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

ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF DISSOLUTION OF GLICLAZIDE IN GLICLAZIDE AND METFORMIN HCL 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
Signature	:	
Date	:	08/09/23
REVIEWED BY		
Name	:	M.VIJAYAKUMAR
Designation	:	AGM-QC
Signature	:	
Date	:	09/09/2023
APPROVED BY		
Name	:	J.YARAN
Designation	:	AGM-QA
Signature	:	
Date	:	11/09/23

Effective Date	:	12/09/2023

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3.0 OBJECTIVE

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

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Dissolution of Gliclazide 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 :

Time intervel	Limit
2nd Hour	NMT 25.0%
5th Hour	Between 30 – 60%
12th Hour	NLT 70%

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

VALIDATION TEAM : 1. C.K.Saravanan

2. S.Bhavyasri

3. E.Meena

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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	G17230801, G1722093	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

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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

Dissolution:

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

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

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 :

Gliclazide (Working standard)

Potassium Di-hydrogen orthophosphate (AR grade)

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Dipotassium hydrogen orthophosphate (AR Grade)

Disodium hydrogen orthophosphate (AR grade)

Orthophosphoric acid (AR grade)

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

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

Sodium hydroxide (AR grade)

Sodium Chloride (AR grade)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Apparatus	:	USP Apparatus II (Paddle) with sinker
Volume	:	900 mL
Dissolution medium	:	Phosphate buffer pH 7.4
Speed	:	100 rpm
Temperature	:	37.0±0.5°C
Time	:	2nd, 5th and 12 Hours

Chromatographic Conditions:

Column	:	Kromasil 100-C18 ,250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	228 nm
Column Temperature	:	Ambient

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Flow Rate : 1.0 mL/min

Injection Volume : 20 µL

Run time : 12 Minutes

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 6.85±0.05 using Orthophosphoric acid. Filter through 0.20micron membrane filter, sonicate and degas.

Preparation of Phosphate buffer (pH = 7.4): (Dissolution medium)

Weigh accurately about 23.8gm of disodium hydrogen orthophosphate, 1.9gm of Potassium dihydrogen orthophosphate and 80gm of Sodium Chloride in 500ml with water, shake and sonicate to dissolve completely and finally make the solution to 10 liters of water. Adjust pH to 7.4 using 0.5 M Sodium Hydroxide solution.

Preparation of Standard solution:

Weigh accurately and transfer about 66 mg of Gliclazide working standard into 100ml volumetric flask. Add about 5 ml of Methanol, sonicate to dissolve and dilute up to mark with dissolution medium and mix. Further dilute 5 ml of this solution to 50 ml with dissolution medium and mix well. (**Concentration**:0.066mg/ml of Gliclazide)

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 and inject. (**Concentration**:0.066mg/ml of Gliclazide)

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(After withdrawing aliquot at each interval, then add same volume of dissolution medium to maintain 900 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:

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	Dissolution medium (Blank)	1
2	Standard solution	5
3	Sample solution	6
4	Standard solution (Bracketing standard)	1 Each after every 6 sample injection

System suitability:

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

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

3) The % RSD for the retention time of 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 Gliclazide peak obtained with the replicate injections of standard solution should not more than 2.00

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5) The % RSD for the retention time of 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 Gliclazide peak obtained with the replicate injections of standard solution and bracketing standard solution should not more than 2.00

Calculations:

Calculate % drug release of Gliclazide as follows:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} + D$$

Where,

AT	=	Peak area response of Gliclazide peak obtained with sample solution.
AS	=	Average peak area response of Gliclazide peak obtained with replicate injections of standard solution
WS	=	Weight of Gliclazide working standard in mg.
P	=	Potency of Gliclazide working standard in % on as such basis.
LC	=	Label claim of Gliclazide in mg/tablet.
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.

$$CFn = \frac{Dn}{900} X10$$

Where,

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

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Calculation for corrected results:

For 2nd Hour = D2
For 5th Hour = D5+CF1
For 12th Hour = D12+CF2+CF1

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	**Accuracy (Recovery)**
5	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
6	**Stability of analytical solution**
7	**Filter paper study**
8	**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 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 Injections
1	Blank (Dissolution medium)	1
2	Standard solution	5

Evaluate the following system suitability parameters:

1) % RSD of area of Gliclazide peak in five replicate standard injections.

2) Theoretical plates for Gliclazide peak in standard injection.

3) Tailing factor for Gliclazide peak in standard injection.

Acceptance Criteria:

1) % RSD of area for Gliclazide peak in five replicate standard injections should not more than 2.0%.

2) Theoretical plates for Gliclazide peak in standard injection should not less than 2000.

3) Tailing factor for Gliclazide peak in standard injection should not more than 2.0.

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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.

S.No.	Description of solution	No. of injections
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Blank (Dissolution medium)	1
4	Gliclazide Working standard	1
5	Metformin Hydrochloride working standard	1
6	Blank (Dissolution medium)	1
7	Plain placebo for Glide-M 60/500	1
8	Plain placebo for Glide-M 60/850	1
9	Plain placebo with Gliclazide Working standard	1
10	Plain placebo with Metformin HCL Working standard	1

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S.No.	Description of solution	No. of injections
11	Plain placebo + Gliclazide WS + Metformin HCL WS	1
12	Blank (Dissolution medium)	5
13	Test preparation for Glide-M 60/500 B.No: G1722093	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 of analyte should be pass. According to Lab solution software.

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%, 25%, 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.

Gliclazide:

Linearity Stock solution	66.00	1	1	1	1	660ppm (con. ppm)
	100	1	1	1	1	

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Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	6.60	5	500	6.60
25%	16.50	5	200	16.50
50%	33.00	5	100	33.00
75%	49.50	7.5	100	49.50
100%	66.00	5	50	66.00
125%	82.50	2.5	20	82.50
150%	99.00	7.5	50	99.00

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Dissolution medium)	1
2	Level – 1 (10%)	3
3	Blank (Dissolution medium)	1
4	Level – 2 (25%)	3
5	Blank (Dissolution medium)	1
6	Level – 3 (50%)	3
7	Blank (Dissolution medium)	1
8	Level – 4 (75%)	3
9	Blank (Dissolution medium)	1
10	Level – 5 (100%)	3
11	Blank (Dissolution medium)	1
12	Level – 6 (125%)	3
13	Blank (Dissolution medium)	1
14	Level – 7 (150%)	3

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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 ($r^{2)}$ should not be less than 0.995.

2) To conclude the range. % RSD for peak area of linearity level of 10%, 25%, 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.

Sequence shall be in following provisional manner

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S.No.	Description of solution	No. of Injections
1	Blank (Dissolution medium)	1
2	Standard solution	5
3	Blank (Dissolution medium)	1
4	Level – 1 Set – 1 (5%)	1
5	Level – 1 Set – 2 (5%)	1
6	Level – 1 Set – 3 (5%)	1
7	Blank (Dissolution medium)	1
8	Level – 2 Set – 1 (50%)	1
9	Level – 2 Set – 2 (50%)	1
10	Level – 2 Set – 3 (50%)	1
11	Blank (Dissolution medium)	1
12	Level – 3 Set – 1 (100%)	1
13	Level – 3 Set – 2 (100%)	1
14	Level – 3 Set – 3 (100%)	1
15	Blank (Dissolution medium)	1
16	Level – 4 Set – 1 (150%)	1
17	Level – 4 Set – 2 (150%)	1
18	Level – 4 Set – 3 (150%)	1
19	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 5%, 50%, 100% and 150% of targeted concentration) to placebo. Prepare the recovery samples in triplicate for each level.

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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 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	Blank (Dissolution medium)	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.

9.5.2 METHOD PRECISION

Purpose:

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

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Study design:

To demonstrate the method precision, analyze six sample units preparations 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.

For 60/500mg:

S.No.	Description of solution	No. of Injections
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
9	Standard solution (BKT)	1

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For 60/850mg:

S.No.	Description of solution	No. of Injections
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
9	Standard solution (BKT)	1

Acceptance Criteria :

% RSD for dissolution of six sample units should not be more than 5.0.

9.5.3 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. Analyze six sample units preparations 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.

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Study Design:

Sequence shall be in following provisional manner.

For 60/500mg:

S.No.	Description of solution	No. of Injections
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
9	Standard solution (BKT)	1

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For 60/850mg:

S.No.	Description of solution	No. of Injections
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
9	Standard solution (BKT)	1

Acceptance criteria:

1) % RSD for Dissolution of six sample units preparations should not be more than 5.0.

2) Cumulative % RSD for dissolution of twelve sample units 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 sample solution as per the methodology and store at room temperature. Chromatograph these solution at regular intervals by using same solution. Calculate the % difference of analyte peak area for standard preparations with that of initial. The study may be stopped if 2 consecutive failure of standard solution.

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Sequence shall be in following provisional for Standard preparation.

S.No.	Description of solution	No. of Injections
1	Blank (Dissolution medium)	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 standard and sample 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, PVDF and Whatman membrane filter paper. Against that of unfiltered (Centrifuged).

Sequence shall be in following provisional manner.

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S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution	5
3	Sample solution –Unfiltered sample (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	Sample solution –Filter Set 1 (0.45µ PVDF membrane)	1
8	Sample solution –Filter Set 2 (0.45µ PVDF membrane)	1
9	Sample solution –Filter Set 3 (0.45µ PVDF membrane)	1
10	Sample solution –Filter Set 1 (Whatman membrane)	1
11	Sample solution –Filter Set 2 (Whatman membrane)	1
12	Sample solution –Filter Set 3 (Whatman membrane)	1
13	Standard solution (BKT)	1

Acceptance criteria:

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

9 .8 ROBUSTNESS:

Purpose:

To establish the robustness of the analytical method.

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

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

a) Wave length change by ± 3nm (i.e. 225nm and 231nm)

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

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

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d) Effect of variation in dissolution media volume:

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

e) Effect of variation in dissolution medium pH variation:

Effect of variation in dissolution medium pH variation ±0.2% pH.

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

Acceptance criteria:

System suitability should comply for each variable.

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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
WS	:	Working standard

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11.0 REVISION HISTORY:

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