

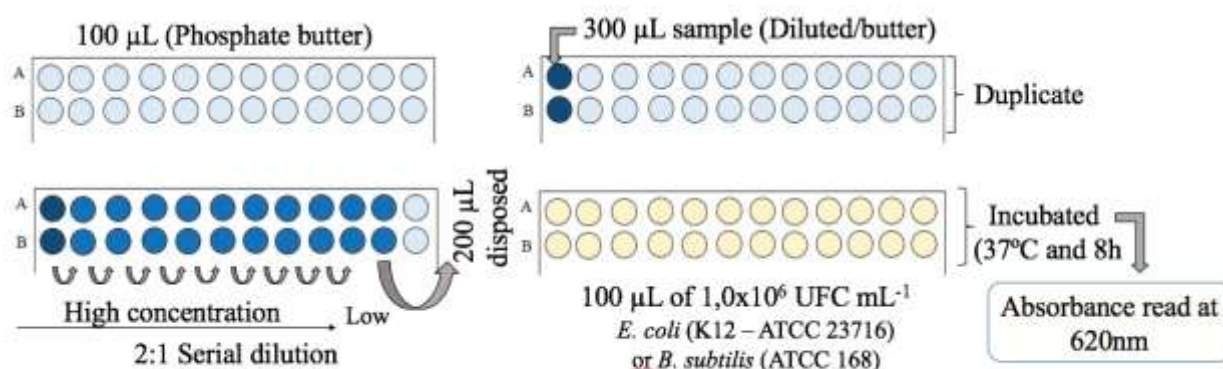
## Supplementary Information

### Fluoroquinolones in Hospital Wastewater: Analytical Method, Occurrence, Treatment with Ozone and Residual Antimicrobial Activity Evaluation

Caio Rodrigues-Silva,<sup>ID</sup>\*,<sup>a</sup> Rafael S. Porto,<sup>a</sup> Sabrina G. dos Santos,<sup>a</sup> Jerusa Schneider<sup>b</sup>  
and Susanne Rath\*,<sup>a</sup>

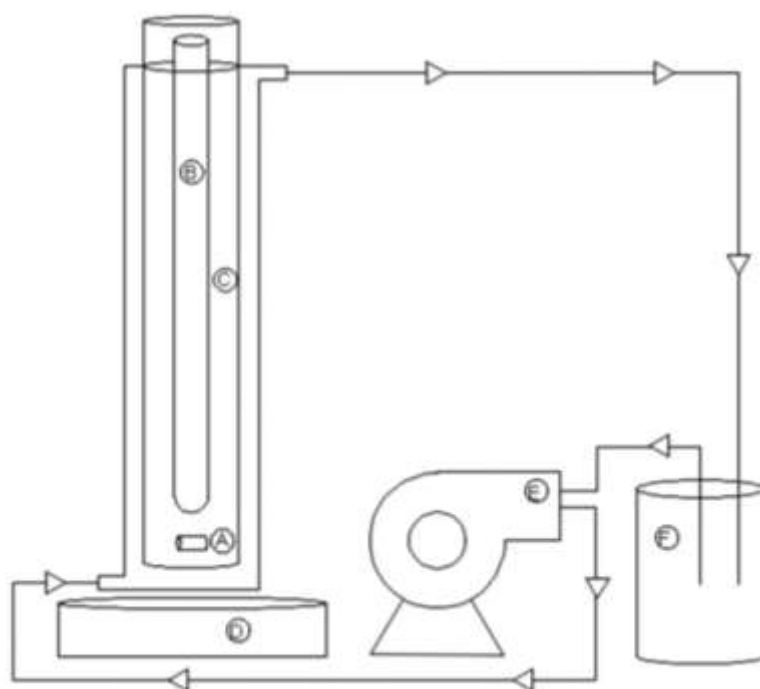
<sup>a</sup>Departamento de Química Analítica, Instituto de Química, Universidade Estadual de Campinas, P.O. Box 6154, 13083-970 Campinas-SP, Brazil

<sup>b</sup>Faculdade de Engenharia Civil, Arquitetura e Urbanismo, Universidade Estadual de Campinas, P.O. Box 6143, 13083-889 Campinas-SP, Brazil

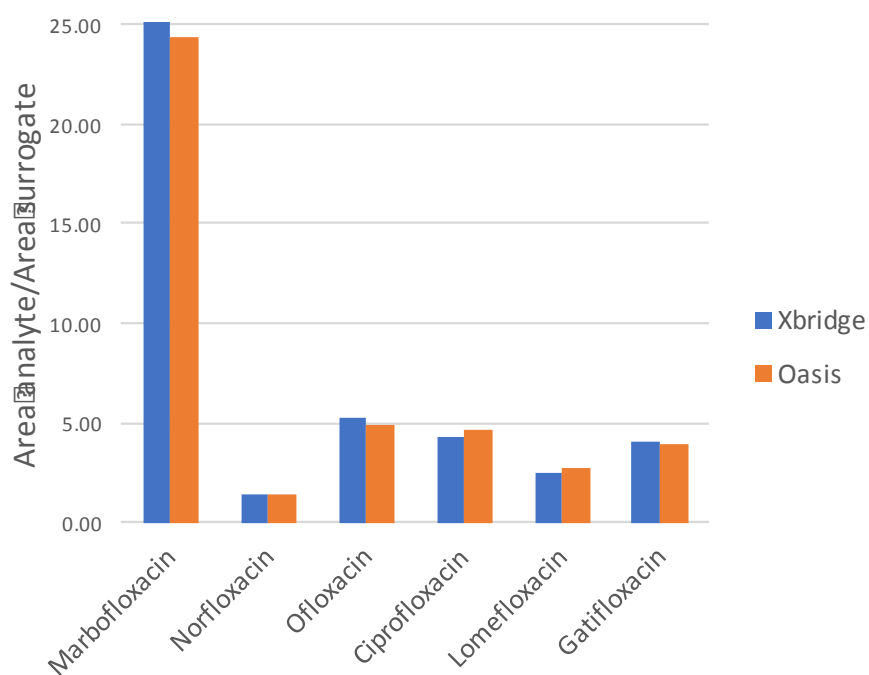


**Figure S1.** Antimicrobial activity assay scheme. An aliquot of the sample (300 µL) was added to the first well of the first row of a 96-well microtiter plate; then, 100 µL of  $1 \text{ mmol L}^{-1}$  phosphate buffer (pH 8) was added to all wells of two rows of the plate, except the well that contained the sample. Subsequently, a 22-member 3:2 serial dilutions series was performed by dosing 200 µL of the solution contained in the first well into the next (second) well and repeating this sequence until the 23<sup>rd</sup> well. The last well (i.e., the 24<sup>th</sup> well) was left with the buffer solution only, where the bacteria growth was observed in the absence of contact with the sample (blank well). After the serial dilution, all wells of the row plate were inoculated with 100 µL of a  $1.0 \times 10^6$  CFU mL<sup>-1</sup> of the bacteria culture (Gram-positive bacteria *Bacillus subtilis*, ATCC 168, or the Gram-negative bacteria *Escherichia coli* K12, ATCC 23716). The plate was sealed and incubated at 100 rpm (Marconi, model MA-420) agitation for 8 h at 37 °C. After incubation, the absorbance of each sample well was measured at 620 nm using a SpectraMax microplate reader (Molecular Devices). The data treatment is thoroughly described in Caianelo *et al.*<sup>1</sup>

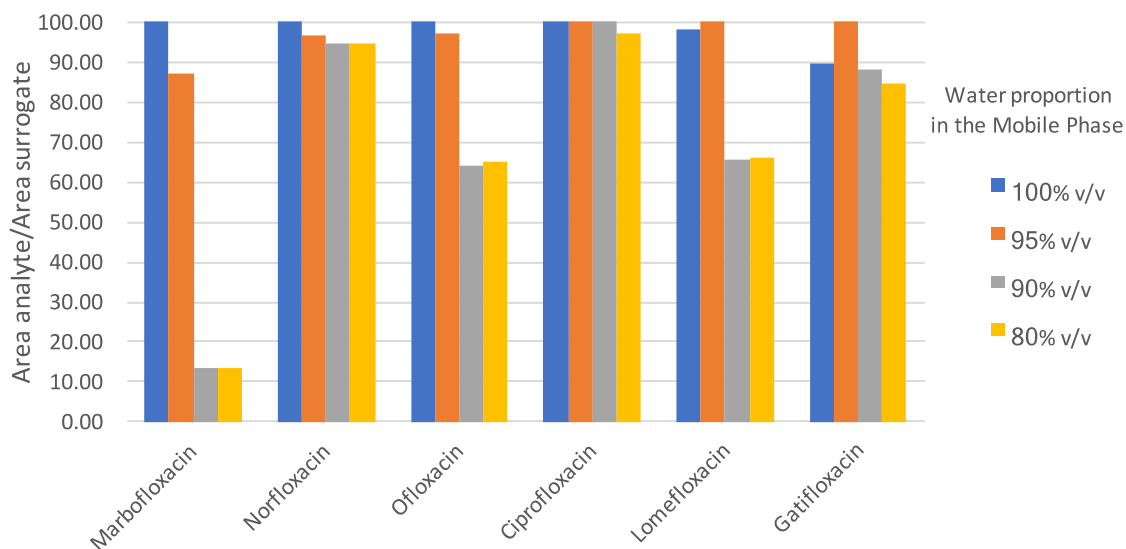
\*e-mail: mr.caito@gmail.com; susanne.rath@gmail.com



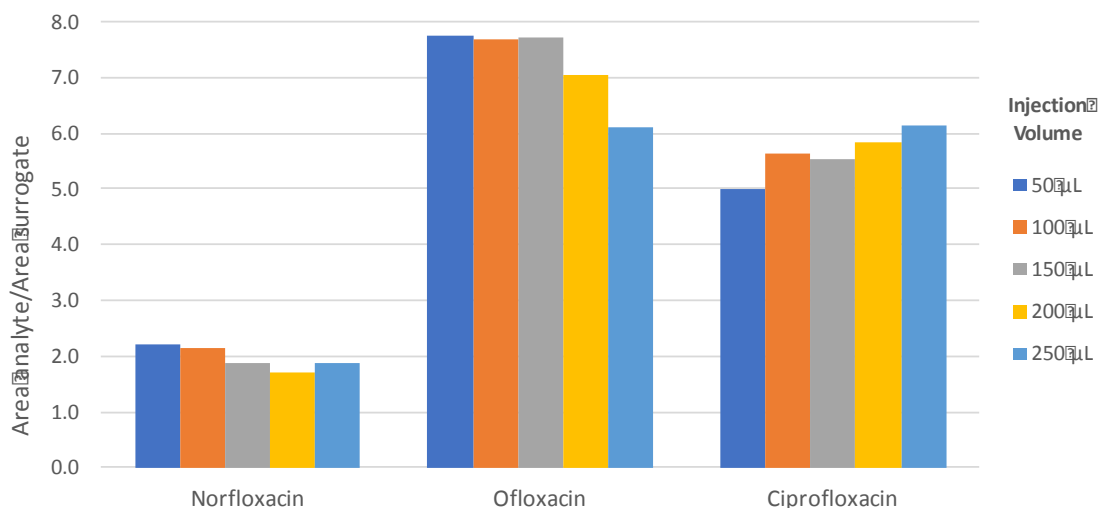
**Figure S2.** Experimental system used in the degradation studies: A and D: magnetic stirrer, B: quartz tube: UV-C lamp support; C: reactor; E: pump; F: reservoir.



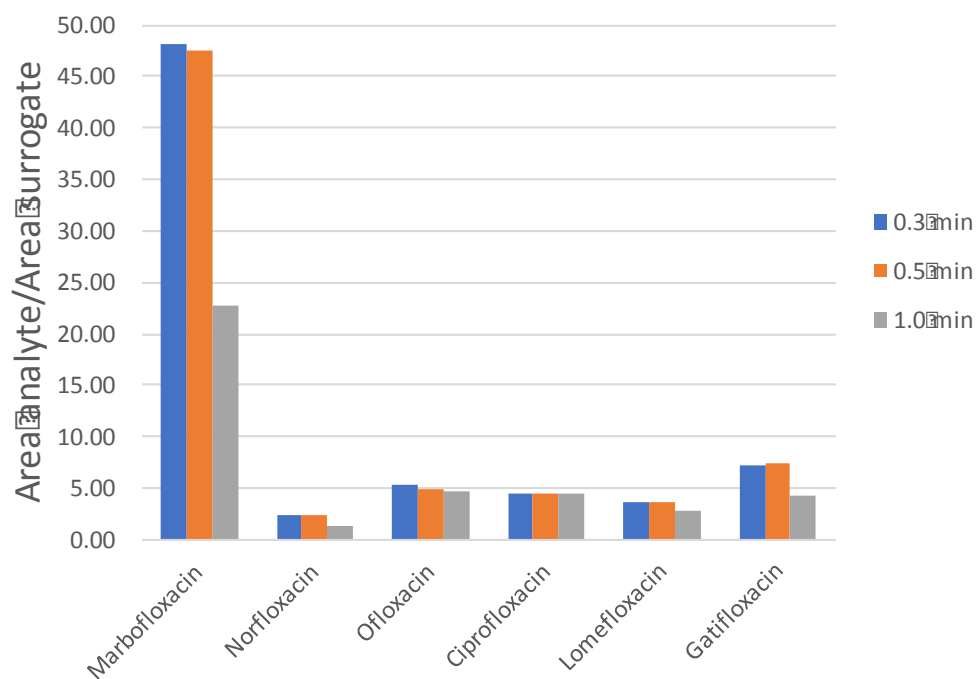
**Figure S3.** Average ( $n = 3$ ) area ratio of each analyte *per* internal standard on the HLB Oasis and C18 XBridge sorbents. Volume injected: 250  $\mu\text{L}$  of samples, containing all analytes at 17.4  $\text{ng mL}^{-1}$  and surrogate (ciprofloxacin- $d_8$ ) at 11.5  $\text{ng mL}^{-1}$ .



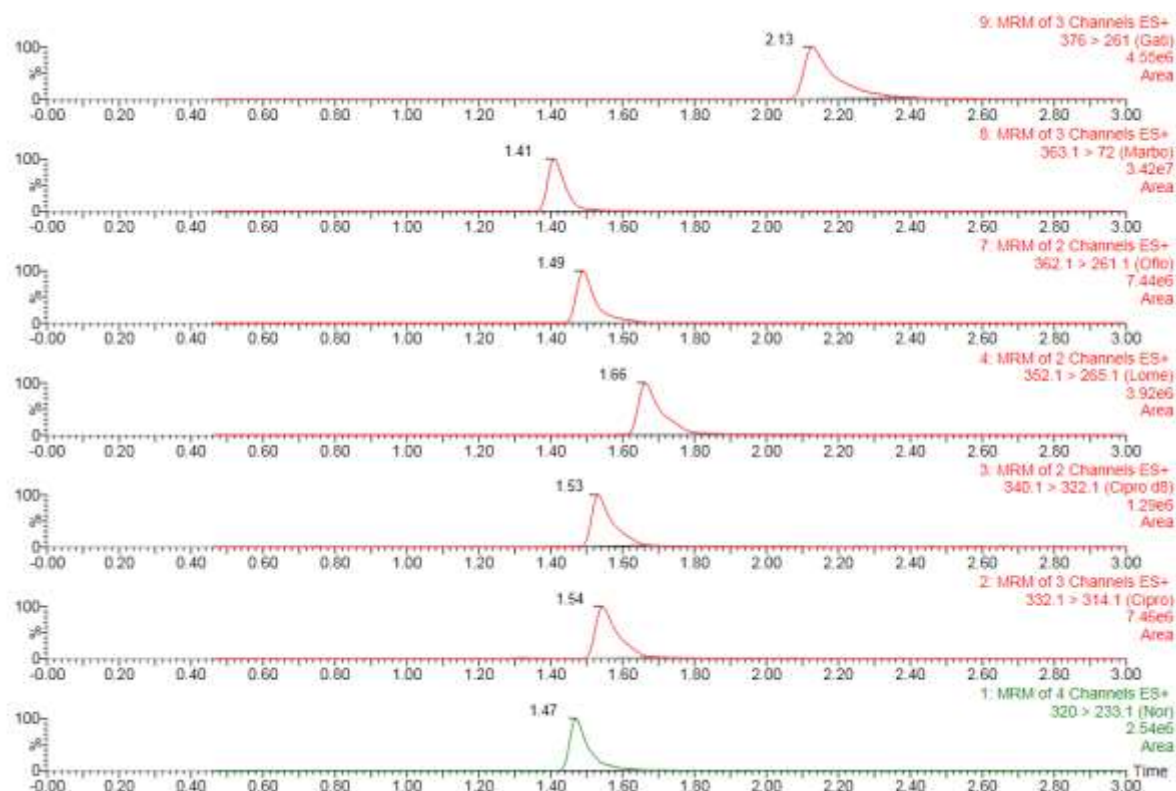
**Figure S4.** Area ratio of the chromatographic peaks (analyte/internal standard) using different water proportions in the mobile phase water:methanol, with 0.1% formic acid. (a) 100:0 v/v; (b) 95:5 v/v; (c) 90:10 v/v; (d) 80:20 v/v. Volume injected: 250  $\mu\text{L}$  of samples, containing all analytes at  $17.4 \text{ ng mL}^{-1}$  and surrogate (ciprofloxacin- $d_8$ ) at  $11.5 \text{ ng mL}^{-1}$ .



**Figure S5.** Evaluation of the injection volume. Concentration of all analytes in the samples:  $17.4 \text{ ng mL}^{-1}$  and surrogate (ciprofloxacin- $d_8$ ):  $11.5 \text{ ng mL}^{-1}$ .

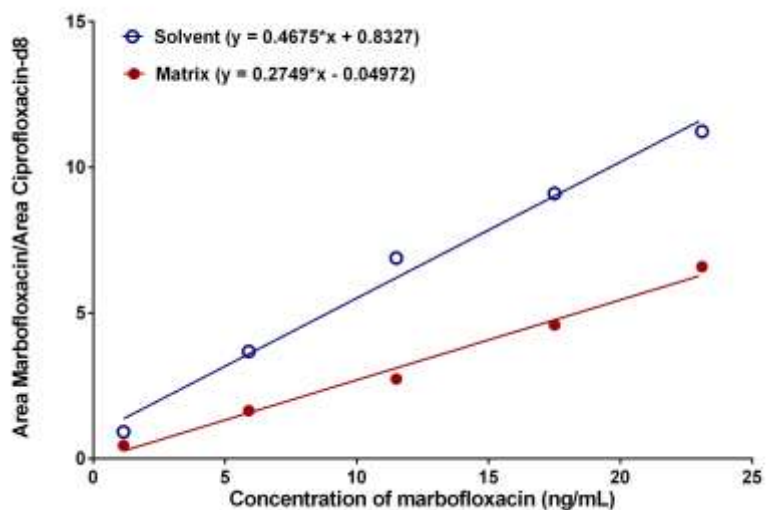


**Figure S6.** Area ratio of the analyte/internal standard with different sample loading times. Volume injected: 250  $\mu\text{L}$  of samples, containing all analytes at 17.4  $\text{ng mL}^{-1}$  and surrogate (ciprofloxacin- $d_8$ ) at 11.5  $\text{ng mL}^{-1}$ .

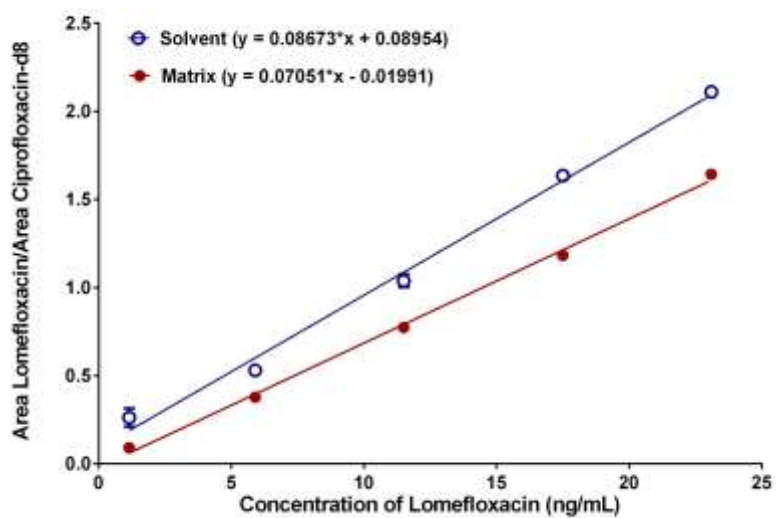


**Figure S7.** SRM chromatograms of gatifloxacin, marbofloxacin, ofloxacin, lomefloxacin, ciprofloxacin- $d_8$ , ciprofloxacin and norfloxacin.

(a) Calibration curves of Marbofloxacin using Ciprofloxacin-d8 as internal standard

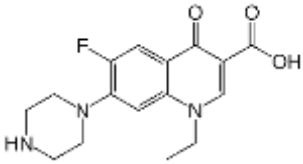
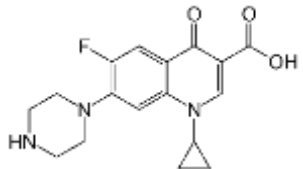
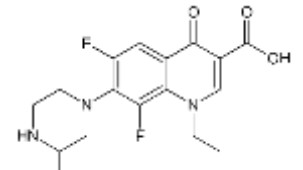
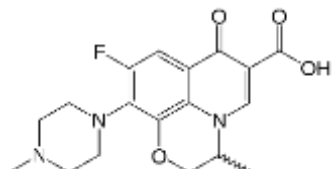
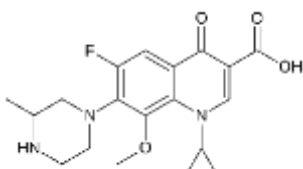
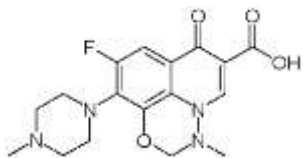


(b) Calibration curves of Marbofloxacin using Ciprofloxacin-d8 as internal standard



**Figure S8.** Calibration curves for (a) marbofloxacin and (b) lomefloxacin in solvent (blue) and in a fortified hospital wastewater sample (red), showing the matrix effect. Ciprofloxacin- $d_8$  was added as surrogate.

**Table S1.** Chemical structures and physicochemical properties of fluoroquinolones

Compound	Chemical structure	pK <sub>a</sub>	log Kow	Solubility in water / (mg mL <sup>-1</sup> )
Norfloxacin <sup>2</sup>		6.14; 8.31	0.46	0.28
Ciprofloxacin <sup>3</sup>		6.09; 8.74	0.28	30.0
Lomefloxacin <sup>4</sup>		5.82; 9.30	–	27.2
Ofloxacin <sup>5</sup>		5.97; 9.28	–0.39	10.8
Gatifloxacin <sup>6</sup>		6.43; 8.72	2.6	60.0
Marbofloxacin <sup>7</sup>		5.51; 8.38	–0.08	< 1

## References

1. Caianelo, M.; Rodrigues-Silva, C.; Maniero, M. G.; Guimarães, J. R.; *Environ. Sci. Pollut. Res.* **2017**, *24*, 6288.
2. <https://pubchem.ncbi.nlm.nih.gov/compound/norfloxacin>, accessed on January 15, 2018.
3. <https://pubchem.ncbi.nlm.nih.gov/compound/ciprofloxacin>, accessed on January 15, 2018.
4. <https://pubchem.ncbi.nlm.nih.gov/compound/lomefloxacin>, accessed on January 15, 2018.
5. <https://pubchem.ncbi.nlm.nih.gov/compound/ofloxacin>, accessed on January 15, 2018.
6. <https://pubchem.ncbi.nlm.nih.gov/compound/gatifloxacin>, accessed on January 15, 2018.
7. <https://pubchem.ncbi.nlm.nih.gov/compound/marbofloxacin>, accessed on January 15, 2018.