

Electrochemical Method Development for Enalapril Determination in a Pharmaceutical Formulation

Abdulaziz Amro, Muneer Fadr

Department of Chemistry, Faculty of Science, Taibah University, Al Madinah Al Munawwarah, Kingdom of Saudi Arabia

Abstract

Aims: The present study focuses on a comparison between three voltammetric methods for enalapril assay in a pharmaceutical formulation, in addition to optimization of voltammetric analysis parameters such as working electrode and supporting electrolyte. **Materials and Methods:** Glassy carbon (GC) working electrode and KNO_3 (1 M) supporting electrolyte exhibited the best performance compared to other working electrodes and supporting electrolytes used in this study. **Results and Discussion:** Voltammograms of enalapril exhibited two oxidation peaks at 1.4 V and 1.75 V. Square wave voltammetry (SWV) showed the lowest limit of detection (LOD) and limit of quantitation (LOQ) with values of 90.1 and 247 $\mu\text{g/mL}$ LOD and LOQ, respectively. Furthermore, SWV exhibited the lowest relative standard deviation values of 2.33 and 3.87% for inter and intraday analysis, respectively. SWV showed high-performance in recovery study of commercially available enalapril tablets ANGIOTEC 20 mg[®] reach 99.06%. **Conclusion:** Accuracy, precision, and detection limit of SWV are the best for enalapril analysis compared to other methods in this study.

Key words: Cyclic voltammetry, differential pulse voltammetry, enalapril, square wave voltammetry

INTRODUCTION

Enalapril IUBAC name is (2S)-1-[(2S)-2-[[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino]propanoyl]pyrrolidine-2-carboxylic acid [Figure 1]. Enalapril is majorly used as a medication for high blood pressure related to angiotensin-converting enzyme inhibitor drug class. It is also used for congestive heart failure. Furthermore, enalapril is prodrug of enalaprilate which is formed by biotransformation (hydrolysis) of enalapril.^[1]

Due to its importance, different methods have been used for enalapril assay in pharmaceutical formulation and biological samples. Several studies use chromatographic methods for enalapril assay *in vivo* and *in vitro* samples.^[2-7] Spectrophotometric and atomic absorption spectrometric methods were also used for the analysis of enalapril maleate.^[8] ¹H-NMR spectroscopic was applied for enalapril quantitation in pharmaceutical preparations.^[9]

Electroanalytical methods are remarkable alternatives of spectroscopic and chromatographic

methods because of their high selectivity, sensitivity, simplicity, and low running and instrumentations costs.^[10-14] Furthermore, electroanalytical methods are green analytical method according to type and amount of chemicals used in analysis. Mehmeti *et al.*^[15] used screen printed working electrode and amperometric sensing for electrochemical determination of enalapril in pharmaceutical tablets. Elmali *et al.* applied differential pulse polarographic method for the determination of enalapril maleate.^[16] Gusakova and Ivanovskaya have applied anodic stripping polarography for the quantitation of enalapril in blood serum.^[17] Valezi *et al.* used multiwalled carbon nanotubes paste electrode as working electrode for square wave voltammetry (SWV) determination of amlodipine and enalapril with cationic surfactant.^[18] Farghaly *et al.* studied the voltammetric behavior of enalapril using square wave cathodic stripping voltammetry

Address for correspondence:

Abdulaziz Amro, Department of Chemistry, Faculty of Science, Taibah University, Al Madinah Al Munawwarah, Kingdom of Saudi Arabia. Tel.: +966590990428. E-mail: abdulazizamro@yahoo.com

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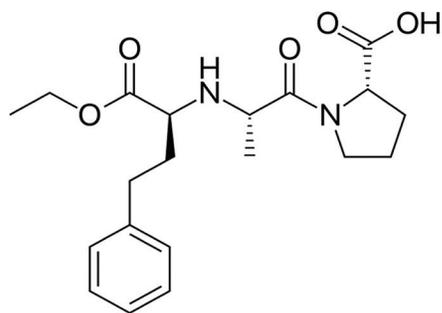


Figure 1: Enalapril structure

with carbon paste working electrode in pharmaceutical dosage forms and biological fluids.^[1]

The present work focuses on comparison between voltammetric methods for enalapril assay in a pharmaceutical formulation, in addition to the optimization of voltammetric analysis parameters such as supporting electrolyte and working electrode.

MATERIALS AND METHODS

Materials

The standard pharmaceutical formulation of enalapril was provided by Hikma Pharmaceuticals company (Jordan). A commercial enalapril ANGIOTEC 20 mg[®] tablets manufactured by Jordanian Pharmaceutical Manufacturing company, Jordan. Potassium nitrate (KNO₃) of ACS reagent grade brought from Fluka[™] (USA).

Phosphate buffer supporting electrolyte of pH 6.8 (NaH₂PO₄/H₃PO₄) was prepared by dissolving 24 g of NaH₂PO₄·H₂O in 800 mL of deionized water, then 85% H₃PO₄ has been added to reach pH 6.8, after that deionized water is used to complete volume to 1.0 L.

Standard solutions

Stock solutions of enalapril were prepared from enalapril powder standard dissolved in supporting electrolyte solutions. Supporting electrolyte solutions were also used in the dilution of stock solutions to prepare the working standard solutions.

Preparation of sample solutions

The ANGIOTEC 20 mg[®] tablets were weighed and ground by mortar and pestle, after that certain mass of powder was dissolved in the supporting electrolyte solution. Then, undissolved substance was removed by simple filtration. After that, supporting electrolytes were used for washing

filter paper several times; then, they were used to complete solution volume.

Apparatus

Potentiostat (Metrohm Autolab) PGSTAT 204 is used for voltammetric analysis of enalapril samples. Glassy carbon (GC), Au, and Pt are used as working electrodes, with Ag/AgCl (3 M KCl) as reference electrode and platinum (Pt) sheet as counter electrode.

Statistical methods

$$\text{Limit of detection} = yB + 3sB$$

$$\text{Limit of quantitation} = yB + 10sB$$

Where, yB: Blank signal

sB: Standard deviations of the blank

$$\text{Relative standard deviation (RSD)} = 100 \frac{s}{\bar{x}}$$

Where, S: Standard deviation

\bar{x} : Mean.

RESULTS AND DISCUSSION

Optimization of voltammetric analysis parameters

Parameters affecting voltammetric analysis performance of enalapril were studied. Pt, Au, and GC have been used as working electrodes for enalapril determination, cyclic voltammograms of Figure 2 indicated that enalapril is an electroactive compound, GC working electrode exhibits the best performance compared to other working electrodes, enalapril showed quasi-reversible cycle when GC was used as working electrode and KNO₃ (1 M) supporting electrolyte, broad oxidation peak indicated the presence of more than 1 oxidation step. According to cyclic voltammograms of Figure 2c and d, KNO₃ (1 M) showed higher performance than phosphate buffer as supporting electrolyte.

Optimization of enalapril voltammetric analysis method

According to the optimization of voltammetric analysis parameters, GC is used as working electrode and KNO₃ (1 M) as supporting electrolyte for the optimization of voltammetric analysis method. Enalapril of 0.4–2.0 mg/mL concentrations has been analyzed by CV, SWV, and DPV, Figures 3-5. SWV and DPV voltammograms indicated the presence of two oxidation peaks at 1.4 V and 1.75 V but later are sharper which have been used for the quantitation of enalapril in all applied voltammetric methods. Standard calibration curve

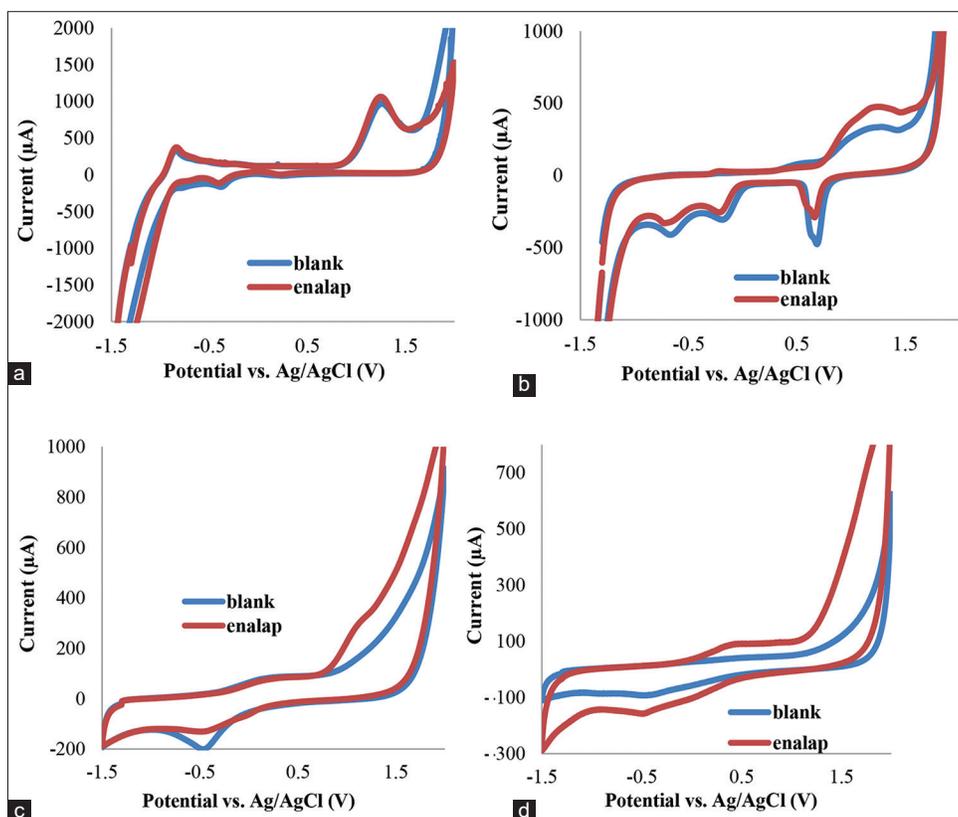


Figure 2: Cyclic voltammograms of enalap 2.0 mg/mL, scan rate 0.1 V/s (a) Pt working electrode with KNO₃ (1 M) supporting electrolyte, (b) Au working electrode with KNO₃ (1 M) supporting electrolyte, (c) GC working electrode with phosphate buffer supporting electrolyte, (d) GC working electrode with KNO₃ (1 M) supporting electrolyte

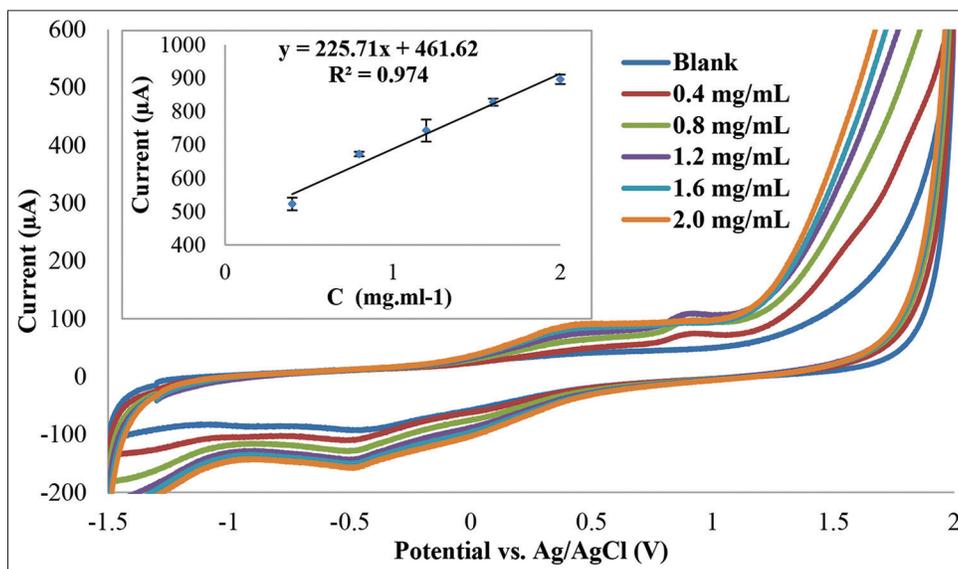


Figure 3: CV study of enalap (0.4–2.0 mg/ml), GC working electrode, KNO₃ (1 M) supporting electrolyte, scan rate 0.1 V/s, each concentration has been done triplicate

of SWV showed the best correlation coefficient R^2 value of 0.9877 compared to CV and DPV, as shown in Table 1, the slope of standard calibration curves of Figures 3-5 reflects the sensitivity of the method, which indicated that CV is the most sensitive compared to other used methods.

Limit of detection (LOD) and Limit of quantification (LOQ)

Table 1 shows LOD and LOQ of enalap determination by CV, SWV, and DPV which have been calculated based on

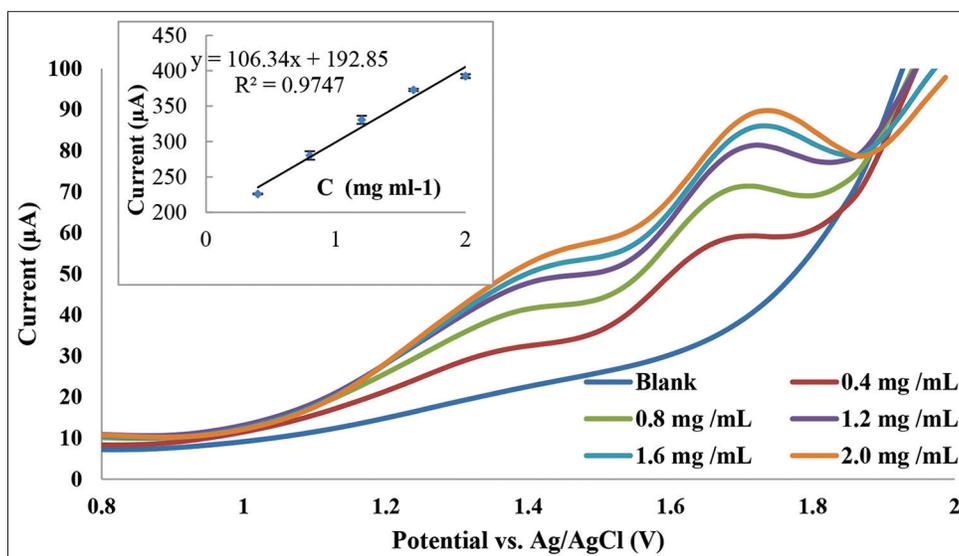


Figure 4: SWV study of enalap (0.4–2.0 mg/ml), GC working electrode, KNO_3 (1 M) supporting electrolyte, each concentration has been done triplicate

Table 1: Linearity of enalap (0.4–2.0 mg/mL, KNO_3 1 M)

Method	LR	R ²	LOD (µg/mL)	LOQ (µg/mL)
CV	$y = 225.71x + 461.62$	0.9740	114	330
SWV	$y = 111.34x + 188.85$	0.9877	90.1	247
DPV	$y = 19.875x + 54.543$	0.9632	140	380

LR: Linear regression, R²: Correlation coefficient, LOD: Limit of detection, LOQ: Limit of quantification

signal-to-noise ratio of 3 and 10, respectively. Supporting electrolyte anodic current peaks were measured for different methods at 1.75 V [Table 1]. The lowest LOD and LOQ were exhibited by SWV with values of 90.1 and 247 µg/mL LOD and LOQ, respectively.

Precision and accuracy

Intraday repeatability and interday reproducibility are used to measure the precision of methods applied in this study. 0.8 mg/mL of pure enalap in 1 M KNO_3 supporting electrolyte was used in precision study. RSD of inter and intraday analysis for enalap of studied methods in Table 2 showed that SWV has the lowest RSD values, which indicated that SWV has the best precision compared to other used methods in this study.

Accuracy of voltammetric methods was determined using 1.0 mg/mL enalap prepared from commercially available enalapril tablets ANGIOTEC 20 mg[®]. The average of three replicates measurements by each used method was recorded. The recovery based on standard calibration curves of each method reflects the accuracy. According to the recoveries exhibited in Table 3, only the recovery of SWV falls within the accepted range.

Table 2: Precision of enalap (0.8 mg/mL, KNO_3 1 M)

Method	Intraday RSD%	Interday RSD%
CV	2.86	4.15
DPV	6.81	8.92
SWV	2.33	3.87

Intraday (repeatability) RSD: Relative standard deviation of triplicate determinations on the same day, interday (reproducibility) RSD: Relative standard deviation of 3 consecutive days. RSD: Relative standard deviation, DPV: Differential pulse voltammetry, CV: Cyclic voltammetry, SWV: Square wave voltammetry

Table 3: Accuracy and precision of commercial preparation of enalap ANGIOTEC 20 mg[®] GC electrode (KNO_3 1M)

Method	Statistical parameters	1.0 mg/mL
CV	Found±SD	1.071±0.038
	Recovery %	107.1
	RSD %	3.55
DPV	Found±SD	0.842±0.066
	Recovery %	84.2
	RSD %	7.89
SWV	Found±SD	0.991±0.036
	Recovery %	99.06
	RSD %	3.65

SD: Standard deviation of triplicate determinations, RSD: Relative standard deviation, Recovery=Found/added*100, DPV: Differential pulse voltammetry, CV: Cyclic voltammetry, SWV: Square wave voltammetry

Table 4 shows statistical comparison in accuracy, precision, and LOD between SWV of the present work and previous studies methods used for enalapril analysis. Comparison exhibited comparable accuracy and precision with previous

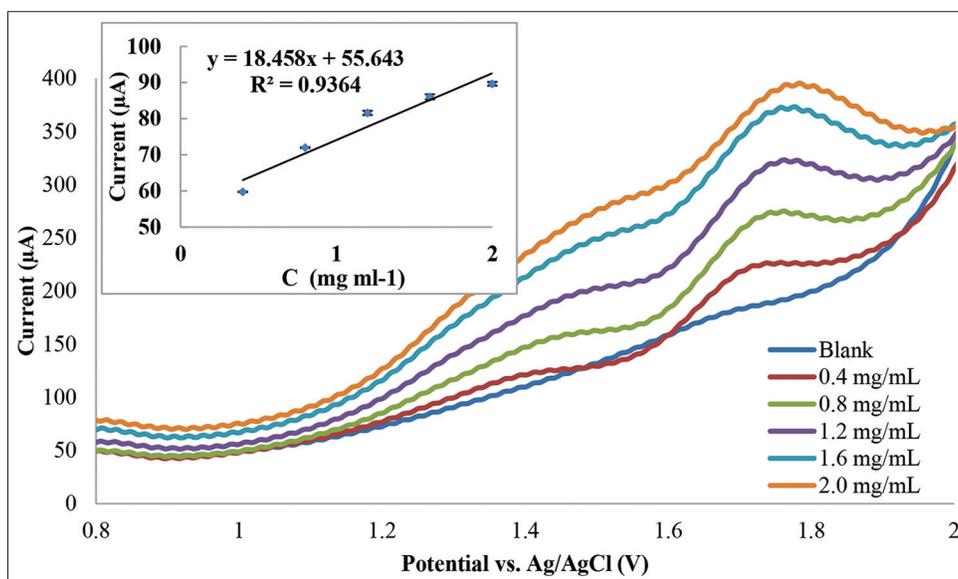


Figure 5: DPV study of enalap (0.4–2.0 mg/ml), GC working electrode, KNO_3 (1 M) supporting electrolyte, each concentration has been done triplicate

Table 4: Comparison in LOD, precession, and accuracy between the present work and other used methods for the determination of enalapril

Method	LOD	Precession (%)	Recovery (%)	References
LCMS	0.1 ng/mL	3.5–3.8	97.2	[6]
Spectrophotometry	1.412 $\mu\text{g/mL}$	1.85–1.98.	98.52	[8]
NMR	-	1.7	98.8	[9]
CV screen printed electrode	0.34 $\mu\text{g/mL}$	1.50	102	[15]
SWV carbon paste electrode	0.31 $\mu\text{g/mL}$	3.4	96	[18]
SWV GC electrode	90.1 $\mu\text{g/mL}$	2.33–3.87	99.06	Present work

LOD: Limit of detection, LCMS: Liquid chromatography–mass spectrometry, CV: Cyclic voltammetry, SWV: Square wave voltammetry, GC: Glassy carbon

electrochemical, chromatographic, and spectroscopic methods.

CONCLUSION

According to statistical results, SWV considered the optimum voltammetric method for enalapril analysis compared to other methods in this study, since it has shown the best performance in accuracy, precession and detection limit. GC working electrode and KNO_3 (1 M) supporting electrolyte showed good performance for enalapril analysis. Advantages of SWV shown in this study make it suitable alternative of chromatographic and spectroscopic method.

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