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

ANALYTICAL METHOD VALIDATION REPORT 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 REPORT APPROVAL SHEET

PREPARED BY		
Name	:	K. SARAVANAN
Designation	:	Dy. Manager - QC
Signature	:	
Date	:	30/01/2024
REVIEWED BY		
Name	:	M. VIJAYAKUMAR
Designation	:	GM - QC
Signature	:	
Date	:	31/01/2024
APPROVED BY		
Name	:	S. YARAN
Designation	:	AGM - QA
Signature	:	
Date	:	01/02/2024

Effective Date	:	02/02/2024

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Title	Analytical Method Validation Report 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 VALIDATION : 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 standard, 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	B.No: NA	Not applicable
Working standard Paracetamol	W.S.No: ST/WS/23/012	99.9%
Phenylephrine Hydrochloride	W.S.No: ST/WS/23/045	100.0%
Chlorphenamine Maleate	W.S.No: ST/WS/23/044	100.0%
Ascorbic Acid	W.S.No: ST/WS/23/042	99.9%

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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:

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

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

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

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 VALIDATED PARAMETERS:

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 VALIDATION RESULTS:

9.1 SYSTEM SUITABILITY:

Study Summary:

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

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

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

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

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

Results are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Paracetamol	Resolution for Ascorbic acid and Chlorpheniramine Maleate	Resolution for Chlorpheniramine Maleate and Phenylephrine Hcl	Resolution for Phenylephrine Hcl and Paracetamol
Theoretical Plates	NLT 2000	15219	4.149	3.700	20.917
Tailing Factor	NMT 2.0	1.06			
% RSD	NMT 2.0	0.127			

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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 be less than 2.0.

Result and Conclusion:

The System suitability 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 and sample are 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 analyte should be pass (peak purity value should not less than 0.950) according to Lab solution software.

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Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
1	Blank	Blank peaks	No peak	Not applicable
2	Resolution solution	Ascorbic Acid	2.965	1.000
		Chlorpheniramine Maleate	3.583	1.000
		Phenylephrine Hcl	4.150	1.000
		Paracetamol	8.874	1.000
3	Standard preparation	Paracetamol	8.875	1.000
4	Blank	Blank peaks	No peak	Not applicable
5	Paracetamol Working standard	Paracetamol	8.875	1.000
6	Phenylephrine Hcl Working standard	Phenylephrine Hcl	4.152	1.000
7	Chlorpheniramine Maleate Working standard	Chlorpheniramine Maleate	3.583	1.000
8	Ascorbic Acid Working standard	Ascorbic Acid	2.958	1.000

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Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
9	Paracetamol Working standard + Chlorpheniramine Maleate Working standard + Phenylephrine Hcl Working standard + Ascorbic Acid Working standard	Ascorbic Acid	2.957	1.000
		Chlorpheniramine Maleate	3.575	1.000
		Phenylephrine Hcl	4.141	1.000
		Paracetamol	8.853	1.000
10	Blank	Blank peaks	No peak	Not applicable
11	Plain placebo	Placebo peaks	No peak	Not applicable
12	Placebo + Paracetamol Working standard	Paracetamol	8.859	1.000
13	Placebo + Phenylephrine HCl Working standard	Phenylephrine Hcl	4.150	1.000
14	Placebo + Chlorpheniramine Maleate Working standard	Chlorpheniramine Maleate	3.580	1.000
15	Placebo + Ascorbic Acid Working standard	Ascorbic Acid	2.964	1.000

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Sr.No	Sample ID	Peak Name	Retention time	Peak Purity index
16	Placebo + Paracetamol Working standard + Chlorpheniramine Maleate Working standard + Phenylephrine Hcl Working standard + Ascorbic Acid Working standard	Ascorbic Acid	2.962	1.000
		Chlorpheniramine Maleate	3.579	1.000
		Phenylephrine Hcl	4.145	1.000
		Paracetamol	8.854	1.000
17	Blank	Blank peaks	No peak	Not applicable
18	Test preparation B.No.G17231228	Paracetamol	8.860	1.000

Results and Conclusion:

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

9.3 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 3A and 3B.

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Table 3A. LOD and LOQ determination for Paracetamol

Paracetamol		Residual output		
Conc. (ppm) (x-axis)	**Average area (y-axis)**	**Observation**	**Predicted Y**	**Residuals**
0.301	6130	1	6259.909188	-128.2425214
1.503	31253	2	31038.68355	214.6497863
2.254	46124	3	46525.41752	-401.084188
3.006	61595	4	62012.1515	-416.8181624
3.757	79395	5	77498.88547	1896.447863
4.508	91821	6	92985.61944	-1164.952778
Slope	20611	**Standard deviation**		1034

Table 3B: Limit of Detection and Limit of Quantitation for Paracetamol

Concentration		%	ppm
Observed	LOD	0.099	0.166
	LOQ	0.301	0.502
Determined	LOD	0.020	0.033
	LOQ	0.040	0.067

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Results and conclusion:

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

9.4 INTERFERENCE FROM DEGRADANTS (Forced degradation)

In order to prove specificity of method, further degradation was carried out and peak purity of Dapoxetine peak was monitored.

9.4.1 Acid Degradation:

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, cool and 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. Filter through 0.45µ nylon filter.

9.4.2 Alkali degradation:

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, cool and 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. Filter through 0.45µ nylon filter.

9.4.3 Oxidative Degradation:

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, cool and add 5ml of 30% Hydrogen peroxide solution and heat on water bath at 80°C for 30 minutes, cool and make up to volume with diluent. Filter through 0.45µ nylon filter.

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Acceptance criteria:

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

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.

Table 4: Peak purity (Chemical degradation)

S.No	Sample name	Single maximum unknown impurity	Total impurities	Peak purity
1	Acid Degradation	0.02	0.06	1.000
2	Alkali Degradation	0.98	2.47	1.000
3	Oxidative Degradation	0.03	0.03	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.

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9.5 PRECISION:

9.5.1 Method Precision:

Study summary:

Six sample preparations are analyzed as per the method. The results are tabulated in Table 5.

Acceptance criteria:

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

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

Table 5: Method precision

Preparation No.	Single maximum Unknown Impurity (%)	Total unknown impurity (%)
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	NIL	NIL
% RSD	NIL	NIL

Results and Conclusion: *The method precision results are well within the acceptance criteria indicates the precision of the analytical method.*

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9.5.2 Intermediate Precision:

Study summary:

Six sample preparations are 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 6.

Table 6: Intermediate precision

Preparation No.	Single maximum Unknown Impurity (%)	Total unknown impurity (%)
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	NIL	NIL
% RSD	NIL	NIL

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 7.

Acceptance criteria:

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

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

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

Table 7: Cumulative % RSD

Parameter	Single Unknown Impurity (%)	Total impurity (%)
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	NIL	NIL
% RSD	NIL	NIL

Result and Conclusion:

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

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9.6 LINEARITY AND RANGE:

Study design:

Linearity for Paracetamol is 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 8 for Linearity and Table 9 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 for 10%, 50%, 75%, 100%, 125% and 150% should be not more than 2.0.

Table 8: Linearity Table for Paracetamol

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
10%	0.301	6130
50%	1.503	31253
75%	2.254	46124
100%	3.006	61595
125%	3.757	79395
150%	4.508	91821
Slope		20611
CC		0.998
Sqaured R		0.9989
Intercept		63.90

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Fig.1 : Liner Graph for Paracetamol

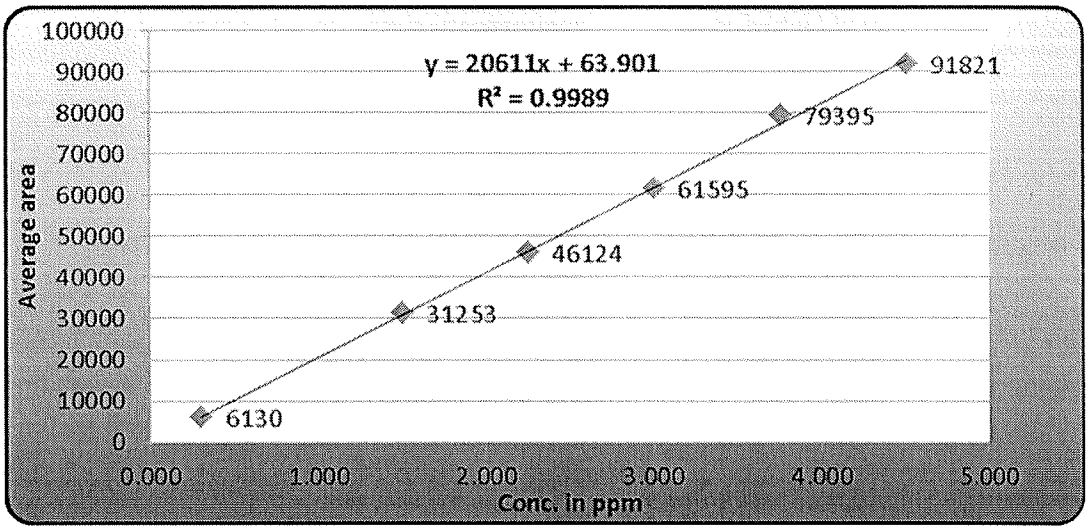

Table:9 Range for Paracetamol

Linearity Levels (%)	% RSD for Paracetamol
10%	0.741
50%	0.177
75%	0.203
100%	0.137
125%	0.057
150%	0.101

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 10% to 150% should be not more than 2.0.

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9.7 STABILITY OF ANALYTICAL SOLUTION:

Study design:

Sample preparation:

Sample preparation is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 10A.

Table 10A: Stability of sample solution for Paracetamol

Time in hours	Area of Sample solution	Absolute % Difference
Initial	13463570	Not applicable
14	13463627	0.00
17	13467257	-0.03
20	13562237	-0.73
24	13458987	0.03
28	13468058	-0.03
38	13448568	0.11
42	13457810	0.04
45	13453311	0.08
48	13449031	0.11
Mean	13469246	-0.05
% RSD	0.248	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 ±10%.

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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 10B.

Table 10B: Stability of standard solution for Paracetamol

Time in hours	Area of Standard solution	Absolute % Difference
Initial	68953	Not applicable
14	68987	-0.05
17	68816	0.20
20	69267	-0.45
24	69072	-0.17
28	69064	-0.16
38	68871	0.12
42	68956	0.00
45	69041	-0.13
48	68961	-0.01
Mean	68999	-0.07
% RSD	0.180	Not applicable

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.*

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9.8 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method is performed by filtering sample solution through 0.45µ Nylon and 0.45µ PVDF membrane filter against that of unfiltered (centrifuged) sample. The results are tabulated in Table 11.

Table 11: Filter paper study for Sample solution

Filter study	Single maximum unknown Impurity (%)	%difference from unfiltered sample	Total unknown impurities (%)	%difference from unfiltered sample
UNFILTERED (CENTRIFUGED)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-I (0.45µ NYLON FILTER)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-II (0.45µ NYLON FILTER)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-III (0.45µ NYLON FILTER)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-I (0.45µ PVDF FILTER)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-II (0.45µ PVDF FILTER)	Not Detected	Not applicable	Not Detected	Not applicable
FILTER SET-III (0.45µ PVDF FILTER)	Not Detected	Not applicable	Not Detected	Not applicable

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Acceptance criteria:

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

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

Results and conclusions:

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

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

9.9 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 ±5.0^{0}C (i.e 25^{0}C to 35^{0}C)

The results are tabulated in table 12.

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Table 12: Robustness of analytical method

Parameter	Single maximum unknown impurity in %	Total unknown impurities in %	Tailing Factor	Theoretical plate	RSD %
Low wavelength (217nm)	Not detected	Not detected	1.07	15303	0.442
High wavelength (223nm)	Not detected	Not detected	1.06	14981	0.255
Low flow rate (0.9ml/minute)	Not detected	Not detected	1.06	15612	0.719
High flow rate (1.1ml/minute)	Not detected	Not detected	1.05	13595	0.303
Column Oven temperature 25°C	Not detected	Not detected	1.06	13937	2.045
Column Oven temperature 35°C	Not detected	Not detected	1.05	13375	0.334
% RSD	NIL	NIL	NA	NA	NA

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%.

Result and Conclusion:

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

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10.0 SUMMARY:

No	Validation parameter	Acceptance criteria	Results
1	System suitability	1) % RSD of area of each Paracetamol peak in six replicate standard low load solution injections should not be more than 5.0.	0.127
		2) Theoretical plate for Paracetamol peak standard low load solution injection should not be less than 2000.	15219
		3) Tailing factor for Paracetamol peak in standard low load solution injection should not be more than 2.0.	1.06
		4) Resolution between Ascorbic acid and Chlorpheniramine Maleate should not be less than 2.0	4.149
		5) Resolution between Chlorpheniramine Maleate and Phenylephrine Hcl should not be less than 2.0	3.700
		6) Resolution between Phenylephrine Hcl and Paracetamol should not be less than 2.0	20.917
2	Specificity Interference from blank, placebo, impurities and placebo spiked with analyte.	1) No significant interference from blank and placebo. 2) Peak purity of analyte peak and each impurity peak should be pass. (Peak purity should not be less than 0.950 according to lab solution software.	Blank and Placebo peaks are not interfere with in test preparation and Peak purity passes within specified limits

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No	Validation parameter	Acceptance criteria	Results
3	Determination of limit of detection and limit of quantitation	LOD: NMT 0.02% LOQ: NMT 0.04%	LOD in% LOD in ppm 0.099 0.166 LOQ in% LOQ in ppm 0.020 0.033
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 index NLT 0.950	There is No any interference due to degradants with analyte in stressed samples and Peak purity is passes according to Lab solution software.

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No	Validation parameter	Acceptance criteria	Results
5	Precision 1) Method Precision	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.	0.00 0.00
	2)Intermediate Precision	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.	0.00 0.00 0.00

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S.No	Verification parameter	Acceptance criteria	Results
6	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: 0.9989** Level %RSD 10% : 0.741 50% : 0.177 75% : 0.203 100% : 0.137 125% : 0.057 150% : 0.101
7	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 is stable upto 48 hours at room temperature.
8	Filter paper study (0.45µ Nylon and 0.45µ PVDF Filter)	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)	i) There is no any interference due to Filter paper in test solution. ii) Results are complies as per the acceptance criteria.

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S.No	Verification parameter	Acceptance criteria	Results
9	**Robustness**		
	(i) Flow rate change	i) % RSD for single maximum unknown impurities above 0.1% should be not more than 20%.	0.00
	(ii) Wavelength change	ii) % RSD for total unknown impurities above 0.1% should be not more than 15%.	0.00
	(iii) Column oven temperature change		

11.0 CONCLUSION:

Validation studies have been conducted for Related substances in Paracetamol, Phenylephrine Hydrochloride, Chlorphenamine Maleate and Ascorbic Acid Sachet for the parameters of System suitability, Specificity, Determination of LOD and LOQ, forced degradation, Method Precision, Intermediate Precision, Solution stability, Linearity and Range, Robustness and Filter paper study 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.

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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
RS	:	Reference standard

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13.0 REVISION HISTORY:

Report No.	Effective date	Reason for Review
ST/AMVRR/23/037	02/02/2024	New Report prepared.