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

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2.0 REPORT APPROVAL SHEET

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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

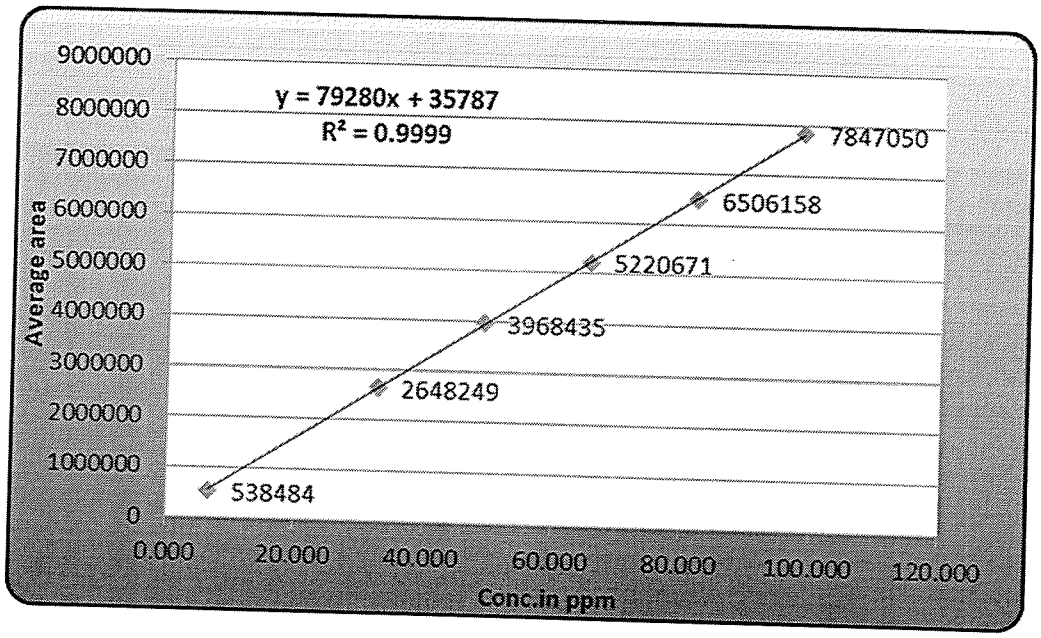

Table 3B: Linearity Table for Phenylephrine

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

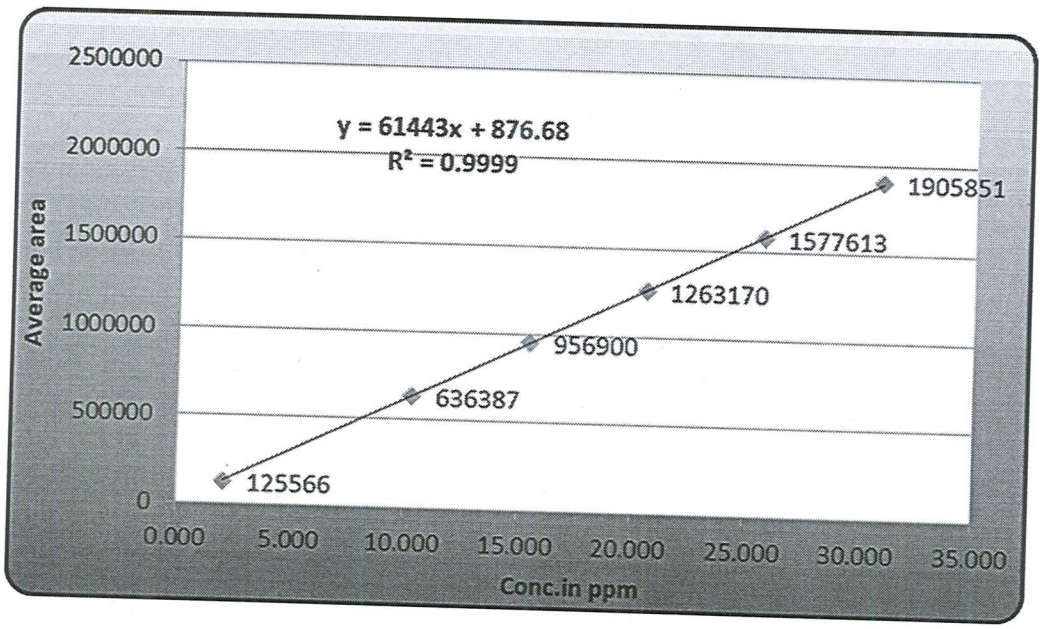

Table 3C: Linearity Table for Chlorphenamine

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

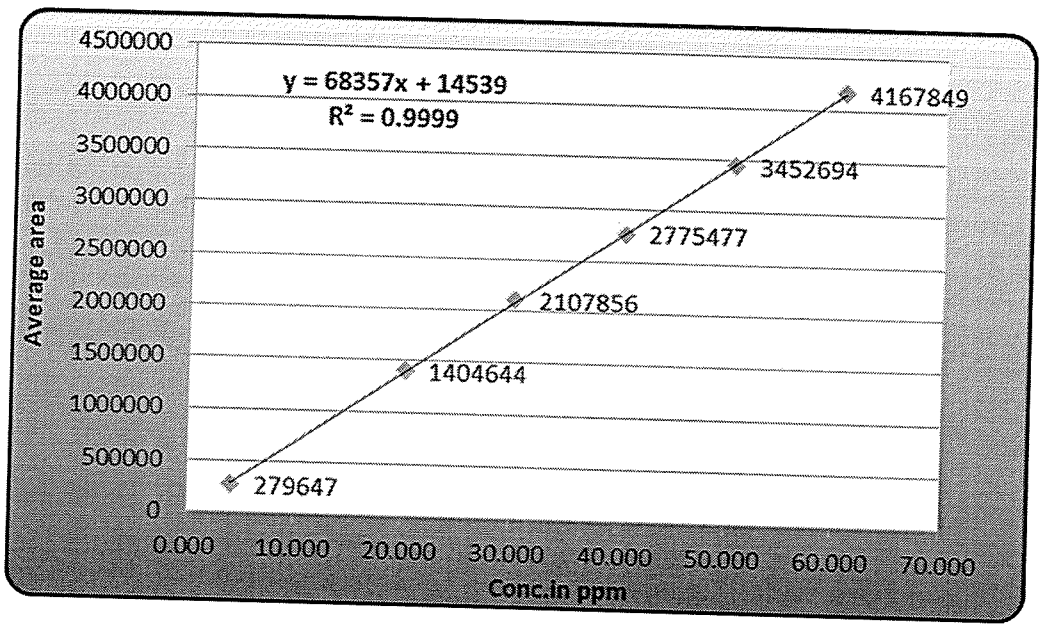

Table:4 Range for Paracetamol, Phenylephrine and Chlorphenamine

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.