WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

New Analytical Method Development and Validation of Atorvastatin By RP-HPLC Method

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ABSTRACT

Atrovastatin is an HMG-CoA reductase inhibitor that reduces cholesterol synthesis. The purpose of this project was to create something that was accurate, exact, particular, and repeatable. For Atrovastatin, a quick, specific stability indicating reverse phase high performance liquid chromatography method was developed and validated. The HPLC analysis used a reversed phase C18 (150 x 4.6mm, 5µm) column with an Acetonitrile:water (30:70) mobile phase and a flow rate of 0.9 ml/min. The method was verified for precision, accuracy, linearity, and limit of detection (LOD) and limit of quantitation (LOQ) in accordance with regulatory criteria (LOQ). The method's percentage RSD for precision and accuracy was determined to be less than 2%. The procedure was discovered to be linear.

KEYWORDS: Atrovastatin, HPLC, validated.

INTRODUCTION

Atrovastatin (3,5-dihydroxy – 7 – ((1R,2S,6S,8R,8aR) – 6 – hydroxyl – 2 – methyl – 8 – [(2S) -2-methyl butanoyl] oxy – 1,2,6,7,8,8a-hexahydronaphthalen-1-yl) oxy – 1,2,6,7,8,8a- hex heptanoic acid is a kind of heptanoic acid. HMG, a substituent of the natural substrate of HMG-CoA reductase, is structurally identical to atorvastatin. In contrast to its parent chemical, mevastatin, and statins like lovastatin and simvastatin, atorvastatin does not require in vivo activation. Its hydrolyzed lactone ring resembles the reductase's tetrahedral intermediate, allowing it to bind with far more affinity than its normal substrate. The bicyclic part of atorvastatin binds to the active site's coenzyme A section. The lipid-lowering action of atorvastatin sodium is achieved in two ways. First, it causes modest decreases in intracellular cholesterol pools due to its reversible suppression of HMG-CoA reductase activity. The number of LDL-receptors on cell surfaces increases, resulting in improved receptor-mediated degradation and clearance of circulating LDL. Second, atorvastatin reduces LDL formation by decreasing the synthesis of VLDL, a precursor to LDL, in the liver.

MATERIALS AND METHODS

Chemicals and reagents: Sura labs, a bulk producer, provided an active pharmaceutical ingredient sample of Atrovastatin (Hyderabad, India). Merck provided the HPLC grade Acetonitrile (ACN) and doubly Distilled Water (Darmstadt, Germany)

Instrumentation: The HPLC system was made up of a WATERS system with a Prominence PDA detector and the Empower 2 Solution software. This method used a C18 (150 mm x 4.6 mm) 5m analytical column.

Preparation of mobile phase:

Prepare the mobile phase by mixing 700 mL HPLC water in a 1000 mL beaker with 300 mL Acetonitrile (30%), degassing for 5 minutes in an ultrasonic water bath, and filtering through a 0.45 filter under vacuum filtration.

Atorvastatin Standard and Sample Solution Preparation:

Standard Solution Preparation:

To make the volume up to the mark, accurately weigh and transfer 10 mg of Atorvastatin working standard into a 10 ml clean dry volumetric flask, add around 7 ml of Diluent, and sonicate to dissolve it completely.

(Stock answer) Pipette 2.5 mL of Atorvastatin from the aforesaid stock solution into a 50 mL volumetric flask and dilute with diluents to the desired concentration (primary solution). Pipette 3 mL of Atorvastatin from the primary solution into a 10 mL volumetric flask and dilute with diluent to the desired concentration.

Preparation of the Sample Solution: Accurately weigh and transfer 10 mg eq.valent of Atorvastatin Tablet powder into a 10 ml clean dry volumetric flask, add roughly 7 ml of Diluent and sonicate to completely dissolve it, then make up the volume with the same solvent. Pipette 2.5 mL of the

aforementioned stock solution into a 50 mL volumetric flask and dilute to the desired concentration with diluents. Pipette 3 mL of Atorvastatin from the primary solution into a 10 mL volumetric flask and dilute with diluents to the desired concentration..

CHROMATOGRAPHIC CONDITIONS

Table:1

PARAMETERS	CONDITIONS
Mobile Phase	Acetonitrile:water (30:70)
Column (Stationary Phase)	Symmetry $C1_8$ (4.6 x 100 mm, 3.5µm, Make: Phenomenax)
Flow rate (ml/min)	0.9ml/min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20µl
Detection wavelength (nm)	300nm
Retention Time (min)	2.248 min
Plate count	2,615
Tailing factor	1.423

RESULTS AND DISCUSSION

Method development and optimization

A new isocratic reverse-phase high performance liquid chromatography with UV-detection at 300nm was developed for quantitative determination of Atorvastatinin bulk form. The mobile phase used was of Acetonitrile and water. The chromatographic method was performed Symmetry $C1_8$ (4.6 x 100 mm, 3.5µm, Make: Phenomenax) at a flow rate of 0.9 ml/min. Column Temperature was set a ambient temperature and injection volume was 20µl.

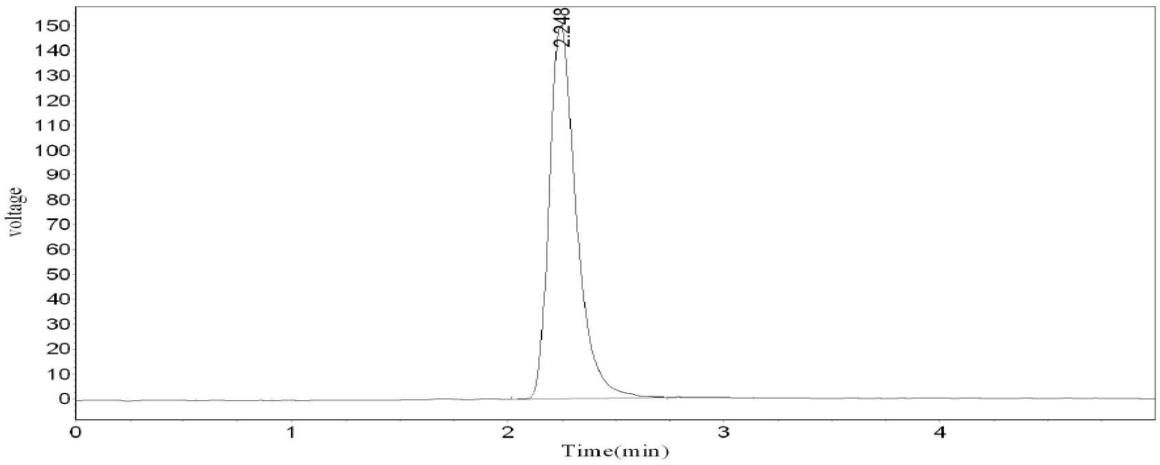

Figure: 1. Chromatogram for optimized trail 7

After conducting total 7 trials 7th trail shows proper peak, tailing, plate count and baseline in the chromatogram. So it's optimized chromatogram.

Accuracy:

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found. Inject the standard solution, Accuracy -50 %, Accuracy -100 % and Accuracy -150 % Solutions. The Amount found and Amount added for atorvastatin and the individual recovery and mean recover were calculated.

Table:2 Accurcy for Atrovastatin

Accuracy	Area	Amt added	Amt found	% recovery	Mean recovery
50%	1944859	22.5	22.3	97.46%	**99.4%**
100%	2607924	30	29.91	99.4%	
150%	3296181	37.5	37.8	101.3%	

Linearity: The linearity study was performed for concentration range of 5-30ppm of Atrovastatin of and the correlation coefficient was found to be 0.999 (NLT 0.999).

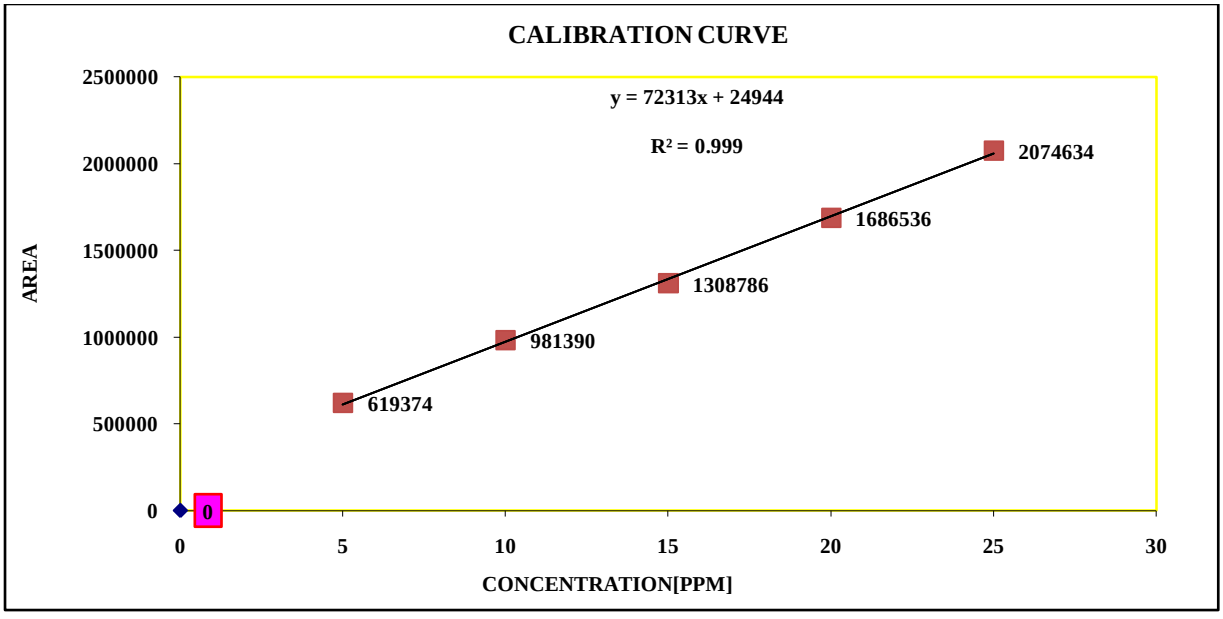

Figure: 2. Calibration curve of Atrovastatin

Precision: The repeatability of the method was checked by repeated analysis of the formulation for six times with the same concentration. The amount of drug present in the formulation was calculated. The percentage RSD value was calculated. %RSD for sample should be NMT 2

Table:3 Results of method precision for Atrovastatin

INJECTION	atorvastatin AREA
Injection1	1283904
Injection2	1287461
Injection3	1282427
Injection4	1304270
Injection5	1333463
Average	1298305
Standard Deviation	21498.59
%RSD	1.66

Intermediate Precision/Ruggedness: The Robustness of the method was established by changing the parameters like Flow rate and mobile phase composition. The small changes in system suitability parameters were with in the limits which ensures that the method developed can withstand slight changes in the experimental conditions and produce results with good reproducibility and repeatability

Table:4 Ruggedness for Atrovastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate countCount	USPTailing
1	Atrovastatin	5.452	121609	54804	9009.0	1.1
2	Atrovastatin	5.446	121822	54903	9131.5	1.1
3	Atrovastatin	5.493	121953	55996	9015.7	1.0
4	Atrovastatin	5.484	121988	56102	8987.3	1.0
5	Atrovastatin	5.419	121989	55577	9070.5	1.0
6	Atrovastatin	5.406	122282	55808	9047.6	1.0
Mean			121940.3			
Std. Dev.			2216.8			
% RSD			0.4			

Robustness

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Atrovastatin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Atrovastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Table:5 Robustness for Atrovastatin

S. no	Drug name	Flow (ml/min)	Area	Height	USP plate count	USP Tailing
1	Atrovastatin	Less (0.7)	126644	58074	9364	1.02
		Actual(0.8)	123002	56127	9118	1.03
		More (0.9)	125918	53155	7559	0.98

Limit of detection and limit of quantification

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The detection limit (LOD) was found to be 0.429µg/ml.

The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10).The quantitative limit (LOQ) was found to be 1.432µg/ml. In case of LOD and LOQ the S/N ratio were found to be with in the limits.

CONCLUSION

For the determination of Atorvastatin in bulk form by isocratic mode elution, a simple, rapid, cost-effective, and accurate RP-HPLC technique was devised. The designed solvent system and analytical conditions resulted in satisfactory separation in a short run time. The linearity, precision, accuracy, and specificity of the RP-HPLC method were all proved to be good. As a result, the established RP-HPLC method can be used for routine analysis while looking at Atorvastatin. All of the characteristics for the medication Atorvastatin had passed the ICH method validation requirements. The RP-HPLC approach, on the other hand, is thought to be more specific and sensitive. It is desirable to develop methods capable of analyzing a large number of samples in a short amount of time with good robustness, accuracy, and precision without the need for any prior separation step for routine analytical purposes. The assay findings of bulk form as well as laboratory generated mixtures by devised procedures showed good agreement. We came to the conclusion that all of the provided methods are a decent strategy for obtaining trustworthy findings and are suitable for routine Atorvastatin estimate. The proposed technique's percent RSD was determined to be less than 2%, indicating its ability to remain unaffected by minor but deliberate adjustments in method parameters and indicating its reliability in routine use. The validation parameter's result shows that the analytical technique is appropriate for its intended purpose and meets the ICH standards. As a result, it is suggested that it be employed in routine release and stability sample testing.

Acknowledgment :

The authors are thankful to Dr. K. Venktaramana principal of A.S.N Pharmacy college for providing the facilities to carry out this project.

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