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Artificial Neural Networks for the Simultaneous Spectrophotometric Determination of Amoxicillin Trihydrate, Metronidazole and Pantoprazole in pharmaceutical Formulations.

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Abstract

Amoxicillin Trihydrate, Metronidazole, and Pantoprazole have been spectrophotometric anticipated in drugs by utilization artificial Neural Networks and UV-Visible spectroscopy. Three components' spectra of absorption had been recorded within the 200-400 nm wavelength variety with a 1 nm interval. The standardization models were carefully tested at numerous levels of concentration the usage of artificial tertiary aggregate spectra (organized utilizing orthogonal strategy). For building and testing models, 3 layers feed-forward neural networks with the lower backward-spread set of rules (B.P) were used. numerous parameters had been optimized, consisting of the neuron quantity in the hidden layer, learning rate, and range of epochs. The RMSE for each analyte inside the actual pattern turned into 0.25, 0.34, and 0.43 for Amoxicillin Trihydrate, Metronidazole, and Pantoprazole, respectively. The results found out a high degree of agreement between predicted and actual values of concentration. The projected approach is a modest, properly-described, and appropriate technique for figuring out the concentrations of Amoxicillin Trihydrate, Metronidazole, and Pantoprazole in tablets.

Keywords: Artificial Neural Networks, Amoxicillin Trihydrate, Metronidazole, Pantoprazole, and pharmaceutical formulations.

1. Introduction

Amoxicillin Trihydrate, which biochemical structure is displayed in (Fig. 1A), is considered as broad-spectrum, β-lactam anti-microbial that is managed orally It has a place to the foremost endorsed drugs and is delivered on a large scale around the world [1]. It is an anti-microbial phenolic β-lactam with noteworthy movement against both Gram-positive and Gram-negative microbes. This anti-microbial is extensively utilized to manage irreversible illnesses in animals and humans, for example, nausea bronchitis, skin and throat infections, and pneumonia, ear infections, and urinary tract infection [2]. Amoxicillin slaughters microbes by interferometer with the union of the bacterial cell divider [3]. As a result, the bacterial cell wall is debilitated, the cell swells and after that breaks. Amoxicillin is promptly hydrolyzed by the staphylococcal penicillinase [4]. It has been perceived that AMT has no biodegradation susceptibility and AMT has little rate of metabolism in human being leading to its excretion in un-metabolized formula by about 80-90% from the body of the humans [5]. Metronidazole, a nitroimidazole, is still a first-line treatment for infections associated with gastrointestinal tract inflammatory syndromes, comprising Clostridium difficile induced colitis [6]. MET is a synthetic antimicrobial derived from azomycin, nitroimidazole, as shown in (Fig. 1B), which is formed by the Proteobacteria and Actinobacteria genera. That complex was first utilized to treat trichomonas's, which is the protozoan Trichomonas vaginitis induced infection, in 1959. Furthermore, MET has been shown to be operational in treating vomiting and hepatic boils caused by Entamoeba histolytic, the protozoan parasite of the intestine. It was likewise effective in contradiction of additional intestinal parasite called Giardia lamblia that is responsible for epigastric pain and malabsorption [7]. The mechanism of action of metronidazole has not been fully elucidated. However, its nitro group reduction by anaerobic organisms appears to be responsible for the cytotoxic and antimicrobial effects. Pantoprazole Sodium (PPZS) is a proton pump inhibitor (PPI) that is widely used to reduce acid production by restricting the enzyme in the stomach wall that

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produces acid, as shown in (Fig. 1C). Acid is required for the formation of most ulcers in the esophagus, stomach, and duodenum, and lowering acid with PPIs drugs prevents ulcers and allows any ulcers that do exist to heal [8,9].

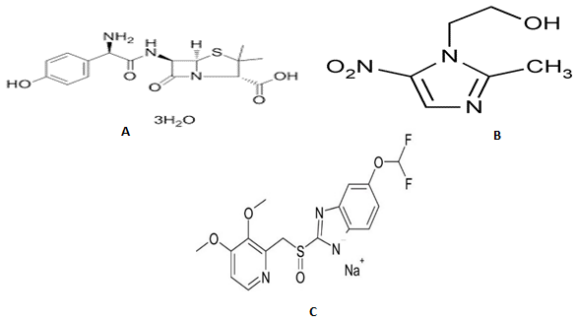

Fig. 1. Chemical structure of Amoxicillin Trihydrate (A), Metronidazole (B) and Pantoprazole Sodium (C)

Recent methods for determination of AMT include liquid chromatography [10,11], voltammetry determination [12, 13], flow injection analysis by utilization of ultraviolet revealing, chemiluminescent flow analysis [14], conductometric and potentiometric [15] and various spectrophotometric methods [16-19]. An overview of the writings discovered that numerous HPLC (high performance liquid chromatographic) have been conveyed for the determination of AMT [20–22] and MET [23–26] and in serum or plasma. even though the technique of HPLC has been stated for the concurrent metronidazole and amoxicillin willpower in the plasma of the humans by utilization of liquid extraction, this technique calls for using a gradient elution assay and detectors with two specific wavelengths [27].High performance liquid chromatographic techniques had been pronounced for the pantoprazole willpower in serum or plasma, inclusive of enantiomer separation with direct injection [28, 29] and pattern education tactics with column switching [30], and an High performance liquid chromatographic approach has been advanced for the instantaneous AMT, PPZS and MET willpower in the plasma of humans. Numerous excessive HPLC strategies were posted for the man or woman assessment of MET and AMT in biotic models [31-35].

Several methods have been used for PPZS determination in pharmaceutical dosage forms and biological fluids either alone or in combination with other co-administered drugs including spectrofluorimetry [36], high performance liquid chromatography, capillary electrophoresis [37,38] and electrochemical methods [39]. Hydrophilic interaction liquid Chromatography (HILIC) accompanied by using mass spectrometric detection approach changed into evolved for the willpower of metronidazole and amoxicillin in the serum of the humans [40].

1.1. Methodology

Artificial Neural Network will be an artificial intelligence technique type, which takes after natural apprehensive organization in having the capacity to discover the association between yields and inputs. Artificial Neural Network is composed of elements called artificial neurons that are interconnected through associations called weights. usually it network is balanced, grounded totally on a contrast of the yield and the goal, till the ANN yield suits the goal.

Regularly numerous enter/target sets are utilized to teach a network. ANN functions a great advantage over the traditional multivariate strategies in modeling linear and non-linear relationship between variables. There are several papers that depict the application of ANN on linear and non-linear facts [41,42].

The type of ANN applied in this paper is feed-forward show which became prepared with the back propagation of errors learning calculation, the back propagation ANN is utilized in signal processing, facts optimization, spectra and calibration elucidation and prediction. it is formed of 3 layers, the layer of input in which the input information is delivered (e.g. spectrophotometry absorbance).

Those inputs are surpassed to second hidden level where inputs are rectified and balanced with the aid of weights and after that eventually exceeded to outside maximum layer (yield level) to furnish yields (e.g. concentration). The institutions (masses) among layers are surpassed frontward (from enter to yield layer), thus it is named feed-forward ANN. The expected concentrations are as equated with real concentrations and the difference between them is known as the error that is again proliferated (and so referred to as feed-forward ANN with the returned proliferation of mistakes learning set of rules) to arrange yet again to be reduced thru assist weights alteration. ANN is repeated numerous times in such manner until the error comes to a minimal range.

2.Experimental

2.1. Apparatus

A double-beam ultraviolet observable spectrophotometer (shimadzu, japan) type UV-1650 pc with one centimeter path length quartz cell, linked to an IBM-well-matched computer. The speed of the wavelength-scanning is 2800 nm/min and the spectral

bandwidth was 2 nm. A short wavelength (254 nm) ultraviolet lamp.

The whole raptness spectral measurements were prepared by utilization of JASCO v-530 (UV-VIS) spectrophotometer (Japan), with band width 2 nm and speed of scanning of about 400 nm per minute, equipped with 10 millimeter matched quartz cells.

A digital pH meter (JEANWAY 3510) was adjusted by utilization of buffering medias and then utilized for measurements of pH.

2.2. Software

Artificial Neural Network become applied in MATLABW 7.1.0.246 (R14) the usage of Toolbox of the Neural Network. the F check and t check were achieved by using the Microsoft EXCEL. The whole measurements had been done the use of intel core™ i5-2400, 3.10 GHz, RAM of about 4.00 GB beneath Microsoft home windows 7. The spectrophotometric measurements have been carried out at room temperature and all solutions have been organized at the same day earlier than that of the evaluation.

2.3. Reagents and chemicals

The AMT, MET and PPZS were kindly delivered by the National Organization for Drug Control and Research (NODQAR). National Organization for Drug Control and research (Cairo, Egypt). The purities of PPZS, AMT, MET were 99.78%, 99.5%, and 99.5% respectively. AMT was obtained from North China Pharmaceutical Company (NCPC). MET was obtained from Amriya Pharmaceuticals. PPZS was kindly delivered by AL-Rowad Industrial pharmaceutical company (RPIC).

2.4.Preparation of AMT, MET and PPZS standard solutions

Stock solutions of 100 µg/ml become organized through transferee adequate weighed of AMT, MET and PPZS in 25 ml 0.1M HCl then complete the suitable volume (100 ml) with the same solvent, the stock solutions had been in a fridge and double distilled water become applied in the course of all experiments.

2.5.Preparation of pharmaceutical tablets sample solutions

Ten tablets were finely powdered and an appropriate portion comparable to the median mass of one tablet) was dissolved in 100 ml of 0.1M HCl. It was mechanically shaken for a period of 20 min and filtered into a 250 ml calibrated flask. The residue was washed twice with the same solvent and diluted to the volume. After filtration, the obtained clear solution was balanced to the volume of 100 ml with the same solvent. This solution was further diluted to get the appropriate concentration for the UV measurements.

2.6. Urine sample analysis

Urine spiked with AMT, MET and PPZS obtained by following procedure; an aliquot of pure AMT, MET and PPZS was added into 10 mL urine sample. A 1 mL of the resulting urine solution was mixed with 5 ml (1 M) sodium hydroxide buffer. The mixture was rotated for 25 min and centrifuged at 2500 rpm for 10 min. Subsequent residue was dissolved in universal buffer into a 10 mL volumetric flask and diluted to the mark with buffer solution.

3.Results and Discussion

3.1. Calibration procedures

Standard solutions aliquots equal to a 100 µg ml^{-1} of AMT, MET and PPZS were transported discretely into a chain of 10 ml volumetric flasks and the samples had been adulterated to the capacity with the volume with 0.1N HCl and combined nicely. The absorption spectra zero order of the organized solutions had been documented from (200-400) nm towards water as a blank and kept inside the computer. The absorbance changed into calculated at 230 nm for determination of AMT and 280 nm for determination of MET and PPZS, respectively. The graphs of calibration were fashioned through connecting the stated wavelength absorbance to the conforming AMT, MET and PPZS concentrations respectively.

The equations of regression for the information were calculated.

3.2. ANN technique

The AMT, MET and PPZS absorption spectra overlapping without a doubt, as described in Fig.2. That truly designated that concurrent willpower of additives through UV–Vis direction spectrophotometry couldn't yield dependable outcome for quantitative analysis of tertiary mixture of AMT, MET and PPZS.

The multivariate calibration requires a suitable experimental design of the standard composition of calibration set to provide the best prediction. The orthogonal array design method was used to construct the calibration set. Application of four-level orthogonal array design led to construction and optimization of the ANN model. therefore, set of tertiary artificial mixes existing in random ratio were set, one set with 25 samples for ANN version optimization and production (Table 1), and one set with 8 samples were employed as an independent test to evaluate the quality of the model.

3.3. the effect of pH

The pH effect changed into calculated for the spectral departure. The sample changes in pH had no influence in decreasing or separation of overlying spectra. Because of their mutual interference, concurrent dedication of the tertiary mix of AMT,

MET and PPZS isn't always viable by utilizing classical spectrophotometric strategy.

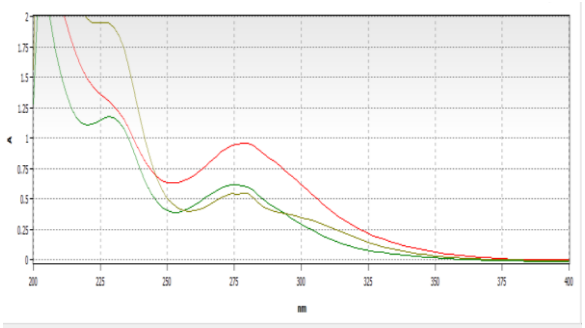

Fig. 2. Absorbance spectra of AMT 20 µg ml^{-1} (I), MET 20 µg ml^{-1} (II) and PPZS(III) 20 µg ml^{-1} in 0.1N HCl.

Table 1. the calibration sample Composition.

Sample	Concentration (µg/ml)		
	AMT	MET	PPZS
1	1.6	1.0	2.1
2	0.7	1.8	1.7
3	0.2	1.8	2.1
4	1.6	0.1	1.2
5	0.7	0.5	0.3
6	0.2	1.0	1.2
7	1.1	0.1	1.7
8	2.0	1.4	2.1
9	0.2	0.5	0.8
10	1.6	1.8	0.8
11	0.7	0.1	2.1
12	2.0	1.8	0.3
13	0.2	1.4	1.7
14	1.1	1.8	1.2
15	1.1	0.5	2.1
16	1.6	1.4	0.3
17	0.2	0.1	0.3
18	1.6	0.5	1.7
19	0.7	1.4	1.2
20	1.1	1.4	0.8
21	2.0	0.5	1.2
22	0.7	1.0	0.8
23	1.1	1.0	0.3
24	2.0	1.0	1.7
25	2.0	0.1	0.8

3.4. ANN models Training and optimization

The standardization information gotten from investigational have been collected in a matrix information by Microsoft office Excel (Ver. 2016) means and conveyed to MATLAB (Ver. 7.1). A limited printed software in MATLAB surroundings turned into utilized to create the BP-artificial neural network with sigmoidal revolution feature within the knobs. The net included 3 layers, specifically, solitary input level with forty enter knots that signify the absorbance concentrations calculated at 25 exclusive wavelengths from every spectrum. Hidden layer turned into formed of 4 optimized process elements and yield layer with a single output node that contained the concentration of component. Although, a neural network has the property to model multiple responses simultaneously, it is recommended that one model only generate one response at a time and therefore our network had a single output node.

Network optimization was performed by changing each time one of the internal parameters of ANN such as the number of nodes in the hidden layer, number of epochs and learning rate while keeping rest of them constant. The proper number of nodes in the hidden layer was determined by training ANN with different number of nodes in hidden layer, it has a great effect on the prediction result. Fig. 3. shows the variation of Sum- Square Error (SSE) value of the network when the number of node in hidden layer changed. The result shows that, by increasing the number of nodes for AMT, MET and PPZS, the SSE value increasing and then decreasing.

To study the ability of established ANN in prediction of individual analysis found in synthetic and real mixtures, eight tertiary synthetic mixture test samples were analyzed using proposed method. The predicted concentration of the analytes in each sample was then compared with the known concentration of the analytes in the respective sample; the predicted results are given in Table (2). The plots of the predicted concentration versus actual values are shown in Fig. 4. for AMT, MET and PPZS, as well as the line equations and R2 values.

Performances of these neural network is compared by their generalization accuracy and convergence speed. Fig. 5 shows the error versus number of iteration for training of neural network. As in the above descriptions, the reliable results for the quantitative prediction of AMT, MET and PPZS in samples were obtained by the application of ANN approach.

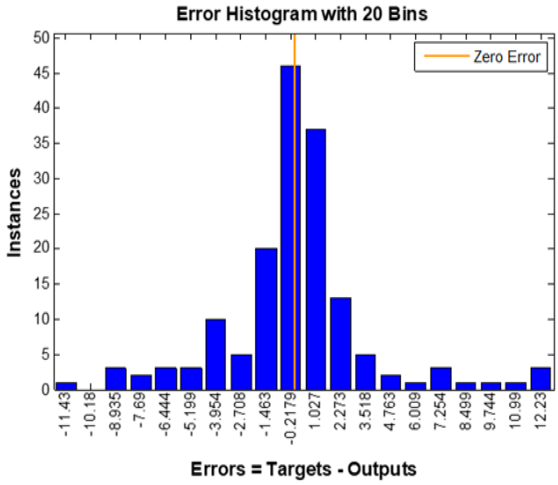

Fig. 3. the relation between nodes number in the hidden layer vs. errors for tertiary mixture.

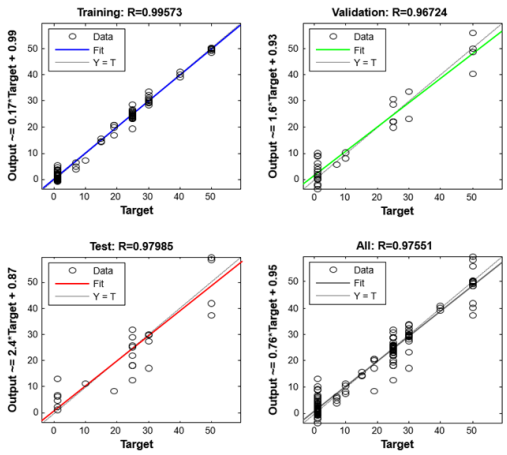

Fig. 4. Predicted concentration plots vs. real concentration for tertiary mixture by ANN (µg ml^{-1}).

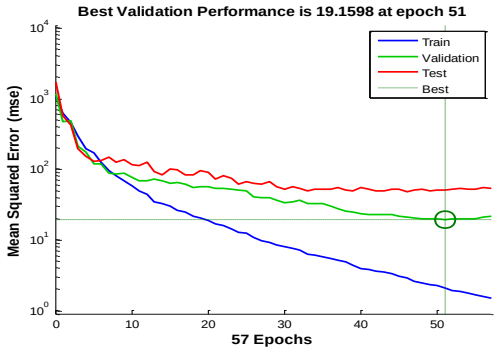

Fig. 5. Error performance for training of ANN with actual inputs.

3.5. End result for result sample

By utilizing the prepared tablet solutions explained within the above section "Preparation of real samples", ANN were applied to the simultaneous quantitative determination of AMT, MET and PPZS in tablets. The obtained experimental results of three drugs in marketed tablets were shown in (Table 3). additionally, a comparison of the spectra from the AMT, MET and PPZS in standard and drug formulation solutions appears comparative spectral design in Fig.2. The statistical parameters, specifically, percent relative standard deviation, root mean square error is appeared in (Table 3).

3.6. Determination of AMT, MET and PPZS in pharmaceutical preparations and biological fluids

In order to appear the analytical applicability of the proposed methods, to begin with calibration curve obtained from ANN model at pH 2 were applied assurance of AMT, MET and PPZS in real samples (pharmaceutical formulations) and complex matrices, i.e. urine. The results showed that satisfactory recovery for AMT, MET and PPZS could be obtained using the prescribed methods. Results of the determination is summarized in (Table 4).

The data obtained by these methods reveal the capability of the methods for assurance of AMT, MET and PPZS in real samples such as pharmaceutical formulations and complex matrices such as urine without considerable error. The average recoveries in pharmaceutical formulation and complex matrix (urine) are summarized in (Table 4).

ANOVA

The results achieved by means of the proposed method were compared with utilization of one-way ANOVA test. The measured F-standards (P = 0.05) had been less than the tabularized F-standards. No crucial variations had been perceived among the effects of the proposed technique at 95% confidence limit. The conforming consequences are summarized in (Table 5). Results of the study recommend that, there are no critical errors in simultaneous determination of AMT, MET and PPZS by proposed methods.

Table 2. Validation set configuration, their forecast by the artificial neural network model and statistical parameters.

Sample	**Actual**			**Prediction**			**Recoveries (%)**		
	AMT	**MET**	**PPZs**	**AMT**	**MET**	**PPZs**	**AMT**	**MET**	**PPZs**
1	2	2	2	2.20	1.80	1.80	110.00	90.00	90.00
2	3	3	3	2.85	3.31	3.30	95.00	110.33	110.00
3	4	6	5	3.84	6.11	5.07	96.00	101.83	101.4
4	5	8	9	5.05	8.40	9.34	101.00	105.00	103.77
5	6	9	9	5.80	9.32	9.52	96.66	103.55	105.77
6	7	10	8	6.66	10.12	8.21	95.14	101.2	102.62
7	8	12	10	7.50	11.55	9.88	93.75	96.25	98.8
8	10	14	9	9.87	14.21	8.91	98.7	101.5	99.00
Mean Recovery (%)							98.23	101.20	101.42
RMSE*							0.25	0.34	0.43

Table 3. Determination of AMT, MET and PPZS by utilization of artificial neural network model in pharmaceutical formulation.

	Taken AMT	Taken MET	Taken PPZS	Found AMT	Found MET	Found PPZS	Recovery AMT	HPLC	Recovery MET	HPLC	Recovery PPZS	HPLC
	2	14	17	1.93	13.87	16.88	96.61	100.6	99.07	99.15	99.29	99.86
	16	5	17	15.75	4.91	17.07	98.45	100.5	98.11	98.05	100.4	100.2
	20	5	12	20.45	5.08	12.08	102.3	102.4	101.58	101.82	100.7	100.92
Mean							99.11	101.2	99.67	99.59	100.1	100.33
Variance							8.31	1.07	3.75	3.21	0.56	0.29
Observations							3		3		3	
df							2		2		2	
F							7.77		1.17		1.93	
t Stat							1.16		0.06		0.35	
F Critical one-tail	19											
T Critical two-tail	2.776											

* Each result is the average of three different separate determinations. The theoretical values of t and F are 2.78 and 19, Figures in parentheses are the tabulated t and F values respectively at P= (0.05).

Table 4. Determination of AMT, MET and PPZS in urine by using artificial neural network model.

Sample	AMT		MET		PPZS		Recovery %	HPLC*
	Actual	prediction	Actual	prediction	Actual	prediction		
1	30	29.31	90	91.17	20	19.31	99.95	99.96
2	30	31.33	90	89.49	20	21.22	99.76	100.2
3	30	29.44	90	88.62	20	19.44	99.95	100.31
F-test								5.56
t-test								0.75
RSD	0.29		0.42		0.29			
RMSE	1.06		1.33		1.06			

* Each result is the average of three different separate determinations. The theoretical values of t and F are 2.78 and 19, Figures in parentheses are the tabulated t and F values respectively at P= (0.05).

Table 5. The ANOVA outcomes by means of applying of the proposed technique to the actual pattern.

Sample	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	HPLC*
Urine sample 1	1.60	1.52 2.21	95.00	95.99
Urine sample 2	2		110.5	110.66
F-test				0.80
t-test				0.75

df, degree of freedom, the theortical standards of t and F are 2.78 and 19, correspondingly at sureness boundary at 95% confidence limit and 5 degrees of freedom (p=0.05)

4. Conclusion

This study confirmed the high potential of convenience for ANN methods combined with UV–Vis spectrophotometer for simultaneous quantitative analysis of tertiary mixture Amoxicillin Trihydrate,8 Metronidazole and Pantoprazole Sodium in pharmaceutical formulation. Chemo- metrics calibration techniques in spectral analysis have been broadly utilized in quality control of drugs in mixture and multicomponent formulations with overlapping spectra, as separation procedures in the drug determinations are not required. Although other methods such as chromatographic methods can be used to determine these components in pharmaceuticals, they can be utilized to determine these components in pharmaceuticals, they are both more time expending and expensive than the method here developed. The results obtained in this paper empower us to apply the proposed strategy for the simultaneous determination of **AMT, MET and PPZS** in tablets without making utilize of any pretreatment procedure. The validation of the proposed methods according to the

ICH guidelines proved the applicability and great value of these methods for routine application in quality control laboratories for the simultaneous analysis of **AMT, MET and PPZS** in their pure powder and dosage forms without earlier separation or excipient interference.

Acknowledgments

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