

Supplementary Information

Effect of Environmental Factors on the Oxidative Transformation of Cephalosporin Antibiotics by Manganese Dioxides

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Text S1. Chemicals and standards

All cephalosporin compounds were purchased from Sigma-Aldrich and Fluka and had a purity over 98%. Individual stock solutions in concentration of 100-1000 mg/L were prepared in deionized water and stored at 4°C in amber glass bottles for a maximum of 14 days. The compounds and instruments are listed in Table S1 and Table S2.

Table S1

Compounds	Suppliers	purities
Cefotaxime sodium	Fluka	≥98%
Cephapirin sodium	Fluka	≥98%
Cephradine	Sigma-Aldrich	≥99%
Cephalexin hydrate	Sigma-Aldrich	100%
Cephazolin sodium	Fluka	≥98%
Potassium permanganate	J.T.Baker	99%
Manganese(II) sulfate monohydrate	J.T.Baker	99.9%
Oxalic acid	Sigma-Aldrich	≥99%
Sodium chloride	Nacalai tesque	≥99.5%
Sodium acetate	Nacalai tesque	≥98%
Acetic acid	J.T.Baker	100%
Formic Acid	Riedel-deHaen	≥98%
Ammonium Acetate	Sigma	ACS grade
Methanol	Mallinckrodt Baker	LC grade
Potassium hydrogen phthalate	J.T.Baker	99.95%
2-Amino- α -(methoxyimino)-4-	Sigma-Aldrich	97%

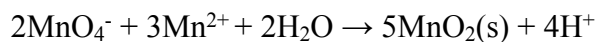
Compounds	Suppliers	purities
thiazoleacetic acid		
4-mercaptopyridine	Sigma-Aldrich	95%
2-(methylthio)pyridine	Fluka	≥ 95 %
(4-pyridylthio)acetic acid	Sigma-Aldrich ^{CPR}	95 %
3-(N-morpholino)propanesulfonic acid (MOPS)	Sigma-Aldrich	≥ 99.5 %
<i>N</i> -Cyclohexyl-2-aminoethanesulfonic acid (CHES)	Sigma-Aldrich	≥ 99 %

Table S2

Instrument	Model type
High performance liquid chromatography, HPLC	Agilent Technologies, 1200 module
Tandem mass spectrometry	Applied Biosystems, API 4000 -Electrospray ionization, ESI -Triple quadrupole mass
Atomic absorption spectrometer	AAS, Perkin, AA800
TOC analyzer	Systematic instrument, Model 1010
Microtox acute toxicity test	Model 500 Analyzer

Text S2. δ -MnO₂ synthesis

The method of δ -MnO₂ synthesis was proposed by Murray ¹. The stoichiometry of δ -MnO₂ preparation was shown below:



First of all, 0.1M KMnO₄, 0.1M NaOH, and 0.1 M MnCl₂ was prepared with N₂ - sparged reagent water which is sparged for 2 hours. Then, 80 ml of 0.1M KMnO₄ and 160 ml 0.1M of NaOH were added into 1.64 L DI-water, and sparge with N₂ gas for another half hour. Subsequently, 120 ml of 0.1M MnCl₂ was added drop-wise to the solution throughout 3 hours and the solution was kept constantly stirred. After the MnO₂ particles settled, we removed the supernatant and replaced with DI-water several times until the aqueous supernatant reached the conductivity less than 2 $\mu\text{S}\cdot\text{cm}^{-1}$. Finally, the volume of MnO₂ suspension was adjusted to about 1L and stored at 4°C. The concentration of MnO₂ was then determined by atomic absorption spectrometer.

The SEM micrographs, particle size distribution and X-ray diffraction (XRD) patterns of δ -MnO₂ were provided in the supporting information of our previous work ².

Reference

1. Murray, J. W., The surface chemistry of hydrous manganese dioxide. *J Colloid Interf Sci* **1974**, *46*, (3), 357-371.
2. Hsu, M.-H.; Kuo, T.-H.; Chen, Y.-E.; Huang, C.-H.; Hsu, C.-C.; Lin, A. Y.-C., Substructure Reactivity Affecting the Manganese Dioxide Oxidation of Cephalosporins. *Environmental Science & Technology* **2018**, *52*, (16), 9188-9195.

Table S3 The HPLC gradient for the five cephalosporins and byproduct analysis.

Time (min)	Flow rate ($\mu\text{L}/\text{min}$)	HPLC gradient of mobile phase	
		A (%)	B (%)
0	1000	100.0	0.0
0.5	1000	100.0	0.0
3.5	1000	60.0	40.0
4.0	1000	5.0	95.0
5.5	1000	5.0	95.0
6.0	1000	100.0	0.0
7.0	1000	100.0	0.0

Table S4 Mass spectrometry parameters of the compounds in this study.

Compounds	Retention time (min)	Precursor ion (m/z)	DP (Volt)	Quantitation			Confirmation		
				Product ion (m/z)	CE (Volt)	CXP (Volt)	Product ion (m/z)	CE (Volt)	CXP (Volt)
Cefotaxime (CTX)	6.18	[M+H] ⁺ =456	40	324	18.3	9.8	396	16.9	11
Cephapirin (CFP)	5.55	[M+H] ⁺ =424	36	152	30	12.3	292	21.5	7.2
Cephradine (CFD)	6.23	[M+H] ⁺ =350	32	158	13	13	176	28.9	7.5
Cephalexin (CFX)	6.16	[M+H] ⁺ =348	27	158	12.8	8.1	174	18.9	12.9
Cefazolin (CFZ)	5.46	[M+H] ⁺ =455.2	21	156	23	8	323	15	8

Text S3 Total organic carbon (TOC) and toxicity measurements.

The total organic carbon (TOC) content was measured with an OI Analytical model 1030 TOC analyzer (OI Analytical, College Station, TX, USA). All samples were sparged with nitrogen gas to remove inorganic carbon and used phosphoric acid to acidify. Potassium hydrogen phthalate was used as a calibration standard.

Acute toxicity was tested using the Microtox system and was performed with *Vibrio fischeri* using a Model 500 Analyzer (Microbics Corp., Carlsbad, CA, USA). The Microtox system measures decrease in the light released by luminescent marine bacteria and calculates the effects of different toxicant concentrations, which can influence the enzymatic activity of *Vibrio fischeri* and reduce bioluminescence (Parvez et al. 2006). Samples were adjusted to pH 7 before the test, and the acute toxicity was measured by mixing the samples with bacteria for 5 or 15 minutes. Then, the EC₅₀ value was calculated as the percentage (% V/V) of the sample that caused a 50% reduction in bioluminescence. The toxicity of the sample was directly indicated using toxicity units (TU, $TU=100/EC_{50}$).

Table S5 Effect of MnO₂ loading, initial CFP concentration and solute pH on k_{init}. The experimental data were fitted to pseudo-first-order kinetic for the initial 40 minutes (initial reaction rate constant k_{init}, half-life t_{1/2}) for CFP.

	k _{init} (h ⁻¹)	t _{1/2} (h)	R ²
MnO₂ loading ([CFP]₀ = 100 µg/L, pH = 5)			
1 mg/L	-	-	-
2 mg/L	0.02	33.2	0.955
4 mg/L	0.05	13.9	0.967
CFP initial concentration ([MnO₂]₀ = 4 mg/L, pH = 5)			
50 µg/L	0.059	11.7	0.99
100 µg/L	0.05	13.9	0.992
500 µg/L	0.019	36.5	0.948
Dissolved NOM ([MnO₂]₀ = 4 mg/L, [CFP]₀ = 100 µg/L, pH = 5)			
0.1 mg-C/L	0.026	26.9	0.756
1 mg-C/L	0.014	48.8	0.897
Solute pH ([MnO₂]₀ = 4 mg/L, [CFP]₀ = 100 µg/L)			
pH 4	1.15	0.62	0.990
pH 5	0.045	15.33	0.984
pH 7	-	-	-
pH 9	-	-	-
Dissolved cations ([MnO₂]₀ = 4 mg/L, [CFP]₀ = 100 µg/L, pH = 5)			
Mn ²⁺ (1 µM)	0.029	23.9	0.902
Mn ²⁺ (5 µM)	0.006	117.5	0.988
Ca ²⁺ (200 µM)	0.025	27.7	0.992
Ca ²⁺ (2000 µM)	0.014	48.1	0.934
Mg ²⁺ (200 µM)	0.044	15.6	0.987
Mg ²⁺ (2000 µM)	0.021	33.0	0.971
Fe ³⁺ (1 µM)	0.061	11.4	0.953

Fe ³⁺ (10 μM)	0.026	26.7	0.949
without cations	0.050	13.9	0.967

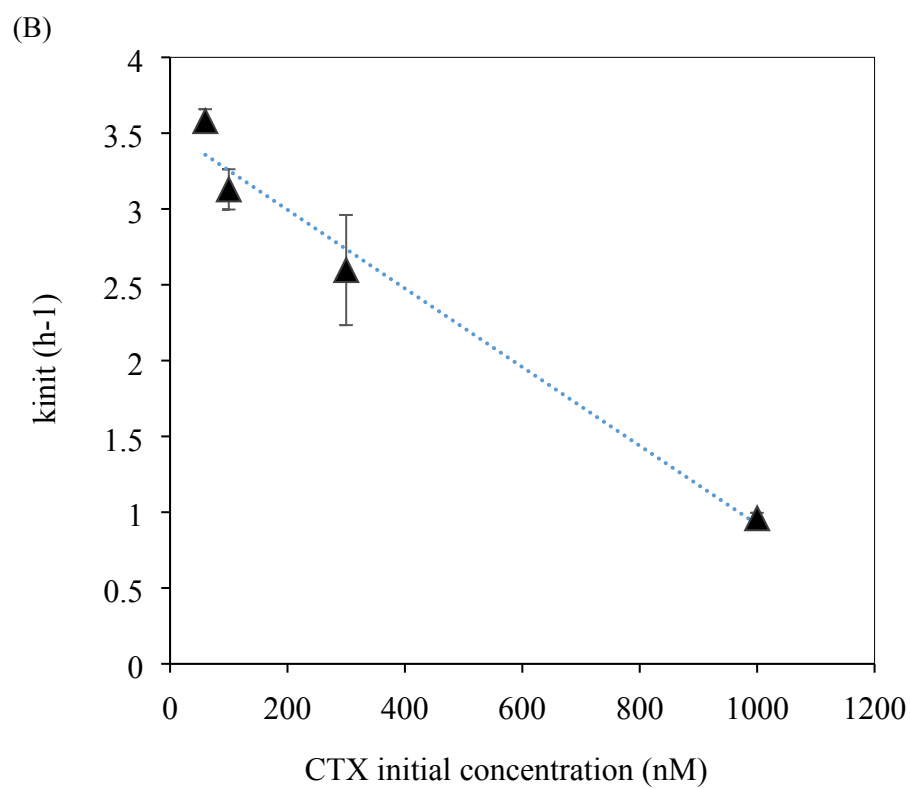
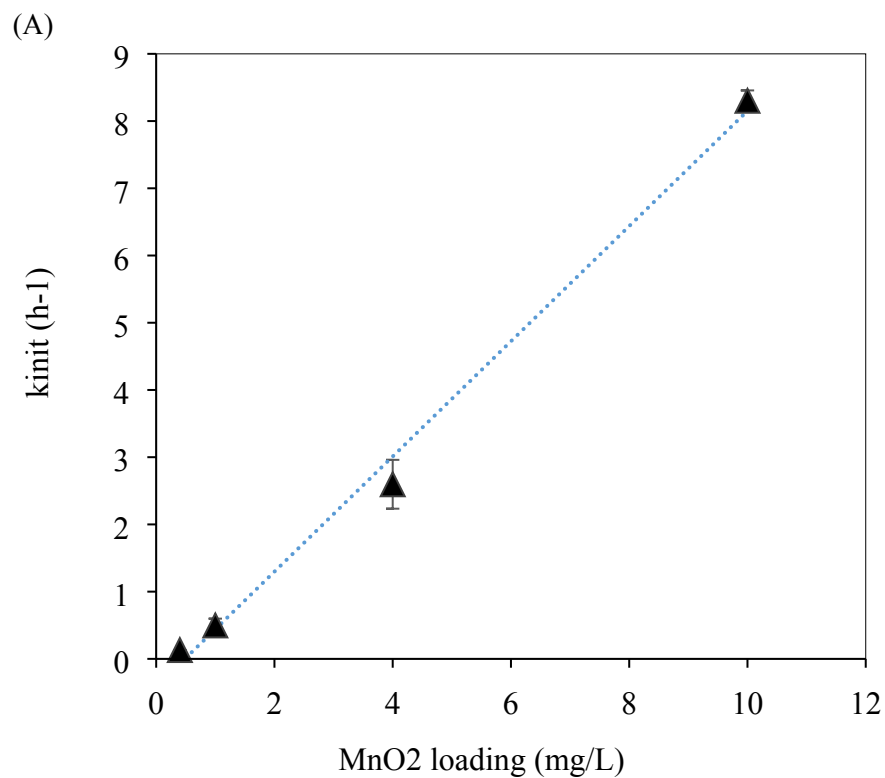


Fig. S1 Effect of (A) MnO₂ loading ($[CTX]_0 = 300$ nM) and (B) CTX initial concentration on initial rate constant k_{init} at pH 4.

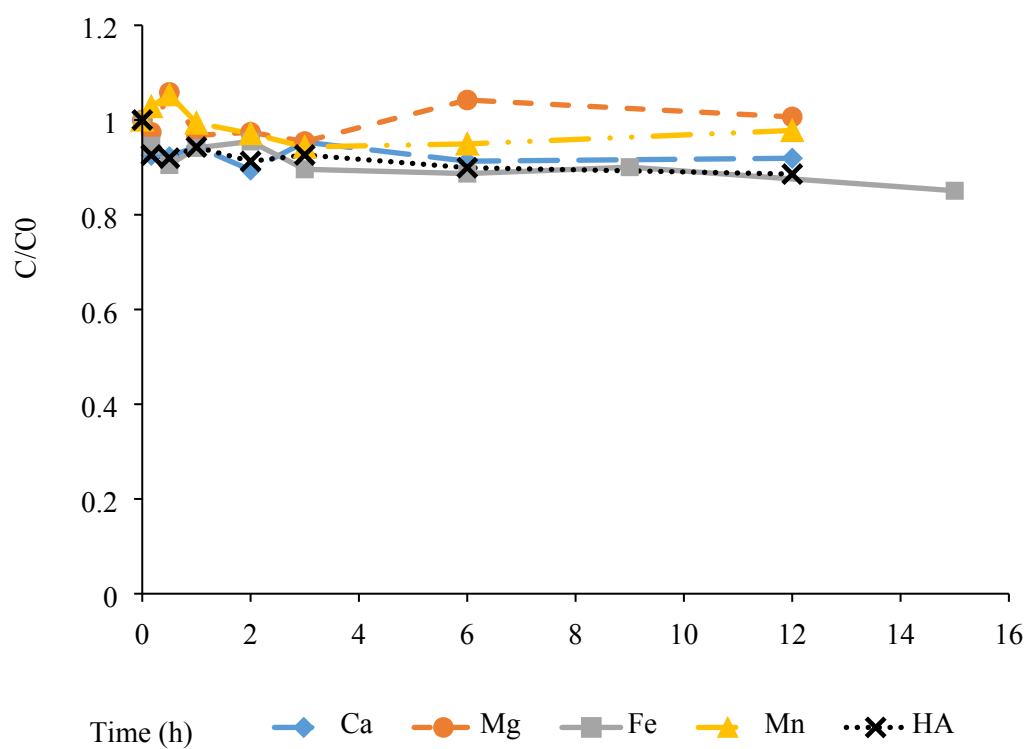
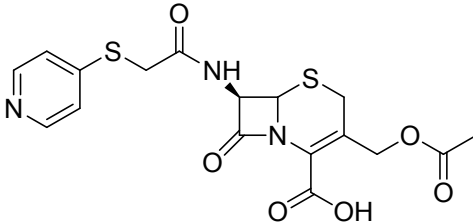
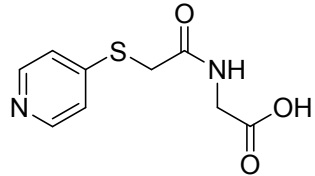
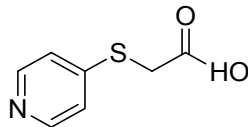
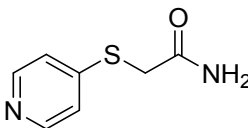
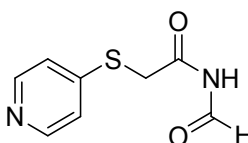
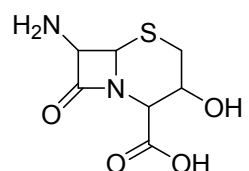
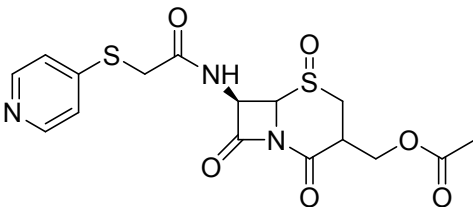
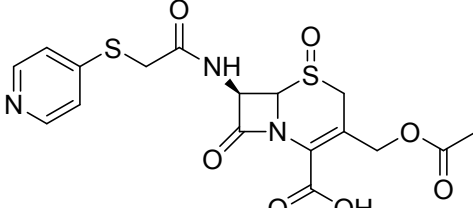
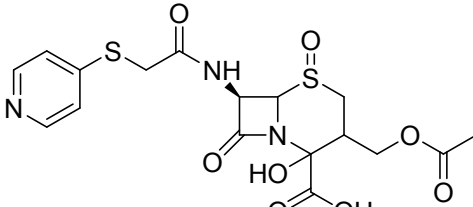
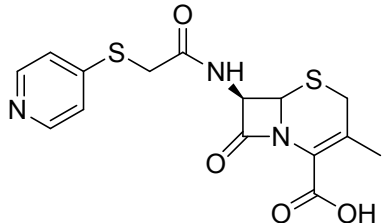
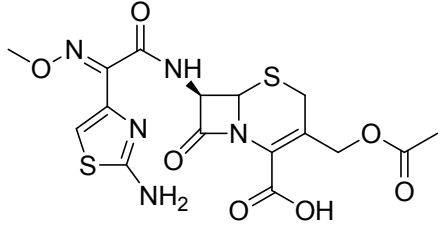
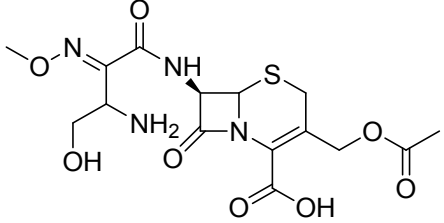
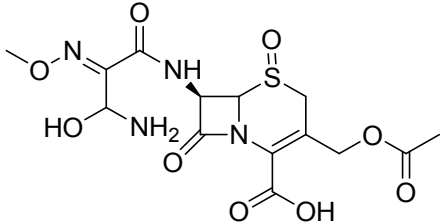
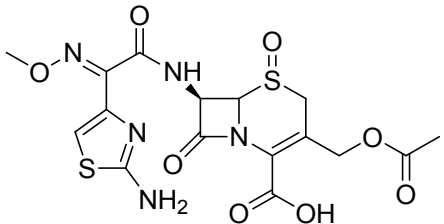


Fig. S2 Effect of coexisting metal ions and natural organic matter on the hydrolysis of CTX at pH 4. ($[CTX]_0 = 3 \times 10^{-7}$ M, $[NaCl]_0 = 0.01$ M)

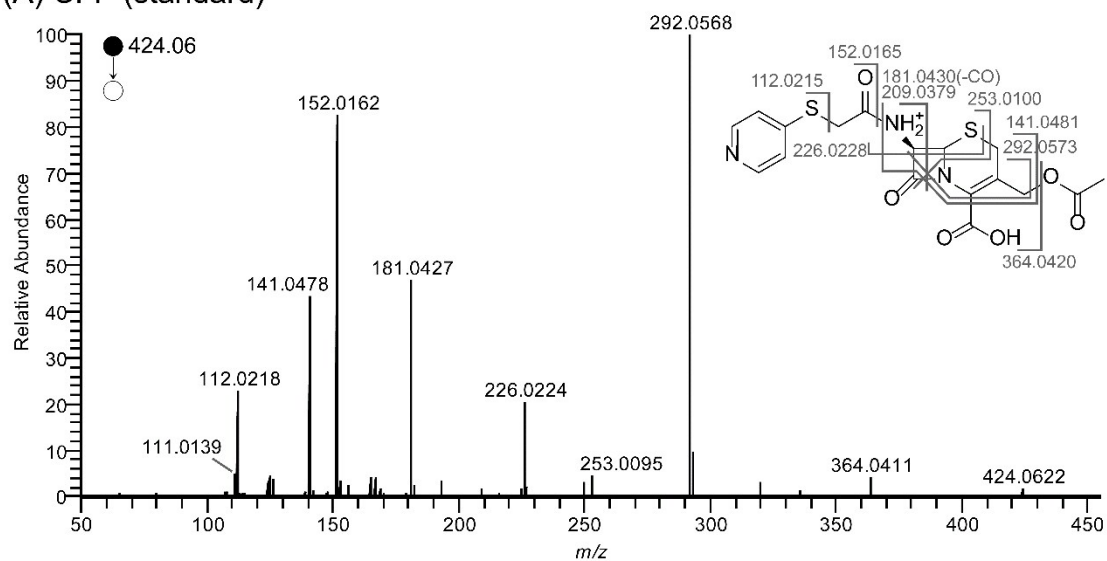
Table S6 The HR-MS/MS proposed oxidation byproducts of CFP and CTX during MnO₂ oxidation.

Name	Structure	Chemical formula of the [M+H] ⁺ ion	Observed [M+H] ⁺ (m/z)	Calculated [M+H] ⁺ (m/z)	Mass error (ppm)	Retention time (min)	HR-MS/MS annotation
CFP		C ₁₇ H ₁₈ O ₆ N ₃ S ₂ ⁺	424.0632	424.0633	0.24	3.57	Fig. S3(A)
CFP-1		C ₉ H ₁₁ O ₃ N ₂ S ⁺	227.0486	227.0485	0.44	1.26	Fig. S3(B)
CFP-2 (4-PA)		C ₇ H ₈ O ₂ NS ⁺	170.0272	170.0270	1.18	1.26	Fig. S3(C) Fig. S4(A)
CFP-3 (4-PA-NH)		C ₇ H ₉ ON ₂ S ⁺	169.0432	169.0430	1.18	1.17	Fig. S4(B)
CFP-4		C ₈ H ₉ O ₂ N ₂ S ⁺	197.0382	197.0379	1.52	1.29	Fig. S4(C)

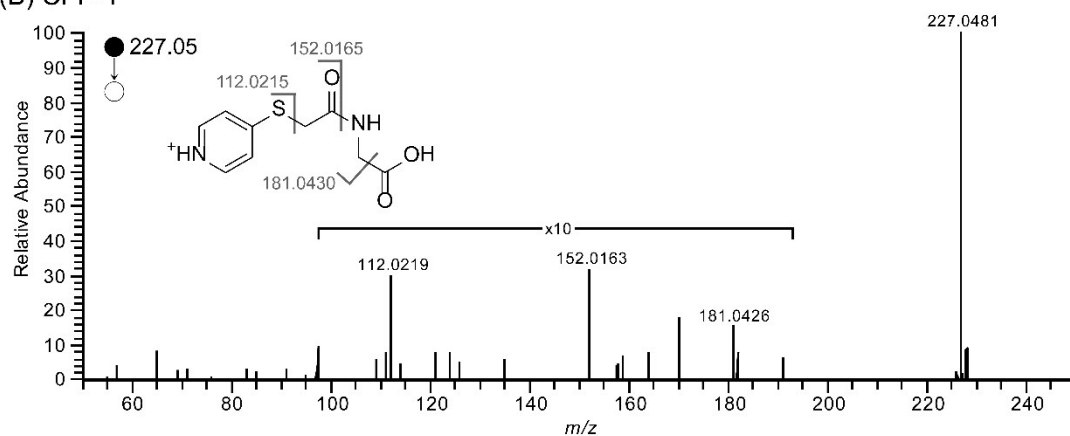
CFP-5		$C_7H_{11}O_4N_2S$	219.0438	219.0434	1.83	3.50	Fig. S5(A)
CFP-6		$C_{16}H_{17}O_6N_3S_2^+$	412.0635	412.0632	0.73	3.43	Fig. S5(B)
CFP-7		$C_{17}H_{18}O_7N_3S_2^+$	440.0586	440.0581	1.14	1.47	Fig. S5(C)
CFP-8		$C_{17}H_{19}O_8N_3S_2^+$	458.0690	458.0686	0.87	3.20	Fig. S6(A)
CFP-9		$C_{15}H_{16}O_4N_3S_2^+$	366.0578	366.0577	0.27	3.52	Fig. S6(B)

CTX		$C_{16}H_{18}N_5O_7S_2^+$	456.0641	456.0642	-0.22	3.57	Fig. S7(A)
CTX-1		$C_{15}H_{21}O_8N_4S^+$	417.1075	417.1076	-0.24	4.26	Fig. S7(C)
CTX-2		$C_{14}H_{19}O_9N_4S^+$	419.0867	419.0866	0.24	3.77	Fig. S8(A)
CTX-3		$C_{16}H_{18}O_8N_5S_2^+$	472.0594	472.0591	0.64	3.32	Fig. S8(B)

(A) CFP (standard)



(B) CFP-1



(C) CFP-2 (4-PA; standrad)

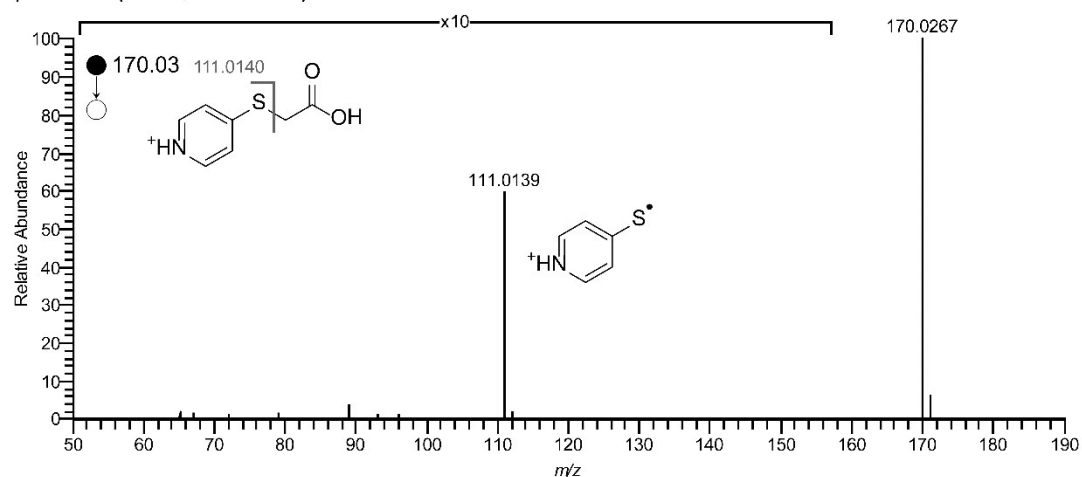
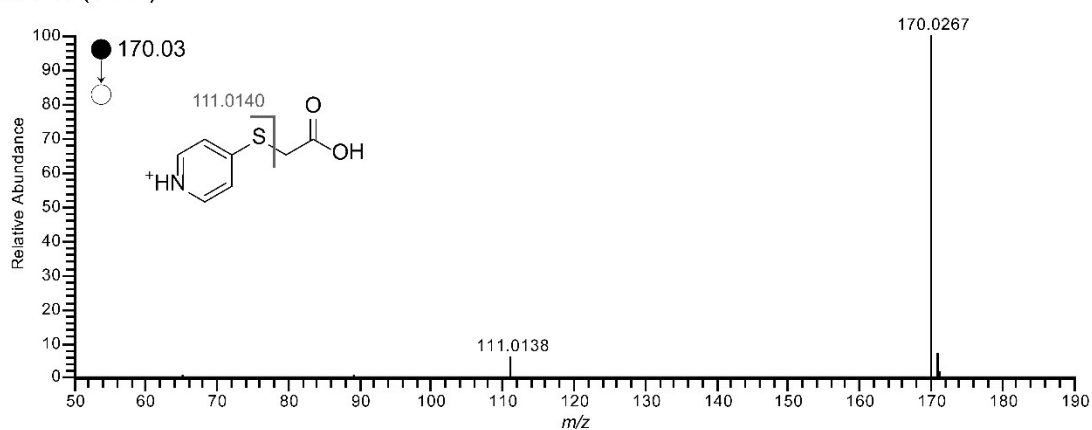
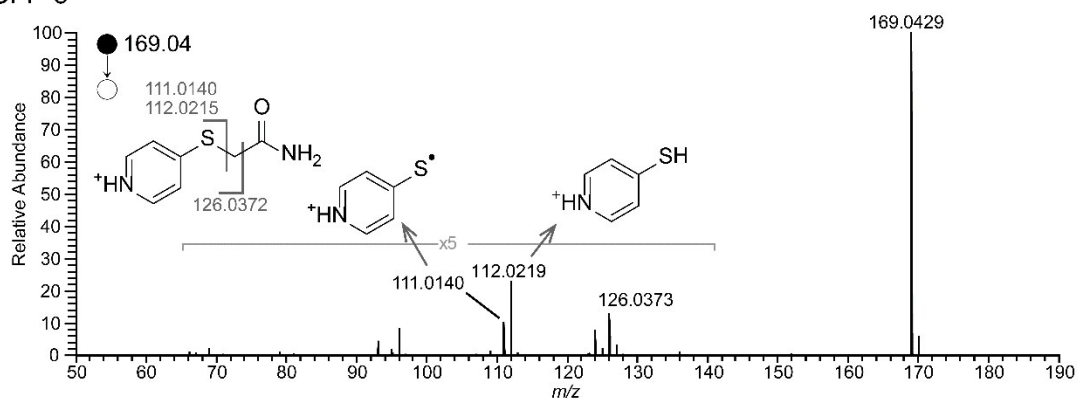


Fig. S3 HR-MS/MS annotations of (A) CFP (standard), and the oxidation byproducts (B) CFP-1 and (C) CFP-2 (4-PA; standard).

CFP-2 (4-PA)



CFP-3



CFP-4

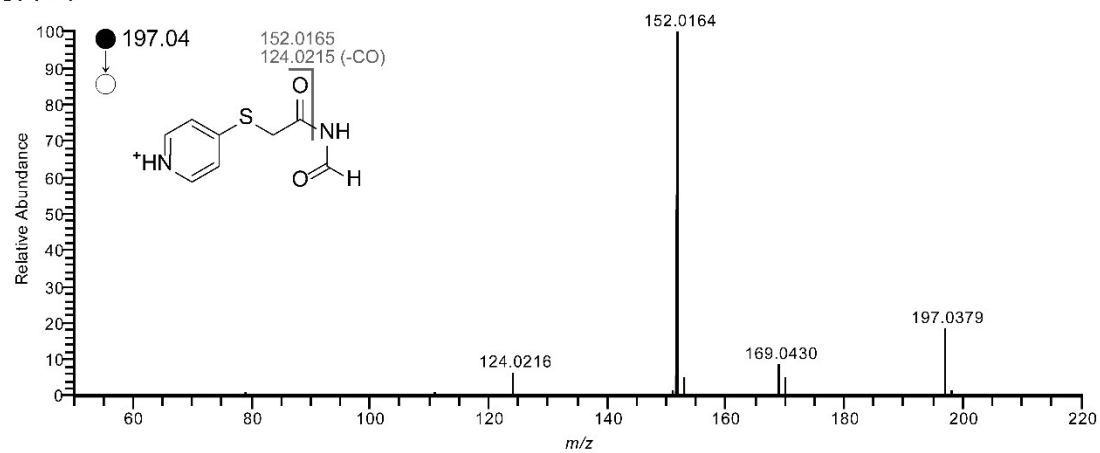
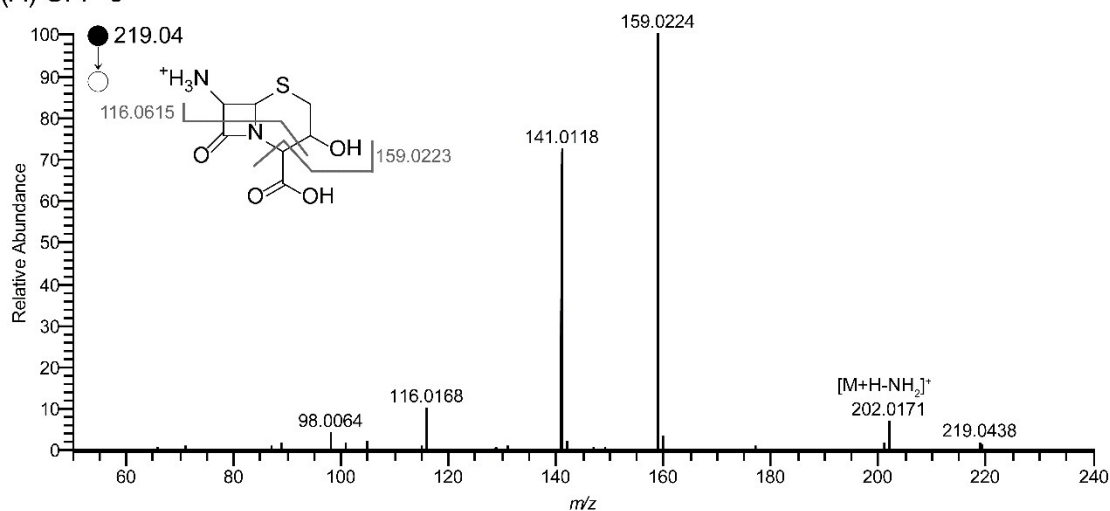
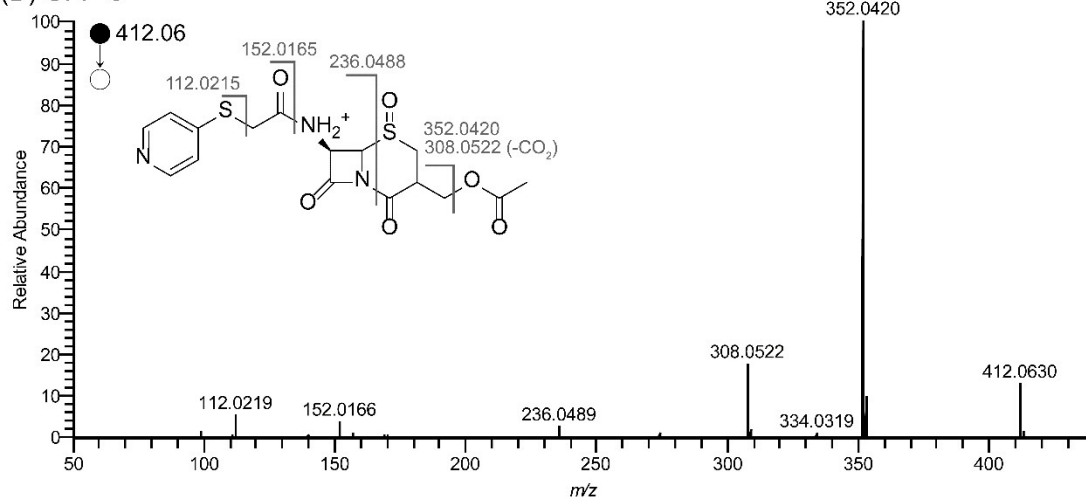


Fig. S4 HR-MS/MS annotations of the oxidation byproducts of CFP: (A) CFP-2, (B) CFP-3 and (C) CFP-4.

(A) CFP-5



(B) CFP-6



(C) CFP-7

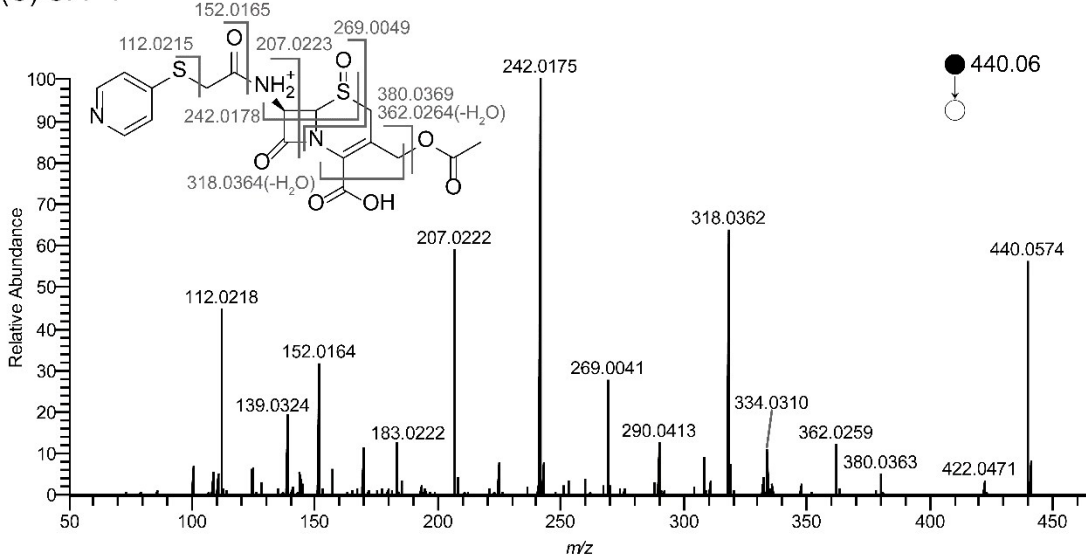
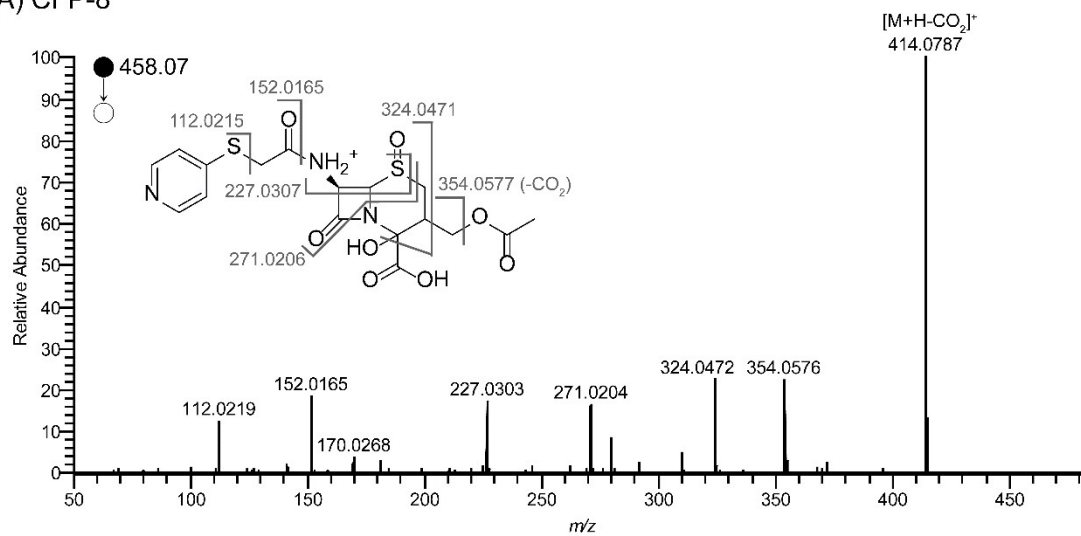


Fig. S5 HR-MS/MS annotations of the oxidation byproducts of CFP: (A) CFP-5, (B) CFP-6 and (C) CFP-7.

(A) CFP-8



(B) CFP-9

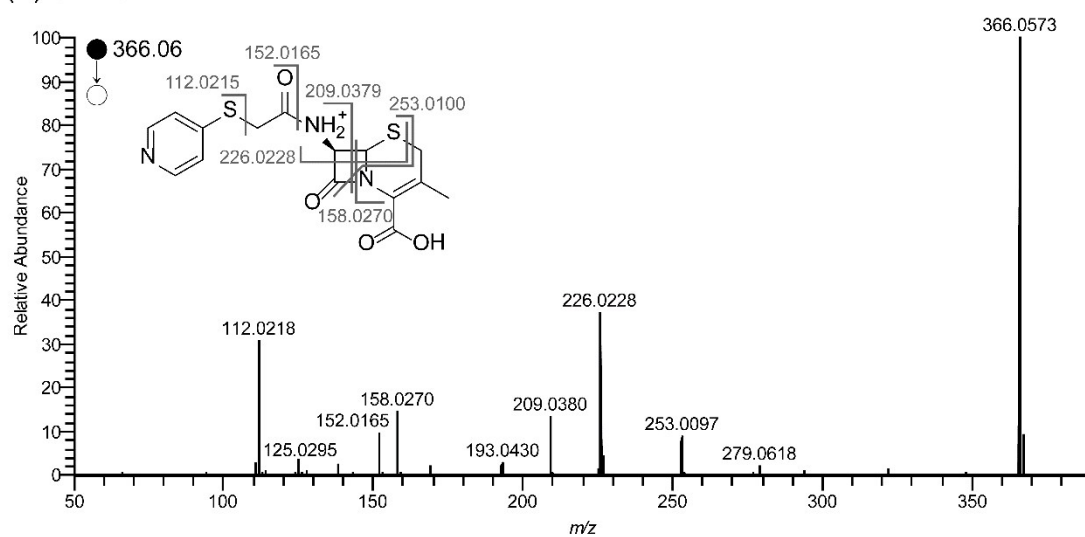
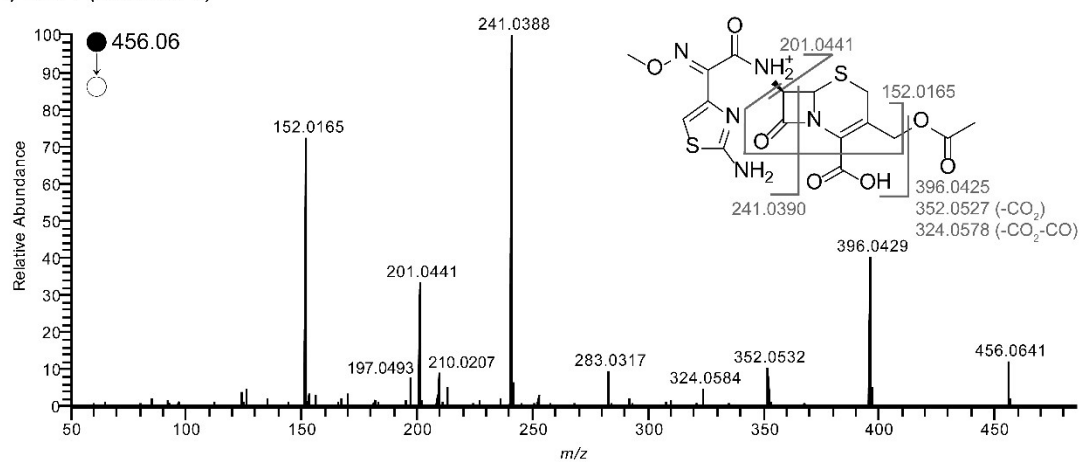
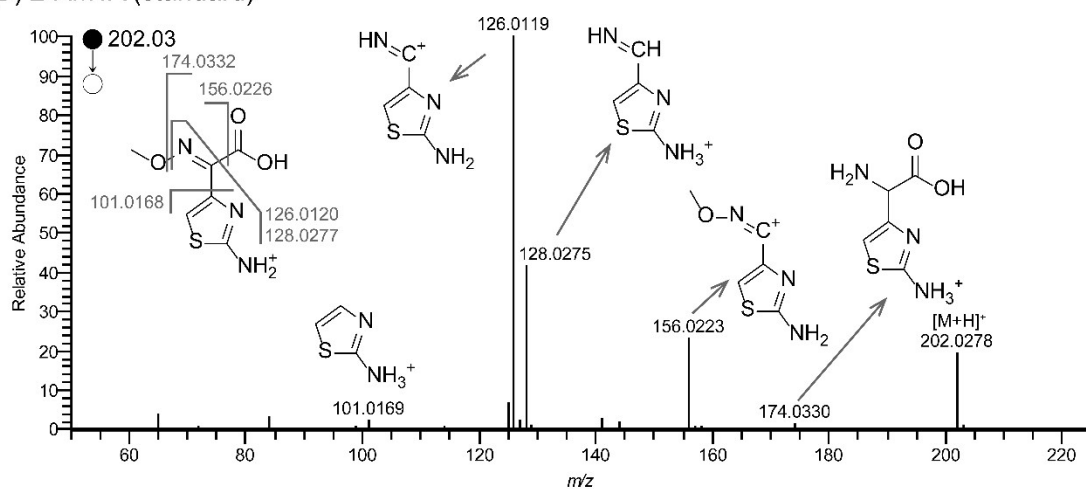


Fig. S6 HR-MS/MS annotations of the oxidation byproducts of CFP: (A) CFP-8 and (B) CFP-9.

(A) CTX (Standard)



(B) 2-AMTA (standard)



(C) CTX-1

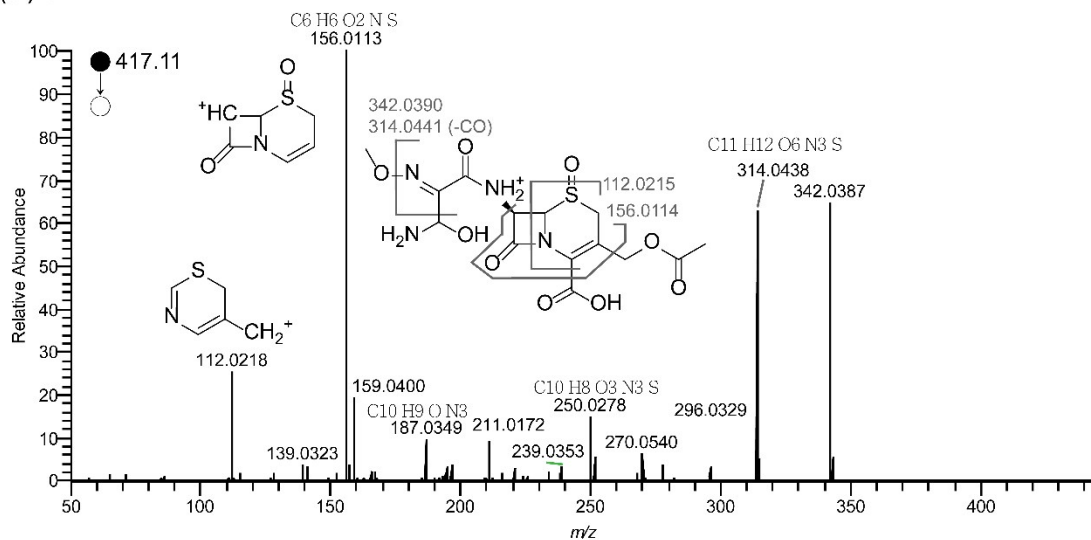
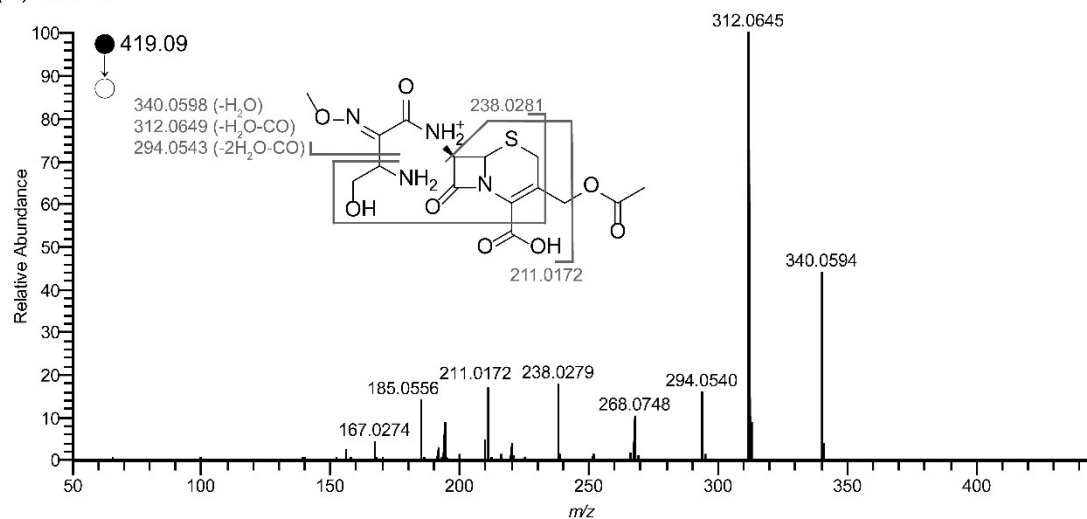


Fig. S7 HR-MS/MS annotations of (A) CTX, (B) 4-AMTA, and (C) the oxidation byproduct of CTX, CTX-1.

(A) CTX-2



(B) CTX-3

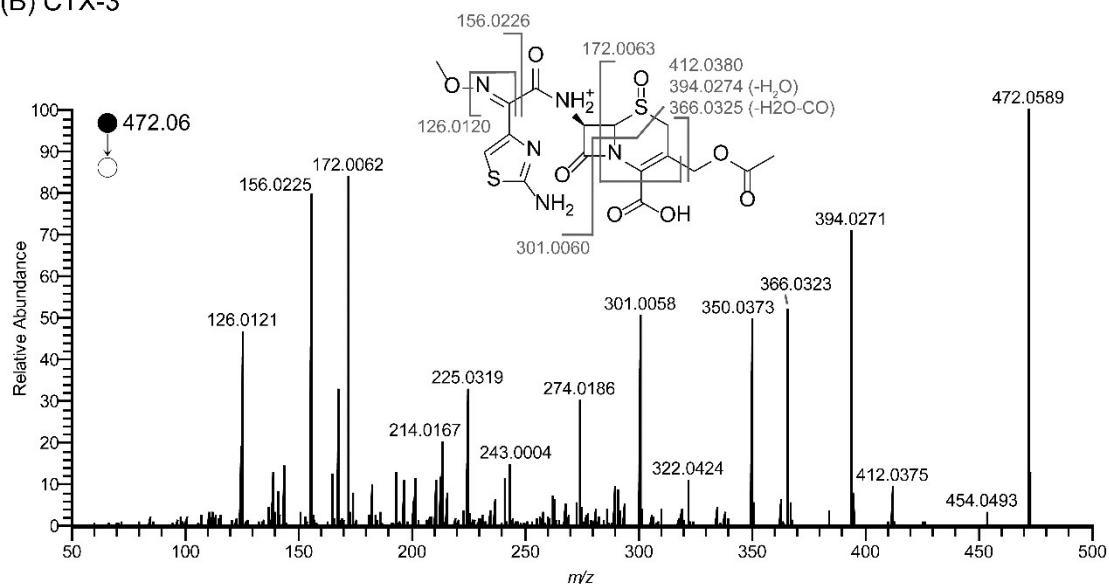


Fig. S8 HR-MS/MS annotations of and the oxidation byproduct of CTX: (A) CTX-2 and (B) CTX-3

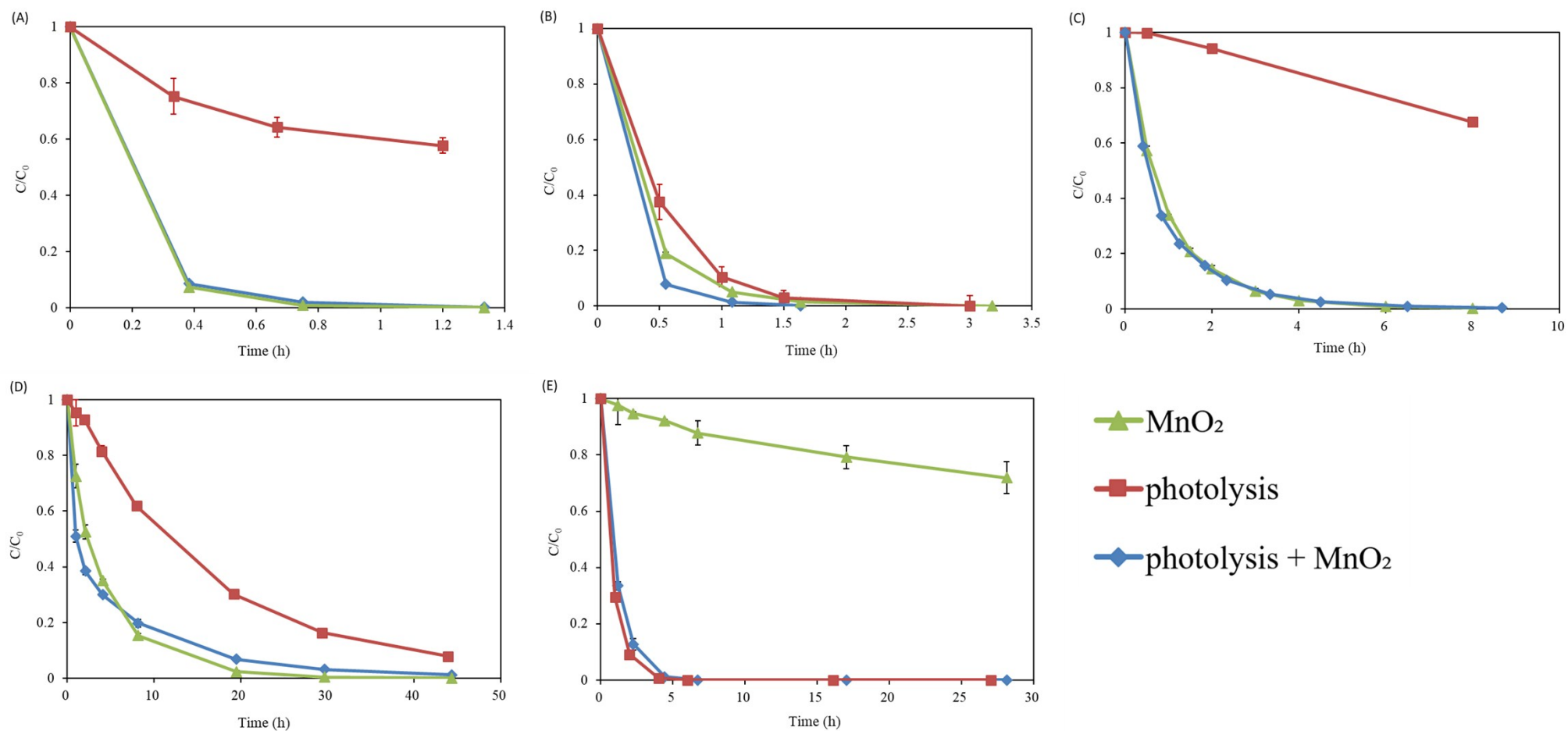


Fig. S9 Degradation rates of (A) CTX, (B) CFP, (C) CFD, (D) CFX and (E) CFZ under oxidation and irradiation at pH 4 ([cephalosporin] = 3×10^{-7} M, $[\text{MnO}_2]_0 = 4$ mg/L, irradiation = 700 W/m^2)