PROTOCOL	
Title	Analytical Method Validation Protocol For the test of Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet
Protocol No.	ST/AMVRP/23/037

ANALYTICAL METHOD VALIDATION PROTOCOL FOR THE TEST OF RELATED SUBSTANCES IN PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE, CHLORPHENAMINE MALEATE AND ASCORBIC ACID SACHET (GRIPEX)

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

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1.0 INDEX

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2.0 PROTOCOL APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Dy. Manager - QC
Signature	:	
Date	:	04/01/2024
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM - QC
Signature	:	
Date	:	05/01/2024
APPROVED BY		
Name	:	S. AKSAN
Designation	:	AGM - QA
Signature	:	
Date	:	06/01/2024

Effective Date	:	08/01/2024

PROTOCOL	
Title	Analytical Method Validation Protocol For the test of Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet
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3.0 OBJECTIVE

To validate the method for the test of Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet.

4.0 GENERAL INFORMATION

REFERENCE : In-House

TYPE OF VALIDATION : Validation of non-pharmacopoeial method

TEST TO BE VALIDATED : Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet.

COMPOSITION : Each 4.5gm Sachet contains:

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

BATCH NO : G17231228

SPECIFICATION LIMIT : Single maximum unknown impurity: Not more than 0.5%
Total unknown impurities: Not more than 1.0%

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

VALIDATION TEAM : 1. S.Elavarasan

2. S. Bhavyasri

3. C.Albin jose

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5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

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

NAME OF THE MATERIAL	ID NO/BATCH NO	POTENCY/PURITY
Sample	B.No: G17231228	Not Applicable
Plain Placebo	Not Applicable	Not Applicable
Working standard Paracetamol	To be mentioned in report	To be mentioned in report
Phenylephrine Hydrochloride	To be mentioned in report	To be mentioned in report
Chlorphenamine Maleate	To be mentioned in report	To be mentioned in report
Ascorbic Acid	To be mentioned in report	To be mentioned in report

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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-2030C 3D Prominence i

High performance liquid chromatograph with UV detector

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

High performance liquid chromatograph with PDA 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:

C8 4.6mmx250mm, 5µ

Solvents and chemicals with grade:

Paracetamol (Working standard)

Ascorbic acid (Working standard)

Chlorphenamine Maleate (Working standard)

Phenylephrine HCL (Working standard)

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Potassium dihydrogen orthophosphate (AR grade)

Orthophosphate (AR grade)

Methanol (HPLC grade)

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

Hydrochloric acid (AR grade)

Sodium hydroxide (AR grade)

30% Hydrogen peroxide (AR grade)

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic condition:

Column description	:	C8 4.6mmx250mm, 5µ (Kromasil column is suitable)
Flow rate	:	1.0ml/min
Detector	:	220nm
Column temperature	:	30°C
Injection volume	:	10µl

Preparation of Buffer solution:

Weigh accurately and transfer about 6.8g of Potassium dihydrogen orthophosphate to 1000ml glass beaker. Add about 500ml of water, shake and sonicate to dissolve completely and finally make the solution to 1000ml with water. Adjust the pH to 3.0±0.05 with Orthophosphoric acid.

Preparation of Mobile phase:

Mix 860ml of butter solution and 140ml Methanol. Filter through 0.20µ membrane filter and degas.

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Preparation of diluent:

Use mobile phase as such.

Preparation of blank:

Inject mobile phase as such.

Preparation of placebo solution:

Weigh accurately and transfer about 0.348g Placebo powder into 100ml volumetric flask. Add about 20ml of diluent sonicate for 10 minutes with intermittent shaking to dissolve and dilute up to the volume with diluent. Filter sufficient quantity of this solution through 0.45µ syringe filter.

Preparation of Resolution stock solution:

Weigh accurately and transfer about 116.0mg of Ascorbic acid Working standard, 46.0mg of Chlorphenamine Maleate Working standard and 23.0mg of Phenylephrine Hydrochloride into 50ml volumetric flask. Add about 20ml of diluent, sonicate for 10 minutes with intermittent shaking to dissolve and dilute up to the volume with diluent. Dilute 10ml of this solution to 50ml with diluent and mix well. (**Concentration:** 0.464mg/ml of Ascorbic acid, 0.184mg/ml of Chlorphenamine maleate, 0.092mg/ml of Phenylephrine Hcl).

Preparation of Resolution solution:

Weigh accurately and transfer about 60.0mg of Paracetamol Working standard into 100ml volumetric flask. Add 10ml of Resolution stock solution and 50 ml of diluent, sonicate to dissolve and dilute up to mark with diluents mix well and inject.

(**Concentration:** Ascorbic acid: 0.046 mg/ml, Chlorphenamine Maleate: 0.0184 mg/ml, Phenylephrine Hcl: 0.0092 mg/ml and Paracetamol: 0.6mg/ml)

Preparation of Standard low load solution:

Weigh accurately and transfer about 60.0mg of Paracetamol Working standard into 100ml volumetric flask. Add about 50ml of diluents, sonicate to dissolve and dilute upto mark with diluents. Dilute 5ml of this solution to 100ml with diluent and mix well. Further dilute 5ml of this solution 50ml with diluent. Mix well and inject. Prepare the duplication for similarity factor. (**Concentration:** 0.003 mg/ml of Paracetamol).

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Preparation sample solution:

Weigh accurately and transfer about 0.415g of sample powder into 100ml volumetric flask. Add about 50ml of diluent, sonicate for 10 minutes with intermittent shaking to dissolve and dilute up to the volume with diluent. Filter sufficient quantity of this solution through 0.45µ syringe filter. (**Concentration:** Ascorbic acid 0.046 mg/ml, Chlorphenamine maleate: 0.0184 mg/ml, Phenylephrine Hcl: 0.0092 mg/ml and Paracetamol: 0.6mg/ml)

Procedure:

Equilibrate the chromatographic system with mobile phase till a stable baseline is obtained. Separately inject equal volumes (10µl) of solutions as per sequence of injections in to the chromatograph and record the peak area responses for the major peaks and check for the system suitability requirements.

Injection sequence:

S. No	Sample Name	No. of injections
1	Diluent (Blank)	1
2	Resolution solution	1
3	Standard low load solution	6
4	Blank solution	1
5	Placebo solution	1
6	Sample solution	1
7	Bracketing standard low load solution	1 Each after every 6 sample injection

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System suitability;

1) The Resolution between the peaks corresponding to

(i) Ascorbic acid and Chlorphenamine
(ii) Chlorphenamine and Phenylephrine
(iii) Phenylephrine and Paracetamol obtained with Resolution should not be less than 2.0.

2) The theoretical plates for the peaks of Paracetamol obtained with standard low load solution should not be less than 2000.

3) The symmetry factor for the peaks of Paracetamol obtained with standard low load solution should not be more than 2.0.

4) The %RSD for the retention time of peaks of Paracetamol obtained with replicate injection of standard low load solution should not be more than 1.0.

5) The %RSD of peak area response for the peaks of Paracetamol obtained with replicate injection of standard low load solution should not more than 5.0.

6) The %RSD for the retention time of peaks of Paracetamol obtained with replicate injection of standard low load solution and bracketing standard low load solution should not be more than 1.0.

7) The %RSD of peak area response for the peaks of Paracetamol obtained with replicate injection of standard low load solution and bracketing standard low load solution should not be more than 5.0.

Calculation:

Calculate the % content of single maximum unknown impurity by following formula:

$$= \frac{AT_I}{AS} \times \frac{WS}{100} \times \frac{5}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{P}{100} \times \frac{AFW}{LC} \times 100$$

Calculate the % content of total unknown impurities by following formula:

$$= \frac{AT_T}{AS} \times \frac{WS}{100} \times \frac{5}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{P}{100} \times \frac{AFW}{LC} \times 100$$

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Where,

AT_I	=	Peak area response of single maximum unknown impurity obtained with sample solution.
AT_T	=	Peak area response of total unknown impurities obtained with sample solution.
AS	=	Average peak area response of Paracetamol peak obtained with replicate injections of standard low load solution
WS	=	Weight of Paracetamol Working standard in mg.
WT	=	Weight of Sample taken in mg.
P	=	Potency of Paracetamol working standard (on % as is basis).
AFW	=	Average fill weight of sachet in mg.
LC	=	Label claim of Paracetamol in mg.

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8.0 PARAMETERS TO BE VALIDATED:

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

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

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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	System suitability solution	1
3	Standard low load solution	6

Acceptance criteria:

1) % RSD of peak area of Paracetamol in Six replicate Standard low load solution injections should not be more than 5.0.

2) Tailing factor for Paracetamol peak in Standard low load solution injection should not be more than 2.0.

3) Theoretical plate for Paracetamol peak in standard low load solution injection should not be less than 2000.

4) Resolution between Ascorbic acid and Chlorpheniramine, Chlorpheniramine and Phenylephrine Hcl, Phenylephrine Hcl and Paracetamol should not be less than 2.0.

9.2 SPECIFICITY (SELECTIVITY)

9.2.1 Interference from blank and Placebo

Study design:

Sequence shall be in following provisional manner.

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No.	Description of solution	No. of injections
1	Blank (Diluent)	1
2	Resolution Solution	1
3	Standard preparation	5
4	Blank	1
5	Paracetamol Working standard	1
6	Phenylephrine Hcl Working standard	1
7	Chlorpheniramine Maleate Working standard	1
8	Ascorbic Acid Working standard	1
9	Paracetamol Working standard + Chlorpheniramine Maleate Working standard + Phenylephrine Hcl Working standard + Ascorbic Acid Working standard	1
10	Blank	1
11	Plain placebo	1
12	Placebo + Paracetamol Working standard	1
13	Placebo + Phenylephrine HCl Working standard	1
14	Placebo + Chlorpheniramine Maleate Working standard	1
15	Placebo + Ascorbic Acid Working standard	1
16	Placebo + Paracetamol Working standard + Chlorpheniramine Maleate Working standard + Phenylephrine Hcl Working standard + Ascorbic Acid Working standard	1
17	Blank	1
18	Test preparation B.No.G17231228	1

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

Acceptance criteria:

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

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

9.3 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.

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

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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.

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

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.

9.4 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 30 minutes.
Alkali degradation	Exposure to 5N NaOH and heat on water bath 80°C for 30 minutes.
Oxidative degradation	Exposure to 30% H2O2 and heat on water bath 80°C for 30 minutes.

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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	6
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

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).

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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.

9.5 PRECISION:

9.5.1 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 single maximum unknown impurity and total unknown impurities for the same. Evaluate the method precision by computing the percentage and relative standard deviation of the Related substance results.

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No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	System suitability solution	1
3	Blank (Diluent)	1
4	Standard low load solution	6
5	Blank (Diluent)	1
6	Sample solution -1	1
7	Sample solution -2	1
8	Sample solution -3	1
9	Sample solution -4	1
10	Sample solution -5	1
11	Sample solution -6	1
12	Standard low load solution (BKT)	1

Acceptance criteria:

1) % RSD for single maximum unknown impurity above 0.1% in six sample preparations should be not more than 15.

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

9.5.2 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.

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Analyze six sample preparations as per the methodology representing a single batch and determine the Related substance for the same. Evaluate the intermediate precision by computing the percentage and relative standard deviation of the assay results.

No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	System suitability solution	1
3	Blank (Diluent)	1
4	Standard low load solution	6
5	Blank (Diluent)	1
6	Sample solution -1	1
7	Sample solution -2	1
8	Sample solution -3	1
9	Sample solution -4	1
10	Sample solution -5	1
11	Sample solution -6	1
12	Standard solution (BKT)	1

Acceptance criteria:

1) % RSD for single maximum unknown impurity above 0.1% in six sample preparations should be not more than 15.

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

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

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9.6 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 using the Paracetamol standard low load solution.

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

Paracetamol:

Linearity Stock solution	75	5	1	1	1	37.5 (con. ppm)
	100	100	1	1	1	

Lin level	Exp conc (ppm)	Lin Stock Vol (ml)	Dil to (ml)	Calc conc (ppm)
10%	0.30	2	250	0.30
50%	1.50	2	50	1.50
75%	2.25	3	50	2.25
100%	3.00	4	50	3.00
125%	3.75	5	50	3.75
150%	4.50	6	50	4.50

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.

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Note:

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

Sequence shall be in following provisional

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:

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%, 125% and 150% levels should be not more than 2.0.

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9.7 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 by using same solvent. Calculate the % difference of single maximum unknown impurity and total unknown impurity 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	System suitability solution	1
3	Standard low load solution (Initial)	6
4	Placebo Preparation	1
5	Sample solution (Initial)	1
6	Standard low load solution (Interval)	1
7	Sample solution (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%.

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9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of the analytical method shall perform by filtering sample 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	System suitability solution	1
3	Standard low load solution	6
4	Sample preparation –Unfiltered (Centrifuge)	1
5	Sample preparation –Filter Set I (0.45µ Nylon Filter)	1
6	Sample preparation –Filter Set II (0.45µ Nylon Filter)	1
7	Sample preparation –Filter Set III (0.45µ Nylon Filter)	1
8	Sample preparation – Filter set I (0.45µ PVDF membrane filter)	1
9	Sample preparation – Filter set II (0.45µ PVDF membrane filter)	1
10	Sample preparation – Filter set III (0.45µ PVDF membrane filter)	1

Acceptance criteria:

i) For % RSD of single maximum unknown impurities above 0.1%: % difference should ± 20 against that unfiltered. (Centrifuged)

ii) For % RSD of Total unknown impurities >0.1%: % difference should ±15 against that unfiltered. (Centrifuged)

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9.9 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 low load solution	6
3	Sample Preparation	1
According to each variable		
No.	**Description of solution**	**No. of Injections**
1	Blank (Diluent)	1
2	Standard low load solution	6
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)

Acceptance criteria:

i) % RSD for single maximum unknown impurities above 0.1% should be not more than 20%.

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

PROTOCOL	
Title	Analytical Method Validation Protocol For the test of Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet
Protocol No.	ST/AMVRP/23/037

10.0 ABBREVIATION:

mg	:	Milligram
No	:	Number
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

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11.0 REVISION HISTORY:

Protocol No.	Effective date	Reason for Review
ST/AMVRP/23/037	08/01/2024	New Protocol prepared.