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RESEARCH ARTICLE

STABILITY-INDICATING RP-HPLC METHOD FOR ESTIMATION OF ATORVASTATIN CALCIUM IN SOLID DOSAGE FORM

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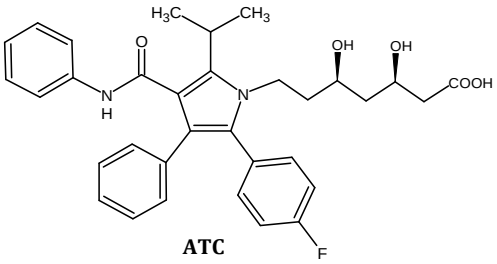
In present investigation, a stability indicating RP-HPLC method for estimation of Atorvastatin calcium in solid dosages form is developed and validated. The chromatographic separation was achieved on Phenomenax Luna C_{18} (50 × 4.6 mm,5 μm) column using a mobile phase consisting of methanol:acetonitrile:water in the ratio of 70:20:10 % *v/v*, at a flow rate of 1.0 ml/min and UV detection at 256 nm. The linearity of the proposed method for Atorvastatin Calcium was 2-10 μg/ml (r^2= 0.999) and retention time for Atorvastatin calcium was found to be 1.9223. The method was validated for accuracy, repeatability, reproducibility, robustness and system suitability. LOD and LOQ of Atorvastatin calcium were found to be 1.218 μg/ml and 4.060 μg/ml respectively. The stability studies of Atorvastatin calcium were conducted and the degradation characteristics were found to be much more prominent in alkaline hydrolysis (alkaline stress condition).

Key words: Atorvastatin calcium, RP-HPLC, Stability-indicating assay, Validation.

INTRODUCTION

Atorvastatin calcium (ATC) (**Figure 1**) is calcium salt of (*βR*, 8*R*)-2-(4-fluorophenyl)- *α,δ*-dihydroxy-5 - (1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl] - 1*H*-pyrrole-1-heptanoic acid trihydrate. ATC is a HMG CoA reductase inhibitor, a member of the drug class known as statins which is commonly used for lowering blood cholesterol (IP, 2007; Jadhav *et al* 2010).

Atherosclerotic Vascular disease is a condition in which there is an artery wall thickness as a result of accumulation of fatty materials such as cholesterol. It affects mostly arterial blood vessels, inflammatory response in walls of arteries commonly referred to as hardening of arteries. It is caused by formation of multiple plaques with in arteries. Drugs like Atorvastatin calcium has a highly beneficial effect on all lipid parameters and is more effective in reduction of cholesterol level (EP, 2005; Jat *et al* 2012).



ATC

Fig. 1. Chemical structure of Atorvastatin calcium (ATC)

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy. Instability of pharmaceuticals can cause a change in physical, chemical, pharmacological

and toxicological properties of the active pharmaceutical ingredients (API), thereby affecting its safety and efficacy. Hence, the pharmacists should take cognizance of various factors such as drug stability, possible degradation products, mechanisms and routes of degradation and potential interactions with excipients utilized in the formulation to ensure the delivery of their therapeutic values to patients.

In order to assess the stability of a drug product, one needs an appropriate analytical methodology, so called the stability indicating methods which allow accurate and precise quantitation of the drug, its degradation products and interaction products, if any (Janardhanan *et al* 2011).

In continuation of efforts made by researchers for development of RP-HPLC methods for determination of drugs (Prasanthi *et al* 2011; Bhimavarapu *et al* 2011; Basaveswara Rao *et al* 2012a; 2012b; Chhabra and Banerjee, 2013), the present work was aimed at the development of a simple, rapid, accurate, specific and economic RP-HPLC stability indicating method for the estimation of Atorvastatin calcium in bulk and tablet dosages form. The method was further validated as per ICH guidelines for the parameter like precision, accuracy, sensitivity, and linearity.

MATERIAL AND METHODS

Samples

Atorvastatin calcium was provided by Rightaid Laboratories, Hyderabad, India. The pharmaceutical formulation Mactor™F (Label claim: Atorvastatin calcium - 10 mg) used in this study is procured from local market of Bareilly, Uttar Pradesh.

Reagents

Methanol, water and acetonitrile used were of HPLC grade. All other reagents used for the forced degradation studies were of analytical grade. Sodium hydroxide was procured from Qualigens fine chemicals, Mumbai.

Instruments

HPLC system SHIMADZU-LC 20AD, Injector (Helminton syringe, 20 μl), Sonicator, pH meter, Vaccum filter pump, mobile phase reservoir, water bath, sample filtration assembly and glasswares were used throughout the experiment. Phenomenex Luna C_{18} (250 × 4.6 mm, 5 μm) column was used as a stationary phase.

Preparation of standard stock solution

Accurately weighed quantity of about 50 mg of Atorvastatin calcium was taken in 50 ml volumetric flask and dissolved in sufficient quantity of methanol, sonicated for 10 min and diluted to 50 ml with the same solvent to get the concentration of 1000 μg/ml. The stock solution was filtered through 0.45 μm membrane filters. From this, 5 ml solution was pipetted out in 50 ml volumetric flask and volume was made up with methanol (100 μg/ml).

Preparation of mobile phase

Mobile phase was prepared by mixing of methanol, acetonitrile and water in the ratio of 70:20:10. The mobile phase is then, sonicated using ultrasonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of tablet for assay

Twenty Atorvastatin calcium (10 mg Atorvastatin) tablets were weighed and powdered. A portion equivalent to 10 mg was weighed into 100 ml clean and dry volumetric flask followed by addition of about 70 ml of methanol, sonicated for 20 min and volume was made upto the mark with methanol, mixed well and filtered through 0.45 μm membrane filters. First few ml filtrate was discarded and then 5 ml of filtrate was pipette out and diluted to 50 ml with methanol.

Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120% of the test concentration as per ICH guidelines). A known amount of drug was added to preanalyzed capsule powder and percentage recoveries were calculated. The results of recovery studies were satisfactory.

Method validation

The analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness and recovery (ICH Q2(R1), 1996).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was constructed in range of 5-25 μg/ml for ATC.

Accuracy
Accuracy was studied by adding two different amounts (corresponding to 80%, 100% and 120% of the test preparation concentrations) of ATR to the placebo preparation and comparing the actual and measured concentrations.

Precision
The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the RSD %. The intermediate (interday) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions.

LOD and LOQ
The LOD and LOQ of ATC are calculated by mathematical equation:

LOD = 3.3 × standard deviation ÷ slope

LOQ = 10 × standard deviation ÷ slope

The LOD of Atorvastatin calcium was found to be 1.281 μg/ml and the LOQ of Atorvastatin calcium was found to be 4.060 μg /ml.

Robustness
Robustness of proposed method was performed by changing HPLC analyst and remaining conditions (solvent, dilution, HPLC) were same.

Forced degradation
Forced degradation studies were performed on Atorvastatin calcium to prove the stability indicating property of the method. The stress conditions employed for degradation study includes light exposure, acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH), and thermal degradation. The duration of time selected for degradation studies was 6 h. The photolytic degradation was performed by exposing the solid drugs to sunlight for 12 h. The concentration of 100 μg/ml of each of Atorvastatin calcium was prepared using respective solvents (NaOH, HCl, methanol) separately (ICH Q1A(R2), 2003).

Acid hydrolysis
Solutions for acid degradation studies were prepared in methanol (2 μg/ml) and 10 ml of 0.1 M hydrochloric acid solution was added and kept at room temperature (22 °C).

Base hydrolysis
Solutions for base degradation studies are prepared in methanol (2 μg/ml) and 100 ml of 0.1 M sodium hydroxide was added in both dilutions and kept at room temperature (22 °C) and the resultant solutions were analyzed.

Photostability studies
Fifty mg of drug was weighed and kept in the sun light for 12 h. After that, the solutions for photostability studies were prepared in methanol. The dilutions (2μg/ml) were prepared and analyzed.

Thermal degradation
Fifty mg of drug was weighed and kept in the oven and temperature was maintained at 80°C for 3 h. After this, the solutions for thermal studies were prepared in methanol and the dilutions (2 μg/ml) were prepared and analyzed.

Statistical analysis
Means, standard deviation (SD), relative standard deviation (RSD) and linear regression analysis were calculated using Microsoft Excel 2007.

RESULTS AND DISCUSSION
HPLC method with UV detection for analysis of Atorvastatin calcium in a tablet formulation (Mactor™F) was developed and validated. The analytical conditions were selected after testing the different condition affecting HPLC analysis. The best peak shape was obtained by the use of methanol, acetonitrile and water. The optimized mobile phase enabled good resolution of Atorvastatin calcium and of compounds generated during forced degradation. Atorvastatin was eluted in 1.979 min (**Figure 2**).

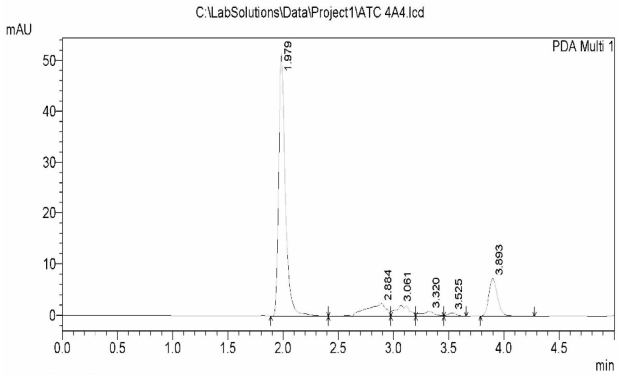


Fig. 2. Chromatogram of Atorvastatin calcium

Atorvastatin calcium showed linearity in the range of 2-10 μg/ml (**Table 1**).

Table 1. Linearity of ACE for RP-HPLC method

Conc.	Peak Area						Mean±SD
	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Dilution 5	Dilution 6	
2	120670	118598	126409	129093	123705	124564	123839.8±3801.674
4	214375	221083	220846	228831	217846	212365	219224.3±5845.319
6	310462	312069	307716	313395	327863	330349	316975.7±9617.13
8	419426	411858	413785	416047	414030	420996	416023.7±3539.481
10	517339	499216	504562	547135	521546	545728	522587.7±2018.31

The linear regression equation was y = 49715x + 21442, correlation coefficient was r^2= 0.999 for Atorvastatin calcium. (**Figure 3**) where x is the concentration in μg/ml and y is the peak area in absorbance units.

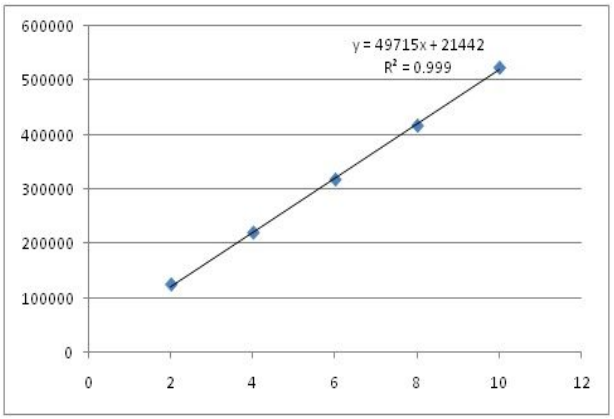


Fig. 3. Calibration graph of Atorvastatin calcium

The percentage recovery value obtained was within standard limit of 98% to 101% for the method which confirmed that the method was accurate and free from any interference of excipients (**Table 3**).

Table 3. Result of recovery study for RP-HPLC method

% added	Drug	Mean%±SD	%RSD
80%	ACE (10 mg)	100.38±0.0019	0.0012
100%	ACE (10 mg)	100.98±0.0010	0.0042
120%	ACE (10 mg)	99.96±0.0015	0.0014

The newly developed analytical method was validated according to ICH guidelines. The result was satisfactory as shown in **Table 4**. After that the forced degradation studies was performed successfully by ICH guideline Q1A(R2), result is summerized in **Table 5**. The results of the stress studies indicated the specificity of the method that has been developed. After exposure of ATC solutions to stress conditions, an assay of ATC was performed on the resultant solutions. Typical chromatograms obtained for these analyses are shown in **Figure 4-7**.

Table 4. Validation parameter for HPLC

S. No.	Parameter (units)	ATC
1.	Linearity	2-10 μg/ml
2.	Accuracy (80%)	100.38±0.0019
	Accuracy (100%)	100.98±0.0010
	Accuracy (120%)	99.96±0.0015
3.	Interday precision	
	1st day	102.54% ± 0.0044*
	2nd day	101.98% ± 0.0103*
	3rd day	102.11% ± 0.0023*
4.	Intraday precision	
	1st h	102.20% ± 0.0012*
	2nd h	101.22% ± 0.0029*
	3rd h	102.04% ± 0.0048*
5.	LOD	1.218 (μg/ml)
6.	LOQ	4.060 (μg/ml)
7.	Robustness	100.21% ± 0.0020*

Table 5. Forced degradation studies

Condition	ATC	ATC
Acid degradation	92.61	7.39
Base degradation	79.15	20.85
Thermal degradation	85.35	14.65
Photolytic degradation	56.36	43.64

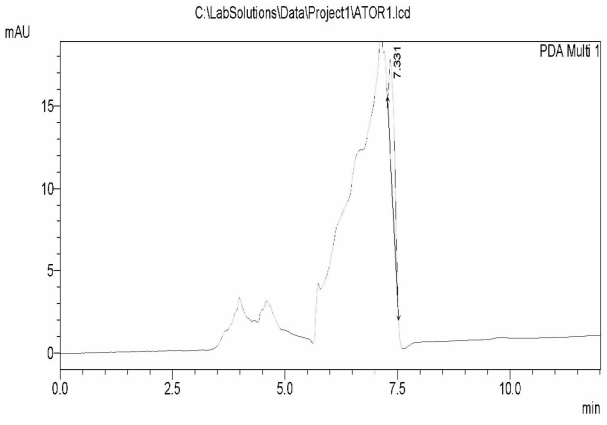


Fig. 4. Acidic degradation of atorvastatin calcium

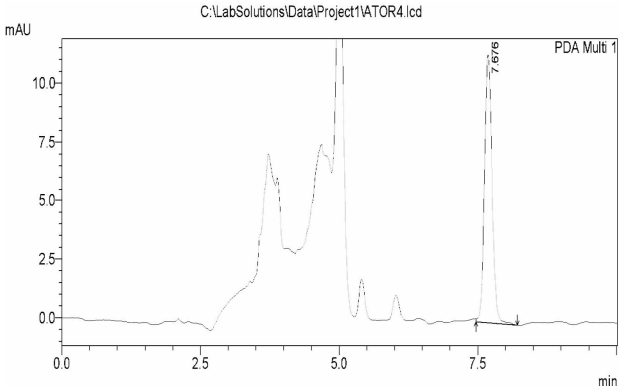


Fig. 5. Basic degradation of atorvastatin calcium

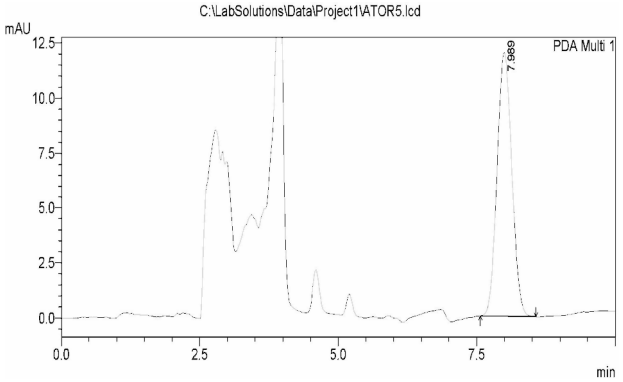


Fig. 6. Thermal degradation of atorvastatin calcium (ATC)

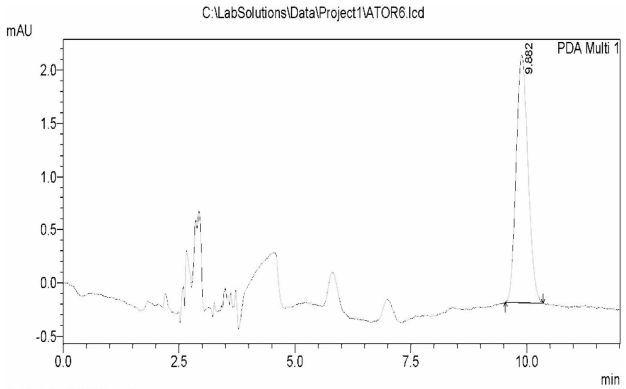


Fig. 7. Neutral degradation of atorvastatin calcium (ATC)

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Atorvastatin calcium in bulk as well as in pharmaceutical preparation. Statistical analysis of the results confirmed high accuracy and good precision.

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REFERENCES

Basaveswara Rao MV, Prasanthi V, Sivanadh M, Venkata Rao G. Newer RP-HPLC method for the determination of doxazosin in human plasma and formulation. *Bull. Pharm. Res.* 2012a;2(1):1-4.

Basaveswara Rao MV, Nagendrakumar AVD, Sivanadh M, Venkata Rao G. Validated RP-HPLC method for the estimation of telmisartan in tablet formulation. *Bull. Pharm. Res.* 2012b;2(2):50-5.

Bhimavarapu R, Chitra KP, Meda H, Kanikanti D, Anne M, Gowthami N. Forced degradation study of paracetamol in tablet formulation using RP-HPLC. *Bull. Pharm. Res.* 2011;1(3):13-7.

Chhabra GS, Banerjee SK. Stability indicating assay method development and validation of dronedarone hydrochloride in its bulk form by RP-HPLC. *Bull. Pharm. Res.* 2013;3(2):58-65.

European Pharmacopoeia (EP), 5th Edition, European Pharmacopoeia Commission, Strasbourg, France: 2005; pp. 1581-2.

ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and methodology Q2(R1), Geneva, Nov 6, 1996.

ICH Harmonised Tripartite Guideline. Stability testing of new drug substances and products Q1A(R2), Geneva, Feb 6, 2003.

Indian Pharmacopoeia (IP), Indian Pharmacopoeia Commission, Ghaziabad, Ministry of Health & Family Welfare, Govt. of India: 2007; pp. 2131-3.

Jadhav SD, Bhatia MS, Thamake SL, Pishawikar SA. Spectrophotometric methods for estimation of Atorvastatin calcium from tablet dosages forms. *Int. J. PharmTech. Res.* 2010;2(3):1948-53.

Janardhanan VS, Manavalan R, Valliappan K. Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms. *Int. J. Drug Dev. Res.* 2011;3(4):323-35.

Jat RK, Sharma S, Chhipa RC, Singh R, Alam I. Quantitative estimation of fenofibrate in bulk drug and tablets by UV-Vis spectroscopy. *J. Drug Deliv. Therap.* 2012;2(3): 129-31.

Prasanthi V, Mary K, Narasimha Raju CH, Basaveswara Rao MV. Development and validation of new RP-HPLC method for determination of acetyl sulfisoxazole in bulk and pharmaceutical dosage forms. *Bull. Pharm. Res.* 2011; 1(1):47-53.

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