

Supplementary Information

Degradation of Levofloxacin by *Porostereum spadiceum*: from genome to application

Amal Ben Ayed ^{1,2,*}, Imen Akroud ^{1,2}, Karima Staita ^{1,2}, Quentin Albert ^{2,3}, Stéphane Greff ⁴, Charlotte Simmler ⁴, Steven Ahrendt ⁵, Kurt LaButti ⁵, Anna Lipzen ⁵, Guifen He ⁵, Emily Savage ⁵, Jean Armengaud ⁶, Mélodie Kielbasa ⁶, David Navarro ^{2,3}, Elodie Drula ^{2,7,8}, Annick Turbé-Doan ², Emmanuel Bertrand ², Anne Lomascolo ², Delphine Chaduli ^{2,3}, Craig B. Faulds ², Mohamed Chamkha ⁹, Amina Maalej ⁹, Kerrie Barry ⁵, Igor V Grigoriev ^{5,10,11}, Francis Martin ¹², Héla Zouari-Mechichi ¹, Giuliano Sciara ², Tahar Mechichi ¹ and Eric Record ^{2,*}

¹ Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, Laboratoire de Biochimie et de Génie enzymatique des lipases, 3038 Sfax, Tunisia; amal.benayed@enis.tn (A.B.A.); akroutimen1@gmail.com (I.A.); hela.zouari@isbs.usf.tn (H.Z.-M.); tahar.mechichi@enis.rnu.tn (T.M.)

² Aix-Marseille Université, INRAE, UMR1163, Biodiversité et Biotechnologie Fongiques, 13288 Marseille, France; quentin.albert@univ-amu.fr (Q.A.); elodie.drula@inrae.fr (E.D.); david.navarro@inrae.fr (D.N.); annick.doan@univ-amu.fr (A.T.-D.); emmanuel.bertrand@univ-amu.fr (E.B.); anne.lomascolo@univ-amu.fr (A.L.); delphine.chaduli@inrae.fr (D.C.); craig.faulds@univ-amu.fr (C.B.F.); giuliano.sciara@inrae.fr (G.S.); eric.record@inrae.fr (E.R.)

³ Aix-Marseille Université, INRAE, UMR1163, CIRM-CF, 13288 Marseille, France

⁴ Aix Marseille Université, CNRS, IRD, Avignon Université, IMBE, UMR 7263, Station Marine d'Endoume, Rue de la Batterie des Lions, 13007 Marseille, France; stephane.greff@imbe.fr (S.G.); charlotte.simmler@imbe.fr (C.S.)

⁵ US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA; sahrendt0@lbl.gov (S.A.); klabutti@lbl.gov (L.L.); alipzen@lbl.gov (A.L.); ghe@lbl.gov (G.H.); EmilySavage@lbl.gov (E.S.); kwbarry@lbl.gov (K.B.); ivgrigoriev@lbl.gov (I.V.G.)

⁶ Université Paris-Saclay, Département Médicaments et Technologies pour la Santé, CEA, INRAE, SPI, 30200 Bagnols-sur-Cèze, France; jean.armengaud@cea.fr (J.A.); melodie.kielbasa@cea.fr (M.K.)

⁷ Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique, Aix-Marseille Université, 13288 Marseille, France.

⁸ USC AFMB, Institut National de Recherche Agronomique, 13288 Marseille, France.

⁹ Université de Sfax, Centre de Biotechnologie de Sfax, Laboratoire des Bioprocédés Environnementaux, 3063 Sfax, Tunisia ; mohamed.chamkha@cbs.rnrt.tn (M.C.); maalejamina@yahoo.fr (A.M.)

¹⁰ Environmental Genomics and Systems Biology, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA.

¹¹ Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA, USA.

¹² Université de Lorraine, INRAE, UMR1136, Interactions Arbres/Microorganismes, Champenoux, 54280, France; francis.martin@inrae.fr (F.M.)

* Correspondence to : Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, Laboratoire de Biochimie et de Génie enzymatique des lipases, 3038 Sfax, Tunisia (A.B.A.) and Aix-Marseille Université, INRAE, UMR1163, Biodiversité et Biotechnologie Fongiques, 13288 Marseille, France (E.R.)

E-mail address: amal.benayed@enis.tn and eric.record@inrae.fr

Figure S1. Bootstrap consensus tree of *Porostereum spadiceum* based on the maximum likelihood method. Bootstrap values are indicated in the branches, and all strains are shown with their accession numbers in brackets. Strain BS34 is indicated by the black circle.

The neighbor-joining method was used to determine evolutionary history (Saitou and Nei, 1987). The bootstrap consensus tree computed from 500 replicates was used to plot the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions replicated in less than 50% of bootstrap replicates were trimmed. Percentage of replicate trees grouping the associated taxa in the bootstrap test (500 replicates) was indicated next to the branches, and the maximum composite likelihood method was used to calculate evolutionary distances expressed as number of base substitutions per site (Felsenstein, 1985; Tamura et al., 2004). This analysis included 9 nucleotide sequences. All ambiguous positions in each pair of sequences were removed (pairwise removal option). The final dataset contained 650 positions. Evolutionary analyses were performed using MEGA11 software (Tamura et al., 2021).

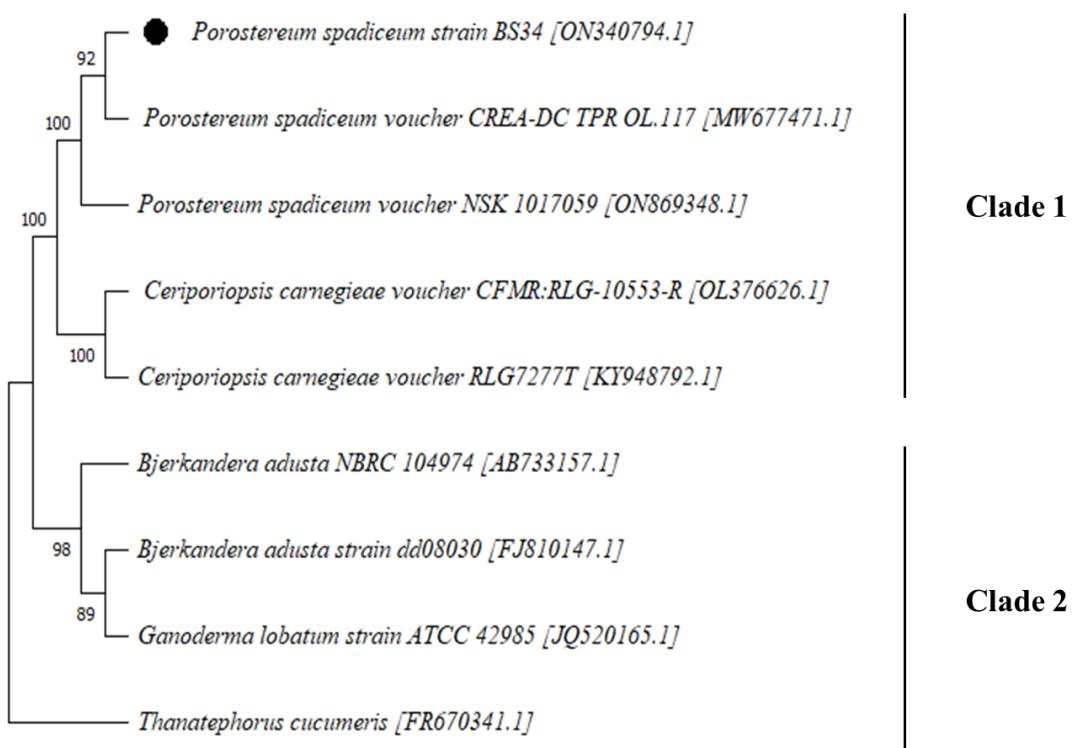


Figure S2. UV chromatograms of levofloxacin samples after 4, 7, and 10 days of culture in the presence of *Porostereum spadiceum*. LEV = Levofloxacin. Numbers are degradation products of levofloxacin (see Table 4 in the main manuscript). Letters are *P. spadiceum* metabolites.

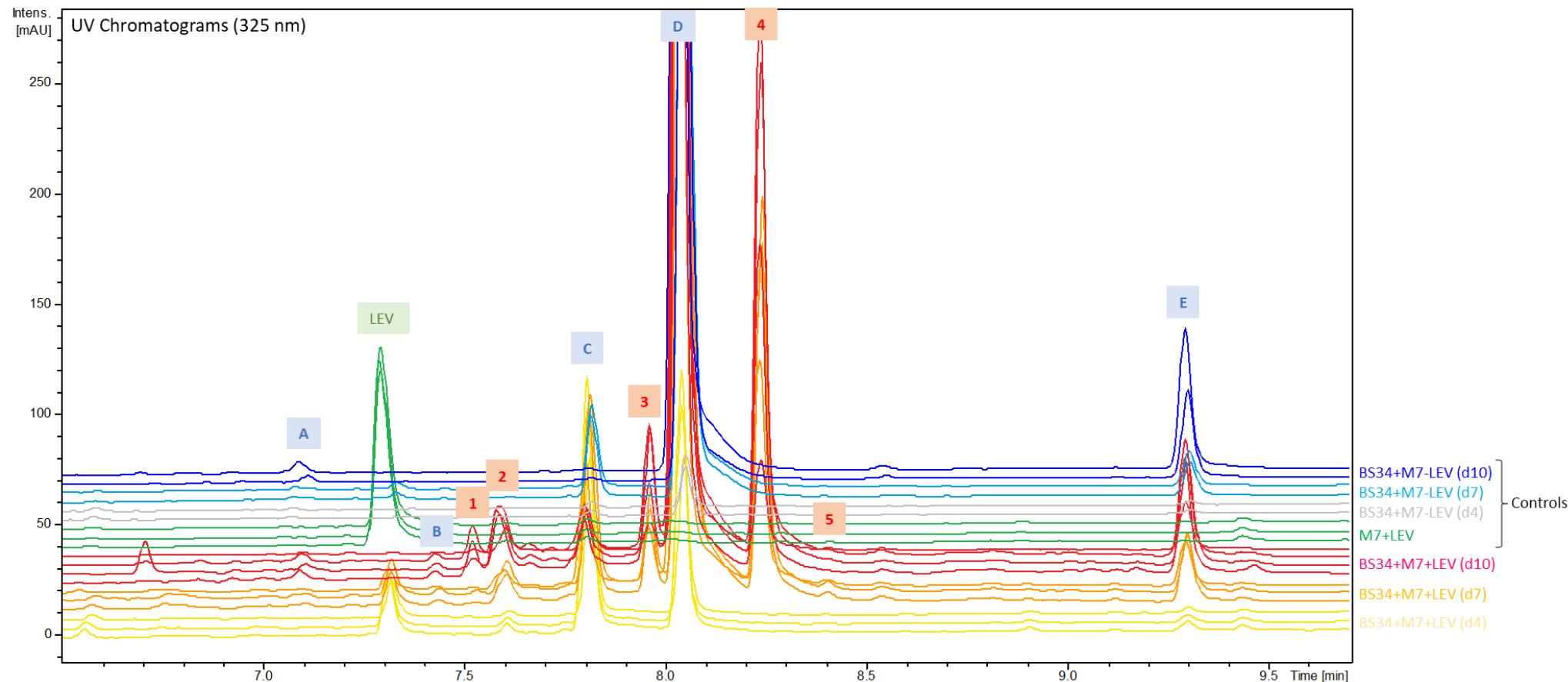


Figure S3. Chemical formula and fragmentation mass spectrum of levofloxacin (at a collision energy of 40 eV).

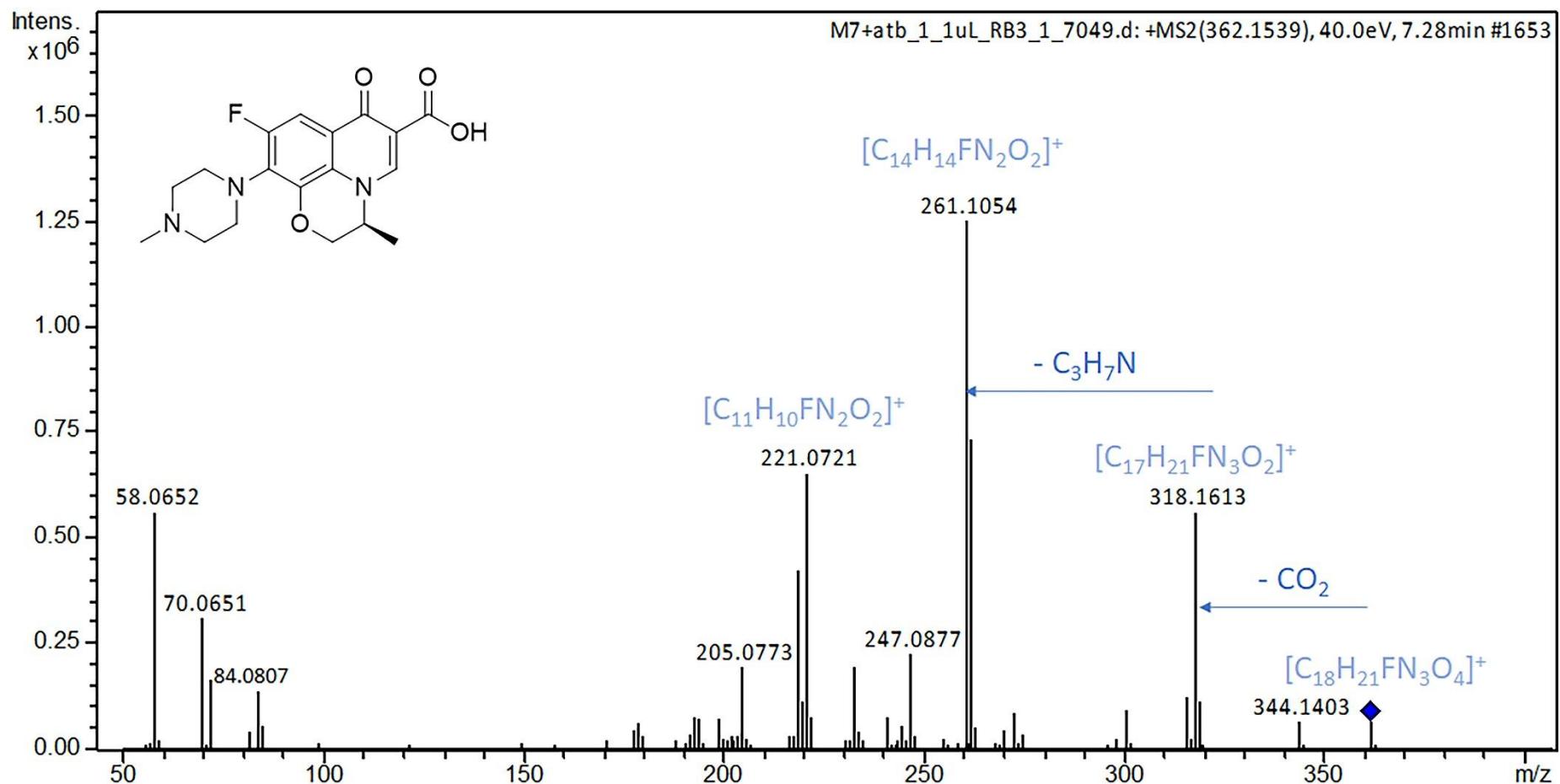


Figure S4. The fragmentation mass spectrum of levofloxacin degradation product #1 (collision energy 40 eV), a minor product detected on day 10 in the UV chromatogram at 325 nm (See Table 4 of the main manuscript).

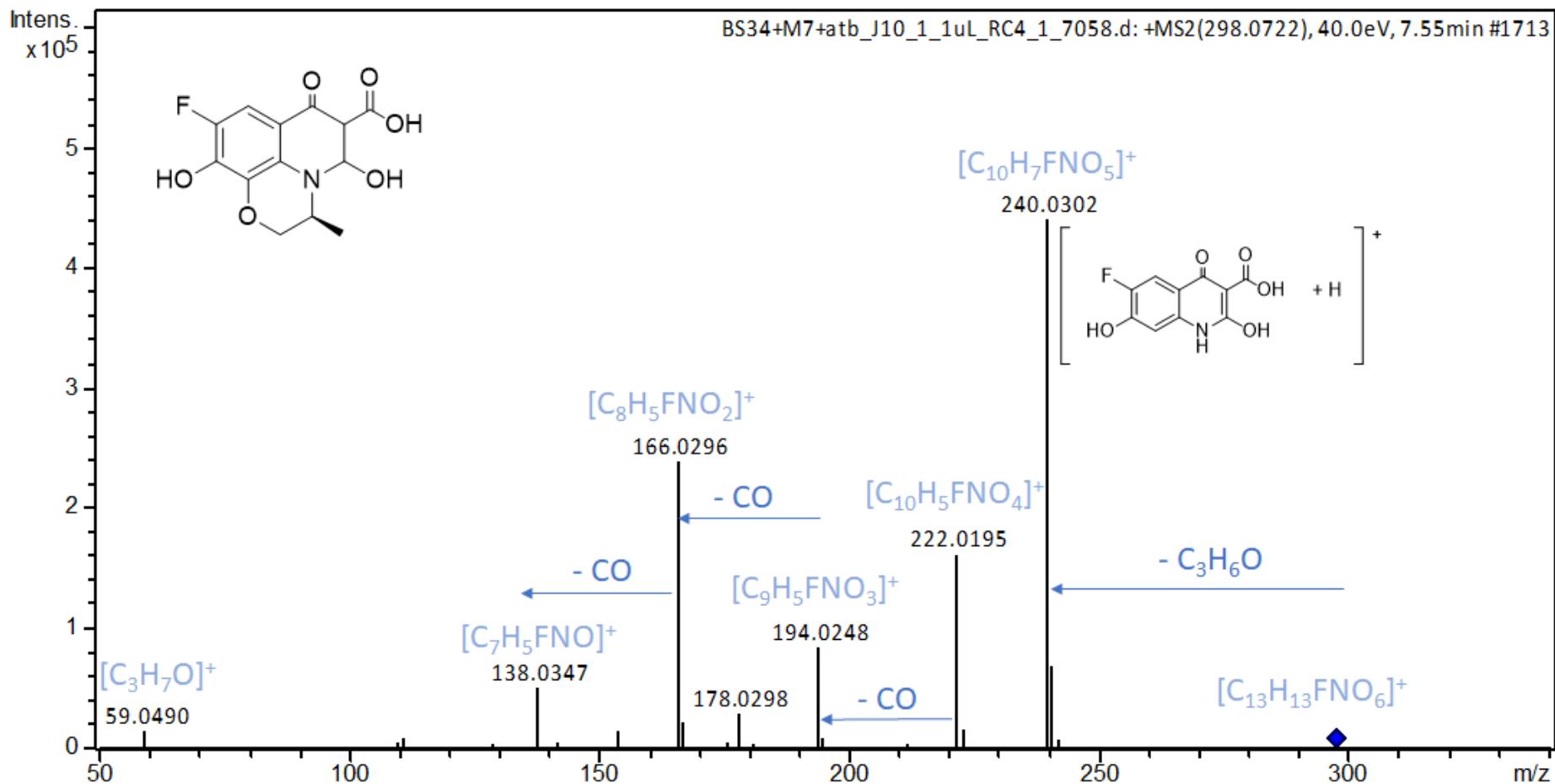


Figure S5. The fragmentation mass spectrum of levofloxacin degradation product #2 (collision energy 40 eV), a minor product detected at days 7 and 10 in the UV chromatogram at 325 nm (See Table 4 of the main manuscript).

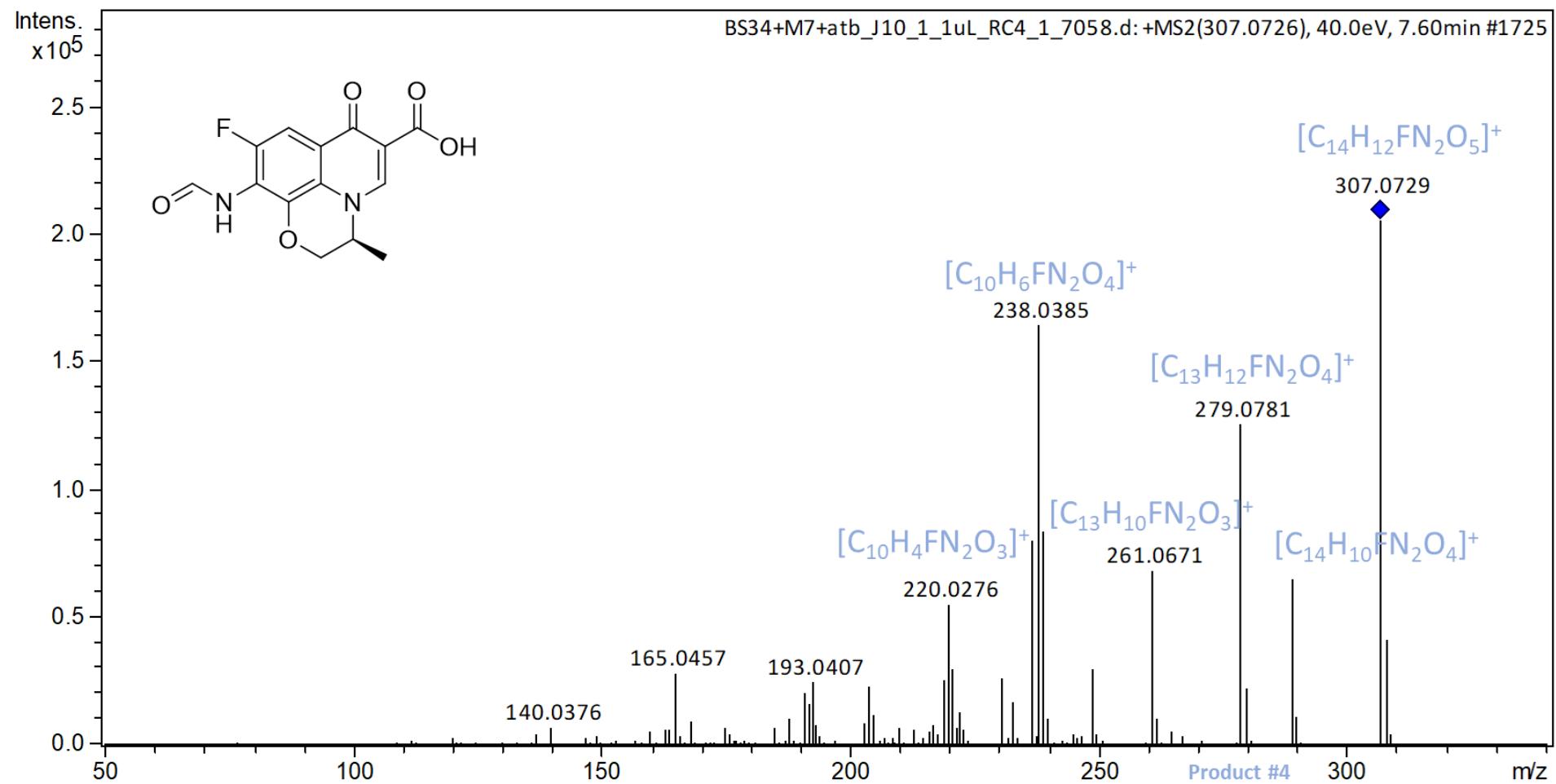


Figure S6. The fragmentation mass spectrum of levofloxacin degradation product #3 (collision energy 40 eV), detected at days 7 and 10 in the UV chromatogram at 325 nm (See Table 4 of main manuscript).

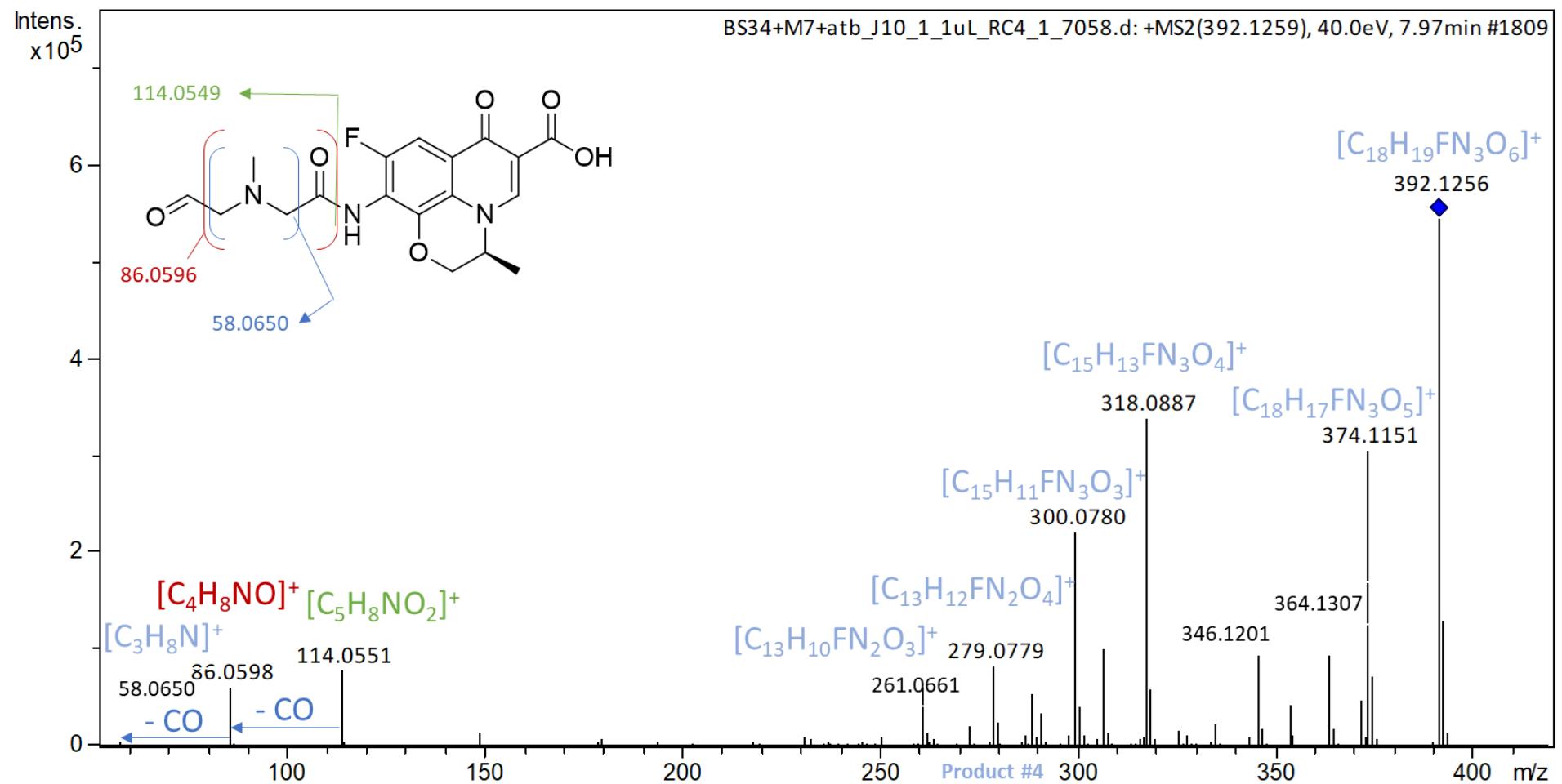


Figure S7. The fragmentation mass spectrum of levofloxacin degradation product #4 (collision energy 40 eV), a major product detected at days 7 and 10 in the UV chromatogram at 325 nm (See Table 4 of main manuscript).

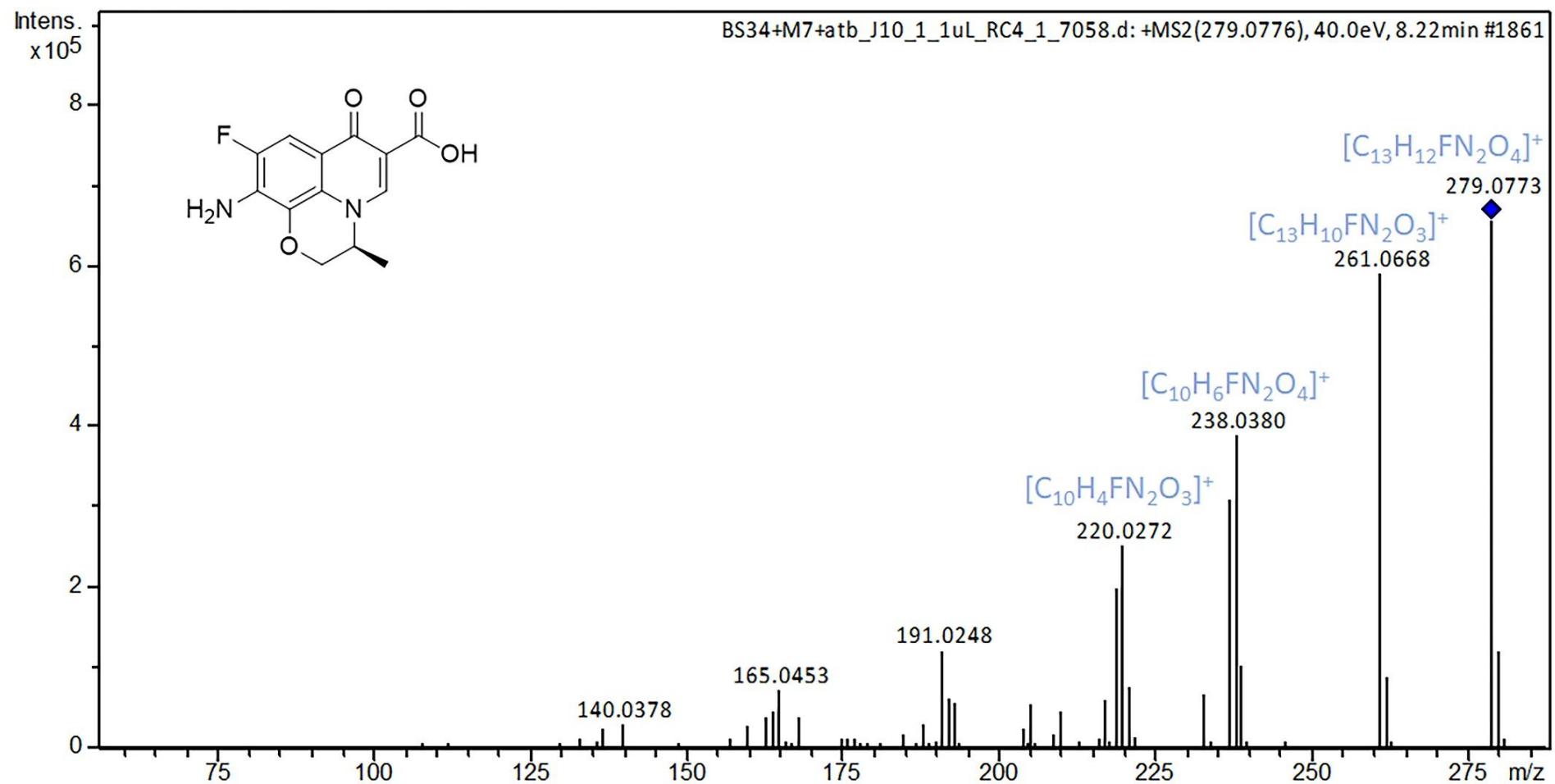


Figure S8. The fragmentation mass spectrum of levofloxacin degradation product #5 (collision energy 40 eV), detected at trace levels at days 7 and 10 in the UV chromatogram at 325 nm (See Table 4 of main manuscript).

