An extended siderophore suite from *Synechococcus* sp. PCC 7002 revealed by LC-ICPMS-ESIMS

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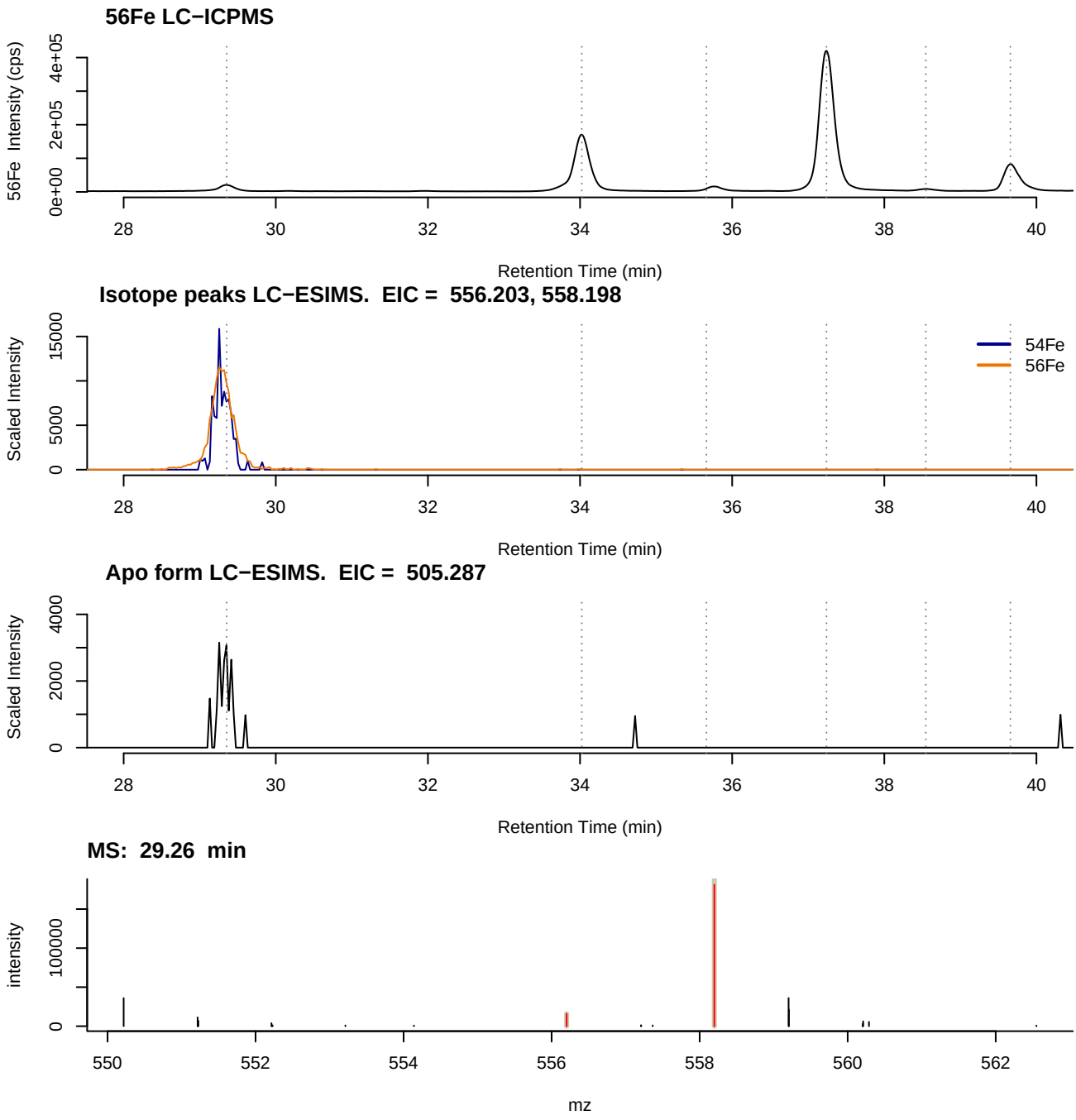
Supplementary Information:

Figure SI-1: Chromatograms and mass spectra of the 29 curated isotope pairs identified by algorithm. **(Top panel)** ^{56}Fe LC-ICPMS chromatogram. Dotted lines indicate the retention times of the six synechobactins. **(Second panel)** extracted ion chromatograms from positive mode LC-ESIMS runs. Blue lines correspond to the light iron isotopologue [M+$^{54}Fe^{3+}$-2H^{+}] that were identified by the isotope algorithm, and orange lines correspond to the heavy iron isotopologue [M+$^{56}Fe^{3+}$-2H^{+}]. The intensity of the heavy iron isotopologue has been scaled by the natural abundance ratio of ^{56}Fe/^{54}Fe (divided by 15.7) so that the isotopologues overlap. **(Third panel)** extracted ion chromatograms of the Apo (iron free) monoisotopic m/z [M+H^{+}]. **(Bottom panel)** MS1 spectra of the Fe-complex. The red lines highlight the peaks corresponding to the ^{54}Fe and ^{56}Fe isotopologues that are plotted in the second panel. The theoretical Fe isotope pattern (Mass M_1-M_2=1.995 m/z, Intensity M_1/M_2=15.7) for the ion pair is displayed as gray bars.

Supplementary Information Figure 1-1



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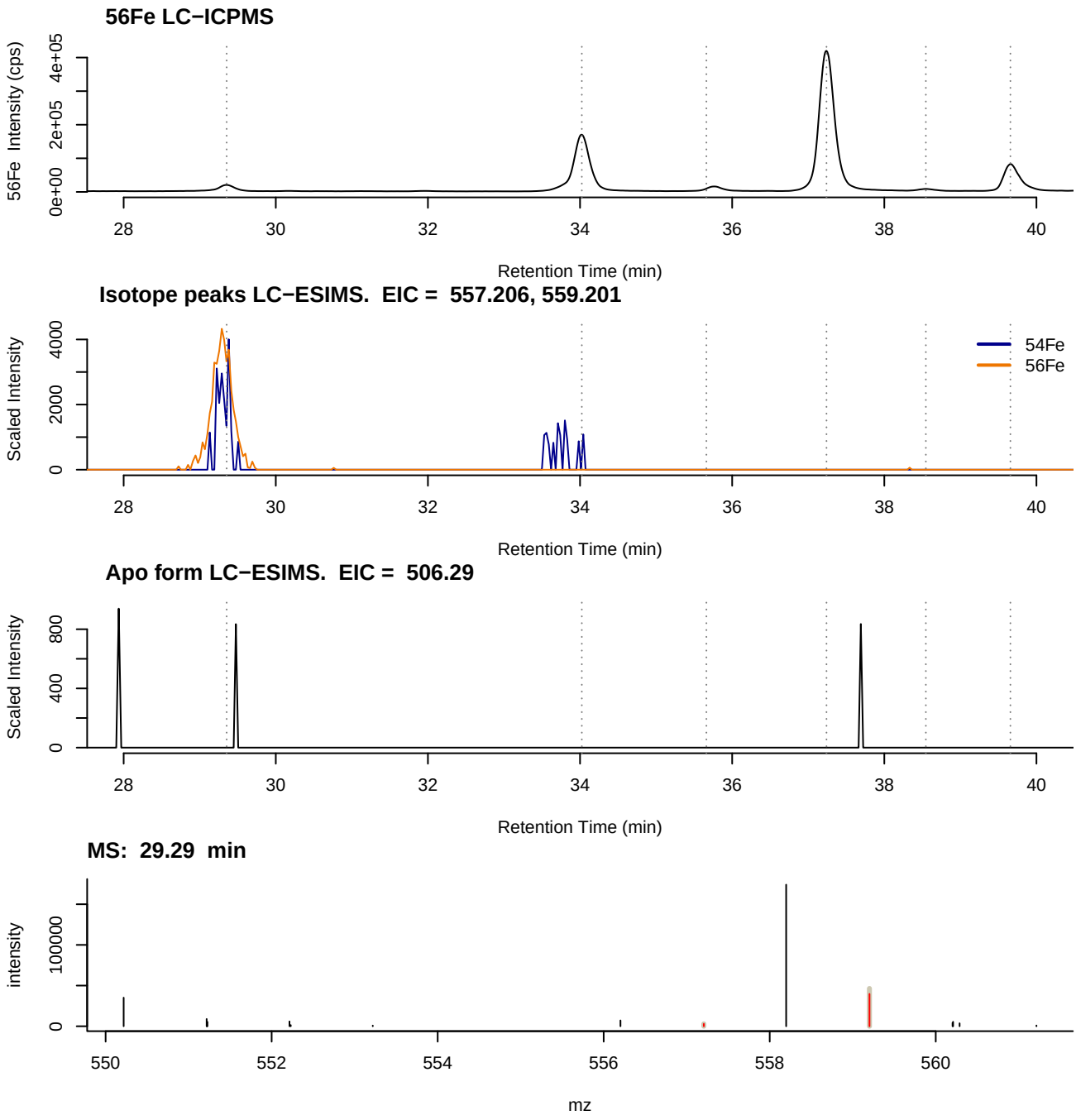

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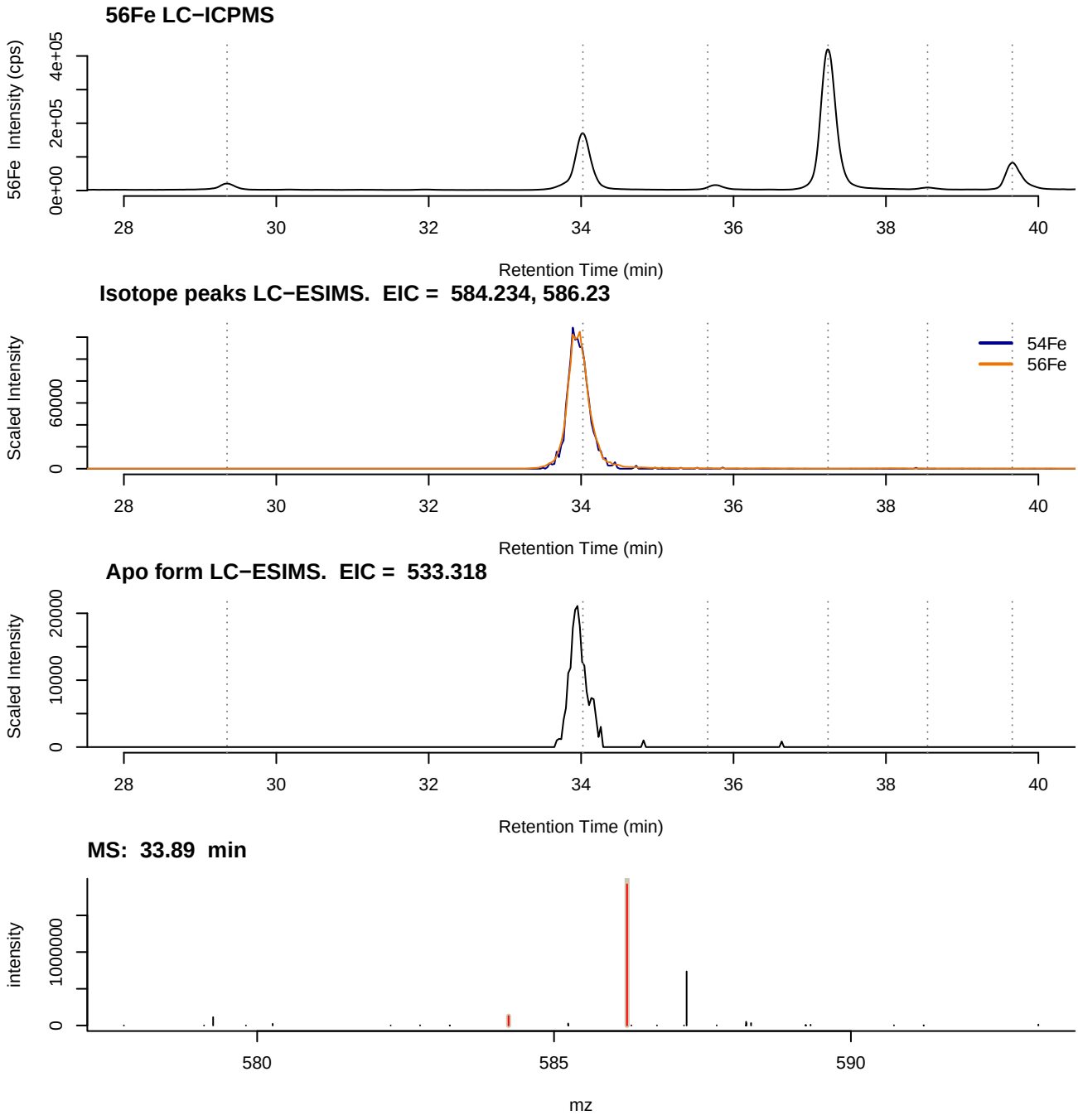

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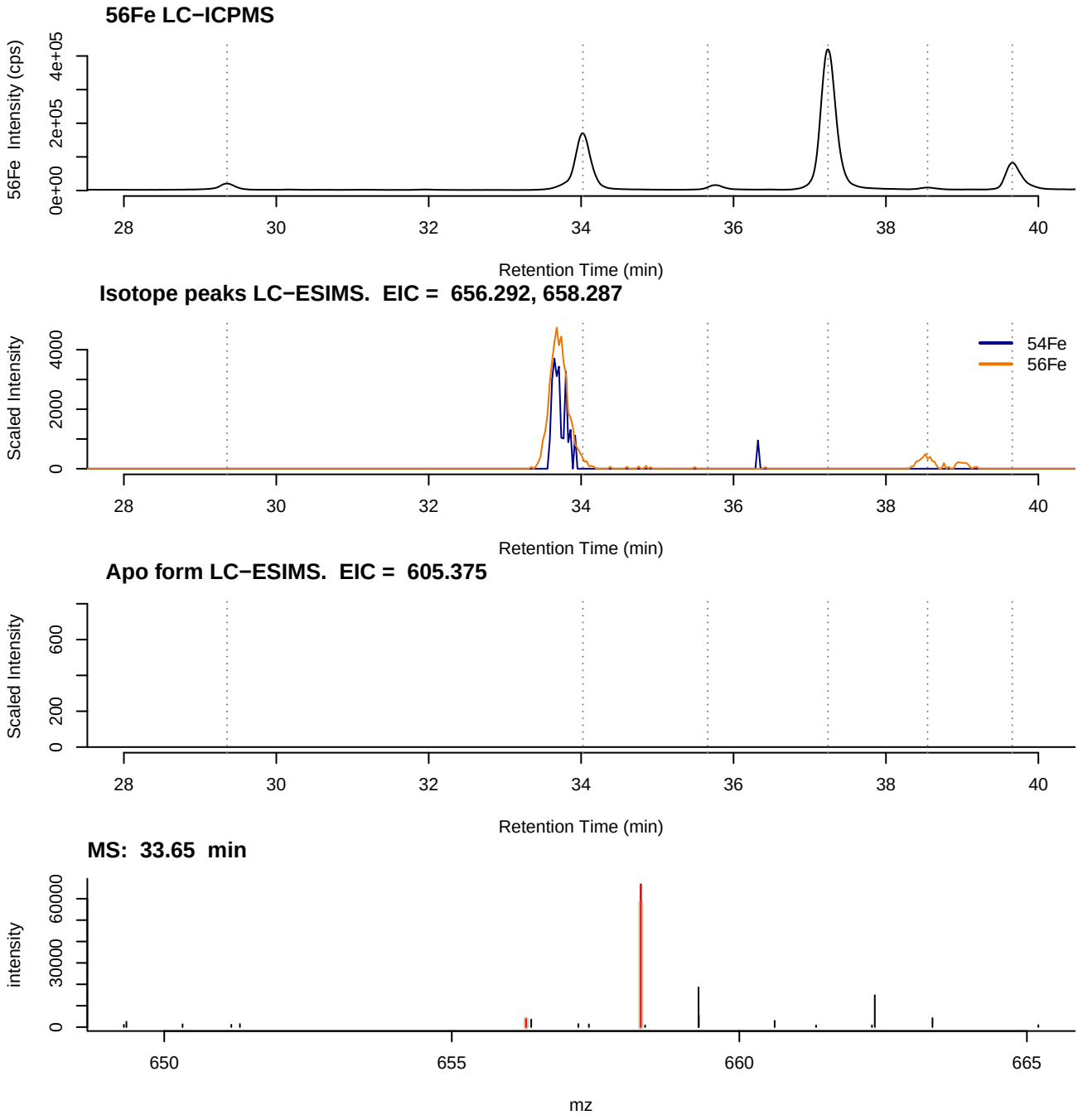

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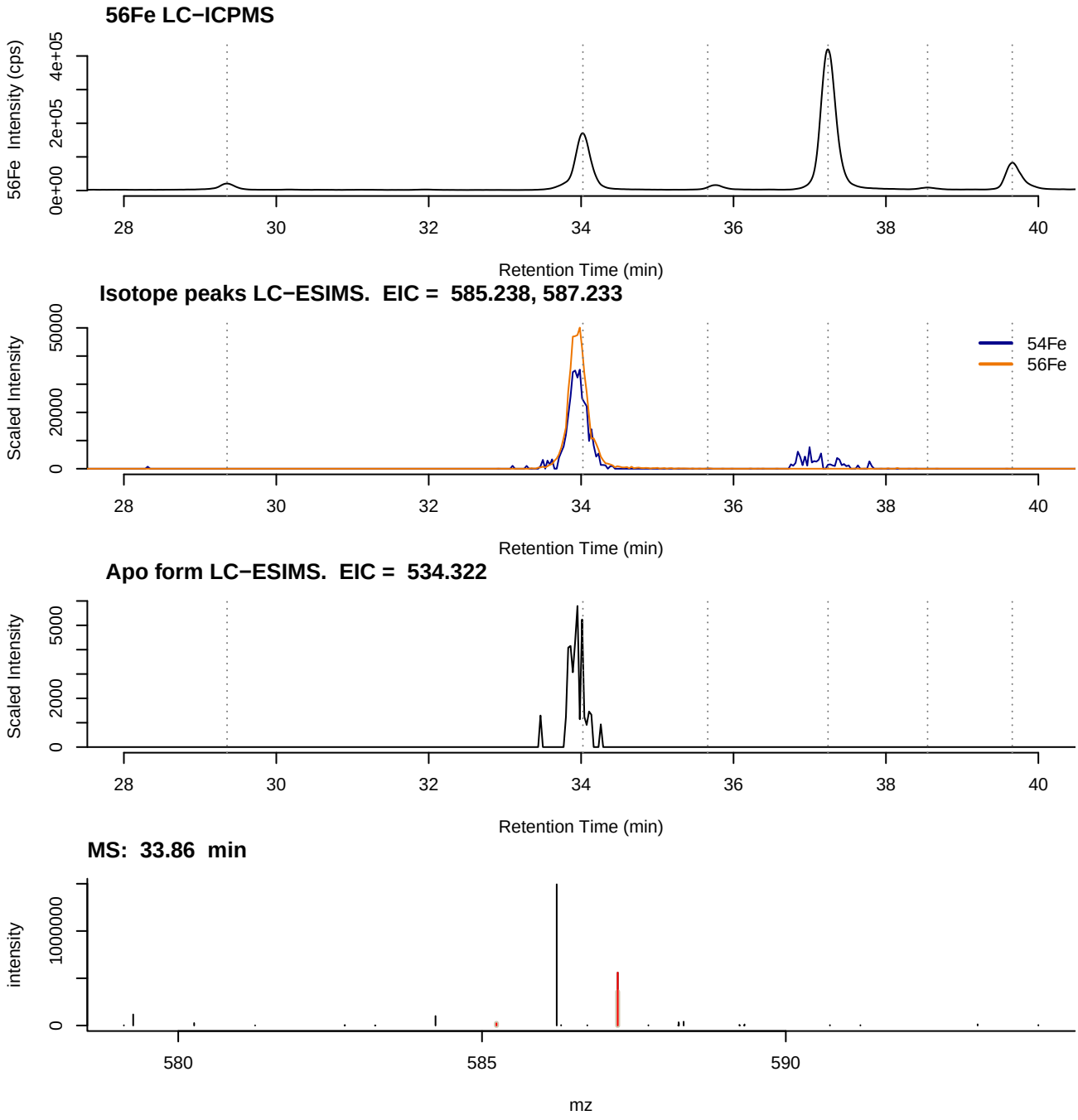

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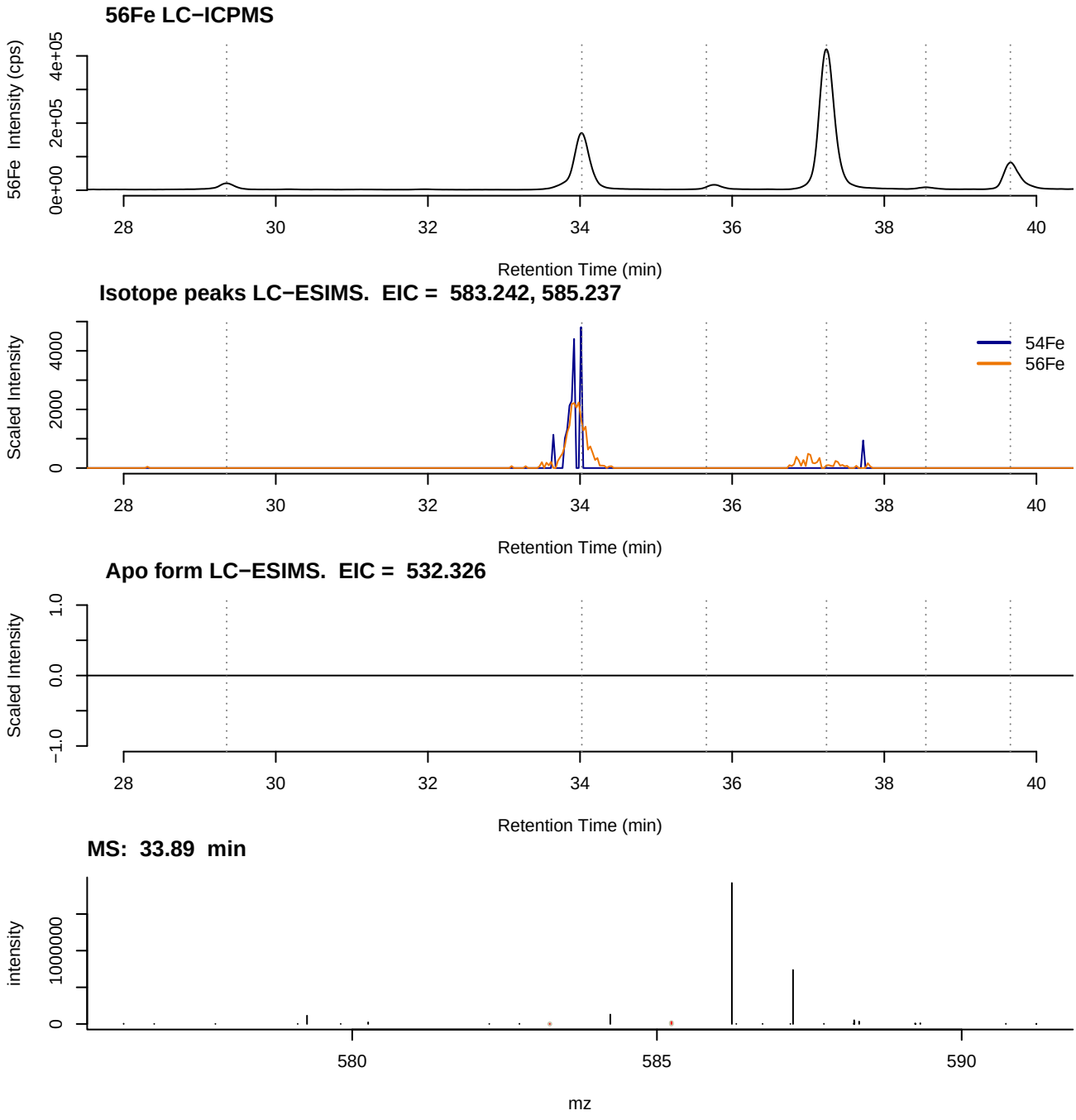

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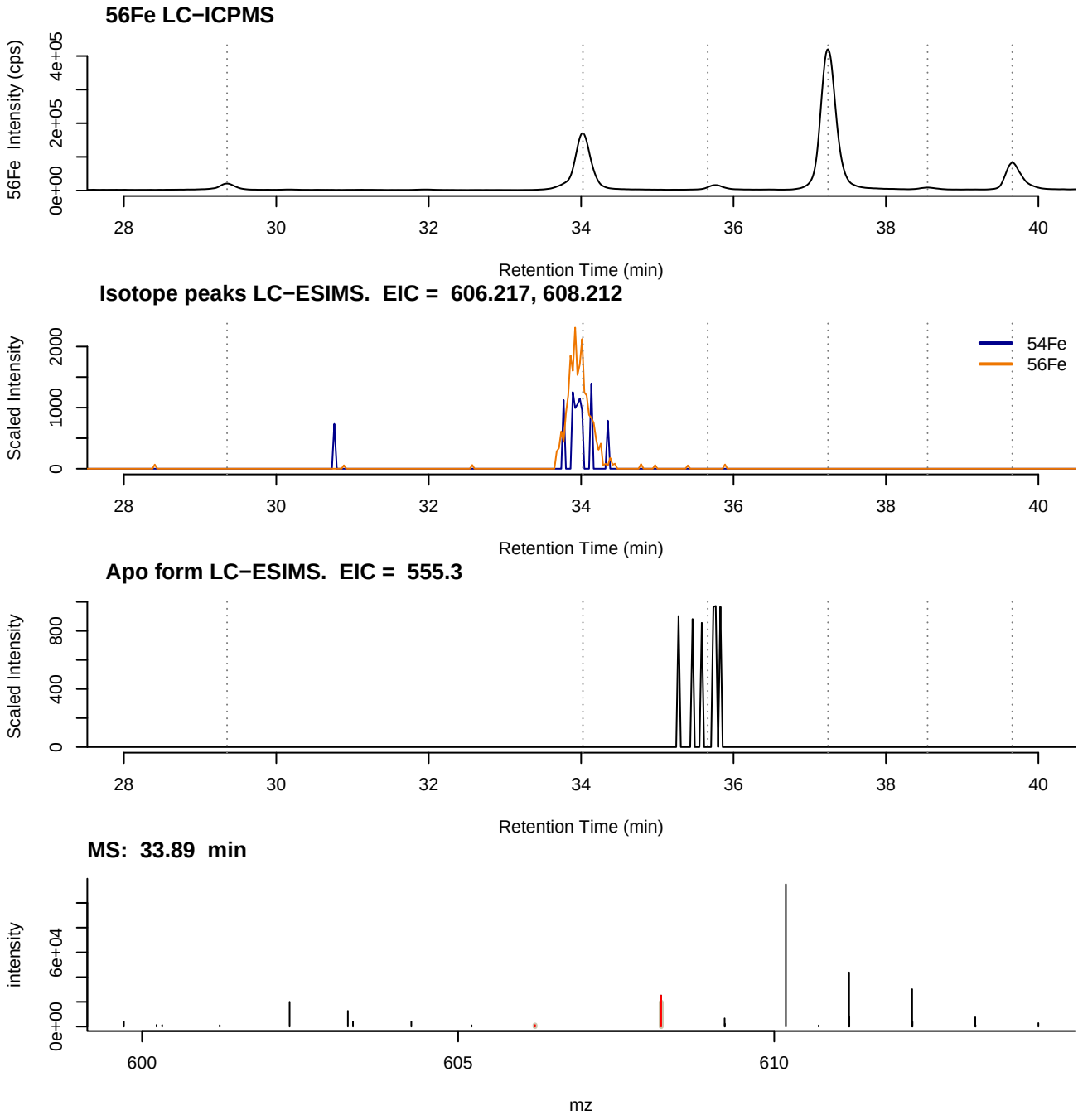

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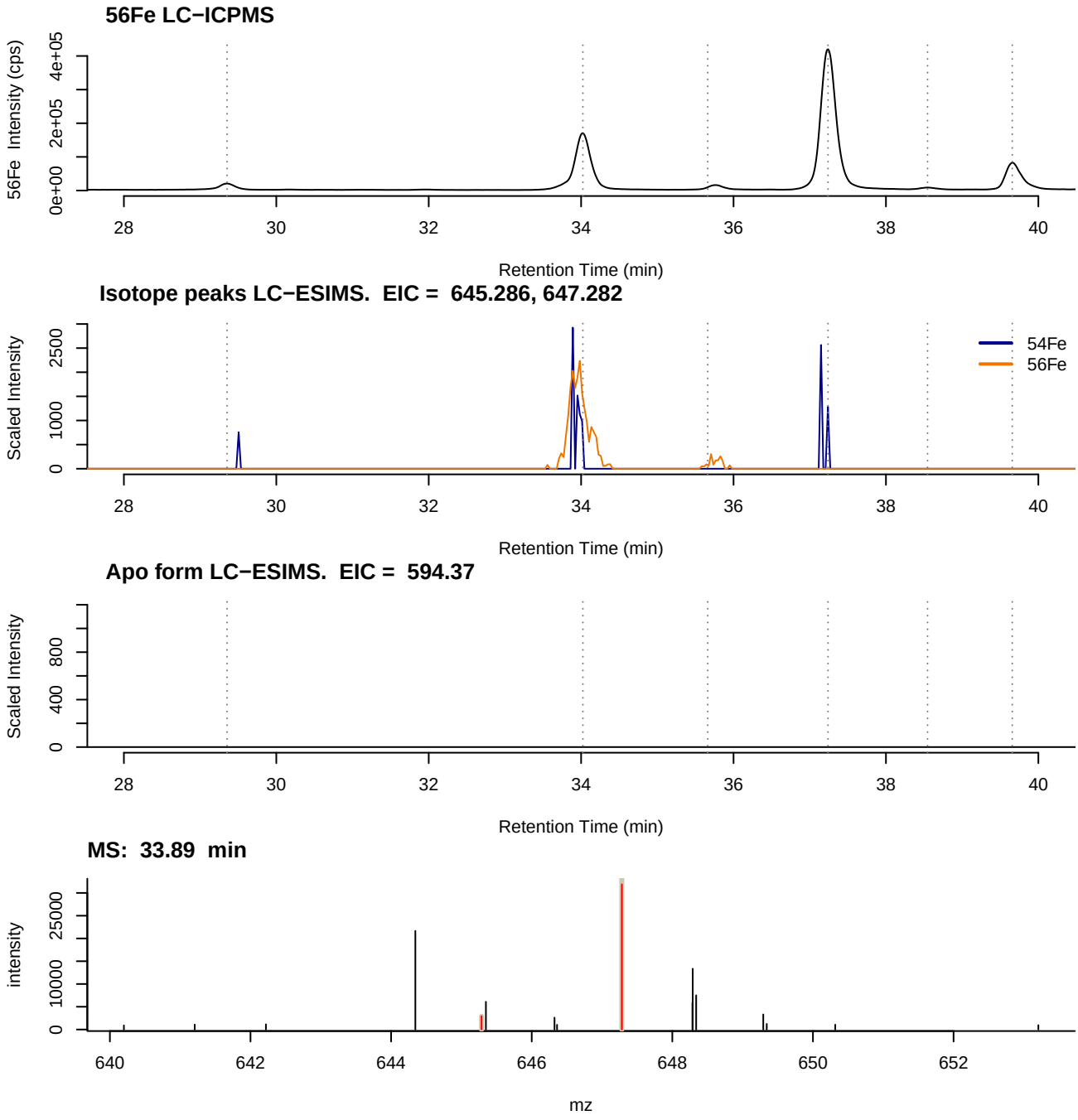

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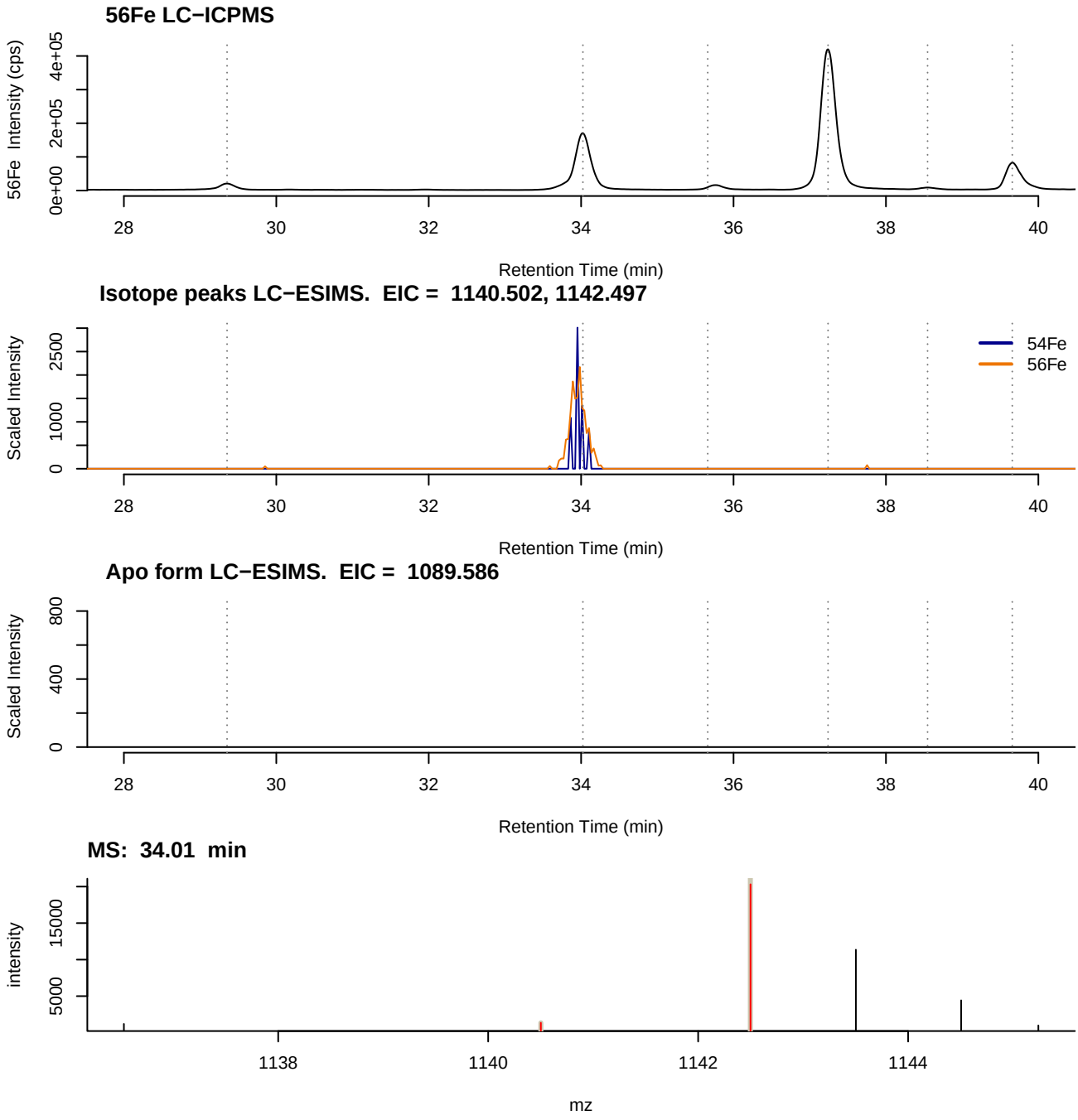

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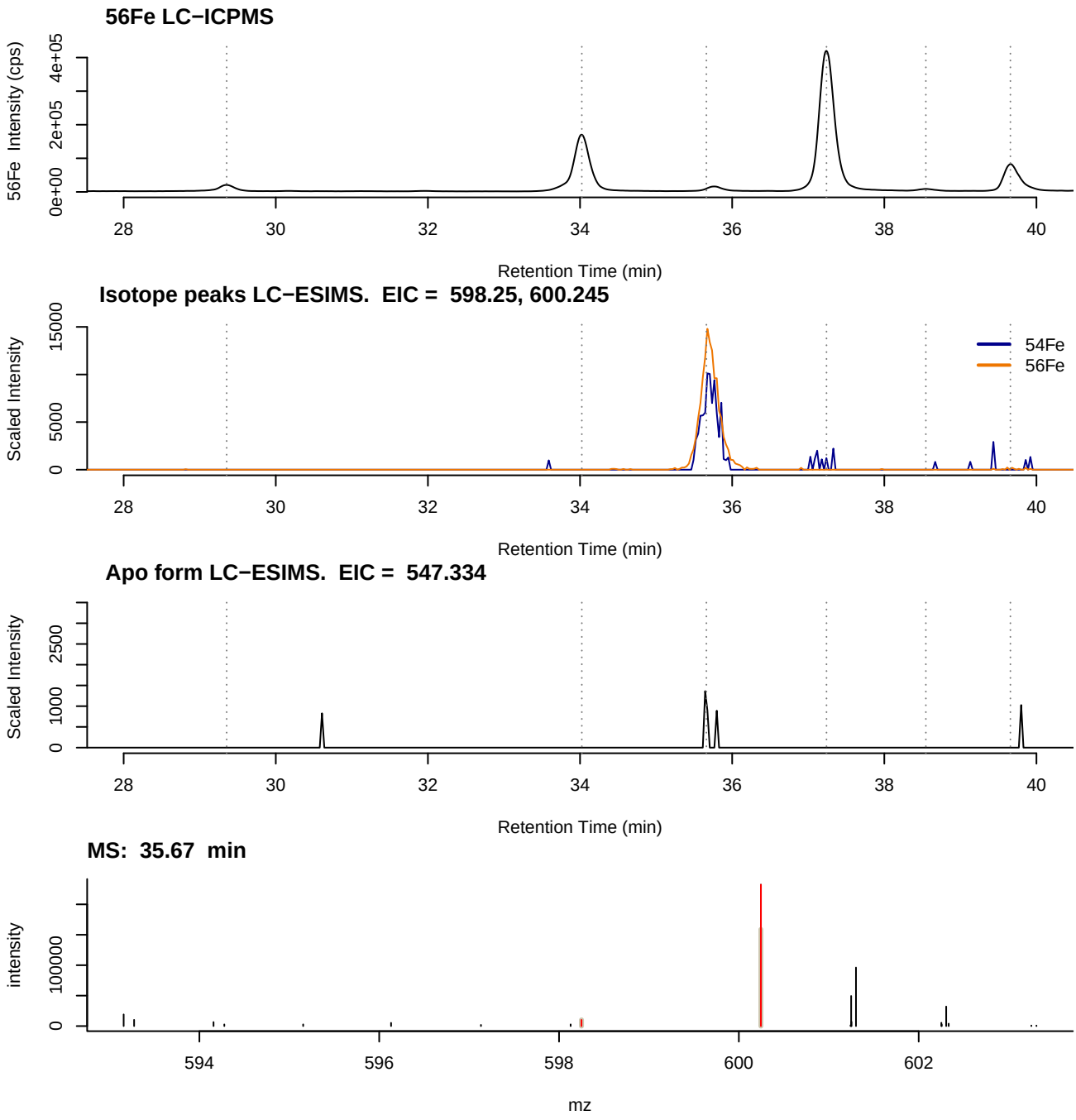

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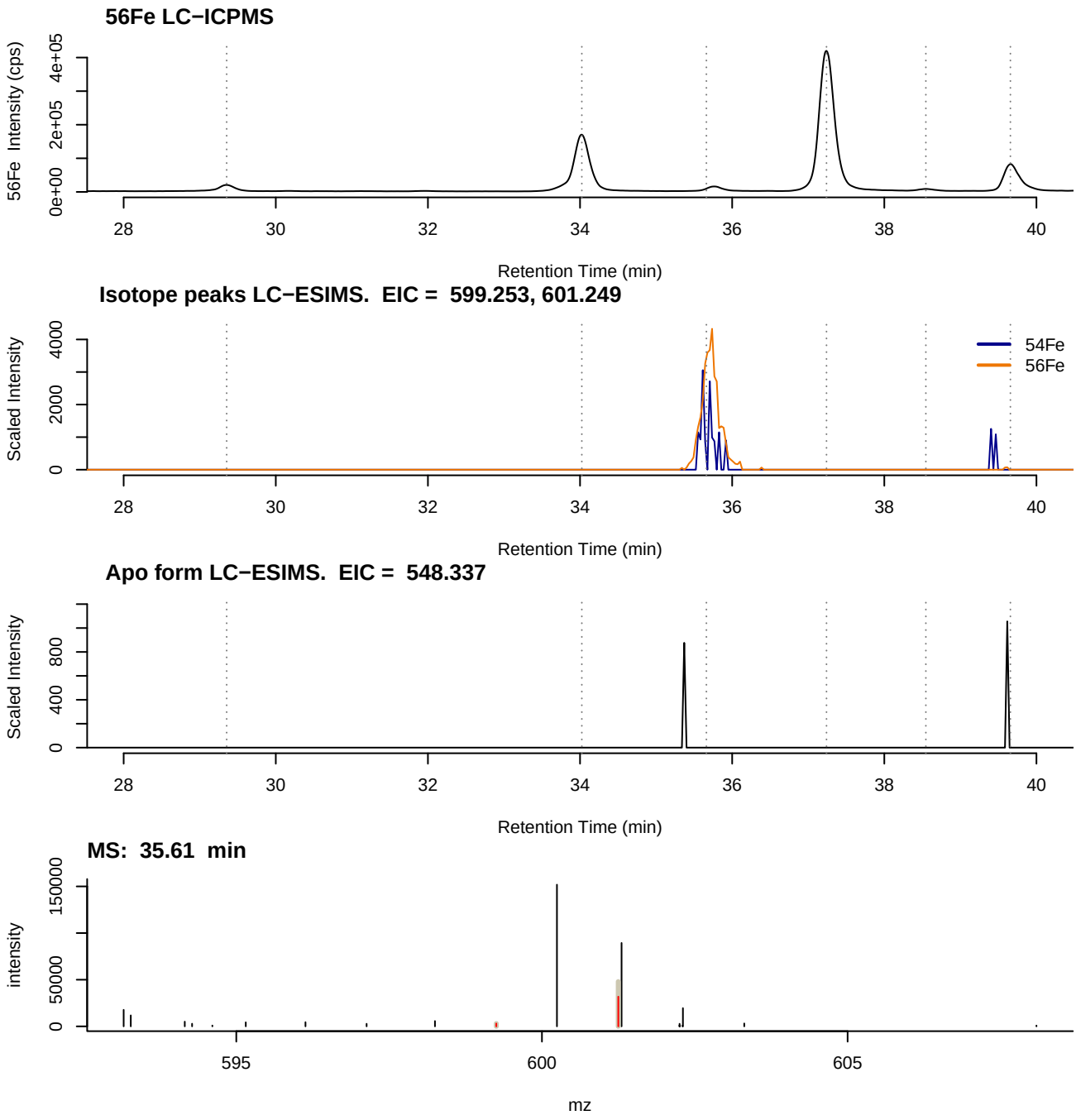

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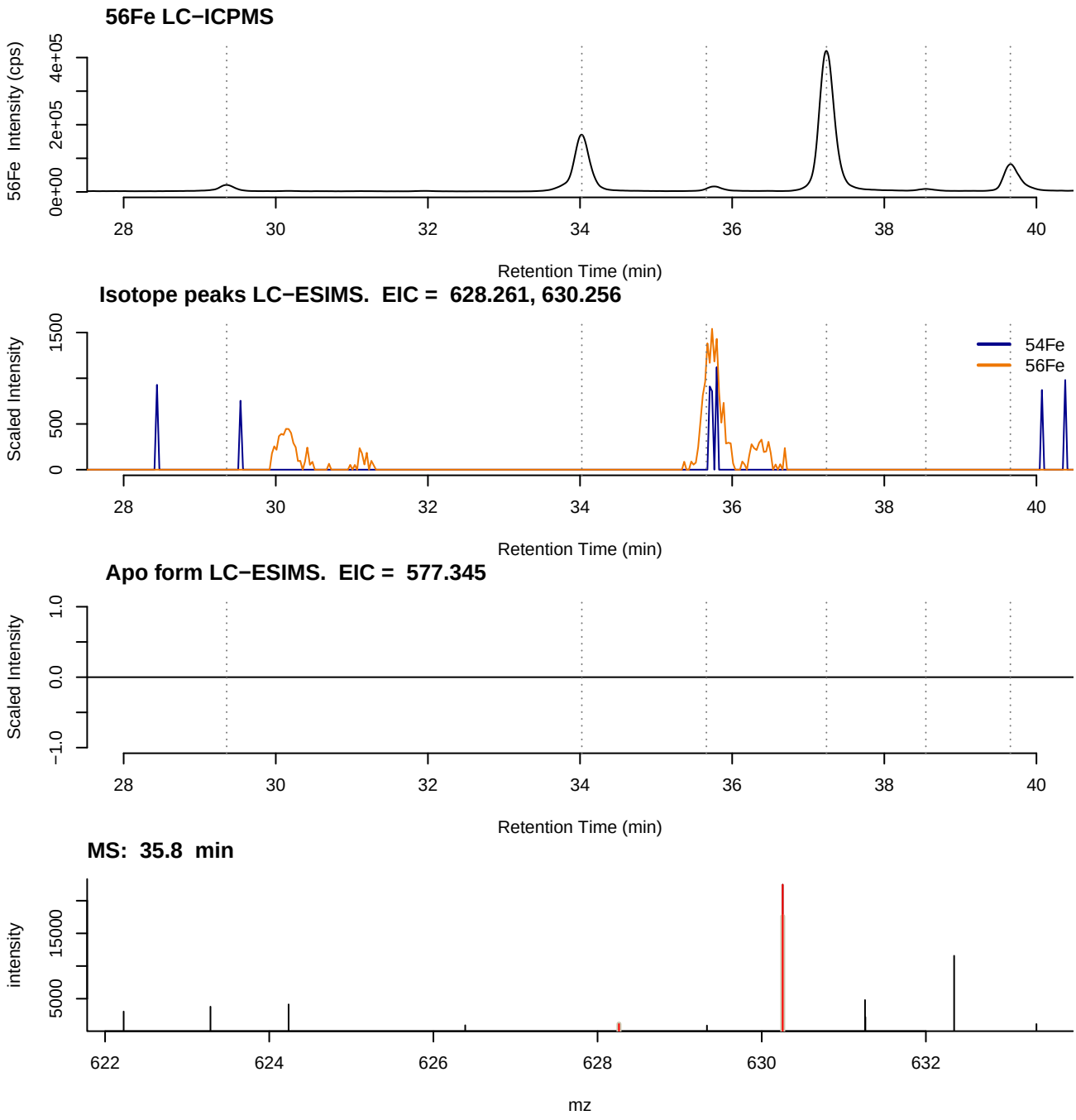

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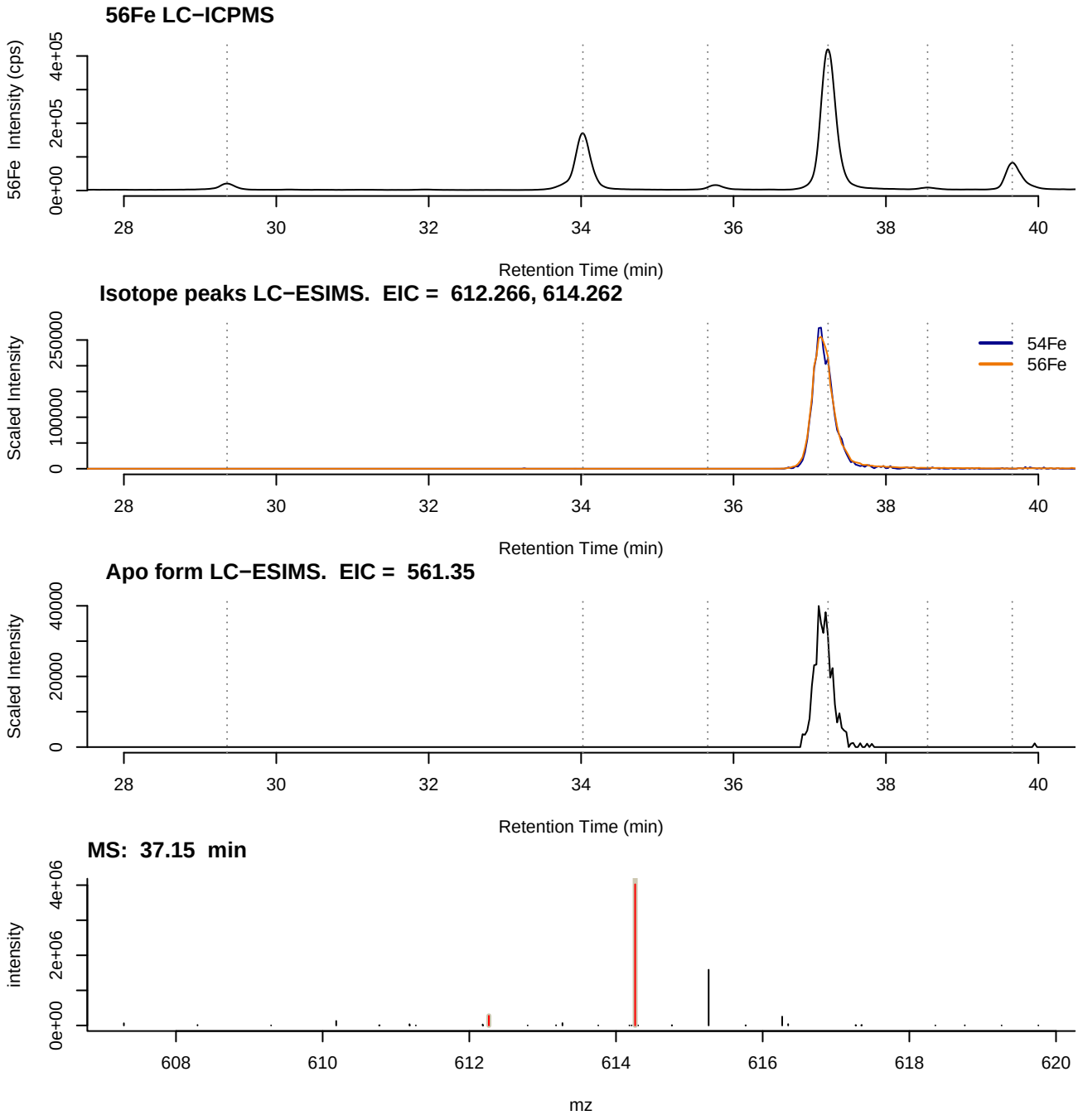

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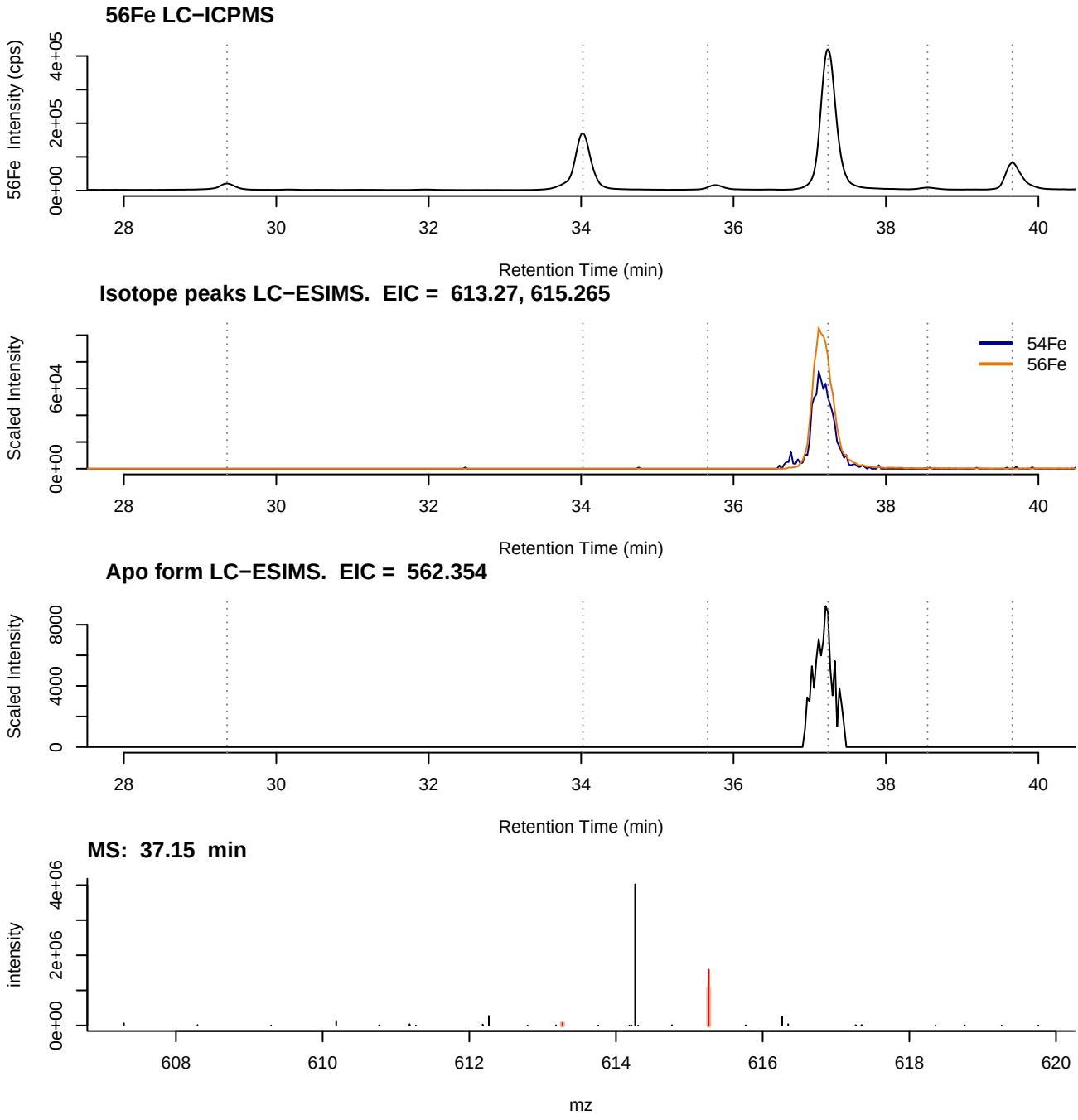

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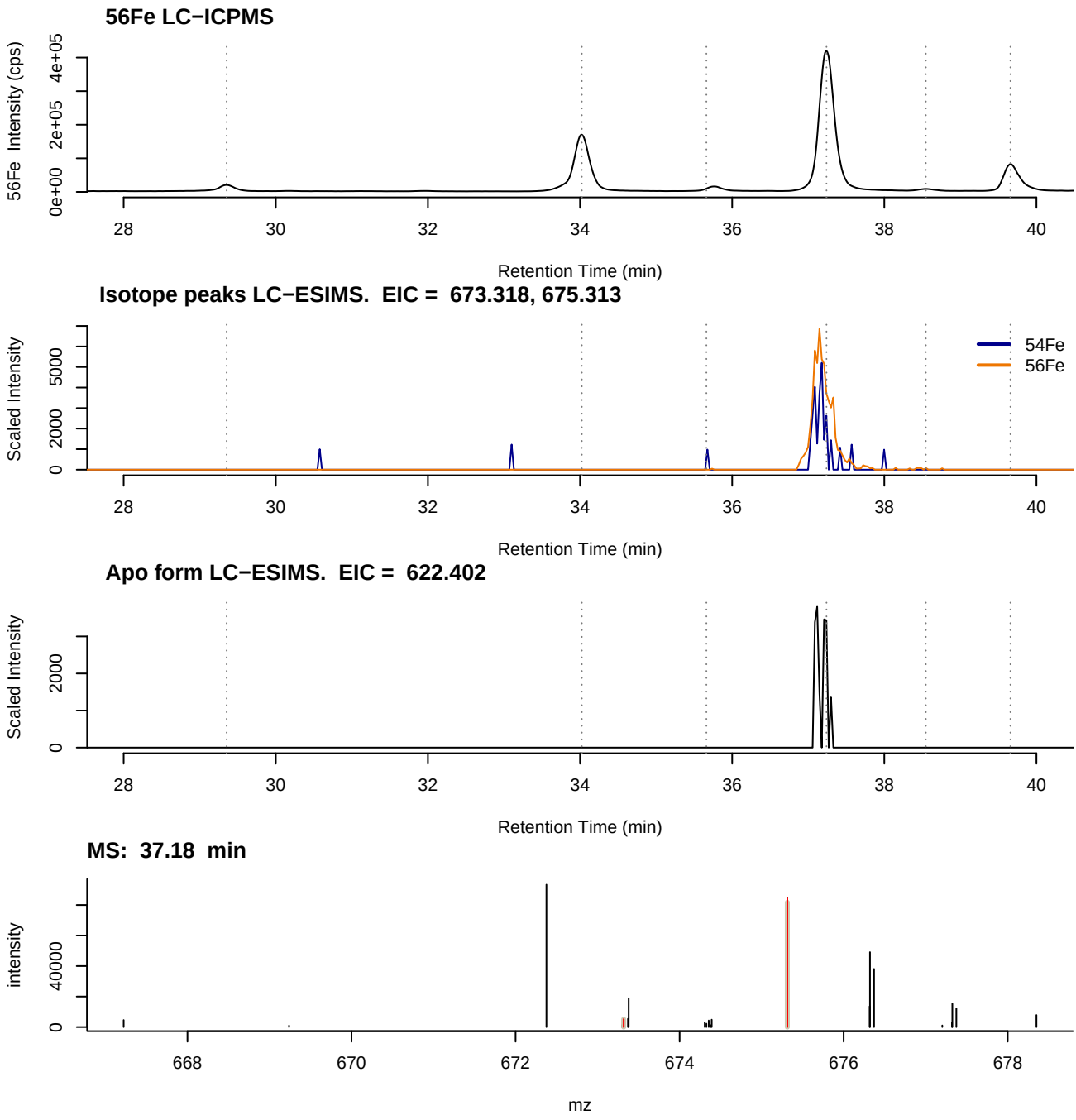

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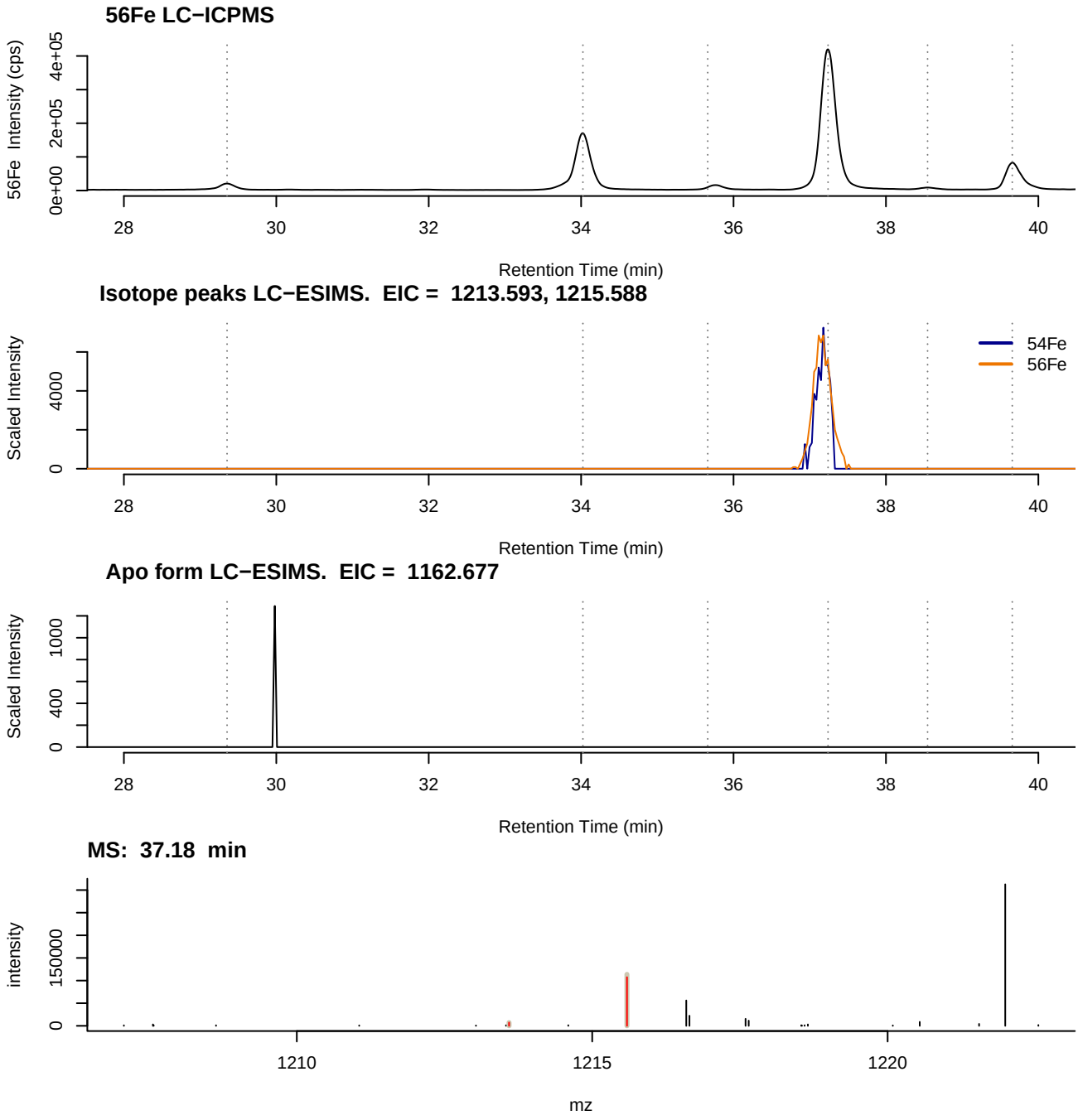

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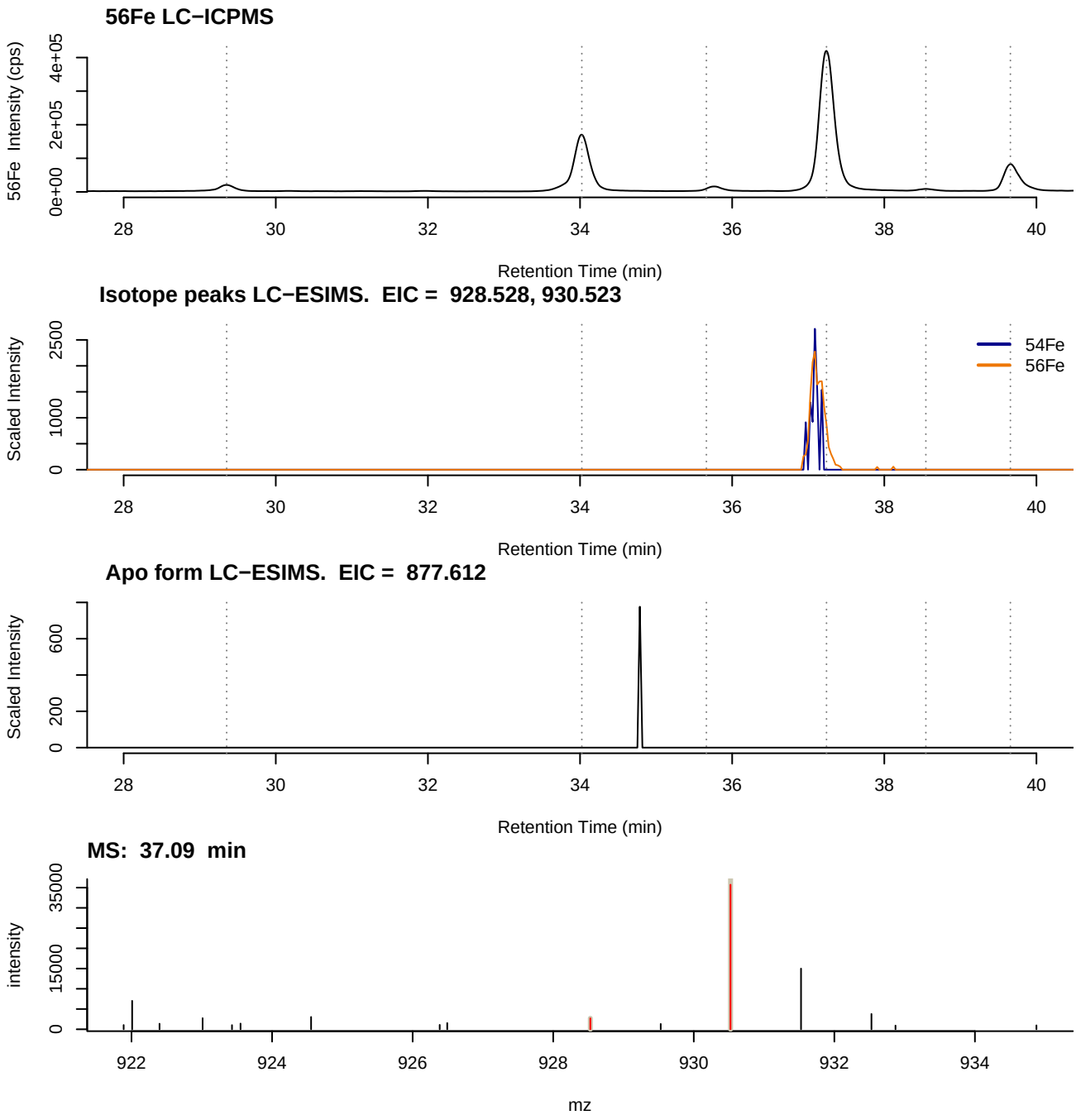

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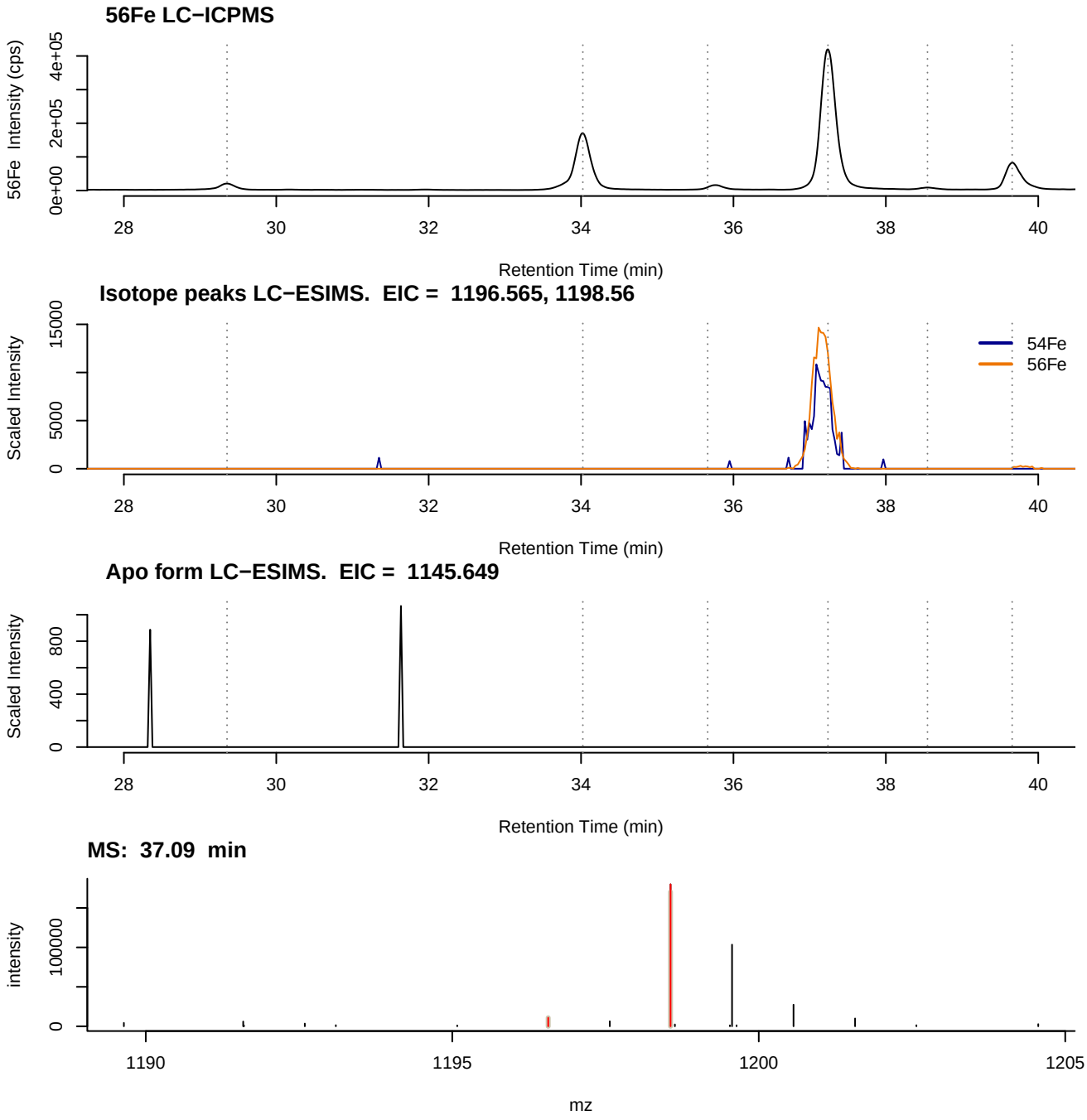

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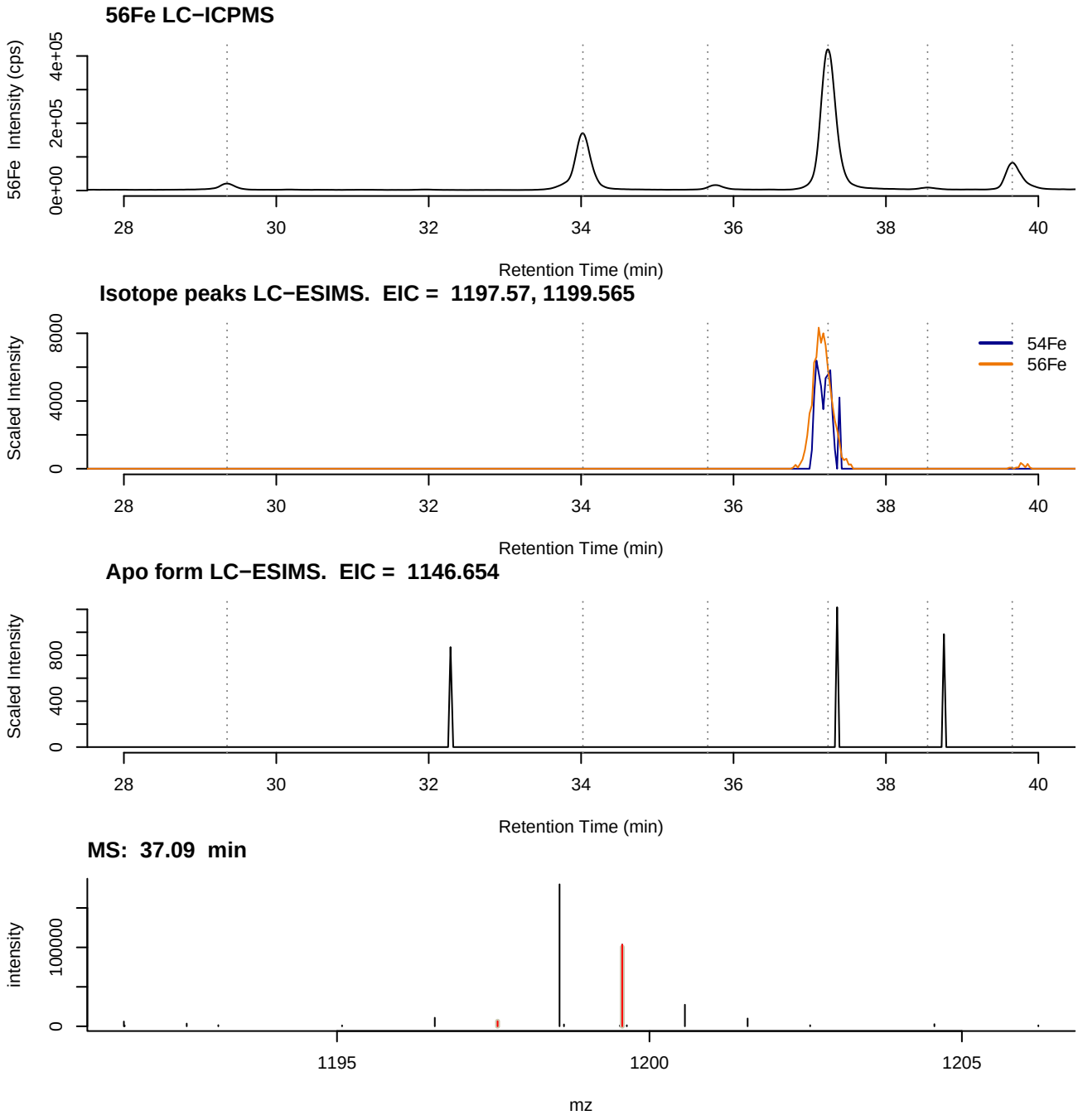

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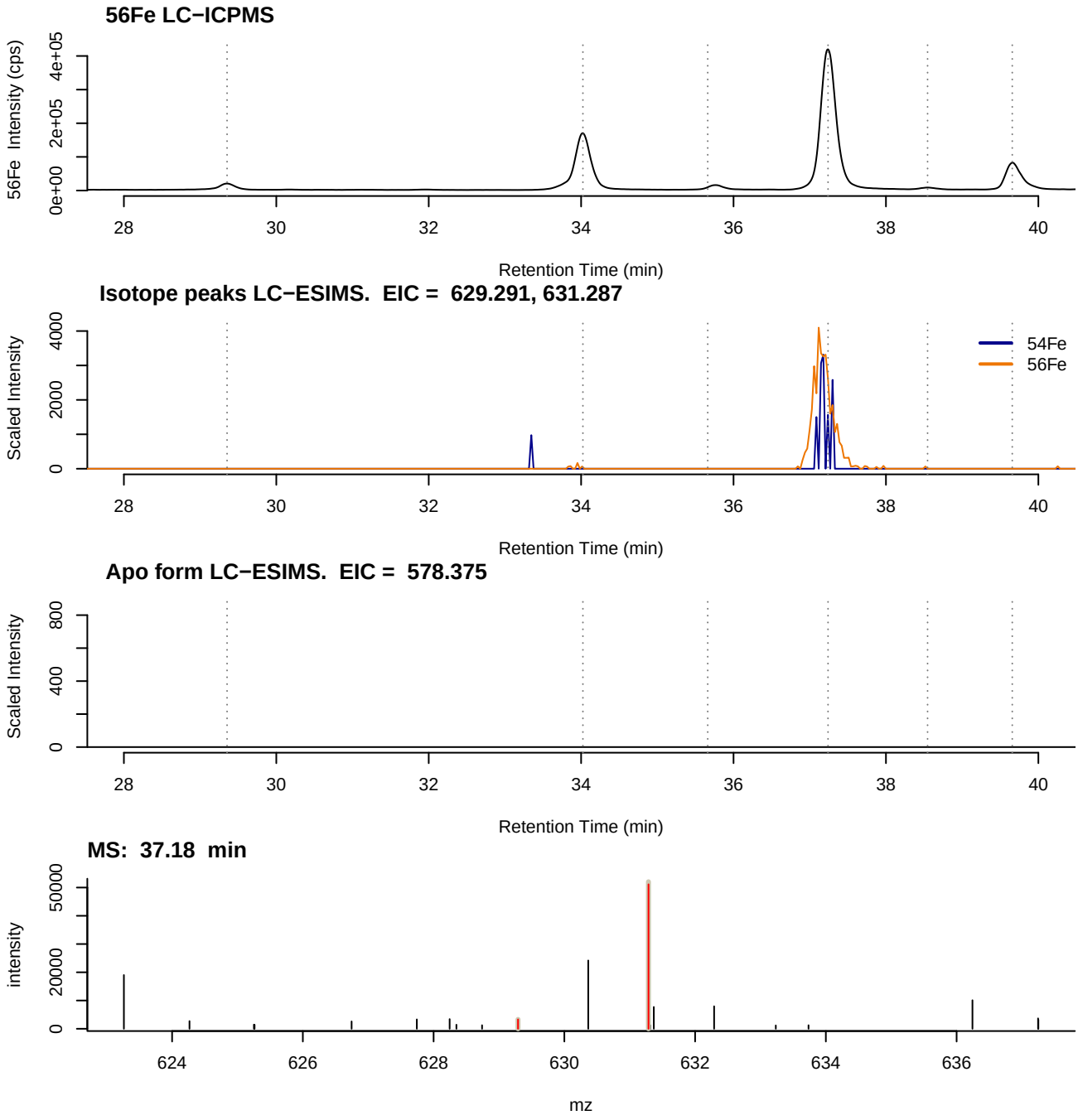

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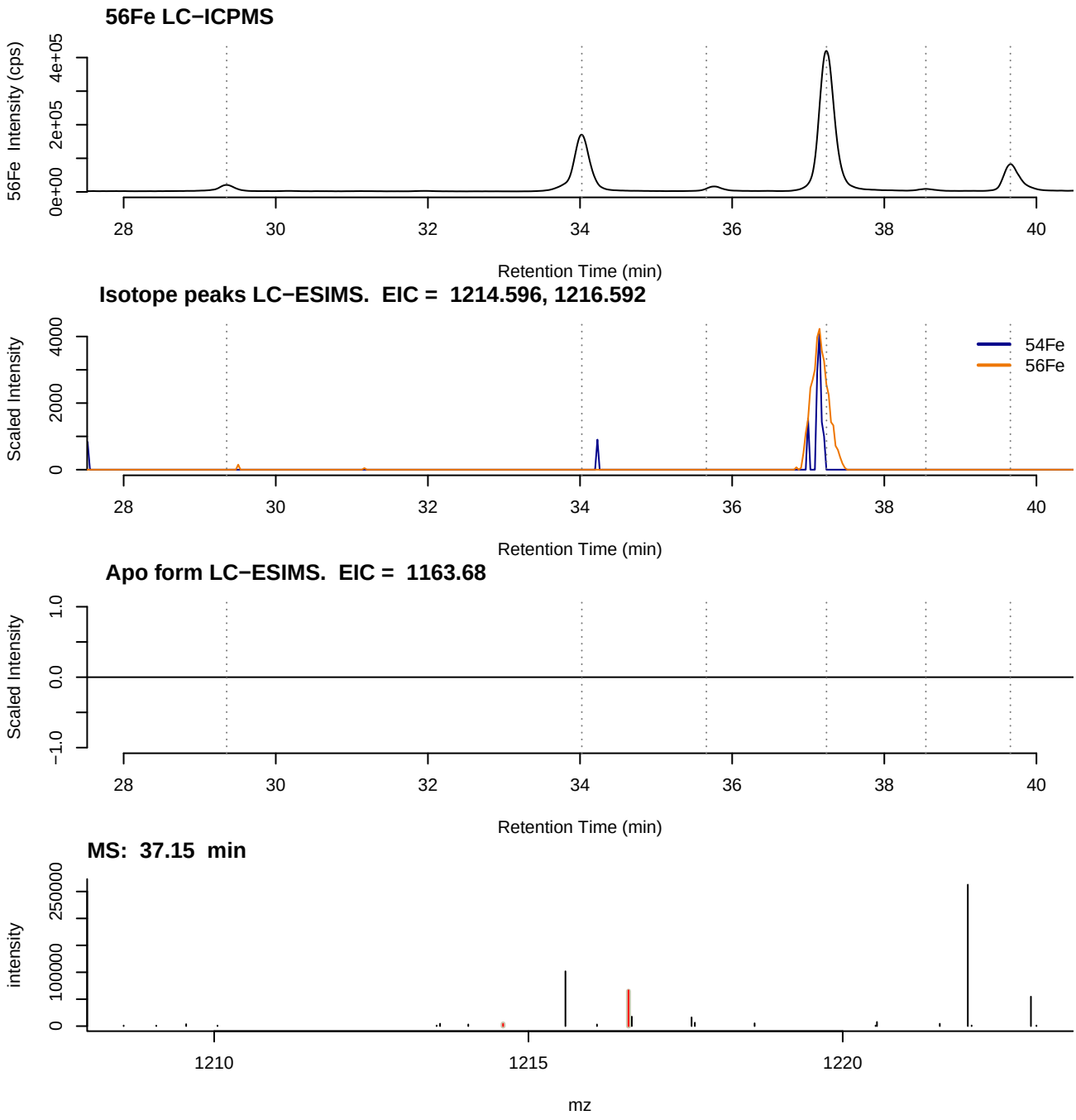

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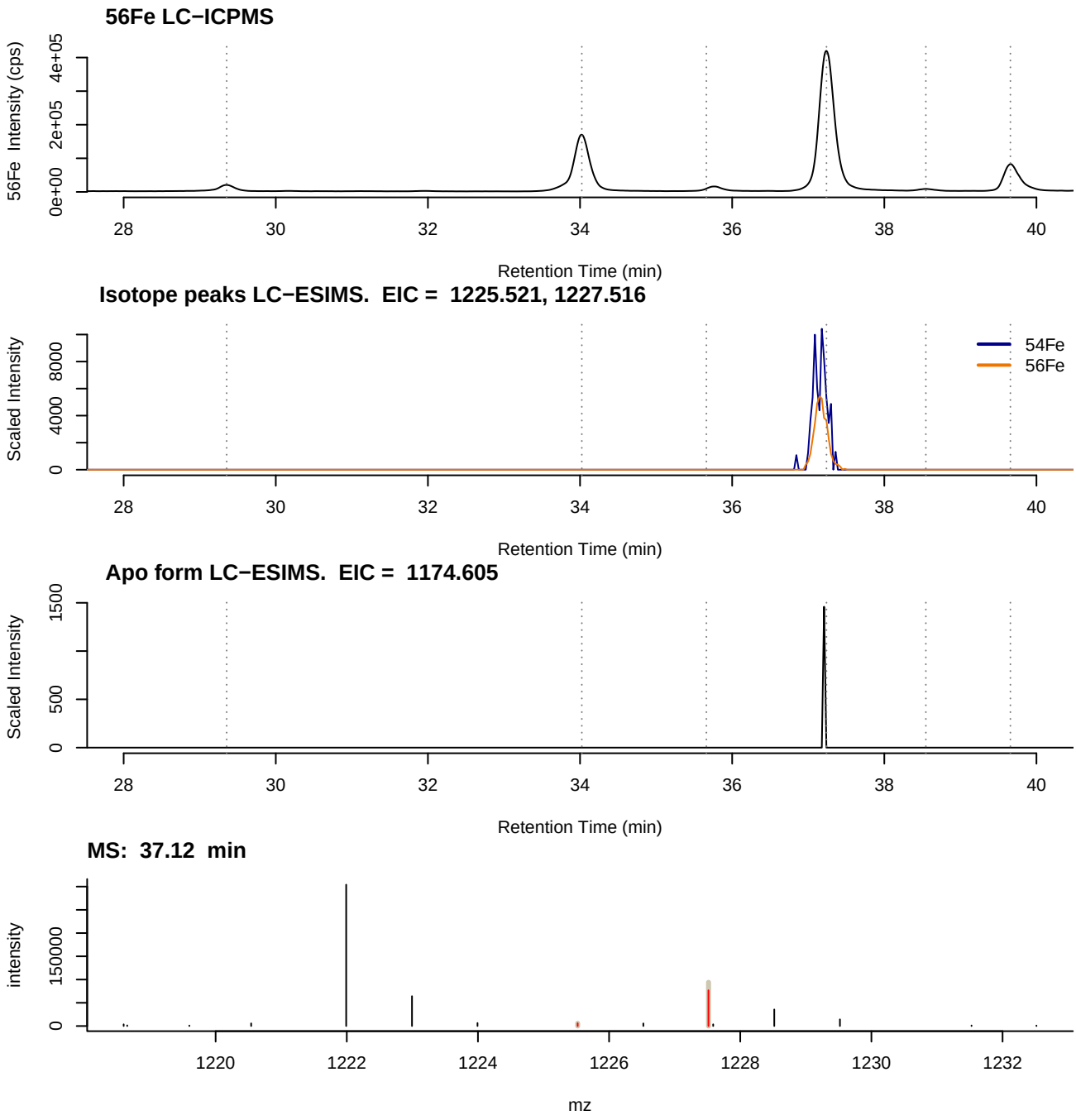

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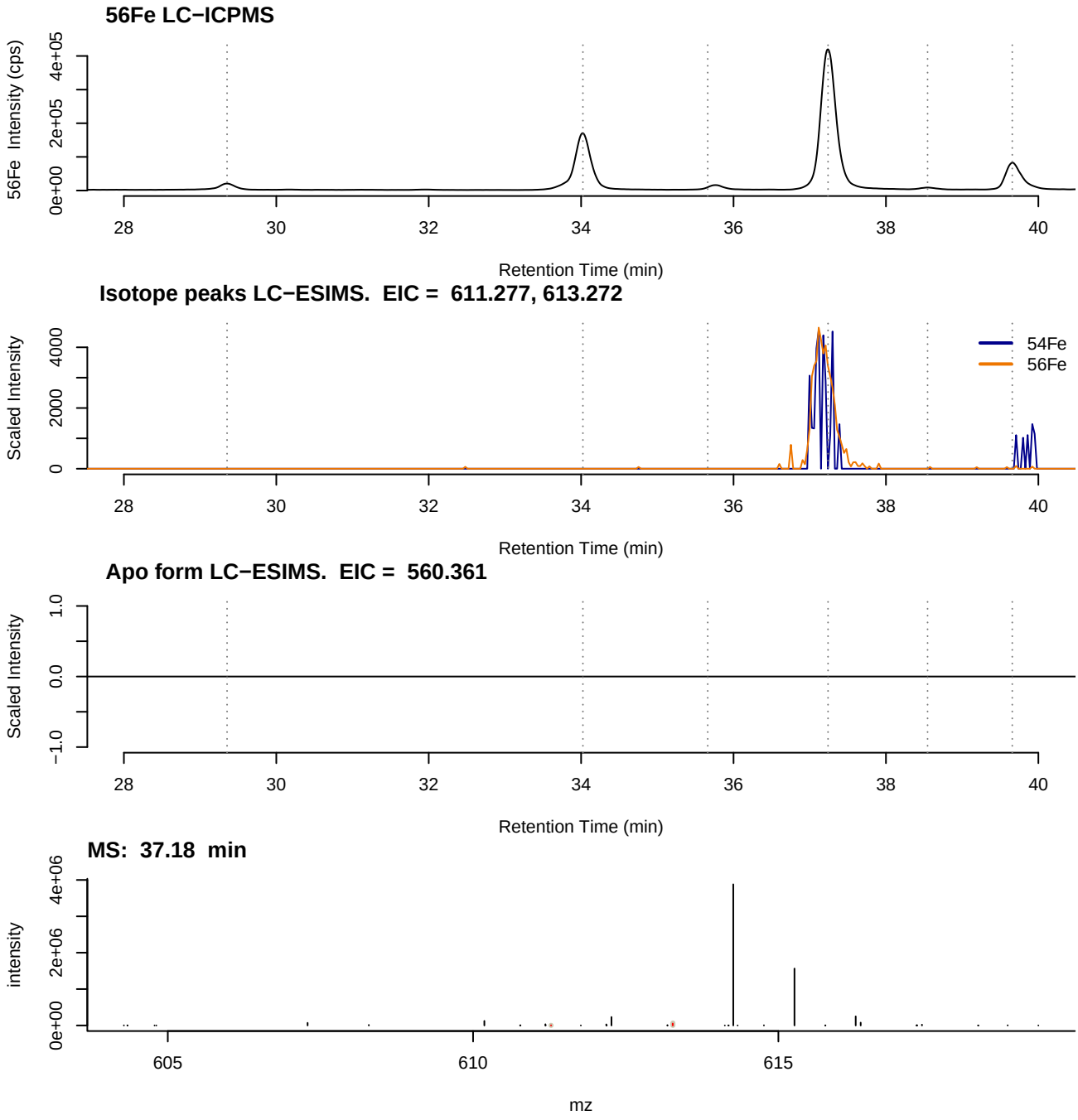

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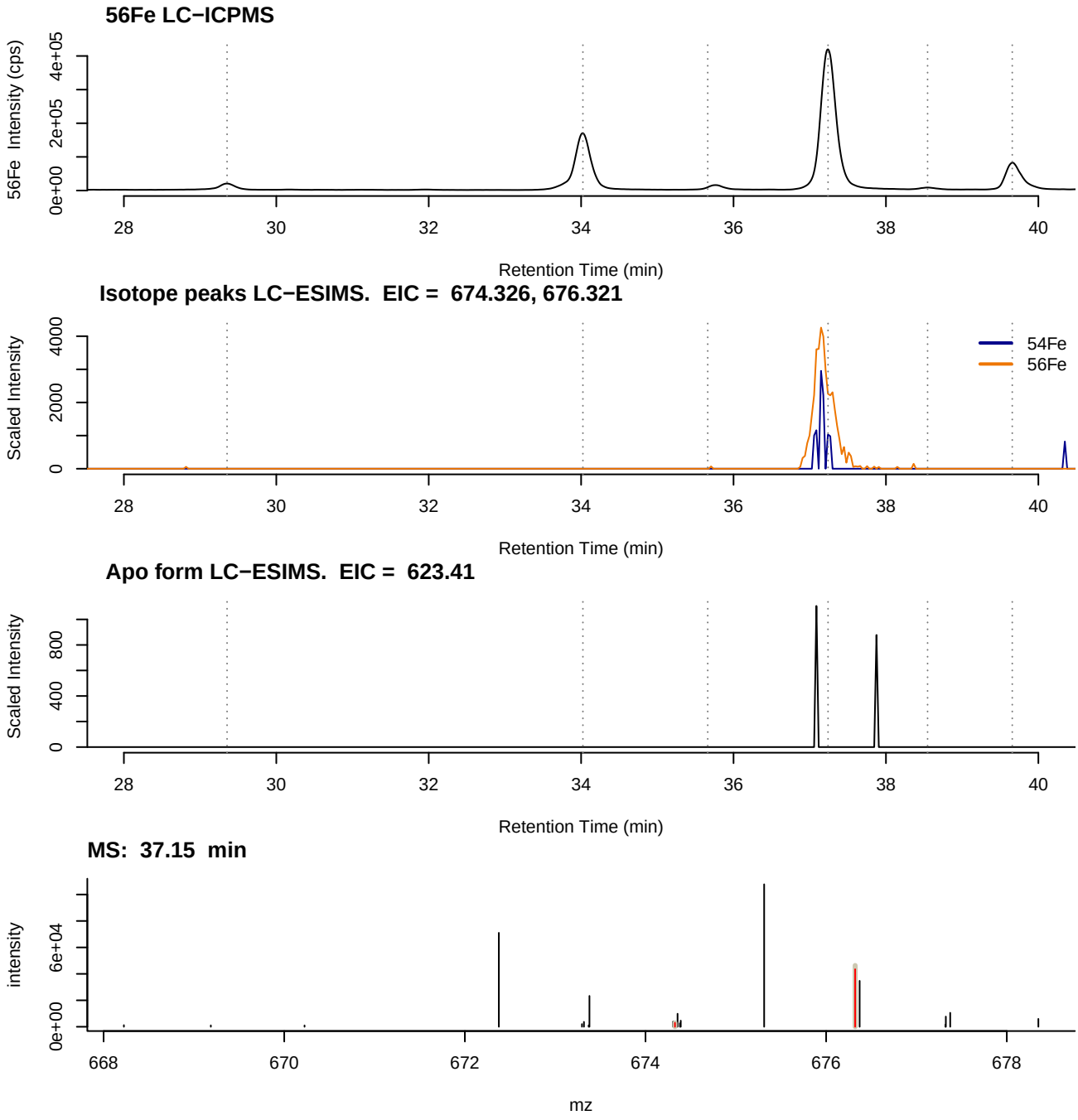

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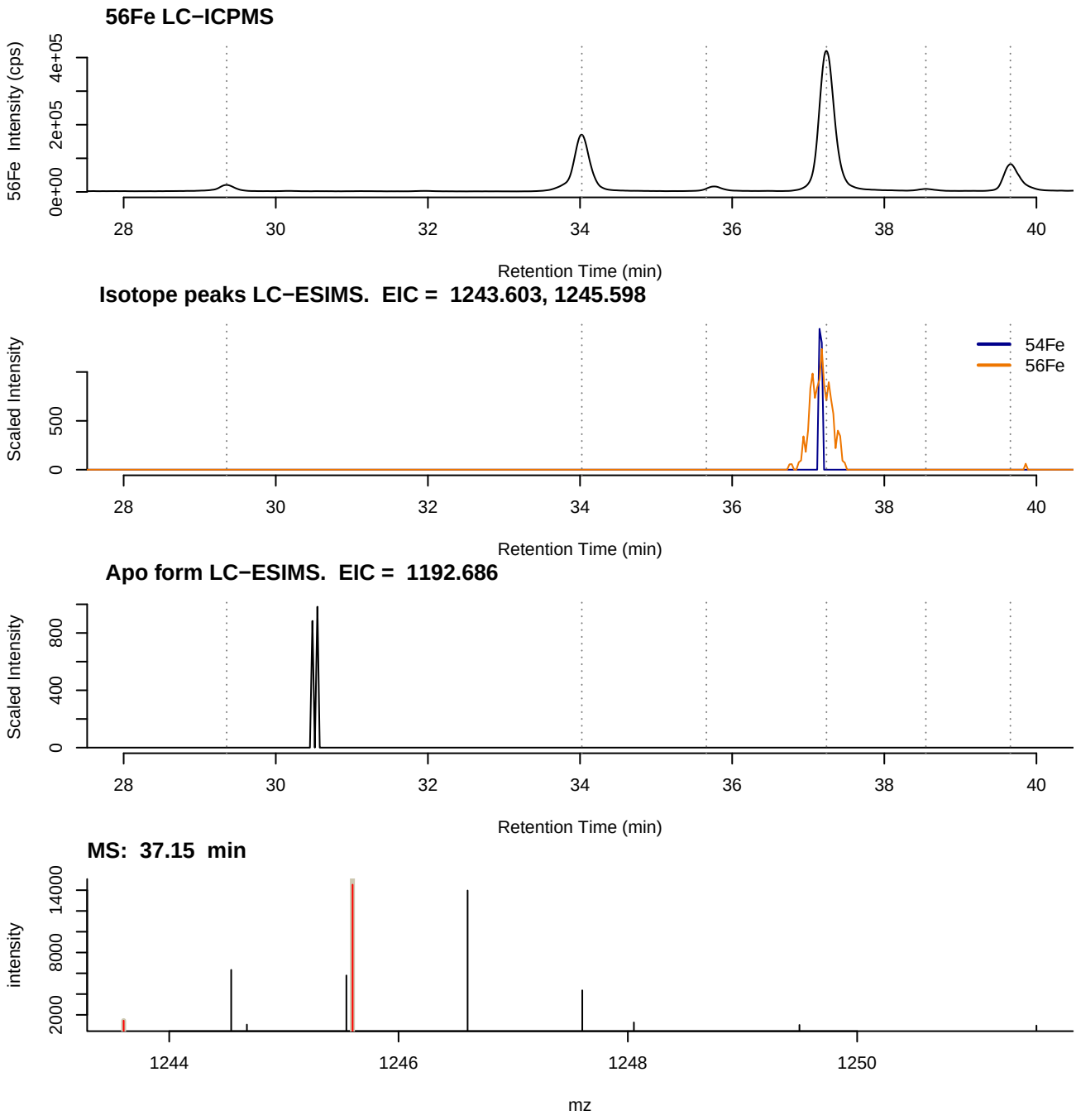

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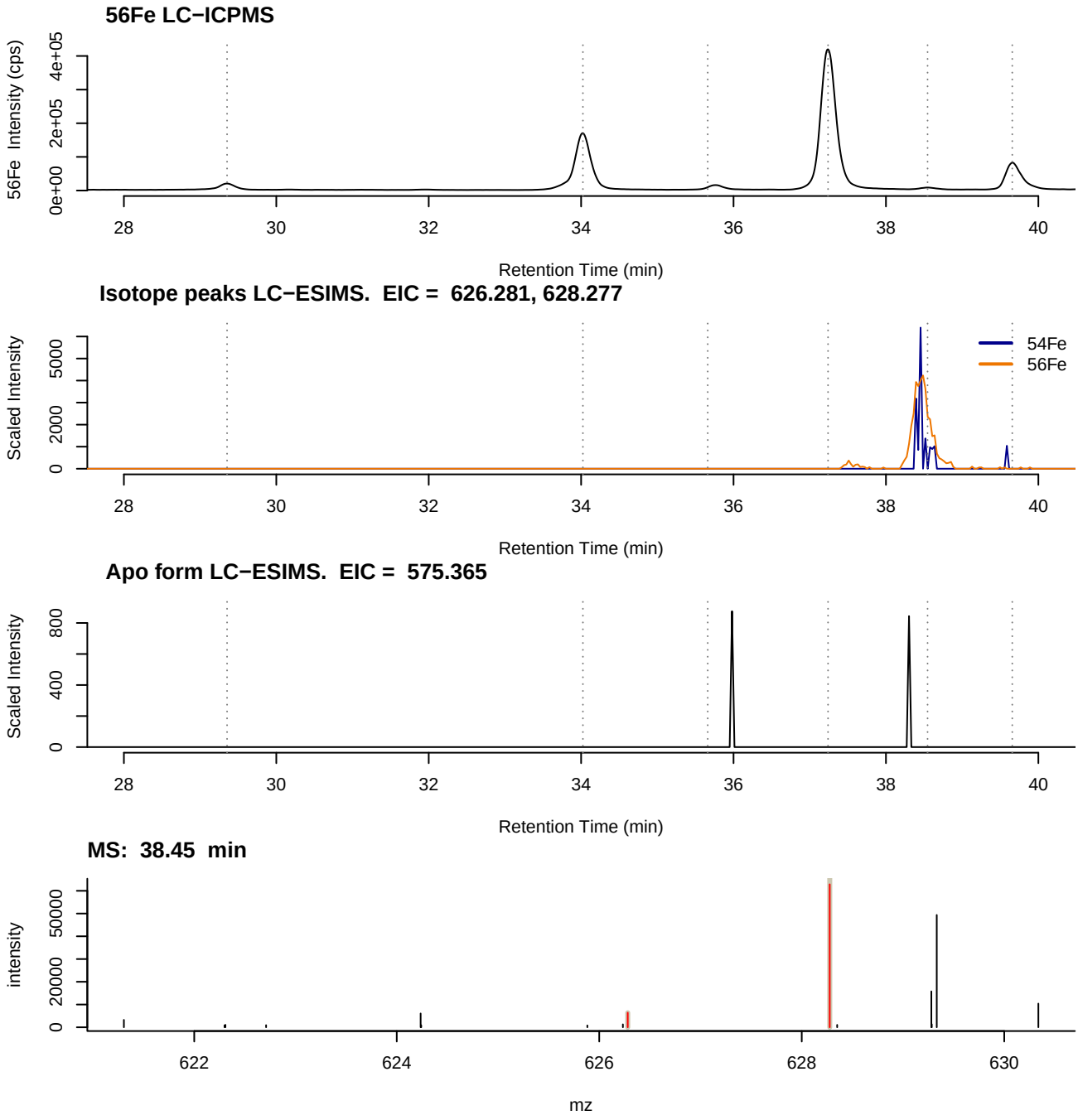

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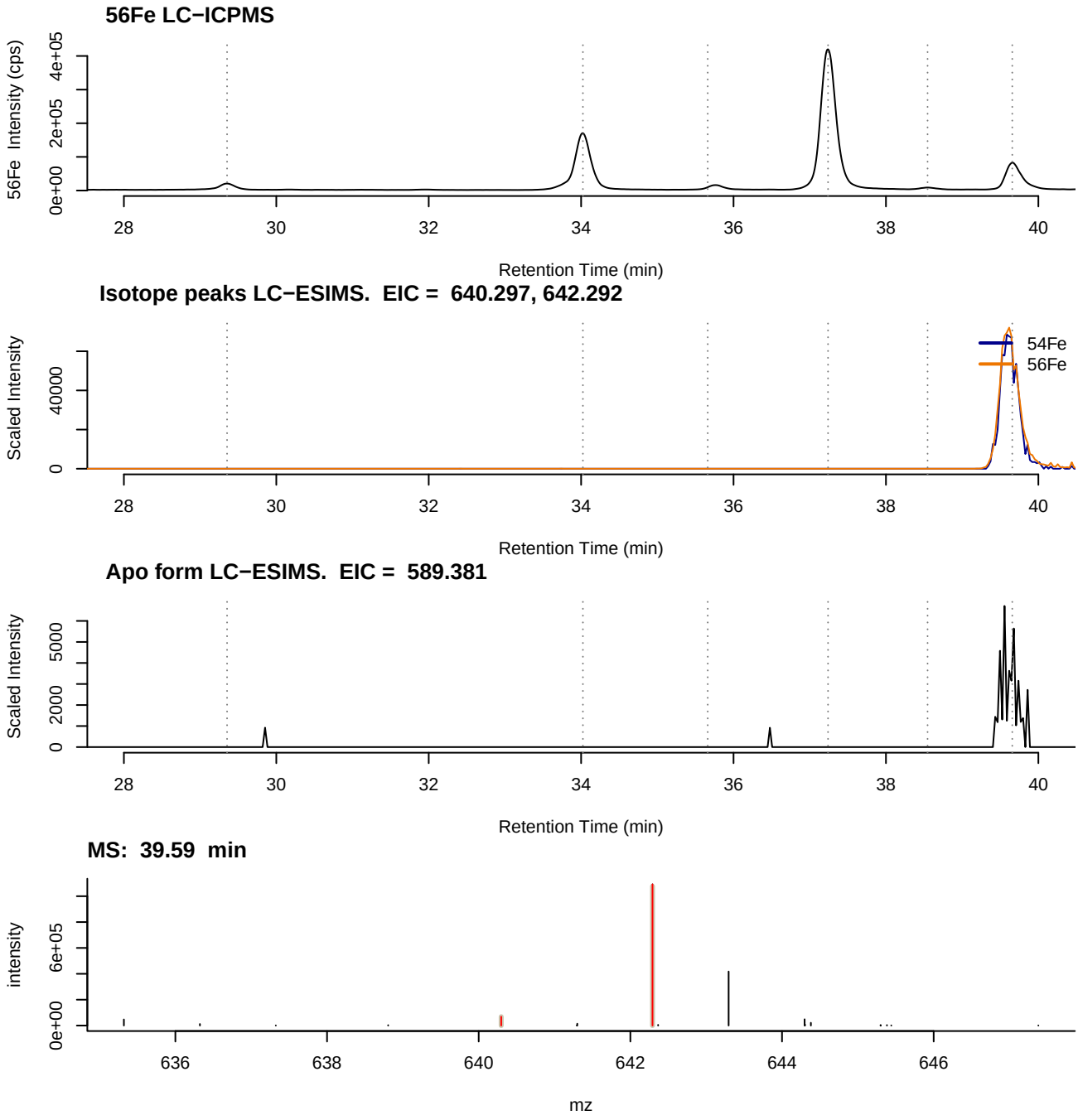

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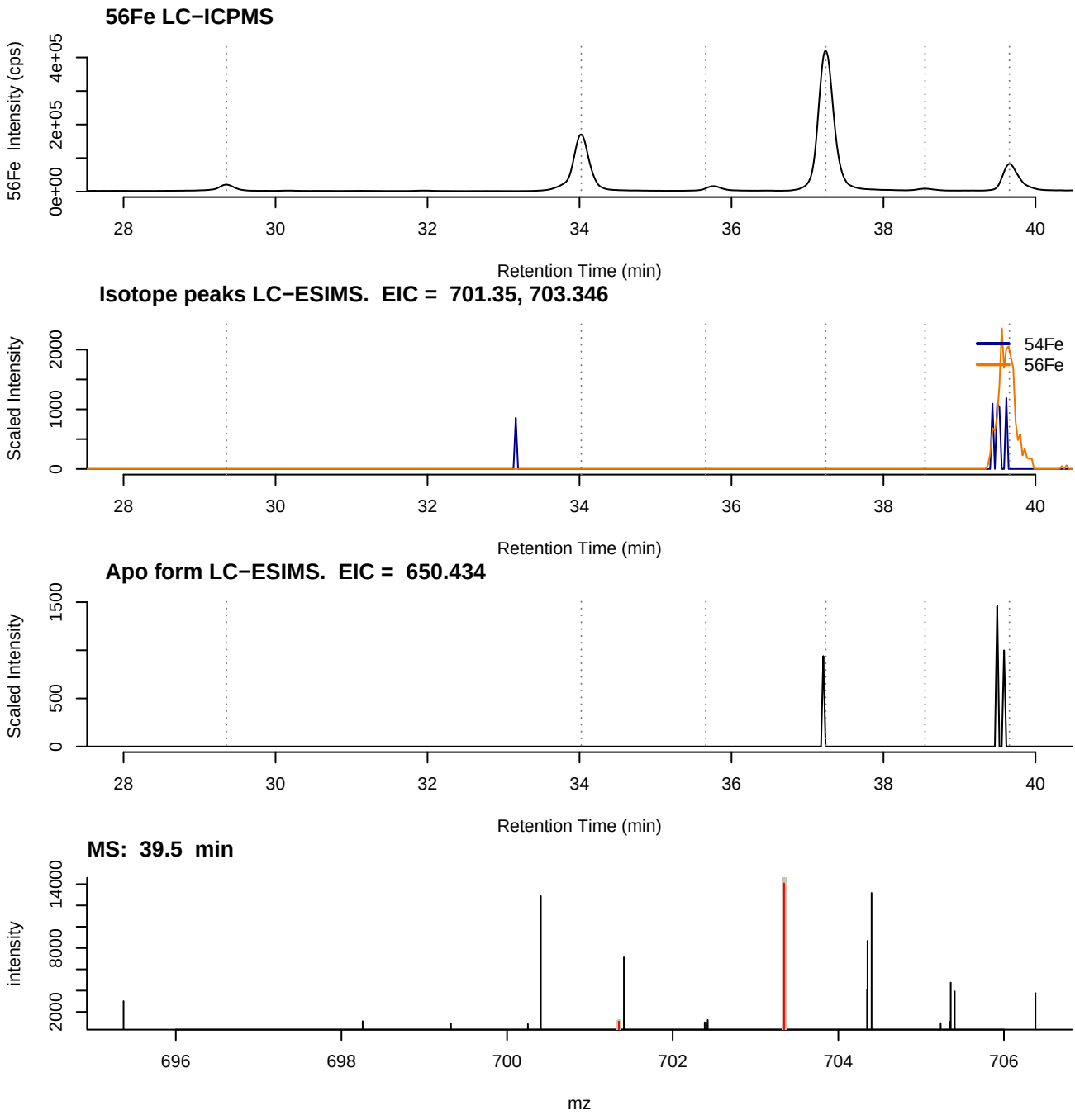

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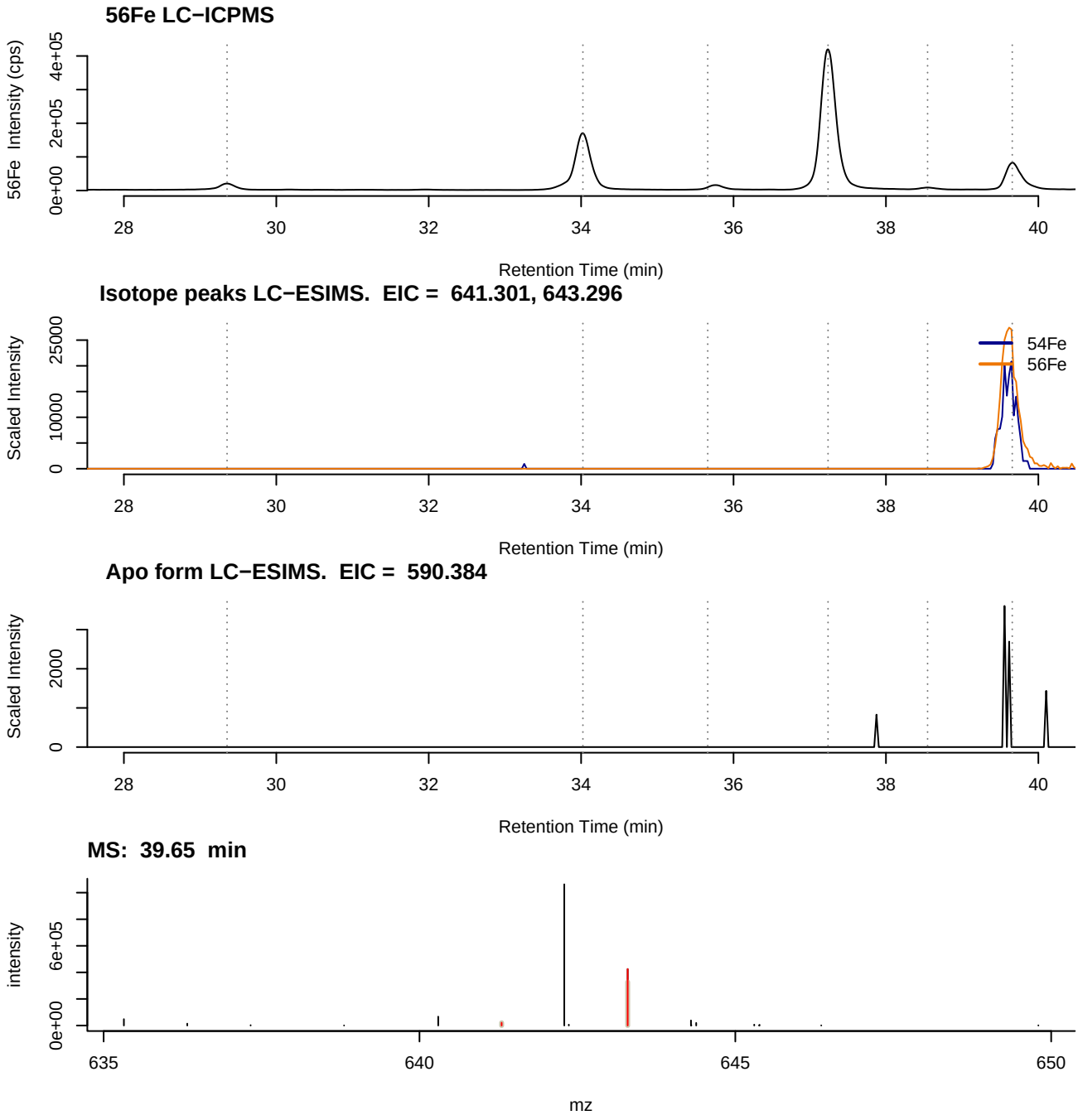

Table SI-1: Complete list of curated isotope pairs identified by algorithm

Retention Time (min)	^{54}FeL m/z	Max ^{54}FeL intensity (cps)	^{56}FLe m/z	Max ^{56}FeL intensity (cps)	Detected with narrow intensity ratio tolerance (±25%) ?
29.3	556.203	15865	558.198	181282	Y
29.3	557.206	2958	559.202	39739	Y
33.9	584.235	128678	586.230	1922865	Y
33.6	656.292	3707	658.287	66731	Y
33.9	585.238	22767	587.234	560587	Y
33.9	583.242	1075	585.238	26840	
33.9	606.217	1255	608.211	25138	
33.9	645.287	2925	647.282	31892	
34.0	1140.502	1348	1142.499	20312	Y
35.7	598.250	10117	600.245	232330	Y
35.6	599.253	3057	601.249	31863	Y
35.8	628.261	1122	630.256	22456	
37.1	612.266	274030	614.261	4023540	Y
37.1	613.270	66696	615.263	1588548	Y
37.2	673.318	5209	675.314	84421	
37.2	1213.593	7249	1215.588	107493	Y
37.1	928.528	2713	930.523	35734	Y
37.1	1196.565	10849	1198.561	179926	Y
37.1	1197.570	6392	1199.564	103510	Y
37.2	629.291	3316	631.287	51143	Y
37.1	1214.596	4136	1216.592	66421	
37.1	1225.521	5997	1227.515	76948	Y
37.2	611.277	4396	613.270	52233	
37.1	674.326	2953	676.321	43493	
37.1	1243.603	1442	1245.599	14531	
38.5	626.282	6402	628.276	62850	
39.6	640.297	68580	642.292	1091913	Y
39.5	701.350	1098	703.345	14075	
39.6	641.301	20860	643.296	425004	

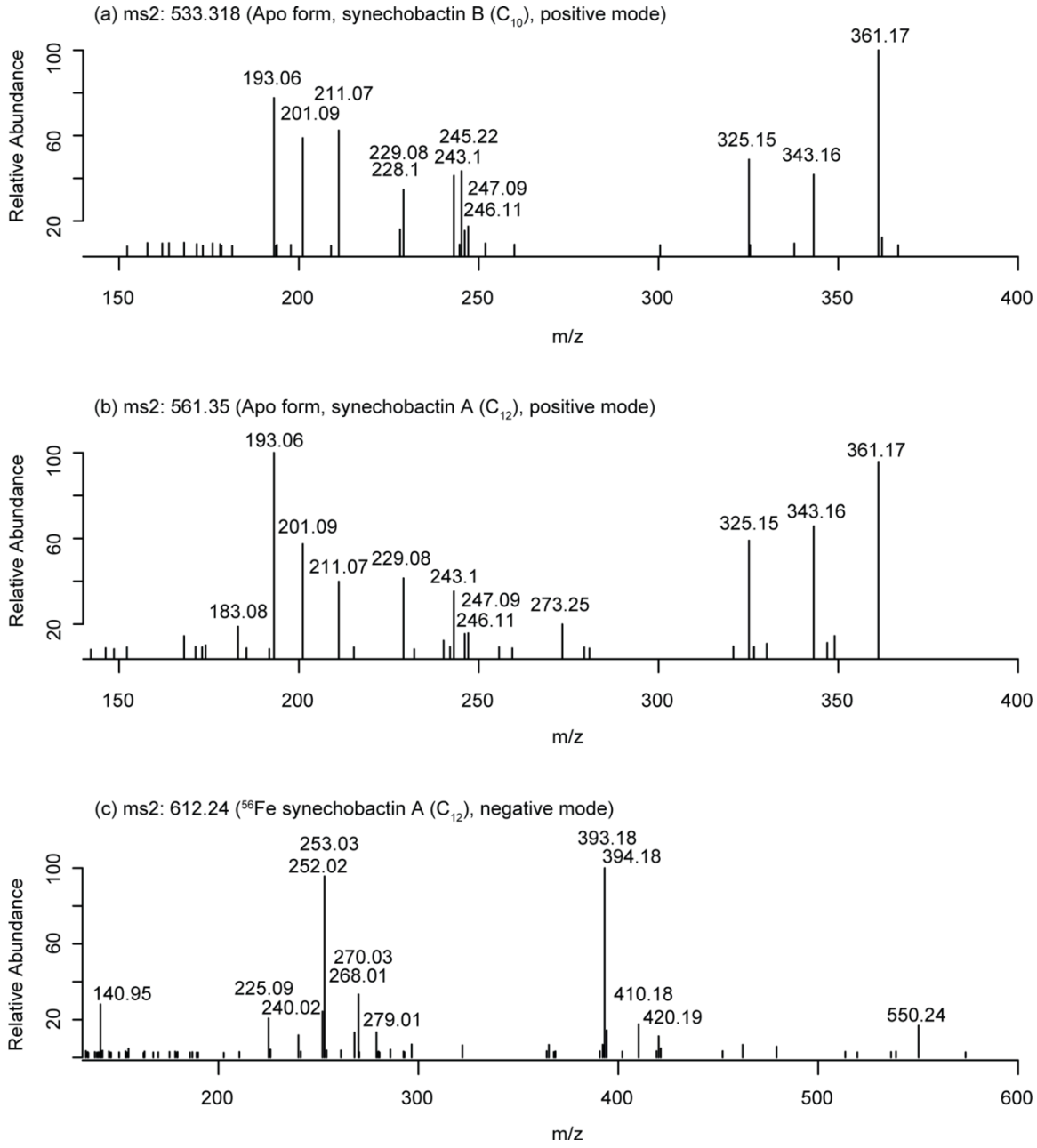

Figure SI-2: (a-b) Top and middle panels show the positive mode MS2 spectra of apo (iron-free) form [M+H^+] of synechobactin B and synechobactin A. (c) Bottom panel shows the negative mode MS2 spectra of the iron bound form of synechobactin A [M+^{56}Fe-4H^+]. Data was collected on the low mass resolution ion trap detector using an isolation window of ±2 m/z

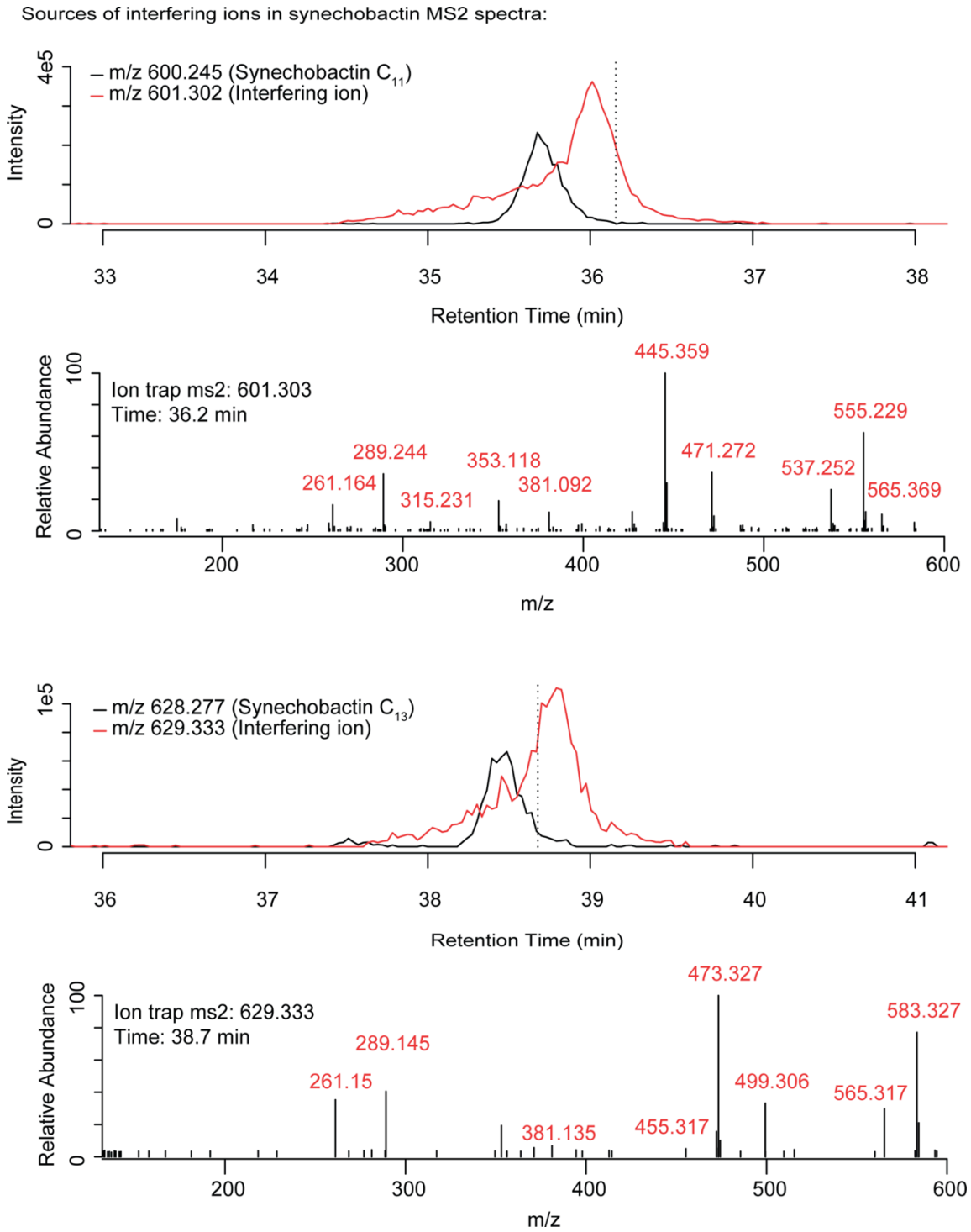

Figure SI-3: EIC and MS2 spectra of interfering ions. The upper panel shows the extracted ion chromatogram (EIC) of synechobactins (a) C_{11} and (b) C_{13} in black and the EIC of the coeluting ion that interferes with the MS2 spectra in red. The vertical dashed lines indicate the retention time at which MS2 spectra of the interfering ion (shown in the lower panel) was collected on the low mass resolution ion trap detector using an isolation window of ±2 m/z. The red labels in the lower panels correspond to the interfering MS2 ions that appear in the spectra in Figure 4 of the text.

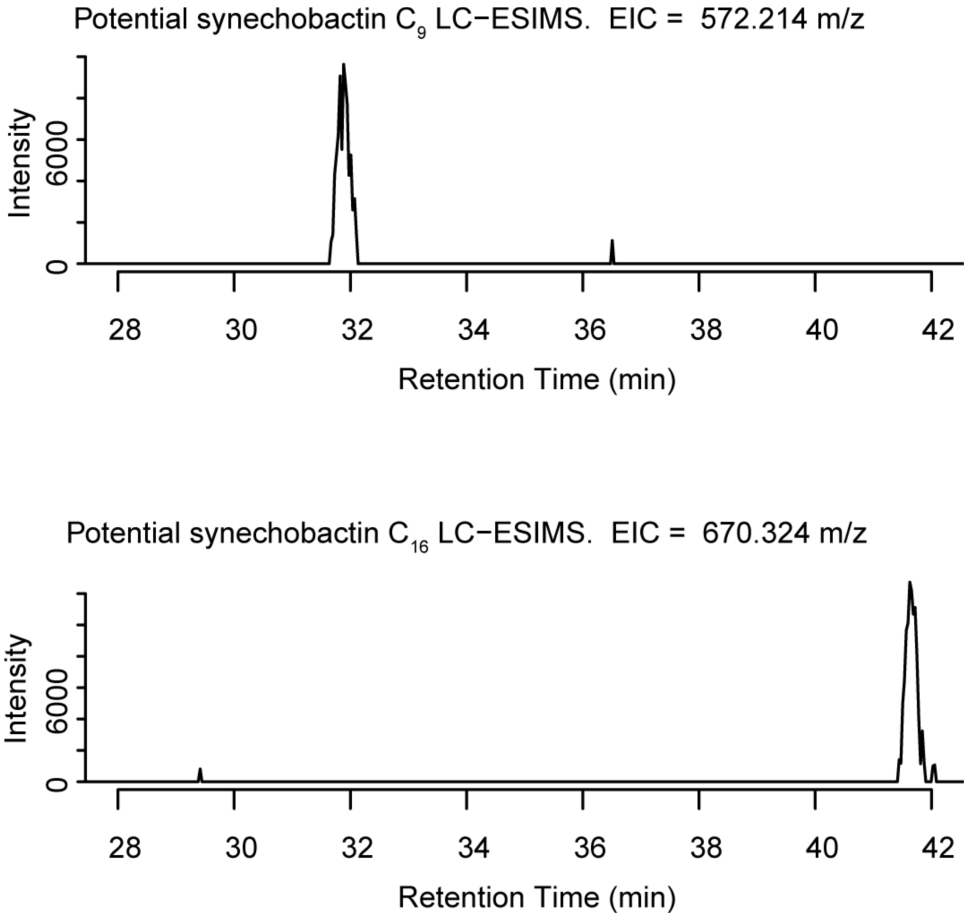

Figure SI-4: Extracted ion chromatograms (EIC) of m/z 670.323 and 572.214, which correspond to the ^{56}Fe monoisotopic mass [$M+^{56}Fe^{3+}-2H^{+}$] of synechobactin C_9 and synechobactin C_{16}.

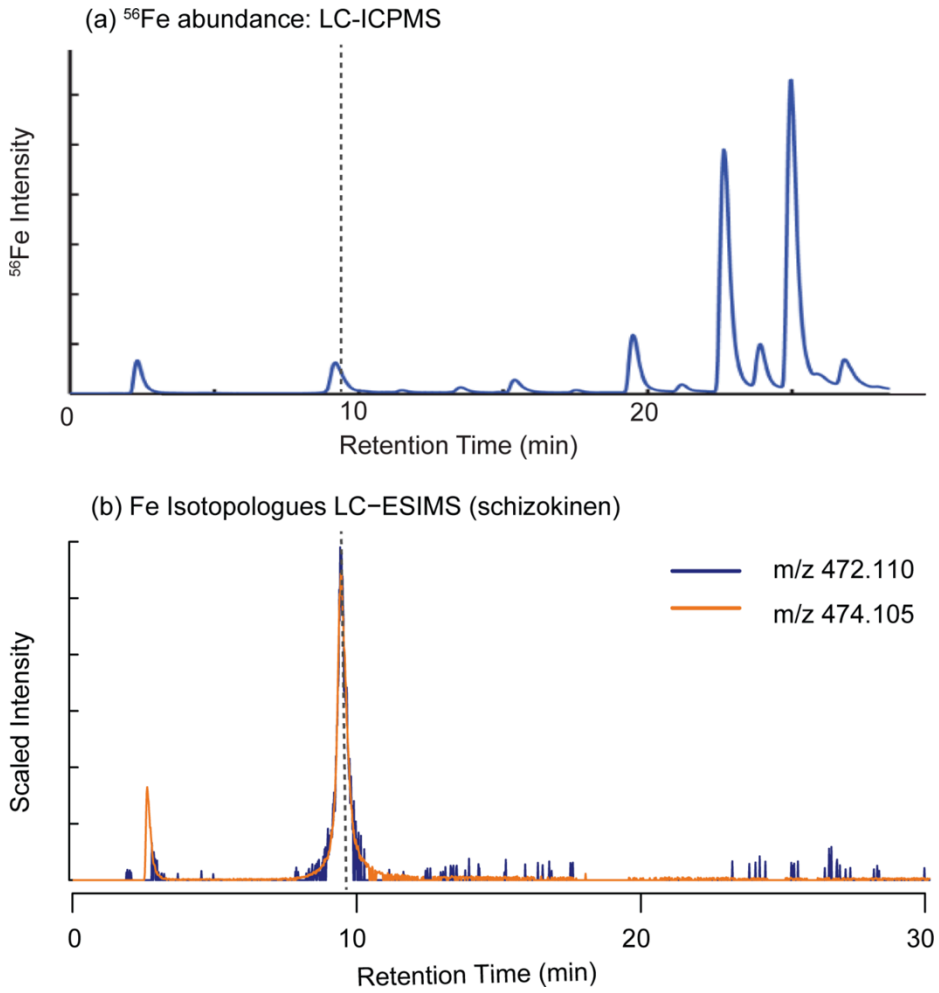

Figure SI-5: Schizokinen production by Synechococcus sp. PCC 7002. LC-MS chromatograms of *Synechoccoccus* sp. PCC 7002 media extract collected during a previous study (*Boiteau et al.,* 2013). (a) ^{56}Fe LC-ICPMS chromatogram. The peak at 10 minutes corresponds to schizokinen produced by *Synechococcus* sp PCC 7002 in this experiment (b) extracted ion chromatograms from positive mode LC-ESIMS run. The blue line correspond to the light iron isotopologue of schizokinen [M+$^{54}Fe^{3+}$-2H^{+}] that were identified by the isotope algorithm, and the orange line corresponds to the heavy iron isotopologue [M+$^{56}Fe^{3+}$-2H^{+}]. The intensity of the heavy iron isotopologue has been scaled by the natural abundance ratio of ^{56}Fe/^{54}Fe (divided by 15.7) so that the isotopologues overlap. Compounds were separated with a 20 minute chromatographic gradient from H_2O to MeOH using 5 mM ammonium formate as a buffer and a 3 μm particle size 2.1x150 mm C18 column (Hamilton).