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STRESS DEGRADATION STUDY OF PITAVASTATIN BY LC-ESI/MS/MS

A. Ramesh Babu[1], Murali Mohan Bhandi[2], Harika Batula[3], R. Srinivas[4]

[1,2,3,4]National Centre for Mass Spectrometry,
CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, (India)

ABSTRACT

Degradation pathway for pitavastatin calcium is established by using LC-ESI-MS method. Pitavastatin was subjected to hydrolytic, photolytic, thermal and oxidative stress conditions as per ICH guidelines Q1A (R2). Pitavastatin showed significant degradation under acidic, alkaline and oxidative stress condition. No degradation products were observed in neutral, thermal and photolytic condition. A total of five degradation products (DPs) were formed. Efficient chromatographic separation of the DPs was achieved on a Waters C-18 column (4.6 X 250mm, 5µm) using mobile phase consisting of acetonitrile and 0.1% of formic acid delivered in gradient mode. Most probable structures of DPs were proposed based on their m/z values in the LC/ESI mass spectra and supported by LC/ESI/MS/MS in combination with accurate mass measurements.

Keywords: Pitavastatin; Stress degradation, Degradation Products; LC/ESI/MS/MS.

I INTRODUCTION

Pitavastatin (PIT), (3R, 5S, 6E)-7-[2-cyclopropyl-4-(pfluorophenyl)-3-quinolyl]-3, 5-dihydroxy-6-heptenoic acid, used as the calcium salt in the treatment of hyperlipidemia, is a novel, fully synthetic statin, which has a more potent cholesterol-lowering action than other drugs in its class. Like the other statins, it is an inhibitor of HMG-CoA reductase, the enzyme that catalyses the first step of cholesterol synthesis [1-4].

Literature survey revealed that, there are some analytical methods reported for determination of pitavastatin in plasma and biological fluids [5-6]. Few analytical methods such as spectrophotometry [7], HPLC [8], HPTLC [9], and UPLC [10] have been reported for estimation of pitavastain. A thorough literature survey revealed that no information exists in the literature on the degradation pathway and fragmentation pattern of pitavastin and its degradation products under mass spectral condition. Therefore, the present study focuses on the identification and structural characterization of the degradation products of pitavastin by using LC-ESI/MS/MS. Stress degradation studies of pitavastatin is carried out according to ICH guidelines [11-12].

II EXPERIMENTAL

2.1. Materials

Pitavastatin was obtained as gift sample from NIPER, Hyderabad. Analytical reagent (AR) grade sodium hydroxide (NaOH) was purchased from S.D. Fine-Chem Ltd. (Mumbai, India), hydrochloric acid (HCl), HPLC grade methanol (MeOH) and acetonitrile (ACN) from Merck Specialties Pvt. Ltd. (Mumbai, India) and

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hydrogen peroxide (H_2O_2) from Qualigens Fine Chemicals Pvt. Ltd. (Mumbai, India). Ultra pure water obtained from Millipore water purification system (Molsheim, France) was used throughout the studies.

2.2. Equipment and Chromatographic condition

Characterization of degradation products was carried out using ion trap LC-MS^n (Thermo Scientific). The chromatographic separation was achieved on a C18 column by using a mixture of acetonitrile–0.1% formic acid as mobile phase in gradient mode. The proposed structures of degradation products were established using LC/MS/MS LCQ Advantage Max ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source. The data acquisition was under the control of Xcalibur software (Thermo Finnigan).

2.3. Stressed degradation studies

Stress studies are performed according to ICH guidelines, Pitavastatin (PIT) is subjected to acid, base and neutral hydrolysis and thermal, oxidative and photolytic stress conditions. Stress degradation studies of PIT were carried out under the ICH prescribed conditions of hydrolysis (acidic, alkaline, and neutral), photolysis, oxidation and thermal stress. Acidic, basic and neutral hydrolysis was carried out by refluxing PIT with 2N HCl for 24h, 2N NaOH for 24h and water for 24 h, respectively at room temperature. Oxidative study was carried out with 6% H_2O_2 at room temperature for 72 h. The photo stability study was carried out by exposing PIT to 1.2×10^6 lux hr of fluorescent light and 200 Watt hr m^{-2} UV light in a photo stability chamber. For thermal degradation study, PIT was spread over Petri dish and kept at 60° C for 10 days.

2.4. Sample preparation

All stress samples were diluted 10 times with the mobile phase. All degradation studies were carried out with a drug concentration of 1 mg mL^{-1}. All solutions were filtered through 0.22 µm pore size nylon 66 membrane filter before LC-MS analysis.

III RESULTS AND DISCUSSION

The separation of PIT and its degradation products was achieved on a Waters C-18 column (4.6 X 250mm, 5µm) using the mobile phase consisting of acetonitrile (A) and 0.1% of formic acid in water (B) in gradient mode at flow rate of 0.4 mL/min. Figures 1-3 shows the LC-ESI-MS total ion and extracted ion chromatograms of unreacted drug, degradation products formed under acidic, basic and oxidative stress conditions. A total of three degradation products are formed under these conditions. DP-I is formed both in acidic and basic stress condition. DP-III and DP-IV were formed under oxidative stress condition.

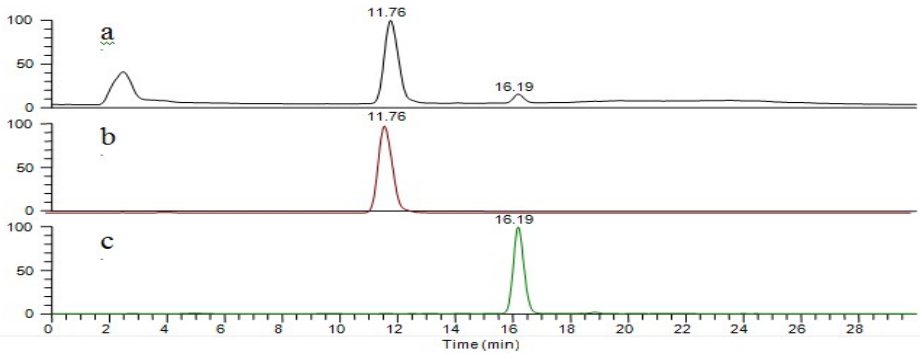

Figure1: LC/ESI/MS total ion chromatogram of the drug and its degradation products (a), extracted ion chromatogram of Drug (b) and DP-I (c) under acid degradation condition.

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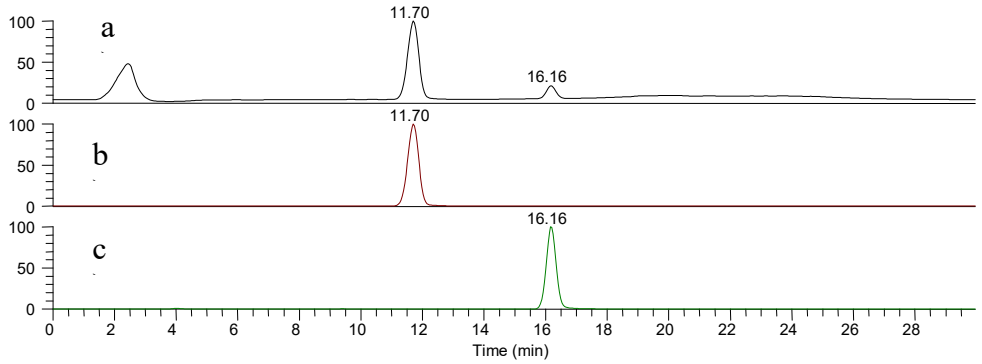

Figure 2: LC/ESI/MS total ion chromatogram of the drug and its degradation products (a), extracted ion chromatogram of Drug (b) and DP-I (c) under base degradation condition.

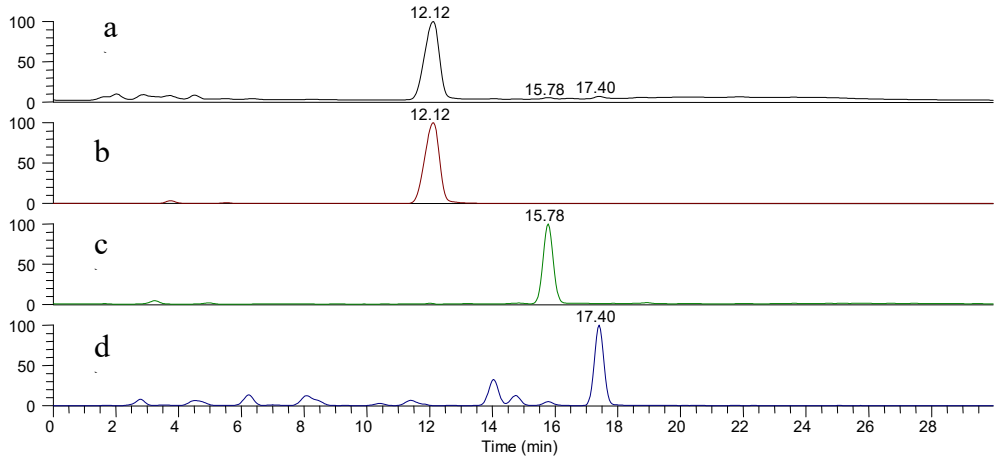

Figure 3: LC/ESI/MS total ion chromatogram of the drug and its degradation products (a), extracted ion chromatogram of Drug (b), DP-III (c) and DP-IV (d) under oxidative stress condition.

In addition to DP-I, other degradation products like DP-II and DP-V are observed in acidic degradation and DP-V is observed in basic stress condition when methanol is used as solvent (fig 4 and 5).

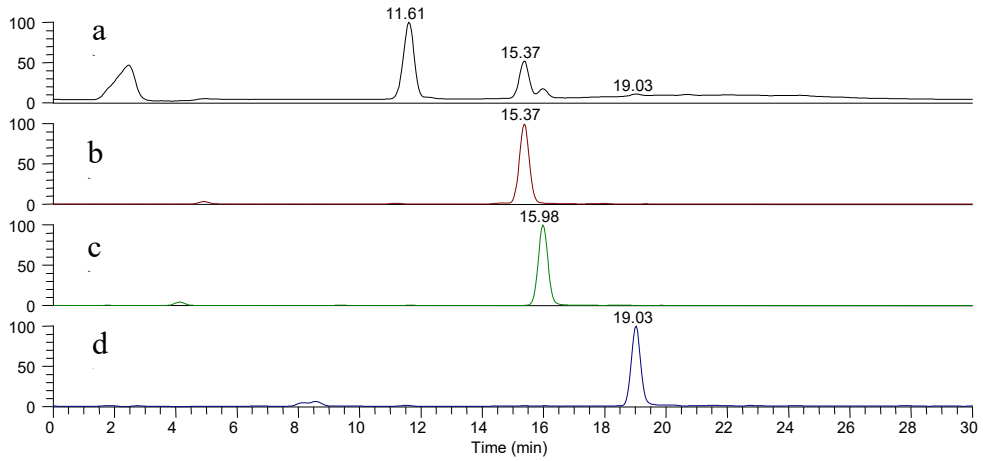

Figure 4: LC/ESI/MS total ion chromatogram of the drug and its degradation products (a), extracted ion chromatogram of DP-V (b), DP-I (c) and DP-II (d) under acidic stress condition. (When MeOH is used as solvent).

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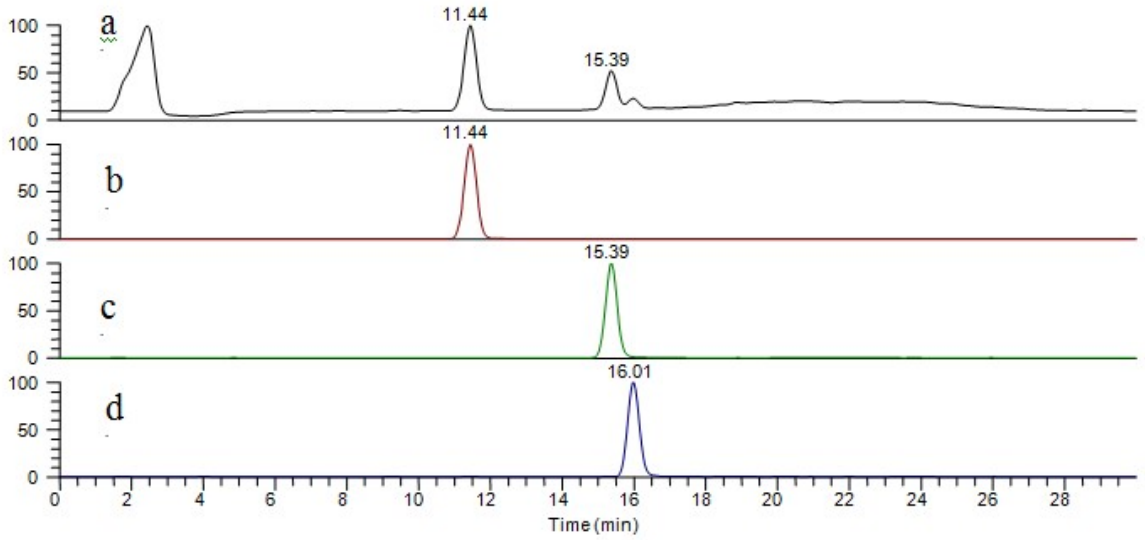

Figure 5: LC/ESI/MS total ion chromatogram of the drug and its degradation products (a), extracted ion chromatogram of drug (b), DP-V (c) and DP-I (d) under base stress condition. (When MeOH is used as solvent).

3.1. ESI-MS/MS of PIT

To investigate the degradation behavior of pitavastatin, MS/MS fragmentation of its [M+H]+ was examined. The positive ion ESI-MS of PIT shows an abundance $[M+H]^+$ ion at *m/z* 422 and its MS/MS spectrum displays the fragment ions at *m/z* 404, m/z 386, *m/z* 362, *m/z* 318 and *m/z* 290 (Figure 6 and Scheme 1). The ion at m/z 404 is formed due to loss of water from the parent ion (m/z 422). The base peak appears at *m/z* 290 and is also observed in most of the degradation products. Based on the fragmentation pattern of the pitavastatin the structures and fragmentation pattern of degradation products are established.

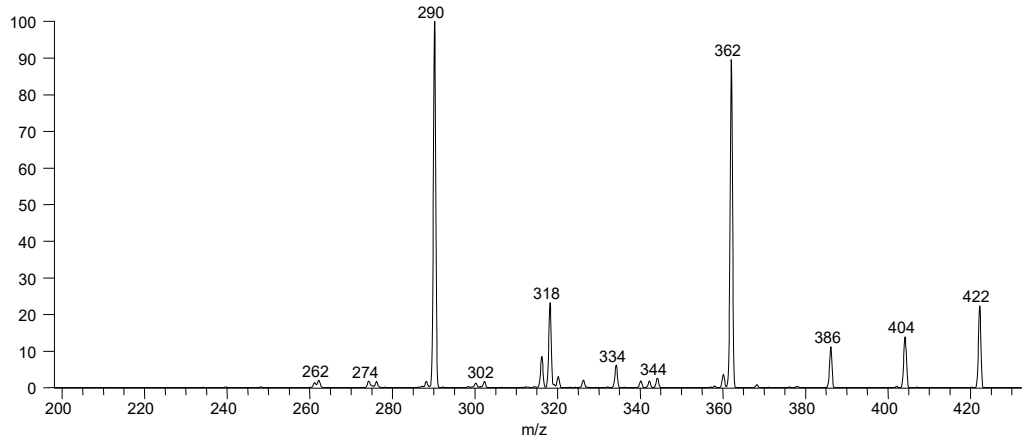

Figure 6: ESI/MS/MS Spectrum of Pitavastatin (*m/z* 422)

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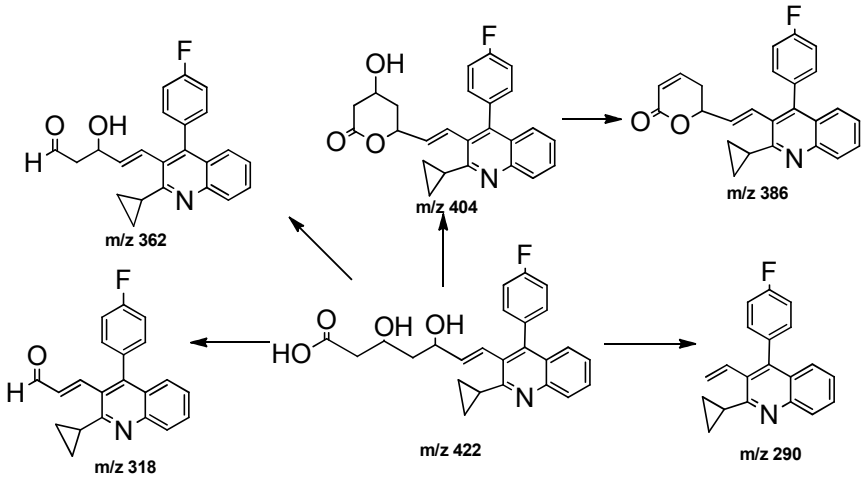
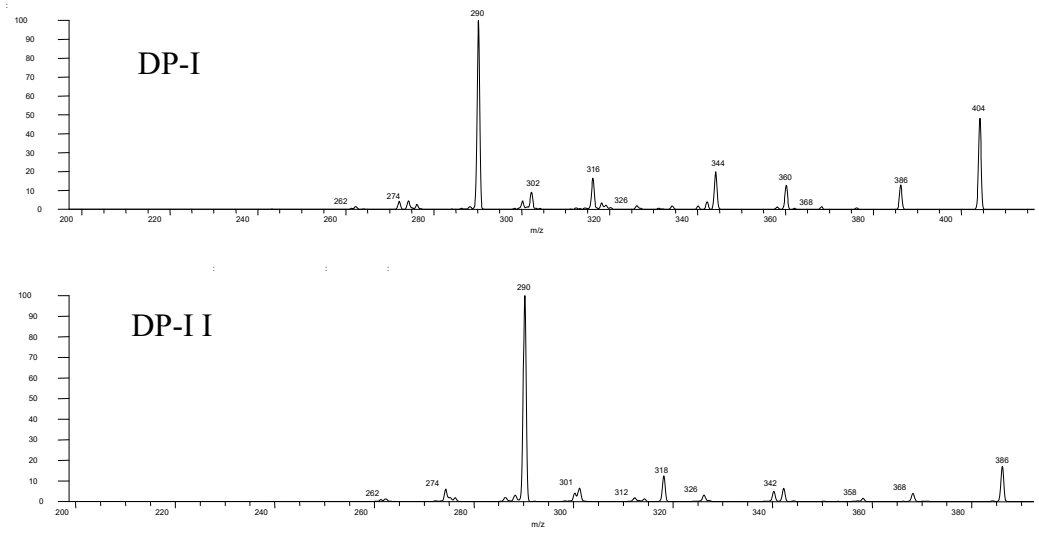


Scheme 1: Proposed fragmentation pathway of Pitavastatin (*m/z* 422) (charges on the ions are missing)

3.2. Characterization of DPs by LC/MS/MS To establish the structures of DPs, ESI/MS/MS of their protonated ions were carried. The spectra are compared with the MS/MS spectrum of protonated pitavastatin. The LC-ESI-MS spectrum of DP-I shows $[M+H]^+$ ion at *m/z* 404. Its MS/MS spectrum exhibits the product ions at *m/z* 290, *m/z* 386, *m/z* 360 and *m/z* 344. Based on ESI/MS/MS spectra, lactone containing structure is proposed for DP-I (Scheme 2) f The ESI/MS/MS spectrum of protonated DP-II shows a product ion at *m/z* 290. The LC/MS spectrum of DP-III shows an ion at *m/z* 438 which is 16 units mass higher than PIT indicating that its an N-Oxide of the drug,. Its MS/MS spectrum shows product ions at *m/z* 420 and 402. The LC/ESI/MS spectrum of DP-IV shows an ion at *m/z* 420 (N-Oxide of lactone form) and its MS/MS spectrum shows an ion at *m/z* 288. The LC/ESI/MS spectrum of DP-V shows a product ion at *m/z* 436 (methanol adduct) and its MS/MS spectrum shows product ions at *m/z* 418, m*/z* 400, *m/z* 362 and *m/z* 290 (Fig 7). The proposed structures and its fragmentation pattern of DPs are given in scheme 2 and 3 respectively.



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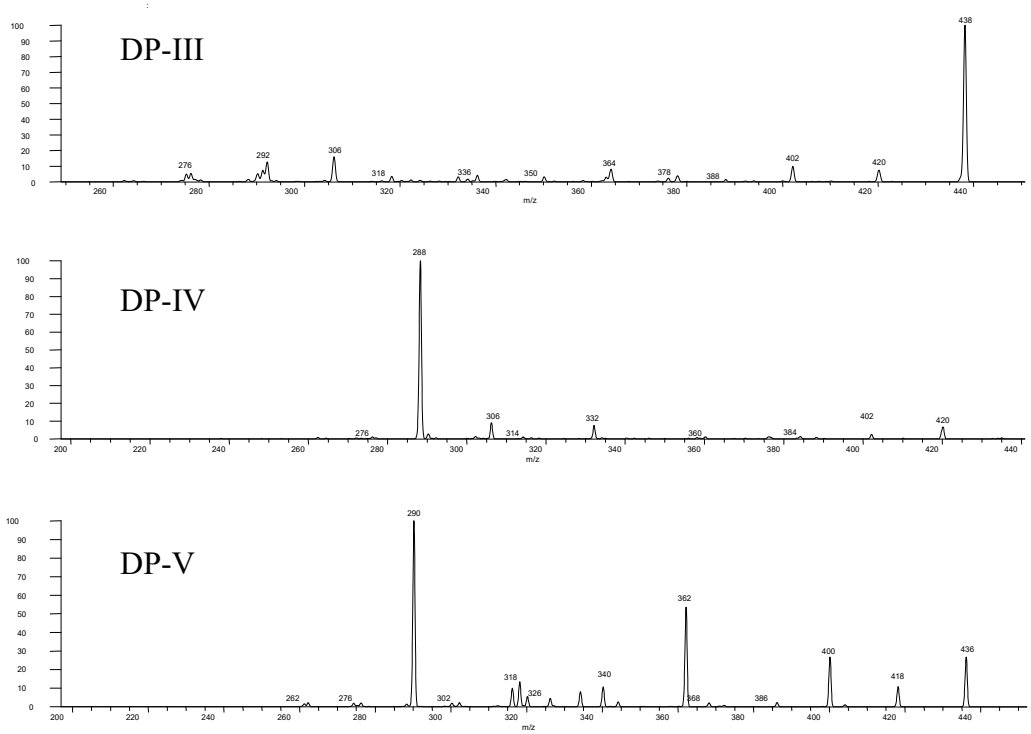
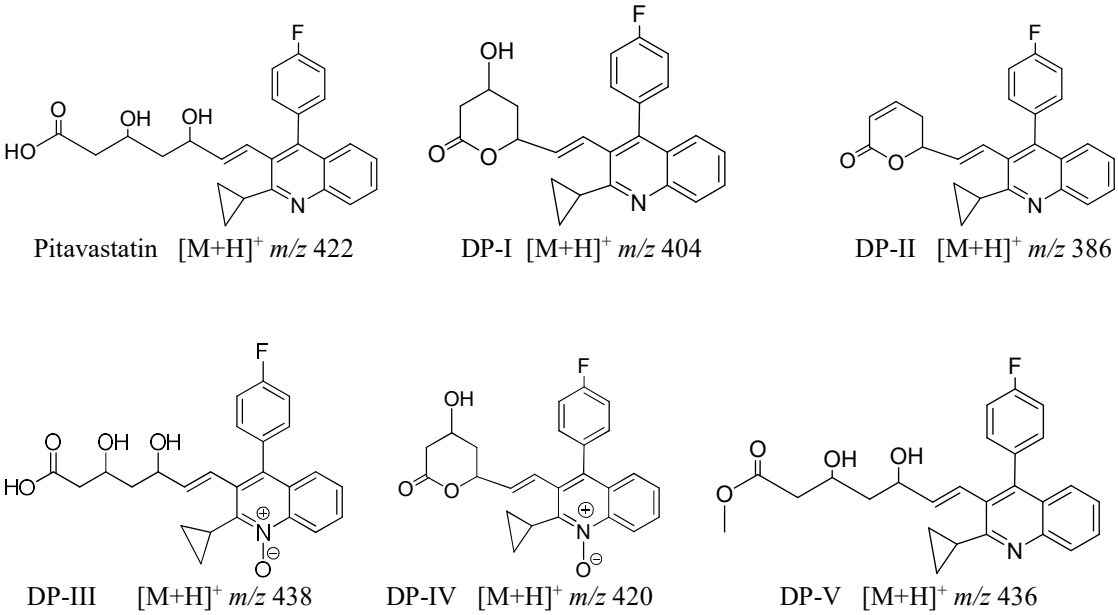


Figure 7: ESI/MS/MS Spectra of Degradation Products (DP-I to DP-V)



Scheme 2: Structure of Pitavastatin and Proposed Structures of its Degradation Products (DP-I to DP-V)

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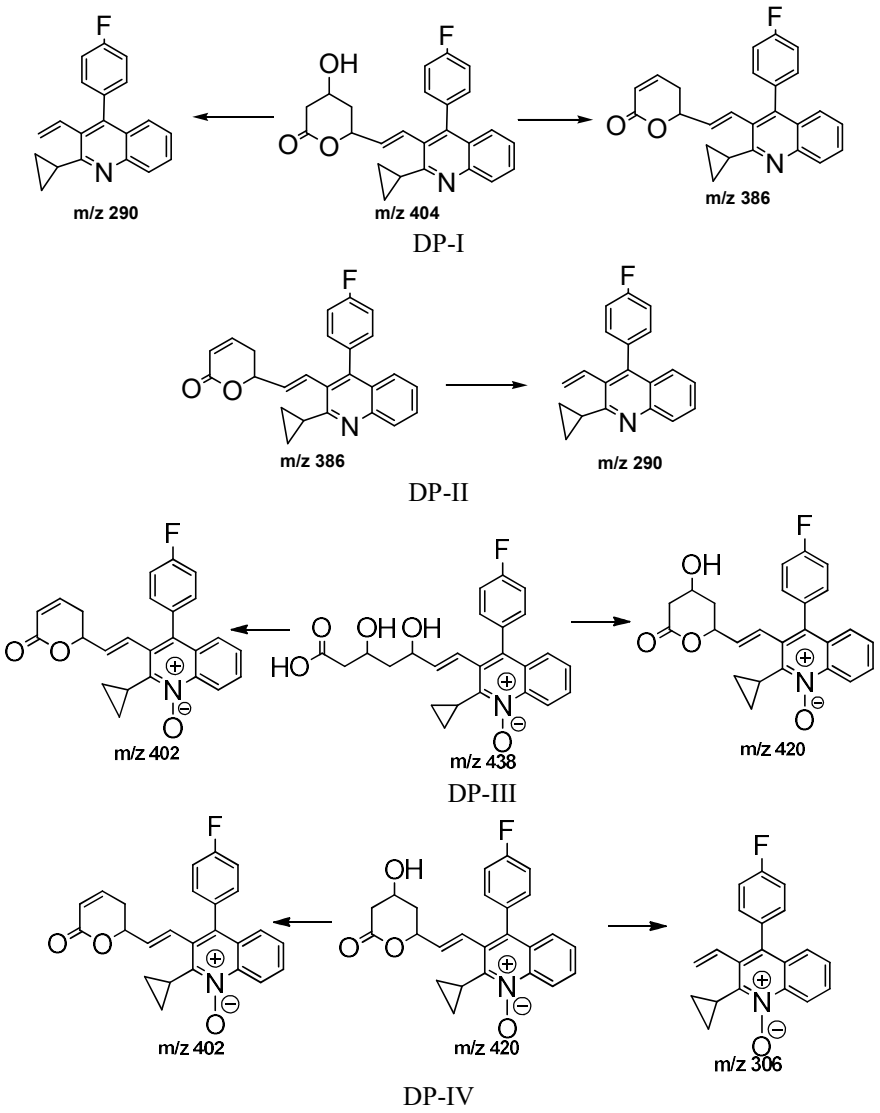

Scheme 3: Proposed Fragmentation Pathway of Degradation Products

IV CONCLUSION

The degradation pathway of pitavastatin is established by LC/ESI/MS/MS method as per the ICH guidelines. Pitavastatin undergoes significant degradation under acidic, basic and oxidative stress stress conditions. A total of five degradation products were observed and their structures were proposed based on LC/ESI/MS/MS of their protonated ions

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