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Inversely Calibrated Curvilinear Artificial Neural Network Model for Simultaneous Assay of Ternary Cardiovascular Drug Mixture

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ABSTRACT

Novel chemometric design, tailored for pre-clinical multiple drug screening, goals for bioanalytical future scope. A highly sensitive, non-linear multivariate Artificial Neural Network (ANN) is developed and applied for simultaneous spectrophotometric determination of three commonly concomitant cardiovascular drugs in a laboratory made mixtures and spiked human plasma samples. Ticagrelor, Irbesartan, and Hydrochlorothiazide have been simultaneously quantified in the curvilinear ranges of 0-30 µg/mL, 0-10 µg/mL, and 0-3 µg/mL respectively. Highly overlapping Near UV absorption spectra of three drugs, in the region of 215-280 nm, have been recorded 1-nm range in synthetic ternary mixtures and trained iteratively. By inversely relating the concentration matrix (*x*-block) with its corresponding absorption one (*y*-block), gradient-descent back-propagation ANN calibration could be computed and optimized. All proposed mathematical modeling was manipulated using MATLAB® 2007, reaching down to sixth order exponential Mean Square Error, MSE. To validate, an independent set of ternary synthetic mixtures has been constructed and examined, where excellent recovery results have been obtained. Furthermore, the application of the suggested model to varying ratios of synthetic ternary mixtures as well as spiked plasma samples has resulted in accurate, precise, and robust estimations with no background interference. ANN method was compared to a reference HPLC method; Student's *t*-test and *F*-variance ratio were calculated and showed the insignificant difference. This chemometric approach is an eco-friendly green assay, time-saving, and economic method. It initiates a pathway for clinical drug screening through affordable spectroscopic instrumentation.

Keywords: *Artificial Intelligence; UV-Spectrophotometry; Ticagrelor; Irbesartan; Hydrochlorothiazide; spiked plasma; Non-linear range.*

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1. INTRODUCTION

Although Spectrometric drug assays are still the most applicable in quality control laboratories, their application is highly limited at the demand of high sensitivity and resolving much complicated overlaps. Intelligent spectral data analysis is no longer welfare for analysts **[1]**. Statistical data analysis, artificial intelligence, mathematical optimization, and machine learning are core competencies of chemometric trials for drug analysis in multicomponent complex

formulations **[2, 3]**. Besides, being ecofriendly and green methods **[4]**, Artificial neural networks (ANNs) or connectionist systems work like human brains, collect joined units or nodes known as artificial neurons, which handle the neurons in the human brain. Each junction, like synapses in real brains, can send a signal to other neurons. In ANN manipulations, the "signal" is data and each neuro signal is calculated by non-linear function **[5]**.

ANN abilities, as a machine learning computational mathematical pattern, is classified a subcategory of; function approximation, regression analysis, data processing, classification, sequential decision making, and reaching robotics control. Predictive analytics by the ANN calibration model can be efficiently applied for non-linear relationships, quantitative analysis of complex pharmaceutical matrices, and highly overlapped spectral data. Yet unresolved data sets could be identified using ANNs **[6, 7]**. ANNs have processed in vitro in vivo correlations **[8, 9]**. Networks have also been applied to pharmacokinetic data sets **[10]** and different pharmaceutical drug combinations have been assayed by ANNs **[11-13]**.

Antihypertensive treatment reduces the risk of cardiovascular infarctions. Recent cardiac guidelines recommend combination therapy, rather than monotherapy **[14]**. Antithrombic agents synergize with antihypertensive combinations for long term treatment **[15]**. Alternatively, screening for potential drug-drug interactions, contraindications, or both, and by making therapeutic recommendations aimed at achieving optimal response without increasing the potential for adverse drug reactions, especially among elderly patients and those with multiple medical conditions. High demand for accurate and sensitive analysis as well as being economic, affordable, and green one.

The antihypertensive, Irbesartan (IRB), or 2-butyl-3-[[2′-(1H-tetrazole-5-yl)[1,1′-biphenyl]-4-yl]-methyl1-3-diazaspiro-[4,4]-non-1-en-4-one, **(Fig. 1)**, is an angiotensin II blocker. It blocks AT1 receptors, reducing the effects of angiotensin II **[16]**.

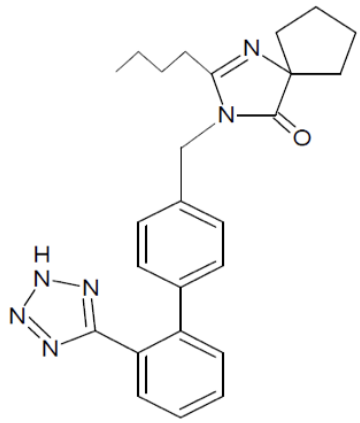

Fig. 1. Chemical Structure of Irbesartan

The antihypertensive, Hydrochlorothiazide (HCT), or 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide, **(Fig. 2)**, promotes excretion of sodium and chloride through the kidney. It also prevents stone formation **[16]**.

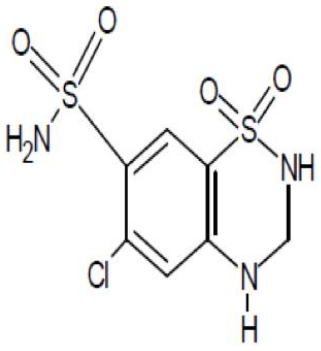

Fig. 2. Chemical Structure of Hydrochlorothiazide

The orally active antithrombic, Ticagrelor (TICA), or (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3H-[1,2,3]-triazolo [4,5-d]

pyrimidin-3-yl]-5-(2-hydroxyethoxycyclo pentane-1,2-diol 2, was FDA approved 2011 **(Fig. 3)**. It inhibits platelet activation and aggregation mediated by the P2Y12 ADP-receptor1. Thus it lowers the rate of thrombotic cardiovascular infarction in patients with acute coronary syndrome **[16]**.

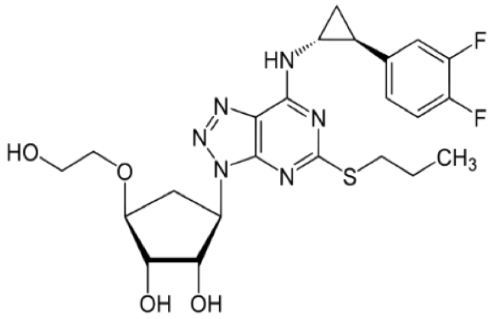

Fig. 3. Chemical Structure of Ticagrelor

Literature states different analytical techniques for IRB determination using UV-Spectrophotometry **[17]**, and RP-HPLC **[18]**. Also, determined in human plasma by LC **[19]**. In presence of HCT, IRB has been determined spectrophotometrically **[20]**, RP-HPLC **[21, 22]**, and thermometrically **[23]**. Both HCT and IRB mixture was simultaneously quantified in biological fluids **[24, 25]**. Other IRB combinations have been assayed chromatographically **[26, 27]**. HCT, as a single component, has been determined UV-spectrophotometrically **[28]**, and LC **[29]**. HCT combination mixtures have been resolved either by LC **[30]** or thermometrically **[31]**. TICA has been assayed by UV-spectrophotometry **[32]**. As well as in plasma samples, it has been determined by LC-MS **[33]** and LC-MS-MS **[34]**.

Literature reveals the simultaneous determination of IRB, HCT, and TICA ternary mixtures by RP-LC, and spectrophotometrically **[35, 36]**. Meanwhile, the present work is the first to use iterative neural modeling for the three drugs' non-linear UV spectra, starting at zero µg/mL quantification. The proposed model permits their determination in plasma samples spectrophotometrically without prior chromatographic separation, coming over the latter references.

2. MATERIALS AND METHODS

2.1. Apparatus

Thermo Spectronic UV-Vis spectrophotometer connected to Harvest computer system was used. Absorption spectra were measured in 1-cm quartz cells. The absorbance data was displayed on EXCEL sheets and processed using MATLAB software.

2.2. Materials and Reagents

TICA was purchased from AstraZeneca. IRB and HCT were obtained from Accord-UK LTD, London.

2.3. Standard Solutions

Standard stock solutions, 100 µg/mL, of each of TICA, IRB, and HCT were separately and accurately prepared in ethanol. Different aliquots were micropippetted from each stock solution to form a set of 90 standard ternary mixtures. A wide range of drug concentrations was stated in each synthetic mixture as in **Table 1**.

2.4. Preparation of Spiked Plasma Samples

Plasma from the blood bank (Biuret, Lebanon) was purchased and kept at −20 °C. Gentle heating and shaking were required at the time of analysis. K3 EDTA was added for protein precipitation. Here, 400 µL of plasma samples were taken separately into a serial tube and vortexed for 3 min after being spiked with ternary mixture solution (in ethanol) at different concentration ratios. Then, the total amount of ethanol was brought to 1 mL by evaporation under a stream of nitrogen and vortexed for 3 min. The mixtures were centrifuged for 20 min at 4500 rpm, the supernatants were carefully

separated using a Pasteur pipette and analyzed.

Table 1. The ANN training set; 90 different ternary mixture solutions of TICA, IRB and HCT

Set No.	TICA µg/mL	IRB µg/mL	HCT µg/mL	Set No.	TICA µg/mL	IRB µg/mL	HCT µg/mL
1	5	1	10	**46**	25	0	9
2	5	1.5	9	**47**	25	1	8
3	5	2	8	**48**	25	1.5	7
4	5	2.5	7	**49**	25	2	6
5	5	3	6	**50**	25	3	5
6	5	0	5	**51**	30	1	4
7	5	1	4	**52**	30	1.5	3
8	5	1.5	3	**53**	30	2	2
9	5	2	2	**54**	30	2.5	1
10	5	3	1	**55**	30	3	0
11	10	1	0	**56**	30	0	10
12	10	1.5	10	**57**	30	1	9
13	10	2	9	**58**	30	1.5	8
14	10	2.5	8	**59**	30	2	7
15	10	3	7	**60**	0	2.5	6
16	10	0	6	**61**	3	3	5
17	10	1	5	**62**	3	0	4
18	10	1.5	4	**63**	3	1	3
19	10	2	3	**64**	3	1.5	2
20	10	3	2	**65**	3	2	1
21	15	1	1	**66**	3	2.5	0
22	15	1.5	0	**67**	3	3	10
23	0	2	10	**68**	3	0	9
24	15	2.5	9	**69**	3	1	8
25	15	3	8	**70**	3	1.5	7
26	15	0	7	**71**	23	2	6
27	15	1	6	**72**	23	2.5	5
28	15	1.5	5	**73**	23	3	4
29	15	2	4	**74**	23	0	3
30	15	3	3	**75**	23	1	2
31	20	1	2	**76**	23	1.5	1
32	20	1.5	1	**77**	23	2	0
33	20	2	0	**78**	23	2.5	10
34	20	2.5	10	**79**	23	3	9
35	20	3	9	**80**	23	0	8
36	20	0	8	**81**	12	1	7
37	20	1	7	**82**	12	1.5	6
38	20	1.5	6	**83**	12	2	5
39	20	2	5	**84**	12	2.5	4
40	20	3	4	**85**	12	3	3
41	25	1	3	**86**	12	0	2
42	25	1.5	2	**87**	12	1	1
43	25	2	1	**88**	12	1.5	0
44	25	2.5	0	**89**	12	2	10

45	0	3	10	**90**	12	2.5	5

ANN; Artificial Neural Network – TICA; Ticagrelor – IRB; Irbesartan – HCT; Hydrochlorothiazide addition of organic solvent + centrifugation + evaporation + addition of mobile phase)

2.5. Construction of ANN Model

Modeling access related both the concentrations of the ternary drug mixture (TICA, IRB, and HCT) and their corresponding absorbance values, in a wide non-linear range, independent of Beer's law. As a start, the multivariate ternary model was constructed based on a training set; 90 mixtures of standard drugs, followed by ANN optimization through a predictive five-level three-factor design. These 135 sample mixtures were split into 90 training mixtures (for building the models) and 45 validation mixtures (for measuring the predictive power of the model). The concentration set; training set, of 90 synthetic mixtures containing TICA, IRB, and HCT in the concentration range of 0-30 µg/mL, 0-10 µg/mL, and 0-3 µg/mL, respectively in ethanol were prepared. Their absorption spectra (A; x-block, conc.; y-block) of the mixture set were plotted and recorded (66-wavelength points) in the spectral range of 215-280 nm **(Fig. 4)**.

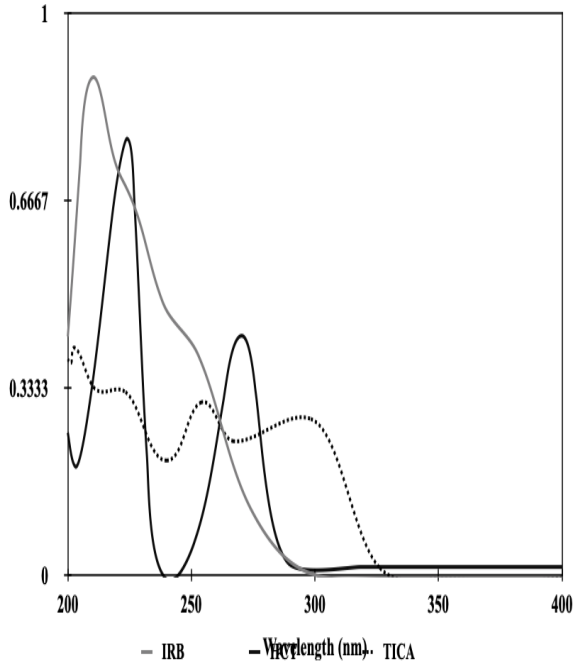

Fig. 4. Absorption curves of 9 µg.mL^{-1} TICA (….), 6 µg.mL^{-1} HCT (___ black line), and 1.25 µg.mL^{-1} IRB (grey line) in ethanol

ANN chemometric calibration was computed in a non-linear relationship between triple vector (3 concentration set) and their corresponding A readings (y-block).

2.6. ANN Optimization

Various topological networks were iteratively run for optimization. A training network of 120 neurons in the input layer, 50 neurons in hidden layers, and three outputs for the calibration and prediction steps was found to be suitable for the construction of ANN calibration for the simultaneous quantitative prediction of the three co-administrated drugs in laboratory prepared mixtures and spiked plasma samples.

2.7. Study for ANN Optimization parameters

Transfer function; it is chosen according to the nature of trained data. Being non-linear (A vs conc). Correlation, Log sigmoid activation function has been used for hidden and output layers.

Gradient descent training neural network has been backpropagated **(Fig. 5)**. Thus, the mean square error, MSE, between the network output and the actual values was minimized.

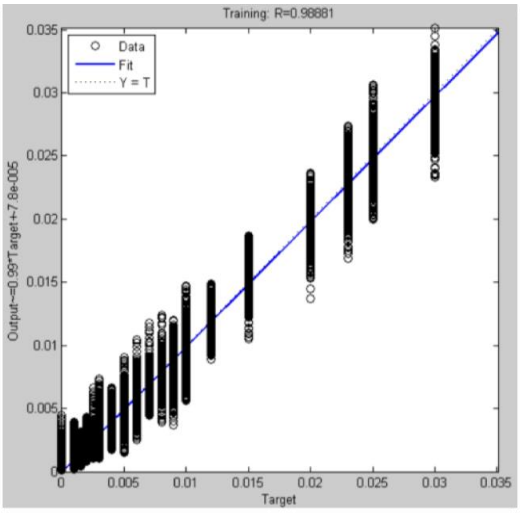

Fig. 5. Training Plot of the proposed Artificial Neural Network

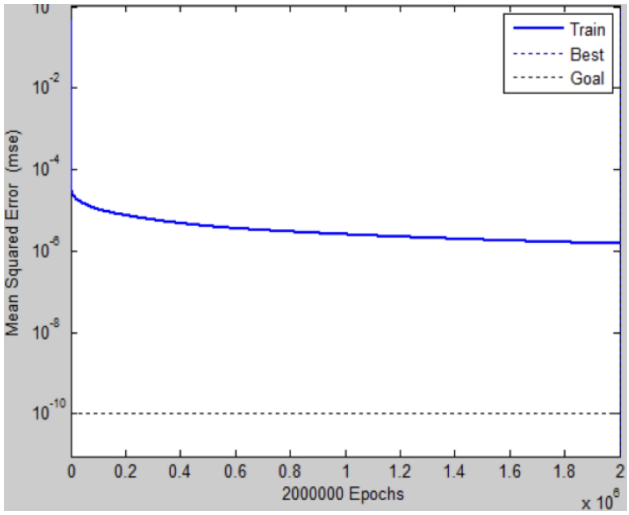

Fig. 6. Regression Plot of the proposed Artificial Neural Network

The learning rate initialized for the network was 0.5. The learning coefficient (Lc) masters the connection weights variation during the learning phase. Hidden neurons number (HNN) affected the convoluted performance of the output error function during the learning process.

Different training functions showed no significant difference in their performance (i.e. root mean square error of prediction (RMSEP) was not affected). M-training algorithm was chosen.

Iterative propagations were run to optimize regression of the targeted values versus the real outputs. As shown, **Fig. 6** is an illustrative regression plot taken for one of these run propagations.

Upon application of an optimized calibration model, Mean Square Error (MSE) and network Epochs were correlated. The least MSE recorded a value of 1.49 $x10^{-6}$ as shown in the "Performance Plot" of the proposed network **(Fig. 7)**.

3. Results and Discussion

3.1. Method Validation

To validate the predictability of the present model, ANN was run to estimate the concentration of three analytes in 45 synthetic mixtures (validation set) containing different ratios of drugs within the previously trained ranges. Validation and training sets are independent. Satisfactory results have been obtained for all mixtures. Mean recovery results and relative standard deviations are shown in **Table 2**.

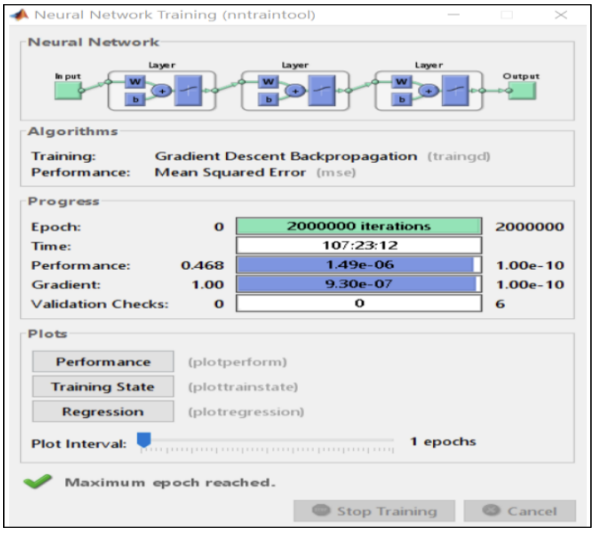

Fig. 7. Performance Plot of the proposed Artificial Neural Network

3.2.1. Analysis of Laboratory made mixtures

Laboratory made mixtures were prepared and analyzed by the proposed ANN method. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD, and RSD % **(Table 3)**. This table also represents a statistical comparison between the assay of TICA, IRB, and HCT in their synthetic mixtures by the proposed assisted ANN method and a developed HPLC method **[35]**, using the student's t-test and the variance ratio F-test. Since the calculated t- and F- values for each drug did not exceed the theoretical ones **[37, 38]**, this indicated no significant difference between the applied methods for determination of the three drugs in a laboratory made mixtures.

3.2. Application

Table 2. Recovery results of ANN validation set; 45 synthetic ternary mixtures of TICA, IRB and HCT

Set No.	Concentration (µg/mL)						Recovery (%)		
	Actual			Predicted					
	TICA	IRB	HCT	TICA	IRB	HCT	TICA	IRB	HCT
1	5	1	10	5.1	1	10	102	100	100
2	5	2	8	5	2.1	8	100	105	100
3	5	3	6	5.1	3.1	6	102	103.3	100
4	5	1	4	5	1	3.9	100	100	97.5
5	5	2	2	5	1.9	2.1	100	95	105
6	10	1.5	10	10	1.4	10	100	93.3	100
7	10	2.5	8	10	2.5	8	100	100	100
8	10	0	6	9.7	0	6	97	100	100
9	10	1.5	4	10	1.5	3.8	100	100	95
10	10	3	2	10.3	2.9	2	103	96.7	100
11	15	1.5	0	15	1.5	0	100	100	100
12	15	2.5	9	14.9	2.5	8.6	99.3	100	95.6
13	15	0	7	15	0	7	100	100	100
14	15	1.5	5	15	1.5	5	100	100	100
15	15	3	3	14.5	3	3	96.7	100	100
16	20	1.5	1	20.6	1.5	1	103	100	100
17	20	2.5	10	20.1	2.6	9.7	100.5	104	97
18	20	0	8	20	0	7.9	100	100	98.7
19	20	1.5	6	20	1.5	6	100	100	100
20	20	3	4	20.2	3.2	4.1	101	106.7	102.5
21	25	1	3	25.4	1	3	101.6	100	100
22	25	2	1	25.4	1.9	1	101.6	95	100
23	25	3	10	25.3	3	9.8	101.2	100	98
24	25	1	8	25.3	1	8.1	101.2	100	101.2
25	25	2	6	25.2	2.1	6	100.8	105	100
26	30	1	4	30.3	1	4.2	101	100	105
27	30	2	2	30.3	2.1	2.1	101	105	105

28	30	3	0	30.1	3	0	100.3	100	100
29	30	1	9	30.2	1	9.1	100.7	100	101.1
30	30	2	7	30.3	2	7	101	100	100
31	3	3	5	3	3	4.8	100	100	96
32	3	1	3	3	1	3	100	100	100
33	3	2	1	3	2	1	100	100	100
34	3	3	10	3	3	10.1	100	100	101
35	3	1	8	3	1	8.1	100	100	101.2
36	23	2	6	23.1	2	6.2	100.4	100	103.3
37	23	3	4	23	3	3.8	100	100	95
38	23	1	2	23.2	1	2	100.8	100	100
39	23	2	0	23.1	2	0	100.4	100	100
40	23	2.5	10	23.2	2.4	10.1	100.8	96	101
41	23	0	8	23.3	0	7.9	101.3	100	98.7
42	12	1.5	6	12	1.5	6.1	100	100	101.7
43	12	2.5	4	12.1	2.5	4.1	100.8	100	102.5
44	12	1.5	0	12.1	1.5	0	100.8	100	100
45	12	2.5	5	12.1	2.4	4.7	100.8	96	94
						Mean	100.46	100.26	99.91
						RSD	1.11	2.51	2.36

ANN; Artificial Neural Network – TICA; Ticagrelor – IRB; Irbesartan – HCT; Hydrochlorothiazide – RSD; Relative Standard Deviation

Table 3. Assay results for TICA, IRB and HCT in their laboratory made mixtures using the proposed ANN method

Ratio TICA+IRB+HCT µg/mL 9:1.5:5	**Mean Recovery ± SD[a] RSD %[b] Er %[c]**	
	HPLC (230 nm)	**ANNs**
TICA		
Mean Recovery ± SD[a]	98.76 ± 0.45	99.85 ± 1.13
RSD %[b]	0.45	0.45
Er %[c]	-1.01	-1.01
****t-test**	—	2.01
****F-test**	—	6.3
IRB		
Mean Recovery ± SD[a]	98.99 ± 0.87	100.05 ± 1.53
RSD %[b]	0.87	0.87
Er %[c]	-1.01	-1.01
****t-test**	—	1.35
****F-test**	—	3.09
HCT		
Mean Recovery ± SD[a]	99.08 ± 0.61	98.23 ± 1.21
RSD %[b]	0.61	0.61
Er %[c]	-0.92	-0.92
****t-test**	—	1.42
****F-test**	—	3.93

ANN; Artificial Neural Network – TICA; Ticagrelor – IRB; Irbesartan – HCT; Hydrochlorothiazide
[a] Mean ± SD for the five determinations; [b]% Relative standard deviation; [c]% Relative error
**Theoretical values of t- and F- at P = 0.05 are 2.13 and 6.93, respectively

3.2.2. Analysis of Spiked Plasma Samples

Absorbance data of ternary mixture (full scan), in three spiked human plasma samples, was applied to the ANN calibration model. Each sample was scanned for three successive times, satisfactory results were obtained **(Table 4)**.

3.3. Conclusion

As it is known, analysts tend to use linear calibration systems. On the other hand, non-linear calibration models are necessary for the spectral quantitative analysis of complex pharmaceutical matrices due to small deviations from linearity, some interactions due to excipients or interfering components, and the need for high sensitivity. The proposed method used a feed-forward back propagation neural network to predict blood serum concentration levels of TICA, IRB, and HCT in spiked plasma samples. The results of the study show that the neural network has the predictive capability and able to accurately predict drug concentration levels in spiked human plasma making it interchangeable tools for effectively estimating concentration levels. In addition to accuracy, the neural network application has the advantage of producing results empirically, without the need for developing statistical prediction models.

Table 4. Assay results for TICA, IRB and HCT mixtures in spiked plasma samples using the proposed ANN method

Concentration Added µg/mL			concentration found µg/mL			Mean Recovery ± SD[a] RSD %[b] Er %[c]		
TICA	IRB	HCT	TICA	IRB	HCT	TICA	IRB	HCT
30	3	10	30.30	3.00	10.30	101.00±1.01 1.00 1.00	100.00±0.9 0.90 0.00	103.00±1.00 0.97 3.00
30	2.5	6	30.20	2.45	5.87	100.70±0.85 0.84 0.70	98.00±1.41 1.44 -2.00	97.80±1.21 1.23 -2.20
5	1	2	5.10	1.01	1.99	102.00±0.55 0.54 2.00	101.00±0.31 0.31 1.00	99.50±0.81 0.81 -0.50

ANN; Artificial Neural Network – TICA; Ticagrelor – IRB; Irbesartan – HCT; Hydrochlorothiazide
[a] Mean ± SD for the five determinations; [b]% Relative standard deviation; [c]% Relative error

List of Abbreviations

ANN, Artificial Neural Network; TICA, Ticagrelor; IRB, Irbesartan; HCT, Hydrochlorothiazide; HNN, hidden neurons number; Lc, Learning coefficient; RMSEP, Root mean square error of prediction; MSE, Mean square error.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

All laboratory requirements; instrumentation, reagents, and solvents were funded by Beirut Arab University, Lebanon.

Authors' contributions

A.A.G pointed out the idea of the assay, gave the plan of work, and revised the output data.

M.F.K controlled the mathematical manifestations, arranged the chemometric steps (practical and data handling), and wrote the final manuscript.

M. J. worked out all practical experiments and prepared for manuscript writing by summing up tables and figures.

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