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A Review On: Process Validation and Quality Parameters of Atorvastatin Calcium

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ABSTRACT

Atorvastatin is a selective competitive inhibitor of HMG CoA reductase. It reduces total cholesterol, low density lipoprotein (LDL). HMG CoA reductase catalyzes the HMG CoA to mevolanate, which is the limiting step in cholesterol biosynthesis. It also reduces the VLDL cholesterol and triglyceride. The present research work focused process validation for Atorvastatin 10 mg. Validation is best viewed as an impartment and integral part of cGMP. Validation is therefore one element of quality assurance programs associated with a particular process. Quality cannot be assured only by doing finished product testing and in process monitoring but it should be built into the manufacturing process. So, building of quality require a special attention to a few factors like selection of material, process design, control variables, in process control and finished product testing. In this study three initial batch of Atorvastatin Calcium with same amount, method, equipment & validation criteria were taken. Various critical parameters during identification, determination of sodium content, determination of volatile matter, enantiomeric purity by HPLC, bulk and tapped density stages were identified and evaluated as per validation protocol. The outcomes of the entire process indicate that process validation data provides a high degree of assurance that the manufacturing process will produce a product meeting its predetermined specification and quality attributes.

Keywords- Validation, Lipoprotein, cGMP, Quality parameters.

I. INTRODUCTION

Atorvastatin (AVT), as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutarylcoenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, an early rate limiting step in cholesterol biosynthesis. Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. Atorvastatin calcium([R-(R*,R*)]-2-(4-fluorophenyl)-β,γ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1Hpyrrol- 1-heptanoic acid, hemi-calcium salt). Is a white to off-white crystalline powder that is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and slightly soluble at pH 7.4 phosphate buffers and acetonitrile, slightly soluble in ethanol and freely soluble in methanol. [(1-3)]

Chemical Structure of Atorvastatin Calcium: The empirical formula of atorvastatin calcium is **$(C_{33}H_{34}FN_2O_5)2Ca•3H_2O$** and its molecular weight is 1209.42.

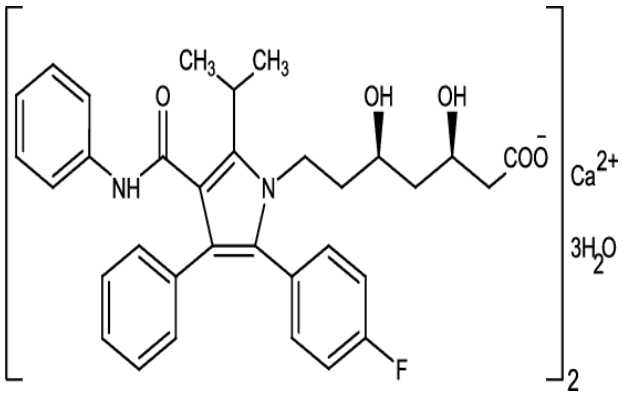

Fig.1: Chemical Structure of Atorvastatin Calcium

II. MATERIAL AND METHOD FOR PROCESS OF VALIDATION OF ATORVASTATIN CALCIUM

Materials: Working standards of ATC (potency = 95.30%) and HPLC grade Acetonitrile, Methanol, Tetrahydrofuran, glacial acetic acid and Ammonium acetate. Water was purified with Milli-Q Millipore system. All the solvents and solutions were filtered through a 0.45µ.

Method: The HPLC system (waters e2695) used for analysis consisted of autosampler, UV detector with EM Power software for data acquisition and processing. The

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chromatographic separation was performed on C18 column (150 mm x 3 mm, 3µ particle size) with isocratic condition at ambient temperature. The analysis was performed at flow rate 1.5 ml/min. Quantification was achieved with UV detection at 246 nm. Retention time of Atorvastatin Calcium found to be 5.70 ± 0.10 minute. Analytical balance and digital pH meter of Mettler Toledo was used for analysis purpose. [4-7]

Table 1: Chromatographic Conditions

HPLC System	Waters e2695
Software	EM Power
Detector	UV-detector
Wavelength	246nm
Pump	Isocratic pump
Stationary phase	Silica gel
Mobile phase	0.02M ammonium acetate buffer (Ph 4.0± 0.05)
Flow rate	1.5ml/ml
Injection volume	20µl
Diluent	Methanol: water (80:20v/v)
Column temperature	24 degrees Celsius

Standard Solution Preparation: A). To prepare a stock solution for assay, weight accurately equivalent to 50 mg of Atorvastatin working standard and transferred into 100 ml volumetric flask, to this 50 ml diluent was added to dissolve the substance by sonication for 5 minutes and volume was made up to the mark by diluent (solution A).
Method Validation: The developed method was validated according to International Council for Harmonisation (ICH) (Q2) B guidelines for validation of analytical procedures. As per the ICH guidelines the method validation parameters checked were linearity, accuracy, precision, assay, LOD, LOQ and robustness.
Linearity: For constructing calibration curve, series of four dilutions in the concentration range 10-80 (20, 40, 60 and 80) µg/ml for ATC was taken. Linearity curves for ATC is shown in figure 2. The method shows good linear response in the concentration range 10-60µg/ml for ATC(r2=0.9997). [8-12]

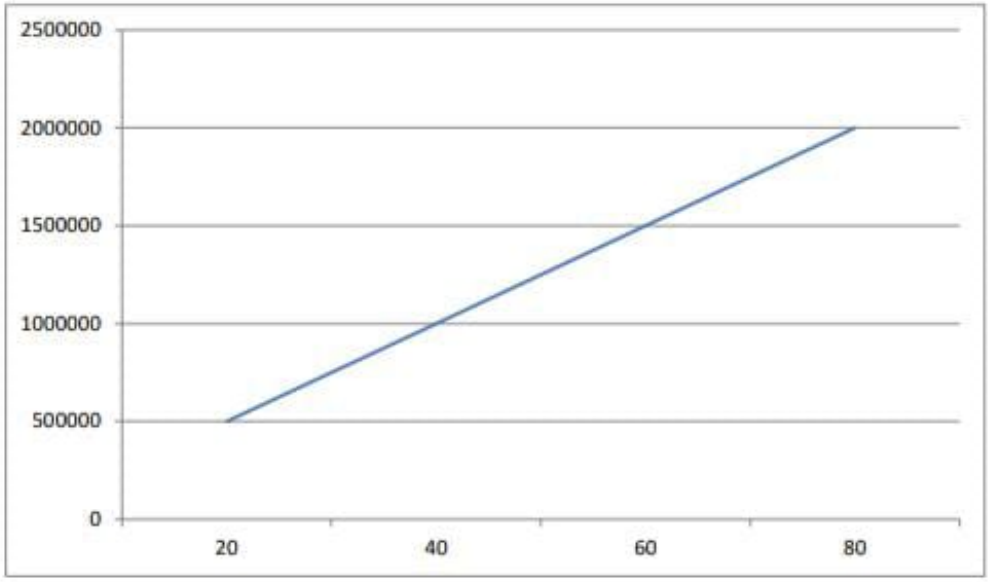

Fig. 2: Calibration Curve

Accuracy: The accuracy of the method was determined by calculating recovery of ATC a by the standard addition method. The accuracy of the analytical method was assessed by determination of recovery for three concentrations (corresponding to 80,100 and 120% of test solution concentration). For each concentration, two sets were prepared. The mean recovery and % RSD of recoveries of ATC and was reported. The results of recovery of ATC with the %RSD are given in below table 2

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Table 2: Accuracy data of ATC

Accuracy level	Set No	Amount added(µg/ml)	Amount founded(µg/ml)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80%	1	80	79.85	99.2	100.005%	0.612	0.609
	2	80	80.60	100.81			
100%	1	100	98.20	98.2	99.25%	1.111	1.119
	2	100	100.30	100.3			
120%	1	120	118.25	98.5	99.05%	0.586	0.592
	2	120	119.50	99.6			

Precision: The precision of analytical method express the degree of agreement among individual test when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision is considered at three levels that is system precision, method precision (repeatability) and intermediate precision (reproducibility). System precision the system precision of the instrument was checked by repeatedly injecting (n =3) standard solutions of the ATC and under the chromatographic condition and calculate the % RSD of peak area which should not be more than 2%.

Method precision (Repeatability): The method precision of the analytical method was determined by analysed three sets of sample preparation against the same standard. Assay of all three-sample preparation was determined and mean of assay, standard deviation and %RSD for the same was calculated. [13-16]

Intermediate Precision (Reproducibility): Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different instrument under same experimental conditions. Assay of all replicate sample preparation was determined and mean assay, standard deviation and %RSD for the same was calculated. The method was found to be precise and %RSD was found to be less than 2% was shown in below tables3,4,5,6.

Table 3: Data of system precision study

Sr. No.	Area of ATC	Mean	Standard deviation	%RSD
1	1755613			
2	1761832	1758828.33	3164.804	0.181
3	1759040			

Table 4: Data of method precision study

S. No.	Wt of Sample in mg	Avg. Area of ATC	% Assay of ATC	Mean	SD	%RSD
1	667.60	1837081	99.9			
2	673.50	1845030	100.9	100.1	1.129	1.129
3	672.58	1819618	99.6			

Table 5: Data of intermediate precision

S. No.	Weight of sample in mg	Avg. area of ATC	% Assay of ATC	Mean	Standard Deviation	%RSD
1	676.60	1807024	100.4			
2	674.10	1779869	99.2	100.5	1.197	1.195
3	671.32	1774613	101.9			

Table 6: Assay of ATC

Drugs	Amount claim(mg)	Amount of drug estimated(mg)	%Amount found
ATC	10.0	10.06	100.6

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III. ROBUSTNESS

The robustness of the method was established by introducing small changes in various parameters like, pH of mobile phase, flow rate, wavelength, column temperature and mobile phase composition. The robustness of the method was evaluated by calculating % assay of test solution which is not more than ± 2.0% from mean value of method precision and system suitability parameters meets the requirements. Robustness was evaluated by varying different parameters. The results of these variations are given below;

Table 7: Robustness of ATC

Parameters	Variation	ATC Retention Time(min)	Assay (%)
Flow rate(ml/min)	1.1	6.54	100.63
	1.7	5.70	100.03
pH	3.5	5.65	99.32
	4.2	5.70	99.92
Column temperature(0C)	22	5.66	99.54
	24	5.64	98.63
Wavelength(nm)	244	5.69	101.84
	248	5.71	99.38

Limit of detection and limit of quantification: Limit of Detection (LOD) is the lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of Quantification (LOQ) is the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD can be calculated according to formula LOD = 3.3 (SD/M) and LOQ = 10(SD/M).

The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1.

Table 8: LOD and LOQ results of ATC

Drugs	LOD (µg/ml)	LOQ (µg/ml)
ATC	0.230	0.690

Quality Parameters of ATC

a) Appearance: Atorvastatin is a member of pharmaceutical class known as statins used for lowering blood cholesterol. It is white in colour. [17-20]

b) Identification: IR spectroscopy is used for both quantitative and qualitative analysis. IR spectrophotometer is used to detect functional group. When a sample (ATC 20mg) is placed on IR spectrophotometer it absorbs infrared radiations and starts to vibrate and then give rise to a packed infrared absorption spectrum. This spectrum is specific for each molecule absorbing infrared radiations.

Fig. 3: IR Spectrophotometer

Results:

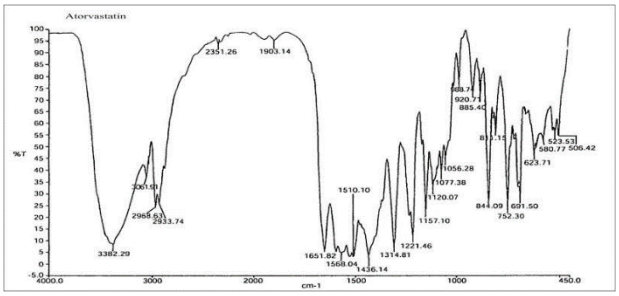

Fig.4: IR absorption spectrum of ATC

c) Solubility test: It is generally carried out to conclude the capability of compounds to dissolve in a solvent**.** Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

d) Enantiomeric purity by HPLC: A simple, rapid isocratic chiral hplc method has been developed for the

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separation of (S, S)-Atorvastatin from (R, R)-Atorvastatin enantiomer in bulk drug form (API form). A simple way to separate enantiomers is to use a chiral column (a variant of column chromatography). Firstly solvent (mobile phase) is degassed for eliminating the bubbles. It is passed through the pump with a uniform pressure. The sample is injected into the mobile phase flow stream. It passes through the stationary phase identified by the detectors and recorded.

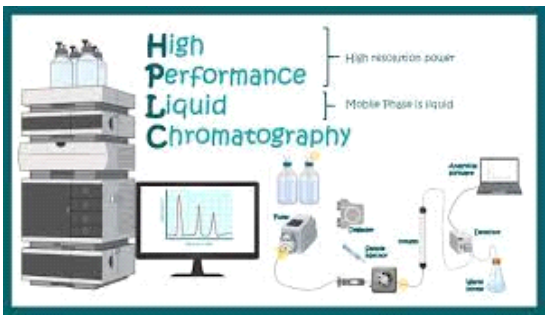

Fig.5: HPLC

Result: Normal phase chromatographic separation was achieved on chiral stationary phase, Chiral pak AD-H (250 mm × 4.6 mm H)) column at 30°C temperature. Flow rate was kept at 1.0 mLmin-1. The elution time was - 8.0 min and the resolution (R5) between the enantiomers are greater than 2.5. Interestingly (S, S) - form of Atorvastatin peak was eluted prior to the (R, R) - form of Atorvastatin. The limit of detection (LOD) and limit of quantification (LOQ) for the (S, S) Atorvastatin were 0.18µg mL-1 and 0.60µg mL-1 respectively, for a 10µL load of the sample.

e) Determination of volatile matter: Halogen moisture analyzer is a LOD method used to determine the total volatile matter. In halogen moisture analyzer we put our sample (ATC 20mg) in the oven and it heats the sample and tells us about all the volatile matter like water content, any liquid and solvents.

Table 9: Quantity of volatile matter in Atorvastatin calcium

Tests	Specifications	Units	Results
Water content	3.5 to 5.5	%w/w	4.7
Residual solvent			
Methyl tertiary butyl ether	Not more than 5000	ppm	284
Methanol	Not more than 3000	ppm	BQL

PSD analysis: It is used to determine and reports information about the size and range of particles present inside the sample.

Fig.6: PSD analyzer

Table 10: Particle size analysis of Atorvastatin calcium

Test	Specifications	Units	Results
Particle size distribution dry method			
-D90	Not more than 10	µm	6
-D50	Not more than 3	µm	2
-D10	Not more than 2	µm	1

IV. CONCLUSION

The developed method was suitable for the simultaneous estimation of Atorvastatin Calcium in bulk dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH of mobile phase, column temperature, wavelength etc. A good peak symmetry, resolution for ATC was obtained with mobile phase Buffer (pH 4.0 ± 0.05): ACN: THF (40:40:1.4 v/v/v) at a flow rate 1.5 ml/min. The wavelength of detection selected was 246 nm. The retention time of Atorvastatin Calcium was about 5.70 ± 0.20. A validated RP-HPLC method has been developed for the determination of Atorvastatin calcium in bulk dosage form. The developed method is simple, rapid, linear, accurate, precise and specific. Results from the validation experiments showed that the method is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Atorvastatin calcium and in bulk dosage form. And all the tests performed assure us that our product complies with pharma European monograph 2191 manufactured according to ICH Q7 GMP for APIs. And the material produced in accordance with the specifications currently in force for this product grade.

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