—Regular Articles—

Determination of Paracetamol and Orphenadrine Citrate in Pharmaceutical Tablets by Modeling of Spectrophotometric Data Using Partial Least-Squares and Artificial Neural Networks

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The estimation of paracetamol and orphenadrine citrate in a multicomponent pharmaceutical dosage form by spectrophotometric method has been reported. Because of highly interference in the spectra and the presence of non-linearity caused by the analyte concentrations which deviate from Beer and Lambert's law, partial least-squares (PLS) and artificial neural networks (ANN) techniques were used for the calibration. A validation set of spiked samples was employed for testing the accuracy and precision of the methods. Reasonably good recoveries were obtained with PLS for paracetamol and the use of an ANN allowed the estimation of orphenadrine citrate, a minor component which could not be adequately modeled by PLS. Three production batches of a commercial sample were analysed, and there was statistically no significant difference ($P<0.05$) between the results with the proposed method and those obtain with the official comparative method.

Key words——paracetamol; orphenadrine citrate; partial least-squares; artificial neural networks; spectrophotometry

INTRODUCTION

One of the determination techniques most frequently used in pharmaceutical analysis is UV-VIS spectrophotometry because of the robustness, ease of operation, rapid response as well as low purchase and maintenance costs. However, the lack of specificity of the UV-absorption of components in a multicomponent drug formulation usually hinders the application of this technique, due to spectral overlap. More recently, a group of methods known as 'multivariate calibration' such as multiple linear regression (MLR), principal component regression (PCR) and partial least-squares (PLS) have been found to play important roles in analytical chemistry and they are capable of analysing and modeling hundreds of experimental data, making it possible to determine multiple components of interest simultaneously. Among them, PLS has found important applications in pharmaceutical analysis.[1] This method is useful for the resolution of complex mixtures of analytes and the spectra of which are strongly overlapping.[2] Although PLS assumes a linear relationship between the measured sample concentrations and the intensity of its absorption bands, small deviations from linearity are acceptable as they can readily be suppressed by including additional modeling factors.[3] However, in the presence of substantial non-linearity, PLS tends to give large prediction errors and calls for more robust models such as artificial neural networks (ANN).[4]

Artificial neural networks are computer methods that simulate learning and generalization behavior of the human brain through data modeling and pattern recognition for complicated multidimensional problems. A significant difference between an ANN model and a statistical model is that the ANN can generalize the relationship between independent and dependent variables without a specific mathematical function. Thus, an ANN works well for solving nonlinear problems of multivariate and multi-response systems. The ANN has been used in a variety of disciplines, such as chemistry and chemical engineering. For example, the ANN technique has been applied to several analytical methods, such as nuclear magnetic resonance,[5] high-performance liquid chromatography,[6] infrared spectroscopy,[7] mass spectroscopy,[8] and UV-VIS spectroscopy.[9] It has also proved useful for resolving mixtures of analytes giving deviate signal form Beer and Lambert's Law in spectrophotometric techniques.[10]

Paracetamol (4-acetamidophenol) is an effective

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analgesic and antipyretic for treatment of minor, non-inflammatory conditions in patients who are prone to gastric symptoms.[11] Orphenadrine citrate ((*RS*)-(dimethyl-2-(2-methylbenz-hydroxy) ethyl) amine citrate) is employed as skeletal muscle relaxant.[12] Thus, tablets containing paracetamol (PAR) and orphenadrine citrae (OPC) show combined analgesic, antipyretic and skeletal muscle relaxing actions. This combination is widely used in Thailand. Even though there are many reports of the quantitative determination of paracetamol and orphenadrine citrate separately[13–16] or in combination with other drugs,[17–19] no spectrophotometric method has been reported for the simultaneous determination of these two compounds in pharmaceutical tablets.

In this report, we study the possibility of the estimation of paracetamol and orphenadrine citrate in pharmaceutical dosage form, which has highly difference in proportions of two active ingredients by PLS and ANN. Each tablet contains paracetamol 500 mg and orphenadrine citrate 35 mg (in the ratio about 14 : 1 (PAR : OPC)). When both active constituents are to be simultaneously estimated with a single spectrum measurement, the major analyte (PAR) would be present in concentrations which deviate from Beer and Lambert's law and the minor analyte (OPC) would be in concentration near to the noise level. In our study, PAR can be estimated with PLS method. On the other hand, OPC requires the use of an ANN since it is apparent non-linearity that cannot be adequately modeled by PLS.

MATERIALS AND METHODS

Apparatus and Software Electronic absorption measurements were carried out on a Shimadzu UV160A spectrophotometer connected to a computer loaded with Shimadzu UVPC software, using quarts cells with a 1-cm path length. The absorption spectra of all test and reference solutions were recorded each 1 nm in the range 200–300 nm. The obtained data were processed by a Pentium IV computer having 512 MB for RAM (Windows XP operating system). The PLS was performed by PLS_Toolbox 2.0[20] under MATLAB 7.0[21] and the ANN was implemented in MATLAB 7.0 using the additional Neural Network Toolbox.[21]

Chromatographic measurements were carried out in a high-performance liquid chromatography Shimudzu LC-20AT, equipped with single pump, a 243-nm detector, manual injector (20 μl) and a 4.6-mm$\times$ 25-cm column that contains packing ODS (C-18). The mobile phase was 75% of water and 25% of methanol.

Reagents All experiments were performed with pharmaceutical-grade PAR and OPC and analytical-grade reagents. Tablets containing PAR and OPC were kindly supplied by manufacturer (SEA PHARM Co., Ltd., Thailand). The preparations contain 500 mg of PAR, 35 mg of OPC and excipients (EXP) such as lactose, corn starch, magnesium stearate, microcrystalline cellulose and sodium starch glycolate. Stock solutions of PAR (150 and 170 mg L^{-1}), OPC (12.5 and 25 mg L^{-1}) and EXP (50 mg L^{-1}) were prepared by dissolving accurately weighed amounts of the drugs in methanol-water (1:3, v/v).

Calibration Set Artificial, a training set of 22 samples corresponding to central composite design with three centre samples and 10 pure samples was built to be used as calibration set for PLS. The compositions of the mixtures used in the calibration set were summarized in Table 1. For the application of ANN, there are constraints concerning the number of samples, which at times may be limiting the development of an ANN model. The number of adjustable parameters (synaptic weights) is such that the calibration set is rapidly overfitted if too few training pairs are available leading to loss of generalization ability. Therefore, calibration set of 77 samples corresponding to central composite design with three centre samples and mixture design of PAR and OPC in a matrix of excipients was used. A representative distribution of the concentration of PAR and OPC

Table 1. Composition of the Calibration Set for PLS

No.	PAR (mg L^{-1})	OPC (mg L^{-1})	No.	PAR (mg L^{-1})	OPC (mg L^{-1})
1	30	2	12	20	2
2	10	2	13	10	0
3	20	1	14	15	0
4	20	3	15	20	0
5	25	1.5	16	25	0
6	25	2.5	17	30	0
7	15	1.5	18	0	1
8	15	2.5	19	0	1.5
9	20	2	20	0	2
10	20	2	21	0	2.5
11	20	2	22	0	3

across the range is shown in Fig. 1. This set was composed of 4 groups. Each group was prepared on different days in order to take into account the maximum possible variability on the data. The set of 77 samples was randomly divided into a training set (65 samples) and a monitoring set (12 samples). All samples were obtained by serial dilution of the stock solutions in 25-ml volumetric flasks with methanol-water (1:3, v/v). The concentrations of PAR were varied from 0 to 35 mg L^{-1} and the concentrations of OPC were varied from 0 to 5 mg L^{-1}.

Spiked Sample Preparations One set of five synthetic samples on each compound (no.1–5 for PAR, no.6–10 for OPC) were prepared, using spike placebo technique, to validate the PLS model and ANN model. In Table 2 the compositions of these synthetic mixtures have been summarized.

Commercial Sample Preparation Three production batches of a commercial pharmaceutical formulation were evaluated. In this case, groups of 20 tablets were weighed, finely powdered and mixed. Portions of the powder equivalent to about 170 mg of PAR and 12 mg of OPC were accurately weighed and transferred to 250 ml volumetric flasks using 150 ml of methanol-water (1:3, v/v). After being continuously shaken for 20 min, the flasks were made up to volume with the same solvent, and the solids were filtered, then, 5 ml aliquots were transferred from each flask to 100 ml volumetric flasks and completed to volume with the same solvent. Each batch was analysed in three replicate.

RESULTS AND DISCUSSION

Spectral Features The highly interference of the individual spectra of two drugs at equal concentrations (12 mg L^{-1}) are shown in Fig. 2. The spectra at the same solvent, but with analyte concentrations corresponding to sample diluted 1/15000: PAR, 33 mg L^{-1}, and OPC, 2.3 mg $L^{-1}$are shown in Fig. 3. As can be seen in Fig. 3, the minor analyte's concentration (OPC) is near to the noise level (absorbance is under 0.05), while that for the major analyte (PAR)

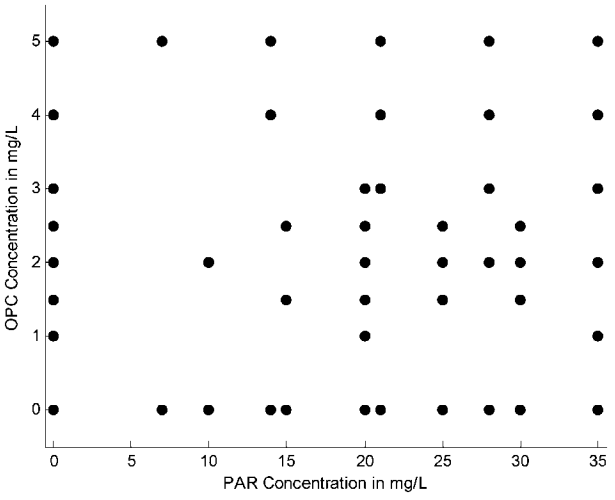

Fig. 1. Distributed Concentration of Synthetic Mixture Design for Testing the Neural Networks

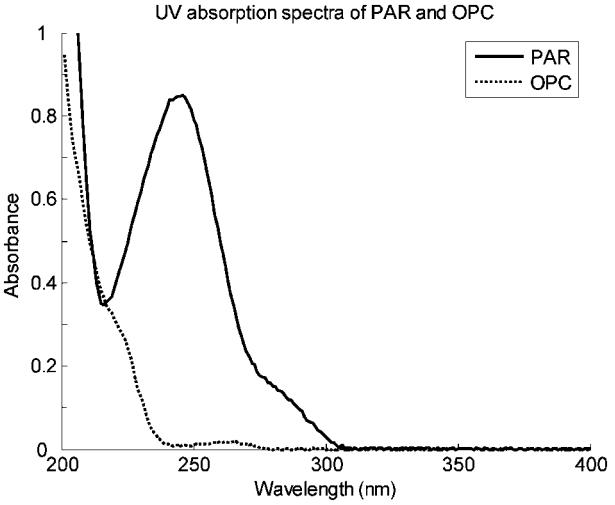

Fig. 2. Spectra of PAR and OPC (both at 12 mg L^{-1}) in Methanol/Water (1:3, v/v) Solution

Table 2. Composition of the Spiked Samples

No.	PAR (mg L^{-1})	OPC (mg L^{-1})	EXP (mg L^{-1})	No.	PAR (mg L^{-1})	OPC (mg L^{-1})	EXP (mg L^{-1})
1	10.70	1.86	4.51	6	26.51	1.30	4.77
2	16.05	1.86	4.51	7	26.51	1.94	4.77
3	21.40	1.86	4.51	8	26.51	2.59	4.77
4	26.75	1.86	4.51	9	26.51	3.23	4.77
5	32.10	1.86	4.51	10	26.51	3.88	4.77

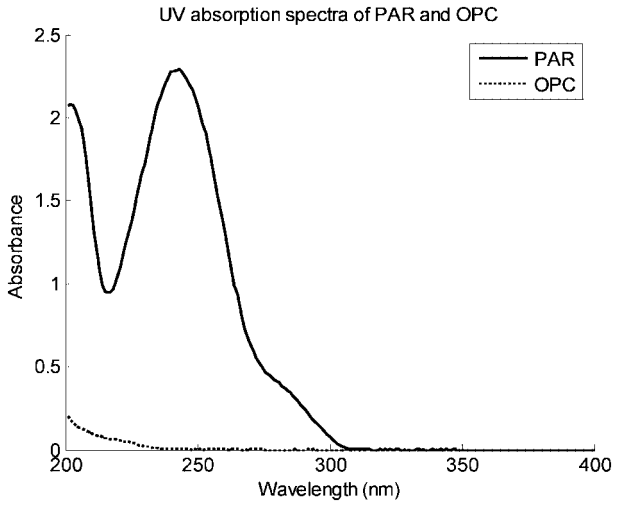

Fig. 3. Spectra in Concentrations Obtained when the Sample was Diluted 1/15000: PAR, 33 mg L^{-1} and OPC, 2.3 mg L^{-1}

is near to a concentration level, which deviates from Beer and Lambert's law (absorbance's rang between 2 and 2.5). Therefore, in order to simultaneously estimate both constituents, it may introduce to deviations of the absorbance/concentration linearity for the major and the minor analytes.

PLS Modeling In the case of PLS, the absorption spectra for the calibration samples were recorded in the wavelength range of 200–300 nm and then subjected to PLS analysis. Although PLS is usually considered as a full spectrum method, literature shows a growing tendency to perform variable selection before multivariate regression, in order to improve its predicting ability. Many different procedures have been published for wavelength selection.[22] In this study, we have employed a moving window strategy to the calibration set in order to find the location of the minimum calibration variance[23]. Even though the technique is very necessary, it should not be blindly applied. It should be checking the overlapping of the spectroscopic signal of the analytes at hand after selecting an appropriate spectral region. In our case, the selected regions were 211–235 nm for PAR and 206–220 nm for OPC because the absorbance in the other ranges were reached a concentration level, which deviates from Beer and Lambert's law (absorbances are over 2 or under 0.05). Once the optimum spectral ranges were selected, a cross-validation method using leave one out, was applied to select the number of principal components (PCs). The cross-validation procedure consists of systematically removing one of the training samples in turn, and using only the remaining ones for construction of the latent factors and regression. The predicted concentrations were then compared with the actual ones for each of calibration samples, and the root mean square of error of cross-validation (RMSECV) was calculated. The RMSECV was computed in the same manner, each time a new principal component was added to the PLS model. The method described by Haaland and Thomas[24] was used for selecting the optimum number of PCs. Three PCs and 7 PCs were found suitable for PLS models of PAR and OPC, respectively. The calibration PLS models were established by PLS_Toolbox 2.0 program with these optimum parameters. Table 3 and 4 show the result obtained when applying each PLS model for PAR and OPC to the spiked samples.

It can be seen from Table 3 and 4 that the statistical parameters are good for PAR, but those are poor for OPC due to severe interference occurring between OPC and more concentrated PAR. Thus, ANN technique could be used for the estimation of OPC to handle this intrinsically non-linearity.

ANN Modeling When the presence of non-linearity was found and cannot be modeled by linear model such as PLS, one can apply ANN. Although ANN are also able to deal with a linear behavior and can often improve the results in comparison with a linear model, they are calibration techniques especially constructed to model non-linear information. In this application, the set of 77 samples was used as calibration set for ANN. This set was randomly divided into a training set (65 samples) and a monitoring set (12 samples). The calibration ANN model was established by MATLAB Neural Network Toolbox 2.0 program. This ANN model consisted of three layers of neurons or nodes, which were the basic computing units: the input layer with a number of active neurons corresponding to the scores, one hidden layer with a number of active neurons, and the output layer with one active neuron corresponding to the scaled concentration of the component of interest. The neurons were fully connected in a hierarchical manner, *i.e.* the outputs of one layer of nodes were used as inputs for the next layer and so on. The nodes in the input layer transfer the input data to all nodes in hidden layer. These nodes calculate a weighted sum of the inputs that is subsequently subjected to a non-linear transformation:

Table 3. Results Obtained for the Analysis of PAR when Applying PLS Model to the Spiked Samples

Spiked sample	Actual (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
1	10.70	10.83	101.21
2	16.05	15.98	99.56
3	21.40	21.46	100.28
4	26.75	26.47	98.95
5	32.10	31.90	99.38
Mean recovery			99.88
r^2			0.9998

Table 4. Results Obtained for the Analysis of OPC when Applying PLS Model to the Spiked Samples

Spiked sample	Actual (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
6	1.30	1.58	121.54
7	1.94	2.72	140.21
8	2.59	2.91	112.36
9	3.23	3.65	113.00
10	3.88	3.02	77.84
Mean recovery			112.99
r^2			0.6387

$$o_j = f\left[\sum_{i=1}^{I}(s_i w_{ij} + w_{bj})\right] \quad (1)$$

where s_i is the input to node i in the input layer, I is the number of nodes in the input layer, w_{ij} (weights) are the connections between each node i in the input layer and each node j in hidden layer, w_{bj} is the bias to node j and o_j is the output of node j in hidden layer, and f is a non-linear function. In this work we have used the tan-sigmoid function (Eq. (2)) to increase the convergence speed.[25]

$$f(x) = \frac{e^x - e^{-x}}{e^x + e^{-x}} \quad (2)$$

The tan-sigmoid hidden layer is critical as it allows the network to learn non-linear relationships between inputs and outputs. Linear functions are used in both the input and output layers. The learning process was carried out through the back-propagation algorithm. The back-propagation network learns by calculating an error between desired and actual output and propagating this error information back to each node in the network. This back-propagation error is used to drive the learning at each node. The process of changing the weight of the connections to achieve some desired result is called learning or adaptation.

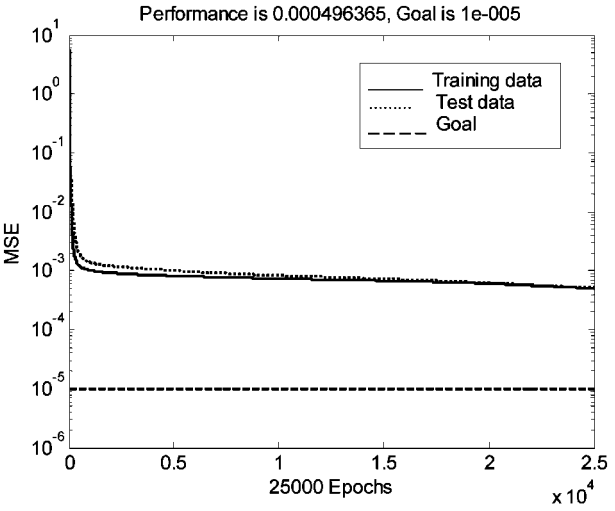

Fig. 4. Evolution of Training and Test Errors *versus* the Number of Epochs for OPC at Learning Rate 0.4 and Momentum 0.7

In the present work the number of neurons in the hidden layer, momentum and learning rate were optimized. At this point, the mean square error (MSE) was calculated, each time a new node was added to the hidden layer at arbitrary learning rate, momentum and the number of iterations. The number of neurons at the hidden layer, which has the minimum MSE value, was selected as the optimum number. After this step, the learning rate was varies from 0.1 to 0.9, and for each learning rate the momentum was examined from 0.1 to 0.9. A total of 81 networks were designed in this way. Each network was trained with training set, but it was subsequently stopped before it learns idiosyncrasies present in the training data by searching the minimum MSE for the test set (monitoring set). Finally, the number of the neurons at the hidden layer with the use of optimized momentum and learning rate was determined. Figure 4 shows the stopping point, which was obtained at 25000 epochs for best ANN found for OPC. The summary specifications for the network created for the calibrations were listed in Table 5.

For accuracy studies, by recovery, five spiked samples were analysed. The recovery values of OPC, obtained using ANN calibration model are shown in Table 6. They illustrate the reasonable good recovery values in most of the samples analysed.

Analysis of Tablet Dosage Form Once upon optimized PLS model for PAR and ANN model for OPC, a commercial sample was analysed in according with the foregoing procedures. The intermediate pre-

Table 5. Artificial Neural Network Specifications and Parameters

Parameter	OPC
Wavelength range	211–300
Input nodes	4
Hidden nodes	4
Output nodes	1
Learning rate	0.4
Momentum	0.7
Hidden layer transfer function	Tan-sigmoid
Output layer transfer function	Linear
Optimum number of iterations	25000

Table 6. Results Obtained for the Analysis of OPC when Applying ANN Model to the Spiked Samples

Spiked sample	Actual (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
6	1.30	1.38	106.63
7	1.94	1.96	100.72
8	2.59	2.57	99.51
9	3.23	3.19	98.82
10	3.88	3.89	100.36
Mean recovery			101.21
r^2			0.9988

Table 7. Precision for Concentrations on Difference Days (n =6 Determinations on Each Day)

Day	PAR (PLS method)			OPC (ANN method)		
	Mean	SD	RSD	Mean	SD	RSD
1	99.02	1.108	1.119	100.98	2.695	2.668
2	99.33	0.849	0.855	101.88	2.082	2.044
3	98.71	1.769	1.792	99.83	2.894	2.898
4	99.59	1.762	1.769	98.51	2.016	2.047
5	99.38	1.737	1.748	101.12	3.382	3.345
6	99.23	1.446	1.457	98.87	2.205	2.230
Mean	99.21			100.20		
SD	0.306			1.345		
RSD	0.309			1.342		

Table 8. Statistical Analysis of the Results Obtained by the Proposed Method and the Official Method for the Analysis of Both Active Ingredients in Commercial Sample

Item	PAR		OPC	
	PLS method	Official method	ANN method	Official method
Batch A	99.21	99.34	101.04	99.77
Batch B	99.46	98.25	101.24	99.54
Batch C	101.68	102.24	102.34	101.99
Calculated t	0.325		2.779	
Critical t[a]	4.303		4.303	

[a] Tabulated t-values (p=0.05, df=2)

cision of the proposed methods was evaluated over six consecutive days by performing six absoption spectrophotometric records each day and was expressed as the standard deviation (SD) and relative standard deviation (RSD). In Table 7, the results illustrate that the repeatability for both active ingredients on each day is satisfactory.

The proposed methods were applied to the determination of PAR and OPC in pharmaceutical tablets for three production batches (batches A, B and C). The average content of PAR was between 99.21 and 101.68 percent of the labeled amount (%LA) and the average content of OPC was between 101.04 and 102.34%LA. The United States pharmacopeia 26th ed. (USP 26), the content limits for PAR and OPC are between 90.0 and 110.0%LA and between 93.0 and 107.0%LA, respectively. The obtained results of these drugs gave rise to acceptable percentage of the labeled amount.

Statistical analysis of the results obtained by the suggested spectrophotometric procedures has been also carried out. Table 8 shows the results of paired t-test for a comparison of the proposed procedures with the official procedures. The official methods for PAR and OPC were followed to USP 26 methods[13]. The calculated t-values are less than their corresponding tabulated ones, indicating no significant differences between the suggested procedures and the reference procedures. The suggested procedures surpass the comparing ones so they can be used to determine PAR and OPC in pharmaceutical tablets.

CONCLUSIONS

This work illustrated the potential of the estimation of two drugs in tablet preparations, which have highly interference of the spectra and have the presence of non-linearity cause by the analyte concentrations, which deviate from Beer and Lambert's law. A validation set of spiked samples and a commercial sample were employed for testing the accuracy and precision of the methods. Reasonably good recoveries were obtained with PLS for PAR and the use of an ANN allowed the estimation of OPC, a minor component, which could not be adequately modeled by PLS. In

ANN, too many samples were used to train the network. This makes it expensive in time and resources to develop for use in routine work. Thus, PLS method should be expected to give the best of choice in data set where linear response is observed. However, when non-linear response is present, artificial neural networks may be capable of giving superior performance for spectrophotometric calibration. Due to the rapid calculation of the predictive results of very large samples, ANN approach is an effective choice for developing new analytical methods for in-process control in pharmaceutical manufacturing, which concern with several evaluated samples.

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