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Simultaneous Determination of Amlodipine and Rosuvastatin in Pharmaceutical Preparations by Square Wave Voltammetry

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ABSTRACT

Objectives: In this work, a new, rapid, simple, precise and specific method has been developed for the simultaneous determination of amlodipine (AML) and rosuvastatin (ROS) in pharmaceutical preparations by square wave voltammetry (SWV). **Methods:** Electrochemical behavior and simultaneous voltammetric determination of AML and ROS were investigated using platinum disk electrode. Validation parameters such as specificity, linearity, accuracy, precision, ruggedness, stability, limit of quantification and limit of detection were studied according to the ICH Guidelines. **Results:** The linearity of this developed method was established in the concentration range of 5-40 µg/mL for AML and ROS, respectively. The precision was less than 1.88 and 1.93 %, determined from quality control samples for AML and ROS, and accuracy was within 1.69 and 1.97 % in terms of relative error, respectively. The percentage recovery obtained for AML and ROS in pharmaceutical preparations were 99.5 and 100.2 %, respectively. Limit of detection and quantification for AML were 0.70 and 2.10 µg/mL, for ROS 0.80 and 2.40 µg/mL, respectively. **Conclusion:** The developed SWV method can be used for routine analysis of AML and ROS in pharmaceutical preparations.

Keywords: *Amlodipine; Rosuvastatin; Square wave voltammetry; Validation*

INTRODUCTION

Hyperlipidemia and hypertension are major risk factors for the development of atherosclerosis and its associated conditions such as ischemic cerebrovascular disease, coronary heart disease and peripheral vascular disease[1-3].

Amlodipine (AML) **(Figure 1a),** a calcium antagonist, is prescribed for the treatment of hypertension and angina pectoris. It has a long elimination half-life and large volume of distribution. Rosuvastatin (ROS) **(Figure 1b),** a synthetic statin, was developed for the treatment of hyperlipidaemia[4,5].

The dose dependent peak plasma concentration reached 3-5 h after oral administration of a 10- to 80-mg dose[6-8]. The combination of AML and ROS exerts more beneficial effects on cardiomyocyte hypertrophy and fibrosis[9,10].

In literature, a few methods using HPLC were reported for the simultaneous determination of AML and ROS in pharmaceutical preparations respectively[11-13].

Electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there are, in most, instances no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques[14-17]. Despite the analytical importance no report has been published till today on the voltammetric study of the electrochemical oxidation of AML and ROS in non-aqueous media.

Therefore, this paper describes a new SWV method for the simultaneous determination of AML and ROS. The SWV method was aimed at developing an easy and rapid assay method for AML and ROS without any time consuming sample preparation steps for routine analysis.

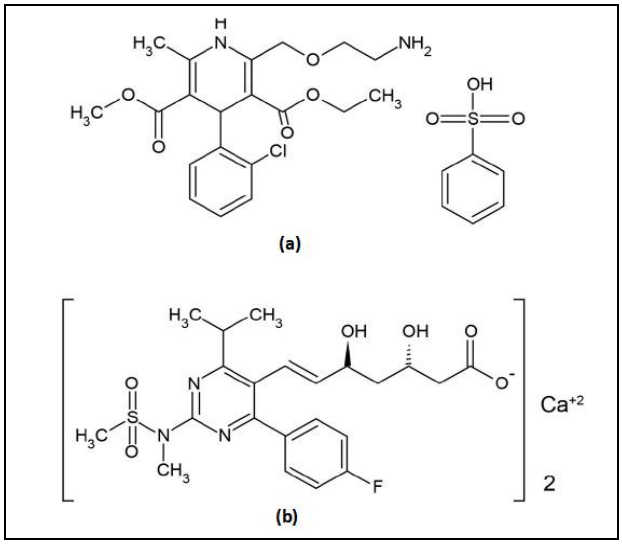

Figure 1. Chemical structures of AML (a) and ROS (b)

MATERIALS AND METHODS

Chemicals

Standard samples of AML (purity 99.06 %) and ROS calcium (purity 99.55 %) respectively were obtained, from Sigma (St. Louis, MO, USA). Rosucor® film coated tablet was obtained from pharmacy (Erzurum, Turkey).

Voltametric system

Electrochemical experiments were performed on a Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. All measurements were carried out with a standard three-electrode arrangement. In this study, platinum working disk (Pt) electrode, platinum wire and Ag/AgCl/KCl (3.0 M) reference electrode were used. For analytical conditions, SWP pulse amplitude 25 mV, frequency 15 Hz, potential step 4 mV were used.

Preparation of the standard and quality control solutions

The stock standard solutions of 100 µg/mL AML and ROS were prepared in 0.1 M $LiClO_4$/acetonitrile. After, standard solutions were prepared as 5-40 µg/mL (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL) for AML and ROS. The quality control samples were prepared 7.5, 17.5 and 37.5 µg/mL for the AML and ROS

RESULTS AND DISCUSSION

Electrochemical behavior of AML and ROS

The electrochemical behaviors of AML and ROS were investigated at the Pt disc electrode in anhydrous acetonitrile solution containing 0.1 M $LiClO_4$ as the supporting electrolyte by using cyclic voltammetry (CV). The electrochemical behavior of AML and ROS on Pt was investigated by use of CV. **Figure 2** shows the CV profile of the electrochemical oxidation of AML and ROS at 50 µg/mL concentration in 0.1 M $LiClO_4$/acetonitrile solution at the Pt electrode.

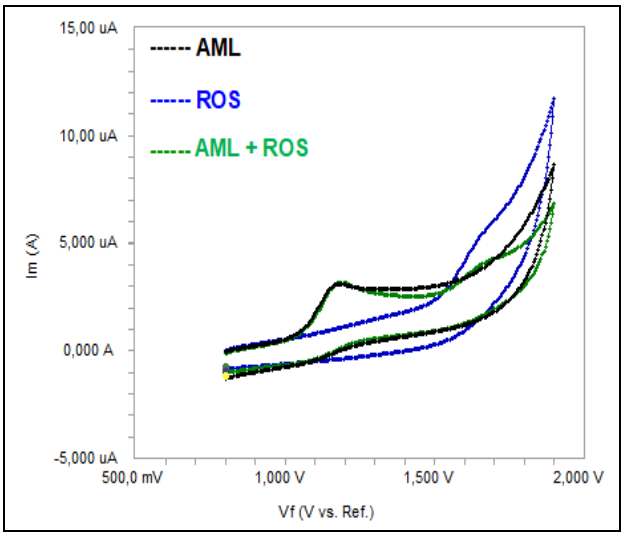

Figure 2. CV voltammogram for the oxidation of AML (50 µg/mL) and ROS (50 µg/mL) in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s.

As seen in the **Figure 3**, AML and ROS exhibited only one well-defined oxidation peak at 1.15 V and 1.61 V, respectively.

Method validation

Parameters such as specificity, linearity, precision, accuracy, limit of detection, limit of quantification, ruggedness, recovery and stability parameters were investigated according to the ICH guidelines[18,19]

Specificity

The specificity of the method was investigated for the excipients, AML and ROS. Voltammograms of AML and ROS are given in **Figures 4** and **5.**

Linearity

The linearity of AML and ROS was studied between 5-40 µg/mL concentration range. The calibration curves were evaluated by its correlation coefficients. The calibration equation, correlation coefficient, the standard deviations of the slope and intercept of the calibration curves were in **Table 1** and **Figures 6** and **7.**

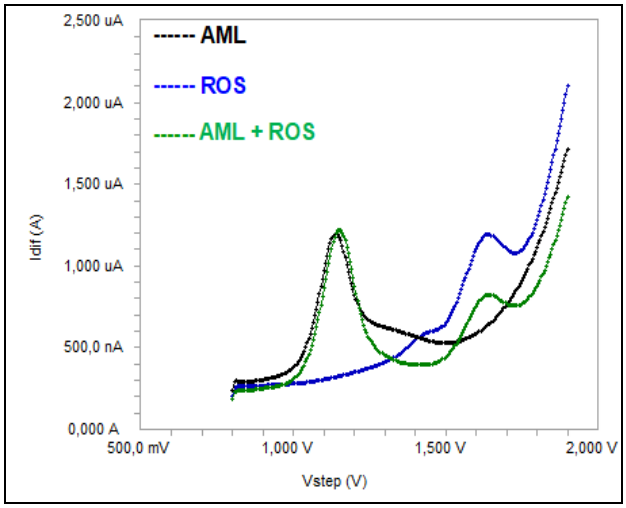

Figure 3. DPV voltammogram of AML (20 µg/mL) and ROS (20 µg/mL) in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s.

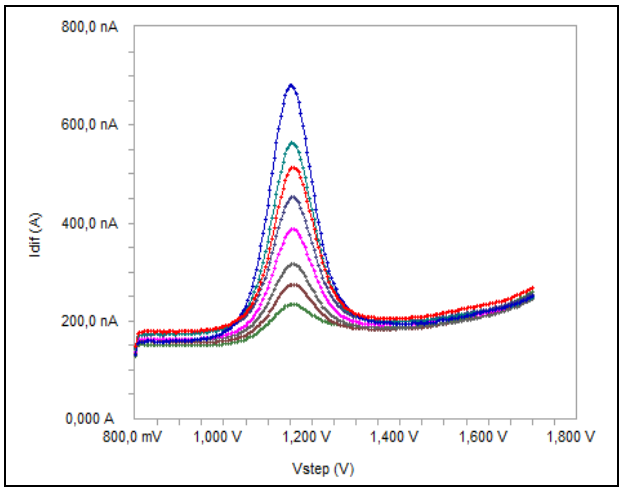

Figure 4. SWV voltammograms for different concentrations of AML in acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL)

Precision and accuracy

The precision was determined by intra-day and inter-day repeatability. The accuracy of method was assessed as the percentage relative error. Three different concentrations (7.5, 17.5 and 37.5 µg/mL) were analyzed six time in one day for intra-day precision and accuracy, and once daily for three days for inter-day precision and accuracy. The results are summarised in **Table 1.**

Limits of detection (LOD) and quantitation (LOQ)

For SWV measurements, LOD and LOQ of AML and ROS were determined using calibration standards. The LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n=6)[20]. The results were given in **Table 1.**

Stability

To determine the stability of AML and ROS standard solutions in the refrigerator and at room temperature, AML and ROS solutions of 10, 20 and 30 µg ml^{-1} concentrations and stock solution were stored in the refrigerator and at room temperature for two days. Then, the stability measurements were carried out. The results were evaluated by comparing these measurements with those of standards and expressed as percentage deviation. The stability of AML and ROS solutions were determined by keeping them for three days in the refrigerator and for two days in room temperature. A significant change in concentration (recovery = 100 ± 2.1%) were not found under both conditions. In addition to this, stock solutions were found to be stable for a week in refrigerator.

Ruggedness

In this study, the SWP determination of AML and ROS was carried out by a different analyst in same instrument with the same standard. The results showed no statistical differences between different operators suggesting that the developed method was rugged.

Application of Method

Ten tablets of AML and ROS (Rosucor®) were accurately weighed and powdered. For the SWV method, an amount of this powder corresponding to one tablet AML and ROS content was weighed and accurately transferred into 100 mL calibrated flask and 50 mL of 0.1 M $LiClO_4$/acetonitrile was added and then the flask was sonicated to 10 min at room tempature. The flask was filled to volume with 0.1 M $LiClO_4$/acetonitrile. The resulting solutions in both the cases were filtered through Whatman filter paper no 42 and suitably diluted to get final concentration within the limits of linearity for the respective proposed method. In this work, SWV method was applied for the simultaneous determination of AML and ROS from their pharmaceutical preparation **(Figure 8).**

To determine the accuracy of the SWV method and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The results are summarised in **Table 2.**

Table 1. Linearity of AML and ROS

Parameters	SWV	
	AML	ROS
Potential (V)	1.15	1.61
Linearity (µg/mL)	5-40	5-40
Slope	12.69	11.892
Intercept	185.36	161.68
R	0.9948	0.9907
Sa	18.16	12.24
Sb	0.725	0.813
LOD (µg/mL)	0.70	0.80
LOQ (µg/mL)	2.10	2.40
Intra-day precision (RSD%)[a]	1.88	1.64
Inter-day precision (RSD%)[a]	1.97	1.97
Intra-day accuracy (% relative error)	-1.69	1.78
Inter-day accuracy (% relative error)	1.97	1.96

RSD: Relative standard deviation, [a]Average of six replicate determinations, Sa: Standard deviation of intercept of regression line, Sb: Standard deviation of slope of regression line, R: Coefficient of correlation, LOD: Limit of detection, LOQ: Limit of quantification

Table 2. Recovery of AML and ROS in pharmaceutical preparation

	SWV	
	AML	ROS
Labeled claim (mg)	5	20
Amount found (mg)[a]	4.96	20.06
RSD %	1.39	1.97
Bias%	-0.80	0.30
Added (mg)	10	10
Found (mg)	9.95	10.02
Recovery %	99.5	100.2
RSD % of recovery	1.84	1.79

[a] *Each value is the mean of six experiments*

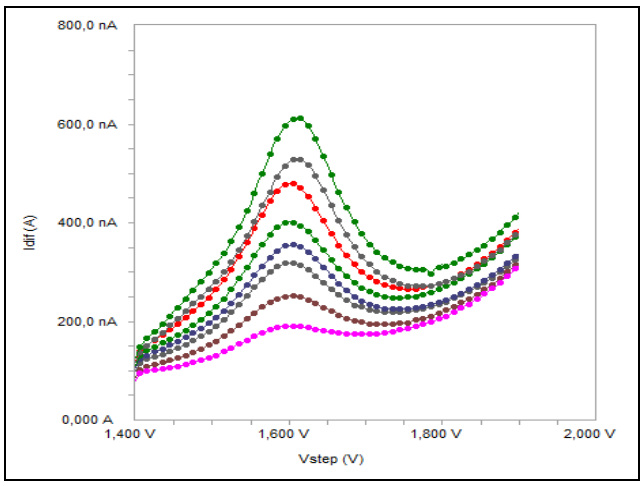

Figure 5. SWV voltammograms for different concentrations of ROS in acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL)

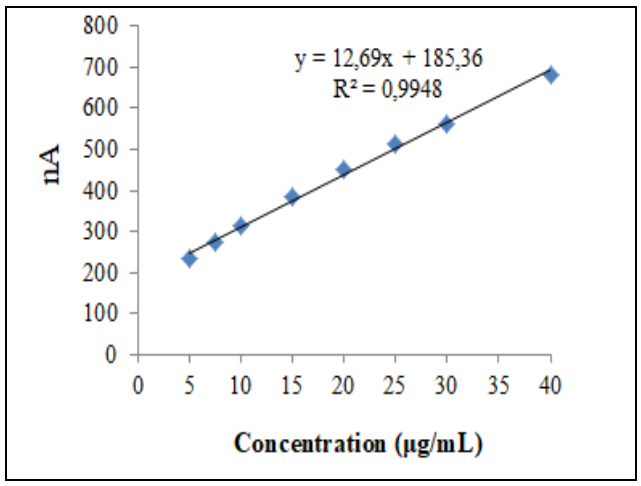

Figure 6. The linearity of AML

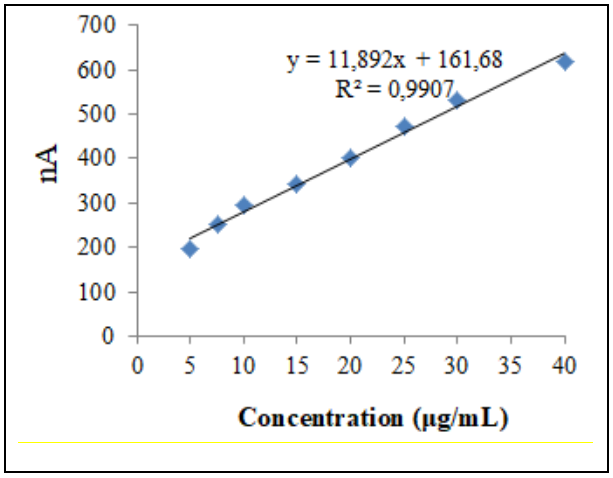

Figure 7. The linearity of ROS

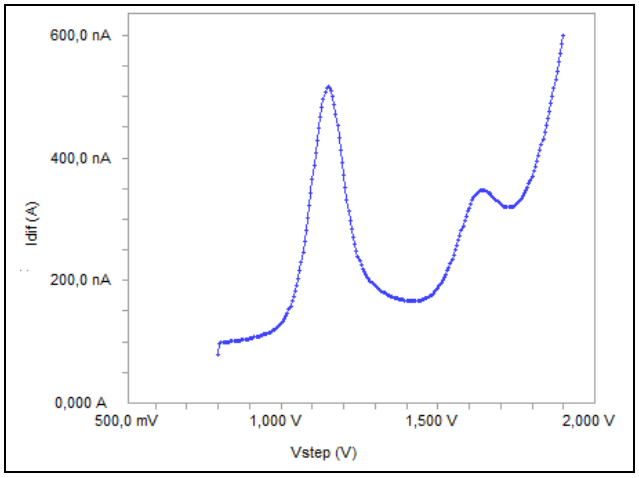

Figure 8. SWV voltammogram of Rosucor® film coated tablet (25 µg/mL)

CONCLUSION

In the present work, a new and simple SWV method has been developed for the simultaneous quantitation of AML and ROS in pharmaceutical preparations and the method was validated. The proposed SWV method is accurate, precise and specific. Therefore, the proposed method can be used effectively, without separation and interference, for routine analysis of AML and ROS in pure form and its pharmaceutical preparations.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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