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Research article

Development, validation and stability study of UV spectrophotometric method for determination of Repaglinide in bulk and pharmaceutical dosage forms

Mishra J.[1*], Nayak S.K[1], Sahoo S.K[2]

[1]College of pharmaceutical Sciences, Puri.
[2]Royal college of pharmaceutical Sciences & Health Sciences, Berhampur.

Abstract

A Simple, fast and reliable spectroscopic method has been developed for development, validation and stability study of Repaglinide in the pharmaceutical dosage forms. The quantitative determination of the drug was carried out by using of double beam UV-Visible spectrophotometer at wavelength 237nm. Calibration graph constructed at wavelength 237nm was linear in concentration range of 10µg/ml to 90µg/ml with correlation co-efficient 0.999. This method was validated as per ICH guidelines and can be used for determination Repaglinide in the pharmaceutical dosage forms.

Key words: Repaglinide, UV-Visible double beam spectrophotometer, Validation.

***Corresponding Author: Mishra J.,** College of pharmaceutical Sciences, Puri.

Introduction

Repaglinide chemically (S)-2-Ethoxy-4- [2-[[3-methyl-1-[2-(1 piperidinyl) phenyl] butyl] amino]-2-oxoethyl] benzoic acid is a meglitinide analogue oral hypoglycemic designed to normalize meal time glucose excursions [1]. It lowers the blood glucose level by stimulating the release of insulin from pancreas. It achieves this by closing ATP–dependant potassium channels in the membrane of the beta cells. This depolarizes the beta cell leads to opening of calcium channels & resulting calcium influx induces insulin secretion. A survey of literature reveals that good analytical methods are not available for the drugs like repaglinide [2]. Even though very few methods of estimation of above drugs i.e. meglitinide analogue are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selective and simplicity etc. [3]. The present work is to develop a simple and accurate method for the determination of repaglinide by UV spectrophotometric methods in bulk and their pharmaceutical dosage forms. [4].

Materials and methods

Instrument

1. SHIMADZU-UV-1700 PHARMASPEC UV-VIS Spectrophotometer
2. Afcoset ER 200A electronic balance.
3. SIGMA-200/A SUPER balance.

4. Ultra Sonicator (ENERTECH).
5. Borosil glass wares.

Chemicals

1. Drug Name: REPAGLINIDE
2. Solvent: Methanol.
3. Distilled water.

Scanning and determination of maximum wavelength (λ_{max})

In order to ascertain the wavelength of maximum absorbance for the drug repaglinide at different concentrations ranging from 10 µg/ml - 90 µg/ml in methanol and they were scanned within the wavelength range of 200-400nm taking methanol as blank. The calibration curve of repaglinide at different concentrations of 10µg/ml to 90µg/ml at different wavelengths is shown in Figure No. 2. The absorption curves showed characteristic absorption maxima at 237nm for repaglinide.

Preparation of stock solution

Standard stock solution of repaglinide was prepared by dissolving 10mg of drug in 10ml of methanol to get a concentration of 1mg/ml or 1000 µg/ml. From the above solutions 1ml was taken in 10ml volumetric flask and volume was made up with methanol to get a concentration of 100µg/ml. This solution was taken as stock solution.

Preparation of working standard solution and construction of standard graph

From the stock solution 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml and 9ml was pipette out to into 9 separate 10ml volumetric flask and the volume was made up with methanol to get a working standard solution of concentration ranging from 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml and 90µg/ml. These solutions were screened at wavelength 237nm and the data were given in the table no 1. Taking this data into consideration a standard graph was constructed by taking concentration in the X-axis and Absorbance in the Y-Axis as in Figure No.1.

Preparation of test solutions

For analysis of commercial formulations, 20 tablets were weighed and powdered and powder of equivalent to 10mg of repaglinide were transferred into 50ml volumetric flasks and dissolved in methanol to get 100µg/ml solutions. Then the solution was sonicated for 15 min and filtered and further dilutions were made with methanol to get the concentrations within the linearity range of respective drugs and measured the absorbance at wavelength 237 nm for solution against methanol as blank. 1ml, 1.5ml and 2ml were taken and made up to 10ml. The drug content in each tablet was estimated by using the standard graph. The results were shown in Table no.2.

Stability study

Stability testing is a routine procedure performed on drug substances and products. It is involved at various stages of product development. Testing under more gentle conditions (those recommended for long-term shelf storage), and slightly elevated temperatures, can be used to determine a product's shelf life and expiration dates [5].

In these types of studies, the product is analyzed at intervals for various parameters, which may include assay of the active ingredient, measurement of known degradation products, dissolution time, appearance, etc. Additionally, samples from production lots of approved products are retained for stability testing in case of product failure in the field. Retained samples can be tested alongside returned samples to ascertain if the problem was manufacturing or storage related [6].

Stress degradation studies

Photolytic degradation

Specific amount of bulk drug repaglinide was weighed accurately & putted into the UV chamber for three days. After three days 10mg

drug was weighed and made 1000µg/ml solution with specified solvent i.e. methanol [7]. Then an appropriate concentration was prepared & absorbance was measured in UV spectrophotometer as shown in Figure No.3.

Thermal degradation

A specific amount of Bulk drug was taken in a Petridis which was previously cleaned & dried then the Petridis along with bulk drug was put it into the oven for 48 hrs then it was taken out & weighed 10mg then prepare 1000ppm solution with Methanol[8]. After this a required concentration was made & absorbance was measured in UV spectrophotometer and shown in Figure No.4.

Acid degradation

0.1N HCl was taken in a 10 ml volumetric flask then accurately weighed 10mg bulk drug repaglinide was dissolved in it. To make soluble the repaglinid*e*, few drops of Methanol was added then volume is made by 0.1N HCl. Then the solution was refluxed for 4 hrs then from this solution 1ml was taken & the volume was made with Methanol [9]. After this absorbance is measured by scanning the prepared Solution of required concentration in U.V Spectrophotometer, and the result are shown in Figure No.5.

Alkali Degradation

Accurately weighed 10mg bulk drug was taken in 10ml volumetric flask. Then the volume was made with 0.1N NaoH. Then this solution was refluxed for 4 hrs then from it 1ml was taken out and volume was made upto the mark[10]. Then absorbance was measured by scanning the prepared solution of required concentration in a U.V spectrophotometer and result are shown in Figure No.6.

Oxidation with H_2O_2

Specific amount of bulk drug was weighed accurately, 2-3 drop of Methanol was added to make the drug soluble then the volume was made up by 3% H_2O_2 & kept in a dark place for 42 hrs, the sample was taken out & then the required concentration was prepared & then it was scanned in U.V spectrophotometer [11] and the graph was shown in Figure No.7.

Validation of Proposed Method

Linearity study

A calibration curve was constructed at optimum experimental conditions using absorbance value versus concentration in the range 10ppm to 90ppm. It has shown linear relationship with the regression equation Y=0.026x-0.001, where 'Y' is the intercept at wavelength 237nm and 'x' is the concentration of the sample in µg/ml high value of correlation coefficient (r=0.999) indicates good linearity and adherence of the method to Beer's law [12].

Table 1. Calibration Table of UV-Vis Spectrophotometric Method for Repaglinide

Conc. of drug (µg/ml)	Absorbance (n=6)	C.V
0	0	0
10	0.278	0.08
20	0.521	0.06
30	0.767	0.04
40	1.098	0.03
50	1.350	0.01
60	1.614	0.02
70	1.888	0.02
80	2.157	0.23
90	2.389	0.27

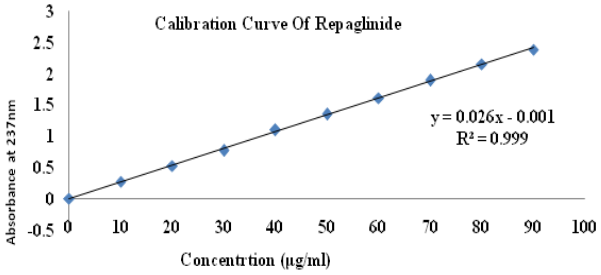

Figure 1. Calibration curve of repaglinide with solvent methanol at 237nm

Precision

Intraday and inter day precision were performed by analyzing sample solution on the same day and on the different days at specific time intervals [13]. The results are shown in the following Table No 2.

Table 2. Interday & Intraday Data of UV-Vis Spectrophotometric Method for Repaglinide

Concentration Of drug (µg/ml)	Observation concentration of drug (mcg/ml)	
	Inter day	Intra day
EUREPA 1 mg	0.463±.018	0.452±.015
REGAN 1 mg	0.466±.019	0.460 ±.016

Accuracy / Recovery Study

To determine the accuracy of the proposed method recovery studies were carried out adding different amounts (80%, 100%, and 120%) of the bulk sample of repaglinide to the previously analyzed solution of formulation of concentration 20 mg/ml. The results were shown in Table No 3. [14].

Table 3. Accuracy Data of UV-Vis Spectrophotometric Method for Repaglinide

Amount of drug added (mcg) to Solution of formulation.	Recovery form tablet formulation	
	Mean (±SD) Amount (mg) Found	Mean(±SD)% Recovery
16	0.962 ±.065	96.2±0.074
20	0.985±.056	98.5±0.086
24	0.992±.027	99.2±.024

Limit of detection and Limit of Quantification

Limit of detection (LOD) and Limit of quantification (LOQ) of repaglinid*e* was calculated by using equation given in the ICH guidelines. The result of the same is shown in the Table No 4. [15].

Table 4. LOD & LOQ of UV-Vis Spectrophotometric Method for Repaglinide

S. no	Parameters	S.D*	b**	Formula	Calculation
1	LOD	0.043021	0.026	$3.3(S.D/b)$	5.4603
2	LOQ	0.043021	0.026	$10(S.D/b)$	16.5465

S.D*=Standard Deviation, b**= slop (from calibration curve)

Results

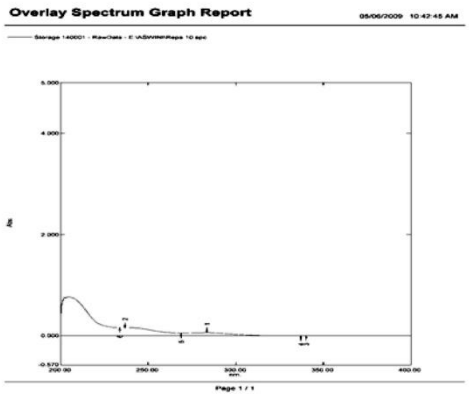

Figure 2. Scanning and determination of maximum wavelength (λ_{max})

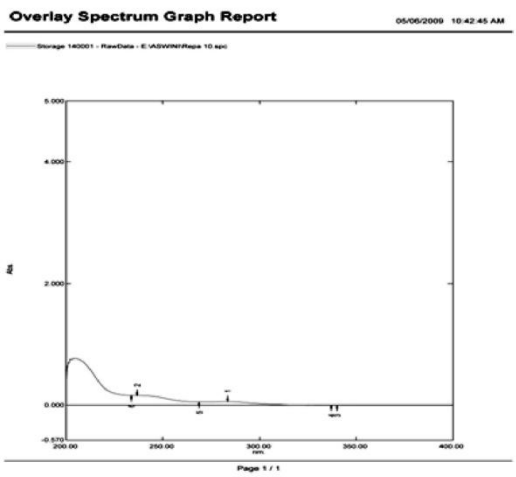

Figure 3. Model spectra for *Repaglinide* in UV chamber at wavelength 237nm

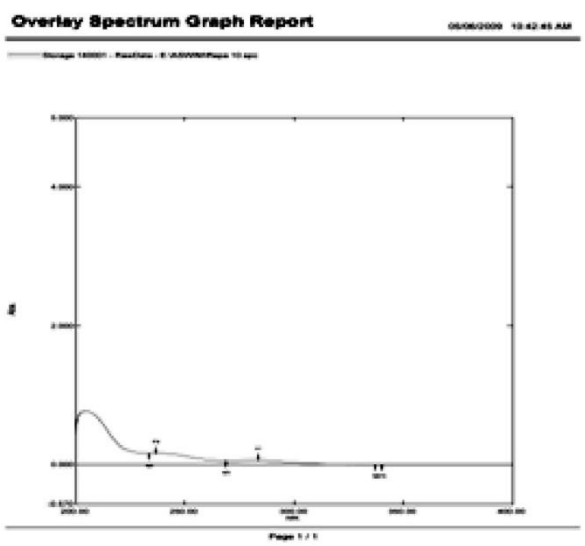

Figure 4. Model spectra for *Repaglinide* in oven chamber at wavelength 237nm

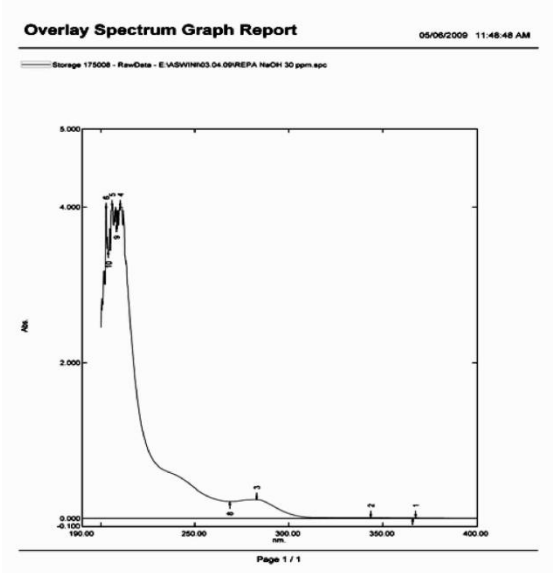

Figure 6. Model spectra for *Repaglinide* in 0.1N NaOH at wavelength 237nm

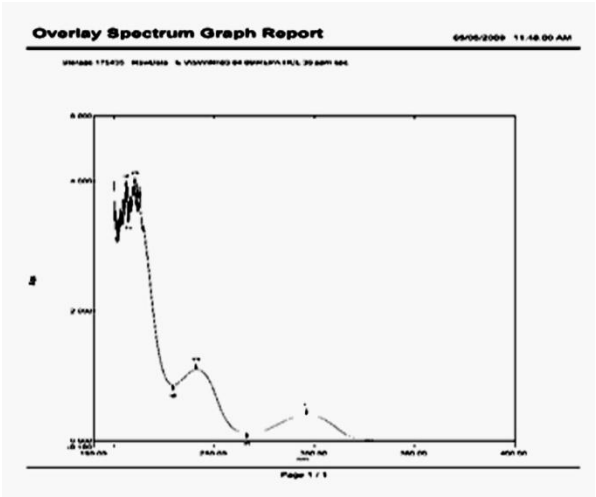

Figure 5. Model spectra for *Repaglinide* in 0.1N HCL at wavelength 237nm

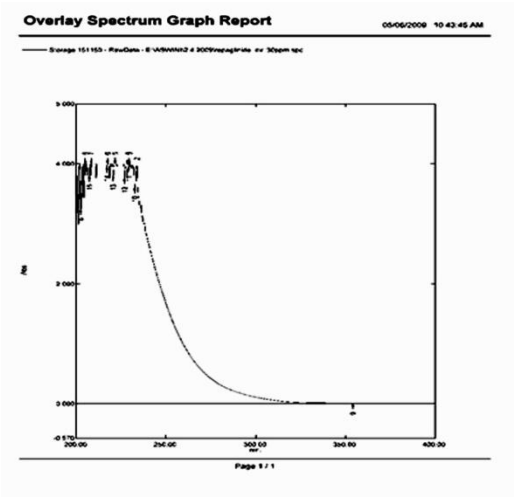

Figure 7. Model spectra for *Repaglinide* in H2O2 at wavelength 237nm

Discussion

From the optical characteristics of the proposed method it was found that *repaglinide* obeys linearity within the concentration range of 10 – 100 mcg/ml. From the results shown in tables it was found that the % R.S.D. is less than 2% which indicates that the method has good reproducibility. From the result shown in accuracy tablet was found that the percentage recovery values of pure drug from the pre analyzed solution of the formulation were in between 96 – 98% which indicates that the method is accurate and also reveals that the commonly used excipients and additives in the pharmaceuticals formulations were not interfering in the proposed method. The study conducted shows that there is degradations of the drug under the stress condition like, Photolytic degradation, thermal degradation, acid degradation, alkali degradation and oxidation with 3% $H_2 O_2$.

Table 5. Summary of Stress Degradations Results

Stress Condition	Time	% Assay of Active Substance	% assay of degraded product	MASS BALANCE
Alkali Hydrolysis (0.1N NaOH)	4 Hour	72.65	27.66	100.31
Acid Hydrolysis (0.1N HCL)	4 Hour	72.03	28	100.03
Oxidation (3% H_2O_2)	42 Hour	48.52	35	99.99
Dry heat (60^0C)	42 Hour	71.78	28.23	100.01
Photolytic degradation (237nm)	42 Hour	77.6	22.4	100

Conclusion

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulation without interference of excipients and the other additives. Hence, this method can be used for routine determination of repaglinide in bulk samples and pharmaceutical formulations. The proposed the method for stability study shows that there is appreciable degradation found in stress conditions of repaglinide.

The proposed UV-Spectrophotometric method has been evaluated over the linearity, accuracy, precision, specificity, LOD and LOQ and proved to be convenient and effective for the quality control and stability studies of repaglinide. A new simple analytical method has been developed to be applied for the evaluation of the stability of repaglinide and quantify repaglinide and its degradation products in a solid premix dosage forms.

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