UNIVERSITY OF SOUTH ALABAMA
MASS SPECTROMETRY RESEARCH FACILITY

Basic Data Processing with Thermo Xcaliber Qual Browser

CALCULATING THE THEORETICAL M/Z

Data is reported in m/z format (mass/charge). It is important to use exact mass, not average mass.

Symbol	Mass
C	12.000000
H	1.007825
N	14.003074
O	15.994915
S	31.972071
P	30.973760
Na	22.989770
K	38.963707
Fe	55.934939
Fluorine	19.008762
Boron	11.009305
Deuterium	2.014102
13C	13.003355
15N	15.000109
18O	17.999160
Protonation	1.007276
Deprotonation	-1.007276

Table 2. Common adduct ions

Cationized adducts (positive mode)	
$[M+NH_4]^+$	M+18
$[M+Na]^+$	M+23
$[M+CH_3OH+H]^+$	M+33
$[M+K]^+$	M+39
$[M+CH_3CN+H]^+$	M+42

Table 1. Common neutral losses

Loss	Fragment
15	CH_3
18	H_2O
19	F
28	CO
29	C_2H_5 or CHO
35	Cl
46	NO_2
59	C_3H_7O, $COOCH_3$ or CH_2COOH
77	C_6H_5

Monitor the retention time as well as the mass accuracy of the compound you are measuring for verification, as other compounds can have very similar RTs and masses.

CALCULATING MASS ACCURACY

$$\text{Mass accuracy (ppm)} = \frac{\text{observed mass} - \text{theoretical mass}}{\text{theoretical mass}} \times 10^6$$

The mass accuracy of the high resolution instruments should be 5-10ppm or better.

ISOTOPIC DISTRIBUTION

On a mass spectrum of an element, each peak corresponds to a different isotope. The carbon 13 isotope peak appears at approximately one mass unit higher (the actual mass delta 1.00335) than the carbon 12 ion peak. The intensity of these isotopes is proportional to the relative abundance of the naturally occurring isotopes. The relative abundance of the two isotopes is 12C ≈ 98.89% and 13C ≈ 1.11%.

TIC VS BASE PEAK

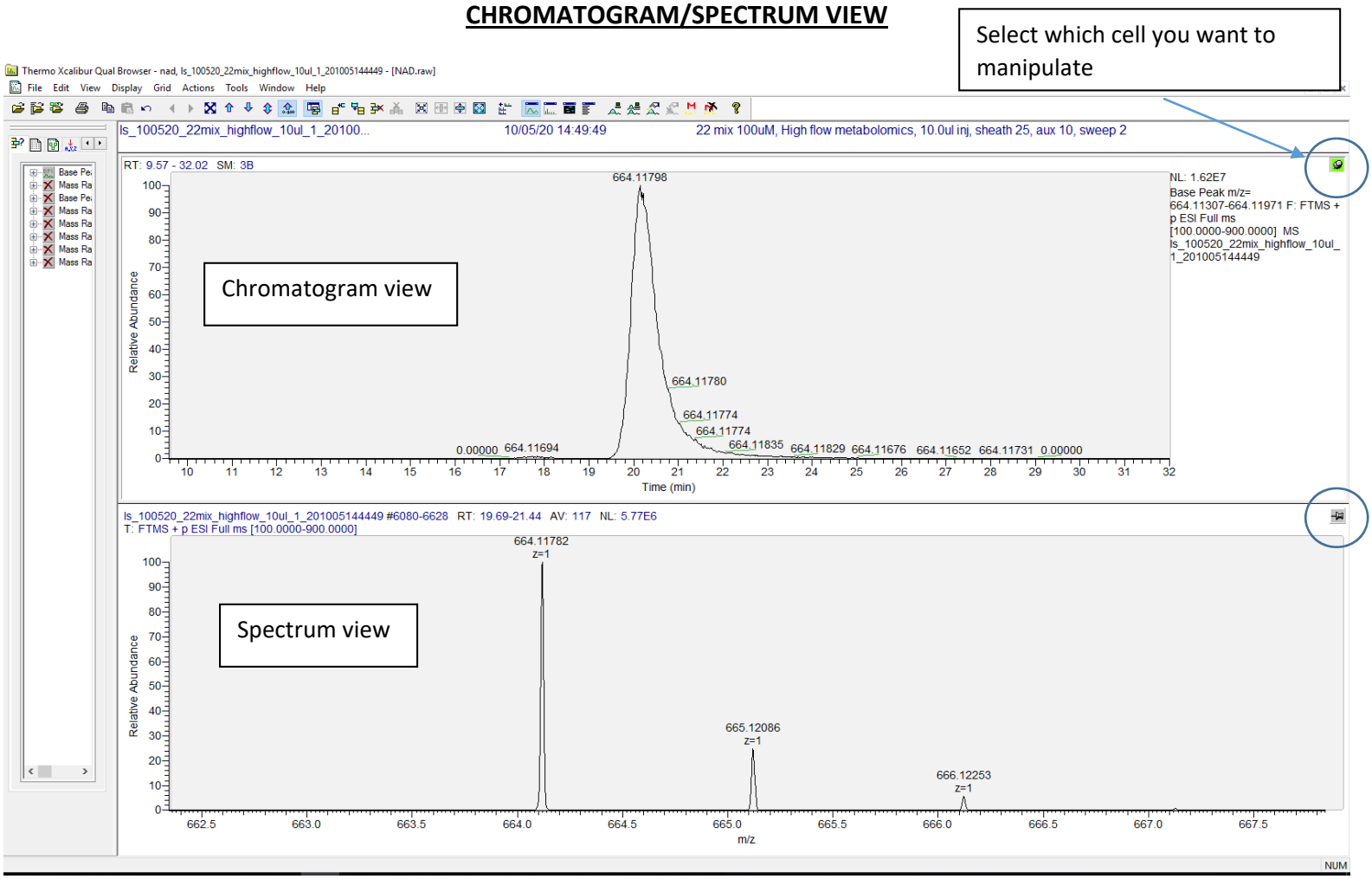
A TIC chromatogram represents the summed intensities of all the ions in the scanned mass range (mass spectrum) plotted against the chromatographic retention time. Each peak in the TIC represents one or more eluting compounds, which can be identified from the mass spectra recorded across the peak.

Base peak chromatograms show the ion intensities of the most intense ions for each time point in the chromatogram.

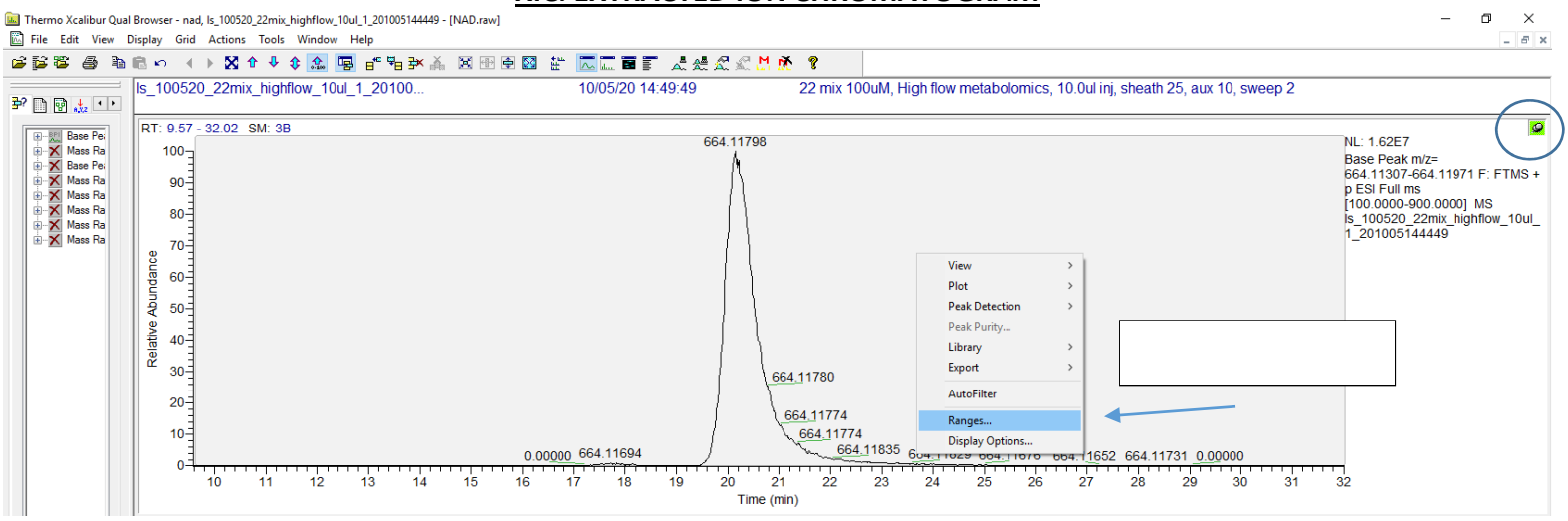
MS/MS FRAGMENTATION

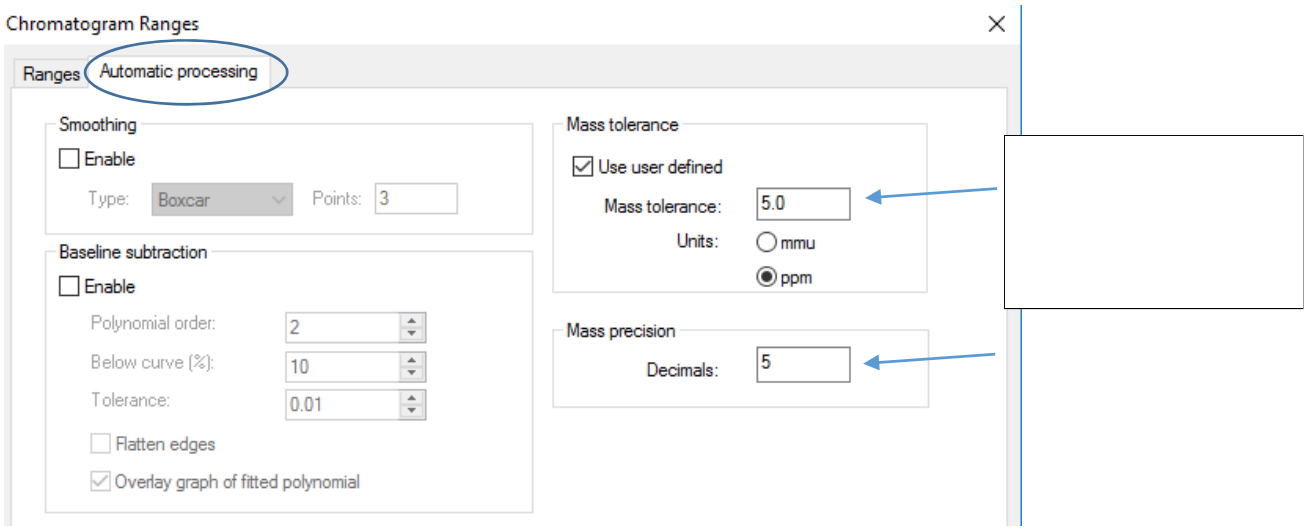
In an MS/MS experiment, an ion from the mass spectrum is selected for fragmentation while all other masses are discarded. This can be targeted or non-targeted. The selected ion, called a precursor (parent) ion, is then collided with a neutral background gas. In the LTQ XL instrument, CID fragmentation is performed with helium. In the Q Exactive, HCD fragmentation is performed with nitrogen. As a result of the collisions, the precursor ion is broken into fragments called product ions.

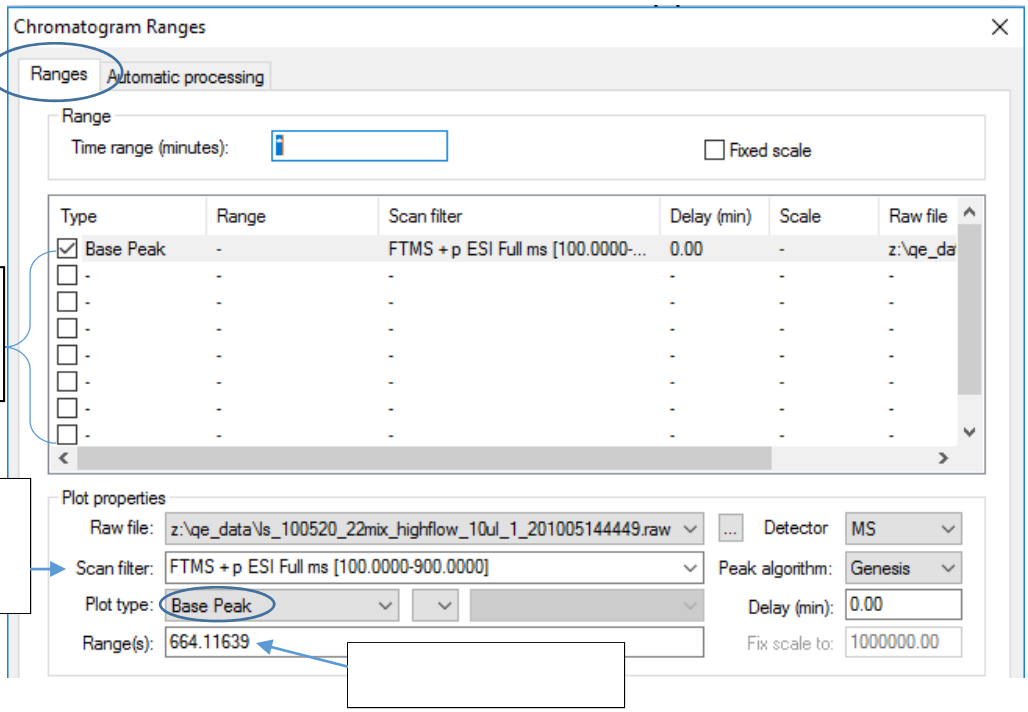
CHROMATOGRAM/SPECTRUM VIEW



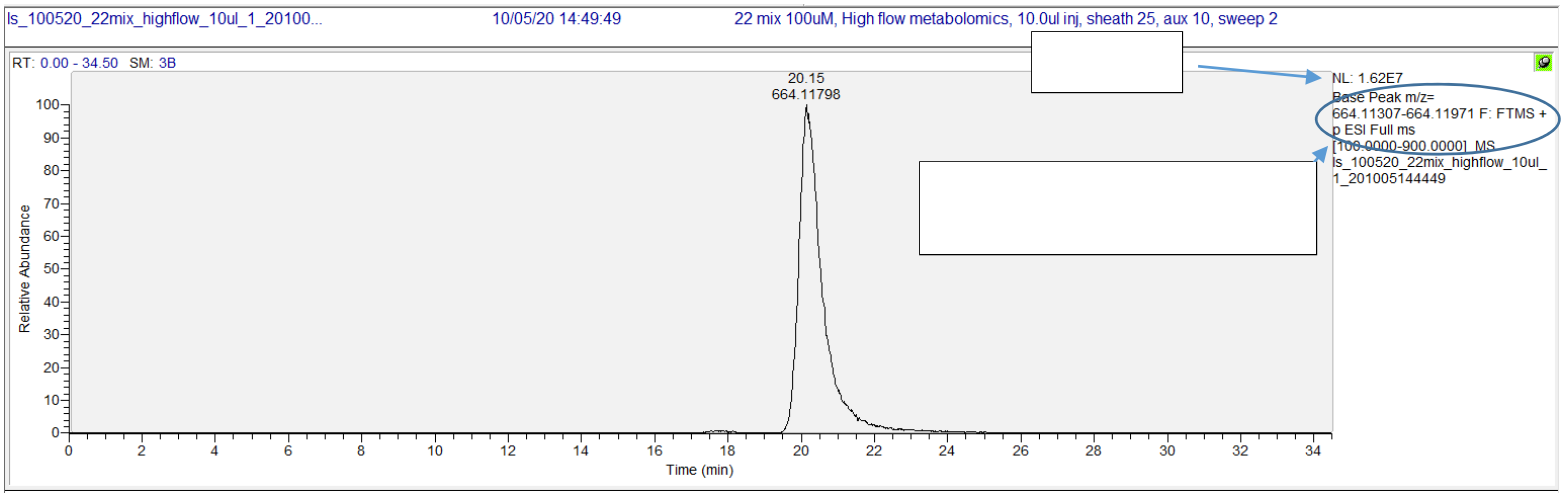
XIC: EXTRACTED ION CHROMATOGRAM







Tick multiple lines to view more than one at once

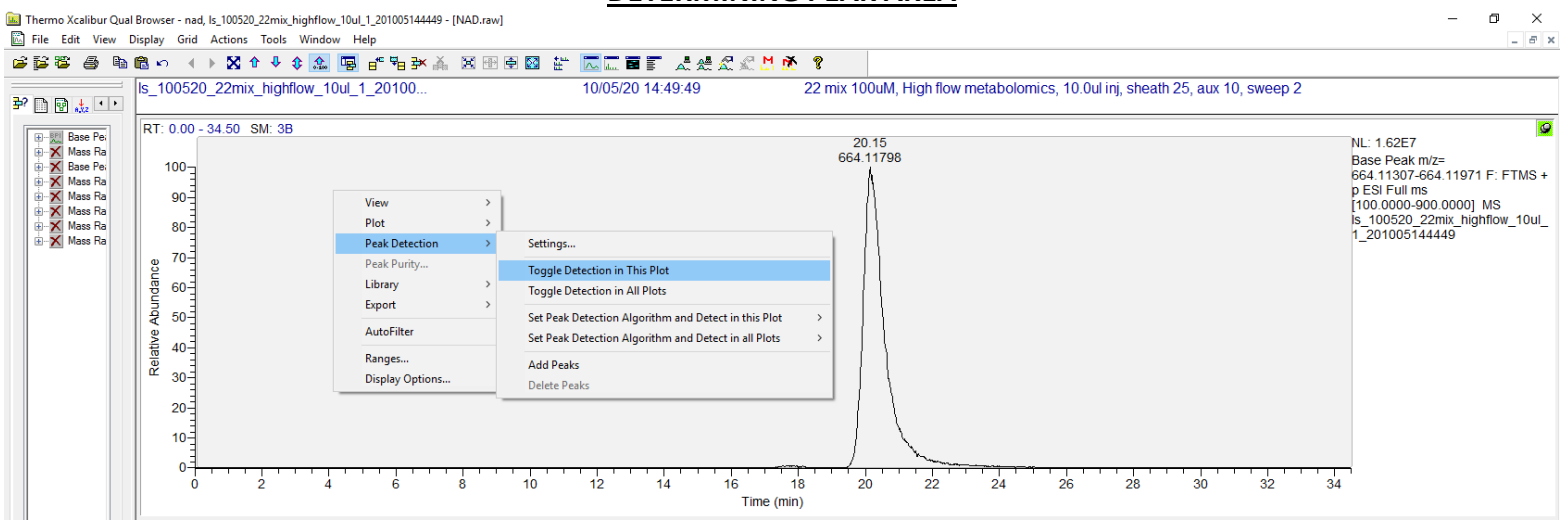


XIC for NAD, 664.11m/z @ 20.15min. The Y axis is plotting relative abundance, and the X axis is retention time. This is a standard, but in a complex sample matrix there can be multiple peaks within your 5-10ppm mass window across the span of the analysis, which is why it is important to also know retention time and fragmentation pattern as well (if necessary).

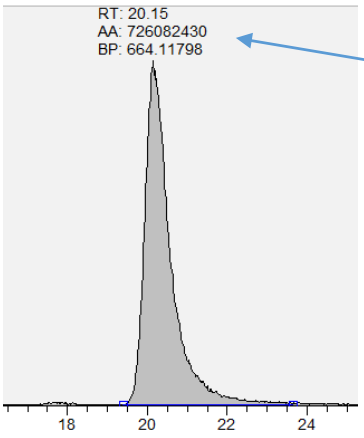
Left click and drag the cursor to only show a limited time range.

Notice the base peak intensity of 1.62E7. The intensity is normalized to the largest peak in the selected time range. This gives an idea of how concentrated the compound is compared to other compounds in the sample, but can be affected by things such as ionization efficiency and peak shape. This should not be compared across instruments, and should not be used for quantitative purposes. Peak area is more appropriate.

DETERMINING PEAK AREA

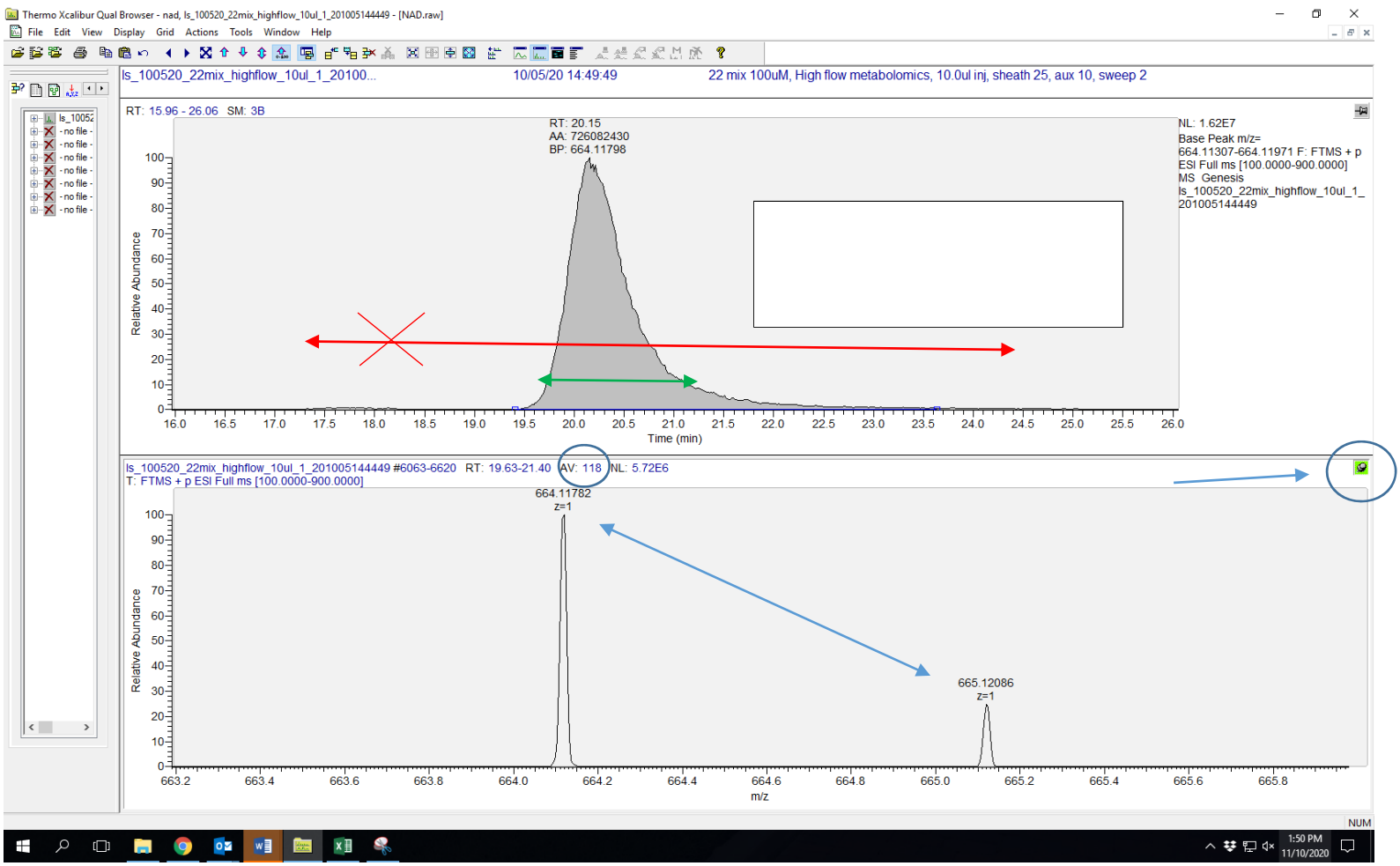


To determine the peak area, right click in the chromatogram, select peak detection > toggle detection in this plot. This will automatically incorporate the peak for you. This can be done manually as well for peaks that are not so well defined and the software does not pick them up.



The integrated peak becomes filled in and the area is displayed above the peak. This can be used for relative quantitation.

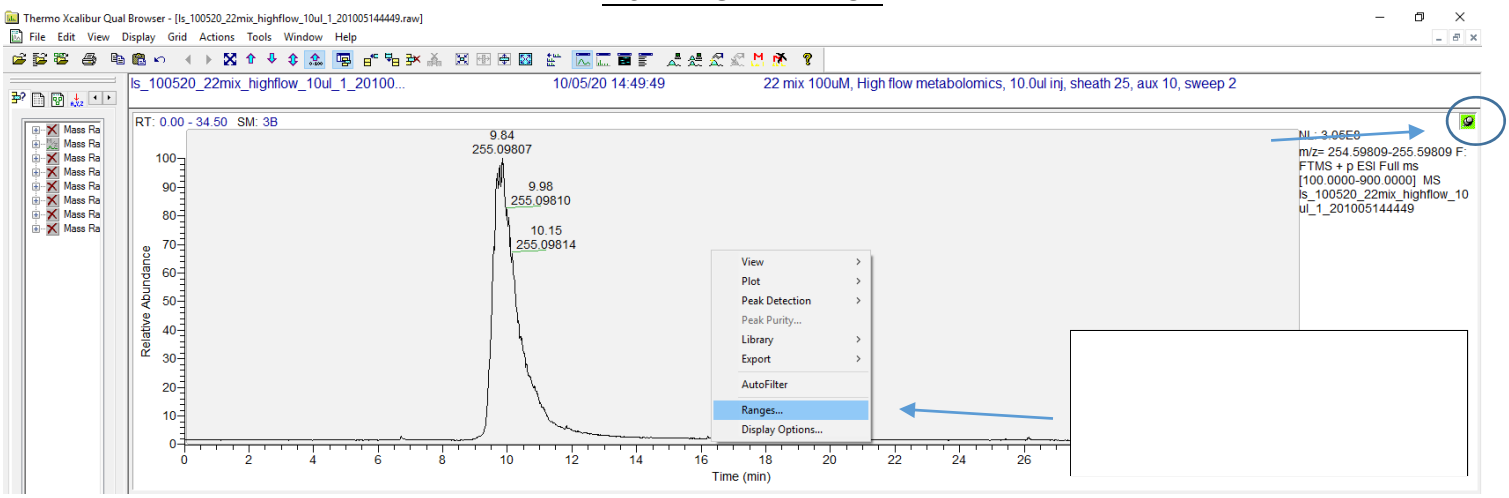
VIEWING THE MS1 SPECTRUM

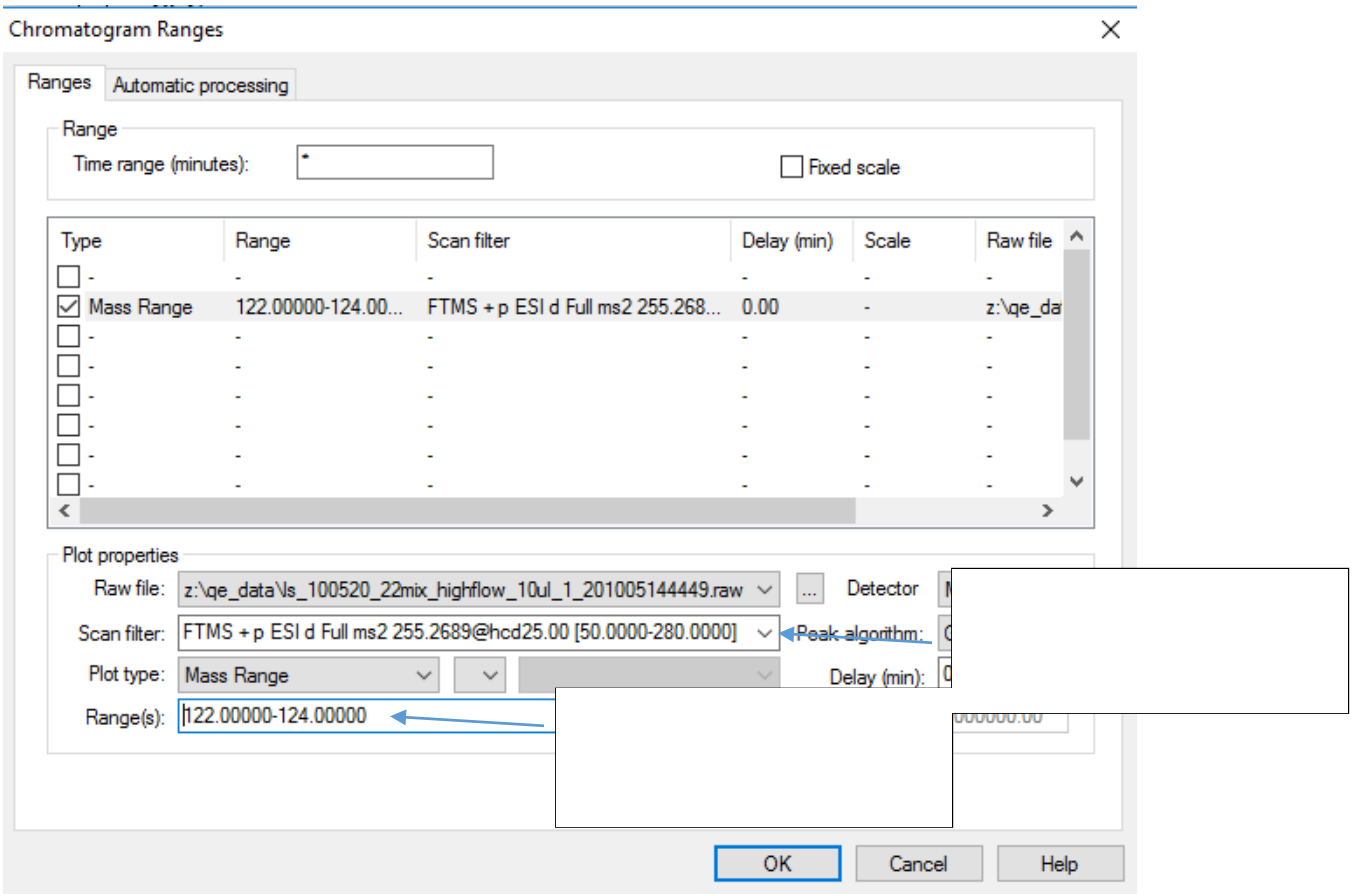


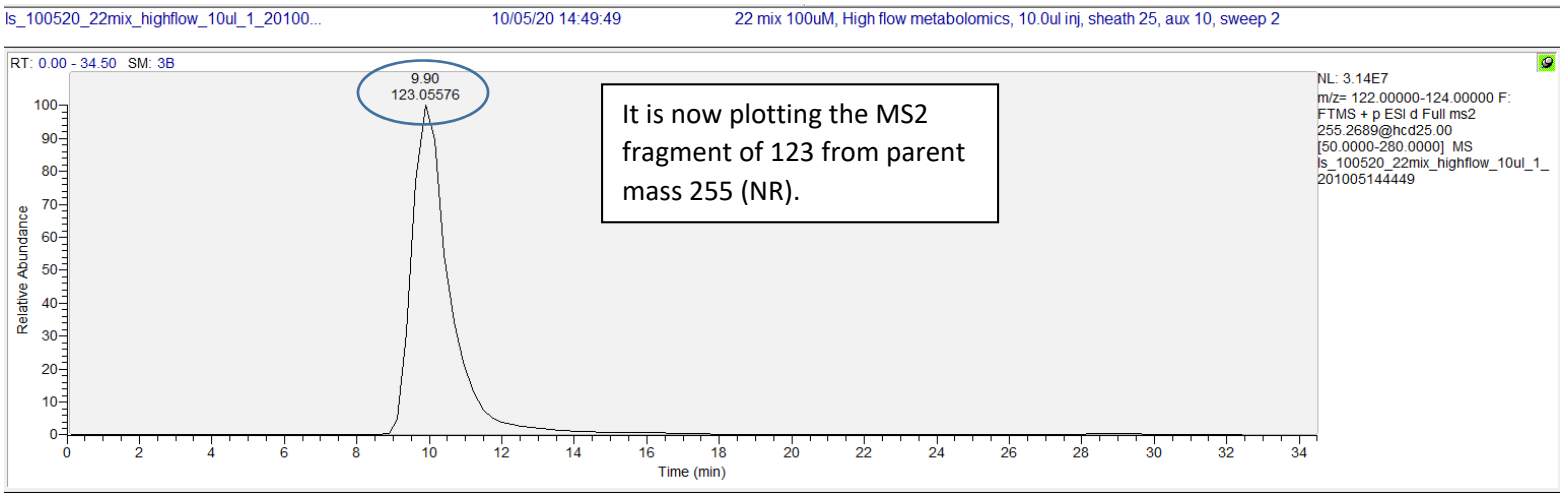
Pin the cell on the bottom to manipulate the spectrum. Left click and drag across the peak in the chromatogram above to average everything under that peak, giving you an averaged spectrum showing mass and charge. **Do not average outside of the peak**. Because you averaged data from the MS1 full scan, it is providing the MS1 spectrum. For the spectrum above, 118 scans were averaged to provide the spectrum. Because the full scan was averaged, everything in the mass range of the full scan within the time range that you averaged will be shown. You can average compounds that were analyzed in SIM also if you used the SIM filter in your chromatogram view, and your averaged spectrum would show the mass range within the SIM scans. To zoom in on your compound, left click and drag the cursor. You can also right click, go to ranges and manually set the mass range you want to see.

Because NAD has 21 carbons, theoretically the 13C isotopic peak should be ~23% of the parent peak. Always note the charge state and isotopic distribution in the spectrum for further verification of your compound.

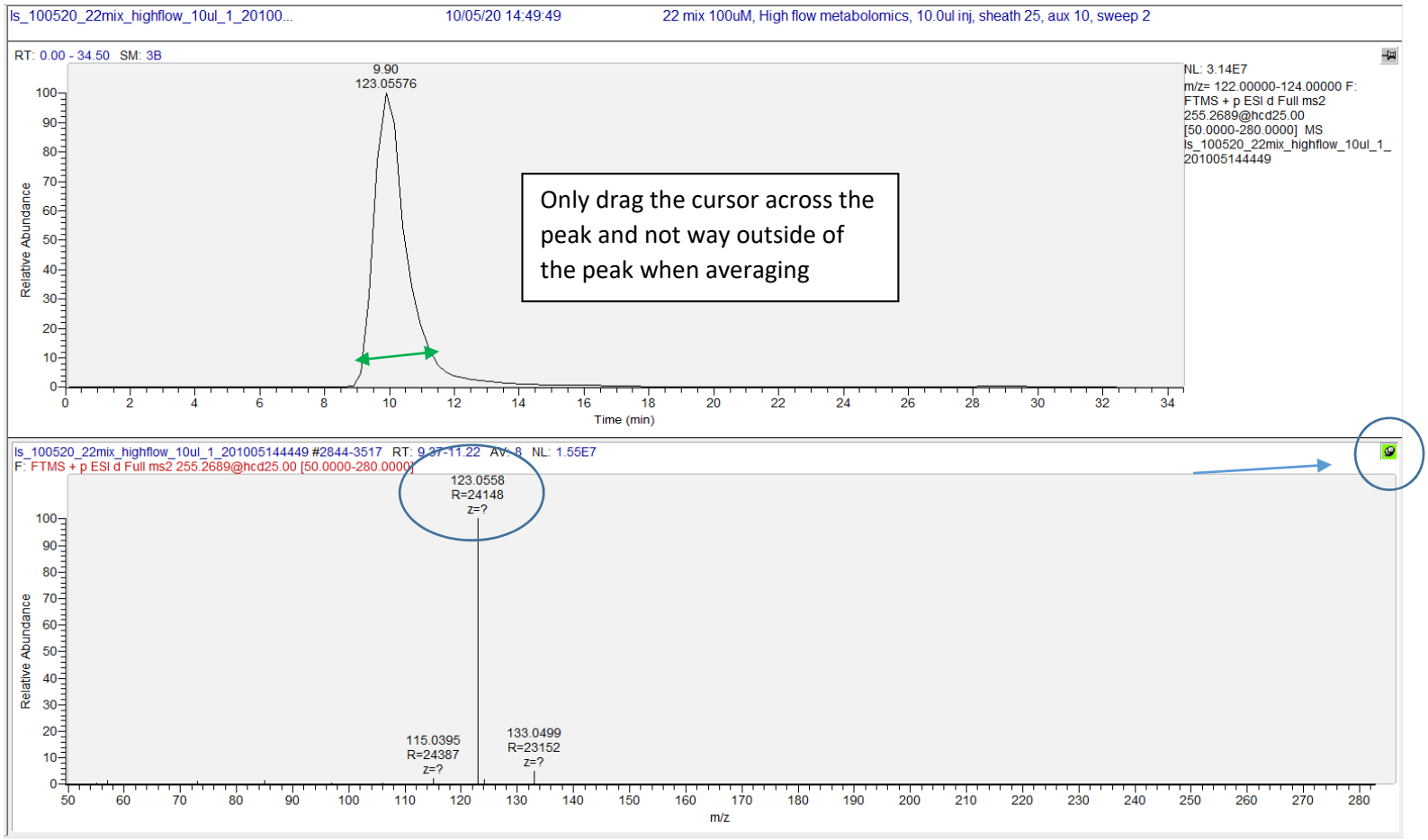
MS2 FRAGMENTATION







The top chromatogram trace is now plotting the MS2 fragment of 123 from parent mass 255 (NR). If you did not specify a particular mass or mass range, it will plot the most intense fragment ion instead.



Just as you did to look at the averaged MS1 spectrum, to show the MS2 spectrum (fragmentation of your parent ion), pin the cell on the bottom to manipulate the spectrum. Left click and drag across the peak in the chromatogram above to average everything under that peak, giving you an averaged spectrum showing mass and charge. **Do not average outside of the peak**. Because you averaged data from the MS2 scan, it is providing the MS2 spectrum.

The major fragment ion from NR is 123, which is what is shown above. Depending on the compound and the fragmentation, you can have multiple fragment ions.

MS2 data can be targeted to a list you specify, or performed in a data dependent manner by selecting the top # of ions in each full scan.

For quantitation purposes, you can choose to use the area of the MS2 rather than the area of the MS1 peak. This is done in the same manner as integrating the peak area for MS1 data. Right click and select peak detection, and toggle detection in this plot. This makes the data more specific to your compound and provides further validation that you are in fact looking at your compound of interest, but this is not required.