

Xevo Q-ToF Resolution and Accuracy

Mass accuracy

Accurate mass is the experimentally measured mass value

Exact mass is the calculated mass based on adding up the masses of each atom in the molecule

Mass error = (accurate mass) - (exact mass)

Mass error in parts per million (ppm) = (mass error / exact mass) * 10^6

When calculating the exact mass, use the actual atomic weights of the atom not the nominal mass. Eg. Use 14.0031 for ^{14}N not 14, use 15.9949 for ^{16}O not 16. Using the actual atomic weights of atoms ensures a high confidence for any accurate mass measurements.

Mass Resolution

Mass resolution = (ion mass)/(mass peak width at half height)

The resolution of a peak depends on the mass being analyzed, signal to noise ratio and instrumental parameters e.g. acquisition time.

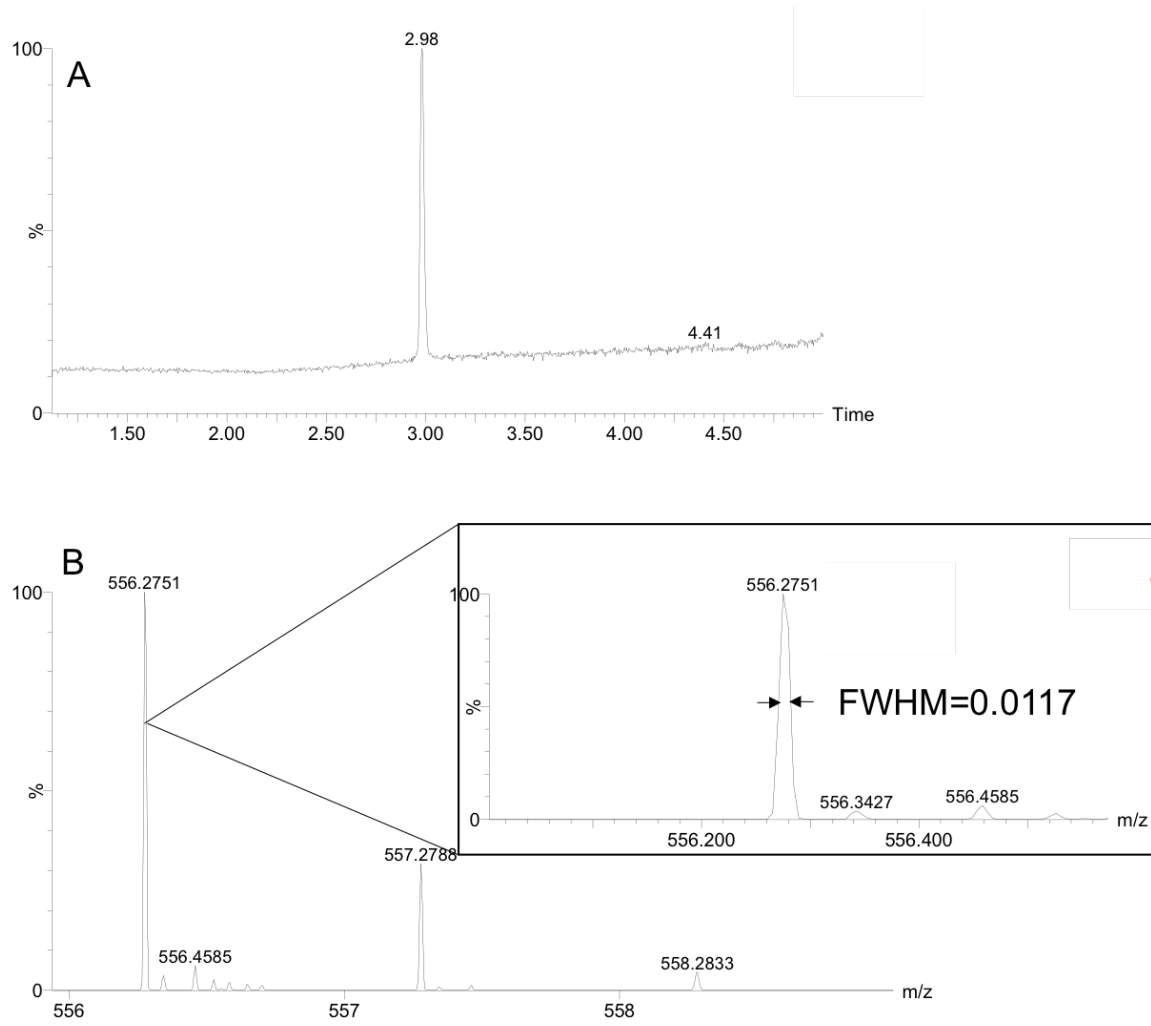
The presence of isotopes at their natural abundances gives rise to an isotopic distribution for detected masses. For small molecules and small peptides use the monoisotopic mass; which is the first peak of the isotope distribution.

The following example was acquired on the Xevo using the default peptide method with no lock mass. Leucine Enkephalin ($\text{C}_{28}\text{H}_{37}\text{N}_5\text{O}_7$) has exact monoisotopic mass of 556.2771 (M+H). The observed monoisotopic peak was 556.2751 (Figure 1 B) Mass error = $556.2771 - 556.2751 = 0.002$. Mass error in ppm = $(0.002/556.2771) * 10^6 = -3.6$ ppm. The FWHM of the monoisotopic peak is 0.0117 (Figure 1B) therefore the resolution of the peak is $556.2751/0.0117 = 47,544$

For proteins, use the centroid of the isotopic envelope to provide an average mass value and use the whole isotopic envelope as a measure of resolution. Since in bigger proteins the isotopic peaks will not be resolved anyway.

Figure 2A shows the total ion chromatogram of ~45KD protein analyzed on the Xevo using the default protein method. The calculated mass of the protein is 45,780Da. The observed mass is 45,781Da. The mass error is $45,781 \pm 1$ Da. Figure 2B is the MS data showing the charge state envelope. The peak labeled 818 has a charge state of 56 and the isotopic envelop of that charge state has a FWHM of $0.3 * 56 = 16.8$. The resolution of the 818 isotopic envelope is $45,781/16.8 = 2725$.

Max Ent 1 deconvolution increases signal to noise ratio and also enhances peak resolution. The Max Ent 1 deconvoluted mass peak of the ~ 45KD protein has a resolution of 5,722 (Figure 3).



$$R = 556.2751 / 0.0117 = 47,544$$
$$\text{Mass error} = (556.2751 - 556.2771) / 556.2771 * 10^6 = - 3.6 \text{ ppm}$$

Figure 1: A) TIC and B) MS spectra of Leucine Enkephalin analyzed on the Xevo using the default peptide method with no lock mass.

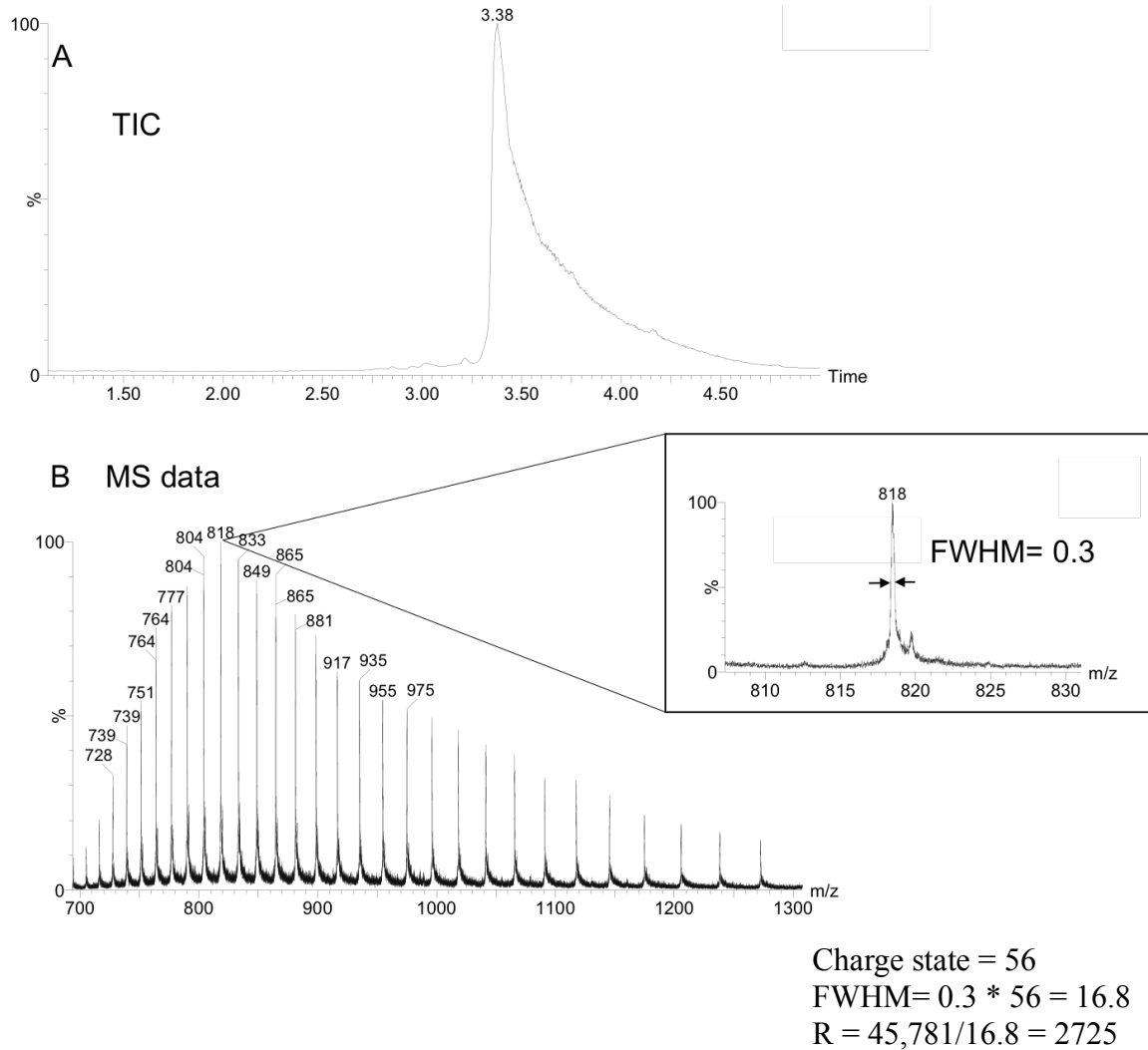


Figure 2: TIC and MS spectra of a ~45KDa protein analyzed on the Xevo using the default protein method.

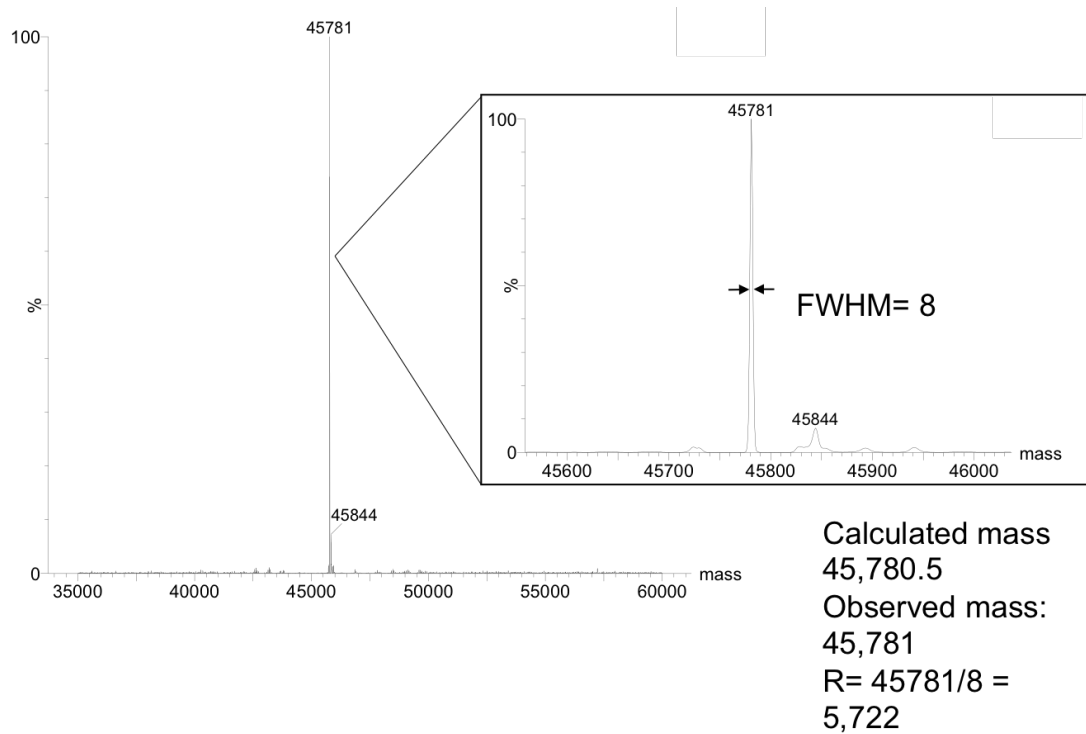


Figure 3: Max Ent1 deconvoluted spectrum of ~45KDa protein

Mass accuracy of the Xevo per Manufacturer: The mass measurement accuracy of the instrument, in resolution mode, will be better than 1 ppm RMS, based on 10 consecutive repeat measurements of the $[M + Na]^+$ ion of raffinose (m/z 527.1588), using a suitable choice of lock mass.

Mass resolution of the Xevo per Manufacturer: Resolution Mode: $>32,500$ FWHM measured on the $[M + 6H]^{6+}$ isotope cluster from bovine insulin (m/z 956) at a data acquisition rate of 30 spectra per second.