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Structural identification and characterization of potential degradants of zotarolimus on zotarolimus-coated drug-eluting stents

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ABSTRACT

Identification and characterization of unknown zotarolimus impurities on zotarolimus-coated drug-eluting stents is an important aspect of product development since the presence of impurities can have a significant impact on quality and safety of the drug product. Four zotarolimus degradation products have been characterized by LC/UV/PDA, LC/MS, LC/MS/MS and NMR techniques in this work. Zotarolimus drug substance and zotarolimus-coated stents were subjected to degradation under heat, humidity, acid or base conditions. The HPLC separation was achieved on a Zorbax Eclipse XDB-C8 column using gradient elution and UV detection at 278 nm. All four impurities generated through the degradation were initially analyzed by LC/MS and/or LC/MS/MS for structural information. Then the isolation of these degradants was carried out by semi-preparative HPLC method followed by freeze-drying of the collected fractions. Finally the degradants were studied by ^{1}H and ^{13}C NMR spectrometry. Based on LC/MS, ^{1}H NMR and ^{13}C NMR data, the structures of these impurities were proposed and characterized as zotarolimus ring-opened isomer (**1**), zotarolimus hydrolysis product, 16-O-desmethyl ring-opened isomer (**2**) and zotarolimus lower fragment (**3**). Degradants **1**, **2** and **3** have been observed on degraded zotarolimus-coated stent products.
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1. Introduction

Zotarolimus (ABT-578), a synthetic tetrazole derivative of rapamycin (sirolimus) with molecular formula of $C_{52}H_{79}N_5O_{12}$ (Fig. 1), belongs to the class of macrolide compounds. This class of compounds has potent anti-inflammatory, antiproliferative and immunosuppressive properties [1–3]. Some of them have been used on drug-eluting stents to prevent angioplasty-induced neointimal formation in human coronary arteries. For example, sirolimus is used as the active pharmaceutical ingredient on *Cypher*™ stents [4]. On Xience V® stents, the active pharmaceutical ingredient is everolimus [5]. Zotarolimus has also been used as the active pharmaceutical ingredient on drug-eluting stents, such as on *Endeavor*™ and *ZoMaxx*™ [6–7]. This class of macrolide compounds has two functional molecular domains: (1) left part of the molecule serving as the binding domain, responsible for the binding to the intracellular cytosolic receptor; (2) right part of the molecule serving as the effector domain, contributing to the specific biological activity [8–9].

For zotarolimus, the binding domain has active adjacent carbonyl groups at C_8–C_9 and hemiketal 6-member ring, which can lead to the isomerization and tautomerization of the molecule. The effector domain features the triene moiety, which can introduce radical-initiated auto-oxidation of the compound. It is well known that this type of compound usually needs to be protected by an anti-oxidant such as butylated hydroxytoluene (BHT) during production and storage [10]. The ester bond of the compound can also undergo ester hydrolysis/dehydration or β-elimination to form a ring-opened isomer. Zotarolimus has 15 chiral centers and several reactive sites as discussed above. The presence of impurities and degradants in active pharmaceutical ingredients can have a significant impact on the quality and safety of final drug products.

Literature studies reveal several different studies on the separation and detection of zotarolimus, zotarolimus process impurities and equilibrium isomers. Christians *et al.* reported a specific LC/MS/MS method for the quantitation of zotarolimus in blood and tissue samples [11]. After protein precipitation treatment, the samples were injected into HPLC system and extracted online for LC/MS analysis. Sirolimus was used as internal standard for quantitation. There are several other papers reporting the quantitation of zotarolimus in whole blood samples using liquid–liquid extraction and LC/MS [12–13]. Dhaon *et al.* isolated and characterized the two major isomeric forms of zotarolimus, the major 6-member pyran form and a minor 7-member oxepane form as shown in Fig. 1 [14]. These two forms are typically in equilibrium with each other in 10:1 ratio. Chen *et al.* demonstrated the use of normal phase and reverse

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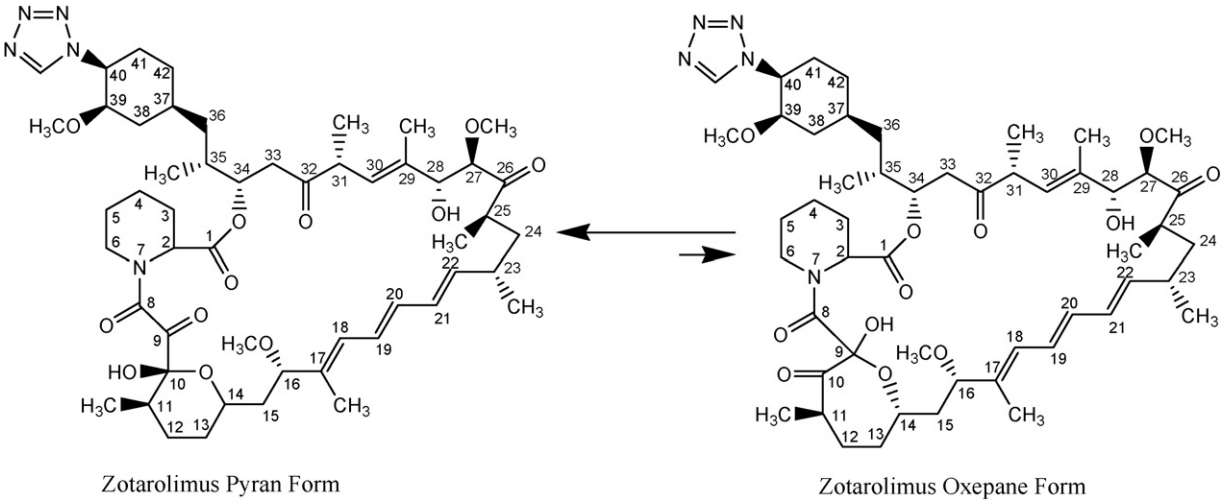


Fig. 1. Chemical structure of zotarolimus in pyran form and oxepane form.

phase HPLC/UV/MS methods for detecting and quantitation of starting materials, process impurities and isomers from zotarolimus synthesis process [15]. Their reverse phase HPLC method can separate 25 major starting materials and process related impurities such as rapamycin, N2 isomer, ditetrazole of zotarolimus, ring-opened isomer and reaction intermediates. A complimentary normal phase HPLC method is used for the separation of epimeric impurities and aqueous-sensitive reactive species. Even though the major degradation pathways for sirolimus have been reported [16–18], very little information was available for zotarolimus degradation pathways and degradation products. Identifying zotarolimus degradation products on zotarolimus-coated drug-eluting stents was of critical interest to ensure product quality, since degradants have a significant impact on safety, efficacy, product storage and handling.

In this paper, we report the degradation of zotarolimus and zotarolimus-coated drug-eluting stents under various conditions and HPLC separation of these degradants. The chemical structures of the degradants are illustrated by the combination techniques of HPLC coupled with a diode array detector (DAD), HPLC/MS, HPLC/MS/MS and NMR.

2. Experimental

2.1. Materials and reagents

All aqueous solutions including the HPLC mobile phase were prepared with in-house purified water. HPLC grade acetonitrile was obtained from Burdick and Jackson (Muskegon, MI). Ammonium acetate, phosphoric acid and ammonium hydroxide were purchased from J. T. Baker (Phillipsburg, NJ). Acetic acid was obtained from EMD (Gibbstown, NJ). Absolute ethanol was purchased from Aaper (Shelbyville, KY). DMSO-d_6 was obtained from Sigma–Aldrich (St. Louis, MO). Zotarolimus drug substance was made in Abbott Global Pharmaceutical Research and Development internally. Zotarolimus-coated stents are *Zomaxx*™ drug-eluting stents obtained from Abbott Vascular Devices (Redwood City, CA).

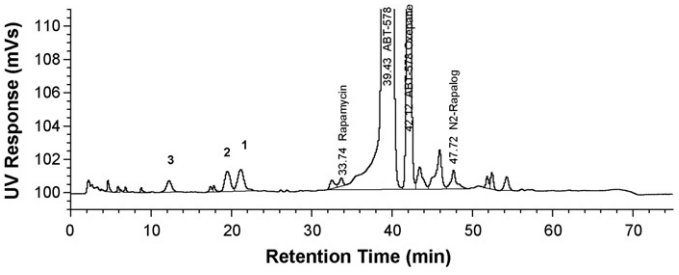


Fig. 2. HPLC Chromatogram of thermally degraded *ZoMaxx* stents.

2.2. High performance liquid chromatography and conditions

Agilent 1100 series HPLCs with single wavelength detector and DAD multi-wavelength detector were used for this work. The typical HPLC system consisted of G1322A model degasser, G1311A model pump, G1329A model autosampler, G1316A model HPLC column

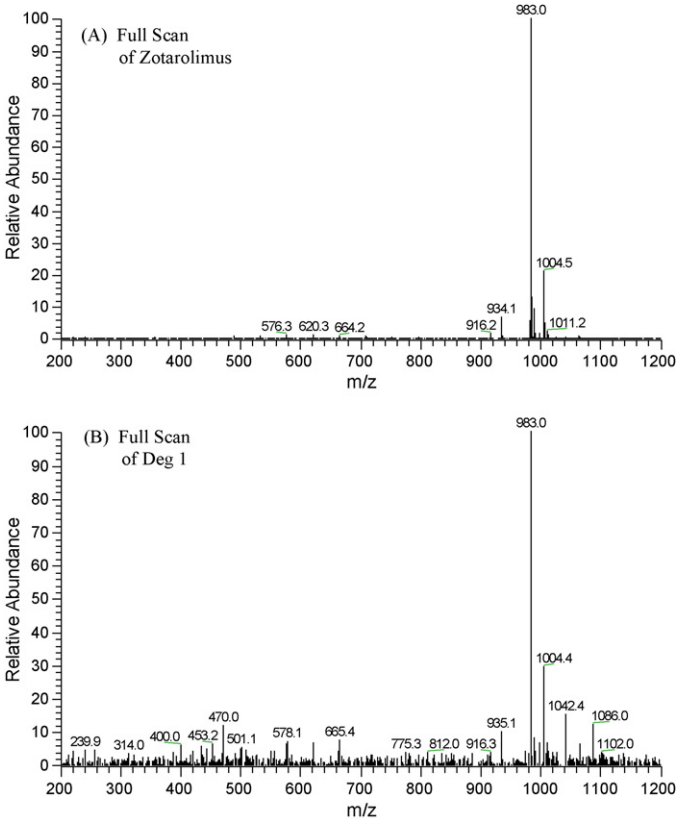


Fig. 3. (A) Positive ESI-MS spectrum of zotarolimus with retention time of 39.4 min, (B) Positive ESI-MS spectrum of degradant **1** with retention time of 21.2 min.

compartment and G1314A model UV detector (single wavelength), or G1315A model DAD detector (multi-wavelength). Chromatographic data were acquired and processed using Atlas 2003R1 data acquisition system (Thermo Electron Corp.). Chromatographic separations were performed on a Zorbax (Agilent Technologies, USA) Eclipse XDB-C8 column (4.6 mm × 250 mm, 5 μm particle size). The separation was achieved with initial equilibration of 50% Mobile Phase A (10 mM pH 3.8 ammonium acetate buffer) and 50% Mobile Phase B (100% acetonitrile), and a slow gradient of 50% to 70% Mobile Phase B from 0 min to 65 min. After 65 min the gradient was brought back to the initial conditions and the analytical column was reconditioned for 9 min. The flow rate was maintained at 1.0 ml min^{-1} with UV detection at 278 nm. Sample injection volume was 200 μl and column temperature was maintained at 45 °C. Samples are chilled to about 4 °C on the autosampler tray. For semi-preparative HPLC analysis, a Zorbax preparative Eclipse XDB-C8 column (9.4 mm × 250 mm, 5 μm particle size) was used. The flow rate was increased to 4.2 ml min^{-1} to maintain the same separation and the injection volume was increased to 400 μl. A Hitachi L-5200 fraction collector (Hitachi, Japan) was connected to Agilent HPLC

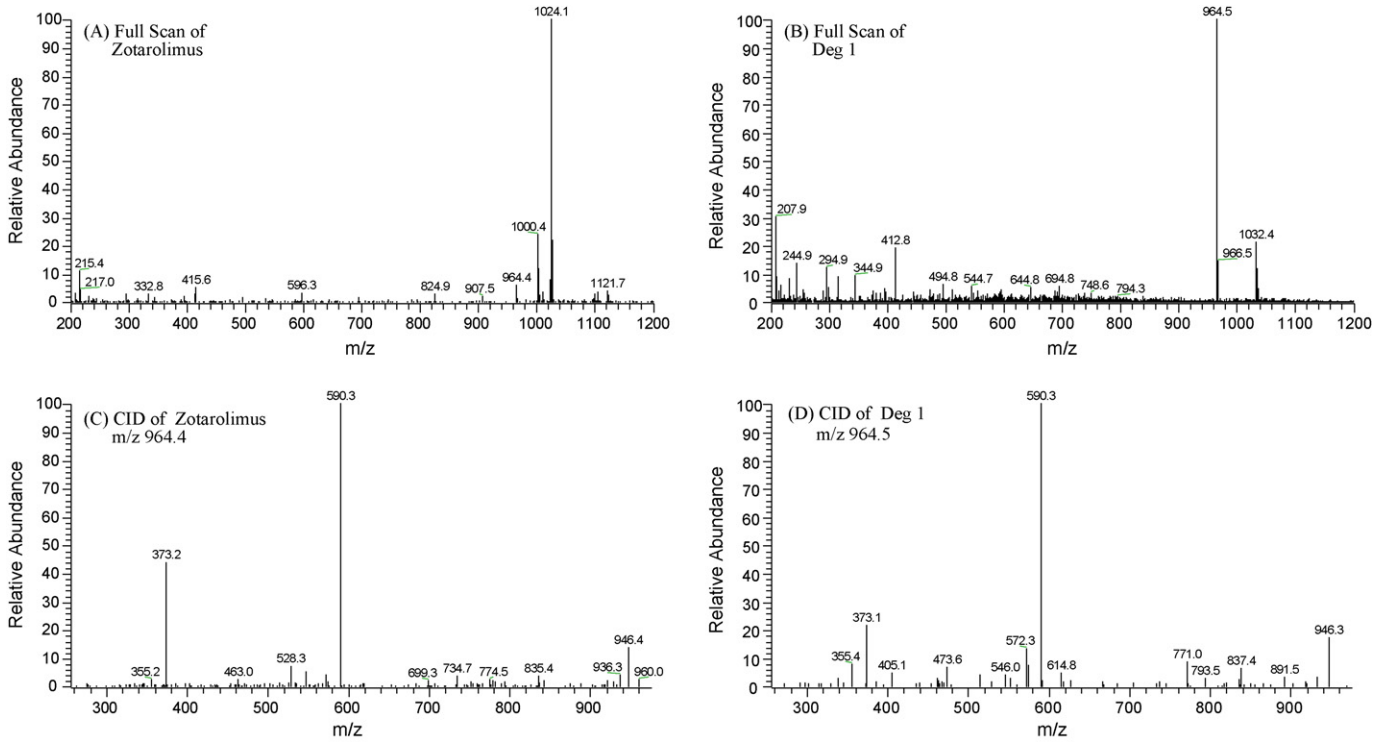


Fig. 4. (A) Negative ESI-MS spectra of zotarolimus with retention time of 39.4 min, (B) Negative ESI-MS spectra of degradant **1** with retention time of 21.2 min, (C) Negative ESI-LC/MS/MS of zotarolimus, (D) Negative ESI-LC/MS/MS of degradant **1**.

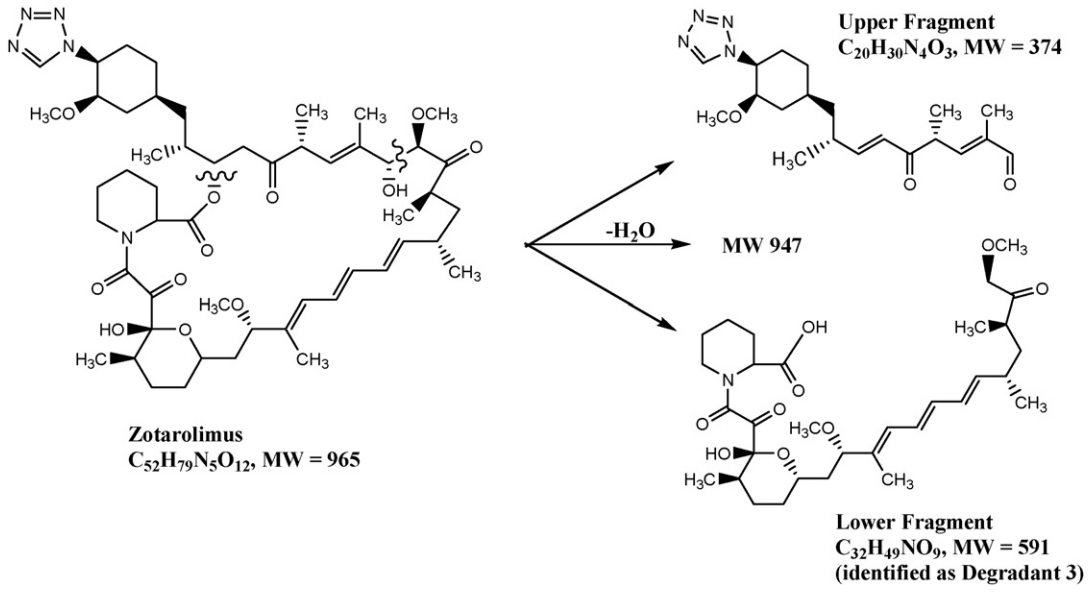
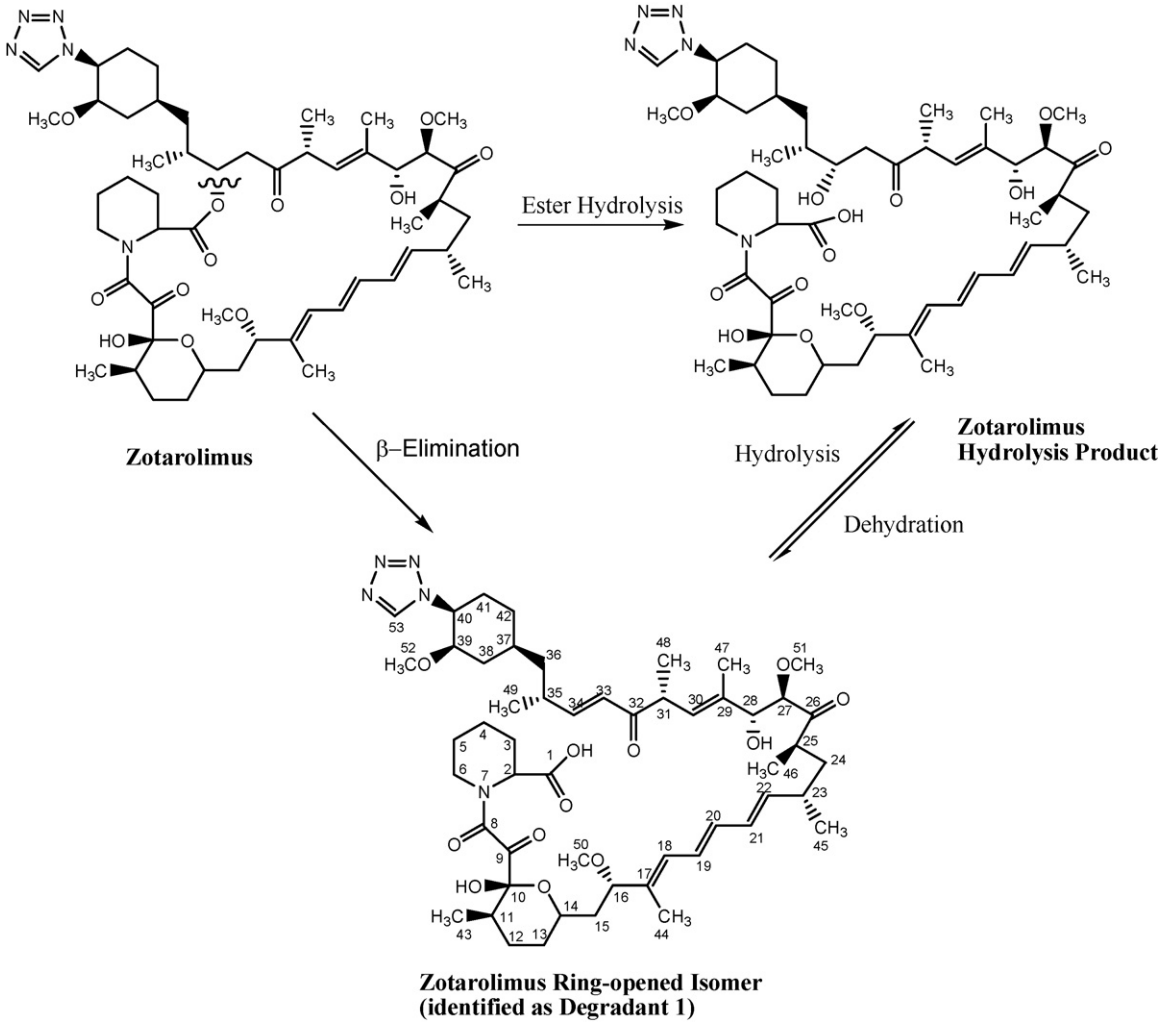


Fig. 5. Proposed fragmentation pathways of zotarolimus.

system to collect desired fractions. Freezone 1 l benchtop freeze-dry system from Labconco Corp. (Kansas City, MO) was used to freeze-dry the collected HPLC fractions.

2.3. Liquid chromatography–mass spectrometry (LC/MS)

The LC/MS studies for impurity identification were carried out on LCQ (ThermoFinnigan, USA) system coupled with an Agilent 1100 series HPLC. The HPLC was composed of a quaternary pump, degasser, autosampler, and column oven as described in previous section. The chromatographic conditions were identical to that described above. The HPLC effluent was introduced into electrospray ionization (ESI) source of the LCQ mass spectrometer for analysis. MS data were acquired in either positive or negative ion mode. The capillary voltage was set at 4.5 kV. The capillary source temperature was set at 250 °C. Nitrogen was used as sheath and auxiliary gas. The range of m/z acquired was from 150 to 2000.



Scheme 1.

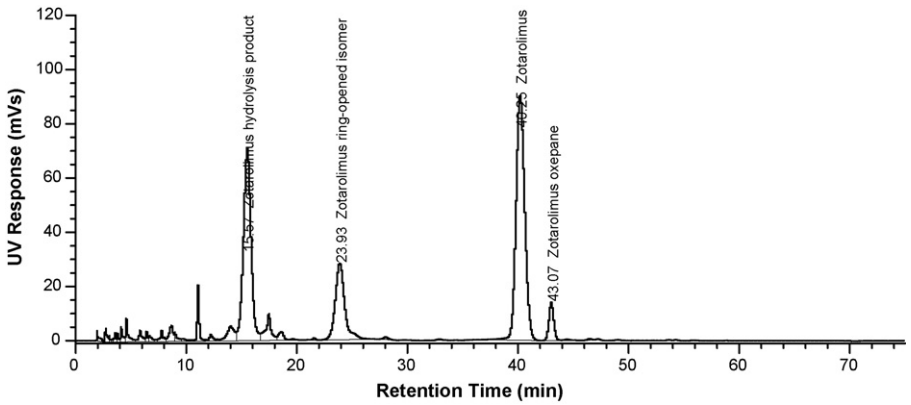


Fig. 6. HPLC chromatogram of degraded zotarolimus drug substance under 37 °C and pH 8 conditions.

2.4. Generation and purification of degradants

2.4.1. Degradant **1** and zotarolimus hydrolysis product

About 200 mg of zotarolimus was dissolved in 20 ml ethanol and further diluted with 400 ml of 0.1 M pH 8 ammonium acetate buffer. The mixture was incubated in a 37 °C water bath for 24 h. After the reaction, the suspension was filtered for preparative HPLC purification. The semi-preparative HPLC system was used to isolate and purify degradant **1** and zotarolimus hydrolysis product. The collected fractions were dried with lab scale freeze drier.

2.4.2. Degradant **2**

About 400 mg of zotarolimus drug substance was added into a 25 ml volumetric flask and dissolved in 8 ml ethanol and diluted to volume with 50:50 (v:v) pH 2 phosphoric acid: acetonitrile medium. The solution was aged at room temperature for about 3 days. The eluent of degradant **2** peak was collected from about 60 injections using semi-preparative HPLC system. The collected fractions were pooled and lyophilized to dryness for NMR characterization.

2.4.3. Degradant **3**

About 1 gram of zotarolimus drug substance was transferred into a 25 ml volumetric flask, dissolved in 8 ml ethanol and diluted to volume with 35:65 (v:v) 10 mM pH 4 ammonium acetate buffer:acetonitrile medium. The solution was then degraded in an 85 °C oven for about 7 h. The eluent containing degradant **3** was collected from about 50 HPLC injections. The collected fractions were pooled and lyophilized to dryness.

2.5. NMR spectroscopy

NMR spectra were acquired in DMSO-d_6 at 25 °C on a Varian Inova Unity 500 MHz spectrometer equipped with a cold probe using the VNMR 6.1 C software package. The standard ^{1}H and ^{13}C spectra were collected with a 45° pulse flip angle. A series of two-dimensional spectra were acquired to determine the structure of degradants. The pulse programs of the gDQCOSY, gHSQC, gHMBC, and ROESY experiments were taken from the Varian software library. Proton chemical shifts were referenced relative to the residual ^{1}H signal of DMSO-d_6 at 2.50 ppm and carbon chemical shifts were referenced to the DMSO-d_6 at 39.5 ppm.

3. Results and discussion

3.1. Degradation, purification and identification of degradants

ZoMaxx stents are subjected to accelerated degradation in 40 °C oven with 75% relative humidity control for six months. An example chromatogram of related substances analysis of the degraded sample is shown in Fig. 2. Three major degradants were observed and labeled as **1**, **2** and **3** at about 0.4%, 1.6% and 1.8%, respectively. Some late-eluting peaks are also seen in the chromatogram and they are synthetic process related impurities. An HPLC system coupled with diode array detector was used to study the origin of these impurities. The UV profiles of all three impurities exhibit similar UV profile as of zotarolimus, indicating that these impurities are zotarolimus related degradants. As discussed in detail in the following sections, LC/MS and LC/MS/MS were then used to obtain molecular weight and structural information of these degradants. Structures are proposed based on LC/MS/MS data. To confirm the proposed structures, these degradants were isolated, purified and then characterized using ^{1}H NMR and ^{13}C NMR techniques. Because of the limited amount of zotarolimus on ZoMaxx stents and limited number of stents that can be used for the further degradation study, the degradants were generated using zotarolimus drug substance.

3.2. Structural elucidation of ring-opened isomer, hydrolysis product and scheme of formation

LC/MS and LC/MS/MS were used to analyze degradant **1** in degraded stent samples. Both positive and negative ion ESI mass spectra were recorded. The positive ion ESI spectrum of the impurity showed peaks at *m*/*z* 983.0, 1004.4, corresponding to the

Table 1
^{1}H and ^{13}C NMR assignment for degradant **1**.

No.	Group[a]	^{1}H (ppm)[b,d]	^{13}C (ppm)[c,d]
1	C=O	–	172.0(171.7)
1	OH	13.0	–
2	CH	4.94 (4.39)	50.7 (55.8)
3	CH_2	1.59, 2.16 (1.67, 2.13)	26.2 (27.0)
4	CH_2	1.69,1.32	20.8 (20.8)
5	CH_2	1.68, 1.28 (1.61, 1.35)	24.2 (24.6)
6	CH_2	3.51, 3.23 (4.25,2.82)	38.1 (43.8)
8	C=O	–	166.5 (167.3)
9	C=O	–	199.8 (200.2)
10	C	–	99.4 (99.1)
10	OH	6.41	–
11	CH	2.09 (2.02)	34.8 (34.6)
12	CH_2	1.48	26.4
13	CH_2	1.57, 1.24	30.7
14	CH	3.74 (3.72)	66.3 (66.2)
15	CH_2	1.66, 1.44 (1.73, 1.45)	39.7 (39.2)
16	CH	3.63	82.9
17	C	–	136.7 (137.0)
18	CH	6.14 (6.10)	128.4 (127.8)
19	CH	6.43	127.2 (127.3)
20	CH	6.24	133.0 (132.9)
21	CH	6.17	130.1
22	CH	5.57	139.5 (139.3)
23	CH	2.27	34.2 (34.1)
24	CH_2	1.57, 1.08	38.7
25	CH	2.75	40.4 (40.5)
26	C=O	–	213.3 (213.4)
27	CH	3.76	86.0 (85.9)
28	CH	3.96	76.4
28	OH	5.24	–
29	C	–	137.6 (137.7)
30	CH	5.23	126.6
31	CH	3.62	43.4
32	C=O	–	200.1
33	CH	6.16	126.6
34	CH	6.68	152.1
35	CH	2.43	33.2
36	CH_2	1.36	42.1
37	CH	1.50	32.1
38	CH_2	1.76, 1.20	31.9
39	CH	3.63	77.5
40	CH	5.14	56.3
41	CH_2	2.18, 1.89	26.7
42	CH_2	1.48	26.4 (26.5)
43	CH_3	0.70 (0.72)	15.4
44	CH_3	1.61	10.5 (10.9)
45	CH_3	0.98	21.4
46	CH_3	0.92	14.6 (14.5)
47	CH_3	1.70	12.0
48	CH_3	1.04	16.3
49	CH_3	1.00	19.6
50	OCH_3	3.03 (3.04)	55.1 (55.3)
51	OCH_3	3.16	57.6
52	OCH_3	3.25	55.8
53	CH	9.32	144.9

[a] The numbering scheme for the degradant **1** is shown in Scheme 1.
[b] Relative to the residual signal of DMSO-d_6 assigned to 2.50 ppm.
[c] Relative to DMSO-d_6 assigned to 39.5 ppm.
[d] The values in parentheses are assignments for the *cis* rotamers, where these can be distinguished. Some of the *cis*/*trans* assignments may be switched due to the similar concentrations of *trans* and *cis* rotamers in solution.

adduct ions of $[M+NH_4]^+$ and $[M+K]^+$, where M = 965. The negative ESI spectrum of the impurity showed deprotonated molecular ion peak at *m*/*z* 964.5, which corresponds to $[M-H]^-$. The positive ion ESI spectrum of zotarolimus also showed peaks at *m*/*z* 983.0 and 1004.5, corresponding to the adduct ions of $[zotarolimus+NH_4]^+$ and $[zotarolimus+K]^+$. The major peak of zotarolimus in negative ion ESI spectra is $[M+CH3COO]^-$ at *m*/*z* 1024.1. Fig. 3 is the positive ESI spectra of zotarolimus and degradant **1**. Degradant **1** has the same molecular weight as that of zotarolimus. Further LC/MS/MS fragmentation was performed for both degradant **1** and zotarolimus using negative ion ESI mode. As shown in Fig. 4, the major fragments for zotarolimus are at *m*/*z* 590.3 and 373.2, which can be attributed to ester bond cleavage and C27–C28 bond cleavage. Fragment at *m*/*z* 946.4 corresponds to a loss of water. The zotarolimus fragmentation pathways are illustrated in Fig. 5. Interestingly, the major fragmentation pattern for degradant **1** is very similar to that of zotarolimus. The most intense fragment ion is at *m*/*z* 590.3 and two other major fragment ions have *m*/*z* 373.1 and 946.3. Similar fragmentation pathways have been reported for rapamycin degradation in the literature [17]. Degradant **1** is proposed as zotarolimus ring-opened isomer or open ring acid formed by ester hydrolysis and dehydration or through β-elimination (Scheme 1).

To confirm the proposed structures by NMR, a reaction was set up to degrade zotarolimus to generate ring-opened isomer as

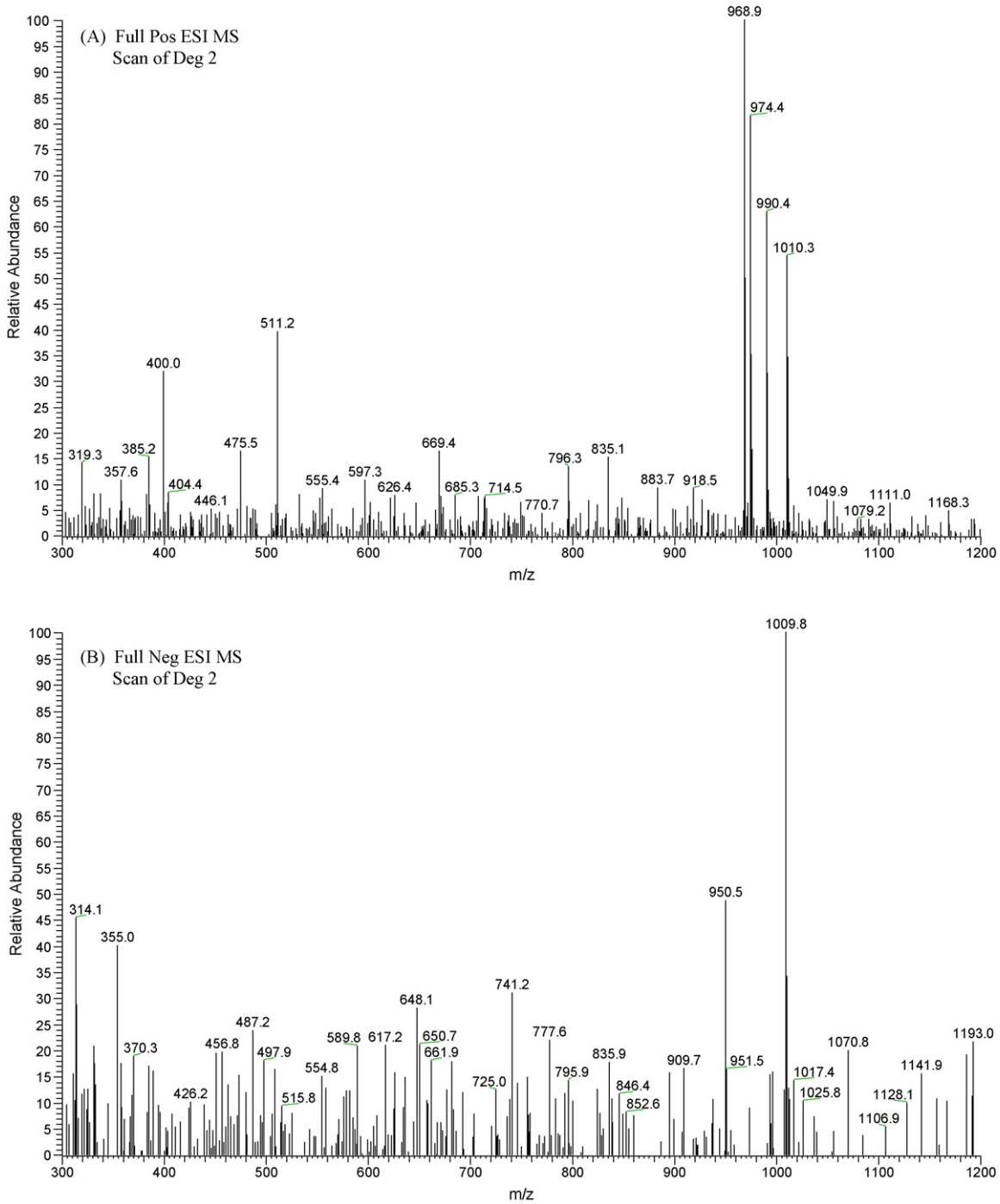


Fig. 7. (A) Positive ESI-MS spectra of degradant **2** with retention time of 19.5 min, (B) Negative ESI-MS spectra of degradant **2** with retention time of 19.5 min.

described in section 2.4.1. Zotarolimus drug substance was used because the amount of zotarolimus drug on each stent is very small, usually less than 0.3 mg per stent, and the limited number of stents can be used for the degradation study. The reaction suspension was first analyzed by HPLC for its degradation profile and the LC/MS/MS structural analysis was performed. As shown in Fig. 6, two major degradation peaks were observed with retention time (RT) of about 57 min and 23.93 min, the later peak with relative retention time of 0.59 corresponds to ring-opened isomer or degradant **1**. Positive and negative ion ESI LC/MS indicated that the dominant degradant at RT 15.57 min has a molecular weight of 983, corresponding to [zotarolimus+H_2O], and the degradant at RT 23.93 min with a molecular weight of 965. A semi-preparative HPLC system was used to isolate and purify these two degradants. The collected fractions from semi-preparative HPLC were dried and characterized using NMR.

The detailed NMR study and full assignment of protons and carbons for zotarolimus and rapamycin has been reported [19–20]. Zotarolimus is a tertiary cyclic amide and two amide bond rotamers are observed in DMSO-d_6 solution. The predominant rotamer is the *trans* conformation. The dried solid of degradant **1** was dissolved in DMSO-d_6 and ^{1}H, ^{13}C, gDQCOSY, gHSQC, gHMBC, and ROESY spectra were acquired. There are two sets of peaks at 1:1 ratio which are from amide bond rotamers. The rotamer ratio of this compound is quite different from zotarolimus pyran form [19], which indicates the opening of the 31-membered ring in the molecule. Also the amount of *cis* rotamer was increased dramatically in the presence of base. This phenomenon is similar to the report for the ring-opened acid form of rapamycin isomer [21]. Through analyzing all the correlations in two-dimensional spectra (gDQCOSY, gHSQC, gHMBC, and ROESY), the structure was determined to be a ring-opened acid form of zotarolimus isomer as shown in Scheme 1. The ^{1}H and ^{13}C chemical shifts are listed in Table 1 (see Scheme 1 for nomenclature).

The zotarolimus hydrolysis product was also identified through two-dimensional NMR spectra (gDQCOSY, gHSQC, and gHMBC). A hydroxyl group was observed at 5.52 ppm which connects to carbon 34. The proton and carbon chemical shifts of position 34 are 3.73 ppm and 70.5 ppm, respectively, which are also consistent with hydroxyl group attached. Position 33 is a CH_2 with proton chemical shifts at 2.22 ppm and 2.53 ppm and carbon chemical shift at 43.8 ppm. The rest of NMR signals are similar to those of degradant **1**.

The identified structures of ring-opened isomer and hydrolysis product confirmed the proposed degradation pathways for zotarolimus. The zotarolimus hydrolysis product has not been observed on zotarolimus-coated stent products and degraded zotarolimus-coated stents, which indicates that the degradation of zotarolimus on stent to ring-opened isomer is mainly through β-elimination pathway. The degradation via ester hydrolysis followed by dehydration dominates in aqueous environment.

3.3. Structural elucidation of desmethyl zotarolimus

LC/MS study was carried out for degradant **2** generated in the above degraded stent samples using positive and negative ion ESI mass spectrometric techniques. Fig. 7 shows the ESI LC/MS spectra for the sample. Under positive ion ESI, the major peak observed is an adduct ion $[M+NH_4]^+$ at m/z 968.9, where M is equal to 951. Other adduct ions, $[M+Na]^+$ and $[M+K]^+$ at m/z 974.4 and 990.4, were also detected. Under negative ion ESI, peaks detected were $[M+CH_3COO]^-$ and $[M-H]^-$ at m/z 1009.8 and 950.5, respectively.

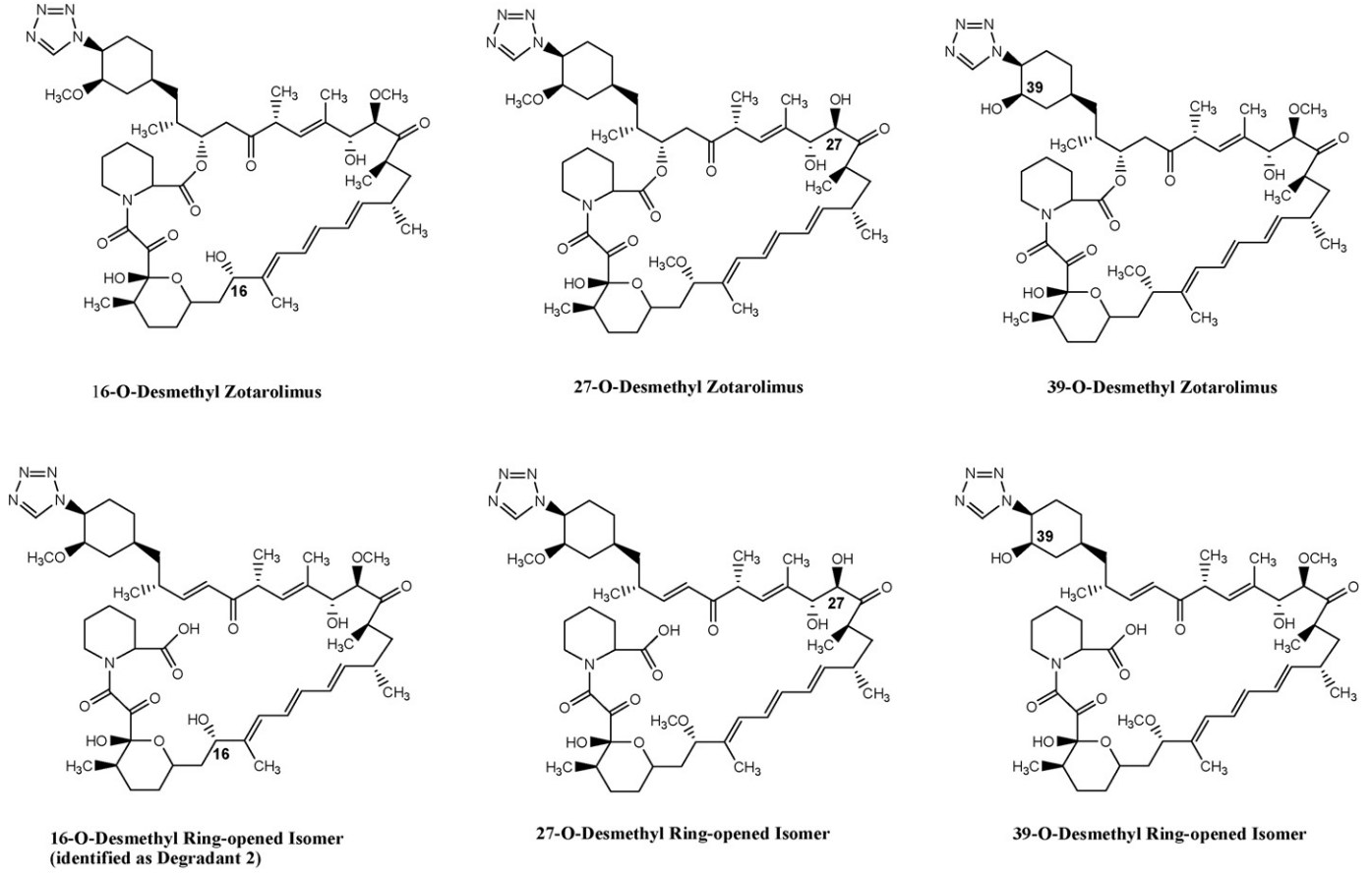


Fig. 8. Six proposed structures of degradant **2**.

This impurity is proposed to have a molecular weight of 951, corresponding to a loss of CH_2 from zotarolimus. Assuming that desmethyl elimination occurs by the conversion of a $-OCH_3$ group to a –OH group, then three sites in zotarolimus could give rise to a molecular weight of 951 degradant. Fig. 8 depicts zotarolimus degradants with the methoxy-to-hydroxyl conversion at C_{16}, C_{27} or C_{39} (top row). Each of these possible degradants is depicted together with a ring-opened form (bottom row). The complex nature of zotarolimus will further introduce analogous structures of epimers and oxepane isomers of this group of possible structures.

Thorough NMR analysis of pure compound is needed to narrow down the list of possible structures for this degradant. Again it is not feasible to generate a larger amount of pure compound on stent product; therefore zotarolimus drug substance was used for further preparative scale work. Following the procedure described in Section 2.4.2, degradant **2** was observed in HPLC chromatogram and confirmed by LC/MS analysis. Several milligrams of the pure compound were obtained from the purification procedure and then analyzed by NMR.

The pure compound was dissolved in DMSO-d_6 and ^1H, ^{13}C, gDQCOSY, gHSQC, and gHMBC spectra were acquired. The OCH_3 at position 50 (see Scheme 1 for nomenclature) that connects to position 16 (3.03 ppm for proton and 55.1 ppm for carbon in degradant **1**) has disappeared. The hydroxyl group attached to position 16 was observed at 4.90 ppm and carbon chemical shift of position 16 was

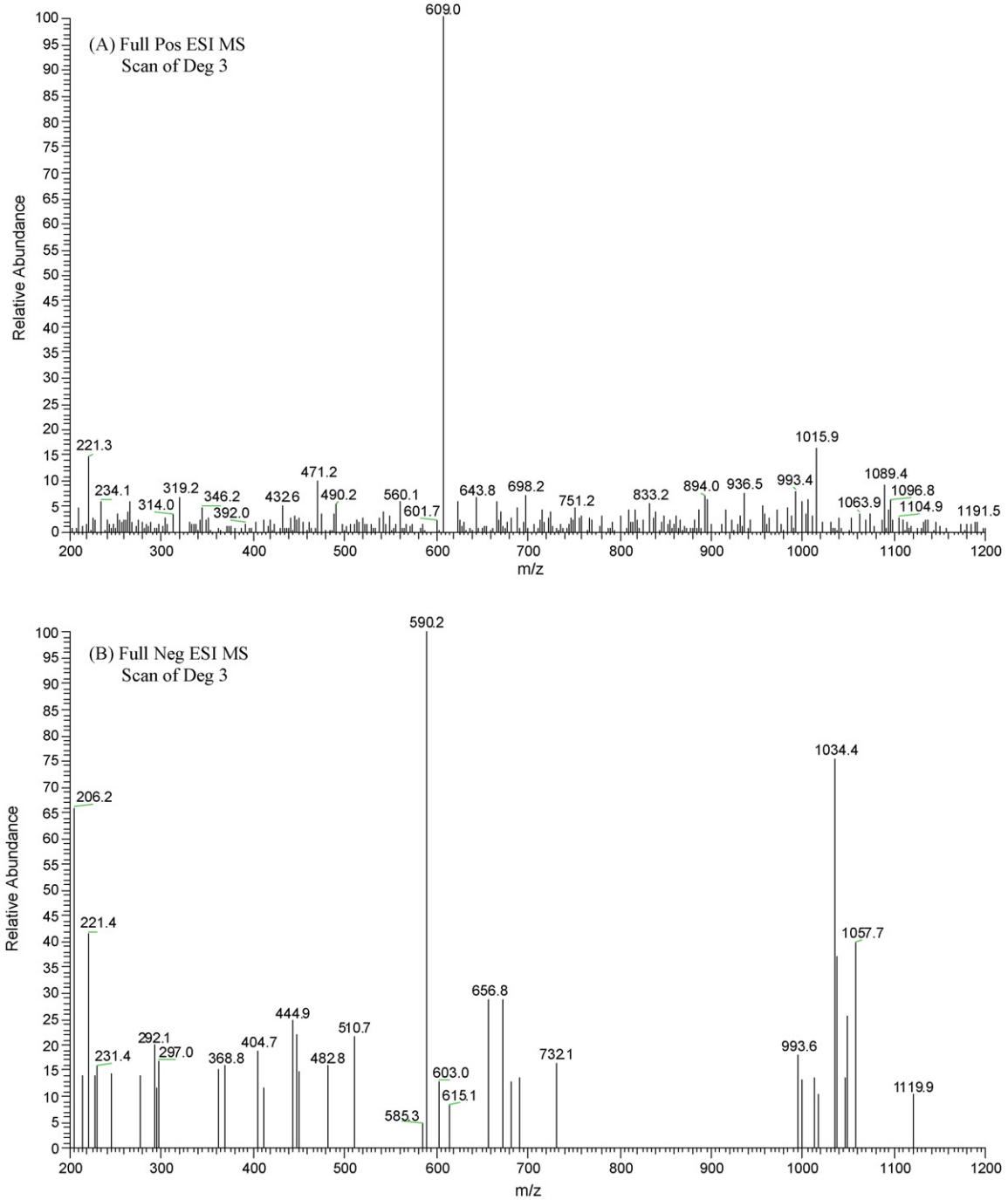


Fig. 9. (A) Positive ESI-MS spectra of degradant **3** with retention of 12.3 min, (B) Negative ESI-MS spectra of degradant **3** with retention time of 12.3 min.

moved from 82.9 ppm in degradant **1** to 69.6 ppm. Therefore the NMR spectra are consistent with the 16-O-desmethyl ring-opened zotarolimus isomer.

3.4. Structural elucidation of zotarolimus lower fragment

LC/MS study was carried out for degradant **3** in the degraded stent sample. Fig. 9 shows the positive and negative ion ESI spectra for the peak. The major peak under positive ion ESI is an adduct ion $[M+NH_4]^+$ at m/z 609.0, where M = 591. The major peak observed under negative ion ESI is the deprotonated ion $[M-H]^-$ at m/z 590.2. This impurity has a molecular weight of 591 as indicated by LC/MS analysis and its structure is proposed to be lower fragment of zotarolimus as illustrated in Fig. 5.

A procedure, as described in Section 2.4.3, was developed in our laboratory to generate pure compound for NMR structural confirmation. The ^{1}H, gDQCOSY, gHSQC and gHMBC spectra of degradant **3** in DMSO-d_6 were acquired. Comparing to the spectra of degradant **1** (see nomenclature in Scheme 1), there are missing signals of tetrazole, position 28 to 42, methyls 47 to 49, and O-methyl 52. The position 27 is a CH_2 with chemical shifts at 75.3 ppm for carbon, and 4.08 and 4.16 ppm for protons. The O-methyl at position 51 was shifted to 3.25 ppm for proton and 58.2 ppm for carbon. The rest of the signals from position 1 to 26, methyls 43 to 46, and O-methyl 50 have the similar chemical shifts compared to those of degradant **1**. Therefore, the spectra are consistent with the lower fragment structure shown in Fig. 5.

4. Conclusions

In this work, degradation study of zotarolimus and zotarolimus-coated stents under different pH, moisture and temperature conditions were performed. Three possible major zotarolimus degradation products on zotarolimus-coated stent products were identified, isolated and characterized by a combination of analytical and preparative HPLC, LC/MS, LC/MS/MS and NMR techniques. The chemical structures of the three degradation products have been identified as ring-opened isomer, 16-O-desmethyl ring-opened isomer and MW 591 low fragment of zotarolimus, which were not discussed in literature until now. Zotarolimus hydrolysis product, a potential zotarolimus degradant in an aqueous medium, was also characterized. The study also pointed out that it is very important to protect zotarolimus-coated stent products from moisture and heat.

Similarly, the approaches and techniques described in this work can be applied to study of the degradation products of other macrolide compounds and their respective drug-eluting stents.

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