

Strategy for Manual Interpretation of Peptide Fragmentation Mass Spectra

1. Compute the protonated molecular weight for the peptide – generally from the observed doubly-charged precursor ion ($[M+H]^+$ = ion m/z * charge – (charge-1)). EX: If the observed precursor ion has an m/z of 389.8 and is doubly-charged, the peptide MW should be around $389.8 * 2 - 1 = 778.6$ (see mass spectrum below, which will be used as an example).
2. Divide the $[M+H]^+$ by 110, the average mass of an amino acid, to figure out approximately how many amino acids may be included. $778.6/110 = 7.078$ for our example, therefore assume between 6 and 8 amino acids are likely.
3. If the mass spectrometer used yields low mass immonium ions ($NH_2=CHR^+$), inspect the low mass region to get an idea of which amino acids might be included in the peptide. This will not work for an ion trap mass spectrometer, since the low mass region is generally excluded. Immonium ion masses range from m/z 30 – 159. In our example there is an ion at 120 which suggests that either oxidized methionine (Mo) or phenylalanine (F) is included somewhere in the peptide.
4. The mass window an ion can fall in is between 57 and 186 amu.
5. If possible, look for the largest y -type ion. This will appear in the mass window below the parent ion. You will also need to account for the proton. In our example this should be between $778.6-57 = 721.6$ and $778.6-186 = 592.6$. The possibilities are 665.2, 647.2 and 632.3 in our example. $779-665-1=113$ (L/I); $779-647-1=131$ (M) ; $779-632-1=146$ (nothing). The largest y ion will correspond to the smallest b ion and the largest b ion will compliment the smallest y ion.
6. If the sample was digested with trypsin, look in the low mass end of the spectrum (if available) for an ion at 147 (K) or 175(R) which will be the smallest y ions. In our example K is the smallest y ion.
7. If you identified the largest y ion, try to find the next largest y ion. In our example, we would look from $665-57 = 608$ and $665-186=479$ if the largest y ion is L/I. The possibilities are 575, 557, 518, 500. Note that a mass difference of 17 corresponds to loss of NH_3 and of 18 is loss of water, thus concentrate on the ions at 575 and 518 as more likely. $665-575=90$ (nothing); $665-518=147$ (F). Since we know F is in the sequence, this is likely the second largest y ion. Checking the other possibility (which may not really be necessary since it differs by 18 from 665), we get $647-518=129$ (E); $647-575=72$ (nothing); $647-476=171$ (nothing). Noting that the largest b ion can be found offset by 19 from the precursor ion and should be K, we expect the largest b ion to be at 632.
8. Try to find the second b ion (generally the first b ion is not observable). In our example this should be either L/IF or L/IE. Look at the chart. L/IF is 261. L/IE is 243. We have 261 in our spectrum so the sequence is L/I FK thus far.
9. Continue going through the spectrum, looking in the mass window below the peaks identified and trying to validate with the complimentary ion set. So, in our example, the window from 518 is 461 – 332. Choices are 348, 381, 431, 458. Results are nothing, 137 (H), 87 (S), nothing. Going up from 261 for the b ions, we would expect either $261+137=398$ or $261+87=348$. We only have a peak at 348, therefore the b_3 ion is S. From 431, the possibilities are between 374-245 and the only peak the yields a result is 303. $431-303=128$ (K/Q). The complimentary b ion

should be at $348+128=476$. If the peptide is tryptic, we would most likely see a cleavage at K, therefore the residue is most likely a Q. Now our sequence is L/I F S Q..K. Working from the b4 ion at 476, look for peaks between 533 and 662. Possibilities are 575 and 632, assuming 647 is a loss of water. $575-476=99$ (V); $632-476=156$ (R). R is unlikely since the peptide should be tryptic, but we can double check. From the y ion at 303, we have $303-99=204$ and $303-156=147$. But 147 is the y1 ion so the b5 ion should be V. So, we have L/I F S Q V.K. Adding up the masses we have 1 (for the proton) + $113+147+87+128+99+128+19$ (for the C terminus)=722. $779-722=57$ (G). So, $575+57=632$, validating the assignment for b6.

10. Add up the masses to see if they come to the precursor ion mass. For our example, $1+113+147+87+128+99+57+128+18=778$. Why not 779? This is due to rounding, since we rounded up on the precursor mass and rounded down on the product ion masses. Display the sequence and the m/z values for the expected and observed amino acid residue fragment peaks. Usually we bold or underline the observed ones. In our example they were all observed:

b	114	261	348	476	575	632	778	
	L/I	F	S	Q	V	G	K	
	778	665	518	431	303	204	147	y

