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# Determination and Method Validation for Metformin Hydrochloride Drug Content from Pharmaceutical Product by Reversed Phase High Performance Liquid Chromatography

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**ABSTRACT**

*Reversed phase high performance liquid chromatography (RP-HPLC) method was developed for the determination of metformin hydrochloride from marketed tablets. Chromatographic Separation of metformin hydrochloride has been done with $C^{18}$ column. The mobile phase methanol-water was used in the ratio 70:30 (v/v). The HPLC instrument was equipped with dual pump and UV-VIS detector. The active ingredient of the drug content metformin hydrochloride was optimized at 216 nm. Drug content was determined at the flow rate 1.0 mL/min. The retention time of analyte was 6.38 min. The linearity range was 5-100 µg/mL. The linearity of the method was obtained ($R^2$=0.997). The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.03 and 0.09 µg/mL, respectively. The recoveries of the drug content were found at the three concentration level 99.80, 99.90 and 99.80 %, respectively. Hence, all these results shown that the method validation can be used for routine analysis.*

**Keywords:** Metformine Hydrochloride, RP-HPLC.

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## INTRODUCTION

Chemically metformin is 1-carbamimidamido-N, N-dimethylmethanimidamide. Metformin is an oral biguanide antihyperglycemic drug. It is used for the treatment of diabetes mellitus (type 2). Diabetes is categorised into type 1 and type 2. Type 1 is related to insulin-dependent diabetes and type 2 is related to non-insulin-dependent diabetes. Metformin hydrochloride is also used in the treatment of polycystic ovary syndrome. It helps reducing low-density lipoprotein cholesterol and triglyceride levels and is not at all associated with weight gain. It improves the sensitivity of hepatic and peripheral tissue [1].

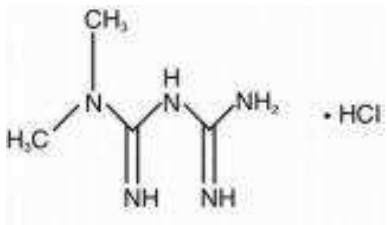


**Fig No.1: structure of metformin hydrochloride**

Several methods have been reported of method validation of metformin with different separation techniques [2-4].

## MATERIALS AND METHODS

### Experimental

### Chemicals and reagents

The 99.7% pure drug of metformin hydrochloride, HPLC grade methanol, distilled water and 0.45 nylon filter membrane were purchased from Merck India Ltd. Mumbai.

### Instrumentation

A binary pump CYBERLAB$^{TM}$ HPLC chromatograph was used for analysis. The separation was on reversed phase $C_{18}$-column. The analyte was monitored with UV detector at 232 nm. The HPLC was operated at isocratic elution mode with 70:30 (v/v) methanol-water mobile phase. The flow rate of elution was 1.0 mL/min. An ultrasonic sonicator was used for the degassing of mobile phase, standard solution and sample solution.

### Preparation of mobile phase

A mixture of methanol /water in the ratio of 70:30 was used as mobile phase. The mobile phase was filtered through a 0.45µm nylon membrane and degassed by sonication.

### Preparation of Metformin hydrochloride stock solution

A 100 ppm stock solution was prepared for the preparation of serial dilutions. Metformin hydrochloride (10 mg) was transferred into a 100 mL volumetric flask and mixed with mobile phase and made up to meniscus, and then it filtered through 0.45µm nylon filter membrane.

### Preparation of sample solution

The 10 tablets of Metformin hydrochloride were weight and crushed by mortar piston. The crushed tablets were mixed well, and then about 10 mg were transferred in to a small conical flask and extracted with mobile phase. The extract was filtered into a 100 ml volumetric flask and the volume was made up to 100 ml. Achieved aliquots were covered the working concentration range 100 ppm.

### Preparation of calibration curve

A calibration curve was constructed to evolution the linearity. The calibration curve was plotted between average peak area and its drug concentration (µg/mL) levels. The serial dilutions 5, 10, 20, 25 and 50 and 100 µg/mL were prepared from the stock solution of Metformin hydrochloride. A total volume of 10 ml was maintained with mobile phase. These different serial dilutions were filtered through a 0.45µm nylon membrane. The each solution of 20 µL was injected into the column in thrice replication.

### Method validation

The describe method was validated according to ICH guidelines with respect to linearity, specificity, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

### Linearity

As per ICH guidelines the linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity can be calculated by using following equation as per ICH guidelines [5-7].

$$y = mx + b$$

Where *m* and *b* are constants (parameters), the constant *m* determines the slope or gradient of that line, and the constant term *b* determines the point at which the line crosses the *y*-axis, otherwise known as the y-intercept.

### Specificity

Specificity was determined with excipient of formulated tablets. An equivalent weight was taken and solution prepared similarly to the sample solution. The prepared solution was determined as per the describe method. After determination was not reported any interference with excipients at the retention time of the peaks of metformin hydrochloride. Therefore, it is concluded that the method is specific.

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives+number of false positives}}$$

**Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the proposed methods was checked by recovery studies, by addition of standard drug solution to reanalyzed sample solution at three different concentration levels 5, 10 and 25 µg/mL. The chromatograms were recorded and the percentage recovery was calculated.

Accuracy can be calculated by using following equation as per ICH guidelines.

$$\text{Percentage Recovery} = \frac{\text{Peak Area of the Drug in Standard}}{\text{Peak Area of the Drug in Sample Mix.}} \times 100$$

**System suitability test**

The reproducibility of sample was checked of the system to measurement of peak area and was carried out using three replicates of same concentration of standard and sample, respectively.

**Limit of detection (LOD)**

According to ICH guidelines the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
Limit of detection can be calculated using following equation as per ICH guidelines.

$$\text{Limit of Detection} = 3.3 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

**Limit of quantification (LOQ)**

The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantization limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$\text{Limit of Quantisation} = 10 \times \frac{\text{Standard deviation of the Peak Area of the Drug}}{\text{Slope of the Corresponding Calibration Curve}}$$

## RESULTS

**Selection of mobile phase**

It is a basic need of high performance liquid chromatography (HPLC) technique. The mobile phase have been selected to check the mobile phase at various composition with the solvent of HPLC grade water and methanol 70:30, 60:40, 50:50, 40:60 and 30:70 (v/v) on reversed phase $C_{18}$- column with the wave length 232 nm. The mobile phase methanol and water in the ratio of 70:30 (v/v) are selected, thus at this mobile phase suitable retention time and peak area was obtained.

**Chromatographic Conditions**

The HPLC analysis has done with mobile phase water and methanol in the ratio of 70:30 (v/v). The separation and identification of the drug content have been done at the flow rate of 1.0 mL/min. The wavelength of drug content 232 nm has been obtained by scanned method. These all parameters showed a well-defined chromatographic separation within a run time of about 7.7 min. The retention time of Metformin hydrochloride was 6.38 min.

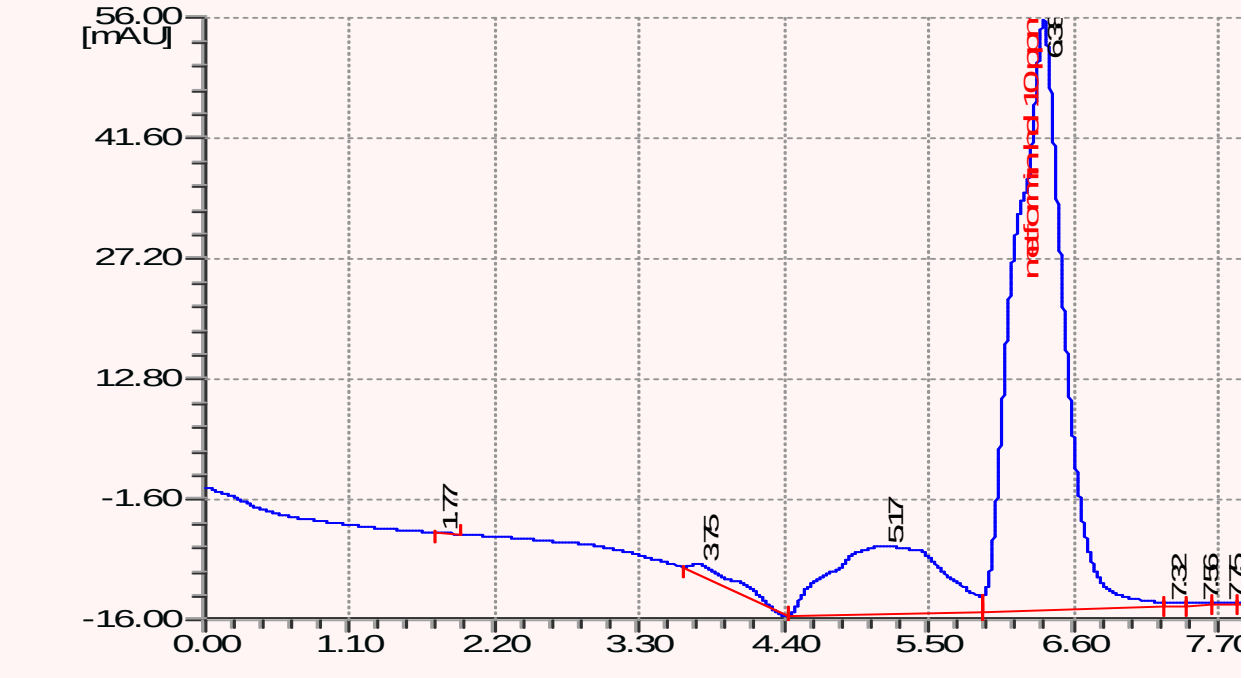


**Fig. 2. Chromatogram of Metformin hydrochloride**

**Specificity**

The specificity of the method was determined by checking the interference with the components from placebo, No interference was observed for any of the components like excipients. For the repeatability of the peaks and retention time the required temperature was 18 $^0$C [8]. The above Chromatogram is showing that the specificity of the method (Fig. 2).

**System stability test**

The System suitability test was performed to stabilization the chromatographic conditions. The test was performed by injecting the standard mixture in thrice replication. The various parameters Retention Time ($R_t$), Tailing Factor ($T_f$), Resolution Factor ($R_f$), and Theoretical Plates ($T_p$) were computed, as reported by USP and International conference harmonized guidelines (ICH). The all parameters were statistically calculated. The CV % to the retention, tailing factor, resolution factor and theoretical plates were reported 1.87, 2.20, 2.60 and 2.45. The calculated system suitability parameters were shown in the following table.

**Table 1. The results for system suitability test**

| Sl. No | Parameter | Mean value | CV in Percentage (%) |
|---|---|---|---|
| **1** | Retention Time | 6.38 | 1.87 |
| **2** | Tailing factor | 0.95 | 2.20 |
| **3** | Resolution factor | 1.17 | 2.60 |
| **4** | Theoretical plates | 2991.99 | 2.45 |

*CV=coefficient variance*

*The calculated CV % value of all parameters are less than 10 (<10) [9], thus all CV% values are significant.*

**Linearity**

The detector response for the proposed method determined to be linear over the range. The five concentration levels 5, 10, 20, 25 and 50 and 100 µg/mL were prepared and injected. The calibration curve was plotted between drug concentrations level and peak area (average value) for HPLC. The linearity of the method was evaluated by linear regression analysis. The following results are obtained on the analysis of the data:

**Table 2. Results for linearity**

| Sl. No | Parameter | RP-HPLC |
|---|---|---|
| 1 | Correlation Range | 5-100 |
| 2 | Liner equation | Y=14679x+16229 |
| 3 | Regression coefficient | $R^2$ =0.997 |

The correlation range was determined for HPLC 5-100 µg/mL, respectively. Regression coefficient was calculated 0.997 which significant at level of 0.05 % error. Hence the above calculated data are showing that the detector response is Linear

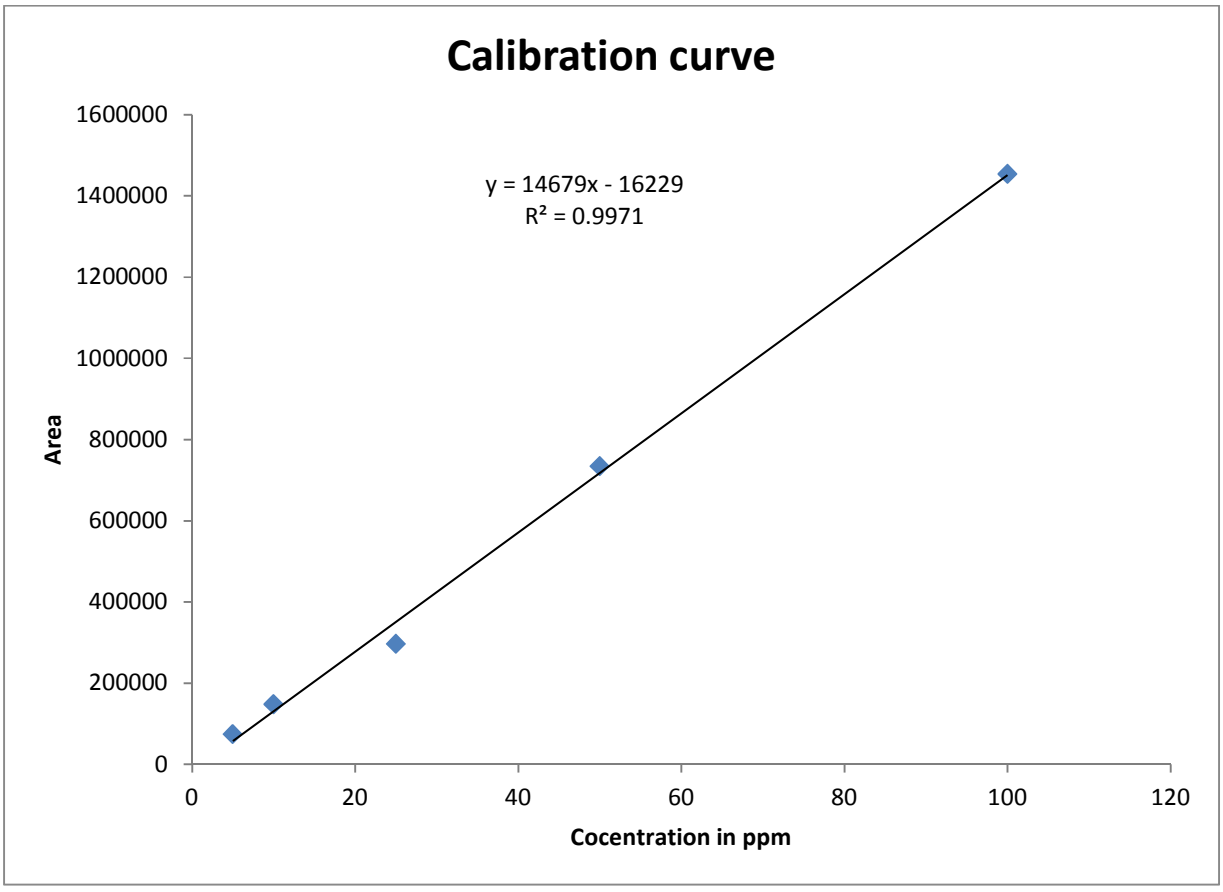


**Fig. 3. The Calibration curve for RP-HPLC method**

**Limits of Detection (LOD) and Limit of Quantification (LOQ)**

The limit of detection (LOD) and limit of quantification (LOQ) were determined by calculating signal to noise ratio for metformine hydrochloride 3 and 10 respectively. The limit of detection (LOD) and limit of quantification (LOQ) values for HPLC were found 0.03 µg/mL and 0.09 µg/mL.

**Table 3. Limit of detection (LOD) and limit of quantification (LOQ)**

| Sl. No | Parameters | RP-HPLC |
|---|---|---|
| **1** | Limit of Detection (LOD) | 0.03µg/ml |
| **2** | Limit of Quantification (LOQ) | 0.09µg/ml |

**Accuracy**

The accuracy of the method validation was determined by recovery method. The recovery was calculated in percentage for HPLC method 99.80, 99.90 and 99.80 at three concentrations level 5, 10 and 25 µg/ml.

**Table 4. Results of recovery experiment by HPLC Method**

| Sl. No | Drug added in µg | Recovery in µg | Recovery in % |
|---|---|---|---|
| **1** | 5 | 4.99 | 99.80 |
| **2** | 10 | 9.99 | 99.90 |
| **3** | 25 | 24.95 | 99.80 |

The recovery results were show good co-relation with adding Metformin hydrochloride drug content. Thus, experimental method was success for the quantitative determination of Metformin hydrochloride.

## DISCUSSION

The system suitability study is indicates that the applied method was suitable for the analysis. Wave length selection is the primary need for the chromatographic analysis. To selection the wave length for metformin hydrochloride was investigated in order to determine a suitable wavelength for the assay evaluation. The suitable wave length was found 232 nm. The selection of mobile phase is an important secondary basic need for chromatographic analysis. The mobile phase was select under isocratic chromatographic mode. The recovery results were showed good accuracy with less than one coefficient variation percentage in both methods.

## CONCLUSION

The development of method and validation for Metformin Hydrochloride by techniques reverse phase-high performance liquid chromatography (RP-HPLC) was successfully applied in the laboratory for the determination of Metformin Hydrochloride content from the marketed tablets (single dose). Hence, the above method can be recommended for simultaneous determination of Metformin Hydrochloride from formulated tablets for routine analysis.

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