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METHOD DEVELOPMENT AND VALIDATION OF ATORVASTATIN BY USING RP-HPLC IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

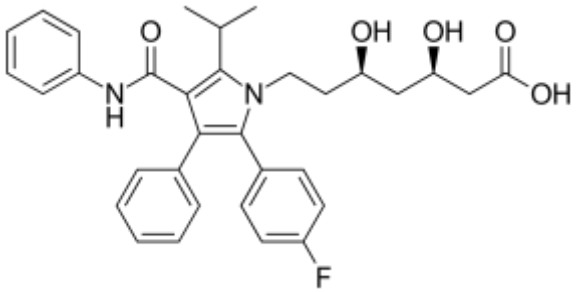
This work describes a strategy for the systematic development of High-performance liquid chromatographic (HPLC) methods for the method development and validation of Atorvastatin in its fixed dose. HPLC is an analytical tool which is able to detect, separate and quantify the drug, its various impurities and drug related degradants that can form on synthesis or storage. It involves the understanding of chemistry of drug substance and facilitates the development of analytical method. A number of chromatographic parameters were evaluated in order to optimize the method. An appropriate mobile phase, column, column temperature, wavelength and gradient must be found that affords suitable compatibility and stability of drug as well as degradants and impurities. Method development and validation for the determination of Atorvastatin in its fixed dose drug product is by RP-HPLC. In this work, simple, rapid, accurate and sensitive analytical methods have been developed and validated. This method was developed by using C18 column with a mobile phase consisting of Methanol and Water in the ratio of 95:5. The flow rate was adjusted at 0.8 ml/min, injection volume 20µL, with UV-Detector the maximum absorption peak (λmax) is at 240 nm. Column was maintained at ambient temperature(25ºC), and retention time was found to be 2.9min. Under the Optimized conditions, Beer's law correlating the Area (Y) with concentration (X) was obeyed in the range of 900 to 1000 µg/ml. Assay was obtained as 98% for Atorvastatin. Regression equation for Atorvastatin is y=19.567x-15761.Correlation coefficient of Atorvastatin was found to be 0.9902.

INTRODUCTION

Description

Atorvastatin belongs to a group of drugs called HMG CoA reductase inhibitors, or "statins." Atorvastatin is used together with diet to lower blood levels of "bad" cholesterol (low-density lipoprotein, or LDL), to increase levels of "good" cholesterol (high-density lipoprotein, or HDL), and to lower triglycerides (a type of fat in the blood).Atorvastatin is used to treat high cholesterol, and to lower the risk of stroke, heart attack, or other heart complications in people with type 2 diabetes, coronary heart disease, or other risk factors.[1] It is on the World Health Organization's List of Essential Medicines.[2]

Structure:



Systematic (IUPAC) name

(3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]3,5-dihydroxyheptanoic acid

Physiochemical Data:

Formula : C33H35FN2O5

Molecular weight : 558.640 Da

Mechanism of Action

Several landmark studies demonstrate that the use of statins is associated with both a reduction in LDL levels and CVD(Cardio Vascular Disease) risk.[3,4,5,6,7,8] Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes. With ultracentrifugation, these complexes separate into HDL (high-density lipoprotein), IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein) fractions. Triglycerides (TG) and cholesterol in the liver are incorporated into VLDL and released into the plasma for delivery to peripheral tissues. LDL is formed from VLDL and is catabolized primarily through the high-affinity LDL receptor. Clinical and pathologic studies show that elevated plasma levels of total cholesterol (total-C), LDL-cholesterol (LDL-C), and apolipoprotein B (apo B) promote human atherosclerosis and are risk factors for developing cardiovascular disease, while increased levels of HDL-C are associated with a decreased cardiovascular risk.[9]

HPLC:

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.[10]

Reverse phase chromatography is established on the partition equilibrium between the non-polar (hydrophobic) stationary phase and the polar mobile phase, where the polarity of the mobile phase is higher than the stationary phase.[11]

EXPERIMENT:

Instrumentation: The waters HPLC system comprising of an isocratic pump, auto sampler and UV detector was utilized. Sonicator was used for enhancing dissolution of analyte in the diluent. A digital weighing balance was used for weighing all the materials. Pipettes and Beakers used were made of Borosilicate glass.

Materials: The working standard Atorvastatin was provided by Thermos Fisher Scientific India Pvt. Ltd. Marketable Atorvastatin tablets of 10mg and 20mg were brought from local pharmacies. HPLC grade methanol and water obtained from Thermos Fisher Scientific India Pvt.Ltd

Chromatographic Conditions: The HPLC system was operated isocratically with the C18 column using a mobile phase composition of Methanol and Water in the ratio of 95:5% v/v at a flow rate of 0.8 mL/min and the retention time was 2.9 min within a run time at 10 min. The Atorvastatin was detected and quantified at 240 nm.

RESULTS AND DISCUSSION:

Method development and optimisation

Column chemistry solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent in the mobile phase), additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized, so there was no interference with the Atorvastatin peak from solvent. To investigate the appropriate wavelength for the determination of Atorvastatin, UV-visible spectra in the range of 200-400 nm were obtained from a solution of the drug in the mobile phase. From the UV spectra obtained, the wavelength selected for

monitoring the drug was 240 nm. It was observed that, there was no interference from the mobile phase or baseline disturbance at 240 nm. Therefore it was concluded that 240nm was the most accurate wavelength.

Symmetrical peaks are obtained for Atorvastatin. Typical chromatograms obtained from a bulk and from a solution of drug are illustrated in fig1& 2. The retention time was 2.9 min .

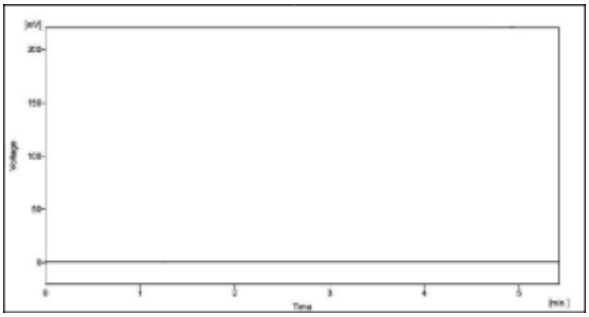


Figure 1 Chromatogram obtained from blank

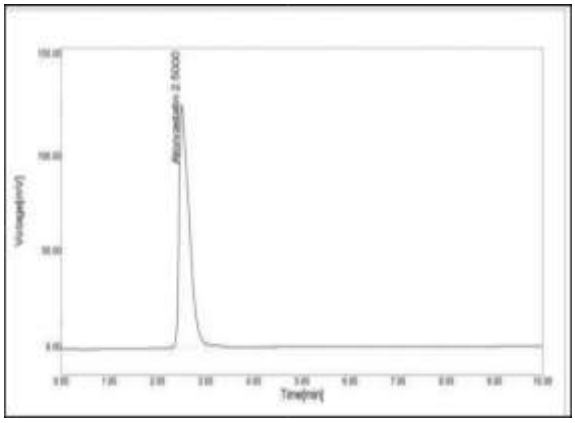


Figure 2 Chromatogram obtained from Atorvastatin solution

Method Validation

The method was validated as per ICH Q2(R1) guidelines13. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

1. Linearity:

Accurately weighed and transferred 50 mg of Atorvastatin working standard into a 10 ml clean dry volumetric flask. added about 5 ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further, pipetted 1 ml of the above stock solution into a 10 ml volumetric flask and diluted up to 10ml with diluent.(1000 µg /ml) (Stock solution).

Preparation of standard dilutions

Methanol is used as a diluent. From the stock solution of concentration 1000µg/ml pipetted out the required volumes of concentration as 980µg/ml, 960µg/ml, 940µg/ml, 920µg/ml, 900µg/ml.

Chromatograms for Linearity:

Chromatograms Linearity

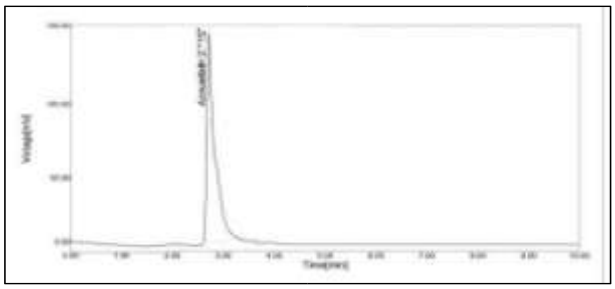


Figure 3:Chromatogram sLinearity 1

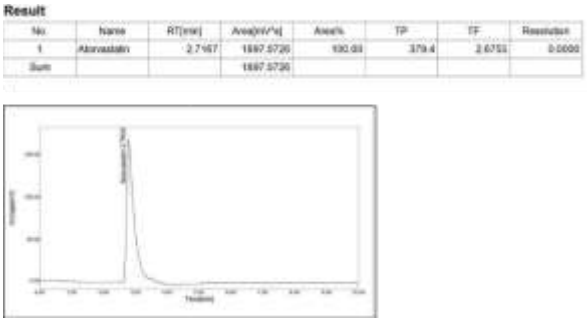


Figure 4: Chromatograms for linearity 2

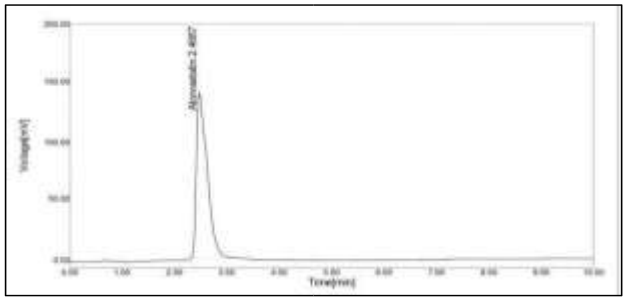


Figure 5: Chromatograms for Linearity 3

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	3.0000	2957.7349	100.00	1596.2	1.5544	0.0000
Sum			2957.7349				

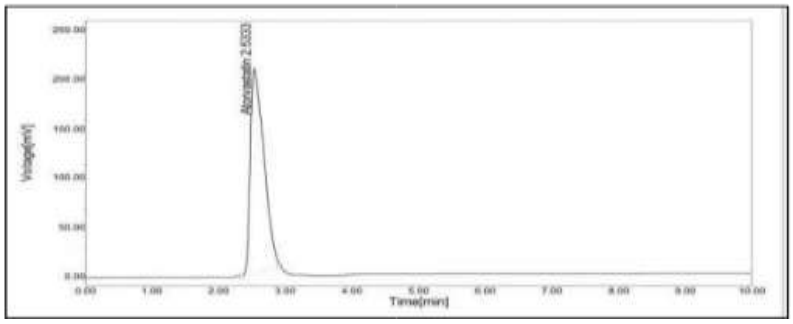


Figure 7: Chromatograms for Linearity 5

Result

No.	463lame	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.5333	3463.5237	100.00	691.6	1.8657	0.0000
Sum			3463.5237				

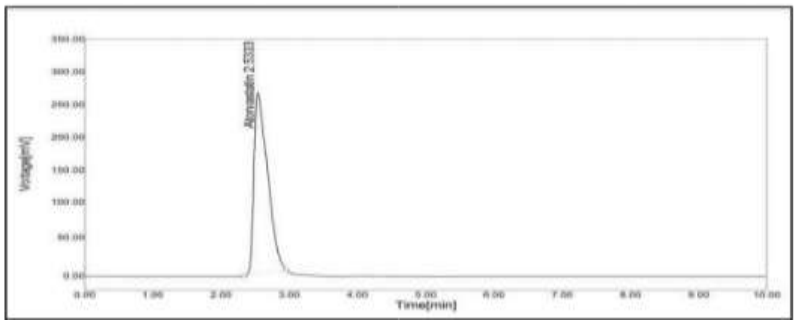


Figure 8 : Chromatograms for Linearity 6

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.5333	3845.3750	100.00	779.8	2.0091	0.0000
Sum			3845.3750				

Table 1 Area of different concentration of Atorvastatin

S. No	Atorvastatin	
	Concentration (µg/ml)	Area
1	900	3845
2	920	3463
3	940	2957
4	960	2516
5	980	2291
6	1000	1897

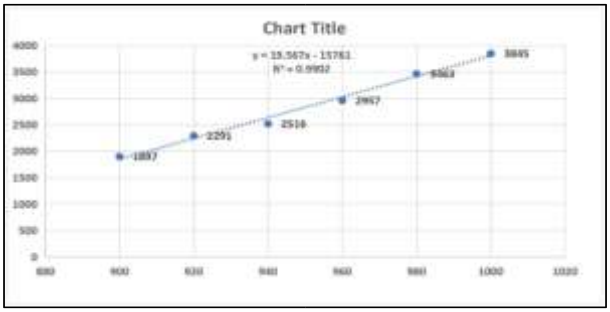


Figure 9 Calibration Graph

Parameters	Atorvastatin
Slope (m)	19.567
Intercept (c)	15761
Correlation coefficient (R^2)	0.9902

Table 2 Analytical performance parameters of Atorvastatin

Precision:

The middle concentration level from standard stock solution from 1000µg/ml to 900µg/ml like 940µg/ml has been taken. Now the standard solution was injected 6 times and measured the area for all 6 injections in HPLC. The % RSD for the area of 6 replicate injections was found to be within the specified limits.

Acceptance criteria: The %RSD for the area of 6 standard injections results should not be more than 2%.

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.5000	2317.3271	100.00	744.0	2.1338	0.0000
Sum			2317.3271				

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.5333	2130.4236	100.00	743.2	1.9855	0.0000
Sum			2130.4236				

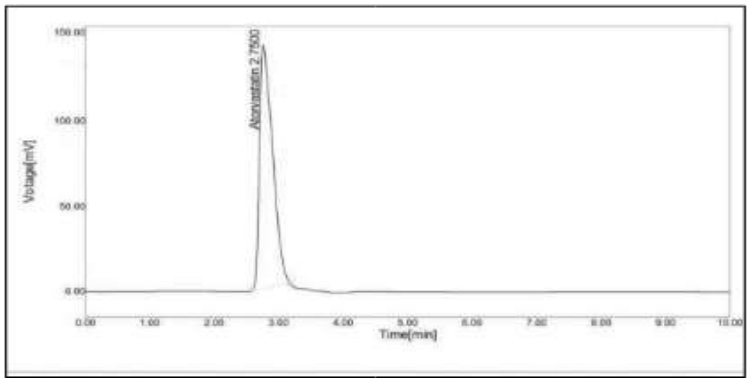


Figure 15 Chromatogram for Precision 6

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.7500	2516.3201	100.00	904.0	2.0461	0.0000
Sum			2516.3201				

Injection	Area
Injection-1	2317
Injection-2	2401
Injection-3	2646
Injection-4	2634
Injection-5	2130
Injection-6	2516

Table 3 Results of Precision for Atorvastatin

Average	2440
Standard Deviation	14644
%RSD	0.1667

Table 4 RSD Results for Atorvastain

Limit of Detection (LOD):

It is done by taking 25% of the lowest concentration and further diluting it by adding 4ml Methanol. Inject it thrice. Determine the Retention time and area. Here the lowest concentration was found to be 900µg/ml. Hence it is injected thrice after diluting it with Methanol and the areas are determined for 3 of them respectively.

Estimation of Atorvastatin in Tablets:

Accurately weighed 1 tablet from 3 brands of Atorvastatin crushed in mortar and pestle and transferred sample into a 10 ml clean dry volumetric flask. Added about 10mL of diluent and sonicated it up to 3 mins to dissolve it completely and volume is made up to the mark with the same solvent. Then it is filtered through 0.45-micron injection filter. (Stock solution)

Sample was found to be highly concentrated, then further pipetted 1 ml of the above stock solution into a 10 ml volumetric flask and diluted up to 5ml with the diluent.

Brands used for the Sample Preparation

Aztor : 20mg

Atrocord : 10mg

Atrobest : 20mg

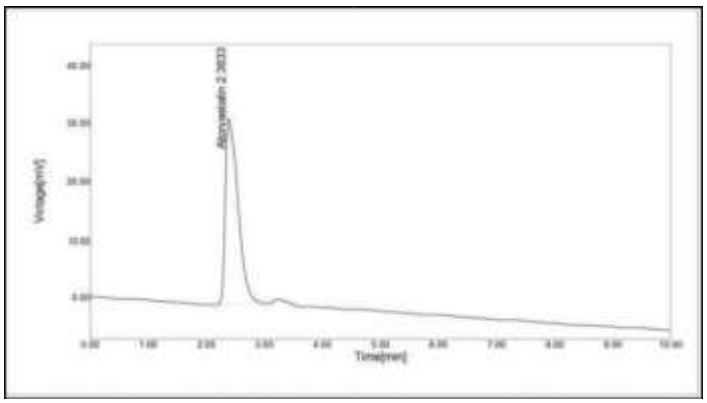


Figure 16 Chromatogram for LOD

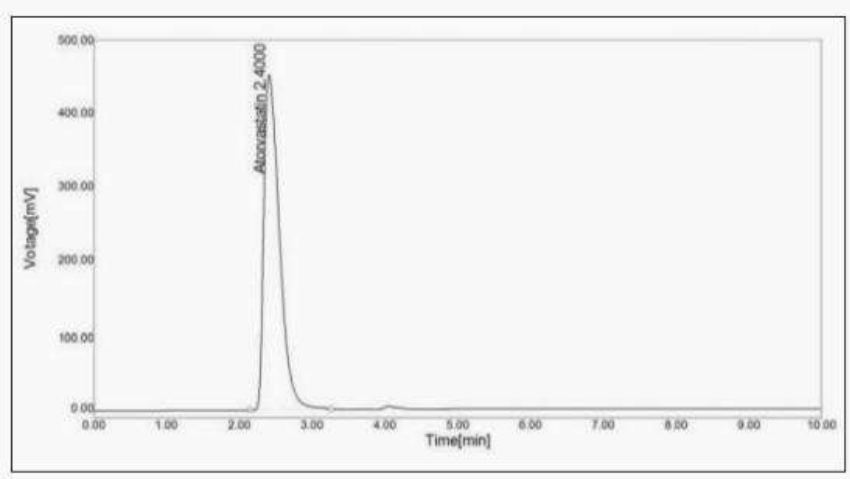
Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.7500	2516.3201	100.00	904.0	2.0461	0.0000
Sum			2516.3201				

Limit Of Detection	Area
LOD 1	381
LOD 2	401
LOD 3	380

Table No: 5 Results for Limit of Detection

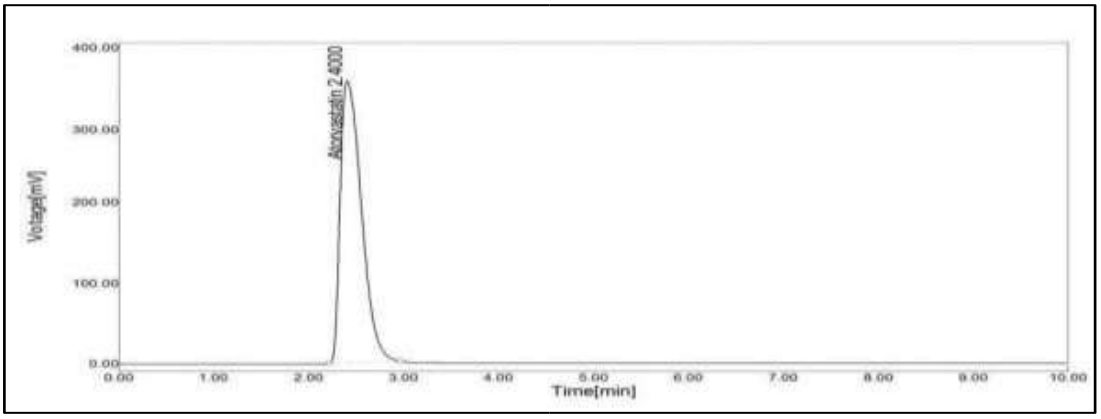
Chromatograms for Samples



Chromatogram for Sample1

Result

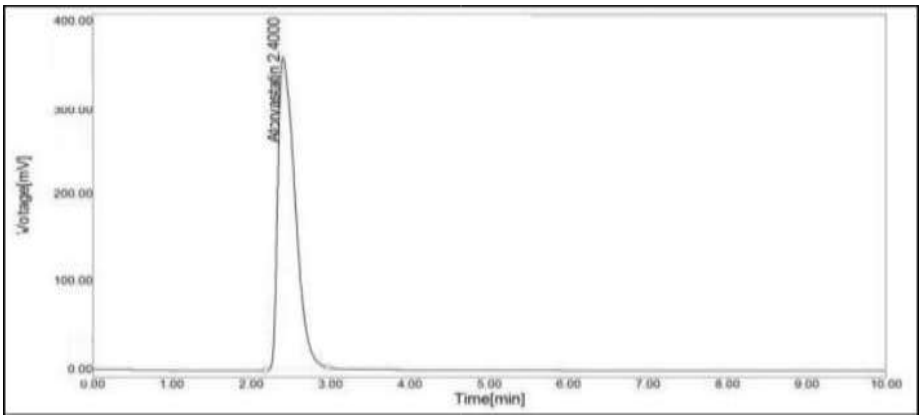
No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.4000	21667.6235	100.00	733.0	1.8950	0.0000
Sum			21667.6235				



Chromatograms for Sample 2

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.4000	19768.9014	100.00	590.6	1.8627	0.0000
Sum			19768.9014				



Chromatograms for Sample 3

Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Atorvastatin	2.4000	20768.4355	100.00	700.2	1.8801	0.0000
Sum			20768.4355				

CONCLUSION:

It can be concluded that the proposed method was simple, selective, sensitive, accurate, precise and rapid for the estimation of Atorvastatin in a short analysis time. The method was proved to be superior to most of the reported methods. The mobile phase is simple to prepare and economical. The sample recovery in the formulations were in good agreement with their respective label claims and they suggested non-interference of formulation in the estimation. Hence this method can easily be adopted as an alternative method to the reported one for the routine determination of Atorvastatin depending upon the nature of the ingredients present in the sample. The estimation of Atorvastatin was done by RP-HPLC. The assay of Atorvastatin was performed with tablets and the % assay was found to be 98.99%which show that the method is useful for routine analysis.The linearity of Atorvastatin was found to be linear with a correlation coefficient of 0.999,which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1667 for Atorvastatin which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.1667 for Atorvastatin which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery which should be in the range of 98.0% - 102.0%. The total recovery was found to be 98% for Atorvastatin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

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