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UV Spectrophotometric Determination of Allopurinol and Benzbromarone in their Binary Mixture using Artificial Neural Networks and Genetic Algorithm- Artificial Neural Networks

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Abstract: *Chemometric assisted spectrophotometric models were suggested for the selective quantitative determination of allopurinol and benzbromarone without previous separation. Multivariate artificial neural networks and genetic algorithm artificial neural networks were developed. The proposed methods were validated and successfully applied for the determination of the drugs in their commercial preparation.*

Keywords: *Allopurinol, artificial neural networks, benzbromarone, genetic algorithm, multivariate calibration methods, pharmaceutical tablets.*

1. INTRODUCTION

Allopurinol (ALP), **figure (1),** is (1, 5-Dihydro-4H-Pyrazolo [3, 4-d] pyrimidin-4-one).[(1)]It is used in gout and hyperuricemia to inhibit the enzyme xanthine oxidase, thus preventing the oxidation of hypoxanthine to xanthine and xanthine to uric acid resulting in the reduction of urate and uric acid concentrations in plasma and urine. [(2)]

Benzbromarone (BENZ), **figure (2),** is (3, 5-dibromo-4-hydroxyphenyl)- (2-ethyl-3-benzofuranyl) methanone. [(1)] It is a uricosuric drug that reduces plasma concentrations of uric acid by blocking renal tubular reabsorption.[(2)]

Combination of ALP and BENZ has the advantages of greater therapeutic effect than with either drug alone. This combination causes manifold reduction in uric acid concentrations in plasma and urine as compared to double dose of the individual drug when used alone. Also, this combination helps to decrease the dose of each active ingredient, and as a result, decreases the side effects of each of component if given separately in high doses. [(3)]

Reviewing the literature in hand, only four reports have been published for determination of the studied mixture, the first and second reports manipulated UV spectrophotometric methods. [(3,4)] The third and fourth reports manipulated TLC-densitometric and RP-HPLC methods. [(5,6)]

The developed work aimed to develop and validate simple and sensitive chemometric assisted spectrophotometric methods for the simultaneous determination of ALP and BENZ in powdered forms, laboratory prepared mixtures and in pharmaceutical formulation namely, artificial neural networks and genetic algorithm artificial neural networks. The proposed methods have been optimized and validated as per the International Conference on Harmonization (ICH) guidelines ICH, and were found to comply with the acceptance criteria. [(7)]

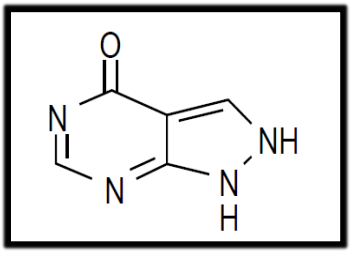

Figure3. *Structural formula of ALP*

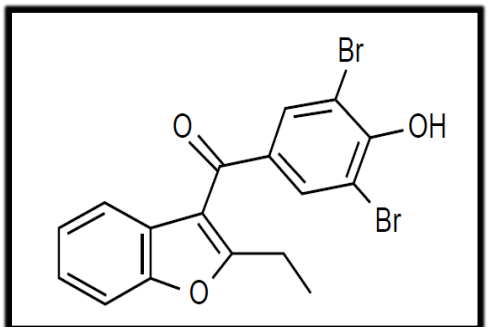

Figure4. *Structural formula of BENZ*

2. EXPERIMENTAL

2.1. Materials

2.1.1. Pure Samples

Pure ALP and BENZ standard samples (99.36 and 99.92 % for ALP and BENZ, respectively), were kindly supplied by Global Napi Pharmaceuticals, 2nd Industrial Zone, 6th of October City- Egypt.

2.1.2. Pharmaceutical Preparation

Alloben® tablets (100/25) (B.NO. B 10601) labeled to contain 100 mg ALP + 25 mg BENZ and were manufactured by Global Napi Pharmaceuticals, 2nd Industrial Zone, 6th of October City- Egypt, were purchased from a local market.

2.1.3. Chemicals and Reagents

Methanol, analytical grade (El-Nasr Company, Egypt).

2.1.4. Apparatus

- Shimadzu UV-Visible 1800 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. Scans were carried out in the range from 200 to 400 nm at 0.1 nm intervals.
- Sonicator (Q sonica, LLC,53 Church Hill Road Newtown, CT.U.S.A).

2.1.5. Standard Solutions

Stock standard solutions for both ALP and BENZ containing 1000 µg/mL for each drug were prepared separately in methanol. Working standard solutions of both drugs (100 µg/mL) were obtained by dilution of the respective stock solutions with methanol.

2.1.6. Software

- UV-Probe personal spectroscopy software version 2.1. (Shimadzu).
- All chemometric methods were implemented in Matlab R2013b (8.2.0.701).
- All models were carried out by PLS toolbox software version 2.1.

2.2. Procedures

2.2.1. Experimental Design

Experimental design has been carried out using multilevel multifactor for construction of the calibration and validation sets [8]. 25 samples were constructed based on five-levels, two factors. These 25 samples were prepared by transferring different volumes of ALP and BENZ from their standard solutions (100 µg/ mL) into 10-mL volumetric flasks and completed to volume with methanol. The choice of concentrations was based on the linearity range, (2-16 µg/ mL) of ALPand (1-16 µg/ mL) of BENZ, and the ratio of the two drugs in the pharmaceutical preparation. The central level of the experimental design was 10 µg/ mL of both ALP and BENZ. The absorption spectra of different ratios of the prepared mixtures were recorded over the wavelength range 200-400 nm with 1 nm interval. The region from 200-219 nm accounted for the rejection of this part from the spectra.

ANN and GA-ANN models were constructed by transferring the recorded spectral data to Matlab® version R2013b (8.2.0.701), together with PLS-Toolbox 2.1. Software to apply the data analysis. 13 mixtures of this design were used as a calibration set and the other 12 mixtures were used as a validation set. The predictability of the proposed multivariate models was evaluated.

2.2.2. Procedure for Pharmaceutical Preparation

Ten **Alloben®** tablets were accurately weighed and finely powdered, then a quantity equivalent to 100 mg of ALP and 25 mg of BENZ were extracted three times with 25 ml of methanol by mixing well for 10 minutes by vigorous shaking then the prepared solution was sonicated for 20 minutes, finally the prepared solution has been filtered through Whatman filter paper No. 41 into 100 ml-volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and the volume was made up to the mark with methanol to obtain stock solution (1000/250 µg/mL). Working solution (100/25 µg/mL) was obtained by further dilution with methanol, and then analyzed using the general procedures of the proposed methods.

2.2.3. Reported Method

Dual wavelength, in which two wavelengths were selected for each drug in such a way that the difference in absorbance was zero for the second drug. At wavelengths 238.2 and 261.2 nm ALP had equal absorbance values; therefore, these two wavelengths have been used to determine BENZ; on a similar basis 253 and 274.4 nm were selected to determine ALP.

3. RESULTS AND DISCUSSIONS

In the present study, artificial neural networks and genetic algorithm artificial neural networks chemometric assisted spectrophotometric methods were applied for the determination of ALP and BENZ in their binary mixture in bulk powder and pharmaceutical preparation.

3.1. Spectral Characteristics

The zero-order absorption spectra of ALP and BENZ, as shown in **figure (3)**, show severe overlap, which did not permit direct determination of them. So, the previously mentioned chemometric methods have been developed to resolve this overlapping and enable determination of ALP and BENZ without previous separation.

3.2. Optimization of Experimental Conditions

Initially constructing the calibration set for the binary mixture of the studied drugs was done to design the described models. The proposed methods were optimized with the aid of five-levels, two factors experimental design [8] resulting in 25 sample mixtures. These 25 sample mixtures **table (1)** were split to two groups. The first group was 13 mixtures which were used to construct a calibration set. Another group was 12 mixtures were to compute the predictive benefits of the model. The choice of concentrations was based on the linearity range of each drug and the ratio of the two drugs in the pharmaceutical preparation. The selected wavelength range and the used spectral mode enhance the quality of the analysis. So, the recorded spectral data was pre-processed and the region 200-219 nm was rejected due to non-absorbance behavior, this results in 181 variables.

For the optimization of a neural network, a trial and error method has to be used to find the best neural network architecture. Selecting the optimum parameter values for constructing a network is not an easy task. In fact, the parameters are mutually related, so a compromise must usually be adopted. The error function RMSEP used as criterion for finalizing the learning process [9].

The output layer is the concentration matrix of each component. The hidden layer consists of just a single layer which has been considered sufficient to solve similar or more complex problems. Moreover, more hidden layers may cause over fitting.

Among other ANN parameters, the hidden neurons number which is related to the converging performance of the output error function during the learning process. A grid search was done. The resulting error in prediction was the optimization criterion in this selection procedure. Another parameter that should be optimized carefully is the transfer function pair. Choosing of transfer function depends on the nature of data being analyzed. In this work, purelin–purelin transfer function was implemented due to linear correlation between absorbance and concentration. The learning rate controls the degree at which connection weights are modified during the learning phase. For

optimization of these parameters, many experiments have been done to improve the model performance. The values of the optimized ANN parameters for ALP and BENZ were shown in **table (2)**

However, with the aim of increasing the quality of the calibration, wavelengths selection was performed, in such a way that uninformative variables were eliminated. This variables selection was carried out using the GA procedure. The GA was run on 181 variables for ALP and BENZ using a PLS with the maximum number of latent variables determined by cross-validation on the model containing all the variables. The adjusted GA parameters were shown in **table (2)**.

GA reduced absorbance matrix to about 38.674 and 29.281% of the original matrix (70 and 53 variables for ALP and BENZ, respectively). The selected variables were used as input layer to ANN model. A large number of nodes in the input layer of the network (wavelengths) increase the CPU time for ANN modeling. GA allowed the use of less number of neurons (shorter training time) than those used in the network built with the raw data.

Recoveries, mean, SD, RMSEC and RMSEP and other validation parameters ALP and BENZ in both models were shown in **table (3)**

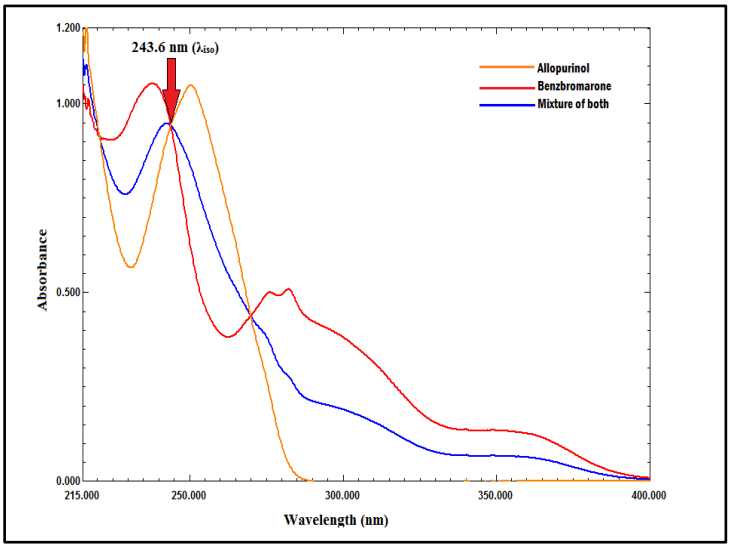

Figure3. *Absorption spectra of ALP (16 µg/mL), BENZ (16 µg/mL) and their mixture (8+8 µg/mL) of each.*

3.3. Pharmaceutical Application

The proposed chemometric assisted spectrophotometric methods were applied for the determination of ALP and BENZ in **Alloben®** tablets. Satisfactory results were obtained in good agreement with the label claimed, indicating no interference from excipients and additives. The obtained results were statistically compared to those obtained by the reported method [3]. No significant differences were found by applying student's *t*-test and *F* value at 95% confidence level [10], indicating good accuracy and precision of the proposed method for the analysis of the studied drugs in their pharmaceutical dosage form, as shown in **table (4)**.

Table1. *Experimental design of concentrations of ALP and BENZ mixtures used in the chemometric assisted spectrophotometric methods*

No. of Mix	ALP (µg/mL)	BENZ (µg/mL)
1	10	10
2	10	8
3	8	8
4	8	12
5	12	9
6	9	12
7	12	10
8	10	9
9	9	9
10	9	11
11	11	12

12	12	11
13	11	10
14	10	12
15	12	12
16	12	8
17	8	11
18	11	8
19	8	10
20	10	11
21	11	11
22	11	9
23	9	8
24	8	9
25	9	10

The shaded rows represent the calibration set.

Table2. *Optimized parameters of ANNs and GA-ANNs for ALP and BENZ*

Methods	ANNs		GA-ANNs	
Drug	ALP	BENZ	ALP	BENZ
Architecture	181-7-1	181-6-1	70-5-1	53-4-1
Hidden neurons number	7	6	5	4
Transfer functions	Purelin – Purelin			
Learning rate	0.1	0. 1	10	10
Training function	TRAINLM			

Table3. *% Recoveries, mean, SD, RMSEC and RMSEP for ALP and BENZ in the calibration and the validation samples by ANNs and GA-ANNs models*

Mixtures	Calibration set				Validation set			
	ALP		BENZ		ALP		BENZ	
	ANN	GA-ANN	ANN	GA-ANN	ANN	GA-ANN	ANN	GA-ANN
1	100.00	98.74	99.99	100.07	102.17	100.23	100.25	101.69
2	100.00	99.76	99.98	100.33	100.25	99.34	100.00	98.21
3	100.00	99.37	100.00	98.93	100.56	98.03	104.42	97.02
4	104.71	99.65	103.35	100.19	98.82	97.97	98.57	98.41
5	100.00	97.96	99.97	100.28	100.67	99.06	100.18	97.98
6	100.00	98.33	100.03	100.28	102.67	97.54	99.81	101.63
7	100.00	99.81	100.00	100.15	96.03	98.93	100.25	99.36
8	100.00	98.79	99.08	100.23	101.00	100.05	100.75	102.09
9	100.25	101.17	96.79	99.82	99.82	100.68	104.03	100.95
10	100.00	100.49	96.26	99.92	98.00	98.66	99.84	98.50
11	100.21	100.44	100.02	100.20	101.09	99.38	101.64	101.43
12	99.14	99.32	99.95	99.50	100.25	100.89	98.31	100.79
13	100.00	100.14	99.98	99.83	-------	---------	----------	--------
Mean	100.33	99.54	99.65	99.98	100.11	99.23	100.67	99.84
SD	1.3429	0.919	1.700	0.396	1.809	1.083	1.877	1.773
RMSE	0.165	0.104	0.180	0.036	0.182	0.134	0.194	0.179

* *RMSEC* ** *RMSEP*

Table4. *Determination of ALP and BENZ in Alloben® tablets by the proposed spectrophotometric and reported methods*

Parameters	ANN		GA-ANN		Reported method [3]	
	ALP	BENZ	ALP	BENZ	ALP	BENZ
n*	5	5	5	5	5	5
Average (%Recovery)	99.70	101.59	101.17	101.57	99.99	100.68
%RSD	1.284	0.661	0.595	1.559	1.340	1.570
Student's *t*-test (2.306)**	0.349	1.188	1.794	0.892		
F value (6.388)**	0.913	0.181	0.202	1.003		

**Number of samples*

** *The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)*

4. CONCLUSION

The proposed multivariate calibration methods were simple, rapid, sensitive and precise and could be easily applied in quality-control laboratories for the simultaneous determination of ALP and BENZ in pure bulk powders. Moreover, these methods could be applied for dosage form analysis as well as in pure powder form without any preliminary separation step.

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