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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
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107.

1.0 INDEX

2.0 PROTOCOL APPROVAL SHEET

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Date	:	06/10/2022
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Signature	:	
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****

ANALYTICAL METHOD VALIDATION

ANALYTICAL METHOD VALIDATION PROTOCOL

FOR

THE TEST OF ASSAY OF PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE

IN

PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE AND ASCORBIC ACID POWDER

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Thirubuvanai, Puducherry-605 107.

1.0 INDEX

INDEX		PAGE No.

2.0 PROTOCOL APPROVAL SHEET

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

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Signature :

Date : 06/10/2022

Reviewed by : **AGM-QC**

Name : M. VIJAYA KUMAR

Signature :

Date : 06/10/2022

Approved by : **GM-QA**

Name : A. G. ICANNAN

Signature :

Date : 07/10/2022

Effective Date : 10/10/2022

3.0 OBJECTIVE:

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

4.0 SCOPE:

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

5.0 GENERAL INFORMATION:

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

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

COMPOSITION : Each 4.5gm sachet contains:

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

BATCH NO : ST/T/S-1322

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

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

VALIDATION TEAM : 1. C.Albin jose

2. L.Parthasarathi

3. R.Vignesh

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, COLUMN, SOLVENTS AND CHEMICALS USED FOR VALIDATION WORK:

Instruments:

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2030C 3D Prominence i

High performance liquid chromatograph with UV visible detector

Make : Shimadzu, Model : LC 2030 Prominence i

Analytical Balance

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

pH:

Make: Eutech instruments, Model No: pH 700

Column:

Inerstil ODS 3V, 250 mm X 4.6 mm, 5µm (or) equivalent

Reagents, chemicals and Working standard with grade:

Paracetamol BP (Working standard)

Phenylephrine Hydrochloride BP (Working standard)

Chlorphenamine Maleate (Working standard)

1-Heptanesulphonic acid sodium salt (AR grade)

Orthophosphoric acid (AR grade)

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

Acetonitrile (HPLC grade)

Methanol (HPLC grade)

8.0 DESCRIPTION OF ANALYTICAL METHOD:

Preparation of Buffer solution:

Weigh and dissolve about 2.0g of 1-heptane sulphonic acid sodium salt in 1000 mL of Milli-Q water. And adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter and degas.

Preparation of Mobile Phase A:

Use buffer solution as mobile phase A.

Preparation of Mobile Phase B:

Use acetonitrile as mobile phase B.

Preparation of Diluent:

Prepare a degassed mixture of buffer and methanol in the ratio of 50:50 *v/v*.

Chromatographic Conditions:

Column	:	Inerstil ODS 3V, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	UV at 220 nm
Column Temperature	:	30°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	50 µL
Run time	:	20 Minutes

Preparation of Blank Solution:

Use diluent as blank.

Note: Keep all the prepared standard and sample solutions on bench top for 10minutes before further using for dilution / filtration.

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	80	20
5.0	80	20
8.0	50	50
14.0	50	50
14.01	80	20
20.0	80	20

Preparation of Standard Stock Solution-1:

Weigh and transfer accurately about 40 mg of Phenylephrine hydrochloride WS into a 200 mL clean, dry volumetric flask. Add 140 mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix.

Preparation of Standard Stock Solution-2:

Weigh and transfer accurately about 40 mg of Chlorphenamine maleate WS and 65mg of Paracetamol WS into a 100 mL clean dry volumetric flask. Add 70 mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix.

Preparation of Standard Solution:

Transfer each 5 mL of standard stock solution-1, standard stock solution-2 and into a 50 mL volumetric flask. Dilute up to the volume with diluent and mix.

Preparation of Sample solution-A (For Phenylephrine & Chlorphenamine maleate):

Transfer and mix the contents of not less than 5 sachets. Weigh and transfer the sample equivalent to 650mg of Paracetamol into a 500 mL volumetric flask add about 340 mL of diluent and sonicate for 20 minutes with intermittent shaking. Cool to room temperature and dilute up to the volume with diluent and mix. Filter through 0.45µm PVDF filter.

Preparation of Sample solution-B (For Paracetamol):

Transfer 5 mL of above Sample solution-A in to a 100 mL volumetric flask and dilute up to the volume with diluent and mix.

Procedure:

Inject diluent as blank solution. Inject Standard solution in five replicates, Inject Sample solution-A and Sample solution-B in duplicates into the chromatograph. Record the chromatograms and measure the responses for the major peaks.

The retention times for Paracetamol, Phenylephrine and Chlorphenamine were about 4.0 minutes, 6.7 minutes and 10.4 minutes respectively and it's for information purpose only.

System suitability:

Theoretical plate	: NLT 2000 for Paracetamol, Phenylephrine and Chlorphenamine peak.
Tailing factor	: NMT 2.0 for Paracetamol, Phenylephrine and Chlorphenamine peak.
Relative standard Deviation	: NMT 2.0% for five replicate standard injection of Paracetamol, Phenylephrine and Chlorphenamine.

Inject 50µl of the above solution as per following sequence.

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (Blank)	1
2	Standard solution	5
3	Sample solution-A	2
4	Sample solution-B	2
5	Bracketing standard	1 Each after every 6 sample injection

Calculate the assay of Paracetamol in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Paracetamol in Sample solution B.

AS = Average area of peak due to Paracetamol in standard preparation.

WS = Weight of Paracetamol working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Paracetamol working standard in % on as such basis.

Calculate the assay of Paracetamol in % as follows:

$$= \frac{mg/sachet}{LC} \times 100$$

LC = Label claim of Paracetamol in mg/sachet.

Calculate the assay of Phenylephrine Hydrochloride in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Phenylephrine Hydrochloride in Sample solution A.

AS = Average area of peak due to Phenylephrine Hydrochloride in standard preparation.

WS = Weight of Phenylephrine Hydrochloride working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Phenylephrine Hydrochloride working standard in % on as such basis.

Calculate the assay of Phenylephrine Hydrochloride in % as follows:

$$= \frac{\text{mg/sachet}}{\text{LC}} \times 100$$

LC = Label claim of Phenylephrine Hydrochloride in mg/sachet.

Calculate the assay of Chlorphenamine maleate in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Chlorphenamine maleate in Sample solution A.

AS = Average area of peak due to Chlorphenamine maleate in standard preparation.

WS = Weight of Chlorphenamine maleate working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Chlorphenamine maleate working standard in % on as such basis.

Calculate the assay of Chlorphenamine maleate in % as follows:

$$= \frac{\text{mg/sachet}}{\text{LC}} \times 100$$

LC = Label claim of Chlorphenamine maleate in mg/sachet.

9.0 VALIDATION RESULTS:

9.1 SYSTEM SUITABILITY TEST:

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 (Diluent)	1
2	Standard preparation	5

Evaluate the following system suitability parameters:

1) % RSD of area of Paracetamol, Phenylephrine and Chlorphenamine peak in five replicate standard injections.

2) Theoretical plates for Paracetamol, Phenylephrine and Chlorphenamine peak in standard injection.

3) Tailing factor for Paracetamol, Phenylephrine and Chlorphenamine peak in standard injection.

Acceptance Criteria:

1) % RSD of area for Paracetamol, Phenylephrine and Chlorphenamine peak in five replicate standard injections should not more than 2.0%.

2) Theoretical plates for Paracetamol, Phenylephrine and Chlorphenamine peak in standard injection should not less than 3000.

3) Tailing factor for Paracetamol, Phenylephrine and Chlorphenamine peak in standard injection should not more than 2.0.

9.2 SPECIFITY:

"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 (Diluent)	1
2	Standard preparation	5
3	Plain placebo	1
4	Paracetamol working Standard	1
5	Phenylephrine HCL working Standard	1
6	Chlorphenamine Maleate working Standard	1
7	Plain placebo with Paracetamol	1
8	Plain placebo with Phenylephrine Hcl	1
9	Plain placebo with Chlorphenamine Maleate	1
10	Plain placebo with Chlorphenamine, Phenylephrine Hcl and Paracetamol	1
11	Test preparation-Soln-A	1
12	Test preparation-Soln-B	1

Acceptance criteria:

i) There should not be any interference due to blank, Placebo peak with analyte.
ii) Peak purity should not be less than 0.995 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 give 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% to 150% of targeted concentration.

Sequence shall be in following provisional manner.

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

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 peak area of linearity level of 10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.

9.4 INTERFERENCE FROM DEGRADANT (forced degradation)

Study design:

To evaluate the interference from degradants, carry out a forced degradation study by stressing the test preparation under the following maximum stress conditions.

Degradation	Stress Condition
Acid degradation	Exposure to 5ml of 5N HCL and Heat on water bath at 80°C for 30 minutes.
Alkali degradation	Exposure to 5ml of 5N NaOH and Heat on water bath at 80°C for 30 minutes.
Oxidative degradation	Exposure to 5ml of 30% H_2O_2 and Heat on water bath at 80°C for 30 minutes.

Sequence shall be in following provisional manner, For forced chemical degradation:

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample Solution (A&B) (As such)	2
4	Sample Solution (A&B) (Acid degradation)	2
5	Sample Solution (A&B) (Alkali degradation)	2
6	Sample Solution (A&B) (Oxidative degradation)	2
7	Standard preparation (Bracketing)	1

Chromatograph the samples of chemical and physical forced degradation into HPLC system equipped with diode array detector and evaluate the peak purity for the analytes in stressed samples and the degradation profiles under each stressed condition.

Acceptance Criteria:

1) There should not be any interference due to degradants with analyte in stressed samples.

2) The desired degradation should be 10-30% in acid, alkali and oxidative degradation, (if possible).

3) If about 10% to 30% degradation is not achieved by applying above stressed condition, same shall be documented and reported.

4) Peak purity should not be less than 0.950 according to Lab solution software.

9.5 ACCURACY STUDY (RECOVERY STUDY)

"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

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Blank (Diluent)	1
4	Level – 1 Set – 1 (50%)	1
5	Level – 1 Set – 2 (50%)	1
6	Level – 1 Set – 3 (50%)	1
7	Blank (Diluent)	1
8	Level – 2 Set – 1 (100%)	1
9	Level – 2 Set – 2 (100%)	1
10	Level – 2 Set – 3 (100%)	1
11	Blank (Diluent)	1
12	Level – 3 Set – 1 (150%)	1
13	Level – 3 Set – 2 (150%)	1
14	Level – 3 Set – 3 (150%)	1
15	Standard preparation (Bkt)	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.

9.6 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) 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 (Diluent)	1
2	Standard preparation	5

Acceptance criteria:

% RSD of area of analyte peak in five replicate standard injections should not be more than 2.0.

(ii) 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 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.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample Solution (A&B) -1	2
4	Sample Solution (A&B) -2	2
5	Sample Solution (A&B) -3	2
6	Sample Solution (A&B) -4	2
7	Sample Solution (A&B) -5	2
8	Sample Solution (A&B) -6	2
9	Standard preparation (BKT)	1 (after six sample injection)

Acceptance criteria:

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

(iii) Intermediate Precision (Ruggedness):

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. Analyse six sample 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.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample Solution (A&B) -1	2
4	Sample Solution (A&B) -2	2
5	Sample Solution (A&B) -3	2
6	Sample Solution (A&B) -4	2
7	Sample Solution (A&B) -5	2
8	Sample Solution (A&B) -6	2
9	Standard preparation (BKT)	1 (after six sample injection)

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.

9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Prepare Standard and sample solution as per the methodology and store at Ambient temperature. Chromatograph this solution at regular intervals for 48 hours by using same diluent. Calculate the % difference of analyte peak area for standard and test preparations with that of initial. The study may be stopped if 2 consecutive failure of sample solution.

Sequence shall be in following provisional

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Standard preparation(A&B) (Initial)	1
4	Sample solution (A&B) (Initial)	1
5	Standard preparation (Time interval)	1
6	Sample solution (A&B) (Time interval)	1

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%.

9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering test solution through 0.45µ PVDF filter against that of unfiltered.

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank	1
2	Standard preparation	5
3	Sample solution (A&B) –Unfiltered (Centrifuge)	1
4	Sample solution (A&B) –Filter Set 1 (0.45µ PVDF filter)	1
5	Sample solution (A&B) –Filter Set 2 (0.45µ PVDF filter)	1
6	Sample solution (A&B) –Filter Set 3 (0.45µ PVDF filter)	1
8	Standard preparation	1

Acceptance criteria:

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

9.9 ROBUSTNESS:

Purpose:

To establish the robustness of the analytical method.

Study Design:

The robustness of the analytical method can be established 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 (Diluent)	1
2	Standard preparation	5
3	Sample solution (A&B)	2
4	Bracketing standard	1

According to each variable		
S.No.	**Description of solution**	**No. of Injections**
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample solution (A&B)	2
4	Bracketing standard	1

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

a) Flow rate change by ±10% mean (i.e 1.1 ml/min and 1.3 ml/minute)

b) Wave length change by ± 3nm (i.e. 217nm and 223nm)

c) Column oven Temperature change by ± 5.0 (i.e. 25°C and 35°C)

Acceptance criteria:

System suitability should comply for each variable and % of drug not differ ±2% from mean assay value of method precision.

10.0 PARAMETERS TO BE VALIDATED:

No	Validation parameters
1.	System suitability
2.	Specificity (Selectivity) (i) Interference from blank and placebo (ii) Interference from degradants (Forced degradation) a) Acid degradation b) Alkali degradation c) Oxidative degradation
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 a) Flow rate change b) Wavelength c) Temperature change

Note: More than one parameter can be performed at once with relevant sequence having common system suitability with bracketing standard.

11.0 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
NaoH	:	Sodium Hydroxide
H_2O_2	:	Hydrogen Peroxide

12.0 REVISION HISTORY:

Protocol No.	Effective date	Reason for Review
ST/AMVAP/017	10/10/2022	New Protocol prepared.

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

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.

INDEX

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

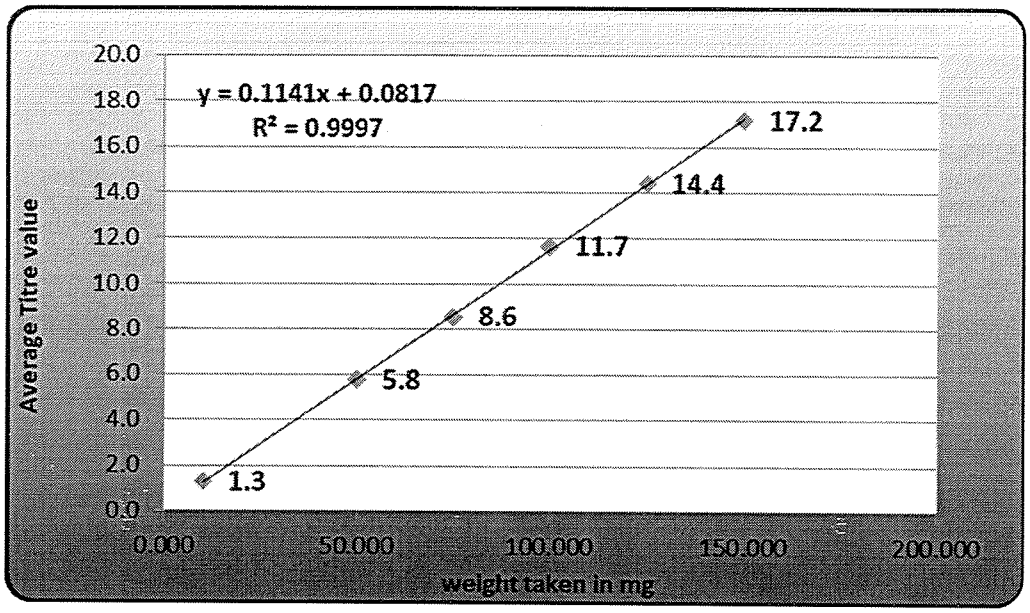

Table:3 Range for Ascorbic acid

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.

ANALYTICAL METHOD VALIDATION

ANALYTICAL METHOD VALIDATION REPORT

FOR

THE TEST OF ASSAY OF PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE

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.

INDEX

S.NO	INDEX	PAGE No.

2.0 REPORT APPROVAL SHEET

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

Name : K. SARAVANAN

Signature :

Date : 09/12/2022

Reviewed by : **AGM-QC**

Name : M. V. JAYAKUMAR

Signature :

Date : 09/12/2022

Approved by : **GM-QA**

Name : A. G. ICANNAN

Signature :

Date : 10/12/2022

Effective Date : 12/12/2022

3.0 OBJECTIVE:

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

4.0 SCOPE:

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

5.0 GENERAL INFORMATION:

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST VALIDATED : Assay of Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate 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/S-1322

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

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

VALIDATION TEAM : 1. C.Albin jose

2. T.Maruthi

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, COLUMN, SOLVENTS AND CHEMICALS USED FOR VALIDATION WORK:

Instruments:

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2030C 3D Prominence i

High performance liquid chromatograph with UV visible detector

Make : Shimadzu, Model : LC 2030 Prominence i

Analytical Balance

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

pH:

Make: Eutech instruments, Model No: pH 700

Column:

Inerstil ODS 3V, 250 mm X 4.6 mm, 5µm (or) equivalent

Reagents, chemicals and Working standard with grade:

Paracetamol (Working standard)

Phenylephrine Hydrochloride (Working standard)

Chlorphenamine Maleate (Working standard)

1-Heptanesulphonic acid sodium salt (AR grade)

Orthophosphoric acid (AR grade)

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

Acetonitrile (HPLC grade)

Methanol (HPLC grade)

8.0 DESCRIPTION OF ANALYTICAL METHOD:

Preparation of Buffer solution:

Weigh and dissolve about 2.0g of 1-heptane sulphonic acid sodium salt in 1000 mL of Milli-Q water. And adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter and degas.

Preparation of Mobile Phase A:

Use buffer solution as mobile phase A.

Preparation of Mobile Phase B:

Use acetonitrile as mobile phase B.

Preparation of Diluent:

Prepare a degassed mixture of buffer and methanol in the ratio of 50:50 *v/v*.

Chromatographic Conditions:

Column	:	Inerstil ODS 3V, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	UV at 220 nm
Column Temperature	:	30°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	50 µL
Run time	:	20 Minutes

Preparation of Blank Solution:

Use diluent as blank.

Note: Keep all the prepared standard and sample solutions on bench top for 10minutes before further using for dilution / filtration.

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	80	20
5.0	80	20
8.0	50	50
14.0	50	50
14.01	80	20
20.0	80	20

Preparation of Standard Stock Solution-1:

Weigh and transfer accurately about 40 mg of Phenylephrine hydrochloride WS into a 200 mL clean, dry volumetric flask. Add 140 mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix.

Preparation of Standard Stock Solution-2:

Weigh and transfer accurately about 40 mg of Chlorphenamine maleate WS and 65mg of Paracetamol WS into a 100 mL clean dry volumetric flask. Add 70 mL of diluent and sonicate to dissolve. Dilute up to the volume with diluent and mix.

Preparation of Standard Solution:

Transfer each 5 mL of standard stock solution-1, standard stock solution-2 and into a 50 mL volumetric flask. Dilute up to the volume with diluent and mix.

Preparation of Sample solution-A (For Phenylephrine & Chlorphenamine maleate):

Transfer and mix the contents of not less than 5 sachets. Weigh and transfer the sample equivalent to 650mg of Paracetamol into a 500 mL volumetric flask add about 340 mL of diluent and sonicate for 20 minutes with intermittent shaking. Cool to room temperature and dilute up to the volume with diluent and mix. Filter through 0.45µm PVDF filter.

Preparation of Sample solution-B (For Paracetamol):

Transfer 5 mL of above Sample solution-A in to a 100 mL volumetric flask and dilute up to the volume with diluent and mix.

Procedure:

Inject diluent as blank solution. Inject Standard solution in five replicates, Inject Sample solution-A and Sample solution-B in duplicates into the chromatograph. Record the chromatograms and measure the responses for the major peaks.

The retention times for Paracetamol, Phenylephrine and Chlorphenamine were about 4.0 minutes, 6.7 minutes and 10.4 minutes respectively and it's for information purpose only.

System suitability:

Theoretical plate	: NLT 2000 for Paracetamol, Phenylephrine and Chlorphenamine peak.
Tailing factor	: NMT 2.0 for Paracetamol, Phenylephrine and Chlorphenamine peak.
Relative standard Deviation	: NMT 2.0% for five replicate standard injection of Paracetamol, Phenylephrine and Chlorphenamine.

Inject 50µl of the above solution as per following sequence.

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (Blank)	1
2	Standard solution	5
3	Sample solution-A	2
4	Sample solution-B	2
5	Bracketing standard	1 Each after every 6 sample injection

Calculate the assay of Paracetamol in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Paracetamol in Sample solution B.

AS = Average area of peak due to Paracetamol in standard preparation.

WS = Weight of Paracetamol working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Paracetamol working standard in % on as such basis.

Calculate the assay of Paracetamol in % as follows:

$$= \frac{mg/sachet}{LC} \times 100$$

LC = Label claim of Paracetamol in mg/sachet.

Calculate the assay of Phenylephrine Hydrochloride in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Phenylephrine Hydrochloride in Sample solution A.

AS = Average area of peak due to Phenylephrine Hydrochloride in standard preparation.

WS = Weight of Phenylephrine Hydrochloride working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Phenylephrine Hydrochloride working standard in % on as such basis.

Calculate the assay of Phenylephrine Hydrochloride in % as follows:

$$= \frac{\text{mg/sachet}}{\text{LC}} \times 100$$

LC = Label claim of Phenylephrine Hydrochloride in mg/sachet.

Calculate the assay of Chlorphenamine maleate in mg/sachet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{500}{WT} \times \frac{P}{100} \times AFW$$

Where,

AT = Average area of peak due to Chlorphenamine maleate in Sample solution A.

AS = Average area of peak due to Chlorphenamine maleate in standard preparation.

WS = Weight of Chlorphenamine maleate working standard in mg.

WT = Weight of sample taken in mg.

AFW = Average fill weight of sachet in mg.

P = Potency of Chlorphenamine maleate working standard in % on as such basis.

Calculate the assay of Chlorphenamine maleate in % as follows:

$$= \frac{\text{mg/sachet}}{\text{LC}} \times 100$$

LC = Label claim of Chlorphenamine maleate in mg/sachet.

9.0 VALIDATION RESULTS:

9.1 SYSTEM SUITABILITY:

Study Summary:

Five replicates of standard preparation were injected into HPLC and following system suitability parameters are evaluated.

1) Theoretical plate for Paracetamol, Phenylephrine and Chlorphenamine peaks.

2) Tailing Factor for Paracetamol, Phenylephrine and Chlorphenamine peaks.

3) % RSD of area of five replicate standard injections

Results are tabulated in Table 1.

Table 1: System suitability for Paracetamol, Phenylephrine and Chlorphenamine

System Suitability Parameter	Limit	Paracetamol	Phenylephrine	Chlorphenamine
Theoretical Plates	NLT 2000	4758	7245	78548
Tailing Factor	NMT 2.0	1.267	1.209	1.468
% RSD	NMT 2.0	0.049	0.031	0.307

Result and Conclusion:

The System suitability test result are well within the acceptance criteria and the study concludes the suitability of analytical system for the analysis.

9.2 SPECIFICITY

Interference from blank and placebo

Study Summary:

Blank, standard, placebo and placebo spiked with analyte and sample were analyzed as per the method to examine the interference of blank and placebo with Paracetamol, Phenylephrine and Chlorphenamine peaks.

Peak purity of the analyte peak and the representative chromatograms of blank, standard, placebo, placebo spiked with analyte and sample are attached.

Results are tabulated in Table 2.

Acceptance criteria:

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

2) Peak purity should not less than 0.995 accordingly to lab solution software.

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Blank	No Peak	No Peak	Not Applicable
2	Standard preparation	Paracetamol	3.801	1.000
		Phenylephrine HCL	6.795	1.000
		Chlorphenamine Maleate	10.886	1.000
3	Plain placebo	No Peak	No Peak	Not Applicable
4	Paracetamol	Paracetamol	3.811	1.000
5	Phenylephrine HCL	Phenylephrine HCL	6.799	1.000
6	Chlorphenamine Maleate	Chlorphenamine Maleate	10.889	1.000
7	Plain placebo with Paracetamol	Paracetamol	3.808	0.999
8	Plain placebo with Phenylephrine Hcl	Phenylephrine HCL	6.781	1.000
9	Plain placebo with Chlorphenamine Maleate	Chlorphenamine Maleate	10.889	1.000

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
10	Plain placebo with Chlorphenamine, Phenylephrine Hcl and Paracetamol	Paracetamol	3.811	1.000
		Phenylephrine HCL	6.741	1.000
		Chlorphenamine Maleate	10.878	1.000
11	Test preparation-Solution-A	Chlorphenamine Maleate	10.890	1.000
		Phenylephrine HCL	6.760	1.000
12	Test preparation-Solution-B	Paracetamol	3.811	0.999

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with Paracetamol, Phenylephrine and Chlorphenamine peak in test preparation and Peak purity passes within specified limits. Hence method is selective and specific.

9.3 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Paracetamol, Phenylephrine and Chlorphenamine 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%). Replicate injections of these solutions are injected and checked for Linearity and Range. The results are tabulated in Table 3A, 3B, 3C for Linearity and Table 4 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 3A: Linearity Table for Paracetamol

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	6.558	538484
50%	32.788	2648249
75%	49.182	3968435
100%	65.576	5220671
125%	81.970	6506158
150%	98.364	7847050
Slope		79280
CC		0.999
Sqaured R		0.9999
Intercept		35787

Fig.1 : Liner Graph for Paracetamol

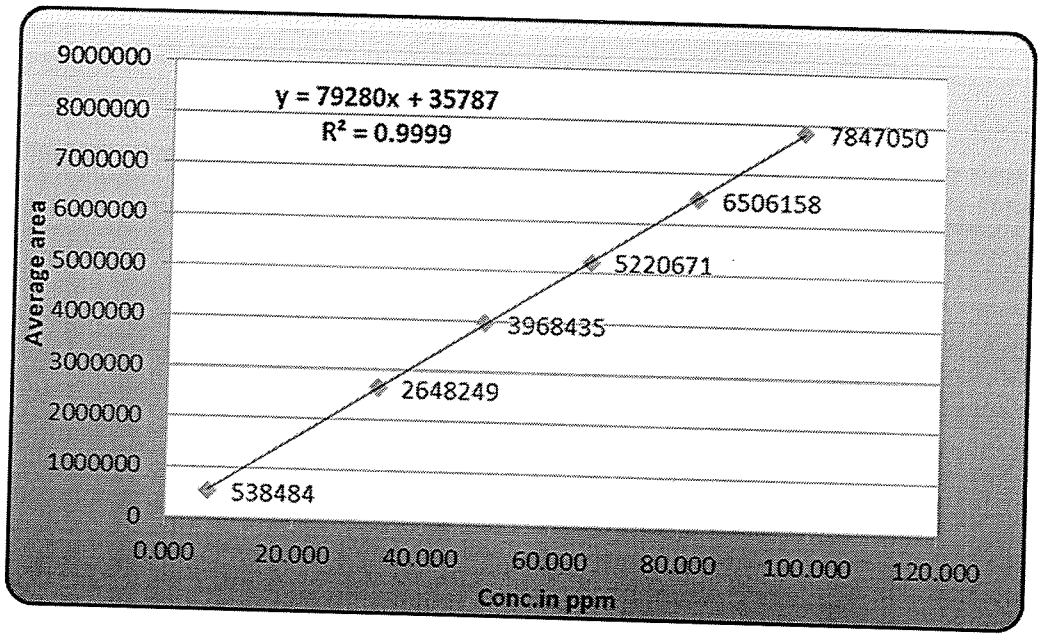

Table 3B: Linearity Table for Phenylephrine

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	2.062	125566
50%	10.308	636387
75%	15.462	956900
100%	20.616	1263170
125%	25.770	1577613
150%	30.924	1905851
Slope		61443
CC		0.999
Sqaured R		0.9999
Intercept		876.68

Fig.2 : Liner Graph for Phenylephrine

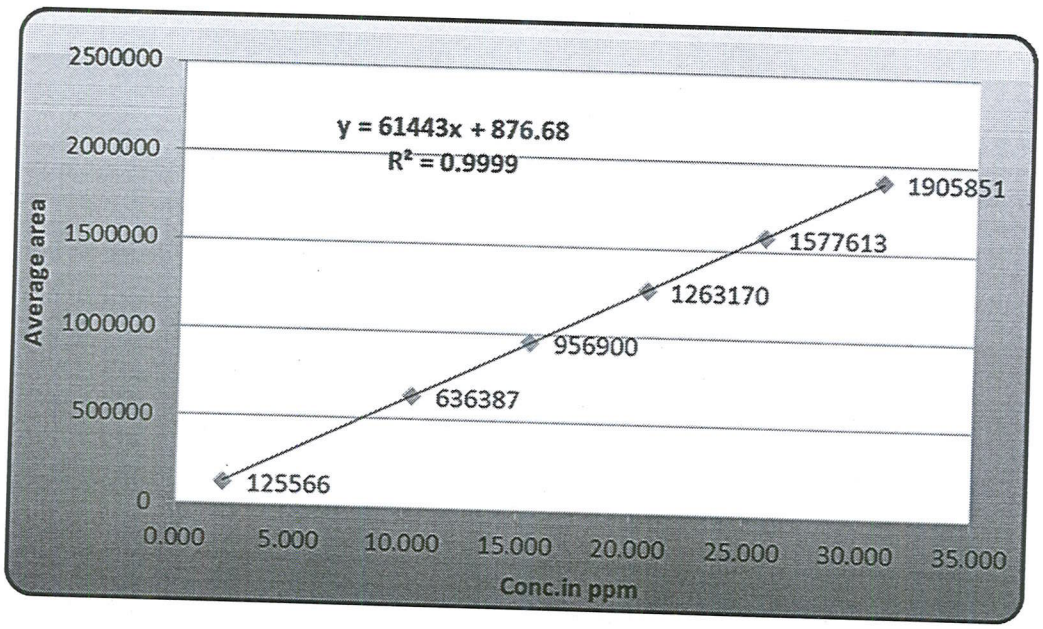

Table 3C: Linearity Table for Chlorphenamine

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	4.045	279647
50%	20.224	1404644
75%	30.336	2107856
100%	40.448	2775477
125%	50.560	3452694
150%	60.672	4167849
Slope		68357
CC		0.999
Sqaured R		0.9999
Intercept		14539

Fig.3 : Liner Graph for Chlorphenamine

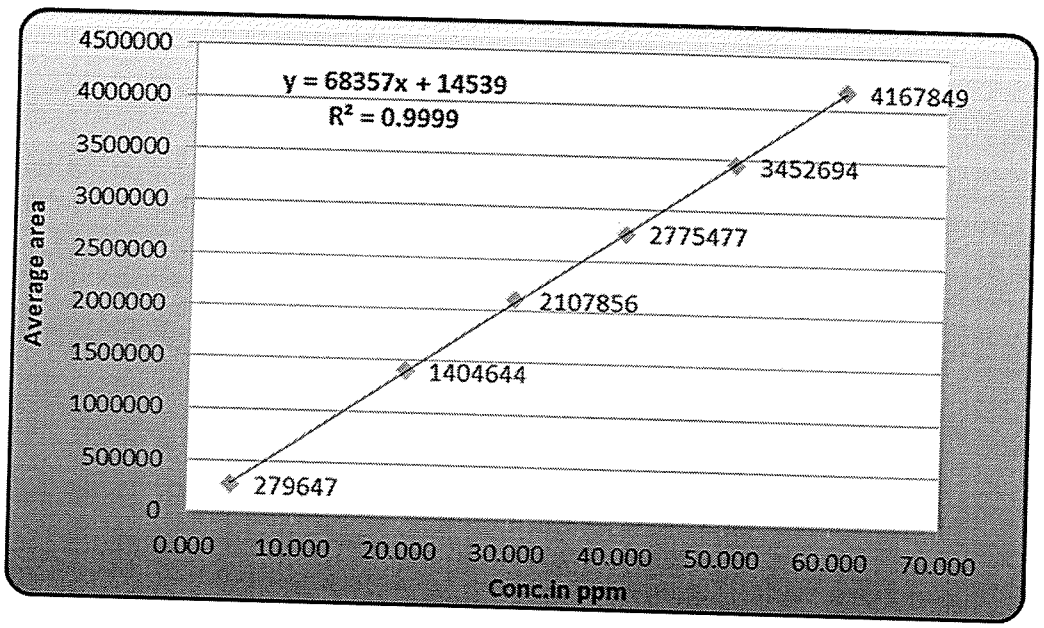

Table:4 Range for Paracetamol, Phenylephrine and Chlorphenamine

Linearity Levels (%)	% RSD for Paracetamol	% RSD for Phenylephrine	% RSD for Chlorphenamine
10%	0.019	0.118	0.181
50%	0.039	0.113	0.027
75%	0.088	0.055	0.115
100%	0.029	0.011	0.050
125%	0.062	0.126	0.039
150%	0.016	0.207	0.191

Result and Conclusion:

Squared correlation coefficient and Range, %RSD of areas at 10%, 50%, 75%, 100%, 125 & 150% levels within limits.

9.4 INTERFERENCE FROM DEGRADANTS (Forced degradation)

In order to prove specificity of method, further degradation was carried out and peak purity of Paracetamol, Phenylephrine HCL and Chlorphenamine peak was monitored.

a) Acid Degradation:

Solution A:

Transfer and mix the contents of not less than 5 sachets. Weigh and transfer the sample equivalent to 650mg of Paracetamol into a 500 mL volumetric flask add about 340 mL of diluent and sonicate for 20 minutes with intermittent shaking. Add 5ml of 5N Hydrochloric acid and sonicate for 20minutes with intermittent shaking Cool and neutralized with 5ml of 5N Sodium hydroxide and Dilute to volume with diluent and mix. Filter through 0.45µ PVDF filter paper.

Solution B:

Transfer 5ml of solution A in to a 100ml volumetric flask and dilute up to volume with diluent and mix.

b) Alkali degradation:

Solution A:

Transfer and mix the contents of not less than 5 sachets. Weigh and transfer the sample equivalent to 650mg of Paracetamol into a 500 mL volumetric flask add about 340 mL of diluent and sonicate for 20 minutes with intermittent shaking. Add 5ml of 5N Sodium hydroxide and sonicate for 20minutes with intermittent shaking Cool and neutralized with 5ml of 5N Sodium hydroxide and Dilute to volume with diluent and mix. Filter through 0.45µ PVDF filter paper.

Solution B:

Transfer 5ml of solution A in to a 100ml volumetric flask and dilute up to volume with diluent and mix.

c) Oxidative Degradation:

Solution A:

Transfer and mix the contents of not less than 5 sachets. Weigh and transfer the sample equivalent to 650mg of Paracetamol into a 500 mL volumetric flask add about 340 mL of diluent and sonicate for 20 minutes with intermittent shaking. Add 5ml of 30% Hydrogen peroxide solution and sonicate for 20minutes with intermittent shaking Cool and dilute to volume with diluent and mix. Filter through 0.45µ PVDF filter paper.

Solution B:

Transfer 5ml of solution A in to a 100ml volumetric flask and dilute up to volume with diluent and mix.

Acceptance criteria:

i) There should not be any interference due to degradants with analyte in stressed sample.

ii) The desired degradation should be 10-30% in acid, alkali and oxidation degration, (if possible).

iii) If about 10% to 30% degradation is not achieved by applying above stressed condition. Same shall be documented and reported.

iv) Peak purity should not be less than 0.950 according to Lab solution software.

Table 5: Peak purity (Chemical degradation)

S.No	Sample name	Peak name	Assay in (%)	Degradation in %	Peak purity index
1	Sample as such	Paracetamol	99.1	NA	1.000
		Phenylephrine Hcl	97.3	NA	1.000
		Chlorphenamine	101.2	NA	1.000
2	Sample – Acid 5ml of 5N Hydrochloric acid and sonicate for 20minutes	Paracetamol	93.8	5.3	1.000
		Phenylephrine Hcl	64.4	32.9	1.000
		Chlorphenamine	94.7	6.5	1.000
3	Sample – Base 5ml of 5N Sodium hydroxide and sonicate for 20minutes	Paracetamol	92.3	6.8	1.000
		Phenylephrine Hcl	62.2	35.1	1.000
		Chlorphenamine	96.5	4.7	0.999
4	Sample – peroxide 5ml 30% Hydrogen peroxide and sonicate for 20minutes	Paracetamol	93.9	5.2	1.000
		Phenylephrine Hcl	96.6	0.7	1.000
		Chlorphenamine	90.0	11.2	1.000

Result and Conclusion:

There is No any interference due to degradants with analyte in stressed samples and Peak purity was passes According to Lab solution.

9.5 ACCURACY STUDY (RECOVERY STUDY)

Study Summary:

Known quantity of Paracetamol, Phenylephrine HCL and Chlorphenamine 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 6A, 6B and 6C for Paracetamol, Phenylephrine HCL and Chlorphenamine respectively to demonstrate the accuracy of the method.

The mean % recovery at each level for Paracetamol, Phenylephrine HCL and Chlorphenamine should be 98.0 to 102.0.

Table 6A: Accuracy for Paracetamol

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	98.65	*98.66*	0.070
	2	98.73		
	3	98.59		
100%	1	99.01	98.93	0.165
	2	98.75		
	3	99.04		
150%	1	98.10	98.32	0.317
	2	98.18		
	3	98.68		

Table 6B: Accuracy for Phenylephrine HCl

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	101.15	101.58	0.414
	2	101.59		
	3	102.00		
100%	1	99.32	98.95	0.360
	2	98.60		
	3	98.94		
150%	1	101.48	101.26	0.289
	2	101.38		
	3	100.93		

Table 6C: Accuracy for Chlorphenamine

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	98.22	98.76	0.534
	2	98.78		
	3	99.28		
100%	1	100.59	100.47	0.674
	2	99.74		
	3	101.08		
150%	1	98.29	98.66	0.470
	2	98.52		
	3	99.18		

Result and Conclusion:

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

9.6 PRECISION:

(i) System precision

Study summary:

Five replicate injections of standard preparation were injected into the HPLC system. The area response for Paracetamol, Phenylephrine and Chlorphenamine Peaks along with % RSD are tabulated in Table 7

Acceptance criteria:

% RSD of area of analyte peak in Five replicate standard injections should not be more than 2.0.

Table 7: System precision

Injection No.	Paracetamol	Phenylephrine	Chlorphenamine
1	5071091	1227523	2710886
2	5069883	1228106	2719096
3	5069892	1227976	2712960
4	5075239	1227441	2719453
5	5072826	1227183	2710557
Mean	5071786	1227646	2714590
% RSD	0.045	0.031	0.161

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for the replicate injections indicates the system precision of HPLC system used.

(ii) Method Precision:

Study summary:

Six Assay preparations of sample were analyzed as per the method. The Assay of Paracetamol, Phenylephrine and Chlorphenamine is calculated. The results are tabulated in Table 8.

Acceptance criteria:

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

Table 8: Method precision for Paracetamol, Phenylephrine HCL and Chlorphenamine

No. of Preparation	Paracetamol	Phenylephrine	Chlorphenamine
1	101.0	95.2	99.7
2	98.7	96.0	98.7
3	97.4	97.3	103.2
4	98.9	99.0	101.5
5	99.5	98.2	101.9
6	98.8	98.0	102.0
Mean	99.1	97.3	101.2
% RSD	1.189	1.475	1.637

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.

(iii) Intermediate Precision (Ruggedness):

Study summary:

Six Assay preparations of sample were analyzed as per the method by different analyst using different instrument and different column on different day. The assay of Paracetamol, Phenylephrine and Chlorphenamine is calculated. The results are tabulated in Table 9 and cumulative results are tabulated in Table 10.

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 9: Intermediate precision for Paracetamol, Phenylephrine and Chlorphenamine

No. of Preparation	Paracetamol	Phenylephrine	Chlorphenamine
1	97.2	96.2	99.6
2	97.9	98.6	98.0
3	97.6	101.9	98.6
4	97.5	99.6	98.2
5	97.4	99.7	99.3
6	97.7	98.8	97.5
Mean	97.6	99.1	98.5
% RSD	0.249	1.871	0.811

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 10.

Table 10: Cumulative % RSD for Paracetamol, Phenylephrine and Chlorphenamine

Parameter	Paracetamol	Phenylephrine	Chlorphenamine
Method Precision	101.0	95.2	99.7
	98.7	96.0	98.7
	97.4	97.3	103.2
	98.9	99.0	101.5
	99.5	98.2	101.9
	98.8	98.0	102.0
Intermediate Precision	97.2	96.2	99.6
	97.9	98.6	98.0
	97.6	101.9	98.6
	97.5	99.6	98.2
	97.4	99.7	99.3
	97.7	98.8	97.5
Mean	98.3	98.2	99.9
% RSD	1.147	1.886	1.854

Result and Conclusion:

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

9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Sample solution:

Sample preparation were prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 11A, 11B and 11C.

Table 11A: Stability of sample solution for Paracetamol

Time in hours	Area of Paracetamol peak	Absolute % Difference
Initial	4925079	Not applicable
2	4925345	-0.01
4	4928358	-0.07
6	4930168	-0.10
8	4932849	-0.16
10	4929737	-0.09
12	4908573	0.34
16	4947677	-0.46
20	4982290	-1.15
24	4966235	-0.83
28	4936853	-0.24
32	4958588	-0.68
36	4947666	-0.46
40	4959520	-0.69
44	4966015	-0.82
Mean	4942997	-0.39
% RSD	0.408	Not applicable

Table 11B: Stability of sample solution for Phenylephrine HCL

Time in hours	Area of Phenylephrine HCL peak	Absolute % Difference
Initial	1165981	Not applicable
2	1165416	0.05
4	1165174	0.07
6	1162951	0.26
8	1161638	0.37
10	1161012	0.43
12	1153456	1.09
16	1161420	0.39
20	1161276	0.41
24	1162495	0.30
28	1161262	0.41
32	1162011	0.34
36	1159390	0.57
40	1161765	0.36
44	1162439	0.30
Mean	1161846	0.38
% RSD	0.253	Not applicable

Table 11C: Stability of sample solution for Chlorphenamine

Time in hours	Area of Chlorphenamine peak	Absolute % Difference
Initial	2670400	Not applicable
2	2671178	-0.03
4	2669563	0.03
6	2660606	0.37
8	2663302	0.27
10	2668062	0.09
12	2664088	0.24
16	2652937	0.66
20	2653169	0.65
24	2651393	0.72
28	2660799	0.36
32	2658650	0.44
36	2645749	0.93
40	2664706	0.21
44	2652746	0.67
Mean	2660490	0.40
% RSD	0.295	Not applicable

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

Standard solution:

Standard preparation were prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 12A, 12B and 12C.

Table 12A: Stability of standard solution for Paracetamol

Time in hours	Area of Paracetamol peak	Absolute % Difference
Initial	3223629	Not applicable
2	3228202	-0.14
4	3227028	-0.11
6	3226423	-0.09
8	3226648	-0.09
10	3223569	0.00
12	3220281	0.10
16	3234620	-0.34
20	3239548	-0.49
24	3233798	-0.31
28	3231066	-0.23
32	3233265	-0.30
36	3224184	-0.02
40	3225424	-0.06
44	3224404	-0.02
Mean	3228139	-0.15
% RSD	0.163	Not applicable

Table 12B: Stability of standard solution for Phenylephrine HCl

Time in hours	Area of Phenylephrine HCl peak	Absolute % Difference
Initial	1219178	Not applicable
2	1223704	-0.37
4	1220297	-0.09
6	1217218	0.16
8	1219763	-0.05
10	1217414	0.14
12	1220624	-0.12
16	1219779	-0.05
20	1225065	-0.48
24	1227808	-0.70
28	1225894	-0.55
32	1226790	-0.62
36	1221998	-0.23
40	1222884	-0.30
44	1224314	-0.42
Mean	1222182	-0.26
% RSD	0.273	Not applicable

Table 12C: Stability of standard solution for Chlorphenamine Maleate

Time in hours	Area of Chlorphenamine Maleate peak	Absolute % Difference
Initial	2747054	Not applicable
2	2748152	-0.04
4	2744905	0.08
6	2750153	-0.11
8	2750671	-0.13
10	2740710	0.23
12	2741408	0.21
16	2761349	-0.52
20	2757052	-0.36
24	2756651	-0.35
28	2747570	-0.02
32	2756336	-0.34
36	2755310	-0.30
40	2751333	-0.16
44	2753930	-0.25
Mean	2750839	-0.15
% RSD	0.218	Not applicable

Results and conclusions:

The Standard solution and Sample solution was stable upto 44 hours at ambient temperature.

9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method was performed by filtering test solution through 0.45µ PVDF membrane filter against that of unfiltered sample. The results were tabulated in Table 13A, 13B and 13C.

Table 13A: Filter paper study for Sample solution of Paracetamol

Filter study	Assay in (%)	% difference from unfiltered sample
UNFILTERED SAMPLE (CENTRIFUGED)	96.8	Not applicable
FILTER SET-I (0.45µ PVDF FILTER)	96.6	0.21
FILTER SET-II (0.45µ PVDF FILTER)	95.7	1.15
FILTER SET-III (0.45µ PVDF FILTER)	96.3	0.52

Table 13B: Filter paper study for Sample solution of Phenylephrine HCL

Filter study	Assay in (%)	% difference from unfiltered sample
UNFILTERED SAMPLE (CENTRIFUGED)	103.4	Not applicable
FILTER SET-I (0.45µ PVDF FILTER)	103.4	0.00
FILTER SET-II (0.45µ PVDF FILTER)	103.2	0.19
FILTER SET-III (0.45µ PVDF FILTER)	103.5	-0.10

Table 13C: Filter paper study for Sample solution of Chlorphenamine Maleate

Filter study	Assay in (%)	% difference from unfiltered sample
UNFILTERED SAMPLE (CENTRIFUGED)	99.2	Not applicable
FILTER SET-I (0.45µ PVDF FILTER)	98.7	0.51
FILTER SET-II (0.45µ PVDF FILTER)	98.8	0.40
FILTER SET-III (0.45µ PVDF FILTER)	98.7	0.51

Acceptance criteria:

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

Results and conclusions:

The % difference on filtered sample (0.45µ PVDF) within limit against that of unfiltered.

9.9 ROBUSTNESS:

Study Summary:

Five replicate injections of standard preparation and duplicate injections of test preparation were injected varying different chromatographic conditions as per protocol. System suitability parameters and mean assay difference with respect to assay value in method precision were calculated. The results are tabulated in table 14A, 14B and 14C Paracetamol, phenylephrine HCL and Chlorphenamine peaks respectively.

Table 14A: Robustness of analytical method for Paracetamol

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Flow rate 1.1ml/min	5140	1.260	0.340	99.1	97.9	1.20
Flow rate 1.3ml/min	4552	1.248	0.230		97.2	1.90
Wavelength 217nm	4840	1.253	0.224		97.4	1.70
Wavelength 223nm	4801	1.254	0.267		97.5	1.60
Low oven Temperature 25°C	4660	1.255	0.065		97.4	1.70
High oven Temperature 35°C	4959	1.260	0.383		97.3	1.80

Table 14B: Robustness of analytical method for Phenylephrine HCL

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Flow rate 1.1ml/min	7178	1.186	0.126	97.3	98.8	-0.10
Flow rate 1.3ml/min	6660	1.174	0.262		98.8	-1.40
Wavelength 217nm	6929	1.173	0.163		97.4	-1.50
Wavelength 223nm	6852	1.175	0.255		98.7	-1.50
Low oven Temperature 25°C	6613	1.174	0.157		98.5	-1.20
High oven Temperature 35°C	7294	1.183	0.361		99.2	-1.90

Table 14C: Robustness of analytical method for Chlorphenamine Maleate

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Flow rate 1.1ml/min	64660	1.414	0.138	101.1	99.8	1.30
Flow rate 1.3ml/min	69998	1.399	0.208		100.1	1.00
Wavelength 217nm	65566	1.381	0.134		101.0	0.10
Wavelength 223nm	66365	1.405	0.282		99.9	1.20
Low oven Temperature 25°C	67289	1.406	0.138		100.6	0.50
High oven Temperature 35°C	71273	1.417	0.253		99.4	1.70

Acceptance criteria:

1) Theoretical plates for Paracetamol, phenylephrine HCL and Chlorphenamine peaks should be NLT 2000
2) Tailing Factor for Paracetamol, phenylephrine HCL and Chlorphenamine peaks should be NMT 2.0.
3) % RSD of area of analyte in replicate standard injections should be NMT 2.0.
4) % Assay of analyte should not differ by ±2.0 to that of method precision.

Result and Conclusion:

Each chromatographic variation System suitability parameters are within limits. % Difference of assay within limits at each variation.

10.0 SUMMARY:

No	Validation parameter	Acceptance criteria	Results
1	**System suitability**	1) % RSD of area of analyte in five replicate standard injections should not be more than 2.0.	Paracetamol:0.049 Phenylephrine HCl:0.031 Chlorphenamine Maleate: 0.307
		2) Theoretical plate should be not less than 2000.	Paracetamol:4758 Phenylephrine HCl:7245 Chlorphenamine Maleate: 78548
		3) Tailing factor should not be more than 2.0.	Paracetamol:1.267 Phenylephrine HCl:1.209 Chlorphenamine Maleate: 1.468
2	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	1) There should not be any interference due to blank and placebo with analyte. 2) Peak purity of analyte should 0.995.	Blank peaks, Placebo peaks are not interfere with Paracetamol, Phenylephrine maleate and Chlorphenamine peak in test preparation and Peak purity passes within specified limits.
3	**Linearity and Range**	1) R^2 Should be NLT 0.995	**Squared correlation coefficient for** Paracetamol:0.999 Phenylephrine HCl:0.999 Chlorphenamine Maleate: 0.999

10.0 SUMMARY:

No	Validation parameter	Acceptance criteria	Results
	Linearity and Range	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.	**Paracetamol:** Level %RSD 10% : 0.019 50% : 0.039 75% : 0.088 100% : 0.029 125% : 0.062 150% : 0.016 **Phenylephrine HCL:** Level %RSD 10% : 0.118 50% : 0.113 75% : 0.055 100% : 0.011 125% : 0.126 150% : 0.207 **Chlorphenamine:** Level %RSD 10% : 0.181 50% : 0.027 75% : 0.115 100% : 0.050 125% : 0.039 150% : 0.191

10.0 SUMMARY:

No	Validation parameter	Acceptance criteria	Results
4	**Interference from degradants (Forced degradation)**	1) There should not be any interference due to degradants with analyte and impurity in stressed samples. 2) The desired degradation should be 10-30% in acid, alkali and oxidation degradation, (if possible). 3) If about 10% to 30% degradation is not achieved by applying above stressed condition, same shall be documented and reported. 4) Peak purity of analyte peak each impurity peak (above LOQ/0.1% level of test concentration whichever is higher) should be pass (Peak purity should not be less than 0.950 according to Lab solution.	There is No any interference due to degradants with analyte in stressed samples and Peak purity was passes According to Lab solution.
5	**Accuracy** (Recovery)	The mean % recovery at each level should be 98.0 to 102.0.	**Paracetamol:** Level %Recovery 50% : 98.66 100% : 98.93 150% : 98.32 **Phenylephrine HCl:** Level %Recovery 50% : 101.58 100% : 98.95 150% : 101.26

SUMMARY:

No	Validation parameter	Acceptance criteria	Results
			Chlorphenamine: Level %Recovery 50% : 98.76 100% : 100.47 150% : 98.66
6	**Precision** 1) System Precision	%RSD of area of analyte peaks in five replicate standard injections should not be more than 2.0	Paracetamol:0.045 Phenylephrine HCl:0.031 Chlorphenamine maleate: 0.161
	2) Method Precision	%RSD of Assay of six preparations should not be more than 2.0	Paracetamol:1.189 Phenylephrine HCl:1.475 Chlorphenamine maleate:1.637
	3)Intermediate Precision	1) % RSD for assay of six preparations should not be more than 2.0	Paracetamol:0.249 Phenylephrine HCl:1.871 Chlorphenamine Maleate:0.811
		2) Cumulative %RSD for assay of twelve preparations (of method and intermediate precision) should not be more than 2.0.	Paracetamol:1.147 Phenylephrine HCl:1.886 Chlorphenamine:1.854
7	**Stability for analytical solution**	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%.	The Standard solution and Sample solution was stable up to 44 hours at ambient temperature.
8	**Filter paper study (0.45µ PVDF)**	The % difference on filter solution should not differ ±2.0 against that of unfiltered.	The % difference on filtered sample (0.45µ PVDF) within limit against that of unfiltered.

Summary:

No	Validation parameter	Acceptance criteria	Results
9	**Robustness** (i) Flow rate change (ii) Wavelength change (iii) Temperature Change	System suitability parameters should comply.	Each chromatographic variation System suitability parameters are within limits. % Difference of assay within limits at each variation.

11.0 CONCLUSION:

Validation studies have been conducted for Assay of Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic acid sachet for the parameters of system suitability, specificity, system precision, method precision, Intermediate precision, Robustness, Linearity and range and accuracy, Filter paper study by using the proposed method. The data is complied 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
No	:	Number
ml	:	Milliliter
%	:	Percentage
ID	:	Identification
API	:	Active pharmaceutical ingredient
HPLC	:	High performance liquid chromatography
B.NO	:	Batch number
WS.NO	:	Working standard number
mm	:	Millimeter
µm	:	Micrometer
min	:	Minutes
°C	:	Degree centigrade
nm	:	Nanometer
RSD	:	Relative standard deviation
µl	:	Micro litre

13.0 REVISION HISTORY:

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