International Journal of Drug Development & Research | July-September 2012 | Vol. 4 | Issue 3 | ISSN 0975-9344 | Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

Development and Validation of RP-HPLC Method for the Estimation of Repaglinide in Bulk Drug and Pharmaceutical Formulation

***Pingale P. L., Nandasana P. V.**
School of Pharmacy & Technology Management, SVKM's NMIMS, Shirpur, Dhule-425 405, Maharashtra, India

Abstract

A new Reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Repaglinide in bulk drug and pharmaceutical formulations. Optimum separation was achieved in 3 minutes using C18 column (100×4.6) mm×5µ Kromasil ODS and mobile phase was (methanol: phosphate buffer pH 3.0) ratio in gradient flow. Elution was accomplished using a flow rate of (1mL/min). Detection was carried out using a UV detector set at 242 nm. A linear relationship between mean peak area and concentration of Repaglinide was observed in the range 1-5 µg/mL. Intra-day and Inter-day precision, accuracy, robustness and system suitability of the method have been established according to the current ICH guidelines. The developed method was successfully applied to the determination of Repaglinide in pharmaceutical formulations. Accuracy, evaluated by means of the recovery study was found within the range as per the limits. No interference was observed from black as well as placebo. The proposed method was successfully employed for the determination of Repaglinide in various pharmaceutical preparations. The proposed method was found to be simple, precise, accurate, rapid and cost effective for the determination of Repaglinide in pure form and its dosage form.

*Corresponding author, Mailing address:
Pingale P. L
Mail ID: prashant.pingale@gmail.com

Key words:
Repaglinide; RP-HPLC; ICH guidelines.

How to Cite this Paper:
***Pingale P. L., Nandasana P. V.** "Development and Validation of RP-HPLC Method for the Estimation of Repaglinide in Bulk Drug and Pharmaceutical Formulation" Int. J. Drug Dev. & Res., July-September 2012, 4(3): 247-252

Article History:----------
Date of Submission: 27-06-2012
Date of Acceptance: 11-07-2012
Conflict of Interest: NIL
Source of Support: NONE

1. Introduction

Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically 2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-1-yl) phenyl]butyl]carbamoyl} methyl)benzoic acid (Fig 1). It reduces the fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood sugar by increasing the amount of

insulin released by the pancreas. Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion. Tablets containing 0.5, 1 and 2 mg of Repaglinide are available for oral administration [1,2]. It is official in USP [11] which describes liquid chromatographic method for its quantitation. A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations and biological samples, which include visible spectrophotometric [3,4,5], HPLC [6,7,8] and electrochemical methods [9]. The purpose of the present study was to develop a simple, sensitive, accurate and precise RP-HPLC method for the determination of Repaglinide in pharmaceutical formulations. The developed method has been validated by evaluation of the system suitability, linearity, precision, accuracy and robustness as per ICH guidelines [10]. The validated method was applied to the commercially available pharmaceutical formulations containing Repaglinide.

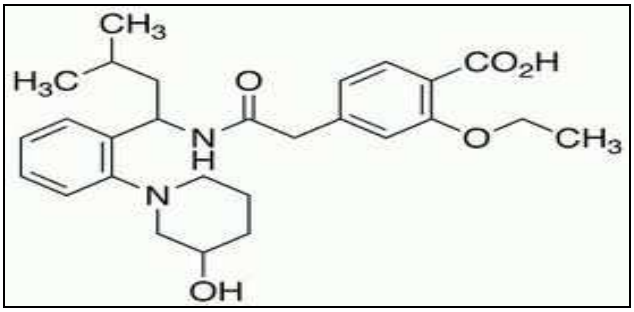

Figure 1: Structure of Repaglinide

A new method for the HPLC determination of Repaglinide is described in this paper. The method is substantially simpler, faster, cost effective and more sensitive.

2. Experimental

2.1 Apparatus

A HPLC (Perkin Elmer Binary LC Pump 200B/250) equipped with an inbuilt solvent degasser, Series 200 Pump, Series 200 UV/VIS detector and Kromasil ODS C_{18} column was used with total Chrome Navigator Software.

2.2 Reagent and Materials

HPLC grade Methanol (Sigma Aldrich), AR grade O-Phosphoric acid (Spectrochem Pvt. Ltd), GR grade Hydrochloric acid (Merck Ltd.), GR grade Sodium hydroxide (Merck Ltd.), Phosphoric acid (Merck Ltd.) and distilled water filtered through a 0.45 µm filter (millipore) were used.

2.2.1 Diluent solution

Dilution was prepared by mixing Methanol.

2.2.2 Solvent system

The solvent system employed for chromatography consisted of gradient flow of Methanol: Phosphate buffer pH 3.

2.2.3 Repaglinide standard stock solution

Pharmaceutical grade Repaglinide, certified to be 99.8% pure was procured from Torrent pharmaceuticals Ltd. A stock standard containing 1mg/mL repaglinide solution was prepared by dissolving accurately weighed 100 mg of pure drug in the 25mL of methanol and diluting to 100 mL with the diluent in a calibrated flask.

2.2.4 Repaglinide working standard solution

From the above stock solution (500µg/ml), an accurately measured 0.05, 0.1, 0.15, 0.2, and 0.25 ml was transfer into separate 50 ml volumetric flask and final volume was adjusted with methanol upto mark to prepare 1 - 5 µg/ml solutions.

2.2.5 Sample solution

Weigh and finely powder 20 tablets. Transfer exactly equivalent to 0.5 mg of Repaglinide to a 50 ml volumetric flask. Add about 60 ml of methanol and sonicate for 15 minutes and make up volume with methanol. From this solution take 1ml and dilute upto 10ml with methanol. This solution was injected for HPLC determination.

2.3 Optimization of the solvent system

Varying compositions of Methanol: Phosphate buffer (pH 3.0 adjusted with phosphoric acid) 70 : 30, 80 : 20, 85 : 15, and 90 : 10 v/v were evaluated as mobile phase in order to achieve good peak shape and short run time. Finally, Methanol: Phosphate buffer (pH 3.0, adjusted with phosphoric acid) gradient method was used.

2.4 Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reversed-phase Kromasil ODS C_{18} column (100 × 4.6 mm, 5µ) using a mobile phase consisting of Methanol: Phosphate buffer pH 3 gradient flow at a flow rate of 1 ml min^{-1}. The detector wavelength was set at 242 nm as determined by Perkin Elmer Lambda 25 UV/VIS spectrometer.

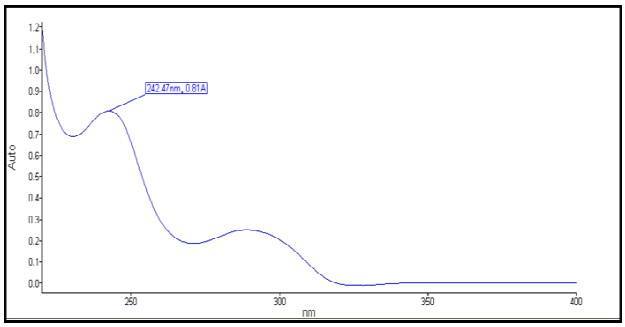

Figure 2: λ_{max} of Repaglinide by Perkin Elmer Lambda 25UV/VIS Spectrometer

2.5 Validation

2.5.1 Method Validation:

In order to determine the adequate resolution and reproducibility of the proposed method, suitability parameters including retention time, plate number and tailing factor were investigated, and were found to be 3.81 min, 4055 and 1.0, respectively, which indicate the method suitability.

2.5.2 Linearity and Range:

Working standard solutions equivalent to 1 to 5µg/mL Repaglinide were prepared by appropriate dilution of stock standard solution (1mg/mL) with the diluent solution. 20 µl aliquot of each solution was injected on to the column for five times. The peak area and retention time was recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of Repaglinide. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the mean peak area - concentration data.

2.5.3 System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. For System suitability study six samples of 3µg/ml were injected and RT, AUC, No of theoretical plate and tailing factor were calculated for System suitability test.

2.5.4 Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amount of Repaglinide was added to a pre quantified sample solution and the amount of Repaglinide was estimated by measuring the peak area ratio and by fitting these values to the straight line equation of straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Repaglinide was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve Accuracy was evaluated at three different concentrations equivalent to 80, 100 and 120% of the active ingredient by calculating the recovery of Repaglinide with (%) RSD.

2.5.5 Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. The intra-day precision of the method was determined for both peak area and retention time by repeat analysis (three identical injections) at three concentration levels. Repeatability of sample measurement was carried out in three different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate inter-day precision. The

inter-day precision was established by performing the analysis over a 3-day period on solution prepared freshly on each day.

2.5.6 Repeatability

Repeatability is the result of the method operating over short time interval (within a day) under the same conditions. The peak area of 3.0 µg/ml drug solution was analysed six times on the same day. The %RSD was calculated for the resultant peak area and retention time.

2.5.7 Robustness

For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH (3.2 and 2.8), in the flow rate (0.8 and 1.2 ml/min) and changing the wavelength (243 and 241 nm).

3. Result and Discussion

3.1 Method Development

The method utilising Methanol: Water as mobile phase yielded disturbed base line, asymmetric peak and interference was present in standard peak whereas with Methanol: Buffer pH 3.0 (80:20) Peak asymmetric tailing was found, more retention time was observed. Procedure utilising Methanol: Buffer pH 3.0 (85:15) as mobile phase also yielded tailing whereas with Methanol: Buffer pH 3.0 (gradient flow) yielded sharp peak.

Table 1: Optimized gradient flow

Time period(min)	Mobile phase ratio
0 - 0.5	85:15
0.5 - 3.5	95:05
3.5 - 5.5	90:10
5.5 - 7.5	80:20
7.5 - 10.5	85:15

During the development of the method, a number of variations were tested. The pH, buffer concentration, Methanol concentration and flow rate were chosen to give a symmetric peak with good resolution. With a mobile phase Methanol: Buffer pH 3.0 (gradient flow) well resolved symmetric peak was obtained.

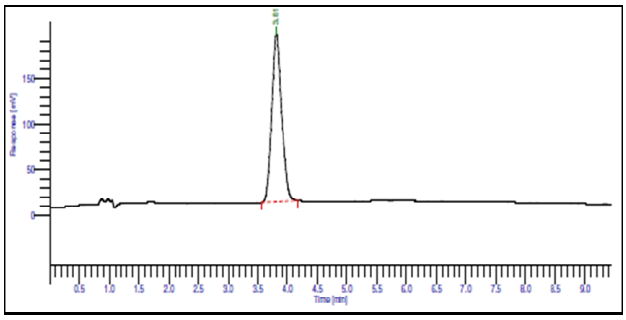

Figure 3: Chromatogram of Repaglinide

3.1.1 Linearity

Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range 1-5 µg/mL. Using the regression analysis, the linear equation, Y = -2161 + 45683 X, was obtained, where Y is the mean peak area and X concentration in µg/mL. The Linearity co-efficient of mean response of replicate determination plotted against respective concentration was found to be 0.999. The % RSD for peak are a response of five replicates was 0.564.

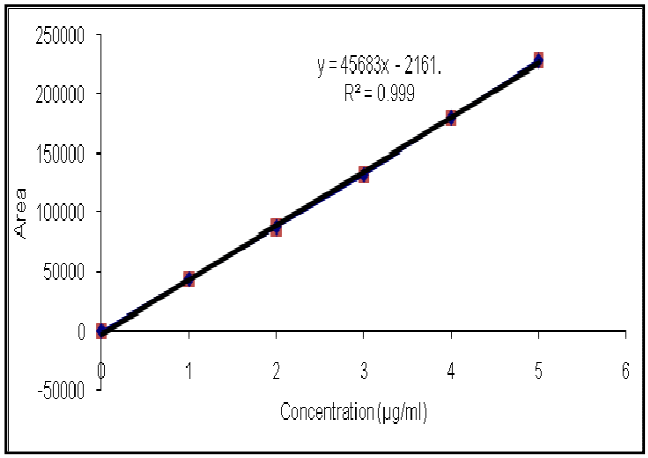

Figure 4: Linearity curve of Repaglinide

3.1.2 System suitability:

3.1.2.1 AUC and RT

The data for AUC and RT of Repaglinide is depicted in Table 2. %RSD was observed below 2 which satisfy the requirement of .

3.1.2.2 Number of theoretical plates (N)

The data for number of theoretical plates of Repaglinide was depicted in Table 2. Number of theoretical plates observed for was 4082.

3.1.2.3 Tailing factor (Tf)

The data for tailing factor of Repaglinide was depicted in Table 2. Tailing factor obtained for Repaglinide was 0.98.

Table 2: System suitability study (3µg/ml, n=6)

Parameter	R.T.	AUC	No. of T.P.	Tf
Mean	3.80	131717	4082	0.98
S.D.	0.03	758.67	26.93	0.01
%RSD	0.81	0.58	0.66	1.40

3.1.3 Accuracy

The accuracy was assessed by analyzing the pharmaceutical formulation (Eurepa- 1mg, Torrent Pharmaceuticals Ltd.) containing the Repaglinide and calculated the percent recovery of the active ingredient. The accuracy of method was calculated at three concentrations such as 80, 100 and 120 µg mL^{-1} in triplicate, which was found within the range of 98 to 102% as per ICH guidelines. Mean % recovery (mean ± SD) was found to be 99.46 ± 0.31, indicating that the co-formulated substances such as talc, starch, gum acacia, lactose, dextrose, hydroxyl methyl cellulose, sodium alginate and magnesium stearate did not interfere in the assay.

Table 3: Recovery study (n = 3)

Amount added (µg/ ml)	Amount recovered (µg/ ml)	% Recovered
1	0.9961	99.61
3	2.9732	99.10
5	4.9538	99.07

3.1.4 Precision

The low %RSD values indicate the ruggedness of the method.

Table 4: Precision study (n = 3)

Conc. (µg/ml)	Mean Peak area	SD	%RSD
Inter-day Precision			
2	89421.08	859.61	0.96
3	131715.31	737.92	0.56
4	164260.25	2697.74	1.64
Intra-day Precision			
2	88138.59	798.50	0.90
3	134368.02	655.78	0.48
4	172061.53	1725.18	1.00

3.1.5 Repeatability

The %RSD value of the repeatability study was found less than 2, which was shown in **Tab.5.**

Table 5: Repeatability study (n = 6)

Concentration	% RSD[a]	%RSD[b]
3 µg ml^{-1}	1.28	0.75

[a]Based on peak area

[b] Based on retention time

3.1.6 Robustness

The robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH, in the flow rate and changing the wavelength and results of %RSD was found less than 2 which indicates robustness of developed method.

Table 6: Robustness study under a variety of conditions

Parameter	Buffer pH 3.2	Buffer pH 2.8
Avg AUC	130953.33	133192.33
Stdev	706.82	1157.97
%RSD	0.53	0.87
	Wavelength 243 nm	**Wavelength 241 nm**
Avg AUC	130758.33	131345
Stdev	429.81	1063.17
%RSD	0.32	0.81
	Flow 0.8ml/min	**Flow 1.2ml/min**
Avg AUC	132091.66	133145
Stdev	1097.74	1290.86
%RSD	0.83	0.96

4. Conclusion

The developed method was found to be simple, sensitive and selective, accurate, precise, repeatable, rapid and cost effective for analysis of Repaglinide in

market formulation without any interference from the excipients. The method was successfully used for determination of Repaglinide in a pharmaceutical formulation.

5. Acknowledgement

The authors are thankful to Torrent Pharmaceuticals LTD., Ahmedabad for providing the pure sample of Repaglinide.

6. References

1) Goyal RK. Elements of pharmacology. ed 16, B.S.Shah Prakashan, Ahmedabad, India 2006-07; pp 171-182.

2) Patel DR, Patel LJ, Patel MM. Development and Validation of stability indicating method for the determination of Repaglinide in Pharmaceutical dosage form using HPLC. Int. J. ChemTech Res. 2011; 3(2); 539-546.

3) Smita S, Mukesh CS. UV-Visible spectrophotometry of Repaglinide in bulk and in formulation by using methyl orange as reagents. J. Iran. Chem. Res. 2011; 4: 69-76

4) Prameela R, Bala SC, Archana N, Siva TP, Aruna B. Determination of Repaglinide in Pharmaceutical Formulations by RP-HPLC Method. J. App. Sci. Res. 2009; 5(10): 1500-1504.

5) Kaushal N, Jain S, Tiwary AK. Development of spectrofluorimetric and HPLC methods for In vitro analysis of Repaglinide. Ind. J. Pharm Sci. 2010; 72(2): 240-244.

6) Meeta AJ, Saurabh SP. Estimation of Repaglinide in bulk and tablet dosage forms by HPTLC method. Int. J. Pharm and Pharm Sci. 2009; 1(1): 21.

7) Abu BR, Suhaimi MA, Ahmad I, Ismail Z, Siew HG. Method development and validation of repaglinide in human plasma by HPLC and its application in pharmacokinetic studies.

a. J. Pharm. Biomed. Anal. 2007; 43: 1831–1835.

8) Jie Z, Feng G, Xin G, Yan-tong SB, Jing-kai GN, Paul JF. Determination of Repaglinide in human plasma by high-performance liquid chromatography–tandem mass spectrometry. Acta Pharm. Sinica B. 2011; 1(1): 40–45.

9) Krishna RJ. Babu JM, Vijayavitthal TM, Eswaraiah S, Satyanarayana MR. Impurity profile study of Repaglinide. J. Pharm. Biomed. Anal. 2003; 32: 461-467.

10) International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1): 2005.

11) United States Pharmacopoeia 30. Validation of Compendial Methods. Rockville MD USA. United States Pharmacopoeial Convention Inc. 2007; 1225.