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Research Article

MULTIVARIATE CHEMOMETRIC MODELS APPLIED FOR SIMULTANEOUS DETERMINATION OF BROMHEXINE AND GUAIFENESIN IN PURE FORM AND IN PHARMACEUTICAL PREPARATION; A COMPARATIVE STUDY

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ABSTRACT

Objective: Experimental design of different synthetic mixtures of bromhexine and guaifenesin in different ratios were constructed.The zero-order absorption spectra of these prepared mixtures have been recorded and used for building first reported four multivariate chemometric methods for simultaneous determination of bromhexine and guaifenes in in their pure and pharmaceutical dosage forms.**Methods:** namely; partial least squares and artificial neural network have been applied for the quantitative analysis of the studied drugs. **Results:** the application of genetic algorithm to partial least squares and artificial neural network has been done and greatly increased the precision and predictive ability of the methods.**Conclusion:** The four methods have been successfully applied for determination of both drugs in their pharmaceutical preparation without any preliminary separation steps.

Key words: Bromhexine; guaifenesin; chemometry; overlapped spectra.

INTRODUCTION

Bromhexine hydrochloride (BRM) is 2,4-Dibromo-6-[[cyclohexyl (methyl) amino] methyl] aniline hydrochloride **(Figure 1)**. It is a white powder, freely soluble in methanol, ethanol and slightly soluble in water. Its molecular weight is 412.60[1].It's a synthetic derivative of the herbal active ingredient vasicine. It's a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus[2].

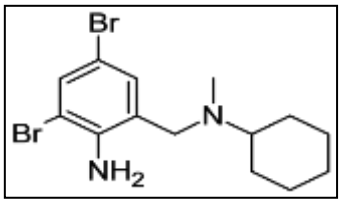

Fig. 1: Structural formula of Bromhexine

Guaifenesin (GUA) ;(Figure 2) Guaifenesin is (2RS)-3-(2-methoxyphenoxy) propane-1,2-diol.It is a white fine powder ,sparingly soluble in water; soluble in alcohol. Its molecular weight is 198.2 and its molecular formula is $C_{10}H_{14}O_4$ [1]. Its increase the volume and reduce the viscosity of tenacious sputum and is used as an expectorant for productive cough[2]. Literature survey revealed that bromhexine and guaifenesin is official in British Pharmacopoeia (BP) [3].

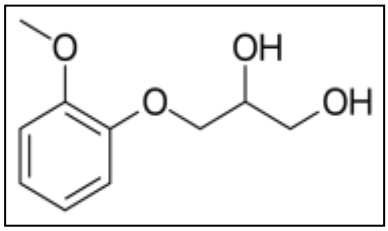

Fig. 2: Structural formula of Guaifenesin.

Literature survey revealed that various analytical methods such as spectrophotometry [4-7] HPLC [8-11], have been reported for determination of bromhexine (BRM) and guaifenesin (GUA) in bulk drug formulations or combination with other drugs. Hence the objective of the present work is to develop simple, precise and accurate methods for the simultaneous determination of bromhexine and guaifenesin in capsules formulation.

To the best of our knowledge there is no reported chemometric method available for simultaneous determination of bromhexine and guaifenesin. Hence, the aim of this work was to develop accurate and precise chemometric methods for simultaneous determination of BRM and GUS in their dosage form. The developed methods are partial least squares (PLS-1) with application of genetic algorithm (GA-PLS-1) and artificial neural network (ANN) with application of genetic algorithm (GA-ANN).

EXPERIMENTAL

Instruments

Shimadzu UV-Vis. 1800 *Spectrophotometer*, (Tokyo, Japan), equipped with 10 mm matched quartz cells was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 1 nm interval.

Software

UV-Probe personal spectroscopy software version 2.1.(SHIMADZU).

All chemometric methods were implemented in Matlab R2013b (8.2.0.701).

PLS, ANN and application of GA were carried out by using PLS toolbox software version 2.1. in conjugation with neural network toolbox

The student *t*-test and F value were performed using Microsoft-Excel.

All calculations were performed using a Quad core CPU, 1.47 GHz, 4.00 GB of RAM under Microsoft Windows 7 ™.

Materials and Reagents

- Pure bromhexine hydrochloride (99.6 %) was kindly supplied by Arab Drug Company (ADCO), Egypt**.**

- Pure guaifenesin (99.7 %) was kindly supplied by Arab Drug Company (ADCO), Egypt**.**
- Pharmaceutical preparation: Muclear®capsules (Batch no. N 129936), manufactured by Rameda pharmaceutical company. It is labelled to contain (8 mg of BRM and 100 mg of GUA) per capsule and purchased from local pharmacy.
- Methanol, analytical grade was purchased from (El-Nasr Pharmaceutical Chemicals Co. Abu- Zabaal, Cairo, Egypt).

Standard solutions

Standard solution of bromhexine

A standard solution of bromhexine(100 µg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of methanol and complete to 100 ml with the same solvent. Working solution (10 µg/ml) was obtained by dilution of the stock solution with methanol.

Standard solution of Guaifenesin

- A standard solution of guaifenesin (100µg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of the methanol and complete to 100 ml with the same solvent.

PROCEDURES

Experimental design

A 5 levels, 2 factors experimental design was used in which 1, 2, 3, 4 or 5 mL aliquots of working solutions of BRM (10 µg/ml) equivalent to (1, 2, 3, 4 and 5 µg/mL) and1.25, 2.5, 3.75, 5 and 6 mL aliquots of standard solution of GUA equivalent to (12.5, 25, 37.5, 50 and 62.5 µg/ mL) of GUA were combined and diluted to 10 mL with methanol resulting in 25 mixtures[12]. The central level of the design is 3µg/mL and 37.5µg/mL for BRM and GUA respectively. The chosen concentrations for each compound are based on their linearity and the ratio between both compounds involved in their pharmaceutical preparation. The concentrations details are given in **Table 1.**

Table 1: The 5-level, 2-factor experimental design shown as concentrations of the mixture components in µg/mL.

Mixture number	BRM	GUA
1	3	37.5
2	3	12.5
3	1	12.5
4	1	62.5
5	5	25.0
6	2	62.5
7	5	37.5
8	3	25.0
9	2	25.0
10	2	50.0
11	4	62.5
12	5	50.0
13	4	37.5
14	3	62.5
15	5	62.5
16	5	12.5
17	1	50.0
18	4	12.5
19	1	37.5
20	3	50.0
21	4	50.0
22	4	25.0
23	2	12.5
24	1	25.0
25	2	37.5

The shaded rows represent the validation set.

The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-350 nm with 1 nm interval thus the produced spectral data matrix has 25 rows representing different samples and 151 columns representing wavelengths (25 x 151).Thirteen mixtures of this design were used as a calibration set and the other twelve mixtures were used as a validation set to test the predictive ability of the developed multivariate models.

Application of the method to pharmaceutical preparation

Contents of ten capsules of Muclear® (8/100 mg) was finely powdered and an amount equivalent to (10 mg of BRM and 125 mg of GUA) was extracted three times with 25 mL of methanol, filtered into 100 mL volumetric flask then the volume was adjusted with methanol to obtain a solution labelled to contain (100 µg/mL of BRM and 1250 µg/mL of GUA). This solution was diluted to obtain solution labelled to contain (10 µg/mL of BRM and 125 µg/mL of GUA).The spectra of these solutions were scanned from 200 to 400 nm, stored in the computer and analysed by the proposed methods.

RESULTS AND DISCUSSION

Spectroscopic techniques can supply the analyst with a large data within a short period of time. Coupling the spectral data with chemometric models enhance the quality of the spectral information and making this combined technique into a powerful and highly convenient analytical tool. To date there is no reported spectrophotometric method for the simultaneous analysis of BRM and GUA. This has prompted the authors to apply different chemometric methods, especially PLS, GA-PLS, ANN, GA-ANN for simultaneous analysis of the studied drugs. These described methods have higher prediction power, providing maximum relevant information and analyzing a large number of samples in a short period of time with higher degree of accuracy and precision.

The UV spectra of BRM and GUA show sever overlap(**Figure 3)**, which creates difficulty in the simultaneous analysis of this mixture. Therefore, multivariate calibration methods were applied to predict the concentrations of BRM and GUA in both calibration and validation sets as well as in their pharmaceutical formulation.

GA searches the solution space of a function through the use of simulated evolution. It solves the optimization problem by exploring all regions of the potential solutions and exponentially exploiting promising areas through mutation, crossover, and selection operation applied to individuals in the populations. A critical issue of successful GA performance is the adjustment of GA parameters [13]. In order to avoid the risk of over fitting, a number of independent short runs were done and the results of all the runs were taken into consideration to obtain the final model. Doing this, a much more consistent (and less over fitted) solution can be obtained [14, 15]. The adjusted GA parameters with the lowest mean square error were shown in **Table 2.**

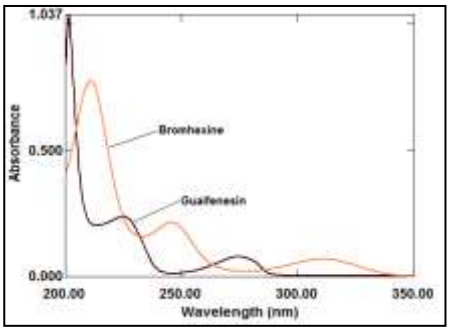

Fig. 3: Zero order absorption spectra of (7 µg/mL) BRM and (10 µg/mL) GUA.

Partial least squares (PLS) and applying genetic algorithm (GA-PLS)

PLS-1 is a widely used regression method. It is known that information from the concentrations values is introduced into the calculation of the so-called latent variables, which are linear combinations of the original variables. PLS-1 method was run on the calibration data of absorption spectra. To select the number of factors in the PLS-1 algorithm, a cross validation (CV) method leaving out one sample at a time was applied using calibration set of 13 calibration spectra. RMSECV (Root Mean Squared Error of Cross Validation) was recalculated upon addition of each new factor to the

PLS-1. Then number of factors was selected based on Haaland and Tomas criteria [16]. It was found that two factors were sufficient for modelling both BRM and GUA.

Table 2: Parameters of genetic algorithm.

Parameter	Value	
	BRM	GUA
Population size	32	32
Maximum generations	42	48
Mutation rate	0.005	0.005
The number of variables in a window (window width)	3	2
Per cent of population the same at Convergence	100	100
% Wavelengths used at initiation	50	50
Crossover type	Double	Double
Maximum number of latent variables	2	2
Cross validation	Random	Random
Number of subsets to divide data into for cross validation	5	5
Number of iterations for cross validation at each generation	2	2

However, to increase the quality and improve the calibration, the variables selection technique namely genetic algorithm (GA) was performed; by its application the un-informative variables were excluded. The predictability of both models was tested by validation set and it was found that the PLS-1 model constructed after removing the un-informative variables is more robust and simpler with lower root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). This is surely due to the fact that the un-informative wavelengths have been excluded. The percentage % recoveries, RSD (relative standard deviation) and RMSEP values of the validation set for PLS and GA-PLS models are listed in **Table 3**.

The GA was run on 151 variables for BRM and GUA using a PLS with the optimum number of LVs determined by cross validation on the model containing all the variables. GA reduced absorbance matrix to about 49.6 % of the original matrix of BRM and 48.4 % of GUA. The whole parameters involved upon application of GA on PLS model are shown in (**Figure 4)** and (**Figure 5)** for BRM and GUA respectively.

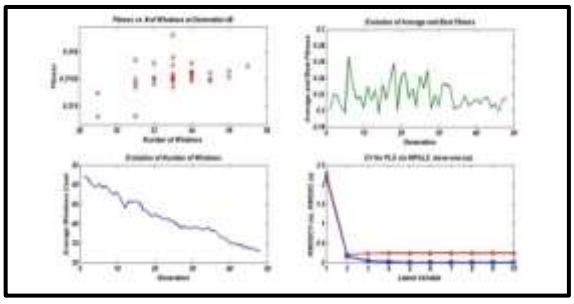

Fig. 4: The whole parameters involved in application of GA on PLS model for BRM.

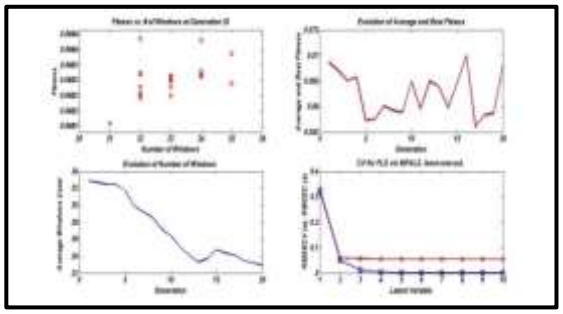

Fig.5: The whole parameters involved in application of GA on PLS model for GUA

Artificial neural network (ANN) and applying genetic algorithm (GA-ANN)

ANNs are a type of computational models simulating the biological neural networks. They composed of an inter-connected group of artificial neurons. To optimize a neural network, we have to use the trial and error method to find out the best neural network architecture. [17, 18] Choosing the values of optimum parameters to construct the network are not an easy task because the parameters are mutually related.

The output layer resembles the concentration vector of one component. The hidden layer consists of single layer which is sufficient to solve similar or more complex problems. Moreover, more hidden layers may cause over-fitting. The hidden neurons number is one of the most important parameters among other ANN parameters that must be adjusted. This parameter is related to the converging performance of the output error function during the learning process.

Transfer function pairs also an important parameter that should be adjusted carefully. Choosing of transfer function based on the nature of data to be analysed. In the present work, purelin-purelin transfer function was used due to the linear correlation between absorbance and concentration. The learning rate controls the degree at which connection weights are modified during the learning phase. The optimized parameters values of the ANN for BRM and GUA were shown in **Table 4.**

ANNs show better RMSEP than PLS-1 which may be due to the fact that ANNs is a type of artificial intelligence where there is less chance for over-fitting than that may occur in PLS calibrations. % recoveries, % RSD and RMSEP values of the validation set for ANN and GA-ANN models are listed in **Table 3**.

The application of the ANN on the raw data after using the variable selection technique GA shows improvement of the results. A large number of nodes in the input layer of the network (wavelengths) increase the CPU time for ANN modelling. GA allowed the use of less number of neurons (shorter training time) than those used in the network utilized the raw data

Table 3: **Validation parameters of the proposed methods.**

Mixture number	PLS		GA-PLS		ANN		GA-ANN	
	BRM	GUA	BRM	GUA	BRM	GUA	BRM	GUA
2	101.89	100.6	100.04	100.24	100.85	100.86	101.35	100.65
4	97.53	99.19	101.36	98.79	100.89	98.29	100.61	99.61
6	98.25	98.16	99.25	100.36	100.06	100.63	99.39	100.36
8	100.62	100.56	100.32	100.98	98.26	101.36	100.34	100.17
10	99.65	100.76	100.89	99.77	99.65	100.31	99.91	100.06
12	100.69	98.57	99.09	98.29	100.43	98.9	100.34	99.24
14	100.14	99.67	98.74	98.36	100.99	99.33	99.61	99.37
16	99.84	100.17	100.34	99.18	99.19	99.67	100.39	99.51
18	100.29	99.97	100.29	100.35	100.68	99.68	100.22	100.37
20	100.39	98.98	101.79	99.64	100.98	99.91	100.38	99.97
22	99.28	96.63	99.95	99.24	99.35	99.46	100.35	100.98
24	100.96	96.19	101.94	98.11	99.96	98.31	101.32	99.61
Mean (%R)	99.96	99.12	100.33	99.44	100.11	99.73	100.35	99.99

%RSD	1.185	1.524	1.022	0.941	0.862	0.962	0.577	0.539
RMSEP	0.0399	0.1978	0.0374	0.1731	0.0372	0.1538	0.0348	0.1335

Table 4: Optimized parameters of ANN.

Method	ANN		GA-ANN	
Drug	BRM	GUA	BRM	GUA
Architecture	151-10-1	151-7-1	75-4-1	73-4-1
Hidden neurons number	10	7	4	3
Transfer functions	Purelin-Purelin			
Learning rate	0.1	0.1	10	10
Training function	TRAINLM			

Analysis of pharmaceutical sample

The proposed procedure was applied for determination of both BRM and GUA in Muclear® capsules. Satisfactory results were obtained in good agreement with the label claim. The obtained results were statistically compared to those obtained by the reported method [8]. No significant differences were found by applying two tail student *t*-test and F-test at 95% confidence level [19], indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **Table 5.**

Table 5: Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of BRM and GUA in Muclear® capsules.

Method	Drug	Mean	N*	S.D	% RSD	*t***	F**
PLS	**BRM**	100.01	**5**	0.943	0.943	0.40(2.31)	1.07(6.39)
	GUA	99.79		1.106	1.109	0.59(2.31)	1.31(6.39)
GA-PLS	**BRM**	99.81		0.867	0.868	0.08(2.31)	1.26((6.39)
	GUA	99.73		0.981	0.984	0.54(2.31)	1.67(6.39)
ANN	**BRM**	99.73		0.590	0.592	0.07(2.31)	2.72(6.39)
	GUA	99.65		0.906	0.909	0.43(2.31)	1.96(6.39)
GA-ANN	**BRM**	99.83		0.790	0.792	0.11(2.31)	1.52(6.39)
	GUA	100.01		0.857	0.857	0.97(2.31)	2.19(6.39)
Reported method [8]	**BRM**	99.77		0.974	0.976	-------	-------
	GUA	99.35		1.268	1.276	-------	-------

***No. of experimental. **The values in the parenthesis are tabulated values of t and F at (p= 0.05).[8] HPLC using C_{18} column, mobile phase was acetonitrile: methanol: buffer [30:25:45 v/v], pH 4.2) at a flow rate (1mL/min) and UV detection at 220 nm.**

CONCLUSION

In this study, first reported, accurate and precise multivariate chemometric models were developed for simultaneous determination of BRM and GUA.

The developed methods have the advantages of being sensitive, time saving and low cost unlike HPLC procedure which is time consuming and of high cost.

Application of GA on PLS and ANN models enhance the results with respect to RMSEP. The developed methods can be applied for routine and analysis of bromhexine and guaifenesin in its pure forms and in capsules.

Conflict of interest

The authors declare that they have no conflict of interest.

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