Research Article

Bioanalytical RP-HPLC Method Development and Validation for the Determination of Metformin Hydrochloride in Spiked Human Plasma

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ABSTRACT

Bioanalytical method development is the process of creating a procedure to enable a compound of interest to be identified and quantified in a biological matrix. A simple, selective, rapid, precise, and economical Reverse-Phase HPLC method has been developed and validated for the determination of Metformin hydrochloride in spiked human Plasma. It is an orally administered biguanide derivative used to lower blood glucose concentrations in patients with non-insulin-dependent diabetes mellitus. The method was carried out with an Agilent HPLC with a PDA detector. ZORBAX Eclipse Plus C18 column (4.6 x 100 mm, 3.5 µm) is used at a flow rate of 2.0 mL/min. Detection was carried out at 235 nm. The mobile phase consisting of a mixture of Acetonitrile and Sodium dodecyl sulphate (0.01M) in a ratio of 40:60% v/v, respectively. The pH of the buffer was maintained at 5.1. The retention time of Metformin hydrochloride was found at 4.152 min, with a maximum run time of 8 minutes. The developed method was validated according to USFDA guidelines. For linearity testing, the concentration range of Metformin hydrochloride is 1-6 µg/mL with R^2 = 0.9989. These bioanalytical validations play a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic studies of Metformin Hydrochloride.

Keywords: Bioanalytical RP-HPLC Method, Human plasma, Metformin hydrochloride and Validation.

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INTRODUCTION

Methods of measuring drugs in biological media are becoming increasingly important for the study of bioavailability & bioequivalence studies, quantitative evaluation of drugs and their metabolites, new drug development, clinical pharmacokinetics, research in basic biomedical and pharmaceutical sciences and therapeutic drug monitoring. Metformin hydrochloride is chemically 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride[1].

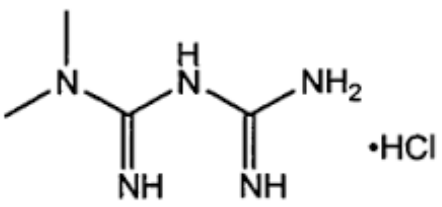

Figure 1: Chemical structure of Metformin hydrochloride

It decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.

Literature survey revealed that few analytical methods have been reported for estimation of Metformin hydrochloride individually or in combination with other drugs. The reported methods include RP-HPLC[2-5], HPLC[6-8], UHPLC–MS/MS[9]. There are no reports as per our knowledge that methods developed for the analysis of this analyte in spikes human plasma. The present study was aimed to develop a simple, sensitive, precise and accurate bioanalytical method for the estimation of Metformin hydrochloride in spiked human plasma. The developed method was validated according to USFDA[10] guidelines.

MATERIALS AND METHODS

Chemicals

Metformin hydrochloride was obtained as a gift sample from HIQ Pharma Labs Hyderabad, Telangana. Acetonitrile (HPLC Grade), Methanol (HPLC Grade) were procured from Fisher Scientific and milli-Q water was from SG Series Compact Pretreatment Module. AR Grade Sodium dodecyl sulphate, Ammonium citrate was purchased from Fisher Scientific. Human Plasma sample was procured from Dr. Lal Path Labs, Hyderabad.

Instrument

The high-pressure liquid chromatographic system utilized was an Agilent high-pressure liquid chromatograph 1260 series with the GI311C quaternary pump, ZORBAX Eclipse Plus C18 (4.6 x 100 mm, 3.5µm) (made in USA) and a diode array detector G1315D was utilized. Ezchrome elite software was used for chromatography data acquisition, processing and control of HPLC chromatograph. Digital pH meter (systronics model-802), an electronic balance (Shimadzu TX223L), a sonicator (spectral lab, model UCB 40) were used in this study.

Preparation of Metformin hydrochloride Standard Stock Solution

Stock solution(1mg/mL) and appropriate dilutions of metformin were prepared in methanol and stored at +4˚C no change in stability over the period of two weeks were observed. Working standards 1-6 µg/mL solutions were prepared in methanol as diluent.

Optimized extraction procedure

After taking trials with different methods of extraction, protein precipitation method was optimized with acetonitrile as a precipitating agent for the extraction of metformin from the plasma and following procedure was used. 100 µL of standard drug solution was taken in a micro centrifuge tube and 100µL of Human plasma added, mixed on the vortexed for 2 minutes. To that mixture, 300µL acetonitrile was added and centrifuged for 10 min at 6,000 RPM. at 37C. 0.2 mL of supernatant was transferred in to auto sampler vial and 0.2 mL of reconstitution solution was added and injected in to HPLC system.

METHOD VALIDATION

The method performance was evaluated for accuracy, linearity and stability during stress conditions which include stock solution stability.

Linearity and Range

Working solution of various concentrations was injected under the operating chromatographic condition and peak area of each drug were calculated at 235 nm. The calibration curves were constructed using simple linear regression between peak area and concentrations. The range of solution has been decided according to correlation coefficient of regression equation.

Precision

The precision of this method was evaluated by the % CV at different concentration levels corresponding to LQC, MQC and HQC. Intraday and inter day precision was evaluated in 3 replicate batches of different concentrations (2, 4 and 6 µg/mL).

Recovery

The accuracy of the method was performed by calculating % recovery for the different concentration levels of drug. The samples of three concentration levels prepared as LQC, MQC and HQC by standard addition method.

Stability studies

The stability of Metformin hydrochloride in solution and plasma sample was evaluated using two concentration level (LQC and HQC i.e. 2 and 6 µg/mL). The stability of Metformin was also evaluated in deep freezing at -20^{0}C for 12 hrs. The plasma stock solution was kept at freezer and after stressed to three freeze thawing cycles (for 24 hrs. per cycle). All samples described above were compared to freshly prepared quality control samples of Metformin hydrochloride at the same concentration level.

RESULTS AND DISCUSSION

Optimization of Chromatographic conditions

The chromatographic conditions were optimized in order to provide good system suitability parameters. The mobile phase was selected on the basis of its polarity and different trials were taken. Acetonitrile was selected as an organic modifier, because lower column efficiency was observed using methanol. Finally, a mobile phase consisting Sodium dodecyl sulphate (pH5.1): Acetonitrile (60:40% v/v) at a flow rate of 2mL/min was selected. The retention time of Metformin hydrochloride was found to be 4.152 min. The optimized chromatographic conditions and chromatogram of Analyte and system suitability parameters are listed in table 1 and Figure 3.

Table 1: Optimized chromatographic conditions and system suitability parameters of selected method.

S. No.	Condition	Details
1	Column	ZORBAX Eclipse Plus C18 (4.6 x 100mm, 3.5µm)
2	Mobile phase	Sodium dodecyl sulphate : Acetonitrile (60:40% v/v)
3	Flow rate	2.0 mL/min.
4	Column temperature	28°C
5	Volume of injection	20 µL
6	Detection wavelength	235 nm
7	Theoretical plate	2300
8	Retention time	4.152 min.
9	Tailing factor	1.29
10	Run time	8 min.

Typical chromatograms of drug free human plasma and spiked drug-plasma of Metformin hydrochloride are shown in figure2 and 3, respectively. The retention time of Metformin was found at 4.152 min indicating this method is faster than then reported methods. The typical column efficiency expressed as the number of

theoretical plates was found to be 2300.

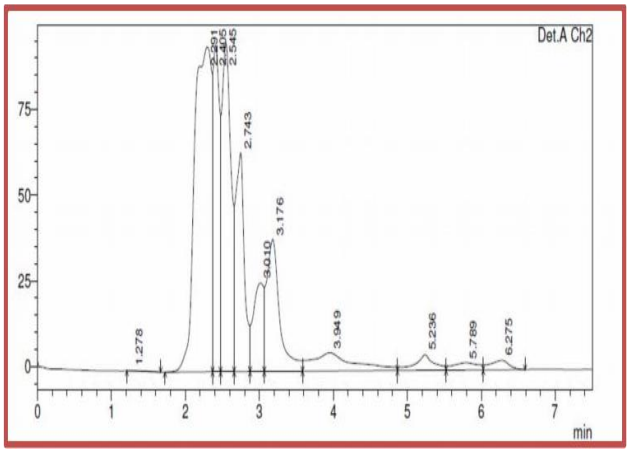

Figure 2: Chromatogram of blank human plasma

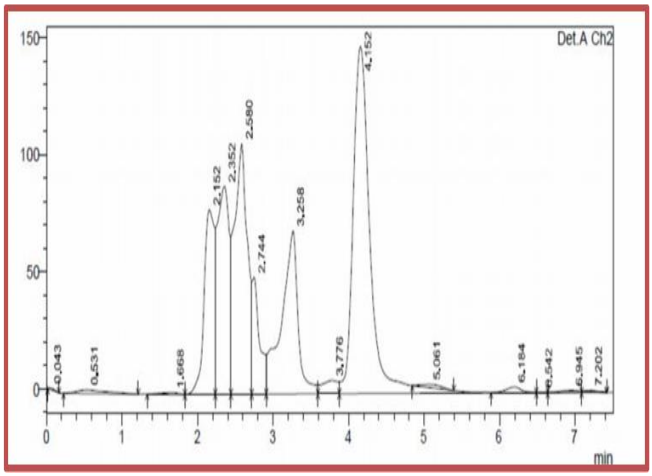

Figure 3: Chromatogram of spiked human plasma with Metformin hydrochloride

Linearity and Range

The calibration curve was found to be linear in the range 1-6 µg/mL (R^2 = 0.9989) and equation is y = mx+c, where y represents the area of Metformin hydrochloride and x represents concentration of Metformin in µg/mL. Linearity results of proposed method was shown in Figure 4.

Recovery

The recovery for the Metformin hydrochloride was determined by spiking known amount of drug into drug free human plasma to obtain three different concentrations covering the low medium and high ranges of the calibration curves. The recovery was calculated by comparing the Peak areas of the drug with those obtained from the pure standard in mobile phase at the same concentrations. The Mean percentage recovery of the Metformin hydrochloride ranges from 97.46% to 99.73%. the obtained results are within acceptable limits. The results of the recovery studies were shown in the Table 2.

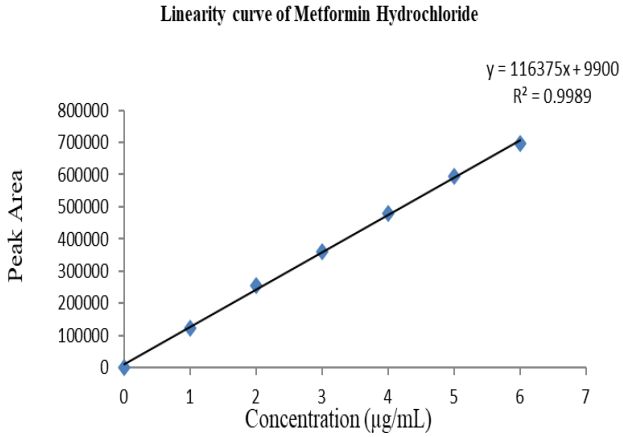

Figure 4: Calibration curve of Metformin

Table 2: Results of Accuracy study

	LQC	MQC	HQC
Mean (µg/mL) *	1.94	3.98	5.98
% Mean	94.46	99.76	99.73
S.D.	0.00471	0.00471	0.00471
% CV	0.242	0.118	0.078

**(N=5 and Concentrations are given in µg/mL)*

Stability Studies

The results of all stability studies are within acceptance criteria. The results of stock solution studies confirmed stability of stock solution.

Table 3: Results of stability studies

Stability	Parameter	Concentrations	
Stock solution stability	Mean Concentration (µg/ml)	1.94	5.98
	%Mean stability	97.1	98.74
	S.D.	0.00418	0.00707
	% CV	0.216	0.178
Freeze-thaw stability 3 cycles/24 hrs.	Mean Concentration (µg/ml)	1.92	5.85
	%Mean stability	96.02	97.5
	S.D.	0.00408	0.00691
	% CV	0.212	0.174

(N=3 and Concentrations are given in µg/ml)

CONCLUSION

The work describes in this paper deals with analysis of Metformin hydrochloride using RP-HPLC method in human plasma. The Linearity and recovery of the method met the acceptance criteria laid down in guideline for industry, Bioanalytical method validation, USFDA. Sufficient stability of both LQC and HQC was shown to allow for completion of sample analysis in clinical trials. From the results, we can conclude that developed method is simple, accurate, rapid and precise. Thus, it can be used for routine analysis of Metformin hydrochloride in human plasma.

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