International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319-6718, ISSN (Print): 2319- 670X
www.ijpsi.org Volume 14 Issue 3 ǁ May –June, 2025 ǁ PP. 107-116

Development Of New Analytical Method and Validation of Atorvastatin and Amlodipine in Pure and Pharmaceutical Formulation by RP-HPLC

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ABSTRACT: *The development and validation of a new analytical method for the simultaneous quantification of atorvastatin and amlodipine in pure and pharmaceutical formulations have been successfully achieved using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). The method was optimized to enhance accuracy and efficiency, utilizing a Waters HPLC system equipped with an auto-sampler and a PDA Detector 996 model. A Phenomenon Gemini C18 column (4.6 mm × 150 mm, 5.0 µm) was employed for the separation of the analytes at a column temperature of 38°C.*

The mobile phase consisted of methanol and acetone in a 32:68 v/v ratio, and the flow rate was set to 1.0 mL/min. Detection was performed at a wavelength of 240 nm, with an injection volume of 20 µL. The chromatographic run time was optimized to 10 minutes to ensure efficient analysis.

The method demonstrated excellent performance in terms of accuracy, precision, specificity, and linearity. Validation parameters were thoroughly evaluated, including the determination of limits of detection (LOD) and quantitation (LOQ), robustness, and stability. The validated method provides a reliable and efficient tool for the routine analysis of atorvastatin and amlodipine in both pure substances and pharmaceutical formulations. This RP-HPLC method ensures compliance with regulatory standards and supports quality control in pharmaceutical manufacturing.

KEYWORDS:*RP-HPLC (Reverse Phase High-Performance Liquid Chromatography), Atorvastatin, Amlodipine, Method Development, Method Validation*

Date of Submission: 26-05-2025 Date of acceptance: 07-06-2025

I. INTRODUCTION

The term "chromatography," which comes from the Greek words "chroma" and "graphene," was first used by Russian chemist and botanist MichealTswett (1872–1919) to refer to his work on the separation of coloured plant pigments into bands on a column of chalk and other materials like polysaccharides, sucrose, and insulin.Chromatography is a technique that separates a mixture's constituent parts using an adsorbent column in a flowing system. Russian scientist named Tswett in 1906, the adsorbent material, also known as the stationary phase, has evolved over time to include paper, immobilized liquids, gels, thin layers of solids adhered to glass plates, and solid particles arranged in columns. "Chromatography is a physical separation technique where the substance to be separated is divided into two phases, one of which is stationary and the other moves in a specific direction" (IUPAC)[1].

Types of Chromatography

Chromatography can be classified as either gas chromatography (GC) or liquid chromatography (LC) based on whether the mobile phase is a liquid or a gas. A liquid mobile phase is used in two other modes besides these, but it is transported through the porous stationary phase by either (a) capillary forces, as in planar chromatography (also known as Thin-Layer Chromatography, or TLC), or (b) electro osmotic flow, as in Capillary Electro Chromatograph[2-6]

High Performance Liquid Chromatography (HPLC)

The late Professor Csaba Horvath first used the abbreviation HPLC to refer to the fact that high pressure was utilized to create the flow needed for liquid chromatography in packed columns in his 1970 Pittcon paper. Initially, pumps could only handle 500 psi [35 bars] of pressure. This was known as HPLC, or high-pressure liquid chromatography. Technology made a huge jump in the early 1970s. With the addition of

enhanced injectors, detectors, and columns, these new HPLC equipment could achieve pressures of up to 6,000 psi [400 bars][7].

Atorvastatin LDL cholesterol levels are lowered when atorvastatin inhibits HMG-CoA reductase, the enzyme in the liver that produces cholesterol. Watch out for medications that are metabolized by CYP3A4, such as erythromycin and ketoconazole, as they may raise the risk of myopathy when taken with gemfibrozil. (r)4-fluorophenyl-2--7-2-(4-fluorophenyl) phenyl-6-(1-methylethyl) A thiazole with the chemical formula C33H35F2O5, 4-yl-2,3-dihydroxybutanoic acid has a bioavailability of roughly 14%. Ethanol and methanol dissolve it, but water practically insoluble. Utilized to minimize triglycerides and LDL cholesterol as well as to lower cardiovascular risk[8].

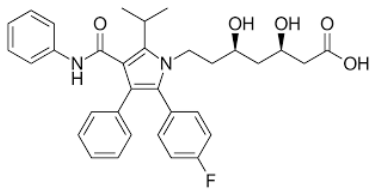

Figure 1; Structure of Atorvastatin.

Amlodipine Inhibits calcium ion influx into vascular smooth muscle and cardiac muscle, leading to vasodilation and reduced blood pressure. Caution with other antihypertensives, CYP3A4 inhibitors (e.g., ketoconazole), and cyclosporine.3-Ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-1,4-dihydro-6-methyl-2,6-dioxo-3,5-pyridine-dicarboxylate with the molecular formula C20H25ClN2O5 and has bioavailability approximately about 60-65%. Slightly soluble in water, soluble in methanol and ethanol. Used for the treatment of hypertension and angina.

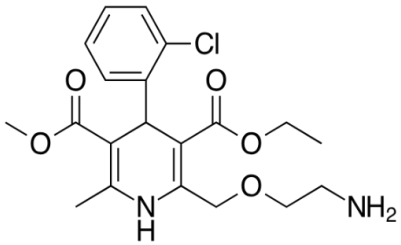

Figure 2; Structure of Amlodipine.

According to the literature determination of amlodipine and atorvastatin in the presence of their acidic degradation products in tablets. Two methods were developed for separation and quantitation of amlodipine (AML) and atorvastatin (ATV) in the presence of their acidic degradation products. The first method was a simple isocratic RP-HPLC method while the second was capillary electrophoresis (CE). Degradation products were obtained by acidic hydrolysis of the two drugs and their structures were elucidated for the first time by IR and MS spectra. Degradation products did not interfere with the determination of either drug and the assays were therefore stability-indicating[9-10].

II. EXPERIMENTAL METHODS

Table 1: Instruments used

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detectors.
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

Table 2: chemicals used

S.No	Chemical	Brand names
1	Atorvastatin	Procured from Sun Pharma, provided by Sura Pharma labs
2	Amlodipine	Procured from Sun Pharma, provided by Sura Pharma labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

METHOD DEVELOPMENT:

Preparationofstandardsolution: Accurately weigh and transfer 10 mg of Atorvastatin and Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.Further pipette 2.25ml of the above Atorvastatin and 0.45ml of the Amlodipine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonein proportion 32:68 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra.Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDADetector 996 model.
Column	:	Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm)
Column temperature	:	38˚C
Mobile phase	:	Methanol: Acetone (32:68v/v)
Flow rate	:	1ml/min
Wavelength	:	240 nm
Injection volume	:	20µl
Run time	:	10 min

METHOD VALIDATION

Preparation of mobile phase:

Accurately measured 320ml (32%) of HPLC Methanol and 680ml of Acetone (68%) were mixed and degassed in a digital ultrasonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Atorvastatin and Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.5ml of the above Atorvastatin and 0.3ml of the Amlodipine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

PRECISION:

Preparation of Atorvastatin and Amlodipine Product Solution for Precision: Accurately weigh and transfer 10 mg of Atorvastatin and Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.5ml of the above Atorvastatin and 0.3ml of the Amlodipine stock solutions into

a 10ml volumetric flask and dilute up to the mark with Diluent.The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution: Accurately weigh and transfer 10 mg of Atorvastatin and Amlodipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.25ml of the above Atorvastatin and 0.15ml of the Amlodipine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

III. RESULTS AND DISCUSSION

Trail 1:

Mobilephase:Methanol: Water (75:25%v/v)
Column :Symmetry C18 (4.6 ×150mm, 5µm particlesize)
Flowrate : 1.2ml/min
Wavelength :248 nm
Columntemp :38ºC
InjectionVolume : 9 µl
Run time : 9 minutes

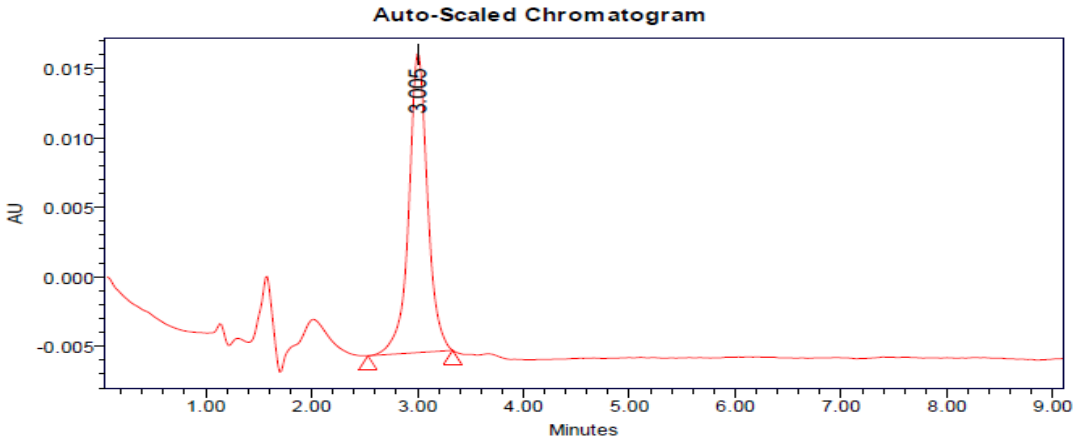

Fig 1: chromatogramfor trail-1

Table3: Peakresultsfor trail-1

S.No	PeakName	R_t	Area	Height	USP Resolution	USP Tailing	USP Platecount
1	Amlodipine	3.005	23432	1234		5.1	675

Observation: InaseparationofAtorvastatin and Amlodipinepeak was obtainedonlyforone compound becausethere may beless solubility. So, wegoforfurthertrails.

Trail 2:

Mobilephase : Methanol: Water (30:70 % v/v)
Column : PhenomenexGemini C18 (4.6×250mm 5µm)
Flowrate : 1.0 ml/min
Wavelength : 248 nm
Columntemp : 38ºC
InjectionVolume : 20 µl
Run time : 14 minutes

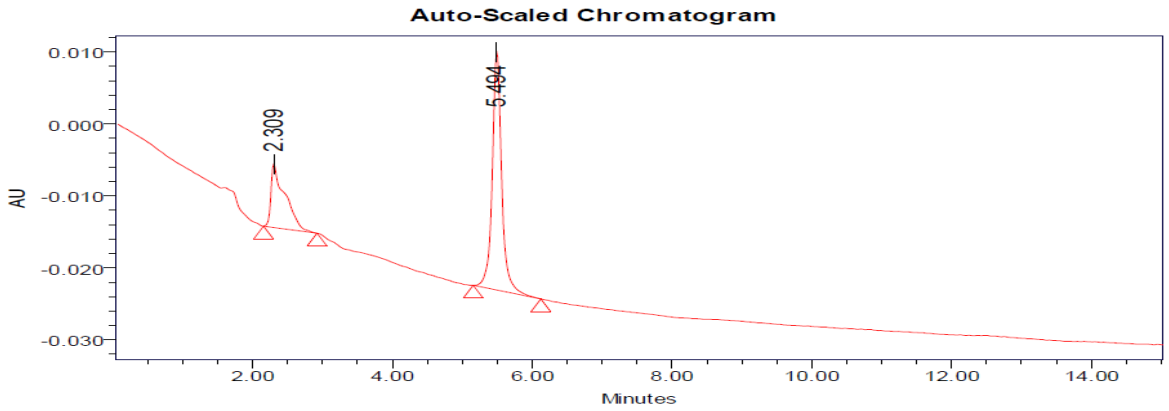

Fig2- chromatogramfortrail -2

Table:4–Peakresultsfortrail -2

S. No	Peakname	R_t	Area	Height	USP Resolution	USP Tailing	USP platecount
1	Atorvastatin	2.309	123459	8872		2.72	154
2	Amlodipine	5.494	310282	33205	8.62	1.25	9682

Observation: Thistrialshowverylessplatecountandsamplepeaksarenotwellseparated, somoretrialswererequiredforobtaininggoodpeaks.

Systemsuitability:

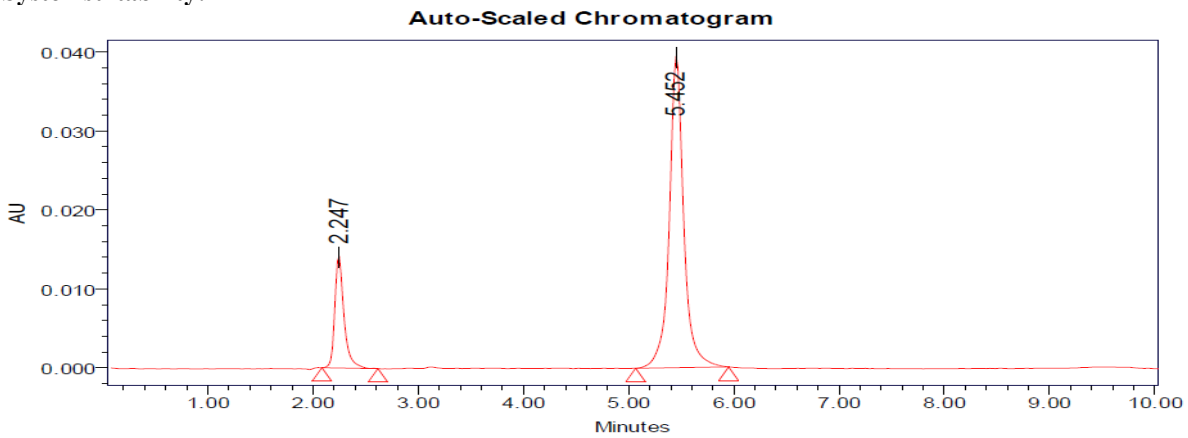

Fig3: Chromatogramshowinginjection -1

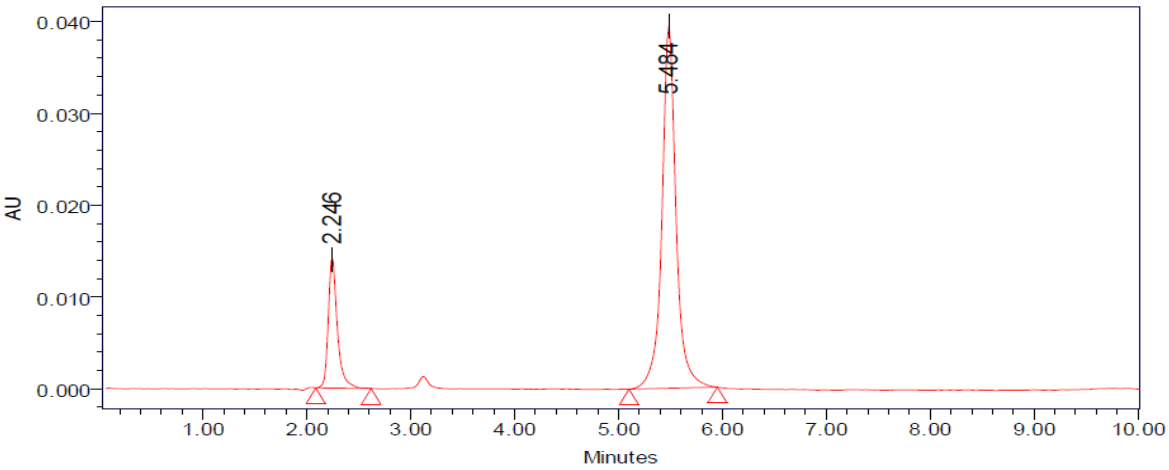

Fig 4: Chromatogramshowinginjection -2

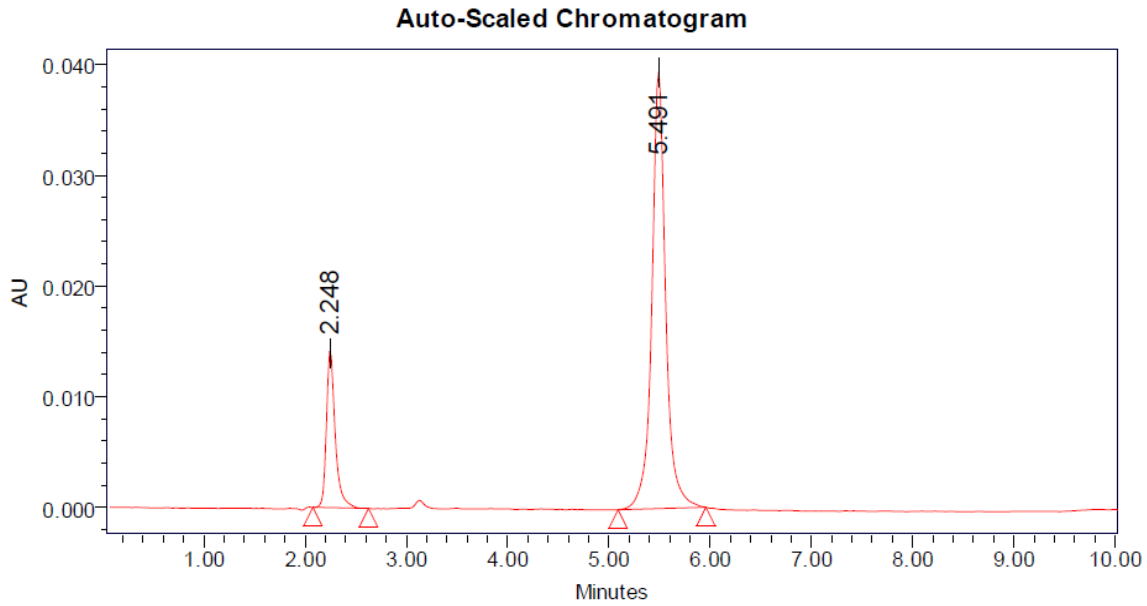

Fig5: Chromatogramshowinginjection -3

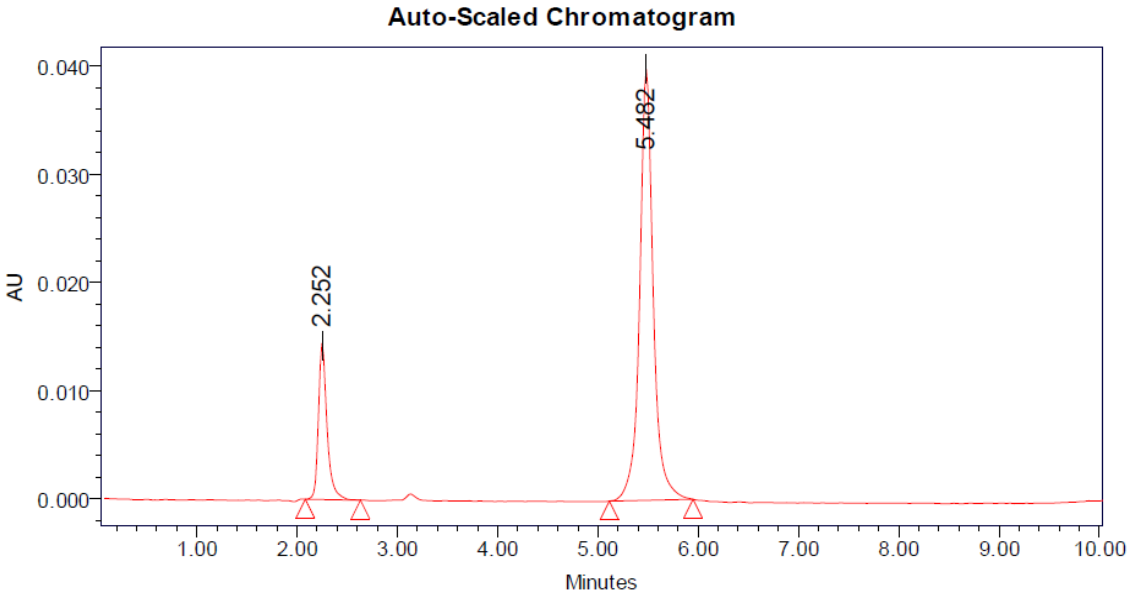

Fig6: Chromatogramshowinginjection -4

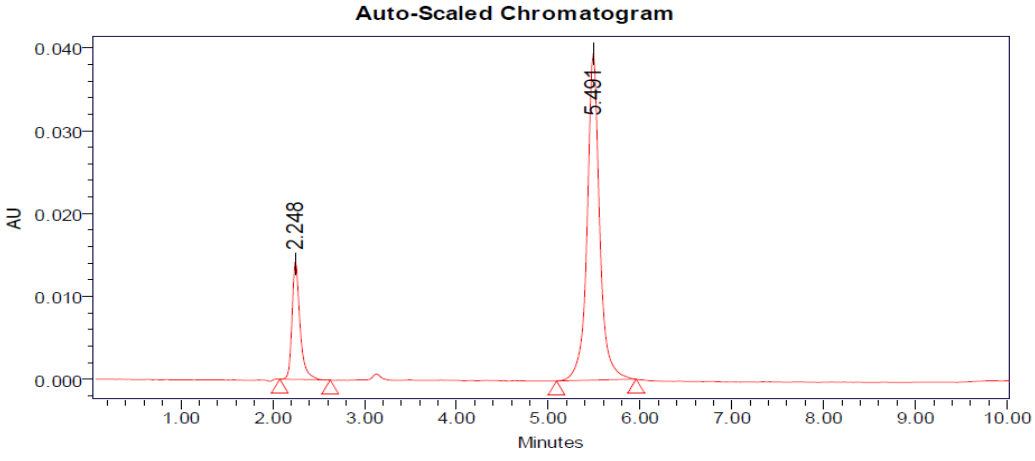

Fig 7: Chromatogramshowinginjection -5

Table 5: Resultsofsystemsuitabilityfor Atorvastatin

S no	Name	Rt	Area	Height	USP platecount	USP Tailing
1	Atorvastatin	2.247	86092	14051	5506	1.36
2	Atorvastatin	2.246	85626	14025	5674	1.2
3	Atorvastatin	2.248	85557	14132	5298	1.2
4	Atorvastatin	2.252	86141	14306	5032	1.0
5	Atorvastatin	2.248	86557	14152	5812	1.33
Mean			85994.6			
Std. Dev			410.662			
% RSD			0.4			

Acceptancecriteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtainedis withinthelimit, hencethemethodissuitable.

Table 6:ResultsofsystemsuitabilityforTelmisartan

S.No	Name	Rt	Area	Height	USP platecount	USP Tailing	USP Resolution
1	Amlodipine	5.452	376065	39373	9146	1.04	15.0
2	Amlodipine	5.484	373325	39429	9024	1.5	15.5
3	Amlodipine	5.491	373435	39403	9167	1.2	15.3
4	Amlodipine	5.482	375113	39745	9076	1.1	15.1
5	Amlodipine	5.491	373435	39403	9327	1.2	15.2
Mean			374274.6				
Std. Dev			1247.001				
% RSD			0.3				

Acceptancecriteria:

- %RSD for sampleshould be NMT 2
- The %RSD forthestandardsolutionisbelow 1, whichiswithinthelimitshencemethod is precise.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Atorvastatin:

Table 7: chromatogram for linearity concentration-25 µg/ml of Atorvastatin& 125µg/ml of Amlodipine

Concentration (µg/ml)	Average P eakArea
30	51080
40	92208
50	139140
60	180998
70	223920

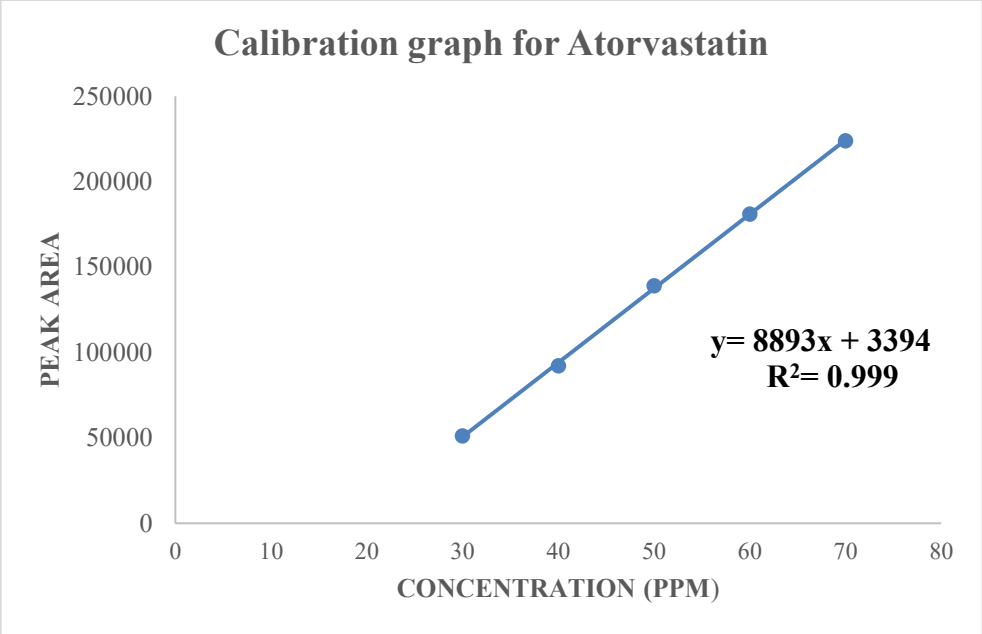

Fig 8: Calibration graph for Atorvastatin

LINEARITY PLOT:
TheplotofConcentration (x) versustheAveragePeakArea (y) dataofAtorvastatinis a straight Line.
Y = mx + c
Slope (m) = 8893
Intercept (c) = 3394
CorrelationCoefficient (r) = 0.999
AMLODIPINEIDATION CRITERIA: The responselinearityisverifiedifftheCorrelationCoefficientis 0.99 orgreater.
CONCLUSION: CorrelationCoefficient (r) is 0.99, andtheinterceptis 3394.

Amlodipine

Table 8: chromatogram for linearity concentration-125µg/ml of Amlodipine

Concentration (µg/ml)	AveragePeakArea
10	224573
20	441895
30	635379
40	842226
50	1041381

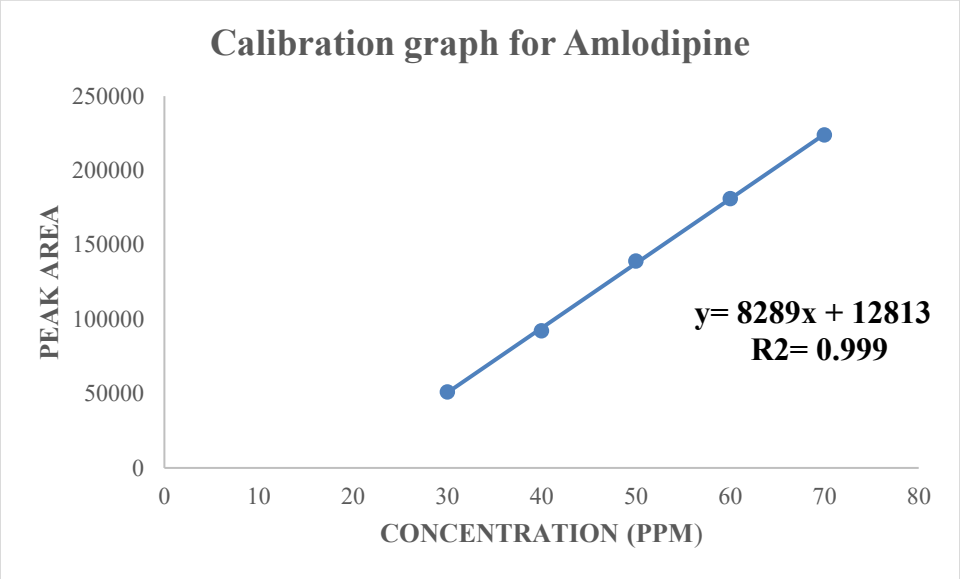

Fig 9: Calibration graph forAmlodipine

LINEARITY PLOT:

TheplotofConcentration (x) versustheAveragePeakArea (y) dataofAmlodipineis a straightline.

Y = mx + c

Slope (m) = 8289

Intercept (c) = 12813

CorrelationCoefficient (r) = 0.999

AMLODIPINEIDATION CRITERIA: TheresponselinearityisverifiedifftheCorrelationCoefficientis 0.99 orgreater.

CONCLUSION: Correlation Coefficient (r) is 0.99, andtheinterceptis 12813.

IV. SUMMARY AND CONCLUSION

A novel Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous quantification of atorvastatin and amlodipine in both pure and pharmaceutical formulations. The method was optimized using a Waters HPLC system with an auto-sampler and a PDA Detector 996 model. A Phenomenex Gemini C18 column (4.6 mm × 150 mm, 5.0 µm) was used, with the column maintained at 38°C. The mobile phase comprised methanol and acetone in a 32:68 v/v ratio, with a flow rate of 1.0 mL/min. Detection occurred at 240 nm, and the injection volume was 20 µL, with a total run time of 10 minutes.

The newly developed method exhibited high accuracy, precision, specificity, and linearity. Comprehensive validation was conducted, assessing limits of detection (LOD) and quantitation (LOQ), robustness, and stability. The method proved to be reliable and efficient for routine analysis, meeting regulatory standards and enhancing quality control in pharmaceutical manufacturing.

The RP-HPLC method developed for atorvastatin and amlodipine provides a robust and efficient approach for their simultaneous quantification in pure and pharmaceutical formulations. By employing optimized chromatographic conditions and a validated analytical process, this method ensures accurate and reliable results. Its successful validation underscores its suitability for routine quality control and compliance with regulatory requirements. This method represents a significant advancement in pharmaceutical analysis, offering a practical tool for ensuring the quality and efficacy of atorvastatin and amlodipine in various formulations.

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