Research Article

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HPLC METHOD DEVELOPMENT OF ATORVASTATIN BY RP-HPLC IN ITS BULK DOSAGE FORMS

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ABSTRACT
A fast, simple, sensitive, precise, accurate and reproducible Reverse phase high performance chromatographic method was developed and validated for the analysis of atorvastatin in bulk dosage forms. The separation was conducted by using C-18 RP-HPLC column, which was maintained at ambient temperature. The mobile phase consisting of potassium di-hydrogen ortho phosphate, methanol and acetonitrile in the ratio 30:20:50 was delivered at a rate of 1 ml/min. The analysis was detected by using UV detector at the wave length of 240nm.The method is validated for its Precision, Limit of quantitiation (LOQ), Limit of detection (LOD), linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/ml (r^2 =0.999). The retention time for Atorvastatin was found to be 8.320±25min.Limit of quantification of the method is 0.189 µg/ml and limit of detection is0.086 µg/ml.
KEY WORDS: Atorvastatin, C-18 column, RP-HPLC, Acetonitrile

INTRODUCTION
Atorvastatin is chemically known as (3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl]-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxy heptanoic acid[1]. Atorvastatin selectively and competitively inhibits the hepatic enzyme hydroxy methyl glutaryl Co A reductase(HMG CO A).As HMG CO A reductase is responsible for converting HMG Co A to mevalonate. This results in a decrease in mevalonate, a precursor of cholesterol and a subsequent decrease in hepatic cholesterol levels and increase in uptake of LDL and cholesterol[2]. The Various methods are used for the determination of drugs in pharmaceutical formulations. These methods include titrimetry, flourimetry, UV spectrophotometry, infra red spectroscopy, differential scanning calorimetry (DSC), chromatographic methods etc. Among these, chromatographic methods are frequently used for the qualitative & quantitative analysis of drug substances, drug products and raw materials. This paper describes a fast, sensitive, rapid and accurate method for developed and validated the analysis of atorvastatin in bulk dosage forms by using Reverse phase-High performance liquid chromatography(RP-HPLC)[3,4].The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [5,6.]

MATERIALS AND METHODS
Instrumentation
Quaternary isocratic HPLC (Younglin HPLC YL9000 series) with YL 9110 Pump and with "autochrom 3000" software and UV-Vis detector YL9120, electronic balance (Shimadzu) was used for weighing the samples, were injected on to HPLC system using Hamilton micro syringe.
Reagents and chemicals
Atorvastatin was obtained from FDC limited Goa and acetonitrile and water employed for the preparation of mobile phases were of HPLC grade (Qualigens fine chemicals, Mumbai). All the other chemicals and solvents viz. Phosphate buffer, methanol, Acetonitrile are of ambient grade.
Chromatographic conditions
The mobile phase for the proposed method, potassium di hydrogen ortho phosphate, methanol and acetonitrile (30:20:50) was filtered through a 0.45-µ m membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column inertsil C 18 column (250 × 4.6mm) at a flow rate, 1.0 ml/min. The run time was set at 10 min the column temperature was maintained at room temperature. Prior to injecting the drug solution in to the column, the column was equilibrated for atleast 1 hour with the mobile phase flowing through the system. The eluent was monitored at 240 nm. The data was stored and analyzed with the soft ware "autochrom-3000" (youngling).

Selection of mobile phase
The solution of Atorvastatin was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally Phosphate buffer, methanol and acetonitrile (30:20:50) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Atorvastatin.

Preparation of mobile phase
Mobile phase comprised of Potassium di- hydrogen ortho phosphate. (Adjusted to P^H 3.5±0.05 with Ortho phosphoric acid), methanol and acetonitrile (30:20:50), diluent (pH 7) used was water. Mobile phase was filtered through a 0.45-µ m membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.0 ml/min), which yield a column back pressure of 653-720 psi. Run time was set as 10 min; column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 240 nm and data were acquired, stored and analyzed with the software"Autochro-3000" (Young Lin).

EVALUATION OF ANALYTICAL METHODS
Linearity
Aliquots ranging from 10-100μg/ml were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Atorvastatin, the higher concentration range was used to improve signal to noise. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100μg/ml for Atorvastatin.
Precision
Five sets of aliquots with same concentration (50 μg/ml) were prepared and these solutions were analyzed to record any intra and inter day variations in the results.
Limit of Detection (LOD)
The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

LOD is calculated from the formula,

$$LOD = \frac{3.3\sigma}{S}$$

LOD = 0.086µg/ml

Where,

σ = standard deviation of the response

S = slope of the calibration curve

Limit of quantification (LOQ)

The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy.

LOQ is calculated from formula,

$$LOQ = \frac{10\sigma}{S}$$

Where,

σ = standard deviation of the response

S = slope of calibration curve

RESULTS AND DISCUSSION

The calibration graph was found to be linear in the range of 10-100 µg/mL (Table.1 & 2) (Fig.1-6) .When the Atorvastatin solution was analyzed by the proposed RP-HPLC method, in intra and inter day variation studies, Inter day and intraday precision was determined by analyzing the drug sample at three different concentration levels. The results are presented in the form of %RSD which is shown (Tables3A &3B) that the proposed HPLC method was highly precise. Limit of quantification of the method is 0.189µg/ml and limit of detection is0.086 µg/ml (Table.4). The drug content in the Capsules was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

CONCLUSION

The drug content in the Capsules was quantified using the proposed analytical method. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

ACKNOWLEDGEMENT

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TABLE NO. 1 RESOLUTION OF DRUG MATERIAL

Drug	RT (min)	Peak Area	Height	Plates	HETP
ATRVOSTATIN	8.32	3798546	43314	5689	0.0456

TABLE NO. 2 LINEARITY OF ATORVASTATIN

S.No	Concentration (µg/ml)	Retention Time (min)	Peak Area
1	10	8.322	868456
2	25	8.323	2364251
3	50	8.325	3798546
4	75	8.326	5556486
5	100	8.328	7213945

TABLE NO. 3A INTRA-DAY PRECISION FOR ATORVASTATIN

Concentration (µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3765485			
50	3894562			
50	3785469	3785469.5	52007.66	0.12
50	3845621			
50	3798546			

TABLE NO. 3B INTER DAY PRECISION OF ATORVASTATIN

Concentration (µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3873448			
50	3883621			
50	3785469	3785469	56735.38	0.14
50	3923748			
50	3924568			

TABLE NO. 4 LIMIT OF DETECTION AND QUANTIFICATION (LOQ&LOD)

Limit of Detection	Limit of Quantification
LOD = 0.086µg/ml	0.189µg/ml

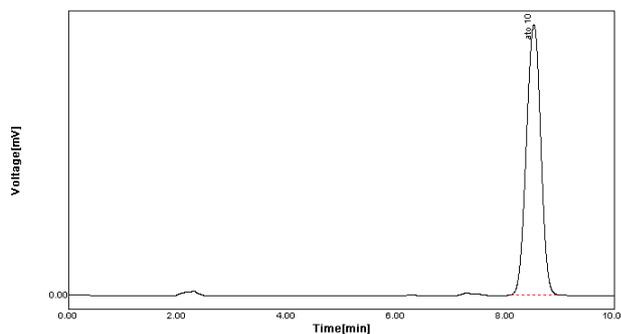**Figure 1**: atypical chromatogram for atrovastatin (10µg/ml) standard solution

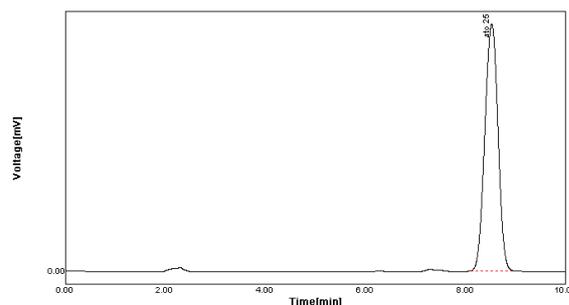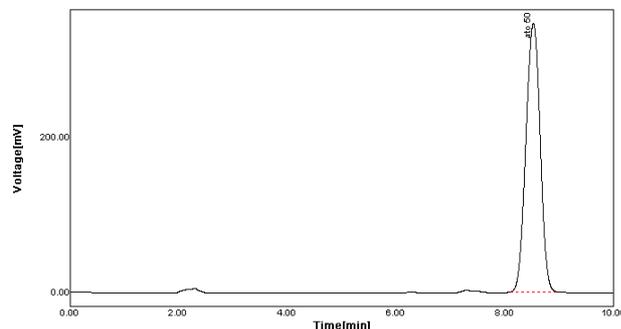**Figure 2: a typical chromatogram for atorvastatin (25µg/ml) standard solution**

Figure 3: a typical chromatogram for atorvastatin (50µg/ml) standard solution
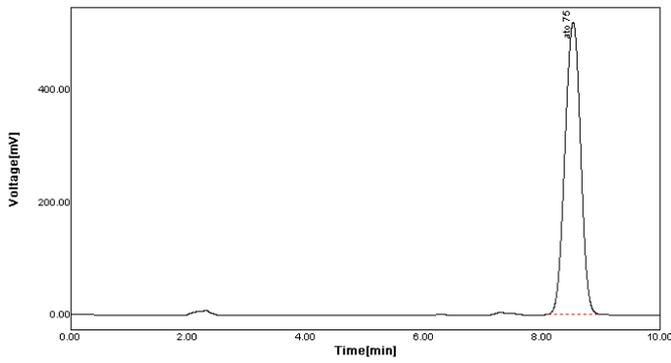
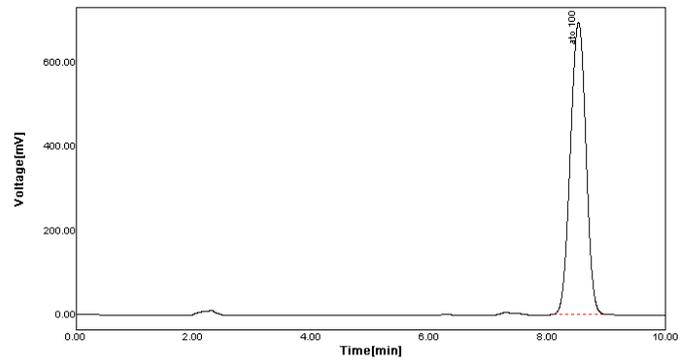**Figure 4: a typical chromatogram for atorvastatin (75µg/ml) standard solution**

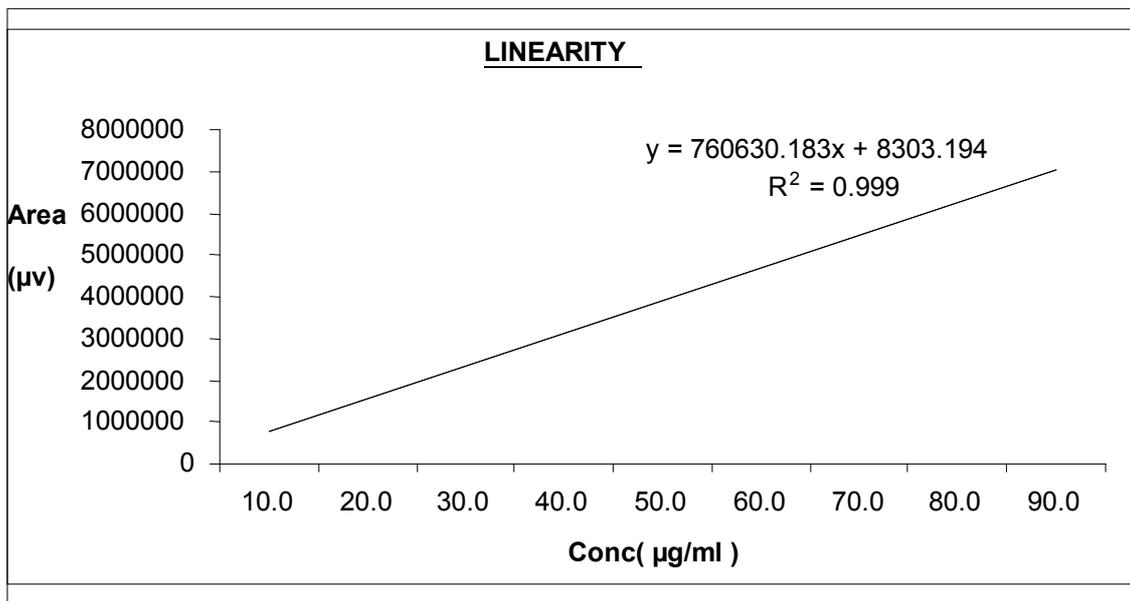**Figure 5: a typical chromatogram for atorvastatin (100µg/ml) standard solution**

LINEARITY

$y = 760630.183x + 8303.194$

$R^2 = 0.999$

Figure: 6 Calibration curve of atrovastatin

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