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In-vivo studies of metformin modified release formulations

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ABSTRACT

The current study was undertaken to conduct *in vivo* studies and to establish the new validated bioanalysis for the determination of Metformin present in Blood Plasma by using the Reverse Mode-LC Method. The separation of the Metformin was carried out on Reverse mode LC using Shimadzu® LC - 10AT with the following Stationary Phase: Kromasil octadecyl silane column (25 cm x 4.6 mm i.d., 5μm) Eluent: Cyanomethane: 25 mM Pentane Sulfonic acid of pH 3.5. ratio 09:91 % v/v with 1.0 ml/min flow rate has been fixed, and this has been measured at 232 nm, and the sample volume will be 10 ul using Rheodyne 7725i injector. Based on the method established for Metformin, the drug peak is well resolved at 11.11 min and validated as per US FDA guidelines with respect to linearity, accuracy, precision, robustness ruggedness, and stability. The calibration curve was found to be linear over a range of 0.025 –1 μg/mL (r2 = 0.9999). The method has proved high sensitivity and specificity. Established method have been used to quantify the Pharmacokinetic parameters like C_{max}, T_{max}, AUC_{0-t} & AUC0-∞, K_{eli}, and $t_{1/2}$ studied and the values for reference formulation (660.05$\pm$91.52 ng/ml, 4.46 $\pm$1.10 h, 8280.41 $\pm$ 1356.39 g.h/ml, 9200.31$\pm$ 1569.26 ng.h/ml, 0.11$\pm$0.03 h^{-1}, and 6.96$\pm$1.53 h respectively) and the test formulation (705.06$\pm$102.58 ng/ml, 4.13$\pm$0.74 h, 8185.21$\pm$2101.56 g.h/ml, 8946.39$\pm$ 2457.66 ng.h/ml, 0.12$\pm$0.03 h^{-1}, and 6.06$\pm$1.61 h, respectively) were compared and found to be biologically equivalent. Based on the Pharmacokinetic and statistical analysis Test formulation of Metformin Hydrochloride containing 500 mg Metformin Hydrochloride (modified release formulations) is biologically equivalent to that of the Reference.

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INTRODUCTION

Metformin chemically N, N-Dimethyl-imido-dicarbonimidic diamide is an antidiabetic drug belonging to biguanide class that benefits the control of glucose in patients with type 2 diabetes by lowering both basal and postprandial plasma glucose level (Fun, 2003). It is slowly and incompletely absorbed from the GIT with a bioavailability of 50-60 %. (Bristol-Myers, 2002; Hawari *et al.*, 2007). Metformin is available as immediate-release formulation as well as modified-release formulations. Whenever want to release the product in the market concern company should study bioequivalence studies Bioequivalence (BE) studies are

concentrated to investigate the pharmacokinetic parameters of two pharmaceutical formulations of the same drug and to demonstrate the equivalence of their pharmacokinetic parameters (Davit *et al.*, 2013; Marathe *et al.*, 2000) For example, it involves a comparison between test (T) and reference drug formulation (R), where T and R can vary, depending on the comparison to be performed. BE studies can be assessed via plasma or urine data using the following parameters: (AUC) or the cumulative amount of drug excreted in the urine, Maximum concentration (C_{max}), or the rate of drug excretion in urine and Time of maximum concentration (T_{max}). The present study was undertaken to establish the new method for the quantification of Metformin in blood plasma and validated the developed method as per the US FDA guidelines as well as after validation, is to perform in vivo studies (Marathe *et al.*, 2000; Najib *et al.*, 2002) to prove the equivalence of test Metformin concerning the reference Metformin modified release dosage form.

MATERIALS AND METHODS

Solvents and Chemicals used

HPLC quality cyanomethane, AR grade of Pentane sulphonic acid, and ortho-phosphoric acid were procured from S.D. Fine Chemicals. HPLC quality aqua was obtained from the Milli-Q Reverse Osmosis system, and the working standards of Metformin were obtained from Tablets India, Chennai.

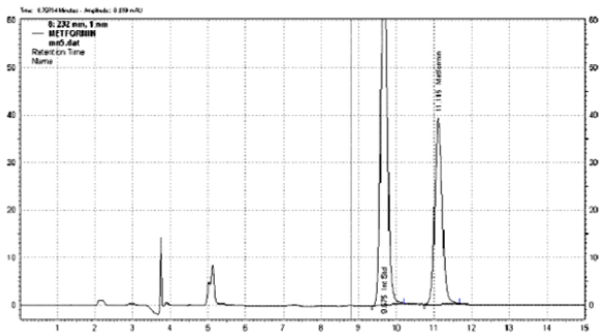

Figure 1: Typical chromatogram of standard metformin solution

Instruments and accessories used for the analysis

I. Waters gradient HPLC, Shimadzu LC-10AT –VP gradient HPLC, Agilent HPLC 1100, HPLC system were used for the analysis

II. Solid-phase Extractor

III. Following make of octadecyl silane analytical column with 25 cm length and particle size of 5μ, used

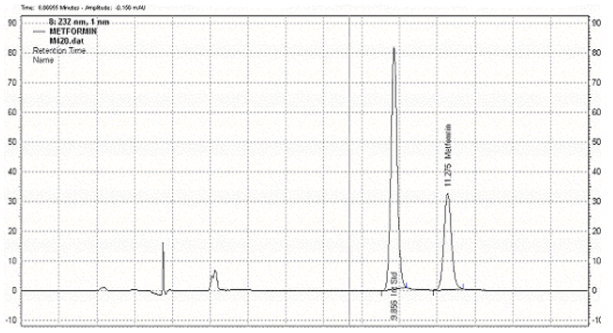

Figure 2: Typical chromatogram of sample metformin solution

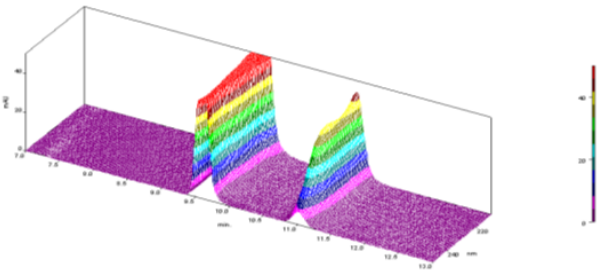

Figure 3: PDA chromatogram of metformin solution

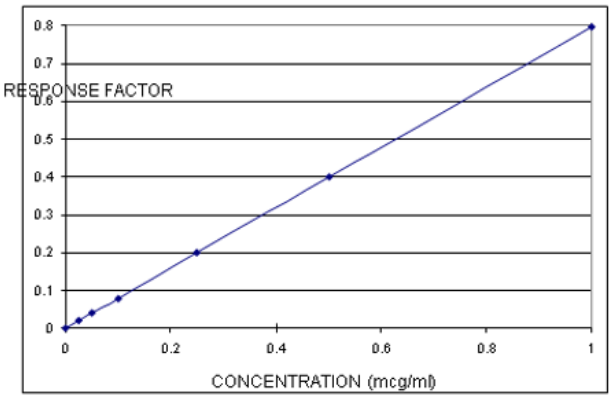

Figure 4: calibration curve for metformin

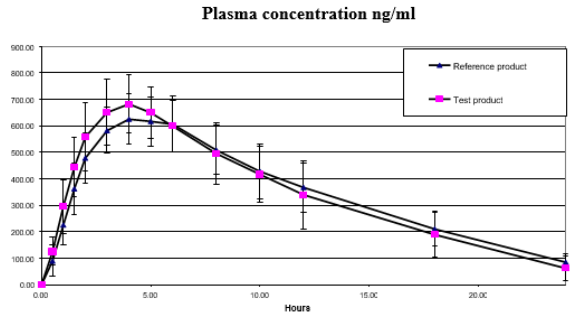

Figure 5: Mean Concentration: Time Curve for Metformin Modified Release formulation

Table 1: Recovery Studies

Level	Amount of metformin added (ng/ml)	Amount of metformin recovered (ng/ml) in a plasma sample	Recovery (%)	Amount of metformin recovered (%) in Mobile phase	Relative Recovery (%)
Level-I	25	23.91 ± 0.655	Mean: 95.64 CV: 2.62 N: 6	Mean: 99.045 CV: 1.138 N: 6	96.56
Level-II	500	479.42 ± 5.108	Mean: 95.88 CV: 1.02 N: 6	Mean: 98.891 CV: 1.017 N: 6	96.95
Level-III	1000	963.89 ± 15.128	Mean: 96.38 CV: 0.60 N: 6	Mean : 98.805 CV: 1.550 N: 6	97.54

Table 2: Linearity and range for metformin

Concentration of Metformin (mcg/ml)	Concentration of Metformin (mcg/ml)	Response Factor (RSD)
0.025	2.5	0.0199
0.050	2.5	0.0399
0.100	2.5	0.0797
0.250	2.5	0.1994
0.500	2.5	0.3990
1.000	2.5	0.7976

Table 3: System suitability studies for metformin

S.No	Parameters	Metronidazole (IS)	Metformin
1	Theoretical Plate	31243	22131
2	Resolution factor	2.21	
3	Asymmetric factor	1.03	1.01
4	LOD(ng/ml)	2	5
5	LOQ(ng/ml)	2	5

Table 4: Summary of results (n=24) for metformin

S. No	Parameters	Test formulation	Reference formulation	% Ratio
1.	AUC_{0-t} (ng.h/ml)	8185.21± 2101.56	8280.41± 1356.39	98.85
2.	AUC_{0-inf} (ng.h/ml)	8946.39± 2457.66	9200.31± 1569.26	97.24
3.	C_{max} (ng/ml)	705.06± 102.58	660.05± 91.52	106.82
4.	t_{max} (h)	4.13± 0.74	4.46± 1.10	92.52
5.	k_{eli} (h-1)	0.12± 0.03	0.11± 0.03	116.23
6.	$t_{\frac{1}{2}}$ (h)	6.06± 1.61	6.96± 1.53	87.03

such as – Lichrospher C18, Phenomenex Luna C18, and Kromasil C18.

Separation conditions

A Shimadzu® LC - 10AT HPLC / Waters HPLC systems was used for the analysis.

Stationary Phase: Kromasil octadecyl silane column (25 cm x 4.6 mm i.d., 5μ) Eluent: cyanomethane: 25 mM pentane sulfonic acid of pH 3.5, ratio 09:91 % v/v with 1.0 ml/min flow rate has been fixed, and this has been measured at 232nm, and the sample volume will be 10 ul using Rheodyne 7725i injector. The eluent was passed through a 0.22 membrane to remove the fibers and degassed by vibration technique. The experiments were performed out at ambient temperature.

Preparation of Metformin standard stock solution

Constituted 1 mg/ml solution by weighing 100mg of Metformin and dissolved and made the volume with 100ml of a mixture of cyanomethane and aqua. Labeled the container and stored below 8°C. [Figures 1, 2 and 3]

Preparation of Metformin standard solution

Constituted 10 ml each of 0.025, 0.050, 0.100, 0.250, 0.500, and 1 μg/ml of Metformin standard solutions using the Metformin standard stock solution and eluent and preserved at –20º $\pm$ 2 ºC until analysis.

Preparation calibration curve samples

Constituted 10 ml each of 0.100, 0.200, 0.400, 1, 2, and 4 mcg/ml of Metformin calibration curve samples using the Metformin standard stock solution and eluent and preserved at –20º $\pm$ 2 ºC until analysis. Pipetted 0.5 ml of the calibration curve sample into a 2.0 ml tube, 0.5 ml of 10 mcg/ml of internal standard solution, 0.5 ml of blank plasma, and 0.5 ml of a precipitating agent were added to the centrifuge tube. Vortexed the mixture for 5 min and centrifuged at 3500 rpm for 10 min. The supernatant layer has separated after centrifugation of CC samples and preserved for experimentation purpose. [Figure 4]

Preparation of quality control (QC) samples

Constituted 100 ml each of 0.100, 1.00, and 4.00 mcg/ml of Metformin Quality Control samples using the Metformin standard stock solution and mobile phase and stored at –70 $\pm$ 2 ºC until analysis. Transferred 0.5 ml of the Quality control sample into a 2.0 ml tube and then 0.5 ml of 10 mcg/ml of internal standard solution, 0.5 ml of blank plasma and 0.5 ml of a precipitating agent were added. Vortexed this mixture for 5 min and centrifuged the mixture at the speed of 3500 rpm for 10 min. Separated the supernatant layer and preserved for the experimentation purpose.

Preparation of blank plasma

Transferred 1 ml of blank plasma into a 2.0 ml tube and 0.5 ml of 10 mcg/ml of internal standard solution and 0.5 ml of a precipitating agent were added. Vortexed the resulting solution for 5 min and centrifuged the mixture at 3500 rpm for 10 min. After vortexing the solution, two layers were formed. Separated the supernatant layer and used for the analysis.

Preparation of plasma samples

Transferred 1 ml of plasma samples obtained from the volunteers into a 2 ml tube and 0.5 ml of 10 mcg/ml of internal standard solution and 0.5 ml of a precipitating agent were added like blank preparation. Vortexed the resulting solution for 5 min and centrifuged the mixture at 3500 rpm for 10 min. The supernatant solution was separated, and this layer was used for analysis [Figure 2]

Order of analysis

Injected 10 μl of each sample in the following order

Metformin standard solution, a blank solution containing the plasma, Calibration curve samples, Quality control samples, and plasma sample. Injected the Standard solutions, CC, QC, and Plasma sample solutions to the instrument with the above optimized chromatographic conditions and recorded the chromatograms for further process. The quantification of the chromatogram is performed using the response factor of the drug to an internal standard. The calibration curves are plotted routinely for spiked plasma containing Metformin and internal standard during the process of pre-study validation and in-study validation

RESULTS AND DISCUSSION

Validation of developed method as per USFDA bioanalytical method validation

Selectivity and sensitivity, accuracy [Table 1], and precision studies were observed as per the USFDA guidelines. All the values are confirmed within the criteria.

Linearity studies

Linearity studies have been studied by using the different concentrations of standard solutions of the metformin was prepared and analyzed along with the internal standard. After the analysis, the peak areas and response factors were calculated. As per the USFDA guidelines, the linearity and range were

calculated, and it showed the r2 value within the range. The results are presented in [Table 2].

Study of plasma sample stability

The stability studies of plasma samples spiked with Metformin were subjected to three Freeze-thaw cycles, Short term stability at ambient temperature for 3 hrs, and Long term stability at – 70± 2 º C over three weeks. No degradation was found in both short term and long term stability studies.

System suitability

The parameters, namely column efficiency, resolution, peak asymmetry factor, and capacity factor for the standard solutions was calculated [Table 3].

Limit of detection (LOD) & limit of quantitation (LOQ)

Determination of the LOD and LOQ has been calculated based on the signal-to-noise ratio. The data shows that the developed methods have adequate sensitivity. The values obtained demonstrated the suitability of the system for the analysis of the Metformin in plasma. [Table 3]

Robustness and ruggedness

Robustness and ruggedness studies established as per the US-FDA guidelines by changing the pH, flow rate, and it fell within the prescribed criteria.

Pharmacokinetic studies

The Pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-t} K_{eli}, and $t_{1/2}$ were calculated, and the blood level data of the Reference and Test formulation was studied, compared, and tabulated. Oral administration of a single dose of the Reference and the Test formulation in the fasting state exhibited Measurable Metformin in blood levels in all the volunteers from 0.50 hr onwards and noticed up to 18 hours in both the drug formulation. Established method have been used to quantify the Pharmacokinetic parameters like C_{max}, T_{max}, AUC_{0-t} & AUC0-∞, K_{eli}, and $t_{1/2}$ studied and the values for reference formulation (660.05±91.52 ng/ml, 4.46 ±1.10 h, 8280.41 ± 1356.39 g.h/ml, 9200.31± 1569.26 ng.h/ml, 0.11±0.03 h^{-1}, and 6.96±1.53 h respectively) and the test formulation (705.06±102.58 ng/ml, 4.13±0.74 h, 8185.21±2101.56 g.h/ml, 8946.39± 2457.66 ng.h/ml, 0.12±0.03 h^{-1}, and 6.06±1.61 h, respectively) [Table 4] and mean plasma concentrations[Figure 5] were compared and found to be biologically equivalent.

CONCLUSIONS

The developed and validated analytical method is accurate, precise, and it is linear as well as a selective and sensitive method for the quantification of Metformin. Based on the Pharmacokinetic data, namely, AUC_{0-t}, T_{max}, C_{max}, k_{eli}, Half-life and AUC_{0-inf} of the reference and the test formulations and their statistical analysis, Test formulation of Metformin Hydrochloride containing 500 mg Metformin Hydrochloride (modified release formulations) is biologically equivalent to that of the Reference.

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