Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 2| Issue 4 |OCT-DEC |2012|277-283

Research Article
Pharmaceutical Sciences

A NEW SIMPLE RP – HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND GLICLAZIDE TABLET DOSAGE FORM

EDIGA SASI KIRAN GOUD[*1], V.KRISHNA REDDY [1], CHANDRA K SEKHAR[2]*

*[*1]Research Scholar, Department of Chemistry, Sri Krishna Devaraya University, Anantapur, India*

[1] Department of Chemistry, Sri Krishna Devaraya University, Anantapur, India

[2]Quality Control, Bio-Leo Analytical Labs India Pvt Ltd, Prasanthinagar, Kukatpally, Hyderabad, India.

*Corresponding Author Email: sasikiranediga@gmail.com

ABSTRACT

A new simple fast economical reverse phase high performance liquid chromatographic method was developed for the determination of Metformin Hcl [MFH] and Gliclazide [GZ] in bulk and dosage form. The separation was eluted on a RP-Select B C_{18} column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of phosphate buffer and acetonitrile in a ratio of 60:40 v/v at a flow rate of 1.0ml/min. The detection was made at 261 nm. The retention times were 3.26min for [MFH] and 6.07min for [GZ]. Calibration curve was linear over the concentration range of 125-750 µg/ml for (MFH) and 20 to 120 µg/ml for [GZ]. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

KEYWORDS

Method development and validation, Gliclazide, Tablets, C_{18} column, RP-HPLC.

1. INTORDUCTION

Metformin HCl is 1, 1 dimethyl guanide hcl and Gliclazide is 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[4-methylphenyl) sulphonyl] urea[1]. MFH and GZ are official in Indian Pharmacopoeia [1].but there is no official method for the combination. Both drugs in combination of tablet dosage form in the ratio of 500:80 mg MFH: GZ. As per literature survey many methods have been reported the estimation of MFH and GZ individually or in combination with some other drugs[2-6]. With this present proposed method both MFH and GZ estimates simple and economical in tablet formulation.

2. MATERIAL AND METHODS

2.1 Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a RP-Select B C_{18} column (250 mm x 4.6 mm ; 5µ) and 2489 UV-Visible detector, autoinjector, autosampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile, water was purchased from Ranbaxy, India, and Potassium dihydrogen phosphate, ortho phosphoric acid AR grade were purchased from SD Fine Chem Mumbai, India were used in the study.

2.2 Drug Samples

The reference samples were obtained from M/s. Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the formulation samples were purchased from local market.

2.3 Mobile phase

A mixture of phosphate buffer pH 6.6 and acetonitrile in the ratio 60:40 v/v was filtered through 0.45µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics india, Mumbai before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 30°C and the detection of the drug was carried out at 261nm.

2.4 Preparation of stock and working standard solution of Metformin and Gliclazide

About 500mg of Metformin HCl and 80 mg of Gliclazide was weighed accurately on Sartorius semi micro balance model-CPA225D and transfers in to 100ml volumetric flask the solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution of 500 µg/ml MFH AND 80 µg/ml GZ.

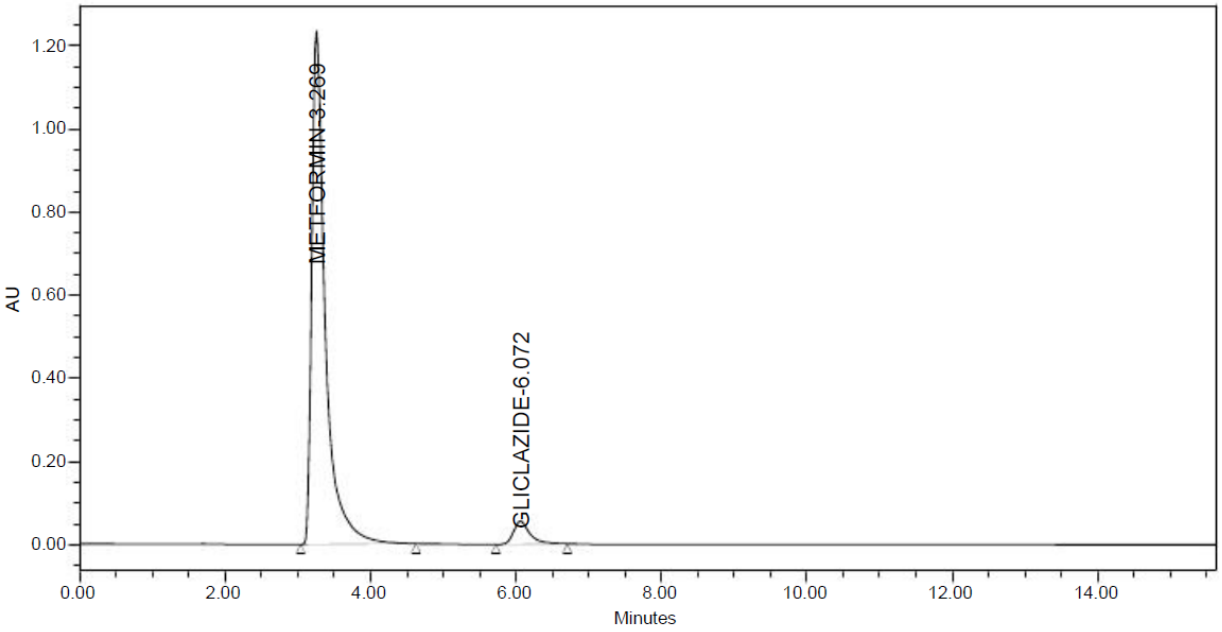
2.5 Sample Preparation

Weighed accurately previously weighed and crushed 20 tablets powder equivalent to 500mgof MFH and 80mg of GZ transferred to 100ml volumetric flask make up to the mark with mobile phase sonicated and filtered through whattsman filter paper. Further dilute 10 ml to 100 ml with mobile phase.

2.6 Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working standard dilution of MFH and GZ in the range of 125-750 µg/ml and 20 to 120 µg/ml respectively. 20µl quantity of the dilution was injected each time in to the column. The drug in the elutes was monitored at 261 nm and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. This regression equation was later used to estimate the amount of MFH and GZ in pharmaceutical dosage form. A representative chromatogram for the separation of MFH and GZ presented in **Fig.1**

Figure 1: Chromatogram of MFH (500mcg/ml) & GZ (80mcg/ml).



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	METFORMIN	3.269	15967825	1234745	94.90		1.66	3835
2	GLICLAZIDE	6.072	858431	55375	5.10	8.37	1.46	3951

2.7 System Suitability Testing

The system suitability parameters such as Theoretical plates, tailing Factor and resolution were performed to verify the system is adequate for the analysis to be performed. The results are performed in **Table 1**.

Table 1: System suitability parameters

Parameters	Metformin	Gliclazide
Tailing Factor	1.66	1.46
Theoretical plates	3835	3951
Resolution	--	8.37
LOD(µg/ml)	2.3138	0.4368
LOQ(µg/ml)	7.0116	1.3236

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of MFH and GZ in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_{18} stationary phase. A mixture of Phosphate buffer pH 6.6: acetonitrile in a proportion of 60:40 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for MFH was 3.26 ± 0.1 min and for GZ was 6.07±0.1min. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of MFH and GZ were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 0.999) was observed for MFH and (r^2=0.999) was observed for GZ. The regression concentration and areas are given in **Table 2**. And the regression characters are given in **Fig 2&3**. When test solutions were analyzed by the proposed method for finding out intra and inter-day variation, low co-efficient of variation was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

Table 2: Calibration data of the proposed method

Metformin HCl		Gliclazide	
Conc (mcg/ml)	Mean Area	Conc (mcg/ml)	Mean Area
125	3992846	20	212603
250	7986848	40	429239
375	11965648	60	645098
500	15967825	80	858431
625	19968784	100	1074235
750	23566128	120	1260649

Figure 2: Linearity of Metformin

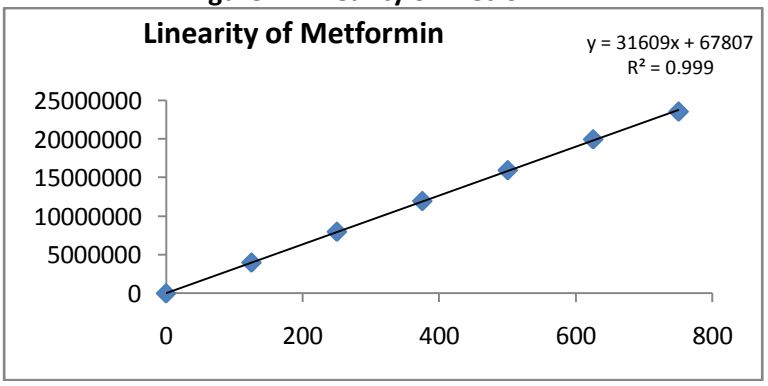
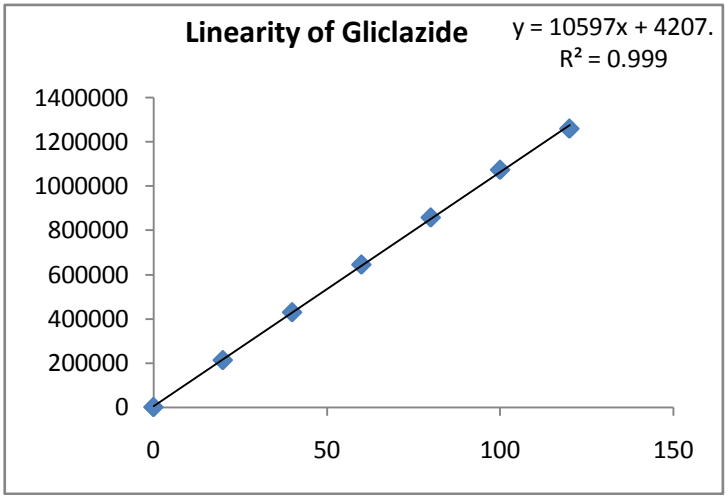


Figure 3 : Linearity of Gliclazide



High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The drug content in tablets was quantified using the proposed analytical method are given in **Table 3**.

Table 3: Accuracy data (Triplicate values at 50, 100 &150 percent levels)

	Amount taken (µg)	Amount found (µg)	Percent Recovery	Percentage of mean recovery
Metformin	250	250.12	100.48	100.48
	500	499.56	99.91	99.91
	750	749.1	99.88	99.88
Gliclazide	40	40.06	100.15	100.15
	80	80.23	100.29	100.29
	120	119.8	99.83	99.83

***Each value is a mean of three readings**

The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in **Table 4**. The lowest value of LOD and LOQ as obtained by the proposed method by calculated using 3.3xstdev/slope for LOD and 10xstdev/slop for LOQ. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit system suitability parameters were studied with six replicates standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plate are in the acceptable limits.

Table 4: Robustness Study

Drug name	Variations	Chromatographic parameters				
		Retention time	Area	Height	Theoretical plates	Asymmetry
Metformin	Buffer change ± 5% 55% v/v 60%v/v 65% v/v	 2.621 3.268 3.918	 15856459 15956543 16015254	 1229856 1234654 1245645	 3889 3845 3824	1.64 1.65 1.67
	Change in flow rate at ±0.1ml/min 1.flow rate at 0.90ml/min 2.flow rate at 1.0ml/min 3.flow rate at 1.10ml/min	 3.635 3.262 2.935	 15689456 15866453 15989562	 1231025 1234056 1238456	 3956 3902 3859	 1.65 1.62 1.60
Gliclazide	Buffer change ± 5% 55% v/v 60%v/v 65% v/v	 5.454 6.072 6.787	 842567 848621 850415	 55126 55468 55897	 3999 3958 3921	1.41 1.45 1.49
	Change in flow rate at ±0.10ml/min 1.flow rate at 0.90ml/min 2.flow rate at 1.0ml/min 3.flow rate at 1.10ml/min	 6.798 6.074 5.469	 841243 845621 850659	 54985 55654 56054	 3898 3968 3989	 1.46 1.44 1.42

The system precision was established by six replicate injections of the standard solution containing analytes of interest. The values of relative standard deviation were found within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found within the limit, indicating the injection repeatability of the method. The results were presented in **Table 5&6**.

The diluted preparations of marketed tablets were injected in duplicate and the results were calculated and presented in **Table 7**.

Table 5: Precision Study

Metformin			Gliclazide	
S.No.	**RT**	**Area**	**RT**	**Area**
1	3.265	15902160	6.073	850645
2	3.266	15942541	6.072	854266
3	3.264	15956425	6.07	852345
4	3.265	15953060	6.072	852339
5	3.267	15964680	6.072	854327
6	3.267	15936895	6.073	853380
Avg	3.265667	15942627	6.072	852883.7
Stdev	0.001211	22163.07	0.001095	1402.773
%RSD	**0.04**	**0.14**	**0.02**	**0.16**

Table 6 : Method Precision study

Metformin			Gliclazide	
S.No.	**RT**	**Area**	**RT**	**Area**
1	3.269	15921346	6.072	858648
2	3.267	15903564	6.07	845986
3	3.265	15932568	6.073	846980
4	3.268	15861458	6.071	850126
5	3.266	15892542	6.071	851016
6	3.267	15946459	6.073	854274
avg	3.267	15909656	6.071667	812073
stdev	0.001414	30558.55	0.001211	4713.786
%RSD	**0.04**	**0.19**	**0.02**	**0.58**

Table 7: Assay Results

Drug	Amount present/tablet	% of Assay
Metformin	499.2 mg	99.84
Gliclazide	79.94 mg	99.93

The specificity of the HPLC method was determined by the complete separation of MFH and GZ. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation at 80°C. The method does not permit detection of degradation product for MFH and GZ.

Hence it can be concluded that the proposed HPLC method is evident very fast and economical compared to the literature available.

ACKNOWLEDGEMENT

The authors are thankful to M/s Bio-Leo Analytical Labs India (P) Ltd, Hyderabad for providing standards of Metformin and Gliclazide and facilities. The authors are also thankful to Department of Chemistry, Sri Krishna Devaraya University, Anantapur, India for encouragement.

REFERENCES

1. Indian Pharmacopoeia. The Indian Pharmacopoeia Commision. Vol.II, Ghaziabad 2010; 1657-1658 & 1416-1417.
2. Narasimha swamy lakka, Nishant Goswami, solubility and dissolution profile studies of gliclazide in pharmaceutical formulations by RP-HPLC IRJP, 2012, 3(6) : 126-129.
3. T.Ramanji Reddy,D.Dachina Moorthi, K.B.Chandra sekhar. Development of RP-HPLC method for Metformin and Repaglinide in rat plasma, IJPPS, 2012, 4(2) : 311-313.
4. Kanij Fatema, Md.zakir Rahman, Tasnuva Haque, Mohammad abul kalam azad, and Md.selim reza, Development and validation of a simple method for simultaneous estimation of Metformin hydrochloride and gliclazide in tablets by using RP-HPLC, Dhaka Univ J.Pharm Sci , 2010, 9(2) : 83-89.
5. M.Gandhimathi, K.Anandakumar, A.Cheriyan and T.K.Ravi, Simultaneous estimation of Metformin and Gliclazide in tablets using RP-HPLC, IJPS, 2003, 530-531.
6. Dalia rashad El-Wasseef, Simultaneous determination of Metformin, Netaglinide and Gliclazide in pharmaceutical preparations using micellar chromatography, International journal of Biomedical science, 2012, 8(2) : 144-151.

***Corresponding Author:**
EDIGA SASI KIRAN GOUD
Department of Chemistry,
Sri Krishna Devaraya University,
Anantapur, Andhra Pradesh, India,
Email: sasikiranediga@gmail.com