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Research Article

Validated RP-HPLC method development for the simultaneous estimation of antidiabetic drugs

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Abstract

A force degradation profile of Metformin HCL & Glimepiride in combine tablet dosage form on RP-HPLC was developed using Grace RP-C18 (4.6 x 150 mm, 5 µm) in an gradient mode with mobile phase comprising of Acetonitrile: Dihydrogen Pott.Phosphate (pH 2.5 using 0.1% OPA) The flow rate was 0.7 mL/ min and effluent was monitored at 242 nm.. The retention times were found to be 2.06 min for MET and 5.80 min for GLIM. The assay shows a linear dynamic range of 250.0- 1250.0 µg/mL for MET and 1.0-5.0 µg/mL for GLIM. The calibration curves were linear (r^2 = 0.999 for MET and r^2 = 0.998 for GLIM) over the entire linear range. Mean % recovery was found to be 99.80 % for MET and 98.93 % for GLIM with % RSD was NMT 2.0 for both estimations which fully agrees with system suitability which is in good agreement with labeled amount of formulation. The % RSD for Intra- Day & Inter-Day Precision was NMT than 2.0 for both the drugs. The developed method was validated as per ICH guidelines.

Keywords: MET, GLIM, Pot.Phospate, RP-HPLC, Assay method, Method Validation.

1. Introduction

HPLC is a physical separation technique carried out in the liquid phase in which a sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). An online detector monitors the concentration of each separated component in the column effluent and generates a chromatogram. HPLC is the most widely used analytical technique for the quantitative analysis of pharmaceuticals, bio molecules, polymers, and other organic compounds1-9. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. The parameters for method validation as defined by ICH (International Conference on Harmonization) guidelines are Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantitation, Linearity, Range, Robustness and Ruggedness [2]. From the literature review [7-16] it has been found that only few analytical methods for the above combination have been reported. Therefore the attempt is made to develop simple, accurate, precise, rapid and economical RP-HPLC method for determination of Metformin HCL (MET) and Glimepiride (GLIM) in combine dosage form. Metformin HCL [Fig. 1] Chemically is [*N,N*-dimethyl imidodicarbonimidic-diamide hydrochloride]. It is white to almost white powder used as anti- diabetic having solubility in methanol and water, sparingly soluble in acetone the pKa is 9.6. While Glimepiride [Fig. 2] chemically is 4ethyl 3methyl [2, 4methylcyclohexyl) carbamoylsulfomoyl, phenyl, ethyl 5-oxo-24-pyrrole 1-carboxamide. It is white to yellowish white crystalline and practically odorless used as anti-diabetic having solubility in methanol and insoluble in water, slightly soluble in ethylene chloride [5, 6,19, 20].

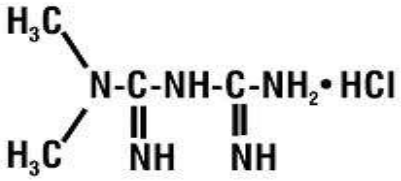

Fig. 1 Chemical Structure of Metformin HCL

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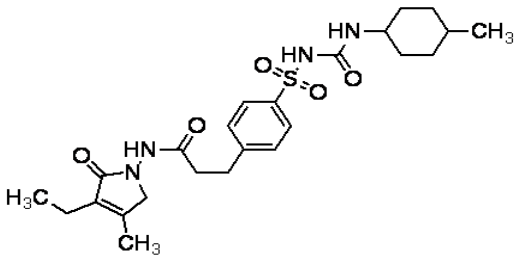

Fig. 2 Chemical Structure of Glimepiride

2. Experimentals

2.1 Reagents & Chemicals

MET (5 gm) supplied as gift sample by Cipla Pharma Ltd. (Mumbai, India) and its claimed purity was 99.4% and GLIM (5 gm) supplied as gift sample by Ranbaxy Laboratories Limited (Haryana), India and have 99.6 % purity.The marketed formulation Metpride (Alkem Laboratories) was purchased from the local market containing MET 500.0 mg and GLIM 2.0 mg and all the chemicals used were are of analytical grade.

2.2 Instruments

HPLC System of Younglin Quaternary pump with UV- VIS detector (UV 730 D) Software Autochro 3000. Analytical balance of Wensar-PGB100 ESSAE, Electronic Weighting Scale, India (microanalytical balance) was used for weighing purpose also the pH meter of Digisun electronics model 700 was used and Ultrasonicator Servewell instruments model RC-SYSTEM MU-1700 used for sonication purpose..

2.3 Preparation of Standard Solutions

Preparation of Standard Solutions

Standard Stock Solution (A) Accurately weighed quantity of MET (250.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 5000.0 µg/mL The resultant solution was then sonicated for 10.0 min in Ultrasonicator.

Standard Stock Solution (B) Accurately weighed quantity of GLIM (10.0 mg) was transferred to 10.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 200.0 µg/mL The resultant solution was then sonicated for 10.0 min in Ultrasonicator.

Working Standard Solution (C) 1.0 mL of solution (A) and 1.0 mL of solution (B) was transferred to 10.0 mL volumetric flask and then the volume was made up to the mark with mobile phase to get final concentration of (500.0 µg/mL of MET & 2.0 µg/mL of GLIM) respectively. The resultant solution was then sonicated for 10.0 min in Ultrasonicator.

2.4 Optimization of Mobile Phase and Chromatographic Conditions

Procedure

The chromatographic conditions were set as per the trial of various optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in the Rheodyne injector (20.0 µL) and the respective chromatograms were recorded. Various mobile phases were tried by combinations and also by varying column, different flow rate, column temperature and type of buffers with varying pH and solvents.

Mobile phases were tried as follows:

- Trial -1 MEOH: 0.05 %TEA (50:50, v/v), pH 2.5 with 0.1% OPA.
- Trial -2 MEOH: 0.05 %TEA (50:50, v/v), pH 2.5 with 0.1% OPA.
- Trial -3 MEOH: 0.05 %TEA (50:50, v/v), pH 2.5 with 0.1% OPA.
- Trial -4 MEOH: 0.05% TEA (30:70, v/v), pH 2.5 with 0.1% OPA.
- Trial-5 MEOH: 0.05% TEA (30:70, v/v), pH 2.5 with 0.1% OPA.
- Trial-6 MEOH: 0.05% TEA (30:70, v/v), pH 2.5 with 0.1% OPA.
- Trial-7 ACN: Pot.Phospate (50:50, v/v), pH 2.5 with 0.1% OPA.
- Trial-8 ACN: Pot.Phospate (20:80, v/v), pH 2.5 with 0.1% OPA.
- Trial-9 ACN: Pot.Phospate (70:30, v/v), pH 2.5 with 0.1% OPA.
- Trial-10 ACN: Pot.Phospate (70:30, v/v), pH 2.5 with 0.1% OPA.
- Trial 11- ACN: Pot.Phospate (70:30, v/v), pH 2.5 with 0.1% OPA.

Above mentioned various mobile phases were tried. The mobile phase which containing Acetonitrile: Pot. Phospate (70.0:30.0, v/v), pH 2.5 with 0.1% OPA, injection volume-20.0 µL flow rate of 0.7 mL/min was selected, due to its high resolving power, sensitivity and suitability, for the determination of MET and GLIM. The chromatogram is shown in **Figure 3**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

Chromatographic Parameters

Column : Grace C 18(150×4.6 mm, 5 µ)
Flow Rate : 0.7 mL/Min
Wavelength : 242.0 nm
Injection Volume : 20.0 µL
Column Oven Temperature: Ambient
Run Time : 10.0 Min
Mobile Phase : ACN: Pot.Phospate (70.0:30.0 V/V)
PH of buffer : 2.5 (Adjusted with 0.1% OPA)

System Suitability Studies

System suitability is a Pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be carried out. It is performed to ensure that the system is operating properly and read to deliver results with acceptable accuracy and precision. The tests were performed by collecting data from five replicate injections of standard solutions.

Procedure The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Five replicate injections of mixed working standard solution (C) were injected in to the system, the chromatograms were recorded for both the drugs and the results are shown in **Table 1 & 2.**

Analysis of Marketed Formulation

Preparation of Standard Solutions

Standard solutions prepared as per the methodology adopted for system suitability studies.

Preparation of Sample Solution

Take the powder weight of tablet equivalent to 250.0 mg of MET in 100.0 mL of volumetric flask and add sufficient mobile phase and sonicate it for 15.0 min. Make up the volume up to the mark with mobile phase and filtered it with 0.24µ to get 5000.0 µg/mL and 200.0 µg/mL of MET and GLIM respectively. Take 0.05 mL of GLIM and 1.0 mL of MET from above solution of GLIM and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 1.0 µg/mL GLIM & 250.0 µg/mL MET.

Procedure Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured.

Mixed Standard Solutions

Working Standard Solution (C) 2.0 mL of solution (A) and 0.1 mL of solution (B) was transferred to 10.0 mL volumetric flask and then the volume was made up to the mark with mobile phase to get final concentration of (500.0 µg/mL of MET & 2.0 µg/mL of GLIM) respectively. The resultant solution was then sonicated for 10.0 min in Ultrasonicator.

The amount of drug in a Tablet was calculated using following formula

$$\% \text{ Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

Where,

At = Area count for sample solution
As = Area count for standard solution
Ds = Dilution factor for standard
Dt = Dilution factor for sample
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)

The results are shown in **Table 3.**

Analysis of Marketed Formulation

Preparation of Standard Solutions

Prepared as per the methodology adopted for laboratory mixtures

Preparation of Sample Solutions

Take the powder weight of tablet equivalent to 250.0 mg of MET in 100.0 mL of volumetric flask and add sufficient mobile phase and sonicate it for 15.0 min. Make up the volume up to the mark with mobile phase and filtered it with 0.24µ to get 5000.0 µg/mL and 200.0 µg/mL of MET and GLIM respectively. Take 0.05 mL of GLIM and 1.0 mL of MET from above solution of GLIM

and MET respectively in a 10.0 mL volumetric flask and make up the volume up to the mark with mobile phase to get 1.0 µg/mL GLIM & 250.0 µg/mL MET.

Procedure Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug in a Tablet was calculated using following formula

$$\text{mg/Tablet} = \frac{AT1 \times WS1 \times Ds \times P1}{AS1 \times WT \times Dt} \times \text{Avg. wt}$$

Where,

AT1 = Average area of MET/GLIM peaks in Test chromatogram
AS1 = Average area of MET/GLIM peaks in Standard chromatogram
Ds = Dilution factor for standard
Dt = Dilution factor for test
P1 = Potency of working standards of MET/GLIM of % w/w basis
Avg. wt = Average weight of 10 Tablets

Further calculate the amount of MET/GLIM present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/Tablet)} \times 100}{\text{Label claim of MET/GLIM}}$$

The results are shown in **Table 4**, while chromatogram is shown in **Figure 4.**

Method Validation

1. Linearity

Preparation of Standard Solutions

MET Standard Stock Solution (A) Accurately weighed quantity of MET (250.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (5000.0 µg/mL of GLIM). The resultant solution was then sonicated for 10.0 min in ultrasonicator.

GLIM Standard Stock Solution (B) Accurately weighed quantity of GLIM (10.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of (200.0 µg/mL of GLIM). The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Mixed Standard Solutions aliquots portions of 1.0 to 5.0 mL from the standard stock solutions (A & B) were transferred to five 10.0 mL volumetric flasks and then volume was made up to the mark with mobile phase to get 5 different mixed standard solutions having concentrations of (1.0:250.0,2.0:500.0,3.0:750.0,4.0:1000,5.0:1250 µg/mL of GLIM & MET) respectively. The resultant solutions was then sonicated in ultrasonicator for 10.0 min

Procedure Equal volumes (20.0 µL) of 5 mixed standard solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. Then calibration curve (Peak area *vs.* concentration) was plotted and it is shown in **Figure 5 & 6**. The observations are shown in **Table 5.**

2. Accuracy

Preparation of Standard Solutions Standard solutions of (GLIM & MET) were prepared at the level of 80 %, 10.00 %, 120 %.

Preparation of Sample Solution To the pre analysed sample solution (1.0 µg/mL of GLIM & 250 µg/mL of MET) a known amount of standard solutions of pure drugs (GLIM & MET) were added in different levels i.e. 80%, 10.00 %, 120%. The results of recovery studies shown in **Table 6**. The percent recovery was then calculated by using formula;

$$\% \text{ Recovery} = \frac{E_w - B}{C} \times 100$$

Where,

E_w = Total drug estimated (mg)

B= Amount of drug contributed by pre analyzed Tablet powder (mg)

C= Weight of pure drug added (mg)

3. Precision

3.1 Intra-Day Precision

It was determined by analyzing the 3 different solutions having concentration **(**1.0:250,2.0:500 & 5.0:1250 µg/mL of GLIM & MET respectively) at 3 different times over a period of day.

3.2 Inter-Day Precision

It was determined by analyzing the 3 different solutions having concentration **(**1.0:250, 2.0:500 & 5.0:1250 µg/ mL of GLIM & MET respectively) at 3 days over a period of week.

Procedure Equal volumes (20.0 µL) of these solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak areas, retention time of major peaks were measured. The results are shown in **Table 7 & 8.**

4. Specificity

Specificity is an ability to measures accurately and specifically the analyte of interest in the other components that may be expected to be present in the sample matrix.

Preparation of Standard Solutions The standard solutions were prepared as per the methodology adopted for laboratory mixtures.

Preparation of Sample Solution Sample solution of marketed formulation was prepared as per the methodology adopted for marketed formulation analysis.

Procedure Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. Along with this the interference between the active ingredient and its excipient was also checked. The corresponding chromatograms are shown in **Figure 12 & 13.**

5. Robustness

Preparation of Sample Solution Sample solution of marketed formulation was prepared as per the methodology adopted for marketed formulation analysis.

Procedure Equal volume (20.0 µL) of sample solution was injected separately after equilibrium of stationary phase. Then deliberate variation in method parameters such as flow rate (<0.2mL/min), change in detection wavelength (<2.0 nm) was carried out. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. The results are shown in **Table 9** chromatograms are shown in **Figure 14 & 15.**

3. Results and discussion

3.1 Optimization of Mobile Phase and Chromatographic Conditions

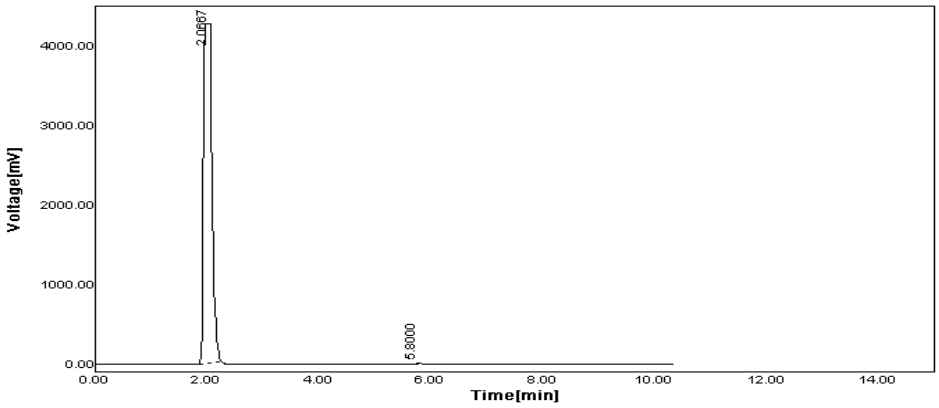

Fig. 3 Optimized Chromatogram of MET & GLIM

Observation: Good resolution with minimized tailing and proper peak shape and system suitability was observed within the limits. Hence the above chromatographic parameters are finalized.

System Suitability Studies

Table 1 Result of System Suitability Studies for (MET)

Sr. No	Area Reproducibility	Retention Time	Tailing Factor	Theoretical Plates
1	9315.22	2.000	1.6071	1137.4
2	9316.12	2.031	1.5998	1137.2
3	9318.25	2.103	1.6039	1136.9
4	9321.29	2.003	1.6069	1137.6
5	9322.15	2.041	1.6055	1137.3
Mean	9318.606	2.0354	1.6046	1137.28
%RSD	0.019	0.096	1.026	1.812
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2000**

Observation: All the parameters of system suitability are observed within the limits for MET.

Table 2 Results of System Suitability Studies for (GLIM)

Sr. No	Area Reproducibility	Retention Time	Tailing Factor	Theoretical plates
1	235.9	5.650	1.346	7532.0
2	240.41	5.630	1.314	7532.3
3	235.80	5.636	1.252	7494.2
4	235.17	5.618	1.293	7531.9
5	236.25	5.632	1.340	7532.1
Mean	236.706	5.6332	1.309	7524.5
%RSD	0.527	0.318	1.117	0.4006
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2000**

Observation: All the parameters of system suitability are observed within the limits for GLIM.

Analysis of Marketed Formulation

Table 4 Results of Marketed Formulation Analysis

Sr. No.	Concentration in µg/ml		Peak Area	
	MET	GLIM	MET	GLIM
1	250.0	1	3150.73	82.87
2	500.0	2	6243.20	171.37
3	750.0	3	9295.39	240.17
4	1000.0	4	12134.77	311.92
5	1250.0	5	15366.00	393.37
Slope	12.12	76.15		
Intercept	141.3	11.46		
Correlative Coefficient (r^2)	$R^2 = 0.999$	$R^2=0.998$		

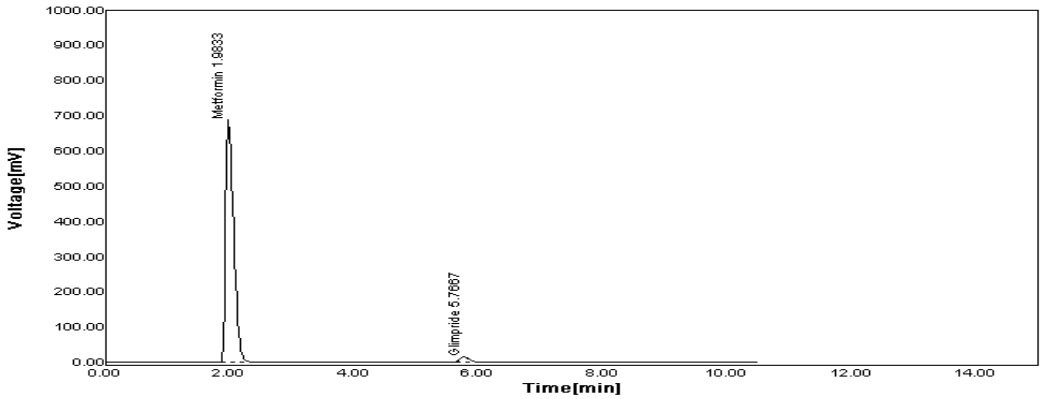

Fig. 4 Chromatogram of Marketed Formulation

The proposed method was applied to the determination of MET & GLIM in marketed formulation the **mean % amount** found was **99.0 (MET) & 98.12 (GLIM)** with **% RSD** values is **NMT 2.0%** indicates the developed method was successfully applied for analysis of marketed formulation. All the results found are in good agreement with the label content of marketed formulation.

Method Validation

1. **Linearity**

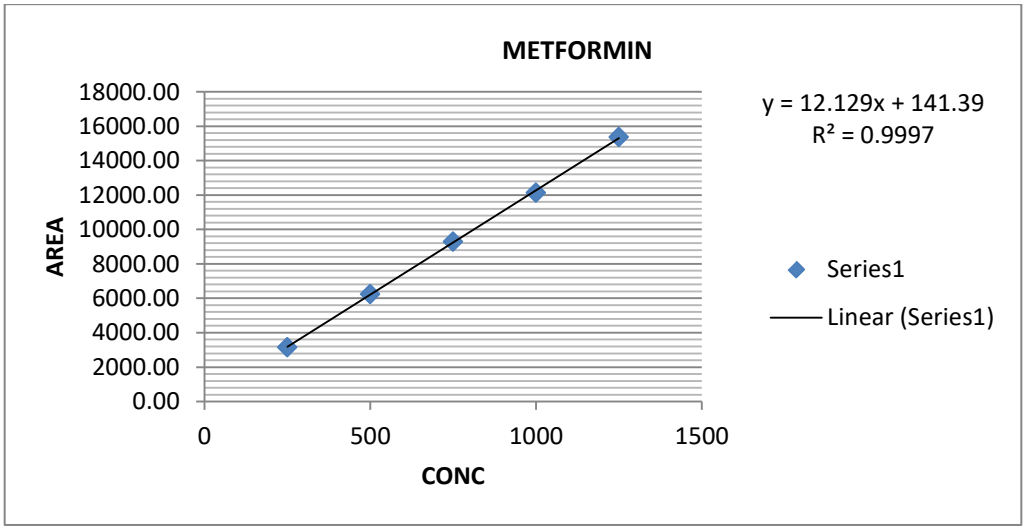

Fig. 5 Calibration Curve of MET

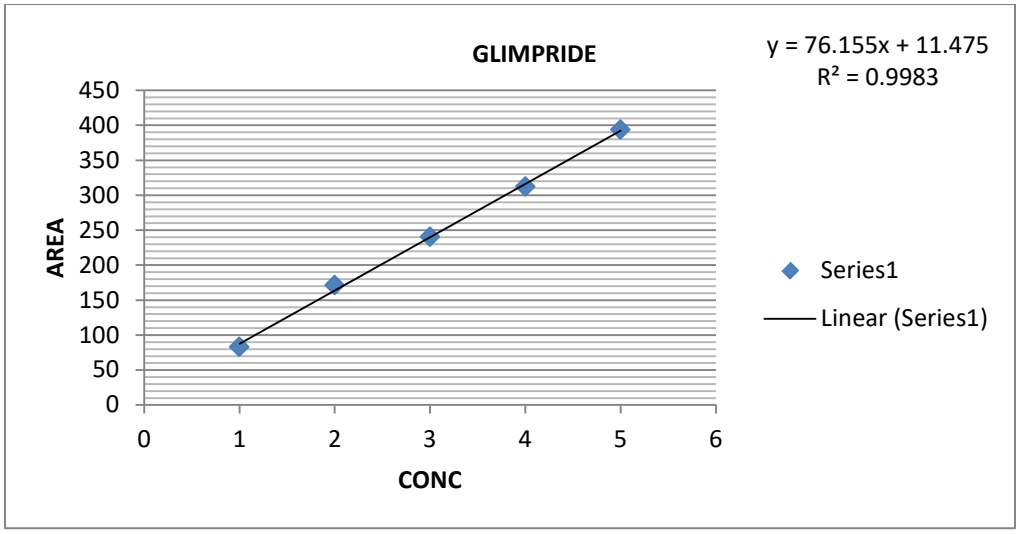

Fig. 6 Calibration Curve of GLIM

Table 5 Linearity Studies of MET & GLIM

Sr. No.	Concentration in µg/ml		Peak Area	
	MET	GLIM	MET	GLIM
1	250.0	1	3150.73	82.87
2	500.0	2	6243.20	171.37
3	750.0	3	9295.39	240.17
4	1000.0	4	12134.77	311.92
5	1250.0	5	15366.00	393.37
Slope	12.12	76.15		
Intercept	141.3	11.46		
Correlative Coefficient (r2)	$R^2 = 0.999$	R^2=0.998		

In both calibration curves the r^2 value was found to be **0.999** which nearly equals to unity. The regression equation for MET was **y = 12.12x + 141.3** while for GLIM it was **y = 76.15x + 11.47.** It indicates the capability of developed method to estimate both the drugs over the desired concentration range.

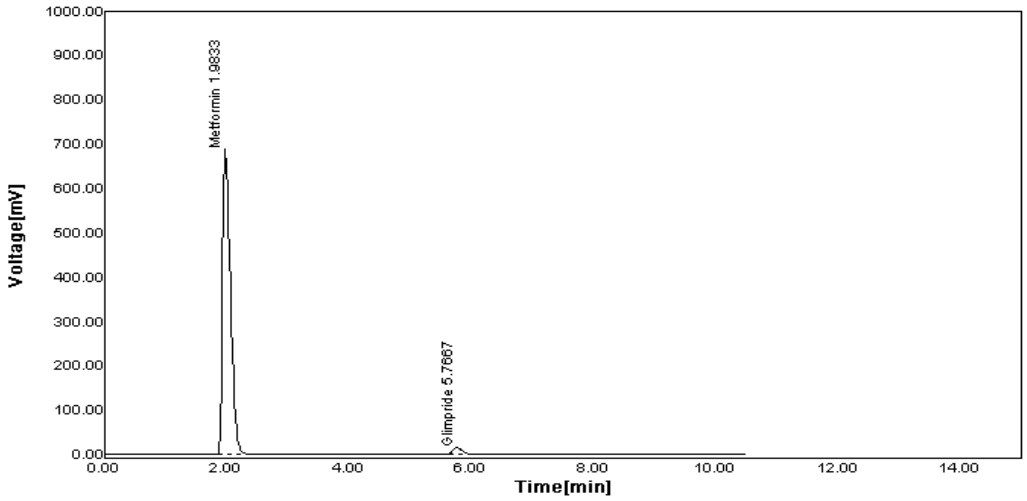

Fig. 7 Linearity Chromatogram for (250.0 µg/mL of MET & 1.0 µg/mL of GLIM)

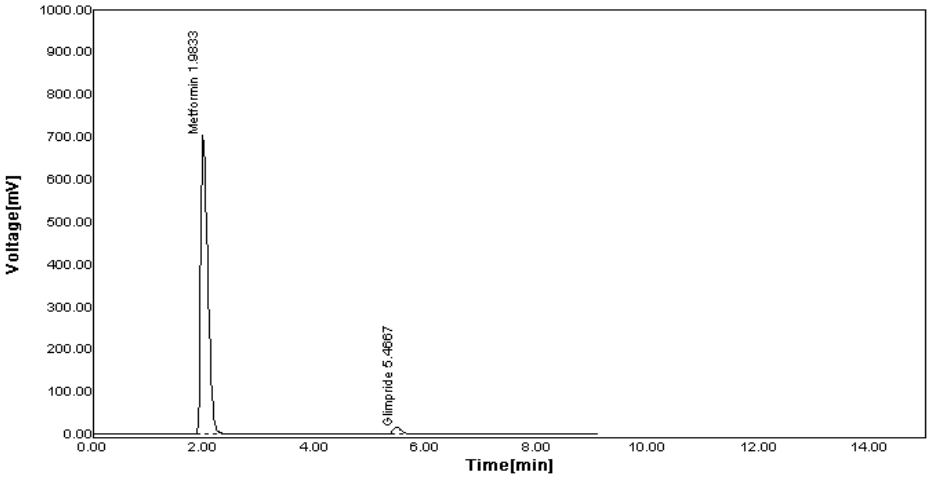

Fig. 8 Linearity Chromatogram for (500.0 µg/mL of MET & 2.0 µg/Ml of GLIM)

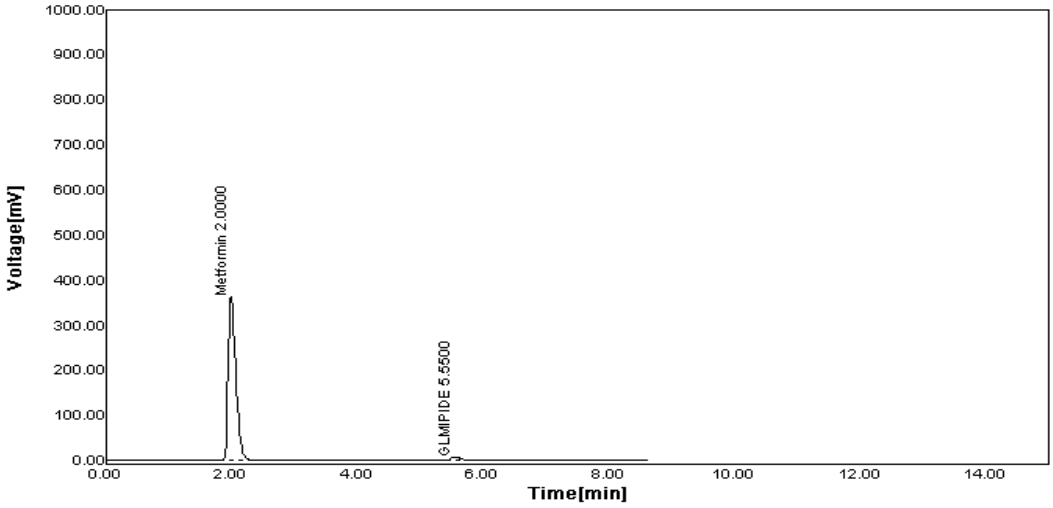

Fig. 9 Linearity Chromatogram for (750.0 µg/mL of MET & 3.0 µg/mL of GLIM)

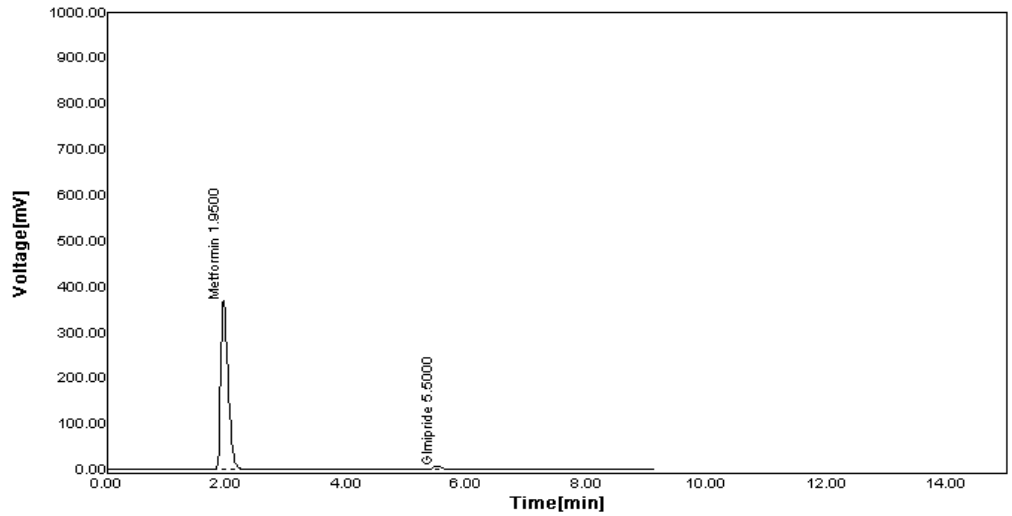

Fig. 10 Linearity Chromatogram for (750.0 µg/mL of MET & 4.0 µg/mL of GLIM)

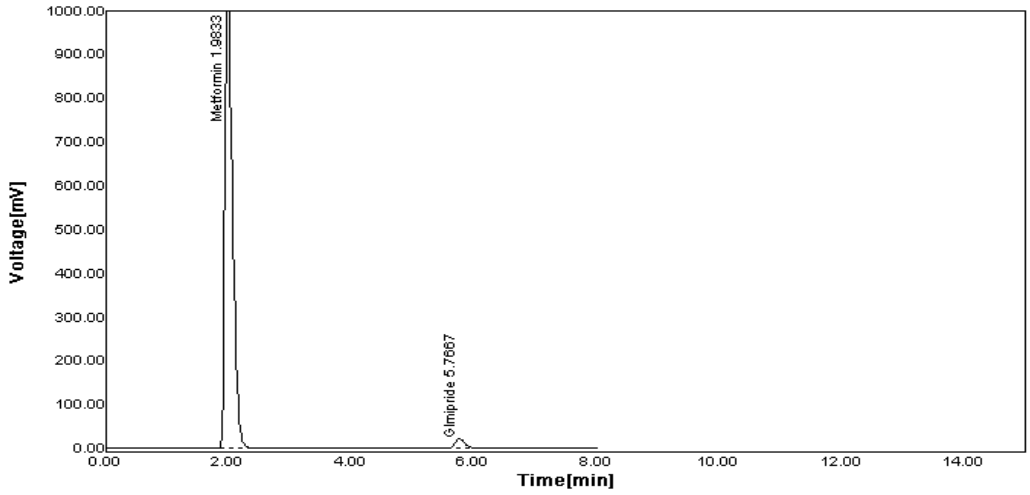

Fig. 11 Linearity Chromatogram for (1000.0 µg/mL of MET & 5.0 µg/mL of GLIM)

2. Accuracy

This is performed on the basis of recovery studies by standard addition method. Standard solutions of pure drugs (MET & GLIM) were added in different levels i.e. 80%, 100 %, 120%.

Table 6 Results of Recovery Studies

Level of % Recovery	Amount present (mg/tab)		Amount taken (µg/mL)		Amount of Std. Drug Added(µg/mL)		Total Amount Recovered (µg/mL)		%Recovery	
	MET	GLIM	MET	GLIM	MET	GLIM	MET	GLIM	MET	GLIM
80%	500	2.0	250	1.0	400	1.6	413.84	320.42	103.46	100.13
	500	2.0	250	1.0	400	1.6	414.35	319.89	103.28	99.97
	500	2.0	250	1.0	400	1.6	413.95	319.60	103.52	100.05
100%	500	2.0	250	1.0	500	2.0	515.75	321.04	103.15	100.32
	500	2.0	250	1.0	500	2.0	507.53	323.28	101.50	101.02
	500	2.0	250	1.0	500	2.0	510.30	322.16	102.33	100.67
120%	500	2.0	250	1.0	600	2.4	589.98	332.59	98.32	103.93
	500	2.0	250	1.0	600	2.4	593.10	327.49	98.85	102.34
	500	2.0	250	1.0	600	2.4	592.30	330.04	98.59	103.14

% mean recoveries were found with **% RSD** for **MET & GLIM** which fully agrees with system suitability. This showed that, the proposed HPLC method for the determination of MET and GLIM in a tablet was found to be sufficiently **accurate**.

3. Precision

3.1 Intra- Day Precision

Table 7 Results of Intra- Day Precision Studies for MET

Sr. No	Observations	% Drug estimation		
		Intra-day	Inter-day	Different Analyst
1	I	99.512	98.922	98.943
2	II	99.522	99.347	99.620
3	III	99.612	99.524	99.855
Mean		99.548	99.264	99.472
±S.D.		0.0550	0.309	0.493
%R.S.D.		0.0553	0.312	0.495

3.2 Inter- Day Precision

Table 8 Results of Inter- Day Precision Studies for GLIM

Sr. No	Observations	% Drug estimation		
		Intra-day	Inter-day	Different Analyst
1	I	98.625	98.754	98.915
2	II	99.639	99.615	99.609
3	III	99.542	99.794	99.867
Mean		99.268	99.387	99.463
±S.D.		0.910	0.895	0.773
%R.S.D.		0.917	0.901	0.777

Ruggedness was determined as Intra-day, Inter-day & Different Analyst. % amount of drugs were found with **% RSD (NMT than 2%)** which was in agreement with system suitability. Therefore, the proposed HPLC method for the determination of MET and GLIM in a tablet was found to be sufficiently **rugged.**

4. Specificity

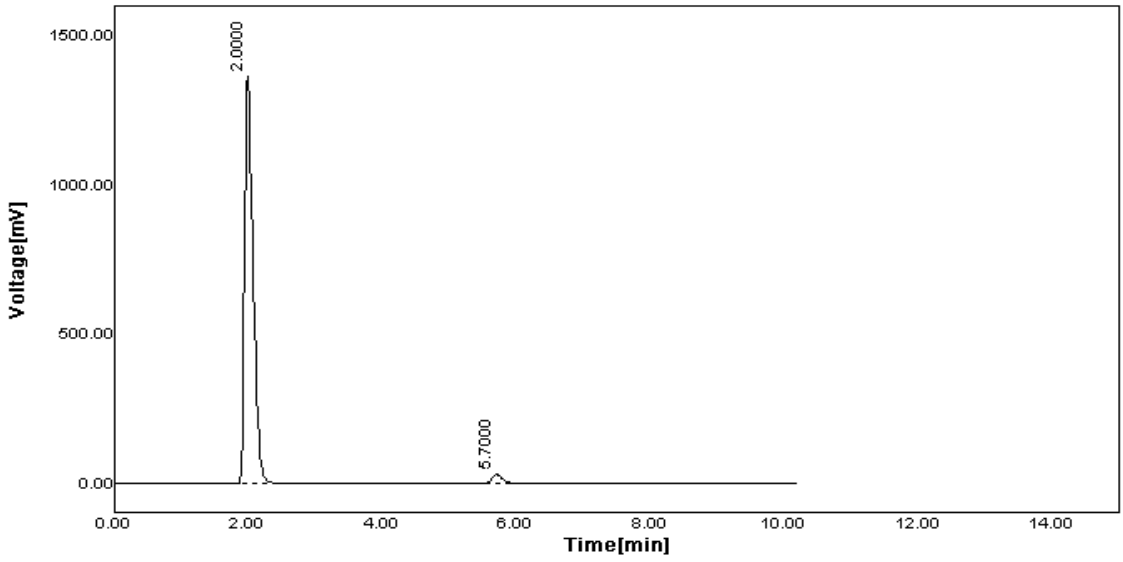

Fig. 12 Chromatogram of MET & GLIM Working Standards

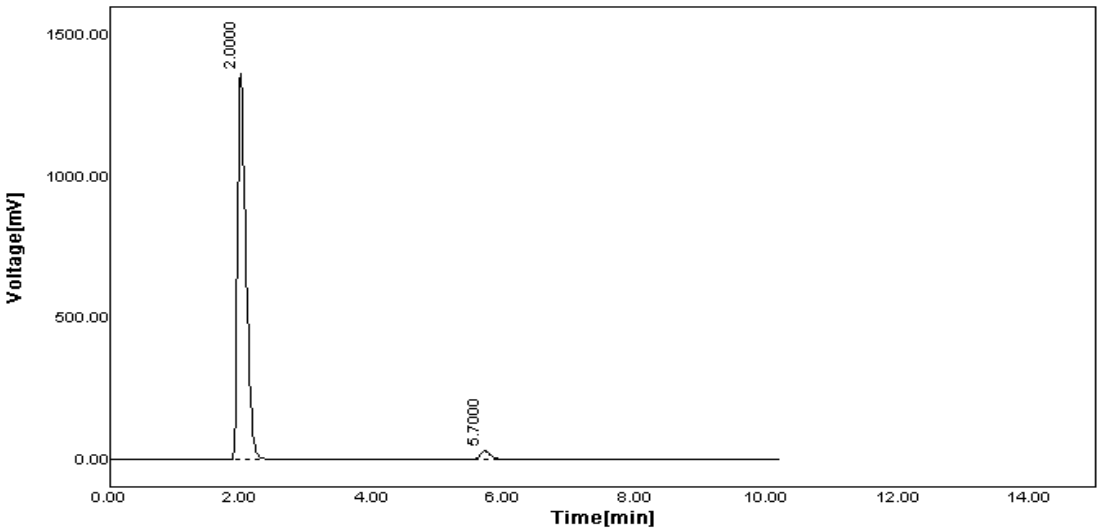

Fig. 13 Chromatogram of Marketed Formulation (Specificity)

In the chromatogram obtained with working standard and marketed formulation solution interference is not observed at the retention time of any peak. Therefore, the proposed HPLC method for the determination of MET and GLIM in a tablet was found to be **specific.**

5. Robustness

Table 9 Results of Robustness Studies for MET

Condition	Mean	±SD n=3	%RSD
Change in flow rate (± 0.1 ml)	5388.89	11.36	0.21
Change mobile Phase(±1%)	5417.68	8.50	0.16
Change in detection wavelength (± 1nm)	5744.15	44.95	0.78

Table 10 Results of Robustness Studies for GLIM

Condition	Mean	± SD n=3	%RSD
Change in flow rate (± 0.1 ml)	143.05	2.57	1.79
Change mobile Phase (±1%)	143.26	1.27	0.88
Change in detection wavelength (± 1 nm)	151.54	1.73	1.14

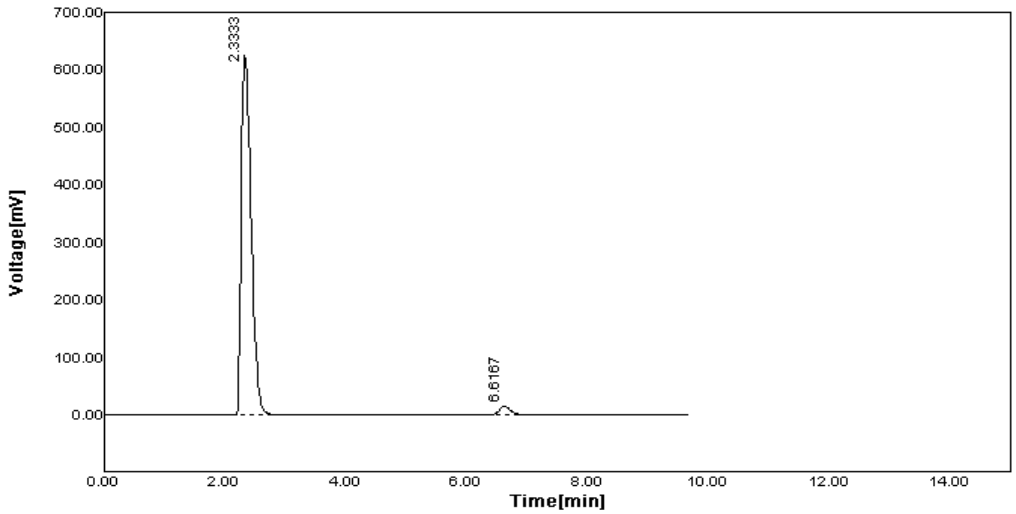

Fig. 14 Chromatogram of Robustness (<0.1mL/min)

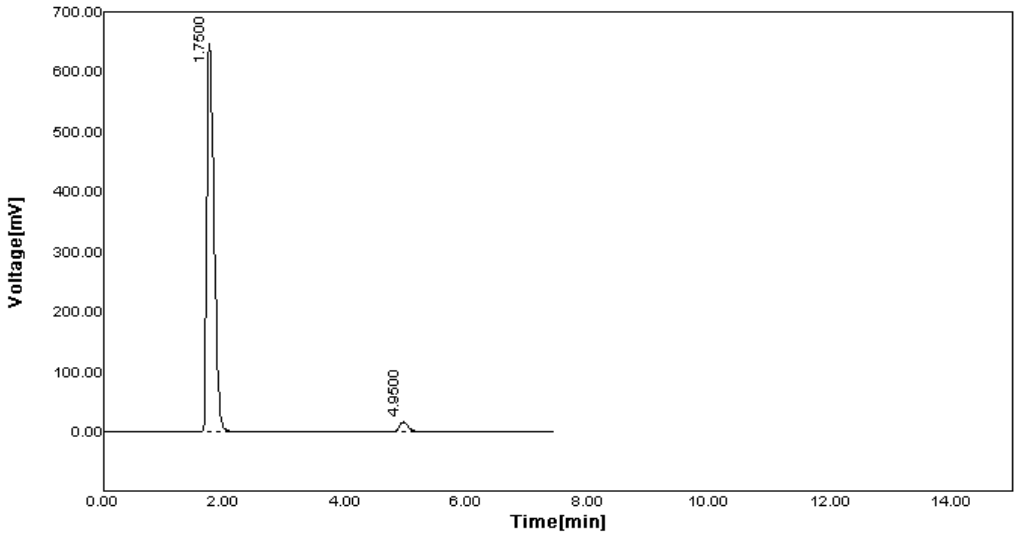

Fig. 15 Chromatogram of Robustness (<2.0 nm)

The results of assay of test solution were not affected by varying the conditions. They fully agree with the results obtained under original conditions. The **% RSD** for **(Retention time, Peak area and % Amount Found)** was not more than **2%** for both (MET & GLIM) which was in agreement with system suitability. Hence the proposed HPLC method for the determination of MET and GLIM in a tablet was found to be **robust.**

4. Conclusion

The developed RP-HPLC method was found to be highly specific. The developed RP-HPLC method was found to be linear over wider concentration range. The developed RP-HPLC method was found to be simple, accurate, sensitive, precise, specific, economical and rapid. The developed RP-HPLC method shows the good resolution between MET and GLIM within the run time of 10 min. The developed RP-HPLC method is very simple involving no complicated sample preparations. Therefore the developed RP-HPLC method can be applied for routine quantitative and qualitative analysis of MET and GLIM in bulk and pharmaceutical formulations like tablets. The developed RP-HPLC method was validated as per the ICH guidelines.

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