Original Article

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Validated spectrophotometric methods for determination of some oral hypoglycemic drugs

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ABSTRACT: Four accurate, precise, rapid, reproducible, and simple spectrophotometric methods were validated for determination of repaglinide (RPG), pioglitazone hydrochloride (PGL) and rosiglitazone maleate (RGL). The first two methods were based on the formation of a charge-transfer purple-colored complex of chloranilic acid with RPG and RGL with a molar absorptivity 1.23 × 10^3 and 8.67 × 10^2 l•mol^{-1}•cm^{-1} and a Sandell's sensitivity of 0.367 and 0.412 µg•cm^{-2}, respectively, and an ion-pair yellow-colored complex of bromophenol blue with RPG, PGL and RGL with molar absorptivity 8.86 × 10^3, 6.95 × 10^3, and 7.06 × 10^3 l•mol^{-1}•cm^{-1}, respectively, and a Sandell's sensitivity of 0.051 µg•cm^{-2} for all ion-pair complexes. The influence of different parameters on color formation was studied to determine optimum conditions for the visible spectrophotometric methods. The other spectrophotometric methods were adopted for demtermination of the studied drugs in the presence of their acid-, alkaline- and oxidative-degradates by computing derivative and pH-induced difference spectrophotometry, as stability-indicating techniques. All the proposed methods were validated according to the International Conference on Harmonization guidelines and successfully applied for determination of the studied drugs in pure form and in pharmaceutical preparations with good extraction recovery ranges between 98.7-101.4%, 98.2-101.3%, and 99.9-101.4% for RPG, PGL, and RGL, respectively. Results of relative standard deviations did not exceed 1.6%, indicating that the proposed methods having good repeatability and reproducibility. All the obtained results were statistically compared to the official method used for RPG analysis and the manufacturers methods used for PGL and RGL analysis, respectively, where no significant differences were found.

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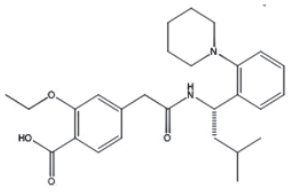
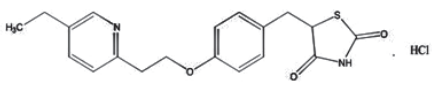
1. Introduction

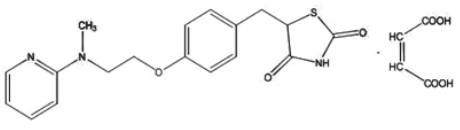
For many years pharmacological agents such as sulphonylureas and biguanides were the mainstay of oral treatment for type II diabetes. Target control is achieved with these medications for some patients only, however; secondary failure is relatively common. Thus, the introduction of newer agents such as meglitinides (repaglinide; RPG) and thiazolidinediones (pioglitazone and rosiglitazone) has been welcomed (*1*). RPG acts by stimulating insulin secreation of β-cells of the pancreas, while both pioglitazone hydrochloride (PGL) and rosiglitazone maleate (RGL), which exert their glucose-lowering effect by binding to peroxisome proliferator-activated γ receptors, thus increasing the receptor sensitivity to insulin (*2*). Structures of RPG, PGL, and RGL are illustrated below.



Repaglinide



Pioglitazone hydrochloride



Rosiglitazone maleate

Many analytical methods have been reported for the quantitative estimation of RPG in pharmaceutical preparations and biological samples (*3-5*) which include visible spectrophotometric (*6,7*), HPLC (*8,9*) and electrochemical methods (*10*). PGL and its metabolites have been determined in biological fluids and pharmaceutical preparations by HPLC with UV detection (*11-13*), reversed phase TLC (*14*), liquid chromatography coupled with mass spectrometry (*15*), and spectrometry (*16*). On the other hand, RGL in pharmaceutical preparations and human plasma has been determined by HPLC with UV detection (*17-21*), HPTLC (*22*), TLC (*23*), and liquid chromatography coupled with mass spectrometry (*24*).

The aim of this study is to develop and validate simple, rapid, sensitive, and reliable spectrophotometric methods for accurate quantitation of RPG, PGL, and RGL *via* 'charge-transfer and ion-pair' complexation reactions and stability-indicating assays using 'derivative and pH-induced difference spectrophotometry'. All the proposed methods were successfully applied for routine quality control analysis of the mentioned drugs in raw material and in their pharmaceutical preparations unaffected by interference from excipients.

2. Materials and Methods

2.1. *Chemicals and reagents*

RPG and PGL were kindly supplied by Amoun Pharmaceutical Co. (Cairo, Egypt) and certified to contain 99.99% and 99.95%, respectively. Diarol® tablets, batch number 1018, were labeled to each contain 2 mg of RPG and Actozone® tablets, batch number 3543, were labeled to each contain 45 mg of PGL. RGL was kindly supplied by Apex Pharma (Cairo, Egypt) and certified to contain 99.99%. Rosizone® tablets, batch number MT0410208, were labeled to each contain 4 mg of RGL.

Methanol and acetonitrile were purchased from Honeywell Riedel-de Haen, Seelze, Germany. Chloranilic acid, bromophenol blue, hydrochloric acid (35.4%), sodium hydroxide, potassium hydrogen phthalate, chloroform, hydrogen peroxide (30%), and ethanol were from BDH Chemicals, Poole, UK. All chemicals and reagents used throughout this work were spectroscopic analytical grade. Bi-distilled water was used throughout the whole work and indicated by the word "water".

2.2. *Instruments*

A Hewlett-Packard HP 8452A Diode Array Spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) connected to an IBM compatible computer and HP laser printer was used. The bundled software was UV-Visible ChemStation Rev. A.08.03, Agilent Technologies, Santa Clara, CA, USA. The spectral bandwidth was 0.2 nm and the wavelength scanning speed was 2,800 nm•min^{-1}. The absorption spectra of the reference and the test solutions were recorded in 1.0-mL quartz cells at 25.0°C using conditions of '$\Delta\lambda$ = 4 nm and scaling factor of 10 for first derivative (D^1)' and '$\Delta\lambda$ = 8 nm and scaling factor of 100 for second and third derivative (D^2 and D^3, respectively)'. A sonicator (Model RK 100H DVE GS; Bandelin Sonorex, Berlin, Germany) and a pH-meter equipped with a combined glass electrode (Jenway, Essex, UK) were used.

2.3. *Standard solutions*

2.3.1. *Standard solutions of drugs studied*

For the charge-transfer method, RPG and RGL stock standard solutions with a concentration of 1.0 mg•mL^{-1} in acetonitrile were prepared, which were also used as working standard solutions. For the other three spectrophotometric methods, stock standard solutions of RPG, PGL, and RGL with concentrations of 1.0 mg•mL^{-1} in methanol were prepared, which were further diluted with methanol to obtain concentrations of 0.1 mg•mL^{-1} to be used as working standard solutions.

2.3.2. *Standard solutions of reagents used for charge-transfer and ion-pair methods*

0.1% (w/v) chloranilic acid (CLA) in acetonitrile was used for the charge-transfer method and 0.1% (w/v) bromophenol blue (BPB) and phthalate buffers, pH 2.4 and 2.2, (*25*) were for the ion-pair method.

2.3.3. *Standard solutions of degradates for stability-indicating spectrophotometric methods*

Three standard solutions of degradates, *i.e.* acid-, alkaline-, and oxidative degradation products of RPG, PGL, and RGL, were prepared. Ten mg of each compound were mixed with 50 mL of 2 M HCl, 2 M NaOH, and 30% H_2O_2 for acid-, alkaline-, and oxidative degradation, respectively, followed by heating in a thermostatic water-bath at 80°C for 24 h. After cooling, the mixtures for the acid- and alkaline-degradated-solutions were neutralized with 5 M NaOH and 5 M HCl, respectively. The final concentrations of all the degradated-solutions were adjusted to 0.1 mg•mL^{-1} with methanol.

2.4. *Charge-transfer method*

Aliquots of RPG and RGL working standard solutions were mixed with 3.0 and 2.0 mL of 0.1% CLA in a series of 10-mL volumetric flasks and then diluted with acetonitrile to obtain a concentration range of 50-325 and 50-300 µg•mL^{-1}, respectively. The absorbance of

the produced purple-colored charge-transfer complex was measured at 518 nm against a reagent-blank at room temperature. Calibration curves were constructed and the regression equation was then computed.

2.5. *Ion-pair method*

Into three separating funnels, aliquots of RPG, PGL, and RGL working standard solutions were separately transferred and mixed with 4.0 mL of phthalate buffer, pH 2.4 for RPG and PGL, and pH 2.2 for RGL. The solutions were then mixed with 3.0 mL of 0.1% BPB reagent solution. The produced yellow-colored ion-pair complexes were extracted twice with 4 mL chloroform and allowed to stand for clear separation of the two phases. The chloroformic layer was then passed through anhydrous sodium sulfate and diluted with chloroform in 10-mL volumetric flasks to obtain a concentration range of 5-35 $\mu g \cdot mL^{-1}$. The absorbance of the produced colored-complexes was measured at 414 nm, 416 nm and 415 nm, respectively, against a reagent blank at room temperature. Calibration curves were constructed and the regression equation was then computed.

2.6. *Stability-indicating spectrophotometric methods*

2.6.1. *Derivative spectrophotometric (D^n) method*

From standard working solutions, aliquots were transferred into a series of 10 mL-volumetric flasks and diluted with methanol. RPG was determined in a concentration range of 5-75 $\mu g \cdot mL^{-1}$ in the presence of its acid-, alkaline- and oxidative-degradates, where the values of the first derivative (D^1) amplitudes were computed at 263.79, 264.33 and 304.84 nm, respectively. PLG was determined in a concentration range of 5-60 $\mu g \cdot mL^{-1}$ in the presence of its acid- and alkaline-degradates, where the values of the first derivative (D^1) were computed at 253.35 and 284.05 nm, respectively, and the values of the second derivative (D^2) were computed at 276.31 nm in a concentration range of 5-75 $\mu g \cdot mL^{-1}$ in the presence of its oxidative-degradates. RGL was determined in a concentration range of 5-70 $\mu g \cdot mL^{-1}$ in the presence of its acid-, alkaline- and oxidative-degradates, where the values of the second derivative (D^2) amplitudes were computed at 307.95, 287.73, and 325.67 nm, respectively. Calibration curves were constructed and the regression equation was then computed.

2.6.2. *pH-induced difference spectrophotometric (DD^n) method*

From standard working solutions, aliquots were transferred into two sets of 10-mL volumetric flasks and diluted with either 0.1 M HCl or 0.1 M NaOH. ΔA spectra were computed by placing the acid solution in the reference beam and the alkaline solution in the sample beam. RPG was determined in a concentration range of 5-65 $\mu g \cdot mL^{-1}$ in the presence of its acid- and alkaline-degradates, where the values of the first derivative of ΔA spectra (DD^1) were computed at 258.04 and 261.82 nm, respectively, while the second derivative of ΔA spectra (DD^2) values were computed at 252.80 nm in a concentration range of 5-75 $\mu g \cdot mL^{-1}$ in the presence of its oxidative-degradates. PGL was determined in a concentration range of 5-80 $\mu g \cdot mL^{-1}$ in the presence of its acid- and alkaline-degradates, where the values of the first derivative of ΔA spectra (DD^1) were computed at 242.81 and 243.41 nm, respectively, and the values of the second derivative ΔA spectra (DD^2) were computed at 253.12 nm in a concentration range of 5-75 $\mu g \cdot mL^{-1}$ in the presence of its oxidative-degradates. RGL was determined in a concentration range of 5-70 $\mu g \cdot mL^{-1}$ in the presence of its acid-, alkaline-, and oxidative-degradates, where the values of second derivative ΔA spectra (DD^2) were computed at 272.00 nm in the presence of its alkaline-degradates and those of the third derivative of ΔA spectra (DD^3) amplitudes were computed at 275.90 and 267.40 nm in the presence of its acid- and oxidative-degradates, respectively. Calibration curves were constructed and the regression equation was then computed.

2.7. *Assays of the pharmaceutical preparations by the proposed methods and application of standard addition techniques*

Sixty tablets of Diarol®, 10 tablets of Actozone®, and 30 tablets of Rosizone® were individually weighed to get the average weight of the tablets, respectively. For the charge-transfer method, a sample of the powdered tablets containing 50 mg of RPG or RGL was transferred to 50-mL volumetric flasks and sonicated for 20 min with 30 mL acetonitrile. The solution was brought to 50 mL with the same solvent and then filtered to prepare stock working solutions with a concentration of 1.0 $mg \cdot mL^{-1}$. Aliquots of the filtrate were further diluted with the same solvent and then subjected to the procedure for the charge-transfer method as described above. For other spectrophotometric methods, a sample of the powdered tablets containing 25 mg of RPG, PGL, or RGL was transferred to 250-mL volumetric flasks and sonicated for 20 min with 200 mL methanol. The solution was brought to 250 mL with the same solvent and then filtered to prepare stock working solutions with a concentration of 0.1 $mg \cdot mL^{-1}$. Aliquots of the filtrate were further diluted with the same solvent and then subjected to the procedures for ion-pair and stability-indicating spectrophotometric methods as described above.

To check the validity of the proposed methods, the standard addition technique was applied. For the

charge-transfer method, a sample of the powdered tablets containing 5 mg of RPG or RGL was accurately weighed and mixed with 5, 10, 15, 20, and 25 mg of the corresponding pure drug. Each spiked sample of RPG and RGL was transferred to a 25-mL volumetric flask, and sonicated for 20 min with 20 mL acetonitrile. The mixtures were diluted with the same solvent and filtered to get five spiked solutions from each pharmaceutical preparation in a concentration range of 0.4-1.2 mg•mL^{-1}. From each spiked solution, 2.5 mL was transferred to a 10-mL volumetric flask and then subjected to the procedure for the charge-transfer method as described above. For ion-pair and stability-indicating spectrophotometric methods, a sample of the powdered tablets containing 5 mg of RPG, PGL, or RGL was accurately weighed and mixed with 5, 10, 15, 20, 25 mg of the corresponding pure drug. Each spiked sample of RPG, PGL, and RGL was transferred to a 100-mL volumetric flask, and sonicated for 20 min with 75 mL methanol. The mixtures were then diluted with the same solvent and filtered to get five spiked solutions from each pharmaceutical preparation in a concentration range of 0.1-0.3 mg•mL^{-1}. One mL each and 1.5 mL each of spiked solutions was subjected to the procedure for the ion-pair method and stability-indicating spectrophotometric methods, respectively, as described above.

3. Results

3.1. *Development of charge-transfer and ion-pair methods*

3.1.1. *Absorption spectra*

Absorption spectra of charge-transfer complexes formed by RPG and CLA (Figure 1A) and RGL and CLA (Figure 1B) and those of ion-pair complexes formed by RPG and BPB (Figure 1C), PGL and BPB (Figure 1D), and RGL and BPB (Figure 1E) were measured against reagent-blanks. Both charge-transfer complexes showed maximum absorbance at 518 nm (Figures 1A and 1B). In contrast, the ion-pair complexes showed maximum absorbance at 414, 416, and 415 nm for RPG-BPB, PGL-BPB, and RGL-BPB, respectively. The influence of different parameters on color formation was studied to determine optimum conditions for the visible spectrophotometric methods.

3.1.2. *Choice of solvent*

In order to select the suitable solvent for charge-transfer complex formation, the reaction of RPG and RGL with CLA was performed in different solvents. Acetonitrile showed super priority over chloroform, 2-propanol, dichloroethane, 1,4-dioxan, methanol, and ethanol, as the complex formed in these solvents had a low molar absorptivity. Furthermore, acetonitrile was considered as an ideal solvent for CLA (π-acceptor), because it offered a maximum sensitivity which was attributed to its high dielectric constant that promotes maximum yield of the complex (*26*). For the ion-pair method, the effect of several organic solvents such as chloroform, carbon tetrachloride, ethyl acetate, diethylether, toluene, and dichloromethane were tried for effective extraction of the colored species from the aqueous phase. Chloroform was found to be the most suitable solvent for extraction of ion-pair complexes from the aqueous solutions. It yielded maximum absorbance intensity and considerably lower extraction ability for the reagent blank and it was also observed that only double extraction was adequate to achieve a quantitative recovery of the complex.

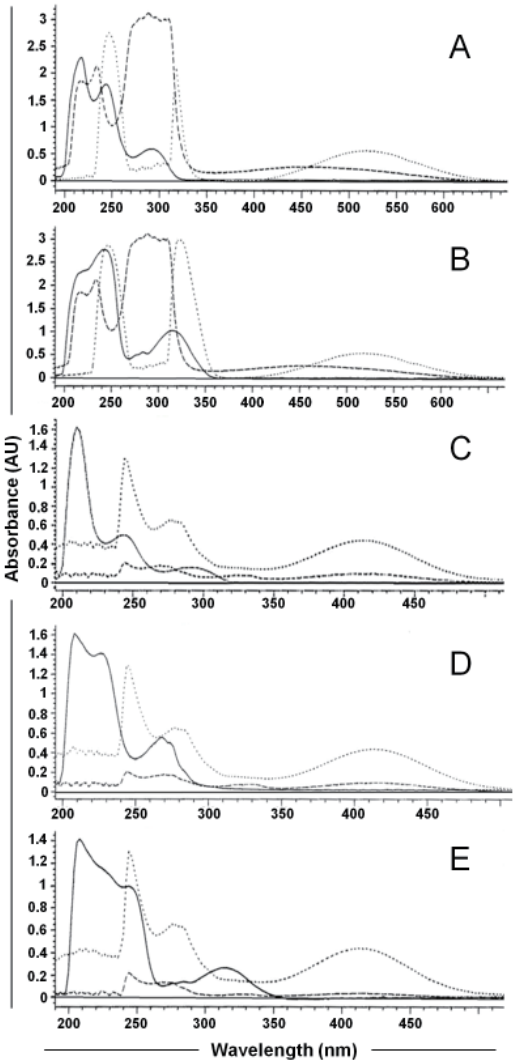


Figure 1. Absorption spectra of various charge-transfer and ion-pair complexes examined. (A) RPG, solid line; CLA, dashed line; RPG-CLA charge-transfer complex, dotted line. **(B)** RGL, solid line; CLA, dashed line; RGL-CLA charge-transfer complex, dotted line. **(C)** RPG, solid line; BPB, dashed line; RGL-BPB ion-pair complex, dotted line. **(D)** PGL, solid line; BPB, dashed line; PGL-BPB ion-pair complex, dotted line. **(E)** RGL, solid line; BPB, dashed line; RGL-BPB ion-pair complex, dotted line.

3.1.3. *Reagent concentration*

Figure 2A shows the effect of CLA concentration (by volume) on the quantitativeness of its reaction with RPG and RGL. It was found that, when various concentrations (by volume) of CLA solution were added to fixed concentrations of the studied drugs, 3.0 and 2.0 mL of 0.1% (w/v) CLA solutions were found to be effective volumes for quantitative determination of RPG and RGL, respectively. Figure 2B shows the effect of BPB concentration (by volume) on the intensity of the color-developed when reacted with RPG, PGL, and RGL. It was found that, when various concentrations (by volume) of BPB solution were added to fixed concentrations of the studied drugs, 3.0 mL of 0.1% (w/v) BPB solution was adequate to obtain a stable product for quantitative determination of RPG, PGL, and RGL.

3.1.4. *Effect of reaction time and temperature*

Optimum reaction time was investigated by following color development at ambient temperature. As shown in Figure 3A, the relationship between time and absorbance showed that the reaction was instantaneous and stable up to 2 h for the produced charge-transfer complexes. For ion-pair complexes, complete color intensity was attained after 2 min of mixing with chloroform and stable up to 2 h (Figure 3B). Figures 3C and 3D shows the relationship between temperature and absorbance, where raising the temperature to 30°C had no effect on the formation of both charge-transfer and ion-pair complexes, but the absorbance started to decay above 30°C.

3.1.5. *Effect of pH and volume of phthalate-buffer on the ion-pair complex formation*

The effect of pH on ion-pair complex formation was studied by extracting the yellow-colored complexes in the presence of phthalate-buffer at various pH between 2.0-4.0. As shown in Figure 4A, the relationship between pH and the absorbance showed maximum color intensity and consequently a higher absorbance at pH 2.4 for RPG and PGL and at pH 2.2 for RGL. Also, the stability of the formed color-complexes was achieved without affecting the absorbance by using 4.0 mL of phthalate buffers at the chosen pH-values, where maximum absorbance and reproducible results were obtained (Figure 4B).

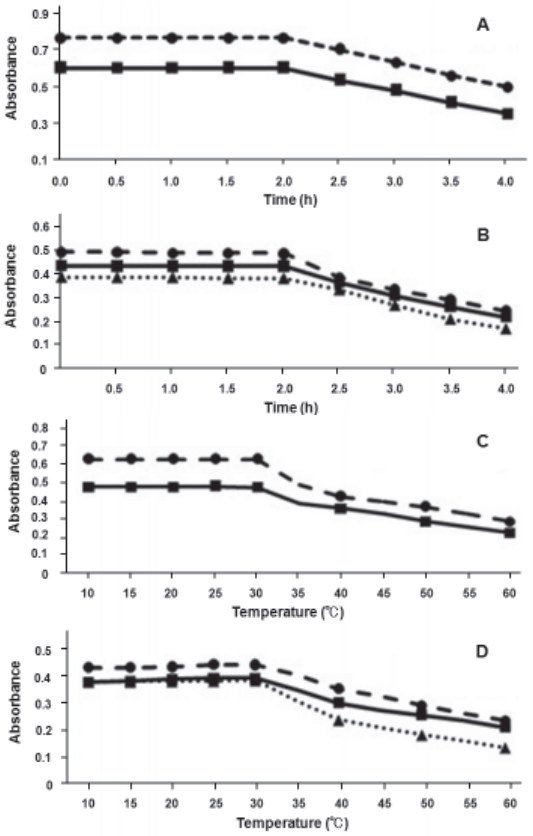


Figure 3. Effect of reaction time or temperature to form various complexes. (**A** and **C**) Charge-transfer complex formation of RPG (dashed line) and RGL (solid line); (**B** and **D**) Ion-pair complex formation with RPG (dashed line), PGL (dotted line), and RGL (solid line).

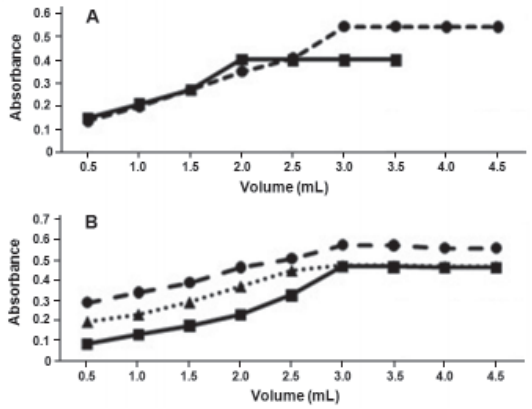


Figure 2. Effect of CLA and BPB volumes in reaction mixtures for complex formation with RPG, PGL and RGL. (A) Effect of CLA volume for charge-transfer complex formation with RPG (dashed line) and RGL (solid line). **(B)** Effect of BPB volume for ion-pair complex formation with RPG (dashed line), PGL (dotted line), and RGL (solid line).

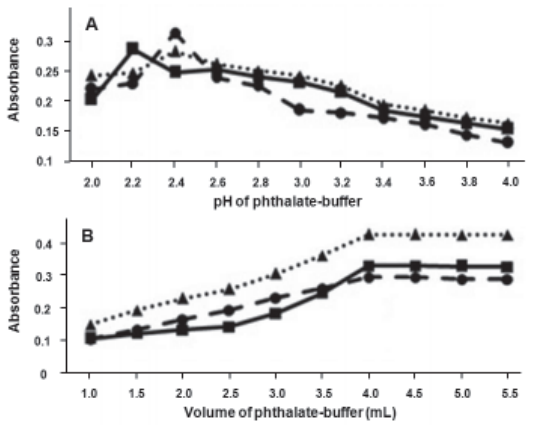


Figure 4. Effect of pH (A) and volume (B) of phthalate-buffer on ion-pair complex formation. Dashed line, RPG; dotted line, PGL; solid line, RGL.

3.1.6. *Stoichiometric relationship*

Job's method of continuous variation (*27*) was applied in order to ascertain the stoichiometry of the reactions of charge-transfer and ion-pair complex formation, where equimolar solutions (1.0×10^{-3}) of each drug, of CLA and BPB were used.

As shown in Figure 5A, the results obtained from Job's method suggested that 1:1 (drug:π-acceptor) charge-transfer complexes were formed through complete electron transfer from RPG or RGL as an electron donor to CLA as an electron acceptor with the formation of intensely colored radical ions in the polar solvent acetonitrile. The deduced scheme is shown in Figure 5C. This finding was anticipated by the presence of one basic electron-donating center (nitrogen atom) present in RPG and RGL structures, while PGL lacks this basic center and consequently failed to form a charge transfer complex when reacted with CLA as a π-acceptor.

Reaction-stoichiometry for ion-pair complexes was found to be a good 1:1 approximation (drug:reagent) ratio which were formed through the electrostatic attraction between positive protonated RPG^+, PGL^+, or RGL^+ and negative BPB^- (Figure 5B). The deduced extraction equilibrium is shown in Figure 5D. In this scheme, $Drug^+$ and BPB^- represent the protonated oral hypoglycemic drugs studied and the anion of the dye, respectively, and the subscripts (aq) and (org) refer to the aqueous and organic phases, respectively.

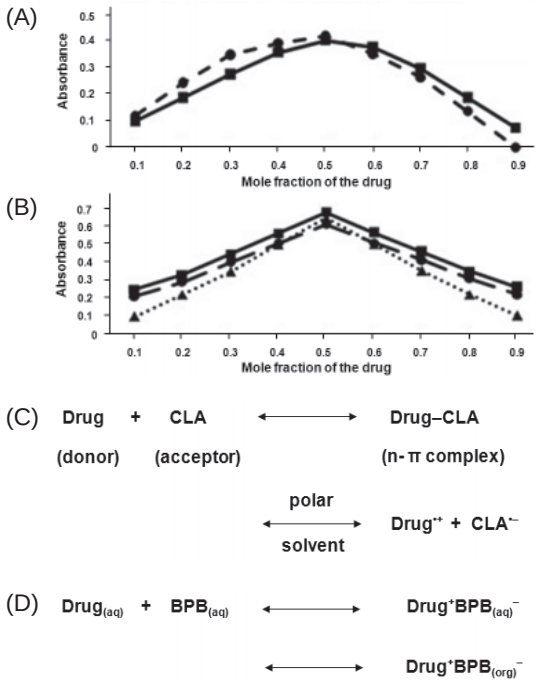


Figure 5. Job's method graphs for charge-transfer and ion-pair complex formations (A, B); Deduced scheme of charge-transfer and ion-pair complex formation and the extraction equilibrium (C, D). A, charge-transfer complex formation of RPG (dashed line) and RGL (solid line); **B**, ion-pair complex formation with RPG (dashed line), PGL (dotted line), and RGL (solid line); **C**, charge-transfer complex formation; **D**, Ion-pair complex formation.

3.2. *Development of stability-indicating spectrophotometric methods*

3.2.1. *Derivative spectrophotometry method (D^n)*

The UV-spectra of the oral hypoglycemic drugs under study and their acid-, alkaline- and oxidative-degradates are shown in Figure 6A$_I$-6I$_I$, where zero order determination for the drugs was not permitted in the presence of their degradates. Therefore, derivative spectrophotometric methods were adopted, where zero-crossing points for acid-, alkaline-, and oxidative-degradates of each studied drug are indicated. The first derivative spectrophotometric method (D^1) permitted a selective determination of RPG in the presence of its acid-, alkaline-, and oxidative-degradates at 263.8, 264.3, and 304.8 nm, respectively (Figures 6A$_{II}$-6C$_{II}$), and PGL in the presence of its acid- and alkaline-degradates at 253.4 and 284.1 nm, respectively (Figures 6D$_{II}$ and 6E$_{II}$). Also, the second derivative spectrophotometric method (D^2) permitted an excellent determination of PGL in the presence of its oxidative-degradates at 276.3 nm (Figure 6F$_{II}$), and RGL in the presence of its acid-, alkaline-, and oxidative-degradates at 308.0, 287.7, and 325.7 nm, respectively (Figures 6G$_{II}$-6I$_{II}$).

3.2.2. *pH-induced difference spectrophotometric method (DD^n)*

Change in the absorption spectra of the intact drugs under investigation, by using acid and alkaline media, was used as a stability-indicating study. The direct UV measurement of ΔA spectra were not suitable for assaying the studied drugs in the presence of their degradates, since there was severe overlap between spectra of the drugs and the degradates (Figure 7A$_I$-7I$_I$). Thus, first, second, and third derivative of ΔA spectra were adopted, where zero-crossing points for the acid-, alkaline-, and oxidative-degradates of each studied drug are indicated, respectively. First derivative [DD^1] of ΔA spectra was computed for determination of RPG in the presence of its acid- and alkaline-degradates at 258.0 and 261.8 nm, respectively (Figures 7A$_{II}$ and 7B$_{II}$), and PGL in the presence of its acid- and alkaline-degradates at 242.8 and 243.4 nm, respectively (Figures 7D$_{II}$ and 7E$_{II}$). Second derivative of ΔA spectra [DD^2] was computed for determination of RPG and PGL in the presence of their oxidative-degradates at 252.8 and 253.1, respectively (Figures 7C$_{II}$ and 7F$_{II}$). RGL was also determined in the presence of its acid- and oxidative-degradates at 275.9 and 267.4 nm by computing third derivative [DD^3] of ΔA spectra (Figures 7G$_{II}$ and 7H$_{II}$) and in the presence of its alkaline-degradates at 272.0 nm by computing second derivative [DD^2] of ΔA spectra (Figure 7I$_{II}$).

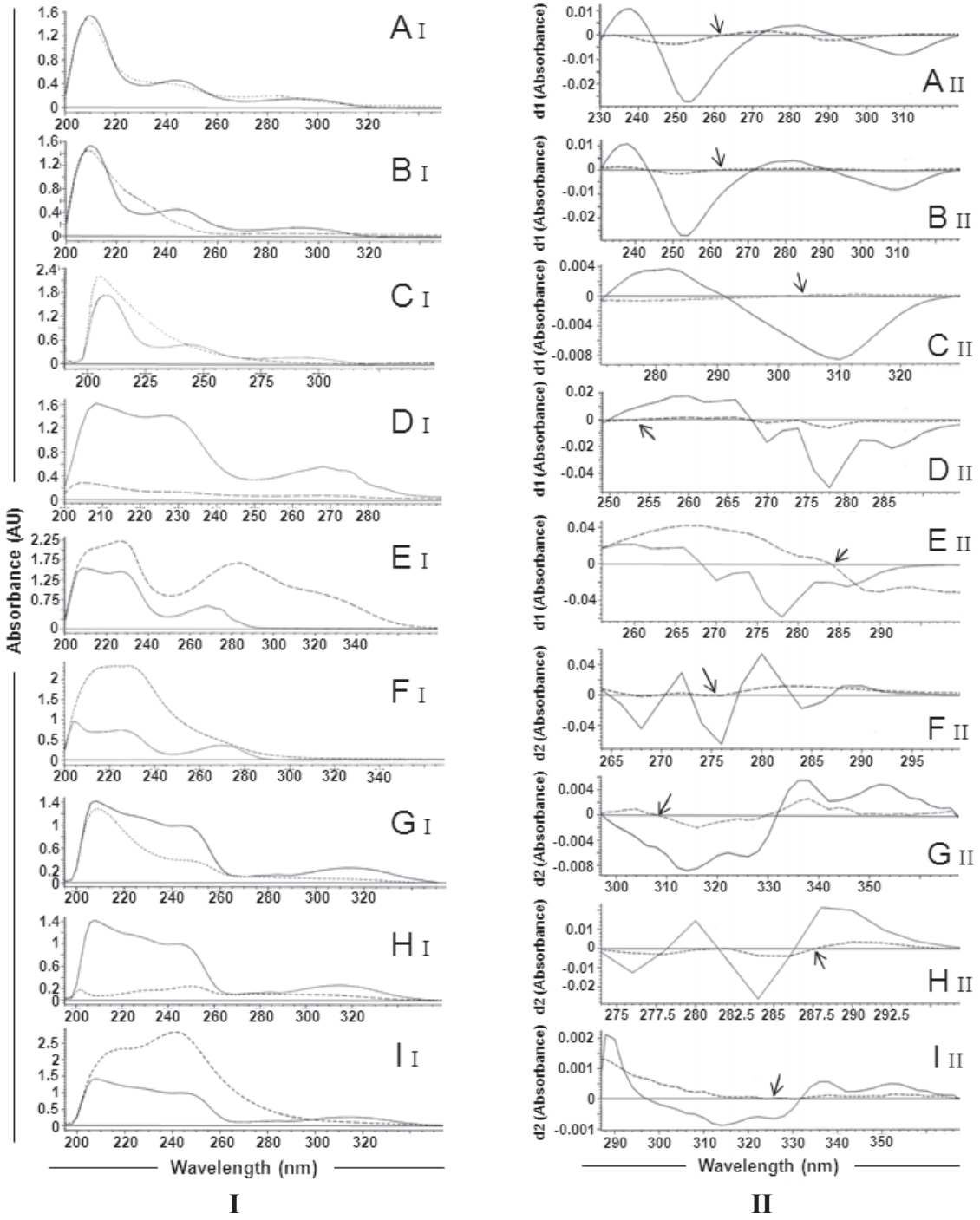


Figure 6. (I) UV-spectra of RPG, PGL, RGL, and their acid-, alkaline-, and oxidative-degradates. AI, RPG (solid line) and its acid-degradate (dashed line); **B**I, RPG (solid line) and its alkaline-degradate (dashed line); **C**I, RPG (solid line) and its oxidative-degradate (dashed line); **D**I, PGL (solid line) and its acid-degradate (dashed line); **E**I, PGL (solid line) and its alkaline-degradate (dashed line); **F**I, PGL (solid line) and its oxidative-degradate (dashed line); **G**I, RGL (solid line) and its acid-degradate (dashed line); **H**I, RGL (solid line) and its alkaline-degradate (dashed line); **I**I, RGL (solid line) and its oxidative-degradate (dashed line). **(II) First (D^1) or second (D^2) derivative spectra of RPG, PGL, RGL, and their acid-, alkaline-, and oxidative-degradates. A**II, first (D^1) derivative spectra of RPG (solid line) and its acid-degradate (dashed line); **B**II, first (D^1) derivative spectra of RPG (solid line) and its alkaline-degradate (dashed line); **C**II, first (D^1) derivative spectra of RPG (solid line) and its oxidative-degradate (dashed line); **D**II, first (D^1) derivative spectra of PGL (solid line) and its acid-degradate (dashed line); **E**II, first (D^1) derivative spectra of PGL (solid line) and its alkaline-degradate (dashed line); **F**II, second (D^2) derivative spectra of PGL (solid line) and its oxidative-degradate (dashed line); **G**II, second (D^2) derivative spectra of RGL (solid line) and its acid-degradate (dashed line); **H**II, second (D^2) derivative spectra of RGL (solid line) and its alkaline-degradate (dashed line); **I**II, second (D^2) derivative spectra of RGL (solid line) and its oxidative-degradate (dashed line).

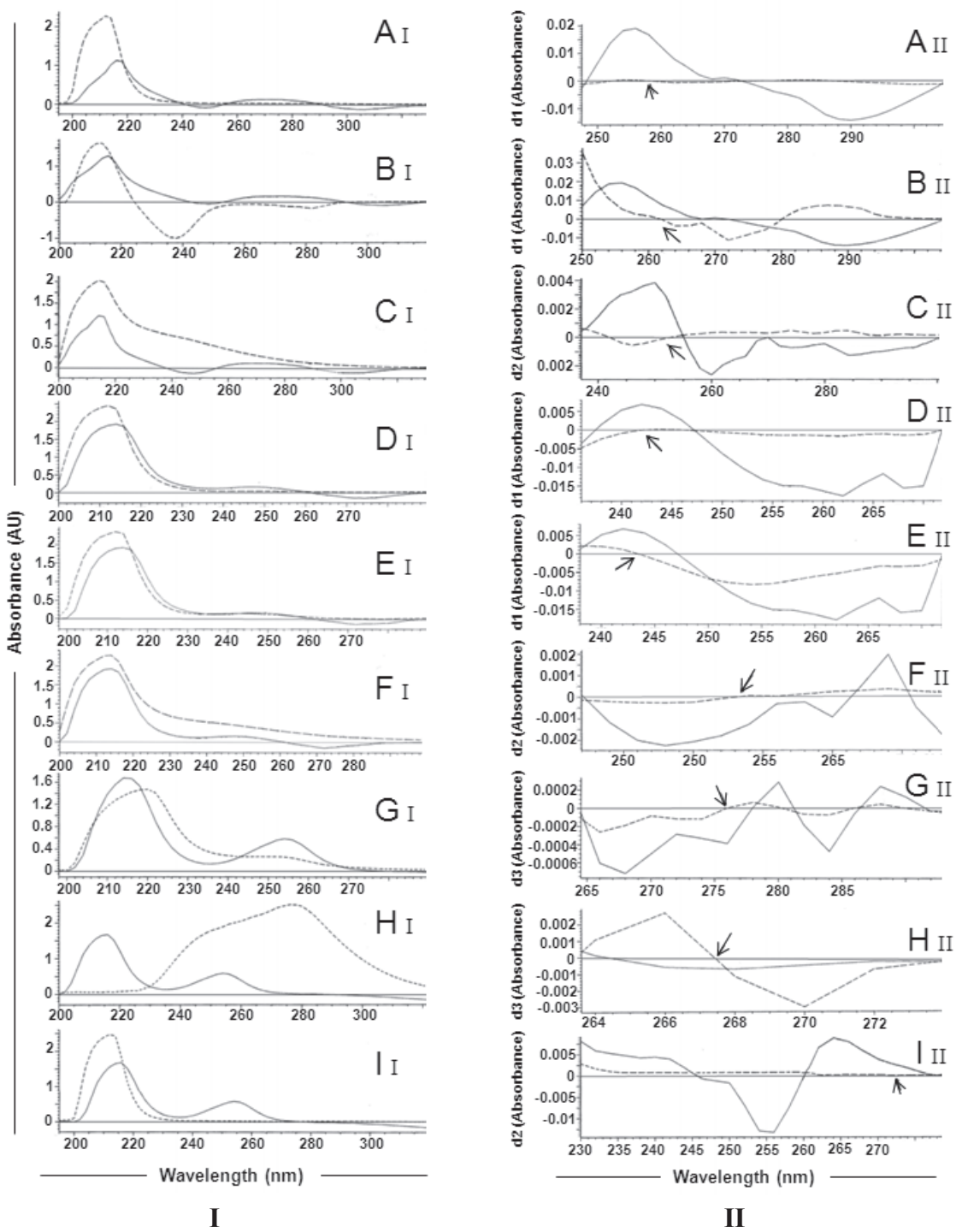


Figure 7. (I) ΔA spectra of RPG, PGL, RGL, and their acid-, alkaline-, and oxidative-degradates. AI, RPG (solid line) and its acid-degradate (dashed line); **B**I, RPG (solid line) and its alkaline-degradate (dashed line); **C**I, RPG (solid line) and its oxidative-degradate (dashed line); **D**I, PGL (solid line) and its acid-degradate (dashed line); **E**I, PGL (solid line) and its alkaline-degradate (dashed line); **F**I, PGL (solid line) and its oxidative-degradate (dashed line); **G**I, RGL (solid line) and its acid-degradate (dashed line); **H**I, RGL (solid line) and its alkaline-degradate (dashed line); **I**I, RGL (solid line) and its oxidative-degradate (dashed line). **(II) First (DD^1), second (DD^2), or third (DD^3) derivative of ΔA spectra of RPG, PGL, RGL, and their acid-, alkaline-, and oxidative-degradates. A**II, first (DD^1) derivative of ΔA spectra of RPG (solid line) and its acid-degradate (dashed line); **B**II, first (DD^1) derivative of ΔA spectra of RPG (solid line) and its alkaline-degradate (dashed line); **C**II, second (DD^2) derivative of ΔA spectra of RPG (solid line) and its oxidative-degradate (dashed line); **D**II, first (DD^1) derivative of ΔA spectra of PGL (solid line) and its acid-degradate (dashed line); **E**II, first (DD^1) derivative of ΔA spectra of PGL (solid line) and its alkaline-degradate (dashed line); **F**II, second (DD^2) derivative of ΔA spectra of PGL (solid line) and its oxidative-degradate (dashed line); **G**II, third (DD^3) derivative of ΔA spectra of RGL (solid line) and its acid-degradate (dashed line); **H**II, third (DD^3) derivative of ΔA spectra of PGL (solid line) and its oxidative-degradate (dashed line); **I**II, second (DD^2) derivative of ΔA spectra of PGL (solid line) and its alkaline-degradate (dashed line).

3.3. *Method validation*

Validation parameters according to International Conference on Harmonization (ICH) guidelines (*28*) are summarized in Tables 1-2. In the adopted spectrophotometric methods, the limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ = 3.3 for LOD and 10 for LOQ. SDa is the standard deviation of the intercept, and b is the slope. Three different concentrations of each studied drug (in the linear range) were analyzed by the proposed

Table 1. Validation parameters for charge-transfer and ion-pair spectrophotometric methods

Validation parameters	Charge-transfer method		Ion-pair method		
	RPG; 518.00 nm	RGL; 518.00 nm	RPG; 414.00 nm	PGL; 416.00 nm	RGL; 415.00 nm
Linearity (μg•mL^{-1})	50-325	50-300	5-35	5-35	5-35
Slope	0.00273	0.00243	0.01958	0.01950	0.01957
Intercept	0.01426	0.04705	0.05515	0.05229	0.03418
Correlation coefficient (*r*)	0.9998	0.9996	0.9997	0.9998	0.9997
LOD (μg•mL^{-1})	4.22	5.96	0.49	0.47	0.56
LOQ (μg•mL^{-1})	12.80	18.06	1.50	1.42	1.76
Precision					
Intra-day Mean (%)	99.46	99.52	99.87	98.82	100.85
R.S.D. (%)	0.396	1.558	0.881	1.565	0.662
Inter-day Mean (%)	99.45	99.62	99.82	98.84	100.70
R.S.D. (%)	0.431	1.520	1.124	1.591	0.549
Ruggedne [R.S.D. (%)]	0.755	0.716	0.710	0.607	1.340
Robustness [R.S.D. (%)]	0.539	0.581	0.760	0.725	1.493

Table 2. Validation parameters for the proposed stability-indicating spectrophotometric methods. A, Validation parameters for derivative spectrophotometric method (D^n); **B**, Validation parameters for pH-induced difference spectrophotometric (DD^n) method.

Table 2A

Validation parameters	RPG			PGL			RGL		
	D^1 at 263.79 nm	D^1 at 264.33 nm	D^1 at 304.84 nm	D^1 at 253.35 nm	D^1 at 284.05 nm	D^2 at 276.31 nm	D^2 at 307.95 nm	D^2 at 287.73 nm	D^2 at 325.67 nm
Linearity (μg•mL^{-1})	5-75	5-75	5-75	5-60	5-60	5-75	5-70	5-70	5-70
Slope	0.00046	0.00043	0.00037	0.00052	0.00119	0.00021	0.00003	0.00010	0.00004
Intercept	–0.00040	–0.00056	–0.00029	–0.00121	–0.00376	0.00093	0.00005	–0.00020	–0.00012
Correlation coefficient (*r*)	0.9998	0.9998	0.9998	0.9998	0.9999	0.9997	0.9999	0.9997	0.9997
LOD (μg•mL^{-1})	0.90	0.88	0.78	0.83	0.53	0.95	0.55	1.02	0.98
LOQ (μg•mL^{-1})	2.73	2.66	2.37	2.52	1.60	2.88	1.67	3.10	2.98
Precision									
Intra-day Mean (%)	100.29	100.93	99.13	99.02	99.33	99.12	100.70	100.58	100.33
R.S.D. (%)	0.987	1.248	0.286	0.221	0.728	1.143	0.167	0.129	0.771
Inter-day Mean (%)	100.27	100.75	98.99	99.72	99.06	98.98	100.61	100.80	100.36
R.S.D. (%)	1.142	1.315	0.237	0.539	0.861	1.007	0.570	0.963	1.514
Ruggedness [R.S.D. (%)]	0.404	0.634	0.802	0.460	0.770	0.845	0.663	0.412	0.429

Table 2B

Validation parameters	RPG			PGL			RGL		
	DD^1 at 258.04 nm	DD^1 at 261.82 nm	DD^2 at 252.80 nm	DD^1 at 242.81 nm	DD^1 at 243.41 nm	DD^2 at 253.12 nm	DD^3 at 275.90 nm	DD^2 at 272.00 nm	DD^3 at 267.40 nm
Linearity (μg•mL^{-1})	5-65	5-65	5-75	5-80	5-80	5-75	5-70	5-70	5-70
Slope	0.00083	0.00038	0.00010	0.00035	0.00033	0.00007	0.00002	0.00008	0.00004
Intercept	0.00016	0.00032	0.00016	-0.00053	-0.00053	0.00008	-0.00005	0.00013	-0.00002
Correlation coefficient (*r*)	0.9997	0.9996	0.9997	0.9997	0.9997	0.9998	0.9997	0.9997	0.9997
LOD (μg•mL^{-1})	0.98	1.08	1.02	1.09	0.96	0.73	0.92	1.04	1.04
LOQ (μg•mL^{-1})	2.98	3.26	3.08	3.30	2.90	2.22	2.78	3.14	3.16
Precision									
Intra-day Mean (%)	100.63	101.30	100.30	100.02	99.53	98.94	99.79	101.51	100.65
R.S.D. (%)	0.853	0.499	1.623	0.967	0.797	0.367	0.615	0.144	0.914
Inter-day Mean (%)	100.30	101.01	100.10	98.68	99.56	98.93	100.16	101.56	100.76
R.S.D. (%)	0.841	0.723	1.636	0.163	0.849	0.364	0.880	0.281	0.950
Ruggedness [R.S.D. (%)]	0.410	0.489	0.355	0.479	0.630	0.338	0.477	0.590	0.710
Ruggedness [R.S.D. (%)]	0.435	0.316	0.250	0.457	0.642	0.355	0.413	0.434	0.556

spectrophotometric methods in three independent series on the same day (intra-day precision) and three consecutive days (inter-day precision) within each series and every concentration was examined three times. The R.S.D.% values of intra- and inter-day studies showed that the intermediate precision of the proposed methods were satisfactory (Tables 1-2). When ruggedness of the adopted spectrophotometric methods was assessed by applying the procedures using two different sources of solvents, *i.e.* methanol and acetonitrile, and results obtained were found to be reproducible since R.S.D.% did not exceed 2%. Robustness of the spectrophotometric procedures was determined by evaluating the influence of small variations of experimental variables: CLA concentration (charge-transfer method), BPB concentration and pH of phthalate buffer (ion-pair method) and HCl and NaOH concentration used in pH-induced difference spectrophotometric method; where the capacity of the method remained unaffected by small deliberate variations. The results obtained from both ruggedness and robustness provided an indication of the reliability of the proposed methods during routine work.

Solution stability was evaluated, in which the standard solutions and the reagents solutions were subjected to long term (8 days) stability studies. The stability of the solutions kept at 4°C or room temperature was studied by comparing their recoveries with freshly prepared solutions. It was found that solutions kept at 4°C were stable up to 7 days while those kept at room temperature were stable for only 3 days (data not shown).

Degradation behaviors of the studied drugs were investigated by the proposed stability-indicating spectrophotometric methods, where RPG, PGL, and RGL were determined in solutions containing different amounts of their acid-, alkaline-, and oxidative-degradates by D^n and DD^n spectrophotometric methods. The recovery% and R.S.D.% proved a high specificity of the adopted stability-indicating methods (Table 3), where the studied hypoglycemic drugs could be determined in the presence of their degradates (up to 90%).

Molar absorptivity values of the charge-transfer method for RPG and RGL with CLA were found to be 1.23×10^3 and 8.67×10^2 l•mol^{-1}•cm^{-1}, respectively, and those of the ion-pair method for RPG, PGL, and RGL with BPB were found to be 8.86×10^3, 6.95×10^3, and 7.06×10^3 l•mol^{-1}•cm^{-1}, respectively. Sandell's sensitivity (S) represents the number of micrograms of the determinant per milliliter of a solution having an absorbance (A) of 0.001 for a path length (l) of 1-cm (*29*). Thus, $S = 10^{-3}/a = \mu g \cdot cm^{-2}$ where, a is the specific absorptivity and its value (in mL•g^{-1}•cm^{-1}) corresponds to determination in a cuvette with a path length of 1-cm. Also, a = (b/molecular weight of the drug under study) × 1,000, where b = molar absorptivity = A/Cl, where C is the molar concentration of the determinant and l = 1-cm path length. Sandell's sensitivity was found to be 0.367 and 0.412 μg•cm^{-2} for the charge-transfer method of RPG and RGL with CLA, respectively, and 0.051 μg•cm^{-2} for the ion-pair method for all hypoglycemic drugs under study with BPB.

Table 3. Specificity of the proposed stability-indicating spectrophotometric methods. A, Specificity of the proposed derivative spectrophotometric (D^n) method; **B**, Specificity of the proposed pH-induced difference spectrophotometric (DD^n) method.

Table 3A

Laboratory-prepared mixture		% Recovery[b] of RPG			% Recovery[b] of PGL			% Recovery[b] of RGL		
Intact drug (μg•mL^{-1})	Degradate[a] (μg•mL^{-1})	D^1 at 263.79 nm	D^1 at 264.33 nm	D^1 at 304.84 nm	D^1 at 253.35 nm	D^1 at 284.05 nm	D^2 at 276.31 nm	D^2 at 307.95 nm	D^2 at 287.73 nm	D^2 at 325.67 nm
45.00	5.00	98.34	98.14	98.79	101.23	99.68	99.33	100.78	100.42	100.46
35.00	15.00	99.13	98.38	99.07	101.00	99.47	98.93	101.09	100.62	99.72
25.00	25.00	99.25	98.50	99.17	101.43	99.70	99.52	101.72	101.97	100.25
15.00	35.00	99.26	99.12	99.27	101.36	99.87	99.59	101.94	100.09	99.11
5.00	45.00	99.38	99.44	101.01	101.27	100.20	98.51	101.62	101.13	99.86
Mean (%)		99.07	98.71	99.46	101.26	99.78	99.18	101.43	100.84	99.88
R.S.D. (%)		0.423	0.543	0.890	0.163	0.272	0.456	0.473	0.726	0.522

Table 3B

Laboratory-prepared mixture		% Recovery[b] of RPG			% Recovery[b] of PGL			% Recovery[b] of RGL		
Intact drug (μg•mL^{-1})	Degradate[a] (μg•mL^{-1})	DD^1 at 258.04 nm	DD^1 at 261.82 nm	DD^2 at 252.80 nm	DD^1 at 242.81 nm	DD^1 at 243.41 nm	DD^2 at 253.12 nm	DD^3 at 275.90 nm	DD^2 at 272.00 nm	DD^3 at 267.40 nm
45.00	5.00	98.95	101.12	98.64	98.16	98.18	100.41	99.87	101.02	101.70
35.00	15.00	99.69	101.45	99.51	98.51	98.10	100.05	100.40	101.44	100.60
25.00	25.00	99.77	101.50	99.86	98.50	98.38	99.62	101.91	101.16	101.57
15.00	35.00	99.85	101.84	100.44	98.45	98.34	99.73	101.63	100.91	101.77
5.00	45.00	100.98	100.82	101.38	98.55	98.20	100.40	101.51	99.86	101.02
Mean (%)		99.85	101.35	99.97	98.44	98.24	100.04	101.06	100.88	101.33
R.S.D. (%)		0.730	0.384	1.027	0.162	0.119	0.365	0.871	0.598	0.499

[a] In presence of acid, alkaline and oxidative-degradates of each studied oral hypoglycemic drug, respectively.
[b] Mean of three determinations.

The accuracy of the proposed methods was demonstrated by recovery experiments, using a standard addition technique, where the percentage of R.S.D.s can be considered to be very satisfactory. The analytical results of the pharmaceutical preparations and the standard addition technique of the studied drugs by the proposed spectrophotometric methods are summarized in ¶ Tables S1-S4, suggesting that there is no interference from any excipients present normally in tablets.

All the obtained results were statistically compared to the official method used for RPG analysis and the manufacturer methods used for PGL and RGL analysis, respectively. As shown in ¶ Table S5, no significant differences were found.

4. Discussion

The aim of this study was to develop simple, fast, validated, and economical methods for analysis of RPG, PGL, and RGL in pure forms and in their pharmaceutical preparations. Two selective, simple, and less time consuming spectrophotometric methods were described for analyzing RPG and RGL using CLA reagents and RPG, PGL, and RGL using BPB reagents. The proposed stability-indicating methods (derivative and pH-induced difference spectrophotometry) provided accurate, specific, and reproducible quantitative analysis of the studied drugs in the presence of their acidic, alkaline, and oxidative degradation products. ICH guidelines were followed throughout the study for method validation and stress testing. The high recovery percentage and low relative standard deviation suggested high accuracy and precision of the proposed methods. Moreover, the adopted methods are easy, applicable to a wide range of concentration, besides being less time consuming, highly cost-effective and depending on simple and available reagents, thus offering economical and acceptable methods for the routine quality control analysis of drugs in bulk powder and in their pharmaceutical preparations without interference from common excipients.

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