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RESEARCH ARTICLE

Spectrophotometric Method and its Validation for Repaglinide in its Bulk and Formulation Dissolution Samples Including Stress Studies

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ABSTRACT

Repaglinide has been estimated by UV-Visible spectrophotometer in bulk and pharmaceutical dosage form using an easy, affordable, precise, accurate, and quick method. Studies on forced degradation and dissolution have also been carried out. The diluent used for estimation of repaglinide was Acetonitrile (ACN): Water (70:30). The lambda max was found at 244 nm with ACN: Water (70:30) as diluent. Linearity was obtained in the range of 2–70 µg/ml. The proposed method's recovery rate was found to be 98.5%. The values of %RSD studies were found to be <2%. 0.1109 µg/ml and 0.336 µg/ml were LOD and LOQ for the developed method, respectively. All the degradation parameters indicate that the drug degradation was within the limits. Dissolution of the drug was done according to IP and it was also found to be linear. All the studies were found to be within the limits. Using a UV-Visible Spectrophotometer, the proposed method can be successfully used to measure repaglinide in tablet dosage forms and bulk forms and also for knowing the quality control of repaglinide.

Keywords: Bulk, Dissolution, Forced degradation, Pharmaceutical dosage form, Repaglinide, UV-visible spectroscopy, Validation

INTRODUCTION

Repaglinide tablet is used to treat patients with high blood glucose levels. Repaglinide is used to treat persons with diabetes as well as those who have non-insulin-dependent diabetic mellitus.[1] Repaglinide is a member of the meglitinide class of insulin secretagogues, which has a short half-life. Attaching to pancreatic cells, it promotes the release of insulin. Postprandial glucose levels are reduced.[2-4] Repaglinide drug should only be taken with a meal, if any meal has been skipped, then the drug should also be skipped. Without a meal, the repaglinide tablet should not be taken. Weight gain caused by meglitinides is also less when compared to sulfonylurea and insulin. About 1 month of therapy is needed earlier than a decrease in fasting blood glucose that is visible.[1,5] Since its action depends on the presence of glucose, the risk of hypoglycemia is less when compared to sulfonylurea and insulin. Meglitinides have more advantages when compared to other drugs. Compare to metformin, sulfonylureas, and thiazolidinediones, meglitinides have been proven to be more effective in decreasing post-prandial glucose levels. From orally administered repaglinide, 8% is eliminated in urine and 90% is excreted through feces. Figure 1 shows the structure of repaglinide. Diabetic cases are increasing day by day in all age groups so that's why repaglinide has been selected for improving its linearity and lowering the LOD, and LOQ levels to improve the sensitivity of the method by UV-Visible spectrophotometer.[6-9]

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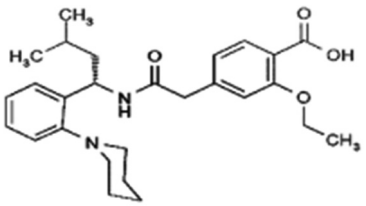

Figure 1: Structure of repaglinide

Molecular formula: $C_{27}H_{36}N_2O_4$
Molecular weight: 452.5857g/mol

MATERIALS AND METHODS

Apparatus and instrument

Double-beam UV-Visible spectrophotometer "Elico Sl 210," digital analytical balance, UV chamber, and ultrasonic water bath were used. Pipettes, beakers, measuring cylinders, and volumetric flasks were used.

Chemicals and reagents

The pharma company offered metformin and repaglinide drug samples as gift samples. The medication EUROPA (Repaglinide) was bought from a neighboring pharmacy. Analytical-grade materials were utilized throughout the experiment.

METHOD DEVELOPMENT

Solvent selection

Acetonitrile (ACN), methanol, ethanol, and ACN: Water (70:30) were selected as the solvent after considering the solubility and stability factor of repaglinide.

Standard solution preparation

Standard solution of repaglinide was prepared by taking 10 mg in 10 ml volumetric flask make up to 10 ml with diluents such as ACN, methanol, ethanol, and ACN: Water (70:30) to get 1000 µg/ml make up to the mark with suitable diluents. Pipette out 1 ml from 1000 µg/ml and take in 10 ml Volumetric flask, make up to mark with diluent to get 100 µg/ml concentration (Working Standard). One milliliter was taken from a working standard solution in a 10 ml volumetric flask make up to mark with diluent to get 10 µg/ml.

Wavelength determination

To determine the wavelength, the standard solution containing 10 µg/ml of repaglinide was scanned between 200 and 400 nm. ACN: Water (70:30) was selected as solvent as it gave a good peak with λ_{max} at 244 nm. Figure 2 shows the λ_{max} of repaglinide.

Calibration curve preparation

Repaglinide stock solution was diluted to achieve a concentration in the range of 70 g/ml. The calibration curve between concentration (x-axis) and absorbance was plotted after the absorbances were measured using the diluent as a blank (y-axis). The calibration curve of repaglinide is shown in Figure 3.

Assay

Twenty tablets were precisely weighed and the weight was recorded. Next, the tablets were crushed into a fine powder in a mortar, and the weight equivalent to 10 mg of the drug was taken and transferred to a 10 ml volumetric flask. The medicine should then be dissolved with small amounts of diluent before being made up to the mark, then diluted once more up to the linearity range. At 244 nm, sample solution absorbances were measured. The drug concentration was then obtained using the formula below.

$$\text{Concentration of sample} = \left(\text{Absorbance of sample} \div \text{Absorbance of Standard}\right) \times \text{Concentration of Standard}$$

Validation parameters

ICHQ2 (R1) guidelines were followed for analytical method validation. The following is the validation parameters performed for the repaglinide drug.

Linearity

Standard solution Preparation:
Pipette out 0.2, 0.4, 0.6.,8, 1.01.2, 1.4, 1.6, 1.8, and 2.0 ml from 100 ppm stock solution and transfer

to separate 10 ml volumetric flasks and make up to the mark with diluent to yield 2, 4, 6, 8, 10, and 12 ppm solution, respectively. The results of linearity are shown in Table 1.

Precision

A homogenous sample of a sufficient number of aliquots is assayed to produce statistically accurate values of SD or %RSD. The results of linearity are shown in Table 1.

%RSD = (SD of measurement/mean value of measurement) × 100

$$\sigma = \sqrt{\frac{\sum (x_i - \mu)^2}{N}}$$

Limit

%RSD should be <2%

Results of precision are shown in Table 2.

Accuracy

The accuracy was determined by spiking standard solution to sample solution at three concentrations, that is, 50, 100, and 150. %RSD was computed.

%Recovery = (Absorbance of Sample/Absorbance of Standard) × 100

Results of accuracy are shown in Table 3.

Robustness

The robustness of an analytical procedure is determined by checking the absorbance at ±nm from the fixed wavelength. The ±nm from the fixed wave length. Three aliquots of a standard solution containing 10 g/ml were made, and they were scanned at fixed wavelengths of 1 nm and 2 nm.

Limit

%RSD was found to be within the limits, that is, <2%

Results of robustness are shown in Table 4.

Ruggedness

The absorbance was examined in a variety of ways, including by several analyzers and using various instruments. In this method, absorbance of the same solution is checked by two different analysts and %RSD was calculated.

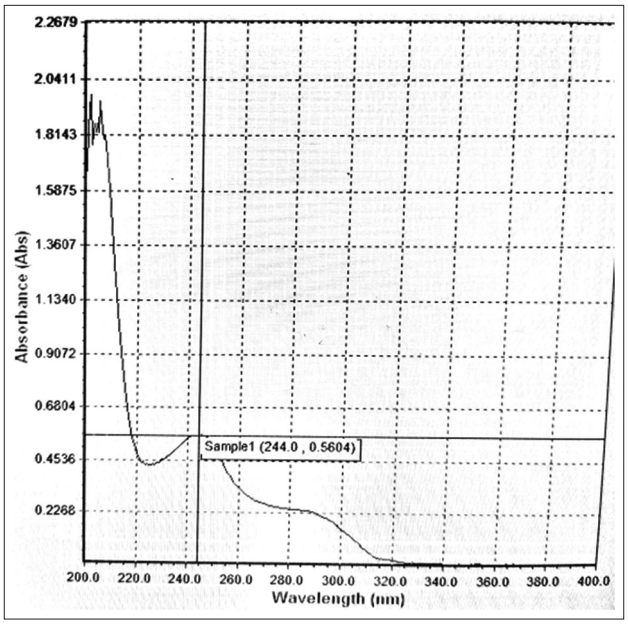

Figure 2: Repaglinide shows maximum absorbance at 244nm

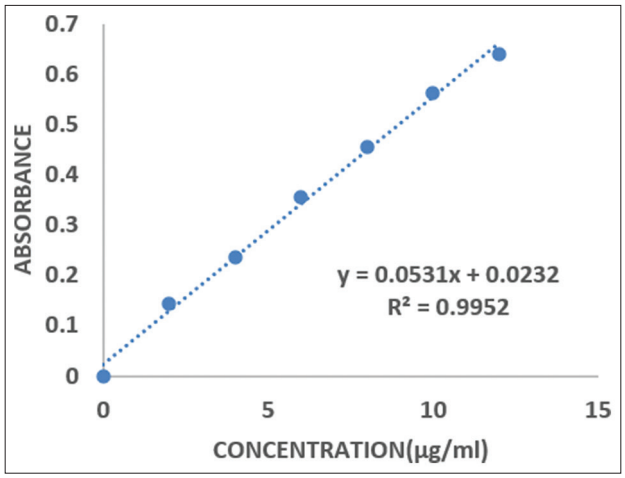

Figure 3: Calibration curve of repaglinide

Table 1: Results of linearity

Concentration (µg/ml)	Absorbance (nm)
2	0.0764
4	0.1784
6	0.2679
8	0.3576
10	0.456
12	0.5502
15	0.6395
20	0.7966
25	0.8395
30	0.7005
40	0.9502
50	1.245
60	1.895
70	2.005

Table 2: Results of Precision

Concentration(µg/ml)	Absorbance (nm)
10	0.456
10	0.4552
10	0.4562
10	0.4601
10	0.4566
10	0.4568
Average	0.456816667
Standard deviation	0.001553938
RSD%	0.340166584

Table 3: Results of accuracy

% Level	Absorbance (nm)	% Recovery	Mean % Recovery
50% (2ppm+4ppm)	0.8197	97.58%	97.71%
	0.8191	97.73%	
	0.8199	97.82%	
100% (4ppm+4ppm)	0.9832	98.64%	98.59%
	0.9821	98.53%	
	0.9830	98.62%	
150% (6ppm+6ppm)	1.1398	99.49%	99.34%
	1.1368	99.23%	
	1.1378	99.31%	

Table 4: Results of robustness

Concentration(µg/ml)	243 nm	244 nm	245 nm
10	0.3877	0.456	0.4266
10	0.3877	0.4552	0.4377
10	0.3829	0.4562	0.4367
10	0.3867	0.4601	0.43212
10	0.3866	0.4566	0.4353
10	0.3809	0.4568	0.4352
Average	0.385416667	0.456816667	0.433936667
Standard deviation	0.002588704	0.001553938	0.003705409
RSD%	0.671663765	0.340166584	0.853905476

Limit

%RSD was found to be within the limits, that is, <2%.

Results of ruggedness are shown in Table 5.

Table 5: Results of ruggedness

Concentration(µg/ml)	Analyst-1(nm)	Analyst-2(nm)
10	0.3877	0.456
10	0.3877	0.4552
10	0.3829	0.4562
10	0.3867	0.4601
10	0.3866	0.4566
10	0.3809	0.4568
Average	0.385416667	0.456816667
Standard deviation	0.002588704	0.001553938
RSD%	0.671663765	0.340166584

LOD

The lowest amount of analyte in a sample that can be identified but not necessarily quantitated under the specified experimental setting is referred to as the LOD of an analytical process.

LOD = 3.3 × SD/slope.

SD = Standard deviation

LOQ

It is the smallest amount of analyte in the sample that can be quantitatively determined under the specified experimental circumstances with acceptable precision and accuracy.

LOQ = 10× SD/slope.

SD = Standard deviation

Forced degradation studies

Acid hydrolysis

To the 1 ml of standard, stock solution (100 µg/ml) add 1ml of 1N HCL and be allowed to stand for 3 h. Then, neutralize using 1N NaOH and dilute with solvent to give a 10 µg/ml solution of standard repaglinide. This 10 µg/ml solution was scanned in UV-spectrophotometer at 244 nm.

Base hydrolysis

To the 1 ml of standard, stock solution (100 µg/ml) was taken in two different 10 ml volumetric flasks add 1ml of 1N NaOH in 1 volumetric flask and 0.1N NaOH in another volumetric flask. Both the flask were allowed to stand for 3 h, then neutralize using 1N HCl and 0.1N HCl, respectively. Dilute with solvent to give a 10 µg/ml solution of standard repaglinide. This 10 µg/ml solution was scanned in UV-spectrophotometer at 244 nm.

Peroxide hydrolysis

1.0 ml of an aliquot from standard stock solution(100 µg/ml) add 1 ml of 30% hydrogen peroxide was added, and then, the solution is allowed to stand for 6 h and the contents were diluted to get a final concentration of 10 µg/ml of

repaglinide and this solution was scanned in UV at 244 nm.

Heat degradation

15 mg sample was weighed and placed in an oven at 45–70°C from this sample prepare 1000 ug/ml of solution. Then, 1 ml of the above solution was taken and transferred in 10 ml of volumetric flask and the volume made up to the mark with diluent. This solution was scanned at 244 nm.

Bench top hydrolysis

One milliliter from stock solution was taken into a 10 ml volumetric flask and made up to the mark with diluent and this solution was scanned at an initial, 24, and 48 h at 244 nm.

The results of forced degradation studies are shown in Table 6. Figure 4 depicts the forced degradation graph.

Dissolution

Dissolution is the *in vivo* process to know the drug release. The dissolution process repaglinide tablet 2 mg (EUROPA) given in IP has been followed in this experiment. The medium prepared for dissolution should have the pH 5.0 buffer. 10.2 g of citric acid monohydrate was accurately weighed and taken in a 1000 ml volumetric flask. Then, weigh 18.2 g of dibasic sodium phosphate dihydrate then add small amounts of water to dissolve these ingredients. After all the ingredients have been thoroughly dissolved makeup to the mark with water. From this, 900 ml was taken in a dissolution basket. USP apparatus 2 is used for dissolution. The process was done at 75 rpm for 30 min. The samples were aliquoted every 5 min. The results of dissolution are shown in Table 7. The dissolution studies graph is shown in Figure 5. Table 8 shows the summary of results.

RESULTS AND DISCUSSION

Limit of Detection

LOD = 3.3 × SD/slope
=3.3 × 0.001553/0.0462
=0.1109 µg/ml

Table 6: Results of forced degradation

Degradation Type	Concentration reagent	Time	%Degradation
Acid	1N HCL, 0.1NHCL	3hrs	19.2%, 14.3%
Base	1N NaOH, O.1N NaOH	3hrs	11.4%, 10.4%
Oxidative hydrolysis	30% H_2O_2	30 mins, 1hrs, 2hrs	13.2%
Thermal hydrolysis	40,50,60°C	1hrs	4.23%
UV degradation	Exposure to Drugs in UV Light	24hrs	8.42%

Table 7: Results of dissolution

Time (mins)	Absorbance (nm)
0	0
5	0.2763
10	0.2923
15	0.3121
20	0.3454
25	0.3468
30	0.466

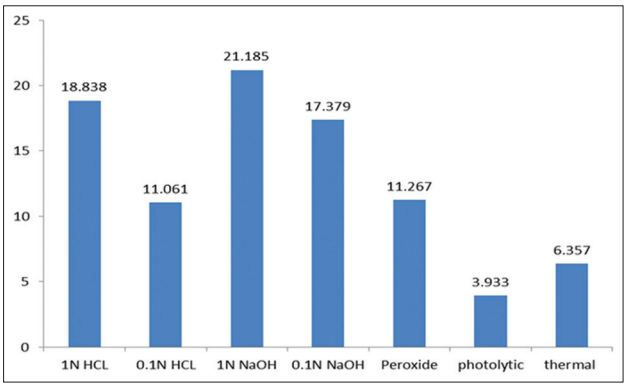

Figure 4: Forced degradation studies

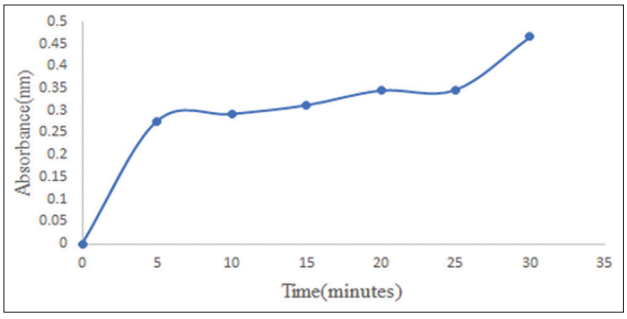

Figure 5: Dissolution studies of repaglinide tablet

Limit of Quantitation

LOQ = 10 × SD/slope
= 10 × 0.001553/0.0462
= 0.33614 µg/ml

Table 8: Summary of results

Parameters	Repaglinide
Linearity range	0-70µg/ml
Slope	0.034
Standard Deviation	0.001553
%RSD	0.3401
LOD	0.1109
LOQ	0.3361
%Assay	98.24

Assay

%Assay = (abs of sample/abs of std) × (conc of std/conc of sample) × 100

= (0.4532/0.456) × (10/10) × 100

= 98.24%

CONCLUSION

For the estimation of repaglinide in bulk as well as the tablet dosage form, a straightforward approach has been developed. A method has been developed and validated according to ICHQ2 (R1) guidelines. All the validation parameters have been performed and all the parameters were found to be within the limits. %Assay=98.24% of repaglinide is present in tablets, which was also under the limit according to Indian pharmacopeia. The above-developed method can be applied successfully for estimation of repaglinide in bulk and tablet. The dissolution process was also carried out according to IP and the drug release was found to be linear that the graph of drug release was depicted in the results section. Forced degradation results showed that the drug degraded was also within the limits. Hence, the method can also be used for quality control purposes.

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