

Research Article

A Validated Reversed-Phase HPLC Method for the Determination of Atorvastatin Calcium in Tablets

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Abstract

A Reversed-Phase Liquid Chromatographic (RP-LC) assay method was developed for the quantitative determination of atorvastatin calcium in the presence of its degradation products. The assay involved an isocratic elution of atorvastatin calcium in a LiChroCART[®] 250*4 mm HPLC Cartridge LiChrospher[®] 100 RP-18 (5 μm) column using a mobile phase consisting of 0.1% acetic acid solution: acetonitrile (45:55, v/v), pH = 3.8. The flow rate was 0.8 mL/min and the analytes monitored at 246 nm. The assay method was found to be linear from 8.13 to 23.77 μg/mL. All the validation parameters were within the acceptance range. The developed method was successfully applied to estimate the amount of atorvastatin calcium in tablets.

Keywords: Atorvastatin calcium; RP-HPLC; Tablets assay

Introduction

Atorvastatin, 1*H*-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-, calcium salt (2:1), trihydrate [R-(R',R')]-; (Figure 1) is a statin group medicine that reduces the level of serum cholesterol; thus it is used to treat hypercholesterolemia. Independent of the cholesterol-lowering property of statins they also have anti-inflammatory and immunomodulating effects. It is rapidly absorbed from the gastro-intestinal tract. It has low absolute bioavailability of about 12% due to presystemic clearance in the gastro-intestinal mucosa and/or first pass metabolism in the liver, its primary site of action. Atorvastatin is metabolized by cytochrome P450 3A4 to a number of compounds which are also active inhibitors of HMG-CoA reductases. The mean plasma elimination half-life of inhibitory activity for HMG-CoA reductases is approximately 20 to 30 hours due to the contribution of the active metabolites. It is 98% bound to plasma proteins. Atorvastatin is excreted as metabolites, primarily in the bile [1].

Several HPLC methods were reported in the literature for the quantitative determination of atorvastatin calcium in biological samples alone [2-4], and with another active drug substance [5-9]. Most of the analytical techniques for atorvastatin calcium described in the literature are based on the liquid chromatographic determination of this drug alone in pharmaceutical formulations [10-13] with another active drug substance [14-18]. Other analytical techniques such as spectroscopy [19-23], MALDI Mass spectrometry imaging [24] electrochemical [25] and capillary electrophoresis [26] has also been described. A reversed phase LC with UV detection for the quantitation of atorvastatin calcium in bulk material is described in United States Pharmacopeia [27].

The purpose of this work was to develop a procedure for the quantitation of atorvastatin calcium and its separation, mainly, from its related substances. In addition, forced degradation studies of atorvastatin calcium were performed to provide an indication of the specificity of the method. The method was also applied to four

commercial formulations of the Argentinean market. The method was validated following the analytical performance parameters suggested by International Conference on Harmonization (ICH) [28].

Materials and Methods

Atorvastatin calcium trihydrate (98.8% calculated with reference to the dried substance) was purchased in Saporiti, Argentina. Acetonitrile and Methanol used were HPLC grade, Sintorgan (Buenos Aires, Argentina). Acetic acid was AR grade Sintorgan (Buenos Aires, Argentina). Distilled water was passed through a 0.45 μm membrane filter.

Equipment

The HPLC system consisted of a dual piston reciprocating Spectra Physics pump (Irvine, CA, United States, Model ISO Chrom. LC pump), a UV-Vis Hewlett Packard detector (Model 1050), a Hewlett Packard integrator (Loveland, CO, United States, Series 3395) and a Rheodyne injector (Model 7125).

Chromatographic conditions

The experiment was performed on a LiChroCART[®] 250*4 mm HPLC Cartridge LiChrospher[®] 100 RP-18 (5 μm) Merck (Darmstadt, Germany). The separation was carried out under isocratic elution with 0.1% acetic acid solution: acetonitrile (45:55, v/v), pH = 3.8. The flow rate was 0.8 mL/min. The wavelength was monitored at 246

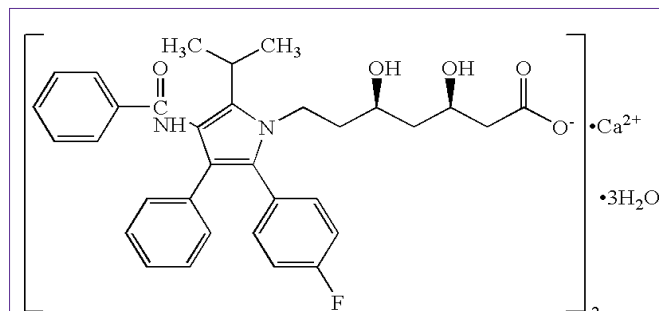


Figure 1: Atorvastatin Calcium.

nm, and the injection volume was 20 μ l. The HPLC was operated at ambient temperature. Under these conditions, the retention time (t_r) of atorvastatin calcium was approximately 6.3 min.

Preparation of standard solution

An accurately weighed quantity of 25 mg of atorvastatin calcium was placed into a 100 mL volumetric flask, dissolved in 5 mL of methanol and taken to volume with mobile phase. Then, 4 mL were withdrawn in a 100 mL volumetric flask. The volume was made with mobile phase (Conc 20 μ g/mL). The solutions were passed through a 0.45 μ m nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota, USA).

Sample preparation

Approximately 25 mg of atorvastatin calcium raw material was placed into a 100 mL volumetric flask, dissolved in 5 mL of methanol and taken to volume with mobile phase. Then, 4 mL were withdrawn in a 100 mL volumetric flask. The volume was made with mobile phase (Conc 20 μ g/mL). The solutions were passed through a 0.45 μ m nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota, USA).

Preparation of comercial formulations

Twenty tablets were weighed and finely powered and an accurately weighed powder sample equivalent to one tablet was transferred to a 50 ml volumetric flask; 10 ml of methanol was added and the flask was kept in an ultrasonic bath during 5 min. The mixture was then diluted to 50 ml with mobile phase. 1 mL was withdraw in a 10 mL volumetric flask and diluted to volume with mobile phase. The solutions were passed through a 0.45 μ m nylon membrane filter before injection (25 mm disposable filter; Cat. N° R04SP02500 Osmonics Inc., Minnesota USA).

Method validation

System suitability: The Relative Standard Deviations (RSD) values of the peak area, tailing factor, retention time, capacity and theoretical plates were the chromatographic parameters selected for the system suitability test [27].

Specificity: Forced degradation studies were performed to evaluate the specificity of the method. Degraded samples were prepared by refluxing 1.25 mg/mL atorvastatin calcium working standard with acid (1N hydrochloric acid), base (1N NaOH), water, 30% hydrogen peroxide and refluxing for 1 hour. The drug was subjected to thermal degradation in solution state in a closed container in an oven at 80°C for 2 h and to photochemical degradation, (a solution was transferred to a container and exposed to daylight for 96 h). After each degradation treatment, samples were allowed to cool at room temperature and diluted, to the same concentration as that of the standard solution, after being neutralized. Further, samples were analyzed using the methodology and the chromatographic conditions described.

Linearity: The linearity solutions were prepared at five concentrations levels from 40 % (w/v) to 125 % (w/v) of analytes concentration. Triplicate injections of 20 μ L were made and chromatograph under the conditions described above. The drug was evaluated and peak areas were recorded. A calibration curve was plotted by taking the peak area on y-axis and respective concentration

of drug on x-axis. The calibration curve was constructed and evaluated by its coefficient of determination (r^2) and by least-squares linear regression analysis.

Precision and accuracy: Six replicated of standard solution were analyzed to assess system precision. Both reproducibility and accuracy studies were evaluated by carrying out nine independent assays at concentration levels of 80, 100 and 120 % (w/v) (3 samples each) of a commercial formulation of atorvastatin calcium. The amount of atorvastatin calcium recovered was calculated.

Robustness: The robustness was performed by deliberately changing the chromatographic conditions. The relative organic portion ratio of the eluent was varied by 45 to 55 %, while pH was adjusted to 2.8 and 4.3. The RSD, retention time, tailing, and theoretical plates were evaluated.

Results and Discussion

The described reverse-phase liquid chromatography method was developed to provide a rapid quality control determination of atorvastatin calcium in tablets. Validation of the method was performed according to ICH. This method uses a simple mobile phase. All samples were analyzed using the assay chromatographic conditions described.

The analytical column was equilibrated with the eluting solvent system used. After an acceptably stable baseline was achieved, the standards and then the samples were analyzed.

The system suitability results were calculated according to the USP 35 <621> (27) from typical chromatograms. 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 six replicate analyses of the same working solution. The Relative Standard Deviation (RSD) obtained was 0.6%. Peak asymmetry or tailing factor, T, was calculated as $T = W_{0.05} / 2f$; where $W_{0.05}$ is the distance from the leading edge to the tailing edge of the peak, measured at 5% of the peak height from the baseline and f is the distance from the peak maximum to the leading edge of the peak. The tailing factor did not exceed 1.5 The RSD of peak area response and retention time showed the satisfactory repeatability of the system (< 1.5%) (Table 1).

Degradation was indicated in the stressed sample by a decrease in the expected concentration of the drug and increased levels of degradation products. Atorvastatin calcium was degraded to different products under acid, base, oxidation, hydrolysis and photolysis (Table 2). In addition, there was no interference regarding the retention time of atorvastatin calcium and its degradation products.

Table 1: System Suitability.

Parameter	Minimum value	Maximum value	Average	RSD (%)
Retention time	6.292	6.331	6.312	0.44
Area	6924058	7088160	7006109	1.66
Capacity	2.146	2.165	2.155	0.62
Asimetry factor	0.98	1.00	0.99	1.43
Theoretical plates	2534	2565	2550	0.86

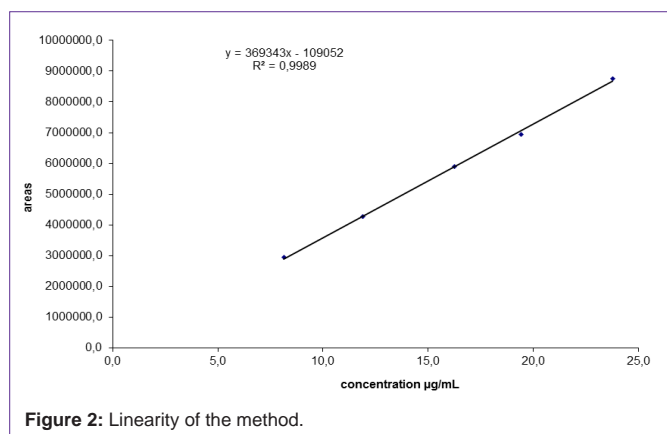


Figure 2: Linearity of the method.

Table 2: Selectivity.

Condition	Time (h)	% of Atorvastatin	RRT of degradation products
Acid (1 N HCl, reflux)	1	18.5	1.34, 1.43, 2.14
Base (1 N NaOH, reflux)	1	79.3	0.39, 0.46, 0.57
Hydrogen peroxide 100 vol (reflux)	1	7.8	0.33, 0.43, 0.72, 0.78, 1.68, 2.90
Water (reflux)	1	85.9	0.38
Heat dry, 80°C (solution)	2	97.9	Non detected
Daylight exposure	96	56.6	0.77, 1.35, 1.64

* RRT: Relative Retention Time

The linearity of the HPLC method was determined by analysis of three replicates of five concentrations of standard solutions (ranging from 8.13 and 23.77 µg/mL). The calibration curve showed good linearity over the concentration range. The correlation coefficient ("r") value was 0.9994. Typically, the regression equation for the calibration curve was found to be $y = 369342.9x - 109051.8$. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value that was not statistically ($p = 0.05$) different from zero (Figure 2 and Table 3).

The precision is usually expressed as the RSD of a series of measurements. The reproducibility and accuracy studies were evaluated by recovery studies with 9 samples of one commercial formulation studied ($n = 3$ for 80%, 100% and 120%) indicated that the mean recovery was 99.4 %, and the RSD was 0.63%.

Method accuracy was also demonstrated by plotting the amount of atorvastatin calcium found against the amount present in the sample, both expressed in mg. Linear regression analysis rendered slopes not significantly different from 1 (t test $p=0.05$), intercepts not

Table 3: Linearity.

% of nominal value	Concentration (µg/mL)	Average peak area response	RSD %
40	8.13	2938581	0.6
60	11.88	4266208	1.2
80	16.26	5889101	0.9
100	19.42	6949213	0.3
125	23.77	8759624	0.1
Slope ^a	369342.9 ± 929563,0		
Intercept ^b	-109051.8 ± 15630862,6		

^aConfidence limits of the slope ($p=0.05$)

^bConfidence limits of the intercept ($p= 0.05$)

Table 4: Precision and Accuracy.

% of nominal value	Added Amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n=3)	RSD (%)
80	20.2	20.1	99.5	99.0	0.48
	20.8	20.5	98.8		
	21.0	20.7	98.6		
100	26.2	26.1	99.7	99.4	0.80
	25.2	24.8	98.5		
	24.7	24.7	100.0		
120	31.1	31.4	100.1	99.9	0.32
	30.2	30.2	100.0		
	29.7	29.5	99.5		
Mean (n=9)				99.4	0.63

Table 5: Robustness.

Mobile Phase	RT	Tailing factor	N
0.1% Acetic Acid solution –Acetonitrile (45:55, v/v) pH: 3.8	6.30	1.11	2545
0.1% Acetic Acid solution - Acetonitrile (45:55, v/v) pH: 2.8	6.57	1.00	2767
0.1% Acetic Acid solution - Acetonitrile (45:55, v/v) pH: 4.3	6.01	1.00	2312
0.1% Acetic Acid solution - Acetonitrile (55:45, v/v)	15.24	1.33	14865
0.1% Acetic Acid solution - Acetonitrile (50:50, v/v)	9.16	1.00	5365

Table 6: Commercial formulations assay.

Brand	Average %	RSD%
A	92.1	1.11
B	105.0	0.53
C	92.9	1.54
D	98.6	0.82

significantly different from zero (t test $p=0.05$), and $r = 0.9992$. The experimental t of the recovery percentage was also studied, showing a value of 2.00, below the 2.306 established in the tabulated t (95% level of probability, 8d.f) (Table 4).

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 and percentage of acetonitrile in the mobile phase.

The effect on retention time, theoretical plates and tailing factor can be seen in Table 5. A decrease in acetonitrile proportion increases both retention time and theoretical plates. It was found that retention time of atorvastatin calcium ranges from 0.3 cm/min by pH changes.

The results of the evaluation of the four market products can be in Table 6.

Conclusion

A straightforward, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of atorvastatin calcium in tablets. The method is very simple and specific, as the peak is well separated from its impurities, which makes it especially suitable for routine quality control analysis work.

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