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Electrochemical Study of 17β-estradiol and its Determination in Pharmaceutical Preparations using Square Wave Voltammetry

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Abstract: In the present study, the electroanalytical behavior of 17β-estradiol was investigated using cyclic voltammetry. The procedure was based on 17β-estradiol being electrochemically oxidized at a platinum electrode in non-aqueous solutions. At 1.47 V, the oxidation peak was noted. It was discovered that 17β-estradiol's oxidation was diffusion-controlled. Additionally, a quick and easy square wave voltammetry method was developed and validated in this work to determine 17β-estradiol in pharmaceutical preparations. The calibration curve was linear at 5 and 30 µg/mL concentrations. The precision was given by relative standard deviation and was less than 3.36%. Accuracy was given with relative error and did not exceed 2.54%. In pharmaceutical preparations, 17β-estradiol had an average recovery of 100.3%. Under the chosen experimental conditions, no interference was found. The suggested method is highly accurate and precise. Therefore, the method applies to measuring 17β-estradiol in pharmaceutical formulations.

Keywords: 17β-estradiol, Voltammetry, Validation, Analysis.

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1. INTRODUCTION

The most substantial naturally-occurring human estrogen is 17β-estradiol (Figure 1) [1]. This hormone, also known chemically as 1,3,5(10)-estratrien-3,17-diol, is the most potent among endogenous estrogen steroids, including estrone and estriol. 17β-estradiol is primarily responsible for the development of secondary sexual characteristics, the formation of breast and reproductive epithelia, as well as the maturation of long bones.

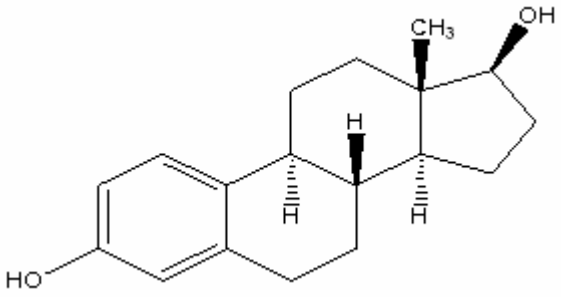


Figure 1: Chemical structure of 17 β-estradiol.

The primary application of 17β-estradiol and its semi-synthetic esters is as menopausal hormone replacement. Additionally, they can serve as alternative treatments for primary ovarian failure or female hypogonadism. When 17β-estradiol levels decline during menopause, it often results in vascular instability, increased incidence of heart disease, and heightened risk of osteoporosis (2).
Various techniques for determining 17β-estradiol have been published, including spectrophotometry (3) and high-performance liquid chromatography (4-9). Furthermore, many gas chromatography-mass spectrophotometry (GC-MS) techniques for quantifying 17β-estradiol and its metabolites have been documented (10-16).

A comprehensive literature survey has revealed a broad spectrum of chromatographic techniques for detecting 17β-estradiol in human plasma. However, the reported methods can be hindered by endogenous interference, potential drug loss during re-extraction, labor-intensive and time-consuming processes for preparing and extracting plasma samples, and the requirement of expensive equipment.
Developing a new method for calculating medication dosage in pharmaceutical dosage

forms is vital. Electroanalytical techniques, which often do not require derivatization and are less prone to matrix effects than other analytical techniques, have been utilized to identify numerous medicinal compounds. Another application of electrochemistry involves elucidating electrode mechanisms. The redox properties of drugs can provide insights into their pharmacological potency, in vivo redox activities, or metabolic fate.

Despite the analytical significance of the electrochemical behavior and oxidation mechanism of 17β-estradiol, no research on its voltammetric oxidation in non-aqueous media has been published. Given that experimental and operational parameters directly influence the electrochemical process and the voltammetric response of pharmaceuticals, investigating how 17β-estradiol oxidizes in aprotic environments would be intriguing. However, the voltammetry method has not yet been utilized to assess 17β-estradiol using a platinum electrode quantitatively. The primary objective of this work was to develop a novel square wave voltammetry (SWV) method for swiftly and accurately evaluating 17β-estradiol in pharmaceutical preparations without requiring labor-intensive extraction or evaporation procedures prior to drug testing.
This study describes SWV methods using a platinum disc electrode to determine 17β-estradiol via simple, quick, and selective processes that have been thoroughly validated. Additionally, the technique was effectively applied to evaluate the consistency of formulation content and to quantitate a commercially available 17β-estradiol medication for quality control.

2. EXPERIMENTAL SECTION

2.1. Chemicals

17β-estradiol standard (98≥ purity), lithium perchlorate ($LiClO_4$), and acetonitrile were purchased from Sigma (Germany). Estrofem tablet that included 2 mg 17β-estradiol was purchased from a pharmacy (Erzurum, Turkey).

2.2. Electrochemical Instrumentation

Using the software PHE 200 and PV 220, electrochemical experiments were carried out on a Gamry Potentiostat Interface 1000. The single-compartment electrochemical cell used for all tests has a conventional three-electrode setup. Platinum wire was the counter electrode, and a platinum disk was the working electrode. The reference electrode for each potential was Ag/AgCl/KCl (3.0 M). The SWV was operated at pulse amplitudes of 25 mV, 10 Hz, 4 mV potential step, and 0.1 V/s scan rate.

2.3. Preparation of Standard Solutions

In 0.1 M $LiClO_4$/acetonitrile, the stock standard solution of 17β-estradiol (100 µg/mL) was prepared. This stock solution was used to prepare working standard solutions. The concentrations of the standard solutions were 5, 7.5, 10, 15, 20, 25, and 30 µg/mL. The QC solutions were created at 7.5, 12.5, and 27.5 µg/mL concentrations.

3. RESULTS AND DISCUSSION

3.1. Development and Optimization of the Method

The electrochemical behavior of 17β-estradiol was studied at the Pt disc electrode. An acetonitrile solution with 0.1 M $LiClO_4$ was the supporting electrolyte in cyclic voltammetry. Figure 2 depicts a typical cyclic voltammogram for 100 µg/mL 17β-estradiol at 0.1 V/s scan rate. The oxidation peak was seen in the anodic sweep at 1.47 V.

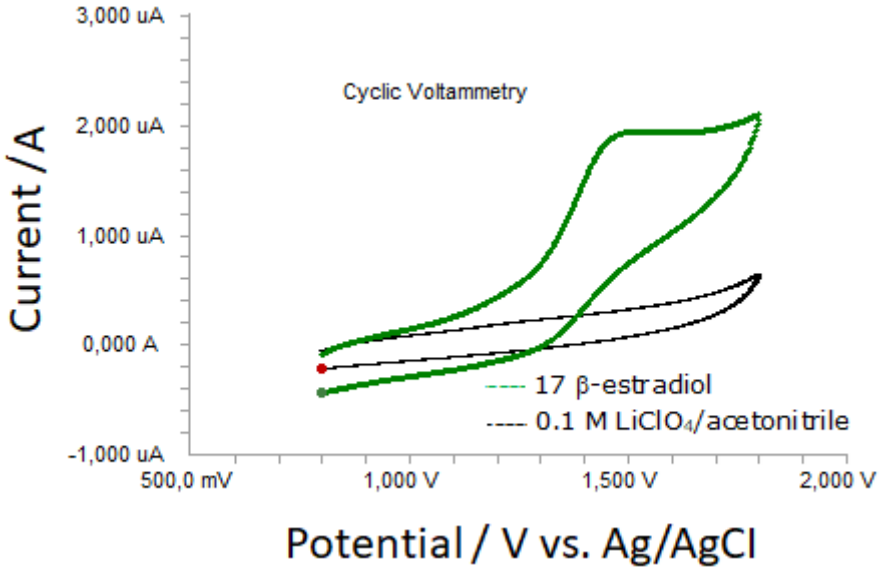


Figure 2: Cyclic voltammogram of 17β-estradiol (30 µg/mL)

The influence of scan rate on the anodic peak currents and peak potentials was investigated in the range of 0.01-1 V/s (Figure 3).

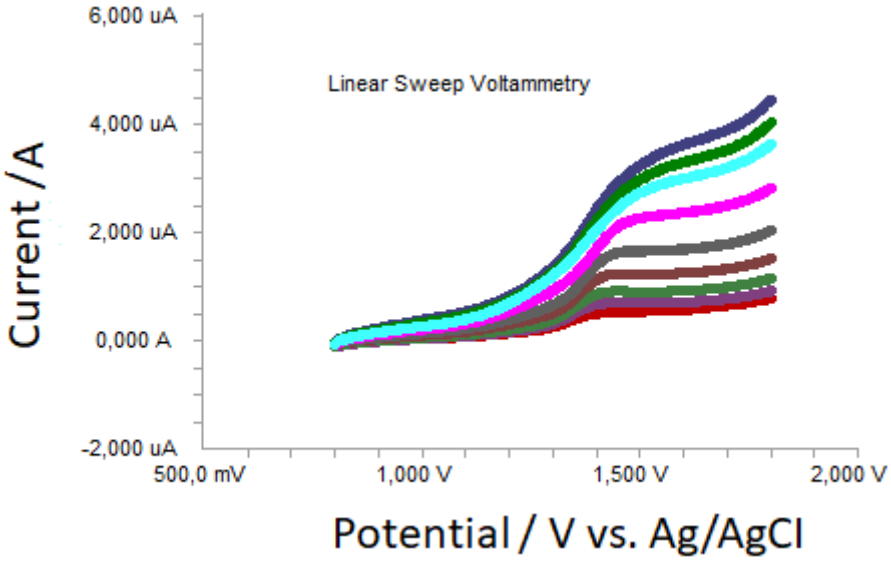
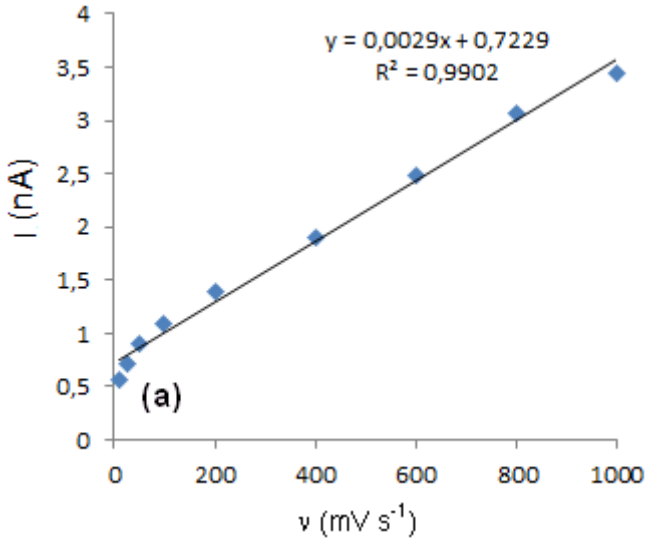


Figure 3: Linear sweep voltammograms of 30 µg/mL 17β-estradiol as a function of scan rate

Figure 4a,b shows the linear sweep voltammograms for 17β-estradiol as a function of scan rate. However, at 17β-estradiol concentrations of 30 µg/mL, the logarithm of peak currents against the logarithm of scan rates graphs display straight lines with a slope of 0.39 (Figure 4c), which is close to the predicted value of 0.5 anticipated for an ideal diffusion-controlled electrode process (17).

In order to accomplish this, the *log I-log v* curve is more suitable; therefore, a diffusional process for the peak should be considered. These findings show that the redox species readily diffuse from the solution instead of precipitating onto the electrode surface. This phenomenon can be brought on by either a lack of product adhesion to the electrode surface or the solubility of the intermediate species in acetonitrile.



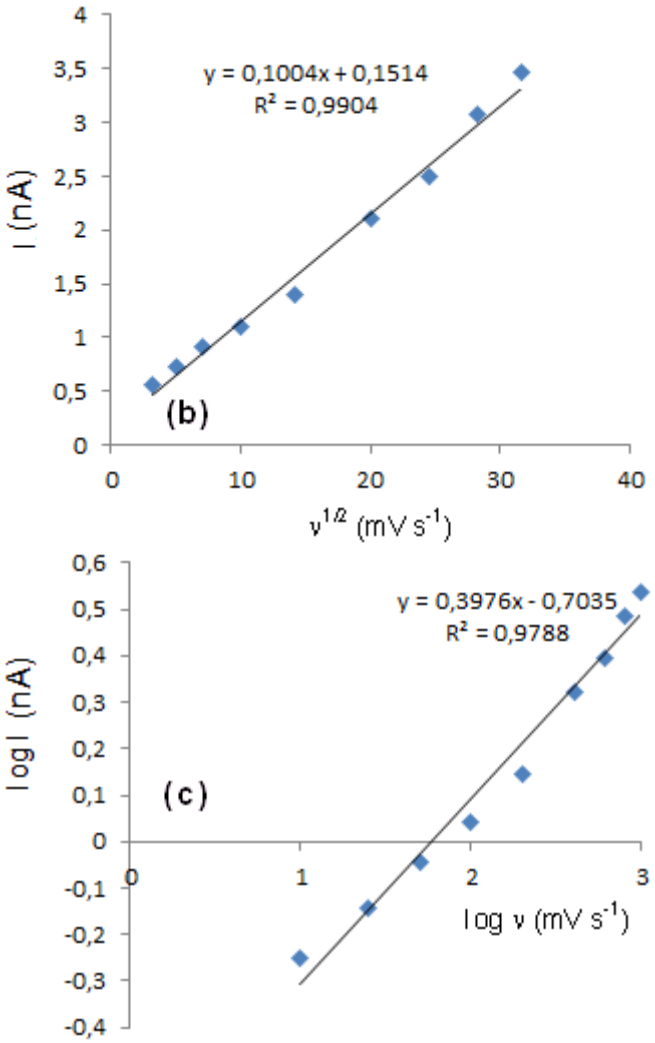


Figure 4(a-c): Peak current dependence on scan rate (30 µg/mL).

Figure 3 shows the oxidation peak potential (Epa) movement for peaks toward higher positive values as the scan rate increases. The equation below (18) describes the relationship between the peak potential and scan rate,

$$E_{pa} = E^{0'} + RT/\left[(1-\alpha)n_a F\right]\left[0.78 + \ln\left(D^{1/2}k_s^{-1}\right) - 0.5\ln RT/\left[(1-\alpha)n_a F\right]\right] + RT/\left[(1-\alpha)n_a F\right]/2\ln v$$

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. The plots of the oxidation peak potentials against *ln v* demonstrate a linear connection in accordance with this equation (Figure 5).

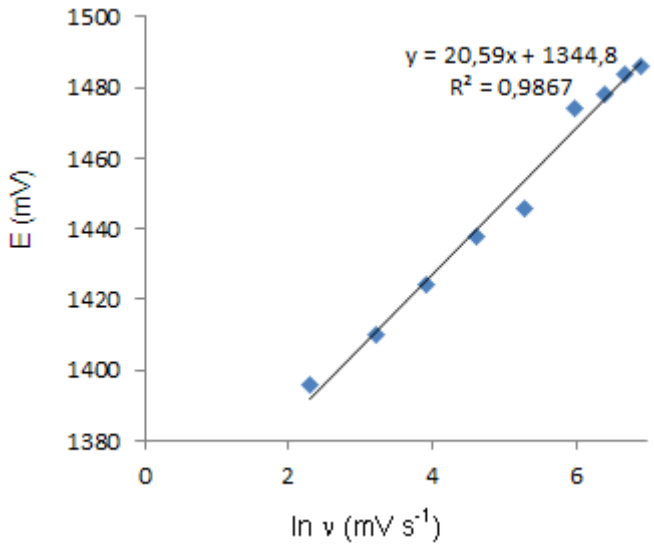


Figure 5: Dependence of the 17β-estradiol anodic peak potentials on the scan rate

The slope indicates that the highest value of αn is 9.41. Additionally, this value shows that the electron transfer processes are entirely irreversible. This outcome demonstrates that the chemical step is a charge transfer and a quick following reaction.

3.2. Validation of the Method

ICH Q2B guidelines were followed while determining the validation parameters (19). These criteria include specificity, linearity, precision, accuracy, recovery, the limit of detection (LOD), limit of quantification (LOQ), ruggedness, and stability.

3.2.1. Specificity

In this study, it was investigated the potential interferences of common excipients and additives. The control samples were prepared and examined. There is no evidence of any interference from these chemicals at the concentrations in dosage forms. The excipient employed in this formulation was one that the pharmaceutical industry employs most frequently. The method's specificity was examined by examining for any interference from common tablet ingredients like talc, lactose, sodium chloride, titanium dioxide, and magnesium stearate. These exceptions had no adverse effects on the suggested method. The procedure might be specific in accordance with the findings of the analysis.

3.2.2. Linearity

Standard solutions at concentrations of 5, 7.5, 10, 15, 20, 25, and 30 µg/mL were prepared for SWV (Figure 6). Plotting the 17β-estradiol concentration versus peak current responses allowed constructing the calibration curve for the 17β-estradiol (Figure 7).

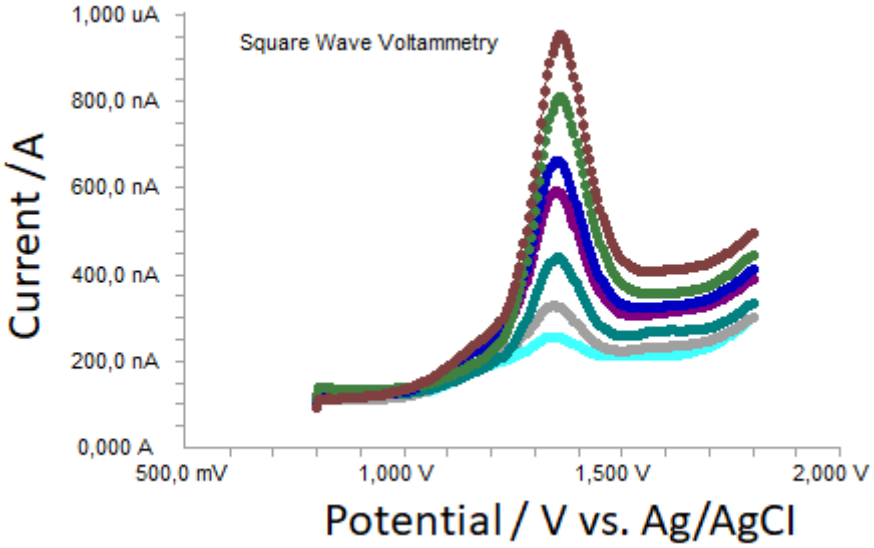


Figure 6: SWV voltammograms of 17 β-estradiol.

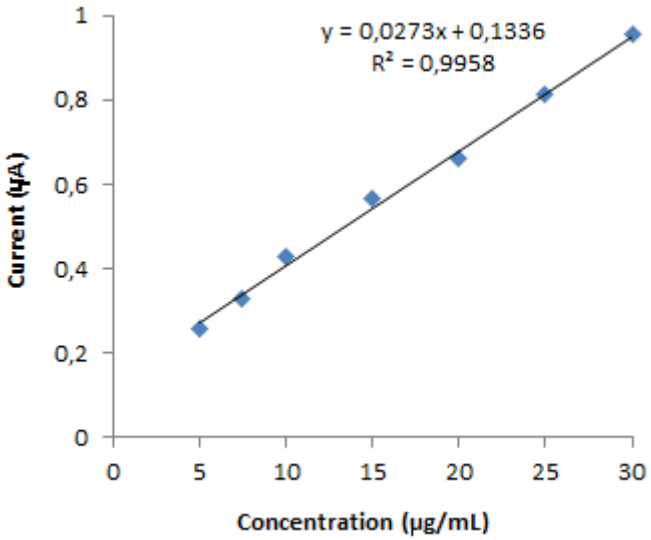


Figure 7: Calibration curve of 17 β-estradiol.

All calibration curves' correlation coefficients (r) were consistently higher than 0.99. Using the least squares method and the Microsoft Excel® application, the linear regression equations were derived and described in Table 1.

Table 1: Linearity of 17 β-estradiol.

Parameters	17β-estradiol
Linearity range (µg/mL)	5-30
Slope	0.0273
Intercept	0.1336
Correlation coefficient	0.9958
LOD (µg/mL)	1.00
LOQ (µg/mL)	3.00

3.2.3. Precision and accuracy

The precision and accuracy of the square wave voltammetry (SWV) method were evaluated for intra-day and inter-day measurements using quality control (QC) samples. Intra-day precision and accuracy were assessed by analyzing the QC samples on the same day. Inter-day precision and accuracy were evaluated by comparing the assays conducted on two different days. The results showed that the intra-day accuracy ranged from 1.09% to 1.33%, and the precision ranged from 0.95% to 3.36% (Table 2). These findings indicate that the SWV method demonstrates good accuracy and precision in this study.

Table 2: Precision and accuracy of 17 β-estradiol.

	Intra-day			Inter-day		
Added (µg/mL)	**Found ± SD[a]**	**Precision % RSD[b]**	**Accuracy[c]**	**Found ± SD[a]**	**Precision % RSD[b]**	**Accuracy[c]**
7.5	7.6 ± 0.205	2.70	1.33	7.4 ± 0.142	1.92	-1.33
12.5	27.2 ± 0.914	3.36	-1.09	26.8 ± 0.821	3.06	-2.54
27.5	44.5 ± 0.424	0.95	-1.11	45.9 ± 0.532	1.16	2.00

3.2.4. Recovery

At three different concentrations, the recovery was examined to investigate the impacts of formulation interference. The recoveries were carried out by mixing pre-analyzed samples of 17β-estradiol tablets with a known quantity of pure medicines. The recoveries were calculated by comparing the amounts extracted from the spiked samples with the actually added concentrations. The results are listed in Table 3.

Table 3: Recovery of 17β-estradiol in tablets (n=6).

Tablet	Added (µg/mL)	Found ± SD	%Recovery	%RSD
Estrofem (10 µg/mL)	5	4.9 ± 0.131	98.0	2.67
	15	14.9 ± 0.312	99.3	2.09
	25	25.3 ± 0.684	101.2	2.70

3.2.5. LOD and LOQ

The suggested technique's LOD and LOQ values were calculated using calibration standards. LOD and LOQ values were calculated as 3.3 /S and 10 /S, respectively (19). In this equation, S is the calibration curve's slope and is the y-intercept's standard deviation (n=6). The results are summarized in Table 1.

3.2.6. Ruggedness

A separate analyst used the same instrument and standard solution in this study to assess the concentration of 17β-estradiol (Table 4). No statistically significant discrepancies between the operators were found in the results, indicating the ruggedness of the developed method.

Table 4: Results of another analyst's studies of 17β-estradiol (n=6).

Method	Added (µg/mL)	Found (µg/mL) (Mean±SD)	% Recovery	% RSD
SWV	5	5.1 ± 0.12	102.0	2.35
	15	14.9 ± 0.21	99.3	1.41
	35	35.1 ± 1.07	100.2	3.04

3.2.7. Stability

The stability of 17β-estradiol stock solution was examined over a period of at least 72 hours. Furthermore, 17β-estradiol standard solutions were stable for 72 hours at 4 and -20 ºC refrigeration temperatures and ambient temperature. The 17β-estradiol accuracy is within the acceptable range of 90 to 110% (Table 5). There are no major 17β-estradiol breakdown products under these circumstances.

Table 5: 17 β-estradiol's stability at various temperatures (n = 6).

Added (µg/mL)	Room temperature 24 h (Mean ± SD)	Room temperature 72 h (Mean ± SD)	Refrigeratory +4 °C, 72 h (Mean ± SD)	Frozen -20 °C, 72 h (Mean ± SD)
15	100.7 ± 2.57	100.3 ± 1.71	101.2 ± 1.67	98.6 ± 3.71
30	98.9 ± 1.77	98.9 ± 2.16	100.2 ± 1.96	98.7 ± 2.73
45	99.6 ± 2.19	101.2 ± 2.37	98.8 ± 2.27	101.7 ± 3.09

3.3. Procedure for Pharmaceutical Preparations

Estrofem tablet containing 2 milligrams of 17β-estradiol was precisely weighed and finely powdered. A suitable amount of powder was dissolved in 50 mL of 0.1 M $LiClO_4$/acetonitrile. Then, the final volume was made up to 100 mL in a balloon flask. Whatman filter (paper no 42) was used to filter the tablet solutions after they had been properly diluted in order to provide a final concentration that was within the linearity constraints of the SWV method (Figure 8). The calibration curve determined the drug concentration for 17β-estradiol (Table 6).

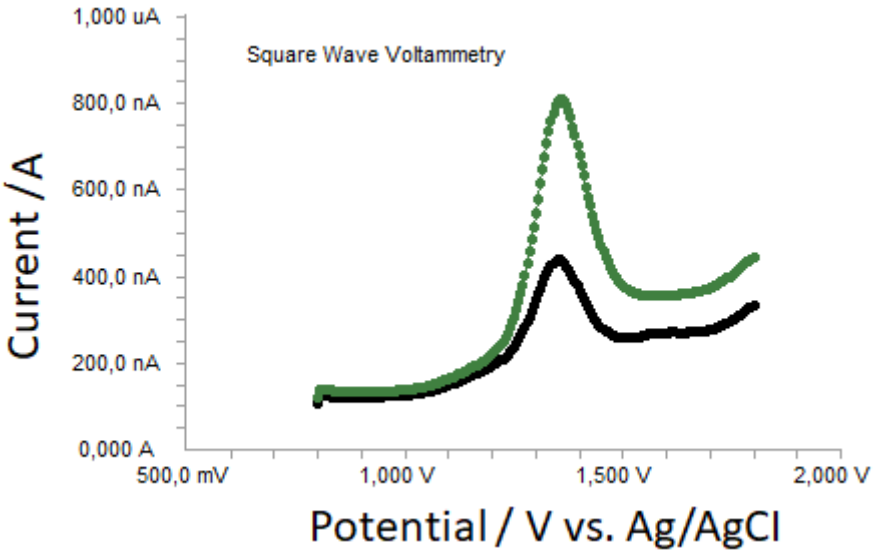


Figure 8: The voltammograms of Estrofem tablet containing 17 β-estradiol.

For the analysis of associated compounds in pure 17β-estradiol and the assay of 17β-estradiol in pharmaceutical dosage form (tablet), the United States Pharmacopoeia (20) has recommended the liquid chromatography (HPLC) method. The method suggested uses a stainless steel column (5 µm, 4.6 mm, 250 mm i.d.) with UV detection (280 nm) and a mobile phase of 2,2,4-trimethylpentane-n-butyl chloride-methanol (45:4:1, v/v) at a flow rate of 2 mL/min. In contrast, the previously described HPLC technique (5) uses methanol as the mobile phase instead of buffered systems.

The proposed SWV approach was relatively quick compared to previously published and authorized methods for estimating 17β-estradiol in pharmaceutical formulations (3,10,11). The sample recoveries in a formulation aligned with the claims made on the corresponding labels. Additionally, the reported methods (3) and the new SWV method were statistically evaluated using the F-test. The computed F-values do not exceed the theoretical values at a 95% confidence level (Table 6).

Table 6. Comparison of the proposed and reported methods for determination of 17 β-estradiol.

Parameters	SWV	Reported Method (3) (Spectrophotometry)	Reported Method (3) (HPLC)
Mean (Recovery %)	100.3	101.6	100.2
SD	1.29	0.038	0.060
%RSD	1.28	0.52	0.60
Variance	1.66	0.270	0.360
F-test	3.07		

SD: Standard deviation, RSD: relative standard deviation, Ho is acceptable (P > 0.05) since there is no statistically significant difference between the three methods.

The analytical findings in this investigation showed that the level of the active ingredient in the medicine is within the pharmacopeia's recommended range. The developed method was practical, accurate, and adaptable to drug dose forms. Therefore, the developed SWV method can be advised for the routine QC analyses of 17β-estradiol in pharmaceutical preparations.

4. CONCLUSION

In the current work, the CV method has been used to examine the electrochemical behavior of 17β-estradiol in non-aqueous media. Additionally, a quick, accurate, specific, and precise SWV method was developed and validated in the Study for detecting 17β-estradiol in pharmaceutical formulations. Voltammetry runs for one minute. The method enables the speedy analysis of a large number of samples. As a result, the method can regularly examine 17β-estradiol in both its formulations and pure form.

5. CONFLICT OF INTEREST

The authors state that they did not have a conflict of interest.

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