

# High Resolution Mass Spectrometry Elucidation of Captopril's Ozonation and Chlorination By-Products

Frederico Jehár Oliveira Quintão<sup>1</sup>, Geraldo Célio Brandão<sup>2</sup>, Silvana de Queiroz Silva<sup>1</sup>, Sérgio Francisco Aquino<sup>1</sup>, Robson José de Cássia Franco Afonso<sup>1\*</sup>

<sup>1</sup>Programa de Pós-Graduação em Engenharia Ambiental (ProAmb), Universidade Federal de Ouro Preto, Ouro Preto, Brazil

<sup>2</sup>Programa de Pós-Graduação em Ciências Farmacêuticas (CiPharma), Universidade Federal de Ouro Preto, Ouro Preto, Brazil

Email: fredjoq@gmail.com, celiobrandao@ef.ufop.br, silvana@iceb.ufop.br, sergio@iceb.ufop.br, \*robsonafonso@iceb.ufop.br

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## Abstract

The article evaluated the degradation of the captopril in aqueous solution after ozonation and chlorination. The process was continuously monitored focusing on the identification, mass spectrometry and elucidation of its by-products by applying direct infusion and high performance liquid chromatography, electrospray ionization high resolution mass spectrometry, in the negative ion mode. The cytotoxicity of its by-products solutions were evaluated with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. It was observed through that after 30 min of ozonation and chlorination, there was complete oxidation of captopril, *i.e.*, 100% removal efficiency. At these conditions, the rate of mineralization, by total organic carbon, was only 7.63% for ozonation and 6.40% for chlorination, evidencing the formation of degradation by-products. Ten captopril by-products were identified and their respective chemical structures elucidations are proposed. The treated samples and their by-products were nontoxic to HepG2 cells by MTT assay.

## Keywords

Chlorination, Ozonation, Captopril, High-Resolution Mass Spectrometry, Liquid Chromatography, Characterization of By-Products, MTT Assay

## 1. Introduction

The pharmaceutically active compounds are an essential part of modern human and veterinary medicine. The uncontrolled use of these compounds through anthropogenic sources causes their accelerated introduction into the environ-

ment and can be a potential risk for aquatic and terrestrial organisms [1]. The molecules are absorbed, distributed, metabolized, and excreted at their unchanged form and as metabolites [2]. Often they are excreted slightly transformed only or even unchanged, mostly conjugated to polar molecules [3] [4]. However, several studies have revealed that they are not quantitatively removed in conventional wastewater treatment processes. Due to their persistence in these secondary effluents, as well as in surface waters, and since these aquatic streams could be latter used as drinking water sources, they constitute a potential risk to human health [5].

Chlorine is the most commonly used oxidizer in water treatment plants around the world. Its wide use is justified by its disinfectant action besides being a strong oxidant. In addition to these factors, the chlorination process has low cost and frees residual chlorine in distribution networks, ensuring water quality until its consumption [6]. Chlorination can occur at one or two points in the water treatment plant, early in the process, conducting a pre-oxidation of organic matter and/or in the end as a disinfectant [7]. Chlorine is able to react quickly with pharmaceutically active compounds, which are toxic compounds and exhibit carcinogenic activity on animals and humans [6].

Ozone (O<sub>3</sub>) is a very powerful oxidizing agent which is commonly used in water treatment, particularly in continental Europe. Due to its high oxidation potential, ozone is widely used in drinking water treatment for disinfection, color removal, taste and odor control, decrease of disinfection by-products formation, biodegradability increase, and also for effective degradation of many organic contaminants [8] [9] [10]. The oxidation of organic compounds during ozonation can occur via ozone or hydroxyl radicals or a combination of both. Ozone is an electrophile with high selectivity to oxidize organic micropollutants. Ozone reacts mainly with double bonds, activated aromatic systems, and non-protonated amines [11]. Product formation from the ozonation of organic micropollutants has only been established for a few compounds [12].

Captopril (CP; 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-L-proline), as shown in **Figure 1**, is an angiotensin converting enzyme inhibitor (iACE). It is used in the management of hypertension, in heart failure, after myocardial infarction, and in diabetic nephropathy [13]. It is largely excreted in the urine, 40% - 50% as unchanged drug, the rest as disulfide and other metabolites [14] [15].

Pharmaceuticals have been developed to produce a biological effect, so their residues, metabolites, and degradation products released in the environment can cause different ecotoxicological effects that are difficult to predict, especially in complex matrices [16]. The MTT assay is a sensitive, quantitative, and reliable colorimetric assay that measures viability of cells. Here the MTT assay was used, with HepG2 cells, to determine the cytotoxicity of the chlorination and ozonation by-products solutions.

The main objectives of this work are 1) identify the water soluble transformation products of captopril generated from the chlorination and ozonation in ultrapure water 2) propose a degradation route of captopril during chlorination

and ozonation process and evaluate their toxicity against HepG2 cells. In this experiments, the structure elucidation of the transformation products was performed using High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (HPLC/HRMS) system via an electrospray ionization interface (ESI). The by-products solutions were submitted to MTT assay, with HepG2 cells, to determine their cytotoxicity.

## 2. Experiments

### 2.1. Chemicals

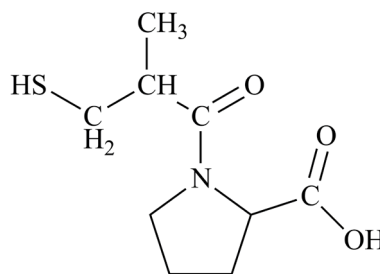
Captopril ( $C_9H_{15}NO_3S$ , nominal mass 217.2867), chemical structure of which is shown in **Figure 1**, was purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents for analytical determinations were acetonitrile (HPLC grade, JT Baker) and ultrapure water. Ultrapure water, from a Millipore Milli-Q system (Milford, MA, USA), was employed to prepare all the solutions.

Standard solution of sodium hypochlorite at 10% w/v was provided by SEM-AE (Municipal Water and Sewage Service) in Ouro Preto, Minas Gerais, Brazil. Ozone gas was generated using an ozone generator of electrical discharge with production capacity of 3 g  $O_3$ /h (Ozone Generator, model O & L3.0RM, Ozone & Life Industry, São José dos Campos-SP, Brazil) from oxygen feed gas (oxygen purity 99.99%).

### 2.2. Ozonation Experiments

Aqueous solutions of ozone were prepared by continuously bubbling ozone gas into Milli-Q ultrapure water through bottled gas scrubber for no less than 10 minutes at room temperature ( $20^\circ\text{C} - 23^\circ\text{C}$ ). The quantification of ozone occurred by direct spectrophotometric measurement at 260 nm ( $CO_3 \text{ (mg}\cdot\text{L}^{-1}) = 14.59 \times \text{Absorbance}$  [17]). The ozonation experiments were performed in bench scale with amber bottles of 20 mL capacity. Initially a volume of the CP solutions were added into the flask to a final concentration of  $10 \text{ mg}\cdot\text{L}^{-1}$  [18]. After preparation of the ozone solution, it was added to the bottles a volume of solution which contained the final concentration of ozone ( $8 \text{ mg}\cdot\text{L}^{-1}$ ). Each bottle used had a contact time of 0, 5, 10, 15 and 30 minutes.

1



**Figure 1.** Chemical structure of captopril.

### 2.3. Chlorination Experiments

The chlorination experiments were performed in bench scale with amber bottles of 20 mL capacity. The stock CP solution ( $100 \text{ mg}\cdot\text{L}^{-1}$ ) was prepared separately using ultrapure water (Milli-Q) and 2 mL of this solution were added into the amber bottles (final concentrations of  $10 \text{ mg}\cdot\text{L}^{-1}$  of CP). The solutions were stirred for 10 minutes before the degradation tests. All tests were performed at room temperature, which varied between  $22^\circ\text{C}$  and  $23^\circ\text{C}$ . In the chemical oxidation reaction system it was collected the first sample (time 0) and subsequently an amount of sodium hypochlorite was added to achieve a final concentration of  $10 \text{ mg}\cdot\text{L}^{-1}$ . After specific contact time, sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ )  $10 \text{ mg}\cdot\text{L}^{-1}$  were added to quench the residual hypochlorite and stop the reactions. The chlorination was accomplished over a period of 30 minutes. Samples were collected in amber glass bottles at the times of 0, 5, 10, 15 and 30 minutes.

The aliquots collected from the chlorination and ozonation tests were also maintained under identical conditions until the Total Organic Carbon (TOC) and mass spectrometry analyses. Although the CP concentration of  $10 \text{ mg}\cdot\text{L}^{-1}$  used in this study was much higher than those typically found in the environment, it was chosen to facilitate the subsequent mass spectrometry analysis, eliminating the steps of extraction and pre-concentration of the samples, thereby minimizing errors related to sample preparation.

### 2.4. TOC Analyses

The analysis of total organic carbon (TOC) was based on the determination of  $\text{CO}_2$  produced through the oxidation of organic matter in the samples, and was carried out on a TOC analyzer (Shimadzu, model TOC-L, Kyoto, Japan). The TOC content of each collected aliquot was obtained by the indirect method which corresponds to the difference between the total carbon and inorganic carbon values. The TOC-L equipment employs a combustion ( $680^\circ\text{C}$ ) catalytic oxidation method with Non Dispersive Infra Red (NDIR) detection which allows the TOC analysis in a wide range ( $4 \text{ }\mu\text{g}/\text{L}$  to  $30,000 \text{ }\mu\text{g}/\text{L}$ ) with a low limit of detection ( $4 \text{ }\mu\text{g}/\text{L}$ ) [19].

### 2.5. Direct Infusion High Resolution Mass Spectrometry

The direct infusion analyses were carried out in a high resolution mass spectrometer (IT-TOF; Shimadzu Corporation, Kyoto, Japan), a hybrid ion trap and time of flight, equipped with an electrospray (ESI) ionization source operating in negative mode ( $-3.5 \text{ kV}$ ). The samples were directly introduced into the ESI source via the HPLC autosampler (SIL 30AC; Shimadzu Corporation, Kyoto, Japan). The mass spectrometer parameters are presented in **Table 1** [19] [20].

### 2.6. Liquid Chromatography Coupled to Mass Spectrometry

The evolution, formation, and degradation of each by-product during the photodegradation and photolysis processes were monitored by high performance liquid chromatography coupled to the hybrid mass spectrometry system. The

liquid phase chromatograph was equipped with a binary pump (Nexera LC-30AD; Shimadzu Corporation, Kyoto, Japan) and an autosampler (SIL 30AC; Shimadzu Corporation, Kyoto, Japan). The mass spectrometer parameters were the same as described above. The samples were introduced into the ESI source by injecting 8  $\mu\text{L}$  of sample via the HPLC autosampler. For separation, it used a Nucleosil® 100-5 CN column (250 mm  $\times$  4.6 mm  $\times$  5  $\mu\text{m}$  particle diameter); and as mobile phases, it used water (A) and acetonitrile (B), both contained 0.1% formic acid, at a flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$ . The mobile phase was split so that the flow became 0.2  $\text{mL}\cdot\text{min}^{-1}$  prior entering the electrospray capillary at the mass spectrometer. The gradient elution program used in separation of *CP by-products* are presented in **Table 2** [20] [21] [22].

## 2.7. Cytotoxicity Assay (MTT)

The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product that is insoluble in water [23] [24]. Viable cells are able to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formation which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead following a toxic damage, cannot transform MTT. The amount of these crystals can be determined spectrophotometrically and hence the number of living cells in the sample. These features can be taken as advantage of cytotoxicity or cell proliferation assays, which are widely used in toxicology [23] [25] [26].

**Table 1.** Mass spectrometer parameters.

Parameters	Conditions
Temperature of interface	200°C
Temperature of curved desolvation line (CDL)	200°C
Nebulizer gas ( $\text{N}_2$ ) flow rate	1.5 $\text{L}\cdot\text{min}^{-1}$
Drying gas pressure	100 kPa
mass-to-charge ( $m/z$ ) range	100 - 600
Sample volume injecting	15 $\mu\text{L}$
Flow rate	0.2 $\text{mL}\cdot\text{min}^{-1}$
Mobile phase	acetonitrile with 0.1 % of formic acid

**Table 2.** Gradient elution program used in separation of *CP by-products*.

Time	Solvent ratio	
	A (water)	B (acetonitrile)
0 min	70 %	30%
16 min	10%	90%
22 min	70 %	30% B
27 min	70 %	30% B

The cytotoxicity of CP and its transformation products were determined with 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay according to Mosmann (1983) and Zegura *et al.* (2009) [23] [25]. HepG2 cell monolayers were trypsinized, washed with culture medium, plated in 96-well flat-bottomed plates ( $4 \times 10^4$  cells per well) and incubated in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. After a 24h incubation, serial dilutions of the CP samples (0.01, 0.25, 0.50, 0.75 and 1.00 mg·L<sup>-1</sup>) made in phosphate buffered saline (PBS) were added to appropriate wells, and the plates were incubated for an additional 20 h. After this time, the supernatants were removed from the wells, and MTT (28 µl of a 5 mg/mL solution in PBS) was added to each well; the plates were incubated for 90 min at 37°C; then, DMSO (130 µL) was added to each well to dissolve the formazan crystals. After shaking the plates to ensure complete dissolution of formazan, the optical density was determined at 490 nm in a multiwell spectrophotometer (Spectra max340PC-Molecular Devices) [21] [22]. The cell survival (viability) was determined by comparing the absorbance of the wells containing the cells treated with MTF solutions (treated and untreated) to the cells exposed to a negative control (culture media with cells). Positive control was done by pure DMSO. An initial solution of sodium hypochlorite 1 mg L<sup>-1</sup> was neutralized by sodium thiosulfate and used as blank. A 30% reduction in the viability of a given sample was considered as a positive cytotoxic response. These experiments were also performed in triplicate.

### 3. Results and Discussion

#### 3.1. CP Degradation and Mineralization

Changes in CP (negative ion  $m/z$  216.0700) areas during ozonation and chlorination were monitored by high performance liquid chromatography coupled to the hybrid mass spectrometry system. The ozonation and chlorination promoted high degree of CP degradation, with 100% removal efficiencies after 5 min of exposure. Total organic carbon content (TOC/TOC<sub>0</sub>) as a function of their reaction time are presented in **Figure 2**.

Ozone is unstable in water, however, the unique feature of ozone is its decomposition into hydroxyl radicals ( $\bullet$ OH), which are the strongest oxidants in water. In water,  $\bullet$ OH reacts fast with CP and the by-products formed from the reaction of  $\bullet$ OH also reacts fast with CP. Ozonation may lead to a complete removal of organic compounds, but it does not lead to the complete mineralization of organics, which results in the formation of carboxylic acids, carbonyl compounds, and many others by-products [8] [10] [12].

One hypothesis for CP removal by chlorination can also be attributed to the fact that reduced sulfur moieties can easily be oxidized in presence of chlorine. In the case of thiol-containing compounds, as captopril, thiols oxidation leads, mainly, to disulfide and sulfonic acid [6] [27].

Although impressive CP degradation rates of 100% were achieved when using both systems, the TOC data revealed that CP was not mineralized to a similar extent, even after a treatment time as long as 30 min. For instance, the highest

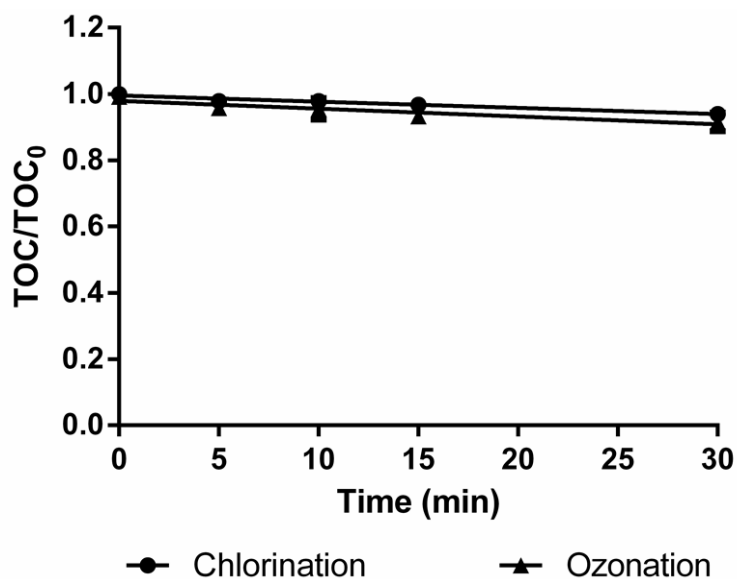
mineralization rate was only 7.63% and it was achieved upon the application of the ozonation. Moreover, the mineralization rates achieved through chlorination were lower (6.4%). These findings indicate that whereas most of the original CP was not mineralized, recalcitrant by-products were generated under these oxidative conditions.

### 3.2. Identification of CP By-Products: Proposal of a Degradation Route

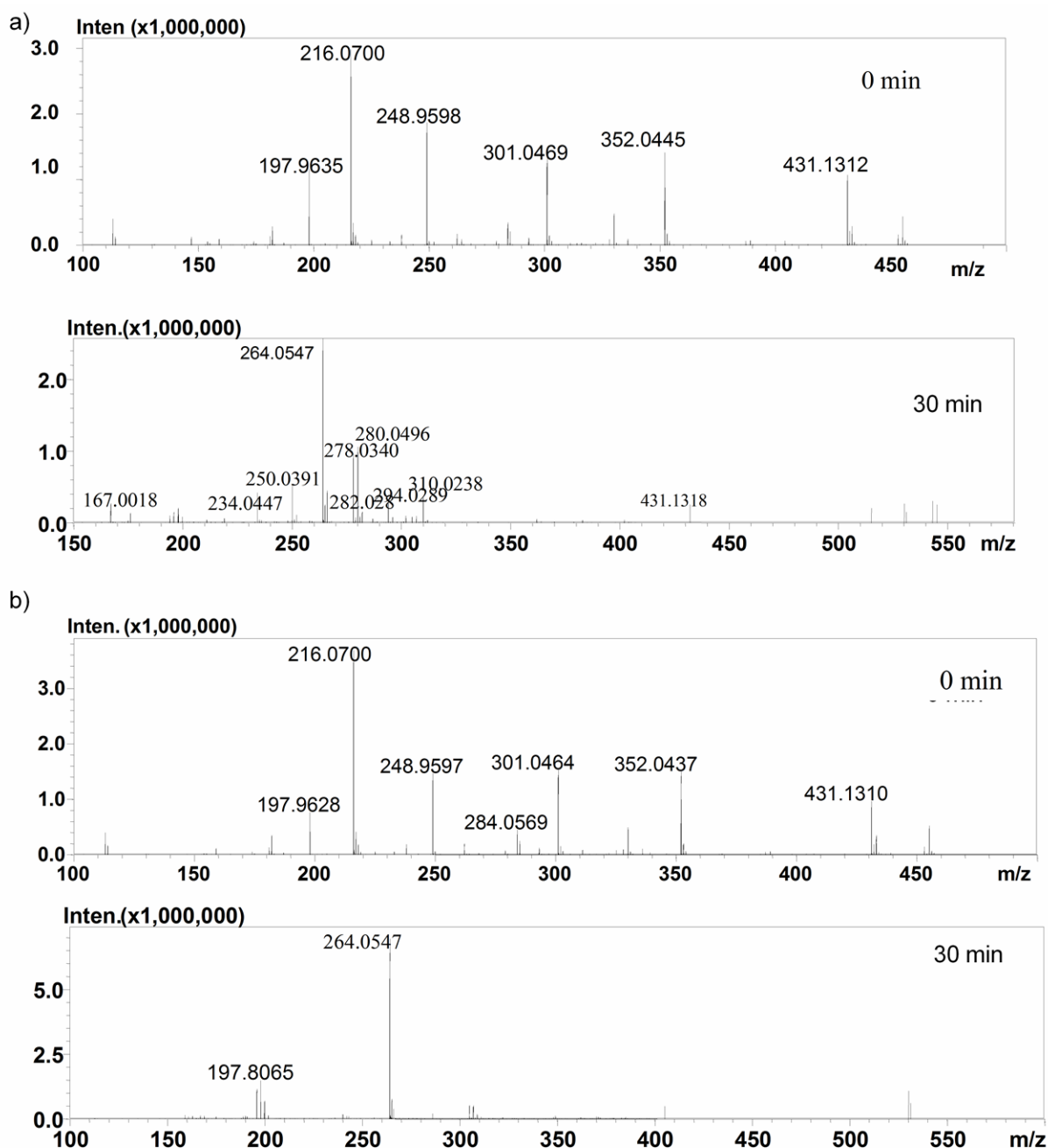
The aliquots collected during the ozonation and chlorination experiments were analyzed by [ESI(-)-HRMS] and HPLC/HRMS. Examples of the mass spectra and extracted-ion chromatogram (EIC) containing peaks from the CP and its by-products are shown on **Figure 3** and **Figure 4**, respectively. The mass spectra recorder in times of 0 and 30 min are depicted in **Figure 3**.

**Figure 3** clearly shows that whereas the CP ( $m/z$  216.0700) is fully consumed after 30 min of ozonation and chlorination experiments, the total carbon content in solution remained practically unchanged (**Figure 2**). The identification of the main by-products was carried out using the software formula predictor from Shimadzu, in order to propose a plausible degradation route. These ESI(-)-HRMS and HPLC/HRMS results allowed the detection of thirteen by-products, which elemental compositions were assigned based on the accurate mass measurements (<5 ppm) provided by the TOF analyzer, **Figure 3(a)** and **Figure 3(b)** [28].

After a meticulous analysis of these data, it was possible to propose the molecular formula for the thirteen degradation by-products of CP detected in their deprotonated form, as shown in **Table 3**. A maximum error of 3.00 ppm between the experimental and theoretical accurate masses of CP and its proposed by-products was observed.



**Figure 2.** Total organic carbon content (TOC/TOC<sub>0</sub>) as a function of ozonation time. TOC<sub>0</sub> = 4.415 mg·L<sup>-1</sup>.

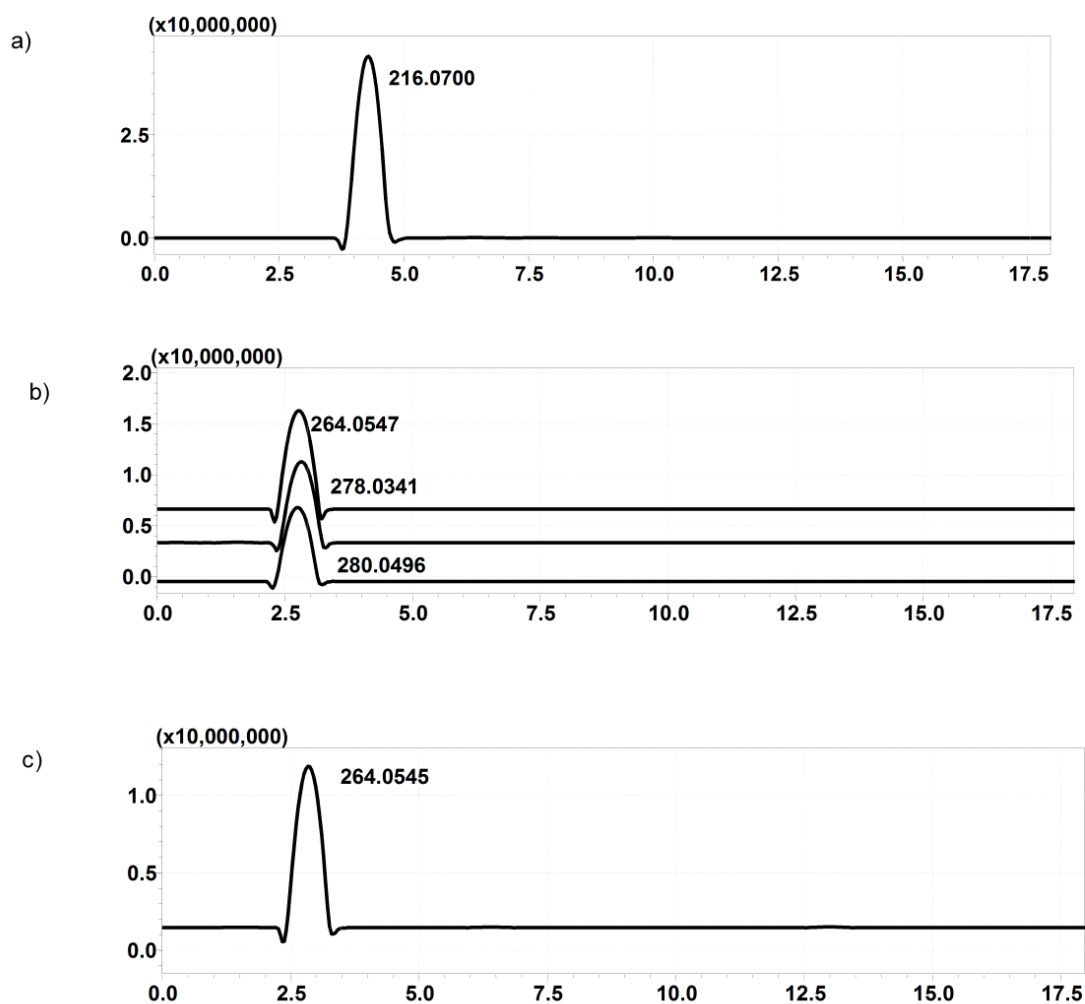


**Figure 3.** High resolution mass spectra (HRMS) recorded for aliquots (0 and 30 min) of the (a) ozonation and (b) chlorination.

Based on these results as well as on the well-known reactivity of hydroxyl radical towards organic molecules in aqueous medium and isotopic ratio (Figure 5), a route for CP ozonation (Figure 6) and CP chlorination (Figure 7) could be proposed.

The by-product 1 ( $C_9H_{15}NO_6S$ ,  $m/z$  264.0547) can be formed from the oxidation of CP thiol group, and such oxidation probably happened because of sulfur's ability of expanding its octet. The degradation progress follows different routes. The by-product 2 ( $C_9H_{15}NO_7S$ ,  $m/z$  280.0496) was probably formed after a hydroxylation of the intermediate 1, followed by its successive oxidation

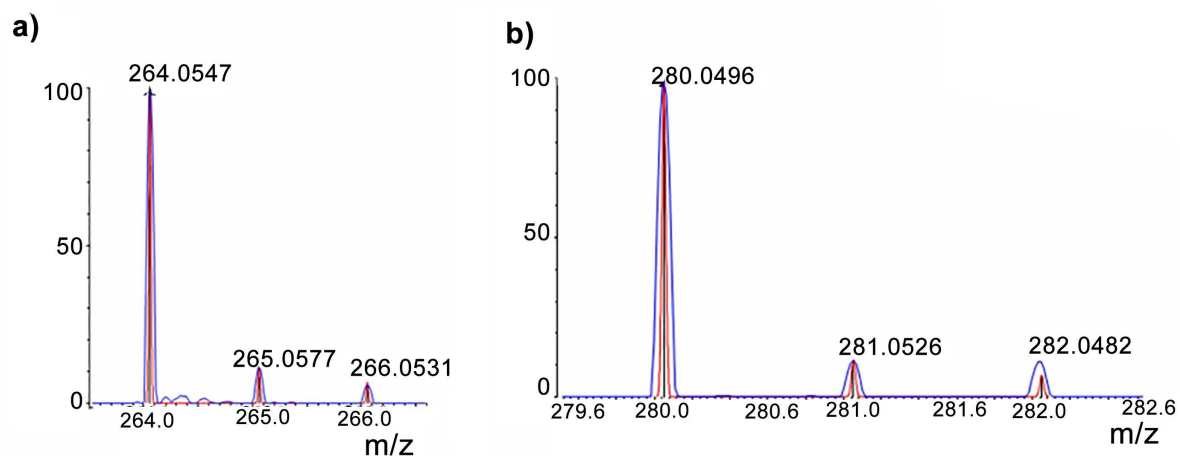




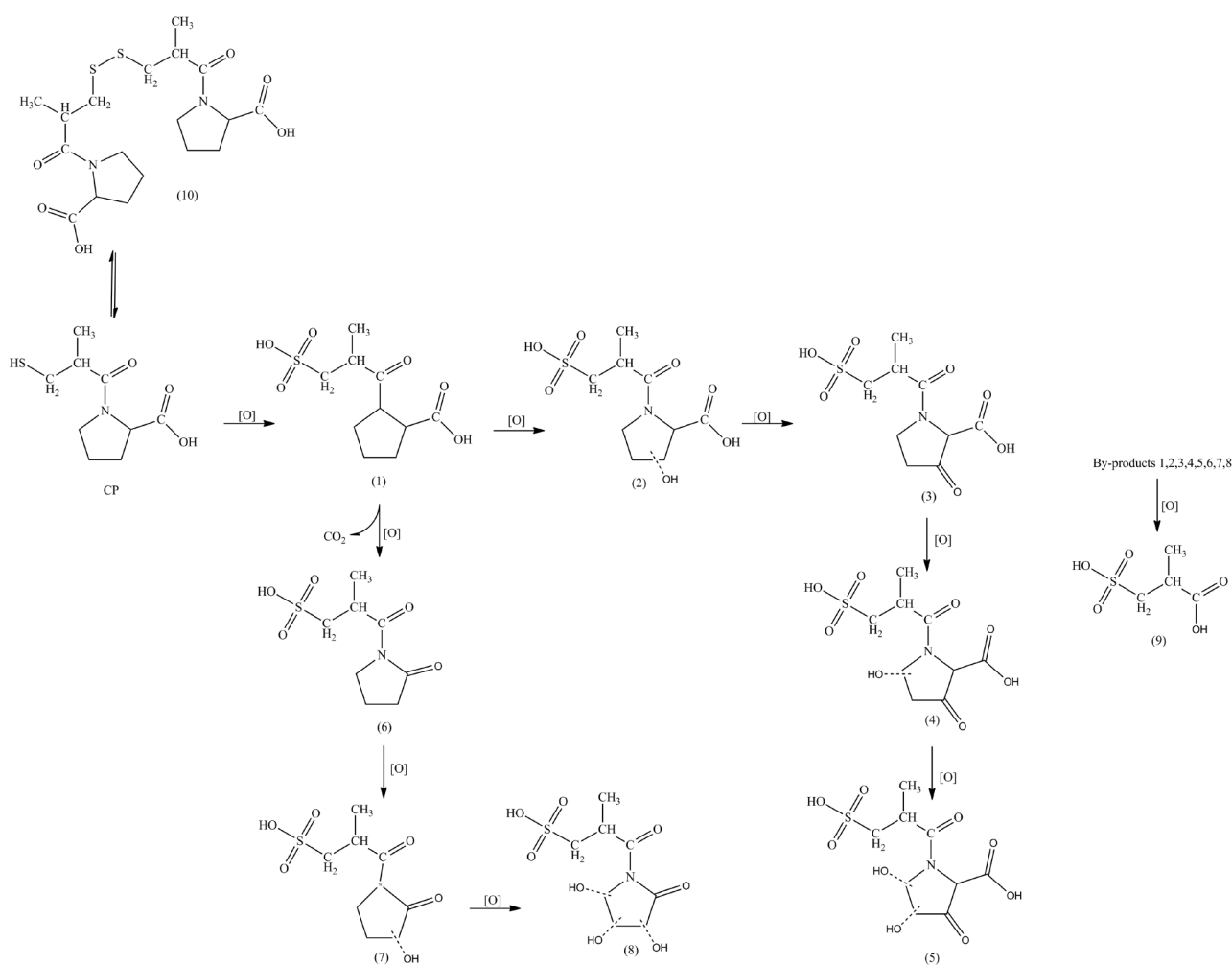
**Figure 4.** Extracted-ion chromatogram (EIC) using a mass window of  $\pm 3.00$  mDa from the CP (0 min) and by-products (30 min). To facilitate visualization, only 3 of the 10 intermediates are plotted. (a) Captopril; (b) ozonation by-products ; (c) chlorination by-products.

**Table 3.** Molecular formulae (calculated from HRMS data) of CP and its main by-products

Compound	Molecular Formula	Molecular Mass	(M-H) <sup>-</sup> theoretical	(M-H) <sup>-</sup> Experimental	Error (ppm)	Double bond equivalence
CP	C <sub>9</sub> H <sub>15</sub> NO <sub>3</sub> S	217.0773	216.0700	216.0700	0	3
1	C <sub>9</sub> H <sub>15</sub> NO <sub>6</sub> S	265.0620	264.0547	264.0547	0	3
2	C <sub>9</sub> H <sub>15</sub> NO <sub>7</sub> S	281.0569	280.0496	280.0496	0	3
3	C <sub>9</sub> H <sub>13</sub> NO <sub>7</sub> S	279.0413	278.0340	278.0340	0	4
4	C <sub>9</sub> H <sub>13</sub> NO <sub>8</sub> S	295.0362	294.0289	294.0286	1,50	4
5	C <sub>9</sub> H <sub>13</sub> NO <sub>9</sub> S	311.0311	310.0238	310.0241	1.61	4
6	C <sub>8</sub> H <sub>13</sub> NO <sub>6</sub> S	235.0514	234.0442	234.0442	0	3
7	C <sub>8</sub> H <sub>13</sub> NO <sub>6</sub> S	251.0464	250.0391	250.0390	0.37	3
8	C <sub>8</sub> H <sub>13</sub> NO <sub>8</sub> S	283.0362	282.0289	282.0284	2.21	3
9	C <sub>4</sub> H <sub>6</sub> O <sub>8</sub> S	168.0092	167.0020	167.0018	0.80	1
10	C <sub>18</sub> H <sub>28</sub> N <sub>2</sub> O <sub>6</sub> S <sub>2</sub>	432.1389	431.1316	431.1318	1.00	6

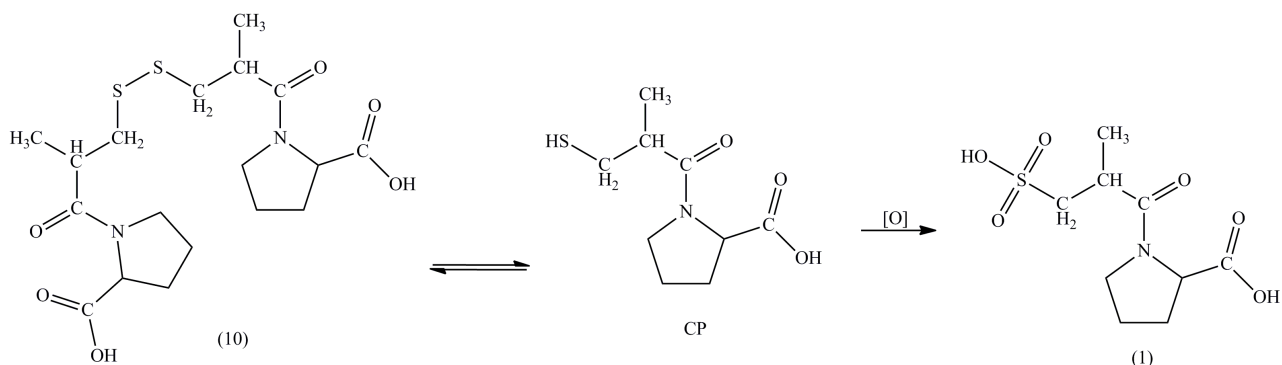


**Figure 5.** Theoretical (red) and experimental (blue) isotope ratio for the by-product 1 and 2.



**Figure 6.** Possible transformation pathways followed by captopril after ozonation.

forming a ketone group in by-product **3** ( $C_9H_{13}NO_7S$ ,  $m/z$  278.0340) [19]. The by-products **4** ( $C_9H_{13}NO_8S$ ,  $m/z$  294.0289) and **5** ( $C_9H_{13}NO_9S$ ,  $m/z$  310.0238), which have an additional hydroxyl group, are probably formed from oxidation



**Figure 7.** Possible transformation pathways followed by captopril after chlorination.

of by-products 3 and 4, respectively. In sequence, the by-product **6** ( $C_8H_{13}NO_5S$ ,  $m/z$  234.0442) was probably formed after a decarboxylation of the intermediate **1** followed by its successive oxidation (forming a hydroxyl and then a ketone group) given that the medium is very oxidative. The by-product **7** ( $C_8H_{13}NO_6S$ ,  $m/z$  250.0391) has another hydroxyl group in the ring when compared to by-product **6**. The by-product **8** ( $C_8H_{13}NO_8S$ ,  $m/z$  282.0289), and it has two hydroxyl groups in the ring when compared to by-product **7**. The acyclic intermediate **9** ( $C_4H_8O_5S$ ,  $m/z$  167.0020) could be generated from intermediates (**1-8**). The by-product **10** ( $C_{18}H_{28}N_2O_6S_2$ ,  $m/z$  431.1316) can also be oxidized to form the CP ( $C_9H_{15}NO_3S$ ,  $m/z$  216.0700).

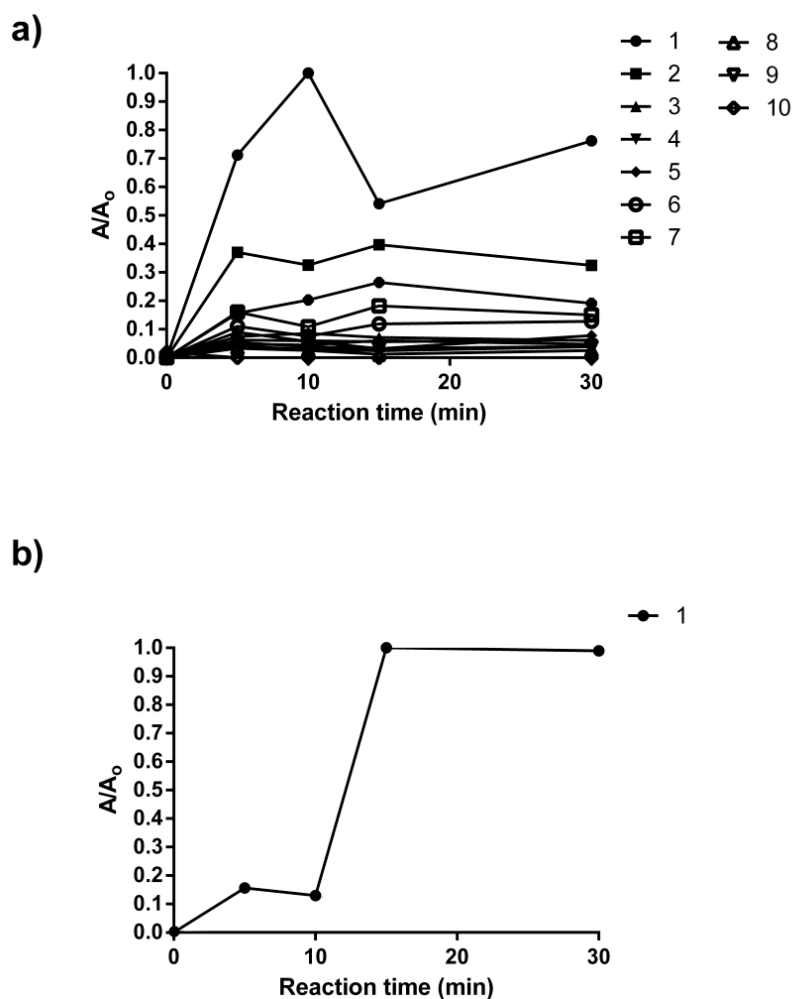
In the case of captopril thiol group, the oxidation mainly leads to sulfonic acid (by-product **1**) and disulfide (by-product **10**; detection was not possible) [6] [27]. It is noteworthy that the intermediate **1** has been previously reported by Mahmoud and Kummerer (2012), who studied the photodegradation and the aerobic biodegradation of CP and its dimer (captopril disulfide) in aqueous solution. The intermediate **1**, **3**, **6** and **7** has been previously reported by Freitas and co-authors (2017) who studied the photodegradation of CP in aqueous solution after oxidation induced by photolysis and photocatalysis [21].

The evolution, formation, and degradation of each intermediate (**1 - 10**) during the ozonation and chlorination processes, were monitored by HPLC/HRMS. The results from EIC were analyzed to build plots of the temporal accumulation of intermediates for all processes evaluated (Figure 8). Hence, for the ozonation system practically all intermediates remained in solution after 30 min of exposure (Figure 8(a)). For the chlorination system (Figure 8(b)) only the intermediates **1** remained in solution after 30 min of reaction. In both systems it was observed an increase in the relative concentrations of by-products. Therefore the data presented in Figure 8 corroborates the results of TOC analysis and are in agreement with the proposed degradation route.

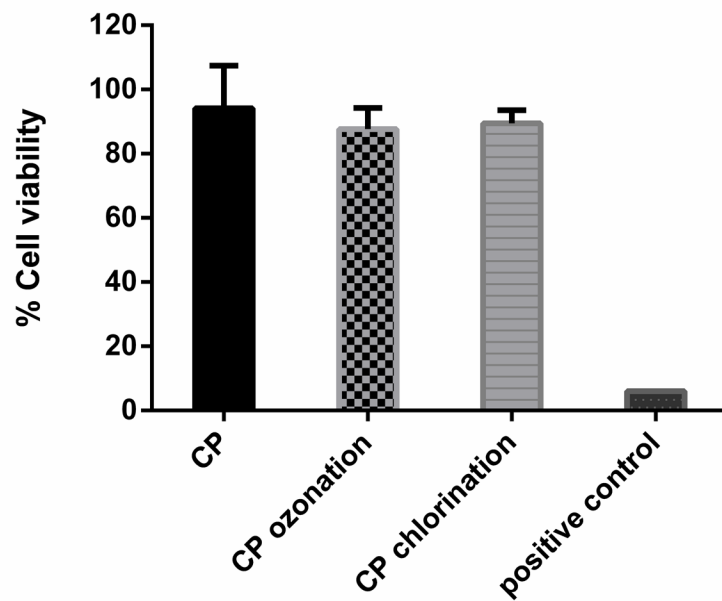
### 3.3. Cytotoxicity Assay (MTT)

Cell survival (viability) was determined by comparing the absorbance of the wells containing the cells treated with CP solutions (treated and untreated) to

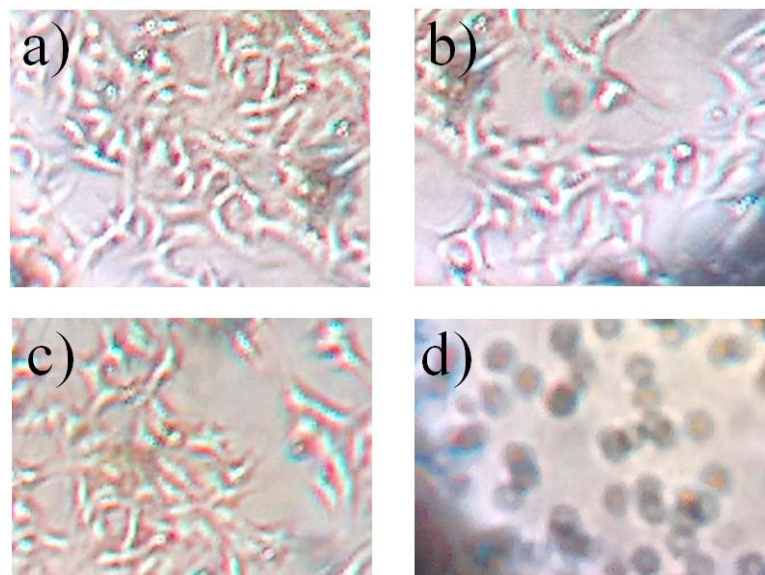
the cells exposed to a negative control (culture media with cells). The results were expressed in percentage of cell viability. It has to be noted that in order to avoid overestimation or underestimation of the toxicity of a substance, incubations with various concentrations were required to be able to accurately determine the cytotoxicity. Since none of the diluted samples tested decreased cell viability, **Figure 9** shows cell viability data for undiluted (at the highest tested concentration, *i.e.*,  $1 \text{ mg}\cdot\text{L}^{-1}$ ) CP samples before and after degradation experiments. In addition, the cells were exposed to DMSO, considered as positive controls. **Figure 10** shows cell morphologies before and after the treatment of by-products. It can be seen that the morphology of the cells treated with the by-products solutions were not different when compared to the cells treated with CP. Cells treated with the positive control showed the spherical morphology thus showing that they are dead.



**Figure 8.** Plots of the relative areas of the degradation products of CP (1 - 10) versus time for the systems: The areas of the by-products were determined by HPLC/HRMS directly from the aliquots collected at assorted times (one determination for each aliquot). A and A<sub>0</sub> refer to the chromatographic peak areas at reaction times t and 0, respectively. A value of 1.0 was attributed to the maximum relative concentration of each by-product. (a) Ozonation and (b) Chlorination.



**Figure 9.** Graph of MTT assay of CP untreated and after 30 min of degradation tests showing the % cell viability HepG2 cells after exposure to concentrations [ $1.0 \text{ mg}\cdot\text{L}^{-1}$ ] of the samples. Values are expressed as mean and SD.



**Figure 10.** Cell morphologies before and after the treatment: (a) before treatment; (b) after exposure ozonation by-products; (c) after exposure chlorination by-products and (d) after exposure positive control.

Cells viability exposed to all samples (raw and treated CP solutions) were the same as that of control cells. Values are expressed as mean and 95% confidence interval for the mean. This shows that there was no decrease of cells viability after their exposition to CP or its degradation by-products. Considering a 30% reduction in cell viability as cytotoxic effect [25], it can be seen that none of the samples tested at the highest concentration showed toxicity to HepG2 cells. Un-

fortunately no reference on toxicity effects of CP or its by-products were found in the literature for comparison.

#### 4. Conclusion

The chlorination and ozonation were efficient in promoting the degradation of captopril in an aqueous solution, but not in causing its mineralization. The persistent by-products formed under these conditions were correctly assigned by the application of HPLC/HRMS. Results from HPLC/HRMS analyses allowed the detection and characterization of the 10 by-products, all of them persistent even after an exposure time as long as 30 min. These by-products were proposed to be formed via a prompt reaction with hypochlorous acid or attack of hydroxyl radicals on the original substrate as well as on the subsequent intermediate compounds. Based on these results a route for the ozonation and chlorination of CP, which includes its successive oxidation in the aqueous medium, could be proposed. One can also conclude that the samples after the degradations experiments were not cytotoxic to HepG2 cells at MTT assay. Thus, these findings underline the fact that toxicity analyses of treated samples and identification of possible by-products are essential in any research that deals with water treatment. Future works need to be done to optimize the oxidative conditions in an attempt to enhance high mineralization degree and avoid the accumulation of by-products in the treated water.

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