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Original Article

QUANTITATION OF METFORMIN IN URINE BY RP-HPLC METHOD AND ITS APPLICATION IN PHARMACOKINETICS

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ABSTRACT

Objective: To develop and validate an easy and sensitive HPLC method for quantitation of metformin in urine.

Methods: The technique involved deproteinisation of urine sample with methanol and analysis of the supernatant the usage of Zorbax 300–SCX, 4.6 X 150 mm ID, 5 µm particle size and UV detection at a wavelength of 233 nm.

Results: The assay was specific for metformin and linear from 1.25 to 50.0µg/ml. The relative standard deviation of intra-and inter-day assays was lesser than 7%. The recovery of metformin from urine ranged from 97-103%.

Conclusion: An easy and sensitive HPLC approach for quantitation of metformin in urine had been developed. Due to its simplicity in sample preparation and instrumentation, this technique can be used for pharmacokinetic studies of metformin in urine samples.

Keywords: HPLC, Metformin in urine, Anti-diabetic drug, Pharmacokinetics

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INTRODUCTION

Metformin HCL (N, N-dimethyl imido dicarbonimidic diamide hydrochloride) (MET) is the first desire of oral hypoglycemic drug used within the remedy of non-insulin structured diabetes Mellitus (DM), through enhancing glycemic control thereby stops gluconeogenesis and glycogenolysis [1]. Being an uncrowned queen, MET serves justice for the reason that FDA approved aid of United States in 1995 until date, which has paved its manner to good-sized boom in medical use [2]. Metformin is absorbed slowly submit oral administration, approximately 60% is eliminated in urine as such within 24 h, and almost 30% of the drug dosage is non-absorbed and excreted in faeces [2]. Urine is a non-invasive sample series technique and the estimation of drug tiers in urine is simpler when in comparison to plasma and different organic body fluids that are complex [3].

Metformin is an extremely polar and small molecule, which has first-rate solubility in water and relatively poor lipid solubility. Therefore, it can be retained in reverse phase (RP) HPLC columns. Numerous methods are available to quantitate MET in urine. They encompass capillary electrophoresis with the detector of contactless conductivity [4], voltammetric approach [5], and cation alternate HPLC [6], reverse phase HPLC [7-9] and liquid chromatography mass spectrometry (LCMS) [10]. These methods had complicated sample extraction technique and improved run time.

In gasoline chromatography, chemical derivatization was applied [11-13]. FT-IR (Fourier Transformation Infrared) and mass spectroscopic techniques [14] and electrochemiluminescence coupled with capillary electrophoresis [15] are other existing methodologies. However, the complexity is levelled up in these techniques, though they prove to be advantageous in terms of sensitivity.

Hence, LC is preferred mostly in biological laboratories. Although LCMS methodology had been implied for MET quantification, and techniques are efficient for selectivity and sensitivity with brief retention time, these techniques are too luxurious in growing international locations and aid poor settings.

We developed a simple HPLC method with simple sample extraction and preparation procedure using a conventional RP Zorbax SCX Column to estimate urine MET [16]. Similar to this method, in particular to the matrix difference, we have developed and validated a specific methodology for urine MET.

MATERIALS AND METHODS

Metformin

Pure MET Hydrochloride was a kind gift from M/s Aarthi Drugs Ltd. Methanol (99.80% purity), Acetonitrile (99.9% purity), Potassium dihydrogen phosphate (99.5% purity), Dipotassium hydrogen orthophosphate (99% purity) were purchased from M/s Qualigens (India) Ltd. Deionized water was processed through a water purification system (Siemens, Germany). Urine sample was obtained from normal healthy volunteers, Chennai, India.

Chromatographic system

HPLC instrumentation (Shimadzu Corporation, Kyoto, Japan) consisted of pumps (LC-20AD), photodiode array detector (SPDM20A) and automatic sampler (SIL20AC-HT) with constructed gadget controller. Lab solutions software program was used for collection and acquisition of data. The analytical column used was Zorbax 300–SCX, 4.6 X 150 mm ID, 5 µm particle length (Agilent, USA) covered by way of a well-suited protect column. An isocratic mobile phase is a mixture of 10 mmol phosphate buffer (1.625 gm of KH2PO4 and 0.3 gm of K2HPO4 in 1000 ml of MQ Water, pH 4.8) and acetonitrile in the ratio of 55: 45 (v/v) was used to split the analyte from the endogenous additives. Before the preparation of the mobile phase, the solvents had been degassed using a Millipore vacuum pump. The PDA detector was tuned at the wavelength of 233 nm. The chromatogram was run for 8 min at 1.3 ml flow rate per min. The column temperature turned into at 30◦C. Unknown concentrations had been derived from linear regression evaluation vs. Concentration curve. The linearity changed into established the usage of estimates of correlation coefficient (r).

Preparation of standard solution

A stock solution (1 mg/ml) was prepared by dissolving MET HCL in methanol. The working standards of MET in concentrations ranging from 1.25µg/ml to 50µg/ml were prepared in pooled urine.

Sample preparation

To 900 ul of distilled water, 100 µl of urine was added (1:10 dilution). To 400 µl of calibration standards or test samples, 450 µl of methanol was added and the contents had been vortexed vigorously for 3 min and centrifuged at 10,000 RPM for 10 min. 75 µl of the supernatant was injected into the HPLC column

Method validation parameters

Method validation were carried out as per ICH guidelines [17]

Accuracy and linearity

The accuracy and linearity of MET standards were evaluated by means of measuring a fixed of standards starting from 50 to 1.25µg/ml. By processing each standard in multiples for six consecutive days, the intra-day and inter-day variations were decided.

Precision

The precision of this method was calculated by analyzing different concentrations of MET in pooled urine samples in replicates on three successive days.

Recovery

On three unique events, the recuperation experiments were done. Known concentrations of MET (2.5, 10 and 50µg/ml) had been made in pooled urine and were spiked with lower, median and maximum concentrations of standards. The percentage of drug restoration from urine samples was calculated by dividing the difference in MET concentrations with the aid of the estimated concentration.

Specificity

Interference from endogenous compounds was ruled out by investigating blank pooled urine samples. Interference from anti-TB drugs-rifampicin, isoniazid, ethambutol, pyrazinamide, levofloxacin, cycloserine, ethionamide, rifapentine, anti-retroviral tablets–nevirapine, efavirenz, lamivudine, stavudine, zidovudine and anti-diabetic capsules-sulphonyl ureas–glibenclamide at a concentration of 10.0µg/ml were evaluated.

Limit of detection (LOD) and quantitation (LOQ)

The lower restriction of quantification became the lower maximum attention of the analyte that would be determined with proper precision and accuracy. These values have been envisioned mathematically from the standard curve equations. LOD calculations were done by the usage of the formula 3.3 x o/S, where o is the usual deviation of Y-axis intercepts and S is the slope of the calibration curve.

Clinical application

We have applied this method for the estimation of urine MET in diabetes mellitus patients who were part of a pharmacokinetic study. These patients received MET 500 mg and the urine samples collected after administration (0-8 h). Informed written consent was obtained from them prior to sample collection.

RESULTS

Under the chromatographic conditions described above, MET became well separated and seen as a discrete peak inside the representative chromatograms of extracted urine standards 50, 5.0 and 1.25 µg/ml and an extracted pattern from DM patient (fig. 1-5). No peak was observed at the retention time of MET (fig. 1E) in the blank urine sample. In the prevailing approach, urine MET concentrations ranging from 1.25–50 µg/ml were checked for linearity. These concentrations span the range of therapeutic interest. The suggest correlation coefficient (r), coefficient of determinants (R2), slope and intercept values have been 0.998, 0.999, 0.9893,14026 and 4321.4, respectively (fig. 6). The linearity and reproducibility of the various standards used for building calibration graphs for urine MET are given (table 1). The intra-and inter-day relative standard deviation (RSD) for requirements containing 1.25–50 µg/ml ranged from 0.1% to 4.1% and 0.4% to 0.6% respectively. The accuracy of urine MET concentrations ranged from 96% to 107%.

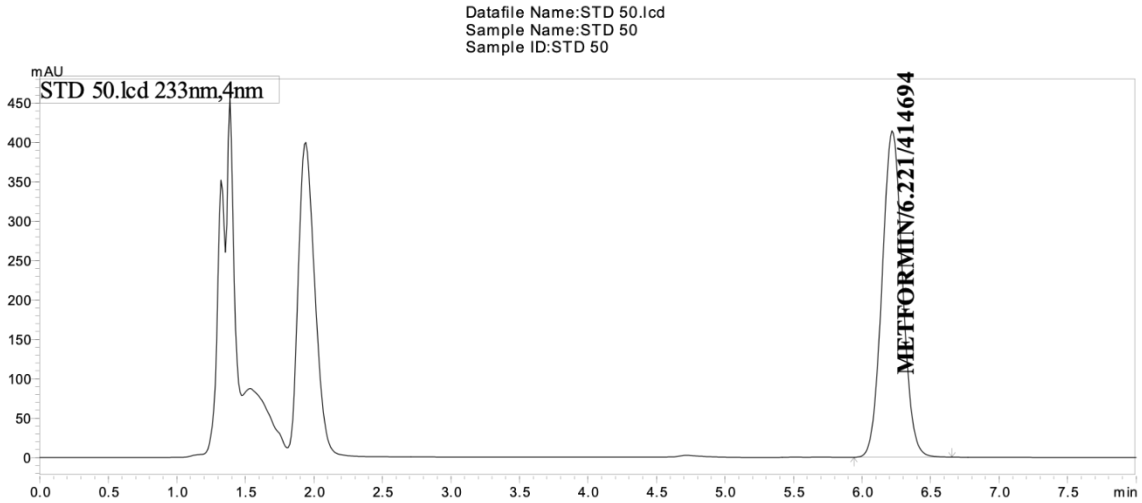

Fig. 1: Chromatogram of extracted urine metformin standard 50.0 µg/ml

Table 1: Linearity and reproducibility of urine metformin

Standard concentration (µg/ml)	Mean peak height+SD (%RSD)	
	Within day (n=6)	Between day (n=6)
50	690130.8+12575.99 (1.82)	709577.8+2575.7 (0.36)
25	361542+377.2 (0.1)	354458.5+23489.37 (6.63)
10	143415.3+120.01 (0.08)	146787.5+5221.56 (3.56)
5	72206+1015.9 (1.41)	75866.3+1799.58 (2.37)
2.5	36604.5+1512.3 (4.13)	35947.3+351.8 (0.98)
1.25	17782.5+113.5 (0.64)	19008+1182.82 (6.22)

SD =Standard deviation, RSD= Relative standard deviation, n=number, µg/ml =microgram per milliliter

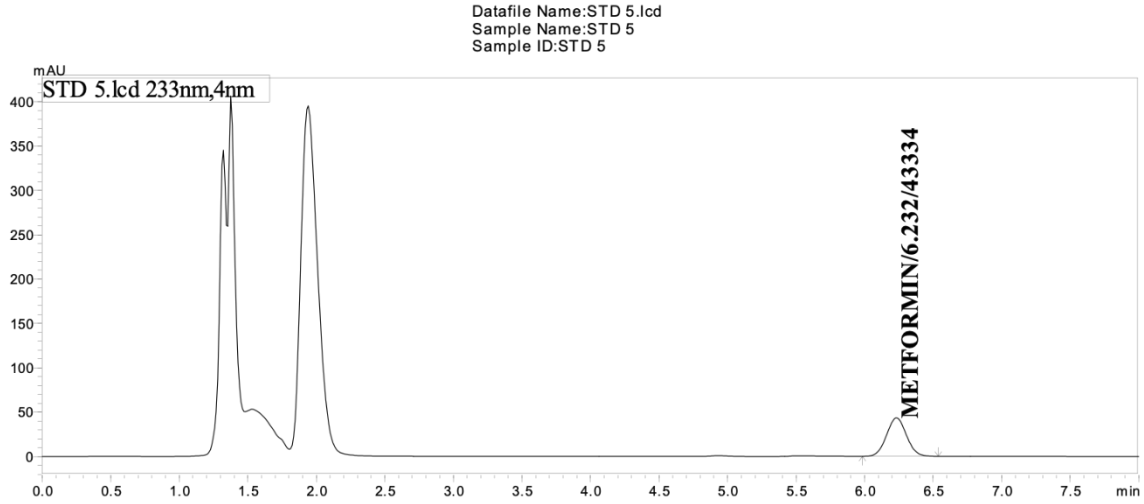

Fig. 2: Chromatogram of extracted urine metformin standard 5.0 µg/ml

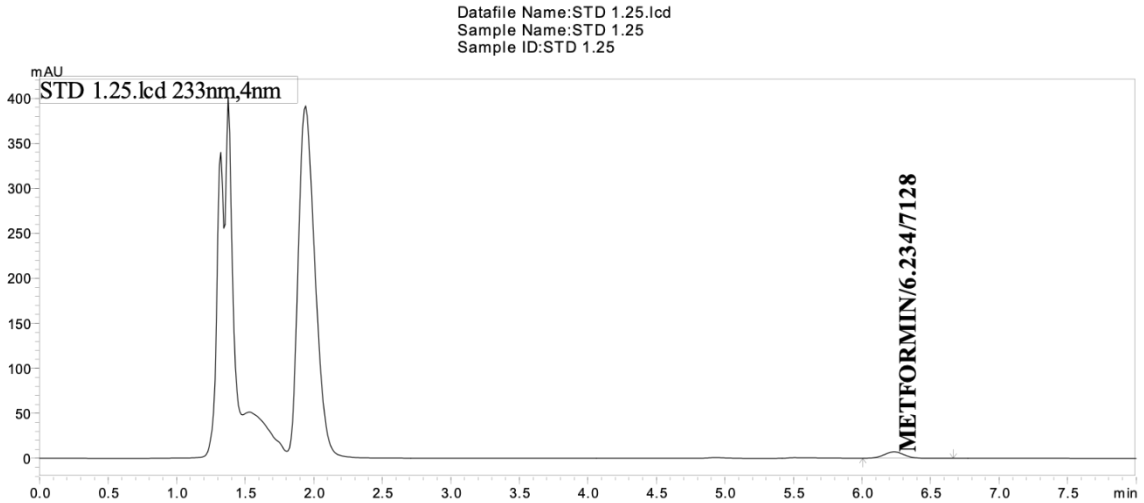

Fig. 3: Chromatogram of extracted urine metformin standard 1.25 µg/ml

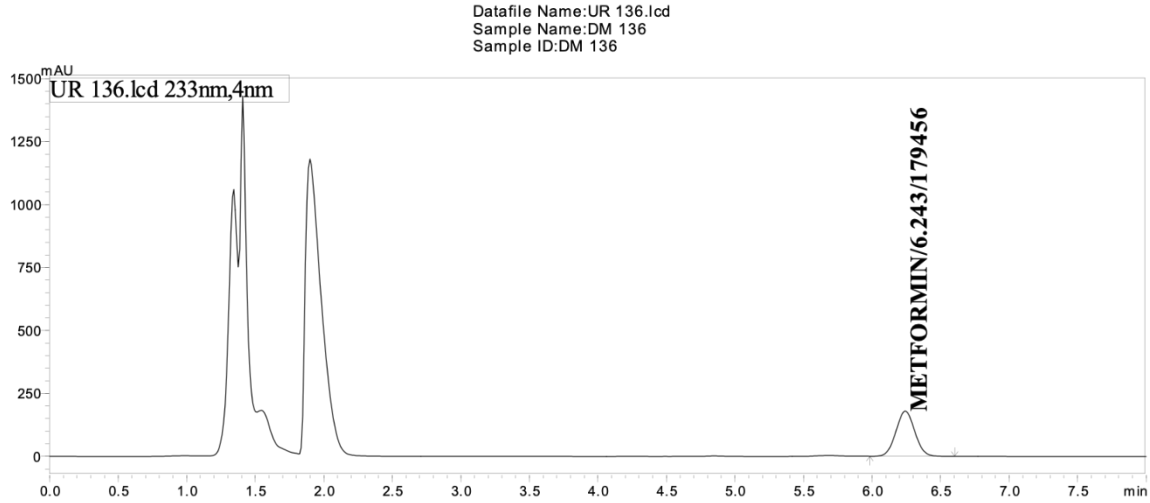

Fig. 4: Chromatogram of extracted urine metformin from a DM patient

The precision of the method was checked by analyzing three urine samples containing various concentrations of MET (table 2). The relative standard deviation (RSD) for those samples ranged from 97% to 104%, respectively. The LOD and LOQ predicted mathematically from the standard curve equation were 50 and 1.25µg/ml, respectively. This method reliably removed interfering substances from urine, yielding a recovery for MET that ranged from 97 % to 103% (table 3). Interference from endogenous compounds changed into investigated by way of measuring blank urine samples.

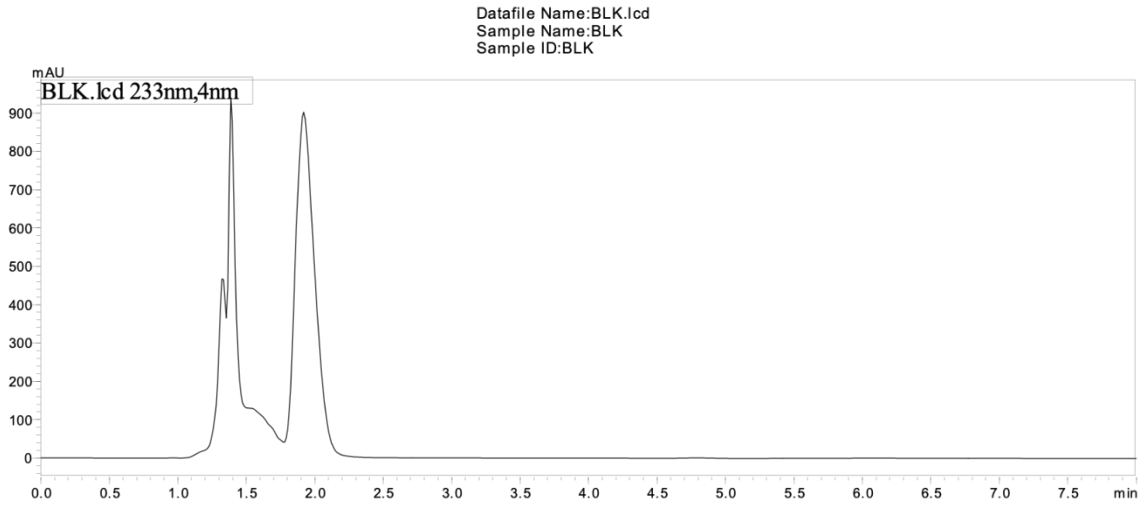

Fig. 5: Chromatogram of extracted blank urine

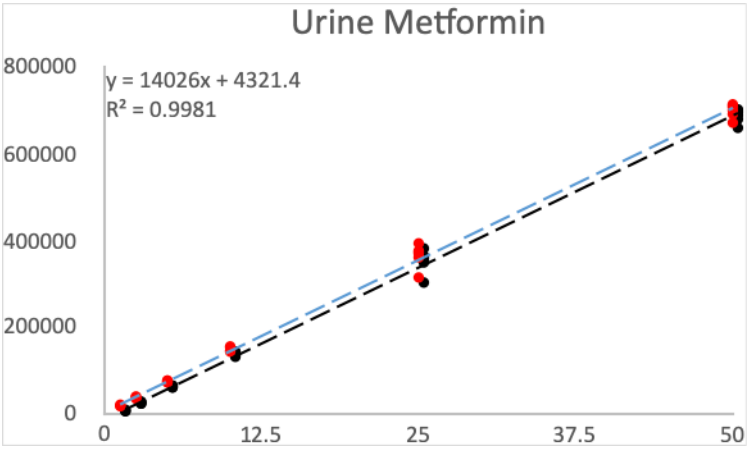

Fig. 6: Calibration curve of urine metformin

Table 2: Recovery of urine metformin

Base	Added (µg/ml)	Actual (µg/ml)	Obtained (µg/ml)	Recovery (%)
50	12.5	30	30.91	103
10	5	7.5	7.28	97
2.5	1.25	1.875	1.83	97

µg/ml =microgram per milliliter

Table 3: Precision of urine metformin

Actual concentration (µg/ml)	Found concentration (µg/ml) mean+SD	% RSD
50	49.7+0.27	99
10	10+0.01	100
1.25	1.3+0.01	104

SD =Standard deviation, RSD =Relative standard deviation, n =number, µg/ml =microgram per millilitre

Interference from certain anti-TB drugs such as Rifampicin, Rifabutin, Rifapentine, Pyrazinamide, Moxifloxacin, Lipoic acid, Levofloxacin, Ethionamide, Delamanid, Clofazimine, Bedaquiline, Cycloserine Isoniazid, anti-retroviral drugs like Efavirenz, anti-hypertensive drugs like Nor-verapamil, Verapamil and anti-diabetic drugs-Sulphonylureas are Glipizide, Glibenclamide, Glimmered were assayed and this method was specific for urine MET and none of these drugs showed any interference. The urine was collected from two diabetic patients was analyzed. The mean percentage of urine MET concentration in DM patients was 54%.

DISCUSSION

Many of the present methods rent one-of-a-kind techniques for sample extraction together with the acetone precipitation approach [18], solid section extraction [19], ion-pair strong section extraction [20, 21], liquid-liquid ion-pair extraction [22-23], liquid-liquid extraction [24], protein precipitation and extraction into one step [25]. The extraction of the sample is quite complicated and time taking because of the polar nature of MET. The run time of the prevailing methods was greater than 9 min while our developed

method has a shorter runtime of eight minutes [26]. Even the injection quantity of the sample became higher in most of the methods. Therefore, the technique developed involved simple extraction, which stays smooth, less tedious and the compound of interest become extracted in the usage of organic solvent.

We present a simple HPLC method for detection of urine MET using lesser quantity of sample. The calibration curve turned into linear in the span of 1.25–50µg/ml (r2 =0.998). The validation parameters are desirable for the estimation of the analyte in urine samples. We bear in mind this technique to be precise, accurate and reproducible. The developed technique discovered to be less complicated to carry out than the posted techniques for determination of MET in urine.

CONCLUSION

We have defined a simple, touchy, and specific HPLC approach for the quantitation of MET in urine. The advantageous method became tested as per Bioanalytical approach validation pointers. The technique concerned a simple pattern dilution procedure that's precise, reproducible and economic for the quantitation of MET in urine. The run time becomes 8 min and could be proved as better opportunity for steeply-priced liquid chromatographic-mass spectrometric technique. Urine MET method developed may be apt for pharmacokinetic and bioavailability studies.

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AUTHORS CONTRIBUTIONS

Dr. A. K. Hemanth Kumar: Literature search, manuscript preparation, Ms. Mary Rebecca: Method development and validation, Ms. V. Sudha: Method development and validation. Mr. A. Vijayakumar: Method validation and data preparation.

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Nil

CONFLICTS OF INTERESTS

None

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