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Full Length Research Paper

Stability-indicating high performance liquid chromatographic determination of atorvastatin calcium in pharmaceutical dosage form

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A simple, selective, precise and stability-indicating high-performance liquid chromatographic (HPLC) method of analysis of Atorvastatin Calcium in pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed-phase C_{18} column (250 x 4.6 mm), 5 µ with a mobile phase consisting of a mixture of Methanol: Acetonitrile: Phosphate Buffer solution in the ratio (45:45:10). Flow rate was 1 mL / min. Detection was carried out at 246 nm. The retention time of Atorvastatin was 6.98 min. Atorvastatin Calcium was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 52.20 to 156.60 µg/mL. The value of correlation coefficient, slope and intercept were, 0.9999, 36.02 and 26.45, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Key words: Atorvastatin, chromatography, stability indicating, degradation.

INTRODUCTION

Atorvastatin Calcium, chemically, [R - (R*, R*) – 2 - (4 – flurophenyl) – - dihydroxy - 5 - (1- methylethyl) - 3 - phenyl - 4 - [(phenylamino) carbonyl] - 1H- pyrrole - 1- heptonicacid - calcium salt (2:1) trihydrate. (Figure 1) is used as HMG-Co-A reductase inhibitor. Literature survey reveals that UV, HPLC, and HPTLC for determination of content uniformity and simultaneous estimation of Atorvastatin is reported (Syed et al., 2007; Mishra et al., 2007; Raja et al., 2006; Khan and Jain, 2006; Sahur and Patel, 2006 Chaudhari et al., 2006; Erturk et al., 2003), but there is no stability indicating high-performance liquid chromatography (HPLC) method for the determination of Atorvastatin from its tablets, as its Pharmaceutical dosage form

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidetion is one of the required tests (ICH, 1993, 1996). The hydrolytic and the photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of Atorvastatin Calcium is HPLC.

The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Atorvastatin Calcium in the presence of its degradation products and related impurities as per ICH guideline.

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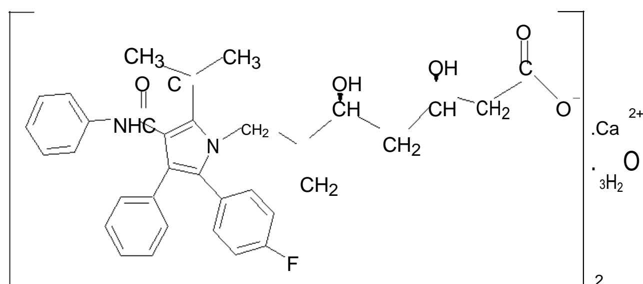

ATORVASTATIN CALCIUM

Figure 1. Chemical Structure of Atorvastatin Calcium.

Experimental

Reagents and Materials

Atorvastatin Calcium was supplied by INTAS PHARMA and tablets (Label Claim: 10 mg per tablet, Product Name: Atorec-10 and Manufacturer: Windlas Biotech limited was procured from the market. Methanol, Acetonitrile and water (HPLC grade), and Potassium dihydrogen orthophosphate AR Grade, Orthophosphoric acid LR Grade, Water HPLC Grade were used.

Instrument used

The HPLC used was a Jasco HPLC LC -2000 PLUS series with UV photodiode array detector and Borwin software, Japan was used for all the experiments unless specified otherwise. The column used was XTerra ® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA).

METHODOLOGY

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium dihydrogen orthophosphate in water. Adjust pH of solution to 6.85 ± 0.05 with orthophosphoric acid) in the ratio (45: 45:10) at a flow rate of 1 mL / min. Detection was carried out at 246 nm. A 78. 3 µg / mL Atorvastatin solution of standard and sample preparation was injected. The injection volume was 20 µL for assay and degradation level.

Standard preparation

50 mg of Atorvastatin Calcium working standard was accurately
50 mg of Atorvastatin Calcium working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase.

Sample preparation

20 tablets were weighed and finely powdered. Blend equivalent to 40 mg of Atorvastatin was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and diluted up to the mark with mobile phase. The solution was mixed well and centrifuged at 2500 RPM for 10 min.

Preparation of calibration graph

The linearity of response for Atorvastatin Calcium assay method was determined by preparing and injecting solutions with concentrations of about 52.20 to 156.60 µg/mL of Atorvastatin Calcium.

Method validation

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (78.3 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (78.3 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Atorvastatin.

Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Atorvastatin working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\% \text{ Recovery} = \frac{\% \text{ Amount recovered}}{\% \text{ Sum Level}}$$

Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ± 2.0%.

Analysis of marketed formulation

Weigh and finely powder not less than 20 tablets. Transfer blend equivalent to 40 mg of Atorvastatin to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

Forced degradation studies

Preparation of acid and based- induced degradation product

Tablet powder equivalent to 40 mg of Atorvastatin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Preparation of hydrogen peroxide - induced degradation product

Tablet powder equivalent to 40 mg of Atorvastatin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Photodegradation product

Tablet powder equivalent to 40 mg of Atorvastatin (previously kept in UV light for 24 h) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Thermal degradation product

Tablet powder equivalent to 40 mg of Atorvastatin was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room tempe-rature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Atorvastatin and its degradants in pharmaceutical dosage form is given in Table 4 The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples (Figures 3 to 7) is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

Detection of the related impurities

Weigh and finely powder not less than 20 tablets. Transfer blend equivalent to 40 mg of Atorvastatin calcium to a 50 mL volumetric flask. Add about 30 mL of mobile phase and sonicate for 20 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 rpm for 10 min. Inject the clear supernatant solution into the HPLC system.

RESULTS AND DISCUSSION

Method of development

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra ® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C_8 gave good peak shape but a lower retention with low peak purity. XTerra ® RP18 column had given a good peak shape with response at affordable retention time with peak purity of Atorvastatin on higher side. Also, two different pH were tried on XTerra ® RP18 column, these were pH 6.85 and 7.85. These pH had minimal effect on Atorvastatin retention time, tailing factor, peak purity and theoretical plates. The chroma-tographic conditions finally comprised of a mobile-phase in the ratio of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium dihydrogen orthophosphate in water. Adjust pH of solution to 6.85 ± 0.05 with ortho-phosphoric acid) in the ratio (45:45:10) at a flow rate of 1 mL / min using XTerra ® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 246 nm.

Calibration curve

These results indicate that the response is linear over the range of 41.56 to 124.67 µg/mL of Atorvastatin with coefficient of regression, R^2, value as 0.9998. The value of correlation coefficient, slope and intercept were, 0.9998, 36.02 and 26.45, respectively.

Validation of the method

Precision

The %RSD for repeatability of sample preparation is 0.95%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. Table 1 represents the precision of method.

Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Atorvastatin ranges from 100.41% to 101.52% with mean recovery of 100.75% and % relative standard deviation of 0.41%. The recovery of Atorvastatin by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0%.

Table 1. Method precision of Atorvastatin Calcium

Sample Preparation	% Assay Atorvastatin	% Deviation From Mean Assay value Atorvastatin
1	99.79	1.80
2	97.86	-0.16
3	98.04	0.02
4	97.26	-0.78
5	97.26	-0.77
6	97.92	-0.11
Mean	98.02	
± SD	0.93	
%RSD	0.95	

Table 2. Ruggedness and robustness of Atorvastatin calcium.

Parameter	Normal (Original)	Changed conditions
Column make	XTerra ® RP 18,250 x 4.6 mm, 5 μ (Waters, Ireland)	Lichrosphere CN, 250 x 4 mm, 5 μ (E. Merck, Germany)
Flow Rate	1 mL/min	1.2 mL/min
Mobile Phase Composition	(pH 6.85) Methanol: Acetonitrile: Buffer (KH_2PO_4) (45:45:10)	(pH 6.50) Methanol:Acetonitrile:Buffer (KH_2PO_4) (40:50:10)
Pump	Jasco PU-2080 plus series	Shimadzu 2010
Detector	Jasco UV-2075 plus series,	Shimadzu 2010
Analyst	Audumbar	Zahid
%Assay Atorvastatin	98.02%	98.05%
% Deviation from mean assay value obtained in method precision studies		
for Atorvastatin: 0.03%		

Table 3. Recovery of Atorvastatin calcium.

Sample Preparation	% Simulated Dosage Nominal	% Sum Level	% Amount Recovered	% Recovery
Preanalysed Sample				98.02
1	80	80.46	81.18	100.90
2	80	79.98	81.19	101.52
1	100	100.90	101.52	100.61
2	100	101.08	101.50	100.41
1	120	121.24	121.85	100.50
2	120	121.04	121.70	100.54
Mean				100.75
+ Standard Deviation				0.41
% Relative Standard Deviation				0.41

Table 2 represents the accuracy of the method.

Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst The deliberate aforementioned changes in parameters alters the result of Atorvastatin 0.03% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. Table 3 represents the ruggedness and robustness of the method.

Table 4. Stressed study data of Atorvastatin Calcium.

S. No.	Condition	% Assay	% degradation	
		Atorvastatin	Single maximum	Total
1.	No stress treatment (control sample)	98.02	Nil	Nil
2.	Acid	98.19	0.05	0.09
3.	Alkali	98.77	0.04	0.07
4.	H_2O_2	100.32	0.03	0.05
5.	UV	96.22	0.25	0.49
6.	Thermal	99.07	0.03	0.03

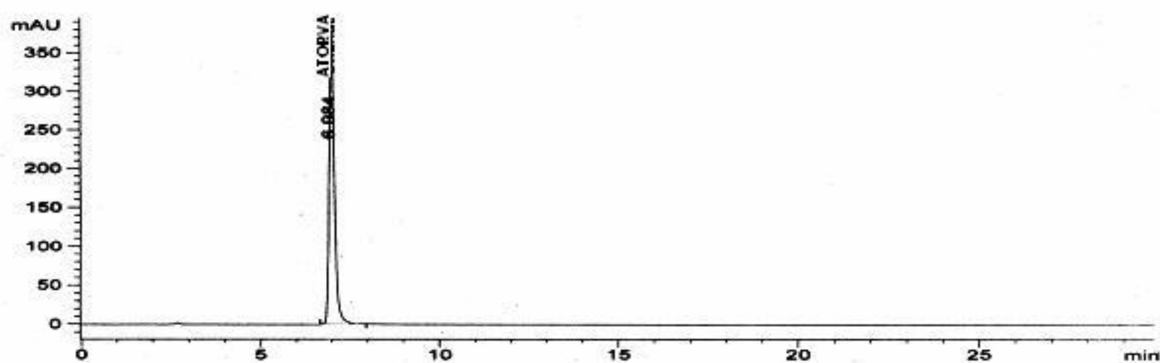

Figure 2. Typical chromatogram of Atorvastatin.

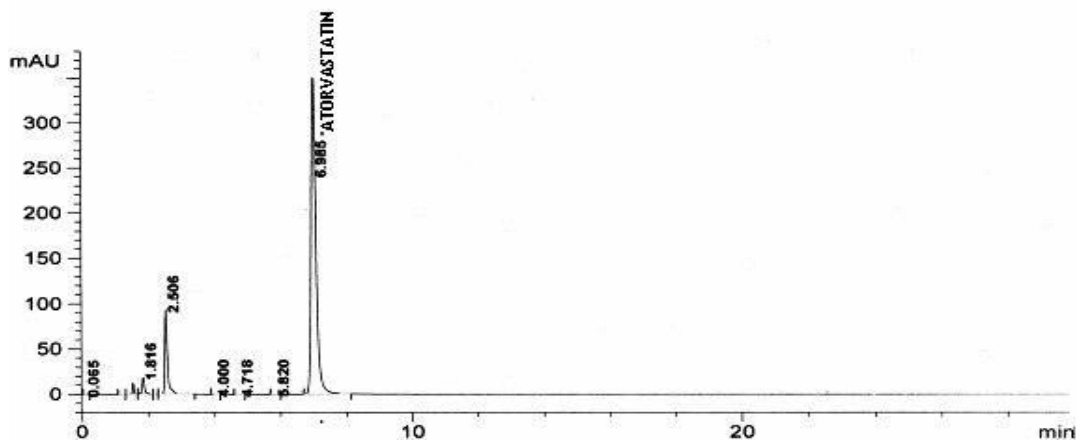

Figure 3. The chromatogram of acid degraded sample.

Analysis of the marketed formulation

The drug content was found to be 98.02% with a % RSD of 0.95%. It was noted that no degradation of Atorvastatin had occurred in the marketed formulation that were analysed by this method. The low RSD value indicated the suitability of this method for routine analysis of Atorvastatin in pharmaceutical dosage form.

Stability- indicating property

The chromatogram of no stress treatment sample (as control) showed no additional peak (Figure 2). The chromatogram of acid degraded sample showed additional peaks at retention time (RT) of 4.00, 4.72 and 5.82 min, respectively (Figure 3). The chromatogram of alkali degraded sample showed additional peaks at RT of 4.72

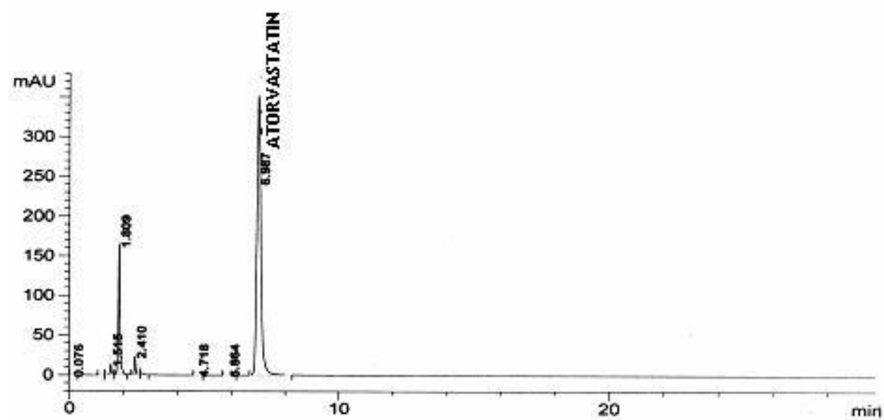

Figure 4. The chromatogram of alkali degraded sample**.**

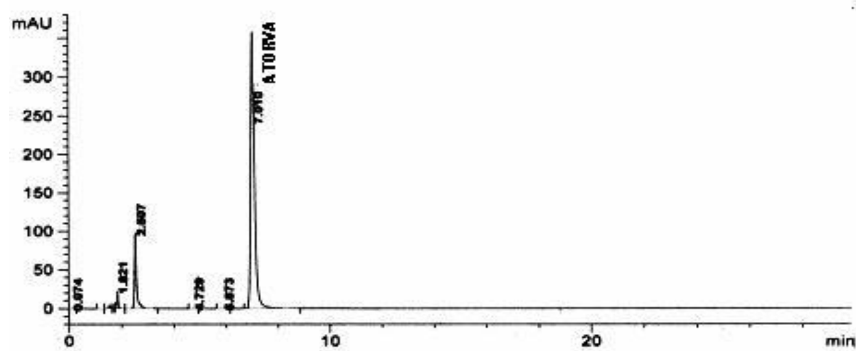

Figure 5. The chromatogram of hydrogen peroxide degraded sample.

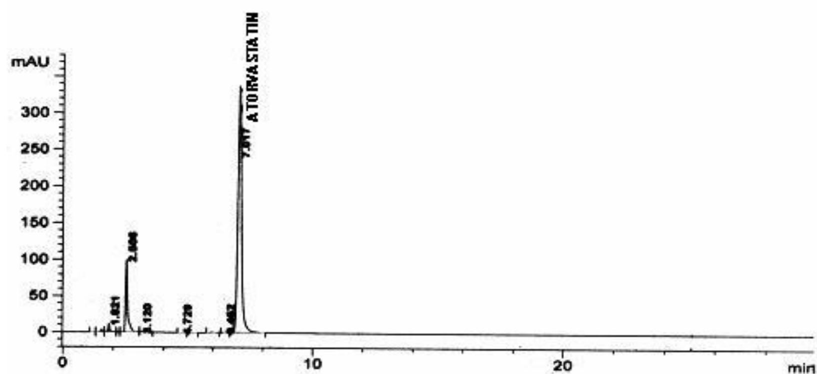

Figure 6. The chromatogram of UV degraded sample.

min and 5.86 min, respectively (Figure 4). The chromategram of hydrogen peroxide degraded sample showed additional peaks at RT of 4.73 and 5.87 min respectively (Figure 5). The chromatogram of UV degraded sample showed additional peaks at RT of 3.12, 4.74, 5.94 and 6.47 min, respectively (Figure 6). The chromatogram of thermal degraded sample showed additional peak at RT of 4.7 min (Figure 7). Rest of the peaks, if any, were

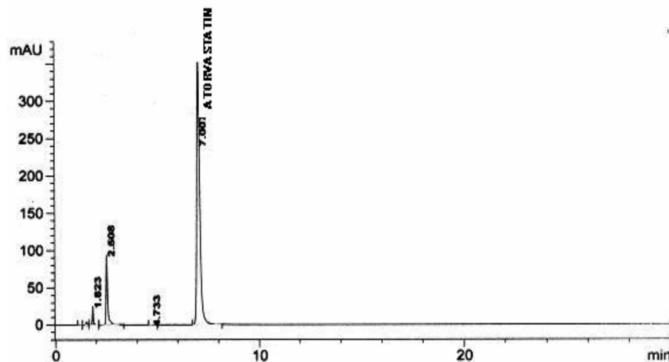

Figure 7. The chromatogram of thermal degraded sample.

from its blank or placebo in each of these specified conditions. In each forced degradation samples were additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation, hydrogen peroxide degradation, UV degradation and thermal degradation. The lower RT of the degraded component indicated that they were more polar than the analyte itself.

Detection of the related impurities

The sample solution showed no additional peak other than principal peak. Hence, related impurities are not present in the market sample.

Conclusion

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of Atorvastatin Calcium in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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