International Journal of Pharmaceutical Sciences and Drug Research 2019; 11(5): 187-193

RESEARCH ARTICLE

ISSN: 0975-248X
CODEN (USA): IJPSPP

Isolation, Identification and Characterization of Degradation Impurity of Atorvastatin in Fixed Dose Combination of Atorvastatin and Ezetimibe

Rajesh Desai[1*], Suresh Koradia[2]

[1]*School of Science, RK University, Rajkot, Gujarat, India*
[2]*Shree M. & N. Virani Science College, Rajkot, Gujarat, India*

Copyright © 2019 Rajesh Desai *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

ABSTRACT

The objective of this study is to isolation and characterization of unknown degradation product of Atorvastatin calcium in combination formulation product with Ezetimibe by using modern techniques of separation and characterization. An unknown impurity is generating during a forced degradation study of Atorvastatin and Ezetimibe fixed-dose combination tablets. By using the gradient reversed-phase high-pressure liquid chromatographic method, unknown degradation impurity was detected and quantified in the range of 0.05% to 0.2% of Atorvastatin. The impurity was enriched by extreme oxidation degradation of Atorvastatin and isolated through preparative HPLC. The structure of the impurity was characterized by mass and NMR spectrum.

Keywords: Atorvastatin calcium, Ezetimibe, HPLC, Mass, NMR, Degradation.

DOI: 10.25004/IJPSDR.2019.110506 **Int. J. Pharm. Sci. Drug Res. 2019; 11(5): 187-193**

***Corresponding author**: Mr. Rajesh Desai
Address: School of Science, RK University, Rajkot, Gujarat, India
E-mail ✉: rdesai777@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 15 July, 2019; **Revised:** 13 August, 2019; **Accepted**: 23 August, 2019; **Published:** 25 September, 2019

INTRODUCTION

Hypertension is the most common disease worldwide; this is a major risk factor for cardiovascular disease. Most of the population is suffering from higher blood pressure and obesity with a high level of bad cholesterol (i.e. LDL, triglycerides). A fixed-dose combination product of Atorvastatin and Ezetimibe Tablets was used as a hypertensive drug and its helps to reduce the LDL cholesterol level in blood. High risk of the heart disease can be reduce by decreasing the LDL cholesterol and triglyceride level by using this combination product and it helps to reduce the risk of coronary heart disease and similar critical conditions. [1]

Chemical name of Atorvastatin is (βR, δR)-2-(4-Fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt trihydrate. [2] Appearance of Atorvastatin calcium is a white crystalline powder. The chemical and physical property of the compound shows it is soluble in dimethyl sulfoxide, practically insoluble in water, alcohol, and dichloromethane. Molecular formula is $C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$. [2] Appearance of Ezetimibe is a white, crystalline powder. The chemical and physical property of the compound is shows it is soluble in alcohol and acetone. Ezetimibe is practically insoluble in water. [3]

Combination of the product is available in the market and few methods for determination of impurity of the combination product by high-pressure liquid chromatography are available. Impurity profiling of the combination product is critical to its safety assessment and formulation process. [4-13] To separate all possible degradation impurities a specific and selective reverse phase chromatographic method was developed and force degradation study was planned. Several degradation products were generated during the degradation study of combination product which is known and reported. Some of the unknown degradations are not observed increased potential. In oxidative degradation with hasher condition, one impurity was increased which can be a hazard with regards to toxicity issue. The present study provides isolation, characterization, and identification of the unknown degradation product which is related to Atorvastatin. The unknown impurity was isolated by preparative HPLC and characterized by LCMS and NMR techniques. Literature survey shows an analytical method for related impurities by HPLC for Atorvastatin [14-18], Ezetimibe [19-21] and for combination product of Atorvastatin and Ezetimibe is available to determine related impurities of Atorvastatin and Ezetimibe.

The presence study is important for identify potential degradation impurity in current marketed product and can control this impurity in formulation as a good product life cycle approach. Also, it will help in new drug development activity to save overall time for identification and characterization of degradation products. The present study provides the isolation, characterization, and identification of the unknown degradation product of the Atorvastatin, which is characterized by LCMS and NMR spectrums.

MATERIALS AND METHODS

Material and reagents

The raw material of Atorvastatin calcium trihydrate API, Ezetimibe API, Atorvastatin and Ezetimibe combination tablets ware received from a well-known pharmaceutical company.

Solution preparation

Mobile phase A

20mM Ammonium Acetate buffer pH 5.0

Approximately weighed and transferred 3.08 g ammonium acetate into 1000 ml water and dissolved it. Adjust pH of 5.0 with diluted glacial acetic acid.

Mobile phase B

The mobile phase was prepared by proper mixing of acetonitrile (950 ml) and THF (Tetrahydrofuran) (50 ml).

Diluent preparation

The diluent was prepared by proper mixing of water (200 ml) and acetonitrile (800 ml).

Sample preparation for forced degradation study

A 10 tablets of Atorvastatin and Ezetimibe crushed into a fine powder and transferred into 100 ml volumetric flask, added 20 ml of diluent and sonicated for 30 minutes. 5 ml 30% H2O2 solution and 2 ml of 1N NaOH solution was added and kept on room temperature for 7 days. After 7 days 50 ml diluent was added in a volumetric flask and sonicated for 15 minutes. Cooled to room temperature and made volume up to the mark. Filtrate the sample through 0.45µ PVDF filter and analyzed in HPLC system.

High-performance liquid chromatography (HPLC analytical)

A Waters HPLC system with a quaternary pump, photo-diode array detector, and auto-sampler has been used for analysis. A reversed-phase liquid chromatography gradient method used to perform a forced degradation study of related substances method for Atorvastatin and Ezetimibe combination tablets. A YMC pack ODS-AQ, 250 × 4.6 mm, 3µm column was used for analysis with a flow rate of 0.6 ml/minutes. The analyte peaks were monitored and detection wavelength 244 nm. A mixture of 20mM acetate buffer pH 4.5 and acetonitrile in the ratio of 70:30%v/v was used as mobile phase A. Prepared 20mM acetate buffer pH 4.50 ± 0.05 and mixed with acetonitrile in the ratio of 350:650 v/v and used as a mobile phase B. The mobile phase gradient was started at 05% of B up to 10.00 minutes and increased to 15% B within 10 minutes then increased to 50% of B within 10 minutes then increased up to 80% within 20 minutes. The injection volume was 20µl. Diluent prepared by proper mixing of water and acetonitrile in the ratio of 20:80% v/v.

High-performance liquid chromatography (Preparative HPLC)

Preparative HPLC is the technique which is used for isolation and purification of the compound. Shimadzu LC10 purification system was used to get good recovery with pure compund, with PDA detector and the flow rate is 5 ml/min with. An ODS-C18, 250 mm × 21.2 mm × 10µm reverse phase silica column was employed for the separation unknown impurity. The mobile phase used for the separation was 20 mM ammonium acetate buffer as a mobile phase A and acetonitrile as mobile phase B, with the detection of 244 nm. The mobile phase gradient was started at 25% of B and increased to 65% B within 80 minutes then increased to 90% of B within 3 minutes.

MASS spectroscopy (LC-MS/MS)

The analysis was conduct in positive ionization mode with an electrospray interface in the range of 100-550 m/z. The parameters for the capillary and Rf voltage were 80 V with nebulizer gas air at a pressure of 35 psi and curtain gas as nitrogen at a pressure of 10 psi.

The mass compatible method was developed to identify the unknown impurity of Atorvastatin. An YMC pack ODS, 250 × 4.6 mm, 5µm column was used for analysis with a flow rate of 0.9 ml/minutes. The analyte peaks were monitored and detection wavelength 244 nm. 20mM acetate buffer pH 5.0 was used as mobile phase A. Mobile phase prepared by

proper mixing of acetonitrile and THF (tetrahydrofuran) in the ratio of 95:5% v/v. The gradient was started at 25% of B and increased to 65% B within 80 minutes then increased to 90% of B within 3 minutes. The injection volume was 10µl. A mixture of water and acetonitrile in the ratio of 20:80% v/v was used as a diluent.

NMR Spectroscopy

NMR spectra were obtained from a Bruker spectrometer using tetramethylsilane (TMS) as an internal standard in MeOD. Chemical shifts were in ppm concerning TMS. Coupling constants were in Hz.

RESULTS AND DISCUSSION

During the forced degradation study of Atorvastatin and Ezetimibe tables in the presence of oxidative agent such as hydrogen peroxide in basic condition, degradation impurity was generated. The reversed-phase HPLC method for related substances test was developed for Atorvastatin and Ezetimibe tablets and validated as per ICH. The sample was stressed in oxidative condition and analyzed in a validated HPLC method for related substances of Atorvastatin and Ezetimibe Tablets. In HPLC chromatogram degradation impurity was observed at relative retention time 1.84 (Fig. 1). Based on the UV spectrum of unknown peak at RRT-1.84, it was concluded that the peak of unknown impurity at RRT-1.84 is a degradation product of Atorvastatin (Fig. 2).

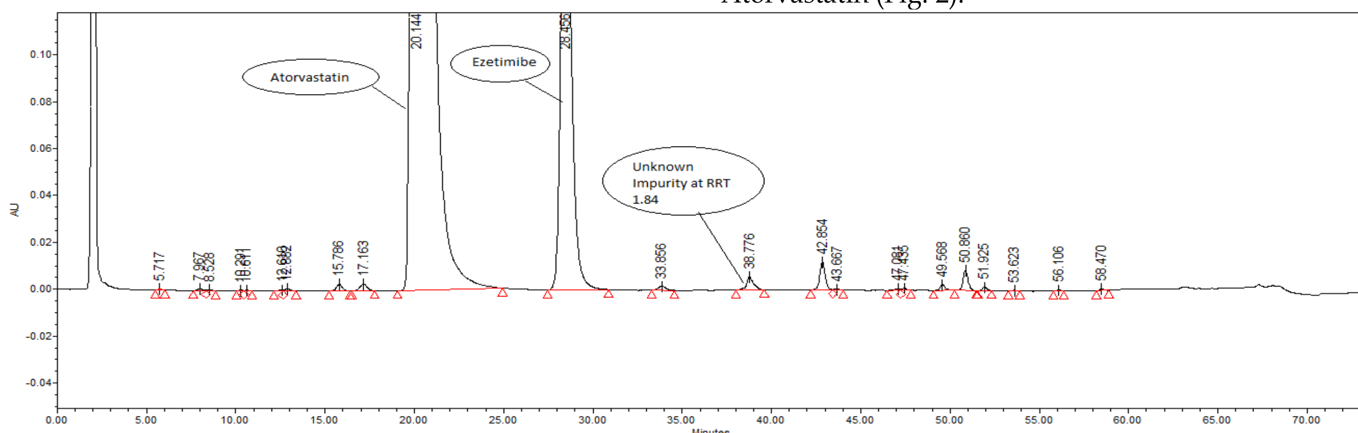


Fig. 1: Oxidative stress sample of Atorvastatin and Ezetimibe Tablets analyzed in HPLC system (Unknown degradation at eluted at relative retention time 1.84)

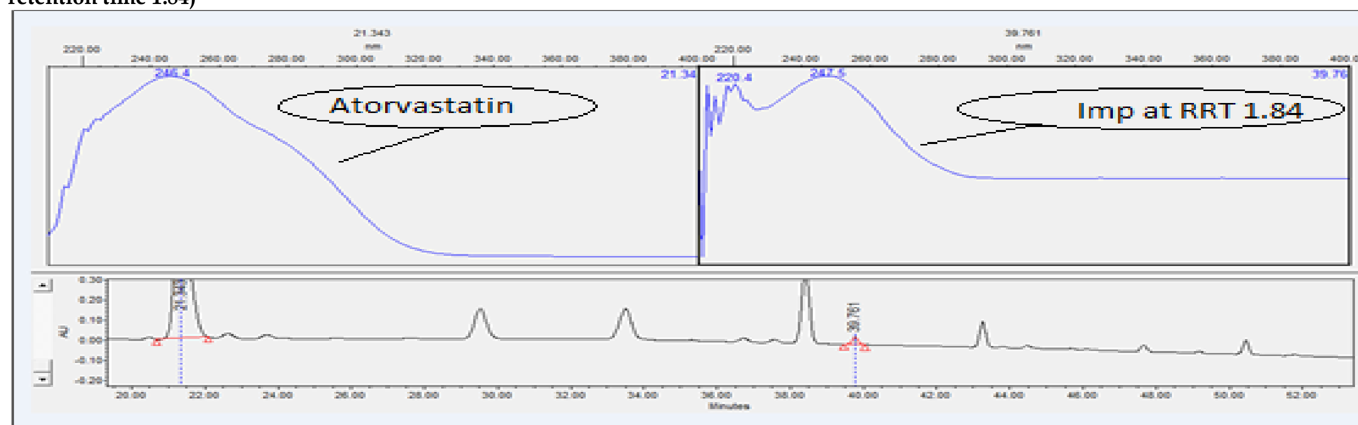


Fig. 2: UV spectrum of Atorvastatin and unknown impurity at RRT-1.84

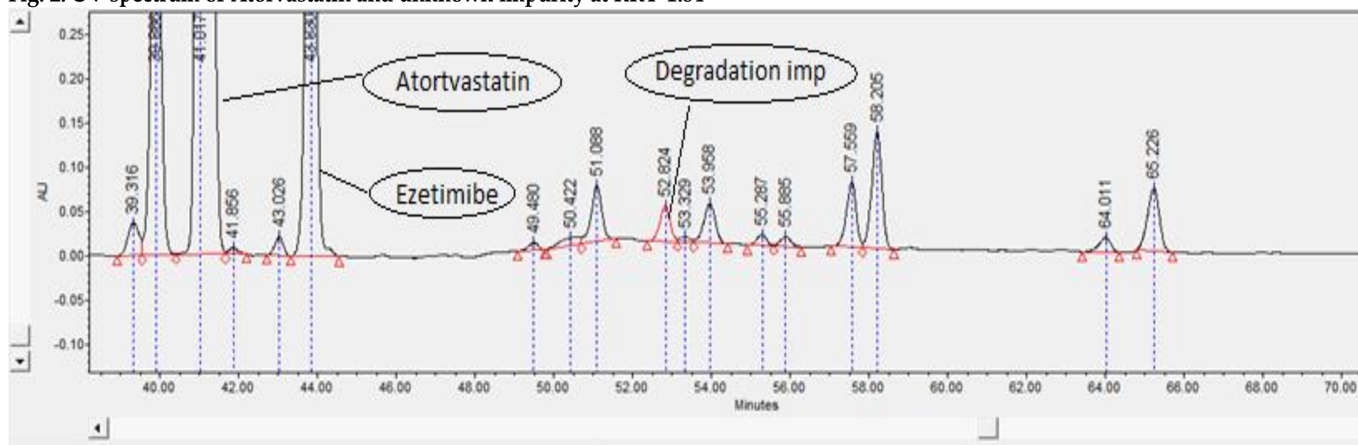


Fig. 3: Chromatogram of oxidative degradation sample in mass compatible HPLC method.

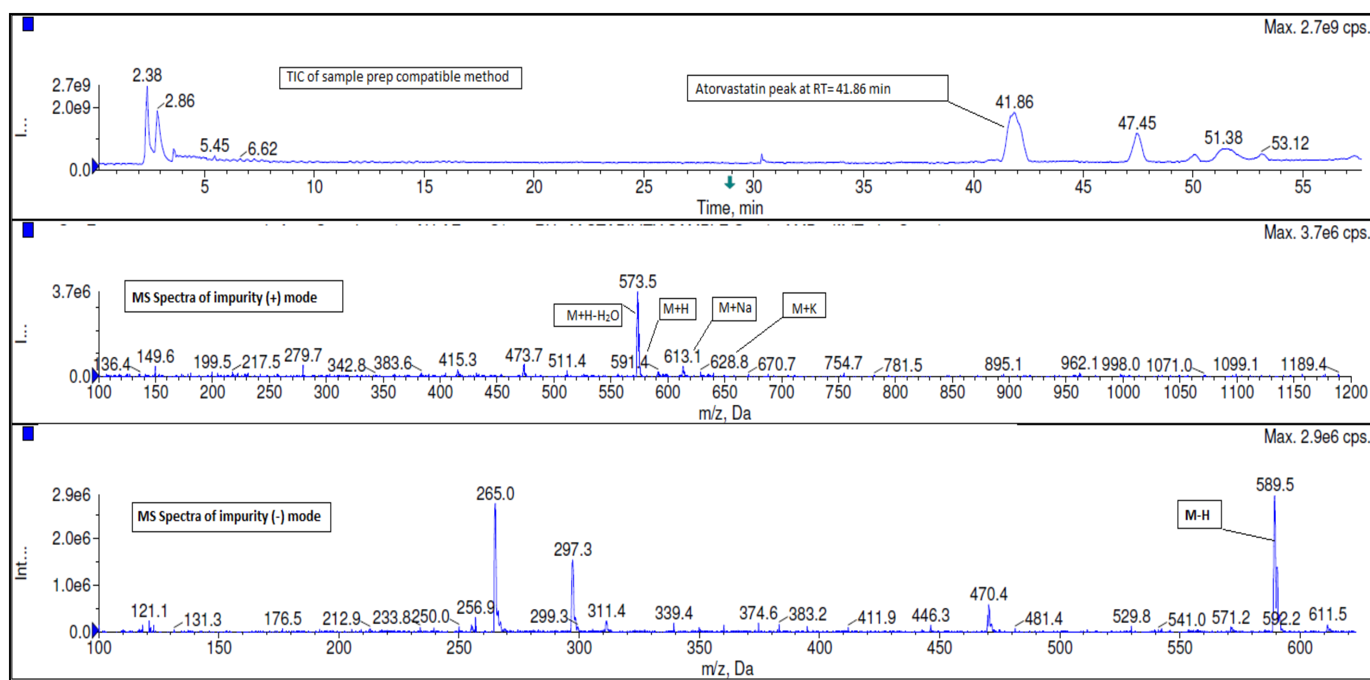


Fig. 4: LCMS/MS spectrum of degradation sample.

The oxidative degradation sample of Atorvastatin and Ezetimibe tablets was injected in the system to mass compatible HPLC method and the peak of interest eluting at 53.824 minutes (Fig. 3).

To identify the unknown impurity generated in Atorvastatin and Ezetimibe tables, the sample was injected in the LCMS/MS system to ascertain the unknown peak of interest eluting at retention time 52.824 (RRT-1.29) using the same chromatographic condition (Fig. 4).

The molecular ion peak of unknown impurity corresponding to RRT = 1.29 was deduced to be m/z = 589.5 in negative mode and m/z = 591.4 in positive mode.

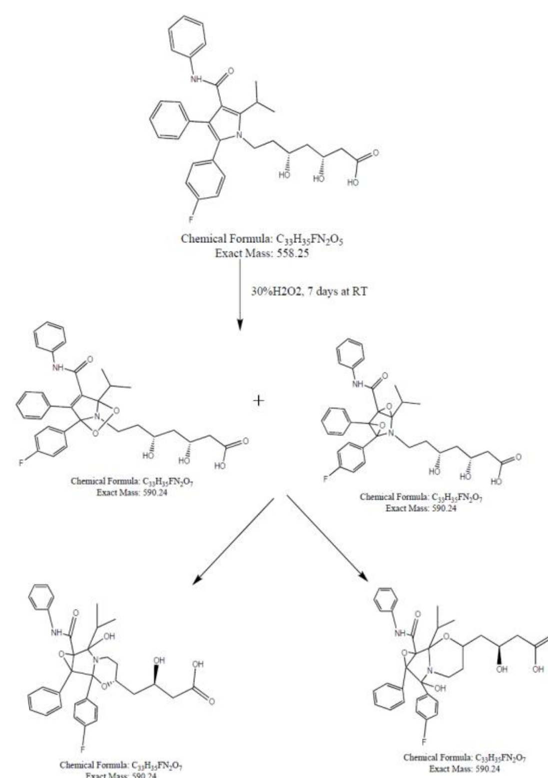
The oxidative degradation of atorvastatin forms endoperoxide from pyrrole ring. MSMS fragmentation of the Atorvastatin shows the probable degradation (Fig. 6). Further, this impurity also showed loss of water molecule M-18 = 573.5, M+Na = m/z = 613.1 and M+K = m/z = 628.8 corresponding to sodium and potassium ion adducts respectively. The most intense peak of 573.5 if the formation of the cyclic derivative of Atorvastatin. Daughter ion spectral data is also shown in Fig. 4 which confirms many fragments. Its details are shown in Fig. 5. Three common cleavage Sites are depicted which are common fragments of unknown impurity with atorvastatin (Table 1).

Table 1: Fragment mass from the spectral study of degradation impurity of atorvastatin.

Fragment mass of unknown impurity	375.17amu	120.04amu	480.21amu
Fragment mass of Atorvastatin	438.21amu	397.17amu	361.17 and 447.21amu

Probable structure of the impurity was derived by LCMS/MS fragmentation and to confirm the structure of the degradation impurity, the impurity was isolated by preparative HPLC. The chromatogram and spectra are shown to isolate the degradation compound, impurity was enriched and reparative HPLC method was developed. The impurity was isolated by preparative HPLC techniques. Approximate 9.070 mg material was isolated and used for further characterization study.

Mass and NMR of the isolated material were done to confirm the structure of impurity.



Scheme 1: Possible oxidative degradation of atorvastatin

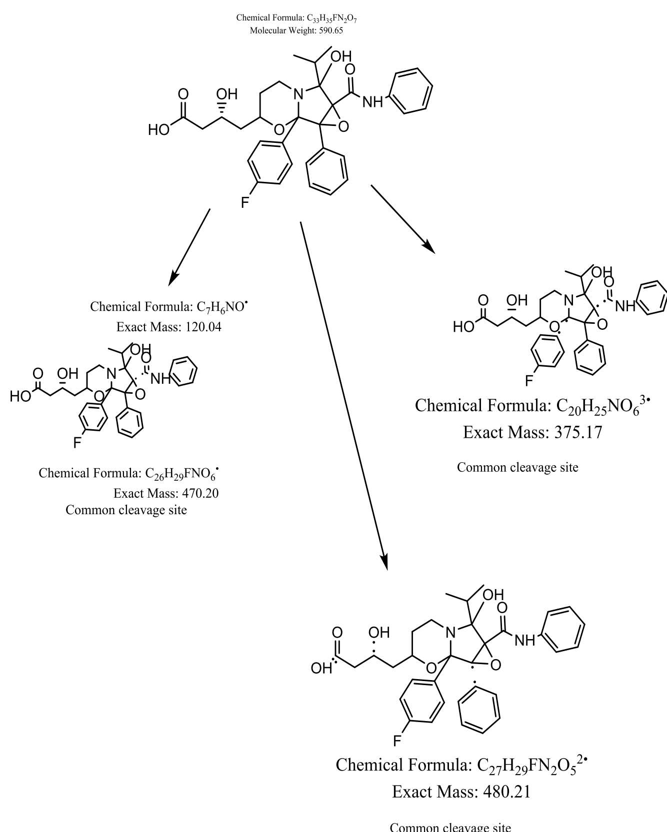


Fig. 5: MSMS fragmentation of unknown impurity at RRT-1.29

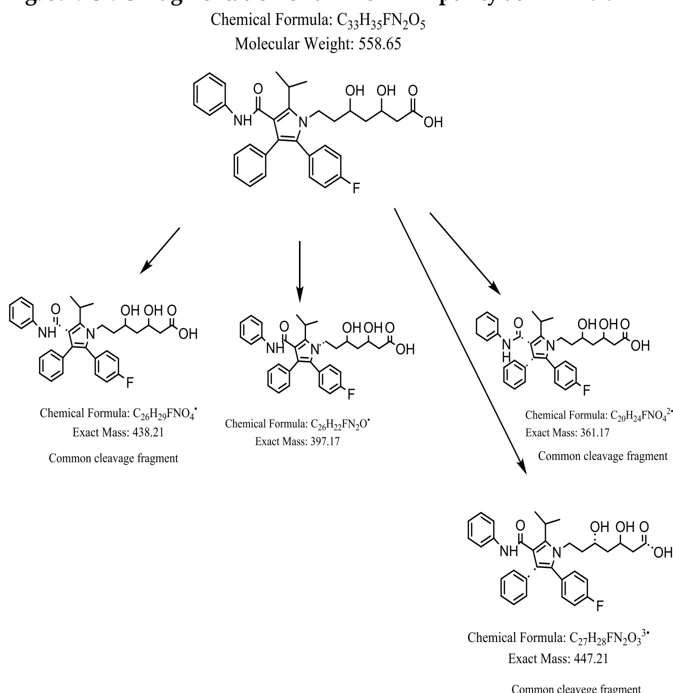


Fig. 6: MSMS fragmentation of unknown impurity at Atorvastatin

Mass analysis

The MS spectrum was taken in positive and negative mode co-relates the spectrum obtained (Figure 8 and 9) where molecular ion peak (m/z = 591.6) is lesser in intensity than base peak (m/z = 573.4) in positive mode. This is attributed to the fact that loss of water molecule is more prone from proton attached to the cyclo propyl group in MS instrument because of temperature and voltages applied leading to greater intensity of m/z = 573.4. In negative ion mode, a similar pattern was observed. Molecular ion peak is clearly visible m/z = 589.4 in MS spectra.

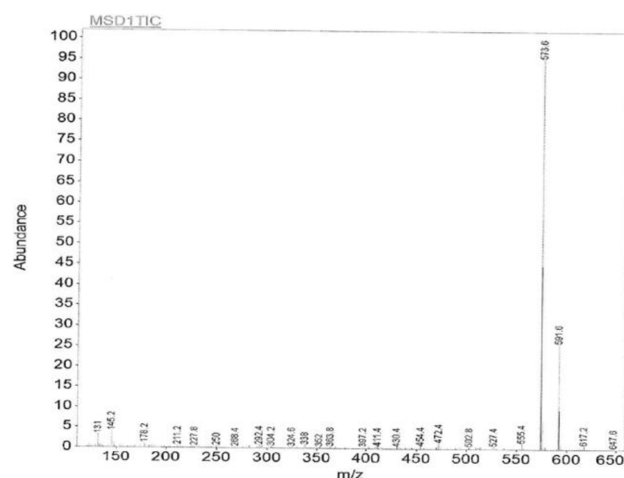


Fig. 7: MS spectrum of unknown impurity (Positive mode)

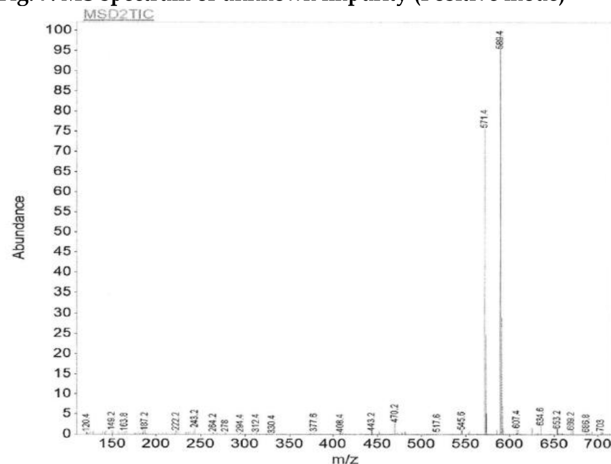


Fig. 8: MS spectrum of unknown impurity (Negative mode)

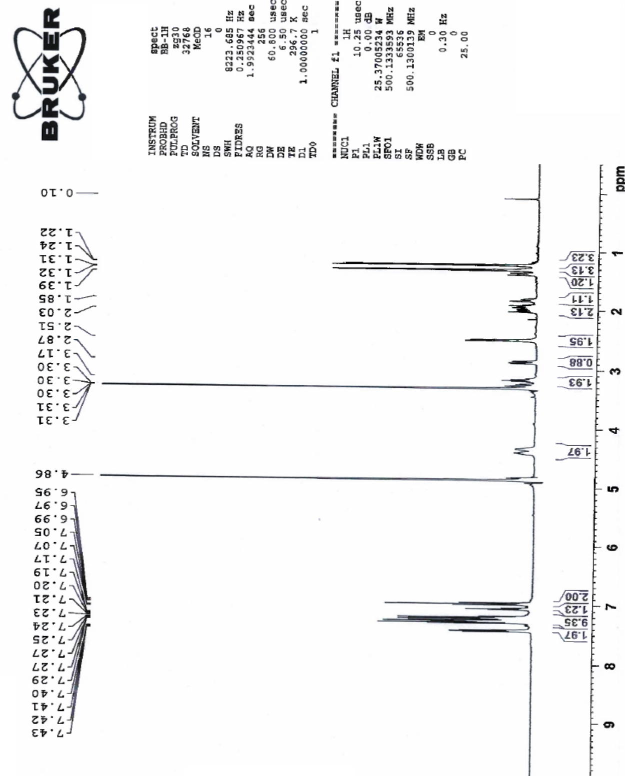


Fig. 9: ¹H NMR spectrum of unknown impurity

1H NMR spectrum analyzed in MeOD shows signals as mentioned below table,

Table 2: NMR shift

Proton	Chemical shift in PPM	Proton group
6	1.22 - 1.24 and 1.31 - 1.32	-CH3, -CH3
1	1.39	CH
1	1.85	CH
2	2.03	-CH2
2	2.51	-CH2
1	2.87	-CH (Pyrol ring)
2	3.17	-CH2
2	4.51	-CH2
2	6.96 - 6.99	Ar-CH
2	7.05, 7.07	Ar-CH
9	7.17- 7.29	Ar-CH
2	7.40 – 7.43	Ar-CH

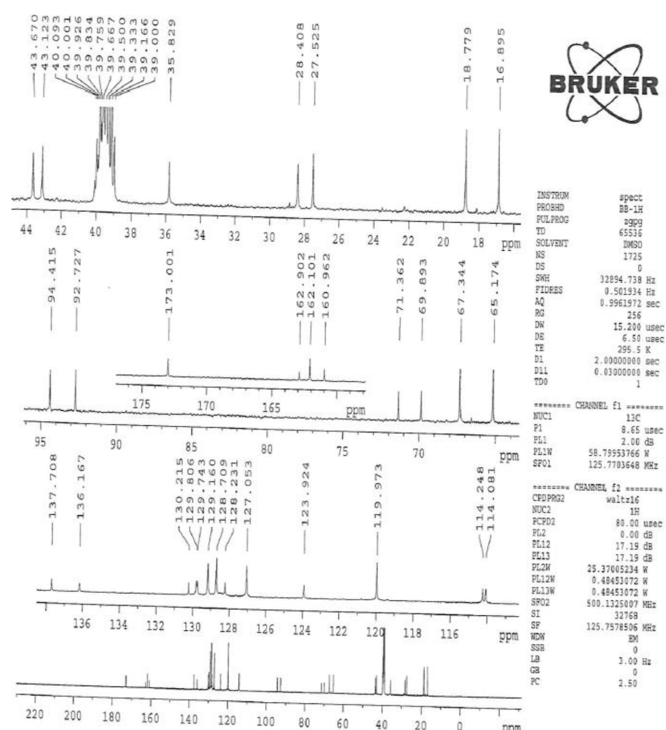


Fig. 10: ^{13}C NMR spectrum of unknown impurity

^{13}C NMR spectrum shows signals 68.990 ppm, 72.298 ppm, 93.174 ppm and 94.758 ppm corresponding to pyrrole C-5′,C-4′,C-3′ and C-2′ respectively in the ^{13}C NMR spectrum as shown below. This confirms the breakage of 5 membered pyrrole ring of atorvastatin leading to an upfield shift of these signals.

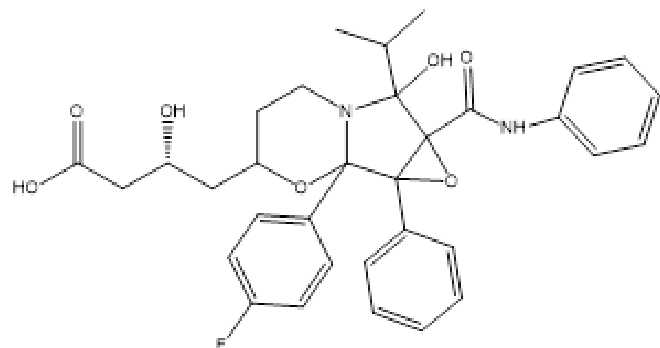


Fig. 11: Chemical structure of unknown impurity at RRT-1.84

DISCUSSION

Degradation condition for oxidative stress was optimized to enrich the unknown impurity at RRT-1.84 in Atorvastatin and Ezetimibe Tablets. Unknown degradation impurity is generating by oxidative degradation of Atorvastatin, which was identified by UV spectrum of the impurity and active drug. To isolate an unknown oxidative degradation impurity by preparative HPLC, the method was developed and the compound was isolated. The isolated impurity was analyzed by LCMS/MS with the mass compatible method. Based on the fragmentation pattern and probable degradation pattern shows two unstable structural isomeric compounds which is formed the cyclic analog of the Atorvastatin by the cyclization to the pyrrole ring. The isolated unknown impurity of atorvastatin was determined by quantitative NMR spectroscopy to be 4-[1b-(4-Fluoro-phenyl)-6-hydroxy-6-isopropyl-1aphenyl-6a-phenylcarbamoyl-hexahydro-1,2-dioxa-5a-aza-cyclopropa[a]inden-3-yl]-3-(R)-hydroxy-butyric acid. The isolated, identified and characterized impurity was injected in the HPLC method and included in validation study to prove precision, linearity, the accuracy of the impurity in the developed method.

REFERENCES

1. Leiter LA, Bays H, Conard S, et al. Efficacy and Safety of Ezetimibe Added on to Atorvastatin (40 mg) Compared With Uptitration of Atorvastatin (to 80 mg) in Hypercholesterolemic Patients at High Risk of Coronary Heart Disease. Am J Cardiol. 2008;102(11):1495-1501.
2. https://wenku.baidu.com/view/35a9708f9ec3d5bbfd0a74f3?pcf=2&re=view.
3. Filip K, Bańkowski K, Sidoryk K, *et al*. Physicochemical characterization of ezetimibe and its impurities. J Mol Struct. 2011; 991(1-3):162-170.
4. Kumar P, Ghosh A, Chaudhary M. Stability Indicating Method Development for Simultaneous Estimation of Ezetimibe and Atorvastatin in Pharmaceutical Formulations by RP-HPLC. Pharm Anal Acta. 2012;03(06):164.
5. Rajasekaran A, Sasikumar R, Dharuman J. Simultaneous RP-HPLC method for the stress degradation studies of atorvastatin calcium and ezetimibe in multicomponent dosage form. Ars Pharm. 2011;52(3):12-18.
6. Yusuf SM, Samparna S, Yallareddy K, Pavani B, Sivakala T. Analytical method development and validation of atorvastatin and clopidogrel in tablet dosage form by RP-HPLC. EJPMR 2017;4(4):553-558.
7. Kadav AA, Vora DN. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. J Pharm Biomed Anal. 2008;48(1):120-126.
8. Talluri MVNK, Kalyankar A, Ragampeta S. Synchronized separation of atorvastatin—an antihyperlipidemic drug with antihypertensive, antidiabetic, antithrombotic drugs by RP-LC for determination in combined formulations. J Pharm Anal. 2012;2(4):285-292.
9. Suresh R, Manavalan R, Valliappan K. Developing and optimizing a validated RP-HPLC method for the analysis of amlodipine and ezetimibe with atorvastatin in pharmaceutical dosage forms applying response surface methodology. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(3):550-558.
10. Londhe SV, Deshmukh RS, Mulgund SV, Jain KS. Development and Validation of a Reversed-phase HPLC Method for Simultaneous Determination of Aspirin,

Atorvastatin Calcium and Clopidogrel Bisulphate in Capsules. Indian J. Pharm. Sci., 2011;73(1):23-29.
11. Hafez HM, Elshanawany AA, Abdelaziz LM, Mohram MS. Development of a Stability-Indicating HPLC Method for Simultaneous Determination of Amlodipine Besylate and Atorvastatin Calcium in Bulk and Pharmaceutical Dosage Form. Pharm Anal Acta. 2014; 5(9):316.
12. https://sg.inflibnet.ac.in/bitstream/10603/27294/7/07_chapter%202.pdf.
13. Dubey S, Kumar Pandey R, Shankar Shukla S. Impurity Profiling and Drug Characterization: Backdrop and Approach. Indo Am. J P Sci. 2018;(04):2499-2515.
14. Nigovic B, Mornar A, Sertic M. A Review of Current Trends and Advances in Analytical Methods for Determination of Statins: Chromatography and Capillary Electrophoresis. In: Chromatography - The Most Versatile Method of Chemical Analysis. InTech; 2012.
15. Gupta LK. Spectroscopic characterization and quantitative determination of atorvastatin calcium impurities by novel HPLC method. Spectrochim Acta Part A Mol Biomol Spectrosc. 2012; 97:495-501.
16. Ertürk S, Sevinç Aktaş E, Ersoy L, Fıçıcıoğlu S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm Biomed Anal. 2003;33(5):1017-1023.
17. Sonje VM, Kumar L, Meena CL, *et al*. Atorvastatin Calcium. Profiles Drug Subst Excipients Relat Methodol. 2010;35:1-70.
18. Kumar KK, Rao CK. A Validated Stability Indicating RP-UPLC Method for Atrovastain Calcium. American Journal of Analytical Chemistry. 2012;3:392-399.
19. K. Gajjar A. Impurity Profiling: A Case Study of Ezetimibe. Open Conf Proc J. 2011;2(1):108-112.
20. Sistla R, Tata VSSK, Kashyap YV, Chandrasekar D, Diwan P V. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. J Pharm Biomed Anal. 2005;39(3-4):517-522.
21. Gajjar AK, Shah VD. Isolation and structure elucidation of major alkaline degradant of Ezetimibe. J Pharm Biomed Anal. 2011;55(1):225-22.

HOW TO CITE THIS ARTICLE: Somwanshi SV, Thonte SS. Isolation, Identification and Characterization of Degradation Impurity of Atorvastatin in Fixed Dose Combination of Atorvastatin and Ezetimibe. Int. J. Pharm. Sci. Drug Res. 2019; 11(5): 187-193. **DOI: 10.25004/IJPSDR.2019.110506**