ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF DISSOLUTION OF METFORMIN HYDROCHLORIDE IN GLICLAZIDE AND METFORMIN HYDROCHLORIDE SUSTAINED RELEASE TABLETS

(60/850mg)

Site Address: SAFETAB LIFE SCIENCE
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

1.0 INDEX

2.0 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	S. SANTHI
Designation	:	ASST. MANAGER - QC
Signature	:	
Date	:	07/12/23
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM - QC
Signature	:	
Date	:	07/12/2023
APPROVED BY		
Name	:	S. Maran
Designation	:	AGM - QA
Signature	:	
Date	:	08/12/2023

Effective Date	:	09/12/2023

3.0 OBJECTIVE

To validate the method for test of Dissolution of Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release tablets by UV-VIS Spectrophotometer.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopoeial method

TEST TO BE VALIDATED : Dissolution of Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release tablets

COMPOSITION : Each Uncoated bilayered Sustained Release tablet contains:

Content	Strength
Gliclazide BP	60mg
Metformin Hydrochloride BP	850mg

BATCH NO : G1723081

SPECIFICATION LIMIT :

Time in interval	Limit
1st Hour	Between 20 – 40%
3rd Hour	Between 45 – 65%
10th Hour	NLT 85%

VALIDATION STUDY : QC-Laboratory, Safetab Life Science, Puducherry-605107

VALIDATION TEAM : 1. V.Vignesh

2. K.Ragavan

5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No, Potency of the reference/working std, test samples, to be used during validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	B.No: G17230801	Not applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Gliclazide BP	To be mentioned in report	To be mentioned in report
Metformin Hydrochloride BP	To be mentioned in report	To be mentioned in report

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, SOLVENTS AND CHEMICALS TO BE USED:

Instruments:

UV VIS Spectrophotometer

Make : Shimadzu, Model : UV-1700

Analytical Balance

Make : Sartorius, Model : Quintix-125D-1OIN

Dissolution:

Make : Electro lab, Model No : EDT-14LX

Make : Electro lab, Model No : EDT-08LX

pH meter:

Make : Eutech, Model No : PH 700

Chemicals/Reagents/Standards:

Metformin Hydrochloride (Working standard)

Monobasic potassium phosphate (AR grade)

Sodium Hydroxide (AR grade)

Water (Purified)

7.0 DESCRIPTION OF ANALYTICAL METHOD:

Dissolution parameters:

Apparatus	:	Apparatus II (Paddle) with sinker
Volume	:	1000 mL
Dissolution medium	:	Phosphate buffer pH 6.8
Speed	:	100 rpm
Temperature	:	37.0±0.5°C
Time	:	1st, 3rd and 10th Hours

Preparation of Phosphate buffer (pH 6.8):

Weigh accurately about 68 gm of Monobasic potassium phosphate in 500ml with water, shake and sonicate to dissolve completely and finally make the solution to 10 liters of water. Adjust pH to 6.8 using 0.2 N sodium hydroxide solution.

Preparation of Standard solution:

Weigh accurately and transfer about 42.5 mg of Metformin Hydrochloride Working standard into 100ml volumetric flask. Add about 30 ml of dissolution medium, sonicate to dissolve and dilute up to mark with dissolution medium and mix. Further dilute 5ml of this solution to 250 ml with dissolution medium and mix. (Concentration: 0.0085 mg/ml)

Preparation of Sample solution:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each vessel. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter sufficient quantity of this solution through 0.45micron, PVDF syringe filter.

Further dilute 1ml of this filtrate to 100 ml with dissolution medium and Mix.
(Concentration: 0.0085 mg/ml)

(After withdrawing aliquot at each interval, then add same volume of dissolution medium to maintain 1000 ml volume in dissolution vessel)

(Aliquot withdrawal position: - from the mid-way zone between the top surface of dissolution medium and top of rotating paddle and 1 cm away from vessel wall.)

Procedure:

Measure the absorbance of resulting Standard solution (5 replicates) and sample solution at 233 nm and calculate % dissolution.

Calculation:

Calculate the % drug release of Metformin hydrochloride as follows:

$$= \frac{TAB}{SAB} \times \frac{WT}{100} \times \frac{5}{250} \times \frac{1000}{1} \times \frac{100}{1} \times \frac{P}{100} \times \frac{100}{LC} + D$$

Where,

TAB	=	Absorbance of Metformin Hydrochloride in sample solution.
SAB	=	Absorbance of Metformin Hydrochloride in Standard solution.
WT	=	Weight of Metformin Hydrochloride working standard in mg.
P	=	Potency of Metformin Hydrochloride working standard (% on as such basis).
LC	=	Label claim of Metformin Hydrochloride in mg.
D	=	Sum of correction factor for all previous time points.

Calculation for correction factor:

Calculate the correction factor (CFn) at each time point by using the following formula.

$$CFn = \frac{Dn}{1000} X\ 10$$

Where,

Dn = % Labeled amount of Metformin Hydrochloride Dissolved at respective time point.

Calculation for corrected results:

For 1st Hour = D1
For 3rd Hour = D3+CF1
For 10th Hour = D10+CF2+CF1

8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for validation.

S.No.	VALIDATION PARAMETERS
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Linearity and Range**
4	**Accuracy (Recovery)**
5	**Precision** i) Method precision ii) Intermediate Precision
6	**Stability of analytical solution**
7	**Filter paper study**
8	**Robustness** i) Effect of dissolution apparatus speed ii) Effect of variation in dissolution media volume. iii) Effect of variation in nanometer. iv) Effect of Variation in pH

Note: More than one parameter may be performed at once with relevant sequence having common system suitability with bracketing preparation.

9.0 DETAILS OF VALIDATION PARAMETERS:

9.1 SYSTEM SUITABILITY:

Purpose:

To establish system suitability as per methodology.

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No of Absorption
1	Blank (Dissolution medium)	1
2	Standard solution	5

Evaluate the following system suitability parameters.

%RSD of Absorption of Metformin Hydrochloride in five replicate absorbance of standard preparation.

Acceptance Criteria:

%RSD of absorption of Metformin Hydrochloride in five replicate absorption of standard preparation should not be more than 2.0.

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and Placebo

"The specificity is the ability of an analytical procedure to measure accurately an analyte in presence of componenets that may be expected present in sample matrix".

Purpose:

To demonstrate that the blank and placebo not interfering with the analyte peak.

Study Design:

Sequence shall be in following provisional manner.

No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Standard solution for Metformin 850 mg	1
3	Blank (Dissolution medium)	1
4	Standard solution for Gliclazide	1
5	Placebo for Metformin 850mg	1
6	Placebo for Gliclazide	1
7	Placebo+ Gliclazide Working standard	1
8	Placebo+ Metformin Hcl Working standard	1
9	Metformin 850mg -Test solution	1

Note:

Blank and placebo preparation shall be prepared by spiking of drugs at target concentration.

Acceptance criteria:

1) There should not be any interference due to blank, Placebo absorption with analyte.

9.3 LINEARITY AND RANGE:

"The linearity of the analytical method is its ability to elecit test results data directly proportional to the concentration of the analyte in samples within given range".

Purpose:

To Establish the linearity of analyte within the specified range.

Study Design:

To demonstrate the linearity and range of analytical method over the range of 10%, 50%, 75%, 100%, 125% and 150% of targeted concentration.

Linearity stock solution, linearity level, expected concentration, linearity stock dilution and calculated concentration are tabulated below.

Linearity Stock solution	125	10	1	1	1	125.00 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	1.00	2	250	1.00
50%	5.00	2	50	5.00
75%	7.50	3	50	7.50
100%	10.00	4	50	10.00
125%	12.50	5	50	12.50
150%	15.00	6	50	15.00

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Level – 1 (10%)	3
3	Blank (Dissolution medium)	1
4	Level – 2 (50%)	3
5	Blank (Dissolution medium)	1
6	Level – 3 (75%)	3
7	Blank (Dissolution medium)	1
8	Level – 4 (100%)	3
9	Blank (Dissolution medium)	1
10	Level – 5 (125%)	3
11	Blank (Dissolution medium)	1
12	Level – 6 (150%)	3

Plot a graph of concentration (at X-axis) versus average peak area of analyte (at Y-axis). Evaluate the squared correlation coefficient (r^2), correlation coefficient (r), residual sum of square, slope and Y-intercept.

Acceptance criteria:

1) To conclude the linearity, the squared correlation coefficient should not be less than 0.995.

2) To conclude the range. % RSD for absorbance of linearity level of 10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.

9.4 ACCURACY (RECOVERY)

"The accuracy of an analytical method is the closeness of results obtained by that method to the true value. Accuracy may often be expressed as present recovery by the dissolution of known, add amount of analyte".

Purpose:

To establish the accuracy of the analytical method in the specified range.

Study design:

To demonstrate the accuracy of the analytical method, prepare recovery samples by spiking known quantities of drug (at level 10%, 50%, 100%, 150% of targeted concentration) to placebo. Prepare the recovery samples in triplicate for each level.

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Standard solution	1
3	Level – 1 Spl – 1 (10%)	1
4	Level – 1 Spl – 2 (10%)	1
5	Level – 1 Spl – 3 (10%)	1
6	Blank (Dissolution medium)	1
7	Level – 2 Spl – 1 (50%)	1
8	Level – 2 Spl – 2 (50%)	1
9	Level – 2 Spl – 3 (50%)	1
10	Blank (Dissolution medium)	1
11	Level – 3 Spl – 1 (100%)	1
12	Level – 3 Spl – 2 (100%)	1
13	Level – 3 Spl – 3 (100%)	1
14	Blank (Dissolution medium)	1
15	Level – 4 Spl – 1 (150%)	1
16	Level – 4 Spl – 2 (150%)	1
17	Level – 4 Spl – 3 (150%)	1

Acceptance criteria:

The mean % recovery at each level should be 95.0 to 105.0.

9.5 PRECISION

"The Precision of an analytical procedure express the closeness of the agreement (Degree of factor) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed condition. Precision may be considered repeatability and reproducibility"

9.5.1 METHOD PRECISION

Purpose:

To establish the repeatability of test results obtained by the analytical method.

Study design:

To demonstrate the method precision, analyze six sample units as per the methodology representing a single batch and determine the dissolution for the same.

Evaluate the method precision by computing the percentage and relative standard deviation of the dissolution results.

S.No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Sample solution-1	1
4	Sample solution-2	1
5	Sample solution-3	1
6	Sample solution-4	1
7	Sample solution-5	1
8	Sample solution-6	1

Acceptance Criteria:

% RSD for Dissolution of six test unit should not be more than 5.0 at final time point and meet the specification limit.

9.5.2 INTERMEDIATE PRECISION

Purpose:

To demonstrate the reproducibility of test results obtained by the analytical method for the variability of instrument. Analyze six sample units as per the methodology representing a single batch and determine the dissolution for the same. Evaluate the intermediate precision by computing the percentage and relative standard deviation of the dissolution results.

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Sample solution-1	1
4	Sample solution-2	1
5	Sample solution-3	1
6	Sample solution-4	1
7	Sample solution-5	1
8	Sample solution-6	1

Acceptance criteria:

1) % RSD for Dissolution of six sample preparations should not be more than 5.0 and meet the specification limit.

2) Cumulative % RSD for dissolution of twelve preparations (of method precision and intermediate precision) should not be more than 10.0%.

9.6 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Prepare standard and Test solution as per the methodology and store at room temperature Measure the absorbance solution at regular intervals. Calculate the % difference of absorbance for standard and Test preparations with that of initial. The study may be stopped if 2 consecutive failure of standard solution.

Sequence shall be in following provisional.

For Sample:

No.	Description of solution	No. of absorbance
1	Sample solution (Initial)	1
2	Sample solution 2nd hours	1
3	Sample solution 4th hours	1
4	Sample solution 6th hours	1
5	Sample solution 16th hours	1
6	Sample solution 20th hours	1
7	Sample solution 24th hours	1
8	Sample solution 28th hours	1
9	Sample solution 32nd hours	1
10	Sample solution 40th hours	1
11	Sample solution 44th hours	1
12	Sample solution 48th hours	1

For standard:

No.	Description of solution	No. of absorbance
1	Standard solution (Initial)	1
2	Standard solution 2nd hours	1
3	Standard solution 4th hours	1
4	Standard solution 6th hours	1
5	Standard solution 16th hours	1
6	Standard solution 20th hours	1
7	Standard solution 24th hours	1
8	Standard solution 28th hours	1
9	Standard solution 32nd hours	1
10	Standard solution 40th hours	1
11	Standard solution 44th hours	1
12	Standard solution 48th hours	1

Acceptance criteria:

The sample and standard solution shall be considered stable for the final period till which the area difference between initial and next periodic interval should not be more than ±2%.

9.7 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering sample solution through 0.45µ Nylon membrane filter, 0.45µ PVDF membrane filter and Whatman filter against that of unfiltered (centrifuged) sample.

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of absorbance
1	Blank (Dissolution medium)	1
2	Standard solution	1
3	Sample solution –Unfiltered (Centrifuge)	1
4	Sample solution –Filter Set 1 (0.45µ Nylon membrane filter)	1
5	Sample solution –Filter Set 2 (0.45µ Nylon membrane filter)	1
6	Sample solution –Filter Set 3 (0.45µ Nylon membrane filter)	1
7	Blank (Dissolution medium)	1
8	Sample solution –Filter Set 1 (0.45µ PVDF membrane filter)	1
9	Sample solution –Filter Set 2 (0.45µ PVDF membrane filter)	1
10	Sample solution –Filter Set 3 (0.45µ PVDF membrane filter)	1
11	Blank (Dissolution medium)	1
12	Sample solution –Filter Set 1 (Whatman filter)	1
13	Sample solution –Filter Set 2 (Whatman filter))	1
14	Sample solution –Filter Set 3 (Whatman filter))	1

Acceptance criteria:

The % difference on filter solution should not differ ±2.0 against that of unfiltered (centrifuged) sample.

9.8 ROBUSTNESS:

Purpose:

To establish the robustness of the analytical method.

Study Design:

The robustness of the analytical method can be establish by demonstrating its reliability against deliberate changes in chromatographic conditions.

Sequence shall be in following provisional manner.

As such		
No.	**Description of solution**	**No. of absorbance**
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Sample solution	6
According to each variable		
No.	**Description of solution**	**No. of absorbance**
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Sample solution	6

Following variable shall be done according to deliberate changes in chromatographic parameters.

9.8.1 Effect of dissolution apparatus speed:

To demonstrate the effect of apparatus speed, carryout the dissolution study on six test preparations with ±4% of the apparatus speed. Prepare six test solutions on drug product.

Determine % dissolution, average % dissolution of six dosage units and % relative standard deviation of dissolution results.

9.8.2 Effect of variation in dissolution media volume:

To demonstrate the effect of dissolution media volume, carryout the dissolution study on six test preparations with ±1% of the dissolution medium volume. Prepare six test solutions on drug product.

Determine % dissolution, average %dissolution of six dosage units and % relative standard deviation of dissolution results.

9.8.3 Effect of variation in nanometer:

To demonstrate the effect of nanometer, carryout the dissolution study on six test preparation with ± 2nm.

9.8.4 Effect of variation in pH:

To demonstrate the effect of Nonometer, carryout the dissolution study on six test preparation with ±0.2 pH of the Dissolution medium. Prepare six test solutions on drug product.

Acceptance criteria:

1. %Dissolution result shall meet the specification.
2. Relative standard deviation of %dissolution results should not be more than 5.0%.
3. % of dissolution results should not differ by ±5.0% to that of method precision.

10.0 ABBREVIATION:

mg	:	Milligram
S.No	:	Serial Number
ml	:	Milliliter
%	:	Percentage
ID	:	Identification
API	:	Active pharmaceutical ingredient
UV	:	Ultra Violet Spectrophotometer
B.NO	:	Batch number
mm	:	Millimeter
µm	:	Micrometer
min	:	Minutes
°C	:	Degree centigrade
nm	:	Nanometer
RSD	:	Relative standard deviation
WS	:	Working standard

11.0 REVISION HISTORY:

Protocol No.	Effective date	Reason for Review
ST/AMVDMP/23/022	09/12/2023	New Protocol prepared.

ANALYTICAL METHOD VALIDATION REPORT FOR THE TEST OF DISSOLUTION OF METFORMIN HYDROCHLORIDE IN GLICLAZIDE AND METFORMIN HYDROCHLORIDE SUSTAINED RELEASE TABLETS (60/850mg)

Site Address: SAFETAB LIFE SCIENCE
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

1.0 INDEX

2.0 REPORT APPROVAL SHEET

PREPARED BY		
Name	:	S. SANTHI.
Designation	:	ASST. MANAGER - QC
Signature	:	
Date	:	13/01/24
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM - QC
Signature	:	
Date	:	13/01/2024
APPROVED BY		
Name	:	S. Maran
Designation	:	AGM - QA
Signature	:	
Date	:	18/01/2024

Effective Date	:	19/01/2024

3.0 OBJECTIVE

To validate the method for test of Dissolution of Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release tablets by UV-VIS Spectrophotometer.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopoeial method

TEST VALIDATION : Dissolution of Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release tablets

COMPOSITION : Each Uncoated bilayered Sustained Release tablet contains:

Content	Strength
Gliclazide BP	60mg
Metformin Hydrochloride BP	850mg

BATCH NO : G17230801

SPECIFICATION LIMIT :

Time in interval	Limit
1st Hour	Between 20 – 40%
3rd Hour	Between 45 – 65%
10th Hour	NLT 85%

VALIDATION STUDY : QC-Laboratory, Safetab Life Science, Puducherry-605107

VALIDATION TEAM : 1. V.Vignesh

2. K.Ragavan

5.0 DETAILS OF STANDARD,SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., test samples to be used during Validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	B.No: G17230801	Not applicable
Plain Placebo	B.No: NA	Not applicable
Working standard Gliclazide BP	WS. No: ST/WS/22/040	100.0% (As is basis)
Metformin Hydrochloride BP	WS. No: WS/MEF/22/01	100.2% (As is basis)

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, SOLVENTS AND CHEMICALS TO BE USED :

Instruments:

UV VIS spectrophotometer

Make : Shimadzu, Model : UV-1700

Analytical Balance

Make : Sartorius, Model : Quintix-125D-1OIN

Dissolution:

Make : Electro lab, Model No : EDT-14LX

Make : Electro lab, Model No : EDT-08LX

pH meter:

Make : Eutech, Model No : PH 700

Chemicals/Reagents/Standards:

Metformin Hydrochloride (Working standard)

Monobasic potassium phosphate (AR grade)

Sodium Hydroxide (AR grade)

Water (Purified)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Apparatus	:	Apparatus II (Paddle) with sinker
Volume	:	1000 mL
Dissolution medium	:	Phosphate buffer pH 6.8
Speed	:	100 rpm
Temperature	:	37.0±0.5°C
Time	:	1st, 3rd and 10th Hours

Preparation of Phosphate buffer (pH 6.8):

Weigh accurately about 68 gm of Monobasic potassium phosphate in 500ml with water, shake and sonicate to dissolve completely and finally make the solution to 10 liters of water. Adjust pH to 6.8 using 0.2 N sodium hydroxide solution.

Preparation of Standard solution:

Weigh accurately and transfer about 42.5 mg of Metformin Hydrochloride Working standard into 100ml volumetric flask. Add about 30 ml of dissolution medium, sonicate to dissolve and dilute up to mark with dissolution medium and mix. Further dilute 5ml of this solution to 250 ml with dissolution medium and mix. (Concentration: 0.0085 mg/ml)

Preparation of Sample solution:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each vessel. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter sufficient quantity of this solution through 0.45micron, PVDF syringe filter.

Further dilute 1ml of this filtrate to 100 ml with dissolution medium and Mix. (Concentration: 0.0085 mg/ml)
(After withdrawing aliquot at each interval, then add same volume of dissolution medium to maintain 1000 ml volume in dissolution vessel)

(**Aliquot withdrawal position:** - from the mid-way zone between the top surface of dissolution medium and top of rotating paddle and 1 cm away from vessel wall.)

Procedure:

Measure the absorbance of resulting Standard solution (5 replicates) and sample solution at 233 nm and calculate % dissolution.

Calculation:

Calculate the % drug release of Metformin hydrochloride as follows:

$$= \frac{TAB}{SAB} \times \frac{WT}{100} \times \frac{5}{250} \times \frac{1000}{1} \times \frac{100}{1} \times \frac{P}{100} \times \frac{100}{LC} + D$$

Where,

TAB	=	Absorbance of Metformin Hydrochloride in sample solution.
SAB	=	Absorbance of Metformin Hydrochloride in Standard solution.
WT	=	Weight of Metformin Hydrochloride working standard in mg.
P	=	Potency of Metformin Hydrochloride working standard (% on as such basis).
LC	=	Label claim of Metformin Hydrochloride in mg.
D	=	Sum of correction factor for all previous time points.

Calculation for correction factor:

Calculate the correction factor (CFn) at each time point by using the following formula.

$$CFn = \frac{Dn}{1000} X\ 10$$

Where,

Dn = % Labeled amount of Metformin Hydrochloride Dissolved at respective time point.

Calculation for corrected results:

For 1st Hour = D1
For 3rd Hour = D3+CF1
For 10th Hour = D10+CF2+CF1

8.0 VALIDATION PARAMETERS:

Following parameters shall be selected for Validation	
S.No.	VALIDATION PARAMETERS
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Linearity and Range**
4	**Accuracy (Recovery)**
5	**Precision** i) Method precision ii) Intermediate Precision
6	**Stability of analytical solution**
7	**Filter paper study**
8	**Robustness** i) Effect of dissolution apparatus speed. ii) Effect of variation in dissolution media volume. iii) Effect of variation in nanometer. iv) Effect of variation in pH

Note: More than one parameter may be performed at once with relevant sequence having common system suitability with bracketing preparation.

9.0 VALIDATION RESULTS :

9.1 SYSTEM SUITABILITY TEST:

Study Design:

Five replicates absorbance of standard preparation are taken from UV-VIS spectrophotometer and following system suitability parameter are evaluated.

% Relative standard deviation for five replicate absorbance of standard should not be more than 2.0.

Results are tabulated in Table 1.

Table 1: System suitability for Metformin Hydrochloride

S.NO	Absorbance of Metformin Hydrochloride
Blank	0
1	0.660
2	0.658
3	0.659
4	0.659
5	0.658
Average	0.659
RSD %	0.127

Result and Conclusion:

The results are well within the acceptance criteria and the study concludes the suitability of analytical system for the analysis.

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and placebo

Study Design:

Blank, standard, placebo and placebo spiked with analyte and sample are analyzed as per the method to examine the interference of blank and placebo with Metformin Hydrochloride absorbance.

Results are tabulated in Table 2.

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Absorbance
1	Blank	Not Applicable	0.000
2	Standard solution for Metformin 850 mg	Metformin	0.659
3	Blank	Not Applicable	0.000
4	Standard solution for Gliclazide	Gliclazide	0.001
5	Placebo for Metformin 850mg	Not Applicable	0.003
6	Placebo for Gliclazide	NA	0.001
7	Placebo+ Gliclazide Working standard	Gliclazide	0.001
8	Placebo+ Metformin Hcl Working standard 850mg	Metformin	0.662
9	Metformin 850-Test solution	Metformin	0.658

Results and Conclusion:

Blank and Placebo peaks are not interfere with Metformin Hydrochloride absorbance in test preparation.

9.3 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Metformin Hydrochloride are prepared over the range of 10% to 150% concentration with respect to target concentration (i.e. 10%, 50%, 75%, 100%, 125% and 150%). The results are tabulated in Table 3 for Linearity and Table 4 for Range.

Acceptance criteria:

1) The squared correlation coefficient should not be less than 0.995.

2) Determine the linearity levels 10%, 50%, 75%, 100%, 125%& 150% should not be more than 2.0 for Metformin Hydrochloride absorbance.

Table 3: Linearity Table for Metformin hydrochloride

Linearity Levels (%)	Final Conc.(ppm)	Avg. Absorbance of Metformin Hydrochloride
10%	1.000	0.082
50%	5.001	0.398
75%	7.501	0.607
100%	10.002	0.803
125%	12.502	1.016
150%	15.002	1.207
Slope		0.0807
CC		1.000
Sqaured R		0.9999
Intercept		0.0009

Fig.1 : Liner Graph for Metformin Hydrochloride

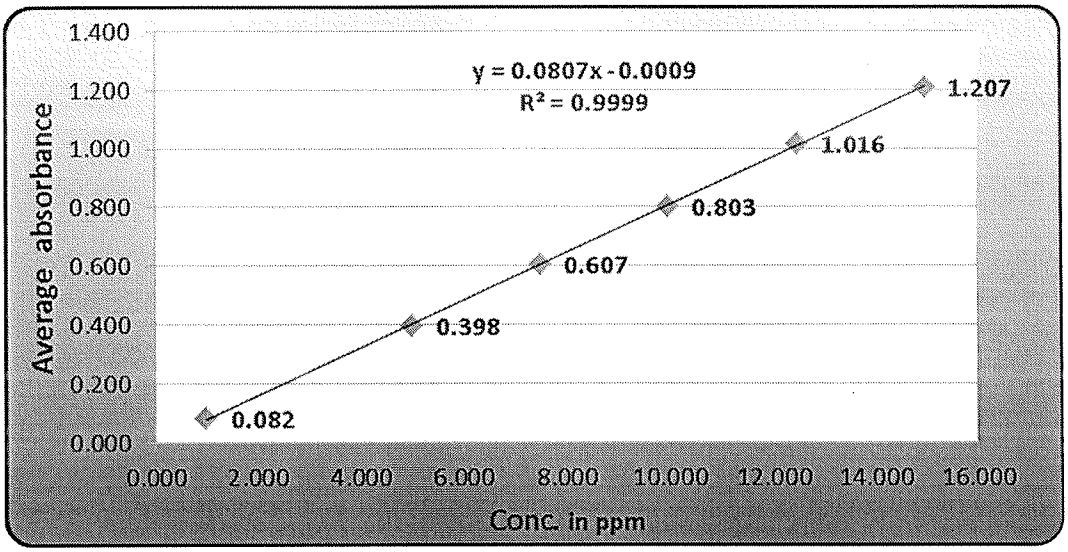

Table:4 Range for Metformin Hydrochloride

Linearity Levels (%)	% RSD
10%	0.701
50%	0.251
75%	0.252
100%	0.125
125%	0.057
150%	0.172

Result and Conclusion:

Squared correlation coefficient and % RSD range value is well within the specified limit.

9.4 ACCURACY (RECOVERY)

Study Design:

Known quantity of Metformin Hydrochloride Working standard are spiked with placebo at four different levels (at level of 10%, 50%, 100% and 150% of targeted concentration).

Prepared the recovery samples in triplicates for each level. The samples are analyzed as per the proposed method. The results are tabulated in Table 5 for Metformin Hydrochloride respectively to demonstrate the accuracy of the method.

Acceptance criteria:

The mean % recovery at each level for Metformin Hydrochloride should be 95.0 to 105.0.

Table 5: Accuracy for Metformin Hydrochloride

Recovery level	Sample No.	% Recovery	Mean	% RSD
10%	1	104.74	102.7	1.855
	2	101.00		
	3	102.24		
50%	1	100.75	100.7	0.623
	2	101.25		
	3	100.00		
100%	1	100.37	100.4	0.190
	2	100.62		
	3	100.25		
150%	1	100.50	100.4	0.083
	2	100.33		
	3	100.42		

Result and Conclusion:

All the results are well within the acceptance criteria and results indicate that the method is accurate and precise.

9.5 PRECISION:

9.5.1 Method Precision:

Study summary:

Six Dissolution preparations of sample are analyzed as per the method. The Dissolution of Metformin Hydrochloride is calculated. The results are tabulated in Table 6.

Acceptance criteria:

% RSD for Dissolution of six sample preparations should not be more than 5.0 at final time point and meet the specification limit at individual limit.

Table 6: Method precision for Metformin Hydrochloride

No. of Preparation	Dissolution of Metformin Hydrochloride
1	97.77
2	100.33
3	100.74
4	98.47
5	100.17
6	98.82
Mean	99.4
% RSD	1.20

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for drug release indicates the precision of the analytical method.

9.5.2 Intermediate Precision:

Study summary:

Six Dissolution preparations of sample are analyzed as per the method by different analyst using different instrument and different day. The Dissolution of Metformin Hydrochloride is calculated. The results are tabulated in Table 7 and cumulative results are tabulated in Table 8.

Acceptance criteria:

1) % RSD for Dissolution of six test preparations should not be more than 5.0 at final time point and meet the specification limit at individual limit.

2) Cumulative % RSD for Dissolution of twelve sample preparations of (method and intermediate precision) should not be more than 10.0% at final time point and meet the specification limit at individual limit.

Table 7: Intermediate precision for Metformin Hydrochloride

No. of Preparation	Dissolution of Metformin HCL
1	99.34
2	100.84
3	101.03
4	98.38
5	97.93
6	99.78
Mean	99.5
% RSD	1.27

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 8.

Table 8: Cumulative % RSD for Metformin Hydrochloride

Parameter	Dissolution of Metformin HCL
Method Precision	97.77
	100.33
	100.74
	98.47
	100.17
	98.82
Intermediate Precision	99.34
	100.84
	101.03
	98.38
	97.93
	99.78
Mean	99.5
% RSD	1.18

Result and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for drug release indicates the precision of the method.

9.6 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Sample and Standard solution:

Standard and sample preparation are prepared as per the proposed method and take the absorption from UV-VIS spectrophotometer initially and calculate the result in percentage at various time intervals and data tabulated in Table 9.

Table 9: Stability of sample and standard solution for Metformin hydrochloride

Time in hours	Absorbance of Standard solution	Absorbance of Sample solution	Final time point dissolution in %	Absolute % Difference
Initial	0.659	0.655	99.03	Not applicable
2	0.660	0.653	98.58	0.46
4	0.659	0.656	99.18	-0.15
6	0.659	0.655	99.03	0.00
16	0.657	0.655	99.33	-0.30
20	0.659	0.654	98.88	0.15
24	0.658	0.653	98.88	0.15
28	0.658	0.654	99.03	0.00
32	0.657	0.653	99.03	0.00
40	0.659	0.654	98.88	0.15
44	0.657	0.652	98.88	0.16
48	0.656	0.651	98.87	0.16
Mean	0.658	0.654	99.0	0.07
% RSD	0.181	0.218	0.189	Not applicable

The standard and sample solution shall be considered stable for the final period till which the area difference between initial and next periodic interval should be not more than ±2%.

Results and conclusions:

The Standard solution and sample solution is stable upto 48 hours at room temperature.

9.7 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method is performed by filtering test solution through 0.45µ Nylon membrane, 0.45µ PVDF membrane and Whatman filter against that of unfiltered. (Centrifuged) sample. The results are tabulated in Table 11.

Table 11: Filter paper study for Sample solution of Metformin Hydrochloride

Filter study	Sample Absorbance	Metformin Hydrochloride content in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	0.662	100.1	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	0.661	99.9	0.15
Filter Set-2 (0.45µ Nylon membrane)	0.662	100.1	0.00
Filter Set-3 (0.45µ Nylon membrane)	0.659	99.6	0.46
Filter Set-1 (0.45µ PVDF membrane)	0.660	99.8	0.30
Filter Set-2 (0.45µ PVDF membrane)	0.658	99.5	0.61
Filter Set-3 (0.45µ PVDF membrane)	0.658	99.5	0.61
Filter Set-1 (Whatman filter)	0.695	105.1	-4.75
Filter Set-2 (Whatman filter)	0.696	105.2	-4.89
Filter Set-3 (Whatman filter)	0.697	105.4	-5.02

Acceptance criteria:

The % difference on filter solution should not differ ±2.0 against that of unfiltered.(Centrifuged)

Results and conclusions:

The % difference on filtered sample (0.45µ Nylon and PVDF membrane filter) within limit against that of unfiltered. (Centrifuged sample)

9.8 ROBUSTNESS:

Study Design:

Five replicate absorbance of standard preparation and six absorbance of sample preparation are taken from UV-VIS spectrophotometer at different dissolution profile as per protocol. System suitability parameters and mean dissolution difference with respect to dissolution value in method precision are calculated. The results are tabulated in table 12 Metformin HCL absorbance respectively.

Table 12: Robustness of analytical method for Metformin Hydrochloride

Variation's of Parameters	RSD %	Metformin Dissolution in %	Mean Dissolution value of method precision	% Difference
Low wavelength (231nm)	0.71	99.8	99.4	-0.40
High wavelength (235nm)	1.17	100.1		-0.70
Low Volume(990ml)	1.19	99.1		0.30
High Volume(1010ml)	0.98	98.7		0.00
Low Rpm(96rpm)	1.59	98.9		-0.10
High Rpm (104rpm)	1.34	100.2		-0.80
Low pH (6.6pH)	1.32	99.6		-0.20
High pH (7.0pH)	1.03	99.6		-0.20

Acceptance criteria:

1) % Dissolution result shall meet the specification.

2) Relative standard deviation of % dissolution results should not be more than 5.0%

3) % of dissolution results should not differ by ±5.0% to that of method precision

Result and Conclusion:

Each chromatographic variation System suitability parameters are within limits. % Difference of dissolution within limits at each variation.

10.0 SUMMARY:

S.No	Validation parameter	Acceptance criteria	Results
1	System suitability	% RSD of absorbance of analyte in five replicate standard absorbance should not be more than 2.0.	0.127
2	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	Prepare the blank and placebo solutions as per the method and checked the absorbance at 233nm.	Blank, Placebo solutions are not interfere with Metformin Hydrochloride in sample preparation.
3	Linearity and Range	1) R^2 Should be NLT 0.995 2) To conclude the range, %RSD for peak area of linearity level-10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.	Squared correlation coefficient for 0.9999

Level	%RSD
10%	0.701
50%	0.251
75%	0.252
100%	0.125
125%	0.057
150%	0.172

S. No	Validation parameter	Acceptance criteria	Results
4	Accuracy (Recovery)	The mean % recovery at each level should be 95.0 to 105.0.	Level %Recovery **Metformin HCL:** 10% : 102.7 50% : 100.7 100% : 100.4 150% : 100.4
5	**Precision** i) Method Precision	%RSD of Dissolution of six sample preparations should not be more than 5.0, at final time point and meet the specification limit at individual limit	1.20
	ii) Intermediate Precision	1) % RSD for Dissolution of six sample preparations should not be more than 5.0, at final time point and meet the specification limit at individual limit.	1.27
		2) Cumulative %RSD for Dissolution of twelve preparations (of method and intermediate precision) should not be more than 10.0. at final time point and meet the specification limit at individual limit.	1.18

S.No	Validation parameter	Acceptance criteria	Results
6	Stability for analytical solution	The sample and standard solution shall be considered stable for the final period till which the absorbance difference between initial and next periodic interval should not be more than ±2%.	The Standard and Sample solution is stable upto 48hours at room temperature.
7	Filter paper study (0.45µ Nylon, PVDF and Whatman)	The % difference on filter solution should not differ ±2.0 against that of unfiltered. (Centrifuged)	The % difference on filtered sample 0.45µ Nylon, PVDF membrane filter within limit against that of unfiltered. (Centrifuged)
8	Robustness (i) Wavelength change (ii) RPM change (iii) Volume Change (iv) pH Change	System suitability parameters should comply.	Each chromatographic variation System suitability parameters are within limits. % Difference of dissolution within limits at each variation.

11.0 CONCLUSION:

Validation studies have been conducted for Dissolution of Metformin hydrochloride in Gliclazide and Metformin hydrochloride tablets for the parameters of system suitability, specificity, Method precision, Intermediate precision, Linearity and range and accuracy, Filter paper study, solution stability and Robustness by using the proposed method. The data is complied and found satisfactory with the analytical method for all the parameters analysed. Hence it is concluded that the method can be used for regular analysis.

12.0 ABBREVIATION:

mg	:	Milligram
No	:	Number
ml	:	Milliliter
%	:	Percentage
ID	:	Identification
API	:	Active pharmaceutical ingredient
UV VIS	:	Ultraviolet Spectrophotometer
B.NO	:	Batch number
WS.NO	:	Working standard number
mm	:	Millimeter
µm	:	Micrometer
min	:	Minutes
°C	:	Degree centigrade
nm	:	Nanometer
RSD	:	Relative standard deviation
µl	:	Micro litre

13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVDMR/23/022	19/01/2024	New Report prepared.