

Estimation and pharmacokinetics of metformin in human volunteers

D. Bhavesh*, G. Chetan, K. M. Bhat¹ and Shivprakash

Synchron Research Services Private Limited. Ahmedabad – 380 054

¹Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal 576 104

E – mail : bhavesh@synchronresearch.com

ABSTRACT

A simple and rapid HPLC assay method for the estimation of metformin in human plasma was developed and validated. The method totally eliminates the extraction procedure. The plasma proteins were precipitated using perchloric acid : acetonitrile (50%v/v) mixture and the supernatant liquid was removed, dried under nitrogen, reconstituted in mobile phase and injected into the HPLC system. The separation was achieved with a cationic exchange column (Hihchrom, 250X4.6mm) with mobile phase of methanol: potassium di-hydrogen orthophosphate buffer (0.1M, pH 3.5) mixture 46 : 54 % v/v and elevated temperature of 40 °C. Detection was by UV detector at 236nm. The retention time (RT) observed are at around 13 and 16 minutes for metformin and phenformin respectively. The response was linear over a range of 30-5000 ng ml⁻¹. The same method was used for the bioequivalence study of two metformin formulations in healthy, human, Indian, male volunteers.

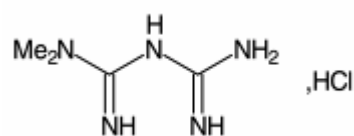
INTRODUCTION

Metformin hydrochloride is an oral biguanidine, which reduces the elevated blood glucose concentration in patients with diabetes but does not increase insulin secretion. It does not lower the blood glucose in non-diabetic subjects¹. Augmentation of muscular glucose uptake and utilization, and reduction of increased hepatic glucose production through an antigluconergic action explain the blood glucose lowering effect^{2,3}. Metformin is safe⁴ and not teratogenic⁵ in many of the species studied.

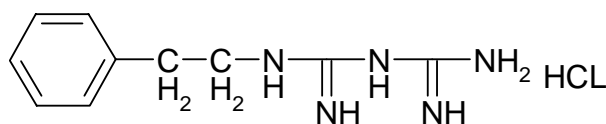
Oral bioavailability of metformin is about 50 - 60% and fecal recovery is about 30%⁶. The rate of absorption was slower than that of elimination, which resulted in a plasma concentration profile of “flip-flop” type for oral metformin⁷.

Many HPLC methods for the analysis of metformin in plasma are reported. But most of the methods use either ion pair reagent^{8,9,10} or cation exchange column¹¹. Some methods reported require elaborate sample preparation^{12,13}. Though, these methods are sensitive and reproducible, RP-HPLC method for the estimation of metformin in human plasma are found

to be more suitable. The same method has also been utilized for the Bioequivalence study of metformin hydrochloride formulations.



Metformin hydrochloride



Phenformine hydrochloride

MATERIALS AND METHODS

Metformin and phenformin were obtained from Medrich Sterilab, Bangalore, India. Methanol and acetonitrile of HPLC Grade and potassium dihydrogen orthophosphate, glacial acetic acid, ammonium hydroxide and perchloric acid of GR Grade were purchased from E. Merck (India) Ltd., Mumbai.

Chromatographic conditions:

Analysis was performed using Merck Hitachi System containing a pump (L-7100), UV-Visible Detector (L-7400), auto sampler (L-7200) with peltier cooler, column oven (L-7350). The data processing was done

with the help of MHSM software through D-7000 interface.

The analytical column used was cation exchange column (NC1005SA, 250X4.6mm Hihchrom). The mobile phase was a mixture of methanol and buffer at the ratio of 46:54% v/v. The buffer was 0.1M potassium dihydrogen orthophosphate of pH 3.5 adjusted with glacial acetic acid or ammonium hydroxide. The flow rate was maintained at 1.0 ml min⁻¹ and the eluents were monitored at 236nm. The column was maintained at 40⁰C.

Pharmacokinetic Studies

The test drug metformin hydrochloride (850 mg) of Medrich Sterilab, India and the reference Glycolphage (850 mg) of Lipha Pharma, UK was procured from Medrich Sterilab, India and both test and reference tablet were administered to 12 healthy human male Indian volunteers in a double blind, randomized, cross over design. The washout period was seven days. The volunteers were selected on pre set inclusion-exclusion criteria. The volunteers were screened for vital signs, blood and urine analysis before enrolment. The tablets were administered with 240 ml of potable water at ambient temperature. 7 ml of blood samples were withdrawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 18.0, 24.0 hours post dose. The samples were stored at -20⁰C for analysis. A concentration time curve was plotted and Area Under Curve (AUC) was calculated by linear trapezoidal rule (AUC_{0-∞}). Maximum Plasma Concentration (C_{max}) and Time to achieve the maximum concentration (t_{max}) was obtained directly from the concentration time curve without interpolation. All the pharmacokinetic and statistical data were calculated using the software *Kinetica*. (Innaphase, USA)

Sample Extraction

100µl of metformin hydrochloride solution of appropriate concentration and 100µl of phenformin hydrochloride solution (20µg ml⁻¹) were added to 900 µl of drug free plasma contained in a clean 5 ml Ria Vial and was properly mixed. To this 50 µl of protein precipitating agent (perchloric acid : acetonitrile 50%v/v each) was added and was vortexed for 30 seconds. After centrifugation at 3000 rpm for 10 minutes, 700 µl of the supernatant was evaporated to dryness at 45⁰C under nitrogen. The residue was

reconstituted in 100 µl of mobile phase and 80 µl of this was injected to the HPLC system.

Method validation

The linearity of the method was investigated by serially diluting a stock solution of metformin (in methanol; 1.0 mg/ml) with drug free plasma to concentrations in the range 30-5000 ng/ml and subjecting 100 µl of each of these solutions to the proposed assay method. Calibration curves were constructed by plotting the ratio of peak height of metformin to phenformin (Internal Standard) against the concentration of metformin added.

Analyte recovery was determined by comparing the ratio of peak height of metformin to internal standard for the standard preparations against those of same preparations in mobile phase. Interday assay reproducibility was assessed over a period of 4 days at 100, 3000 and 4750 ng/ml concentration. Intraday analysis was determined upon replicate analysis of 8 check samples at same concentrations.

RESULTS

The precision of the retention times observed for metformin and Internal Standard (n=6), expressed as RSD were less than 0.5%. The representative chromatograms of blank human plasma and overlay chromatograms of three Quality Controls plasma with metformin and Internal Standard shown in figure 1 and figure 2 respectively indicate that there are no endogenous interfering components co-eluting with metformin or the Internal Standard.

Linear regression results for calibration curves performed on 4 different days showed mean correlation coefficients (r²) of more than 0.99 and slope of 1082.75± 5.62 .

Table1 summarizes the assessment of both interday and intraday reproducibility of the method. Data presented in table 1 are the coefficients of variability (CV%) for each check sample processed. The extraction yield (recovery) was calculated by comparing extracted samples with unextracted samples at two different concentration levels. The data is given in table 2. Absolute recovery of metformin from plasma was found to be greater than 72%.

Evaluation of the effect of short-term storage of extracted plasma samples on the standard curve characteristics and chromatographic behaviour of

Table 1: Intraday and Interday variability of the assay of quality control samples at three concentration levels.

Added concentration of ng/ml	Intra Day			Inter Day		
	Mean analysed ± S.D. ng/ml (n=24)	%CV	%Bias	Mean analysed ± S.D. ng/ml (n=24)	%CV	%Bias
100	105.24±11.03	10.48	-5.23	98.97±9.94	10.05	1.03
3000	2941.70±79.6	2.70	1.94	2897.64±120.76	4.04	0.41
4750	4749.03±128.03	2.70	0.02	4805.03±139.69	2.90	-1.15

Table 2: Percent yield in Human Plasma (Metformin)

Sr. No.	% Yield (recovery)	
	100 ng/ml	4750 ng/ml
1	76.48%	66.72%
2	85.18%	73.18%
3	81.69%	77.06%
Mean analysed ± S.D.	81.12±4.38	72.32±5.22

Table 3: Freeze-Thaw effect on Metformin Recovery

Concentration (ng/ml)	Mean (n = 3)	% CV
100	99.60	3.20
4750	4403.56	3.19

Table 4: Pharmacokinetic Data of Metformin

Parameter	Test	Reference	Ratio	90% C.I.
AUC ₀₋₂₄ (ng h ⁻¹ ml ⁻¹)	5320.0808±1130.43	5119.178±2052.981	1.202	107.08-114.02
AUC _{0-inf} (ng h ⁻¹ ml ⁻¹)	5721.8925±1189.5	5841.848±2266.634	1.119	101.62-106.76
C _{max} (ng ml ⁻¹)	769.0368±177.75	773.682±207.807	1.0834	97.39- 103.34

metformin and Internal Standard were also performed. Regression analysis of the standard curve data gave correlation coefficients and values for the slope and Y-intercept within the same order of magnitude following storage of samples at -20⁰ C from 1 - 4 days. The chromatographic behaviour was also unaffected by storage of extracted plasma samples in auto sampler at 20⁰C for 12 h. Freeze-thaw analysis of 3 cycles did not show any major degradation of metformin. The results are shown in table 3. Overlay graph of mean concentration v/s time curve of the two formulations (Test and Reference) is shown in figure 3. The AUC

from 0 to 24 h was determined by linear trapezoidal rule. The extrapolation to infinity was calculated using a linear regression model from the terminal data points in the elimination phase that has been log transformed. Maximum concentration achieved (C_{max}) was obtained directly from the measured concentration without interpolation. Assuming the multiplicative models expected medians of these parameters of the test and reference formulations were computed and presented in table 4 as their ratios. The confidence intervals suitable for bioequivalence testing were found well within the range of 0.8-1.25.

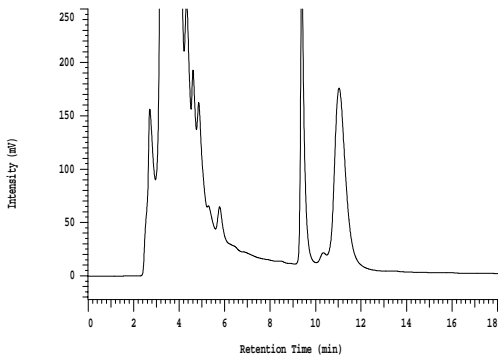


Figure 1: Representative chromatogram of blank human plasma

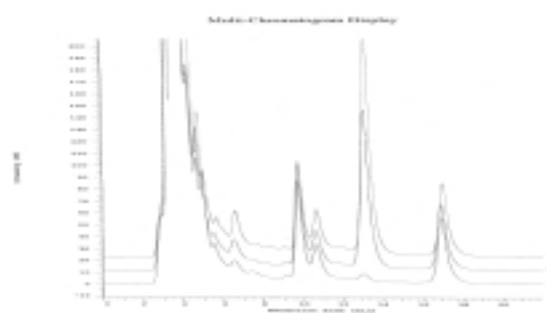


Figure 2: Overlain chromatogram of three Quality Controls

Legends:

- a _____ Chromatogram of LQC
- b _____ Chromatogram of MQC
- c ----- Chromatogram of HQC

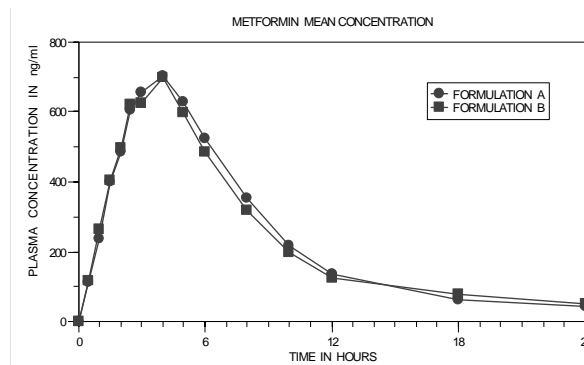


Figure 3: Mean concentration v/s time curve of the two formulations

CONCLUSION

The HPLC assay method described here is simple, precise and accurate for quantitation of metformin in human plasma. The sensitivity, simplicity and rapidity of the method were the main advantages of the method. The method was cross checked by different analyst and equipment and was found to be rugged and robust. The method can be conveniently used for the therapeutic monitoring and pharmacokinetic studies of metformin.

REFERENCES

1. Hermann L. S. Clinical Pharmacology of biguanides, In: Kulhmann I, Plus W editor. Hand Book of Experimental Pharmacology. Hiedelberg: Springer Veelog; 1995. p. 374 - 407.

2. Bailey C. J. Biguanides and NIDDM. Diabetes Care .1992 Jun; 15(6): 755-772.
3. Hermann L. S. Metformin: a review of its pharmacological properties and therapeutic use. Diabete metab. 1979 Sep; 5(3): 233-45.
4. Duval D. 1959 Pharmacological study of N,N,Dimethyl guanyl guanidine (LA 6023), Therapie. 1959; 14(1): 70-78.
5. Denno K. M., Saddle T. W. Effects of biguanidine class of oral hypoglycemic agents on mouse embryogenesis. Teratology. 1994 April; 49(4): 260-266.
6. Tucker G. T., Casey C, Philips P. J, Connor H, Ward J. D, Wooda H. F. 981 Metformin kinetics in healthy subjects and in patients with diabetes mellitus. British J. of clinical pharmacology. 1981 Aug; 12(2): 235-246.

7. Pentikainen P. J., Neuvonen P. J., Pentilla A. Pharmacokinetics of Metformin after intravenous and oral administration to man. *Eur. J. Clin. Pharmacol.* 1979 Sep; 16(3): 683-693.
8. Zarghi A., Foroustan S. M, Shafaati A, Khoddam A. Rapid determination of Metformin in human plasma using ion-pair HPLC. *J. Pharm Biomed Anal.* 2003 Feb. 5; 31(1), 197-200.
9. Zhang M, Moore G. A, Lever M, Gardiner S. J, Kirkpatric C. M, Begg E. J. Rapid and simple high-performance liquid chromatography assay for the determination of Metformin in human plasma and breast milk. *J. Chromatogr B Analyt Technol Biomed Life Sci.* 2002 Jan 5; 766(1) 175-179.
10. Cheng C. L, Chou C. H. Determination of Metformin in human plasma by high-performance liquid chromatography with spectrophotometric determination. *J. Chromatogr B Biomed Sci Appl.* 2001 Oct. 5; 762(1) 51-58.
11. Bonfigli A. R, Manfrini S, Gregorio F, Testa R, Testa I, De Sio G, Coppa G. Determination of plasma Metformin by new cation exchange HPLC technique. *Ther Drug Monit.* 1999 June; 21(3), 330-334.
12. Vesterqvist O, Nabbie F, Swanson B. Determination of Metformin in plasma by high-performance liquid chromatography after ultrafiltration. *J. Chromatogr B Biomed Sci Appl.* 1998 Sept. 5; 716(1-2) 299-304.
13. Zhouping Wang, Zhujan Zhang, Zhifeng Fu, Wanfen Luo, Xiao Zhang. Sensitive Flow injection Chemiluminescence Determination of Metformin based on N-Bromosuccinimide-flourescein System. *Analytical Letters.* 2001 Dec; 36(12), 2683-2697.
