



## Full Length Article

# Validation and application of the square wave voltammetry method for the electrochemical determination of eszopiclone in pharmaceutical formulations and human biological fluids using glassy carbon electrode

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## ARTICLE INFO

## Keywords:

Square wave voltammetry  
Cyclic voltammetry  
Differential pulse voltammetry  
Reduction  
Eszopiclone  
GC electrode

## ABSTRACT

A buffer solution of Britton-Robinson (B-R) pH 6.5 was utilized to validate and apply a square wave voltammetry (SWV) method for the electrochemical determination of Eszopiclone (ESP) in pharmaceuticals and biological samples. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were employed in the initial study of the voltammetric behavior of the ESP compound. The rotating glassy carbon (GC) indicator electrode (2.0 mm<sup>2</sup>), Pt auxiliary electrode, and Ag/AgCl reference electrode were used for ESP determination. ESP was analyzed to produce a sharp reduction peak at a potential of -750 mV. The best shape and high sensitivity of SWV cathodic current for ESP, were observed using GC electrode, B-R, pH 6.5, accumulation time ( $t_{acc}$ ) of 60-second, accumulation potential ( $E_{acc}$ ) of -0.1 V, amplitude voltage of 150 mV, frequency of 15 Hz, scan rate of 150 mV s<sup>-1</sup>, and stirrer rate of 1000 round per minute (rpm). The electroanalytical performance of the SWV method was evaluated through study of repeatability, stability, calibration curve, recovery, and limits of detection and quantification (LOD, LOQ). Under the optimal conditions, the SWV response was linear in the range from  $3 \times 10^{-6}$  to  $5 \times 10^{-5}$  mol/L ( $n = 10$ ). The SWV method achieved a LOD of  $1.9 \times 10^{-8}$  mol/L (7.5 ppb), and a LOQ of  $6.41 \times 10^{-8}$  mol L<sup>-1</sup> (24.93 ppb). The validated SWV method was appeared the good repeatability, sensitivity and stability for the determination of ESP. It was given a very stable SWV current for 90 min, with a 0.141 % relative standard deviation (RSD%). The SWV method was applied to the determination of ESP in pharmaceutical tablets and biological samples.

## 1. Introduction

A sedative called Eszopiclone (ESP) is used to treat both acute and persistent insomnia. It is a non-benzodiazepine hypnotic, a type of sleeping pill that induce relaxation to help with falling asleep and staying asleep for a set period. The United State Food and Drug Administration (FDA) approved ESP in 2004 as the first drug for treating insomnia in adults. It is available in pharmacies in 1.0, 2.0, and 3.0 mg film-coated tablets and can be used for short- or long-term treatment a sleep insomnia for adults. Oral ESP quickly enters the body and is vastly distributed through tissues of body, such as the brain. ESP chemically known as 5S and S-isomer zopiclone, (5S)-6-(5-chloropyridin-2-yl) -7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b] pyrazin-5-yl 4-methylpiperazine -1-carboxylate. It has a molecular formula of C<sub>17</sub>H<sub>17</sub>ClN<sub>6</sub>O<sub>3</sub> with a molecular weight of 388.9 g mol<sup>-1</sup> (Monti and Pandi-Perumal, 2007, Rosner et al., 2018, Halas and Pharm, 2006, Wessell et al., 2005).

Eszopiclone has been identified using various analytical techniques,

including spectrophotometry (Chennaiah et al., 2012, Patel et al., 2013), chromatography (Bhusari and Dhaneshwar, 2021, Anandakumar et al., 2010, Liu et al., 2020, Ravi et al., 2014, Shaikh et al., 2014), spectrophotometry with chromatography (Pandya et al., 2013, Panchal and Maheshwari, 2017), IR spectroscopy (Liu et al., 2014), thermodynamic and gravimetry (Liu et al., 2016).

SWV is a frequently used method in various fields, including the analysis of caffeine in beverages (Tefera et al., 2016), analysis of pharmaceutical formulations (Afzali et al., 2020, Faria et al., 2019, Alghamdi, 2016, Karastogianni and Girousi, 2022, Kokoskarova et al., 2023, Neven and Emad, 2022, Biya et al., 2023), metals (Mekassa et al., 2018, Alshawi et al., 2022, Leon-Medina et al., 2022), and dyes (Hayat et al., 2022, Yahya et al., 2022).

The current research aims to develop a rotating glassy carbon (GC) indicator electrode-based method for quick, sensitive, and selective square wave voltammetry for the quantitative analysis of ESP in commercial tablets and human fluids. The study focus on the simplicity,

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<https://doi.org/10.1016/j.jksus.2024.103408>

Received 17 January 2024; Received in revised form 20 August 2024; Accepted 21 August 2024

Available online 22 August 2024

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sensitivity, affordability and time-saving aspects of SWV methodology compared to other analytical methods. The accuracy and precision of the SWV approach were validated via comparison of its voltammetric results with UV spectrophotometry utilizing the statistical “F-test”. This research is developed the SWV method to estimate ESP drug and had never done as like it.

## 2. Materials and methods

### 2.1. Materials

The starting material of ESP was obtained from Qasim Pharmaceutical Company (Spimaco) in Buraydah, KSA, in a powder form. A 50 mL volumetric flask containing a mixture of phosphate buffer (pH 3) and distilled H<sub>2</sub>O (1:10) was used to prepare the ESP stock solution. The buffer solutions (carbonate, phosphate, acetate, and B-R) were prepared using chemicals as described by “Alghamdi et al. (2014)”, for the purpose of determining ESP and monitoring its reduction reactions.

### 2.2. Methods

The voltammetric applications of SWV, CV and DPV used a 797 VA equipment, (Switzerland made) for ESP drug analysis. This apparatus included auxiliary electrode of Pt, reference electrode of Ag / AgCl (KCl), and indicator electrode of GC. The acidity and basicity of ESP solutions were measured and controlled using pH meter of model pH 211. The Labofuge 200 device, manufactured in Germany, was used to centrifuge the biological fluids.

### 2.3. Procedures

Firstly, the electrochemical cell (10 mL capacity) is typically cleaned using ethanol and distilled water before any experiment. The square wave reduction was applied in the potential range from 0.0 to -1.0 Volt. Nitrogen gas was used to purge all buffer solutions to eliminate O<sub>2</sub> gas. To achieve optimal electro-voltammetric signal shape and high current for ESP determination, the B-R pH 6.5 solution,  $t_{acc}$  of 60-second,  $E_{acc}$  of -0.1 V, frequency of 15 Hz, scan rate of 150 mVs<sup>-1</sup>, amplitude voltage of 150 mV, and 1000-rpm were chosen. All voltammetric measurements were carried out at room temperature (25 °C).

#### 2.3.1. Preparation of ESP tablets

Pharmaceutical tablets labeled “Zonam® 3 mg (Eszopiclone)” were obtained from the pharmaceutical plant “SPIMACO” in Al-Qassim, KSA. Ten tablets were crushed and weighed using a sensitive balance to prepare  $1 \times 10^{-3}$  mol L<sup>-1</sup> of ESP tablets in a volumetric flask of 25 mL. Then, the filtrate of solution was used to apply the SWV method for the determination of ESP content by recovery.

#### 2.3.2. Preparation of biological fluids

The half-mL samples of human urine and plasma were mixed with  $5 \times 10^{-6}$  mol L<sup>-1</sup> of ESP. To this mixture, 1.0 mL of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 1.0 mL of CH<sub>3</sub>OH, and 0.1 mL of NaOH were added in a tube of centrifuge (Alghamdi, 2014). After centrifuging the mixture for eight minutes at 5000 rpm, the filtrate was placed in the electrochemical cell. Thereafter, SWV method was applied to estimate ESP content in the biological fluids.

## 3. Results

### 3.1. Electrochemical observations

The DPV and CV techniques were applied to evaluate the voltammetric behavior of  $4 \times 10^{-5}$  mol L<sup>-1</sup> of ESP compound as shown in Figs. 1 and 2, respectively. The resulting voltammetric signals confirmed the suggestion of the electro-voltammetric reduction of the carbonyl

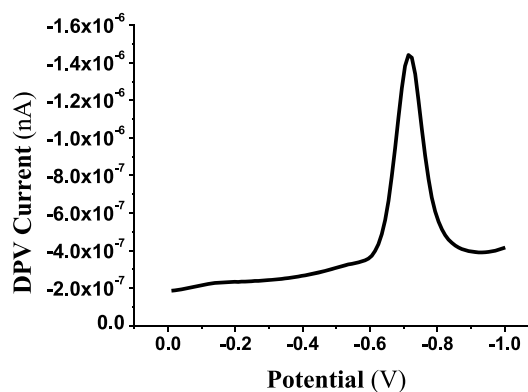


Fig. 1. Differential pulse voltammetric reduction behavior of  $4 \times 10^{-5}$  mol L<sup>-1</sup> of ESP ( $I_c = 1100$ nA).

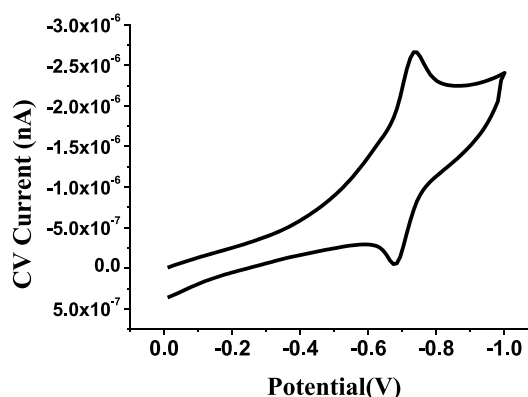


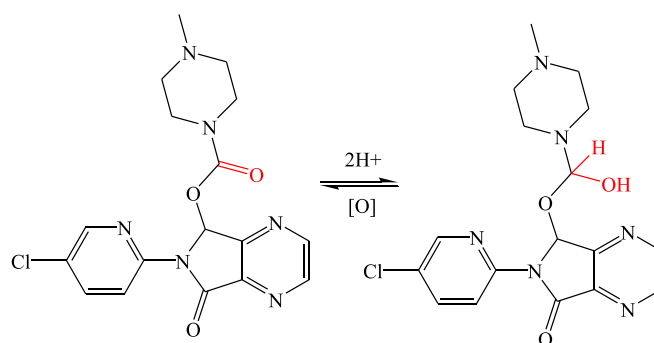
Fig. 2. Cyclic voltammetric reduction behavior of  $4 \times 10^{-5}$  mol L<sup>-1</sup> of ESP ( $I_c = 1040$ nA).

(C=O) to the alcohol (OH—C—H) functional groups (Morrison and Boyd, 1992) as proposed in Scheme 1.

The CV technique was utilized to study the reversibility of the cathodic peak for the determination of ESP within the range of 20–120 mV s<sup>-1</sup> ( $n = 6$ ). The relationship between the logarithm of scan rate ( $\log \nu$ ) and the logarithm of current ( $\log I$ ) was examined, resulting in a slope of 0.85, which approached a value of 1.00. This value (0.85) confirmed the involvement of an adsorption process of ESP onto the surface of the GC electrode (Laviron, 1981).

Furthermore, the multi-cyclic voltammetry method was used to measure  $4 \times 10^{-5}$  mol L<sup>-1</sup> of ESP for five sweep rates. The resulting reduction signals decreased suddenly after the first scan. This CV approach confirmed the saturation of ESP onto the GC surface.

Finally, the SWV technique was applied and recorded a well-defined



Scheme 1. Eszopiclone's chemical structure and the proposed electrochemical mechanism.

reduction peak at  $-750$  mV potential for the electro-determination of  $1 \times 10^{-5}$  mol L $^{-1}$  of ESP as shown in Fig. 3.

### 3.2. Analytical parameters

In order to optimize the experimental conditions and achieve the highest sensitivity for the SWV method, the effect of buffer solutions, pH, scan rate,  $t_{acc}$ ,  $E_{acc}$ , frequency, amplitude voltage, and stirrer rate on the reduction response of  $5 \times 10^{-6}$  mol L $^{-1}$  of ESP was studied.

#### 3.2.1. Effect of buffers and pH

Here, the SWV method was used to determine ESP using various buffers, including acetate at pH 3, carbonate at pH 10, phosphate at pH 3, and B-R at pH 3, 7, and 10 as shown in Fig. 4. The B-R buffer at pH 7 was recorded a highest current and was used for subsequent experiments. Additionally, the pH effect was studied over the range of 5- 8.5 ( $n = 8$ ) using the B-R buffer, as depicted in Fig. 5. A pH value of pH of 6.5 gave a high current, and was therefore chosen for further experiments.

#### 3.2.2. Effect of $t_{acc}$ and $E_{acc}$

The  $t_{acc}$  was investigated over the time range of 0.0 – 105 s. A 60-second of  $t_{acc}$  was given a high cathodic current, therefore the optimal  $t_{acc}$  value (60 s) was chosen for further studies. Additionally,  $E_{acc}$  was assessed over the range of  $-0.8$  to  $+0.8$  V. The highest current was recorded at  $-0.1$  V  $E_{acc}$ , wherefore this value was chosen for subsequent experiments.

#### 3.2.3. Effect of frequency and amplitude

The frequency (Hz) was studied in the range of 5-40 Hz. A frequency of 15 Hz was identified as producing a high sensitivity of reduction signal and was chosen for subsequent work. Additionally, the amplitude voltage was monitored over the range of 10-200 mV. A high reduction current was observed at an amplitude of 150 mV, so this value was selected for further experiments.

#### 3.2.4. Effect of scan rate and stirrer

The scan rate was investigated over the range of 50-300 mVs $^{-1}$ . A scan rate of 150 mVs $^{-1}$  was given a high reduction peak, leading to its selection for further studies. Furthermore, the stirrer rate was examined over the stirrer range of 0.0-3000 rpm. A stirrer operating at 1000 rpm demonstrated the highest SWV current, prompting its choice for continued research.

#### 3.2.5. Effect of rotating indicator electrodes

The gold (Au), silver (Ag), glassy carbon (GC), and (UT) graphite electrodes were used to determine ESP. The gold, and silver electrodes failed to produce a SWV signal at  $-750$  mV. The UT graphite electrode showed an overlapping peak around the reduction potential. The glassy

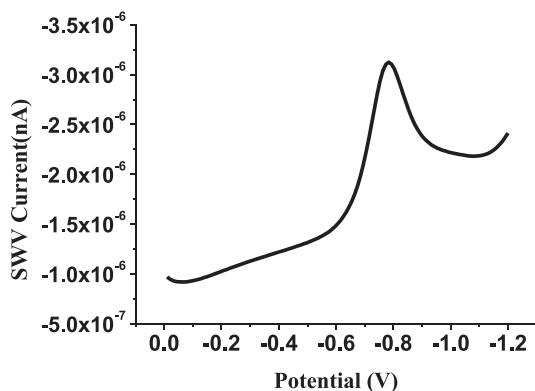


Fig. 3. Reduction of  $1 \times 10^{-5}$  mol L $^{-1}$  of ESP using square wave voltammetry under optimal conditions ( $I = 865$  nA).

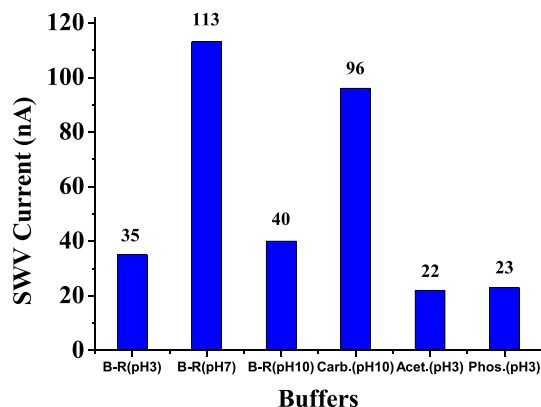


Fig. 4. Effect of buffers on SWV reduction current for  $5 \times 10^{-6}$  mol L $^{-1}$  of ESP.

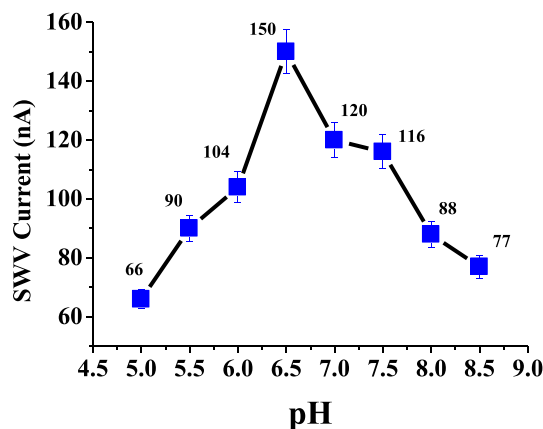


Fig. 5. Effect of pH on SWV reduction current for  $5 \times 10^{-6}$  mol L $^{-1}$  of ESP in B-R buffer.

carbon electrode was the only one that recorded a high current (387 nA) and the best SWV shape. It was selected as the best electrode for determining ESP in pharmaceutical and human fluids.

#### 3.2.6. Effect of some interferences

In general, certain substances such as lactose, sucrose, and starch, were added to the standard drug materials for patients use. These additives could affect the SWV response for ESP determination by competing with the ESP cathodic response on the GC indicator electrode surface. Interferences at folds of 2.0, 5.0, 10.0, and 50 were added to a  $5 \times 10^{-6}$  mol L $^{-1}$  of ESP solution. At high folds (50), lactose and starch had adverse effects of 10 % and 8 % on the cathodic current of ESP. In contrast, sucrose also had negative effects of 11.3 %, 17.6 %, 20 %, and 25 % on the cathodic current of ESP.

### 3.3. SWV validation

According to the criteria set by the United State Food and Drug Administration (FDA) (US-FDA, Guidelines, 2018), the SWV method was validated for calibration curve, LOD, LOQ, repeatability, recovery and stability.

#### 3.3.1. Calibration curve

Under optimal conditions, the correlation between square wave voltammograms and ESP concentration was monitored in the concentration range of  $3 \times 10^{-6}$ – $5 \times 10^{-5}$  mol L $^{-1}$  as shown in Fig. 6. The linearity (calibration curve) of the SWV method was investigated for ten cathodic measurements ( $n = 10$ ), to give analytical results with a correlation coefficient of 0.999 ( $r^2$ ), as listed in Table 1. On the other hand,

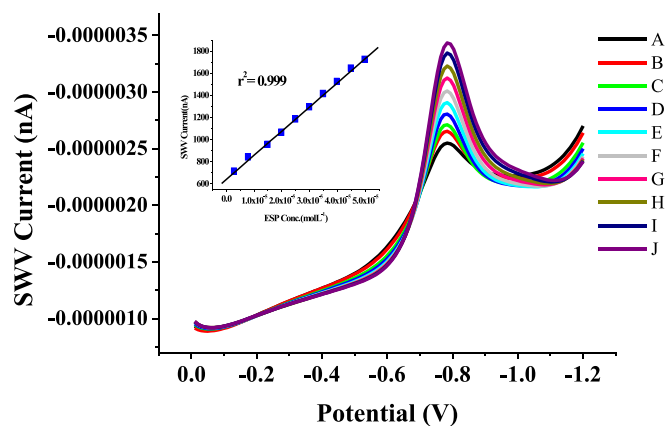


Fig. 6. SW-Voltammograms of ESP using calibration curve under optimal conditions over the range of  $3 \times 10^{-6}$ – $5 \times 10^{-5}$  mol L<sup>-1</sup> [A:  $3 \times 10^{-6}$ , B:  $8 \times 10^{-6}$ , C:  $1.5 \times 10^{-5}$ , D:  $2 \times 10^{-5}$ , E:  $2.5 \times 10^{-5}$ , F:  $3 \times 10^{-5}$ , G:  $3.5 \times 10^{-5}$ , H:  $4 \times 10^{-5}$ , I:  $4.5 \times 10^{-5}$ , J:  $5 \times 10^{-5}$  mol/L].

Table 1

Analytical data for ESP determination by SWV under optimal conditions.

Calibration curve		Parameters	
ESP Concentration (mol L <sup>-1</sup> )	SWV current (nA)		
$3.0 \times 10^{-6}$	709	Range (mol/L)	$3.0 \times 10^{-6}$ – $5.0 \times 10^{-5}$
$8.0 \times 10^{-6}$	837	Correlation coefficient <sup>a</sup>	0.999
$1.5 \times 10^{-5}$	950	Equation	$Y = 2.2 \times 10^7 C + 639.5$
$2.0 \times 10^{-5}$	1060		
$2.5 \times 10^{-5}$	1180	Y	I (nA)
$3.0 \times 10^{-5}$	1290		
$3.5 \times 10^{-5}$	1410	n (number of measurements)	10
$4.0 \times 10^{-5}$	1520		
$4.5 \times 10^{-5}$	1640	Slope	$2.2 \times 10^7$
		Intercept	639.5
		LOD <sup>b</sup>	7.5 ppb
		LOQ <sup>b</sup>	24.93 ppb
$5.0 \times 10^{-5}$	1720		

<sup>a</sup> LOD: "Limit of detection can be calculated at a signal-to-noise ratio of 3".

<sup>b</sup> LOQ: "Limit of quantification can be calculated at a signal-to-noise ratio of 10".

the linearity of UV-Vis spectrophotometry; as a reference method, was investigated during the concentration range ( $2 \times 10^{-5}$ – $1 \times 10^{-4}$  mol L<sup>-1</sup>; n = 5) to give 0.9999  $r^2$  correlation coefficient and linear equation of Abs =  $1.36 \times 10^4 C + 0.065$ .

Table 2

Results of SWV Recovery and SWV-UV Repeatability for ESP under optimal conditions.

ESP concentration (mol L <sup>-1</sup> )		SWV Current (nA)	UV (Abs)	RSD %	Recovery% ( $5.0 \times 10^{-6}$ mol L <sup>-1</sup> )
SWV	UV	785	0.740	SWV 0.141	UV 0.1214
		785	0.741		
$5.0 \times 10^{-6}$	$5.0 \times 10^{-5}$	786	0.742	Mean ± SD 0.7414 ± 0.001	Mean 110
		787	0.742		
		787	0.742		
		788	0.742		
"F-test" variances significance		788	Mean ± SD 0.7414 ± 0.001	SDV ± 1.1	± 1.414
				$F_{\text{calc}}^d = 1.33$	
				$F_{\text{crit}}^e (\alpha^a = 0.05; N^b = 9; n^c = 4) = 6.00$	
				$F_{\text{calc}} < F_{\text{crit}}$	

a: Level of confidence.

b: Number of concentrations.

c: Number of replicates for each concentration

d: F-test calculated value.

e: F-test critical value.

### 3.3.2. Repeatability, stability and recovery

The repeatability, stability, and recovery of ESP of  $5 \times 10^{-6}$  mol L<sup>-1</sup> were investigated under optimal parameters. The cathodic response remained stable through the 90-minute analytical period. Repeatability was assessed through six SWV measurements, to give 0.141 % (RSD%). Recovery was studied through five SWV measurements of the ESP drug, yielding a result of 110 % ± 1.1 SD. The results for repeatability and recovery were summarized in Table 2, for ESP determination using SWV and UV spectrophotometry techniques.

### 3.3.3. LOD and LOQ

The LOD was estimated to be  $1.9 \times 10^{-8}$  mol L<sup>-1</sup> (7.5 ppb), while the LOQ was found to be  $6.41 \times 10^{-8}$  mol/L (24.93 ppb). The developed SWV approach was discovered to be significantly more sensitive than previous analytical techniques for detecting ESP in pharmaceuticals. For example, the PR-HPLC method reported ESP levels of 54 and 132 ppb, respectively, according to the LOD and LOQ (Ravi et al., 2014).

## 4. Discussion

Using the validated SWV method, the presence of the eszopiclone molecule was swimmingly determined in both pharmaceuticals and biological samples.

### 4.1. Application to pharmaceutical tablet

The SWV method was used to determine the content of ESP in commercial tablets "Zonam® 3 mg (Eszopiclone)", with a recovery of  $5 \times 10^6$  mol L<sup>-1</sup> of ESP. The recovery was calculated based on five additions of  $3 \times 10^{-6}$  mol L<sup>-1</sup> of ESP to the medication solution. The average recovery was found to be 105 ± 0.63 % (see Table 3).

Table 3

Validated SWV results for ESP determination in commercial tablets and biological fluids.

	Recovery %		
	Zonam® 3 mg ESP Tablet	Urine	Plasma
Recovered	104	104	99
Conc. of ESP ( $5.0 \times 10^{-6}$ mol/L)	105	105	100
	105	105	101
	105	106	102
	106	106	103
	105	105.2	101
	±0.63	±0.75	±1.414

## 4.2. Application to biological fluids

The SWV method was utilized to estimate the concentration of ESP in urine and plasma samples by recovering  $5 \times 10^{-6}$  mol L<sup>-1</sup> of ESP. The recoveries for the five additions of  $3 \times 10^{-6}$  mol L<sup>-1</sup> of ESP stock solution to the biological samples are summarized in Table 3. The mean recoveries were reported as  $105.2 \pm 0.75$  % for urine sample and  $101 \pm 1.414$  %, for plasma sample.

On the other hand, the results obtained from square wave voltammetry (SWV) were compared to results from spectrophotometric method, which served as the reference method for determining ESP content in pharmaceutical tablets. The comparison showed that there was no considerable variance between all results obtained from UV-Vis spectrophotometry and SWV methods, confirmed the statistical valuation using the “F-test” for all analytical data. The calculated “F-test” value in this study was 1.33, which fell below the crucial value of 6.00 at a 95 % confidence level. Therefore, it can be concluded that there was no noticeable difference in accuracy between the spectrophotometry and SWV methods that were validated.

## 5. Conclusion

This study validated the square wave voltammetry approach for the determination of ESP in pharmaceutical formulations and biological samples using a GC indicator electrode. Under optimal conditions, the SWV method can accurately determine the concentration of ESP drug within a linear scope of  $3 \times 10^{-6}$ – $5 \times 10^{-5}$  mol L<sup>-1</sup>. One advantage of this study is the simple approach used to prepare human biological fluids, resulting in several characteristics that validate the SWV method. The electrochemical results highlighted additional benefits, such as affordability, speed, simplicity, and sensitivity of the SWV method. The analysis of ESP by SWV was found to be accurate and precise, with validation conducted in accordance with FDA requirements. The findings of the LOD and LOQ values further supported the SWV method's sensitivity in determining ESP. A comparison between the SWV and reference UV-Vis spectrophotometry methods was made using “F-test”.

## Disclosure of funding

This research did not receive any funds.

## CRediT authorship contribution statement

**Ali F. Alghamdi:** Validation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

The author would like to express gratitude to Spimaco-KSA, Mr. Abdulwase'a Alshaikh, Miss Rahaf Almatrafy, Mrs. Afnan Almatrafy, and the Qassim Pharmaceutical Plant in Buraydah for their assistance in completing this research.

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