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New spectral approaches to the simultaneous quantitative resolution of a combined veterinary formulation by ANN and PCA-ANN methods

Erdal Dinç[1,*], Dumitru Baleanu[2,3] and Nigar Şen Köktaş[2]

[1] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100, Tandoğan, Ankara, Turkey, e-mail: dinc@pharmacy.ankara.edu.tr
[2] Department of Mathematics and Computer Sciences, Faculty of Arts and Sciences, Çankaya University, 06530 Balgat, Ankara, Turkey
[3] National Institute for Laser, Plasma and Radiation Physics, Institute of Space Sciences, Magurele-Bucharest, P.O. Box, MG-23, R 76911, Romania

*Corresponding author

Abstract

The simultaneous spectral prediction of levamisole (LEV) and triclabendazole (TRI) in combined veterinary formulation was performed by the new chemometric methods, artificial neural network (ANN) and principal component analysis-artificial neural network (PCA-ANN). Despite the overlapping spectra of LEV and TRI in the same wavelength region, the proposed methods do not use any separation procedure for the analysis of the related compounds. Good precision and accuracy were observed for the applications of the proposed artificial neural network models to an independent binary mixture set consisting of the active compounds. These methods were successfully applied for the chemometric quantitation of a veterinary formulation of LEV and TRI.

Keywords: artificial neural networks; levamisole; principal component analysis; triclabendazole.

Introduction

Principal component analysis represents a useful statistical technique that has application in several fields such as face recognition and image compression; chemometrics is a common technique for finding patterns in data of high dimension.

The determination of levamisole (LEV) in its different sample forms together with other active compounds and their metabolites have been previously analyzed using HPLC (Sakamoto et al. 2002, Sari et al. 2004, Palma et al. 2006, Tyrpenou and Xylouri-Frangiadaki 2006) and LC-MS/MS (Dreassi et al. 2001, De Ruyck et al. 2002, De Baere et al. 2003). For triclabendazole (TRI) in samples in the presence of other compounds or its metabolites, the corresponding analysis was done by making use of HPLC (Negro et al. 1992, Takeba et al. 2000).

Recently the spectrophotometric simultaneous determination of LEV and TRI in tables was investigated by principal component regression and partial least squares chemometric methods (Pektas et al. 2008).

Currently, artificial neural networks (ANNs) are being applied to many areas of science for the purpose of calibration and recognition of patterns. The ANN approach is a system based on the operation of biological neural networks. As described above, ANNs have many applications in analytical chemistry such as calibration recognition pattern. This indicates the flexibility and versatility properties of the ANN method in the quantitative resolution of the complex mixture in the presence of interference and non-linear relationships. In addition to this, ANN approaches in different designs with transfer functions have been applied to the electrochemical and spectral data sets for the chemometric quantitations or predictions of target metals and active compounds.

In this study, ANN without and with principal component analysis (PCA) was used for the simultaneous quantitation of LEV and TRI in veterinary binary mixtures by using spectrophotometric data sets for the calibration and predictions steps. Next, these two methods of ANN application will be explained. Firstly, ANN for the training set was applied to the original absorption data, and secondly to the corresponding PCA data sets for ANN and PCA-ANN calibrations. The capacity of the applicability of the above two approaches was observed by analyzing the synthetic veterinary samples and a good recovery was reported.

Materials and methods

Apparatus and software

In our research for registration of the absorption spectra, a Shimadzu UV-160 double beam UV-VIS (Shimadzu Corporation, Kyoto, Japan) spectrophotometer equipped with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used.

In the first step the absorption data was transferred to EXCEL. After that the transferred vectors data corresponding to the concentration set and sample set were processed by ANN and PCA-ANN methods. All data treatments, regressions and statistical analysis were performed by using EXCEL (Microsoft Windows, USA) and Matlab 7.0 software (The MathWorks, Inc., Natick, MA, USA).

Commercial veterinary formulation

In this paper, a commercial veterinary preparation (BESTAN® Oral Tablet, Vilsan Pharm. Ind., Ankara, Turkey) was investigated. Its declared content contains 600 mg TRI, 375 mg LV HCl per tablet. LEV and TRI were obtained as a donation from Vilsan Pharm. Ind.

Standard solutions

Stock solution of LEV and TRI were prepared by dissolving 25 mg of each veterinary drug in 100 ml calibrated flask in the solvent system consisting of methanol and 0.1 M NaOH (75:25, v/v). A concentration set of 25 mixture solutions consisting of LEV and TRI in the concentration range of 5.0–25.0 and 1.0–11.0 μg/ml was symmetrically prepared from the prepared stock solution, respectively.

For the confirmation of the validity and applicability of the proposed methods and independent validation set of 10 synthetic veterinary mixtures containing LEV and TRI were prepared by using the above veterinary stock solutions.

Sample solution preparation

In total, ten tablets were accurately weighed and powdered in a mortar. A sample containing LEV and TRI equivalent to 1/8 tablet content was dissolved in the solvent consisting of methanol and 0.1 M NaOH (75:25, v/v) and made up in 100 ml calibrated flasks. The content of the flask was mechanically shaken for 25 min and filtrated into a 100-ml volumetric flask through a 0.45 μm membrane filter. The final solution was diluted to the working concentration range. After the procedure was repeated ten times, the absorption spectra of sample solution were recorded to apply the PCA and PCA-ANN calibrations.

Results and discussion

In this study, the application of a new chemometric approach was carried out for the quantitation of a combination of two veterinary drugs named LEV and TRI. For this purpose, the absorption spectra of target veterinary drugs were registered between 225 nm and 270 nm.

The same spectral registration procedure was also applied to all samples. Figure 1 indicates the absorption spectra of LEV and TRI with their binary veterinary mixture. The simultaneous quantitation of LEV and TRI is not possible owing to the overlapping spectra of two veterinary drugs in the wavelength region of 225–270 nm. For this reason, it is required to find a suitable quantitation method and thus ANN and PCA-ANN calibration models were applied to this quantitation. It was found that ANN and PCA-ANN applications give a good quantitation for LEV and TRI in their synthetic veterinary and commercial veterinary samples without using an additional separation.

The effect of tablet excipients on the analysis is considered in the presence of the above-mentioned overlapping spectra

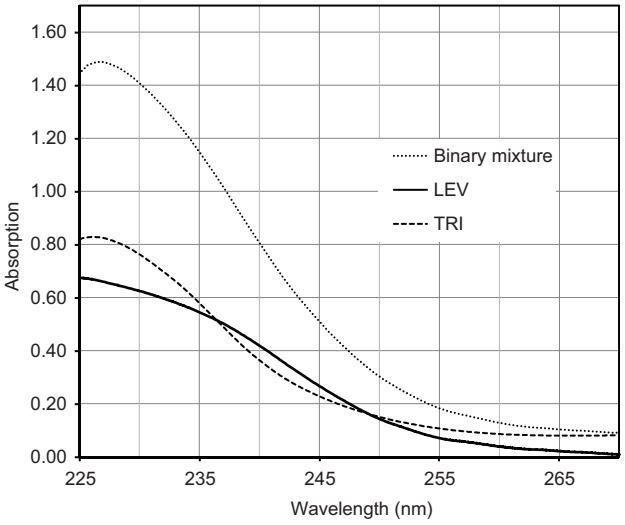

Figure 1 Absorption spectra of TRI (6) and LEV (20), and their binary mixtures.

in the same wavelength region. Against the above restricted analysis conditions, the ANNs based on the use of the original absorbance data set and their principal components were applied to the simultaneous quantitative prediction of LEV and TRI in samples without requiring a chemical separation procedure.

In the application of PCA-ANN and ANN calibrations, the concentration data set consisting of mixture solutions in the different concentration ranges between 5.0–25.0 μg/ml of LEV and 1.0–9.0 μg/ml of TRI was symmetrically prepared considering the quantities of drugs in tablets. The concentration data set for the above chemometric calibrations is illustrated in Table 1. The absorbance values of the concentration data set measured at the wavelength set 225.0–270.0 nm corresponds to an absorbance data set in a 401×25 matrix. PCA-ANN and ANN calibrations were obtained from the relationship between the concentration data set and corresponding absorbance data set.

Artificial neural network (ANN)

It is known that an ANN operates by making relationships between many different processing elements, each analogous to a single neuron in a biological brain. These neurons can be physically constructed or they can be simulated by a digital computer. Each neuron takes many input signals, and after that, based on an internal weighting system, produces a single output signal that is typically sent as input to another neuron.

Table 1 ANN topologies for the applied calibration methods.

	ANN	PCA-ANN
Input	401	3
Hidden	5	2
Output	2	2
Transfer functions	Tansig/purelin	Tansig/purelin

The neurons are tightly interconnected and organized into different layers. The input layer receives the input; the output layer produces the final output. It is known that one or more hidden layers are sandwiched in between the other two.

Principal component analysis (PCA)

PCA denotes a mathematical procedure for eigenvector-based multivariate analyses. PCA transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables denoted as principal components. The method is used to reduce the dimension of the data set by identifying new meaningful underlying variables. If a multivariate data set can be seen as a set of coordinates in a high-dimensional data space, PCA supplies the user with a lower-dimensional picture from the most informative point of view. The first principal component carries much of the variability in the data and the succeeding component that is uncorrelated to former ones carries the remaining variability.

Chemometric method application

In this application, the performances of two ANNs with different input sets are compared. The topology of these networks is presented in Table 2. In the first stage, the ANN calibration set was directly applied to the original absorption data and the calibration performance is tested by analyzing the validation set. A data set having the dimension 401×25 (spectra×sample) was presented to the ANN as an input vector. The validation set with the dimension 401×10 is also supplied during the training phase for early stopping of the training to prevent overfitting. A two layer ANN was created with tansig activation function in the first hidden layer and purelin activation function in the output layer. With this structure rapid convergence of the inputs was supplied and linear mapping of the outputs decreased the generalization error.

In the second application, the dimension of the calibration, the validation and sample datasets were reduced by the PCA method. A data set of the size 25×401 (observations×variables) was presented to PCA routine and three outputs were gathered. The first output is coefficient matrix with the size 401×401, each column consisting of coefficients for one principal component. The columns were in order of decreasing component variance. The second output is the matrix of principal component scores. The scores were the data formed by transforming the original data into the space of the principal components. The third output is a vector containing the eigenvalues of the covariance matrix of input. It is observed that the first five principal components represent 99.6% of the total variance. Therefore, the first five columns of score values were used to compose new data sets for learning and testing. An input data set of the size 5×25 and validation data set of the size 5×10 is presented to the PCA-ANN, which is a two-layered neural network with tansig and purelin activation functions in hidden and output layers, respectively. Performances of these two neural networks were compared by generalization accuracy of them and convergence speed. The errors vs. number of iteration for training of neural networks are shown in Figures 2 and 3.

As in the above descriptions, the reliable results for the quantitative evaluation of LEV and TRI in samples were obtained by the applications of PCA-ANN and ANN calibrations.

Table 2 A concentration set consisting of the mixtures LEV and TRI substances.

No.	Concentration set (μg/ml)		No.	Concentration set (μg/ml)	
	LEV	TRI		LEV	TRI
1	5	1	14	15	9
2	5	3	15	15	11
3	5	6	16	20	1
4	5	9	17	20	3
5	5	11	18	20	6
6	10	1	19	20	9
7	10	3	20	20	11
8	10	6	21	25	1
9	10	9	22	25	3
10	10	11	23	25	6
11	15	1	24	25	9
12	15	3	25	25	11
13	15	6			

Validity of PCA-ANN and ANN

Validity and applicability of the proposed PCA-ANN and ANN methods were tested by using the binary mixtures of LEV and TRI. Mean recoveries and relative standard deviations for the two ANN calibrations were calculated and are shown in Table 3. These assay results indicate that these two methods are suitable for simultaneous quantitative evaluation of the above mentioned compounds in samples.

To test the proposed calibrations, an independent set of the validation set in Table 3 was analyzed and used for the calculations of the standard error of prediction (SEP) in the validation step. The standard error of calibration (SEC) for calibration samples (n=25) and the errors of prediction (SEP)

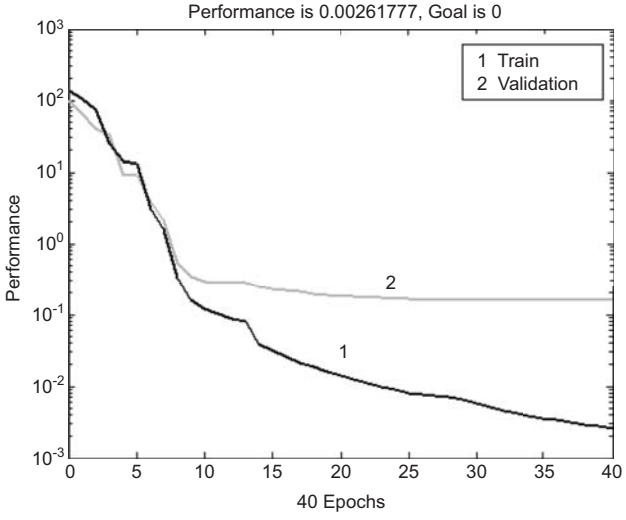

Figure 2 Error performance for training of ANN with actual inputs.

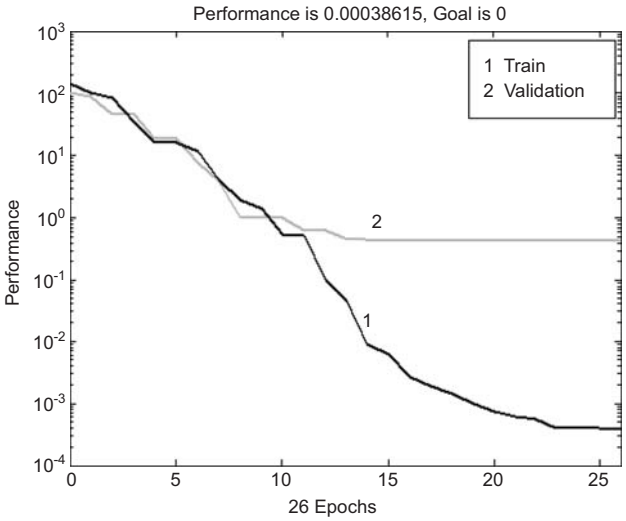

Figure 3 Error performance for training of ANN with PCA applied inputs.

for prediction samples (n=10) were calculated for PCA-ANN and ANN methods and their values are presented in Table 3. In Table 3, the results of linear regression analysis were applied to the relationships between the actual and predicted concentrations in the calibration and validation sets. Their statistical results are illustrated in Table 4.

Sample analysis

In this study, ANN and PCA-ANN chemometric methods were applied to the simultaneous quantitative prediction of the amounts of LEV and TRI in tablets. The obtained predicted results of LEV and TRI compounds in marketed tablets are shown in Table 5. A good coincidence was reported between the results obtained by the application of the ANN and PCA-ANN methods. The statistical parameters, namely, standard deviation, percent relative standard deviation, standard errors and confidence limits (p=0.05) are presented in Table 5. Two chemometric approaches give successful determination results in spite of the effect of tablets excipients and in the presence of the overlapping spectra of the related compounds.

Table 4 Statistical parameters in the calibration and prediction steps.

Parameter	ANN		PCA-ANN	
	LEV	TRI	LEV	TRI
Calibration				
SEC	0.0741	0.1478	0.0630	0.1394
Slope	1.0000	1.0001	0.9999	0.9999
Intercept	0.0038	0.0045	0.0020	0.0006
r	1.0000	1.0000	1.0000	1.0000
Prediction				
SEP	0.6667	0.3528	0.8408	0.4328
Slope	1.0591	0.9887	1.0668	0.9727
Intercept	–0.3212	0.1852	–0.4831	0.3181
r	0.9993	0.9997	0.9935	0.9992

Conclusion

During the past decade ANN has been used in the area of chemometrics in order to identify and to eliminate the compounds interactions as well as the sample matrix effect.

As can be seen from the chemometric analysis results, both ANN and PCA-ANN methods performed well on the calibration set. PCA-ANN performed better then ANN for the validation set, which is more important for quality of a classification algorithm. In addition, the convergence speed corresponding to ANN was found shorter as seen in training graphs.

Table 3 Recovery results obtained by the application of ANN and PCA-ANN to the validation set consisting of LEV and TRI drugs.

Validation set (μg/ml)		Predicted concentration (μg/ml)				Recovery (%)			
LEV	TRI	ANN		PCA-ANN		ANN		PCA-ANN	
		LEV	TRI	LEV	TRI	LEV	TRI	LEV	TRI
5	9.6	4.83	9.43	4.95	9.34	96.6	98.2	99.0	97.3
10	9.6	9.508	9.69	9.731	9.74	95.1	100.9	97.3	101.5
15	9.6	14.78	9.865	14.47	9.74	98.5	102.8	96.5	101.5
20	9.6	19.41	9.62	19.69	9.57	97.1	100.2	98.5	99.7
25	9.6	24.18	9.44	24.03	9.63	96.7	98.3	96.1	100.3
6	1	6.03	0.98	6.174	0.97	100.5	98.0	102.9	97.0
6	3	6.104	2.85	6.06	2.95	101.7	95.0	101.0	98.3
6	6	6.08	5.83	5.98	5.926	101.3	97.2	99.7	98.8
6	9	6.12	8.85	6.08	8.78	102.0	98.3	101.3	97.6
6	11	6.091	10.86	6.08	10.81	101.5	98.7	101.3	98.3
					Mean	99.1	98.8	99.4	99.0
					SD	2.60	2.13	2.29	1.64
					RSD	2.62	2.15	2.30	1.66

RSD, relative standard deviation; SD, standard deviation.

Table 5 Analysis results obtained by applying ANN and PCA-ANN to tablets.

No.	ANN		PCA-ANN	
	LEV	TRI	LEV	TRI
1	369.2	621.6	361.6	605.9
2	370.2	602.3	377.9	586.3
3	373.8	607.7	374.0	598.1
4	368.5	613.0	369.4	603.4
5	369.5	618.4	366.2	595.5
6	364.6	599.1	367.0	589.5
7	383.1	614.1	367.8	584.2
8	376.8	614.1	371.7	606.9
9	381.2	591.7	370.9	608.8
10	375.7	606.6	368.6	594.4
Mean	373.3	608.9	369.5	597.3
SD	5.93	9.21	4.50	8.82
RSD	1.59	1.51	1.22	1.48
SE	1.87	2.91	1.42	2.79
CL	1.26	1.96	0.96	1.88

CL, confidence level (p=0.05); SE, standard error. Claim label: 600 mg TRI, 375 mg LEV per tablet.

The obtained results open the possibility to apply the proposed technique for the simultaneous determination of LEV and TRI in tablets without any pretreatment technique.

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