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Behavior of Granisetron at Glassy Carbon Electrode and its Determination in Pure form, Pharmaceutical form and Biological Fluids using Square Wave and Differential Pulls Voltammetry

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Abstract

A sensitive electro analytical method for determination of Granisetron hydrochloride has been investigated on the basis of the enhanced electrochemical response at glassy carbon electrode during oxidation of Granisetron hydrochloride, Cyclic voltammetric undergo one irreversible anodic peak at Ep= 674 mV in Britton - Robinson (BR) (pH 9.0). Cyclic voltammetric study indicated that the oxidation process is irreversible and adsorption controlled. The number of exchanged electrons in the electro -oxidation process was obtained; Differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were studied and a linear calibration obtained from: 75 x 10^{-8} – 32 x 10^{-7} M and 16 x 10^{-8} – 85 x 10^{-8} M respectively. The RSD for five measurements were found 0.388 %, 0.391% for DPV and SWV respectively. Precision and accuracy of the developed method was checked by recovery studies. The method was applied to determine Granisetron hydrochloride in pure form, pharmaceutical formulations, and human urine sample and compared with official methods.

Keywords: Granisetron hydrochloride; Voltammetry; Differential pulse; cyclic voltammetry; Electrochemical, GCE, Pharmaceuticals; Human serum

Introduction

Granisetron is 1-methyl-N-((1R, 3r, 5S)-9-methyl-9-azabicyclo [3.3.1]nonan-3-yl)-1H-indazole-3-carboxamide hydrochloride with a molecular weight of 348. 87. The empirical formula of Granisetron is $C_{18}H_{24}N_4O$ •HCl and its chemical structure is shown in figure 1 [1-6].

A serotonin 5-HT_3 receptor antagonist used as an antiemetic to treat nausea and vomiting following chemotherapy. Its main effect is to reduce the activity of the vagus nerve, which is a nerve that activates the vomiting center in the medulla oblongata. It does not have much effect on vomiting due to motion sickness. This drug does not have any effect on dopamine receptors or muscarinic receptors. The literature reveals that several methods have been reported for determination of GRS in pharmaceutical preparations and biological fluids. These include high performance liquid chromatography (HPLC) using DAD (diode-array detection), [7] Fluorescence, spectrophotometer [8,9] ultraviolet (UV) tandem mass spectrometric and electrochemical detectors [10-16]. A flow-injection chemiluminescence method has been reported f or the determination of granisetron [17-23]. Voltammetric methods have proved to be very sensitive for the determination of wide variety organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids [24-27].

This is due to their simple, reliable, low cost and fast (relatively short analysis time compared with the other techniques). The aims of this work are to establish the experimental conditions, to investigate the oxidation mechanism of Granisetron hydrochloride and to optimize the conditions for the determination and Validation

of the proposed method of this compound in pharmaceutical dosage forms and human serum using cyclic, differential pulse (DPV) and square wave (SWV) voltammetric techniques at a glassy carbon electrode (GCE). The diffusion nature of the drug at the GCE surface forms the bases for the electroanalytical determination of Granisetron in tablets and biological fluids such as urine. In addition, the method can be considered as a stability-indicating assay the proposed method is based on the oxidation of 1, 4-dihydropyridine group (Figure 1).

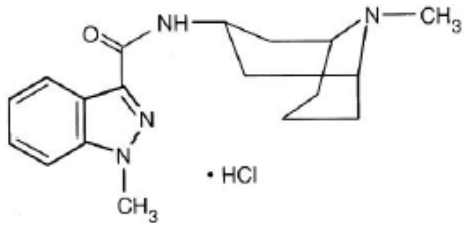

Figure 1: Chemical structure of granisetron hydrochloride.

Experimental

Apparatus

All voltammetric experiments were carried out using a Metrohm computrace voltammetric analyzer model 797 VA with Software Version 1.0 (Metrohm Switzerland) is the name of the control software for the PC-controlled 797 VA Computrace System for voltammetric analysis. With a three-electrode configuration: glassy carbon disc electrode as working electrode (mini glassy carbon disk electrode of the active zone: 2.8 mm, for ELCD 641/656), a Ag/AgCl (3 ML-1 KCl) as reference electrode and a platinum wire counter electrode were used. A digital pH/mV meter (JEANWAY 3510) with a glass combination electrode was used for the preparation of the buffer solution. A micropipette (Eppendorf-multipette plus) was used throughout the present experimental work.

Reagents

Granisetron hydrochloride was supplied from Otsuka maser Pharmaceutical, Egypt. Stock solutions of 10^{-3} M was prepared by dissolving an appropriate weighed of Granisetron in 25ml distilled water then complete the appropriate volume (100ml) with distilled water . The stock solution was stored in a refrigerator. Britton - Robinson (BR) buffer solutions (2.0-12) [28] were used as supporting electrolyte. All solutions were prepared by using analytical grade reagents in distilled water.

Working electrodes

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode (GCE) was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

Preparation of tablet sample assay

Ten tablets of granisetron hydrochloride were crushed into a fine powdered in a mortar. A sufficient amount of powder, for preparing a stock solution of 10^{-3}M was accurately weighed and transferred into a 25 ml calibrated flask and completed to the volume with methanol. It was sonicated for 5 min to provide complete dissolution. The content was allowed to settle after stirring magnetically for 5.0 min. The sample solution was filtered through a whatman no.42 filter paper. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte. This solution was then transferred to a voltammetric cell and DP and SW voltammograms were recorded. The drug content in per capsule was determined referring to the related regression equations.

The optimizations

To obtain the optimum pH, an appropriate amount of granisetron hydrochloride working standard solution 10^{-3} M was placed in the electrolytic cell, which contained 25 ml of BR buffer solution and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values (2.0-12) and the optimum pH was obtained

The study the effect of scan rate (υ) on the peak current (Ip) of Granisetron hydrochloride, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Granisetron hydrochloride standard solution 2.5 x 10^{-5M}, and the cyclic voltammograms were recorded at different scan rates over the scan range 10 - 250 mV/s. Plot log Ip versus log υ to know the nature of the process, diffusion controlled process or adsorption controlled process.

To study the effect of accumulation time, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Granisetron hydrochloride standard solution 2.5 x 10^{-5} M to select times with stirring at 1200 rpm at open circuit condition. After accumulation, the cyclic voltammograms were recorded then plot the peak current (Ip) versus time to obtain the optimum accumulation time.

The optimum instrumental conditions for the determination of Granisetron hydrochloride by using DPV and SWV methods were chosen from a study of the variation of the peak current with pulse amplitude (pulse width and scan rate). During the study, each parameter was changed while the others were kept constant: pulse amplitude over the range of 30-100 mV, pulse width 30-80 ms, and scan rate 20-250mV/s.

General procedure

Supporting electrolyte BR buffer (25 ml) was placed in the voltammetric cell and the required volume of standard Granisetron hydrochloride solution was added by micropipette. The solution was continuously stirred at 1200 rpm when accumulation potential (usually open circuit conditions) was applied for a certain time to the working electrode. At the end of accumulation period, the stirring was stopped, and after 5.0 sec rest period was allowed for the solution to become quiescent. The used drug was determined by using DPV and SWV methods. Aliquots of the drug solution of 10^{-3} M were introduced into the electrolytic cell and the procedure was repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at room temperature.

Preparation of urine sample

Determination of Granisetron hydrochloride in spiked urine samples, 1.0 ml of urine mixed with 24 ml of buffer of the optimum pH, without treatment then transferred to the voltammetric cell, and Carrey out the differential pulse voltammetric procedure as described above for the pure drug.

Results and Discussion

Electrochemical behavior of Granisetron hydrochloride at GCE

To understand the voltammetric process occurring and reversible behavior of Granisetron hydrochloride redox reactions on the glassy carbon electrode cyclic voltammetry was carried out. Another two different techniques were developed for the quantitative determination of Granisetron hydrochloride based on DPV and SWV methods. (Figure 2) shows the continuous cyclic voltammograms of 2 X 10^{-6} M granisetron hydrochloride on the GCE electrode at scan rate 100 mV/s in pH 9.0 BR buffer. During the first cycle appears to be an electro active drug. It was oxidized on glassy carbon electrode between 0 and1.0 V, producing one well-defined sensitive oxidation peak irreversible oxidation peak appears at 0.667 V on the anodic sweep. In the successive cycles, without the reduction peak, indicating an irreversible electrochemical process.

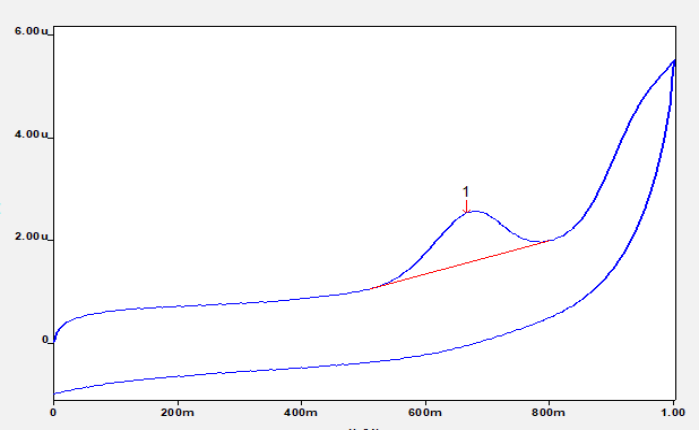

Figure 2: Cyclic voltammetric response of 2 X 10-6 M GRH at GCE electrode in pH 9.0 BP buffer. Scan rate is 100 mVs-1.

Effect of Ph

The effect of pH on the electrode reaction was investigated by Cyclic Voltammetry using Britton Robinson buffer (BR) at various pH ranging from 2 to 10. Figure 3 shows that the anodic peak current and potential was dependent on the pH. The anodic peak current increased with increasing pH in the range of pH 4.0 – 10.0, the Ip versus pH plot shows that peak current reaches a maximum value at pH 9. This means the maximum oxidation response of GRH appeared at pH 9.0, so pH 9 B-R buffer solution was used as optimum pH throughout all experiments. Figure 4 Shows Ep as a function of pH, with the increase of buffer pH the oxidation peak moved to the negative potential, indicating that proton take part in the electrode reaction. The linear regression equation between the oxidation peak potential and the buffer pH was calculated as Ep (V) = -0.067 pH + 1.2731, (R2 = 0 .9939).

Which is close to that given by the Nernstian equation for equal number of electrons and protons transfer processes. The slope value of 67 mV /pH indicated that the one electron is accompanied by one proton involved in the electrode reaction [28,29].

And indicated cyclic voltammetric measurements showed an irreversible nature of the oxidation process, since no reduction wave was observed on the cathodic branch.

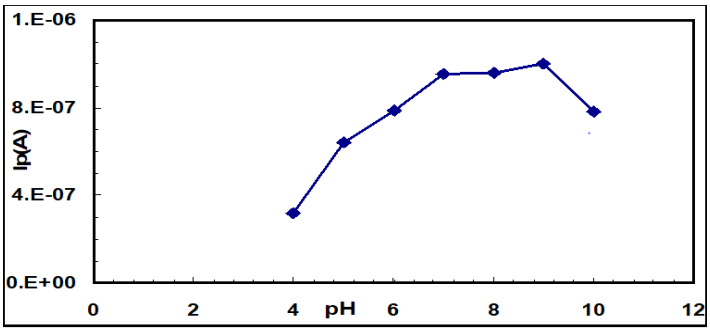

Figure 3: Effect of pH on peak current of 2 X 10-6 M GRH solution in B-R buffer at CPE paste and GCE at a scan rate 100 mV/s.

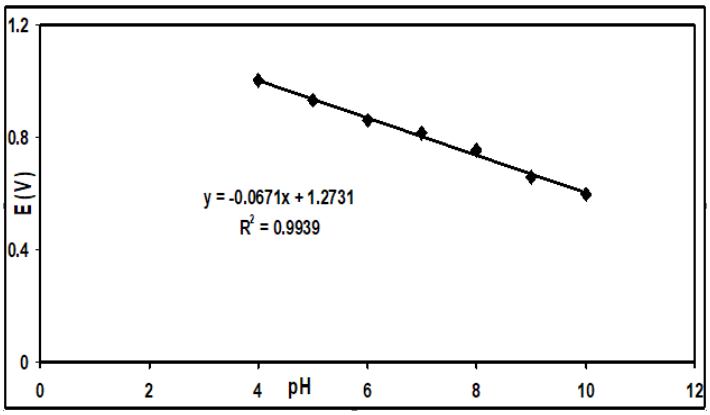

Figure 4: Plot Relationship of peak potential of GRH 2 X 10-6 M with electrolyte solution Ph 9 at GCE electrode.

Effect of scan rate

The effect of scan rate (ν) on the peak currents Ip and the peak potential Ep of GRH was studied. A linear relationship was observed between the oxidation peak current Ip and the square root of the scan rate According to the Randles–Sevcik equation showed a linear relation with a significant correlation coefficient of 0.996 Indicating diffusion controlled [30] process, what is expected for catalytic systems and it is advantageous for quantitative determination. in order to confirm that GRH oxidation occurred by an electro catalytically process the dependence of the parameter Ip/ ν1/2 on scan rate was plotted, as shown in (Figure 5). According to Nicholson and Shain [31], in (Figure 6) a non-linear relationship of the plot Ip/ ν1/2 against ν exhibit the characteristic shape of a typical EC (electrochemical-chemical) catalytic process [32].

Ip = (2.99x105) **n**(αna)1/2ACD1/2**υ**1/2

Ip (μ A) = 0.7389 υ1/2 (mVs-1)1/2 – 4.28, R2 = 0.996

Additionally, it is also found that the Ep value for the peak shows a linear variation with logarithmic scan rate (Figure 6). These findings suggest that the anodic processes, in the monomer oxidation potential range, are under diffusion control, and the corresponding system is completely irreversible [33].

With an increase in scan rate, the peak potential shifted to a more positive value, and a linear relationship was observed in the range 0.025 to 0.3 Vs-1 as shown in the equation can be expressed as.

Ep = 0.093 log υ + 0.746, r = 0.9925

As for an irreversible electrode process, according to Laviron, Ep is defined by the following equation [34].

$$E = E0 + \left[\frac{2.303RT}{\alpha nF}\right]\log\left[\frac{RTK}{\alpha nF}\right] + \left[\frac{2.303RT}{\alpha nF}\right]\log\upsilon$$

where α is the transfer coefficient, k0 the standard heterogeneous rate constant of the reaction, n the number of electron transferred, υ the scan rate, and E0 is the formal redox potential. Other symbols have their usual meanings. Thus value of αn can be easily calculated from the slope of Ep vs. log υ. In this system, the slope is 0.0671, taking T = 298, R = 8.314, and F = 96480, the αn was calculated to be 0.652. The value of α is 0.5. Further, the number of electron (n) in the electro oxidation of GRH was calculated to be 1.47~1. The value of k0 and E0 can be determined from the intercept of the previous plot (Figures 7-12).

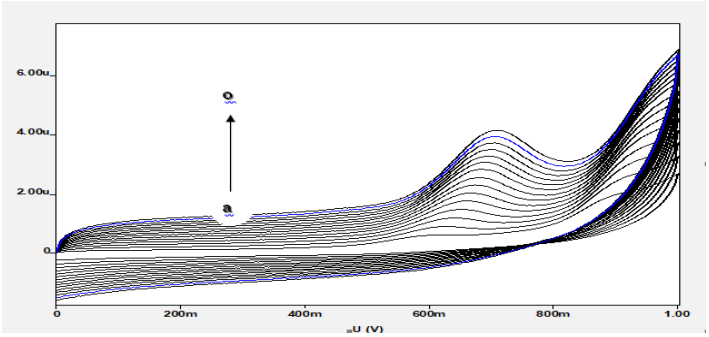

Figure 5: Scan rate (υ) (0.02 - 0.3 VS-1) in BR buffer of pH 9 of 2 X 10-6 M GRH solution at GCE.

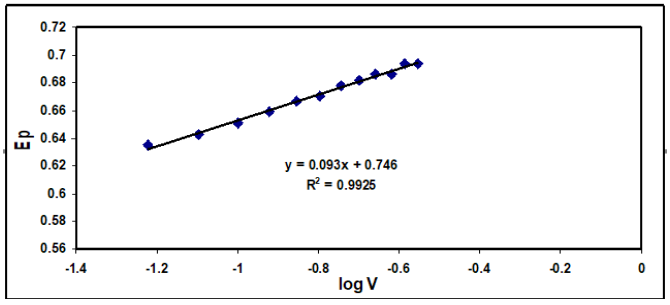

Figure 6: The variation of anodic peak potential Ep of 2 X 10-6 M GRH versus Log (υ) derived from the voltammetric data.

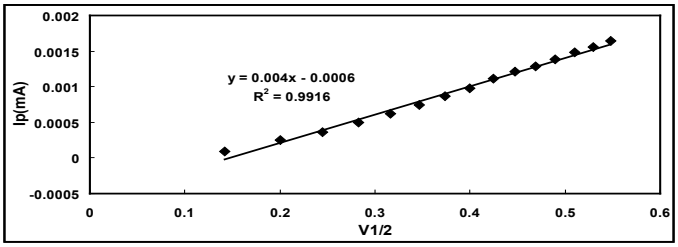

Figure 7: The variation of anodic peak current, Ip of 2 X 10-6 M GRH versus. square root of potential scan rate, V1/2

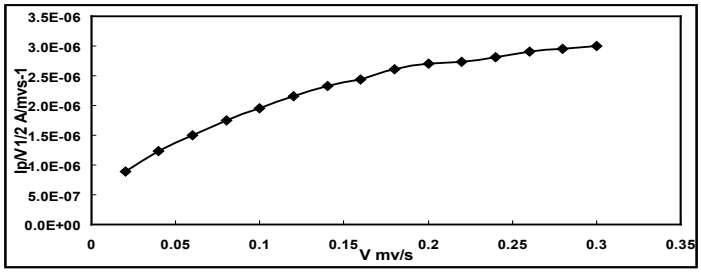

Figure 8: Plot of Ip/ v1/2 against scan rate ν for GRH 2 × 10− 6 M. Supporting electrolyte: B-R buffer (0.04 M) pH = 9.

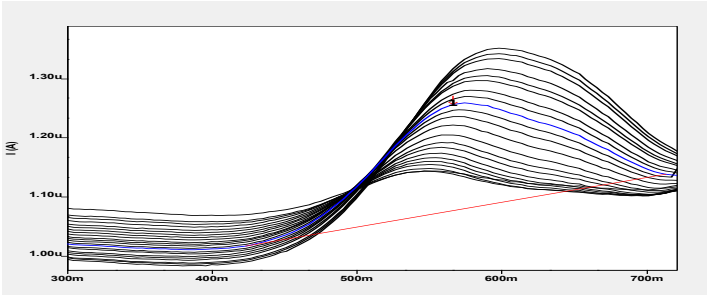

Figure 9: Differential pulse voltammograms (DPVs) obtained for a GRH on GCE in B–R buffer (pH 9) by changing the concentration of GRH.

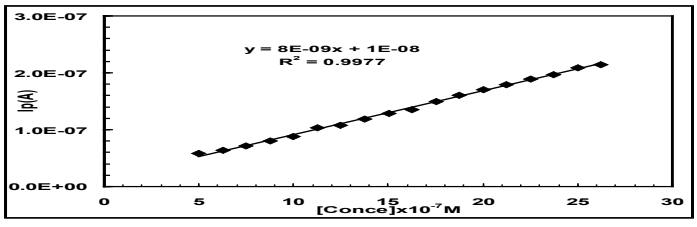

Figure 10: Calibration curve of 7.50 x 10-7 – 3.2 x 10-6M GRH drug at GCE electrode by using DPV method, pulse amplitude 50 mV and scan rate of 10 mVs-1, and scan rate of 10 mVs-1.

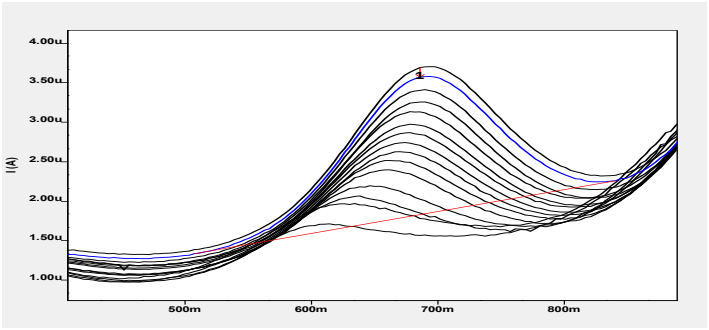

Figure 11: SWV voltammograms of 16 x 10-7 – 5.8 x 10-6M GRH drug at GCE electrode.

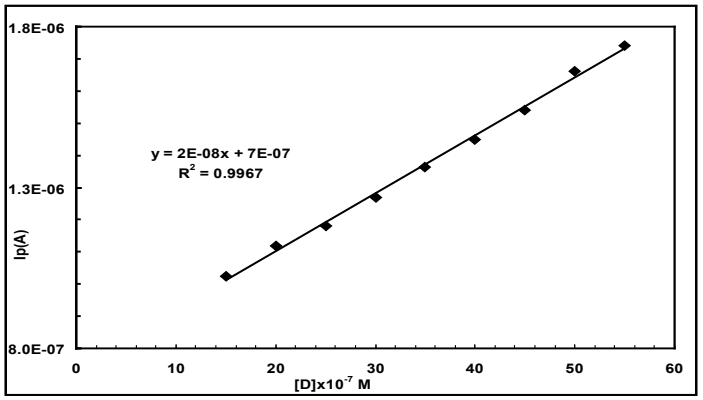

Figure 12: Calibration curve of 16 x 10^{-7} – 5.8 x 10-6M GRH drug at GCE electrode by using SWV method, Frequency 50 Hz, pulse amplitude 19.99 mV and scan rate 19.84 mVs-1.

Calibration curve

In two cases of the DPV and SWV the calibration curve was obtained with the GRH using the optimized experimental conditions adding aliquots consequently of GRH standard solution to the supporting electrolyte, the resulting voltammograms shown in (Figures 9, 11) reveal that while the peak potential remained almost constant at 0.56 V, the current increased with GRH concentration. A good linear correlation was obtained between current Ip of GRH electrochemical response and its concentration in the range 7.50 x 10^{-7} – 3.2 x 10^{-6}M and 16 x 10^{-7} – 5.8 x 10 M for DPV and SWV respectively. The standard curve was linear with correlation coefficient (r) not less than 0.9977 and 0.9967 for DPV and SWV respectively. The regression analysis data was calculated at 95% confidence and the regression equations as follow.

Ip = 8E-09C + 1E-08, r = 0.9977 DPV

Ip = 2E-08C + 7E-07, r = 0.9967 SWV

The detection limit (DL) and the quantification limit (QL) were calculated according to the equations: DL = 3 SD/B and QL = 10S b/B, where SD is the standard deviation of the coordinate from the line of best fit (linear coefficient) and B is the slope (angular coefficient) of this line the result listed in (Table 1).

	DPV	**SWV**
Linearity range (M)	$7.50 \times 10^{-7} – 3.2 \times 10^{-6}$	$16 \times 10^{-7} – 5.8 \times 10^{-6}$
Slope (AM−1)	8.00E-09	2.00E-08
Intercept (A)	1.00E-08	7.00E-07
Correlation coefficient (r)	0.9977	0.9967
Detection limit (µg/ml)	0.0302	0.0555
Quantification limit (µg/ml)	0.101	0.185
Error*%	0.123	0.121
RSD %	0.388	0.391

* Average of six determinations.

Table 1: Characteristics of GRH calibration plots using proposed voltammetric.

Analytical applications

Following the electro analysis procedure described above, the validity of the developed method for the determination of GRH in pharmaceutical formulation. The GRH content of commercially available capsules (Table 2) was determined directly by the proposed DPV and SWV methods after the required dissolving and filtration steps. Five aliquot of the dissolved sample were diluted to the required concentration level. As can be seen from (Table 2), the analytical results achieved by the proposed DPV and SWV procedure were in good agreement with those reported in the literature for the analysis of the same pharmaceutical capsules [35]. The agreement of the obtained result was tested by the paired F-test statistical [36]. The variances of both analytical methods were found to be not differing significantly.

Interferents	Labeled content	**Proposed method ±%RSD, n=5**		**Official method***	**F-test**		**T-test**	
		DPV	SWV	±%RSD, n=5	DPV	SWV	DPV	SWV
Kytril	1mg glaxo	100.01 ± 0.8	100.05 ± 0.2	100.01± 2.1	1.3	1.45	2.52	2.13
	3mg glaxo	100.2 ± 0.77	100.03± 0.77	99.52 ± 0.97	1.44	1.74	2.1	2.21
EM_EX	1mg amoun	100.21 ± 1.1	100.14 ± 1.05	99.67± 1.41	1.27	2.18	2.1	2.08
G-Setron	1.2mg amoun	100.93 ± 1.21	100.53 ± 0.35	99.98 ±0.99	1.44	1.36	1.47	1.22
granistron	1mg amoun	100.1 ± 0.59	100.2 ± 0.48	100.1 ± 1.02	1.87	1.47	2.48	2.31

Table 2: Evaluation of the accuracy and precision of the proposed and official methods for the determination of GRH. In its pharmaceutical forms at GCE using DPV and SWV [37].

Spiked human urine

GRH in spiked human urine samples was successfully determined by the described voltammetric methods (DPV and SWV) without the necessity for extraction of the drug to the analysis. Representative DPV and SWV voltammograms of GRH in spiked human urine recorded under the optimum operational conditions of the described voltammetric methods. No interfering peaks were observed in the blank human urine sample within the studied potential range. A quantitative analysis can be carried out by adding the standard solution of GRH into the detect system of urine samples, and the peak linearly increased in height. The calibration graph was used for the determination of spiked GRH in urine samples. The detection results of three urine samples obtained are listed in Table 3. The recovery determined was in the range from 99.5% to 100.28% and the RSD are given in (Table 3) [38].

Urine	Spiked (µM)	Detected(a) (µ M)		Recovery%		RSD%	
		DPV	SWV	DPV	SWV	DPV	SWV
Sample 1	0.2	0.199	0.201	99.5	101	1.07	0.93
Sample 2	0.5	0.501	0.499	100.2	100	0.85	0.87
Sample 3	0.7	0.698	0.702	99.71	100	0.91	0.94

(a)Mean average of five determinations.

Table 3: Determination of GRH in urine samples.

Interference studies

Under the same optimum experimental conditions, potential interferences such as some ions and organic compounds were investigated by addition of possible interferent to mixture solution containing 20 µM GRH. It was observed that the common ions such as K+, Na+, CO32−and NO3−did not interfere with GRH. No interfering peaks were also recorded around the peak potentials excess of common urinary compounds such as glucose, microcrystalline cellulose, urea and lactose. These mean that good selectivity of the proposed method and offer the promising possibilities for determination of GRH by SWV and DPV.

Conclusion

The voltammetric anodic peak of GRH at pH 3–11.0 most probably is due to diffusion–desorption process. The electrochemical process is irreversible and pH dependent. A validated differential pulse and square wave voltammetric procedure was developed and successfully applied to the estimation of GRH in bulk drug, tablet and human urine samples. For the analysis in the presence of biological material standard addition method is preferred. Two voltammetric methods have been developed for the determination of GRH in tablet dosage form and biological sample. The principal advantage of the proposed methods, over the official HPLC, one is that the excipients do not interfere and the separation procedure is not necessary. In summary, it is concluded that the proposed voltammetric techniques have the advantages of being simpler, faster, more selective and less expensive than official HPLC procedure. The proposed methods are rapid, requiring less than 5 min to run sample, and involves no sample preparation other than dissolving and transferring an aliquot to the supporting electrolyte. The developed methods are also selective and sensitive enough to determine GRH in commercial preparations and urine samples without any interference from the additives and endogenous substances.

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