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Research Article

MATHEMATICAL SIMULTANEOUS DETERMINATION OF ROSUVASTATIN CALCIUM AND PROPRANOLOL HYDROCHLORIDE USING DIFFERENT CHEMOMETRIC TECHNIQUES

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ABSTRACT

Introduction: Simultaneous mathematical determination of rosuvastatin calcium and propranolol hydrochloride was described using different methods. The mathematical determination involves different chemometric models, namely, partial least square (PLS-1) as traditional model and artificial neural network as advanced model with and without variable selection procedure (genetic algorithm) .**Experimental:** A two factor, 5-level experimental design was established resulting in 25 mixtures with variable ratios of the drugs. Thirteen mixtures were used as a calibration set and the last twelve mixtures were used as a validation set to validate the ability of the prediction. **Conclusion:** The methods were used for quantitative analysis of the drugs in raw materials and pharmaceutical dosage form. The validity of the proposed methods was assessed using the standard addition technique.

Keywords: Chemometric technique; partial least squares; genetic algorithm; artificial neural network.

INTRODUCTION

Rosuvastatin calcium(ROS) is a synthetic lipid - lowering agent for oral administration.It has the chemical name of (3R, 5S, 6E)-7-[4-(P-flurophenyl)-6-isopropyl-2-(N-methylmethane sulfonamide)-5-pyrimidinyl]-3,5-dihydroxy-6-heptenoic acid [1], as shown in Figure 1. The molecular formula ofROS is $(C_{22}H_{27}FN_3O_6S)_2Ca$ and the molecular weight is 1001.14. It is a white amorphous powder that is sparingly soluble in water and methanol, and slightly soluble in ethanol.It is a competitive inhibitor of HMG-CoA reductase enzymethat is used inthe treatment of dyslipidemia and improve the lipid profile of the patient with hyperchloestrolemia.

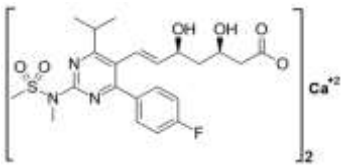

Fig. 1: Chemical structure of rosuvastatin calcium (ROS).

Propranolol hydrochloride (PRO) is non-selective beta-adrenergic receptor blocking agent with the chemical name of 1-[(1-methylethyl)amino]-3-(1-napthalenyloxy)-hydrochloride[1] as shown in Figure 2. The molecular formula of PRO is $(C_{16}H_{22}NO_2Cl)$ and the molecular weight is 259.34. It is a white crystalline solid that is readily soluble in water, methanol and ethanol. It is used for the treatment of high blood pressure, a number of types of irregular heart rate, thyrotoxicosis, capillary hemangioma, performance anxiety and the complication associated with angina or previous heart attacks [3-5].

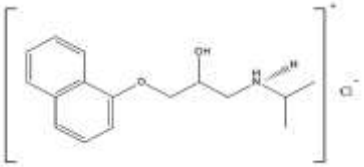

Fig. 2: Chemical structure of propranolol hydrochloride (PRO).

Determination of the ingredients in a pharmaceutical preparation becomes more difficult as the number of components in the mixture increases. Chemometric (multivariate calibration) techniques are employed for the analysis of multicomponent samples. All of the chemometric spectral analysis techniques are useful for the resolution of spectral bands overlapping in quantitative determination without chemical pre-treatment or graphical procedure of spectra such as derivative and ratio spectra derivative.

Chemometric models (partial least squares PLS-1 and artificial neural network ANN) were applied via handling the UV spectral data. The variable selection (genetic algorithm GA) was applied to enhance the predictive power of these chemometric models.

ANN is advanced model which is a type of artificial intelligence method that resembles the biological nervous system in having the capability to find the relationship between input and output. The outputs (predicted concentrations) are compared with targets (actual concentrations), and the difference is called error [6].

Reviewing the literature on the simultaneous determination of both ROS and PRO revealed that only Vierordt's and absorbance ratio methods were reported [7].

The aim of the present work was to develop simple and accurate methods for the simultaneous determination of ROS and PRO in pure and pharmaceutical forms.

EXPERIMENTAL

Materials and reagents:

Pure ROS (99.35%) and PRO (99.55%) were kindly supplied by National Organization for Drug Control and Research, Giza, Egypt. Rosuprol® tablet was purchased from the Jordanian pharmaceutical market Batch No. SP33142RP (labeled to contain 10 mg rosuvastatin calcium and 10 mg propranolol hydrochloride per tablet). Ethanol (HPLC grade) was obtained from Sigma-Aldrich, Germany pharmaceutical company.Whatman filter paper No. 40.

Instruments:

Shimadzu UV-Visible 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells.

Software

UV-Probe personal spectroscopy software version 2.21 was used. All chemometric methods were implemented in Matlab®R2013b (8.2.0.701). PLS, GA-PLS, ANN, GA-ANN were carried out by using PLS toolbox software version 2.1 in conjunction with a Neural Network Toolbox.

Standard solution preparation

Stock standard solutions of ROS and PRO (100 µg/mL) were prepared by weighing accurately 10 mg of each drug powder and transferred separately in 100-ml volumetric flask and dissolved in 50 ml of ethanol and the volume was completed to the mark with the same solvent. Working standard solutions of each drug (10 µg/mL) were prepared by accurately transferring 10 mL of stock solutions of both drugs in two separate 100-ml volumetric flask and diluted to the mark with ethanol.

Pharmaceutical sample preparation

Ten tablets were accurately weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred to a 100-mL volumetric flask to which50 ml of ethanol was added. The flask was sonicated for 15 min. Then filtered into 100-ml volumetric flask through, then the residue was washed twice with ethanol. All the filtrate and washings were collected and the volume was adjusted to 100 ml with ethanol to obtain solution of 100 µg/ml of each ROS and PRO.

Procedure

Experimental design for chemometric models

Zero order absorption spectra of ROS and PRO (14 µg/mL) solutions were recorded against ethanol as a blank over a range of 200-400 nm, as shown in Figure 3.A 2-factor, the 5-levels were established using 5 concentration levels for each interested drug, resulting in 25 mixtures [8].The central level of the design is 14 µg/mL for each interested drug which, based on the calibration range of each drug. The regions from 350 to 400 nm were not involved in experimental design data. Thirteen mixtures were used as a calibration set and the last twelve mixtures were used as a validation set to validate the predictive ability of the developed chemometric models.

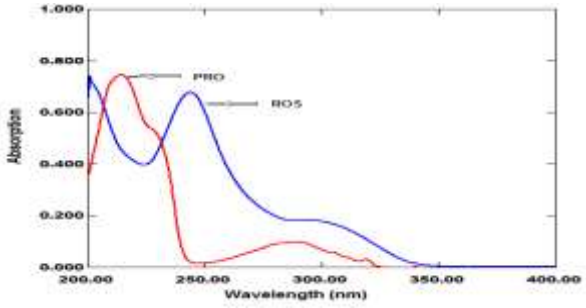

Fig. 3: Zero order absorption spectra of ROS (14 µg/ml) and PRO (14 µg/ml).

RESULTS AND DISCUSSION

The literature survey reveals that chemometric methods have not been reported for the simultaneous determination of ROS and PRO. Thus, The application of both chemometric techniques for the simultaneous determination of both drugs was of interest.

Chemometric models:

In recent years, chemometric calibrations such as classical least-squares (CLS), inverse least squares (ILS), principle component regression (PCR) and partial least squares (PLS)have been applied to the analysis of analytical data obtained from many instruments[9-11].

The core points of the application of chemometric technique in this work were to develop simple and accurate methods for the simultaneous determination of ROS and PRO and showed the effect of variable selection on the improvement of thepredictive power of the interested chemometric methods by application of genetic algorithms (GA) technique, since GA could be used successfully as avariable selection technique [12,13]. The adjustment of GA parameters is an important issue of successful GA performance [14].

The construction of the calibration matrix for ROS and PRO mixture has been developed. The chemometric models were optimized with respect to the 5-level, 2-factor design resulting in 25 sample mixtures. These sample mixtures were divided into two groups; 13 training mixtures (odd numbers of sample) for building the calibration set and 12 validation mixtures (even numbers of samples) for measuring the predictive power and validation of these models, the concentrations details were demonstrated in [Table 1]. The adjusted GA parameters were shown in [Table 2].

Table 1: The experimental design of the concentrations of ROS and PRO mixtures used in the chemometric methods

Mix. No.	ROS	PRO
1	14	14
2	14	11.2
3	11.2	11.2
4	11.2	16.8
5	16.8	12.6
6	12.6	16.8
7	16.8	14
8	14	12.6
9	12.6	12.6
10	12.6	15.4
11	15.4	16.8
12	16.8	15.4
13	15.4	14
14	14	16.8
15	16.8	16.8
16	16.8	11.2
17	11.2	15.4
18	15.4	11.2
19	11.2	14
20	14	15.4
21	15.4	15.4
22	15.4	12.6
23	12.6	11.2
24	11.2	12.6
25	12.6	14

The shaded rows represent the validation set.

Table 2: Parameters of the genetic algorithms

Parameter	Value
Population size	36
The number of variables in a window (window width)	2
%Wavelengths used at initiation	50
Maximum generation	32
% of population the same at convergence	100
Mutation rate	0.005
Maximum number of LVs	2
Cross over type	Single
Cross validation	Random
The number of subsets to divide data into for cross validation	4
The number of iterations for cross validation at each generation	2

The wavelength range had to be selected optimally to control the quality of multi-component analysis [15]. The wavelengths used to be in the range 200 – 350 nm for both ROS and PRO, respectively. The wavelengths more than 350 nm were not used since, both drugs do not absorb in this region.

Partial Least Square (PLS-1)

PLS-1 method was applied for a relation between of the interested drugs (ROS and PRO) and the latent variable of data matrix [16]. PLS-1 deals with the whole spectrum and the information about the concentration of only one component to create the latent variables

(LVs) used by the model without application of a variable selection [17, 18].

The optimization of the number of LVs is a critical issue in PLS-1 method, since extra LVs increases the possibility of the known problem of overfitting, while if the number of LVs was too small, the useful data that could be necessary for the calibration would be discarded. Leave one out (LOO) cross validation [19] can be applied to predict the optimum number of PLS components. The method for Haaland and Thomas [20] was developed for selecting the optimum number of the factors, since this method has significant selection of optimum models, while there is no significant difference between the corresponding root mean square error of cross validation RMSECV and the minimum RMSECV.

The training set (calibration data) was used for constructing PLS-1 model. RMSECV was calculated for each drug in the method (recalculated on addition of new factor to the PLS-1) for examining the error in the predicted concentrations and indicates both of precision and accuracy of the predictions **[Figure 4,5].** GA was applied as a variable selection technique to enhance the predictive power of PLS-1 method by eliminating uninformative variables and select the most informative to enhance the interpretability.

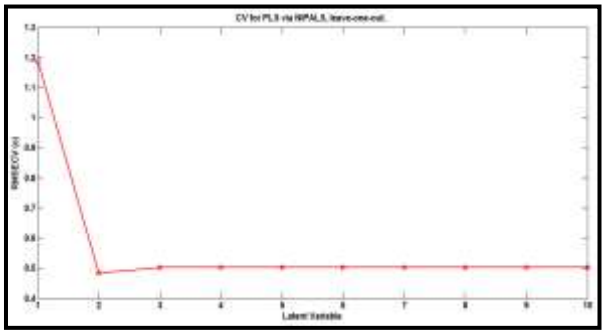

Fig. 4: RMSECV plot of the calibration set as a function of the optimum LVs for ROS.

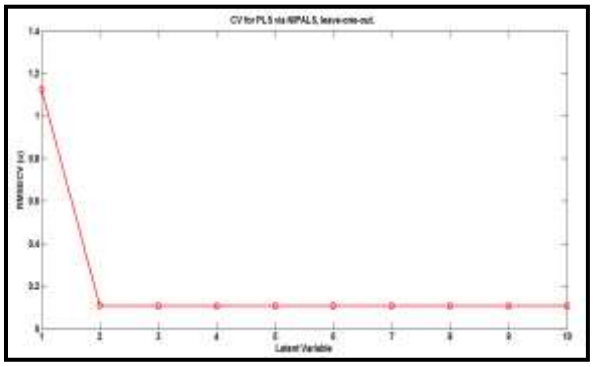

Fig. 5: RMSECV plot of the calibration set as a function of the optimum LVs for PRO.

ANN and GA-ANN

The technique of ANN is the inherent ability to create arbitrary non-linear boundaries represented as neurons between input and output layers. The architecture of ANN could be divided into three components: Input layer, hidden layer and output layer. There are two important issues in the network learning: The estimation error and the training time. These issues may be affected by two factors: The first one is the factor related to the architecture of the neural network, which includes the number of hidden nodes, number of hidden layers and values of learning parameters, the second factor is that related to the training set which, include the number of training pattern, inaccuracy of input data and preprocessing of data. GA might be useful in reducing the absorbance matrix hence, the large number of nodes in the input layer increases the training time for ANN modeling. In addition to reducing the absorbance matrix of input data, GA allows the elimination of irrelevant data, such as noise or redundancies which involved in the data matrix. Thus, both of ANN and GA-ANN had been applied in this work to test for improvement of prediction.The output layer is the concentration matrix of one component. The hidden layer is the single layer which had been sufficient to resolve that similar or more complex issue since, more hidden layers overfitting [21].

ANN parameters had been optimized for each drug as shown in [Table 3], Purelin-Purelin transfer function was developed in the proposed methods due to linear correlation among absorbances and concentrations. ANN was trained by different training function and there were no significant differences in performances.Levenberg-Marquardt back propagation TRAINLIM) was applied as it is time saving.

Table 3: Optimized Parameters of ANNs.

Method	**ANN**		**GA-ANN**	
	ROS	**PRO**	**ROS**	**PRO**
Architecture	151-10-1	151-7-1	87-3-1	87-3-1
Hidden neuron number	10	7	3	3
Training function	TRAINLIM			
Adapting learning function	LEARNGDM			
Transfer functions	Purelin-Purelin			
Learning coefficient	0.001	0.001	0.001	0.001
Learning coefficient decrease	0.001	0.001	0.001	0.001
Learning coefficient increase	100	100	100	100

Calibration of the methods

The proposed chemometric methods were implemented in the calibration data using optimized parameters. The concentrations of each drug (ROS and PRO) in the calibration set (13 mixtures) were calculated. A linear correlation was obtained among predicted concentrations of each drug and that of actual concentartions [Table 4].

Validation of the methods

The validation set was encountered in training step. ANN stops when Mean Square Error (MSE) of calibration set was decreased and that of validation set increased, to avoid overfitting problem.The validation set (12 mixtures) was analyzed to validate the proposed methods, [Table 5].

ANN and GA-ANN show better RMSEC and RMSEP than that of PLS-1[Table 4,5], this may be due to the fact that ANN is an artificial intelligence where there is less chance for overfitting than that of PLS-1.

Table 4: Determination of ROS and PRO in the calibration set of the proposed methods:

Conc. (µg/mL)		PLS-1		GA-PLS-1		ANN		GA-ANN	
ROS	PRO	ROS % Recovery[a]	PRO	ROS % Recovery[a]	PRO	ROS % Recovery[a]	PRO	ROS % Recovery[a]	PRO
14	14	98.86	99.00	99.53	98.10	101.43	98.54	101.29	99.25
11.2	11.2	101.02	99.21	101.16	99.37	100.65	100.32	100.29	99.87
16.8	12.6	100.82	100.54	99.41	100.81	99.37	99.51	99.72	99.90
16.8	14	100.27	99.95	99.65	101.01	100.00	101.60	99.35	100.53
12.6	12.6	98.65	98.95	99.10	99.89	100.24	100.17	101.03	99.38
15.4	16.8	99.61	101.91	99.35	99.64	99.35	99.64	99.35	99.64
15.4	14	101.05	99.58	99.49	101.43	99.20	99.85	99.20	98.92

16.8	16.8	98.94	101.28	100.49	100.49	99.78	100.27	99.78	100.09
11.2	15.4	101.15	99.81	101.25	100.91	100.00	99.51	100.63	99.64
11.2	14	101.07	99.14	99.44	101.20	100.00	100.68	98.69	101.53
15.4	15.4	100.33	100.85	101.10	100.19	102.60	99.34	101.30	99.73
12.6	11.2	98.67	99.04	99.90	99.89	99.35	99.82	99.51	100.35
12.6	14	100.30	99.24	101.03	99.49	100.28	100.86	100.28	100.57
Mean		100.06	99.89	100.07	100.19	100.17	100.01	100.03	99.96
%RSD		0.988	0.972	0.808	0.921	0.951	0.775	0.842	0.680
RMSEC[b]		0.134	0.147	0.108	0.132	0.145	0.109	0.116	0.095

[a]Average of three determinations. [b]Root Mean Square Error of calibration.

Table 5: Determination of ROS and PRO in the validation set of The proposed methods:

Conc. (µg/mL)		PLS-1		GA-PLS-1		ANN		GA-ANN	
ROS	PRO	ROS	PRO	ROS	PRO	ROS	PRO	ROS	PRO
		% Recovery[a]		% Recovery[a]		% Recovery[a]		% Recovery[a]	
14	11.2	98.81	99.03	101.37	101.71	101.43	99.10	101.14	100.88
11.2	16.8	101.75	100.97	101.42	99.46	100.89	99.66	100.44	99.06
12.6	16.8	98.97	101.21	101.57	99.99	101.02	99.00	100.14	100.79
14	12.6	99.51	99.48	100.65	99.93	102.14	99.39	101.71	98.60
12.6	15.4	99.85	100.08	99.43	99.54	99.63	100.46	99.95	100.59
16.8	15.4	99.60	100.88	100.56	99.44	99.82	99.65	99.82	99.65
14	16.8	98.62	101.52	100.14	99.52	98.57	100.50	98.50	100.08
16.8	11.2	101.43	100.20	99.99	99.38	99.40	100.23	99.40	100.05
15.4	11.2	100.37	99.74	99.07	101.40	99.61	100.88	100.06	101.33
14	15.4	99.20	100.42	100.36	99.68	100.71	100.65	100.71	100.26
15.4	12.6	101.76	100.16	99.46	99.58	99.22	98.42	99.48	99.77
11.2	12.6	99.62	98.74	99.55	101.19	99.02	101.22	101.07	100.43
Mean		99.96	100.20	100.30	100.07	100.12	99.93	100.20	100.12
%RSD		1.125	0.857	0.843	0.851	1.088	0.857	0.878	0.775
RMSEP[b]		0.152	0.129	0.114	0.100	0.144	0.110	0.120	0.101

[a]Average of three determinations. [b]Root Mean Square Error of Prediction.

Application to the pharmaceutical preparation:

The proposed chemometric methods were applied for the determination of ROS and PRO in their commercial tablets, Rosuprol® tablets [Table 6], and showed satisfactory results for the simultaneous determination of ROS and PRO in a good agreement with label claim.The obtained results were statistically compared to those obtained by the reported absorbance ratio method [7]. The calculated t and F values indicate that there were no significant difference between the proposed and the reported methods. The obtained results assure that the excipients did not interfere with proposed methods.

Table 6: Determination of ROS and PRO in Rosuprol® tablets by the proposed chemometric methods:

Value	Rosuprol® tablets									
	PLS-1		GA-PLS-1		ANN		GA-ANN		Reported method	
	ROS	PRO	ROS	PRO	ROS	PRO	ROS	PRO	ROS	PRO
N	5	5	5	5	5	5	5	5	5	5
Mean	99.80	100.43	100.39	99.96	99.91	100.25	99.88	99.90	100.95	101.49
SD	1.022	0.786	1.044	0.851	1.058	0.823	1.172	0.774	1.178	1.310
%RSD	1.024	0.782	1.040	0.851	1.059	0.821	1.173	0.775	1.167	1.290
Student's *t*-test (2.306)*	0.803	0.618	0.899	0.713	0.452	0.582	0.627	0.726	-	-
*F*value (6.388)*	1.329	1.059	1.273	1.243	1.240	1.161	1.011	1.028	-	-

CONCLUSION

The developed chemometric method (PLS-1, GA-PLS, ANN and GA-ANN) have been presented as a powerful method

To resolve the binary mixture of ROS and PRO, and can be simultaneously determined in both their powder and pharmaceutical dosage form with acceptable results.

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