

Interpretation of the Product Ion Spectra of Tryptic Peptides

The Nine-Step Strategy for Interpretation

1. *Inspect the low-mass region for immonium ions.* The first step in the interpretation is to inspect the low-mass region of the spectrum, noting the presence of any immonium ions and the amino acid composition that they indicate.
2. *Inspect the low-mass region for the b_2 -ion.* In the second step of the interpretation, the low-mass region of the spectrum is inspected to identify the b_2 -ion, generally recognizable by the b_2 -ion/ a_2 -ion pair separated by 28 Da. By using Table 4.2, the possible two-amino acid-combinations indicated by the b_2 -ion are noted. The m/z of the b_2 -ion is then used to calculate the m/z of the corresponding y_{n-2} -ion, and the high-mass region of the product ion spectrum is inspected to identify this ion.
3. *Inspect the low-mass region for the y_1 -ion.* The third step of the interpretation is to assign the C-terminal amino acid. The low-mass region of the spectrum is inspected to identify the y_1 -ion at either m/z 147, for C-terminal lysine peptides, or m/z 175, for C-terminal arginine peptides. The m/z of the y_1 -ion is then used to calculate the m/z of the b_{n-1} -ion, and the high-mass region of the product ion spectrum is inspected to identify that ion, if present.
4. *Inspect the high-mass region to identify the y_{n-1} -ion.* The fourth step of the interpretation is to attempt to assign the N-terminal amino acids from combinations indicated by the b_2 -ion. The high-mass region of the spectrum is scrutinized to identify the y_{n-1} -ion, if present. The list of possible amino acid combinations derived from the b_2 -ion limits the possible residue masses to consider. If an ion is identified, the m/z of that ion is used to calculate the residue masses of the first two amino acids and to assign those peptides.
5. *Extend the y -ion series toward lower m/z .* Working with the residue masses listed in Table 4.1, begin to extend the y -ion series backwards (toward lower m/z) from the y_{n-2} -ion. As a y -ion is identified calculate the m/z of the corresponding b -ion and identify that ion in the spectrum. Work towards extending the y -ion series from the y_{n-2} ion to the y_1 -ion.
6. *Extend the b -ion series toward higher m/z .* If progress extending the y -ion series falters, use the residue masses listed in Table 4.1 to extend the b -ion series from the last identified b -ion. As any b -ions are identified, use the m/z of that ion to calculate the m/z of the corresponding y -ion and identify that ion in the spectrum.
7. *Calculate the mass of the peptide.* When the interpretation of the spectrum is complete, calculate the mass of the proposed peptide sequence and check its agreement with the measured mass.
8. *Reconcile the amino acid content with spectrum data.* Check that the amino acid content agrees with the immonium ions observed. Also consider the charge state of the peptide in terms of the presence of histidine, and internal lysine or arginine residues.
9. *Attempt to identify all ions in the spectrum.* Work to identify the other ions in the spectrum based on the proposed peptide sequence and pay particular attention to the ions from the loss of H_2O , NH_3 , and $HSOCH_3$; any doubly charged ions; and any ions due to internal cleavages.

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