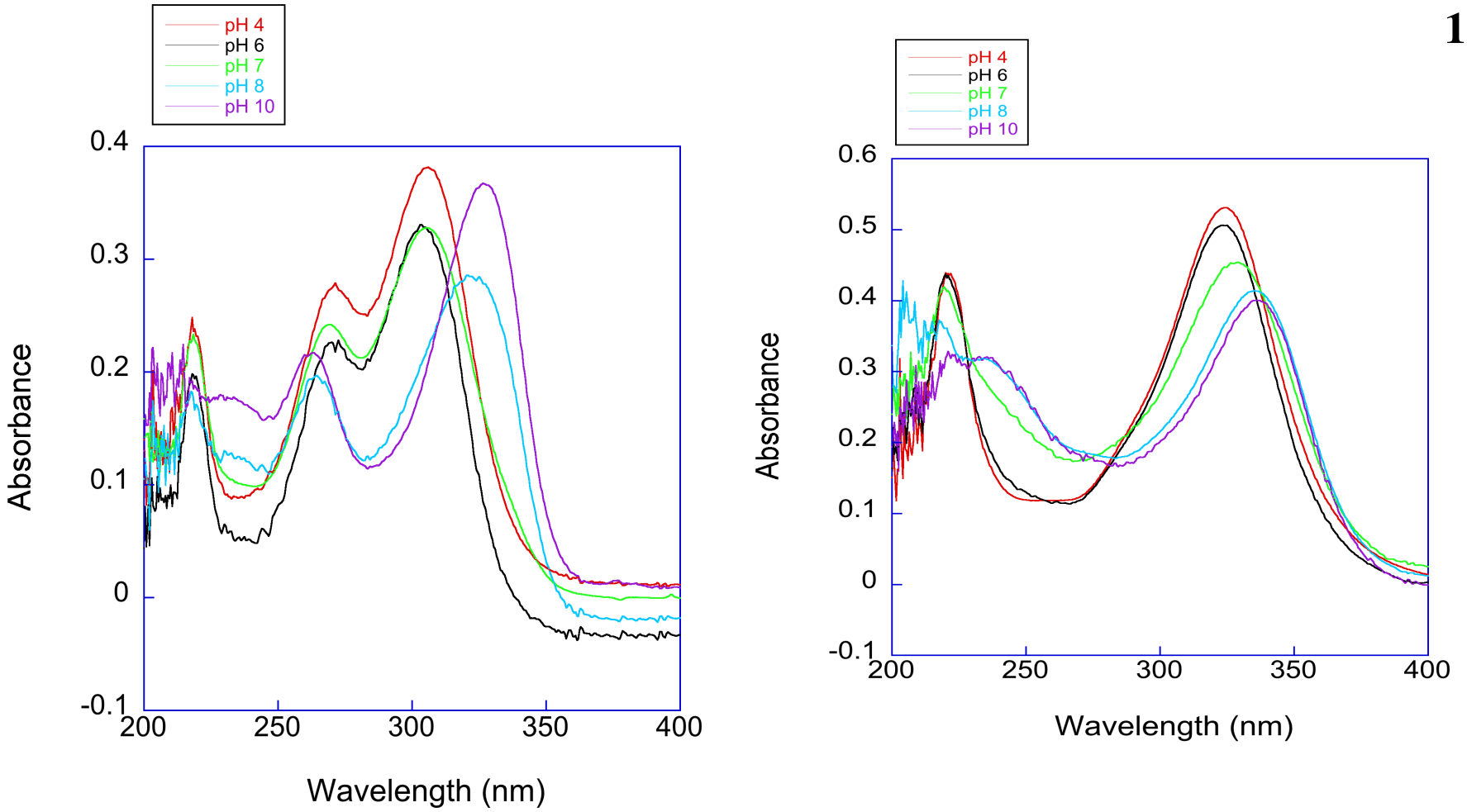


Figure S1: UV-vis scan of 25 μM s^2c^5Ura (left) or 25 μM se^2c^5Ura (right) as a function of pH.

Notes to Supplemental Data: For each reaction described for the ^{1}H-NMR experiments in Figures 6-10, a separate experiment was conducted such that the reaction was allowed to proceed for 2 min, after which it was flash frozen and then lyophilized. The solid was then submitted for LCMS analysis. Each peak in the chromatogram corresponds to a compound identified by ESI-MS. Each peak is either labeled with the name of the compound along with the corresponding m/z value, or with a mass spectrogram shown as an inset figure with peaks labeled with the corresponding structure and m/z value. The legend to each figure identifies the identity of the compounds along with the corresponding m/z value. The **red trace** in the LC is a water blank and the **blue trace** in the LC represents the injection from the experiment. In each blue trace there is a peak at ~1.5 min that is from the buffer. A dehydration reaction (loss of 18 amu) occurs for all of the uracil derivatives as identified by the mass spectra.

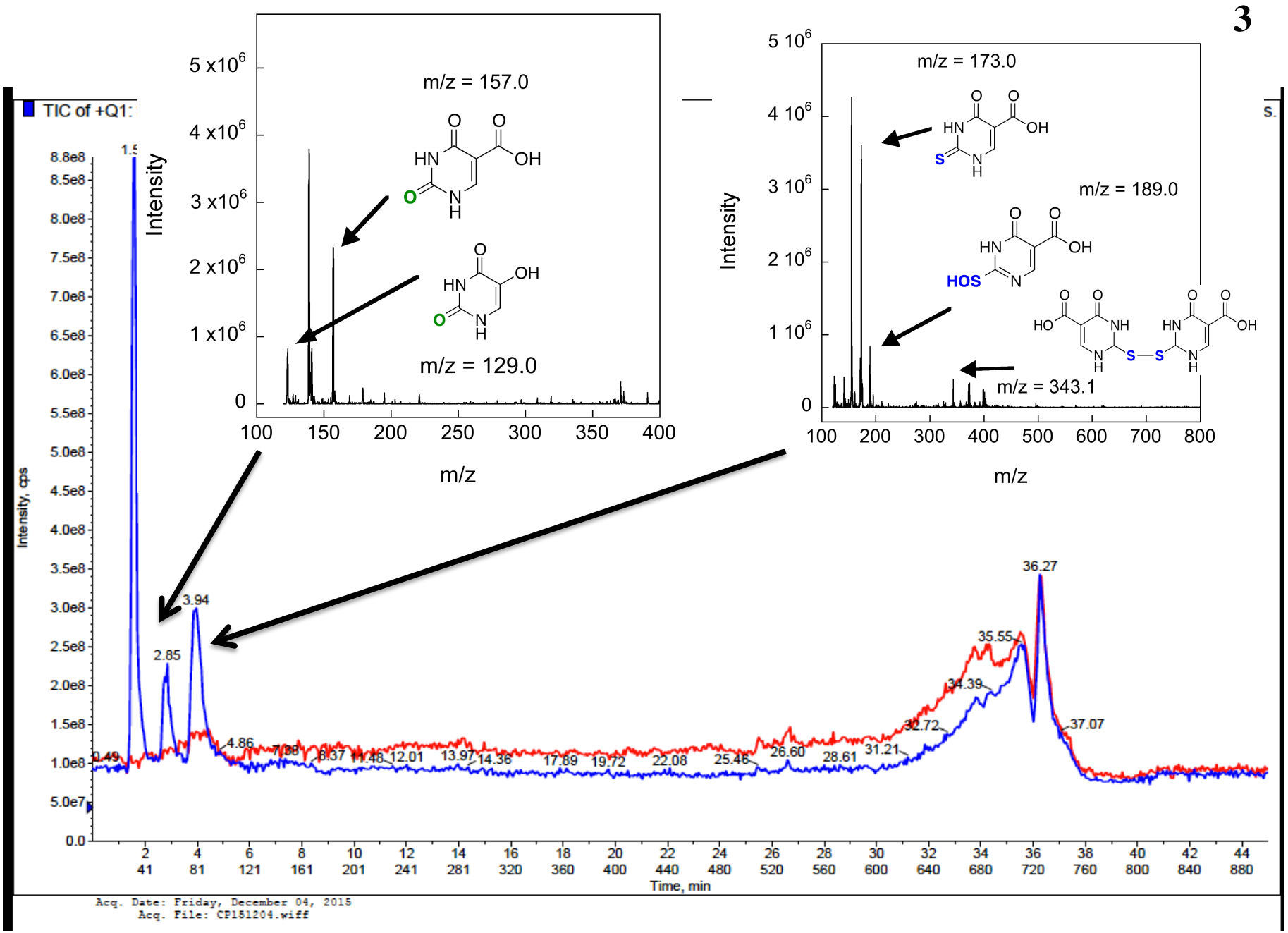

TIC of +Q1:
Intensity, cps
Time, min
Acq. Date: Friday, December 04, 2015
Acq. File: CP151204.wiff
m/z = 157.0
m/z = 129.0
m/z = 173.0
m/z = 189.0
m/z = 343.1
Intensity
m/z
HOS

Figure S2 (previous slide): 10 mM s^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min and then flash frozen. The reaction was then lyophilized and submitted for LCMS analysis. The peak at 2.85 min in the HPLC trace was a mixture of c^5Ura (m/z = 157.1) and the decarboxylated c^5Ura analog (m/z = 129.0 – structure shown) as identified in the mass spectrogram. The peak at 3.94 min in the HPLC trace contained a mixture of s^2c^5Ura (m/z = 173.0), the sulfenic acid form of s^2c^5Ura (m/z = 189.0), and the disulfide form of s^2c^5Ura (m/z = 343.1) as identified in the mass spectrogram.

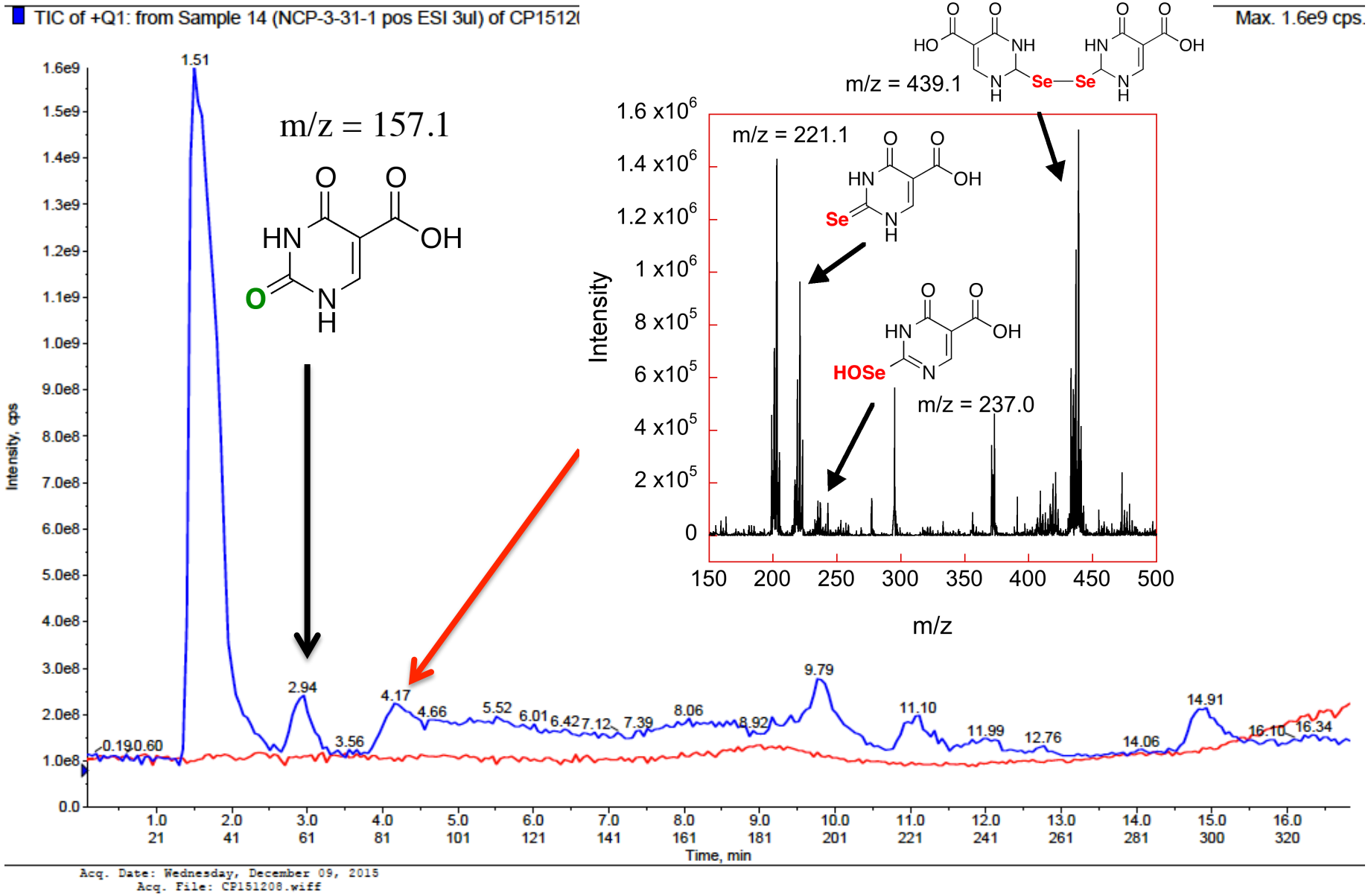
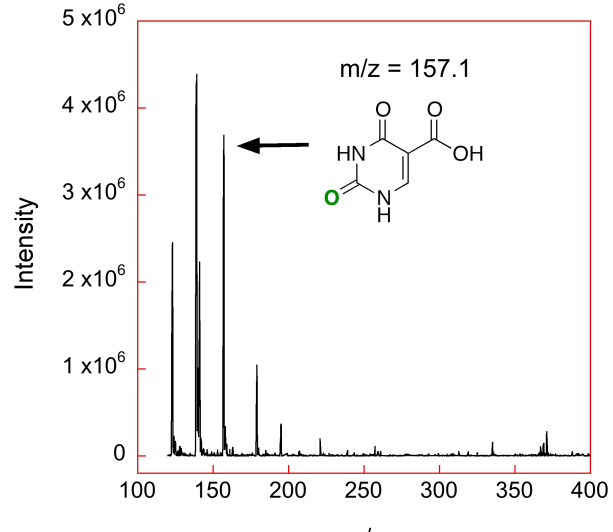
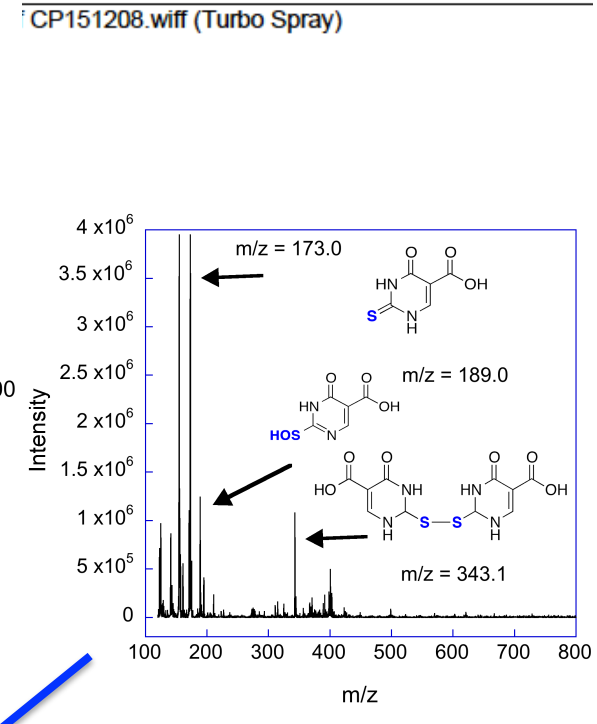
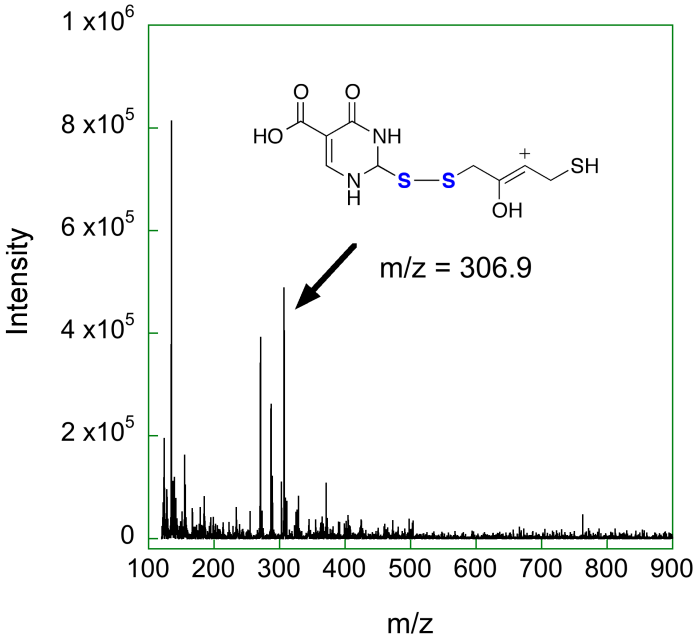

TIC of +Q1: from Sample 14 (NCP-3-31-1 pos ESI 3ul) of CP1512(
Max. 1.6e9 cps.
m/z = 157.1
m/z = 439.1
m/z = 221.1
m/z = 237.0
HOSe
Intensity
m/z
Intensity, cps
Time, min
1.51
2.94
3.56
4.17
4.66
5.52
6.01
6.42
7.12
7.39
8.06
8.92
9.79
11.10
11.99
12.76
14.06
14.91
16.10
16.34
0.19
0.60
Acq. Date: Wednesday, December 09, 2015
Acq. File: CP151208.wiff

Figure S3 (previous slide): 10 mM se^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.94 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 4.17 min in the HPLC trace contained a mixture of se^2c^5Ura (m/z = 221.1) and the diselenide form of se^2c^5Ura (m/z = 439.1) as identified in the mass spectrogram. The unique isotopic pattern of selenium was clearly visible in the mass spectrogram. There were a number of other small peaks in the LC trace that could not be identified.


m/z = 157.1
Intensity
m/z


CP151208.wiff (Turbo Spray)
m/z = 173.0
m/z = 189.0
m/z = 343.1
Intensity
m/z


m/z = 306.9
Intensity
m/z

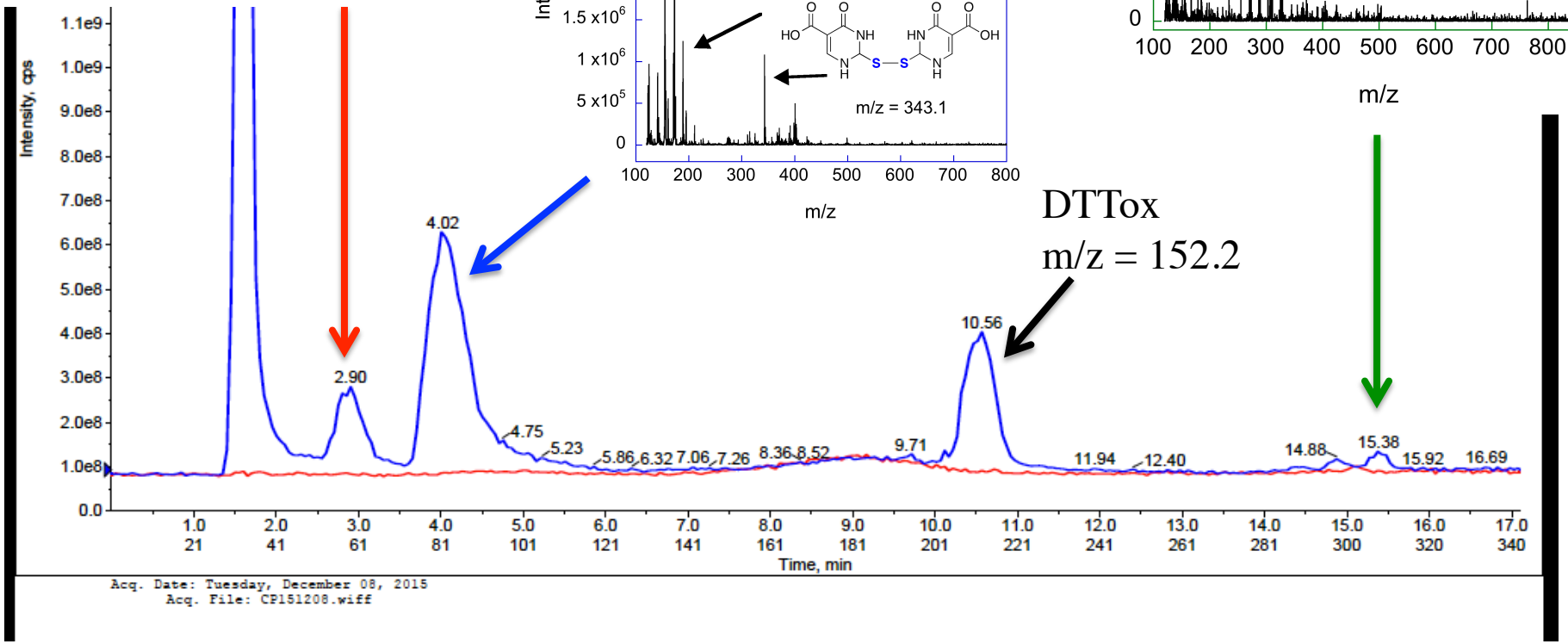

DTTox
m/z = 152.2
Intensity, cps
Time, min
Acq. Date: Tuesday, December 08, 2015
Acq. File: CP151208.wiff

Figure S4 (previous slide): 10 mM s^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer, pH 7.4 for 2 min, after which 10 mM DTT (dissolved in the same buffer) was added and the reaction was allowed to proceed for a further 2 min. The reaction was then flash frozen, lyophilized, and then submitted for LCMS analysis. The peak at 2.90 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 4.02 min in the HPLC trace contains a mixture of s^2c^5Ura (m/z = 173.0), the sulfenic acid form of s^2c^5Ura (m/z = 189.0), and the disulfide form of s^2c^5Ura (m/z = 343.1) as identified in the mass spectrogram. The peak at 10.56 min in the HPLC trace was identified as the oxidized form of DTT (m/z = 152.2 – mass spectrogram not shown). Last, the peak at 15.38 min in the HPLC trace is identified as the mixed disulfide between s^2c^5Ura and DTT (m/z = 306.9).

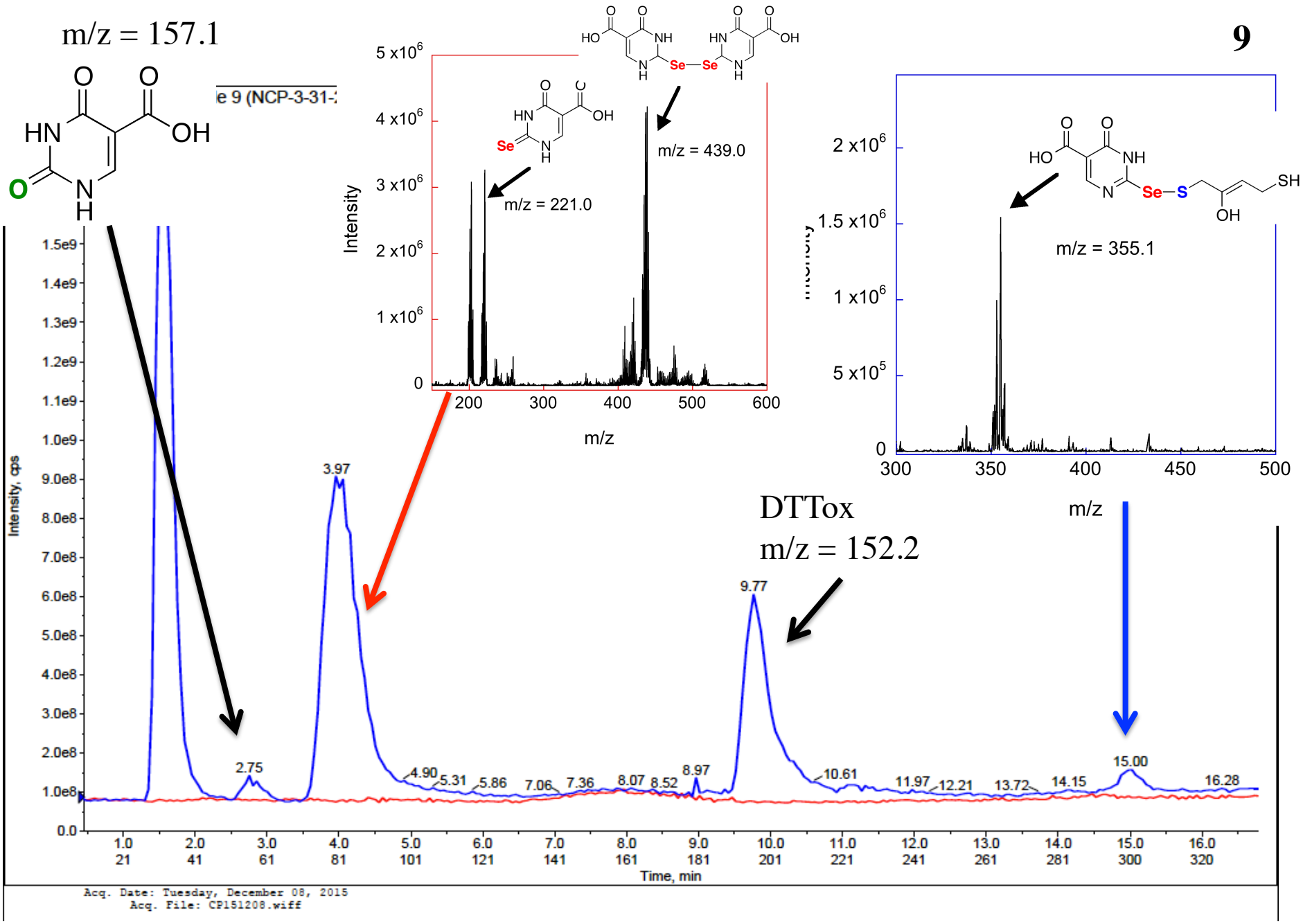

m/z = 157.1
e 9 (NCP-3-31-
m/z = 439.0
m/z = 221.0
Intensity
m/z
m/z = 355.1
DTTox
m/z = 152.2
m/z
Intensity, cps
Time, min
2.75
3.97
4.90
5.31
5.86
7.06
7.36
8.07
8.52
8.97
9.77
10.61
11.97
12.21
13.72
14.15
15.00
16.28
Acq. Date: Tuesday, December 08, 2015
Acq. File: CP151208.wiff

Figure S5 (previous slide): 10 mM se^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min, after which 10 mM DTT (dissolved in the same buffer) was added and the reaction was allowed to proceed for a further 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.75 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 3.97 min in the HPLC trace contains a mixture of se^2c^5Ura (m/z = 221.0) and the diselenide form of se^2c^5Ura (m/z = 439.0) as identified in the mass spectrogram. The peak at 9.77 min in the HPLC trace was identified as the oxidized form of DTT (m/z = 152.2). The peak at 15 min in the HPLC trace was identified as the mixed selenosulfide between se^2c^5Ura and DTT (m/z = 355.1 as shown in the mass spectrogram of the inset).

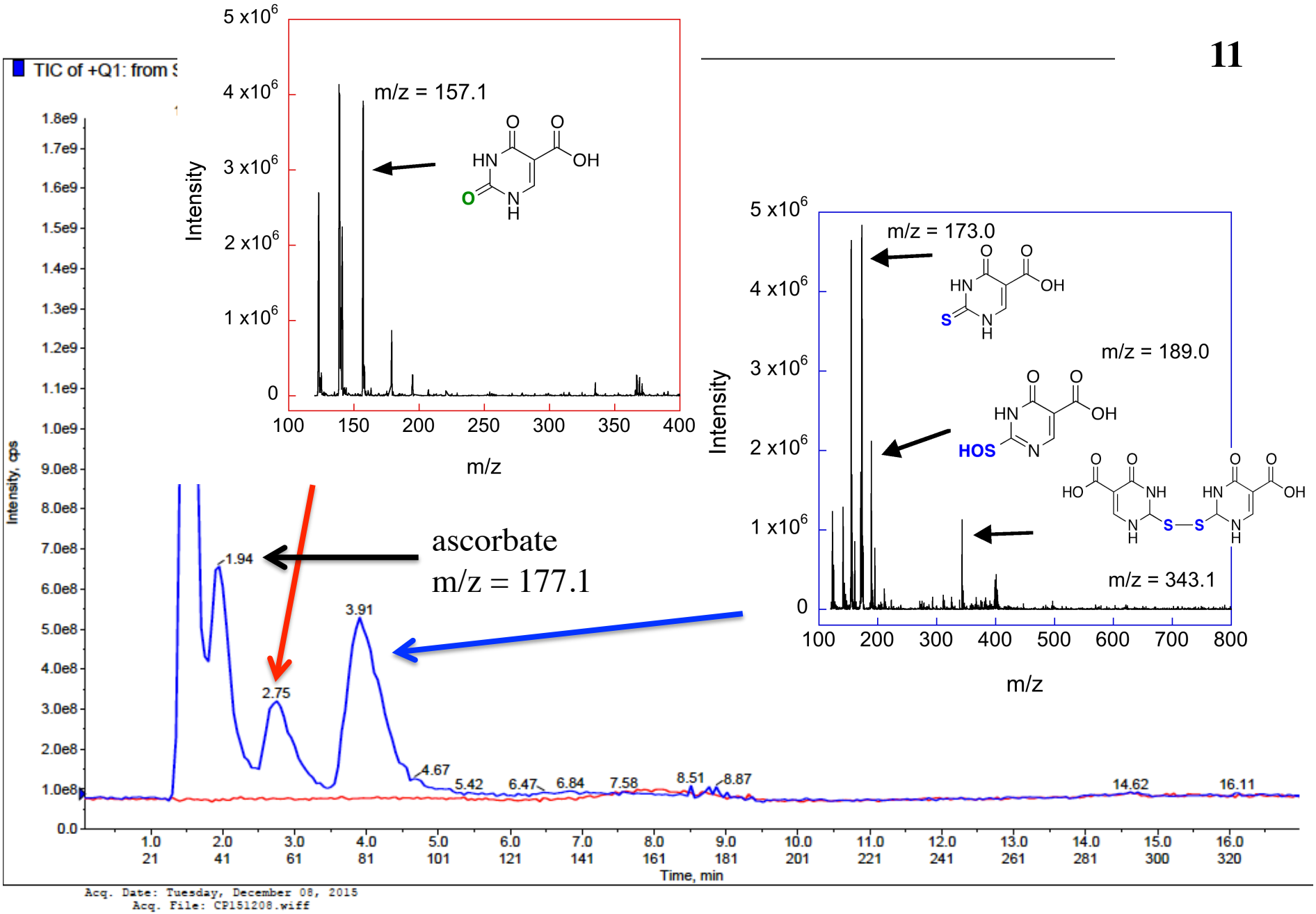

TIC of +Q1: from S
Intensity, cps
1.8e9
1.7e9
1.6e9
1.5e9
1.4e9
1.3e9
1.2e9
1.1e9
1.0e9
9.0e8
8.0e8
7.0e8
6.0e8
5.0e8
4.0e8
3.0e8
2.0e8
1.0e8
0.0
1.94
2.75
3.91
4.67
5.42
6.47
6.84
7.58
8.51
8.87
14.62
16.11
1.0 21
2.0 41
3.0 61
4.0 81
5.0 101
6.0 121
7.0 141
8.0 161
9.0 181
10.0 201
11.0 221
12.0 241
13.0 261
14.0 281
15.0 300
16.0 320
Time, min
Acq. Date: Tuesday, December 08, 2015
Acq. File: CP151208.wiff
ascorbate
m/z = 177.1
Intensity
5 x10^6
4 x10^6
3 x10^6
2 x10^6
1 x10^6
0
m/z = 157.1
HN
O
O
OH
N
H
100 150 200 250 300 350 400
m/z
Intensity
5 x10^6
4 x10^6
3 x10^6
2 x10^6
1 x10^6
0
m/z = 173.0
HN
S
O
O
OH
N
H
m/z = 189.0
HOS
HN
O
O
OH
N
m/z = 343.1
HO
NH
S
S
HN
OH
100 200 300 400 500 600 700 800
m/z

Figure S6 (previous slide): 10 mM s^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min, after which 10 mM ascorbate (dissolved in the same buffer) was added and the reaction was allowed to proceed for a further 2 min. The reaction was then flash frozen, lyophilized, and then submitted for LCMS analysis. The peak at 1.94 min in the HPLC trace was identified as ascorbate (m/z = 177.1). The peak at 2.75 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 3.91 min in the HPLC trace contained a mixture of s^2c^5Ura (m/z = 173.0), the sulfenic acid form of s^2c^5Ura (m/z = 189.0), and the disulfide form of s^2c^5Ura (m/z = 343.1) as identified in the mass spectrogram.

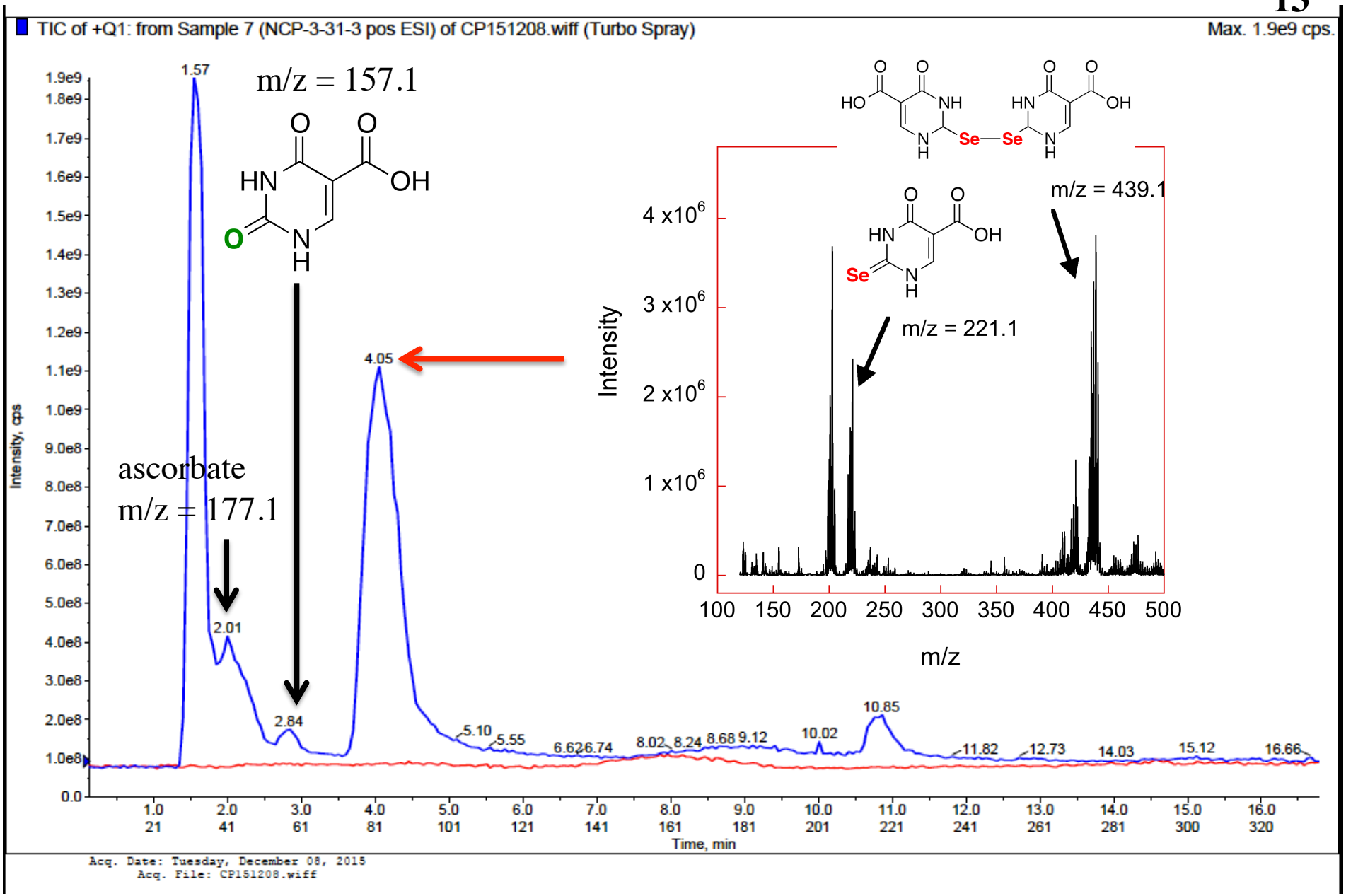

TIC of +Q1: from Sample 7 (NCP-3-31-3 pos ESI) of CP151208.wiff (Turbo Spray)
Max. 1.9e9 cps.
m/z = 157.1
ascorbate
m/z = 177.1
m/z = 439.1
m/z = 221.1
Intensity
m/z
Intensity, cps
Time, min
1.57
2.01
2.84
4.05
5.10
5.55
6.62
6.74
8.02
8.24
8.68
9.12
10.02
10.85
11.82
12.73
14.03
15.12
16.66
Acq. Date: Tuesday, December 08, 2015
Acq. File: CP151208.wiff

Figure S7 (previous slide): 10 mM se^2c^5Ura was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min, after which 10 mM ascorbate (dissolved in the same buffer) was added and the reaction was allowed to proceed for a further 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.10 min in the HPLC trace was identified as ascorbate (m/z = 177.1). The peak at 2.84 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 4.05 min in the HPLC trace contains a mixture of se^2c^5Ura (m/z = 221.1) and the diselenide form of se^2c^5Ura (m/z = 439.1) as identified in the mass spectrogram. The peak at 10.85 min in the HPLC trace could not be identified by mass analysis.

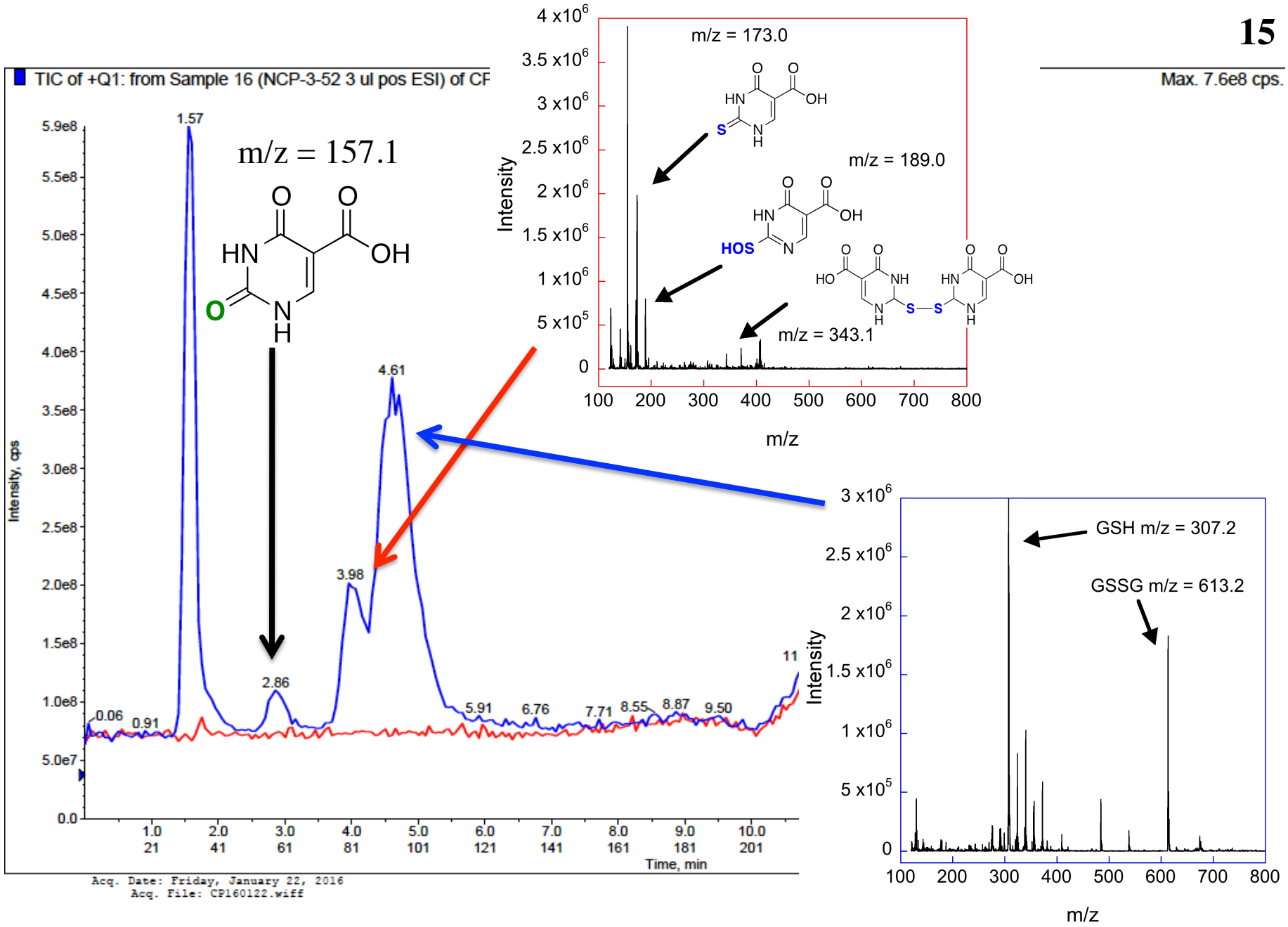

TIC of +Q1: from Sample 16 (NCP-3-52 3 ul pos ESI) of CF
Max. 7.6e8 cps.
m/z = 157.1
m/z = 173.0
m/z = 189.0
m/z = 343.1
GSH m/z = 307.2
GSSG m/z = 613.2
Intensity, cps
Intensity
m/z
Time, min
1.57
4.61
3.98
2.86
0.06
0.91
5.91
6.76
7.71
8.55
8.87
9.50
11
Acq. Date: Friday, January 22, 2016
Acq. File: CP160122.wiff

Figure S8 (previous slide): 10 mM s^2c^5Ura that was **preincubated** with 10 mM GSH, was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.86 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 3.98 min in the HPLC trace contained a mixture of s^2c^5Ura (m/z = 173.0), the sulfenic acid form of s^2c^5Ura (m/z = 189.0), and the disulfide form of s^2c^5Ura (m/z = 343.1) as identified in the mass spectrogram. The peak at 4.61 min in the HPLC trace was identified as a mixture of GSH (m/z = 307.2) and GSSG (m/z = 613.2). GSH = reduced glutathione and GSSG = oxidized glutathione.

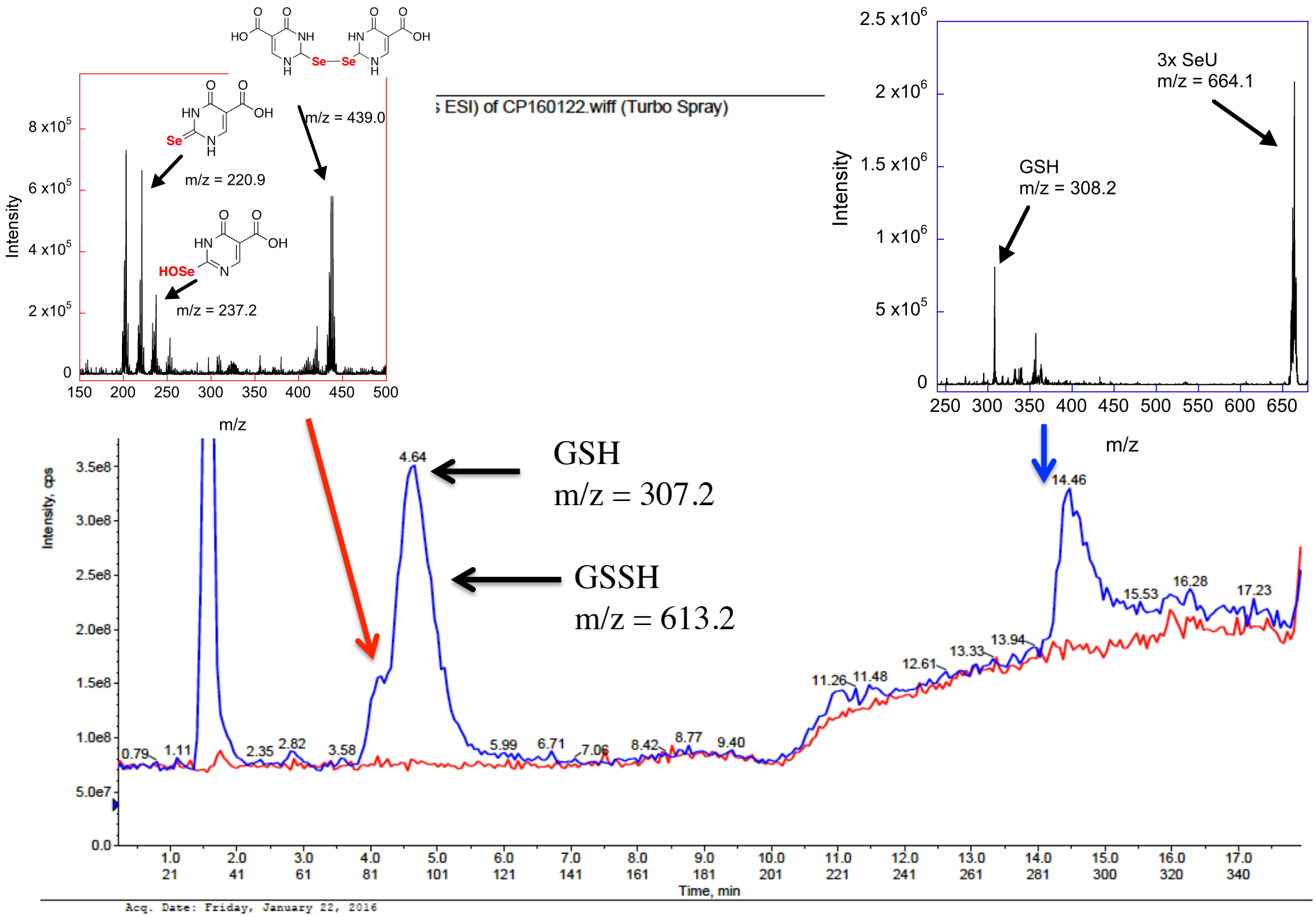

ESI) of CP160122.wiff (Turbo Spray)
m/z = 439.0
m/z = 220.9
m/z = 237.2
Intensity
m/z
3x SeU
m/z = 664.1
GSH
m/z = 308.2
GSH
m/z = 307.2
GSSH
m/z = 613.2
Intensity, cps
Time, min
Acq. Date: Friday, January 22, 2016

Figure S9 (previous slide): 10 mM se^2c^5Ura that was **preincubated** with 10 mM GSH, was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 4.10 min in the HPLC trace contained a mixture of se^2c^5Ura (m/z = 221.0), the selenenic acid form of se^2c^5Ura (m/z = 237.1), and the diselenide form of se^2c^5Ura (m/z = 439.0) as identified in the mass spectrogram. The peak at 4.64 min in the HPLC trace was identified as a mixture of GSH (m/z = 307.2) and GSSG (m/z = 613.2). GSH = reduced glutathione and GSSG = oxidized glutathione. The peak at 14.46 min in the HPLC trace was tentatively identified as a trimer of se^2c^5Ura (m/z = 646.1). We cannot be definitive about the identification, although the selenium isotope pattern is clearly visible in the mass spectrogram.

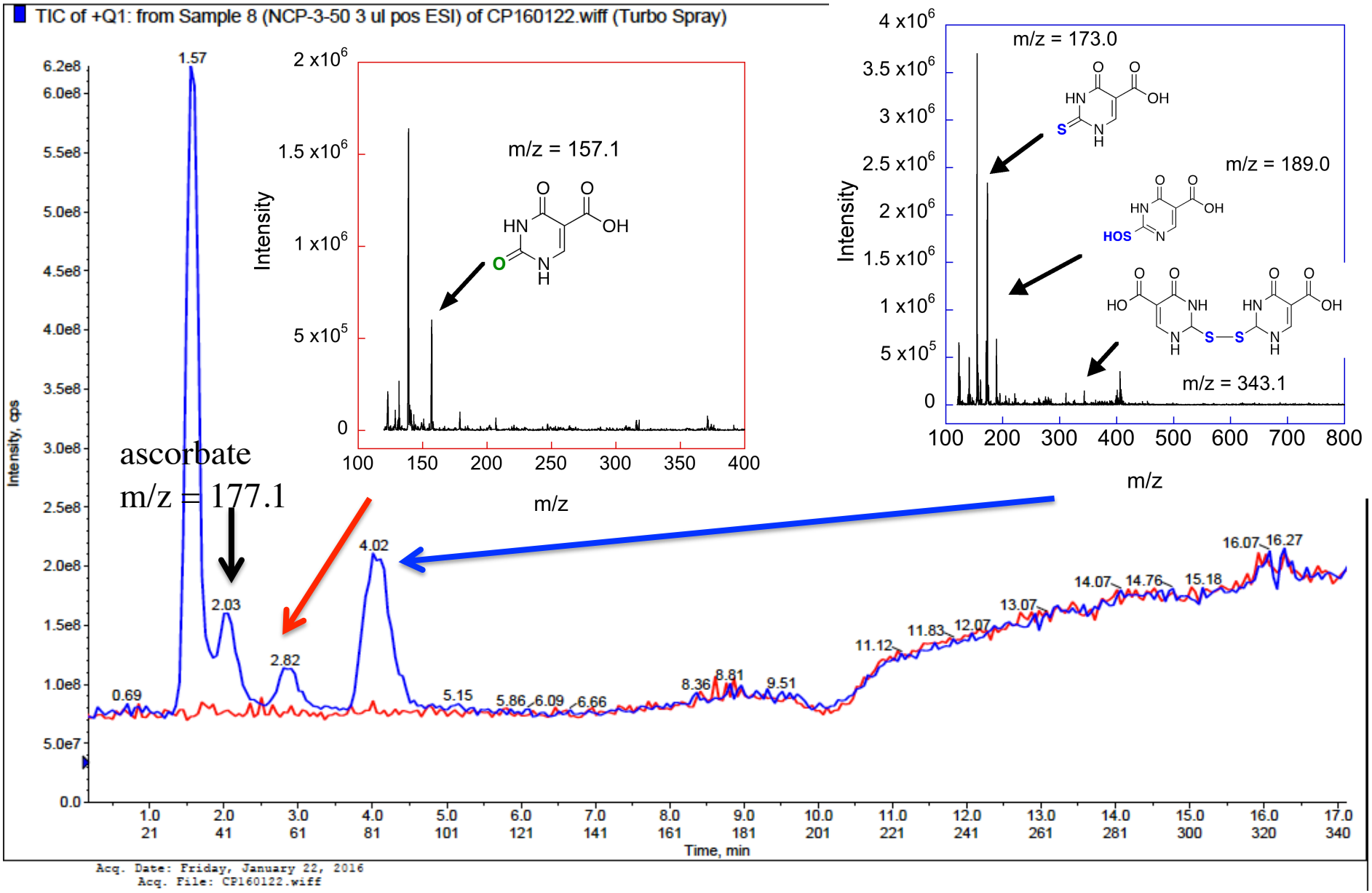

TIC of +Q1: from Sample 8 (NCP-3-50 3 ul pos ESI) of CP160122.wiff (Turbo Spray)
Intensity, cps
Time, min
ascorbate
m/z = 177.1
m/z = 157.1
m/z = 173.0
m/z = 189.0
m/z = 343.1
Intensity
m/z
Acq. Date: Friday, January 22, 2016
Acq. File: CP160122.wiff

Figure S10 (previous slide): 10 mM s^2c^5Ura that was **preincubated** with 10 mM ascorbate, was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.03 min in the HPLC trace is identified as ascorbate (m/z = 177.1). The peak at 2.82 min in the HPLC trace was identified in the mass spectrogram as c^5Ura (m/z = 157.1). The peak at 4.02 min in the HPLC trace is identified as a mixture of s^2c^5Ura (m/z = 173.0), the sulfenic acid form of s^2c^5Ura at (m/z = 189.0), and the disulfide form of s^2c^5Ura (m/z = 343.1) as identified in the mass spectrogram.

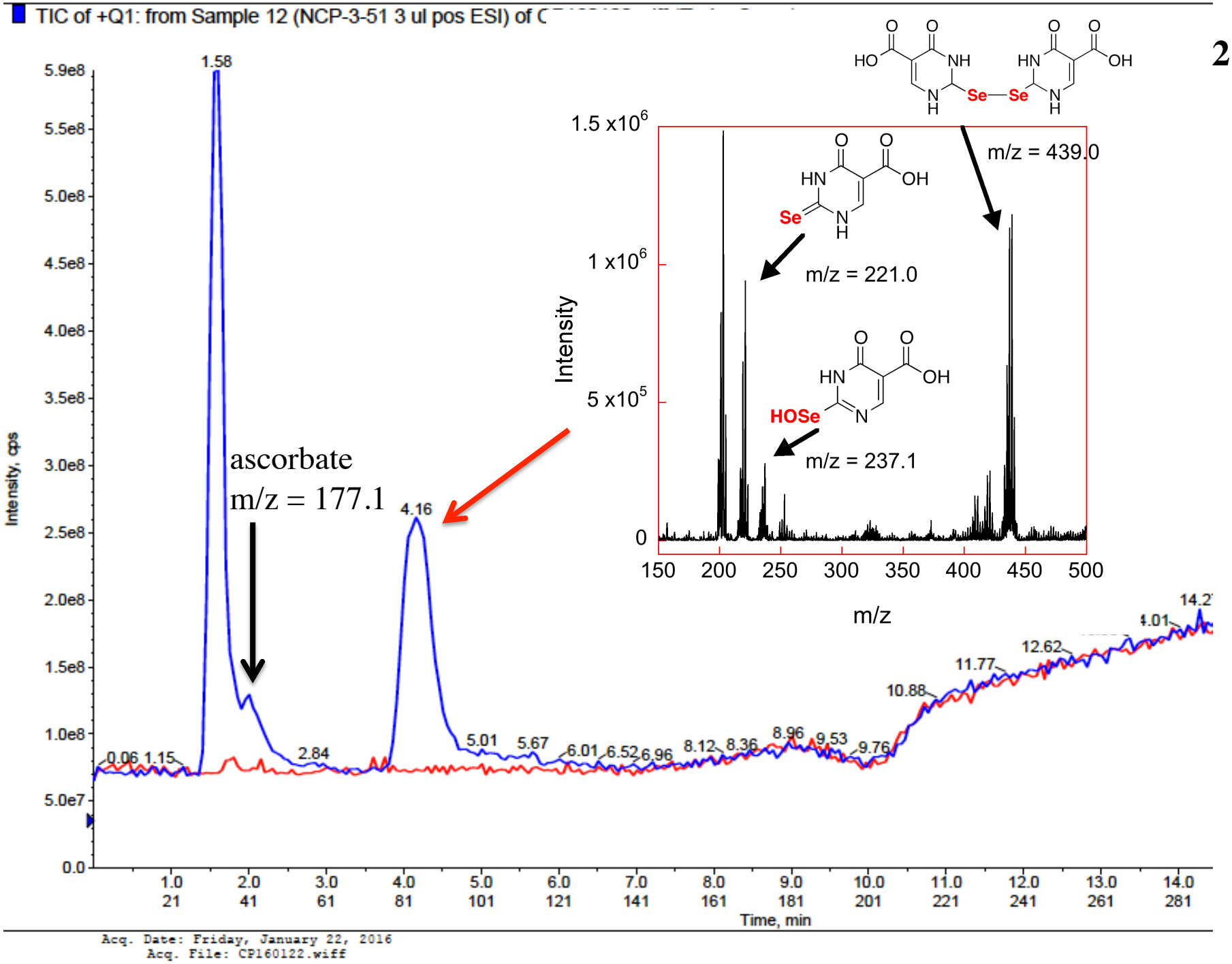

TIC of +Q1: from Sample 12 (NCP-3-51 3 ul pos ESI) of C
Intensity, cps
5.9e8
5.5e8
5.0e8
4.5e8
4.0e8
3.5e8
3.0e8
2.5e8
2.0e8
1.5e8
1.0e8
5.0e7
0.0
1.58
4.16
ascorbate
m/z = 177.1
0.06
1.15
2.84
5.01
5.67
6.01
6.52
6.96
8.12
8.36
8.96
9.53
9.76
10.88
11.77
12.62
14.01
14.2
1.0 21
2.0 41
3.0 61
4.0 81
5.0 101
6.0 121
7.0 141
8.0 161
9.0 181
10.0 201
11.0 221
12.0 241
13.0 261
14.0 281
Time, min
Acq. Date: Friday, January 22, 2016
Acq. File: CP160122.wiff
1.5 x10^6
1 x10^6
5 x10^5
0
Intensity
150 200 250 300 350 400 450 500
m/z
m/z = 439.0
m/z = 221.0
m/z = 237.1
HO
NH
HN
OH
Se
Se
HOSe
N
H
O

Figure S11 (previous slide): 10 mM se^2c^5Ura that was **preincubated** with 10 mM ascorbate was treated with 10 mM H_2O_2 in 100 mM potassium phosphate buffer pH 7.4, for 2 min. The reaction was then flash frozen, lyophilized, and submitted for LCMS analysis. The peak at 2.01 min in the HPLC trace was identified as ascorbate (m/z = 177.1). The peak at 4.16 min in the HPLC trace was identified as a mixture of se^2c^5Ura (m/z = 221.0), the selenenic acid form of se^2c^5Ura (m/z = 237.1), and the diselenide form of se^2c^5Ura (m/z = 439.0) in the mass spectrogram.

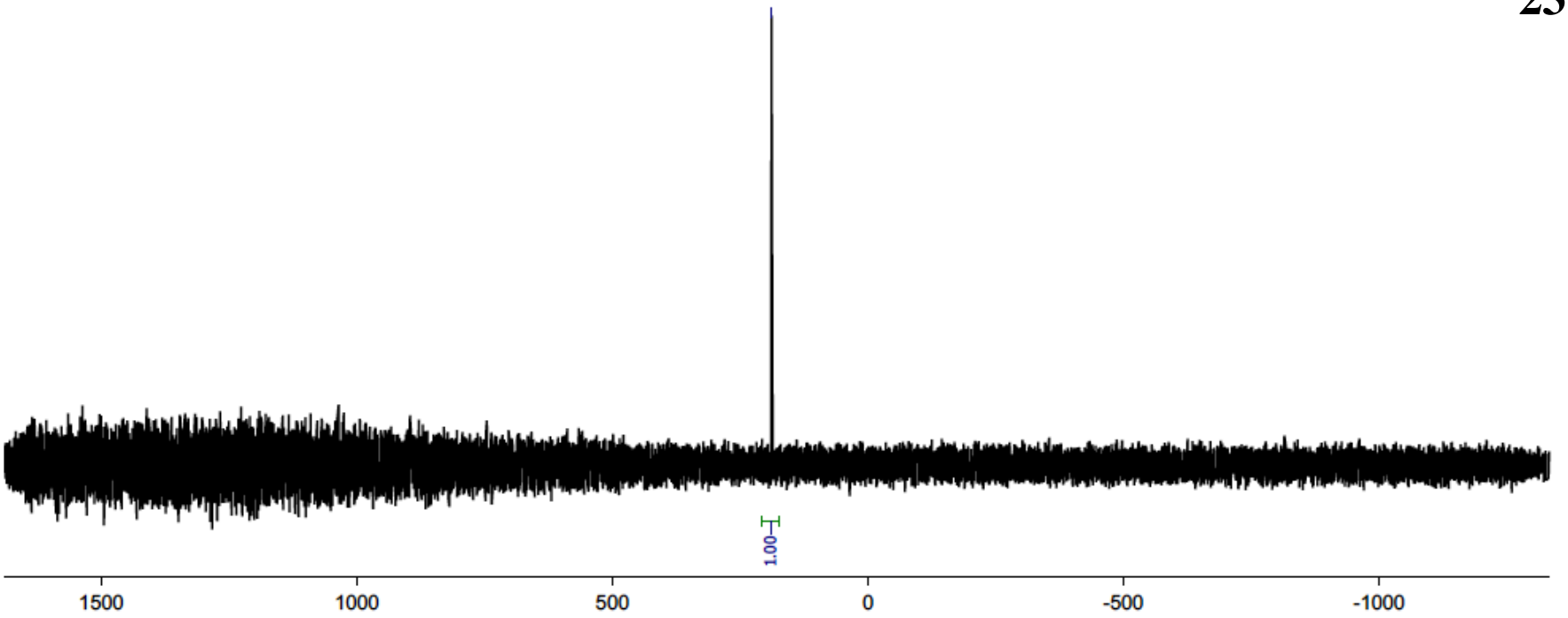


Figure S12: Full ^{77}Se-NMR spectrum of the reaction shown in Figure 12 of the text after 18 hours of reaction. Only the resonance corresponding to se^2c^5Ura is visible at 188.5 ppm.