Calculating Molecular Mass by ESI/nESI Mass Spectrometry

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One of the advantages of mass spectrometry is providing accurate and precise molecular mass of biomolecules. Mass spectrometry determines the mass-to-charge (*m/z*) ratio of the analyte. A mass spectrum is a plot of ion abundance versus *m/z*.

In electrospray (ESI) or nanoelectrospray (nESI) ionization, ions have a distribution of charge at several acidic or basic sites due to protonation or deprotonation, respectively. Multiple peaks can be observed due to the different charge states. The multiple charging allows us to analyze high-molecular-weight molecules even with mass spectrometers that have limited *m/z* ranges.

Based on the *m/z* values of adjacent peaks observed, we can calculate the protein molecular mass (m) and corresponding charge state (z) for each peak. Figure 1 shows an example of an ESI positive mode mass spectrum of an unknown analyte. We can calculate the molecular mass (m) of the molecule with multiple charge states in this ESI spectrum by using the following steps.

Shortcut:

$$\frac{(m/z)_A}{(m/z)_B - (m/z)_A} \approx z_B$$

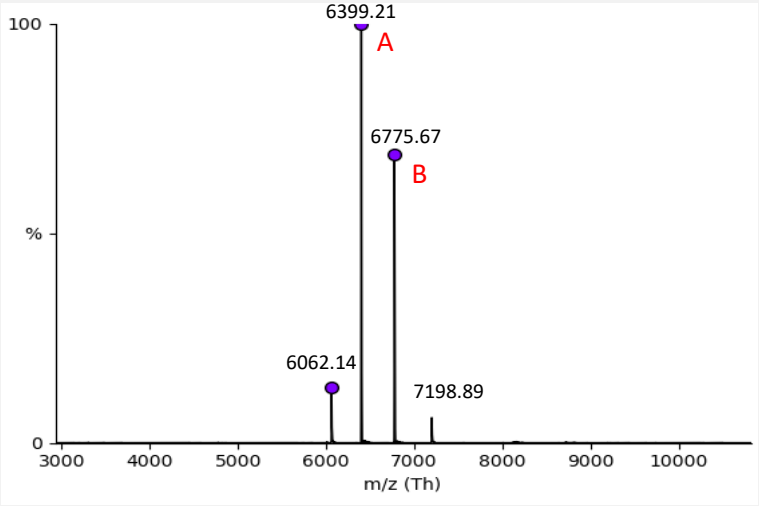

Figure 1: ESI *m/z* spectrum of an unknown molecule obtained in positive mode.

1. Assume that ions' charges are caused by protonation (H^+). Thus, each extra H^+ adds one extra mass unit.

2. Peak A and B are two adjacent peaks that have $(m/z)_A < (m/z)_B$.

3. Assign *z* as the charge state of peak B, thus, adjacent peak A has charge state *z*+1

$$Peak\ B: \frac{m+z}{z} = 6775.67\ (A)\ \&\ Peak\ A: \frac{m+z+1}{z+1} = 6399.21\ (B)$$

4. Solve these two equations:

$$\frac{m+z}{z} = 6775.67\ (A) \rightarrow m = 6775.67z - 1z\ (C)$$

$$\frac{m+z+1}{z+1} = 6399.21\ (B) \rightarrow m = 6398.21z + 6398.21\ (D)$$

$$Combine\ (B)\ \&\ (D):\ 6774.67z = 6398.21z + 6398.21$$

$$6774.67z - 6398.21z = 6398.21 \rightarrow z = 17$$

$\because z = 17$

$$\therefore m\ of\ Peak\ B = 6775.67 \times 17 - 17 = 115{,}169\ (Da)$$

$\because z\ of\ peak\ A = 17 + 1 = 18$

$$\therefore m\ of\ Peak\ A = 6399.21 \times 18 - 18 = 115{,}168\ (Da)$$

5. Average mass based on 4 peaks = 115,166 (Da).