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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR AMLODIPINE BESYLATE USING RP-HPLC

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Abstract:

Amlodipine Besylate is a calcium channel blocker commonly prescribed for the management of hypertension and angina pectoris. Accurate and reliable analytical methods are essential for quality control in pharmaceutical formulations. This study focuses on the development and validation of a novel High-Performance Liquid Chromatography (HPLC) method for the quantitative estimation of Amlodipine Besylate in tablet dosage forms. The chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 65:35 v/v, at a flow rate of 1.0 mL/min. Detection was carried out at 238 nm using a UV detector. The method was validated according to ICH Q2 (R1) guidelines, covering parameters such as linearity, accuracy, precision, specificity, robustness, and system suitability. The linearity of the method was established in the range of 10–60 µg/mL with a correlation coefficient (R^2) of 0.999. The method demonstrated acceptable accuracy (recovery between 98.5%–101.5%), precision (RSD < 2%), and robustness under varied experimental conditions. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.53 µg/mL and 1.61 µg/mL, respectively. The developed HPLC method proved to be simple, sensitive, precise, and suitable for routine analysis of Amlodipine Besylate in pharmaceutical dosage forms.

Keywords: *Amlodipine Besylate, HPLC, Method Development, Validation, ICH Guidelines, Tablet Dosage Form*

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1. INTRODUCTION:

Amlodipine besylate is a long-acting calcium channel blocker that inhibits calcium influx into vascular smooth muscle and cardiac cells, leading to vasodilation and reduced myocardial contractility. It is widely used to treat hypertension and angina.

Analytical methods play a crucial role in both scientific research and industry by enabling the identification, quantification, and structural analysis of compounds. These methods ensure product quality, safety, and compliance across multiple sectors (1).

Precision refers to the consistency of repeated measurements under the same conditions, while **sensitivity** reflects a method's ability to detect low analyte concentrations vital in fields such as clinical diagnostics, environmental monitoring, and pharmaceuticals.

Accuracy denotes how close results are to the true value, and both precision and accuracy are enhanced by proper calibration, quality control, and validation (2).**Technological advancements** in instrumentation such as improved spectrophotometers, chromatographs, and mass spectrometers have significantly increased sensitivity and reduced errors (3).**Chromatographic techniques**, such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), are widely used for separating and analyzing complex mixtures. HPLC, in particular, provides high-resolution separation in the liquid phase and is essential in pharmaceutical quality control (4, 5).

Applications of Analytical Techniques:

- **Chemistry:** Identification and quantification using HPLC, GC, UV-Vis, and IR spectroscopy.
- **Biology:** Analysis of biomolecules using mass spectrometry and electrophoresis.
- **Environmental Science:** Detection of pollutants using GC-MS and spectroscopic methods.
- **Pharmaceuticals:** Ensuring drug purity and potency using chromatography and mass spectrometry.
- **Materials Science:** Structure analysis using XRD and characterization via spectroscopy.
- **Forensics:** Detection of toxins and DNA analysis through chromatography, spectroscopy, PCR.
- **Clinical Diagnostics:** Disease monitoring through blood tests, immunoassays, and mass spectrometry.
- **Industrial Quality Control:** Product safety assurance via chromatography, spectroscopy, and electrochemical methods (6, 7, 8).

Validation Parameters

1.Precision:

Indicates the consistency of results when the method is repeated on the same sample. Expressed as standard deviation or %RSD (9).

2.Accuracy:

Measures how close the test results are to the true value. Recovery should be between 99–101% (10).

3.Specificity:

Assesses the method's ability to identify the analyte in the presence of impurities, degradation products, or excipients. Verified through forced degradation studies (11–15).

4.Linearity and Range:

Confirms the method produces results proportional to analyte concentration. Linearity is accepted when $r^2 \geq 0.999$. The range defines the concentration limits within which the method is accurate and precise (16).

5.Ruggedness:

Evaluates reproducibility under varied conditions (e.g., different operators, instruments, labs). Confirms method reliability across typical variables (17, 18).

6.Robustness:

Demonstrates the method's stability against small deliberate changes in conditions like temperature, pH, and mobile phase composition (19).

Table No.1: Characteristics to be validated in HPLC and their Acceptance criteria: (20)

Characteristics	Acceptance criteria
Accuracy/trueness	Recovery 98-102% (individual) with 80, 100,120% spiked
Precision	RSD < 2%
Repeatability	RSD < 2%
Intermediate Precision	RSD < 2%
Specificity / Selectivity	No interference
Detection Limit	S/N > 2 or 3
Quantitation Limit	S/N > 10
Linearity	Correlation coefficient r > 0.999
Range	80 –120 %
Sample solution stability	> 24 h or >12 h

Drug profile

Amlodipine is a drug used to treat angina (chest discomfort) and high blood pressure. It is a member of the class of medications known as calcium channel blockers, which improve blood flow by relaxing blood arteries.

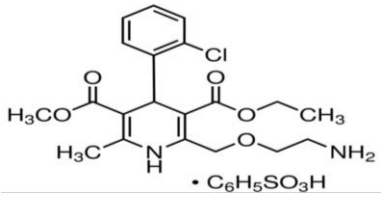

Figure no 1. Structure of Amlodipine

Chemical name of amlodipine: (RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

Table no 2.

Parameters	Value
Molecular weight	408.88
Appearance	White to off white crystalline Powder
Solubility	Soluble in organic solvent like methanol ethanol
Melting point	199-201^{0}C
Density	1.18g/cm^3
pka	8.6
Half-life	30 to 50 hours
Absorbance	240nm
Bioavailability	60 to 65 %

Experimental work

1 Material and Instruments:

1.1 Materials:

Leben Pharmaceuticals provided the medications utilized in this study as a free sample.

A. Details of Pure drug:

Table3. Details of pure API

Drug	Supplied by	Quantity	Purity (Assay)
Amlodipine	Leben Pharma Pvt.Ltd.	10 g	99.02 % w/w

B. Marketed Preparation:

Table 4: Details of marketed Preparation

Brand Name	Mfd by	Content	Quantity
Amlodipine Besylate Tablet IP	**Megma Health Care Pvt.Ltd**	Amlodipine besylate	5mg

C. Reagent and chemicals:

- Acetonitrile (HPLC Grade)
- Methanol
- Water (HPLC Grade)
- Potassium dihydrogen phosphate
- Orthophosphoric acid
- Triethylamine

1.2 Instruments

Table no.5: list of instruments

Sr. No	Instrument	Make	Model
1	**UV-Visible Spectrophotometer**	Thermo Electron	Double beam carry-07 Bio
2	**HPLC**	1.Waters 2.Waters	486 UV Detector 996 PDA Detector
3	**pH Meter**	Hanna	-
4	**Balance**	Citizen	CY 104 (Micro Analytical Balance)

2. Determination of wavelength maxima and beers lamberts law study using ultraviolet visible spectroscopy

Preparation of standard solution:

Weigh a known quantity of pure amlodipine precisely (10 milligrams, for example). It should be dissolved in an appropriate solvent, usually phosphate buffer (pH 7.4) or methanol. To create a stock solution, dilute to a known volume (for example, 100 mL).

Extra Distillation

For UV analysis, dilute the stock to a workable concentration (e.g., 10 µg/mL).

Depending on the solvent and pH, amlodipine's λmax can vary significantly, although it usually ranges between 238 and 246 nm.

3. Determination of wavelength of maxima for amlodipine

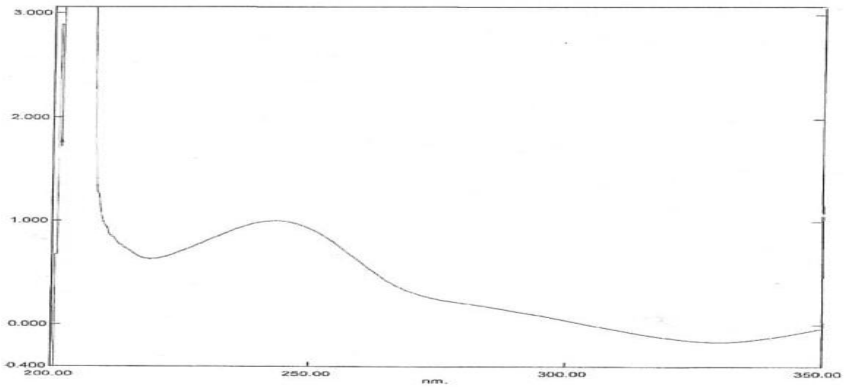

Fig no 2 .: Wavelength maxima for amlodipine

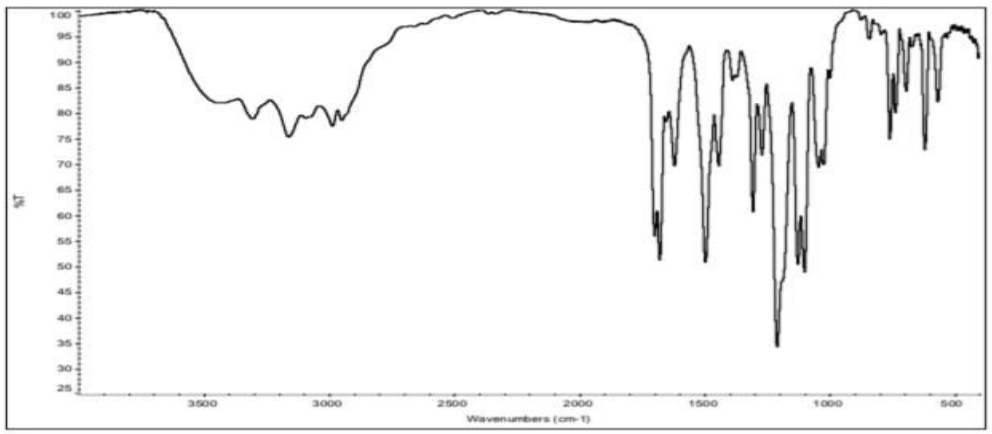

Figure no.3 IR spectra of amlodipine

A) Method development:

a) Selection of Common solvent (Diluents):

In order to prepare stock solutions and develop spectrum properties of pharmaceuticals, acetonitrile of HPLC grade was chosen as a common solvent. Acetonitrile was then used to make further dilutions from stock solutions. Following an evaluation of both medications' solubility in various solvents, the choice was decided.

a) Amlodipine standard stock solution:

2.5 mg of precisely weighed amlodipine was added to a 10-milliliter volumetric flask and dissolved in acetonitrile. To get the final concentration of 250 ppm, the volume was finally adjusted using diluent.

b) Standard preparation (10µg/ml)

Stock solution (1 ml) of amlodipine was pipette out and transferred to 10 ml volumetric flask and made volume up to the mark with diluent.

c) Sample preparation

After calculating the average mass of 20 tablets, they were ground into powder and precisely weighed. A certain amount of powdered tablet equivalent to 10 mg of amlodipine transferred to 100 ml volumetric flask, 60 ml was diluent added, sonicated for 20 minutes with shaking and volume make up with diluent. After cooling to room temperature and adding diluent to make up the volume, 1 ml of the solution was diluted with 10 ml of diluent in a 10-ml volumetric flask. A 0.45 µ Millipore filter was used to filter the resultant solution; the filtrate was collected after the first few milliliters were discarded.

Chromatographic parameters:

HPLC system

Parameter

Column: ODS

Flow rate: 1.0 ml/ minute

Wave length: 237 nm

Injection volume: 20 microliter

Buffer: 0.05 M Ammonium Acetate in water

Mobile phase:

Table 6: Gradient program

Sr.No	Buffer pH 3.3	Acetonitrile	Time (min)
Step 1.	98	2	2
Step 2.	20	80	10
Step 3.	98	2	14

Acetonitrile (98:02) and 0.01M sodium hydrogen phosphate buffer (pH 3.3) make up the mobile phase. Step 1 of the three-step gradient program was created using 98% (by volume) buffer and 2% acetonitrile at a flow of 1 ml for the first two minutes. The technique concluded with step 3 after reaching the initial concentration of 98% buffer and acetonitrile. Step 2 began with gradient changes to 20% buffer and 80% acetonitrile for the next 10 minutes. The method's overall flow rate was 1 milliliter per minute.

Mobile phase-buffer:

After precisely weighing 1.78g of disodium hydrogen phosphate, dissolving it in a small amount of HPLC-grade water, adding 1% triethylamine, and finally using ortho-phosphoric acid to bring the pH down to 3.3, the volume was completed.

Preparation of diluent:

Acetonitrile of HPLC grade were selected as common solvent for manufacture of stock solution and developing spectrum properties of medicines, additional dilutions from stock solutions were made in the acetonitrile

System suitability test:

System appropriateness, a pharmacopoeia requirement, is used to confirm that the chromatographic system's resolution and repeatability are sufficient for analysis. Using the software empower pro, three replicate injections of standard solutions were collected for the experiments on an HPLC (Waters 996).

4. Method development

Methodology

When five or more distinct known proportionate concentrations are prepared in ascending order, linearity is demonstrated. To estimate the known quantities, the various concentrations are analyzed. Absorbance vs. conc. is shown on a graph to determine the linear relationship and evaluate the degree of linearity using a regression line.

Table no7: Coefficient Correlation: Alodipine 10 mg

% Concentration	Actual Conc. in ppm	Area	Coefficient Correlation
80	40.00	27144161	0.99996
90	45.00	34126786	
100	50.00	41202064	
110	55.00	48487244	
120	60.00	55343418	

Validation Acceptance Criteria

Within the specified range, the absorbance should be proportionate to the test conc. The $\mathbf{R^2}$ correlation should not be less than 0.998.

- ✓ Relative standard deviation for peak area less than 2%
- ✓ Theoretical plates more than 2000.
- ✓ Tailing Factor between 0.85 to 2.

Mean % Assay and RSD

Table no8: Linearity and range

	CHEMIST A	CHEMIST B	CHEMIST C	RSD %
% of Amlodipine	99.44%	99.35%	99.43%	0.05%

It is expected that the assay's relative standard deviation will be less than 2.0%.

Specificity & Selectivity:

Make sure that every analytical technique carried out enables an accurate statement of the analyst's impurity content. One blank solution, such as diluents used in standard and sample preparation, should be injected after the column has stabilized. The placebo solution is then injected.

Table no9: Specificity and linearity

No. Of Injection	Sample ID	Interference
1	Blank	-----
1	Placebo	-----
5	Standard	No interference
3	Sample	No interference

RESULT AND CONCLUSION:

Establishing a straightforward, accurate, precise, and reliable reversed-phase high-performance liquid chromatographic (RP-HPLC) approach for the quantitative analysis of amlodipine besylate in pharmaceutical formulations was the main goal of the method development process. In order to attain acceptable resolution, peak shape, and retention duration, a number of chromatographic parameters have to be systematically evaluated and optimized during the technique development process.

1. Selection of Analytical Wavelength

Using a UV-visible spectrophotometer, amlodipine besylate was scanned in the UV range (200–400 nm). To ensure optimal sensitivity, the wavelength at which the greatest absorbance (λ max) was observed 237 nm was chosen for detection throughout the analysis.

2. Selection of Chromatographic Column

A reverse-phase C18 column (250 mm × 4.6 mm, 5 µm) was chosen because of the compound's non-polarity. Because of its robust hydrophobic interactions with the analyte, which resulted in sufficient retention and enhanced resolution, this column was determined to be appropriate.

3. Mobile Phase Selection

Initial experiments were conducted at varying pH levels using different combinations of aqueous buffers (phosphate, acetate) and organic solvents (acetonitrile and methanol). The best peak shape and resolution were found to be obtained using a combination of acetonitrile and 0.02 M phosphate buffer (pH adjusted to 3.0 using orthophosphoric acid).

It was discovered that the ideal mobile phase ratio of acetonitrile to phosphate buffer was 65:35 (v/v). This combination produced a well-defined peak with a decent theoretical plate count and little tailing.

4. pH Optimization

The pH of the mobile phase has a major impact on the retention and peak shape of amlodipine because it is a basic molecule. After evaluating a range of pH values from 3.0 to 5.0, the ideal value of 3.0 was determined to offer superior peak symmetry and stability.

5. System Suitability Parameters

System suitability experiments were conducted to guarantee chromatographic performance prior to method validation. The approach revealed:

About 5.8 minutes is the retention time.

Tailing factor: less than two

Plates in theory: > 2000% Area and retention time RSD: less than 2%

These metrics attested to the method's dependability and appropriateness for more testing.

Blank Peak

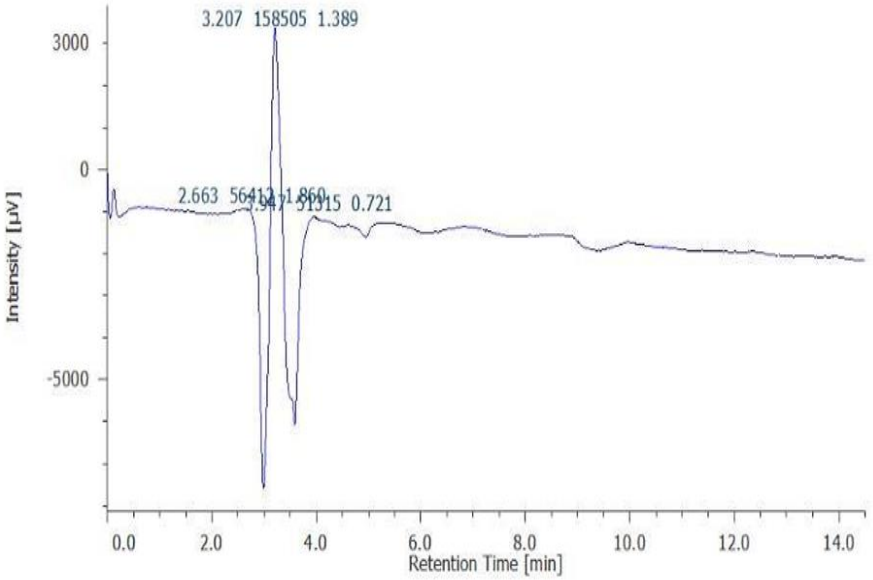

Figure 2: Blank chromatogram

Amlodipine Besylate:-

10 mg of amlodipine besylate should be added to a 10 ml volumetric flask, followed by 5 ml of ACN and 5 minutes of sonication. The kit can then be filled with 10 ml of ACN in a 10 ml volumetric flask.

After making a 10 ml stock solution with an ACN, take 1 ml of the solvent from the stock solution and put it in a 10 ml volumetric flask. You can then make up to 10 ml with blank by sonicating 1 ml of blank, which is made with ACN and water, for 5 minutes.

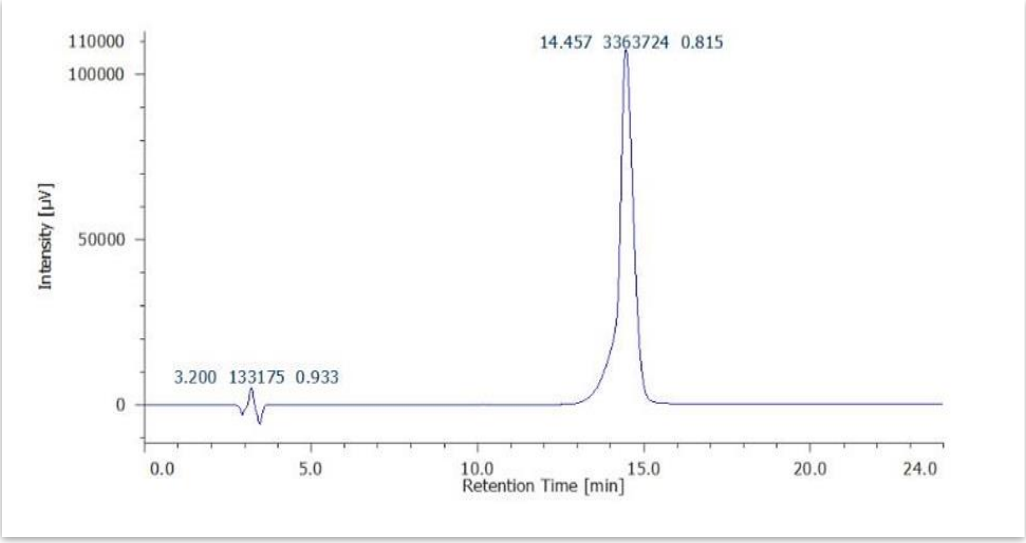

Figure no 3: HPLC chromatogram of Amlodipine Besylate

6. Method development:

Various experiments were conducted using different columns, mobile phases, and detectors in an effort to find a suitable symmetric peak with shorter retention duration.

Table no 10: Method development

Sr.No.	Mobile phase	pH	Flow Rate(ml/min)	Column	λ (nm)	Retention time	Observation
1	Water :Acetonitrile	**-**	**1.0**	**C18**	**238**	**6.5**	**Broad peak, high tailing, poor resolution**
2	**KH2**PO4 Buffer: Acetonitrile (50:50)	**4.5**	**1.0**	**C18**	**238**	**5.2**	**Improved shape, reduced tailing**
3	**KH2**PO4 Buffer: Acetonitrile (40:60)	**3.0**	**1.0**	**C18**	**238**	**4.5**	**Sharper peak, better symmetry**
4	**KH2**PO4 Buffer: Acetonitrile (35:65)	**3.0**	**1.0**	**C18**	**238**	**4.2**	**Best resolution, ideal peak shape**
5	Optimized mobile phase (35:65)	**3.0**	**1.0**	**C8**	**238**	**3.1/poor**	**C8: Short RT, fronting; Phenyl : poor shape**
6	Optimized mobile phase	**3.0**	**0.8/1.0/1.2**	**C18**	**238**	**5.5/4.2/3**	**1.0ml/min optimal for balance of time and resolution**
7	Optimized mobile phase	**3.0**	**1.0**	**C18**	**238**	**4.2**	**Λ max at 237 confirmed for best sensitivity**
8	Optimized mobile phase	**3.0**	**1.0**	**C18**	**238**	**4.2**	**20 ul injection gave optimal response**

Table no 11: Coefficient Correlation: Alodipine 10 mg

% Concentration	Actual Conc. in ppm	Area	Coefficient Correlation
80	40.00	27144161	0.99996
90	45.00	34126786	
100	50.00	41202064	
110	55.00	48487244	
120	60.00	55343418	

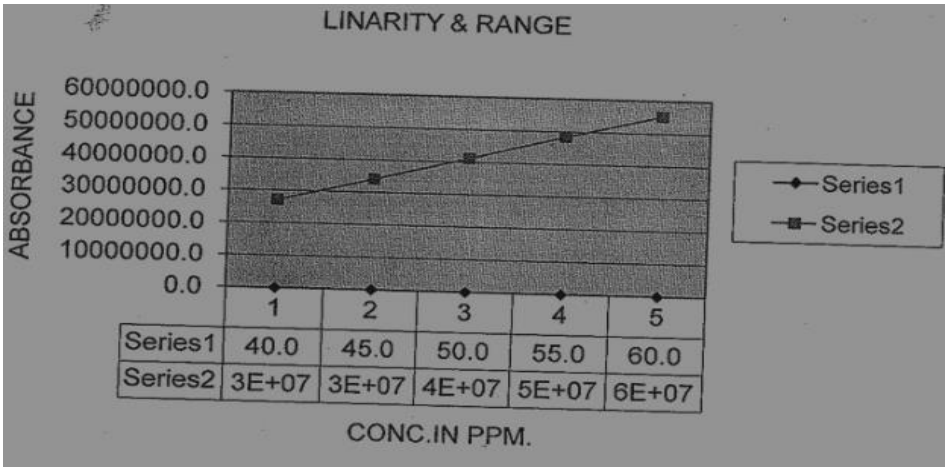

Figure no 4: linearity peak

Table no 12. Precision data of Amlodipine Besilate eq. to Amlodipine

Sr. No.	Final Conc. (ppm)	Assay (% of Amlodipine)
1	Final Conc. 50ppm of Amlodipine	100.05
2	Final Conc. 50ppm of Amlodipine	99.98
3	Final Conc. 50ppm of Amlodipine	99.5
4	Final Conc. 50ppm of Amlodipine	98.68
5	Final Conc. 50ppm of Amlodipine	100.1
6	Final Conc. 50ppm of Amlodipine	98.9
Average (%)		99.54%
RSD (%)		0.62%

Weight taken for accuracy solution.

Accuracy (By Recovery)

80 % 8 mg of Amlodipine Besilate eq. to Amlodipine

100 % 10.0mg of Amlodipine Besilate eq. to Amlodipine

120 % 12.0 mg of Amlodipine Besilate eq. to Amlodipine

Amlodipine Besilate eq. to Amlodipine

Table no 13: Recovery Results

Level (Approx) (%)	Recovered Assay (%)	Conc. (mg of Amlodipine Besilate eq. to Amlodipine / tab)	Target Conc. (mg of Amlodipine Besilate eq. to Amlodipine / tab)	Recovery (%)	Deviation (%)	Mean Recovery (%)	RSD (%)
80	80.01	8.010	8.0	100.01	- 0.001	99.7 %	0.200%
100	99.19	9.959	10.0	99.19	+ 0.81		
120	119.89	11.995	12.0	99.9	+ 0.1		

Ruggedness (InterdayPrecision)

Table no14: Mean % Assay and RSD

	CHEMIST A	CHEMIST B	CHEMIST C	RSD %
% of Amlodipine	99.44%	99.35%	99.43	0.05%

Specificity & Selectivity:

Table no15: Specificity and range

Blank	-----
Placebo	-----
Standard	No interference
Sample	No interference

CONCLUSION:

HPLC Method Development for Amlodipine and Chlorthalidone

A novel, precise, accurate, and stability-indicating HPLC method was developed and validated for the estimation of Amlodipine. The method used a C18 column with a mobile phase of **[KH2PO4 Buffer: Acetonitrile (35:65)]**, flow rate of **[1.0ml/min]**, and detection at **[238 nm]**. Validation followed ICH guidelines, confirming specificity, linearity, accuracy, precision, sensitivity, and robustness.

For simultaneous estimation, a combined HPLC method for Amlodipine Besylate and Chlorthalidone was optimized using a Finepak SIL C18T-5 JASCO column. The mobile phase comprised **Acetonitrile: Methanol: Water with 0.2% triethylamine**, pH adjusted to 3.0 using formic acid. Detection wavelengths were **215 nm** (Amlodipine) and **240 nm** (Chlorthalidone). The method achieved a resolution of ~9 between the two drugs.

Calibration was performed using standard/external methods. Linearity was observed in the 5–25 µg/mL range for both drugs, following Beer's Law. The method was validated using doses of **40 mg Amlodipine Besylate** and **12.5 mg Chlorthalidone**.

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