PROTOCOL	
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ANALYTICAL METHOD VALIDATION PROTOCOL

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 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	S. SANTHI
Designation	:	ASST. MANAGER - QC
Signature	:	
Date	:	01/09/23
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	AGM-QC
Signature	:	
Date	:	02/09/2023
APPROVED BY		
Name	:	[illegible]
Designation	:	AGM - QA
Signature	:	
Date	:	04/09/23

Effective Date	:	06/09/2023

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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 TO BE VALIDATED : 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 (as applicable).

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	To be mentioned in report	To be mentioned in report
Metformin Hydrochloride BP	To be mentioned in report	To be mentioned in report

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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 detector

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

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.

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.

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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)

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)

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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.

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

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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.

Calculate the assay of Gliclazide in % as follows:

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

LC = Label claim of Gliclazide in mg/tablet.

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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:

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

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

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8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for Validation	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and placebo
3	**Linearity and Range**
4	**Interference from degradant (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** (i) Wavelength change (ii) Flow rate change (iii) column oven Temperature change

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 DETAILS OF VALIDATION PARAMETERS:

9.1 SYSTEM SUITABILITY TEST:

Purpose:

To establish system suitability as per methodology.

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard preparation	5

Evaluate the following system suitability parameters:

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

Acceptance Criteria:

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 peak area response of Metformin and Gliclazide peak obtained with the replicate injections of standard solution should not more than 2.00

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 placebo not interfering with the analyte peak.

Study Design :

Sequence shall be in following provisional manner.

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S.No.	Description of solution	No. of injections
1	Mobile phase (Blank)	1
2	Standard preparation	5
3	Metformin Hydrochloride Working standard	1
4	Gliclazide Working standard	1
5	Mobile phase (Blank)	1
6	Plain placebo for Glide-M 60/500	1
7	Plain placebo for Glide-M 60/850	1
8	Plain placebo for Glide-M 60/850 with Gliclazide WS	1
9	Plain placebo for Glide-M 60/850 with Metformin HCL WS	1
10	Plain placebo for Glide-M 60/850 with Metformin HCl WS and Gliclazide WS	1
11	Mobile phase (Blank)	1
12	Test preparation for Glide-M 60/500 B.No: G1722093	1
13	Mobile phase (Blank)	1
14	Test preparation for Glide-M 60/850 B.No: G17230801	1

Acceptance Criteria :

i) There should not be any interference due to blank, Placebo peak with analyte.

ii) Peak purity index Not less than 0.995 according to Lab solution software.

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9.3 LINEARITY AND RANGE:

"The linearity of the analytical method is it's 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% to 150% of targeted concentration.

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

For Metformin Hydrochloride:

Linearity Stock solution	850.00	1	1	1	1	8500.00 (con. ppm)
	100	1	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	170.0	5	250	170.0
50%	850.0	10	100	850.0
75%	1275.0	7.5	50	1275.0
100%	1700.0	5	25	1700.0
125%	2125.0	5	20	2125.0
150%	2550.0	15	50	2550.0

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For Gliclazide:

Linearity Stock solution	60.00	1	1	1	1	600.00 (con. ppm)
	100	1	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	12.0	5	250	12.0
50%	60.0	10	100	60.0
75%	90.0	7.5	50	90.0
100%	120.0	5	25	120.0
125%	150.0	5	20	150.0
150%	180.0	15	50	180.0

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Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Level – 1 (10%)	3
3	Mobile phase (Blank)	1
4	Level – 2 (50%)	3
5	Mobile phase (Blank)	1
6	Level – 3 (75%)	3
7	Mobile phase (Blank)	1
8	Level – 4 (100%)	3
9	Mobile phase (Blank)	1
10	Level – 5 (125%)	3
11	Mobile phase (Blank)	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:

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

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

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9.4 INTERFERENCE FROM DEGRADANTS (forced degradation)

Study design:

To evaluate the interference from degradants, carry out a forced degradation study by stressing the test preparation under the following maximum stress conditions.

Degradation	Stress Condition
Acid degradation	Exposure to 5ml of 5N HCL and heat on water bath at 80°C for 30minutes
Alkali degradation	Exposure to 5ml of 5N NaOH and heat on water bath at 80°C for 30minutes
Oxidative degradation	Exposure to 5ml of 30% H2O2 and heat on water bath at 80°C for 30minutes

Sequence shall be in following provisional manner, For forced chemical degradation:

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution (As such)	2
4	Sample solution (Acid degradation)	2
5	Sample solution (Alkali degradation)	2
6	Sample solution (Oxidative degradation)	2
7	Standard solution (Bracketing)	1

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Chromatograph the samples of chemical and physical forced degradation into HPLC system equipped with diode array detector and evaluate the peak purity for the analytes in stressed samples and the degradation profiles under each stressed condition.

Acceptance Criteria:

1) There should not be any interference due to degradants with analyte in stressed samples.

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

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

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

9.5 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 assay of known, add amount of analyte".

Purpose:

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

Sequence shall be in following provisional manner

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S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Mobile phase (Blank)	1
4	Level – 1 Set – 1 (50%)	1
5	Level – 1 Set – 2 (50%)	1
6	Level – 1 Set – 3 (50%)	1
7	Mobile phase (Blank)	1
8	Level – 2 Set – 1 (100%)	1
9	Level – 2 Set – 2 (100%)	1
10	Level – 2 Set – 3 (100%)	1
11	Mobile phase (Blank)	1
12	Level – 3 Set – 1 (150%)	1
13	Level – 3 Set – 2 (150%)	1
14	Level – 3 Set – 3 (150%)	1
15	Standard solution (Bkt)	1

Study design:

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

Acceptance criteria:

The mean % recovery at each level should be 98.0 to 102.0.

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9.6 PRECISION

9.6.1 SYSTEM PRECISION

Purpose:

To establish the precision of the HPLC system being used for the analysis.

Study Design :

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5

Acceptance Criteria :

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

9.6.2 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 preparations as per the methodology representing a single batch and determine the assay for the same. Evaluate the method precision by computing the percentage and relative standard deviation of the assay results.

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For 60mg/500mg:

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution-1	2
4	Sample solution-2	2
5	Sample solution-3	2
6	Standard solution (BKT)	1
7	Sample solution-4	2
8	Sample solution-5	2
9	Sample solution-6	2
10	Standard solution (BKT)	1

For (60mg/850mg):

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution-1	2
4	Sample solution-2	2
5	Sample solution-3	2
6	Standard solution (BKT)	1
7	Sample solution-4	2
8	Sample solution-5	2
9	Sample solution-6	2
10	Standard solution (BKT)	1

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Acceptance Criteria :

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

9.6.3 INTERMEDIATE PRECISION

Purpose:

To demonstrate the reproducibility of test results obtained by the analytical method for the variability of instrument, column (different lot no) analyst and day. Analyse six sample preparations as per the methodology representing a single batch and determine the assay for the same. Evaluate the intermediate precision by computing the percentage and relative standard deviation of the assay results.

Study Design :

Sequence shall be in following provisional manner.

For 60mg/500mg:

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution-1	2
4	Sample solution-2	2
5	Sample solution-3	2
6	Standard solution (BKT)	1
7	Sample solution-4	2
8	Sample solution-5	2
9	Sample solution-6	2
10	Standard solution (BKT)	1

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For (60mg/850mg):

S.No.	Description of solution	No. of Injections
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution-1	2
4	Sample solution-2	2
5	Sample solution-3	2
6	Standard solution (BKT)	1
7	Sample solution-4	2
8	Sample solution-5	2
9	Sample solution-6	2
10	Standard solution (BKT)	1

Acceptance criteria:

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

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

9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Prepare Standard and sample solution as per the methodology and store at room temperature. Chromatograph this solution at regular intervals by using same diluent. Calculate the % difference of analyte peak area for standard and sample preparations with that of initial. The study may be stopped if 2 consecutive failure of sample solution.

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Sequence shall be in following provisional

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution	5
3	Standard solution (Initial)	1
4	Sample solution - (Initial)	1
5	Standard solution (Time interval)	1
6	Sample solution - (Time interval)	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%.

9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering sample solution through 0.45µ Nylon filter against that of unfiltered (Centrifuged).

Sequence shall be in following provisional manner.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Gliclazide and Metformin Hydrochloride in Gliclazide and Metformin Hydrochloride Sustained Release Tablets
Protocol No.	ST/AMVAP/23/022

For 60/500:

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution	5
3	Sample solution –Unfiltered sample (Centrifuged)	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	Standard solution (Bracketing standard)	1

For 60/850:

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution	5
3	Sample solution –Unfiltered sample (Centrifuged)	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	Standard solution (Bracketing standard)	1

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Acceptance criteria:

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

9.9 ROBUSTNESS:

Purpose:

To establish the robustness of the analytical method.

Study Design:

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

Sequence shall be in following provisional manner.

As such		
S.No.	**Description of solution**	**No. of Injections**
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution	1
4	Standard solution (Bracketing standard)	1
According to each variable		
S.No.	**Description of solution**	**No. of Injections**
1	Mobile phase (Blank)	1
2	Standard solution	5
3	Sample solution	1
4	Standard solution (Bracketing standard)	1

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

a) Flow rate change by ±10% mean (i.e 0.9 ml/min and 1.1 ml/minute)

b) Wave length change by ± 3nm (i.e. 262nm and 268nm)

c) Column oven Temperature change by ± 5.0ºC (i.e. 20ºC and 30ºC)

Acceptance criteria:

System suitability should comply for each variable and % of drug not differ ±2% from mean assay value of method precision.

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10.0 ABBREVIATION:

mg	:	Milligram
S.No	:	Serial Number
ml	:	Milliliter
%	:	Percentage
ID	:	Identification
API	:	Active pharmaceutical ingredient
HPLC	:	High performance liquid chromatography
B.NO	:	Batch number
mm	:	Millimeter
µm	:	Micrometer
min	:	Minutes
°C	:	Degree centigrade
nm	:	Nanometer
RSD	:	Relative standard deviation
µl	:	Micro litre
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H_2O_2	:	Hydrogen Peroxide
WS	:	Working standard

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11.0 REVISION HISTORY:

Protocol No.	Effective date	Reason for Review
ST/AMVAP/23/022	06/09/2023	New Protocol prepared.