Evolution of Absorbance Spectra of Ozonated Wastewater and Its Relationship with the Degradation of Trace-Level Organic Species

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Table of Contents

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Materials and Methods

Reagents: LC-MS grade water, methanol and acetonitrile were purchased from EMD Chemicals (Gibbstown, NJ, USA). LC-MS grade formic acid, mass spectrometric grade ammonium acetate, pCBA acid and selected PPCPs (bisphenol-A, carbamazepine, ibuprofen, iopromide and propranolol) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Internal standards included carbamazepine-d_{10}, ibuprofen-d_3, and bisphenol-A-d_4 supplied by C/D/N Isotopes Inc. (Quebec, Canada). KH_2PO_4 and NaOH were purchased from JT Baker (Phillipsburg, NJ, USA). Ultrapure water (18 MΩ cm) was produced by the Milli Q apparatus (Millipore, USA). Stock solutions were prepared by accurately weighing and dissolving requisite amounts of each compound in Milli Q water to yield a 1 mg/l concentration. Methanol was not used for the dissolution of the target compounds in order to prevent the impact of the solvent on ozone decay and $OH^{\bullet}$ concentrations. Phosphate buffer stock was prepared by dissolving 68.1 g KH_2PO_4 and 11.7 g NaOH in 1 L water. 20 mL of this solution was added to 1 L sample to obtain 0.001 M phosphate buffer at pH 7.0. pCBA stock solution (100 mg/L) was prepared by dissolving 10 g in 100 mL ultrapure water.

Wastewater characterization: A sample of secondary effluent (before disinfection) from a wastewater treatment site in Seattle was collected in a pre-cleaned, 10-L polypropylene container that had been thoroughly washed and rinsed successively with DI water and ultrapure water. Upon arrival to the laboratory, wastewater was filtered through a 0.45 μM nylon filter and stored at 4°C for no more than a week until used in experiments The

wastewater was analyzed for the following parameters: DOC 9.6 mg/L, pH 7.6, ammonia 22.0 mg/L NH_3-N, nitrate 66.2 mg/L, and nitrite 1.8 mg/L. Because the sample was filtered before water quality analyses, the TOC was expected to be primarily in dissolved form.

Ozonation experiments: Ozone was generated using an Airox Minipak ozonator (Pollution Control Industries Inc, Conn, USA) that was fed with oxygen. Experiments were performed in 125 mL Erlenmeyer flasks. Ozone stock solution concentrations and dissolved O_3 residuals were measured using the standard indigo method (2). In experiments with varying O_3 concentrations, aliquots of O_3 stock solutions (that had 9-11 mg/L of ozone) were added to achieve initial ozone concentrations of 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L O_3. The total volume of sample was 120 mL. After 30 minutes of exposure, residual ozone was quenched with sodium sulfite and the samples were processed to determine their UV spectra and PPCP concentrations. UV spectra of the samples were recorded and 100 mL of each sample were processed by SPE. These experiments used wastewater spiked with 1 µg/L of each PPCP species, which were spiked from 1 mg/L stock solution.

Additional experiments were performed with non-spiked wastewater exposed to a continuous flow of ozone that yielded an average study-state concentration of 1.5 mg/L O_3. Oxygen flow and voltages of ozone generator were optimized in order get 1.5 mg/L of average ozone concentration in the sample. Ozone concentrations in the sample were

(2) Clesceri, L. S., Greenberg, A. E., Eaton, A. D., Eds. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, 1998.

measured with indigo method at the reaction times of 5, 10 and 20 min. and found the average concentration is 1.5 mg/L ozone. Experiments were performed in 1 L amber glass bottles completely filled with wastewater. 80 mL sample aliquots were collected at exposure times 0, 0.5, 1, 2, 4, 8, 10, 12, 15 and 20 minutes. Residual ozone was quenched with sodium sulfite. UV spectra of the samples were recorded and 50 mL of each aliquot were extracted by SPE and analyzed for PPCPs. A similar experiment was conducted in presence of 75 µg/L pCBA to evaluate the OH· exposure.

***Analytical methods*:** Absorbance spectra were recorded using a PerkinElmer Lambda 18 spectrophotometer. Nitrate and nitrite concentrations were determined with a ICS-3000 ion chromatograph (Dionex Corporation, Sunnyvale, CA, USA) equipped with a AS-18 Ionpack column (250 x 2.0 mm id) preceded by a guard column (50 x 2.0 mm id) with the same packing material was used for the separation. Analysis was done at ambient temperature at 0.25 mL/min flow rate of 25 mM NaOH mobile phase.

PPCP analyses were performed with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system that included a Shimadzu Prominence HPLC (Kyoto, Japan) coupled with a 4000 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA, USA). Target compounds were determined using ESI ionization (both positive and negative modes). The compounds were separated on an Inertsil ODS-3 C_{18} column (150 x 2.0 mm ID, 5 µm particle size) preceded by a guard column (10 x 3.0 mm ID, 5 µm particle size) of same stationary phase. A binary gradient consisting of 0.1% formic acid in water (A) and 1:1 methanol-acetonitrile (B) at a 0.3 mL/min flow rate was used for ESI positive mode. The gradient started with 2% B up to 2 min, followed by a linear gradient of 90% B within 17 min, keeping the isocratic conditions up to 22 min. and

initial conditions were reached within 22.1 min followed the re-equilibration up to 30 min. For ESI negative mode, 2 mM ammonium acetate in water (A) and 1:1 methanol-acetonitrile (B) at a flow rate of 0.3 mL/min was used. The gradient started with 10% B followed by a linear gradient of 90% B within 17 min, keeping the isocratic conditions up to 22 min. and initial conditions were reached within 22.1 min followed the re-equilibration up to 30 min. An injection volume of 20 µL was used for both ionization modes.

The analytes were quantified using multiple reaction monitoring (MRM) mode. Information concerning the parent and daughter ions and compound-dependent parameters is presented in Table S1. Two daughter ions were given for each compound except ibuprofen and triclosan due to the lack of prominent second daughter ions. The MRM transition with parent to first daughter ion was used for quantification of the analyte whereas the transition with parent to second daughter ion was used for qualification of the analyte. The source dependant parameters are compiled in Table S2. The analysis of pCBA was performed using LC-MS/MS with negative ionization mode. Column and mobile phases were same as those used for ESI-negative mode of PPCP analysis, with different gradient program. The gradient starts with 10% B followed by linear gradient of 90% B within 5 minutes, followed by an isocratic step up to 8 min, came back to initial condition within 8.1 min and followed the re-equilibration up to 15 min. The parent and daughter ions 155.0 and 111.0 respectively were used for pCBA quantification. pCBA was analyzed with concentrating the samples.

Solid phase extraction used for sample preparation was carried out according to method developed by Vanderford et al. (3) with slight modifications. Analytes were extracted in batches of six samples using hydrophilic-lipophilic balance (HLB, 500 mg/6 mL) cartridges from Waters Corp. (Milford, MA, USA) at sample pH on an AutoTrace automated SPE system (Caliper Life Sciences, Hopkinton, MA, USA). The SPE cartridges were sequentially preconditioned with 5 mL methyl tertiary butyl ether (MTBE), 5 mL methanol and 5 mL reagent water. 50 to 100 mL sample was loaded onto the cartridges at a 15 mL/min flow rate, then the cartridges were rinsed with 5 mL 5% methanol in reagent water, and dried with a stream of nitrogen for 20 min. The compounds were then eluted off of the cartridges with 5 mL 10/90 v/v methanol/ MTBE followed by 5 mL methanol. The eluent was evaporated to dryness under a gentle stream of nitrogen and then reconstituted with 500 µL of water-methanol mixture (4:1 v/v, spiked with 10 µg/L of internal standards), resulting in 100- to 200- fold concentration of analytes. The samples were analyzed by LC-MS/MS for PPCPs.

(3) Vanderford, B. J.; Pearson, R. A.; Rexing, D. J.; Snyder, S. A. Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry. *Anal. Chem*, **2003**, 75, 6265-6274.

Table S1 Parent and daughter ions, and compound dependent parameters for selected compounds.

Compound	Retention Time (min)	M.W	Precursor ion (m/z)	Product ions (m/z)	DP (V)	CE (eV)	CXP (V)	MDL ng/L
ESI-positive mode								
Atenolol-d_7	6.8	273.3	274.1 $[M+H]^+$	145.1	70	38	10	
Atenolol	6.8	266.3	267.1 $[M+H]^+$	145.1	70	38	10	0.1
				190.1	70	38	10	
Iopromide	7. 9	791.4	792.0$[M+H]^+$	573.1	90	37	16	0.6
				559.0	90	37	26	
Acetaminophen	8.1	151.16	152.1 $[M+H]^+$	110.1	60	22	20	0.1
				93.0	60	22	20	
Trimethoprim	8.2	290.32	291.1 $[M+H]^+$	230.1	85	33	18	0.05
				123.1	85	33	18	
Propranolol	10.8	259.31	260.1$[M+H]^+$	116.1	70	27	8	0.04
				183.1	70	27	10	
Sulfamethoxazole	11.5	253.28	254.1 $[M+H]^+$	156.1	60	23	10	0.05
				108.0	60	23	10	
Carbamazepine-d_{10}	14.3	246.3	247.1$[M+H]^+$	204.1	70	30	14	
Carbamazepine	14.4	236.27	237.0$[M+H]^+$	194.1	70	30	15	0.02
				192.1	70	30	15	
Atrazine	15.4	215.7	216.0 $[M+H]^+$	174.1	60	25	15	0.02
				96.1	60	25	15	
DEET	15.6	191.27	192.1 $[M+H]^+$	119.1	65	25	8	0.02
				91.0	65	25	8	
Diclofenac	18.5	296.14	296.0 $[M+H]^+$	215.1	50	28	15	0.12
				250.0	50	28	15	
ESI-negative mode								
Naproxen-d_3	11.9	233.3	232.1 $[M-H]^-$	188.1	-40	-10	-15	
Naproxen	11.9	230.26	229.0 $[M-H]^-$	185.0	-40	-10	-15	0.4
				170.0	-40	-10	-15	
Ibuprofen-d_3	15.2	209.26	208.1 $[M-H]^-$	164.1	-50	-12	-7	
Ibuprofen	15.2	206.26	205.1 $[M-H]^-$	161.0	-50	-12	-7	0.2
Gemfibrozil	16.8	250.3	249.1 $[M-H]^-$	121.0	-55	-15	-10	0.01
				127.0	-55	-15	-10	
Triclosan	16.8	288.5	287.0 $[M-H]^-$	35	-50	-30	-5	0.2

DP: Declustering potential, CE: Collision energy, CXP: Collision cell exit potential
MDL: Method detection limit

Table S2 Source dependent parameters used in LC/MS experiments

Parameter	ESI positive	ESI negative
Curtain gas	15	15
Ion source gas 1	50	50
Ion source gas 2	50	50
Collision gas	medium	medium
Ion spray voltage	5000 V	-4500 V
Temperature	500^{o}C	500^{o}C
Probe X-axis position	5	5
Probe Y-axis position	5	5

Note: The units for curtain gas, ion source gas 1 and ion source gas 2 are arbitrary.

Figures

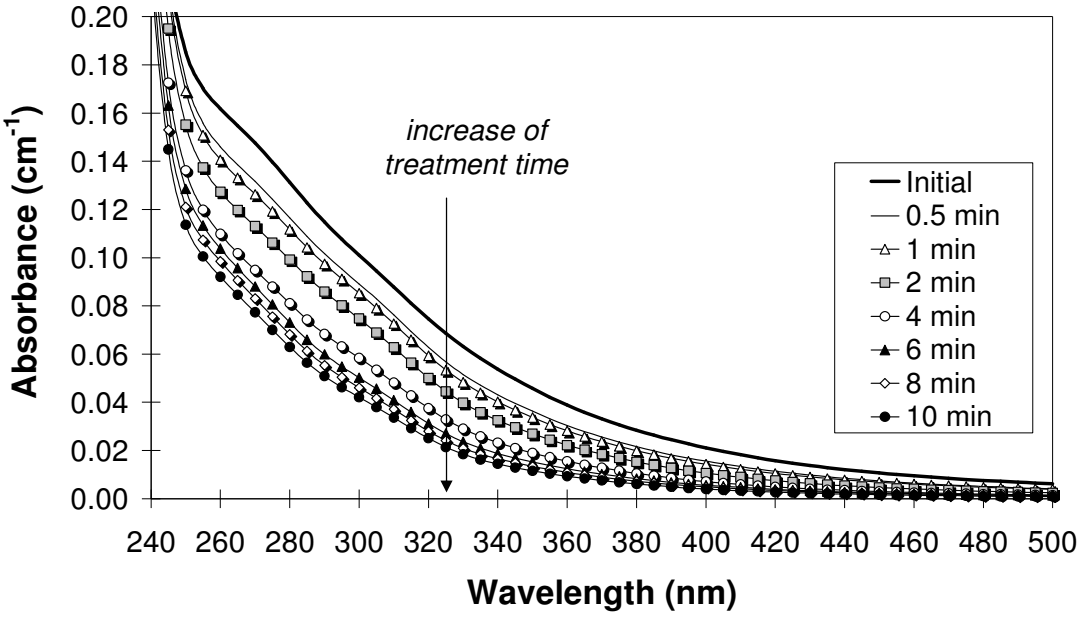

Figure S1 Evolution of the absorbance spectra of wastewater ozonated at varying treatment times. Steady state ozone dose 1.5 mg/L.

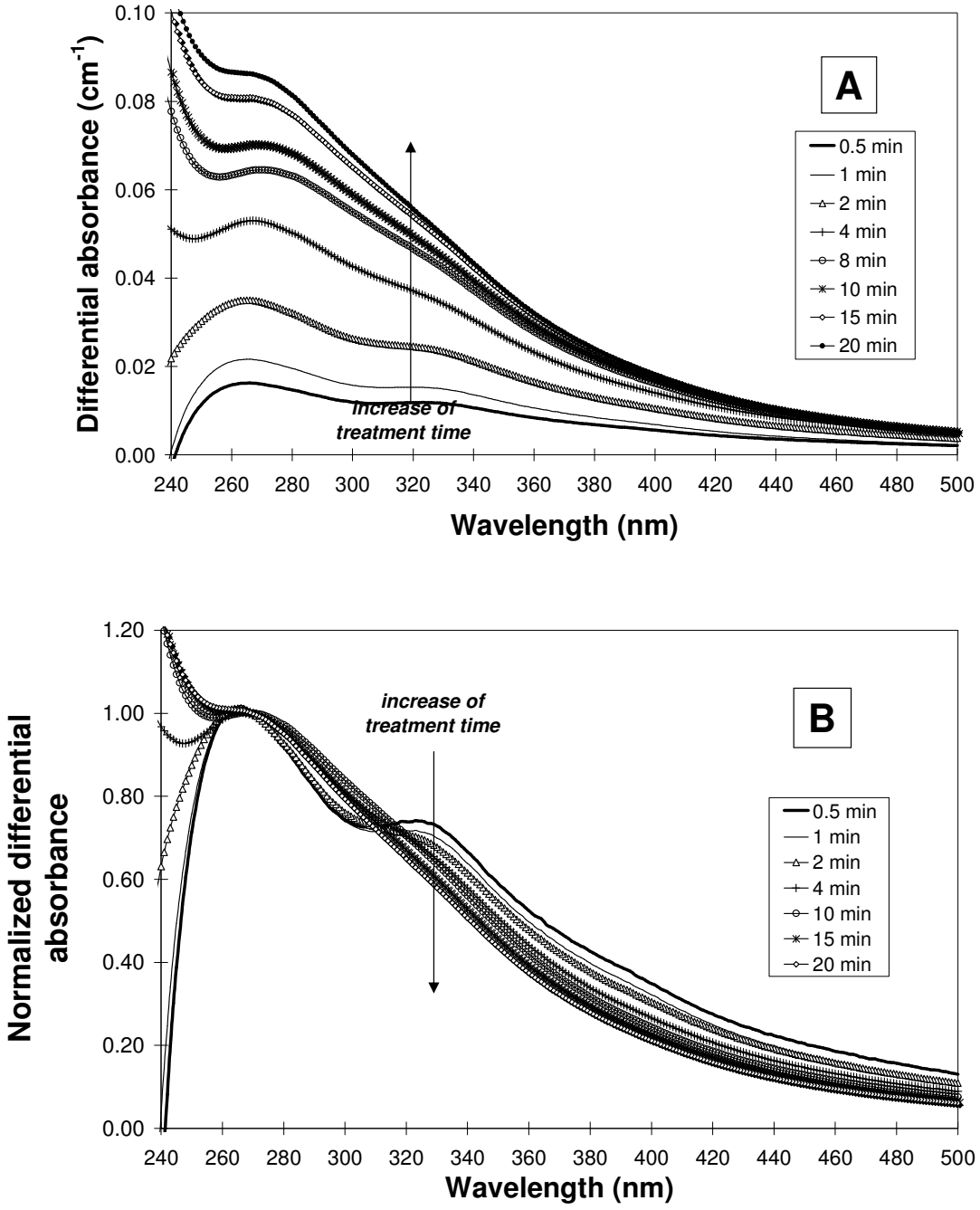

Figure S2 (A) Differential absorbance and (B) normalized differential absorbance spectra of ozonated wastewater. Steady state ozone dose 1.5 mg/L, varying treatment times.

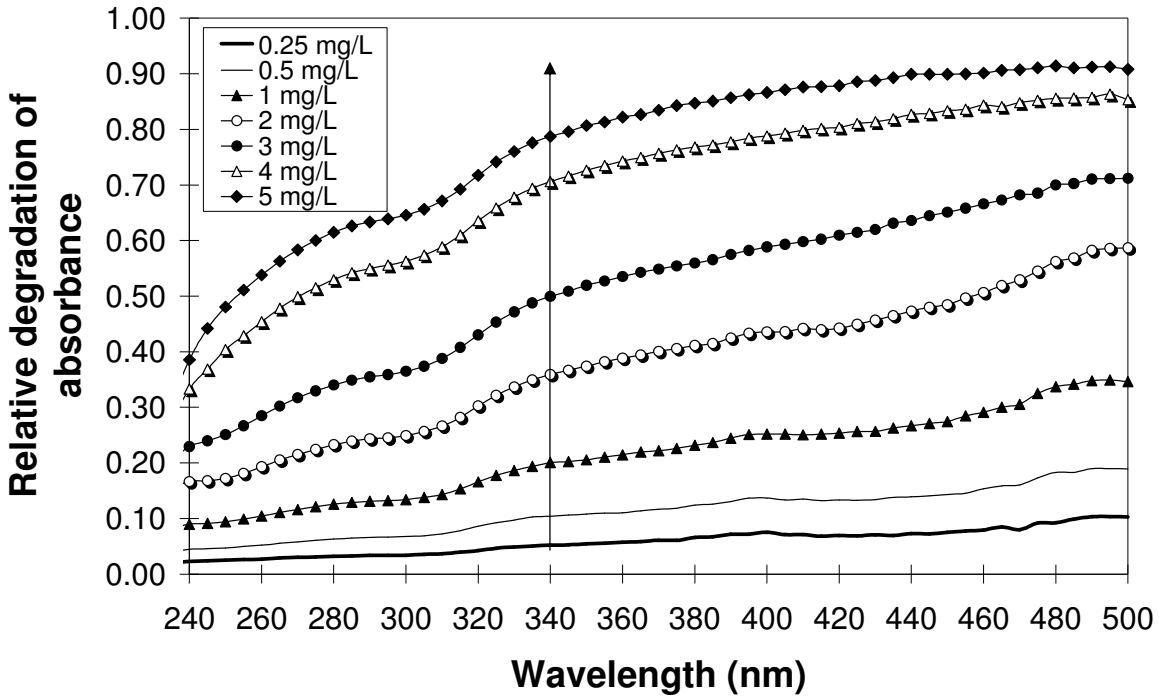

Figure S3 Relative changes of the absorbance of wastewater ozonated using varying initial ozone concentrations.

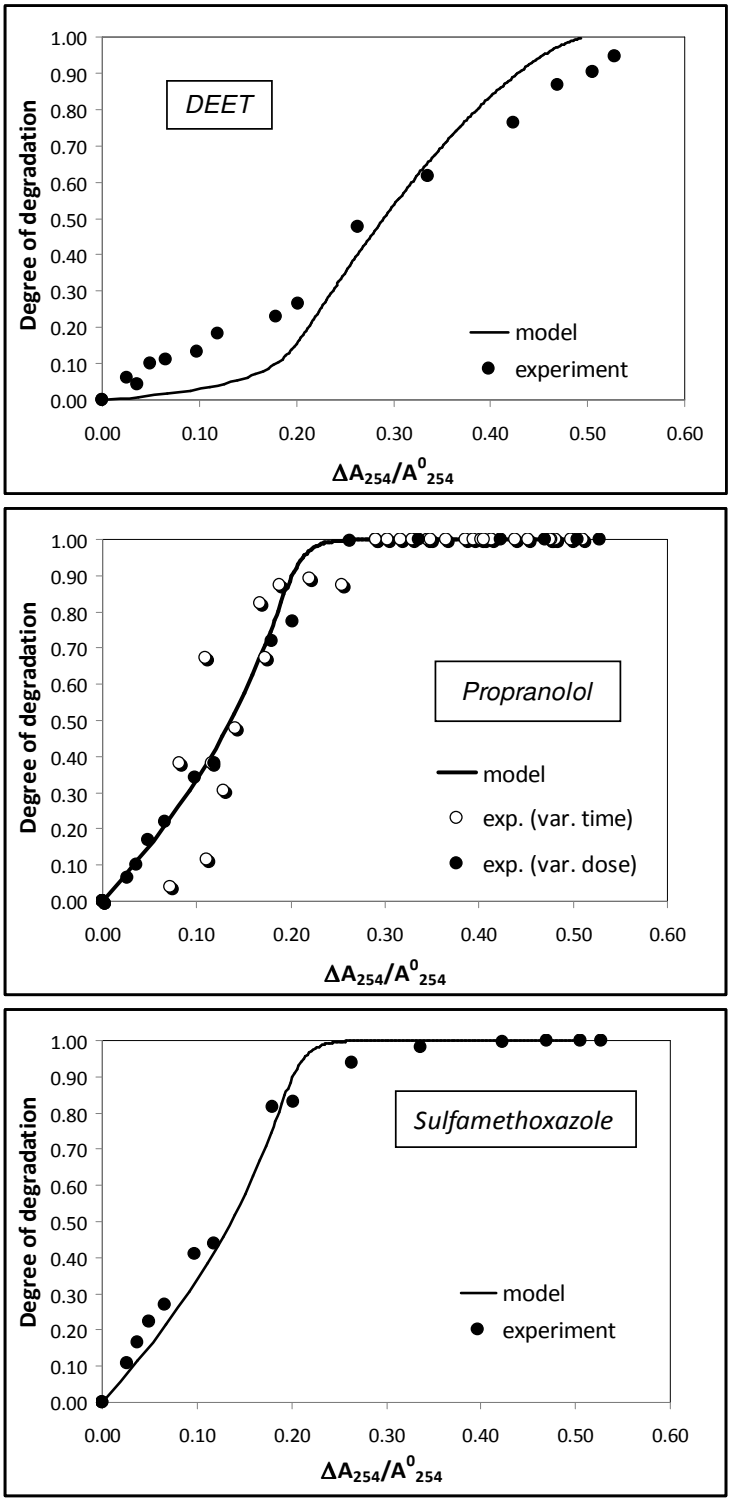

Figure S4 Correlations between relative changes of absorbance of ozonated water and degradation of DEET, propranolol and sulfamethoxazole.

Compilation of published kinetic rates used to calculate apparent oxidation constants

Table S3 Published intrinsic rates of reactions of selected pharmaceuticals with ozone and hydroxyl radical.

Compound	pK	k_{03}, $M^{-1}s^{-1}$	Species	Reference	$k_{OH.}$, $M^{-1}s^{-1}$	Reference
Atenolol	9.6	1.1E+02 6.3E+05	$HAten^+$ Aten	(1) (1)	7.1E+09	(2)
Carbamazepine		3.0E+05		(3)	8.8E+09	(3)
DEET		1.0E+01			5.0E+09	(4)
Diclofenac	4.2	1.0E+06 6.8E+05	HDiclof $Diclo^-$	(3) (5)	7.5E+09	(3)
Gemfibrozil	4.7	2.0E+03			1.0E+10	(6)
Ibuprofen	4.9	9.6E+00	HIbupr	(3)	6.5E+09	(8)
Iopromide		8.0E-01		(3)	3.3E+09	(3)
Naproxen	4.2	2.0E+05	$Napr^-$	(7)	9.6E+09	(8)
Propranolol	9.5	1.0E+05	Propr	(1)	1.0E+10	(1)
Sulfamethoxazole	5.7	4.7E+04 5.7E+05	HSulf $Sulf^-$	(9) (9)	8.5E+09	(10)
Trimethoprim	3.2 7.1	3.3E+04 7.4E+04 5.2E+05	H_2Trim^{2+} $HTrim^+$ Trim	(9) (9) (9)	6.9E+09	(9)

References in Table S3

Number	Source
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(2)	Song, W.; Cooper, W. J.; Mezyk, S. P.; Greaves, J.; Peake, B. M. Free radical destruction of beta-blockers in aqueous solution. *Environmental Science and Technology* **2008**, 42, 1256-1261.
(3)	Huber, M. M.; Canonica, S.; Park, G. Y.; Von Gunten, U. Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. *Environmental Science and Technology* **2003**, 37, 1016-1024.
(4)	Song, W.; Cooper, W. J.; Peake, B. M.; Mezyk, S. P.; Nickelsen, M. G.; O'Shea, K. E. Free-radical-induced oxidative and reductive degradation of N,N'-diethyl-m-toluamide (DEET): Kinetic studies and degradation pathway. *Water Research* **2009**, 43, 635-642.
(5)	Sein, M. M.; Zedda, M.; Tuerk, J.; Schmidt, T. C.; Golloch, A.; Von Sonntag, C. Oxidation of diclofenac with ozone in aqueous solution. *Environmental Science and Technology* **2008**, 42, 6656-6662.

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(8)	Packer, J. L.; Werner, J. J.; Latch, D. E.; McNeill, K.; Arnold, W. A. Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquatic Sciences* **2003**, 65, 342-351.
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Derivation of differential equations relating the degradation of EfOM chromophores and PPCP species

Variables and notations

A	Absorbance of wastewater
A_F	Absorbance of unreactive wastewater chromophores
C	Concentration of trace-level organic contaminant C (e.g., pCBA, ibuprofen etc.)
Γ_{OH/O_3}	Apparent ratio of concentrations of hydroxyl radicals and ozone that have affected the concentrations of substrates S_1, S_2 and target species C throughout the treatment.
ε_1	Molar extinction coefficient of site S_1
ε_2	Molar extinction coefficient of site S_2
k_C^{OH}	intrinsic rate of second-order reaction between contaminant C and OH· radicals
$k_{S_1}^{OH}$	intrinsic rate of second-order reaction between site S_1 and OH· radicals
$k_{S_2}^{OH}$	intrinsic rate of second-order reaction between site S_2 and OH· radicals
$k_C^{O_3}$	intrinsic rate of second-order reaction between contaminant C and ozone

$k_{S_1}^{O_3}$	intrinsic rate of second-order reaction between site S_1 and ozone
$k_{S_2}^{O_3}$	intrinsic rate of second-order reaction between site S_2 and ozone
κ_C	apparent rate of second-order reaction of degradation of contaminant C by ozone
κ_{S_1}	apparent rate of second-order reaction of degradation of site S_1 by ozone
κ_{S_2}	apparent rate of second-order reaction of degradation of site S_2 by ozone
r_{C/S_2}	Ratio of kinetic constants κ_C and κ_{S_2}
r_{S_1/S_2}	Ratio of kinetic constants κ_{S_1} and κ_{S_2}
S_1	Concentration of kinetically fast EfOM chromophores that react readily with O_3 and OH· species
S_2	Concentration of EfOM chromophores that are less kinetically active than reactive sites S_1.

Differential equations relating changes of absorbance of wastewater and degradation of trace-level contaminant C

The degradation of sites S_1 and S_2 by OH· and O_3 can be described by the following equations:

$$\frac{d[S_1]}{dt} = -\left(k_{S_1}^{OH}[OH^\bullet] + k_{S_1}^{O_3}[O_3]\right)[S_1] \quad \text{(SI-1)}$$

$$\frac{d[S_2]}{dt} = -\left(k_{S_2}^{OH}[OH^\bullet] + k_{S_2}^{O_3}[O_3]\right)[S_2] \quad \text{(SI-2)}$$

The consumption of a target PPCP species C is likewise described as

$$\frac{d[C]}{dt} = -\left(k_{C}^{OH}[OH^\bullet] + k_{C}^{O_3}[O_3]\right)[C] \quad \text{(SI-3)}$$

The absorbance of wastewater is at any moment of time is assumed to reflect of sites S_1, S_2 and non-reactive EfOM chromophores:

$$A = \varepsilon_1[S_1] + \varepsilon_2[S_2] + A_F \quad \text{(SI-4)}$$

Obviously, before the start of reaction, the absorbance of light by EfOM is defined as

$$A_0 = \varepsilon_1[S_1^0] + \varepsilon_2[S_2^0] + A_F \quad \text{(SI-5)}$$

Because the interpretation of the changes of component C as a function of absorbance is equivalent to interpreting changes of components S_1 and S_2 and accounting for their contributions using equation 6, the above expressions can be presented as a function of any of these sites, for instance S_2:

$$\frac{d[S_1]}{d[S_2]} = \frac{\left(k_{S_1}^{OH}[OH^{\bullet}] + k_{S_1}^{O_3}[O_3]\right)[S_1]}{\left(k_{S_2}^{OH}[OH^{\bullet}] + k_{S_2}^{O_3}[O_3]\right)[S_2]} = \frac{\left(k_{S_1}^{OH}\frac{[OH^{\bullet}]}{[O_3]} + k_{S_1}^{O_3}\right)[S_1]}{\left(k_{S_2}^{OH}\frac{[OH^{\bullet}]}{[O_3]} + k_{S_2}^{O_3}\right)[S_2]} \quad \text{(SI-6)}$$

$$\frac{d[C]}{d[S_2]} = \frac{\left(k_{C}^{OH}\frac{[OH^{\bullet}]}{[O_3]} + k_{C}^{O_3}\right)[C]}{\left(k_{S_2}^{OH}\frac{[OH^{\bullet}]}{[O_3]} + k_{S_2}^{O_3}\right)[S_2]} \quad \text{(SI-7)}$$

At least in some conditions the ratio of concentrations of hydroxyl radicals and ozone designated as $\Gamma_{OH/O_3} = [OH\cdot]/[O_3]$ can be assumed to be nearly-constant. However, because equations SI-6 and SI-7 describe the change of concentrations of S_1 and C as a function of S_2, the assumption that Γ_{OH/O_3} is nearly constant is not necessarily applicable to any instant of wastewater treatment but it rather reflects an integral of changes of S_1, S_2 and C throughout any particular duration of treatment.

Actual Γ_{OH/O_3} values are likely to be site-specific and affected by the pH, carbonate, concentration and properties of EfOM and other components of any particular wastewater. Thus, the assumption of near-stability of Γ_{OH/O_3} needs to be examined in more detail for a representative range of wastewater. Still, the introduction of dimensionless Γ_{OH/O_3} values allows rewriting equations SI-6 and SI-7 to introduce apparent second-order rates of the degradation by ozone of the reactive chromophores in EfOM and target compounds. These apparent rates of the oxidation of species C, EfOM substrates S_1 and S_2 and ratios of these constants are defined below:

$$\kappa_C = k_C^{OH}\Gamma_{OH/O_3} + k_C^{O_3} \quad \text{(SI-8)}$$

$$\kappa_{S_1} = k_{S_1}^{OH}\Gamma_{OH/O_3} + k_{S_1}^{O_3} \quad \text{(SI-9)}$$

$$\kappa_{S_2} = k_{S_2}^{OH}\Gamma_{OH/O_3} + k_{S_2}^{O_3} \quad \text{(SI-10)}$$

$$r_{C/S_2} = \frac{\kappa_C}{\kappa_{S_2}} \quad \text{(SI-11)}$$

$$r_{S_1/S_2} = \frac{\kappa_{S_1}}{\kappa_{S_2}} \quad \text{(SI-12)}$$

In the above equations, r_{S_1/S_2} and r_{C/S_2} are dimensionless ratios of the apparent kinetic rates of and the oxidation of site S_1 and component C, respectively, to the reaction rate that corresponds to the engagement of site S_2. Expressions SI-6 to SI-12 can be rearranged to yield a differential equation relating the change of absorbance and that of the concentration of reactive site S_2 and the target species as shown below.

Specifically, expression SI-6 yields the differential equation that relates changes of the concentration of EfOM reactive site S_1 with those of site S_2:

$$\frac{d[S_1]}{d[S_2]} = \frac{\kappa_{S_1}[O_3][S_1]}{\kappa_{S_2}[O_3][S_2]} = \frac{\kappa_{S_1}[S_1]}{\kappa_{S_2}[S_2]} = r_{S_1/S_2}\frac{[S_1]}{[S_2]} \quad \text{(SI-13)}$$

The integration of equation SI-13 yields the following expressions

$$\int_{S_1^0}^{S_1}\frac{d[S_1]}{[S_1]} = r_{S_1/S_2}\int_{S_2^0}^{S_2}\frac{d[S_2]}{[S_2]} \quad \text{(SI-14)}$$

$$\frac{[S_1]}{[S_1^0]} = \left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} \tag{SI-15}$$

$$[S_1] = [S_1^0]\left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} \tag{SI-16}$$

Therefore, expression SI-4 for the absorbance of EfOM can be rewritten as

$$\varepsilon_1[S_1^0]\left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} + \varepsilon_2[S_2] = A - A_F \tag{SI-17}$$

To determine the relationship between changes of absorbance and those of target component C, we recall that

$$\frac{dA}{dt} = \varepsilon_1 \frac{d[S_1]}{dt} + \varepsilon_2 \frac{d[S_2]}{dt} \tag{SI-18}$$

Division of expression SI-3 by equation SI-18 results in the following expression

$$\frac{d[C]}{dA} = -\frac{\kappa_C[O_3][C]}{\varepsilon_1 d[S_1] + \varepsilon_2 d[S_2]} \tag{SI-19}$$

Taking into account expressions SI-9 to SI-12, it can be rewritten as

$$\begin{aligned}\frac{d[C]}{dA} &= \frac{\kappa_C[O_3][C]}{\varepsilon_1\kappa_{S_1}[O_3][S_1] + \varepsilon_2\kappa_{S_2}[O_3][S_2]} = \\ &= \frac{\kappa_C[C]}{\varepsilon_1\kappa_{S_1}[S_1] + \varepsilon_2\kappa_{S_2}[S_2]} = \frac{r_{C/S_2}[C]}{\varepsilon_1 r_{S_1/S_2}[S_1] + \varepsilon_2[S_2]}\end{aligned} \tag{SI-20}$$

$$\frac{d[C]}{[C]} = \frac{r_{C/S_2}dA}{\varepsilon_1 r_{S_1/S_2}[S_1] + \varepsilon_2[S_2]} \tag{SI-21}$$

Taking into account equation SI-16, equation SI-21 can be presented in a different form

$$d[\ln C] = \frac{r_{C/S_2} dA}{\varepsilon_1 r_{S_1/S_2} \left[S_1^0\right] \left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} + \varepsilon_2 [S_2]} \quad \text{(SI-22)}$$

Equation SI-22 can be numerically integrated simultaneously with equation SI-17 that explicitly defines the concentration of site S_2 as a function of the absorbance of EfOM measured at any moment of reaction:

$$\varepsilon_1 \left[S_1^0\right] \left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} + \varepsilon_2 [S_2] = A - A_F \quad \text{(SI-23)}$$

$$\int_{C_0}^{C} d[\ln C] = r_{C/S_2} \int_{A_0}^{A} \frac{dA}{\left(\varepsilon_1 r_{S_1/S_2} \left[S_1^0\right] \left(\frac{[S_2]}{[S_2^0]}\right)^{r_{S_1/S_2}} + \varepsilon_2 [S_2] \right)} \quad \text{(SI-24)}$$