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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2.0 PROTOCOL APPROVAL SHEET

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Date : 07/10/2022

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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

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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****