Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Ball milling - a new concept for predicting degradation profiles in active pharmaceutical ingredients

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1. General Information

The ball milling experiments were carried out with a Retsch MM400 (Retsch GmbH, Retsch-Allee 1-5, 42781 Haan, Deutschland) ball mill. ZrO_2-Y (zirconia dioxide stabilised with Yttria) milling jars (10 mL) and one ZrO_2-Y milling ball (10 mm, 3.1 g) were used as milling equipment.

HPLC measurements were conducted with an Agilent Technologies (1100 series) instrument using an Agilent Zorbax Eclipse Plus C8 column (double-endcapped, carbon load 7%, 250 × 4.6 mm, 5 µm particles). The mobile phases A and B were H_2O and MeCN, both containing 0.1% formic acid (V/V), respectively. The gradient elution was performed as follows: 0 - 10 min: 20% B, 10 - 40 min: 20→80% B, 40 - 45 min: 80% B, 45 - 48 min: 80→20% B, 48 - 50 min: 20% B, at a constant flow rate of 1 mL/min. The injection volume was 15 µL, the temperature during separation was maintained at 25 °C. The detection wavelength was λ = 235 nm.

For filtration of reaction mixtures, qualitative filter circles type 2020 DIN 53137 with 5-13 µm pore size were used.

For evaporation of solvents, Heidolph Laborota 4000 (Heidolph Instruments GmbH & Co.KG, Walpersdorfer Str. 12, 91126 Schwabach, Germany) was used.

The powder X-ray diffraction pattern was measured with a Bruker D8 Discover powder diffractometer (DaVinci Design; Bruker AXS GmbH, Karlsruhe, Germany) using copper radiation. The scan was run from 3° to 50° 2θ. A step size of 0.020° was adjusted with a counting time of 2.00 s for each step.

Table S1. Sources and suppliers of chemicals used for this study. All chemicals were used as received.

Chemical	**Supplier**	**Purity, Notes**
Clopidogrel hydrogensulfate (**Clp**)	Pen Tsao Chemical Industry Ltd.	according to Chinese Pharmacopoeia
Water (H_2O)	Sigma-Aldrich Chemie GmbH, Eschenstr. 5, 82024 Taufkirchen, GER	HPLC grade
Acetonitrile (ACN)	Sigma-Aldrich Chemie GmbH	HPLC grade (≥ 99.9%)
Silica gel (SiO_2)	Fisher Scientific GmbH	For LC, 60-200 µm, 60A
$KMnO_4$	Sigma-Aldrich Chemie GmbH	97%
KNO_3	Sigma-Aldrich Chemie GmbH	>99%
Oxone®	Sigma-Aldrich Chemie GmbH	$2\ KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$

Experimental details:

Solid state degradation studies were done by ball milling mixtures of clopidogrel hydrogensulfate (**Clp**) and the oxidant in a mixer mill. For this, 100 mg of **Clp**, 250 mg of SiO_2 and one ZrO_2-Y ball (*d* = 10 mm) were placed in a ZrO_2-Y jar and one equivalent of the respective oxidant was added (25 mg for KNO_3, 38 mg for $KMnO_4$, and 73 mg for Oxone®, respectively). The jar was closed, transferred to the ball mill and the mixture was allowed to react at a frequency of 30 Hz for 1, 5, 10, and 15 minutes, respectively. The jar was opened thoroughly rinsed with approximately 10-15 mL of MeCN, followed by filtration through a paper filter. The filtrate was concentrated to dryness in vacuum to produce oily mixtures. For HPLC analysis, 1 mg/mL **Clp** equivalents were weighed directly and dissolved in mobile phase A/B 4:1 (v/v).

2. XRD analysis of clopidogrel hydrogensulfate

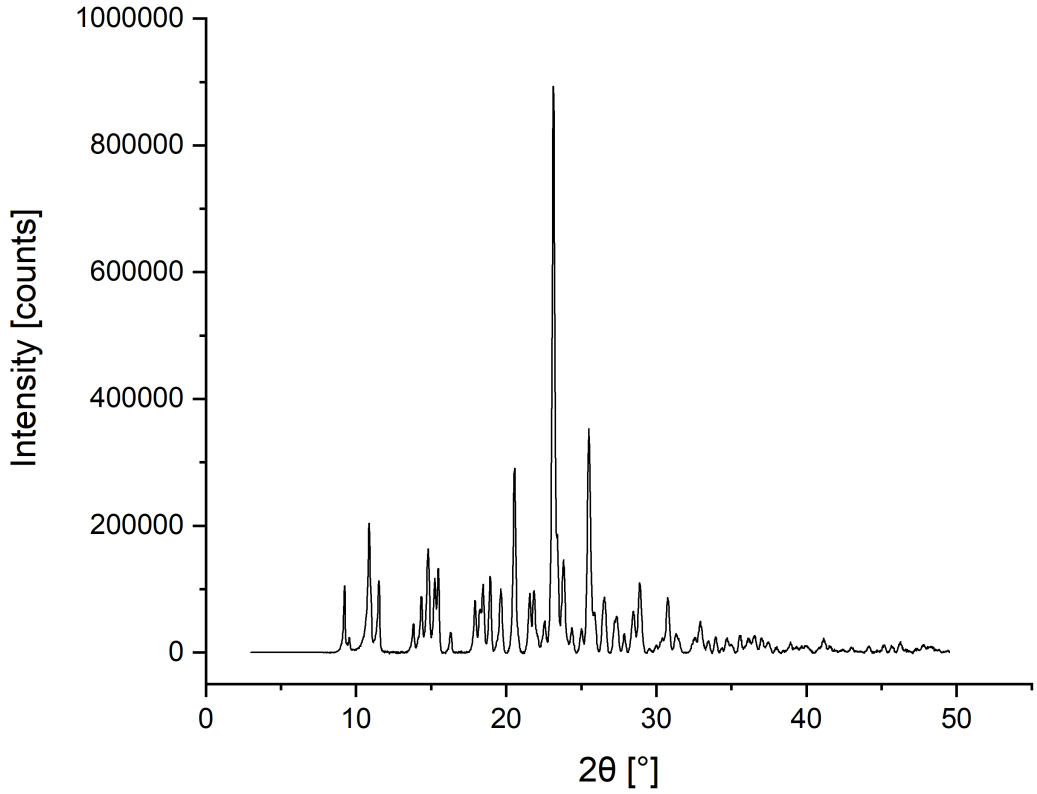


Figure S1. PXRD analysis of **Clp** used for mechanochemical studies, confirming the presence of polymorph I.[1]

3. Optimization of the HPLC method

The optimized method was carried out on an Agilent Zorbax Eclipse Plus C8 column (double-endcapped, carbon load 7%, 250 × 4.6 mm, 5 µm particles). The mobile phases A and B were H_2O and MeCN, both containing 0.1% formic acid (V/V), respectively. The gradient elution was performed as follows: 0 - 10 min: 20% B, 10 - 40 min: 20→80% B, 40 - 45 min: 80% B, 45 - 48 min: 80→20% B, 48 - 50 min: 20% B, at a constant flow rate of 1 mL/min. The injection volume was 15 µL, the temperature during separation was maintained at 25 °C. The detection wavelength was λ = 235 nm.

The optimized HPLC method was validated according to ICH Q2(R1). Therefore, a CLP stock solution of 5 mg/mL was prepared in a solvent mixture of mobile phase A and B (80:20 V/V) and diluted with solvent mixture to achieve the desired CLP concentration. Linearity was evaluated within the working range of 100 - 1000 µg/mL at five approximately equidistant concentrations ($y = 17.227x + 100.78$, $R^2 = 0.9999$). Intra- and interday precision were determined by measuring CLP concentrations of 100, 500 and 1000 µg/mL on two consecutive days. Accuracy was evaluated by spiking a sample with known concentrations of CLP (100, 250 and 500 µg/mL) and determining the recovery. The resulting recoveries ranging from 103.6 - 104.5% (mean value 104.1%, RSD < 0.25%). The determination of the limit of detection (LOD) and the limit of quantification (LOQ) was based on a signal-to-noise ratio (S/N) of 3 and 10, respectively, by measuring a diluted CLP solution of known concentration. The noise was determined from a chromatogram of a blank solution over the distance of 5 times the width at half-height of the CLP peak. For 15 µL injection volume, the LOD was 0.46 µg/mL and the LOQ was 1.36 µg/mL. The stability of a CLP test solution was determined, which was stored for 6 days at 4 °C, protected from light. All validation measurements were performed in triplicates.

To improve the occurring tailing, the acid component of mobile phases A and B was replaced in the optimized HPLC method. Instead of 0.1% formic acid, 0.1% trifluoroacetic acid was added.

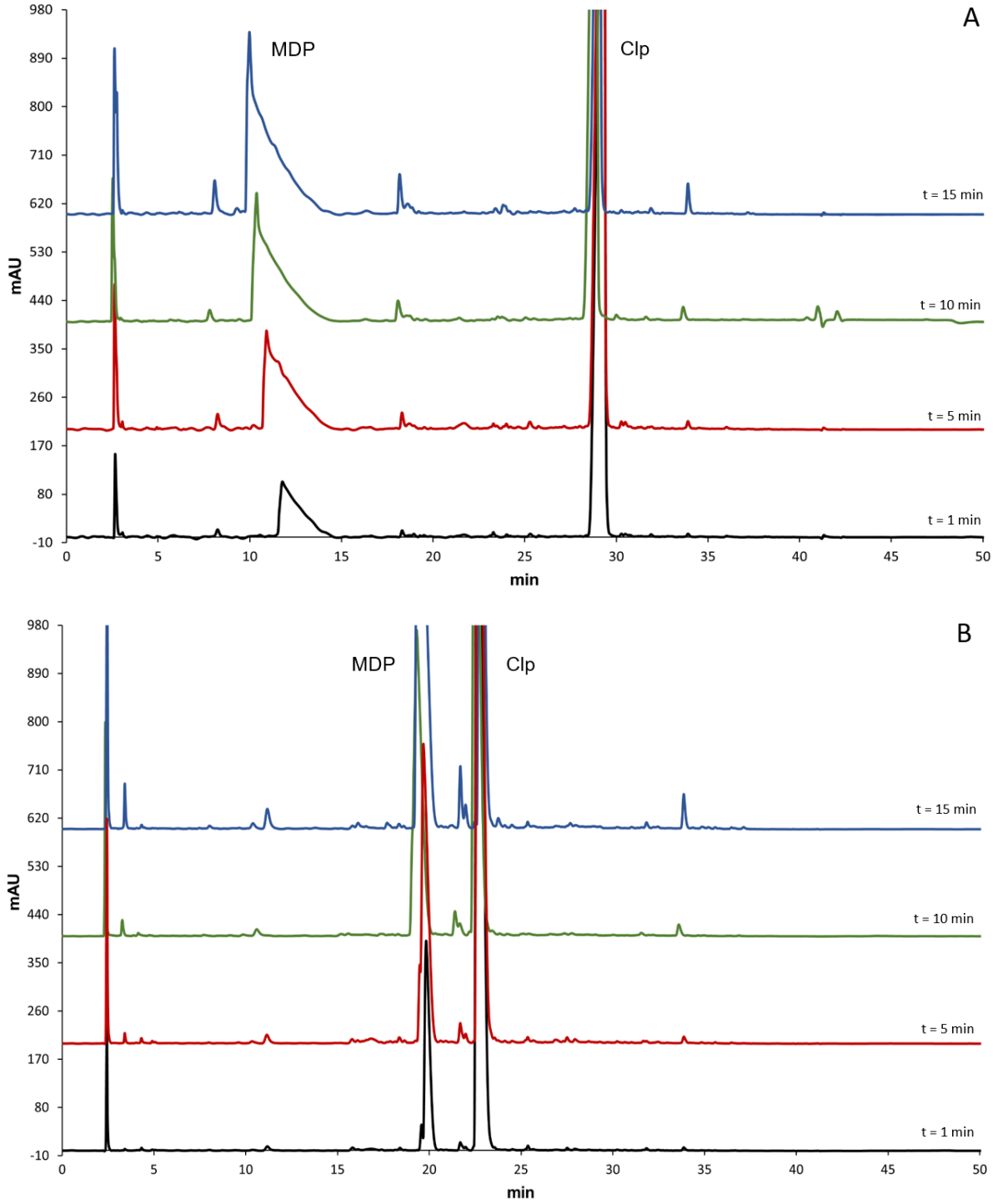


Figure S2. Overlay of the chromatograms of samples stressed with KNO_3 for t = 1 min, t = 5 min, t = 10 min and t = 15 min. (A) Chromatogram obtained with 0.1% formic acid in mobile phases A and B; (B) Chromatogram obtained with 0.1% trifluoroacetic acid in mobile phases A and B. MDP: main degradation product.

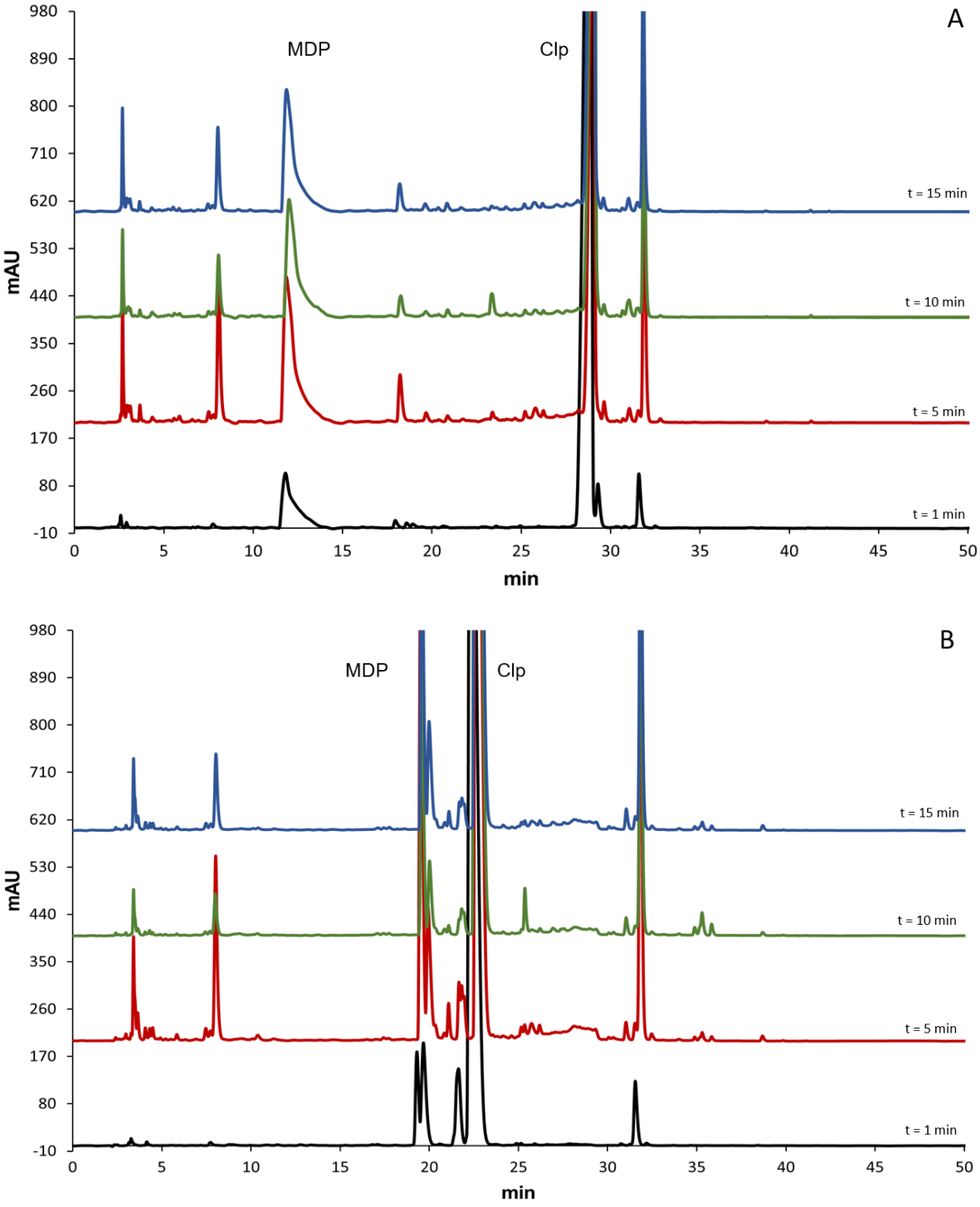


Figure S3. Overlay of the chromatograms of samples stressed with $KMnO_4$ for t = 1 min, t = 5 min, t = 10 min and t = 15 min. (A) Chromatogram obtained with 0.1% formic acid in mobile phases A and B; (B) Chromatogram obtained with 0.1% trifluoroacetic acid in mobile phases A and B. MDP: main degradation product.

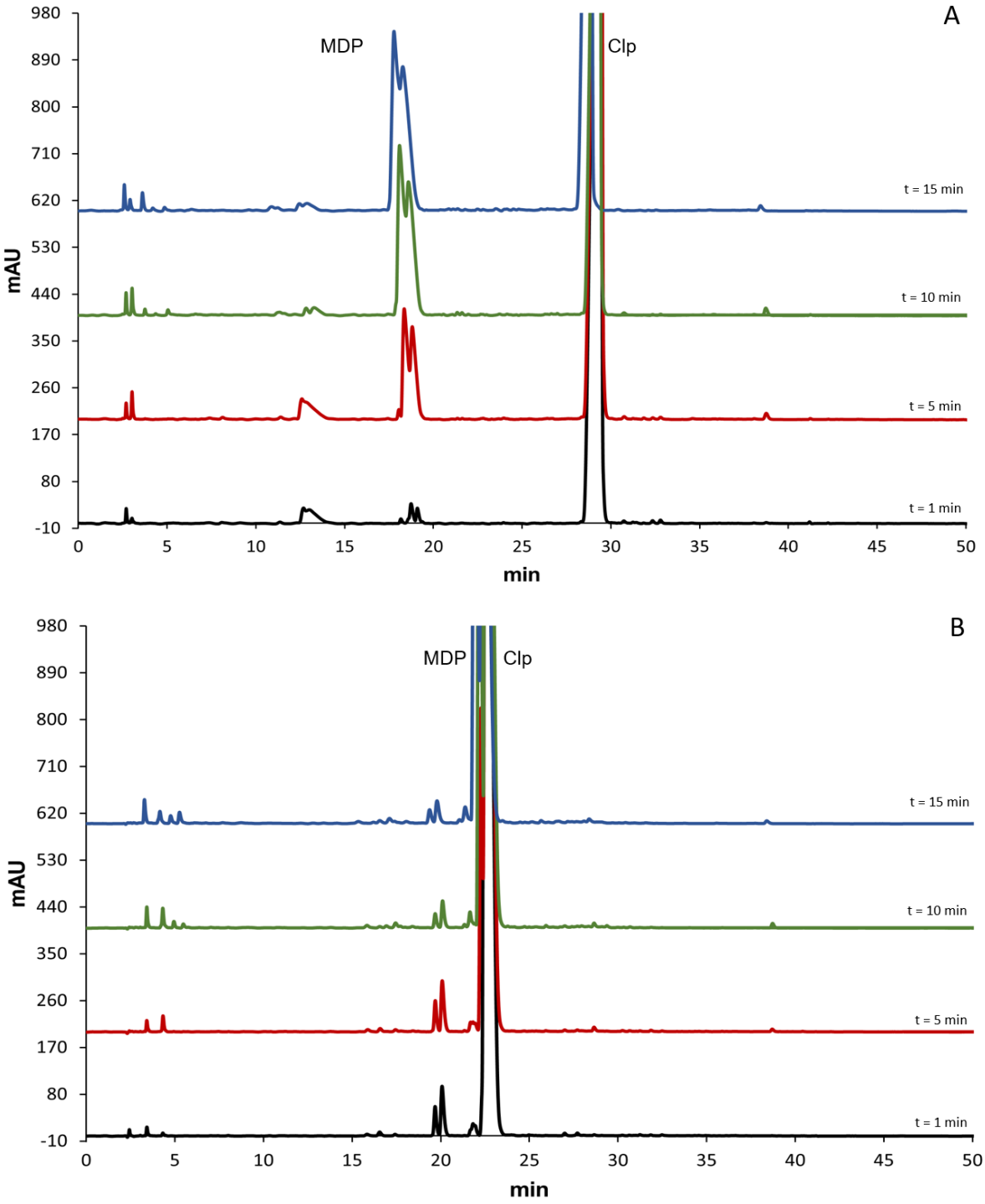


Figure S4. Overlay of the chromatograms of samples stressed with Oxone® for t = 1 min, t = 5 min, t = 10 min and t = 15 min. (A) Chromatogram obtained with 0.1% formic acid in mobile phases A and B; (B) Chromatogram obtained with 0.1% trifluoroacetic acid in mobile phases A and B. MDP: main degradation product.

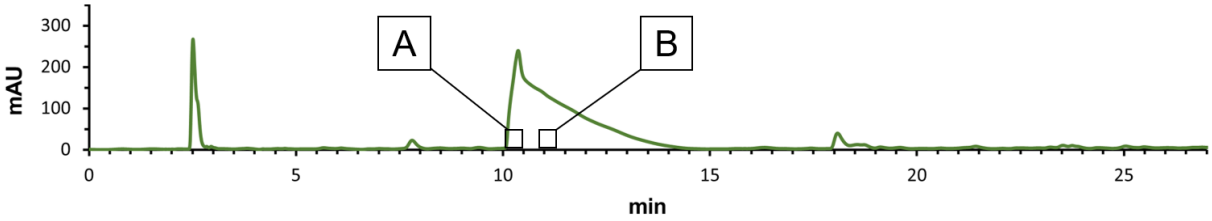


Figure S5. Section of the chromatogram of the sample stressed with KNO_3 for *t* = 10 min and formic acid added to the mobile phase. Marked with A and B are respectively the areas below the peak of the main degradation product for which the UV spectra are shown.

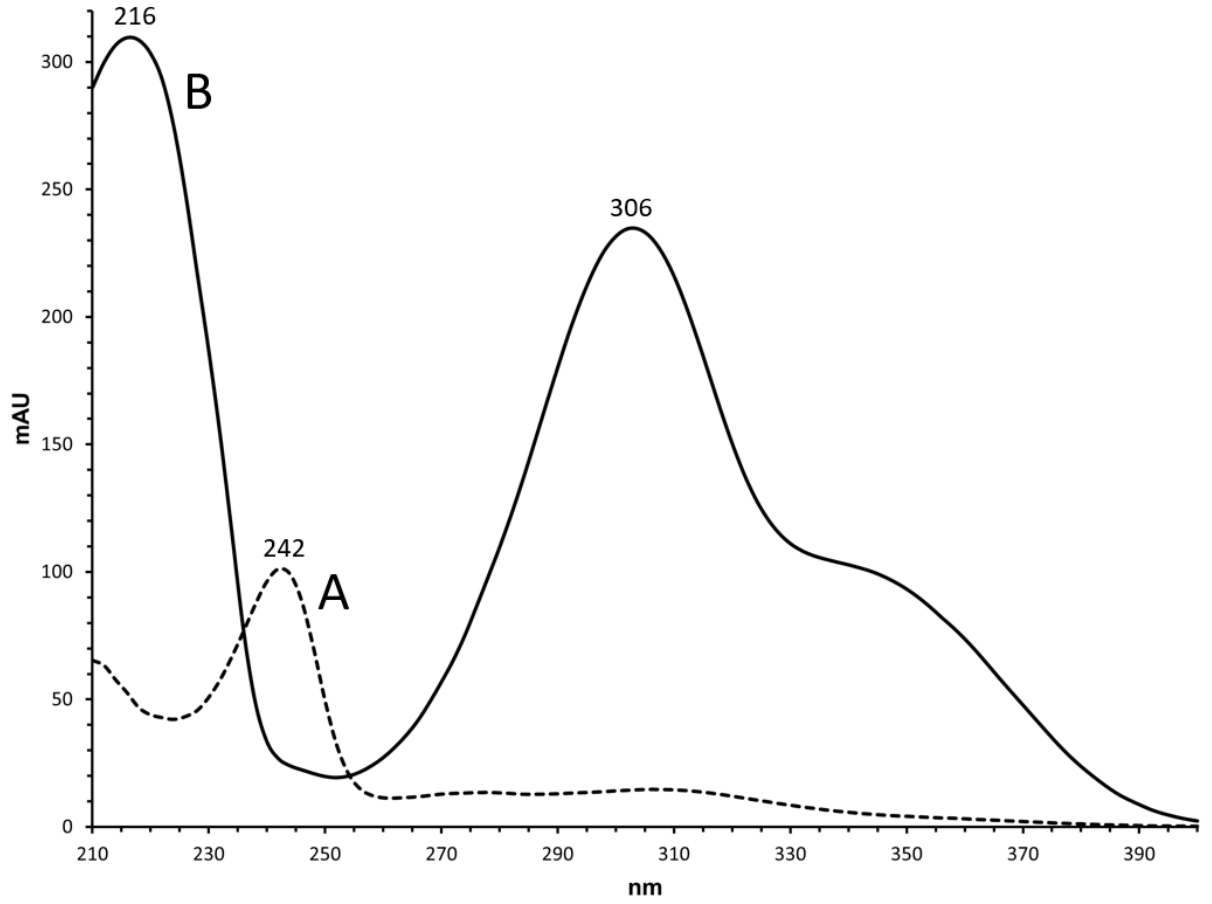
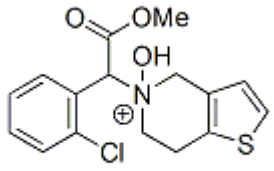
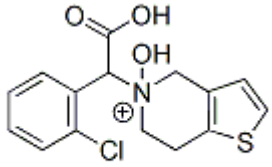
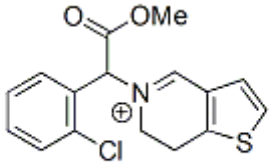
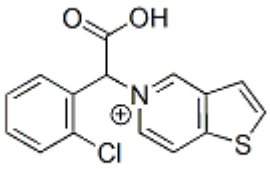
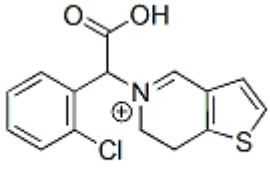
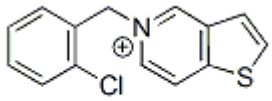
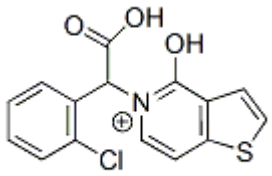
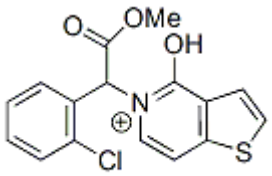
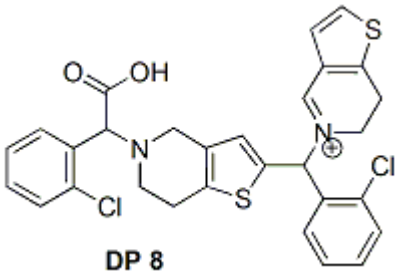


Figure S6. Overlay of the UV spectra under the peak of the main degradation product of the sample stressed with KNO_3 for *t* = 10 min. (A) UV spectrum at the retention time of 10.135 min with an absorption maximum at 242 nm; (B) UV spectrum at the retention time of 11.395 min with absorption maxima at 216 nm and 306 nm.

4. Overview of previous degradation studies of Clp

Table S2. Overview of relevant publications on oxidative degradants of **Clp**.

Applied conditions	Structures identified	Reference
6% aqueous H_2O_2 Room temperature 5 days	 **Degradation Product III** ***(5)***	2
	 **Degradation Product II** *(product of hydrolysis of **5**)*	
5% H_2O_2 in water (5 mL) 0.5 mL MeOH 60°C 3 hours	 **Clopidogrel related compound D** ***(3)***	3,4
3% H_2O_2 in water (20 mL) Acetonitrile 60°C 1 hour	Hydrolytic degradation product identified and confirmed, no structural elucidation for oxidation products	5
Neutral (solid API without stressor) or solid API with solid Na_2CO_3 40°C/75% rH 3 months	 **DP 1** *(product of hydrolysis of **2**)*	6
	 **DP 2** *(product of hydrolysis of **3**)*	

Applied conditions	Structures identified	Reference
	 **DP 3**	
	 **DP 6** *(product of hydrolysis of **4**)*	
	 **DP 7** ***(4)***	
	 **DP 8**	
30% H_2O_2 (1 mL in 50 mL solution) Reflux 1 hour	No oxidative degradation observed	7
2% H_2O_2 Room temperature 48 hours	No oxidative degradation observed	8

5. MS analysis

LC-MS analysis was done using solutions that were prepared by dissolving the oily samples obtained after ball milling in 3 mL of MeCN/H_2O (1:1). We present representative LC-MS data for mechanochemical oxidation of clopidogrel hydrogensulfate using one equivalent KNO_3, $KMnO_4$, and oxone® after a reaction time of 10 minutes.

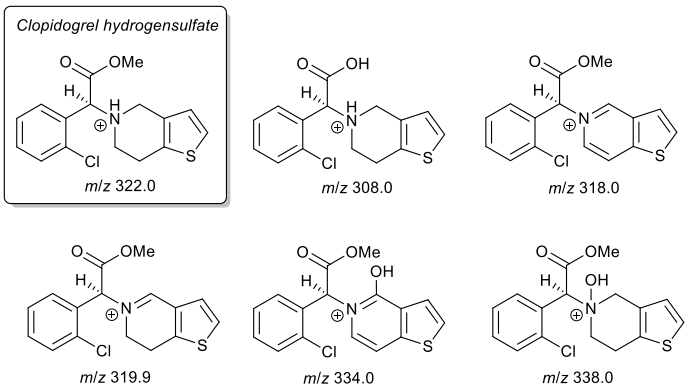
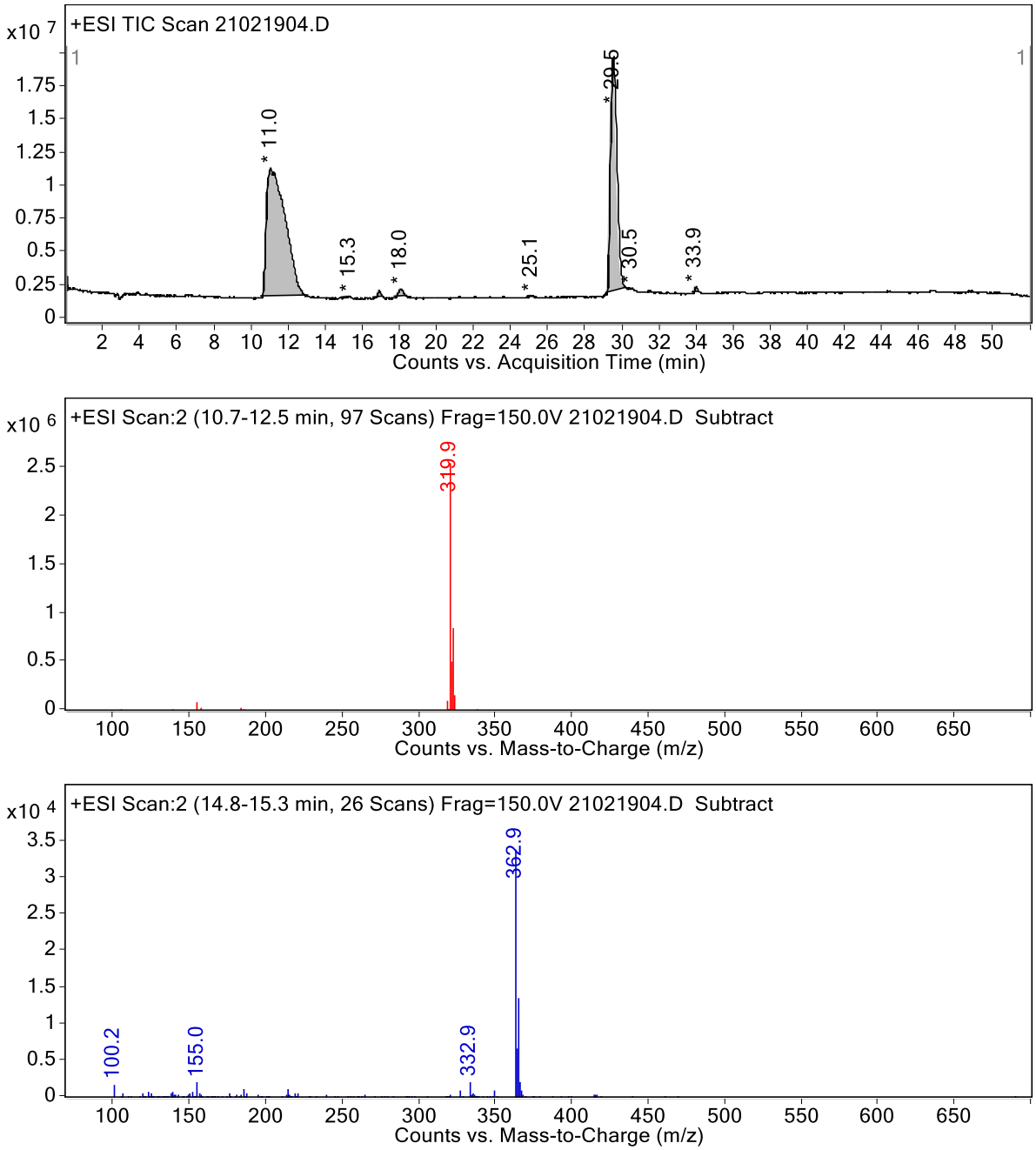
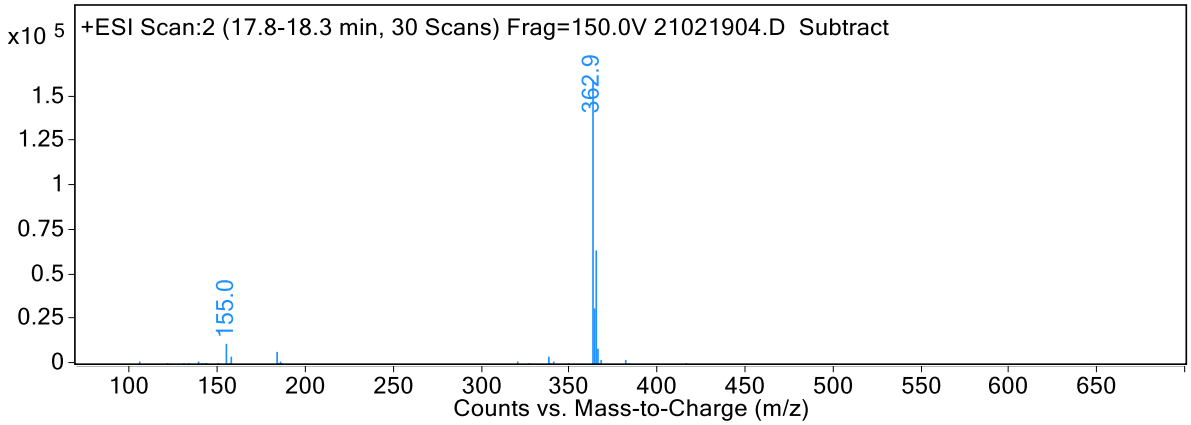
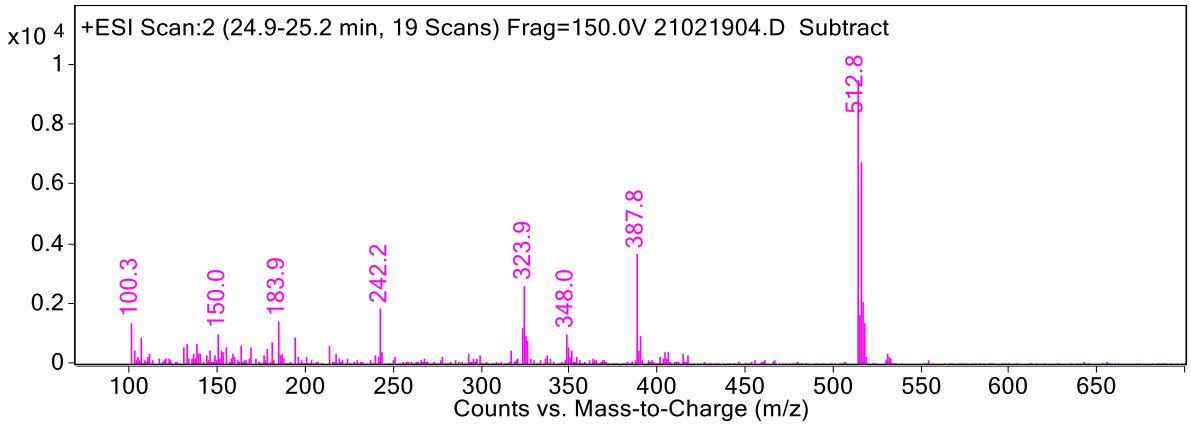


Figure S7. Summary of degradants formed during mechanochemical oxidative degradation of clopidogrel hydrogensulfate.

Figure S8. ESI+ MS data recorded after mechanochemical oxidation of clopidogrel hydrogensulfate with KNO_3, *t* = 10 min. The first plot shows LC-MS data, all other plots show MS data at given retention times.




+ESI Scan:2 (17.8-18.3 min, 30 Scans) Frag=150.0V 21021904.D Subtract
x10 5
1.5
1.25
1
0.75
0.5
0.25
0
362.9
155.0
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (24.9-25.2 min, 19 Scans) Frag=150.0V 21021904.D Subtract
x10 4
1
0.8
0.6
0.4
0.2
0
512.8
387.8
323.9
348.0
242.2
183.9
150.0
100.3
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)

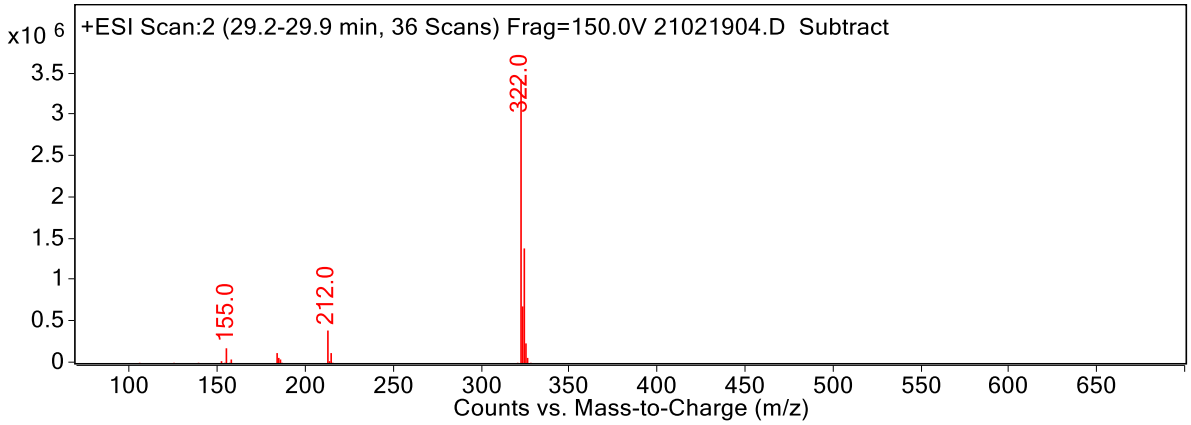
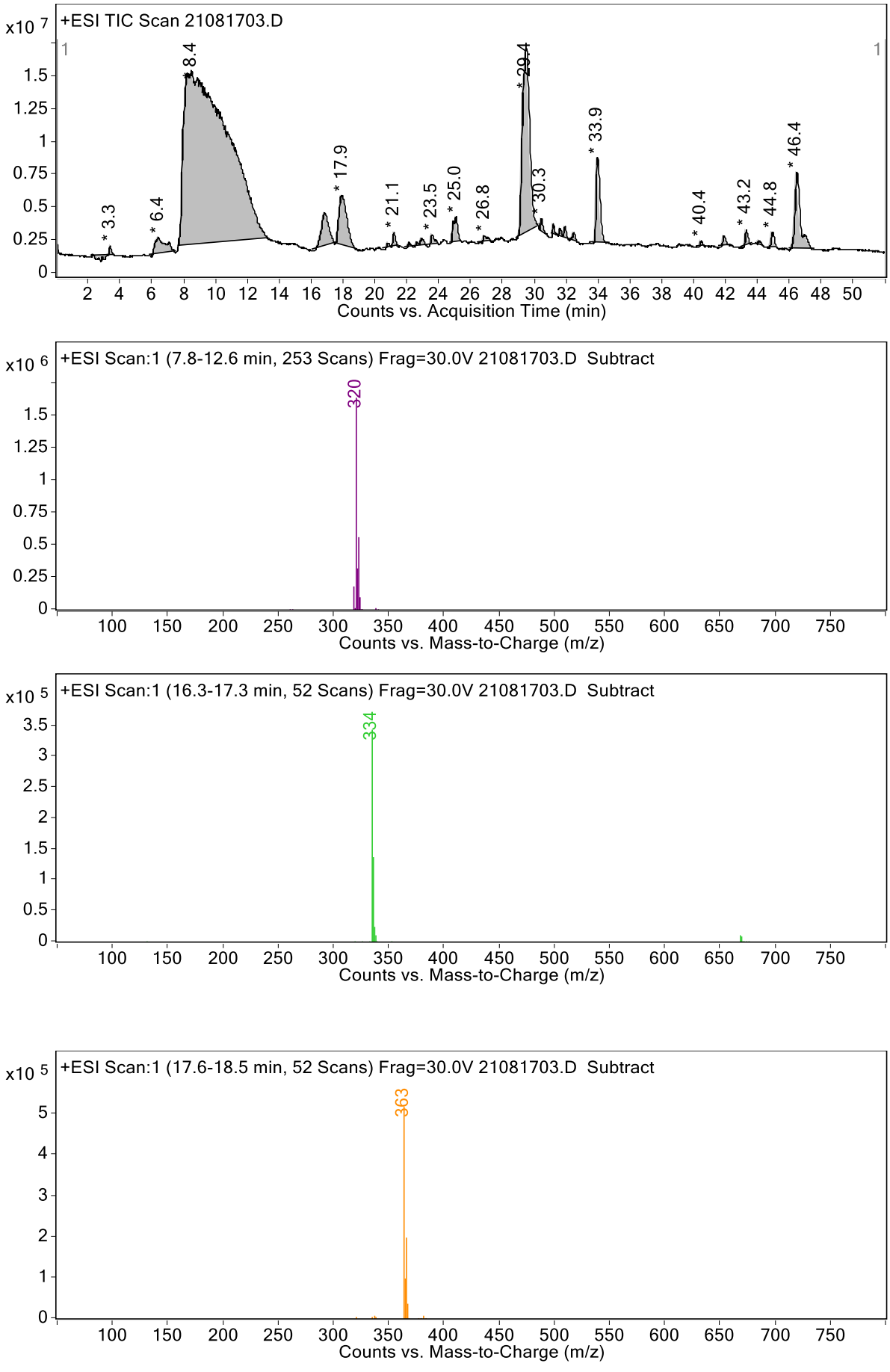
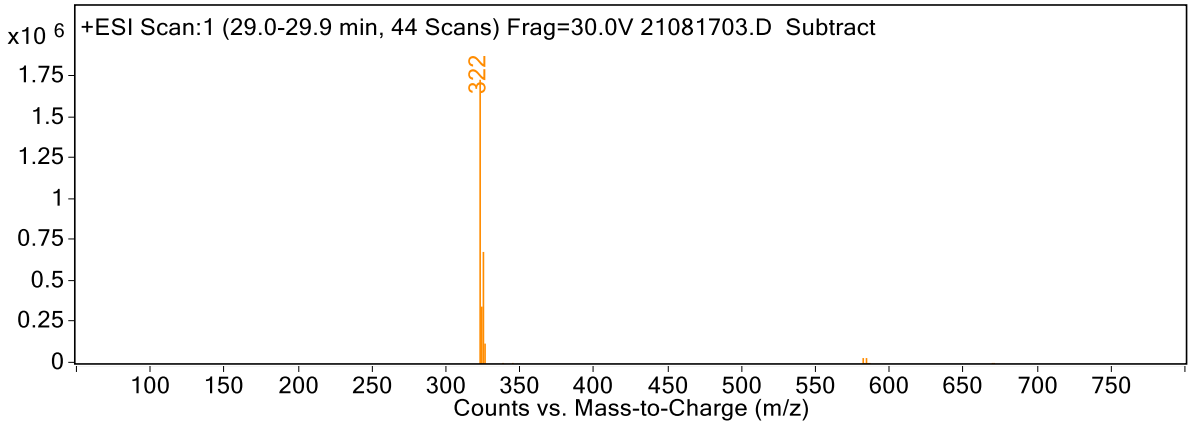

+ESI Scan:2 (29.2-29.9 min, 36 Scans) Frag=150.0V 21021904.D Subtract
x10 6
3.5
3
2.5
2
1.5
1
0.5
0
322.0
212.0
155.0
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)

Figure S9. ESI+ MS data recorded after mechanochemical oxidation of **Clp** with KNO_3, *t* = 120 min.




+ESI Scan:1 (29.0-29.9 min, 44 Scans) Frag=30.0V 21081703.D Subtract
x10 6
1.75
1.5
1.25
1
0.75
0.5
0.25
0
322
100 150 200 250 300 350 400 450 500 550 600 650 700 750
Counts vs. Mass-to-Charge (m/z)

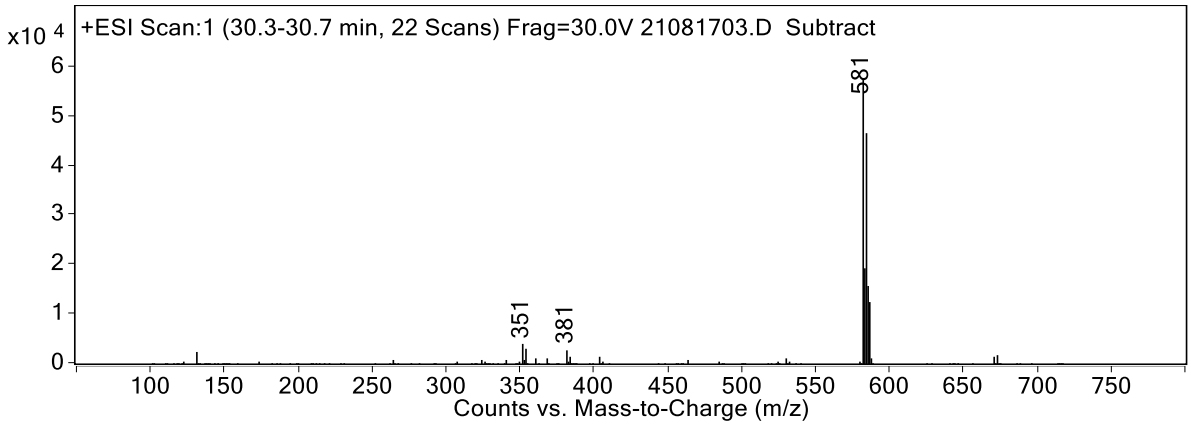
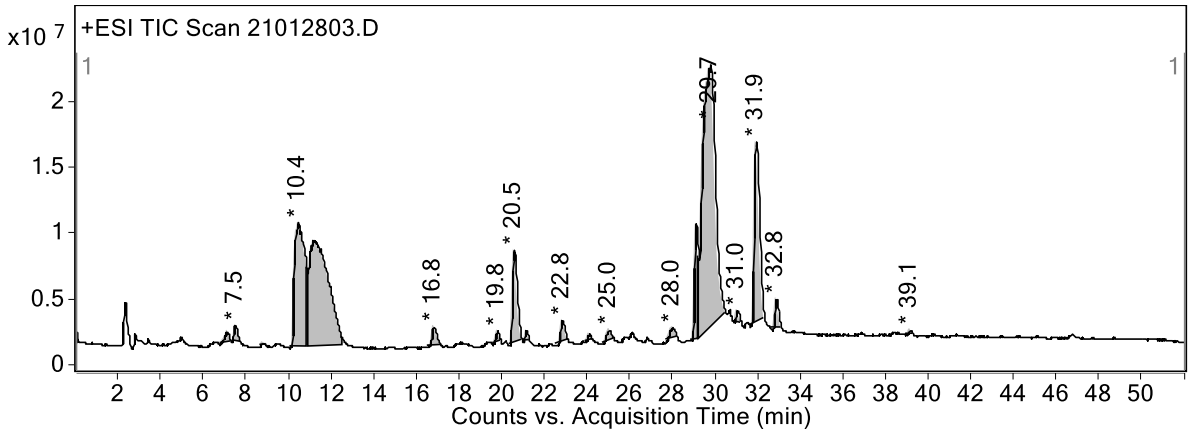
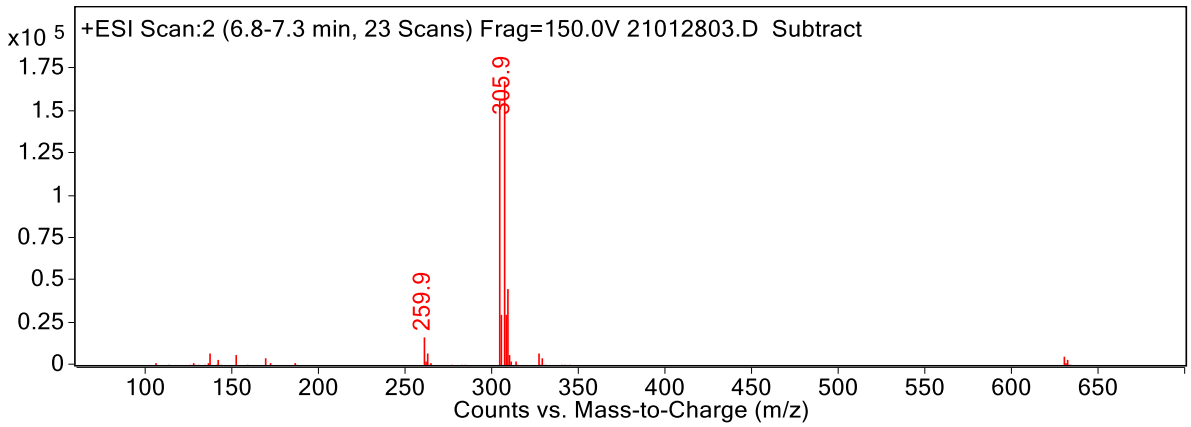
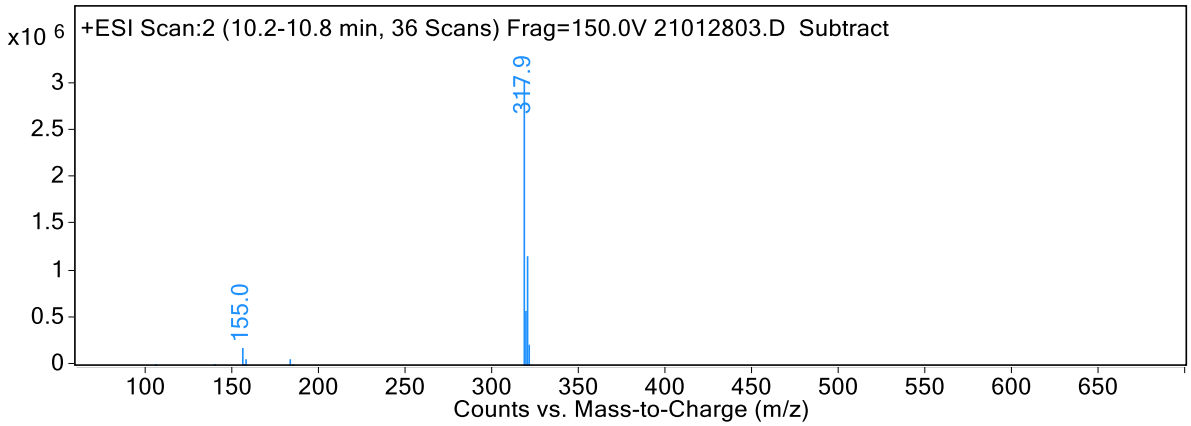
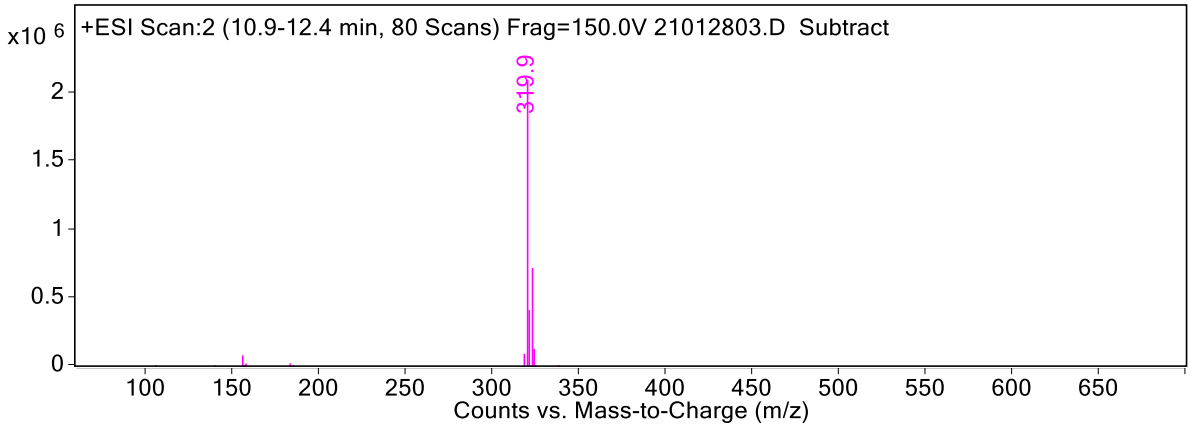

+ESI Scan:1 (30.3-30.7 min, 22 Scans) Frag=30.0V 21081703.D Subtract
x10 4
6
5
4
3
2
1
0
581
351
381
100 150 200 250 300 350 400 450 500 550 600 650 700 750
Counts vs. Mass-to-Charge (m/z)

Figure S10. ESI+ MS data recorded after mechanochemical oxidation of clopidogrel hydrogensulfate with $KMnO_4$, *t* = 10 min. The first plot shows LC-MS data, all other plots show MS data at given retention times.





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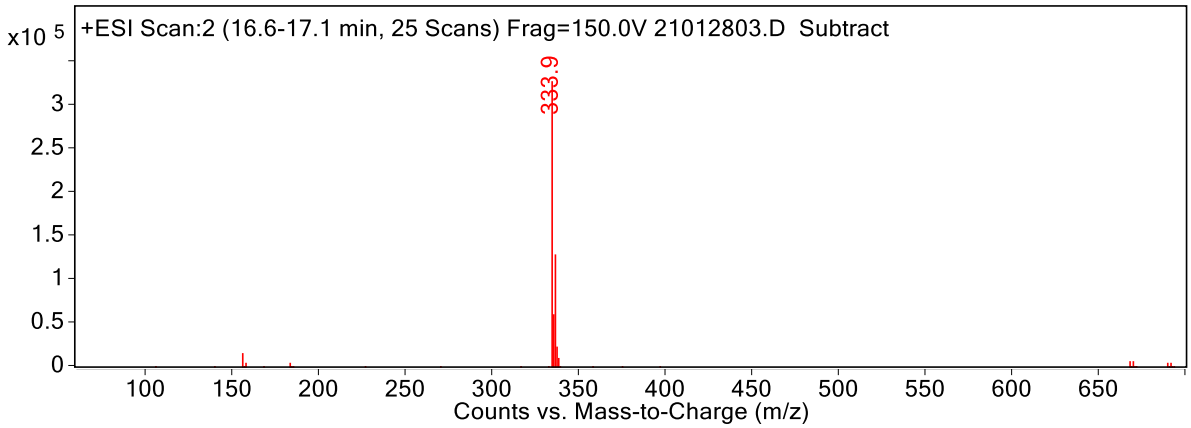
x10 6
319.9
Counts vs. Mass-to-Charge (m/z)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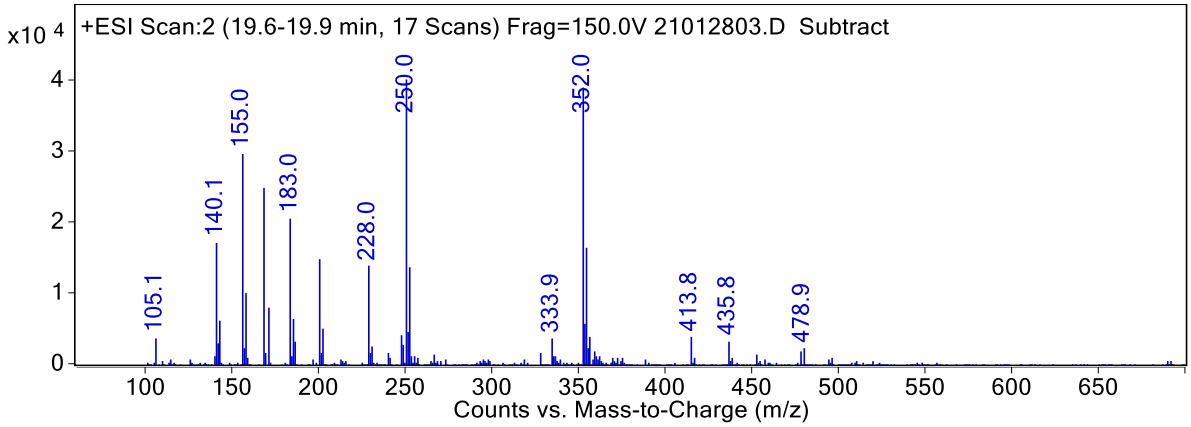
x10 5
333.9
Counts vs. Mass-to-Charge (m/z)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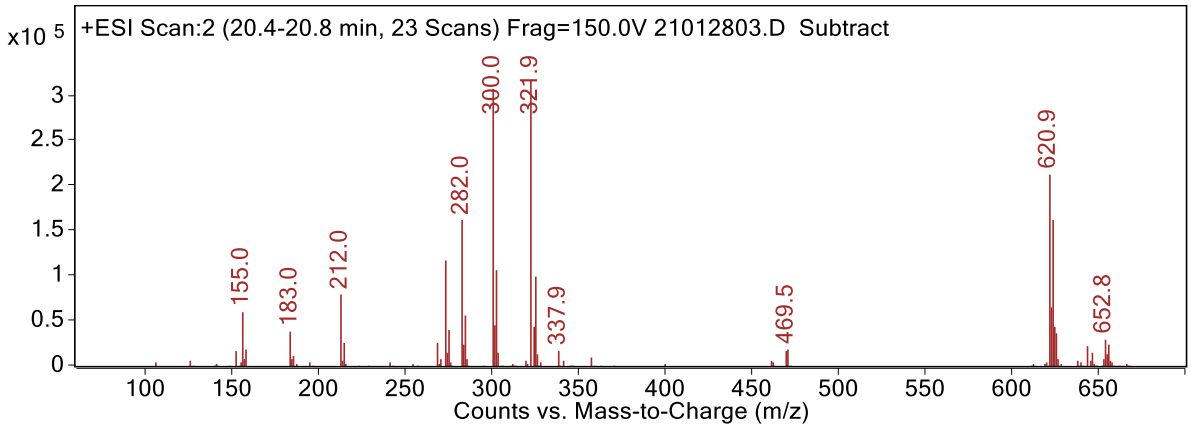
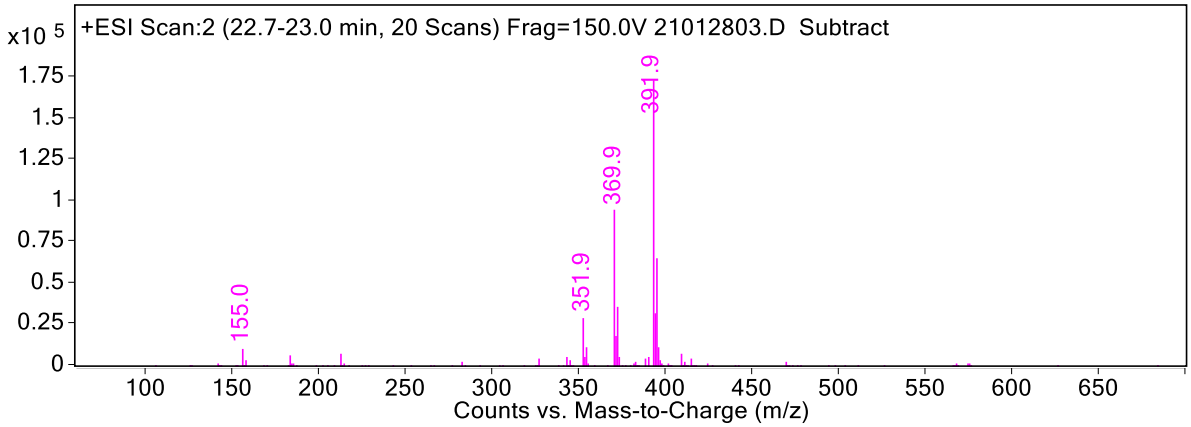
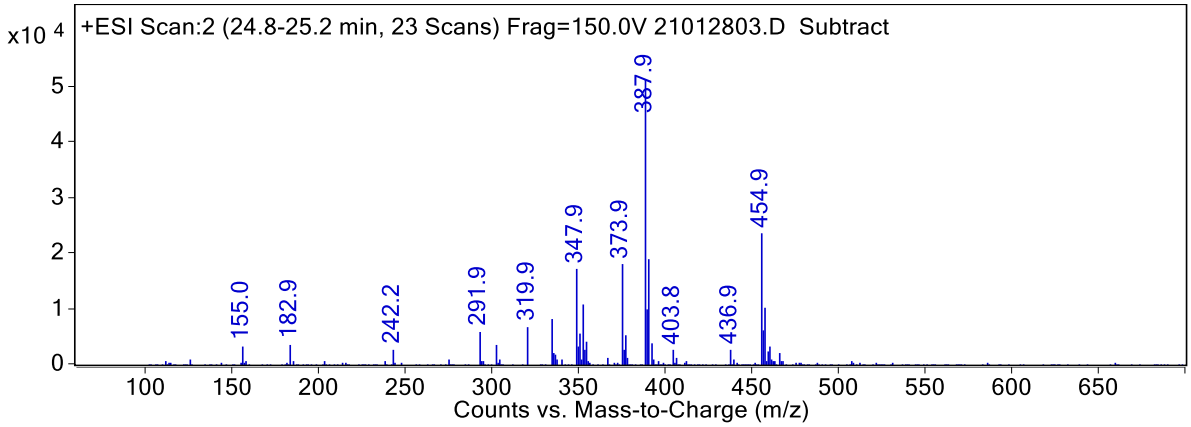
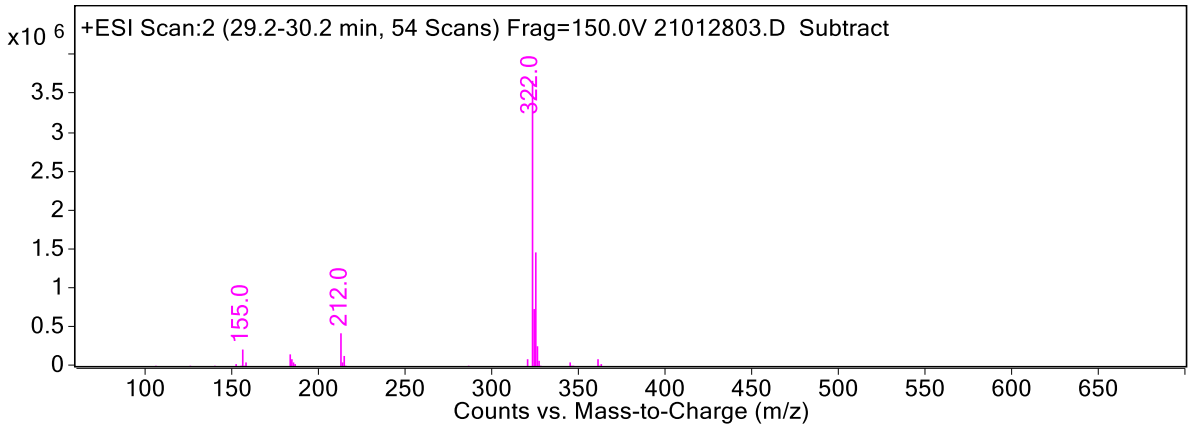
x10 4
105.1
140.1
155.0
183.0
228.0
250.0
333.9
352.0
413.8
435.8
478.9
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (20.4-20.8 min, 23 Scans) Frag=150.0V 21012803.D Subtract
x10 5
155.0
183.0
212.0
282.0
300.0
321.9
337.9
469.5
620.9
652.8
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (22.7-23.0 min, 20 Scans) Frag=150.0V 21012803.D Subtract
x10 5
1.75
1.5
1.25
1
0.75
0.5
0.25
0
155.0
351.9
369.9
391.9
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (24.8-25.2 min, 23 Scans) Frag=150.0V 21012803.D Subtract
x10 4
5
4
3
2
1
0
155.0
182.9
242.2
291.9
319.9
347.9
373.9
387.9
403.8
436.9
454.9
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (29.2-30.2 min, 54 Scans) Frag=150.0V 21012803.D Subtract
x10 6
3.5
3
2.5
2
1.5
1
0.5
0
155.0
212.0
322.0
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)

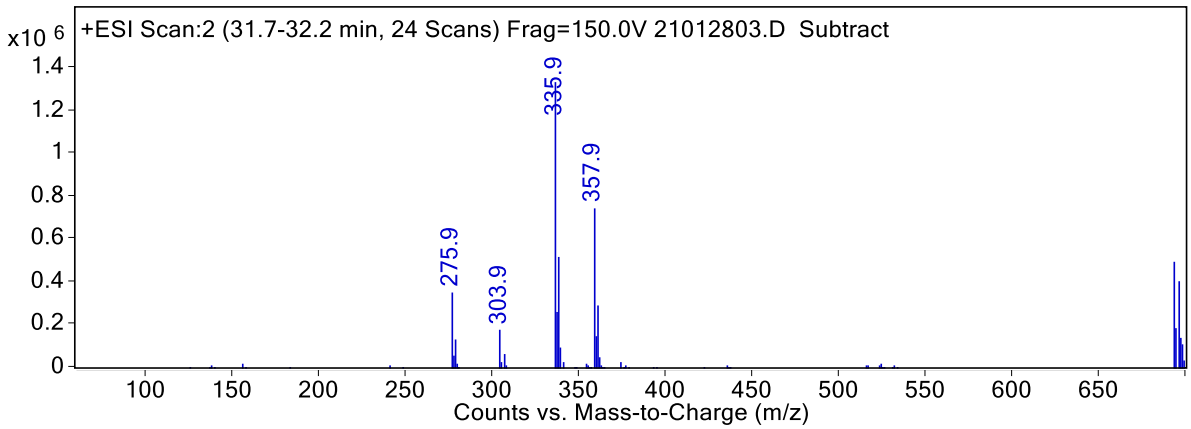
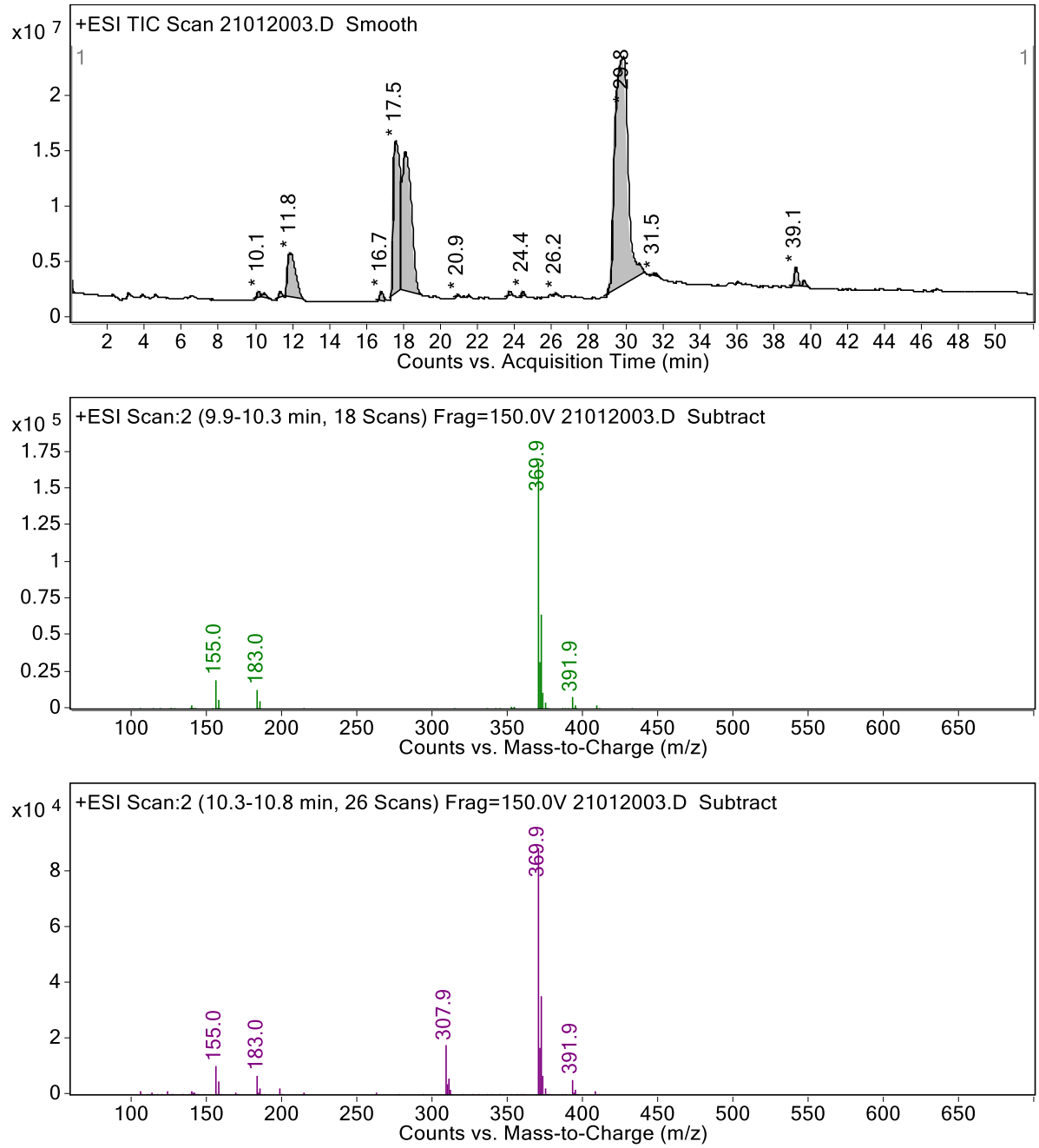
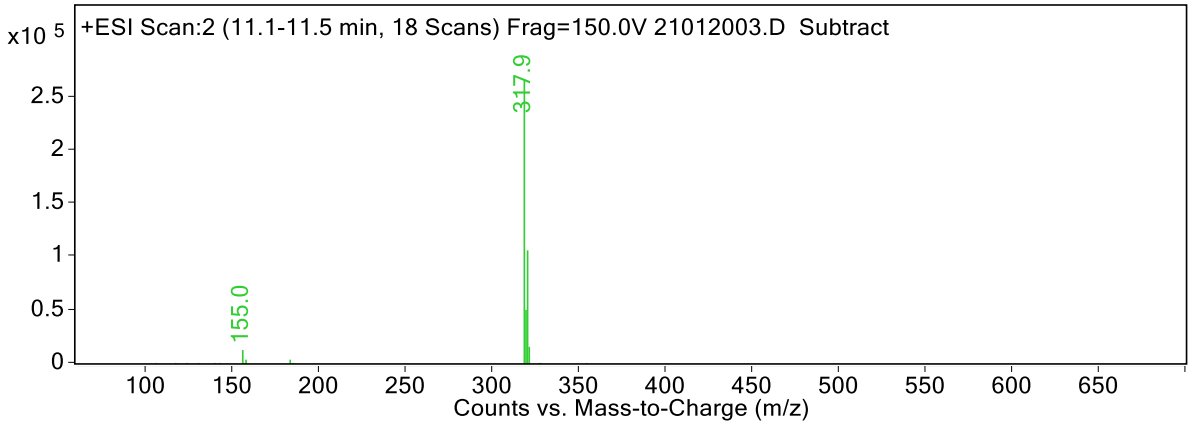
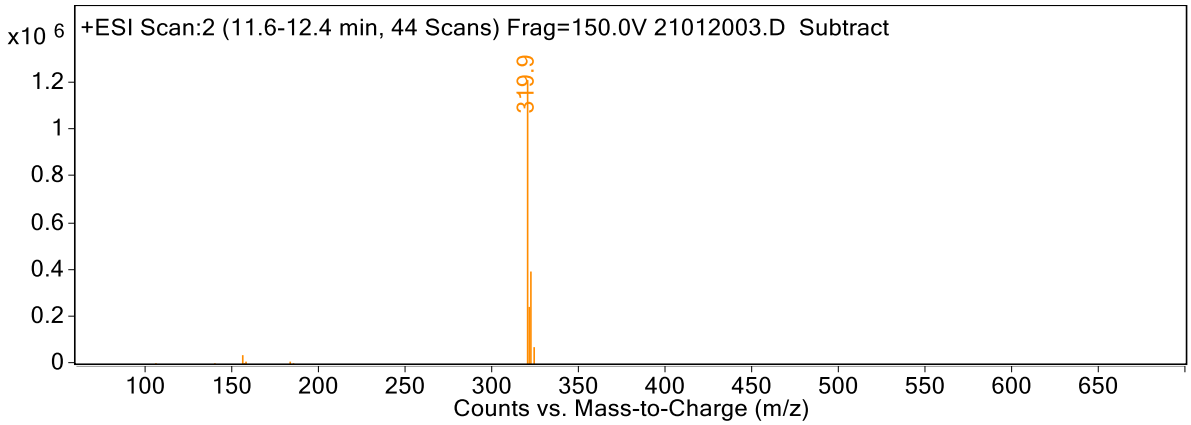
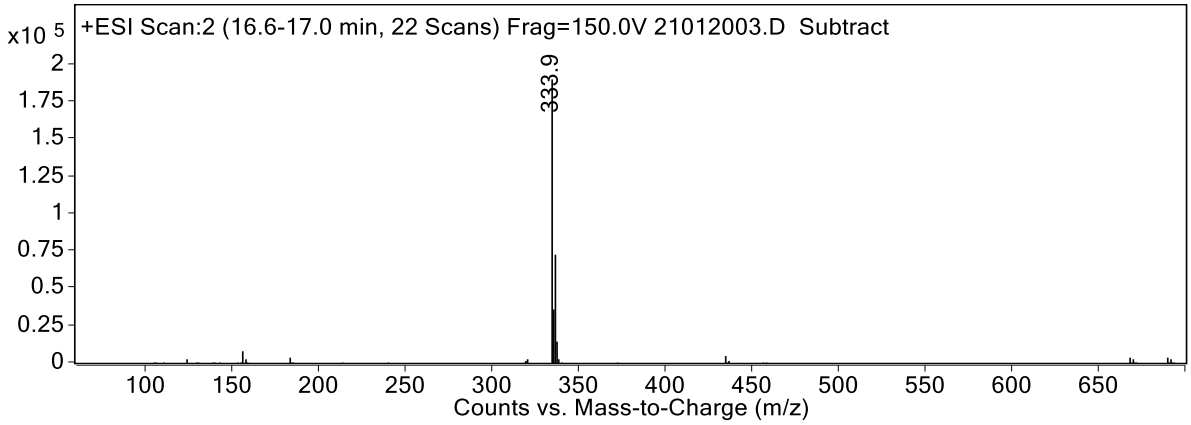

+ESI Scan:2 (31.7-32.2 min, 24 Scans) Frag=150.0V 21012803.D Subtract
x10 6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
275.9
303.9
335.9
357.9
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)

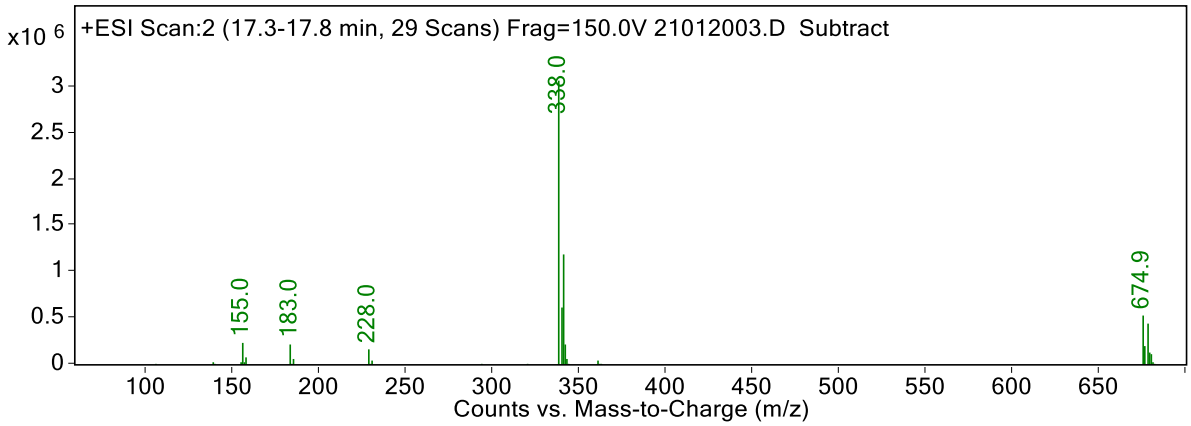
Figure S11. ESI+ MS recorded after mechanochemical oxidation of clopidogrel hydrogensulfate with oxone®, *t* = 10 min. The first plot shows LC-MS data, all other plots show MS data at given retention times.

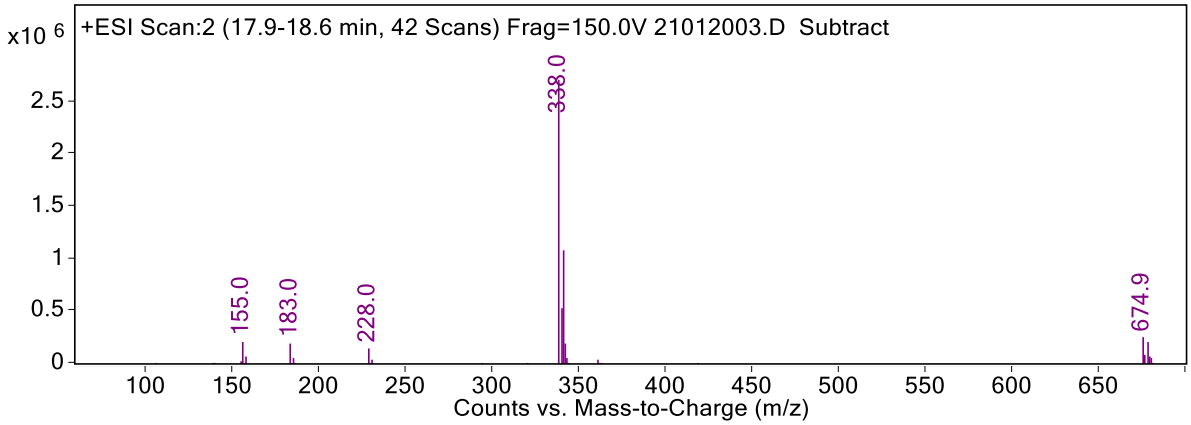


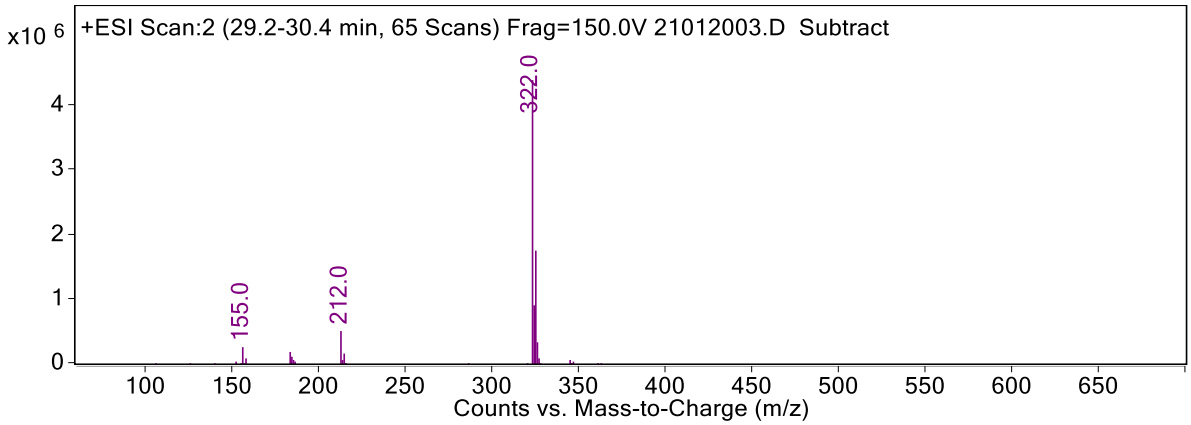

+ESI Scan:2 (11.1-11.5 min, 18 Scans) Frag=150.0V 21012003.D Subtract
x10 5
317.9
155.0
Counts vs. Mass-to-Charge (m/z)

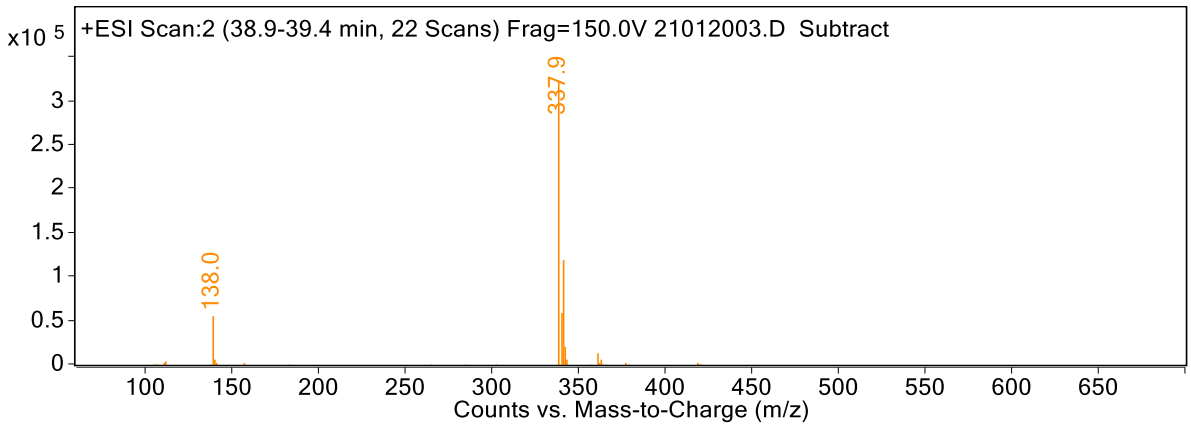

+ESI Scan:2 (11.6-12.4 min, 44 Scans) Frag=150.0V 21012003.D Subtract
x10 6
319.9
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (16.6-17.0 min, 22 Scans) Frag=150.0V 21012003.D Subtract
x10 5
333.9
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (17.3-17.8 min, 29 Scans) Frag=150.0V 21012003.D Subtract
x10 6
338.0
155.0
183.0
228.0
674.9
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (17.9-18.6 min, 42 Scans) Frag=150.0V 21012003.D Subtract
x10 6
2.5
2
1.5
1
0.5
0
338.0
155.0
183.0
228.0
674.9
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (29.2-30.4 min, 65 Scans) Frag=150.0V 21012003.D Subtract
x10 6
4
3
2
1
0
322.0
155.0
212.0
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)


+ESI Scan:2 (38.9-39.4 min, 22 Scans) Frag=150.0V 21012003.D Subtract
x10 5
3
2.5
2
1.5
1
0.5
0
337.9
138.0
100 150 200 250 300 350 400 450 500 550 600 650
Counts vs. Mass-to-Charge (m/z)

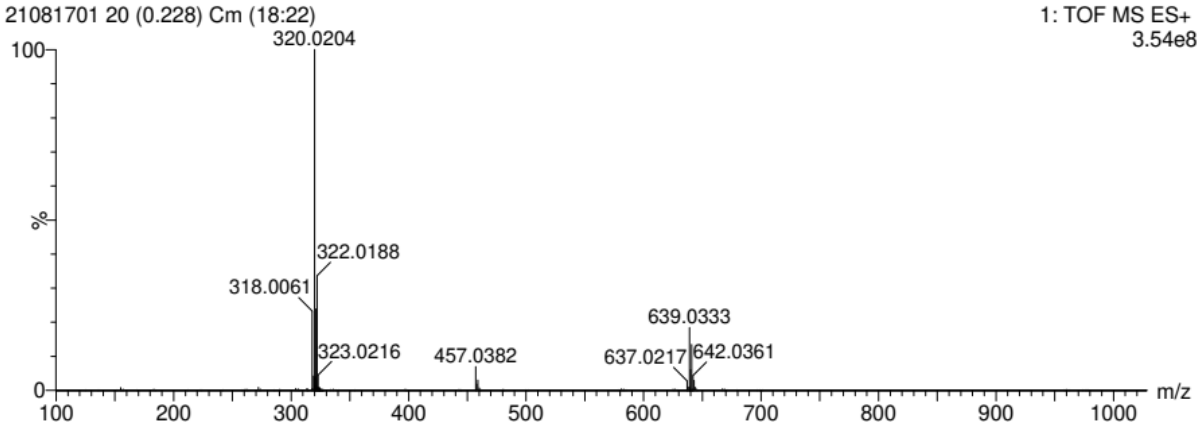


Figure S12. HRMS (ESI-TOF) of compound **3**, obtained after ball milling of **Clp** with SiO_2 and one equivalent of KNO_3 for 10 minutes. The exact mass is 320.05120 $g \cdot mol^{-1}$.

6. IR spectra

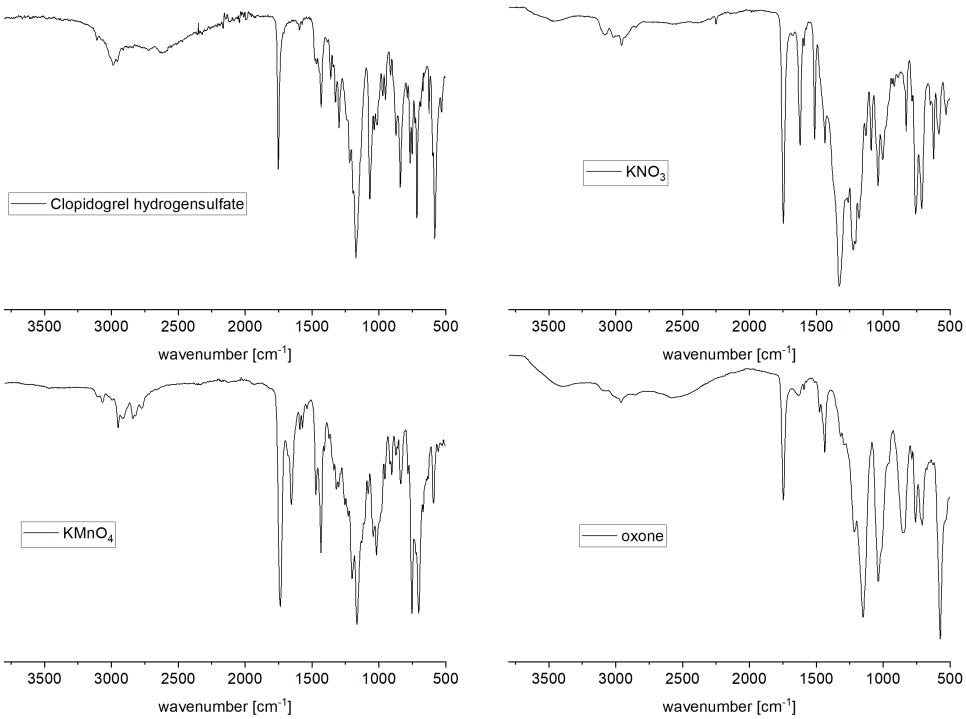


Figure S13. Comparison of ATR-IR spectra of pure **Clp** and product mixtures obtained after ball milling of **Clp** with SiO_2 and the respective oxidant for 15 minutes (after extraction from SiO_2 and residual oxidant using MeCN/H_2O).

7. NMR spectra

7.1 KNO_3

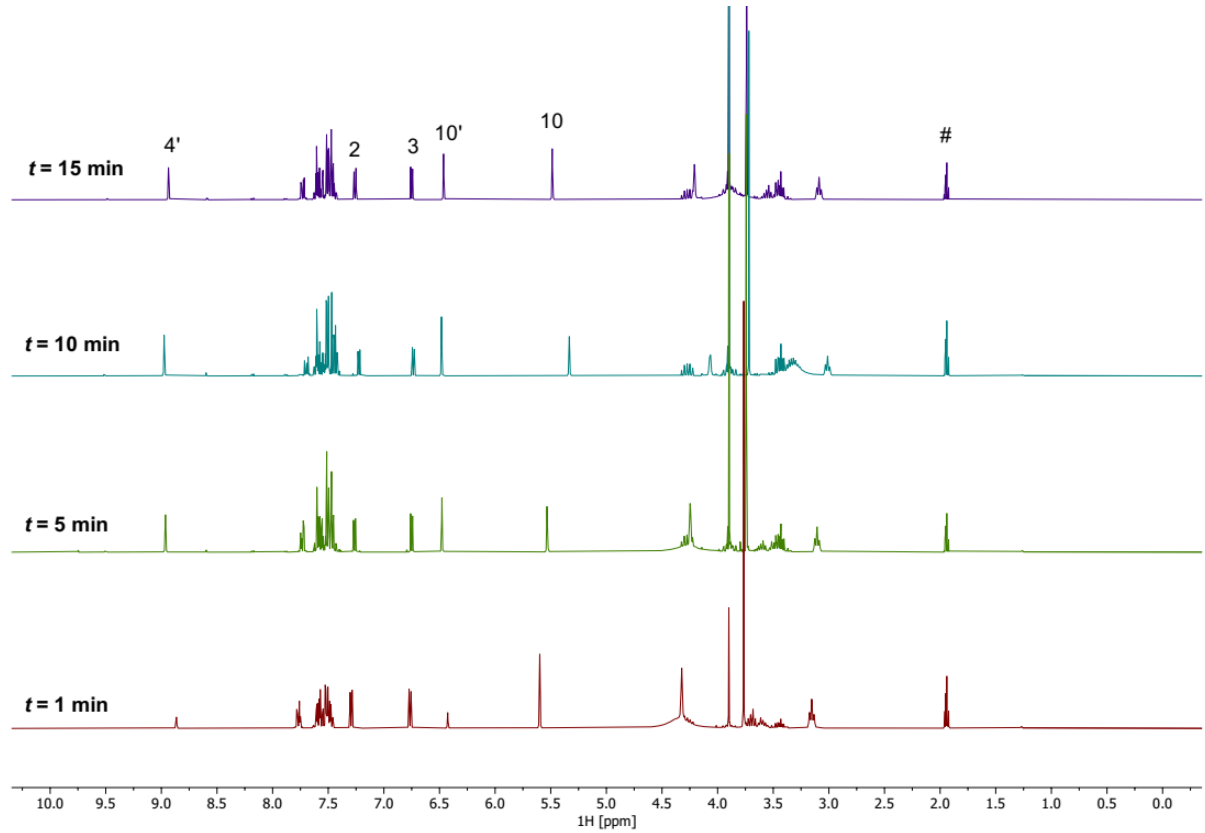


Figure S14. Comparison of ^{1}H NMR spectra, recorded after ball milling of **Clp** with SiO_2 and one equivalent of KNO_3 for 1, 5, 10, and 15 minutes (CD_3CN, 25 °C, 400 MHz). Legend for peak assignments shown in Figure S14, # CHD_2CN.

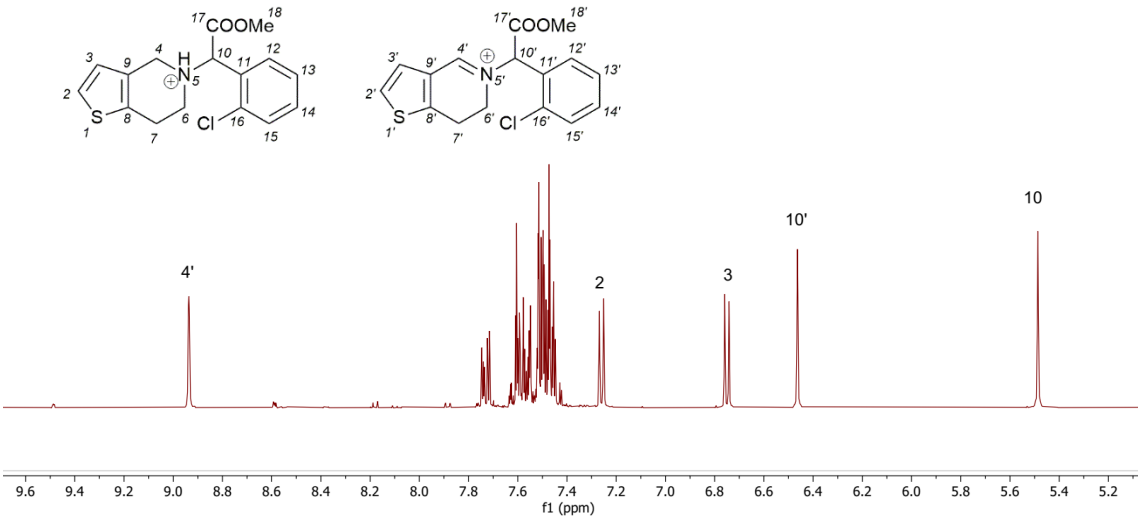


Figure S15. ^{1}H NMR spectrum (enlarged view of Figure S13, CD_3CN, 25 °C, 400 MHz), recorded after ball milling of **Clp** with SiO_2 and one equivalent of KNO_3 for 15 minutes showing the peak assignment for **Clp** (left) and compound **3** (right).

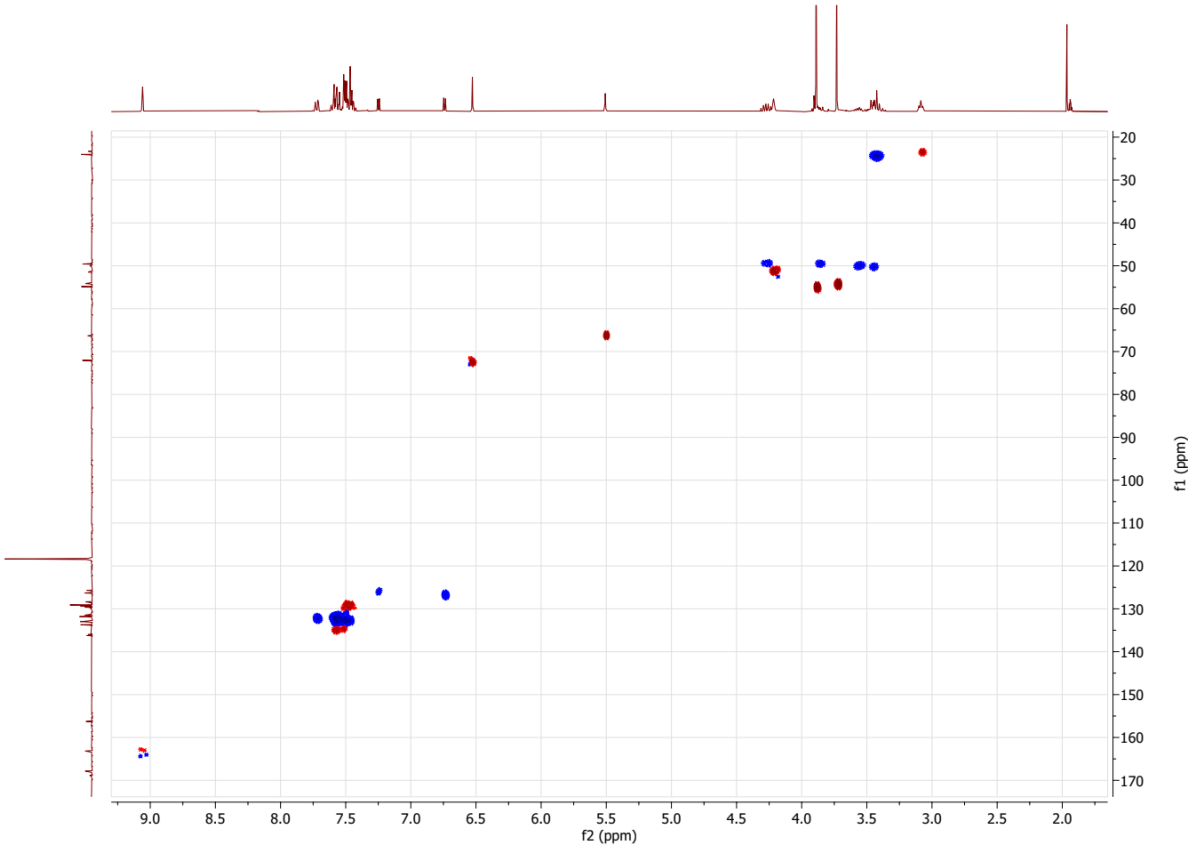


Figure S16. ^{1}H-^{13}C HSQC spectrum, recorded after ball milling of **Clp** with SiO_2 and one equivalent of KNO_3 for 15 minutes (CD_3CN, 25 °C, 400 MHz).

7.2 $KMnO_4$

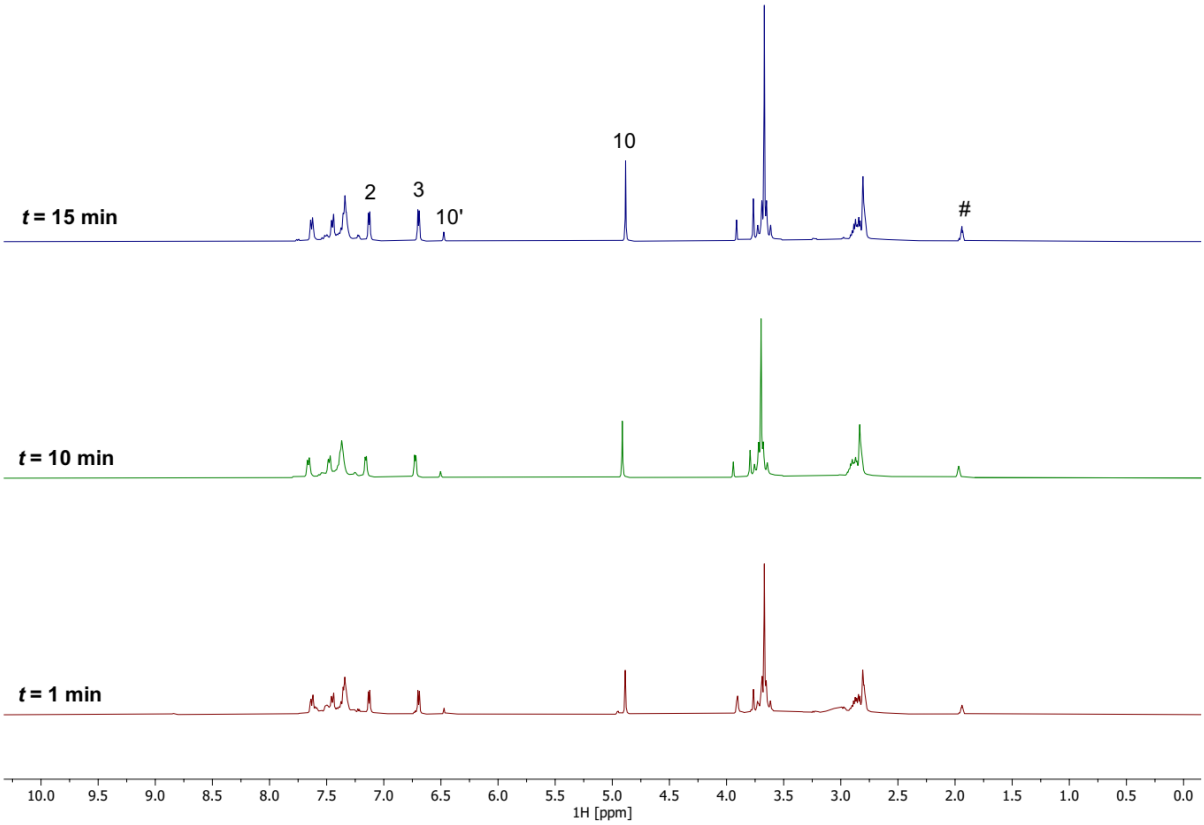


Figure S17. Comparison of ^{1}H NMR spectra, recorded after ball milling of **Clp** with SiO_2 and one equivalent of $KMnO_4$ for 1, 10, and 15 minutes (CD_3CN, 25 °C, 400 MHz). Legend for peak assignments shown in Figure S14, # CHD_2CN.

Note: The spectrum recorded after 5 minutes showed very broad resonances, likely due to the presence of larger amounts of paramagnetic impurities that originate from the oxidant and are therefore not shown here.

7.3 Oxone®

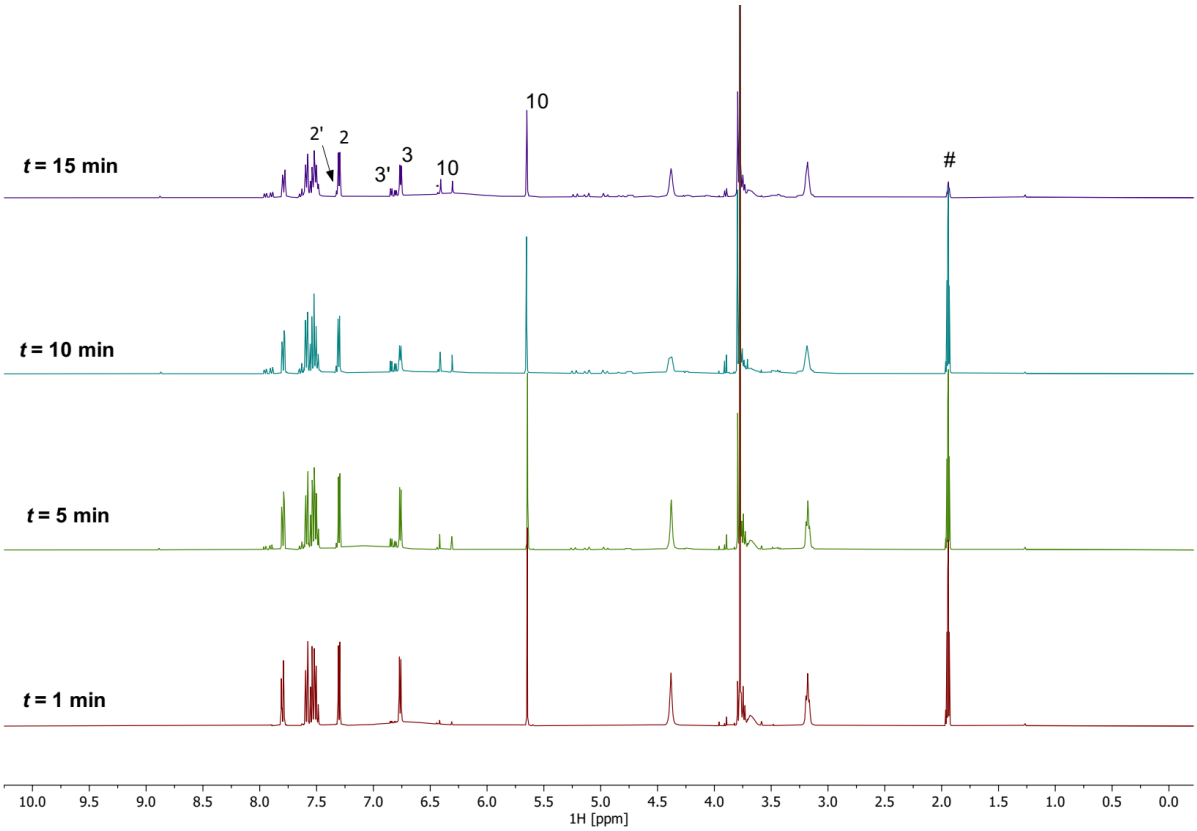


Figure S18. Comparison of ^{1}H NMR spectra, recorded after ball milling of **Clp** with SiO_2 and one equivalent of oxone® for 1, 5, 10, and 15 minutes (CD_3CN, 25 °C, 400 MHz). Legend for peak assignments shown in Figure S18, # CHD_2CN.

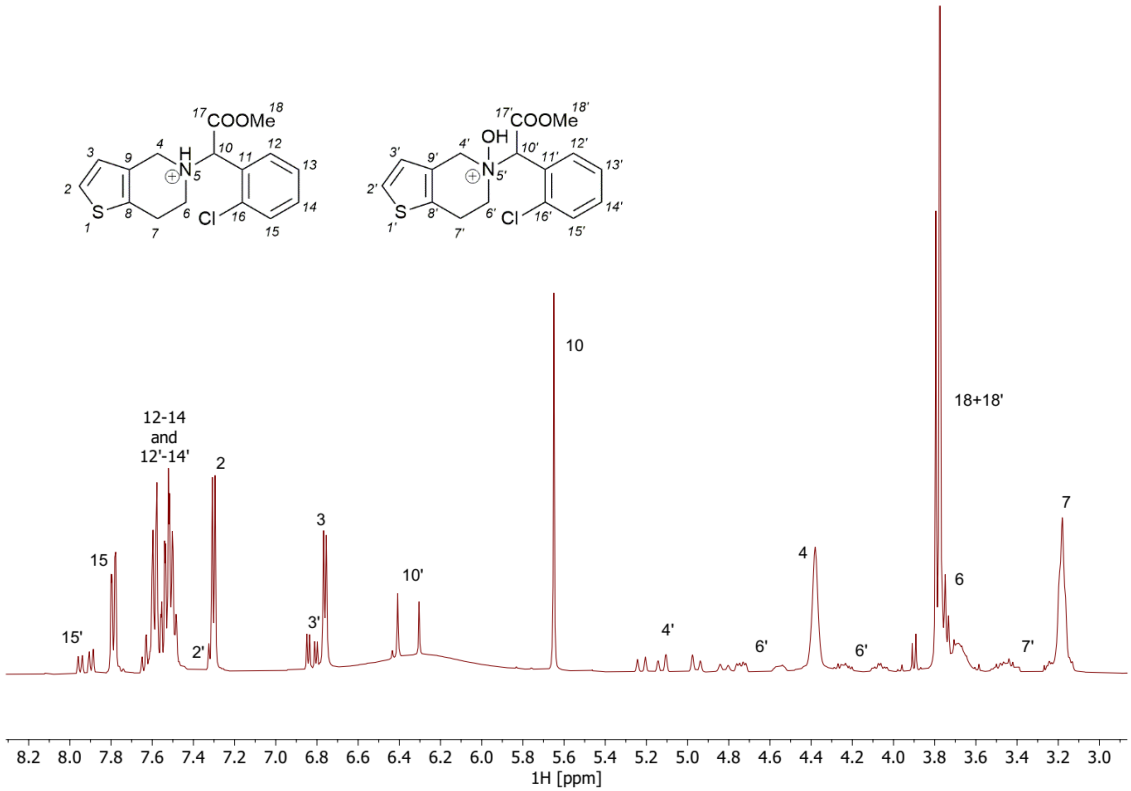


Figure S19. ^{1}H NMR spectrum (enlarged view of Figure S17), recorded after ball milling of **Clp** with SiO_2 and one equivalent of oxone® for 15 minutes showing the peak assignment for **Clp** and *N*-oxide **5** (two diastereomers) (CD_3CN, 25 °C, 400 MHz).

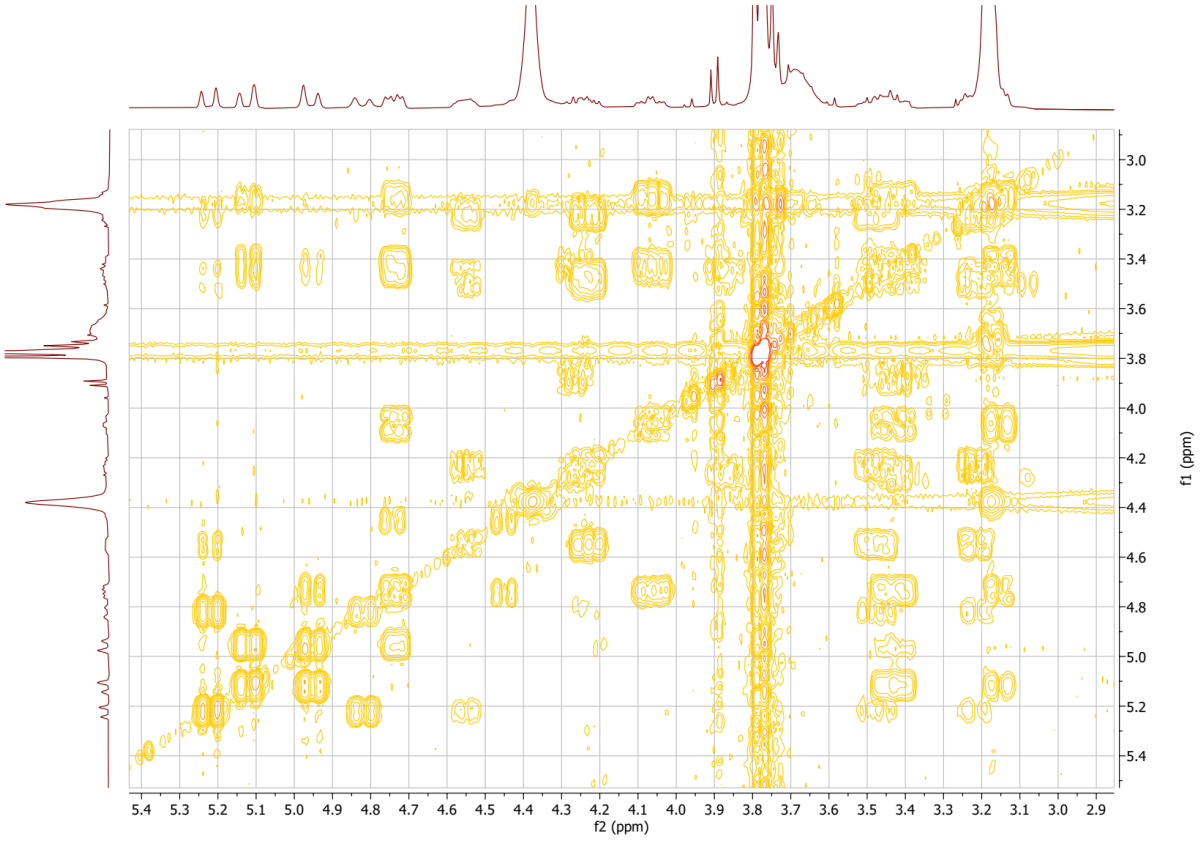


Figure S20. ^{1}H COSY NMR spectrum, recorded after ball milling of **Clp** with SiO_2 and one equivalent of oxone® for 15 minutes, used for peak assignment for **Clp** and *N*-oxide **5** (CD_3CN, 25 °C, 400 MHz).

8. References

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