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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD BASED ON CHARGE TRANSFER COMPLEX FORMATION FOR THE DETERMINATION OF REPAGLINIDE AND ROSIGLITAZONE MALEATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A rapid and accurate spectrophotometric method has been developed for the separate determination of repaglinide (RPG) and rosiglitazone maleate (RGZ) in bulk and tablet forms. The procedures were validated according to the international conference on harmonization (ICH) guidelines and the recovery study was performed by standard addition technique. *p*-Chloranilic acid was found to form a charge-transfer complex in a stoichiometric 1:1 ratio with both drugs of maximum absorbance at 530 nm. Beer's law was obeyed over the concentration range 100 – 500 µg/mL and 100 – 800 µg/mL for RPG and RGZ; respectively. All the ICH validation parameters were met for the procedure. No significant statistical difference between the developed and reference HPLC methods was found. The proposed method is suitable for the quality control analysis of RPG and RGZ in pure and pharmaceutical dosage forms.

Keywords: *p*-chloranilic acid, Charge transfer complex, Spectrophotometric determination

INTODUCTION

Repaglinide (RPG); (S)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino)-2-oxoethyl]benzoic acid and rosiglitazone maleate (RGZ); (RS)-5-[4-(2-[methyl(pyridin-2-yl)amino]-ethoxy)benzyl]thiazolidine-2,4-dione are oral anti-diabetic agents (Figures 1 and 2). RPG is a member of the meglitinide family which stimulates the release of insulin from pancreatic ß cells in presence of glucose. Members of this family have fast absorption rate and rapid onset action[1,2,3]. They interact with the ATP-sensitive potassium (K+ATP) channels on pancreatic ß cells causing depolarization of the ß cell membrane which opens calcium channels leading to calcium influx and insulin secretion[1]. RGZ belongs to the thiazolidinediones family and is a ligand of peroxisome proliferator-activated cytoplasmic receptor. Activation of this receptor regulates the transcription of insulin-responsive genes leading to an increase in glucose uptake by muscle cells. RGZ is also known to increase adipogenesis [1, 4].

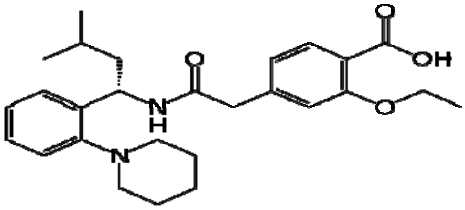

Fig.1: Repaglinide (RPG)

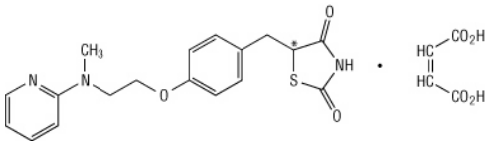

Fig. 2: Rosiglitazone (RGZ)

HPLC determination of RPG has been described in United States Pharmacopeia (USP) and elsewhere [5, 6, 7]. Electrochemical and visible spectrophotometric methods have been reported for the quantitative determination of RPG [8, 9, 10]. RGZ has been determined by UV spectroscopy and chromatography [11, 12, 13].

Visible spectrophotometry is still considered a convenient and low cost method for the determination of pharmaceuticals in bulk and dosage forms. *p*-chloranilic acid, as a π-acceptor has been successfully utilized for the spectrophotometric determination of a variety of electron donating basic compounds. It has been used in the estimation of some cardiovascular, antihistaminic, and psychotropic drugs [14,15,16]. π –Acceptors react with compounds containing basic nitrogenous or carboxylic groups (n-donors) to form charge transfer complexes or radical anions that can be measured spectrophotometrically [17].

We have developed simple, accurate, and rapid quantitative methods for determination of RPG and RGZ based on charge transfer complexation with *p*-chloranilic acid. These methods can be applied for the routine analysis of both drugs in quality control laboratories.

MATERIALS AND METHODS

Apparatus

Biotech-Engineering DV82 UV-VIS(UK) and Jasco 760 (Japan) spectrophotometers with 1 cm quartz cuvettes were used for the determination of RPG and RGZ; respectively.

Materials

Pure standards

Pure RPG and RGZ were obtained from the manufacturing companies (Novonordisk and APEX pharma, respectively) and were 99.9 and 99.89 % pure as stated by the manufacturer. The RPG and RGZ standard solutions were prepared with concentration of 2.0 mg/mL in methanol and 1.0 mg/mL in ethanol; respectively.

Pharmaceutical dosage form

Commercially available Novonorm®2mg and Rosizone®4 mg tablets were used.

Chemicals and reagents

Double-distilled water and analytical grade reagents were used throughout the procedures. Ethanol and methanol were of HPLC grade obtained from Sigma-Aldrich (Germany). *p*-Chloranilic acid (Sigma-Aldrich, Germany) was prepared as 3.96 mg/mL (0.0189 M) solution in methanol for RPG determination and in ethanol for RGZ determination. Calcium sulphate (Al Gomhoria, Egypt) was used as a desiccating agent.

Optimum conditions of the reaction

Reaction conditions were optimized, including volume of the *p*-chloranilic acid reagent required for a complete reaction, time for

complete reaction, and stability of the formed color. Stoichiometry of the reactions between RPG and RGZ with *p*-chloranilic acid was studied by applying Job's method [18, 19].

Method validation

Linearity

Aliquots from standard solutions equivalent to (1 – 5) and (1 – 8) mg of RPG and RGZ; respectively, were separately transferred into two series of 10 mL volumetric flasks. 3 mL of *p*-chloranilic acid solution was added to each of the RPG flasks, while 1 mL was added to the RGZ series. The volume was completed to 10 mL with methanol for RPG and ethanol for RGZ. Absorbance was measured at 530 nm against reagent blank. Calibration curves were plotted relating absorbance at 530 nm to the corresponding concentration of the analyzed drug (RPG and RGZ) and regression equations were computed.

Accuracy

Aliquots from the RPG and RGZ standard solutions equivalent to (1 – 5) and (1 – 8) mg, respectively, were separately transferred into two series of 10-mL volumetric flasks and the analysis described under linearity were followed. Absorbance was recorded at 530 nm and used for calculating the recovery percentage of each drug applying the corresponding regression equation.

Precision (Repeatability)

Six samples of RPG and RGZ each of final concentration 300µg/mL were analyzed for testing the repeatability of the assay and the SD of the analytical response (absorbance at 530 nm) was calculated [20].

Limit of detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated based on the standard deviation of the analytical response (absorbance at 530 nm) and the slope of the calibration curve using the equations LOD = 3.3 σ/S and LOD = 10 σ/S, where σ is the SD of the response and S is the slope of the calibration curve [20].

Determination of RPG and RGZ in commercial tablets

Twenty tablets of Novonorm® 2 mg (RPG) and Rosizone® 4 mg (RGZ) were separately weighed and powdered. Weight equivalent to 25 mg of the powdered tablets were separately transferred into two separating funnels each containing 20 mL water and extracted with chloroform (10 mL X 4) for 15 min. The combined chloroform extracts were collected in a separate beaker after passing through calcium sulphate and evaporation in a water bath. The residues was re-dissolved in (3 mL X 3) methanol for RPG and ethanol for RGZ, separately transferred into two 25-mL volumetric flask and the volume was completed to 25 mL with the corresponding solvent for each drug. 1 mg/mL solutions of RPG in methanol and RGZ in ethanol were obtained. Procedures were completed as described under accuracy. Recovery study was performed using standard addition technique.

RESULTS AND DISCUSSION

The solution of *p*-chloranilic acid in methanol or ethanol exhibits λ_{max} at 435 nm. The addition of either RPG solution in methanol or RGZ solution in ethanol to these solutions produced an immediate change of the orange color of *p*-chloranilic acid into violet color which can be measured spectrophotometrically at 530 nm (Figure 3). The addition of *p*-chloranilic acid (π-acceptor) to either RPG or RGZ which have a lone pair of electrons (n-donors) results in the formation of charge transfer complexes.

Figures 4A and 5A show that the addition of 3 mL and 1 mL of *p*-chloranilic acid (0.0189 M) solution to RPG and RGZ solutions, respectively, produced the maximum color intensity at 530 nm

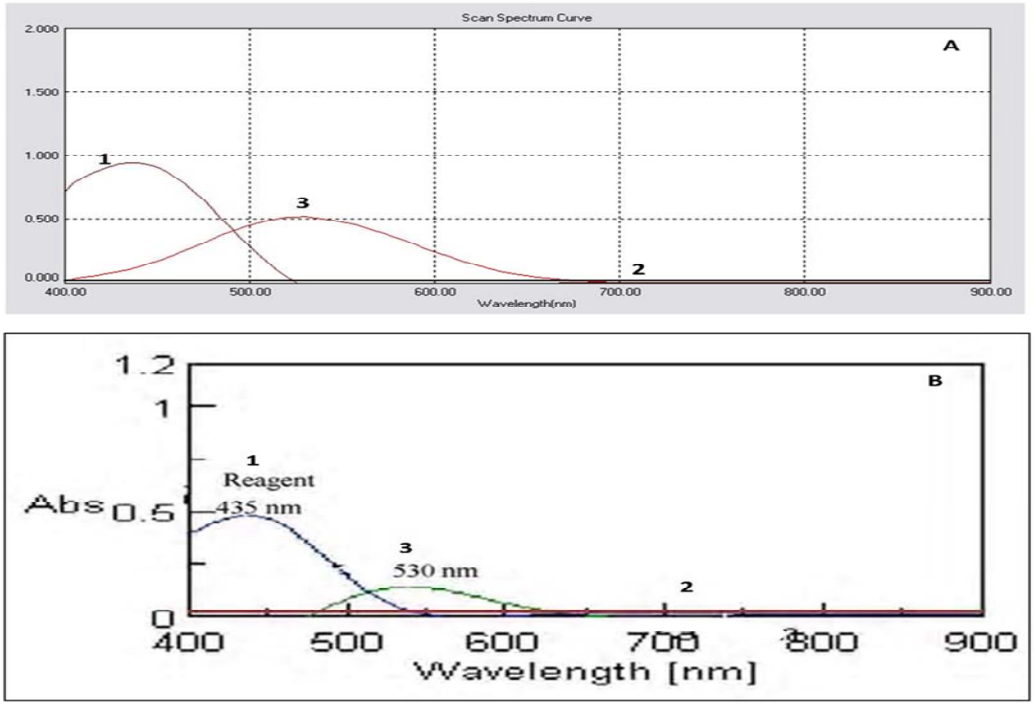

Fig.3: Absorbance spectra of RPG and RGZ. A) 1: Reagent blank (3.96 mg/mL); 2: Pure drug (200 µg/mL) in methanol; 3: *p*-chloranillic acid (3.96 mg/mL)-RPG (200 µg/mL) charge transfer complex. B) 1: Reagent blank (1.18 mg/mL); 2: Pure RGZ (100 µg/mL) in ethanol; 3: *p*-chloranillic acid (1.18 mg/mL)-RGZ (100 µg/mL) charge transfer complex.

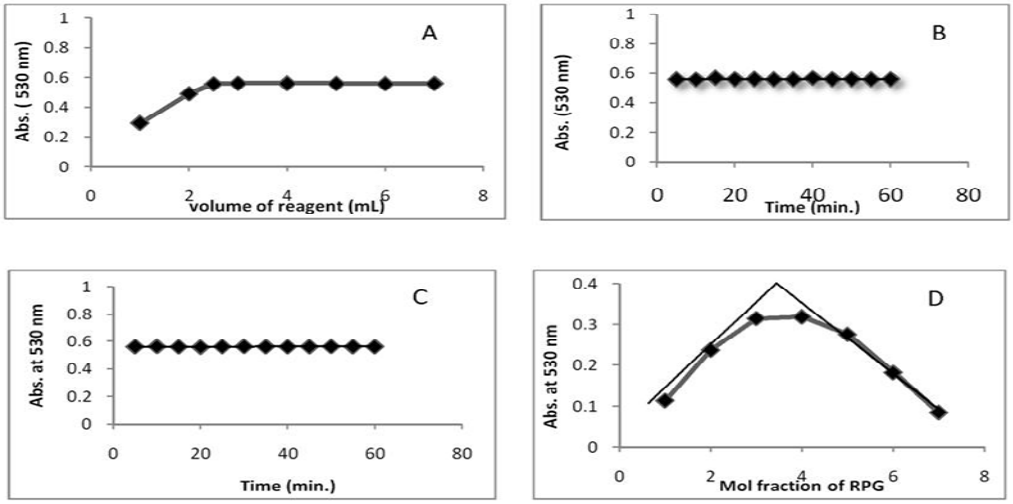

Fig.4: Optimization of reaction conditions for RPG. A) Effect of volume of the *p*-chloranillic acid (3.96 mg/mL) on its reaction with RPG (400 µg/mL). B) Time of the reaction of RPG 400 µg/mL with *p*-chloranilic acid (3.96 mg/mL). C) Stability of the formed RPG (400 µg/mL)/*p*-chloranillic acid (3.96 mg/mL charge transfer complexes. D) Determination of the stoichiometry of the reaction of RPG and *p*-chloranilic acid by the continuous variation method using 2 mM solutions.

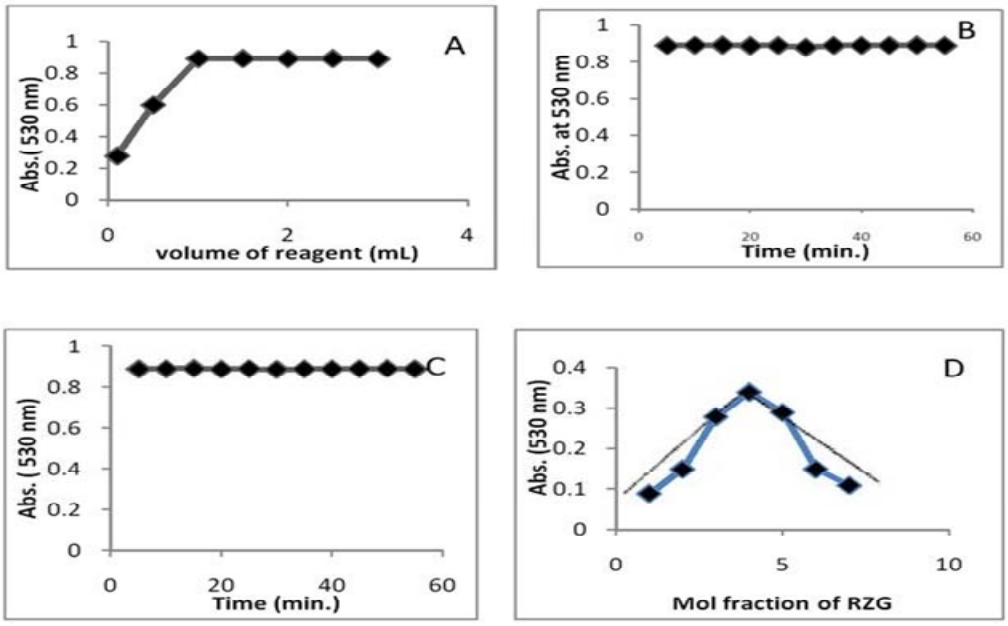

Fig. 5: Optimization of reaction conditions for RGZ. A) Effect of volume of the *p*-chloranillic acid (3.96 mg/mL) on its reaction with RGZ (700 µg/mL). B) Time of the reaction of RPG 400 µg/mL with *p*-chloranilic acid (3.96 mg/mL). C) Stability of the formed RGZ (700 µg/mL)/*p*-chloranillic acid (3.96mg/mL) charge transfer complexes. D) Determination of the stoichiometry of the reaction of RGZ and *p*-chloranilic acid by the continuous variation method using 2 mM solutions.

Table 1: Characteristic parameters of the regression equation of *p*-chloranilic acid method for determination of RZG and RPG

Parameter	RPG	RGZ
Calibration range (µg/mL)	100 – 500	100 – 800
Correlation coefficient	0.996	0.999
Slope	1.6×10^{-3}	1.3×10^{-3}
Intercept	- 0.04	- 0.03
SE	1.8×10^{-2}	6.2×10^{-3}
LOD (µg/mL)	21.20	20.70
LOQ (µg/mL)	64.24	62.73

Table 2: Accuracy of the presented methods(*) and statistical comparison with the reported methods

	RPG*	Reference method for RPG [5]***	RGZ*	Reported method for RGZ [11]***
Accuracy%	100.48 ± 1.01	100.40 ± 1.00	99.83 ± 0.55	100.20 ± 0.77
SD	1.01	1.00	0.55	0.77
RSD	1.01	1.00	0.55	0.77
Variance	1.02	1.00	0.30	0.59
F-test	1.02(6.39)**		1.97(6.39)**	
t-test	0.13(2.31)**		0.88(2.31)**	

** Values between parentheses represent tabulated F and t at p=0.05; *** The USP HPLC method was applied for RPG and a reported HPLC method was applied for RGZ[5,11].

Figures 4B and 5B show that a maximum intensity of the violet color was obtained immediately after addition of the reagent solution on solutions of both drugs. The color intensity remained stable for 60 min (Figure 4C and 5 C).

Job's method of continuous variation showed that the reaction between either RPG or RGZ with *p*-chloranilic acid occurred in 1:1 ratio (Figure 4D and 5D).

The calibration curves were linear for RPG over the range of 100 – 500 µg/mL and RGZ over the range of 100 – 800 µg/mL. Regression equations were computed and correlation coefficients were 0.996 and 0.999 for RPG and RGZ; respectively. Parameters of the regression equations are presented in table 1.

The method was found to be accurate with average accuracy percentage of 100.48 ± 1.01 and 99.83 ± 0.55 for RPG and RGZ; respectively (Table2). In the precision study, the SD of the analytical response (absorbance at 530 nm) were within the acceptable limits indicating good precision of the methods (0.010 for RPG and 0.008 for RGZ). LOD were 21.20 and 20.70 µg/mL and LOQ were 64.24 and 62.73 µg/mL for RPG and RGZ; respectively. Statistical comparison between the described methods and reported methods showed no significant difference with respect to accuracy and precision (Table2).

The method was also used for determination of RPG and RGZ in the pharmaceutical dosage form (tablets). However, an extraction step of the drug from the corresponding tablets was required to remove interference of the tablet additives. Average recovery percentages of 99.2 and 99.3 for RPG and RGZ, respectively were obtained. Using standard addition technique, the average recovery percentages of pure drug added to tablet extract were 101.02 ± 1.6 and 99.92 ±1.5 for RPG and RGZ; respectively, indicating no interference from tablet matrices.

CONCLUSION

We have developed and validated simple, rapid and accurate spectrophotometric methods for the quantitative determination of RPG and RGZ in bulk and in pharmaceutical dosage forms. These methods are based on the formation of charge transfer complexes between RPG and RGZ with *p*-chloranilic acid which can be measured spectrophotometrically at 530 nm. These methods are simpler and cheaper than other methods including HPLC and can be applied for routine quality control applications for both drugs.

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