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Ozonation of acebutolol in aqueous solution: Ozonation by-products and degradation pathway

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ABSTRACT

Ozonation is one of the common methods for pollutant removal and disinfection in water treatment. The reaction between the ozone and organic pollutants involves complex oxidation pathway which may lead to the formation of various ozonation by-products. In some cases, toxic ozonation by-products may be produced. In this study, the ozonation by-products of acebutolol, one of the common β-blockers, were identified. The mechanisms for the transformation of acebutolol during ozonation were elaborated.

In this study, ozonation was carried out at pH 2 (with the presence of radical scavenger), 7 and 12, in order to study the role of the ozone (O_3) and hydroxyl radical (·OH) in the transformation of acebutolol. Structure elucidation of the ozonation by-products was carried out using HPLC coupled with quadrupole time-of-flight high resolution mass spectrometry. Sixteen ozonation by-products were identified, of which fifteen have never been reported elsewhere. Based on the detected ozonation by-products, acebutolol can be degraded by both O_3 and ·OH. When the O_3 itself becomes a reactive species, the transformation of the acebutolol proceeded via the reaction between the O_3 and the aromatic ring of the acebutolol to form the aromatic ring hydroxylated and aromatic ring opening by-products. Without the radical scavenger, ozonation at pH 7 and 12 showed that the transformation of the acebutolol proceeded via the involvement of the ·OH and different aliphatic chain degraded and hydroxylated by-products were detected.

1. Introduction

In recent years, the presence of pharmaceuticals in the environment has been a growing major worldwide concern. Due to its extensive use as human and veterinary medicine, pharmaceuticals have been introduced continuously into the environment and their impact on living organisms is now becoming an important issue [11]. Various pharmaceutical residues have been frequently detected in various water samples such as the effluents of wastewater treatment plants, surface water as well as drinking water [12,15,19]. These results indicate that the currently available water treatment facilities are unable to completely remove all pharmaceutical residues.

For water treatment, ozonation remains one of the most common methods for the pollutant removal and disinfection in Europe, USA and North America [1]. The complete mineralization of the organic pollutants via ozonation always involved high operational costs. The removal of organic pollutants by ozonation also involved complex oxidation pathways which could lead to the formation of various ozonation by-products. In some cases, toxic ozonation by-products might be formed [9]. Following the discharge of incompletely treated effluents, toxic ozonation by-products may emerge in the environment. As ozonation is a commonly employed water treatment method, the evaluation and determination of ozonation by-products are an important consideration.

β-Blockers are widely used pharmaceuticals in human medicine for the treatment of cardiovascular diseases [6,18]. This study focused on the ozonation of acebutolol as it has been frequently detected in the various water samples such as surface water as well as the influents and effluents of wastewater treatment plants [8,10,14]. Ozonation has been proven to be an efficient method for the removal of different β-blockers such as acebutolol, atenolol, metoprolol and propranolol according to kinetic studies [3]. Based on our literature review, the ozonation by-products of atenolol [13,16], metoprolol [4,17] and propanolol [5] have been reported. Although a few common ozonation breakdown products of β-blockers have been reported by Quispe et al. [13] in the ozonation of acebutolol, however the ozonation by-products of acebutolol have not been fully explored. The main objectives of this study were (i) to identify the water soluble ozonation by-products of acebutolol and (ii) to elaborate the transformation mechanisms of acebutolol during ozonation.

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2. Materials and methods

2.1. Materials

Acebutolol and sodium phosphate monobasic were obtained from Sigma (St. Louis, USA). Phosphoric acid and all the HPLC grade solvents were purchased from Merck (Darmstadt, Germany). Formic acid for LCMS was obtained from Fluka (Steinheim, Germany). Sodium phosphate dibasic and *tert*-butanol were obtained from Ridel-de Haën (Seelze, Germany). All the stock solutions were prepared by dissolving acebutolol in ultrapure deionized water (Elga, Buckinghamshire, UK).

2.2. Ozonation of acebutolol

The details of the procedure have been described in our previous study [16,17]. Briefly, the experiment was conducted under heterogeneous conditions where the O_3 with an output of 0.7 g/h and flow rate of 290 mL/min was bubbled through a 1 L solution of 100 mg/L acebutolol in a 1 L cylindrical jacketed beaker through a dispersion tube. Ozone was produced from purified oxygen (99.8%) using the OZX5K model O_3 generator (Enaly Trade Co., Ltd., Shanghai, China). Experiments were performed at pH 2, 7 and 12 at 25 °C. The pH was adjusted using phosphate buffer. Solutions were stirred during the ozonation process with a magnetic stirrer. In order to evaluate the reaction between the O_3 and acebutolol, ozonation at pH 2 was performed in the presence of *tert*-butanol (100 mM). Then, 1 mL of the aliquots of the reaction mixture was withdrawn at suitable time intervals. The reaction mixtures were immediately bubbled with nitrogen gas for the O_3 residue removal. 1 μL of the aliquot was subjected to instrumental analysis.

2.3. Instrumental analysis

The ozonated acebutolol solutions were analyzed using 6500 accurate mass quadropole time-of-flight mass spectrometer bearing with electrospray ionization (ESI) source coupled to 1200 series rapid resolution LC system (Agilent Technologies, Santa Clara, USA). A ZORBAX Rapid Resolution High Throughput (RRHT) SB C18 column (2.1 × 100 mm, 1.8 μm particle size), was selected for the separation. The column was thermostatted at 35 °C. Water (A) and acetonitrile (B) containing 0.1% of formic acid were used as the eluents. The A/B ratio was changed from 90/10 to 0/100 in 5 min at the flow rate of 0.25 mL min^{-1}. The QTOF-MS system was operated in the 4 GHz high-resolution mode. Ions were generated 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 10 L min^{-1}. Analyses were performed in ESI positive ion mode using the following setting: nebulizer at the operating pressure of 40 psi, Vcap voltage of 4000 V, fragmentor voltage of 90 V, skimmer voltage of 65 V, and the collision energy was fixed at 20 V. A sprayer with a reference solution was used as the continuous calibration in the 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 working in the MS mode and also in the MS/MS mode for transformation by-products identification. The recorded full-scan and MS/MS data was processed using Agilent MassHunter Workstation Software.

3. Results and discussion

3.1. Reaction pathway of acebutolol in ozonation

Dissolved O_3 is unstable in water and it tends to decompose to form ·OH [20]. Therefore, during ozonation, the organic compounds can react directly with both the O_3 and ·OH. The pH has been identified as one of the main factors that influence the decomposition of O_3 in water. Under acidic condition, pH has a slight effect on the rate of O_3 decomposition, whereas under basic conditions, the rate increases significantly [2]. In order to study the influence of O_3 in the degradation pathway of acebutolol, the experiment was performed at pH 2 in the presence of *tert*-butanol as the radical scavenger. In addition, experiments were also conducted at pH 7 and 12 to study the role of the ·OH in the ozonation of acebutolol.

3.2. Reactions between ozone and acebutolol during ozonation

In this experiment, structure elucidation of the ozonation by-products was performed using High Performance Liquid Chromatography (HPLC) coupled with Quadropole-Time-Of-Flight-high resolution Mass Spectrometry (QTOF-MS). This instrument produces MS and MS/MS with high mass accuracy [7]. Such an accurate mass can provide high assurance of the precise the molecular formula and the nominal mass for the ionized organic analytes and its fragment ions. Analyses were performed by comparing the mass spectrometric data of the initial acebutolol solution as the control sample with the data obtained from the ozonated solutions that were withdrawn at consecutive times.

The MS/MS fragmentation of the protonated acebutolol with the quasi-molecular ion, $[M+H]^+$, of *m*/*z* 337.2106 is presented in Fig. A1a. A fragment ion at *m*/*z* 319 was formed as a result of the loss of one water (H_2O) molecule from the $[M+H]^+$ ion. The ion at *m*/*z* 260 was formed through the loss of the isopropylamine group from the ion at *m*/*z* 319. Further fragmentation of the ion at *m*/*z* 319 through the loss of acetyl group formed the fragment ion at *m*/*z* 218. The ion at *m*/*z* 116 represents carbocation of 1-(isopropylamino)propan-2-ol. Table 1 shows the proposed ozonation by-products formed during ozonation at pH 2 with the presence of *tert*-butanol as the radical scavenger. These seven proposed ozonation by-products represent the oxidation products resulting from the reaction between the O_3 and acebutolol. The reaction under this condition was found to proceed through deacetylation, hydroxylation and aromatic ring opening reactions. Among the ozonation by-products, Ace-368a was the only detected dihydroxylated acebutolol. For the Ace-368a with the $[M+H]^+$ ion at *m*/*z* 369.2012, a mass difference of 32 Da as compared to acebutolol corresponded to the addition of two OH groups. The presence of the fragment ion at *m*/*z* 134 which represents the unmodified 2-hydroxy-3-(isopropylamino)propoxy chain of the acebutolol indicated the addition of the OH groups to the aromatic ring (Fig. A1b). Other hydroxylated by-products such as Ace-326, Ace-342a, Ace-360 and Ace-376 were formed through the elimination of the acetyl group from the aromatic ring of acebutolol. The $[M+H]^+$ ion of Ace-326 and Ace-342a show additional 32 and 48 Da when compared with the calculated $[M+H]^+$ ion of deacetylated-acebutolol (*m*/*z* 295.2016) and corresponded to the presence of the additional two and three OH groups to the deacetylated-acebutolol, respectively (Fig. A1c and d). Ace-360 and Ace-376 were proposed to be the by-products of the Ace-342a and Ace-326, respectively. When compared the $[M+H]^+$ ion of Ace-360 with the calculated $[M+H]^+$ ion of the corresponding tetrahydroxylated deacetylated-acebutolol (*m*/*z* 359.1813), a mass different by 2 Da was observed (Fig. A1e). Therefore, it is proposed that two OH groups were added to each C8 and C9 of the Ace-326. The $[M+H]^+$ ion of the Ace-376 revealed an additional 16 Da from the $[M+H]^+$ ion of Ace-360. This result indicated an additional one OH group was added to the Ace-360 for the formation of Ace-376. A comparison of the fragmentation pathway showed that the ion at *m*/*z* 234.1336, *m*/*z* 262.1274 and *m*/*z* 290.1238 in the MS/MS spectrum of Ace-376 (Fig. A1f) were found