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Simultaneous Determination of Amlodipine and Hydrochlorothiazide in Pharmaceutical Preparations by Differential Pulse Voltammetry Method

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ABSTRACT

Objectives: In this study, a new, fast and reliable differential pulse voltammetry (DPV) method was developed and validated for the simultaneous determination of amlodipine (AML) and ***hydrochlorothiazide*** (HCT) in pharmaceutical preparations. **Methods**: Electrochemical behavior and simultaneous voltammetric determination of AML and HCT were investigated using glassy carbon electrode. Validation parameters such as specificity, linearity, accuracy, precision, ruggedness, stability, limit of quantification and limit of detection were studied according to the International Conference on Harmonisation (ICH) Guidelines. **Results**: The linearity of this developed method was established in the concentration range of 2.5-30 μg/mL for AML and HCT, respectively. The precision was less than 5.34 and 2.91 %, determined from quality control samples for AML and HCT, and accuracy was within 2.57 and 3.60 % in terms of relative error, respectively. The percentage recovery obtained for AML and HCT in pharmaceutical preparations were 99.6 and 100.1 %, respectively. Limits of detection and quantification for AML and HCT were 0.80 and 2.40 µg/mL, respectively. **Conclusion**: The developed DPV method can be used for routine analysis of AML and HCT in pharmaceutical preparations.

Keywords: *Amlodipine; Differential pulse voltammetry;* Hydrochlorothiazide*; Validation*

INTRODUCTION

Hypertension is major risk factor 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. Hydrochlorothiazide (HCT) **(Figure 1b)** is a diuretic drug of the thiazide class, which acts by inhibiting the ability of the kidneys to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance[4,5].

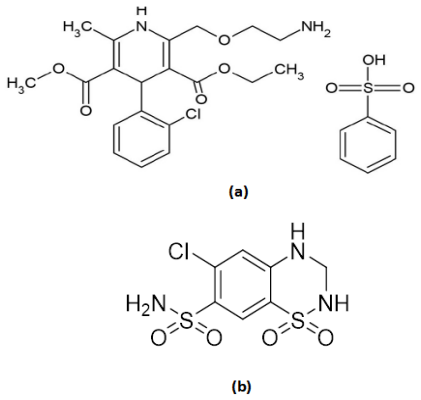

Figure 1. Chemical structures of AML (a) and HCT (b)

Several methods have been reported for the determination of AML alone and its combination are by

UV[6], voltametry[7], mass spectrophotometry[8,9] and HPLC[10]. There are many published methods for the determination of HCT in tablets using spectrophotometry[11], fluorodensistometry[12] and gas chromatography[13].

On extensive survey of literature, no DPV method is reported till date for the simultaneous determination of AML and HCT in pure and pharmaceutical dosage forms. We wanted to develop a new DPV method for the simultaneous determination of AML and HCT in pharmaceutical preparations. The method was aimed at developing an easy and rapid assay method for AML and HCT without any time consuming sample preparation steps for routine analysis, to be adopted in quality control and drug testing laboratories, and at the same time ensure satisfactory recovery during drug determination from pharmaceutical formulations.

In the proposed method, there is no need to extract the drug from the formulation excipient matrix thereby decreasing the error in quantitation. Formulation sample can be directly used after dissolving and filtration. The developed method was used to determine the total drug content in commercially available tablets of AML and HCT.

MATERIALS AND METHODS

Chemicals

Standard samples of AML and HCT (purities ≥ 98%) were obtained from Sigma (St. Louis, MO, USA). Cardofix Plus 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, glassy carbon electrode, platinum wire and Ag/AgCl/KCl (3.0 M) reference electrode were used. Operating conditions for DPV were pulse amplitude 50 mV, pulse width 50 ms and scan rate 20 mV/s.

Preparation of the standard and quality control solutions

The stock standard solutions of 100 µg/mL AML and HCT were prepared in 0.04 M Britton-Robinson buffer solution (pH 5.0). After, standard solutions were prepared as 2.5-30 µg/mL (2.5, 5, 10, 15, 20, 25 and 30 µg/mL) for AML and HCT. The quality control samples were prepared 7.5, 17.5 and 27.5 µg/mL for the AML and HCT.

RESULTS AND DISCUSSION

Electrochemical behavior of AML and HCT

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].

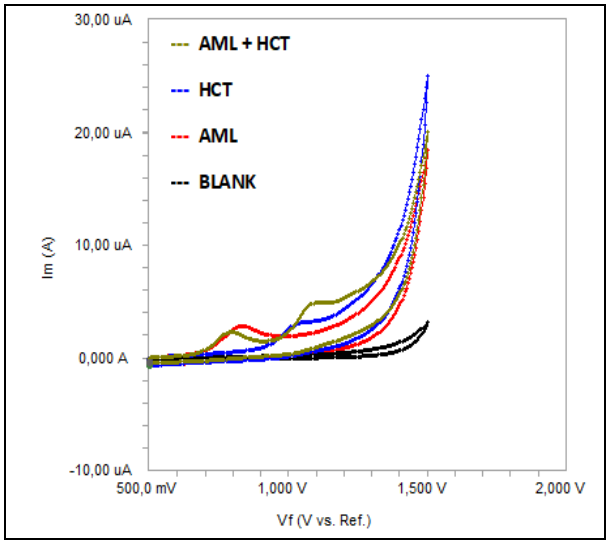

Figure 2. CV voltammogram for the oxidation of AML (50 µg/mL) and HCT (50 µg/mL) in 0.04 M Britton-Robinson buffer solution (pH 5.0) at glassy carbon electrode, scan rate: 0.1 V/s.

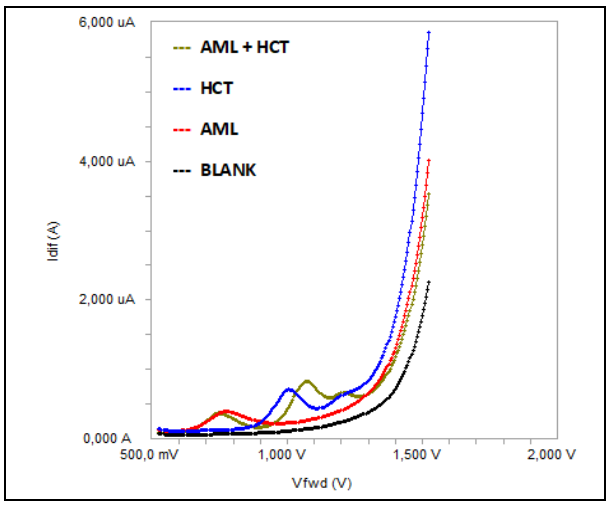

Figure 3. DPV voltammogram of AML (20 µg/mL) and HCT (20 µg/mL) in 0.04 M Britton-Robinson buffer solution (pH 5.0) at glassy carbon electrode, scan rate: 0.1 V/s.

The electrochemical behaviors of AML and HCT were investigated at the glassy carbon electrode in 0.04 M Britton-Robinson buffer solution (pH 5.0) as the supporting electrolyte by using cyclic voltammetry (CV). The electrochemical behavior of AML and HCT on glassy carbon electrode was investigated by use of CV. **Figure 2** shows the CV profile of the electrochemical oxidation of AML and HCT at 50 µg/mL concentration in 0.04 M Britton-Robinson buffer solution (pH 5.0) at the glassy carbon electrode.

As seen in the **Figure 3**, AML and HCT exhibited only one well-defined oxidation peak at +0.80 V and +1.03 V, respectively.

Method validation

Parameters such as specificity, linearity, precision, accuracy, limit of detection, limit of quantification, recovery and stability parameters were investigated according to the International Conference on Harmonisation (ICH) guidelines[18,19].

Specificity

The effects of common excipients and additives were tested for their possible interferences in the assay of AML and HCT. The simulated and placebo samples were prepared and analyzed. The presence of titanium dioxide, ***sodium chloride,*** talc, lactose, starch, and magnesium stearate did not appear interfere in the results of the analysis. Voltammograms of AML and HCT are given in **Figures 4,5.**

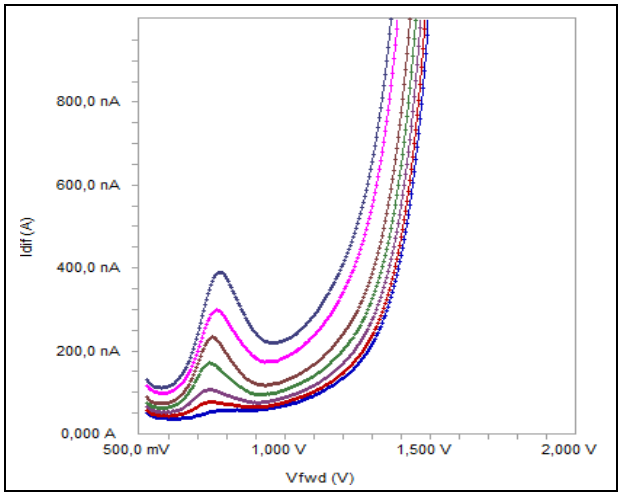

Figure 4. DPV voltammograms for different concentrations of AML in 0.04 M Britton-Robinson buffer solution (pH 5.0) (2.5, 5, 10, 15, 20, 25 and 30 µg/mL)

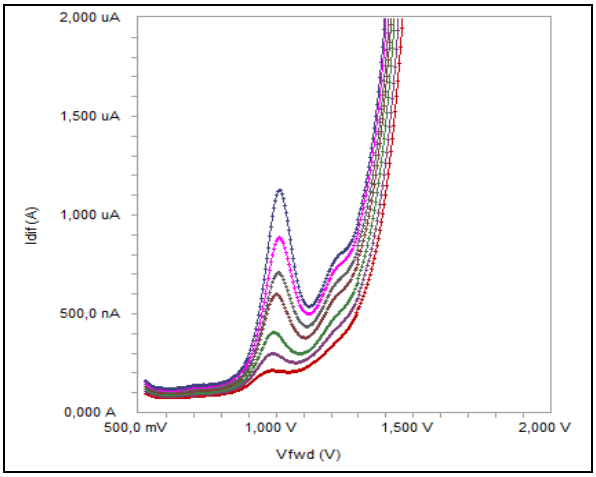

Figure 5. DPV voltammograms for different concentrations of HCT in 0.04 M Britton-Robinson buffer solution (pH 5.0) (2.5, 5, 10, 15, 20, 25 and 30 µg/mL)

Linearity

The linearity of AML and HCT was studied between 2.5-30 µg/mL concentration range (**Figures 6,7)**. Calibration curves were constructed for AML and HCT standard by plotting the concentration of compound versus peak current responses. The results was summarized in **Table 1.**

Table 1. Linearity of AML and HCT

Parameters	DPV	
	AML	**HCT**
Potential (V)	+0.80	+1.03
Linearity (µg/mL)	2.5-30	2.5-30
Slope	0.0264	0.0638
Intercept	0.1364	0.3811
R	0.9986	0.9941
Sa	1.726	0.234
Sb	0.312	0.712
LOD (µg/mL)	0.80	0.80
LOQ (µg/mL)	2.40	2.40
Intra-day precision (RSD %)[a]	5.34	2.61
Inter-day precision (RSD %)[a]	4.92	2.91
Intra-day accuracy (% relative error)	2.57	3.60
Inter-day accuracy (% relative error)	-5.88	6.29

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

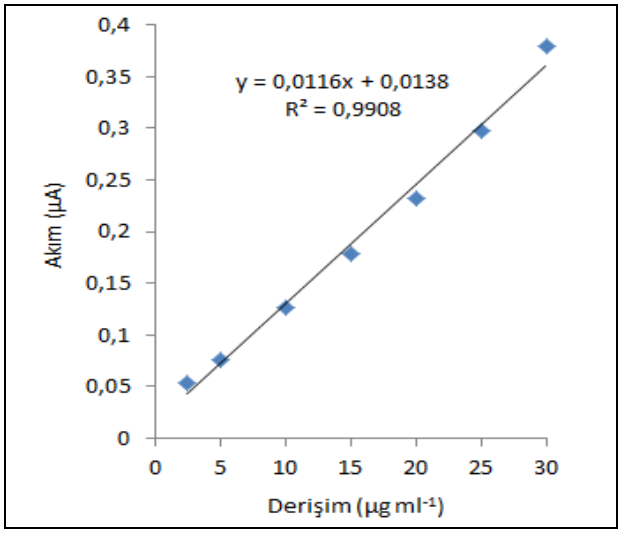

Figure 6. The linearity of AML

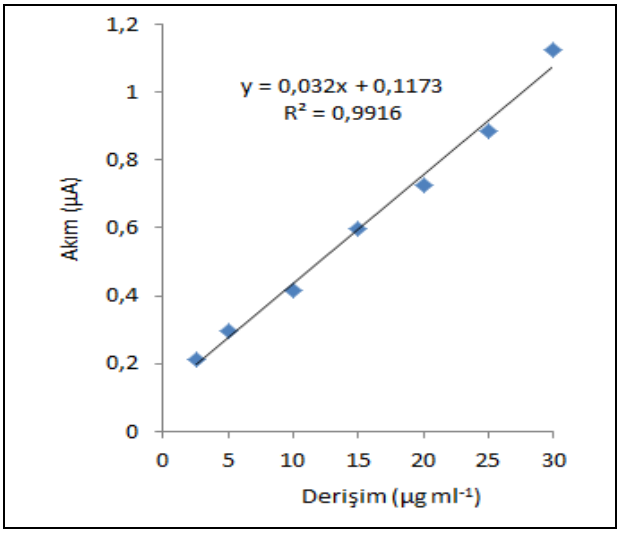

Figure 7. The linearity of HCT

Precision and accuracy

Precision and accuracy of the assay method were determined for both intra-day and inter-day variations using the six times analysis of the quality control (QC) samples. The results are summarised in **Table 1.**

Limits of detection (LOD) and quantification (LOQ)

For DPV measurements, LOD and LOQ of AML and HCT 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 evaluate the stability of AML and HCT, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of calibration curve for different temperature and times. These solutions were stored at room temperature, refrigerator (4 °C) and frozen (-20 °C) temperature for 24 h and 72h. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation. AML and HCT were found as stable at room temperature, 4 and -20 °C for at least 72h. The stability of AML and HCT was obtained within the acceptance range of 90-110%.

Recovery

To determine the accuracy of the DPV 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 tablet form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The proposed method when used for extraction and subsequent estimation of both drugs from pharmaceutical dosage form after spiking with 80, 100 and 120 % of additional drug afforded recovery of 99.6-100.1 %.

Ruggedness

Five sets of experiments for these drugs were carried out using two different analysts; no significant difference was obtained between the results in this study. The standard deviation of peak currents was calculated for parameter and % RSD was found to be less than 2%. The low value of % RSD value indicated ruggedness of the method.

Application of Method

Ten tablets of AML and HCT (Cardofix Plus) were accurately weighed and powdered. For the DPV method, an amount of this powder corresponding to one tablet AML and HCT content was weighed and accurately transferred into 100 mL calibrated flask and 50 mL of 0.04 M Britton-Robinson buffer solution (pH 5.0) was added and then the flask was sonicated to 10 min at room temperature. The flask was filled to volume with 0.04 M Britton-Robinson buffer solution (pH 5.0). The resulting solutions in both the cases were filtered through Whatman filter paper no 42. DPV method was applied for the simultaneous determination of AML and HCT from their pharmaceutical preparation **(Figure 8 and Table 2).**

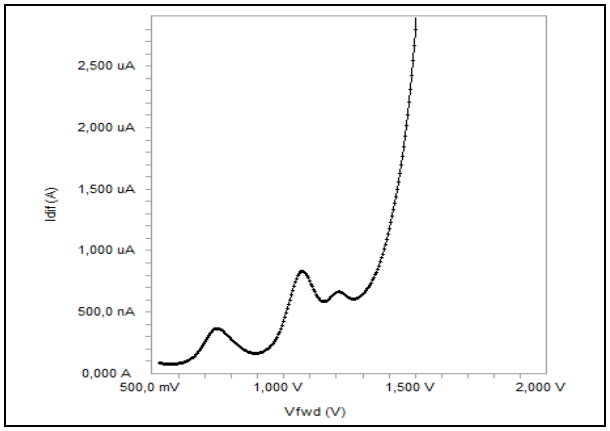

Figure 8. DPV voltammogram of Cardofix Plus tablet (25 µg/mL)

Several methods have been reported for the determination of AML in pharmaceutical preparations alone and its combination are by UV and HPLC. Malesuik *et. al.*[21] developed isocratic HPLC and spectrophotometric methods for quantitative determination of AML in tablets and compounded capsules. The analyses was performed by using mobile phase composed of 0.1 % (v/v) ortho-phosphoric acid (pH 3.0)-acetonitrile (60: 40, v/v) at a flow rate of 1 mL/min. Mohammadi *et. al.*[22] developed isocratic stability indicating HPLC method for the simultaneous determination of atorvastatin and AML in commercial tablets. Separation was achieved using a mobile phase consisting of acetonitrile-0.025 M NaH_2PO_4 buffer (pH 4.5) (55:45, v/v) at a flow rate of 1 mL/min at 237 nm. Dongre *et. al.*[23] developed a specific HPLC method for the simultaneous determination of metoprolol succinate and AML besylate in dosage form. The mobile phase consisted of aqueous triethylamine and acetonitrile in the ratio of 85:15 (v/v) at a flow rate of 1 mL/min. Detection using HPLC would be a more sensitive approach but is costly and not yet available for every laboratory.

Table 2. Recovery of AML and HCT in pharmaceutical preparation

	DPV	
	AML	**HCT**
Labeled claim (mg)	10	12.5
Amount found (mg)[a]	9.96	12.51
Bias %	-0.40	0.10
Recovery %	99.6	100.1
RSD % of recovery	2.65	1.26

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

CONCLUSION

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

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Conflict of Interest

The authors declare that they don't have any conflict of interest.

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