



GENERIC HEALTHCARE PRIVATE LIMITED

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ANNEX-II

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TITLE

Analytical Method Validation Report Layout

REPORT

Title


Analytical Method Validation Report For test of Dissolution of Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release Tablets

Report No.

ST/AMVDMR/23/022


**ANALYTICAL METHOD  
VALIDATION REPORT  
FOR  
THE TEST OF DISSOLUTION OF  
METFORMIN HCL IN GLICLAZIDE  
AND METFORMIN HCL SUSTAINED  
RELEASE TABLETS  
(GLIDE-M 60/850)**

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED  
Plot No.A-67 to 72, PIPDIC Electronic Park,  
Thirubuvanai, Puducherry-605 107

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## 2.0 REPORT APPROVAL SHEET

PREPARED BY		
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Designation	:	AGM-QA
Signature	:	
Date	:	18/01/2024

Effective Date	:	19/01/2024
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### 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

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

SPECIFICATION LIMIT :

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. V.Vignesh  
2. K.Ragavan

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## 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
<u>Working standard</u> 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)



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## 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-10IN

### 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)

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### 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	: 1 <sup>st</sup> , 3 <sup>rd</sup> and 10 <sup>th</sup> 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)

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**(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.**

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

Where,

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

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**Calculation for corrected results:**

For 1<sup>st</sup> Hour = D1

For 3<sup>rd</sup> Hour = D3+CF1

For 10<sup>th</sup> Hour = D10+CF2+CF1




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## 8.0 VALIDATION PARAMETERS :

Following parameters shall be selected for Validation	
S.No.	Validation Parameter
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.

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## 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.*



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## 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

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Sr.No	Sample ID	Peak Name	Absorbance
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.

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#### 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**

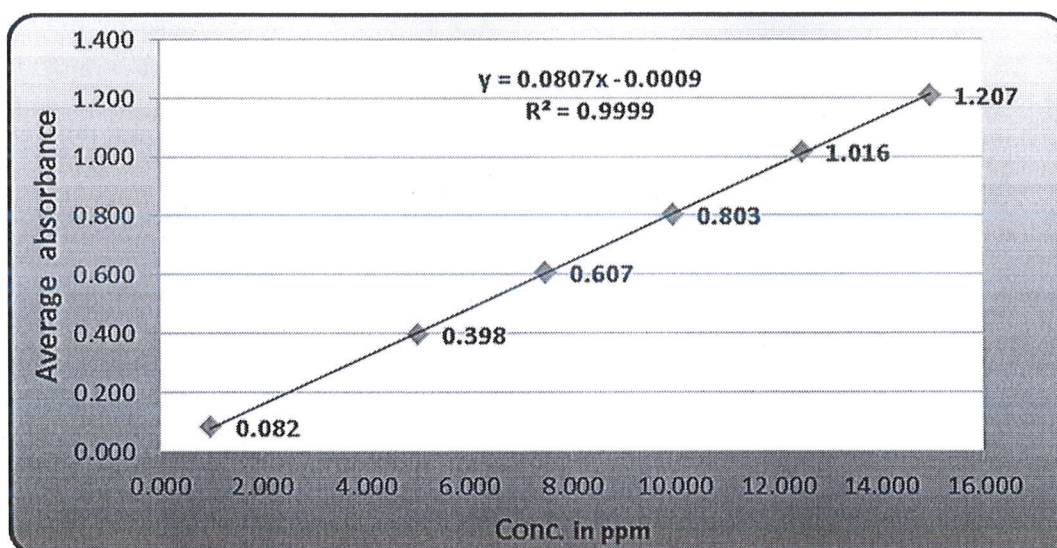
Linearity Levels (%)	Final Conc.(ppm)	Avg. Absorbance of Metformin Hydrochloride
10%	1.000	0.082
50%	5.001	0.398
75%	7.501	0.607
100%	10.002	0.803
125%	12.502	1.016
150%	15.002	1.207
Slope		0.0807
CC		1.000
Sqaured R		0.9999
Intercept		0.0009



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**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.*

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#### 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.

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**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.*



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## 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.*

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### 9.5.2 Intermediate Precision (Ruggedness):

#### 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.

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**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.*



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## 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

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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  $\pm 2\%$ .

#### 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 0.45 $\mu$  Nylon membrane, 0.45 $\mu$  PVDF membrane and Whatman filter against that of unfiltered. (Centrifuged) sample. The results are tabulated in Table 11.



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**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  $\pm 2.0$  against that of unfiltered.(Centrifuged)



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### 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

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**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  $\pm 5.0\%$  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.*



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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	<b>Specificity</b> 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 <sup>2</sup> 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 <table><tr><th>Level</th><th>%RSD</th></tr><tr><td>10%</td><td>0.701</td></tr><tr><td>50%</td><td>0.251</td></tr><tr><td>75%</td><td>0.252</td></tr><tr><td>100%</td><td>0.125</td></tr><tr><td>125%</td><td>0.057</td></tr><tr><td>150%</td><td>0.172</td></tr></table>	Level	%RSD	10%	0.701	50%	0.251	75%	0.252	100%	0.125	125%	0.057	150%	0.172
Level	%RSD																
10%	0.701																
50%	0.251																
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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 <b>Metformin HCL:</b> 10% : 102.7 50% : 100.7 100% : 100.4 150% : 100.4
5	<b>Precision</b>		
	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

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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 $\pm 2\%$ .	The Standard and Sample solution is stable upto 48hours at room temperature.
7	Filter paper study (0.45 $\mu$ Nylon, PVDF and Whatman)	The % difference on filter solution should not differ $\pm 2.0$ against that of unfiltered. (Centrifuged)	The % difference on filtered sample 0.45 $\mu$ 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.



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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



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