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Research Article

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Application of PCA-ANN models to spectrophotometric data for quantitative analysis of a hydrochlorothiazide and amiloride hydrochlorothiazide in pharmaceutical formulations

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ABSTRACT

A multivariate calibration-prediction techniques, principal component artificial neural networks (PCA- ANN) were applied to the spectrometric multicomponent analysis of the drug containing hydrochlorothiazide (HCT) and amiloride hydrochlorothiazide (AMH) without any separation step. The selection of variables was studied. A series of synthetic solution containing different concentrations of HCT and AMH were used to check the prediction ability of the PCA-ANN. The results obtained in this investigation strongly encourage us to apply these techniques for a routine analysis and quality control of the drug.

Keywords: hydrochlorothiazide, amiloride hydrochlorothiazide, spectrometry, multivariate calibration

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INTRODUCTION

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, HCT) is a diuretic in the body which helps to prevent the absorption by the body of excess salt leads to fluid retention. Hydrochlorothiazide thiazide diuretics among the group. In the context of widely used drugs hydrochlorothiazide another name that is used to lower high blood pressure hypertensive drugs is the mostly used. Hydrochlorothiazide in the body sodium, chlorine, and quickly drop your blood pressure by allowing the water to be thrown quickly and allows to achieve a balance. While doing it helps the body by lowering blood pressure to return to normal.

Amiloride hydrochloride (N-amidino-3,5-diamino-6-chloropyrazine-2-carboxiamide hydrochloride, AMH) is chemically known type diuretic or other drugs antikaliuretik that has nothing to do with a pirazi-carbonyl - guadin agent is a mixture. Is the salt of a medium strength base (pKa: 8.7). Amiloride hydrochloride in the use of medical drugs as weak natriuretic, diuretic, and showing the antihypertensive effect is a preventive medicine sodium. For the body amiloride, a diuretic or thiazide showed that urinary excretion of magnesium is decreased when used alone [1].

The binary mixtures of HCT - AMH are widely used in diuretic and antihypertensive pharmaceutical formulations. The literature contains abundant references to methods for determining both compounds individually and in mixtures with other compounds. Thus, there are UV spectroscopy [2-6], high-performance liquid chromatography [7-12], liquid chromatography-tandem mass spectrometry [13] and electrophoretic [14] methods for their quantitation in mixtures with other analytes or in complex matrices.

Mostly preferred compared to other common methods spectrophotometric methods in pharmaceutical analysis is simpler and more convenient. However, the strong overlap of spectral bands exhibited by mixtures of active principles and some excipients in the UV region, usually constraints the application of such methodologies and these cases often require the use of separation methods prior to spectrophotometric quantification of the analytes interest. To overcome such difficulties, refers to advanced chemometric methods in recent years. Looking at the literature, various chemometric methods are used to overcome these difficulties. Among them, multivariate calibration

techniques such as derivative spectroscopy [15,16], absorbance ratio spectroscopy [17], differential pulse polarography with PLS calibration [18], UV spectrophotometry with PLS [19], PCR and PLS [20] etc.

When the literature is examined, these substances for the PCA-ANN the application area of the method were observed to have been, and for this reason in this study, HCT-AMH in pharmaceutical samples for the analysis of the mixture of PCA-ANN it was decided that the implementation of the method.

Chemometrics is one of the most important tools applied frequently to optimization of analytical methods. Its advantage is a reduction in the number of experiments that need be executed resulting in lower reagent consumption and considerably less laboratory work. Furthermore, the chemometric methods allow the development of mathematical models. These models evaluate statistical significance of the main factors and interactions between them. If there is significant interaction between factors, the optimal conditions indicated by the univariate studies will be different from correct results of the multivariate optimization. The larger the interaction effects, the greater the difference that will be found using univariate and multivariate optimization strategies. So the univariate procedure may fail since the effect of one variable can be dependent on the level of other variables involved in the optimization. That is why multivariate optimization schemes involve designs for which the levels of all variables are changed simultaneously.

Principal component analysis-artificial neural network was proposed by Gemperline et all [20] in order to improve training speed and decrease the overall calibration error. This method is performed principal component analysis data obtained from the spectrum before applying and then used in a neural network as a basic component in the original data matrix.

In this study a PCA-ANN calibration method was used for resolution of mixtures HCT-AMH by UV spectrophotometry. Furthermore, the proposed method is applied to determine HCT-AMH in available pharmaceutical preparation, their contents being 50 and 5 mg per tablet respectively.

EXPERIMENTAL SECTION

Apparatus and software

An absorbance measurement was carried out by using a Shimadzu (Model UV-1700) UV-Visible spectrometer (Shimadzu, Kyoto, Japan), equipped with 1 cm matched quartz cells, and was used for spectrometric measurements. Application of PCA algorithm was supported by the software package "Minitab® 16". The software is dedicated to both multivariate analysis and experimental design and is equipped with several multivariate methods. It allows to optimize the calibration models and to develop validation procedures.

The back-propagation neural network algorithm two layers were used in MATLAB (version 8.1, Math Work Inc.) using NN toolbox. All programs were run on a Pentium, personal computer, with windows XP home edition.

Commercial product

A commercial pharmaceutical product (Moduretic® Oral tablet, Fako Pharm. Ind., Istanbul, Turkey) was purchased from local resources and assayed. Its declared content was as follows: Hydrochlorothiazide 50 mg and Amiloride hydrochloride 5 mg, in each capsule.

Chemicals

Hydrochlorothiazide and amiloride hydrochloride were kindly donated by the pharmaceutical industries and were used without further purification. All solvents and reagents were of analytical reagent grade (Sigma and Fluka).

Standard solutions

Stock solution 50 mg/100 mL HCT and AMH in methanol + water mixture were used to set up the calibration set samples. A concentration set of 28 mixture solutions consisting of HCT and AMH in the concentration range of 5.0 - 35.0 and 0.5 – 3.5 µg/mL for HCT and AMH in the same solvent were symmetrically prepared from the prepared stock solutions respectively (Fig.1). Symmetric set of calibration is preferred. The reason for this is to minimize errors in calibration may occur during analysis. To check the proposed methods we used an independent validation set consisting of the synthetic mixture solutions of HCT and AMH in the above working concentration ranges.

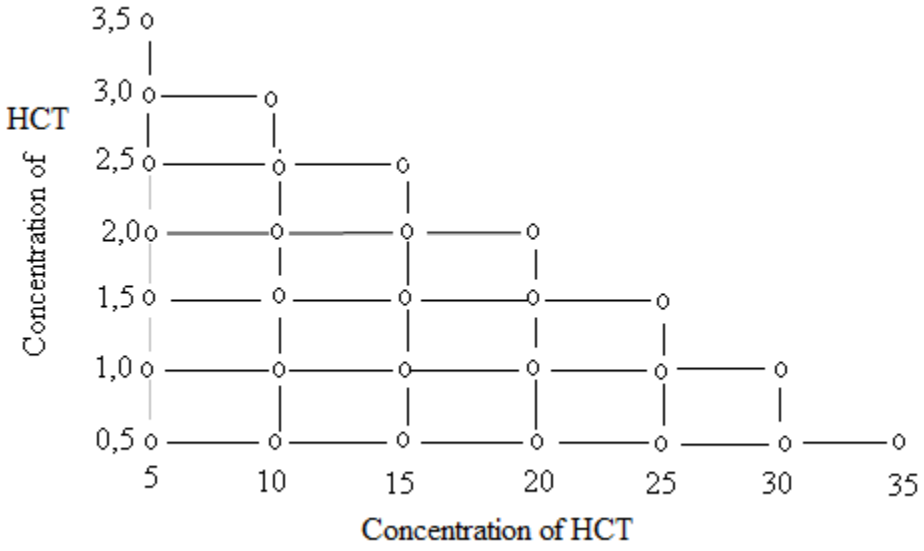

Figure 1. Concentration set design for the preparation of PCA-ANN calibrations

Sample solutions

Pharmaceutical formulations were assayed by weighing the content of 20 tablets and reducing them a fine powder. An amount exactly corresponding to the average tablet weight was suspended in methanol + water mixture and made up to a volume of 100 mL calibrated flasks. The suspension of the flask was mechanically shaken for 30 min and filtrated into a 100 mL volumetric flask through a 0.45 µm membrane filter. The final tablet solution was diluted to the working concentration range. The absorption spectra of these sample solution were recorded for the application of the PCA-ANN calibrations.

PCA-ANN algorithm

The PCA-ANN algorithm which was developed and was used in this research can be summarized as follows.

130 experimental data points based on absorption spectra were selected. PCA was used to assess the internal dimensionality of the problem and to extract a linear combination. In the presented PCA, we started 28x130 correlation matrix. The dominant principal components (PCs) were used as variables for each sample. A feed-forward ANN model with two layers of nodes was constructed. The artificial neuron is the building component of ANN designed to simulate the function of biological neuron. The arriving signals, called inputs, multiplied by the connection weighted (adjusted) are first summed (combined) and then passed through a transfer function to output that neuron. The activation function is the weighed sum of the neuron's inputs and the most commonly used transfer function is sigmoid function.

RESULTS AND DISCUSSION

Experimental design of the calibration matrix

Figure 2 shows the absorption spectra for HCT and AMH and their mixture in methanol + water mixture. A concentration set of 28 mixture solutions consisting of HCT and AMH in the concentration range of 5.0 - 35 and 0.5 – 3.5 µg/mL for HCT and AMH in the same solvent were symmetrically prepared from the prepared stock solutions respectively (Fig.1). Symmetric set of calibration is preferred. The reason for this is to minimize errors in calibration may occur during analysis. To check the proposed methods we used an independent validation set consisting of the synthetic mixture solutions of HCT and AMH in the above working concentration ranges.

Optimization of networks variables

A training set of 28 samples was taken (Fig. 1). The concentration of HCT was varied between 5 - 30 µg mL^{-1}, and that of AMH was varied between 0.5 - 3.5 µg mL^{-1}. The spectral range of 200 - 400 nm, with 200 data points per spectrum, was selected as the spectral zone which gave the maximum spectral information for investigating the components of interest (Fig. 2).

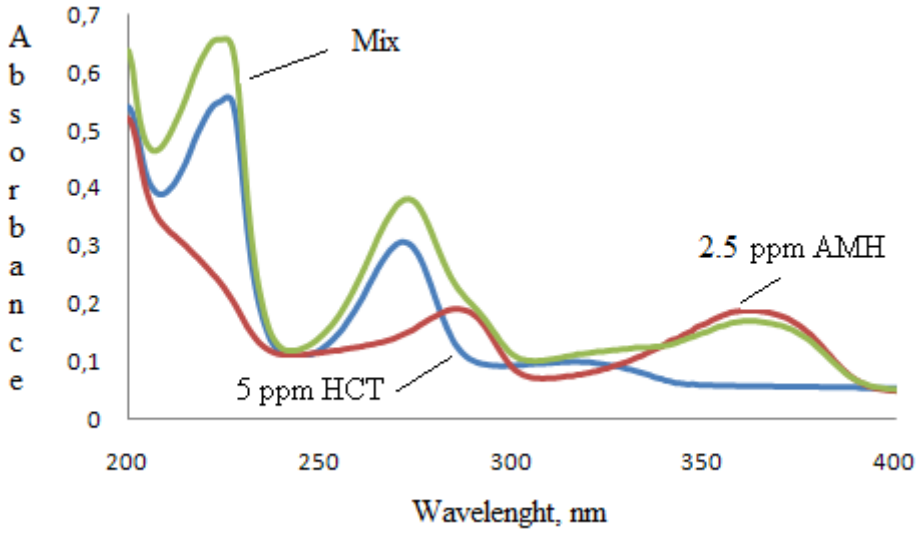

Figure 2. Original absorption spectra of 5 µg/mL HCT, 2.5 µg/mL AMH and their mixture in methanol + water

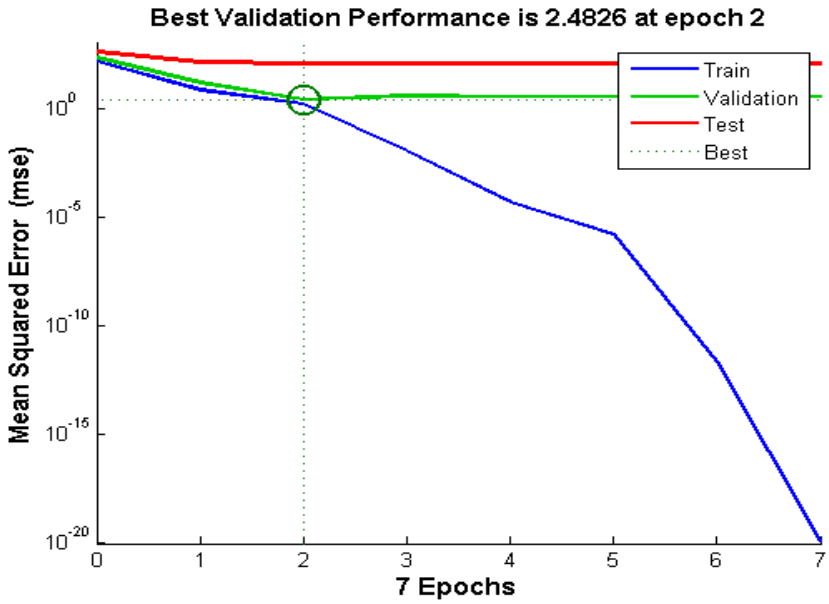

Figure 3. Error performance for training of PCA-ANN applied inputs

Method application

The experiment dimension of the calibration, validation and sample data sets are reduced by PCA method. A data set of size 28x130 (observations x variables) is presented PCA routine and three output are collected. The first output is coefficient 130 x130 each column consisting of coefficients for one principal component. The second output is the matrix of principle component scores which are 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. Since the first six components of the absorbance found to be responsible for 99.97% since the change in profits was continued using these six components of the process. An input dataset of size 6x28 and validation 6x14 is presented to the PCA-ANN, which is a two-layered neural network with sigmoid transfer functions in hidden and output layers. Figure 3 shows the error versus number of iteration for training of neural networks.

Statistical parameters

The application competence of a calibration model can be explained in several ways. We can also examine these results numerically. One of the best ways to do this, by examining the predicted residual error sum of squares (PRESS). To calculate PRESS we compute the errors between the expected and predicted values for all the samples, square them, and sum them together.

$$\text{PRESS} = \sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2 \quad (1)$$

Strikingly speaking, this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. If we want correctly compare PRESS values for data sets that contain differing numbers of samples, we should convert to standard error of prediction (SEP), which is given by following formula.

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2}{n}} \quad (2)$$

Where C_i^{added} the added concentration of drug is, C_i^{found} is the found concentration of drug and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. Often, however, the performance of the calibration model varies depending on the analyte level.

The standard error of calibration denoted by SEC represents another important quantity and is given us by

$$\text{SEC} = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2}{n}} \quad (3)$$

The values of PRESS, SEP and SEC were calculated and the results are presented in Table 1. In the same table, the statistical parameters between actual and predicted concentrations of HCT and AMH substances in mixtures were found by using the experimental data in the calibration and prediction steps.

Table 1. Statistical parameters for PCA-ANN

Step	Parameter	HCT	AMH
Calibration	SEC	0.5141	0.0026
	PRESS	0.2643	0.0900
	Slope	1.0003	1.0007
	Intercept	0.0176	0.0084
	r	0.9988	0.9953
Prediction	SEP	0.5394	0.0510
	Slope	1.0166	0.9815
	Intercept	0.1259	0.0041
	r	0.9966	0.9970

Table 2. Recovery results obtained in synthetic mixtures for PCA-ANN method

Mixtures added (µg/mL)		Recovery (%)	
HCT	AMH	HCT	AMH
5	3.2	100.56	99.58
10	3.2	101.52	101.04
15	3.2	100.92	99.94
20	3.2	100.22	100.02
25	3.2	101.38	100.03
30	3.2	99.94	101.20
35	3.2	99.56	101.58
8	0.5	99.98	99.88
8	1.0	99.82	101.26
8	1.5	99.96	100.20
8	2.0	100.29	99.75
8	2.5	100.38	99.00
8	3.0	99.88	100.60
8	3.5	101.20	100.32
Mean		100.40	100.31
RSD[a]		0.60	0.71

a: Relative Standard Deviation

Method validation

The validation of PCA-ANN method have been done by their performance for obtaining reliable results of analysis. Therefore, 14 synthetic mixtures containing HCT and AMH in different concentrations levels as shown in Table 2 were prepared as an independent validation set. The percentage recoveries and relative standard deviations were indicated in Table 2. In the recovery study, the numerical values were found satisfactory for the validity PCA-ANN. The reliable accuracy and higher precision in application of both compounds. During the process of the analysis, interference and systematical errors were absent.

To test the selectivity of the methods, the standard of HCT and AMH was added to the tablet solution. This procedure was repeated five times for each concentration level. During the process no interference of the excipients formulation was reported. Therefore, PCA- ANN method apply to proposed in this study are appropriate for the determination of HCT and AMH compounds in the tablets. The recovery results presented in Table 3.

Table 3. Recovery results obtained standard addition technique by the proposed PCA-ANN method

		Recovery (%)	
Added to tablet (µg/mL)			
HCT	AMH	HCT	AMH
3.0	6.0	99.36	99.78
6.0	12.0	99.00	99.12
9.0	18.0	99.88	98.32

Analysis of commercial pharmaceutical

Determination results obtained by the application of PCA-ANN calibrations to the tablet solutions containing HCT and AMH in Moduretic® Oral tablet formulations were summarized in Table 4. The analysis obtained from three methods was found satisfactory for the quantitative analysis of commercial tablet. Moreover, proposed procedures gave results in agreement with the labeled drugs content when applied on pharmaceutics.

Table 4. Assay results for the commercial pharmaceutical formulation (mg/tablet)

Sample No	HCT	AMH
1	50.10	5.02
2	50.17	5.01
3	49.78	4.99
4	50.12	5.01
5	49.28	4.94
6	50.22	4.94
7	49.99	4.99
8	50.13	4.97
9	49.98	5.01
10	49.87	4.98
Mean	49.96	4.99
RSD	0.28	0.22

Claim label: 50 mg HCT and 5 mg AMH/tablet

CONCLUSION

The chemometric technique in spectrometric analysis, PCA-ANN was proposed for the simultaneous determination of HCT and AMH in their binary mixtures. These techniques were applied with great success to commercial pharmaceutical tablets. The resolution of highly overlapping drug mixtures was achieved by the use of PCA-ANN technique. A selection of working wavelength having high correlation values with concentration due to interference coming from matrix sample or additional analytes outside the working range. The proposed chemometric techniques can be applied for the routine analysis of drug in the tablet formulation without any a priori chemical separation and without time consuming.

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