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Electrochemical Determination of Ipragliflozin in Pure Form and in Spiked Human Plasma on a Glassy Carbon Electrode

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Ipragliflozin, a highly potent and selective sodium glucose cotransporter II inhibitor, is an effective blood glucose lowering drug in patients with type 2 diabetes mellitus by promoting urinary glucose excretion. The present work represents the first electrochemical determination of ipragliflozin that depends on the oxidation of sulfur atom present in its structure. Cyclic wave and differential pulse voltammetry were applied by scanning potential over range of 0 to 2.8 V vs the reference electrode Ag/Ag^{+} in non-aqueous medium. The method was developed and validated in accordance with the guidelines of the International Council for Harmonisation (ICH). With a detection limit of 1.98×10^{-6} M, the method was considered to be linear in the range of 7.5×10^{-6}–1×10^{-3} M. The method was then efficiently applied for the determination of ipragliflozin in spiked human plasma. The method proved to be an excellent green analysis according to analytical eco-scale for greenness assessment.
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Diabetes mellitus is a serious, chronic disease that belongs to the class of metabolic disorders characterized by chronic hyperglycemia caused by insulin secretion deficiencies, insulin activity or both. According to the International Diabetes Federation, nearly half a billion people worldwide are living with diabetes, and by 2030 the number is projected to rise by 25%. There are two main types of diabetes: Type-I which is an autoimmune disorder that leads to the destruction of the pancreatic Beta-cells and Type-II which is caused by the combined effects of dysfunctional beta pancreatic cells and insulin resistance[1]. Diabetes must be well controlled as the prolonged hyperglycemia can cause numerous complications as diabetic retinopathy, nephropathy and neuropathy. That's why the treatment of diabetes is one of the critical issues to be reviewed. Type-I is mainly treated with insulin while Type-II requires the use of antidiabetic agents along with some lifestyle changes.[2]

Ipragliflozin (IPG), (1 s)-1,5-anhydro-1-C-{3-[(1-benzothiophen-2-yl)methyl]-4-fluorophenyl}-D-glucitol, is a once-daily orally administered anti-diabetic drug, its chemical structure is shown in Fig. 1. It acts by inhibiting the sodium glucose co-transporter-II present in the renal proximal tubules. Those transporters are responsible for about 90% of glucose reabsorption so their inhibition facilitates glucose excretion in urine and lowers blood glucose level.[3] It is worth mentioning that the additional benefit of this class is that it has a mechanism of action that is entirely independent of insulin action and depends only on blood glucose levels so that the risk for hypoglycemia is reduced as well as the risk of over-stimulation of pancreatic beta cells.[4,5]

Few analytical methods, including UV spectrophotometry[6] high-performance liquid chromatography with UV detection[7–9] and LC-MS/MS[10,11] have been implemented for the quantitative analysis of IPG.

Although the good selectivity and limit of detection of the reported methods, they are time-consuming and involve high cost instruments and relatively complex procedures. Electrochemical techniques have been used in recent decades to determine organic chemicals in various types of samples, particularly in the pharmaceutical industry.[12–41] In particular, voltammetry is easy, convenient and considered a cost-effective technique that is characterized by the short time of analysis and its ability to produce accurate, precise and reliable results. The chemical structure of IPG includes benzothiophene moiety which can be oxidized so facilitates its determination using electrochemical analytical methods.

To the best of our knowledge, there are no published methods for the electrochemical estimation of IPG. This work represents the first electrochemical-based method for the analysis of IPG in pure form and in spiked human plasma by using differential pulse voltammetry, so it can be used routinely in quality control laboratories. Moreover, knowing the oxidation potential value can be correlated with the stability of the drug,[42] the aim of this study was also to investigate the electrochemical behavior of IPG hence improving its stability. Glassy Carbon electrode (GCE) was used as an indicator electrode as it is both chemically and electrochemically very inert and fairly reproducible.[43]

Experimental

Materials and reagents.—Ipragliflozin was obtained from BaoJi Guokang Bio-technology co., Ltd (with certified purity 99.90% ± 0.01). Acetonitrile and methanol of HPLC grade were supplied from Fisher Scientific (Loughborough, Leicestershire, UK). Lithium perchlorate of analytical grade was chosen as supporting electrolyte and was obtained from CDH Chemicals, India. Polishing kit was purchased from CH instruments, Inc. (Texas, USA) for the mechanical polishing of GCE using 1.0 μm, 0.3 μm, and 0.05 μm alumina polishing powder and sonicated in water to remove any fine powder form the GCE surface just prior to voltammetric measurements. Blank human plasma was supplied from the Holding Company for Biological Products and Vaccines (VACSERA, Egypt).

Apparatus.—All voltammetric measurements were performed using a PC-controlled electrochemical analytical workstation (Metrohm Autolab potentiostat/ galvanostat PGSTAT204) supplied with NOVA software for electrochemistry. The used reference electrode was Ag/Ag^{+}, while a Pt wire was utilized as a counter electrode. The working electrode used was GCE that was purchased from CH Instruments, Inc. part number: CHI104, (Texas, USA). For the analysis of spiked human plasma, specimens were first mixed using vortex (VELP Scientifica, Europe), followed by centrifugation utilizing the Centurion K241R centrifuge (UK) and then evaporation with the help of a rotary vacuum concentrator connected to a vacuum pump (DVP TYRO 12, Germany), in addition to a solvent trap (CHRIST CT 02-50, Germany) as well as a rotor (CHRIST RVC 2-18 CDplus, Germany).

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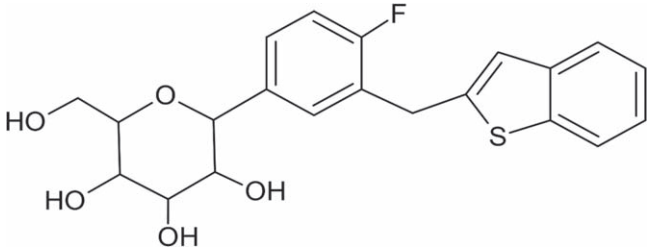

Figure 1. Chemical structure of Ipragliflozin.

Procedure.— *Standard solution.*—Stock standard solution of IPG was prepared by accurately weighing 40.5 mg of pure drug and transferring to 100-ml volumetric flask. Lithium perchlorate was added as supporting electrolyte (0.05 M) in acetonitrile to obtain IPG solution of concentration equals to 10^{-3} M.

Operational conditions of electrochemical measurements.—The cyclic wave voltammograms were collected by scanning potential over range of −0.1 to 2.8 V starting from 0 using GCE vs the reference electrode Ag/Ag^{+}. All determinations were carried out at room temperature. While for DPV measurements, voltammograms were recorded by scanning potential over range of 0 to 2.8 V, scan rate 10 mV s^{-1}, sample width 17 ms, pulse amplitude 50 mV, pulse width (modulation time) 50 ms, pulse period (interval) 500 ms and quiet time 5 s.

Construction of calibration curve.—Different aliquots of IPG were transferred from stock solution to 25-ml volumetric flasks then volumes were completed using 0.05 M lithium perchlorate dissolved in acetonitrile to produce the desired final concentrations of 7.5×10^{-6}, 1×10^{-4}, 2.5×10^{-4} and 5×10^{-4} M. Cyclic wave and differential pulse voltammograms were generated in the range from 0 to 2.8 V. Calibration curve and regression equations were determined by plotting current peak height recorded for each sample against its corresponding concentration.

Method Validation

The developed approach was validated in accordance with ICH guidelines in terms of linearity, specificity, accuracy, precision, detection and quantification limits[44] to confirm that the method is fit for the intended use.

Linearity.—Linearity was examined under optimum electrochemical conditions, by analyzing five different IPG concentrations. Linear correlation was obtained between the current peak height and their corresponding concentrations over the concentration range of $7.5 \times 10^{-6} \times 10^{-3}$ M.

Specificity.—Specificity was assessed by the ability of the method to determine IPG without any interference from the plasma matrix.

Accuracy.—The analytical method accuracy is an indication that the actual value and the reported value are in close agreement. It is demonstrated as percentage recovery of the studied drug's various levels of standard solutions.

Precision.—Both intraday and interday precision have been assessed. The intraday precision is normally assessed on the same day by quantifying three different analyte concentrations within the linearity range. While, upon analyzing three different concentrations of the drug on three different days, the interday precision was evaluated. Results were reported as percentage relative standard deviation (%RSD).

Limit of detection (LOD) and limit of quantification (LOQ).—

$$LOD = 3.3^{*}\sigma/S$$

$$LOQ = 10^{*}\sigma/S$$

Where, σ is the calculated standard deviation of blank response and S is the slope of the obtained calibration curve.

Application Procedure

Plasma assay procedure.—A stock standard solution of 0.05 M IPG was prepared by dissolving an accurately weighed 202.5 mg of IPG pure powder in 10.0 ml methanol from which different working standard solutions were prepared. 500.0 μl was withdrawn from each working solution and added separately to 500.0 μl plasma in a 10-ml centrifuge tubes. Protein precipitation was accomplished by adding acetonitrile (1.5 ml). The mixtures were blended by vortex mixer for 5 min at 3000 rpm followed by centrifugation for 15 min at 6000 rpm. The supernatant was transferred and evaporated to complete dryness by means of the rotary vacuum concentrator at 60 °C and 1500 rpm for 4 h. Samples were reconstituted in 25.0 ml of 0.05 M lithium perchlorate dissolved in acetonitrile to obtain final desired concentrations of 7.5×10^{-6}, 1×10^{-5}, 5×10^{-5}, 5×10^{-4} and 10^{-3} M. Differential pulse voltammograms were generated for those spiked samples in the range from 0 to 2.8 V.

The measured current peak heights of those concentrations were used to construct a calibration curve against their corresponding concentrations. The accuracy and the precision were also investigated for this method. The accuracy was assessed by calculating the percentage recovery of three different concentrations while the precision was evaluated by measuring the %RSD of three determinations of three different concentrations on the same day for the intraday precision and on three successive days for the interday one.

Results and Discussion

The electrochemical oxidation of IPG at a glassy carbon electrode surface in acetonitrile as non-aqueous solvent that contains lithium perchlorate as the supporting electrolyte was investigated using cyclic and differential pulse voltammetry between 0 and 2.8 V against Ag/Ag^{+} reference electrode.

Electrochemical behavior of IPG.—Figure 2 represents the acquired differential pulse voltammogram, which shows the presence of three chemically irreversible anodic peaks on a direct scan ($E_{pI} = 0.7$ V/Ag/Ag^{+}, $E_{pII} = 1.5$ V/Ag/Ag^{+}, $E_{pIII} = 2.1$ V/Ag/Ag^{+}). Those peaks could be associated with various pathways. The first peak probably involves the oxidation of the sugar part of IPG, while the second and third are probably linked to the oxidation of the sulfur atom to sulfoxide and sulfone, respectively. As the reported

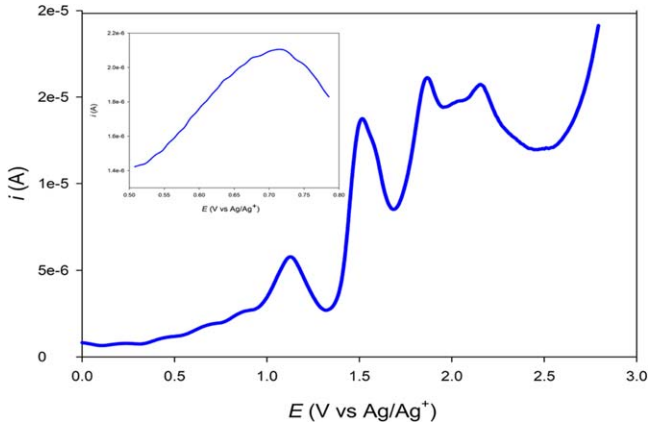

Figure 2. Differential pulse voltammogram of 1 mM ipragliflozin in acetonitrile + 0.05 M lithium perchlorate at glassy carbon electrode. The inset: The peak at 0.7 ($E_{pI} = 0.7$ V/Ag/Ag^{+}).

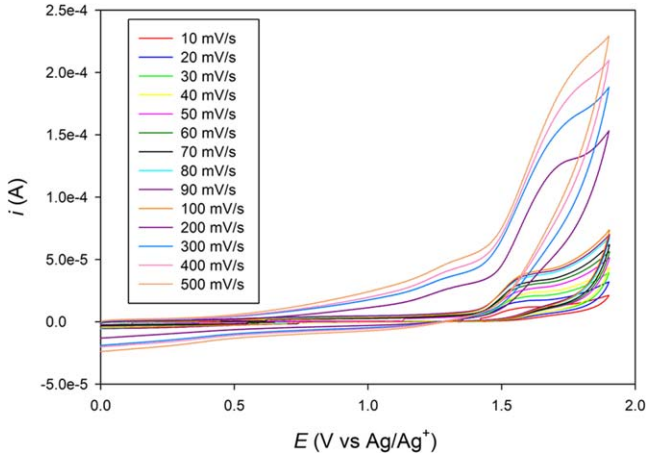

Figure 3. Cyclic voltammograms of 1 mM of ipragliflozin at glassy carbon electrode as a function of scan rate (10–500 mV s^{-1}).

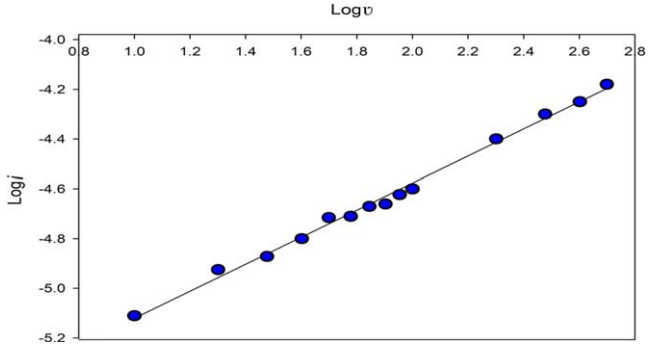

Figure 4. Plot of logarithm of anodic peak current of ipragliflozin (Log *i*) vs the logarithm of scan rate (Log υ).

method[45] was focusing on the electrochemical oxidation of dibenzothiophene which is structurally related to IPG, our results are in accordance with the findings of the peaks obtained.

By investigating the mechanism of the oxidation of other structurally related compounds (i.e. dibenzothiophene), it is apparent that oxidation can differ according to the solvent used being protic or aprotic one[45] in addition to the type of the electrode used. In a reported method,[45] the electrode used was GCE and the determinations were performed in acetonitrile in presence or absence of water, the oxidation products were sulfoxide and sulfone. While in another reported method,[46] the electrochemical oxidation was carried out on platinum electrodes in dry acetonitrile, which enabled them to obtain sulfonium dimers as products instead of sulfone and sulfoxide derivatives. Alternatively, the ability of the platinum electrode to oxidize adsorbed thiophene to produce SO_4^{2-} and CO_2 was demonstrated.[47] Therefore, to obtain a full mechanism of the oxidation of the studied drug, we need further investigations.

Effect of scan rate.—Figure 3 illustrates the effect of scan rate (10−500 mV s^{-1}) on the anodic oxidation peak of 1 mM of IPG. It reveals that the potential is slightly shifted to more positive side that confirms that E_p depends on the scan rate. Figure 4 displays a plot of log peak current (i_p) against log scan rate, a straight line can be observed with the following equation of regression: $\log i = 0.6215 \log \upsilon - 5.7984$ ($R^2 = 0.9757$), which assumes the slope is higher than the theoretical value of 0.50 which predicts that the anodic reaction at the electrode surface is a process controlled by diffusion in addition to some adsorption character. In addition, with the following regression equation, $i = -1E\text{-}05 + 4E\text{-}06\ \upsilon^{1/2}$ ($R^2 = 0.9741$), the peak current (*i*) was found to be directly proportional to the square root of the scan rate ($\upsilon^{1/2}$) over the range of scan rate from 10 to 100 mV s^{-1}, Fig. 5, and more than 100 mV s^{-1} as the scan rate, the current departs from this correlation, proving that the oxidation of IPG at GCE is controlled by mixed adsorption/diffusion processes.[48]

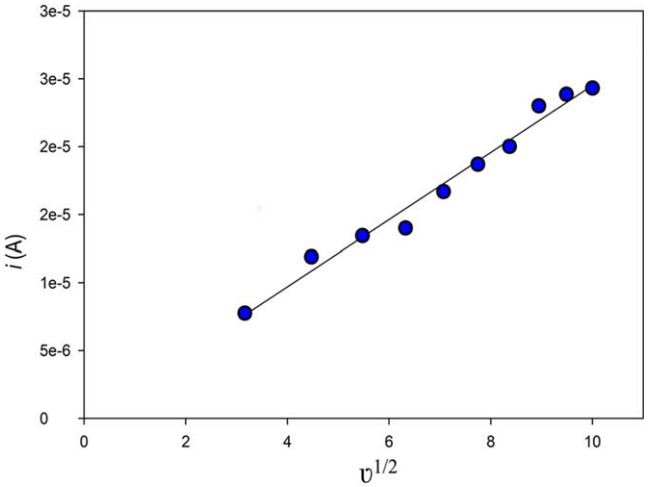

Figure 5. Plot of anodic peak current (i_p) of IPG in function of the square root of scan rate ($\upsilon^{1/2}$).

Table I. Assay and validation parameters obtained by using the developed differential pulse voltammetric method

Parameter	Ipragliflozin
Linearity range	$7.5 \times 10^{-6} - 1 \times 10^{-3}$ M
Slope	0.0096
Intercept	4×10^{-6}
Correlation coefficient	0.9992
Accuracy (mean ± SD)	99.65 ± 1.51
LOD	1.98×10^{-6} M
LOQ	6.01×10^{-6} M
Precision (%RSD)	
Intraday precision—Repeatability	1.12
Interday precision—Intermediate precision	1.19

Method validation.—The method was observed to be linear within the range of $7.5 \times 10^{-6} - 10^{-3}$ M under the optimum electrochemical conditions, with a correlation coefficient of 0.9992. The linearity results are represented in Table I and Fig. 6. LOD and LOQ were calculated using the previously mentioned equations. By examining three different levels of standard solutions of IPG and calculating the percentage recovery, the suggested method was found to be accurate. The results of LOD, LOQ and accuracy are all shown in Table I.

The studied method was also considered to be specific due to its ability to efficiently detect IPG with uniform peaks without any interference from the plasma matrix as shown in Fig. 7.

By assessing the intraday and interday precision, the % RSD values were within the accepted range which is less than 2%, indicating that the suggested method is precise. The percentage relative standard deviations were calculated and recorded in Tables II and III.

Analysis of spiked human plasma.—Sample preparation step is crucial in totally extracting the drugs and minimizing the impact of plasma proteins and other plasma matrix components that might interfere with the determination of the drug of interest. Regarding the ratio of volume of plasma to plasma proteins precipitating agent (acetonitrile in our method), the best results were achieved upon adding

Table II. Results of the intraday precision performed on the same day by the proposed electrochemical method.

Concentration (M)	Mean Peak Height	Standard deviation	% RSD
1.00×10^{-4}	4.86×10^{-6}	6.03×10^{-8}	1.24
5.00×10^{-4}	8.72×10^{-6}	1.1×10^{-7}	1.26
1.00×10^{-3}	1.33×10^{-5}	1.15×10^{-7}	0.87

Table III. Results of the interday precision performed on three different days by the proposed electrochemical method.

Concentration (M)	Day	Mean of triplicate on the same day	Mean Peak Height of three days	Standard deviation	% RSD
1.00×10^{-4}	Day 1	4.90×10^{-6}	4.92×10^{-6}	2.52×10^{-8}	0.51
	Day 2	4.95×10^{-6}			
	Day 3	4.92×10^{-6}			
5.00×10^{-4}	Day 1	8.66×10^{-6}	8.57×10^{-6}	1.28×10^{-7}	1.49
	Day 2	8.62×10^{-6}			
	Day 3	8.42×10^{-6}			
1.00×10^{-3}	Day 1	1.34×10^{-5}	1.36×10^{-5}	2.13×10^{-7}	1.57
	Day 2	1.35×10^{-5}			
	Day 3	1.38×10^{-5}			

Table IV. Results of analysis of the spiked plasma samples with different concentrations of ipragliflozin.

Parameter	Ipragliflozin spiked in plasma
Linearity range	7.5×10^{-6}–1×10^{-3} M
Slope	0.0118
Intercept	5×10^{-7}
Correlation coefficient	0.9969
Accuracy (mean ± SD)	100.45 ± 1.87
LOD	1.61×10^{-6} M
LOQ	4.89×10^{-6} M
Precision (%RSD)	
Intraday precision—Repeatability	1.61
Interday precision—Intermediate precision	1.73

1.5 ml of acetonitrile to 0.5 ml plasma. Table IV shows the results of the analysis of spiked human plasma samples. The results of accuracy and precision are represented in Tables V and VI, respectively, and the suggested method has proven to be accurate and precise.

As the lowest concentration that our method can detect is less than 7.5×10^{-6} M which is lower than the C_{max} value as previously reported[49] so the suggested method can be effectively applied for the analysis of IPG in real human plasma samples.

Greenness Assessment of the Procedure Using the Analytical Eco-Scale

The analytical community has focused over the past few decades on preventing or minimizing the use of hazardous chemicals and solvents in various analytical research methods that have been identified to be extremely hazardous to human health and the environment. The analytical Eco-Scale was proposed as a comprehensive approach for the assessment of the greenness of analytical method. Eco-scale estimation is focused on assigning penalty points to any factor that does not correspond to perfect green technique. The estimation of penalty points is governed by four essential factors of the analytical method: quantity of chemicals used, the potential hazards of the chemicals and instruments used, energy usage and waste generation. The computed estimate is rated on a scale, where the perfect green procedure has eco-scale value of 100, more than 75 proving excellent green analysis, more than 50 indicating acceptable green analysis and fewer than 50 demonstrating inadequate green analysis.[50] The calculated Eco-Scale for our proposed method was 84 as shown in Table VII, which indicates that our proposed method is excellent green analysis.

Table V. Results of the proposed DPV method accuracy in spiked human plasma samples.

Prepared concentration (M)	Found concentration (M)	Recovery %
2.50×10^{-5}	2.54×10^{-5}	101.69
2.50×10^{-4}	2.53×10^{-4}	101.36
7.50×10^{-4}	7.37×10^{-4}	98.31

Table VI. Results of the proposed DPV method precision in spiked human plasma samples.

Prepared concentration (M)	% RSD intraday	% RSD interday
5.00×10^{-5}	1.67	1.65
5.00×10^{-4}	1.55	1.56
1.00×10^{-3}	1.60	1.99

Conclusions

The proposed method represents the first electrochemical determination of IPG using GCE. Many advantages were offered by this

Table VII. The Penalty Points calculated using the analytical eco-scale for the proposed method.

Hazard	Penalty points for the proposed method
Type of reagent	
Acetonitrile	(more than 100 ml) = 12
Lithium Perchlorate	(Less than 10 gm) = 4
Instrument	
Energy consumption	(Less than or equal to 0.1 kWh per sample)= 0
Occupational Hazard	(None) = 0
Waste production	(None) = 0
Total penalty points	16
Analytical Eco-Scale total score	100–16 = 84
	Excellent green analysis

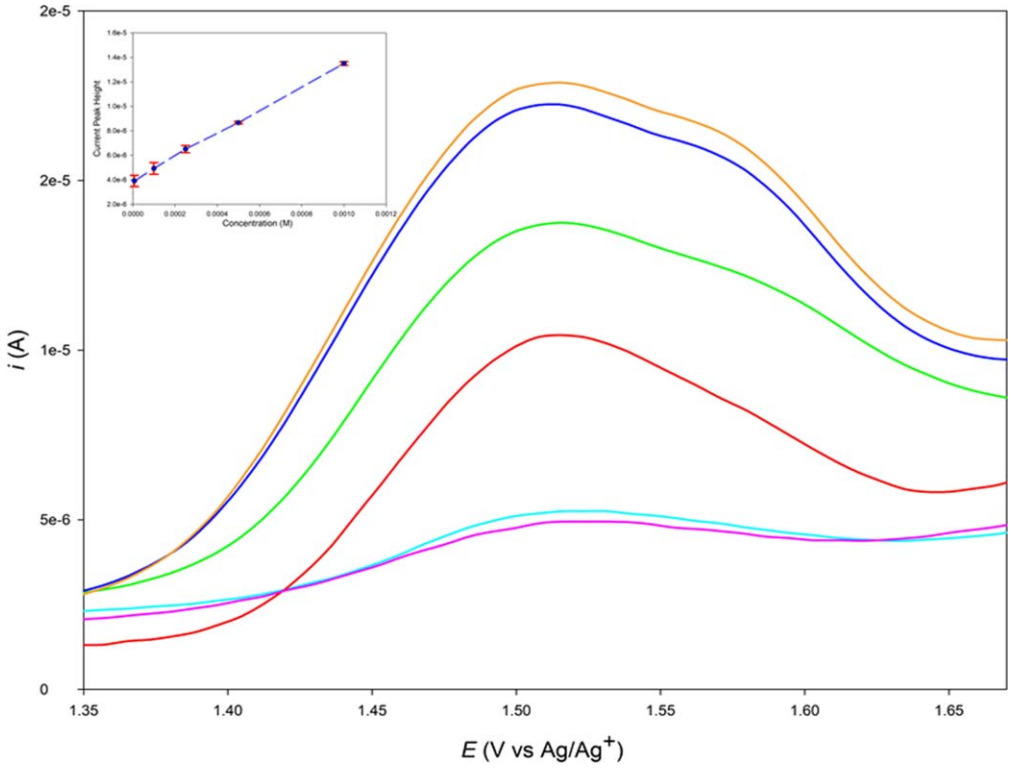

Figure 6. Differential pulse voltammograms of different concentrations of ipragliflozin. The inset: the calibration curve of ipragliflozin.

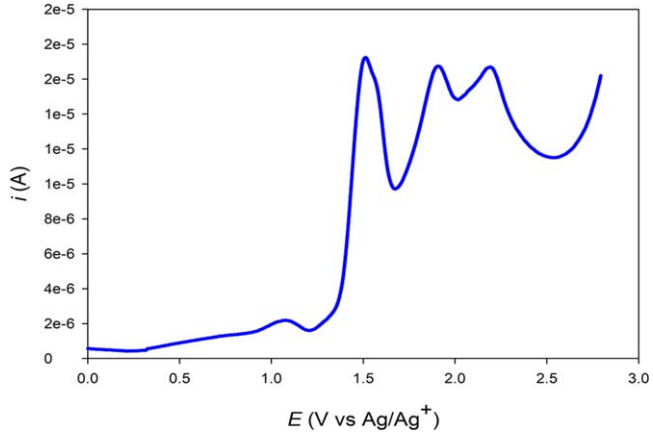

Figure 7. Differential pulse voltammogram of 1 mM ipragliflozin in spiked human plasma at glassy carbon electrode.

method as short analysis time, simple procedure, no need for expensive instrumentation as well as being an excellent green analytical method. The proposed procedure was capable of assessing IPG in bulk form as well as in spiked human plasma without any interference from the components of plasma matrix. The method can detect low concentrations in micromolar range that can permit its future application for the pharmacokinetics studies.

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