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

# ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF DISSOLUTION OF METFORMIN HYDROCHLORIDE IN GLICLAZIDE AND METFORMIN HYDROCHLORIDE 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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## 1.0 INDEX

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## 2.0 PROTOCOL APPROVAL SHEET

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

| | | |
|---|---|---|
| Effective Date | : | 09/12/2023 |

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

COMPOSITION : Each Uncoated bilayered Sustained Release tablet contains:

| Content | Strength |
|---|---|
| Gliclazide BP | 60mg |
| Metformin Hydrochloride BP | 850mg |

BATCH NO : G1723081

SPECIFICATION LIMIT :

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

VALIDATION STUDY : QC-Laboratory, 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 | Not Applicable | Not Applicable |
| Working standard<br>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, SOLVENTS AND CHEMICALS TO BE USED:

**Instruments:**

UV VIS Spectrophotometer

Make : Shimadzu, Model : UV-1700

**Analytical Balance**

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

**Dissolution:**

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

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

**pH meter:**

Make : Eutech, Model No : PH 700

**Chemicals/Reagents/Standards:**

Metformin Hydrochloride (Working standard)

Monobasic potassium phosphate (AR grade)

Sodium Hydroxide (AR grade)

Water (Purified)

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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 | : | 1st, 3rd and 10th Hours |

**Preparation of Phosphate buffer (pH 6.8):**

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

**Preparation of Standard solution:**

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

**Preparation of Sample solution:**

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

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

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

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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.**

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

Where,

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

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**Calculation for corrected results:**

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

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## 8.0 PARAMETERS TO BE VALIDATED:

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

### 9.1 SYSTEM SUITABILITY:

**Purpose:**

To establish system suitability as per methodology.

**Study Design:**

Sequence shall be in following provisional manner.

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

**Evaluate the following system suitability parameters.**

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

**Acceptance Criteria:**

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

### 9.2 SPECIFICITY (SELECTIVITY)

#### 9.2.1 Interference from blank and Placebo

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

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**Purpose:**

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

**Study Design :**

**Sequence shall be in following provisional manner.**

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

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***Note:***

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

**Acceptance criteria:**

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

**9.3 LINEARITY AND RANGE:**

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

**Purpose:**

To Establish the linearity of analyte within the specified range.

**Study Design:**

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

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

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

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

Sequence shall be in following provisional manner.

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

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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 should not be less than 0.995.

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

**9.4 ACCURACY (RECOVERY)**

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

**Purpose**:

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

**Study design:**

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

Sequence shall be in following provisional manner.

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

**Acceptance criteria:**

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

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## 9.5 PRECISION

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

### 9.5.1 METHOD PRECISION

**Purpose:**

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

**Study design:**

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

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

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

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**Acceptance Criteria:**

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

### 9.5.2 INTERMEDIATE PRECISION

**Purpose:**

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

**Study Design:**

Sequence shall be in following provisional manner.

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

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**Acceptance criteria:**

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

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

**9.6 STABILITY OF ANALYTICAL SOLUTION:**

**Study design:**

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

Sequence shall be in following provisional.

**For Sample:**

| No. | Description of solution | No. of absorbance |
|---|---|---|
| 1 | Sample solution (Initial) | 1 |
| 2 | Sample solution 2$^{nd}$ hours | 1 |
| 3 | Sample solution 4$^{th}$ hours | 1 |
| 4 | Sample solution 6$^{th}$ hours | 1 |
| 5 | Sample solution 16$^{th}$ hours | 1 |
| 6 | Sample solution 20$^{th}$ hours | 1 |
| 7 | Sample solution 24$^{th}$ hours | 1 |
| 8 | Sample solution 28$^{th}$ hours | 1 |

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| No. | Description of solution | No. of absorbance |
|---|---|---|
| 9 | Sample solution 32$^{nd}$ hours | 1 |
| 10 | Sample solution 40$^{th}$ hours | 1 |
| 11 | Sample solution 44$^{th}$ hours | 1 |
| 12 | Sample solution 48$^{th}$ hours | 1 |

**For standard:**

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

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**Acceptance criteria:**

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

**9.7 FILTER PAPER STUDY:**

**Study design:**

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

Sequence shall be in following provisional manner.

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

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**Acceptance criteria:**

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

**9.8 ROBUSTNESS:**

**Purpose:**

To establish the robustness of the analytical method.

**Study Design:**

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

Sequence shall be in following provisional manner.

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

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

**9.8.1 Effect of dissolution apparatus speed:**

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

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

**9.8.2 Effect of variation in dissolution media volume:**

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

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

**9.8.3 Effect of variation in nanometer:**

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

**9.8.4 Effect of variation in pH:**

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

**Acceptance criteria:**

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

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## 10.0 ABBREVIATION:

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

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## 11.0 REVISION HISTORY:

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