Received: 23 April 2020 | Revised: 15 June 2020 | Accepted: 21 June 2020
DOI: 10.22034/ecc.2020.109382
Eurasian Chemical Communications
SAMI

FULL PAPER

Simultaneous determination of rosuvastatin and amlodipine in binary mixtures by differential pulse voltammetry and HPLC methods

Bilal Yilmaz* |**Nurullah Yilmaz**

Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, Turkey

***Corresponding Author:**
Bilal Yilmaz
Email: yilmazb@atauni.edu.tr
Tel.: +90 (442) 1315213

This research study discusses two rapid, sensitive and specific methods for simultaneous determination of rosuvastatin (ROS) and amlodipine (AML) in pharmaceutical preparations using the differential pulse voltammetry (DPV) and high-performance liquid chromatography (HPLC). Electrochemical behavior and simultaneous voltammetric determination of ROS and AML were investigated using the platinum disk electrode. HPLC was also developed for the comparison. The flow rate of the mobile phase was 1.0 mL/min and all the detections were carried out at 225 nm using the UV detection. The calibration curve was established over the concentration range of 0.5-4 µg/mL for DPV and 0.1-2 µg/mL for HPLC. The intra- and inter-day relative standard deviation was less than 2.96 and 3.07% for DPV and HPLC, respectively. Limits of quantification were determined as 0.21 and 0.06 µg/mL for DPV and HPLC, respectively. Both the drugs along with their degradation products were separated in less than 8 min. No interference was found from tablet excipients at the selected assay conditions. The methods were applied for the quality control of the commercial ROS and AML drug.

KEYWORDS

Rosuvastatin; amlodipine; differential pulse voltammetry; HPLC; validation.

Introduction

Hypertension is a major risk factor for developing the atherosclerosis and its associated conditions such as ischemic cerebrovascular disease, coronary heart disease and peripheral vascular disease[1-3]. Rosuvastatin (ROS) (Figure 1-a), is used for 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]. Amlodipine (AML) (Figure 1-b) is prescribed for the treatment of hypertension and angina pectoris.

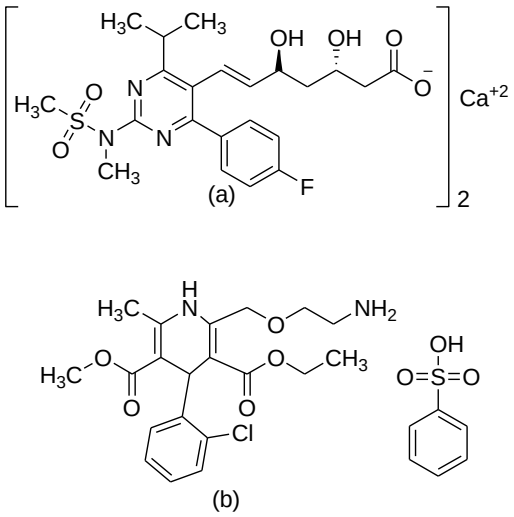

FIGURE 1 Chemical structures of ROS (a) and AML (b)

It has a long elimination half-life and the large volume of distribution. The combination of ROS and AML exerts more beneficial effects on cardiomyocyte hypertrophy and fibrosis [9, 10]. To our knowledge, no scientific papers regarding the simultaneous determination of ROS-AML by use of DPV method have been published. In literature, a few methods using HPLC method applied for the simultaneous determination of these drugs have been reported [11-13]. The reported methods although utilized isocratic elution with low retention times of both the analytes but they lack stress testing on the drugs and therefore unable to separate degradation products. We also focused our attention to develop and validate a simple and precise stability indicating HPLC method for the concurrent determination of ROS and AML.

The development of a new method is important for the determination of the drug amount in pharmaceutical preparations. Redox reactions of drugs in invitro conditions can inform us about what kind of reaction they can get in the body after are taken into the body [14-16]. Despite the analytical importance of the electrochemical behavior and oxidation mechanism of ROS-AML, no report has been published on the voltammetric study of the electrochemical oxidation of ROS-AML in nonaqueous media. It is well known that the experimental and instrumental parameters directly affect the electrochemical process and voltammetric response of drugs. Consequently, it is of a great importance to investigate the oxidation process of the ROS-AML in aprotic media.

Therefore, this study discussed two new DPV and HPLC methods for the simultaneous determination of ROS-AML. The DPV method was aimed at developing an easy and rapid assay method for ROS-AML without any time consuming sample preparation steps for routine analysis. HPLC method was attempted to demonstrate the utility of UV detection for the simultaneous determination of ROS-AML with simple sample preparation and reasonable analysis time with high precision. Also, the developed methods were used to determine the total drug content in commercially available tablets of ROS-AML.

Experimental

Materials

ROS calcium (99.55%) and AML (99.06%) standards were obtained from the Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, lithium perchlorate ($LiClO_4$) and other chemicals were purchased from Fluka. Rosucor® film coated tablet was obtained from pharmacy (Erzurum, Turkey).

Voltammetric and chromatographic system

Electrochemical experiments were performed on a Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. All the measurements were carried out in a single-compartment electrochemical cell with a standard three-electrode arrangement. A platinum disk (Pt) and a platinum wire were used as the working and the counter electrodes, respectively. All potentials were reported versus Ag/AgCl/KCl (3.0 M) reference electrode at room temperature. Operating conditions for DPV were pulse amplitude 50 mV, pulse width 50 ms and scan rate 20 mV/s.

HPLC analysis was carried out on an Agilent 1260 series HPLC system was used for the method development and validation studies. This chromatographic system was equipped with a quaternary pump (G7111A), auto injector (G7129A), and UV detector (G71144A). The separations were performed at 25 °C using Ace C_{18} (250×4.60 mm ID, 5µm) analytical column.

Preparation of standard and quality control solutions

For the DPV method, the stock standard solutions of ROS and AML were prepared in 0.1 M $LiClO_4$/acetonitrile to a concentration of

100 µg/mL. For the HPLC method, the stock solutions of ROS and AML were prepared in methanol solution to a concentration of 100 µg/mL. Standard solutions were prepared as 0.5-4 µg/mL (0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 µg/mL) for DPV and 0.05-2 µg/mL (0.10, 0.25, 0.50, 1.0, 1.5 and 2.0 µg/mL) for the HPLC method. The quality control (QC) samples were prepared by adding aliquots of standard working solution of ROS and AML to final concentrations of 0.60, 1.25 and 2.75 µg/mL for the DPV and 0.15, 1.25 and 1.75 µg/mL for the HPLC.

Procedure for pharmaceutical preparations

Ten tablets of ROS and AML (Rosucor®) accurately weighed and powdered. For the DPV method, an amount of this powder corresponding to one tablet ROS and AML 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 temperature. 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. For the HPLC method, an appropriate volume of the filtrate was diluted further with methanol so that the concentration of ROS and AML in the final solution was within the working range, and then analyzed by HPLC.

Results and discussion

Electrochemical behavior of ROS and AML

Electrochemical behaviors of the ROS and AML 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 ROS and AML on Pt was investigated by use of CV. Figure 2 demonstrates the CV profile of the electrochemical oxidation of ROS and AML at 10 and 5.0 µg/mL concentration in 0.1 M $LiClO_4$/acetonitrile solution at the Pt electrode, respectively. It is clear that the electrochemical reactions of these compounds at the Pt are irreversible. As can be seen in Figure 2, ROS and AML exhibit only one well-defined oxidation peak at 1.65 V and 1.19 V, respectively, without the presence of any cathodic peak on the reverse scan.

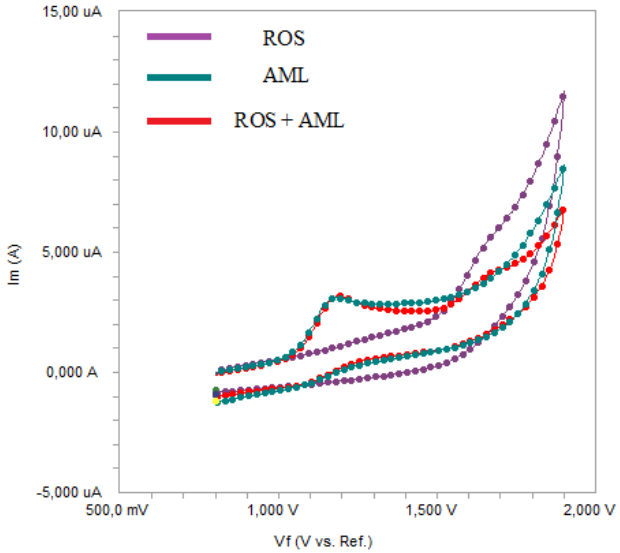

FIGURE 2 CV voltammogram for the oxidation of ROS (10 µg/mL and AML (5 µg/mL) in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s

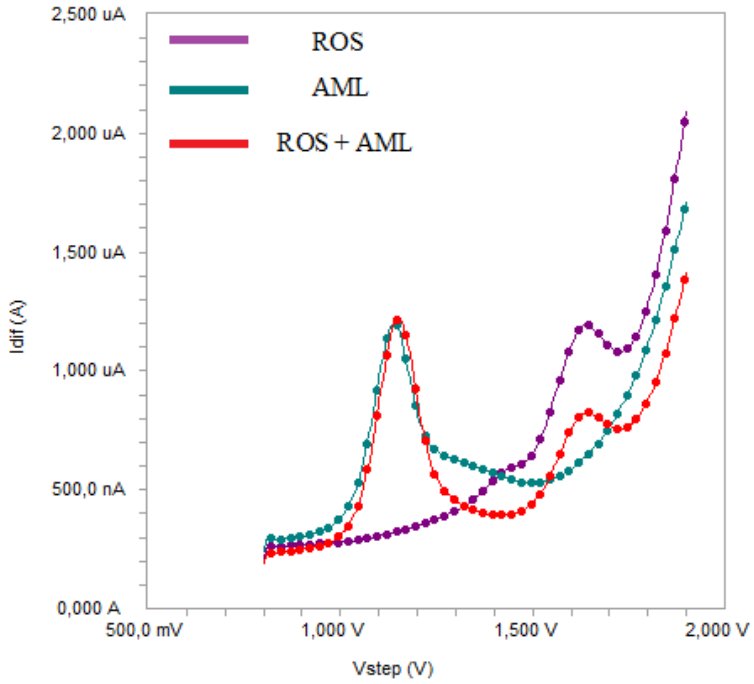

FIGURE 3 DPV voltammogram of ROS (10 µg/mL and AML (10 µg/mL) in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s

In order to gain a deeper insight into the voltammetric waves, the effect of scan rate on the anodic peak currents (I_m) and peak potentials (E_p) was studied in the range of 0.01-1 V/s of the potential scan rates in $LiClO_4$/acetonitrile solution containing 5 µg/mL concentration of ROS and AML. A plot of the logarithm of the peak current versus the logarithm of the scan rate for ROS and AML gave a straight line with a slope of 0.468 and 0.449, respectively. If this curve is linear, diffusion or adsorption process can be expected due to the slope value. If the slope is nearly 0.5, diffusion process can be expected [17].

These results suggested that, the redox species are diffusing freely from solution and not precipitating onto the electrode surface. The reason for this behavior may be due to the solubility of the intermediate species in acetonitrile or poor adherence of products on the electrode surface.

Optimization of HPLC conditions

It was difficult to set chromatographic conditions that produced sharp peak shape and adequate response for ROS and AML due to their different physicochemical properties. These parameters should be suitably monitored to produce the better resolution from endogenous components which in turn affect sensitivity and reproducibility of the analytical method. Once the above mentioned parameters were optimized the flow rate, column temperature and buffer type and concentration can be altered for optimal response. For this reason, an isocratic mobile phase system consisting of acetonitrile:water with the addition of 0.1% H_3PO_4 (40:60; v/v) was selected. The injection volume was 10 µL and the mobile phase flow rate was kept constant at 1 mL/min. Different conditions, for instance, analytical columns, mobile phase composition, flow rate, and column temperature were varied to obtain efficient separation between ROS and AML for HPLC experiments.

Different acetonitrile ratios, such as 35 to 50, were tested and 40% (v/v) acetonitrile was approved for all studies. No interferences with other compounds that originated from

excipients were observed even with this percentage. Different acidic and basic additives were added for the preparation of buffer. In order to arrange the total separation time, flow rate was altered between 0.75 and 1.25 mL/min. The best resolution was achieved at 1.0 mL/min. The temperature of the column oven and detection wavelength was adjusted to 30 °C and to 225 nm, respectively, for all compounds. Final optimized conditions were as follows: mobile phase composition consisting of a mixture of acetonitrile/water (40:60; v/v), containing 0.1% H3PO4, (pH 3.0). This mobile phase composition was found to be optimal for symmetrical peaks as well as to achieve minimal background noise.

Method validation

To ensure the optimization of the methods in light of the standardization rules, we developed these methods along with the process of validation. The assay methods were evaluated through determination of specificity, linearity, accuracy, precision, limit of detection, limit of quantification, recovery and the stability was investigated by analyzing the pure ROS and AML solution and drug samples [18,19].

Specificity

ROS and AML were determined by simultaneously changing their equal concentrations (Figure 4 and 5).

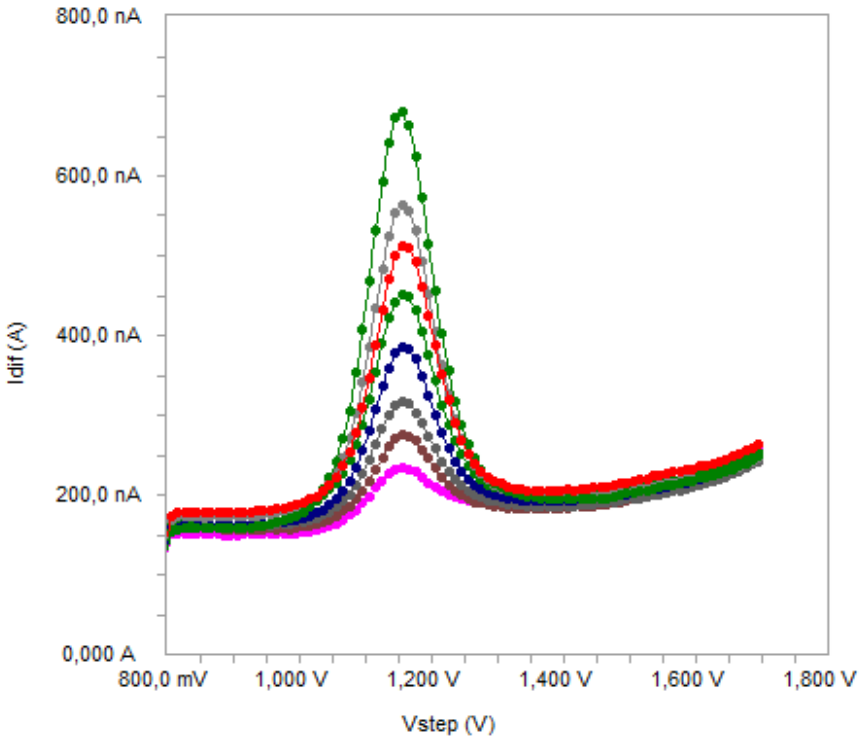

FIGURE 4 DPV voltammograms for different concentrations of AML in acetonitrile solution containing 0.1 M $LiClO_4$ (0.5, 0.75, 1.0, 1.5, 2, 2.5, 3 and 4 µg/mL)

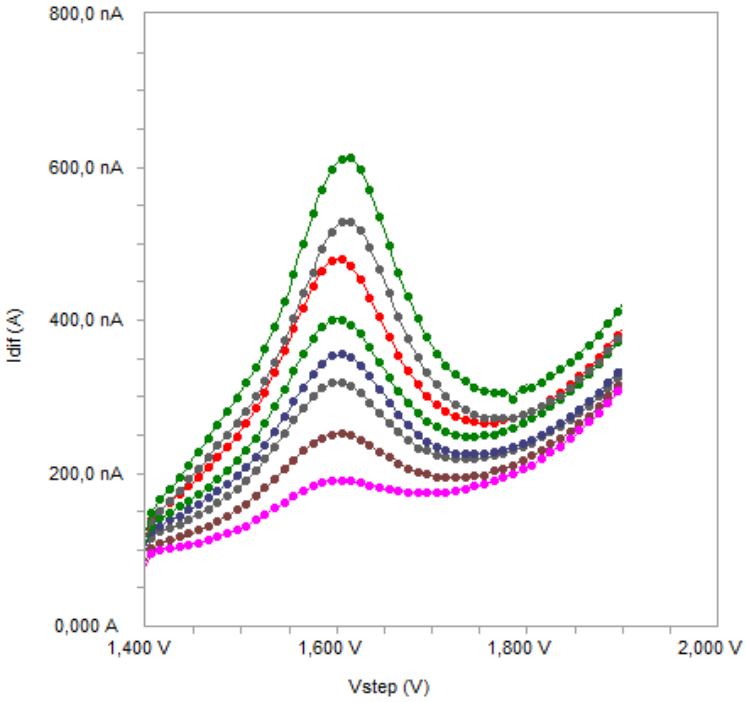

FIGURE 5 DPV voltammograms for different concentrations of ROS in acetonitrile solution containing 0.1 M $LiClO_4$ (0.5, 0.75, 1.0, 1.5, 2, 2.5, 3 and 4 μg/mL)

For chromatographic separations, the retention times were obtained 5.4 min for AML and 7.4 min for ROS, being extremely stable among injections (Figures 6 and 7).

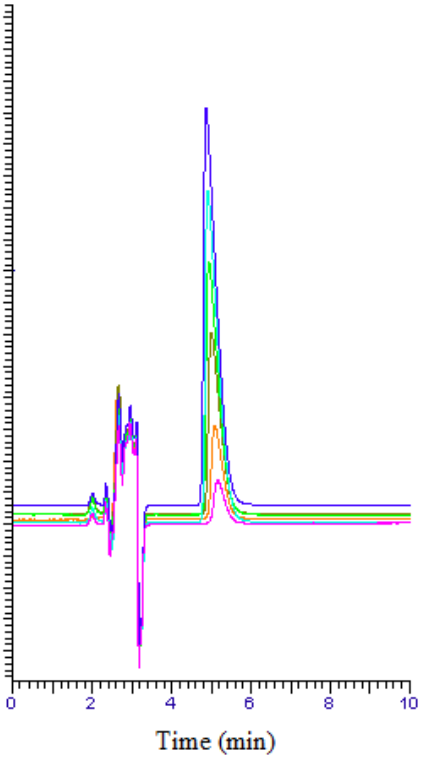

FIGURE 6 HPLC chromatograms of AML (0.10, 0.25, 0.50, 1.0, 1.5, and 2 μg/mL)

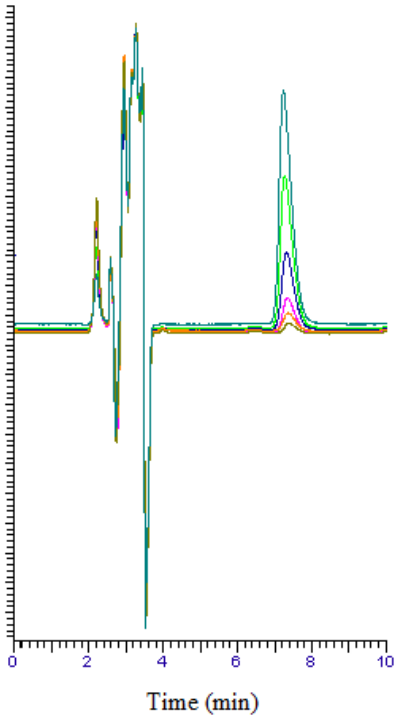

FIGURE 7 HPLC chromatograms of ROS (0.10, 0.25, 0.50, 1.0, 1.5 and 2 µg/mL)

Linearity

For DPV and HPLC measurements, the solutions were prepared by dilution of the stock solution of ROS and AML to reach a concentration range of 0.5-4 µg/mL (0.5, 0.75, 1.0, 1.5, 2, 2.5, 3 and 4 µg/mL) and 0.10-2 µg/mL (0.10, 0.25, 0.50, 1.0, 1.5 and 2 µg/mL), respectively. Calibration curves were constructed for ROS and AML standards by plotting the concentration of ROS and AML versus voltammogram and peak area response. The calibration curve constructed was evaluated by its correlation coefficient. The correlation coefficient (r) of all the calibration curves were consistently greater than 0.99. The results are shown in Table 1.

TABLE 1 The linearity of ROS and AML

Parameters	DPV		HPLC	
	AML	ROS	AML	ROS
Measured potential (V)	1.19	1.61	-	-
Linearity (µg/mL)	0.5-4	0.5-4	0.1-2	0.1-2
Slope	126.5	121.1	6.763	5.324
Intercept	186.1	160.3	27.26	32.67
R	0.995	0.991	0.999	0.998
Sa	2.95	2.83	0.045	0.106
Sb	0.765	1.624	0.024	0.041
LOD (µg/mL)	0.07	0.07	0.02	0.06
LOQ (µg/mL)	0.21	0.21	0.02	0.06
Intra-day precision (RSD%)[a]	1.98	3.27	1.36	2.11
Inter-day precision (RSD%)[a]	2.84	3.07	1.94	2.49
Intra-day accuracy (% relative error)	1.12	2.14	1.16	1.43
Inter-day accuracy (% relative error)	2.69	2.47	2.18	1.97

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

Precision and accuracy

The precision of the DPV and HPLC methods was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by analyzing QC samples six times per day, at three different concentrations which were QC samples. The intermediate precision was evaluated by analyzing the same samples once daily for two days. The relative standard deviation (RSD) of the predicted concentrations from the regression equation was taken as precision. The accuracy of this analytic method was assessed as the percentage relative error. These results are presented in Table 1.

Limits of detection (LOD) and quantitation (LOQ)

For DPV measurements, LOD and LOQ of ROS and AML 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].

For HPLC measurements, the LOD and LOQ of ROS and AML were determined by injecting progressively low concentration of the standard solution under the chromatographic conditions. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as LOQ. The LOD was defined as a signal/noise ratio of 3:1. The LOD and LOQ for DPV were 0.07 and 0.21 μg/mL, for HPLC 0.020 and 0.060 μg/mL, respectively. Among the two methods, HPLC is more sensitive than DPV (Table 1).

Recovery

To determine the accuracy of the DPV and HPLC methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding a known amount of pure drugs to pre-analyzed samples of commercial dosage forms. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 2.

TABLE 2 Recovery of ROS and AML in pharmaceutical preparation

	DPV		HPLC	
	AML	**ROS**	**AML**	**ROS**
Labeled claim (mg)	5	20	5	20
Amount found (mg)[a]	4.95	20.08	5.04	5.03
RSD%	2.43	1.84	2.73	2.81
Bias%	-0.60	0.40	0.53	0.69
Added (mg)	10	10	10	10
Found (mg)	9.94	10.03	10.08	10.12
Recovery%	99.4	100.3	100.9	101.2
RSD% of recovery	2.512	2.91	1.31	1.97

[a] Each value is the mean of six experiments

Forced degradation studies

Stress studies were performed to evaluate the specificity of the method [19]. All the samples were diluted with mobile phase to give a final concentration of 1.0 μg/mL and filtered through 0.45 μm nylon filter before injection.

Acidic degradation

Acidic degradation was performed by treating the drug solution mixture (containing each of 1.0 μg/mL ROS and AML) with 0.1 M hydrochloric acid for 30 min in a thermostat maintained at 80 °C. The drug solution mixture was cooled, neutralized with 0.1 M

sodium hydroxide and then diluted with mobile phase as per the requirement and 10 µL of the solution was injected into the HPLC system.

Alkaline degradation

Alkaline degradation was performed by treating the drug solution mixture (containing each of 1.0 µg/mL ROS and AML) with 0.1 M sodium hydroxide for 30 min in a thermostat maintained at 80 °C. The drug solution mixture was cooled, neutralized with 0.1 M hydrochloric acid and then diluted with mobile phase as per the requirement and 10 µL of the solution was injected into the HPLC system (Figure 8).

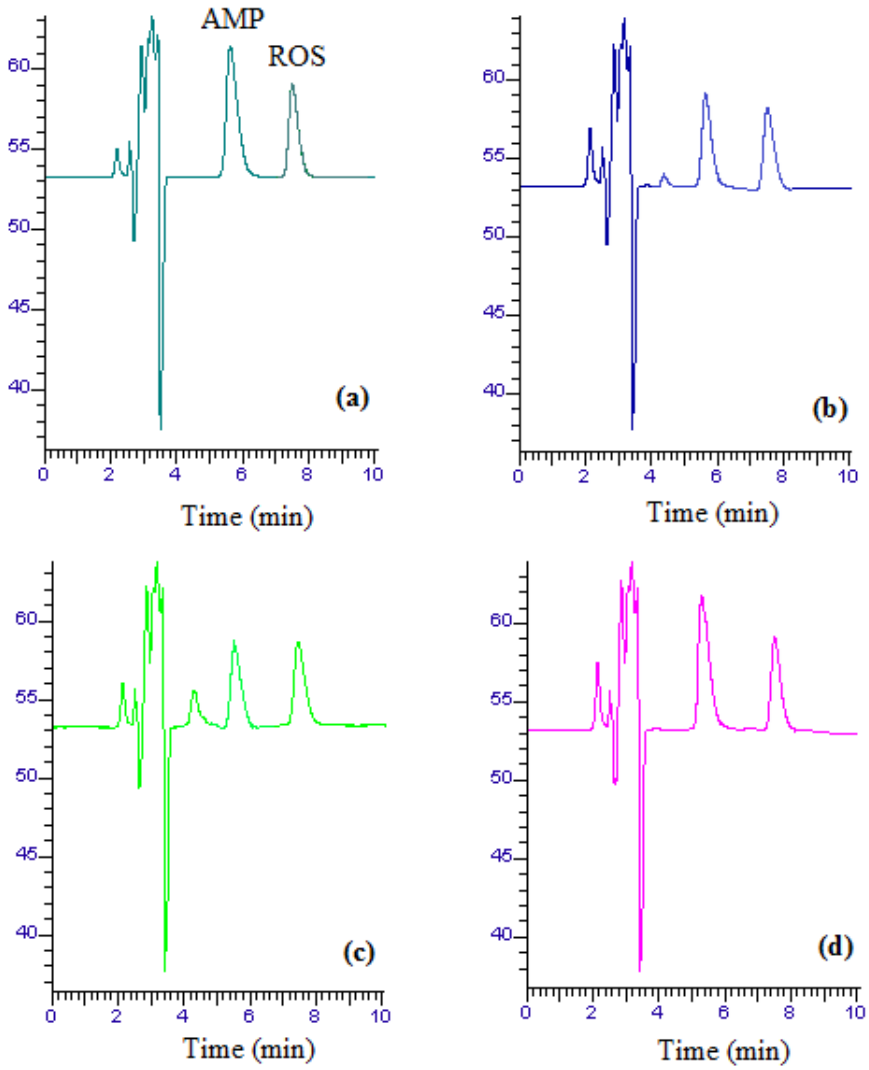

FİGURE 8 Typical Chromatograms of ROS and AML on acidic (a), alkali (b), oxidation (c) and thermal (d) degradations

Oxidation degradation

Oxidation degradation was performed by treating the drug solution mixture (containing each of 1.0 µg/mL ROS and AML) with 30% H_2O_2 for 30 min in a thermostat maintained at 80 °C. The drug solution mixture was cooled and then diluted with mobile phase as per the requirement and 10 µL of the solution was injected into the HPLC system.

Thermal degradation

The drug solution mixture (containing each of 1 µg/mL ROS and AML) was in a thermostat maintained at 80 °C for 10 h, cooled and 10 µL

of the solution was injected into the HPLC system.

The specificity of the developed method can be determined from the stress studies and the percentage drug recovery was calculated from the peak area of the resultant chromatograms. 30.26 % of ROS has undergone alkaline degradation. The carboxylic acid group present in the ROS chemical structure is highly responsible for the alkaline degradation. AML also has undergone alkaline degradation (43.96 %) and the -CO_2H, -CO_2R, amide group present in the chemical structure may be responsible for it. During the oxidation an extra peak was observed at 4.36 min. During the acidic, oxidative and thermal degradations the percentage of decomposition was found to be less than 20.0 %. The results obtained during the stress degradation conditions are presented in Table 3.

TABLE 3 Stability of ROS and AML in solution (n=3)

Stress conditions		% Drug recovery	% Drug decomposed	Theoretical plates	Tailing factor	% RSD
ROS	Standard drug					0.57
	Acidic degration	98.93	1.07	26081.4	1.08	0.42
	Alkaline degration	69.74	30.26	25983.7	1.09	0.85
	Oxidative degration	92.62	7.38	26127.2	1.08	0.74
	Thermal degration	99.64	0.36	26047.2	1.08	0.21
AML	Standard drug					0.35
	Acidic degration	80.92	19.08	38604.2	1.08	0.23
	Alkaline degration	56.04	43.96	39011.8	1.03	0.54
	Oxidative degration	89.44	11.56	38886.1	1.02	0.56
	Thermal degration	96.21	3.79	38375.7	1.04	0.62

System suitability

The suitability of the HPLC system was tested before each stage of validation. The system suitability parameters for all the degradation studies were shown in Table 3. The number of theoretical plates (N) is used to determine the performance and effectiveness of the column. The efficiency of a column can be measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Columns with N ranging from 5,000 to 100,000 plates / meter are ideal for a good system. Efficiency can be calculated by using the formula: $N=5.54\ [R_t/W_{h/2}]^2$

Where 'W' is the peak width, 'h' is the height of the peak and 'R_t' is the retention time of the drug peak. The theoretical plates were found to be more than 2000 and the tailing factor was less than <1.5 -2 or <2 indicating that the method is more selective and specific.

Comparison of methods

In this work, both DPV and HPLC methods were applied for the simultaneous determination of ROS and AML from their pharmaceutical preparations (Figures 9 and 10).

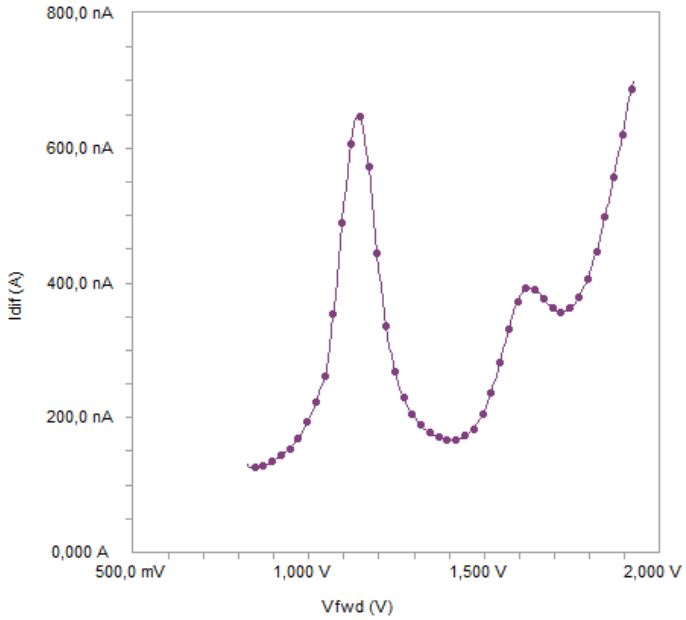

FIGURE 9 DPV voltammogram of Rosucor® film coated tablet (5 μg/mL)

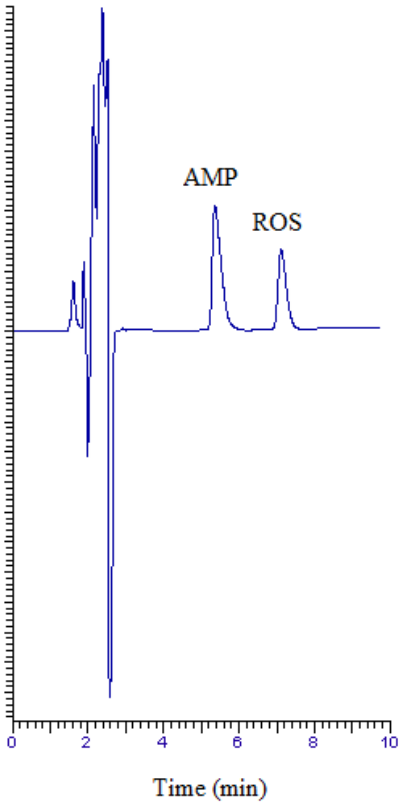

FIGURE 10 HPLC chromatogram of Rosucor® film coated tablet (5 μg/mL)

Electrochemical behaviors of ROS and AML were also assessed and the electrochemical process was found to be irreversible and controlled by diffusion. By using DPV method, simultaneous determination of both compounds was achieved. Under optimized conditions, current responses of both compounds significantly increased. The repeatability results of the voltammetric responses are in good agreement with the

validation requirements (RSD< 3%). Recovery experiments also showed that the proposed method was not affected from the matrix in the pharmaceutical dosage form. The results of ROS and AML were compared to published papers (Table 4). According to the results, the most sensitive responses were obtained in this study. From the analytical point of view, the developed isocratic HPLC method has advantages when compared to published papers. First of all, in the present study, two compounds including ROS and AML were well separated. The solvent consumption of the present work is less than those in the already published methods [12, 13]. Furthermore, the present work was fully validated according to the ICH guidelines.

TABLE 4 Comparison of proposed and reported methods for determination of ROS and AML

Compound	Linear range (µg/mL)	LOD (µg/mL)	Reference
AMLP	0.57-39.7	0.34	[21]
AML	13.8-19.6	-	[22]
ROS	0.10-24.1	0.013	[23]
ROS	0.20-10	0.07	[24]
ROS	0.27-26.5	0.034	[25]
Simulteneous determination of ROS and AML	0.5-4	0.21	Present study (DPV)
Simulteneous determination of ROS and AML	0.05-2	0.020	Present study (HPLC)

Banerjee *et al*. [13] used 10-cm analytical column for the separation of two compounds using the gradient conditions and the separation time was found to be very close to that of the present study, and both of them were completed in 5 min. Tajane *et al*. [12] completed their analysis in 6 min for two compounds [12]. The resolution factor for the present study was higher than those for the published ones. From the sensitivity point of view, the proposed methods were found to be more sensitive than the reported ones. In this study, the LOD values were reported as 0.11 and 0.06 µg/mL for ROS and AML, respectively [12]. In our proposed method, LOD values were found as 0.020 and 0.060 µg/mL in HPLC method, respectively. The present method has the following advantages over the reported methods. Calibration curves of ROS and AML were linear over the concentration range of 0.10-2.0 µg/mL which is as good as or superior to that reported in other papers [21-26]. For all the concentrations studied, intra- and inter-day RSD values were ≤3.07% and for all concentrations of ROS and AML the relative errors were ≤2.69%. These results are well within the acceptance limits [27-32].

Conclusion

In the present work, two new methods were developed and validated for simultaneous routine determination of ROS and AML in pharmaceutical preparations. All the proposed methods were found to be accurate, precise, specific, and sensitive. They found to be better than previously reported methods. This was due to their wide range of linearity, use of an economical, readily available, and greener solutions and lack of extraction procedures. Both the DPV and HPLC methods involved a sensitive, simple, rapid, cost-effective process for the simultaneous quantification of ROS and AML in pharmaceutical preparations without the necessity of sample pretreatment, and time consuming evaporation or extraction steps prior to analysis. All the proposed methods were found to be suitable for quality control laboratory, where economy and time are essential. High percentage recoveries revealed

that, the proposed methods are free from interferences of the commonly used excipient and additives in pharmaceutical preparations.

Acknowledgements

This study was supported by a Grant from the Ataturk University Research Foundation (Project no: TYL-2017-6214).

Orcid:
Bilal Yilmaz: https://orcid.org/0000-0002-8574-7570

References

[1] J. Shepherd, *Am. J. Cardiol.,* **1998**, *82,* 57T-59T.
[2] D. Williams, J. Feely, *Clin. Pharmacokinet.,* **2002**, *41,* 343-370.
[3] J.W. Jukema, A.H. Zeinderman, A.J. Van Boven, J.H. Reiber, A. Van Der Laarse, K.I. Lie, A.V. Bruschke, *Arterioscler Thromb. Vasc. Biol.*, **1996**, *16,* 425-430.
[4] A.G. Olsson, F. McTaggart, A. Raza, *Cardiovasc. Drug Rev.,* **2002**, *20,* 303-328.
[5] J.W. Blasetto, E.A. Stein, W.V. Brown, R. Chitra, A. Raza, Am. J. Cardiol., **2003**, *91,* 3C-10C.
[6] P.D. Martin, A.L. Dane, O.M. Nwose, D.W. Schneck, M.J. Warwick, *J.Clin. Pharmacol.,* **2002**, *42,* 1116-1121.
[7] P.D. Martin, P.D. Mitchell, D.W. Schneck, *Br. J. Clin. Pharmacol.,* **2002,** *54,* 472-477.
[8] P.D. Martin, M.J. Warwick, A.L. Dane, C. Brindley, T. Short, *Clin. Ther.,* **2003**, *25,* 2553-2563.
[9] J.W. Jukema, J.W. Van Der Hoorn, *Expert Opin. Pharmacother.,* **2004**, *5,* 459-468.
[10] B.Y. Kang, W. Wang, P. Palade, S.G. Sharma, J.L. Mehta, *J. Cardiovas. Pharmacol.,* **2009**, *54,* 327-334.
[11] N. Karadas Bakirhan, M. Gumustas, B. Uslu, S.A. Ozkan, *Ionics* **2016**, *22,* 277-288.
[12] D. Tajane, A.M. Raurale, P.D. Bharande, A.N. Mali, A.V. Gadkari, V.R. Bhosale, *J. Chem. Pharm. Res.,* **2012**, *4,* 2789-2794.
[13] S.K. Banerjee, N.M. Vasava, *Bull. Pharm. Res.,* **2013**, *3,* 29-33.
[14] G.B. El-Hefnawey, I.S. El-Hallag, E.M. Ghoneim, M.M. Ghoneim, *J. Pharm. Biomed. Anal.,* **2004**, *34,* 75-86.
[15] P. Corti, G. Corbini, P. Gratteri, S. Furlanetto, S. Pinzauti, *Int. J. Pharm.,* **1994**, *11,* 83-87
[16] A. Radi, T. Elmogy, *IL Farmaco,* **2005,** *60,* 43-46.
[17] United States Pharmacopeia 32 (USP 32) Revision; United States Pharmacopeia Convention, Rockville, MD, USA, **2009**.
[18] I.C.H, Stability testing of new drug substances and products, Q1A(R2). International Conference on Harmonization; IFPMA, Geneva, **2003**.
[19] E. Laviron, L. Roullier, C. Degrand, *J. Electroanal. Chem.,* **1980**, *112,* 11-23.
[20] B. Yilmaz, D. Ekinci, *Rev. Anal. Chem.,* **2011**, *30,* 187-193.
[21] E. Arkan, Z. Karimi, M. Shamsipur, R. Saber, *J. Reports Pharmaceutical Sci.,* **2014**, *3,* 99-107.
[22] Z.Z. Stoiljkovic, M.L. Avramov Ivic, S.D. Petrovic, D.Z. Mijin, S.I. Stevanovic, U.C. Lacnjevac, A.D. Marinkovic, *Int. J. Electrochem. Sci.,* **2012**, *7,* 2288-2303.
[23] A.A. Ramadan, H. Mandil, N. Ghazal, *Int. J. Pharmacy Pharmaceutical Sci.,* **2014**, *6,* 128-133.
[24] S. Altınoz, B. Uyar, *Anal. Methods,* **2013**, *5,* 5709-5716.
[25] T.A. Silva, H. Zanin, F.C. Vicentini, E.J. Corat, O. Fatibello-Filho, *Sensors Act. B Chemical*, **2015**, *218,* 51-59.
[26] M. Ashfaq, T. Akhtar, G. Mustafa, M. Danish, S.N. Razzaq, M.F. Nazar, *Braz. J. Pharm. Sci.,* **2014**, *50,* 629-638.
[27] US DHHS. FDA and CDER. Guidance for industry: bioanalytical method validation. Rockville (MD): US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, **2001**.

[28] Guideline on bioanalytical method validation. Science and Medicinal Health. European Medicines Agency (EMEA). EMEA/CHMP/EWP/192217/2009, **2011**.
[29] ICH, Topic Q2A validation of analytical procedures, methodology, PMP/ICH/281/95.
[30] C.M Riley , T.W. Rosanske, Development and validation of analytical methods. Elsevier; New York, **1996**.
[31] M.E. Swartz, I.S. Krull, Analytical method development and validation. Marcel Dekker, New York, **1997**.
[32] The United States Pharmacopoeia. 24th revision, Easton, Rand Mc Nally Taunton, **2000**.

How to cite this article: Bilal Yilmaz*, Nurullah Yilmaz. Simultaneous determination of rosuvastatin and amlodipine in binary mixtures by differential pulse voltammetry and HPLC methods. *Eurasian Chemical Communications*, 2020, 2(8), 881-894. **Link:** http://www.echemcom.com/article_109382.html