Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
PubMed (National Library of Medicine): ID: (101671502)
Research Article
Volume 8, Issue 3: May-June: 2019, 61-64
ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

SPECTROPHOTOMETRIC ESTIMATION OF METFORMIN IN HUMAN PLASMA

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Article Info: Received 10 May 2019; Accepted 02 June. 2019
DOI: https://doi.org/10.32553/jbpr.v8i3.609
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Conflict of interest statement: No conflict of interest

ABSTRACT:
To develop a simple, selective and sensitive spectrophotometric method and validated for the determination of metformin in human plasma. The method was applied for the determination of metformin from pharmaceutical preparations and plasma samples and after spiking with metformin. The results were checked by standard addition method. A number of pharmaceutical additives and plasma matrix did not affect the determination of metformin. The proposed method was found to be accurate and precise for routine estimation of metformin hydrochloride in human plasma.
Keywords: Metformin, spectrophotometric, standard addition, plasma, pharmaceutical preparation

INTRODUCTION

Metformin HCl (1,1-dimethylbiguanide HCl), first developed in 1957, is one of the most commonly used oral anti-hyperglycemic agents for the treatment of Type II diabetes mellitus. It is currently recommended as first-line therapy in overweight or obese patients with this condition (1). However high concentration of metformin in plasma has been associated with an increase in the incidences of lactic acidosis, particularly in patient in acute renal failures. The methods for the quantitation of metformin from pharmaceutical preparations and biological fluids include spectrophotometry [5, 6], gas chromatography [7-9], high performance liquid chromatography-tendom mass-spectrometry (HPLCMS-MS) [10], reversed phase HPLC [11, 12], ion pair HPLC [13, 14]. The spectrophotometric methods are simple and required sensitivity and selectivity could be achieved. Regarding sample preparation from biological fluids, extraction and clean-up of the sample is a critical first step in bioanalysis and requires high selectivity. Organic liquid-liquid extraction is a simple and effective method of affording sample cleanup for most analytes. Unfortunately, in the case of metformin, this approach is challenging because of the drug's polar characteristics. Herein, a method that successfully employs the liquid-liquid extraction of metformin and capable of measuring metformin in small volumes with high sensitivity.

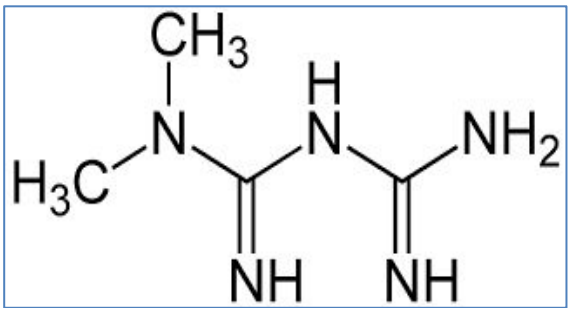

Figure 1: Chemical structure of metformin HCL

MATERIAL AND METHODS:

Chemicals and reagents:

metformin HCL were obtained as gift sample from pharmaceutical Laboratories Ltd. Perchloric acid, Acetonitrile and water used were of analytical grade (FINAR limited, hyderabad, India).. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Instrumentation

The proposed work was carried out on a ELICO UV-visible spectrophotometer (model SL 210), which possesses a silicon photodiode detector with a1 cm quartz matched cell. All weighing was done on electronic balance (Wensar HPB 220).

Selection of Solvents:

The water solubility of metformin HCL was found to be1.38mg/ml, log p value -1.8 and pKa (strongly basic) 12.33 On the basis of solubility study perchloric acid and Acetonitrile was selected for extraction.

Preparation of Standard Stock Solution of Metformin:

Metformin Stock Solution

An accurately weighed quantity of Metformin (100 mg) was taken in 100 mL volumetric flask and dissolved in water (50 -mL) with the help of ultrasonication for about 5 min. Then the volume was made up to the mark using methanol to get metformin standard stock solution (1 mg / mL).

Metformin Working Standard Solution

Metformin standard stock solution10mLwas diluted to 100 mL using to get working standard solution 100 µg / mL.

Preparation of biosample:

500µL volume of plasma or erythrocyte from individuals not recieving metformin was transferred to 1.5mLpolyproplylene microextraction tube. Extraction was performed by adding 50µL of metformin working standard to the tube and shaking for 60 seconds, followed by centrifugation at 10000g for 10 minutes, the whole organic layer was separated into another tube, then 200µL of perchloric acid 10% was added, the mixture was vortexed and centrifuged at 10000g for 5 minutes. From the organic layer 0.1mL of extract was transferred to 10 mL volumetric flask and volume was made upto 10mL with distilled water. The absorbance of sample is determined using water as blank solution at 233nm.

Determination of λ Max of Metformin HCL:

An appropriate aliquot portion of metformin (1 mL) was transferred to 10 mL volumetric flask, the volume was made up to the mark using distilled water to obtain 10ppm solution of Metformin. Drug solution were scanned between 200 nm to 400 nm. Metformin shows **λ** max at 233nm.

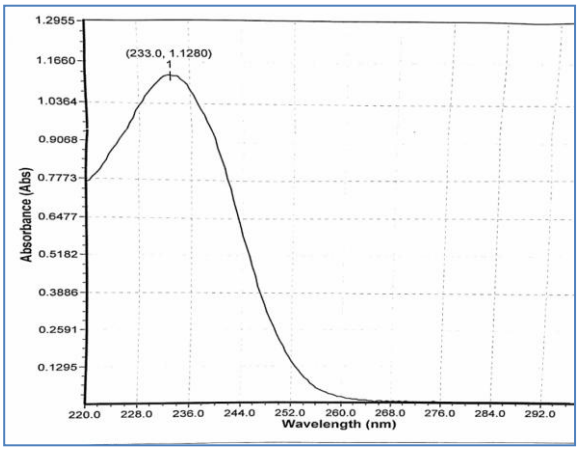

Figure 2: spectra of metformin HCL

Linearity Studies:

An accurately measured aliquot portion of working standard solution of was transferred to seven separate 10 mL volumetric flasks. The volume was made up to the mark using 77%ss v/v methanol to obtain concentrations (10-160 µg/mL). Absorbance of these solutions was measured at 274 nm, (Table1) Calibration curve was plotted, Absorbance Vs Concentration as shown in (Fig. 1).

Table 1: Regression and Optical characteristics of metformin

Parameters	Values
Beer's law limit (µg/mL)	20-100µglml
Correlation Coefficient (r)	0.997
Regression equation	
Slope	0.203
Intercept	0.230

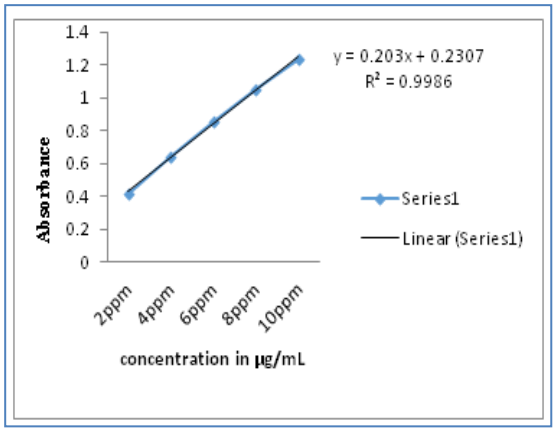

Figure 1: calibration plot of metforminHCL

Accuracy [Recovery Study

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the sample to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula. % Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

The results are reported in (Table 41).

Table 2: Recovery Study.

Drug in solution µg/ml	%recovery±S.D
40.10	100.04±0.021
60.02	99.56±0.056
80.05	99.59±0.032

S.D. =Standard Deviation

Robustness

The robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrometric condition for determination of Metformin HCl in biosample . The Robustness data for variations in wavelength of detections (±5nm) and the absorbance and its analytical performance parameters of Metformin HCl were shown in Table

Table 3: Robustness Study.

Wavelength in nm	metformin 20 µg/mL	
	Absorbance found in µg/mL Mean S.D. (n=3)	% R.S.D.
238	0.4202 0.001	0.0010
228	0.4109±0.019	0.0069

ACKNOWLEDGEMENT:

The authors are grateful to the authorities of RBVRR Women's College of Pharmacy, hyderabad for the facilities.

REFERENCES:

1. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 1996;30:359-
2. Simple hplc-uv method for determination of metformin in human plasma and erythrocytes application to therapeutic drug monitoring , International Journal of Pharmacy and Pharmaceutical Sciences 2015-ISSN-0975-1491
3. Willard HH and Merritt II. Instrumental method analysis. New Delhi: CBS Publication and Distribution; 2001
4. Sethuraman S, Adhakrishnan K, Venkateswarlu V, Sravani M, Ramathulasi G, Bhanuteja S. Estimation And Degradation Monitoring Of Cefadroxil In Pharmaceutical Dosage Form By Using UV-Spectroscopy. Asian Journal of Research in Biological and Pharmaceutical Sciences. 2014; 2(1):27-33. 17.
5. Tache F, Albu M. Specificity of an Analytical HPLC Assay Method of Metformin Hydrochloride. Revue Roumaine De Chimie. 2017;52(6):603–609.
6. 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;716:299-304.
7. Karkhanis VV, Anand Kumar DC, Patel P. Development and Validation of UV Spectrophotometric Method for Estimation of Glipizide in Bulk and Pharmaceutical Dosage Forms. Int J Pharmaceutical Science and Res. 2013;4(5):1865-67
8. Amini H, Ahmadiani A, Gazerani P. Determination of metformin in human plasma by high-performance liquid chromatography. J Chromatogr B: Anal Technol Biomed Life Sci 2005;824:319-22. 21
9. Cheng CL, Chou CH. Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. J Chromatogr B: Biomed Sci Appl 2001;762:51-8. 19.
10. Chatwal GR and Sham KA. Instrumental method of chemical analysis. 5th ed. Mumbai: Himalaya Publishing House; 2002;626-636. 20.
11. Beckett AH and Stenlake JB. The Practical Pharmaceutical Chemistry. Part II. New Delhi: CBS Publishers and Distributors; 1997. 21.

12. Lloyd RS, Joseph JK, Joseph LG. Practical HPLC Method Development. 2nd ed.York: Wiley Wiley-Interscience publication; 1997.

13. Narasimha Rao D, Chandana M. Method Development and Validation of RP-HPLC Method for Simultaneous Analysis of Three-Component Tablet Formulation containing Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glibenclamide. Int J Pharm Tech Research. 2012;4(3):948-956

14. Neelima K, Prasad YR. Analytical method development and validation of metformin, voglibose, glimepiride in bulk and combined tablet dosage. Pharmaceutical Methods. 2014;5(1):27-33.

15. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Rapid determination of metformin in human plasma using ion-pair HPLC. J Pharm Biomed Anal 2003; 31: 197-200 and Bioanalytical Chemistry. 2011; 1(3): 70-76.

16. Canadian Diabetes Association 2008 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada. Can J Diab 2008; 32: S1-S201.

17. Porta V, Schramm SG, Kano EK, Koono EE, Armando YP, Fukuda K, Serra CH. HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies. J Pharm Biomed Anal 2008; 46: 143-147

18. Dey S, Kalyani K, Samyuktha B, Sahoo SK, Mohapatra S, Murthy PN, Kumar D. Development And Validation Of A UV-Vis Spectrophotometric Method For The Estimation And Degradation Monitoring of Metformin In Bulk And Pharmaceutical Dosage Forms. Int J Chem Res. 2010;1(1):29-34

19. Deveaux M, Dallet P, Quintin C, Nisse P, Gosset D. Ultra-short determination of metformin in plasma by hydrophilic interaction liquid chromatography. Ann Toxicol Anal 2002;14:125-8.Lalau JD, Lacroix C, Couragon P, Decagney B, Rigaud JP, Bleichner G, et al. Role of metformin accumulation in metformin-associated lactic acidosis. Diabetes Care 1995;18:779-84