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Development and validation of stability indicating high performance liquid chromatography method for determination of Atorvastatin calcium in the presence of its degradation products

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ABSTRACT

The aim of this study was to optimize chromatographic method for the analysis of atorvastatin calcium and its degradation products using an experimental design approach. The purpose of a screening design is to identify the factors that have significant effects on the chromatographic responses and for this purpose we applies 2^3 factorial design. The present study describes simple, accurate, precise and cost effective HPLC method for estimation atorvastatin calcium, the mobile phase used consists of acetonitrile: orthophosphoric acid 0.1%(66:34 v/v), detection wavelength 246nm. A linear response was observed in the range of 6.4 µg/ml-9.6 µg/ml with œrrelation coefficient of 0.999, this method can be used as stability indicating method.

Key words: Atorvastatin Calcium HPLC method development, factorial design.

INTRODUCTION

Atorvastatin calcium is chemically [R-(R*, R*)]-2-(4-flurophenyl) - β, δ-dihydroxy-5- (1-methylethyl)-3-phenyl-4 [(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt trihydrate. Atorvastatin calcium is an inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis. [1.2]

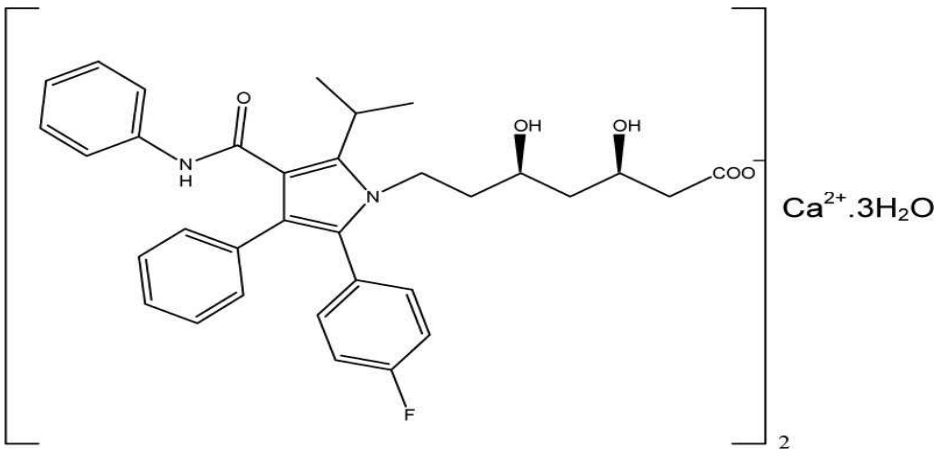

Figure 1. Chemical structure of Atorvastatin Calcium

Method validation can be define as (ICH). Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristic.

Method validation study include system suitability, linearity, precision, accuracy, specificity, robustness, limit of detection, limit of quantification and stability of sample, reagents, instruments.

The aim of this investigation was to establishing a new simple and sensitive method that could be used in analysis degradation products of Atorvastatin calcium.

MATERIALS AND METHODS

Atorvastatin calcium working standard 99.86%was provided by Cadila Healthcare Limited .Tablet of atorvastatin calcium 20mg label claim manufactured by MICRO Labs Limited 92.Sipcot INDIA .HPLC grade of Acetonitrile, orthophosphoric acid 85 % from SD fine-chem limited(India), Methanol and hydrogen peroxide 30% from Merck India limited and 0.45 µm nylon membrane filter was obtained from meds com distributor fze Span.

Chromatography:
The chromatographic system used to perform development and validation of this assay method consisted of an Sykam pump S2100 solvent delivery system, auto sampler S5200 sample injector, S3210 UV/VIS detector and UV-visible double beam spectrophotometric (UV-1800 SHIMADZU Limited Japan.

Chromatographic analysis was performed on Kromasil 100-5 C1825cmx4.6mm E82860 i.d., 5µm particle (column). Separation was achieved using mobile phase consist of 0.1% orthophosphoric acid: Acetonitrile (66:34) solution at flow rate 1.0ml/min. The eluent was monitored using UV detector at wavelength 246nm. The column was maintained at ambient temperature and injection volume of 20µl was used. the mobile phase was filtered through 045µm filter prior to use.

Preparation of mobile phase:
Mobile phase consist of 0.1% orthophosphoric acid: Acetonitrile (66:34) .The mobile phase was degassed by sonication before use.

Preparation of standard solution:
A stock standard solution containing 1.0mg/ml of atorvastatin calcium was prepared by weighing 100mg of atorvastatin and transferring to 100ml volumetric flask, the volumes were made up to the mark with methanol. To prepare the working solution(1000µg/ml for atorvastatin calcium) the stock standard was diluted with diluent.

Preparation of diluents: 90:10 of acetonitrile and water.

Specificity: [3.4]
Placebo of the tablets, equivalent to the sample weighing was taken and solution prepared similarly to the sample solution. The solution was analyzed as per the proposed method. Sample solution was also analyzed as per proposed method.

Linearity and range:
Different aliquots,0.16,0.18,0.20,0.22 and 0.24ml of stock solution were taken in a series of 25 ml volumetric flasks and diluted up to the mark with diluent to get required concentration range of 80 %to 120%. The solution were then filtrated through 0.45µm nylon filter and injected into HPLC system.

Accuracy:
0.18,0.2 and 0.22ml of the stock solution was taken in triplicate in nine 25 ml volumetric flasks. And about 0.233g of placebo in it. Then sufficient diluent was added to the flasks and sonicated for 20 min to dissolve. The volume was made up with diluent and mixed. The solution were filtrated through 0.45µm and injected into HPLC system. The chromatograms were recorded and the percentage recovery was calculated.

Precision:
For the precision study, precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) in triplicate. Repeatability refers to use of the analytical procedure over a short period of time that was evaluated by assaying the QC samples the same day. Intermediate precision was assessed by comparing the assays on different days (3 days) and % RSD calculated.

Robustness:
The robustness was measured by injecting duplicate injections of standard and three- sample solution in single at each different condition with respect to the control. The robustness as a measure of method capacity to remain unaffected by small, but deliberate change in chromatographic condition is studied by testing influence of small change in flow rate±0.1 ml/min, composition of acetonitrile in mobile phase ±1.0v/v and wavelength ±2.0nm.

Stability in analytical solution:
A sample solution 8µg/ml of drug were prepared by taken 0.18ml of standard solution in 25 ml volumetric flask and kept at room temperature. It was analyzed initially and at different time intervals day 1 and day 2.

Stress degradation study:
Stress degradation study was carried out on the Atorvastatin calcium hydrolytic and oxidation degradation.

Sample solutions of the drug was prepared in 0.1M sodium hydroxide, 0.1M hydrochloric acid and 3% hydrogen peroxide for thermal degradation. The samples were heated on boiling water bath for 45 minutes, cool to room temperature and diluted to volume with diluent. Photolytic degradation was carried by expositing the sample to UV radiation at 254nm for 16 hours all solutions were filtered through 0.45um filter and injected into the HPLC system proposed method.

Application of method to dosage form:
The development and validated HPLC method was applied for determination atorvastatin calcium from dosage form. Atorvastatin calcium tablet of 20mg strength from MICRO Labs Limited 92.Sipcot evaluated. The tablets were powdered and powder equivalent to 20mg of drug was weight. The weighted sample placed in extraction flask and methanol was added to extract the drug. The suspension was sonicated for 10 min .The supernatant was diluted suitably to obtain 10µg/ml concentration. The solution was injected into HPLC and analyzed for content.

RESULTES AND DISCUSION

In this work HPLC method for determination of Atorvastatin calcium in tablets and their degradation products was developed and validated. The optimization of the method was done by selected of suitable solvents such as methanol and acetonitrile, different columns C8, C18, detection wavelength and analyte concentration. The detection wavelength of 246nm was selected after scanning the standard solution over range 200-400nm by using UV detector.

The traditional approach to HPLC optimization is to perform an experiment by trial and error or by change one control variable at time; such method can frequently require a very large number of experiments to identify the optimal condition. Recently computer assessed to HPLC separation has addressed the problem using factorial design strategies.

In this work a three factors with two level was applied to predict the retention behavior of Atorvastatin calcium and optimize their isocratic elution using acetonitrile as organic modifier and buffer as mobile phase.

An eight- run, 2^3 factorial design of three factors at two level was set up to standardize the spectrographic condition which are likely to be employed .Percentage of acetonitrile in the organic phase (X1), proportion of orthophosphoric acid 0.1 %(X2) and flow rate (X3) as per 2^3 factorial design are represented in the table Factors and their corresponding levels as per 2^3 factorial design.

X1, X2 and X3 are independent factors whereas Y1, Y2 and Y3 are dependent factors.

Factors and their corresponding levels as per 2^3 factorial design Independent factors table 2:

Table 1: factor and their employed as per 2^3 factorial design

Factor	Level	
	-1	+1
Acetonitrile %	65	70
Buffer V/V	35	40
Flow rate ml/min	1.0	1.5

Table 2: Matrix for three factors (number of runs =8)

Experiment	Factor X1	Factor X2	Factor X3
Run1	-1	-1	-1
Run2	1	-1	-1
Run3	-1	1	-1
Run4	1	1	-1
Run5	-1	-1	1
Run6	1	-1	1
Run7	-1	1	1
Run8	1	1	1

-1 and 1 are symbols representing different levels in factorial design notation.

Table 3: Chromatographic conditions employed as per 2^3 factorial design

Exp No	X1	X2	X3	Result		
				Y1	Y2	Y3
1	65	35	1.0	5.52	1.44	7508
2	70	35	1.0	5.10	1.54	6321
3	65	40	1.0	6.28	1.27	8760
4	70	40	1.0	6.08	1.48	7367
5	65	35	1.5	3.78	1.36	4447
6	70	35	1.5	3.56	1.58	4556
7	65	40	1.5	4.35	1.46	5087
8	70	40	1.5	3.92	1.36	4808

[

Regression analysis:

Most statistical software packages used are Minitab17, SPSS 22, Prism 6.0 or even Microsoft Excel are used analyze data.

The targeted response parameters were statistically analyzed by appalling linear regression model, the significant terms ($P \leq 0.05$). The results of analysis of variance models generated by general linear model.

Table 4: Calibration curve of Atorvastatin calcium

Con(µg/ml)	Peak area at 246nm			
6.4	Inj1	Inj2	Inj3	Mean
	183.4	183.19	183.76	183.45
7.2	202.40	201.11	203.47	203.33
8.0	226.16	224.83	226.76	225.92
8.8	251.84	250.17	249.48	250.50
9.6	275.85	274.16	275.79	275.27
Atorvastatin			Slope	28.503
			Intercept	0.2739
			r	0.9998
			Std. Erro	1.603
			Intercept	
			Slope	0.2173

The obtained adjusted R^2 were within acceptable limit of $R^2 \geq 0.80$, indicating that the experimental data were a good fit to the equation [2.5].

$$Y = ß_0+\beta_1 X_1 +\beta_2X_2+\beta_3X_3+ \beta_4X_1X_2+ \beta_5X_2X_3+ \beta_6X_1X_3+ \beta_7X_1X_2X_3.$$

Where, Y is the level of the measured response, ß0 is the intercept, β_1 to β_7 are the regression coefficients, X_1, X_2 and X_3 stand for the main effects, $X_1 X_2$, $X_2 X_3$ and $X_1 X_3$ are the two- way interaction between the main effects and $X_1 X_2 X_3$ is the three-way interact between the main effect.

All the factors(X1,X2 and X3) were found to be significant(p value less than ≤ 0.05 for response of retention time (Y1).

Response models with p –value and statistical parameters, R^2 =0.977, Adjusted R^2=0.9473 and RSD=0.16221.

Buffer solution in the mobile phase so the positive sign indicate direct correlation between response and factor, while a minus shows decreasing response with increasing factor. P – Values for all factors, X1(0.05), X2(0.004) and X3(0.00).

Linearity:
The linearity study was performed and the correlation coefficient of atorvastatin calcium was found to be 0.9998.

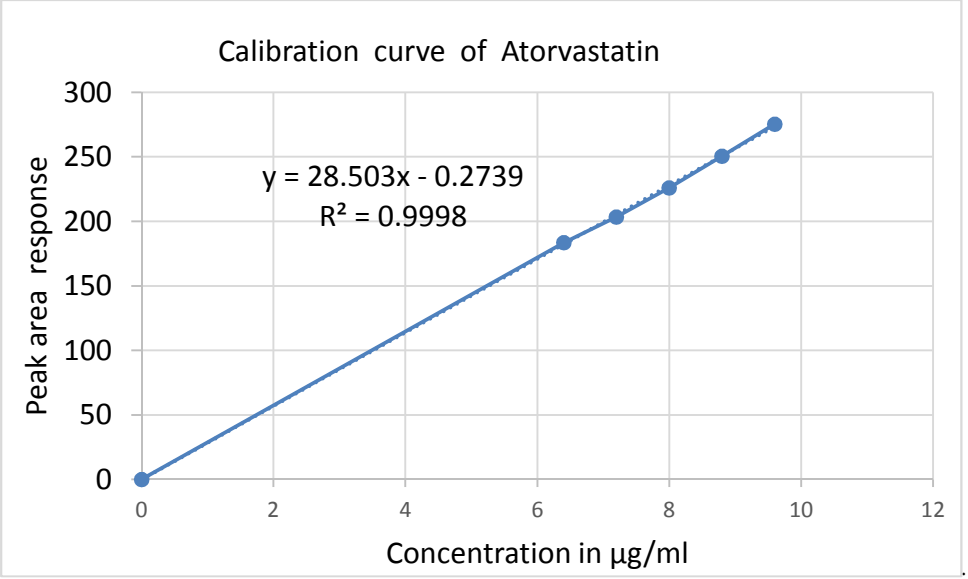

Figure 2: Linearity curve of Atorvastatin calcium

The accuracy of the method was assessed by determination of recovery for three concentration covering the range of the method. The amount of atorvastatin calcium was recovered in the presence of placebo interference, was calculated. The mean recovery of Atorvastatin was 100.49% which is satisfactory and result shown in table 2.

Table 5: Recovery for Atorvastatin calcium

Sample level	Actual amount added mg	Amount recovery	% Recovery
80	20	20.15	100.70
80	20	20.10	100.50
80	20	20.18	100.90
100	20	19.87	99.37
100	20	20.37	101.83
100	20	19.76	98.80
120	20	20.20	101.00
120	20	20.07	100.35
120	20	20.19	100.98
		Mean±SD	100.49±0.86

Under experimental conditions described, the recovery indicated that the method was suitable for its intended purpose .The specificity of the method was determined by checking for interference with the drug from placebo component and also specificity evaluated by checking the peak purity of the analyte during the stress testing study.. Indicated that no interference with the analyte peak result. Shown table 3. And figure 11 System suitability the results obtained show that the HPLC system is capable providing high recovery.

Table 6: System suitability data for Atorvastatin

No	Peak area reproducibility at 246nm	Retention time-min	Asymmetry 5 %	Theoretical plate
1	249.9690	4.983	1.25	7739
2	251.0010	4.983	1.25	6115
3	252.7550	4.983	1.25	6115
4	251.2100	4.983	1.25	6115
5	255.1990	5.00	1.25	6156
6	254.4840	5.00	1.25	7791
Mean	252.4360	4.988	1.25	6671.80
RSD	0.822%	0.175%		
Limit	NMT 2%	NMT 1%	NMT2%	NLT 2000

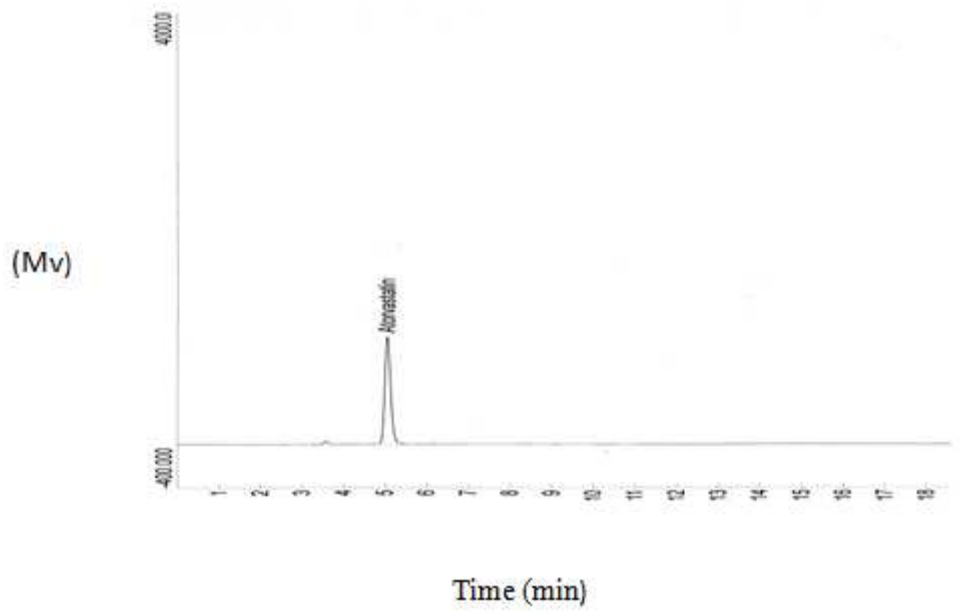

Figure 4: Chromatogram of peroxide effect of Atorvastatin calcium

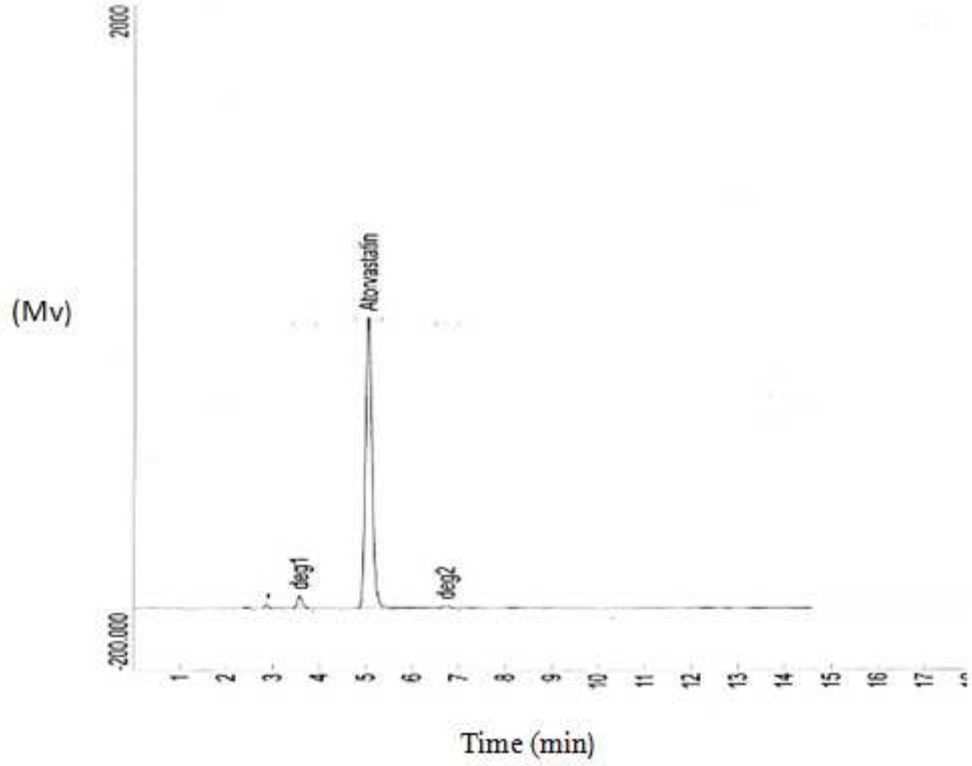

Figure 5: Chromotogram of acid Degradatin of Atorvastatin calcium

Precision of the method were done in the %RSD for repeatability was found 0.82% And intermediate precision 0.29.From the data obtained the method was found to be precise, the accuracy of the method was assessed by determination of recovery for three concentration covering the range of the method .

The robustness of the method was assessed by changing of the wavelength, flow rate and mobile phase composition. System suitability data were found to be satisfactory during variation of analytical conditions.

Assay value after 24 hours indicate that the method was accurate and precise.

No interference from placebo was observed at retention time of the drug peak, atorvastatin calcium peak is pure and don't have any co eluting peak there for it is conducted that the method is specific.

RSD meet the acceptance criteria, it conducted that sample is stable in analytical solution for at least 48 hr.

For stress testing under different conditions, the major degradation up to 80.42% under acidic condition table7. and figure 5, under alkaline condition atorvastatin calcium there's no degradation observed. The drug was approximately 4.36% degradated under oxidizing condition figure 9. The drug was degradated 0.05% under thermal condition and4.16 % degradation occurred under UV radiation at 254nm.

Table 7: degradation of stress testing of Atorvastatin calcium

S.NO	Condition	% Assay of atorvastatin calcium	% degradation	
			Single maximum	Total
1	No stress treatment(control sample)	99.89	Nil	Nil
2	Acid	19.567	55.128	80.42
3	Alkali	100.4	Nil	Nil
4	H_2O_2	95.55	3.19	4.36
5	UV	95.70	4.16	4.16
6	Thermal	99.84	0.06	0.06

Precision [4]

The main reason of study precision is to establish that promoted RP-HPLC is accurate for analyzing atorvastatin calcium in pharmaceutical formulation as well as bulk forms.

The precision (system method) of the proposed method was evaluated by carrying out six independent assays of test sample. RSD 0.484% of six assay value obtained was calculated .The intermediate precision was carry out by analyzing the sample in different days.

Table 8: Intra-day precision of Atorvastatin calcium

Repeatability precision		Retention time	Peak area at 246nm	Assay
Sample	Concentration			
Atorvastatin	8µg/ml	4.783	233.12	99.96
		4.883	233.95	100.31
		4.883	234.99	100.76
		4.883	233.15	99.97
		4.883	232.37	99.63
		4.866	231.76	99.40
RSD		0.82	0.49	0.484

Table 9: Inter –day precision of atorvastatin calcium

Intermediate precision		Inject	Peak area at 246nm	%Assay
Sample	Concentration			
Atorvastatin	8µg/ml	1	243.78	100.5
		2	242.24	99.86
		3	243.1	100.22
		4	241.93	99.73
		5	242.20	99.84
		6	242.14	99.82
RSD			0.29	0.29

Robustness: [1]

Robustness of the method was determined by small deliberate changes in flow rate, and wavelength. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

Table 10: change of wavelength

Injection	Change in wavelength±2.0nm	% Estimation of Atorvastatin
1	244nm	99.40
2	246nm	100.79
3	248nm	99.60
Mean		99.93
RSD%		0.73

Table 11: Change of flow rate

Flow rate(ml/min)			Peak area	RSD %	t_R	% RSD
Original	Used	Level				
1.0	0.9	-0.1	354.384	0.36	5.533	0.00
	1.0	0	326.4726	0.47	5.00	0.00
	1.10	+0.1	283.440	0.75	4.410	0.21

Table 12: Change of mobile phase composition

Mobile phase Composition			Peak area	RSD%	t_R	% RSD
Original	Used	Level				
66:34	65:35	-1.0	325.748	0.11	5.00	0.00
	66:34	0.0	331.930	0.50	4.910	0.188
	67:33	+1.0	331.010	1.62	4.883	0.00

Stability of Solution

The standard and sample solution of an atorvastatin have reasonably good stable over 24hours.

Table 13: Solution stability

Time	Peak area of standard solution at 246nm	Peak area of sample solution at 246nm	% estimation
Zero time	260.49	266.20	99.99
24 hours	255.8	256.14	100.80
48	245.6	248.11	100.00
Mean			100.30
RSD			0.38%

Table 14: Results of analysis of Atorvastatin calcium

Drug	n	Amount claimed(mg/tablet)	Amount found (mg/tablet)	Recovery	RSD
Atorvastatin	6	20	20.14	100.40	0.40

The assay value for marketed formulation was found to be within the limits ,as listed in table 13 , the low RSD value indicated the suitability of the method for routine analysis of Atorvastatin in pharmaceutical dosage form.

Table 15: Summary of validation parameters

Parameters	Observations
Specificity	No interference was found w.r.t excipients
Linearity r^2	0.9998
Range	80 -120% of test concentration
Precision(RSD) a)Repeatability(n=6) (system precision) b)Intermediate precision (inter-day analyst) (n=6)	 0.484 0.290
Solution Stability	0.38
Accuracy(% recovery)	100.49±0.86
Stability in analytical solution	Stable
Stress degradation	The peaks were pure without any interference
Robustness(overall RSD)	Less than 2%
a) Change of wavelength 244nm 248nm b) Change in flow rate c) 0.9ml/min d) 1.0ml/min e) 1.1 ml/min f) Change in mobile phase composition g) 65:35 h) 66:34 i) 67:33	 0.730 0.36 0.47 0.75 0.112 0.50 1.62

CONCLUSION

All the above factors lead to conclusion that the proposed method is simple, accurate, precise, sensitive, robust and cost effective and can be applied as stability indicating method for estimation of atorvastatin calcium in bulk and marketed formulation.

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