REPORT	
Title	Analytical Method Validation Report For test of Assay of Gliclazide and Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release Tablets
Report No.	ST/AMVAR/23/022

ANALYTICAL METHOD VALIDATION REPORT FOR THE TEST OF ASSAY OF GLICLAZIDE AND METFORMIN HYDROCHLORIDE IN GLICLAZIDE AND METFORMIN HYDROCHLORIDE SUSTAINED RELEASE TABLETS (GLIDE-M 60/500 AND 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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Date	:	18/09/23

Effective Date	:	20/09/2023

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3.0 OBJECTIVE

To validate the method for test of assay of Gliclazide and Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopoeial method

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

COMPOSITION : This Validation Report is applicable for both strength

Each Uncoated bilayered Sustained Release tablet contains:

Content	Strength
Gliclazide BP	60mg
Metformin Hydrochloride BP	500mg and 850mg

BATCH NO : G17230801, G1722093

SPECIFICATION LIMIT : 90.0% to 110.0% of the labeled claim

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

VALIDATION TEAM : 1. C.K.Saravanan

2. C.Albin Jose

3. E.Meena

4. S.Gomathi

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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/placebo to be used during Validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	B.No: G17230801, G1722093	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)

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

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

High performance liquid chromatograph with UV visible detector

Make : Shimadzu, Model : LC-2050C

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PH 700

COLUMN :

Kromasil 100-C18 ,250 mm X 4.6 mm, 5µm (or) equivalent

SOLVENTS AND CHEMICALS WITH GRADE :

Metformin Hydrochloride (Working standard)

Gliclazide (Working standard)

Potassium dihydrogen orthophosphate (AR grade)

Dipotassium hydrogen orthophosphate (AR grade)

Acetonitrile (HPLC grade)

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Purified Water (Milli-Q water (or) equivalent)

Orthophosphoric acid (HPLC grade)

Hydrochloric acid (AR grade)

Sodium Hydroxide (AR grade)

30% Hydrogen Peroxide solution (AR grade)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column	:	Kromasil 100-C18 ,250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	265 nm
Column Temperature	:	Ambient
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL
Run time	:	25 Minutes
Diluent-1	:	Buffer Preparation

Buffer Preparation:

Weigh accurately about 2.96gm of potassium dihydrogen orthophosphate and 0.54gm of dipotassium hydrogen orthophosphate in 1000 ml glass beaker. Add about 500 ml of water, shake and sonicate to dissolve completely and finally make the solution 1000 ml with water.

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Preparation of Mobile phase:

Mix 550 ml of buffer solution and 450 ml Acetonitrile, adjust the pH 3.0 using Orthophosphoric acid. Filter through 0.20micron membrane filter, sonicate and degas.

Preparation of Standard solution: (For 60/500)

Weigh accurately and transfer about 60mg of Gliclazide working standard and 500mg of Metformin Hydrochloride working standard into 100ml volumetric flask. Add about 50 ml of Mobile phase, sonicate to dissolve and dilute up to mark with Mobile phase and mix. Further dilute 5 ml of this solution to 25 ml with mobile phase and mix well. (**Concentration**:0.12mg/ml for Gliclazide and 1.00mg/ml for Metformin Hydrochloride)

Preparation of Standard solution: (For 60/850)

Weigh accurately and transfer about 60mg of Gliclazide working standard and 850mg of Metformin Hydrochloride working standard into 100ml volumetric flask. Add about 50 ml of Mobile phase, sonicate to dissolve and dilute up to mark with Mobile phase and mix. Further dilute 5 ml of this solution to 25 ml with mobile phase and mix well. (**Concentration**:0.12mg/ml for Gliclazide and 1.70mg/ml for Metformin Hydrochloride)

Preparation of sample powder:

Weigh accurately 20 tablets and make the powder by using mortar and pestle. Use the same for preparation of sample solution. Calculate the average weight by taking weight of 20 tablets taken above and use the same for calculation.

Preparation of Sample solution: (For 60/500)

Weigh accurately and transfer the sample powder eq.to 60mg of Gliclazide into 100ml clean and dry volumetric flask, add about 45ml of Acetonitrile and sonicate for 10 minutes with intermittent shaking cool and then add 30ml of diluent-1, sonicate for 10 minutes with intermittent shaking cool and dilute upto mark with diluent-1. Filter through 0.45µ syringe filter. Further dilute 5ml of this solution to 25ml with mobile phase and mix well. (**Concentration**:0.12mg/ml for Gliclazide and 1.00mg/ml for Metformin Hydrochloride)

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Preparation of Sample solution: (For 60/850)

Weigh accurately and transfer the sample powder eq.to 60mg of Gliclazide into 100ml clean and dry volumetric flask, add about 45ml of Acetonitrile and sonicate for 10 minutes with intermittent shaking cool and then add 30ml of diluent-1, sonicate for 10 minutes with intermittent shaking cool and dilute upto mark with diluent-1. Filter through 0.45µ syringe filter. Further dilute 5ml of this solution to 25ml with mobile phase and mix well. (**Concentration:**0.12mg/ml for Gliclazide and 1.70mg/ml for Metformin hydrochloride)

Procedure:

Equilibrate the chromatographic system with mobile phase till a stable baseline is obtained. Separately inject equal volumes (20 µl) of solutions as per Sequence of injections into the chromatograph and record the peak area responses for the major peaks and check for the System suitability requirements.

Injection sequence:

S. No	Sample Name	No. of injections
1	Mobile phase (blank)	1
2	Standard solution	5
3	Sample solution	2
4	Bracketing standard	1 Each after every 6 sample injection

System suitability:

1) The tailing factor for the peak of Metformin and Gliclazide obtained with standard solution should not more than 2.0.

2) The column efficiency for the peak of Metformin and Gliclazide obtained in the chromatogram of Standard solution should not less than 2000.

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3) The % RSD for the retention time of Metformin and Gliclazide peak obtained with the replicate injections of standard solution should not more than 1.00

4) The % RSD for the peak area response of Metformin and Gliclazide peak obtained with the replicate injections of standard solution should not more than 2.00

5) The % RSD for the retention time of Metformin and Gliclazide peak obtained with the replicate injections of standard solution and bracketing standard solution should not more than 1.00

6) The % RSD for the peak area response of Metformin and Gliclazide peak obtained with the replicate injections of standard solution and bracketing standard solution should not more than 2.00.

Calculations:

Calculate the assay of Gliclazide in mg/tablet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times AW$$

Where,

AT	=	Average area of peak due to Gliclazide in sample solution.
AS	=	Average area of peak due to Gliclazide in standard solution.
WS	=	Weight of Gliclazide working standard in mg.
DS	=	Dilution factor for standard solution.
DT	=	Dilution factor for sample preparation.
WT	=	Weight of sample taken in mg.
AW	=	Average weight of tablet in mg.
P	=	Potency of Gliclazide working standard in % on as such basis.

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Calculate the assay of Gliclazide in % as follows:

$$= \frac{\text{mg/tablet}}{\text{LC}} \times 100$$

LC = Label claim of Gliclazide in mg/tablet.

Calculate the assay of Metformin Hydrochloride in mg/tablet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times AW$$

Where,

AT = Average area of peak due to Metformin Hydrochloride in sample solution.

AS = Average area of peak due to Metformin Hydrochloride in standard solution.

WS = Weight of Metformin Hydrochloride working standard in mg.

DS = Dilution factor for standard solution.

DT = Dilution factor for sample preparation.

WT = Weight of sample taken in mg.

AW = Average weight of tablet in mg.

P = Potency of Metformin Hydrochloride working standard in % on as such basis.

Calculate the assay of Metformin Hydrochloride in % as follows:

$$= \frac{\text{mg/tablet}}{\text{LC}} \times 100$$

LC = Label claim of Metformin Hydrochloride in mg/tablet.

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8. VALIDATED PARAMETERS :

Following parameters shall be selected for validation	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** Interference from blank and placebo
3	**Linearity and Range**
4	**Interference from degradation (Forced Degradation)**
	i) Acid degradation ii) Alkali Degradation iii) Oxidative Degradation
5	**Accuracy (Recovery)**
6	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
7	**Stability of analytical solution**
8	**Filter paper study**
9	**Robustness**

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 of standard preparation are injected into HPLC and following system suitability parameters are evaluated.

1) The tailing factor for the peak of Metformin and Gliclazide obtained with standard solution should not more than 2.0.

2) The column efficiency for the peak of Metformin and Gliclazide obtained in the chromatogram of Standard solution should not less than 2000.

3) The % RSD for the peak area response of Metformin and Gliclazide peak obtained with the replicate injections of standard solution should not more than 2.00.

Results are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Gliclazide	Metformin HCL
Theoretical Plates	NLT 2000	17564	2108
Tailing Factor	NMT 2.0	1.104	1.427
% RSD	NMT 2.0	0.204	0.113

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Result and Conclusion:

The System suitability test result 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 Gliclazide and Metformin Hydrochloride peaks.

Peak purity of the analyte peak and the representative chromatograms of blank, standard, placebo, placebo spiked with analyte and sample are attached.

Results are tabulated in Table 2.

Acceptance criteria:

1) There should not be any interference due to blank, placebo peak with analyte.

2) Peak purity index is not less than 0.995 accordingly to lab solution software.

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Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Mobile phase (Blank)	Blank peak	No peak	Not Applicable
2	Standard preparation	Gliclazide	1.911	0.998
		Metformin HCL	16.99	1.000
3	Metformin HCL Working standard	Metformin HCl	1.922	0.998
4	Gliclazide Working standard	Gliclazide	16.947	1.000
5	Mobile phase (Blank)	Blank peaks	Refer chromatogram	Not applicable
6	Plain placebo for Glide-M 60/500	Placebo peaks	Refer chromatogram	Not applicable
7	Plain placebo for Glide-M 60/850	Placebo peaks	Refer chromatogram	Not applicable
8	Plain placebo for Glide-M 60/850 with Gliclazide Working standard	Gliclazide	16.948	1.000
9	Plain placebo for Glide-M 60/850 with Metformin HCL Working standard	Metformin HCl	1.928	0.997
10	Plain placebo for Glide-M 60/850 with Metformin HCl working standard and Gliclazide WS	Metformin HCl	1.921	0.998
		Gliclazide	16.947	1.000

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Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
11	Mobile phase (Blank)	Blank peak	No peak	Not Applicable
12	Test preparation for Glide-M 60/500 B.No: G1722093	Metformin HCl	1.913	0.997
		Gliclazide	16.950	1.000
13	Mobile phase (Blank)	Blank peaks	Refer chromatogram	Not applicable
14	Test preparation for Glide-M 60/850 B.No: G17230801	Metformin HCl	1.917	0.997
		Gliclazide	16.937	1.000

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with Gliclazide and Metformin Hydrochloride peak in sample preparation and Peak purity passes within specified limits. Hence method is selective and specific.

9.3 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Gliclazide and Metformin Hydrochloride Working standard are prepared over the range of 10% to 150% concentration with respect to target concentration (i.e. 10%, 50%, 75%, 100%, 125% and 150%). Replicate injections of these solutions are injected and checked for Linearity and Range.

The results are tabulated in Table 3A and 3B 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) To conclude the range % RSD for peak areas of linearity levels 10%, 50%, 75%, 100%, 125%& 150% should not be more than 2.0.

Table 3A: Linearity Table for Gliclazide

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	12.010	28394
50%	60.050	139947
75%	90.075	209426
100%	120.100	274522
125%	150.125	339832
150%	180.150	401461
Slope		2221.5
CC		0.9997
Sqaured R		0.9994
Intercept		5482.8

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Fig 1. : Liner Graph for Gliclazide

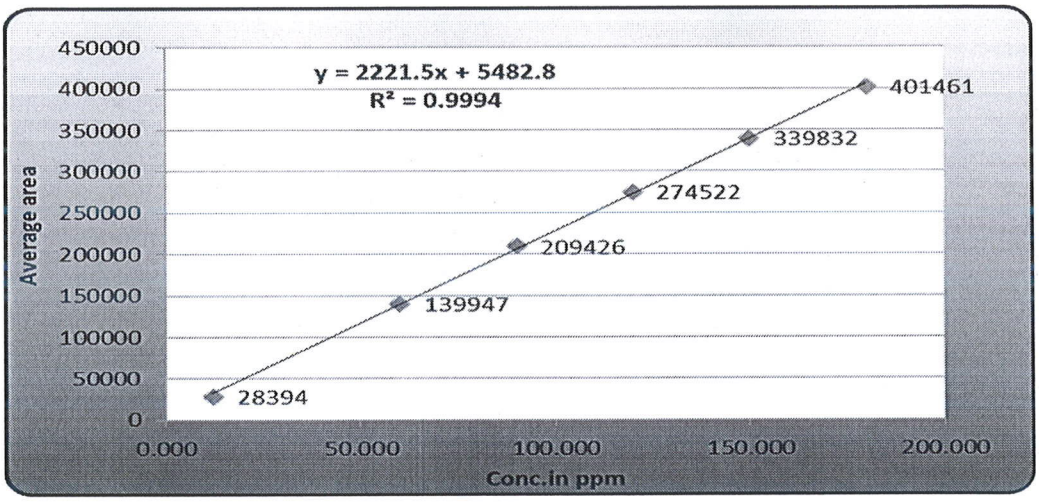

Table 3B: Linearity Table for Metformin HCL

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	170.048	150286
50%	850.240	624839
75%	1275.360	901203
100%	1700.480	1141310
125%	2125.600	1385838
150%	2550.720	1589042
Slope		604.75
CC		0.9977
Sqaured R		0.9954
Intercept		91305

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Fig 2. : Liner Graph for Metformin HCL

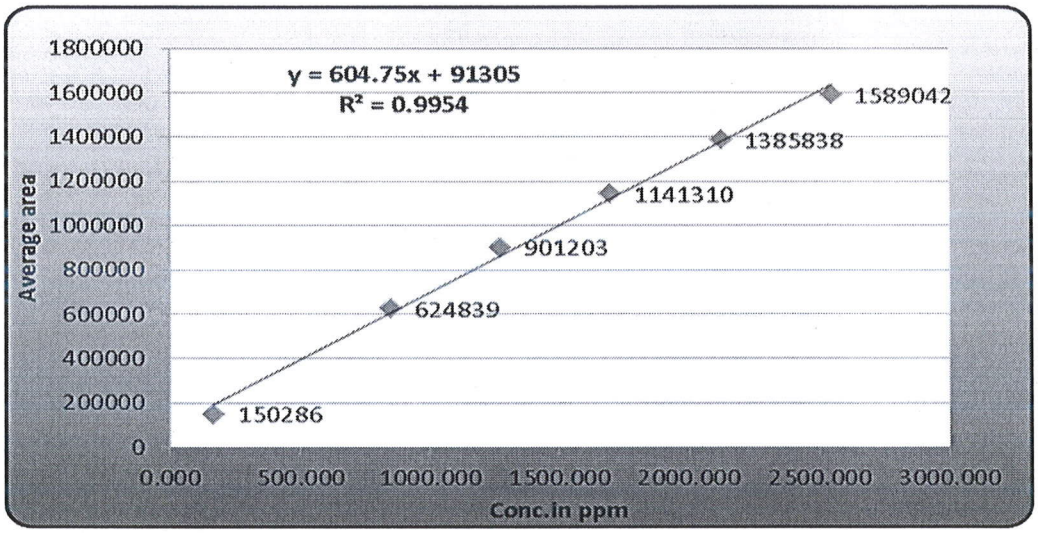

Table:4 Range for Gliclazide and Metformin HCL

Linearity Levels (%)	% RSD for Gliclazide	% RSD for Metformin HCL
10%	0.979	0.114
50%	0.200	0.052
75%	0.896	0.034
100%	0.187	0.180
125%	0.244	0.072
150%	0.335	0.045

Result and Conclusion:

Squared correlation coefficient and Range, %RSD of areas at 10%, 50%, 75%, 100%, 125 & 150% levels within limits.

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9.4 INTERFERENCE FROM DEGRADANTS (Forced degradation)

In order to prove specificity of method, further degradation is carried out and peak purity of Gliclazide and Metformin Hydrochloride peak is monitored.

a) Acid Degradation:

Weigh 20 tablets and crush to fine powder. Weigh accurately and transfer the sample powder eq.to 60mg of Gliclazide into 100ml clean and dry volumetric flask, add about 45ml of Acetonitrile and sonicate for 10 minutes with intermittent shaking cool and then add 30ml of diluent-1, sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 5N Hydrochloric acid and heat on water bath at 80°C for 60minutes Cool and neutralized with 5ml of 5N Sodium hydroxide and Dilute to volume with diluent-1 and mix. Filter through 0.45µ syringe filter. Further dilute 5ml of this solution to 25ml with mobile phase and mix well.

b) Alkali degradation:

Weigh 20 tablets and crush to fine powder. Weigh accurately and transfer the sample powder eq.to 60mg of Gliclazide into 100ml clean and dry volumetric flask, add about 45ml of Acetonitrile and sonicate for 10 minutes with intermittent shaking cool and then add 30ml of diluent-1, sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 5N Sodium hydroxide and heat on water bath at 80°C for 60minutes Cool and neutralized with 5ml of 5N Hydrochloric acid and Dilute to volume with diluent-1 and mix. Filter through 0.45µ syringe filter. Further dilute 5ml of this solution to 25ml with mobile phase and mix well.

c) Oxidative Degradation:

Weigh 20 tablets and crush to fine powder. Weigh accurately and transfer the sample powder eq.to 60mg of Gliclazide into 100ml clean and dry volumetric flask, add about 45ml of Acetonitrile and sonicate for 10 minutes with intermittent shaking cool and then add 30ml of diluent-1, sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 30% Hydrogen peroxide solution and heat on water bath at 80°C for 60minutes Cool and dilute to volume with diluent-1 and mix. Filter through 0.45µ syringe filter. Further dilute 5ml of this solution to 25ml with mobile phase and mix well.

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Acceptance criteria:

i) There should not be any interference due to degradants with analyte in stressed sample.

ii) The desired degradation should be 10-30% in acid, alkali and oxidation degration, (if possible).

iii) If about 10% to 30% degradation is not achieved by applying above stressed condition. Same shall be documented and reported.

iv) Peak purity should not be less than 0.950 according to Lab solution software.

Table 5A: Peak purity Chemical degradation for Gliclazide

S.No	Sample name	Peak name	Assay in (%)	Degradation in %	Peak purity index
1	Sample as such	Gliclazide (Method Precision)	100.2	Not applicable	Not applicable
2	Acid degradation	Gliclazide	61.9	38.32	1.00
3	Alkali degradation	Gliclazide	100.0	0.23	1.00
4	Oxidative Degradation	Gliclazide	94.0	6.24	1.00

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Table 5B: Peak purity Chemical degradation for Metformin Hydrochloride

S.No	Sample name	Peak name	Assay in (%)	Degradation in %	Peak purity index
1	Sample as such	Metformin HCL (Method Precision)	97.9	Not applicable	Not applicable
2	Acid degradation	Metformin HCL	93.9	4.05	0.99
3	Alkali degradation	Metformin HCL	80.5	17.41	0.99
4	Oxidative Degradation	Metformin HCL	100.2	-2.30	0.99

Result and Conclusion:

There is No any interference due to degradants with analyte in stressed samples and Peak purity was passes According to Lab solution software.

9.5 ACCURACY (RECOVERY)

Study Design:

Known quantity of Gliclazide and Metformin HCL working standard are spiked with placebo at three different levels (at level of 50%, 100% and 150% of targeted concentration).

Prepared the recovery samples in triplicate for each level. The samples are analyzed as per the proposed method. The results are tabulated in Table 5A and 5B for Gliclazide and Metformin HCL respectively to demonstrate the accuracy of the method.

The mean % recovery at each level for Gliclazide and Metformin HCL should be 98.0 to 102.0.

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Table 5A: Accuracy for Gliclazide

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	99.61	100.23	0.637
	2	100.21		
	3	100.88		
100%	1	101.16	100.84	0.299
	2	100.80		
	3	100.56		
150%	1	100.48	100.04	0.392
	2	99.73		
	3	99.90		

Table 5B: Accuracy for Metformin HCL

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	100.63	100.63	0.041
	2	100.67		
	3	100.59		
100%	1	98.32	98.48	0.158
	2	98.49		
	3	98.63		
150%	1	98.52	98.53	0.015
	2	98.54		
	3	98.55		

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Result and Conclusion:

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

9.6 PRECISION:

9.6.1 SYSTEM PRECISION

Study design:

Five replicate injections of standard preparation are injected into the HPLC system. The area response for Gliclazide and Metformin Hydrochloride Peaks along with % RSD are tabulated in Table 6.

Acceptance criteria:

% RSD of area of analyte peak in five replicate standard injections should not be more than 2.0.

Table 6: System precision

Injection No.	Gliclazide	Metformin HCL
1	290502	1123345
2	291534	1121012
3	290256	1123667
4	290386	1124404
5	289976	1122907
Mean	290531	1123067
% RSD	0.204	0.113

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Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for the replicate injections indicates the system precision of HPLC system used.

9.6.2 Method Precision:

Study Design:

Six Assay preparations of sample are analyzed as per the method. The Assay of Gliclazide and Metformin HCL is calculated. The results are tabulated in Table 7A and 7B.

Acceptance criteria:

% RSD for Assay of six sample preparations should not be more than 2.0.

Table 7A: Method precision for Gliclazide and Metformin HCL (60/500mg)

No. of Preparation	Drug release % of Gliclazide	Drug release % of Metformin HCL
1	99.5	97.8
2	100.1	98.7
3	98.9	98.3
4	96.7	96.7
5	97.2	97.6
6	97.6	98.7
Mean	98.3	98.0
% RSD	1.39	0.78

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Table 7A: Method precision for Gliclazide and Metformin HCL (60/850mg)

No. of Preparation	Drug release % of Gliclazide	Drug release % of Metformin HCL
1	101.3	98.3
2	100.9	99.5
3	102.2	99.6
4	98.4	96.2
5	98.9	97.0
6	99.5	96.6
Mean	100.2	97.9
% RSD	1.49	1.52

Results and Conclusion:

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

9.6.3 Intermediate Precision (Ruggedness):

Study summary:

Six Assay preparations of sample are analyzed as per the method by different analyst using different instrument and different column on different day. The assay of Gliclazide and Metformin hydrochloride is calculated. The results are tabulated in Table 8A and 8B and cumulative results are tabulated in Table 9A and 9B.

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Acceptance criteria:

1) % RSD for Assay of six sample preparations should not be more than 2.0.

2) Cumulative % RSD for Assay of twelve sample preparations (of method and intermediate precision) should not be more than 2.0.

Table 8A: Intermediate precision for Gliclazide and Metformin HCL (60/500mg)

No. of Preparation	Drug release % of Gliclazide	Drug release % of Metformin HCL
1	101.3	98.6
2	100.4	98.4
3	100.2	98.2
4	99.8	98.2
5	99.6	98.1
6	99.2	98.1
Mean	100.1	98.3
% RSD	0.73	0.19

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Table 8B: Intermediate precision for Gliclazide and Metformin HCL (60/850mg)

No. of Preparation	Drug release % of Gliclazide	Drug release % of Metformin HCL
1	100.9	99.5
2	100.2	99.0
3	96.6	97.9
4	98.5	100.3
5	96.5	99.7
6	97.9	101.7
Mean	98.4	99.7
% RSD	1.85	1.28

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 9A and 9B.

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Table 9A: Cumulative % RSD for Gliclazide and Metformin HCL (60/500mg)

Parameter	Drug release % of Gliclazide	Drug release % of Metformin HCL
Method Precision	99.5	97.8
	100.1	98.7
	98.9	98.3
	96.7	96.7
	97.2	97.6
	97.6	98.7
Intermediate Precision	101.3	98.6
	100.4	98.4
	100.2	98.2
	99.8	98.2
	99.6	98.1
	99.2	98.1
Mean	99.21	98.12
% RSD	1.40	0.57

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Table 9B: Cumulative % RSD for Gliclazide and Metformin HCL (60/850mg)

Parameter	Drug release % of Gliclazide	Drug release % of Metformin HCL
Method Precision	101.3	98.3
	100.9	99.5
	102.2	99.6
	98.4	96.2
	98.9	97.0
	99.5	96.6
Intermediate Precision	100.9	99.5
	100.2	99.0
	96.6	97.9
	98.5	100.3
	96.5	99.7
	97.9	101.7
Mean	99.32	98.78
% RSD	1.85	1.64

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Result and Conclusion:

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

9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Sample solution:

Sample preparation is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 10.

Table 10: Stability of sample solution for Gliclazide and Metformin HCl

Time in hours	Gliclazide Sample area	Absolute % Difference	Metformin HCl Sample area	Absolute % Difference
Initial	288061	Not applicable	1089282	Not applicable
4	287169	0.31	1098586	-0.85
6	285270	0.98	1093160	-0.35
8	283283	1.69	1095978	-0.61
10	284014	1.42	1098486	-0.84
12	283284	1.69	1099988	-0.97
18	284168	1.37	1110065	-1.87
24	283651	1.55	1107503	-1.65
Mean	284863	1.29	1099131	-1.02
%RSD	0.641	Not applicable	0.628	Not applicable

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Acceptance criteria:

The 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%.

Standard solution:

Standard preparation is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 11.

Table 11: Stability of standard solution for Gliclazide and Metformin HCl

Time in hours	Gliclazide Standard area	Absolute % Difference	Metformin HCl Standard area	Absolute % Difference
Initial	290531	Not applicable	1123067	Not applicable
4	290566	-0.01	1130787	-0.68
6	287769	0.96	1124093	-0.09
8	285302	1.83	1125768	-0.24
10	287356	1.10	1128266	-0.46
12	285718	1.68	1129120	-0.54
18	286425	1.43	1141670	-1.63
24	285934	1.61	1130787	-0.68
Mean	287450	1.23	1129195	-0.62
%RSD	0.723	Not applicable	0.515	Not applicable

Results and conclusions:

The Standard solution and Sample solution was stable upto 24 hours at room temperature.

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9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method is performed by filtering sample solution through 0.45µ Nylon membrane filter against that of unfiltered (centrifuged) sample. The results are tabulated in Table 12A and 12B.

Table 12A: Filter paper study for Sample solution of Gliclazide and Metformin HCL (60/500mg)

Filter study	Gliclazide Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	239754	99.1	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	240674	99.5	-0.38
Filter Set-2 (0.45µ Nylon membrane)	236121	97.6	1.50
Filter Set-3 (0.45µ Nylon membrane)	237427	98.1	0.96

Filter study	Metformin HCL Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	714711	97.6	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	718550	98.1	-0.52
Filter Set-2 (0.45µ Nylon membrane)	710237	97.0	0.61
Filter Set-3 (0.45µ Nylon membrane)	716955	97.9	-0.31

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Table 12B: Filter paper study for Sample solution of Gliclazide and Metformin HCL (60/850mg)

Filter study	Gliclazide Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	234759	102.8	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	235286	103.1	-0.23
Filter Set-2 (0.45µ Nylon membrane)	232844	102.0	0.84
Filter Set-3 (0.45µ Nylon membrane)	231066	101.2	1.62

Filter study	Metformin HCL Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	1108223	98.5	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	1095068	97.4	1.17
Filter Set-2 (0.45µ Nylon membrane)	1092342	97.1	1.41
Filter Set-3 (0.45µ Nylon membrane)	1090956	97.0	1.54

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 membrane) within limit against that of unfiltered. (Centrifuged).

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9.9 ROBUSTNESS:

Study Design:

Five replicate injections of standard preparation and duplicate injections of sample preparation are injected varying different chromatographic conditions as per protocol. System suitability parameters and mean assay difference with respect to assay value in method precision were calculated. The results are tabulated in table 13A and 13B for Gliclazide and Metformin HCL peaks respectively.

Table 13A: Robustness of analytical method for Metformin HCL

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 3.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Low wavelength (262nm)	1974	1.51	0.200	97.9	97.6	0.30
High wavelength (268nm)	2071	1.57	0.147		96.4	1.48
Low flow rate (0.9ml/minute)	2192	1.53	0.052		97.2	0.66
High flow rate (1.1ml/minute)	1958	1.53	0.057		97.4	0.48
Low Temperature (20°C)	1954	1.56	0.082		99.4	-1.48
High Temperature (30°C)	2171	1.49	0.226		97.8	0.15

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Table 13B: Robustness of analytical method for Gliclazide

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 3.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Low wavelength (262nm)	18016	1.11	0.657	100.2	98.5	1.72
High wavelength (268nm)	17861	1.12	0.804		99.3	0.90
Low flow rate (0.9ml/minute)	18782	1.11	0.609		99.6	0.61
High flow rate (1.1ml/minute)	17410	1.11	0.922		101.9	-1.66
Low Temperature (20ºC)	16967	1.13	0.848		101.6	-1.44
High Temperature (30ºC)	18724	1.11	0.859		101.8	-1.62

Acceptance criteria:

1) Theoretical plates for Gliclazide and Metformin HCL peaks should be NLT 1500
2) Tailing Factor for Gliclazide and Metformin HCL peaks should be NMT 2.0.
3) % RSD of area of analyte in replicate standard injections should be NMT 2.0.
4) % Assay of analyte should not differ by ±2.0 to that of method precision.

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Result and Conclusion:

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

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10.0 SUMMARY:

S.No	Validation parameter	Acceptance criteria	Results
1	System suitability	1) % RSD of area of analyte in Five replicate standard injections should not be more than 2.0.	Gliclazide:0.204 Metformin HCL:0.113
		2) Theoretical plate should be not less than 2000.	Gliclazide:17564 Metformin HCL:2108
		3) Tailing factor should not be more than 3.0.	Gliclazide:1.104 Metformin HCL:1.427
2	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	1) There should not be any interference due to blank and placebo with analyte. 2) Peak purity of analyte should pass	Blank and Placebo peaks are not interfere with Gliclazide and Metformin HCL peak in sample preparation and Peak purity passes within specified limits.
3	Linearity and Range	1) R^2 Should be NLT 0.995	Squared correlation coefficient for Gliclazide:0.9994 Metformin HCL:0.9954

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<table>
<tr><th>S.No</th><th>Validation parameter</th><th>Acceptance criteria</th><th>Results</th></tr>
<tr><td></td><td>Linearity and Range</td><td>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.</td><td>
<table>
<tr><th>Level</th><th>Glicla Zide %RSD</th><th>Metfor Min HCL %RSD</th></tr>
<tr><td>10%</td><td>0.979</td><td>0.114</td></tr>
<tr><td>50%</td><td>0.200</td><td>0.052</td></tr>
<tr><td>75%</td><td>0.896</td><td>0.034</td></tr>
<tr><td>100%</td><td>0.187</td><td>0.180</td></tr>
<tr><td>125%</td><td>0.244</td><td>0.072</td></tr>
<tr><td>150%</td><td>0.335</td><td>0.045</td></tr>
</table>
</td></tr>
<tr><td>4</td><td>Interference from degradants (Forced degradation)</td><td>1) There should not be any interference due to degradants with analyte and impurity in stressed samples.

2) The desired degradation should be 10-30% in acid, alkali and oxidation degradation, (if possible).

3) If about 10% to 30% degradation is not achieved by applying above stressed condition, same shall be documented and reported.</td><td>There is no any interference due to degradants with analyst in stressed sample and peak purity was passes according to lab solution.</td></tr>
</table>

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S.No	Validation parameter	Acceptance criteria	Results
		4) Peak purity of analyte peak each impurity peak should be pass (Peak purity should not be less than 0.950 according to Lab solution.	
5	Accuracy (Recovery)	The mean % recovery at each level should be 98.0 to 102.0.	Level %Recovery **Gliclazide:** 50% : 100.23 100% : 100.84 150% : 100.04 **Metformin HCL:** 50% : 100.63 100% : 98.48 150% : 98.53
6	**Precision** 1) System Precision	%RSD of area of analyte peaks in five replicate standard injections should not be more than 2.0.	Gliclazide:0.204 Metformin HCL:0.113

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S.No	Validation parameter	Acceptance criteria	Results
	2) Method Precision	%RSD of Assay of six sample preparations should not be more than 2.0.	**For 60/500mg:** Gliclazide:1.39 Metformin HCL:0.78 **For 60/850mg:** Gliclazide:1.49 Metformin HCL:1.52
	3)Intermediate Precision	1) % RSD for assay of six preparations should not be more than 2.0.	**For 60/500mg:** Gliclazide:0.73 Metformin HCL:0.19 **For 60/850mg:** Gliclazide:1.85 Metformin HCL:1.28
		2) Cumulative %RSD for assay of twelve preparations (of method and intermediate precision) should not be more than 2.0.	**For 60/500mg:** Gliclazide:1.40 Metformin HCL:0.57

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S.No	Validation parameter	Acceptance criteria	Results
			For 60/850mg: Gliclazide:1.85 Metformin HCL:1.64
7	Stability for analytical solution	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%.	The Standard and Sample solution was stable upto 24hours at room temperature.
8	Filter paper study (0.45µ Nylon)	The % difference on filter solution should not differ ±2.0 against that of unfiltered. (Centrifuged)	The % difference on filtered sample 0.45µ Nylon within limit against that of unfiltered (Centrifuged).
9	Robustness (i) Wavelength change (ii) Flow rate change (iii) Column oven Temperature Change	System suitability parameters should comply.	Each chromatographic variation System suitability parameters are within limits. % Difference of assay within limits at each variation.

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11.0 CONCLUSION:

Validation studies have been conducted for Assay of Gliclazide and Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release Tablets for the parameters of system suitability, specificity, Degradation, System Precision, 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
HPLC	:	High performance liquid chromatography
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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REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVAR/23/022	20/09/2023	New Report prepared.