

Electrochemical System for Determination of Esmolol Hydrochloride Using Square Wave Adsorptive Stripping Voltammetry

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A simple, validated, sensitive and reliable electrochemical method for determination of esmolol hydrochloride (ESM) has been described. The cathodic behavior of ESM onto a hanging mercury drop electrode (HMDE) was evaluated using phosphate buffer of pH 7.2. The square wave adsorptive stripping voltammetric (SW-AdSV) method exhibited a peak current over a concentration range of 1.0×10^{-8} - 1.0×10^{-4} mol L⁻¹ ($r = 0.9992$). The low limits of detection and quantification were evaluated as 5.0×10^{-9} mol L⁻¹ and 1.0×10^{-8} mol L⁻¹, respectively. Method applications were concerned with the detection of ESM in its injections and biological samples. The recoded results were calculated in the term of percentage recoveries and they were 99.8 ± 0.2 for ESM injection, while for spiked serum and urine was found to be 98.8 ± 0.7 and 98.9 ± 1.1 , respectively. The introduced method was validated and the data were assessed using t-student's test and F-test. Compatible results were recorded with those obtained from other published methods.

Keywords: Esmolol hydrochloride; Voltammetric determination; Pharmaceutical preparations; Biological fluids; Adsorptive stripping voltammetry

1. INTRODUCTION

Esmolol hydrochloride (ESM) is a cardio selective beta-adrenergic blocker, used in the treatment of acute supraventricular tachycardia [1]. It is chemically known as methyl (RS)-3-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} propanoate. ESM is used during surgery to prevent tachycardia by decreasing the force and rate of heart contractions. Figure 1 illustrates the chemical structure of ESM. Owing to, the overdose of ESM may cause both cardiac and central nervous system side effects such as bradycardia, cardiac arrest, respiratory depression, mood disturbance and coma [2].

Therefore, it is very important to determine ESM levels in biological fluids such as human serum and urine as well as in pharmaceutical preparations.

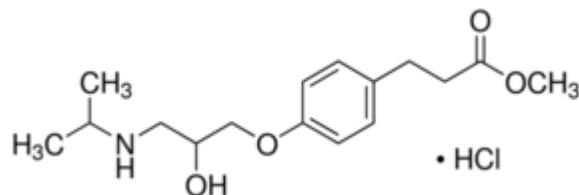


Figure 1. Chemical structure of esmolol hydrochloride

ESM was detected using different analytical methods. Spectroscopic methods such as spectrophotometry were published as clear in references [3-6]. Chromatographic separation and determination of ESM using high performance liquid chromatography was also reported [7-9]. Other chromatographic methods coupled with mass spectrometry were concerned with the detection of ESM such as gas chromatography-mass spectrometry [10] and liquid chromatography-mass spectrometry [11]. While, a unique capillary zone electrophoresis method [12] for detection of ESM was described in literature survey. Other spectroscopic methods were reported for detection of ESM including spectrophotometry [13-17]. Since the chromatographic techniques need a high skill operator's specific pretreatment of samples and expensive instrumentations, square wave- adsorptive stripping voltammetry is considered as a simple, efficient electrochemical method of drug analysis that exhibited an adsorptive properties of the working electrode.

Additionally, the electrochemical method of analysis is very simple, less expensive, less time consuming, and no extensive pretreatment of samples is required. As seen in literature survey, no adsorptive stripping voltammetric detection method of ESM has been reported yet. Therefore, this study aimed to introduce a simple, accurate and validated electrochemical detection of ESM in its bulk powder, pharmaceutical formulations and spiked biological samples.

2. EXPERIMENTAL

2.1. Chemicals and reagents

The selected drug ESM in pure grad was purchased from Sigma Aldrich, (Hamburg, Germany). The pharmaceutical preparation in the form of injection Brevibloc[®] 10 mg mL⁻¹ was purchased from local drug stores. Boric acid 99.9 %, sodium acetate > 99.0 %, potassium mono-hydrogen phosphate > 99.0 %, potassium di-hydrogen phosphate > 99.0 % and sodium bicarbonate 99.5 % were purchased from (Winlab, Market Harborough, UK), while, (BDH, London, UK) provided all the following chemicals such as glacial acetic acid 99.7 %, phosphoric acid > 85 % and sodium carbonate > 99.0 %. The employed urine samples were provided by healthy volunteers and (Multi-Serum Normal, Randox Laboratories, Crumlin, Antrim, UK) is a commercial source of human serum samples.

2.2. Apparatus

All electrochemical behavior of ESM was investigated using 797 VA Computrace connected with 843 pump station (Metrohm, Switzerland). The device was in conjunction with Dell PC and hp LaserJet Cp1525 color printer. The system was controlled with VA Computrace software version 2.0). The pH adjustment was performed using HANNA 211- pH meter (Made in Romania).

2.3. Method of analysis

2.3.1. Analysis of standard drug solution of ESM

The analysis of the standard drug solution of ESM by the selected adsorptive stripping voltammetric technique was carried out at room temperature by adding 25 mL of phosphate buffer at pH 7.2 as supporting electrolyte in a clean and dry voltammetric cell followed by adding accurate amounts of drug test solutions. Firstly, nitrogen gas was used to purge the solutions with continuous stirring for 5 min. While, the drug test solution was stirred for about 25 s, the accumulation potential of -0.6 V vs. silver-silver chloride electrode was applied. Then the stripping was stopped after about 20 s and the cathodic scan was performed over the range of 0.0 to -2.0 V.

2.3.2. Analysis of ESM injection solution

The content of three ESM vials Brevibloc[®] each 10 mL vial contains 100 mg ESM were transferred into a 100-mL volumetric flask and diluted with the supporting electrolyte to obtain 9.0×10^{-3} mol L⁻¹ of ESM solution. The desired testing solutions were prepared in the range of 1.0×10^{-8} - 1.0×10^{-4} mol L⁻¹ by serial dilutions using phosphate buffer. Then SW-AdSV was applied for ESM detection.

2.3.3. Analysis of ESM in biological fluids

The spiking technique was performed for detection of ESM in biological samples. About 500 μ L of serum or urine was spiked with different aliquots of the investigated drug in centrifuged tubes and vortex for 10 min. A simple and rapid elimination of most possible interfering species was made using precipitation process as previously reported [18]. After centrifugation at 2500 rpm for 30 min, the clear solution was filtered using 0.2 μ m membrane filter. 0.2 mL of the solution was transferred to a 20-mL volumetric flask and diluted with phosphate buffer as supporting electrolyte at pH 7.2. The pH of urine samples were adjusted using phosphate buffer to pH 7.2 and directly subjected to electrochemical analysis. ESM was then determined using SW-AdSV method.

3. RESULTS AND DISCUSSION

3.1. The cathodic behavior of ESM

The cathodic behavior of $5.0 \times 10^{-5} \text{ mol L}^{-1}$ of ESM was monitored by cyclic voltammetry in the presence of a supporting electrolyte phosphate buffer of pH 7.2 at the HMDE. A sharp and single cathodic peak was recorded at potential -1.43 V which can be assigned to the reduction of the carbonyl group in the compound. Owing to no anodic peak was achieved; we can attribute that to the irreversible nature of the electrode (Figure 2).

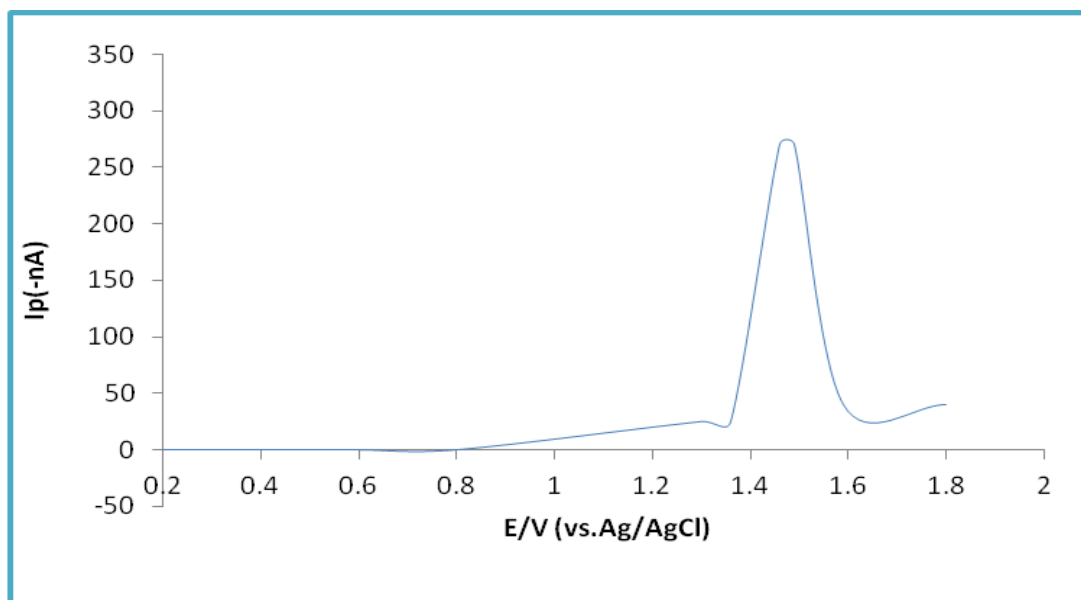


Figure 2. Cyclic voltammograms of $5.0 \times 10^{-5} \text{ mol L}^{-1}$ esmolol hydrochloride: phosphate buffer pH 7.2, the recorded potential -1.43 V

3.2. Optimization of electrochemical conditions

3.2.1. Effect of pH

As we know, the kind of supporting electrolyte and its pH were greatly affecting the SW-AdSV method. Therefore, to evaluate the sensitivity of adsorptive stripping procedure for ESM detection, several kinds of supporting electrolyte solutions with different pH values were examined using cyclic voltammetry after $t_{\text{acc}} = 45 \text{ s}$ and at 0.0 V accumulation potential. These include 0.1 mol L^{-1} of each of Britton-Robinson (pH = 2-10), acetate buffer (pH = 4-6), phosphate buffer (pH = 5.8-8), and sodium citrate buffer (pH = 1-5). Among these supporting electrolyte solutions, it was noticed that the best reduction response as a higher peak current signal was observed by using phosphate buffer of pH 7.2, which was chosen for further studies.

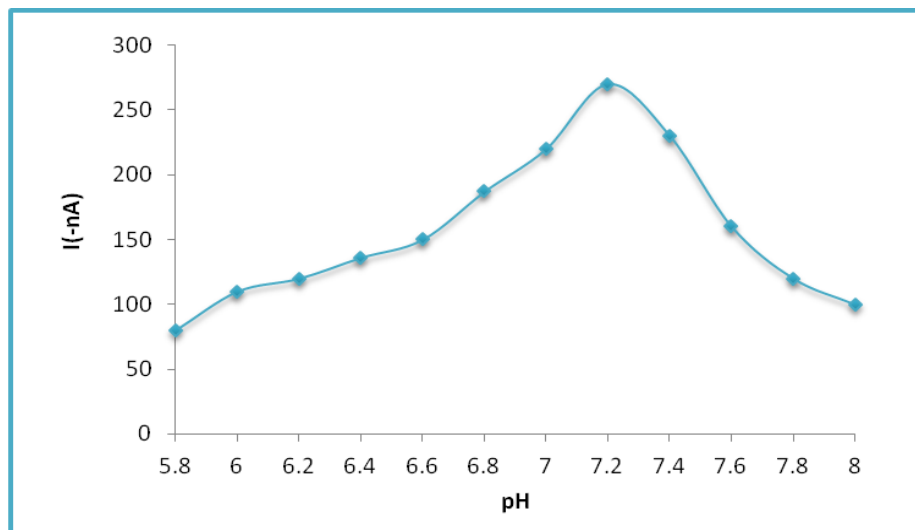


Figure 3. Effect of pH on square wave-adsorptive stripping voltammetry peak current: Esmolol hydrochloride sample $5.0 \times 10^{-5} \text{ mol L}^{-1}$, phosphate buffer of pH 5.8-8 after accumulation period 45 s at accumulation potential = -0.6 V

Figure 3, demonstrated the stripping voltammetric peak current signal as a function of pH over a range of 6-8. At the beginning a gradual increase in the peak current was observed and then achieved a maximum value at pH 7.2. While, by changing pH values the voltammetric peak potential is not influenced which revealed that E_p was pH independent.

3.2.2. Effect of accumulation time and accumulation potential

Since the interfacial accumulation of the drug onto the surface of the working electrode is considered as one of the critical parameters which should be optimized, the accumulation time of ESM $5.0 \times 10^{-5} \text{ mol L}^{-1}$ was investigated over the range of 0-160 s.

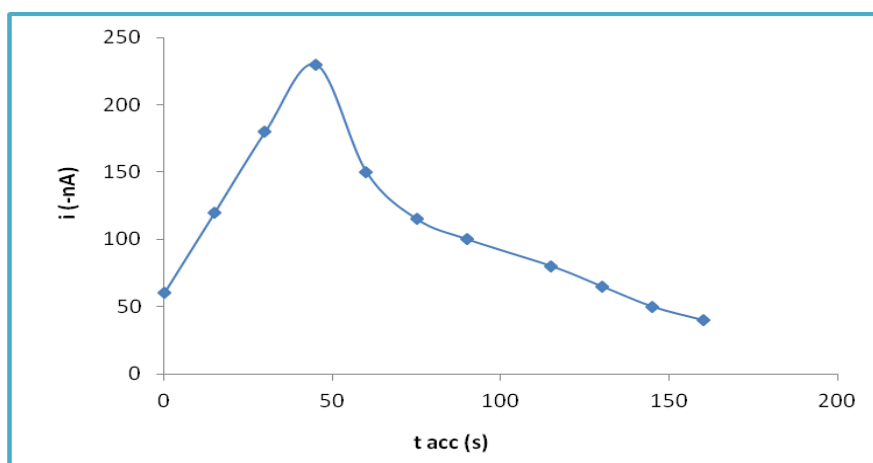


Figure 4. Effect of accumulation time on the stripping voltammetric peak current of $5.0 \times 10^{-5} \text{ mol L}^{-1}$ esmolol hydrochloride in the presence of phosphate buffer of pH 7.2 and accumulation potential -0.6 V

As depicted in Figure 4, the obtained peak current – accumulation time (i_p-t_{acc}) was increased gradually over the range of 0-45, and then peak current was decreased due to the saturation of the HMDE. So that 45 s is considered as the accumulation time. Furthermore, the preconcentration potential of ESM was examined over the accumulation potential range of 0.2 to -1.6 V at 45 s accumulation time and it was indicated that -0.6 V was selected as optimum accumulation potential.

3.2.3. The selection of scan rate

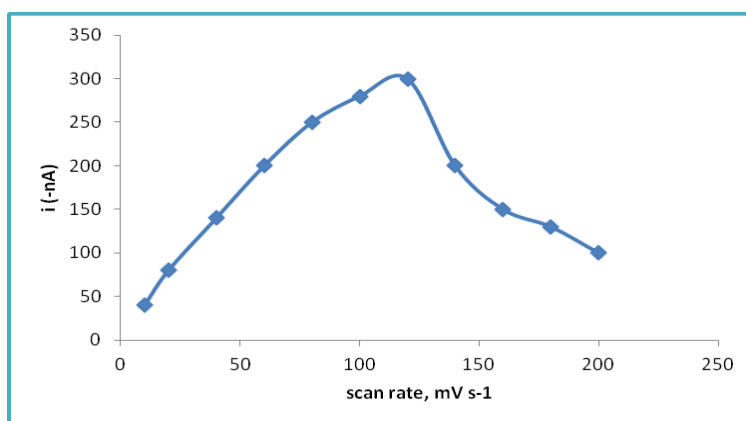


Figure 5. Effect of scan rate on the stripping voltammetric peak current of 5.0×10^{-5} mol L⁻¹ esmolol hydrochloride in the presence of phosphate buffer of pH 7.2 and accumulation time 45 s and accumulation potential -0.6 V

The cathodic SW-AdSV for the estimation of ESM was studied using the scan rate in the range of 10- 200 mV s⁻¹. We can observe that the peak intensity was directly proportional to the scan rate over the range of 20-120 mV s⁻¹. Hence, the value 120 mV s⁻¹ was taken for further experiments (Figure 5).

3.2.4. Effect of pulse amplitude

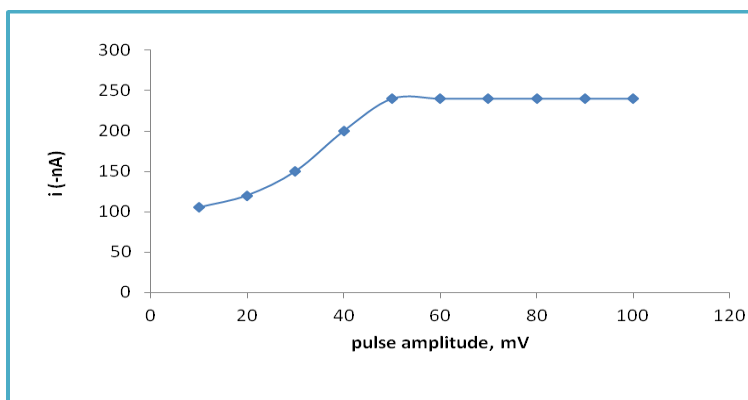


Figure 6. Effect of pulse amplitude on the stripping voltammetric peak current of 5.0×10^{-5} mol L⁻¹ esmolol hydrochloride in phosphate buffer of pH 7.2, accumulation time 45 s, accumulation potential -0.6 V and scan rate 120 mV s⁻¹

The volumetric current intensity was influenced by the pulse amplitude and it was tested over the range of 10-100 mV. As depicted in Figure 6, using 50 mV pulse amplitude gave maximum peak current and was selected for further studies. While, evaluating the maximum SW-AdSV signal, the voltammetric peak current signal was tested over a range of 10-60 Hz and it was clear that 30 Hz is the ideal value for this parameter (Figure 7).

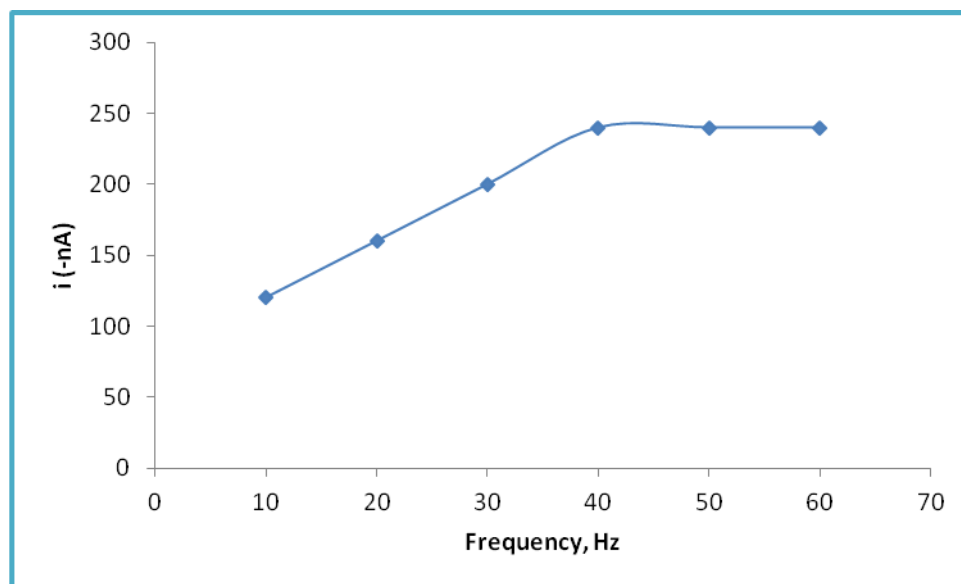


Figure 7. Effect of frequency on the stripping voltammetric peak current of 5.0×10^{-5} mol L⁻¹ esmolol hydrochloride in phosphate buffer 7.2, accumulation time 45 s, accumulation potential -0.6 V, scan rate 120 mV s⁻¹ and pulse amplitude 50 mV

3.2.5. Effect of other operating parameters

The size of the adsorption area of hanging mercury drop electrode and the efficiency of the adsorption of ESM were tested. A linear peak current intensity was obtained over the range of 0.2-0.8 mm² drop size area. The efficiency of the adsorption stripping rate was over the range of 0-2500 rpm. Therefore, the optimal value of size adsorption area 0.8 and 2500 rpm as stripping rate speed were chosen.

3.3. Method validation

The SW-AdSV method was validated to be suitable for detection of ESM using the ICH guidelines [19]. Method validation was performed using different parameters which addressed below.

3.3.1. Linearity

Under optimal conditions, it was clarified that linear relationship was obtained over a concentration range of 5.0×10^{-8} - 1.0×10^{-4} mol L⁻¹ (Figure 8). Least square method was applied.

$$I_p = 75.172 C - 244.1 \quad (n = 6) \quad r = 0.9992$$

For our information, the cathodic peak current was evaluated in nano amperes (I_p), the concentration of ESM test solution C is expressed by (mol L^{-1}) of ESM and r represented the correlation coefficient.

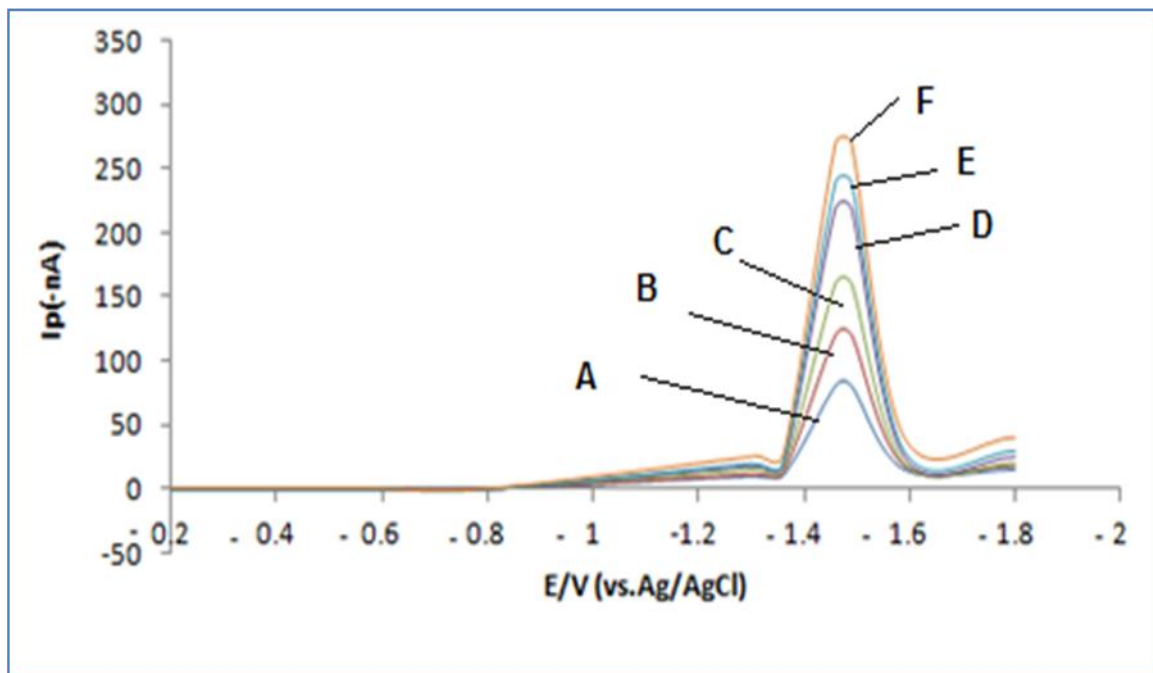


Figure 8. Square-wave adsorptive stripping voltammograms of esmolol hydrochloride in phosphate buffer 7.2, accumulation time 45 s, accumulation potential -0.6 V, drug concentrations A: 5.0×10^{-8} , B: 1.0×10^{-7} , C: 1.0×10^{-6} , D: 5.0×10^{-6} , E: 1.0×10^{-5} and F: 1.0×10^{-4} mol L^{-1}

3.3.2. Limits of detection and quantification

The lower limits of detection LOD and quantifications LOQ were found to be 1.4×10^{-8} mol L^{-1} and 5.0×10^{-8} mol L^{-1} , respectively. These values were calculated as standard deviation divided by slope and multiplying by 3.3 and 10 for LOD and LOQ, as displayed in the following equations: $3.3 S_a/b$ and $10 S_a/b$, respectively.

3.3.3. Accuracy and precision

The accuracy and precision of the proposed method were evaluated by testing three different concentrations of ESM three times in five successive days. The obtained data were calculated as % RSD as presented in Table 1.

It was clear that the % RSD were 0.2% and 0.6% for inter-day and intra-day, respectively, indicating high precision.

Table 1. Accuracy and precision data of the determination of ESM using SW-AdSV method in terms of inter-day and intra-day assay (n =3)

	Taken Conc. mol L⁻¹	Found Conc. mol L⁻¹	% Recovery
Inter-day assay	1.0×10^{-8}	1.02×10^{-8}	99.9
	1.0×10^{-6}	1.05×10^{-6}	99.7
	1.0×10^{-4}	1.00×10^{-4}	100.0
Mean ± SD	99.9±0.2		
n	3		
*%SE	0.11		
%RSD	0.20		
Intra-day assay	1.0×10^{-8}	1.00×10^{-8}	100.0
	1.0×10^{-6}	1.09×10^{-6}	99.3
	1.0×10^{-4}	1.12×10^{-4}	98.8
Mean ± SD	99.4±0.6		
n	3		
*%SE	0.35		
%RSD	0.60		

$$* \%SE = SD/\sqrt{n}$$

3.3.4. Robustness

The robustness of the developed SW-AdSV method for detection of ESM was tested by carrying small changes in method parameters such as pH, applied potential, scan rate and accumulation time. It was found that the method is robust at pH 7.2.

3.3.5. Ruggedness

To study the ruggedness of the described method, the analysis of ESM was calculated three times for three successive days using different operator and different device. The %RSD was evaluated and it was found to be 1.2 % revealing that the proposed method can be repeated for detection of the investigated drug with high precision and hence the method is considered as rugged.

3.3.6. Selectivity and recovery studies

The selectivity of the proposed method for detection of ESM was checked in the presence of any interfering substances which may be co-formulated substances such as glacial acetic acid, sodium acetate tri-hydrate, sodium chloride, using the standard addition method. Furthermore, the proposed method of analysis was applied for detection of ESM in the presence of some common ions such as Na⁺, K⁺, Ca²⁺ and Mg²⁺ and amino acids such as histidine, glycine and tryptophan. The summarized

results in Table 2, clarified that the developed electrochemical method can be used for detection of ESM in the presence of such interfering species without any interfering effect.

Table 2. Standard addition method for detection of ESM in the presence of interfering substances using SW-AdSV method

ESM Solution	Standard addition method			
	Added* Conc. mol L ⁻¹	Found Conc. mol L ⁻¹	% Recovery	%RSD
	1.0 × 10 ⁻⁸	1.02 × 10 ⁻⁸	99.9	0.5
2.5 × 10 ⁻⁸	2.57 × 10 ⁻⁸	99.8	0.9	
5.0 × 10 ⁻⁸	5.13 × 10 ⁻⁸	99.9	1.1	
1.0 × 10 ⁻⁷	1.05 × 10 ⁻⁷	99.4	1.5	
2.5 × 10 ⁻⁷	2.63 × 10 ⁻⁷	99.6	0.8	
5.0 × 10 ⁻⁷	5.62 × 10 ⁻⁷	99.2	0.2	
1.0 × 10 ⁻⁶	1.00 × 10 ⁻⁶	100.0	1.3	
2.5 × 10 ⁻⁶	2.69 × 10 ⁻⁶	99.3	0.7	
5.0 × 10 ⁻⁶	5.49 × 10 ⁻⁶	99.1	0.4	
1.0 × 10 ⁻⁵	1.02 × 10 ⁻⁵	99.8	1.4	

*ESM drug solution in the presence of interfering substances

3.4. Analytical applications

Table 3. Analytical results of the determination of esmolol hydrochloride in its bulk powder and Brevibloc[®] injection using SW-AdSV method

SW-AdSV method			Reference Method [4]	SW-AdSV method			Reference Method [4]
Pure Drug				Brevibloc [®] injection 100 mg/vial			
Taken Conc. mol L ⁻¹	Found Conc. mol L ⁻¹	% Recovery		Taken Conc. mol L ⁻¹	Found Conc. mol L ⁻¹	% Recovery	
1.0 × 10 ⁻⁸	1.05 × 10 ⁻⁸	99.8	99.2±0.6 6 0.36 0.24	1.0 × 10 ⁻⁸	1.02 × 10 ⁻⁸	99.9	99.5±0.3 6 0.09 0.12
1.0 × 10 ⁻⁷	1.09 × 10 ⁻⁷	99.4		1.0 × 10 ⁻⁷	1.00 × 10 ⁻⁷	100.0	
5.0 × 10 ⁻⁷	5.37 × 10 ⁻⁷	99.5		5.0 × 10 ⁻⁷	5.13 × 10 ⁻⁷	99.8	
1.0 × 10 ⁻⁶	1.02 × 10 ⁻⁶	99.8		1.0 × 10 ⁻⁶	1.05 × 10 ⁻⁶	99.7	
1.0 × 10 ⁻⁵	1.00 × 10 ⁻⁵	100.0		1.0 × 10 ⁻⁵	1.07 × 10 ⁻⁵	99.4	
1.0 × 10 ⁻⁴	1.12 × 10 ⁻⁴	98.8		1.0 × 10 ⁻⁴	1.00 × 10 ⁻⁴	100.0	
Mean ± SD	99.6±0.4			99.8±0.2			
n	6		6				
Variance	0.16		0.04				
%SE*	0.16		0.08				
t-test	1.386(2.228)**		2.080(2.228)**				
F-test	2.25(5.05)**		2.25(5.05)**				

* %SE = SD/√n

**Figures in parentheses are the tabulated values of t- and F- testes at 95% confidence limit [20]

Using the described SW-AdSV electrochemical method which proposed above, the drug of interest ESM was evaluated firstly in its bulk material. The obtained % recovery was 99.6±0.4.

Furthermore, the selected drug was investigated in its dosage form such as Brevibloc[®] injection, each vial labeled to contain 100 mg mL⁻¹. The obtained results gave percentage recovery with a standard deviation of 99.8±0.2 as shown in Table 3.

Table 4. Analytical results of the determination of esmolol hydrochloride in biological samples such as human serum and urine using SW-AdSV method

SW-AdSV method			SW-AdSV method		
Serum			Urine		
Taken Conc. mol L ⁻¹	Found Conc. mol L ⁻¹	% Recovery	Taken Conc. mol L ⁻¹	Found Conc. mol L ⁻¹	% Recovery
1.0 × 10 ⁻⁸	1.29 × 10 ⁻⁸	98.6	1.0 × 10 ⁻⁸	1.00 × 10 ⁻⁸	100.0
1.0 × 10 ⁻⁷	1.09 × 10 ⁻⁷	99.4	1.0 × 10 ⁻⁷	1.12 × 10 ⁻⁷	99.3
5.0 × 10 ⁻⁷	5.75 × 10 ⁻⁷	99.0	5.0 × 10 ⁻⁷	5.49 × 10 ⁻⁷	99.4
1.0 × 10 ⁻⁶	1.09 × 10 ⁻⁶	99.3	1.0 × 10 ⁻⁶	1.32 × 10 ⁻⁶	98.0
1.0 × 10 ⁻⁵	1.34 × 10 ⁻⁵	97.4	1.0 × 10 ⁻⁵	1.02 × 10 ⁻⁵	99.8
1.0 × 10 ⁻⁴	1.12 × 10 ⁻⁴	98.8	1.0 × 10 ⁻⁴	1.29 × 10 ⁻⁴	97.3
Mean ± SD	98.8±0.7		98.9±1.1		
n	6		6		
Variance	0.49		1.21		
%SE*	0.29		0.44		

$$* \%SE = SD/\sqrt{n}$$

The obtained data were assessed statistically using t-students test and variance F-test [20]. The achieved results were in good agreement with those obtained from a previously published method [4] which is based on a spectrophotometric detection of ESM using Ce (IV) as an oxidizing agent in the presence of amaranth dye, the detection carried out at 523 nm. In addition, the SW-AdSV method was applied for detection of ESM in bio-samples such as serum and urine. The spiking technique was used and the results were reported in Table 4.

4. CONCLUSION

The cathodic behavior of ESM onto HMDE was studied in this work. The SW-AdSV method was employed for estimation of ESM in its pure material, injections and bio-samples such as serum and urine. The high sensitivity and selectivity of the proposed method was proved by assessing the obtained results statistically and compared them with other results obtained from a previously published method. A linear relationship was plotted between ESM concentrations and the current response, which demonstrated excellent features, such as low detection and quantification limits, high repeatability and reproducibility. Additionally, the SW-AdSV method is very fast, inexpensive with no need for sample pretreatment. The proposed method was fully validated with respect to ICH

guidelines and excellent data were recorded indicating the suitability of using the electrochemical SW-AdSV for estimation of ESM in different matrices.

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