ISSN : 0974-7419 Volume 10 Issue 3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(3) 2011 [188-203]

Validated spectrophotometric methods for determination of some oral hypoglycemic used drugs

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Received: 17th August, 2010 ; Accepted: 27th August, 2010

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 "charge-transfer purple-colored complex, between chloranilic acid (CLA) and (RPG and RGL) with molar absorptivity 1.23×10^3 and 8.67×10^2 $L.mol^{-1}.cm^{-1}$ and sandell's sensitivity 0.367 and 0.412 $\mu g.cm^{-2}$, respectively" and "ion-pair yellow-colored complex between bromophenol blue (BPB) and (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 sandell's sensitivity 0.051 $\mu g.cm^{-2}$ for all ion-pair complexes". The influence of different parameters on the color formation was studied to determine the optimum conditions for the visible spectrophotometric methods. The other spectrophotometric methods were adopted for determination of the studied drugs in presence of their acid, alkaline and oxidative-degradates by computing derivative and pH-induced difference spectrophotometry, as stability-indicating methods. All the proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for determination of the studied drugs in pure form and in pharmaceutical preparations with good extraction recoveries ranges between 98.71-101.35 %, 98.24-101.26 % and 99.88- 101.43 % for RPG, PGL and RGL, respectively. Results of relative standard deviation did not exceed 1.636 %, 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 manufacturer methods used for PGL and RGL analysis, respectively, where there is No significant differences were found.

KEYWORDS

Repaglinide;
Pioglitazone hydrochloride;
Rosiglitazone maleate;
Charge-transfer complex;
Ion-pair complex;
Derivative spectrophotometry;
Difference spectrophotometry.

INTRODUCTION

For many years pharmacological agents such as sulphonylureas and biguanides were the mainstay of oral treatment of 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) and thiazolidinediones (pioglitazone and rosiglitazone) has been welcome[1]. Repaglinide (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 receptors gamma (PPARγ), thus increasing the receptor sensitivity to insulin[2].

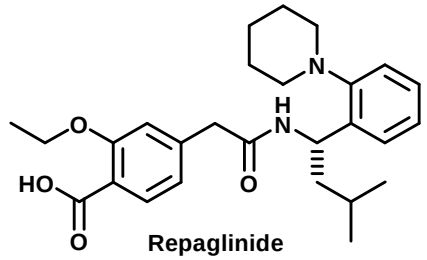
Many analytical methods have been reported for the quantitative estimation of (RPG) in pharmaceutical preparations and biological samples[3-5] which include visible spectrophotometric[6,7], HPLC[8,9] and electrochemical methods[10]. (PGL) and its metabolites have been determined in biological fluids 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 a 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 assay using 'derivative and pH-induced difference spectrophotometry'. All the proposed methods were successfully applied for the routine quality control analysis of the mentioned drugs in raw material and in their pharmaceutical preparations unaffected by interference from excipients.

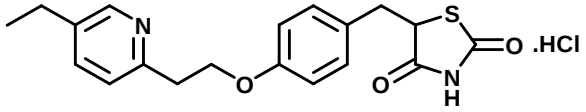
MATERIALS AND METHODS

Chemicals and reagents

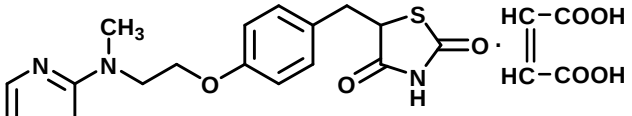
Repaglinide and pioglitazone hydrochloride were kindly supplied by Amoun pharmaceutical company and certified to contain 99.99% and 99.95%, respectively. Diarol® tablets: batch number: 1018, each tablet was labeled to contain 2 mg repaglinide and Actozone® tablets: batch number: 3543, each tablet was labeled to contain 45 mg pioglitazone hydrochloride. Rosiglitazone maleate was kindly supplied by Apex pharmaceutical company and certified to contain 99.99%. Rosizone® tablets: batch number: MT0410208, each tablet was labeled to contain 4 mg Rosiglitazone maleate.



Repaglinide



Pioglitazone hydrochloride



Rosiglitazone maleate

Water (bi-distilled), methanol (Riedel-de Haen), acetonitrile (Riedel-de Haen), chloranilic acid (BDH), bromophenol blue (BDH), potassium hydrogen phthalate (El-Nasr Pharmaceutical Co.), chloroform (El-Nasr Pharmaceutical Co.), hydrochloric acid 35.4 % (BDH); 0.1M, 0.2M, 2M and 5M aqueous solutions, sodium hydroxide (BDH); 0.1M, 2M and 5M aqueous solutions, hydrogen peroxide 30 % (El-Nasr Pharmaceutical Co.) and 96 % ethanol (El-Nasr Pharmaceutical Co.).

All chemical and reagents used through this work are of spectroscopic analytical grade. Bi-distilled water is used throughout the whole work and is indicated by the word 'water'.

Instruments

Hewlett Packard HP 8452A Diode Array Spectrophotometer connected to an IBM compatible computer and HP Laser printer is used. The bundled software is UV-Visible chemstation Rev. A.08.03 [71] copyright® Agilent Technologies 95-00. The spectral bandwidth is 0.2 nm and the wavelength scanning speed was 2800.0 nmmin^{-1}. The absorption spectra of the reference and the test solutions are recorded in 1.0-ml quartz cells at 25.0°C, using '$\Delta\lambda$ = 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)'. Bandelin Sonorex RK 100H DVE GS (gepüfte sicherheit) Sonicator. A (Jenway 3310, UK) pH-meter, equipped with combined glass elec-

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trode for pH adjustment.

Standard solutions

(1) Standard solutions of the studied drugs

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

(2) Standard solutions of the used reagents (for charge-transfer and ion-pair methods)

0.1 % (w/v) CLA in acetonitrile and [0.1 % (w/v) BPB and phthalate buffers "pH 2.4 and 2.2"][25] were used for charge-transfer and ion-pair methods, respectively.

(3) Standard solutions of the degradates (for stability indicating spectrophotometric methods)

Three standard degradated-solutions "acid, alkaline and oxidative" of RPG, PGL and RGL, were prepared by mixing 10 mg of each separately with fifty mls of "2 M HCl, 2 M NaOH and 30% H_2O_2, respectively", heating in thermostatic water-bath at 80°C for 24 hours, cooling, [neutralizing the media with "5 M NaOH and 5 M HCl for the acid and alkaline degradated-solutions, respectively"] and then complete the volume for all the degradated-solutions with methanol to obtain a final concentration of (0.1 mg.ml^{-1}).

Procedures

(1) For 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 to the volume 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.

For ion-pair method

Into three separating funnels, aliquots of (RPG, PGL and RGL) working standard solutions were separately transferred, 4.0 ml of phthalate buffer pH 2.4 and 2.2 for [(RPG and PGL) and RGL] and then 3.0 ml of 0.1% BPB reagent solution were added. 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 sulphate and diluted to the volume with chloroform in 10 ml volumetric flasks to obtain a concentration range of 5-35 μg.ml^{-1}. The absorbance of the produced colored-complexes was measured at 414 nm, 416 nm and 415 nm against a reagent blank at room temperature, respectively. Calibration curves were constructed and the regression equation was then computed.

For stability-indicating spectrophotometric methods

(1) Derivative spectrophotometric (D^n) method

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

(2) pH-induced difference spectrophotometric (DD^n) method

From standard working solutions, aliquots were transferred into two sets of 10 ml volumetric flasks which

were then diluted with 0.1M [HCl and NaOH], respectively. ΔA spectra were computed by placing the acid solution in the reference beam and the alkaline solution in the sample beam. RPG can be determined in a concentration range of 5-65 $\mu g.ml^{-1}$ in presence of its acid and alkaline-degradates, where the values of the first derivative of ΔA spectra (DD^1) were computed at 258.04 nm and 261.82 nm, respectively, while second derivative of ΔA spectra (DD^2) values were computed at 252.80 nm in a concentration range of 5-75 $\mu g.ml^{-1}$ in presence of its oxidative-degradates. PGL can be determined in a concentration range of 5-80 $\mu g.ml^{-1}$ in presence of its acid and alkaline-degradates, where the values of the first derivative of ΔA spectra (DD^1) were computed at 242.81 nm 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 $\mu g.ml^{-1}$ in presence of its oxidative-degradates. While RGL can be determined in a concentration range of 5-70 $\mu g.ml^{-1}$ in 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 presence of its alkaline-degradates and the values of the third derivative of ΔA spectra (DD^3) amplitudes were computed at 275.90 nm and 267.40 nm in presence of its acid and oxidative-degradates, respectively. The calibration curves were constructed and the regression equation was then computed.

Assay of the pharmaceutical preparations by the proposed methods and application of standard addition technique

Sixty tablets from Diarol®, ten tablets from Actozone® and thirty tablets from Rosizone® were individually weighed to get the average weight of the tablets, respectively. For charge-transfer method, a sample of the powdered tablets, claimed to contain 50 mg of RPG and RGL was transferred separately to 50 ml volumetric flasks, sonicated for 20 minutes with 30 ml acetonitrile, then the volume was brought to 50 ml with same solvent and filtered to prepare stock working solutions, each having a concentration 1.0 $mg.ml^{-1}$. Aliquots of the filtrate were further diluted with same solvent, then proceeds as described under (2.4.1). For other spectrophotometric methods, a sample of the powdered tablets, claimed to contain 25 mg of RPG, PGL and RGL was transferred separately to 250 ml volumetric flasks, sonicated for 20 minutes with 200 ml methanol, then the volume was brought to 250 ml with same solvent and filtered to prepare stock working solutions, each having a concentration 0.1 $mg.ml^{-1}$. Aliquots of filtrate were further diluted with same solvent and then proceeds as described under (2.4.2 and 2.4.3) for ion-pair and stability-indicating spectrophotometric methods, respectively.

To check the validity of the proposed methods, the standard addition technique was applied. For charge-transfer method, a sample of the powdered tablets, claimed to contain 5 mg of RPG and RGL, respectively was accurately weighed and mixed with [5, 10, 15, 20 and 25 mg] of pure drug, separately. Each spiked sample of (RPG and RGL) was transferred to 25 ml volumetric flask, sonicated for 20 minutes with 20 ml acetonitrile then the volume was adjusted with same solvent and filtered, to get five spiked solutions from each pharmaceutical preparation in a concentration range (0.4-1.2 $mg.ml^{-1}$). From each spiked solution, 2.5 ml was transferred to 10 ml volumetric flask then proceeds as described under (2.4.1). For ion-pair and stability-indicating spectrophotometric methods, a sample of the powdered tablets, claimed to contain 5 mg of RPG, PGL and RGL, respectively was accurately weighed and mixed with [5, 10, 15, 20 and 25 mg] of pure drug, separately. Each spiked sample of (RPG, PGL and RGL) was transferred to 100 ml volumetric flask, sonicated for 20 minutes with 75 ml methanol then the volume was adjusted with same solvent and filtered, to get five spiked solutions from each pharmaceutical preparation in a concentration range (0.1-0.3 $mg.ml^{-1}$). For ion-pair method, 1.0 ml is taken from each spiked solution and then proceeds as described under (2.4.2), while for stability-indicating spectrophotometric methods, 1.5 ml is taken from each spiked solution and then proceeds as described under (2.4.3).

RESULTS

Method development

(1) Charge-transfer and ion-pair methods

The absorption spectra of charge-transfer complexes formed between (RPG and RGL) and CLA and

the ion-pair complexes formed between (RPG, PGL and RGL) and BPB were measured against reagent-blanks (Figure 1-5). The charge-transfer complexes show maximum absorbance at 518 nm for (RPG and RGL), respectively. The ion-pair complexes show maximum absorbance at 414 nm, 416 nm and 415 nm for (RPG, PGL and RGL), respectively. The influence of different parameters on the color formation was studied to determine the optimum conditions for the visible spectrophotometric methods.

(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 made 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 low molar absorptivity. Furthermore, acetonitrile was considered as an ideal solvent for CLA (π-acceptor), this because it offered a maximum sensitivity which was attributed to its high dielectric constant that promotes maximum yield of the complex[26]. While for 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 aqueous phase. Chloroform was found to be the most suitable solvent for extraction of ion-pair complexes from the aqueous solutions, yielding 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.

(3) Reagent concentration

Figure 6 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 added to a fixed concentrations of the studied drugs, 3.0 ml and 2.0 ml of 0.1 % CLA solution (w/v) were found to be the effective volumes for the quantitative determination of the mentioned drugs, respectively. Figure 7 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 added to a fixed concentrations of the studied drugs, 3.0 ml of 0.1% BPB solution (w/v) was adequate to obtain a stable product for quantitative determination of RPG, PGL and RGL, respectively.

(4) Effect of time and temperature

The optimum reaction time was investigated by following the color development at ambient temperature, where the relationship between time and absorbance represented in figure 8 which shows that the reaction is instantaneous and stable up to two hours for the produced charge-transfer complexes. While, for ion-pair complexes, complete color intensity was attained after two minutes of mixing with chloroform and stable up to two hours as shown in figure 9. Figure 10 and 11 show the relationship between temperature and absorbance, where raising the temperature up to 30 °C has no effect on the formed complexes (either charge-transfer or ion-pair), but the absorbance starts to decay above 30°C.

(5) Effect of phthalate-buffer (pH and volume) on the ion-pair complex formation

The effect of pH was studied by extracting the yellow-colored complexes in the presence of phthalate-buffer at various pH (2.0-4.0), where the relationship between pH and the absorbance represented in figure 12 which shows a maximum color intensity and consequently a higher absorbance at pH 2.4 and 2.2 for (RPG and PGL) and RGL, respectively. Also, the stability of the formed color-complexes without affecting the absorbance was achieved by using 4.0 ml of phthalate buffers at the chosen pH-values, where a maximum absorbance and reproducible results were obtained as shown in figure 13.

(6) Stoichiometric relationship

Job's method of continuous variation[27] has been applied in order to ascertain the stoichiometry of the reaction between [(RPG and RGL) and CLA] and [(RPG, PGL and RGL) and BPB], respectively, where equimolar solutions (1.0×10^{-3}) of each drug, CLA and BPB were used.

The results obtained from job's method represented in figure 14, which indicate that 1:1 (drug : π-acceptor) charge-transfer complexes are formed through com-

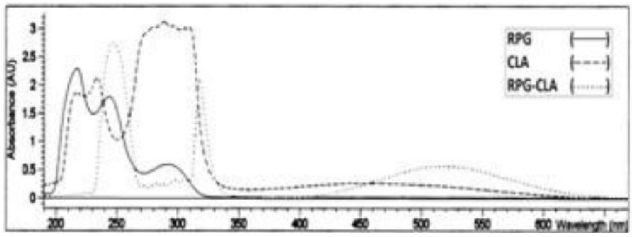

Figure 1 : UV-Vis spectra of RPG, CLA and RPG-CLA charge-transfer complex

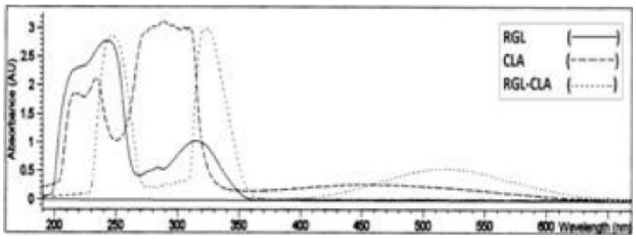

Figure 2 : UV-Vis spectra of RGL, CLA and RGL-CLA charge-transfer complex

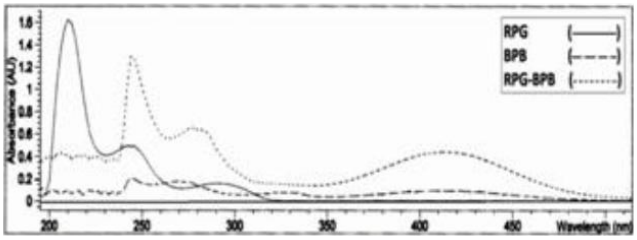

Figure 3 : UV-Vis spectra of RPG, BPB and RPG-BPB ion-pair complex

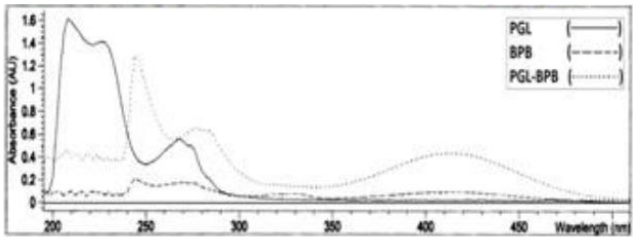

Figure 4 : UV-Vis spectra of PGL, BPB and PGL-BPB ion-pair complex

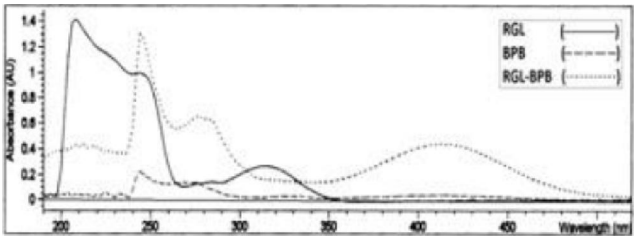

Figure 5 : UV-Vis spectra of RGL, BPB and RGL-BPB ion-pair complex

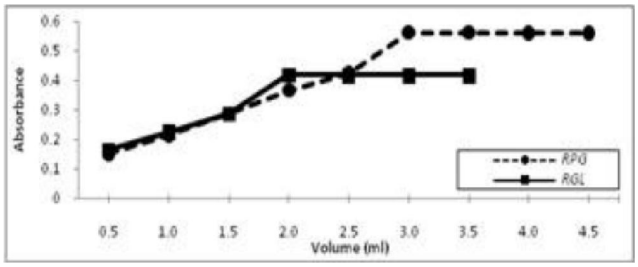

Figure 6 : Effect of CLA concentration (by volume)

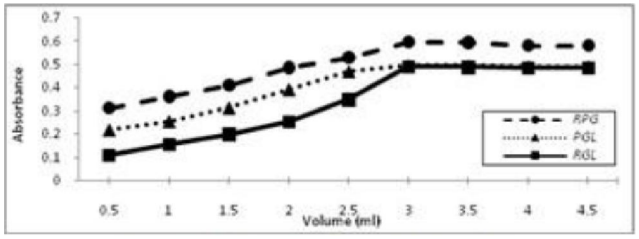

Figure 7 : Effect of BPB concentration (by volume)

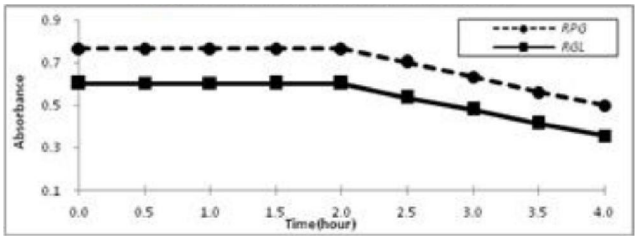

Figure 8 : Effect of time on charge-transfer complexes

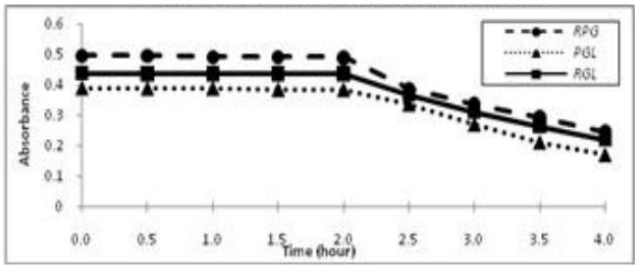

Figure 9 : Effect of time on ion-pair complexes

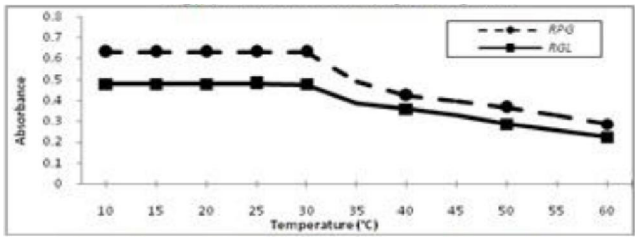

Figure 10 : Effect of temperature on charge-transfer complexes

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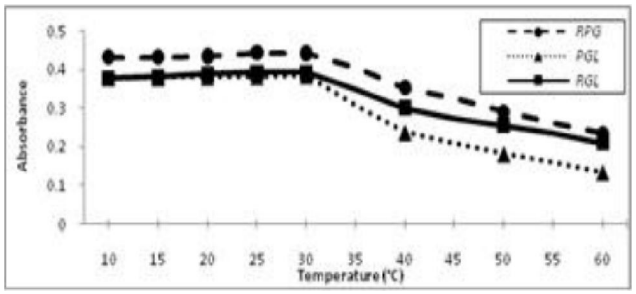

Figure 11 : Effect of temperature on ion-pair complexes

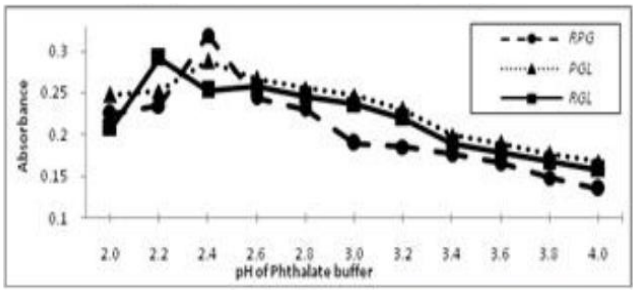

Figure 12 : Effect of pH of phthalate buffer on ion-pair complexes

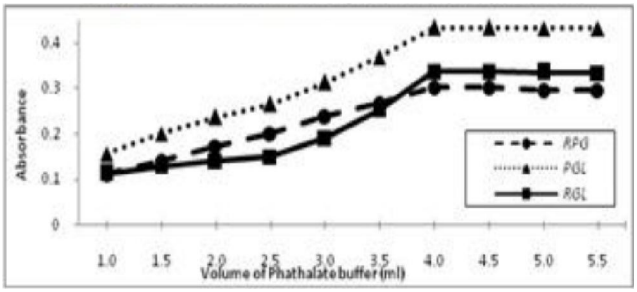

Figure 13 : Effect of phthalate buffer (at the chosen pH)

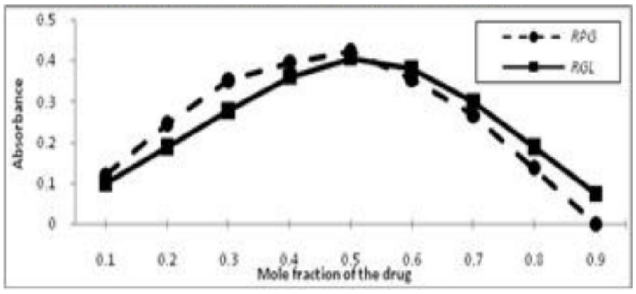

Figure 14 : Job's method graph for the reaction with CLA

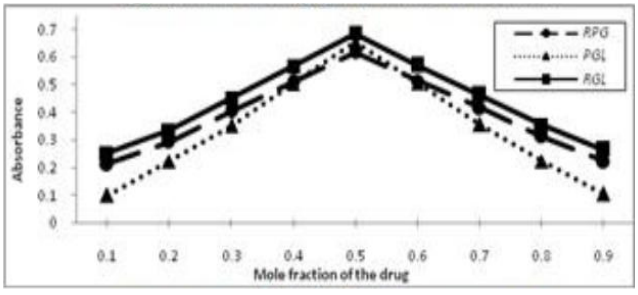

Figure 15 : Job's method graph for the reaction with BPB

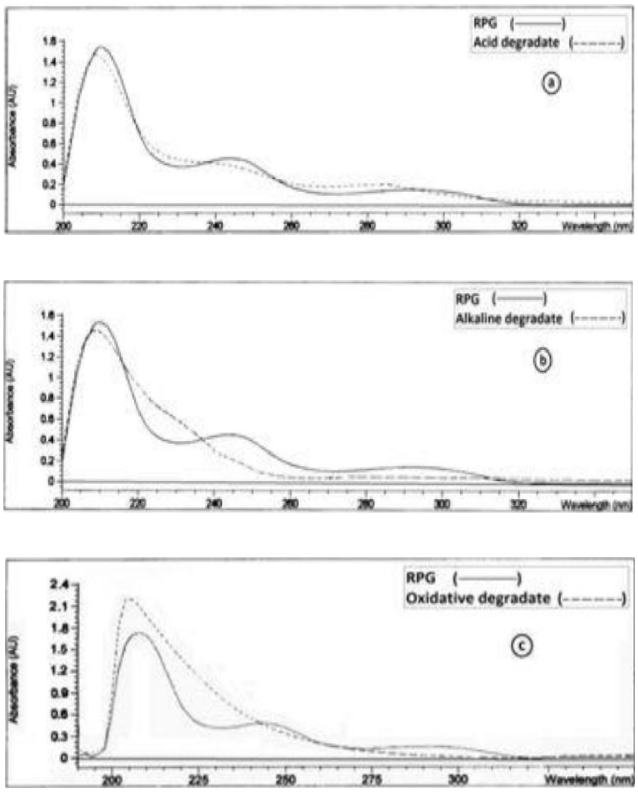

Figure 16 : UV- spectra of RPG and its acid (a), alkaline (b) and oxidative (c) degradates

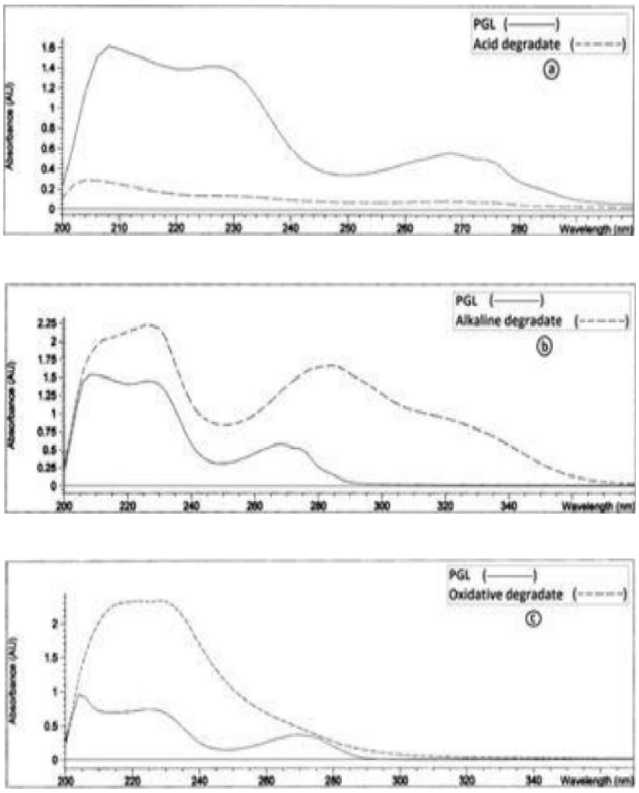

Figure 17 : UV- spectra of PGL and its acid (a), alkaline (b) and oxidative (c) degradates

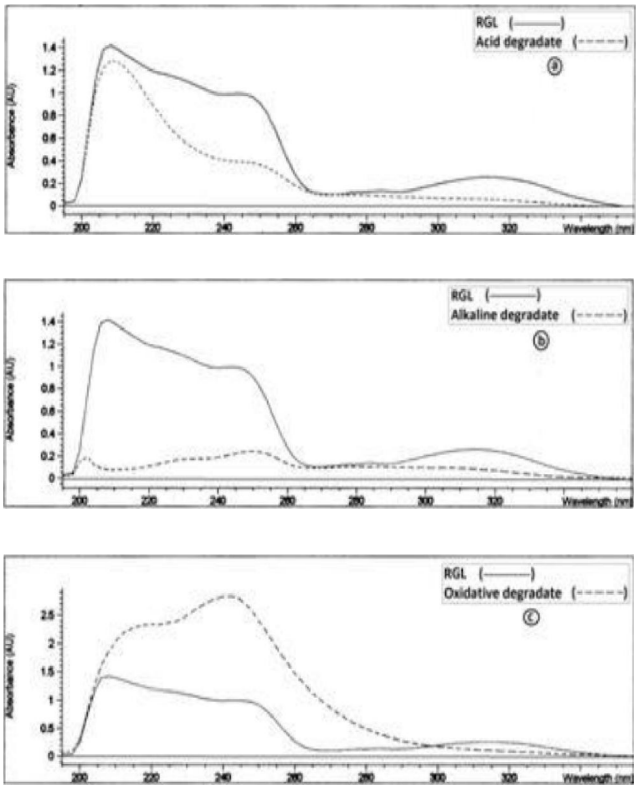

Figure 18 : UV- spectra of RGL and its acid (a), alkaline (b) and oxidative (c) degradates

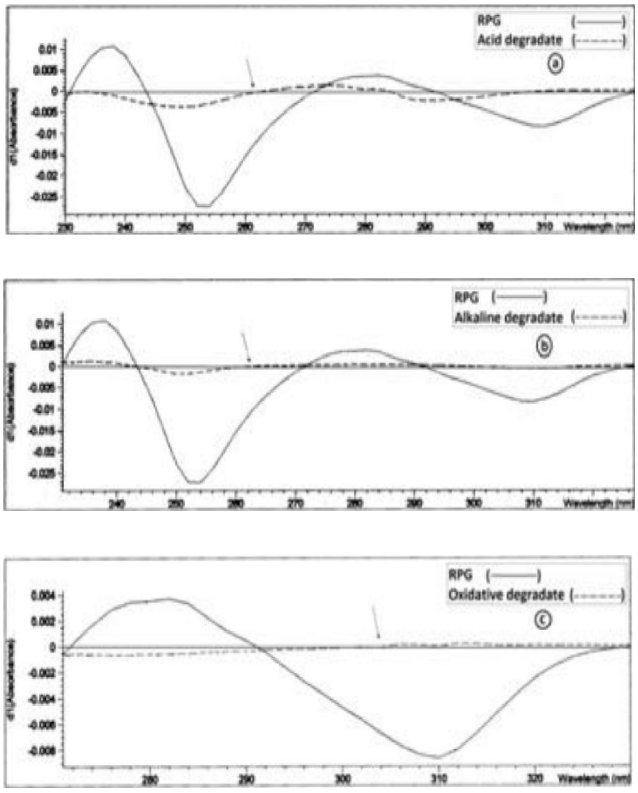

Figure 19 : First (D^1) derivative spectra of RPG and its acid (a), alkaline (b) and oxidative (c) degradates

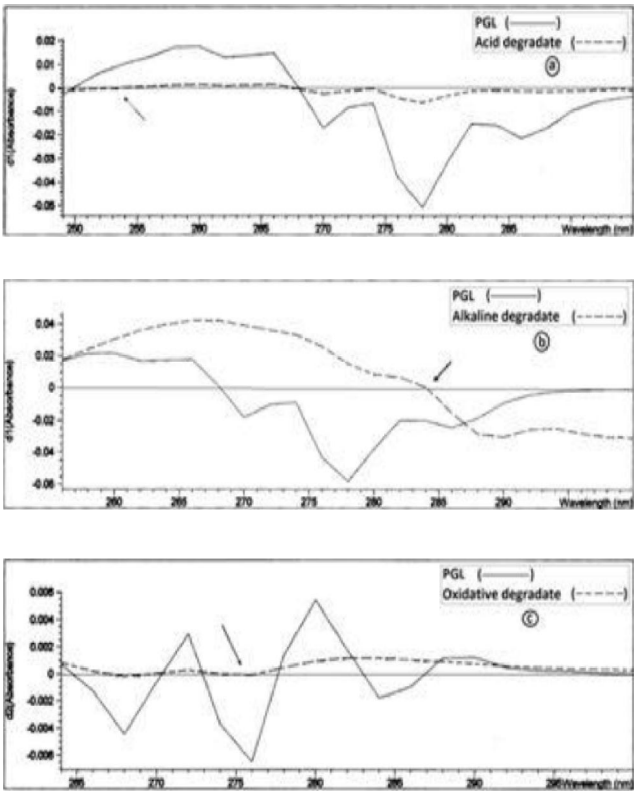

Figure 20 : First (D^1) and second (D^2) derivative spectra of PGL and its [acid (a) and alkaline (b)] and oxidative(c) degradates

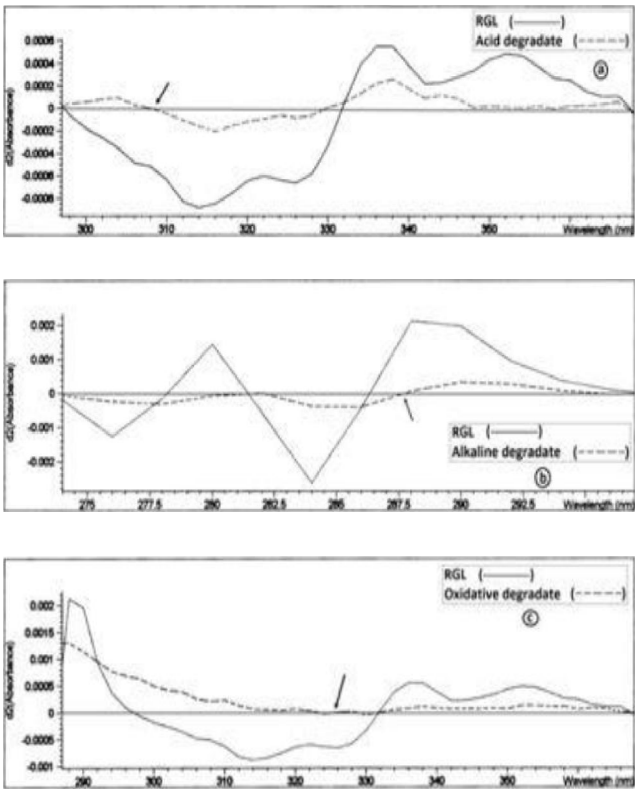

Figure 21 : Second derivative spectra (D^2) of RGL and its acid (a), alkaline (b) and oxidative (c) degradates

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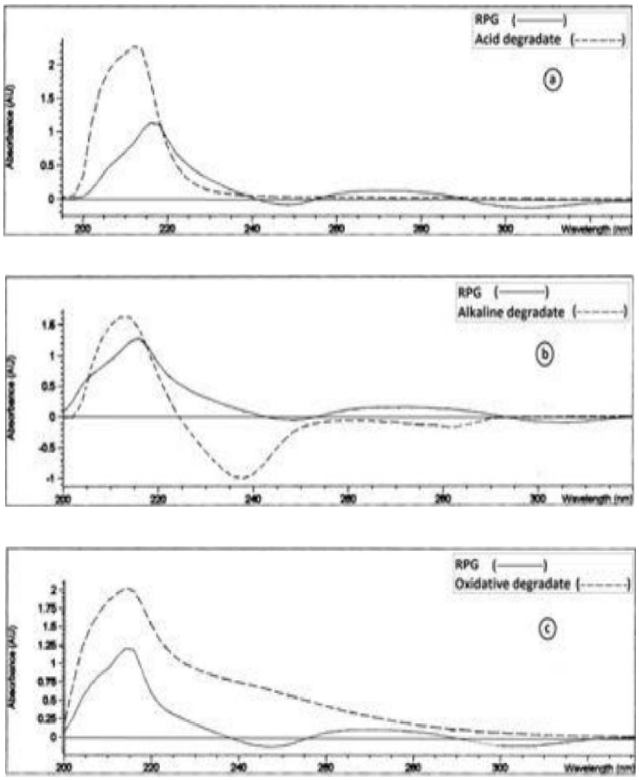

Figure 22 : ΔA spectra of RPG and its acid (a), alkaline (b) and oxidative (c) degradates

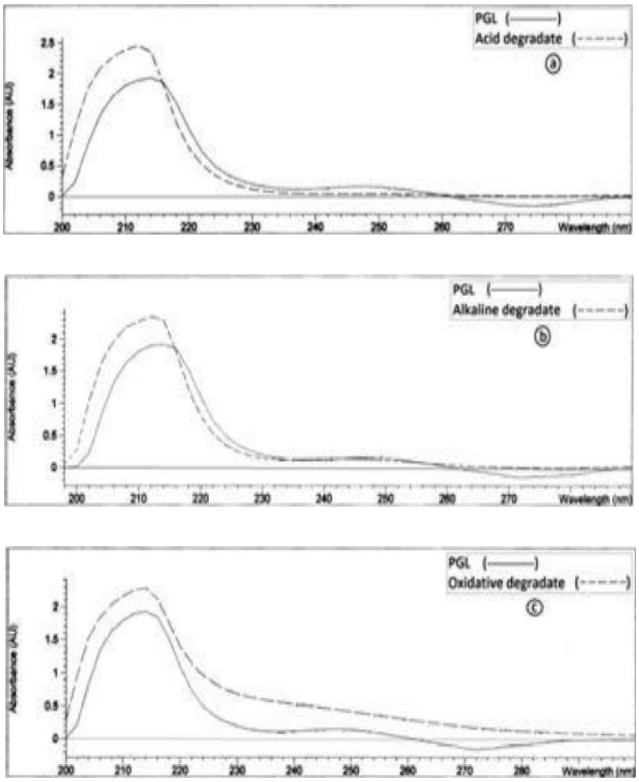

Figure 23 : ΔA spectra of PGL and its acid (a), alkaline (b) and oxidative (c) degradates

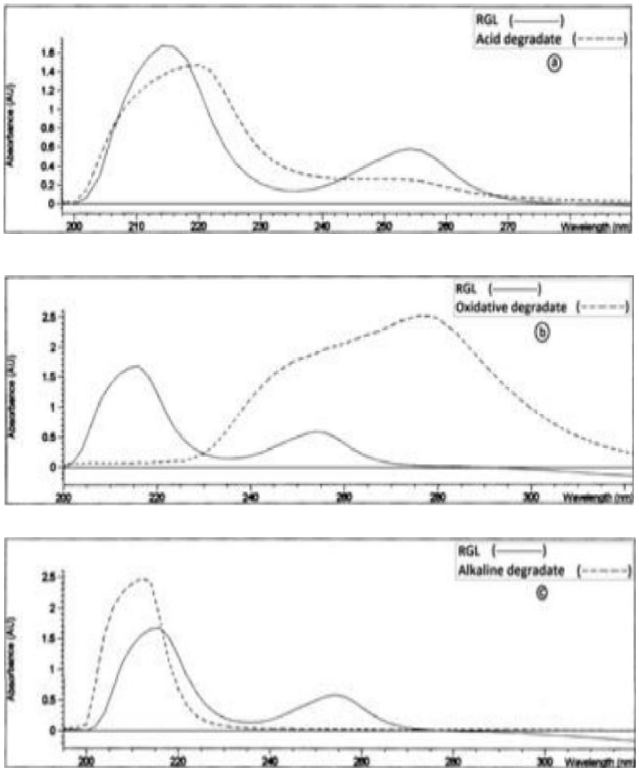

Figure 24 : ΔA spectra of RGL and its acid (a), oxidative (b) and alkaline (c) degradates

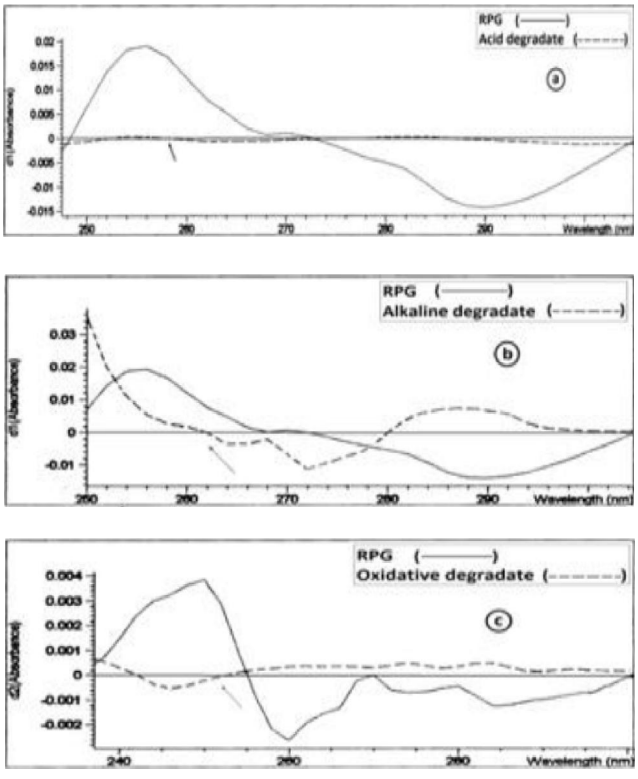

Figure 25 : First (DD^1) and second (DD^2) derivative of ΔA spectra of RPG and its [acid (a)and alkaline (b)] and oxidative(c) degradates

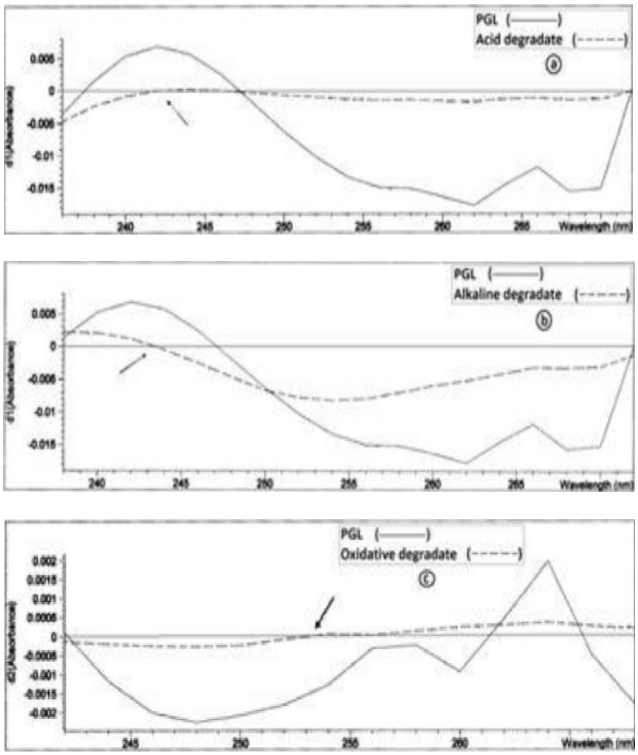

Figure 26 : First (DD[1]) and second (DD[2]) derivative of ΔA spectra of PGL and its [acid (a) and alkaline (b)] and oxidative(c) degradates

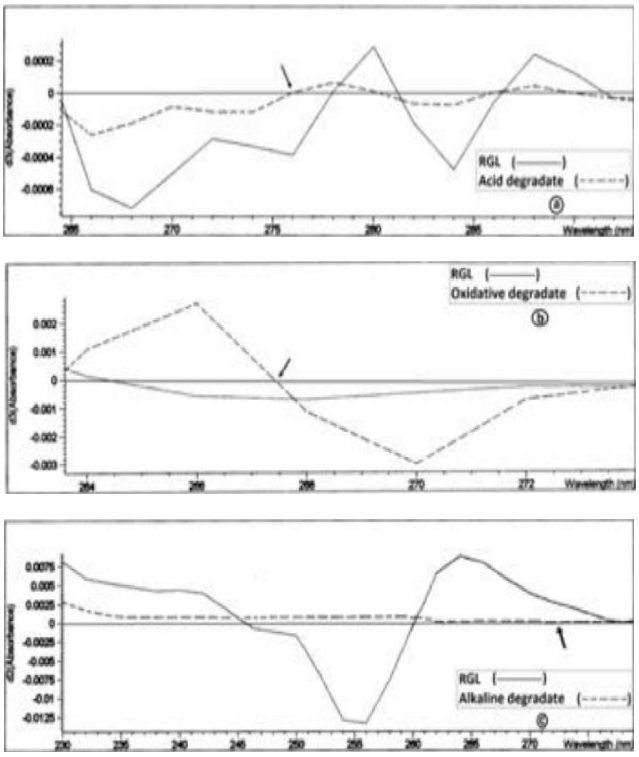

Figure 27 : Third (DD[3]) and second (DD[2]) and derivative of ΔA spectra of RGL and its [acid (a) and oxidative (b)] and alkaline (c) degradates

plete 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 polar solvent (acetonitrile), according to the following scheme:

$$\underset{\text{donor}}{\text{Drug}} + \underset{\text{acceptor}}{\text{CLA}} \longleftrightarrow \underset{(n\text{-}\pi)\text{ complex}}{\text{Drug—CLA}} \xleftrightarrow[\text{solvent}]{\text{polar}} \text{Drug}^{\cdot+} + \text{CLA}^{\cdot-}$$

This finding was anticipated by the presence of one basic electron-donating center (nitrogen atom) present in RPG and RGL structure, while PGL suffers from absence of this basic center and consequently failed to form charge transfer complex when reacted with CLA as a π-acceptor.

While, reaction-stoichiometry for ion-pair complexes was found to be a good approximation 1:1 ratio (drug/reagent) which are formed through the electrostatic attraction between positive protonated RPG^+, PGL^+ or RGL^+ and negative BPB^- as shown in figure 15. The extraction equilibrium can be represented as follows:

$$\text{Drug}_{(aq)}^{+} + \text{BPB}_{(aq)}^{-} \leftrightarrow \text{Drug}^{+}\,\text{BPB}_{(aq)}^{-} \leftrightarrow \text{Drug}^{+}\,\text{BPB}_{(org)}^{-}$$

where $Drug^+$ and BPB^- represent the protonated studied oral hypoglycemic drugs and the anion of the dye, respectively and the subscripts (aq) and (org) refer to the aqueous and organic phases, respectively.

Stability-indicating spectrophotometric methods

(1) Derivative spectrophotometry method (D^n)

The UV-spectra of the oral hypoglycemic drugs under study and their acid, alkaline and oxidative-degradates showed overlapping [Figure 16(a, b and c), 17(a, b and c) and 18(a, b and c)], which would not permit zero order determination of them in presence of their degradates. So, derivative spectrophotometric methods were adopted, where zero-crossing point for acid, alkaline and oxidative-degradates of each studied drug was indicated, respectively. The first derivative spectrophotometric method (D^1) permitted a selective determination of RPG in the presence of its acid, alkaline and oxidative-degradates at 263.79 nm, 264.33 nm and 304.84 nm, respectively as shown in figure 19 (a, b and c), and PGL in the presence of its acid and alkaline-degradates at 253.35 nm and 284.05 nm, respectively as shown in figure 20 (a and b). Also, second derivative spectrophotometric method (D^2) permitted an excellent determination of PGL in the pres-

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TABLE 1(a) : 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 ($\mu g.ml^{-1}$)	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 ($\mu g.ml^{-1}$)	4.22	5.96	0.49	0.47	0.56
LOQ ($\mu g.ml^{-1}$)	12.80	18.06	1.50	1.42	1.76
Precision					
Intra day Mean (%)	99.46	99.52	99.87	98.82	100.85
Intra day RSD (%)	0.396	1.558	0.881	1.565	0.662
Inter day Mean (%)	99.45	99.62	99.82	98.84	100.70
Inter day RSD (%)	0.431	1.520	1.124	1.591	0.549
Ruggedness [RSD (%)]	0.755	0.716	0.710	0.607	1.340
Robustness [RSD (%)]	0.539	0.581	0.760	0.725	1.493

TABLE 1(b) : Validation parameters for derivative spectrophotometric method [D^n]

Validation parameter	RPG with its degradates			PGL with its degradates			RGL with its degradates		
	Acid	Alkaline	Oxidative	Acid	Alkaline	Oxidative	Acid	Alkaline	Oxidative
	D^1at 263.79 nm	D^1at 264.33 nm	D^1at 304.84 nm	D^1at 253.35 nm	D^1at 284.05 nm	D^2at 276.31nm	D^2at 307.95 nm	D^2at 287.73 nm	D^2at 325.67 nm
Linearity ($\mu g.ml^{-1}$)	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 ($\mu g.ml^{-1}$)	0.90	0.88	0.78	0.83	0.53	0.95	0.55	1.02	0.98
LOQ ($\mu g.ml^{-1}$)	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
Intra day RSD (%)	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
Inter day RSD (%)	1.142	1.315	0.237	0.539	0.861	1.007	0.570	0.963	1.514
Ruggedness [RSD (%)]	0.404	0.634	0.802	0.460	0.770	0.845	0.663	0.412	0.429

ence of its oxidative-degradates at 276.31 nm as shown in figure 20(c), and RGL in the presence of its acid, alkaline and oxidative-degradates at 307.95 nm, 287.73 nm and 325.67 nm, respectively as shown in figure 21 (a, b and c).

(2) pH-induced difference spectrophotometric method (DD^n)

The change in the absorption spectra of the intact drugs under investigation, by using acid and alkaline media could be used as a stability-indicating study. The direct UV measurement of ΔA spectra were not suitable for assaying the studied drugs in presence of their degradates, due to severe overlapping, as shown in figure 22 (a, b and c), 23 (a, b and c) and 24 (a, b and c). Thus, first, second and third derivative of ΔA spectra were adopted, where zero-crossing point for the acid, alkaline and oxidative-degradates of each studied drug were indicated, respectively. First derivative [DD^1] of ΔA spectra was computed for determination of RPG and PGL in presence of their acid and alkaline-degradates at '258.04 nm and 261.82 nm' and '242.81 nm and 243.41 nm', respectively as shown in figure 25 (a and b) and figure 26 (a and b), while second derivative of ΔA

TABLE 1(c) : Validation parameters for pH-induced difference spectrophotometric [DD^n] method

Validation parameters		RPG with its degradates			PGL with its degradates			RGL with its degradates		
		Acid	Alkaline	Oxidative	Acid	Alkaline	Oxidative	Acid	Alkaline	Oxidative
		DD^{1}at 258.04nm	DD^{1}at 261.82nm	DD^{2}at 252.80nm	DD^{1}at 242.81nm	DD^{1}at 243.41nm	DD^{2}at 253.12nm	DD^{3}at 275.90nm	DD^{2}at 272.00nm	DD^{3}at 267.40nm
Linearity (μgml^{-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 (μgml^{-1})		0.98	1.08	1.02	1.09	0.96	0.73	0.92	1.04	1.04
LOQ (μgml^{-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
	RSD (%)	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
	RSD (%)	0.841	0.723	1.636	0.163	0.849	0.364	0.880	0.281	0.950
Ruggedness [RSD (%)]		0.410	0.489	0.355	0.479	0.630	0.338	0.477	0.590	0.710
Robustness [RSD (%)]		0.435	0.316	0.250	0.457	0.642	0.355	0.413	0.434	0.556

TABLE 2(a) : Derivative spectrophotometric [D^n] method

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 304.84nm	D^{1}at 253.35nm	D^{1}at 284.05nm	D^{2}at 276.31nm	D^{2}at 307.95nm	D^{2}at 287.73nm	D^{2}at 325.67nm
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
RSD (%)		0.423	0.543	0.890	0.163	0.272	0.456	0.473	0.726	0.522

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

TABLE 2(b) : pH-induced difference spectrophotometric [DD^n] method

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 258.04nm	DD^{1}at 261.82nm	DD^{2}at 252.80nm	DD^{1}at 242.81nm	DD^{1}at 243.41nm	DD^{2}at 253.12nm	DD^{3}at 275.90nm	DD^{2}at 272.00nm	DD^{3}at 267.40nm
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
RSD (%)		0.730	0.384	1.027	0.162	0.119	0.365	0.871	0.598	0.499

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

spectra [DD^2] was computed for determination of last mentioned two drugs in presence of their oxidative-degradates at '252.80 nm and 253.12 nm', respectively as shown in figure 25 (c) and 26 (c). RGL can be also determined in presence of its 'acid and oxidative-degradates' and its alkaline-degradates at '275.90 nm

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TABLE 3 : Quantitative determination of the studied drugs in their pharmaceutical preparations by the proposed spectrophotometric methods

Pharmaceutical preparation	Claimed amount per tablet	Charge-transfer method	Ion-pair method	Derivative spectrophotometric method[a]			pH-induced difference spectrophotometric method[a]		
Diarol® tablets, B.N.: 1018	2 mg RPG	518.00nm	414.00nm	D^1at 263.79nm	D^1at 264.33nm	D^1at 304.84nm	DD^1at 258.04nm	DD^1at 261.82nm	DD^2at 252.80nm
Amount found (%)		99.75	99.03	99.30	99. 80	100.71	99.45	100.21	99.73
RSD (%)		0.666	0.958	0.712	1.294	1.143	0.541	0.746	0.994
Actozone® tablets, B.N.: 3543	45 mg PGL	---	416.00nm	D^1at 253.35nm	D^1at 284.05nm	D^2at 276.31nm	DD^1at 242.81nm	DD^1at 243.41nm	DD^2at 253.12nm
Amount found (%)			98.41	100.33	99.74	99.20	99.81	99.41	99.72
RSD (%)			0.670	0.869	1.241	0.428	1.004	0.989	0.787
Rosizone® tablets, B.N.: MT0410208	4 mg RGL	518.00nm	415.00nm	D^2at 307.95nm	D^2at 287.73nm	D^2at 325.67nm	DD^3at 275.90nm	DD^2at 272.00nm	DD^3at 267.40nm
Amount found (%)		100.22	101.29	100.86	99.85	99.43	100.98	101.39	101.54
RSD (%)		0.610	0.632	0.635	0.399	1.243	0.767	0.133	0.276

[a]for acid, alkaline and oxidative-degradates of each studied oral hypoglycemic drug, respectively

TABLE 4(a) : Charge-transfer and ion-pair spectrophotometric methods

Pharmaceutical preparation ($\mu g.ml^{-1}$)	Authentic added ($\mu g.ml^{-1}$)	Charge-transfer method		Pharmaceutical preparation ($\mu g.ml^{-1}$)	Authentic added ($\mu g.ml^{-1}$)	Ion-pair method		
		%Recovery[a] of RPG	%Recovery[a] of RGL			%Recovery[a] of RPG	%Recovery[a] of PGL	%Recovery[a] of RGL
50.00	50.00	98.38	100.93	5.00	5.00	98.25	100.30	101.47
50.00	100.00	99.03	98.95	5.00	10.00	98.01	99.41	100.93
50.00	150.00	99.33	101.21	5.00	15.00	99.85	98.21	101.13
50.00	200.00	99.77	100.09	5.00	20.00	98.81	98.66	99.98
50.00	250.00	100.74	100.02	5.00	25.00	99.61	98.44	99.37
Mean (%)		99.45	100.24	Mean (%)		98.91	99.00	100.57
RSD (%)		0.885	0.885	RSD (%)		0.810	0.852	0.837

[a]Mean of three determinations

TABLE 4(b) : Derivative spectrophotometric [D^n] method

Pharmaceutical preparation ($\mu g.ml^{-1}$)	Authentic added ($\mu g.ml^{-1}$)	% Recovery[a] of RPG			% Recovery[a] of PGL			% Recovery[a] of RGL		
		D^1at 263.79nm	D^1at 264.33nm	D^1at 304.84nm	D^1at 253.35nm	D^1at 284.05nm	D^2at 276.31nm	D^2at 307.95nm	D^2at 287.73nm	D^2at 325.67nm
7.50	7.50	98.34	98.14	98.79	101.23	99.68	99.33	100.78	100.42	100.46
7.50	15.00	99.13	98.38	99.07	101.00	99.47	98.93	101.09	100.62	99.72
7.50	22.50	99.25	98.50	99.17	101.43	99.70	99.52	101.72	101.97	100.25
7.50	30.00	99.26	99.12	99.27	101.36	99.87	99.59	101.94	100.09	99.11
7.50	37.50	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
RSD (%)		0.423	0.543	0.890	0.163	0.272	0.456	0.473	0.726	0.522

[a]Mean of three determinations

and 267.40 nm' and 272.00 nm by computing third derivative [DD^3] and second derivative [DD^2] of ΔA spectra as shown in figure 27 (a and b) and (c).

Method validation

ICH guidelines[28] for validation method were followed, where all validation parameters were shown in

TABLE 4(c) : pH-induced difference spectrophotometric [DD^n] method

Pharmaceutical preparation ($\mu g.ml^{-1}$)	Authentic added ($\mu g.ml^{-1}$)	% Recovery[a] of RPG			% Recovery[a] of PGL			% Recovery[a] of RGL		
		DD^1at 258.04nm	DD^1at 261.82nm	DD^2at 252.80nm	DD^1at 242.81nm	DD^1at 243.41nm	DD^2at 253.12nm	DD^3at 275.90nm	DD^2at 272.00nm	DD^3at 267.40nm
7.50	7.50	98.95	101.12	98.64	98.16	98.18	100.41	99.87	101.02	101.70
7.50	15.00	99.69	101.45	99.51	98.51	98.10	100.05	100.40	101.44	100.60
7.50	22.50	99.77	101.50	99.86	98.50	98.38	99.62	101.91	101.16	101.57
7.50	30.00	99.85	101.84	100.44	98.45	98.34	99.73	101.63	100.91	101.77
7.50	37.50	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
RSD (%)		0.730	0.384	1.027	0.162	0.119	0.365	0.871	0.598	0.499

[a]**Mean of three determinations**

TABLE 5 : Statistical comparison[a] between the proposed Spectrophotometric methods and the official and manufacturer methods for determination of the studied drugs

Parameters	Method of comparison	Charge-transfer method	Ion-pair method	Derivative spectrophotometric method			pH-induced difference spectrophotometric method		
	Official method[b] (RPG)	518.00 nm	414.00 nm	D^1at263.79 nm	D^1at264.33 nm	D^1at304.84 nm	DD^1at258.04 nm	DD^1at261.82 nm	DD^2at252.80 nm
Mean ± S.D.	100.50 ± 0.975	99.75 ± 0.664	99.44 ± 0.873	99.63 ± 0.669	99.52±0.710	100.19±0.665	99.58±0.692	100.24±0.923	99.73±0.527
t-test	---	1.56	1.99	1.80	2.20	0.65	1.89	0.48	1.69
F-test	---	2.16	1.25	2.12	1.89	2.15	1.98	1.12	3.43
Parameters	**Manufacturer method[c] (PGL)**		**416.00 nm**	**D^1at253.35 nm**	**D^1at284.05 nm**	**D^2at276.31 nm**	**DD^1at242.81 nm**	**DD^1at243.41 nm**	**DD^2at253.12 nm**
Mean ± S.D.	100.73 ± 0.897	---	100.11 ± 0.452	99.97 ± 0.455	99.90 ± 0.468	99.60 ± 0.803	99.92 ± 0.758	99.78 ± 0.853	100.27 ± 0.547
t-test	---		1.51	1.84	2.01	1.70	1.69	1.88	1.07
F-test	---		3.94	3.89	3.67	1.25	1.40	1.11	2.69
Parameters	**Manufacturer method[d] (RGL)**	**518.00 nm**	**415.00 nm**	**D^2at307.95 nm**	**D^2at287.73 nm**	**D^2at325.67 nm**	**DD^3at275.90 nm**	**DD^2at272.00 nm**	**DD^3at267.40 nm**
Mean ± S.D.	100.35 ± 0.958	100.22 ± 0.611	99.36 ± 0.536	99.99 ± 0.811	99.65 ± 0.939	99.51 ± 0.476	100.16 ± 0.763	99.49 ± 0.732	99.32 ± 0.831
t-test	---	0.27	2.20	0.70	1.28	1.93	0.37	1.75	1.99
F-test	---	2.46	3.19	1.39	1.04	4.05	1.58	1.71	1.33

[a]**The theoretical values of t and F at P = 0.05 are (2.23) and (5.05), respectively where n=6.** [b]**The Official Method for RPG determination; C18 column (125×4.6 mm), Methanol: Buffer"monobasic potassium phosphate solution(1 in 1000)" [adjust with phosphoric acid to a pH 2.5] (80:20) as a mobile phase; Temperature = 45°C; "UV detection at 240 nm".** [c]**The Manufacturer Method obtained from Amoun Pharmaceutical Company for PGL determination; C18 column (250×4.6 mm), Acetonitrile: 1 M Ammonium acetate : Glacial acetic acid (25:25:1) as a mobile phase; "UV detection at =269", respectively.** [d]**The Manufacturer Method obtained from Apex Pharmaceutical Company for RGL determination; C18 column (2504.6 mm), Potassium dihydrogen phosphate buffer (pH= 3.0): Acetonitrile: Methanol (65: 25: 10) as a mobile phase; "UV detection at 235 nm"**

TABLE 1 (a, b and c). In the adopted spectrophotometric methods, the limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ = kSDa/b, where k=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 spectrophotometric methods in three independent series in the same day (intra-day precision) and three consecutive days (inter-day precision) within each series every concentration was examined three times. The RSD % values of intra- and inter- day studies showed that the intermediate precision of the proposed methods were satisfactory. The ruggedness of the adopted spectrophotometric methods was assessed by applying the procedures using two different sources of solvents; methanol and acetonitrile supplied from Riedel-de Haen and Fisher; results obtained were found to be reproducible as RSD did not exceed 2 %. Robustness of the spectrophotometric

procedures was determined by evaluating the influence of small variation 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 for 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 in refrigerator and those kept on bench was studied by performing the experiments and estimate their recoveries then compared with those of freshly prepared solutions. It was found that solutions kept in refrigerator are stable up to 7 days while that kept on bench are stable for only 3 days.

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 as shown in TABLE 2 (a and b), where the studied hypoglycemic drugs could be determined in the presence of their degradates (up to 90 %).

Molar absorptivity value of charge-transfer method for (RPG and RGL) with CLA was found as 1.23×10^3 and 8.67×10^2 ($L.mol^{-1}.cm^{-1}$), respectively and that of ion-pair method for (RPG, PGL and RGL) with BPB was found as 8.86×10^3, 6.95×10^3 and 7.06×10^3 ($L.mol^{-1}.cm^{-1}$), respectively. Sandell's sensitivity[29] (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. Thus, $S = 10^{-3}/a = \mu g\ cm^{-2}$ where, a is the specific absorptivity and its value (in $ml\ g^{-1}\ cm^{-1}$) correspond to the determinant in a cuvette with an optical length of 1-cm. Also, a = (b/molecular weight of the drug under study)×1000, 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 $\mu g.cm^{-2}$ for charge-transfer method of (RPG and RGL) with CLA, respectively and 0.051 $\mu g.cm^{-2}$ for ion-pair method for all hypoglycemic drugs under study with BPB.

The accuracy of proposed methods was demonstrated by recovery experiments, using standard addition technique, where the percentage of RSDs 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 were summarized in TABLE 3 and TABLE 4(a, b and c), respectively, 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, where there is No significant differences were found as shown in TABLE 5.

DISCUSSION

The aim of this study was to develop simple, fast, validated and very economic methods for analysis of RPG, PGL and RGL in pure forms and in their pharmaceutical preparations. Two selective, simple and less time consuming visible spectrophotometric methods were described for analyzing (RPG and RGL) and (RPG, PGL and RGL) using CLA and BPB reagents, respectively. The proposed stability-indicating methods (derivative and pH-induced difference spectrophotometry) provide 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 reflect the 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 economic 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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