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RESEARCH ARTICLE

Ozonation of metoprolol in aqueous solution: ozonation by-products and mechanisms of degradation

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Abstract This study investigated the degradation pathway of metoprolol, a widely used β-blocker, in the ozonation via the identification of generated ozonation by-products (OPs). Structure elucidation of OPs was performed using HPLC coupled with quadrupole time-of-flight high-resolution mass spectrometry. Seven OPs were identified, and four of these have not been reported elsewhere. Identified OPs of metoprolol included aromatic ring breakdown by-products; aliphatic chain degraded by-products and aromatic ring mono-, di-, and tetrahydroxylated derivatives. Based on the detected OPs, metoprolol could be degraded through aromatic ring opening reaction via reaction with ozone (O_3) and degradation of aliphatic chain and aromatic ring via reaction with hydroxyl radical (•OH).

Keywords Ozone · β-blocker · Hydroxyl radical · Aromatic ring opening · Pharmaceuticals

Introduction

The occurrence of pharmaceuticals and their metabolites in the aquatic environment is an issue of growing concern (Benitez et al. 2009). Generally, pharmaceuticals that are consumed by human are not completely metabolized and are excreted unchanged in urine and feces. These pharmaceuticals may end up in the municipal sewage treatment plants. Since no municipal treatment plants are equipped with the specific facility for the removal of pharmaceuticals (USEPA 2010), untreated pharmaceuticals have been frequently detected in the effluents of the municipal sewage treatment plants (e.g., Bueno et al. 2012; Gracia-Lor et al. 2012). In order to treat these pollutants, various chemical oxidation methods such as chlorination (e.g., Acero et al. 2010; Homem and Santos 2011; Rodil et al. 2012), ozonation (e.g., Lee Homem and Santos 2011; Tay et al. 2011a, b), and advanced oxidation processes (e.g., Homem and Santos 2011; Bińk and Sobera-Madej 2012) have been developed and frequently evaluated. Among the chemical treatment methods, ozonation has emerged as one of the important technologies for the removal of a wide range of organic pollutants in water treatment industry (Ikehata et al. 2006; Sowmya 2008). O_3 with the redox potential of 2.07 V is a strong oxidizing reagent (Beltrán 2004). O_3 is unstable in water; it tends to decompose and forms highly reactive •OH as a secondary oxidant. During ozonation, •OH can react non-selectively with most of the organic compounds in water, whereas O_3 with its electrophilic nature, can react selectively with electron-rich reaction site of organic compounds. Therefore, organic compounds in water may be degraded by both O_3 and •OH through a series of oxidation and radical reactions. On the other hand, toxic OPs can be produced when pollutants are not completely treated (Ikehata et al. 2006). These OPs may become new chemical entities and emerge in the environment following the discharges of incompletely treated effluent. Thus, it is necessary to identify the OPs and understand the breakdown process of these pollutants during ozonation.

Among the pharmaceuticals, β-blockers are one of the most commonly detected pharmaceuticals in the environment (Corcoran et al. 2010). Metoprolol is one of the most commonly used β-blockers for the treatment of cardiovascular diseases (Carlberg et al. 2004; Huang et al. 2007). According to the pharmacokinetics study (Medsafe 2009), about 5 % of

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metoprolol is excreted unchanged after oral administration and it has been detected in various water samples such as influent and effluent of wastewater, surface water, and sewer (Hernando et al. 2007). The main objectives of this study are to identify the OPs of metoprolol and to elaborate the degradation mechanism of metoprolol during ozonation. Some OPs of metoprolol have been reported by Benner and Ternes (2009); however, in this study, another four OPs of metoprolol were identified and the mechanisms of metoprolol degradation were proposed in detailed. Structure elucidation of OPs was performed using HPLC coupled with quadrupole time-of-flight high-resolution mass spectrometry (QTOF-HRMS). MS and MS/MS obtained from QTOF-HRMS is unique in its high typical mass accuracy (1–2 mmu) (Ferrer and Thurman 2003), and such features can provide high assurance of correct nominal masses and also empirical formula for OPs and its fragment ions.

Materials and methods

Chemicals

(±)-Metoprolol (+)-tartrate salt (Molecular mass = 684.8 $gmol^{-1}$) with >99 % purity was obtained from Sigma (USA). Stock solution was prepared by dissolving (±)-metoprolol (+)-tartrate salt in ultrapure deionized water (Elga, USA). All solvents used were of HPLC-grade (Merck, Germany) and were used without purification. Formic acid for LCMS was purchased from Fluka (Germany). *tert*-Butanol (*t*-BuOH) with ≥99.5 % purity was purchased from Sigma-Aldrich (USA).

Ozonation of metoprolol

The detail of the procedure was given in a previous study (Tay et al. 2011b). Briefly, the experiment was performed in heterogeneous conditions where O_3 with the output of 0.70 gh^{-1} and the flow rate of 290 $mL\,min^{-1}$ was bubbled into a 100 mgL^{-1} metoprolol solution in a 1,000 mL cylindrical jacketed beaker through gas dispersion tube (Pyrex, USA). In order to evaluate the contribution of O_3 in the degradation of metoprolol, ozonation was also performed in the presence of *t*-BuOH (100 mM) as radical scavenger (Benner and Ternes 2009). O_3 was produced from purified oxygen (99.8 %) by an OZX03K model O_3 generator (Enaly Trade Co. Ltd., Canada). Reaction temperature was maintained at 25.0±0.1 °C by using a circulating water bath. Ozonation of metoprolol was performed without pH adjustment (pH 8.3). Solutions were stirred during ozonation by using a magnetic stirrer. An aliquot of 1 mL reaction mixture was withdrawn every 2 min and flushed immediately by nitrogen gas for O_3 residues removal; 1 μL of aliquot was then injected into the QTOF HPLC/ESI-MS/MS for analysis.

Instrumental analysis

The reaction mixtures were analyzed using Agilent 6500 accurate mass quadropole time-of-flight mass spectrometer bearing with electrospray ionization (ESI) source coupled with Agilent 1200 series rapid-resolution LC system (Tay et al. 2011b). A ZORBAX rapid-resolution high-throughput SB C18 column (2.1 × 100 mm, 1.8 μm particle size) was selected for separation. The column was thermostatted at 35 °C. Water (A) and acetonitrile (B) containing 0.1 % formic acid were used as eluents. A/B ratio was changed from 90/10 to 10/90 in 5 min and was maintained at 10/90 for further 3 min. The flow rate was maintained at 0.25 $mL\,min^{-1}$.

QTOF-MS system was operated in the 4 GHz high-resolution mode. Ions were produced using an electrospray ion source with Agilent Jet Stream Technology. Parameters for the Agilent Jet Stream Technology were the superheated nitrogen sheath gas at the temperature of 300 °C and flow rate of 11 $L\,min^{-1}$. Analyses were performed in ESI-positive ion mode using the following setting: nebulizer at the operating pressure of 35 psig, Vcap voltage of 3,500 V, fragmentor voltage of 125 V, skimmer voltage of 65 V, nozzle voltage of 1,000 V, and the collision energy was fixed at 15 V. A sprayer with a reference solution was used as continuous calibration in positive ion using the following reference masses: *m/z* 121.0509 and 922.0098. The QTOF-MS instrument was used as a TOF-MS system operated in the MS mode and MS/MS mode for transformation by-products identification. The recorded full-scan and MS/MS data was processed using Agilent MassHunter Workstation Software.

Results and discussion

Identification of OPs

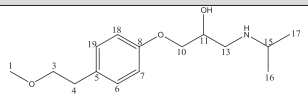
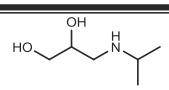
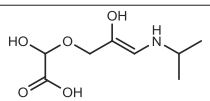
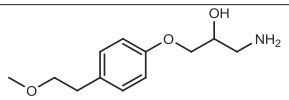
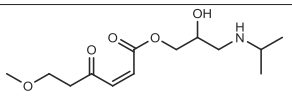
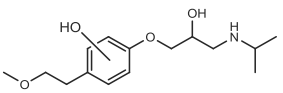
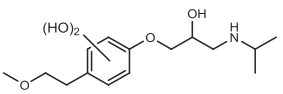
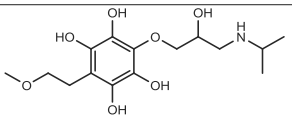
Ozonation of metoprolol was performed without pH adjustment. This operating condition is different from the method reported by Benner and Ternes (2009), and it was selected in order to compare the OPs that were generated using different ozonation method. In order to differentiate the OPs generated by the reaction of metoprolol with O_3 and •OH, ozonation was performed in the presence and absence of *t*-BuOH as a radical scavenger. Analyses were carried out by comparing the mass spectrometric data of the initial metoprolol solution as a control sample with the data obtained of the sample withdrawn at consecutive ozonation time. Structure elucidation of OPs was performed based on their fragmentation patterns in MS/MS spectrum, where protonated molecule, $[M + H]^+$, was selected as precursor ion for the product ion scan. In this study, seven OPs were identified (Table 1), and the MS/MS spectra are presented in

Electronic supplementary material (ESM) (Fig. S1). MS/MS fragmentation of protonated metoprolol, $[M + H]^+$ at *m/z* 268.1899 is illustrated in Fig. S1a. Fragment ions at *m/z* 250.1782 and *m/z* 226.1427 were formed through the loss of water molecules and propane group from the precursor ion *m/z* 268.1899, respectively. Other significant peaks are *m/z* 116.1059, *m/z* 191.1058, *m/z* 159.0788, and *m/z* 133.0632 representing carbocation of 1-(isopropylamino)prop-1-en-2-ol, 1-(2-methoxyethyl)-4-(prop-1-enyloxy)benzene, 1-(prop-1-enyloxy)-4-vinylbenzene, and (2-methoxyvinyl)-benzene, respectively. The OPs of the metoprolol can be divided according to their structures as hydroxylated metoprolol, aliphatic chain degraded by-products, and aromatic ring opening by-products.

Hydroxylated by-products

Hydroxylated metoprolol with the $[M + H]^+$ at *m/z* 284.1857, *m/z* 300.1800, and *m/z* 332.1698 were detected. Addition of 16, 32, and 64 amu from the $[M + H]^+$ of metoprolol indicated the addition of one, two, and four hydroxyl groups (OH) to metoprolol for the formation of monohydroxylated (MT-283), dihydroxylated (MT-299), and tetrahydroxylated (MT-331) metoprolol (Fig. S1b–d of the ESM). For MT-283, the peak at *m/z* 248.1637 was formed through the loss of methoxymethane group from the precursor ion *m/z* 284.1857. Further fragmentation of *m/z* 248.1637 ion through the loss of ethene group formed *m/z* 224.1271. The peak at *m/z* 207.1023 was produced

Table 1 Metoprolol and its OPs

$[M+H]^+$	Elemental composition of $[M+H]^+$	Measured exact mass (*m/z*)	Calculated exact mass (*m/z*)	Error (ppm)	Proposed structure (Label, retention time)	Reaction condition	
						Without radical scavenger	With radical scavenger
268.1899	$C_{15}H_{26}NO_3^+$	267.1837	267.1834	-0.96	 (Metoprolol, 6.328 min)	-	-
134.1187	$C_6H_{16}NO_2^+$	133.1115	133.1103	-9.17	 (MT-133, 0.981 min)	√	√
206.1033	$C_8H_{18}NO_5^+$	205.0960	205.0950	-4.77	 (MT-205, 1.072 min)	√	√
226.1438	$C_{12}H_{20}NO_3^+$	225.1366	225.1365	-0.47	 (MT-225, 5.058 min)	√	×
274.1658	$C_{13}H_{24}NO_5^+$	273.1585	273.1576	-3.27	 (MT-273, 3.110 min)	√	√
284.1861	$C_{15}H_{26}NO_4^+$	283.1789	283.1784	-1.91	 (MT-283, 3.182 min)	√	×
300.1811	$C_{15}H_{26}NO_5^+$	299.1739	299.1733	-2.10	 (MT-299, 2.693 min)	√	√
332.1705	$C_{15}H_{26}NO_7^+$	331.1632	331.1631	-0.30	 (MT-331, 1.537 min)	√	×

through the loss of water molecule and isopropylamine group (Fig. S1b of the ESM) from the precursor ion. The presence of *m*/*z* 116.1064 and *m*/*z* 133.0650 ion showed that both of the aliphatic side chain of metoprolol remained unchanged. For MT-299, the presence of *m*/*z* 167.0697 ion, which was formed through the loss of 2-hydroxy-3-(isopropylamino)propoxy chain from the precursor ion $[M + H]^+$ at *m*/*z* 300.1800, indicated the addition of two OH groups to the aromatic ring of metoprolol (Fig. S1c of the ESM). For MT-331, the peak at *m*/*z* 300.1433 and *m*/*z* 199.0613 were found to be 31.9894 and 31.9914 amu higher than the peak of *m*/*z* 268.1539 and *m*/*z* 167.0697 of MT-299 (Fig. S1d of the ESM). This result indicated that the additional two OH groups were added to the aromatic ring of MT-299. In a previous study (Benner and Ternes 2009), the formation of aliphatic chain hydroxylated metoprolol through the hydroxylation of secondary amine group was reported. According to von Gunten (2003), hydroxylation of amine group in ozonation reaction is more likely to occur at the non-protonated amine. In this study, experiments were performed at pH below pK_a of metoprolol (9.7) (Benner and Ternes 2009) and the pH was found to decrease from 8.3 to 4.1 after 10 min of ozonation for both scavenged and non-scavenged conditions. Under this condition, protonated metoprolol is the predominant species and the formation of hydroxylamine is not likely to occur.

Among the aromatic ring hydroxylated metoprolol, only MT-283 has been reported by Benner and Ternes (2009). According to the detected hydroxylated by-products, higher diversity of hydroxylated by-products was generated when the ozonation was performed under non-scavenged condition (Table 1). Therefore, as reported by Benner and Ternes (2009), the formation of hydroxylated by-products is more likely to be the predominant reaction pathway when ozonation was performed under non-scavenged condition. Since hydroxylation of metoprolol can occur through the reaction with O_3 and •OH, mechanism of the hydroxylation based on both O_3 and •OH pathway was proposed and presented in Fig. 1. It is proposed that the electron-rich aromatic ring of metoprolol can react with O_3 by 1,3-cycloaddition (Pathway **I-1**) and electrophilic substitution (Pathway **I-2**) reactions. In addition, the aromatic ring of metoprolol can react with •OH through the addition reaction (Pathway **II**).

Aliphatic chain degraded by-products

MT-133 and MT-225 are the aliphatic chain breakdown products of metoprolol. MT-133 (Fig. S1e) showed a prominent product ion at *m*/*z* 116.1057 that was produced through the elimination of one water molecule from the precursor ion $[M + H]^+$ at *m*/*z* 134.1187. Tay et al. (2011b) also reported MT-133 in the ozonation of atenolol. For MT-225 with $[M + H]^+$ at *m*/*z* 226.1390, the peaks at *m*/*z* 121.0652, *m*/*z* 133.0641, *m*/*z* 159.0800, and *m*/*z* 191.1083 are the most predominant product ions in the MS/MS spectrum (Fig. S1f). Since the precursor ion of MT-225 is one of the product ions of metoprolol, product ions similar to the parent compounds were observed.

It is proposed that both MT-133 and MT-225 are formed through •OH reaction, as the formation of these two OPs involved cleavage of carbon–carbon and carbon–oxygen bond, respectively. MT-133 was found in both scavenged and non-scavenged conditions, whereas MT-225 only occurred in non-scavenged condition. The presence of MT-133 in scavenged condition might due to the incomplete suppression of •OH by *t*-BuOH (Benner and Ternes 2009). The mechanism for the formation of MT-225 is proposed to start with the formation of aliphatic chain monohydroxylated metoprolol (*A*) (Fig. 2, Pathway **I**). Then, hydrogen abstraction of *A* forms radical *B*. Radical *B* can react with •OH to form intermediate *C*. *C* then reacts with •OH to form radical

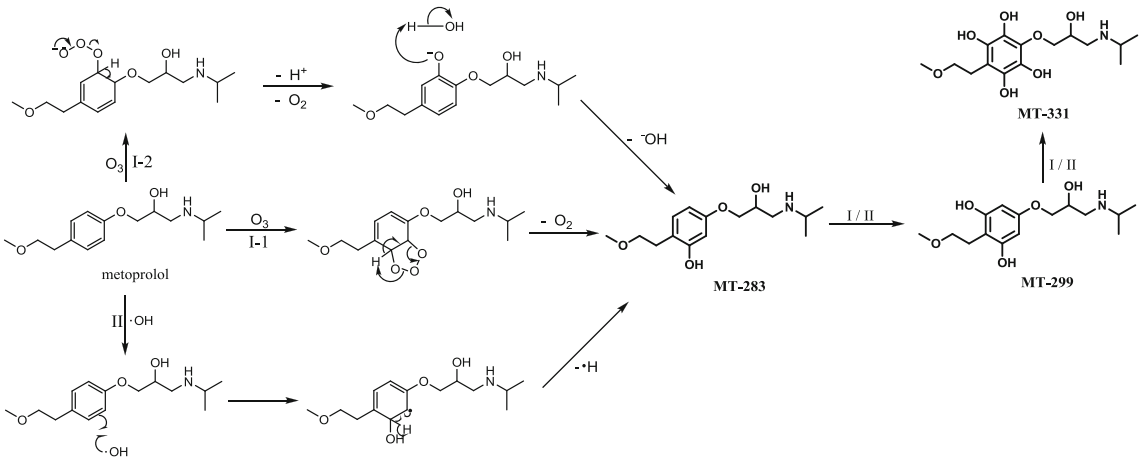


Fig. 1 Mechanism of the hydroxylation of aromatic ring of metoprolol

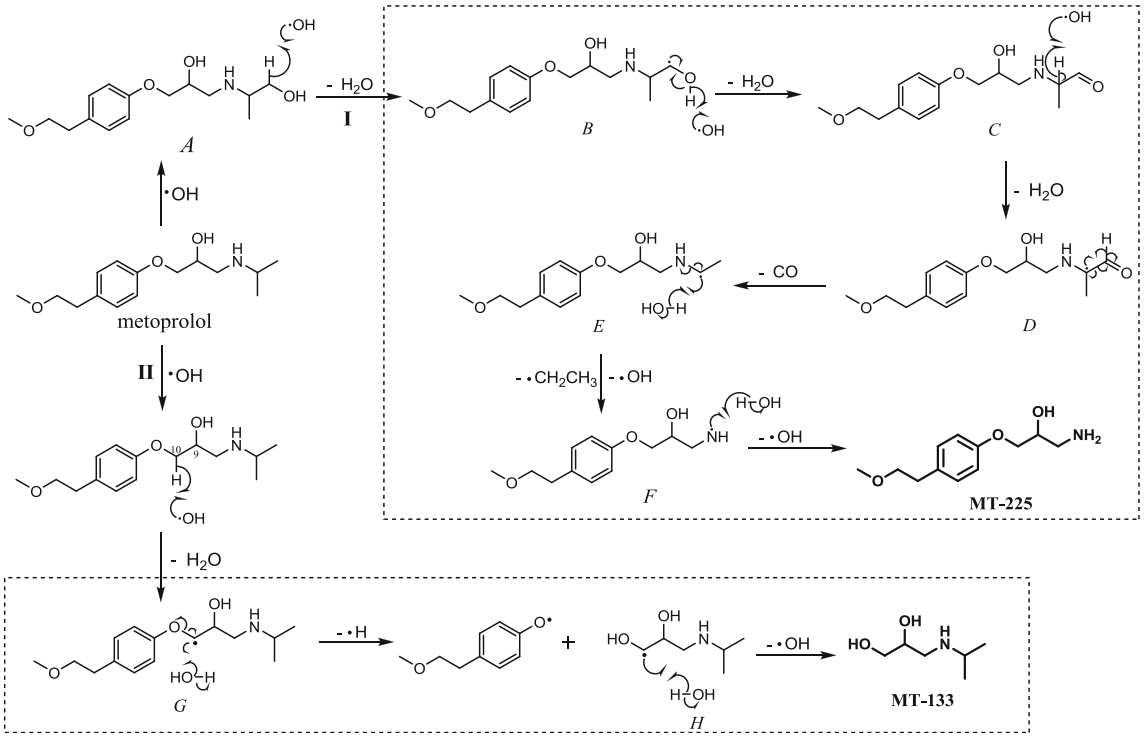


Fig. 2 Proposed mechanism for the reaction at the side chain of metoprolol

D. Rearrangement of *D* through the loss of CO forms radical *E*. Rearrangement of *E* through the elimination of ethyl radical forms radical *F*. *F* then reacts with water to form MT-225. Formation of MT-133 is proposed to start with the hydrogen abstraction at C_{10} to form radical *G*. Rearrangement of *G* through the breakdown of C_{10}–O_9 bond forms radical *H*. *H* then reacts with water to form MT-133 (Fig. 2, Pathway **II**).

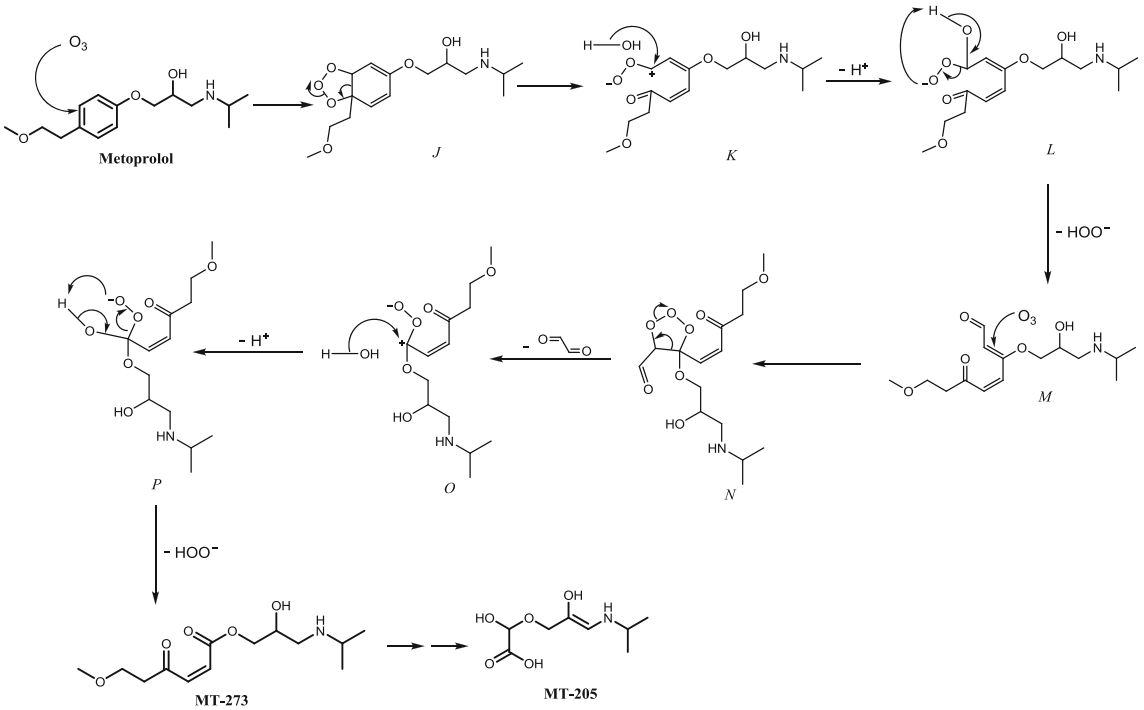


Fig. 3 Proposed mechanism for the formation of MT-273 through the aromatic ring opening reaction

Aromatic ring opening by-products

MT-205 and MT-273 are aromatic ring opening by-products of metoprolol. These products were formed from the breakdown of the aromatic ring of metoprolol. MT-273 has been reported previously by Benner and Ternes (2009). For MT-273, the peak at *m/z* 215.0926 was generated from the loss of 59.0729 amu from the precursor ion $[M + H]^+$ at *m/z* 274.1658, indicating the presence of isopropylamine side chain in MT-273 (Fig. S1g of the ESM). MT-205 is the breakdown by-product of MT-273. This transformation by-product with the $[M + H]^+$ at *m/z* 208.1178 produced two important product ions at *m/z* 134.1177 and *m/z* 162.1138 (Fig. S1h of the ESM). Product ion at *m/z* 134.1177 indicated the presence of 3-(isopropylamino)propane-1,2-diol group in MT-205. The peak at *m/z* 162.1138 was formed through the loss of formic acid, indicating the presence of carbonyl group in this compound.

MT-205 and MT-273 were detected in both scavenged and non-scavenged conditions, indicating the importance of O_3 in the aromatic ring opening reaction. Aromatic ring opening reaction can proceed through the direct reaction between aromatic ring of organic compounds with O_3 (Benner and Ternes 2009). The detailed reaction mechanisms are presented in Fig. 3. The reaction is proposed to start with the reaction of O_3 with the aromatic ring of metoprolol. This reaction leads to the formation of intermediate *J*. Rearrangement of *J* through the breakdown of C–C bond forms *K*, the first aromatic ring opening intermediate. *K* then reacts with water to form *L*. Inter-molecular rearrangement of *L* through the elimination of perhydroxyl anion (HOO^-) forms intermediate *M*. The presence of *M* has been reported by Benner and Ternes (2009). *M* can further react with O_3 to form intermediate *N*. Rearrangement of *N* through the elimination of oxalaldehyde forms *O*. *O* then reacts with water to form *P*. Inter-molecular rearrangement of *P* through the elimination of HOO^- forms MT-273.

Conclusions

In this experiment, ozonation of metoprolol was performed in order to determine the possible OPs. Seven degradation by-products were identified and four of these OPs of metoprolol (MT-133, MT-205, MT-299, and MT-331) have not been reported elsewhere. Based on the detected OPs, metoprolol can be degraded via aromatic ring opening reaction through the reaction with O_3 and the degradation of aliphatic chain and aromatic ring via the reaction with •OH.

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