Supporting material II

Integrated CZE-ESI-MS/MS system with an immobilized trypsin microreactor for online digestion and analysis of picogram amounts of RAW 264.7 cell lysate

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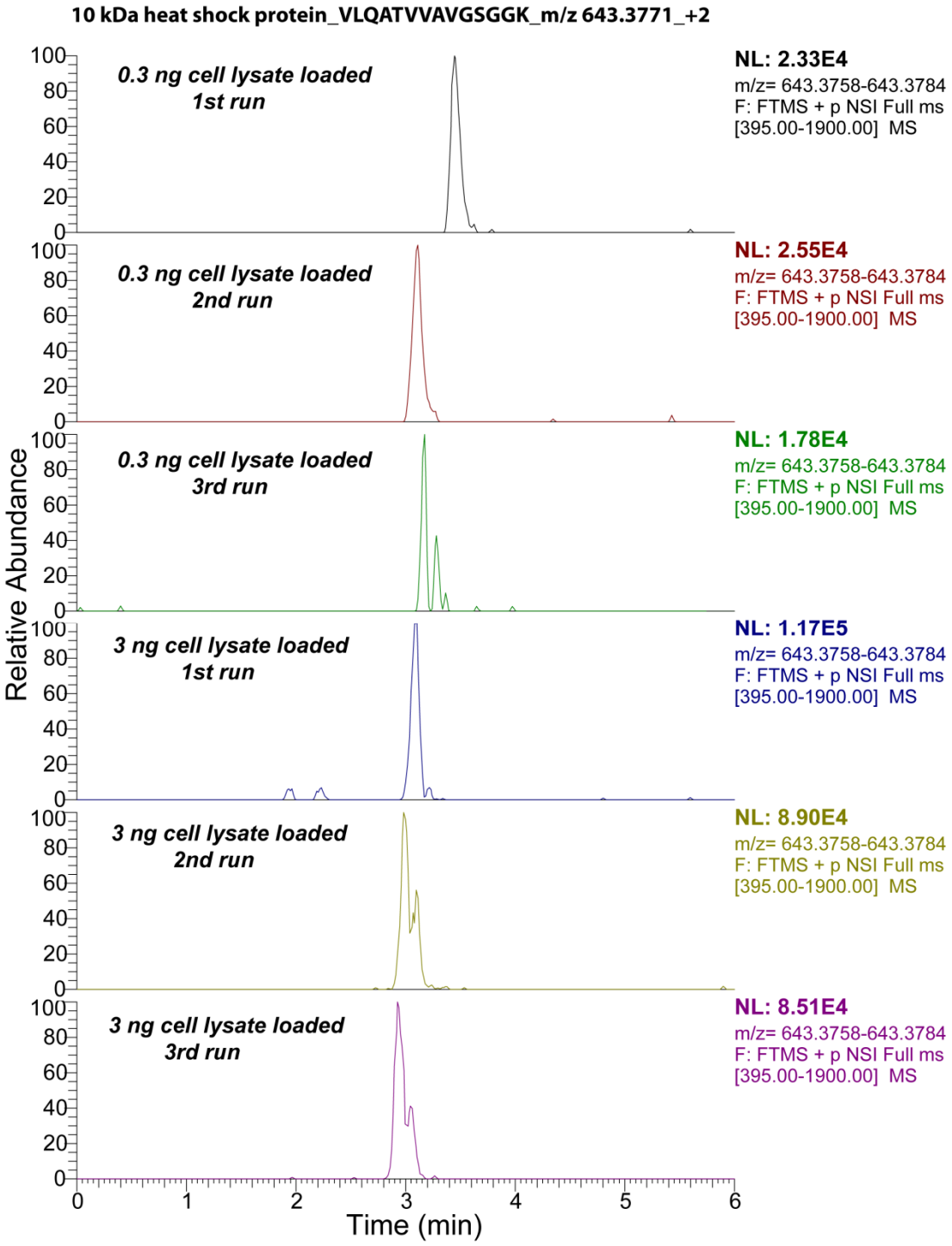
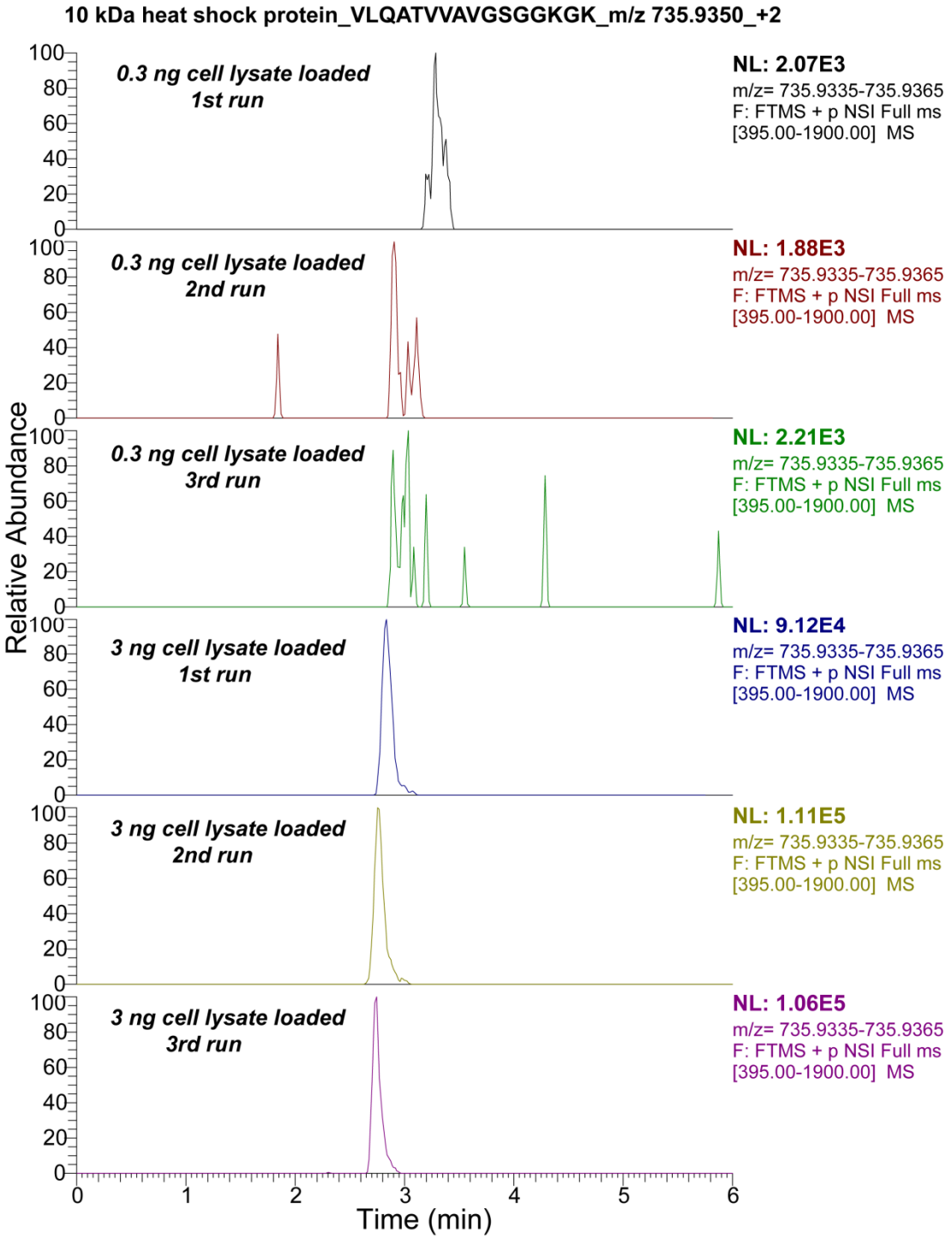
Part I S-Figure 1 and S-Figure 2



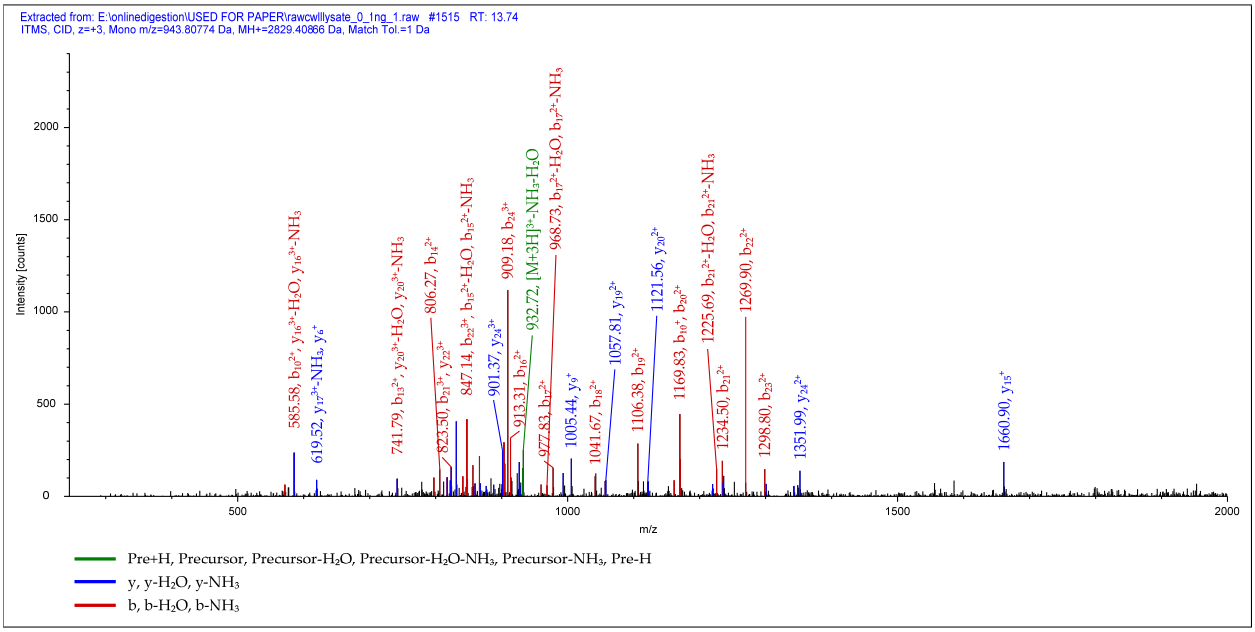
S-Figure 1 Triplicate extracted ion electropherograms of a peptide (VLQATVVAVGSGGK) that was only identified in the tandem spectra from 0.3 ng cell lysate. The peptide's ion intensity was extracted with mass tolerance as 2 ppm, and Gaussian smoothing with 5 points was applied.

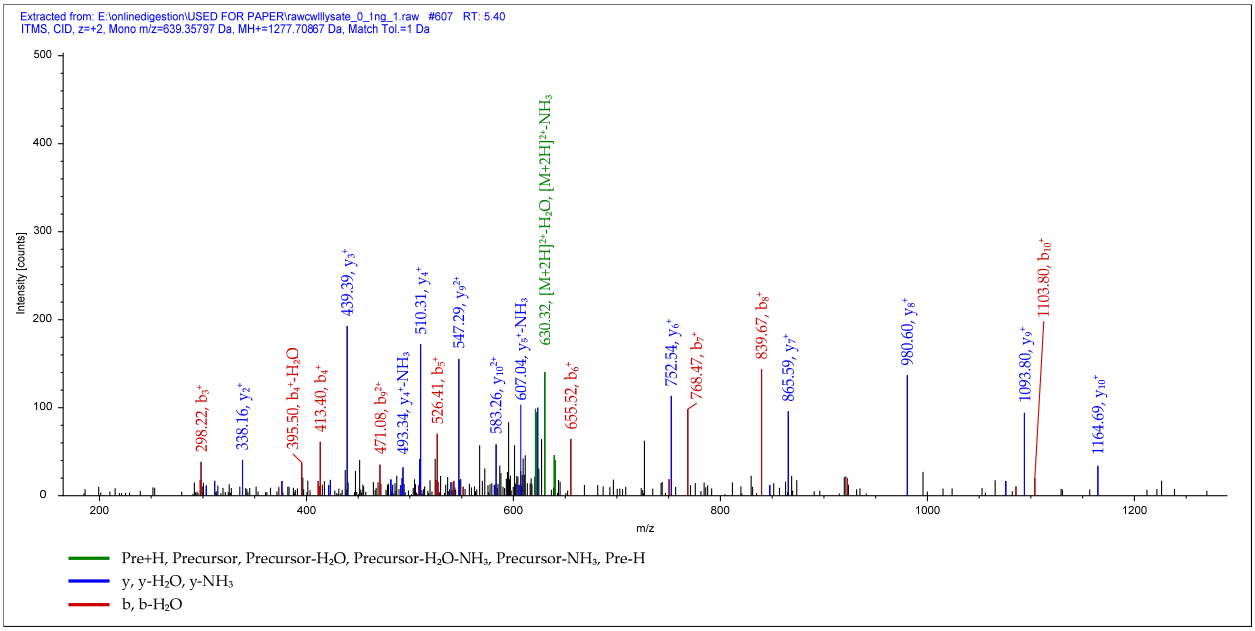


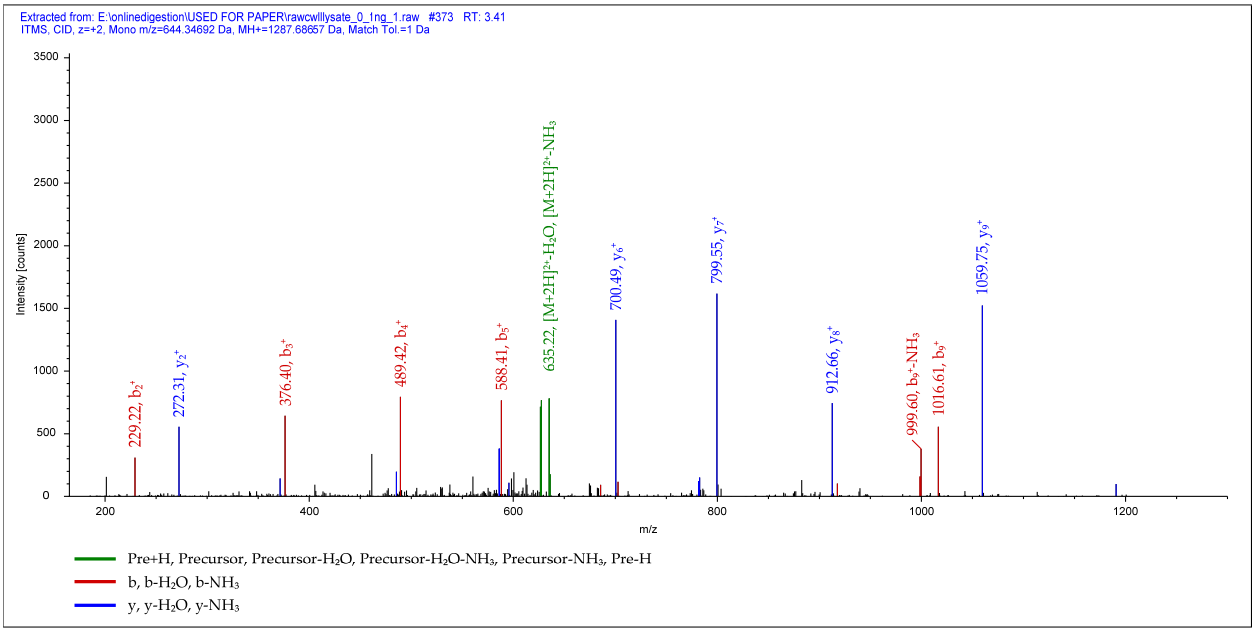
S-Figure 2 Triplicate extracted ion electropherograms of a peptide (VLQATVVAVGSGGKGK) that was only identified in the tandem spectra from 3 ng cell lysate. The peptide was extracted with mass tolerance as 2 ppm, and Gaussian smoothing with 5 points was applied.

Part II Annotated tandem spectra of identified peptides from 0.3 ng and 3 ng RAW 264.7 cell lysate analyzed by the integrated CZE-ESI-MS/MS system in triplicate runs

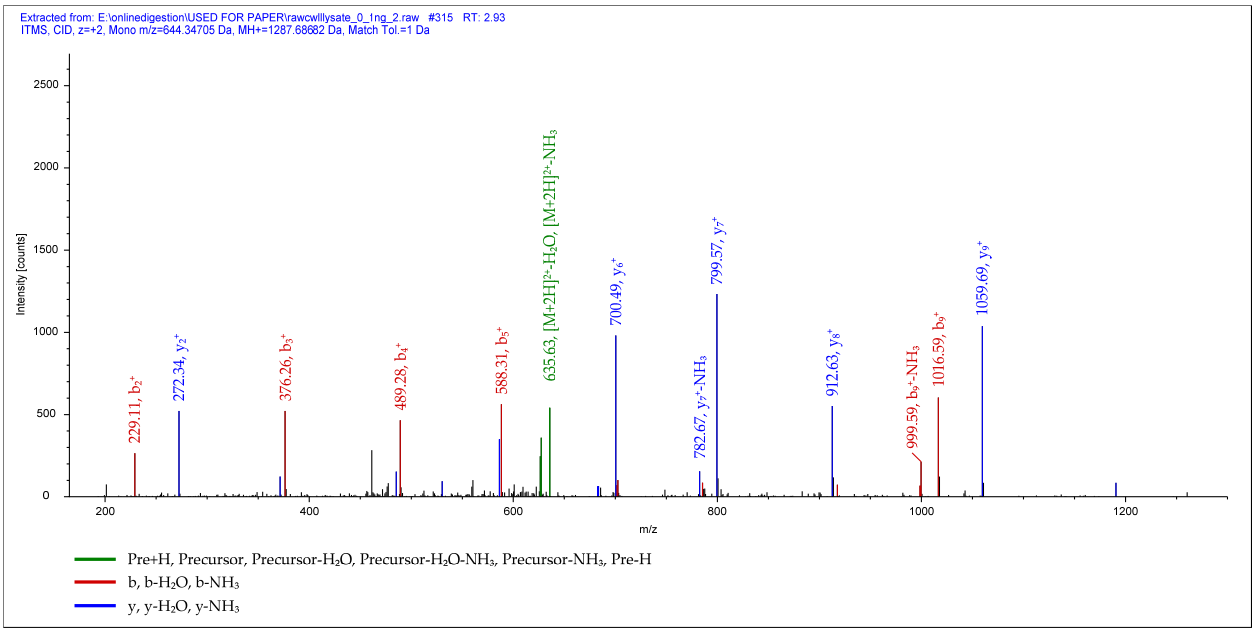
0.3 ng RAW 264.7 cell lysate_1st run

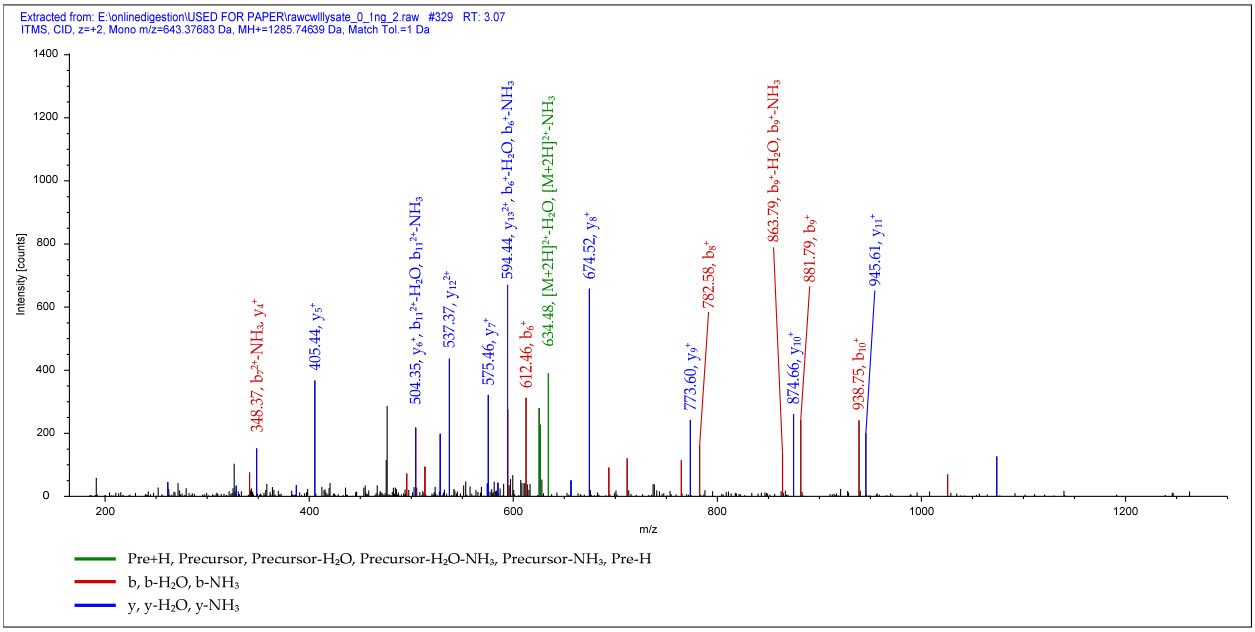




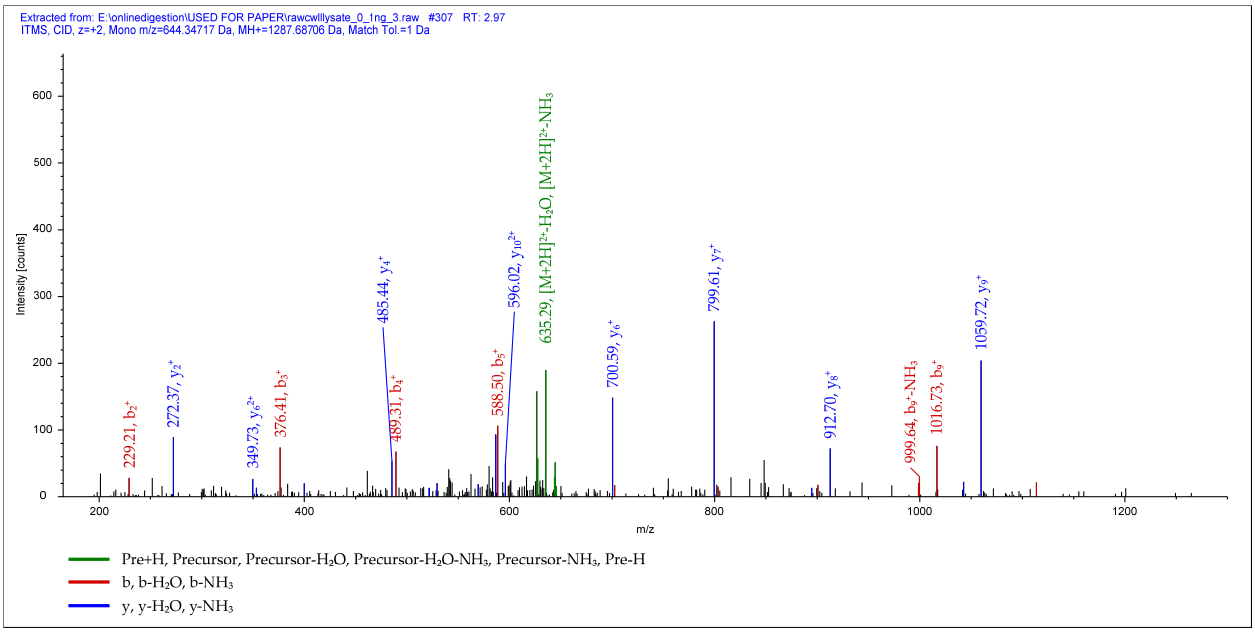


0.3 ng RAW 264.7 cell lysate_2nd run

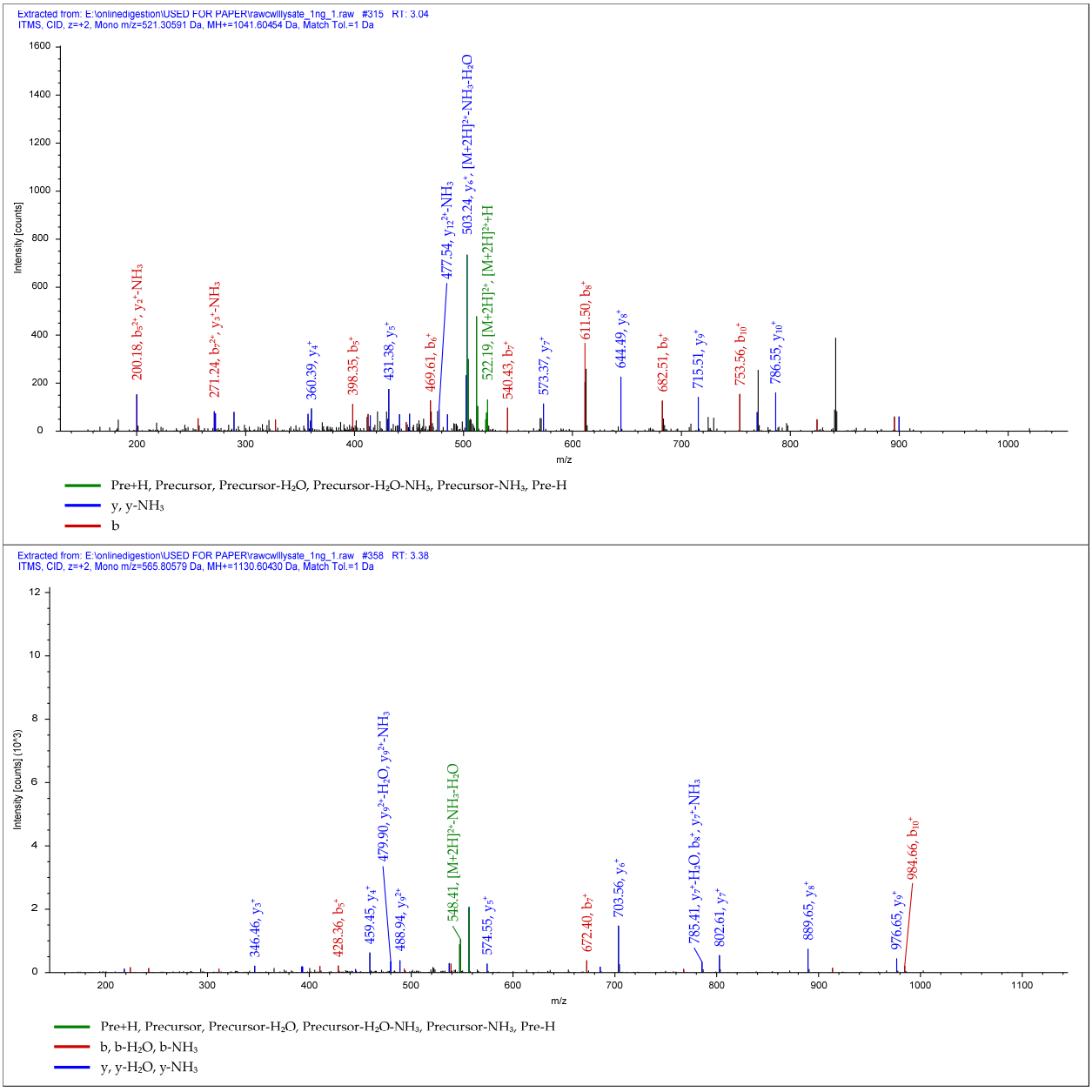


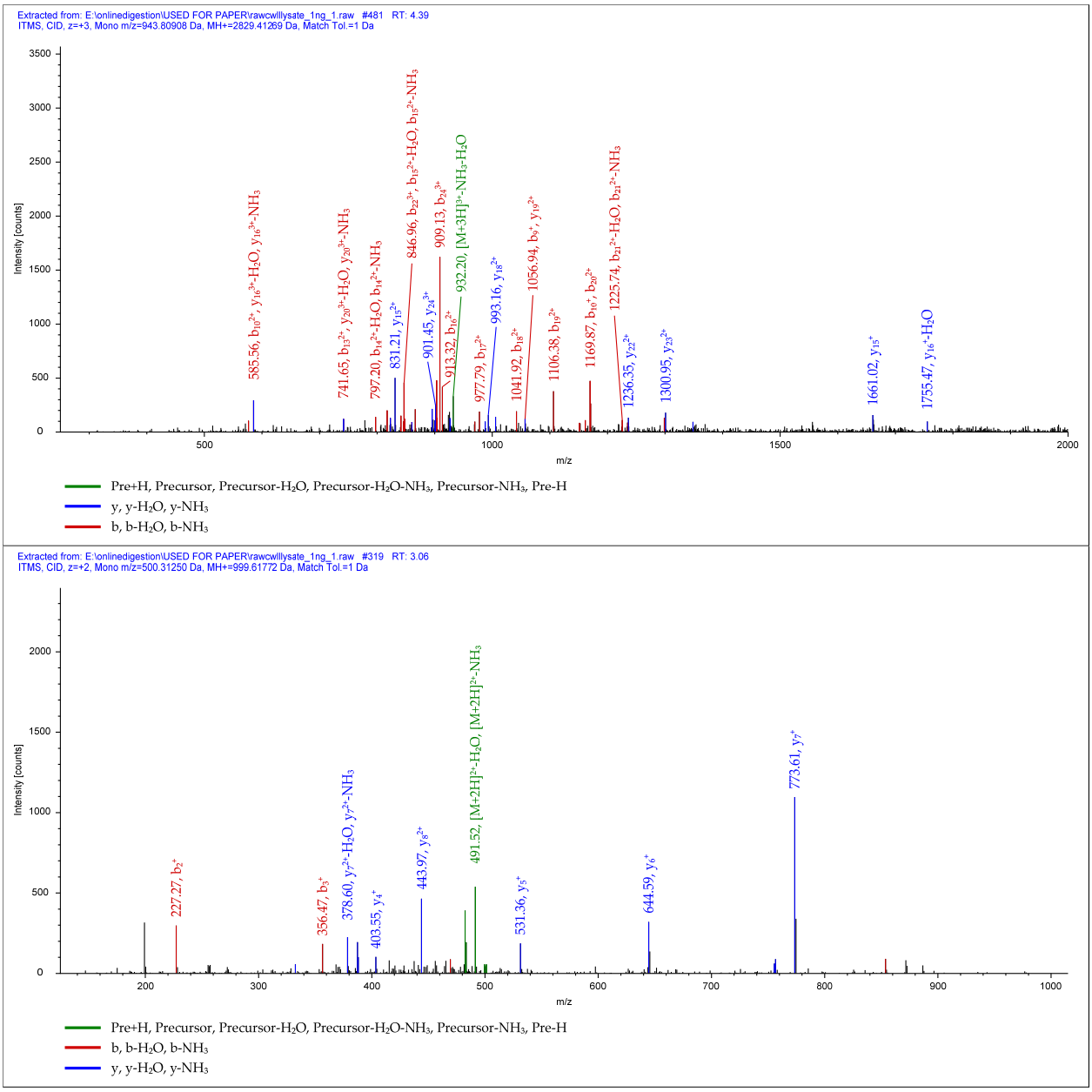


0.3 ng RAW 264.7 cell lysate_3rd run



3 ng RAW 264.7 cell lysate_1st run

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Intensity [counts]
m/z
585.56, b10 2+, y16 3+-H2O, y16 3+-NH3
741.65, b13 2+, y20 3+-H2O, y20 3+-NH3
797.20, b14 2+-H2O, b14 2+-NH3
831.21, y15 2+
846.96, b22 3+, b15 2+-H2O, b15 2+-NH3
901.45, y24 3+
909.13, b24 3+
913.32, b16 2+
932.20, [M+3H]3+-NH3-H2O
977.79, b17 2+
993.16, y18 2+
1041.92, b18 2+
1056.94, b9 +, y19 2+
1106.38, b19 2+
1169.87, b10 +, b20 2+
1225.74, b21 2+-H2O, b21 2+-NH3
1236.35, y22 2+
1300.95, y23 2+
1661.02, y15 +
1753.47, y16 +-H2O
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O, b-NH3

Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcwlllysate_1ng_1.raw #319 RT: 3.06
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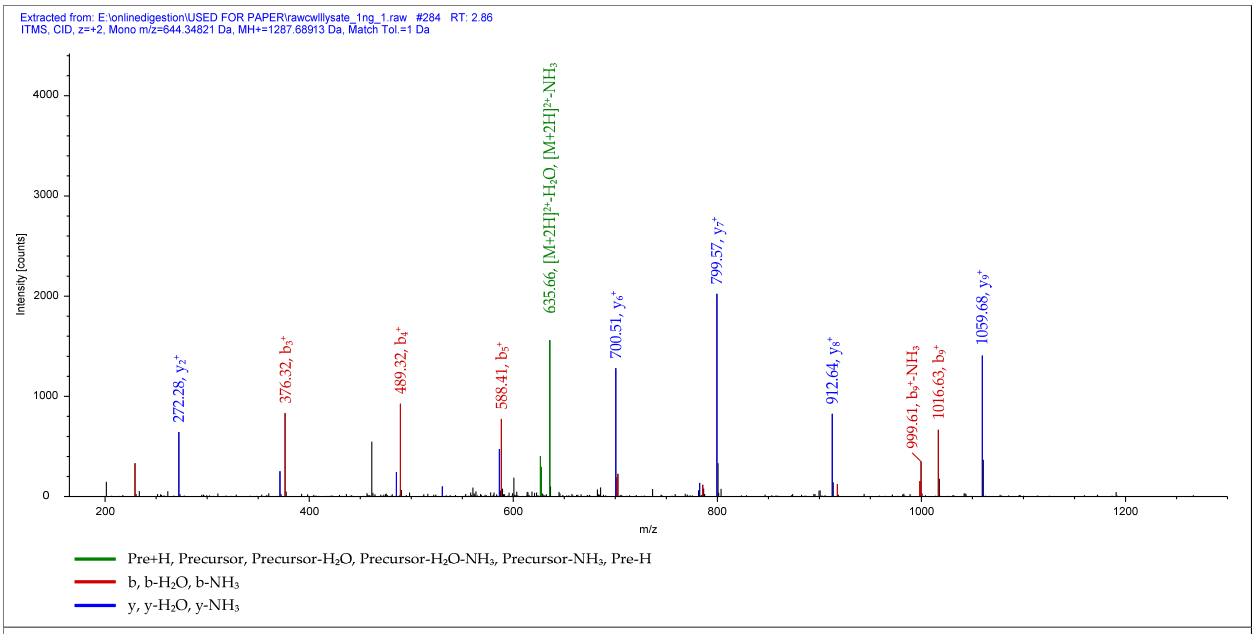
Intensity [counts]
m/z
227.27, b2 +
356.47, b3 +
378.60, y7 2+-H2O, y7 2+-NH3
403.55, y4 +
443.97, y8 2+
491.52, [M+2H]2+-H2O, [M+2H]2+-NH3
531.36, y5 +
644.59, y6 +
773.61, y7 +
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3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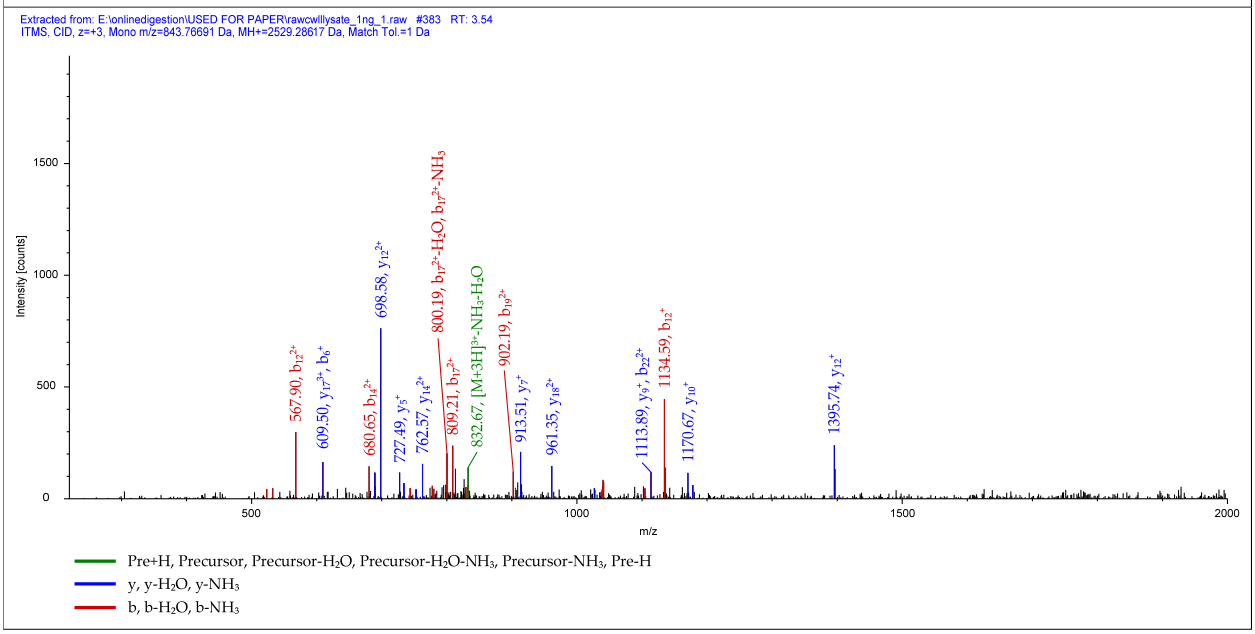
Intensity [counts]
m/z
272.28, y2+
376.32, b3+
489.32, b4+
588.41, b5+
635.66, [M+2H]2+-H2O, [M+2H]2+-NH3
700.51, y6+
799.57, y7+
912.64, y8+
999.61, b9+-NH3
1016.63, b9+
1059.68, y9+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3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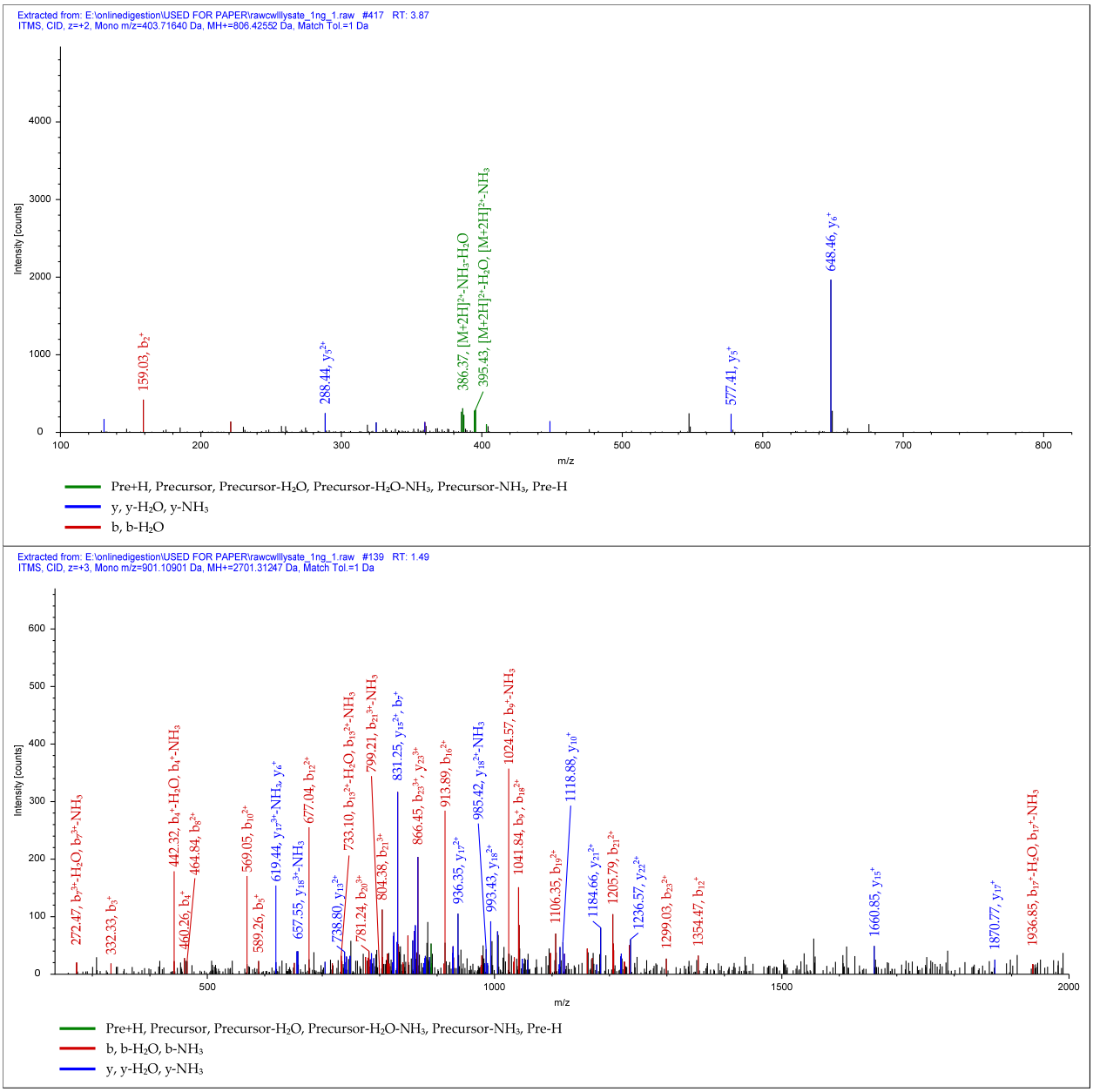
Intensity [counts]
m/z
567.90, b12 2+
609.50, y17 3+, b6+
680.65, b14 2+
698.58, y12 2+
727.49, y5+
762.57, y14 2+
800.19, b17 2+-H2O, b17 2+-NH3
809.21, b17 2+
832.67, [M+3H]3+-NH3-H2O
902.19, b19 2+
913.51, y7+
961.35, y18 2+
1113.89, y9+, b22 2+
1134.59, b12+
1170.67, y10+
1395.74, y12+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O, b-NH3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Intensity [counts]
m/z
159.03, b2+
288.44, y52+
386.37, [M+2H]2+-NH3-H2O
395.43, [M+2H]2+-H2O, [M+2H]2+-NH3
577.41, y5+
648.46, y6+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O
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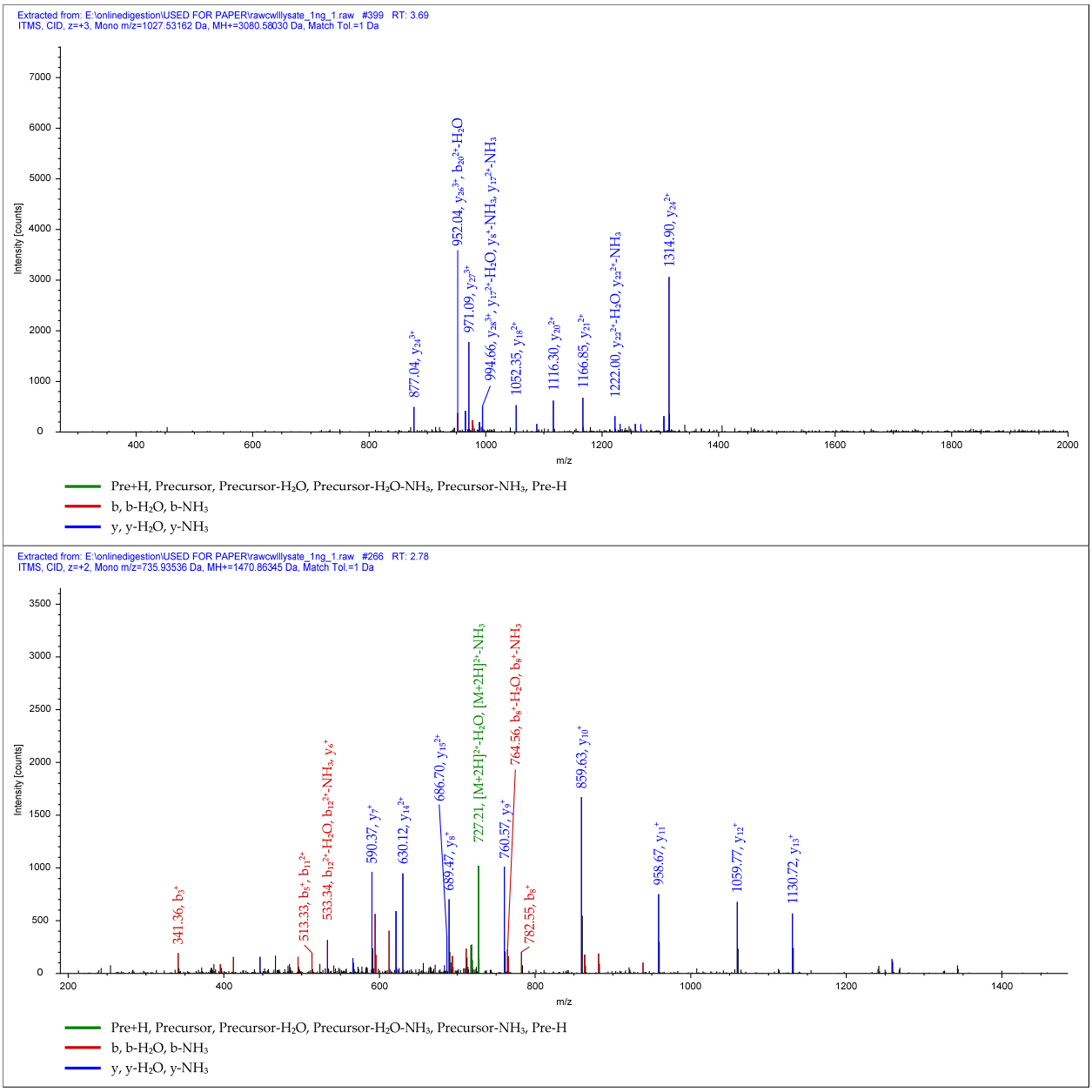
ITMS, CID, z=+3, Mono m/z=901.10901 Da, MH+=2701.31247 Da, Match Tol.=1 Da
Intensity [counts]
m/z
272.47, b73+-H2O, b73+-NH3
332.33, b33+
442.32, b4+-H2O, b4+-NH3
460.26, b4+
464.84, b82+
569.05, b102+
589.26, b52+
619.44, y122+-NH3, y6+
657.55, y183+-NH3
677.04, b122+
738.80, y132+
733.10, b132+-H2O, b132+-NH3
781.24, b203+
799.21, b212+-NH3
804.38, b213+
831.25, y152+, b72+
866.45, b253+, y233+
913.89, b162+
936.35, y172+
985.42, y162+-NH3
993.43, y182+
1024.57, b92+-NH3
1041.84, b92+, b182+
1106.35, b192+
1118.88, y102+
1184.66, y212+
1205.79, b212+
1236.57, y222+
1299.03, b252+
1354.47, b122+
1660.85, y152+
1870.77, y172+
1936.85, b172+-H2O, b172+-NH3
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3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Intensity [counts]
m/z
877.04, y24 3+
952.04, y26 3+, b20 2+-H2O
971.09, y27 3+
994.66, y28 3+, y17 2+-H2O, y8 +-NH3, y15 2+-NH3
1052.35, y18 2+
1116.30, y20 2+
1166.85, y21 2+
1222.00, y22 2+-H2O, y22 2+-NH3
1314.90, y24 2+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3
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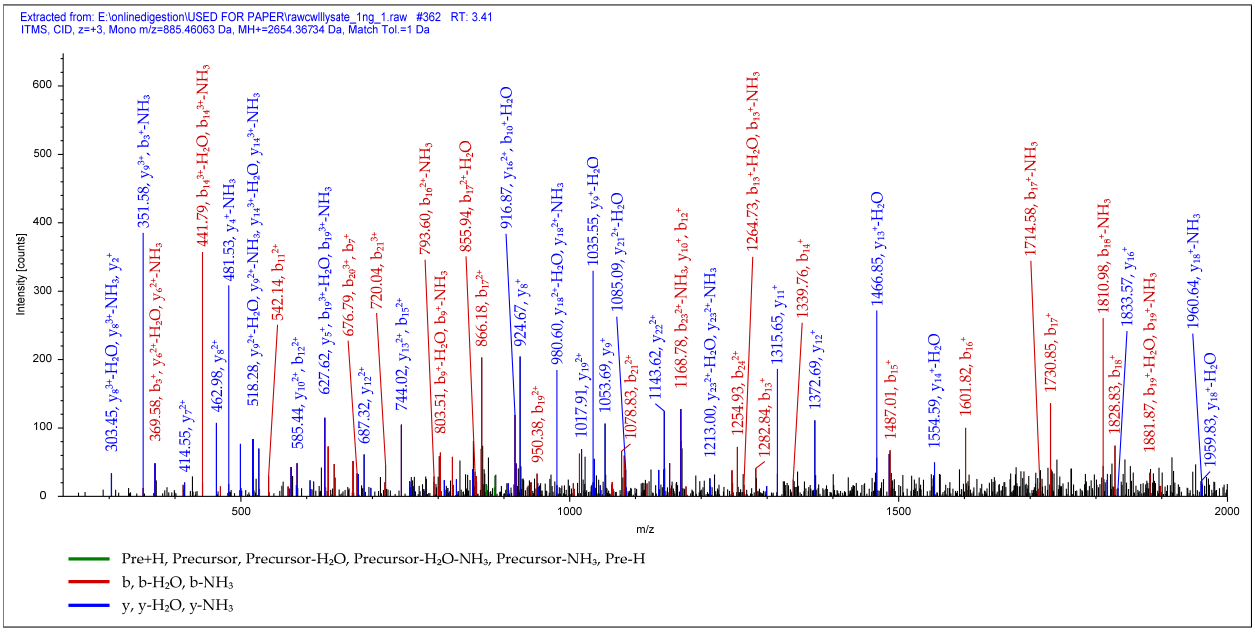
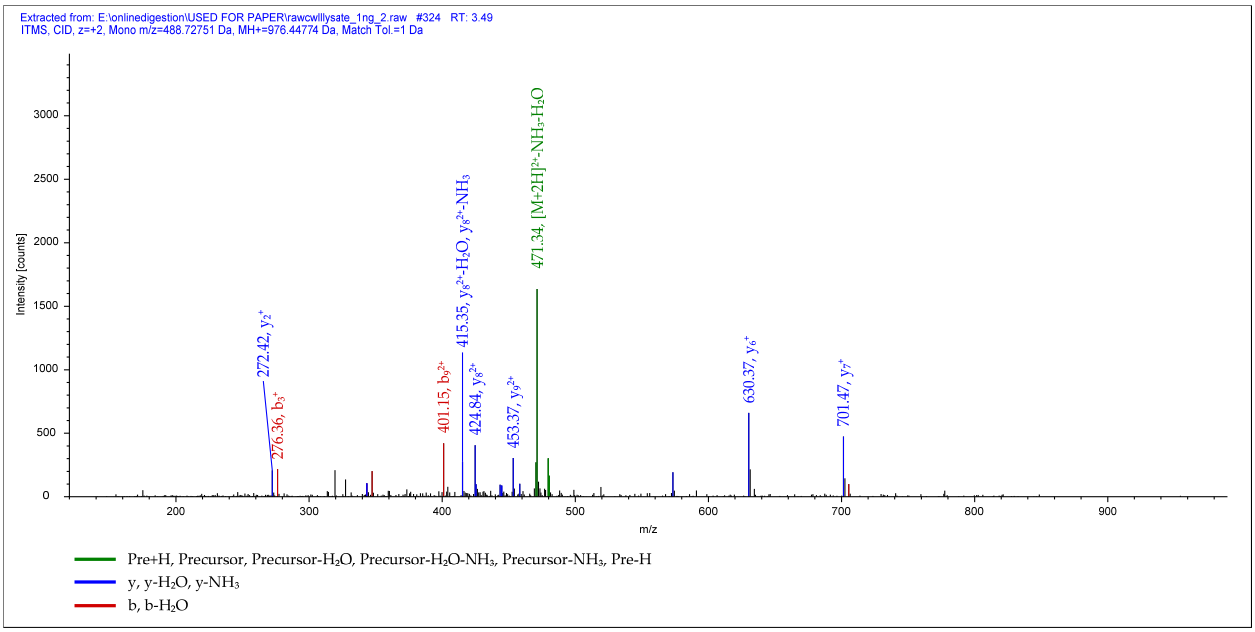
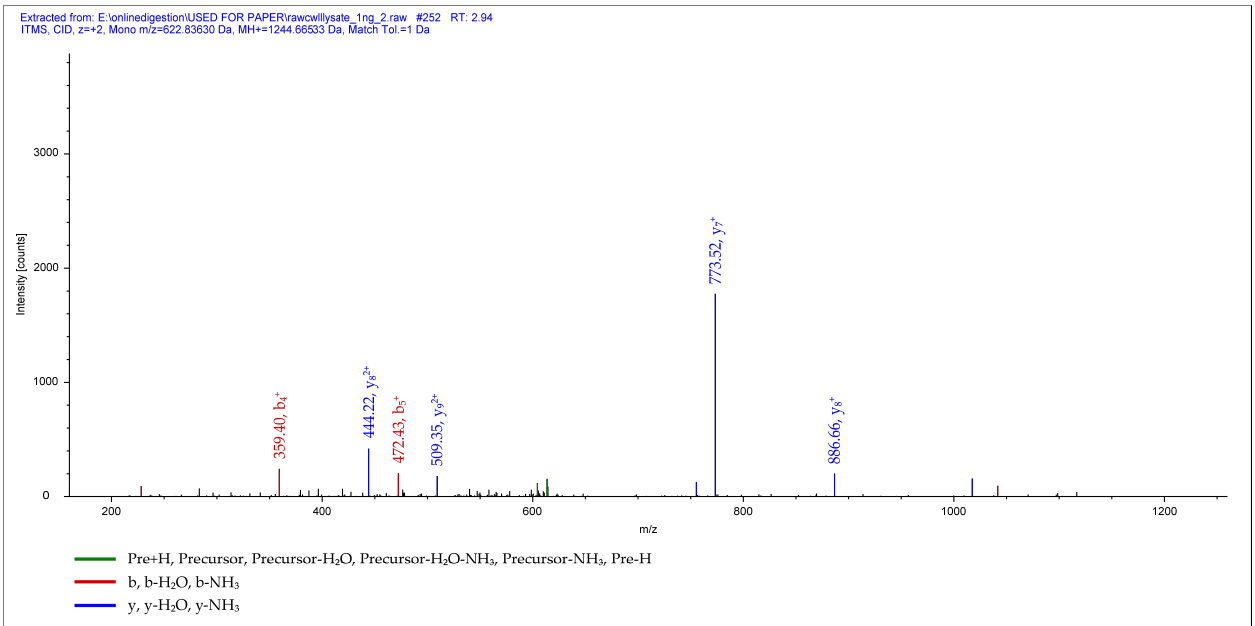
ITMS, CID, z=+2, Mono m/z=735.93536 Da, MH+=1470.86345 Da, Match Tol.=1 Da
Intensity [counts]
m/z
341.36, b3 +
513.33, b5 +, b11 2+
533.34, b12 2+-H2O, b12 2+-NH3, y6 +
590.37, y7 +
630.12, y14 2+
686.70, y15 2+
689.47, y8 +
727.21, [M+2H]2+-H2O, [M+2H]2+-NH3
760.57, y9 +
764.56, b8 +-H2O, b8 +-NH3
782.55, b8 +
859.63, y10 +
958.67, y11 +
1059.77, y12 +
1130.72, y13 +
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3



3 ng RAW 264.7 cell lysate_2nd run

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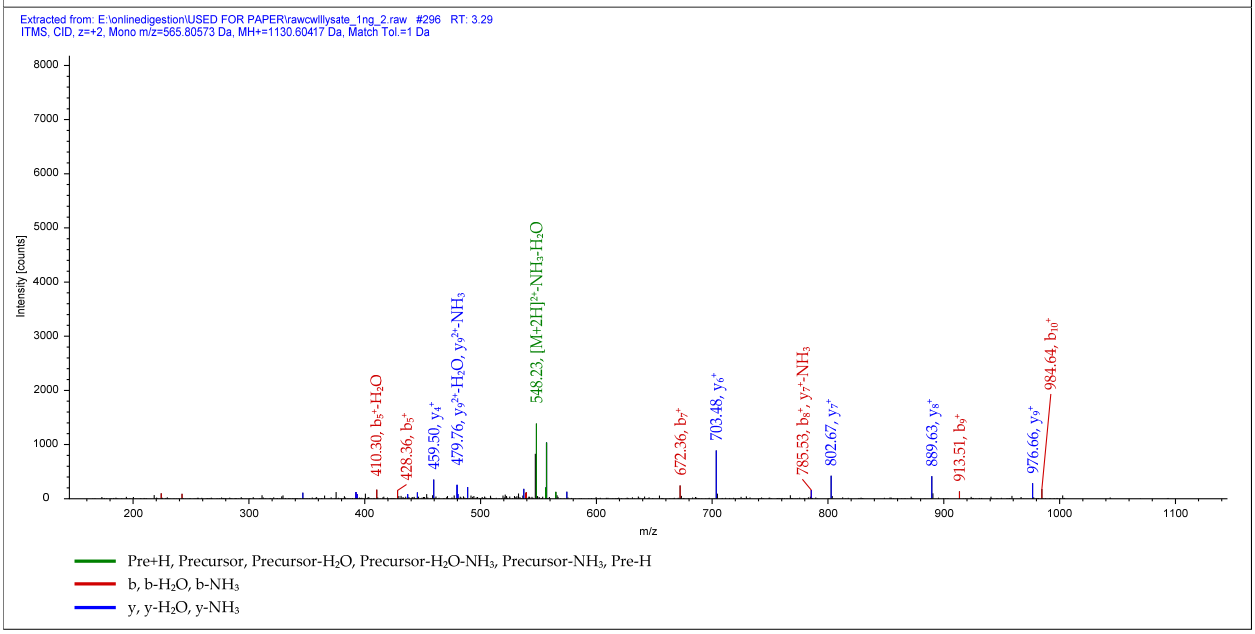
Intensity [counts]
m/z
359.40, b4+
444.22, y8 2+
472.43, b5+
509.35, y9 2+
773.52, y7+
886.66, y8+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3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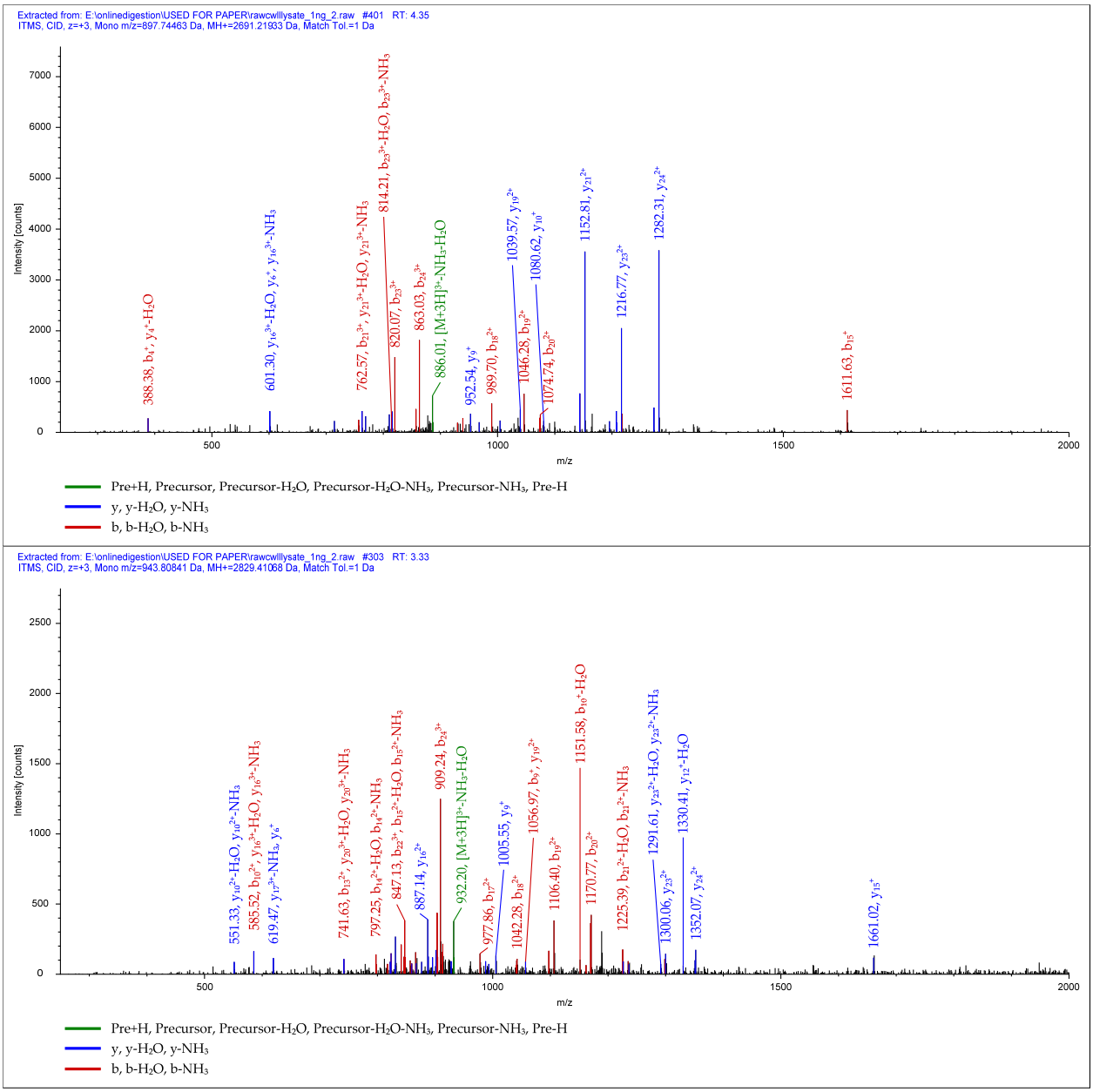
Intensity [counts]
m/z
410.30, b5*-H2O
428.36, b5*
459.50, y4+
479.76, y9 2+-H2O, y9 2+-NH3
548.23, [M+2H]2+-NH3-H2O
672.36, b7+
703.48, y6+
785.53, b8*, y7*-NH3
802.67, y7+
889.63, y8+
913.51, b9+
976.66, y9+
984.64, b10+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3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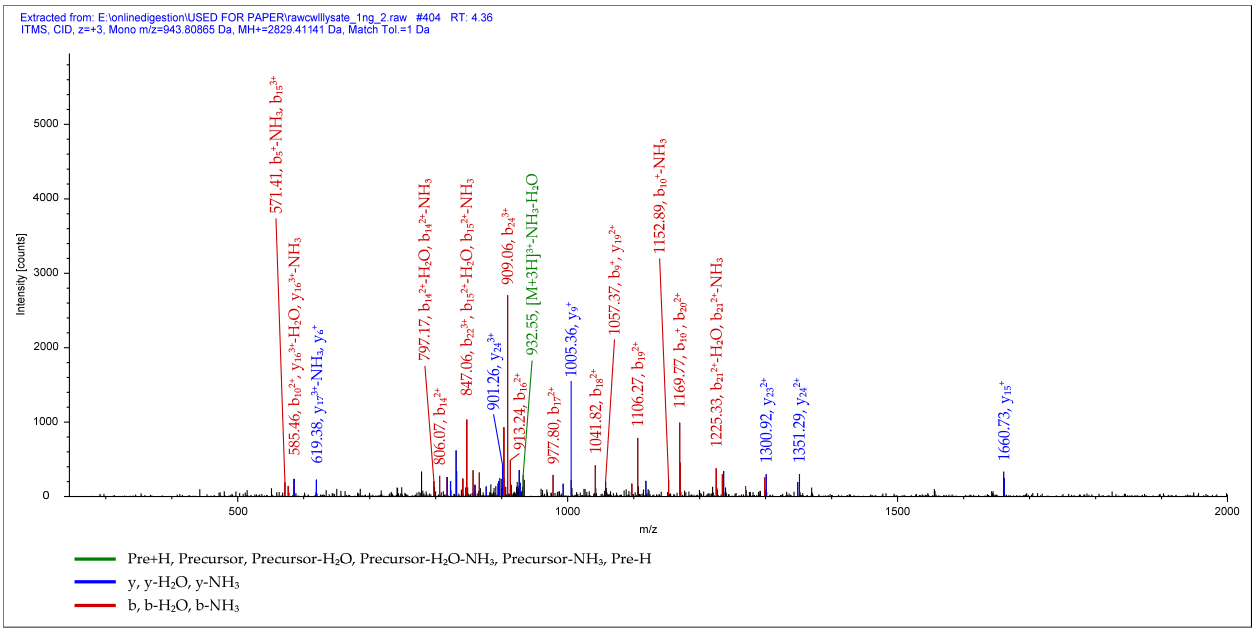
Intensity [counts]
m/z
388.38, b4*, y4*-H2O
601.30, y16 3+-H2O, y6*, y16 3+-NH3
762.57, b21 3+, y21 3+-H2O, y21 3+-NH3
814.21, b23 3+-H2O, b23 3+-NH3
820.07, b23 3+
863.03, b24 3+
886.01, [M+3H]3+-NH3-H2O
952.54, y9+
989.70, b18 2+
1039.57, y19 2+
1046.28, b19 2+
1074.74, b20 2+
1080.62, y10+
1152.81, y21 2+
1216.77, y23 2+
1282.31, y24 2+
1611.63, b15+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O, b-NH3
Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_2.raw #303 RT: 3.33
ITMS, CID, z=+3, Mono m/z=943.80841 Da, MH+=2829.41068 Da, Match Tol.=1 Da
Intensity [counts]
m/z
551.33, y10 2+-H2O, y10 2+-NH3
585.52, b10 2+, y16 3+-H2O, y16 3+-NH3
619.47, y17 3+-NH3, y6*
741.63, b13 2+, y20 3+-H2O, y20 3+-NH3
797.25, b14 2+-H2O, b14 2+-NH3
847.13, b22 3+, b15 2+-H2O, b15 2+-NH3
887.14, y16 2+
909.24, b24 3+
932.20, [M+3H]3+-NH3-H4O
977.86, b17 2+
1005.55, y9+
1042.28, b18 2+
1056.97, by*, y19 2+
1106.40, b19 2+
1151.58, b10+-H2O
1170.77, b20 2+
1225.39, b21 2+-H2O, b21 2+-NH3
1291.61, y23 2+-H2O, y23 2+-NH3
1300.06, y23 2+
1330.41, y12+-H2O
1352.07, y24 2+
1661.02, y15+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O, b-NH3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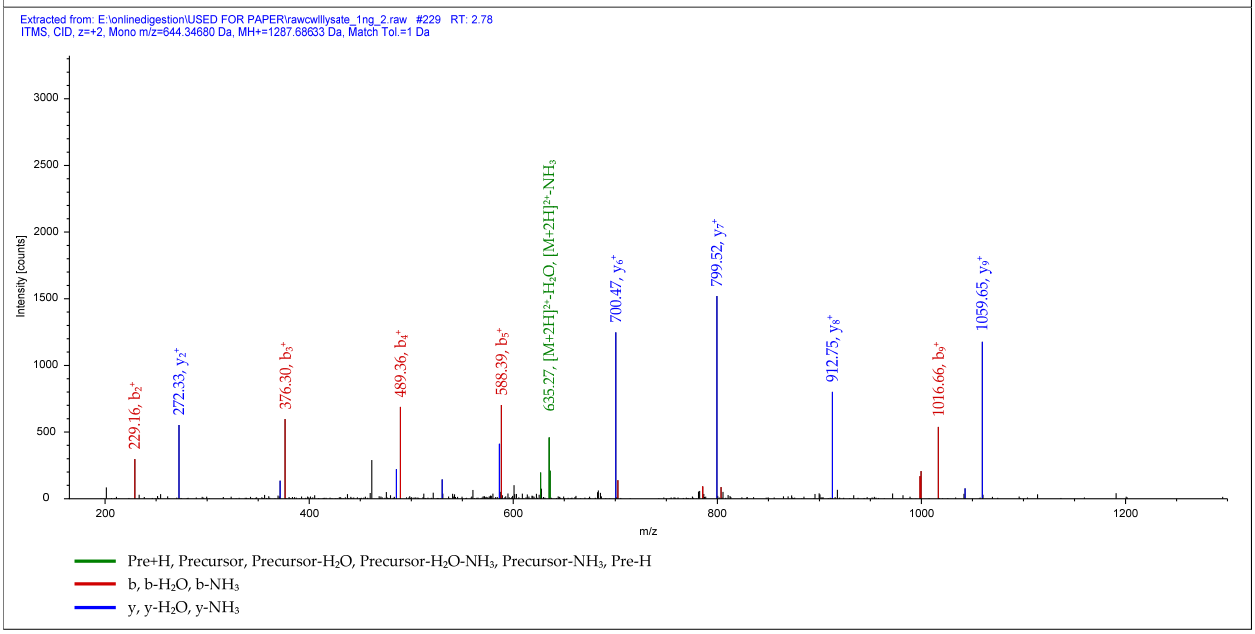
Intensity [counts]
m/z
571.41, b5+-NH3, b15 3+
585.46, b10 2+, y15 3+-H2O, y15 3+-NH3
619.38, y17 2+-NH3, y6+
797.17, b14 2+-H2O, b14 2+-NH3
806.07, b14 2+
847.06, b22 3+, b13 2+-H2O, b13 2+-NH3
901.26, y24 3+
909.06, b24 3+
913.24, b16 2+
932.55, [M+3H]3+-NH3-H2O
977.80, b17 2+
1005.36, y9+
1041.82, b18 2+
1057.37, b9+, y19 2+
1106.27, b19 2+
1152.89, b10+-NH3
1169.77, b10+, b20 2+
1225.33, b21 2+-H2O, b21 2+-NH3
1300.92, y23 2+
1351.29, y24 2+
1660.73, y15+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
y, y-H2O, y-NH3
b, b-H2O, b-NH3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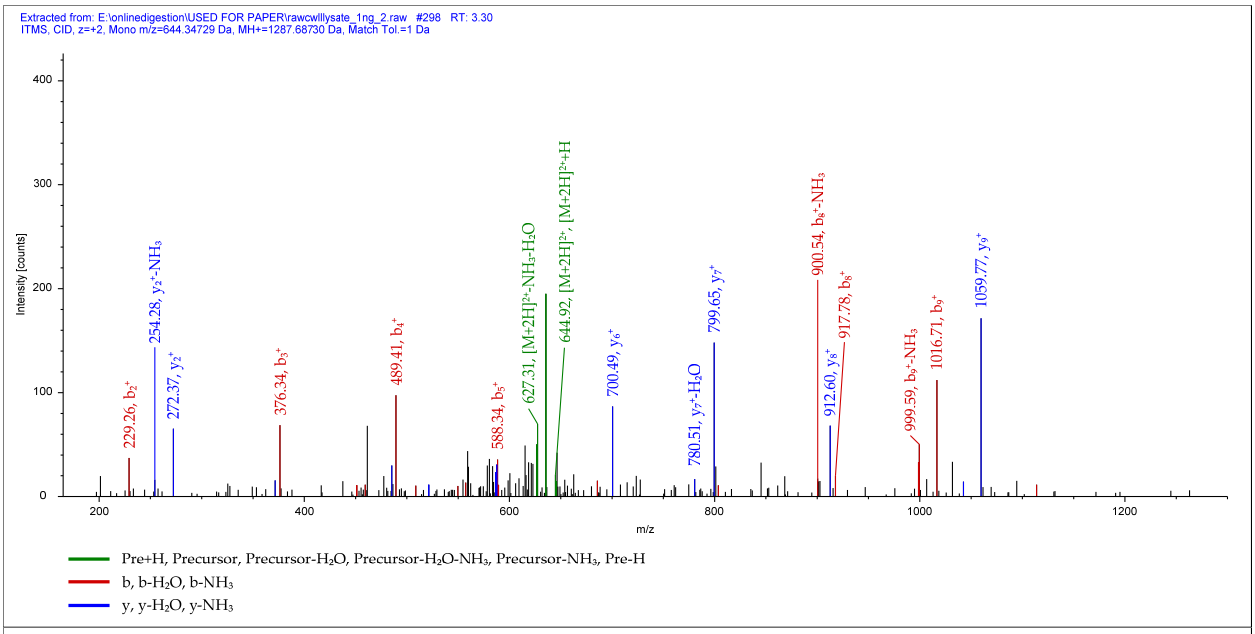
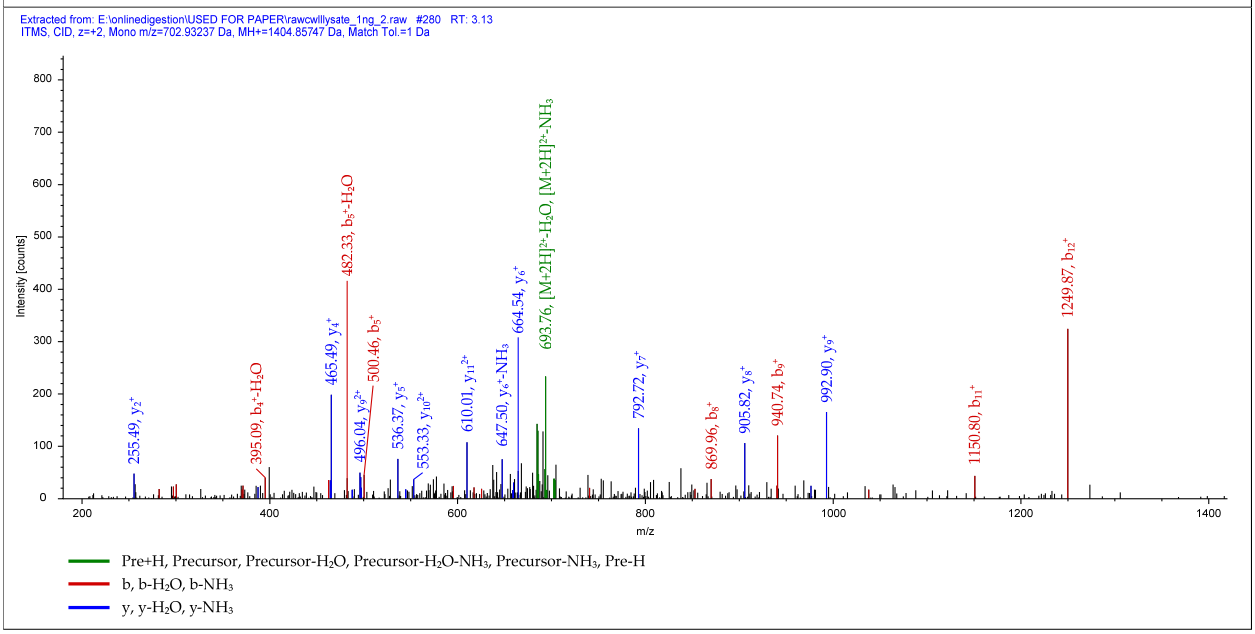
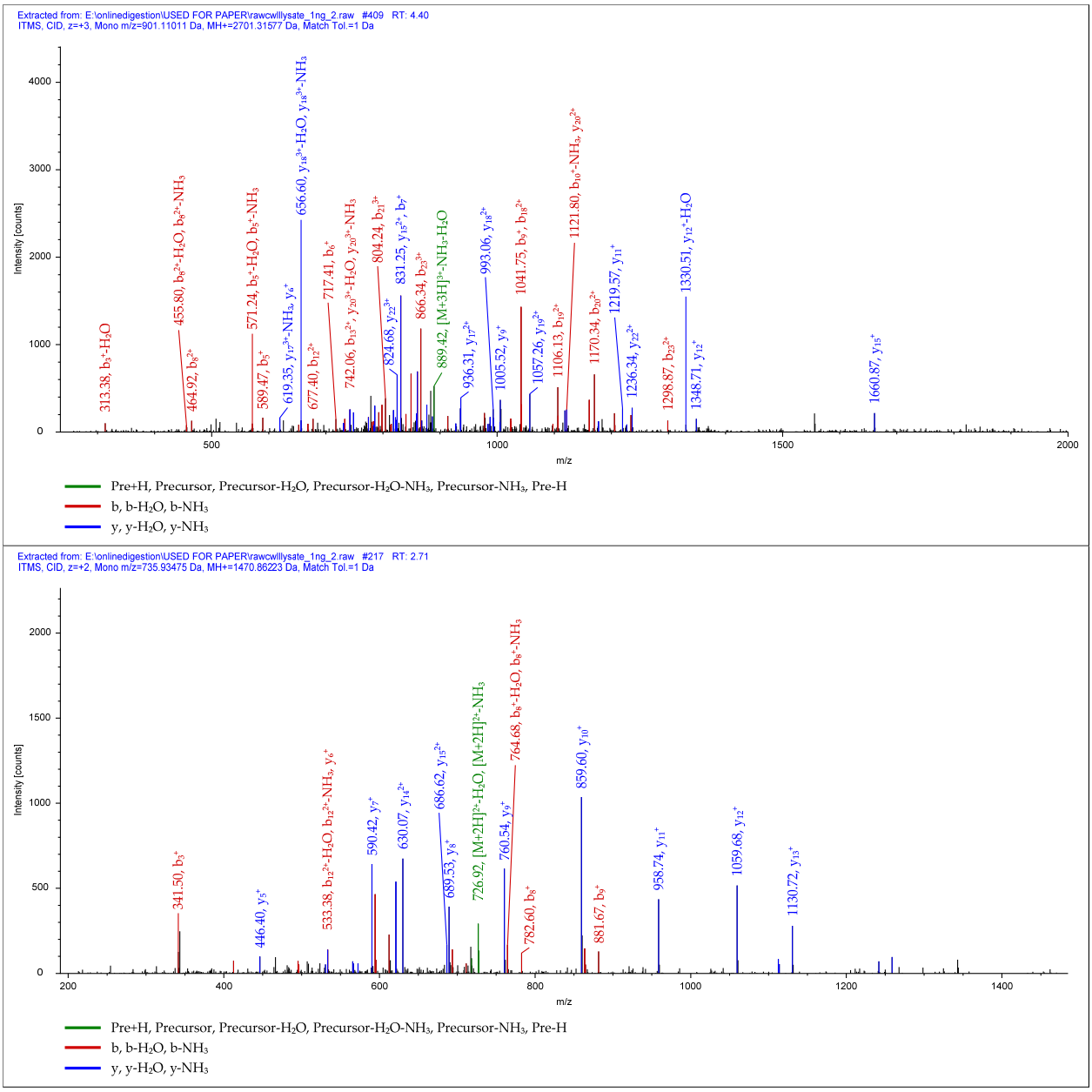
ITMS, CID, z=+2, Mono m/z=644.34680 Da, MH+=1287.68633 Da, Match Tol.=1 Da
Intensity [counts]
m/z
229.16, b2+
272.33, y2+
376.30, b3+
489.36, b4+
588.39, b5+
635.27, [M+2H]2+-H2O, [M+2H]2+-NH3
700.47, y6+
799.52, y7+
912.75, y8+
1016.66, b9+
1059.65, y9+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

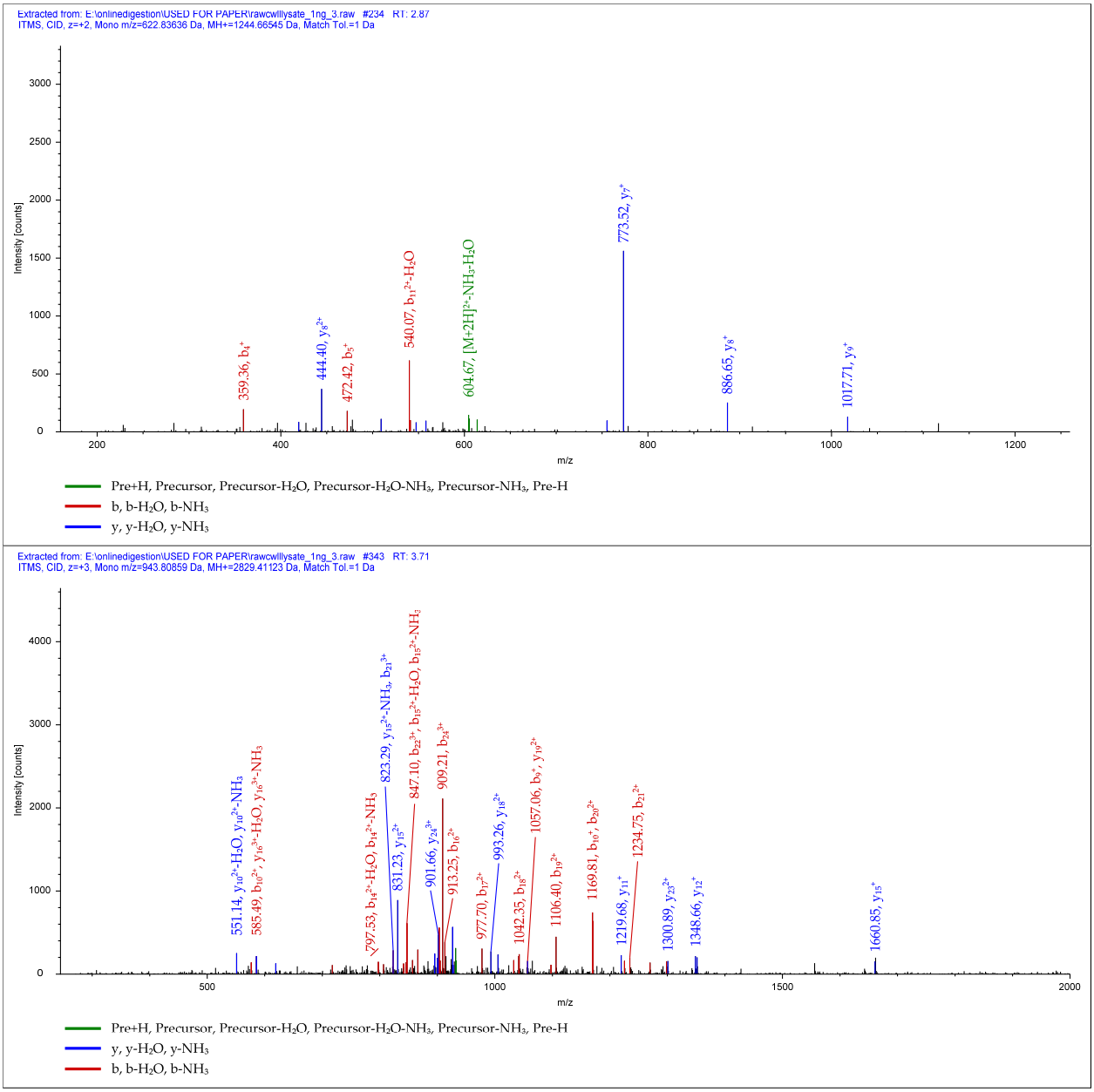

Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_2.raw #298 RT: 3.30
ITMS, CID, z=+2, Mono m/z=644.34729 Da, MH+=1287.68730 Da, Match Tol.=1 Da
Intensity [counts]
m/z
229.26, b2+
254.28, y2+-NH3
272.37, y2+
376.34, b3+
489.41, b4+
588.34, b5+
627.31, [M+2H]2+-NH3-H2O
644.92, [M+2H]2+, [M+2H]2+-H
700.49, y6+
780.51, y7+-H2O
799.65, y7+
900.54, b8+-NH3
912.60, y8+
917.78, b8+
999.59, b9+-NH3
1016.71, b9+
1059.77, y9+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

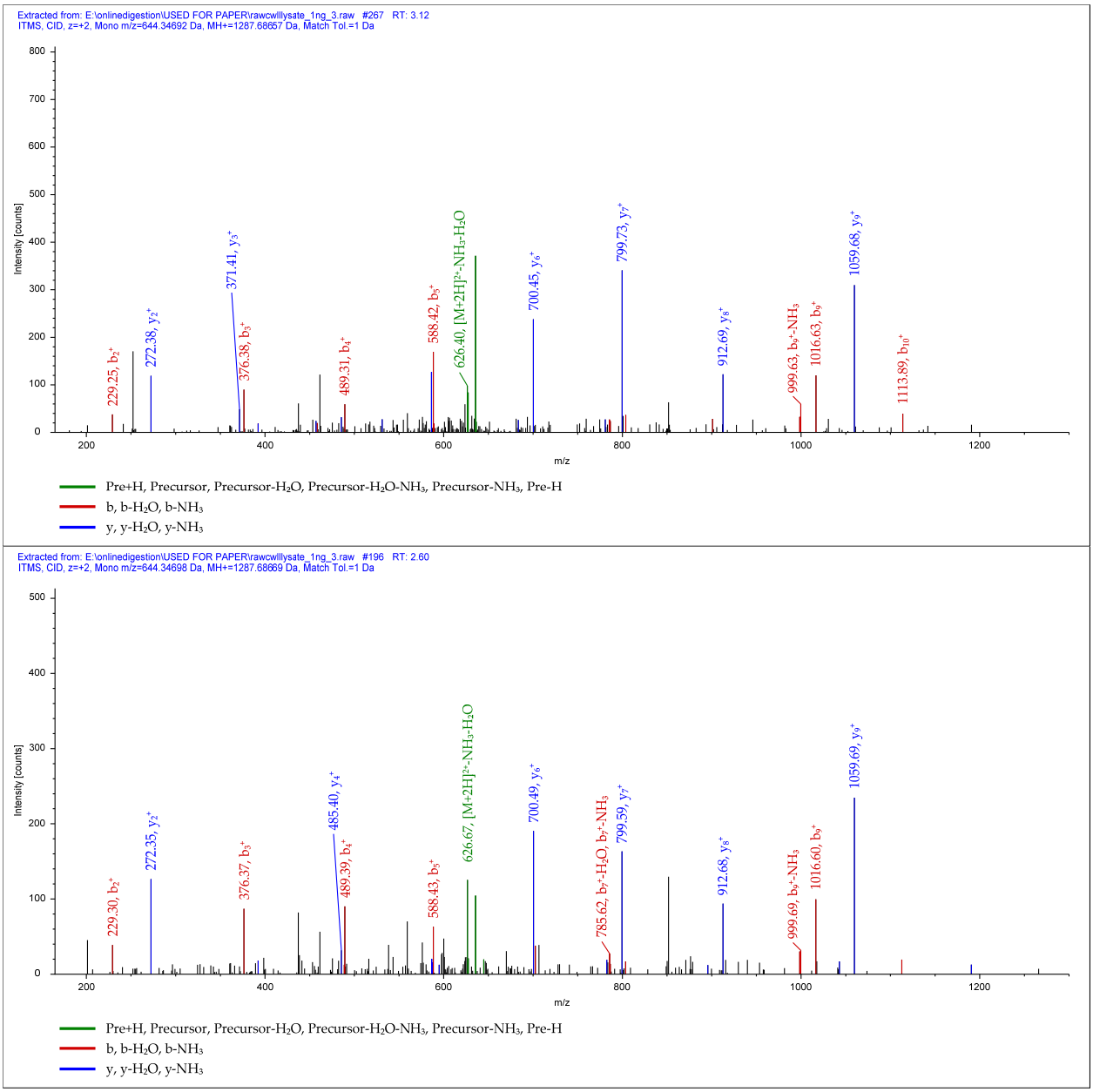

Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_2.raw #280 RT: 3.13
ITMS, CID, z=+2, Mono m/z=702.93237 Da, MH+=1404.85747 Da, Match Tol.=1 Da
Intensity [counts]
m/z
255.49, y2+
395.09, b4+-H2O
465.49, y4+
482.33, b5+-H2O
496.04, y9 2+
500.46, b5+
536.37, y5+
553.33, y10 2+
610.01, y11 2+
647.50, y6+-NH3
664.54, y6+
693.76, [M+2H]2+-H2O, [M+2H]2+-NH3
792.72, y7+
869.96, b8+
905.82, y8+
940.74, b9+
992.90, y9+
1150.80, b11+
1249.87, b12+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3


Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_2.raw #409 RT: 4.40
ITMS, CID, z=+3, Mono m/z=901.11011 Da, MH+=2701.31577 Da, Match Tol.=1 Da
Intensity [counts]
m/z
313.38, b3+-H2O
455.80, b82+-H2O, b82+-NH3
464.92, b82+
571.24, b52+-H2O, b52+-NH3
589.47, b5+
619.35, y172+-NH3, y6+
656.60, y182+-H2O, y182+-NH3
677.40, b122+
717.41, b6+
742.06, b132+, y202+-H2O, y202+-NH3
804.24, b212+
824.68, y222+
831.25, y152+, b7+
866.34, b232+
889.42, [M+3H]3+-NH3-H2O
936.31, y172+
993.06, y182+
1005.52, y9+
1041.75, b9+, b182+
1057.26, y102+
1106.13, b102+
1121.80, b102+-NH3, y202+
1170.34, b202+
1219.57, y11+
1236.34, y222+
1298.87, b232+
1330.51, y12+-H2O
1348.71, y12+
1660.87, y15+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

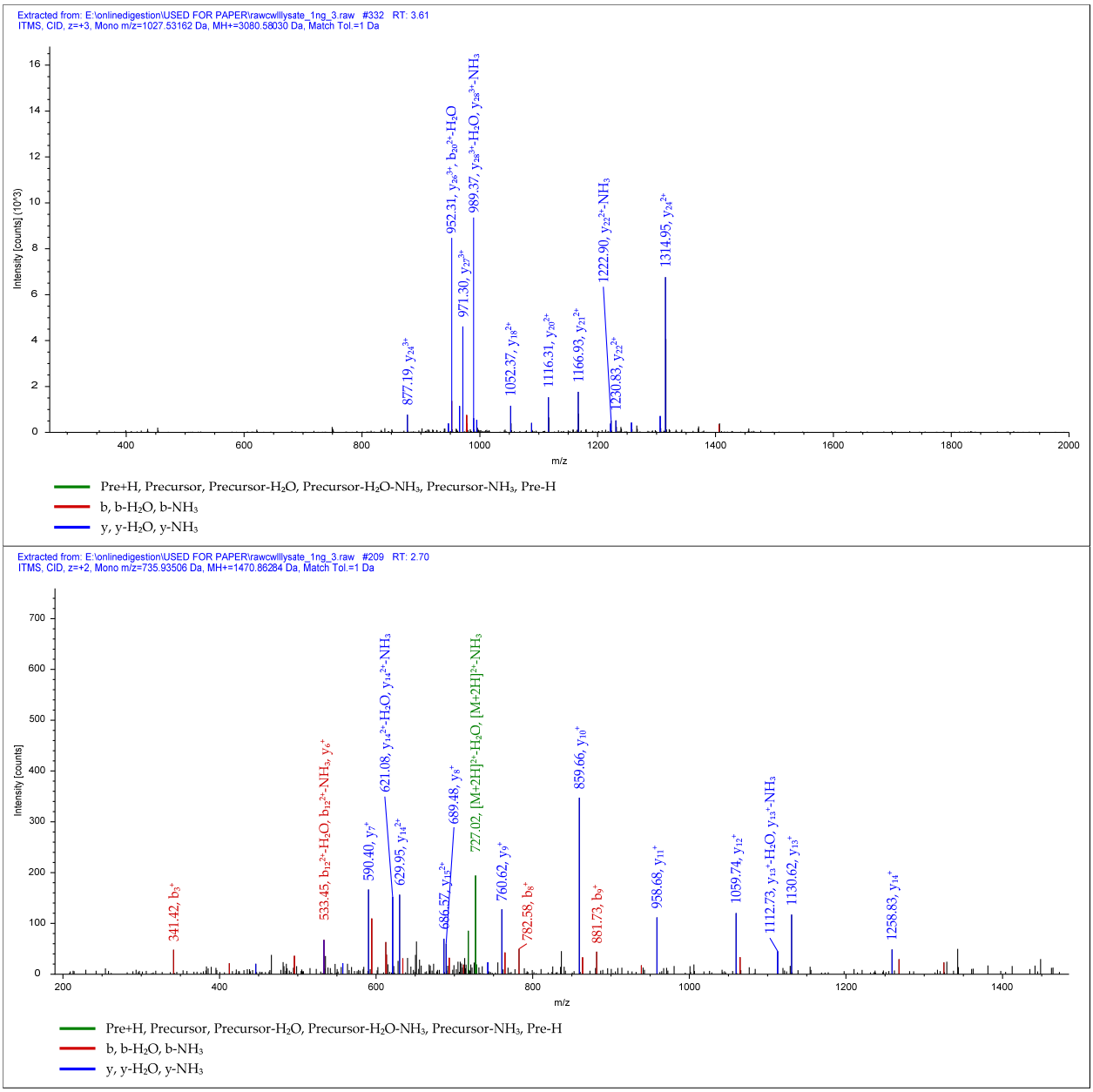
Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_2.raw #217 RT: 2.71
ITMS, CID, z=+2, Mono m/z=735.93475 Da, MH+=1470.86223 Da, Match Tol.=1 Da
Intensity [counts]
m/z
341.50, b3+
446.40, y5+
533.38, b122+-H2O, b122+-NH3, y6+
590.42, y7+
630.07, y142+
686.62, y152+
689.53, y8+
726.92, [M+2H]2+-H2O, [M+2H]2+-NH3
760.54, y9+
764.68, b8+-H2O, b8+-NH3
782.60, b8+
859.60, y10+
881.67, b9+
958.74, y11+
1059.68, y12+
1130.72, y13+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

3 ng RAW 264.7 cell lysate_3rd run

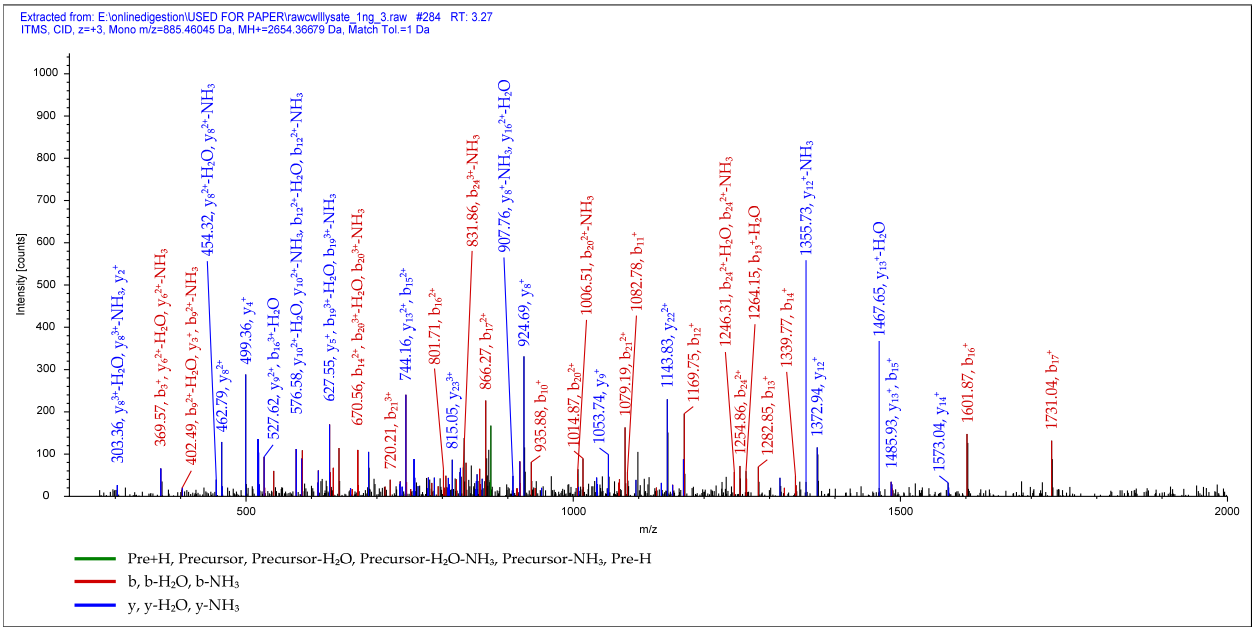



Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_3.raw #267 RT: 3.12
ITMS, CID, z=+2, Mono m/z=644.34692 Da, MH+=1287.68657 Da, Match Tol.=1 Da
Intensity [counts]
m/z
229.25, b2+
272.38, y2+
371.41, y3+
376.38, b3+
489.31, b4+
588.42, b5+
626.40, [M+2H]2+-NH3-H2O
700.45, y6+
799.73, y7+
912.69, y8+
999.63, b9+-NH3
1016.63, b9+
1059.68, y9+
1113.89, b10+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_3.raw #196 RT: 2.60
ITMS, CID, z=+2, Mono m/z=644.34698 Da, MH+=1287.68669 Da, Match Tol.=1 Da
Intensity [counts]
m/z
229.30, b2+
272.35, y2+
376.37, b3+
485.40, y4+
489.39, b4+
588.43, b5+
626.67, [M+2H]2+-NH3-H2O
700.49, y6+
785.62, b7+-H2O, b7+-NH3
799.59, y7+
912.68, y8+
999.69, b9+-NH3
1016.60, b9+
1059.69, y9+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3


Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_3.raw #332 RT: 3.61
ITMS, CID, z=+3, Mono m/z=1027.53162 Da, MH+=3080.58030 Da, Match Tol.=1 Da
Intensity [counts] (10^3)
m/z
877.19, y24 3+
952.31, y26 3+, b20 2+-H2O
971.30, y27 3+
989.37, y28 3+-H2O, y28 3+-NH3
1052.37, y18 2+
1116.31, y20 2+
1166.93, y21 2+
1222.90, y22 2+-NH3
1230.83, y22 2+
1314.95, y24 2+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3

Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcw\lysate_1ng_3.raw #209 RT: 2.70
ITMS, CID, z=+2, Mono m/z=735.93506 Da, MH+=1470.86284 Da, Match Tol.=1 Da
Intensity [counts]
m/z
341.42, b3+
533.45, b12 2+-H2O, b12 2+-NH3, y6+
590.40, y7+
621.08, y14 2+-H2O, y14 2+-NH3
629.95, y14 2+
686.57, y16 2+
689.48, y8+
727.02, [M+2H]2+-H2O, [M+2H]2+-NH3
760.62, y9+
782.58, b8+
859.66, y10+
881.73, b9+
958.68, y11+
1059.74, y12+
1112.73, y13+-H2O, y13+-NH3
1130.62, y13+
1258.83, y14+
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3


Extracted from: E:\onlinedigestion\USED FOR PAPER\rawcwlllysate_1ng_3.raw #284 RT: 3.27
ITMS, CID, z=+3, Mono m/z=885.46045 Da, MH+=2654.36679 Da, Match Tol.=1 Da
Intensity [counts]
m/z
303.36, y8 3+-H2O, y8 3+-NH3, y2 +
369.57, b5 +, y6 2+-H2O, y6 2+-NH3
402.49, b6 2+-H2O, y3 +, b6 2+-NH3
454.32, y8 2+-H2O, y8 2+-NH3
462.79, y8 2+
499.36, y4 +
527.62, y6 2+, b16 3+-H2O
576.58, y10 2+-H2O, y10 2+-NH3, b12 2+-H2O, b12 2+-NH3
627.55, y5 +, b19 3+-H2O, b19 3+-NH3
670.56, b14 2+, b20 3+-H2O, b20 3+-NH3
720.21, b21 3+
744.16, y13 2+, b15 2+
801.71, b16 2+
815.05, y23 3+
831.86, b24 3+-NH3
866.27, b17 2+
907.76, y8 +-NH3, y16 2+-H2O
924.69, y8 +
935.88, b10 +
1006.51, b20 2+-NH3
1014.87, b20 2+
1053.74, y9 +
1079.19, b21 2+
1082.78, b11 +
1143.83, y22 2+
1169.75, b12 +
1246.31, b24 2+-H2O, b24 2+-NH3
1254.86, b24 2+
1264.15, b13 +-H2O
1282.85, b13 +
1339.77, b14 +
1355.73, y12 +-NH3
1372.94, y12 +
1467.65, y13 +-H2O
1485.93, y13 +, b15 +
1573.04, y14 +
1601.87, b16 +
1731.04, b17 +
Pre+H, Precursor, Precursor-H2O, Precursor-H2O-NH3, Precursor-NH3, Pre-H
b, b-H2O, b-NH3
y, y-H2O, y-NH3