INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF METFORMIN AND REPAGLINIDE DOSAGE FORM BY USING RP- HPLC

S.Rajeswari, K.Harika

Department of Pharmaceutical Analysis, Kakinada Institute of Technology and Science, Divili, Peddapuram, Andhra Pradesh, India

ABSTRACT

The estimation of Repaglinide and Metformin was done by RP-HPLC. The assay of Repaglinide and Metformin was performed with tablets and the % assay was found to be 99.97 and 100.01 which shows that the method is useful for routine analysis. The linearity of Repaglinide and Metformin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.25 and 0.24 for Repaglinide and Metformin which shows that the method is precise.

Key Words: Repaglinide, Metformin, RP-HPLC

Author for correspondence
S.Rajeswari,
Department of Pharmaceutical Analysis,
Kakinada Institute of Technology and Science,
Divili, Peddapuram, Andhra Pradesh, India.
Email: gmangadevi6@gmail.com

INTRODUCTION

Quality is important in every product or service, but it is vital in medicine as it involves life. Unlike other consumer goods, there can be and there is no second quality. Therefore analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities, to assure that a drug product meets the established standard, is a stable and will continue to meet purported quality throughout its shelflife (1). These methods should be selective and sensitive to monitor the known and unknown impurities, have to be written in a format such that they can be produced over a period of time and from laboratory to laboratory, i.e. these methods should be validated. Analytical methods are required to characterize drug substance and drug product composition during all phases of pharmaceutical development. Early phase methods must support changes in synthetic routes and dosage form and elucidate the structures and levels of impurities. In later phases, goals change to the development of rapid and robust methods for release and stability evaluation. Analysis includes a wide range of simple and instrumental analytical methods, but the most widely most used analytical methods for quality assurance are spectroscopy and chromatography based. Most quantitative analysis require, measuring specified components in the presence of sample matrix and /or related substances, therefore isolation or separation of the components are required preceding quantitative analysis. In such cases chromatographic techniques are used for quantitative analysis. In cases where matrix interference is not observed quantitative

measurements are made using spectroscopic or titration methods directly. For the present studies analytical methods based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), Reserved Phase and Normal Phase High Performance Thin Layer Chromatography HPTLC and Infra Red Spectrophotometry have been developed (2, 3). The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories (4). Repaglinide and Metformin are used widely in type 2 anti diabetic, and it is proved to be safe when used in combinaion. The earlier literature reveals the analytical methods like UV and HPLC were reproted for determination of these drugs individually and other combinations. Therefore the present study has been undertaken in order to develop new, simple, rapid, efficient and reproducible method for the analysis of Repaglinide and Metformin.

MATERIALS AND METHODS

Wave length selection

UV spectrum of 10 µg / ml Repaglinide and Metformin in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 242. At this wavelength both the drugs show good absorbance.

Assay (5, 6)

Standard Solution Preparation

Accurately weigh and transfer 2mg of Repaglinide & 500mg of Metformin working standard into a 100ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of Repaglinide & Metformin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Repaglinide & Metformin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 2mg of Repaglinide & 500mg Metformin equivalent weight of the sample into a 100ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of Repaglinide & Metformin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Repaglinide & Metformin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Repaglinide & Metformin peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSION

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown in fig-1, 2 and table-1

Table-1 Results of Assay for Repaglinide and Metformin

	Label Claim (mg)	% Assay
Repaglinide	2	99.97
Metformin	500	100.01

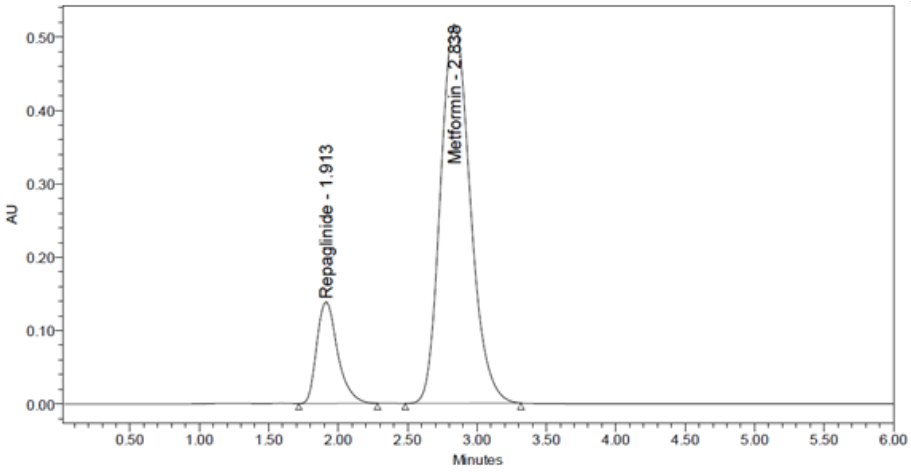

Fig-1 Chromatogram for Standard

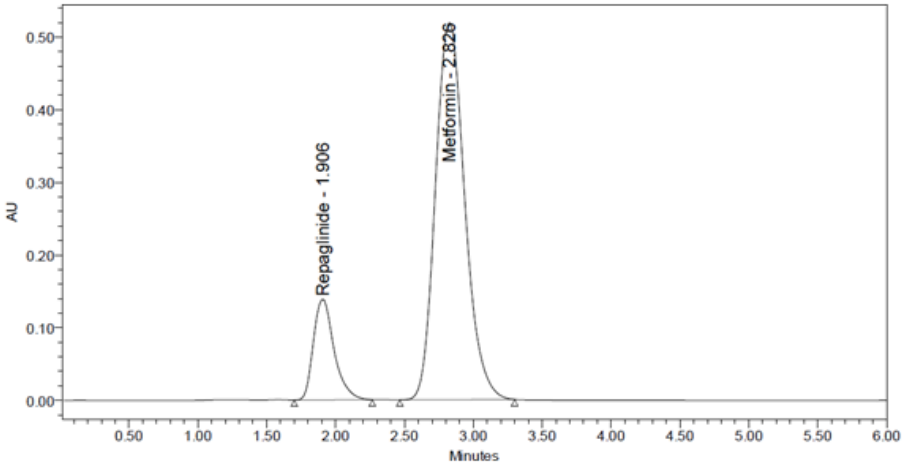

Fig-2 Chromatogram for Sample

The linearity range was found to lie from 0.2µg/ml to 1µg/ml of Repaglinide, 50µg/ml to 250µg/ml 0f Metformin (table-2, fig-3 and 4).

Table-2 Area of different concentration of Repaglinide and Metformin

S. No	Repaglinide		Metformin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	0.2	615618	50	3160168
2	0.4	851274	100	5596971
3	0.6	1050645	150	7747596
4	0.8	1274352	200	10306499
5	1	1515639	250	12430844

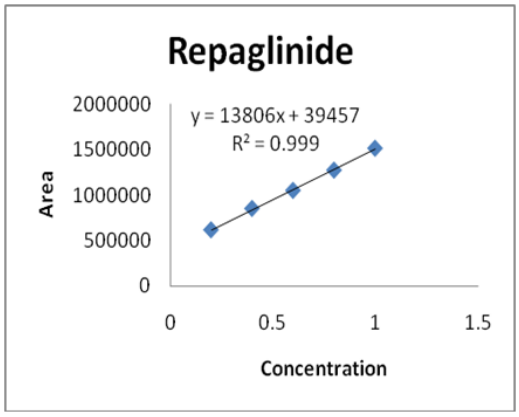

Fig-3 Calibration graph for Repaglinide

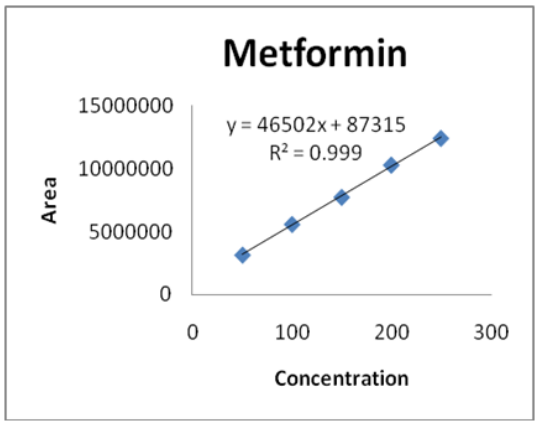

Fig-4 Calibration graph for Metformin

The %RSD for the standard solution is below 1, which is within the limits hence method is precise (Table-3 and 4).

Table-3 Results of Precision for Repaglinide

Injection	**Area**
Injection-1	1453371
Injection-2	1455487
Injection-3	1456408
Injection-4	1459838
Injection-5	1462389
Average	1457499
Standard Deviation	3593.8
%RSD	0.25

Table-4 Results of Precision for Metformin

Injection	**Area**
Injection-1	7839785
Injection-2	7852682
Injection-3	7857421
Injection-4	7873389

Injection-5	7888982
Average	7862452
Standard Deviation	19092
%RSD	0.24

The percentage recovery was found to be within the limit (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate. The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

CONCLUSION

The estimation of Repaglinide and Metformin was done by RP-HPLC. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 1.7 and 0.32 for Repaglinide and Metformin which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.39% and 101.97% for Repaglinide and Metformin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Repaglinide was found to be 2.97 and 9.97 and LOD and LOQ for Metformin was found to be 2.98 and 9.98. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

REFERENCES

1. A *Handbook of modern pharmaceutical analysis, Separation Science and Technology*.In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol. 3,p 2.
2. *Handbook of modern pharmaceutical analysis, Separation Science and Technology*. In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol. 3, p 383.
3. *Handbook of modern pharmaceutical analysis, Separation Science and Technology*. In Ahuja, S.; Scypinski, S., Eds. Academic Press, USA: 2001; Vol. 3, p 346.
4. Beckett, A. H.; Stenlake, J. B. *Practical pharmaceutical chemistry*. In CBS publishers and distributors, New Delhi: 1997; Vol.2, p 163.
5. ICH Q2A, *Validation of Analytical Procedures: Definitions and Terminology*, Geneva, 1995, in 2005 incorporated in Q2(R1).
6. ICH Q2B, *Validation of Analytical Procedures: Methodology*, adopted in 1996, Geneva Q2B, in 2005 incorporated in Q2(R1).