International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

DETERMINATION OF 17 β-ESTRADIOL IN PHARMACEUTICAL PREPARATIONS BY LINEAR SWEEP VOLTAMMETRY METHOD

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ABSTRACT

In this study, simple, fast and reliable linear sweep voltammetry (LSV) method was developed and validated for determination of 17 β-estradiol in pharmaceutical preparation. The proposed method was based on electrochemical oxidation of 17 β-estradiol at platinum electrode in acetonitrile solution containing 0.1 M $LiClO_4$.The well-defined an oxidation peak was observed at 1.47 V. The calibration curve was linear for 17 β-estradiol at the concentration range of 5-30 µg/mL for LSV method. Intra- and inter-day precision values for 17 β-estradiol were less than 4.63, and accuracy (relative error) was better than 3.45%. The mean recovery of 17 β-estradiol was 100.8% for pharmaceutical preparation. No interference was found excipient at the selected assay conditions. The proposed method is highly sensitive, precise and accurate and can be used for the reliable quantitation of 17 β-estradiol in pharmaceutical dosage form.

Keywords: 17 β-estradiol, Cyclic voltammetry, Linear sweep voltammetry, Validation

INTRODUCTION

17 β-estradiol (Figure 1) is the most potent of the natural human estrogens [1]. 17 β-estradiol, chemically 1, 3, 5 (10)-estratrien-3, 17 β-diol, is the most potent estrogen of a group of endogenous estrogen steroids which includes estrone and estriol. It is responsible for the growth of breast and reproductive epithelia, maturation of long bones, and development of secondary sexual characteristics.

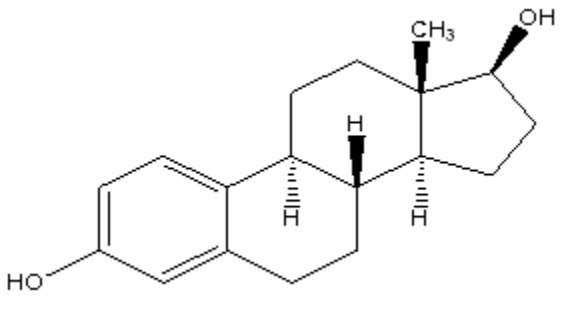

Figure 1: Chemical structure of 17 β-estradiol

17 β-estradiol and its semi-synthetic esters are primarily used as menopausal hormones. It may also be used as replacement therapy for female hypogonadism or primary ovarian failure. The decrease of 17 β-estradiol at menopause is often accompanied by vascular instability and rise in incidence of heart disease and an increasing risk of osteoporosis [2].

Several methods have been reported for determination of 17 β-estradiol including voltametric [3], high performance liquid chromatography [4-9]. Many other gas chromatography-mass spectrophotometry (GC-MS) methods have been published quantifying 17 β-estradiol and its metabolites [10–14]. 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 [15-20]. Despite the analytical importance of the electrochemical behavior and oxidation mechanism of 17 β-estradiol, no

report has been published on the voltammetric study of the electrochemical oxidation of 17 β-estradiol 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 17 β-estradiol in aprotic media. Therefore, the goal of this work was the development of new a LSV method for the direct determination of 17 β-estradiol in pharmaceutical preparation 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 17 β-estradiol employing LSV method at platinum disc electrode. Besides, the method was successfully applied for the quality control of a commercial 17 β-estradiol quantify the drug and to check the formulation content uniformity.

MATERIALS AND METHODS

Chemical and reagents:

17 β-estradiol was purchased from Sigma (St. Louis, Mo, USA). 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. L***ithium perchlorate*** ($LiClO_4$) were purchased from Fluka and used as received without further purification. Estrofem tablet containing 2 mg 17 β-estradiol was obtained from pharmacy (Erzurum, Turkey).

Electrochemical instrumentation:

Electrochemical experiments were performed on a GamryPotentiostat 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.

Preparation of the standard and quality control solutions:

The stock standard solution of 17 β-estradiolwas 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 5-30 μg/mL for LSV. The quality control (QC) solutions were prepared by adding

aliquots of standard working solution of 17 β-estradiol to final concentrations of 7.5, 12.5 and 27.5 µg/mL for LSV.

Procedure for pharmaceutical preparations:

Ten tablets of Estrofem (contained 2 mg 17 β-estradiolper tablet) were accurately weighed and finely powdered. An amount of powdered tablet equivalent to about 2 mg of 17 β-estradiol was accurately weighed and dissolved with 0.1 M $LiClO_4$/acetonitrilee. 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. The drug content of 17 β-estradiolwas calculated from the current potential curve.

RESULTS AND DISCUSSION

Voltammetric behavior of 17 β-estradiol:

The electrochemical behavior of 17 β-estradiolwas 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 voltammogramof 30 µg/mL 17 β-estradiolrecorded 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 1.47 V.

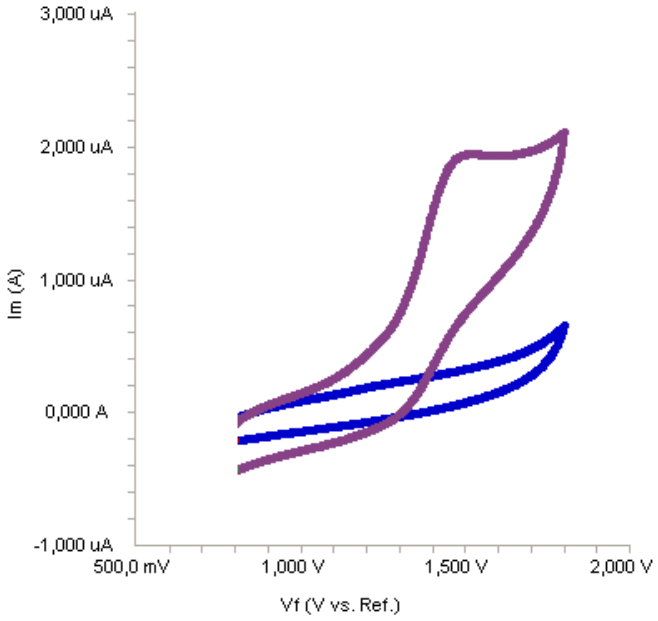

Figure 2. Cyclic voltammogram for the oxidation of 30 µg/mL 17 β-estradiol in acetonitrile containing 0.1 M $LiClO_4$ at Pt disk electrode, scan rate: 0.1 V/s.

Validation of the method:

The validation was carried out by establishing specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), stability, recovery according to ICH Q2B recommendations [21, 22].

Specificity:

The effects of common excipients and additives were tested for their possible interferences in the assay of 17 β-estradiol. The simulated and placebo samples were prepared and analyzed. It has not been determined any interference of these substances at the levels found in dosage forms. Excipient that was used in this preparation was the most commonly used by the pharmaceutical industry. The specificity of the method was investigated by observing any interference encountered from the common tablet excepients such as titanium dioxide, ***sodium chloride,*** talc, lactose, starch, and magnesium stearate. These excepients did not interfere with the proposed method.

Linearity:

Standard solutions were prepared as 5-30 µg/mL (5, 7.5, 10, 15, 20, 25 and 30 µg/mL) for LSV (Figure 3).

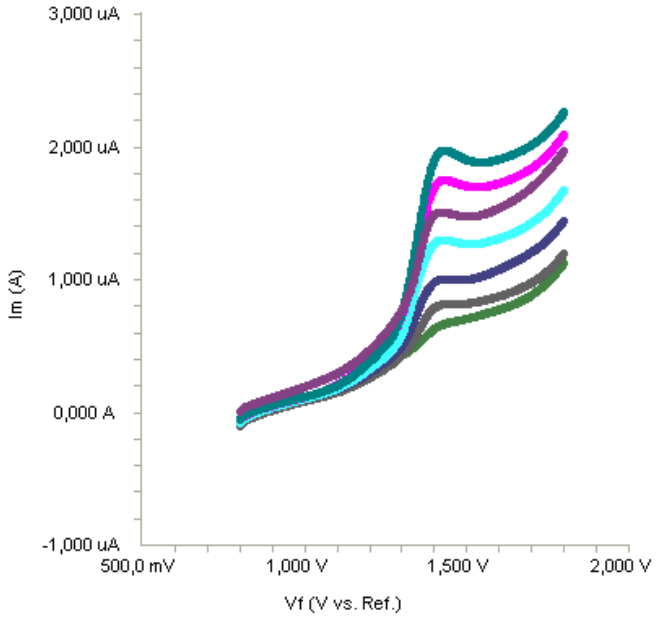

Figure 3: Linear sweep voltammograms for different concentrations of 17 β-estradiolin acetonitrile solution containing 0.1 M $LiClO_4$ (5, 7.5, 10, 15, 20, 25 and 30 µg/mL).

The linearity of peak current response versus concentration for 17 β-estradiolwas studied between concentration range of 5-30 μg/mL. The calibration curve constructed was evaluated by its correlation coefficient. The calibration equation from six replicate experiments, y = 0.0524x+0.4209 (r = 0.9933), demonstrated the linearity of the method. Standard deviations of the slope and intercept for the calibration curves were 0.009 and 0.0238 respectively.

Accuracy and precision:

Accuracy of the assay method was determined for both intra-day and inter-day variations using 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. Intermediate precision was assessed by comparing the assays on different days (2 days). The intra-day accuracy ranged from 2.38% to 3.45% and precision from 3.44% to 4.63%. The results obtained from intermediate precision (inter-day) also indicated a good method precision.

Limits of detection (LOD) and quantification (LOQ):

The LOD and LOQ of 17 β-estradiolby the proposed method was 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) [22]. The LOD and LOQ values for 17 β-estradiolwere found to be 1.50 and 4.5 μg/mL, respectively.

Stability:

Stability studies indicated that the samples were stable when kept at room temperature, 4 ^{0}C and -20 ^{0}C refrigeration temperature for 24 h (short-term) and refrigerated at 4 and -20 ^{0}C for 72 h (long-term). The results of these stability studies are within the acceptance range of 90-110%.

Recovery:

To determine the accuracy of the LSV 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 drug form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 1.

Commercial preparation		Estrofem tablet (5 µg/mL)		
Method	**Added (µg/mL)**	**Found±SD (µg/mL)**	**Recovery (%)**	**RSD [a] (%)**
LSV	5	4.95±0.192	99.0	3.88
	10	10.14±0.263	101.4	2.59
	25	25.53±0.631	102.1	2.47

Table 1: Recovery values of 17 β-estradiol pharmaceutical preparation

SD: Standard deviation of six replicate determinations,RSD: Relative standard deviation

[a]Average of six replicate determinations

CONCLUSION

The electrochemical behavior of 17 β-estradiolhas been studied in nonaqueous media by CV and LSV voltammetry methods. Besides, in the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise LSV method for the determination of 17 β-estradiolin pharmaceutical preparation was developed and validated. The method described has been effectively and efficiently used to analyze 17 β-estradiolpharmaceutical preparations 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 method can be used effectively without separation for routine analysis of 17 β-estradiolin pure form and its formulations.

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