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Method Development and Validation of Related Substances in Atorvastatin Calcium Amorphous by HPLC

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ABSTRACT

A rapid high performance liquid chromatographic method was developed and validated for determination of Atorvastatin related substances (9 impurities), and degradation products in bulk drugs. The chromatographic separation was achieved on a polar RP 80 A (Synergic) (250mm×4.6mm), 4µcolumn by employing a gradient elution with Water–Acetonitrile–Formic acid-Tetrahydrofuran as the mobile phase in a shorter run time of 75 min. The flow rate was 1.1 mL min^{-1} and the detection wavelength was 254 nm. The drug substance was subjected to stress studies such as hydrolysis, oxidation, photolysis, and thermal degradation, and considerable degradation was observed in acidic hydrolysis, oxidative, thermal, and photolytic stress conditions. The method was validated as per ICH guidelines.

Keywords: Atorvastatin calcium, HPLC Validation, Degradation studies, Forced degradation, related substances.

INTRODUCTION

Atorvastatin calcium, chemically (4R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-4-phenyl (phenylcarbamoyl) -1 *H*-pyrrol-1-yl]-4,5-dihydroxyheptanoic acid calcium salt (2:1) (Fig-1), is an inhibitor of the 4-hydroxy-4-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 between 10 and 80 mg per day is used to reduce the raised lipid levels in patients with primary hyperlipidemia (familial and non-familial) or combined hyperlipidemia [1–3].

Several analytical methods that have been reported for the individual determination of Atorvastatin in pharmaceutical formulations which include methods such as RP-HPLC [4], spectrophotometric method [5, 6], LC-MS [7], HPTLC [8], UV Spectrophotometric methods [9, 10]. Since no HPLC method is reported for simultaneous estimation of nine related substances in Atorvastatin calcium drug, an attempt has been made to develop and validate HPLC method for the analysis of Atorvastatin calcium and related substances. The proposed method was optimized and validated as per the ICH guidelines [11].

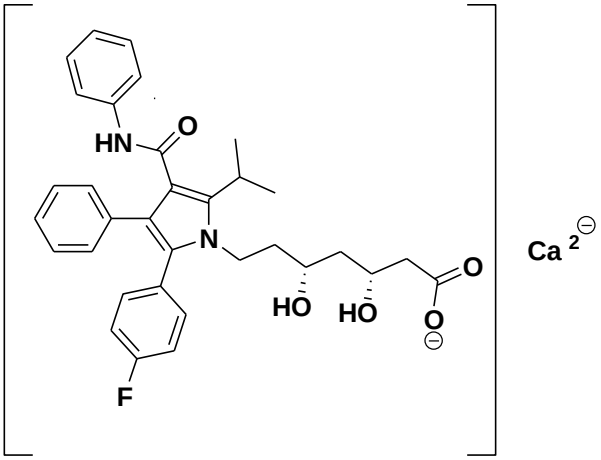

Fig. 1 Atorvastatin calcium

MATERIALS AND METHODS

Chemicals and reagents: Atorvastatin calcium and nine process related impurities were received as complementary gift from SimSon Pharma and USP, Mumbai, India. HPLC grade tetrahydrofuran**,** acetonitrile and hydrogen peroxide were purchased from Merck. Lower grade ammoniumformate, ammonium acetate, formic acid, sodium hydroxide and hydrochloric acid were purchased from Merck. High pure water was prepared by using Millipore Milli Q plus water purification system.

Instrumentation: For method development and method validation used HPLC instruments are Waters alliance and Agilent-1260 Infinity Series, injector, column oven and PDA Detector. Data acquisition was done by using Empower 2 and Chromeleon software.

Chromatographic conditions: Chromatographic separation was achieved by gradient elution of the mobile phase containing solution A, solution B and solution C. Mobile phase composition was maintained as showed in the Table-2 and the flow rate of the mobile phase was 1.1 mL/min. Separation was performed on Synergic polar RP 80 A (250mm×4.6mm), 4µ column with photo diode array detector (PDA) and detector wavelength was kept at 254 nm for monitoring the separation. Injection volume at 15µL, column oven temperature at 40°C and run time at 75 min.

Preparation of buffer: 2.84 g of ammonium formate and 0.45 g of ammonium acetate were weighed and transferred to a 950 mL of water, dissolved and adjusted the pH to 5.0 with diluted formic acid and diluted with water to 1000mL.
Preparation of solution-A: Acetonitrile and buffer were mixed in the ratio of 33:67 (v/v).
Preparation of solution-B: Acetonitrile was used as solution B
Preparation of solution-C: Stabilizer free tetrahydrofuran solvent was used as solution of C
Preparation of Diluent: Acetonitrile, stabilizer free tetrahydrofuran and buffer were mixed in the ratio of 60:5:35.

Preparation of system suitability solution: 1.25 mg of each working standards of Atorvastatin related compound-A, Atorvastatin related compound-B, Atorvastatin related compound-H and Atorvastatin related compound-I were weighed accurately and transferred to a 50 mL volumetric flask. 30 mL of diluent was added and sonicated to dissolve and made up to volume with diluent. Transferred 1mL of this

solution to 10 mL volumetric flask, containing about 5 mg of Atorvastatin calcium working standard, added 7 mL of diluent, sonicated to dissolve and made up to volume with diluent.

Preparation of standard solution: 30 mg of Atorvastatin calcium working standard was weighed accurately and transferred into 200mL volumetric flask, 100 mL of diluent was added and sonicated to dissolve and made up to volume with diluent. Further diluted 1 mL of this to 100 mL with diluent.

Preparation of test solution: 25 mg of sample accurately weighed and transferred to a 50 mL volumetric flask, added 30 mL of diluent sonicated to dissolve and diluted to made up to volume with diluent.

RESULTS AND DISCUSSION

Method Development: The present proposed HPLC method is aimed to develop chromatographic system capable of eluting and resolving Atorvastatin calcium from its process related impurities and degradation products that comply with the general requirements for system suitability. In this method the development is mainly focused on resolution and run time, due to more number of related substances are present in the drug.

Based on literature survey and considering the structures of Atorvastatin and its related substances method development has started with Zorbax SB Phenyl, 250x4.6 mm, 5µm, with mobile phase containing a mixure of 0.03M ammoniumformate (pH adjusted to 5.0 with formic Acid): Acetonitrile with the gradient as (time (min)/% solution B): 0/10, 15/20, 50/60, 60/30, 70/10, 75/10 at flow rate 1.0 mL/·min with the diluent of buffer and acetonitrile in the ratio of 65:35 v/v. In this case the noisy of baseline, longer retention times and poor peak shapes were observed.

To reduce baseline noise and longer retention times selected Phenomenox Cyno Column Synergi Polar RP 80A, mobile phase A is made up with buffer and acetonitrile in the ratio of 60:40 and mobile phase B is acetonitrile. From this trail noisy baseline was reduced but poor peak shapes were observed. In the presence of Synergi Polar Column and using the above same mobile phase the peak shapes are good but longer retention times and poor resolution was observed. When THF is used as a Mobile Phase C with very low concentrations (3%) the resolution was good but longer retention time was observed.

After making many logical gradient trails, finally in the presence of mobile phase A, B and C, Synergi Polar Column and different chromatographic conditions and gradient program (Shown in Table-1 and 2 respectively) separation was achieved between drug-degradation products and drug known impurities.

Table1. Chromatographic Parameters **of** High Performance Liquid Chromatograph equipped with UV- detector

Column	:	Synergi polar RP 80 A (250mm×4.6mm), 4µ
Column temperature	:	40°C
Sample compartment temperature	:	4°C
Flow rate	:	1.1 mL min^{-1}.
Injection volume	:	15 µL.
Wave Length	:	254 nm
Run time	:	75 min.

Table - 2 Gradient Programs

Sr. No.	Time (minutes)	Solution A (%)	Solution B (%)	Solution C (%)
1	0	91	0	9
2	15	91	6	3
4	20	82	16	2
4	25	82	16	2
5	50	32	66	2
6	55	32	66	2
7	65	91	0	9
8	75	91	0	9

Method Validation

Precision: (System precision Repeatability) Six replicate injections of standard solution were made into HPLC system. The mean, standard deviation (SD) and % RSD for the peak areas of Atorvastatin were calculated. The % RSD is 0.93.

Method precision: Six samples of single batch of Atorvastatin calcium amorphous spiked with known impurities at specification limit were prepared, because in control sample of all impurities were not detected, and analyzed as per the test method. The % RSD of individual impurities and total impurities were quantified and the results are tabulated in Tables-3a (six impurities) and 3b (three impurities, highest individual impurity and total impurities).

Ruggedness (Intermediate precision): Ruggedness of the method has been verified by analyzing the six samples, spiked with known impurities at specification limit of same batch used for method precision, as per test method by different analysts using different instrument and different column on a different day. The % RSD for individuals and total impurities of above six results was calculated and compared with the method precision results. The results are tabulated in tables-3a and 3b. The % RSD for individual impurities and overall %RSD was under acceptance criteria (i.e. less than 10)

Table - 3a. Ruggedness data (Atorvastatin and related impurities)

S. No.	Atorvastatin Diamino (% w/w)		Atorvastatin related compound A (% w/w)		Atorvastatin related compound B (% w/w)		Atorvastatin 4-deoxy-hept-2-enoic acid (% w/w)		Atorvastatin related compound H (% w/w)		Atorvastatin epoxy Tetrahydrofuran analog (% w/w)	
	Analyst		Analyst		Analyst		Analyst		Analyst		Analyst	
	I	II	I	II	I	II	I	II	I	II	I	II
1	0.244	0.237	0.276	0.283	0.235	0.233	0.125	0.140	0.164	0.152	0.151	0.130
2	0.247	0.243	0.274	0.285	0.229	0.238	0.124	0.150	0.158	0.155	0.144	0.129
4	0.244	0.246	0.269	0.290	0.224	0.238	0.117	0.148	0.160	0.153	0.148	0.134
4	0.248	0.243	0.282	0.287	0.232	0.230	0.127	0.144	0.158	0.154	0.148	0.135
5	0.244	0.243	0.275	0.289	0.226	0.239	0.127	0.152	0.157	0.157	0.159	0.138
6	0.244	0.247	0.275	0.283	0.220	0.228	0.122	0.147	0.151	0.152	0.153	0.133
Mean	0.245	0.243	0.275	0.286	0.228	0.234	0.124	0.147	0.158	0.154	0.151	0.133
SD	0.0018	0.0035	0.0042	0.0030	0.0055	0.0047	0.0038	0.0043	0.0042	0.0019	0.0052	0.0033
%RSD	0.73	1.44	1.54	1.05	2.41	2.01	2.06	2.93	2.66	1.23	3.44	2.48

Overall Mean	0.244	0.281	0.231	0.135	0.156	0.142
Overall SD	0.0029	0.0067	0.0060	0.0127	0.0038	0.0100
Overall %RSD	1.19	2.38	2.60	9.41	2.44	7.04

Table 3b. Ruggedness data (Atorvastatin and related impurities)

S. No.	Atorvastatin ethyl ester (% w/w)		Atorvastatin related compound D (% w/w)		Atorvastatin related compound I (% w/w)		Highest individual impurity (% w/w)		Total impurities (% w/w)	
	Analyst		Analyst		Analyst		Analyst		Analyst	
	I	II	I	II	I	II	I	II	I	II
1	0.159	0.148	0.163	0.176	0.148	0.144	0.045	0.054	1.954	1.992
2	0.156	0.150	0.158	0.175	0.152	0.144	0.047	0.055	1.936	2.022
3	0.154	0.148	0.156	0.180	0.150	0.145	0.044	0.053	1.907	2.035
4	0.162	0.149	0.160	0.176	0.151	0.143	0.048	0.052	1.961	2.011
5	0.156	0.149	0.157	0.180	0.149	0.149	0.052	0.054	1.951	2.049
6	0.154	0.147	0.154	0.172	0.147	0.146	0.044	0.053	1.912	2.009
Mean	0.157	0.149	0.158	0.177	0.150	0.145	0.047	0.054	1.937	2.020
SD	0.0031	0.0010	0.0032	0.0031	0.0019	0.0021	0.0031	0.0010	0.0227	0.0203
%RSD	1.97	0.67	2.03	1.75	1.27	1.45	6.60	1.85	1.17	1.00
Overall Mean	0.153		0.167		0.147		0.050		1.978	
Overall SD	0.0049		0.0101		0.0030		0.0042		0.0479	
Overall %RSD	3.20		6.05		2.04		8.40		2.42	

Specificity: Blank, standard solution, sample solution spiked with known impurities at specification limit and individual impurity solution were injected into the HPLC system. There was no interference from the blank at the retention time of Atorvastatin and known impurities peak. The chromatogram of spiked sample reveals that the impurities were well separated and also the peak purity data reveals that there were no co-eluting peaks found in chromatogram of blank (Fig. 2) and in system suitability solution chromatogram (Fig. 3) there is no interference of impurities at the retention time of analyte peak. The chromatograms of standard solution and sample spiked with known impurities are shown in fig. 4 and fig. 5 respectively.

The retention time (RT), relative retention time (RRT) and purity data are compiled in table 4. No peaks were eluted at the retention time of Atorvastatin and known impurities in blank solution. Peak purity for Atorvastatin and known impurities are passed (Purity angle should not be more than Purity threshold or Match factor should not be less than 980). This method is specific for the determination of related substance in Atorvastatin calcium amorphous.

Table 4. RT, RRT and Peak Purity data

Name of the peak	RT (min)	RRT	Purity Angle	Purity Threshold
Atorvastatin Diamino	9.544	0.57	2.175	2.743
Atorvastatin related compound A	14.639	0.87	1.755	2.182
Atorvastatin related compound B	15.844	0.94	1.533	2.210
Atorvastatin 3-deoxy-hept-2-enoic acid	27.138	1.61	2.818	3.532
Atorvastatin related compound H	34.484	2.05	1.756	2.433
Atorvastatin epoxy Tetrahydrofuran analog	36.734	2.18	2.619	3.506
Atorvastatin ethyl ester	38.094	2.26	1.452	2.211
Atorvastatin related compound D	39.941	2.37	1.712	2.514
Atorvastatin related compound I	50.543	3.00	1.497	2.319
Atorvastatin	16.825	1.00	0.015	1.006

RRT calculated with respect to retention time of Atorvastatin peak.

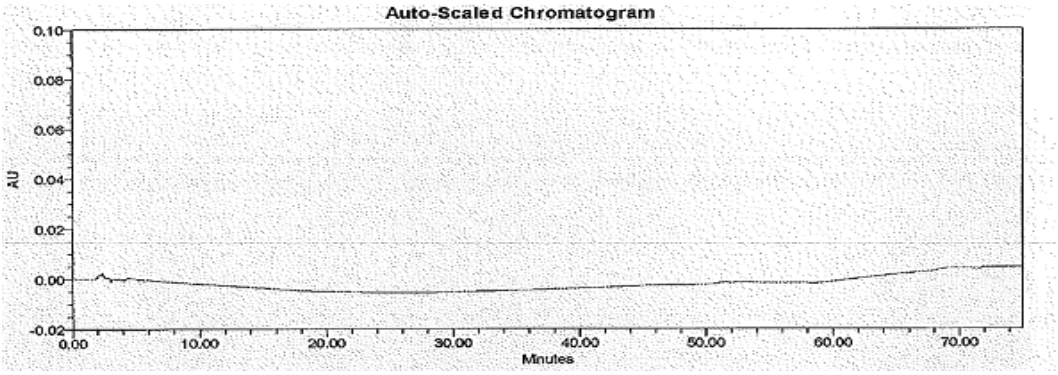

Fig. 2 HPLC chromatogram of Blank

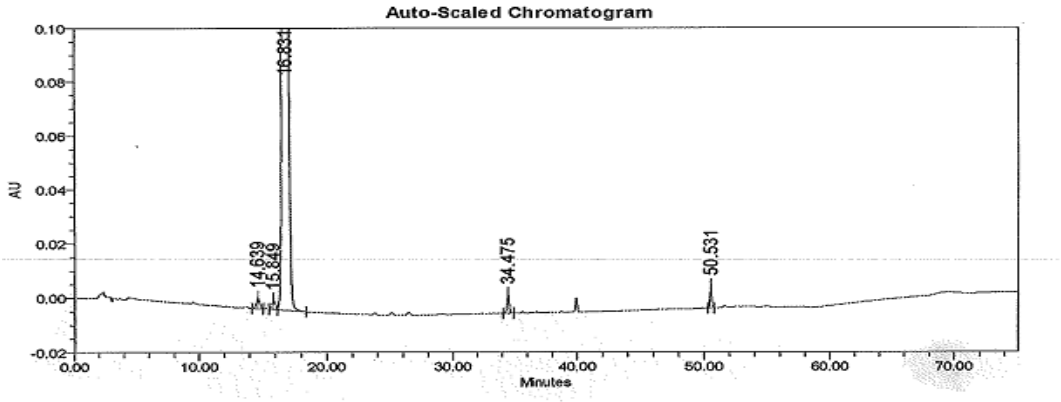

Fig. 3 HPLC chromatogram of System suitability solution

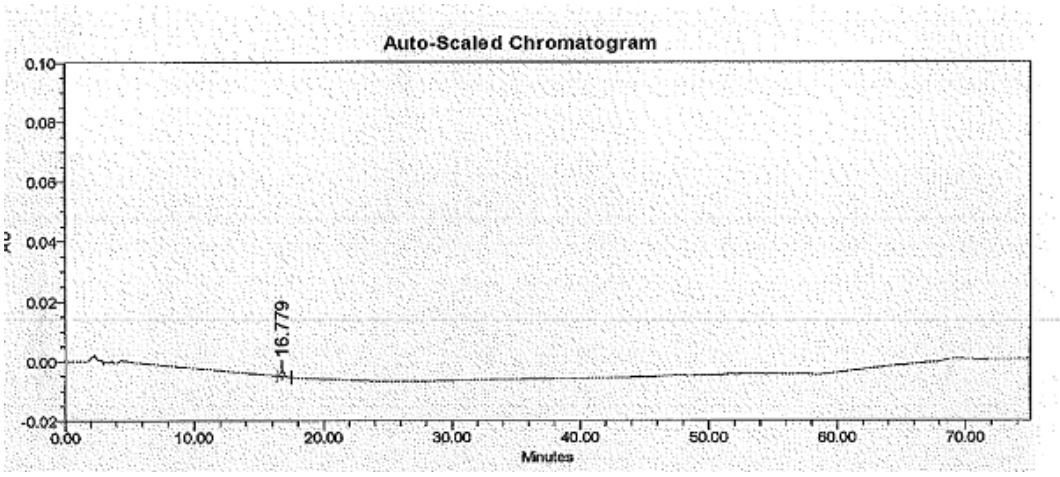

Fig. 4. HPLC chromatogram of standard solution

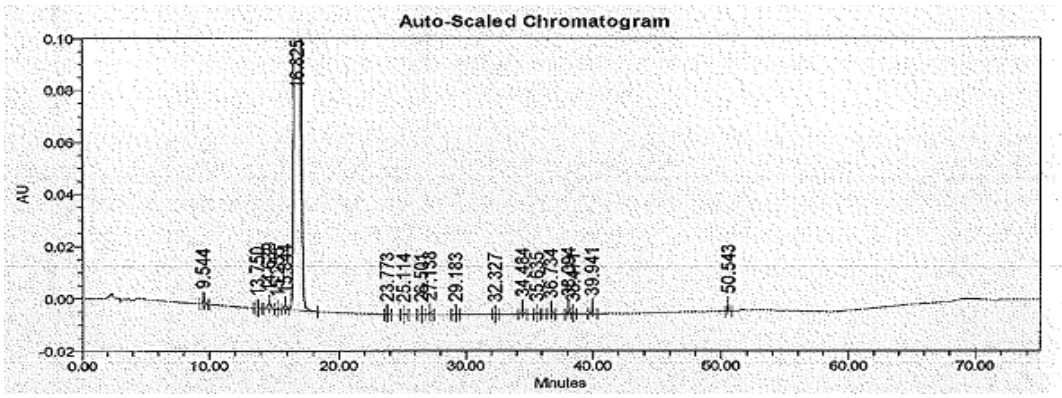

Fig. 5. HPLC chromatogram of sample solution spiked with known impurity

Stability in analytical solution: Standard solution and sample spiked with the known impurities at specification level were injected into HPLC system initially and different time intervals up to 52 h at 4°C and 25°C and determined the cumulative % RSD for the peak areas. For 4°C and 25°C the solution was kept in sample compartment.

As the cumulative %RSD for the peak area of known impurities, highest unknown impurity, total impurities and Atorvastatin are within the acceptance criteria at 4°C, hence the standard solution and spiked sample solution are stable for about 52 h at 4°C and 41 h at 25°C respectively.

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were predicted for the known impurities and Atorvastatin calcium amorphous from the linearity using the following formula.
LOD = 3.3 x Residual standard deviation/slope,
LOQ = 10 x Residual standard deviation/slope

Predicted LOD value of all impurities detection not found hence lowest detected value considered as LOD and three times of LOD value considered as LOQ and these values of LOD & LOQ were verified by giving six replicate injections of solution containing Atorvastatin diamino, Atorvastatin related compound-A, Atorvastatin related compound-B, Atorvastatin 4-deoxy-hept-2-enoic acid, Atorvastatin related compound-H, Atorvastatin epoxy tetrahydrofuran analog, Atorvastatin ethyl ester, Atorvastatin related compound-D, Atorvastatin related compound-I and Atorvastatin calcium amorphous. LOD and LOQ

values are summarized in table- 5 and fig. 6 represent the chromatogram of LOQ solution. For LOD and LOQ as the % RSD for the peak area of Atorvastatin and known Impurities were within the acceptance criteria (% RSD should not be more than 3.3 for LOD and 10 for LOQ). Hence method is precise at LOQ level.

Table 5. Summary of LOD and LOQ

Name	LOD (µg/mL)	LOD (%w/w)	LOQ (µg/mL)	LOQ (%w/w)
Atorvastatin Diamino	0.061	0.012	0.182	0.036
Atorvastatin related compound A	0.072	0.014	0.217	0.043
Atorvastatin related compound B	0.073	0.015	0.220	0.044
Atorvastatin 4-deoxy-hept-2-enoic acid	0.056	0.011	0.168	0.034
Atorvastatin related compound H	0.036	0.007	0.107	0.021
Atorvastatin epoxy Tetrahydrofuran analog	0.039	0.008	0.117	0.023
Atorvastatin ethyl ester	0.038	0.008	0.114	0.023
Atorvastatin related compound D	0.032	0.006	0.097	0.019
Atorvastatin related compound I	0.036	0.007	0.109	0.022
Atorvastatin	0.059	0.012	0.177	0.035

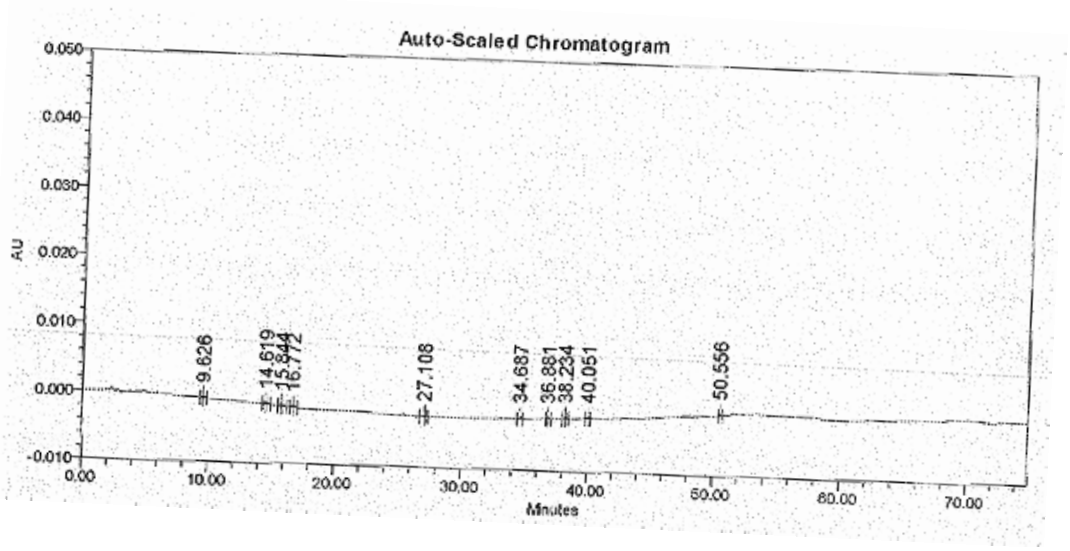

Fig. 6 HPLC chromatogram of LOQ solution

Linearity: Linearity for Atorvastatin diamino, Atorvastatin related compound-A, Atorvastatin related compound-B, Atorvastatin 4-deoxy-hept-2-enoic acid, Atorvastatin related compound-H, Atorvastatin epoxy tetrahydrofuran analog, Atorvastatin ethyl ester, Atorvastatin related compound-D, Atorvastatin related compound-I was performed from about LOQ to 150 % of specification limit with respect to test concentration (Concentration depicted was based on taking 0.4% for each of Atorvastatin related compound-A & Atorvastatin related compound-B and 0.15 % for others known impurity of test concentration (500 µg mL^{-1}) as 100%). A graph was plotted with concentration on X axis and peak area on Y-axis for impurities and correlation coefficient was determined and the relative response factor (RRF) values for known impurities were calculated. The results are tabulated in tables - 6a, 6b and 6c and figs. 7 and 8 shown the linearity graphs of

Atorvastatin related compound-H and Atorvastatin. The Correlation coefficient is within the acceptance criteria i.e. not less than 0.99.

Table 6a. Linearity data

% Level	Atorvastatin diamino		Atorvastatin related compound A		Atorvastatin related compound B		Atorvastatin 4-deoxy hept-2-enoic acid	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
5	0.03827	0	0.07298	1287	0.07385	2557	0.03550	0
8	0.06124	1371	0.11677	2728	0.11816	2914	0.05679	1498
10	0.07655	2265	0.14597	4438	0.14770	3934	0.07099	1604
40	0.22964	4344	0.43790	11544	0.44311	12239	0.21297	5045
50	0.38274	7271	0.72984	19622	0.74851	20107	0.35495	8791
80	0.61239	11669	1.16774	34791	1.18162	35151	0.56793	13525
100	0.76548	14597	1.45967	42742	1.47702	43342	0.70991	16866
120	0.91858	17802	1.75161	50122	1.77243	51146	0.85189	20500
150	1.14822	21828	2.18951	63651	2.21553	62989	1.06486	25379
CC	0.9994		0.9995		0.9994		0.9999	
Slope	18741.634		29396.943		28898.152		23875.683	
Intercept	316.106		-707.426		-184.342		28.083	
RSS	489631.4		4506144.5		4600938.2		141393.6	
RRF	0.62		0.98		0.96		0.79	

Table 6b. Linearity data

% Level	Atorvastatin related compound H		Atorvastatin epoxy tetrahydrofuran		Atorvastatin ethyl ester		Atorvastatin related compound D	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
5	0.03639	1245	0.03884	1191	0.03814	1157	0.03246	1114
8	0.05823	1681	0.06215	1127	0.06102	1909	0.05194	1688
10	0.07278	1942	0.07769	1511	0.07628	2603	0.06493	2008
40	0.21835	5869	0.23306	5398	0.22883	6499	0.19478	6197
50	0.36392	10624	0.38843	8640	0.38138	10592	0.32463	10625
80	0.58226	18626	0.62149	15218	0.61021	17756	0.51941	16783
100	0.72783	22604	0.77687	18738	0.76277	21667	0.64926	20599
120	0.87340	27429	0.93224	22690	0.91532	26341	0.77911	24657
150	1.09175	44084	1.16530	28759	1.14415	32660	0.97389	40759
CC	0.9995		0.9995		0.9998		0.9999	
Slope	31604.587		24757.678		28455.693		31600.391	
Intercept	-357.069		-343.191		128.338		107.960	
RSS	1310997.5		858830.0		394294.8		187965.2	
RRF	1.05		0.82		0.94		1.05	

Table 6c. Linearity data

% Level	Atorvastatin related compound H		Atorvastatin	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
5	0.03772	1176	0.07456	2094
8	0.06035	1506	0.11929	3203
10	0.07543	1998	0.14911	3820
40	0.22630	6059	0.44734	13289
50	0.37717	10674	0.74556	22371
80	0.60348	16602	1.19289	35404
100	0.75435	20434	1.49112	43388
120	0.90522	24497	1.78934	52099
150	1.13152	30384	2.23668	68553
CC	0.9998		0.9994	
Slope	26922.472		30125.556	
Intercept	102.426		-465.669	
RSS	366194.1		5986117.3	
RRF	0.89		1.00	

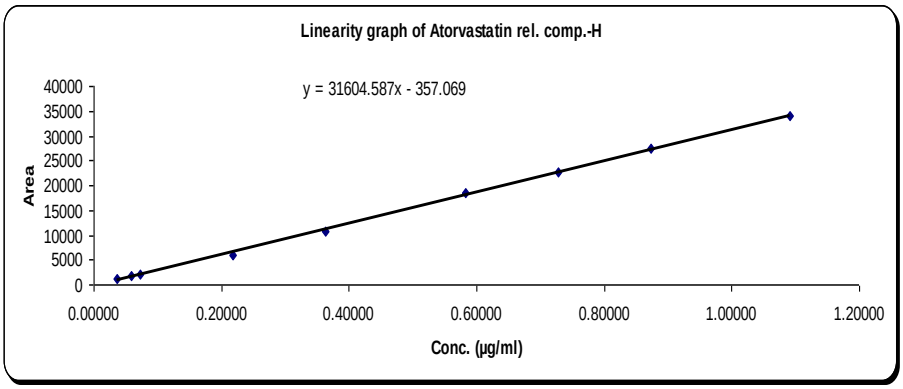

Fig. 7 Linearity graph for Atorvastatin related compound-H

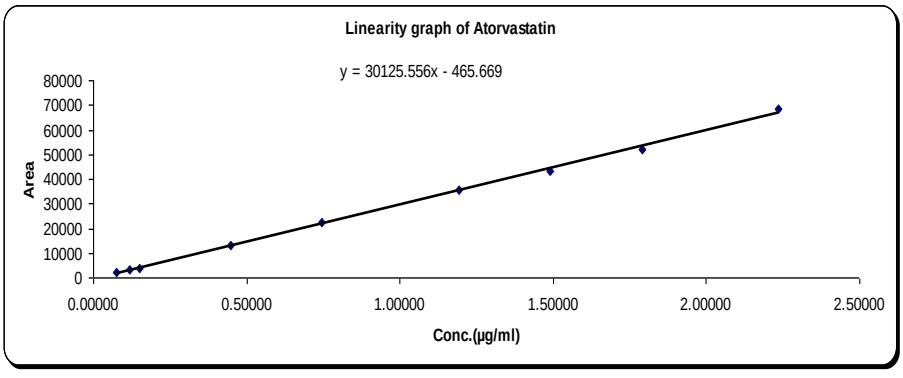

Fig. 8 Linearity graph for Atorvastatin

Based on above linearity graphs, the detector response for impurities of Atorvastatin calcium amorphous is directly proportional to concentration ranging from LOQ to 150% of specification limit with respect to test concentration.

Accuracy as recovery: Known amount of Atorvastatin diamino, Atorvastatin related compound-A, Atorvastatin related compound-B, Atorvastatin 4-deoxy-hept-2-enoic acid, Atorvastatin related compound-H, Atorvastatin epoxy tetrahydrofuran analog, Atorvastatin ethyl ester, Atorvastatin related compound-D and Atorvastatin related compound-I spiked in the sample solutions at about 40 %, 100 % and 150 % of specification limit with respect to test concentration were analyzed. Total amount present in the spiked sample was calculated. Amount recovered was calculated by subtracting the amount already present in the sample from amount found. From amount recovered and amount added, % recovery was calculated. The final recovery results are satisfactory and within the acceptance criteria i.e. the overall average % recovery is in between 75 and 125 with RSD less than 5%. The analytical method meets the pre-established acceptance criteria for recovery. Hence the method is accurate

Robustness: The Robustness of the method was evaluated by changing the following small deliberate variations in the method. Also inject the sample solution spiked with known impurities to verify the retention time of impurities. The relative retention time of impurities are tabulated in table- 7.

1. Flow rate (-10 % and +5%) (I & II)
2. Column oven temperature (± 5°C) (III & IV)
3. pH of buffer (± 0.1 unit) (V & VI)
4. Wavelength (± 2 nm) (VII & VIII)
5. Organic content (± 1 %) (IX & X)
6. Specificity (XI)

After incorporating the above modifications, the RRTs for known impurities were comparable in the conditions described in this report, hence the method is robust.

Table 7. Robustness RRT Comparison data

Name of the peak	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Atorvastatin Diamino	0.56	0.57	0.58	0.55	0.60	0.56	0.57	0.57	0.56	0.55	0.57
Atorvastatin related comp. A	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Atorvastatin related comp. B	0.94	0.95	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Atorvastatin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Atorvastatin 4-deoxy-hept-2-enoic acid	1.64	1.50	1.67	1.56	1.67	1.60	1.61	1.61	1.62	1.52	1.61
Atorvastatin related comp. H	2.12	1.83	2.17	1.92	2.28	2.01	2.05	2.05	2.06	1.88	2.05
Atorvastatin epoxy Tetrahydrofuran analog	2.26	1.94	2.32	2.05	2.41	2.14	2.18	2.18	2.19	1.98	2.18
Atorvastatin ethyl ester	2.34	2.00	2.42	2.11	2.50	2.22	2.26	2.26	2.27	2.04	2.26
Atorvastatin related comp. D	2.46	2.10	2.52	2.22	2.61	2.32	2.37	2.37	2.39	2.15	2.37
Atorvastatin related comp. I	3.11	2.62	3.22	2.79	3.27	2.94	3.00	3.00	3.02	2.69	3.00

Forced degradation: Forced degradation study was carried out by treating the sample under the following different conditions in the presence of acid, alkali, peroxide, photolytic, thermal, hydrolytic and humidity. The forced degradation studies and peak purity results were tabulated in table- 8.

Acid degradation: Sample was treated with 4 mL of 1N hydrochloric acid at room temperature for 15 min and neutralized the solution with 1N sodium hydroxide. Treated sample solution was diluted and analyzed as per the test method. In this case there was no significant degradation in presence of acid medium and the drug was most stable. The acid degraded sample chromatogram was shown in Fig. 9.C

Alkali degradation: Sample was treated with 4 mL of 1N Sodium hydroxide solution by heating at 90°C for 72 h and neutralized the solution with 1N hydrochloric acid. Treated sample solution was diluted and analyzed as per the test method. In this case there was no significant degradation in presence of alkali medium and the drug was most stable.

Peroxide degradation: Sample was treated with 2 mL of Hydrogen peroxide solution (40%) at 90°C for 5 min, then diluted and analyzed as per the test method. In this case there was no significant degradation in presence of peroxide and the drug was most stable in oxidative conditions. The peroxide degraded sample chromatogram was shown in fig. 10.

Photolytic degradation: Sample was exposed to UV light of 200Watt-h/sq-mtr and fluorescent light of 1200 KLux-Hrs. Treated sample was analyzed as per the test method. In this case there was no significant degradation in presence of photolytic and the drug was most stable in UV light.

Thermal degradation: Sample was kept in oven at 105°C for about 119 h. Treated sample was analyzed as per the test method. In this case no significant degradation was observed and the drug was most stable in thermal condition.

Hydrolytic degradation: Sample was treated with 5mL of water by heating at 90°C for about 72 h. Treated sample was analyzed as per the test method. In this case there was no significant degradation in presence of water and the drug was most stable

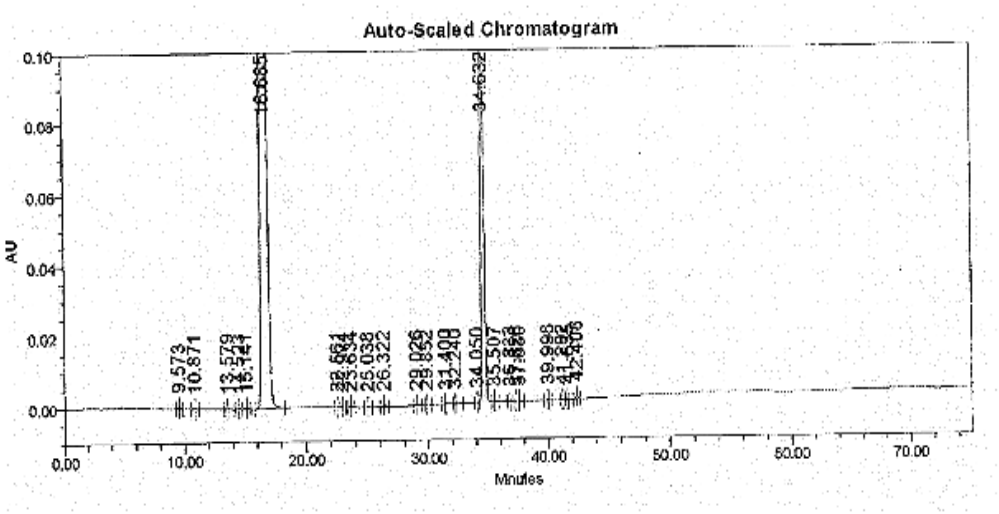

Fig. 9 HPLC chromatogram of Acid stressed sample

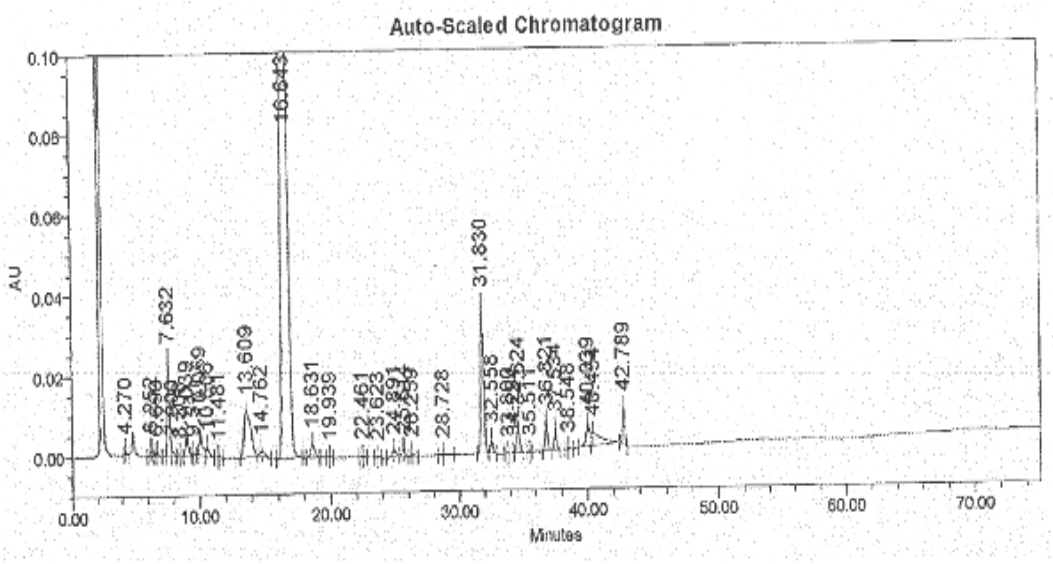

Fig. 10 HPLC chromatogram of peroxide stressed sample

Table 8. Forced degradation data

Name of impurity	Control sample (Unstressed sample)	Peroxide stressed sample (2mL,40% H_2O_2,5min. at 90°C)	Acid stressed sample (4mL, 1N HCl, 15min at RT)	Light stressed sample (UV stressed)	Base stressed sample (4mL, 1N NaOH, 72h at 90°C)	Hydrolytic stressed sample(5mL H_2O, 72h at 90°C	Heat stressed sample (119 h at 105°C)
Atorvastatin Diamino	0.108	0.097	0.069	0.095	0.057	0.085	0.097
Atorvastatin related compound A	0.018	0.262	0.023	0.017	0.020	0.021	0.011
Atorvastatin related compound B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Atorvastatin 4-deoxy-hept-2-enoic acid	0.000	0.000	0.000	0.000	0.176	0.014	0.056
Atorvastatin related compound H	0.018	0.662	15.235	0.061	0.042	0.069	0.293
Atorvastatin epoxy Tetrahydrofuran analog	0.000	0.829	0.022	0.148	0.047	0.000	0.040
Atorvastatin ethyl ester	0.000	0.032	0.000	0.000	0.039	0.110	0.010
Atorvastatin related compound D	0.048	0.770	0.028	0.531	0.012	0.020	0.540
Atorvastatin related compound I	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Any other individual impurity	0.074	3.966	0.091	0.369	0.829	0.343	0.484

Total impurities	0.611	17.407	15.847	1.969	2.282	1.312	2.658
% Degradation	Not applicable	16.796	15.236	1.358	1.671	0.701	2.047

APPLICATIONS

HPLC method could be used for the routine analysis of production samples and also to check the stability of bulk samples of Atorvastatin calcium during its storage.

CONCLUSIONS

The developed one is a rapid HPLC method for the related substances and assay determination of Atorvastatin calcium is linear, precise, accurate and specific. The method was validated following the requirements of ICH guidelines and the results were satisfactory. The developed stability indicating HPLC method could be used for the routine analysis of production samples and also to check the stability of bulk samples of Atorvastatin calcium during its storage.

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