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Analytical Method Development and Validation of Metformin by using RP-HPLC System

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Abstract: *A precise and reliable high-performance liquid chromatography (HPLC) method was developed and validated for the quantitative determination of metformin in pharmaceutical formulations. The method employed a reverse-phase chromatographic separation using a C18 column, with a mixture of mobile phase. Detection was performed at a wavelength of 236 nm. This study presents a comprehensive theoretical and practical framework for the development and validation of a high-performance liquid chromatography (HPLC) method for the quantitative analysis of metformin in pharmaceutical formulations. The theoretical aspects of HPLC, including chromatographic theory, column chemistry, and detector principles, are discussed in relation to the development of a robust and reliable analytical method. The validated method can be readily applied for the routine analysis of metformin in pharmaceutical products, ensuring the quality and purity of the active pharmaceutical ingredient.*

Keywords: Reverse phase-HPLC (RP-HPLC), Limit of detection, Limit of quantification, Method validation, System suitability, Relative standard deviation, Specificity, Linearity, Range, Precision, Intermediate precision, Accuracy, Solution stability and system suitability

I. INTRODUCTION

HPLC offers several advantages, including high sensitivity, accuracy, and precision, making it an ideal technique for the analysis of metformin. HPLC can be used for the separation, identification, and quantification of metformin and its related substances. The development of a reliable and robust HPLC method for metformin analysis is crucial to ensure the quality and purity of the active pharmaceutical ingredient. A well-validated HPLC method can help to detect impurities, degradation products, and other related substances, which is essential for maintaining the safety and efficacy of metformin-containing pharmaceutical products.

The Shimadzu HPLC is a high-performance liquid chromatography system designed for the separation, identification, and quantification of various compounds. It consists of a solvent delivery system, an autosampler, a column oven, and a detector, which work together to provide accurate and reliable results. The Shimadzu HPLC is equipped with advanced features such as high-pressure gradient capabilities, precise temperature control, and a user-friendly interface. It is widely used in various fields, including pharmaceuticals, biotechnology, environmental monitoring, and food safety, for the analysis of complex samples and the detection of trace levels of impurities.

Analytical chemistry uses specific techniques and tools to separate, measure, and identify the sample in order to assist determine the quality, purity, and safety of chemicals and medications. It aids in both quantitative and qualitative examination of the sample; in the former, it establishes the sample's quality and purity, while in the latter, it identifies the concentration of the sample, or the quantity of the anticipated material contained within it. High-Performance Liquid Chromatography, or HPLC, is primarily used to separate, identify, and quantify the constituents of a mixture.

Metformin hydrochloride, also known as 1-carbamimidamido-N,N- metformin dimethylmethanimidamide hydrochloride, is an antidiabetic medication that belongs to the biguanide class of antihyperglycemic agents. It works by increasing glucose absorption and decreasing hepatic glucose synthesis, which also helps to avoid cardiovascular

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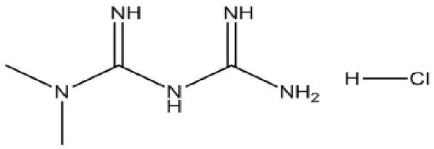
system problems. It also lowers triglyceride and low-density lipoprotein cholesterol levels and is used to treat PCOS issues.



Metformin hydrochloride

Fig. 1: Structure of metformin HCl

Chemical name: 1-carbamimidamido-N, N-dimethyl-methanimidamide hydrochloride
Molecular Formula: $C_4H_{12}ClN_5$
Molecular Weight: 165.62
Appearance: White or almost white crystalline powder
State: solid
Melting point: 223-226 °C
pKa: 12.4
Category: Biguanide class of anti-hyperglycemic agent.
Materials and Techniques:
Collection of Drug -
The drug material i.e. Metformin were collected from the Arny Analytics, Nashik,Maharashtra, during the month of march in the year 2025.

Instrument- RP-HPLC System

Requirements : Following list of Reagents/Standards/Equipments was required during method validation.
Working Standard Details:

Name of Standard	Metformin

List of Reagents and Standards:

Sr. No.	**Reagents and Standards**	**Grade**	**Make**
1	Potassium dihydrogen orthophosphate	AR	SD fine-chem limited
2.	Acetonitrile	AR	Fisher Scientific
3.	Water	HPLC	Fisher Scientific

List of Instruments / Equipments:

Sr. No.	**Instruments / Equipments**	**Instruments / Equipments Make**
1.	HPLC System	Shimadzu, LC-2010C_{HT}
2.	Software	LC Solution
3.	Sonicator	Life Care Instruments Pvt. Ltd.
4.	Analytical Balance	WabMan
5.	HPLC Column :C18, (4.6 mm x 25-cm), 5µm	Waters

Methodology: Assay by HPLC
Specification of Metformin 50mg (% Assay): Between 90.0% to 110.0% .
The % Assay in Metformin 50mg was determined by HPLC according to the Following parameters:

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Typical Chromatographic Conditions:

Equipment	Shimadzu (LC-2010C_{HT})
Column	HPLC Column : C18,(250 x 4.6 mm),5µm
Flow Rate	1.0 ml / min
Wavelength	233 nm
Injection volume	20 µl

Mobile phasepreparation: Water and Acetonitrile were combined in a 70:30 ratio to create the mobile phase.

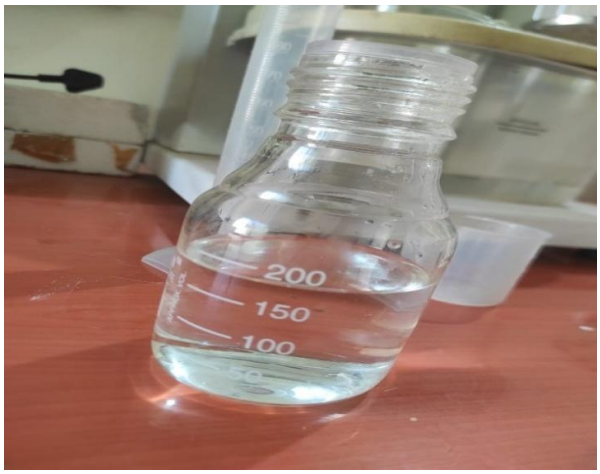

Fig. No: 1 – Mobile Phase Preperation

Standard solution preparation: weighing 50 mg of the working standard sample, metformin HCl, roughly, transferring it to a 100 ml volumetric flask, dissolving it in mobile phase solution, and then adding more solution to get the desired volume.

Fig.No: 2 – 50mg Metformin

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Fig.No: 3 – Sample Preperation

Sample solution: Transfer 10 ml of the produced standard solution to a 25 ml volumetric flask and diluting it with mobile phase until it reached the desired level.

Validation of the developed method

The developed RP-HPLC technique for Metformin HCl in the tablet dosage form was validated in accordance with ICH recommendations. The characteristics such as accuracy, linearity and range, specificity, robustness, system appropriateness, and intermediate precision (ruggedness) were all validated.

II. RESULTS AND DISCUSSION

New method was developed for the assay studies of Metformin HCl .The following are conditions stabilized.

Table 1: Chromatographic conditions

Chromatographic mode	**Isocratic (70:30) Buffer: Acetonitrile**
Detector wavelength	232 nm
Flow rate	0.5 ml/min
Injection volume	20µl
Column	Shimadzu C_{18} column (5µm×4.6×250 mm).
Column oven	25 °C
Run time	10 min

1. SPECIFICITY: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Acceptance criteria:

The relative standard deviation for Metformin should not be more than 2.0%

There is no interference of blank with the principal peaks i.e. Metformin .

Observation Table:

System Suitability observed during Specificity

	Area
No of Inj	**Metformin**
1	1123484
2	1123274
3	1122697
Average	**1123152**
STDEV	407.512
%RSD	**0.04**

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Specificity

	R.T.
Sample	**Metformin**
Blank	
Standard Solution	3.395
Sample Solution	3.390

Conclusion:
There was no interference observed in Standard & Sample due to Blank.

2. 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. This is sometimes termed trueness.
The accuracy of an analytical method is defined as the closeness between the observed values with actual or true value for a specific concentration. Accuracy - closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference sample..
Spike the placebo with 80 % of the standard concentration.
Spike the placebo with 100 % of the standard concentration.
Spike the placebo with 120 % of the standard concentration.
Calculate % Recovery for every solution at each level.
Acceptance Criteria:
The % recovery of the Metformin for each injection of each concentration must be between 98 % and 102%.
Observation Table: Accuracy of Metformin

Test Solutions				
Level	**mg of drug**	**Metformin**	**mg of drug Recovered**	**% Recovery**
80%	0.40000	905782	0.403108	100.78
100%	0.50000	1117183	0.497189	99.44
120%	0.60000	1360440	0.605448	100.91
			Average	**100.37**
			Std.dev	0.81
			%RSD	0.81

Conclusion:
The % recovery of the Metformin for each injection of each concentration is 100.78% , 99.44% & 100.91% respectively and mean recovery is 100.37% , which is within the acceptance criteria, hence the method for % assay determination of Metformin is accurate.

3. PRECISION: Precision is a measurement of degree of Reproducibility of analytical method and it will be expressed in terms of % relative standard for the area and retention time of Solution prepared. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It is usually
specified in terms of standard deviation (SD) or relative standard deviation (RSD) is as follows and calculated by formulas

SD =i=1nXi-X2n-1

RSD (%) = 100SDX

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Acceptance criteria: The relative standard deviation for Metformin should not be more than 2.0%

Observation Table:

System Suitability observed during Precision

Standard Solution	
	Area
No. of Inj.	**Metformin**
1	1131058
2	1131304
3	1131959
Average	**1131440**
SDTEV	465.715
%RSD	**0.04**

Sample Results..

SR. No.	**Area**	**% Assay**
	Metformin	**Metformin**
1	1138024	100.58
2	1138321	100.61
3	1138196	100.60
Average	**1138180**	**100.60**

Acceptance criteria: % Assay of Metformin is NLT 90.0% And NMT 110.0%.

Conclusion: % Assay is NLT 90.0% and NMT 110.0% So the method for assay of Metformin 100mg is precise.

4. INTERMEDIATE PRECISION:

Precision under analysis repeatability conditions i.e. conditions where independent test results were obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

	Standard Solution
	Area
No. of Inj.	**Metformin**
1	1123497
2	1123534
3	1123464
Average	**1123498**

	Area	**% Assay**
Sr. No.	**Metformin**	**Metformin**
1	1127819	100.38
2	1127533	100.36
3	1128237	100.42
Average	**1127863**	**100.39**

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	% Assay	Difference
Precision %Assay	100.60	
Intermediate Precision %Assay	100.39	0.21

(Difference = Intermediate Precision % Assay –Precision %Assay)
Acceptance criteria: % Assay of Metformin is NLT 90.0% And NMT 110.0%.
Conclusion:% Assay is NLT 90.0% and NMT 110.0% So the method for assay of Metformin 100 mg Tablet is precision

7. **LINEARITY &RANGE:** Weigh 500 mg of Metformin in 100 ml of volumetric flask sonicate and dissolve and dilute to volume with Mobile phase. Further dilute 1.0ml of standard to 50 ml with Mobile phase.

Linearity Solutions:
Linearity Solution (80ppm Metformin) : Further dilute 0.8ml of standard to 50 ml with Mobile phase.
Linearity Solution (90ppm Metformin) : Further dilute 0.9ml of standard to 50 ml with Mobile phase.
Linearity Solution (100ppm Metformin): Further dilute 1.0ml of standard to 50 ml with Mobile phase.
Linearity Solution (110ppm Metformin): Further dilute 1.1ml of standard to 50 ml with Mobile phase.
Linearity Solution (120ppm Metformin): Further dilute 1.2ml of standard to 50 ml with Mobile phase.

Observation Table:
Linearity of Metformin

Avg. Area	Conc in ppm	Conc. of Solution
0	0.0	
915241	400.0	80
1026947	450.0	90
1141634	500.0	100
1254159	550.0	110
1370242	600.0	120

Metformin	
Y intercept	**395.5893**
Slope	**11411.6989**
Corelation Coefficient	**1.0000**

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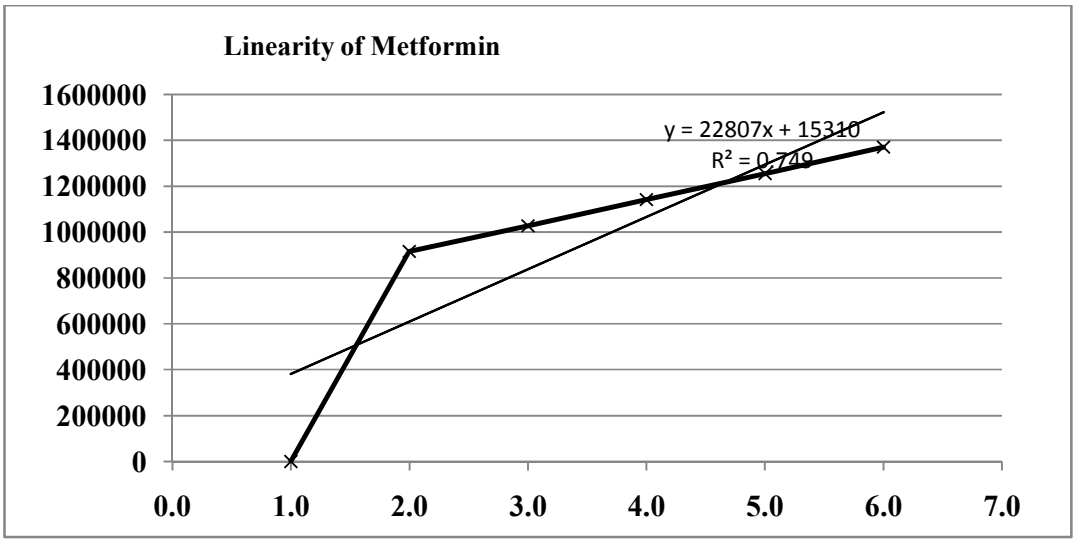

Conclusion: The obtained results are within the range and coefficient of correlation for the standard curve is 0.9996 which is within acceptance criteria of 1.0000,hence the method is linear within given range.

8. STABILITY OF SOLUTION:

The solution stability of Metformin in the assay method was carried out by leaving the working standard in tightly capped volumetric flasks at room temperature for 24 hrs. The assay sample is also prepared and kept for stability up to 24 hours.

Acceptance Criteria: Stability of solution: Difference between Initial solution & different Interval is NMT 2.0

Solution Stability of Metformin 100mg)

Standard Solution	
	Area
No. Of Inj.	**Metformin**
1	1115718
2	1115620
3	1115790
Average	**1115709**

	Area	**% Assay**
.	**Metformin**	**Metformin**
8th hr	1118199	100.22
24 Hr	1116070	100.03

	%Assay	**%Assay Difference**
	Metformin	**Metformin**
Initial	100.60	
8thHr	100.22	0.38
24Hr	100.03	0.57

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Conclusion: The sample solutions of Metformin 100mg are stable up to 24 hours at room temperatur

9**. ROBUSTNESS** : The robustness of an analytical procedure is the characteristic of its stability with respect to small variations of the system parameters possible under real conditions. This stability is usually evaluated in terms of RSD of the results of analyses compared to analogous data obtained using strictly observed conditions according to the validation analytical procedure.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Variability in wavelength:

Changed in wavelength (± 5nm) & performed the analysis by using the same chromatographic conditions.

Acceptance Criteria:

Difference between normal Assay results and wavelength change: NMT 2.0

Variability in mobile phase flow rate:

Changed in mobile phase flow rate (± 10%) & performed the analysis by using the same chromatographic conditions. Prepared the standard solution and injected into the chromatographic conditions.

Acceptance Criteria:

Difference between normal Assay results and mobile phase flow rate change: NMT 2.0

Difference between normal Assay results and Change in wavelength: NMT 2

Observation Table: Robustness for Standard Solution Peak Area Metformin

Sr. No	**Wavelength**		**Flow rate**	
	230 nm	**240nm**	**0.9 ml/min**	**1.1 ml/min**
1	1057298	1266359	1239743	1014467
2	1069024	1270872	1240677	1015198
3	1067483	1274040	1240819	1013120
Average	**1064602**	**1270424**	**1240413**	**1014262**

Observation Table: Robustness for Metformin

Sr. No	**Precision**	**Wavelength**		**Flow rate**	
		230 nm	**240nm**	**0.9 ml/min**	**1.1 ml/min**
1	100.58	100.28	100.92	99.82	99.00
2	100.61	100.34	100.98	99.99	99.29
3	100.60	100.12	100.69	99.98	99.17
Average	**100.60**	**100.25**	**100.86**	**99.93**	**99.15**
Difference	**0.00**	**0.35**	**0.26**	**0.67**	**1.45**

Evaluation of Data: Following is the summarized report of the validation study of % Assay of Metformin in Metformin 12.5mg 8.0mg Tablet

VALIDATION PARAMETER		**RESULTS**	**ACCEPTANCE CRITERIA**
Specificity		There was no interference observed in Standard & Sample due to Blank	Blank should not be interfering in the Standard & Sample Solutions.
Precision	Metformin	% Assay	% Assay is NLT 90.0% And NMT 110.0%
		100.60%	
Intermediate Precision	**Method Precision**	% Assay	% Assay is NLT 90.0% And NMT 110.0%
		100.39%	
	Difference	0.21	%Assay Difference NMT 2.0

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Solution Stability	Metformin	0.57	Difference should not be more than 2.0
Accuracy	**Mean Recovery**	100.37%	Between 98.0 % to 102.0 %
Linearity	**Correlation Coefficient**	1.0000	NLT 0.99
	Y-Intercept	395.5893	NA
	Slope	11411.6989	NA
Limit of Detection		0.043ppm	
Limit of Quantation		0.131ppm	
Robustness		Metformin	% Assay difference is less than 2.0
	Normal		
	Wavelength 230 nm	0.35	
	Wavelength 240 nm	0.26	
	Flow rate 0.9 ml/min	0.67	
	Flow rate 1.1 ml/min	1.45	

Keywords: NA-Not Applicable, NMT-Not More Than, NLT-Not Less Than, RSD-Relative Standard Deviation.

III. CONCLUSION

The suggested approach is accurate, precise, quick, reproducible, and particular. All of the examined method validation parameters showed good data, indicating that the technique was fully and properly verified. It was discovered that the devised approach was reliable for both metformin isolation and quantification.

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