MASTER COPY

ANALYTICAL METHOD VALIDATION

ANALYTICAL METHOD VALIDATION PROTOCOL

FOR

THE TEST OF ASSAY OF ASCORBIC ACID

IN

PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE AND ASCORBIC ACID POWDER

Site Address: Safetab Life Science
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1.0 INDEX

2.0 PROTOCOL APPROVAL SHEET

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Date	:	07/10/2022
Effective Date	:	10/10/2022

3.0 OBJECTIVE:

To validate the method for test of assay of Ascorbic acid in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid powder by Titrimetric method.

4.0 SCOPE:

The scope of this protocol is to evaluate the acceptability of analytical method used for Ascorbic acid content by Titrimetry for the assay determination in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid powder. This protocol shall define the procedure, Documentation refer the acceptance criteria to be used in determination of assay by Titrimetry Method.

5.0 GENERAL INFORMATION:

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST VALIDATED : Assay of Ascorbic acid in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid powder

COMPOSITION : Each 4.5g sachet contains:

Content	Strength
Paracetamol BP	650mg
Phenylephrine hydrochloride BP	10mg
Chlorphenamine Maleate BP	20mg
Ascorbic acid BP	50mg

BATCH NO : ST/T/C-1322

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

VALIDATION PLACE : QC-Laboratory, Safetab Life science, Puducherry

VALIDATION TEAM : 1. A.Priyanka

2. E.Meena

6.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED FOR VALIDATION WORK:

NAME OF THE MATERIAL	ID NO./BATCH NO	POTENCY/PURITY
Sample	To be mentioned in report	To be mentioned in report
Plain placebo	To be mentioned in report	To be mentioned in report
Working standard Paracetamol BP	To be mentioned in report	To be mentioned in report
Phenylephrine Hydrochloride BP	To be mentioned in report	To be mentioned in report
Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Ascorbic acid BP	To be mentioned in report	To be mentioned in report
API Paracetamol BP	To be mentioned in report	To be mentioned in report
Phenylephrine Hydrochloride BP	To be mentioned in report	To be mentioned in report
Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Ascorbic acid BP	To be mentioned in report	To be mentioned in report

7.0 DETAILS OF INSTRUMENTS, SOLVENTS AND CHEMICALS USED FOR VALIDATION WORK:

Analytical Balance

Make : Sartorius, Model : BSA224S-CW

pH:

Make: Eutech instruments, Model No: pH 700

Solvents and chemicals with grade:

Potassium iodide (AR grade)

Sodium carbonate (AR grade)

Hydrochloric acid (AR grade)

Starch (AR grade)

Potassium bromate (Primary standard)

Iodine (AR grade)

Glacial acetic acid (AR grade)

Sulfuric acid (AR grade)

Sodium thiosulphate (AR grade)

Water (AR grade)

8.0 DESCRIPTION OF ANALYTICAL METHOD:

Preparation of 0.1M sodium thiosulfate solution:

Weigh and transfer accurately 25.0 g of potassium iodide and 0.2 g of sodium carbonate into 1000 mL volumetric flask. Add about 400 mL of purified water and sonicate to dissolve. Make up to the volume with purified water and mix.

Preparation of 2M hydrochloric acid solution:

Transfer accurately 17 mL of hydrochloric acid and dilute to 100 mL with purified water.

Preparation of starch solution:

Weigh and transfer accurately 1 g of starch into a 200 mL beaker, add 100 mL of boiling water, and dissolve.

Use freshly prepared starch solution.

Standardization of 0.1M sodium thiosulfate solution:

Weigh accurately 0.200 g of potassium bromate, transfer into a 250 mL of volumetric flask add 50 mL of purified water swirl to dissolve and make up to the volume with purified water. Transfer 50 mL of above solution to a 250 mL conical flask. Add 2 g of potassium iodide and 3 mL of 2 M hydrochloric acid solution.

Titrate with 0.1 M sodium thiosulfate solution using starch solution, added towards the end point of the titration, as indicator until the blue colour is discharged.

1 mL of 0.1 M sodium thiosulfate solution is equivalent to 0.002784 g of $KBrO_3$.

Calculation:

Calculate the actual molarity of 0.1 M Sodium thiosulfate as follows,

$$\text{Actual Molarity} = \frac{\text{Weight of Potassium bromate(g)} \times 50}{\text{Titer value} \times 0.002784 \times 250}$$

Preparation of 0.05 M Iodine:

Weigh and transfer accurately 20.0 g of potassium iodide and 12.6 g of Iodine into 1000 mL volumetric flask. Add about 700 mL of purified water and sonicate for 30 minutes with intermittent shaking (ensure iodine balls dissolved completely). And make up to the volume with purified water and mix.

Preparation of dilute acetic acid solution:

Transfer accurately about 5.7 mL of glacial acetic acid and dilute to 100 mL with purified water and mix.

Standardization of 0.05 M Iodine:

Transfer accurately 10 mL of 0.05M Iodine solution into a conical flask, add 1 mL of dilute acetic acid, 40 mL of purified water and add 1 mL of starch solution and titrate against 0.1 M sodium thiosulfate solution until the disappearance of violet blue color. Perform a blank determination for correction.

Calculation:

Calculate the actual molarity of 0.05 M iodine as follows,

$$\text{Actual Molarity} = \frac{M_1 x V_1 x\ 0.05}{V_2 x\ 0.1}$$

Where,

M_1	:	Molarity of titrant
V_1	:	Volume of 0.05 M Iodine taken (mL)
V_2	:	Titer volume (mL)

Preparation of dilute sulfuric acid solution:

Transfer accurately about 5.7 mL of sulfuric acid and dilute to 100 mL with purified water and mix well.

Procedure:

Transfer the contents of not less than 5 sachets. Weigh accurately and transfer sample equivalent to 100 mg of ascorbic acid into a 250 mL conical flask. Add 10 mL of dilute sulfuric acid and sonicate for 15 minutes with intermittent shaking. Ensure to disperse sample completely. Add 80 mL of purified water and sonicate for 15 minutes with intermittent shaking add 1.0 mL of starch solution as indicator. Titrate against 0.05M iodine solution until the appearance of dark violet blue colour as end point. Perform a blank determination for correction.

Each mL of 0.05 M iodine equivalent to 8.81 mg of ascorbic acid.

Calculation:

Calculate the content of Ascorbic acid (mg) as follows,

$$\text{Ascorbic acid (mg) per sachet} = \frac{\text{Titer value x Actual strength of Iodine x 8.81 x Avg fill Wt. (mg)}}{\text{Weight of the sample taken (mg) x 0.05}}$$

$$\text{Ascorbic acid (\%) per sachet} = \frac{\text{Content in of Ascorbic acid (mg/Sachet)}}{\text{Label claim of Ascorbic acid (mg/sachet)}} \times 100$$

9.0 VALIDATION RESULTS:

9.1 SPECIFITY:

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

Purpose:

To demonstrate that the placebo are not interfering with the analyte end point.

Study design:

Sequence shall be in following provisional manner.

No.	Description of solution	No. of Titration
1	Blank	1
2	Plain Placebo preparation	1
3	Plain placebo with Ascorbic acid	1
4	Plain placebo with Paracetamol	1
5	Plain placebo with Phenylephrine Hcl	1
6	Plain placebo with Chlorphenamine Maleate	1
7	Test preparation	1

Acceptance criteria:

There should not be any interference from blank and Placebo sample.

9.2 LINEARITY:

"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 give range ".

Purpose:

To Establish the linearity of content 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.

Sequence shall be in following provisional manner.

No.	Description of solution	No. of Titration
1	Blank	1
2	Level – 1 (10%)	2
3	Level – 2 (50%)	2
4	Level – 3 (75%)	2
5	Level – 4 (100%)	2
6	Level – 5 (125%)	2
7	Level – 6 (150%)	2

Plot a graph of concentration (at X-axis) versus titre value (at Y-axis). Evaluate the squared correlation coefficient (r^2), correlation coefficient (r), residual sum of square, slope and Y-intercept.

Acceptance criteria:

To conclude the linearity, the squared correlation coefficient should not be less than 0.995

9.3 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

No.	Description of solution	No. of Titration
1	Blank	1
2	Level – 1 Set – 1 (50%)	1
3	Level – 1 Set – 2 (50%)	1
4	Level – 1 Set – 3 (50%)	1
5	Level – 2 Set – 1 (100%)	1
6	Level – 2 Set – 2 (100%)	1
7	Level – 2 Set – 3 (100%)	1
8	Level – 3 Set – 1 (150%)	1
9	Level – 3 Set – 2 (150%)	1
10	Level – 3 Set – 3 (150%)	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.

Acceptance criteria:

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

9.4 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"

(i) Method Precision:

Purpose:

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

Study design:

To demonstrate the method precision, analyze six test 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.

No.	Description of solution	No. of Titration
1	Blank	1
2	Test preparation-1	1
3	Test preparation-2	1
5	Test preparation-3	1
6	Test preparation-4	1
7	Test preparation-5	1
8	Test preparation-6	1

Acceptance criteria:

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

(ii) Intermediate Precision (Ruggedness):

Purpose:

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

Study design:

To demonstrate the method precision, analyze six test 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.

No.	Description of solution	No. of Titration
1	Blank	1
2	Test preparation-1	1
3	Test preparation-2	1
5	Test preparation-3	1
6	Test preparation-4	1
7	Test preparation-5	1
8	Test preparation-6	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.

10.0 PARAMETERS TO BE VALIDATED:

No	Validation parameters
1.	Specificity
2.	Linearity
3.	Accuracy (recovery)
4.	Precision (i) Method precision (ii) Intermediate precision

11.0 ABBREVIATION:

mg	:	Milligram
g	:	Gram
RSD	:	Related Standard Deviation
ml	:	Milliliter
%	:	Percentage
NLT	:	Not less than

12.0 REVISION HISTORY:

Protocol No.	Effective date	Reason for Review
ST/AMVAAP/017		New Protocol prepared.

**** END OF THE DOCUMENT****