WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 769-779. Research Article ISSN 2277– 7105

METHOD DEVLOPMENT AND VALIDATION OF METFORMN AND GLIMEPIRIDE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

Somnath Gaikwad[1*], Dr.Laxman Kavale[2], Namrata Bhirud[3], Rahinj Bebi[4], Nitin Girawale[5], Mayur Gandhi[6]

M.V.P. Samaj's College of Pharmacy, Near K.T.H.M. Campus, Gangapur road, Nashik-422002, Maharashtra, India.

Article Received on
26 August 2014,

Revised on 21 Sept 2014,
Accepted on 16 Oct 2014

***Correspondence for Author**
Dr. Somnath Gaikwad
M.V.P. Samaj's College of Pharmacy, Near K.T.H.M. Campus, Gangapur road, Nashik-422002, Maharashtra, India.

ABSTRACT

The present work was undertaken with an objective to develop an accurate, simple, precise and reliable method for estimation of Metformin and Glimepiride in their combined dosage form. An RP-HPLC method was developed and validated for the determination of Metformin and Glimepiride in tablet dosage form. The analytes were resolved by using gradient programme. Hypersil Gold C18 column (4.6 x 250 mm, 5 µ) is used for separation and injection volume was 20 µL, mobile phase used is Methanol: Phosphate buffer (pH-3), at a flow rate of 0.8ml/min, on HPLC auto-sampler system containing UV-visible detector with Workstation Software. The detection of Metformin and Glimepiride were carried out at 237 nm and 228 nm respectively and isobestic point at 241 nm. The method gave the good resolution and suitable retention time. The results of analysis in all the method were validated as per ICH guidelines.

KEYWORDS: Metformin, Glimepiride, RP-HPLC, Validation, Gradient system.

INTRODUCTION

Metformin hydrochloride {1, 1-dimethylbiguanide} was an Antihyperglycemic agent that improves glucose tolerance in patients with type II Diabetes, lowering both basal and postprandial plasma glucose. Metformin hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. [1]

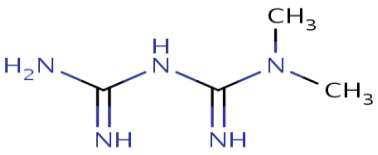

Fig.1. Structure of Metformin

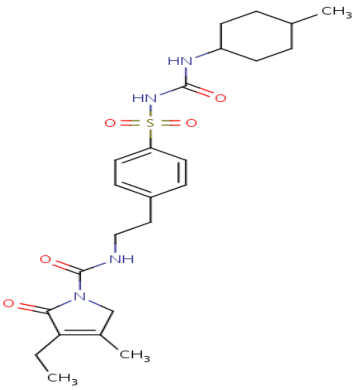

Fig.2. Structure of Glimepiride

The mechanism of action of Glimepiride {3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl) carbamoyl] amino} sulfonyl) phenyl] ethyl}-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide} in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin. [2]

The market survey revealed that Metformin and Glimepiride in combination are recently introduced in market as tablet dosage form. It is mainly indicated in diabetes mellitus. Literature survey revealed that Metformin and Glimepiride are official in U.S.P. and B.P. Although there are many methods reported for estimation of these drugs singly. However, no method is so far reported for simultaneous estimation of these drugs in combined dosage form. The present work was undertaken with an objective to develop an accurate, simple, precise and reliable method for estimation of these two drugs in their combined dosage form.[5-10]

MATERIALS AND METHODS

Chemicals and Reagents: Pure Metformin and Glimepiride were obtained as gift samples from Glenmark Pharmaceutical Ltd. Sinnar, Dist. Nasik. HPLC grade Acetonitrile and Methanol from Merck, AR grade ortho phosphoric acid from Qualigens, and AR grade Potassium Dihydrogen Orthophosphate from Emplura, UV-1100 Shimadzu, HPLC, Sonicator from PCI.

Instrumentation and Optimized Chromatographic Conditions: Separation was performed with Waters HPLC equipped with a pump 2695, auto sampler and UV detector. HPLC workstation software was applied for data collecting and processing. The separation was achieved on Hypersil Gold C18 column (250 mm x 4.6 mm, 5 µ). The mobile phase consisted of Methanol and Potassium Dihydrogen Phosphate buffer (pH adjusted to 3.0 with Phosphoric acid). The gradient programme is used for separation. The flow rate was 0.8 mL/min and UV detection was performed at 241 nm. The injection volume was 20 µL and all the experiments were performed at temperature 30^0C. The run time was set at 10.20 min. Mixture of Methanol and Water in the ratio of 10:30% v/v is used as a solvent which is sonicated to degas. HPLC grade water was obtained from a Milli – Q water purification system.

Gradient Program

Table no.1: Mobile Phase Gradient Program

Time (min)	Mobile Phase A	Mobile phase B
0	50	50
2	80	20
4	70	30
6	80	20
8	60	40

Solutions and Sample Preparation

Preparation of Standard Stock Solution: Standard stock solution of Metformin and Glimepiride was prepared by dissolving 100 mg of Metformin and 10 mg of Glimepiride respectively in 100 mL mobile phase. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Glimepiride respectively.

Preparation of Test Solution: Test stock solution of Metformin and Glimepiride was prepared by dissolving about 2.0 g of test sample (Brand Name: GLIMY M2) (which is

equivalent to 100 mg Metformin and 10 mg of Glimepiride) into 100 ml volumetric flask. The solutions are sonicated to dissolve the drugs. Further these solutions were diluted to prepare concentrations of 500 µg/ml and 2 µg/ml of Metformin and Glimepiride respectively.

Preparation of Potassium Dihydrogen Phosphate Buffer: Weighed about 272.1 mg of Potassium Dihydrogen phosphate and dissolved in 100 ml of water and pH was adjusted to 3.0 with Phosphoric acid and then filtered through 0.45 µ nylon membrane filter.

Method Validation

The following parameters were considered for the analytical method validation.

1. Similarity factor
2. System Suitability
3. Linearity
4. Precision
5. Accuracy
6. Range
7. Robustness
8. Limit of Detection
9. Limit of Quantitation[3-4]

1) Similarity Factor

Two standard Solutions were prepared by standard procedure, and result obtained by using HPLC.

$$\text{Similarity factor} = \frac{\text{Weight of standard 1}}{\text{Weight of standard 2}} \times \frac{\text{Area of Standard 2}}{\text{Area of Standard 1}}$$

Limit :(0.98 – 1.02)

It is observed that the results were within limit, so the similarity factor passed.

Table No. 2: Result for Similarity factor of Metformin and Glimepiride.

Component Name	1st Standard		2nd Standard		Observation
	Weight	Area	weight	Area	
Metformin	500	19569382	500	19545658	0.998
Glimepiride	2	28590	2	28343	0.991

2) System Suitability

System suitability was determined by three replicate injections of the system suitability solution. The acceptance criteria's are relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0. The system suitability results obtained for Metformin and Glimepiride is summarized in Table No.3 and 4. The typical Chromatogram of Metformin and Glimepiride was shown in Figure 2. It was observed that the method complies with system suitability parameters. Hence, it can be concluded that the system suitability parameter meets the requirement of method validation.

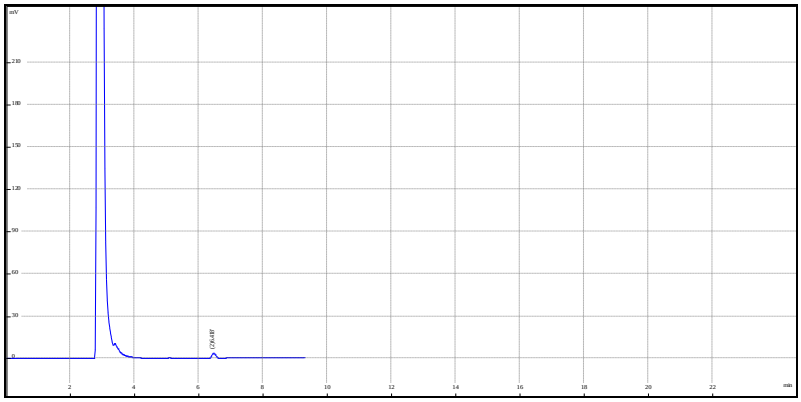

Fig. no. 2: Chromatogram of system suitability Metformin and Glimepiride

Table No.3: Result for system suitability test of Metformin.

Sample Name	Retention Time (min)	Area	Plate Count	Tailing
Standard 1	2.888	19569382	6679	1.22
Standard 2	2.458	19558642	7123	1.21
Standard 3	2.785	19554532	6123	1.17
Mean		19560852		
S.D		7667.705		
% RSD		0.03919924		

Table No.4: Result for system suitability test of Glimepiride.

Sample Name	Retention Time(min)	Area	Plate Count	Tailing
Standard 1	7.15	28590	11422	1.14
Standard 2	7.213	27495	12193	1.17
Standard 3	7.412	28465	11323	1.19
Mean		84550		
S.D		599.381626		
% RSD		0.7089079		

3) Linearity

Linearity was performed by diluting standard stock solution. The final concentration of Metformin from 250 to 1250 µg/ml and Glimepiride from 1 to5 µg/ml were used. 20 µl of each sample injected in duplicate for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The method was found to be linear for Metformin and Glimepiride. The correlation coefficient of the plot for Metformin and Glimepiride were found to be 0.9675and 0.9923 respectively.

Table No. 5: Result for Linearity of Metformin and Glimepiride.

Sr. No.	Concentration range for Metformin (ppm)	Peak Area	Concentration range for Glimepiride (ppm)	Peak Area
1	250	19569382	1	28590
2	500	25684461	2	135452
3	750	29801008	3	219765
4	1000	32398390	4	288402
5	1250	35378099	5	363894
Slope		15333		82356
Correlation coefficient		0.9675		0.9923

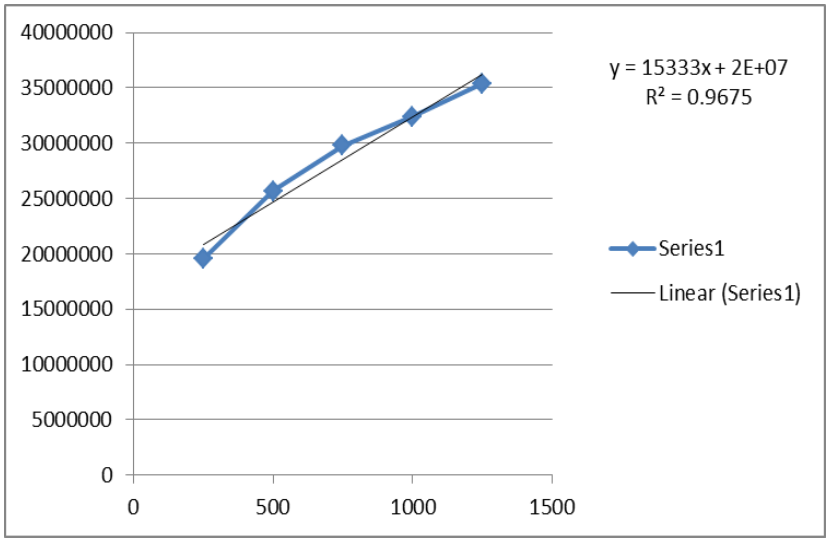

Fig.No.3: Linearity plot of Metformin

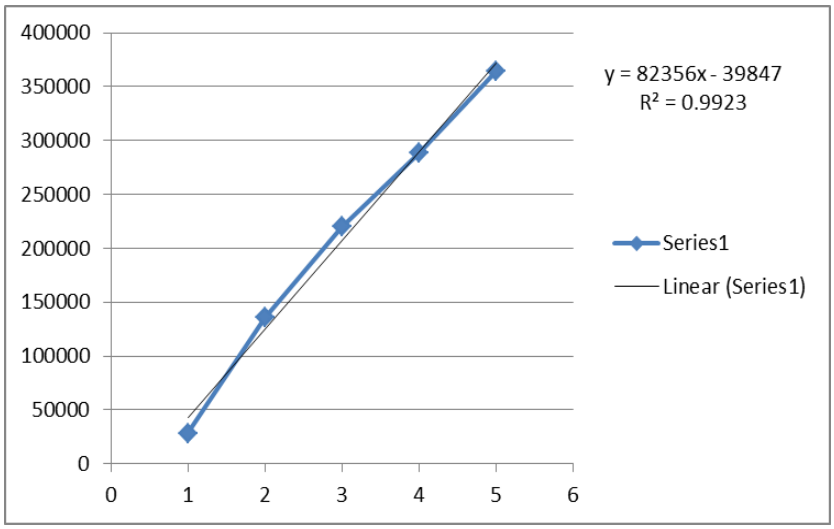

Fig.No.4: Linearity plot of Glimepiride

4) Precision

In precision, a homogenous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results for single aliquots. To each six 100 ml flask, 2 gm sample of Metformin and Glimepiride were transferred and %RSD of assay was calculated. Acceptance Criteria is % RSD for the six determinations shall be NMT 2.0. The % RSD for Metformin and Glimepiride were found to be 1.92 and 1.89 respectively which indicates that method is precise.

Table No. 6: Results of Method Precision of Metformin and Glimepiride.

Injection	Metformin		Glimepiride	
S. NO	Retention Time	Average	Retention Time	Average
	(min)	Area	(min)	Area
1	**2.88**	25684461	6.384	135452
2	2.953	24498785	6.456	140085
3	2.808	24968458	6.125	135685
4	2.645	25635483	6.513	134526
5	2.124	24854249	6.173	134248
6	2.487	25489755	6.993	132456
Mean		25188531.8		135408.667
S.D.		484226.12		2560.89123
%RSD		1.92240708		1.8912314

5) Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was assessed by recovery studies of Metformin and Glimepiride in the dosage form at three concentration levels. Spiked known quantity of Metformin and Glimepiride Standard at 50%, 100% and 150% level into the placebo.

Analyses of samples were done in triplicate for each level. From the results, % recovery was calculated. Acceptance criteria's are the % RSD for % recovery for all spike levels shall be NMT 2.0, the % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount. The mean recoveries of Metformin and Glimepiride were found to be 101.74% and 100.47% respectively that shows there is no interference from excipients and the mean % RSD were found to be 0.867 and 1.3 respectively which indicate that method is accurate.

Table no. 7: Result for Accuracy of Metformin.

Sr. No.	Metformin	Amount Recoverd (µg)	%Recovery	Avarage Recovery	% RSD
1	50%	194.005 193.24 193.99	100.1 99.7 100.7	101.1666	0.5
2	100%	387.45 388.45 388.76	101.8 102.57 102.76	102.37	0.5
3	150%	582.45 582.88 582.006	100.59 103.62 100.92	101.71	1.6
			Mean	101.74	0.867

Table no. 8: Result for Accuracy of Glimepiride.

Sr. No.	Glimepiride	Amount Recoverd (µg)	%Recovery	Avarage Recovery	% RSD
1	50%	0.7652 0.7762 0.7775	98.03 100.3 100.7	99.68	1.4
2	100%	1.558 1.575 1.45	100.51 102.71 99.1	100.77	1.8
3	150%	2.456 2.475 2.4121	100.8 101.1 101.0	100.96	0.15
			Mean	100.47	

6) Range

Range inferred from the data of linearity and recovery experiments. The range for Metformin and Glimepiride were found to be 250-1250 ppm and 1-5 ppm respectively.

7) **Robustness**

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The robustness of the developed method was established in differently and deliberately by varied chromatographic conditions e.g. Change in flow rate and pH. The robustness was checked by changing the flow rate 0.72 and 0.88 ml/min and the mobile phase pH 2.8 and 3.2 for Metformin and Glimepiride respectively. The corresponding data for Metformin and Glimepiride was shown in Table No 9 & 10 respectively.

Table-9: Metformin % RSD by change in Mobile phase flow rates, by change in mobile phase Ph.

By Change In	Area	%RSD	Limit
Flow rate of mobile phase 0.72mL/min	25465853	1.1072	
Flow rate of mobile phase 0.88mL/min	24568796	0.5977	NMT 2.0
Mobile phase pH at 2.8	25698588	0.5718	
Mobile phase pH at 3.2	24857985	0.3367	NMT 2.0

Table-10: Glimepiride % RSD by change in Mobile phase flow rates, by change in mobile phase pH.

By Change In	Area	%RSD	Limit
Flow rate of mobile phase 0.72mL/min	134526	0.5652	
Flow rate of mobile phase 0.88mL/min	132589	0.673	NMT 2.0
Mobile phase pH at 2.8	125645	1.2103	
Mobile phase pH at 3.2	135425	1.2659	NMT 2.0

8) Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOD = 3.3XSD/S

Where, SD= Standard deviation

S= Slope

Table no. 11: Result for LOD of Metformin and Glimepiride.

Content	LOD (µg/ml)
Metformin	0.41688
Glimepiride	1.026

9) Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. A typical signal-to-noise ratio is 10:1. It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

LOQ = 10 X S.D./S

Where, SD= Standard deviation

S= Slope

Table no. 12: Result for LOQ of Metformin and Glimepiride.

Content	LOQ (µg/ml)
Metformin	1.2632
Glimepiride	3.109

RESULT AND DISCUSSION

From the optimised method and above observations the present work is successfully carried out for the development and validation of Metformin and Glimepiride in tablet dosage form and found to be suitable for the simultaneous estimation by using RP-HPLC method. The developed method was validated as per ICH guideline for Accuracy, Precision, Linearity, Range, LOD, LOQ, Robustness etc.

CONCLUSION

The present work involved the development of accurate, precise, simple and suitable RP-HPLC method for estimation of the drugs in multicomponent formulations. Hence the present study was undertaken with an objective of developing suitable, sensitive and simple analytical method like RP-HPLC method for simultaneous estimation of both drugs in their combined dosage form. The proposed method is found to be accurate, precise, linear, specific and robust.

REFERENCES

1. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, 2007; 1382.
2. USP-NF, Validation of Compendial Procedures, General Chapters, 2010; 1: 734-36.
3. ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, FDA, 1995; 1: 60.

4. ICH Guidelines Q2B validation of analytical procedure: Methodology International Conference on Harmonization of Technical Requirement for Registration of Pharmaceutical's for Human use Geneva, Switzerland, 1996.
5. US FDA, General principles of validation, Rockville, MD, Center for Drug Evaluation and Research (CDER), May 1987.. US FDA, Guidelines for submitting samples and analytical data for method validation, Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services, Feb. 1987.
6. S. AbuRuz,J. Millershipb, J. McElnay**.** The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma, Journal of Chromatography B, 2005; 817:277–286.
7. S. Rao, Polagania, B. N. Rao, R Gajulab,G. Venkateswarlu. developed simultaneous determination of atorvastatin,metformin and glimepiride in human plasma by LC–MS/MS and its application to a human pharmacokinetic study, 2008.
8. J. Yao, Y.Q. Shi, Z.-Rong Li, S.H. Jin. Studied development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs, March, 2007.
9. P. Kovarıkova, J. Klimes, J. Dohnal, L.Tisovska**.** HPLC study of glimepiride under hydrolytic stress conditions, 2009.
10. F. Al-Rimawi**.** Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV, 2010.