International Journal of Research in Advent Technology, Vol.7, No.4S, April 2019
E-ISSN: 2321-9637
Available online at www.ijrat.org

Hplc Method Validation Of Metformin Hcl In Bulk And Extended Release Tablet Dosage Form As Per Usp

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Abstract- Metformin HCL in bulk and extended release tablet dosage form has been validated for different parameters as per USP and ICH guidelines. The chromatograms were developed using buffer solution 0.5 gm/ltr of sodium heptane sulfonate and 0.5gm/ltr of sodium chloride in water, adjusted with 0.06 M phosphoric acid to a pH of 3.85. the mobile phase used is Acetonitrile and Buffer solution 1:9 proportion. Column – L1 of size 2.9mm × 30cm, 10 µm packing was used as stationery phase. The detection was carried out at 233 nm with flow rate 1 ml/ min. The drug was validated according to USP guidelines for linearity, precision, range, ruggedness, robustness, accuracy and system suitability. The linearity of metformin hydrochloride shows the good values within the standard limits with correlation coefficient 0.999 and slope 75.307. The result shows the good intra-day precision with %RSD 0.8672, 0.8743, 0.8652 within the Acceptable limits. The result shows the good inter-day precision with %RSD 1.2632, 1.0499, 1.1512 within the Acceptable limits. The range of Metformin Hydrochloride shows the good values within the standard limits. (NMT 2.0%). System suitability parameters shows good results with high accuracy of results like number of theoretical plates 4212 which is above the limit specified (NLT 2000), and good retention time 4.320. From the results it is concluded that the method yielded high recoveries with good linearity and precision and the method have good approach for obtaining reliable results and found to be suitable for the routine analysis of Metformin sustained release tablet.

Index Terms- Chromatograms, Mobile phase, Stationary phase, Linearity, Precision, Range, Ruggedness, Robustness, Accuracy and System Suitability.

1. INTRODUCTION

Metformin (dimethyl biguanide) is a synthetic analog of the natural product guanidine, whose history as a treatment for diabetes can be traced to medieval times. Metformin has surpassed the sulfonylureas as the most prescribed oral agent for T2D in the US. In the major European markets, metformin is the second most prescribed agent after glyburide. The widespread acceptance of metformin evolved after the realization that lactic acidosis was not a major problem in individuals with normal renal function. Phenformin, a structurally similar analog of metformin, was previously withdrawn from the market in many countries due to its propensity to induce lactic acidosis.

Metformin is recommended as a first-line therapy in newly diagnosed individuals, and can be used in combination with an insulin secretagogue (sulfonylurea or meglitinide), thiazolidinedione, glucosidase inhibitor, or insulin. When used as a monotherapy; metformin decreases HbA1C by 1.5-2.0%, increases insulin sensitivity, does not promote weight gain, and has an acceptable side effect profile.

Mechanism of Action

Metformin Hydrochloride is not a hypoglycemic agent but antihyperglycemic agent. It lowers blood glucose concentration in presence of hyperglycemia but does not decrease it below normal range. Metformin Hydrochloride decreases hepatic glucose production and decreases insulin requirement for glucose disposal, decreases intestinal absorption of glucose, increases uptake of glucose from the blood into the tissues. Metformin hydrochloride improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas Metformin Hydrochloride has no effect on pancreatic insulin secretion and is not effective in absence of insulin. It has been noted that Metformin Hydrochloride does not lower blood glucose concentrations in non-obese and non-diabetic individuals.

Uses

Antidiabetic agent; lowers plasma glucose levels and improves insulin sensitivity. Metformin Hydrochloride Inhibits hepatic gluconeogenesis via activation of the LKB1/AMPK pathway. It also displays antiproliferative effects in cancer cell lines. It is also used in the treatment of polycystic ovary syndrome, and has been investigated for other diseases where insulin resistance may be an important factor.

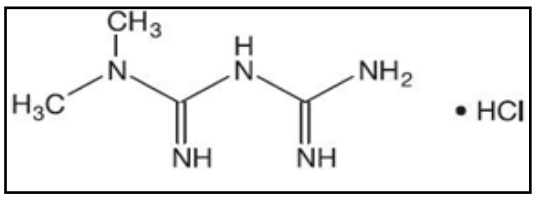


Figure 1.1

Structure of Metformin Hydrochloride

USP defines, it is often necessary to validate methods to determine if their average results or variabilities differ by an amount that is deemed important. The goal of the method validation is to generate adequate data to evaluate the equivalency of method over a range of concentrations. Work based on comparison of various parameters to be evaluated. The present work is based on the analytical assay and method validation parameters as per official books i.e. USP. The standard books have described the assay and validation procedures for the Metformin sustain release tablet, extended release tablet respectively. For validation selected method is HPLC method and is validated for specificity, linearity and range, precision, accuracy, robustness and system suitability according to USP and ICH guidelines.

2. MATERIAL AND METHODS

The following chromatographic conditions were established and were kept constant throughout the experimentation

Table No. 2.1
Specifications for HPLC

Sr. No.	Specification	Details
1.	HPLC	Shimadzu CBM-20A/20Alite
2.	Column – L_1	2.9mm × 30cm, 10 µm packing
3.	Column temp.	30^0
4.	Flow rate	1 ml/ min.
5.	Software	LC-Solution
6.	Detector	UV- 218 nm

Reagents And Chemicals

Table No. 2.2
Specifications for Chemicals & Reagents

Sr. No.	Reagents and Chemicals	Details
1.	Sodium heptane sulfonate	HPLC Grade
2.	Sodium chloride	AR Grade
3.	Phosphoric Acid	HPLC Grade
4.	Acetonitrile	HPLC Grade
5.	HPLC water	HPLC Grade
6.	Distilled Water	Double distilled

Preparation of Buffer Solution

0.5 gm/ltr of sodium heptane sulfonate and 0.5 gm/ltr of sodium chloride in water. Before final dilution adjusted with 0.06 M phosphoric acid to a pH of 3.85.

Mobile phase

Acetonitrile and Buffer solution 1:9 proportion. (Note: to improve the separation, the composition of acetonitrile and buffer solution may be changed to 1:19, if necessary.)

Dilution: 1.25% solution of acetonitrile in water.

Standard solution

Taken (L/4000) mg/ml of USP Metformin hydrochloride RS in Diluent, where L is the labeled quantity, in mg of metformin hydrochloride in each tablet.

Sample stock Solution

Finely powdered NLT 10 tablets, transferred powder equivalent to the average tablet weight to a homogenization vessel, and added 500ml of 10% acetonitrile solution. Alternately homogenized and allowed soaking, the suggested homogenization sequence as follows.

Homogenized the sample using five pulses, each of 5 sec, at about 20,000 rpm, and soaked for 2 min. repeat these steps two additional times.

Sample solution

Passed a portion of the Sample Stock Solution through a suitable filter of 0.45µm pore size. Discarded the first 3 ml of filtrate, transferred 25 ml 0f the filtrate to a 200 ml volumetric flask. And diluted with water to volume 200ml.

Acceptance Criteria

Metformin Hydrochloride Sustained release tablet should contain not less than 90.0 % and not more than 110.0 % of Metformin hydrochloride.

Preparation of Stock solution for Validation

Finely powdered NLT 10 tablets, transferred powder equivalent to the average tablet weight (750 mg) to a homogenization vessel, and added 500ml of 10% acetonitrile solution. Alternately homogenized and allowed to soak, the suggested homogenization sequence as follows.

Homogenized the sample using five pulses, each of 5 sec, at about 20,000 rpm, and allowed soaking for 2 min. repeated these steps two additional times. Passed a portion of the Sample Stock Solution through a suitable filter of 0.45 µm pore size. Discarded the first 3 ml of filtrate, transferred 25 ml 0f the filtrate to a 200 ml volumetric flask. And diluted with water to volume 200ml.

This was taken as a 100% concentration solution. Solutions containing Metformin Hydrochloride of five different concentrations (50%, 75%, 80%, 100%, 120%, 125% and 150% of target concentration) were prepared in the same way for validation purpose.

3. PREPARATION OF SAMPLE SOLUTIONS

Preparation of 50% solution containing 12.5ml of MET

Pipette out 12.5ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 75% solution containing 18.75ml of MET

Pipette out 18.75ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 80% solution containing 20ml of MET

Pipette out 20ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 100% solution containing 25ml of MET

Pipette out 25ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 120% solution containing 30ml of MET

Pipette out 30ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 125% solution containing 31.25ml of MET

Pipette out 31.25ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

Preparation of 150% solution containing 37.5ml of MET

Pipette out 37.5ml filtrate from the filtrated sample stock solution and transferred to 200 ml volumetric flask, and diluted with water to make up volume 200ml.

4. RESULTS AND DISCUSSION

HPLC Assay Method As Per USP

USP assay was performed by using HPLC as described in the standard book. Table no. 4.1 shows the detail of assay solutions. The solution taken for the experimental procedure was prepared in triplicate and chromatogram plotted standard and sample solutions which are shown in Figure no. 4.1 and 4.2. The resultant areas are noted, and from this data the % assay was calculated in accordance with standard area. From this data Mean, SD and %RSD were calculated.

The %RSD observed 1.0822 was within the limit. And the % purity was found to be 98.2029% which was also within the standard limit.

Table No. 4.1
Assay Data

Sr. No.	% Solution Concentration	Area	% assay	Mean	SD	% RSD
1.	100	739.221	98.2029	**98.2029**	**1.0627**	**1.0822**
2.	100	747.221	99.2657			
3.	100	731.221	97.1402			

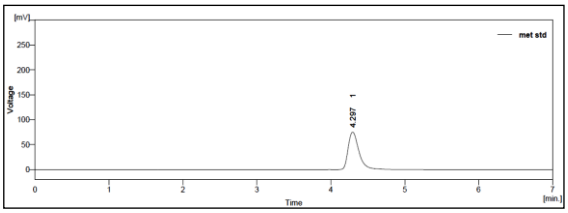


Figure No. 4.1
Chromatogram of Standard Solution

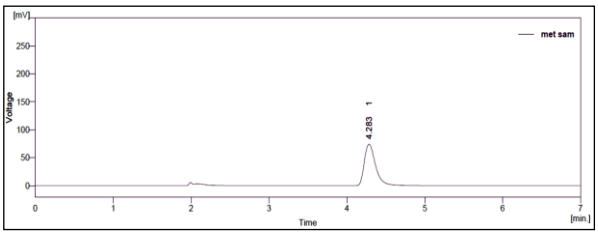


Figure No. 4.2

Chromatogram of Sample Solution

Linearity Study

Linearity study was performed by testing series of sample solution of Metformin Hydrochloride. Table no. 4.2 shows the variable concentrations ranging from 5µg/ml to 15µg/ml i.e. the solution having 50% to 150% concentration of drug sample. The 5 samples were tested for the linearity study. The calibration graph was constructed peak area vs. the drug concentration as shown in Figure No. 4.3.

The linearity of metformin hydrochloride shows the good values within the standard limits with

correlation coefficient 0.999 and slope 75.307. as shown in graph no 4.1

Table No. 4.2
Linearity Data

Sr. No.	Concentration (µg/ml)	Retention time (min.)	Injection volume (µL)	USP plate count	Area
1.	5.0	4.307	10	4370	376.085
2.	7.5	4.290	10	4337	562.897
3.	10.0	4.293	10	4343	571.992
4.	12.5	4.310	10	4377	944.243
5.	15.0	4.283	10	4323	1126.751
Mean					**716.393**
Slope					**75.307**
SD					**2.5360**
Correlation Coefficient (r^2)					**0.9999**

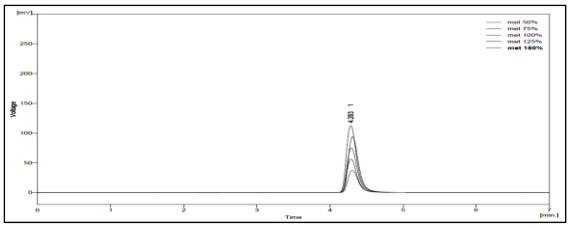
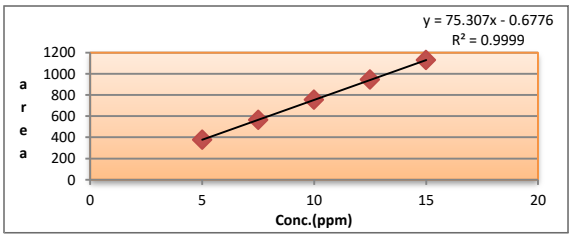


Figure No. 4.3
Overlay Chromatogram of Linearity Study



Graph No. 4.1
Graph of Linearity Study

Precision Study
Intra-Day Precision

Three sample preparations of homogeneous mixture of Metformin Hydrochloride were prepared and estimated for content as per methodology for 50%, 100% and 150% strengths of samples in one day

The results along with the %RSD of area shows the acceptable level of precision for the Metformin Hydrochloride. The result shows the good precision with %RSD 0.8672, 0.8743, 0.8652 within the Acceptable limits as described in table no. 4.3

Table No. 4.3
Intra-Day Precision Data

Sr. No.	% Solution Concentration	Retention time	Area	Mean	SD	% RSD
1.	50	4.320	379.482	**375.9546**	**3.2605**	**0.8672**
2.	50	4.300	373.051			
3.	50	4.303	375.331			
4.	100	4.283	746.785	**752.3003**	**6.5774**	**0.8743**
5.	100	4.307	759.58			
6.	100	4.290	750.536			
7.	150	4.293	1136.931	**1126.3793**	**9.7463**	**0.8652**
8.	150	4.277	1117.714			
9.	150	4.283	1124.493			

Table No. 4.4
USP Inter-Day Precision Data

Sr. No.	% Solution Concentration	Retention time	Area	Mean	SD	% RSD
1.	50	4.290	371.172	**376.2076**	**4.7523**	**1.2632**
2.	50	4.320	380.614			
3.	50	4.313	376.837			
4.	100	4.283	476.785	**754.2806**	**7.9195**	**1.0499**
5.	100	4.313	762.565			
6.	100	4.300	753.492			
7.	150	4.270	1117.769	**1130.1593**	**13.0110**	**1.1512**
8.	150	4.303	1143.713			
9.	150	4.290	1128.996			

***Inter-Day Precision*:**

Three sample preparations of homogeneous mixture of Metformin Hydrochloride were prepared

and estimated for content as per methodology for 50%, 100% and 150% strengths of samples in three days.

The results along with the %RSD of area shows the acceptable level of precision for the Metformin Hydrochloride. As specified in table no 4.4 The result shows the good precision with %RSD 1.2632, 1.0499, 1.1512 within the Acceptable limits.

Range Study

When the range for the validation of analytical procedures was investigated, 80 to 120 of specified limit of testing were considered. Range studies for Metformin Hydrochloride were recommended to be performed at the 80% and 120% levels of label claim. Study performed by using the 6 replicates of each specified concentrations. Area observed and from this data %RSD calculated.

The %RSD of Metformin Hydrochloride was found to be 0.9997 and 0.9989 for 80% and 120% concentration solutions respectively as specified in table no 4.5. The range of Metformin Hydrochloride shows the good values within the standard limits. (NMT 2.0%)

Table No. 4.5
USP Range Data

Sr. No.	Concentration (µg/ml)	Area	Mean	SD	% RSD
80% - Solution of Lower Concentration					
1.	8	613.874	**605.8553**	**6.0570**	**0.9997**
2.	8	610.431			
3.	8	603.469			
4.	8	608.248			
5.	8	597.632			
6.	8	601.478			
120% - Solution of Higher Concentration					
1.	12	903.124	**909.7162**	**9.0872**	**0.9989**
2.	12	917.992			
3.	12	915.879			
4.	12	918.976			
5.	12	897.232			
6.	12	905.094			

Recovery (Accuracy Study)

Series of standard preparations of 80µg/ml, 100µg/ml and 120µg/ml of Metformin Hydrochloride were prepared in triplicate of the working concentration. The solutions prepared tested and area was noted. The resultant recoveries and the % recovery were calculated. From this data Mean, SD and %RSD were calculated.

The mean recovery is 100.3088%, 100.0031% and 100.5207% and calculated %RSD is 0.3781%, 1.1350% and 0.9951% for 80µg/ml, 100µg/ml and 120µg/ml of Metformin Hydrochloride solutions respectively. The percentage recovery is well within limit and also %RSD is within the acceptable criteria. (NMT 2.0%) as described in table no. 4.6.

Table No. 4.6
Recovery Data

Sr. No	% Solution Concentration	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Mean	SD	% RSD
1.	80	8.0	7.9956	99.9461	**100.3088**	**0.3793**	**0.3781**
2.	80	8.0	8.0562	100.7028			
3.	80	8.0	8.0221	100.2774			
4.	100	10.0	9.8913	98.9130	**100.0031**	**1.1350**	**1.1350**
5.	100	10.0	10.1178	101.1783			
6.	100	10.0	9.9918	99.9180			
7.	120	12.0	11.9383	99.4860	**100.5207**	**1.0003**	**0.9951**
8.	120	12.0	12.1779	101.4828			
9.	120	12.0	12.0711	100.5933			

System Suitability Study

System suitability was performed by using 100% concentration solution of Metformin Hydrochloride and system suitability stock solutions as described in the USP standard procedure. The earlier prepared solutions were observed for the results during the testing.

System suitability parameters shows good results with high accuracy of results like number of theoretical plates 4212 which is above the limit specified (NLT 2000), and good retention time 4.320, having asymmetry within the limit 1.667 which is within standard limit specified (NMT 2) and %RSD

also shows the good results 1.0822 which is within the standard limit specified (NMT 2%).

The whole study parameters shows good results as per specified limit which gave permission to carry out analysis of other parameters as specified in table no 4.7

Table No. 4.7
System Suitability Data

Sr. No.	System Suitability Parameters	Results Observed
1.	Retention time	4.320
2.	Theoretical plate	4212
3.	Asymmetry	1.667
4.	%RSD	1.0822

Robustness Study

It was measured by its capacity to remain unaffected by small but deliberate change in method parameters and provides an indication of its reliability in normal usage. The method parameters studied for HPLC robustness study are the variation in flow rate, mobile phase composition, pH, temperature as specified in table no 4.8

Variation in Flow Rate:

It was determined by multiple injections of homogeneous test solutions of Metformin Hydrochloride by changing the flow rate ± 0.2 ml/min i.e. 0.8 ml/min and 1.2 ml/min on same instrument under the same operating conditions. The injections were done in triplet manner and mean, SD and %RSD were calculated.

The result shows method is robust for Metformin Hydrochloride as it shows good results for %RSD 0.8614, 0.5499 which is well within acceptance limit with change in flow rate +0.2ml/min and -0.2ml/min respectively.

Variation in Mobile Phase Composition

It was determined by multiple injections of homogeneous test solutions of Metformin Hydrochloride by changing the mobile phase composition i.e. 0.8:9.2 and 1.2:8.8 of acetonitrile and buffer solution on same instrument under the same operating conditions. The injections were done in triplet manner and mean, SD and %RSD were calculated.

The result shows method is robust for Metformin Hydrochloride as it shows good results for %RSD 0.8628, 1.0047 which is well within acceptance limit with change in mobile phase composition +0.2ml and -0.2ml respectively.

Variation in pH:

It was determined by multiple injections of homogeneous test solutions of Metformin Hydrochloride by changing the pH of mobile phase i.e. 3.83 and 3.87 of acetonitrile and buffer solution on same instrument under the same operating conditions. The injections were done in triplet manner and mean, SD and %RSD were calculated.

The result shows method is robust for Metformin Hydrochloride as it shows good results for %RSD 0.8648, 1.0490 which is well within acceptance limit with change in pH of mobile phase +0.2 and -0.2 respectively.

Table No. 4.8 USP Robustness Data

Sr. No.	Retention time (Min.)	Area	Mean	SD	% RSD
Change in flow rate +0.2					
1.	4.090	719.006	**712.029**	**6.1341**	**0.8614**
2.	4.080	707.483			
3.	4.073	709.598			
Change in flow rate -0.2					
1.	4.523	784.504	**787.494**	**4.3304**	**0.5499**
2.	4.513	792.46			
3.	4.500	785.518			
Change in mobile phase +0.2					
1.	4.307	757.303	**750.2917**	**6.4737**	**0.8628**
2.	4.280	744.541			
3.	4.287	749.031			
Change in mobile phase -0.2					
1.	4.307	761.067	**753.0463**	**7.5658**	**1.0047**
2.	4.280	746.037			
3.	4.297	752.035			
Change in pH +0.2					
1.	4.303	755.785	**748.777**	**6.4755**	**0.8648**
2.	4.280	743.015			
3.	4.287	747.531			
Change in pH -0.2					
1.	4.280	743.015	**750.768**	**7.8757**	**1.0490**
2.	4.307	758.761			
3.	4.293	750.528			

5. CONCLUSION

The present work done was based on the analytical assay and method validation parameters as per official book i.e. USP. Standard book have described the assay and validation procedures for the Metformin extended release tablet. Selected method was HPLC method and validated for specificity, linearity, range, precision, accuracy, ruggedness robustness and system suitability according to USP and ICH guidelines. From the results it is concluded that standard method yielded high recoveries with good linearity and precision and have good approach for obtaining reliable results and found to be suitable for the routine analysis of Metformin sustained release tablet.

The USP method can be easily carried out at laboratory level and also industrial level for better results, ease of process also reduces cost per sample and reduces time of testing. The overall results shows that the USP described HPLC method was validated according to USP and ICH guidelines that offering simple and economical mean to monitor quality formulations. From the validation parameters we can conclude that this method is simple, economic, precise, accurate, selective, specific and iterative.

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