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SELECTIVE AND SENSITIVE DETERMINATION OF REPAGLINIDE IN PHARMACEUTICALS BY VOLTAMMETRIC AND LC METHODS

Mehmet GUMUSTAS,[a,b] Gokce COSKUN[a] and Sibel A. OZKAN[a,*]

[a] Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100 Tandogan, Ankara, Turkey
[b] Hitit University, Science & Literature Faculty, Department of Chemistry, 19040 Corum, Turkey

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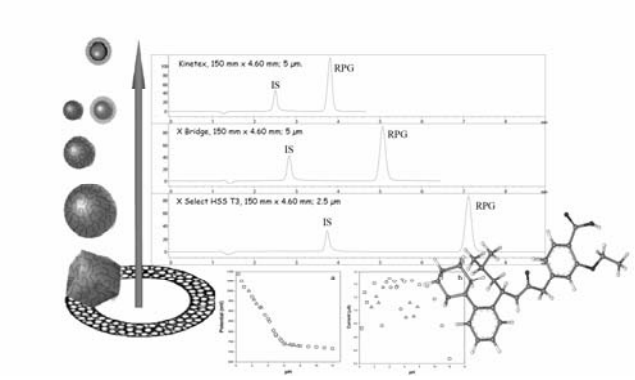
Detailed electrochemical study and novel voltammetric and LC methods are presented for the determination of repaglinide (RPG) in pharmaceuticals. Repaglinide in methanol: buffer solution (20:80; v/v) shows one or two anodic responses on glassy carbon electrode between pH 0.3 and 12.0 depending on the pH values. The HPLC and UPLC methods were developed using core shell columns with mobile phase consisting of 50:50; ACN:Water (0.05 % TFA; at pH: 3.0) (v/v) with UV detection at 215 nm. Finally, the proposed methods were successfully applied for the determination of RPG in pharmaceutical dosage forms. The proposed voltammetric and LC methods allow a number of cost and time saving benefits. RPG was also exposed to thermal, photolytic, oxidative stress, acid–base catalyzed hydrolyses, and the stressed samples were detected by the proposed RP-HPLC method.



INTRODUCTION

Repaglinide (RPG) (Scheme 1) is a new generation oral antidiabetic drug. It lowers blood glucose levels by stimulating the release of insulin from the pancreas. RPG is rapidly eliminated from the blood stream with a half-life of approximately 1 hour. [1-3]

Several methods have been reported for the determination of RPG in bulk, and its pharmaceutical dosage forms, and/or biological fluids.[4-12] Some of these techniques are time consuming because of expensive sample pretreatment and beyond the reach of small laboratories.

LC and voltammetric techniques have been used for the determination of a wide range of drug active compounds with the advantages that, in most instances, there is no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of electrode mechanism. Hence, redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity. [13-20]

From the analytical point of view the importance of the electrochemical behavior and mechanism of RPG, there is no report on electrode processes of the substance in literature. Therefore, the aim of this study is to establish the experimental conditions to investigate the oxidation behavior of RPG by cyclic and linear sweep voltammetry. Also, up to date, no detailed reports on the forced degradation study on these

* Corresponding author: ozkan@pharmacy.ankara.edu.tr, tel.: +903122033175, fax: +90 312 203 1081

compounds under the conditions of hydrolysis (across wide pH range), oxidation, dry heat, and photolysis are available. An ideal stability indicating chromatographic method should be able to resolve the drug from its potential impurities and degradation products. It also requires that analytical test procedures for samples should be stability-indicating, and they should be fully validated.[18-22] Therefore, the aim of the reported research was also the development of a stability indicating study. Hence, related tests were performed on RPG substance as per International Conference on Harmonization (ICH) guidelines.[23]

The goal of this study was to develop and validate different assay methods that had less run times, included simple and automatic sample processing procedures, had a lower limit of quantification (LOQ) that may be adequate for sensitive pharmaceutical studies and had sample volume requirements that would accommodate the small sample sizes. To this end, we chose to develop and validate electroanalytical and LC methods that would use small sample volume, and require simple sample preparation. Also, the proposed methods gave us an opportunity to work with less amount of organic waste and sample, and to be able to provide faster analysis time with better peak shapes and higher resolution values.

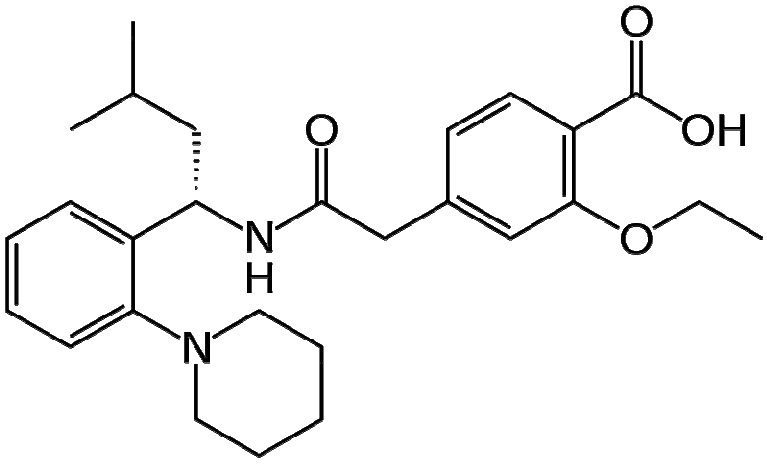
RESULTS AND DISCUSSION

Electroanalytical study

No detailed electrochemical information was available concerning the solid electrode behavior of the working substance. Therefore, several measurements with varied electroanalytical methods like linear sweep, cyclic, square wave and differential pulse voltammetry were investigated by different supporting electrolytes and buffers in order to obtain some information. RPG was electrochemically oxidized in a broad pH range (0.3–12.0) using a glassy carbon (GC) disc electrode. The cyclic voltammetric behavior of RPG yielded one well-defined peak in strongly acidic solution such as 0.2 M H_2SO_4 (Fig. 1a). As the pH increased, two peaks were observed (Fig. 1b-1d). Cyclic voltammetric measurements showed an irreversible nature of the oxidation process (Fig. 1). By reversing at +1.70 V, no reduction wave or peak corresponding to the anodic peaks was observed on the cathodic branch. In the repetitive cyclic voltammetric method, it was observed that at the second and higher cycles, the RPG peaks decreased. This phenomenon may be partly attributed to the consumption of adsorbed RPG on the electrode surface.

The effects of pH on peak potential and peak intensity were studied using CV, DPV and SWV techniques separately. However, the E_p-pH and I_p-pH results were given using DPV results in Fig. 2a and 2b, respectively, in order to obtain better signal. The peak potential of the oxidation process moved to less positive potential and the second oxidation peak was obtained by raising the pH. The plot of the first peak potential versus pH showed two linear segments between the ranges of 0.3-6.0 and 6.0-12.0. These linarities can be expressed by the following equations in all supporting electrolytes using the DPV technique:

$$Ep\ (mV) = 1045.9 - 59.88\ pH;\ r : 0.981$$
(between pH 0.3 and 6.0)
$$Ep\ (mV) = 746.22 - 5.75\ pH;\ r : 0.992$$
(between pH 6.0 and 12.0)



Scheme 1 – Molecular structure of REP.

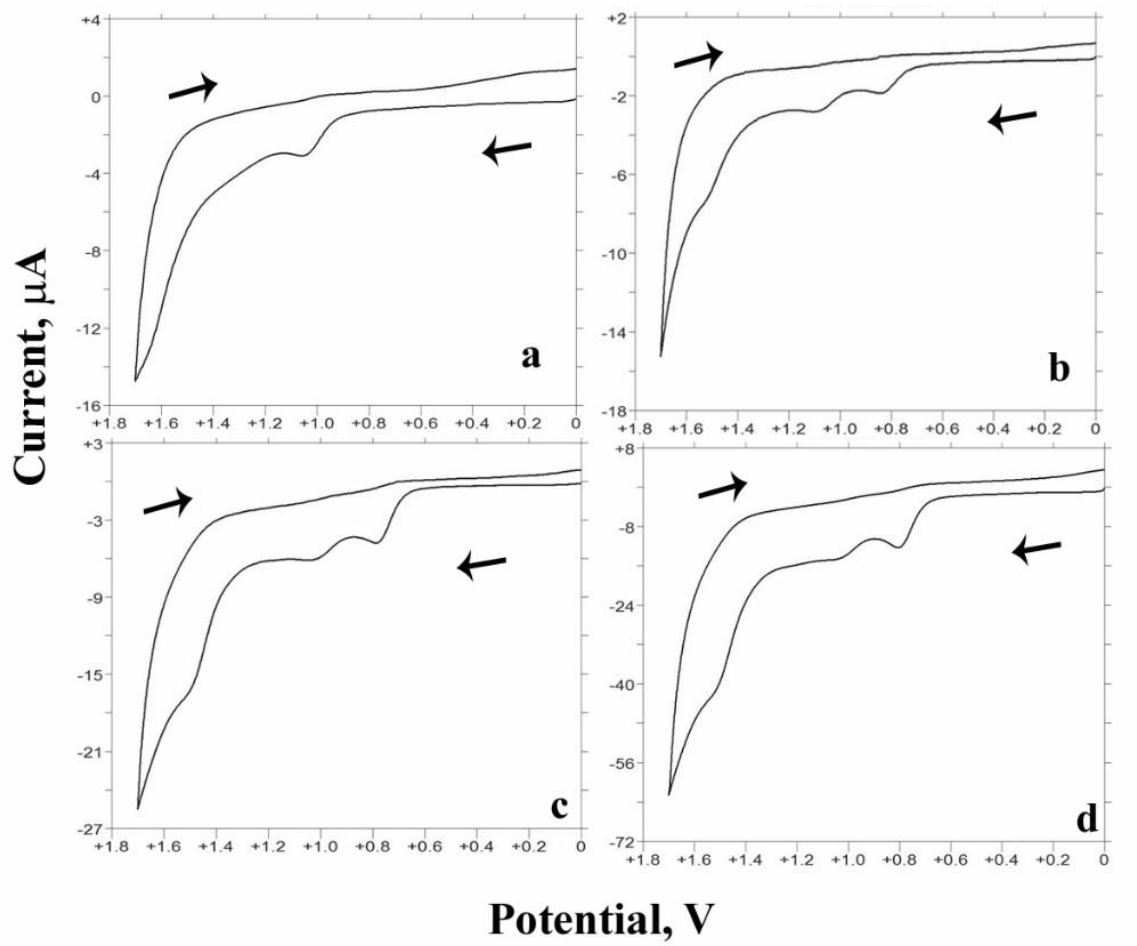


Fig. 1 – Cyclic voltammograms of 45.3 μg.mL^{-1} RPG in 0.2 M H_2SO_4 **(a)** and Britton-Robinson buffers at pH 4.0 **(b)**; at pH 5.0 **(c)**; at pH 9.5 **(d).** Scan rate 100 mV s^{-1}.

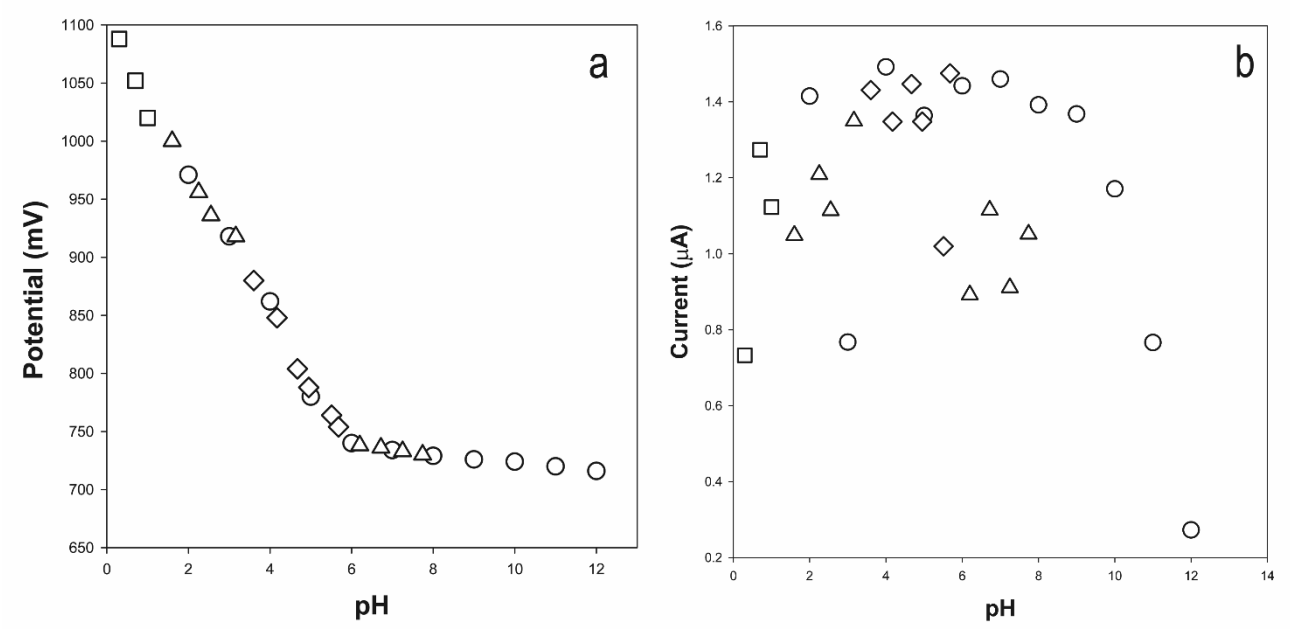
The second equation and Fig. 2a showed that the peak potential almost becomes pH independent (between pH 6.0 and 12.0). The acidic pKa of RPG is 3.9, and the pKa with amine is 6.0. [24] The intersection points of the curves are close to the pKa_2 value of piperidinyl molecule present in RPG molecule which has pKa as 6.0. [24] RPG has another intersection point at pH 3.9. It can be explained by changes in protonation of the acid–base functions in the molecule. The influence of pH on the signal (current) of RPG at glassy carbon disc electrode was also studied. The Ip versus pH plot (Fig. 2b) shows that peak current is maximum in the acidic media. The experimental results showed that the shapes of the curves were better in 0.2 M H_2SO_4 than in other media. Hence, 0.2 M H_2SO_4 was chosen with respect to sharp response and better peak shape for the pharmaceutically active compound assay in its dosage form.

The effects of the potential scan rate between 5.0-1000 mV.s^{-1} on the peak current and potential of RPG were evaluated. A 170 mV positive shift in the peak potential confirmed the irreversibility of the oxidation process. Scan rate studies were carried out to assess whether the processes at the glassy carbon electrode was under diffusion or adsorption control. From the scan rate studies, a linear dependence was found between the peak intensity Ip (μA) and the square root of the scan rate $v^{1/2}$ (mV.s^{-1}). This value demonstrates the diffusional behavior. The equation is noted below in 0.2 M H_2SO_4:

$$ip\ (\mu A) = 0.19\ v^{1/2}(mVs^{-1}) + 0.11,\ r = 0.999\ (n = 10)$$

$$\log ip\ (\mu A) = 0.46 \log v\ (mVs^{-1}) - 0.60,\ r = 0.999\ (n = 10)$$

A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.46, very close to the theoretical value of 0.50, which is expressed for an ideal reaction to the diffusion controlled electrode process. [25]



a b

Fig. 2 – Effects of pH on RPG (45.30 μg.mL^{-1}) Ep (peak potentials) **(a)**; and Ip (peak currents) **(b)**. (□) 0.1 M H_2SO_4; (Δ) 0.2 M phosphate buffer; (◊) 0.2 M acetate buffer; (○) 0.04 M BR buffer using DPV technique.

The Tafel plots (log i vs E) were obtained with a scan rate of 5 mV.s^{-1} beginning from a steady-state potential in 0.2 M H_2SO_4. *α*n value of anodic reaction from the slope of the linear part of this plot was found to be 0.36. The exchange current density (i_o) is 1.58×10^{-12} A.cm^{-2} for this system. These values together with the absence of cathodic waves in cyclic voltammetry (Fig. 1) indicated the irreversibility of the oxidation reaction.

Considering the results above and bearing the electrochemical behavior of piperidine in mind, the parent molecule of RPG at GC electrode,[12] we may assume that the oxidation process may be located on the nitrogen group in the piperidine moiety of the molecule. As reported for other piperidine compounds,[17,26] cyclic voltammograms of RPG obtained with 100 mV.s^{-1} presents an irreversible oxidation process at all pH values (Fig. 1) attributed to the oxidation of the nitrogen group of the piperidine moiety of RPG. The oxidation of the nitrogen group in the piperidine moiety has been followed by two-step oxidation mechanism. RPG was electrochemically oxidized by 2 e$^-$ process to give piperidine N-oxide at the glassy carbon electrode over the investigated pH range (Fig. 1).

DP and SW Voltammetric Analysis

Different buffers and pH values, namely, sulphuric acid, Britton–Robinson, acetate and phosphate buffers, were examined. The best results with respect to signal enhancement and peak shape accompanied by sharper response were obtained with 0.2 M H_2SO_4. In order to develop sensitive and selective electroanalytical methods of RPG, DPV and SWV techniques were selected. DPV and especially SWV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits.

First of all, the important parameter that affects the shape of the electroanalytical signal, peak current increment over was obtained. For this reason, pulse amplitude, frequency, potential step, pulse width and scan rate were studied. These parameters need to be optimized to give a well-resolved large peak and to give good selectivity and larger sensitivity in the determination. They were used for SWV as: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV; and for DPV as: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV.s^{-1}.

Under selected experimental conditions, the variation of peak current with the RPG concentration was studied by means of DPV and SWV (Fig. 3a and b). Both the peak height and the peak shape were taken in consideration during 0.2 M H_2SO_4. Therefore, two calibration graphs from the standard solution of RPG according to the procedures described above were constructed by using DPV and SWV techniques.

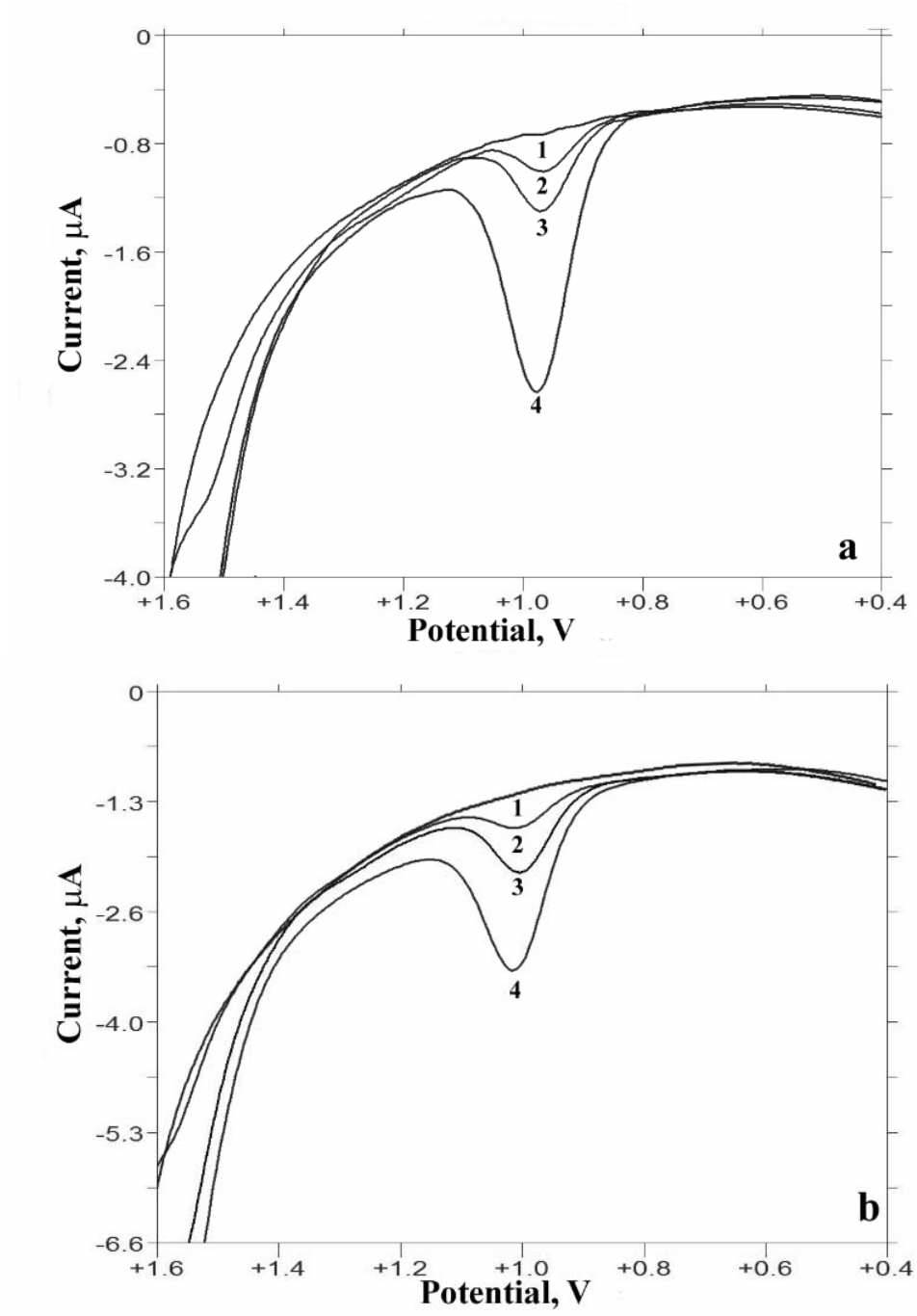


Fig. 3 – **(a)** DP, **(b)** SW voltammograms of RPG in 0.2 M H_2SO_4 using GC electrode. **(1)** blank, **(2)** 9.06 µg.mL^{-1}; **(3)** 27.17 µg.mL^{-1}; **(4)** 90.60 µg.mL^{-1} RPG concentrations.

UPLC and HPLC Analysis

Another objective of this study was to develop sensitive, selective, rapid and fully validated stability indicating HPLC and UPLC procedures for the determination of RPG in pharmaceutical preparations and in stress degradation samples. Therefore, it was of primary importance to investigate a suitable wavelength for the detection of RPG in order to achieve the highest sensitivity.

For the separation, different types of columns were tested such as X Bridge and X Select HSS T3 by Waters, and Kinetex by Phenomenex. All columns that were used in HPLC had the same dimensions as 150 mm x 4.60 mm I.D. with different particle sizes such as 5 µm, 2.5 µm and 5 µm, respectively. Fine and more efficient separations were performed at Kinetex C18 (150 mm x 4.60 mm, 5µm, Phenomenex, USA) analytical column for HPLC as shown in Fig. 4.

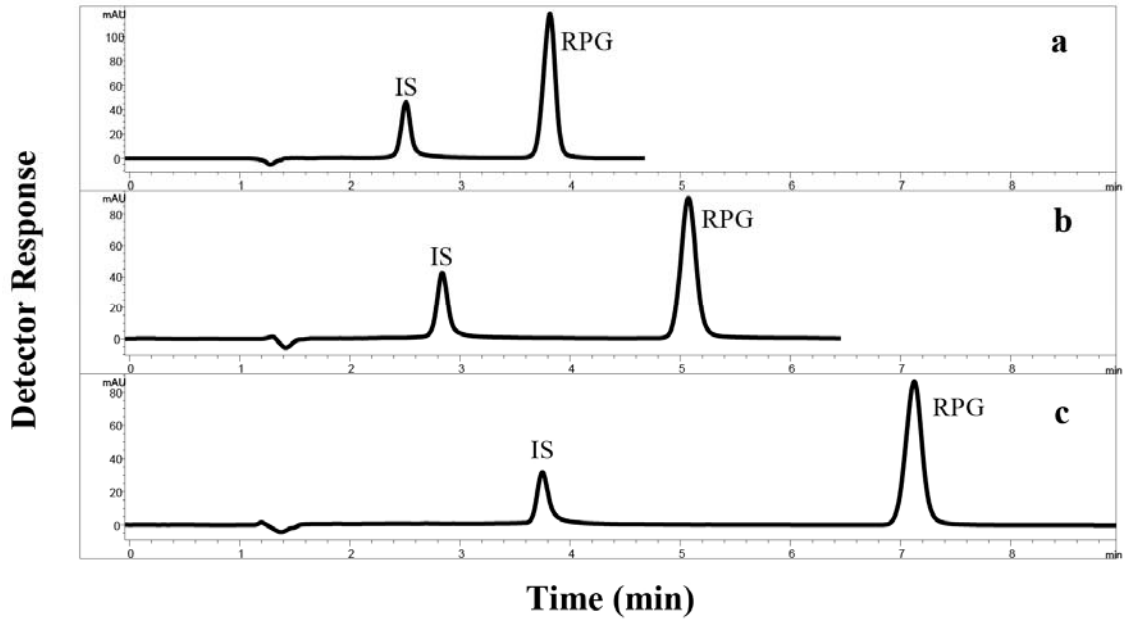


Fig. 4 – Comparison between tested HPLC columns **a)** Kinetex, 150 mm x 4.60 mm; 5 µm. **b)** X Bridge, 150 mm x 4.60 mm; 5 µm. **c)** X Select HSS T3, 150 mm x 4.60 mm; 2.5 µm.

After that result, Kinetex (50 mm x 2.1 mm, 1.3 µm, Phenomenex, USA) was selected for UPLC system as stationary phase for a better comparison and ease of transfer. The selected stationary phase that includes core-shell silica particles was given the better peak shape with faster analysis time (Fig. 4). The mobile phase was chosen after several trials with methanol, acetonitrile, water and different buffer solutions in various proportions at different pH values. Also, experimental conditions, such as pH of the mobile phase, acetonitrile percentage etc. were optimized to provide efficient, accurate, rapid, precise, and reproducible results for the determination of RPG. Chromatographic elution was performed using 50:50; ACN:Water [(0.05 % TFA; pH: 3.0), (v/v)] as the mobile phase at 25 ^{o}C with the flow rate of 1 mL.min^{-1} for HPLC and 0.3 mL.min^{-1} for UPLC. The maximum absorption of RPG was found to be at 215 nm and this wavelength was chosen for the analysis. The injection volumes were used as 10 µL and 2 µL for HPLC and UPLC, respectively.

Using these conditions, the retention times for RPG and IS were observed to be 3.82 and 2.51 min, for HPLC (Fig. 5a and c) and 2.10 and 0.86 min, for UPLC (Fig. 5b and d), respectively. Total time of analysis was less than 4.5 min for HPLC and 2.5 min for UPLC (Fig. 5).

In order to determine the adequate resolution and reproducibility of the proposed methodology, system suitability parameters including retention times, selectivity, resolution factor, tailing factor and plate number (to show the efficiency of the method) were investigated. As an integral part of liquid chromatographic method development, system suitability test results were calculated. [27, 28] It can be defined to ensure that the method can generate results of acceptable accuracy and precision with efficient separation. Generally, at least two of these criteria are required to demonstrate that suitability of the tested system that were carried out on freshly prepared standard stock solutions of RPG. The results are summarized and tabulated in Table 4. The obtained results were in agreement with the United States Pharmacopeial Convention (USP) requirements.[27]

Validation of the proposed methods

The proposed methods were tested for linearity, range, repeatability, reproducibility, ruggedness, selectivity and specificity. According to the tests, it can be said that satisfactory results were obtained and the proposed methods complied with International Conference on Harmonization (ICH) [22] validation guidelines.

On the basis of the electro oxidation of RPG at GC electrode, electroanalytical methods were developed involving DPV and SWV methods for the determination of RPG under investigation (Fig. 3 a and b).

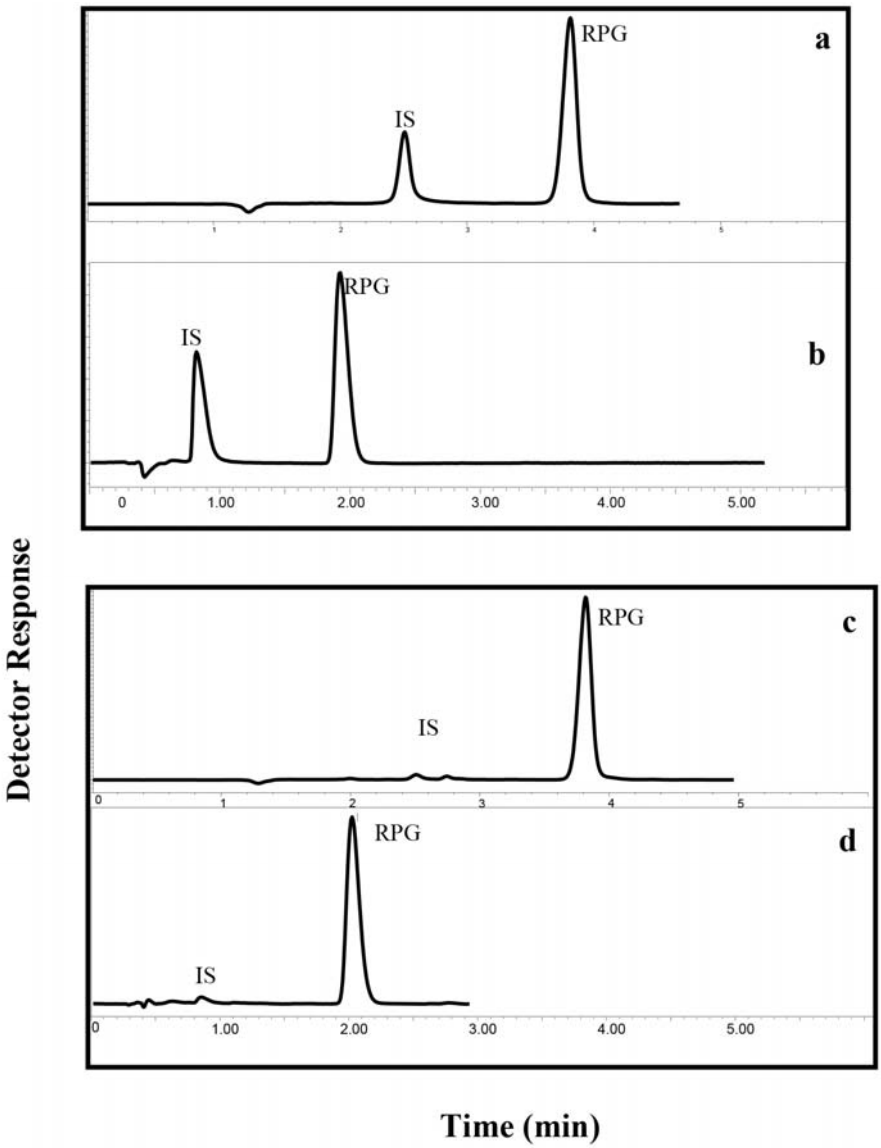


Fig. 5 – HPLC **(a**,**c)** and UPLC **(b**,**d)** chromatograms for the determination of RPG; **(a**,**b)** bulk form 25 μg.mL^{-1} (IS) and 25 μg.mL^{-1} RPG; **(c**,**d)** pharmaceutical preparation 2 μg.mL^{-1} (IS) and 40 μg.mL^{-1}.

A linear relationship in the concentration range between 1.81 and 90.58 μg.mL^{-1} was found by DPV and SWV methods, indicating that the response was diffusion controlled in this range. Above 90.58 μg.mL^{-1} concentration a loss of linearity was probably due to the adsorption of RPG on the electrode surface. Also, linear calibration plots for the assay methods of UPLC and HPLC were obtained over the calibration ranges tested between 0.2 and 300 μg.mL^{-1}. Using the optimum conditions described above, a satisfactory chromatographic peak resolution was obtained from the standard compound in a short analysis time as can be seen in Fig. 5 a and b for HPLC and UPLC, respectively. For both methods, sharp and symmetrical single peaks were obtained with fine resolution between RPG and IS.

The correlation coefficient obtained was greater than 0.999 for all methods. The results showed an excellent correlation between the peak area and concentration of RPG. The detection limit (LOD) and limit of quantification (LOQ) of the procedures (Table 2) were calculated according to the 3 s/m and 10 s/m criteria, respectively, where s is the standard deviation of the response and m is the slope of the related calibration plot.[18-23]

To judge the quality of the method, precision and accuracy were determined. For the precision, the inter-day and intra-day (ruggedness) reproducibility of peak potentials/retention times and peak currents/peak area ratio were tested by repeating five experiments on the selected working concentrations. Obtained experiment values of RPG are expressed in terms of RSD % values

calculated from the data of within-day results. RSD % values were found to be well indicating a good repeatability (Table 2). The ruggedness (intra-day) of assay methods are defined as the degree of reproducibility of the results obtained by analysis of the samples under a variety of normal test conditions such as different days and different lots of reagents etc. The samples of the selected concentration levels of RPG in different days and various lots of reagents were analyzed at same laboratory. The RPG samples were analyzed for 3 consecutive days by performing five measurements on each day. Related parameters are reported in Table 2. The obtained data were within 2.0 % RSD (Table 2). The results indicate an acceptable precision in measures made on the same day and on separate days.

In order to know the applicability of the proposed methods, a commercial tablet formulation containing RPG was studied using all proposed methods (Table 3). Consequently, application of DPV and SWV methods using GC electrode and both chromatographic methods to pharmaceutical preparations is possible after a few simple filtration and dilution steps. Five replicate determinations were made and very well results (Table 3) were obtained for RPG and these results were in good agreement with the labeled claims. The analyses were performed without any interference from the additives present in tablet (Fig. 5c and d).

The accuracy of the method was examined by standard addition to the dosage form. Mean recovery, RSD % and Bias % results for the studied compound were calculated from five replicate analyses (Table 3). The results which were obtained from this investigation showed good precision and accuracy; therefore, we can easily say that excipients in the pharmaceutical formulation interfere with the main signal of RPG in tablets in neither voltammetric nor chromatographic methods. The obtained results were compared using DPV method as the judge method for the statistical evaluations. For this reason Student's *t*- and *F*-ratio tests were selected and they indicated that there were no significant differences among the results obtained by the four methods for the same batch, at the 95% confidence level.

Results of forced degradation studies

Degradation studies were performed with stress conditions of UV light, heating in an oven (at 100 °C), acid and base hydrolysis, and oxidation to evaluate the ability of the proposed method to separate RPG from its possible degradation products. [23, 29] For heating and stressed UV light studies, the study period was 24 hours; for acid and base hydrolysis and oxidation, all samples were mixed well separately using vortex and kept waiting for 60 min, after the application of stress conditions. The drug was exposed in 0.1 M HCl and 0.1 M NaOH for 60 min for acid and alkaline hydrolysis. RPG has shown significant sensitivity towards the treatment of 0.1 N HCl and 0.1 N NaOH. The drug gradually underwent degradation in 0.1 N HCl and 0.1 N NaOH and some unknown degradation compounds were observed using HPLC (Fig. 6-1 b-d) and UPLC (Fig. 6-2 b-d). Also, when using oxidative condition solution, in 3% H_2O_2 were stored for 60 min, a relatively high degradation response and major degradation peak was observed. No major degradation products were observed when the sample was stressed in 254 nm UV light and 100°C heating conditions after 6 h using HPLC (Fig. 6-1 e and f) and UPLC (Fig. 6-2 e and f).

Table 1

System suitability parameters of the proposed LC methods

	UPLC		HPLC		Acceptable criteria
Parameters	**IS**	**RPG**	**IS**	**RPG**	
Retention time (min)	0.86	2.08	2.51	3.82	-
Capacity (k)	1.00	3.65	1.02	2.08	$1 \leq k \leq 10$
Resolution (R_s)	-	6.09	-	7.08	Rs >2.0
Theoretical plates (N) (Plate/Column)	3882	16400	3801	5509	N > 2000
Selectivity factor (α)	-	3.65	-	2.04	$\alpha \geq 1.5$
Tailing factor	0.95	1.05	0.80	1.13	≤2.0

Table 2

Regression data of the calibration lines for quantitative determination of RPG by proposed techniques

Technique	Chromatographic		Electroanalytical	
	UPLC	**HPLC**	**DPV**	**SWV**
Linearity Range (μg.mL^{-1})	0.2-300	0.2-300	1.81-90.58	1.81-90.58
Slope	1.670	0.960	0.017	0.018
Intercept	-0.117	0.598	0.046	0.023
Correlation Coefficient	0.999	0.999	0.999	0.999
SE of slope	1.21×10^{-2}	2.25×10^{-3}	2.17×10^{-4}	1.77×10^{-4}
SE of intercept	1.450	2.73×10^{-1}	7.76×10^{-3}	6.31×10^{-3}
Limit of Detection (μg.mL^{-1})	0.012	0.017	0.278	0.230
Limit of Quantification (μg.mL^{-1})	0.035	0.051	0.842	0.700
Within-day Precision [a] (RSD %)	1.179	0.995	1.370	0.730
Between-day Precision [a] (RSD %)	1.220	1.252	1.830	1.980

[a] Each value is the mean of five experiments

Table 3

Assay results and mean recovery studies of RPG in pharmaceutical dosage forms

Technique	Chromatographic		Electroanalytical	
	UPLC	**HPLC**	**DPV**	**SWV**
Labeled claim (mg)	2.00	2.00	2.00	2.00
Amount found (mg) [a]	2.01	2.00	1.99	1.99
RSD (%) [a]	0.47	0.78	0.50	0.42
Bias (%)	100.31	0.25	0.75	0.30
t_{value}	0.70	0.77	t_{theo}: 2.31	0.85
F_{value}	0.85	0.25	F_{theo}: 6.39	1.45
Added (mg)	1.00	1.00	1.00	1.00
Found (mg) [a]	1.00	1.01	1.00	0.99
Recovery (%)	100.26	100.72	100.27	99.42
RSD % of recovery [a]	0.37	0.44	0.69	0.48
Bias (%)	-0.26	-0.70	-0.27	0.58

[a] Each value is the mean of five experiments

Table 4

Results of hydrolytic, oxidizing, thermal and photolytic stress conditions with using HPLC

Stress Conditions	% Degradation of RPG	
	UPLC	HPLC
HCl (0.1 M)	0.81	0.29
NaOH (0.1 M)	0.72	0.48
H_2O_2 (3 %)	5.85	5.57*
6 hours UV	ND	ND
6 hours 100 ^oC	ND	ND

ND; No degradation; * Peak broadening was detected

Moreover, peak purity test results were checked from DAD detector and the purity factors confirmed that the RPG peak was homogeneous and pure in all the analyzed stress samples. The purity factors were more than the purity thresholds. It indicated that no additional peaks were co-eluted with RPG, confirming the ability of present method to determine the main analyte in the presence of interferences. However, an appropriate blank was injected before analysis of the forced degradation samples.

EXPERIMENTAL

Instrumentation

Electroanalytical experiments were recorded using BAS 100W (Bioanalytical System, USA) electrochemical analyzer with a standard three-electrode configuration. The three electrode system consisted of a glassy carbon (GC) as working, a platinum wire as counter and an Ag/AgCl saturated KCl as reference electrodes. GC working electrode was polished manually with aqueous slurry of alumina powder (Φ = 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad) just before each measurement. Operating conditions for SWV and DPV were indicated above.

Chromatographic separations were performed at 25 ^oC using 50:50; ACN:Water (0.05 % TFA; pH: 3.0) (v/v) as the mobile phase. Detection wavelength was selected as 215 nm. Agilent 1100 series HPLC system (Willmington, DE, USA) was used for method development and validation studies for HPLC. Separations were performed at Kinetex C18 (150 mm x 4.60 mm, 5μm, Phenomenex, USA) analytical column. The flow rate was 1 $mL.min^{-1}$ and the injection volume was 10 μL.

UPLC system consisted of Waters Acquity (Waters, Milford, USA) system and separation was performed using a Kinetex C18 (50 mm x 2.1 mm, 1.3 μm) stationary phase with a flow rate of 0.3 $mL.min^{-1}$ and an injection volume of 2 μL. The controlling of the system and processing of the data were performed by Empower 2 software (Waters, Milford, USA).

Before injections, both HPLC and UPLC systems were conditioned using degassed and filtered mobile phase in about 20 minutes. Samples were filtrated through 0.45 μm syringe filter (Millipore Corporation, Bedford, USA).

Materials and reagents

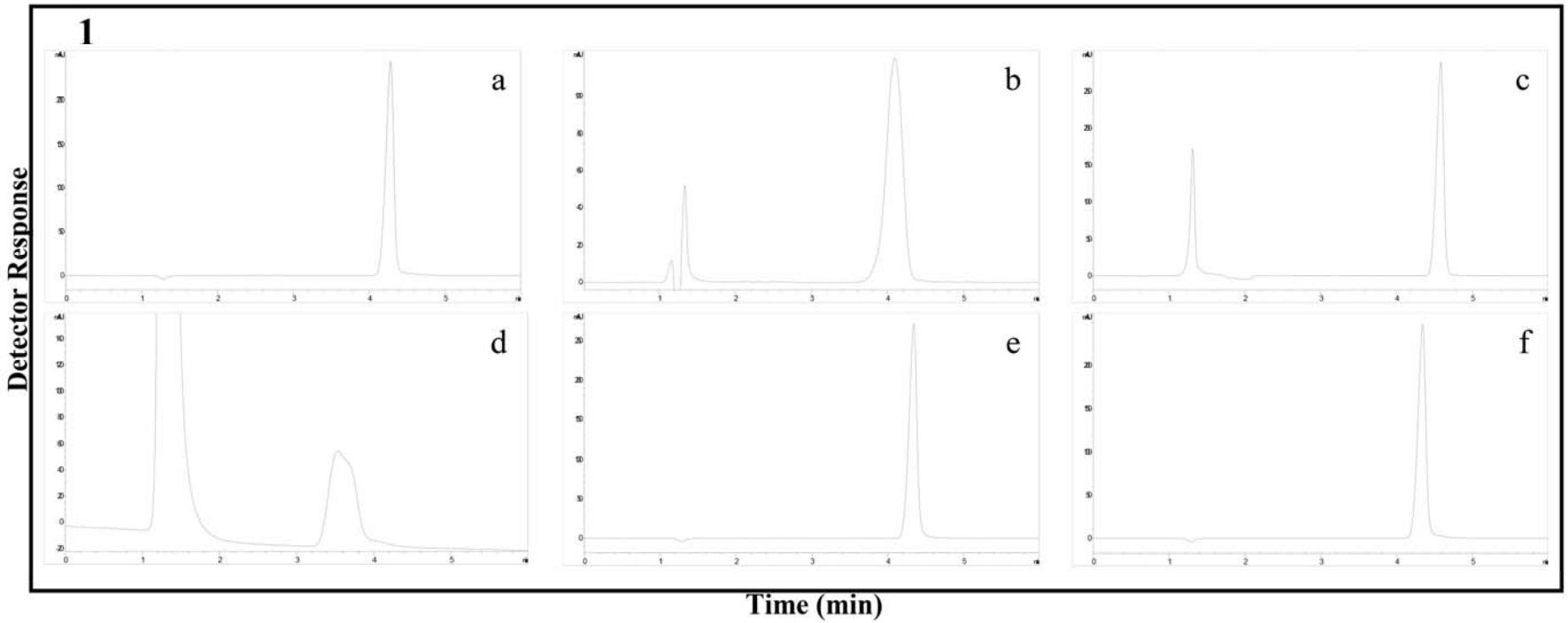
RPG and its pharmaceutical dosage form (Diafree® tablet) were kindly provided by Biofarma (Istanbul, Turkey). All chemicals that were used for the preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma, USA). Stock solutions of RPG (1×10^{-3} M=452.9 $\mu g.mL^{-1}$) were prepared in methanol and preserved in the dark in a refrigerator. All other necessary solutions including water were also prepared using double distilled water.

Four different supporting electrolytes, namely, sulphuric acid (0.1 M and 0.5 M), phosphate buffer (0.2 M; pH 2.0–11.0), acetate buffer (0.2 M; pH 3.5–5.7) and Britton–Robinson buffer (0.04 M; pH 2.0–12.0), were prepared in bidistilled water. The working solutions of the voltammetric measurements were prepared by serial dilution of the stock solution with selected supporting electrolytes and contained a constant amount of (20 %) methanol.

Acetonitrile and methanol were of chromatographic grade purchased from Sigma-Aldrich (Munich, Germany). All other chemicals were of commercially analytical reagent grade. Chromatographically usable water was obtained following distillation in glass and passage through a Milli-Q® system (EMD Millipore, MA, USA), and was used to prepare all the necessary solutions. Trifluoroacetic acid (TFA) was from Merck (Darmstadt, Germany). Sodium hydroxide was purchased from Sigma-Aldrich (Munich, Germany). Working solutions were diluted with the mobile phase for the preparation of the necessary concentrations for chromatographic studies.

Preparation of stock standard solution

Stock standard solution of RPG was prepared in methanol at a concentration of approximately 452.9 $\mu g.mL^{-1}$ for electroanalytical studies and 500 $\mu g.mL^{-1}$ for chromatographic assays. For voltammetric measurements standard solutions were prepared by the dilution of the stock solution with selected supporting electrolyte to give solutions containing RPG in the concentration range of 1.81-90.58 $\mu g.mL^{-1}$. The calibration curve for DPV and SWV analysis was constructed by plotting the peak current against the RPG concentration. The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias %). One example of relative error is the accuracy, which describes the deviation from the expected results. The ruggedness and precision were checked on different days, within day (n= 5), and between days (n= 5) for three different concentrations. RSD % and Bias % were calculated to check the ruggedness and precision of the method.[20-24]


1
a
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Detector Response
Time (min)

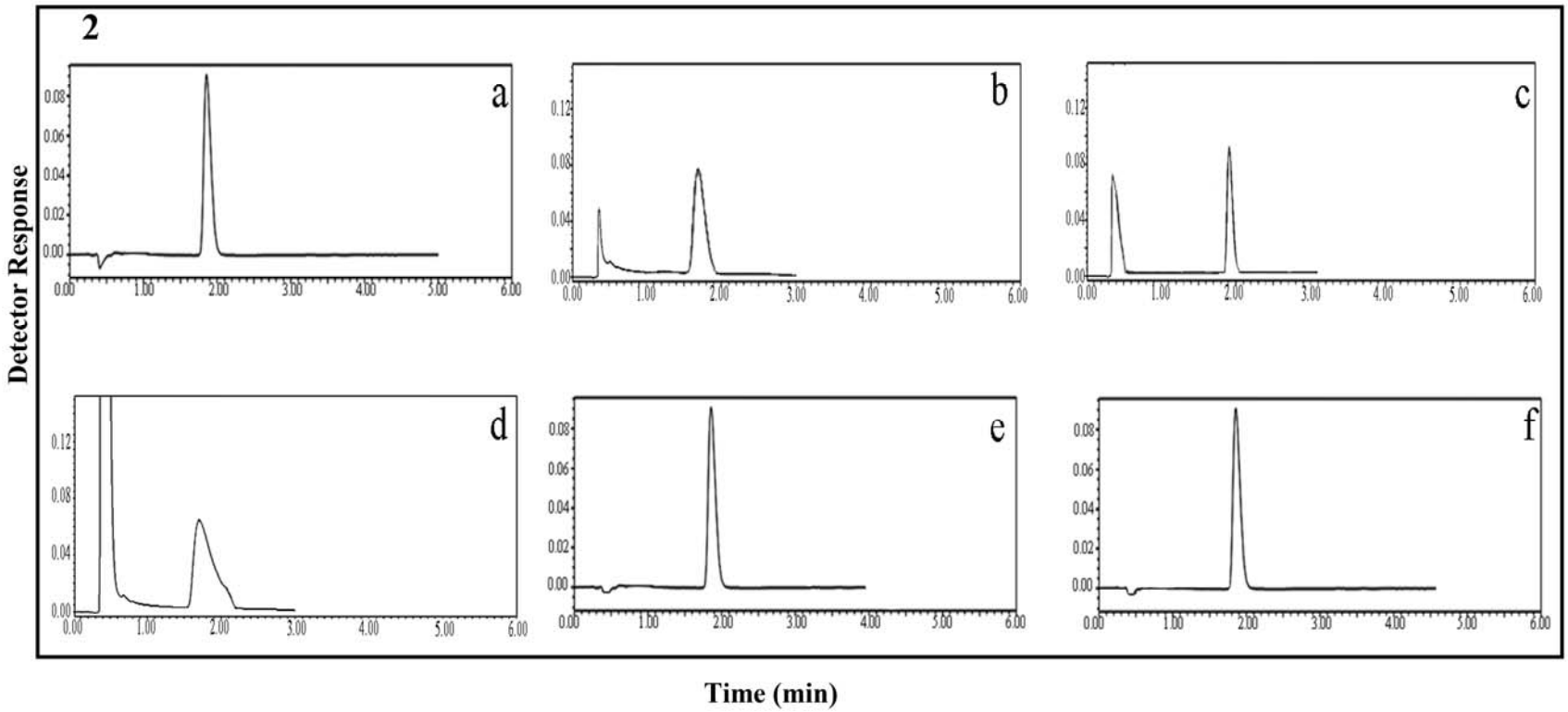

Fig. 6 – **(1)** HPLC and **(2)** UPLC results of 50 µg.mL^{-1} RPG; under stressed conditions: **a)** standard chromatogram, **b)** 0.1 M acid hydrolysis, **c)** 0.1 M alkaline hydrolysis, **d)** oxidative stress with 3 % H_2O_2, **e)** 6 hours UV at 254 nm, **f)** 6 hours dry heat in oven at 100 ^{o}C.

Stock standard solution of RPG and the internal standard (IS) entacapone were prepared in acetonitrile at concentrations of approximately 500 $\mu g.mL^{-1}$ for RPG and 100 $\mu g.mL^{-1}$ for the IS. The concentration range of RPG varied between 0.2 and 300 $\mu g.mL^{-1}$. The concentration of IS was maintained at a constant level of 2.0 $\mu g.mL^{-1}$. The calibration curves for LC analysis were constructed by plotting the ratio of the peak area of the drug to that of the IS against the corresponding drug concentration.

All stock and working solutions were protected from light and preserved in a refrigerator at about 4°C. Also, all solutions were protected from light and were used within 24 h to avoid decomposition. However, current–potential curves of sample solutions recorded 72 h after preparation did not show any appreciable change in assay values.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of excipients, and their potential impurities.[18-23] The specificity of the developed methods for RPG was evaluated in the presence of its pharmaceutical dosage form and degradation products. Forced degradation studies were performed using stress conditions on RPG to provide an indication of the stability indicating property and specificity of the proposed method.

Generation of stressed samples for the establishment of the stability-indicating assay

Degradation studies were performed with stress conditions of UV light, acid and base hydrolysis, oxidation, and heating in an oven (at 100 °C) to evaluate the ability of the proposed method to RPG from its degradation products.[23] For heat and light studies, the study period was over a day; whereas for acid and base hydrolysis and oxidation, it was 1 h. Peak purity tests were performed for RPG by using the DAD detector with the stressed samples. The optimized method was used to study the forced degradation behavior of RPG and may be applied in stability testing of pharmaceutical dosage forms. An appropriate blank was injected before analysis of the forced degradation samples. The reactions were carried out at a drug concentration of 50 $\mu g.mL^{-1}$ for RPG. The stress conditions were as follows: drug solution in 0.1 M HCl, 0.1 M NaOH and 3 % H_2O_2 were exposed for 60 min, some amount of solid form of the RPG was exposed under the 254 nm UV light which was recommended by ICH at room temperature for 6 and 24 h; and thermal stress bulk drugs were subjected to dry heat as a solid form at 100 °C for 6 and 24 h.

Pharmaceutical dosage form assay procedure

Ten Diafree® tablets (each tablet contains 2.0 mg repaglinide) were accurately weighed and finely powdered by pestle in a mortar. An accurate amount of the powder equivalent to the content of one tablet was weighed, transferred into a 100 mL volumetric flask, diluted with methanol, stirred for about 10 min, and then diluted to volume with methanol. The content of the flask were sonicated for 10 min in an ultrasonic bath for complete dissolution. This solution was filtered, and the filtrate was collected in a clean flask. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate solution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte or mobile phase (after addition of a constant amount of IS (2.0 $\mu g.mL^{-1}$)) in order to obtain a final solution. The amount of RPG per tablet was calculated using the related linear regression equation obtained from the calibration curve of pure RPG.

Recovery studies

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of pure RPG were added to the pre-analyzed tablet formulation and the mixtures were analyzed by the proposed methods. After five repeated experiments, the recovery results were calculated using the calibration equation. The resulting amount of RPG was assayed and the obtained results were compared with expected results.

CONCLUSIONS

The detailed electrooxidative behavior of RPG on glassy carbon electrode was established and studied. RPG is irreversibly oxidized at high positive potentials. The proposed methods provide simple, accurate, and reproducible quantitative analysis for the determination of RPG in its tablet dosage form. The SWV method is more rapid and simpler than the DPV method. Besides, the SWV technique has greater sensitivity and accuracy. The proposed voltammetric methods are suitable for routine determination of RPG in its dosage form.

The HPLC and UPLC methods have some advantages such as short run time (<4.5 min for HPLC and <2.5 min for UPLC), low limit of detection, low limit of quantitation, good precision (standard deviation less than 1%) and good resolution between RPG and IS peaks, with symmetric, pure and perfect homogeneity for peaks. When comparing the HPLC and UPLC methods; it can be said that UPLC is more environmental friendly than the HPLC. On the other hand, organic solvent consumption of UPLC less than 6 fold per run, and also analysis time decreased 2 times with nearly the same resolution.

The principal advantage of DPV and SWV techniques over the other techniques is that they may be applied directly to the analysis of pharmaceutical dosage forms without the need for separation or complex sample preparation, since there was no interference from the excipients.

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REFERENCES

1. J. P. Remington, "Remington pharmaceutical sciences", 17th edition, Mack Publishing Co. USA, 1985
2. W. Lund, "The Pharmaceutical Codex", 12th edition, The Pharmaceutical Press, London, 1994.
3. L. L. Brunton, "Goodman and Gilman's: The pharmacological basis of therapeutics", McGraw Hill Press, New York, 2010.
4. C. Madathil, B. Kanakapura and A. Sameer, *Chem. Ind. & Chem. Eng.*, **2011**, *17*, 469-476.
5. A.B. Alkhalidi, M. Shtaiwi and S. Hatim, *J. AOAC Int.*, **2008**, *91*, 530-535.
6. P.S. Jain, M.K. Patel and S.J. Surana, *Acta Chrom.*, **2013**, *25*, 531-544.
7. A.B. Ruzilawati, M.S. Wahab and A. Imran, *J. Pharm. Biomed. Anal.*, **2007**, *5*, 1831-1835.
8. M. Gandhimathi, T.K. Ravi and S.K. Renu, *Anal. Sci.*, **2003**, 1675-1677.
9. N. Kaushal, S. Jain and A.K. Tiwary, *Ind. J. Pharm. Sci.*, **2010**, 240-244.
10. D. Jirovsky, Z. Bartosova and J. Skopalova, *J. Chrom. B*, **2010**, *31*, 3243-3248.
11. R.N. El-Shaheny, *J. Fluor.*, **2012**, *22*, 1587-1594.
12. M.A.N. E-Ries, G.G. Mohamed and A.K. Attia, **2008**, *128*, 171-177.
13. J. Wang, "Electroanalytical techniques in clinical chemistry and laboratory medicine", VCH Publishers, New York, 1988.
14. P.T. Kissinger and W.R. Heineman, "Laboratory techniques in electroanalytical chemistry", 2nd edition, Marcel Dekker, New York, 1996.
15. M.R. Smyth and J.G. Vos (Eds.) "Analytical voltammetry", Vol. XXVII, Elsevier, Amsterdam, 1992.
16. S.A. Ozkan, B. Uslu and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, **2003**, *33*, 155-181.
17. J. Grimshaw, "Electrochemical reactions and mechanism in organic chemistry", Elsevier, New York, 2000.
18. S.A. Ozkan, "Electroanalytical methods in pharmaceutical analysis and their validation", HNB Pub., New York, 2011.
19. M. Gumustas and S. A. Ozkan, *Open Anal. Chem. J.*, **2011**, *5*,1-21.
20. M. Gumustas, S. Kurbanoglu, B. Uslu and S.A. Ozkan, *Chromatographia*, **2013**, *76*, 1365-1427.
21. J. Ermer and J.H. Miller, "Method validation in pharmaceutical analysis", Wiley-VCH, Weinheim, 2005.
22. ICH Guideline (Q2A) (R1) (2005) Validation of Analytical Procedures: Text and Methodology
23. ICH Guideline (Q1AR) (2000) Stability Testing of New Drug Substances and products International Conference on harmonization IFPMA, Geneva
24. http://www.druglib.com/druginfo/prandimet/description_pharmacology/
25. E. Laviron, L. Rouillier and C. Degrand, *J. Electroanal. Chem.*, **1980**, *112*, 11-23.
26. S.A. Ozkan, Y. Ozkan and Z. Sentürk, *Analytica Chim. Acta*, **2002**, *453*, 221-229.
27. The United States Pharmacopeia (The USP 32-NF 27), The Official Compendia of Standards, United States Pharmacopeial Convention, MD, Rockwille, USA, 2009.
28. M. Gumustas, C. Sengel-Turk, C. Hascicek and S.A. Ozkan, *Biomed Chrom*, **2014**, *10*, 1409-1417.
29. M. Gumustas, B. Uslu, S. A. Ozkan and H. Y. Aboul-Enein, *Chromatographia*, **2014**, *77*, 1721-1726.