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Original Article

Estimation of Metformin Hydrochloride by UV Spectrophotometric Method in Pharmaceutical Formulation

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ABSTRACT

A simple and sensitive UV spectrophotometric method has been developed and validated for the estimation of metformin hydrochloride in tablet formulation. Metformin hydrochloride is determined spectrophotometrically at 232 nm using distilled water as solvent. It obeyed Beer's law in the range of 2-10 µg/ml. Percentage recovery of the drug for the proposed method ranged from 102-105% indicating no interference of the tablet excipients. The proposed method was found to be accurate and precise for routine estimation of metformin hydrochloride in bulk and pharmaceutical formulation.

Key words: Metformin hydrochloride, UV Spectrophotometric, Estimation

INTRODUCTION

Metformin hydrochloride (**Figure 1**) is 1, 1-dimethlybiguanide hydrochloride is white crystalline powder, hygroscopic and freely soluble in water, used as hypoglycemic drug. It is official in Indian Pharmacopoeia [1]. It helps to reduce LDL cholesterol and triglyceride levels and is not associated with weight gain; in some people it promotes weight loss. It is the only antidiabetic possibly associated with reduced risk of cardiovascular complications in those patients with type II diabetes mellitus. Metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines. Extensive literature survey reveals that only few methods like HPLC and GC have been reported for estimation of the metformin hydrochloride in pharmaceutical formulations and biological fluids [2-5].

Aim of present work was to develop simple, rapid, economical and reproducible UV spectrophotometric method for determination of drug in pharmaceutical formulations. This paper describes validated UV spectrophotometric method for determination of Metformin hydrochloride in bulk and tablet dosage forms. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [6].

MATERIALS AND METHODS

Instrumentation: A double beam spectrophotometer (Shimadzu-UV-2450 with 10mm path length and slit width variable) was employed for measurement of absorbance.

Chemicals and reagents: The reference standard of metformin hydrochloride was procured as gift sample from USV Limited, Mumbai, India and tablets (**Riomet OD** 500 mg, Ranbaxy labs Ltd) were utilized for the study. Distilled water was used as solvent.

Preparation of stock solution: A standard solution of metformin hydrochloride was prepared by dissolving 100 mg of metformin hydrochloride in 100 ml of distilled water and further diluted with water to get concentration of 100 µg/ml.

Determination of wavelength (λ max): One ml aliquot of stock solution was diluted to 10 ml with water and absorbance was measured in the scanning mode from 200 to 400 nm against water as reference. Wavelength corresponding maxima absorbance in water was found at 232 nm which is depicted in **Figure 2**.

Preparation of standard calibration curve: For the preparation of standard calibration curve, concentrations of 2-10µg were prepared by Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the 100µg/ml solution into a 10ml volumetric flask and made up the volume with water. The absorbance of each solution was measured at 232 nm . The calibration

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curves were plotted over a absorbance against concentration range of solution 2-10 µg/ml **(Figure -3**).

Analysis of tablet formulation: Twenty tablets were weighed, powdered and the powder equivalent to 100 mg of metformin hydrochloride was accurately weighed, dissolved in 100 ml of distilled water, filtered through Whitman filter paper No: 41 and diluted further to get a concentration of 100 µg/ml. The absorbance of sample solution was also measured and the amount of metformin hydrochloride present in tablet formulation was determined by extrapolating from the calibration curve. The results are shown in the **Table 1**.

VALIDATION OF PROPOSED METHOD

Linearity: The linearity of the analytical method according to ICH guidelines was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To determine the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analyzed. The drug showed linearity in the range of 2-10 µg/ml with correlation coefficient 0.999. Linearity data are shown in **Table2.**

Precision: Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability was determined by preparing five replicates of the same concentration of the sample and the absorbance was measured. The Intraday precision study was carried out by preparing a drug solution of same concentration and analyzing it at three different times in a day. The result of precision showed a good reproducibility with percent relative standard deviation less than 2. (**Table 3**)

Accuracy: The accuracy of measurement is defined as the closeness of the measured value to the true value. Accuracy of the proposed method was determined using recovery studies. The recovery study of metformin hydrochloride was carried out by adding different amounts (80%, 100%, and 120%) of the pure drug to the pre analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated. The percentage recovery of metformin hydrochloride was found in the range of 102-105% indicating that there is no interference by the excipients in the method. The method was found to be precise as %RSD values for intraday was found to be less than 2. The results are shown in the **Table** 4.

Robustness: Analysis was carried out at two different wavelengths such as 231 nm and 233 nm, to determine the robustness of the method and the respective absorbance was measured. The result was indicated as %RSD values were found to be less than 2 (**Table 5**).

Ruggedness: Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. Ruggedness was determined by carrying out analysis by three different analysts and the respective absorbance was noted and the result was indicated as % RSD values were found to be less than 2 **(Table 6).**

LOQ and LOD: Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ and LOD were determined using the following equation LOQ-10s/m, LOD-3.3s/m where s is the standard deviation of the response and m is the slope of the related calibration curve. The values of LOQ and LOD were found to be 0.732 and 0.241µg/ml respectively.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slopes, intercepts, correlation coefficients and optical characteristic of the proposed method are shown in Table 2. The proposed method was evaluated by the assay of commercially available tablets containing metformin hydrochloride. Three replicate determinations were performed on the accurately weighed amounts of tablets. For recovery study volume of standard solution of different concentration were added to the fixed volume of sample solution. The recovery study results ranged from 102 to 105% with % RSD values 1.09. Results of recovery studies were found to be satisfactory and indicating no interference of the tablet excipients. The values of LOQ and LOD were 0.732 and 0.241µg/ml shows method was found to be precise. The result of analysis of commercial formulations was found to be 96.48 % with percent R.S.D. values were less than 2, indicating good reproducibility of the method. The robustness and ruggedness studies showed that results are unaffected by small but deliberate changes in method parameters (RSD < 2).

CONCLUSION

The proposed method is simple, accurate, precise sensitive and can be successfully applied for routine quantitative estimation of metformin hydrochloride in bulk and pharmaceutical formulation. It should be used for routine analysis in industry.

Acknowledgments

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Table No.1: Result of analysis of Metformin hydrochloride tablet

Sr no.	Labelled claim mg/ Tablet	Drug found in mg/ Tablet *	% purity	Average
1	500	476.15	95.23	
2	500	483.01	96.61	96.48 %
3	500	488.05	97.61	

- N=5 replicates

Table 2: Optical characteristics for Metformin hydrochloride

Parameters	**Metformin hydrochloride**
Maximum wavelength (nm)	232
Beers law limit in µg/ml	2-10
Correlation coefficient	0.999
Regression equation y= mx+c	0.0842x + 0.0402
Intercept	0.0402
Slope	0.0842
LOQ µg/ml	0.259
LOD µg/ml	0.085

Table No.3: Precision of Metformin hydrochloride

Level of addition	Drug added µg/ml	Tablet solution	Drug found µg/ml	%recovery	SD	%RSD
80%	6	4.8	6.16	102.6		
100%	6	6	6.25	104.1	0.006164	1.09
120%	6	7.2	6.3	105		

SD-standard deviation, RSD-relative standard deviation

Table No.4: Accuracy of Metformin hydrochloride

Sr no.	**Conc** µg/ml	**Absorbance(nm)**	**SD**	**%RSD**
1	6	0.543		
2	6	0.547		
3	6	0.544	0.002179	0.399
4	6	0.547		
5	6	0.548		
		Average =0.546		

Table No.5: Robustness for Metformin hydrochloride (Change in wavelength)

		Variation			
Sr No.	Conc µg/ml	Wavelength	Absorbance (X)	$X - \overline{X}$	$(X - \overline{X})^2$
1	6	231	0.549	-0.008	0.000064
2	6	231	0.546	0.011	0.000121
3	6	231	0.551	0.006	0.000036
4	6	232	0.564	0.007	0.000049
5	6	232	0.567	0.01	0.0001
6	6	232	0.566	0.009	0.000081
7	6	233	0.559	0.002	0.000004
8	6	233	0.555	0.002	0.000004
9	6	233	0.554	0.003	0.000009
		Average($\overline{X}$) =	0.557	SUM =	0.000468
				SD =	0.007648
				% RSD=	1.37

Table No.6: Ruggedness of Metformin hydrochloride (change in analyst)

Sr No.	Conc µg/ml	Variation Wavelength	Absorbance (X)	$X - \bar{X}$	$(X - \bar{X})^2$
1	6	I	0.563	0.003	0.000049
2	6	I	0.557	0.003	0.000009
3	6	I	0.554	0.006	0.000036
4	6	II	0.563	0.003	0.000009
5	6	II	0.569	0.009	0.000081
6	6	II	0.558	0.002	0.000004
7	6	III	0.556	0.004	0.000016
8	6	III	0.561	0.001	0.000001
9	6	III	0.553	0.007	0.000049
		Average($\bar{X}$) =	0.56	SUM =	0.000254
				SD=	0.005634
				%RSD=	1.006

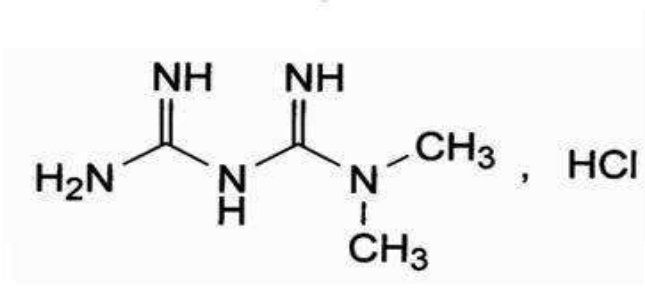

Figure 1: Structure of Metformin Hydrochloride

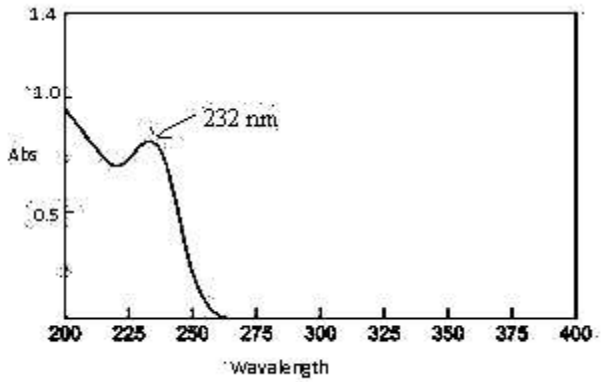

Figure 2 UV spectra of Metformin Hydrochloride at 232 nm

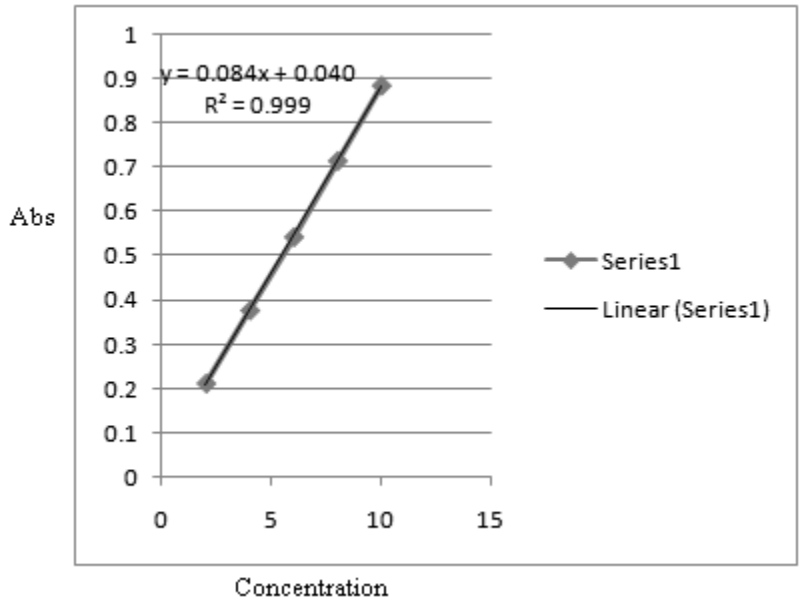

Figure 3 Calibration Curve of Metformin Hydrochloride (2-10 µg/ml) in water

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