Photodegradation of fluoroquinolones in aqueous solution under light conditions relevant to surface waters, toxicity assessment of photoproduct mixtures

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Abstract
Photochemical degradation of fluoroquinolones ciprofloxacin, enrofloxacin and norfloxacin in aqueous solution under light conditions relevant to surface waters at neutral and alkaline pH was found to proceed readily with half-lives between 0.9 and 2.7 min. The products of photochemical degradation identified by HPLC-MS included defluorinated, hydroxylated, and decarboxylated structures as well as structures with opened cyclic structures. For all of the studied substances, the reaction pathways were influenced significantly by the pH of the reaction system, with more products formed at alkaline pH than at neutral pH: the ratios of products in neutral and alkaline pH were 16/26, 9/19, 15/23 for ciprofloxacin, enrofloxacin, and norfloxacin, respectively. The structures of photoproducts and pathways of photochemical degradation are proposed. The antibacterial activities of photoproduct mixtures tested on E. coli and S. epidermidis were significantly higher in comparison to parental antibiotics in the case of both ciprofloxacin and enrofloxacin with p-values less than 0.0001 in most cases. The effect of the photoproducts was shown to be dependent on the pH value of the original antibiotic solutions before photodegradation: for ciprofloxacin, antibacterial activity against E. coli was more notably pronounced with regard to neutral pH photoproducts, while a less significant, or in one case not significant, effect of pH was observed against S. epidermidis; for norfloxacin, antibacterial activity against both E. coli and S. epidermidis was especially high with regard to alkaline pH photoproducts.

Keywords Ciprofloxacin · Enrofloxacin · Norfloxacin · Photodegradation · Antibacterial activity of photoproducts

Introduction

Antibiotics have been increasingly detected in surface waters in the past few decades, and are thus classified as pollutants of emerging significance, though there is limited understanding of their environmental fate and toxicological effects (Van Doorslaer et al. 2014).

Among antibiotics, fluoroquinolones represent a group widely used in both human and veterinary medicine. According to WHO, fluoroquinolones are considered “highest priority critically important antibiotics” (Roth et al. 2019). An estimation of quinolone production and usage from the year 2007 shows an amount of around 50 tons in the form of proprietary products and 70 tons in the form of generic quinolones for the USA, EU, Japan and South Korea, while with respect to China, the estimate reached almost 2000 tons (Sukul and Spiteller 2007). The review article of Frade et al. (2014) provides an overview of environmental concentrations of fluoroquinolones found in surface waters, waste water treatment plants influents and effluents and hospital effluents in European countries, the USA, Japan and China — the concentration range is from tens of ng/l to several μg/l for surface waters as well as for waste-water treatment plant effluents. The highest concentrations, reaching hundreds of μg/l were found in hospital effluents.

Antibiotics generally exhibit no or extremely low biodegradability (Kümmerer et al. 2000; Vasconcelos et al. 2009). Therefore, photochemical degradation is considered a
possibly significant pathway of fluoroquinolone environmental fate (Vasconcelos et al. 2009; Li et al. 2011; Sturini et al. 2012; Sturini et al. 2015; Zhang et al. 2019). The residual antibiotic activity and possible toxic effects of photoproducts have been reported (Sturini et al. 2012; Sturini et al. 2015).

For toxicity assessments, microorganisms belonging to biosafety level 1 are extremely useful and widely adopted. *Escherichia coli* and *Staphylococcus epidermidis* belong to the category of fluoroquinolone-susceptible control strains (Baudry-Simner et al. 2012; Yamada et al. 2008) and are widely used worldwide in studies of antibacterial activity (e.g. Jacobs et al. 2004; Rizzo et al. 2013; Sanhueza et al. 2017) as well as of new approaches to antibiotics applications (Kratochvíl et al. 2018a); Kratochvíl et al. 2018b).

The objectives of this study were: (1) To test whether three widely used fluoroquinolone representants, namely ciprofloxacin, enrofloxacin, and norfloxacin, may undergo photochemical degradation under irradiation relevant to natural short-wavelength conditions and at neutral and mildly alkaline pH values common in natural waters. (2) To reveal whether the degradation pathways and products formed during degradation processes depend on pH. (3) To ascertain whether this degradation leads to complete or partial loss of antibacterial activity.

### Materials and methods

#### Photodegradation procedure

**Sample preparation** Ciprofloxacin, enrofloxacin, and norfloxacin (Sigma-Aldrich, all ≥98%, HPLC) solutions were prepared by the dissolution of 5 mg of the compound in 4 ml HCl (0.1 mol/l), then diluted to 100 ml with Milli-Q® water. The pH values were adjusted by 10 % NaOH, for neutral pH to a range of 7.0–7.13, for alkaline pH to 8.13–8.8.

| Table 1 Details of HPLC-MS analysis |
|-------------------------------------|
| Method Parameter | Specification |
| Column specification | Phenomenex Luna C18 (250 × 4.6 mm; 5 μm) |
| mobile phase | HCOOH in water (66.7 %) and 0.1 % HCOOH in acetonitrile (33.3 %), isocratic elution |
| Flow rate | 1 ml/min |
| Pump pressure | 157 bar |
| Column oven temp. | 30 °C |
| Sample volume | 20 μl |
| Wavelength (channels) | 200 nm, 254 nm, 278 nm |
| Filter bandwidth | 1 nm |
| Data collection rate | 10 Hz |
| EX, EM, EF | Ex 231 nm, Em 328 nm, emission filter 280 nm |
| Peak width | 0.1 nm |
| Data collection rate | 5 Hz |
| IS – type | heated electrospray (HESI II) |
| IS – heater temperature | 350°C |
| IS – sheath gas | 60 arb |
| IS – auxiliary gas | 20 arb |
| IS – sweep gas | 0 arb |
| IS – spray voltage | 3.00 kV |
| IO – capillary temp. | 350°C |
| IO – S-Lens RF level | 60 |
| IO – front lens | −7.5 V |
| IT – records | + c ESI Full MS (50.00–1000.00) + c ESI Full MS2 (125.00–500.00) |

| Table 2 Rate constants of ciprofloxacin, enrofloxacin and norfloxacin in neutral and mildly alkaline pH |
|---------------------------------------------------------------|
| Fluoroquinolone | Rate constant (min⁻¹) |
| Neutral pH (6.8–7.1) | Alkaline pH (8.7–9.1) |
| Ciprofloxacin | 0.701 | 0.558 |
| Enrofloxacin | 0.521 | 0.763 |
| Norfloxacin | 0.341 | 0.254 |

**Fig. 1** Photodegradation pathways of ciprofloxacin at neutral pH (pH = 7.0)
Irradiation Samples containing 3 ml of an antibiotic solution in 1-cm glass cuvettes with PTFE lids were irradiated in a Rayonet reactor with RPR 3000 Å lamps emitting light at a wavelength range of 254–350 nm — light below 300 nm was filtered out by optical glass to imitate short-wavelength solar radiation that reaches the Earth’s surface. Radiant flux was measured using a Lutron UV A light metre, the total power of all the electromagnetic radiation within the wavelength range 320–390 nm (the range detected by the Lutron metre) emitted per unit time was calculated for the irradiated area, the value being 4.5 W.

Analyses of samples The extent of fluoroquinolones photodegradation was determined by HPLC (ThermoScientific Dionex Ultimate, column Phenomenex Kinetex® 5 μm EVO C18, 30 × 2.1 mm, mobile phase 0.1 % HCOOH in water and acetonitrile 1:1) with PDA 3000RS spectrophotometric and FLD 3000RS fluorescence detectors. The identification of products was performed using a HPLC-MS system (Thermo Scientific Ultimate 3000 Rapid Separation Quaternary System coupled with Velos Pro mass spectrometer with dual-pressure linear ion trap). The details of HPLC-MS measurement are summarized in Table 1. Ion chromatography (Dionex ISC 3000, Dionex Ion Pack AS11-HC column 250 mm, mobile phase KOH from EG C III KOH eluent generator with AERS 500 suppressor) was applied for the detection of low-molecular charged products.

Antibacterial activity tests Escherichia coli Seattle 1946 (ATCC 25922) and Staphylococcus epidermidis RP62A
Table 3  Proposed structures of photoproducts of ciprofloxacin at neutral pH (pH = 7.0). All m/z values identified in the positive ion mode (+ESI)

| Name                                           | Formula                                               | m/z  |
|------------------------------------------------|-------------------------------------------------------|------|
| ciprofloxacin                                  | ![ciprofloxacin structure]                           | 332.0|
| product 1                                      | ![product 1 structure]                               | 288.1|
| product 2                                      | ![product 2 structure]                               | 314.1|
| product 3                                      | ![product 3 structure]                               | 346.1|
| product 4                                      | adduct of ciprofloxacin with acetonitrile (part of mobile phase) | 376.1|
| product 5                                      | ![product 5 structure]                               | 290.0|
| Product 6 | ![Structure](image) | 272.0 |
| Product 7 | ![Structure](image) | 229.0 |
| Product 8 | ![Structure](image) | 271.0 |
| Product 9 | ![Structure](image) | 245.0 |
| Product 10 | ![Structure](image) | 328.2 |
| Product 11 | ![Structure](image) | 330.1 |
| Product 12 | ![Structure](image) | 316.1 |
| Product 13 | Structure not identified | 312.1 |

| Anion          | Identification Method | Notes |
|----------------|-----------------------|-------|
| Formate        | Identified with ion chromatography | -     |
| Acetate        | Identified with ion chromatography | -     |
| Fluoride       | Identified with ion chromatography | -     |
Table 4  Proposed structures of photoproducts of ciprofloxacin at alkaline pH (pH = 8.5). Some m/z values detected only in the positive ion mode (+ESI), some only in the negative ion mode (-ESI), several products were detected in both modes

| Name       | Formula | m/z       |
|------------|---------|-----------|
| ciprofloxacin | ![Structure](image) | 332.2 (+ESI) 330.2 (-ESI) |
| product 1  | ![Structure](image) | 393.2 (-ESI) |
| product 2  | ![Structure](image) | 314.2 (+ESI) |
| product 3  | ![Structure](image) | 362.9 (+ESI) |
| product 4  | ![Structure](image) | 345.2 (+ESI) |
| product 5  | ![Structure](image) | 362.9 (+ESI) |
| product 6  | not identified | 226.9 |
| product 7  | ![Structure](image) | 374.9 (-ESI) |
| Product  | Molecular Structure |
|----------|---------------------|
| 8        | ![Molecular Structure](image) | 328.2 (-ESI) 330.2 (+ESI) |
| 9        | ![Molecular Structure](image) | 312.1 (+ESI) |
| 10       | ![Molecular Structure](image) | 284.3 (-ESI) 286.3 (+ESI) |
| 11       | ![Molecular Structure](image) | 243.1 (+ESI) |
| 12       | ![Molecular Structure](image) | Not identified 388.2 (-ESI) |
| 13, 14   | ![Molecular Structure](image) | Not identified, probably stereoisomers (same m/z, different retention times) 380.2 (-ESI) |
| 15       | ![Molecular Structure](image) | 316.1 (-ESI) 318.1 (+ESI) |
| 16       | ![Molecular Structure](image) | 286.2 (-ESI) 288.2 (+ESI) |
| 17       | ![Molecular Structure](image) | 268.2 (+ESI) |
| Product   | Structure | Molecular Mass |
|-----------|-----------|----------------|
| 18        | ![Structure](image) | 274.1 (+ESI)   |
| 19        | ![Structure](image) | 344.2 (-ESI) 346.2 (+ESI) |
| 20        | ![Structure](image) | 328.2 (+ESI)   |
| 21        | ![Structure](image) | 328.2 (+ESI)   |
| 22        | ![Structure](image) | 300.2 (-ESI) 302.2 (+ESI) |
| 23        | ![Structure](image) | 376.2 (-ESI)   |
| 24        | ![Structure](image) | 332.2 (-ESI) 334.2 (+ESI) |
(ATCC 35984) bacterial strains were grown in LB medium overnight at 37 °C with constant agitation. Then, the bacteria were diluted to 200,000 cells per ml of DMEM culture medium supplemented with 10 % fetal calf serum and seeded into 96-well plate. Undiluted, 10-fold diluted and 100-fold diluted samples were added to the bacteria and cultivated at 37 °C and 5 % CO₂ for 3 h. Bacterial viability was quantified by measuring fluorescence upon excitation and emission wavelengths of 550 nm and 590 nm, respectively, after an additional 3 and 6 h of cultivation using alamarBlue™ Cell Viability Reagent (ThermoFisher Scientific). The values from photoproduct-containing samples were normalized to control (antibiotics without photoproducts), i.e. the metabolic activity of the control samples (as a function of bacterial proliferation) is set to 100%. Data are represented as means and standard error of mean (SEM). Differences between samples were evaluated using 2-way analysis of variance followed by Tukey post-hoc test.

Results

The photoinitiated degradation of the three studied substances (ciprofloxacin, enrofloxacin and norfloxacin) followed first order kinetics. Their kinetic characteristics are summarized in Table 2. The values of rate constants are of the same order of magnitude; nevertheless, ciprofloxacin differed from the other two compounds since it has a higher reaction rate at neutral pH while enrofloxacin and norfloxacin were degraded more quickly at alkaline pH. The half-lives of the fluoroquinolones under the experimental conditions were in the range of 0.9 to 2.7 min.

The HPLC analyses revealed the formation of a significant amount of intermediates and final products in all reaction systems. Thus, reaction mixtures at irradiation times providing the most complex reaction mixtures were chosen for HPLC-MS and for antibacterial activity tests.

![Photodegradation pathways of enrofloxacin at neutral pH (pH = 6.9)](image)
Table 5  Proposed structures of photoproducts of enrofloxacin at neutral pH (pH = 6.9). Some m/z values detected only in the positive ion mode (+ESI), some only in the negative ion mode (-ESI), several products were detected in both modes

| Name       | Structure                                | m/z          |
|------------|------------------------------------------|--------------|
| enrofloxacin | ![Structure](image1)                      | 360.2 (+ESI) | 358.2 (-ESI) |
| product 1  | ![Structure](image2)                      | 316.2 (+ESI) | 314.2 (-ESI) |
| product 2  | ![Structure](image3)                      | 342 (+ESI)   |
| product 3  | ![Structure](image4)                      | 394 (-ESI)   |
| product 4  | ![Structure](image5)                      | 179.6 (+ESI) |
| product 5  | ![Structure](image6)                      | 245.1 (+ESI) |
| product 6  | ![Structure](image7)                      | 298.1 (+ESI) |
The reaction schemes of ciprofloxacin in neutral and alkaline solution are demonstrated in Figs. 1 and 2, respectively. The observed m/z values of products and their proposed structures are summarised in Table 3 for neutral pH and in Table 4 for alkaline pH. Table 4 contains additional three low-molecular ions (fluoride, formate and acetate) that were detected in the reaction mixture using ion chromatography. The scheme for neutral pH (Fig. 1) contains the pathways for formation of 13 products. The main degradation mechanisms are defluorination, hydroxylation, decarboxylation, and the opening and further degradation of cyclic structures. In alkaline pH, 26 products were documented, for 22 of them their chemical structure has been proposed. Under these conditions, the double hydroxylated product 1 and protonated structure of product 23 are the main sources for a series of further product formation.

Enrofloxacin at neutral pH (Fig. 3) has the lowest number of products — only nine were found in the reaction mixture, their structures are shown in Table 5. In alkaline medium, 19 products were detectable, for 16 of them structures are proposed (Table 6). The degradation pathways are presented in Fig. 4.

Fig. 5 and Table 7 demonstrate the photochemical degradation of norfloxacin at neutral pH. In this case, 15 products were detected and their structures proposed. Irradiation at alkaline pH led to 23 products (Fig. 6), for 19 of them proposed structures are presented (Table 8).

In all cases, the degradation in alkaline medium leads to a significantly greater amount of products, nevertheless the degradation pathway (defluorination, hydroxylation, decarboxylation, and the opening and further degradation of cyclic structures) was common under all conditions tested.

Antibacterial activity was tested with paternal compounds and mixtures of photoproducts, i.e. irradiated samples with developed intermediates and products profiles, using the same irradiation times as in the HPLC-MS analyses. Results are summarised in Fig. 7.

As can be seen from Fig. 7, the inhibitory activities against the two tested bacteria strains were significantly higher for photoproducts of ciprofloxacin and enrofloxacin in comparison to the parental antibiotics (numbers in the presence of the respective parental antibiotic represent control column in Fig. 7). Ciprofloxacin photoproducts exhibited higher inhibition towards *E. coli* when irradiated at neutral pH, the effect being more pronounced with increasing cultivation time; a lesser significant inhibitory effect was observed in the reaction mixture of ciprofloxacin irradiated at alkaline pH. The inhibitory effects towards *S. epidermidis* were on the other hand evidently higher in photoproduct mixtures irradiated at mildly alkaline pH.

Enrofloxacin photoproducts exhibit an extremely significant decrease in the numbers of bacteria towards both tested species with the effect being more noticeable with regard to photoproducts that were formed by photochemical degradation at mildly alkaline pH.
Table 6  Proposed structures of photoproducts of enrofloxacin at alkaline pH (pH = 8.1). Some m/z values detected only in the positive ion mode (+ESI), some only in the negative ion mode (-ESI), several products were detected in both modes.

| Name           | Structure | m/z     |
|----------------|-----------|---------|
| enrofloxacin   | ![Structure](enrofloxacin.png) | 360.2 (+ESI) 358.2 (-ESI) |
| product 1      | ![Structure](product1.png) | 342.2 (+ESI)    |
| product 2      | ![Structure](product2.png) | 316.2 (+ESI) 314.2 (-ESI) |
| product 3      | ![Structure](product3.png) | 392.2 (+ESI)    |
| product 4      | ![Structure](product4.png) | 334.0 (+ESI) 332.0 (-ESI) |
| product 5      | not identified | 288.1 (-ESI)    |
| product 6      | identical with product 3, only without attached water molecule | 372.2 (-ESI)    |
| product 7      | ![Structure](product7.png) | 330.0 (+ESI) 328.0 (-ESI) |
| product 8      | ![Structure](product8.png) | 356.2 (+ESI)    |
| Product | Identification | Mass (ESI) |
|---------|----------------|------------|
| Product 9 | identical with product 7 + attached water molecule | 350.0 (+ESI) 348.0 (-ESI) |
| Product 10 | ![Structure](image1) | 376.2 (+ESI) |
| Product 11 (ciprofloxacin) | ![Structure](image2) | 332.2 (+ESI) |
| Product 12 | ![Structure](image3) | 346.2 (+ESI) |
| Product 13 | ![Structure](image4) | 300.1 (-ESI) |
| Product 14 | ![Structure](image5) | 328.2 (+ESI) |
| Product 15 | ![Structure](image6) | 288.2 (+ESI) |
| Product 16 | ![Structure](image7) | 314.1 (+ESI) |
| Product 17 | ![Structure](image8) | 358.2 (+ESI) |
| Product 18 | not identified | 328.2 (+ESI) |
| Product 19 | not identified | 350.0 (-ESI) |
Regarding norfloxacin, no differences in the antibacterial activity of photoproduction mixture when compared to norfloxacin itself were discerned towards either tested species.

**Discussion**

The phototransformation of the studied substrates followed first-order kinetics, the values of reaction rate constants are similar to those attained by Babić et al. (2013) and Wammer et al. (2013) for ciprofloxacin and enrofloxacin. They observed a faster photodegradation of norfloxacin, but this may have been caused by a higher concentration of antibiotics in this study in comparison to their experiment, since Babić et al. (2013) noticed a decrease in reaction rate in relation to increasing concentration.

The degradation mechanism of the studied fluoroquinolone antibiotics depends strongly on the pH value of the irradiated reaction mixture. Even though the main degradation pathways involve in all cases processes such as defluorination, hydroxylation, decarboxylation, and the opening and further degradation of the cyclic structures under all conditions tested, the number of products was noticeably higher when the photodegradation was carried out at alkaline pH. The findings are in agreement with the results of Zhang et al. (2019) who revealed the influence of chemical speciation on photochemical transformation of three fluoroquinolones (lomefloxacin, norfloxacin and ofloxacin). In their study, they also observed defluorination, decarboxylation and direct piperazinyl ring oxidation. Generally, it can be concluded that at alkaline pH, hydroxylation prevails due to abundant hydroxyl groups. Nevertheless, the hydroxylated products depend on the character of the antibiotics: with ciprofloxacin and norfloxacin, a substitution of the OH group for the F atom and a direct hydroxylation of the piperazinyl ring and its further oxidation and breakage were observed, whereas in enrofloxacin the OH group was bound to the ethyl group on
Table 7  Proposed structures of norfloxacin at neutral pH (pH = 7.1). Some m/s values detected only in the positive ion mode (+ESI), some only in the negative ion mode (-ESI), one of the products was detected in both modes

| Name      | Structure | m/z     |
|-----------|-----------|---------|
| norfloxacin | ![Structure](image) | 320.0 (+ESI) |
| product 1 | ![Structure](image) | 302.2 (+ESI) |
| product 2 | ![Structure](image) | 276.2 (+ESI) |
| product 3 | ![Structure](image) | 258.2 (+ESI) |
| product 4 | ![Structure](image) | 256.2 (+ESI) |
| product 5 | ![Structure](image) | 228.1 (+ESI) |
| product 6 | ![Structure](image) | 233.2 (+ESI) |
| product 7 | ![Structure](image) | 318.2 (+ESI) |
| Product | Chemical Structure | Mass (ESI) |
|---------|-------------------|------------|
| product 8 | ![Image](product8.png) | 274.2 (+ESI) |
| product 9 | ![Image](product9.png) | 300.2 (+ESI) |
| product 10 | ![Image](product10.png) | 334.2 (-ESI) |
| product 11 | ![Image](product11.png) | 364.2 (-ESI) |
| product 12 | ![Image](product12.png) | 320.2 (-ESI) |
| product 13 | ![Image](product13.png) | 294.1 (+ESI) |
| product 14 | ![Image](product14.png) | 276.1 (+ESI) 274.1 (-ESI) |
| product 15 | ![Image](product15.png) | 259.1 (+ESI) |
the piperazinyl group. Enrofloxacin photodegradation provided ciprofloxacin in both neutral and alkaline pH; the production of ciprofloxacin from enrofloxacin was mentioned in the study of Babić et al. (2013), though their subsequent products differ from the products presented in this study, since their reaction mixture contained humic acid which may affect degradation pathways. The products No 7 and 8 (m/z = 374 and 358, respectively) of enrofloxacin photodegradation at neutral pH were noticed by Wammer et al. (2013). Two of the products of enrofloxacin degraded at alkaline pH were reported by Li et al. (2011), product No 2 (m/s = 316.2 in + ESI) and product No 6 (m/s = 372.2 in + ESI). Product No 13 (m/s = 294.1) was found by Ahmad et al. (2015).

Antibacterial activity tests performed in the study against two bacterial strains came from the assumption (null hypothesis) that photochemical degradation would result in a decrease or complete loss of antibacterial activity. The loss of antibacterial activity was shown to be true for norfloxacin since the mixture of norfloxacin photoproducts exhibited the same inhibitory effect as the antibiotic itself; since the dose of photoproducts mixture for the test was calculated to contain the same amount of parental antibiotics as the antibiotic sample, the result shows conclusively that norfloxacin photoproducts do not contribute to inhibition of the bacterial growth in either tested species. This corresponds with the results of bioluminescent inhibition tests on *Vibrio fischeri* performed by Zhang et al. (2019) in which they observed a significant decrease in toxicity for norfloxacin and ofloxacin.

The null hypothesis about the decrease of antibacterial activity through photochemical degradation must be rejected for the two remaining compounds tested in this study, ciprofloxacin and enrofloxacin. Taking into account the statistical evaluation presented in Fig. 7, only for the pair ciprofloxacin/alkaline photoproducts tested on *E. coli* were the p-values less than or equal to 0.05 and 0.01 for 6 and 9–h incubation, resp; nevertheless, even these values of p-value are considered to be significant for rejecting null hypothesis. In all other cases, the pairs control/photoproducts-p-values were 0.0001, which means there is an extreme statistical significance that photoprodct toxicity to bacteria is notably higher than those of parent antibiotics themselves. The difference between photoproducts formed at neutral and mildly alkaline pH and the extremely significant higher toxicity of photoproducts formed in alkaline solution were proven with regard to enrofloxacin toward both tested species. With regard to ciprofloxacin, photoproducts formed at neutral pH exhibited extremely significant higher antibacterial activity against *E. coli*; against *S. epidermidis*, though...
Table 8  Proposed structures of norfloxacin at alkaline pH (pH = 8.9). Some m/z values detected only in the positive ion mode (+ESI), some only in the negative ion mode (-ESI), several products were detected in both modes

| Name       | Structure | m/z       |
|------------|-----------|-----------|
| norfloxacin| ![Structure](image) | 32.2 (+ESI) 318.2 (-ESI) |
| product 1  | ![Structure](image) | 302.2 (+ESI) |
| product 2  | ![Structure](image) | 276.2 (+ESI) |
| product 3  | ![Structure](image) | 256.2 (+ESI) |
| product 4  | ![Structure](image) | 233.1 (+ESI) |
| product 5  | ![Structure](image) | 364.2 (-ESI) |
| product 6  | ![Structure](image) | 304.2 (+ESI) 302.0 (-ESI) |
| product 7  | not identified | 316.3 (+ESI) |
| product 8  | ![Structure](image) | 286.2 (+ESI) |
| product 9  | ![Structure](image) | 320.2 (-ESI) |
| Product      | Molecular Structure | Mass/Charge Ratio       |
|--------------|---------------------|-------------------------|
| Product 10   | ![Molecular Structure](image1) | 274.1 (-ESI)            |
| Products 11, 12 | not identified     | 336.3 and 354.2, resp. (both -ESI) |
| Product 13   | ![Molecular Structure](image2) | 310.1 (-ESI)            |
| Product 14   | ![Molecular Structure](image3) | 381.1 (-ESI)            |
| Product 15   | ![Molecular Structure](image4) | 292.1 (-ESI)            |
| Product 16   | ![Molecular Structure](image5) | 318.2 (+ESI), 316.2 (-ESI) |
| Product 17   | ![Molecular Structure](image6) | 274.2 (+ESI), 272.2 (-ESI) |
| Products 18, 19, 20 | not identified, products 19 and 20 – stereoisomers (identical m/z, but different retention times) | 352.1; 362.2; 362.2, resp. (all -ESI) |
no significant difference was observed after longer incubation time. This is in agreement with the results of Sturini et al. (2012) who found that the irradiation of four fluoroquinolone antibiotics (ciprofloxacin, danofloxacin, levofloxacin and moxifloxacin) dissolved in untreated river water under solar light led to photoproducts that possessed residual antibacterial activity towards *E. coli* and *S. aureus*. The authors attributed the activity manifestation to the products conserving the fundamentals of the fluoroquinolone structure. Since a significant portion of photoproducts detected in our study retain the basic fluoroquinolone structure, the hypothesis that this structure is responsible for the antibacterial activity may be considered viable. Nevertheless, the increased antibacterial effect in some photoproduct mixtures observed in this study suggests that some modifications of the base structure lead to augmentation of the antibacterial effect when compared to the original antibiotic. Thus, though photodegradation may represent a feasible degradation pathway for fluoroquinolone antibiotics in surface waters, there is even the possibility of production substances possessing enhanced antibacterial
activity which may pose a threat to microorganism populations.

**Conclusions**

Three representatives of fluoroquinolones, ciprofloxacin, enrofloxacin, and norfloxacin, were demonstrated to be readily degraded photochemically under irradiation relevant to short-wavelength solar radiation reaching the Earth surface. In all cases, the photodegradation at neutral and mildly alkaline pH followed first-order kinetics with half-lives in the range from 0.8 to 2.7 min$^{-1}$.

Photodegradation mechanisms and their pathways are strongly dependent on pH; for all antibiotics tested, the number of products and structures of these products formed at neutral or at mildly alkaline pH differ significantly, with more complex reaction schemes occurring at alkaline pH.

Antibacterial activity tests against two bacteria species, *E. coli* and *S. epidermidis*, showed an increased toxicity in their photoproducts compared to parental compounds. For *S. epidermidis*, the rise in toxicity was extremely significant for photoproducts of ciprofloxacin and enrofloxacin under all conditions tested with p-value $\leq 0.0001$. For *E. coli*, the increase in toxicity was similarly extremely significant for the photoproducts of enrofloxacin, and significant (p-value $\leq 0.05$) for longer incubation in the photoproducts of ciprofloxacin.

With respect to norfloxacin, no enhanced antibacterial activity in connection to photochemical degradation was observed.

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**Author contribution** Š. Klementová organised and supervised the research and was a major contributor in writing the manuscript.

M. Poncarová performed photochemical experiments and analysed LC-MS data.

H. Langhansová and J. Lieskovská carried out the testing of antibacterial activity.

D. Kahoun supervised the LC-MS measurements.

P. Fojtíková participated in the HPLC analyses.

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**Data availability** All relevant data generated and analysed during this study are included in the article.

**Declarations**

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

**Competing interests** The authors declare no competing interests.

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