

Original Article

## Development and Validation of Analytical Method for Determination of Metformin Hydrochloride in Bulk and Tablet Dosage Form by Using UV-Visible Spectrophotometer.

**Bhadresh A. Koli\*, Pravin N. Sable.**

Department of Quality Assurance Technique, P.E.Society's Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India.

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### Abstract

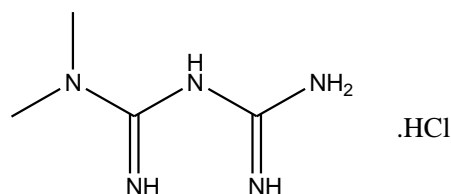
Simple, precise and economical UV methods have been developed for Estimation Metformin hydrochloride in bulk and pharmaceutical dosage form. Solubility analysis was performed with different type of solvent but highest solubility was found in water. Solution of metformin hydrochloride in water was prepared and measured at wavelength maxima 233nm. Linearity for detector response was observed in the concentration range of 2-10µg/ml. Accuracy of the methods was assessed by recovery studies and was found to be 99.02%. The developed method was validated with respect to linearity, precision, accuracy (recovery) and specificity, LOD, LOQ and ruggedness. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of Metformin hydrochloride in commercial pharmaceutical dosage form.

**Keywords:** ICH guideline, Analytical method validation, UV-Spectrophotometer, Metformin.

### 1. Introduction

UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution.<sup>[1]</sup>

Metformin Hydrochloride (MET) is 3-(diaminomethylidene)-1, 1-dimethylguanidine. It is an oral anti-diabetic drug which is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese people and those with normal kidney function.<sup>[2]</sup> Metformin improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis).<sup>[3]</sup>



Metformine hydrochloride

**Fig. 1.** Metformin hydrochloride

Several methods were reported for the estimation of Metformin HCl drugs by UV-spectrophotometry. A comprehensive literature research reveals the lack of a spectrophotometric analytical method for estimation of Metformin HCl in pharmaceutical formulations. A successful attempt was made to develop accurate, precise, specific, Rugged and simple method of analysis for estimation of Metformin hydrochloride in dosage form in water at wavelength maxima 233nm.

\*Corresponding author.

E-mail address: [Bhadresh.koli@gmail.com](mailto:Bhadresh.koli@gmail.com)

(Bhadresh A. Koli)

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## Introduction to validation of analytical method

Validation is concerned with assuring that a measurement process produces valid measurements. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. A measurement process producing valid measurements for an intended application is fit for purpose.<sup>[4]</sup>

ICH parameter for Validation of Analytical method<sup>[5]</sup>

- Accuracy
- Precision
- Specificity
- Limit of Detection
- Limit of Quantitation
- Linearity
- Range
- Ruggedness

## Types of Analytical Procedures to be validated

- Identification tests;
- Quantitative tests for impurities content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Analytical methods need to be validated or revalidated

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix);
- Whenever the method is changed and the change is outside the original scope of the method.

## Materials and Methods

Metformin hydrochloride was generous gift samples from Emcure Pharmaceuticals Limited (Pune, India). Commercial Glycomet tablets containing 500mg of Metformin

hydrochloride were purchased from local market and used within their shelf-life period. All chemicals used during work are of AR grade.

### Instrumentation

A Jasco double beam UV-visible spectrophotometer, Model: V-630, with a fixed bandwidth (1nm) and 1-cm quartz cell was used for Spectral and absorbance measurements. In addition, electronic balance, micropipette and sonicator were used in this study.

### Preparation of stock solution

10mg Metformin hydrochloride drug were dissolved in 50ml Distilled water and was shaken well for 30min. Then distilled water was added to it to adjust the volume up to 100 ml (100µg/ml).

### Preparation test solution

20 tablets were weighed and powdered. Powdered tablet equivalent to 10mg of Metformin hydrochloride was weighed and taken into 100ml volumetric flask containing 50ml of distilled water and shaken well to dissolve it and after that distilled water was added to adjust the volume up to 100ml. filter the solution.

### Solubility Analysis

100 mg Metformin hydrochloride drug was dissolved in 50ml test solvent (table 2) and was shaken well for 30 minute. Then test solvent was added to it to adjust the volume up to 100ml (1000ppm). From that 10 ml was pipette out into 100ml volumetric flask and volume was adjusted up to mark with test solvent (100ppm). From that 1 ml was pipette out into 10ml volumetric flask and volume was adjusted up to mark with test solvent (10ppm).

**Accuracy:** Accuracy of an analysis was determined by systemic error involved. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines.

**Linearity:** The linearity of measurement was evaluated by analyzing different concentration (2,4,6,8,10µg/ml) of the standard solution of Metformin hydrochloride. Result should be expressed in terms of correlation co-efficient.

**Precision:** The reproducibility of the proposed method was determined by performing tablet

assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

## Results and Discussion

### Solubility Analysis

Solubility was performed by using different type of solvent which is mention in table I. Solubility of Metformin in solvent was shown in table II, III, IV, V, VI. Maximum solubility was found in ethanol and water but water is easy to handle and cheap.

### Selection of wavelength

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode using distil water as a blank. Metformin hydrochloride shows  $\lambda_{max}$  at 233nm. The proposed analytical method is simple, accurate and reproducible.

**Range:** Range for Metformin hydrochloride was determine between lowest and highest concentration upto which it followed beers law. Range for Metformin hydrochloride in water was found to be 2 $\mu$ g/ml to 12 $\mu$ g/ml.

**Linearity:** Six points calibration curve were obtained in a concentration range from 2 $\mu$ g/ml to 10 $\mu$ g/ml for Metformin hydrochloride. The absorbance of the drug was found to be linear in the investigation concentration range and the linear regression equation was  $y = 0.077x + 0.023$  with correlation coefficient 0.999 (Table II, Figure VIII).

**Specificity:** Solution of each of the analyte was scan separately and their absorbance is noted. The standard working solution containing a mixture of the component being analyze is also scan and each of analyte peaks is check for its absorbance.

**Precision:** The developed method was found to be precise as the %RSD values for the intraday and interday precision studies were <0.32% and <0.51% respectively (Table III).

**Accuracy:** The result shown that best recoveries (98.27-99.02%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Composition of Placebo Added for Accuracy Study is shown in Table IV.

**Ruggedness:** Concentration was measure at two different instruments and method was found to rugged shown in Table V.

**Limit of Detection:** limit of detection were calculated from three different straight line equations. Minimum quantity to be detected was found to be 0.62ppm.

$$\begin{aligned} \text{LOD} &= 3.3 \times \sigma/s \\ &= 3.3 \times 0.016/0.077 \\ &= 0.062\mu\text{g/ml} \end{aligned}$$

Where  $\sigma$  = standard deviation of intercept

S = slope

**Limit of Quantitation:** Limit of Quantitation was calculated from three different straight line equations. Minimum quantity to be detected was found to be 2.00ppm.

$$\begin{aligned} \text{LOD} &= 10 \times \sigma/s \\ &= 10 \times 0.016/0.077 \\ &= 2.00\mu\text{g/ml} \end{aligned}$$

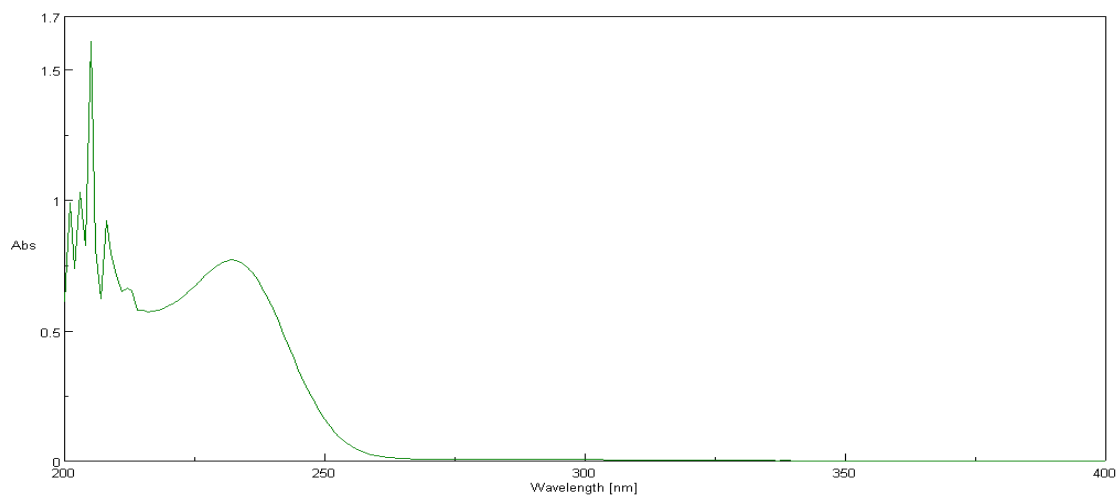
## Conclusion

UV-spectrophotometric methods for Metformin hydrochloride were developed in bulk and tablet dosage form using a different solvent. Maximum solubility was found in water at 233nm. The methods were validated as per ICH guidelines. The standard deviation and percent relative standard deviation calculated for these methods was <1, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise and selective and can be employed successfully for the estimation of Metformin Hydrochloride in bulk and pharmaceutical dosage form.

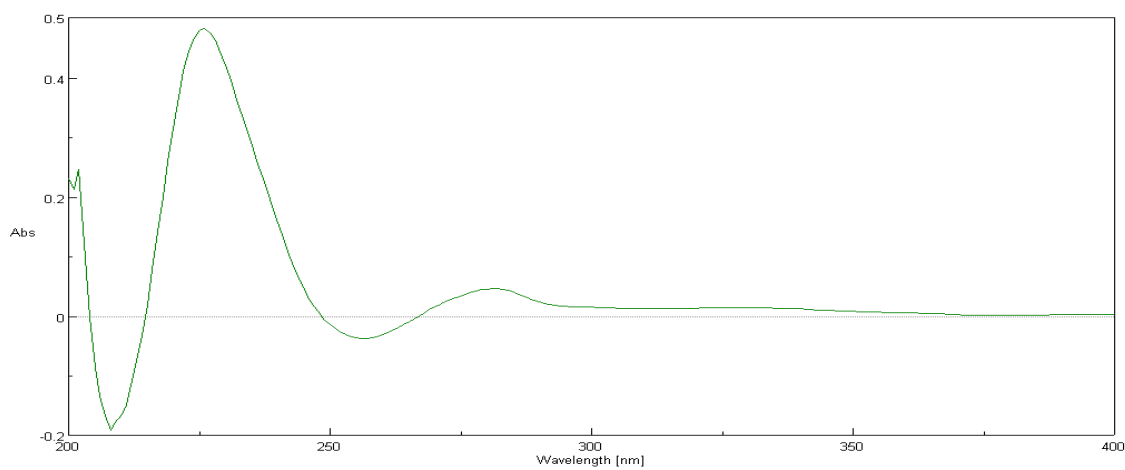
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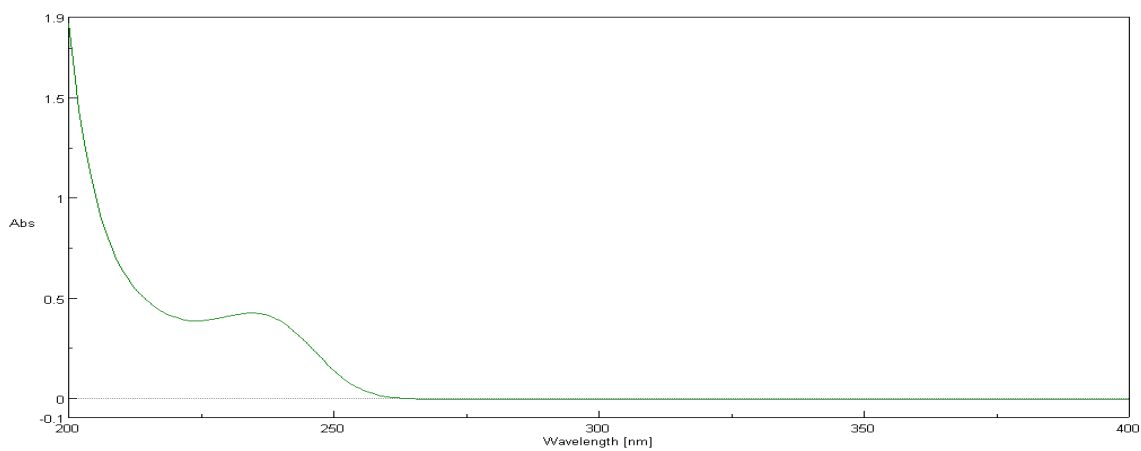




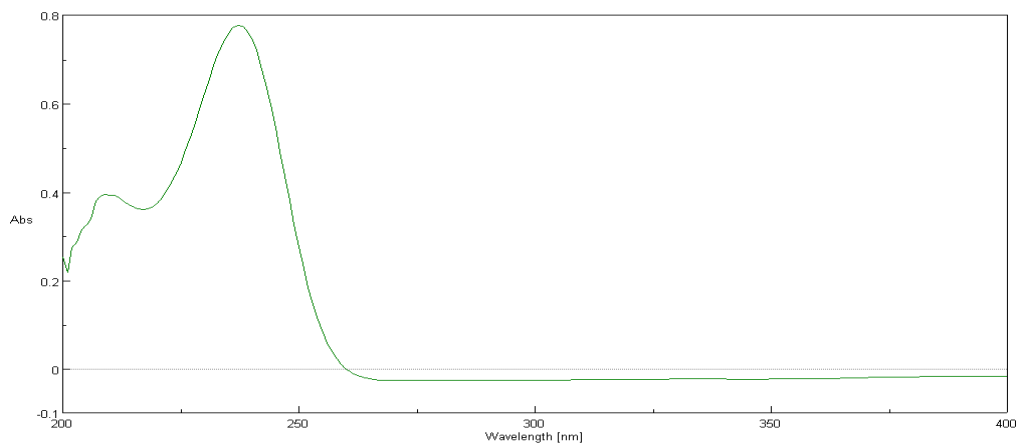
**Fig. 2.** Solubility of Metformin hydrochloride in NaOH.



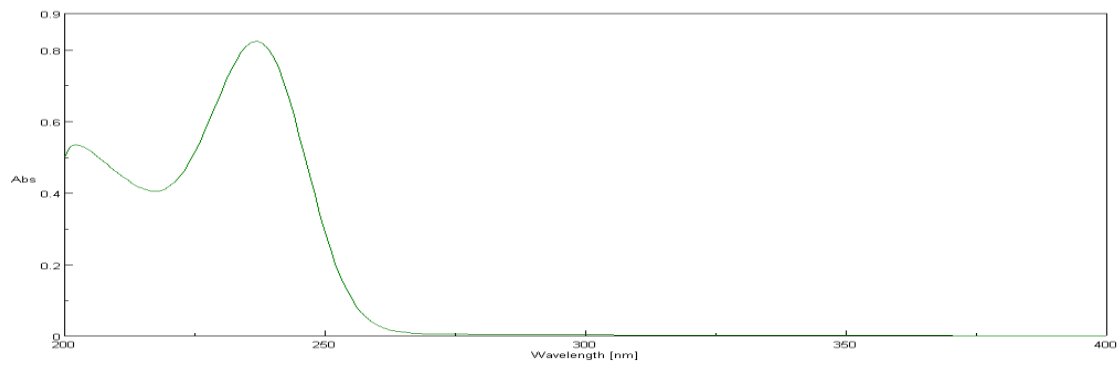
**Fig. 3.** Spectrum of Metformin hydrochloride in HCl.



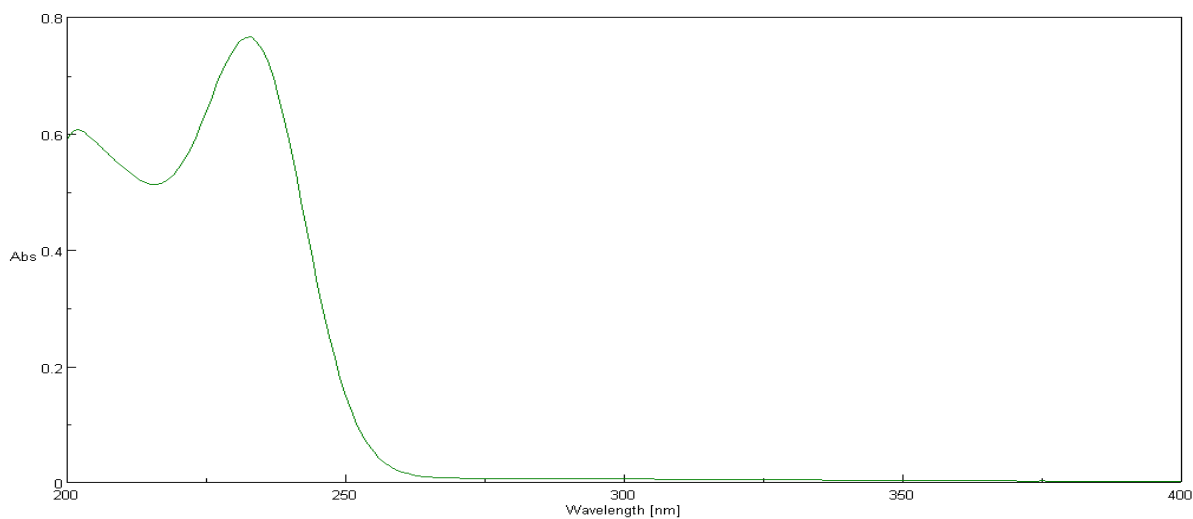
**Fig. 4.** Spectrum of metformin hydrochloride in Acetonitrile.



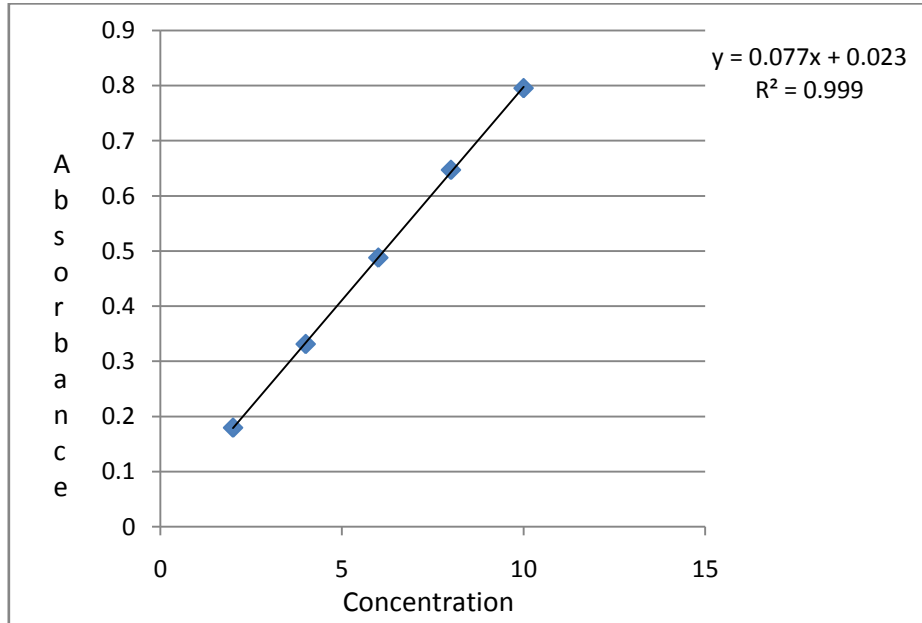
**Fig. 5.** Spectrum of metformin hydrochloride in Ethanol.



**Fig. 6.** Spectrum of metformin hydrochloride in Water.



**Fig. 7.** Spectrum of metformin hydrochloride in Methanol.



**Fig. 8.** Linearity curve for Metformin hydrochloride.

**Table 1.** Solubility of Metformin hydrochloride in various solvent.

Solvent	Wavelength maxima	Absorbance
NaOH	232nm	0.768673
HCL	226nm	0.481611
Acetonitrite	235nm	0.422698
Ethanol	237nm	0.822761
Water	233nm	0.7957

**Table 2.** Linearity of Metformin hydrochloride.

Concentration(ppm)	Absorbance
2	0.1795
4	0.3314
6	0.4882
8	0.6475
10	0.7959

$$y=0.077x+0.023$$

$$R^2 = 0.999$$

**Table 3.** Precision of Metformin hydrochloride.

Sr. No	Percentage Assay	
	Intraday	Interday
1	99.66	98.35
2	99.80	99.02
3	99.79	99.16
4	99.31	99.09
5	98.97	98.69
6	99.97	99.88
<b>Mean</b>	99.51	99.03
<b>SD</b>	0.3228	0.5140
<b>%RSD</b>	0.324	0.519

**Table 4.** Accuracy of Metformin hydrochloride

Recovery Level	% Recovery	Mean % Recovery	Standard Deviation	%RSD
80%	98.58	98.62	0.0282	0.0287
	98.63		0.0070	0.0072
	98.65		0.0212	0.0215
100%	98.28	98.27	0.0071	0.0072
	98.27		0	0
	98.27		0	0
120%	99.06	99.02	0.0283	0.0286
	99.01		0.0071	0.0071
	99.0		0.0141	0.0143

**Table 5.** Ruggedness of Metformin hydrochloride.

Sr. no.	Instrument 1 (Jasco V650)	Instrument 2 (Jasco V530)
1	99.66	99.43
2	99.80	99.28
3	99.79	99.62
4	99.31	99.47
5	98.97	97.41
6	99.97	98.29
<b>Mean</b>	99.51	99.58
<b>S.D</b>	0.3228	0.3731
<b>%RSD</b>	0.3244	0.3746

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