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Development and validation RP-HPLC method for estimation of Repaglinide in pharmaceutical dosage Forms

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Abstract

A simple and rapid reverse phase HPLC method was developed and validated for the estimation of Repaglinide present in pharmaceutical dosage forms. Chromatopak C18 column under reversed-phase partition chromatographic conditions, 4.6mm x 250 mm, 5 µm column (Shimadzu), with mobile phase methanol: ammonium acetate buffer (pH-4) (80:20) at flow rate of 1.0 ml/min was used. The effluent was monitored at 242 nm. Ritonavir was used as internal standard. Calibration standards were prepared by spiking working standard solutions into methanol (5 mL) to yield various concentrations ranges from 0.5 to 200 µg/ml. The total run time was 10 min and the repaglinide and ritonavir were eluted at 6.2 min and 5.2 min respectively. The results showed linearity range was found to be 0.5-200 µg/ml for repaglinide. The proposed method was validated for specificity, sensitivity, accuracy, precision, linearity, LOD, LOQ, system suitability and robustness in accordance with ICH guidelines. Validation revealed that the method is specific, rapid, accurate, precise, reliable, and reproducible. The high recovery and low coefficients of variation confirmed the suitability of the method for the estimation of the drug in the dosage forms.

Key words: RP HPLC, Repaglinide , Ritonavir, Retention time, Validation and Dosage forms.

Introduction

Repaglinide is used in the treatment of type II diabetes; it lowers blood glucose level by stimulating the release of insulin from the pancreas.[1] Repaglinide belongs meglitinide class and chemically it is S(+)2-ethoxy-4-[N-{1-(2-piperidinophenyl)-3-methyl-1-butyl}-aminocarbonylmethyl] benzoic acid.[2] It stimulates the secretion of insulin from beta cells in the pancreas by acting via calcium channels.[3]

Now-a-days the importance of chromatographic techniques is increasing rapidly in the pharmaceutical analysis. Another important field of application of chromatographic methods is the purity testing of final products and intermediates (detection of decomposition products and by-products). As a consequence of the above points, chromatographic methods are occupying an ever-expanding position in the latest editions of the pharmacopoeias and other testing standard monographs. Ruzilawati, *et al*, developed a HPLC method for determination of repaginate concentration in human plasma for pharmacokinetic studies.[4]

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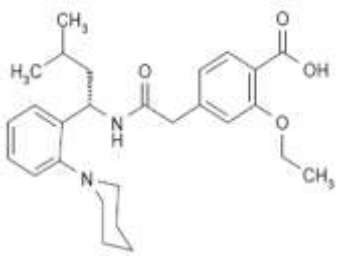
In the present study the authors has objective to develop a RP HPLC method for the easy and rapid estimation of Repaglinide in the pharmaceutical dosage forms with methanol and ammonium acetate buffer as mobile phase and the developed method is validated as per ICH guidelines.[5,6]



Structure of Repaglinide

Material and Methods

Instruments:

The HPLC was performed with a Shimadzu LC-10 AT VP solvent-delivery system, a Shimadzu SPD-10 AVPUV–visible photodiode-array (PDA) detector, a Chromatopak C18 column (250mm x 4.6mm i.d., 5-

□m particles), and a Rheodyne 7725 I universal loop injector of injection capacity 20 mL. The equipment was controlled by a PC workstation and compounds were separated on under reversed-phase partition chromatographic conditions. The flow rate was maintained at 1.0 ml/min and the effluent was monitored at 242 nm. Before analysis both mobile phase and sample solutions were degassed by sonication and filtered through 0.2-□m filter paper.

Standards and Chemicals:

Pure sample of Repaglinide was kindly gifted from Torrent Pharma Ltd (Gujarat, India) and Ritonavir used as an internal standard (IS) were gifts obtained Ranbaxy Laboratories Limited (Mohali, India).; and tablets (Rapilin and Eurepa) were obtained from local market. All the chemicals and reagents used were of HPLC grade and purchased from Spectrochem and SD Fine chemicals, Mumbai, India. Double distilled water was used for entire washing and other procedures while HPLC grade water was used for preparation of solutions (mobile phase).

Method development

Preparation of standard drug solutions:

Standard master stock solution of Repaglinide was prepared by dissolving 20 mg of Repaglinide in 20 mL of volumetric flask containing about 15 mL of methanol. The solution was sonicated for about 20 min and then made up to volume upto 20 mL with methanol. Routine working standard solutions of Repaglinide was prepared by suitable dilution of the stock solution with appropriate solvent. Similarly stock solution of internal standard was prepared by dissolving 10mg of Ritonavir in 10 mL of methanol.

The 0.01 M ammonium acetate solution was prepared by dissolving 0.77g of ammonium acetate in 800 ml water and then makes the final volume up to 1000 ml with water. The pH was adjusted to 4.0 with formic acid.

Chromatographic conditions:

The mobile phase used in this study was a mixture of ammonium acetate buffer (0.01M, pH 4.0) and methanol in ratio of 20:80 v/v. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1ml/min. The column temperature was maintained at 22±10C. The eluents were monitored at 242nm. The identification of the separated Repaglinide and Ritonavir were confirmed by running the chromatograms of the individual compounds under identical conditions. The optimized conditions of were shown in Table 1.

Table1. Optimized chromatographic conditions of Repaglinide & internal std. (Ritonavir)

Parameter	Condition	
	Repaglinide	Ritonavir
Mobile phase	Methanol:0.01M ammonium acetate buffer of pH: 4.0 (80:20 v/v).	Methanol: 0.01M ammonium acetate buffer of pH: 4.0 (80:20 v/v).
Stationary phase	Chromatopak C18 column	Chromatopak C18 column
Flow rate	1ml/min	1ml/min
Runtime	10 min.	10 min.
Column temp.	22 ± 0.2 ºC	22 ± 0.2 ºC
Injection Vol.	20-□L	20-□L
Detection at □	242 nm	242 nm
Retention time	6.2 min.	5.2 min.

Calibration curves of standards:

Calibration standards were prepared by spiking working standard solutions into methanol containing 5 mL volumetric flasks to yield concentrations of 0.5, 1, 2, 5, 7.5, 10, 15, 20, 50, 100, 150, and 200 µg /mL. To the above solutions 20 µg /mL of Ritonavir (internal standard) was added and the final volume was made up to the mark. A 20-µL aliquot was injected in to the analytical column. Quantitative analysis based on peak area measurements as ratios towards the peak area of internal standard. Another standard solution was prepared separately for quality control. All the standard curves were checked with quality control samples.

Validation of developed Method:

Developed method was validated in terms of sensitivity, specificity, linearity, LOQ, LOD, precision, accuracy, robustness and system suitability.

Specificity and selectivity:

The specificity of the method was evaluated with regard to interference due to presence of any other excipients. Four different samples were injected and studied with respective excipients.[5]

Linearity:

The six replicates of analyte were measured to establish the linearity between drug concentration and detector response.

Precision and Accuracy:

For the assessment of the precision of the method, intra-day and inter-day (3 days) measurements of Repaglinide concentration were completed with computation of the coefficient of variation (C.V %) for

replicate samples (n=6) using concentrations of 20, 50 and 100 µg/mL. Both intra-day and inter-day samples were calibrated with standard curves concurrently prepared on the day of analysis.[7]

Intra-day and inter-day accuracy was evaluated by assaying with different concentrations of Repaglinide. Intra-day and inter-day accuracy was assessed by analyzing three samples at each concentration on the same day and mean values of six samples for 3 days respectively. Accuracy was presented as percent error (relative error), [(measured concentration-added concentration) / added concentration] x 100 (%).[6]

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD can be defined as the smallest level of analyte that gives a measurable response. The LOD is based on S/N ratio typically 3.0 for HPLC methods. Six replicates of analyte were measured.

The LOQ was defined as the lowest concentration at which the precision was expressed by relative standard deviation (RSD) is less than 2% and accuracy expressed by relative difference in the measured and true value; it was also less than 2%. In other words, the analyte response is 10 times greater than the noise response. Six replicates of analyte were measured and quantified.

Robustness:

The optimum HPLC conditions set for this method have been slightly modified for samples of Repaglinide (mg/mL) dissolved in the drug matrix as a means to evaluate the method ruggedness. The small changes include: the mobile phase ratio, the flow rate, the detection wavelength, the sonication time, the filtration system and the column.

System suitability:

It concerns that developed method can generate result of acceptable accuracy and precision or not. The system suitability was carried out after the method development and validation have been completed. For this, parameters like plate number (N), tailing factor (k), resolution (R), relative retention time (α), HETP, capacity factor (kI), plates per meter and peak symmetry of samples were measured.

Recovery of Repaglinide in tablets:

Ten tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 20 mg of Repaglinide was extracted with methanol in a 20mL volumetric flask using ultra sonicator. This solution was filtered through Whatmann No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of previously determined linearity. An aliquot of the internal standard was added to the sample solution prior to the dilution so as to give a final concentration of internal standard 20µg/mL. All the determinations were carried out in six replicates.

Results and Discussion

The developed method was used based upon its ability to detect and quantitate Repaglinide with the use of standard HPLC system equipped with UV-Visible (PDA) detector.

Choice of stationary phase and mobile phase:

A non-polar C-18 analytical chromatographic column was chosen as the stationary phase in the present study for the separation and determination of Repaglinide. For the mobile phase several eluting systems were examined and the use of buffer with lower pH was found to be essential. For the optimization of mobile phase, concentration of buffer, pH of buffer and different composition of the mobile phase were examined at different conditions. The optimum composition based on chromatographic response factor (shown in Table 2), was found to be 80:20 (%, v/v) methanol and 0.01 M ammonium acetate buffer, pH 4.0, provided an efficient separation of Repaglinide with sufficient retention time. A flow rate of 1.0 ml/min was found to be optimum from the studied range 0.5-1.5 ml/min as comprise between an optimum retention time, baseline stability and noise. An UV spectrum of Repaglinide was shown in fig1.

Table2. Optimization of mobile phase for the determination of Repaglinide

S. No	Composition of mobile phase	Retention time (min)	Total run time (min)
1	90:10	3.1	10
2	80:20	6.2	10
3	60:40	8.9	10

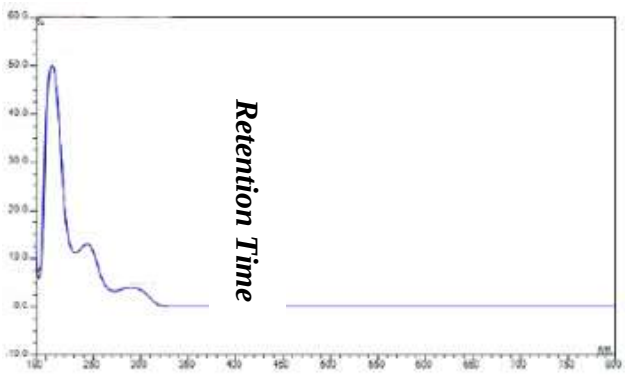


Fig.1 UV Spectrum of Repaglinide at 6.2 Min.

Internal standard approach:

To minimize the variation in results by the contribution of sample preparation, injection variation and column deterioration to the final results, the internal standard mode of quantification was applied. For this several drugs were tested and amongst them Ritonavir was considered as internal standard, because it fulfills the requirements; as it possess similar physicochemical properties, go through all the steps of procedures, and elute close to analyte.

Method validation

Specificity and selectivity:

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 10 min. The chromatograms of Repaglinide alone and mixture of Repaglinide with internal standard were shown in Fig 2 and 3. The figures show that Repaglinide is clearly separated from its internal standard. Thus, the HPLC method presented in this study is selective for Repaglinide.

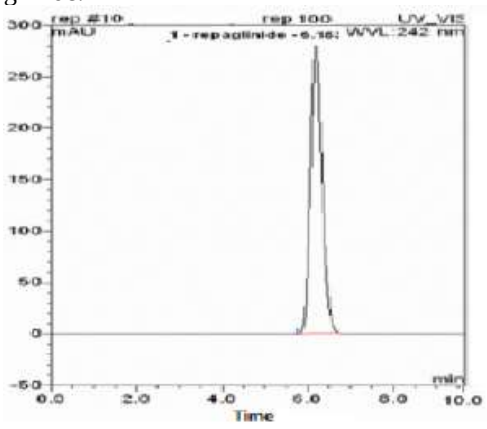


Fig 2. HPLC Chromatogram of REP at 100µg/mL

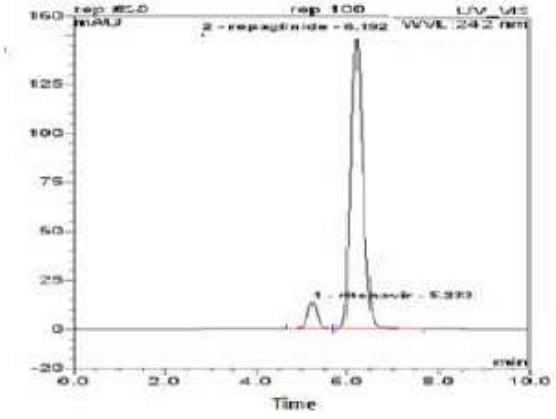


Fig 3. HPLC Chromatogram of RPG with Ritonavir 20µg/mL

Linearity:

The HPLC chromatograms of Repaglinide at 100 µg/mL and Repaglinide with 20 µg/mL of Ritonavir (IS) were shown in fig.2 and fig.3. The elution time was less than 10 min. The standard curve was obtained in the concentration range of 0.5-200µg/mL. The linearity of these methods was evaluated by linear regression analysis, which was calculated by least square method and the data shown in table 3 and in fig 4. The regression equation from 0.5-200(µg/mL) was fond to be Y=35.234X150.642 and R^2=0.9998.

Table 3. Linearity range of Repaglinide.

Conc. (µg/mL)	Area
0.5	18.6
1	40.7
2	72.9
5	186.2
7.5	273.1
10	355.9
15	554.2
20	698.8
50	1832.5
100	3602.3
150	5294.6
200	7024.4

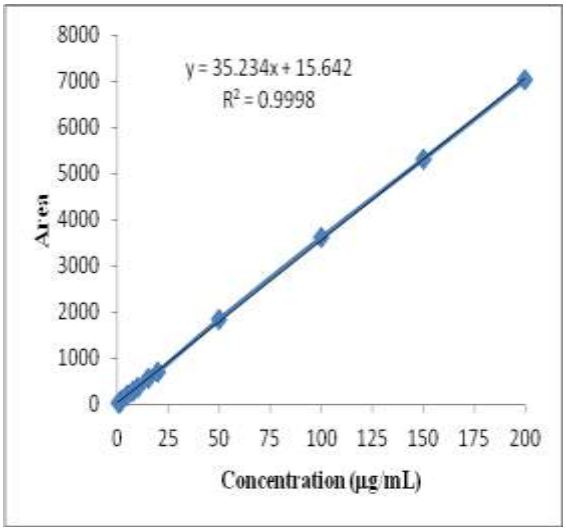


Fig. 4. Calibration curve of Repaglinide using HPLC method

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were observed 5 ng/mL (signal to noise ratio 3) and 17 ng/mL (signal to noise ratio 10) respectively.

Precision and accuracy:

Method of validation regarding reproducibility (precision) and accuracy by replicates injection of standard solution at low, medium and high concentration levels was done and the peak areas were measured in comparison to the peak area of the internal standard.

Inter-day precision and accuracy were conducted during routine procedure over a period of 3 consecutive days while Intra-day precision and accuracy were studied by six replicate measurements at three different concentration levels. Statistical evaluation revealed that relative standard deviation of Ritonavir at different concentration for six injections was less than 2.0. Precision and accuracy data were shown in tables 4 and 5.

Table 4. Precision study data of developed HPLC method

Conc. Taken (□g/mL)	**Intra- day**		**Inter-Day**	
	Conc. Found (□g/mL) ±S.D	% coefficient of variation	Conc. Found (□g/mL))±S.D	% coefficient of variation
20	20.47±0.15	0.34	20.18±0.17	0.52
50	49.78±0.1	0.22	49.57±0.12	0.29
100	99.89±0.13	0.15	99.14±0.1	0.08

Table 5. Accuracy study data of developed HPLC method

Qty. of Drug added (□g)	Mean±S.D qty. (□g) recovered (n=6)	Mean±S.D qty. % recovery
20	20.14±0.12	100.7±0.19
50	49.92±0.1	99.84±0.12
100	101.12±0.16	101.12±0.16

System suitability:

For system suitability study, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), HETP, capacity factor (k^l), plates per meter and peak symmetry of samples. The data were shown in table 6.

Table 6. System suitability parameters of Repaglinide

Parameter	Value
Resolution	2.23
Capacity factor	2.11
Theoritical plates	2747
Tailing factor	0.921
HETP	0.093
Asymmetry	1.03

Robustness:

The tailing factor observed for Repaglinide was always less than 2.0 and the components were well separated under all the changes carried out during study. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

The method was optimized by changing various parameters, like pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of Repaglinide has more dependent on pH of the mobile phase when compared to Ritonavir. The separation of peaks was also dependent on pH of the buffer and the percentage of methanol.

Table 7. Amount of Repaglinide in tablet dosage forms by proposed HPLC method

Marketed Formulation	**Labeled qty (mg)**	**Mean ±SD (Qty mg recovered) n=3**	**Mean ±SD (% of recovery)**
Brand1	2	1.97	98.12± 0.36
Brand2	2	1.95	97.39±0.49

Conclusion

Under the presently prescribed conditions (Table 7), the recoveries of Repaglinide were found to be in range. A very low concentration of buffer (0.01 M ammonium acetate, pH adjusted to 4.0 with formic acid) was used to reduce the tailing of Repaglinide. This method might be useful for determination of Repaglinide in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies. The differences of less than 2.0 % in both intra- and inter-day data reflect the accuracy of the method. The observation of C.V (less than 2.0) in both intra- and inter-day measurements also indicates the high degree of precision. In the present method, we have established a linearity range of 0.5-200 μg /mL; this linearity range covers all the strengths of available brands of Repaglinide in the market. Hence this method can be applied for quantifying the Repaglinide in

pharmaceutical dosage forms and other pharmacokinetic studies.

References

1. M Salman, J S Babu, P C Ray, S Biswas, Naresh Kumar, *United States Patent*, 6686497.
2. United States Pharmacopoeia, USP Convention Inc.: Rockville M.D., Asian Edn., 2006; 1899.
3. Rendell, M. (2004) Advances in Diabetes for the Millennium: Drug Therapy of Type 2 Diabetes. *Med Gen Med 2004, 6, 9.*
4. A B Ruzilawati, M Wahab, A Imran, Z Ismail, S H Gan (2007) "Method development and validation of repaglinide in human by HPLC and its application in pharmacokinetic studies". *Journal of Pharmaceutical and Biomedical Analysis, 43(5): 1831-1835.*
5. ICH, Q2A Validation of Analytical Procedures: Consensus Guidelines; ICH Harmonized Tripartite Guidelines, ICH Secretariat, Geneva, Switzerland, 1994.
6. ICH, Q2B Validation of Analytical Procedures: Methodology, Consensus Guidelines; ICH Harmonized Tripartite Guidelines, ICH Secretariat, Geneva, Switzerland, 1996.
7. Khan RH, Talegaonkar S, Singh RM (2007) "A simple HPLC method for quantitation of repaglinide in tablet dosage form". *Indian Drugs 44(6): 428-433.*

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