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Estimation of Atorvastatin by High Performance Liquid Chromatography in Pure and Pharmaceutical Dosage Forms

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A reverse phase high performance liquid chromatographic method has been developed for the estimation of atorvastatin in pure form and in pharmaceutical formulations. The quantification was carried out using a RPC-18 column in isocratic mode, with mobile phase consisting of acetonitrile, water and triethyl amine in the ratio of 800 : 200 : 0.2 (v/v). Beclomethasone dipropionate was used as an internal standard. The detection was carried out at 248 nm and the linearity was found to be in the range of 3–15 μg/mL. The proposed method was found to be simple, precise, accurate, less time consuming and reproducible for the estimation of atorvastatin in pharmaceutical dosage forms, *i.e.*, tablets.

Key Words: HPLC, Atorvastatin, Pharmaceutical dosage forms.

INTRODUCTION

Atorvastatin calcium (ATS) is a synthetic lipid-lowering agent. Chemically ATS is [R-R*, R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2 : 1) trihydrate. The empirical formula of ATS is $(C_{33}H_{34}FN_2O_5)_2Ca{\cdot}3H_2O$ and its molecular weight is 1209.42. It is indicated during primary hypercholesterolaemia, heterozygous familial hypercholesterolaemia and homozygous familial hyperlesterolaemia. Literature survey reveals that few HPLC methods[1–3] were reported. The proposed method was accurate and precise for the estimation of atorvastatin in bulk as well as in pharmaceutical formulations.

EXPERIMENTAL

An isocratic high performance liquid chromatograph (Shimadzu) with two LC-10AS pumps, variable wavelength programmable UV-Visible detector SPD-10A, Chromatopac integrator C R6 A, 20 μL Rheodyne 7125 loop injector and RP C-18 column (250 × 4.6 mm i.d; particle size 10 μm) were used.

Atorvastatin and Beclomethasone dispropionate were the gift samples from Cipla Labs. Acetonitrile and triethyl amine (Qualigens) used in the study were of AR quality. Triple distilled water was used.

Chromatographic conditions: The chromatographic column used was a 250 × 4.6 mm Techsphere ODS C-18 with 10 μm particles. Both acetonitrile and

triethyl amine were filtered through 0.45-μm membrane filter and sonicated before use. The HPLC equipment was operated at ambient temperature.The attenuation was set at 6 and the range was set at 0.002 AUFS with chart speed of 5 mm/min. The flow rate of the mobile phase was maintained at 0.5 mL /min. Detection was carried out by UV detector at 248 nm and the injection volume was 20 μL.

Preparation of internal standard solution: About 100 mg of Beclomethasone dipropionate reference standard was dissolved in 100 mL of HPLC grade methanol to get 1 mg/mL solution. It was further diluted with mobile phase to prepare an internal standard of 100 μg/mL. The solution was sonicated for 30 min.

Procedure: About 100 mg of pure sample of ATS was weighed accurately and dissolved in 100 mL of HPLC grade water to get 1 mg/mL solution. It was further diluted to prepare a standard solution of 100 μg/mL. The solution was sonicated for 30 min. Subsequent dilutions of this solution were made after addition of beclomethasone dipropionate (100 μg/mL) as an internal standard (IS) to get concentrations of 3–15 μg/mL of ATS and 10 μg/mL of IS in each dilution. the solutions prepared as above were filtered through 0.45-μm membrane filter and then 20 μL of filtrate was injected five times into the column at a flow rate of 0.5 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of ATS in tablet dosage forms.

Estimation of ATS in tablet dosage forms: About 20 tablets were pulverized and the powder equivalent to 100 mg of ATS was weighed and dissolved in 100 mL of HPLC grade water. The insoluble portion was filtered through a 0.45 μm membrane filter. The filtrate was further diluted to prepare a solution of 100 μg/mL and sonicated for about 30 min. From the filtrate, different aliquots (3–15 μg/mL) were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that the concentration of the internal standard in each was 10 μg/mL. The contents of the flask were made up to volume with the mobile phase and mixed well. Each of these solutions (20 μL) was then injective five times into the column. The mean peak area ratios of the drug to the internal standard of five such determinations were calculated and the drug content in the tablets was quantified using the standard graph of the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, rapid, accurate and precise HPLC method for the analysis of ATS in pharmaceutical dosage forms. The retention times for ATS and internal standard (Beclomethasone dipropionate) were 5.77 and 7.12 min, respectively. Each of the samples was injected five times and the same retention times were observed in all cases. The ratio of the peak area of the ATS to peak area of internal standard for different concentrations set up as above were calculated and the average values for five such determinations are shown in

Table-1. A typical chromatogram is shown in Fig. 1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (0.3973).

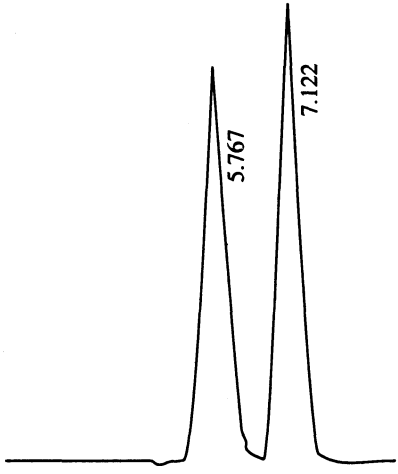

Fig. 1. Model chromatograph of ATS

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Drug concentration (μg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%) (CV)
3.0	0.312	0.39
6.0	0.631	0.43
9.0	0.930	0.37
12.0	1.260	0.38
15.0	1.572	0.44

A good linear relationship (r = 0.9999) was observed between the concentration of the ATS and the respective ratio of peak areas. The calibration equation was found to be Y = 0.00514X + 0.00231 (where Y is the ratio of peak area of drug to that of internal standard, X = concentration of ATS). The intra-day and inter-day variations of the method were determined using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table-2). This shows that the proposed HPLC method is highly precise.

TABLE-2
PRECISION OF THE PROPOSED METHOD

Concentration of ATS (μg/mL)	Observed concentration of ATS (μg/mL)			
	Intra-day		Intra-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
6.0	6.02	0.38	6.04	0.37
9.0	8.99	0.45	9.01	0.42
12.0	12.04	0.43	12.05	0.44

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values are shown in Table-3. About 99.9% of ATS could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

TABLE-3
RESULTS OF RECOVERY STUDY

Amount of drug added (μg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
3.0	3.02	100.6	2.99	99.6
9.0	9.01	100.1	9.02	100.22
15.0	14.98	99.86	15.01	100.06

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of ATS in two different brands of tablets dosage forms is shown in Table-4. The absence of additional peaks in the chromatogram indicates the non-interference of the common excipients used in the tablets. The tablets were found to contain 99.9 to 100.3% of the drug.

TABLE-4
ASSAY OF ATORVASTATIN IN TABLET DOSAGE FORMS

Brand name of the tablet	Labelled amount of drug (mg)	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) amount (mg) found by the reference method[3] (n = 5)
Tablet I	10	9.99 ± 0.04	10.01 ± 0.01
Tablet II	10	10.01 ± 0.01	9.98 ± 0.01

It can be concluded that the proposed HPLC method is simple, sensitive, rapid and reproducible for the analysis of ATS in pharmaceutical dosage forms.

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