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DETERMINATION OF ATORVASTATINE IN PHARMACEUTICAL FORMULATIONS BY REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract
A simple, sensitive and reproducible reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantitative estimation of Atorvastatin calcium (ATOR-C) in the pharmaceutical formulations. Chromatographic separation was achieved on a 250 × 4.6 mm, 5µ, Waters symmetry column. The flow rate was 1mL/min and eluent was monitored by absorbance at 246.0 nm using a mixture of Methanol and Acetonitrile (pH 3.0±0.01) in the ratio of 25:75 (v/v). The retention times of ATOR-C was found to be 5.5 min. Calibration plots were linear in the concentration range of 5-25 µg/mL for ATOR-C calcium. The total run time was 12 min. The proposed method was validated by testing its linearity, recovery, specificity, system suitability, precision (Interday, intraday, analyst and instrument precision), robustness and LOD/LOQ values and it was successfully employed for the determination of ATOR-C in pharmaceutical tablet formulations.
***Keywords*:** HPLC, Acetonitrile, Atorvastatine Calcium, Validation

1. Introduction:

Atorvastatine Calcium (ATOR-C) (Figure 1) is the calcium salt (2:1) trihydrate of [R-(R*,R*)]-2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methylethyl)-3-phenyl4[(phenylamino) carbon yl] lH pyrrole-heptanoic acid. It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme-A-(HMGCoA) reductase. ATOR-C is the most efficacious of the currently available HMG-CoA reductase inhibitors in terms of lowering plasma cholesterol levels by suppressing the hepatic production of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol[1].

A novel formulation ATOR-Ccommercially available, benefit for antihyperlipidemic action. This provides powerful efficacy for day long control of BP and has proven evidence in cardiovascular (CV) outcomes of ATOR-C[2].

Literature review revealed that there are various methods for determination of ATOR-Ccalcium, individually and in combination with other drugs. A variety of analytical methods are reported such as, estimation of enantiomeric of ATOR-C[3], in human serum[4] and its impurity in bulk drugs[5]. The majority of methods reported are liquid chromatography in which ATOR-Cwas estimated simultaneously with ezetimibe[1, 6, 7], fenofibrate[8], aspirin[9, 10], ramipril[11, 12], nicotinic acid[13] and amLodipine [14, 15, 16, 17, 18]. Some stability indicating RP-HPLC methods of ATOR-C and Amlodipine[19] was also reported. Some triple combination of ATOR-C was reported with aspirin and pioglitazone[20].

The present manuscript describes a simple, rapid, precise and accurate isocratic Reversed-phase HPLC method for determination of ATOR-C in the tablet dosage forms[2].

2. Experimental:

a. Chemicals: ATOR-C (101.5%) was obtained from Cipla Pharmaceutical Ltd, Mumbai, India, as gift samples. Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Potassium dihydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) were purchased from E. Merck (India) Ltd. The 0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. Mili-Q water was used throughout the experiment. Tablets were purchased from Indian market containing of ATOR-C10.0 mg per tablet.

b. Instruments: Analysis was performed on a chromatographic system Agilent 1200 series separation module (Japan) equipped with an auto injector (G1329A), Diode array detector SL (G1315C), Quaternary pump (G1311A) and column thermostat (G1316A). Data acquisition was made with Chemstation software. The peak purity was evaluated with DAD detector.

c. Liquid chromatographic conditions: Chromatographic conditions were obtained using

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a stainless steel column (Waters symmetry C_{18} 250mm x 4.6mm 5µm), which was maintained at 40^0C. The analytical wavelength was set at 246 nm and samples of 20µl were injected to HPLC system. The mobile phase was Potassium dihydrogen phosphate (10mM, pH 3.0 adjusted with ortho-phosphoric acid) and acetonitrile in ratio of 60:40 (v/v) at a flow rate of 1.0 mL/min. The mobile phase was filtered through 0.45µm filter and degassed for 10 minutes by sonication.

d. Standard solutions:

Stock standard solutions: An accurately weighed quantity of 10.0 mg of ATOR-C was transferred into a 100.0 mL volumetric flask. Dissolved with 25.0 mL of methanol and diluted to required volume with mobile phase, having the concentration of 100 µg/mL of ATOR-C.

Preparation of working standard: From the standard stock solution 10.0 mL is pipette out into 100.0 mL volumetric flask and made up the volume with mobile phase, having the concentration of 10.0 µg/mL of ATOR-C.

Preparation of laboratory mixture: Accurately weighed quantities of ATOR (≈10 mg) was transferred into a 100.0 mL volumetric flask, than dissolved with 25.0 mL of methanol and diluted to required volume with mobile phase, having the concentration of 100.0 µg/mL of ATOR. An accurately measured 1.0 mL portion of the resultant solution was diluted to 10.0 mL with diluent to obtain a laboratory mixture having concentration similar to marketed formulation.

Sample preparation: Twenty tablets (ATOR, Dr. Reddy's) were weighed and ground to a fine powder. An amount of powder equivalent to 10.0mg of ATOR-C was weighed accurately and transferred into a 100.0 mL A-grade volumetric flask containing 25.0 mL of methanol and sonicated for 30 min to effect complete dissolution of the ATOR-C and diluted upto 100.0 mL with diluent, then the solution was filtered through 0.45 µm membrane filter and 10.0 mL of filtrate taken into 100.0 mL volumetric flask. The aliquot portion of the filtrate was further diluted to get final concentration of 10.0 µg/mL of ATOR-C.

e. Linearity study and Calibration curve: To study the linearity range of component, **s**erial dilutions were made to obtained working standards in the concentration range of ATOR-C (5-25 µg/mL). A graph was plotted as concentration of drugs versus peak area response and results found linear for analytes. From the standard stock solution, a mixed standard of working concentration was prepared containing ATOR-C (10 µg/mL). The system suitability test was performed from five replicate injection of mixed standard solution.

f. Analysis of Laboratory Mixture: In order to established suitability of the proposed method for quantative estimation of ATOR-C in the pharmaceutical formulations, the method was first tried for the estimation of the component in a standard laboratory mixture of two drugs by using equation 1 and 2.

g. Analysis of Marketed Formulation: 20.0 µl of the standard and sample was injected separately and chromatograms are generated. With peak area obtained for standard and sample, the content of ATOR-C in each tablet was calculated using the following equation:

$$\textbf{Amount of drug present in each tablet} = \frac{\text{Sample area} \times \text{Std. Conc.} \times \text{Std. Purity} \times \text{Avg. weight}}{\text{Std. area} \times \text{Sample conc.}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\textbf{Percentage label claim} = \frac{\text{Amount present}}{\text{Label claim}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

h. Recovery study: Recovery studies were performed to validate the accuracy of developed method. For recovery study different concentrations (50%, 100% and 150%) of standard drug was prepared and then its recovery was analyzed.

i. Method validation: The HPLC method was validated in terms of precision, accuracy, specificity and linearity according to ICH guidelines[22].

Accuracy: The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high i.e. 50%, 100% and 150%) of the authentic standards were added to the placebo. The mixtures were extracted as described in section 2d, and were analyzed using the developed HPLC method.

Precision: Precision was determined using nine-independent test solutions (3 concentration/3 replicates). To study precision 80%, 100% and 120% concentration was prepared and three replicate of each concentration was injected. The intermediate precision of the assay method was also evaluated using different analyst different days.

Specificity: Accurately weighed quantities of the tablets powder equivalent to about 10 mg of ATOR was taken in a dry 50.0 mL volumetric flask. Each sample solution was stored under following different relevant small stress

conditions (light, heat, acid/base hydrolysis and oxidation) for sufficient time (24 hrs) to achieve 10 to 30% degradation of the initial sample.

1. Addition of small amount of alkali solution (0.1 N NaOH).
2. Addition of small amount of acid solution (0.1 N HCl).
3. Addition of small amount of oxidative agent (3% H_2O_2).
4. Sample solution was heated 50 ^{0}C on water bath for a sufficient time
5. Sample solution was exposed 600 foot-candle of UV light for a sufficient time.

After 24 hr each treated sample was analyzed and percent labeled claims were calculated by the method using formula under estimation of ATOR by proposed method.

Linearity: Solutions for linearity study were prepared as described in Section 2e. Six replicates of each concentration were injected and results are examined and it was found that calibration curve was linear in the concentration range of 5-25 µg/mL for TELM with correlation coefficient (R^2) 0.999.

LOD and LOQ: The LOD and LOQ for analytes were estimated by SD of injecting a series of dilute solutions of known concentrations.

Ruggedness: Ruggedness was ascertained by getting the sample analyzed from different analysts and carrying out analysis on different days by proposed method.

Robustness: To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The ratio of mobile phase was varied.

3. Results and Discussion:

Optimization of the chromatographic conditions: In order to develop RP-HPLC method for antihyperlipidemic drug ATOR-Cin formulation. The chromatographic conditions were optimized for better resolution by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that Phosphate buffer (10mM Phosphate buffer pH at 3.0) gave better peak shapes than their acetate and citrate counterparts. With methanol as solvent both the peaks showed less theoretical plates and bad peak shapes, on changing to acetonitrile the peak shape improved along with theoretical plates. Further optimization experiments were carried out 30% and 40% of acetonitrile in mobile phase. The best peak shape and maximum separation was achieved with mobile phase composition consisting acetate buffer-acetonitrile (60:40 v/v). The best separation, peak symmetry and reproducibility were obtained on Waters symmetry C_{18}, 250 mm x 4.6 mm, 5 µm column compared to Hypersil ODS C_{18}, 250 mm x 4.6 mm, 5 µm. The optimum wavelength for detecting the analytes was ascertained and found to be 246.0 nm.

The specificity of the HPLC method is illustrated in Figure 2 and Figure 3, where complete separation of ATOR-C was noticed in presence of tablet excipients and its impurities produced by alkali and thermal degradation. There were no interfering peaks of endogenous compounds observed at the retention time of the analytes.

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage recovery obtained for ATOR-C was 100.23.

Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing 80%, 100% and 120% analyses of the working solution.

The intra-day, inter-day, analyst and instruments variability or precision data are summarized in Table 3. The R.S.D of the assay results, expressed as percentage of the label claim, was used to evaluate the method precision. The inter-day precision was also determined by assaying the tablets in triplicate per day. The results indicated the good precision of the developed method.

The developed method was applied to the analysis of ATOR-C in tablet dosage from marketed as ATOCOR (Label claim 10 mg strength, Dr. Reddy's). The results of analysis are given in Table 5 and Figure 4. The contents of marketed tablet dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of ATOR-C in tablet dosage form.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of pH of the mobile phase, flow rate, percentage of acetonitrile in the mobile phase. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of

assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

Conclusion:

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of ATOR-C in tablet formulation. The method is very rapid and specific as both peaks are well separated from its excipients peaks and with the total runtime of 12 min, makes the developed method it's suitable for routine quality control of the selected drugs.

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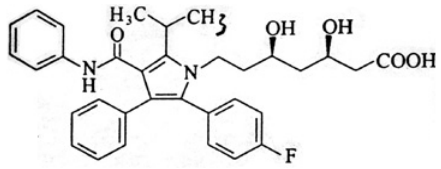

Figure 1 Structure of ATOR-C

Figure 2 Alkali degradation test solution for specificity

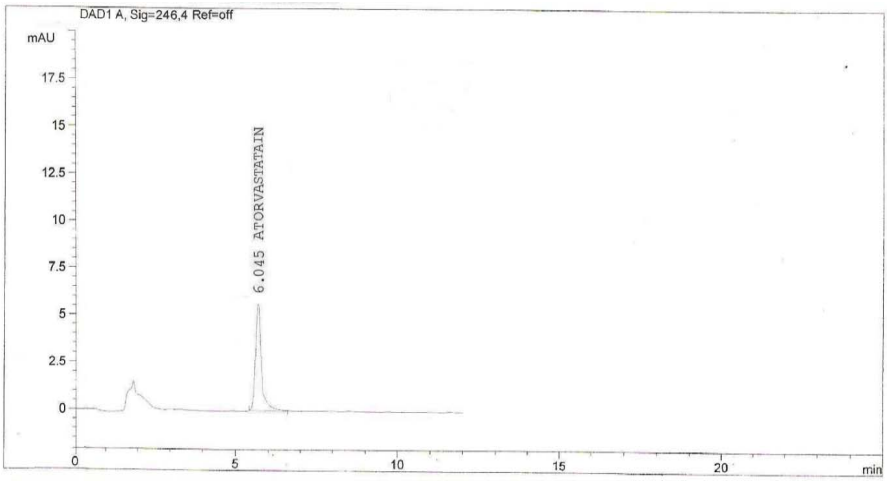

Figure 3 Thermal degradation test solution for specificity

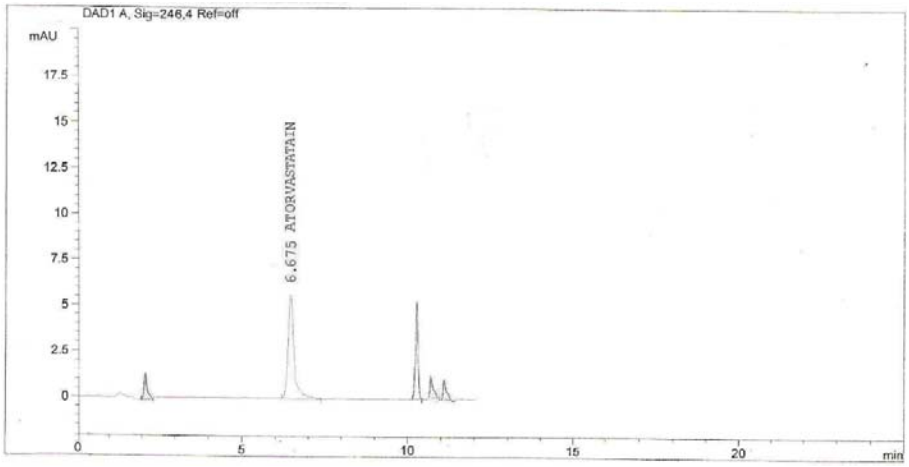

Figure 4 Test solution for assay

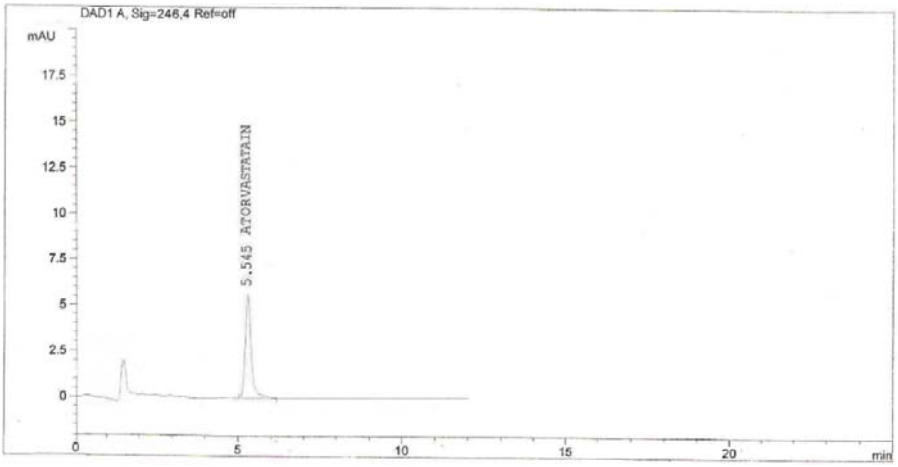

Table 1 Results of recovery analysis of ATOR-C

Compound	Wt. Spiked (%)	Wt. recovered (%)	Recovery (%)	RSD (%) n=3
Atorvastatin	50	50.60	101.2	0.008
	100	99.733	99.73	0.005
	150	149.663	99.77	0.004

Table 2 System suitability Parameter of ATOR-C

Parameters	ATOR-C
Theoretical plates	7425
Peak Height	5.57
Peak Symmetry	0.935
USP tailing	1.026
Width at half height	0.640

Table 3 Results of precision of ATOR-C

Compound	Precision	Mean	RSD (%)
Atorvastatin	Intra day	99.62	0.042
	Inter day	99.64	0.014
	Analyst	99.64	0.014
	Instrument	99.65	0.007

Table 4 Results of robustness study of ATOR-C

Factor	Level	Mean % assay (n=3)	RSD (%)
pH of mobile phase	3	99.6	0.209
	3.2	99.1	0.308
Flow rate (mL/min)	1	99.5	0.058
	1.3	99.2	0.153
% of Acetonitrile	30	99.2	0.209
	40	100.8	0.210

Table 5 Quantitative analysis of marketed formulation of ATOR-C

Tablet Sample	Label Claim (mg)	Amount present (mg/tablet)	%Label Claim	%Deviation
Ator	10	10.15	100.99	+0.99