Quantitative Determination of Metformin in Human Plasma Using High Performance Liquid Chromatography

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Abstract - A simple HPLC assay method for the determination of metformin in human plasma was developed and validated. The plasma proteins were precipitated using perchloric acid: acetonitrile (50%v/v) mixture and the supernatant liquid was removed, and stored at -4^{0}C before analysis. The separation was achieved with Agilent Technologies 1120 Compact LC model of HPLC, column type ODS Hypersil C18 4.6 x 125mm,5um . Mobile phase was Acetonitrile: potassium di-hydrogen orthophosphate: Methanol (13:80:7), buffer with (0.025M, pH 5.6) phosphoric acid. The temperature was ambient and the Detector was UV at 238 nm. The retention time, (RT) were 1.11 and 4.99 minutes for metformin and sulfadoxine respectively. The response was linear over a range of 0.1-3µg ml^{-1}

Key – HPLC, Human, Plasma, Metformin, Method, Quantity

1. INTRODUCTION

Metformin hydrochloride is an oral biguanidine, whichreduces the elevated blood glucose concentration inpatients with diabetes but does not increase insulin secretion.It does not lower the blood glucose in nondiabetic subjects[1].Many HPLC methods for the analysis of metformin in plasma are reported.But most of the methods use either ion pair reagent [2]or cation exchange column [3].Some methods reported require elaborate sample preparations [4].RP-HPLC methodfor the estimation of metformin in human plasma, are found to be more suitable.HPLC is distinguished from traditional liquidchromatography because operational pressures are significantly higher (50–350 bar) [5].

2. METHODOLOGY

2.1 Some of materials;

- Digital weighing balance OHAUS model EP 64 BY Ohaus corporation, Switzerland
- U.V. detector T80 + U.V/Vis spectrometer by PG instrument Ltd U.K
- High Performance Liquid Chromatography; Agilent Technologies, 1120LC series, USA.
- Centrifuge: Heraeus (labafuge 300) D-37520 ostence mated: 2003, serial No40267581, BN: 75003230
- Methanol: Sigma – Aldrich ≥ 99.9% U.K , Mntd: Sept 14, 2011
- Acetnitrile: Sigma – Aldrich ≥ 99.9%, U.K ,Mntd: Sept 14, 2011
- Potassium Dihydrogen phosphate (Buffer) by J.T Baker 99.5% USA
- Metformon HCL reference standard
- Sulphadoxine: Internal standard source: Rambax pharmaceutical Ltd, Lagos.

2.2 Method

The study involved six healthy subjects. All were screened to be free from both diabetic and hypertension cases. They are age 28-45 years, free from liver and kidney diseases.Drug free blood samples at fasting state were taken from the subjects after which, 500mg x 2 of metformin tables was administered with 200ml of water. The subjects were allowed to take food after 2 hrs. 3ml blood samples were withdrawn at 0.0, 0.5, 1.5, 3, 4, 5, 6, 8, hrs. The blood samples were collected inside anticoagulant bottles and stored in a fridge at -4^{0}C.

2.3 Standards preparation

Stock solution of 1 mg/mLmetformin standard was prepared with the diluent (Distilled water 70: 30 methanol).1mL from this stock solution was pipette out and made up to 1000 mL.Serial dilutions were prepared for concentrations 0.01 – 3µg/mL. Internal standard (sulfadoxine) was prepared in similar manner.

3. RESULTS

3.1 Extraction;

The extraction method was adopted and modified from[6]. 100µL of metformin hydrochloride solution of appropriate concentration and 100µL of sulfodoxine solution (20µg/ mL) 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 (perchloricacid : acetonitrile 50%v/v each) was added and was vortexed for 30seconds.After centrifugation at 3000 rpm for 10minutes, 700 µL of the supernatant was evaporated to dryness at 450C. The residue was reconstituted in 100 µL of mobile phase and 20 µL of this was injected to the HPLC system.

Table 1. Percentage Recovery of Metformin (n = 6)

Sample	Concentration ng/ml	Recovery % ± S.D
Metformin	300	97.47 ± 4.2
	500	97.58 ± 6.7

3.2 HPLC conditions

Mobile; Acetonitrile:25mMKH_2P04: Methanol
13 80 7

- Column : ODS Hypersil –C18 4. 6 x 125mm, 5um
- Wavelength: 238nm
- Temperature: ambient
- Flow rate: 1.00mL/min
- Run time : 7 minute
- Injection volume: 20 µL
- pH : 5.8 (adjusted with acetic acid)
- Chromatogram; Metformin Sulfodoxine Retention time (min): 1.1114.999

3.3 Optimization of solvent system

Seven different ratios of solvent systems were tested at 1 mL/min. The system with high capacity factor was adopted (better resolution).

Table 2. Optimization of solvent system

Solvent system			pH	Retention time (min)	
KH_2PO_4:ACN:Methanol				Metf	Sulfadoxine
60	20	20	5.7	2.03	4.93
65	25	10	5.6	1.94	5.08
65	28	7	5.6	1.07	4.70
75	20	5	5.6	1.09	4.84
80	15	5	5.8	1.09	4.88
80	13	7	5.7	1.11	5.00
70	20	10	5.8	2.16	4.95

Metf - Metformin

3.4 Precision of the method

Table 3. Intra and Inter-day Assay variation of Metformin (n = 6)

Sample	Concentration ng/ml	CV %
Intraday run (Metformin)	200	3.4 ± 0.56
	500	1.8 ± 0.87
	1000	0.5 ± 0.64
Inter-day run (Metformin)	200	4.2 ±0.23
	500	3.5 ±0.41
	1000	1.2 ±0.04

CV = Coefficient of Variation, n = Number of samples

3.5 Chromatograms

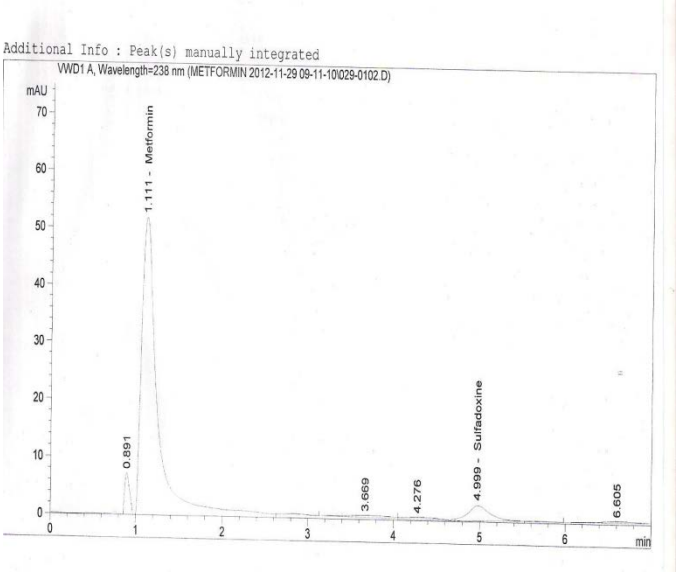

Fig. 1 Chromatograms of metformin and sulfodoxine

3.6 Calibration curve

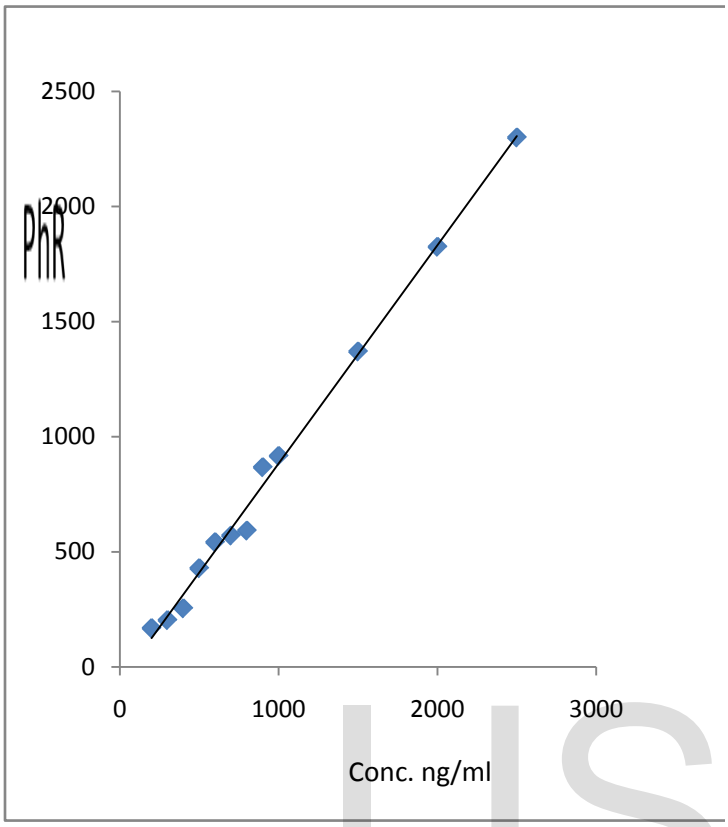

Fig.2 Calibration Curve of Standard metformin

3.7 Pharmacokinetics

Table 5. Pharmacokinetics of metformin (mean, n=6) of healthy subjects.

Pharmacokinetic parameter	Volunteers
Cmax (ng/ml)	1857.67 ±0.169
AUC_{0-8} (ng/ml/h)	8318.89 ±.030
$AUC_{0-\infty}$ (ng/ml/h)	10688.05 ±0.120

4.0 DICUSSION

The recovery observed formetformin and Internal Standard (n=6), expressed asRSD were less than 0.5%. The Linear regression results for calibration curvesperformed on 3 different days showed mean correlationcoefficients (r2) of 0.994.Table 3 shows the assessment of both interday and intraday reproducibility of the method. Similarly Table 2 shows the optimization of the method by randomly selecting 7 different ratio of solvent system. The solvent system with best resolution was chosen. The extraction yield(recovery) was calculated by comparing extractedsamples with unextracted samples at two differentconcentration levels. The data is given in Table 1.Therefore, the method showed good response for metformim and internal standard, sulfodoxine.

5.0 CONCLUSION

The HPLC assay method described here is simple, precise and accurate for quantitation of metformin in human plasma.The method can be conveniently used for the therapeutic monitoring and pharmacokinetic studies of metformin.The sensitivity, simplicity and rapidity of the method were the main advantages of the method.

6.0 REFERNCES

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