PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

ANALYTICAL METHOD VERIFICATION PROTOCOL FOR ASSAY

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Suba
Date	:	17/04/2024
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.R.
Date	:	17/04/2024.
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	17/4/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	17/04/24

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

3.0 OBJECTIVE

To verify the method for the test of Assay of Lolip 10mg & Lolip 20 mg & Lolip 80mg (Atorvastatin Calcium Tablets USP) by HPLC.

S. No	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	195.00 mg
2	20 mg	195.00 mg
3	80mg	175.00 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**To verify the assay test for Lolip tablets 10 mg & Lolip tablets 20 mg & Lolip tablets 80 mg as per United states Pharmacopoeia.**

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

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 Verification (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard	:		:		:	
Placebo (If applicable)	:		:		:	
Sample	:		:		:	
Impurities	:		:		:	

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector

Make : Waters Model : e2695

High performance liquid chromatograph with UV visible detector

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

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Column:

C18, 250 mm x 4.6 mm, 5 µm or Equivalent

Working standard ,Solvents and chemicals with grade:

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC Grade)

Anhydrous citric acid (AR Grade)

Stabilizer-free- tetrahydrofuran (AR Grade)

Ammonium hydroxide (AR Grade)

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C18,250 mm x 4.6 mm, 5 µm or Equivalent
Flow rate	:	1.5 ml / minute
Detector wavelength	:	244 nm
Column oven temperature	:	30°C
Injection volume	:	20 µl

Preparation of Buffer:

Dissolve 9.62 g of anhydrous citric acid in 950 ml of water, adjust with ammonium hydroxide to a pH of 4.0, and dilute with water to 1000 ml.

Preparation of Mobile phase:

Acetonitrile, stabilizer-free tetrahydrofuran, and Buffer (270:200:530)

Preparation of Solution A:

Dissolve 9.62 g of anhydrous citric acid in 900ml of water, adjust with ammonium hydroxide to a pH of 7.4, and dilute with water to 1000ml.

Preparation of Diluent:

Mixture of 1 ml Acetonitrile and 1 ml solution A and mix.

System suitability solution:

Weigh accurately 10 mg of USP Atorvastatin calcium working standard and 1 mg of USP Atorvastatin Related Compound-H RS in a 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Shake mechanically for 30 min or until dissolved. Dilute up to the mark using the same solvent. (Concentration: 0.1 mg/ml of USP Atorvastatin calcium RS and 0.01 mg/ml of USP Atorvastatin related compound H RS).

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

Preparation of Standard solution:(Similarity Factor) 10mg & 20mg & 80mg

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent.

(Concentration: 0.1 mg/ml of Atorvastatin calcium working standard)

Preparation of Standard solution: 10mg & 20mg & 80mg

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent.

(Concentration: 0.1 mg/ml of Atorvastatin calcium working standard)

Preparation of Sample solution for 10 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent. (Concentration: 0.1 mg/ml of atorvastatin Calcium).

Preparation of Sample solution for 20 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.(Concentration: 0.1 mg/ml of atorvastatin Calcium)

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

Preparation of Sample solution for 80 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent.(Concentration: 0.1 mg/ml of atorvastatin Calcium)

Preparation of Placebo solution for 10 mg :

Weigh accurately and transfer accurately 1844 mg of Placebo (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent.

Preparation of Placebo solution for 20 mg :

Weigh accurately and transfer accurately 1732 mg of Placebo (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.

Preparation of Placebo solution for 80 mg :

Weigh accurately and transfer accurately 902 mg of Placebo (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Suitability requirements:

1) The Resolution between the peaks corresponding to Atorvastatin and Atorvastatin related compound-H obtained with standard solution should NLT 5.0
2) The tailing factor for the peak of Atorvastatin with standard solution should NMT 1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate injections of standard solution should NMT 1.00.
4) The similarity factor replicate injections of standard solution and similarity factor standard Solution between 0.98 to 1.02.

Calculations:

1) Calculate the content of Atorvastatin Calcium using following formula for 10mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{25}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Atorvastatin Calcium obtained due to sample solution

B = Average area Atorvastatin Calcium obtained due to standard solution

W1= Weight of Atorvastatin Calcium working standard

W2 = Weight of Atorvastatin Calcium sample

P = % Purity of Atorvastatin Calcium working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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2) Calculate the content of Atorvastatin Calcium using following formula for 20 mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{50}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Atorvastatin Calcium obtained due to sample solution

B = Average area Atorvastatin Calcium obtained due to standard solution

W1= Weight of Atorvastatin Calcium working standard

W2 = Weight of Atorvastatin Calcium sample

P = % Purity of Atorvastatin Calcium working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor

3) Calculate the content of Atorvastatin Calcium using following formula for 80mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{200}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Atorvastatin Calcium obtained due to sample solution

B = Average area Atorvastatin Calcium obtained due to standard solution

W1= Weight of Atorvastatin Calcium working standard

W2 = Weight of Atorvastatin Calcium sample

P = % Purity of Atorvastatin Calcium working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor

PROTOCOL	
Title	**Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)**
Protocol No.	**AMVP/ATC/001**

8.0 PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for Verification	
Sr. No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**Precision** i) System precision ii) Method precision

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from Placebo and Impurities (As applicable)

"The specificity is the ability of an analytical procedure to measure accurately an analyte in presence of components that may be expected present in sample matrix".

Purpose:

To demonstrate that the placebo not interfering with the analyte peak.

Preparation of Standard solution: 10mg & 20mg & 80mg

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent.

(Concentration: 0.1 mg/ml of Atorvastatin calcium working standard)

System suitability solution:

Weigh accurately 10 mg of USP Atorvastatin calcium working standard and 1 mg of USP Atorvastatin Related Compound-H RS in a 100 ml volumetric flask. Add 70 ml of Diluent and Dissolve the substance. Shake mechanically for 30 min or until dissolved. Dilute up to the mark using the same solvent.(Concentration: 0.1 mg/ml of USP Atorvastatin calcium RS and 0.01 mg/ml of USP Atorvastatin related compound H RS)

Preparation of Sample solution for 10 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 Minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent.

(Concentration: 0.1 mg/ml of atorvastatin Calcium).

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

Preparation of Sample solution for 20 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm Pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent. (Concentration: 0.1 mg/ml of atorvastatin Calcium).

Preparation of Sample solution for 80 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm Pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with Diluent.

(Concentration: 0.1 mg/ml of atorvastatin Calcium).

Preparation of Placebo solution for 10 mg :

Weigh accurately and transfer accurately 1844 mg of Placebo (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent.

Preparation of Placebo solution for 20 mg :

Weigh accurately and transfer accurately 1732 mg of Placebo (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

Preparation of Placebo solution for 80 mg :

Weigh accurately and transfer accurately 902 mg of Placebo (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent.

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 Solution	1
4	Placebo Solution 10 mg	1
5	Placebo Solution 20 mg	1
6	Placebo Solution 80 mg	1
7	Lolip 10 mg B.No.G18231027 – 1 to 6	Each Sample 1
8	Lolip 20 mg B.No.G18240412– 1 to 6	Each Sample 1
9	Lolip 80 mg B.No.G18231241– 1 to 6	Each Sample 1
10	Standard Solution (BKT)	1

Acceptance criteria:

1) There should not be any interference due to blank, Placebo peak with analyte.
2) For empower software purity angle shall be lesser than the purity threshold.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

9.2 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"

9.2.1 System Precision

Purpose:

To establish the precision of the HPLC system being used for the analysis.

Preparation of Standard solution: 10mg & 20mg & 80mg

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent. (Concentration: 0.1 mg/ml of atorvastatin Calcium).

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution Similarity Factor	1
3	Standard preparation	6

Acceptance criteria:

1) The Resolution between the peaks corresponding to Atorvastatin and Atorvastatin related compound-H obtained with standard solution should not less than 5.0
2) The tailing factor for the peak of Atorvastatin with standard solution should NMT1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate Injections of standard solution should not more than 1.00.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

4) The similarity factor replicate injections of standard solution and similarity factor Standard solution between 0.98 to1.02.

9.2.2 Method Precision:

Purpose:

To establish the repeatability of test results obtained by the analytical method.

Preparation of Sample solution for 10 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent.

Preparation of Sample solution for 20 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.

Preparation of Sample solution for 80 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent.

NOTE: Repeat the same procedure for another 5 Preparation of Sample Solution.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

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.

Note: Sequence table follow as per specificity parameters.

Acceptance criteria:

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

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

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 liter
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Protocol No.	AMVP/ATC/001

11.0 CONCLUSION

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00			

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

ANALYTICAL METHOD VERIFICATION REPORT FOR ASSAY

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

1.0 INDEX

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Ruba
Date	:	01/07/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.
Date	:	01/07/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	01/07/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	01/07/24.

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

3.0 OBJECTIVE

To verify the method for the test of Assay of Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP) by HPLC.

S. No	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	194.48 mg
2	20 mg	193.28 mg
3	80mg	170.25 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**To verify the assay test for Lolip tablets 10 mg & Lolip tablets 20 mg & Lolip tablets 80 mg as per united states pharmacopoeia.**

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

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 VERIFICATION (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard Atorvastatin Calcium	:	**WS No:** WS/ATC/002	:	94.42 %	:	15/07/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Lolip-10mg Lolip-20mg Lolip-80mg	: : :	 G18231027 G18240412 G18231241	:	COA Attached	:	Not Applicable
Impurities	:	Not Applicable	:	Not Applicable	:	Not Applicable

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector

Make : Waters Model : e2695

High performance liquid chromatograph with UV visible detector

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

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Column:

C18, 250 mm x 4.6 mm, 5 µm Thermo or Equivalent (QC-LC-051)

Working standard ,Solvents and chemicals with grade:

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC Grade)

Anhydrous citric acid (AR Grade)

Stabilizer-free- tetrahydrofuran (AR Grade)

Ammonium hydroxide (AR Grade)

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C18,250 mm x 4.6 mm, 5 µm Thermo or Equivalent
Flow rate	:	1.5 ml / minute.
Detector wavelength	:	244 nm.
Column oven temperature	:	30ºC
Injection volume	:	20 µl

Preparation of Buffer:

Dissolve 9.62 g of anhydrous citric acid in 950ml of water, adjust with ammonium Hydroxide to a pH of 4.0, and dilute with water to 1000ml.

Preparation of Mobile phase:

Acetonitrile, stabilizer-free tetrahydrofuran, and Buffer (270:200:530)

Preparation of Solution A:

Dissolve 9.62 g of anhydrous citric acid in 900ml of water, adjust with ammonium Hydroxide to a pH of 7.4, and dilute with water to 1000ml.

Preparation of Diluent:

Mixture of 1 ml Acetonitrile and 1 ml solution A and mix.

System suitability solution:

Weigh accurately 10 mg of USP Atorvastatin calcium working standard and 1 mg of USP Atorvastatin Related Compound-H RS in a 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Shake mechanically for 30 min or until dissolved. Dilute up to the mark using the same solvent.

(Concentration: 0.1 mg/ml of USP Atorvastatin calcium RS and 0.01 mg/ml of USP Atorvastatin related compound H RS)

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

Preparation of Standard Solution & Similarity factor:

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent.

(Concentration: 0.1 mg/ml of Atorvastatin calcium working standard)

Preparation of Standard solution: 10mg & 20mg & 80mg

Weigh and transfer about 20.70 mg of Atorvastatin calcium working standard to a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, Dissolve make up with same solvent. (Concentration: 0.1 mg/ml of Atorvastatin calcium working standard)

Preparation of Sample solution for 10 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 100 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent. (Concentration: 0.1 mg/ml of atorvastatin Calcium).

Preparation of Sample solution for 20 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.(Concentration: 0.1 mg/ml of atorvastatin Calcium)

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

Preparation of Sample solution for 80 mg :

Weigh accurately and transfer accurately 10 intact tablets (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 Minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent. (Concentration: 0.1 mg/ml of atorvastatin Calcium).

Preparation of Placebo solution for 10 mg :

Weigh accurately and transfer accurately 1844 mg of Placebo (equivalent to 100 mg Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 25 ml of volumetric flask with diluent.

Preparation of Placebo solution for 20 mg :

Weigh accurately and transfer accurately 1732mg of Placebo (equivalent to 200 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 50 ml of volumetric flask with diluent.

Preparation of Placebo solution for 80 mg :

Weigh accurately and transfer accurately 902 mg of Placebo (equivalent to 800 mg of Atorvastatin) in a 200 ml volumetric flask. Add about 100 ml of diluent. Shake for 15 minutes, dilute with same solvent and centrifuge or pass through a suitable filter of 0.45 µm pore size filter. Further dilute 5 ml of this solution in to 200 ml of volumetric flask with diluent.

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
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Procedure:

Equilibrate the chromatographic system with mobile phase till a stable baseline is obtained. Separately inject equal volume (20 µL) of solution as per sequence of injections into the chromatograph and record the peak area responses for the major peaks and check for the system suitability requirements.

Suitability requirements:

1) The Resolution between the peaks corresponding to Atorvastatin and Atorvastatin related compound-H obtained with standard solution should not less than 5.0
2) The tailing factor for the peak of Atorvastatin with standard solution should not more than 1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate injections of standard solution should not more than 1.00.
4) The similarity factor replicate injections of standard solution and similarity factor standard solution between 0.98 to1.02.

Calculation:(ASSAY)

1) Calculate the content of Atorvastatin Calcium using following formula for 10mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{25}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

2) Calculate the content of Atorvastatin Calcium using following formula for 20mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{50}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
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3) Calculate the content of Atorvastatin Calcium using following formula for80mg

$$= \frac{A}{B} \times \frac{W1}{200} \times \frac{200}{W2} \times \frac{200}{5} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Atorvastatin Calcium obtained due to sample solution

B = Average area Atorvastatin Calcium obtained due to standard solution

W1= Weight of Atorvastatin Calcium working standard

W2 = Weight of Atorvastatin Calcium sample

P = % Purity of Atorvastatin Calcium working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor

8.0 PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for Verification	
S.No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**Precision** i) System precision ii) Method precision

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from blank and placebo

Study Design:

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

System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
Tailing Factor	NMT 1.5	1.1
% RSD	NMT 1.0	0.2
Similarity factor	0.98 to 1.02	1.00
Resolution	NLT 5.0	5.0

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
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Table 2: Specificity

S.No	Sample ID	Peak Name	Retention time	Purity Angle	Purity Threshold
1	Blank	No Peak	No Peak	Not applicable	Not applicable
2	Standard solution	Atorvastatin Calcium	11.974	0.038	0.235
3	Placebo for Lolip - 10mg	Placebo peaks	No Peak	Not applicable	Not applicable
4	Placebo for Lolip - 20mg	Placebo peaks	No Peak	Not applicable	Not applicable
4	Placebo for Lolip - 80mg	Placebo peaks	No Peak	Not applicable	Not applicable
5	Sample Solution G18231027- 10 mg	Atorvastatin Calcium	11.999	0.033	0.232
6	Sample Solution G18240412 - 20mg	Atorvastatin Calcium	12.026	0.036	0.232
7	Sample Solution G18231241 - 80mg	Atorvastatin Calcium	12.059	0.035	0.230

Results and Conclusion:

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

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
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9.2 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"

9.2.1 System Precision

Study design:

Six replicate injections of standard preparation were injected into the HPLC system. The area response for Atorvastatin Calcium Peak along with % RSD are tabulated in Table 3.

Acceptance criteria:

% RSD of area of analyte peak in six replicate standard injections should not be more than 2.0.

Table 3: System precision

Injection No.	Atorvastatin Calcium
1	3226650
2	3231720
3	3230677
4	3230276
5	3235005
6	3248178
Mean	3233751
% RSD	0.23

Report	
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Report No.	AMVR/ATC/001

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.

9.2.2 Method Precision:

Study Design:

Six Assay preparations of sample were analyzed as per the method. The Assay of Atorvastatin Calcium is calculated. The results are tabulated in Table 4.

Acceptance criteria:

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

Table 4: Method precision for Atorvastatin Calcium

No. of Preparation	Assay % of Lolip-10 mg	Assay % of Lolip-20 mg	Assay % of Lolip-80 mg
1	101.48	100.35	101.16
2	99.99	100.32	100.25
3	101.03	99.75	101.30
4	101.41	100.36	100.45
5	100.13	100.52	102.28
6	100.26	99.96	102.05
Mean	100.72	100.21	101.25
% RSD	0.66	0.29	0.81

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for assay Values indicate The precision of the analytical method.

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

10.0 ABBREVATION:

mg	:	Milligram
S.No	:	Serial Number
ml	:	Milli liter
%	:	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 liter
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

Report	
Title	Analytical Method Verification Assay Report For Lolip 10mg & Lolip 20mg & Lolip 80mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/001

11.0 CONCLUSION:

Verification studies have been conducted for Assay of Lolip 10 mg & Lolip 20 mg & Lolip 80 mg for the parameters of Specificity, System Precision & Method 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 can be used for regular analysis.

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00	01.07.2024	New Report Prepared	New Report Prepared

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

ANALYTICAL METHOD VERIFICATION PROTOCOL FOR DISSOLUTION

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Subha
Date	:	25/04/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.R.
Date	:	25/04/2024.
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	25/04/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	25/04/24

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

3.0 OBJECTIVE

To verify the method for the test of Dissolution of Lolip 10mg & Lolip 20 mg & Lolip 80mg (Atorvastatin Calcium Tablets USP) by UV

S. No	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	195.00 mg
2	20 mg	195.00 mg
3	80mg	175.00 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**Lolip tablets 10 mg & Lolip tablets 20 mg & Lolip tablets 80 mg as per United states Pharmacopoeia.**

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

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 VERIFICATION (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard	:		:		:	
Placebo (If applicable)	:		:		:	
Sample	:		:		:	
Impurities	:		:		:	

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

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

INSTRUMENTS/EQUIPMENTS:

Ultra-violet spectrophotometer

Make: Shimadzu, Model: UV-1900

Dissolution

Make : Electro lab Model:TDT-08L

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Working standard, Solvents and chemicals with grade

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Monobasic Potassium Phosphate (AR Grade)

Sodium Hydroxide (AR Grade)

Acetonitrile (HPLC grade)

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Medium	:	pH 6.8 Phosphate Buffer
Apparatus	:	Apparatus 2 (paddle)
Volume	:	900 ml
RPM	:	75
Temperature	:	37°C ± 0.5°C
Time	:	15 minutes

Instrumental Conditions:

Mode	:	Ultraviolet-visible spectroscopy
Cell-10mg	:	1.0 cm
Cell-20mg	:	0.5 cm
Cell-80mg	:	0.2 cm
wavelength	:	244 nm.
Blank	:	Medium

Preparation of 0.05 M phosphate Buffer solution:

Dissolve 6.8 g of monobasic potassium Phosphate in 900 ml of water, adjust with 6N Sodium hydroxide to a pH of 6.8 and dilute with Water to 1000 ml.

Diluent:

Mixture of 50 ml of Acetonitrile and 50 ml of water, mix well.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

Preparation of Standard Solution :10mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml Of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further Diluted with 2ml of above solution to 200ml using disso medium.

(Concentration: 0.010 mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution : 20mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml Of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further Diluted with 2 ml of above solution to 100ml using disso medium.

(Concentration: 0.020 mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution : 80mg

Weigh accurately and dissolve 28.4mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further Diluted with 4ml of above solution to 50 ml using disso medium.

(Concentration: 0.088 mg/ml of Atorvastatin Calcium working standard).

Preparation of Sample Solution: 10mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable Centrifuge. (Concentration: 0.011 mg/ml of Atorvastatin Calcium).

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

Preparation of Sample Solution: 20mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37°C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable Centrifuge. (Concentration: 0.022 mg/ml of Atorvastatin Calcium).

Preparation of Sample Solution: 80mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained At 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a Suitable centrifuge. (Concentration: 0.088 mg/ml of Atorvastatin Calcium).

Preparation of Placebo Solution: 10mg

Weigh and transfer 184 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso Medium. Through a suitable centrifuge.

Preparation of Placebo Solution: 20mg

Weigh and transfer 173 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso Medium. Through a suitable centrifuge.

Preparation of Placebo Solution: 80mg

Weigh and transfer 90 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso Medium. Through a suitable centrifuge.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

Calculation:

1) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 10 mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{2}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

2) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 20mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{2}{100} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

3) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 80 mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{4}{50} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

Where,

A = Area of peak obtained due to sample solution

B = Area of peak obtained due to standard solution

P = Purity of Atorvastatin Calcium working standard

CF = Conversion Factor (0.967)

LC = Label claim (mg / tablet)

PROTOCOL	
Title	**Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)**
Protocol No.	**AMVP/ATC/002**

System suitability requirement:

% RSD of analyte absorbance in six replicate standard should not be more than 5.0.

8.0 PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for VERIFICATION	
S.No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Blank and Placebo (as applicable)
2.	**Precision** i) System precision ii) Method precision

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from Blank and Placebo (As applicable)

"The specificity is the ability of an analytical procedure to measure accurately an analyte in Presence of components that may be expected present in sample matrix".

Purpose:

To demonstrate that the placebo not interfering with the analyte Absorbance.

Preparation of Standard Solution for 10mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 2ml of above solution to 200ml using disso medium.

(Concentration: 0.010 mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution for 20mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml Of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further Diluted with 2ml of above solution to 100ml using disso medium. (Concentration: 0.020 Mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution for 80mg

Weigh accurately and dissolve 28.4 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further Diluted with 4ml of above solution to 50ml using disso medium.

(Concentration: 0.088 mg/ml of Atorvastatin Calcium working standard).

PROTOCOL	
Title	**Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)**
Protocol No.	**AMVP/ATC/002**

Study design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of injections
1	Blank (Diluent)	1
2	Dissolution Standard Solution	1
3	Dissolution Placebo Solution 10 mg	1
4	Dissolution Placebo Solution 20 mg	1
5	Dissolution Placebo Solution 80 mg	1
6	Lolip-10 mg Jar-1 to Jar-6	Each 1
7	Lolip-20 mg Jar-1 to Jar-6	Each 1
8	Lolip-80 mg Jar-1 to Jar-6	Each 1

Acceptance criteria:

No significant interference due to blank and placebo.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

9.2 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"

9.2.1 System Precision

Purpose

To establish the precision of the UV system being used for the analysis.

Preparation of Standard Solution :10mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 2ml of above solution to 200ml using disso medium. (Concentration: 0.010 mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution : 20mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 2ml of above solution to 100ml using disso medium. (Concentration: 0.020 mg/ml of Atorvastatin Calcium working standard).

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

Preparation of Standard Solution : 80mg

Weigh accurately and dissolve 28.4mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 4ml of above solution to 50ml using disso medium.

(Concentration: 0.088 mg/ml of Atorvastatin Calcium working standard).

Acceptance criteria

% RSD of analyte absorbance in six replicate standard should not be more than 2.0%

9.2.2 Method Precision:

Purpose:

To establish the precision of the UV system being used for the analysis.

Preparation of Sample Solution: 10mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable centrifuge.

(Concentration: 0.011 mg/ml of Atorvastatin Calcium).

Preparation of Sample Solution: 20mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37°C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable centrifuge.

(Concentration: 0.022 mg/ml of Atorvastatin Calcium).

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

Preparation of Sample Solution: 80mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a Suitable centrifuge.

(Concentration: 0.088 mg/ml of Atorvastatin Calcium).

Study design

To demonstrate the method precision, analyze six sample 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.

Note: Sequence table follow as per specificity parameters.

Acceptance criteria:

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

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

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 liter
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablet USP)
Protocol No.	AMVP/ATC/002

11.0 CONCLUSION

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00			

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

ANALYTICAL METHOD VERIFICATION REPORT FOR DISSOLUTION

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	
Date	:	02/07/2024.
Reviewed By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V.
Date	:	02/07/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	
Date	:	02/07/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	02/07/2024

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

3.0 OBJECTIVE

To verify the method for the test of Dissolution of Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP) by UV.

S. No	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	194.48 mg
2	20 mg	193.28 mg
3	80mg	170.25 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**To verify the Dissolution test for Lolip 10 mg & Lolip 20 mg & Lolip 80 mg as per United states Pharmacopoeia.**

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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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 Verification (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard Atorvastatin Calcium	:	**WS/No:** WS/ATC/002	:	94.42%	:	15/07/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Lolip-10mg Lolip-20mg Lolip-80mg	: : :	G18231027 G18240412 G18231241	:	COA Attached	:	Not Applicable
Impurity	:	Not Applicable	:	Not Applicable	:	Not Applicable

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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6.0 DETAILS OF INSTRUMENTS/EQUIPMENTS,COLUMN,SOLVENTS AND CHEMICALS TO BE USED:

INSTRUMENTS/EQUIPMENTS:

Ultra-violet spectrophotometer

Make: Shimadzu, Model: UV-1900

Dissolution

Make : Electro lab Model:TDT-08L

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Working standard, Solvents and chemicals with grade :

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Monobasic Potassium Phosphate (AR Grade)

Sodium Hydroxide (AR Grade)

Acetonitrile (HPLC grade)

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Medium	:	pH 6.8 Phosphate Buffer
Apparatus	:	Apparatus 2 (paddle)
Volume	:	900 ml
RPM	:	75
Temperature	:	37°C ± 0.5°C
Time	:	15 minutes

Instrumental Conditions:

Mode	:	Ultraviolet-visible spectroscopy
Cell-10mg	:	1.0 cm
Cell-20mg	:	0.5 cm
Cell-80mg	:	0.2 cm
wavelength	:	244 nm.

Preparation of 0.05 M phosphate Buffer solution:

Dissolve 68 g of monobasic potassium Phosphate in 900 ml of water, adjust with 6N sodium hydroxide to a pH of 6.8 and dilute with Water to 10000 ml.

Diluent:

Mixture of 50 ml of Acetonitrile and 50 ml of water, mix well.

Preparation of Standard Solution for 10mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake Mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 2 ml of above solution to 200ml using disso medium. (Concentration: 0.010 mg/ml of Atorvastatin Calcium working standard).

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Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Preparation of Standard Solution for 20 mg

Weigh accurately and dissolve 26 mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 2ml of above solution to 100ml using disso medium.

(Concentration: 0.020 mg/ml of Atorvastatin Calcium working standard).

Preparation of Standard Solution for 80 mg

Weigh accurately and dissolve 28.4mg of Atorvastatin Calcium working standard in a 25 ml of volumetric flask. Add about 15 ml of Diluent to dissolve the substance. Shake mechanically for 10 min or until dissolved. Dilute up to the mark using Diluent. Further diluted with 4ml of above solution to 50ml using disso medium. (Concentration: 0.088 mg/ml of Atorvastatin Calcium working standard).

Preparation of Sample Solution for 10 mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable filter or centrifuge. (Concentration: 0.011 mg/ml of Atorvastatin Calcium).

Preparation of Sample Solution: 20 mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37°C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable filter or centrifuge. (Concentration: 0.022 mg/ml of Atorvastatin Calcium).

Preparation of Sample Solution: 80 mg

Place one tablets in each vessel containing 900 ml of dissolution medium maintained at 37 °C (± 0.5 °C). With draw the aliquot from vessel, at given interval, through a suitable filter or centrifuge. (Concentration: 0.088 mg/ml of Atorvastatin Calcium).

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Preparation of Placebo Solution for 10 mg

Weigh and transfer 184 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso medium through a suitable filter or centrifuge.

Preparation of Placebo Solution for 20 mg

Weigh and transfer 173 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso medium through a suitable filter or centrifuge.

Preparation of Placebo Solution for 80 mg

Weigh and transfer 90 mg of placebo into 1000 mL volumetric flask. Add about 500 mL of disso medium, Sonicate to dissolve and make up to volume With 900ml disso medium through a suitable filter or centrifuge.

Calculation:

1) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 10 mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{2}{200} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

2) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 20 mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{2}{100} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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3) Calculated the content of Atorvastatin Calcium equivalent to Atorvastatin by using following formula for 80 mg

$$= \frac{A}{B} \times \frac{W1}{25} \times \frac{4}{50} \times \frac{900}{1} \times \frac{P}{100} \times \frac{100}{LC} \times CF$$

Where,

A = Area of peak obtained due to sample solution

B = Area of peak obtained due to standard solution

P = Purity of Atorvastatin Calcium working standard

CF = Conversion Factor (0.967)

LC = Label claim (mg / tablet)

System suitability requirement:

% RSD of analyte absorbance in six replicate standard should not be more than 5.0.

8.0 PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for Verification	
Sr. No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**Precision** i) System precision ii) Method precision

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from blank and placebo

Study Design:

Blank, standard, placebo and placebo spiked with analyte and sample were analyzed as per the method to examine the interference of blank and placebo with Lolip tablets

System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
% RSD	NMT 2.0	0.10

Table 2: Specificity

S.No	Description of solution	244 nm
1	Blank	0.00
2	Standard Solution-10 mg	0.385
3	Standard Solution-20 mg	0.390
4	Standard Solution-80 mg	0.599
5	Placebo-10mg	0.00
6	Placebo-20mg	0.00
7	Placebo-80mg	0.00
8	Sample -1(Lolip-10mg)	0.386
9	Sample -1(Lolip-20mg)	0.423
10	Sample -1(Lolip-80mg)	0.604

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with Lolip 10, 20 & 80 mg sample within specified limits. Hence method is selective and specific.

9.2 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"

9.2.1 System Precision

Study design:

Six replicate injections of standard preparation were injected into the UV system.The area response for Atorvastatin calcium Peak along with % RSD are tabulated in Table 3.

Acceptance criteria:

% RSD of analyte absorbance in six replicate standard should not be more than 2.0.

Table 3: System precision

Injection No.	Lolip-10 mg	Lolip-20 mg	Lolip-80 mg
1	0.391	0.384	0.690
2	0.392	0.384	0.690
3	0.392	0.384	0.690
4	0.392	0.384	0.690
5	0.392	0.383	0.690
6	0.392	0.383	0.690
Mean	0.392	0.384	0.690
% RSD	0.104	0.135	0.000

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for the absorbance indicates the system precision of UV system used.

9.2.2 Method Precision:

Study Design:

Six dissolution unit preparations of sample were analyzed as per the method. The dissolution of Lolip 10, 20 & 80 mg is calculated. The results are tabulated in Table 4.

Acceptance criteria:

% RSD for dissolution of six test units should not be more than 5.0.

Table 4: Method precision for Lolip Tablets

No. of Preparation	Dissolution of Lolip-10mg	Dissolution of Lolip-20mg	Dissolution of Lolip-80mg
1	96.70	95.59	93.62
2	92.27	94.91	93.49
3	93.15	94.68	91.42
4	95.37	95.59	91.01
5	94.71	94.00	91.70
6	91.38	94.46	92.80
Mean	93.93	94.87	92.34
% RSD	2.14	0.67	1.20

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

Results and Conclusion:

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

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

10.0 ABBREVATION:

mg	:	Milligram
S.No	:	Serial Number
ml	:	Milli liter
%	:	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 liter
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

Report	
Title	Analytical Method Verification Dissolution Report For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/002

11.0 CONCLUSION:

Verification studies have been conducted for Dissolution of Lolip-10 mg and Lolip-20 mg and Lolip-80 mg tablets for the parameters of specificity, System and Method 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 can be used for regular analysis.

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00	02.07.2024	New Report prepared.	New Report prepared

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

ANALYTICAL METHOD VERIFICATION PROTOCOL FOR RELATED SUBSTANCES

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
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Signature	:	Subha
Date	:	20/04/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.
Date	:	20/04/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	
Date	:	20/4/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	20/04/24.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

3.0 OBJECTIVE

To verify the method for the test of Related Substances of Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP) by HPLC.

S.No.	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	195.00 mg
2	20 mg	195.00 mg
3	80 mg	175.00 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**To verify the Related Substances test for Atorvasatin Calcium Tablet USP as per United States Pharmacopoeia.**

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

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 VERIFICATION (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard	:		:		:	
Placebo (If applicable)	:		:		:	
Sample	:		:		:	
Impurities	:		:		:	

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector

Make : Waters Model : e2695

High performance liquid chromatograph with UV visible detector

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

Dissolution

Make : Electro lab Model:TDT-08L

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Column

C18,4.6 mm x 250 mm, 5 µm or Equivalent

Working standard, Solvents and chemicals with grade

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Monobasic Ammonium Phosphate (AR Grade)

Stabilizer-free tetrahydrofuran (AR Grade)

Methanol (AR Grade)

N,N-Dimethylformamide (AR Grade)

Ammonium Hydroxide (AR Grade)

Acetic Acid (AR Grade)

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic conditions:

Column	:	C18,4.6 mm x 250 mm, 5 µm or Equivalent
Flow rate	:	See the table mentioned below
Wavelength	:	244 nm
Column temperature	:	30°C
Auto sampler	:	10°C
Injection Volume	:	20 µl

Preparation of Diluent :

N, N-Dimethylformamide.

Preparation of Buffer:

Dissolve 5.75 g of monobasic ammonium phosphate in 1000 ml of water. Adjust with dilute acetic acid (10 % v/v) or dilute ammonium hydroxide (10 % v/v) to a pH of 4.3 ± 0.05.

Preparation of Solution A:

Prepare the solution of 925 ml of Acetonitrile and 75 ml of stabilizer- free tetrahydrofuran solution.

Preparation of Solution B:

Mixture of 420 ml of solution-A and 580 ml of buffer. Sonicate and Filter through 0.45 µm membrane filter and degas.

Preparation of Solution C:

Mixture of 600 ml of methanol and 200 ml of solution-A and 200 ml of buffer. Sonicate and Filter through 0.45 micron membrane filter and degas.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
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Gradient program:

Times (Minutes)	Mobile Phase B (% v/v)	Mobile Phase C (% v/v)	Comment
0	100	0	1.8
30	100	0	1.8
45	25	75	1.5
50	25	75	1.5
55	20	80	1.5
58	100	0	1.8
65	100	0	1.8

For the standard solution, the run time is only 30 min. For the system suitability solution and Sample solution, the run time is 65 min.

Preparation of System Suitability solution:

Weigh accurately 5 mg of USP Atorvastatin Related Compound-D RS in 100 ml volumetric flask. Add 50 ml of diluent and dissolve the substance. Sonicate to dissolve if necessary. Dilute up to the mark using the same solvent. Dilute 1 ml of this solution to 100 ml volumetric flask, add weigh accurately 6 mg of USP Atorvastatin Calcium RS and 5 mg of USP Atorvastatin Calcium Compound-B RS and 1 mg of USP Atorvastatin Related Compound-H RS, Add 50 ml of diluent and dissolve the substance. Dilute up to the mark using the same solvent.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

Preparation of Standard solution:

Weight accurately and transfer 50 mg of Atorvastatin calcium working standard in 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Sonicate to dissolve, if Necessary. Dilute up to the mark with Diluent. Further dilute 1 ml of this solution with 100 ml of diluent. (Concentration: 5 µg/ml of USP Atorvastatin calcium working standard).

Preparation of Placebo solution for Lolip 10 mg ,20 mg & 80 mg

Transfer the placebo (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate.

Preparation of Sample solution for Lolip 10 mg,20 mg & 80 mg

Transfer the powder (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate. (Concentration: 1 mg/ml of atorvastatin)

System suitability:

The relative retention times of the all peaks eluting before atorvastatin related Compound-H as given in table are calculated with respect to the atorvastatin peak. The relative retention times of the all peaks eluting after atorvastatin related compound-H are calculated with respect to Atorvastatin related compound-H.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

Suitability requirements:

1) The Resolution between the peaks corresponding to Atorvastatin related compound-B and Atorvastatin obtained with standard solution should not less than 1.4
2) The tailing factor for the peak of Atorvastatin with standard solution should NMT 1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate injections of standard solution should NMT 5.0%
4) The Signal-noise –ratio of Atorvastatin related compound-D not less than 10.0.

Calculations:

Calculate the percentage of each impurity in the portion of tables taken.

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{100} \times \frac{50}{W2} \times \frac{P}{100} \times \frac{100}{LC} \times CF \times AW \times \frac{1}{RRF}$$

Where,

A = Area of peak obtained due to sample solution

B = Area of peak obtained due to standard solution

W1 = Weight of standard Atorvastatin Calcium working standard in mg

W2 = Weight of sample in g

CF = Conversion factor (0.967)

P = Purity of Atorvastatin Calcium working standard

AW = Average Weight of tablet (in g)

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
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Acceptance criteria:

Name	RRT	RRF	Limits
Atorvastatin amide	0.44	--	--
Atorvastatin related compound A	0.84	--	--
Atorvastatin pyrrolidone analog	0.88	0.68	0.5
Atorvastatin related compound B	0.94	--	--
Atorvastatin	1.00	--	--
Atorvastatin related compound C	1.09	--	--
Atorvastatin pyrrolidone lactone	1.62	--	--
Atorvastatin related compound H	1.00	1.18	1.0
Atorvastatin epoxy pyrrolooxazin 6-hydroxy analog	1.06	0.53	0.5
Atorvastatin methyl ester	1.12	--	--
Atorvastatin epoxy pyrrolooxazin 7-hydroxy analog	1.14	0.53	0.5
Atorvastatin epoxy THF analog	1.20	1.12	1.0
Atorvastatin related compound D	1.27	1.12	0.5
Atorvastatin tert-butyl ester	1.49	--	--
Any other unspecified degradation product	--	1.00	0.2
Total degradation products	--	--	4.0

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

8.0 PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for VERIFICATION	
S.No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**Precision** i) Method precision

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

9.1.1 Interference from Placebo and Impurities (As applicable)

"The specificity is the ability of an analytical procedure to measure accurately an analyte in presence of components that may be expected present in sample matrix".

Purpose:

To demonstrate that the placebo not interfering with the analyte peak.

Preparation of System Suitability solution:

Weigh accurately 5 mg of USP Atorvastatin Related Compound-D RS in 100 ml volumetric flask. Add 50 ml of diluent and dissolve the substance. Sonicate to dissolve if necessary. Dilute up to the mark using the same solvent. Dilute 1 ml of this solution to100 ml volumetric flask, add weigh accurately 6 mg of USP Atorvastatin Calcium RS and 5 mg of USP Atorvastatin Calcium Compound-B RS and 1 mg of USP Atorvastatin Related Compound-H RS, Add 50 ml of diluent and dissolve the substance. Dilute up to the mark using the same solvent.

Preparation of Standard solution:

Weight accurately and transfer 50 mg of Atorvastatin calcium working standard in 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Sonicate to dissolve, if Necessary. Dilute up to the mark with Diluent. Further dilute 1 ml of this solution with 100 ml of diluent.

(Concentration: 5 µg/ml of USP Atorvastatin calcium working standard).

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

Preparation of Placebo solution for 10 mg ,20 mg & 80 mg

Transfer the placebo (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate.

Preparation of Sample solution: 10mg & 20mg & 80mg

Transfer the powder (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate. (Concentration: 1 mg/ml of atorvastatin)

Study design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of injections
1	RS Blank	1
2	System suitability Solution	1
3	Standard Solution Similarity Factor	1
4	Standard Solution	6
5	Lolip-10 mg Placebo	1
6	Lolip-20 mg Placebo	1
7	Lolip-80 mg Placebo	1
8	Lolip 10 mg B.No.G18230127 Sample 1-6	Each 1
9	Standard Solution-BKT	1
10	Lolip 20 mg B.No.G18240412 Sample 1-6	Each 1
11	Standard Solution-BKT	1

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

12	Lolip 80 mg B.No.G18231241 Sample 1-6	Each 1
13	Standard Solution-BKT	1

Acceptance criteria:

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

ii) For empower software purity angle shall be lesser than the purity threshold.

9.2 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"

9.2.1 System Precision

Purpose:

To establish the precision of the HPLC system being used for the analysis.

Preparation of Standard solution and Similarity Factor:

Weight accurately and transfer 50 mg of Atorvastatin calcium working standard in 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Sonicate to dissolve, if Necessary. Dilute up to the mark with Diluent. Further dilute 1 ml of this solution with 100 ml of diluent.

(Concentration: 5 µg/ml of USP Atorvastatin calcium working standard).

PROTOCOL	
Title	**Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)**
Protocol No.	**AMVP/ATC/003**

Study Design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard solution Similarity Factor	1
3	Standard preparation	6

Suitability requirements:

1) The Resolution between the peaks corresponding to Atorvastatin related compound-B and Atorvastatin obtained with standard solution should not less than 1.4
2) The tailing factor for the peak of Atorvastatin with standard solution should NMT 1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate injections of standard solution should NMT 5.0%

4) The Signal-noise –ratio of Atorvastatin related compound-D not less than 10.0.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

9.2.2 Method Precision:

Purpose:

To establish the repeatability of test results obtained by the analytical method.

Preparation of System Suitability solution:

Weigh accurately 5 mg of USP Atorvastatin Related Compound-D RS in 100 ml volumetric flask. Add 50 ml of diluent and dissolve the substance. Sonicate to dissolve if necessary. Dilute up to the mark using the same solvent. Dilute 1 ml of this solution to 100 ml volumetric flask, add weigh accurately 6 mg of USP Atorvastatin Calcium RS and 5 mg of USP Atorvastatin Calcium Compound-B RS and 1 mg of USP Atorvastatin Related Compound-H RS, Add 50 ml of diluent and dissolve the substance. Dilute up to the mark using the same solvent.

Preparation of Standard solution:

Weight accurately and transfer 50 mg of Atorvastatin calcium working standard in 100ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Sonicate to dissolve, if Necessary. Dilute up to the mark with Diluent. Further dilute 1 ml of this solution with 100 ml of diluent.

(Concentration: 5 µg/ml of USP Atorvastatin calcium working standard)

Preparation of Placebo solution for 10 mg,20 mg & 80 mg

Transfer the placebo (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

Preparation of Sample solution for 10 mg, 20 mg & 80 mg

Transfer the powder (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate.

(Concentration: 1 mg/ml of atorvastatin)

Study design:

To demonstrate the method precision, analyze six sample preparations as per the methodology representing a single batch and determine the Related substance for the same. Evaluate the method precision by computing the percentage and relative standard deviation of the Related substances results.

Note: Sequence table follow as per specificity parameters.

Acceptance criteria:

i) Individual known and unknown impurity of individual sample and mean of all sample should not be more than specification limit.

ii) Total impurities of individual sample and mean of all samples should not be more than specification limit.

iii) Difference between lowest value and highest value of Individual known and unknown impurity should not be more than 0.1% absolute.

iv) Difference between lowest value and highest value of total impurities should not be more than 0.2% absolute.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

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
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Lolip 10 mg & Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets BP)
Protocol No.	AMVP/ATC/003

11.0 CONCLUSION

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00			

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

ANALYTICAL METHOD VERIFICATION REPORT FOR RELATED SUBSTANCES

Site Address: GENERIC HEALTHCARE PRIVATE LIMITED
R.S. No. 4/3, plot No. 33, Kurumbapet Industrial Estate,
Villianur Commune, Pondicherry- 605009

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

1.0 INDEX

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Ruba
Date	:	08/07/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.
Date	:	08/07/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	08/07/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	08/07/24

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

3.0 OBJECTIVE

To verify the method for the test of Related Substances of Lolip-10 mg ,Lolip-20 mg & Lolip-80 mg (Atorvastatin Calcium Tablet USP) by HPLC.

S. No	Strength of Atorvastatin Calcium Tablets	Average weight in mg
1	10 mg	194.48 mg
2	20 mg	193.28 mg
3	80 mg	170.25 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**USP 2023**
REASON FOR VERIFICATION	:	**To verify the Related Substances test for Atorvastatin Calcium Tablet USP as per United States Pharmacopoeia.**

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

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 verification (as applicable).

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard	:	**WS NO:** WS/ATC/002	:	94.42%	:	15/07/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Lolip-10mg Lolip-20mg Lolip-80mg	: : :	 G18231027 G18240412 G18231241	:	COA Attached	:	Not Applicable
Impurities Atorvastatin Related Com-H Atorvastatin Related Com-D Atorvastatin Related Com-B	: : :	 IM/A/22/045 IM/A/22/085 GL-A1203/001	:	Not Applicable	:	Not Applicable

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector

Make : Waters Model : e2695

High performance liquid chromatograph with UV visible detector

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

Dissolution

Make : Electro lab Model:TDT-08L

Analytical Balance

Make : Shimadzu, Model : AUW220D

pH Meter

Make: Eutech instruments, Model No: pH 700

Column

C18,4.6 mm x 250 mm, 5 µm (or Equivalent) (QC-LC-062)

Working Standard,Solvents and chemicals with grade

Atorvastatin Calcium (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Monobasic Ammonium Phosphate (AR Grade)

Stabilizer-free tetrahydrofuran (AR Grade)

Methanol (AR Grade)

N,N-Dimethyl formamide (AR Grade)

Ammonium Hydroxide (AR Grade)

Acetic Acid (AR Grade)

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic conditions:

Column	:	C18,4.6 mm x 250 mm, 5 µm or Equivalent
Flow rate	:	See the table mentioned below
Wavelength	:	244 nm
Column temperature	:	30°C
Autosampler	:	10°C
Injection Volume	:	20 µl

Preparation of Diluent :

N, N-Dimethylformide.

Preparation of Buffer:

Dissolve 5.75 g of monobasic ammonium phosphate in 1000 ml of water. Adjust with dilute Acetic acid (10 % v/v) or dilute ammonium hydroxide (10 % v/v) to a pH of 4.3 ± 0.05.

Preparation of Solution A:

Prepare the solution of 925 ml of Acetonitrile and 75 ml of stabilizer- free tetrahydrofuran solution.

Preparation of Solution B:

Mixture of 420 ml of solution-A and 580 ml of buffer. Sonicate and Filter through 0.45 µm membrane filter and degas.

Preparation of Solution C:

Mixture of 600 ml of methanol and 200 ml of solution-A and 200 ml of buffer. Sonicate and Filter through 0.45 µm membrane filter and degas.

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Gradient program:

Times (Minutes)	Mobile Phase B (% v/v)	Mobile Phase C (% v/v)	Comment
0	100	0	1.8
30	100	0	1.8
45	25	75	1.5
50	25	75	1.5
55	20	80	1.5
58	100	0	1.8
65	100	0	1.8

For the standard solution, the run time is only 30 min. For the system suitability solution and Sample solution, the run time is 65 min.

Preparation of System Suitability solution:

Weigh accurately 5 mg of USP Atorvastatin Related Compound-D RS in 100 ml volumetric flask. Add 50 ml of diluent and dissolve the substance. Sonicate to dissolve if necessary. Dilute up to the mark using the same solvent. Dilute 1 ml of this solution to 100 ml volumetric flask, add weigh accurately 6 mg of USP Atorvastatin Calcium RS and 5 mg of USP Atorvastatin Calcium Compound-B RS and 1 mg of USP Atorvastatin Related Compound-H RS, Add 50 ml of diluent and dissolve the substance. Dilute up to the mark using the same solvent.

Preparation of Standard solution:

Weight accurately and transfer 50 mg of Atorvastatin calcium working standard in 100 ml volumetric flask. Add 70 ml of Diluent and dissolve the substance. Sonicate to dissolve, if Necessary. Dilute up to the mark with Diluent. Further dilute 1 ml of this solution with 100 ml of diluent. (Concentration: 5 µg/ml of USP Atorvastatin calcium working standard).

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

Preparation of Placebo solution: 10 mg & 20 mg & 80 mg

Transfer the placebo (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate.

Preparation of Sample solution: 10 mg & 20 mg & 80 mg

Transfer the powder (equivalent to about 50 mg of atorvastatin), to a 50 ml volumetric flask. Add 30 ml of diluent and shake mechanically for 15 min. Dilute with diluent to volume and pass the solution through a suitable filter of 0.45-µm pore Size, discarding the first few ml of the filtrate. (Concentration: 1 mg/ml of atorvastatin)

System suitability:

Note: The relative retention times of the all peaks eluting before atorvastatin related Compound-H as given in table are calculated with respect to the atorvastatin peak. The relative retention times of the all peaks eluting after atorvastatin related compound-H are calculated with respect to Atorvastatin related compound-H.]

Suitability requirements:

1) The Resolution between the peaks corresponding to Atorvastatin related compound-B and Atorvastatin obtained with standard solution should not less than 1.4
2) The tailing factor for the peak of Atorvastatin with standard solution should not more than 1.5.
3) The % RSD for the peak area response of Atorvastatin obtained with the replicate injections of standard solution should not more than 5.0%
4) The Signal-noise –ratio of Atorvastatin related compound-D not less than 10.0.

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Calculations:

Calculate the percentage of each impurity in the portion of tables taken.

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{100} \times \frac{50}{W2} \times \frac{P}{100} \times \frac{100}{LC} \times CF \times AW \times \frac{1}{RRF}$$

Where,

A = Area of peak obtained due to sample solution

B = Area of peak obtained due to standard solution

W1 = Weight of standard Atorvastatin Calcium working standard in mg

W2 = Weight of sample in g

CF = Conversion factor (0.967)

P = Purity of Atorvastatin Calcium working standard

AW = Average Weight of tablet (in g)

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Acceptance criteria:

Name	RRT	RRF	Limits
Atorvastatin amide	0.44	--	--
Atorvastatin related compound A	0.84	--	--
Atorvastatin pyrrolidone analog	0.88	0.68	0.5
Atorvastatin related compound B	0.94	--	--
Atorvastatin	1.00	--	--
Atorvastatin related compound C	1.09	--	--
Atorvastatin pyrrolidone lactone	1.62	--	--
Atorvastatin related compound H	1.00	1.18	1.0
Atorvastatin epoxy pyrrolooxazin 6-hydroxy analog	1.06	0.53	0.5
Atorvastatin methyl ester	1.12	--	--
Atorvastatin epoxy pyrrolooxazin 7-hydroxy analog	1.14	0.53	0.5
Atorvastatin epoxy THF analog	1.20	1.12	1.0
Atorvastatin related compound D	1.27	1.12	0.5
Atorvastatin tert-butyl ester	1.49	--	--
Any other unspecified degradation product	--	1.00	0.2
Total degradation products	--	--	4.0

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

8.0PARAMETERS TO BE VERIFIED:

Following parameters shall be selected for Verification	
S.No.	**VERIFICATION Parameter**
1.	**Specificity (Selectivity)** Interference from Placebo and Impurities (as applicable)
2.	**Precision** i) System Precision ii) Method precision

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from Placebo and Impurities (As applicable)

Study Design:

Blank, standard, placebo and placebo spiked with analyte and sample were analyzed as per the method to examine the interference of blank and placebo with Lolip peaks. System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
Resolution	NLT 1.4	1.4
Tailing Factor	NMT 1.5	1.2
RSD %	NMT 5.0%	1.1
Single-to-noise ratio NLT 10	NLT 10	13.2
Similarity factor	0.98 to 1.02	0.99

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Table 2: Specificity

S.No	Sample ID	Peak Name	Retention time	Purity Angle	Purity Threshold
1	Blank	No Peak	No Peak	Not applicable	Not applicable
2	Standard	Atorvastatin Calcium	16.564	0.830	1.285
3	Placebo for Lolip -10 mg	Placebo peaks	No Peak	Not applicable	Not applicable
4	Placebo for Lolip -20 mg	Placebo peaks	No Peak	Not applicable	Not applicable
5	Placebo for Lolip -80 mg	Placebo peaks	No Peak	Not applicable	Not applicable
6	Test preparation G18231027-10 mg	Atorvastatin Calcium	16.662	0.030	0.425
7	Test preparation G18240412-20 mg	Atorvastatin Calcium	18.625	0.019	0.302
8	Test preparation G18231241-80 mg	Atorvastatin Calcium	21.081	0.025	0.296

Results and Conclusion:

From the blank and placebo peaks are not interfere with Atorvastatin Calcium peak in test preparation and Peak purity passes within specified limits. Hence method is selective and specific.

9.2 PRECISION

9.2.1 System Precision :

Study design:

Six replicate injections of standard preparation were injected into the HPLC system. The area response for Atorvastatin Calcium Peak along with % RSD are tabulated in table 3.

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

Acceptance criteria:

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

Table 3: System precision

Injection No.	Atorvastatin Calcium -10 mg	Atorvastatin Calcium -20 mg	Atorvastatin Calcium -80 mg
1	125252	126885	125950
2	124527	126135	126941
3	123753	126994	127016
4	124141	126385	126937
5	123213	125551	126873
6	121368	126856	125611
Mean	123709	126468	126555
% RSD	1.1	0.4	0.5

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.

9.2.2 Method Precision :

Study Design:

Six sample preparations were analyzed as per the method. The results are tabulated in table 3 and 4 and 5

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Table 3

No. of Preparation	Related substances of Atorvastatin Calcium for 10 mg %							
	Atorvastatin pyrrolidone analog	Atorvastatin related com-H	Atorvastatin epoxy pyrrolo oxazin 6-hydroxy analog	Atorvastatin epoxy pyrrolo oxazin 7-hydroxy analog	Atorvastatin epoxy THF analog	Atorvastatin related com-D	Any other unspecified degradation product	Total Impurities
1	0.03	0.25	ND	ND	0.05	0.04	0.07	0.52
2	0.02	0.23	ND	ND	0.08	0.04	0.05	0.50
3	0.03	0.24	ND	ND	0.04	0.04	0.04	0.45
4	0.03	0.26	ND	ND	0.05	0.04	0.05	0.50
5	0.02	0.26	ND	ND	0.07	0.04	0.05	0.52
6	0.03	0.25	ND	ND	0.06	0.04	0.04	0.49
Mean	0.03	0.25	NA	NA	0.06	0.04	0.05	0.50
Lowest and highest % difference	0.01	0.03	NA	NA	0.04	0.00	0.03	0.07

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Table 4

No. of Preparation	Related substances of Atorvastatin Calcium for 20 mg %							
	Atorvastatin pyrrolidone analog	Atorvastatin related com-H	Atorvastatin epoxy pyrrolo oxazin 6-hydroxy analog	Atorvastatin epoxy pyrrolo oxazin 7-hydroxy analog	Atorvastatin epoxy THF analog	Atorvastatin related com-D	Any other unspecified degradation product	Total Impurities
1	0.05	0.07	ND	0.04	ND	0.01	0.04	0.28
2	0.05	0.08	ND	0.05	ND	0.01	0.04	0.26
3	0.05	0.08	ND	0.05	ND	0.02	0.05	0.27
4	0.05	0.06	ND	0.04	ND	0.01	0.04	0.24
5	0.05	0.04	ND	0.04	ND	0.02	0.06	0.25
6	0.05	0.07	ND	0.04	ND	0.02	0.05	0.31
Mean	0.05	0.07	NA	0.04	NA	0.02	0.05	0.27
Lowest and highest % difference	0.00	0.04	NA	0.01	NA	0.01	0.01	0.07

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
Report No.	AMVR/ATC/003

Table 5

No. of Preparation	Related substances of Atorvastatin Calcium for 80 mg %							
	Atorvastatin pyrrolidone analog	Atorvastatin related com-H	Atorvastatin epoxy pyrrolo oxazin 6-hydroxy analog	Atorvastatin epoxy pyrrolo oxazin 7-hydroxy analog	Atorvastatin epoxy THF analog	Atorvastatin related com-D	Any other unspecified degradation product	Total Impurities
1	0.071	0.040	0.051	0.075	ND	0.033	0.054	0.368
2	0.075	0.023	0.044	0.078	ND	0.019	0.041	0.295
3	0.075	0.033	0.058	0.074	ND	0.088	0.036	0.410
4	0.074	0.049	0.025	0.076	ND	0.020	0.037	0.321
5	0.077	0.061	0.048	0.078	ND	0.016	0.032	0.300
6	0.075	0.049	0.058	0.076	ND	0.010	0.035	0.295
Mean	0.075	0.043	0.047	0.076	NA	0.031	0.039	0.332
Lowest and highest % difference	0.006	0.038	0.033	0.004	NA	0.078	0.022	0.115

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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Acceptance criteria:

i) Individual known and unknown impurity of individual sample and mean of all sample should not be more than specification limit.

ii) Total impurities of individual sample and mean of all samples should not be more than specification limit.

iii) Difference between lowest value and highest value of Individual known and unknown impurity should not be more than 0.1% absolute.

iv) Difference between lowest value and highest value of total impurities should not be more than 0.2% absolute.

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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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 liter
HCL	:	Hydrochloric acid
NaoH	:	Sodium Hydroxide
H2O2	:	Hydrogen Peroxide

Report	
Title	Analytical Method Verification Related Substances Report For Lolip 10 mg, Lolip 20 mg & Lolip 80 mg (Atorvastatin Calcium Tablets USP)
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11.0 CONCLUSION

Verification studies have been conducted for Related substances of Lolip-10 mg, Lolip- 20 mg & Lolip-80 mg for the parameters of specificity, system & method 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 can be used for regular analysis.

12.0 REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00	08.07.2024	New Report Prepared	New Report Prepared