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Spectroscopic characterization and quantitative determination of atorvastatin calcium impurities by novel HPLC method

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HIGHLIGHTS

- Identification of possible impurities in atorvastatin calcium drug substance.
- Synthesis and spectroscopic characterization of atorvastatin related compounds.
- Development of single analytical method for quantitative determination with HPLC.
- Method validation, as per international pharmaceutical guidelines.

GRAPHICAL ABSTRACT

Seven, process related impurities were identified by LC-MS in the Atorvastatin Calcium drug substance. These impurities were identified by LC-MS. The structure of impurities was confirmed by modern spectroscopic techniques like ^{1}H-NMR and IR and physicochemical studies conducted by using synthesized authentic reference compounds. The synthesized reference samples of the impurity compounds were used for the quantitative HPLC determination. These impurities were detected by newly developed gradient, reverse phase High Performance Liquid Chromatographic (HPLC) method. The system suitability of HPLC analysis established the validity of the separation. The analytical method was validated according to International Conference of Harmonization (ICH) with respect to specificity, precision, accuracy, linearity, robustness and stability of analytical solutions to demonstrate the power of newly developed HPLC method.

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ABSTRACT

Seven process related impurities were identified by LC–MS in the atorvastatin calcium drug substance. These impurities were identified by LC–MS. The structure of impurities was confirmed by modern spectroscopic techniques like ^{1}H NMR and IR and physicochemical studies conducted by using synthesized authentic reference compounds. The synthesized reference samples of the impurity compounds were used for the quantitative HPLC determination. These impurities were detected by newly developed gradient, reverse phase high performance liquid chromatographic (HPLC) method. The system suitability of HPLC analysis established the validity of the separation. The analytical method was validated according to International Conference of Harmonization (ICH) with respect to specificity, precision, accuracy, linearity, robustness and stability of analytical solutions to demonstrate the power of newly developed HPLC method.

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Introduction

Atorvastatin calcium: (3R,5R)-7-[2-(4-Fluoro-phenyl)-5-isopropyl-3-phenyl-4-phenyl carbamoyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid calcium salt, is an established drug [1,2] under the category of cardio-vascular therapeutic use. Previously the majority of formulations were being made by using "Simvastatin", "Lovastatin" or "Fluvastatin" for the same purpose. But due to a number of side-effects and less effectiveness of these molecules, atorvastatin (calcium salt) was developed and found effective against several cardiac diseases [3]. The drug atorvastatin was first synthesized by an American scientist: Dr. Bruce D. Roth in 1985 when he was working as Senior Scientist with Parke-Davis of Warner-Lambert Company (later on in 2000 it was merged with Pfizer). The method of synthesis prescribed in this article is not concordant of original one; it is alternate, cost effective and comparatively better synthone pathway. The atorvastatin drug substance popularized with the name of "Lipitor" as a drug product is very commonly used cholesterol reducing drug, being used around the globe. The manufacturers of atorvastatin purify the drug substance and evaluate the quality either on TLC or HPLC. Although, however, the United States Pharmacopoeia (USP) had incorporated the monograph of atorvastatin in September 2009, but still impurity related information are deficient and till now there is no literature that describes any analytical technology

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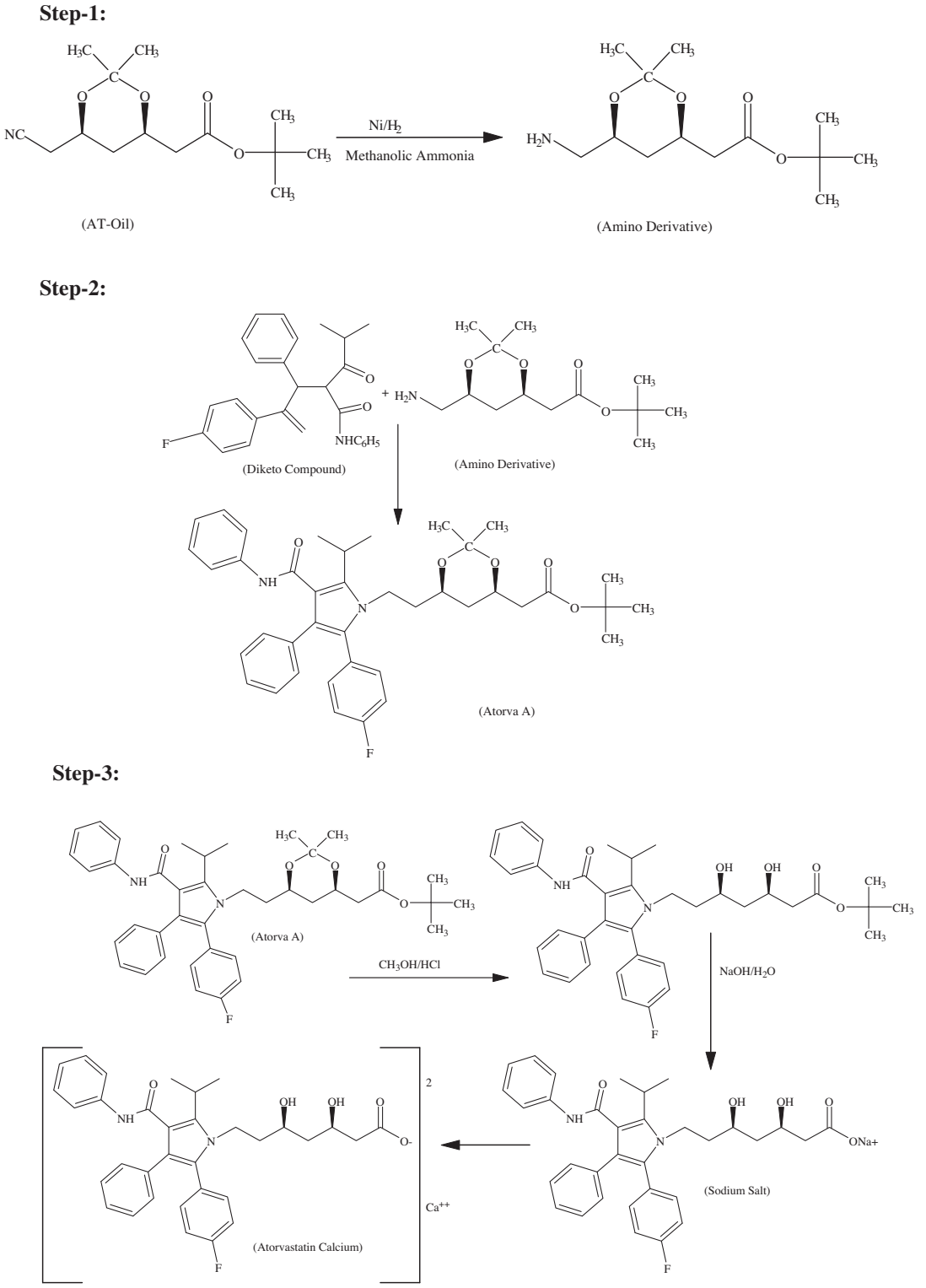


Fig. 1. The synthesis scheme of atorvastatin calcium.

which is able to quantify "Seven" process related impurities. The method presented in this article gives sufficient information about possible impurity and their evaluation to help the chemists in purifying the drug in a better way. Atorvastatin has been formulated as an individual and in several combinations [4].

A few bio-analytical methods are reported in the literature for the quantitative determination of atorvastatin calcium [5,6]. The British Pharmacopoeia [7,8] describes a thin layer chromatography (TLC) method for the quantitative assessment of atorvastatin related substances. Atorvastatin is cholesterol reducing drug and acts as HMG-CoA reductase inhibitory agent, like other drugs i.e. Lovastatin, Simvastatin, etc. and has some common side effects. This article describes the possibility of process related impurities, their structure and HPLC technology to quantify them, by which these

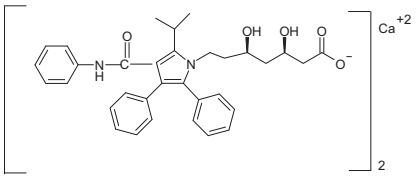
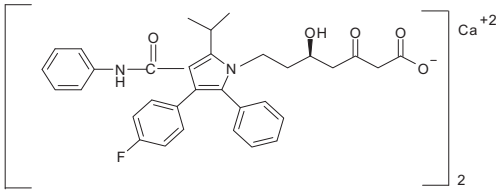
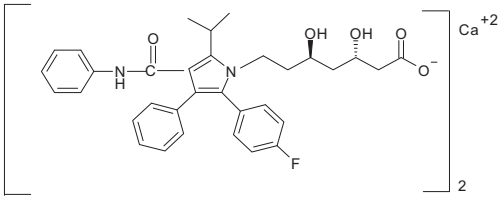
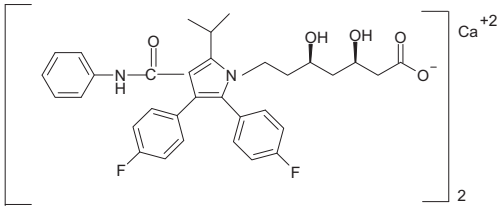
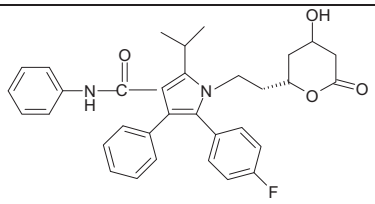
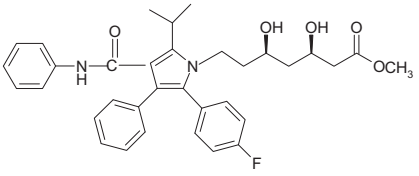
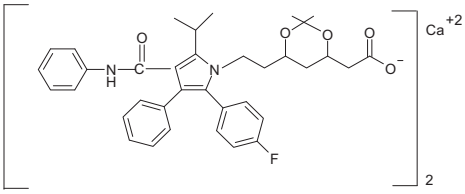
Sr. No.	Name of related compound	Structure
1.	ATVRS-1	
2.	ATVRS-2	
3.	ATVRS-3	
4.	ATVRS-4	
5.	ATVRS-5	
6.	ATVRS-6	
7.	ATVRS-7	

Fig. 2. The structure of atorvastatin calcium related substances.

Table 1
Mass, melting point and elemental analysis data of the compounds.

Compounds	MW (calculated)	MW (observed)	Color	M.P. (°C)	Elemental analysis % found (calculated)		
					C	H	N
ATVRS-1 ($C_{66}H_{70}N_4O_{10}Ca$)	1118	1078	White powder	163	70.92 (70.84)	6.18 (6.26)	5.13 (5.01)
ATVRS-2 ($C_{66}H_{64}N_4O_{10}F_2$ Ca)	1150	1110	White powder	191	68.72 (68.87)	5.43 (5.57)	5.02 (4.87)
ATVRS-3 ($C_{66}H_{68}N_4O_{10}F_2$ Ca)	1155	1114	White powder	193	68.51 (68.63)	5.99 (5.89)	4.99 (4.85)
ATVRS-4 ($C_{66}H_{66}N_4O_{10}F_4$ Ca)	1190	1151	White powder	188	66.70 (66.55)	5.68 (5.55)	4.85 (4.71)
ATVRS-5 ($C_{33}H_{33}N_2O_4F$)	540	541	Off-white powder	159	73.19 (73.33)	6.00 (6.11)	5.36 (5.19)
ATVRS-6 ($C_{34}H_{37}N_2O_5F$)	572	572	Off-white powder	145	71.38 (71.33)	6.43 (6.47)	5.01 (4.90)
ATVRS-7 ($C_{72}H_{76}N_4O_{10}F_2$ Ca)	1234	1194	White powder	187	70.17 (70.02)	6.22 (6.16)	4.63 (4.54)
Atorvastatin calcium ($C_{66}H_{68}N_4O_{10}F_2Ca$)	1155	1114	White powder	160	68.52 (68.63)	6.89 (6.76)	4.92 (4.85)

Table 2
Gradient program of the HPLC analysis.

Sr. No.	Time (minutes)	Mobile phase A%	Mobile phase B%	Curve
1	0	100	0	6
2	10	100	0	6
3	20	85	15	6
4	35	15	85	6
5	47	100	0	6
6	50	100	0	6

can be properly evaluated and an impurity free drug can be manufactured. The evaluation of toxicological impacts of these impurities and their biological effects can be an encouraging part for further research, which is a separate chemistry. Since the impurity profile study of any pharmaceutical substance is a crucial part of process development, it was quite desirable and necessary to develop a reliable method for quantitation of all identified impurities in atorvastatin calcium, in a single shot.

In process development several impurities were detected in crude and pure sample of atorvastatin calcium by using a newly developed reverse phase gradient HPLC method. To get a control on this synthesis process related impurities it was quite compulsory to identify these impurities first. In this regard the LC–MS spectra of atorvastatin samples were recorded and the synthesis process was reviewed. It was observed that there is a possibility of seven impurities to be present in the atorvastatin samples. For the complete identification and confirmation, these all seven impurities have been synthesized, separately and characterized. By using these impurities as reference material the HPLC method has been validated for their quantification.

Experimental

Atorvastatin calcium samples (crude and pure, both) were received from An API manufacturing company. Other chemicals were purchased from Sigma Aldrich and Lancaster/Merck. Solvents used were of Spectroscopic/Chromatographic grade and were used as received. The study has been performed with HPLC (Water Alliance 2695 system equipped with UV detector, quaternary gradient flowing pump, column and sample cooler/heater), Perkin Elmer FT–IR BX-II spectrometer. The MS studies were carried out on Waters Quadrapole ion trap mass spectrophotometer attached with the HPLC system. The melting point of the compounds was measured on "Labindia" made automatic melting point recorder. The used UV spectrophotometer was of "Schimadzu" make while the NMR studies have been performed over the "Bruker" instrument. The microanalysis (CHN) was measured on CHN analyzer which was of "Perkin Elmer" make.

Synthesis of atorvastatin calcium

Atorvastatin was made in three steps [9] (Fig. 1) by using two intermediates, i.e. AT-oil and Diketo compound. These intermediates were readily available in market and were outsourced from China.

Step-1: AT-oil to amino derivative

AT-oil was hydrogenated with hydrogen gas in presence of nickel catalyst in ammonical methanol. After reaction completion, catalyst was filtered off and methanol distilled out, oily product (amino derivative) was obtained which was taken as such, for next stage.

Step-2: atorva A series

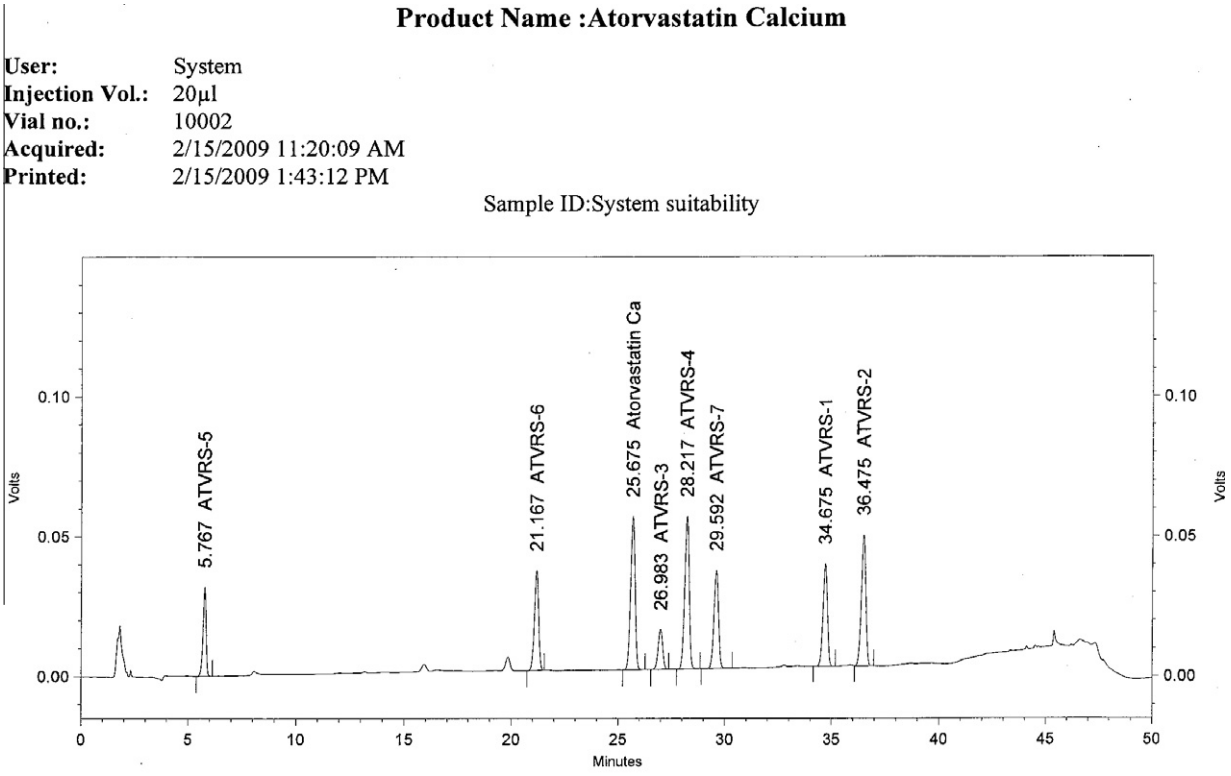
Product amino derivative (output of step-1) and diketo compound (outsourced) were allowed for condensation in the presence of soft acid in the organic solvent after reaction completion, reaction mixture was washed with alkaline water and then sodium chloride solution. Solvent distilled out, product was crystallized in alcoholic solvent and DM water than filtered, dried & further re-crystallized in the same solvent.

Step-3: atorva A to atorvastatin calcium

Atorva A was stirred in methanol and hydrochloric acid solution then this methanolic solution was concentrated and then again stirred. After reaction completion, adjust pH alkaline with sodium hydroxide solution and stirred for several (10–12) h, after reaction completion, methanol was distilled off under reduced pressure. Product is taken in methanol water mixture and washed with organic solvent. Aqueous calcium acetate solution was added, product (atorvastatin calcium) was precipitated out which was filtered, washed with de-mineralized water and dried under vacuum.

Characterization atorvastatin related compounds

All impurities (atorvastatin related substances), synthesized in laboratory (Fig. 2) have been characterized by using physicochemical (color, yield, melting point and CHN analysis) and modern spectroscopic techniques (Mass, IR, ^{1}H NMR, UV and HPLC). The observed value of the CHN%, which found very close to the calculated values, Table 1, mass and IR spectra confirms the proposed structure of these compounds. UV (maxima at 210–240 nm) and NMR analysis further supports the elucidated structure. The HPLC analysis gives the idea of the purity of the compounds. Elucidated



Detector A (210nm) Retention Time	Area	Area %	Name	Theoretical plates	Height	Asymmetry	Resolution
5.767	313424	7.12	ATVRS-5	7930.24	31907	0.87	0.00
21.167	498378	11.31	ATVRS-6	51247.16	35784	0.96	48.65
25.675	811845	18.43	Atorvastatin Ca	67386.30	54892	0.96	11.72
26.983	213045	4.84	ATVRS-3	73996.81	14337	0.95	3.30
28.217	812129	18.44	ATVRS-4	80835.35	54738	0.95	3.11
29.592	524145	11.90	ATVRS-7	89612.30	35195	0.97	3.47
34.675	540040	12.26	ATVRS-1	125034.61	36788	0.97	12.91
36.475	691691	15.70	ATVRS-2	137072.92	46968	0.96	4.58
Totals	4404697	100.00			310609		

Fig. 3. HPLC chromatogram; system suitability of atorvastatin calcium.

Table 3
R^2 values of all impurities evaluated over the concentration linear curve.

Sr. No.	Name of the component	R^2 value (limit not less than 0.99)	Linear equation
1	ATVRS-1	0.9958	$y = 144909x$
2	ATVRS-2	0.9955	$y = 43605x$
3	ATVRS-3	0.9977	$y = 17980x$
4	ATVRS-4	0.9988	$y = 35988x$
5	ATVRS-5	0.9921	$y = 75112x$
6	ATVRS-6	0.9975	$y = 59178x$
7	ATVRS-7	0.9980	$y = 34013x$

structure of the possible impurities in atorvastatin calcium has been shown in Fig. 2.

High performance liquid chromatography

All samples were analyzed on a HPLC by using C-18 250 mm (long) × 4.6 mm (ID), 3.5-micron particle size analytical column was used for chromatographic separation. There were two mobile phases, A and B, were used in timed gradient flow of 1.5 mL per/minute. The mobile phase A consists the phosphate buffer with pH 5.4 and the mobile phase B have the HPLC grade Acetonitrile: Tetrahydrofuran:: 90:10 (v/v). The injection volume was 20 L. The column and sample temperature was maintained at 40 and 6 °C, respectively. The detector was set at 220 nm, throughout the analysis. Gradient program was set shown in Table 2.

Liquid chromatography–mass spectrophotometry

During mass studies the in instrument (as described above), the source voltage was maintained at 3.0 kV and capillary temperature at 240 °C. Helium gas was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the range of m/z 70 to 1500 u.

Infra-red spectrophotometry

The IR spectra of the newly synthesized API and its related substances were recorded in the solid phase as KBr pallets on FTIR spectrometer.

Table 4
Concentration linearity data of all impurities.

Linearity of atorvastatin calcium related substances (impurities)														
Concentration Level	ATVRS-1		ATVRS-2		ATVRS-3		ATVRS-4		ATVRS-5		ATVRS-6		ATVRS-7	
	Conc.	Average area	Conc.	Average area	Conc.	Average area	Conc.	Average area	Conc.	Average area	Conc.	Average area	Conc.	Average area
70.0%	1.40	206,697.72	1.40	61,618.65	1.40	25,390.77	1.40	50,447.48	1.40	110,270.36	1.40	83,863.84	1.40	47,583.72
90.0%	1.80	254,860.30	1.80	76,862.46	1.80	32,418.25	1.80	65,460.76	1.80	133,814.57	1.80	107,592.30	1.80	60,912.68
100.0%	2.00	286,682.00	2.00	85,784.00	2.00	35,314.00	2.00	71,153.00	2.00	149,014.00	2.00	116,316.00	2.00	69,062.00
110.0%	2.20	320,797.16	2.20	96,935.92	2.20	39,904.82	2.20	79,406.75	2.20	163,617.37	2.20	129,808.66	2.20	75,070.39
130.0%	2.60	379,566.97	2.60	114,435.86	2.60	46,791.05	2.60	93,495.04	2.60	195,804.40	2.60	154,467.65	2.60	87,667.30

Results and discussion

HPLC analysis using the above method revealed the presence of seven impurities (ATVRS-1 to ATVRS-7) at RRTs: 1.35, 1.42, 1.05, 1.10, 0.22, 0.82 and 1.15 with respect to principle peak of atorvastatin calcium. The target impurities, under study, are marked as ATVRS-1, ATVRS-2, ATVRS-3, ATVRS-4, ATVRS-5, ATVRS-6 and ATVRS-7, respectively. The typical chromatogram of (composite of all seven impurities and atorvastatin calcium) system suitability, highlighting the retention times of all impurities and their resolution is shown in Fig. 3. The evaluation of spectroscopic results confirms the structure of the compounds as prescribed in respective figures (Figs. 1 and 2).

Validation of HPLC analytical method

The newly developed method for atorvastatin calcium and its related substances was validated as per the ICH guidelines [10] and the guidelines described in United State Pharmacopoeia (USP) [11].

The validation studies [12] were carried out for the analysis of all possible seven impurities in atorvastatin calcium. The system suitability (Fig. 3) was established to demonstrate and verify the chromatographic separation between all the components.

The entire activity of the analytical method validation was carried out under the following heads:

(a) Specificity.
(b) Precision (Intermediate Precision, Method Precision and System Precision).
(c) Accuracy and recovery.
(d) Linearity and range.
(e) Robustness.
(f) Stability of analytical solutions.

Specificity

The ability of analytical method to unequivocally assess the analyte in the presence of other components can be demonstrated by evaluating specificity (system suitability). The specificity of this HPLC method was determined by injecting individual impurity samples and the composite solution of all impurities along-with the atorvastatin calcium drug substance, wherein no interference was observed for any of the components. The chromatograms were checked for the presence of any other/extra peak. The peak purity of these samples was checked on Waters 2998 photodiode array (PDA) detector. The purity of the principle peak and other peaks due to impurities found satisfactory (Observe peak purity was >98%). It suggests that there is no merging of any unknown peak with any known peak. This study confirmed the stability indicating power of the method.

Precision

The system precision was examined by using six replicate injections of a standard solution (composite of all impurities along with atorvastatin calcium). The relative standard deviation (RSD) was calculated for the response area of each component, individually. The observed values were well within the generally acceptable limit of not more than 10%. The method precision was determined by analyzing samples of atorvastatin calcium using six different preparations. The calculated RSD of these results were found to be 1.17%. The intermediate precision was established by performing the method precision with two columns of different lot and by using the solvents of two different lots. The cumulative RSD of both the results was 1.43%.

Accuracy and recovery

The accuracy of the method was determined for the related substances by spiking known amounts of the impurity in atorvastatin calcium standard solution. At three levels that is 80%, 100% and 120%. The observed recovery of impurities (96.3–103.6%) was well within the specified limit of 90–110%.

Linearity

To examine the linearity of the method and to set the range for analysis the composite solution of all components in a range of concentration (70–130%, 5 concentration levels) was injected onto the HPLC in triplicate for each level. To calculate different statistical parameters the regression curve was plotted between the area response and concentration for each component, separately. Linear calibration curve for the related substances method were obtained over the range of 70–130% of standard concentration. Method showed the linear response for all the impurities. The values of calculated co-relation co-efficient (R^2) along-with the linear equation has been shown in Table 3 and corresponding data in Table 4.

Robustness

The robustness of the method has also been established by performing the exercise of method precision with making minor but significant changes in the specified chromatographic conditions. The changes tried were: change in column temperature (2.0 °C), change in flow rate (0.1 mL per minute) and the change in the pH of mobile phase A (0.2). The results obtained with the standard method and altered methods were not significantly differing and were within the acceptance criteria.

Stability of analytical solutions

The freshly prepared analytical standard solution (composite of all impurities and the atorvastatin calcium), as prepared for system precision was injected onto the HPLC and after the intervals of 3, 6, 12 and 24 h. The cumulative RSD of the area of each component, separately, was calculated. The observed value was 1.8% (limit not more than 10%). So it was concluded that the analytical solutions are stable for 24 h at 6 °C.

Conclusion

A new HPLC method was developed for the separation and quantification of possible impurities in atorvastatin calcium bulk drug sample. For the identification and confirmation these impurities were synthesized and characterized. The newly developed HPLC method has also been validated as per the regulatory guidelines; it can be conventionally used for the quantitative determination of related substances in atorvastatin calcium API sample. The analytical method was found to be specific, accurate and precise, linear and robust. It can be used for the routine analysis as well as to monitor the stability studies of the drug substance.

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References

[1] W. Xing, L. Daowei, Li-Guafi, Z. Zhiyi, Eur. J. Pharmacol. 636 (2010) 114–120; S. Tom, Eur. J. Health Eco. 11 (2010) 267–277.
[2] J.L. Venneerstrom, W.Y. Eliss, A.J. Ager, S.L. Andersen, L. Gerena, W.K. Milhous, J. Med. Chem. 35 (1992) 2129–2134;
A. Young, K.J. Yaun, S. Hak, J. Cardiovascular Pharmacol. Therm. 15 (2010) 167–174.
[3] L. Roberto, C. Domenico, M. Roberta, B. Silvia, C. Alessandra, B. Cesare, Int. J. Cardiol. 141 (2010) 68–74.
[4] C. Alvaro, G. Fabiana, D.V. Grasi, S. Simone, H. Marioh, D. Egidiol, Clin. Chim. Acta 411 (2010) 631–637.
[5] S. Erturk, E.S. Aktas, L. Ersoy, S. Ficiciolu, J. Pharm. Biomed. Anal. 33 (2003) 1017–1023.
[6] U. Seshachalam, C.B. Kothapally, J. Liq. Chromatogr. Related Technol. 31 (2008) 714–721.
[7] V.B. Dohalsky, J. Huclova, B. Barrett, B. Nemec, I. Ulc, I. Jelinek, Anal. Bio-anal. Chem. 386 (2006) 275–285.
[8] Chinese Pharmacopoeia, 2 (2000) 530.
[9] M. Kracun, A. Kocijan, A. Bastarda, R. Grahek, J. Plavec, D. Kocjan, J. Pharm. Biomed. Anal. 50 (2009) 729–736.
[10] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedures text and methodology, section Q2 (R1).
[11] United State Pharmacopoeia 34 (2011).
[12] V.G. Dongre, P.P. Karmuse, P.D. Ghugare, M. Gupta, B. Nerukar, C. Shaha, A. Kumar, J. Pharm. Biomed. Anal. 43 (2007) 186–195.