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# Back-Propagation Neural Network Model for Simultaneous Spectrophotometric Estimation of Losartan Potassium and Hydrochlorothiazide in Tablet Dosage.

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**The development of multivariate calibration model with back-propagation neural network using calibration sets constructed from the spectral data of pure components is proposed for the simultaneous estimation of active components, losartan potassium and hydrochlorothiazide in tablet dosage. The calibration sets were designed such that the concentrations were orthogonal and span the possible mixture space fairly evenly. The back-propagation neural network model was optimized with respect to the spectral input, training parameters and topology including transfer functions for each layer so as to yield accurate and precise estimations on model validation. The optimized model showed sufficient robustness even when the calibration sets were constructed from different set of pure spectra of components thus enabling periodical validation of model rapidly and economically. Although the components showed significant spectral overlap, the model could accurately estimate the drugs, with satisfactory precision and accuracy, in tablet dosage with no interference from excipients as indicated by the recovery study results.**

The artificial neural networks (ANNs) are a data processing system consisting of a large number of simple, highly interconnected processing elements inspired by the biological system and designed to simulate neurological processing ability of human brain. Theoretical background information on ANNs can be found elsewhere[1-3]. ANNs have been applied to diverse areas from missile technology to medical diagnosis in performing tasks such as classification, modeling, association, mapping etc. There are reports in literature on application of ANN for determining the spectra-structure relationship[4-8], prediction of secondary and tertiary structure of proteins[9-11], quantitative structure-activity relationship[12-13], pharmacokinetic and pharmacodynamic analysis[14-15], pharmaceutical product development[16-17] and diagnostic and clinical medicine[18-20]. The applications of ANN in the field of chemistry and pharmacy have been reviewed.[21-28] Computationally, the ANN is an approach for handling multivariate and multiresponse data and hence suitable for modeling, i.e. a search for an analytical function that will give a specified n-variable output for any m-variable input[27]. Unlike standard modeling techniques where the mathematical function is required to be known in advance, ANN models do not require knowledge of the mathematical function in advance and are called 'soft models', i.e. the models are able to represent the experimental behaviour of the system when the exact description is missing or too complex[29]. ANNs adapt to any relation between input and output data on the basis of their supervised training. The Characteristics that make ANN systems different from traditional computing are: learning by example, distributed associative memory, fault tolerance and pattern recognition[29]. The flexibility of ANNs and their ability to maintain their performance even in the presence of significant amounts of noise in the input data are highly desirable[2, 25], since perfectly linear and noise free data sets are seldom available in practice, thus making it suitable for multivariate calibration modeling. The authors had earlier published their preliminary findings on the application of ANN for multicomponent sample analysis[30].

Losartan potassium (LST), chemically 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl) [1,1'-biphenyl]-4-yl] methyl]-1H-imidazole-5-methanol, is an angiotensin II receptor

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antagonist.31 Hydrochlorothiazide (HCT), chemically 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide, is a diuretic drug[32]. LST and HCT combination tablet dosage in the ratio of 4:1 is available, for the treatment of hypertension. The literature describes RP-HPLC[33] and spectrophotometric methods[34-35] for simultaneous estimation. This paper presents a rapid and economical method compared to HPLC for routine pharmaceutical quality control of this tablet dosage form by multivariate calibration based on soft modeling using back-propagation neural network.

## MATERIALS AND METHODS

Analytical reagent grade sodium hydroxide was used to prepare 0.1M sodium hydroxide solution in distilled water which then served as solvent for making the stock solutions and all further dilutions of LST, HCT, their standard combination and the tablet powder.

UV absorption measurements were carried out on Shimadzu 1601PC (Japan) double beam spectrophotometer controlled by UVPC software version 3.7, using matched 1.00 cm quartz cells. Class A volumetric glassware such as pipettes and volumetric flasks were used for the purpose of making dilutions. All weights were measured on an electronic balance with 0.01 mg sensitivity. All computations were carried out on a desktop computer with a Pentium 4, 1.8 GHz processor and 256 MB RAM.

UVPC software version 3.7 was used for spectral scanning with the required parameters and saving the spectral data in ASCII format in addition to its native format. Graphical User Interface based application software named "Neuralyzer" was developed in house by the authors, in Java programming language capable of handling all the required tasks towards developing, training, validating the back-propagation neural network (BPNN) models and using them in analysis of the test samples' spectral data. Major features of Neuralyzer include capabilities to generate the training (calibration) and monitoring set, design and configure the BPNN, training the neural network, use the monitoring error to stop training at an appropriate optional point, create a report of the analysis of the external validation spectra along with prediction parameters, analysis of test spectra and report the concentrations of the analytes in the test sample. The trained BPNN model can be saved and loaded into Neuralyzer later at any time for analysis.

Spectra of all the solutions were recorded against a blank solution containing no analytes between 220 to 400 nm. and saved in native and ASCII format.

### Preparation of standard solutions:

Standard solutions of pure LST and HCT were made at different concentration levels ranging from 4 to 22 mg/l for the purpose of linearity determination and to design the calibration matrix from their spectra. The absorbance spectra of the two standards were recorded under the selected experimental conditions and are shown in fig. 1.

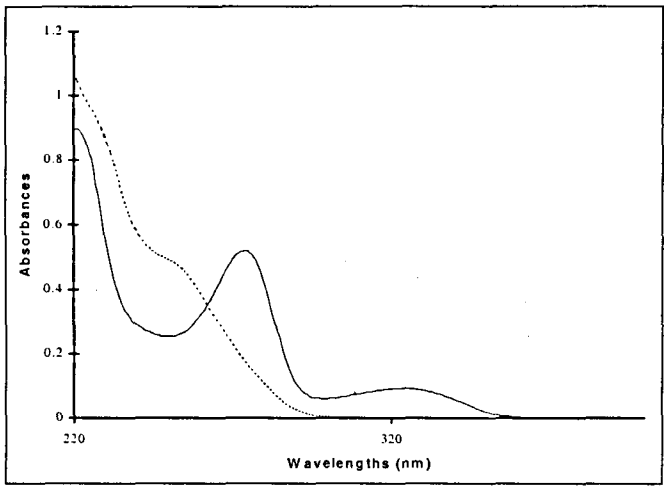


**Fig. 1: UV Spectra of LST and HCT.**

**Overlain spectra of LST (····) at concentration of 16.260 mg/l and HCT (—) at concentration of 9.963 mg/l in 0.1M sodium hydroxide.**

### Synthetic binary mixtures for validation:

A set of 6 stock solutions of pure LST and HCT were prepared on different days, each by separate weighing, in 0.1M sodium hydroxide. Standard mixtures of the components were prepared with the concentrations lying within the known linear absorbance-concentration range by dissolving varying proportions of LST and HCT stock solutions such that the concentration of LST varied between 75 to 125 % of the test level concentration while the minor component HCT varied between 50 to 150 % of its test level concentration. A total of 80 standard mixtures were made in which 55 mixtures had different concentrations and 25 were duplicate dilutions of some of them. The concentrations of components were selected to span the mixture space fairly evenly, as shown in fig. 2.

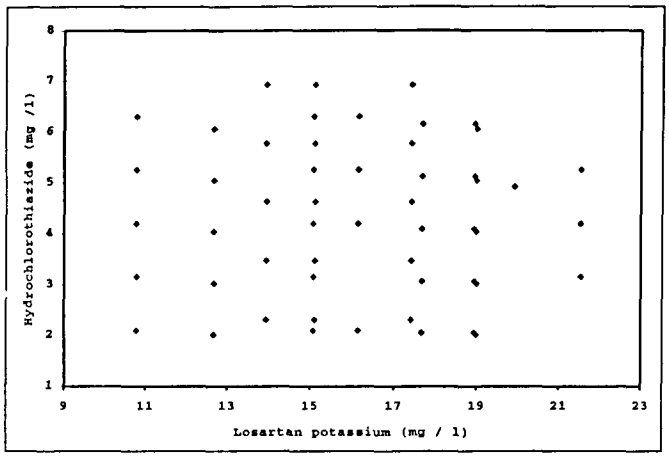


**Fig. 2: Synthetic binary mixture design for the validation of the neural networks.**

***Synthetic binary mixture design for validation of neural* network model. Each point (•) represents a mixture at the respective concentration of the components. Since there are some replicate mixtures, they are overlapped in the plot. The design ensures that the model is validated in a well distributed concentration space especially with regard to chosen analytical level.**

**Analysis of tablet dosage form:**

For the analysis of the active components of the antihypertensive tablet (Tozaar-H, LST 50 mg and HCT 12.5 mg, Torrent Pharmaceuticals Ltd.), twenty tablets were accurately weighed, carefully powdered and mixed. Tablet powder corresponding to the equivalent of 33 mg of LST was dissolved in 0.1M sodium hydroxide solution by sonication for 5 min and made up to 100 ml. The solution was centrifuged and 5 ml of supernatant was diluted to 100 ml. Six replicate dilutions were made for each experiment, repeating the experiment on six different days with freshly weighed tablet powder. For accuracy studies, by recovery, the same tablet powder was used in amounts corresponding to the equivalent of 20 mg of LST (in order to enable spiking up to desired levels). The powder was then mixed with a known quantity of pure LST and HCT and dissolved in 0.1M sodium hydroxide by sonication and made up to 100 ml with the same solvent. The solution was then centrifuged and 5ml of supernatant was diluted to 100 ml. The recovery was performed at five different levels of spiking, in the range of 80 to 120 % of the test level of concentration of the analytes, each in three replicates.

**Calibration data:**

Since the spectra were linearly additive, and no serious baseline problems or interactions found and since majority of chemometric techniques for regression and calibration do assume linear additivity, the process described below was adopted in the design of calibration data set for training the BPNN. Three pairs of pure components' spectra were employed in order to provide a fair simulation, with some degree of experimental variation. A full factorial design was employed to obtain 128 training pairs from each spectral pair representing the mixture space evenly with target concentrations that are orthogonal.

A total of 384 training pairs were thus obtained to constitute the complete calibration set that would be used to train the BPNN. All the target concentrations in the calibration set were then normalized to lie between 0.01 and 1.0. In order to optimize the number and the nature of spectral inputs, several combinations of spectral regions and wavelength points were chosen and calibration sets were designed in the same concentration mixture space with the same pairs of pure spectra using eighteen different

TABLE 1: CALIBRATION DATA SET DESIGN PARAMETERS.[a]

| Set | Wavelength (nm) | | | Inputs |
|---|---|---|---|---|
| | Spectral Region | | Interval | |
| | Upper | Lower | | |
| A | 330 | 230 | 3 | 34 |
| B | 330 | 230 | 4 | 26 |
| C | 330 | 230 | 5 | 21 |
| D | 330 | 230 | 7 | 15 |
| E | 330 | 230 | 9 | 12 |
| F | 330 | 230 | 10 | 11 |
| G | 290 | 235 | 3 | 19 |
| H | 290 | 235 | 4 | 14 |
| I | 290 | 235 | 5 | 12 |
| J | 290 | 235 | 7 | 8 |
| K | 290 | 235 | 9 | 7 |
| L | 290 | 235 | 10 | 6 |
| M | 280 | 240 | 3 | 14 |
| N | 280 | 240 | 4 | 11 |
| O | 280 | 240 | 5 | 9 |
| P | 280 | 240 | 7 | 6 |
| Q | 280 | 240 | 9 | 5 |
| R | 280 | 240 | 10 | 5 |

[a] All sets have 384 training pairs.

parameters as given in Table 1, varying stepwise only the spectral region and the wavelength interval.

**Monitoring data:**

Monitoring data sets are used for the internal validation and terminating the training of the BPNN at an optimum point to prevent over-fitting and retain generalization ability of the network. Monitoring data set of same size was also designed from the same spectra of LST and HCT standard but paired in a different way than the one used for designing calibration data set.

**BPNN design:**

ANN models are built iteratively by successive optimizations of their inputs and topology. In the phase one, BPNNs having three layers with varying input nodes (based on the calibration set employed) twice the input nodes in the hidden layer and two nodes, corresponding to the number of components, in the output layer were used to optimize the best of possible calibration sets. The input and output layer nodes had a linear transfer function while the hidden layer nodes had hyperbolic tangent transfer function decided on the basis of preliminary studies. In phase two, optimization of network architecture was done using the best calibration set selected from phase one study. The selected calibration set was used in the BPNN designs with input nodes corresponding to the calibration set and the two output nodes having linear transfer function. Only the number of nodes in hidden layer and their transfer function was varied this time in a factorial design.

**Training the BPNN:**

BPNNs were trained using the popular gradient descent algorithm which performs a steepest-descent minimization on the error surface in the adjustable parameters hyperspace as described and popularized by Rumelhart and McClelland[1]. All the BPNNs were trained with a learning rate of 0.1[30] and a momentum of 0.5. The training was monitored to prevent memorization (over-fitting) with the corresponding monitoring set after every 100 epochs (since a fairly large monitoring set equal in size to the calibration set was used) and terminated as soon as any of the following criteria was met: (a) the root mean square error of monitoring (RMSEM) rises while the root mean square error of training (RMSET) is lowering (b) the RMSEM rises continuously for 1000 epochs or (c) the decrement between two successive RMSEM is below a pre-specified threshold of 0.1 ppm on the average per epoch, and the network assumed to have stabilized with adequate generalization capacity. The BPNNs were then frozen to prevent further training and preserve the weights.

**Validation of the BPNN:**

All trained and frozen BPNNs were validated for their modeling capability by testing with the spectral data obtained from the standard physical mixture designs as described above. Neuralyzer would then use the trained BPNN model (in its buffer) to obtain the concentrations for each component from the validation spectral files and calculate the root mean square error of prediction (RMSEP) and regression parameters which could be saved to hard disk.

**Tablet Analysis:**

Spectra recorded from the tablet solutions were analyzed by the chosen BPNN model and the concentrations predicted for each solution were used for calculation of the tablet content. Similarly LST and HCT concentrations in the solutions prepared for recovery study were also obtained from the respective spectra and percentage recovery was calculated to determine the accuracy of the method.

## RESULTS AND DISCUSSION

The overlain absorption spectra (fig. 1) show strong spectral overlap, which complicates the determination of the individual drug concentrations from a spectrum of a mixture. When considered separately, concentrations between 2 to 22 mg/l for both LST and HCT were found to be linear, with $R^2$ of 99.99% (goodness of fit) for each, slopes of 0.036 and 0.052, intercepts of 0.0024 and -0.0026 and residual standard deviation about the regression line being 0.0021 and 0.0005, respectively.

There are many pitfalls in the use of calibration models, perhaps the most serious being variability in instrument performance over time. Each instrument has different characteristics and on each day and even hour the response may vary. Therefore it is necessary to reform the calibration model on a regular basis, by running a standard set of samples, possibly on a weekly basis[36]. Like other regression methods, 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 over-fitted if too few training pairs are available leading to loss of generalization ability. Therefore, calibration sets of several hundred training pairs may often be necessary to

get a representative distribution of the concentration across their range. This makes it expensive in time and resources to develop calibration mixtures physically in such large numbers which is rarely possible in routine laboratory studies and justifies our attempt to use mathematically constructed calibration data set from individual spectra of components.

The problem of deciding the best possible input space in terms of the spectral region and intervals was taken up in first phase using the eighteen calibration sets already created and designing BPNNs for each of them as mentioned above. Each BPNN was trained three times with random initialization of weights and the RMSEP was calculated using the validation set, consisting of synthetic binary mixtures, along with RMSEM. All the root mean square errors were calculated using the formula[22]

$$RMS = \sqrt{\sum_{s=1}^{m}\sum_{i=1}^{n}(y_{si} - out_{si})^2 \Big/ m \times n}$$

where $y_{si}$ is the i-th component of the desired target Ys, $out_{si}$ is the i-th component of the output produced by the network for the s-th input vector, m is the number of input vectors or samples and n is the number of output variables. The results are presented in Table 2.

Step-down multiple-stage testing process of successive elimination of calibration sets was adopted by repeatedly subjecting the results to one way ANOVA and Hsu's Multiple Comparisons with the best[37] having the lowest RMSEP, whenever p-value was significantly less than 0.05 was obtained. HSU's Multiple Comparison with best provides confidence intervals for the difference between each mean and the best of the other means which can be used to eliminate levels (treatments) that are not the best. When no significant difference among the calibration set could be found the same process was repeated using the best RMSEM for further round of elimination till no significant difference was found among the sets. At this point four sets H, I, K, L were found to show no significant difference among them. Set H, which had the least mean RSMEP and low standard deviation compared to others, was then chosen from among these.

The calibration set H thus chosen was used to optimize the BPNN configuration in the second phase by a factorial design, with number of hidden neurons (7, 14 or 21) and their transfer function (sigmoid or hyperbolic tangent) as two factors.

Each model of BPNN was trained three times with random initialization of weights and the root mean square error of prediction was calculated using the validation set. Analysis of the results, shown in Table 3, indicated that there was significant difference (p-value < 0.01) with respect to the transfer function used in the hidden layer, sigmoid being better compared to hyperbolic tangent function with respect to RMSEP and was preferred over the hyperbolic tangent transfer function even though it offered

TABLE 2: SELECTION OF OPTIMUM CALIBRATION SET FOR BPNN MODELING.[a]

| Set | Mean RMSEP $x10^{-1}$ | SD $x10^{-2}$ | Mean RMSEM $x10^{-3}$ | SD $x10^{-4}$ | Set | Mean RMSEP $x10^{-1}$ | SD $x10^{-2}$ | Mean RMSEM $x10^{-3}$ | SD $x10^{-4}$ |
|---|---|---|---|---|---|---|---|---|---|
| A | 1.0367 | 1.68 | 1.07 | 1.57 | J | 0.9100 | 0.00.819 | 1.68 | 1.97 |
| B | 1.1133 | 0.764 | 0.997 | 1.14 | K | 1.0100 | 0.458 | 1.23 | 2.29 |
| C | 1.2433 | 3.958 | 1.18 | 0.81 | L | 0.9733 | 0.115 | 1.41 | 1.78 |
| D | 1.1233 | 0.666 | 1.05 | 1.42 | M | 1.1433 | 0.586 | 1.72 | 1.50 |
| E | 1.0733 | 0.551 | 1.63 | 3.49 | N | 1.0833 | 0.586 | 1.62 | 2.06 |
| F | 1.3500 | 1.670 | 1.05 | 1.83 | O | 1.1567 | 0.586 | 1.81 | 0.61 |
| G | 0.9333 | 0.902 | 1.69 | 2.62 | P | 1.1967 | 0.252 | 1.87 | 0.62 |
| H | 0.9233 | 0.666 | 1.31 | 1.14 | Q | 1.2067 | 0.416 | 1.79 | 0.68 |
| I | 0.9900 | 0.889 | 1.42 | 2.90 | R | 1.1000 | 0.608 | 1.70 | 0.31 |

[a] The selection is based on one way analysis of variance in combination with Hsu's multiple comparisons with the best.

TABLE 3: BPNN HIDDEN LAYER CONFIGURATION ANALYSIS.[a]

| Neuron Count | Transfer Function | | | |
|---|---|---|---|---|
| | Sigmoid | | Hyperbolic Tangent | |
| | RMSEP | Epochs | RMSEP | Epochs |
| 7 | 0.060 | 10300 | 0.070 | 4500 |
| 7 | 0.065 | 10800 | 0.066 | 5400 |
| 7 | 0.062 | 11100 | 0.066 | 5900 |
| 14 | 0.061 | 9700 | 0.068 | 4200 |
| 14 | 0.060 | 11000 | 0.070 | 5200 |
| 14 | 0.063 | 11000 | 0.070 | 4300 |
| 21 | 0.064 | 9100 | 0.065 | 3200 |
| 21 | 0.063 | 10400 | 0.074 | 4300 |
| 21 | 0.062 | 10900 | 0.070 | 4900 |

[a] The hidden layer is optimized for the neuron count and transfer function of the nodes in a factorial design with three replicates.

the benefit of less epochs since the time taken was less than 15 minutes in all cases. The limited variation in the number of hidden neurons surprisingly had no significant difference (p-value > 0.05) on the performance of the BPNN in this case. The interaction between the two factors was insignificant (p-value > 0.05). Therefore, a hidden layer with the least neurons from the factorial design and sigmoid transfer function was taken as optimum configuration. Based on these results, the final BPNN model had an input of 14 neurons, an output of 2 neurons, both having linear transfer

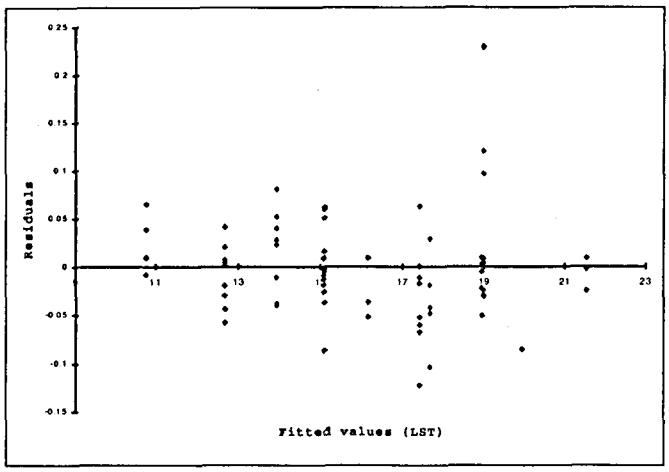


**Fig. 3: Residuals plot for the prediction regression for LST by BPNN.**

**The residuals versus the fitted value for the regression of the predictions made for Losartan potassium by the BPNN model.**

function and a hidden layer of 7 neurons with sigmoid transfer function. The 14 inputs correspond to absorbances at wavelength points 290, 286, 282, 278, 274, 270, 266, 262, 258, 254, 250, 246, 242 and 238 nm.

The optimized BPNN model was validated for its robustness by training the network using three different calibration sets and monitoring sets and investigating their prediction characteristics using the 75 synthetic binary mixtures' spectral data, after eliminating the 5 consistent outliers from a total of 80. The Regression characteristics of the predictions are listed in Table 4 and the residual plots versus fits are shown in fig. 3 and 4 for LST and HCT respectively. These results imply that the BPNN model performed well irrespective of the calibration data set and

TABLE 4: SYNTHETIC BINARY MIXTURE CONCENTRATION PREDICTION REGRESSION PARAMETERS.[a]

| Model | Losartan potassium | | | | Hydrochlorothiazide | | | |
|---|---|---|---|---|---|---|---|---|
| | Slope | Intercept | $R^2$%[b] | SD[c] | Slope | Intercept | $R^2$%[b] | SD[c] |
| BPNN-1 | 1.009 | -0.040 | 99.95 | 0.063 | 1.012 | 0.002 | 99.85 | 0.061 |
| BPNN-2 | 1.003 | -0.032 | 99.96 | 0.059 | 1.010 | -0.036 | 99.87 | 0.055 |
| BPNN-3 | 1.005 | -0.074 | 99.90 | 0.088 | 1.010 | 0.061 | 99.84 | 0.061 |

[a] All regressions are done predicted concentrations against the actual concentrations of the respective components. in all cases the p-value was 0.005. [b] $R^2$ is coefficient of determination and $r^2$% is known as the percentage fit. [c] Estimated standard deviation about the regression line, also known as the residual standard deviation.

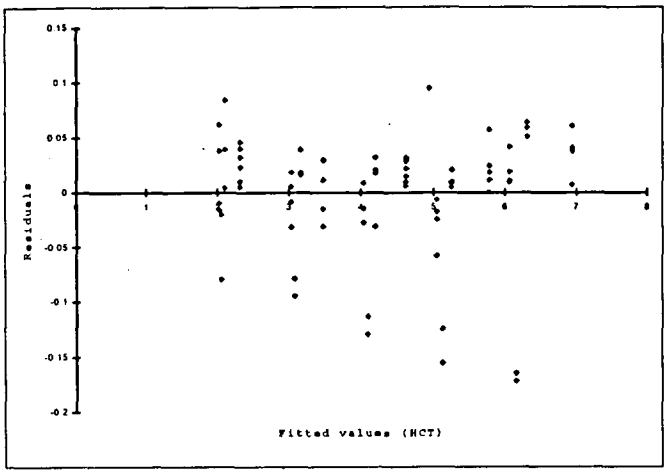


**Fig. 4: Residuals plot for the prediction regression for HCT by BPNN.**

**The residuals versus the fitted value for the regression of the predictions made for Hydrochlorothiazide by the BPNN model.**

hence, rugged enough for periodic calibration necessitated by conditions such as variability in instrument performance.

Spectra obtained from 36 tablet solutions prepared from 6 different weighings as described in the experimental section were analyzed by the BPNN model and the average content was calculated. The results are summarized in Table 5. The accuracy of the method for analysis of tablets was

TABLE 5: RESULTS OBTAINED USING BPNN MODEL TO SIX TABLET SAMPLES OF LST AND HCT[a]

| | LST | HCT |
|---|---|---|
| Sample 1 (mg)[a] | 51.08 | 12.89 |
| Sample 2 (mg)[a] | 49.46 | 12.00 |
| Sample 3 (mg)[a] | 49.97 | 12.14 |
| Sample 4 (mg)[a] | 50.07 | 12.07 |
| Sample 5 (mg)[a] | 50.25 | 12.18 |
| Sample 6 (mg)[a] | 49.21 | 12.08 |
| Average of samples (mg) | 50.01 | 12.23 |
| Relative Standard Deviation | 1.31 | 2.70 |
| Amount on the label (mg) | 50.00 | 12.50 |
| % of the reported content | 100.0 | 97.84 |

[a]Actual concentrations calculated form the content of each component in the tablets by the BPNN model.

further investigated using the recovery studies as described earlier. The mean percentage recovery with BPNN model was 100.0 and 100.4 with relative standard deviation of 1.27 and 1.58 for losartan potassium and hydrochlorothiazide respectively which is very well acceptable and reflects the models reliability without doubt

TABLE 6: RESULTS OBTAINED FOR RECOVERY STUDIES USING THE BPNN MODEL.

| Sample | Analyte content (mg)[a] | | Added (mg)[b] | | Total (mg) | | Found (mg) | | Recovery (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LST | HCT | LST | HCT | LST | HCT | LST | HCT | LST | HCT |
| 1 | 20.94 | 5.12 | 3.98 | 0.99 | 24.92 | 6.11 | 24.96 | 6.14 | 100.16 | 100.49 |
| 2 | 20.66 | 5.05 | 7.97 | 1.97 | 28.63 | 7.02 | 28.64 | 7.03 | 100.03 | 100.14 |
| 3 | 20.64 | 5.05 | 11.95 | 2.96 | 32.59 | 8.01 | 32.22 | 7.93 | 98.86 | 99.00 |
| 4 | 20.61 | 5.04 | 15.94 | 3.95 | 36.55 | 8.99 | 36.20 | 8.95 | 99.04 | 99.56 |
| 5 | 21.01 | 5.14 | 19.92 | 4.94 | 40.93 | 10.08 | 41.77 | 10.38 | 102.05 | 102.98 |
| Mean recovery | | | | | | | | | 100.03 | 100.43 |
| Standard deviation | | | | | | | | | 1.27 | 1.58 |
| Relative standard deviation | | | | | | | | | 1.27 | 1.58 |

[a]The average weight of each tablet used in the analysis is 0.18371g.; each sample consists of separately weighed tablet powder. [b]Five different levels of spiking was done , in the range of 80 to120 % of the test level of concentration of the analytes.

in the analysis of these tablets. The results are shown in Table 6.

Multivariate calibration techniques utilize large number of variables from a problem domain to obtain a solution and hence are highly reliable and robust. ANNs ability to learn and derive absorbance-concentration relationships from presentations of a set of training samples combined with the fundamental principle of distributing information among several weights and nodes renders the ANN model robust with respect to random noise in the input data and allows one to have several ANNs with different topologies converging to qualitatively equivalent results[25]. The BPNN model developed in this study performed well, when tested with spectra recorded on different days and exhibited ruggedness even when different sets of constructed calibration data were used in the model development as indicated by the validation results. The study revealed that back-propagation model can be successfully used for multivariate calibration for the analysis of multicomponent formulations using UV spectrophotometry, economically and rapidly. The BPNN model can be quickly calibrated whenever the spectrophotometer performance characteristics alter using only three pairs of the spectra of the individual components, offering considerable advantage over classical methods.

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