ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE DETERMINATION OF ASSAY OF LACTIC ACID BACILLUS BY MICROBIOLOGICAL ASSAY METHOD

1. PURPOSE:

This Protocol is designed to validate the analytical test procedure for the determination of Assay of Lactic Acid Bacillus as per ICH –Guidelines Q2 (R1): Validation of analytical procedure: Text and Methodology.

2. SCOPE:

This protocol is applicable to the analytical method validation study for the determination of assay of Lactic Acid Bacillus by Microbiological Assay Method.

3. DEFINITIONS/ ABBREVIATION:

RSD	:	Relative Standard Deviation.
W.R.T	:	With respect to
API	:	Active Pharmaceutical Ingredient
ICH	:	International conference on Harmonization

4. RESPONSIBILITY:

Executive – Quality Control is responsible for execution of the protocol and compilation of the task and preparation of the report.

Manager -QC is responsible for reviewing the protocol and report.

Manager -QA is responsible for approving the protocol and report.

5. PROCEDURE :

5.1 VALIDATION PARAMETERS:

1. Precision

1.1 Method precision

1.2 Intermediate precision or Ruggedness

2. Accuracy as recovery

5.2 PRE STUDY-REQUISITES:

Test procedure for assessment of the quality levels of pharmaceutical articles are subject to various requirements according to section 501 of the federal food , drug and cosmetic act , assays and specifications in monograph constitute legal standard . The current good manufacturing practice regulations [21CFR211.194 (a)] require that

test methods, which are used for assessing compliance of pharmaceutical articles which established specifications, must meet proper standards of accuracy & reliability.

All validation experiments will be performed with qualified equipments and trained analysts.

5.3 TEST PROCEDURE:

5.3.1 REAGENTS & PRERQUISITIES:

- 90mm Petri plates
- Glucose Yeast Extract Agar
- 0.9% Saline
- Distilled water
- 25 x150mm Test Tubes
- 100 ml Volumetric Flask
- Vortex Mixer

5.3.2 EQUIPMENTS:

- Vortex Mixer
- Calibrated Weighing Balance
- Water Bath at 75°C
- Micropipette

5.3.3 PERSONEL INVOLVED:

- …….. : Sr. Executive QC
- ……. : Executive QC
- …….. : Sr. Executive QA

Method for the determination of Assay of Lactic Acid Bacillus:

Weight accurately powder of 1gm of Lactic Acid Bacillus and add 100 ml of sterile normal saline solution and homogenize at 12,000 to 15,000 rpm for 7-10 minutes. Mix well the test solution by hand shaking and dilute it further step wise through a series of test tubes (size 25 mm X 150 mm), containing 9 ml of sterile normal saline solution by an appropriate decimal dilution method.

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The final dilution is estimated to produce 30-300 colonies per plates after incubation (Recommended final dilution should 100 X 10^{-6}). The tube containing the final dilution is allowed to stand in water bath at 75° for 30 minutes. Cool the tube immediately to about 45°C and disperse 1ml aliquot in each of 5 sterile petri plates.

Add 15ml of pre sterilised and held at 50°C glucose yeast extract agar medium previously sterilized, melted and cooled 45-50°C to each of the 5 sterile petri plates containing the test solution. Incubate the plates at 37° C for 72 hours after incubation count the number of colonies in each plate.

After incubation count the number of colonies in each plate.

Take average number of colonies and calculate the spore count of lactic acid bacillus.

Calculation:

Take average number of colonies X by the dilution number (dilution factor) represent the viable lactobacillus spores count per gm powder.

6.0 PRECISION:

Precision provides the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple analysis of a homogenous sample.

6.1 Method Precision:

Prepared the sample as mentioned in the assay method and perform the Lactic Acid Bacillus assay in six replicates.

Prepare sample solution six times as per methodology.

Calculate the Average and %RSD of six assay values.

6.2 Intermediate precision (Ruggedness):

This study shall be carried out as per method precision by a different analyst, different day by using different set of sample solution.

ACCEPTANCE CRITERIA:

% RSD Should be Not More Than 10 .

Intermediate precision: The overall RSD of twelve assay value should not be more than 10 %.

7. ACCURACY:

The accuracy (trueness) of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found known as recovery as

% recovery. Prepare the recovery solutions at 80%, 100%, and 120% level as given below and test each concentration in triplicate:

Preparation of recovery solutions:

Spiked Solution at 80 % :-

Accurately weigh about 800 mg of Lactic Acid Bacillus in a 100ml Sterile Normal Saline Solution and homogenize at 12,000 to 15,000 rpm for 7-10 minutes. Mix well the test solution by hand shaking and dilute it further step wise through a series of test tubes (size 25mm X 150mm), containing 9ml of sterile normal saline solution by an appropriate decimal dilution method.

The final dilution is estimated to produce 30-300 colonies per plates after incubation (Recommended final dilution should 100 X 10^{-6}). The tube containing the final dilution is allowed to stand in water bath at 75° for 30 minutes. Cool the tube immediately to about 45°C and disperse 1ml aliquot in each of 5 sterile petri plates.

Add 15ml of pre sterilised and held at 50°C glucose yeast extract agar medium previously sterilized, melted and cooled 45-50°C to each of the 5 sterile petri plates containing the test solution. Incubate the plates at 37° C for 72 hours after incubation count the number of colonies in each plate.

After incubation count the number of colonies in each plate.

Take average number of colonies and calculate the spore count of lactic acid bacillus.

Spiked Solution at 100 %:-

Accurately weigh about 1000 mg of Lactic Acid Bacillus in a 100ml Sterile Normal Saline Solution and homogenize at 12,000 to 15,000 rpm for 7-10 minutes. Mix well the test solution by hand shaking and dilute it further step wise through a series of test tubes (size 25 mm X 150 mm), containing 9 ml of sterile normal saline solution by an appropriate decimal dilution method.

The final dilution is estimated to produce 30-300 colonies per plates after incubation (Recommended final dilution should 100 X 10^{-6}). The tube containing the final dilution is allowed to stand in water bath at 75° for 30 minutes. Cool the tube immediately to about 45°C and disperse 1ml aliquot in each of 5 sterile petri plates.

Add 15ml of pre sterilised and held at 50°C glucose yeast extract agar medium previously sterilized, melted and cooled 45-50°C to each of the 5 sterile petri plates containing the test solution. Incubate the plates at 37° C for 72 hours after incubation count the number of colonies in each plate.

After incubation count the number of colonies in each plate.

Take average number of colonies and calculate the spore count of lactic acid bacillus.

Spiked Solution at 120 % :-

Accurately weigh about 1200 mg of Lactic Acid Bacillus in a 100ml Sterile Normal Saline Solution and homogenize at 12,000 to 15,000 rpm for 7-10 minutes. Mix well the test solution by hand shaking and dilute it further step wise through a series of test tubes (size 25mm X 150mm), containing 9ml of sterile normal saline solution by an appropriate decimal dilution method.

The final dilution is estimated to produce 30-300 colonies per plates after incubation (Recommended final dilution should 100 X 10^{-6}). The tube containing the final dilution is allowed to stand in water bath at 75° for 30 minutes. Cool the tube immediately to about 45°C and disperse 1ml aliquot in each of 5 sterile petri plates.

Add 15ml of pre sterilised and held at 50°C glucose yeast extract agar medium previously sterilized, melted and cooled 45-50°C to each of the 5 sterile petri plates containing the test solution. Incubate the plates at 37° C for 72 hours after incubation count the number of colonies in each plate.

After incubation count the number of colonies in each plate.

Take average number of colonies and calculate the spore count of lactic acid bacillus.

Acceptance Criteria

Recovery for assay from the sample obtained with triplicate test preparations at each level (I.e. about 80%, 100%, 120 % of specification level) should be between 95 and 105 %.

% RSD should be within 10.0.

10. Reporting and evaluation of data

All the raw data shall be taken in the Final report and will be part of the validation exercise. On completion of the validation study, a detailed validation report shall be prepared as a separate document.