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Research Article

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Application of ANN-ICA and PLSR Methodologies for the Simultaneous Spectrophotometric Determination of Analgesic Drugs in Commercial Pharmaceutical

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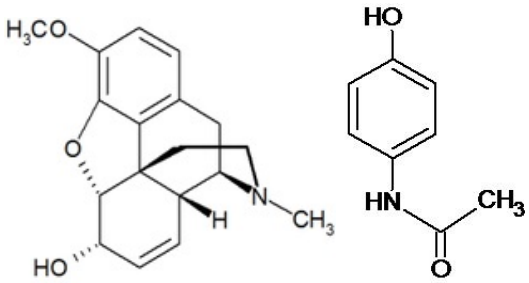
ABSTRACT

In this research, definite, precision and correct spectrophotometric - chemometric techniques were developed for the drugs containing codeine and acetaminophen. The examined commercial pharmaceutical is Geralgine. The used calibration-prediction methods are partial least squares regression (PLSR) and independent component analysis-artificial neural networks (ICA-ANN). A concentration set including binary mixtures of codeine and acetaminophen formed to 25 different concentrations, were synthetic set prepared in 96% ethanol. The precision and accuracy of the developed method were validated by analyzing synthetic mixture containing the examining drug. Eventually, high recoveries and very low relative standard deviations were found.

Keywords: *Independent Component Analysis, Artificial Neural Network, Codeine, Acetaminophen*

INTRODUCTION

Determination of active compound in drugs has become a subject that always pays attention to analytical chemists. Therefore, it is now important to develop fast, reliable, accurate and most inexpensive analytical methods so that the effects of drugs can be detected more accurately. Acetaminophen (ACE), also known as paracetamol, is a well-known analgesic agent [1]. It is often used in adults and especially in children to treat pain and fever, and is the main ingredient of many influenza. Codeine (COD) is an alkaloid in the opium plant [2]. Acetaminophen-codeine tablets are widely used for pain and fever. For this reason, reliable and rapid analytical methods are required according to the importance and necessity of quality control in pharmaceutical preparations. The chemical structures of the materials used in this study are shown in Figure 1.



Codeine Acetaminophen

Fig 1. Structures of the compounds

Consequently, the care in the development of simultaneous analysis methods, which do not improve preliminary separation step, suited for routine pharmaceutical analysis is confirmed. The most frequently used techniques for the determination of codeine and acetaminophen are titrimetry [3], voltammetry [4], spectrophotometry [5,6], chromatography [7,8], GC [9] and TGA [10] methods.

Commonly preferred compared to other widespread methods, spectrophotometric method in pharmaceutical analysis is easier and more useful. Simultaneous determination of pharmaceuticals is difficult to perform by the classical spectrophotometric methods to overlapping spectra. In order to overcome these difficulties, scientists working on these issues have applied advanced chemometric methods in recent years. Some of those include multivariate calibration techniques such as derivative spectroscopy [11,12], differential pulse polarography with PLS calibration [13], absorbance ratio spectroscopy [14], UV spectrophotometry with PLS [15], etc.

Most of these methods are used in the quantitative determination of acetaminophen-codeine mixtures. We did not find out any ICA-ANN chemometric method to determine binary mixture in our literature survey. In this research, two chemometric methods were used to determine COE and ACE at the same time in tablets that did not undergo any separation procedure in a commercial tablet formulation. The chemometric calibration was performed using different concentration mixtures according to the composition of the tablets used in the study. In the validation of the methods, the average recovery (%) and relative standard deviation for each method were calculated. The amounts of codeine and acetaminophen in the tablets worked were calculated by these chemometric methods and the results were compared statistically with each other.

MATERIALS AND METHODS

Apparatus and software

A Shimadzu UV-1700 UV-Visible spectrometer, connected to PS with UV Probe Software was used for all the measurements and data processings. A pair of 1.0 cm quartz cells was used for absorbance measurements. PLS and PCA-ANN methods were implemented with the "STATISTICA" software package.

Drug raw material

A commercial pharmaceutical product (Geralgine-k tablet, Münir Şahin Pharmaceutical Industry, Istanbul, Turkey) was purchased from local resources and tested. Its proclaimed content was as follows: Acetaminophen 500 mg and codeine phosphate 10 mg in per tablet.

Chemicals

All materials used were at the analytical grade.

Standard solutions

Stock solution 50 mg/100 mL codeine and acetaminophen in 96% Ethanol were used to set up the calibration set samples. A concentration set of 25 mixture solutions consisting of codeine and acetaminophen in the concentration range of 5.0 - 25.0 and 8.0 – 40.0 µg/mL for codeine and acetaminophen in the % 96 ethanol solvent were symmetrically prepared from the prepared stock solutions respectively (Figure 2). The reason for symmetric set of calibration is to minimize errors in calibration may occur during analysis. To test the application of the chemometric methods, we used an independent verification set consisting of codeine and acetaminophen synthetic blend solutions at the above working concentration intervals.

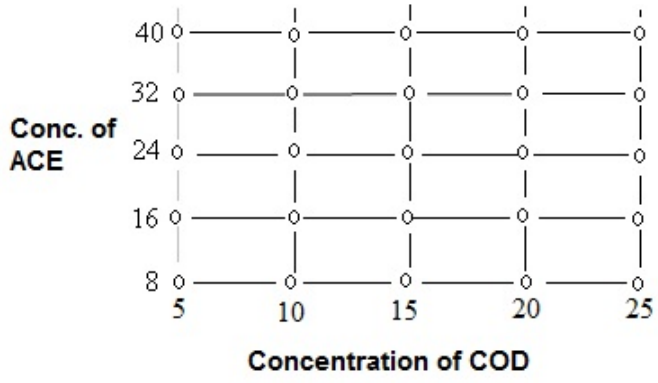


Fig 2. Concentration set used in the running methods

Sample solutions

For this purpose, the contents of 20 tablets were precisely weighed and pulverized. The amount for a tablet was dissolved in 96% ethanol and transferred to 100 mL balloon batches. The prepared solution was shaken for 30 minutes and filtered through 0.45 μm of membrane filter. The solution thus prepared was diluted to the working concentration range. Absorption spectra of the prepared sample solutions were taken and recorded for calibrations of applied chemometric methods.

Chemometric methods

In this study, only the outline of the applied chemometric methods will be explained.

Independent Component Analysis (ICA)

The major aim of ICA is to decompose the factors or components from multivariate signal into independent non-Gaussian signals with minimum information loss. The main difference of ICA from other techniques is that the components are latent variables which cannot be directly acquired. Due to indirect acquisition of data, ICA is also called blind source separation. The blind term represents the source signals are linear mixture of source signals. The main difference between PCA and ICA, is that PCA decorrelates input data based on correlation (covariance) matrix [16]. Besides ICA reduces the higher order statistical relations and dependencies. The error free ICA model could be express by:

$$X= WS$$

where X represents acquired data matrix, S is independent components (ICs) and W is coefficient matrix. There are lots of algorithms to obtain IC that in this study, the fast fixed-point algorithm (FastICA) was used.

Artificial Neural Network (ANN)

ANN, the information processing system developed from neurons, belongs to the family of statistical learning models. ANN takes after computing system like brain neurons interconnected to each other of which switches information between them. These neurons are divided into three-part input layer, hidden layer and output layer and each layer operates in parallel. Implementation of ANN requires a kind of successive phases these are pre-processing of data, network building, training, validation and post processing of data. ANN is suitable for fitting and predicting non-linear functions and recognizing patterns [17,18].

Partial Least Squares (PLS)

PLS is procedure that integrates some features of multiple regression to principle component analysis [19]. When the number of independent variables is very large to predict set of dependent variables, it is particularly useful. PLS is a kind of linear regression but it projects both independent and dependent variables into a new space. PLS is placed on latent variables which are small number of orthogonal factors. Moreover, PLS chooses these latent variables to make available maximum correlation with the set of dependent variables. The general basic multivariate PLS model could be written as follows:

$$X =TP^T+E$$

$$Y = UQ^T+F$$

where $X_{N \times M}$ is the observed data matrix, $Y_{N \times A}$ is the response vector matrix, $T_{N \times L}$ and $U_{N \times L}$ are transformation matrices $P_{M \times L}$, $Q_{M \times L}$ is orthogonal vector matrices, $E_{N \times M}$ is a residual matrix, N is the number of observations, and M is the number of spectral variables.

RESULTS & DISCUSSION

Method development

The overlap spectra of codeine and acetaminophen at the range of 220-280 nm are shown in Fig. 3. Spectrum demonstrates that the classical approach will not allow compounds to be assigned simultaneously. For this reason, we are focusing on applying chemometric methods to binary mixtures of codeine and acetaminophen.

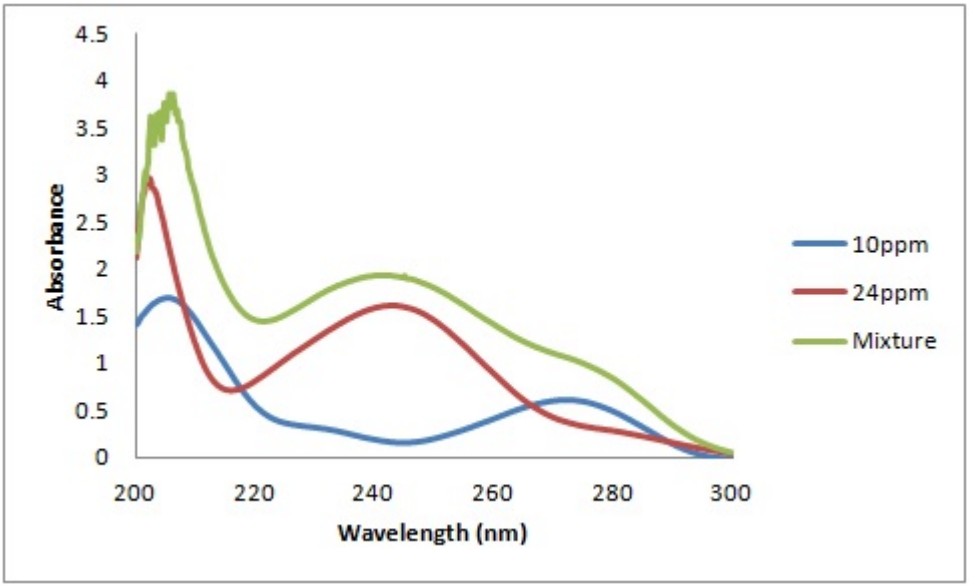


Fig 3. 24 ppm codeine, 10 ppm acetaminophen and their mixtures in 96% ethanol

ICA - ANN and PLS methods

The first thing to do is to identify the quantities of substances in binary mixtures at the same time it is to create the calibration matrix. For this purpose, 25 mixtures were prepared according to the concentration set shown in Fig.2. The set found in this way is shown in Table 1.

Table 1. Calibration based on symmetrical mixture design

Concentration (µg/mL)					
No	COD	ACE	No	COD	ACE
1	5	8	14	15	32
2	5	16	15	15	40
3	5	24	16	20	8
4	5	32	17	20	16
5	5	40	18	20	24
6	10	8	19	20	32
7	10	16	20	20	40
8	10	24	21	25	8
9	10	32	22	25	16
10	10	40	23	25	24
11	15	8	24	25	32
12	15	16	25	25	40
13	15	24			

The spectra of the prepared compounds were measured in the spectral region of 220-280 nm and at intervals of $\Delta\lambda$=0.1 nm. Concentration set and absorption data are designed as y-block (25x2) x-block (25x600) for calibrating the applied chemometric methods. Cross-validation was applied when calibrating, and various factors were tested. As a result of the calculations, the most suitable factor was found as 3.

Thus, the fast ICA was used for data preprocessing on the above data sets, and at the beginning, the input data was quantized by using the fixed point toolbox in STATISTICA. Two new matrices, M^{ICA} (n x I) and M^{ICA}unknown (n x I), with matrices (calibration) and unknown (verification), and then n, calibrated or estimated the set of samples (i express constant numbers). Established calibration model was then used in PLS method.

During the ANN application, a neural network search was performed for the calibration set. Here the first step is to prepare inputs for the neural network. As can be seen from Table 1, this input comes from 25 spectra consisting of 20 points and 25 different concentrations in the calibration set. The main purpose here is to find the most suitable neural network structure in which we achieve the highest achievement results. As a result of these operations, two hidden

layers and two output layers were selected from 5 and 4 neurons respectively. For each layer, the logsig function is selected from the program.

The adequacy of a given model can be explained in several ways. So the results obtained can be explained numerically. From the literature view it can be seen that one of the most important of these paths is the predicted residual error sum of squares (PRESS). We need to use formula 1 to calculate the PRESS value.

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

C_i^{added} = Actual concentration, the added concentration of drug

C_i^{found} = Predicted concentration, the calculated concentration of drug

Clearly, PRESS values are not the only way to normalize results, since not all data sets contain equal numbers of samples. If the PRESS values are compared for sets containing different samples, these values should be converted to the standard estimated error (SEP) value given in formula 2.

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

Where n is the total number of the synthetic mixtures. As a result, SEP is a good parameter for how well the calibration model performs. However, it should also be noted that the model generated changes performance depending on analyte concentration.

Another important parameter, the standard error of calibration (SEP) represents an important quantity and is found in formula 3.

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

All these values are calculated and shown in Table 2 for the study done. Again in the same table, codeine and acetaminophen in the running mixtures are shown in the parameters between actual and estimated concentration values.

Table 2. Statistical parameters for ICA-ANN and PLS

	ICA - ANN		PLS	
Step	Parameter	COD	ACE	COD	ACE
Calibration	SEC	-	-	0.1628	0.1218
PRESS	0.2135	0.2134	0.3974	0.2225	
Slope	1.0057	0.9985	1.0064	1.0008	
Intercept	0.0542	0.092	0.0627	0.0023	
r	0.9996	0.9999	0.9993	0.9998	
Prediction	SEP	0.2472	0.3384	0.2306	0.1725
Slope	0.9182	0.9194	0.1949	0.5393	
Intercept	0.1502	0.0852	0.0021	0.0153	
r	0.9998	0.9999	0.9996	0.9989	

Method validation

The accuracy of the applied chemometric methods was determined by the performance of reliable results obtained from the analyzes performed. For this reason, 15 synthetic mixtures containing different concentrations of codeine and acetaminophen were prepared. The recovery percentages and relative standard deviations from this set are shown in Table 3. As can be seen from Table 3, the numerical values obtained for the applied chemometric methods are very

suitable. Both compounds yielded high accuracy and precision results. During the process of the analysis, interference and systematic errors were absent.

Table 3. Recovery values for the applied chemometric methods

Mixtures added (µg/mL)		Recovery (%)			
		ICA-ANN		PLS	
COD	ACE	COD	ACE	COD	ACE
5	8	101.12	99.58	101.60	99.25
10	16	102.02	100.14	99.70	101.12
15	24	100.04	100.48	98.40	100.41
20	32	100.08	100.24	100.15	100.65
25	40	100.24	101.10	101.08	100.22
15	8	100.02	100.12	101.80	101.75
15	16	100.54	100.18	100.06	99.06
15	24	99.98	100.20	100.26	99.33
15	32	100.00	99.98	99.26	99.62
15	40	100.20	100.28	99.66	100.05
5	24	100.28	101.00	97.00	99.83
10	24	100.42	100.24	100.70	100.37
15	24	100.00	100.32	101.20	99.41
20	24	100.28	100.88	101.45	100.45
25	24	100.00	99.86	99.36	99.91
Mean		100.22	100.36	100.11	100.99
RSD[a]		0.29	0.41	1.28	0.72

RSD[a]: Relative Standard Deviation

The selectivity of the methods applied by adding codeine and acetaminophen standards to the tablet solutions was also tested. This process is repeated five times for each level. No admixture of excipients formulation was reported during the procedure. For all of these reasons, the chemometric methods suggested in the study are considered to be suitable for the identification of codeine and acetaminophen compounds in tablets. The recovery results are presented in Table 4.

Table 4. Recovery values of chemometric methods applied with standard additive technique

Recovery (%)					
Added to tablet (µg/mL)		ICA-ANN		PLS	
COD	ACE	COD	ACE	COD	ACE
4	6	98.88	99.32	96.04	96.66
8	12	99.02	98.66	97.16	97.18
12	18	98.56	99.24	98.66	98.88
Mean		98.82	98.07	97.28	97.57
RSD		0.23	0.36	1.31	1.16

According to the results, ICA-ANN method gives more precise and accurate results based on the PLS method.

Analysis of commercial pharmaceutical

Findings from the chemometric methods were used to calculate the amounts of codeine and acetaminophen in the Geralgine tablet which are shown in Table 5. As can be seen from Table 5, the results obtained from the applied chemometric methods are quite satisfactory. In addition, the proposed chemometric methods can accurately determine the drug content when applied on the drug.

Table 5. Analysis results of commercial drug sample (mg/tablet)

	ICA-ANN		PLS	
No	COD	ACE	COD	ACE
1	9.98	501.02	9.98	502.85
2	9.99	499.58	9.94	498.75
3	10.00	500.04	9.98	501.90
4	10.00	499.86	9.88	502.52
5	10.02	501.02	10.02	499.48
Mean	9.99	500.30	9.96	501.10
RSD	0.01	0.67	0.05	1.86

CONCLUSION

The partial least squares calibration and independent component analysis-artificial neural network methods all successfully applied at the same time were able to identify drugs in synthetic solutions and pharmaceutical formulation. High correlation coefficients and low prediction errors for all data obtained as a result of the studies emphasize the high linear relationship between estimated and actual concentrations. The results obtained with this binary mixture and some ratios of component concentrations show excellent predictive ability with these methods.

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