PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
Protocol No.	AMVP/AML/001

ANALYTICAL METHOD VALIDATION PROTOCOL FOR ASSAY

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

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1.0 INDEX

Prepared By	Sign / Date: M.V.P. 16/09/2023	Authorized By: Head QA	Sign / Date: 16/09/23

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2.0 PROTOCOL APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V.T.
Date	:	16/09/2023
Reviewed By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	R.Subha
Date	:	16/09/2023
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	
Date	:	16/09/2023
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	16/09/2023

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3.0 OBJECTIVE

To validate the method for the test Assay of Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate and Perindopril Erbumine Tablet) by HPLC.

The Amloperin Tablets are manufactured in four strengths as 5/5 mg, 10/10, 5/10 and 10/5 mg wherein the qualitative formula is same, the determination procedure of assay is same for both the strengths for Mobile phase preparation, Standard preparation, system suitability parameters and acceptance criteria are same with only change in sample preparation weight as per the formula weight. The tests as Specificity, Linearity and range, Precision, Intermediate Precision and Accuracy shall be performed on both the strengths (5/5mg and 10/10mg) while Linearity, Robustness, solution stability and Filter evaluation shall be performed on the higher strength as 10/10 mg.

Sr No	Strength of Amloperin Tablets	Average weight in mg
1	5/5 mg	140 mg
2	10/10mg	140 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**In House products**
REASON FOR VALIDATION	:	**To Validate the Amloperin 5/5 mg and 10/10 mg Tablets as per In house method.**

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5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

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

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

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector

Make : 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:

C8,4.6 mm x 250 mm, 5 µm

Working standard, Solvents and chemicals with grade:

Perindopril Erbumine (Working standard)

Amlodipine Besylate (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Potassium dihydrogen orthophosphate (AR Grade)

Orthophosphoric Acid(AR Grade)

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7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C8,4.6 mm x 250 mm, 5 µm
Flow rate	:	1.0 ml / minute.
Detector wavelength	:	210 nm.
Column oven temperature	:	40°C.
Injection volume	:	10 µl.

Preparation of Buffer Solution

Weight accurately about 6.8 g of potassium dihydrogen orthophosphate to 1000 ml glass Beaker. Add about 500 ml of water, shake and sonicate to dissolve completely and finally Make the solution 1000 ml with water.

Preparation of Mobile phase

Mix 590 ml of buffer solution and 410 ml acetonitrile. Sonicate with stirring and adjust the pH 2.6 with orthophosphoric acid. Filter through 0.22µm membrane filter and degas.

Preparation of Standard Solution similarity factor:1

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve And dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix. (Concentration: 0.05 mg/ ml).

Preparation of Standard Solution:2

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve And dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix. (Concentration:0.05 mg/ ml)

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Preparation of sample powder

Weigh accurately 20 tablets and make the powder by using mortar and pestle. Use the same for preparation of sample solution. Calculate the average weight by taking weigh of 20 tablets taken above and use the same for calculation.

Preparation of sample solution for 5/5 mg

Weigh accurately about 10 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up To the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm Syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Procedure :

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

System suitability requirement:

1) The Resolution between the peaks corresponding to Amlodipine and Perindopril Obtained with standard solution should not be less than 2.0.
2) The tailing factor for the peak of Amlodipine and Perindopril obtained with Solution 2 Should not more than 2.0.
3) The % RSD for the peak area response of and Amlodipine and Perindopril Peak obtained with the replicate injections of Solution 2 should not more than 2.0
4) The % RSD for the peak area response of Amlodipine and Perindopril peak obtained with the replicate injections of standard solution and bracketing Solution 2 should not more than 2.0

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5) The column efficiency for the peak of Amlodipine and Perindopril obtained in the Chromatogram of standard solution should not less than 2000.
6) The similarity factor replicate injections of standard solution and similarity factor Standard solution between0.98 to 1.02.

1) Calculated the content of Amlodipine besylate equivalent to amlodipine by using following formula For 5/5 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times F \times \frac{P}{100} \times \frac{100}{5}$$

Where,

A = Peak area response of Amlodpine peak obtained with Sample Solution.
B = Average peak area response of Amlodpine peak obtained with replicate injection Standard solution.
W1 = Weight of Amlodpine standard in mg
W2 = weight of sample in g
Av = Average weight of sample in mg
P = Purity of Amlodpine working standard in %
F = Equivalent factor

2) Calculated the content of Perindopril by using following formula 5/5 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times \frac{P}{100} \times \frac{100}{5}$$

Where,

A = Peak area response of Perindopril peak obtained with Sample Solution.
B = Average peak area response of Perindopril peak obtained with replicate injection Standard solution.
W1 = Weight of Perindopril standard in mg
W2 = weight of sample in mg
Av = Average weight of sample in mg
P = Purity of Perindopril working standard in %

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3) Calculated the content of Amlodipine besylate equivalent to amlodipine by using following formula For 10/10 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times F \times \frac{P}{100} \times \frac{100}{10}$$

Where,

A = Peak area response of Amlodipine peak obtained with Sample Solution.
B = Average peak area response of Amlodipine peak obtained with replicate injection Standard solution.
W1 = Weight of Amlodipine standard in mg
W2 = weight of sample in mg
Av = Average weight of sample in mg
P = Purity of Amlodipine working standard in %
F = Equivalent factor

4) Calculated the content of Perindopril by using following formula 10/10 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times \frac{P}{100} \times \frac{100}{10}$$

Where,

A = Peak area response of Perindopril peak obtained with Sample Solution.
B = Average peak area response of Perindopril peak obtained with replicate injection Standard solution.
W1 = Weight of Perindopril standard in mg
W2 = weight of sample in g
Av = Average weight of sample in mg
P = Purity of Perindopril working standard in %

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8.0 PARAMETERS TO BE VALIDATED:

Following parameters shall be selected for VALIDATION	
Sr. No.	**VALIDATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**Linearity and Range**
3.	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
4.	**Accuracy (Recovery)**
5.	**Solution Stability**
6.	**Robustness**
7.	**Filter evaluation**

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

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9.0 DETAILS OF VALIDATION PARAMETERS

9.1 SPECIFICITY (SELECTIVITY)

Interference from Placebo and Impurities (As applicable)

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

Purpose:

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

Preparation of Standard Solution:

Weigh accurately about 69 mg of amlodipine besylate 50 mg of Perindopril erbumine standard into a 100 ml volumetric flask. Add 70 ml of diluents sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix. (Concentration:0.05 mg/ ml)

Preparation of Plain placebo for 5/5 mg

Weigh accurately about 1300 mg of plain placebo into100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up To the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm nylon syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject:(Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of Plain placebo for 10/10 mg

Weigh accurately about 650 mg of placebo into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

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Preparation of Plain placebo for 5/5 mg +working standard:

Weigh accurately about 1300 mg of plain placebo and 50mg of perindopril erbumine 69mg Amlodipine besylate working standrad into100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the Mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm Syringe Filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and Inject. (Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of Plain placebo for 10/10 mg +working standard:

Weigh accurately about 650 mg of placebo and 50 mg of perindopril erbumine working and 69mg Amlodipine besylate standard into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Preparation of sample solution for 5/5 mg

Weigh accurately about 10 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up To the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm Syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Study design:

Sequence shall be in following provisional manner.

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S.No.	Description of solution	No. of injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Plain placebo 5/5 mg	1
4	Plain placebo 10/10 mg	1
5	Placebo+ Perindopril Erbumine and Amlodipine Besylate Working standard 5/5 mg	1
6	Placebo+ Perindopril Erbumine and Amlodipine Besylate Working standard 10/10 mg	1
7	Sample solution (Amloperin 5/5 mg tablets)	1
8	Sample solution (Amloperin 10/10 mg tablets)	1

Acceptance criteria:

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

9.2 LINEARITY AND RANGE

"The linearity of the analytical method is its ability to elecit test results data directly Proportional to the concentration of the analyte in samples within give range".

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Purpose:

To Establish the linearity of analyte within the specified range.

Preparation (1) of linearity Level: 1 (50%)

Weight accurately 50mg of Perindopril Erbumine and 69mg of Amlodipine Besylate Working standard in 100ml volumetric flask. Add 70 ml standard into 100ml volumetric Flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 100 ml with mobile phase and mix.

Preparation (2) of linearity Level: 2 (75%)

Weight accurately 50mg of Perindopril Erbumine and 69mg of Amlodipine Besylate Working standard in 200 ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 3 ml of this solution to 20 ml with mobile phase and mix.

Preparation of Level: 3 (100%)

Weight accurately 50mg of Perindopril Erbumine and 69mg of Amlodipine Besylate Working standard in 100ml volumetric flask. Add 70 ml standard into 100ml volumetric Flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and mix.

Preparation of Level: 4 (125%)

Weight accurately 50mg of Perindopril Erbumine and 69mg of Amlodipine Besylate Working standard in 100ml volumetric flask. Add 70 ml standard into 100ml volumetric Flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 25 ml of this solution to 200 ml with mobile phase and mix.

Preparation of Level: 5 (150%)

To above the stock solution Preparation (2) in further dilute with 6ml in 20ml volumetric flask.

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Study Design:

To demonstrate the linearity and range of analytical method over the range of 50% to 150% of targeted concentration.

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Level – 1 (50%)	3
3	Blank (Diluent)	1
4	Level – 2 (75%)	3
5	Blank (Diluent)	1
6	Level – 3 (100%)	3
7	Blank (Diluent)	1
8	Level – 4 (125%)	3
9	Blank (Diluent)	1
10	Level – 5 (150%)	3

Plot a graph of concentration (at X-axis) versus average peak area of analyte (at Y-axis). Evaluate the squared correlation coefficient (r2), correlation coefficient (r), Residual sum of square, slope and Y-intercept.

Acceptance criteria:

1) To conclude the linearity, the squared correlation coefficient should not be less than 0.99
2) To conclude the range. % RSD for peak area of linearity level of 50%, 75%, 100%, 125% and 150% should be not more than 2.0.

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9.3 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".

9.3.1 System Precision

Purpose:

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

Preparation of Standard Solution

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluent, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix. (Concentration:0.05 mg/ ml)

Study Design:

Sequence shall be in following provisional manner.

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

Acceptance criteria:

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

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9.3.2 Method Precision:

Purpose:

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

Preparation of Standard Solution

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix.
(Concentration:0.05 mg/ ml)

Preparation of sample solution for 5/5 mg

Weigh accurately about 10 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm Syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.
(Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.
(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

NOTE : Repeat the same procedure for another 5 preparation of sample solution for 5/5 mg & 10/10 mg

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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.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	6
3	Sample preparation-1(5/5 mg)	2
4	Sample preparation-2(5/5 mg)	2
5	Sample preparation-3(5/5 mg)	2
6	Standard preparation (BKT)	1 (after six sample injection)
7	Sample preparation-4(5/5 mg)	2
8	Sample preparation-5(5/5 mg)	2

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9	Sample preparation-6(5/5 mg)	2
10	Standard preparation (BKT)	1 (after six sample injection)
11	Sample preparation-1(10/10 mg)	2
12	Sample preparation-2(10/10 mg)	2
13	Sample preparation-3(10/10 mg)	2
14	Standard preparation (BKT)	1 (after six sample injection
15	Sample preparation-4(10/10 mg)	2
16	Sample preparation-5(10/10 mg)	2
17	Sample preparation-6(10/10 mg)	2
18	Standard preparation (BKT)	1 (after six sample injection

Acceptance criteria:

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

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9.3.3 Intermediate Precision (Ruggedness):

Purpose:

To demonstrate the reproducibility of test results obtained by the analytical method for the variability of instrument, column (different lot no) analyst and day. Analyse six sample preparations as per the methodology representing a single batch and determine the assay for the same. Evaluate the intermediate precision by computing the percentage and relative standard deviation of the assay results.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	6
3	Sample preparation-1(5/5 mg)	2
4	Sample preparation-2(5/5 mg)	2
5	Sample preparation-3(5/5 mg)	2
6	Standard preparation (BKT)	1 (after six sample injection)
7	Sample preparation-4(5/5 mg)	2
8	Sample preparation-5(5/5 mg)	2

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9	Sample preparation-6(5/5 mg)	2
10	Standard preparation (BKT)	1 (after six sample injection)
11	Sample preparation-1(10/10mg)	2
12	Sample preparation-2(10/10mg)	2
13	Sample preparation-3(10/10mg)	2
14	Standard preparation (BKT)	1 (after six sample injection)
15	Sample preparation-4(10/10mg)	2
16	Sample preparation-5(10/10mg)	2
17	Sample preparation-6(10/10mg)	2
18	Standard preparation (BKT)	1 (after six sample injection)

Preparation of Standard Solution

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into a 100 ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve and dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with mobile phase and Mix.
(Concentration:0.05 mg/ ml)

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Preparation of sample solution for 5/5 mg

Weigh accurately about 10 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.
(Concentration: Amlodipine and Perindopril 0.05 mg/ml).

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Acceptance criteria:

1) % RSD for assay of six preparations should not be more than 2.0.
2) Cumulative % RSD for assay of twelve preparations (i.e. method precision and intermediate precision) should not be more than 2.0.

9.4 ACCURACY (RECOVERY)

"The accuracy of an analytical method is the closeness of results obtained by that method to the true value. Accuracy may often be expressed as present recovery by the assay of known, add amount of analyte".

Purpose:

To establish the accuracy of the analytical method in the specified range.

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Preparation of Accuracy 100 % for 5/5 :

Weigh accurately about 69 mg Amlodipine Besylate and 50 mg perindopril Erbumine, 1300 mg of Placebo into 100 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minutes with Intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Preparation of Accuracy 50% for 5/5 :

Weigh accurately about 34.5 mg Amlodipine Besylate and 25 mg perindopril erbumine, 650 mg of Placebo into 100 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minutes with Intermediate shaking to dissolve and dilute up to the mark with diluent and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.025 mg/ml)

Preparation of Accuracy 150% for 5/5:

Weigh accurately about 103.5 mg Amlodipine Besylate and 75 mg perindopril erbumine 1950 mg of Placebo into 200 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minute with Intermediate shaking to dissolve and dilute up to the mark with diluent and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter.Further dilute 6 ml of filtered solution to 20 ml with mobile phase and inject.(Concentration:Amlodipine and Perindopril 0.075 mg/ml)

Preparation of Accuracy 100 % for 10/10 :

Weigh accurately about 69 mg Amlodipine Besylate and 50 mg perindopril Erbumine, 650 mg of Placebo into 100 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minutes with Intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter.Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.05 mg/ml)

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
Protocol No.	AMVP/AML/001

Preparation of Accuracy 50% for 10/10 :

Weigh accurately about 34.5 mg Amlodipine Besylate and 25 mg perindopril erbumine, 325 mg of Placebo into 100 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minutes with Intermediate shaking to dissolve and dilute up to the mark with diluent and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject. (Concentration: Amlodipine and Perindopril 0.025 mg/ml)

Preparation of Accuracy 150% for 10/10:

Weigh accurately about 103.5 mg Amlodipine Besylate and 75 mg perindopril erbumine 975 mg of Placebo into 200 ml volumetric flask. Add about 70 ml diluent sonicate for 10 minute with Intermediate shaking to dissolve and dilute up to the mark with diluent and Mix. Filter sufficient amount of this solution through 0.45 micron syringe filter.Further dilute 6 ml of filtered solution to 20 ml with mobile phase and inject.(Concentration:Amlodipine and Perindopril 0.075 mg/ml)

Sequence shall be in following provisional manner

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Blank (Diluent)	1
4	Level – 1 Set – 1 (50%) (5/5 mg)	1
5	Level – 1 Set – 2 (50%) (5/5 mg)	1
6	Level – 1 Set – 3 (50%) (5/5 mg)	1
7	Blank (Diluent)	1

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
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	8	Level – 2 Set – 1 (100%) (5/5 mg)	1
	9	Level – 2 Set – 2 (100%) (5/5 mg)	1
	10	Level – 2 Set – 3 (100%) (5/5 mg)	1
	11	Blank (Diluent)	1
	12	Level – 3 Set – 1 (150%) (5/5 mg)	1
	13	Level – 3 Set – 2 (150%) (5/5 mg)	1
	14	Level – 3 Set – 3 (150%) (5/5 mg)	1
	15	Standard preparation (Bkt)	1
	16	Level – 1 Set – 1 (50%) (10/10mg)	1
	17	Level – 1 Set – 2 (50%) (10/10mg)	1
	18	Level – 1 Set – 3 (50%) (10/10mg)	1
	19	Blank (Diluent)	1
	20	Level – 1 Set – 1(100%) (10/10mg)	1
	21	Level – 1 Set – 2 (100%) (10/10mg)	1
	22	Level – 1 Set – 3 (100%) (10/10mg)	1
	23	Blank (Diluent)	1
	24	Level – 3 Set – 1 (150%)(10/10 mg)	1

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
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25	Level – 3 Set – 2 (150%)(10/10 mg)	1
26	Level – 3 Set – 3 (150%)(10/10 mg)	1
27	Standard preparation (Bkt)	1

Study design:

To demonstrate the accuracy of the analytical method, prepare recovery samples by spiking known quantities of drug (at level 50%, 100% and 150% of targeted concentration) to placebo. Prepare the recovery samples in triplicate for each level.

Acceptance criteria:

i) Individual %recovery should be between 97.0 to 103.0
ii) The mean % recovery at each level should be 97.0 to 103.0.
iii) % RSD for each level and overall RSD should not be more than 3.0.

9.5 SOLUTION STABILITY

Study design:

Prepare Standard and sample solution as per the methodology and store at room temperature. Chromatograph this solution at regular intervals for 48 hours by using same diluent. Calculate the % difference of analyte peak area for standard and sample preparations with that of initial. The study may be stopped if 2 consecutive failure of sample solution.

Preparation of Standard Solution

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve And dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with Mobile phase and Mix. (Concentration:0.05 mg/ ml)

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
Protocol No.	AMVP/AML/001

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Sequence shall be in following provisional

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation (Initial)	5
3	Sample preparation (Initial)	1
4	Standard preparation (24 Hour)	5
5	Sample preparation (24 Hour)	1
6	Standard preparation (48 Hour)	5
7	Sample preparation (48 Hour)	1

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
Protocol No.	AMVP/AML/001

Acceptance criteria:

The sample and standard solution shall be considered stable for the final period till which the area difference between initial and next periodic interval should not be more than ±2%.

9.6 ROBUSTNESS

Purpose:

To establish the robustness of the analytical method.

Preparation of Standard Solution

Weigh accurately about 69 mg of amlodipine besylate standard and 50 mg of Perindopril erbumine standard into 100ml volumetric flask. Add 70 ml of diluents, Sonicate to dissolve And dilute up to mark with diluents and Mix. Further dilute 5 ml of this solution to 50 ml with Mobile phase and Mix. (Concentration:0.05 mg/ ml)

Preparation of sample solution for 10/10 mg

Weigh accurately about 5 intact tablet into 100 ml volumetric flask. Add about 50 ml of diluent, sonicate for 10 minute with intermediate shaking to dissolve and dilute up to the mark with diluents and Mix. Filter sufficient amount of this solution through 0.45 µm syringe filter. Further dilute 5 ml of filtered solution to 50 ml with mobile phase and inject.(Concentration: Amlodipine and Perindopril 0.05 mg/ml)

Study Design:

The robustness of the analytical method can be established by demonstrating its reliability against deliberate changes in chromatographic conditions.

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
Protocol No.	AMVP/AML/001

Sequence shall be in following provisional manner.

As such		
S.No.	**Description of solution**	**No. of Injections**
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample preparation	2
4	Bracketing standard	1
According to each variable		
S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample preparation	2
4	Bracketing standard	1

Following variable shall be done according to deliberate changes in chromatographic parameters.

a) Flow rate change by ±10% mean (i.e 0.9 ml/min and 1.1 ml/min)

b) Wave length change by ± 3nm (i.e. 207 nm and 213 nm)

c) Column oven Temperature change by ± 5.0 (i.e. 35°C and 45°C)

PROTOCOL	
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Acceptance criteria:

System suitability should comply for each variable and % of drug not differ ±2% from mean assay value of method precision.

9.6 FILTER EVALUATION

Study design:

The filter paper study of the analytical method shall perform by filtering test solution through 0.45 µm Nylon, PVDF filter against that of unfiltered.

Sequence shall be in following provisional manner.

S.No.	Description of solution	No. of Injections
1	Blank (Diluent)	1
2	Standard preparation	5
3	Sample preparation – (0.45µm Nylon Syringe filter)	1
4	Sample preparation –Filter Set 2 (0.45µm PVDF Syringe filter)	1
5	Sample preparation –(Centrifuge)	1
6	Standard preparation_BKT	1

Acceptance criteria:

The % area difference of filter solution should not differ ±2.0 against that of unfiltered.

PROTOCOL	
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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

PROTOCOL	
Title	Analytical Method Validation Assay Protocol For Amloperin 5/5 mg & 10/10 mg (Amlodipine Besylate & Perindopril Erbumine Tablet)
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11.0 CONCLUSION

12.0 REVISION HISTORY

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

Report	
Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
Report No.	AMVR/AML/001

ANALYTICAL METHOD VALIDATION 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 Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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1.0 INDEX

Report	
Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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2.0 REPORT APPROVAL SHEET

Prepared By	:	Analytical Development
Name	:	M.VINOTHINI
Signature	:	M.V
Date	:	26/12/2023
Reviewed By	:	Analytical Development
Name	:	R. SUBADHARSHINI
Signature	:	
Date	:	26/12/2023
Reviewed By	:	Quality Control
Name	:	A. VALLARASAN
Signature	:	
Date	:	26/12/2023
Approved By	:	Quality Assurance
Name	:	R. Stephen
Signature	:	
Date	:	26/12/23.

Report	
Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
Report No.	AMVR/AML/001

3.0 OBJECTIVE

To validate the method for the test Assay of Amloperin 5-5 mg &10-10 mg (Amlodipine besylate & Perindopril erbumineTablet) by HPLC.

The Amlodipine besylate & Perindopril erbumine Tablets are manufactured in two strengths as 5/5 mg and 10/10 mg wherein the qualitative formula is same, the determination procedure of assay is same for both the strengths for Mobile phase preparation, Standard preparation, system suitability parameters and acceptance criteria are same with only change in sample preparation weight as per the formula weight. The tests as Specificity, Precision, Intermediate Precision, and Accuracy shall be performed on both the strengths while Linearity, Robustness, solution stability and Filter evaluation shall be performed on the higher strength as 10/10 mg.

Sr.No	Strength of Amloperin Tablets	Average weight in mg
1	5/5 mg	140.75 mg
2	10/10 mg	140.23 mg

4.0 GENERAL INFORMATION

METHOD REFERENCE	:	**In House products**
REASON FOR VALIDATION	:	**To Validate the Amloperin 5/5 mg and 10/10 mg tablets as per In house method.**

Report	
Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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5.0 DETAILS OF STANDARD, SAMPLES AND PLACEBO TO BE USED

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

Name of Material	:	ID. No./Batch No./Control No.	:	Potency/ Purity	:	Valid Up to
Standard Amlodipine besylate BP		**WS No:** WS/AML/003		99.75 %		12/07/2024
Perindopril erbumine BP		**WS No:** WS/PER/003		99.21 %		01/08/2024
Placebo (If applicable)		Not Applicable		Not Applicable		Not Applicable
Sample Amloperin 5/5 mg Amloperin 10/10 mg		G18230122 G18230116		COA Attached		Not Applicable
Impurities NA		NA		NA		NA

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

INSTRUMENTS/EQUIPMENTS:

High performance liquid chromatograph with PDA detector
Make : 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:

C8 (4.6mm x 250 mm, 5 µm) Cosmosil or equivalent (QC-LC-059)

Working standard,Solvents and chemicals with grade:

Amlodipine besylate (Working standard)

Perindopril erbumine (Working standard)

Purified Water (Milli-Q water)

Acetonitrile (HPLC grade)

Potassium dihydrogen orthophosphate (AR Grade)

Orthophosphric acid (AR Grade)

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Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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7.0 DESCRIPTION OF ANALYTICAL METHOD

Chromatographic Conditions:

Column type	:	C8 (4.6 mm x 250 mm, 5 µm) (Cosmosil C8 is suitable) or Equivalent
Flow rate	:	1.0 ml / minute.
Detector wavelength	:	210 nm.
Column oven temperature	:	40°C.
Injection volume	:	10 µl.

Preparation of Buffer solution:

Weight accurately about 6.8 g of potassium dihydrogen orthophosphate to 1000 ml glass beaker. Add about 500 ml of water , shake and sonicate to dissolve completely and finally Make the solution 1000 ml with water.

Mobile phase :

Mix 590 ml of buffer solution and 410 ml acetonitrile. Sonicate with stirring and adjust the pH 2.6 with orthophosphoric acid. Filter through 0.22 µm

Preparation of Solution 1:Similarity Factor

Weight accurately about 69 mg of Amlodipine besylate standard and 50 mg of Perindopril erbumine standard in to 100 ml volumetric solution add about 70 ml of diluent, sonicate to dissolve and dilute upto the mark with diluent. Further dilute 5 ml of this solution to 50 ml with mobile phase and mix.(concentration:0.05 mg/ml)

Preparation of Solution 2:

Weight accurately about 69 mg of Amlodipine besylate standard and 50 mg of Perindopril erbumine standard in to 100 ml volumetric solution add about 70 ml of diluent, sonicate to dissolve and dilute up to the mark with diluent. Further dilute 5 ml of this solution to 50 ml with mobile phase and mix. (concentration:0.05 mg/ml)

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Preparation of Solution 1 powder:

Weigh accurately 20 tablets and make the powder by using mortar and pestle. Use the same for preparation of sample solution. Calculate the average weight by taking weight of 20 tablets taken above and use the same for calculation.

Preparation of Solution 5/5 mg :

Weight accurately about 10 Intact tablet into 100 ml volumetric flask add about 50 ml diluent, sonicate for 10 minutes with intermediate shaking to dissolve and dilute upto the mark with diluent and mix. Filter the sufficient amount of this solution through 0.45µm syringe filter. Further Dilute 5 ml of above solution into 50 ml of mobile phase and inject.(concentration: Amlodipine and Perindopril 0.05 mg/ml)

Preparation of Solution 10/10mg:

Weight accurately about 5 Intact tablet into 100 ml volumetric flask add about 50 ml diluent, sonicate for 10 minutes with intermediate shaking to dissolve and dilute up to the mark with diluent and mix. Filter the sufficient amount of this solution through 0.45µm syringe filter. Further Dilute 5 ml of above solution into 50 ml of mobile phase and inject.(concentration: Amlodipine and Perindopril 0.05 mg/ml)

Procedure:

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

System suitability requirement:

1) The Resolution between the peaks corresponding to Amlodipine and Perindopril Obtained with standard solution should not be less than 2.0.
2) The tailing factor for the peak of Amlodipine and Perindopril obtained with Solution 2 Should not more than 2.0.
3) The column efficiency for the peak of Amlodipine and Perindopril obtained in the Chromatogram of Solution 2 should not less than 2000.
4) The % RSD for the peak area response of Amlodipine and Perindopril peak obtained With the replicate injections of Solution 2 should not more than 2.00.

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5) The % RSD for the peak area response of Amloperin and Perindopril peak obtained with the replicate injections of standard solution and bracketing standard solution Should not more than 2.00.
6) The similarity factor replicate injections of standard solution and similarity factor Standard solution between0.98 to 1.02.

1) Calculated the content of Amlodipine besylate equivalent to amlodipine by using following formula For 5/5 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times F \times \frac{P}{100} \times \frac{100}{5}$$

Where,

A = Peak area response of Amlodpine peak obtained with Sample Solution.
B = Average peak area response of Amlodpine peak obtained with replicate injection Standard solution.
W1 = Weight of Amlodpine standard in mg
W2 = weight of sample in g
Av = Average weight of sample in mg
P = Purity of Amlodpine working standard in %
F = Equivalent factor

2) Calculated the content of Perindopril by using following formula 5/5 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times \frac{P}{100} \times \frac{100}{5}$$

Where,

A = Peak area response of Perindopril peak obtained with Sample Solution.
B = Average peak area response of Perindopril peak obtained with replicate injection Standard solution.
W1 = Weight of Perindopril standard in mg
W2 = weight of sample in mg
Av = Average weight of sample in mg
P = Purity of Perindopril working standard in %

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3) Calculated the content of Amlodipine besylate equivalent to amlodipine by using following formula For 10/10 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times F \times \frac{P}{100} \times \frac{100}{10}$$

Where,

A = Peak area response of Amlodipine peak obtained with Sample Solution.
B = Average peak area response of Amlodipine peak obtained with replicate injection Standard solution.
W1 = Weight of Amlodipine standard in mg
W2 = weight of sample in mg
Av = Average weight of sample in mg
P = Purity of Amlodipine working standard in %
F = Equivalent factor

4) Calculated the content of Perindopril by using following formula 10/10 mg

$$= \frac{A}{B} \times \frac{W1}{100} \times \frac{5}{50} \times \frac{100}{W2} \times \frac{50}{5} \times Av \times \frac{P}{100} \times \frac{100}{10}$$

Where,

A = Peak area response of Perindopril peak obtained with Sample Solution.
B = Average peak area response of Perindopril peak obtained with replicate injection Standard solution.
W1 = Weight of Perindopril standard in mg
W2 = weight of sample in g
Av = Average weight of sample in mg
P = Purity of Perindopril working standard in %

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Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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8.0 PARAMETERS TO BE VALIDATED :

Following parameters shall be selected for Validation	
Sr. No.	**VALIDATION Parameter**
1.	**Specificity (Selectivity)** i) Interference from Placebo and Impurities (as applicable)
2.	**LINEARITY AND RANGE**
3.	**Precision** i) System precision ii) Method precision iii) Intermediate Precision
4.	**Accuracy (Recovery)**
5.	**STABILITY OF ANALYTICAL SOLUTION**
6.	**ROBUSTNESS**
7.	**FILTER PAPER STUDY**

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

9.0 DETAILS OF VALIDATION 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 carbimazole peaks.

System suitability parameters are tabulated in Table 1.

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Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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Table 1: System suitability

System Suitability Parameter	Limit	Observed Result Perindopril	Observed Result Amlodipine
Tailing Factor	NMT 2.0	1.5	1.5
% RSD	NMT 2.0	0.1	0.2
Theoretical plate	NLT 2000	9183	10877
Resolution	NLT 2.0	8.6	8.6
Similarity factor	0.98 to 1.02	0.99	0.99

Peak purity of the analyte peak and the representative chromatograms of blank, standard, Placebo, placebo spiked with analyte and sample are attached.

Results are tabulated in Table 2.

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.

Table 2: Specificity

Sr.No	Sample ID	Peak Name	Retention time	Purity Angle	Purity Threshold
1	Blank	No Peak	No Peak	Not applicable	Not applicable
2	Standard preparation	Perindopril erbumine	4.083	0.098	0.295
3	Standard preparation	Amlodipine Besylate	5.809	0.034	0.223

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4	Placebo for Amloperin 5/5 mg	Placebo peaks	No Peak	Not applicable	Not applicable
5	Placebo for Amloperin 10/10 mg	Placebo peaks	No Peak	Not applicable	Not applicable
6	Plain placebo + Perindopril WS 5/5 mg	Perindopril erbumine	4.076	0.113	0.288
7	Plain placebo + Amlodipine WS 5/5 mg	Amlodipine Besylate	5.793	0.036	0.223
8	Plain placebo + Perindopril WS 10/10 mg	Perindopril erbumine	4.078	0.103	0.283
9	Plain placebo + Amlodipine WS 10/10 mg	Amlodipine Besylate	5.795	0.044	0.222
10	Sample solution G18230122- 5/5 mg	Perindopril erbumine	4.076	0.119	0.279
11	Sample solution G18230122- 5/5 mg	Amlodipine Besylate	5.794	0.036	0.222
12	Sample solution G18230116-10/10 mg	Perindopril erbumine	4.076	0.091	0.287
13	Sample solution G18230116-10/10 mg	Amlodipine Besylate	5.794	0.037	0.222

Results and Conclusion:

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

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Title	Analytical Method Validation Assay Report For Amloperin 5-5mg &10-10mg (Amlodipine besylate & perindopril erbumine Tablet)
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9.2 LINEARITY AND RANGE:

Study Summary:

Analytical solutions for Amlodipine and Perindopril Working standard were prepared over the range of 50% to 150% concentration with respect to target concentration (i.e. 50%, 75%, 100%, 125% and 150%). Replicate injections of these solutions are injected and checked for Linearity and Range. The results are tabulated in Table 3 for 4 for Linearity and Table 5 for Range.

Table 3: Linearity Table for Perindopril erbumine

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	25.12	266811
75%	37.65	398250
100%	50.14	537233
125%	62.66	670161
150%	75.30	813186
Slope		10884
Sqaured R		0.9999
Intercept		-8968

Report	
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Fig. : Liner Graph for Perindopril erbumine

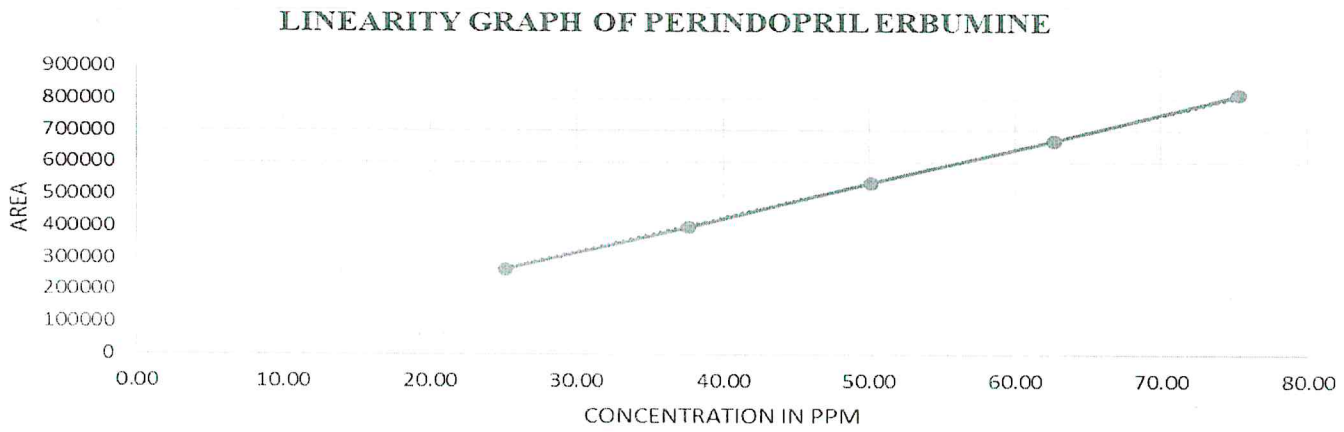

Table 4: Linearity Table for Amlodipine Besylate

Linearity Levels (%)	Conc. in ppm (X- axis)	Avg. Area (Y- axis)
50%	34.56	691537
75%	52.32	1061031
100%	69.54	1394344
125%	87.06	1736447
150%	104.64	2107679
Slope		20056
Sqaured R		0.9999
Intercept		1804

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Fig. : Liner Graph for Amlodipine Besylate

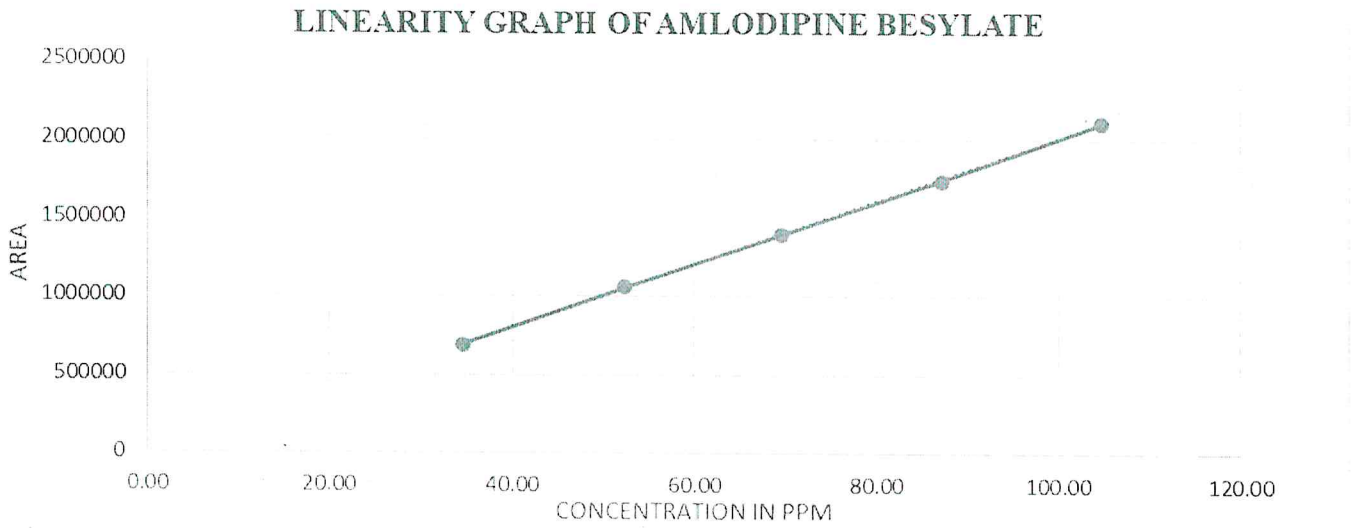

Table:5 Range for Perindopril erbumine and Amlodipine besylate

Linearity Levels (%)	% RSD for Perindopril erbumine	% RSD for Amlodipine Besylate
50%	0.019	0.094
75%	0.022	0.041
100%	0.056	0.097
125%	0.018	0.030
150%	0.017	0.089

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Acceptance criteria:

1) The squared correlation coefficient should not be less than 0.99.
2) To conclude the range % RSD for peak areas of linearity levels 25%, 50%, 75%, 100%, 125%& 150% should not be more than 2.0.

Result and Conclusion:

Squared correlation coefficient and Range, %RSD of areas at 25%, 50%, 75%, 100%, 125 & 150% levels within limits

9.3 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"

9.3.1 System Precision

Study design:

Five replicate injections of standard preparation were injected into the HPLC system. The area response for Amlodipine and perindopril Peak along with % RSD are tabulated in Table 6.

Acceptance criteria:

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

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Table 6: System precision

Injection No.	Perindopril erbumine	Amlodipine besylate
1	521141	1398604
2	521028	1396714
3	520992	1393284
4	521953	1400815
5	521145	1394622
6	520976	1397402
Mean	521206	1396907
% RSD	0.072	0.194

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.

9.3.2 Method Precision:

Study Design:

Six Assay preparations of sample were analyzed as per the method. The Assay of carbimazole is calculated. The results are tabulated in Table 7.

Acceptance criteria:

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

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Table 7: Method precision for Amlodipine and Perindopril

No. of Preparation	Assay of Perindopril -5/5 mg	Assay of Amlodipine -5/5 mg	Assay of Perindopril - 10/10 mg	Assay of Amlodipine - 10/10 mg
1	98.79	100.27	98.95	100.29
2	99.54	100.82	99.29	100.97
3	98.89	100.43	99.57	100.85
4	99.70	101.15	100.80	101.15
5	99.10	100.73	100.91	101.09
6	99.76	101.35	99.64	101.12
Mean	99.30	100.79	99.86	100.91
% RSD	0.43	0.41	0.81	0.32

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.

9.3.3 Intermediate Precision (Ruggedness):

Study summary:

Six Assay preparations of sample are analyzed as per the method by different analyst using different instrument and different column on different day. The assay of Amlodipine and Perindopril is calculated. The results are tabulated in Table 8 and cumulative results are tabulated in Table 9.

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Acceptance criteria:

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

2) Cumulative % RSD for Assay of twelve sample preparations (of method and intermediate precision) should not be more than 2.0.

Table 8: Intermediate precision for Amloperin 5/5 and 10/10

No. of Preparation	Assay of Perindopril-5/5 mg	Assay of Amlodipine-5/5 mg	Assay of Perindopril-10/10 mg	Assay of Amlodipine - 10/10 mg
1	100.77	101.03	101.32	101.61
2	100.36	100.64	100.16	100.48
3	101.03	101.27	101.07	101.40
4	101.32	101.61	100.22	100.48
5	100.73	100.99	100.48	100.79
6	100.58	100.83	100.74	101.05
Mean	100.80	101.06	100.67	100.97
% RSD	0.33	0.34	0.46	0.47

The Cumulative results of Method Precision and Intermediate Precision are tabulated in Table 9

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Table 9: Cumulative % RSD for Amlodipine and Perindopril

Parameter	Assay of Perindopril - 5/5 mg	Assay of Amlodipine- 5/5 mg	Assay of Perindopril- 10/10 mg	Assay of Amlodipine- 10/10mg
Method Precision	98.78	100.27	98.95	100.29
	99.54	100.82	99.29	100.97
	98.89	100.43	99.57	100.85
	99.70	101.15	100.80	101.15
	99.10	100.73	100.91	101.09
	99.76	101.35	99.64	101.12
Intermediate Precision	100.77	101.03	101.32	101.61
	100.36	100.64	100.16	100.48
	101.03	101.27	101.07	101.40
	101.32	101.61	100.22	100.48
	100.73	100.99	100.48	100.79
	100.58	100.83	100.74	101.05
Mean	100.05	100.93	100.26	100.94
% RSD	0.87	0.38	0.75	0.38

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Result and Conclusion:
The results are well within the acceptance criteria and the % RSD observed for drug Release Indicates the precision of the method.

9.4 ACCURACY(RECOVERY)

Study Design:

Known quantity of Amlodipine and Perindopril working standard are spiked with placebo at three different levels (at level of 50%, 100% and 150% of targeted concentration).

Prepared the recovery samples in triplicate for each level and inject only one injection for each sample. The samples are analyzed as per the proposed method. The results are tabulated in Table 9 & 10 for Amloperin 5/5 & 10/10 mg respectively to demonstrate the accuracy of the method.

The mean % recovery at each level for Amlodipine and Perindopril should be 97.0 to 103.0.

Table 10: Accuracy for Amloperin 5/5 mg

Recovery level	Sample No.	% Recovery Perindopril 5/5mg	Mean %	% RSD	% Recovery Amlodipine 5/5mg	Mean %	% RSD
50%	1	100.53	101.20	0.62	99.55	99.94	0.46
	2	101.28			99.81		
	3	101.78			100.45		
100%	1	100.99	101.39	0.34	99.99	100.15	0.15
	2	101.53			100.19		
	3	101.63			100.28		

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150%	1	100.07	100.09	0.27	100.49	101.18	0.71
	2	99.83			101.12		
	3	100.37			101.93		
% Overall RSD		0.710		Overall RSD%		0.718	

Table 11 : Accuracy for Amloperin 10/10 mg

Recovery level	Sample No.	% Recovery Perindopril 10/10mg	Mean %	% RSD	% Recovery Amlodipine 10/10mg	Mean %	% RSD
50%	1	100.29	101.22	0.83	99.38	99.96	0.53
	2	101.94			100.43		
	3	101.43			100.08		
100%	1	100.19	101.03	0.72	99.31	99.81	0.47
	2	101.46			99.88		
	3	101.45			100.25		
150%	1	99.97	100.29	0.73	101.56	101.78	0.32
	2	101.13			102.15		
	3	99.78			101.62		
% Overall RSD		0.78		% Overall RSD		1.02	

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Result and Conclusion:

All the results are well within the acceptance criteria and results indicate that the method Is accurate and precise.

9.5 STABILITY OF ANALYTICAL SOLUTION:

Sample solution:

Sample preparation is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 12.

Table:12 Stability of Analytical Solution

Initial	Assay % of Sample Perindopril-10/10	Absolute % Difference	Assay % of Amlodipine-10/10	Absolute % Difference
Initial	100.78	Not applicable	100.86	Not applicable
24	100.98	-0.2	101.32	-0.46
48	102.28	-1.5	102.72	-1.86

Results and conclusions:

The Standard solution and Sample solution was stable upto 48 hours at room temperature.

9.6 ROBUSTNESS:

Sample solution:

Sample preparation is prepared as per the proposed method and injected into the system initially and at various time intervals and data tabulated in Table 13.

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Table 13: Sample solution for Flow Rate-0.9ml and 1.1 ml Amloperin-10/10

Flow Rate	Assay % of Sample Perindopril-10/10	Absolute % Difference	Assay % of Amlodipine-10/10	Absolute % Difference
As Such	101.58	Not applicable	101.24	Not applicable
0.9 ml	101.84	-0.26	101.76	-0.52
1.1 ml	101.72	-0.14	101.30	-0.06

Table 14: Sample solution for Wave length 207nm and 213nm Amloperin-10/10

Wave Length	Assay % of Sample Perindopril-10/10	Absolute % Difference	Assay % of Amlodipine-10/10	Absolute % Difference
As Such	101.58	Not applicable	101.24	Not applicable
207nm	101.71	-0.13	101.65	-0.41
213nm	101.67	-0.09	101.60	-0.36

Table 15: Sample solution for Temperature 35°C and 45°C Amloperin-10/10

Temperature	Assay % of Sample Perindopril-10/10	Absolute % Difference	Assay % of Amlodipine-10/10	Absolute % Difference
As Such	101.58	Not applicable	101.24	Not applicable
35°C	101.58	0	101.16	0.08
45°C	101.46	0.12	101.28	-0.04

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Acceptance criteria:

System suitability should comply for each variable and % of drug not differ ±2% from mean assay value of method precision.

9.7 FILTER PAPER STUDY:

Study design:

The filter paper study of analytical method is performed by filtering test solution through 0.45µ Nylon membrane filter against that of unfiltered centrifuged sample. The results are tabulated in Table 16.

Table 16: Filter paper study for Sample solution of Amloperin &10/10

Filter study	Assay in % Perindopril 10/10mg	% difference from unfiltered sample	Assay in % Amlodipine 10/10mg	% difference from unfiltered sample
SAMPLE (0.45µm NYLON FILTER)	101.27	Not applicable	101.42	Not applicable
UNFILTER SET-I (CENTRIFUGED)	101.50	-0.23	101.66	0.24
FILTER SET-II (0.45µm PVDF FILTER)	102.16	-0.89	102.37	-0.95

Acceptance criteria:

The % difference on filter solution should not differ ±2.0 against that of unfiltered (centrifuged) sample.

Results and conclusions:

The % difference on filtered sample (0.45 µm NYLON) membrane within limit against that of unfiltered (centrifuged) sample.

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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

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11 CONCLUSION:

Validation studies have been conducted for Assay of Amloperin 5-5 mg & 10-10 mg for the parameters of specificity, Linearity, method precision, Intermediate precision, accuracy, Robustness, Filter paper study and solution stability by using the proposed method. The data is complied 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	26.12.2023	New Report prepared	New Report prepared