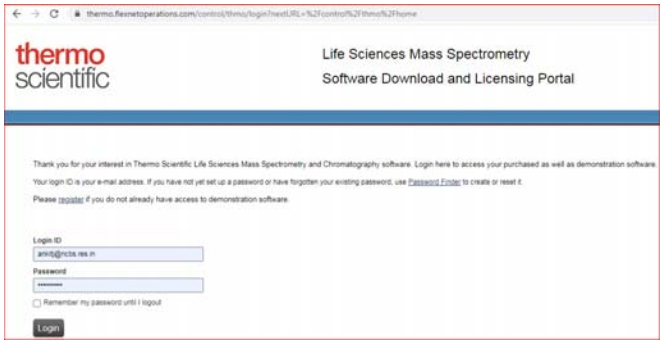
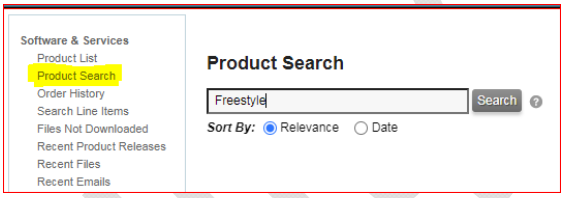
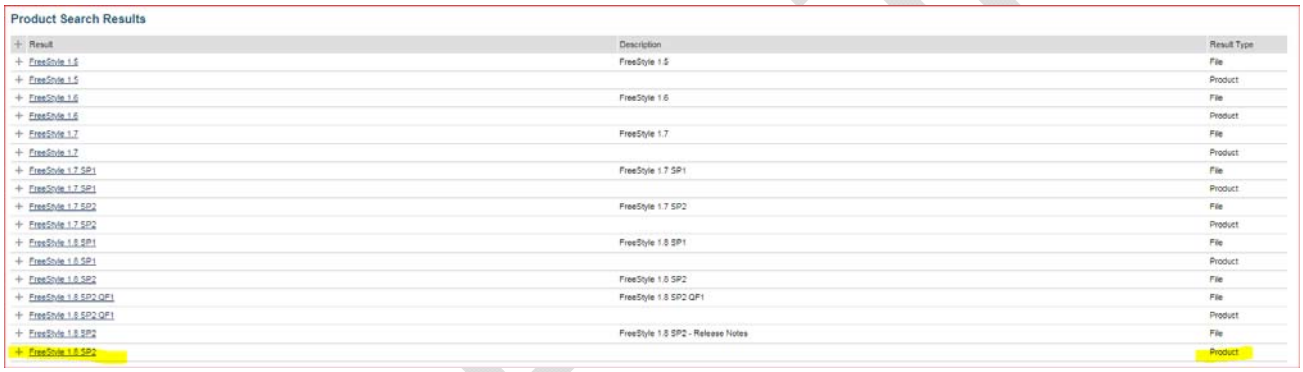
Intact Protein Data Analysis for Thermo Data Only

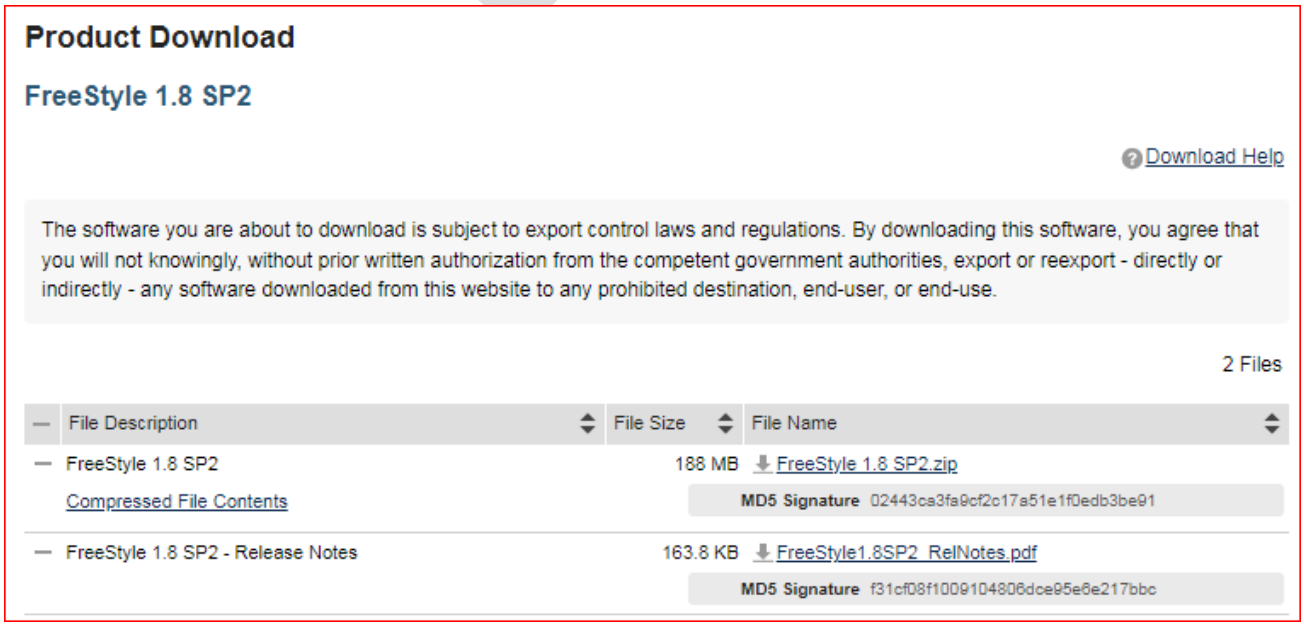
Downloading FreeStyle 1.82 SP2

1. Download 7 Install **FreeStyle 1.8 SP2**
    - Create and login on **https://thermo.flexnetoperations.com**



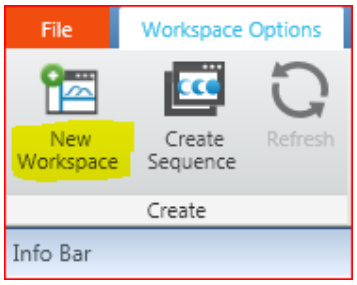




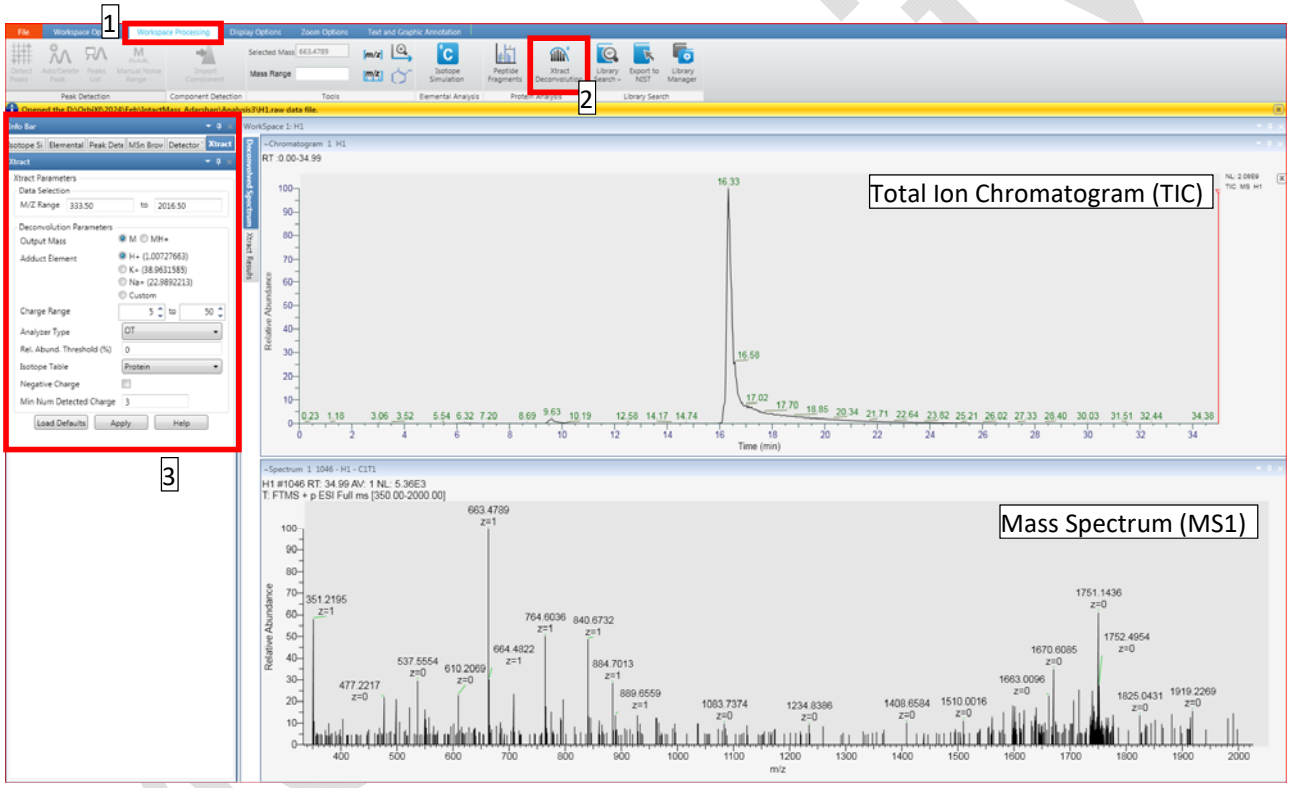


Opening ".raw" from Thermo in FreeStyle 1.82 SP2

1. To open a raw data file or a sequence file Create a new workspace and navigate to the location of the raw file and open



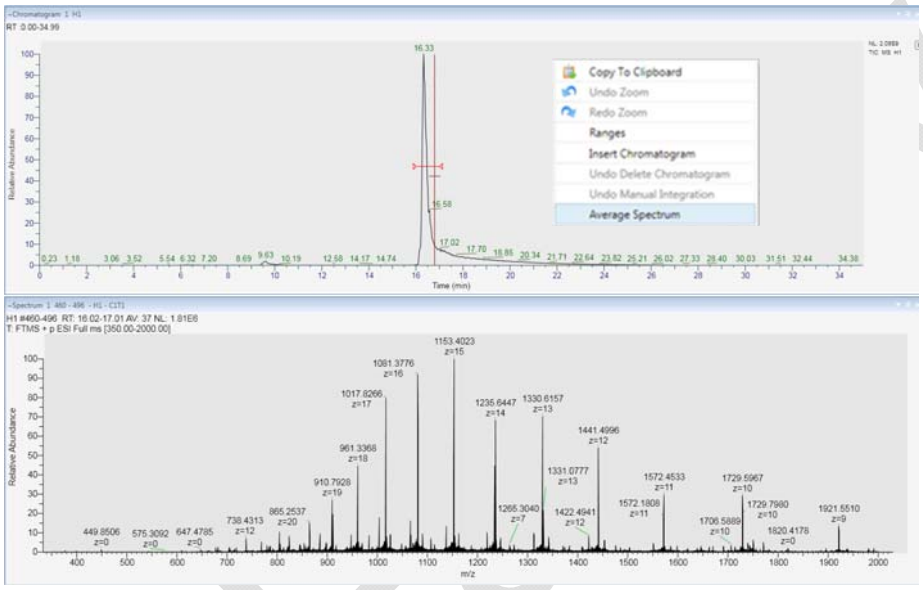
Once you open the raw mass spectrum file the default window would look like this



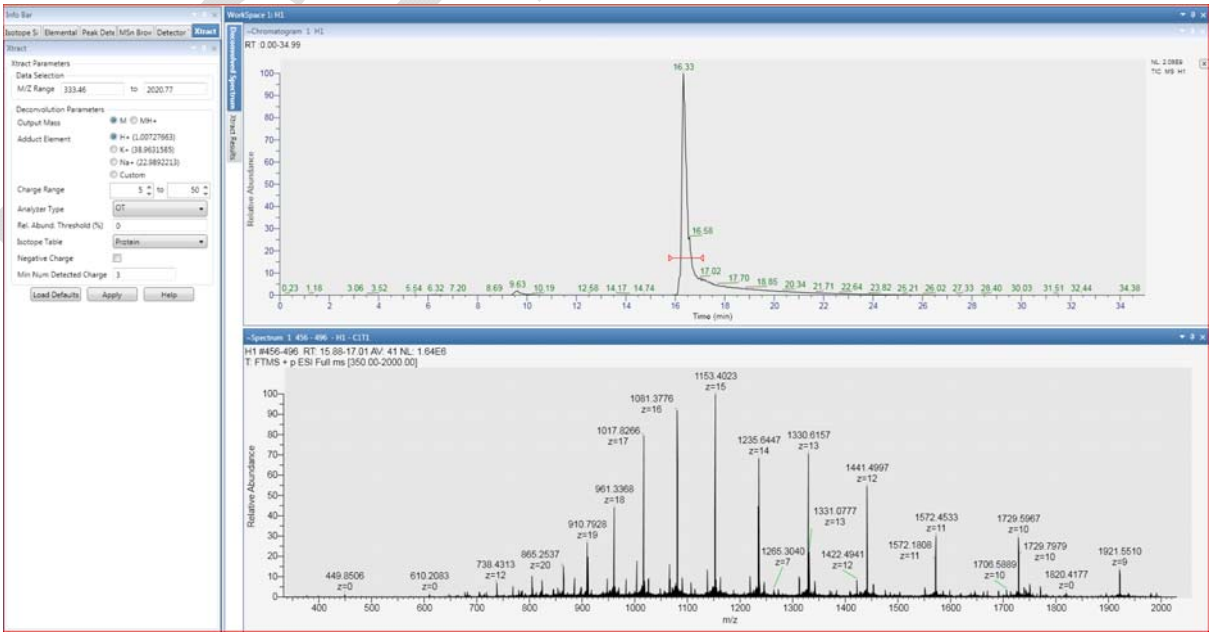
2. Once the raw file is opened, click on Workspace Processing and enable the Xtract Deconvolution tab (refer steps 1-3 in previous image)

Analysis of single chromatographic peak

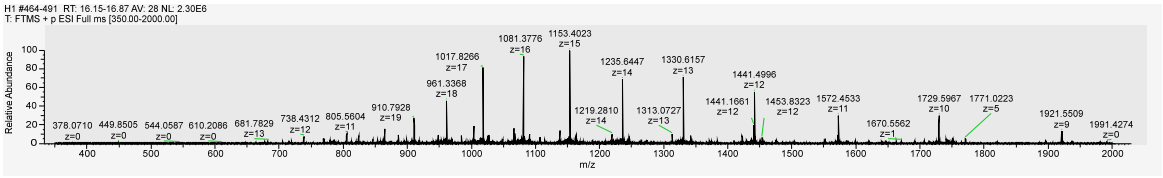
1. For a purified protein a single peak on the chromatographic elution should be observed like in the example above. Multiple MS1 spectrums are recorder by the instrument as the peak eluted from the column. So these MS1 spectrums can be averaged to improve the signal to noise ratio and get an averaged spectrum. To average spectrum right click on the TIC select Average Spectrum, and now select the region of the peak using left click. The MS spectrum window will now show the averaged spectrum of the intact protein peak. The MS1 spectrum shows multiple charge states of the same intact protein for eg. 1017.8 z=17, 1061.3 z=16, 1153.4 z=15 etc

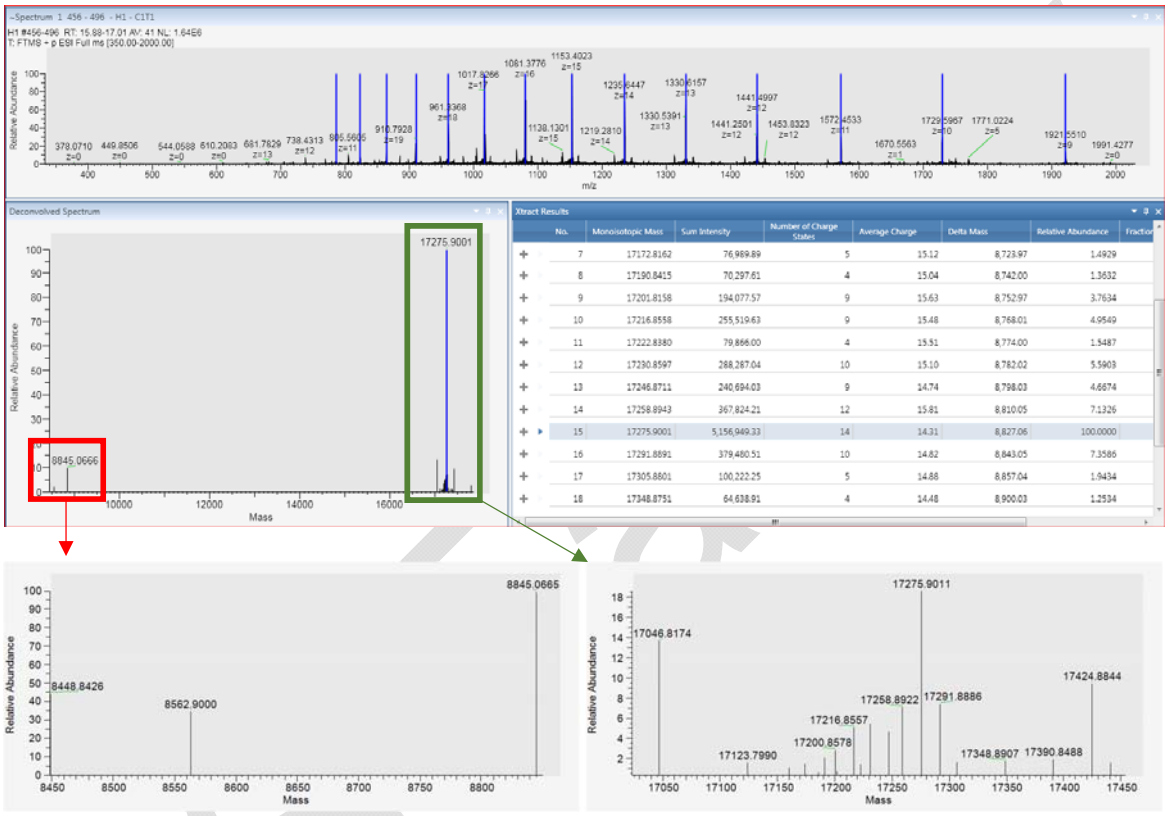


2. Once the spectrum is averaged. Select the MS window by clicking on it. The options in the info bar for the Xtract deconvolution parameters would change accordingly. Use default parameters for Protein isotope model for deconvolution and click apply.



3. The Xtract Algorithm then computes the intact mass from multiple charge states of the protein and assigns the average mass for the protein

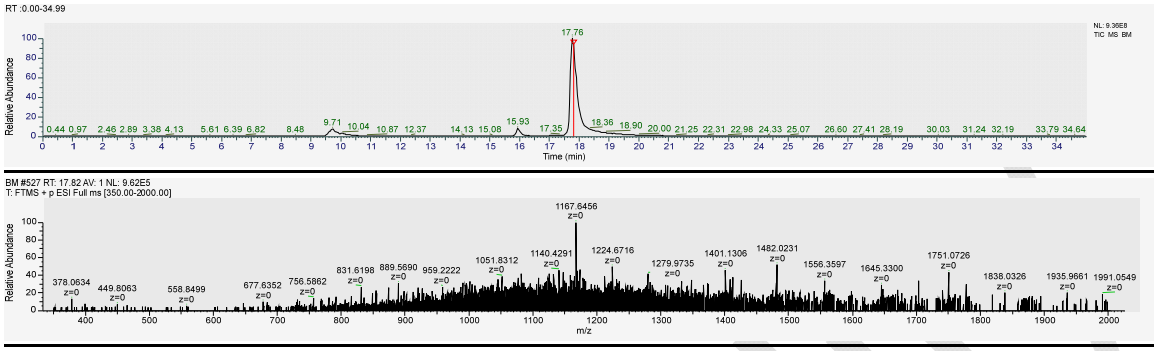




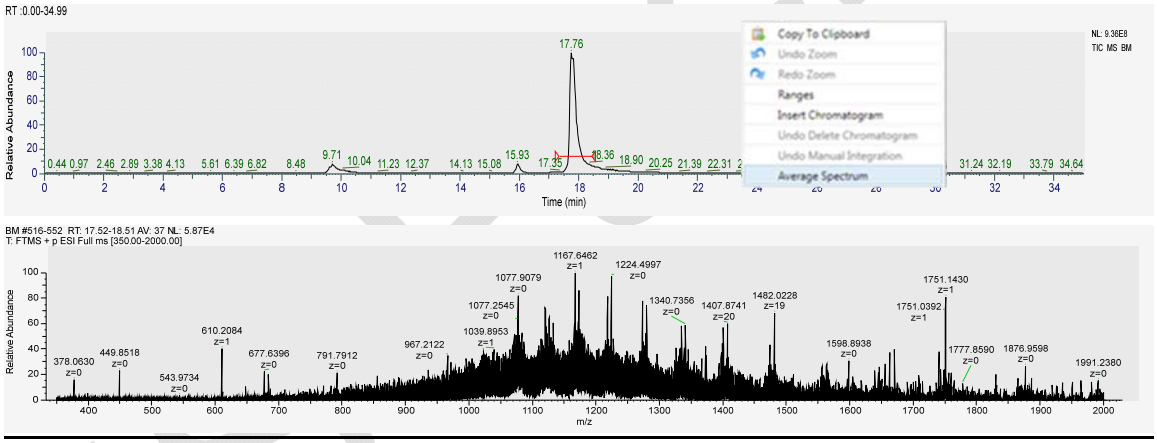
Results show identification of multiple proteoforms of the same proteins with changes in mass values for different modifications in protein structure

Analysis of single chromatographic peak- for Large protein size

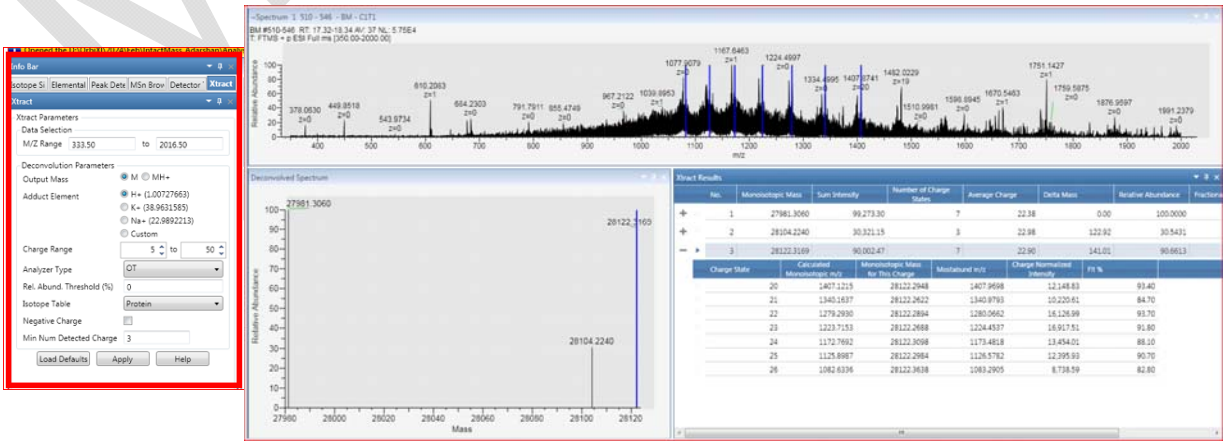
1. For a purified protein a single peak on the chromatographic elution should be observed like in the example above. Multiple MS1 spectrums are recorder by the instrument as the peak eluted from the column.



2. So these MS1 spectrums can be averaged to improve the signal to noise ratio and get an averaged spectrum. To average spectrum right click select **Average Spectrum** and select the chromatographic peak on TIC tab with left click

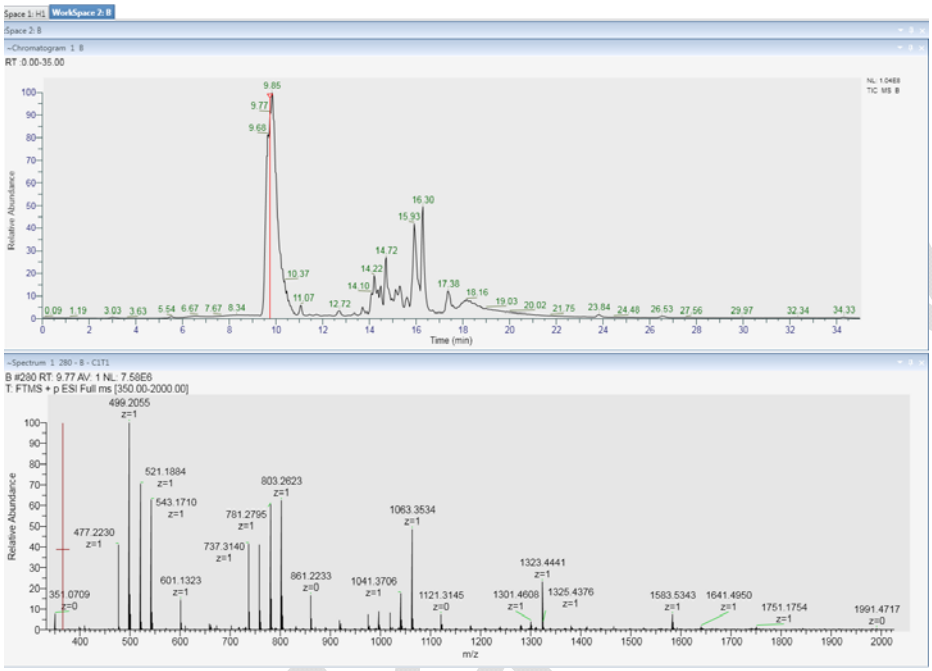


3. **Deconvolution**
   Once the spectrum is averaged select the spectrum tab and change the parameters on the Xtract panel as per requirement and click apply.

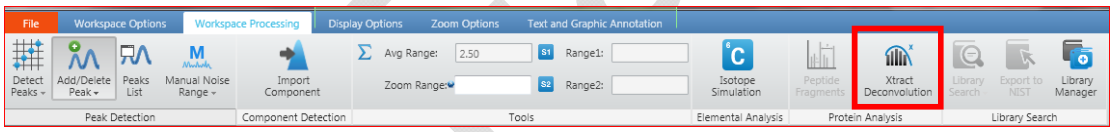


Applying the Xtract Algorithm to a Chromatogram

1. Load the raw file in **FreeStyle 1.8 SP2**
   - Click on the Total Ion chromatogram window to activate it.



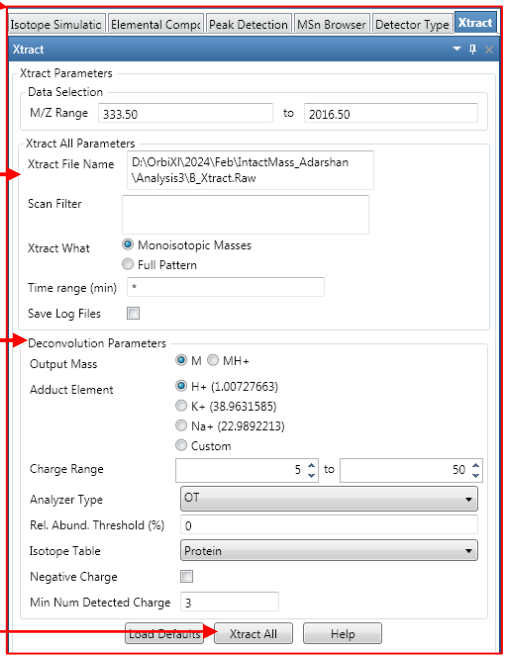
2. Click **Xtract Deconvolution**.

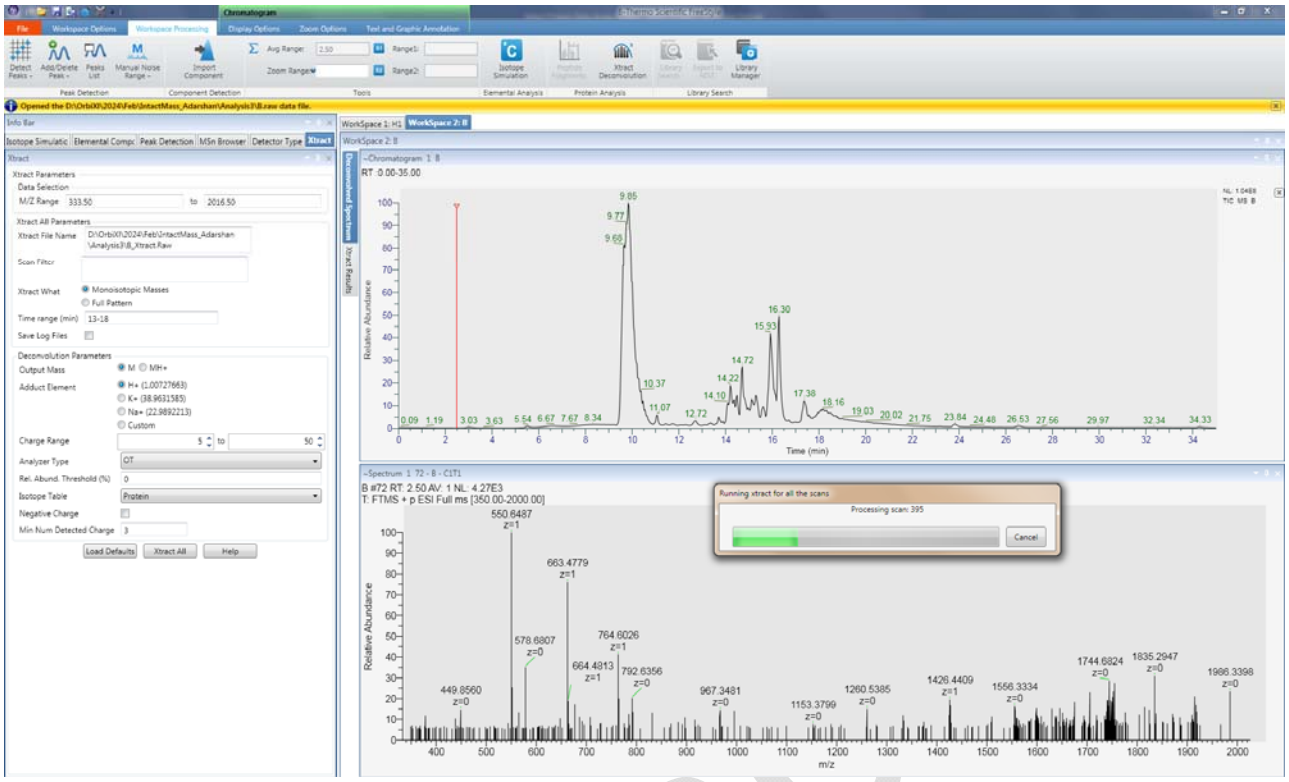


   The application adds the **Xtract** page to the **Info Bar**.

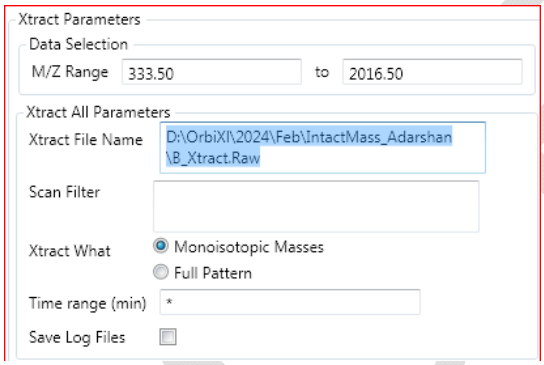
3. Open the **Xtract** page.
   The M/Z Range boxes are populated with the m/z range of the spectrum.

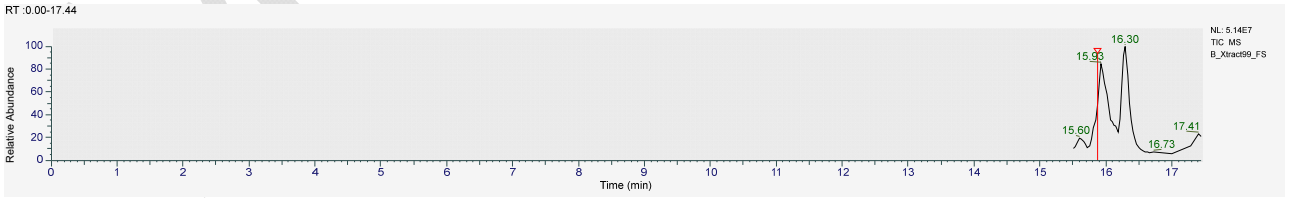
4. When necessary, modify the m/z range and RT by typing the new start and stop values.
5. Review and modify the settings in the Xtract All Parameters area as appropriate.
6. Review and modify the settings in the Deconvolution Parameters area as appropriate.
7. Click Xtract All.
   - The application runs the Xtract algorithm.
   - It can take a few minutes depending on your filters and the amount of data in the specified range.
   - When the deconvolution process completes, the application writes your file to the name and location specified in the Xtract File Name box and records the extraction in the Audit Trail.

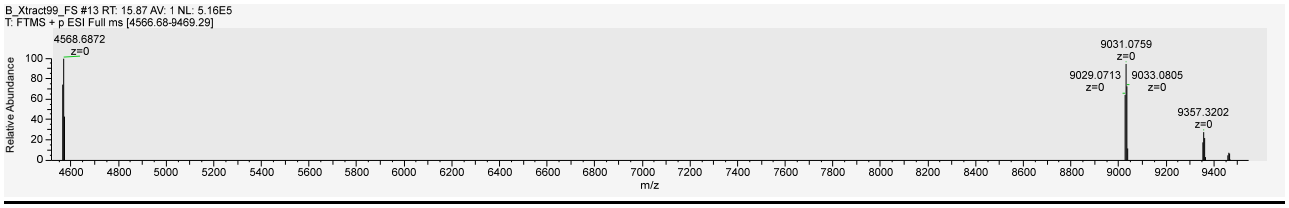


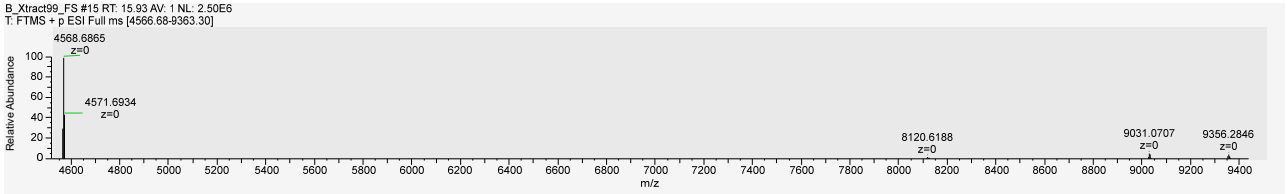


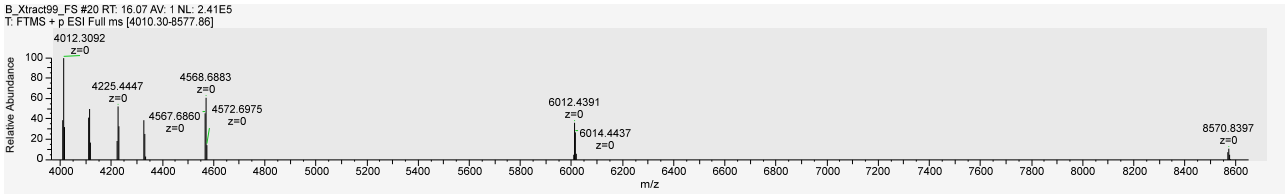
Open new file at the extraction location





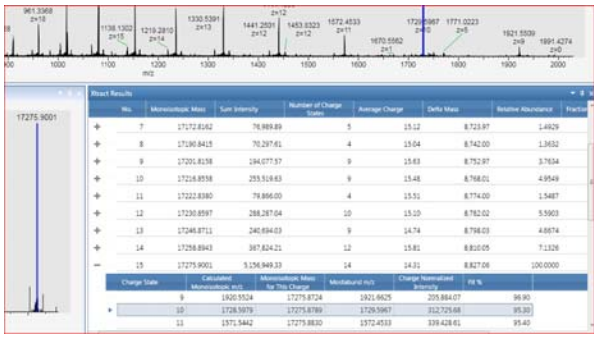


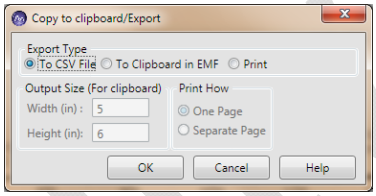

B_Xtract99_FS #15 RT: 15.93 AV: 1 NL: 2.50E6
T: FTMS + p ESI Full ms [4566.68-9363.30]
4568.6865
z=0
4571.6934
z=0
8120.6188
z=0
9031.0707
z=0
9356.2846
z=0
Relative Abundance
m/z


B_Xtract99_FS #20 RT: 16.07 AV: 1 NL: 2.41E5
T: FTMS + p ESI Full ms [4010.30-8577.86]
4012.3092
z=0
4225.4447
z=0
4568.6883
z=0
4567.6860
z=0
4572.6975
z=0
6012.4391
z=0
6014.4437
z=0
8570.8397
z=0
Relative Abundance
m/z

Exporting Results

1. To export the results of the analysis, select **Workspace Options > Export > Export selection As**





	No.	Monoisotopic Mass	Sum Intensity	Number of Charge	Average Charge	Delta Mass	Relative Abundanc	Fractional Abundar	RT Range
2	1	8448.8426	193886.09	8	8.342428508	0	4.25412054	2.200735939	15.923-17.262
3									
4		Charge State	Calculated Monois	Monoisotopic Mass	Mostabund m/z	Charge Normalized	Fit %		
5		5	1690.77599	8448.8334	1691.77661	31428.924	93.8		
6		6	1409.14787	8448.8393	1409.98239	22343.51667	87.7		
7		7	1207.98493	8448.8403	1208.7011	20115.96571	90.8		
8		8	1057.11272	8448.8504	1057.73927	24146.22625	94.7		
9		9	939.76767	8448.8543	940.3267	21407.32111	93.1		
10		10	845.89163	8448.8426	846.39294	28378.685	93.2		
11		11	769.08396	8448.8416	769.53954	36944.60727	95.8		
12		12	705.07757	8448.8437	705.4953	9120.845833	90.2		
13									
14	2	8562.9	151050.55	7	8.159271581	114.0574	3.314251411	1.7145241	15.923-17.262
15									
16		Charge State	Calculated Monois	Monoisotopic Mass	Mostabund m/z	Charge Normalized	Fit %		
17		5	1713.58391	8562.8875	1714.58742	18980.788	84.4		
18		6	1428.15447	8562.8863	1428.99008	32319.96667	94.2		
19		8	1071.36767	8562.8934	1071.99483	29267.105	93.3		
20		9	952.43874	8562.8871	952.99533	25156.84889	95.6		
21		10	857.29559	8562.89	857.79877	21728.744	95		
22		11	779.4512	8562.8898	779.90808	17362.35545	89.1		
23		12	714.58087	8562.8868	714.99915	6234.738333	88.7		
24									
25	3	8845.0665	433328.21	9	8.802919273	396.2239	9.507801405	4.918563086	15.923-17.262
26									

Report contains Calculated Monoisotopic mass, Intensity, Number of charge states detected, Average charge, Relative abundance, fractional abundance and RT where the peak is identified.

2. To copy deconvoluted spectrum zoom in to the mass range of interest, right click and copy