PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	
Date	:	08/05/2024.
Reviewed By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	
Date	:	08/05/2024.
Reviewed By	:	Quality Control
Name	:	A.VALLARASAN
Signature	:	
Date	:	08/05/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	08/05/24

PROTOCOL	
Title	**Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)**
Protocol No.	**AMVP/CIP/001**

3.0 OBJECTIVE

To verify the method for the test of Assay of Boncipro 250 mg & Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Tablets USP) by HPLC.

S. No	Strength of Boncipro Tablets	Average weight in mg
1	250 mg	450.00 mg
2	500 mg	736.00 mg
3	750 mg	1104.00 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**BP 2023**
REASON FOR VERIFICATION	:	**To verify the assay test for Boncipro tablets 250 mg, Boncipro tablets 500 mg & Boncipro tablets 750 mg as per British Pharmacopoeia.**

PROTOCOL	
Title	**Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)**
Protocol No.	**AMVP/CIP/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 Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)**
Protocol No.	**AMVP/CIP/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 (Hypersil BDS) or equivalent

Working standard ,Solvents and chemicals with grade:

Ciprofloxacin HCl (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC Grade)

Othrophosphoric Acid (AR Grade)

Triethylamine (AR Grade)

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C18,250 mm x 4.6 mm, 5 µm (Hypersil BDS) Suitable or Equivalent
Flow rate	:	1.5 ml / minute.
Detector wavelength	:	278 nm.
Column oven temperature	:	40ºC
Injection volume	:	25 µl
Retention Time	:	2 Times of Ciprofloxacin HCl

Preparation of Buffer 0.245 % w/v Solution of Orthophosphoric Acid:

Weigh accurately about 2.45 g of Orthophosphoric acid in 800ml of water, adjust with triethylamine to a pH of 3.0, and dilute with water to 1000ml.

Preparation of Mobile phase:

Acetonitrile, 0.245% w/v Solution of Orthophosphoric Acid (130:870)

Preparation of Standard solution& Similarity Factor for 250mg ,500mg & 750mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase.

(Concentration:0.0011% w/v of Ciprofloxacin HCl)

Preparation of Standard solution for 250mg, 500mg & 750mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase.

(Concentration: 0.0011% w/v of Ciprofloxacin HCl).

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

Preparation of Sample solution for 250 mg :

Weigh accurately and transfer accurately 367 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Sample solution for 500 mg :

Weigh accurately and transfer accurately 297 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Sample solution for 750 mg :

Weigh accurately and transfer accurately 291 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 250 mg :

Weigh accurately and transfer accurately 167 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

Preparation of Placebo solution for 500 mg :

Weigh accurately and transfer accurately 97mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 750 mg :

Weigh accurately and transfer accurately 91 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Suitability requirements:

1) The tailing factor for the peak of Ciprofloxacin obtained with standard solution should NMT
2) The % RSD for the peak area response of Ciprofloxacin peak obtained with the replicate injections of standard solution should NMT 2.0.
3) The similarity factor replicate injections of standard solution and similarity factor standard solution between 0.98 to1.02.

PROTOCOL	
Title	**Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)**
Protocol No.	**AMVP/CIP/001**

Calculations:

1) Calculate the percentage of the labeled amount of Ciprofloxacin HCl in the portion of tablets taken,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{100} X \frac{100}{W2} \times \frac{200}{1} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Ciprofloxacin HCl obtained due to sample solution

B = Average area Ciprofloxacin HCl obtained due to standard solution

W1= Weight of Ciprofloxacin HCl working standard

W2 = Weight of Ciprofloxacin HCl sample

P = % Purity of Ciprofloxacin HCl working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor (0.901)

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

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

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 Standard solution for 250mg , 500mg & 750mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase.

(Concentration:0.0011% w/v of Ciprofloxacin HCl)

Preparation of Sample solution for 250 mg :

Weigh accurately and transfer accurately 367 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase

Preparation of Sample solution for 500 mg :

Weigh accurately and transfer accurately 297 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

Preparation of Sample solution for 750 mg :

Weigh accurately and transfer accurately 291 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 250 mg :

Weigh accurately and transfer accurately 167 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 500 mg :

Weigh accurately and transfer accurately 97 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 750 mg :

Weigh accurately and transfer accurately 91 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

PROTOCOL	
Title	**Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)**
Protocol No.	**AMVP/CIP/001**

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 250 mg	1
5	Placebo Solution 500 mg	1
6	Placebo Solution 750 mg	1
7	Boncipro 250 mg B.No.G18240106 – 1 to 6	Each Sample 1
8	Boncipro 500 mg B.No.G18240201– 1 to 6	Each Sample 1
9	Boncipro 750 mg B.No.G18210221– 1 to 6	Each Sample 1
10	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.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/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 for 250 mg,500 mg & 750 mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase. (Concentration:0.0011% w/v of Ciprofloxacin HCl)

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 similarity factor replicate injections of standard solution and similarity factor standard solution between 0.98 to1.02.

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/001

9.2.2 Method Precision:

Purpose:

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

Preparation of Sample solution for 250 mg :

Weigh accurately and transfer accurately 367 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase

Preparation of Sample solution for 500 mg :

Weigh accurately and transfer accurately 297 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Sample solution for 750 mg :

Weigh accurately and transfer accurately 291 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase

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

PROTOCOL	
Title	Analytical Method Verification Assay Protocol For Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg , Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Protocol No.	AMVP/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

1.0 INDEX

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Suba
Date	:	16/07/2024.
Reviewed By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V.T.
Date	:	16/07/2024.
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	16/07/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	16/07/24

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

3.0 OBJECTIVE

To verify the method for the test of Assay of Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablets BP) by HPLC.

S. No.	Strength of Ciprofloxacin HCl Tablets	Average weight in mg
1	250 mg	459.63 mg
2	500 mg	744.85 mg
3	750 mg	1094.15 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**BP 2023**
REASON FOR VERIFICATION	:	**To verify the assay test for Boncipro-250 mg tablets & Boncipro-500 mg & Boncipro-750 mg as per British Pharmacopoeia.**

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/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	:	**WS No:** WS/CIP/002	:	93.99%	:	08/10/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Boncipro-250mg Boncipro-500mg Boncipro-750mg	: : :	 G18240106 G18240201 G18240221	:	COA Attached	:	Not Applicable
Impurities NA	:	NA	:	NA	:	NA

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/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 Shimadzu Suitable or Equivalent (QC-LC-069)

Working standard ,Solvents and chemicals with grade:

Ciprofloxacin HCL (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC Grade)

Othrophosphoric Acid (AR Grade)

Triethylamine (AR Grade)

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C18,250 mm x 4.6 mm, 5 µm Shimadzu Suitable or Equivalent
Flow rate	:	1.5 ml / minute.
Detector wavelength	:	278 nm.
Column oven temperature	:	40ºC
Run Time	:	2 Times of Ciprofloxacin HCL
Injection volume	:	25 µl

Preparation of Buffer 0.245% w/v Solution of Orthophosphoric Acid:

Weigh accurately about 2.45 g of Orthophosphoric acid in 800ml of water, adjust with triethylamine to a pH of 3.0, and dilute with water to 1000ml.

Preparation of Mobile phase:

Acetonitrile, 0.245% w/v Solution of Orthophosphoric Acid (130:870)

Preparation of Standard solution& Similarity Factor for 250mg,500mg & 750mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase.

(Concentration:0.0011% w/v of Ciprofloxacin HCl)

Preparation of Standard solution for 250 mg ,500 mg & 750 mg

Weigh and transfer about 22.20 mg of Ciprofloxacin HCl working standard to a 100 ml volumetric flask. Add about 50 ml of Mobile phase. Sonicate, dissolve make up with same solvent. Further Dilute 5 ml of this solution to 100 ml with mobile phase.

(Concentration: 0.0011% W/V of Ciprofloxacin HCl).

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

Preparation of Sample solution for 250 mg :

Weigh accurately and transfer accurately 367 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Sample solution for 500 mg :

Weigh accurately and transfer accurately 297 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Sample solution for 750 mg :

Weigh accurately and transfer accurately 291 mg sample powder (equivalent to 200 mg of Ciprofloxacin) in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 250 mg :

Weigh accurately and transfer accurately 167 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

Preparation of Placebo solution for 500 mg :

Weigh accurately and transfer accurately 97mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Preparation of Placebo solution for 750 mg :

Weigh accurately and transfer accurately 91 mg Placebo powder in a 100 ml volumetric flask. Add about 75 ml of mobile phase mix with the ultrasound for 20 minutes, dilute with same solvent. Pass through a suitable filter of Whatman GF/C filter. Further dilute 1 ml of this solution in to 200 ml of volumetric flask with mobile phase.

Suitability requirements:

1) The tailing factor for the peak of Ciprofloxacin obtained with standard solution should NMT 2.
2) The % RSD for the peak area response of Ciprofloxacin peak obtained with the replicate injections of standard solution should NMT 2.0.
3) The similarity factor replicate injections of standard solution and similarity factor standard solution between 0.98 to1.02.

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

Calculations:

1) Calculate the percentage of the labeled amount of Ciprofloxacin HCl in the portion of tablets taken,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{100} X \frac{100}{W2} \times \frac{200}{1} \times \frac{P}{100} \times \frac{100}{LC} \times AW \times CF$$

Where,

A = Area of Ciprofloxacin HCl obtained due to sample solution

B = Average area Ciprofloxacin HCl obtained due to standard solution

W1= Weight of Ciprofloxacin HCl working standard

W2 = Weight of Ciprofloxacin HCl sample

P = % Purity of Ciprofloxacin HCl working standard

LC = Label claim

AW = Average Weight

CF = Conversion Factor (0.901)

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 Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/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 Ciprofloxacin HCl peaks.

System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
Tailing Factor	NMT 2.0	1.2
% RSD	NMT 2.0	0.1
Similarity factor	0.98 to 1.02	1.0

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	Ciprofloxacin HCl	9.527	0.079	0.269
3	Placebo for Boncipro - 250mg	Placebo peaks	No Peak	Not applicable	Not applicable

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

4	Placebo for Boncipro - 500mg	Placebo peaks	No Peak	Not applicable	Not applicable
5	Placebo for Boncipro - 750mg	Placebo peaks	No Peak	Not applicable	Not applicable
6	Sample Solution Boncipro-250mg B.No.G18240106	Ciprofloxacin HCl	9.628	0.090	0.272
7	Sample Solution Boncipro-500mg B.No.G18240201	Ciprofloxacin HCl	9.733	0.089	0.284
8	Sample Solution Boncipro-750mg B.No.G18240221	Ciprofloxacin HCl	9.755	0.082	0.277

Results and Conclusion:

From the Blank and Placebo peaks are not interfere with Ciprofloxacin HCl peak in test preparation and Peak purity passes 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 HPLC system. The area response for Ciprofloxacin HCl Peak along with % RSD are tabulated in Table 3.

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

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.	Ciprofloxacin HCl
1	1179011
2	1174412
3	1175769
4	1177121
5	1178406
6	1177761
Mean	1177080
% RSD	0.146

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 Ciprofloxacin HCl 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.

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

Table 4: Method precision for Ciprofloxacin HCl

No. of Preparation	Assay % of Boncipro-250 mg	Assay % of Boncipro-500 mg	Assay % of Boncipro-750 mg
1	98.80	101.74	102.40
2	103.40	99.32	103.07
3	100.72	103.22	98.89
4	99.64	99.58	100.76
5	103.17	103.51	101.71
6	102.75	103.46	103.52
Mean	101.41	101.81	101.73
% RSD	1.94	1.90	1.67

Results and Conclusion:

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

Report	
Title	Analytical Method Verification Assay Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

10. 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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Hydrochloride Tablets BP)
Report No.	AMVR/CIP/001

11. CONCLUSION

Verification studies have been conducted for Assay of Boncipro-250 mg , Boncipro-500 mg & Boncipro-750 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. REVISION HISTORY

Ver. #	Effective Date	HISTORY OF REVISIONS	
		Reason for change	Summary of change
00	16.07.2024	New Report Prepared	New Report Prepared

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Ruba
Date	:	10/05/2024.
Reviewed By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V.T.
Date	:	10/05/2024.
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	10/05/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	10/05/24

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

3.0 OBJECTIVE

To verify the method for the test of Dissolution of Boncipro-250 mg & Boncipro-500 & Boncipro-750 mg (Boncipro Tablet BP) by HPLC.

S. No	Strength of Ciprofloxacin HCL Tablets	Average weight in mg
1	250 mg	450.00 mg
2	500 mg	736.00 mg
3	750 mg	1104.00mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	BP 2023
REASON FOR VERIFICATION	:	To verify the Dissolution test for Boncipro-250 mg tablets & Boncipro-500 mg tablets & Boncipro-750mg tablets as per British Pharmacopoeia.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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:

Ciprofloxcin HCl (Working standard)

Purified Water (Milli-Q water)

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Medium	:	Water
Apparatus	:	Apparatus 2 (paddle)
Volume	:	900 ml
RPM	:	50
Temperature	:	37°C ± 0.5°C
Time	:	30 Minutes

Instrumental Conditions:

Mode	:	Ultraviolet - Visible spectroscopy
Cell	:	1.0 cm
Blank	:	Medium
Wavelength	:	276 nm

Preparation of Dissolution Medium:

Water

Preparation of standard solution for 250 mg & 500 mg :

Weigh accurately about 27.7 mg of Ciprofloxcin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of Sample solution for 250 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 100 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCl equivalent to Ciprofloxacin in each tablet by using following formula for 250 mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

Preparation of Sample solution for 500 mg:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 200 ml into disso medium.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCl equivalent to Ciprofloxacin in each tablet by using following formula for 500mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

Preparation of standard solution for 750 mg :

Weigh accurately about 36.6 mg of Ciprofloxacin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Preparation of Sample solution for 750 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 250 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCl equivalent to Ciprofloxacin in each tablet by using following formula for 750 mg ,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{2}{100} \times \frac{900}{1} \times \frac{250}{2} \times F \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e. 0.901)

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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

9.0 DETAILS OF VERIFICATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

9.1.1 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 Blank and placebo not interfering with the analyte peak.

Preparation of standard solution for 250 mg & 500 mg :

Weigh accurately about 27.7 mg of Ciprofloxcin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Preparation of Placebo for 250 mg:

Weigh accurately about 209 mg of Placebo into a 1000 ml volumetric flask. Add about 500 ml of dissolution medium, Sonicate to dissolve and dilute up to 900ml with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Preparation of Placebo for 500 mg:

Weigh accurately about 244 mg of Placebo into a 1000 ml volumetric flask. Add about 500 ml of dissolution medium, Sonicate to dissolve and dilute up to 900ml with dissolution medium and Mix. Further dilute 2 ml of this solution to 200 ml with dissolution medium and Mix.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of Placebo for 750mg:

Weigh accurately about 344 mg of Placebo into a 1000 ml volumetric flask. Add about 500 ml of dissolution medium, Sonicate to dissolve and dilute up to 900ml with dissolution medium and Mix. Further dilute 2 ml of this solution to 250 ml with dissolution medium and Mix.

Preparation of Sample solution for 250 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 100 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCl equivalent to Ciprofloxacin in each tablet by using following formula for 250 mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of Sample solution for 500 mg:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 200 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCL equivalent to Ciprofloxacin in each tablet by using following formula for 500mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

Preparation of standard solution for 750 mg :

Weigh accurately about 36.6 mg of Ciprofloxacin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of Sample solution for 750 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 250 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCL equivalent to Ciprofloxacin in each tablet by using following formula for 750 mg ,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{2}{100} \times \frac{900}{1} \times \frac{250}{2} \times F \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e. 0.901)

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Study design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Reading
1	Blank (Diluent)	1
2	Dissolution Standard Solution 250 mg & 500 mg	6
3	Dissolution Placebo Solution 250 mg	1
4	Dissolution Placebo Solution 500 mg	1
5	Dissolution Placebo Solution 750 mg	1
5	Boncipro 250 mg Jar-1 to Jar-6	Each 1
6	Boncipro 500 mg Jar-1 to Jar-6	Each 1
7	Dissolution Standard Solution 750 mg	6
8	Boncipro 750 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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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 for 250 mg & 500 mg :

Weigh accurately about 27.7 mg of Ciprofloxcin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Preparation of standard solution for 750 mg :

Weigh accurately about 36.6 mg of Ciprofloxacin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Acceptance criteria

% RSD of area of analyte peak in six replicate standard injections should NMT 2.0.

9.2.2 Method Precision:

Purpose:

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

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of standard solution for 250 mg & 500 mg :

Weigh accurately about 27.7 mg of Ciprofloxcin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Preparation of Sample solution for 250 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 100 ml into disso medium.

Preparation of Sample solution for 500 mg:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37°C ± 0.5°C. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 200 ml into disso medium

Preparation of standard solution for 750 mg :

Weigh accurately about 36.6 mg of Ciprofloxacin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

PROTOCOL	
Title	Analytical Method Verification Dissolution Protocol For Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/002

Preparation of Sample solution for 750 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 250 ml into disso medium.

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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro 250 mg & Boncipro 500 & Boncipro 750 mg Tablets (Ciprofloxacin Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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 Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

1.0INDEX

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	
Date	:	16/07/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.R.
Date	:	16/07/2024.
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	
Date	:	16/07/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	16/07/24

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

3.0 OBJECTIVE

To verify the method for the test of Dissolution of Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCL Tablet BP) by UV.

S. No	Strength of Ciprofloxacin HCL Tablets	Average weight in mg
1	250 mg	459.63 mg
2	500 mg	744.85 mg
3	750 mg	1094.15 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**BP 2023**
REASON FOR VERIFICATION	:	**To verify the Dissolution test for Boncipro-250 mg tablets & Boncipro-500 mg tablets & Boncipro-750mg tablets as per British Pharmacopoeia.**

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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	:	**WS No:** WS/CIP/002	:	93.99%	:	08/10/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Boncipro-250mg Boncipro-500mg Boncipro-750mg	: : :	 G18240106 G18240201 G18240221	:	COA Attached	:	COA Attached
Impurity NA	:	NA	:	NA	:	NA

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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:

Ciprofloxcin HCL (Working standard)

Purified Water (Milli-Q water)

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

7.0 DESCRIPTION OF ANALYTICAL METHOD

Dissolution parameters:

Medium	:	Water
Apparatus	:	Apparatus 2 (paddle)
Volume	:	900 ml
RPM	:	50
Temperature	:	37°C ± 0.5°C
Time	:	30 Minutes

Instrumental Conditions:

Mode	:	Ultraviolet - Visible spectroscopy
Cell	:	1.0 cm
Blank	:	Medium
Wavelength	:	276 nm

Preparation of Dissolution Medium:

Water

Preparation of standard solution for 250 mg & 500 mg :

Weigh accurately about 27.7 mg of Ciprofloxcin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix.

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

Preparation of Sample solution for 250 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 100 ml into disso medium.

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCL equivalent to Ciprofloxacin in each tablet by using following formula for 250 mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

Preparation of Sample solution for 500 mg:

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 200 ml into disso medium.

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCL equivalent to Ciprofloxacin in each tablet by using following formula for 500mg,

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

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e.0.901)

LC = Label claim.

Preparation of standard solution for 750 mg :

Weigh accurately about 36.6 mg of Ciprofloxacin working standard into a 100 ml volumetric flask. Add about 50 ml of dissolution medium, Sonicate to dissolve and dilute up to mark with dissolution medium and Mix. Further dilute 2 ml of this solution to 100 ml with dissolution medium and Mix

Preparation of Sample solution for 750 mg :

Place the stated volume of dissolution medium of each vessels of the dissolution apparatus. Warm the dissolution medium at 37ºC ± 0.5ºC. Transfer 1 tablet in to each Vessels. Immediately operate the apparatus at specified speed. At the end of specified time interval, withdraw 10 ml of aliquot from each specimen. Filter through PVDF 0.45 micron syringe filter. Further dilute 2 ml to 250 ml into disso medium.

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

Calculation:(Dissolution)

1) Calculated the content released of Ciprofloxacin HCL equivalent to Ciprofloxacin in each tablet by using following formula for 750 mg ,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{2}{100} \times \frac{900}{1} \times \frac{250}{2} \times F \times \frac{P}{100} \times \frac{100}{LC}$$

Where,

A = Absorbance of Ciprofloxacin obtained with sample solution

B = Absorbance of Ciprofloxacin obtained with standard solution

W1 = Weight of Ciprofloxacin standard in mg

P = Purity of Ciprofloxacin working standard in %

F = Equivalent factor (i.e. 0.901)

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 Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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 Ciprofloxacin HCl peaks.

System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
% RSD for 250 mg	NMT 2.0	0.000
% RSD for 500 mg	NMT 2.0	0.000
% RSD for 750 mg	NMT 2.0	0.000

Acceptance criteria:

No significant interference due to blank and placebo.

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

Table 2: Specificity

S.No	Description of solution	276 nm
1	Blank	0.00
2	Standard Solution for 250 mg	0.535
3	Standard Solution for 500 mg	0.535
4	Standard Solution for 750 mg	0.718
5	Placebo for Boncipro-250mg	0.00
6	Placebo for Boncipro-500mg	0.00
7	Placebo for Boncipro-750mg	0.00
8	Sample solution for Boncipro-250mg B.No. G18240106	0.590
9	Sample solution for Boncipro-500mg B.No. G18240201	0.634
10	Sample solution for Boncipro-750mg B.No. G18240221	0.776

Results and Conclusion:

From the Blank and Placebo absorbance are not interfere with Boncipro-250 mg,500 mg & 750 mg sample within specified limits. Hence method is selective and specific.

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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

Study design:

Six replicate absorbance of standard preparation were injected into the UV system. The absorbance response for Boncipro-250mg & 500mg & 750mg along with % RSD are tabulated in Table 3.

Acceptance criteria:

% RSD of area of analyte peak in Six replicate standard injections should NMT 2.0.

Table 3: System precision

Injection No.	Absorbance reading for Boncipro-250 mg	Absorbance reading for Boncipro-500 mg	Absorbance reading for Boncipro-750 mg
1	0.527	0.527	0.719
2	0.527	0.527	0.719
3	0.527	0.527	0.719
4	0.527	0.527	0.719
5	0.527	0.527	0.719
6	0.527	0.527	0.719

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

Mean	0.527	0.527	0.719
% RSD	0.00	0.00	0.00

Results and Conclusion:

The results are well within the acceptance criteria and the % RSD observed for the replicate 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 Ciprofloxacin HCl 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 Ciprofloxacin HCl

No. of Preparation	Dissolution of Boncipro-250 mg	Dissolution of Boncipro-500 mg	Dissolution of Boncipro-750 mg
1	88.74	100.96	100.51
2	92.92	98.71	98.82
3	89.22	101.44	101.42
4	88.42	100.32	100.90
5	87.62	95.81	100.77
6	90.35	98.55	98.17
Mean	89.55	99.30	100.10
% RSD	2.11	2.09	1.29

Report	
Title	Analytical Method Verification Dissolution Report For Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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 Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/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 Boncipro-250mg & Boncipro-500mg & Boncipro-750mg Tablets (Ciprofloxacin HCL Tablet BP)
Report No.	AMVR/CIP/002

11.0 CONCLUSION:

Verification studies have been conducted for Dissolution of Boncipro-250 mg, Boncipro-500 mg & Boncipro-750 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	16.07.2024	New Report prepared.	New Report prepared

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

1.0 INDEX

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	Ruba
Date	:	06/05/2024.
Reviewed By	:	Analytical Development
Name	:	M. VINOTHINI
Signature	:	M.V.P.
Date	:	06/05/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	06/05/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	06/05/24

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

3.0 OBJECTIVE

To verify the method for the test of Related Substances of Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP) by HPLC.

S. No	Strength of Ciprofloxacin Tablets	Average weight in mg
1	250 mg	450.00 mg
2	500 mg	736.00 mg
3	750 mg	1104.00 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	BP 2023
REASON FOR VERIFICATION	:	To verify the Related Substances test for Ciprofloxacin HCl Tablet BP as per British Pharmacopoeia.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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, 250 mm x 4.6 mm, 5 µm (Hypersil BDS) or equivalent

Solvents and chemicals with grade

Ciprofloxacin HCl (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Orthophosphoric Acid (AR Grade)

Triethylamine (AR Grade)

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic conditions:

Column	:	Stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography (5µm). (Hypersil BDS is suitable) or equivalent.
Flow rate	:	1.5 ml/ minute
Wavelength	:	278 nm
Column temperature	:	40 °C
Injection Volume	:	25 µL
Retention time		2 times of Ciprofloxacin hydrochloride peak

Preparation of 0.245 % w/v solution of Orthophosphoric Acid:

Weigh accurately about 2.45 g of Orthophosphoric Acid in 1000 ml volumetric flask, add About 800 ml of water and adjust the pH with Triethylamine having to 3.0. Make volume up to the mark with water.

Preparation of Mobile phase:

Mix 130 volumes of Acetonitrile and 870 volumes of a 0.245% w/v solution of Orthophosphoric Acid .Filter through 0.45 micron membrane filter and degas.

Preparation of Placebo : 250mg & 500mg & 750mg

Weight accurately about (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, Add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

Preparation of Sample Solution 1 for 250 mg , 500mg & 750mg

Weight accurately about sample powder (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Preparation of Solution 2:

Dilute 1 mL of Solution (1) to 20 mL with Mobile Phase and further dilute 1ml to 10 ml with the Mobile Phase.

Preparation of Solution 4:

Dilute 1 mL of Solution (2) to 5 mL with Mobile Phase.

Preparation of Solution 3:

Weight accurately and transfer about 5 mg of Ciprofloxacin impurity standard BPCRS into 50 ml of volumetric flask. Add about 20 ml of mobile phase, sonicate with intermediate shaking to dissolve and dilute up to the volume with mobile phase.(Concentration: 0.1 mg/ml)

Relative Retention time of Impurity

S.No	Name of impurity	RRT	Response Factor (RF)
1	Impurity B	0.6	0.7
2	Impurity C	0.7	0.6
3	Impurity D	1.2	1.4
4	Impurity E	0.4	6.7
5	Impurity F	0.5	

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to ciprofloxacin (retention time about 9 minutes) are: Impurity E, about 0.4; Impurity F, about 0.5; impurity B, about 0.6; Impurity C, about 0.7; and impurity D, about 1.2.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

System Suitability Requirement:

1) In the chromatogram obtained with solution (3), the resolution between the peaks due to impurity B and impurity C is at least 1.3.
2) The signal-to-noise ratio of the principal peak in the chromatogram obtained with solution (4) is at least 70.

Limits:

Identify any peaks in the chromatogram obtained with solution (1) corresponding to impurities B, C, D and E using solution (3) and multiply the area of these peaks by the following correction factors: 0.7, 0.6, 1.4, and 6.7 respectively.

Calculation:

1) Calculate the % Known impurity by using following formula,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} \times \frac{100}{W2} \times \frac{20}{1} \times \frac{P}{100} \times \frac{100}{LC} \times Av \times 0.901$$

Where,

A = Peak area response of known impurity obtained from the sample solution.

B = Peak area response of Ciprofloxacin peak obtained with solution (2).

W1 = Equivalent weight of Ciprofloxacin in mg

W2 = Weight of sample in mg

Av = Average weight of sample in mg

P = % Purity of Ciprofloxacin working standard on as is basis.

LC = label claim

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

2) Calculate the % Any other secondary peak by using following formula,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} \times \frac{100}{W2} \times \frac{20}{1} \times \frac{P}{100} \times \frac{100}{LC} \times Av \times 0.901$$

Where,

A = Peak area response of known impurity obtained from the sample solution.

B = Peak area response of Ciprofloxacin peak obtained with solution (2).

W1 = Equivalent weight of Ciprofloxacin in mg

W2 = Weight of sample in mg

Av = Average weight of sample in mg

P = % Purity of Ciprofloxacin working standard on as is basis.

LC = label claim

Total impurities = Known Impurity + Any other secondary peak

8.0PARAMETERS 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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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 Sample Solution 1 for 250 mg,500 mg & 750 mg

Weight accurately about sample powder (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Preparation of Solution 2:

Dilute 1 mL of Solution (1) to 20 mL with Mobile Phase and further dilute 1ml to 10 ml with the Mobile Phase.

Preparation of Solution 4:

Dilute 1 mL of Solution (2) to 5 mL with Mobile Phase.

Preparation of Solution 3:

Weight accurately and transfer about 5 mg of Ciprofloxacin impurity standard BPCRS into 50 ml of volumetric flask. Add about 20 ml of mobile phase, sonicate with intermediate shaking to dissolve and dilute up to the volume with mobile phase.(Concentration: 0.1 mg/ml)

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

Preparation of Placebo for 250 mg,500 mg & 750 mg

Weight accurately about (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, Add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Study design:

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of injections
1	RS Blank	1
2	RS Solution-3	1
3	RS Solution-2-Standard	1
4	Boncipro GH 250 mg Placebo	1
5	Boncipro GH 500 mg Placebo	1
6	Boncipro GH 750 mg Placebo	1
7	RS Standard-solution-2	1
8	Boncipro GH 250 mg B.NO.G18240106 Sample 1-6	Each 1
9	Boncipro GH 500 mg B.NO.G18240201 Sample 1-6	Each 1
10	Boncipro GH 750 mg B.NO.G18240221 Sample 1-6	Each 1
11	RS Solution-2	Each 1
12	RS Solution-4	Each 1

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

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 Method Precision:

Purpose:

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

Preparation of Sample Solution 1 for 250 mg ,500 mg & 750 mg

Weight accurately about sample powder (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Preparation of Solution 2:

Dilute 1 mL of Solution (1) to 20 mL with Mobile Phase and further dilute 1ml to 10 ml with the Mobile Phase.

Preparation of Solution 4:

Dilute 1 mL of Solution (2) to 5 mL with Mobile Phase.

Preparation of Solution 3:

Weight accurately and transfer about 5 mg of Ciprofloxacin impurity standard BPCRS into 50 ml of volumetric flask. Add about 20 ml of mobile phase, sonicate with intermediate shaking to dissolve and dilute up to the volume with mobile phase.(Concentration: 0.1 mg/ml

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/003

Preparation of Placebo for 250 mg, 500 mg & 750 mg

Weight accurately about (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, Add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

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.

PROTOCOL	
Title	Analytical Method Verification Related Substances Protocol For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin HCl Tablet BP)
Protocol No.	AMVP/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

1.0 INDEX

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	R.Suba
Date	:	17/07/2024.
Reviewed By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V.T.
Date	:	17/07/2024
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	AV
Date	:	17/7/2024
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	17/07/24

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

3.0 OBJECTIVE

To verify the method for the test of Related Substances of Boncipro 250 mg & Boncipro 500 mg & Boncipro 750 mg (Ciprofloxacin Tablet BP) by HPLC.

S. No	Strength of Boncipro Tablets	Average weight in mg
1	250 mg	459.63 mg
2	500 mg	744.85 mg
3	750 mg	1094.15 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	BP 2023
REASON FOR VERIFICATION	:	To verify the Related Substances test for Ciprofloxacin Tablet BP as per British Pharmacopoeia.

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/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/CIP/002	:	93.99%	:	08/10/2024
Placebo (If applicable)	:	Not Applicable	:	Not Applicable	:	Not Applicable
Sample Boncipro-250 Boncipro-500 Boncipro-750	: : :	 G18240106 G18240201 G18240221	:	COA Attached	:	Not Applicable
Impurities Impurity standard	:	3453	:	Not Applicable	:	Not Applicable

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/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 (Shimadzu or Equivalent) (QC-LC-069)

Working Standard and Solvents and chemicals with grade

Ciprofloxacin HCl (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Orthophosphoric Acid (AR Grade)

Triethylamine (AR Grade)

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic conditions:

Column	:	C18,4.6 mm x 250 mm, 5 µm (Shimadzu or Equivalent)
Flow rate	:	1.5 ml/ min
Wavelength	:	278 nm
Column temperature	:	40°C
Injection Volume	:	25 µl
Retention time	:	2 times of Ciprofloxacin hydrochloride peak

Preparation of 0.245 % w/v solution of Orthophosphoric Acid:

Weigh accurately about 2.45 g of Orthophosphoric Acid in 1000 ml volumetric flask, add About 800 ml of water and adjust the pH with Triethylamine having to 3.0. Make volume up to the mark with water.

Preparation of Mobile phase :

Mix 130 volumes of Acetonitrile and 870 volumes of a 0.245% w/v solution of Orthophosphoric Acid .Filter through 0.45 micron membrane filter and degas

Preparation of Placebo for 250 mg, 500 mg & 750 mg

Weight accurately about (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, Add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

Preparation of Sample Solution 1 for 250 mg , 500 mg & 750 mg

Weight accurately about sample powder (equivalent to 0.2 gm of Ciprofloxacin) in to 100 ml volumetric flask, add about 75 ml of mobile phase sonicate for 20 minutes to dissolve and dilute up to the mark with mobile phase. Filter (Whatman GF/C is filter). Dilute 1 ml of the filtrate to 20 ml with the mobile phase.

Preparation of Solution 2:

Dilute 1 mL of Solution (1) to 20 mL with Mobile Phase and further dilute 1ml to 10 ml with the Mobile Phase.

Preparation of Solution 4:

Dilute 1 mL of Solution (2) to 5 mL with Mobile Phase.

Preparation of Solution 3:

Weight accurately and transfer about 5 mg of Ciprofloxacin impurity standard BPCRS into 50ml of volumetric flask. Add about 20 ml of mobile phase, sonicate with intermediate shaking to dissolve and dilute up to the volume with mobile phase. (Concentration: 0.1 mg/ml)

Relative Retention time of Impurity

S.No	Name of impurity	RRT	Response Factor (RF)
1	Impurity B	0.6	0.7
2	Impurity C	0.7	0.6
3	Impurity D	1.2	1.4
4	Impurity E	0.4	6.7
5	Impurity F	0.5	

When the chromatograms are recorded under the prescribed conditions, the relative Retentions with reference to ciprofloxacin (retention time about 9 minutes) are: Impurity E, about 0.4; Impurity F, about 0.5; impurity B, about 0.6; Impurity C, about 0.7; and

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
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impurity D, about 1.2.

System Suitability Requirement:

1) In the chromatogram obtained with solution (3), the resolution between the peaks due to impurity B and impurity C is at least 1.3;
2) The signal-to-noise ratio of the principal peak in the chromatogram obtained with solution (4) is at least 70.

Calculation:

1) **Calculate the % Known impurity by using following formula,**

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} \times \frac{100}{W2} \times \frac{20}{1} \times \frac{P}{100} \times \frac{100}{LC} \times Av \times 0.901$$

Where,

A = Peak area response of known impurity obtained from the sample solution.

B = Peak area response of Ciprofloxacin peak obtained with solution (2).

W1 = Equivalent weight of Ciprofloxacin in mg

W2 = Weight of sample in mg

Av = Average weight of sample in mg

P = % Purity of Ciprofloxacin working standard on as is basis.

LC = label claim

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

2) Calculate the % Any other secondary peak by using following formula,

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} \times \frac{100}{W2} \times \frac{20}{1} \times \frac{P}{100} \times \frac{100}{LC} \times Av \times 0.901$$

Where,

A = Peak area response of known impurity obtained from the sample solution.

B = Peak area response of Ciprofloxacin peak obtained with solution (2).

W1 = Equivalent weight of Ciprofloxacin in mg

W2 = Weight of sample in mg

Av = Average weight of sample in mg

P = % Purity of Ciprofloxacin working standard on as is basis.

LC = label claim

Total impurities = Known Impurity + Any other secondary peak

8.0 PARAMETERS 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) Method precision

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

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 Ciprofloxacin HCl peaks.

System suitability parameters are tabulated in Table 1.

Table 1: System suitability

System Suitability Parameter	Limit	Observed Result
Resolution	At least 1.3	3.2
Signal-to-noise ratio	At least 70.	88.55

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	Placebo for Boncipro - 250 mg	Placebo peaks	No Peak	Not applicable	Not applicable
3	Placebo for Boncipro - 500 mg	Placebo peaks	No Peak	Not applicable	Not applicable
4	Placebo for Boncipro - 500 mg	Placebo peaks	No Peak	Not applicable	Not applicable

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5	Solution-2	Ciprofloxacin HCl	10.663	1.219	1.515
6	Test preparation G18240106-250 mg	Ciprofloxacin HCl	10.681	0.030	0.253
7	Test preparation G18240201-500 mg	Ciprofloxacin HCl	10.861	0.022	0.247
8	Test preparation G18240201-750 mg	Ciprofloxacin HCl	11.094	0.021	0.243

Results and Conclusion:

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

9.2 PRECISION

9.2.1 Method Precision :

Study Design:

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

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Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
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Table 3

No. of Preparation	Related substances of Boncipro-250 mg %			
	Impurity-C	Impurity-E	Any Other Secondary Peak	Total Impurities
1	(0.05) BDL	ND	(0.02) BDL	0.15
2	(0.06) BDL	ND	(0.03) BDL	0.16
3	(0.05) BDL	ND	(0.04) BDL	0.16
4	(0.05) BDL	ND	(0.04) BDL	0.15
5	(0.05) BDL	ND	(0.02) BDL	0.13
6	(0.05) BDL	ND	(0.05) BDL	0.17
Mean	(0.052) BDL	NA	(0.033) BDL	0.15
Lowest and highest % difference	0.01	NA	0.02	0.04

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

Table 4

No. of Preparation	Related substances of Boncipro-500 mg %			
	Impurity-C	Impurity-E	Any Other Secondary Peak	Total Impurities
1	(0.04) BDL	ND	(0.05) BDL	0.18
2	(0.04) BDL	ND	(0.03) BDL	0.16
3	(0.04) BDL	ND	(0.04) BDL	0.17
4	(0.04) BDL	ND	(0.05) BDL	0.17
5	(0.04) BDL	ND	(0.04) BDL	0.15
6	(0.05) BDL	ND	(0.04) BDL	0.16
Mean	(0.042) BDL	NA	(0.042) BDL	0.165
Lowest and highest % difference	0.01	NA	(0.02) BDL	0.02

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
Report No.	AMVR/CIP/003

Table 5

No. of Preparation	Related substances of Boncipro-750mg %			
	Impurity-C	Impurity-E	Any Other Secondary Peak	Total Impurities
1	(0.06) BDL	ND	(0.04) BDL	0.12
2	(0.06) BDL	ND	(0.05) BDL	0.13
3	(0.07) BDL	ND	(0.04) BDL	0.13
4	(0.06) BDL	ND	(0.04) BDL	0.12
5	(0.05) BDL	ND	(0.03) BDL	0.10
6	(0.06) BDL	ND	(0.06) BDL	0.12
Mean	(0.060) BDL	NA	(0.043) BDL	0.12
Lowest and highest % difference	(0.02) BDL	NA	(0.03) BDL	0.03

Report	
Title	Analytical Method Verification Related Substances Report For Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
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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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
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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 Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg (Ciprofloxacin Tablet BP)
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11.0 CONCLUSION

Verification studies have been conducted for Related substances of Boncipro-250 mg & Boncipro-500 mg & Boncipro-750 mg for the parameters of specificity & 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	17.07.2024	New Report Prepared	New Report Prepared