



Determination of clopidogrel using square wave voltammetry at a gold electrode

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Abstract: The determination of clopidogrel, an antiplatelet agent, was performed at a gold electrode in pH 3.7 acetate buffer using cyclic voltammetry (CV) and square wave voltammetry (SWV). Each voltammogram was characterized by the well defined peak at approximately 1.0 V. The current of anodic stripping peak exhibited a linear dependence on the clopidogrel concentration in the range from 317.89 to 935.16 $\mu\text{g cm}^{-3}$. The obtained linearity was applied to determine clopidogrel in the tablet form of the pharmaceutical preparation (Plavix[®]). The results were compared to the UV spectrophotometric and HPLC methods.

Keywords: cyclic voltammetry; acetate buffer; tablet; UV, HPLC.

INTRODUCTION

Clopidogrel (CLP), methyl (+)-(*S*)- α -(*o*-chlorophenyl)-6,7-dihydrothieno-[3,2-*c*]pyridine-5(4*H*)-acetate (Fig. 1), is an antiplatelet agent widely used in the prevention of ischemic stroke, myocardial infarction and stroke.^{1,2}

It inhibits platelet aggregation by selectively preventing the binding of adenosine diphosphate (ADP) to its platelet receptor.³ This drug reduces thrombotic events in a wide range of patients (*e.g.*, recent myocardial infarction, established peripheral arterial disease and acute coronary syndrome). Clopidogrel is an inactive prodrug, and a biotransformation by the liver is necessary to induce expression of its anti-aggregating activity.⁴ It is rapidly absorbed and undergoes extensive metabolism after oral administration and its plasma concentration goes down considerably rapidly.⁵

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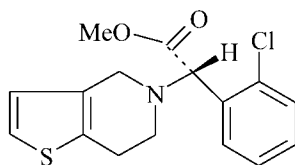


Fig. 1. Structure of clopidogrel.

Analytical methods for the determination of clopidogrel include mostly chromatographic and spectrophotometric techniques: gas chromatography–mass spectrometry (GC–MS),⁶ liquid chromatography–mass spectrometry (LC–MS),^{7–10} high-performance liquid chromatography (HPLC),^{11–13} liquid chromatography,¹⁴ LC–MS/MS,¹⁵ LC–ESI-MS/MS,¹⁶ thin-layer chromatography (TLC),¹⁷ high-performance thin-layer chromatography (HPTLC),¹⁸ UV spectrophotometry.^{19–24} Furthermore, the determination of clopidogrel and its impurities by capillary electrophoresis was reported.²⁵

Electrochemical determination of clopidogrel in standard form and pharmaceutical preparations using the differential pulse voltammetry (DPV) method with a glassy carbon working electrode was recently published.²⁶ Hitherto, a detailed voltammetric determination and electrochemical behaviour of clopidogrel at a gold electrode have not been published.

The aim of this study was to investigate the electrochemical behaviour of clopidogrel using cyclic voltammetry and to present its quantitative determination at a gold electrode in pH 3.7 acetate buffer using square wave voltammetry. Clopidogrel was studied as the pure standard or in the pharmaceutical preparation (Plavix[®]). The square wave voltammetry (SWV) method was applied to determine the amount of clopidogrel in the tablet form of a pharmaceutical preparation (Plavix[®]) and the results were compared with the UV spectrophotometric and HPLC methods.

EXPERIMENTAL

Materials

Clopidogrel bisulphate (CLB) standard was kindly provided by Hemofarm AD, Vršac, Serbia. Plavix[®] tablets containing a 75 mg dose of clopidogrel were obtained from local pharmacies. HPLC grade acetonitrile, methanol, ammonium acetate, sodium bicarbonate, sulphuric acid, sucrose, dichloromethane, sodium dihydrogenphosphate (NaH₂PO₄·H₂O), H₃PO₄ and NaHCO₃ (*p.a.* purity), were obtained from Merck. Diethylamine, analytical grade, and ammonium hydroxide 25 % were obtained from J. T. Baker. All solutions were prepared with water obtained from a Millipore system (18 MΩ cm). Acetate buffer was prepared according to the USP pharmacopoeial procedure.

Cyclic voltammetry

The three electrode electrochemical cell used for the cyclic voltammetry measurements was described in detail previously.^{27,28} The cyclic voltammetry measurements were performed using an Autolab potentiostat/galvanostat Pgstat128N (Metrohm Autolab B.V., The Netherlands).

Polycrystalline gold served as the working electrode, a gold wire was used as the counter electrode and a saturated calomel electrode as the reference electrode. Polycrystalline gold (Pine rotating disc, used as the stationary electrode, surface area 0.500 cm^2) was polished with diamond paste, cleaned with a mixture of water ($18 \text{ M}\Omega \text{ cm}$) and sulphuric acid and further cleaned with deionised water ($18 \text{ M}\Omega \text{ cm}$) in an ultrasonic bath. All the potentials are given vs. SCE. Prior to the addition of the investigated substance, the electrolyte was deoxygenated by purging with nitrogen. All the experiments were performed at room temperature. The voltammetric examinations of all the substances used were performed in acetate buffer solution. The potential was cycled between 0 and 1.5 V at a sweep rate of 50 mV s^{-1} .

Square wave voltammetry

Square wave voltammetry (SWV) measurements were also performed using the Autolab potentiostat/galvanostat. The operating parameters were: step size 2 mV, pulse size 20 mV, frequency 8 Hz and scan rate 15 mV s^{-1} .

Solution preparation for voltammetry

Working standard solutions were prepared daily by dissolving appropriate amounts of CLB in 10 cm^3 of methanol and diluting to 100 cm^3 with acetate buffer (pH 3.7).

Preparation of the standard solutions for the analysis of clopidogrel bisulphate as a content of a solid dosage form

Twenty Plavix[®] tablets were weighed and then the average mass per tablet was determined. The tablets were grounded to a fine powder in a mortar. The required amount of the crushed tablets powder was dissolved in 50 cm^3 of methanol. After sonication for 10 min, the mixture was filtered into a 100 cm^3 volumetric flask. The residue was washed three times with acetate buffer (pH 3.7) and the volume was completed to the mark with the same buffer and stored in dark bottle at $4 \text{ }^\circ\text{C}$. The obtained concentrations were checked by HPLC.

UV spectrophotometric instrument

The spectrophotometric analysis were performed on an Agilent 8453 UV–Vis spectrophotometer with a photodiode array (PDA) detector for measurement of the complete ultraviolet to visible light spectrum. The UV–Vis spectra were recorded in a quartz cuvette of 1 cm path length. The spectrophotometer was connected to a computer with Agilent ChemStation software that was used for all the spectrophotometric measurements and data processing. The samples were tested using a UV–Vis analytical procedure analogous to the procedure for content uniformity determination given in the US pharmacopeia monograph for clopidogrel tablets (USP 36 NF 31).²⁹

Preparation of standard stock solution for spectrophotometric analysis

About 100 mg of clopidogrel bisulphate standard was accurately weighed, transferred to a 50 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol.

Linearity for clopidogrel bisulphate for spectrophotometric analysis

Appropriate aliquots of standard stock solutions were taken in different volumetric flasks and diluted to obtain final concentrations of 0.04, 0.06, 0.08, 0.1, 0.2, 0.4 and 1.0 mg cm^{-3} of clopidogrel bisulphate in Britton Robinson buffer, pH 3.7. The UV spectra of the solutions were recorded and the determination of clopidogrel was realized employing the absorption at 270 nm, the wavelength of maximum absorption.

High performance liquid chromatography (HPLC) – instrumentation and chromatographic conditions

An Agilent HPLC system equipped with 1100 series high pressure binary gradient pump along with pulse damper, a photodiode array detector with an auto liquid sampler handling system was used for the analysis of the samples. The samples were tested using an HPLC analytical procedure analogous to the analytical procedure given in a US pharmacopeia monograph for the assay of clopidogrel tablets and determination of related substances (USP 36 NF 31).²⁹ The HPLC system was equipped with a 4.6 mm × 15 cm analytical column Ultron ES-OVM immobilized with ovomucoid as chiral stationary phase of 5 µm particle size (USP packing designation L57). The column eluent was monitored at a detection wavelength 220 nm. The mobile phase consisted of filtered and degassed mixture of phosphate buffer and HPLC grade acetonitrile in 75:25 ratio. Phosphate buffer was prepared by dissolving 1.36 g of monobasic potassium phosphate in 1000 cm³ of purified water. The chromatography was performed at room temperature at a flow rate of 1.0 cm³ min⁻¹. Data was recorded using OpenLab CDS, Ezchrom edition, software.

Preparation of standard stock solution for HPLC analysis

About 100 mg of clopidogrel bisulphate standard was weighed accurately and transferred to a 100 cm³ volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol.

HPLC linearity for clopidogrel bisulphate

Appropriate aliquots of standard stock solutions were taken in different volumetric flasks and diluted to obtain final concentrations of 0.04, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg cm⁻³ of clopidogrel bisulphate in Britton Robinson buffer, pH 3.7. Equal volumes of 10 µl of the solutions were injected and chromatograms were recorded.

HPLC analysis of clopidogrel bisulphate as a content of a solid dosage form

Twenty tablets of Plavix were weighed and thoroughly powdered. The average of one tablet was weighed, transferred into a 100 cm³ volumetric flask, 50 cm³ of methanol was added and sonicated for 5 min and stirred for 30 min. The solution was diluted with methanol to volume, and mixed. 5 cm³ of this solution was transferred to a flask and diluted with Britton–Robinson buffer, pH 3.7, to 50 cm³. A portion of this solution was passed through a 0.45-µm pore size PTFE membrane filter and discatograms were recorded. The pharmaceutical formulation was analyzed by performing six independent determinations.

RESULTS AND DISCUSSION

Electrochemical determination of clopidogrel in standard form and in pharmaceutical preparations by differential pulse voltammetry on a glassy carbon electrode was recently successfully performed²⁶ and pH 3.7 acetate buffer in mixture with methanol was found to be the optimal electrolyte. The intention was to study the feasibility of the electrochemical determination of clopidogrel as a pure standard and as the content of commercial tablets Plavix[®] by square wave voltammetry on a gold electrode only in pH 3.7 acetate buffer as electrolyte.

The electrochemical behaviour of the pure standard of clopidogrel on gold electrode was first studied by cyclic voltammetry in pH 3.7 acetate buffer. As is presented in Fig. 2, clopidogrel exhibited an apparent oxidative ability in this

electrolyte on a gold electrode. The cyclic voltammogram shows the beginning of anodic activity of clopidogrel in double layer region from 0.2 to 0.6 V. An apparent anodic current maximum appears at 1.0 V before oxide formation on the gold electrode and second one at 1.17 V in the area of oxide formation. The rapid increase of anodic current on gold oxide at 1.30 V in the presence of clopidogrel was observed as well as its shift by 0.1 V to a more negative potential, compared to a clean gold electrode (dashed line in Fig. 2).

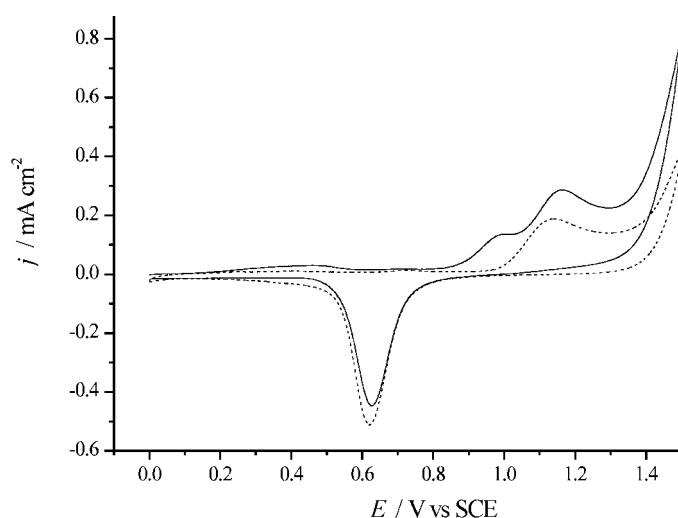


Fig. 2. Cyclic voltammogram of an Au electrode in pH 3.7 acetate buffer, dashed line, and after the addition of clopidogrel (concentration $32.0 \mu\text{g cm}^{-3}$), full line; sweep rate: 50 mV s^{-1} . Only the first sweep is presented.

This voltammetric behaviour indicated the possible successful quantitative determination of clopidogrel using square wave voltammetry.

The application of square wave voltammetry for the quantitative determination of clopidogrel on the gold electrode is presented in Fig. 3. The SW voltammograms for different concentrations of clopidogrel were recorded in pH 3.7 acetate buffer in the potential range from 0 to 1.5 V at a scan rate of 15 mV s^{-1} . Before each scan, the compound was accumulated at the electrode surface at 0 V for 180 s. Under these conditions, the oxidation of clopidogrel proceeded before oxide formation on Au and reached maximum currents at the onset potential of Au oxide formation. Each voltammogram was characterized by a well defined peak at approximately 1.0 V. The currents of anodic stripping peak exhibited a linear dependence on the clopidogrel concentration, as shown in the inset of Fig. 3.

As already mentioned, the successful electrochemical determination of clopidogrel in standard form and pharmaceutical preparations described in literature²⁶ was performed by differential pulse voltammetry on a glassy carbon electrode in

a mixture of pH 3.7 acetate buffer and methanol. In the present study, square wave anodic stripping voltammetry was used to determine clopidogrel at a gold electrode in a similar mixture of acetate buffer (pH 3.7) with methanol in a 50:50 (*V/V*) ratio. However, the results obtained showed inhibition of the gold electrode surface by methanol, resulting in more than two-times smaller anodic currents of clopidogrel oxidation, as presented in Fig. 4. This indicates that further analysis of clopidogrel in Plavix[®] should be continued in acetate buffer (pH 3.7) alone.

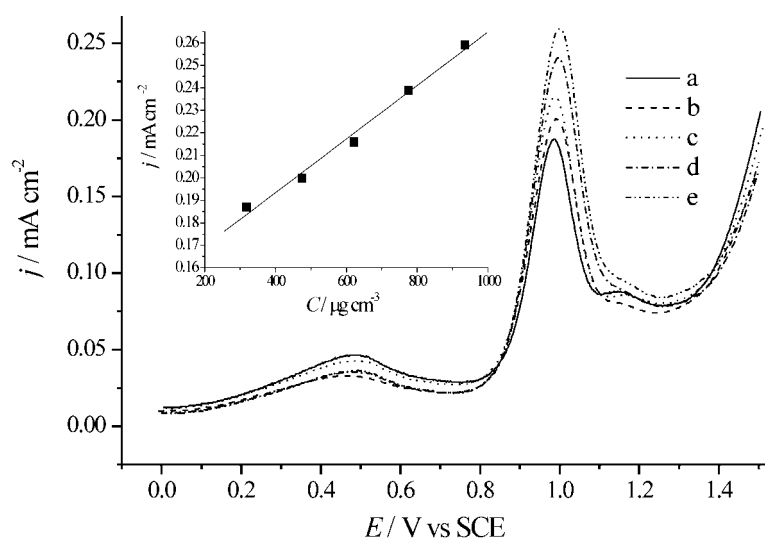


Fig. 3. Square wave anodic stripping voltammograms of clopidogrel at a gold electrode in acetate buffer (pH 3.7). Clopidogrel concentration: a) 317.89, b) 474.49, c) 622.03, d) 775.63 and e) 935.16 $\mu\text{g cm}^{-3}$. Accumulation time: 180 s at $E = 0.0$ V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1} . Inset: The linear dependency of the anodic peak current vs. concentration of clopidogrel.

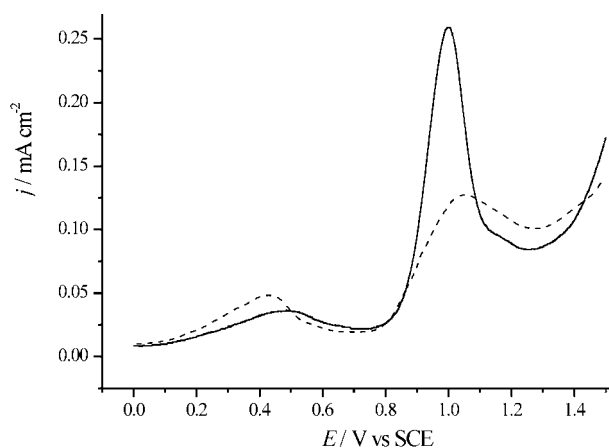


Fig. 4. Square wave anodic stripping voltammograms of 935.16 $\mu\text{g cm}^{-3}$ clopidogrel at a gold electrode in acetate buffer, pH 3.7 (full line), and in acetate buffer (pH 3.7)/methanol, 1:1, *V/V* (dashed line), solutions. Accumulation time: 180 s at $E = 0.0$ V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1} .

The voltammetric behaviour of clopidogrel as the content of commercial tablets Plavix[®] was also in a first stage investigated by cyclic voltammetry. Its voltammetric behaviour even in the presence of the excipients present in Plavix[®] showed the same anodic activity as the standard substance, presented in Fig. 2, with a negligible reduction in the values of the current maximums.

Similarly as for clopidogrel, the oxidation of Plavix[®] at a gold electrode using square wave voltammetry proceeded at potentials before the formation of oxide on the Au. The square wave voltammogram was characterized by a well defined peak at approximately 1.0 V.

The obtained linear relationship between current and concentration of clopidogrel in acetate buffer (pH 3.7) by SW voltammetry within the concentration range 0.317–0.935 mg cm⁻³ (Table I) was used for the determination of clopidogrel in tablet form (Plavix[®]) and the results were compared with those obtained by the UV and HPLC methods. The validation of the SW procedure was carried out by evaluation of the limit of detection (*LOD*), limit of quantification (*LOQ*) and recovery. The *LOD* and *LOQ* were calculated from the calibration curves as kSD/b where $k = 3$ for *LOD* and 10 for *LOQ*, *SD* is the standard deviation of the intercept and b is the slope of the calibration curve.^{30,31} The *LOD* and *LOQ* values for the SW voltammetry method were 117.50 and 391.66 µg cm⁻³, respectively.

TABLE I. Determination of CLP by SW voltammetry (SWV), UV spectrophotometry and HPLC

| Parameter | SWV | UV | HPLC |
|----------------------------------|----------------------------------|-------------|-------------|
| Range / mg cm ⁻³ | 0.317–0.935 | 0.030–0.747 | 0.032–0.962 |
| | Regression equation ^a | | |
| Slope | 0.00012 | 1.81 | 23358783.00 |
| <i>SD</i> of slope | 7.09×10 ⁻⁶ | 0.0003 | 0.0026 |
| Intercept | 0.1457 | 0.0076 | 36117.71 |
| <i>SD</i> of intercept | 0.0047 | 0.0001 | 0.0015 |
| Regression coefficient | 0.9947 | 0.9999 | 0.9999 |
| Recovery, % | 99.6 | 99.94 | 99.57 |
| <i>SD</i> / % | 1.11 | 1.65 | 1.38 |
| <i>LOD</i> / µg cm ⁻³ | 117.50 | 8.56 | 8.24 |
| <i>LOQ</i> / µg cm ⁻³ | 391.66 | 30.46 | 29.28 |

^a $Y = a + bc$, where c is the concentration of clopidogrel, mg cm⁻³; Y is the current per area unit, mA cm⁻², absorbance, A , and peak area unit, mAu, for the SWV, UV and HPLC methods, respectively, and *SD* is the standard deviation

Recovery studies were also performed to judge the accuracy of the SW voltammetry method by performing six measurements at low, intermediate and high CLP concentrations (317.89, 622.03 and 935.16 µg cm⁻³). Mean percentage

recoveries of 99.6 % with relative standard deviation of 1.11 % were found and are presented in Table I.

Tablet solutions of appropriate concentrations were prepared and voltammograms were taken under the same experimental conditions (Fig. 5). The obtained results show that the excipients in the tablet were not electro-active and did not affect the voltammetric analysis. The results of drug analysis by the SW voltammetric technique are compared in Table II with those obtained by the UV-spectrophotometric and high pressure liquid chromatography methods. The analytical results demonstrated that the amount of active substance in the Plavix[®] tablet was within the limits specified in the pharmacopoeia. This indicates that the proposed method could be an alternative to chromatographic and spectrophotometric methods.

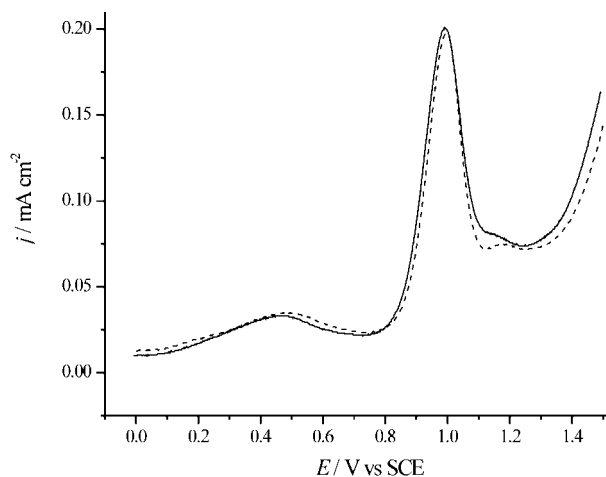


Fig. 5. Square wave anodic stripping voltammograms of clopidogrel standard (full line) and clopidogrel as the content of commercial tablets Plavix[®] (dashed line) at a gold electrode in acetate buffer (pH 3.7). Clopidogrel concentration: 474.49 $\mu\text{g cm}^{-3}$. Accumulation time: 180 s at $E = 0.0$ V; step size 2 mV, pulse size 20 mV, frequency 8 Hz, scan rate 15 mV s^{-1} .

TABLE II. Comparative studies for the determination of clopidogrel in Plavix[®] tablets (75 mg per tablet)

| Parameter | Method | | |
|--------------------------------|--------|------|------|
| | SWV | UV | HPLC |
| Mean content ^a , mg | 74.6 | 76.1 | 75.2 |
| SD / % | 0.8 | 1.1 | 0.3 |

^aEach value is the mean of six experiments

The results obtained in this work show that the SW voltammetry method exhibited an accuracy that recommends it for routine and quality control analysis of the drug in pharmaceutical dosage forms.

CONCLUSIONS

The electrochemical determination of clopidogrel, as standard and in commercial tablets, Plavix[®], was performed at a gold electrode in pH 3.7 acetate buf-

fer using square wave voltammetry. Prior to the determination, their electrochemical behaviour was studied by cyclic voltammetry. It was shown by both techniques that each voltammogram is characterized by a well defined peak at approximately 1.0 V. The linear dependence of currents of the anodic stripping peak on clopidogrel concentration was used to determine the amount of clopidogrel in Plavix[®] tablets. The results demonstrated that the SW voltammetric technique could be used directly, easily and rapidly for the determination of clopidogrel in tablet dosage forms. The SW voltammetric technique was compared to the results of drug analysis by UV and HPLC methods. The analytical results demonstrated that the amount of active substance in the Plavix[®] tablets was within the limits specified in the pharmacopoeia. This indicates that the proposed method could be an alternative to chromatographic and spectrophotometric methods.

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ИЗВОД

ОДРЕЂИВАЊЕ КЛОПИДОГРЕЛА ВОЛТАМЕТРИЈОМ СА ПРАВОУГЛИМ ИМПУЛСИМА НА ЕЛЕКТРОДИ ОД ЗЛАТА

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Клопидогрел је антиромботски агенс из тијенопиридинске класе, који се користи за инхибирање формирања крвних угрушака при лечењу коронарне артеријске болести, периферне васкуларне болести, и цереброваскуларне болести. Електрохемијско понашање клопидогрел стандарда и као састојка таблете Plavix[®] је дефинисано цикличном волтаметријом. Њихово квантитативно одређивање на електроди од злата у ацетатном пуферу, рН 3,7, урађено је волтаметријом са правоугаоним импулсима (SWV). Показано је обема техникама да је сваки волтамограм окарактерисан добро дефинисаним струјним врхом на 1,0 V. На основу линеарне зависности анодних струја од концентрације клопидогрел стандарда одређена је маса клопидогрела у Plavix[®] таблети. Добијени резултати показују да SWV техника може бити коришћена директно, брзо и једноставно за одређивање клопидогрела у таблетама. Резултати добијени SWV волтаметријском техником су поређени са резултатима анализе клопидогрела UV спектрофотометријом и течном хроматографијом са високим перформансама. Аналитички резултати показују да је маса активне супстанце у таблетама Plavix[®] у оквиру лимита спецификованог фармакопејом. То указује да волтаметрија са правоугаоним импулсима може бити успешна алтернатива хроматографским и спектрофотометријским методама.

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