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# A Review on Method Development and Validation of Metformin Hhydrochloride by RP-HPLC Method

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**Abstract:** *The RP-HPLC (reversed phase high-performance liquid chromatography) method has developed to estimate and validate the metformin hydrochloride in tablet dosage form using C18 analytical reverse-phase column. The maximum absorption of metformin hydrochloride was found to be 236.40nm, in methanol, Acetonitrile, water (1:3:6), pumped at a flow rate 0.8ml/min at ambient temperature and the run time was 10 min, the symmetry of the column is 4.6× 150□□, with the particle size of 5mm. The analysis complied with beer's law in the concentration range at 233nm for metformin hydrochloride by RP-HPLC & UV method methanol, acetonitrile, the proposed method was validated as per ICH guidelines parameters like linearity, specificity, precision, accuracy, robustness, degradation studies, absorption maximum, LOD & LOQ. Good recovery results were obtained. The results obtained showed a good agreement with the declared contents in case pharmaceutical formulations. The same is also applied for degradation studies. The proposed method was rapid, accurate, economical selective.*

**Keywords:** validation, metformin hydrochloride, degradation studies, RP-HPLC, UV spectrophotometry

## I. INTRODUCTION

Diabetes mellitus is a type 2 diabetes has a high incidence in the world and a high mortality rate, great impact on people's quality of life and its considered a public health problem. Metformin, N,N-dimethylimidodicarbonimidic diamide hydrochloride. It is a derivative of biguanide hydrochloride, known to possess antihyperglycemic activity because it does not normally cause the hypoglycaemia, it lowers blood sugar levels. The mechanism of action involves the binding of the a-polar biguanide hydrocarbon side chain to membrane phospholipids, evoking a change in the electrostatic surface potential, various metabolic effects are elicited, depending on the target cell, tissue, organ, species and metabolic regulation.

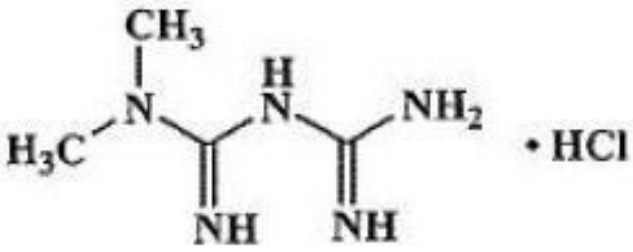


Figure No.1: structure of metformin hydrochloride

It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It has oral bioavailability of 50-60%, under fasting conditions metformin has Pka values of 2.8 and 11.5. the half-life in plasma 6.2-hour, renal clearance value is 510±120ml/min. Approximately 90% of the drug is eliminated in 24 hours in those with healthy renal function and is excreted unchanged in the urine, metformin clearance is approximately 3.5 times that of creatinine clearance including the tubular secretion is the primary mode of metformin UV, RP-HPLC was reported for the determination of metformin.

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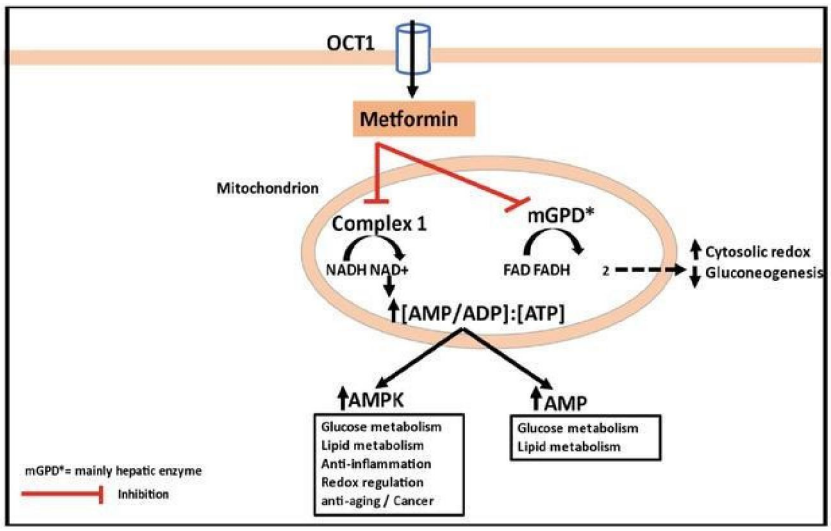


Figure No.2: structure of metformin hydrochloride

**EXPERIMENTAL** The liquid chromatographic equipped with auto sampler and DAD or UV detector with an injecting volume of 20 ml. the analytes were observed
297nm. Chromatographic analysis was performed on Ambient $C_{18}$ having 4.6 × 150nm
i.e. and 5 nm particle size.
Reagents and materials The
drugs used were of AR grade. The drug of metformin in tablet (solid dosage form) were obtained from ESIC supply.

## II. METHOD DEVELOPMENT

Lots of mobile phase and their different proportions were tried and finally was selected as 0.02M Potassium dihydrogen phosphate (KH2PO4), Acetonitrile, Methanol in the ratio of 50:25:25 (v/v/v) at pH 4.3 appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution.

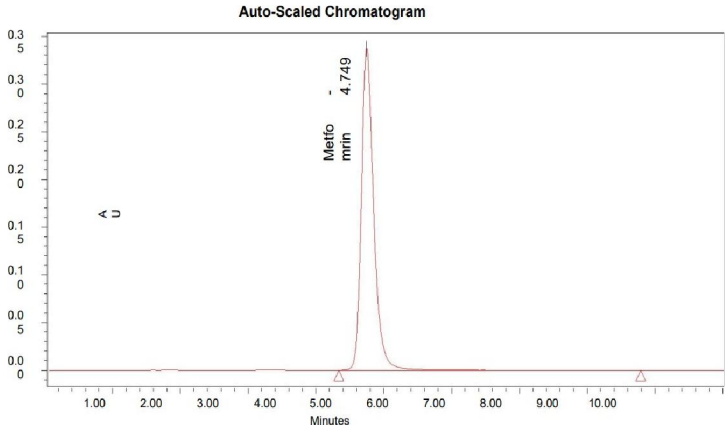


Figure No.3: chromatogram of metformin

## III. SAMPLE PREPARATION

Weigh 5 metformin tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of metformin into a 100 ml volumetric flask. Add about 10ml of dilute and sonicate to dissolve it completely and make up to the mark with diluent. Mix well and filter through 0.45µm filter.
Further pipette out 0.7 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

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**Procedure**

Inject 20 ml of the standard sample into the chromatographic system and measure the area for the metformin peak and calculate the % Assay by using the formulae.

## IV. LINEARITY

According to ICH guidelines linearity should be calculated by taking 5-8 non zeros concentration values and $R^2$ (Correlation Coefficient) should be within the range of 0.995 – 1.0, In this proposed method it was found to be within the limits, table no. 6.3 and figure no 6.3 shows the result.

Table No.1: Linearity

| S. No. | Linearity level | Concentration $(\mu g/ml)$ | Absorbance |
|---|---|---|---|
| **1** | 50 | 0.90 | 0.095 |
| **2** | 75 | 5.00 | 0.295 |
| **3** | 100 | 9.00 | 0.492 |
| **4** | 125 | 14.50 | 0.781 |
| **5** | 150 | 18.00 | 0.933 |

Figure No.4 colour formation for linearity

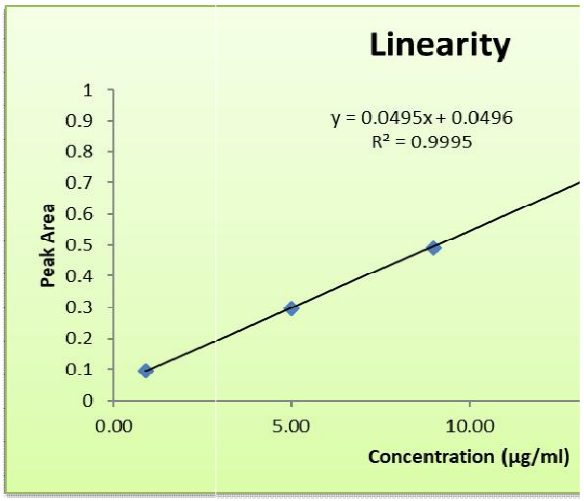


Figure No.5: Linearity for Metformin HCl

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## V. PRECISION

According to ICH guidelines, inter-day and intra-day precision was performed in 3 consecutive days & 3 times in a same day respectively and %RSD in all the cases were found to be less than 2

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Table No.1 inter-day precision

| S. No. | Day 1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|
| | Absorbance | % Assay | Absorbance | % Assay | Absorbance | % Assay |
| 1 | 0.491 | 99.80 | 0.489 | 99.39 | 0.488 | 99.19 |
| 2 | 0.485 | 98.58 | 0.487 | 98.98 | 0.49 | 99.59 |
| 3 | 0.487 | 98.98 | 0.49 | 99.59 | 0.486 | 98.78 |
| 4 | 0.489 | 99.39 | 0.485 | 98.58 | 0.491 | 99.80 |
| 5 | 0.490 | 99.59 | 0.488 | 99.19 | 0.483 | 98.17 |
| 6 | 0.492 | 100.0 | 0.486 | 98.78 | 0.49 | 99.59 |
| Average | 0.49 | 99.39 | 0.49 | 99.09 | 0.49 | 99.19 |
| Std dev | 0.003 | 0.53 | 0.002 | 0.38 | 0.003 | 0.62 |
| % RSD | 0.53 | 0.53 | 0.38 | 0.38 | 0.62 | 0.62 |



| S. No. | Day 1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|
| | Absorbance | % Assay | Absorbance | % Assay | Absorbance | % Assay |
| 1 | 0.491 | 99.80 | 0.489 | 99.39 | 0.488 | 99.19 |
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| 3 | 0.487 | 98.98 | 0.49 | 99.59 | 0.486 | 98.78 |
| 4 | 0.489 | 99.39 | 0.485 | 98.58 | 0.491 | 99.80 |
| 5 | 0.490 | 99.59 | 0.488 | 99.19 | 0.483 | 98.17 |
| 6 | 0.492 | 100.0 | 0.486 | 98.78 | 0.49 | 99.59 |
| Average | 0.49 | 99.39 | 0.49 | 99.09 | 0.49 | 99.19 |
| Std dev | 0.003 | 0.53 | 0.002 | 0.38 | 0.003 | 0.62 |
| % RSD | 0.53 | 0.53 | 0.38 | 0.38 | 0.62 | 0.62 |



Table No.2: Inter-Day Precision

**Degradation studies**

Degradation studies were carried out in 5 different conditions. The % degradation should not be more than 10%/. In this study it was found to be less than 10 %. Table No. 6.10 shows the results of degradation studies.

Table No.7: Degradation Studies

| S. No | Condition | Absorbance | % Assay | % Degradation |
|---|---|---|---|---|
| 1 | Acid (HCl) | 0.45 | 91.46 | 8.54 |
| 2 | Base (NaOH) | 0.448 | 91.06 | 8.94 |
| 3 | $H_2O_2$ | 0.443 | 90.04 | 9.96 |
| 4 | UV | 0.447 | 90.85 | 9.15 |
| 5 | Heat | 0.443 | 90.04 | 9.96 |

## VI. RESULTS AND DISCUSSION

UV Visible spectroscopic method and HPLC are developed and validated as per ICH guidelines. All the methods were found to be simple, sensitive, precise and accurate. These methods were tabulated with one another is given below.

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Table No.8 Summary of UV – Visible spectroscopic method

| Parameter | UV spectroscopy | Visible spectroscopy |
|---|---|---|
| Wavelength | 227.40nm | 270nm |
| Concentration range (µg/ml) | 0-2µg/ml | 0-1 µg/ml |
| Linearity | 0.9982 | 0.9995 |
| Assay | 99.576 | 99.39 |
| Inter-day precision | 0.57 | 0.51 |
| Intra-day precision | 0.41 | 0.52 |
| Accuracy | 99.34% | 98% |
| LOD (µg/ml) | 0.01µg/ml | 0.16µg/ml |
| LOQ (µg/ml) | 0.04µg/ml | 0.53µg/ml |

## VII. CONCLUSION

The review is done based on method developed and validation of metformin hydrochloride. An attempt was developed and validated using different spectrophotometric methods and HPLC method for the estimation of metformin hydrochloride. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines. The method was successfully used for determination of drugs in their pharmaceutical formulation.

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