Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi https://dergipark.org.tr/sdufeffd
Süleyman Demirel University Faculty of Arts and Sciences Journal of Science e-ISSN: 1306-7575
Research Article, 2025, 20(1): 1-9, DOI: 10.29233/sdufeffd.1516938

Simultaneous Quantitation of Amlodipine and Rosuvastatin by Artificial Neural Network

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(Received: 16.07.2024, Accepted: 03.01.2025, Published: 25.05.2025)

Abstract: Artificial neural network (ANN) is a useful tool in chemometrics to model the complex relationship in spectral data resulting in overlapping spectra, spectral interferences, non-linearity, and deviations from Lambert-Beer law. In this work, a new application of ANN was proposed for the simultaneous spectrophotometric quantification of amlodipine (AML) and rosuvastatin (ROS) in tablets despite their overlapping spectra. The relationship between the actual concentrations and absorption data of binary calibration samples was modeled by ANN. The validity of the method was proved by analyzing AML and ROS in their laboratory-made mixtures, intra and, inter-day samples, as well as standard addition samples. The developed method was then applied to the simultaneous quantitation of AML and ROS in commercial tablets and a good agreement was reported between label claim assay results.

Keywords: Chemometrics, Artificial neural network, Amlodipine, Rosuvastatin, Tablet analysis

1. Introduction

Hypertension and hyperlipidemia often occur together in patients due to shared risk factors and the pathophysiological links between them [1]. They also worsen the effects of each other, by disrupting the structure of vascular cells and arteries, increasing platelet activity, and consequently increasing the risk of cardiovascular events [2]. Various medications for the treatment of these diseases are prescribed separately or in combination. Amlodipine and rosuvastatin are two of these drugs [1]. Amlodipine is a calcium channel blocker indicated for hypertension and angina [3]. Rosuvastatin is used to treat dyslipidemia and hypercholesterolemia [4]. The combined use of rosuvastatin and amlodipine in hypertensive patients with dyslipidemia has been proven in scientific studies to effectively reduce blood pressure and LDL-C levels [5].

Simultaneous quantification of combined drugs is an important issue in pharmaceutical research and development laboratories. Spectrophotometric methods are frequently utilized in the pharmaceutical industry due to their ease of use and cost-effectiveness. However, overlapping absorption spectra of multiple drug substances make it difficult to use conventional spectrophotometric methods for drug combinations. To overcome this problem, derivative spectrophotometry and its modified versions [7-10], and multivariate calibration techniques [11-12] have been applied in the literature. However, in the case of deviations from Lambert-Beer law, derivative and multivariate models cannot achieve

an accurate and reliable spectrophotometric analysis. In quantitative mixture analysis, calibration may become non-linear due to high noise or instrumental fluctuations [13]. In such cases, the artificial neural network (ANN) approach can be used to model complex and non-linear chemical data. ANN is a useful tool that resolves complex spectroscopic measurement data and establishes the relationships between the independent and dependent variables directly from raw data. The theoretical background of the ANN model is explained below.

The basic structure of artificial neural networks consists of an input and output layer. Additionally, there are a series of hidden layers between the input and output layers. These hidden layers provide a useful possibility to estimate unknown and complex relations using an iterative training process. The hidden layer(s) using activation functions and numeric weights give a balance to obtain the best relationship between the input and output set [13-15]. For example, Figure 2 shows the architecture of the artificial neural network model to quantify AML and ROS in a mixture.

Applications have shown that ANN offers significant advantages in solving non-linearity and analyzing complex chemical systems. For this reason, ANN serves as an alternative when classical and multivariate calibration methods fail [14-15].

A literature survey reported that different analytical methods for determining AML and ROS alone or in combination with other active pharmaceutical ingredients such as spectrophotometric [16-18], spectrofluorimetric [19-21], HPLC [22-23], UPLC [24-25] and voltametric methods [26-27]. Moreover, the simultaneous analysis of AML and ROS in pharmaceutical formulations using the HPLC-UV [28], HPLC-PDA [29], and tandem mass spectrometric method [30] was reported. However, no spectrophotometric ANN approach was reported for the simultaneous determination of the studied drugs in pharmaceutical tablets.

In this study, the ANN calibration approach was first proposed for the concurrent quantification of AML and ROS in tablets despite their overlapping spectra. The proposed method was tested by analyzing independent sets of their binary mixtures and standard addition samples. Subsequently, the method was utilized for the quantitative analysis of commercial tablets containing both drugs.

2. Material and Method

2.1. Apparatus and software

A Shimadzu UV-2550 (Kyoto, Japan) double-beam UV-VIS spectrophotometer controlled by UV Probe Software (Kyoto, Japan) was used to record the UV spectrophotometric data. The slit width was 2 nm and the light path was 1 cm. The spectra were obtained in the 200-350 nm region with an increment of 0.05 nm. The data vectors of each spectrum were transferred to Microsoft Excel (Microsoft, USA), arranged as data matrices, and were modeled by the ANN approach in Matlab 7.0 (MathWorks, Natick, MA, USA). Modeling, statistical and regression analysis, and figure plots were performed using Matlab.

2.2. Chemicals and reagents

Amlodipine besylate (AML), rosuvastatin calcium (ROS) compounds, and HPLC-grade methanol (a solvent for the spectrophotometric analysis) were supplied by Sigma-Aldrich (Steinheim, Germany). All samples were freshly prepared daily and stored in the dark during the day.

2.3. Standard, calibration, and validation solutions

Both AML and ROS stock solutions were prepared separately by dissolving 10.0 mg AML and 10.0 mg ROS compounds in methanol in a volume of 100 mL. Calibration, validation, and intra-day, and inter-day solutions were prepared from these stock solutions. In the application of the ANN method, 25 standard calibration solutions with a 5^2 full factorial design were prepared in the range of 4.0-36.0 µg/mL for AML and ROS, as indicated in Table 1. Eleven synthetic mixtures containing AML and ROS at different levels within the working concentration range (See Table 2) were prepared similarly. Intra-day and inter-day samples were prepared at three different concentrations (10, 20, and 30 µg/mL for both AML and ROS) as depicted in Table 3. The standard addition samples were prepared in triplicates by adding AML and ROS standard solutions (0, 10, 20, and 30 µg/ mL) to a portion of the commercial tablet sample solution.

Table 1. Calibration set of AML and ROS

Sample no	AML (µg/mL)	ROS (µg/mL)	Sample no	AML(µg/mL)	ROS(µg/mL)
C1	4	4	C14	20	28
C2	4	12	C15	20	36
C3	4	20	C16	28	4
C4	4	28	C17	28	12
C5	4	36	C18	28	20
C6	12	4	C19	28	28
C7	12	12	C20	28	36
C8	12	20	C21	36	4
C9	12	28	C22	36	12
C10	12	36	C23	36	20
C11	20	4	C24	36	28
C12	20	12	C25	36	36
C13	20	20			

2.4. Preparation of Tablet Samples

20 tablets of ROSUCOR PLUS® Tablet (Celtis İlaç Ltd. Şti.) were weighed and the amount corresponding to one tablet was placed in a 100 mL volumetric flask after being thoroughly powdered in a mortar. The flask was filled with methanol, and the powdered sample was mixed with a mechanical stirrer for 20 minutes and then filtered through a syringe filter (Pall Industries, USA). The filtrate was diluted to the working concentration range for the application of the ANN method. The declared contents of the tablet were as follows: 10 mg ROS and 10 mg AML per tablet.

3. Results

3.1. ANN Approach and Its Application

The main problem in the spectral analysis mixtures is the overlap of spectral bands in the operating wavelength region under non-linear conditions, as in our study (see Figure 1). To address this analytical challenge, we used an ANN approach that can establish relationships between variables directly from raw data. ANN can be used to model complex relationships between independent and dependent variables or find patterns in data.

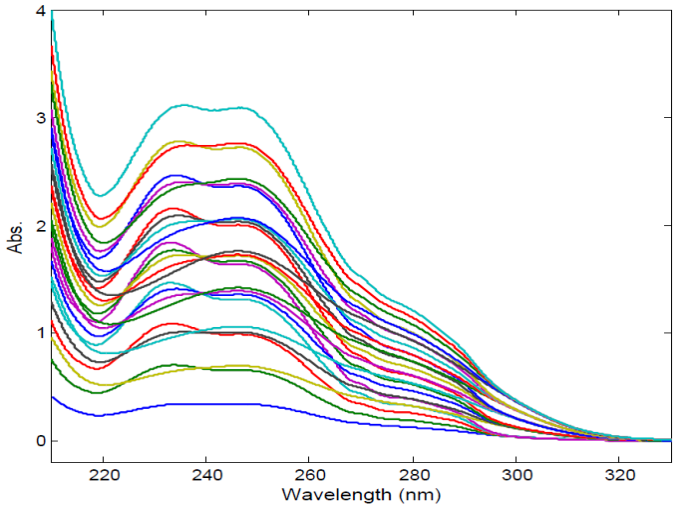


Figure 1. Absorption spectra of 25 binary calibration samples of AML and ROS solutions (C1 to C25 as in Table 1) in methanol

The absorbance spectra of the calibration set of 25 containing AML and ROS, given in Table 1, were recorded in the range of 200-350 nm. the strong spectral overlapping of AML and ROS, depicted in Figure 1, makes it impossible to use univariate linear calibration models for the quantitation of the mentioned drugs. We hypothesized that ANN would be a proper tool for the quantitative estimation of AML and ROS in their mixtures. The actual concentration values and the absorbance values of the calibration set corresponded to x-block and y-block, respectively, in ANN. The nonlinear relationship between the concentration set and their corresponding absorbance values was used to determine the most appropriate ANN calibration model by testing different topological networks and several neuron sizes. A training network with 68 neurons in the input layer, 121 neurons in four hidden layers, and two in the output layer were found to be suitable for calibration and prediction steps. The architecture of the ANN model and the training process monitored in Matlab 7.0 are given in Figure 2.

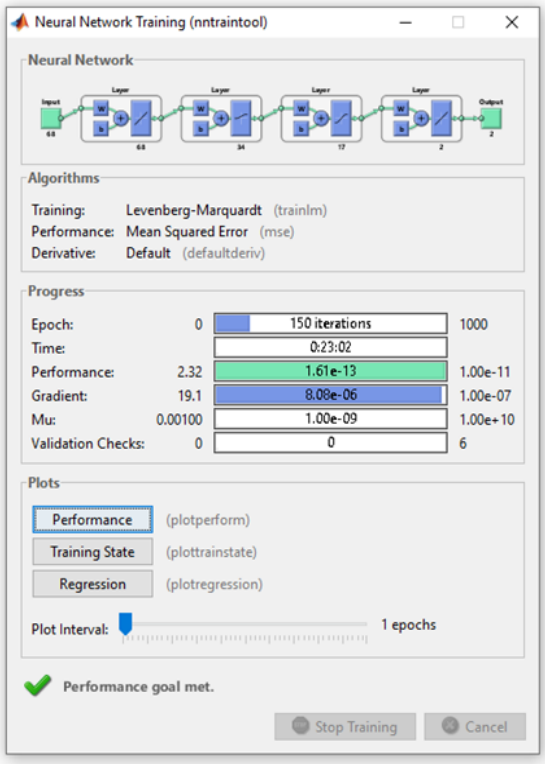


Figure 2. The architecture of the ANN model and the training process

The performance of the training step was evaluated during a back-propagation algorithm using mean square error (MSE), indicating the difference between desired and predicted outputs. Calculated MSE for different number of epochs was calculated and 150 iterations providing lower MSE than the predefined MSE value ($1.00x10^{-11}$) were found appropriate for the training step (See Figure 2). The performance of the training process was also visualized by plotting the actual and predicted concentration values of each drug in the calibration set. As can be seen in Figure 3, the values coincided perfectly.

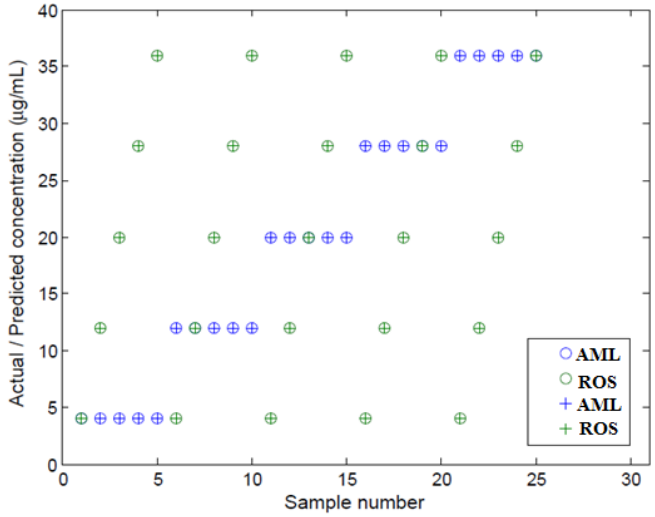


Figure 3. Actual and predicted concentration of AML and ROS in the calibration set

3.2. Validation of ANN Application

To evaluate the performance of the ANN model, an independent test set of 11 binary mixtures was analyzed by the proposed method. Figure 4 and Table 2 indicate the actual and the predicted concentration values of the binary mixtures. Mean recovery and relative standard deviation values were found to be 100.1% and 2.48 for AML and 100.4% and 2.08 for ROS. As can be seen in Table 2, the proposed method achieved high recovery results with good precision.

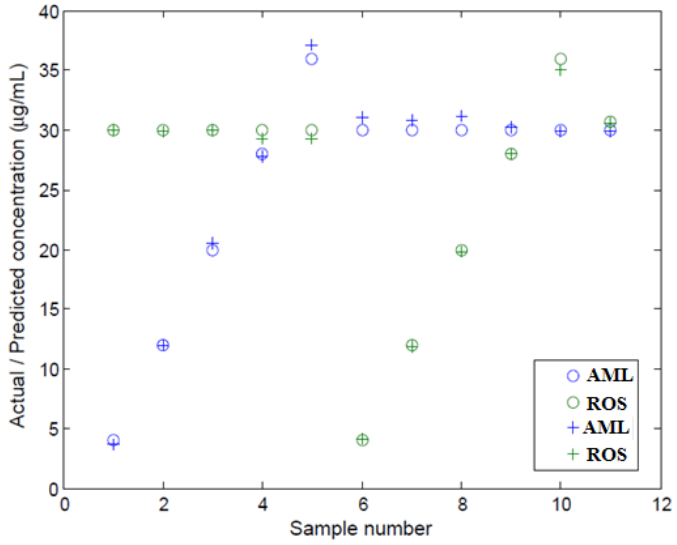


Figure 4. Actual and predicted concentration of AML and ROS in validation samples for recovery studies

Table 2. Recovery results of independent test samples containing different concentrations of AML and ROS by applying the ANN approach

	Actual (µg/µL)		Predicted (µg/µL)		Recovery (%)	
Sample no	**AML**	**ROS**	**AML**	**ROS**	**AML**	**ROS**
1	4	30	4.18	30.05	104.5	100.2
2	12	30	11.69	29.10	97.4	97.0
3	20	30	19.38	29.81	96.9	99.4
4	28	30	27.26	30.77	97.4	102.6
5	36	30	36.43	30.85	101.2	102.8
6	30	4	29.99	3.89	100.0	97.3
7	30	12	29.39	12.24	98.0	102.0
8	30	20	30.25	20.61	100.8	103.0
9	30	28	30.88	27.95	102.9	99.8
10	30	36	30.50	36.35	101.7	101.0
11	30	30	29.97	29.94	99.9	99.8
				Mean	100.1	100.4
				SD	2.48	2.09
				RSD	2.48	2.08

The precision and reproducibility of the ANN approach were evaluated by analyzing the intra-day and inter-day experiments. The average of three results, mean recovery, relative standard deviation, and relative standard error values were given in Table 3. As can be seen in this table, the ANN approach achieved desirable precision and accuracy for the simultaneous analysis of AML and ROS.

Table 3. The recovery results obtained in the intra-day and inter-day samples by the proposed ANN approach

	Added (µg/µL)		Found* (µg/µL		Recovery (%)		RSD (%)		RSE (%)	
	AML	**ROS**	**AML**	**ROS**	**AML**	**ROS**	**AML**	**ROS**	**AML**	**ROS**
Inter-day	10	10	9.88	10.28	98.8	102.8	4.79	1.63	-1.21	2.79
	20	20	19.78	20.16	98.9	100.8	0.73	3.44	-1.11	0.81
	30	30	30.66	29.88	102.2	99.6	5.34	2.70	2.19	-0.41
Intra-day	10	10	9.50	10.39	95.0	103.9	11.33	7.77	-4.96	3.92
	20	20	19.30	19.30	96.5	96.5	2.30	4.14	-3.50	-3.51
	30	30	29.81	30.32	99.4	101.1	2.60	3.05	-0.62	1.08

* n=3 for every concentration level, RSD: relative standard deviation, RSE: relative standard error.

The selectivity of the ANN approach and the effect of excipients of commercial tablets on the simultaneous analysis of binary AML and ROS drugs were determined by the standard addition technique. Standard addition samples were prepared by spiking a fixed volume of tablet solution with the standard AML and ROS solutions at different concentration levels (0 µg/mL, 10 µg/mL, 20 µg/mL, and 30 µg/mL). Samples were prepared three times at each concentration level. The added recovery and relative standard deviation values are listed in Table 4. The results showed that there was no interference of the sample matrix on the analysis of AML and ROS in tablets.

Table 4. The recovery results obtained in the standard addition samples by the proposed ANN approach

	Added (µg/µL)		Found* (µg/µL)		Recovery (%)		RSD (%)	
	AML	**ROS**	**AML**	**ROS**	**AML**	**ROS**	**AML**	**ROS**
Formulation +	10	5	10.12	9.68	101.2	96.8	0.08	0.04
Formulation +	20	20	20.66	20.72	103.3	103.6	0.31	0.27
Formulation +	30	32	30.23	29.70	100.8	99.0	1.29	1.04

RSD: relative standard deviation

3.3. Analysis of Commercial Tablet Formulation

This paper presents the application of a new ANN approach to the simultaneous quantitative analysis of AML and ROS in commercial tablets. Assay results are presented in Table 5. The results provided a good agreement with the reported label claim of the commercial sample. The standard deviation and relative standard deviation given in Table 5 confirm the applicability and performance of the proposed method.

Table 5. Assay results obtained by ANN approach to tablet samples

Exp. no	AML (mg/tablet)	ROS (mg/tablet)
1	9.70	10.31
2	10.15	9.99
3	10.29	10.05
4	10.03	9.90
5	9.87	9.85
6	10.08	9.82
7	10.21	10.02
8	10.15	9.83
9	10.31	9.89
10	10.04	9.75
Mean	10.08	9.94
SD	0.19	0.16
RSD	1.86	1.62

Claimed label: 10 mg ROS and 10 mg AML per tablet.

4. Conclusion

The main aim of this research was to develop a simple, cost-effective, selective, sensitive, and validated method for the analysis of a commercial tablet containing AML and ROS. The developed ANN approach was successfully used for the simultaneous quantification of AML and ROS in both synthetic mixtures and tablet samples. The findings showed that the ANN approach provided accurate predictions and was effective in the quantitative determination of the mentioned drugs in samples without requiring any separation step. This study confirms the practicality and validity of a quick, easy, and reliable ANN method for simultaneously quantifying AML and ROS in synthetic mixtures and commercial tablets without any separation. When the proposed ANN approach was compared with an analytical HPLC reference method [28] in terms of recovery and relative standard deviation, it was found that there were comparable and reasonable results. We conclude that in the pharmaceutical industry and R&D laboratories, the ANN approach can be utilized for quality control and routine analysis of binary combined preparations containing AML and ROS active compounds.

Authorship contribution statement

E. Dinç: Conceptualization, Visualization, Writing, Review and Editing; **Z.C. Ertekin**: Investigation, Formal Analysis, Data Curation, Original Draft Writing, Review and Editing; **A. Üçer**: Investigation, Formal Analysis, Original Draft Writing, Review, and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Committee Approval and/or Informed Consent Information

As the authors of this study, we declare that we do not have any ethics committee approval and/or informed consent statement.

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