Research Article

Drug Testing and Analysis

Received: 26 August 2010 Revised: 28 September 2010 Accepted: 28 September 2010 Published online in Wiley Online Library: 29 December 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.216

Simultaneous spectrophotometric determination of diclofenac potassium and methocarbamol in binary mixture using chemometric techniques and artificial neural networks

Ehab F. Elkady*

In this study, the simultaneous determination of diclofenac potassium (DP) and methocarbamol (MT) by chemometric approaches and artificial neural networks using UV spectrophotometry has been reported as a simple alternative to using separate models for each component. Three chemometric techniques – classical least-squares (CLS), principal component regression (PCR), and partial least-squares (PLS) – along with radial basis function-artificial neural network (RBF-ANN) were prepared by using the synthetic mixtures containing the two drugs in methanol. A set of synthetic mixtures of DP and MT was evaluated and the results obtained by the application of these methods were discussed and compared. In CLS, PCR, and PLS, the absorbance data matrix corresponding to the concentration data matrix was obtained by the measurements of absorbances in the range 260–310 nm in the intervals with $\Delta\lambda = 0.2$ nm in their zero-order spectra. Then, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for the prediction of the unknown concentrations of DP and MT in their mixtures. In RBF-ANN, the input layer consisting of 251 neurons, 9 neurons in the hidden layer, and 2 output neurons were found appropriate for the simultaneous determination of DP and MT. The accuracy and the precision of the four methods have been determined and they have been validated by analyzing synthetic mixtures containing the two drugs. The proposed methods were successfully applied to a pharmaceutical formulation containing the examined drugs.

Keywords: diclofenac potassium; methocarbamol; chemometrics; artificial neural networks; pharmaceutical preparation.

Introduction

Diclofenac, [2-(2,6-dichlorophenylamino)phenyl)]acetic acid (Figure 1A) is a non-steroidal drug with anti-inflammatory, antipyretic and analgesic properties. It is usually found as a sodium or potassium salt.[1] Methocarbamol, 2-hydroxy-3-(2-methoxyphenoxy) propyl carbamate (Figure 1B) is a central muscle relaxant used to treat skeletal muscle spasms. It is the carbamate of guaifenesin. The combination of diclofenac potassium (DP) and methocarbamol (MT) is frequently prescribed to alleviate pain associated with muscle spasm.

Several types of analytical procedures have been proposed for the analysis of diclofenac in pharmaceutical and biological samples. These procedures include potentiometry,[2–4] fluorimetry,[5–7] HPLC either with UV[8–10] or mass spectrometric detection,[11] gravimetry,[12] UV spectrophotometry and partial least- squares (PLS)[13–16] and densitometry.[17]

Methods used for the assay of MT in pharmaceutical and biological samples include GC[18] and HPLC.[19–22] In the United States Pharmacopoeia (USP) 2007, the assay of MT in dosage forms also relies on HPLC determination.[23] Some methods were adopted for the simultaneous determination of MT and paracetamol in combined dosage forms based on spectrophotometry,[24,25] gas liquid chromatography[26,27] and HPLC.[28] Simultaneous determination of DP and MT in ternary mixture with guaifenesin by a RP-LC method has been reported by the same author of the present work.[29]

The UV absorption spectra of DP and MT in methanol at their nominal concentrations ratio in tablets show strong overlap (Figure 2). Thus, direct simultaneous spectrophotometric determination of the two drugs in the mixture is not feasible. To the best of the author's knowledge, no previous article concerning simultaneous spectrophotometric determination of the two drugs has been published. Thus, the main task of this work was to develop and validate simple, accurate, and selective methods based on spectrophotometric measurements and capable of determining the two drugs simultaneously with the help of different chemometric techniques and artificial neural networks.

In recent years, multivariate calibrations, such as classical least-squares (CLS), principal component regression (PCR), and partial least-squares (PLS) started to be applied to the analysis of the analytical data obtained in all the instrumentations. The same methods have been applied to the simultaneous spectrophotometric deter-

* *Correspondence to: Ehab F. Elkady, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St, Cairo 11562, Egypt. E-mail: ehabelkady75@gmail.com*

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St, Cairo 11562, Egypt

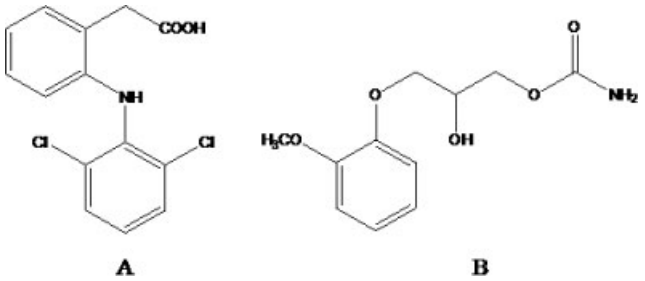

Figure 1. Chemical structures of diclofenac (A) and methocarbamol (B).

mination of drugs in pharmaceutical formulations containing two or more compounds with overlapping spectra.[30,31] The chemometric calibration methods mentioned above have been used extensively in quantitative spectral analysis to obtain selective information from unselective data. The main advantages of these techniques are the higher speed of processing data concerning the values of concentration and absorbance of compounds with strongly overlapping spectra. Besides, the errors of calibration models are minimized by measuring the absorbance values at many points in the wavelength range of the zero-order and derivative spectra. Control analyses on pharmaceutical preparations using multivariate calibration methods have been proven to be a valid alternative to HPLC.[32] Spectrophotometric,[33] spectrofluoremetric,[34] differential pulse polarographic[35] and voltammetric[36] signals have been analyzed by these approaches. This work presents CLS, PCR, and PLS as useful techniques to resolve interference between DP and MT in their zero-order spectra. Although the aforementioned chemometric methods assume a linear relationship between the measured samples' parameters and the intensity of their absorption bands, small deviation from linearity is acceptable and can be readily suppressed by including additional modelling factors. However, in the presence of substantial non-linearity, chemometric approaches such as PLS tend to give large prediction errors and calls for more suitable models. Intrinsically non-linear calibration methods, such as artificial neural networks (ANN), are applicable in such cases.[37]

ANN are computer programs designed to simulate some functions of the human brain using different algorithms, which can learn from experience. ANN analyses are currently recognized as an effective and advantageous way to handle complex data and solve problems of non-linear calibration, pattern recognition, classification, prediction, and other related fields in analytical chemistry. Both linear and non-linear mapping functions can be modelled by suitably configuring the network.[38] Recently, the radial basis function-artificial neural network (RBF-ANN) model has been noted for its simple network structure that avoids lengthy calculations compared to the back propagation-artificial neural network (BP-ANN), and has good robustness, as well as improved sensitivity to noisy data[39]

Experimental

Instrumentation

A double beam ultraviolet/visible spectrophotometer (Shimadzu UV-1650 PC, Tokyo, Japan) connected to an IBM compatible computer and supported with UVPC soft ware version 2.21 (Shimadzu) was used. The chemometric methods, RBF-ANN and data analysis was performed using Matlab™, Version 7 and PLS-Toolbox 2.0.

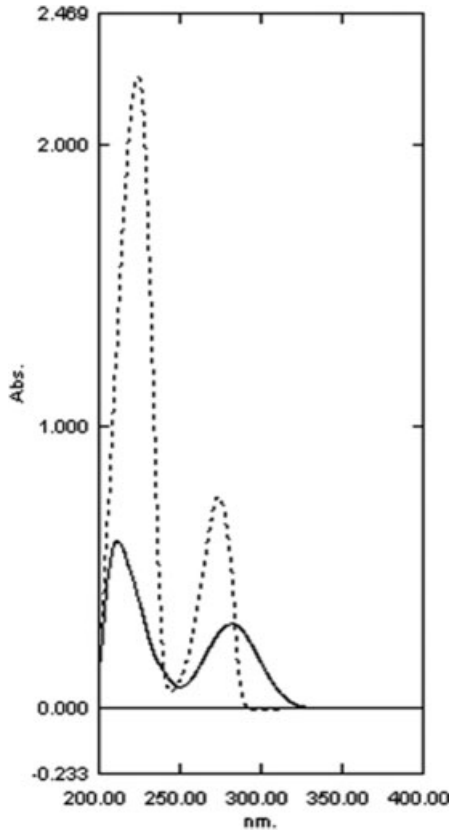

Figure 2. Zero order absorption spectra of DP (8 µg ml-1) (———) and of MT (80 µg ml-1) (- - - -) in methanol.

Reagents and reference samples

Pharmaceutical grade of DP and MT and Dimra® tablets (batch number B0940209) nominally containing DP (50 mg) and MT (500 mg), were kindly supplied by October Pharma Company (6 October City, Egypt) and certified to contain 99.68 and 99.77% of DP and MT, respectively. Methanol used was of analytical grade.

Standard solutions

DP and MT standard solutions

Standard solutions of 0.04 mg ml^{-1} and 0.4 mg ml^{-1} of DP and MT, respectively were prepared in methanol in two separate 100 ml volumetric flasks.

Sample preparation

Twenty tablets were accurately weighed and powdered in a mortar. A quantity of the powdered tablets equivalent to (4 mg) DP and (40 mg) MT was extracted with methanol (3 × 20 ml) and filtered through a Whatman's filter paper into a 100-ml volumetric flask. The residue and filter paper were washed with methanol (3 × 10 ml) and the solution was completed to volume with methanol.

Procedure

Construction of the training set

Nine binary mixtures of DP and MT were prepared by placing different volumes of their standard solutions into a series of 10-ml

Table 1. The concentrations of different mixtures of DP and MT used in the training set

Sample No.	DP Conc. (μg ml^{-1})	MT Conc. (μg ml^{-1})
1	5.6	56
2	5.6	64
3	5.6	72
4	6.4	56
5	6.4	64
6	6.4	72
7	7.2	56
8	7.2	64
9	7.2	72

volumetric flasks (Table 1). The absorbances of these mixtures were measured between 260 and 310 nm at 0.2 nm intervals against methanol as a blank.

Construction of CLS, PCR, and PLS models

Calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for prediction of the unknown concentrations of DP and MT in their binary mixtures. CLS model was constructed with non-zero intercept. To build this model, the computer was fed with the absorbance and concentration matrices for the training set. The calculations to obtain the *K* matrix were carried out. For the PCR and PLS models, the training set absorbance and concentration matrices together with PLS-toolbox 2.0 software were used for calculations.

Selection of the optimum number of factors to build the PCR and PLS models

To select the optimum number of factors in the PLS and PCR algorithms, a cross-validation method leaving out one sample at a time[40] was employed using calibration set of 9 calibration spectra. PLS and PCR calibration on 8 calibration spectra were performed and, using this calibration, the concentration of the sample left out during the calibration process was predicted. This process was repeated 9 times until each training sample had been left out once. The predicted concentrations of the components in each sample were compared with the actual concentrations in this calibration samples and Root-Mean-Square Error of Cross-Validation (RMSECV) was calculated for each method. It indicates both of the precision and accuracy of predictions. It was recalculated upon addition of each new factor to the PLS and PCR models.

$$\text{RMSECV} = \sqrt{\frac{\text{PRESS}}{\text{n}}} \quad (1)$$

where PRESS is the predicted residual error sum of squares and n is the number of calibration samples.[41]

$$\text{PRESS} = \sum (Y_{\text{pred}} - Y_{\text{true}})^2. \quad (2)$$

where Y_{pred} and Y_{true} are predicted and true concentrations in μg ml^{-1}, respectively.

Visual inspection was used for selecting the optimum number of factors. Three factors were found suitable for both PCR and PLS methods as shown in Figures 3 and 4.

Constructing radial basis function-artificial neural network (RBF-ANN)

The same training set (Table 1) used in CLS, PCR, and PLS was used for training the RBF-ANN model created in Matlab™, Version 7. The input layer consisting of 251 neurons, 9 neurons in the hidden layer, and 2 output neurons were found appropriate for the simultaneous determination of DP and MT.

Construction of the validation set

To evaluate the prediction performance of the proposed chemometric models, a set of 12 synthetic validation mixtures was prepared (Table 2), and submitted for prediction by each of the calibration models.

Analysis of DP and MT in Dimra® tablets

The four methods were applied to the determination of DP and MT in commercial tablets. Five replicates determinations were made.

Results and Discussion

CLS, PCR, and PLS

The wavelength range 260–310 nm in the intervals with $\Delta\lambda = 0.2$ nm was chosen as it provided the greatest amount of information about the mixture components. CLS model was constructed with non-zero intercept. The non-zero intercept allows an additional degree of freedom when *k* matrix is calculated. This provides an additional opportunity to adjust the effects of the extraneous substances.[42] The CLS method requires all components in the calibration samples to be known.

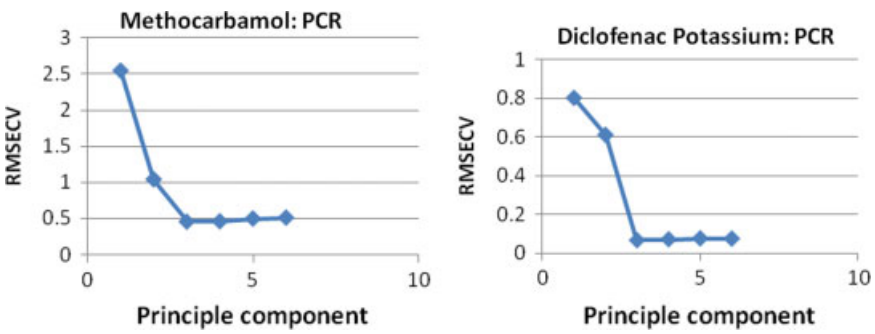

Figure 3. RMSECV plot of a calibration set prediction using cross validation (principal component regression model).

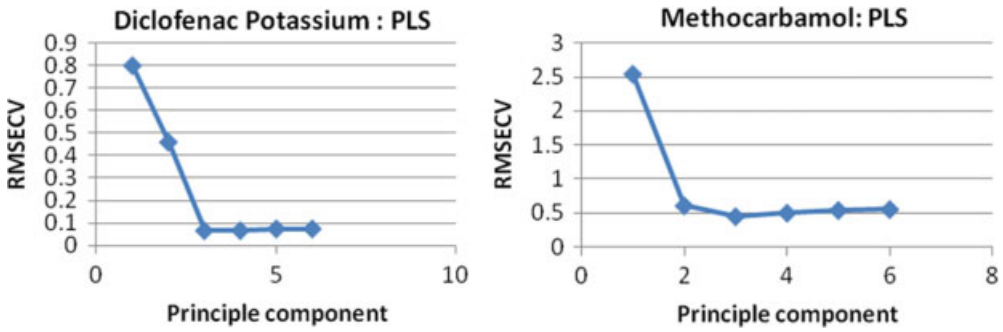

Figure 4. RMSECV plot of a calibration set prediction using cross validation (partial least squares model).

Table 2. Results obtained for the determination of DP and MT in synthetic mixtures by using CLS, PCR, PLS, and RBF-ANN techniques

Sample no.	Concentration (μg ml^{-1})		Found % DP				Found % MT			
	DP	MT	CLS	PCR	PLS	RBF-ANN	CLS	PCR	PLS	RBF-ANN
1	5.6	60	100.33	100.11	100.11	99.49	100.10	100.07	100.07	98.38
2	6.0	56	101.93	101.92	101.92	102.30	102.15	102.16	102.16	101.38
3	6.0	60	101.26	101.08	101.08	100.74	101.43	101.41	101.41	100.33
4	6.0	64	99.52	99.39	99.39	98.72	99.78	99.76	99.76	99.27
5	6.4	60	100.77	100.64	100.64	100.91	100.06	100.04	100.04	99.15
6	6.4	64	101.97	101.83	101.83	102.29	101.04	101.02	101.02	100.65
7	6.4	68	101.12	101.17	101.17	101.82	101.76	101.75	101.75	101.91
8	6.8	60	100.46	100.41	100.41	100.88	99.43	99.43	99.43	98.62
9	6.8	64	100.62	100.56	100.56	100.88	101.29	101.29	101.29	100.82
10	6.8	68	99.66	99.71	99.71	100.41	100.60	100.61	100.61	100.64
11	6.8	72	97.76	97.76	97.76	98.29	98.28	98.27	98.27	98.39
12	7.2	60	99.74	99.71	99.71	99.83	99.79	99.80	99.80	98.97
Mean			100.43	100.36	100.36	100.55	100.48	100.47	100.47	99.88
± S.D.			1.17	1.15	1.15	1.29	1.11	1.11	1.11	1.22

Visual inspection could be used for determining the optimum number of factors.[43] For the PCR and PLS techniques, selection of the optimum number of factors was a very important step before constructing the models. If the number of factors retained was more than the number required, more noise would be added to the data. On the other hand, if the number retained was less than the number required, meaningful data that could be necessary for the calibration might be ignored.

RBF-ANN

Typically in an RBF-ANN (Figure 5), there are three layers: input, hidden, and output. The input layer serves only to distribute inputs to the hidden layer. Each neuron of the hidden layer uses a Gaussian transfer or basis function, instead of the sigmoid one associated with the back propagation model, to account for the non-linearity. A radial basis function is a function whose value depends only on the distance from a centre point c:

$$f(x) = f(|x - c|) \quad (3)$$

The hidden layer in the RBF network consists of an array of nodes that contains a parameter called the 'radial centre' vector. The hidden layer performs a fixed non-linear transformation with non-adjustable parameters. The approximation of the input-output relationship is derived by obtaining a suitable number of nodes in the hidden layer and by positioning them in the input space where the data is most clustered. For each iteration, the position of the radial centres, their width (variation) and their linear weights are modified for each output node. The learning is completed when each radial centre is located as closely as possible to each discrete cluster centre formed from the input space.[39]

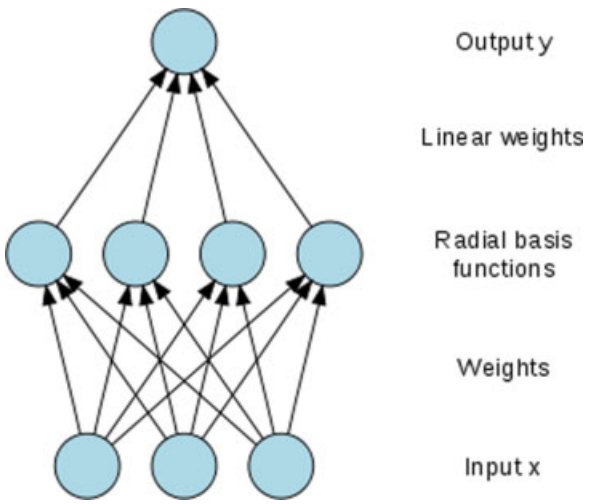

Figure 5. Architecture of radial basis function neural network.

Table 3. Results of the analysis of DP and MT in commercial tablets using CLS, PCR, PLS, and RBF-ANN techniques

Concentration (μg ml^{-1})		Found % DP				Found % MT			
DP	MT	CLS	PCR	PLS	RBF-ANN	CLS	PCR	PLS	RBF-ANN
5.6	56	97.56	97.66	97.48	98.11	98.09	98.09	98.09	101.24
6.0	60	98.17	98.27	98.09	98.29	99.61	99.61	99.61	97.16
6.4	64	98.97	99.07	98.87	99.17	100.99	100.99	100.99	97.83
6.8	68	99.78	99.88	99.66	99.26	100.01	100.01	100.01	98.11
7.2	72	99.40	99.49	99.26	99.55	100.43	100.43	100.43	97.79
Mean		98.78	98.87	98.67	98.88	99.83	99.83	99.83	98.43
± S.D.		0.91	0.90	0.88	0.64	1.10	1.10	1.10	1.61
F-value		0.067				1.579			
p-value		0.977 > 0.05*				0.233 > 0.05*			

* No significant difference between groups by using one-way ANOVA at $p<0.05$.

Twelve binary mixtures for validation were used to evaluate the ability of the four methods to predict the concentrations of DP and MT in their laboratory-prepared synthetic mixtures containing different ratios of both. The ratio of the two drugs in tablets was taken into consideration during the construction of both the training and validation sets. Their numerical values were found to be satisfactory for the validity of all methods, Table 2.

Then, the four proposed methods (CLS, PCR, PLS, and RBF-ANN) were applied to the determination of DP and MT in commercial tablets. Satisfactory results were obtained for both drugs and were in a good agreement with the label claims (Table 3) suggesting no interference from any of the tablets' inactive ingredients. As can be seen in Figure 6, absorption spectrum of the tablet extract is almost identical to that of the binary mixture of DP and MT in the wavelength range of measurements. That is why there was no need to add any of the tablets' excipients to the calibration set to deal with potential interferences in the sample set.

A statistical analysis of the results obtained by the four proposed methods for the determination of DP and MT in tablets was carried out by 'SPSS statistical package, Version 11'. The significant difference between groups were tested by one way ANOVA (F-test) at $p=0.05$ as shown in Table 3. The test ascertained that there was no significant difference between the methods. CLS, which is the simplest model that doesn't need sophisticated software, gave good results that are already non-significant from the others, so it may be the easiest approach to be applied for the determination of DP and MT in binary mixtures and in tablets. Meanwhile, other techniques could be applied for better determinations and comparison. However, it may be more advantageous to apply the RBF-ANN technique due to its ability to overcome any non-linearity that might arise in any other matrix of the two drugs.

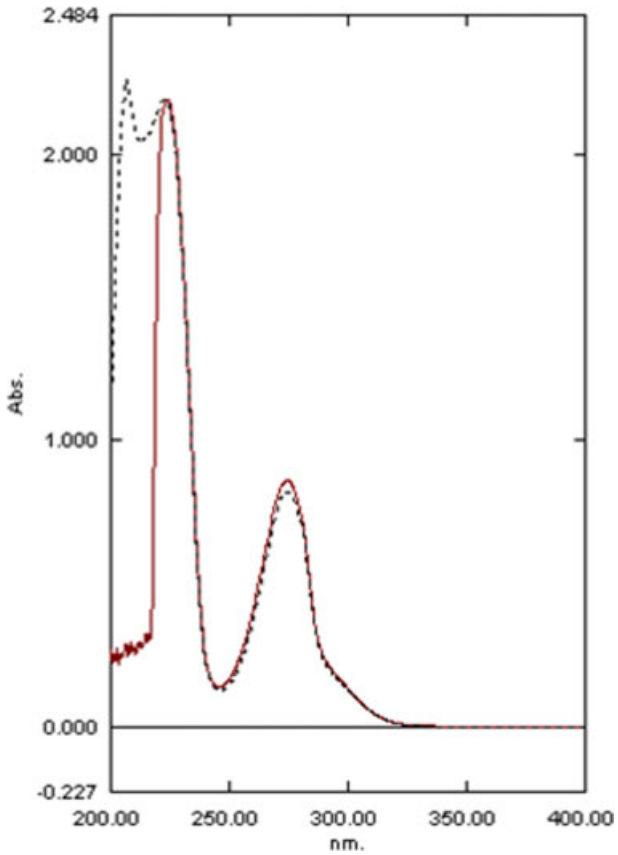

Figure 6. Zero order absorption spectra of a binary mixture of DP (6.4 μg ml-1) and MT (64 μg ml-1) (———) and a Dimra® tablet extract namely containing the same amounts of DP and MT (- - - -) in methanol.

Conclusion

The proposed methods (CLS, PCR, PLS, and RBF-ANN) can be used for simultaneous determination of DP and MT in binary mixtures and pharmaceutical dosage form containing them without interference with each other and without the need for previous physical separation of the two drugs. Multivariate calibration models were built from the spectral and concentration data matrices. Verification of the calibrations, carried out with the aid of a synthetic set of mixtures of the two compounds, produced satisfactory results. Hence, the proposed methods can be used for quality control of the cited drugs in ordinary laboratories.

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