U0126 protects cells against oxidative stress independent of its function as a MEK Inhibitor

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Supplementary Figure Legends

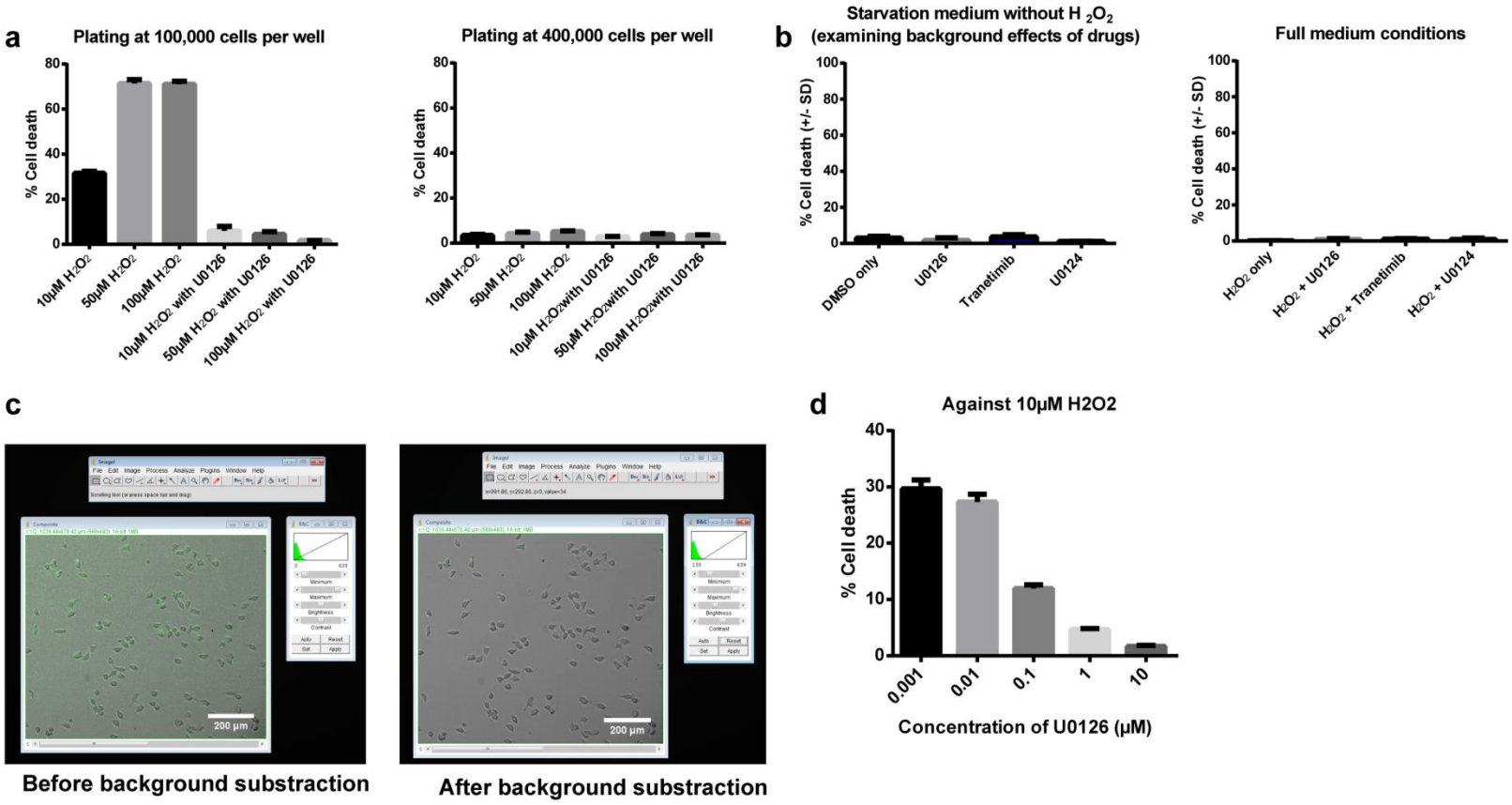
Supp. Fig. 1: (a) Optimization of cell death conditions showed that low cell density at 100,000 cells per 12-well plate well with 10 µM H_2O_2 could elicit cell death in cells. High cell density at 400,000 cells per well does not elicit significant cell death for 10 - 100 µM H_2O_2. **(b)** Control conditions, where cells are serum-starved but without H_2O_2 or cells are provided with full medium while exposed to H_2O_2 showed minimal cell death, indicating no side effect from the small molecules to result in additional cell death. **(c)** Fluorescence is observed in the GFP channel when U0126 is added to PC-12 cells for 2 hours. However upon minimal background subtraction that removes background artefacts in ImageJ, the minimal fluorescence will not be observed and thus should not affect the overall DCHFDA readings. (d) EC-50 curve, calculated from the protective effect exerted by different concentrations of U0126, was detailed via the corresponding percentage of cell death. The EC-50 was seen to be at around 100 nM.

Supplementary Figure 2: Full mass spectra of U0126 and U0124 and their oxidation reactions. **(a)** (i) and (ii) Mass spectrum of U0126 before and 3 hours after addition of 10 equivalence of hydrogen peroxide respectively. **(b)** (i) and (ii) Mass spectrum of U0124 before and 2 hours after addition of 10 equivalence of hydrogen peroxide respectively. Annotated are the key structures to be observed corresponding to their mass to charge ratio (m/z). **(c)** Mass spectrum of U0126 after mixing with hydrogen peroxide and Iron(II) Sulfate heptahydrate. This spectrum shows new peaks as compared with (a).

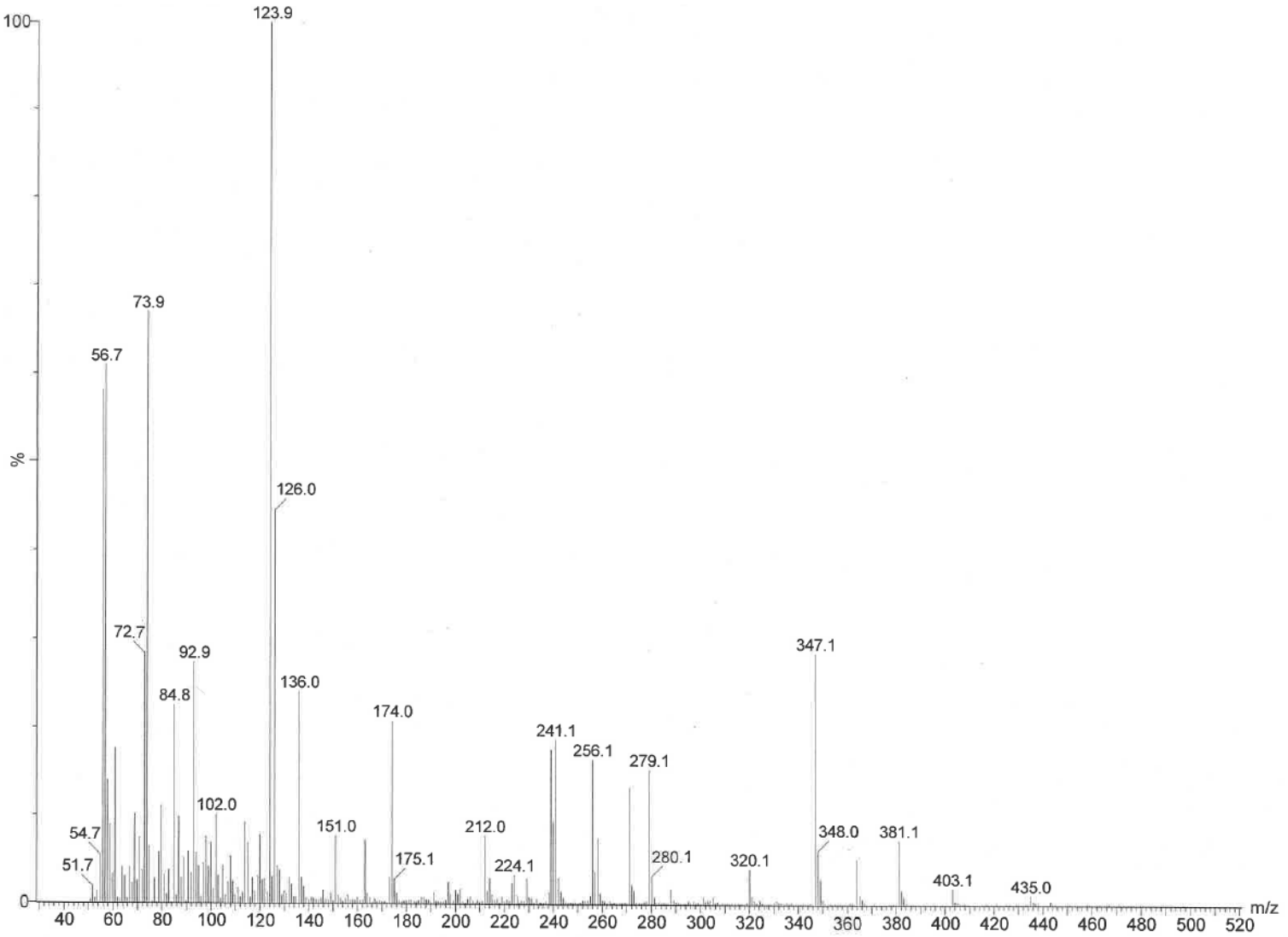
Supplementary Figure 3: Full-range NMR spectra of U0126 and its oxidation reactions. **(a)** ^{1}H NMR of U0126 at 0min, 10 min and 3 hours after addition of 10 equivalence of hydrogen peroxide. **(b)** ^{1}H NMR spectrum of the U0126, H_2O_2 and Fe(II) crude mixture before an aqueous workup procedure to remove iron ions. **(c)** ^{1}H NMR spectrum of the U0126, H_2O_2 and Fe(II) mixture after

removing iron ions. The peaks of the aromatic region shifted upfield, indicating a possible oxidation mechanism with hydroxyl radicals produced via Fenton reaction.

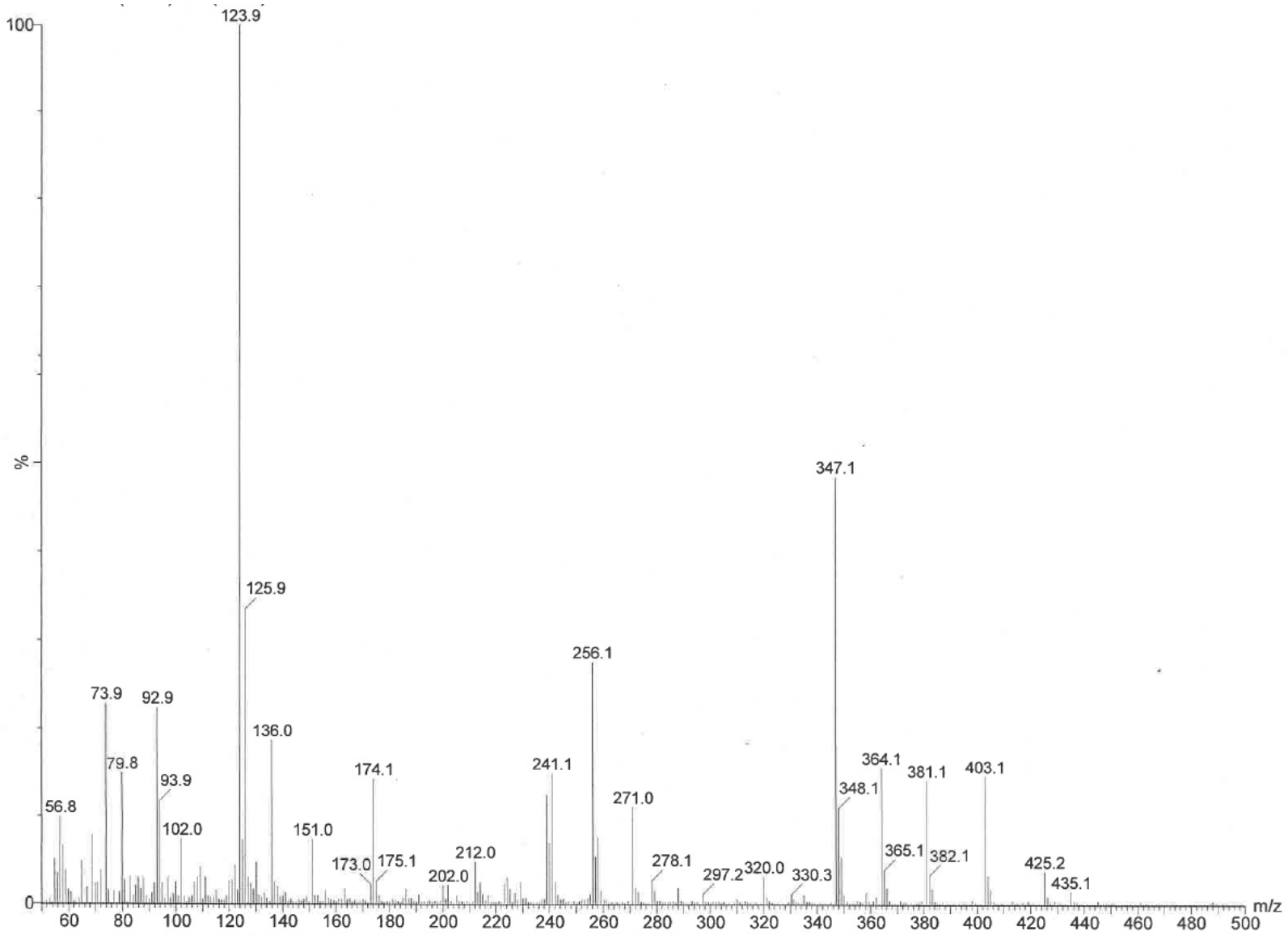
Supp. Fig. 1


a
Plating at 100,000 cells per well
% Cell death
10μM H2O2
50μM H2O2
100μM H2O2
10μM H2O2 with U0126
50μM H2O2 with U0126
100μM H2O2 with U0126
Plating at 400,000 cells per well
b
Starvation medium without H 2O2
(examining background effects of drugs)
% Cell death (+/- SD)
DMSO only
U0126
Tranetimib
U0124
Full medium conditions
H2O2 only
H2O2 + U0126
H2O2 + Tranetimib
H2O2 + U0124
c
200 μm
Before background substraction
After background substraction
d
Against 10μM H2O2
0.001
0.01
0.1
1
10
Concentration of U0126 (μM)

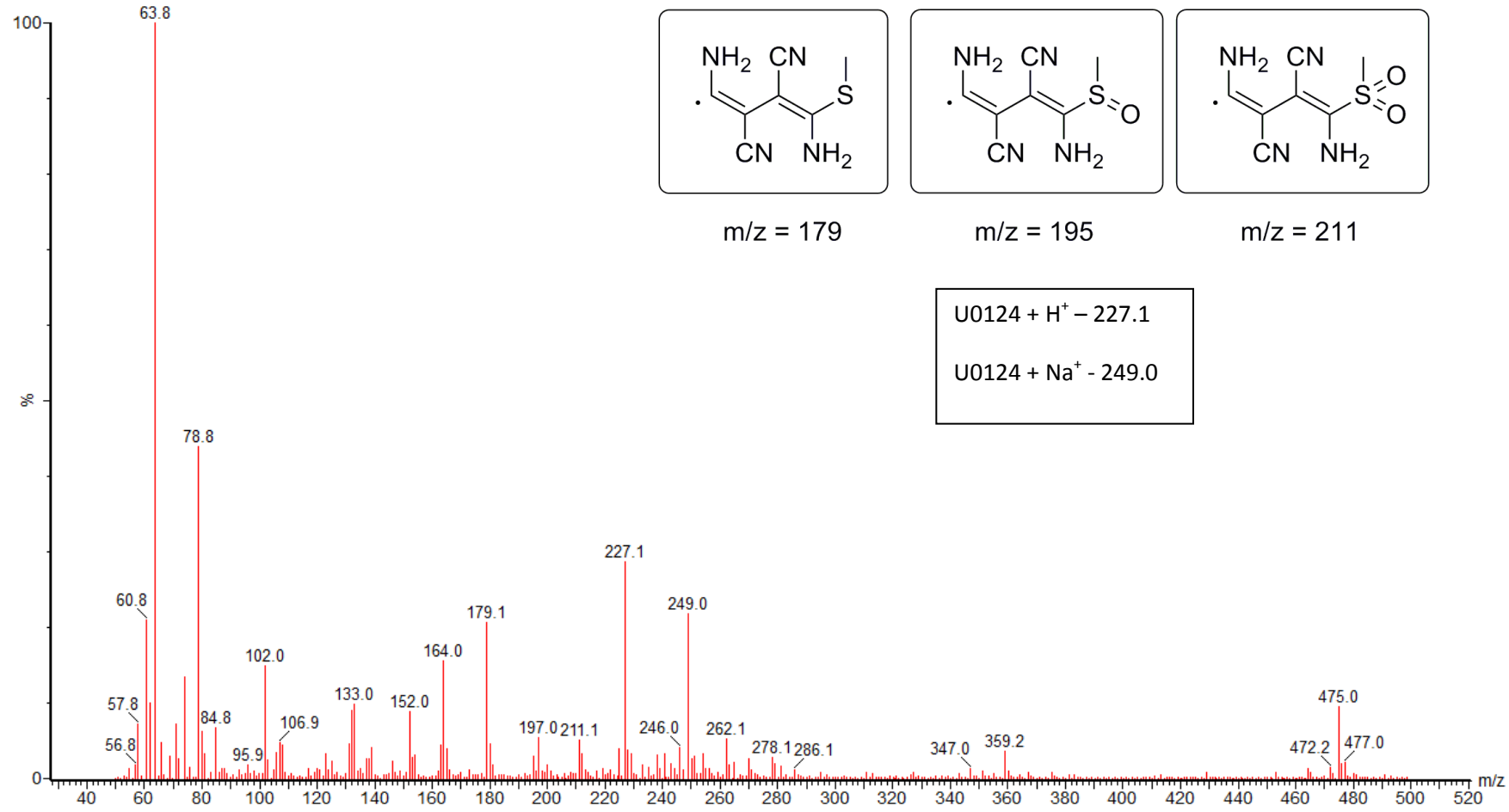
Supp. Fig. 2A (i)


100
%
0
123.9
73.9
56.7
126.0
72.7
92.9
84.8
136.0
174.0
347.1
241.1
256.1
279.1
102.0
54.7
51.7
151.0
212.0
175.1
224.1
280.1
320.1
348.0
381.1
403.1
435.0
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520

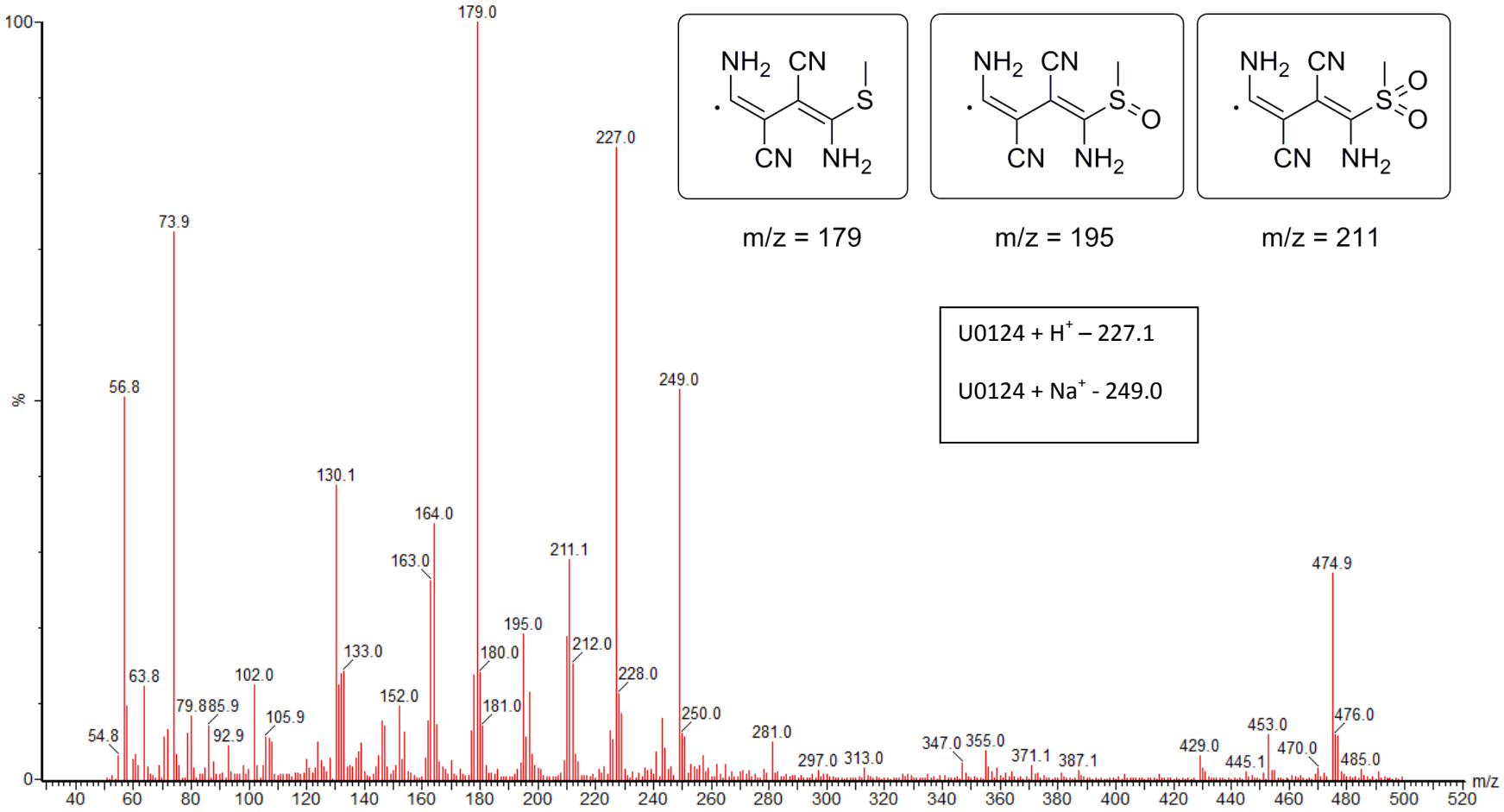
Supp. Fig. 2A (ii)



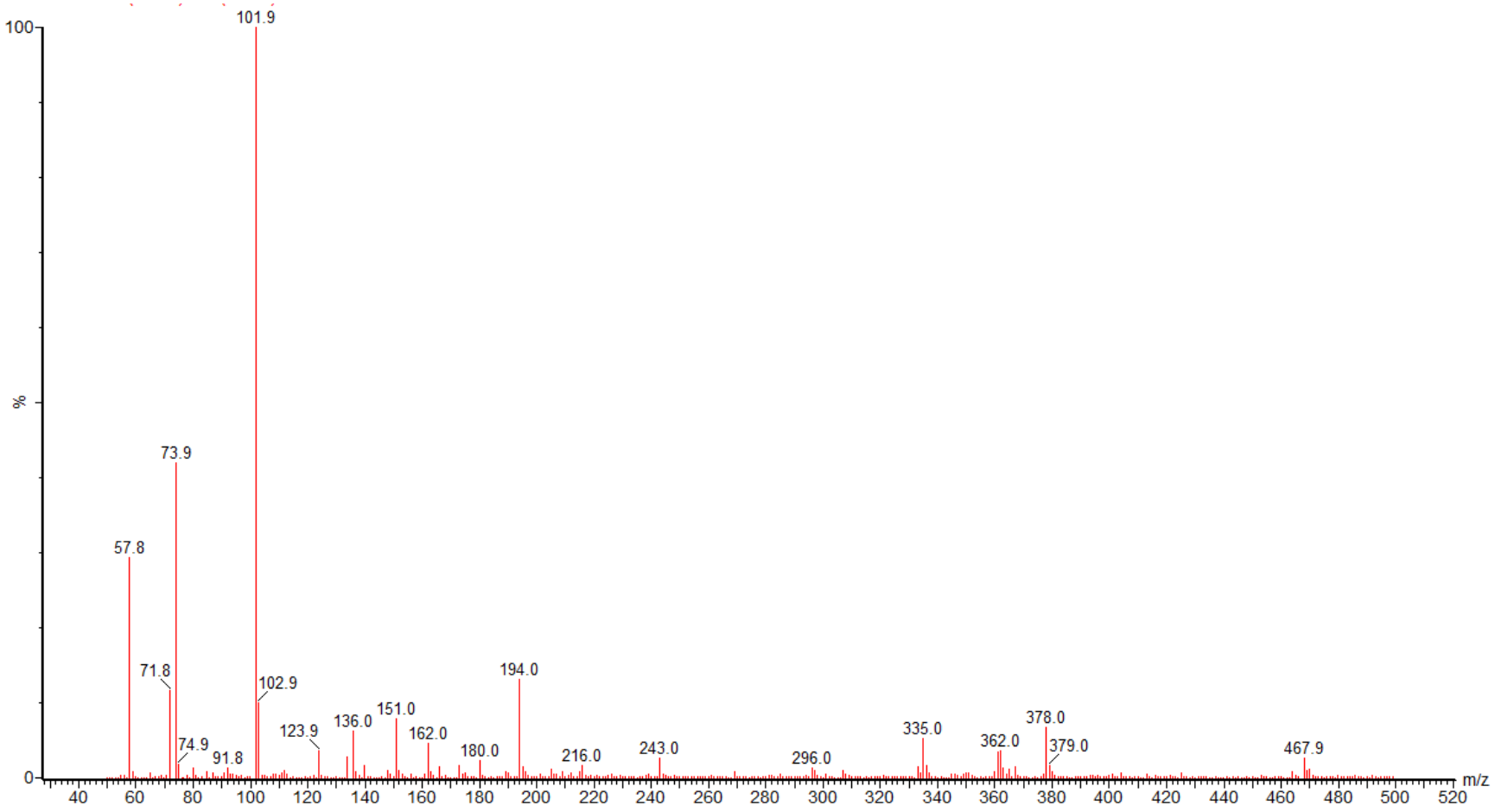
Supp. Fig. 2B (i)


m/z = 179
m/z = 195
m/z = 211
U0124 + H+ – 227.1
U0124 + Na+ - 249.0
100
%
0
m/z
63.8
78.8
60.8
57.8
56.8
84.8
95.9
102.0
106.9
133.0
152.0
164.0
179.1
197.0
211.1
227.1
246.0
249.0
262.1
278.1
286.1
347.0
359.2
472.2
475.0
477.0

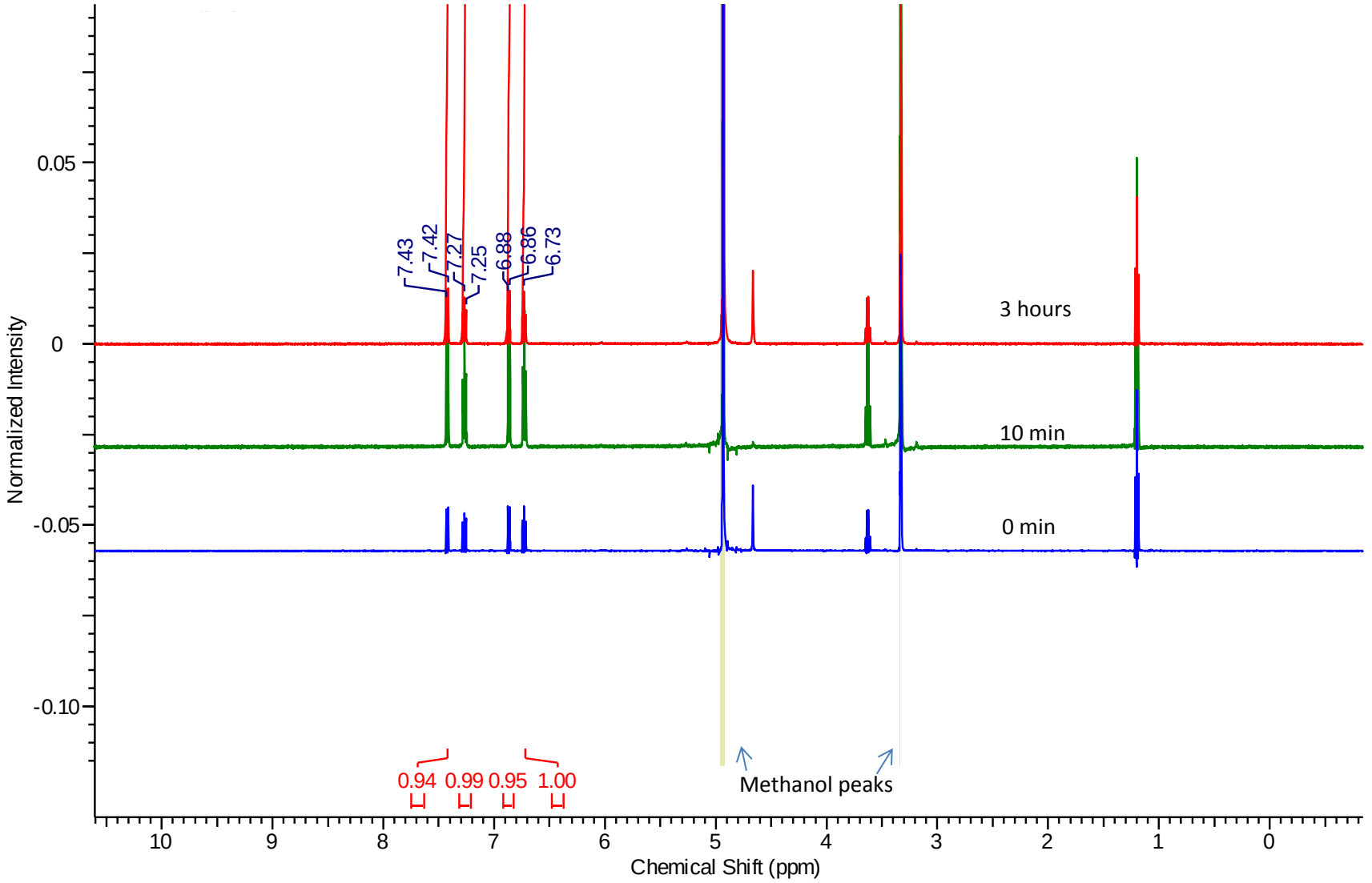
Supp. Fig. 2B (ii)



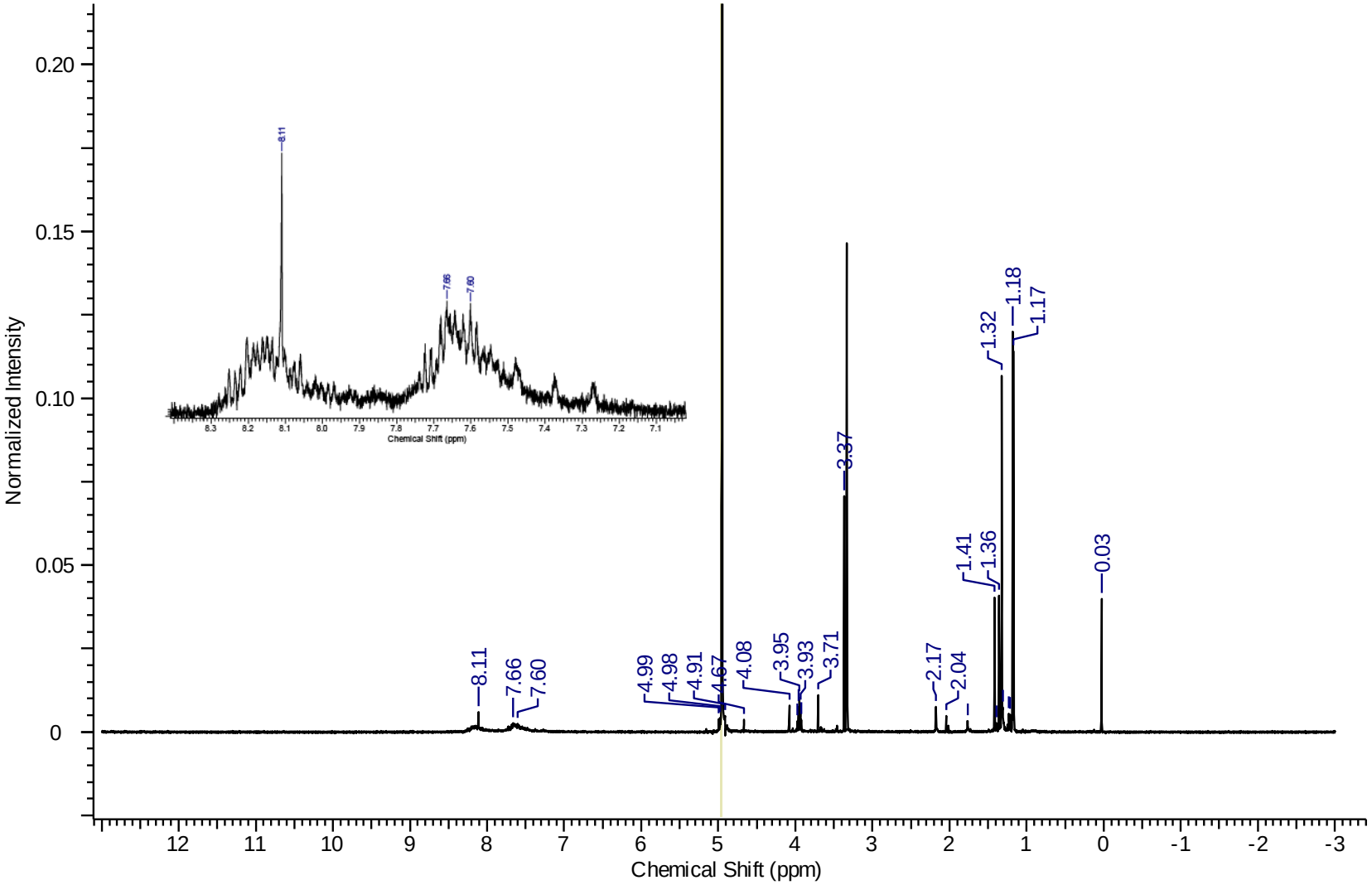
Supp. Fig. 2C


100
%
0
101.9
73.9
57.8
71.8
74.9
91.8
102.9
123.9
136.0
151.0
162.0
180.0
194.0
216.0
243.0
296.0
335.0
362.0
378.0
379.0
467.9
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
m/z

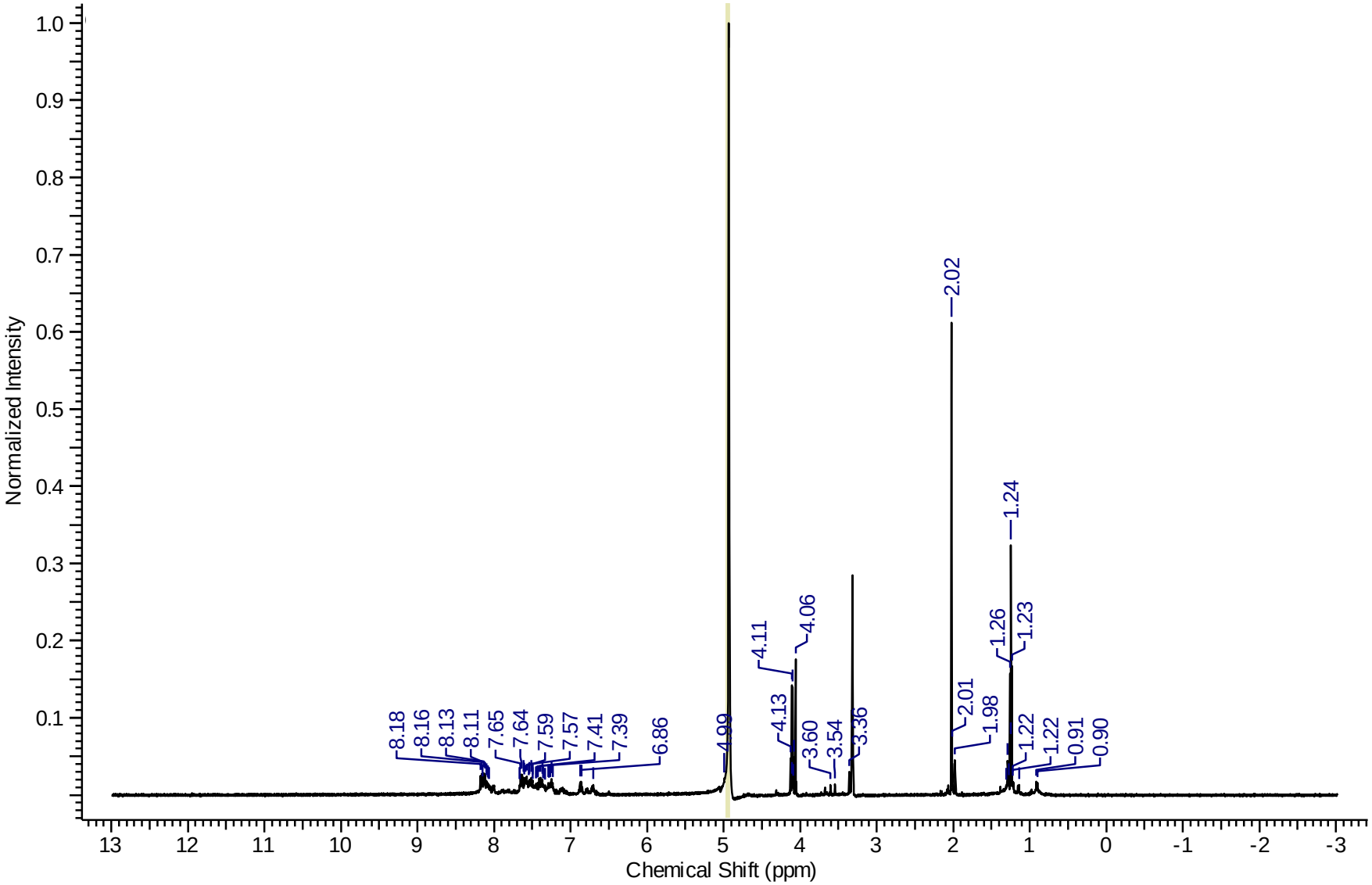
Supp. Fig. 3A


7.43
7.42
7.27
7.25
6.88
6.86
6.73
3 hours
10 min
0 min
0.94
0.99
0.95
1.00
Methanol peaks
Normalized Intensity
0.05
0
-0.05
-0.10
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

Supp. Fig. 3B


Normalized Intensity
0.20
0.15
0.10
0.05
0
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
Chemical Shift (ppm)
8.11
7.66
7.60
4.99
4.98
4.91
4.67
4.08
3.95
3.93
3.71
3.37
2.17
2.04
1.41
1.36
1.32
1.18
1.17
0.03

Supp. Fig. 3C


Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
8.18
8.16
8.13
8.11
7.65
7.64
7.59
7.57
7.41
7.39
6.86
4.99
4.11
4.13
4.06
3.60
3.54
3.36
2.02
2.01
1.98
1.26
1.24
1.23
1.22
1.22
0.91
0.90
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
Chemical Shift (ppm)