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Development and Validation of Rp- Hplc Method for the Estimation of Repaglinide in Bulk and Tablet Dosage FORM

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated of repaglinide in bulk and tablet dosage form. Chromatographic analysis was performed on a C18 column (250x 4.6 mm, 5µm) with a mixture of Acetonitrile: Ammonium formate in the ratio 65:35 as mobile phase, at a flow rate of 1.0 mL min−1. UV detection was performed at 245nm. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The retention times of repaglinide were 4.220.0123 respectively. Calibration plots were linear over the concentration ranges 1–6 µg mL−1 for repaglinide with correlation coefficient ¬¬¬¬ (r2) 0.9996 respectively. The Limit of detection was 0.057 and the quantification limit 0.192 ±g/ml for repaglinide respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.72% to 100.33%. Commercial tablet formulation was successfully analyzed using the developed method and the proposed method is applicable to routine analysis of determination of repaglinide and in bulk and tablet dosage form.

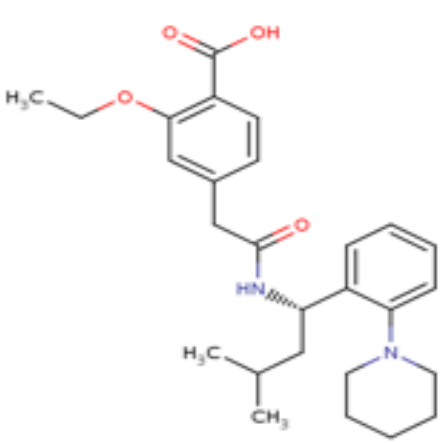
KEYWORDS: Rosiglitazone , RP-HPLC, ICH guidelines

1.INTRODUCTION

Repaglinide class used in the management of type 2 diabetes mellitus. Repaglinide lowers blood glucose levels by stimulating the release of insulin from the pancreas and chemically it is 2-ethoxy-4-[2-[[(1S)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid .

Repaglinide closes ATP-dependent potassium channels in the β -cell membrane by binding at characterizable sites. This potassium channel blockade depolarizes the β-cell, which leads to an opening of calcium channels. The resulting increased calcium influx induces insulin secretion. The ion channel mechanism is highly tissue selective with low affinity for heart and skeletal muscle. A literature survey reveals that various analytical methods like repaglinide by HPLC in pharmaceutical formulation[1,4], Simple HPLC method for the determination of repaglinide in human plasma [2], Simultaneous HPLC estimation of gliclazide and repaglinide in Pharmaceutical Formulations[3] , Quantitative Analysis of repaglinide in Tablets by RP- TLC with Densitometric UV Detection [5]. But these methods are sophisticated,expensive and time consuming when compared to simple HPLC method. There is need for a interest to develop simple,accurate,specific,sensitive, precise and reproduciable HPLC method for the estimation of repaglinide in bulk and its formulation.

Fig. 1: Chemical structure of Repaglinide



2. EXPERIMENTAL

2.1. Materials and Methods:

Pure standard of repaglinide (Assigned purity 99.98%) was obtained as a gift sample from Torrent research center,Gandhinagar. The gift samples were used as standard without further purification. HPLC grade water, methanol (Qualigens), Acetonitrile, Ammonium formate were used throughout the experiment. Commercial pharmaceutical preparation (Pradin) which was claimed to contain 4mg of repaglinide is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2. Instrumentation and chromatographic conditions:

High performance liquid chromatograph, Shimadzu pumpLC-10AT VP equipped with universal injector (Hamilton 25 µL) SPD10A, UV-VIS detector SPD10A-10A VP (Shimadzu) was used. Isocratic elution of mobile phase comprising of Acetonitrile: Ammonium formate in the ratio 65:35 flow rate of 1.0 ml min-1 was performed on C18 column (250x 4.6 mm, 5µm). The effluent was detected at 230 nm. The retention times of repaglinide was 4.22 ± 0.0123 min. The column temperature was maintained at ambient and the volume of injection was 20 µl. Prior to injection of analyte, the column was equilibrated for 30- 40 min with mobile phase.

2.3. Preparation of mobile phase:

The HPLC grade solvents were used for the preparation of mobile phase, isocratic elution of mobile phase comprising of of Acetonitrile: Ammonium formate in the ratio 65:35 were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

2.4. Standard solution

Standard stock solutions 1 mg mL-1 of repaglinide was prepared in methanol and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of repaglinide in over the linearity range from 1 –6 µg/ml.

2.5. Assay in formulation

Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogenious powder and a quantity equivalent to one tablet were weighed in to a 100 ml volumetric flask, dissolve in methanol, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. Further add mobile

phase to obtain an expected concentration of 10µg/ml. All determinations were conducted in triplicate.

3. RESULTS AND DISCUSSIONS

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of repaglinide are shown in (Fig No.1). There was clear resolution between repaglinide with retention time of 4.22 ± 0.0123 minutes respectively.

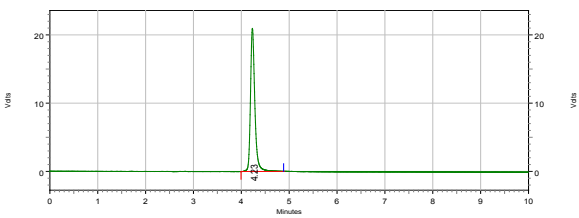


Fig No. 1.Typical chromatogram showing repaglinide

3.1. LINEARITY

The response was determined to be linear over the range of 1 µg/ml to 6 µg/ml (1,2,3,4,5,6) for repaglinide. The solutions were injected into HPLC system. Each of the concentration was injected in triplicate to get reproduciable response. The run time was 10 min and the peak areas were measured. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation. (Fig 2& 3 calibration curve)

$Y_{Repaglinide} = 1573330x + 577936$ Coefficient of correlation (r^2) value = 0.9996

Table No.1 : For Peak Area of Repaglinide

Conc. In µg/mL	1	2	3	4	5	6
Replicate 1	2222861	3655488	5223792	6804548	8467671	10044946
Replicate 2	2254567	3616715	5252395	6806612	8449374	10064042
Replicate 3	2234318	3747713	5297352	6877339	8353710	10149195
Avg	2237249	3673305	5257846	6829500	8423585	10086061
SD	16054.88	67291.99	37081.75	41442.93	61201.16	55503.07
RSD	0.717617	1.831919	0.705265	0.606822	0.726545	0.550295

Figure 2: Calibration curve for Repaglinide

3.2. ACCURACY

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 1 ml, 2.5ml and 4.5 ml of sample drug from repaglinide solution of 10 µg/ml were pipetted into each of three volumetric flasks. To this 0.5 ml of standard drug solution of 10 µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. 20 µl of each solution was injected and chromatograms were recorded. The range was found between 99.72 to 100.33 % respectively. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No.1)

Table No.1 Result of recovery studies

S.No	Conc. taken in (µg/ml)	Std addition in (µg/ml)	Total Conc. found in (µg/ml)*	% recover
1	0.5	0.5	1.00	100.33±0.422
2	0.5	2.5	2.99	99.72±1.44
3	0.5	4.5	5.01	100.28±0.963
Mean ± SD	100.11±0.3349			

3.3. LIMIT OF DETECTION AND QUANTIFICATION

The Limit of detection was 0.057 and the quantification limit was and 0.192 µg/ml for repaglinide respectively which represents that sensitivity of the method is high.

3.4. PRECISION

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 3 µg/ml for repaglinide (n =6) showed a RSD of 0.337% for repaglinide . It was concluded that the analytical technique showed good repeatability.(TableNo.2)

Table No.2 Result of repeatability analysis

S.No.	Conc. (µg/ml)	Peak Area (µV*sec)	Mean ± SD	% RSD
1	REPAGLINIDE 3 µg/ml	5337136	5360335 ±33297.22	0.6211
2		5402661		
3		5340591		
4	5351591			
5		5401782		
6		5328246		

3.5. REPRODUCIBILITY AND RUGGEDNESS

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates. (Table N0.3)

Table N0.3. Results of reproducibility

Parameter	Result observed
	REP
Average Percentage Recovery	101.44%
SD between set of analysis on same date	0.962
SD between set of analysis on different date	1.53
RSD between set of analysis on same date	0.944%
RSD between set of analysis on different date	1.51%

3.6. ROBUSTNESS

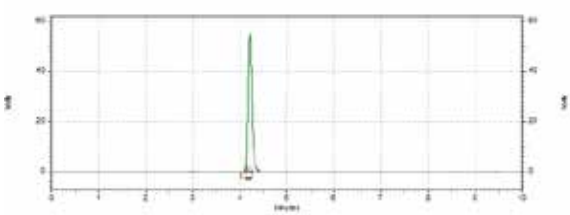
The robustness of the method was determined by delibrate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust.

3.7. SPECIFICITY

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective.

It has been observed that there are no peaks of diluents and placebo at main peak's. Hence, the chromatographic system used for the estimation of repaglinide is very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Fig No.4)

Fig No.4.Chromatogram showing Specificity



3.8. SYSTEM SUITABILITY:

A binary solution of 6 µg $mL^{-1}$of repaglinide (in triplicate) was prepared and same was injected, then the system suitability parameters were calculated from the following chromatogram. (Table No 4)

Table No 4. Results of system suitability parameters

Parameters	Data obtained REP
Number of theortical plates	4547
Symmetry factor/ Tailing factor	1.25

4.CONCLUSION:

The proposed RP-HPLC method is found to be simple,accurate, precise, linear, and specific, and, for quantitative estimation of repaglinide in bulk and its tablet formulation.

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