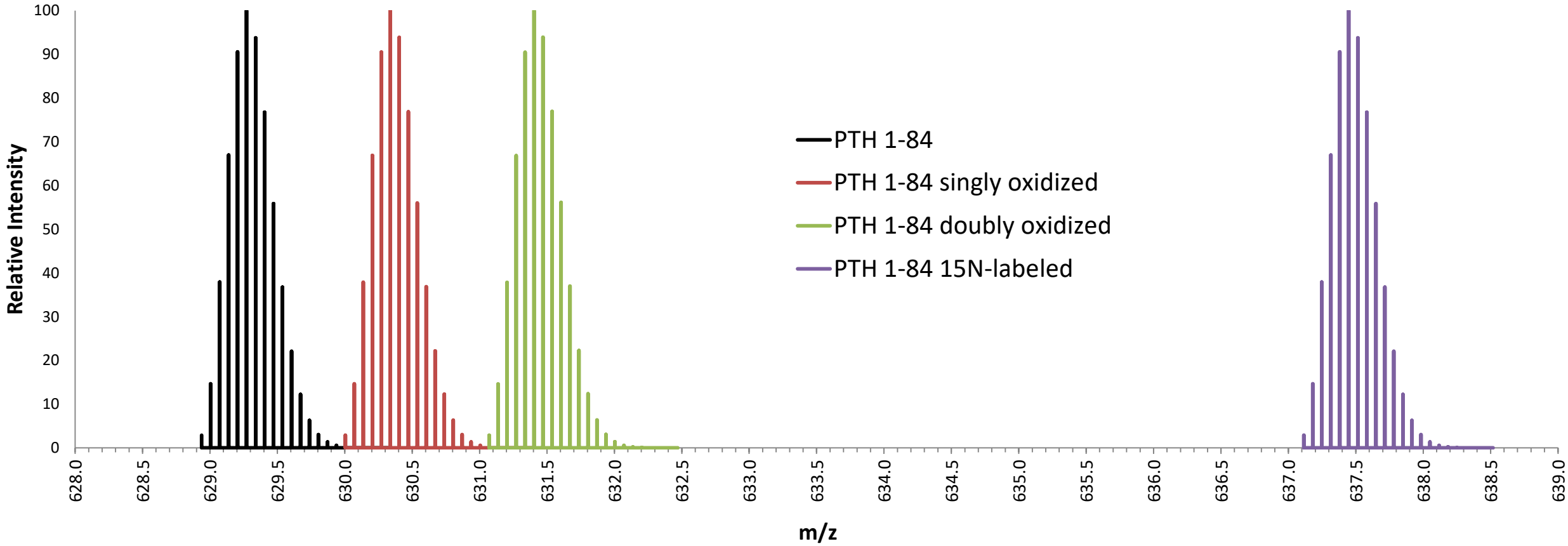
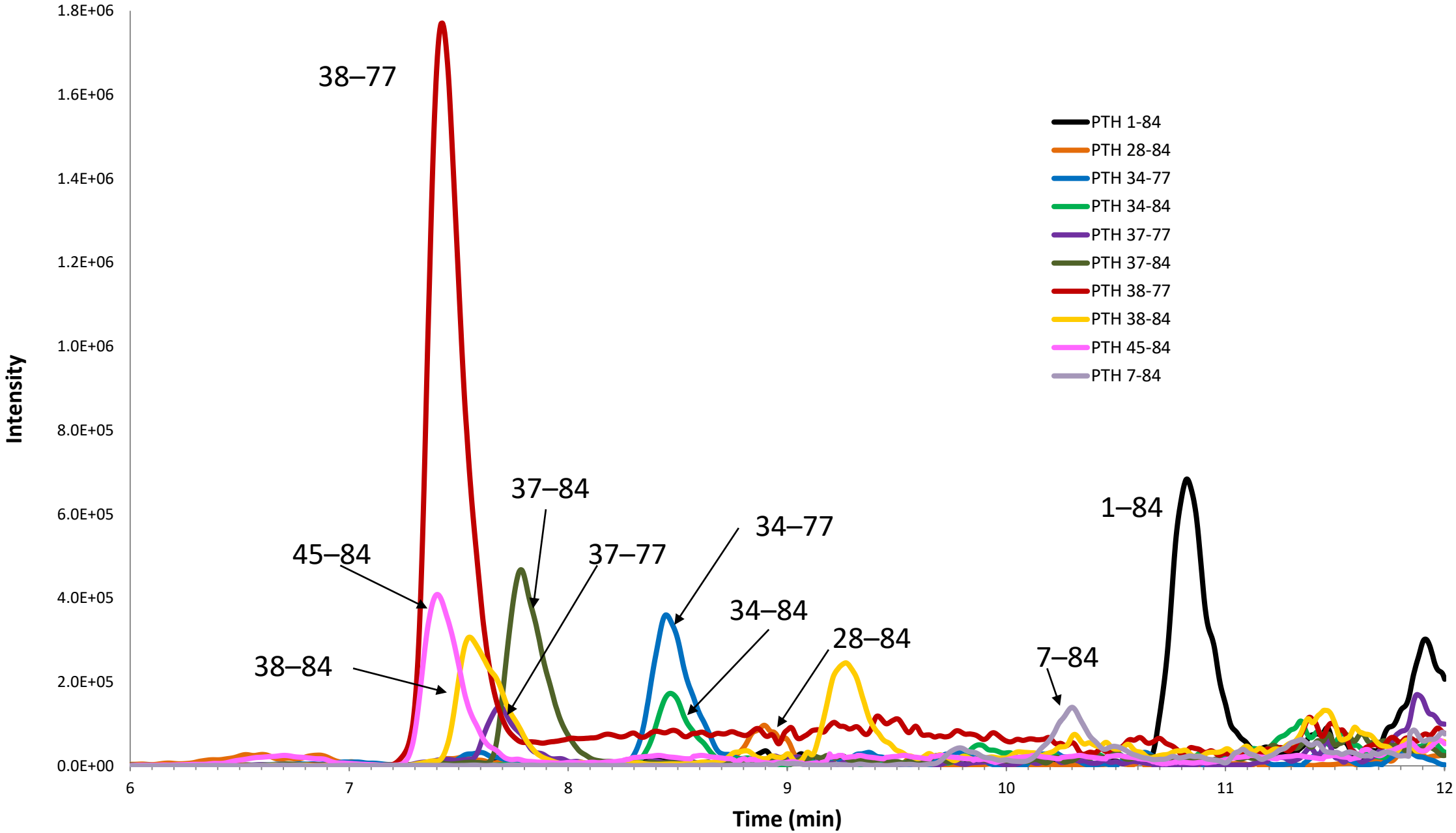
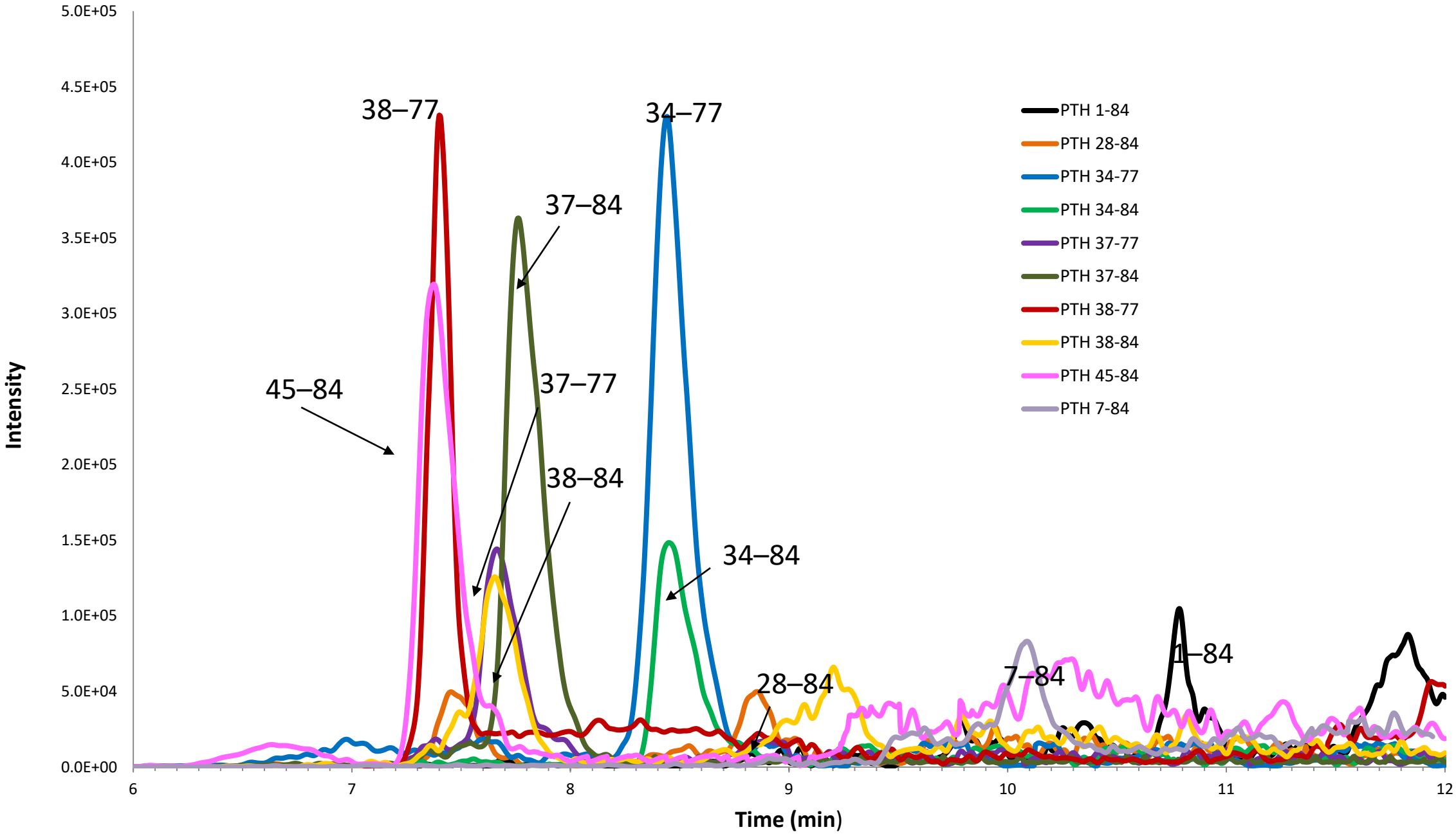


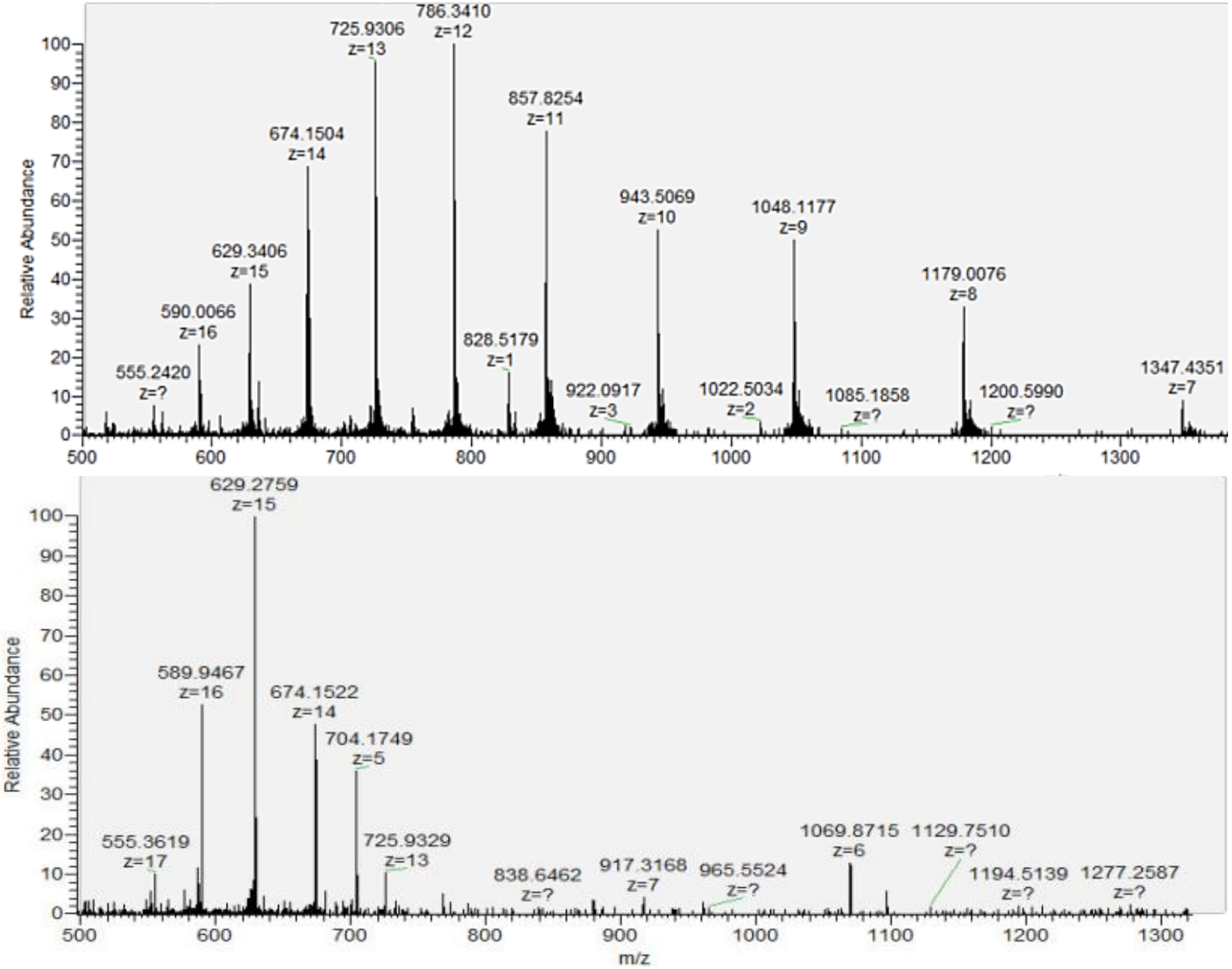
Supplemental Figure 1. Theoretical m/z for the +15 charge state for PTH1-84, U-^{15}N-PTH1-84, and singly-oxidized and doubly-oxidized forms of PTH1-84: PTH1-84M8(O); PTH1-84M18(O); and PTH1-84M8(O)18(O). From each spectral envelope we selected the first 10 m/z which accounts for ~93% of all signal. Due to the minimal overlap of spectral envelopes between the PTH1-84 and singly-oxidized PTH1-84, PTH1-84 could contribute ~0.059% of it's signal to singly-oxidized PTH1-84 which would become significant when PTH1-84 concentrations are ~82,000 pg/mL.



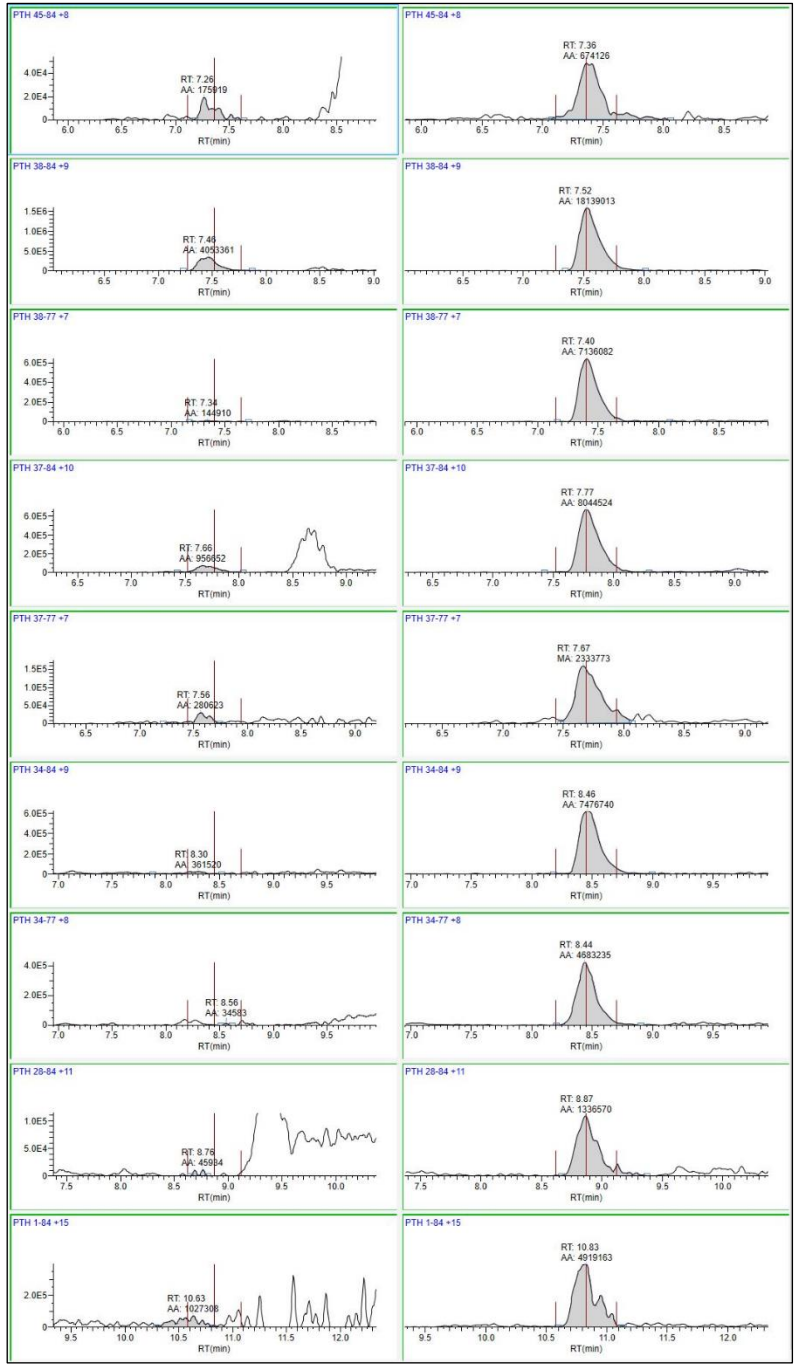
Supplemental Figure 2. Extracted ion chromatogram of a quality control in serum, depicting the lower limits of quantitation for each PTH peptide as described in Supplemental Table 4. PTH peptides were spiked into neat serum with concentrations ranging from 56 to 235 pg/mL. For a list of m/z ions used, refer to Supplemental Table 3.



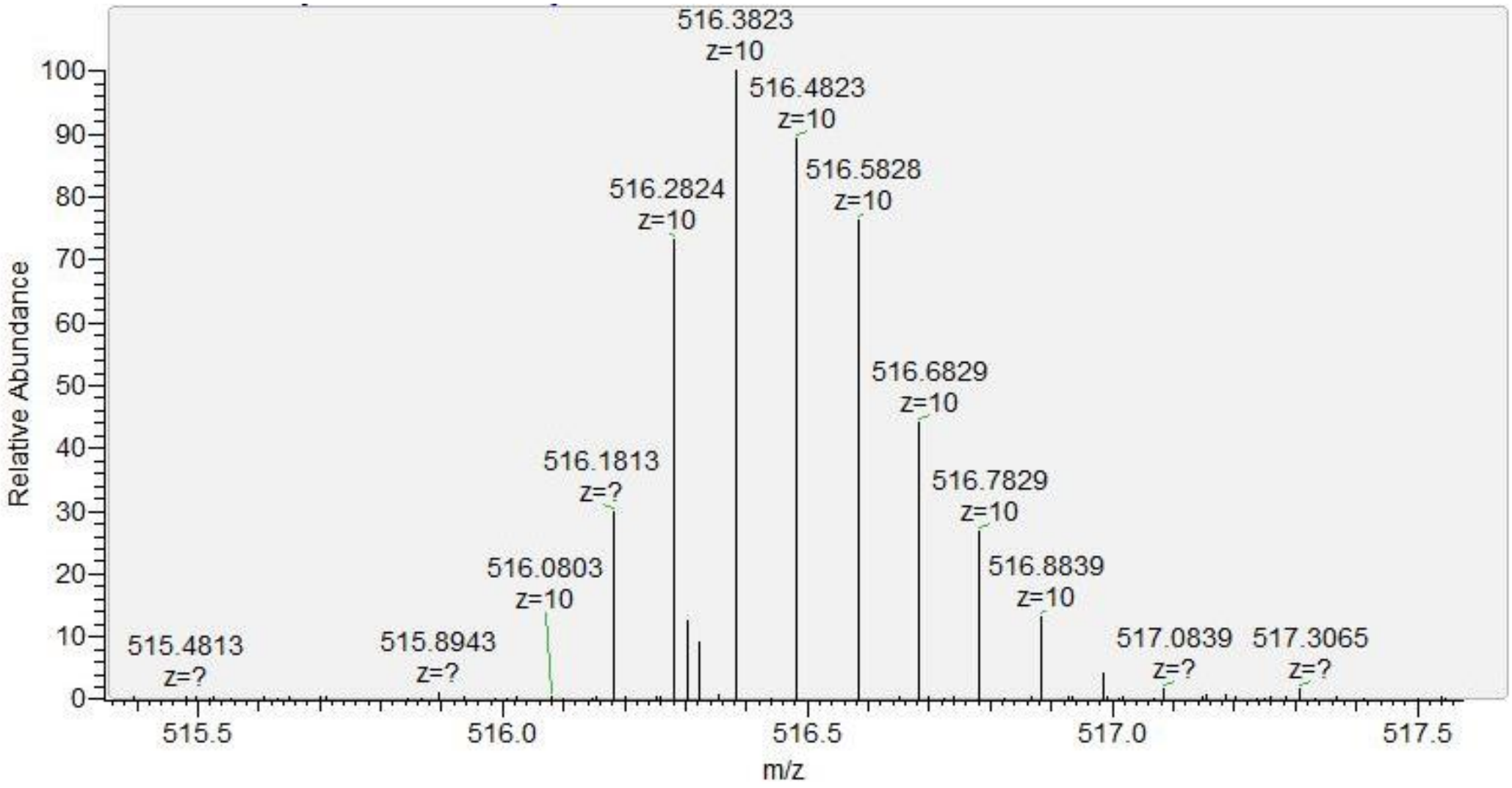
Supplemental Figure 3. Extracted ion chromatogram of a working standard, depicting the limit of detection for each PTH peptide as described in Supplemental Table 4. Concentration range of PTH Peptides was 24-81 pg/mL. For a list of m/z ions used, refer to Supplemental Table 3.



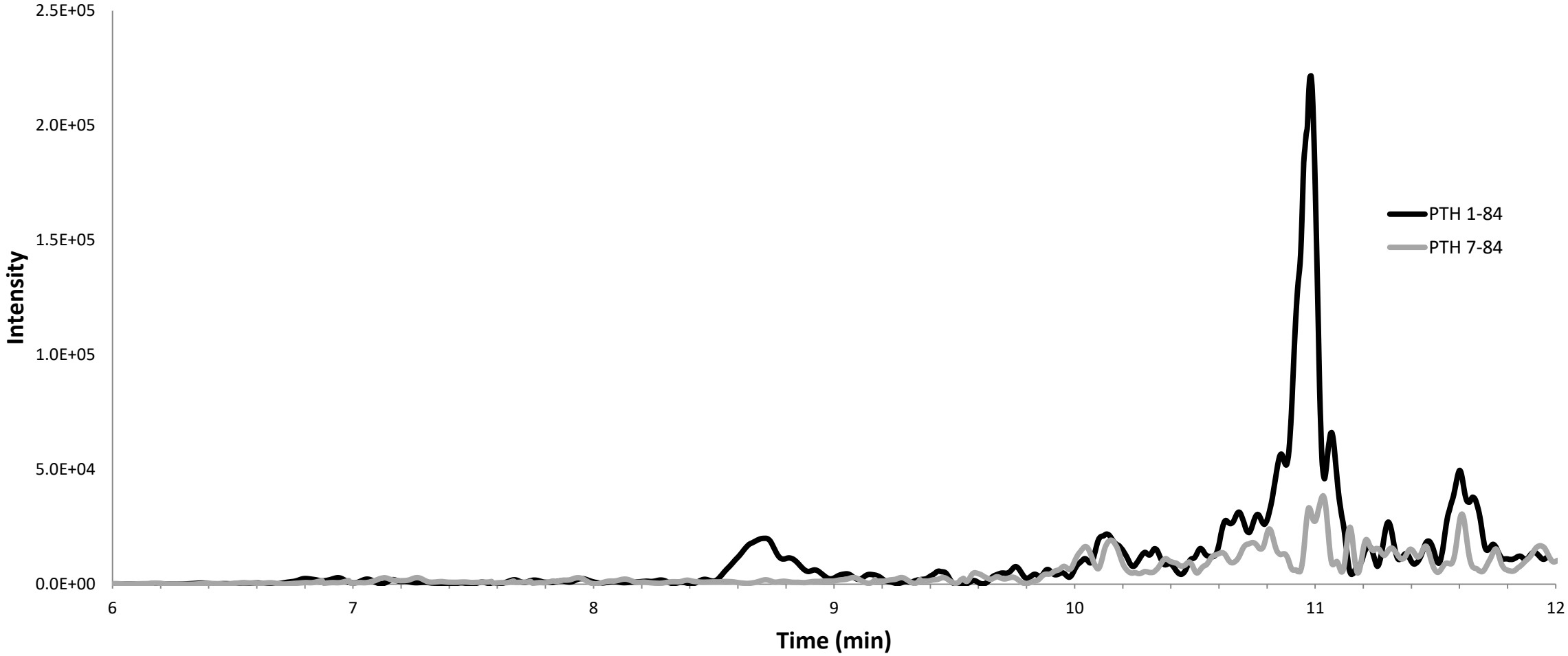
Supplemental Figure 4. Dimethyl sulfoxide (DMSO) as a super-charging agent. Top: Analysis of PTH1-84 using mobile phase without the addition of DMSO which results in PTH1-84 m/z spread over approximately 10 charge states. Bottom: With addition of the super-charging agent DMSO in the mobile phase, the charge states for PTH1-84 are shown to coalesce to approximately 3 with the +15 charge state as the most abundant, increasing signal and thus reducing LLOQ.



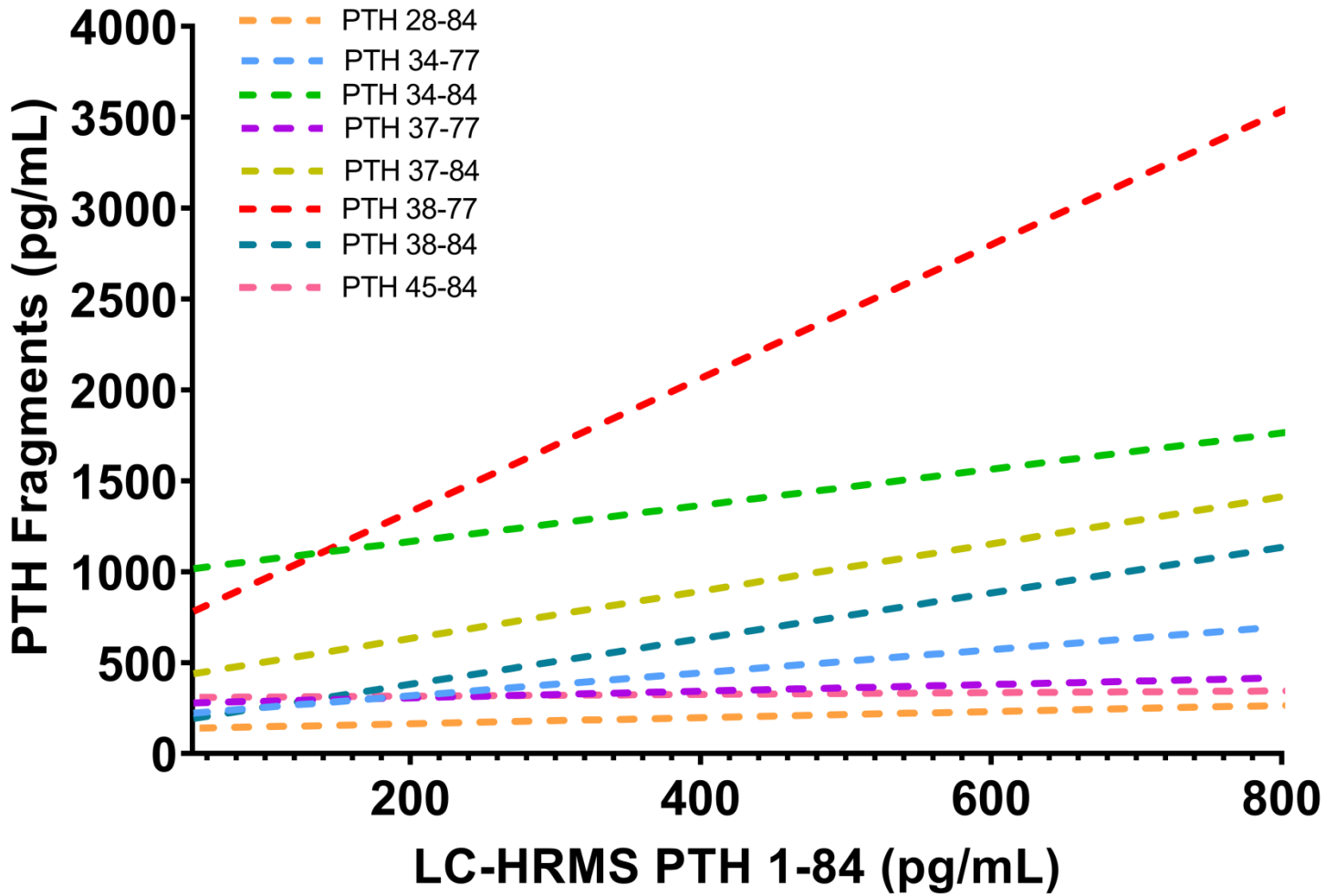
Supplemental Figure 5. Comparative chromatogram of serum samples from a patient with eGFR of 60 (left) versus 20 mL/min/1.73 m^2 (right) for PTH1-84 and each of the observed PTH fragments.



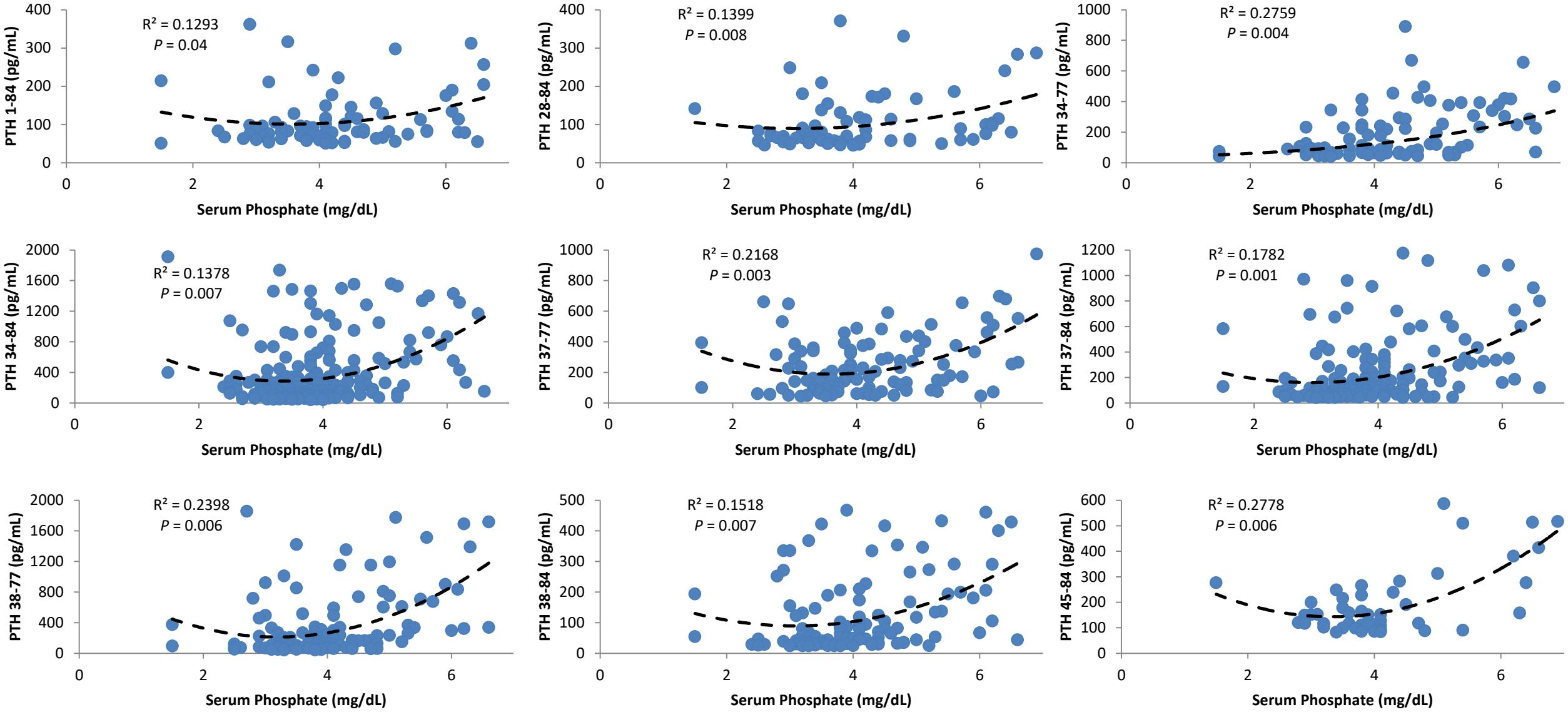
Supplemental Figure 6. Example of PTH37-84 +10 spectral envelope in a serum sample from a patient with eGFR of 30 mL/min/1.73 m^2.



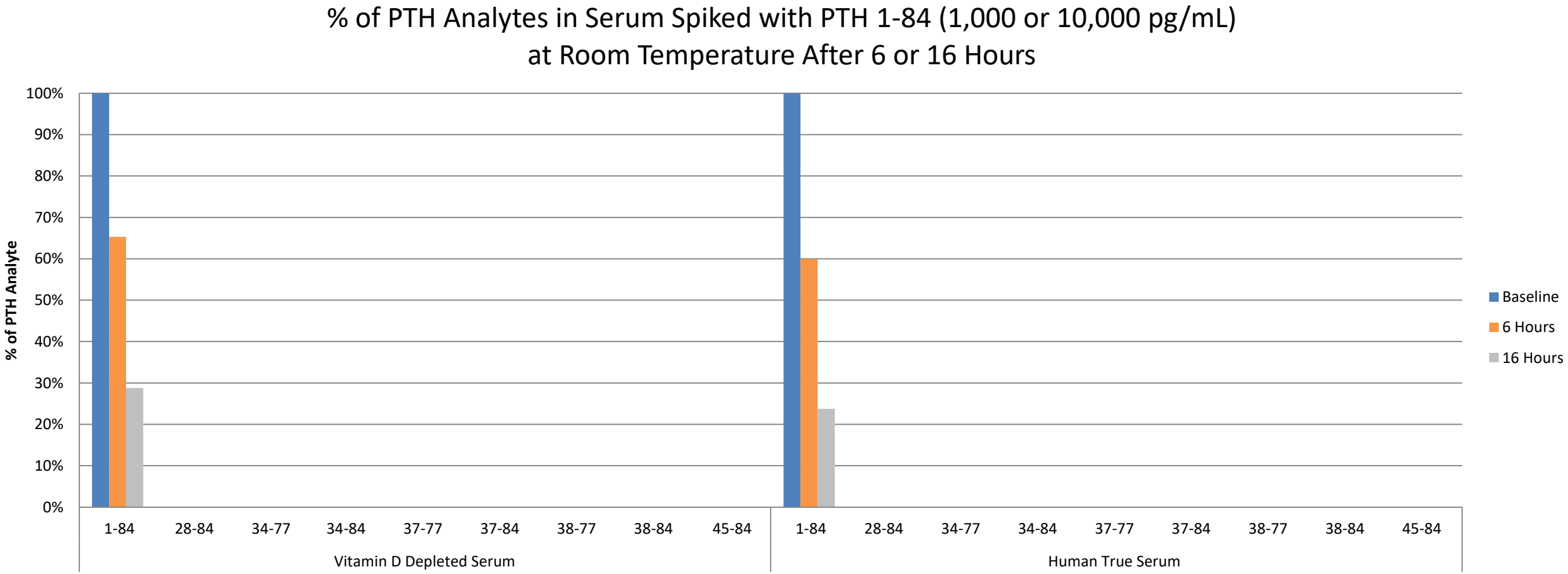
Supplemental Figure 7. Extracted ion chromatograms of PTH1-84 overlaid with PTH7-84 in a patient serum depicting LC-HRMS PTH1-84 at low concentration (86 pg/mL) near the limit of detection (50 pg/mL). PTH7-84 is not detectable at its retention time of ~10.1 minutes.



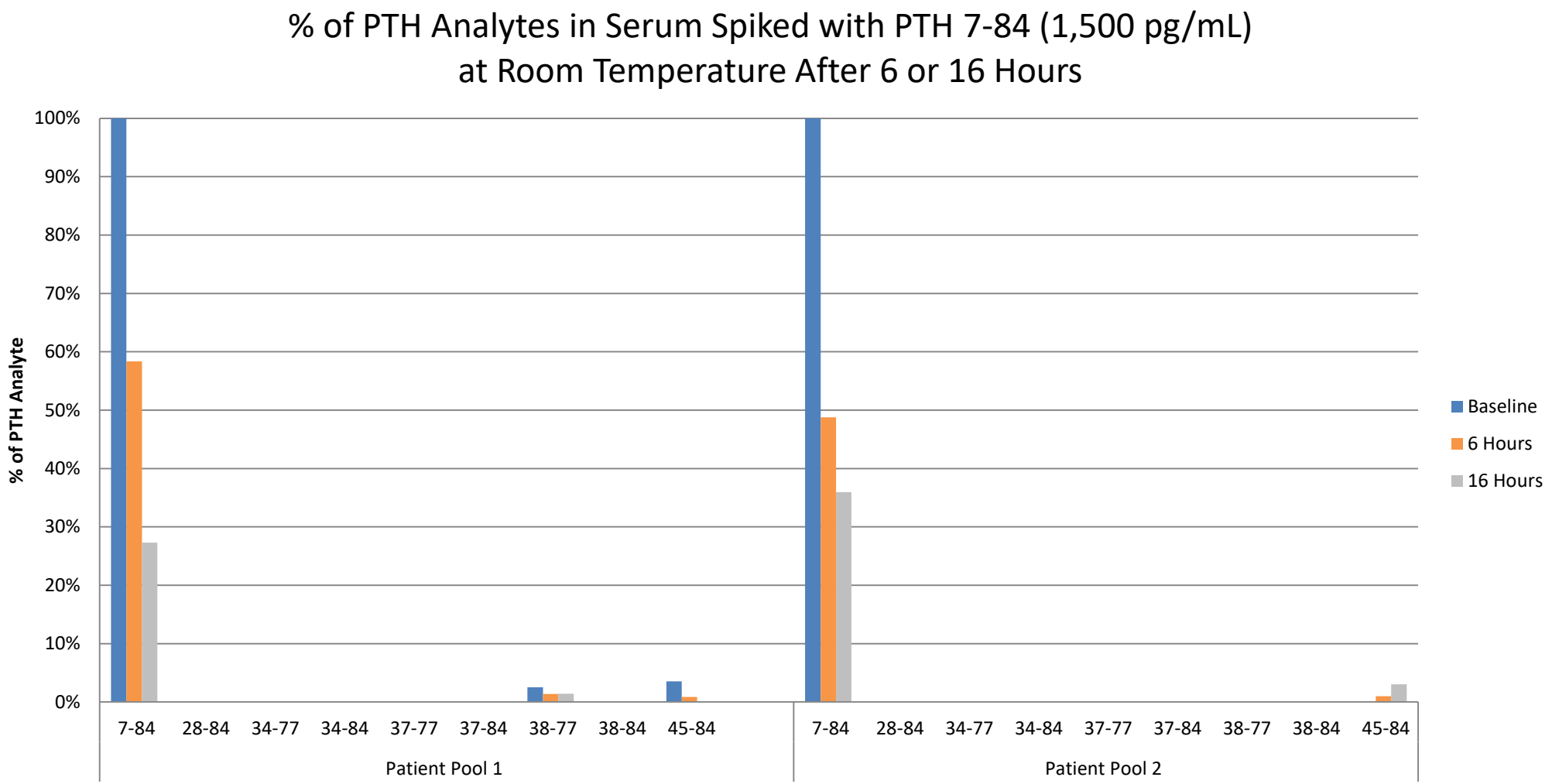
Supplemental Figure 8. Correlations between serum concentrations of LC-HRMS PTH1-84 and PTH fragments: PTH28-84 (P = 0.048), PTH34-77 (P = 0.050), PTH34-84 (P = 0.045), PTH37-77 (P = 0.042), PTH37-84 (P = 0.001), PTH38-77 (P <0.001), PTH38-84 (P <0.001), PTH45-84 (P = 0.047).



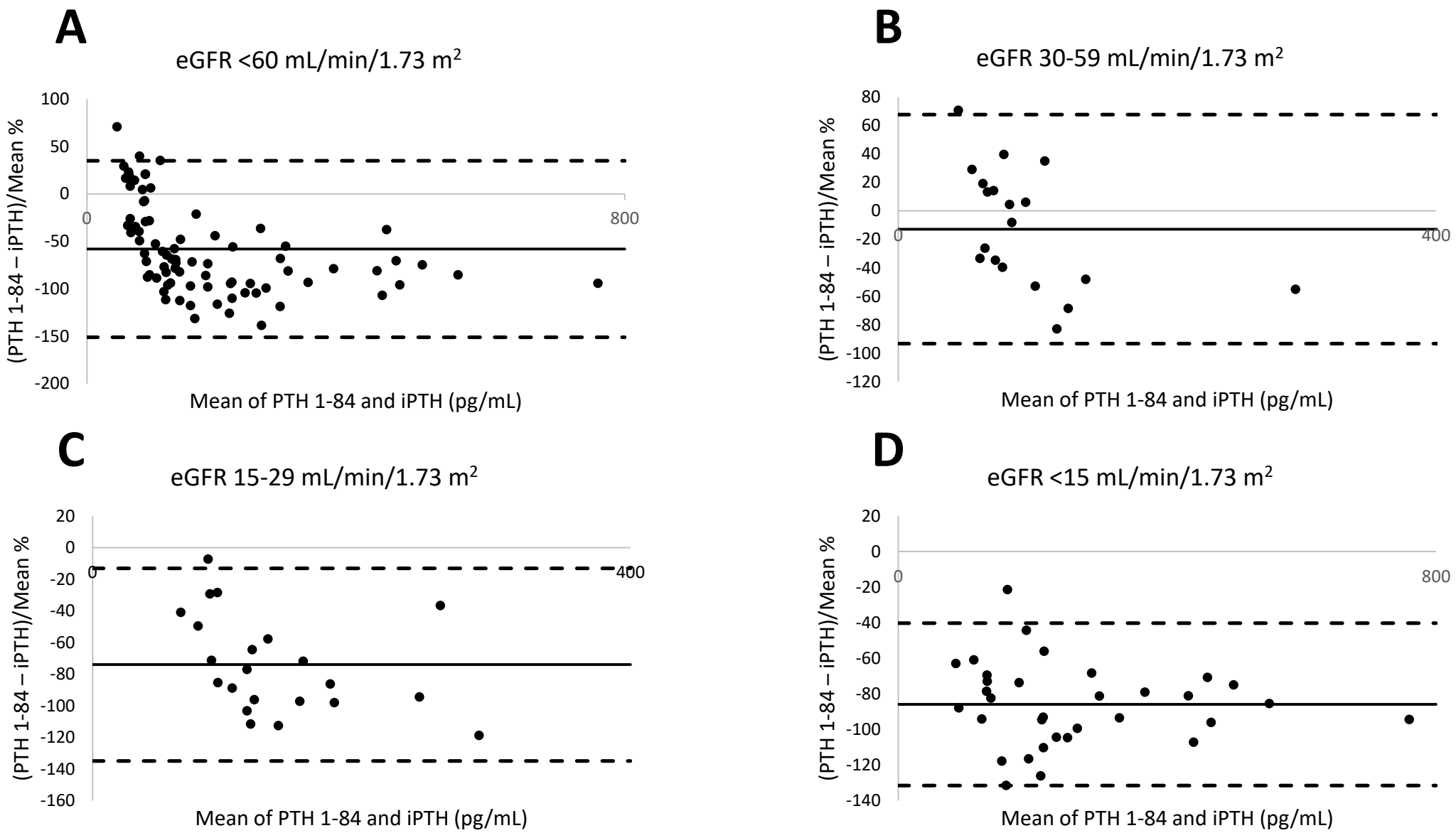
Supplemental Figure 9. Correlations between serum concentrations of phosphate and PTH fragments.



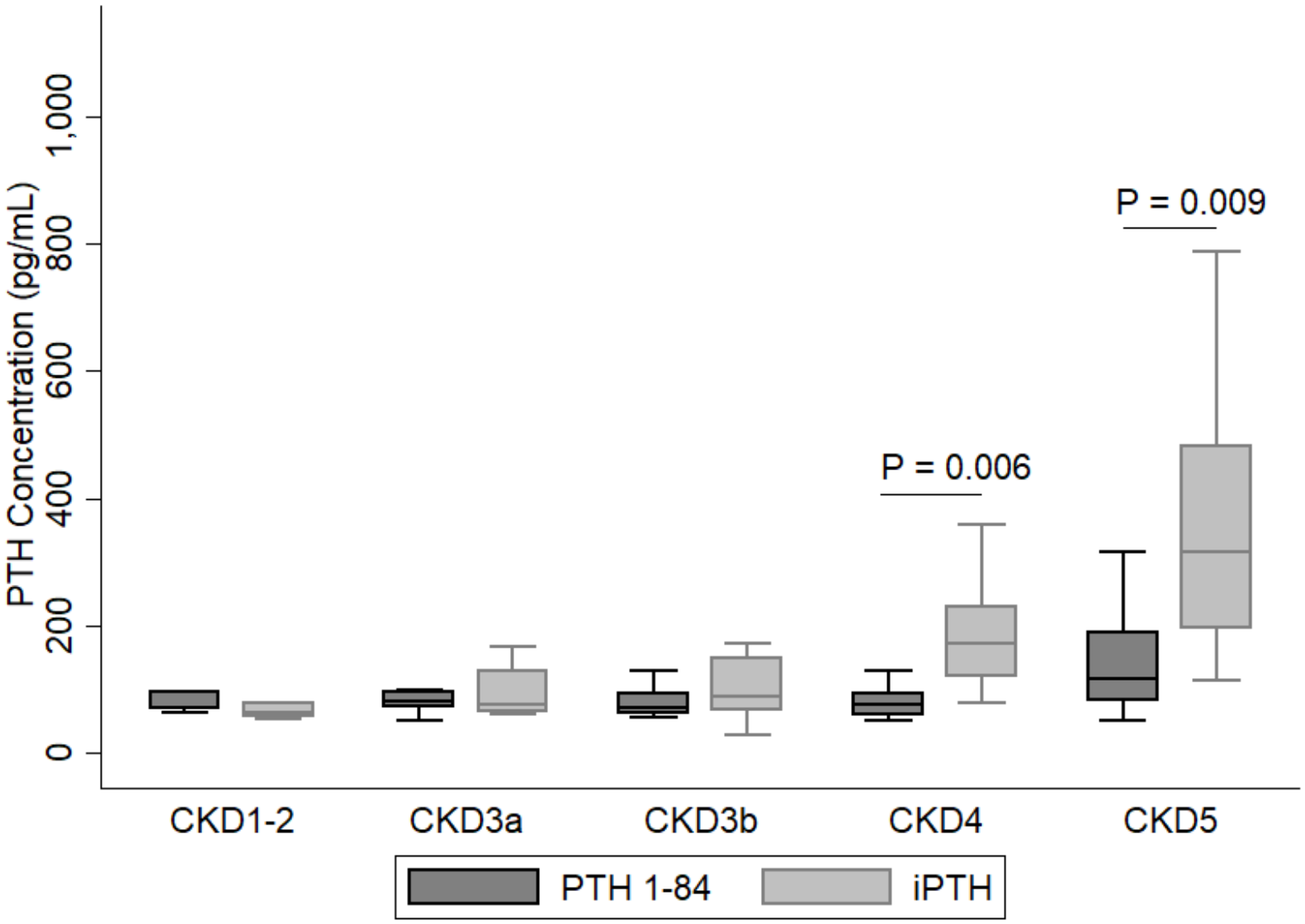
Supplemental Figure 10. Percentage of PTH analytes in serum spiked with PTH1-84 (1,000 or 10,000 pg/mL) at room temperature for 6 or 16 hours. Forced degradation of PTH1-84 in ambient conditions did not generate additional PTH fragments, further confirming the *in vivo* origin of characterized PTH fragments.



Supplemental Figure 11. Percentage of PTH analytes in serum spiked with PTH7-84 (1,500 pg/mL) at room temperature for 6 or 16 hours. Patient pool 1 originates from patient serum samples with eGFR <30 mL/min/1.72m^2 (*n*=7) and patient pool 2 from patients with eGFR >60 mL/min/1.72m^2 (*n*=4). Concentrations of PTH fragments were obtained at baseline, 6 and 16 hours. Data were shown as mean PTH fragment concentrations relative to PTH7-84 at baseline. Forced degradation of PTH7-84 in ambient conditions did not generate additional PTH fragments, further confirming the *in vivo* origin of characterized PTH fragments.



Supplemental Figure 12. Bland-Altman percentage difference plots. The negative bias for the LC-HRMS method compared with an iPTH immunoassay in patients with (A) CKD3-5, (B) CKD3, (C) CKD4, and (D) CKD5 was –58% (95% CI: –150% to 35%), –13% (95% CI: –93% to 68%), –74% (95% CI: –135% to –13%), and –86% (95% CI: –132% to –40%), respectively. Solid black lines represent negative bias. Black dashed lines represent 95% limit of agreement (negative bias±1.96 SD).



Supplemental Figure 13. Comparisons of serum concentrations of LC-HRMS PTH1-84 and Roche iPTH. Data are presented as median [interquartile range]: CKD1-2: 70 (67-95) versus 66 (49-85), *P* = 0.26; CKD3a: 72 (58-93) versus 74 (68-148), *P* = 0.18; CKD3b: 76 (61-95) versus 87 (49-151), *P* = 0.13; CKD4: 77 (58-93) versus 171 (120-229), *P* = 0.006; and CKD5: 115 (81-189) versus 316 (195-483), *P* = 0.009.