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Electrochemical characteristics of zafirlukast and its determination in pharmaceutical formulations by voltammetric methods

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Abstract

Simple, rapid, reliable and fully validated voltammetric methods were developed for the determination of zafirlukast in pharmaceutical formulations, based on its electrochemical reduction at a hanging mercury drop electrode. Its electrochemical behavior in borate buffer (pH 8.0) was investigated using cyclic voltammetry, linear sweep voltammetry and chronoamperometry. The linear sweep voltammetric study of zafirlukast was carried out using glassy carbon eletrode. A well-defined cathodic peak at −1326 mV without the adsorptive accumulation time and at −1312 mV with 20 s of accumulation time versus Ag/AgCl reference electrode in square-wave and square-wave adsorptive stripping voltammetric methods, respectively, was observed. The experimental and instrumental parameters affecting the peak current of zafirlukast were investigated and optimized for the zafirlukast determination. The detection limits of square-wave and square-wave adsorptive stripping voltammetric methods were 50 and 5 ng mL^{-1} with R.S.D. of 6.79 and 5.72%, respectively. The methods showed good sensitivity, accuracy, precision, selectivity, robustness and ruggedness. The proposed methods were applied for the determination of zafirlukast in its pharmaceutical formulations. The results obtained from developed methods were compared with a spectrophotometric method reported in the literature and no significant difference was found statistically.

Keywords: Zafirlukast; Voltammetric method; Electrochemical reduction; Validation; Pharmaceutical formulation

1. Introduction

Asthma is one of the most common chronic disease in children and adults. As long as asthma has been treated as an anti-inflamatory disease, systemic and inhaled corticosteroids have been the most commonly used therapy [1]. The leukotrienes are a family of lipid mediators that are derived from the cell membrane phospholipid arachidonic acid via the 5-lipoxygenase enzyme [2].

Leukotriene receptor antagonists comprise a new class of therapy for the treatment of asthma [3]. Zafirlukast (ZAF), 4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*o*-toylsulphonylbenzamide (Fig. 1), a representative compound from this class, is a selective and competitive orally administered inhibitor of the cysteinyl leukotrienes as LTC_4, LTD_4 and LTE_4 in respiratory tracts [4]. The drug is indicated for the prophylaxis and treatment of mild-to-moderate persistent and chronic asthma [5,6]. ZAF effectively improved symptoms and benefited lung function in asthmatic patients [3,7,8].

ZAF molecule has electroactive groups, but so far, nothing appears to have been published concerning its electrochemical behavior or its voltammetric determination in literature. The most commonly employed techniques such as high-performance liquid chromatography (HPLC) [9–11] and derivative spectrophotometry [11] for the determination of ZAF are mentioned in pharmaceutical formulation and biological samples in literature.

Voltammetric methods are most suitable to investigate the redox properties of the drugs. Owing to the high sensitivity, low cost, simplicity and relatively short analysis time voltammetric techniques are important methods for trace quantification of many organic and inorganic substances [12–14].

The purpose of this study was to develop two new voltammetric methods such as square-wave voltammetry

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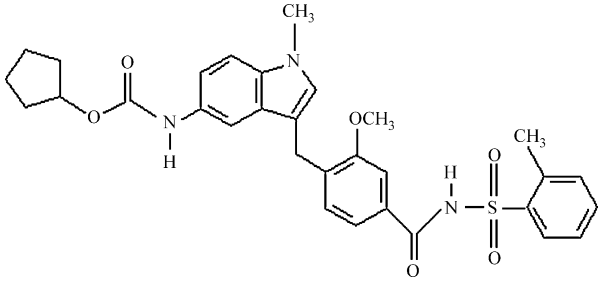

Fig. 1. Chemical structure of ZAF.

(OSWV) and square-wave adsorptive stripping voltammetry (OSWAdSV) for the direct determination of ZAF in its bulk form and pharmaceutical formulations. The method validation studies of these methods were performed according to the evaluation of the validation parameters in these methods. The electrochemical behavior of ZAF was discussed. The diffusion coefficients and number of electrons transferred were determined by employing electrochemical techniques such as cyclic voltammetry (CV), linear sweep voltammetry (LSV) and chronoamperometry (CA). A mechanism for the electrode reaction was proposed. The developed methods were applied to the determination of ZAF in pharmaceutical formulations. The results obtained by the developed methods were compared with the spectrophotometric method in the literature [11].

2. Experimental

2.1. Apparatus

All experiments were performed using a BAS 100 B/W (Bioanalytical System, USA) electrochemical analyzer. A three-electrode system consisted of hanging mercury drop electrode (HMDE) as working electrode, an Ag/AgCl with saturated 3 M KCl as reference electrode and a platinum wire as counter electrode were used (a glassy carbon as working electrode and Ag/AgCl as reference electrode and a platinum wire as counter electrode in LSV method). Before each experiment, the glassy carbon electrode was polished manually with alumina on a smooth polishing cloth. Residual polishing material was removed from the surface after each polishing step by sonication of the electrode in ultrasonic water bath for 5 min and then the electrode was rinsed with water before starting voltammetric scan. The peak heights were automatically or manually measured using the "tangent fit" capability of the instrument. All measurements were performed at room temperature.

2.2. Reagents and solutions

ZAF was kindly provided from Dr. Reddy's Laboratories (Hyderabad, India) and it was used without further purification. Melting point, UV and IR spectra of ZAF were evaluated to check purity and no impurities were found. Accolate Tablets® (20 mg ZAF per tablet) were kindly supplied by Astra Zeneca A.Ş. All solvents and chemicals used were analytical reagent grade.

The stock solution of ZAF (1000 $\mu g\,mL^{-1}$) was prepared in acetonitrile and kept in the dark at 4 °C. The stability of ZAF stock solution (1000 $\mu g\,mL^{-1}$) was tested for two-month period spectrophotometrically and ZAF in acetonitrile solution was stable for at least two months.

Standard solutions of ZAF were prepared daily by appropriate dilution of the stock solution with selected supporting electrolyte.

Different supporting electrolytes, namely borate, phosphate, acetate, Mcilvaine citrate and Britton-Robinson buffers (BR), were used. The supporting electrolytes were prepared in Milli-Q water. The pH of the solutions was adjusted with 0.1 M HCI and 0.1 M NaOH.

2.3. Procedure

2.3.1. General analytical procedure

Two millilitres of the supporting electrolyte was added to electrochemical cell and de-aerated with pure nitrogen for 12 min. The OSWV scan was conducted from −1000 to −1500 mV.

In OSWAdSV method, the required accumulation potential (E_{acc}) of −1000 mV was applied to the Ag/AgCl reference electrode for a selected accumulation time (t_{acc} = 20 s), while the solution was stirred at 400 rpm. After an equilibrium time of 10 s, a negative going scan was performed.

After the voltammogram of supporting electrolyte had been recorded, aliquots of ZAF standard were added and the square-wave voltammetric cycles were repeated using a new mercury drop.

2.3.2. Procedure for Accolate® tablets

Ten tablets of Accolate® were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to the average weight of one tablet was transferred into a 50 mL volumetric flask and 25 mL of acetonitrile was added. The content of the flask was sonicated for 15 min and diluted to volume with acetonitrile. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of clear supernatant and diluting with selected supporting electrolyte. This solution was analyzed as in the general analytical procedure.

The amount of ZAF per tablet was calculated using the calibration curve method.

3. Results and discussion

No previous electrochemical methods were available concerning the redox mechanism of ZAF. Therefore, sev-

eral measurements with different electrochemical techniques such as OSWV, OSWAdSV, CV, LSV and CA were performed in borate buffer (pH 8.0) as supporting electrolyte.

3.1. Optimization of the experimental conditions

Various buffer such as borate, phosphate, acetate, Mcilvaine citrate and BR at different pH values (pH 5–10) were examined as supporting electrolyte. The peak currents of ZAF were increased with the increase in pH of the buffer solutions at pH from 5 to 10. Maximum peak currents were obtained using borate buffer.

The effect of pH of borate buffer on the peak current of ZAF and peak potential was studied over the pH range 6–10. The peak current increased gradually with the increase in pH of the borate buffer (Fig. 2). The peak potential shifted to less negative values with increasing pH. The maximum peak current was obtained at pH 8.0, which was used for the ZAF determination. The square-wave peak current was measured in different ionic strengths of borate buffer (pH 8.0) between 0.025 and 0.20 M. When the ionic strength also increased, the peak current was increased. With respect to the current enhancement, shape and reproducibility, the response is optimum for 0.1 M. Therefore, the optimum condition for square-wave determination of ZAF was 0.1 M borate buffer (pH 8.0), which was chosen for all further measurements. In this condition a well-defined peak of ZAF was observed at −1326 mV for OSWV and −1312 mV for OSWAdSV methods (Fig. 3).

3.2. Optimization of the instrumental parameters

In order to obtain the maximum peak current, the optimum instrumental conditions (frequency (f), scan increment (ΔE) and pulse amplitude (E_{SW})) were studied for 6 and 10 μg mL^{-1} of ZAF in OSWV method and 100 and 200 ng mL^{-1} of ZAF at 20 s accumulation time in OSWAdSV method.

In OSWV method, f was varied from 15 to 300 Hz using ΔE of 5 mV and E_{SW} of 25 mV. A linear relationship

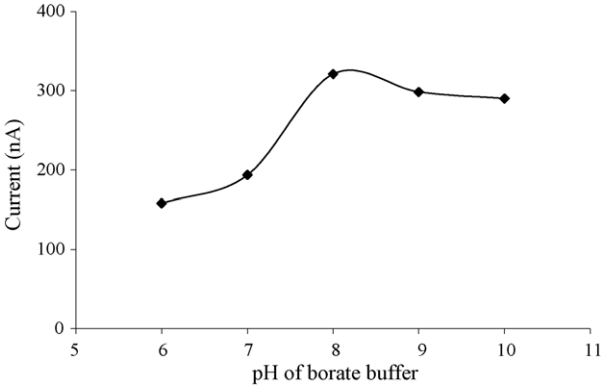

Fig. 2. Effect of pH of borate buffer on the OSW peak current of ZAF (500 ng mL^{-1}) at frequency 70 Hz, scan increment 4 mV and pulse amplitude 25 mV.

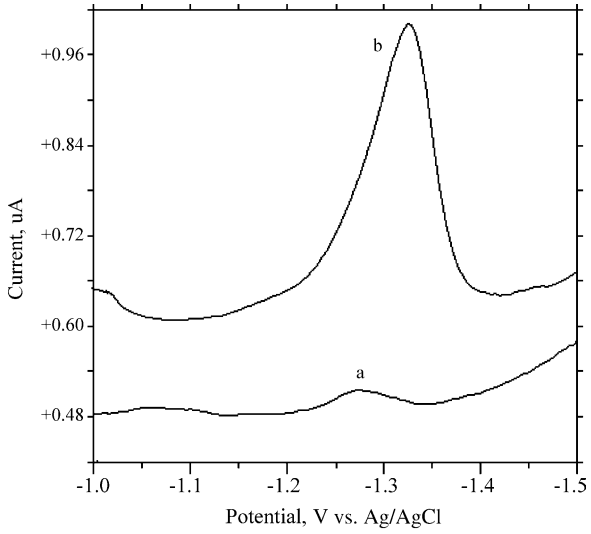

Fig. 3. The voltammograms of 230.00 ng mL^{-1} of ZAF in borate buffer (pH 8.0) using the optimum conditions: (a) OSWV, (b) OSWAdSV methods.

was obtained between the peak current and the f range of 15–90 Hz, and f of 70 Hz was chosen to improve the sensitivity without any distortion of the peak. At f of 70 Hz and E_{SW} of 25 mV, the peak current increased linearly with the ΔE up to 5 mV. The optimal ΔE was chosen as 4 mV. The optimal ΔE was not chosen as 5 mV, because of the reduction peak of ZAF was broad at ΔE of 5 mV. The peak of ZAF at ΔE of 4 mV was sharper than at ΔE of 5 mV.

The peak current increased linearly with the increase of the E_{SW} from 5 to 30 mV. But the E_{SW} was higher than 25 mV, a broad peak was obtained which is not useful for analytical purposes. The width of the peak is also sensitive to pulse height. The narrowest square-wave peak was obtained for E_{SW} of 25 mV; this was an optimal value for analytical determination. Therefore, square-wave optimal conditions of f = 70 Hz, ΔE = 4 mV and E_{SW} = 25 mV, were used in OSWV method.

In OSWAdSV method, at a constant ΔE of 4 mV and E_{SW} of 25 mV, the peak current intensity increased linearly over the f range 15–100 Hz, and the f of 70 Hz was chosen. At f of 70 Hz and the E_{SW} of 25 mV, the peak current increased linearly with the ΔE up to 4 mV and this value was chosen. Although the peak current increased linearly with the increase of the E_{SW} from 5 to 30 mV, a sharper peak was obtained at 25 mV. Thus, E_{SW} of 25 mV was applied. Therefore, the square-wave stripping optimal conditions of f = 70 Hz, ΔE = 4 mV and E_{SW} = 25 mV were used in OSWAdSV method, as these were better for analytical purposes and these values were used in further measurements.

3.2.1. Effect of accumulation potential and accumulation time

The effect of varying accumulation potential (E_{acc}), from −1100 to −800 mV on the cathodic peak current intensity of the OSWAdS voltammogram for 100 and 200 ng mL^{-1} of

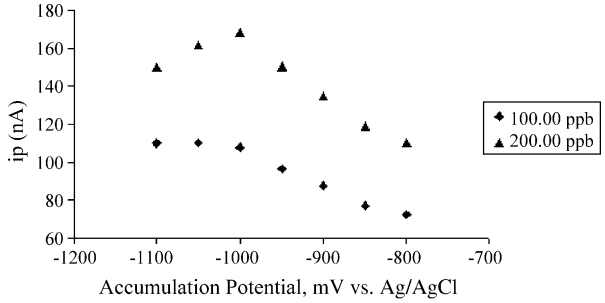

Fig. 4. Effect of the accumulation potential on the OSWAdS peak current response (i_p) for ZAF after 20 s preconcentration time at frequency 70 Hz, scan increment 4 mV and pulse amplitude 25 mV.

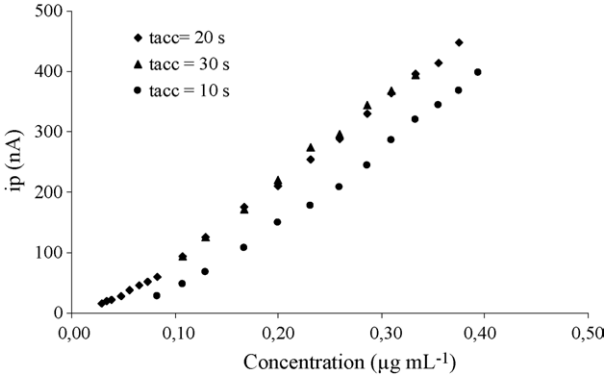

Fig. 5. The calibration curves of ZAF in OSWAdSV method at different accumulation times at accumulation potential of −1000 mV, frequency 70 Hz, scan increment 4 mV and pulse amplitude 25 mV.

ZAF in borate buffer (pH 8.0), followed by pre-concentration for 20 s, was also evaluated (Fig. 4). A maximum of the peak current was obtained at −1000 mV. Hence, an accumulation potential of −1000 mV (versus Ag/AgCl/KCl_s) was used throughout the present study.

The dependence of stripping peak currents on accumulation time (t_{acc}) was studied at −1000 mV of accumulation potential (Fig. 5). The longer the pre-concentration time, the more drug is adsorbed onto the electrode surface and the larger peak current is observed. The peak current increased linearly with increase in the pre-concentrated period. Loss of linearity at higher accumulation times was due to gradual surface saturation (Table 1). The limit of quantification value at accumulation time of 20 s was lower than the other accumulation time. Thus, the accumulation time of 20 s was chosen for further measurements.

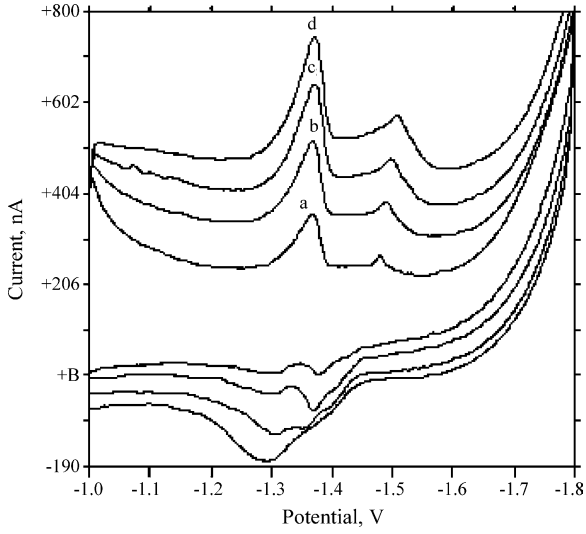

Fig. 6. Cyclic voltammograms of 10.00 μg mL^{-1} ZAF solution on HMDE at different scan rates: (a) 100 mV/s, (b) 200 mV/s, (c) 300 mV/s and (d) 400 mV/s.

3.3. Characterization of the electrode reaction (cyclic and linear sweep voltammetry studies)

The cyclic voltammetric behavior of ZAF (10 μg mL^{-1}) in borate buffer (pH 8.0) at the HMDE showed in the cathodic direction a well-defined peak at −1325 mV (Fig. 6). This peak may be attributed to the saturation of S=O double band through a two-electron process [15–17]. The second peak was observed at −1450 mV. But the peak current of this peak was not increased on increasing the concentration of ZAF and scan rate. The effect of scan rate (ν) on peak current (i_p) was also examined within the range 10–1000 mV/s^{-1}. Plotting log i_p (logarithm of peak current) versus log ν (logarithm of scan rate), gave straight line within the same scan rate range. The slope of 0.5464 of the relationship is close to the theoretical value of 0.5 for diffusion-controlled process ($y = 0.5464 \times -7.894$, $r = 0.9991$) [18].

The reversibility of ZAF reduction was studied by CV method. The observed anodic peak on the reverse scan showed that the reaction was reversible. A plot of peak current versus square root of the scan rate gave a straight line. The peak potentials and shape did not change, when the scan rate was increased. The peak current ratio and the square root of the scan rate did not change with the scan rate. These results showed that the reduction reaction of ZAF may be reversible [19].

Table 1
The obtained regression equation and linearity range for ZAF at different accumulation times in OSWAdSV method

Accumulation time (s)	Regression equation	Correlation coefficient	Linearity range (ng mL^{-1})
10	$y = 1.2822x - 40.154$	0.9969	83.00–394.00
20	$y = 1.6058x - 23.565$	0.9997	30.00–375.00
30	$y = 1.2839x - 109.78$	0.9986	74.00–333.00

Table 2
The calculated values of the number of electrons and diffusion coefficients for ZAF ($n = 6$)

Number of electron transferred	1.84 ± 0.15
Diffusion coefficient from Cottrell's equation (cm^2/s)	$1.85 \times 10^{-6} \pm 1.51 \times 10^{-8}$
Steady-state current for ZAF (A)	$2.01 \times 10^{-9} \pm 3.02 \times 10^{-12}$
Steady-state current for standard (A)	$4.91 \times 10^{-9} \pm 3.40 \times 10^{-12}$
Diffusion coefficient from Cottrell's equation (cm^2/s)	$1.75 \times 10^{-6} \pm 5.75 \times 10^{-8}$

The diffusion coefficient was calculated from Cottrell's equation as the following equation [20]:

$$i = \frac{n\,F\,A\,D^{1/2}C}{\pi^{1/2}t^{1/2}}$$

where i is the current (nA), n is the number of electron transferred per molecule, F is the Faraday's constant (96.485 C/eq), A is the electrode area (cm^2), D is the diffusion coefficient (cm^2/s), C is the concentration (mol/cm^3) and t is the time (s). The experimental Cottrell's slope was determined from the chronoamperometric i_p versus $t^{-1/2}$ plot. The constant potential applied was slightly more cathodic than the cyclic voltammetric E_p from −1400 to −1200 mV. An HMDE with a surface area of 0.0199 cm^2 was employed. The diffusion coefficient was calculated as $1.85 \times 10^{-6} \pm 1.51 \times 10^{-8}$ (Table 2).

The number of electrons was determined by employing LSV method at ultramicro electrode [20]. The steady-state currents for ZAF and potassium hexacyanoferrate(II) trihydrate were measured. The ratio of the steady-state currents for ZAF and standard is:

$$\frac{i_{ZAF}}{i_s} = \frac{n_{ZAF}D_{ZAF}C_{ZAF}}{n_S D_S C_S}$$

where i_{ZAF}, i_S are the steady-state currents (A); n_{ZAF}, n_S are the number of electrons; D_{ZAF}, D_S are the diffusion coefficients (cm^2/s); and C_{ZAF} and C_S (mol/cm^3) are the same concentration of ZAF and standard solution, respectively, in this reaction.

The diffusion coefficient of ZAF was also calculated as the following equation [20]:

$$\frac{n_{ZAF}}{n_S} = \frac{S_{ZAF}^2\, i_S\, C_S}{S_S^2\, i_{ZAF}\, C_{ZAF}}$$

where D_{ZAF}, D_S are the diffusion coefficients (cm^2/s); i_{ZAF}, i_S are the steady-state currents (A); n_{ZAF}, n_S are the number of electrons; S_{ZAF}, S_S are the Cottrell's slopes; and C_{ZAF} and C_S (mol/cm^3) are the same concentration, in the same solvent of ZAF and standard solution, respectively.

The same ultramicro electrode was used to obtain i_{ZAF}, i_S, S_{ZAF} and S_S. In addition, the same solvent and supporting electrolyte were used in measuring i and S for a given reaction. The number of electrons and diffusion coefficients are given in Table 2.

3.4. Validation of the methods

Validation of the proposed methods for the quantitative determination of ZAF was examined via evaluation of linearity range, limit of detection (LOD), limit of quantification (LOQ), repeatability, precision, accuracy, recovery, specificity, robustness, ruggedness and stability.

3.4.1. Linearity range

The applicability of the proposed methods as an analytical method for the determination of ZAF was examined by measuring square peak current as a function of concentration of the bulk form for at least three times under the optimized conditions. The calibration graphs of the peak current versus concentration were found to be linear over the range of 0.15–3.07 and 3.24–10.16 $\mu g\,mL^{-1}$ for OSWV method and 30.00–394.00 $ng\,mL^{-1}$ for OSWAdSV method. The linearity was checked by preparing standard solutions for 15 and 19 different concentrations for OSWV and OSWAdSV methods, respectively. Calibration graphs were constructed using data from these measurements and least-squares were evaluated using linear regression method. The calibration curve is obtained as the following regression equation:

$$i_p\,(nA) = 178.18C\,(\mu g\,mL^{-1}) - 22.135,$$

$$r = 0.9993 (0.15 - 3.07\,\mu g\,mL^{-1}) \quad \text{for OSWV method}$$

$$i_p\,(nA) = 100.82C\,(\mu g\,mL^{-1}) + 205.58,$$

$$r = 0.9993\ (3.24 - 10.16\,\mu g\,mL^{-1}) \quad \text{for OSWV method}$$

$$i_p\,(nA) = 1.6058C\,(ng\,mL^{-1}) - 23.565,\ r = 0.9997$$

for OSWAdSV method

where i_p is the peak current and C is the ZAF concentration, r is the correlation coefficient.

Each point of the calibration graph corresponded to the mean value obtained from 12 independent measurements. The second equation was used in evaluating validation parameters for OSWV method. The amount of ZAF in tablets was calculated using this equation. The r value was found to be significant ($t_c = 96.09 > t_t = 2.16$, $p < 0.05$) for OSWV and $t_c = 291.46 > t_t = 2.11$, $p < 0.05$ for OSWAdSV method. The intercept was not significantly different from zero ($t_c = 0.126$, $p > 0.05$) for the second equation in OSWV method and ($t_c = 0.054$, $p > 0.05$) in OSWAdSV method. In Table 3, the analytical characteristics are summarized for both techniques.

3.4.2. Sensitivity

The calculated LOD values of ZAF at a S/N ratio of 3 were 50.00 $ng\,mL^{-1}$ (R.S.D.% = 6.79) for OSWV and 5 $ng\,mL^{-1}$ (R.S.D.% = 5.72) for OSWAdSV method at an accumulation time of 20 s [21,22].

Table 3
Analytical characteristics of proposed methods ($n = 12$)

	OSWV method	OSWAdSV method
Regression equation	$y = 100.82x + 205.58$	$y = 1.6058x - 23.565$
Standard error of slope	0.45	3.91
Standard error of intercept	1.81	0.44
Correlation coefficient (r)	0.9993	0.9997
Linearity range (ng mL^{-1})	3240.00–10160.00	30.00–394.00
Number of data points	15	19
LOD (ng mL^{-1})	50.00	5.00
LOQ (ng mL^{-1})	146.00	30.00

The LOQ values were determined by analyzing six different standard solutions containing the lowest concentration of ZAF on the calibration curve with acceptable accuracy and precision [23,24]. These values were 146.00 ng mL^{-1} (R.S.D.% = 4.57) for OSWV and 30.00 ng mL^{-1} (R.S.D.% = 2.55) for OSWAdSV methods.

3.4.3. Repeatability

The repeatability of the methods was evaluated by performing 12 repeated measurements for 6.25 μg mL^{-1} of ZAF in OSWV and 130.00 ng mL^{-1} of ZAF in OSWAdSV method under the same optimum conditions [25]. The amount of ZAF was found to be 6.35 ± 0.02 and 130.73 ± 0.49 for OSWV and OSWAdSV methods, respectively. Percentage recoveries of 101.53 ± 0.30 and 100.57 ± 0.38% were achieved with R.S.D.% of 1.03 and 1.29 for OSWV and OSWAdSV methods, respectively. These values indicated that the proposed methods have high repeatability and precision for the ZAF analysis.

3.4.4. Accuracy and precision

The accuracy of a method is the degree of the nearness to the real value of the observed analysis results. The accuracy of the analysis was determined by calculating the percentage relative error between the measured mean concentrations and added concentrations [13,23].

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. The precision of the analysis was determined by calculating the relative standard deviation (R.S.D.%). The precision around the mean value should not exceed 15% of the R.S.D.% [23,26,27].

The accuracy and precision of the proposed methods were investigated by intra-day and inter-day determination of ZAF at three different concentrations of ZAF solution for three times (3.77, 5.46 and 9.84 μg mL^{-1} in OSWV and 48.00, 167.00 and 375.00 ng mL^{-1} in OSWAdSV method) in the linear range. The intra-day studies were performed in one day; inter-day studies were performed in six days over a period one week (Table 4).

The results obtained for intra-day and inter-day accuracy and precision indicates the high accuracy and precision of the proposed methods.

3.4.5. Selectivity and recovery

The selectivity of the optimized methods for the determination of ZAF was examined in presence of some common excipients (e.g., croscarmellose sodium, lactose, microcristallin cellulose, povidone, magnesium stearate, metylhydroxypropylmethyl cellulose and titanium dioxide), added in the same ratio as in pharmaceutical formulation [18,27]. The amounts of 20 mg of ZAF were found to be 20.02 ± 0.04 and 20.11 ± 0.06 for OSWV and OSWAdSV methods, respectively. The mean percentage recoveries in this synthetic mixture of ZAF were found to be 99.45–101.00% and 99.60–101.80% with R.S.D. of 0.55 and 0.80% for OSWV and OSWAdSV methods, respectively. These values showed no significant excipients interference; thus, the procedures were able to determine the amount of ZAF in the presence of excipients. Comparison of the OSW and OSWAdS voltammograms of ZAF standard, synthetic and tablet solutions showed that the peak potential and peak current of ZAF did not change. On the basis of these results, the proposed methods can be considered selective.

In order to evaluate the effect of the presence of the excipients on the proposed methods, the standard addition method was applied. For this reason, appropriate volume of Accolate® tablet solution was added supporting electrolyte. After the voltammogram was recorded, known amounts of standard solutions of ZAF were added and voltammo-

Table 4
Accuracy and precision of ZAF obtained by OSWV and OSWAdSV methods ($n = 6$)

Intra-day				Inter-day		
Added (μg mL^{-1})	Found[a] (μg mL^{-1})	Precision R.S.D.%	Accuracy[b] (Bias%)	Found[a] (μg mL^{-1})	Precision R.S.D.%	Accuracy[b] (Bias%)
OSWV method						
3.77	3.73 ± 0.02	1.61	−1.06	3.79 ± 0.03	1.85	0.53
5.46	5.45 ± 0.02	0.92	−0.18	5.48 ± 0.03	1.28	0.37
9.84	9.80 ± 0.02	0.51	−0.41	9.72 ± 0.02	0.62	−1.22
OSWAdSV method						
48.00	47.14 ± 0.40	2.07	−1.79	47.30 ± 0.42	2.21	−1.46
167.00	167.70 ± 0.70	1.02	0.42	168.97 ± 0.84	1.21	1.18
375.00	375.35 ± 0.95	0.62	0.09	371.94 ± 1.44	0.95	−0.82

R.S.D.: relative standard deviation.
[a] Found = $\bar{x}$ = mean ± S.E.
[b] Accuracy = [(founded − added)/added] × 100.

Table 5
Robustness of OSWV and OSWAdSV methods ($n = 7$)

	Found ($\mu g\,mL^{-1}$)	R.S.D.%
OSWV method		
Standard (6.25 $\mu g\,mL^{-1}$)	6.20 ± 0.03	1.13
pH 7.90	6.28 ± 0.03	1.27
pH 8.10	6.26 ± 0.03	1.28
Initial potential (−1100 mV)	6.24 ± 0.03	1.28
Initial potential (−900 mV)	6.31 ± 0.03	1.27
OSWAdSV method		
Standard (130.00 $ng\,mL^{-1}$)	131.24 ± 0.49	0.99
pH 7.90	129.10 ± 0.54	1.10
pH 8.10	130.23 ± 0.55	1.11
Accumulation potential (−1100 mV)	129.95 ± 0.58	1.19
Accumulation potential (−900 mV)	130.32 ± 0.61	1.24
Accumulation time (15 s)	130.49 ± 0.57	1.16
Accumulation time (25 s)	130.51 ± 0.60	1.21

$\bar{X}$: mean ± S.E. R.S.D.: relative standard deviation.

grams were recorded. The regression equations of standard addition methods were found to be $y = 100.92\,C + 1081.20$, $r = 0.9990$ and $y = 1.6064\,C + 220.33$, $r = 0.9990$ for OSWV and OSWAdSV methods, respectively. There was no difference between the slopes of two methods with calibration curve and standard addition methods. These data showed that there was no interaction of excipients in the analysis of ZAF in pharmaceutical formulations by the proposed methods. Therefore, the calibration curve method, which is easier and quicker than the standard additon method, was used in quantitative analysis of ZAF.

3.4.6. Robustness

The robustness of the proposed methods was examined by evaluating the influence of small variations of some of the most important procedure variables such as pH and initial potential for 6.25 $\mu g\,mL^{-1}$ of ZAF in OSWV method and pH, accumulation potential (E_{acc}) and accumulation time (t_{acc}) for 130.00 $ng\,mL^{-1}$ of ZAF in OSWAdSV method [14,26]. As shown in Table 5, none of these variables significantly affected the assay of ZAF and the proposed methods could be considered robust.

3.4.7. Ruggedness

The ruggedness of the proposed methods was evaluated by applying the developed procedures to assay 6.25 $\mu g\,mL^{-1}$ of ZAF in OSWV method and 130.00 $ng\,mL^{-1}$ of ZAF in OSWAdSV method using the same instrument by two different analysts under the same optimized conditions on different days [14,26] (Table 6). The obtained results were found to be reproducible, since there was no significant difference between the results obtained by the two analysts. Thus, the proposed methods could be considered robust.

3.4.8. Stability

Under the optimum conditions, the stability of 10.00 $\mu g\,mL^{-1}$ of ZAF solution was evaluated by OSWV method. No changes were observed in the peak potential and peak current of ZAF over a period of 6 h.

3.5. Analysis of Accolate® tablets

The optimized voltammetric methods were applied to the determination of ZAF in Accolate® tablets. The amounts of ZAF in tablets were calculated using calibration curve

Table 7
The results of pharmaceutical preparations containing ZAF analyzed by proposed methods ($n = 7$)

OSWV method	OSWAdSV method	Compared method [11]
Found (mg)		
20.14	20.08	19.83
20.06	20.32	20.30
20.22	19.96	19.81
19.99	19.98	20.03
20.00	20.12	20.51
19.94	20.32	21.10
20.03	20.36	20.18
$\bar{x}$: 20.03 ± 0.04	$\bar{x}$: 20.16 ± 0.06	$\bar{x}$: 20.25 ± 0.17
R.S.D.%: 0.50	R.S.D.%: 0.84	R.S.D.%: 2.22
t_c: 9.00 > t_T: 2.00	t_c: 15.00 > t_T: 2.00	
$p > 0.05$	$p > 0.05$	

$\bar{x}$: mean ± S.E. R.S.D.: relative standard deviation (t_c: calculated t value, t_T: tabulated t value, $t_t = 2$).

Table 6
Ruggedness of OSWV and OSWAdSV method (Added of ZAF amount of 6.25 $\mu g\,mL^{-1}$ and 130.00 $ng\,mL^{-1}$ for OSWV and OSWAdSV methods, respectively) ($n = 7$)

OSWV method		OSWAdSV method	
Analyst found ($\mu g\,mL^{-1}$)		Analyst found ($ng\,mL^{-1}$)	
6.25	6.31	132.12	130.07
6.21	6.20	132.96	129.73
6.18	6.25	130.88	131.13
6.31	6.17	131.88	130.54
6.10	6.32	131.69	131.50
6.19	6.25	130.04	131.66
6.13	6.20	129.17	127.27
$\bar{x}$: 6.24 ± 0.02		$\bar{x}$: 130.27 ± 0.57	
R.S.D.%: 0.96		R.S.D.%: 1.16	
$t_c = 7.00 > t_t = 2.00$		$t_c = 6.00 > t_t = 2.00$	
$p > 0.05$		$p > 0.05$	

$\bar{x}$: mean ± S.E. R.S.D.: relative standard deviation (t_c: calculated t value, t_T: tabulated t value, $t_t = 2$).

method. The obtained percentage recoveries and the relative standard deviations based on the average of seven replicate measurements were found (Table 7). The obtained results were compared to those obtained by a reported spectrophotometric method [11]. The statistical comparison of methods was done by Wilcoxon paired test ($t_c = 9 > t_t = 2$, $p > 0.05$ for OSWV method and $t_c = 15 > t_t = 2$, $p > 0.05$ for OSWAdSV method). The experimental values (t_c) did not exceed the therotical ones (t_t), indicating a good agreement with comparison method.

4. Conclusion

Two new, simple, selective, accurate and precise square-wave voltammetric procedures were optimized for determination of ZAF in bulk form and pharmaceutical formulation. This work shows that ZAF can be determined by using voltammetric techniques on the basis of its reduction process over the HMDE.

These voltammetric techniques were applied directly to the analysis of pharmaceutical dosage forms without the need for separation or complex sample preparation such as time-consuming extraction steps prior to the drug analysis. Only centrifugal separation of excipients was used to precipitate the excipients from tablet formulations. The described methods were rapid, requiring less than 2 min to perform.

Moreover the proposed methods described have better sensitivity than HPLC [9,11] and spectrophotometric [11] methods in literature. Comparison of the developed methods and HPLC methods [9,11], shows the advantages of the prior one due to its simplicity, low cost, short time analysis and selectivity. These proposed methods may be preferred to HPLC method for determination of ZAF in pharmaceutical formulations. These methods could be easily used in quality control laboratory for the analysis of ZAF.

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