Research Article

Analytical Method Development of Metformin Hydrochloride in Bulk and Tablet Dosage Form by RP-HPLC Method

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ABSTRACT

A simple, sensitive, rapid, robust and reproducible method for the determination of Metformin Hydrochloride (MET) in bulk and Pharmaceutical Formulation (Tablets) was developed using Reverse Phase High Performance Liquid Chromatographic method (RP-HPLC). The RP-HPLC analysis was performed isocratically on was achieved with 250 x 4.6 mm, i.d 5 µm C-18 column using a mobile phase consisting of Methanol and Water in the ratio of 40:60 v/v, with a flow rate of 1mL/min. The analyte was monitored with UV detector at 233.0 nm. The developed method Metformin Hydrochloride elutes at a run time of 10 min. The proposed method is having linearity in the concentration range from 5 to 100 µg/mL of Metformin Hydrochloride. The present method was validated with respect to system suitability, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), and robustness as per ICH Guidelines. The proposed method can be readily utilized for bulk drug and pharmaceutical formulations.

Keywords: Metformin Hydrochloride (MET), RP-HPLC ,Methanol, Water.

INTRODUCTION

Metformin Hydrochloride is described chemically 3-(diaminomethylidene)-1,1-dimethylguanidine. Metformin decreases glucose production in the liver, increases insulin sensitivity and enhances peripheral glucose uptake. It does not stimulate secretion of endogenous insulin. It is not official in any of the pharmacopoeia and only listed in the Merck Index and Martindale. The complete drug references.[1, 2.] Literature survey reveals that there are few methods like separation and identification of MET metabolite by using RP-HPLC and HPLC-EIMS in human plasma[4]. Hence the objective of the work has been made to develop new RP-HPLC methods for pharmaceutical formations with good accuracy, simplicity, precision and economy[5].

EXPERIMENTAL

Instrument

Agilent HPLC 1120 series containing degasser, binary gradient pump and UV detector is used.

Chemicals and Reagents

Standard gift samples of Metformin procured from Glenmark Pharmaceuticals, Nasik respectively. Methanol (HPLC grade) was obtained from Merck Laboratories Pvt. Ltd., Mumbai.

Chromatographic conditions

The mobile phase consisting of Methanol: Water was filtered before use through 0.4mc membrane filter and was pumped by the dual plunger reciprocating pump (L-7100 Agilent 1120 Compact LC) at a flow rate of 1mL/min in the ratio of 40:60v/v. The separation was carried out on a C_{18} column (5mc, 250(L) x 4.6 i.d., Kromasil). The column temperature was maintained at room temperature. The sample was injected through a Rheodyne injector and was analyzed by variable length detector set at 232.0nm. The data was acquired, stored and analyzed using Ezchrome E-Lite software.

Stock solution

Preparation of Stock Solutions

About 10 mg of MET was accurately weighed and transferred to 100 mL volumetric

flasks respectively and was dissolved in Metha nol: Water (40:60 v/v).

Procedure

The standard stock solution of each drug was suitably diluted with mobile phase to obtain standard solution of different solution was injected six times into the column at a flow rate was kept 1mL/min. The linearity was obtained in the concentration range of 5-100 µg/mL for MET.

Analysis of the marketed formulation (Assay of Tablet)

To estimation of QHF in tablet formulation, twenty tablet i.e. T_1 were weighed. The tablet content was weighed and triturated to fine powder. Tablet powder equivalent to 500 mg of MET was weighed and transferred to a 100mL volumetric flask and dissolved in 50 mL of mobile phase. It was kept for ultrasonication for 45 min. finally the volume was made up to the mark with mobile phase; this was then filtered through Whitman filter paper no.41 and 0.2µ membrane filter get tablet stock solution of the concentration of 100µg/mL. The tablet solutions were further diluted with mobile phase to obtain sample solution within the Beer Lambert's concentration range for the drug solution. A sample solution of concentration 20µg/mL of MET was prepared from the stock solution and injected into the sample injector of HPLC six times (n=6) under the chromatographic condition as was described above. Area of each peak was measured at 232.0 nm. The amount of each drug present in the sample was determined using the peak area of MET present in the pure drug. Figure 01 represents the chromatogram of MET in tablet formulation. The results were statistically evaluated Table 01 & 02.

Validation of HPLC method

The Proposed method was validated as per ICH guidelines [6,7,8].

RESULTS AND DISCUSSION

The goal of this study was to develop a rapid and sensitive RP- HPLC method for the analysis of MET in bulk drug samples and its formulation using the most commonly employed RP-C_{18} with UV detection. The mobile phase consisted of Methanol and Water in the ratio of 40:60 v/v. The run time was set at 10 min and the retention time for MET was 2.59 min (Fig.1). The peak area of the drug was reproducible as indicated by RSD values less than 2%. When the calibration curve for concentration of MET and its respective peak area was plotted, a good linear relationship was observed between the concentration and their respective peak areas in the range of 5-100 µg/mL MET. The results of formulation analysis, recovery studies and its statistical validation data given in Table 01 and Table 03 indicate high degree of precision and accuracy of the proposed method. The results of the validation and system suitability studies are given in Table 05. Hence it can be concluded that the developed RP-HPLC method can be employed successfully for the estimation of MET in both bulk and single component formulation.

CONCLUSION

The developed method was found to be accurate, precise, robust, linear and specific over the given concentration range studied. The method can also be used for routine Q.C analysis of MET in bulk and tablet dosage form.

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Table 01: Analysis of the Tablet formulation (T_1)

S. No.	Amount Present (µg/mL)	Amount Found (µg/mL)	% Amount Found
	MET	MET	MET
1.	500	500.00	100.00
2.	500	499.63	99.26
3.	500	500.36	100.72
4.	500	500.52	101.44
5.	500	500.00	100.00
6.	500	499.99	99.98

Table 2: Statistical Validation of Tablet Analysis

Tablet	% Mean	S.D.*	% R.S.D.*
T_1	100.23	0.7501	0.748

*n=6, Tablet T_1

Table 3: Recovery Studies

Level of % Recovery	Amount of drug present (mg/tablet)	Amount of standard Added (mg)	Total amount recovered (mg)	% Recovery
	MET	MET	MET	MET
80	500	400	399.89	99.97
	500	400	399.90	99.97
	500	400	399.92	99.98
100	500	500	499.96	99.99
	500	500	499.94	99.98
	500	500	499.95	99.99
120	500	600	599.92	99.98
	500	600	599.96	99.99
	500	600	599.99	99.99

*n=3 at each level of recovery

Table 04: Statistical Validation of Recovery Study

Level of % Recovery	% Mean Recovery*	S. D. *	% R.S.D.*
	MET	MET	MET
80	99.97	0.057	0.0570
100	99.98	0.0577	0.05771
120	99.98	0.0057	0.00570

*n= at each level of recovery

Table 05: Validation and system suitability studies

Parameter	MET
Linearity Range(µg/mL)	5-100
Correlation coefficient	0.9980
Limit of detection ((µg/mL)	0.08381
Limit of quantization(µg/mL)	0.25397
Retention of time(min.)	2.59
Precision(%R.S.D) Inter –day (n=6) Intra- day (n=6)	 0.01033 0.4196
Tailing factor	0.97
Theoretical Plates	2100
Mean % Recovery	99.99

Table 06: Robustness evaluation of the RP-HPLC method (n=3*)

S. No.	Chromatogra. Factors	Parameter	Retention Time*(tR)	Tailing factor*(t)	Theoretical plates*
			MET	MET	MET
1	% of Methanol in mobile phase (v/v)	35	2.86	1.01	4430
		40	2.90	1.02	4432
		45	2.95	1.04	4434
		Mean ± SD	2.90± 0.041	1.02± 0.015	4433 ±1.52
2	Flow Rate (mL/min.)	0.90	2.88	1.01	4429
		1.00	2.90	1.02	4430
		1.10	2.92	1.05	4431
		Mean ± SD	2.90±0.021	1.026± 0.02	4430± 1.00
3	Temperature (^{0}C)	24	2.89	1.00	4431
		25	2.90	1.02	4432
		26	2.92	1.04	4433
		Mean ± SD	2.90±0.015	1.02±0.02	4432±1.01
4	Solvent of different lot	First	2.90	1.02	4432
		Second	2.92	1.04	4434
		Mean ± SD	2.91±0.014	1.03±0.01	4433±0.015

Where t_R= Retention time and t=Tailing factor

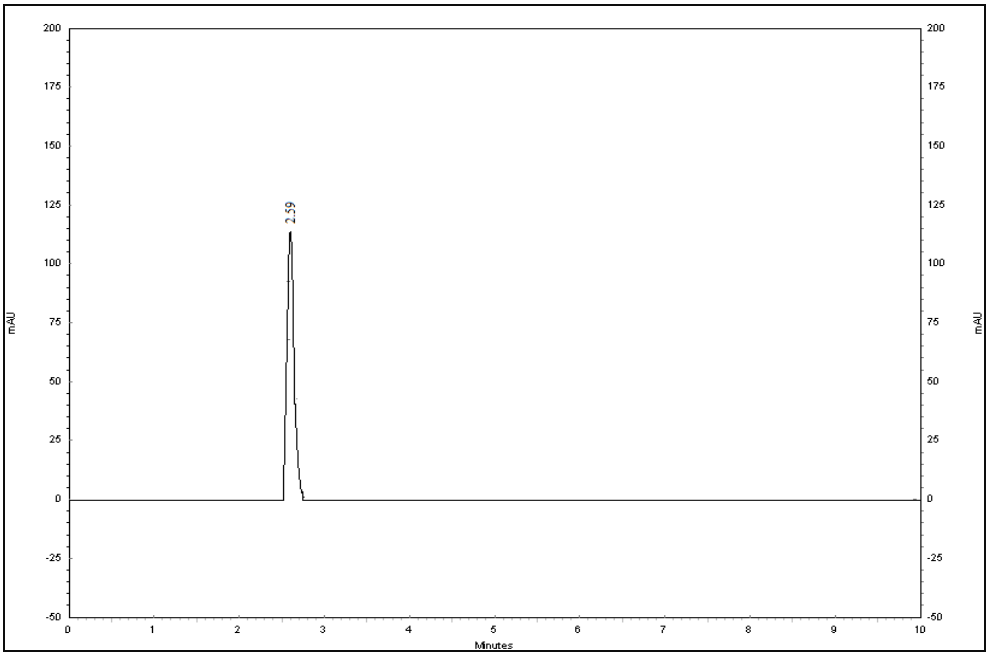

Fig. 1: Chromatogram of standard Metformin Hydrochloride

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