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Research Article

SIMPLE AND SENSITIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HYDROCHLORIDE BY RP-HPLC

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ABSTRACT

A simple, specific, accurate and isocratic reversed phase - HPLC method was developed and subsequently validated for the determination of Metformin Hydrochloride. Separation was achieved with an Inertsil - Extend - C_{18} HPLC column 250mm in length and having an internal diameter of 4.6mm. A mobile phase comprising 10m.mol 1-Octane sulfonic acid: Acetonitrile in the volume ratio of (80:20) was developed. The detection was carried out using a PDA detector set at a wavelength of 232nm. Validation experiments were performed to demonstrate System suitability, specificity, precision, linearity and Range, Accuracy study, stability of analytical solution and robustness. The method was linear over the concentration range of 1-250µg/ml and get the correlation Regration (r^2) 0.9995, showed good recoveries (100.25 - 101.13%), the relative standard deviations of intra and inter-day assay were 99.4% and 99.94% respectively. The method can be used for quality control assay of Metformin Hydrochloride.

Key words: RP-HPLC, Metformin Hydrochloride, Method validation

INTRODUCTION

Metformin hydrochloride (MET) is chemically N, N-dimethylimidodicarbonimidic diamide hydrochloride (1, 1-dimethylbiguanide hydrochloride) which acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity.

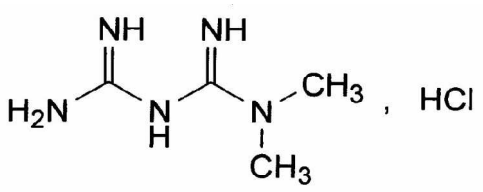

Fig. 1: Chemical Structure of Metformin hydrochloride

It is official in Indian Pharmacopoeia [1], British Pharmacopoeia [2], European Pharmacopoeia [3] and United States Pharmacopeia BP [4]. All the pharmacopoeias describe HPLC method for estimation of MET. A literature survey revealed spectrophotometry [5], HPLC [6-7], LC-MS/MS [8] and LC-electrospray tandem mass spectrometry [9-13] methods for simultaneous estimation of MET in pharmaceutical formulation. And Few UV-Spectrophotometric methods [14, 15], HPLC [16-19] and ion-pair HPLC [20] method have been reported for the estimation of MET. Pharmaceutical validations among these methods undergo the world 'Validation' means 'Assessment' of validity or action of providing effectiveness [21, 22], and validation as per ICH guidelines [23].

MATERIALS AND METHODS

Apparatus

The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 2100 (Cyber scan), the HPLC used is of Water 2695 with PDA- detector. Column used in HPLC is of Inertsil - Extend C_{18} (250 × 4.6mm, packed with 5µm) with a flow rate of 1.0ml/min (isocretic). The mobile phase consists of 10m.mol 1-Octane sulfonic acid: Acetonitrile in the volume ratio of (80:20) pH (3). Mobile phase degassed in a sonicator for about 10minutes. The injection volume is 5µL and the ultra violet detection was at 232nm.

Reagents and solutions

Pure sample of Metformin hydrochloride USP of 100mg and other ingredients such as Acetonitrile and water used were of HPLC and milli-q grade. All other chemicals like 1-Octane sulfonic acid used were of AR grade. Optimized chromatographic conditions are listed in table no.1.

Preparation of standard solution

Accurately weighed 50mg of the Metformin HCl reference standard was transferred to 50mL clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded standard stock solution with concentration 1000µg/ml of Metformin HCl .From the stock solution 5ml was taken and it transferred to the 50mL clean and dry volumetric flask. Then the volume was made up to the mark with the diluent and mixed well. This yielded a standard solution with concentration 100µg/ml was injected.

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution and robustness.

Linearity & range: The Linearity of detector response is established by plotting a graph to concentration versus area of Metformin hydrochloride standard and determining the correlation coefficient. A series of solution of Metformin hydrochloride standard solution in the concentration ranging from about 1µg/ml to 250µg/ml level of the target concentrations were prepared and injected into the HPLC system.

Accuracy: Accuracy for the assay of Metformin hydrochloride tablets is determined by applying the method in triplicate samples of mixture of placebo to which known amount of Metformin hydrochloride standard is added at different levels (80%, 100%, and 120%).

Precision: The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Specificity: The Specificity indicating study of Metformin hydrochloride was undergoes Acid, Alkali and Oxidation degradation Photolysis and Heat condition.

Acid hydrolysis: Sample was treated with 5ml of acid (1N HCl) and kept for 1hr. After 1hr the solution was neutralized with 1N NaOH and analyzed using HPLC.

Oxidation: Metformin hydrochloride solution 100µg/ml was mixed with 5mL of 30% aqueous hydrogen peroxide solution.

Alkali hydrolysis: Sample was treated with 5ml of alkali (1N NaOH) and kept for 1hr. After 1hr the solution was neutralized with 1N HCl and analyzed using HPLC

Photolysis: Samples were kept under UV light for different time intervals (15mins – 7days) and observed by HPLC.

Heat: Samples were heated at 80 ^{0}C for 15mins – 60mins and 220 ^{0}C for 2mins - 5mins and analyzed.

RESULTS AND DISCUSSION

Metformin hydrochloride standard having concentration 100µg/ml was scanned in UV- region between 200-400nm. λ max of Metformin hydrochloride was found to be at 232nm. Metformin hydrochloride Retention time was found to be around 10.785minutes.

The estimation of Metformin hydrochloride tablets was carried out by RP-HPLC using Mobile phase having a composition of 200 volumes of Acetonitrile, 800 volumes of 1-Octane sulfonic acid buffer. The pH was found to be 3. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil-Extend C_{18} (250 × 4.6mm, packed with 5µm). Flow rate of Mobile phase was 1.0ml/min. And all the Optimized chromatographic conditions are listed in table no.1

System suitability parameters such as RSD for six replicate injections was found to be less than 2%, theoretical plates –7187.79, and tailing factor – 1.03. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show System Suitability 0.06% which shows that the method is repeatable.

The acceptance criteria of Method Precision and injection Precision %RSD should be not more than 2.0% and the method show Method Precision 0.6% and injection Precision 0.06% which shows that the method is precise.

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Water 2695 with PDA-detector)
Column	Inertsil-Extend C_{18} (250 × 4.6mm, packed with 5µm) is suitable
Mobile Phase*	1-Octane sulfonic Acid: Acetonitrile (80:20)
Flow rate	1.0ml/min
Detection	PDA at 232nm
Injection volume	5µl
Temperature column	30°C

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 1 to 250µg/ml of target concentration for Metformin hydrochloride standard with a correlation coefficient value is greater than 0.999. The correlation coefficient of (r^2) = 0.999, which shows that the method is capable of producing good response in PDA-detector.

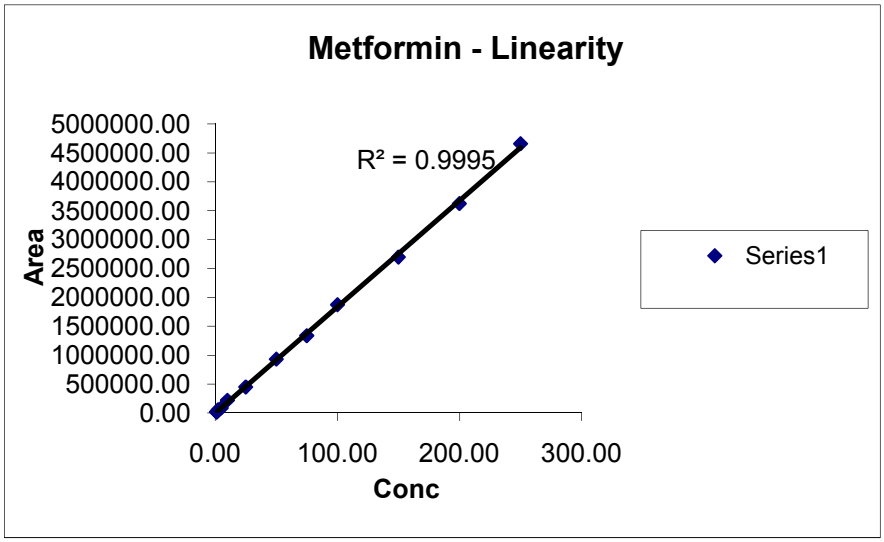

Fig. 2: Linearity of Metformin Hcl

The Accuracy limit is the % recovery should be in the range of 100.25 - 101.13%. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy. And the results of all System suitability parameters are listed in table no.2

The recovery results indicating that the Metformin hydrochloride undergoes Acid, Alkali and Oxidation degradation and drug doesn't undergoes any significant degradation on Photolysis and Heat condition. And the results of all Specificity parameters are listed in table no.3.

Table 2: System suitability parameters

Parameter	Glimepiride hydrochloride
Calibration range (µg/ml)	1-250
Theoretical plates	7187.79
Tailing factor	1.03
Correlation Coefficient(r^2)	0.999
% Recovery	100.25% - 101.13%
System Suitability %RSD	0.06%
Method Precision %RSD	0.6%
Injection Precision %RSD	0.06%

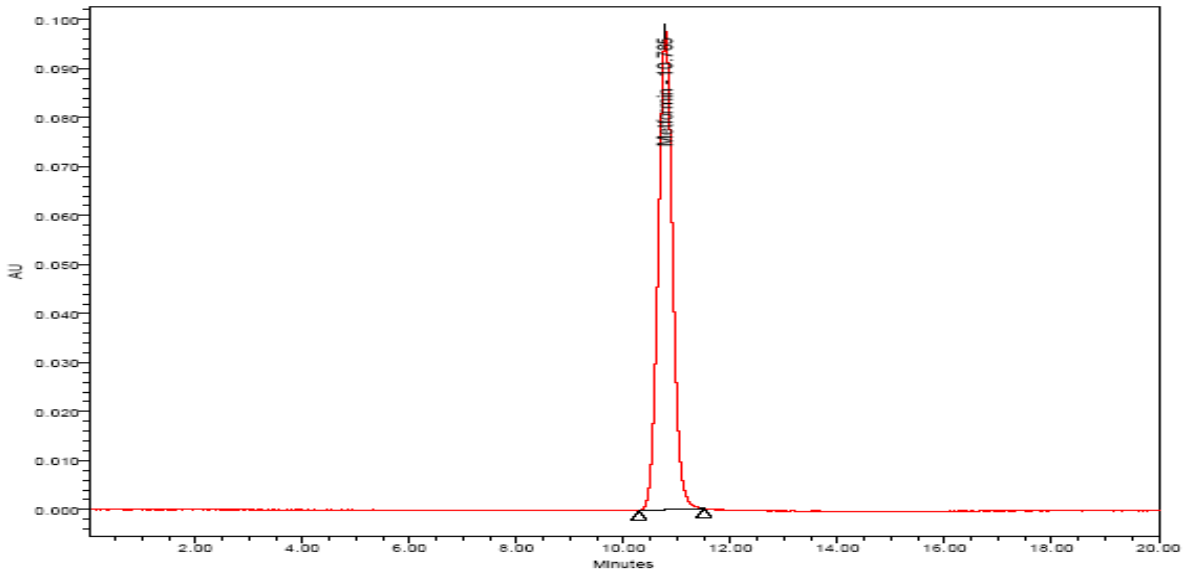

Fig. 3: Standard Chromatogram of Metformin Hcl

Table 3: Specificity parameters

Stress testing	RT	Average area counts	Standard area counts	% Recovery
Acid Hydrolysis				
1N 5ml (1hr)	10.55	1559447	1829709	85.22923591
Alkali Hydrolysis				
1N 5ml (1hr)	10.6	1562935	1829709	85.41986731
Oxidation				
3% H_2O_2	10.5	909380	1829709	49.70079942
Photolysis				
15min	10.7	1841655	1829709	100.6528907
30min	10.7	1815353	1829709	99.21539436
1hr	10.8	1835394	1829709	100.3107051
24hr	10.7	1809604	1829709	98.90119139
48hr	10.6	1807478	1829709	98.78499805
Heat				
80°C (15min)	10.7	1817871	1829709	99.35301187
80°C (30min)	10.8	1818128	1829709	99.36705782
80°C (60min)	10.8	1812204	1829709	99.04329049
220°C (2min)	10.5	1789463	1829709	97.80041526
220°C (5min)	10.4	1790533	1829709	97.8588945

CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. The estimation of Metformin hydrochloride is done by reverse phase HPLC. The mobile phase consists of buffer (800 volumes of 1-Octane sulfonic Acid buffer, and 200 volumes of Acetonitrile. The ratio pH was found to be 3. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes). The detection is carried out using PDA-Detector set at 232nm. The solutions are chromatographer at the constant flow rate of 1.0ml/min. The Retention time for Metformin hydrochloride was around 10.785minutes. Linearity range for Metformin hydrochloride is 1 to 250µg/ml.

The quantitative estimation was carried out on the tablet by RP-HPLC taking a concentration of 100µg/ml. the quantitative results obtained is subjected to the statistical validation. The values of RSD are less than 2.0% indicating the accuracy and precision of the method. The % recovery 100.25% to 101.13% for Metformin hydrochloride. The degradation of Metformin hydrochloride undergoes Acid, Alkali and Oxidation degradation and there was not any significant degradation observed in Photolysis and Heat condition.

The results obtained on the validation parameter met the requirements. It inferred that the method was found to be Simple, Specific, Precision, and Linearity, Proportional i.e. it follows Lambert-Beer's law. The method was found to have a suitable application in routine laboratory analysis with a high degree of Accuracy and Precision.

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