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Research Article

A STUDY TO DETERMINE THE PHARMACOKINETIC PARAMETERS OF NEWLY DEVELOPED SUSTAINED RELEASE METFORMIN DRY SUSPENSION IN RABBITS

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Abstract

A simple, precise and rapid high-performance liquid chromatographic method using ultraviolet detection was developed for the determination of metformin in rabbit plasma. The method entailed direct injection of the plasma sample after deproteination using perchloricacid. The mobile phase comprised 10mM hexane sulphonic acid and 10 mM potassium dihydrogen orthophosphate (pH 3.0) and acetonitrile (85:15, v/v). Analyses were run at a flow-rate of 1.0 ml/min with the detector operating at a detection wavelength of 233 nm. The method is specific and sensitive, with a quantification limit of approximately 2.5µg/ml and a detection limit of 0.625µg/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery value was about 98.25% at HQC, 110.03 % at MQC and 107.46% at LQC while the within-day and between-day coefficient of variation of the assay method were all less than 15%. The calibration curve was linear over a concentration range of 2.5–40 µg/ml.

Keywords: Metformin HCL, Rabbit Plasma. Suspension, Caffeine

1. Introduction

Metformin hydrochloride (1,1-Dimethylbiguanide monohydrochloride) is an oral antihyperglycemic drug which is used for the treatment of type 2 diabetes mellitus. Type 2 diabetes mellitus is insulin dependent diabetes. Metformin belongs to the biguanide class of antidiabetics. Metfromin is either used alone or with other anti-hyperglycemics (such as sitagliptin or sulphonylureas) Structure in Fig-1.

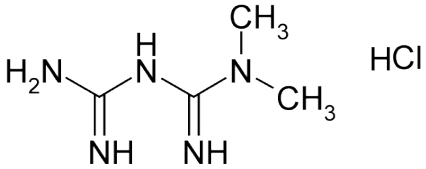

Fig-1: Metformin Hydrochloride.

Metformin it exerts its antidiabetic effects primarily by increasing hepatic insulin sensitivity and the resultant suppression of hepatic glucose output[2]. It may also modestly enhance glucose uptake in peripheral tissues and increase glucose metabolism in the splanchnic bed.[3]

Metformin does not alter location of GLUT4 (the major glucose transporter in skeletal muscle), they enhance GLUT1 transport from intracellular site to plasma membrane. The effect thus differs from that of insulin. It does not stimulate β pancreatic cells. Metformin do not cause insulin release, presence of some insulin is essential for their actions.[1]Potential advantage of metformin weight loss or lack of weight gain.[4]

Metformin is a small highly polar molecule (pKa = 2.8, 11.5, logPoctanol: water = 2.6) which has great solubility in water and poor solubility in lipids so it is not easy to extract it from the aqueous plasma matrix [5,6]. Several analytical methods based on HPLC were reported for the determination of metformin hydrochloride in plasma and urine of human.

Some of these reported methods use gradient mobile phases and solid phase extraction technique which is tedious. Other methods use large quantities of acetonitrile which a costly solvent and others do not internal standard which is very important. So there was a need to develop a method for the determination of metformin from rabbit plasma by using HPLC-UV which is sensitive, specific, fast and economical. The sample preparation method is a single step protein precipitation method hence it is faster and less cumbersome. [7-12]

Caffeine was used as a internal standard in this study. The main reason for using caffeine was its easy availability and that it is cheap. So the method becomes more cost effective.

2. Materials and Methods

Metformin hydrochoride was procured from Aarti Drugs Ltd, Mumbai, India, as a gift sample. Caffeine was used as an internal standard and was purchased from Loba Chemie Pvt Ltd. Analytical grade Hexane Sulphonic acid, Potassium Dihydrogen phosphate, Perchloric acid, Orthophosphoric acid was purchased from Loba Chemie Pvt Ltd. HPLC grade Acetonitrile and Water was purchased from Loba Chemie Pvt Ltd .

HPLC C18 column HiQSil Advance (4.6 mm x 250 mm, 5um diameter) was purchased from Anatek Services Mumbai. The instrument used was SHIMADZU LC 2010, with a quaternary pump and auto sampler and UV detector(operated at 233.2 nm). The column used was C18 HiQsil Advance (4.6 mm x 250 mm, 5um diameter). The temperature of the column was maintained at 40ºC throughout the analysis. Auto sampler temperature was maintained at 15ºC.

2.1 Preparation of Mobile Phase

2.1.1 Solution A: 10mM Hexane Sulphonic Acid and 10mM Potassium Dihydrogen Phosphate Solution (pH 3.0 ± 0.05).Approximately 2851mg of Hexane Sulphonic Acid and 4510 mg of Potassium Dihydrogen Phosphate into 1000 mL volumetric flask was weighed and diluted with HPLC-water up to the mark; pH of the solution was adjusted to 3.0 ± 0.05 with Orthophosphoric Acid.It was then filtered through a 0.2 µ nylon filter paper. The solution is then sonicated in an ultrasonicator for 5 to 10 minutes.

2.1.2 Solution B

Acetonitrile HPLC grade

The mobile phase consisting of 85% Solution A and 15% Solution B was pumped in to the system at a flow rate of 1 ml/min. Peak area was measured and was used for quantitative estimation.

2.1.3 Dilution Solution

A mixture of Acetonitrile (HPLC grade) and HPLC- water in the volume ratio of 50:50 v/v, was prepared as dilution solution. The solution was sonicated in an ultrasonicator for 5 to 10 minutes.

2.2 Preparation of Stock Solution

2.2.1 Preparation of Metformin HCl Stock Solution

About 10 mg of Metformin HCl reference standard was weighed and transferred to 10 mL volumetric flask. It was dissolved in HPLC grade Water and volume was made up with the same to produce approximately of 1000 µg/mL concentration of Metformin HCl. The stock solution of metformin HCl was diluted to give the calibration standards of 2.5, 5, 10, 20, 40 µg/ml. The quality control standards were independently prepared at concentration of 8, 16 and 32µg/ml.

2.2.2Preparation of Caffeine Stock Solution

About 10 mg of caffeine reference standard was weighed and transferred to a 10 mL volumetric flask. It was dissolved in HPLC grade methanol and the volume was made up with the same to produce approximately 1000 µg/mL of Caffeine. The stock dilution of Caffeine of concentration 250µg/mL was prepared in Dilution Solution [mixture of HPLC water: Acetonitrile (50:50) v/v] for preparation of internal standard and stored in refrigerator

2.3 Sample Preparation

The plasma samples were prepared as per the required concentration. 50 µL of caffeine solution which is a Internal standard dilution was added to eppendorf tube except in blank. The sample 380 µL was pipetted into test tube and

vortexed, followed by addition of 40µL of perchloric acid and vortex again. The samples were the centrifuged at 10000 rpm for 5 minutes. The supernatant was filled into vials. Samples were analyzed on HPLC-UV.

3. Validation

3.1 Linearity

A linearity consisted of one processed Blank matrix, One processed Blank matrix with internal standard. Spiked calibration standards from 2.5 to 40 µg/ml linearity were performed in triplicate.

3.2 Precision and Accuracy

Precision and accuracy batch consisted of three low quality control samples (approximately 3 times of the LQC), three middle quality control samples (MQC, ~Middle of Range) three high quality control samples (HQC, ~ 70 to 85% of ULOQ).

3.3 Recovery Studies

Standard calibration curve were constructed by spiking drug free pooled plasma with known amount of metformin in the concentration range of 2.5µg/ml to 40µg/ml. These plasma standards were also used to determine the within day and between day precision and accuracy (n=3). In addition absolute recovery (n-=3) was estimated by comparison with unextracted sample by extracting matrix blank and fortified with the analytes and IS dilutions after extraction, representing a 100 % extraction of QC samples at low, middle and high concentration for analyte and middle concentration for IS. Inject and analyze three sets at each level.

3.4 Freeze Thaw Stability

Three sets of quality control samples (LQC, MQC and HQC) from freezer after at least 24 hours of freezing were withdrawn and thawed at room temperature. When completely thawed the samples were again refreezed. After minimum of 12 hours, these samples were again withdrawn and thawed at room temperature. When these samples were completely thawed they were again refreezed. On the day of stability evaluation (after a minimum of 12 hours), samples were withdrawn and thawed the samples completely at room temperature. All the quality control samples were processed as per the extraction process and analysed.

3.5 Post preparative Stability (Autosampler Stability)

Three sets of LQC, MQC and HQC and process as per extraction procedure, to facilitate injection at proposed stability period were withdrawn. The processed samples were kept in autosampler. Autosampler stability duration as the time of injection of first QC less the time of placing in autosampler was calculated.

4. Animal Study

Animal Study was carried protocol approved by the ethic committee (protocol number). The plasma samples from animal studies were analyzed using the developed bioanalytical method on HPLC.

All the pharmacokinetic calculations were carried out. Pharmacokinetic parameters for metformin hydrochloride, following oral administration, were determined from the plasma concentration time data. The maximum plasma concentration (Cmax) and the corresponding time (tmax) were obtained directly from the individual plasma concentration time data. The area under the plasma concentration time curve (AUC) were estimated by linear trapezoidal rule and extrapolated to infinity using standard techniques.

5. Result and Discussion

5.1 Chromatogram

Chromatogram of drug free plasma samples and spiked calibration samples, Spiked Quality Control samples and post dosing the rabbits are shown in fig A, B and C respectively. From the chromatograms it is understood that the method is selective for Metformin and internal Standard as no endogenous interfering peak of plasma is observed at the retention time of Metformin and Internal Standard.Fig-2, Fig-3, Fig 4.

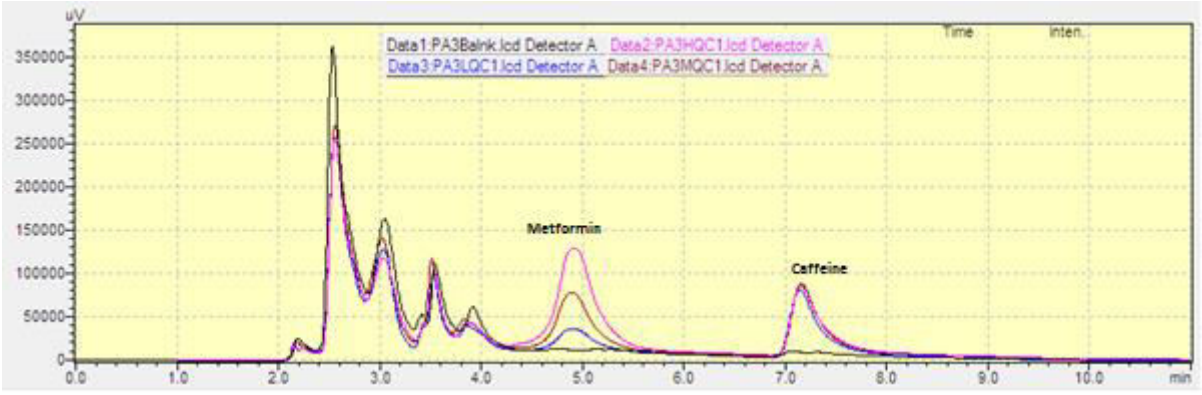

Fig-2: Chromatogram Quality Control Overlay Plot

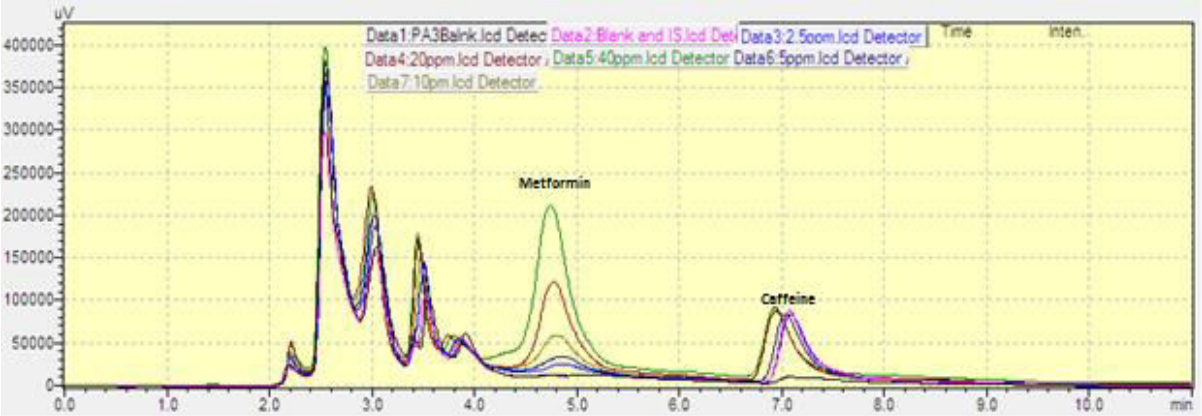

Fig-3: Chromatogram Calibration Curve overlay plot

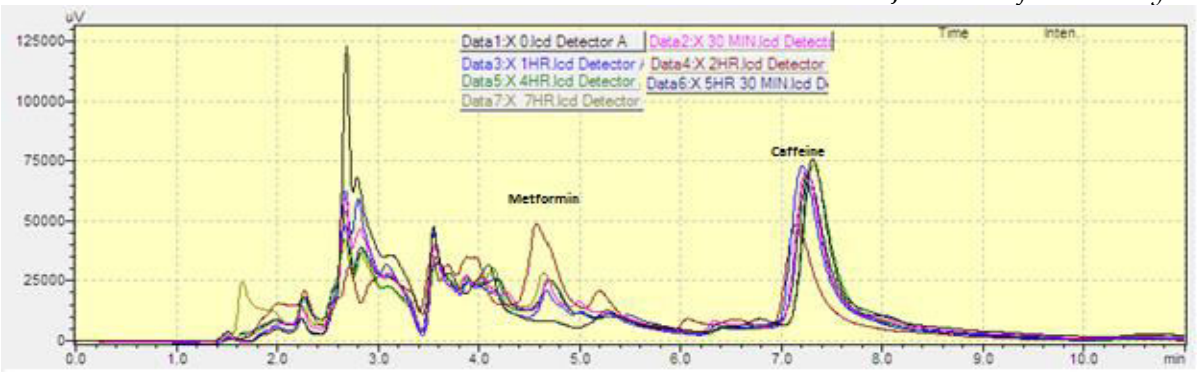

Fig-4: Overlay Cromatograms obtained from plasma drug analysis at the end of 0.5 hrs, 1 hr, 2 hrs, 4 hrs, 5.5 hrs and 7 hrs.

5.2 Validation

5.2.1 Linearity:

The calibration curve was found to be linear in the range of 2.5µg/ml to 40µg/ml. The %CV at each concentration level was found to be less then 9.We would like to claim that the method can detect concentration as low as 0.625µg/ml but was found not to be quantitative in reproducible manner although linearity was found acceptable but it was not found homoscedasticity.The other details are mentioned in the Table-1 and Fig-5.

Table-1: Concentration-response Data for Linearity.

Concentration-response Data for Linearity								
CC ID#	STD A	STD B	STD C	STD D	STD E	Slope	Intercept	R-Square
Nominal Concentration (µg/ml)								
	2.5	5	10	20	40			
CC 01	1.94	4.95	10.56	20.29	39.75	0.608	0.0485	0.9992
CC02	2.45	5.39	10.18	19.17	40.34	0.0586	-0.0995	0.9989
CC03	2.71	4.74	9.20	21.24	39.59	0.0522	0.0132	0.9974
Mean	2.58	5.07	9.69	20.20	39.97			
S.D (+/-)	0.19	0.46	0.69	1.47	0.53			
C.V. (%)	7.22	8.99	7.14	7.27	1.33			
% Nominal	103.06	101.33	96.93	101.02	99.91			
N	3	3	3	3	3			

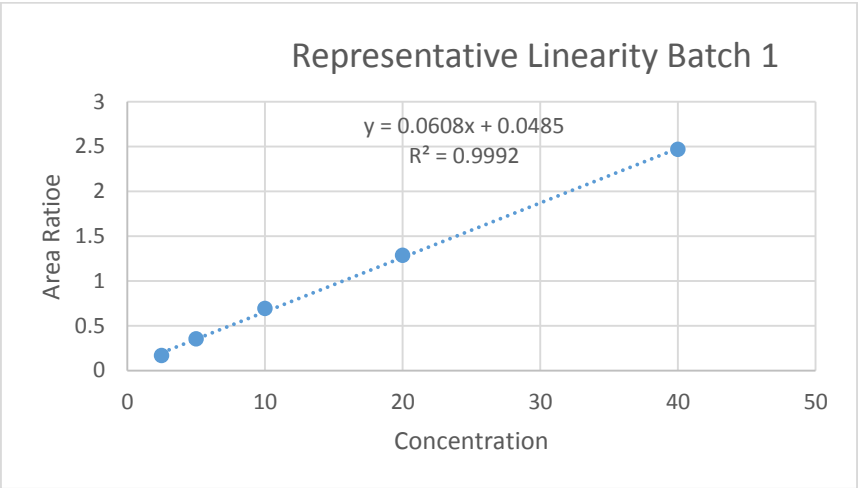

Fig-5: Representative Linearity Batch 1

5.2.2 Precision and Accuracy

The method was found to be Precise and accurate. %CV and Mean for within batch precision and accuracy, Intraday precision, Between Batch/Inter day Precision was found to be less 15 % respectively. The data obtained is presented in the given Table-2, 3, 4, 5, 6.

Table-2: Within Batch Precision and Accuracy for Metformin Hydrochloride for Batch-1.

Within Batch Precision and Accuracy for Metformin HCl			
	HQC	MQC	LQC
PA 1.1	29.59	15.40	9.32
PA 1.2	30.34	12.53	7.86
PA 1.3	28.11	13.11	7.90
MEAN	29.35	13.68	8.36
SD	1.14	1.52	0.83
RSD	3.88	11.12	9.95
%Nominal	91.70	85.50	104.48
N	3	3	3

Table-3: Within Batch Precision and Accuracy for Metformin Hydrochloride for Batch-2.

Within Batch Precision and Accuracy for Metformin HCl			
	HQC	MQC	LQC
PA 2.1	31.10	13.02	8.12
PA 2.2	30.57	15.06	6.95
PA 2.3	27.30	14.65	6.24
MEAN	29.66	14.25	7.10
SD	2.06	1.08	0.95
RSD	6.95	7.57	13.35
%Nominal	92.68	89.03	88.80
N	3	3	3

Table-4: Within Batch Precision and Accuracy for Metformin Hydrochloride for Batch-3.

Within Batch Precision and Accuracy for Metformin HCl			
	HQC	MQC	LQC
PA 3.1	30.07	14.30	7.47
PA 3.2	28.06	14.83	6.44
PA 3.3	30.17	14.20	6.71
MEAN	29.43	14.45	6.87
SD	1.19	0.34	0.53
RSD	4.06	2.34	7.78
%Nominal	91.98	90.29	85.93
N	3	3	3

Table-5: Intraday Precision and Accuracy.

Intra day Precision and Accuracy			
	HQC	MQC	LQC
PA 2.1	31.10	13.02	8.12
PA 2.2	30.57	15.06	6.95
PA 2.3	27.30	14.65	6.24
PA 3.1	30.07	14.30	7.47
PA 3.2	28.06	14.83	6.44
PA 3.3	30.17	14.20	6.71
MEAN	29.55	14.35	6.99
SD	1.51	0.72	0.70
RSD	5.11	5.04	10.02
%Nominal	92.33	89.66	87.36
N	6	6	6

Table-6: Between batch/Inter day Precision and Accuracy.

Between batch/Interday Precision and Accuracy			
	HQC	MQC	LQC
PA 1.1	29.587785	15.403262	9.318321
PA 1.2	30.343426	12.525617	7.859503
PA 1.3	28.105204	13.111191	7.897801
PA 2.1	31.099622	13.024491	8.119826
PA 2.2	30.57421	15.06475	6.949965
PA 2.3	27.298187	14.646739	6.241955
PA 3.1	30.070392	14.304625	7.472041
PA 3.2	28.055847	14.831903	6.441097
PA 3.3	30.174229	14.203034	6.709149
MEAN	29.478767	14.123957	7.445517
SD	1.3269646	1.0077392	0.973651
RSD	4.5014251	7.1349638	13.07701
%Nominal	92.121147	88.274731	93.06897
N	9	9	9

5.2.3 Recovery Studies

The recovery studies we can claim that the recoveries were acceptable at each MCQ level. The data is mentioned in following table-7.

Table-7: Recovery of Metformin HCL from Rabbit plasma.

Recovery of Metformin HCL from Rabbit plasma						
Metformin Rec	Metformin Response		Metformin Response		Metformin Response	
	Extracted(HQC)	Unextracted (HQC)	Extracted (MQC)	Unextracted (MQC)	Extracted (LQC)	Unextracted (LQC)
1	3143400	3127889	1550186	1300992	684052	692611
2	3116304	3247410	1384553	1215457	688856	634978
3	3216304	3243246	1466790	1483811	707591	608554
Mean	3158669.33	3206181.67	1467176.33	1333420.00	693499.67	645381.00

S.D	**51719.09**	**67835.40**	**82817.18**	**137084.46**	**12437.60**	**42983.27**
%C.V	**1.64**	**2.12**	**5.64**	**10.28**	**1.79**	**6.66**
N	**3**	**3**	**3**	**3**	**3**	**3**
%Recovery	**98.52**		**110.03**		**107.46**	

5.2.4 Freeze Thaw stability and Autosampler Stability

The drug was found to be stable in Freeze Thaw cycles and auto sampler at HQC and LQC level it marginally fails at MQC level but we would like to claim that these are errors due to processing. If the total QC levels are considered the batch can be considered to be passing the criteria. Results are mentioned in Table 8 and Table 9.

Table-8: Freeze Thaw Stability Data.

Freez Thaw Cycle 3 Stability Data			
S.No.	**HQC**	**MQC**	**LQC**
	Concentration (µg/mL)		
	32	**16**	**8**
1	**28.73**	**17.27**	**7.11**
2	**27.27**	**15.04**	**6.93**
3	**26.35**	**12.65**	**6.70**
Mean	**27.45**	**14.99**	**6.91**
S.D. (+/-)	**1.20**	**2.31**	**0.20**
C.V. (%)	**4.37**	**15.41**	**2.96**
%Nominal	**85.79**	**93.66**	**86.42**
N	**3**	**3**	**3**

Table-9: Autosampler Stability Data.

Auto Sampler Stability Data			
S.No.	**HQC**	**MQC**	**LQC**
	32	**16**	**8**
1	**28.73**	**17.27**	**5.62**
2	**26.44**	**15.04**	**5.79**
3	**27.27**	**12.65**	**5.69**
Mean	**27.48**	**14.99**	**5.70**
S.D. (+/-)	**1.16**	**2.31**	**0.09**
C.V. (%)	**4.22**	**15.41**	**1.53**
%Nominal	**85.88**	**93.66**	**94.94**
N	**3**	**3**	**3**

6. Animal Study

All the pharmacokinetic calculations were carried out. Pharmacokinetic parameters for metformin hydrochloride, following oral administration, were determined from the plasma concentration time data. The maximum plasma concentration (Cmax) and the corresponding time (tmax) were obtained directly from the individual plasma concentration time data. The area under the plasma concentration time curve (AUC) were estimated by linear trapezoidal rule and extrapolated to infinity using standard techniques. The plasma drug time profiles of all subjects

are as shown in Fig-6. The average plasma drug time profile is shown in Fig-7. Based on log plasma concentration time profile graph, pharmacokinetic parameters were calculated which were as shown in Table-10.

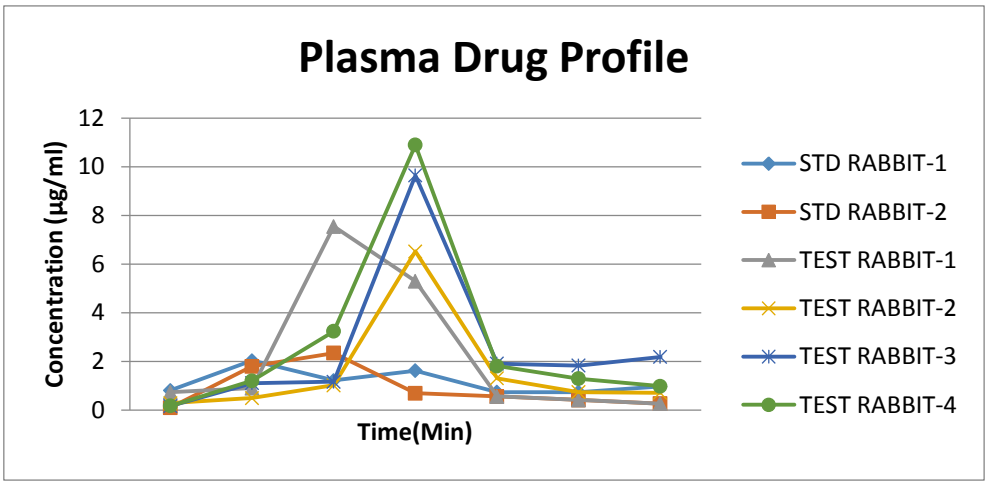

Fig-6: Plasma drug profile of Metformin HCl in rabbit (Standard and Test Formulation).

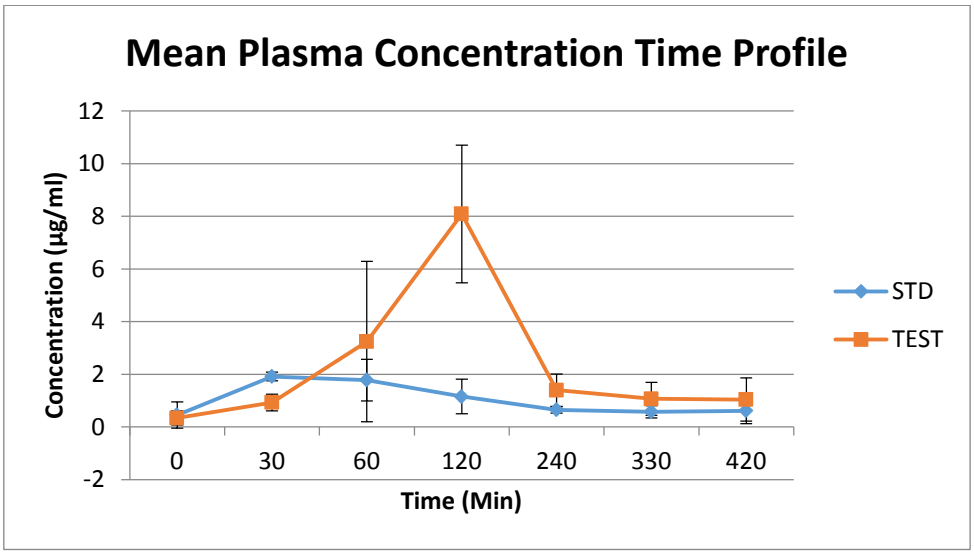

Fig-7: Log plasma concentration time profile.

Table-10: Pharmacokinetic Parameters.

Parameter	Standard	Test
Tmax	30 min	2 hrs
Cmax	1.92	8.09
AUC from 0 to 12 hrs	6.603 mg/Lhr	19.936 mg/Lhr
AUC from 0 to infinity	9.325 mg/Lhr	22.787 mg/Lhr
Elimination rate constant	0.18077 hr^{-1}	0.29493 hr^{-1}
Elimination half life	3.83448 hrs	2.35025 hrs
Relative Bioavailability from 0 to 12 hrs	33.12%	100%

7. Conclusion

We would like to claim that the method developed to estimate metformin using caffeine as internal standard was suitable to analyse the drug concentration from rabbit plasma. The applied method proved that the sustained release suspension was significantly better than the plain drug. Plain drug was used since the sustained released tablet was difficult to ingest in rabbit and sustained release tablet could not be crushed. This formulation is a very good alternative to sustained release tablet.

8. Acknowledgement

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