Research Article

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A NEW METHOD FOR ESTIMATING ATORVASTIN IN TABLET WITH ITS STABILITY STUDIES BY USING RP-HPLC

[*1]Vijya Gopal M, [1]ArabindaPatnaik, [2]Shubhrajit Mantry, [3]Sathees S M

[*1]Samskruti College of Pharmacy, Kondapur, Ghatkeswar, R.R Dist, A.P- 500084, India.

[2]Kottam Institute of Pharmacy, Mahabub nagar, A.P- 509125, India.

[3]Faculty of Pharmacy, JPNES group of institutions, Mahabub nagar, A.P- 509001, India.

Abstract

A simple, precise, accurate, economical and reproducible HPLC method for estimation of atorvastatin in tablet dosage form has been developed. Quantitative HPLC was performed with SHIMADZU LC2010c HT with class-10vp Software with UV-Visible Detector (SPD-IOA), PUMP (LC-IOAT) and (LC-IOATvp). Waters C_8, 5µm, 25cmx4.6mm i.d. column was used in the study. The mobile phase of ACN: phosphate buffer (pH 4) = 6:4, diluents of water: ACN (50: 50) were used for sample preparation in this study. The final pH of mobile phase was 4. The conditions optimized were: flow rate (1 ml/minute), wavelength (246 nm) and run time was 25 min, column temperature was maintained at 50^0C. Retention time was found to be 6.975 min. The linearity was found to be in the concentration range of 10-100 µg/ml. The developed method was evaluated in the assay of commercially available tablets ATORVA-20, 40 containing atorvastatin respectively. The amount of drug in tablet was found to be 20.36, 40.2 mg/tab for the brands. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.67 % was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug. By using the method, stability of the drug has been studied.

Keywords: HPLC, Method validation, ACN, Recovery studies, Precision, Stability studies.

Introduction

It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise[1]. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. According to the literature survey[2-5] it was found that few analytical methods such as (RP-HPLC[5], HPLC[6], UV-Visible analysis[6, 7] and LC-MS[8]) were reported for the estimation of Atorvastatin. The objective of the proposed method is to develop simple and accurate methods for the determination of Atorvastatin by RP-HPLC method in

Author for Correspondence:
Vijya Gopal M,
Samskruti College of Pharmacy,
Kondapur, Ghatkeswar, R.R Dist, A.P- 500084, India.
E-mail: vijaygopalmayuri2@gmail.com

pharmaceutical dosage forms & it's stability indicative studies. Atorvastatin is a synthetic lipid – lowering agent. It is chemically designed as [R – (R*, R*)] – 2- (4- flurophenyl) β-dihydroxy – 5 - (1-methyl ethyl) – 3-phenyl-4-[(phenylamino) carbonyl] -1H-pyrole – 1-heptanoic acid, calcium salt (2:1) trihydrate. Atorvastatin is a synthetic lipid-lowering agent. It is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase enzyme. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate limiting step in the synthesis of cholesterol. The primary site of action of HMG-CoA reductase inhibitors is the liver. Inhibition of cholesterol synthesis in the liver leads to up regulation of LDL-receptors and an increase in LDL-catabolism. There is also some reduction of LDL-production as a result of inhibition of hepatic synthesis of very low density lipoprotein (VLDL), the precursor of LDL-cholesterol.

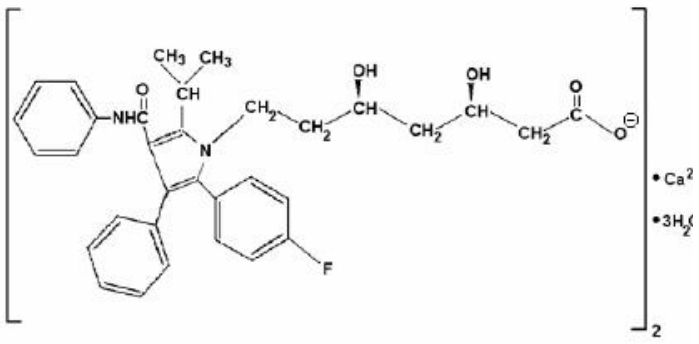

Fig. 01: Structure of atorvastatin calcium

Materials and method

Instruments and Reagents

The chromatographic separation was performed on SCHIMADZU LC2010c HT (Autosampler) with class-10vp Software with Isocratic--Gradient with UV-Visible Detector (SPD-IOA), PDA Detector (PDA-10A), PUMP (LC-IOAT). Waters C_8, 5µm, 25cmx4.6mm i.d. column was used as a stationary phase. P^H Analyzer (ELICO), Electronic Balance (AFCOSET), Ultra Sonicator (ENERTECH) has been used in the work. Atorvastatin. Active Pharmaceutical Ingredient (API) was provided by Startech labs pvt ltd, Madinaguda, Hyderabad. Acetonitrile, Methanol, & water of HPLC grade were from Standard reagents, Hyderabad. Ortho-phosphoric acid (S.D Fine chemicals, Mumbai), Commercial formulations of Atorva-20, 40 (Wexford pharmaceuticals)

Optimised Chromatographic conditions

The mobile phase of ACN: buffer= 6:4, diluents of water: ACN (50: 50) were used in this study. The conditions optimized were: flow rate (1 ml/minute), wavelength (246 nm, monitored by PDA detector) and run time was 25 min, column temperature was maintained at 50^0C. Retention time was found to be 6.975 min. Waters C_8, 5µm, 25cmx4.6mm i.d. column was used as stationary phase in the study.

Preparation of mobile phase

Acetonitrile and buffer were mixed in the ratio of 60:40 and filtered through membrane filter and degassed in a sonicator for 10 minutes. The final pH of the mobile phase was adjusted to 4.

Preparation of buffer (0.01M)

1.3609 gm of Potassium di-hydrogen phosphate in sufficient water to produce 1000ml, pH adjusted to 4.0 with orthophosphoric acid.

Preparation of Standard Drug Solutions

Standard stock solution of a concentration of 100 µg/ml of Atorvastatin was prepared by using diluents (Acetonitrile and water were mixed in the ratio of 50:50)

Preparation of Sample Solutions

Sample solution of a concentration of 100 µg/ml of Atorvastatin was prepared by using diluents. The procedure for preparing the solution is as per the guideline. The graph obtained is shown in fig. 02.

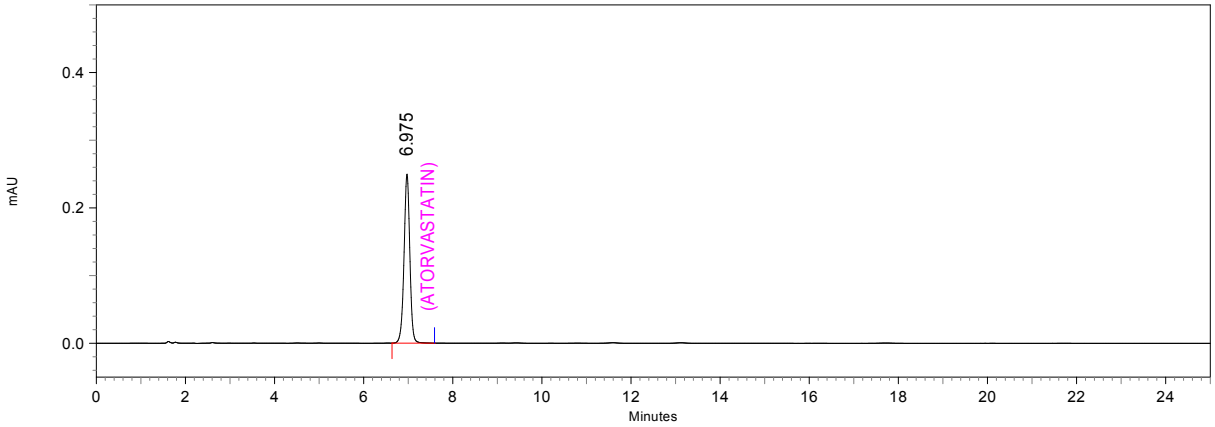

Fig. 02: Chromatogram of atorvastatin (100 ppm) in optimized conditions (RT 6.975 min)

Estimation of Atorvastatin in tablet

The performance test of the method has been conducted on market sample, manufactured by Wexford Laboratories Pvt., Ltd., Brand Name: ATORVA-20, 40 and As per the label claim, the tablets contain 20, 40 of atorvastatin. To estimate these 20 tablets were taken & an equivalent powder weight has been taken which contains 20 mg of atorvastatin (each of these has been taken in separate volumetric flask), than the powders has been dissolved in methanol & made 20ml with the diluents. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and make up with the diluents. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.42 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method. The assay results have been mentioned in table-01.

Table No. 01: Assay of atorvastatin tablets

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Atorva	20	20.363(±0.03)	101.81% (±0.4)
Atorva	40	40.8(±0.09)	102.05% (±0.01)

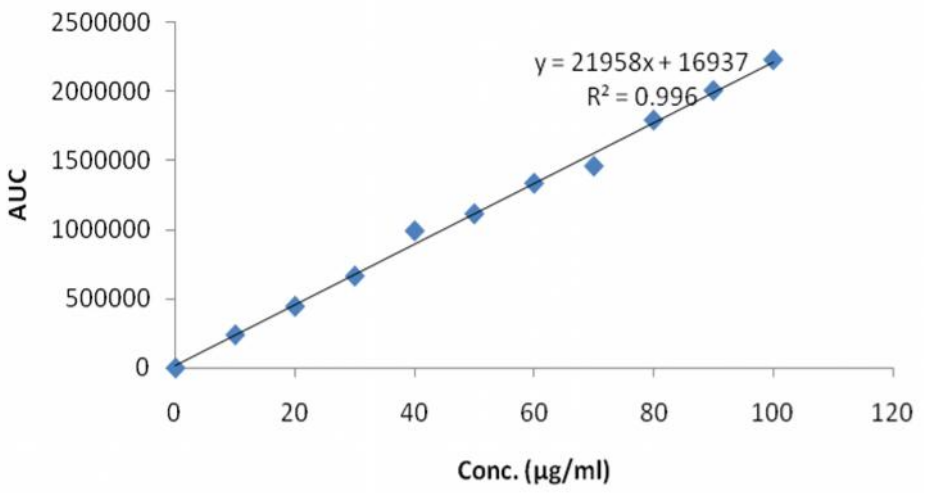

Fig. 03: Calibration curve for atorvastatin

Method Validation

As per the ICH[8] guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantisation.

Preparation of Calibration Curves

Calibration curve was prepared by taking appropriate aliquots of standard Atorvastatin stock solution in different 10 ml volumetric flask and diluted up to the mark with diluents to obtain the final concentrations of 10, 20, 30, 40, 50, 60, 70 ,80, 90, 100 µg/ml of Atorvastatin. Standard solutions (n=6) were injected, the sample volume was 20 µl with a flow rate of 1.0 ml/min., the results shown in fig. 03.

Forced degradation studies:

The protocol was strictly adhered to for forced degradation of atorvastatin Active Pharmaceutical Ingredient (API). The API (atorvastatin) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis and oxidative degradation.

Mother Sample

Before carry out the degradation studies the mother sample was prepared to find out the % degradation. It is prepared as the same method followed in sample or standard preparation.

Acid Hydrolysis

An accurately weighed 20 mg. of pure drug was transferred to a clean & dry 20 ml volumetric flask. To which 1 M HCl was added & make up to the mark & kept for 8 hrs. from that 2 ml was taken in to a 20 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis. The chromatogram obtained has been shown in fig. 04.

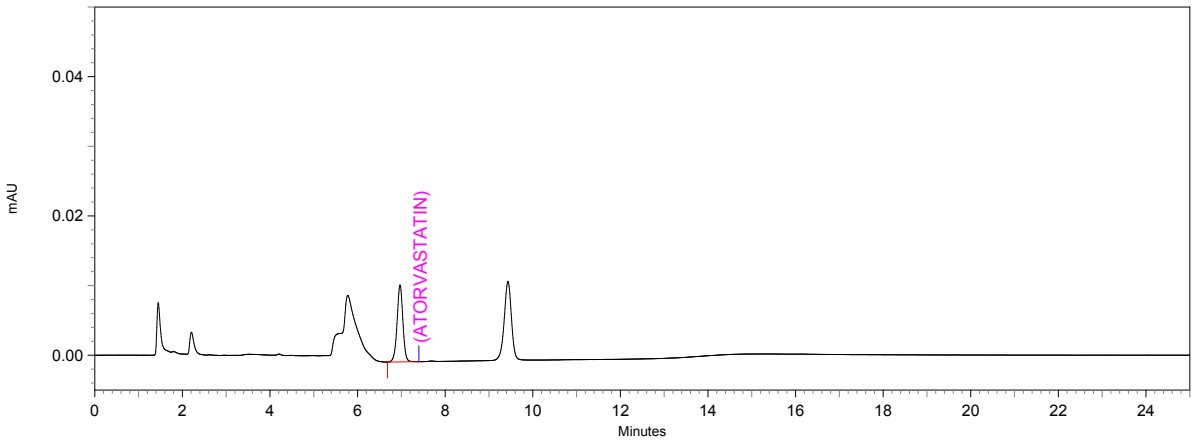

Fig. 04: Chromatogram showing degradation in 0.1 M HCl

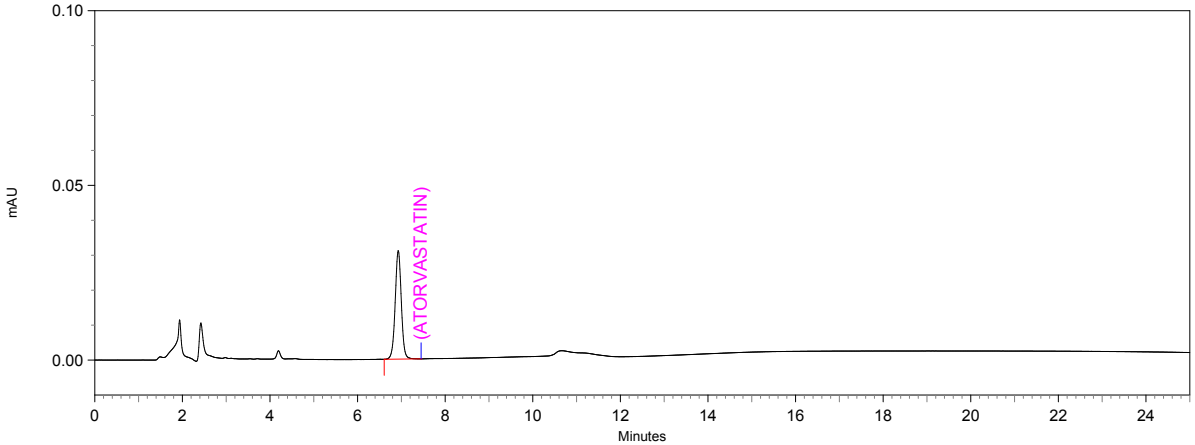

Fig. 05: Chromatogram showing degradation in 0.1 M NaOH

Basic Hydrolysis

An accurately weighed 20 mg. of pure drug was transferred to a clean & dry 20 ml volumetric flask. To which 1 M NaOH was added & make up to the mark & kept for 8 hrs. from that 2 ml was taken in to a 20 ml volumetric flask & make up to the mark with diluent, then injected for HPLC analysis. The chromatogram obtained has been shown in fig. 05.

Oxidation with (30%) H_2O_2

An accurately weighed 20 mg. of pure drug was transferred to a clean & dry 20 ml volumetric flask. To which 30% H_2O_2 was added & make up to the mark & kept for 8 hrs. from that 2 ml was taken in to a 20 ml volumetric flask & make up to the mark with diluent then injected for HPLC analysis. The chromatogram obtained has been shown in fig. 06.

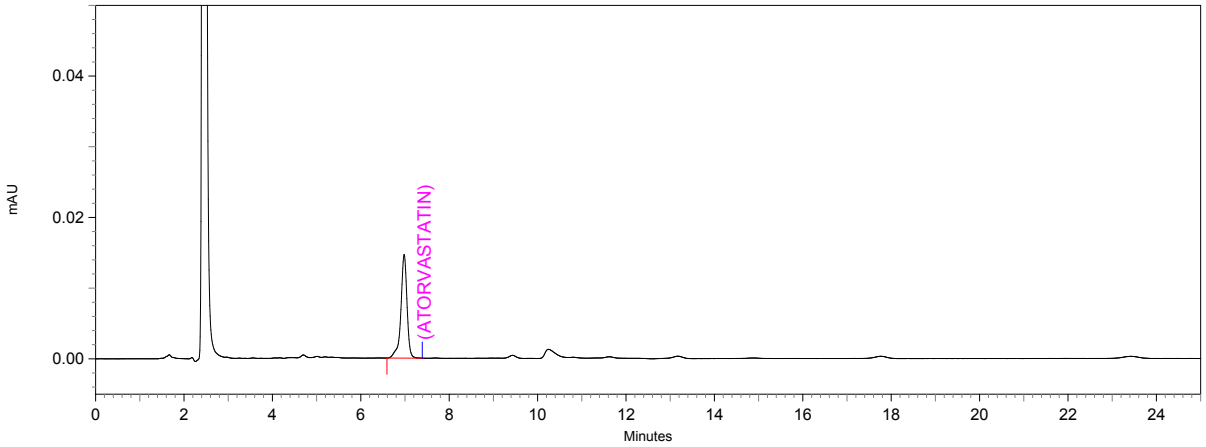

Fig. 06: Chromatogram showing degradation in 3% H_2O_2

Results & discussion

Results of degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Atorvastatin was degraded only in 3% H_2O_2 & temperature stress conditions. The result of forced degradation studies are given in the table-02.

Method Validation

Linearity and Range

The linearity of the method was determined at ten concentration levels ranging from 10-100 µg/ml for atorvastatin. The calibration curve showed good linearity in the range of 10 – 100 µg/ml, for atorvastatin (API) with correlation coefficient (r^2) of 0.995 (Fig. 03). A typical calibration curve has the regression equation of y = 21845x + 24841 for atorvastatin.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery study was carried out by the standard addition method at three levels of 80, 100 and 120%. Each solution was injected in triplicate and the percentage recovery was calculated (Table-03). Recovery was within the range of 100 ± 2% which indicates accuracy of the method.

Precision

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. atorvastatin. (API) The percent relative standard deviations were calculated for atorvastatin (Table-03). The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for atorvastatin (Table-03).

Limit of Detection and Limit of Quantification

The LOD was found to be 0.377 µg/ml and LOQ was found to be 1.143 µg/ml for atorvastatin which represents that sensitivity of the method is high.

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of atorvastatin (API).

Table No. 02: Results of Force Degradation Studies of Atorvastatin Api.

Stress condition	Time (Hrs)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	08	58.36	41.13	99.49
Basic Hydrolysis (0.I M NaOH)	08	88.32	10.42	98.72
Oxidation (3% H_2O_2)	08	97.15	02.05	99.20

Table No. 03: Summary Of Validation Parameters by RP-HPLC Method

Validation parameters		Atorvastatin
Specificity		% interference <0.5 %
Range (µg/ml)	Linear range	10-100 µg/ml
	Working range	0.03-100 µg/ml
	Target range	44,55,60.5 µg/ml
	Target concentration	55 µg/ml
Accuracy (% Recovery) 80, 100, 120		99.67, 99.19, 99.49
Precision (% RSD)	Repeatability	0.865
	Intraday(10,30,100 µg/ml)	1.05, 0.55, 0.18
	Inter day(10,30,100 µg/ml)	0.24, 0.41, 0.18
LOD (µg/ml)		0.03
LOQ (µg/ml)		0.09

Conclusion

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of atorvastatin different chromatographic conditions were applied & the results observed are presented. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here waters C_{18}, 5µm, 25cm x 4.6 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 1M NaOH, IM HCl). The drug was found to be highly soluble in methanol. Drug was sparingly soluble in acetonitrile. Using these solvents with appropriate composition newer methods can be developed and validated. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of atorvastatin in different formulations.

The precision of the method was demonstrated by intra-day and inter-day variation studies. For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and %RSD. The value of %RSD for atorvastatin was found to be 1.05, 0.55, and 0.18 for intra-day studies. The values for inter-day studies were 0.24, 0.41, and 0.18 respectively. This shows that values are not more than 2%, indicates that the developed method is precise.

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Atorvastatin in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

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