World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 8, Issue 3, 2019 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

METHOD DEVELOPED AND VALIDATION OF REPAGLINIDE IN PHARMACEUTICAL DOSAGE FORM

S. Vasantha, SK. Shaila Bhanu, T. Sravani, U. Naga Tejaswi, V. Lalithya Jyothika, N. Lavanya, Nadendla Rama Rao, Shaik Munwar

Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, A.P, INDIA.

Received on: 13-02-2019; Revised and Accepted on: 27-03-2019

ABSTRACT

It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment. The area of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main aspiration for that is to improve the conditions and parameters, which should be followed in the development and validation.

KEYWORDS: *Accurate, Precise, Purity, Validation and Parameters.*

INTRODUCTION

The Plan of the Proposed work includes the Following Steps:

1. To undertake solubility and stability studies of Repaglinide and to develop initial U.V. and chromatographic conditions.
2. Setting up of initial UV and chromatographic conditions for the method development in pure and pharmaceutical dosage forms.
3. Optimization of initial chromatographic and spectro photometric conditions.
4. Analytical method validation of the developed RP- HPLC method.
5. Quantitative determination of Repaglinide in pharmaceutical dosage form using the method developed and validated.

EXPERIMENTAL WORK

Method Development:

Standard & sample preparation for UV-spectrophotometer analysis:

25 mg of Repaglinide standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

***Corresponding author:**

Shaik Munwar

Chalapathi Institute of Pharmaceutical Sciences,
Lam, Guntur, A.P, INDIA.
** E-Mail: pharmacy14443@gmail.com*

DOI: https://doi.org/10.5281/zenodo.2620460

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Repaglinide, so that the same wave number can be utilized in HPLC UV detector for estimating the Repaglinide. While scanning the Repaglinide solution we observed the maxima at 243 nm.

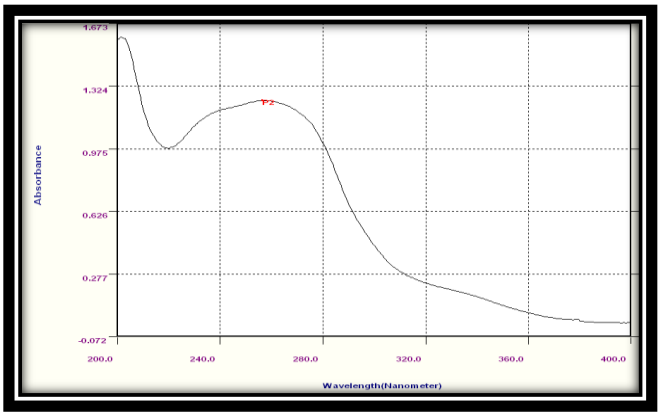

Fig. 1: UV spectrum for repaglinide

Mobile Phase Preparation:

The mobile phase used in this analysis consists of a mixture of Buffer (0.05 M KH_2PO_4 triethylamine & pH adjusted to 6.0 with orthophosphoric acid) and acetronitrile in a ratio of 74: 26.

740 ml of this buffer solution was added and properly mixed with 260 ml of acetronitrile and a homogenous solution is

achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.

Sample & Standard Preparation for the Analysis:

25 mg of Repaglinide standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

The sample was analysed by HPLC by using the above method and a very nicely resolved peak has been obtained at a Retention Time of about 2.96 min. The respective chromatogram is attached in the following page.

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc (Table 2).

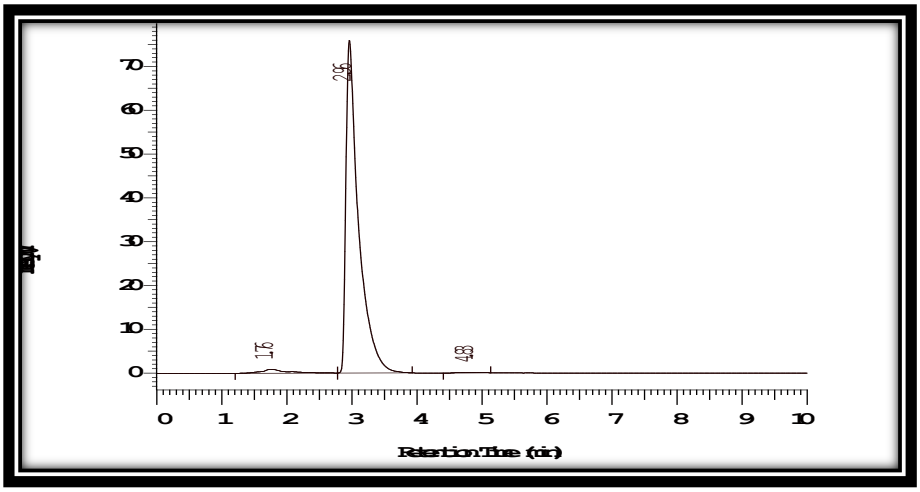

Fig. 2: HPLC spectrum of Repaglinide (10 ppm) in optimized conditions (RT 2.96 min.)

Table No. 1: Peak results

Sr. No:	Name	RT	Area
1	Repaglinide	2.96	1087487

Table No. 2: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Water C_{18}, 5µm, 25cmx4.6mm.	Methanol : Water = 50 :50	0.5 ml/min	243 Nm	Low response	**Method rejected**
Water C_{18}, 5µm, 25cmx4.6mm	ACN : Water = 50 :50	0.5 ml/min	243 Nm	Broken peak	**Method rejected**
Water C_{18}, 5µm, 25cmx4.6mm	ACN: water = 40 : 60	1.0 ml/min	243 Nm	Tailing peak	**Method rejected**
Water C_{18}, 5µm, 25cmx4.6mm	ACN: acetate buffer = 30 : 70	1.0 ml/ min	243 Nm	Broad Peak	**Method rejected**
Water C_{18}, 5µm, 25cmx4.6mm	**ACN : phosphate buffer = 26 : 74**	**1.0 ml/min**	**243 Nm**	**Nice peak**	**Method Accepted**

The Optimum conditions obtained from experiments can be summarized as below:

Table No. 3: Summary of Optimized Conditions

Mobile phase	phosphate buffer(pH 6.0): acetonitrile (74:26)
Wavelength	243nm
Flow rate	1.0 ml/ min.
Run time	05 min.
Column	waters, C-18, (250mm*4.6mmØ)

Method Validation:

1. Accuracy:

Recovery study:

Table No. 4: Accuracy Readings

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.48	Mean= 98.97667% S.D. = 0.200083 % R.S.D.= 0.202152
S_2 : 80 %	8	10	98.48	
S_3 : 80 %	8	10	98.97	
S_4 : 100 %	10	10	99.97	Mean= 99.54% S.D. = 0.33 % R.S.D.= 0.331525
S_5 : 100 %	10	10	99.54	
S_6 : 100 %	10	10	99.11	
S_7 : 120 %	12	10	99.32	Mean= 99.567% S.D. = 0.33 % R.S.D. = 0.331159
S_8 : 120 %	12	10	99.35	
S_9 : 120 %	12	10	100.28	

2. Precision:

Repeatability:

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Repaglinide(API) The percent relative standard deviation were calculated for Repaglinide.

Intra-assay & inter-assay:

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Repaglinide revealed that the proposed method is precise.

3. Linearity & Range:

The calibration curve showed good linearity in the range of 5-15 µg/ml, for Repaglinide (API) with correlation coefficient (r^2) of 0.997 (Fig. 4). A typical calibration curve has the regression equation of y = 46989x + 36232 for Repaglinide.

4. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Repaglinide(API).

5. LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.05 & 0.15 µg/ml respectively.

6. Assay of Repaglinide in Dosage form:

Assay was performed as described in previous chapter. Results obtained are tabulated below table 9.

Table No. 5: Results of Repeatability

HPLC Injection Replicates of Repaglinide	Retention Time	Area
Replicate – 1	2.96	**1025457**
Replicate – 2	2.94	**1003224**
Replicate – 3	2.97	**995798**
Replicate – 4	2.95	**992259**
Replicate – 5	2.97	**998740**
Average	2.958	**1003096**
Standard Deviation	0.013038	**13131.13**
% RSD	**0.440784**	**1.309061**

Table No. 6: Results of intra-assay & inter-assay

Conc. Of Repaglinide (API) (µg/ml)	Observed Conc. of Repaglinide (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.08	0.96	10.03	**0.97**
20	20.04	0.40	30.03	**0.42S**
40	**39.97**	**0.33**	**39.95**	**0.14**

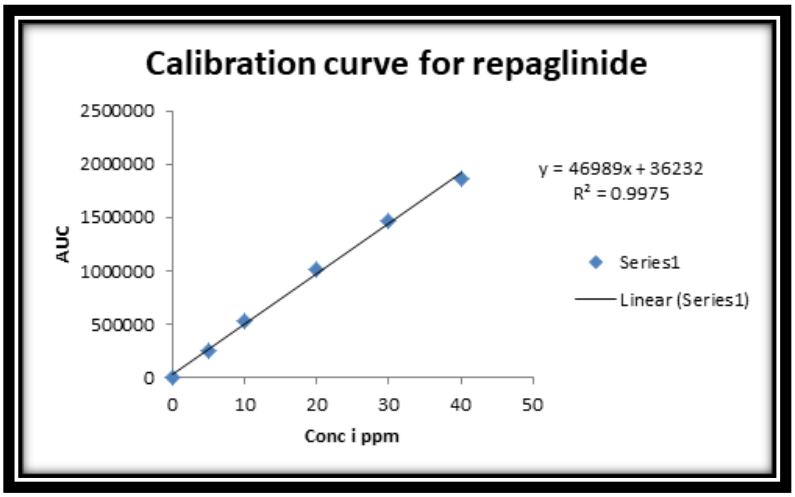

Fig. 3: Calibration curve of Repaglinide

Table No. 7: Results of Linearity

Conc. in µg/ml	AUC n=6
0	0
5	257707
10	536853
20	1012150
30	1473490
40	1871022

Table No. 8: Result of method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.07
Flow (0.9 ml/min)	0.02
Temperature (27⁰C)	0.09
Temperature (23⁰C)	0.13
Wavelength of Detection (225 nm)	0.04
Wavelength of detection (221 nm)	0.01

Table No. 9: Assay of Repaglinide tablets

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Eurepa TAB (Torrent Pharma)	1.0	1.0128 (±0.06)	101.28 (±0.49)

The assay of Eurepa TAB tablets containing Repaglinide was found to be 101.28 %.

RESULT & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Repaglinide, different chromatographic conditions were applied & the results observed are presented in previous chapters.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here waters C_{18}, 5mm, 50 x 4.6 mm i.e. column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl).

The drug was found to be sparingle soluble in acetonitrile, less soluble in water and freely soluble in methanol. Using these solvents with appropriate composition newer methods can be developed and validated.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Repaglinide it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-300 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 ml were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay, which can help in the analysis of Repaglinide in different formulations.

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Repaglinide API.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

The result shows the developed method is yet another suitable method for assay, purity, which can help in the analysis of Repaglinide in different formulations.

REFERENCES:

1. Maryadele J O Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. Whitehouse Station, N.J: Merck and Co. Inc; **2006**;386.
2. Sweetman SC. The Martindale: The Complete Drug Reference. 35th ed. London, UK: Pharmaceutical Press; **2007**;219.
3. Maryadele J O Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals, 14th ed. Whitehouse Station, N.J: Merck and Co. Inc; **2006**;1184.
4. Sweetman SC. The Martindale: The Complete Drug Reference. 35th ed. London, UK: Pharmaceutical Press; **2007**;759.
5. Chaudhari SK. Quintessence of Medical Pharmacology. 1st ed. New Delhi: New Central Book Agency (P) Ltd; **1997**;484.
6. The Indian Pharmacopoeia, Vol. II, Government of India. Delhi: The Controller of Publications; **2010**;1090.
7. The United States Pharmacopoeia, USP 32, NF 27, Vol. 2. Rockville, MD: The United States Pharmacopoeial Convention, Inc; **2009**;1939.
8. British Pharmacopoeia, Vol. I, Stationary Office. London: Medicines and Healthcare Products Regulatory Agency; **2010**;510.
9. JO' Neil. Maryadele. An Encyclopedia of Chemicals, Drugs and Biologicals, *The Merck Index,* 14th edition, Merck and Co. Inc., Whitehouse station, NJ, **2006**; pp. 6253.
10. Budavari S. The Merck Index. 13th edn, An Encyclopedia of Chemicals, Drugs and Biologicals, Published by Merck Research Lab. Division of Merck and co. Inc. whitehouse station, NJ, **2001**;718.
11. United States Pharmacopoeia, 34, NF 29, United States Pharmacopoeial Convention Inc., Rockville MD, **2011**; 905.
12. ICH (Q1B), Harmonized Tripartite Guideline, Stability testing: Photostability Testing of New Drug Substances and Products, In: Proceedings of International conference of Harmonization, Geneva, Switzerland, Nov, **1996**.
13. The complete Drug reference; Martindale, Pharmaceutical press 32 edition;12th pg.
14. Watnabe M, et al. Synthesis and biological activity of methane sulfonamide pyramiding and N-methane sulfonyl pyrrole-substituted 3,5-dihydroxy-heptonates, a novel series of HMG-coa reductase inhibitors. Bioorg Med Chem **1997**;5:437-444.
15. FDA Drug Approvals List [online](cited 26 Aug **2003**).
16. International Conference on Harmonization, Q2A: Text on Validation of Analytical Procedures. Federal Register **1995**;60(40):11260–11262.
17. International Conference on Harmonization, Q2B: Validation of Analytical Procedures: Methodology; Availability. Federal Register **1997**;62(96):27463–27467.

How to cite this article:

Shaik Munwar et al. METHOD DEVELOPED AND VALIDATION OF REPAGLINIDE IN PHARMACEUTICAL DOSAGE FORM. J Pharm Res 2019;8(3):101-105. **DOI: https://doi.org/10.5281/zenodo.2620460**

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil