ASP Analysis Of The First Significant Positive Wild Sample

The following is an appendix to the Food Standards Agency (FSA) Research Project S14017: Pilot study on the incidence of algal toxins (ASP, DSP and PSP) in Scottish Brown Crab (*Cancer pagurus*), hereafter referred to as the main report or by the abbreviation MR. Section 4.3.2.1 of the main report refers to a pooled sample of 20 crabs (sample identification FSA04-011105-Pool-B/W). The approximate collection site can be seen in Appendix 02 (Sampling Areas).

Analysis of this potentially negative material by HPLC-PDA-UV (Figure 1 to Figure 9) revealed the presence of a DA peak in the brown meat that was quantified at a mean value of 25.3µg/g (the regulatory limit is currently 20µg/g). The white meat from this sample did not show any presence of DA. The DA content of this wild sample was confirmed internally by MS/MS and was externally confirmed by Michael Quilliam's group by LC-MS/MS (Table 1 and Figure 10 to Figure 14). Table 1 shows the summary of Results, the retention times and MRM ratios all match – therefore DA is confirmed (Ratio of results Quillum slide 3 AP14000_Scottish DA Summary 051201). The independent verification of the presence of DA confirms the suspicion that wild brown crabs have the ability to accumulate ASP toxins.

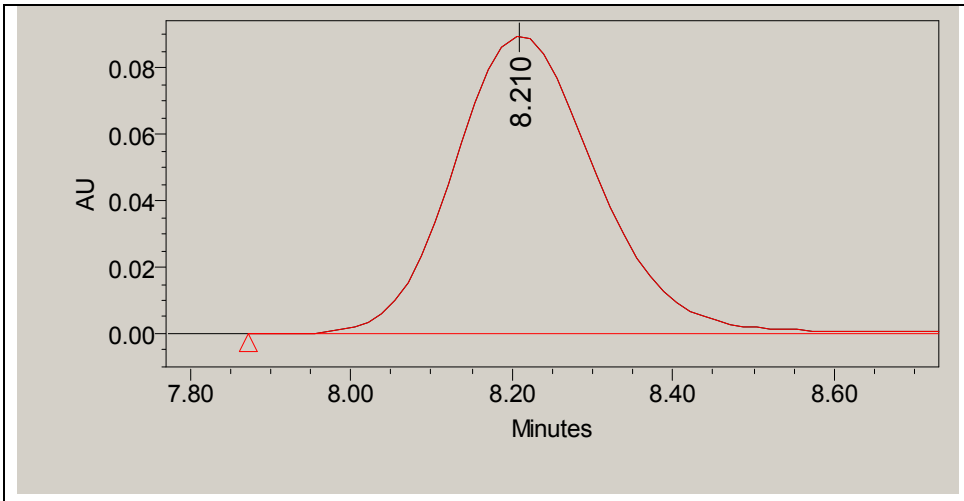


Figure 1: Chromatogram Cal Sol 1

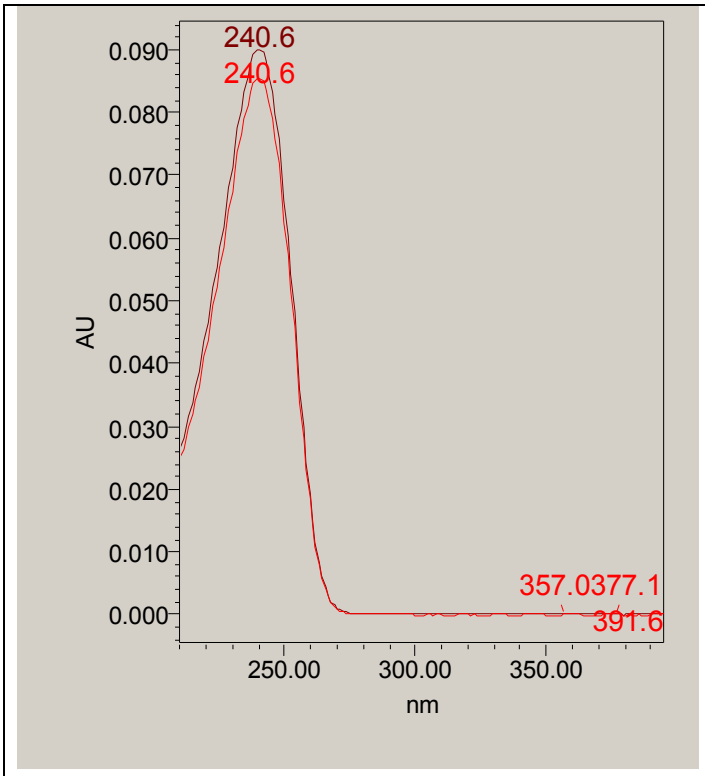


Figure 2: Spectrum for Cal Sol 1 and library DA spectrum. Axis have NOT been normalized

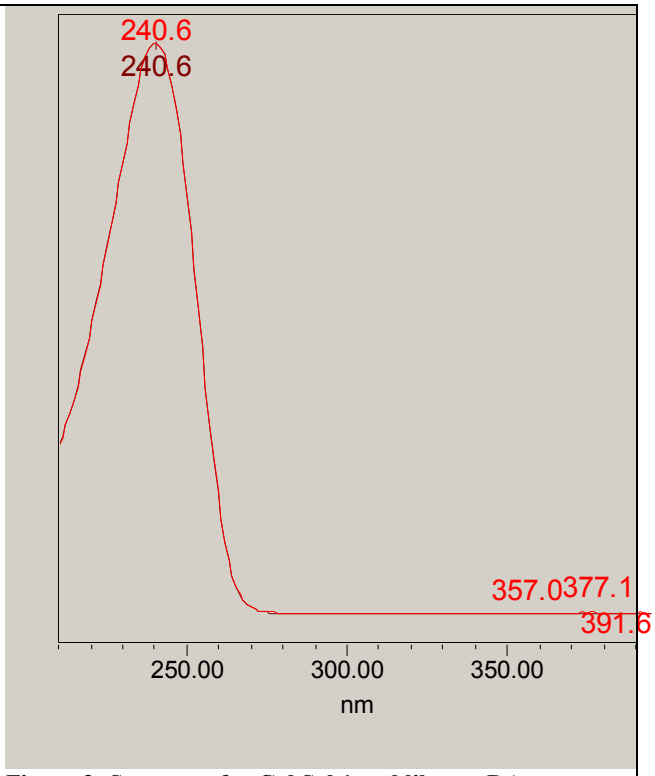


Figure 3: Spectrum for Cal Sol 1 and library DA spectrum. Axis have been normalised

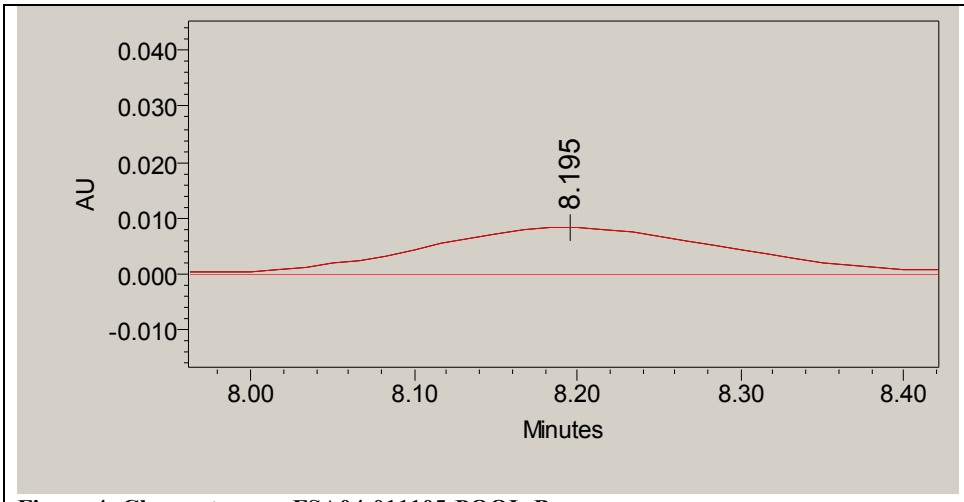


Figure 4: Chromatogram FSA04-011105-POOL-Brown

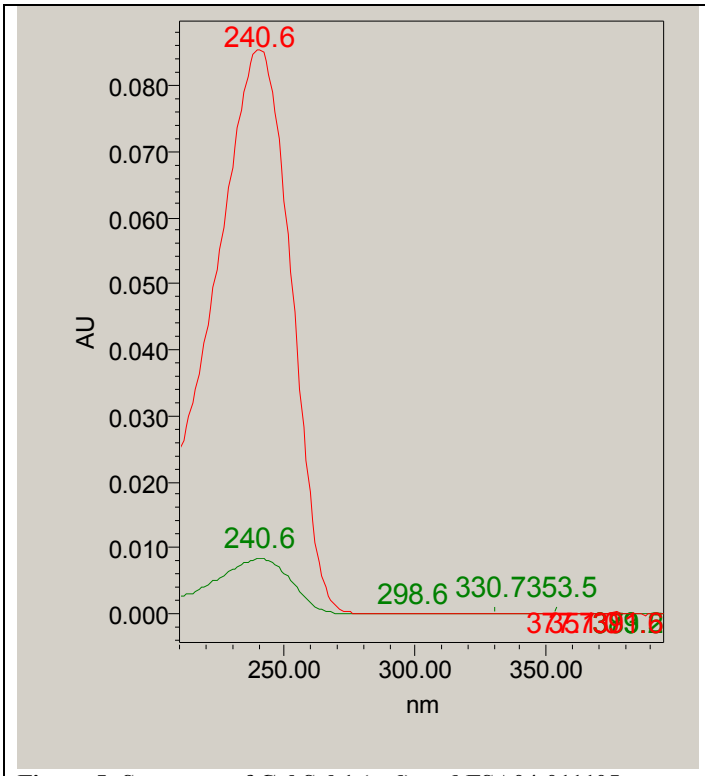


Figure 5: Spectrum of Cal Sol 1 (red) and FSA04-011105-POOL-Brown (green). Axis have NOT been normalized.

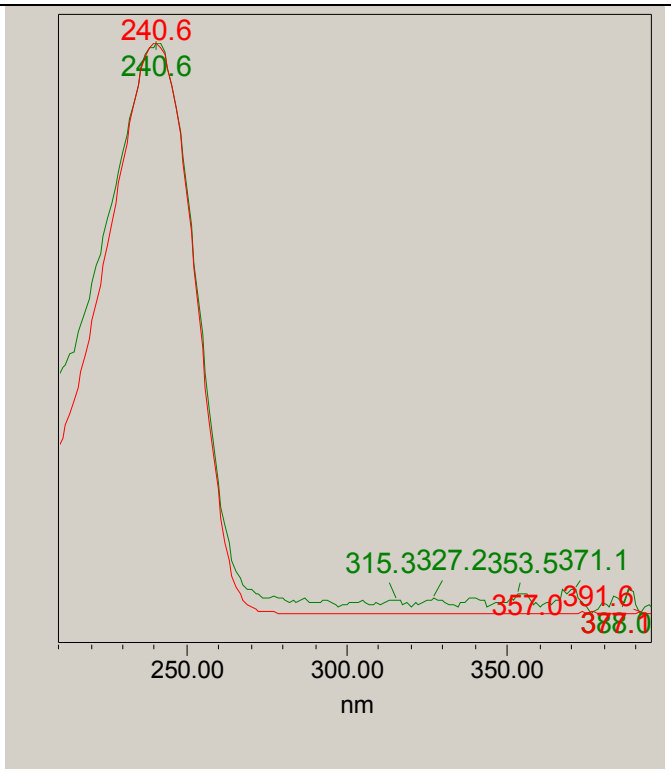


Figure 6: Spectrum of Cal Sol 1 (red) and FSA04-011105 POOL-Brown (green). Axis have been normalized.

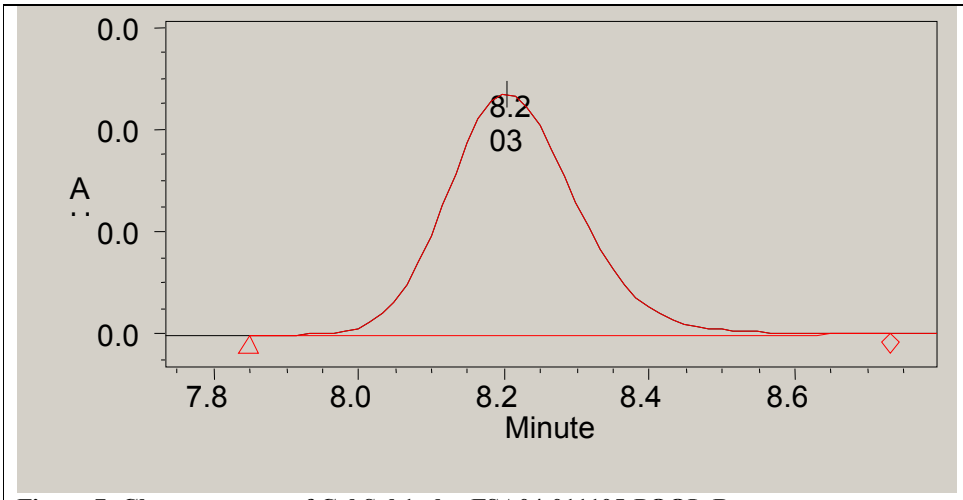


Figure 7: Chromatogram of Cal Sol 1 plus FSA04-011105-POOL-Brown

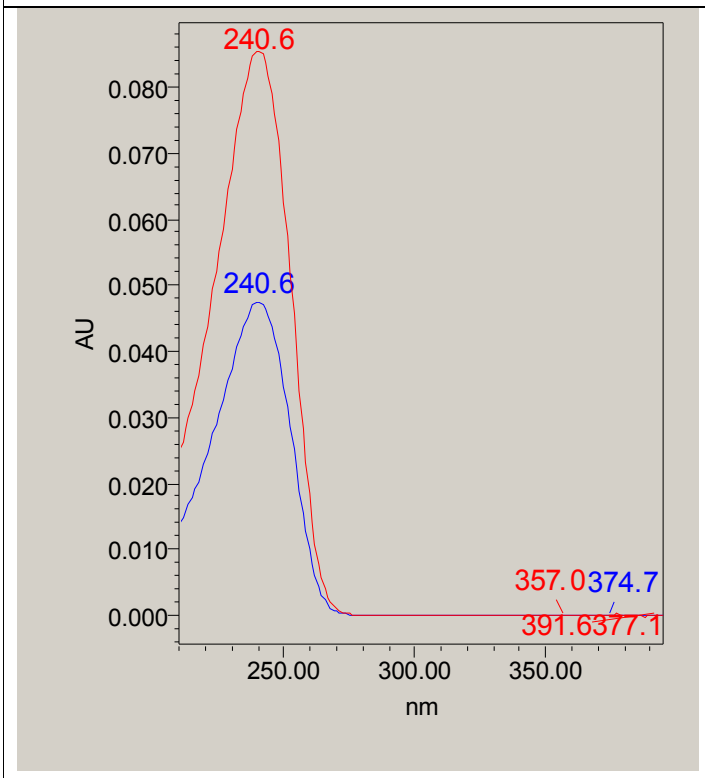


Figure 8: Spectrum of Cal Sol 1 plus FSA04-011105-POOL-Brown (red) and of Cal Sol 1 (blue). The axis have NOT been normalized

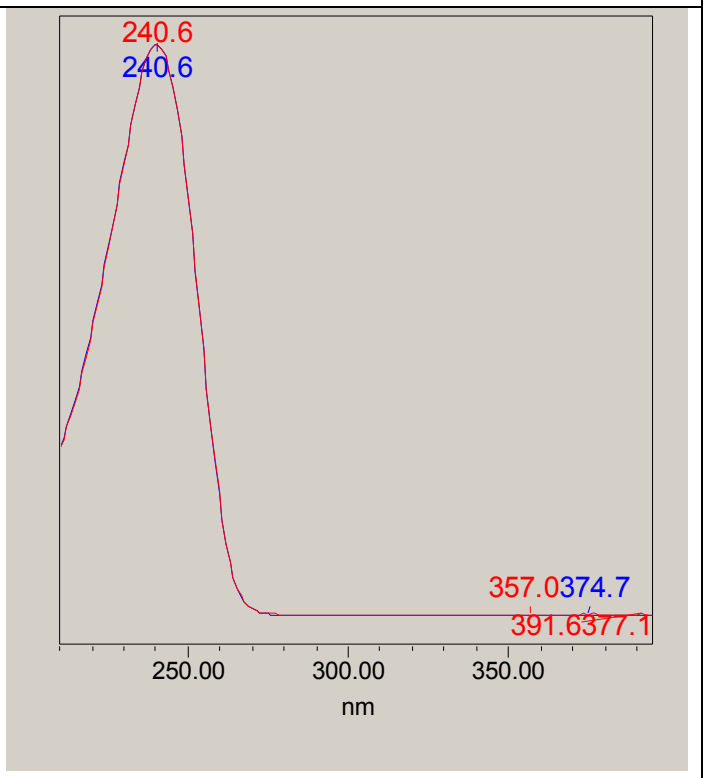


Figure 9: Spectrum of Cal Sol 1 plus FSA04-011105-POOL-Brown (red) and of Cal Sol 1 (blue). The Y axis has been normalized

Table 1: Summary of Results, Retention times and MRM ratios all match – therefore DA is confirmed (Ratio of results Quillum slide 3 AP14000_Scottish DA Summary 051201). FAO 011105 Pool 01 and 02 are two aliquots of FSA04-011105-Pool-B, which were analysed by Quillum.

	5uL FAO 011105 Pool 01	5uL FAO 011105 Pool 02	DA std
312/161	670450	662400	1722800
312/220	275580	275500	723150
312/266	222190	223640	592760
Ratio 1/2	2.43	2.40	2.38
Ratio 1/3	3.02	2.96	2.91
Ratio 2/3	1.24	1.23	1.22

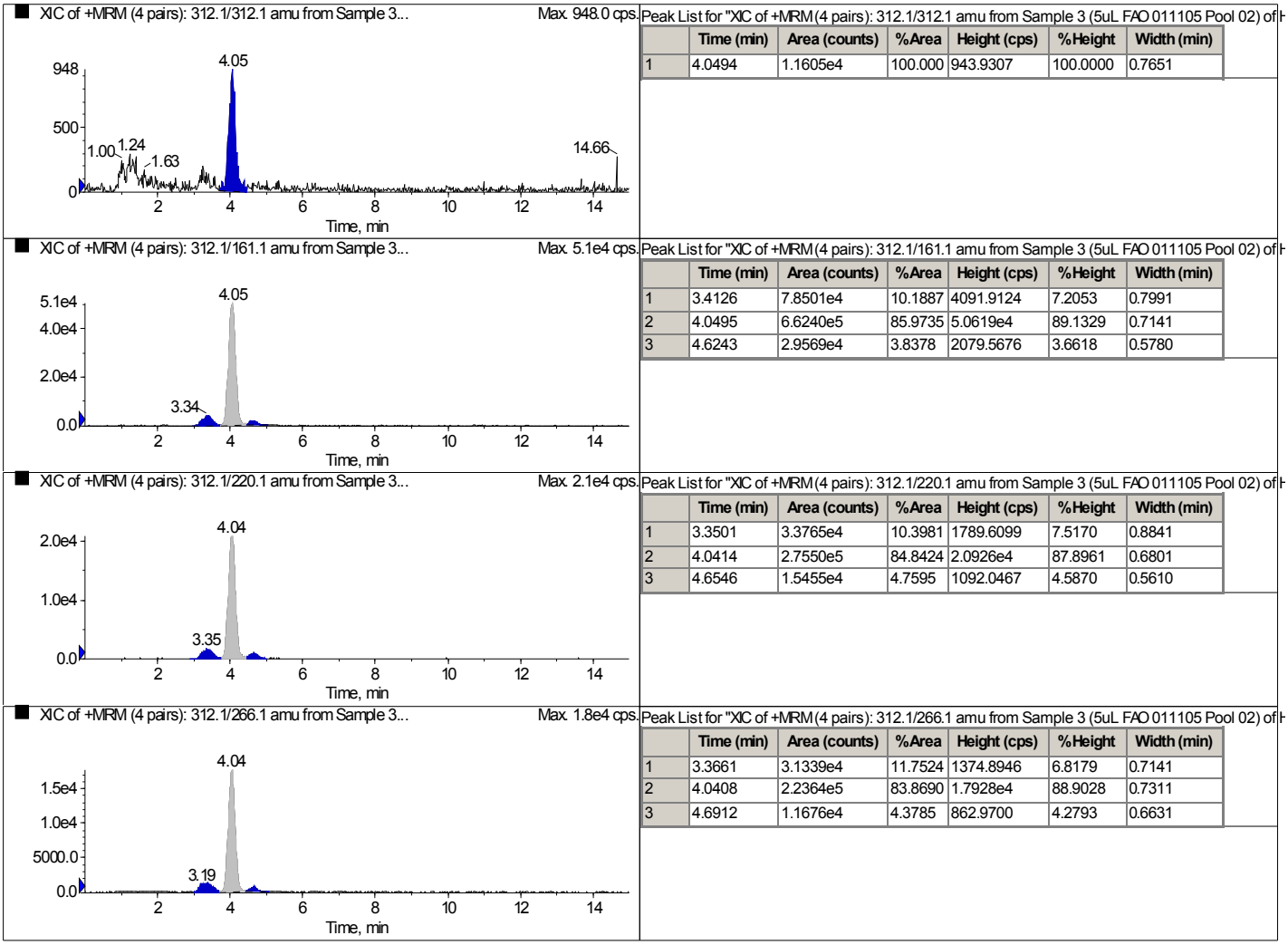
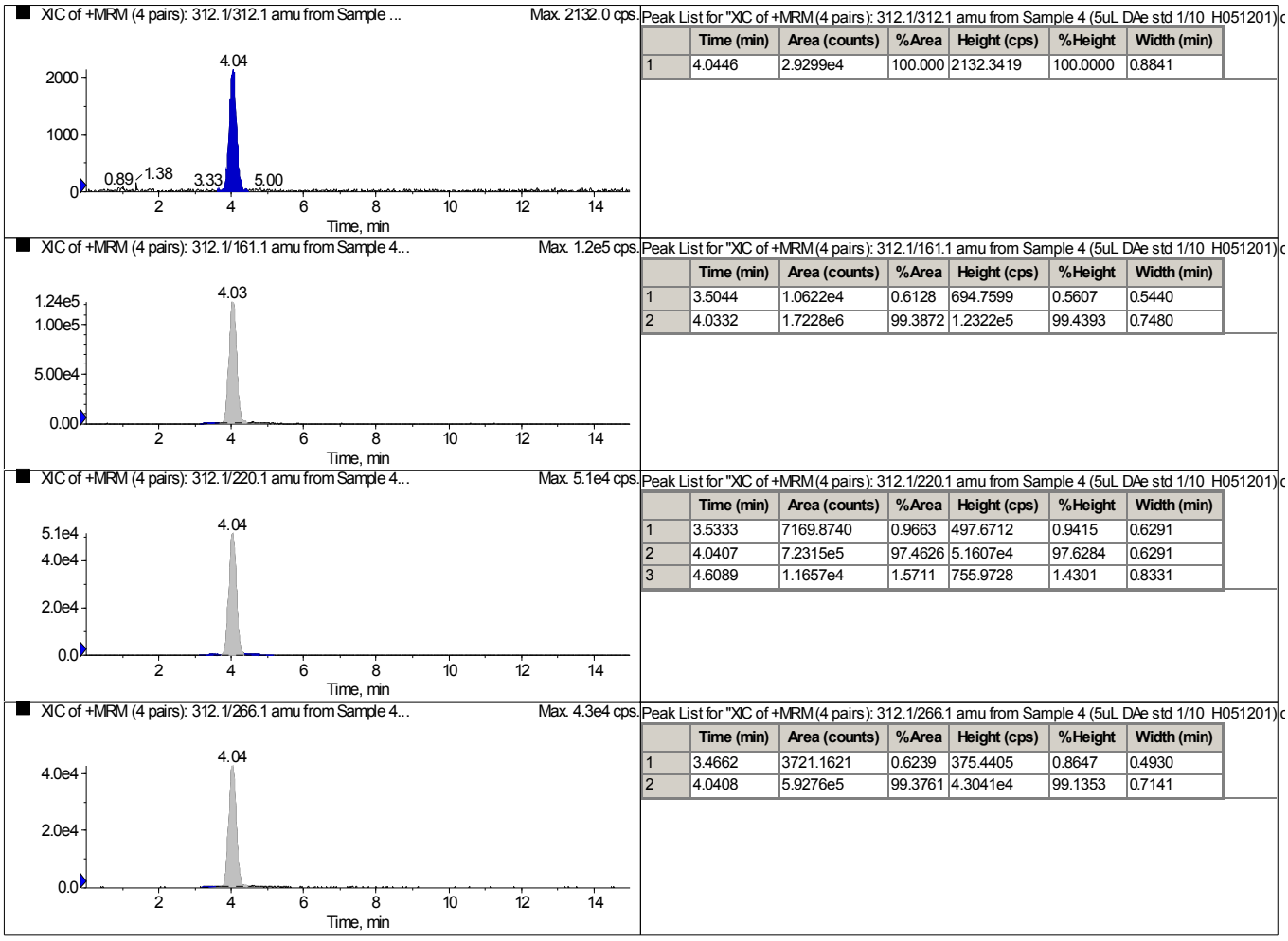


Figure 10: 5uL FAO 011105 Pool 02 (Quillum slide 1 AP14000_Scottish DA Summary 051201)



Peak List for "XIC of +MRM (4 pairs): 312.1/312.1 amu from Sample 4 (5uL DAe std 1/10 H051201)

	Time (min)	Area (counts)	%Area	Height (cps)	%Height	Width (min)
1	4.0446	2.9299e4	100.000	2132.3419	100.0000	0.8841

Peak List for "XIC of +MRM (4 pairs): 312.1/161.1 amu from Sample 4 (5uL DAe std 1/10 H051201)

	Time (min)	Area (counts)	%Area	Height (cps)	%Height	Width (min)
1	3.5044	1.0622e4	0.6128	694.7599	0.5607	0.5440
2	4.0332	1.7228e6	99.3872	1.2322e5	99.4393	0.7480

Peak List for "XIC of +MRM (4 pairs): 312.1/220.1 amu from Sample 4 (5uL DAe std 1/10 H051201)

	Time (min)	Area (counts)	%Area	Height (cps)	%Height	Width (min)
1	3.5333	7169.8740	0.9663	497.6712	0.9415	0.6291
2	4.0407	7.2315e5	97.4626	5.1607e4	97.6284	0.6291
3	4.6089	1.1657e4	1.5711	755.9728	1.4301	0.8331

Peak List for "XIC of +MRM (4 pairs): 312.1/266.1 amu from Sample 4 (5uL DAe std 1/10 H051201)

	Time (min)	Area (counts)	%Area	Height (cps)	%Height	Width (min)
1	3.4662	3721.1621	0.6239	375.4405	0.8647	0.4930
2	4.0408	5.9276e5	99.3761	4.3041e4	99.1353	0.7141

Figure 11: 5uL DAe 1/10 (Quillum slide 2 AP14000_Scottish DA Summary 051201)

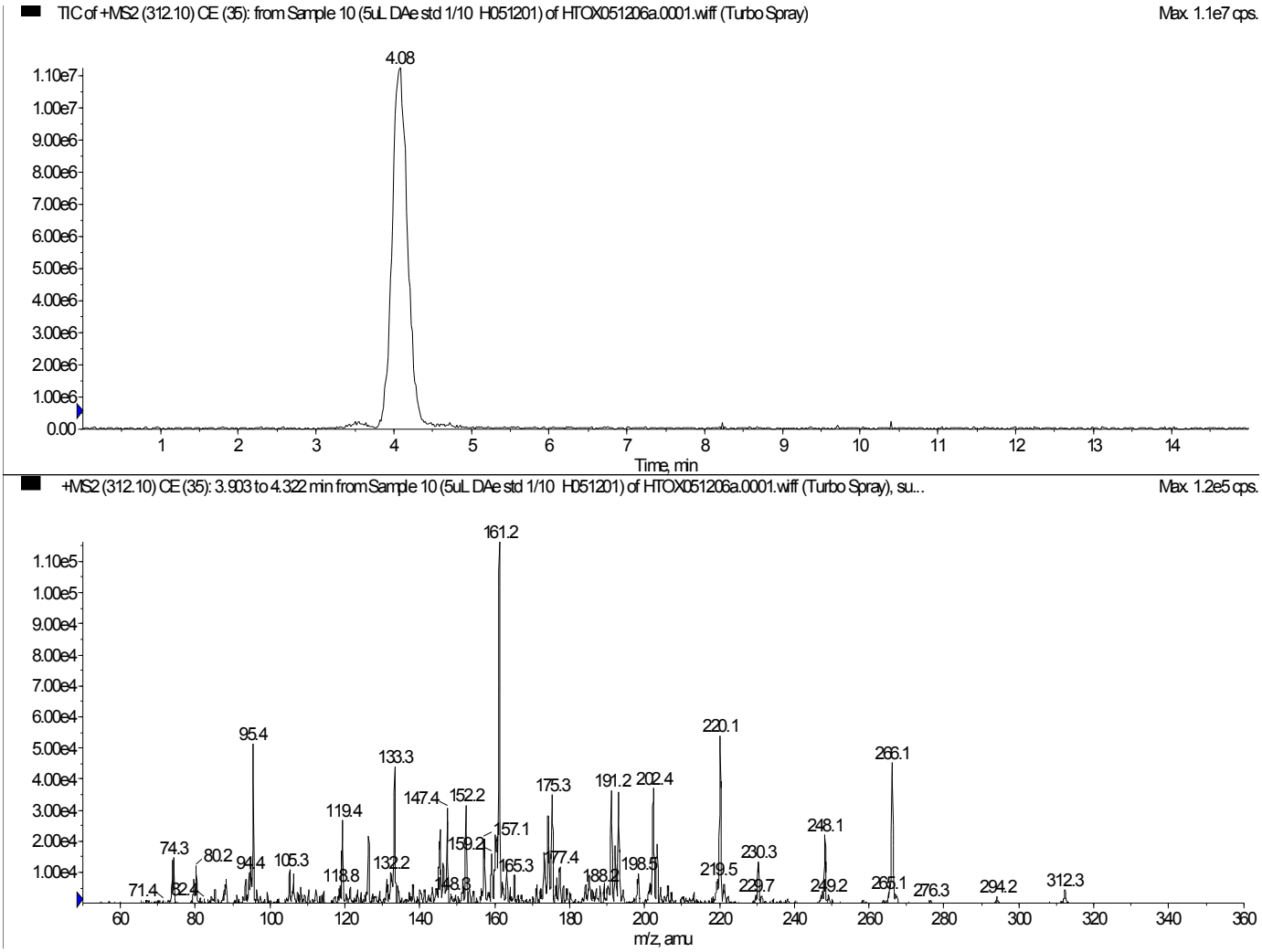


Figure 12: Prod scan m/z 312 for DAe 1/10 (Quillum slide 4 AP14000_Scottish DA Summary 051201)

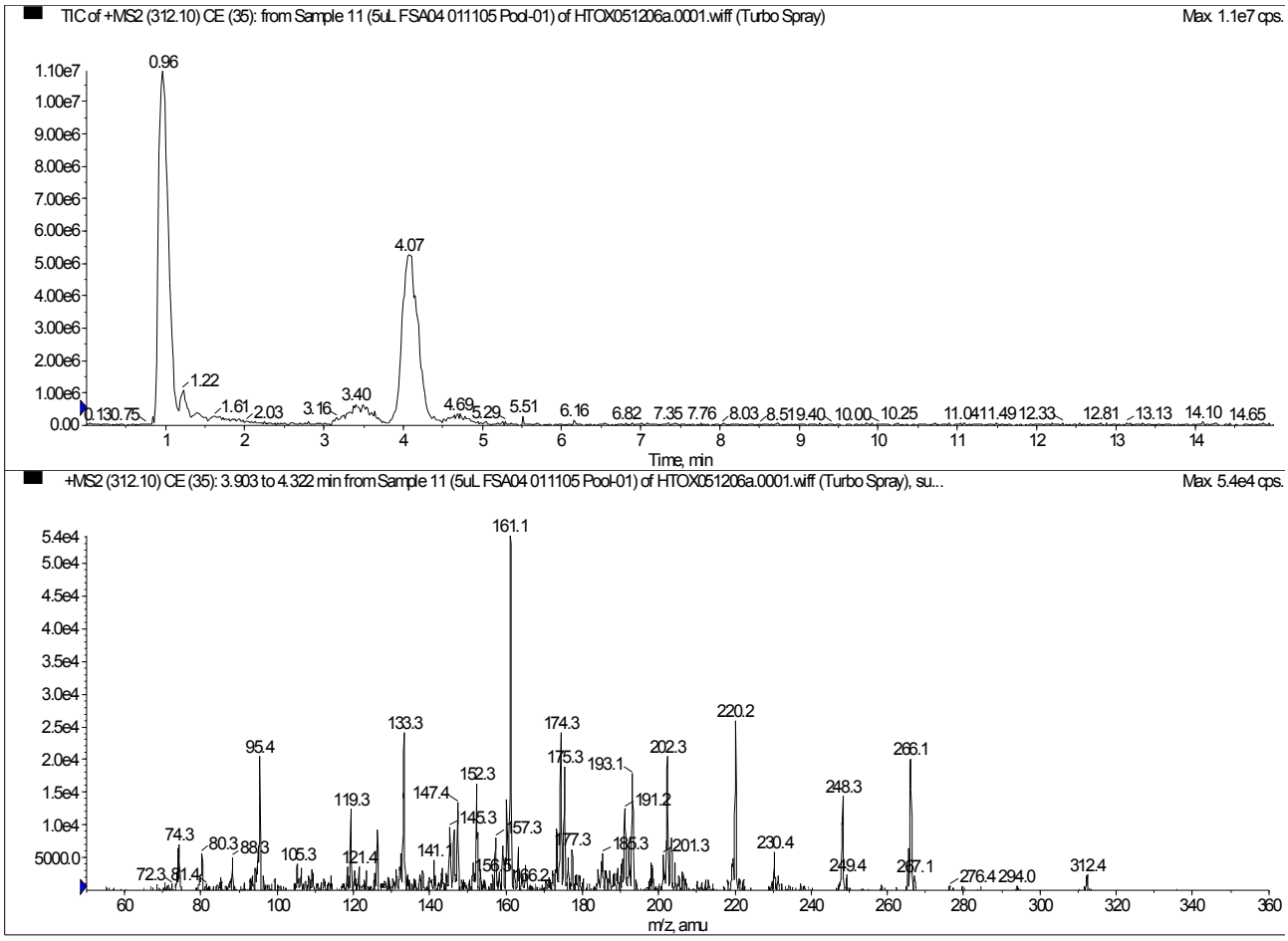


Figure 13: Prod scan m/z 312 for Pool 01(Quillum slide 5 AP14000_Scottish DA Summary 051201)

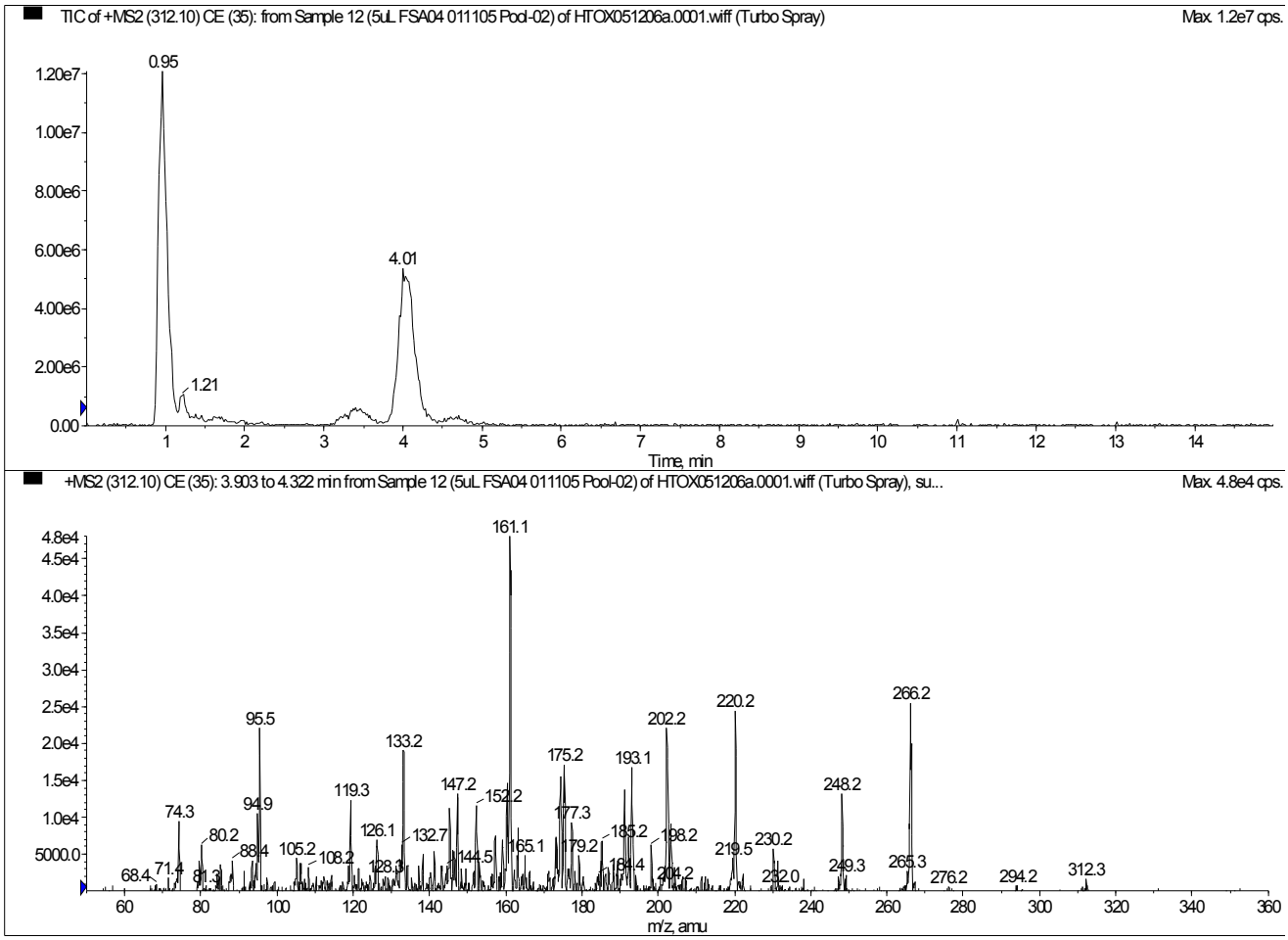


Figure 14: Prod scan m/z 312 for Pool 02 ((Quillum slide 6 AP14000_Scottish DA Summary 051201)