

1 **Exploring the aquatic photodegradation of two ionisable fluoroquinolone**  
2 **antibiotics – gatifloxacin and balofloxacin: Degradation kinetics,**  
3 **photobyprouducts and risk to the aquatic environment**

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10

11 **Abstract:**

12 Fluoroquinolone antibiotics (FQs) are ubiquitous and ionizable in surface waters. Here we  
13 investigate gatifloxacin (GAT) and balofloxacin (BAL), two widely used FQs, and determine  
14 the photochemical reactivity of their respective dissociation species that arise at different pH to  
15 understand the relevance and pathways of phototransformation reactions. Simulated-sunlight  
16 experiments and matrix calculations showed that neutral forms (HFQs<sup>0</sup>) of the two antibiotics  
17 had the highest apparent photolytic efficiency and hydroxyl-radical oxidation reactivity. Based  
18 on the pH-dependent photochemical reactivities, the solar apparent photodegradation half-lives  
19 ( $t_{1/2}$ ) in sunlit surface waters ranged from 14.5 – 169 min and was 1 – 2 orders of magnitude  
20 faster than hydroxyl-radical induced oxidation ( $t_{1/2} = 20.9 – 29.8$  h). The corresponding  
21 pathways were proposed based on the identification of key intermediates using HPLC-ESI-  
22 MS/MS. The apparent photodegradation induced defluorination, decarboxylation, and  
23 piperazinyl oxidation and rearrangement, whereas hydroxyl-radical oxidation caused

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24 hydroxylated defluorination and piperazinyl hydroxylation. The photomodified toxicity of GAT  
25 and BAL was examined using an *Escherichia coli* activity assay. *E. Coli* activity was not  
26 affected by BAL, but was significantly affected by the photo-modified solutions of GAT,  
27 indicating that primary photo-degradates have a comparable or higher antibacterial activity than  
28 the parent GAT. In fresh water and seawater this antibacterial activity remained high for up to  
29 24 h, even after GAT had undergone significant photodegradation (>1 half-life), indicating the  
30 potential impact of this chemical on microbial communities in aquatic systems.

31 **Keywords:** Fluoroquinolones, Dissociation, Phototransformation kinetics, Pathways, Hydroxyl  
32 radicals

33

## 34 1. Introduction

35 The occurrence of antibiotics as micropollutants in surface waters is receiving increasing  
36 attention (Baena-Nogueras et al., 2017; Li et al., 2016; Luo et al., 2011; Schwarzenbach et al.,  
37 2006). Among antibiotics, fluoroquinolones (FQs) are an important class of ‘emerging’  
38 pollutant in surface waters and are considered the fourth largest class of antibiotics with regards  
39 their production and consumption (Sturini et al., 2014) (Van Doorslaer et al., 2014). Once  
40 administered to humans or livestock, antibiotics maybe only partially metabolized and therefore  
41 can enter agricultural runoff waters and wastewaters. Furthermore, some antibiotics may not be  
42 effectively removed during wastewater treatment processes resulting in their occurrence in final  
43 treated effluents and receiving waters (Chen et al., 2013; Liu et al., 2017; Luo et al., 2011; Meng  
44 et al., 2016; Polesel et al., 2016; Schwarzenbach et al., 2006) and this is particularly relevant in  
45 China with their widespread occurrence in coastal areas (Fig. S1). As part of the risk assessment  
46 process it is therefore pertinent to understand the longevity and fate of FQs in aquatic systems.

47 FQs show resistance to hydrolysis and biodegradation in surface waters (Cardoza et al., 2005;  
48 Ge et al., 2010) and therefore photochemical transformation is considered to be an important

49 transformation and loss pathway in the aqueous euphotic zone (Ge et al., 2010) (Sturini et al.,  
50 2015) (Wammer et al., 2013). FQs can absorb sunlight directly and undergo apparent photolysis,  
51 including direct photolysis and self-sensitized **photooxidation** via reactive oxygen species (ROS,  
52 such as hydroxyl radicals,  $\cdot\text{OH}$ ). FQs might also undergo indirect photodegradation or  
53 sensitized photooxidation mediated by the photogenerated  $\cdot\text{OH}$  from dissolved organic matter,  
54 Fe(III) and nitrate (Fisher et al., 2006; Grannas et al., 2014; Mack and Bolton, 1999; Walse et  
55 al., 2004). Furthermore, photomodified FQs demonstrate toxicity to *Vibrio fischeri* and  
56 photoenhanced antibacterial activity to *E. coli*, raising concerns about their impact on aquatic  
57 microorganisms (Ge et al., 2010; Ge et al., 2015; Li et al., 2011).

58 The environmental photochemical transformation of individual FQs is now reported in the  
59 literature (Ge et al., 2010; Ge et al., 2015; Porras et al., 2016; Sturini et al., 2010; Wei et al.,  
60 2013). For example, FQs, such as ciprofloxacin (Ge et al., 2010), danofloxacin (Sturini et al.,  
61 2012), norfloxacin (Niu et al., 2016), marbofloxacin and enrofloxacin (Sturini et al., 2010),  
62 have been found to follow apparent first-order photodegradation kinetics. **Although dissociation**  
63 **of FQs was not fully considered, these previous studies laid a solid foundation for further insight**  
64 **into the phototransformation kinetics. As the molecular structures contain ionisable groups**  
65 **(e.g.,  $-\text{COOH}$  and  $-\text{NH}_n$ ), many FQs are ionisable and will undergo acid-base dissociation**  
66 depending on the environmental pH. Therefore, to assess the environmental photochemical fate  
67 of FQs, the effects of acid-base dissociation and the susceptibility of different ionised forms to  
68 photodegrade should be considered.

69 Several studies have now reported the phototransformation pathways of FQs in pure water,  
70 mainly focusing on their apparent photolysis (Baena-Nogueras et al., 2017; Ge et al., 2010;  
71 Sturini et al., 2010) (Yan and Song, 2014) and hydroxyl-radical oxidation (Ge et al., 2015),  
72 although a variety of chemistries exist depending on the FQ in question and the environmental  
73 conditions (Ge et al., 2010; Ge et al., 2015; Sturini et al., 2010; Wei et al., 2013). Relevant

74 pathways and mechanisms of the concerned FQs are clarified, which is of importance to  
75 understand the risks they may pose. However, for several new widely used FQs, such as  
76 gatifloxacin (GAT) and balofloxacin (BAL) that have been frequently detected in  
77 environmental waters (Bu et al., 2013; Xiao et al., 2008), their phototransformation pathways  
78 still remain unclear. The aim of this study was to clarify the aqueous photodegradation of GAT  
79 and BAL, determining degradation kinetics and the formation of photoproducts over a range of  
80 environmentally-relevant pHs and to investigate changes in their antibacterial activity  
81 following photo-transformation.

82

## 83 **2. Materials and methods**

### 84 **2.1. Reagents and materials**

85 GAT and BAL (both purity 99.0%) were obtained from different suppliers (Table S1).  
86 Acetonitrile and methanol (Tedia Inc.) were of high-performance liquid chromatography  
87 (HPLC) grade. Ultrapure water was prepared using a Milli-Q Millipore system (Waters, USA).  
88 All other reagents used in the experiment were of guaranteed grade. Oasis HLB cartridges  
89 (WAT106202) were purchased from Waters, USA. The supplier and culture of a freeze-dried  
90 wild-type *E. coli* K12 (ATCC 23716) has been provided in one of our recent studies (see Ge et  
91 al., 2015).

### 92 **2.2. Photodegradation experiments**

93 A Pyrex-well cooled and filtered xenon lamp (1 kW) was used to simulate sunlight ( $\lambda > 290$   
94 nm). Aqueous FQ solutions ( $C_0 = 10 \mu\text{M}$ ), pipetted into quartz tubes and placed into a merry-  
95 go-round apparatus, were irradiated and sampled over each irradiation period. A 380 nm cut-  
96 off filter ( $\lambda > 380 \text{ nm}$ ) was obtained from Beijing Lighting Research Institute of China and used  
97 when needed. To compare the FQ apparent photolysis rate constants ( $k_{\text{AP,FQs}}$ ) under different  
98 pHs, phosphate buffers were used to adjust the solution pH and the data were fitted with the

99 first-order-kinetic equation. The photolytic quantum yields ( $\Phi_{AP}$ ) were determined under the  
 100 same conditions. Furthermore, to investigate the hydroxyl-radical oxidation reactivities, the  
 101 bimolecular rate constants ( $k_{\cdot OH, FQs}$ ) for the reaction between FQs and  $\cdot OH$  were determined  
 102 according to the competition kinetic method (Ge et al., 2015).

103 For each FQ, individual  $k_{AP,i}$  or  $k_{\cdot OH,i}$  of the fully protonated ( $H_2FQs^+$ ), neutral ( $HFQs^0$ ), and  
 104 anionic forms ( $FQs^-$ ) were fitted by Matrix (1):

$$105 \quad \begin{matrix} \text{pH}=3 \\ \left( \begin{array}{ccc} \delta_{H_2FQs^+} & \delta_{HFQs^0} & \delta_{FQs^-} \\ M & & M \\ M & & M \end{array} \right) \begin{matrix} \left( \begin{array}{c} k_{H_2FQs^+} \\ k_{HFQs^0} \\ k_{FQs^-} \end{array} \right) \\ \\ \end{matrix} \\ \text{pH}=11 \end{matrix} = \begin{matrix} \left( \begin{array}{c} k_{FQs} \\ M \\ M \end{array} \right) \\ \text{pH}=3 \\ \\ \text{pH}=11 \end{matrix} \quad (1)$$

106 where  $\delta_i$  represents the fraction of each FQ form ( $i$ ) and the values are referred to Fig. S2,  $k_{FQs}$   
 107 is  $k_{AP,FQs}$  or  $k_{\cdot OH,FQs}$  at different pH, and  $k_{H_2FQs^+} - k_{FQs^-}$  represent the  $k_{AP,i}$  or  $k_{\cdot OH,i}$  of the three  
 108 FQ forms to be fitted.

109 The irradiated solutions were sampled to investigate phototransformation products and the  
 110 corresponding photomodified toxicity of GAT and BAL. A Waters UPLC was employed to  
 111 analyze the FQ concentrations. Solid-phase extraction and LC-MS/MS were used to enrich and  
 112 identify the intermediates, respectively. The analysis is further detailed in the Supplementary  
 113 material.

### 114 2.3. Antibacterial activity bioassay

115 Antibacterial activity changes induced by the apparent photolysis were examined with  
 116 *Escherichia coli* (*E. coli*). To better understand environmental risks, the experiment was carried  
 117 out for photolyzed FQs in pure water and natural waters. The assay was conducted following  
 118 the Chinese standard method QB/T 2738-2012 (Ge et al., 2015). The plated inhibition zone  
 119 diameters (mm) were measured and recorded as antibacterial activity indexes (Tao et al., 2010).  
 120 Each run was conducted in triplicate and one-way analyses of variance (ANOVAs) were used  
 121 to assess any changes in antibacterial activity through phototransformation of the parent FQs.

122

### 123 3. Results and Discussion

#### 124 3.1. Apparent photolytic kinetics of neutral and dissociated FQ species.

125 No significant loss of GAT and BAL was observed in the dark controls, eliminating the  
126 possibility of thermal and hydrolytic degradation. The FQs were observed to degrade relatively  
127 quickly (minutes to hours) under the simulated solar irradiation, and followed pseudo-first-  
128 order kinetics with the data conforming to a linear regression ( $r^2 > 0.95$ ) of  $\ln(C/C_0)$  vs time ( $t$ ).  
129 As shown in Fig. 1, their apparent photolytic rate constants ( $k_{AP,FQs}$ ) were found to be pH-  
130 dependent. With the increase of pH from 3 to 11,  $k_{AP,FQs}$  of the FQs initially increased with peak  
131 values at pH = 8 (Fig. 1), followed by a decline at higher pHs (>8). The UV-vis absorption  
132 spectrum also varied at different pH conditions (Fig. S3). Previous studies have also shown that  
133  $k$  for other FQs, including ciprofloxacin and sarafloxacin, varied with pH (Ge et al., 2010;  
134 Porras et al., 2016; Wei et al., 2013). To clarify the pH effect,  $k_{AP,i}$  of the individual dissociation  
135 species was determined.

136

137 Fig. 1. Effect of pH on the apparent photodegradation rate constants of gatifloxacin and balofloxacin ( $C_0 =$   
138  $10 \mu\text{M}$ ). Error bars represent one standard error.

139

140 Based on the matrix calculations,  $k_{AP,i}$  for  $\text{H}_2\text{FQs}^+$ ,  $\text{HFQs}^0$  and  $\text{FQs}^-$  were determined for both  
141 GAT and BAL respectively (Table S2). Furthermore, the corresponding quantum yield ( $\Phi_{AP,i}$ )  
142 of each dissociation species was determined and these values are listed in Table 1. The neutral  
143  $\text{HFQs}^0$  photodegraded the fastest (Table S2) and had the highest photolytic efficiency (Table  
144 1), followed by  $\text{H}_2\text{FQs}^+$  and  $\text{FQs}^-$ . Based on the  $\Phi_{AP,i}$  values,  $\delta_i$  values (Fig. S2) and the method  
145 of Leifer (Leifer, 1988; OECD, 1997), environmental rate constants ( $k_{AP,E}$ ) and half-lives ( $t_{AP,E}$ )  
146 were calculated according to Eqs. 2 and 3 for the solar photodegradation of the FQs in sunlit  
147 surface waters and at  $45^\circ$  N latitude for summer and winter, respectively:

148 
$$k_{AP,E} = \sum \delta_i [2.303 \Phi_{AP,i} \sum (Z_{\lambda} \epsilon_{\lambda,i})] \quad (2)$$

149 
$$t_{AP,E} = \frac{\ln 2}{k_{AP,E}} \quad (3)$$

150 where  $Z_{\lambda}$  is tabular solar photon flux at noon of summer and winter, assuming continuous  
 151 irradiation (Leifer, 1988; OECD, 1997), and  $\epsilon_{\lambda,i}$  is the molar extinction coefficient for the  
 152 individual FQ species (Fig. S4). As listed in Table 1, the calculated  $t_{AP,E}$  values clearly depend  
 153 on the pH and the seasonal solar irradiance. Compared to the estimated solar photodegradation  
 154 half-lives of the FQs in our previous study (Ge et al., 2010), the current method of determining  
 155  $t_{AP,E}$  (that takes into account pH and the major speciated forms of the FQs) is likely to be a  
 156 better reflection of FQ fate in environmental waters.

157  
 158 Table 1. Apparent photolytic quantum yields ( $\Phi_{AP,i}$ ) for the different dissociation FQ species, and the  
 159 corresponding environmental half-lives ( $t_{AP,E}$  at 45° N latitude) based on apparent photolysis including direct  
 160 and self-sensitized photodegradation in sunlit surface waters.

161  
 162 **3.2. Hydroxyl-radical oxidation kinetics of different dissociation FQ species.**

163 The determined values for the bimolecular reaction rate constants ( $k_{\cdot OH, FQs}$ ) between the FQs  
 164 and aqueous hydroxyl radicals ( $\cdot OH$ ) under different pHs are listed in Table S3. The  $k_{\cdot OH, FQs}$   
 165 values were found to be pH-dependent and to exhibit a maximum value at pH 7. As the FQs  
 166 underwent two acid-base processes at given pH values, the hydroxyl-radical oxidation  
 167 reactivity of each protonation state was quantified. As shown in Table 2, the fitting results  
 168 indicated that HFQs<sup>0</sup> reacted the fastest with  $\cdot OH$ , followed by FQs<sup>-</sup> and H<sub>2</sub>FQs<sup>+</sup>.

169 In sunlit surface waters,  $\cdot OH$  are ubiquitous and the most important transient oxidants with  
 170 concentrations ranging from 10<sup>-17</sup> to 10<sup>-15</sup> M (Cooper et al., 1989). Based on these  $\cdot OH$   
 171 concentrations, the environmental half-lives ( $t_{OH,E}$ ) for the FQ reactions with  $\cdot OH$  were  
 172 estimated. The results (Table 2) indicate that  $t_{OH,E}$  are dependent on surface water pH.  
 173 Compared to the environmental apparent photolysis half-lives (Table 1), the  $t_{OH,E}$  values are 1

174 – 2 orders of magnitude higher, suggesting that this is not the dominant loss pathway for the  
175 FQs. However, the hydroxyl-radical oxidation is expected to be more effective at inducing the  
176 degradation of FQs due to the lower selectivity of  $\cdot\text{OH}$ , which can oxidize almost all classes of  
177 organic chemicals (Ge et al., 2015) (Keen and Linden, 2013; Li et al., 2014; Mill, 1999).

178  
179 Table 2. The bimolecular rate constants ( $k_{\cdot\text{OH},i}$ ) for the reaction between the different FQ species and  $\cdot\text{OH}$ ,  
180 and the corresponding environmental half-lives in sunlit surface waters ( $t_{\text{OH,E}}$ ).

181

### 182 **3.3. Pathways for apparent photolysis and aqueous hydroxyl-radical oxidation.**

183 Several studies have reported the major photodecomposition products and pathways of the  
184 FQs rather than GAT and BAL specifically (Ge et al., 2010; Ge et al., 2015; Sturini et al., 2010;  
185 Wei et al., 2013). In this study, ten and four main organic intermediates were identified for their  
186 apparent photodegradation and hydroxyl-radical induced oxidation, respectively, in pure water  
187 (Table S4 and Fig. S5).  $\text{F}^-$  and  $\text{HCOO}^-$  were also identified as two molecular ion products, and  
188 total organic carbon of irradiated FQ solutions was determined (Table S5 and Fig. S6). Among  
189 the apparent photolytic intermediates of GAT, there were two abundant photoproducts with  
190 molecular weights ( $MW$ ) of 335 and 373, for which the responses in ESI (+) MS mode were  
191 most significant. BAL had one significant intermediate ( $MW = 292$ ). As for their photooxidation  
192 via  $\cdot\text{OH}$ , only several products was generated, with  $MW$  of 373 and 363 more abundant for  
193 GAT and BAL, respectively.

194 According to these photoproducts, phototransformation pathways for BAL and GAT are  
195 proposed as shown in Fig. 2. The apparent photodegradation of GAT involves five main  
196 pathways:  $\text{N}^1$ -decyclopropyl, demethoxy, defluorination, decarboxylation, and piperazinyl  
197 oxidation and rearrangement. BAL photodegrades via three main pathways: defluorination,  
198 decarboxylation, and piperazinyl reactions, including  $\text{C}^3$ -deaminomethyl, oxidation and



199 rearrangement. As for their reactions with  $\cdot\text{OH}$ , they are both oxidised through hydroxylated  
200 defluorination and piperazinyl hydroxylation. Furthermore, the hydroxylation of BAL leads to  
201 piperazinyl cleavage and oxidation.

202 The piperazinyl reactions of GAT and BAL result in the same intermediate ( $MW = 292$ ). For  
203 GAT and BAL, no decarboxylated intermediate was observed although decarboxylation  
204 probably occurred as the  $\text{HCOO}^-$  ions were measured in the solutions. Similarly, no  
205 defluorinated intermediate was found for the apparent photolysis of GAT. Piperazinyl N4-  
206 dealkylation, decarboxylation or defluorination are primary important pathways for other FQs,  
207 such as ciprofloxacin and enrofloxacin (Ge et al., 2010) (Wei et al., 2013). When further  
208 photodegraded, the FQs and their intermediates may suffer from piperazinyl reactions (Yan and  
209 Song, 2014) (Wei et al., 2013) (Niu et al., 2016). It is noteworthy that the piperazinyl reactions  
210 have been reported in some advanced oxidation processes (AOP) of FQs (Dodd et al., 2005;  
211 Martin et al., 2015; Michael et al., 2013; Paul et al., 2007; Zhang and Huang, 2005). Therefore,  
212 the results from this study would support the understanding of the AOP during water treatment  
213 techniques.

214  
215 Fig. 2. Main transformation pathways for apparent photolysis (black arrows) and hydroxyl-radical oxidation  
216 (red arrows) of gatifloxacin and balofloxacin.

### 218 3.4. Antibacterial activity changes caused by photodegradation in different waters

219 The antibacterial activity of the two FQs was firstly assayed, and the results are shown in Fig.  
220 S7. GAT showed notable antibacterial activity to *E. coli*, while BAL was found to show no  
221 activity. Thus, further studies were carried out with GAT only and results are given in Fig. 3.  
222 The evolution of antibacterial activity of the FQ photolytic solutions was similar in natural  
223 waters compared to pure water (Fig. 3). Moreover, the antibacterial activity of the light  
224 irradiated solutions did not decrease significantly ( $p > 0.05$ ) in the initial photodegradation

225 period ( $0 - t_{1/2}$ ). This can be attributed to the formation and accumulation of primary  
226 degradation intermediates that appear to possess antibacterial activity. Based on the apparent  
227 photolytic pathways (Fig. 2), photoreactions did not transform the core aromatic backbone of  
228 the GAT molecule, so the primary intermediates may behave similarly with regards to their  
229 antibacterial activity to *E. coli*. This would indicate that in environmental waters antibacterial  
230 activity would continue for a period of time, even after GAT had been subject to significant  
231 photo-transformation in the water column.

232  
233 Fig. 3. Changes in antibacterial activity of gatifloxacin (GAT, 10  $\mu\text{M}$ ) due to photochemical degradation in  
234 different waters. Note that the units on the y-axis are millimeters (mm), representing the agar plated bacteria-  
235 free zone diameter. The more potent the antibacterial effect then the larger the *E. coli*-free zone.

236

#### 237 4. Conclusion

238 This study provides a detailed examination of the aqueous photo-transformation of two  
239 commonly used FQs, examining the susceptibility of the major dissociated forms of two FQ  
240 antibiotics (BAL and GAT) to undergo photo-transformation. Of three individual dissociated  
241 species, the neutral forms of BAL and GAT (HFQs<sup>0</sup>), present predominantly in [most  
242 environmental waters with pH range from 6 to 9](#), had the highest apparent photolytic efficiency  
243 and most reactive hydroxyl-radical oxidation potential. Therefore, in the euphotic zone of  
244 surface waters, photochemical transformation will contribute significantly to their loss.  
245 Apparent photolysis was observed to induce defluorination, decarboxylation, and piperazinyl  
246 oxidation and molecular rearrangement, whereas hydroxyl-radical oxidation caused  
247 hydroxylated defluorination and piperazinyl hydroxylation in both chemicals, based on the  
248 tentative identification of key photo-intermediates. According to an *E. coli*. activity assay, these  
249 photointermediates, notably for GAT and potentially for other FQs, have similar antibacterial  
250 activity to the parent chemical, implying that while GAT may photodegrade quite rapidly (on

251 the order of hours) in sunlit surface waters, antimicrobial activity will persist for considerably  
252 longer. This raises concerns over the impact of FQs on natural microbial populations in the  
253 wider aquatic environment.

254

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## 260 **Appendix A. Supplementary data**

261 Supplementary data to this article can be found online at  
262 [http://dx.doi.org/10.1016/\\*\\*\\*\\*\\*.2018.06.087](http://dx.doi.org/10.1016/*****.2018.06.087).

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