	Protocol Number		**Preparation Date**	
	Version Number		**Revision Date**	

Product / Material	
Active Ingredient	
Types of Analytical Procedures	
Batch No.	

Approvals

Responsibility	Designation	Name	Signature	Date
Prepared by				

Responsibility	Designation	Name	Signature	Date
Reviewed by				

Responsibility	Designation	Name	Signature	Date
Approved by				

	Prepared by	Reviewed by	Approved by
Sign & Date			
Name/ Designation			

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Sign & Date			
Name/ Designation			

1.0 PRE-VALIDATION/ VERIFICATION REQUIREMENTS.

Chemicals, such as reagents and standards, should be available in sufficient quantities, accurately identified, sufficiently stable and checked for purity. Other materials and consumables, for example, chromatographic columns, should be qualified to meet the column's performance criteria. Validation on the analytical procedure should be performed with homogeneous samples, and validation data should be obtained by repeatedly analyzing aliquots of a homogeneous sample, each of which has been independently prepared according to the analytical method procedure.

1.1 ANALYTICAL EQUIPMENT QUALIFICATION.

Before undertaking the validation study, it is necessary to verify that the analytical system is adequately designed, maintained, qualified.

1.1.1 INSTALLATION QUALIFICATION (IQ).

Documented Validation that all key aspects of an installed High performance Liquid chromatography system (Shimadzu) adhere to the approved design specification and that the recommendations of the Shimadzu have been suitably considered.
Protocol Number; _____

1.1.2 OPERATIONAL QUALIFICATION (OQ).

Operational Validation carried out after installation that shows High Performance Liquid chromatography system (Shimadzu performs in accordance with (Shimadzu) specifications and process requirements and that the appropriate GMP systems (e.g. training, calibration, and maintenance, etc.) are in place.
Protocol Number; _____

1.1.3 PERFORMANCE QUALIFICATION (PQ).

Performance Qualification carried out after Operational (vendor) that shows the tests carried out for the Performance Qualification of the HPLC are in complete agreement with the required limits and criteria. Method being used for the determination of test Methods produces consistent, reproducible and reliable results therefore it is suitable for its intended purpose. I-e (Quantification and Qualification).
Protocol Number; _____

1.1.4 CALIBRATION STATUS OF EQUIPMENT.

Equipment was calibrated and bears calibration sticker of the external calibrator.
Calibration Date: _____

Next Calibration Date _____ _____

Calibration Certificate No. _____

Calibrated by: _____

1.2 STABILITY OF THE ANALYTE(S) IN THE SOLUTIONS.

Method development process shows that it generates reproducible, consistent, the stability of the analyte(s) in the solutions, and that of mobile phase give reliable results.

1.3 FACILITIES:

The validation of the method of Determination will be carried out in the Chemistry Lab "Name of organization"

1.4 IDENTIFICATION OF MACHINE/ EQUIPMENT USED:

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High Performance Liquid Chromatography / Analytical Balance.

Sr. #	Instrument Name	Make & Model	Serial No.	Lab Identification No.
01	PUMP			
02	Auto Sampler			
03	Column Oven			
04	CBM			
05	PDA Detector			
06	Analytical balance			
07	HPLC Column			

1.5 TRACEABILITY OF MATERIAL OR PRODUCT USED FOR STUDY:

Sr. #	Type of material	Name	Manufacturer of material / Product	Lot No. / Batch No.	Expiry
01	Reference Standard.				
02	Raw Material				
03	Raw Material				
04	Tablets				
05	Tablets				
06	Tablets				
07	Tablets				

1.6 CHEMICALS / GLASSWARE/ APPARATUS USED:

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Sr. #	Name	Type / uncertainty	Manufacturer	Lot No. / Batch No. / Serial No.	Expiry
01	Methanol				
02	Water				
03	Nylon Filters (M.P)				-
04	Syringe Filters				-
05	50ml volumetric flasks.				-
06	1.0 ml Graduated pipet.				-
07	3.0 ml Gradated pipet.				-
08	5.0 ml Gradated pipet.				-
09	Glass Beakers 50,100,500ml				-
10	Magnetic Stirrer			-	-
11	Spatula(s)			-	-
12	Weighing Boats / Butter papers				
12	Motor & pastel			-	-
13	Sonicate bath				
14	Filtration Syringe				
15	Auto sampler HPLC Vials				

2.0 SCOPE

3.0 OBJECTIVE

4.0 METHOD TO BE VARIFIED/ VALIDATE.

Use HPLC Work instruction for HPLC Operations. Work Instruction #_____

Use Analytical Balance Work instruction for weighing. Work Instruction # _

Test	Specification / Method	Reference
Quantification / Identification	**CHROMATOGRAPHIC CONDITIONS**	
	Column	
	Mode	
	flow rate	
	Column temperature.	
	Wavelength	
	Injection Volume	
	Run Time	
	Injection Procedure	
	System Suitability	
	Mobile Phase	
	Diluent	

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STANDARD SOLUTION PREPRATION:

SAMPLE SOLUTION PREPRATION:

INJECTION PROCEDURE:

CALCULATIONS:

Where ;

90%-110% of the labelled amount of

Precautions:

1. Use Pyrex type glassware and broken glassware should not be used.
2. During analysis temperature should be in between 20-25°c.
3. Use dried glassware and rinse with solvent to be used before use.
4. Use HPLC grade solvents only.
5. All samples must be filtered with 0.45µ Filter.
6. Always degas the mobile phase, as air bubbles may tend to form during solvent mixing or during temperature or pressure changes. Air bubbles may cause pump malfunctions and detector signal noise.
7. Never run the column dry. Make sure there is enough solvent in the reservoir.
8. Use a 0.2µ sized syringe filter for sample. Always filter your sample before injection.

Method Reference: In-House

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Sign & Date			
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5.0 SPECIFICITY:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

5.1 Identification Tests.

Specificity ensure the identity of an analyte.

Methodology: specificity is demonstrated by the ability to discriminate between compounds of closely related structures, or by comparison to known reference materials.

5.2 Purity Test

Specificity ensure that all the analytical procedures (Method) performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Methodology: Impurities available**:** specificity is demonstrated by using spiking the drug substance or product with appropriate levels of impurities.

5.3 Assay (content or potency):

To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Methodology: specificity is demonstrated by using spiked samples to show that method results are unaffected by the presence of impurities or excipients etc.

Placebo Interference

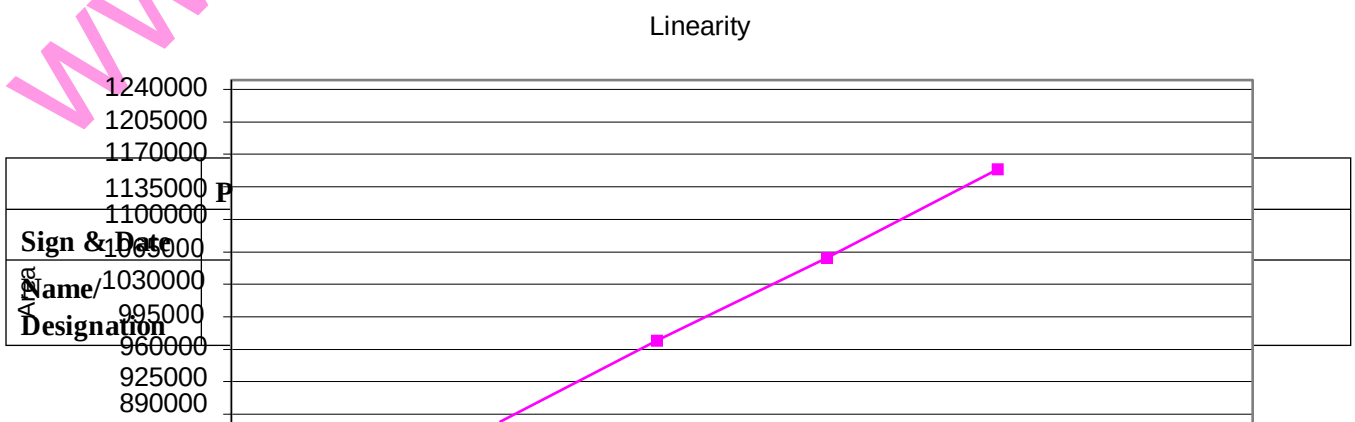
Sr. #	Excipients Name	Peak Time	Peak Area

Acceptance Criteria	**The inactive material did not show significant absorbance / peak at λ max 228 nm.**
	Known degradation products, synthetic impurities and sample matrix present in the commercial product do not interfere with the determination of the active constituent.

6.0 LINEARITY & RANGE.

Prepare a range of standards containing at least five different concentrations of analyte in expected working range. Verify that the method provides acceptable precision, accuracy, and linearity when applied to samples at the extreme as well as within range. For assay 80-120%, determination of impurity 50-120%, content uniformity 70-130%, Dissolution ±20% of over specified range of the test concentration.

Concentration %	Concentration mg/ml	Sample 1	Sample 2	Sample 3	Avg. Peak area



Slope = 9400.39

Parameters	Criteria	Result	Conclusion Qualified√ - Disqualified×
Correlation coefficient	NLT 0.9990		☐Qualified ☐Not Qualified
y-intercept Criteria	NMT 2.0%		☐Qualified ☐Not Qualified

7.0 ACCURACY AND PRECISION

7.1 ACCURACY AND RECOVERY

In the study of accuracy / recovery, Known amount of sample corresponding to three concentration levels i.e. 50%, 100 % and 150 % were taken. Results were obtained using nine determinations over three concentration levels (50%, 100%, and 150 %) and three replicate of each concentration. 50 percent is (lowest concentration) and 150 percent (highest concentration) of the expected working range.

The average of individual recovery was found with in ±2 % of the theoretical amount.

Acceptance criteria: (theoretical amount ± 4%)

Level %	Peak Area of Replicate (Samples)	Peak Area of Replicate (Standard)	%age Recovery	Average Recovery
50 %				
100%				
150%				

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Limits	Criteria		Results	Conclusion Qualified√ - Disqualified×
	For 50 % Level	48 – 52 %	49.64%	☐Qualified ☐Not Qualified
	For 100 % Level	96 – 104 %	100.12 %	☐Qualified ☐Not Qualified
	For 150 % Level	144 – 156 %	153.82 %	☐Qualified ☐Not Qualified

7.2 REPEATABILITY PRECISION (System Precision).

Six replicates of a 100% test solution and measure the responses peak of each sample. The relative standard deviation of the six readings should not be greater than 1%.

S. No.	Observations	
	Retention Time (min)	Peak Area
1	2.847	9188940
2	2.841	9149437
3	2.834	9140982
4	2.837	9121143
5	2.840	9104771
6	2.836	9045541
Mean	2.8392	9125135.66667
Standard Dev.	0.004622409	48329.34105
Relative Standard dev (%RSD)	0.162808659	0.52963

Acceptance Criteria	Criteria	Result	Conclusion Qualified√ - Disqualified×

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	RSD < 1.0 %		☐Qualified ☐Not Qualified

7.3 INTERMEDIATE PRECISION (Method Precision).

The Precision of a method is determined by assaying aliquots of a homogeneous sample to be able to calculate statistically significant estimates of standard deviation or relative standard deviation. Assay should be of samples that have all gone through the entire analytical procedure from sample preparation through final analysis.

Typical variations to be studied include days, analysts, equipment, etc.

7.3.1 WITHIN DAYS AND BETWEEN DAYS VARIATION (Reproducibility)

Day(s)	Peak Area of Sample	Peak Area of Standard	Assay%	Average Assay %	STDV	%RSD
01						
02						
03						

	Criteria	Day (s)	Result	Conclusion Qualified√ - Disqualified×
Acceptance Criteria				☐Qualified ☐Not Qualified
				☐Qualified ☐Not Qualified
	RSD < %			☐Qualified ☐Not Qualified

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Sign & Date			
Name/ Designation			

7.3.2 ANALYST TO ANALYST VARIATION (RUGGEDNESS).

Analyst(s)	Peak Area of Sample	Peak Area of Standard	Assay%	Average Assay %	STDV	%RSD
JB						
AJ						

7.3.3 EQUIPMENT TO EQUIPMENT VARIATION (RUGGEDNESS).

Analyst(s)	Peak Area of Sample	Peak Area of Standard	Assay%	Average Assay %	STDV	%RSD
JB						
AJ						

Acceptance Criteria	Criteria	Analyst(s)	Result	Conclusion Qualified√ - Disqualified×
	RSD < %			☐Qualified ☐Not Qualified
				☐Qualified ☐Not Qualified
	Criteria	Equipment	Result	Conclusion Qualified√ - Disqualified×
				☐Qualified ☐Not Qualified
				☐Qualified ☐Not Qualified

	Prepared by	Reviewed by	Approved by
Sign & Date			
Name/ Designation			

8.0 ROBUSTNESS:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters to ensure reliability during normal usage (e.g., a small change in the composition of an HPLC mobile phase ± 2 %, column temperature ± 5 ºC, reagents, pH of buffer of an HPLC mobile phase 0.1 pH units and Injection volume by + 2 µL.

Flow rate	Peak area	Avg. Peak Area	SD	%RSD
1.1				
1.3				
1.5				
Mobile Phase composition	**Peak area**	**Avg. Peak Area**	**SD**	**%RSD**
Column Oven	**Peak area**	**Avg. Peak Area**	**SD**	**%RSD**
pH Buffer	**Peak area**	**Avg. Peak Area**	**SD**	**%RSD**

Injection volume	Peak area	Avg. Peak Area	SD	%RSD

Acceptance Criteria for Robustness	Criteria	Parameters	Result	Conclusion Qualified√ - Disqualified×
	RSD < %	Flow Rate		☐Qualified ☐Not Qualified
		MP Composition		☐Qualified ☐Not Qualified
		Column Oven		
		pH buffer		☐Qualified ☐Not Qualified
		Injection Volume		☐Qualified ☐Not Qualified

9.0 LIMIT OF DETECTION (LOD).

The detection limit of an analytical procedure is the lowest analytical concentration at which an analyte(s) could be detected qualitatively. LOD can be calculated at levels approximating the LOD according to the formula LOD =3.3(SD/S). Where SD: Standard deviation and S: Slope

Sample	Conc. %	Conc. (mg/ml)	Avg. Peak Area	Standard Deviation	Slope	Conc. (ug/ml) at LOD 3.3 x std.dev /slop	Area at LOD
1	80						
2	90						
3	100						
4	110						
5	120						

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Name/ Designation			

10.0 LIMIT OF QUANTITATION (LOQ).

LOQ is the lowest concentration of an analyte in a sample that can be determined (quantitated) with acceptable precision and accuracy under stated operational conditions of the method. LOQ can be calculated at levels approximating the LOQ according to the formula LOQ = 10(SD/S). Where SD: Standard deviation and S: Slope

Sample	Conc. %	Conc. (mg/ml)	Avg. Peak Area	Standard Deviation	Slope	Conc. (ug/ml) at LOQ 10 x std.dev /slop	Area at LOD
1	80						
2	90						
3	100						
4	110						
5	120						

11.0 SYSTEM SUITABILITY

System Suitability is the checking of a system to ensure system performance before or during the analysis of Unknowns. SST accomplished by summarizing data on reproducibility, efficiency, tailing and resolution for replicate injections.

	Creteria	Parameters	Day(s) SST	Avg. SST	Conclusion Qualified√ - Disqualified×
Acceptance Criteria for SST	RSD ≤ 1%	Inj. Precision for Peak Area (n=6)			☐Qualified ☐Not Qualified
	Rs= ≥ 2.0	Resolution (R1)			☐Qualified ☐Not Qualified
	T= ≤ 2.0	Tailing Factor (T)			☐Qualified ☐Not Qualified

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	N= ≥ 2.0	Capacity Factor (k)			☐Qualified ☐Not Qualified
	N= ≥ 2000	Theoretical Plates (N)			☐Qualified ☐Not Qualified

12.0 OBSERVATIONS / DEVAITIONS:

Protocol Section No:		Page No:		Deviation #:	
Protocol Requirements					
State the protocol requirements:					
Deviation Description					

12.1 JUSTIFICATION FOR ACCEPTANCE:

Deviation Cause/ Investigation

12.2 IMPACT ON OPERATIONS:

Does this Deviation Potentially Impact Distributed Product? (Yes/No)		If so Reference Investigation and/or CAPA No.	
Recommended Corrective Action			
Results of any corrective action taken			

12.3 DEVIATION REPORT WRITTEN BY:

Originator/Function	Name	Signature	Date

	Prepared by	Reviewed by	Approved by
Sign & Date			
Name/ Designation			

	Protocol Number		**Preparation Date**	
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Submitted By			

13.0 CONCLUSIONS

14.0 POST APPROVAL:

Responsibility	Designation	Name	Signature	Date
Written By				
Reviewed By				
Approved By				

	Prepared by	Reviewed by	Approved by
Sign & Date			
Name/ Designation			

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- WhatsApp Group Link: https://chat.whatsapp.com/HY09XT4iEL07oCE4Am3JUW