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Development And Validation Of Two Stability-Indicating Uv-Spectrophotometric Methods For The Determination Of Repaglinide In Bulk And Dosage Forms

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Abstract: Two stability indicating UV-spectrophotometric methods were developed and validated for the determination of repaglinide with its degradation products. Assay of repaglinide in 0.1M NaOH and 0.1M HCl were achieved at 216nm and 243nm respectively. The correlation was in the range of 1.0 – 25 µg mL^{-1} (r = 0.9999) for 0.1M NaOH and 2.0 – 40 µg mL^{-1} (r = 0.9999) for 0.1M HCl with molar absorptivity values of 2.02 × 10^4 L mol^{-1} cm^{-1} and Sandell sensitivity of 0.0224 µg cm^{-2} for 0.1M NaOHand 1.17 × 10^4 L mol^{-1} cm^{-1} and Sandell sensitivity of 0.0386 µg cm^{-2} for 0.1M HCl. The detection limit and quantification limit are calculated to be (0.13, 0.39 µg mL^{-1}) and (0.18 and 0.54 µg mL^{-1}) for 0.1M NaOH and 0.1M HCl respectively. The method was validated for accuracy, precision, robustness, selectivity and applied for marketed formulations with less than 2% relative error and standard deviation.
Keywords: Repaglinide, determination, forced degradation, UV spectrophotometry, formulations.

INTRODUCTION AND EXPERIMENTAL

Stability testing and stress testing (forced degradationstudies) are critical components of drug development strategy[1].Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used[2,3].The studies help us to understand the mechanism of a drug'sdecomposition, which further helps in obtaining informationon physical and chemical factors that result in instability[4].These factors are then controlled to stabilize the drugor drug formulation, resulting in increased shelf-life orimproved efficacy. According to International Conference on Harmonization (ICH) guideline Q1A (R2), the stability testing of drug substances should be carried out under different stress conditions such as hydrolysis, oxidation, photolysis and thermal degradation to validate the stability-indicating supremacy of analytical methods used for the analysis of stability of drugs[5].The standard conditions for photo stability testing are described in ICH guideline Q1B[6]. These tests allow accurate and precise quantification of drugs and their degradation products and interaction products.

Repaglinide (RPG), chemically known as (*S*)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl] butylamino)-2-oxoethyl]benzoic acid (Fig.1) is a new carboxy methyl benzoic acid derivative. It is a novel glucose regulator for the treatment type-2 diabetes mellitus[7].RPG is unusual in that it has a rapid onset of action and a short duration of action. Repaglinide is extensively metabolised by the cytochrome P450 (CYP) system to inactive metabolites, and the main route of excretion of repaglinide and its metabolites is via bile into faeces[8].The drug is official in USP[9] which describes liquid chromatographic method for its quantification. From the literature survey, it is revealed that very a few methods have been reported for the determination of RPG in pure form and dosage form; and include high performance liquid chromatography[10,11,12], reversed phase thin layer chromatography[13], charge transfer spectroscopy[14],and differential pulse polarography[15].However, the reported methodsare not simple and require expensive equipments. Besides, not many institutions can afford to procure and maintain such instruments. The availability of a simple and inexpensive UV spectrometry with sensitivity and selectivity will be very useful for the determination of RPG in pharmaceutical dosage forms.

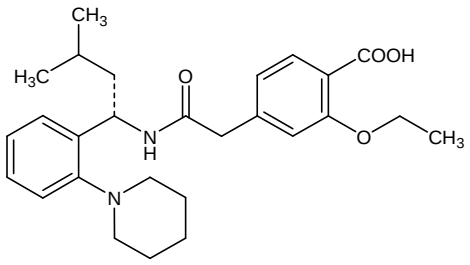


Figure 1. Chemical structure of RPG

Four UV spectrophotometric methods have also been reported in the literature to determine RPG in different solvent media. Firsttwo methods[16] involve the measurement of absorbance of ethanolic solution of repaglinide and metformin drug mixture in direct measurement and Q-absorbance ratio. Both methodsobey Beer's law in the range 4 – 24 µg mL^{-1}RPG. Third method[17] is a comparative study of first-derivative spectrophotometry and HPLC which have a linear range of 1 – 35 µg mL^{-1}. Rajput et al [18] has determined RPG by measuring the absorbance at 281.2 nm in methanol with a linear range of 1-200µg mL^{-1}.

So far, to our knowledge, no stability indicating assay has been reported for the determination of RPG in the presence of its degradants using the ICH approach of stress testing. The proposed UV methods are very less expensive and more accurate compared with the reported methods.The focus of the study was to develop a simple, rapid, accurate and precise stability indicating UV-spectrophotometric method for the determination of RPG in tablet dosage form.

Apparatus

Shimadzu Pharmaspec 1700 UV/Visible spectrophotometer was used for absorbance measurements.

Materials

All chemicals used were of analytical reagent grade. Doubly-distilled water was used to prepare solutions wherever required. Hydrogen peroxide (H_2O_2), hydrochloric acid and sodium hydroxide were purchased from Merck (Mumbai, India). Repaglinide pure compound (99.5%) was kindly supplied by Torrent Pharmaceuticals Ltd, Ahmedabad, India, as a gift. Eurepa-1 and Eurepa-2 tablets were purchased from local commercial sources.

Reagents

Sodium hydroxide (5M) solution was prepared by dissolving required amount of the pellets in water. This solution was diluted to 0.1 M and standardized and used as solvent to dissolve drug. Hydrochloric acid (5M) was prepared by appropriate dilution of concentrated acid (Sp. gr. 1.18)with water. A 5% solution of H_2O_2 was prepared by diluting required volume of the commercially available 30% reagent with water.

Standard drug solution

Standard stock solutionsof 200 µg mL^{-1}RPGwere prepared by dissolving 20 mg of pure RPG in 0.1M NaOH and 0.1M HClseparately and diluted to 100 mL with the respective solvent in calibrated flasks. The solutions were further diluted to obtain 40 and 20 µg mL^{-1}RPG and used for assay.

General procedures

Preparation of calibration curve

Into a series of 10 mL calibration flasks, aliquots of standard drug solution equivalent to 1.0 – 25 µg $mL^{-1}$and 2.0 – 40 µg mL^{-1}RPG were accurately transferred and the volume were made up to the mark with 0.1 M NaOHand 0.1M HCl, respectively. The absorbance of each solution was then measured at 216 nm and 243nm against0.1 M NaOHand 0.1M HCl respectively.

Calibration curves were prepared by plotting the absorbance *versus* concentration of drug. The concentration of the unknown was read from the

respective calibration curve or computed from the regression equation derived using the Beer's law data.

Assay procedure for marketed tablets

Fifty Eurepa 2 tablets containing RPG (2 mg/tablet) and Hundred Eurepa 1 tablets containing RPG (1mg/ml) were weighed and pulverized. The amount of tablet powder containing 20 mg RPG was transferred into a 100 mL volumetric flasks. The content was shaken well separately with about 50 mL of 0.1 M NaOH or 0.1M HCl for 20 min and the extract was diluted to the mark with the same solvent. It was filtered using Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and a subsequent portion was diluted to get a working concentration of 20 µg mL^{-1} and 40 µg mL^{-1} respectively and subjected to analysis following the general procedure described earlier.

Forced degradation studies

Two 200 µg mL^{-1}RPG stock standard solutions were prepared in 0.1 M NaOH and 0.1M HCl separately. Five milliliters each of this solution were accurately transferred to separate 50 mL volumetric flasks. Five milliliters each of water, 5 M HCl, 5 M NaOH or 5% H_2O_2 were added to the flasks separately and the flasks were heated for 2 h on a water bath maintained at 80 °C. Then the solutions were cooled and neutralized by adding base or acid, the volume in each flask was brought to the mark with the respective diluents, and absorbance measured at 216 nm and 243 nm respectively. Solid state thermal degradation was carried out by exposing pure drug to dry heat at 105° C for 4 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2million lux hours in a photostability chamber. The sample after exposure to heat and light was used to prepare 20 µg $mL^{-1}$and 40 µg mL^{-1}solutions in 0.1 M NaOH and 0.1M HCl respectively, and the absorbanceswere measured.

Method validation

Linearity, limits of detection and quantification

Linearity was established by analyzing standard solutions containing 1.0 – 25.0 µg mL^{-1}RPG for method A and 2-40 µg mL^{-1} RPG for method B. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated from the calibration curve as k SD/b where k = 3.3 for LOD and 10 for LOQ, SD is the standard deviation of the blank absorbance values and b is the slope of the calibration curve.

Intra-day and inter-day accuracy and precision

The intra-day and inter-day precisions of the proposed methods were determined by measuring the absorbance seven times on the same day and on five different days using three different concentration of RPG (10, 15 and 20 µg mL^{-1}in 0.1M NaOH and 20, 30 and 40 µg mL^{-1} in 0.1M HCl). From the absorbance values obtained, concentration was calculated, and the results were expressed as percentage relative standard deviation (RSD). The accuracy was evaluated as percentage relative error (%RE) between the found and taken concentrations.

Robustness and ruggedness

Robustness of the method was determined by deliberately varying the wavelength of detection at three levels (216±1 nm for 0.1M NaOH and 243±1 for 0.1M HCl).Triplicate analysis was carried out for three different concentrations of RPG(10, 15 and 20 µg mL^{-1}in0.1M NaOH and 20, 30 and 40 µg mL^{-1}in 0.1M HCl)and the %RSD values were evaluated for each concentration. For robustness, a triplicate analysis was performed by four different analysts, using four different cuvettes for the same concentrations of RPG. The concentration of RPG found in each case was calculated. The inter-mediate precision expressed as RSD was evaluated.

RESULTS AND DISCUSSION

Spectral characteristics

The RPG solutions in 0.1M NaOH and 0.1M HCl prepared separatelywere scanned in the wavelength range from 200 to 400 nm. Maximum absorbance of RPG was obtained at 216nm for 0.1M NaOH and 243nm for 0.1M HCl, and at this wavelength both the solvents havesignificant absorbance(fig 2a, 2b).Therefore, further investigation for the analysis of RPG was carried out at 216 and 243 nmanalytical wavelength (λ_{max}) against 0.1M NaOH and 0.1M HClrespectively as blank.

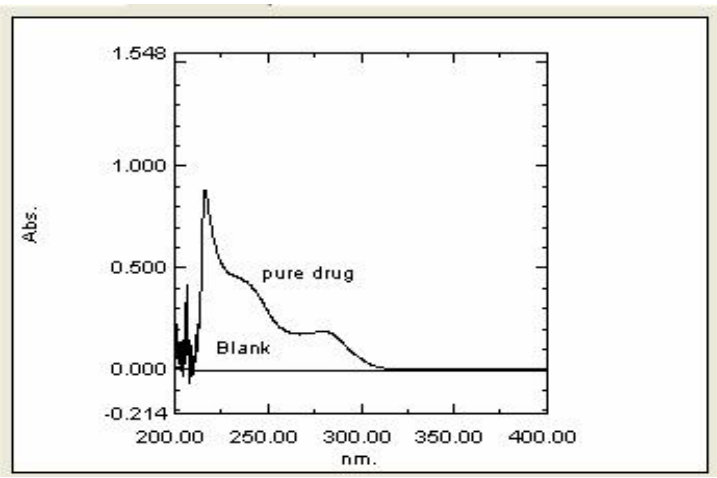


Figure 2a.UV absorption overlay spectra ofRPG in 0.1 MNaOH and B. 0.1 M NaOH as blank.

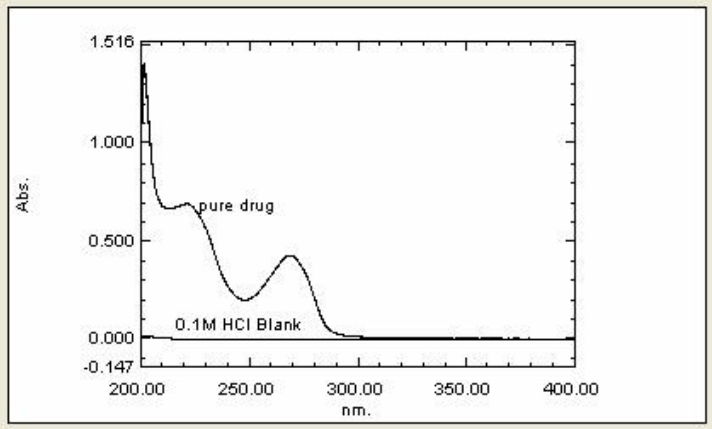
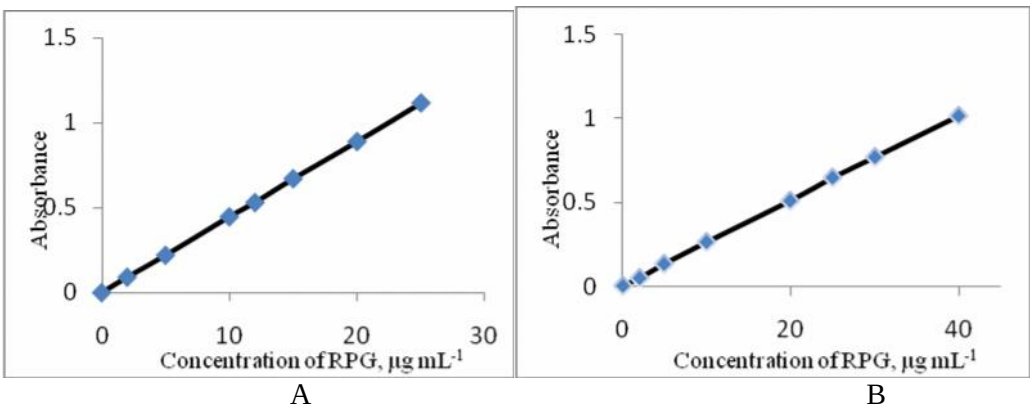


Figure 2b.UV absorption overlay spectra of: RPG in 0.1 MHCl and 0.1 M HCl as blank.

Method validation

Linearity

Calibration plot was linear over 1.0 – 25.0µg $mL^{-1}$for 0.1M NaOH and 2-40 µg mL^{-1} for 0.1M HCl .The slope (b), intercept (a) and correlation coefficient (r) valuesfor both solvents were evaluated by using the method of least squares and were found to be -0.0443, 0.0012 and 0.9999, respectivelyfor method Aand 0.0254, 0.0053 and 0.9999 for method B. The uncertainties with the y-axis (S_y), intercept (S_a) and slope (S_b) were also calculated. These results are presented in Fig-3 and Table 1.



A B

Figure 3.Linearity curve of: A.RPG in 0.1 MNaOH and B. 0.1 M HCl.

Sensitivity, limits of detection and quantification

Optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values are calculated. The limits of detection (LOD) and quantification (LOQ) are also calculated and all these dataare also presented in Table 1.

Precision

The results of intra-day and inter-day analysis of the sample are given in Table 2. As evident, %RSD values of the data obtained were all below 3% .The %RSD values indicated that the proposed method is sufficiently precise.

Accuracy

As shown from the data presented in Table 2, the relative error between the taken and found concentrations of RPG is <2% indicating that the proposed method is very accurate for the assay of RPG.

Robustness and ruggedness

The assay was repeated at different wavelengths and the results as presented in the table 3, clearly indicate that the results were unaffected by the change in wavelength of detection. The insignificant differences in absorbance values demonstrated the good robust nature of the proposed method. Intermediate precision values (%RSD) were in the range 0.13–2.86% indicating acceptable ruggedness. These results are presented in Table 3.

Application to tablet analysis

Commercial RPG tablets were analyzed using the developed methods and also by a reference published method[19].The method describes a HPLC determination of RPG ammonium phosphate buffer and methanol with pH 4 mobile phase.The results obtained were compared statistically by the Student's t-test and the variance-ratio F-test[20]. The calculated t- and F- values did not exceed the tabulated values of 2.77 (t) and 6.39 (F) at the 95 % confidence level and for four degrees of freedom (Table 4), indicating closesimilarity between the proposed methods and the reference method with respect to accuracy and precision.

Recovery study

To further ascertain the accuracy and reliability of the proposed method, recovery experiments were performed *via* standard-addition procedure. Pre-analyzed tablet powder was spiked with pure RPG at three different levels and the total was found by the proposed method. Each determination was repeated three times. The percent recovery of pure RPG added was within the permissible limits indicating the absence of inactive ingredients in the assay. These results are as illustrated in Table 5.

Table 1.Sensitivity and regression parameters

Parameter	Value (Method-A)	Value (Method-B)
λ_{max}, nm	216	243
Linear range, µg mL^{-1}	1.0 – 25.0	2.0 – 40.0
Molar absorptivity(ε), L $mol^{-1}cm^{-1}$	2.02×10^4	1.17×10^4
Sandell sensitivity*, µg cm^{-2}	0.0224	0.0386
Limit of detection (LOD), µg mL^{-1}	0.13	0.18
Limit of quantification (LOQ), µg mL^{-1}	0.39	0.54
Intercept (a)	0.0012	0.0053
Slope (b)	0.0443	0.0254
S_y	0.0520	0.0041
S_a	0.0228	0.0027
S_b	2.5×10^{-4}	1.2×10^{-4}
Regression coefficient (r)	0.9999	0.9999

*Limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **Y=a+bX, Where Y is the absorbance, X is concentration in µg/ml, a is intercept and b is slope

Table 2.Evaluation of intra-day and inter-day precision and accuracy

		Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
	RPG taken, µg mL^{-1}	RPG found, µg mL^{-1}	%RE	%RSD	RPG found, µg mL^{-1}	%RE	%RSD
Method-A	10.0	10.02	0.19	0.19	9.98	0.11	0.15
	15.0	15.06	0.42	0.13	15.05	0.39	0.13
	20.0	20.04	0.22	0.44	19.97	0.13	0.09
Method-B	20.0	19.78	1.12	0.18	19.98	1.00	2.48
	25.0	24.99	0.01	0.23	24.99	0.83	1.76
	30.0	29.96	0.14	0.05	29.96	0.75	1.58

%RE.Percent relative error, %RSD.relative standard deviation

Table 3.Results of robustness and ruggedness expressed as intermediate precision (% RSD)

	RPG taken, µg mL^{-1}	Robustness(%RSD), Wavelength, nm			Ruggedness	
		215	216	217	Inter-analysts (%RSD),(n=4)	Inter-cuvettes (%RSD),(n=4)
Method-A	10	1.13	1.11	0.14	0.64	1.04
	15	0.22	0.23	2.02	0.52	2.86
	20	0.18	0.21	1.11	0.76	2.24
		Robustness (%RSD), Wavelength, nm				
		242	243	244		
Method-B	20	1.09	2.12	0.56	0.24	2.44
	25	0.34	0.24	1.88	0.13	2.45
	30	2.33	2.30	0.90	0.91	2.26

Table 4.Results of analysis of Eurepa-2 and Eurepa-1 tablets by the proposed method and statistical comparison of the results with the reference method

Tablet brand name	Nominal amount (mg/tablet)	Found* (Percent of label claim ± SD)		
		Reference method	Method A	Methood B
Eurepa-1	1	103.6±0.52	102.8±0.96 t = 1.71 F = 3.41	103.9±0.99 t = 0.63 F = 3.62
Eurepa-2	2	98.54±0.68	99.9±1.2 t = 2.29 F = 3.11	100.1±1.35 t = 2.43 F = 3.94

*Mean value of 5 determinations.

Tabulated t-value at the 95 % confidence level and for four degrees of freedom is 2.77.Tabulated F-value at the 95 % confidence level and for four degrees of freedom is 6.39.

Table 5.Results of recovery experiment *via* standard-addition method

	Tablet	RPG in tablet, µg mL^{-1}	Pure RPG added, µg mL^{-1}	Total found, µg mL^{-1}	Pure RPG recovered (Percent±SD*)
Method-A	Eurepa-1	9.92	5.00	15.01	100.6±1.65
		9.92	10.00	20.51	103.0±0.35
		9.92	15.00	25.20	101.1±1.46
	Eurepa-2	9.88	5.00	14.92	100.3±1.62
		9.88	10.00	20.26	101.9±0.85
		9.88	15.00	25.33	101.8±1.06
Method-B	Eurepa-1	9.92	5.00	15.12	101.3±1.77
		9.92	10.00	20.14	101.1±1.45
		9.92	15.00	25.34	101.7±1.23
	Eurepa-2	9.88	5.00	15.34	103.1±1.11
		9.88	10.00	20.35	102.4±0.90
		9.88	15.00	25.44	102.3±1.76

*Mean value of three determinations.

Table 6. Results of forced degradation studies

Parameters studied	Method-A			Method-B		
	RPG taken, µg mL^{-1}	RPG found*, µg mL^{-1}	%Recovery of RPG ±SD	RPG taken, µg mL^{-1}	RPG found*, µg mL^{-1}	%Recovery of RPG ±SD
Acid hydrolysis	20.00	28.76	143.80±0.66	20.00	20.31	101.55±0.26
Alkaline hydrolysis	20.00	20.06	100.30±0.82	20.00	15.06	75.30±0.83
Neutral hydrolysis	20.00	20.16	100.80±0.76	20.00	20.85	104.25±0.47
Oxidative degration	20.00	0.98	9.51±0.54	20.00	10.98	54.90±0.65
Thermal degradation	20.00	20.16	100.80±0.68	20.00	20.63	103.1±0.78
Photo degradation	20.00	20.65	103.25±0.51	20.00	20.16	100.80±0.53

*Mean value of 3 determinations

Stability indicating property

The stability indicating property of the drug was studied by a forced degradation study. The RPG was subjected to acid, neutral, base and hydrogen peroxide induced degradation in solution state, and photo and thermal degradation in solid state. The study was performed by measuring the absorbance of RPG solution only after subjecting to forced degradation. From the response, the percentage recovery of RPG was calculated in each case and is presented in Table 6. The results revealed that, there was almost no change in the absorbance of RPG solution resulting from base, neutral,thermal andphoto degradationfor RPG in 0.1M NaOH. It degraded significantly when cooked with acid and H_2O_2. Similarly, RPG doesn't show any degradation with acid, neutral, thermal and photo degradation in 0.1M HCl solution and showed significant degradation with basic and H_2O_2mediums.The absorption spectra was recorded for this degraded RPG and there was almost completely diminished signal was observed. This confirms that RPG is susceptible to oxidative degradation.

CONCLUSIONS

Two simple, sensitive and stability indicating UV-spectrophotometric methods have been developed and validated. Stress studies showed that the drug in 0.1M NaOHundergoes extensive degradation in oxidative and acidic conditions and stable underneutral, photolytic, and thermal conditions.Simultaneously the drug is stable with neutral, thermal and photolitic0.1M HCl and degrades significantly in alkaline and oxidative conditions.These are the first ever reported stability indicating UV method for the quantification of repaglinide. The proposed method is highly sensitive and rapid, and requires no organic solvents or any additional reagents. Furtherthe method is free from any tedious procedural or extraction steps. The instrument employedis very easy to handle and no expertise personnel is required. The proposed method was successfully applied to the commercial tablets containing RPG as active ingredient. This study is a typical example of development of a stability indicating assay, established following the recommendations of ICH guidelines. The method can be used to determine the purity of the drug available from various sources and in stability studies.

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