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ANALYSIS

VALIDATION OF HPLC METHOD FOR DETERMINATION OF ATORVASTATIN IN TABLETS AND FOR MONITORING STABILITY IN SOLID PHASE

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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. Separation of atorvastatin was successfully achieved on a C-18 column utilizing water – acetonitrile at the volumetric ratio of 48:52, adjusted to pH 2.0 with 80% *ortho*-phosphoric acid. The detection wavelength was 245 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. The degradation kinetic of atorvastatin at 363 K in a relative humidity of 76.4% was observed to be autocatalytic first order reaction. The kinetic parameters were as follows: k (where k represents the velocity constant; s^{-1}) = (1.42 ± 0.19) 10^{-6}; $t_{0.5}$ (where $t_{0.5}$ represents the time needed for a 50% decay of atorvastatin; days) = 32.82 ± 0.9; $t_{0.1}$ (where $t_{0.1}$ represents the time needed for a 10% decay of atorvastatin; days) = 13.86 ± 0.8.

Keywords: atorvastatin (ATO), oxazepam (IS), HPLC method, stability

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 (see 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 (9, 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 (analysis time < 8 min), selective, linear, precise and sensitive HPLC method with the UV detection was applied for determination of ATO in tablets and for evaluation of its stability in the solid phase.

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, Model LC-6A, Shimadzu, equipped with UV-VIS detector Model SPO-6A V, Shimadzu, integrator Model RGA Chromatopac Shimadzu. Injections were carried out using a 100 µL loop at room temperature.

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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.

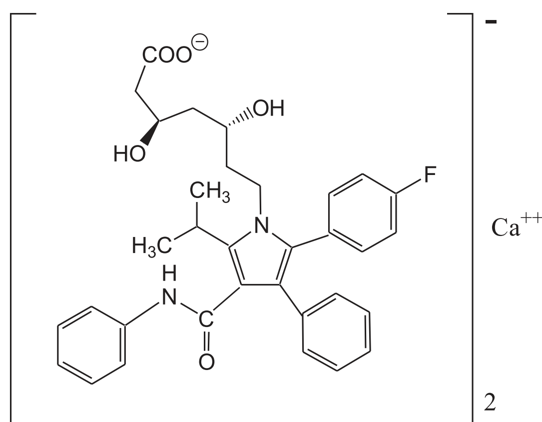


Figure 1. Structure of atorvastain (ATO).

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: $P_{ATO}/P_{IS} = f(t)$; where P_{ATO} is the area of ATO signal and P_{IS} represents the values of IS (oxazepam).

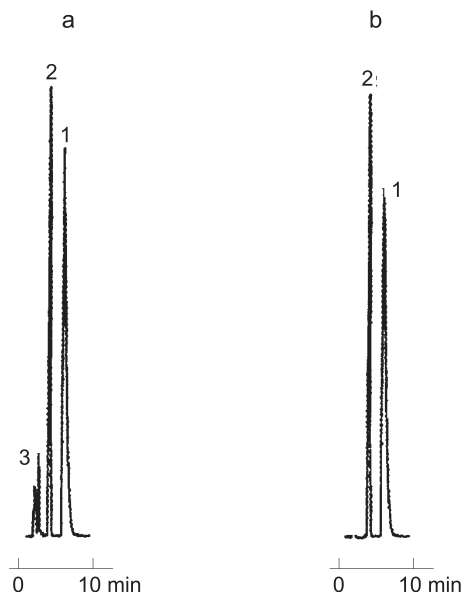


Figure 2a. HPLC chromatogram for the analysis of degradation solution of ATO (363 K, RH 76,4%). Peak 1: ATO; peak 2: internal standard (oxazepam); peak 3: product of degradation. Chromatographic conditions are described in the text.

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

RESULTS AND DISCUSSION

HPLC method development

In the initial trials the following columns were used: LiChrospher ODS, LiChrosorb ODS, Spherisorb ODS and Hypersil OS as the stationary phase and methanol – water (20:80, v/v) (mobile phase 1) and methanol – water (50:50, v/v) (mobile phase 2) as the mobile phases. Mobile phase 1 have been rejected due to a lack of ATO signal on chromatogram. When samples of ATO were analyzed using LiChrospher ODS, LiChrosorb ODS, Spherisorb ODS and Hypersil OS column and a mobile phase 2, peaks shape were not good and retention time was ~25 min, therefore organic modifier concentration was changed from 50% to 70%, but no improvement was observed. Subsequent attempts were made by lowering of pH of the mobile phase (using 80% *ortho*-phosphoric acid) and replacement of methanol by acetonitrile. In both cases, marked improvement was observed. Eventually, a mobile phase composition of water : acetonitrile, adjusted to pH 2.0 with 80% *orto*-phosphoric acid (48:52, v/v) gave the best results. During these studies injection volume was 100 µL and the mobile phase flow rate was constant at 1.5 mL/min. The analytical wavelength was 245 nm.

Several substances were tested to find a suitable IS for the analysis, and oxazepam was found to be suitable, since it has similar solubility and has retention time close to that of ATO.

Validation of the method

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

Selectivitity

This method was selective for the ATO (t_R about 6.5 min), as well as for the internal standard (oxazepam – t_R about 5.0 min), in the presence of degradation product (t_R about 1.5 min). The selectivity of HPLC method is illustrated in Figure 2a.

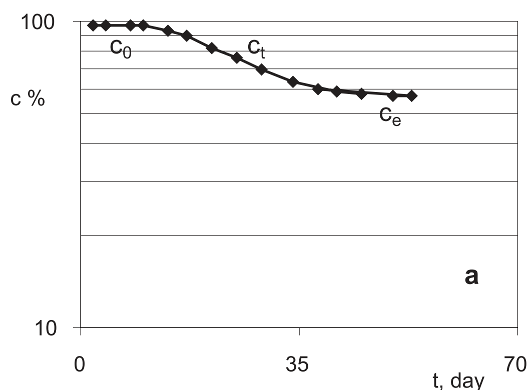
The typical excipients included in the drug formulation do not interfere with selectivity of the method (see Figure 2b). The analysis of the chromatogram of ATO, its degradation product and IS, revealed the following efficiencies of the column: for ATO N = 2600, degradation product N = 4986, and IS N = 1494 (where N represents theoretical plate number). The separation factors between ATO and oxazepam (IS) = 3.15; ATO and product of degradation = 16.9.

Precision and accuracy

The repeatability of the method was examined by injecting the solution consisting of ATO (0.1 mg/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%.

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



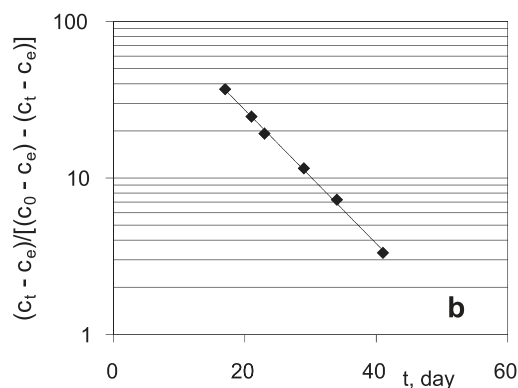


Figure 3a. Diagram presenting changes in concentration of ATO in following conditions: a humid atmosphere (RH = 76.4%) at temp. 363 K; where c_0,- initial concentration of ATO at time t_0, c_t and c_e concentration of ATO at time t and t_e, respectively.
Figure 3b. Semilogarithmic plot $(c_t – c_e)/[(c_0 – c_e) – (c_t – c_e)] = f(t)$ for degradation of ATO in solid phase (RH = 76.4%; temp. 363 K).

Table 1. Accuracy and precision for ATO assay.

Parameter	Intraday			Inter-day; all days (n = 30)
	Day 1 (n = 10)	Day 2 (n = 10)	Day 3 (n = 10)	
	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			
Measured concentration (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	99.97 ± 0.50
SD	0.0013	0.0015	0.0016	0.0015
RSD (%)	0.668	0.731	0.812	0.737
	Added concentration 0.4000 mg/mL			
Measured concentration (mg/mL)	0.4008 ± 0.002	0.4003 ± 0.002	0.3997 ± 0.002	0,4003 ± 0.002
Recovery (%)	100.22 ± 0.63	100.08 ± 0.56	99.93 ± 0.52	100.08 ± 0.57
SD	0.003	0.003	0.003	0.003
RSD (%)	0.742	0.787	0.723	0.751

Note: SD – standard deviation; RSD – relative standard deviation

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

Parameter	Intraday			Inter-day; all days (n = 30)
	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 ± Δb)	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

Note: RSD – relative standard deviation; LOD – limit of detection; LOQ – limit of quantitation

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 nonsignificant and the correlation coefficient closed to unity 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 3. Results obtained in determination of ATO in tablets (SORTIS).

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

Note: SORTIS® was labeled to contain 10 mg Atorvastatin, per one dose; n = 10; SD, standard deviation

Solution stability

The stability of standard and sample preparations were 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.

Application to stability

The proposed chromatographic method was successfully applied to the evaluation stability of ATO in the solid phase.

The analysis of the concentration change of ATO, indicates that in the atmosphere of increased humidity (RH = 76.4%) and temperature at 363 K, decomposition of ATO followed an autocatalytic reversible reaction. The relationship c (%) = f(t) (see Figure 3a) was characterized by a sigmoidal curve. The rate constant relative to substrate concentrations was calculated from the equation:

$$\ln (c_t - c_e)/[(c_0 - c_e) - (c_t - c_e)] = -k \times t + C,$$

where c_0, c_t and c_e represent the substrate concentrations at t = 0, t and t_e; , k – degradation constant of ATO; C denotes the constant related to the induction period.

Semilogarithmic plot of the equation: $(c_t - c_e)/[(c_0 - c_e) - (c_t - c_e)] = f(t)$ characterizing the acceleration period were linear, and its slope was equal to degradation constant of ATO (see Figure 3b). The degradation constant was calculated by means of the last squares method. The kinetic parameters of the degradation of ATO in pure form were as follows: $k = (1.42 \pm 0.19)\ 10^{-6}\ s^{-1}$; $t_{0.1}$ = (13.86 ± 0.8) days and $t_{0.5}$ = (32.82 ± 0.9) days.

The value $t_{0.1}$ and $t_{0.5}$ for the autocatalytic reaction were calculated from the following equations: $t_{0.1} = (b\text{-}2.197)/k$ and $t_{0.5} = b/k$; where $t_{0.1}$ and $t_{0.5}$ represent the time needed for 10% and 50% decay of ATO, respectively; b stands for the value of the ordinate for t = 0 in dependence of $\ln (c_t - c_e)/[(c_0 - c_e) - (c_t - c_e)] = f(t)$, k is the velocity constant of the reaction of degradation of ATO (s^{-1}).

CONCLUSION

An isocratic high performance liquid chromatography method has been successfully applied to a routine study of quality control of ATO in tablets and to evaluate its stability in the solid phase. Furthermore, the typical excipients included in the pharmaceutical product do not interfere with the selectivity of the method.

A water – acetonitrile mixture adjusted to pH 2.0 with 80% *ortho*-phosphoric acid (48:52), at a flow rate 1.5 mL/min was found to be an appropriate mobile phase, allowing adequate and rapid separation of ATO, its product of degradation, excipients and oxazepam as IS. Each analysis required no more than 8 min. This HPLC method is simple, accurate, precise, specific and could separate drug from degradation product and IS.

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