Drug Metabolism & Toxicology

Huang et al., J Drug Metab Toxicol 2015, 6:3
http://dx.doi.org/10.4172/2157-7609.1000181

Research Article Open Access

Ozone-Oxidation Products of Ibuprofen and Toxicity Analysis in Simulated Drinking Water

Haoping Huang[1], Guoguang Liu[1*], Wenying Lv[1], Kun Yao[1], Yapu Kang[1], Fuhua Li[1] and Longli Lin[2]

[1]*College of Environmental Science and Engineering, Guangdong University of technology, Guangzhou, China*

[2]*College of chemistry and chemical engineering, Bijie University, Guizhou, China*

[*]**Corresponding author:** Guoguang Liu, College of Environmental Science and Engineering, Guangdong University of technology, Guangzhou-510006, China, Tel: +81-3-3762-4151; Fax: +81-3-3298-0045; Email: liugglwy@163.com

Received date: March 14, 2015, **Accepted date:** April 20, 2015, **Published date:** April 29, 2015

Abstract

In this study, we report the simulation of the ozone disinfection of drinking water in the presence of ibuprofen, its oxidative degradation products, and the acute toxicity analysis by luminescent bacteria bioassay. The results showed that the ibuprofen oxidation followed first-order kinetics. The ultra-performance liquid chromatography quadrupole time-of-flight mass (UPLC/Q-TOF-MS) analysis showed that the major oxidation products of ibuprofen were as follows: (i) 4-ethylbenzaldehyde, (ii) 2-[4-(1,2-dihydroxypropyl)-2-hydroxy carboxylic acid, (iii) 1-(4-ethylphenyl)-2-methylpropanol, (iv) 1-(4-ethylphenyl)-2-methylpropanone, (v) 2-[4-(1-hydroxy-2-methylpropyl)phenyl]isobutyl propionate, and (vi) 4-ethylbutyl benzene. A reaction mechanism for the ibuprofen oxidation was proposed based on the oxidative degradation products. The photobacterium phosphoreum toxicity tests showed that the toxicity of the ibuprofen oxidation products is higher than ibuprofen. Thus, the ozone-oxidation products of ibuprofen generate a higher risk of acute toxicity.

Keywords: Ibuprofen; Toxicity; Ozone; Drinking water disinfection; Photobacterium phosphoreum

Introduction

In recent years, the wide use of pharmaceutical and personal care products (PPCPs) has significantly increased the PPCP pollution in drinking water [1,2]. Because of strong biological activity of PPCPs [3,4] they are likely to cause various diseases and harm to human health [5,6].

Ibuprofen, a new generation nonsteroidal anti-inflammatory drug [7], is widely used for low toxicity, high efficacy, and less side effects than aspirin and paracetamol [8,9]. It enters aquatic ecosystem through a variety of ways, affecting human health [10-12], and has been detected in drinking water [13-16].

Ozone is commonly used to disinfect drinking water [17,18]. Ozone generates strong oxidizing hydroxyl radicals to kill harmful bacteria in water and oxidatively degrade the organic pollutants into small molecules. Currently, ozone oxidation treatment is being used for antibiotics, hormones, and other pollutants [19,20].

In this study, we experimentally investigated the degradation dynamics of ibuprofen by ozone oxidation. The structures of ibuprofen oxidation products were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS), and the reaction mechanism was elucidated. The biological acute toxicity of the ibuprofen oxidation products was evaluated by luminous bacteria method. This study has practical significance for understanding the transformation of ibuprofen in drinking water disinfection process and its effect on the safety of drinking water [21,22].

Experimental

Instruments and chemicals

The instruments and chemicals used in this study were as follows: electronic balance (AL104, Mettler Toledo), pHs-3C pH meter (Shanghai Jing Branch Industrial), ozone generator (CY-H500), Liquid Chromatography (LC-20AT, Shimadzu), AS20500BDT-I-type ultrasonic cleaning instrument (Auto Science), Smart2 pure ultrapure water/water integrated system (TKA, Germany), and Ultra-Performance Liquid Chromatography (UPLC) combined with time-of-flight tandem quadrupole mass (Q-TOF-MS) spectrometer (ACQUITY/Q-TOFmicro, Waters, USA). Ibuprofen (≥ 99%), methanol, and acetonitrile were of HPLC grade (Shanghai Scientific Instrument and Ann Spectrum). Sulfuric acid, sodium acetate, and sodium chloride (Sinopharm) were of analytical reagent grade. Luminescent bacteria were obtained from the Institute of Soil Science, China. All the experiments were conducted in ultrapure water.

Experimental method

Confection of ibuprofen: Weigh 1.000 g ibuprofen powder and was added into a 1000 ml volumetric flask containing approximately 980 ml of ultrapure water under ultrasonication till a constant volume of 1000 ml, ultrasonicated for 10 min, and stored at 4°C. Then a 1000 mg l^{-1} stock solution of ibuprofen in acetonitrile was prepared. A 1.25 ml 1000 mg L^{-1} stock solution of ibuprofen was added dropwise into a 250 ml volumetric flask containing approximately 230 ml of ultrapure water under ultrasonication till a constant volume of 250 ml, ultrasonicated for 3 min, and a 5.00 mg•l^{-1} ibuprofen solution was prepared.

Ozone oxidation reaction: Ozone (0.06 L/min) was added to the reaction mixture (200 ml Ibupfofen(IBU)), and the solution was

magnetically stirred at room temperature. The samples were taken out at different time intervals and analyzed by UPLC/Q-TOF-MS. Each experiment was conducted three times in order to eliminate the experimental error, and the results were averaged.

Toxicity test: According to the national standard method, light-emitting bacteria T_3 (Souchong lyophilized powder, indicator organisms) was used to measure the acute toxicity of ibuprofen oxidation products in every 15 min. (Water quality-Determination of the acute toxicity-Luminescent bacteria test, GB/T15441-1995).

Analytical Methods

LC analysis

Ibuprofen and its oxidation products were analyzed by UPLC/Q-TOF-MS (11). The chromatographic conditions were as follows: column: Zorbax Eclipse XDB-C18 (2.1 × 150 mm^2, 5 μm); temperature: 30°C, mobile phase: acetonitrile/acetic acid buffer solution (50:50 v/v containing 0.3% acetic acid, pH 3.0), photodiode array detector, 263 nm wavelength, flow rate: 0.2 ml min^{-1}, injection volume: 4 μL, elution time: approximately 12.50 min. The retention times were used for the qualitative analysis, and the external standard method was used for the quantitative analysis.

Qualitative analysis

The UPLC/Q-TOF-MS spectrometer conditions were as follows: eluent: 0.3% acetic acid and acetonitrile gradient in table 1, injection volume: 10 μL, negative ion mode (ESI+), capillary voltage (cone): 3500 v, cone voltage (sample): 30 V, source temperature: 100°C, anti-blowing temperature (desolvation): 350°C, cone anti-gas flow(cone): 50 L/h, desolvation gas flow (des): 500 L/h, and scanning range: 100-800 m/z.

Time	Flow	A%	B%
0.00	0.2	90	10
2.00	0.2	72	28
4.00	0.2	50	50
6.00	0.2	50	50
8.00	0.2	30	70
10.00	0.2	0	100
10.20	0.2	90	10

Table 1: Mobile phase gradient.

Results and Discussion

Ozone oxidation of ibuprofen

The samples of ibuprofen oxidation products (5 mg/L, pH 5) for the UPLC/Q-TOF-MS analysis were taken out at 1, 2, 4, 6, 10, 15 and 20 min intervals, and the results (Ln C vs. t) are shown in Figure 1. The ibuprofen oxidation was consistent with the following kinetic equation: Ln (C/C_0)=-0.0115t-0.00978, R =0.9951.

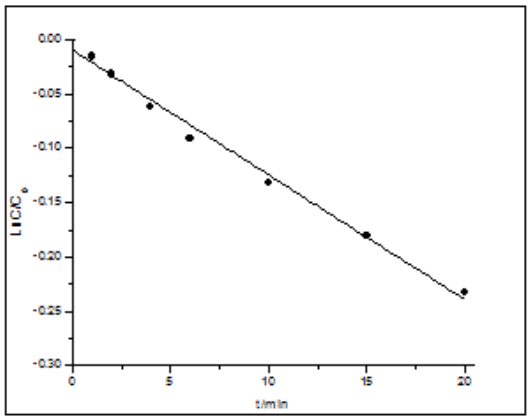

Figure 1: First-order kinetics for oxidation of ibuprofen.

LC/MS/MS analysis

The ibuprofen oxidation products were analyzed by mass spectrometry, as shown in Figure 2, eight significant peaks were obtained, and the molecular weights are listed in Table 2. The mass fragmentations of the eight peaks are shown in Table 2 [23].

From the structure of ibuprofen and oxidation basics of organic compounds [24-26], the mass spectrum of ibuprofen was analyzed as follows:

Firstly, the exact molecular weight of peak 1 was 133.0670, and the calculated elementary composition was C_9H_9O. The tandem mass spectral data showed its fragment at m/z 105. Thus, the tert-butyl side chain was oxidized to an aldehyde group and the COOH group was removed. While peak 2 was 239.0913 and the calculated elementary composition was $C_{12}H_{15}O_5$. The tandem mass spectral data showed its fragments at m/z 223, 206, 195, and 149. Thus, the tert-butyl side chain and the aromatic ring were hydroxylated.

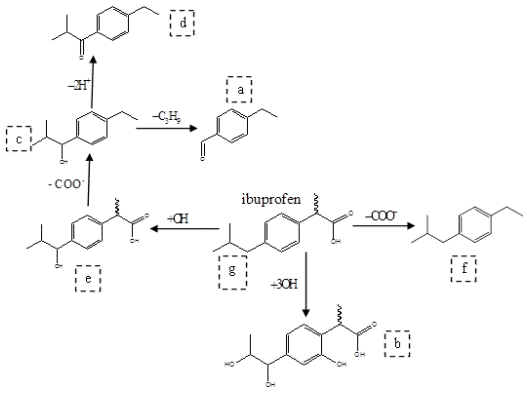

Figure 2: Total ion current of ibuprofen oxidation products under positive ion mode

S.No.	Retention time	Detected ion	Mass (m/z)	Theoretical Value (m/z)	Main ions of MS2	Elementary Composition
1	3.49	[M − H]+	133.0670	133.0653	133 [M − H], 105 [M − H − 28]	C_9H_9O
2	3.68	[M − H]+	239.0913	239.0919	239 [M − H], 223 [M − H − 16], 206 [M − H − 16 − 17], 195 [M − H − 44], 149	$C_{12}H_{15}O_5$
3	3.68	[M − H]+	177.1278	177.1279	177	$C_{12}H_{17}O$
4	3.99	[M − H]+	177.1276	176.1279	177	$C_{12}H_{17}O$
5	4.37	[M − H]+	175.1100	175.1123	175	$C_{12}H_{15}O$
6	4.80	[M − H]+	221.1167	221.1178	221 [M − H], 177 [M − H − 44], 134 [M − H − 44 − 43]	$C_{13}H_{17}O_3$
7	6.04	[M − H]+	473.2163	473.2175	473, 255, 216	$C_{26}H_{33}O_8$
8	6.78	[M − H]+	205.1235	205.1229	205 [M − H], 161 [M − H − 44]	$C_{13}H_{17}O_2$

Table 2: Mass spectrometry data and mass fragments for ibuprofen oxidation products

Secondly, the exact molecular weights of peaks 3 and peak 4 were 177.1278 and 177.1276, respectively, and the calculated elementary composition was $C_{12}H_{17}O$. The tandem mass spectral data showed no other fragments. Thus, peaks 3 and 4 were for the same substance, where one of the side chains was hydroxylated and the other side chain was decarboxylated.

Thirdly, the peak 5 exact molecular weight was 175.1100, and the calculated elementary composition was $C_{12}H_{15}O$. The tandem mass spectra showed no other fragments. Thus, the tert-butyl side chain was hydroxylated and peak 6 was 221.1167; the calculated elementary composition was $C_{13}H_{17}O_3$. The tandem mass spectra showed its fragments at m/z 177 and 134.

Lastly, the exact molecular weight of peak 7 was 473.2163, and the calculated elementary composition was $C_{26}H_{33}O_8$. The tandem mass spectra showed its fragments at m/z 255 and 216. While peak 8 was 205.1235 and calculated elementary composition was $C_{13}H_{17}O_2$. The tandem mass spectra showed its fragment at m/z 161. Thus, it was confirmed to be ibuprofen.

The tandem mass spectra of ibuprofen oxidation products are shown in Figure 3 and 4; only the tandem mass at m/z 175 and 177 had parent ions. The tandem mass of the other oxidation products was relatively simple, mainly the loss of functional groups such as carboxyl, keto, and side chain.

The UPLC/Q-TOF-MS analysis [27-29] indicated that the major ibuprofen oxidation products were as follows: (i) 4-ethylbenzaldehyde, (ii) 2-[4-(1,2-dihydroxypropyl)-2-hydroxy carboxylic acid,(iii) 1-(4-ethylphenyl)-2-methylpropanol,(iv)1-(4-ethylphenyl)-2-methylpropanone, (v) 2-[4-(1-hydroxy-2-methylpropyl)phenyl]isobutyl propionate, and (vi) 4-ethylbutyl benzene.

Based on the molecular weight, structure, and mass spectra, the path of the ibuprofen oxidation in the simulation of ozone disinfection in drinking water is shown in Figure 3.

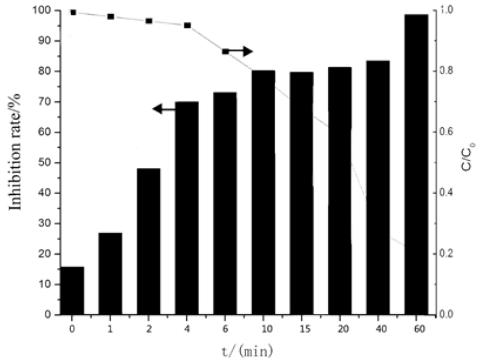

Figure 3: Photobacterium phosphoreum inhibition.

Acute toxicity analysis of oxidation products by photobacterium phosphoreum

As shown in Figure 3, the *P. phosphoreum* T_3 luminous inhibition rate of ibuprofen (I%) was 15.7%. The rate gradually increased with the progress in the oxidation reaction. Within 1 min of the ibuprofen oxidation, the inhibition rate increased with increasing time; however, the inhibition rate was relatively small. As shown in Figure 4, the main product was D with less known toxicity. The concentration of D decreased with increasing time, and the products A, B, C and E were obtained. The *P. phosphoreum* toxicity increased with increasing oxidation time.

Within 2 min to 20 min oxidation time, the *P. phosphoreum* inhibition rate increased from 48.5% to 81.3%. The inhibition rate reached 98% when the oxidation time was extended to 60 min, and high concentrations of A, B, C and E were obtained, indicating that these products were highly toxic.

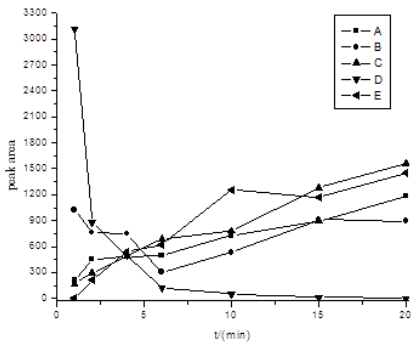

Figure 4: Variation in ibuprofen oxidation products.

The ibuprofen oxidation products generated a higher risk of acute toxicity. In the beginning of the oxidation reaction, less amount of intermediate was formed, mainly because of the degradation of ibuprofen. Therefore, the inhibition rate of *P. phosphoreum* was relatively low. The oxidation of this intermediate increased with time. After 4 min, high toxicity was observed, and the *P. phosphoreum* inhibition rate increased to 70%. The inhibition rate increased with increasing amount of ibuprofen oxidation products.

Conclusion

The simulation of the ozone disinfection of drinking water showed that ibuprofen ozonation followed a quasi-kinetic equation.

The UPLC/Q-TOF-MS analysis indicated that the major oxidation products of ibuprofen were as follows: (i) 4-ethylbenzaldehyde, (ii) 2-[4-(1,2-dihydroxypropyl)-2-hydroxy carboxylic acid, (iii)1-(4-ethylphenyl)-2-methylpropanol,(iv) 1-(4-ethylphenyl)-2-methylpropanone, (v) 2-[4-(1-hydroxy-2-methylpropyl)phenyl]isobutyl propionate, and (vi) 4-ethylbutyl benzene. The *P. phosphoreum* toxicity tests showed that the ibuprofen oxidation products generated a higher risk of acute toxicity.

Acknowledgement

The authors would like to thank the Natural Science Foundation of China (No.21377031 project) for the financial support provided for the realization of this study. We also thank Yanmei Zhong for UPLC/Q-TOF-MS analysis and Xialing Ji for helping prepare the experiment.

References

1. Nakada N, Shinohara H, Murata A, Kiri K, Managaki S, et al. (2007) Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. Water Research 41: 4373-4382.
2. Khetan SK, Collins TJ (2007) Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry. Chem Rev 107: 2319-2364.
3. Xia K, Bhandari A, Das K, Pillar G (2005) Occurrence and Fate of Pharmaceuticals and Personal Care Products (PPCPs) in Biosolids. J Environ Qual 34: 91-104.
4. Lindström A, Buerge IJ, Poiger T, Bergqvist PA, Müller MD, et al. (2002) Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. Environ Sci Technol 36: 2322-2329.
5. Loos R, Gawlik BM, Locoro G, Rimaviciute E, Contini S, et al.(2009) EU-wide survey of polar organic persistent pollutants in European river waters. J Environ. Pollu. 157:561-568.
6. Jolanta D, Agata KW, Jacek N (2004) Fate and Analysis of Pharmaceutical Residues in the Aquatic Environment. Critical Reviews in Analytical Chemistry 34: 51-67.
7. Hutt AJ, Caldwell J (1983) The metabolic chiral inversion of 2-arylpropionic acids-a novel route with pharmacological consequences. J Pharm Pharmacol 35: 693-704.
8. Buser HR, Poiger T, Muller MD (1999) Occurrence and Environmental Behavior of the Chiral Pharmaceutical Drug Ibuprofen in Surface Waters and in Wastewater. Environ Sci Technol 33: 2529-2535.
9. Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. J Water Res 32: 3245–3260
10. Metcalfe CD, Koenig BG, Bennie DT, Servos M, Ternes TA, et al. (2003) Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environ Toxicol Chem 22: 2872-2880.
11. Batt AL, Kim S, Aga D.S (2007) Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations J. Chemosphere 68: 428–435
12. Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H (2006). Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Research 17: 3297-3303.
13. Carballa M, Omil F, Lema JM (2007) Calculation Methods to Perform Mass Balances of Micropollutants in Sewage Treatment Plants. Application to Pharmaceutical and Personal Care Products (PPCPs). Environ Sci Technol 41: 884-890.
14. Lindberg RH, Olofsson U, Rendahl P, Johansson MI, Tysklind M, et al. (2006) Behavior of fluoroquinolones and trimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. Environ Sci Technol 40: 1042-1048.
15. Al-Rifai JH, Gabelish CL, Schäfer AI (2007) Occurrence of pharmaceutically active and non-steroidal estrogenic compounds in three different wastewater recycling schemes in Australia. Chemosphere 69: 803-815.
16. Duong HA, Pham NH, Nguyen HT, Hoang TT, Pham HV, et al. (2008) Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. J Chemosphere 72: 968–973.
17. Huber MM, Canonica S, Park GY, von Gunten U (2003) Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. Environ Sci Technol 37: 1016-1024.
18. Klavarioti M, Mantzavinos D, Kassinos D (2009) Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. J Environ Int 5: 402-417.
19. Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, et al. (1999) Behaviour and occurrence of estrogens in municipal sewage treatment plants—I. Investigations in Germany, Canada and Brazil. Sci Total Environ 225: 81-90.
20. Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. J Pharm Pharmacol 37: 1-12.
21. Roefer P, Snyder S, Zegers RE, Rexing DJ, Fronk JL (2000) Endocrine-Disrupting Chemicals in a Source Water. J AWWA 92: 52-58.
22. Trussell RR (2001) Endocrine Distruptors and the Water Industry (PDF) J AWWA 9358–65.
23. Huang C, Sedlak DL (2001)Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. J Environ Toxicol Chem 20:133–139.

24. Laganà A, Bacaloni A, Fago G,Marino A (2000). Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry. Rapid Communications in Mass Spectrometry 14: 401-407.
25. Moder M, Schrader S, Winkler M, Popp P (2000) Solid-phase microextraction-gas chromatography-mass spectrometry of biologically active substances in water samples. J Chromatogram 873: 95-106.
26. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, et al. (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. J Environ Sci Technol 36: 1202-1211.
27. Santos JL, Aparicio I, Alonso EJ (2007) Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain) Environ. Int 33:596–601.
28. Stumpf M, Ternes TA, Wilken R-D, Rodrigues SV, Baumann W J Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. (1999) Sci Total Environ 225: 135–41.
29. Buser HR, Poiser T, Müller MD (1998) Occurrence and fate of the pharmaceutical drugdiclofenac in surface waters: rapid photodegradation in a Lake. J Environ Sci Technol. 32: 3449-3456.