Supplementary Materials

Study of Oxidation of Ciprofloxacin and Pefloxacin by ACVA: Identification of Degradation Products by Mass Spectrometry and Bioautographic Evaluation of Antibacterial Activity

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2. Materials and Methods

2.8 UHPLC/MS/MS Analysis

The UHPLC-MS/MS system consisted of a Waters ACQUITY UPLC (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C_{18} column; 2.1 × 100 mm, and 1.7 μm particle size, equipped with Acquity UPLC BEH C18 VanGuard pre-column; 2.1 × 5 mm, and 1.7 μm particle size. The column was maintained at 40 °C and eluted under gradient conditions using from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL·min^{-1}. Eluent A: 0.1% water solution of formic acid; eluent B: 0.1% solution of formic acid in acetonitrile. Chromatograms were recorded while using Waters eλ PDA detector. The spectra were analysed in 200–700 nm range with 1.2 nm resolution and sampling rate 20 points/s.

MS detection settings of Waters TQD mass spectrometer were as follows: source temperature 150 °C, desolvation temperature 350 °C, desolvation gas flow rate 600 L·h^{-1}, cone gas flow 100 L·h^{-1}, capillary potential 3.00 kV, and cone potential 30 V. Nitrogen was used for both nebulizing and drying gas. The data were obtained in a scan mode ranging from 50 to 1000 *m*/*z* in time 0.5 s intervals; eight scans were summed up to get the final spectrum. In MS/MS experiments collision energy was set to 50 eV and argon was used as a collision gas. Data acquisition software was MassLynx V 4.1 (Waters (Milford, MA, USA)).

3. Results

3.1. Optimization of Chromatographic Conditions and Method Validation

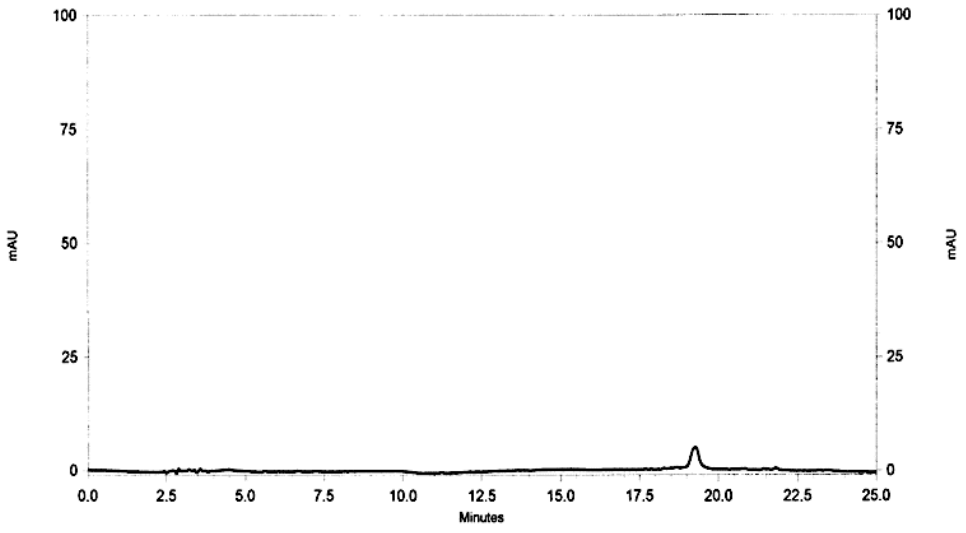

Figure S1. Chromatogram of the 4,4′-azobis(4-cyanopentanoic acid) (ACVA) control sample after incubation for 60 h at 60°C recorded at 277 nm.

3.4. Identification of Oxidation Products

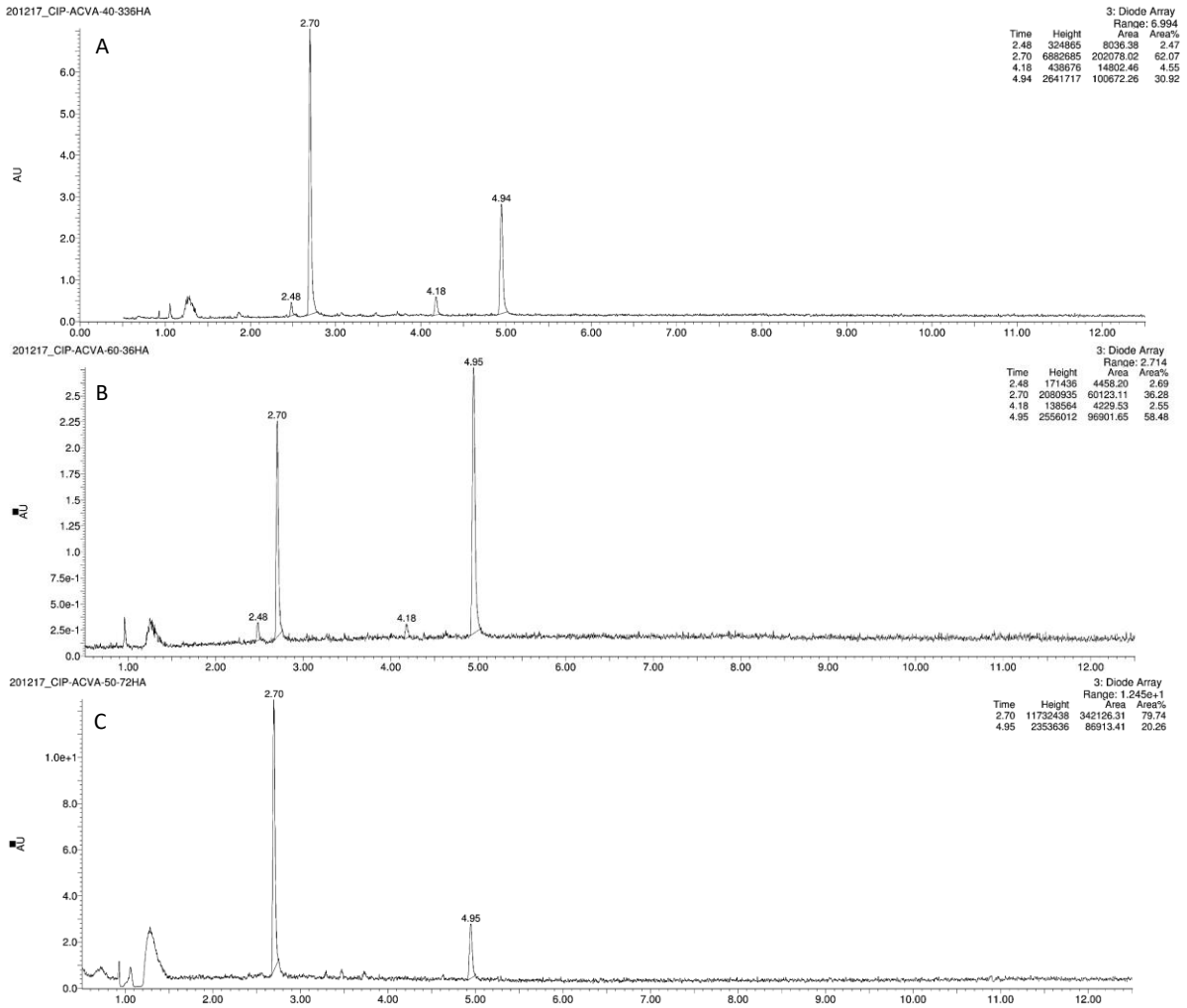

Figure S2. Chromatograms of methanolic solution of ciprofloxacin after oxidation by ACVA: (A)—40 °C and 336 h of incubation, (B)—50 °C and 72 h of incubation, (C)— 60 °C and 36 h of incubation.

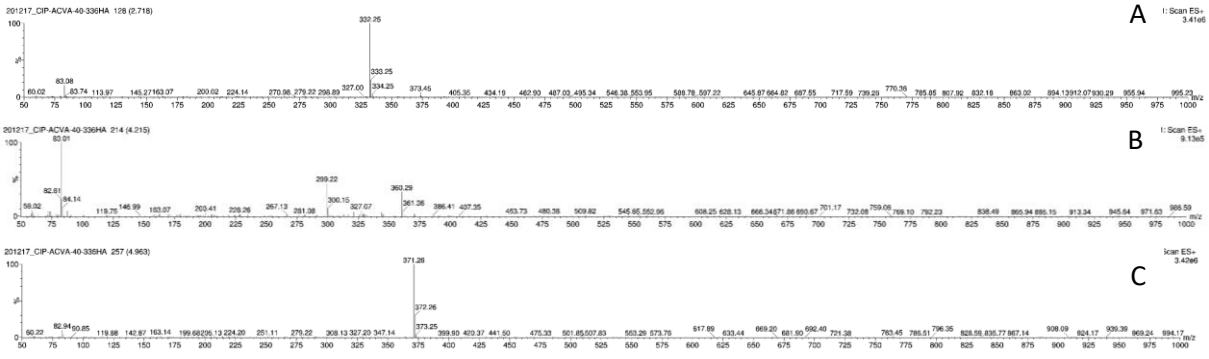

Figure S3. MS spectra of the ciprofloxacin (A) and their degradation (B, C) products after reaction with ACVA.

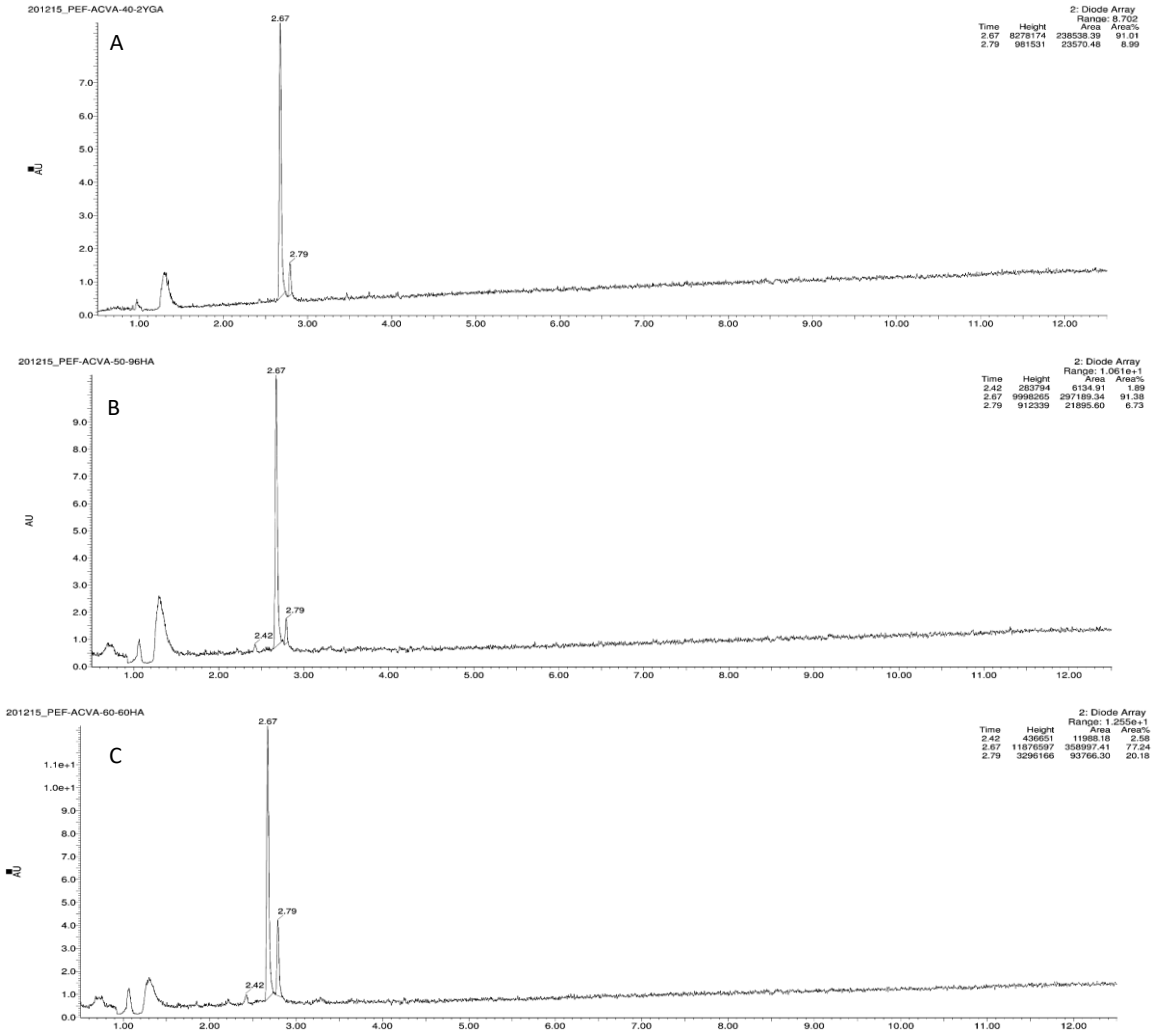

Figure S4. Chromatograms of methanolic solution of pefloxacin after oxidation by ACVA: (A)—40 °C and 336 h of incubation, (B)—50 °C and 96 h of incubation, (C)—60 °C and 60 h of incubation.

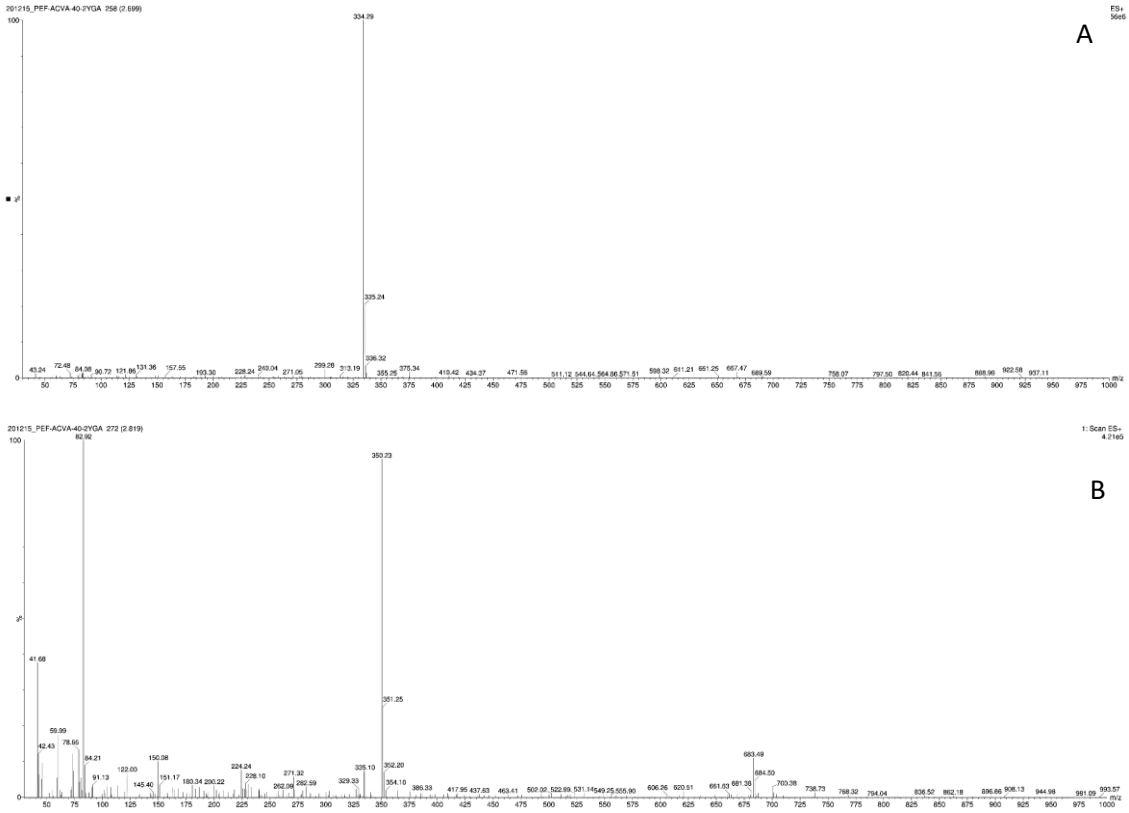

Figure S5. MS spectra of the pefloxacin (A) and their degradation product (B) after reaction with ACVA.