ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Research Article / JAPR
Ylimza and Akcay, 2020, 4 (4), **139-146**

Journal of Advanced Pharmacy Research

Determination of Ivabradine by Linear Sweep, Square Wave and Differential Pulse Voltammetric Methods Using Platinum Electrode in Pharmaceutical Preparation

Bilal Yilmaz *, Bilge Kagan Akcay

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

***Corresponding author:** Bilal Yilmaz, Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, Turkey. Tel.+90 4422315213 Fax: +904422315201*

Email address: yilmazb@atauni.edu.tr

Submitted on: 14-05-2020; Revised on: 23-06-2020; Accepted on: 24-06-2020

To cite this article: Yilmaz, B.; Akcay, B. K. Determination of Ivabradine by Linear Sweep, Square Wave and Differential Pulse Voltammetric Methods using Platinum Electrode in Pharmaceutical Preparation. *J. Adv. Pharm. Res.* **2020**, *4* (4), 139-146. DOI:10.21608/aprh.2020.30205.1107

ABSTRACT

Objectives: In this study, simple, fast and reliable cyclic voltammetry (CV), linear sweep voltammetry (LSV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) methods were developed and validated for determination of ivabradine in pharmaceutical preparations. **Methods**: The proposed methods were based on electrochemical oxidation of ivabradine at platinum electrode in acetonitrile solution containing 0.1 M $LiClO_4$. The well-defined oxidation peak was observed at 1.34 V. **Results**: The calibration curves were linear for ivabradine at the concentration range of 5-40 μg/mL for LSV, SWV and DPV methods, respectively. Intra- and inter-day precision values for ivabradine were less than 3.12, and accuracy (relative error) was better than 4.78%. The limits of detection values ranged from 1.20 to 1.60 μg/mL and quantification from 3.60 to 4.80 μg/mL. The mean recovery of ivabradine was 99.9 % for pharmaceutical preparation. No interference was found from tablet excipients at the selected assay conditions. **Conclusion**: Developed methods in this study are accurate, precise and can be easily applied to Coralan tablet as pharmaceutical preparation.

Keywords: *Ivabradine; Tablet; Validation; Voltammetry*

INTRODUCTION

Ivabradine hydrochloride is 3-[3-[(7S)-3,4-dimethoxy-7-bicyclo[4.2.0]octa-1,3,5-trienyl] methyl-methylamino]propyl]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-onehydro chloride (**Figure 1**). It is a therapeutic agent used for the symptomatic treatment of chronic stable angina pectoris in patients with normal sinus rhythm who cannot take beta blockers. It is also indicated in combination therapy with beta-blockers in patients inadequately controlled by betablocker alone and whose heart rate exceeds 60 beats per minute[1].

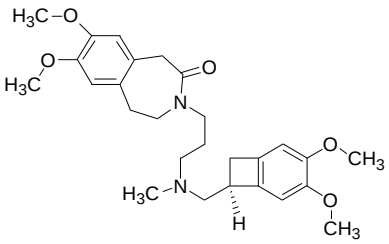

Figure 1. Chemical structure of ivabradine

Several HPLC methods have been reported for the determination of ivabradine in urine, plasma and

formulations with fluorimetric detection[2], mass spectrophotometric detection[3] and UV detection[4-6], respectively. The reported methods were influenced by interference of endogenous substances and potential loss of drugs in the re-extraction procedure and involving lengthy, tedious and time-consuming plasma sample preparation and extraction processes and requiring a sophisticated and expensive instrumentation.

The development of a new method capable of determining drug amount in pharmaceutical dosage forms is important. 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. Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity.

The first paper related to electrochemical investigation of ivabradine has been reported by Attia and et al.[7]. In this paper, the determination of ivabradine based on modification with multiwalled carbon nanotubes using sodium dodecyl sulfate as micellar medium has been studied. The reproducibility of the proposed method was done by two different analysts using the same procedures for analysis of ivabradine (9.9 x 10^{-6} mol/L). The recovery values were 99.65% and 100.48% for the first and the second analyst, respectively. The method is also comprehensive method using expensive chemicals.

Despite the analytical importance of the electrochemical behavior and oxidation mechanism of ivabradine, no report has been published on the voltammetric study of the electrochemical oxidation of ivabradine 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 would be interest to investigate the oxidation process of ivabradine in aprotic media.

Therefore, the goal of this work was the development of new LSV, SWV and DPV methods for the direct determination of ivabradine in pharmaceutical preparations without any time-consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated simple, rapid, selective and sensitive procedures for the determination of ivabradine employing LSV, SWV and DPV methods the platinum disc electrode. Besides, the methods were successfully applied for the quality control of commercial ivabradine tablet form to quantify the drug and to check the formulation content uniformity.

MATERIAL AND METHODS

Chemical and reagents

Ivabradine hydrochloride (99.0% purity) was obtained from Sigma (Germany). Acetonitrile (Fluka for HPLC analysis) was purified by drying with calcium hyride, followed by distillation from phosphorus pentoxide. After purification in order to eliminate its water content as much as possible, it was kept over molecular sieves. ***Lithium perchlorate*** ($LiClO_4$) were purchased from Fluka and used as received without further purification. Coralan tablet (Batch number: 1003, Batch size: 100,000 tablets, Expiry date: April 2015) was purchased from the local pharmacy (Erzurum, Turkey).

Electrochemical instrumentation

Electrochemical experiments were performed on a Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. All measurements were carried out in a single-compartment electrochemical cell with a standard three-electrode arrangement. A platinum disk with an area of 0.72 cm^2 and a platinum wire were used as the working and the counter electrodes, respectively. The working electrode was successively polished with 1.0, 0.3 and 0.05 μm alumina slurries (Buehler) on microcloth pads (Buehler). After each polishing, the electrode was washed with water and sonicated for 10 min in acetonitrile. Then, it was immersed into a hot piranha solution (3:1, H_2SO_4, 30% H_2O_2) for 10 min, and rinsed copiously with water. All potentials were reported versus Ag/AgCl/KCl (3.0 M) reference electrode (BAS Model MF-2078) at room temperature. The electrolyte solutions were degassed with purified nitrogen for 5 min before each experiment and bubbled with nitrogen during the experiment. Operating conditions for SWV were pulse amplitude 25mV, frequency 10 Hz, potential step 4mV; and for DPV were pulse amplitude 50 mV, pulse width 50 ms, scan rate 100 mV/s.

Preparation of the standard and quality control solutions

The stock standard solution of ivabradine was prepared in 0.1 M $LiClO_4$/acetonitrile to a concentration of 100 µg/mL. Working standard solutions were prepared from the stock solution. Standard solutions were prepared as 5-40 μg/mL for LSV, SWV and DPV. The quality control (QC) solutions were prepared by adding aliquots of standard working solution of ivabradine to final concentrations of 7.5, 25 and 37.5 µg/mL for LSV, SWV and DPV.

Procedure for pharmaceutical preparations

Ten 10 tablets of ivabradine (7.5 mg Coralan tablet) were accurately weighed and powdered. An amount of this powder corresponding to one tablet

ivabradine 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 methods. The drug content of ivabradine tablet was calculated from the current potential curves.

Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 15.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

Voltammetric behavior of ivabradine

The electrochemical behavior of ivabradine was investigated at the Pt disc electrode in acetonitrile solution containing 0.1 M $LiClO_4$ as the supporting electrolyte by using cyclic voltammetry (CV). **Figure 2** shows a typical cyclic voltammogram of 10 µg/mL ivabradine recorded under these conditions for the scan rate of 0.1 V/s. In the anodic sweep, an oxidation peak is seen at about potential of 1.34 V. Upon reversing the potential scan, no reduction peak corresponding to this oxidation wave is observed, indicating the irreversible nature of the electrode reactions.

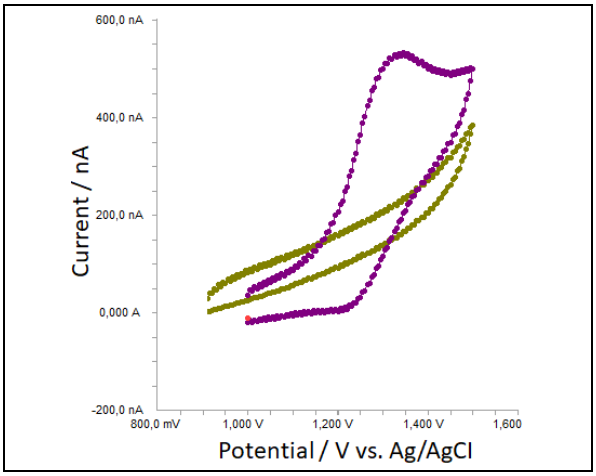

Figure 2. Cyclic voltammogram for the oxidation of 10 µg/mL ivabradine in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s.

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 0.005-1.00 V/s of the potential scan rates in acetonitrile solution containing 10 µg/mL concentration of ivabradine **(Figure 3).**

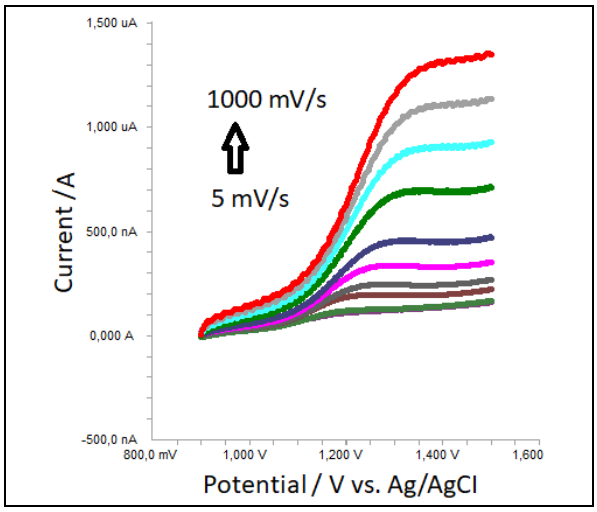

Figure 3. Linear sweep voltammograms for the oxidation of 10 µg/mL ivabradine in acetonitrile containing 0.1 M $LiClO_4$ as a function of scan rate (5, 10, 25, 50, 100, 200, 400, 600, 800 and 1000 mV/s).

The representative linear sweep voltammograms obtained at Pt electrode for 10 µg/mL ivabradine as a function of the scan rate are presented in **Figure 4.** Scan rate dependency experiments show that the peak currents for peak vary linearly with the scan rate (ν) **(Figure 4a,b),** which points out the adsorption-controlled process. However, the plots of logarithm of peak currents versus logarithm of scan rates for 10 µg/mL concentration of ivabradine display straight lines with 0.4229 slope **(Figure 4c),** which are close to theoretical value of 0.5 expected for an ideal diffusion-controlled electrode process[8].

log I_m-*log* v curve is more eligible for this aim, therefore, a diffusional process for peak should be considered. These results suggest 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. As shown in **Figure 3,** the oxidation peak potential (E_{pa}) for peaks shift toward more positive values with increasing scan rate. The relationship between the peak potential and scan rate is described by the following equation[9], and from the variation of peak potential with scan rate αn_a can be determined, where α is the transfer coefficient and n_a is the number of electrons transferred in the rate determining step.

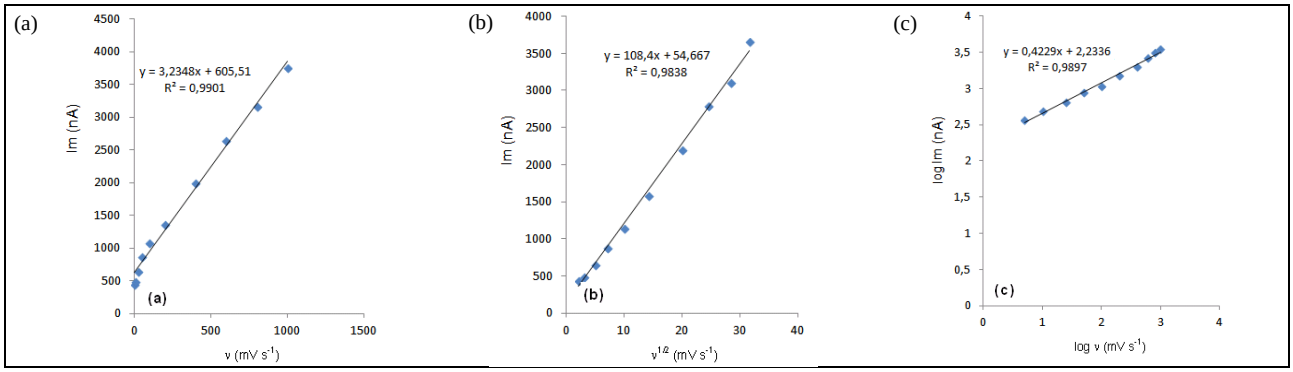

Figure 4(a-c). Dependence of peak current on the scan rate (10 µg/mL).

$$E_{pa} = E^{0\prime} + RT/[(1-\alpha)n_aF]\left[0.78 + ln\left(D^{1/2}k_s^{-1}\right) - 0.5\,ln\,R\,T/[(1-\alpha)n_aF]\right] + RT/[(1-\alpha)n_aF]/2\,ln\,\nu$$

According to this equation, the plots of the peak potentials versus ln ν for oxidation peak show linear relationship **(Figure 5).** The slope indicate the value of αn_a is 0.75 for peak. Also, this value obtained indicate the total irreversibility of the electron transfer processes. This result show that the chemical step is a fast following reaction coupled to a charge transfer.

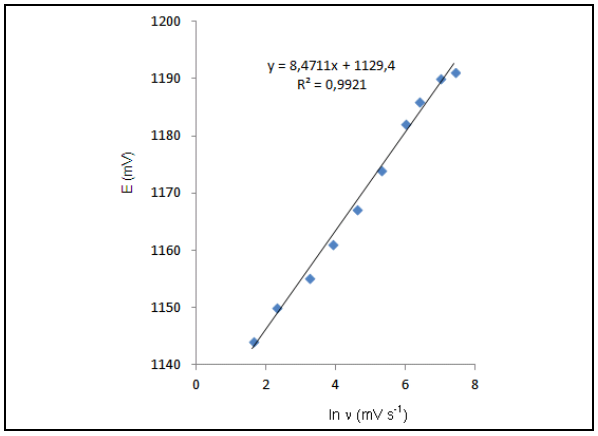

Figure 5. Dependence of anodic peak potentials of voltammetric peak for the oxidation of 10 µg/mL ivabradine on the scan rate.

Validation of the method

The validation was carried out by establishing specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), recovery, ruggedness and stability according to ICH Q2B recommendations[10].

Specificity

Excipients (magnesium carbonate, mannitol, crospovidone, calcium stearate, methylcellulose, polyvidone, titanium dioxide, ferric oxide, propylene glycol, eudragit, sodium dodecyl sulfate, polysorbate 80 and triethyl citrate) were added to the drug for recovery studies, according to the manufacturer's batch formulas for 7.5 mg ivabradine per tablet. The mean percentage recovery of 15 and 30 µg/mL ivabradine showed no significant excipient interference; thus the procedures were able to assay ivabradine in the presence of excipients, and hence it can be considered specific.

Linearity

Standard solutions were prepared as 5-40 µg/mL (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL) for LSV, SWV and DPV **(Figures 6-8).**

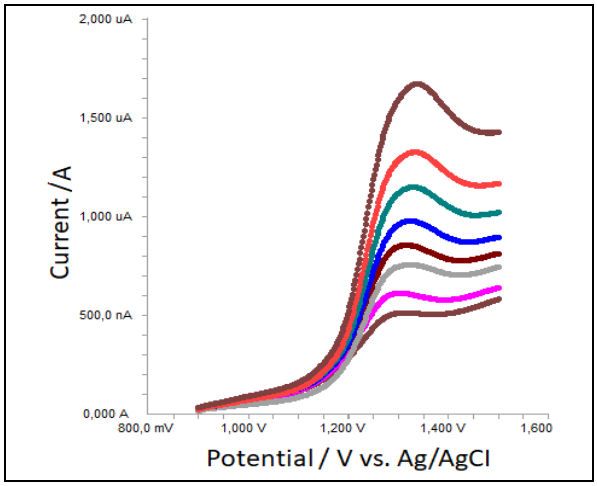

Figure 6. Linear sweep voltammograms for different concentrations of ivabradine in acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL).

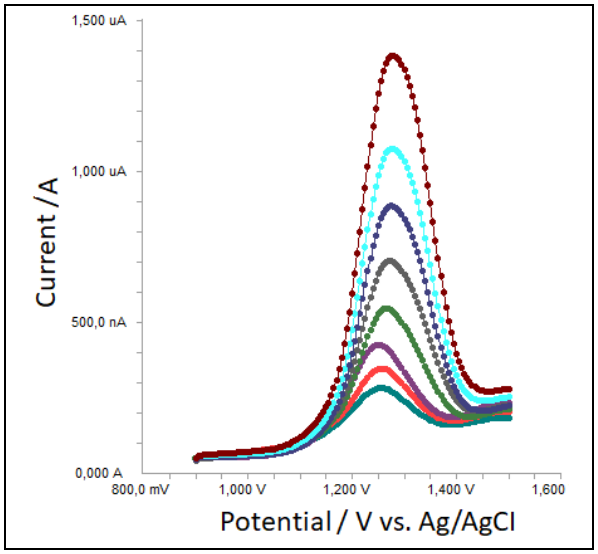

Figure 7. Square wave voltammograms for different concentrations of ivabradine in acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL).

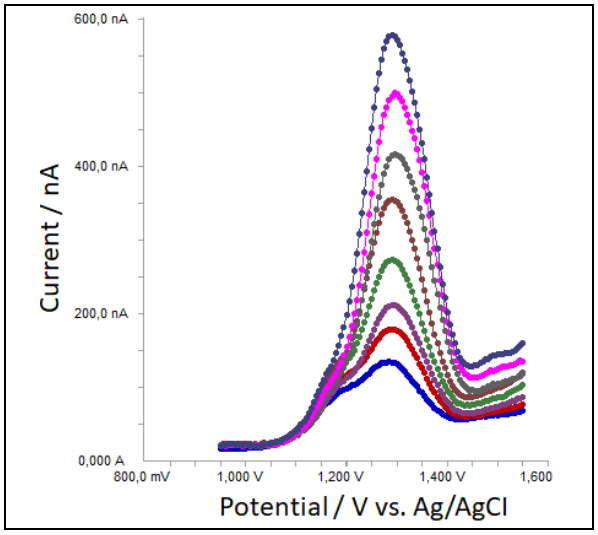

Figure 8. Differential pulse voltammograms for different concentrations of ivabradine in acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25, 30 and 40 µg/mL).

Calibration curves were constructed for ivabradine standard by plotting the concentration of compound versus peak current responses. The calibration curves were evaluated by its correlation coefficients. The correlation coefficients *(r)* of all the calibration curves were consistently greater than 0.99. The linear regression equations were calculated by the least squares method using Microsoft Excel® program and summarized in **Table 1.**

Accuracy and precision

Accuracy of the assay methods were determined for both intra-day and inter-day variations using the six times analysis of the quality control (QC) samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday). Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. The intra-day accuracy ranged from 0.44% to 3.12% and precision from 1.38% to 4.78% **(Table 2).**

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ of ivabradine by the proposed methods were determined using calibration standards. 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)[10]. The LOD and LOQ values of the methods were summarized in **Table 1.**

Recovery

To determine the accuracy of the LSV, SWV and DPV 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 known amount of pure drugs to pre-analyzed samples of commercial tablet. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in **Table 3**.

Ruggedness

In this study, the LSV, SWV and DPV determination of ivabradine were carried out by a different analyst in same instrument with the same standard **(Table 4).** The results showed no statistical differences between different operators suggesting that the developed method was rugged.

Stability

To evaluate the stability of ivabradine, 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, refrigeratory (4 °C) and frozen (-20 °C) temperature for 24h and 72h. Stability measurements were carried out with LSV, SWV and DPV method. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and ivabradine was found as stable at room temperature, 4 and -20 °C for at least 72h.

Table 1. Linearity of ivabradine

Method	Range (µg/mL)	LR[a]	R	LOD	LOQ
LSV	5-40	y=31.836x+375.75	0.9942	1.6	4.8
SWV	5-40	y=31.845x+99.455	0.9964	1.3	3.9
DPV	5-40	y=13.096x+80.477	0.9903	1.2	3.6

[a] Based on three calibration curves, LR: Linear regression, R: Coefficient of correlation, y: Peak current, x: Ivabradine concentration (µg/mL), LOD: Limit of detection, LOQ: Limit of quantification

Table 2. Precision and accuracy of ivabradine

Method	Added (µg/mL)	Intra-day Found±SD[a] (µg/mL)	Intra-day Accuracy	Intra-day Precision RSD%[b]	Inter-day Found±SD (µg/mL)	Inter-day Accuracy[c]	Inter-day Precision RSD%[b]
	7.5	7.47±0.10	-0.44	1.38	7.45±0.10	-0.67	1.41
LSV	25	24.83±0.98	-0.67	3.96	24.67±0.82	-1.33	3.31
	37.5	36.67±1.03	-2.21	2.81	36.17±0.98	3.54	2.71
	7.5	7.38±0.12	-1.55	1.58	7.38±0.12	-1.55	1.58
SWV	25	25.00±0.89	0.04	3.58	24.50±0.55	-2.00	2.23
	37.5	36.33±1.03	-3.12	2.84	36.67±1.21	-2.21	3.29
	7.5	7.47± 0.14	-0.44	1.83	7.37±0.08	-1.78	1.11
DPV	25	25.33±1.21	1.33	4.78	24.67±0.82	-1.33	3.31
	37.5	36.83±1.17	-1.79	3.18	36.17±0.98	-3.68	2.71

[a] SD: Standard deviation of six replicate determinations, [b] RSD: Relative standard deviation, [c] Accuracy: (%relative error) (found-added)/addedx100

Comparison of methods

Successive cyclic voltammogram of ivabradine obtained in acetonitrile solution containing 0.1 M $LiClO_4$ at a scan rate of 100 mV/s are shown in Figure 2. The cyclic voltammogram of 10 µg/mL ivabradine exhibits a single anodic peak. The study of the influence of scan rate shows that the peak current changes linearly with scan rate. The role of adsorption is further supported by the sharp form of the main anodic peak and by the dependence of the peak current on scan rate (v). For diffusion current the plot of $log\ i_p$ as a function of log v should have a slope of 0.5 and for a purely adsorption current a slope of 1.0[11]. The regression of log i_p vs log v gave a slope value of 0.4229, indicating that the oxidation current is of diffusional nature. On the other hand, as scan rate was increased from 10 to 1000 mV/s, the peak potential shifted toward more positive potential as expected for an irreversible oxidation process[12]. The value of αn, product of transfer coefficient and number of electrons transferred in the rate-determining step, was determined from treatment ($log\ i\ vs\ E$) of the voltammetric curves. The value obtained (0.42) shows the total irreversibility of the electron transfer process. It was also demonstrated by the linear relationship obtained between the peak potential (E_p) and the logarithm of scan rate in the range 10-1000 mV/s. Based on the voltammetric behavior of ivabradine, a quantitative method was developed. To select the best electrochemical method, the anodic peak obtained by LSV, SWV and DPV were compared with each other. In order to develop a voltammetric method for determination of the ivabradine, we selected the LSV, SWV and DPV techniques, since the peaks were sharper and better defined at lower concentration of ivabradine than those obtained by linear sweep voltammetry with a lower background current, resulting in improved resolution. SWV and DPV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection and determination limits[13-15].

Voltammetry has been recently proposed as a promising new analytical method for electrochemical detection of drugs. Owing to the high sensitivity, low cost, simplicity of instrumentation and short analysis time voltammetric techniques are important methods for pharmaceutical analysis[16-18].

LSV, SWV and DPV methods were applied for the determination of the commercial tablet **(Table 3).** The results show that high reliability and reproducibility of two methods. The best results were statistically compared using the t-test. At 95% confidence level, the calculated t-values do not exceed the theoretical values **(Table 5).**

Table 3. Recovery values of ivabradine in pharmaceutical preparation

Pharmaceutical preparation		LSV			SWV			DPV		
	Added (μg/mL)	Found ± SD (Mean ±SD)	Recovery (%)	RSD[a] (%)	Found ± SD (Mean ±SD)	Recovery (%)	RSD[a] (%)	Found ± SD (Mean ± SD)	Recovery (%)	RSD[a] (%)
Coralan (5 μg/mL)	5	5.2 ± 0.21	104.0	4.04	5.1 ± 0.18	102.0	3.53	5.1 ±0.22	102.0	4.31
	15	14.6 ± 0.28	97.3	1.92	14.8 ± 0.25	98.7	1.69	14.7 ± 0.34	98.0	2.31
	35	35.4 ± 0.73	101.1	2.06	35.2 ± 1.67	100.6	4.74	35.9 ± 1.24	102.6	3.45

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]Average of six replicate determinations

Table 4. The results of analyses of ivabradine by a different analyst[a]

Method	Added (μg/mL)	Found (μg/mL) (Mean ± SD)	% Recovery	% RSD[a]
LSV	5	4.9 ± 0.13	98.0	2.65
	15	14.8 ± 0.27	98.7	1.82
	35	35.4 ± 0.73	101.1	2.06
SWV	5	5.1 ± 0.18	102.0	3.53
	15	14.8 ± 0.25	98.7	1.69
	35	35.2 ± 1.67	100.6	4.74
DPV	5	5.2 ± 0.21	104.0	4.04
	15	14.6 ± 0.28	97.3	1.92
	35	35.6 ± 1.02	101.7	2.87

[a]*Mean measurements of six replicate determinations*

Therefore, there is no significant difference between LSV, SWV and DPV voltammetry methods. At the same time, the results of the proposed LSV, SWV and DPV methods were evaluated statistically as compared with a spectrophotometric method (Table 5)[19]. According to the results of t - and F-tests, the variances between the methods were found to be insignificant at 95% probability level, indicating that no significant differences exist between the performances of the methods regarding their accuracy and precision.

CONCLUSION

In conclusion, in this study, the electrochemical behavior of ivabradine has been studied in nonaqueous media by CV, LSV, SWV and DPV methods. It has concluded that there is a completely diffusion-controlled current process which isn't affected by adsorption phenomenon. Besides, in the present report, simple, rapid, sensitive, reliable, specific, accurate and precise LSV, SWV and DPV methods for the determination of ivabradine in pharmaceutical preparation was developed and validated. The method described has been effectively and efficiently used to analyze ivabradine pharmaceutical tablets without any interference from the pharmaceutical excipients. The voltammetric run time of 1 min allows the analysis of a large number of samples in a short period of time. Therefore, the proposed methods could possibly be applied for the determination of ivabradine in pharmaceutical samples as well as for quality control laboratories.

Acknowledgement

This study was supported by a Grant from Ataturk University Research Foundation (Project Number:2014/35).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Parfitt, K. Martindale. The complete drug reference. London: Pharmaceutical Press, **2007,** p.1185.
2. Klippert, P.; Jeanniot, J. P.; Polve, S.; Lefevre, C.; Merjan, H. Determination of ivabradine and its N-demethylated metabolite in human plasma and urine, and in rat and dog plasma by a validated high-performance liquid chromatography method with fluorescence detection. *J. Chromatogr. B* **1996**, *719*, 125-133.
3. Bouchard, F. M.; Simonin, G.; Bossant, J.M.; Neyret, B.C. Simultaneous determination of ivabradine and its metabolites in human plasma by liquid chromatography-tandem mass spectroscopy. *J. Chromatogr. B,* **2000**, *745*, 261-269.
4. Shweta, M.; Amit, P. K.; Anurekha, J. Quantitative determination and validation of ivabradine HCL by stability indicating RP-HPLC method and spectrophotometric method in solid dosage form. *Eurasian J. Anal. Chem.* **2010**, *5* (1), 53-62.
5. Snitha, S.; Srinivasan, B.P. Development and Validation of RP-HPLC method for the estimation of ıvabradine hydrochloride in tablets. *Indian J. Pharm. Sci*. **2010**, *72* (5), 667-471.
6. Selva, K. P.; Pandiyan, K.; Rajagopal, K. Development and validation of rapid RP-HPLC method for dissolution release of ivabradine hydrochloride in solid oral dosage form. *World J. Pharm. Pharmaceut. Sci.* **2014**, *3* (7), 1877-1888.
7. Attia A. K.; Abo-Talib N.F.; Tammam M. H. Voltammetric determination of ivabradine hydrochloride using multiwalled carbon nanotubes modified electrode in presence of sodium dodecyl sulfate. *Adv. Pharm. Bull.* **2017,** *7* (1), 151-157.
8. Laviron, E.; Roullier, L.; Degrand, C. A multilayer model for the study of space distributed redox modified electrodes: Part II. Theory and application of linear potential sweep voltammetry for a simple reaction. *J. Electroanal. Chem.* **1980**, *112*, 11-23.
9. Yilmaz, B.; Ekinci, D. Voltammetric behavior of carvedilol in non-aqueous media and its analytical determination in pharmaceutical preparations. *Rev. Anal. Chem.* **2011,** *30*, 187-193.
10. The European Agency for the Evaluation of Medicinal Products. ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, **1996**.
11. Gosser, D.K. Cyclic Voltammetry, VCH, New York, **1994.**
12. Bard, A. J.; Faulkner, L. R. Electrochemical Methods: Fundamentals and Applications, second ed., Wiley, New York, **2001.**
13. Wang, J. (Ed.) Analytical Electrochemistry, 2nd ed., Wiley/VCH Publishers, New York, **2000.**
14. Kissenger, P.T.; Heineman, W.R. (Eds.) Laboratory Techniques in Electroanalytical Chemistry, second ed., Marcel Dekker, New York, **1996.**
15. Wang, J. (Ed.) Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine, VCH Publishers, New York, **1996.**
16. El-Hefnawey, G.B.; El-Hallag, I. S.; Ghoneim, E. M.; Ghoneim, M. M. Voltammetric behavior and quantification of the sedative-hypnotic drug chlordiazepoxide in bulk form, pharmaceutical formulation and human serum at a mercury electrode. *J. Pharm. Biomed. Anal.* **2004**, *34*, 75-86.
17. Corti, P.; Corbini, G.; Gratteri, P.; Furlanetto, S.; Pinzauti, S. Determination of some quinolones in tablets, human plasma and urine by differential pulse polarography. *Int. J. Pharm.* **1994**, *111*, 83-87.
18. Smirnova, L. A.; Tolkachev, B.E.; Ryabukha, A.F. Suchkov, E. A.; Kuznetsov, K. A.; Magnitskaya, O. V.; Petrov, V. I. Quantitative determination of ivabradine and its N-demethylated metabolite in volunteer blood and urine. *Pharm. Chem. J.* **2019,** *51*, 275-278
19. Mitesh, H. M.; Kalpana, G. P.; Purvi, A. S. Validated stability-indicating high performance thin layer chromatographic method for determination of ivabradine hydrochloride in bulk and marketed formulation: An application to kinetic study. *Bulletin Fac. Pharm. Cairo Uni.* **2013**, *51*, 233-241.