PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF RELATED SUBSTANCES OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

2.0 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Dy. Manager - QC
Signature	:	
Date	:	04/10/2023
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM-QC
Signature	:	
Date	:	05/10/2023
APPROVED BY		
Name	:	J. MARAN
Designation	:	AGM-QA
Signature	:	
Date	:	06/10/2023

Effective Date	:	09/10/2023

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

3.0 OBJECTIVE

To validate the method for test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

SPECIFICATION LIMIT : (i) Single maximum unknown impurity: NMT 0.20%
(ii) Total impurities:0.50%

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

VALIDATION TEAM : 1. S.Elavarasan
2. S. Bhavyasri
3. C.Albin jose

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., test samples / placebo to be used during validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	To be mentioned in report	COA Attached
Plain Placebo	To be mentioned in report	Not applicable
Working standard Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report
API Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED:

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C Prominence i

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

Column:

Kromasil C8, 250 mm X 4.6 mm, 5µm (or) equivalent

Solvents and chemicals with grade:

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine Anhydrous (Working standard)

Paracetamol (Working standard)

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Methanol (HPLC grade)

Potassium Di-hydrogen orthophosphate

Orthophosphoric acid

Acetonitrile (HPLC grade)

Hydrochloric Acid (AR grade)

Sodium Hydroxide (AR grade)

Hydrogen Peroxide 30% (AR grade)

Plain placebo

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column	:	Kromasil C8, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	220 nm
Column Temperature	:	30°C
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µl
Run time	:	15 minutes for Standard solution 40 minutes for Blank, System suitability solution, placebo solution and Sample solution
Retention time	:	About 3.5 minutes for Chlorphenamine maleate, about 4.1 minutes for Phenylephrine Hydrochloride, about 8.4 minutes for Paracetamol and about 20.5 minutes for Caffeine

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Preparation of Buffer:

Weigh accurately about 6.8g of potassium Di-hydrogen orthophosphate in 1000 mL of Purified water, sonicate to dissolve. Adjust pH 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

Preparation of Mobile phase:

Prepare a degassed mixture of buffer and methanol in the ratio 85:15 v/v.

Preparation of Diluent:

Prepare a degassed mixture of water and methanol in the ratio 80:20 v/v.

Preparation of Placebo solution:

Weigh accurately and transfer about 55 mg of Plain Placebo into 100mL volumetric flask. Add 50 mL of diluent and sonicate for 10 minutes with intermittent shaking, cool and make up to volume with diluent and mix. Further dilute 5 mL of above solution into 50 mL with diluent. Filter through 0.45µ PVDF filter.

Preparation of Standard solution:

Weigh accurately and transfer about 60 mg of Paracetamol working standard into a 100 mL volumetric flask. Add 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. Dilute 1 mL of this solution to 100 mL with diluent and mix. Further dilute to 5 mL of above solution into 50 mL with diluent and mix. (**Concentration:** 0.0006 mg/ml)

Preparation of system suitability stock solution:

Weigh accurately and transfer about 180 mg of Caffeine working standard, 12 mg of Chlorphenamine maleate working standard and 30 mg of Phenylephrine hydrochloride working standard into a 100 mL volumetric flask. Add 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. (**Concentration:** Caffeine:1.8mg/ml, Chlorphenamine maleate:0.12mg/ml, Phenylephrine Hcl:0.3mg/ml)

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Preparation of system suitability solution:

Weigh accurately and transfer about 30 mg of Paracetamol working standard into a 100 mL volumetric flask. Add 1 mL of system suitability standard stock solution and 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. (**Concentration:** Paracetamol:0.3mg/ml, Caffeine:0.018mg/ml, Chlorphenamine maleate:0.0012mg/ml)

Test preparation:

Weigh 20 tablets and calculate the average weight and make powder by using mortar and pestle. Weigh and transfer sample powder equivalent to 300 mg of Paracetamol, into a 100 mL volumetric flask. Add about 50 mL of diluent and sonicate for 10 minutes with intermittent shaking dilute up to the volume with diluent and mix. Further dilute 10 mL of above solution into 100 mL with diluent. Filter through 0.45µ PVDF filter.

Inject 20µL of the above solution (blank, system suitability solution, standard, placebo and sample) as per following sequence and measure the area due to any unknown impurity peak.

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (blank)	1
2	System suitability solution	1
3	Standard solution	5
4	Placebo Preparation	1
5	Test preparation	1
6	Bracketing standard	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Calculation:

Single maximum unknown impurity:

$$= \frac{AT_I}{AS_T} \times \frac{WS}{100} \times \frac{1}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{100}{10} \times AW \times \frac{P}{100} \times \frac{100}{500}$$

Where,

AT_I	=	Area of peak due to Single maximum unknown impurity in test preparation.
AS_T	=	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.
AW	=	Average weight of tablet in mg.
P	=	Potency of Paracetamol working standard in % on as such basis.

Total impurities:

$$= \frac{AT_T}{AS_T} \times \frac{WS}{100} \times \frac{1}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{100}{10} \times AW \times \frac{P}{100} \times \frac{100}{500}$$

Where,

AT_T	=	Area of peak due to Total impurities in test preparation.
AS_T	=	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.
AW	=	Average weight of tablet in mg.
P	=	Potency of Paracetamol working standard in % on as such basis.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for validation.	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Degradation**
	i) Acid degradation ii) Alkali Degradation iii) Oxidative Degradation
4	Determination of Limit of Detection and Limit of Quantitation
5	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
6	LOQ Precision Study and Observation at LOD
7	Linearity and Range
8	Stability of analytical solution
9	Filter paper study
10	Robustness

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.0 DETAILS OF VALIDATION PARAMETERS:

9.1 SYSTEM SUITABILITY:

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:

i) % RSD area of Paracetamol peak in five replicate standard injections should not more than 5.0.

ii) Theoretical plates for Paracetamol peak in five replicate standard injection should not less than 2000.

iii) Tailing factor for Paracetamol peak in six standard injection should not more than 2.0.

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and Placebo

Study design:

Sequence shall be in following provisional manner.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

No.	Description of solution	No. of injections
1	Blank (Diluent)	1
2	System suitability solution	1
3	Standard Preparation	5
4	Plain placebo	1
5	Phenylephrine HCL- Std	1
6	Chlorphenamine Maleate-Std	1
7	Paracetamol-Std	1
8	Caffeine- Std	1
9	Plain placebo + Phenylephrine HCL + Paracetamol + Caffeine + Chlorphenamine std	1
10	Test preparation	1

Note: Chromatograph the above samples into HPLC system equipped with diode array detector and evaluate the peak purity for the analytes in standard preparation, Placebo preparation and sample preparation and Impurity.

Acceptance criteria:

1) No significant interference from blank, placebo peak with analyte.

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.3 INTERFERENCE FROM DEGRADANTS (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 5N HCL and heat on water bath 80°C for 60 minutes.
Alkali degradation	Exposure to 5N NaOH and heat on water bath 80°C for 60 minutes.
Oxidative degradation	Exposure to 30% H2O2 and heat on water bath 80°C for 60 minutes.

Sequence shall be in following provisional manner.

For chemical forced degradation:

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Plain Placebo preparation	1
4	Sample preparation (As such)	1
5	Blank (Diluent)	1
6	Sample preparation (Acid degradation)	1
7	Blank (Diluent)	1
8	Sample preparation (Alkali degradation)	1
9	Blank (Diluent)	1
10	Sample preparation (Oxidative degradation)	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Chromatograph the samples of chemical 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 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 and each impurity peak (above LOQ to 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.

9.4 DETERMINATION OF LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

Study design:

To determine the limit of detection and limit of quantitation, analyze an appropriate number of diluted solutions of actives. Prepare the linearity solutions from lowest possible concentration to that of specification limit level concentration.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

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 peak area of analytes (at Y-axis). Measure the residual standard deviation of response and slope through regression technique. From the linearity data, calculate the limit of detection and quantitation, using the following formula.

$$\text{Limit of detection} = \frac{3.3\sigma}{S} \quad \text{and} \quad \text{Limit of quantitation} = \frac{10\sigma}{S}$$

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Where,

σ = Residual standard deviation of response
S = Slope of calibration curve.

Note:

- Based on above results if LOD and LOQ are found below 0.02% and 0.04% respectively, report the LOD value as 0.02% and LOQ value as 0.04% (and consider them for further experiments i.e. LOQ precision, Observation at LOD, and linearity)
- Based on above results if LOD and LOQ are found more than 0.02% and 0.04% respectively, report the LOD and LOQ value.

9.5 PRECISION:

9.5.1 System precision:

Study design:

Sequence shall be in following provisional manner.

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5

Acceptance criteria:

i) % RSD of area of Paracetamol peak in Five replicate standard injections should not be more than 5.0%.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.5.2 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.

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Plain placebo preparation	1
4	Sample preparation-1	1
5	Sample preparation-2	1
6	Sample preparation-3	1
7	Sample preparation-4	1
8	Sample preparation-5	1
9	Sample preparation-6	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Acceptance criteria:

1) % RSD for impurities from LOQ to 0.1% in six preparations should be not more than 20.

2) % RSD for impurities above 0.1% in six preparations should be not more than 15.

3) % RSD for total impurities in six preparations should be not more than 10.

9.5.3 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.

Analyze 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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Plain placebo	1
4	Sample preparation-1	1
5	Sample preparation-2	1
6	Sample preparation-3	1
7	Sample preparation-4	1
8	Sample preparation-5	1
9	Sample preparation-6	1
10	Bracketing standard	1

Acceptance criteria:

1) % RSD for impurities between LOQ to 0.1% in six preparations should not be more than 20.

2) % RSD for impurity above 0.1% in six preparations should not be more than 15.

3) % RSD for total impurities in six preparations should not be more than 10.

4) Cumulative % RSD of total impurities of method precision and intermediate precision should not be more than 10.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.6 LOQ PRECISION STUDY AND OBSERVATION AT LOD:

Study Design:

Demonstrate the verification of LOD and Precision of LOQ study by establishing LOD and LOQ level concentration. Verify LOD level and evaluate the precision at LOQ concentration by computing % RSD of area observed in LOQ Preparation.

Note:

Based on LOD & LOQ determination results if LOD and LOQ are found below 0.02% and 0.04% respectively, consider the LOD value as 0.02% and LOQ value as 0.04%. If LOD and LOQ are found more than 0.02% and 0.04% respectively, then report the document.

Sequence shall be in following provisional manner.

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard Preparation	5
3	LOD Verification standard	5
4	LOQ Precision standard	5
5	Bracketing standard	1

Acceptance criteria:

1) To conclude the LOD verification, the peak at LOD level should be visually detected in all five replicates.

ii) To conclude the LOQ precision, % RSD for peak area of LOQ level should be not more than 10.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.7 LINEARITY AND RANGE:

Study design:

Demonstrate the linearity and range of analytical method over the range of 10% to 150% of specification level concentration. Prepare linearity solutions by spiking known active to placebo.

Linearity stock solution, linearity level, expected concentration, linearity stock dilution and calculated concentration are tabulated below.

Linearity Stock solution	30.91	5	1	1	1	7.73ppm (con. ppm)
	200	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	0.060	2	250	0.062
50%	0.30	2	50	0.31
75%	0.45	3	50	0.46
100%	0.60	4	50	0.62
125%	0.75	5	50	0.77
150%	0.90	6	50	0.93

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 I, residual sum of square, slope and Y-Intercept.

Note:

Preparation of linearity solutions of active shall be done on specification limit.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Sequence shall be in following provisional

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

Acceptance criteria:

1) To conclude the linearity, the squared correlation coefficient (r^2) should not be less than 0.995.

2) To conclude the range, %RSD for of area at 10%, 50%, 75%, 100% and 150% levels should be not more than 2.0.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.8 STABILITY OF ANALYTICAL SOLUTION:

Study design:

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

Sequence shall be in following provisional

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation (Initial)	5
3	Placebo Preparation	1
4	Sample preparation (Initial)	1
5	Standard preparation (Interval)	1
6	Sample preparation (Interval)	1

Acceptance criteria:

The sample and standard preparation shall be considered stable for the final period till which the area difference between initial and next periodic interval should not be more than ±10%.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.9 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering test solution through specified filter against that of unfiltered (centrifuge).

Sequence shall be in following provisional manner.

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
2	Plain placebo preparation	1
3	Sample preparation –Unfiltered (Centrifuge)	1
4	Sample preparation –Filter Set 1 (0.45µ PVDF Filter)	1
5	Sample preparation –Filter Set 2 (0.45µ PVDF Filter)	1
6	Sample preparation –Filter Set 3 (0.45µ PVDF Filter)	1
7	Sample preparation –Filter Set 1 (0.45µ Nylon Filter)	1
8	Sample preparation –Filter Set 2 (0.45µ Nylon Filter)	1
9	Sample preparation –Filter Set 3(0.45µ Nylon Filter)	1

Acceptance criteria:

i) For impurities between 0.05-0.1%: % difference should ± 20 against that unfiltered.

ii) For Total impurities >0.1%: % difference should ±15 against that unfiltered.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

9.10 ROBUSTNESS:

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

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample Preparation	1

According to each variable

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample Preparation	1

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

a) Flow rate change by ±10% (i.e 0.90ml/min and 1.1ml/min)

b) Wavelength change by ± 3nm (i.e

217nm to 223nm)

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

Acceptance criteria:

i) % RSD for impurities between LOQ-0.1% should be not more than 20%.

ii) % RSD for impurities above 0.1% should be not more than 15%.

10.0 ABBREVATION:

mg	:	Milligram
No	:	Number
COA	:	Certificate of analysis
ml	:	Milliliter
%	:	Percentage
ID	:	Identification
LOD	:	Limit of detection
LOQ	:	Limit of quantitation
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
i.e.	:	That is

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVRP/23/005

11.0 REVISION HISTORY:

Specification No.	Effective date	Reason for Review
ST/AMVRP/23/005	09/10/2023	New Protocol prepared.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

ANALYTICAL METHOD VALIDATION REPORT FOR THE TEST OF ASSAY OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

1.0 INDEX

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

2.0 REPORT APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Asst. Manager-QC
Signature	:	
Date	:	27/09/2023
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	AGM-QC
Signature	:	
Date	:	28/09/2023
APPROVED BY		
Name	:	S. MARAN
Designation	:	ASM-QA
Signature	:	
Date	:	29/09/2023

Effective Date	:	30/09/2023

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

3.0 OBJECTIVE

To validate the method for test of assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

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

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. M.Bavyasri
2. S.Suganthi
3. C.Albin jose

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

5.0 DETAILS OF STANDARD,SAMPLES AND PLACEBO TO BE USED.

Mention the name and Batch No., Potency of the reference/working std., Impurities standard, test samples/placebo to be used during validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	G17230824	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Chlorphenamine Maleate BP	W.S.No: ST/WS/22/039	99.7% (As such basis)
Phenylephrine HCL BP	W.S.No: 2-WS15IC	99.8% (As such basis)
Caffeine BP	W.S.No: ST/WS/22/038	99.7% (As such basis)
Paracetamol BP	W.S.No: ST/WS/23/012	99.9% (As such basis)
API Chlorphenamine Maleate BP	AR.No:MSP/2223/QCG/QCR/00994	100.29% (As such basis)
Phenylephrine HCL BP	AR.No:MSP/2223/QCG/QCR/01040	99.54% (As such basis)
Caffeine BP	AR.No:MSP/2223/QCG/QCR/00973	99.77% (As such basis)
Paracetamol BP	AR.No:MSP/2223/QCG/QCR/01049	99.94% (As such basis)

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED :

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C Prominence i

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

COLUMN :

Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm

SOLVENTS AND CHEMICALS WITH GRADE :

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine (Anhydrous) (Working standard)

Paracetamol (Working standard)

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Potassium Dihydrogen orthophosphate (AR grade)

Orthophosphoric acid (AR grade)

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

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Hydrochloric acid (AR grade)

Sodium Hydroxide (AR grade)

Hydrogen Peroxide (AR grade)

Plain placebo

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column	:	Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm
Wave length	:	220 nm
Column Temperature	:	40°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	20 µL
Run time	:	30.01 Minutes
Retention time	:	About 4.6 minutes for Phenylephrine Hydrochloride, about 10.5 minutes for Paracetamol, about 12.7 minutes for Caffeine and about 14.8 minutes for Chlorphenamine maleate,

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Preparation of Buffer:

Weigh accurately about 6.8 g of potassium Di-hydrogen orthophosphate in 1000 mL of milli-Q water, sonicate to dissolve. Adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	100	0
6.0	100	0
7.0	70	30
9.0	70	30
10.0	45	55
25.0	45	55
27.0	100	0
30.01	100	0

Preparation of Mobile phase-A:

Prepare a degassed mixture of buffer and acetonitrile in the ratio 95:5 *v/v*.

Preparation of Mobile phase-B:

100% Methanol

Preparation of Diluent:

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Preparation of Standard stock solution:

Weigh accurately and transfer about 32 mg of Chlorphenamine maleate working standard, 80 mg of Phenylephrine Hydrochloride working standard and 24 mg of Caffeine working standard into a 200 mL volumetric flask. Add 120 mL of diluent and sonicate to dissolve and make up to volume with diluent and mix (mcg/ml).(**Concentration** For Chlorphenamine maleate:0.16mg/ml, Phenylephrine Hydrochloride:0.4mg/ml, Caffeine:0.005mg/ml)

Preparation of Standard solution:

Weigh accurately and transfer about 20 mg of Paracetamol working standard into a 200 mL volumetric flask. Add 120 mL of diluent and sonicate to dissolve and add 10 mL of standard stock solution (Chlorphenamine, Phenylephrine and Caffeine) and make up to volume with diluent and mix (mcg/ml). **Concentration:** (For Paracetamol:0.1mg/ml) (For Chlorphenamine maleate:0.008mg/ml, Phenylephrine Hydrochloride:0.02mg/ml, Caffeine:0.06mg/ml)

Test preparation:

Preparation of Sample Solution-A: (For Chlorphenamine maleate and Phenylephrine Hydrochloride)

Weigh accurately 20 tablets and make powder by using morter and pestle. Weigh and transfer sample powder equivalent to 500 mg of Paracetamol, into a 250 mL volumetric flask. Add about 170 mL of diluent and sonicate for 30 minutes with intermittent shaking. Make up to the volume with diluent and mix and Centrifuge this solution at 3000rpm for 10minutes.

Preparation of Sample Solution-B:(For Paracetamol and Caffeine)

Further dilute 5 mL of above solution to 100 mL with diluent and mix.

Procedure:

Inject the solutions as mentioned below and measure the responses of the peaks due to Paracetamol, Phenylephrine, Chlorphenamine maleate, Caffeine.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (blank)	1
2	Standard preparation	5
3	Sample solution-A	2
4	Sample solution B	2
5	Bracketing standard	1

System suitability:

Theoretical plate count	:	NLT 2000 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.
Tailing factor	:	NMT 2.0 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.
Relative standard deviation	:	NMT 2.0% for five replicate injections of Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Calculations:

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{P}{100} \times AW$$

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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.
AW = Average weight of tablet in mg.
P = Potency of Chlorphenamine maleate working standard in % on as such basis.

Calculate the assay of Chlorphenamine maleate in % as follows:

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

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

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{P}{100} \times AW$$

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.
AW = Average weight of tablet in mg.
P = Potency of Phenylephrine Hydrochloride working standard in % on as such basis.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Calculate the assay of Phenylephrine Hydrochloride in % as follows:

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

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

Calculate the assay of Caffeine in mg/tablet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AW$$

Where,

AT	=	Average area of peak due to Caffeine in Sample solution B.
AS	=	Average area of peak due to Caffeine in standard preparation.
WS	=	Weight of Caffeine working standard in mg.
WT	=	Weight of sample taken in mg.
AW	=	Average weight of tablet in mg.
P	=	Potency of Caffeine working standard in % on as such basis.

Calculate the assay of Caffeine in % as follows:

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

LC = Label claim of Caffeine in mg/tablet.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{250}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AW$$

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.

AW = Average weight of tablet in mg.

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

Calculate the assay of Paracetamol in % as follows:

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

LC = Label claim of Paracetamol in mg/tablet.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

8. VALIDATED PARAMETERS :

Following parameters shall be selected for validation.	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and placebo
3	**Linearity and Range**
4	**Interference from Degradation (Forced Degradation)**
	i) Acid degradation ii) Alkali Degradation iii) Oxidative Degradation
5	**Accuracy**
6	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
7	**Stability of analytical solution**
8	**Filter paper study**
9	**Robustness** i) Flow rate change ii) Wavelength change iii) Oven temperature change

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

9.0 VALIDATION RESULTS :

9.1 SYSTEM SUITABILITY:

Study Design:

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

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

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

3) % RSD of area of five replicate standard injections

Results are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Phenylepherine Hydrochloride	Paracetamol	Caffeine	Chlorphenamine
Theoretical Plates	NLT 2000	8068	138502	90799	113797
Tailing Factor	NMT 2.0	1.09	1.21	1.03	1.07
% RSD	NMT 2.0	0.319	0.078	0.624	0.873

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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from Blank and Placebo

Study Design:

Blank, standard, placebo and placebo spiked with analyte and sample are analyzed as per the method to examine the interference of blank and placebo with Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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 index should not be less than 0.995 accordingly to lab solution software.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Blank	Blank peak	Not applicable	Not applicable
2	Standard preparation	Phenylephrine	4.33	1.000
		Paracetamol	10.28	1.000
		Caffeine	12.36	1.000
		Chlorphenamine	14.38	1.000
3	Plain placebo	Placebo peaks	Not applicable	Not applicable
4	Placebo + Phenylephrine- Std	Phenylephrine	4.32	1.000
5	Placebo + Paracetamol- Std	Paracetamol	10.29	1.000
6	Placebo + Caffeine- Std	Caffeine	12.37	1.000
7	Placebo + Chlorphenamine- Std	Chlorphenamine	14.37	1.000

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
8	Plain placebo + Phenylephrine + Paracetamol + Caffeine + Chlorphenamine std	Phenylephrine	4.33	1.000
		Paracetamol	10.31	1.000
		Caffeine	12.40	1.000
		Chlorphenamine	14.36	1.000
9	Test preparation -A	Phenylephrine	4.31	1.000
		Chlorphenamine	14.36	1.000
10	Test preparation -B	Paracetamol	10.29	1.000
		Caffeine	12.37	1.000

Results and Conclusion:

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

9.3 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Working standard are 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 and 3D for Linearity and Table 4 for Range.

Acceptance criteria:

1) The squared correlation coefficient should not be less than 0.999.

2) To conclude the range % RSD for peak areas of linearity levels 10%, 50%, 75%, 100%, 125% and 150% should not be more than 2.0.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 3A: Linearity Table for Chlorphenamine Maleate

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	0.813	20912
50%	4.067	112188
75%	6.101	169419
100%	8.134	230098
125%	10.168	285569
150%	12.201	347234
Slope		28612
CC		0.999
Sqaured R		0.9998
Intercept		3586.6

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Fig.1 : Liner Graph for Chlorphenamine Maleate

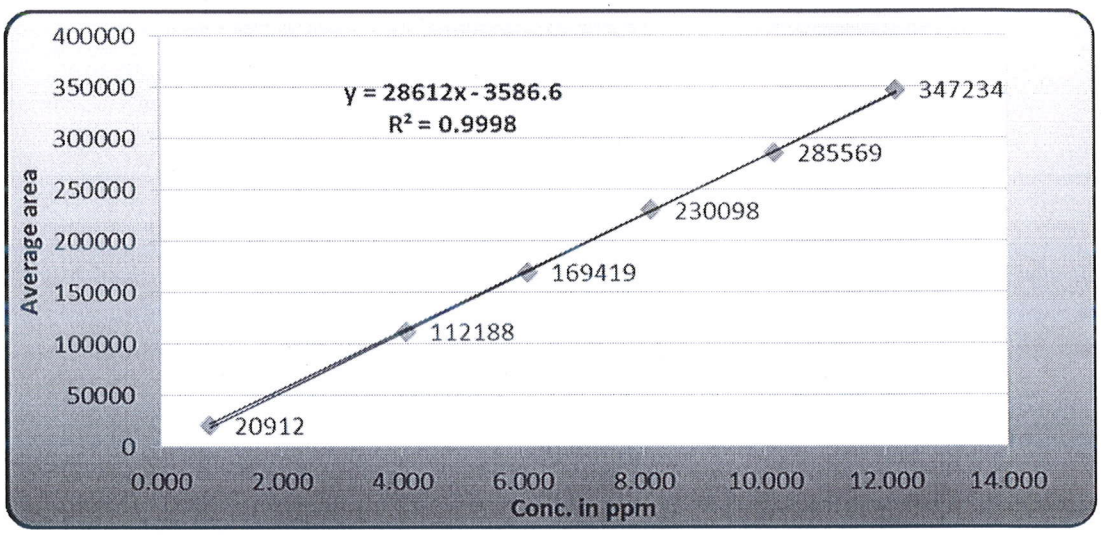

Table 3B: Linearity Table for Phenylephrine Hydrochloride

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	2.003	36881
50%	10.014	166464
75%	15.021	246810
100%	20.028	333872
125%	25.035	410487
150%	30.042	494764
Slope		16334
CC		0.999
Sqaured R		0.9999
Intercept		3486.9

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Fig.2 : Liner Graph for Phenylephrine Hydrochloride

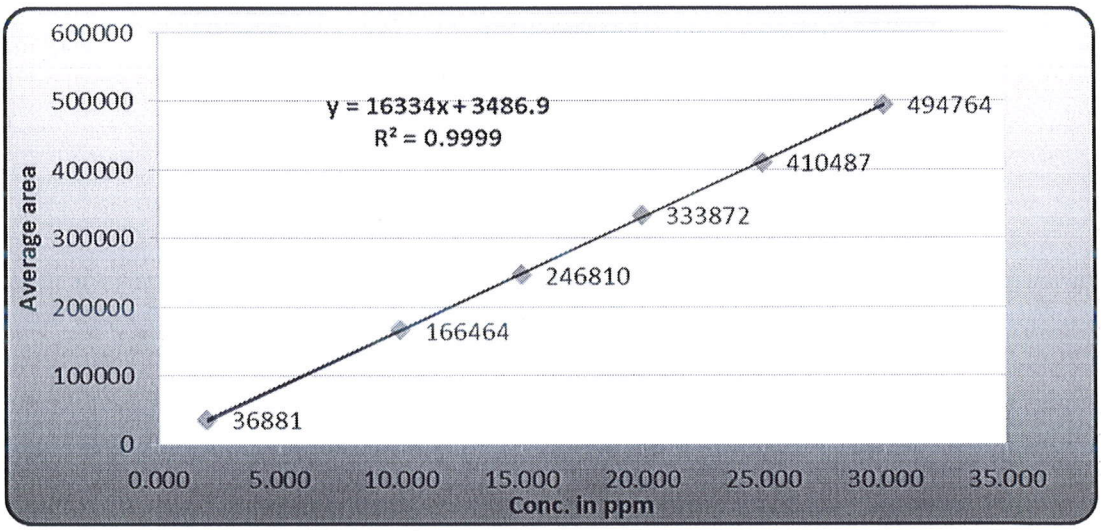

Table 3C: Linearity Table for Caffeine

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	0.603	28918
50%	3.013	150598
75%	4.520	225497
100%	6.026	306167
125%	7.533	378469
150%	9.039	453291
Slope		50423
CC		0.999
Sqaured R		0.9999
Intercept		1115.4

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Fig.3 : Liner Graph for Caffeine

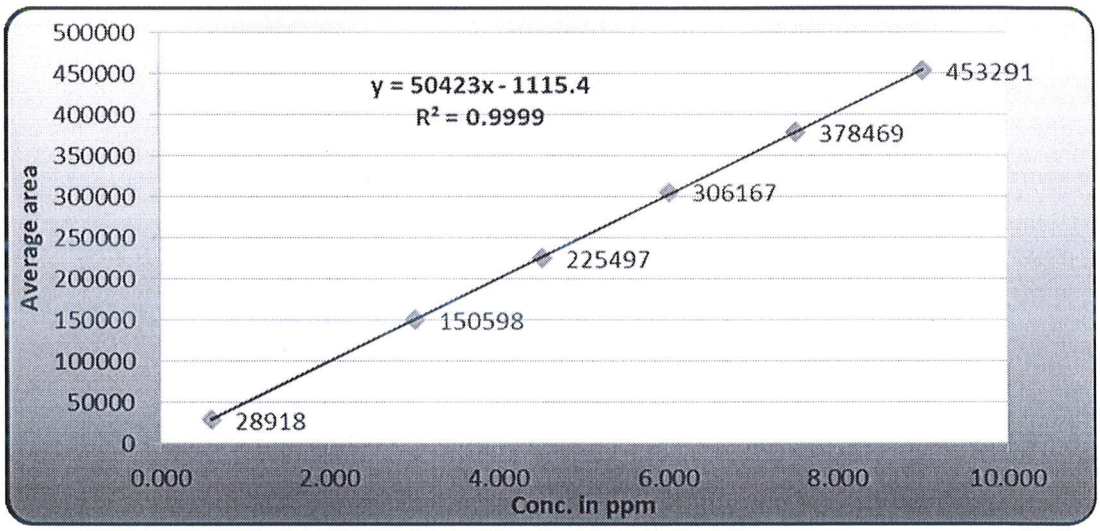

Table 3D: Linearity Table for Paracetamol

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	10.019	332561
50%	50.096	1548465
75%	75.144	2280728
100%	100.192	3064001
125%	125.240	3762282
150%	150.288	4491482
Slope		29670
CC		0.999
Sqaured R		0.9998
Intercept		53118

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Fig.4: Liner Graph for Paracetamol

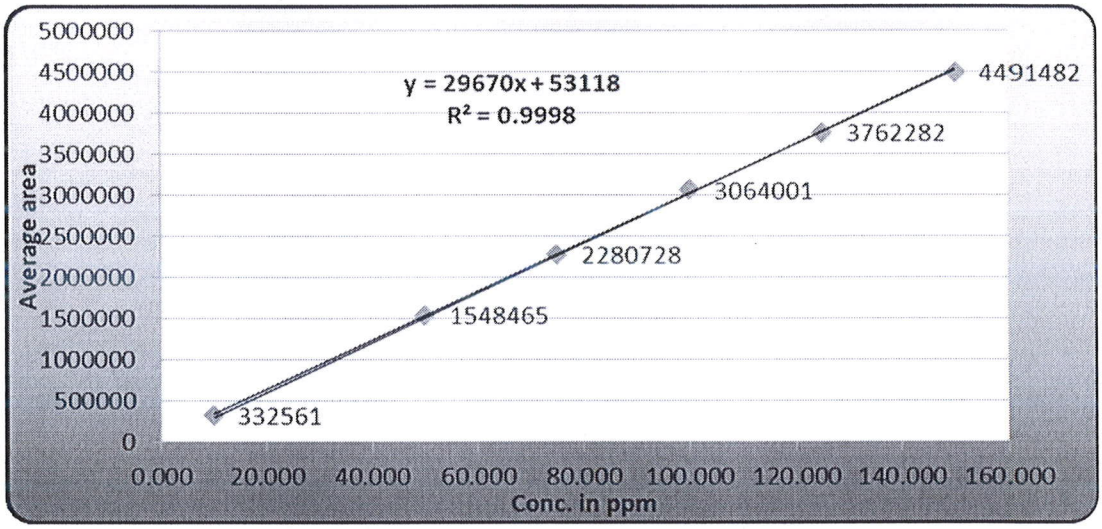

Table:4 Range for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol

Linearity Levels (%)	Chlorphenamine Maleate	Phenylephrine Hydrochloride	Caffeine	Paracetamol
10%	1.301	0.130	0.451	0.067
50%	0.135	0.415	0.179	0.048
75%	0.122	0.891	0.152	0.115
100%	0.234	0.111	0.132	0.015
125%	0.290	0.351	0.160	0.115
150%	0.381	0.249	0.107	0.071

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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 is carried out and peak purity of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak are monitored.

9.4.1 Acid Degradation:

Sample solution A:

Take not less than 20 tablets and make powder by using morter and pestle. Weigh and transfer sample powder equivalent to 500 mg of Paracetamol, into a 250 mL volumetric flask. Add about 170 mL of diluent and sonicate for 30 minutes with intermittent shaking cool. Add 5ml 5N Hydrochloric acid and heat on water bath at 80°C for 30minutes. Cool and neutralized with 5ml of 5N Sodium hydroxide and dilute to volume with diluent and mix. Centrifuge this solution at 3000RPM for 10 minutes.

Sample solution B:

Further dilute 5 mL of above solution to 100 mL with diluent and mix. Filter through 0.45µ nylon filter.

9.4.2 Alkali degradation:

Sample solution A:

Take not less than 20 tablets and make powder by using morter and pestle. Weigh and transfer sample powder equivalent to 500 mg of Paracetamol, into a 250 mL volumetric flask. Add about 170 mL of diluent and sonicate for 30 minutes with intermittent shaking cool. Add 5ml 5N Sodium hydroxide and heat on water bath at 80°C for 30minutes.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Cool and neutralized with 5ml of 5N Hydrochloric acid and dilute to volume with diluent and mix. Centrifuge this solution at 3000RPM for 10 minutes.

Sample solution B:

Further dilute 5 mL of above solution to 100 mL with diluent and mix.

9.4.3 Oxidative Degradation:

Sample solution A:

Take not less than 20 tablets and make powder by using morter and pestle. Weigh and transfer sample powder equivalent to 500 mg of Paracetamol, into a 250 mL volumetric flask. Add about 170 mL of diluent and sonicate for 30 minutes with intermittent shaking cool. Add 5ml of 30% Hydrogen peroxide solution and heat on water bath at 80°C for 30minutes Cool and dilute to volume with diluent and mix. Centrifuge this solution at 3000RPM for 10 minutes.

Sample solution B:

Further dilute 5 mL of above solution to 100 mL 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 0.995 according to Lab solution software.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 5: Peak purity (Chemical degradation)

S.No	Sample name	Peak name	Assay in (%)	Degradation in %	Peak purity index
1	Sample as such (Method Precision)	Phenylephrine Hcl	98.1	No peak	No peak
		Paracetamol	96.6		
		Caffeine	100.1		
		Chlorphenamine	97.6		
2	Acid degradation	Phenylephrine Hcl	98.6	-0.50	1.000
		Paracetamol	101.2	-4.59	1.000
		Caffeine	100.5	-0.41	1.000
		Chlorphenamine	92.1	5.52	1.000
3	Alkali degradation	Phenylephrine Hcl	96.8	1.26	0.995
		Paracetamol	100.8	-4.16	1.000
		Caffeine	90.1	9.96	1.000
		Chlorphenamine	90.4	7.17	1.000
4	Oxidative Degradation	Phenylephrine Hcl	98.1	0.01	1.000
		Paracetamol	97.1	-0.46	1.000
		Caffeine	97.7	2.36	1.000
		Chlorphenamine	90.7	6.92	1.000

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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 software.

9.5 ACCURACY (RECOVERY)

Study Design:

Known quantity of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol working standard are 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 and inject only one injection for each sample. The samples are analyzed as per the proposed method. The results are tabulated in Table 6A, 6B, 6C and 6D for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol respectively to demonstrate the accuracy of the method.

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 6A: Accuracy for Chlorphenamine Maleate

Recovery level	Sample No.	% Recovery	Mean %	% RSD
50%	1	98.28	98.8	0.527
	2	98.74		
	3	99.32		
100%	1	98.15	98.4	0.200
	2	98.39		
	3	98.54		
150%	1	98.19	98.7	0.516
	2	99.20		
	3	98.57		

Table 6B: Accuracy for Phenylephrine Hydrochloride

Recovery level	Sample No.	% Recovery	Mean %	% RSD
50%	1	101.42	101.0	0.339
	2	100.88		
	3	100.78		
100%	1	101.24	101.4	0.136
	2	101.31		
	3	101.51		
150%	1	99.41	99.3	0.141
	2	99.14		
	3	99.33		

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 6C: Accuracy for Caffeine

Recovery level	Sample No.	% Recovery	Mean%	% RSD
50%	1	98.97	98.9	0.063
	2	98.96		
	3	98.86		
100%	1	101.33	101.2	0.144
	2	101.04		
	3	101.18		
150%	1	101.28	101.3	0.214
	2	101.48		
	3	101.05		

Table 6D: Accuracy for Paracetamol

Recovery level	Sample No.	% Recovery	Mean%	% RSD
50%	1	101.89	101.9	0.015
	2	101.87		
	3	101.89		
100%	1	99.67	99.7	0.250
	2	100.01		
	3	99.53		
150%	1	98.12	98.2	0.150
	2	98.09		
	3	98.36		

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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:

9.6.1 SYSTEM PRECISION

Study design:

Five replicate injections of standard preparation are injected into the HPLC system. The area response for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 6: System precision

Injection No.	Phenylephrine Area	Paracetamol Area	Caffeine assay Area	Chlorphenamine Area
1	338920	3173550	317580	237498
2	340085	3177313	315350	233089
3	340014	3178557	312863	234491
4	339287	3177668	313629	233129
5	337425	3180343	313177	232350
Mean	339146	3177486	314520	234111
% RSD	0.319	0.078	1.030	1.070

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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

9.6.2 Method Precision:

Study Design:

Six Assay preparations of sample are analyzed as per the method. The Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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

No. of Preparation	Phenylephrine assay in %	Paracetamol assay in %	Caffeine assay in %	Chlorphenamine assay in %
1	97.6	97.5	101.2	97.8
2	98.0	95.0	99.2	97.4
3	98.8	97.7	101.3	97.2
4	99.3	97.0	100.3	98.4
5	97.6	96.1	99.3	97.7
6	97.2	96.3	99.6	97.2
Mean	98.1	96.6	100.1	97.6
% RSD	0.840	1.070	0.912	0.491

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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

9.6.3 Intermediate Precision (Ruggedness):

Study summary:

Six Assay preparations of sample are analyzed as per the method by different analyst using different instrument and different column on different day.

The assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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

No. of Preparation	Phenylephrine assay in %	Paracetamol assay in %	Caffeine assay in %	Chlorphenamine assay in %
1	100.5	98.9	99.8	94.3
2	101.9	99.6	101.1	98.4
3	99.5	98.2	99.2	97.2
4	101.0	98.7	99.6	96.1
5	102.1	98.9	100.3	95.7
6	99.2	98.6	99.3	97.4
Mean	100.7	98.8	99.88	96.52
% RSD	1.193	0.468	0.715	1.505

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 9.

Table 10: Cumulative % RSD

Parameter	Phenylephrine assay in %	Paracetamol assay in %	Caffeine assay in %	Chlorphenamine assay in %
Method Precision	97.6	97.5	101.2	97.8
	98.0	95.0	99.2	97.4
	98.8	97.7	101.3	97.2
	99.3	97.0	100.3	98.4
	97.6	96.1	99.3	97.7
	97.2	96.3	99.6	97.2
Intermediate Precision	100.5	98.9	99.8	94.3
	101.9	99.6	101.1	98.4
	99.5	98.2	99.2	97.2
	101.0	98.7	99.6	96.1
	102.1	98.9	100.3	95.7
	99.2	98.6	99.3	97.4
Mean	99.4	97.7	100.0	97.1
% RSD	1.690	1.422	0.794	1.220

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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

Table 11A: Stability of sample solution for Chlorphenamine Maleate

Time in hours	Area of Sample solution	Absolute % Difference
Initial	230023	Not applicable
7	226869	1.39
12	230754	-0.32
18	233578	-1.52
24	230953	-0.40
28	233223	-1.37
Mean	230900	-0.44
% RSD	1.054	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 11B: Stability of sample solution for Phenylephrine Hydrochloride

Time in hours	Area of Sample solution	Absolute % Difference
Initial	354216	Not applicable
7	348377	1.68
12	348543	1.63
18	348636	1.60
24	348422	1.66
28	358593	-1.22
Mean	351131	1.07
% RSD	1.229	Not applicable

Table 11C: Stability of sample solution for Caffeine

Time in hours	Area of Sample solution	Absolute % Difference
Initial	314935	Not applicable
7	315992	-0.33
12	314916	0.01
18	314123	0.26
24	313875	0.34
28	315260	-0.10
Mean	314850	0.03
% RSD	0.244	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 11D: Stability of sample solution for Paracetamol

Time in hours	Area of Sample solution	Absolute % Difference
Initial	3047697	Not applicable
7	3034014	0.45
12	3029142	0.61
18	3018256	0.98
24	3003799	1.46
28	3014673	1.10
Mean	3024597	0.92
% RSD	0.515	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 is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 12A, 12B, 12C and 12D.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 12A: Stability of standard solution for Chlorphenamine Maleate

Time in hours	Area of Standard solution	Absolute % Difference
Initial	238375	Not applicable
7	234006	1.87
12	240038	-0.69
18	241429	-1.26
24	238050	0.14
28	235223	1.34
Mean	237854	0.28
% RSD	1.183	Not applicable

Table 12B: Stability of standard solution for Phenylephrine Hydrochloride

Time in hours	Area of Standard solution	Absolute % Difference
Initial	358595	Not applicable
7	352669	1.68
12	352559	1.71
18	352631	1.69
24	354308	1.21
28	363684	-1.40
Mean	355741	0.98
% RSD	1.273	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 12C: Stability of standard solution for Caffeine

Time in hours	Area of Standard solution	Absolute % Difference
Initial	319933	Not applicable
7	323162	-1.00
12	322841	-0.90
18	322225	-0.71
24	322461	-0.78
28	321125	-0.37
Mean	321958	-0.75
% RSD	0.377	Not applicable

Table 12D: Stability of standard solution for Paracetamol

Time in hours	Area of Standard solution	Absolute % Difference
Initial	3164169	Not applicable
7	3120262	1.41
12	3125555	1.24
18	3116606	1.53
24	3113068	1.64
28	3112988	1.64
Mean	3125441	1.49
% RSD	0.626	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Results and conclusions:

The Standard solution and Sample solution was stable upto 28 hours at room temperature.

9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method is performed by filtering test solution through 0.45µ Nylon membrane and 0.45µ PVDF filter against that of unfiltered (centrifuged) sample. The results are tabulated in Table 13A, 13B, 13C and 13D

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

Filter study	Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	235157	96.2	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	233133	95.4	0.87
Filter Set-2 (0.45µ Nylon membrane)	233548	95.6	0.69
Filter Set-3 (0.45µ Nylon membrane)	233518	95.6	0.70
Filter Set-1 (0.45µ PVDF membrane)	234249	95.9	0.39
Filter Set-2 (0.45µ PVDF membrane)	233622	95.6	0.66
Filter Set-3 (0.45µ PVDF membrane)	234015	95.8	0.49

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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

Filter study	Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	368830	100.3	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	367812	100.1	0.28
Filter Set-2 (0.45µ Nylon membrane)	370259	100.7	-0.39
Filter Set-3 (0.45µ Nylon membrane)	367612	100.0	0.33
Filter Set-1 (0.45µ PVDF membrane)	365516	99.4	0.91
Filter Set-2 (0.45µ PVDF membrane)	365847	99.5	0.82
Filter Set-3 (0.45µ PVDF membrane)	365314	99.4	0.96

Table 13C: Filter paper study for Sample solution of Caffeine

Filter study	Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	316163	100.6	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	311923	99.2	1.36
Filter Set-2 (0.45µ Nylon membrane)	312412	99.4	1.20
Filter Set-3 (0.45µ Nylon membrane)	313536	99.7	0.84
Filter Set-1 (0.45µ PVDF membrane)	312293	99.3	1.24
Filter Set-2 (0.45µ PVDF membrane)	312760	99.5	1.09
Filter Set-3 (0.45µ PVDF membrane)	312527	99.4	1.16

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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

Filter study	Area of sample solution	Assay in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	3061269	100.7	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	3013828	99.2	1.57
Filter Set-2 (0.45µ Nylon membrane)	3026447	99.6	1.15
Filter Set-3 (0.45µ Nylon membrane)	3026663	99.6	1.14
Filter Set-1 (0.45µ PVDF membrane)	3019603	99.4	1.38
Filter Set-2 (0.45µ PVDF membrane)	3023125	99.5	1.26
Filter Set-3 (0.45µ PVDF membrane)	3024479	99.5	1.22

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 Nylon and PVDF) membrane within limit against that of unfiltered centrifuged sample. Hence both filter are suitable for filtration.

9.9 ROBUSTNESS:

Study Design:

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 14A: 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
Low wavelength (217nm)	112789	1.12	1.728	97.6	96.5	1.10
High wavelength (223nm)	113811	1.10	0.109		98.5	-0.90
Low flow rate (1.1ml/minute)	110598	1.08	0.180		97.1	0.50
High flow rate (1.3ml/minute)	117784	1.10	0.971		98.3	-0.70
Column oven Low Temperature (35ºC)	98771	1.07	0.341		96.4	1.20
Column oven High Temperature (45ºC)	123207	1.18	1.213		97.6	0.00

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 14B: Robustness of analytical method for Phenylephrine Hydrochloride

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Low wavelength (217nm)	7071	1.08	0.537	98.1	98.7	-0.60
High wavelength (223nm)	6898	1.08	0.155		98.5	-0.40
Low flow rate (1.1ml/minute)	7259	1.07	0.150		99.7	-1.60
High flow rate (1.3ml/minute)	6463	1.06	0.500		97.6	0.50
Column oven Low Temperature (35ºC)	6401	1.09	0.025		98.5	-0.40
Column oven High Temperature (45ºC)	7177	1.08	0.453		98.6	-0.50

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 14C: Robustness of analytical method for Caffeine

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)	Assay % (Method precision)	Mean %Assay	Absolute % Difference
Low wavelength (217nm)	81223	1.08	0.182	100.1	99.8	0.30
High wavelength (223nm)	81623	1.08	0.066		99.7	0.40
Low flow rate (1.1ml/minute)	79744	1.07	0.036		100.5	-0.40
High flow rate (1.3ml/minute)	83103	1.08	0.267		100.3	-0.20
Column oven Low Temperature (35ºC)	71913	1.08	0.047		100.4	-0.30
Column oven High Temperature (45ºC)	88153	1.09	0.571		100.2	-0.10

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

Table 14D: 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
Low wavelength (217nm)	102677	1.14	0.166	96.6	97.8	-1.20
High wavelength (223nm)	102369	1.14	0.019		98.2	-1.60
Low flow rate (1.1ml/minute)	123090	1.20	0.054		98.5	-1.90
High flow rate (1.3ml/minute)	65939	1.03	0.071		98.4	-1.80
Column oven Low Temperature (35ºC)	117172	1.20	0.032		98.5	-1.90
Column oven High Temperature (45ºC)	34089	0.82	0.968		98.2	-1.60

Acceptance criteria:

1) Theoretical plates for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peaks should be NLT 2000.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

2) Tailing Factor for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

10.0 SUMMARY:

S.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.	**Chlorphenamine Maleate**:0.873 **Phenylephrine Hydrochloride**: 0.319 **Caffeine:** 0.624 **Paracetamol:** 0.078
		2) Theoretical plate should be not less than 2000.	**Chlorphenamine Maleate**: 113797 **Phenylephrine Hydrochloride**: 8068 **Caffeine:** 90799 **Paracetamol:** 138502
		3) Tailing factor should not be more than 2.0.	**Chlorphenamine Maleate:**1.07 **Phenylephrine Hydrochloride**:1.09 **Caffeine:**1.03 **Paracetamol:** 1.21

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results
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 pass	Blank and Placebo peaks are not interfere with Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak in test preparation and Peak purity passes within specified limits.
3	Linearity and Range	1) R^2 Should be NLT 0.999	Squared correlation coefficient for **Chlorphenamine Maleate:**0.9998 **Phenylephrine Hydrochloride:** 0.9999 **Caffeine:** 0.9999 **Paracetamol:** 0.9998

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

<table>
<tr><th>S.No</th><th>Validation parameter</th><th>Acceptance criteria</th><th>Results</th></tr>
<tr><td></td><td>Linearity and Range</td><td>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.</td><td>Chlorphenamine Maleate:
<table><tr><th>Level</th><th>%RSD</th></tr><tr><td>10%</td><td>1.301</td></tr><tr><td>50%</td><td>0.135</td></tr><tr><td>75%</td><td>0.122</td></tr><tr><td>100%</td><td>0.234</td></tr><tr><td>125%</td><td>0.290</td></tr><tr><td>150%</td><td>0.381</td></tr></table>Phenylephrine HCL:
<table><tr><th>Level</th><th>%RSD</th></tr><tr><td>10%</td><td>0.130</td></tr><tr><td>50%</td><td>0.415</td></tr><tr><td>75%</td><td>0.891</td></tr><tr><td>100%</td><td>0.111</td></tr><tr><td>125%</td><td>0.351</td></tr><tr><td>150%</td><td>0.249</td></tr></table></td></tr>
</table>

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results

Caffeine:

Level	%RSD
10%	0.451
50%	0.179
75%	0.152
100%	0.132
125%	0.160
150%	0.107

Paracetamol:

Level	%RSD
10%	0.067
50%	0.048
75%	0.115
100%	0.015
125%	0.115
150%	0.071

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.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 0.995 according to Lab solution software.	There is No any interference due to degradants with analyte in stressed samples and Peak purity passes according to Lab solution software.
5	Accuracy (Recovery)	The mean % recovery at each level should be 98.0 to 102.0.	Level %Recovery **Chlorphenamine Maleate:** 50% : 98.8 100% : 98.4 150% : 98.7

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results
			Phenylephrine HCL: 50% : 101.0 100% : 101.4 150% : 99.3 **Caffeine:** 50% : 98.9 100% : 101.2 150% : 101.3 **Paracetamol:** 50% : 101.9 100% : 99.7 150% : 98.2
6	**Precision** 1) System Precision	%RSD of area of analyte peaks in five replicate standard injections should not be more than 2.0.	**Chlorphenamine Maleate:** 1.070 **Phenylephrine Hydrochloride:** 0.319

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results
			Caffeine: 1.030 **Paracetamol:** 0.078
	2) Method Precision	%RSD of Assay of six preparations should not be more than 2.0.	**Chlorphenamine Maleate:**0.491 **Phenylephrine Hydrochloride:** 0.840 **Caffeine:** 0.912 **Paracetamol:** 1.070
	3)Intermediate Precision	1) % RSD for assay of six preparations should not be more than 2.0.	**Chlorphenamine Maleate:** 1.505 **Phenylephrine Hydrochloride:** 1.193 **Caffeine:** 0.715 **Paracetamol**: 0.468

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results
		2) Cumulative %RSD for assay of twelve preparations (of method and intermediate precision) should not be more than 2.0.	**Chlorphenamine Maleate:** 1.220 **Phenylephrine Hydrochloride:** 1.690 **Caffeine:** 0.794 **Paracetamol:** 1.422
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 upto 28hours at room temperature.
8	Filter paper study (0.45µ Nylon and PVDF)	The % difference on filter solution should not differ ±2.0 against that of unfiltered.	The % difference on filtered sample 0.45µ Nylon and PVDF within limit against that of unfiltered (centrifuged).

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

S.No	Validation parameter	Acceptance criteria	Results
9	Robustness (i) Flow rate change (ii) Wavelength change (iii) Column oven 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 Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablet for the parameters of system suitability, specificity, Degradation, System Precision, method precision, Intermediate precision, Linearity and range and accuracy, Filter paper study and solution stability and Robustness 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.

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

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

REPORT	
Title	Analytical Method Validation Report For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets.
Report No.	ST/AMVAR/23/005

13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVAR/23/005	30/09/2023	New Report prepared.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

ANALYTICAL METHOD VALIDATION REPORT FOR THE TEST OF DISSOLUTION OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

1.0 INDEX

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

CONTENTS

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

2.0 REPORT APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Dy. Manager-QC
Signature	:	
Date	:	16/10/2023
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM-QC
Signature	:	
Date	:	17/10/2023
APPROVED BY		
Name	:	S. MARAN
Designation	:	AGM-QA
Signature	:	
Date	:	18/10/2023

Effective Date	:	19/10/2023

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

3.0 OBJECTIVE

To validate the method for test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

SPECIFICATION LIMIT : Not less than 80%

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. M. Bhavyasri

2. S. Suganthi

3. C.Albin jose

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

5.0 DETAILS OF STANDARD,SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., test samples/placebo to be used during Validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	G17230824	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Chlorphenamine Maleate BP	W.S.No: ST/WS/22/039	99.7% (As such basis)
Phenylephrine HCL BP	W.S.No: 2-WS15IC	99.8% (As such basis)
Caffeine BP	W.S.No: ST/WS/22/038	99.7% (As such basis)
Paracetamol BP	W.S.No: ST/WS/23/012	99.9% (As such basis)
API Chlorphenamine Maleate BP	AR.No:MSP/2223/QCG/QCR/00994	100.29% (As such basis)
Phenylephrine HCL BP	AR.No:MSP/2223/QCG/QCR/01040	99.54% (As such basis)
Caffeine BP	AR.No:MSP/2223/QCG/QCR/00973	99.77% (As such basis)
Paracetamol BP	AR.No:MSP/2223/QCG/QCR/01049	99.94% (As such basis)

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED :

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C.

High performance liquid chromatograph with PDA detector

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

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

COLUMN :

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

SOLVENTS AND CHEMICALS WITH GRADE :

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine Anhydrous (Working standard)

Paracetamol (Working standard)

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Potassium Dihydrogen orthophosphate (AR grade)

Orthophosphoric acid (AR grade)

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

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Sodium Hydroxide (AR grade)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Apparatus	:	Apparatus 2 (Paddle)
Medium	:	900ml of PH 6.8 Phosphate buffer
Time	:	45 minutes
Speed	:	75 RPM
Temperature	:	37° C ± 0.5 ° C

Preparation of Dissolution medium:

Dissolve 68 gm of Potassium dihydrogen phosphate and 9.8 gm of Sodium hydroxide pellets in 10 liters of purified water and mix well. Adjust pH 6.8±0.05 with dilute Sodium hydroxide or dilute Orthophosphoric acid and mix well.

Preparation of Buffer:

Weigh accurately about 6.8 g of potassium Di-hydrogen orthophosphate in 1000 mL of Milli-Q water, sonicate to dissolve. Adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Chromatographic Conditions:

Column	:	Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	220 nm
Column Temperature	:	40°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	20 µL
Run time	:	30.01 Minutes
Retention time	:	About 4.6 minutes for Phenylephrine Hydrochloride, about 10.5 minutes for Paracetamol, about 12.7 minutes for Caffeine and about 14.8 minutes for Chlorphenamine maleate,

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	100	0
6.0	100	0
7.0	70	30
9.0	70	30
10.0	45	55
25.0	45	55
27.0	100	0
30.01	100	0

Preparation of Mobile phase-A:

Prepare a degassed mixture of buffer and acetonitrile in the ratio 95:5 *v/v*.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Preparation of Mobile phase-B:

100% Methanol

Preparation of Diluent:

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

Preparation of Standard stock solution:

Weigh accurately and transfer about 28 mg of Chlorphenamine maleate working standard and 68 mg of Phenylephrine Hydrochloride working standard and 83 mg of Caffeine working standard into a 250 mL volumetric flask, add 20 mL of diluent and sonicate to dissolve and make up to volume with dissolution medium and mix. (**Concentration:** For Chlorphenamine maleate:0.112mg/ml, For Phenylephrine HCL:0.272mg/ml, for Caffeine:0.332mg/ml)

Preparation of Standard solution:

Weigh accurately and transfer about 22 mg of Paracetamol working standard into a 200 mL volumetric flask. Add 20 mL of diluent and sonicate to dissolve and add 4 mL of Standard stock solution and make up to volume with dissolution medium and mix. (**Concentration**: For Chlorphenamine maleate:0.00224mg/ml, For Phenylephrine HCL:0.00544mg/ml, for Caffeine:0.00664mg/ml) and Paracetamol:0.11mg/ml)

Test Preparation :

Preparation of sample solution(A) (For Chlorphenamine maleate and Phenylephrine Hydrochloride)

Set the dissolution parameters and place one tablet into each vessel individually containing 900 mL of dissolution medium, immediately start the apparatus. At the end of specified time withdraw the sample and filter through 0.45µ PVDF filter.

Preparation of Sample Solution-B:(For Paracetamol and Caffeine)

Further dilute 10 mL of above filtered solution to 50 mL with Dissolution medium and mix.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Procedure:

Inject the solutions as mentioned below and measure the responses of the peaks due to Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine.

Injection sequence:

S. No	Sample Name	No. of injections
1	Dissolution medium (blank)	1
2	Standard preparation	5
3	Sample solution A (1 injection each)	6
4	Sample solution B (1 injection each)	6
5	Bracketing standard	1 (After every 6 injections)

System suitability:

Theoretical plate count : NLT 2000 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Tailing factor : NMT 2.0 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Relative standard deviation : NMT 2.0% for five replicate injections of Paracetamol, Phenylephrine hydrochloride, Chlorphenamine maleate, Caffeine peak.

Calculations:

Calculate % drug release of Chlorphenamine maleate as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Where,

AT = 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.

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

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

Calculate % drug release of Phenylephrine Hydrochloride as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = 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.

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

LC = Label claim of Phenylephrine hydrochloride in mg/tablet.

Calculate % drug release of Caffeine as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{50}{10} \times \frac{P}{100} \times \frac{100}{LC}$$

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Where,

AT = Area of peak due to Caffeine in Sample solution B.
AS = Average area of peak due to Caffeine in standard preparation.
WS = Weight of Caffeine working standard in mg.
P = Potency of Caffeine working standard in % on as such basis.
LC = Label claim of Caffeine in mg/tablet.

Calculate % drug release of Paracetamol as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{900}{1} \times \frac{50}{10} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = 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.
P = Potency of Paracetamol working standard in % on as such basis.
LC = Label claim of Paracetamol in mg/tablet.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

8. VALIDATED PARAMETERS :

Following parameters shall be selected for validation	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Linearity and Range**
4	**Accuracy**
5	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
6	**Stability of analytical solution**
7	**Filter paper study**
8	**Robustness**

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

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

9.0 VALIDATION RESULTS :

9.1 SYSTEM SUITABILITY TEST:

Study Design:

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

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

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

3) % RSD of area of five replicate standard injections

Results are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Phenylepherine	Paracetamol	Caffeine	Chlorphenamine
Theoretical Plates	NLT 2000	10116	124691	117074	109475
Tailing Factor	NMT 2.0	1.11	1.19	1.07	1.11
% RSD	NMT 2.0	0.243	0.028	0.166	0.914

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Result and Conclusion:

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

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from Blank and Placebo

Study Summary:

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

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

Results are tabulated in Table 2.

Acceptance criteria:

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

2) Peak purity of analyte should be pass. (Peak purity value should not less than 0.950) by lab solution software.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Blank	Blank peak	NIL	Not applicable
2	Standard preparation	Phenylephrine	4.567	1.000
		Paracetamol	10.319	1.000
		Caffeine	12.386	1.000
		Chlorphenamine	14.691	1.000
3	Plain placebo	Placebo peaks	NIL	Not applicable
4	Phenylephrine Working standard	Phenylephrine	4.51	0.999
5	Paracetamol Working standard	Paracetamol	10.28	1.000
6	Caffeine Working standard	Caffeine	12.32	1.000
7	Chlorphenamine Working standard	Chlorphenamine	14.63	1.000

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
8	Plain placebo + Phenylephrine + Paracetamol + Caffeine + Chlorphenamine std	Phenylephrine	4.52	1.000
		Paracetamol	10.28	1.000
		Caffeine	12.31	1.000
		Chlorphenamine	14.62	1.000
9	Test preparation -A	Phenylephrine	4.53	1.000
		Chlorphenamine	14.64	1.000
10	Test preparation -B	Paracetamol	10.28	1.000
		Caffeine	12.32	1.000

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with Principal peak in sample preparation and Peak purity passes within specified limits. Hence method is selective and specific.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

9.3 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Working standard were prepared over the range of 10% to 150% concentration with respect to target concentration (i.e. 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 and 3D for Linearity and Table 4 for Range.

Acceptance criteria:

1) The squared correlation coefficient should not be less than 0.995.

2) % RSD for peak areas of linearity levels 50%, 75%, 100%, 125%& 150% should not be more than 2.0 for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peaks.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 3A: Linearity Table for Chlorphenamine Maleate

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	1.126	34796
75%	1.689	50593
100%	2.252	66427
125%	2.815	83516
150%	3.378	101218
Slope		29444
CC		0.999
Sqaured R		0.9993
Intercept		1002.9

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Fig.1 : Liner Graph for Chlorphenamine Maleate

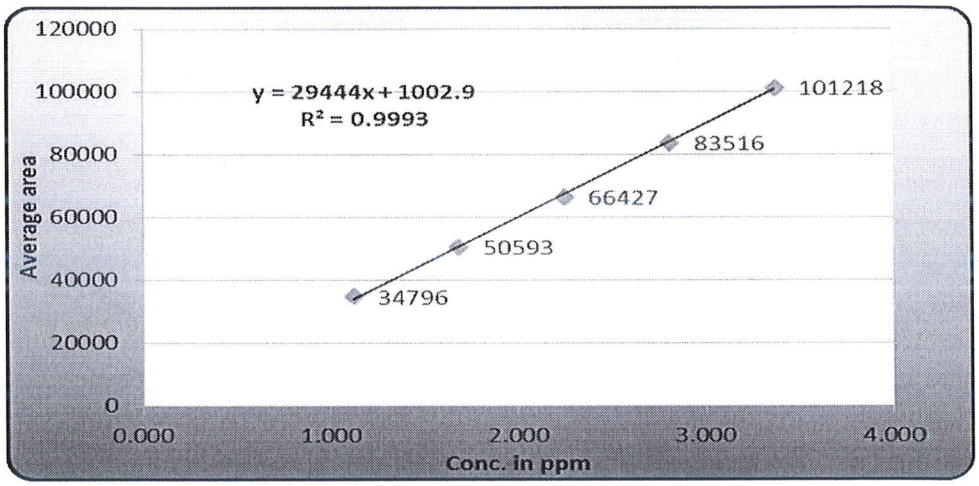

Table 3B: Linearity Table for Phenylephrine Hydrochloride

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	2.720	68848
75%	4.080	102495
100%	5.440	134970
125%	6.800	169099
150%	8.160	203378
Slope		24681
CC		0.999
Sqaured R		0.9999
Intercept		1492.3

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Fig.2 : Liner Graph for Phenylephrine Hydrochloride

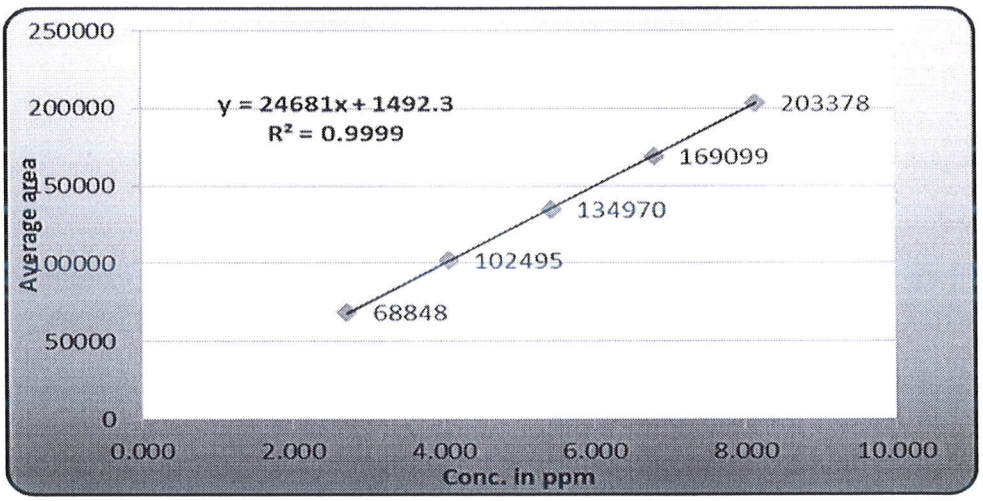

Table 3C: Linearity Table for Caffeine

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	3.339	156619
75%	5.009	232637
100%	6.678	308172
125%	8.348	386597
150%	10.017	468047
Slope		46530
CC		0.999
Sqaured R		0.9998
Intercept		312.47

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Fig.3 : Liner Graph for Caffeine

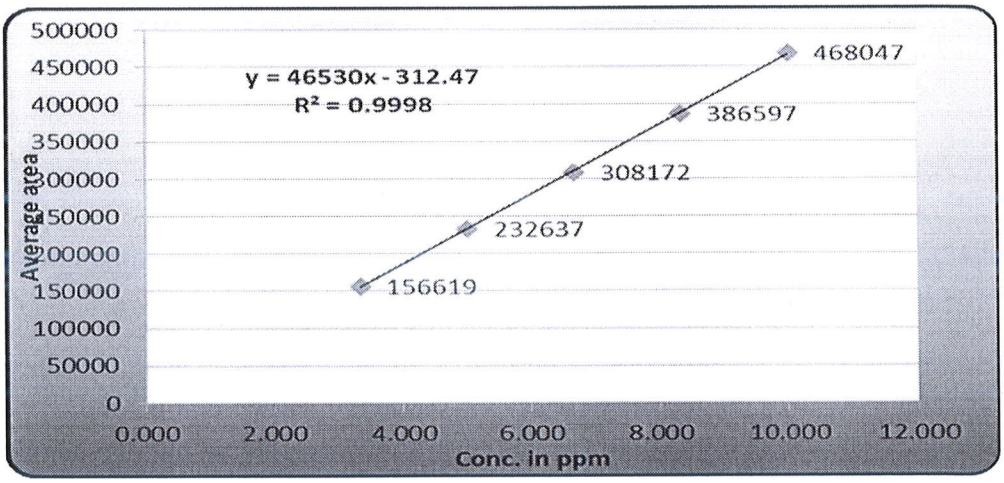

Table 3D: Linearity Table for Paracetamol

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	55.048	1748354
75%	82.572	2577568
100%	110.096	3400219
125%	137.620	4249964
150%	165.144	5140116
Slope		30722
CC		0.999
Sqaured R		0.9997
Intercept		40876

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Fig.4 : Liner Graph for Paracetamol

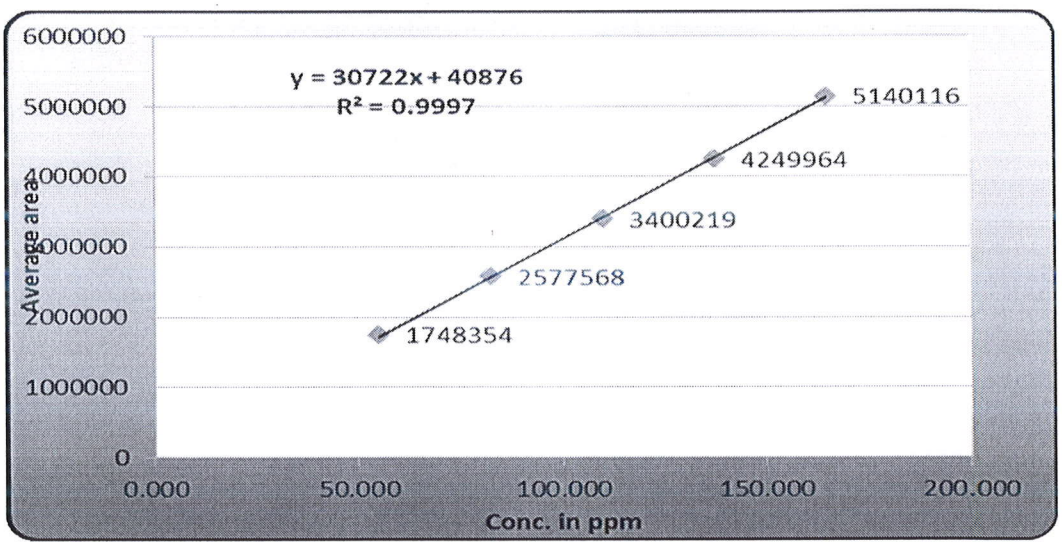

Table:4 Range for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol

Linearity Levels (%)	Chlorphenamine Maleate	Phenylephrine Hydrochloride	Caffeine	Paracetamol
50%	1.857	0.026	0.514	0.044
75%	0.112	0.016	0.045	0.005
100%	0.858	0.057	0.016	0.022
125%	0.530	0.096	0.044	0.008
150%	0.435	0.077	0.044	0.020

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Result and Conclusion:

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

9.4 ACCURACY STUDY (RECOVERY STUDY)

Study Design:

Known quantity of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol 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 and inject only one injection for each sample. The samples were analyzed as per the proposed method. The results are tabulated in Table 5A, 5B, 5C and 5D for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol respectively to demonstrate the accuracy of the method.

The mean % recovery at each level for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol should be 95.0 to 105.0.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 5A: Accuracy for Chlorphenamine Maleate

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	99.65	97.7	1.728
	2	96.76		
	3	96.68		
100%	1	99.28	97.6	1.470
	2	96.89		
	3	96.71		
150%	1	100.78	101.3	0.441
	2	101.50		
	3	101.60		

Table 5B: Accuracy for Phenylephrine Hydrochloride

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	98.86	99.2	0.841
	2	100.21		
	3	98.67		
100%	1	100.03	99.9	0.271
	2	99.63		
	3	100.14		
150%	1	100.41	100.0	0.339
	2	99.87		
	3	99.79		

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 5C: Accuracy for Caffeine

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	99.25	98.6	0.668
	2	97.94		
	3	98.48		
100%	1	99.13	99.0	0.331
	2	99.17		
	3	98.59		
150%	1	99.79	99.6	0.380
	2	99.19		
	3	99.89		

Table 5D: Accuracy for Paracetamol

Recovery level	Sample No.	% Recovery	Mean	% RSD
50%	1	102.06	101.7	0.266
	2	101.60		
	3	101.59		
100%	1	99.71	99.8	0.150
	2	99.75		
	3	99.99		
150%	1	99.69	99.3	0.671
	2	98.48		
	3	99.58		

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Result and Conclusion:

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

9.5 PRECISION:

9.5.1 SYSTEM PRECISION

Study design:

Five replicate injections of standard preparation were injected into the HPLC system. The area response for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Peaks along with % RSD are tabulated in Table 6.

Acceptance criteria:

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

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 6: System precision

Injection No.	Phenylephrine Dissolution in %	Paracetamol Dissolution in %	Caffeine Dissolution %	Chlorphenamine Dissolution in %
1	141150	3471587	319944	64556
2	141829	3478514	319149	66500
3	141585	3480358	319021	65320
4	141446	3479602	318803	66065
5	141305	3478736	318518	65278
Mean	141463	3477759	319087	65544
% RSD	0.184	0.101	0.168	1.152

Results and Conclusion:

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

9.5.2 Method Precision:

Study Design:

Six Dissolution preparations of sample were analyzed as per the method. The Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol is calculated. The results are tabulated in Table 7.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Acceptance criteria:

% RSD for Dissolution of Six sample preparations should not be more than 5.0.

Table 7: Method precision

No. of Preparation	Phenylephrine Dissolution in %	Paracetamol Dissolution in %	Caffeine Dissolution %	Chlorphenamine Dissolution in %
1	98.87	99.56	99.06	98.23
2	100.63	101.43	101.28	99.05
3	97.39	101.53	101.28	99.68
4	94.22	100.47	103.64	95.84
5	95.51	96.49	96.54	91.68
6	93.28	96.37	92.86	91.53
Mean	96.6	99.3	99.1	96.0
% RSD	2.92	2.36	3.92	3.80

Results and Conclusion:

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

9.5.3 Intermediate Precision (Ruggedness):

Study summary:

Six Dissolution preparations of sample were analyzed as per the method by different analyst using different instrument and different column on different day.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

The Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol is calculated. The results are tabulated in Table 8 and cumulative results are tabulated in Table 8.

Acceptance criteria:

1) % RSD for Dissolution of Six sample preparations should not be more than 5.0.

2) Cumulative % RSD for Dissolution of twelve sample preparations of (method and intermediate precision) should not be more than 5.0%.

Table 8: Intermediate precision

No. of Preparation	Phenylephrine Dissolution in %	Paracetamol Dissolution in %	Caffeine Dissolution %	Chlorphenamine Dissolution in %
1	96.93	100.29	99.69	93.76
2	106.27	104.49	102.16	93.06
3	96.49	100.72	97.14	92.95
4	101.66	97.55	95.86	92.77
5	94.94	99.78	98.46	94.45
6	95.96	99.97	102.19	102.76
Mean	98.7	100.5	99.3	95.0
% RSD	4.43	2.25	2.62	4.08

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 9.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 9: Cumulative % RSD

Parameter	Phenylephrine Dissolution in %	Paracetamol Dissolution in %	Caffeine Dissolution in %	Chlorphenamine Dissolution in %
Method Precision	98.87	99.56	99.06	98.23
	100.63	101.43	101.28	99.05
	97.39	101.53	101.28	99.68
	94.22	100.47	103.64	95.84
	95.51	96.49	96.54	91.68
	93.28	96.37	92.86	91.53
Intermediate Precision	96.93	100.29	99.69	93.76
	106.27	104.49	102.16	93.06
	96.49	100.72	97.14	92.95
	101.66	97.55	95.86	92.77
	94.94	99.78	98.46	94.45
	95.96	99.97	102.19	102.76
Mean	97.7	99.9	99.2	95.5
% RSD	3.76	2.28	3.18	3.80

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Result and Conclusion:

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

9.6 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 10A, 10B, 10C and 10D.

Table 10A: Stability of sample solution for Chlorphenamine Maleate

Time in hours	Area of Sample solution	Absolute % Difference
Initial	65033	Not applicable
5	64944	0.14
7	65241	-0.32
9	64235	1.24
12	64137	1.40
Mean	64718	0.61
% RSD	0.771	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 10B: Stability of sample solution for Phenylephrine Hydrochloride

Time in hours	Area of Sample solution	Absolute % Difference
Initial	127669	Not applicable
5	127777	-0.08
7	127605	0.05
9	127477	0.15
12	127632	0.03
Mean	127632	0.04
% RSD	0.085	Not applicable

Table 10C: Stability of sample solution for Caffeine

Time in hours	Area of Sample solution	Absolute % Difference
Initial	310171	Not applicable
5	310898	-0.23
7	310050	0.04
9	310248	-0.02
12	310754	-0.19
Mean	310424	-0.10
% RSD	0.121	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 10D: Stability of sample solution for Paracetamol

Time in hours	Area of Sample solution	Absolute % Difference
Initial	3379760	Not applicable
5	3378986	0.02
7	3377156	0.08
9	3372746	0.21
12	3338928	1.22
Mean	3369515	0.38
% RSD	0.514	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 11A, 11B, 11C and 11D.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 11A: Stability of standard solution for Chlorphenamine Maleate

Time in hours	Area of Standard solution	Absolute % Difference
Initial	67258	Not applicable
5	66508	1.13
7	66328	1.40
9	66553	1.06
12	66032	1.86
Mean	66536	1.36
% RSD	0.681	Not applicable

Table 11B: Stability of standard solution for Phenylephrine Hydrochloride

Time in hours	Area of Standard solution	Absolute % Difference
Initial	136094	Not applicable
5	134422	1.24
7	133784	1.73
9	134045	1.53
12	133532	1.92
Mean	134375	1.60
% RSD	0.756	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 11C: Stability of standard solution for Caffeine

Time in hours	Area of Standard solution	Absolute % Difference
Initial	319637	Not applicable
5	317898	0.55
7	317943	0.53
9	318578	0.33
12	318327	0.41
Mean	318477	0.46
% RSD	0.171	Not applicable

Table 11D: Stability of standard solution for Paracetamol

Time in hours	Area of Standard solution	Absolute % Difference
Initial	3436107	Not applicable
5	3436638	-0.02
7	3436413	-0.01
9	3437039	-0.03
12	3437063	-0.03
Mean	3436652	-0.02
% RSD	0.012	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Results and conclusions:

The Standard solution and Sample solution was stable upto 12 hours at room temperature.

9.7 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method was performed by filtering test solution through 0.45µ Nylon and PVDF filter against that of unfiltered sample. The results were tabulated in Table 12A, 12B, 12C and 12D

Table 12A: Filter paper study for Sample solution of Chlorphenamine Maleate

Filter study	Area of sample solution	Dissolution in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	64510	97.4	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	65766	99.3	-1.91
Filter Set-2 (0.45µ Nylon membrane)	64482	97.4	0.04
Filter Set-3 (0.45µ Nylon membrane)	65262	98.5	-1.15
Filter Set-1 (0.45µ PVDF membrane)	63738	96.2	1.21
Filter Set-2 (0.45µ PVDF membrane)	64131	96.8	0.59
Filter Set-3 (0.45µ PVDF membrane)	63662	96.1	1.33

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 12B: Filter paper study for Sample solution of Phenylephrine Hydrochloride

Filter study	Area of sample solution	Dissolution in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	131859	94.9	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	132548	95.4	-0.52
Filter Set-2 (0.45µ Nylon membrane)	132198	95.2	-0.26
Filter Set-3 (0.45µ Nylon membrane)	132157	95.2	-0.23
Filter Set-1 (0.45µ PVDF membrane)	131916	95.0	-0.04
Filter Set-2 (0.45µ PVDF membrane)	132194	95.2	-0.25
Filter Set-3 (0.45µ PVDF membrane)	132582	95.5	-0.55

Table 12C: Filter paper study for Sample solution of Caffeine

Filter study	Area of sample solution	Dissolution in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	306154	97.5	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	306225	97.5	-0.02
Filter Set-2 (0.45µ Nylon membrane)	305670	97.4	0.16
Filter Set-3 (0.45µ Nylon membrane)	305593	97.3	0.18
Filter Set-1 (0.45µ PVDF membrane)	311588	99.2	-1.74
Filter Set-2 (0.45µ PVDF membrane)	306802	97.7	-0.21
Filter Set-3 (0.45µ PVDF membrane)	306166	97.5	0.00

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 12D: Filter paper study for Sample solution of Paracetamol

Filter study	Area of sample solution	Dissolution in %	% difference from unfiltered sample
Unfiltered sample (Centrifuged)	3420029	99.1	Not applicable
Filter Set-1 (0.45µ Nylon membrane)	3424877	99.3	-0.14
Filter Set-2 (0.45µ Nylon membrane)	3419843	99.1	0.01
Filter Set-3 (0.45µ Nylon membrane)	3419816	99.1	0.01
Filter Set-1 (0.45µ PVDF membrane)	3483389	101.0	-1.82
Filter Set-2 (0.45µ PVDF membrane)	3429750	99.4	-0.28
Filter Set-3 (0.45µ PVDF membrane)	3423778	99.2	-0.11

Acceptance criteria:

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

Results and conclusions:

The results are well within the acceptance criteria and the study proved the compatibility of filter paper.

9.8 ROBUSTNESS:

Study Design:

Standard preparation were injected varying different chromatographic conditions as per protocol. System suitability parameters and mean dissolution difference with respect to dissolution value in method precision were calculated. The results are tabulated in table 13A, 13B, 13C, 13D, 13E and 13F Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peaks respectively.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

The sample Preparations are injected variation difference dissolution condition as per protocol. The results are tabulated in table 14A, 14B, 14C and 14D.

Table 13A: Robustness of analytical method for Chlorphenamine Maleate

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)
Low wavelength (217nm)	108047	1.10	0.446
High wavelength (223nm)	106943	1.07	0.139
Low flow rate (1.1ml/minute)	103130	1.05	0.438
High flow rate (1.3ml/minute)	109074	1.08	0.978
Column oven Low Temperature (35ºC)	86284	1.02	0.430
Column oven High Temperature (45ºC)	124984	1.08	0.709

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 13B: Robustness of analytical method for Phenylephrine Hydrochloride

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)
Low wavelength (217nm)	10222	1.00	0.037
High wavelength (223nm)	10461	1.08	0.085
Low flow rate (1.1ml/minute)	10885	1.03	0.125
High flow rate (1.3ml/minute)	9727	1.04	0.027
Column oven Low Temperature (35ºC)	10011	1.09	0.041
Column oven High Temperature (45ºC)	10580	1.11	0.099

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 13C: Robustness of analytical method for Caffeine

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)
Low wavelength (217nm)	108034	1.06	0.119
High wavelength (223nm)	108696	1.06	0.069
Low flow rate (1.1ml/minute)	132185	1.09	0.145
High flow rate (1.3ml/minute)	85085	1.04	0.071
Column oven Low Temperature (35ºC)	120780	1.08	0.132
Column oven High Temperature (45ºC)	92897	1.05	0.127

Table 13D: Robustness of analytical method for Paracetamol

Parameter	Theoretical Plates (NLT 2000)	Tailing Factor (NMT 2.0)	% RSD (NMT 2.0)
Low wavelength (217nm)	131313	0.03	0.030
High wavelength (223nm)	133100	1.20	0.027
Low flow rate (1.1ml/minute)	119763	1.18	0.009
High flow rate (1.3ml/minute)	138750	1.22	0.046
Column oven Low Temperature (35ºC)	107674	1.16	0.027
Column oven High Temperature (45ºC)	148716	1.24	0.030

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 14A: Robustness of analytical method

Chromatographic variation's	Phenylephrine Dissolution in %	Mean value of Method precision	Paracetamol Dissolution in %	Mean value of Method precision
Low volume Dissolution Medium	102.51	96.7	98.19	99.3
	102.27		96.52	
	99.48	% Difference	99.21	% Difference
	95.96		98.18	
	91.06	-2.1	96.52	1.3
	101.60		99.20	
Average	**98.8**	**Not applicable**	**98.0**	**Not applicable**
% RSD	**4.57**		**1.24**	

Chromatographic variation's	Caffeine Dissolution in %	Mean value of Method precision	Chlorphenamine Dissolution in %	Mean value of Method precision
Low volume Dissolution Medium	96.02	99.1	95.79	96.0
	93.06		91.60	
	93.54	% Difference	95.11	% Difference
	95.97		91.00	
	92.84	4.9	93.24	3.1
	93.53		90.65	
Average	**94.2**	**Not applicable**	**92.9**	**Not applicable**
% RSD	**1.54**		**2.34**	

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 14B: Robustness of analytical method

Chromatographic variation's	Phenylephrine Dissolution in %	Mean value of Method precision	Paracetamol Dissolution in %	Mean value of Method precision
High volume Dissolution Medium	94.31	96.7	96.68	99.3
	99.73		98.43	
	95.53	**% Difference**	97.03	**% Difference**
	97.20		96.66	
	86.28	2.3	98.50	1.9
	93.41		97.00	
Average	**94.4**	Not applicable	**97.4**	**Not applicable**
% RSD	**4.84**		**0.88**	

Chromatographic variation's	Caffeine Dissolution in %	Mean value of Method precision	Chlorphenamine Dissolution in %	Mean value of Method precision
High volume Dissolution Medium	97.11	99.1	95.86	96.0
	94.69		95.19	
	95.61	**% Difference**	91.58	**% Difference**
	97.25		89.10	
	95.24	3.2	84.84	4.5
	95.69		92.37	
Average	**95.9**	**Not applicable**	**91.5**	**Not applicable**
% RSD	**1.07**		**4.47**	

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 14C: Robustness of analytical method

Chromatographic variation's	Phenylephrine Dissolution in %	Mean value of Method precision	Paracetamol Dissolution in %	Mean value of Method precision
Low pH Dissolution Medium	101.78	96.7	103.75	99.3
	96.49		103.67	
	101.05	% Difference	103.58	% Difference
	97.26		103.50	
	98.47	-1.9	103.50	-4.3
	96.33		103.64	
Average	**98.6**	**Not applicable**	**103.6**	**Not applicable**
% RSD	**2.38**		**0.096**	

Chromatographic variation's	Caffeine Dissolution in %	Mean value of Method precision	Chlorphenamine Dissolution in %	Mean value of Method precision
Low pH Dissolution Medium	103.52	99.1	96.76	96.0
	103.68		92.73	
	103.09	% Difference	95.47	% Difference
	103.25		94.24	
	103.17	-4.2	101.47	-0.1
	103.32		95.7	
Average	**103.3**	**Not applicable**	**96.1**	**Not applicable**
% RSD	**0.22**		**3.11**	

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Table 14D: Robustness of analytical method

Chromatographic variation's	Phenylephrine Dissolution in %	Mean value of Method precision	Paracetamol Dissolution in %	Mean value of Method precision
High pH Dissolution Medium	92.81	96.7	98.01	99.3
	96.39		98.21	
	101.22	% Difference	97.52	% Difference
	97.43		96.67	
	98.63	-0.5	96.43	2.2
	96.94		95.88	
Average	**97.2**	**Not applicable**	**97.1**	**Not applicable**
% RSD	**2.84**		**0.96**	

Chromatographic variation's	Caffeine Dissolution in %	Mean value of Method precision	Chlorphenamine Dissolution in %	Mean value of Method precision
High pH Dissolution Medium	98.8	99.1	90.89	96.0
	99.02		91.24	
	97.01	% Difference	96.33	% Difference
	96.88		93.17	
	95.46	2.0	100.88	1.4
	95.17		95.28	
Average	**97.1**	**Not applicable**	**94.6**	**Not applicable**
% RSD	**1.66**		**3.95**	

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

Acceptance criteria:

1) Theoretical plates for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peaks should be NLT 2000.

2) Tailing Factor for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peaks should be NMT 2.0.

3) % RSD of area of five replicates standard injections should be NMT 2.0.

4) % Dissolution result shall meet the specification.

5) Relative standard deviation of % dissolution results should not be more than 5.0%.

6) The difference in average % dissolution with actual dissolution medium volume and with changed dissolution medium volume shall NMT 5.0.

Result and Conclusion:

Each chromatographic variation System suitability parameters are within limits.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

10.0 SUMMARY:

S.No	Validation parameter	Acceptance criteria	Results
1	System suitability	1) % R SD of area of analyte in five replicate standard injections should not be more than 2.0.	**Chlorphenamine Maleate**:0.914 **Phenylephrine Hydrochloride**:0.243 **Caffeine:** 0.166 **Paracetamol:** 0.028
		2) Theoretical plate should be not less than 2000.	**Chlorphenamine Maleate**: 109475 **Phenylephrine Hydrochloride**: 10116 **Caffeine:** 117074 **Paracetamol:** 124691
		3) Tailing factor should not be more than 2.0.	**Chlorphenamine Maleate:** 1.11 **Phenylephrine Hydrochloride**: 1.11 **Caffeine:** 1.07 **Paracetamol:** 1.19

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
2	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	There should not be any interference due to blank, impurity and placebo with analyte.	Blank and Placebo peaks are not interfere with Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak in test preparation and Peak purity passes within specified limits.
3	Linearity and Range	1) R^2 Should be NLT 0.999	Squared correlation coefficient for **Chlorphenamine Maleate:**0.9993 **Phenylephrine Hydrochloride:** 0.9999 **Caffeine:** 0.9998 **Paracetamol:** 0.9997

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

<table>
<tr><th>S.No</th><th>Validation parameter</th><th>Acceptance criteria</th><th>Results</th></tr>
<tr><td></td><td>Linearity and Range</td><td>2) To conclude the range, %RSD for peak area of linearity level-50%, 75%, 100%, 125% and 150% should be not more than 2.0.</td><td>Chlorphenamine Maleate:
<table>
<tr><th>Level</th><th>%RSD</th></tr>
<tr><td>50%</td><td>1.857</td></tr>
<tr><td>75%</td><td>0.112</td></tr>
<tr><td>100%</td><td>0.858</td></tr>
<tr><td>125%</td><td>0.530</td></tr>
<tr><td>150%</td><td>0.435</td></tr>
</table>
Phenylephrine HCL:
<table>
<tr><th>Level</th><th>%RSD</th></tr>
<tr><td>50%</td><td>0.026</td></tr>
<tr><td>75%</td><td>0.016</td></tr>
<tr><td>100%</td><td>0.057</td></tr>
<tr><td>125%</td><td>0.096</td></tr>
<tr><td>150%</td><td>0.077</td></tr>
</table></td></tr>
</table>

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
			Caffeine:

Level	%RSD
50%	0.514
75%	0.045
100%	0.016
125%	0.044
150%	0.044

Paracetamol:

Level	%RSD
50%	0.044
75%	0.005
100%	0.022
125%	0.008
150%	0.020

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
4	Accuracy (Recovery)	The mean % recovery at each level should be 95.0 to 105.0.	Level %Recovery **Chlorphenamine Maleate:** 50% : 97.7 100% : 97.7 150% : 101.3 **Phenylephrine HCL:** 50% : 99.2 100% : 99.9 150% : 100.0 **Caffeine:** 50% : 98.5 100% : 98.9 150% : 99.6 **Paracetamol:** 50% : 101.7 100% : 99.8 150% : 99.3

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
5	**Precision** 1) System Precision	%RSD of area of analyte peaks in six replicate standard injections should not be more than 2.0.	**Chlorphenamine Maleate:**1.152 **Phenylephrine Hydrochloride:**0.184 **Caffeine:**0.168 **Paracetamol:**0.101
	2) Method Precision	%RSD of dissolution of six preparations should not be more than 5.0.	**Chlorphenamine Maleate:** 3.80 **Phenylephrine Hydrochloride:** 2.92 **Caffeine:** 3.92 **Paracetamol:** 2.36
	3)Intermediate Precision	1) % RSD for dissolution of six preparations should not be more than 5.0.	**Chlorphenamine Maleate:** 4.08 **Phenylephrine Hydrochloride:** 4.43 **Caffeine:** 2.62 **Paracetamol**: 2.25

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
		2) Cumulative %RSD for dissolution of twelve preparations (of method and intermediate precision) should not be more than 5.0%.	**Chlorphenamine Maleate:** 3.80 **Phenylephrine Hydrochloride:** 3.76 **Caffeine**: 3.18 **Paracetamol:** 2.28
7	Stability for analytical solution	The standard and sample 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 upto 12hours at room temperature.
8	Filter paper study (0.45µ Nylon and PVDF)	The % difference on filter solution should not differ ±2.0 against that of unfiltered (Centrifuged).	The % difference on filtered sample 0.45µ Nylon and PVDF within limit against that of unfiltered (Centrifuged).

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

S.No	Validation parameter	Acceptance criteria	Results
9	Robustness (i) Flow rate change (ii) Wavelength change (iii) Column oven Temperature Change (iv) Dissolution medium volume change (v) Dissolution medium pH change	System suitability parameters should comply.	Each chromatographic variation System suitability parameters are within limits.

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

11.0 CONCLUSION:

Validation studies have been conducted for Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablet for the parameters of system suitability, specificity, System Precision, method precision, Intermediate precision, Linearity and range and accuracy, Filter paper study and solution stability and Robustness 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

REPORT	
Title	Analytical Method Validation Report For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVDR/23/005

13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVDR/23/005	19/10/2023	New Report prepared.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

ANALYTICAL METHOD VALIDATION REPORT FOR THE TEST OF RELATED SUBSTANCES OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

1.0 INDEX

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

CONTENTS

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

2.0 REPORT APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Dy. Manager - QC
Signature	:	
Date	:	28/10/2023
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM - QC
Signature	:	
Date	:	30/10/2023
APPROVED BY		
Name	:	S. MARAN
Designation	:	AGM - QA
Signature	:	
Date	:	31/10/2023

Effective Date	:	01/11/2023

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

3.0 OBJECTIVE

To Validate the method for test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST VALIDATION : Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

SPECIFICATION LIMIT : (i) Single maximum unknown impurity: NMT 0.20%
(ii) Total impurities:0.50%

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. S.Elavarasan
2. S. Bhavyasri
3. C.Albin jose

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

5.0 DETAILS OF STANDARD,SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., Impurities standard, test samples/placebo to be used during Validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	G17230824	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Chlorphenamine Maleate BP	WS.No: ST/WS/22/039	99.7% (as such basis)
Phenylephrine HCL BP	WS.No: 2-WS15IC	99.8% (as such basis)
Caffeine BP	WS.No: ST/WS/22/038	99.7% (as such basis)
Paracetamol BP	WS.No: ST/WS/23/012	99.9% (as such basis)
API Chlorphenamine Maleate BP	AR.No:MSP/2223/QCG/QCR/00994	100.29% (As such basis)
Phenylephrine HCL BP	AR.No:MSP/2223/QCG/QCR/01040	99.54% (As such basis)
Caffeine BP	AR.No:MSP/2223/QCG/QCR/00973	99.77% (As such basis)
Paracetamol BP	AR.No:MSP/2223/QCG/QCR/01049	99.94% (As such basis)

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED :

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

Column:

Kromasil C8, 250 mm X 4.6 mm, 5µm (or) equivalent

Solvents and chemicals with grade:

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine Anhydrous (Working standard)

Paracetamol (Working standard)

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Potassium Di-hydrogen orthophosphate

Orthophosphoric acid

Acetonitrile (HPLC grade)

Methanol (HPLC grade)

Hydrochloric Acid (AR grade)

Sodium Hydroxide (AR grade)

Hydrogen Peroxide 30% (AR grade)

Plain placebo

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column	:	Kromasil C8, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	220 nm
Column Temperature	:	30°C
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µl
Run time	:	15 minutes for Standard solution 40 minutes for Blank, System suitability solution, placebo solution and Sample solution
Retention time	:	About 3.5 minutes for Chlorphenamine maleate, about 4.1 minutes for Phenylephrine Hydrochloride, about 8.4 minutes for Paracetamol and about 20.5 minutes for Caffeine

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Preparation of Buffer:

Weigh accurately about 6.8g of potassium Di-hydrogen orthophosphate in 1000 mL of Purified water, sonicate to dissolve. Adjust pH 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

Preparation of Mobile phase:

Prepare a degassed mixture of buffer and methanol in the ratio 85:15 *v/v*.

Preparation of Diluent:

Prepare a degassed mixture of water and methanol in the ratio 80:20 *v/v*.

Preparation of Placebo solution:

Weigh accurately and transfer about 55 mg of Plain Placebo into 100mL volumetric flask. Add 50 mL of diluent and sonicate for 10 minutes with intermittent shaking, cool and make up to volume with diluent and mix. Further dilute 5 mL of above solution into 50 mL with diluent. Filter through 0.45µ PVDF filter.

Preparation of Standard solution:

Weigh accurately and transfer about 60 mg of Paracetamol working standard into a 100 mL volumetric flask. Add 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. Dilute 1 mL of this solution to 100 mL with diluent and mix. Further dilute to 5 mL of above solution into 50 mL with diluent and mix. (**Concentration:** 0.0006 mg/ml)

Preparation of system suitability stock solution:

Weigh accurately and transfer about 180 mg of Caffeine working standard, 12 mg of Chlorphenamine maleate working standard and 30 mg of Phenylephrine hydrochloride working standard into a 100 mL volumetric flask. Add 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. (**Concentration:** Caffeine:1.8mg/ml, Chlorphenamine maleate:0.12mg/ml, Phenylephrine Hcl:0.3mg/ml)

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Preparation of system suitability solution:

Weigh accurately and transfer about 30 mg of Paracetamol working standard into a 100 mL volumetric flask. Add 1 mL of system suitability standard stock solution and 50 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. (**Concentration:** Paracetamol:0.3mg/ml, Caffeine:0.018mg/ml, Chlorphenamine maleate:0.0012mg/ml)

Test preparation:

Weigh 20 tablets and calculate the average weight and make powder by using mortar and pestle. Weigh and transfer sample powder equivalent to 300 mg of Paracetamol, into a 100 mL volumetric flask. Add about 50 mL of diluent and sonicate for 10 minutes with intermittent shaking dilute up to the volume with diluent and mix. Further dilute 10 mL of above solution into 100 mL with diluent. Filter through 0.45µ PVDF filter.

Inject 20 µL of the above solution (blank, system suitability solution, standard, placebo and sample) as per following sequence and measure the area due to any unknown impurity peak.

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (blank)	1
2	System suitability solution	1
3	Standard solution	5
4	Placebo Preparation	1
5	Test preparation	1
6	Bracketing standard	1

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Calculation:

Single maximum unknown impurity:

$$= \frac{AT_I}{AS_T} \times \frac{WS}{100} \times \frac{1}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{100}{10} \times AW \times \frac{P}{100} \times \frac{100}{500}$$

Where,

AT_I	=	Area of peak due to Single maximum unknown impurity in test preparation.
AS_T	=	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.
AW	=	Average weight of tablet in mg.
P	=	Potency of Paracetamol working standard in % on as such basis.

Total impurities:

$$= \frac{AT_T}{AS_T} \times \frac{WS}{100} \times \frac{1}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{100}{10} \times AW \times \frac{P}{100} \times \frac{100}{500}$$

Where,

AT_T	=	Area of peak due to Total impurities in test preparation.
AS_T	=	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.
AW	=	Average weight of tablet in mg.
P	=	Potency of Paracetamol working standard in % on as such basis.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

8. VALIDATED PARAMETERS :

Following parameters shall be selected for VALIDATION	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Degradation**
	i) Acid degradation ii) Alkali Degradation iii) Oxidative Degradation
4	**Determination of limit of detection and limit of quantitation**
5	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
6	**LOQ Precision study & Observation at LOD**
7	**Linearity and Range**
8	**Stability of analytical solution**
9	**Filter paper study**
10	**Robustness**

Note: More than one parameter may be performed at once with relevant sequence having Common system suitability with bracketing preparation.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

9.0 VALIDATION RESULTS :

9.1 SYSTEM SUITABILITY TEST:

Study Summary:

Blank and five replicate injection of standard preparation were injected into HPLC and following system suitability parameters are evaluated.

1) Theoretical plate for Paracetamol peak.

2) Tailing Factor for Paracetamol peak.

3) % RSD of area of replicates injections of standard.

Results are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Paracetamol
Theoretical Plates	NLT 2000	13496
Tailing Factor	NMT 2.0	1.129
% RSD	NMT 2.0	0.694

Acceptance criteria:

i) % RSD area of Paracetamol peak in five replicate standard injections should not more than 5.0.

ii) Theoretical plates for Paracetamol peak in five replicate standard injection should not less than 2000.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

iii) Tailing factor for Paracetamol peak in six standard injection should not more than 2.0.

Result and Conclusion:

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

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and Placebo

Study Summary:

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

Peak purity of the analyte peak and the representative chromatograms of blank, standard, placebo and sample are attached. Results are tabulated in Table 2.

Acceptance criteria:

i) There should not be any interference due to blank and placebo peak with analyte.

ii) Peak purity of analyte should be pass (peak purity value should not less than 0.950) by Lab solution software.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Blank	Blank peak	NIL	Not applicable
2	System suitability Solution	Chlorphenamine	3.87	0.999
		Phenylephrine	4.49	1.000
		Paracetamol	9.74	0.991
		Caffeine	26.95	1.000
3	Standard preparation	Paracetamol	9.76	1.000
4	Plain placebo	Placebo peaks	Not applicable	Not applicable
5	Phenylephrine HCL-Std	Phenylephrine HCL	4.49	1.000
6	Chlorphenamine Maleate-Std	Chlorphenamine	3.87	1.000
7	Paracetamol-Std	Paracetamol	9.74	0.993
8	Caffeine- Std	Caffeine	26.95	1.000

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
9	Plain placebo + Phenylephrine + Paracetamol + Caffeine + Chlorphenamine std	Chlorphenamine	3.87	1.000
		Phenylephrine	4.49	1.000
		Paracetamol	9.74	0.991
		Caffeine	26.97	1.000
10	Test preparation	Chlorphenamine	3.88	1.000
		Phenylephrine	4.50	1.000
		Paracetamol	9.77	0.996
		Caffeine	27.04	1.000

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with retention time of principle peak in test preparation and Peak purity passes within specified limits. Hence method is selective and specific.

9.3 INTERFERENCE FROM DEGRADANTS (Forced degradation)

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

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

a) Acid Degradation:

Weigh 20 tablets and calculate the average weight and make powder by using mortar and pestle. Weigh and transfer sample powder equivalent to 300 mg of Paracetamol, into a 100 mL volumetric flask. Add about 50 mL of diluent and sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 5N Hydrochloric acid and heat on water bath at 80°C for 30 minutes, cool and neutralized with 5ml of 5N Sodium hydroxide and make up to volume with diluent. Further dilute 10ml of above solution 100ml with diluent. Filter through 0.45µ PVDF filter.

b) Alkali degradation:

Weigh 20 tablets and calculate the average weight and make powder by using mortar and pestle. Weigh and transfer sample powder equivalent to 300 mg of Paracetamol, into a 100 mL volumetric flask. Add about 50 mL of diluent and sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 5N Sodium hydroxide and heat on water bath at 80°C for 30 minutes, cool and neutralized with 5ml of 5N Hydrochloric acid and make up to volume with diluent. Further dilute 10ml of above solution 100ml with diluent. Filter through 0.45µ PVDF filter.

c) Oxidative Degradation:

Weigh 20 tablets and calculate the average weight and make powder by using mortar and pestle. Weigh and transfer sample powder equivalent to 300 mg of Paracetamol, into a 100 mL volumetric flask. Add about 50 mL of diluent and sonicate for 10 minutes with intermittent shaking cool. Add 5ml of 30% Hydrogen peroxide solution and heat on water bath at 80°C for 30 minutes and make up to volume with diluent. Further dilute 10ml of above solution 100ml with diluent. Filter through 0.45µ PVDF filter.

Acceptance criteria:

i) There should not any interference due to degradants with analyte and impurity in stressed samples.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

ii) The desired degradation should be 10-30% in acid, alkali and oxidation degradation, (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 of analyte and each impurity peak (above LOQ/0.1% level of test concentration whichever is higher) should be pass. (Peak purity should not less than 0.950 according to Lab solution.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 3: Peak purity (Chemical degradation)

S.No	Sample name	Single maximum unknown impurity	Total impurities	Peak Name	Peak purity
1	Acid Degradation	1.06	1.09	Chlorphenamine	1.000
				Phenylephrine	0.999
				Paracetamol	0.995
				Caffeine	1.000
2	Alkali Degradation	4.05	4.15	Chlorphenamine	0.994
				Phenylephrine	0.999
				Paracetamol	0.997
				Caffeine	0.999
3	Peroxide Degradation	0.13	0.20	Chlorphenamine	0.998
				Phenylephrine	1.000
				Paracetamol	0.990
				Caffeine	1.000

Results and conclusion:

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

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

9.4 DETERMINATION OF LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

Study design:

The detection and quantitation limit for Paracetamol was determined by the technique of regression plot of residuals against linearity concentration. The data is tabulated in table 4A and 4B.

Table 4A. LOD and LOQ determination for Paracetamol

Paracetamol		Residual output		
Conc. (ppm) (x-axis)	**Average area (y-axis)**	**Observation**	**Predicted Y**	**Residuals**
0.062	51508	1	51253.51603	254.150641
0.309	252248	2	257282.4853	-5034.151923
0.464	397612	3	386050.591	11561.40897
0.618	506033	4	514818.6968	-8785.696795
0.773	645187	5	643586.8026	1600.530769
0.927	772759	6	772354.9083	403.7583333
Slope	833181	**Standard deviation**		6914

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 4B: Limit of Detection and Limit of Quantitation for Paracetamol

Concentration		%	ppm
Observed	LOD	0.009	0.027
	LOQ	0.028	0.083
Determined	LOD	0.009	0.027
	LOQ	0.028	0.083

Results and conclusion:

Based on above results LOD and LOQ for Paracetamol is less than 0.02% and 0.04% respectively.

9.5 PRECISION:

9.5.1 System precision:

Study design:

Five replicate injections of standard preparation were injected into the HPLC system. The area response for Peaks along with % RSD are tabulated in Table 5.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 5: System precision

Injection No.	Area of Paracetamol
1	31643
2	31013
3	30861
4	31381
5	31269
Mean	31233
% RSD	0.984

Acceptance criteria:

% RSD of area of analyte peak in six replicate standard injections should not more than 5.0.

Results and Conclusion:

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

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

9.5.2 Method Precision:

Study summary:

Six sample preparations were analyzed as per the method. The results are tabulated in Table 6.

Acceptance criteria:

1) % RSD for impurities from LOQ to 0.1% in six preparations should be not more than 20.
2) % RSD for impurities above 0.1% in six preparations should be not more than 15.
3) % RSD for impurities in six preparations should be not more than 10.

Table 6: Method precision

Preparation No.	Single maximum unknown impurity in %	Total impurities in %
1	Not detected	Not detected
2	Not detected	Not detected
3	Not detected	Not detected
4	Not detected	Not detected
5	Not detected	Not detected
6	Not detected	Not detected
Mean	0.00	0.00
% RSD	0.00	0.00

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Results and Conclusion:

The method precision results are well within the acceptance criteria indicates the precision of the analytical method.

9.5.3 Intermediate Precision (Ruggedness):

Study summary:

Six sample preparations were analyzed as per the method by different analyst using different instrument and different column on different day. The results are tabulated in Table and cumulative results are tabulated in Table 7.

Table 7: Intermediate precision

Preparation No.	Single maximum unknown impurity in %	Total impurities in %
1	Not detected	Not detected
2	Not detected	Not detected
3	Not detected	Not detected
4	Not detected	Not detected
5	Not detected	Not detected
6	Not detected	Not detected
Mean	0.00	0.00
% RSD	0.00	0.00

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 8.

Acceptance criteria:

1) % RSD for impurities from LOQ to 0.1% in six preparations should be not more than 20.

2) % RSD for impurities above 0.1% in six preparations should be not more than 15.

3) % RSD for total impurities in six preparations should be not more than 10.

4) Cumulative % RSD of Total impurities of method precision and intermediate precision should be not more than 10.0.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 8: Cumulative % RSD

Parameter	Single maximum unknown impurity in %	Total impurities in %
Method Precision	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
Intermediate Precision	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
	Not detected	Not detected
Mean	0.00	0.00
% RSD	0.00	0.00

Result and Conclusion:

The Cumulative results are well within the acceptance criteria indicates the precision of the analytical method.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

9.6 LOQ PRECISION STUDY&OBSERVATION AT LOD:

Study Design:

Five replicate injections of solutions at previously calculated LOD and LOQ concentration level were injected into the HPLC system as per the proposed method. The area responses of the analyte along with % RSD are tabulated in Table 9.

Limit of Detection level and Limit of quantitation level:

Peak of Paracetamol visually detected in all injections. Limit of quantitation and Limit of detection levels are tabulated in table 9.

Table 9:Limit of Quantitation Level

No. of injections	LOD VERIFICATION		LOQ PRECISION	
	Retention time	Area	Retention time	Area
1	9.06	1195	9.04	7566
2	9.05	1300	9.04	7376
3	9.04	1347	9.04	7190
4	9.04	1333	9.03	7434
5	9.03	1313	9.02	7345
Mean	9.044	1298	9.034	7382
% RSD	0.095	4.646	0.057	1.850

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Acceptance criteria:

1) To conclude the LOD verification, the peak at LOD level should be visually detected in replicates.

2) To conclude the LOQ precision, %RSD for peak of LOQ level should be not more than 10.

Result and conclusion:

The results are well with in acceptance criteria and the %RSD observed of the replicates indicates the reproducibility and hence the precision of limit of quantitation.

9.7 LINEARITY AND RANGE:

Study design:

Linearity for Paracetamol was determined in the concentration range of 10%, 50%, 75%, 100%, 125% and 150% of limit level. The area responses against the corresponding concentration are tabulated in table 10 for Linearity and Table 11 for Range.

Acceptance criteria:

i) To conclude the linearity, the squared correlation coefficient (r^2) should not be less than 0.995.

ii) To conclude the range, % RSD for peak area of linearity level LOQ should be not more than 10.0 and for 10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 10: Linearity Table for Paracetamol

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	0.062	51508
50%	0.309	252248
75%	0.464	397612
100%	0.618	506033
125%	0.773	645187
150%	0.927	772759
Slope		833181
CC		0.999
Sqaured R		0.9993
Intercept		253.7

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Fig. : Liner Graph for Paracetamol

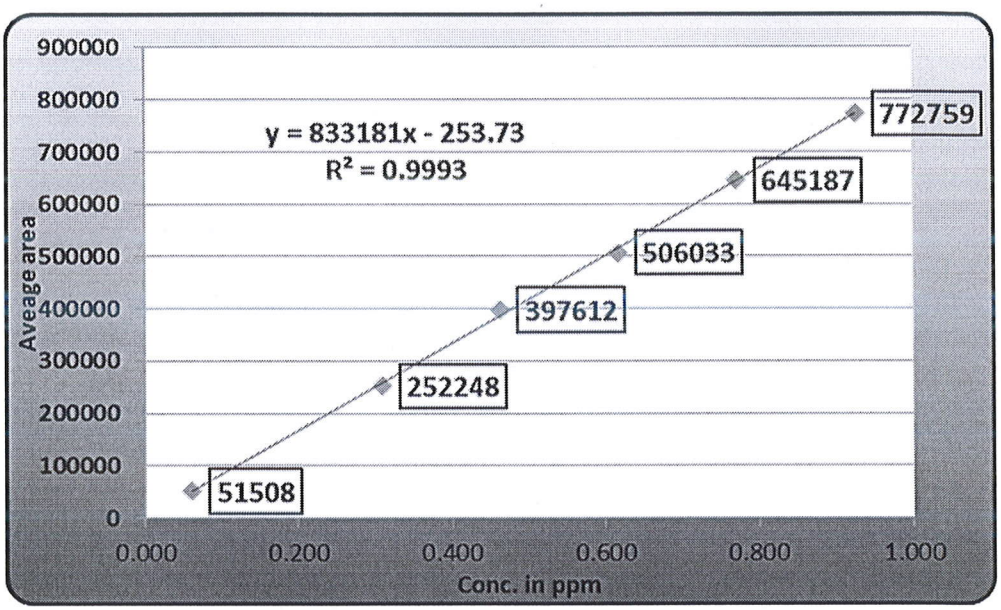

Table:11 Range for Paracetamol

Linearity Levels (%)	% RSD for Paracetamol
10%	0.345
50%	0.066
75%	0.190
100%	0.099
125%	0.021
150%	0.012

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Result and Conclusion:

Squared correlation coefficient value is well within the limit hence the test for linearity passes and % RSD for peak area of linearity level of LOQ should be not more than 10 and for 10% to 150% should be not more than 2.0.

9.8 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Sample preparation:

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 12A.

Table 12A: Stability of sample solution for Paracetamol

Time in hours	Area of Sample solution	Absolute % Difference
Initial	11542322	Not applicable
4	11522274	0.17
8	11485690	0.49
14	11448894	0.82
18	11430194	0.98
22	11436392	0.93
26	11418301	1.09
30	11421104	1.06
36	11419693	1.07
42	11426212	1.02
48	11420689	1.07
Mean	11451979	0.87
% RSD	0.388	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

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 12B.

Table 12B: Stability of standard solution for Paracetamol

Time in hours	Area of Standard solution	Absolute % Difference
Initial	39921	Not applicable
4	40894	-2.38
8	41024	-2.69
14	41565	-3.95
18	42160	-5.31
22	42037	-5.03
26	41961	-4.86
30	41984	-4.91
36	42242	-5.49
42	42501	-6.07
48	42576	-6.24
Mean	41715	-4.69
% RSD	1.927	Not applicable

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

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 be not more than ±10%.

Results and conclusions:

Sample and standard solution was stable up to 48 hours at room temperature.

9.9 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method was performed by filtering test solution through 0.45µ Nylon and PVDF filter against that of unfiltered (centrifuged) sample. The results were tabulated in Table 13.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Table 13: Filter paper study for Sample solution

Filter study	Single maximum unknown impurity (%)	%difference from unfiltered sample	Total impurities (%)	%difference from unfiltered sample
Unfiltered sample (Centrifuged)	Not detected	Not applicable	Not detected	Not applicable
Filter set-1(0.45µ PVDF)	Not detected	Nil	Not detected	Nil
Filter set-2(0.45µ PVDF)	Not detected	Nil	Not detected	Nil
Filter set-3(0.45µ PVDF)	Not detected	Nil	Not detected	Nil
Filter set-1(0.45µ Nylon)	Not detected	Nil	Not detected	Nil
Filter set-2(0.45µ Nylon)	Not detected	Nil	Not detected	Nil
Filter set-3(0.45µ Nylon)	Not detected	Nil	Not detected	Nil

Acceptance criteria:

i) For impurities between 0.05-0.1%: % difference should ±20 against that unfiltered.
ii) For impurities >0.1%: % difference should ±15 against that unfiltered.

Results and conclusions:

** There is no any interference due to Filter paper in test solution.*

** Results are complies as per the acceptance criteria.*

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

9.10 ROBUSTNESS:

Study design:

The robustness of the method was determined by analyzing standard solution under the following variable shall be done according to deliberate changes in chromatographic parameters.

a) Flow rate change by ±10% (i.e 0.9ml/min and 1.1ml/min)
b) Wavelength change by ± 3nm (i.e 217nm to 223nm)
c) Column oven temperature change by ±5.0ºC (i.e 25ºC to 35ºC)

The results are tabulated in table 14.

Table 14: Robustness of analytical method

Parameter	Any individual impurity in %	Total impurities in %	Tailing Factor	Theoretical plate	RSD %
Low wavelength (217nm)	Not detected	Not detected	1.13	13631	0.369
High wavelength (223nm)	Not detected	Not detected	1.14	13607	0.427
Low flow rate (0.9ml/minute)	Not detected	Not detected	1.14	14427	0.653
High flow rate (1.1ml/minute)	Not detected	Not detected	1.13	12933	0.795
Column Oven temperature 25°c	Not detected	Not detected	1.13	13456	3.950
Column Oven temperature 35°c	Not detected	Not detected	1.16	13600	1.215
% RSD	NIL	NIL	NA	NA	NA

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

Acceptance criteria:

i) % RSD for individual impurities between LOQ-0.1% should be not more than 20%.

ii) % RSD for Total impurities above 0.1% should be not more than 15%.

Result and Conclusion:

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

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

10.0 SUMMARY:

S.No	Validation parameter	Acceptance criteria	Results
1	System suitability	1) % RSD of area of each Paracetamol peak in five replicate standard injections should not be more than 5.0.	0.694
		2) Theoretical plate for Paracetamol peak standard injection should not be less than 2000.	13496
		3) Tailing factor for Paracetamol peak in standard injection should not be more than 2.0.	1.129
2	**Specificity** Interference from blank, placebo and placebo spiked with analyte.	1) No significant interference from blank and placebo. 2) Peak purity of analyte peak and 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.	Blank and Placebo peaks are not interfere with in test preparation and Peak purity passes within specified limits
3	Interference from degradants (Forced degradation)	1) There should not be any interference due to degradants with analyte and impurity in stressed samples.	There is no interference due to degradants with analyte and impurity in stressed samples.

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

S.No	Validation parameter	Acceptance criteria	Results
		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 harsher 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.	Peak purity was passes according to lab solution.
4	Determination of limit of detection and limit of quantitation	(Record the value)	**Paracetamol:** LOD in% LOD in ppm 0.009 0.027 LOQ in% LOQ in ppm 0.028 0.083

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

S.No	Validation parameter	Acceptance criteria	Results
5	**Precision** 1) System Precision	%RSD of area of analyte in six Replicate injections should not be more than 5.0 in standard preparation.	0.984
	2) Method Precision	1) % RSD for impurities LOQ to 0.1% in six preparations should not be more than 20. 2) % RSD for impurities above 0.1% in six preparations should not be more than 15. 3) % RSD for impurities in six preparations should not be more than 10.	**For Paracetamol:** Single maximum unknown impurity: 0.000 Total impurities: 0.000
	3)Intermediate Precision	1) % RSD for impurities LOQ to 0.1% in six preparations should not be more than 20. 2) % RSD for impurities above 0.1% in six preparations should not be more than 15. 3) % RSD for impurities in six preparations should not be more than 10.	**For Paracetamol:** Single maximum unknown impurity: 0.000 Total impurities: 0.000

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

S.No	Verification parameter	Acceptance criteria	Results
		4) Cumulative % RSD Any individual impurities and total impurities of method precision and intermediate precision should not be more than 10.	**For Paracetamol:** Single maximum unknown impurity: 0.000 Total impurities: 0.000
6	Observation at LOD & LOQ Precision	1) To conclude the LOD verification, the peak at LOD level should be visually detected in replicates. 2) To conclude the LOQ precision, % RSD for peak area of LOQ level should be not more than 10.	4.646 1.850
7	Linearity and range	1) To conclude the linearity, the squared correlation coefficient should not be less than 0.995 2) %RSD of areas at 10%, 50%, 75%, 100%, 125% & 150% levels should be Not more than 2.0.	**Squared correlation coefficient:**0.9993 Level %RSD 10% : 0.345 50% : 0.066 75% : 0.190 100% : 0.099 125% : 0.021 150% : 0.012

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

S.No	Validation parameter	Acceptance criteria	Results
8	**Stability of 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 ±10%.	Standard and sample solution was stable upto 48 hours at room.
9	**Filter paper study (0.45µ Nylon Filter)**	i) For impurities between 0.05-0.1%: % difference should be ±20. ii) For impurities >0.1%: % difference should be ±15.	i) There is no any interference due to Filter paper in test solution. ii) Results are complies as per the acceptance criteria.
10	**Robustness** (i) Flow rate change (ii) Wavelength change (iii) Column oven temperature change	i) % RSD for impurities between LOQ-0.1percent should not be more than 20%. ii) % RSD for impurities above 0.1% should not be more than 15%.	Single maximum unknown impurity: 0.00 Total impurities: 0.00

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

11.0 CONCLUSION:

Validation studies have been conducted for Related substances of Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets for the parameters of System suitability, Specificity, System precision, Method Precision, Intermediate Precision, Solution stability, Determination of LOD and LOQ Linearity and Range, Robustness, Filter paper study and forced degradation and Observed at LOD and LOQ Precision by using the proposed method. The data is complies and found satisfactory with the analytical method for all the parameters analysed, Hence it is concluded that the method in precise and accuracy and 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

REPORT	
Title	Analytical Method Validation Report For test of Related substances of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Report No.	ST/AMVRR/23/005

13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVRR/23/005	01/11/2023	New Report prepared.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF ASSAY OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

2.0 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Asst. Manager - QC
Signature	:	
Date	:	06/09/2023
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	AGM-QC
Signature	:	
Date	:	07/09/2023
APPROVED BY		
Name	:	S. MARAN
Designation	:	AGM - QA
Signature	:	
Date	:	08/09/2023

Effective Date	:	09/09/2023

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

3.0 OBJECTIVE

To validate the method for test of assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

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

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. M.Bavyasri
2. S.Suganthi
3. C.Albin jose

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., Impurities standard, test samples/placebo to be used during Validation.

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	To be mentioned in report	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report
API Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED:

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with PDA detector

Make : Shimadzu, Model : LC-2050C 3D Prominence i

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C Prominence i

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

COLUMN :

Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm

SOLVENTS AND CHEMICALS WITH GRADE:

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine (Anhydrous) (Working standard)

Paracetamol (Working standard)

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Potassium Dihydrogen orthophosphate (AR grade)

Orthophosphoric acid (AR grade)

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

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Hydrochloric acid (AR grade)

Sodium Hydroxide (AR grade)

Hydrogen Peroxide (AR grade)

Plain placebo

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column	:	Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm
Wave length	:	220 nm
Column Temperature	:	40°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	20 µL
Run time	:	30.01 Minutes
Retention time	:	About 4.6 minutes for Phenylephrine Hydrochloride, about 10.5 minutes for Paracetamol, about 12.7 minutes for Caffeine and about 14.8 minutes for Chlorphenamine maleate.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Preparation of Buffer:

Weigh accurately about 6.8g of potassium Di-hydrogen orthophosphate in 1000 mL of milli-Q water, sonicate to dissolve. Adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	100	0
6.0	100	0
7.0	70	30
9.0	70	30
10.0	45	55
25.0	45	55
27.0	100	0
30.01	100	0

Preparation of Mobile phase-A:

Prepare a degassed mixture of buffer and acetonitrile in the ratio 95:5 *v/v*.

Preparation of Mobile phase-B:

100% Methanol

Preparation of Diluent:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Preparation of Standard stock solution:

Weigh accurately and transfer about 32 mg of Chlorphenamine maleate working standard, 80 mg of Phenylephrine Hydrochloride working standard and 24 mg of Caffeine working standard into a 200 mL volumetric flask. Add 120 mL of diluent and sonicate to dissolve and make up to volume with diluent and mix (mcg/ml).(**Concentration** For Chlorphenamine maleate:0.16mg/ml, Phenylephrine Hydrochloride:0.4mg/ml, Caffeine:0.005mg/ml)

Preparation of Standard solution:

Weigh accurately and transfer about 20 mg of Paracetamol working standard into a 200 mL volumetric flask. Add 120 mL of diluent and sonicate to dissolve and add 10 mL of standard stock solution (Chlorphenamine, Phenylephrine and Caffeine) and make up to volume with diluent and mix (mcg/ml). **Concentration:** (For Paracetamol:0.1mg/ml) (For Chlorphenamine maleate:0.008mg/ml, Phenylephrine Hydrochloride:0.02mg/ml, Caffeine:0.06mg/ml)

Test preparation:

Preparation of Sample Solution-A: (For Chlorphenamine maleate and Phenylephrine Hydrochloride)

Weigh accurately 20 tablets and make powder by using morter and pestle. Weigh and transfer sample powder equivalent to 500 mg of Paracetamol, into a 250 mL volumetric flask. Add about 170 mL of diluent and sonicate for 30 minutes with intermittent shaking. Make up to the volume with diluent and mix and Centrifuge this solution at 3000rpm for 10minutes (mcg/ml).

Preparation of Sample Solution-B:(For Paracetamol and Caffeine)

Further dilute 5 mL of above solution to 100 mL with diluent and mix.

Procedure:

Inject the solutions as mentioned below and measure the responses of the peaks due to Paracetamol, Phenylephrine, Chlorphenamine maleate, Caffeine.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (blank)	1
2	Standard preparation	5
3	Sample solution-A	2
4	Sample solution B	2
5	Bracketing standard	1

System suitability:

Theoretical plate count : NLT 2000 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Tailing factor : NMT 2.0 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Relative standard deviation : NMT 2.0% for five replicate injections of Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.

Calculations:

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{P}{100} \times AW$$

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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.
AW = Average weight of tablet in mg.
P = Potency of Chlorphenamine maleate working standard in % on as such basis.

Calculate the assay of Chlorphenamine maleate in % as follows:

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

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

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{P}{100} \times AW$$

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.
AW = Average weight of tablet in mg.
P = Potency of Phenylephrine Hydrochloride working standard in % on as such basis.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Calculate the assay of Phenylephrine Hydrochloride in % as follows:

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

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

Calculate the assay of Caffeine in mg/tablet as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{10}{200} \times \frac{250}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AW$$

Where,

AT	=	Average area of peak due to Caffeine in Sample solution B.
AS	=	Average area of peak due to Caffeine in standard preparation.
WS	=	Weight of Caffeine working standard in mg.
WT	=	Weight of sample taken in mg.
AW	=	Average weight of tablet in mg.
P	=	Potency of Caffeine working standard in % on as such basis.

Calculate the assay of Caffeine in % as follows:

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

LC = Label claim of Caffeine in mg/tablet.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{250}{WT} \times \frac{100}{5} \times \frac{P}{100} \times AW$$

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.
AW = Average weight of tablet in mg.
P = Potency of Paracetamol working standard in % on as such basis.

Calculate the assay of Paracetamol in % as follows:

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

LC = Label claim of Paracetamol in mg/tablet.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for validation.	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and placebo
3	**Linearity and Range**
4	**Interference from Degradation (Forced degradation)**
	i) Acid degradation ii) Alkali Degradation iii) Oxidative Degradation
5	**Accuracy (Recovery)**
6	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
7	**Stability of analytical solution**
8	**Filter paper study**
9	**Robustness** i) Flow rate change ii) Wavelength change iii) Oven temperature change

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

9.0 DETAILS OF VALIDATION PARAMETERS:

9.1 SYSTEM SUITABILITY:

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 Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak in five replicate standard injections.

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

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

Acceptance Criteria:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

2) Theoretical plates for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak in standard injection should not less than 2000.

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

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and placebo

"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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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	Placebo + Phenylephrine- Std	1
5	Placebo + Paracetamol- Std	1
6	Placebo + Caffeine- Std	1
7	Placebo + Chlorphenamine- Std	1
8	Plain placebo + Phenylephrine + Paracetamol + Caffeine + Chlorphenamine std	1
9	Test preparation -A	1
10	Test preparation -B	1

Acceptance Criteria :

i) There should not be any interference due to blank, Placebo peak with analyte.

ii) Peak purity of analyte should be pass. according to Lab solution software.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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.

Linearity stock solution, linearity level, expected concentration, linearity stock dilution and calculated concentration are tabulated below.

For Chlorphenamine Maleate:

Linearity Stock solution	40.67	25	1	1	1	101.68ppm (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	0.800	2	250	0.8134
50%	4.00	2	50	4.067
75%	6.00	3	50	6.1005
100%	8.00	4	50	8.134
125%	10.00	5	50	10.1675
150%	12.00	6	50	12.201

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

For Phenylephrine Hydrochloride:

Linearity Stock solution	100.14	25	1	1	1	250.35 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	2.00	2	250	2.0028
50%	10.00	2	50	10.014
75%	15.00	3	50	15.021
100%	20.00	4	50	20.028
125%	25.00	5	50	25.035
150%	30.00	6	50	30.042

For Caffeine:

Linearity Stock solution	30.13	25	1	1	1	75.32 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	0.6	2	250	0.6026
50%	3.00	2	50	3.013
75%	4.5	3	50	4.5195
100%	6.0	4	50	6.026
125%	7.5	5	50	7.5325
150%	9.0	6	50	9.039

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

For Paracetamol:

Linearity Stock solution	125.24	2	1	1	1	10.019 (con. ppm)
	100	250	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	10	2	250	10.0192
50%	50	2	50	50.096
75%	75	3	50	75.144
100%	100	4	50	100.192
125%	125	5	50	125.24
150%	150	6	50	150.288

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

Sequence shall be in following provisional manner.

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

Acceptance criteria:

(i) To conclude the linearity, the squared correlation coefficient should not be less than 0.999

(ii) To conclude the range, % RSD for peak area of linearity level of 50%, 100% and 150% should be not more than 2.0.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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 30minutes
Alkali degradation	Exposure to 5ml of 5N NaOH and heat on water bath at 80°C for 30minutes
Oxidative degradation	Exposure to 5ml of 30% H2O2 and heat on water bath at 80°C for 30minutes

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 preparation (As such)	2
4	Sample preparation (Acid degradation)	2
5	Sample preparation (Alkali degradation)	2
6	Sample preparation (Oxidative degradation)	2
7	Standard preparation (Bracketing)	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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 0.995 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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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 and inject only one injection for each samples.

Acceptance criteria:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

9.6 PRECISION

9.6.1 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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

9.6.2 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-1	2
4	Sample solution A-2	2
5	Sample solution A-3	2
6	Sample solution A-4	2
7	Sample solution A-5	2
8	Sample solution A-6	2
9	Standard preparation (BKT)	1
10	Sample solution B-1	2
11	Sample solution B-2	2

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

S.No.	Description of solution	No. of Injections
12	Sample solution B-3	2
13	Sample solution B-4	2
14	Sample solution B-5	2
15	Sample solution B-6	2
16	Standard preparation (BKT)	1

Acceptance Criteria :

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

9.6.3 INTERMEDIATE PRECISION

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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample solution A-1	2
4	Sample solution A-2	2
5	Sample solution A-3	2
6	Sample solution A-4	2
7	Sample solution A-5	2
8	Sample solution A-6	2
9	Standard preparation (BKT)	1
10	Sample solution B-1	2
11	Sample solution B-2	2
12	Sample solution B-3	2
13	Sample solution B-4	2
14	Sample solution B-5	2
15	Sample solution B-6	2
16	Standard preparation (BKT)	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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Prepare Standard and sample solution as per the methodology and store at room temperature. Chromatograph this solution at regular intervals for 28 hours by using same diluent. Calculate the % difference of analyte peak area for standard and sample 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 (Initial)	1
4	Sample solution A (Initial)	1
5	Sample solution B (Initial)	1
6	Standard preparation (Time interval)	1
7	Sample solution A (Time interval)	1
8	Sample solution 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%.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering the sample solution through 0.45µ Nylon filter and 0.45µ PVDF membrane filter against that of unfiltered (Centrifuged samples).

Sequence shall be in following provisional manner.

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

Acceptance criteria:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

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

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

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

c) Column oven Temperature change by ± 5.0°C (i.e. 35°C and 45°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 ABBREVATION:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Assay of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVAP/23/005

RSD	:	Relative standard deviation
µl	:	Micro litre
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H_2O_2	:	Hydrogen Peroxide
Mcg	:	Microgram
ppm	:	Parts per Million

11.0 REVISION HISTORY:

Specification No.	Effective date	Reason for Review
ST/AMVAP/23/005	09/09/2023	New Protocol prepared.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF DISSOLUTION OF CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL IN CHLORPHENAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE AND PARACETAMOL TABLETS (LITACOLD FLU)

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
Plot No.A-67 to 72, PIPDIC Electronic Park,
Thirubuvanai, Puducherry-605 107

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

1.0 INDEX

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

2.0 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	K-SARAVANAN
Designation	:	Asst. Manager - QC
Signature	:	
Date	:	19/09/2023
REVIEWED BY		
Name	:	M.VIJAYAKUMAR
Designation	:	GM-QC
Signature	:	
Date	:	20/09/2023
APPROVED BY		
Name	:	S.MARAN
Designation	:	AGM - QA
Signature	:	
Date	:	20/09/2023

Effective Date	:	21/09/2023

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

3.0 OBJECTIVE

To validate the method for test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets by HPLC.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopeial method

TEST TO BE VALIDATED : Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol tablets

COMPOSITION : Each uncoated tablet contains:

Content	Strength
Chlorphenamine Maleate BP	2mg
Phenylephrine Hydrochloride BP	5mg
Caffeine (Anhydrous) BP	30mg
Paracetamol BP	500mg

BATCH NO : G17230824

SPECIFICATION LIMIT : Not less than 80%

VALIDATION STUDY : QC-Laboratory, Generic Healthcare Private Limited, Puducherry-605107

VALIDATION TEAM : 1. M. Bhavyasri
2. S. Suganthi
3. C.Albin jose

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

Mention the name and Batch No., Potency of the reference/working std., test samples/placebo to be used during Validation

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	To be mentioned in report	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report
API Chlorphenamine Maleate BP	To be mentioned in report	To be mentioned in report
Phenylephrine HCL BP	To be mentioned in report	To be mentioned in report
Caffeine BP	To be mentioned in report	To be mentioned in report
Paracetamol BP	To be mentioned in report	To be mentioned in report

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS, COLUMN, SOLVENTS AND CHEMICALS TO BE USED:

INSTRUMENTS/EQUIPMENTS :

High performance liquid chromatograph with UV detector

Make : Shimadzu, Model : LC-2050C.

High performance liquid chromatograph with PDA detector

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

Analytical Balance:

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

pH:

Make: Eutech instruments, Model No: PC 700

COLUMN :

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

SOLVENTS AND CHEMICALS WITH GRADE :

Chlorphenamine Maleate (Working standard)

Phenylephrine Hydrochloride (Working standard)

Caffeine Anhydrous (Working standard)

Paracetamol (Working standard)

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Potassium Dihydrogen orthophosphate (AR grade)

Orthophosphoric acid (AR grade)

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

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Sodium Hydroxide (AR grade)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Apparatus	:	Apparatus -2 Paddle
Medium	:	900ml of PH 6.8 Phosphate buffer
Time	:	45 minutes
Speed	:	75 RPM
Temperature	:	37° C ± 0.5 ° C

Preparation of Dissolution medium:

Dissolve 68 gm of Potassium dihydrogen phosphate and 9.8 gm of Sodium hydroxide pellets in 10 liters of purified water and mix well. Adjust pH 6.8±0.05 with dilute Sodium hydroxide or dilute Orthophosphoric acid and mix well.

Preparation of Buffer:

Weigh accurately about 6.8 g of potassium Di-hydrogen orthophosphate in 1000 mL of Milli-Q water, sonicate to dissolve. Adjust pH to 3.0±0.05 with Orthophosphoric acid. Filter through 0.45µ membrane filter.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Chromatographic Conditions:

Column	:	Inertsil ODS-3V, 250 mm X 4.6 mm, 5µm (or) equivalent
Wave length	:	220 nm
Column Temperature	:	40°C
Flow Rate	:	1.2 mL/min
Injection Volume	:	20 µL
Run time	:	30.01 Minutes
Retention time	:	About 4.6 minutes for Phenylephrine Hydrochloride, about 10.5 minutes for Paracetamol, about 12.7 minutes for Caffeine and about 14.8 minutes for Chlorphenamine maleate,

Gradient Program:

Time	Mobile phase A %	Mobile phase B%
0.01	100	0
6.0	100	0
7.0	70	30
9.0	70	30
10.0	45	55
25.0	45	55
27.0	100	0
30.01	100	0

Preparation of Mobile phase-A:

Prepare a degassed mixture of buffer and acetonitrile in the ratio 95:5 *v/v*.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Preparation of Mobile phase-B:

100% Methanol

Preparation of Diluent:

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

Preparation of Standard stock solution:

Weigh accurately and transfer about 28 mg of Chlorphenamine maleate working standard and 68 mg of Phenylephrine Hydrochloride working standard and 83 mg of Caffeine working standard into a 250 mL volumetric flask, add 20 mL of diluent and sonicate to dissolve and make up to volume with dissolution medium and mix. (Concentration: For Chlorphenamine maleate:0.112mg/ml, For Phenylephrine HCL:0.272mg/ml, for Caffeine:0.332mg/ml)

Preparation of Standard solution:

Weigh accurately and transfer about 22 mg of Paracetamol working standard into a 200 mL volumetric flask. Add 20 mL of diluent and sonicate to dissolve and add 4 mL of Standard stock solution and make up to volume with dissolution medium and mix. (Concentration: For Chlorphenamine maleate:0.00224mg/ml, For Phenylephrine HCL:0.00544mg/ml, for Caffeine:0.00664mg/ml) and Paracetamol:0.11mg/ml)

Test Preparation :

Preparation of sample solution (A) (For Chlorphenamine maleate and Phenylephrine Hydrochloride)

Set the dissolution parameters and place one tablet into each vessel individually containing 900 mL of dissolution medium, immediately start the apparatus. At the end of specified time withdraw the sample and filter through 0.45µ PVDF filter.

Preparation of Sample Solution-B:(For Paracetamol and Caffeine)

Further dilute 10 mL of above filtered solution to 50 mL with Dissolution medium and mix.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Procedure:

Inject the solutions as mentioned below and measure the responses of the peaks due to Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine.

Injection sequence:

S. No	Sample Name	No. of injections
1	Dissolution medium (blank)	1
2	Standard preparation	5
3	Sample solution A (1 injection each)	6
4	Sample solution B (1 injection each)	6
5	Bracketing standard	1 (After every 6 injections)

System suitability:

Theoretical plate count	:	NLT 2000 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.
Tailing factor	:	NMT 2.0 for Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine maleate, Caffeine peak.
Relative standard deviation	:	NMT 2.0% for five replicate injections of Paracetamol, Phenylephrine hydrochloride, Chlorphenamine maleate, Caffeine peak.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Calculations:

Calculate % drug release of Chlorphenamine maleate as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = 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.

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

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

Calculate % drug release of Phenylephrine Hydrochloride as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = 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.

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

LC = Label claim of Phenylephrine hydrochloride in mg/tablet.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Calculate % drug release of Caffeine as follows:

$$= \frac{AT}{AS} \times \frac{WS}{250} \times \frac{4}{200} \times \frac{900}{1} \times \frac{50}{10} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = Area of peak due to Caffeine in Sample solution B.
AS = Average area of peak due to Caffeine in standard preparation.
WS = Weight of Caffeine working standard in mg.
P = Potency of Caffeine working standard in % on as such basis.
LC = Label claim of Caffeine in mg/tablet.

Calculate % drug release of Paracetamol as follows:

$$= \frac{AT}{AS} \times \frac{WS}{200} \times \frac{900}{1} \times \frac{50}{10} \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

AT = 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.
P = Potency of Paracetamol working standard in % on as such basis.
LC = Label claim of Paracetamol in mg/tablet.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for Validation	
Sr. No.	**VALIDATION PARAMETER**
1	**System suitability**
2	**Specificity (Selectivity)** i) Interference from blank and Placebo
3	**Linearity and Range**
4	**Accuracy**
5	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
6	**Stability of analytical solution**
7	**Filter paper study**
8	**Robustness**

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

9.0 DETAILS OF VALIDATION PARAMETERS:

9.1 SYSTEM SUITABILITY:

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 (Dissolution medium)	1
2	Standard preparation	5

Evaluate the following system suitability parameters:

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

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

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

Acceptance Criteria:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

2) Theoretical plates for Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol peak in standard injection should not less than 2000.

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

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from Blank and Placebo

"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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

S.No.	Description of solution	No. of injections
1	Blank (Dissolution medium)	1
2	Standard preparation	5
3	Plain placebo	1
4	Phenylephrine Working standard	1
5	Paracetamol Working standard	1
6	Caffeine Working standard	1
7	Chlorphenamine Working standard	1
8	Plain placebo + Phenylephrine HCL+ Paracetamol + Caffeine + Chlorphenamine Working standard	1
9	Sample solution - A	1
10	Sample solution - B	1

Acceptance Criteria :

i) There should not be any interference due to blank, Placebo peak with analyte.

ii) Peak purity of analyte should be pass. According to Lab solution software.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

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 50% to 150% of targeted concentration.

Linearity stock solution, linearity level, expected concentration, linearity stock dilution and calculated concentration are tabulated below.

For Chlorphenamine Maleate:

Linearity Stock solution	22.4	25	1	1	1	28.0 (con. ppm)
	200	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
50%	1.12	2	50	1.12
75%	1.68	3	50	1.68
100%	2.24	4	50	2.24
125%	2.80	5	50	2.80
150%	3.36	6	50	3.36

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

For Phenylephrine Hydrochloride:

Linearity Stock solution	27.20	25	1	1	1	68.0 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
50%	2.72	2	50	2.72
75%	4.08	3	50	4.08
100%	5.44	4	50	5.44
125%	6.80	5	50	6.80
150%	8.16	6	50	8.16

For Caffeine:

Linearity Stock solution	33.2	25	1	1	1	83.00 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
50%	3.32	2	50	3.32
75%	4.98	3	50	4.98
100%	6.64	4	50	6.64
125%	8.30	5	50	8.30
150%	9.96	6	50	9.96

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

For Paracetamol:

Linearity Stock solution	137.5	2	1	1	1	1375 (con. ppm)
	100	50	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
50%	55.00	2	50	55.00
75%	82.50	3	50	82.50
100%	110.00	4	50	110.00
125%	137.50	5	50	137.50
150%	165.00	6	50	165.00

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Sequence shall be in following provisional manner.

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

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 ($r^{2)}$ 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.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

9.4 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 dissolution 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 (Dissolution medium)	1
2	Standard preparation	5
3	Blank (Dissolution medium)	1
4	Level – 1 Set – 1 (50%)	1
5	Level – 1 Set – 2 (50%)	1
6	Level – 1 Set – 3 (50%)	1
7	Blank (Dissolution medium)	1
8	Level – 2 Set – 1 (100%)	1
9	Level – 2 Set – 2 (100%)	1
10	Level – 2 Set – 3 (100%)	1
11	Blank (Dissolution medium)	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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

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 95.0 to 105.0.

9.5 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"

9.5.1 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 (Dissolution medium)	1
2	Standard preparation	5

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Acceptance Criteria :

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

9.5.2 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 dissolution for the same. Evaluate the method precision by computing the percentage and relative standard deviation of the dissolution results.

S.No.	Description of solution	No. of Injections
1	Blank (Dissolution medium)	1
2	Standard preparation	5
3	Sample solution A&B-1	1
4	Sample solution A&B -2	1
5	Sample solution A&B -3	1
6	Sample solution A&B -4	1
7	Sample solution A&B -5	1
8	Sample solution A&B -6	1
9	Standard preparation (BKT)	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Acceptance Criteria :

% RSD for dissolution of six sample should not be more than 5.0.

9.5.3 INTERMEDIATE PRECISION

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. Analyze six sample preparations as per the methodology representing a single batch and determine the dissolution for the same. Evaluate the intermediate precision by computing the percentage and relative standard deviation of the dissolution results.

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Dissolution medium)	1
2	Standard preparation	5
3	Sample solution A & B - 1	1
4	Sample solution A & B - 2	1
5	Sample solution A & B - 3	1
6	Sample solution A & B - 4	1
7	Sample solution A & B - 5	1
8	Sample solution A & B - 6	1
9	Standard preparation (BKT)	1

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Acceptance criteria:

1) % RSD for Dissolution of six sample preparations should not be more than 5.0.

2) Cumulative % RSD for dissolution of twelve preparations of (method precision and intermediate precision) should not be more than 5.0%.

9.6 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Prepare standard and sample solution as per the methodology and store at room temperature. Chromatograph these solution at regular intervals for 12 hours by using same solution. Calculate the % difference of analyte peak area for standard preparations with that of initial. The study may be stopped if 2 consecutive failure of standard solution.

Sequence shall be in following provisional for Standard preparation.

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Acceptance criteria:

The standard and sample 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%.

9.7 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering test solution through 0.45µ Nylon filter paper and 0.45µ PVDF membrane filter. Against that of unfiltered (Centrifuged)

Sequence shall be in following provisional manner.

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

Acceptance criteria:

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

9 .8 ROBUSTNESS:

Purpose:

To establish the robustness of the analytical method.

Study Design:

The robustness of the analytical method can be establish 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 (Dissolution medium)	1
2	Standard preparation	5
According to each variable		
S.No.	**Description of solution**	**No. of Injections**
1	Blank (Dissolution medium)	1
2	Standard preparation	5

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

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

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

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

c) Column oven Temperature change by ± 5.0°C (i.e. 35°C and 45°C)

d) Effect of variation in dissolution media volume:

To demonstrate the effect of dissolution media volume, carryout the dissolution study on six test preparations with ±1% of the dissolution medium volume. Prepare six sample solutions on drug product.

Determine % dissolution, average % dissolution of six dosage units and % relative standard deviation of dissolution results.

e) Effect of dissolution medium pH Variation:

To demonstrate the effect of Dissolution medium pH Variation, carry out the dissolution study on six test preparation with ±0.2 pH Variation.

Acceptance criteria:

System suitability should comply for each variable.

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

10.0 ABBREVATION:

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

PROTOCOL	
Title	Analytical Method Validation Protocol For test of Dissolution of Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol in Chlorphenamine Maleate, Phenylephrine Hydrochloride, Caffeine and Paracetamol Tablets
Protocol No.	ST/AMVDP/23/005

11.0 REVISION HISTORY:

Specification No.	Effective date	Reason for Review
ST/AMVDP/23/005	21/09/2023	New Protocol prepared.