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Validation of HPLC Method for Determination of Atorvastatin in Tablets and Identify Diketone Impurity by LC-Mass

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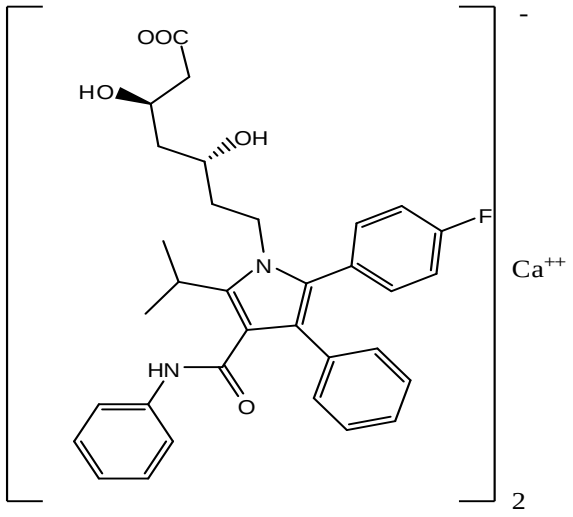
Abstract: A rapid high performance liquid chromatographic method was developed and validated for determination of atorvastatin in pharmaceutical dosage forms and for evaluation of its stability in the solid phase. The identify the diketone impurity by the LC-MS. Separation of atorvastatin was successfully achieved on a C-18 column utilizing the mobile phase A contains a mixture of buffer, acetonitrile and tetrahydrofuran in the ratio of 70:25:5 v/v/v and mobile phase B contains a mixture of buffer, acetonitrile and tetrahydrofuran in the ratio of 25:70:5 v/v/v, 1.54 gm of ammonium acetate in 1000 mL of water and adjust pH to 4.0±0.05 with glacial acetic acid, this solution used as a buffer. The detection wavelength was 248 nm. The method was validated and the response was found to be linear in the drug concentration range of 0.04 mg/mL 0.4 mg/mL. The mean values ± RSD of the slope and the correlation coefficient were 8.192 ± 0.260 and 0.999, respectively. The RSD values for intra- and interday precision were < 1.00% and 0.90%, respectively.

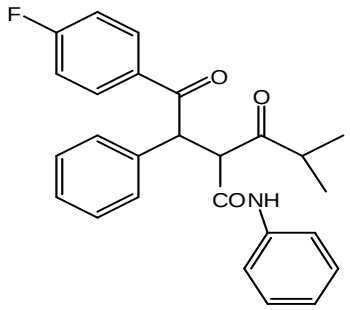
Introduction

Atorvastatin (ATO) is chemically (βR, δR)-2-(4- fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3- phenyl-4-[(phenylamino)carbonyl]-lH-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate and diketo impurity (Figure 1). Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl- coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. Atorvastatin is administered as the calcium salt of the active hydroxyl acid and is used between 10 and 80 mg per day to reduce the raised lipid levels in patients with primary hyperlipidemia (familial and non familial) or combined hyperlipidemia [1-4]. HPLC has been the analytical method of choice for the kinetical study of ATO. Several procedures of chromatographic techniques such as LC/MS/MS, microbore LC/ESI-MS/MS, HPLC with electrospray tandem mass spectrometry and LC methods with UV detector have been tested for the determination of ATO in biological fluids [5-10] and pharmaceutical dosage forms [11, 12]. However, any generally recommended or rapid analytical method for the determination of ATO and simultaneously, for evaluation of its stability in solid state has not yet been described in any pharmacopoeia and literature.

In the present study, a new rapid, validated for determination of atorvastatin in pharmaceutical dosage forms and for evaluation of its stability in the solid phase. The identify the diketone impurity by the LC-MS.



Atorvastatin



Diketo impurity

Fig. 1: Chemical structures of a) Atorvastatin b) Diketo impurity

Experimental

Chemicals and reagents

Atorvastatin (amorphous form) was obtained from Zydus Cadila, tablets of atorvastatin (10 mg of atorvastatin per tablet) were obtained from Parke- Davis, oxazepam and HPLC grade methanol, acetonitrile were purchased from Aldrich.

Instrumentation

The chromatographic system consisted of a pump, Hypersil BDS, C18, 250X4.6mm, 5µm column, UV at 248 nm detectors and analysis was performed on a chromatographic system waters, alliance 2695 HPLC system having 2487 UV detector with empower chromatography software.

Analytical Procedure

Stock and standard calibration solutions Stock solution of ATO (1.2 mg/mL) was prepared in methanol. This solution could be stored at 268 K for over 1 month with no evidence of decomposition. Standards solutions of ATO were prepared with methanol in the range of 0.04 mg/mL 0.4 mg/mL, maintaining the concentration of IS at a constant level of 0.01 mg/mL. Hundred microliters of each solution was injected into the column and chromatograms were recorded. The calibration curve for the HPLC analysis was constructed by plotting the ratio of peak normalization of ATO to IS against concentration. Internal standard (IS; oxazepam) solution 10.0 mg of oxazepam in methanol diluted to 100.0 mL with the same solvent.

Analysis of tablets

Ten tablets were weighed to get the average weight and then powdered. The fine powder, equivalent to 10 mg of ATO, was weighed and transferred into a 25 mL calibrated flask and dissolved using methanol. This mixture was sonicated (15 min) and then filtered through a 0.45 mm membrane filter. After filtration, the appropriate volume (1.0 mL) was taken into a 10 mL flask added to 1.0 mL of IS (oxazepam). All determinations were conducted in triplicate. The amount of ATO was calculated from the related linear regression equations. Conditions of kinetic studies Kinetic studies of conditions were in compliance with recommendations of the International Commission of Harmonisation [13, 14]. Samples of ATO (10.00 mg) were accurately weighed into 5 mL vials. The vials with ATO were placed in desiccators containing aqueous saturated solutions of sodium chloride (relative humidity RH = 76.4%) and inserted in heat chamber set at 363 K. After definite time intervals, determined by rate of degradation, the respective vials were taken out of the chamber, cooled to room temperature, and the contents dissolved in methanol. The so obtained solution was quantitatively transferred into

a measuring flask and made up to total volume of 25.0 mL with methanol. To 1.0 mL of the solution, 1.0 mL of solution of IS was added. The chromatograms were interpreted using the following dependence: PATO/PIS = f (t); where PATO is the area of ATO signal and PIS represents the values of IS (oxazepam).

Results And Discussion

Validation of the method

Validation of HPLC method was in compliance with recommendations of the International Commission of Harmonisation [15-17].

Selectivity

This method was selective for the ATO, as well as for the internal standard (Oxazepam), in the presence of degradation product. The selectivity of HPLC method is illustrated in Figure 2a. The typical excipients included in the drug formulation do not interfere with selectivity of the method (Figure 2b). The analysis of the chromatogram of ATO, its degradation product and IS, revealed the following efficiencies of the column: for ATO N = 2600, degradation product N = 4986, and IS N = 1494 (where N represents theoretical plate number). The separation factors between ATO and oxazepam (IS) = 3.15; ATO and product of degradation = 16.9.

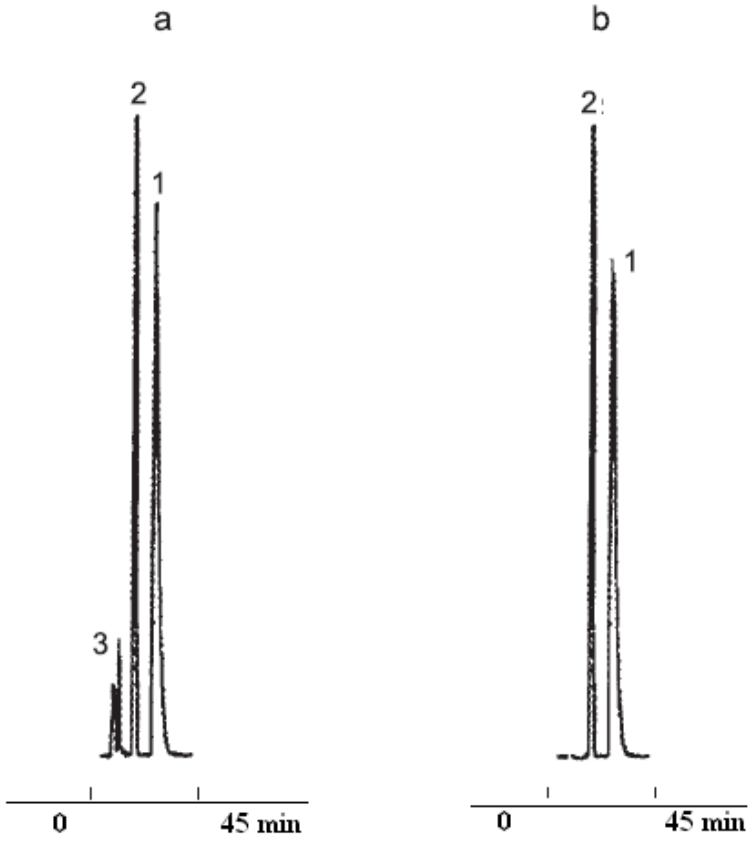


Figure 2a. HPLC chromatogram for the analysis of degradation solution of ATO (363 K, RH 76,4%). Peak 1: ATO; peak 2: internal standard (oxazepam); peak 3: product of degradation. Chromatographic conditions are described in the text.

Figure 2b. HPLC chromatogram of an extract of tablets. Peak 1: ATO; peak 2: internal standard (oxazepam). Chromatographic conditions are described in the text.

Precision and accuracy

The repeatability of the method was examined by injecting the solution consisting of ATO (0.1mg/mL, 0.2 mg/mL and 0.4 mg/mL) and IS (0.1 mg/mL) into the HPLC system for three consecutive days. The results are given in Table 1. The repeatability of the HPLC method was good and precise, and the RSD values were obtained between 0.70% and 1.00%.

Table 1. Accuracy and precision for ATO assay.

Parameter	**Intraday**			**Inter-day**
	Day 1 (n = 10)	Day 2 (n = 10)	Day 3 (n = 10)	(n = 30)
Added concentration 0.1000 mg/mL				
Measured concentration (mg/mL)	0.1009 ± 0.0007	0.1007 ± 0.0006	0.1001 ± 0.0006	0.1006 ± 0.0006
Recovery (%)	100.91 ± 0.73	100.72 ± 0.59	100.13 ± 0.58	100.59 ± 0.63
SD	0.0010	0.0008	0.0009	0.0009
RSD (%)	0.983	0.776	0.885	0.881
Added concentration 0.2000 mg/mL	0.1999 ± 0.001	0.2001 ± 0.001	0.1998 ± 0.001	0.1999 ± 0.001
Recovery (%)	99.95 ± 0.37	100.05 ± 0.54	99.91 ± 0.58	99.91 ± 0.58
SD	0.0013	0.0015	0.0016	0.0015
RSD (%)	0.668	0.731	0.812	0.737

Linearity

The linearity of the method was determined in terms of the correlation coefficient between its ATO and the ratio of peak normalization of ATO to that of IS. The internal standard was added to the solutions under investigation at a constant concentration of 0.1 mg/mL. The calibration range was between 0.04 mg/mL 0.4 mg/mL presented with the equation of

$y = ac + b = (8.192 \pm 0.260) \times c + (0.00232 \pm 0.0010)$.

The intercept b is very small, statistically non-significant and the correlation coefficient closed to r = 0.999. The values obtained showed good linearity. The intraday and inter-day accuracy of method was also examined. The evaluated data are given in Table 2.

Sensitivity

To calculate the limit of quantitation (LOQ) and limit of detection (LOD), signal to noise ratio of 10 and 3, respectively, were used. The results are shown in Table 2.

Table 2. The intraday and inter-day accuracy and linearity of proposed method.

Parameter	**Intraday**			**Inter-day (n = 10)**
	Day 1 (n = 10)	Day 2 (n = 10)	Day 3 (n = 10)	
Slope ($a \pm \Delta a$)	8.132 ± 0.392	8.222 ± 0.172	8.223 ± 0.217	8.192 ± 0.260
intercept ($b \pm \Delta b$)	0.00302± 0.0006	0.00319±0.0019	$(-0.75 \pm 0.6)10^{-3}$	0.00232 ± 0.0010
Correlation coefficient (r)	0.998	0.999	0.999	0.999
RSD of slope *a*	0.170	0.074	0.094	0.113
RSD of intercept b	0.0422	0.0185	0.0233	0.084
N	10	10	10	10
LOQ (mg/mL)	0.015	0.015	0.015	0.015
LOD (mg/mL)	0.035	0.035	0.035	0.035

Solution stability

The stability of standard and sample preparations was determined over 48 h. Standard and sample preparations were stored at ambient temperature under laboratory light conditions. Solutions were analyzed at 0, 24, and 48 h. The results were evaluated for the percent difference from time zero. Less than 1.0% difference was observed, which demonstrates that the standard and sample preparations were stable for up to 48 h, when stored at ambient temperature under laboratory light conditions.

Application to tablets

The present method was applied to the analysis of ATO in three batches of tablets. The results presented in Table 3 are in good agreement with the labeled content. All data represent the average of ten determinations.

Table 3. Results obtained in determination of ATO in tablets.

Batch	Mean (mg) ± SD
1	10.44 ± 0.081
2	10.21 ± 0.045
3	10.32 ± 0.026

LC-Mass conditions

System suitability

Accurately weigh and transfer about 100 mg of diketone standard into 100 mL volumetric flask dissolve and dilute to volume with diluents. Further dilute 5 mL to 50 mL with diluent

Sample preparation

Accurately weigh and transfer about 100 mg of sample into 100 mL volumetric flask dissolve and dilute to volume with diluent. Further dilute 5 mL to 50 mL with diluent.

Chromatographic conditions

Column : Hypersil BDS, C18, 250X4.6mm, 5 µm

Detector : UV at 248nm

The diketone impurity on shows the HPLC chromatogram in Figure 3a and 3b .

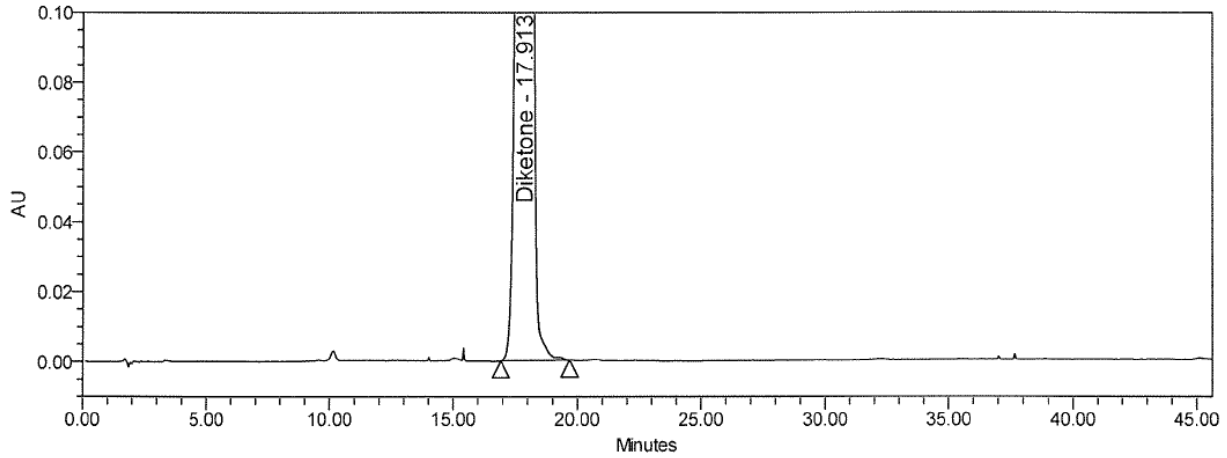


Fig 3a. HPLC chromatogram of Diketone impurity

LC-parameters

Buffer	: 0.02M ammonium acetate pH 4.0 with acetic acid
Column	: Hypersil BDS, C18, 250X4.6mm, 5 µm
Wavelength	: UV at 248nm
Injection volume	: 20 µl
MS/MS parameters	
System	: Applied biosystem
Probe	: ESI
Polarity	: positive
Scan range	: 50-1000 m/z
D.P	: 56 volts
F.P	: 260 volts
Entrance potential	: 10 volts

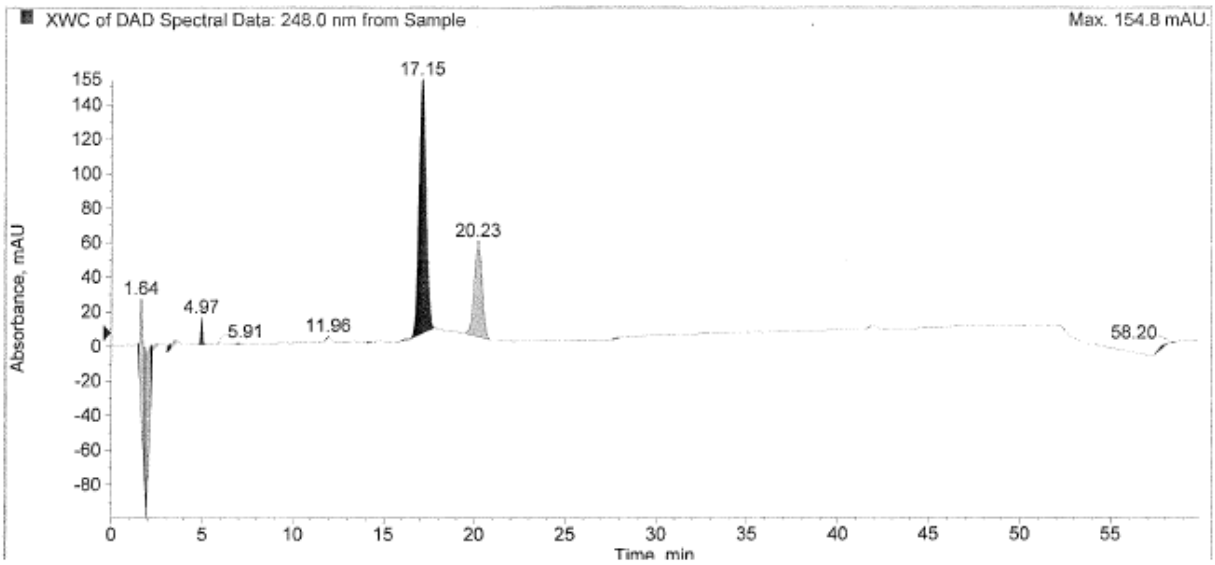


Fig 3b. DAD Spectral data

Conclusion

In summary, a new rapid, validated for determination of atorvastatin in pharmaceutical dosage forms and for evaluation of its stability in the solid phase and identify the diketone impurity by the LC-MS.

Acknowledgments

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