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Evaluation of calibration data in capillary electrophoresis using artificial neural networks to increase precision of analysis

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Abstract

Increase of precision in capillary electrophoresis can be achieved applying suitable markers and evaluating calibration curves and data analysis with artificial neural networks. They are able to account for errors in both *x*- and *y*-axes, nonlinear response of detector and non-linearity of calibration curves eventually. A comparison of the artificial neural networks approach with ordinary least-squares (OLS) and bivariate least-squares regression (BLS) was done. While OLS and BLS give similar results, the method proposed and tested in analysis of several pharmaceutical products yields lower prediction errors than traditional linear least-squares methods and the precision of analysis was found in the range 0.5–1.5% relative.
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1. Introduction

Capillary electrophoresis (CE) is used extensively for drug analysis as an alternative and complementary technique to HPLC with convenient shorter time of analysis. However, precision of analysis is often not sufficient and thus might represent limitation for the use of CE in drug quantitative analysis [1–4].

Several inter-laboratory studies with different mathematical and experimental efforts, including the use of internal standards or of markers have been published in order to eliminate the influence of an electroosmotic flow and improve the injection repeatability [5–8]. Normalization of electropherograms using markers added to the analyzed solution is also one of the benefits of the recommended procedure which is included into an available commercial CE instrumentation of Agilent Technology software [9]. Survey of various possibilities how to improve precision of CE analysis is reviewed by Mayer [10].

Quantification in CE is mostly done from calibration curves and the evaluation of calibration curves is a fundamental step in the drug determination process and method validation. The regression procedure commonly used is based on the linear regression hypothesis being fulfilled by ordinary least-squares (OLS) or, whenever heteroscedasticity is present in the dependent variable, by weighted least-squares (WLS). However, when it is applied to

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real samples evaluation, this procedure has the drawback that regards the concentration (usually represented on the abscissa axis) as being free not only from systematic errors but also of random errors, as well. However, the *x*-axis always includes random errors often of the same magnitude as the *y*-axis. In the event of calibration, consequently the dependence of measured variable on the concentration of determined analyte is neglecting the errors accumulated during solution preparation.

The problem can be solved applying so-called ''bivariate least-squares'' (BLS) that considers errors in both axes and taking into account the uncertainty in the results which both axes may have [11]. This regression procedure looks very convenient, but in actual sense in analytical methods is seldom used because of its increased computational complexity [12] and as commercial programs are not available.

The main limitation of BLS is that the uncertainty associated with the values of the errors on both the *x*- and the *y*-axes has to be estimated, that means that data analysis takes longer and programming of its algorithm is complicated.

Recently, artificial neural networks are increasingly applied in chemistry and in separation science, as well. As for basic review, we refer here to a paper of Gasteiger and Zupan [13]. Artificial neural networks (ANNs) represent so-called ''soft'' modeling, without the need to know or establish a mathematical model [14]. For example, ANNs have successfully been applied in capillary zone electrophoresis [15–17], for modeling in ion chromatography [18], electrokinetic micellar chromatography [19], HPLC [20,21], etc.

In this work the possibility of applying ANNs to the evaluation of calibration graphs and CE analysis is investigated with the aim to treat the random errors in both axes simultaneously and thus to increase the precision in CE. Applying ANNs one can compensate errors in both axes, but also nonlinear response of detector and eventually nonlinearity of the calibration curve, as well.

2. Theory

2.1. Ordinary least-squares regression

Least-squares regression analysis is frequently used to describe the relationship between signal and concentration. All models describing the relationship between y and x can be described by general function Eq. (1):

$$y = f(x, a, b_1, \ldots, b_m) \tag{1}$$

where $a, b_1, \ldots, b_m$ are the parameters of the function. We adopt the convention that x values relate to the independent or controlled variable (e.g., the concentration of a standard) and y values to the dependent variable (the response measurements). This means that the x values have no error. On the condition that error made in preparing the standards are significantly smaller then the measuring errors, this assumption is realistic in calibration problem. Mostly, function (1) is linear and than OLS leads to formulas giving the estimates for the slope and intercept value of a linear regression line [14].

2.2. Bivariate least-squares regression

BLS is the generic name for a set of techniques used for regressing bivariate data, whenever regression method is applied to data containing errors in both axes.

The method consists of minimizing the sum of the weighted residuals, S, expressed in Eq. (2).

$$S = \sum_{i=1}^{n} \frac{(y_i - \hat{y}_i)^2}{w^2} = (n-2)\hat{s}^2 \tag{2}$$

where w is the weighting factor and $\hat{y}_i$ is the estimated value for the y predicted. $\hat{s}^2$ is the estimated value of error, measured in terms of variance, for the set of n experimental data points (x_i,y_i).

This method uses the variance of residuals (s_{Ei}), which can be expressed using the Taylor series, as a weighting factor even when the covariance between variables for each data pair is not equal to zero, cf. Eq. (3):

$$s_{Ei}^2 = w_i = s_{yi}^2 + \hat{b}^2 s_{xi}^2 - 2\hat{b}\ \mathrm{cov}(x_i,y_i) \tag{3}$$

where s_{xi}^2 and s_{yi}^2 stand for the variance of each (x_i,y_i) data point. By minimizing the sum of the weighted residuals in relation to the slope and the intercept, two nonlinear equations are obtained, and by including the partial derivatives of the squared residuals equations can be written in matrix form as Eqs. (4) and (5):

$$R\hat{b} = g \tag{4}$$

$$\begin{bmatrix} \sum_{i=1}^{n} \frac{1}{s_{Ei}^2} & \sum_{i=1}^{n} \frac{x_i}{s_{Ei}^2} \\ \sum_{i=1}^{n} \frac{x_i}{s_{Ei}^2} & \sum_{i=1}^{n} \frac{x_i^2}{s_{Ei}^2} \end{bmatrix} \cdot \begin{bmatrix} \hat{a} \\ \hat{b} \end{bmatrix} = \begin{bmatrix} \sum_{i=1}^{n} \left(\frac{y_i}{s_{Ei}^2} + 1/2 \left(\frac{R_i}{s_{Ei}^2} \right)^2 \frac{\partial s_{Ei}^2}{\partial \hat{a}} \right) \\ \sum_{i=1}^{n} \left(\frac{x_i y_i}{s_{Ei}^2} + 1/2 \left(\frac{R_i}{s_{Ei}^2} \right)^2 \frac{\partial s_{Ei}^2}{\partial \hat{a}} \right) \end{bmatrix} \tag{5}$$

The slope and the intercept, which are components of vector $\hat{b}$ in Eqs. (4) and (5), can be determined by carrying out an iterative process on the following matrix form given in Eq. (6):

$$\hat{b} = R^{-1} g \tag{6}$$

With this method, and assuming that the straight line model is the correct one, the variance–covariance matrix of the calibration straight line coefficients, *B*, is obtained by multiplying the final matrix R^{-1} by the experimental error s^2. As the experimental error is unknown, the estimated value, s^2, expressed in Eq. (2) should be used.

It should be pointed out, if ${s_{Ei}}^2 = 1$ (if all errors are due to the experimental measurement in the ordinate axis), then the expressions obtained are the same as for OLS.

2.3. Artificial neural networks

ANNs are information-processing paradigm inspired by the way of the brain processes information. They emulate some of the observed properties of biological nervous systems and draw on the analogies of adaptive biological learning. The key element of the ANN paradigm is the novel structure of the information processing system, composed of a large number of highly interconnected processing elements (analogous to neurons) and tied together with weighted connections that are analogous to synapses.

In order to translate how the artificial neural network mimics the human brain into our understanding, the processing of information can be divided into three levels. The first level is the *input layer* of which it receives the information about the system; the nodes in the input layer are simple distributive nodes, which do no alter the input value at all. The second level (*hidden layer*) process the information initiated at the input. The third level is the *output layer,* that is the observable response or behavior. The node (neuron) is the basic processing unit in ANNs. The node sums the product of each connection weight (w_{jk}) from a node *j* to the node *k* an input (x_j) to get the value sum for the node *k*, Eq. (7).

$$\text{sum}_k = \sum_i x_j w_{jk} + \gamma \tag{7}$$

where γ is called the bias value. The numbers of nodes in the input and in the output layer are defined by the problem being studied, and they are adjustable parameters.

The sum_k of the weighted inputs is transformed with a linear or nonlinear transfer function, this function is used to get to the output level. Several functions can be used, ''sigmoid function'' is the mostly applied.

Back propagation (BP) is one of the best known training algorithm for neural networks. It has lower memory requirements than others, and usually reaches an acceptable error level quite quickly, although it can then be very slow to converge properly. It can be used on most types of network in Trajan program, although it is most appropriate for training multilayer perceptrons.

Levenberg–Marquardt (LM) is an advanced nonlinear optimization algorithm. It is usually the fastest and most reliable algorithm available. LM can only be used on networks with a single output unit and has space requirements proportional to the square of the number of weights in the network. This effectively precludes its use in networks of great size.

The ANNs ''learn'' by adjusting their weights according to the error. The goal of the training procedure is to change the weights between the layers in a direction that minimizes the error, *E.*

The error *E* of a network, Eq. (8), is defined as the squared difference between the target values (desired output) *t* and the outputs *y* of the output neurons summed over *p* training patterns and *j* output nodes.

$$E = \frac{1}{2} \cdot \sum_p \sum_j (y_{pi} - t_{pj})^2 \tag{8}$$

The error E is minimized according to, e.g., the steepest descent method or other, Eq. (9),

$$\Delta w_{ij}(n) = \eta \cdot \frac{\partial E}{\partial w_{ij}} \quad (9)$$

where η is a positive constant known as the ''learning rate'' and $\Delta w_{ij}(n)$ the current weight change for the weight w_{ij}. The weights are given initially random values at the beginning and then are calculated in an iteration process. These weights are updated by computing the layer errors and the weight changes. The gradient descent method can be enhanced by a ''momentum term'' from the previous weight changes as given in Eq. (10),

$$\Delta w_{ij}(n) = -\eta \cdot \frac{\partial E}{\partial w_{ji}} + \alpha \Delta w_{ji}(n-1) \quad (10)$$

where α (momentum factor) is another constant.

The learning process will stop when the network has reached a proper minimum error. The rate controls the update rate according to the new weights changes and the moments act as stabilisator being aware of the previous weight changes.

During the training, the effect of the learning rate and momentum must be studied. The optimization of these parameters is necessary in order to avoid overfitting.

During the application of ANNs, the goal of net training is to minimize the root mean square error (RMS) given by Eq. (11),

$$\mathrm{RMS} = \sqrt{\frac{\sum_{i=1}^{N}\sum_{j=1}^{M}(t_{ij} - y_{ij})}{M \times N}} \quad (11)$$

where t_{ij} is the element of the matrix $(M \times N)$ for training set or test set, and y_{ij} is the element of the output matrix $(M \times N)$ of the neural network.

3. Experimental

3.1. Reagents

Memantine hydrochloride (MEM), rimantadine hydrochloride (RIM), tetraethylammonium iodide (TEA) and tetrabutylammonium iodide (TBA) were purchased from Sigma (St. Louis, MO, USA), 4-methylbenzylamine was purchased from Fluka (Buchs, Switzerland). Rutin was purchased from Merck (Darmstadt, Germany), ascorbic acid, diphenylguanidinium, and quercetin were from Lachema (Brno, Czech Republic). Rutin tablets: Ascorutin was purchased from Slovakofarma (Hlohovec, Slovak Republic), Wobenzym from Mucos Pharma CZ s.r.o. (Prόhonice, Czech Republic) and Anavenol® was purchased from Léčiva (Praha, Czech Republic). Memantine tablets (Memantine) were purchased from Lachema. Rimantadine tablets (Flumadine) was from Forest Pharmaceuticals (St. Louis, MO, USA) and Rimantadine from Lachema.

All other chemicals were of analytical-grade purity and were obtained from Lachema.

Three times distilled water used to prepare all the solutions was obtained from a quartz distillation stand of Heraeus Quarzschmelze (Hanau, Germany).

3.2. Apparatus and conditions

A SpectraPhoresis 2000 CE System of Thermo Bioanalysis Corporation (CA, USA) equipped with a high-speed scanning detection system and integration software station, version PC 1000 of Thermo Separation Biotechnology Corporation (CA, USA) was employed in the data conversion and evaluation for all experiments. Uncoated fused-silica capillary, 75 μm I.D. of Watrex (Prague, Czech Republic) of 44 cm total length and 36.5 cm length to the detector, was used.

Background electrolyte (BGE), was prepared daily, the solution was filtered through a 0.45-μm filter Spartan®-3 of Schleicher & Schűll (Dassel, Germany) prior to use and degassed in an ultrasonic bath from Branson (Shelton, USA) for 10 min. The capillary was washed daily for 10 min with 0.1 M NaOH, 10 min with deoinized water and 10 min with run buffer.

For the determination of drugs in tablets, every tablet was mixed with a small volume of water and the mixture was placed in an ultrasonic bath for 15 min. The solution was then filtered, a corresponding amount of a standard solution of markers was added and the mixture was made up to the mark in a measuring flask.

Furthermore, between injections the capillary was

washed with the running buffer (1 min) and water (0.5 min) to keep the migration time of the analytes reproducible. The temperature during analysis was maintained constant at 25 °C.

Spectrophotometric measurements were performed with an UV-2 Quartz UV–Vis Unicam spectrophotometer (Cambridge, UK), using a 1-cm quartz cell. A Radelkis OP-208/1 Precision Digital pH meter (Budapest, Hungary) and a Radelkis pH-sensitive combined glass electrode were used for pH measurements.

3.3. ANN software and data processing

ANN software was purchased from Trajan program Neural Network Simulator releases 3.0 D (1998; Trajan Software, Durham, UK) and processed on a Pentium personal computer. For the BLS regression York's program [22] written in FORTRAN 90 and adapted for this problem was used.

4. Results and discussion

The possibility of ANNs to improve the precision of analysis of some important pharmaceutical products will be studied here. The study will be performed on examples of antiviral drugs (memantine, rimantadine) and on rutin analysis.

Memantine hydrochloride (3,5-dimethyl-1-adamantanamine) is a derivative of the decades old anti-influenza drug amantadine. Memantine is used in Germany to treat Parkinson's disease, dementia in the elderly, and to speed the recovery of comatose patients. Memantine may also be useful for prophylaxis of human immunodeficiency virus encephalopathy.

Reichová et al. [23] have developed a CE method for the determination of memantine but the precision was not any excellent.

Rimantadine hydrochloride (α-methyl-1-adamantanemethylamine) is synthetic analog of amantadine; both are antiviral agents used for prophylaxis and treatment of influenza A.

Rutin, 3-{[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-glucopyranosyl]oxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one a flavonoid *O*-glycoside derivate of a quercetin is an important drug used in the treatment of capillary bleeding and increased capillary fragility [24,25].

The pharmaceutical importance of these compounds is the reason why enhancement of the precision of methods for their determination is important.

Quantitative chemical analysis of drugs involved the following steps: (1) CE analysis and determination of peak areas. (2) Adjustment of a mathematical model describing the dependence of y (peak area) at x (concentration). This procedure can be called ''calibration'' and different regression procedures can be used (OLS, BLS and ANN in this case). (3) Evaluation of unknown samples using one of the models for a ''prediction'' of the analyte amount.

The results obtained by OLS and BLS evaluation with those obtained applying ANNs are to be compared.

4.1. CE determination

Generally, for each drug analysis, a total of ≈20 calibration solutions were spiked with markers so that they contained 10 ppm of Marker 1, 10 ppm of Marker 2 and drug in the concentration range 5–15 mg/l, e.g., for memantine analysis.

Diphenylguanidinium (left marker, Marker 1) and quercetin (right marker, Marker 2) for the determination of rutin and tetraethyl ammonium (left marker, Marker 1) and tetrabutylammonium (right marker, Marker 2) for determination of memantine and rimantadine were chosen as internal standards in order to eliminate the influence of an electroosmotic flow and to improve the injection repeatability. Fig. 1 shows the repeatability of memantine analyses.

The samples were measured using CE equipment and peak areas of all peaks were calculated using standard procedures.

The optimal CE conditions for all drug tested are given in Table 1.

4.2. Calibration curves evaluation

4.2.1. Ordinary linear least-squares and bivariate least-squares evaluation of analysis

In order to establish a mathematical model that describes the dependence of peak area of a drug on

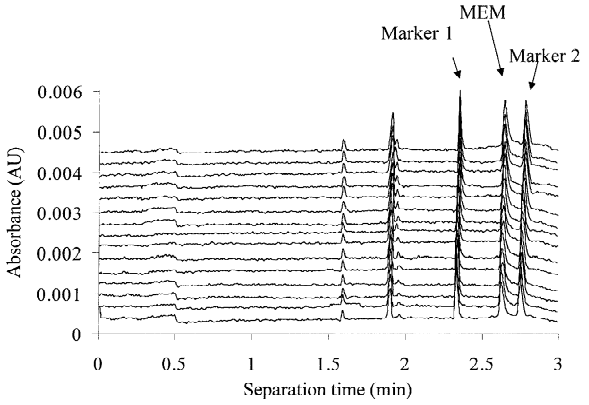

Fig. 1. Example of memantine CE analysis repeatability ($n=15$). The number of analyses was 15, conditions: BGE, 5 mM MBA (20% ethanol), pH 9; hydrodynamic injection for 5 s; separation voltage 20 kV. MEM, peak of memantine, detection at 210 nm.

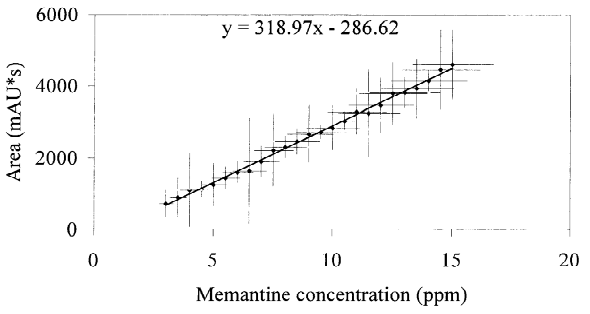

Fig. 2. Calibration curve of memantine. Conditions of analyses were the same as given in Fig. 1. Errors on *x*- and *y*-axis were calculated and the values obtained were multiplied by factor 10. For *y*-axis the errors represent residuals, i.e., the difference between the observed value and the value predicted by OLS. For *x*-axis the residuals represent the difference between the experimental value and the value calculated from the observed *y*-value using regression model.

its concentration the OLS and the BLS analysis were performed.

Fig. 2 shows an example of the calibration curve for memantine. The values of errors for both *x*- and *y*-axes were calculated and are given, as well. The relative errors of analysis obtained are similar for both OLS and BLS (as it would be expected) and they ranges between 1.5 and 6% relative, pointing out limited precision of both of the methods.

4.2.2. Evaluation of data by ANN

The data set containing 20 calibration solutions was divided into the training set and the test set. The data were processed with Trajan program using either Back propagation or Levenberg–Marquardt algorithms. We have used a three-level ANN architecture. The structure of the neural network was first optimized using as input data the values of peak areas of Marker 1, Marker 2, and peaks of the corresponding drug, while drug concentration was the only output. The number of nodes in the hidden layer was changed from 1 to 6. It follows from Fig. 3 that three nodes were sufficient to obtain low RMS values, further increase of the number of nodes does not bring any improvement. The optimal structure found was 3:3:1 (Fig. 4).

The performance of the neural network was tested every hundred or thousands of epochs during the learning in order to avoid overtraining, and the weights for which the value of RMS was minimal (both for the learning and the test set) were recorded. The training conditions were: momentum 0.3, learning rate 0.6. We stopped the training when maximum value of epoch was reached or when no significant improvement for higher value was obtained. The values of errors obtained with the ANN for complete

Table 1
Summary of optimal conditions for all analyzed drugs

	Memantine	Rimantadine	Rutin
BGE	5 mM MBA (20% ethanol)	5 mM MBA (20% ethanol)	15 mM borate
pH	9	9	9.2
Injection	Hydrodynamic injection	Hydrodynamic injection	Hydrodynamic injection
Injection time (s)	5	5	10
Separation voltage (kV)	20	20	20
Detection wavelength (nm)	210	210	260

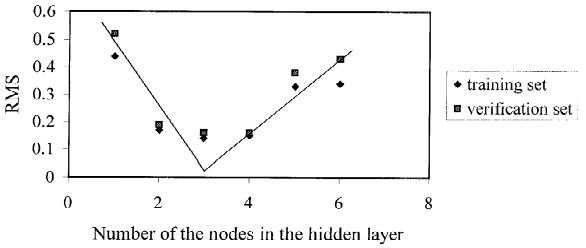

Fig. 3. Search of the optimal ANN structure.

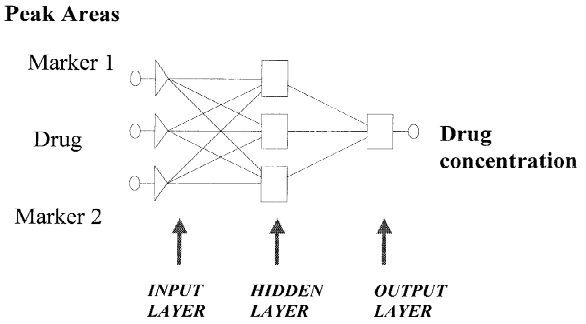

Fig. 4. Optimal ANN structure used for CE quantification of drugs and the organization of input and output data.

training set ranged between 0.5 and 3% relative, and were always lower than the errors obtained using conventional linear calibration curve approach.

4.3. Assay of drug content in tablets

Finally, the proposed method was applied to the quantitative analysis of various commercial oral dosage forms containing the analyzed compounds. Analyses were always taken in the same day as the calibration measurements. During the analysis of all sorts of the tablets no influence of the matrix components was observed.

Data set corresponding to peak areas of each drug (memantine, rimantadine or rutin), Marker 1, and Marker 2, not included in the training set was used as a test set. The selection was done randomly.

The assay and content uniformity for tablet forms of Memantine (memantine), Rimantadine and Flumadine (rimantadine) and Ascorutin®, Wobenzym® and Anavenol® (rutin) predicted by the ANN method including the addition of left and right markers were in all cases much better than the values calculated by the classical OLS or BLS approach.

Table 2
Results of concentration predictions obtained by ANN using this normalization procedure with markers compared those obtained using classical OLS

Tablet	Declared content (mg)	Peak area (AU/s)	Concentration		Content uniformity by OLS (mg)	Difference (pred-exp) (mg)	Content uniformity by ANN (mg)	Difference (pred-exp) (mg)
			by OLS (ppm)	by ANN (ppm)				
Ascorutin 1	20	129 258	106.25	100.2	21.25	1.25	20.04	0.04
Ascorutin 2	20	126 161	103.65	100.4	20.73	0.73	20.08	0.08
Ascorutin 3	20	125 649	103.33	99.16	20.67	0.67	19.83	−0.17
Wobenzym 1	50	128 083	105.30	100.90	52.65	2.65	50.45	0.45
Wobenzym 2	50	132 713	108.95	98.71	54.48	4.48	49.36	−0.64
Wobenzym 3	50	124 751	102.51	100.30	51.26	1.26	50.15	0.15
Anavenol 1	30	128 331	105.41	99.07	31.62	1.62	29.72	−0.28
Anavenol 2	30	133 629	109.79	99.20	32.94	2.94	29.76	−0.24
Anavenol 3	30	130 381	107.07	101.00	32.12	2.12	30.30	0.30
Rimantadine 1	100	2035.6	9.59	9.86	95.9	4.11	9.86	1.41
Rimantadine 2	100	1960.7	9.22	10.05	92.2	7.75	10.05	0.47
Flumadine 1	100	2226.7	10.52	10.19	105.2	5.20	10.19	1.91
Flumadine 2	100	2005.8	9.44	10.08	94.4	5.55	10.08	0.80
Memantine 1	10	2714.4	9.42	9.92	9.42	0.58	9.92	0.08
Memantine 2	10	3044.5	10.44	10.11	10.44	0.44	10.11	0.11
Memantine 3	10	3152.3	10.78	10.32	10.78	0.78	10.32	0.32
Residual						2.63		0.47

Tables 2 and 3 summarise the results of estimated values obtained for analysed drugs under study using, LLS, BLS and ANN methods.

In addition, in order to evaluate the efficiency of method's performance, standard error of the prediction (SEP), as it was defined by Walczak and Wegscheider [26], over the test data set was applied. The SEP over the test data set using the ''traditional'' OLS approach was 3.39, while using direct application of ANN markers method to these data a decrease in the SEP to only 0.68 was reached.

In summary, ANN modeling and prediction using additional markers improves considerably the precision of CE for quantitative purposes of important drugs and commercial medicines.

5. Conclusions

Precision of CE analysis can be improved substantially using suitable markers and evaluating the data of markers and analytes simultaneously to correct for EOF changes and other errors using a soft modeling by artificial neural networks. ANNs are able in this way to compensate for errors in both axes of a calibration curve, nonlinear responses of detector and

Table 3
Comparison of concentration predictions and relative errors for tested pharmaceuticals products evaluated using OLS, BLS and ANN

Tablet	OLS		BLS		ANN	
	Predicted concentration (ppm)	Relative error (%)	Predicted concentration (ppm)	Relative error (%)	Predicted concentration (ppm)	Relative error (%)
Ascorutin 1	106.25	6.25	–	–	100.2	0.2
					100.4	
					99.16	
					100.90	
					98.71	
					100.30	
					99.07	
					99.20	
					101.00	
Ascorutin 2	103.65	3.65	–	–	100.4	0.4
Ascorutin 3	103.33	3.33	–	–	99.16	0.84
Wobenzym 1	105.30	5.30	–	–	100.90	0.9
Wobenzym 2	108.95	8.95	–	–	98.71	1.29
Wobenzym 3	102.51	2.51	–	–	100.30	0.3
Anavenol 1	105.41	5.41	–	–	99.07	0.93
Anavenol 2	109.79	9.79	–	–	99.20	0.8
Anavenol 3	107.07	7.07	–	–	101.00	1.01
Rimantadine 1	9.59±0.17	4.14	9.56±0.17	4.40	9.86±0.11	1.41
						0.47
						1.91
						2.72
						0.84
						1.10
						3.22
						1.15
Rimantadine 2	9.23±0.40	7.75	9.20±0.41	8.00	10.05±0.02	0.47
Flumadine 1	10.52±0.35	5.20	10.48±0.35	4.80	10.19±0.05	1.91
Flumadine 2	9.44±0.11	5.51	9.42±0.11	5.80	10.08±0.03	0.80
Memantine 1	9.42±0.31	5.82	9.45±0.30	5.50	9.92±0.17	0.84
Memantine 2	10.44±0.15	4.43	10.47±0.15	5.30	10.11±0.04	1.10
Memantine 3	9.78±0.33	7.81	9.82±0.33	7.20	10.32±0.12	3.22
Average		5.81		5.86		1.15

eventually non-linearity of the calibration curve. The use of ANNs is easier than using bivariate least-squares algorithm. In addition, the application of the ANN approach is more correct than the use of traditional linear least-squares method neglecting errors on *x*-axis.

Precision of quantitative analysis of three different compounds with pharmaceutical importance 1.15% relative was reached. The method gives in this case acceptable and better prediction errors than the traditional linear least-squares method.

The use of the ANN approach for quantitative CE analysis with suitable markers to the compound of interest appears to be a promising analytical tool for pharmaceutical analysis.

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