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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND GLIMEPIRIDE IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

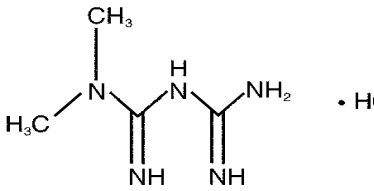
A new Simple, Precise, Fast and Accurate RP-HPLC Method has been developed and validated for simultaneous estimation of Metformin Hydrochloride (MET) and Glimepiride (GLM) in bulk and combined dosage form. The method was carried out on a Agilent CN (250mm × 4.6mm i.d, 5µm) column with mobile phase consisting of Buffer and Acetonitrile in the ratio of 45:55v/v and flow rate of 1ml/min. The detection was carried out at 229 nm. The retention time of MET and GLM were found to be 2.868min and 5.104min respectively. The MET and GLM followed linearity in the concentration range of 0.2-3.03 µg/ml and 10.01-150.15 µg/ml respectively with r^2=0.999 for both MET and GLM .The amount of both drugs estimated by the proposed method was found to be in good agreement with labelled claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. The developed method can be used for routine analysis of titled drugs in combined dosage form.

KEYWORDS: Metformin HCL, Glimepiride; RP-HPLC, Validation, CN column.

INTRODUCTION

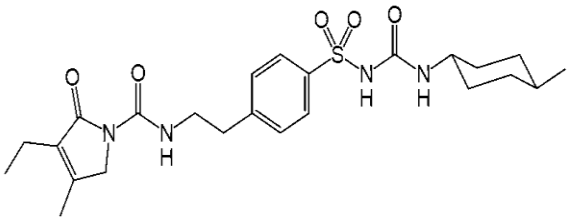
Metformin Hydrochloride (MET) is 1, 1-Dimethylbiguanide hydrochloride and is used in the treatment of diabetes. It is completely different from the hypoglycaemic sulphonamides both in its structure and its mode of action. It possibly interferes with mitochondrial respiratory chains and promotes peripheral glucose utilization by enhancing anaerobic glycol sis or it

enhances binding of insulin to its receptors and potentiates its action. Other explanation is that it suppresses hepatic gluconeogenesis and inhibits intestinal absorption of glucose [1, 2]. Soluble in water (50 mg/ml), ethanol and DMSO (50 mM). Glimepiride i.e. 1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl] phenyl] sulphonyl]-3- *trans*-(4-methylcyclohexyl) urea is a hypoglycaemic agent belonging to the second generation sulfonylurea's. It appears to lower blood glucose by stimulating insulin release from beta cells in the pancreatic islets possibly due to increased intracellular cAMP [3, 4]. Soluble in DMSO (>10 mg/ml), water (<1 mg/ml at 25 °C), ethanol (<1 mg/ml at 25°C). The drugs are official in Indian pharmacopeia and estimated by liquid chromatographic methods [5, 6]. Literature survey reveals simultaneous estimation of drugs in combined dosage forms [7, 8] and also with other combined dosage forms [9-12].



metformin hydrochloride

• HCl



Glimepiride

MATERIALS AND METHODS

Chemicals

Metformin HCL and Glimepiride were purchased from Indian market manufactured by Ranbaxy, Hyderabad. Potassium dihydrogen phosphate, HPLC grade Acetonitrile, O-phosphoric acid were purchased from Merck (Mumbai) and HPLC grade water from cystron laboratories.

Instrumentation

Analysis was performed on waters HPLC Waters, 2695 separation module equipped with PDA detector, Auto sampler and column compartment with Empower 2 software. Other equipment used in the study was analytical balance (DENVER) and P^H meter (EUTECH instrument). Ultra sonic bath (UNICROME ASSOCIATES: UCA-701).

Chromatographic Conditions

Agilent CN column (250 mm × 4.6 mm i.d., 5µm) was used for chromatographic separation. The mobile phase composed of Buffer and Acetonitrile in the ratio of (45:55v/v); at a flow rate of 1.0ml/min with run time 8 min. Mobile phase and sample solutions were filtered

through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 229 nm.

Method Development

Standard stock solutions of 10mg/ml of Metformin HCL and Glimepiride were prepared separately using diluent (Buffer: Acetonitrile - 45:55v/v). The MET stock solution was diluted with diluent to give working standard solution containing 0.2-3.03 µg/ml concentration. Similarly the Glimepiride stock solution was diluted with diluent to give working standard solution in the range 10.01-150.15 µg/ml. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for MET and GLM by injecting nine concentrations of both drugs prepared in diluent and calibration curves were constructed by plotting area against the respective concentrations.

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing Metformin HCL and Glimepiride. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of MET and GLM. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analysed samples solution of MET and GLM, a known amount of standard drug powder of MET and GLM were added at 50, 100, 150% level. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is a procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line.

Robustness was evaluated by making deliberate variations such as variation of wavelength, flow rate and change in mobile phase composition. The robustness of the method was studied for MET and GLM. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by injecting 2µg/ml of MET and 20 µg/ml solutions of GLM, respectively. The system suitability parameters such as resolution, number of theoretical plates and tailing factor were studied. Stability of sample

solution was established by the storage of sample solution at 25^0c for 12hr and 24hrs. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for MET and GLM and compared against fresh sample.

Analysis of Formulation

To determine the content of MET and GLM in injection formulation (MET 500mg, GLM 1mg) an accurately weighed drug powder equivalent to 2500 mg of MET and 5 mg of GLM were transferred into 250mL volumetric flask, dissolved in 150mL of diluent and sonicated for 5 min. After achieving complete solubility of the drug, the volume was made up to the mark using diluent .The solution was filtered through the 0.45 µm nylon syringe filter. From the filtrate a 5mL solution was transferred into 50 mL volumetric flask and volume was made up to the mark with diluent to obtain a concentration of 1µg/mL of MET and 20µg/mL of GLM which was then subjected to proposed method and the amounts of MET and GLM were determined using calibration curves.

RESULTS

The proposed chromatographic system was found suitable for effective separation and quantization of MET (RT 2.86 min) and GLM (RT 5.102 min) with good resolution, peak shapes and minimal tailing. The overlay UV spectra and typical chromatogram were shown in Figures 1 and 2. Both the drugs were found to give linear detector response in the concentration range under study with correlation coefficient of 0.9990 for both MET and GLM. The MET and GLM have followed linearity in the concentration range of 0.2-3.03 µg/ml and 10.01-150.15 µg/ml respectively Figure 3. Percent recoveries for MET and GLM were 100.1-100.4% and 100.3-100.4%. %RSD for tablet dosage form analysis, recovery studies and intra and inter-day precision studies was less than 2. LOD and LOQ were found to be 0.08470µg/ml and 0.26440 µg/ml for MET & 1.221µg/ml and 2.690 µg/ml for GLM.

The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 1.106609 -0.500 and 0.456153-1.212%. As the RSD values were < 2%, the developed method was found to be precise (Table 1). The accuracy of the method studied at three different concentration levels i.e. 50, 100, 150% showed acceptable recoveries in the range of 100.1-100.4%for MET and 100.3-100.4% for GLM (Table2). The LOD for MET and GLM was found to be 0.08470 and 1.221µg/ml respectively. Further the LOQ for MET and GLM was found to be 0.26440 and 2.690 µg/ml respectively. Robustness of the method was studied by making deliberate changes in the chromatographic conditions

like flow rate (± 0.2 ml/min) and mobile phase composition (± 2%). The validation parameters were summarized in (Table 3). The results of robustness study of the developed method was validated by change in flow rate and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4).

When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method. The system suitability parameters such as number of theoretical plates and tailing factor were studied and shown in (Table 3).

Stability of sample solution was established by the storage of sample solution at 25^0c for 6hr, 12hr and sample was reanalysed after 24 hr and assay was determined for the compounds (MET and GLM) and compared against fresh sample. Sample solution did not show any appreciable change in assay value (% RSD<2) when stored at ambient temperature up to 24 hrs. Six replicates of sample solutions containing 1µg/ml for MET and 20µg/ml for GLM were injected for quantitative analysis. The amounts of MET and GLM estimated were found to be 100.6 and 100.5% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

DISCUSSION

The developed RP-HPLC method was found suitable for simultaneous estimation of MET and GLM with good resolution, peak shapes and minimal tailing. The peak areas of the drug were reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.999 showed the stable linear detector response in different concentration ranges of both the drugs. The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for MET and GLM were 100.1-100.4% and 100.3-100.4% respectively, indicating the accuracy of the proposed method. Low LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and %RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same

experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method. The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of MET and GLM from the commercial tablet dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used tablet excipients and buffer used in analysis.

Table 1: Precision of Developed Method.

S. No	Method precision				System precision			
	MET		GLM		MET		GLM	
	RT	Area	RT	Area	RT	Area	RT	Area
1	2.916	1232330	5.108	1182340	2.867	1288159	5.101	1243528
2	2.919	1256364	5.111	1186301	2.870	1285503	5.103	1243036
3	2.918	1234156	5.111	1178235	2.870	1276050	5.100	1236942
4	2.917	1237543	5.108	1185608	2.870	1277479	5.102	1235886
5	2.918	1267907	5.111	1194548	2.869	1284248	5.102	1236994
6	2.920	1259468	5.112	1191455	2.869	1288493	5.103	1240669
Mean	2.918	1247961	5.110167	1186415	2.869	1283322	5.101	1239509
±SD		13810.05		5411.863		4878.058		3054.465
%RSD		1.106		0.456		0.380		0.246

Table 2: Accuracy Data.

% Level of recovery	Area	Amount of sample added (µg/ml)	Amount of API added (µg/ml)	Amount found (µg/ml)	%Recovery	%RSD
			MET			
50%	649485	20	10	30.15	100.5	
	641718	20	10	30.03	100.1	
	654796	20	10	30.18	100.6	0.290
100%	1239288	20	20	40.08	100.2	
	1262093	20	20	40.24	100.6	
	1238035	20	20	40.04	100.1	0.290
150%	1845157	20	30	50.15	100.3	
	1859583	20	30	50.20	100.4	0.330
	1836661	20	30	49.90	99.8	
			GLM			
50%	622145	30	15	45.36	100.8	
	621369	30	15	45.18	100.4	
	635909	30	15	45.00	100	0.400
100%	1192856	30	30	60.24	100.4	
	1213804	30	30	60.00	100	
	1186644	30	30	60.36	100.6	0.320
150%	1785603	30	45	75.075	100.1	
	1793847	30	45	75.525	100.7	
	1770940	30	45	75.00	100	0.360

Table 3: Validation and System Suitability Parameters.

Parameter	MET	GLM
Range (µg/ml)	0.2-3.03	10.01-150.15
Slope	61860	12069
Intercept	61860x - 27362	12069x - 29259
Correlation coefficient (R^2)	0.999	0.999
Retention time	2.860 min	5.102 min
Precision (intra and inter day)% RSD	<2	<2
Accuracy	100.1-100.4	100.3-100.4
LOD(µg/ml)	0.08470	0.22100
LOQ(µg/ml)	0.26440	0.69000
Tailing factor	1.481	1.656
Theoretical plates	4363	6545
Resolution	10.09	

Table 4: influence of flow rate, wavelength and mobile phase Composition on analytical parameters.

Parameter		MET			GLM	
	RT	Area	Tailing	RT	Area	Tailing
Flow rate(±0.2ml/min)						
0.8ml/min	3.596	1572857	1.56	6.763	1490381	1.68
1ml/min	2.868	1237543	1.48	5.103	1243036	1.65
1.2ml/min	2.422	1039537	1.50	4.471	992258	1.62
Mobile phase composition				**(±5%v/v)**		
50:50	2.823	1273181	1.54	6.999	1212579	1.71
45:55	2.918	1237543	1.48	5.111	1194548	1.58
40:60	3.003	1247630	1.48	4.458	1194520	1.56

Table 5: Assay of Commercial Formulation.

Drug	Label claim(mg/tablet)	Calculated value (mg/tablet)	% of Assay
MET	500	500.3	100.2
GLM	1	1.005	100.1

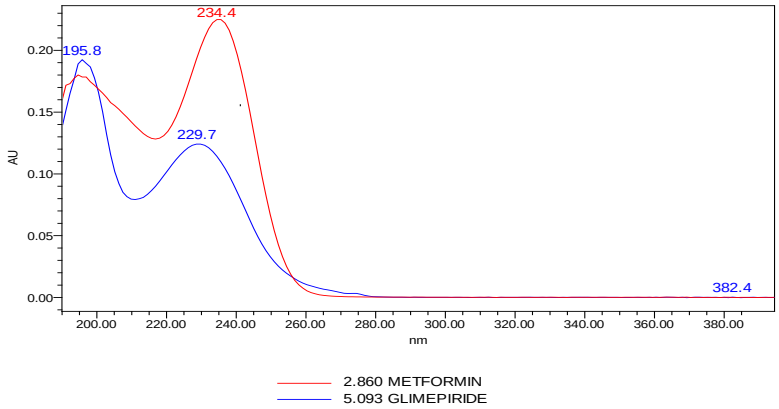


Figure 1: Overlay UV Spectra of Standard MET and GLM.

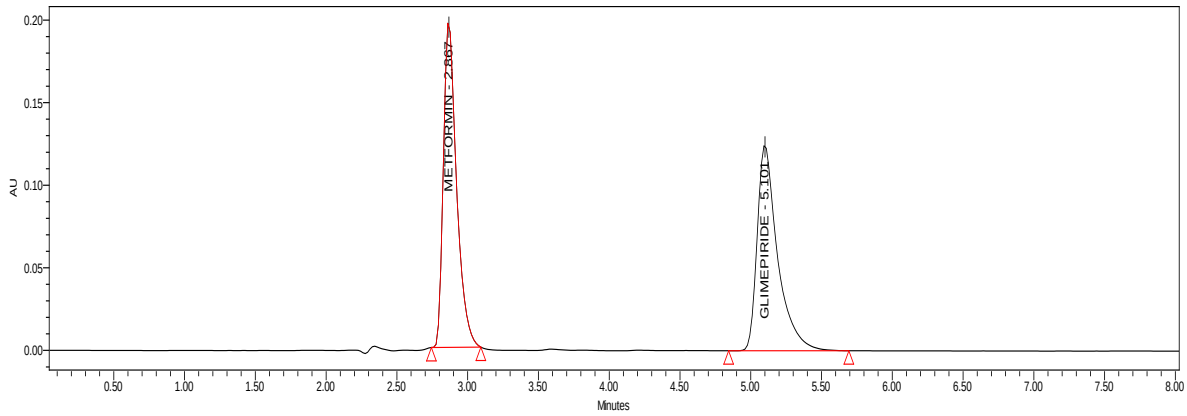
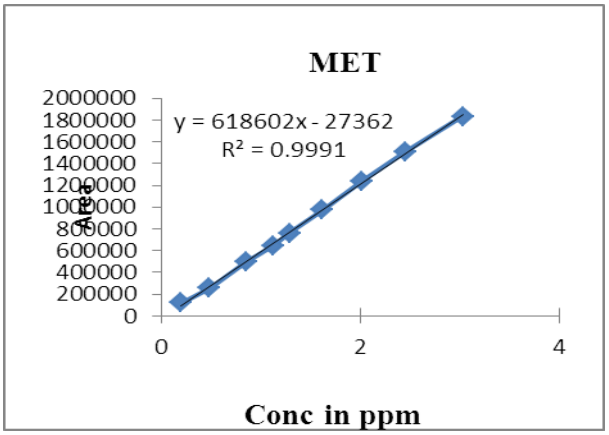


Figure 2: Typical HPLC chromatogram of MET and GLM



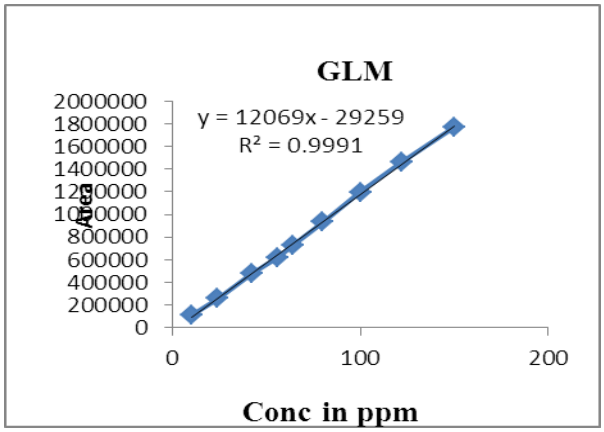


Figure 3: Calibration curves for MET and GLM.

CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of MET and GLM in tablet dosage form for routine quality control analysis.

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