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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND GLICLAZIDE IN BULK AND COMBINED DOASAGE FORM

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ABSTRACT

Objective: The objective of this study was to develop a simple, efficient, specific, precise and accurate Reverse phase High Performance liquid chromatographic method for the simultaneous estimation of Metformin Hydrochloride (MET) and Gliclazide (GLZ) in bulk drugs and combined dosage form.

Methods: The separation method was carried out using reverse phase C18 column; Inertsil ODS – 3V (250 mm x 4.6 mm x 5µm). The mobile phase used was a mixture of Phosphate buffer (1.625 gm of Potassium Di Hydrogen Ortho Phosphate and 0.3 gm of Di Potassium Hydrogen Ortho Phosphate in 550 ml water); pH 4.8 and Acetonitrile in the ratio of 55:45 (v/v) at isocratic mode and eluents were monitored at 234 nm using UV-Visible spectrophotometer as the detector.

Results: With the optimized method, the retention times of MET and GLZ were found to be 2.420 and 4.270 respectively with theoretical plate count and asymmetry as per the ICH limits. The method has shown a good linearity in the concentration range of 60-140µg/ml for Metformin and 3-8µg/ml for Gliclazide with Regression coefficient (R^2) of 0.9956 and 0.9981. The percentage assays were found to be 99.66% and 100.56% respectively for MET and GLZ. Limit of detection and Limit of quantitation values were found to be 1.51µg/ml and 4.59µg/ml for Metformin Hydrochloride, 0.02µg/ml and 0.07µg/ml for Gliclazide respectively. The method was found to be accurate (with percentage mean recoveries 100.141% for Metformin HCl and 100.223% for Gliclazide), precise, robust, stable and specific.

Conclusion: The proposed method was validated in accordance with ICH guidelines and hence can be successfully applied to the simultaneous estimation of MET and GLZ in tablet formulations.

Keywords: Metformin, Gliclazide, Simultaneous estimation, Reverse phase HPLC, Validation.

INTRODUCTION

Metformin HCl is an oral hypoglycaemic antidiabetic drug which comes under the class Biguanides. It is chemically 1, 1-Dimethyl biguanide mono hydrochloride. It is the first line drug for treating Type-2 Diabetes mellitus. Metformin acts by suppressing hepatic gluconeogenesis and glucose output from liver. It is official in USP-2010, BP-2012, and IP-2007 [1, 2, and 3]. Gliclazide is an oral hypoglycaemic antidiabetic drug which comes under the class second generation Sulpfonyl ureas. It is chemically 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl) sulfonyl] urea. Gliclazide acts on Sulfonyl Urea Receptors (SUR1) receptors on the pancreatic β cell membrane; causes depolarization by reducing conductance of ATP sensitive K^+ channels and hence provoke release of insulin from pancreas. It is official in BP-2012 [4].

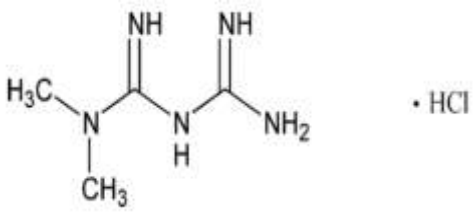

Fig. 1: Chemical structure of Metformin Hydrochloride

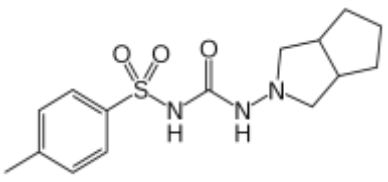

Fig.2: Chemical structure of Gliclazide

For many patients with Type 2 Diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control. In such cases a combination of Metformin Hydrochloride and one of the Sulfonyl ureas is used. The fixed dose combination of Gliclazide (30 mg, 80 mg) and Metformin once or twice daily with meals to a maximum of 4 tablets per day (depending upon the glycaemic control) shows significant efficacy in improving the glycaemic control in Type 2 Diabetes [5].

For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method. Many methods have been reported in the literature for the estimation of Metformin Hydrochloride and Gliclazide individually and in combination [6-13]. However, there is no simple method with shorter run times has been reported for the simultaneous estimation of Metformin Hydrochloride with Gliclazide. The present investigation was aimed at developing a fully validated RP-HPLC method for the simultaneous estimation of Metformin HCl and Gliclazide in bulk and pharmaceutical combined dosage forms that is more economical, simple, precise and accurate than the previous methods.

MATERIALS AND METHODS

Instruments used

The chromatographic determination was performed on Schimadzu (Columbia, MD) HPLC instrument (LC 20AT pump) equipped with UV-Visible detector and Spinchrom CFR software. The different columns were used during method trials such as Inertsil ODS-3V C18 column (250mm×4.6mm, 5µ particle size), Booston C18 (150mmX4.6mm, 5µ), Zodiac C18 (250mm×4.6mm, 5µ), etc. Other equipment used were Schimadzu electronic balance AY220, Global Digital pH meter DPH 500, ultrasonic cleaner (Frontline FS 4, Mumbai, India).

Chemicals and Reagents

Standard gift samples of Metformin Hydrochloride and Gliclazide were obtained from Aurobindo Pharma Hyderabad, India. Acetonitrile, Methanol and water were purchased from Rankem India. Potassium Di Hydrogen Phosphate and Di Potassium Hydrogen Phosphate were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade. The combined dosage formulation selected was RECLIMET OD 30 (Gliclazide Modified Release; not less than 27.0 and not more than 33.0 mg and Metformin Extended Release; not less than 450.0 mg and not more than 550.0 mg) tablets manufactured by Dr. Reddy's Laboratories, Hyderabad, India.

Determination of working detection wavelength

Methanol is selected is selected as the solvent after the solubility studies of both the drugs. The wavelength of maximum absorption (λ_{max}) is determined by scanning 100µg/ml solution of MET and GLZ in solvent using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve has shown a characteristic absorption maxima at 237 nm for Metformin HCl, 228 nm for Gliclazide and at 234 nm same absorbance of 0.524 for both the drugs [Fig. 3] i.e., isobestic point. Thus 234 nm was selected as working detection wavelength for the simultaneous estimation of Metformin HCl and Gliclazide.

Method Development

Initially many method trials were performed using different mobile phases, different columns, and varying chromatographic conditions in attempt to obtain the best separation and resolution between Metformin HCl and Gliclazide**.** The finalized method involved the use of a mixture of Phosphate buffer (1.625 gm of Potassium Di Hydrogen Ortho Phosphate and 0.3 gm of Di Potassium Hydrogen Ortho Phosphate in 550 ml water); pH 4.8 and Acetonitrile in the ratio of 55:45 (v/v) as the mobile phase at isocratic mode and eluents were monitored at 234 nm using UV-Visible spectrophotometer as the detector allowing the adequate separation of both the compounds using the column Inertsil ODS - 3V C18 (250 mm x 4.6 mm x 5µm particle size) at a flow rate of 1.0 ml/min and column temperature 27°C. Sample injection volume was 0.02 ml. Typical chromatograms showing the separation of Metformin HCl and Gliclazide in both standard and sample preparations are shown in Fig. 4 and Fig. 5 and followed by the results in Table 1.

Preparation of Standard solution

Accurately weighed standards of Metformin HCl (50 mg) and Gliclazide (3 mg) were transferred to a 50 ml volumetric flask, dissolved and to the mark with methanol to obtain the standard stock solution of 1000µg/ml MET and 60µg/ml GLZ. From the stock solution an aliquot of 5 ml solution is transferred to 50 ml volumetric flask and diluted to mark with methanol to obtain the working standard solution of 100µg/ml MET and 6µg/ml GLZ.

Preparation of sample solution

Twenty tablets were weighed (average weight 1140 mg) and powdered using mortar and pestle. The quantity of powder equivalent to 500 mg of MET and 30 mg of GLZ i.e., 228 mg was transferred to a 100ml volumetric flask. The content was dissolved in 50 ml methanol, sonicated for 20 minutes to dissolve the drug as completely as possible. The solution was then filtered through 0.45µ Nylon disposable Syringe filter. The volume was then made to mark with methanol. This is standard stock solution. From the standard stock solution, an aliquot of 5ml solution was transferred to a 50 ml volumetric flask and diluted to mark with methanol to obtain 100µg/ml MET and 6µg/ml GLZ.

Assay Method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected five times and the chromatograms were recorded. This procedure was repeated for the sample solution too. The averages of peak areas were determined for standard and sample solutions. The concentration of the drug was calculated using the following formula;

$$\%\text{Assay} = \frac{TA}{SA} \times \frac{SW}{SD} \times \frac{TD}{TW} \times \frac{P}{100} \times \frac{AV}{LC} \times 100$$

Where.,

TA & SA = Average peak area due to sample and standard preparations

SW & TW = Weight of standard and drug sample taken in mg

SD & TD = Dilution of standard and sample preparations

P = Percentage purity of drug standard used.

AV = Average weight of tablets in tablets in mg

LC = Label claim of the drug

The results are shown in Table 2.

Method Validation [14]

System suitability:

The suitability of the chromatographic system was tested before each stage of validation. Six replicates of working standard solution are injected and the chromatograms are recorded. The % Relative Standard Deviation (%RSD) of retention times, asymmetry, theoretical plate count and of peak areas (should not be more than 2%) was determined as shown in Table 3.

Linearity:

The linearity was determined by preparing and injecting the sample solutions in the concentration range of 60-140µg/ml for MET and 3.6-8.4µg/ml for GLZ. These dilutions were prepared by transferring 0.6, 0.8, 1.0, 1.2, 1.4 ml of sample stock solution in to 10 ml volumetric flasks and diluted to mark with methanol. The calibration plots of MET and GLZ are shown in Fig. 6 and Fig. 7 and followed by results in Table 4. The Correlation coefficient was calculated and should not be less than 0.99.

Sensitivity (LOD and LOQ)

The sensitivity of the method for simultaneous estimation of MET and GLZ is estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ are calculated based on the standard deviation of the response and the slope. LOD and LOQ are estimated using the formula;

$$\text{LOD} = 3.3 \times \frac{\sigma}{S} \qquad \text{LOQ} = 10 \times \frac{\sigma}{S}$$

σ = Standard deviation of response

S = Slope of the calibration curve

The results were shown in Table 4.

Accuracy

To the pre-analyzed sample solution, a known amount of standard solution (usually 5-20%) was spiked at three different levels (80%, 100%, and 120%). These solutions were injected in three replicates and Percentage Mean Recoveries are determined for MET and GLZ which should lie between 98-102%. The results are described in Table 5 and Table 6. It is calculated as follows;

$$\text{Obtained concentration} = \frac{\text{Average area of sample}}{\text{Spiked area of standard}} \times \text{conc. of linearity preparation}$$

$$\%\ \text{Recovery} = \frac{\text{Obtained concentration}}{\text{Actual concentration}} \times 100$$

Precision

The precision of the method (Intra-day variation) was determined by repeatedly injecting the sample solution (100µg/ml of Metformin HCl and 6µg/ml Gliclazide) six times. The retention

times and peak areas of six replicates are recorded (Table 7). The precision is expressed as the % RSD of Peak areas and it should not be more than 2%.

Specificity

Commonly used excipients (Starch, Microcrystalline Cellulose and Magnesium Stearate, lactose, etc.) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3, where complete separation of MET and GLZ is seen in presence of tablet excipients and diluent.

Robustness

The robustness of the method is determined under normal operating conditions different conditions such as change in flow rate and detection wave length. 20 µl of standard and sample solutions are injected by varying wavelength (232, 234, 236 nm) and flow rate (0.8 ml/min, 1.0 ml/min, 1.2 ml/min) and the chromatograms are recorded and changes in parameters are observed. The results are shown in Table 8.

RESULTS AND DISCUSSION

Determination of working detection wavelength

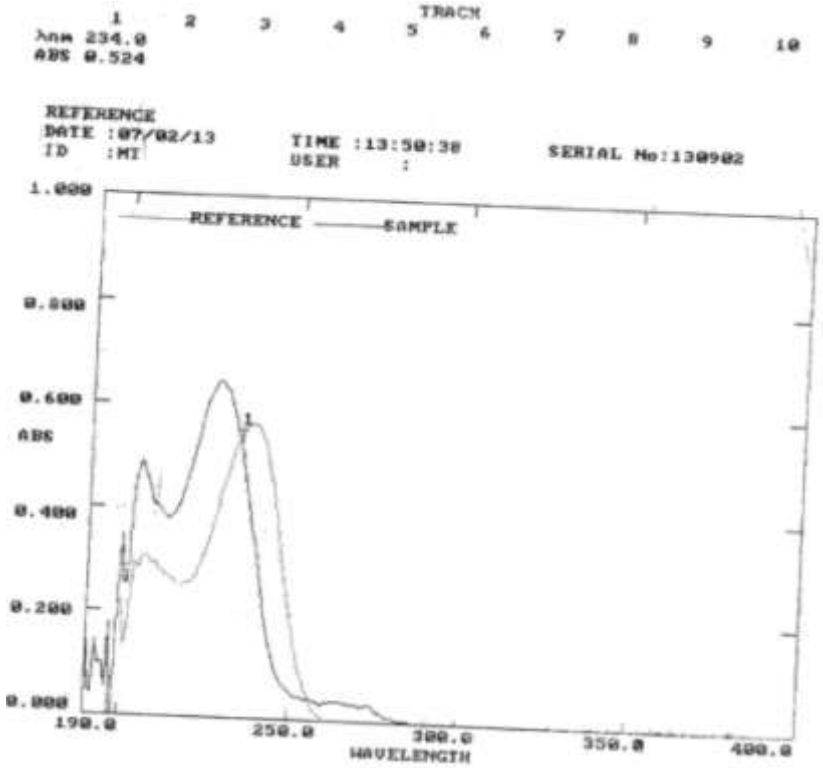

Fig. 3: UV spectrum showing the isobestic wavelength for MET and GLZ

Method Development

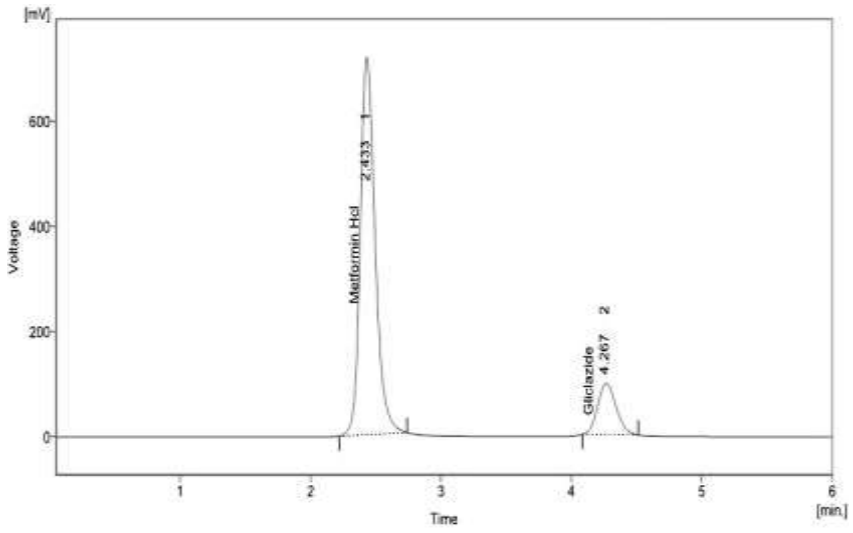

Fig. 4: A typical Chromatogram showing the peaks of MET and GLZ in standard solution for the developed method

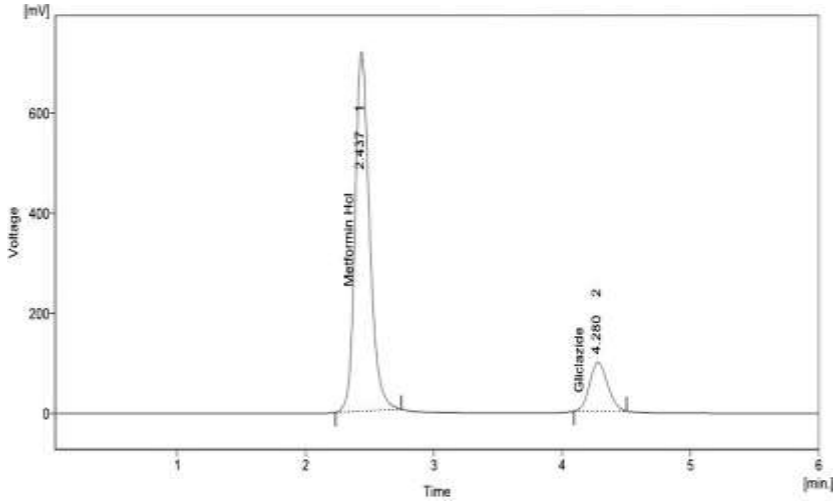

Fig. 5: A typical chromatogram showing the peaks of MET and GLZ in drug sample solution for the developed method

Table 1: Results for chromatograms of MET and GLZ in both standard and sample preparations

Drug	Retention Time(min)	Asymmetry	Area (mV. s)	Height (mV)	Efficiency (th.pl)	th.pl/ L	Resolution
Standard Preparation							
Metformin HCl	2.433	1.414	5673.243	717.311	2798	55960	
Gliclazide	4.267	1.289	953.186	97.140	6924	138480	12.108
Sample Preparation							
Metformin HCl	2.437	1.41	5673.471	718.125	2762	55249	
Gliclazide	4.280	1.105	943.128	97.064	6916	136328	12.142

The retention times of MET and GLZ were found to be 2.420 and 4.270 minutes respectively i.e., shorter elution times when compared with that in other studies with theoretical plate count and asymmetry as per the limits.

Assay

Table 2: Assay results of MET and GLZ

Parameter	Metformin HCl	Gliclazide
Actual amount (Label claim) in mg	500 mg	30 mg
Amount found in mg	498.32	30.168
% Purity	99.664%	100.56%

The percentage assays for Metformin HCl and Gliclazide were found to be 99.66% and 100.56 % respectively.

System Suitability

Table 3: System suitability results for MET and GLZ

Parameter	Metformin HCl	Gliclazide
Retention time (min)*	2.43 $\pm$ 0.24	4.278 $\pm$ 0.20
Peak area*	5670.341 $\pm$ 0.37	964.687 $\pm$ 0.77
Theoretical Plate count*	2796.67 $\pm$ 0.05	6925.167 $\pm$ 0.06
Asymmetry*	1.43 $\pm$ 0.38	1.2535 $\pm$ 0.90

System suitability studies were carried out on the method and %RSD values of retention times, peak areas, asymmetry, and theoretical plate count were found to be less than 2% for both MET and GLZ standards.

Linearity and Sensitivity

Table 4: Linearity and sensitivity data of MET and GLZ

	Metformin HCl		Gliclazide	
Injection	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
1	60	3269.742	3.6	509.077
2	80	4543.96	4.8	764.576
3	100	6258.159	6	1117.518
4	120	7514.107	7.2	1426.863
5	140	8678.012	8.4	1717.216
Regression Equation	y = 68.933x – 840.55		y = 256.55x – 432.23	
R^2	0.9956		0.9981	
LOD	1.51	104.59	0.02	6.27
LOQ	4.59	316.94	0.07	19.00

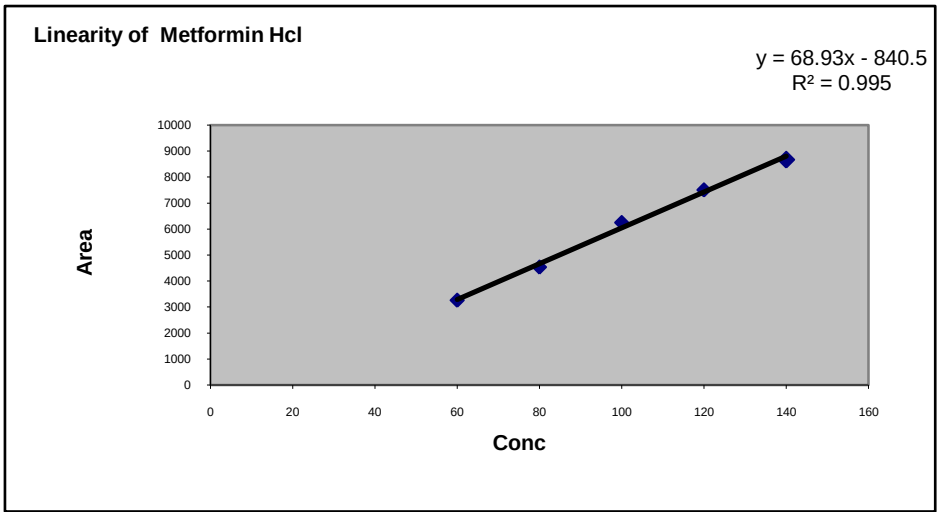

Fig. 6: Calibration curve of MET

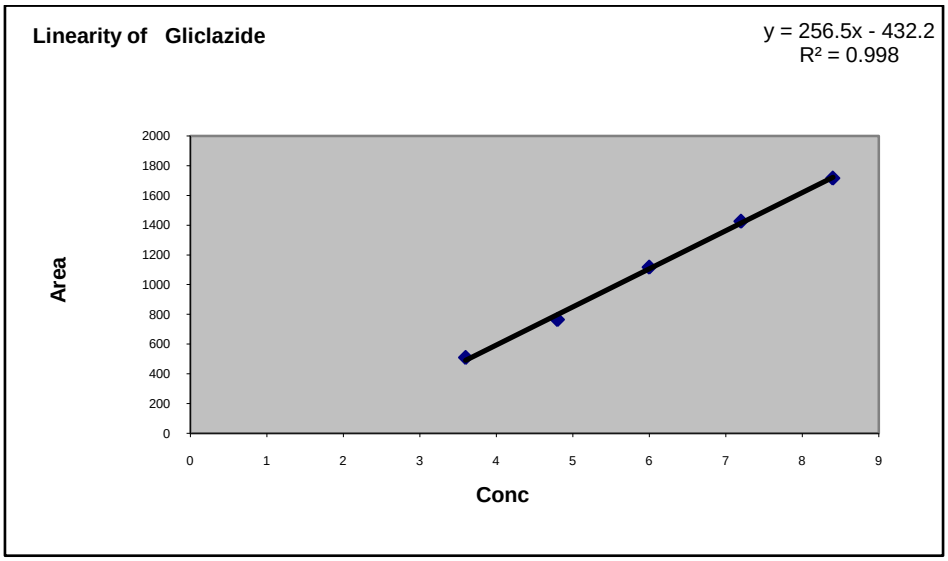

Fig. 7: Calibration curve of GLZ

A linear relationship was evaluated in the concentration range of 60-140µg/ml for MET and 3.6-8.4µg/ml for GLZ with Regression coefficients (R^2) 0.9956 and 0.9981 respectively. The LOD values were found to be 1.51µg/ml for MET and 0.02µg/ml for Gliclazide and LOQ 4.59µg/ml and 0.07µg/ml respectively.

Accuracy (Recovery studies)

Table 6: Recovery data of MET

Concentration of sample taken (µg/ml)	Conc. of standard spiked (µg/ml)	Spiking Standard area	Total conc. (µg/ml)	Total conc. found (µg/ml)	% Recovery Mean (n=3)
80	5	4543.96	85	85.88	101.04
100	5	6258.159	105	106.33	101.26
120	5	7514.107	125	126.39	101.12

Table 7: Recovery data of GLZ

Conc. of sample taken (µg/ml)	Conc. of standard spiked (µg/ml)	Spiking Standard area	Total conc. (µg/ml)	Total conc. found (µg/ml)	% Recovery Mean (n=3)
4.8	0.3	764.576	5.1	5.08	99.54
6	0.3	1117.518	6.3	6.32	100.38
7.2	0.3	1426.863	7.5	7.56	100.75
					100.223

The percentage mean recoveries were found to be 101.141% for MET and 100.223% for GLZ.

Method Precision

Table 5: Results for Method Precision of MET and GLZ

	Metformin HCl		Gliclazide	
Injections	**Retention time**	**Peak area**	**Retention time**	**Peak area**
1	2.443	5650.667	4.293	965.403
2	2.417	5683.849	4.257	956.938
3	2.423	5662.646	4.270	948.278
4	2.423	5679.338	4.263	955.360
5	2.447	5659.977	4.293	951.175
6	2.423	5645.244	4.267	968.288
Mean	2.4293	5663.620	4.274	957.574
SD	0.0124	15.336	0.015	7.858
%RSD	0.51	0.27	0.36	0.82

Method Precision was observed as the %RSD values for the retention times and peak areas of MET and GLZ in both standard and sample preparations were found to be less than 2%.

Robustness

Table 8: Results of Robustness for MET and GLZ

Change in chromatographic conditions		**Retention time (min)**		**Peak area**	
		Metformin HCl	**Gliclazide**	**Metformin HCl**	**Gliclazide**
Flow rate (ml/min)	0.8	3.04	7073.817	5.343	1197.828
	1.0	2.42	5701.197	4.270	991.133
	1.2	2.043	4764.647	3.593	818.306
	Mean	2.5010	5846.554	4.402	1002.422
	SD	0.5034	1161.427	0.882	190.013
	%RSD	20.13	19.87	20.05	18.96
Wavelength (nm)	232	2.45	6139.575	4.293	861.317
	234	2.417	5683.849	4.257	956.938
	236	2.453	4674.949	4.300	949.493
	Mean	2.4400	5499.458	4.283	922.583
	SD	0.0200	749.521	0.023	53.188
	%RSD	0.82	13.63	0.54	5.77

As part of the robustness, deliberate changes in the flow rate and detection wavelength were made to evaluate the impact on the method and retention times were significantly changed.

Ruggedness was established by changing the analysts and environment and not much changes in all the parameters are observed and within the limits. The specificity of the method was established by determining the interferences of peaks of diluent or excipients. These results indicate that the method is sensitive enough to carry out the routine analysis of MET and GLZ combination dosage forms.

CONCLUSION

The proposed R-HPLC method was developed and fully validated as per International Conference on Harmonisation (ICH) Guidelines and found to be applicable for routine quality control analysis for the simultaneous estimation of Metformin HCl and Gliclazide in combination using isocratic mode of elution. The proposed method is highly sensitive, accurate, precise, simple, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control for simultaneous estimation of MET and GLZ in bulk and in combined dosage forms.

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