ANALYTICAL METHOD VALIDATION

ANALYTICAL METHOD VALIDATION REPORT

FOR

THE TEST OF ASSAY OF ASCORBIC ACID

IN

PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE AND ASCORBIC ACID POWDER

Site Address: Safetab Life Science
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107.

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2.0 REPORT APPROVAL SHEET

Prepared by : **Asst.Manager-QC**

Name : K. SARAVANAN

Signature :

Date : 09/12/2022

Reviewed by : **AGM-QC**

Name : M. VIJAYA KUMAR

Signature :

Date : 09/12/2022

Approved by : **GM-QA**

Name : A. G. ICAMMAN

Signature : A. G. I

Date : 10/12/2022

Effective Date : 12/12/2022

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:

This scope of the Report is to evaluate the acceptability of analytical method used for the assay of Ascorbic acid in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid powder by Titrimetric method. This report shall define the procedure, Documentation refer the acceptance criteria to be used in determination of Assay by Titrimetric 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 STUDY : QC-Laboratory, Safetab Life science, Puducherry

VALIDATION TEAM : 1. A.Priyanka

2. E.Meena

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

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	B.No: ST/T/C-1322	COA attached
Plain placebo	B.No: NA	Not applicable
Working standard Paracetamol BP	WS. No: ST/WS/22/011	100.0% (As is basis)
Phenylephrine Hydrochloride BP	WS.No: IAARI/WS/344	98.9% (As is basis)
Chlorphenamine Maleate BP	WS. No: ST/WS/22/039	99.7% (As is basis)
Ascorbic acid BP	WS. No: ST/WS/22/032	100.1% (As is basis)
API Paracetamol BP	B.No:410236	99.7% (As is basis)
Phenylephrine Hydrochloride BP	B.No:2-IL-D-1041121	99.0% (As is basis)
Chlorphenamine Maleate BP	B.No:SLL/C/1021151	99.0% (As is basis)
Ascorbic acid BP	B.No:VP-13080222	100.3% (As is basis)

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 SPECIFICITY

Placebo solutions were prepared by using equivalent weight of placebo present in portion of test preparation as per test method and titrated as per methodology.

Results are tabulated in Table 1.

Acceptance criteria:

There should not be any interference due to blank, placebo peak with analyte.

Table 1: Specificity

Sr.No	Sample ID	Volume of Titration consumed
1	Blank	0.2ml
2	Plain placebo	0.8
3	Plain placebo Ascorbic acid	11.8
4	Plain placebo with Paracetamol	0.8
5	Plain placebo with Phenylephrine Hcl	0.8
6	Plain placebo with Chlorphenamine Maleate	0.8
7	Test preparation	11.9

Results and Conclusion:

There is interference of plain placebo in sample and subtract the interference value from sample titer value. Hence determine the ascorbic acid content.

9.2 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Ascorbic acid Working standard were prepared over the range of 10% to 150% concentration with respect to target concentration (i.e. 10%, 50%, 75%, 100%, 125% and 150%). The sample were analyst as per proposed method. The results are tabulated in Table 2 Linearity and Table 3 for Range.

Acceptance criteria:

1) The squared correlation coefficient should not be less than 0.995.
2) To conclude the range % RSD for peak areas of linearity levels 10%, 50%, 75%, 100%, 125%& 150% should not be more than 2.0.

Table 2: Linearity Table for Ascorbic acid

Linearity Levels (%)	Weight taken in mg (x-Axis)	Titer value (y-axis)
10%	10.350	1.3
50%	50.225	5.8
75%	75.130	8.6
100%	100.065	11.7
125%	125.105	14.4
150%	150.200	17.2
Slope		0.1141
CC		0.999
Sqaured R		0.9997
Intercept		0.0817

Fig.1 : Liner Graph for Ascorbic acid

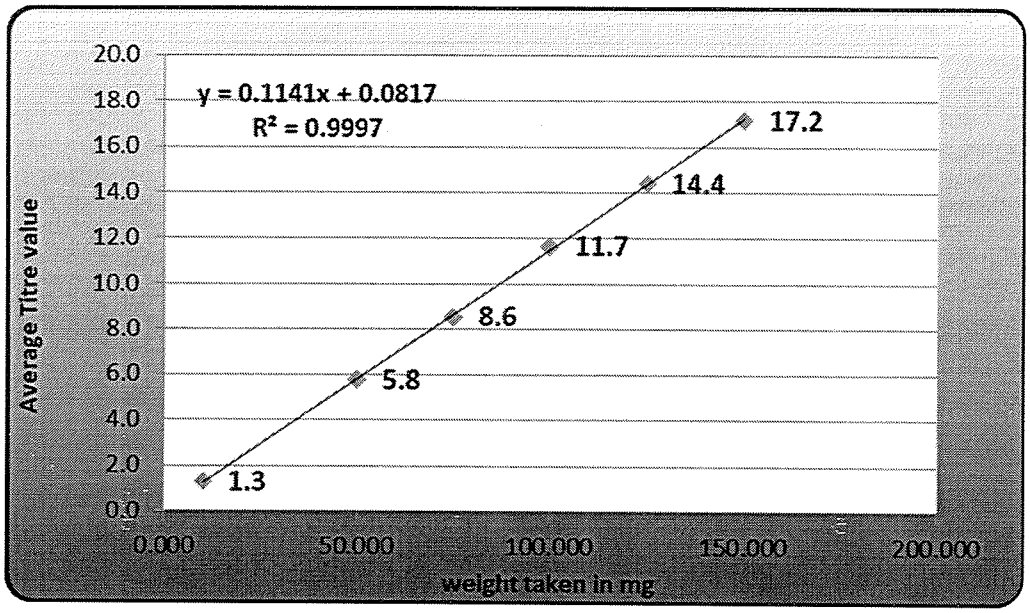

Table:3 Range for Ascorbic acid

Linearity Levels (%)	% RSD for Ascorbic acid
10%	0.000
50%	1.230
75%	0.827
100%	0.607
125%	0.000
150%	0.412

Result and Conclusion:

All the results are well within the acceptance criteria and results indicate that the method is accurate and precise.

9.3 ACCURACY STUDY (RECOVERY STUDY)

Study Summary:

Known quantity of Ascorbic acid working standard were spiked with placebo at three different levels (at level of 50%, 100% and 150% of targeted concentration).

Prepared the recovery samples in triplicate for each level. The samples were analyzed as per the proposed method. The results are tabulated in Table 4 Ascorbic acid respectively to demonstrate the accuracy of the method.

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

Table 4: Accuracy for Ascorbic acid

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	101.53	99.77	1.75
	2	98.02		
	3	99.77		
100%	1	100.85	100.03	0.796
	2	99.97		
	3	99.26		
150%	1	99.96	99.39	1.021
	2	99.99		
	3	98.22		

Result and Conclusion:

All the results are well within the acceptance criteria and results indicate that the method is accurate and precise.

9.4 PRECISION:

(i) Method Precision:

Study summary:

Six Assay preparations of sample were analyzed as per the method. The Assay of Ascorbic acid is calculated. The results are tabulated in Table 5.

Acceptance criteria:

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

Table 5: Method precision for Ascorbic acid

No. of Preparation	Ascorbic acid
1	100.5
2	100.6
3	100.6
4	100.6
5	100.5
6	100.5
Mean	100.6
% RSD	0.05

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for assay values indicates the precision of the analytical method.

(ii) Intermediate Precision (Ruggedness):

Study summary:

Six Assay preparations of sample were analyzed as per the method by different analyst and on different day. The assay of Ascorbic acid is calculated. The results are tabulated in Table 6 and cumulative results are tabulated in Table 7.

Acceptance criteria:

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

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

Table 6: Intermediate precision for Ascorbic acid

No. of Preparation	Ascorbic acid
1	100.6
2	100.6
3	100.6
4	101.4
5	101.4
6	100.6
Mean	100.9
% RSD	0.41

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 7.

Table 7: Cumulative % RSD for Ascorbic acid

Parameter	Ascorbic acid
Method Precision	100.5
	100.6
	100.6
	100.6
	100.5
	100.5
Intermediate Precision	100.6
	100.6
	100.6
	101.4
	101.4
	100.6
Mean	100.7
% RSD	0.32

Result and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for drug release indicates the precision of the method.

10.0 SUMMARY:

No	Validation parameter	Acceptance criteria	Results
1	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	There should not be any interference due to blank and placebo with analyte.	Blank, sample are not interfere with Ascorbic acid in test preparation.
2	**Linearity and Range**	1) R^2 Should be NLT 0.995 2) To conclude the range, %RSD for peak area of linearity level-10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.	**Squared correlation coefficient for** Ascorbic acid:0.9997 **Ascorbic acid:** Level %RSD 10% : 0.000 50% : 1.230 75% : 0.827 100% : 0.607 125% : 0.000 150% : 0.412
3	**Accuracy** (Recovery)	The mean % recovery at each level should be 98.0 to 102.0.	**Ascorbic acid:** Level %Recovery 50% : 99.77 100% : 100.03 150% : 99.39

SUMMARY:

No	Validation parameter	Acceptance criteria	Results
4	**Precision** 1) Method Precision	%RSD of Assay of six preparations should not be more than 2.0	**Ascorbic acid:** 0.05
	2)Intermediate Precision	1) % RSD for assay of six preparations should not be more than 2.0	**Ascorbic acid:** 0.41
		2) Cumulative %RSD for assay of twelve preparations (of method and intermediate precision) should not be more than 2.0.	**Ascorbic acid:** 0.32

11.0 CONCLUSION:

Validation studies have been conducted for Assay of Ascorbic acid in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid powder for the parameters of specificity, Method precision, Intermediate precision, Linearity and range and accuracy, by using the proposed method. The data is compiled and found satisfactory with the analytical method for all the parameters analysed. Hence it is concluded that the method can be used for regular analysis.

12.0 ABBREVIATION:

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

13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVAAR/017	12/12/2022	New Report prepared.