analytes, providing an accurate evaluation of the retention factor *k*, taken as the target property of the QSRR model.

4. Results and discussion

4.1. SPE–HPLC analysis

The proposed sample preparation procedure allows the preconcentration of the analytes by a factor of 2000, 1 l of groundwater being concentrated to a final volume of 500 μl. As a consequence, the detection limit of analysed triazines was as low as 0.010 μg/l for desisopropyilatrazine and desethylatrazine, 0.006 μg/l for simazine and atrazine and 0.018 μg/l for prometon. The repeatability of extraction procedure was tested for the different concentration levels used in the calibration curve of each analyte. The observed recoveries with related SDs are reported in Table 3. The extraction procedure allowed the simultaneous quantification of the five herbicides. No noticeable differences between results of simultaneous and individual analyses were detected.

Table 3
Recoveries (%) of the SPE procedure and, in parentheses, related SDs from three replicate experiments for different spiked levels of the analytes in groundwater

Analyte	Spiked level (μg/l)				
	0.1	0.2	0.3	0.4	0.5
Desisopropylatrazine	85 (4)	87 (4)	95 (5)	97 (5)	95 (5)
Desethylatrazine	87 (4)	91 (4)	92 (5)	90 (5)	93 (2)
Simazine	89 (5)	92 (2)	88 (4)	94 (3)	95 (4)
Atrazine	91 (2)	93 (2)	93 (2)	98 (2)	101 (3)
Prometon	86 (3)	89 (2)	102 (2)	98 (1)	98 (1)

4.2. ANN analysis

A standard procedure in back-propagation ANN analysis is the training and validation of the network by using a set of data (consisting of input variables and target output(s)), that, by means of iterative minimisation of the prediction error, allows to optimise the adjustable parameters of the network (the weights and the biases). A comparison between computed and observed *k* values in training and validation is given in Tables 1 and 2, respectively.

Generalisation ability of the trained ANN was further checked on a third data set (test set). In contrast to the validation set, which is based on the same four triazine herbicides used in the network training, the test procedure evaluates the capability of the network to predict the retention behaviour of a new (unseen) analyte (the triazine herbicide simazine, in this case), not included in training and validation. The test set consisting of 11 data points is given in Table 4 together with the network response.

4.3. Multilinear regression

As an alternative to ANN, a QSRR model was derived by stepwise MLR applied to the variable set used in the training of the neural network (Table 1). Although a relationship including all four descriptors provided the maximum correlation coefficient (*R*) and the minimum SE of the estimate (*s*), the regression coefficient of the descriptor pH resulted to be statistically not significant on 99% confidence level. As a consequence, we decided to exclude this parameter from MLR analysis. The resulting three-descriptor model, as compared with the four-descriptor one, exhibited an almost negligible deterioration of statistical quality of fitting, as revealed

Table 4
Test set and neural network response compared with prediction of the MLR model

Analyte	%MeOH	pH	log K_{ow}	μ (D)	Observed k	Predicted k	
						ANN	MLR
Simazine	65	3.4	2.3	3.76	1.481	1.449	1.587
	45	3.7			6.341	6.216	5.925
	60	3.7			1.982	1.998	2.206
	50	4.0			4.390	4.002	4.262
	55	4.2			2.707	2.773	3.067
	65	4.2			1.390	1.530	1.587
	55	3.2			2.855	2.900	3.067
	70	3.7			1.047	1.098	1.142
	60	4.0			1.895	2.012	2.206
	40	3.7			11.498	10.897	8.235
	35	4.0			14.979	15.577	11.446
					ERR%	4.4	12.8
					R	0.998	0.993

Related correlation coefficients (R) and average percent error (ERR%) are given at the bottom of the table (see text for details).

by comparable R and s figures in fitting and slightly better prediction ability in validation. The final relationship is the following:

$$\log k = 1.267(\pm 0.207) + 0.531(\pm 0.028) \log K_{ow} - 0.0286(\pm 0.0010)\%\text{MeOH} - 0.114(\pm 0.041)\mu$$

with $n = 44$; $R = 0.983$; $s = 0.091$; $F = 383.77$; $p < 10^{-4}$; where numbers in parentheses are SDs of regression coefficients, n is the number of data point used in deriving the regression equation, F is the value F-test of significance and p is the significance level of the equation. Large R and F values indicate adequate fit. Comparison of standardised model coefficients (not shown) reveals that the effect of %MeOH and log K_{ow} on the retention behaviour is comparable and predominant with respect to that of the other descriptors. Calculated k values on the basis of the above relationship are reported in Table 1 and compared with the experimental values. The prediction ability of the QSRR model derived by MLR was separately checked on the validation and test sets previously used in ANN analysis. The predicted k values compared with experimental ones are reported in Tables 2 and 4, respectively.

4.4. Comparison of ANN and MLR models

A graphical comparison of ANN and MLR analyses is given in Figs. 2 and 3, where the log k values calculated by means of the respective models are plotted against the experimental values. In particular, Fig. 2 depicts the response of the two models in the training (or fitting) procedure, whereas Fig. 3 shows the predictive ability of MLR or ANN model when applied to the validation and test set. Inspection of these plots clearly reveals that the prediction of retention values with neural network is superior to MLR. In addition to the correlation coefficient, the overall agreement between observed and predicted k values is quantified by the average percent error (ERR%) reported, for each data set, in the related tables and defined as:

$$\text{ERR\%} = \frac{1}{n}\sum_{i=1}^{n} \text{abs}\left(\frac{t_i - y_i}{t_i}\right) \times 100$$

where n is the number of data in a given set, t_i and y_i are the measured and predicted k values, respectively. The QSRR model developed via ANN exhibits a very good ability in describing the retention behaviour of the selected triazine herbicides both in training and prediction, as witnessed by the high correlation coefficients ($R = 0.997$ or greater) and the relatively low ERR% (4.9, 6.5 and 4.3, for training, validation and test, respectively). On the other hand, the MLR model, although it provided a quite satisfactory correlation both in fitting and prediction, was less accurate than the ANN model. This is proved by the significantly lower R values for

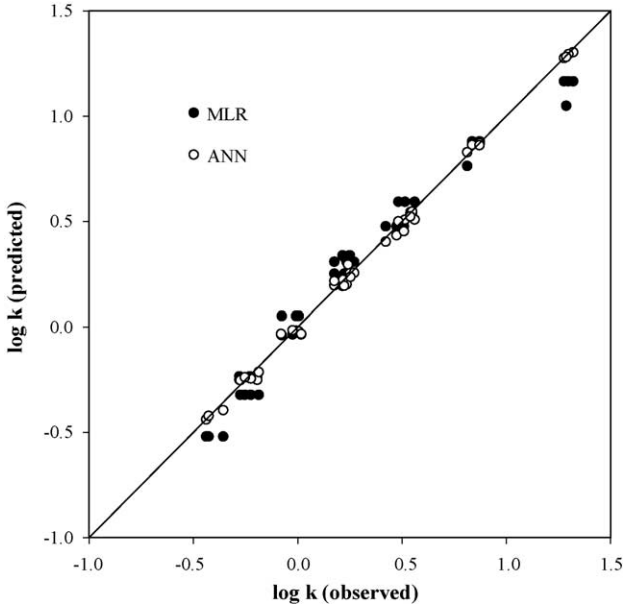


Fig. 2. Comparison of the experimental log k values with the calculated ones from the ANN and MLR models for the training set.

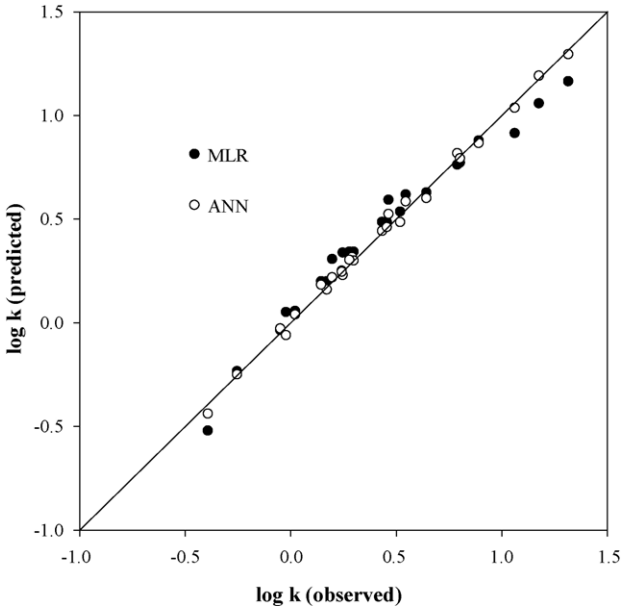


Fig. 3. Comparison of the experimental log *k* values with the predicted ones from the ANN and MLR models for the validation and test sets.

the training and validation set (0.977 and 0.984, respectively). Although *R* values are comparable in the case of the test set, ERR% given by MLR for all three data set is always sensitively higher (15.8, 15.7 and 9.8, respectively) than prediction errors given by the ANN model.

5. Conclusions

In the present study, a set of four descriptors, including both mobile phase and analytes properties, is adopted to build a QSRR model able to describe the retention behaviour of some triazine herbicides in the framework of environmental monitoring by means of reversed-phase HPLC. Our attention has been focused on the effect of mobile phase composition (% of organic modifier of the aqueous phase) and pH, i.e. the experimental parameters most commonly used to optimise the resolution and the analysis time. In addition to these operative parameters, only two descriptors, $\log K_{ow}$ and total dipole moment, are sufficient to account for the effect of the molecular structure of the analyte. In this context, a 4-6-1 feed-forward neural network provides a very accurate QSRR model as proven by the correlation between predicted and observed retention factors better than 0.997 and a relatively low ERR% in prediction (4.9 and 6.5% in training and validation, respectively). For comparison, a MLR model based on three descriptors exhibits a satisfactory prediction ability, but not as good as the ANN response, suggesting the existence of nonlinear relationships between the retention behaviour and some of the selected descriptors. Generalisation ability of the QSRR model is confirmed by its ability in predicting the retention behaviour of simazine, i.e. a solute not included in the set of test molecules used in training and validation of both MLR and ANN. Again, although MLR and ANN provide comparable *R* values, ANN gives a lower ERR% in prediction (4.4% versus 12.8%). Moreover, a simple method for the simultaneous quantification of the five triazine herbicides in groundwater samples was presented. This procedure guarantees high recoveries and good extraction repeatability.

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