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Square-wave voltammetric determination of drospirenone and ethinylestradiol in pharmaceutical dosage form using square wave technique

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RESEARCH ARTICLE

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ABSTRACT

Cathodic voltammetric behaviors of drospirenone and ethinylestradiol were used for the simultaneous determination of both drugs in bulk and in pharmaceutical formulation (Yasmin® tablets) without the interference of excipients. The determinations were made on hanging mercury dropping electrode using square-wave technique in a voltammetric cell containing 10 mL of 0.04 mole/L Britton-Robinson. After every aliquot addition, the solution was stirred for 10 s at 1000 rpm, rested for 10 s then square wave voltammetry mode was ramped from +100 to -1700 mV with scan rate of 100 mV/s, pulse amplitude of 50 mV and measurement time of 5 ms. Several factors such as pH, type of supporting electrolyte, pulse amplitude and scan rate were studied to optimize the condition for voltammetric determination of these drugs. With optimized experimental parameters, a good linearity was obtained for both drugs over a range of 1.36×10^{-6} to 1.91×10^{-7} mole/L and 6.75×10^{-8} to 6.07×10^{-7} mol/L of drospirenone and ethinylestradiol, respectively. Characterization of the proposed method was done according to International Conference on Harmonization, Q2B: Validation of Analytical procedures. The proposed method was statistically compared with the reference method and the results revealed no significant difference regarding accuracy and precision.

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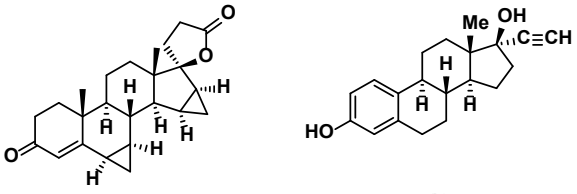
1. Introduction

Drospirenone (Drosp); (17-hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone) (Figure 1) is a progestin medication which can be used in contraceptive oral pills to prevent pregnancy and in menopausal hormone therapy [1]. It binds strongly to the progesterone receptor (PR) and mineralocorticoid receptor (MR), with lower affinity, to the androgen receptor (AR), and very low affinity for the glucocorticoid receptor (GR). It was regarded that Drosp has a pharmacological profile that is very closely related to that of natural progesterone, due to the combination of both progestogenic and anti-mineralocorticoid actions [2,3].

Ethinylestradiol (EE); 17α-ethynylestradiol; 17α-ethynylestra-1,3,5(10)-triene-3,17β-diol (Figure 1) is an orally bioactive estrogen that usually present in many combined formulations of oral contraceptive pills. EE was formerly used for hormone replacement therapy at menopause and for treatment of prostate cancer, and breast cancer. It is mainly utilized in hormone therapies for androgen dependent disorders, acne, hirsutism and seborrhea [4].



(a) (b)

Figure 1. Molecular structures of (a) drospirenone and (b) ethinylestradiol.

Many analytical methods were developed for the determination of Drosp in human plasma or in dosage form

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including the determination of Drosp in human plasma utilizing LC-MS/MS [5,6]. Another RP-HPLC method was developed for the concurrent analysis of 17-beta-estradiol and Drosp in combined dosage form [7]. Moreover, numerous analytical methods were developed for the determination of EE alone in dosage form or in human plasma including spectrophotometric methods [8-15], HPTLC methods [16-18], liquid chromatographic methods [14,19-33], micellar electrokinetic capillary chromatography (MEKC) [34,35], spectrofluorometric methods [36,37] and voltammetric techniques [38,39]. Moreover, few analytical techniques were developed for the simultaneous estimation of the studied drugs in tablet formulations including an HPTLC method [40] and chromatographic methods including an official one in United States Pharmacopoeia (USP) [41-46].

Hitherto, there is no reported method concerning the analysis of this combination by voltammetric technique. So the aim of this work is to develop a sensitive electrochemical method for the simultaneous determination of the two drugs in bulk and in pharmaceutical formulation (Yasmin®tablets, which is labeled to contain 3.00 mg drospirenone and 0.03 mg ethinylestradiol, is used as contraceptive) through electrochemical reduction on a hanging mercury dropping electrode using square wave voltammetry (SWV) technique through several factors. Additionally, the developed method was aimed to be simple without the necessity for sample pre-treatment and/or time-consuming extraction or evaporation steps prior to the analysis.

2. Experimental

2.1. Materials and reagents

2.1.1. Pure samples

Drosp and EE were kindly supplied by NODCAR (El-Haram, Giza, Egypt). Their purities were found to be 99.22±0.931 and 99.89±0.621% for Drosp and EE, respectively, by the official methods [46].

2.1.2. Pharmaceutical dosage form

Yasmin® tablets with batch number 480L, provided by (Bayer PharmaAG, Germany) which is labeled to contain 3.00 mg and 0.03 mg of drospirenone and ethinylestradiol, respectively.

2.1.3. Reagents

All chemicals (NaOH, KCl and $LiCl_3$) were of analytical grade and were purchased from Adwia Pharmaceuticals. Methanol was purchased from Sigma Aldrich (Germany). Double distilled water was used in all work. 0.04 M Britton-Robinson (BR) buffer was prepared by mixing 10 mL volumes of 0.4 mol/L of boric acid, acetic acid and phosphoric acid in a beaker, adjusting the mixture to the desired pH = 5.0-11.0 by drop wise addition of 1.0 mol/L sodium hydroxide solution, then transferring the mixture into a 100 mL volumetric flask and completing to volume with distilled water.

2.1.4. Standard solutions

2.1.4.1. Stock standard solutions

Stock solutions of 3.37×10^{-3} mol/L were prepared by separately dissolving appropriate amounts of Drosp and EE in methanol.

2.1.4.2. Working solutions

Aliquots were accurately transferred from each stock standard solution to 100 mL volumetric flasks and the volumes were completed to the mark with methanol to obtain solutions with the concentration of 3.37×10^{-5} mol/L for Drosp and EE.

2.2. Apparatus

All the measurements were done using Metrohm GA (884 Professional VA) which is equipped with three electrodes. The three electrodes were a reference electrode of Ag/AgCl (3.0 mol/L KCl), a platinum counter electrode and a hanging mercury dropping electrode (HMDE) representing the working electrode. The pH values of solutions were measured using Jenway 3510 meter.

2.3. Procedures

2.3.1. Construction of calibration curve of Drosp and EE

Square-wave voltammetry (SWV) was employed for the determination of both Drosp and EE in bulk powder. Aliquots of Drosp and EE working solutions and working solution of EE solution were transferred into a voltammetric cell containing 10 mL of 0.04 mole/L BR to give the concentration range of 1.36×10^{-6} to 1.91×10^{-7} mole/L and 6.75×10^{-8} to 6.07×10^{-7} mol/L of Drosp and EE, respectively. After every aliquot addition, the solution was stirred for 10 s at 1000 rpm, rested for 10 s then SWV mode was ramped from +100 to -1700 mV with scan rate of 100 mV/s, pulse amplitude of 50 mV and measurement time of 5 ms. The experiment was carried in triplicate for every standard solution addition. The cathodic peak current was plotted versus final concentration to get the calibration curve then the corresponding regression equations were derived.

2.3.2. Application to pharmaceutical formulation

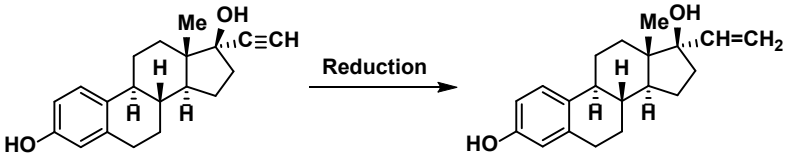
Twenty tablets were triturated and mixed well. An accurate amount of the powder equivalent to the weight of ten tablets was accurately weighed, transferred into a 100 mL volumetric flask and mixed with 50 mL methanol. The solution was sonicated for about 30 min then completed to the final volume with the same solvent and mixed well. The obtained solution was then filtered to prepare a stock solution of 0.8185 mole/L Drosp and 0.0101 mole/L for EE. Then further dilution from this stock was carried out to obtain the working solutions with concentrations of 8.19×10^{-4} and 1.01×10^{-5} mole/L for Drosp and EE, respectively. Then, an aliquot of the clear solution was analyzed according to the proposed voltammetric procedure.

2.3.3. Application of SWV method to the analysis of Drosp and EE (In-vitro dissolution profile)

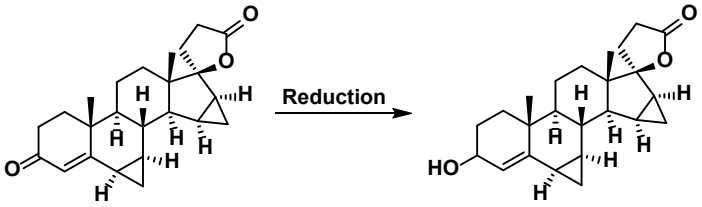
One tablet (Yasmin® tablets) was placed in a vessel of dissolution tester (Pharma Test DT70) containing 900 mL water using Apparatus II (Paddle), with a rotating speed of 50 rpm. Samples were withdrawn after 10, 20 and 30 min and filtered then samples were measured by applying the proposed voltammetric method. The previous method was applied on six different tablets.

3. Results and discussion

EE molecule has acetylenic (alkyne) electroactive group. To our knowledge, a square-wave voltammetric procedure was reported for the determination of EE by accumulation onto a hanging mercury drop electrode in a Britton-Robinson universal buffer of pH = 7.0 [39]. However, there is no reported method used for the determination of Drosp by voltammetric technique.



Scheme 1. The proposed scheme for reduction mechanism of EE.



Scheme 2. The proposed scheme for reduction mechanism of Drosp.

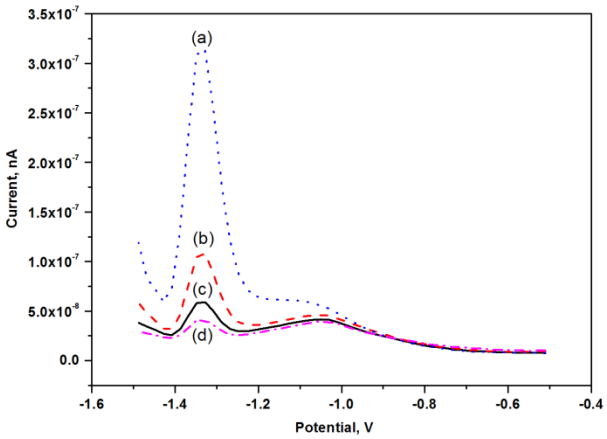


Figure 2. Effect of (a) BR pH = 8.0, (b) 0.1 M phosphate, (c) basic 0.1 M borate buffer and (d) 0.1 M NaOH on differential pulse voltammetric peak potentials for 4 µg/mL Drosp at scan rate 60 mV/s, pulse amplitude 50 mV and stirring 30s.

In our work, HDME was used in a Britton-Robinson universal buffer of pH = 8.0 and was applied successfully for simultaneous determination of Drosp and EE in bulk and pharmaceutical dosage form samples in nanogram concentration as described below. By considering the molecular structure of both Drosp and EE, we suppose cathodic voltammetric behavior for EE which may be due to the reduction of the acetylinic group of EE and cathodic voltammetric behavior for Drosp which may be due to the reduction of carbonyl group of Drosp as illustrated in Schemes 1 and 2.

3.1. Optimization of experimental conditions

Different chemical and electrochemical parameters were investigated thoroughly to study the electrochemical behaviors of Drosp and EE.

3.1.1. Effect of supporting electrolyte and ionic strength

The electrochemical behaviors of Drosp and EE on the HDME were carefully studied in different types of supporting electrolytes like acidic 0.1 mol/L phosphate, basic 0.1 mol/L borate buffer, 0.1 mol/L NaOH and 0.04 M BR buffer. The best voltammetric signals in terms of sensitivity (peak height) and resolution (peak shape) were secured using BR buffer as demonstrated in Figures 2 and 3. Also, the effect of presence of salts like KCl, NaCl and $LiCl_3$ on the peak potential in combination with universal buffer was studied which revealed that the peaks of the studied drugs became very bad and broad so no salt was added to the supporting electrolyte.

3.1.2. Effect of pH

The electrochemical behaviors of the studied drugs were studied over the pH range of 5.0 to 11.0 using differential pulse (DP) sweep and scan rate 0.06 V/s as shown in Figure 4. The stripping voltammetric signal increased steadily over the neutral region and the peak current reached its maximum value at pH = 8.0 which was selected as optimal value for subsequent studies. It is worthy to note that using alkaline BR as a supporting electrolyte resulted in decreasing the currents of the studied drugs and nearly no stripping voltammetric signal was observed especially in EE. The plot of the peak potential versus pH showed one straight line between 5.0 and 11.0, which can be expressed by the following Equations in Britton-Robinson buffer (Figures 5 and 6).

$$Ep = 777.71 - 58.71\ pH \quad r^2 = 0.9993 \quad \text{for Drosp} \qquad (1)$$

$$Ep = 390.86 - 59.34\ pH \quad r^2 = 0.9920 \quad \text{for EE} \qquad (2)$$

The slope is close to the Nernst theoretical value of 59 mV/pH [47] and according to the Equation (3),

$$Ep = E^\circ - \frac{RT}{nF}\ln\frac{[Ox]}{[Red]} \pm \frac{2.303\ \partial RT}{nF}pH \qquad (3)$$

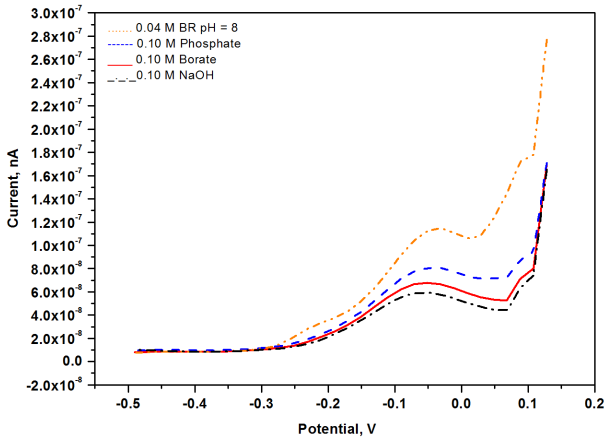


Figure 3. Effect of 0.1 M phosphate, basic 0.1 M borate buffer, 0.1 M NaOH and BR pH = 8.0 on differential pulse voltammetric peak potentials for 80 ng/mL EE at scan rate 60 mV/s, pulse amplitude 50 mV and stirring 30 s.

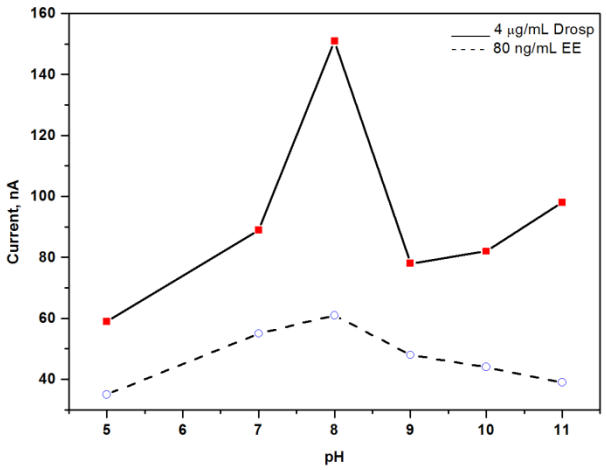


Figure 4. Plotting of pH versus current of 4 µg/mL Drosp solution and 80 ng/mL EE, scan rate 60 mV/s, pulse amplitude 50 mV and stirring 30 s.

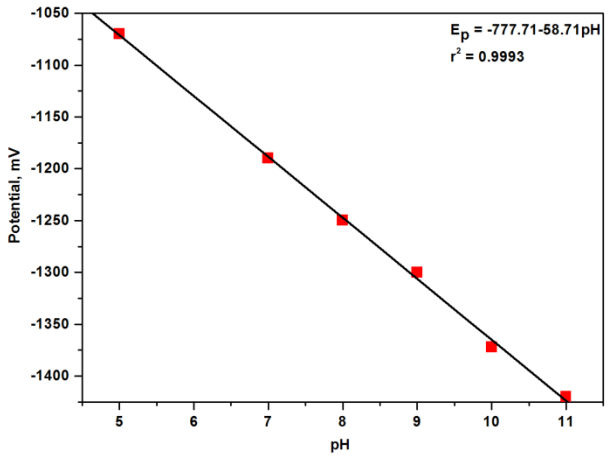


Figure 5. Effect of pH on differential pulse voltammetric peak potentials for 4 µg/mL Drosp in Britton-Robinson buffer at HMDE and scan rate 60 mV/s, pulse amplitude 50 mV, stirring 30s.

Here, E° is standard peak potential in V; [Ox] and [Red] are the equilibrium concentrations of oxidized and reduced species, respectively, ∂ is the number of protons participated in mechanism and n is the number of electrons transferred.

As demonstrated in the above equation, the ratios of protons to electrons participating in the reduction process were calculated as -0.98506 for Drosp and -0.99634 for EE, which were nearly equal to 1, indicating that equal number of protons and electrons participated in the reduction of Drosp and EE.

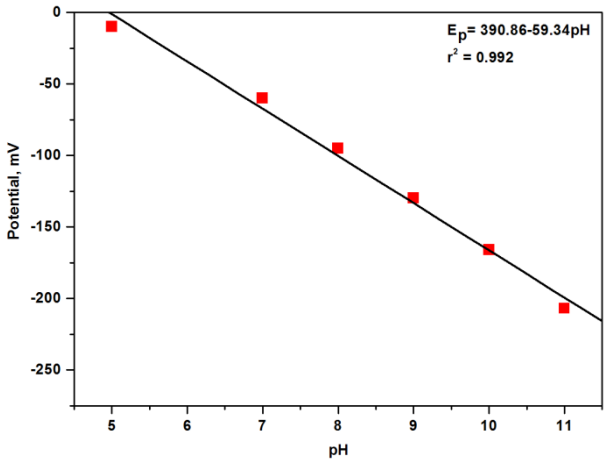


Figure 6. Effect of pH on differential pulse voltammetric peak potentials for 80 ng/mL EE in Britton-Robinson buffer at HMDE and scan rate 60 mV/s, pulse amplitude 50 mV, stirring 30s.

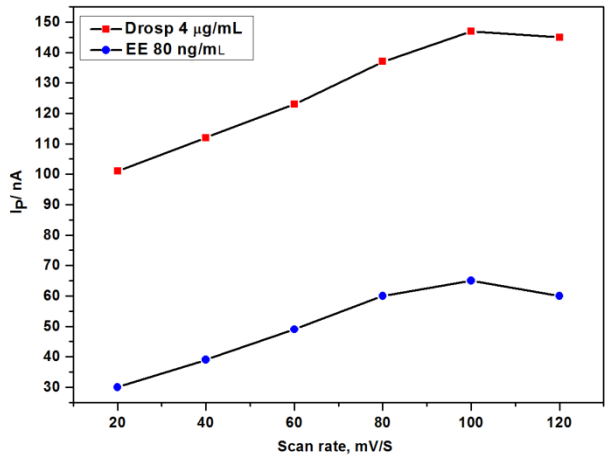


Figure 7. Effect of scan rate on the peak current of 4 µg/mL Drosp and 80 ng/mL EE at DP mode in 0.04 M BR pH = 8.

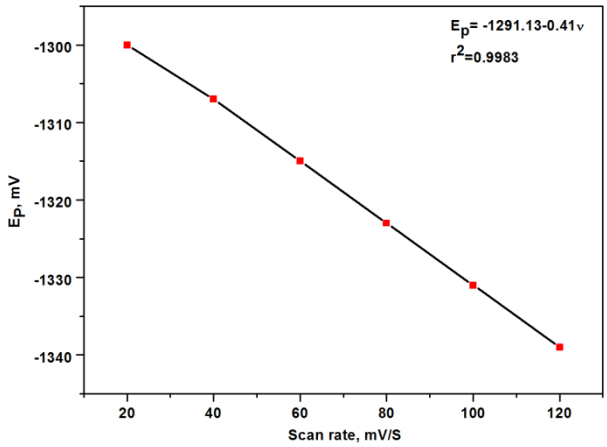


Figure 8. Plotting of potential (Ep, mV) against scan rate (υ, mV/s) of 4 µg/mL Drosp solution in Britton-Robinson, stirring for 30 s on DP mode.

3.1.3. Effect of scan rate

By scanning the effect of different scan rates (from 20 to 120 mV/s) on the peak current of 4 µg/mL Drosp and 80 ng/mL EE at cathodic mode, it was found that 60 mV/s is the most favorable scan rate as it showed the lowest standard deviation (Figure 7).

By plotting the peak potential (E_p, mV) against scan rate (υ, mV/s), the potential was shifted to more negative potential by increasing the scan rate confirming the irreversibility of the reduction electrode reaction of both Drosp and EE at HDME (Figures 8 and 9).

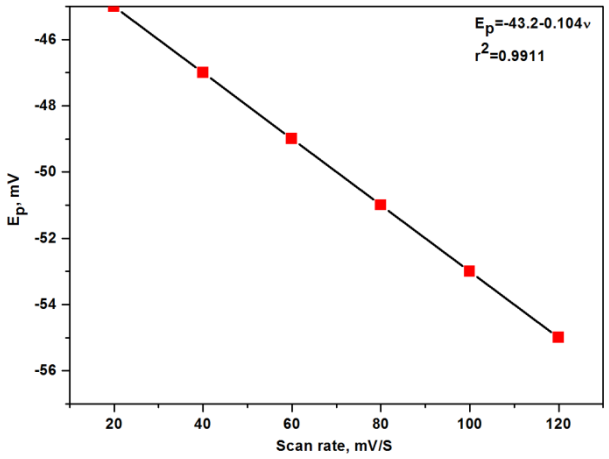


Figure 9. Plotting of potential (Ep, mV) against scan rate (υ, mV/s) of 80 ng/mL EE solution in Britton-Robinson, stirring for 30s on DP mode.

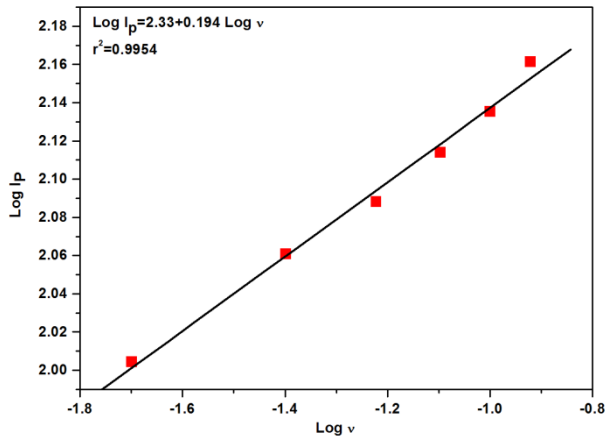


Figure 10. Plot of log current (log Ip) against log scan rate (log υ) of 4 µg/ mL Drosp in 0.04 M BR pH = 8.0, accumulation time for 60 s at accumulation potential -1300 mV with stirring for 10s on DP mode.

From the graphical representations of E_p versus υ for Drosp and EE (Figures 8 and 9), the E° value at HDME can be deduced from the intercept which is equal to -1291.13 mV for Drosp and - 43.20 mV for EE. Subsequently αn value can be calculated from the Equation (4) [48].

$$E_{P_a} - E_{P_{\frac{a}{2}}} = \frac{1.857RT}{\alpha nF} \quad (4)$$

where R is Gas constant, F is Faraday, T is temperature in Celsius At temperature (T) = 25 °C, then

$$E_{P_a} - E_{P_{\frac{a}{2}}} = \frac{47.7}{\alpha n} \quad (5)$$

For Drosp the value of αn was found to be 1.28 and by selecting α value = 0.7 (α ranges from 0.30 to 0.70), thus the number of electrons (n) transferred during the reduction step is equal 1.859 (nearly ≈ 2) which indicates that two electrons were involved in the reduction of the carbonyl group of Drosp on the HDME.

For EE the value of αn was found to be 0.4998 and by selecting α value = 0.3 (α ranges from 0.30 to 0.70), thus the number of electrons (n) transferred during the reduction step is equal 1.666 (nearly ≈ 2) which indicates that two electrons were involved in the reduction of the acetylene group of EE on the HDME.

Scan rate studies were carried out to assess whether the process at the HDME electrode was under diffusion or adsorption controlled process as shown in Figures 10 and 11.

By plotting the log current (I, µA) versus log scan rate (υ, V/s) and extrapolating the line to υ=0, it was found that the logarithm of reduction peak current (log I) is linear to the logarithm of scan rate (log υ) with the linear regression equation

Log I = 2.33 +0.194 log (υ) (r^2 = 0.9954) for Drosp (6)

Log I = 2.28 +0.466 log (υ) (r^2 = 0.9950) for EE (7)

From the values of slope 0.194 and 0.466 for Drosp and EE, respectively, that are less than 0.5, it can be deduced that the electrochemical reduction process of both Drosp and EE at HDME is diffusion controlled process.

3.1.4. Effect of accumulation potential and accumulation time

Scanning the effect of accumulation potential (starting from +50 to -1400 mV) on 4 µg/mL Drosp and 80 ng/mL EE, it was found that the produced currents were nearly the same for both. So, the accumulation potentials of -1300 mV for Drosp and -50 mV for EE were selected.

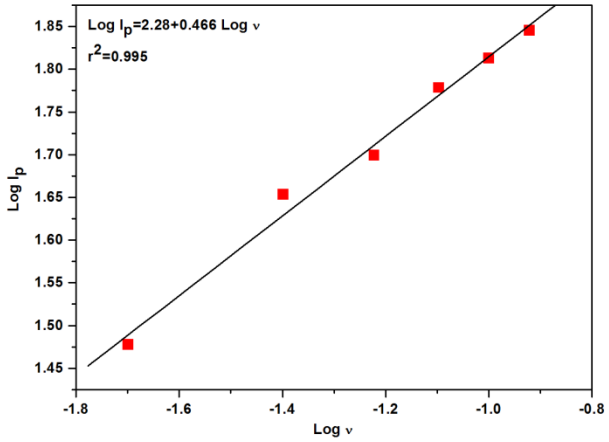


Figure 11. Plot of log current (log Ip) against log scan rate (log υ) of 80 ng/mL EE in 0.04 M BR pH = 8.0, accumulation time for 60 s at accumulation potential -1300 mV with stirring for 10 s on DP mode.

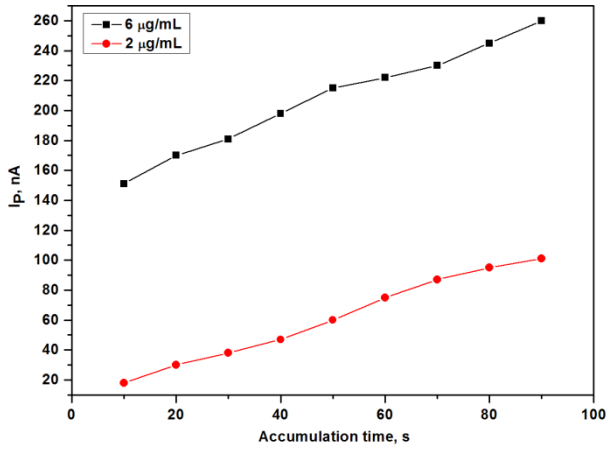


Figure 12. Scan for accumulation time effect on 2 and 6 µg/mL Drosp in 0.04 M BR pH = 8.0 by DP mode and pulse amplitude 50 mV, scan rate 60 mV/s.

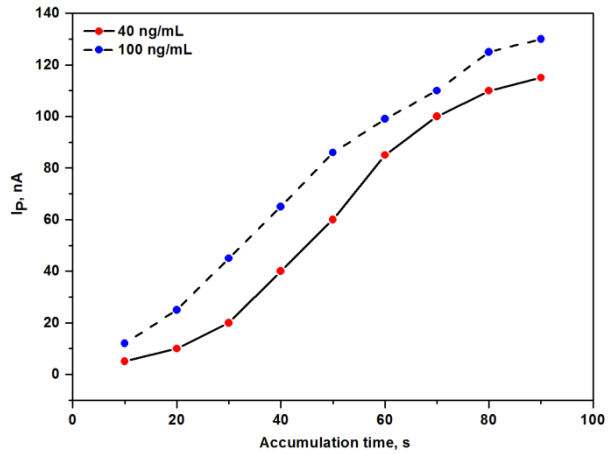


Figure 13. Scan for accumulation time effect on 40 and 100 ng/mL EE in 0.04 M BR pH = 8.0 by DP mode and pulse amplitude 50 mV, scan rate 60 mV/s.

Regarding the effect of accumulation time: Scanning at low concentrations (2 µg/mL and 40 ng/mL for Drosp and EE, respectively) and high concentrations (6 µg/mL and 100 ng/mL for Drosp and EE, respectively) using accumulation potentials -1300 and -50 mV for Drosp and EE, respectively, revealed that 60s showed nearly the lowest standard deviation and gave high current as shown in Figures 12 and 13.

3.1.5. Effect of rotation speed of stir

Scanning the different rotation speeds of stirring the solution, it was found that rotation speed of 600 rpm/min has resulted in current with lowest standard deviation (Figure 14).

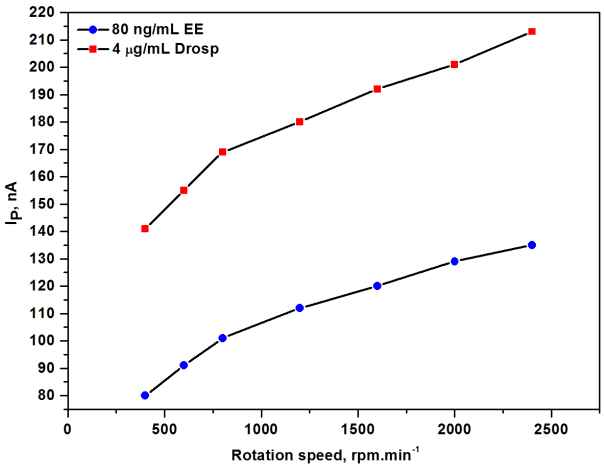


Figure 14. Effect of rotation speed on 4 µg/mL Drosp and 80 ng/mL EE in 0.04 M BR pH = 8.0, scan rate 60 mV/s, accumulation time 60 s at accumulation potential -1300 and -50 mV for Drosp and EE, respectively, with stirring for 10s on DP mode.

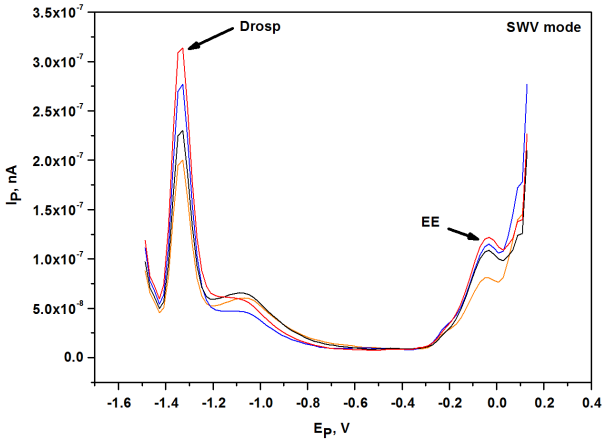


Figure 15. Voltammogram of SWV mode on different concentrations of Drosp and EE, in BR buffer pH = 8.0, stirring 30 s, scan rate 60 mV/s, pulse amplitude 50 mV on HMDE.

3.2. Validation parameters

3.2.1. Linearity and range

Voltammogram of different modes of sweep, differential pulse (DP) and square-wave (SWV) were compared over a potential range from +100 to -1600 mV in the presence of 0.04 M BR buffer pH= 8 with stirring for 30 s then stirring was stopped for 10 s, scan rate of 60 mV/s, and pulse amplitude of 50 mV (Figure 15 and 16). The SWV mode was selected due to its highest response and good linearity and it was applied successfully for determining the active ingredients in pharmaceutical preparations. Calibration curves were constructed as a function of the concentrations of standard Drosp and EE (X) versus their peak currents, (I_P). Calibration curves of Drosp and EE on SWV mode are represented in Figures 17 and 18, respectively. The performance data of the proposed SWV method is presented in Table 1.

3.2.2. Limits of detection and quantitation

The limits of detection (LOD) and the limits of quantification (LOQ) were determined as 3 and 10 times the baseline noise, respectively, following the ICH guidelines [49]. The LODs and LOQs indicate the sensitivity of the method as shown in Table 1.

3.2.3. Accuracy and precision

The accuracy and precision data are presented in Table 2. The intra-day and inter-day data were evaluated by replicate analysis of three different concentrations of authentic drugs three times a day for intra-day precision and for three constitutive days for inter-day precision with the same standard.

3.3. Application of SWV method to the analysis of Drosp and EE in dosage form

Drosp and EE were analyzed successfully in commercial tablets (Yasmin® tablets). Well-defined SWV peaks were obtained and no interferences were observed as represented in Figure 19. It is clear from statistical data that there was no significant difference between the proposed method and official method [46] as shown in Table 3.

3.4. Application of SWV method to the analysis of Drosp and EE (In-vitro dissolution profile)

Dissolution of Yasmin® tablets was done by withdrawal of samples at intervals of 10, 20 and 30 mins, and then samples were measured by SWV method using the aforementioned parameters.

Table 1. Performance data on the proposed SWV mode for determination of Drosp and EE in pure form.

Item	Drosp	EE
Concentration range	0.5-7.0 µg/mL	20.0-180.0 ng/mL
Slope	36.6504	0.6949
Intercept	-0.1033	19.3307
Correlation coefficient(r^2)	0.9993	0.9998
Mean* %	99.89	99.83
±RSD %	1.147	1.086
% Er	0.468	0.443
LOD	0.027 µg/mL	3.58 ng/mL
LOQ	0.081 µg/mL	10.17 ng/mL

Mean* is the average of three determinations.

Table 2. Accuracy and precision data of the proposed SWV method for determination of Drosp and EE in pure form.

Parameter	Drosp					
	Intra-day precision (Repeatability)			Inter-day precision (Intermediate precision)		
Conc. (µg/mL)	1	3	5	1	3	5
% Recovery	101.00	100.33	98.00	98.1	101.00	99.40
	99.50	99.00	100.20	99.50	98.67	101.80
	98.90	98.33	101.00	101.50	101.67	99.80
Mean±SD	99.80±1.082	99.22±1.018	99.73±1.553	99.70±1.709	100.45±1.575	100.33±1.286
±RSD%	1.084	1.026	1.558	1.714	1.568	1.282
% Error	0.625	0.592	0.899	0.989	0.905	0.739
Parameter	**EE**					
	Intra-day precision (Repeatability)			**Inter-day precision (Intermediate precision)**		
Conc. (ng/mL)	30	50	80	30	50	80
% Recovery	98.33	100.12	100.11	98.13	100.04	100.11
	100.03	99.30	99.60	100.03	99.98	100.15
	100.27	98.76	100.05	99.93	100.24	99.56
Mean ± SD	99.54±1.058	99.39±0.685	99.92±0.279	99.36±1.069	100.09±0.136	99.94±0.329
± RSD%	1.062	0.688	0.279	1.076	0.136	0.329
% Error	0.613	0.397	0.162	0.621	0.079	0.190

Table 3. Analysis of Drosp and EE in tablets by the proposed SWV and the official method [46].

Item	Drosp		EE	
	SWV method	Official method	SWV method	Official method
Recovery	98.04	99.96	98.99	100.58
	101.08	99.78	100.08	99.98
	99.25	100.05	99.03	101.02
	100.14	99.34	100.65	99.07
	98.58	98.75	98.37	98.34
Mean %[a]	99.42	99.58	99.42	99.79
SD	1.216	0.537	0.920	1.095
±RSD %	1.223	0.539	0.926	1.097
F-test (6.3882)	5.134		1.415	
t-test (2.306)	0.266		0.5847	

[a] Mean is the average of three determinations. The official method is an HPLC method using solution A (dibasic ammonium phosphate:water (1:24,*v:v*), pH adjusted to 6.8 and acetonitrile with the ratio 1:1 (*v:v*) as a mobile phase andC_{18} column (125×4 mm, 3 µm) as a column and the detection was carried out for drospirenone using UV detector at 270 nm and fluorescence detector at $\lambda_{emission}$ 315 nm and $\lambda_{excitation}$ at 285 nm.

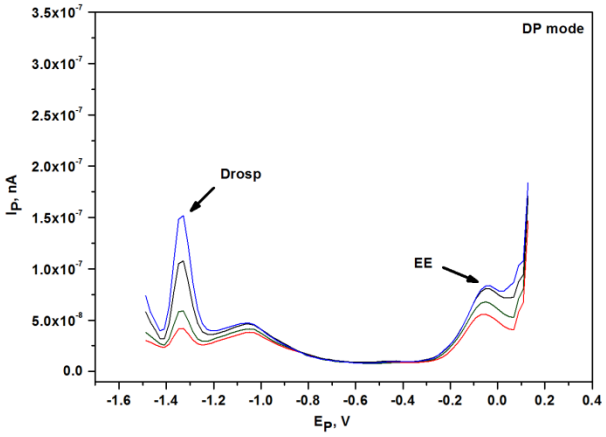


Figure 16. Voltammogram of DP mode on different concentrations of Drosp and EE, in BR buffer pH = 8.0, stirring 30 s, scan rate 60 mV/s, pulse amplitude 50 mV on HMDE.

The percent release was calculated for each time interval by substitution in regression equation of each drug and compared with results measured by the official method [46]. The results have shown no significant difference from the official method (Table 4).

3.5. Statistical analysis

The statistical comparison of the results obtained by the proposed method and the official method [46] was shown in Table 5.

Table 4. Dissolution results of % release of Drosp and EE by the proposed SWV and the official method [46].

Time interval (min)	Drosp % released		EE % released	
	SWV method	Official method	SWV method	Official method
10	55.25	58.09	51.08	54.49
	54.21	57.99	53.09	55.21
	56.68	58.33	52.04	54.59
20	75.28	76.98	73.25	75.20
	77.44	79.23	74.02	74.98
	76.59	77.08	73.11	75.01
30	98.32	99.78	96.09	97.99
	99.98	100.23	98.25	98.07
	97.69	99.14	96.96	97.68

Table 5. Statistical comparison between the results obtained by the proposed method and the official method [46] for the determination of Drosp and EE in pure powder form [a].

Items	Drosp		EE	
	SWV method	Official method	SWV method	Official method
Mean [b]	99.89	99.22	99.83	99.89
±RSD%	1.147	0.931	1.086	0.621
%ER	0.468	0.379	0.443	0.254
Variance	1.313	0.852	1.175	0.385
N	6	6	6	6
Student's t-test [c]	1.124		0.1209	
F value [d]	1.54		3.052	

[a] The official method describes an HPLC method for Drosp and EE where the mobile phase was solution A (dibasic ammonium phosphate:water (1:24,*v*:*v*) pH adjusted to 6.8 and acetonitrile with the ratio 1:1 (*v*:*v*) using C18 column (125×4 mm, 3 µm). Drosp was detected using UV detector at 270 nm while EE was detected using fluorescence detector at $\lambda_{emission}$ 315 nm and $\lambda_{excitation}$ at 285 nm.
[b] Average of three determinations.
[c] The corresponding tabulated value of t equals to 2.228 at p=0.05.
[d] The corresponding tabulated value of F equals to 5.05 at p=0.05.

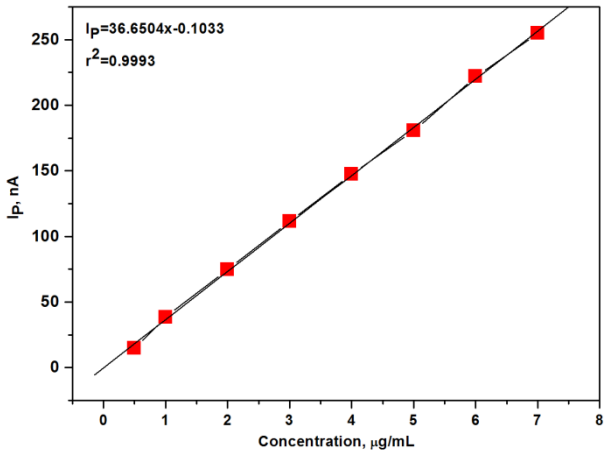


Figure 17. Calibration curve of Drosp, on SWV mode, 30 s rotation at 600 rpm, in 0.04 MBR buffer pH = 8.0, stirring 30 s, scan rate 60 mV/s, pulse amplitude 50 mV, on HMDE.

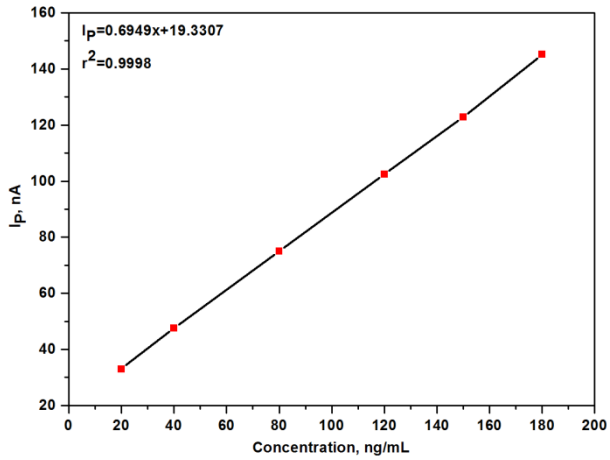


Figure 18. Calibration curve of EE, on SWV mode, 30 s rotation at 600 rpm, in 0.04 M BR buffer pH = 8.0, stirring 30 s, scan rate 60 mV/s, pulse amplitude 50 mV, on HMDE.

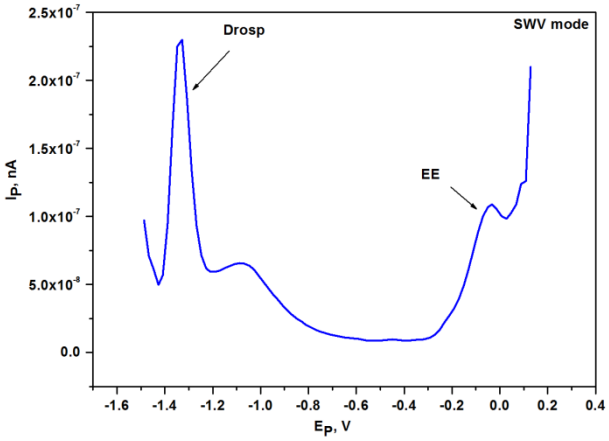


Figure 19. Square-wave voltammogram of Drosp and EE in Yasmin®tablets, using BR buffer pH = 8.0, stirring 30 s, scan rate 60 mV/s, pulse amplitude 50 mV on HMDE.

There is no significant difference between the proposed method and the official method with respect to accuracy and precision as the calculated t and F values were less than the tabulated ones.

4. Conclusion

A simple, rapid and selective method was developed for the simultaneous determination of Drosp and EE in Yasmin® tablets without the need for any prior chemical treatment. This voltammetric method has offered several advantages as very short run-time, low solvent consumption and high sensitivity so it could be applied successfully for the routine analysis of the studied drugs in quality control laboratories.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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References

[1]. Kuhl, H. *Climacteric* **2005**, *8(Supp. 1)*, 3-63.
[2]. Fuhrmann, U.; Krattenmacher, R.; Slater, E. P.; Fritzemeier, K. H. *Contracept.* **1996**, *54(4)*, 243-251.
[3]. Muhn, P.; Fuhrmann, U.; Fritzemeier, K. H.; Krattenmacher, R.; Schillinger, E. *Ann. N. Y. Acad. Sci.* **1995**, *761(1)*, 311-335.
[4]. Sweetman, S. C.; Blake, P. The complete drug reference, London, England, UK: Pharmaceutical Press, 2011.
[5]. Bhaumik, U.; Ghosh, A.; Mandal, U.; Chatterjee, B.; Sarkar, A. K.; Bose, A.; Ray, K. K.; Pal, T. K. *Chromatographia* **2008**, *68(9-10)*, 713-720.
[6]. Pradad, G. R.; Babu, P. S.; Ramana, M. V. *Int. J. Adv. Pharm. Sci.* **2011**, *2(2)*, 239-243.
[7]. Zeynep, A.; Ece, M. Y.; Sevgi, Y.; Samet, A. *Intern. Schol. Res. Not.* **2015**, *3*, 1-7.
[8]. Mohamed, A.; Tawfik, A. S.; Elshabouri, S. R. *J. Pharm. Sci.* **1975**, *64(7)*, 1221-1223.
[9]. Nevado, J. J. B.; Flores, J. R.; Penalvo, G. C. *Anal. Chim. Acta* **1997**, *340(1)*, 257-265.
[10]. Rodr, J.; Rodriguez, J.; Gastaneda, G. *Anal. Sci.* **1997**, *13(6)*, 1029-1032.
[11]. Berzas, J. A.; Rodriguez J. *Analyst* **1997**, *122(1)*, 41-44.
[12]. Sohrabi, M. R.; Emrarian, M.; Javanbakht, M. *J. Appl. Sci.* **2010**, *10(16)*, 1802-1805.
[13]. Sohrabi, M. R.; Abdolmaleki, P.; Esmaeili, E. A. *Spectrochim. Acta A* **2010**, *77(1)*, 107-111.
[14]. Onur, F.; Palabiyik, I. M. *J. Sci. Innov. Res.* **2006**, *2*, 642-650.
[15]. Toral, M. I.; Nacaratte, F.; Nova, F. *Anal. Lett.* **2015**, *48(6)*, 1009-1020.
[16]. Fakhari, A. R.; Khorrami, A. R.; Shamsipur, M. *Anal. Chim. Acta* **2006**, *572(2)*, 237-242.
[17]. Karnik, Y. M.; Gowekar, N. M. *Pharm. Sin.* **2014**, *5(6)*, 22-28.
[18]. Witos, Y. A.; Milz, B.; Spangenberg, B. *JPC-J. Planar Chromat.* **2017**, *30(2)*, 136-141.
[19]. Gluck, J. A. P.; Shek, E. *J. Chromatogr. Sci.* **1980**, *18(11)*, 631-636.
[20]. Strusiak, S. H.; Hoogerheide, J. G.; Gardner, M. S. *J. Pharm. Sci.* **1982**, *71(6)*, 636-640.
[21]. Lane, P. A.; Mayberry, D. O.; Young, R. W. *J. Pharm. Sci.* **1987**, *76(1)*, 44-47.
[22]. Van, D. R.; John, K. J.; Nicholas, J. D. *J. Pharm. Res.* **1987**, *4(1)*, 54-58.
[23]. Laban, A.; Markovic, S.; Stankov, M.; Djurdjevic, P. *Anal. Lett.* **2004**, *37(2)*, 273-282.
[24]. Shou, W. Z.; Jiang, X.; Naidong, W. *Biomed. Chromatogr.* **2004**, *18(7)*, 414-421.
[25]. Wenkui, L.; Ying, H. L.; Austin, C. L.; Shaolian, Z.; Weng, N. *J. Chromatogr. B* **2005**, *825(2)*, 223-232.
[26]. Markopoulou, C. K.; Koundourellis, J. E. *J. Liq. Chromatogr. Relat. Technol.* **2006**, *29(5)*, 685-700.
[27]. Matejicek, D.; Kuban, V. *Anal. Chim. Acta* **2007**, *588(2)*, 304-315.
[28]. Liu, X. F.; Ding, C. G.; Ge, Q. H.; Zhou, Z.; Zhi, X. J. *Acta. Pharm. Sin.* **2010**, *45(1)*, 87-92.
[29]. Xiong, Z.; Sun, X.; Huo, T.; Li, N.; Zheng, Y.; Sun, Y. *Biomed. Chromatogr.* **2010**, *24(2)*, 160-168.
[30]. Patel, M. G.; Sagar, G. V. *Int. J. Uni. Pharm. Life Sci.* **2012**, *2(1)*, 611-622.
[31]. Kanchan, N.; Ramakrishna, K. *J. Chem. Pharm. Res.* **2014**, *6(10)*, 74-80.
[32]. Gandhi, A.; Guttikar, S.; Trivedi, P. *J. Pharm. Anal.* **2015**, *5(5)*, 316-326.
[33]. Seifeldeen, E. M.; Etman, M. A.; Aboul-Enein, H. Y. *J. Liq. Chromatogr. Relat. Technol.* **2016**, *39(3)*, 134-138.

[34]. Berzas, J. J.; Del, C. B.; Castaneda, G.; Pinilla, M. J. *Talanta* **1999**, *50(2)*, 261-268.
[35]. Berzas, J. J.; Rodriguez, J.; Castaneda, G.; Pinilla, M. J. *Chromatographia* **1999**, *49(1)*, 65-70.
[36]. James, T. *J. Pharm. Sci.* **1972**, *61(8)*, 1306-1308.
[37]. Hirai, S.; Hussain, A.; Babhair, S. *J. Pharm. Sci.* **1980**, *69(7)*, 857-859.
[38]. Li, C. *Bioelectrochem.* **2007**, *70(2)*, 263-268.
[39]. Ghoneim, E. M.; El-Desoky, H. S.; Ghoneim, M. M. *J. Pharm. Biomed. Anal.* **2006**, *40(2)*, 255-261.
[40]. Bhusari, V. K.; Dhaneshwar, S. R. *Rev. Anal. Chem.* **2012**, *31(2)*, 123-130.
[41]. Arsova, S. Z.; Pollozhani, A.; Aneta, D. , *Arch. Public Health* **2009,** *1(1)*, 67-74.
[42]. Patel, R. C.; Rathod, D. K.; Rajesh, K. S.; Patel, V. S. *Pharmagene.* **2013**, *1*, 15-20.
[43]. Praveen, C.; Ranganath, M. K.; Divakar, P. *Pharmaceut. Anal. Acta.* **2013**, *4, 231*, 1-5.
[44]. Shaikh, K.; Patil, A.; Katare, S. *J. Trace Anal. Food Drugs* **2013**, *1*, 30-37.
[45]. Singh, A. *Int. J. Chem. Pharm. Anal.* **2014**, *1(3)*, 80-88.
[46]. The United States Pharmacopeia, NF 34, VOL II, U. S. Pharmacopeial Convention, Rockville, MD, 2016.
[47]. Bard, A. J.; Faulkner, L. R. , Fundamentals and Applications, New York, Wiley, 2000.
[48]. Brett, C. M. A.; Brett, A. M. O. , Electrochemistry: Principles, Methods, and Applications, 3rd edition Oxford Science Publications, 1996.
[49]. International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, US FDA, Federal Register, 1997.