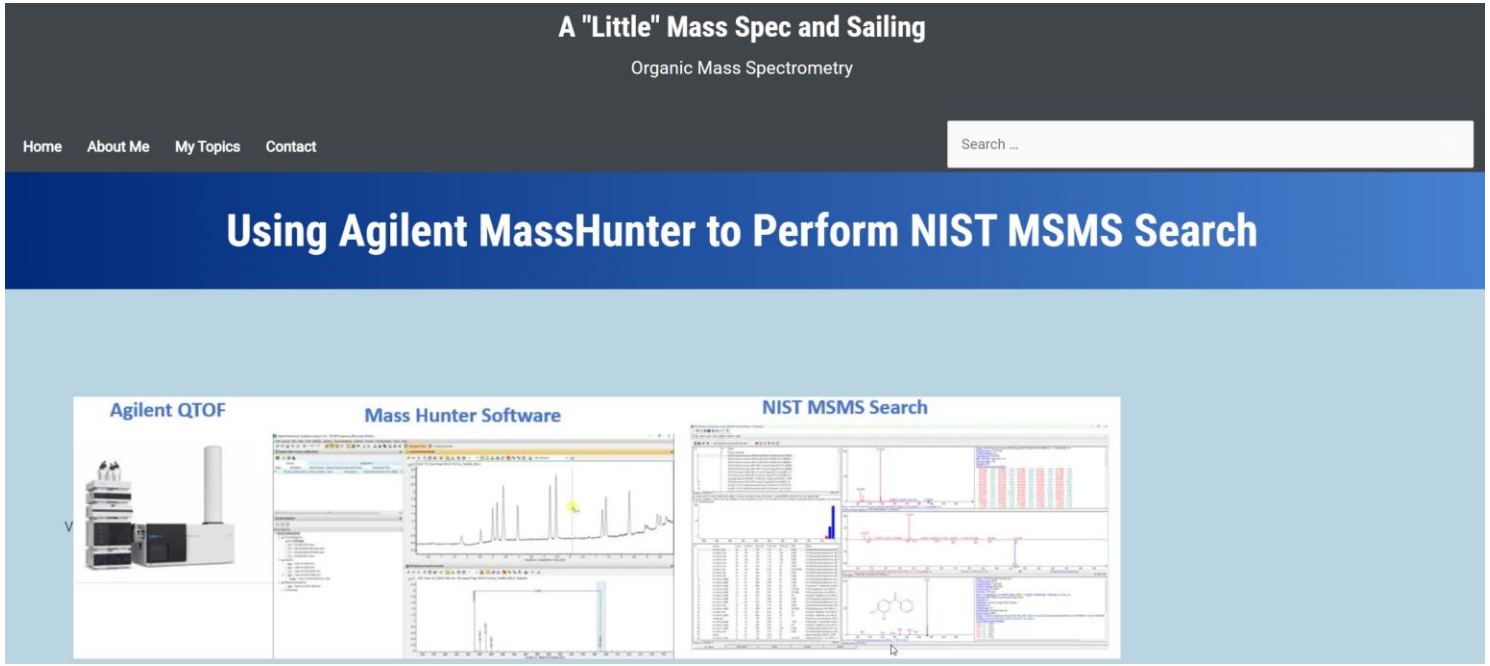
Additional Tips for Processing MSMS Data Files in Agilent MassHunter for NIST MSMS Search



James Little
Mass Spec Interpretation Services
Nov. 27, 2024

https://littlemsandsailing.com/2024/10/using-agilent-masshunter-to-perform-nist-msms-search/
https://littlemsandsailing.com/

Additional Tips Summary

- Quick overview of basics repeated plus types of searches
- Importance of manually entering precursor ion if not imported (***occasional problem***)
- Extracting and processing different collision energies
- Walking chromatogram or what some call the “Walking Man”
- Expanding chromatograms or spectra

Additional Useful Links:

- *NIST Library Setup*, critical to setup correctly and then save settings
- *Types of MSMS Searches*, a part of the library setup document
- *Hybrid Searches*, table linking delta mass values in search results to structure and training material
- *Detailed Training NIST MSMS Search*

Overview of Process

- This is ***only*** an overview of the MassHunter software
- For Details, see *more complete* training on the internet
- Also, learn more in depth the use of NIST MS Search software

MassHunter YouTube Training Videos

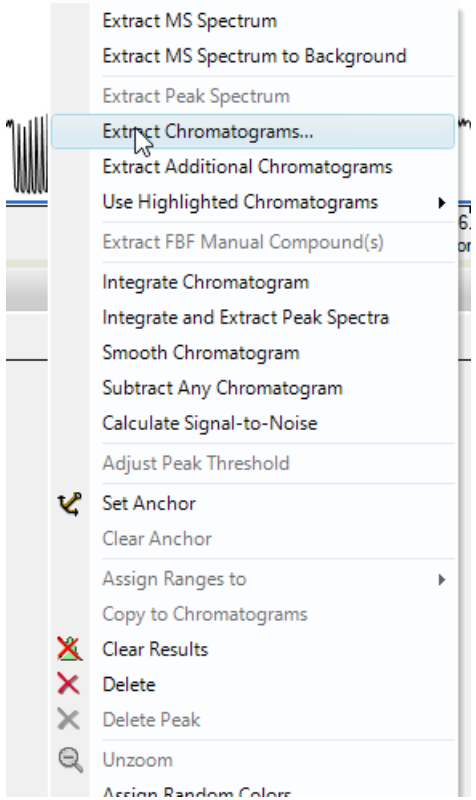
MassHunter Qualitative Analysis Training Video EP01-Basic Navigation
MassHunter Qualitative Analysis Training Video EP02-Extracting Ion Chromatograms
MassHunter Qualitative Analysis Training Video EP03-Other Miscellaneous Tips

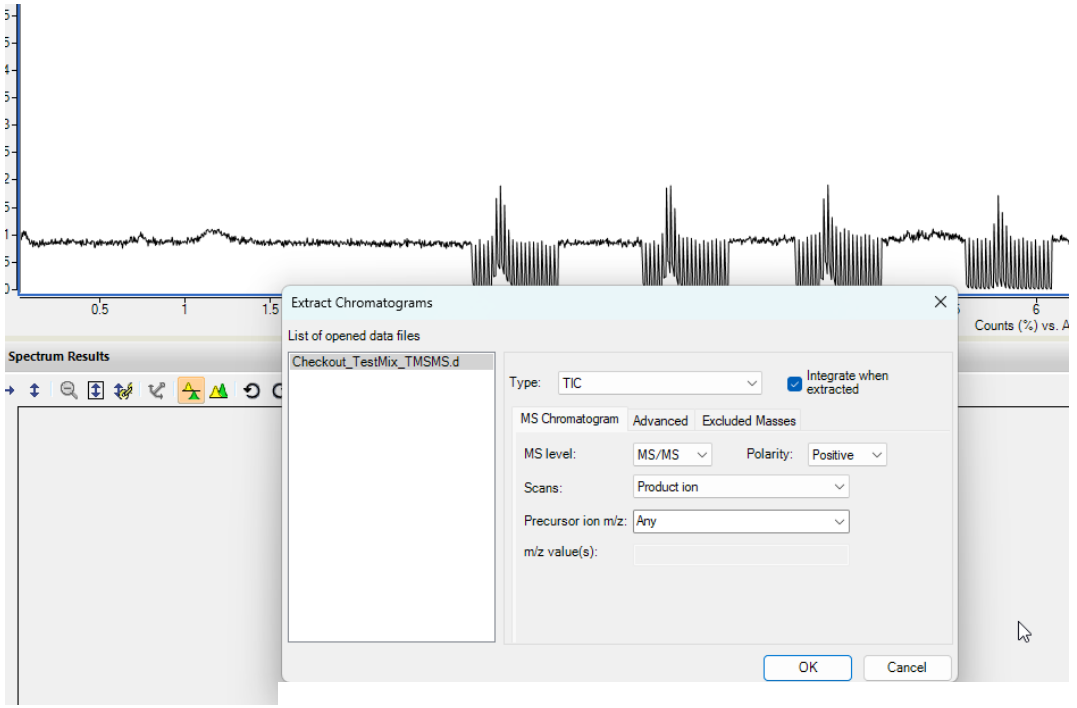
NIST MSMS Full Course

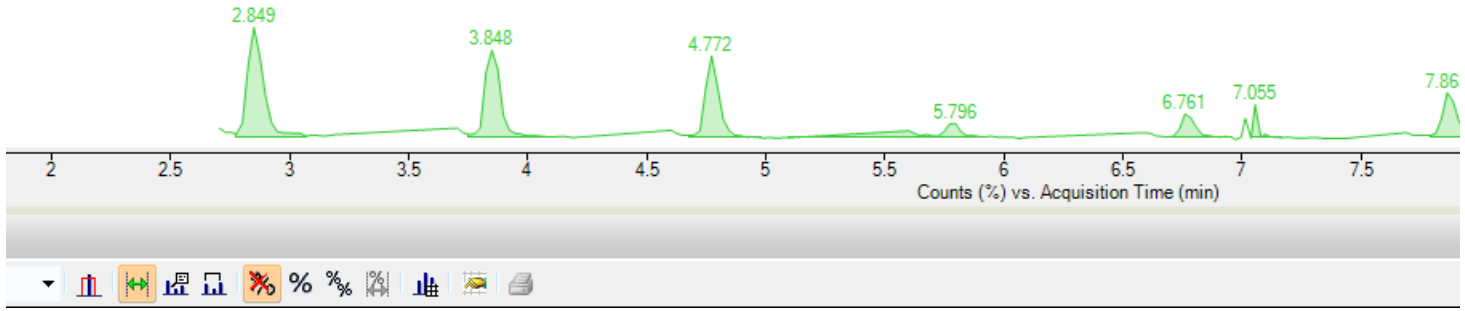
https://littlemsandsailing.com/2020/12/lcms-unknown-identification-with-nist-search-using-msms-libraries/

Extracting MSMS Trace

- ***Right click*** on total ion chromatogram first opened
- Select Extract Mass Spectrum with MS Level of MS/MS

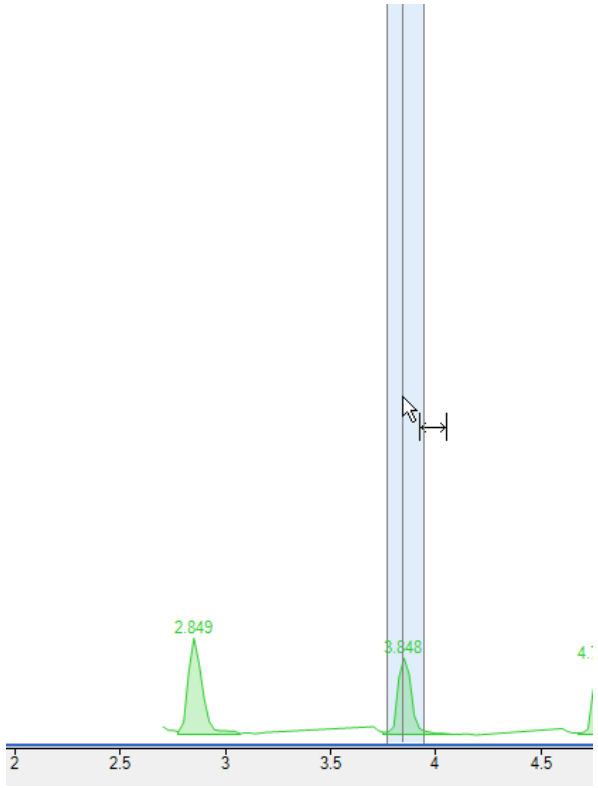


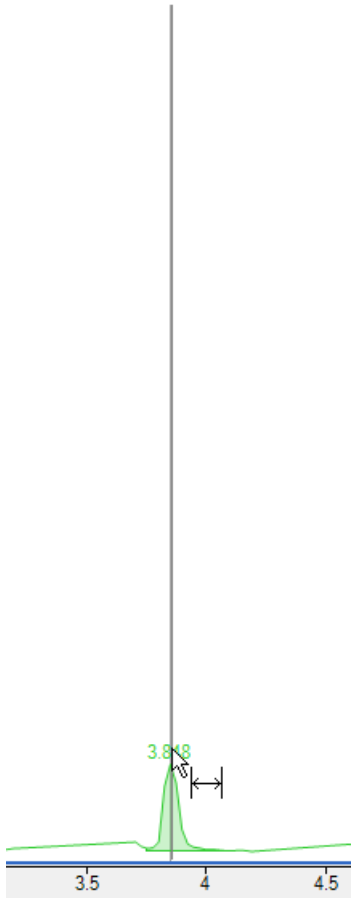


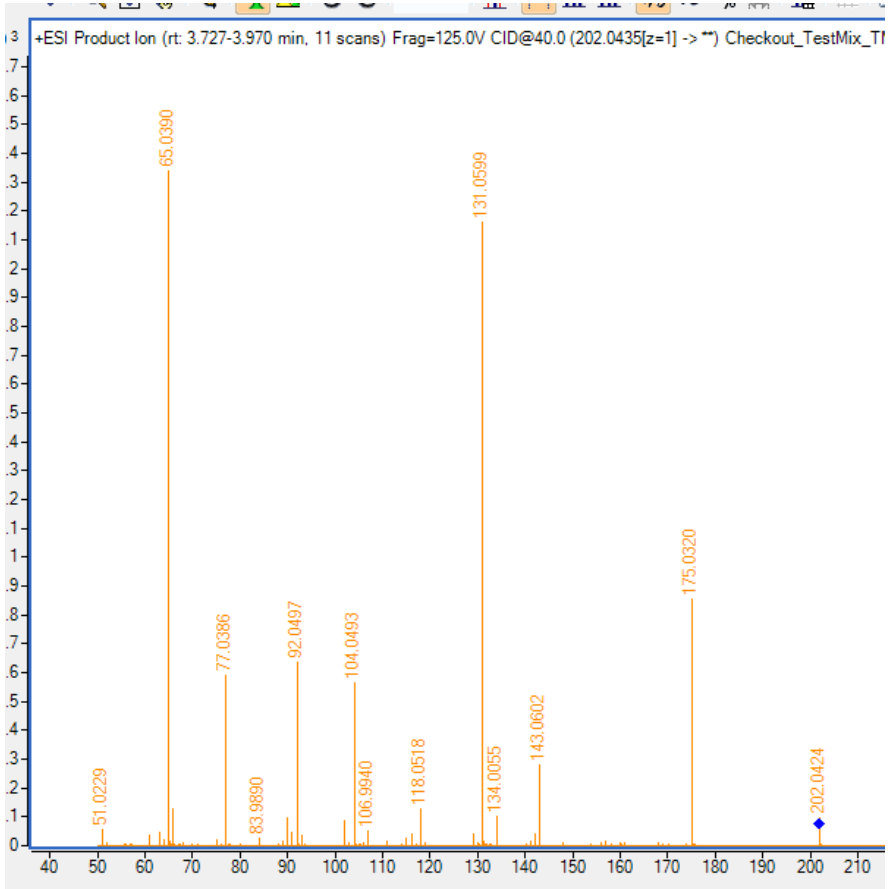


Obtaining MSMS Spectrum

- Left click and drag to get a group of spectra to average, then ***double left click*** in the blue box to obtain spectrum in extract MSMS chromatogram
- For single spectrum, just ***double left click*** on peak of interest in extracted MSMS chromatogram

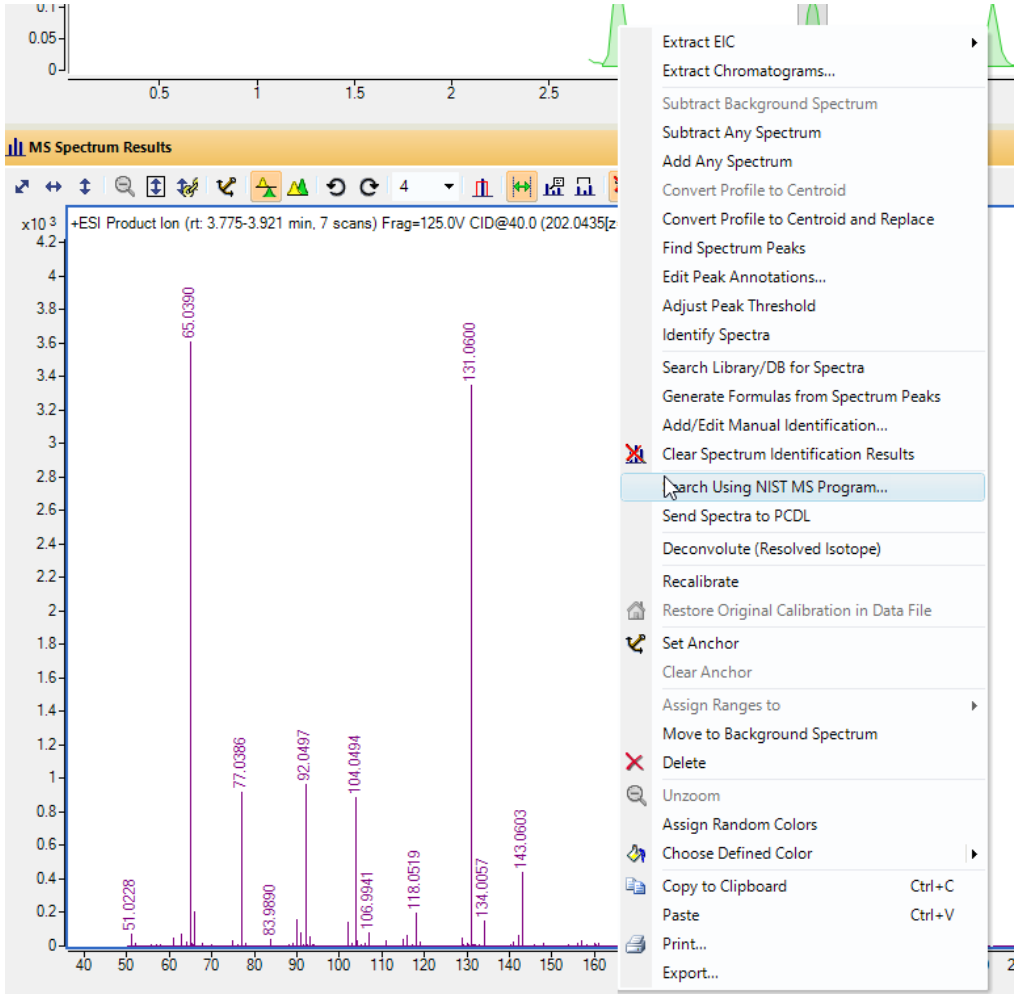


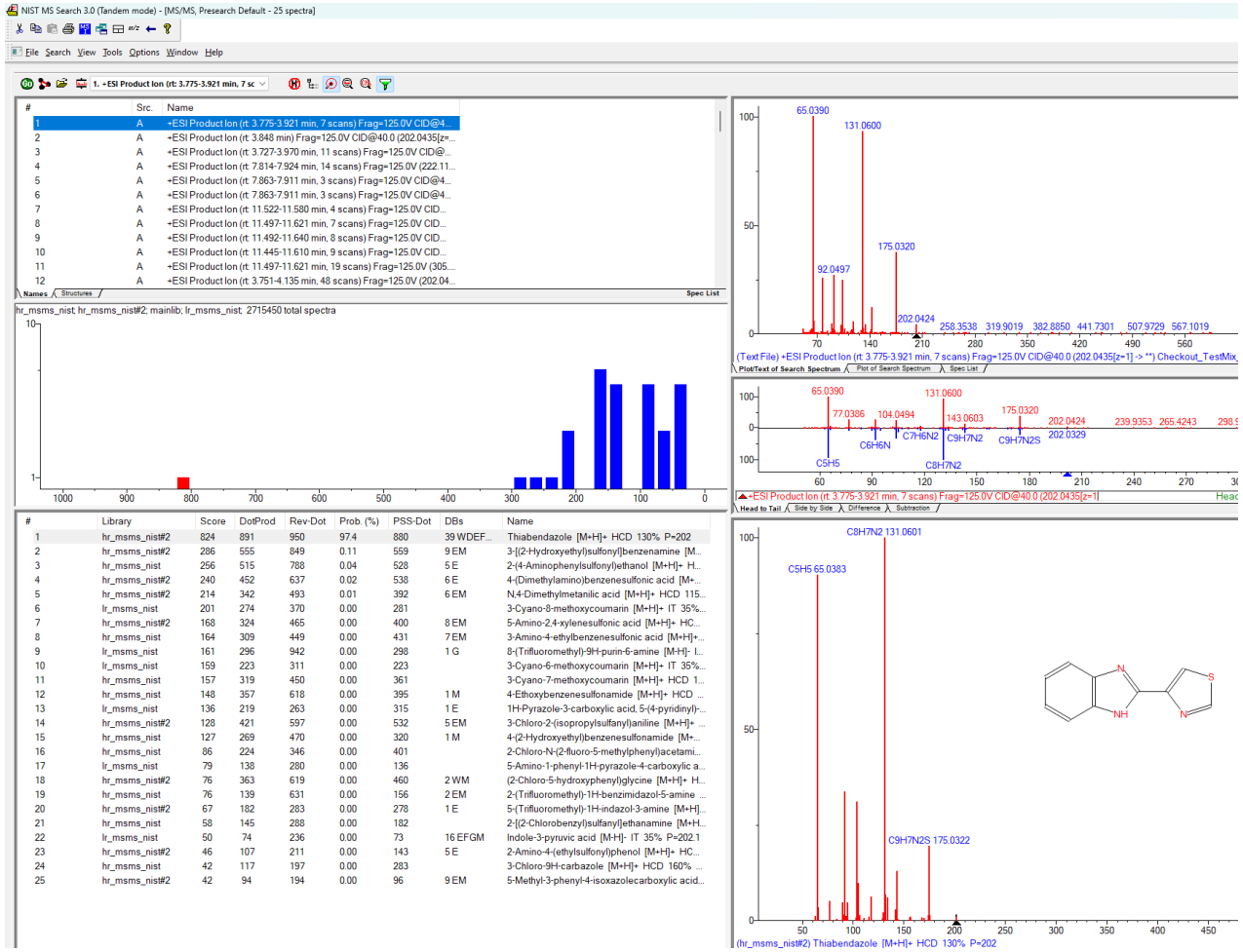




Sending MSMS Spectrum to NIST Search

- ***Right click*** within MSMS spectrum and select Search Using NIST MS Program option

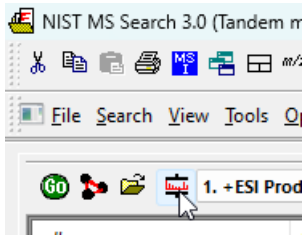


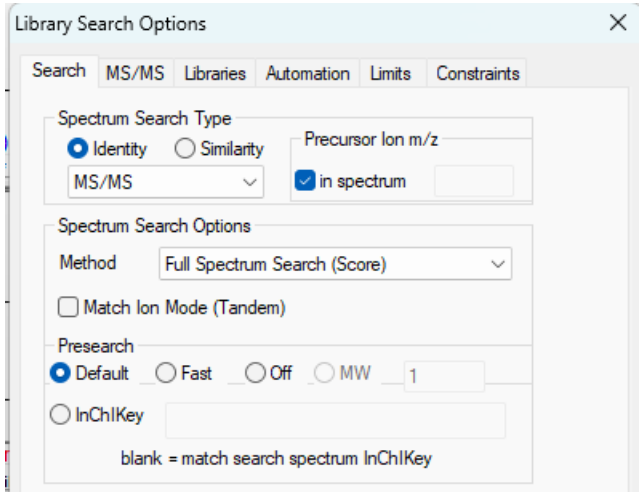


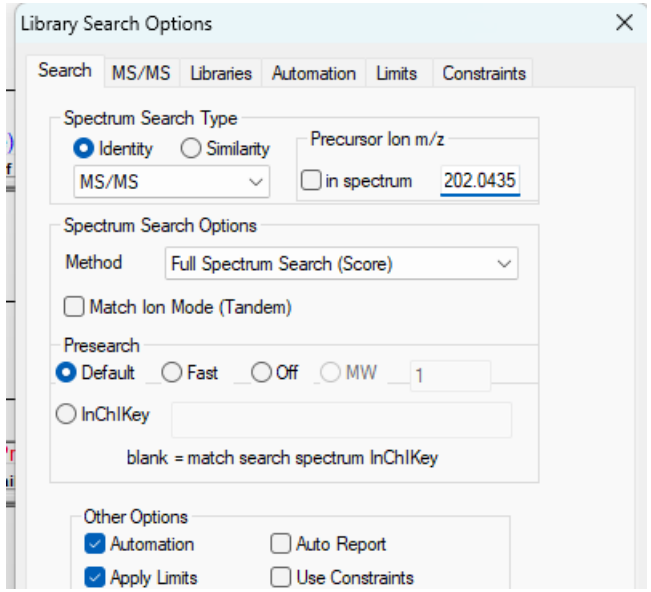
Occasional Problem with MSMS Precursor Ion *Not Exported* to NIST Search from MassHunter

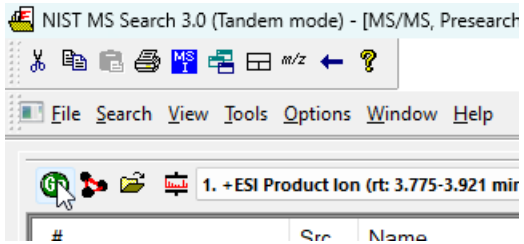
- Normally, the Precursor m/z will be exported to NIST search
- Very important for MS/MS Search which is limited by this value
- If not exported, must enter manually
- Click on MS search options icon
- Unclick the in spectrum option
- Enter value, then perform search again with Go Icon

Name: +ESI Product Ion (rt: 3.775-3.921 min, 7 scans) Frag=125.0V CID@40.0 (202.0435[z=1] -> **) Checkout_TestMix_TMSMS.d
Instrument type: Q-TOF MS
Collision energy: 40 V
Precursor m/z: 202.0435
MW: N/A ID#: 97 DB: Text File
Spectrum type: ms2
Ionization: ESI



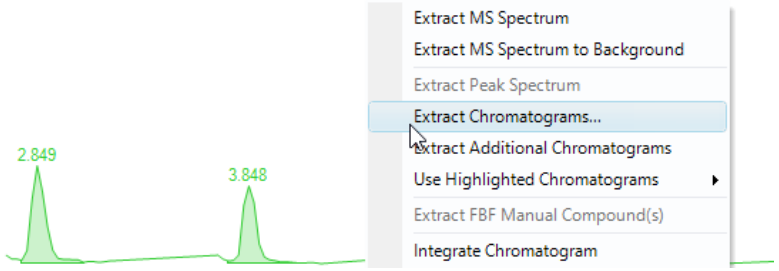


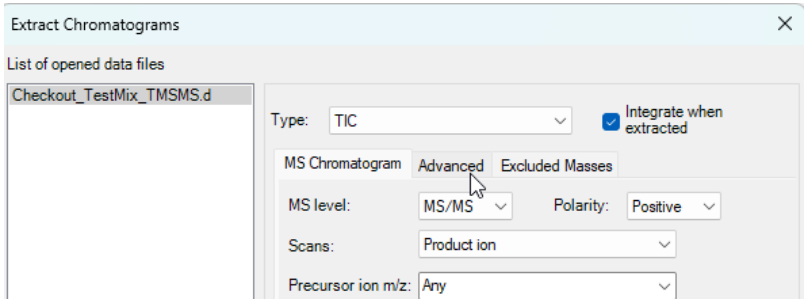


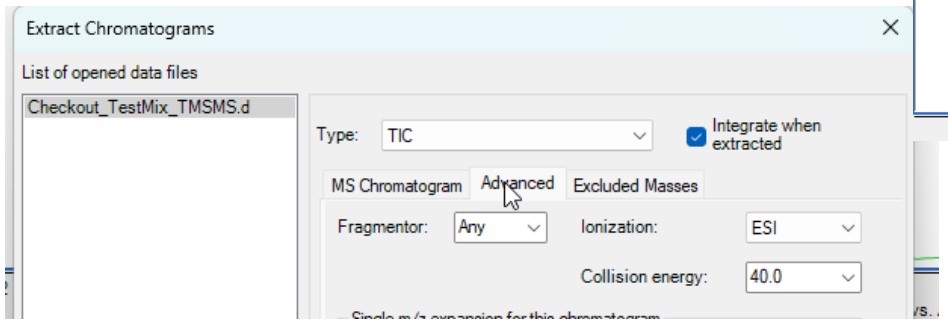


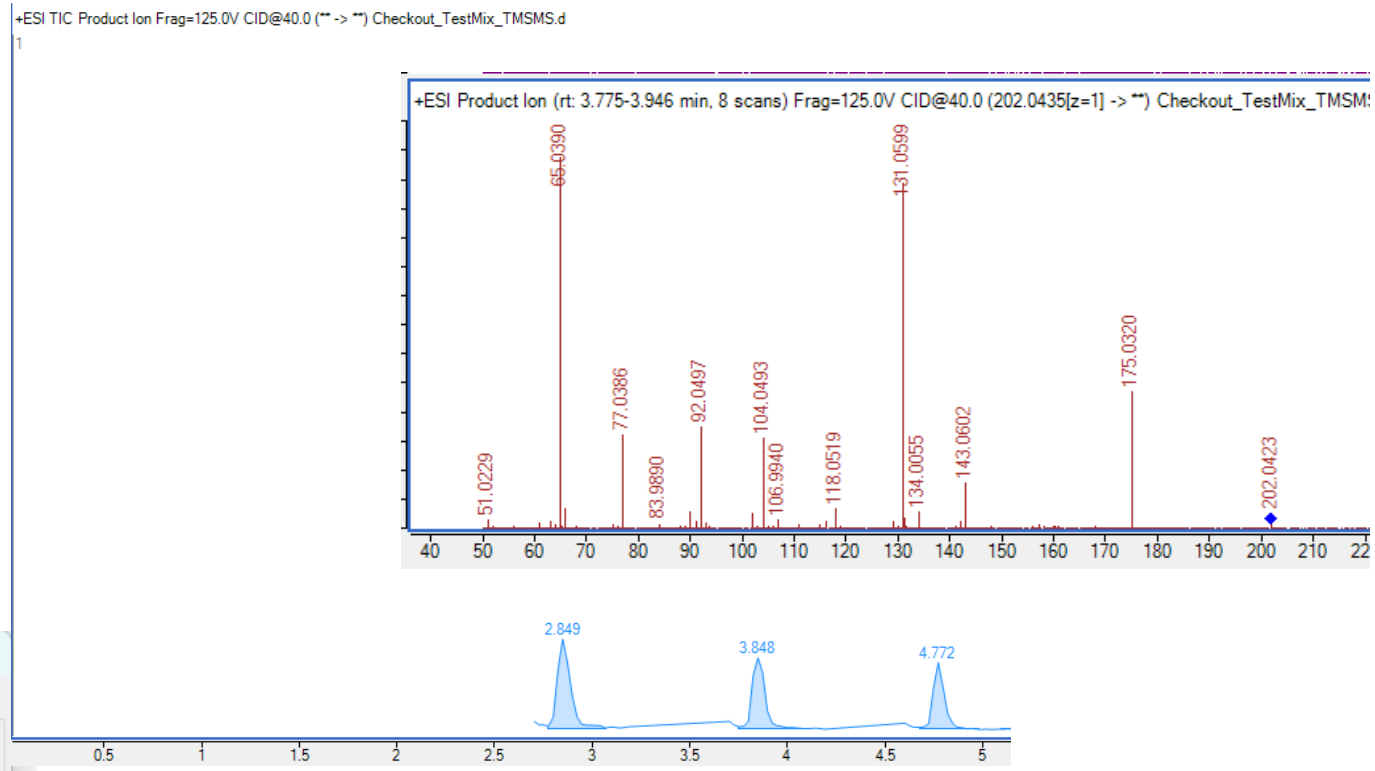
Obtaining Spectra at Specified Collision Energy

- When averaging a group of spectrum from extracted MSMS chromatogram
- One gets the average energy spectrum
- Not necessarily a bad approach
- To get specified energy, ***right click*** on chromatogram box and select Extract Chromatograms
- ***Left click*** on advanced tab and select ESI (if not doing APCI) and select collision energy from choices
- Another chromatogram will be part of the top window
- Just use scroll on mouse to step between the chromatograms displayed
- Can get the MSMS spectrum using the standard approach



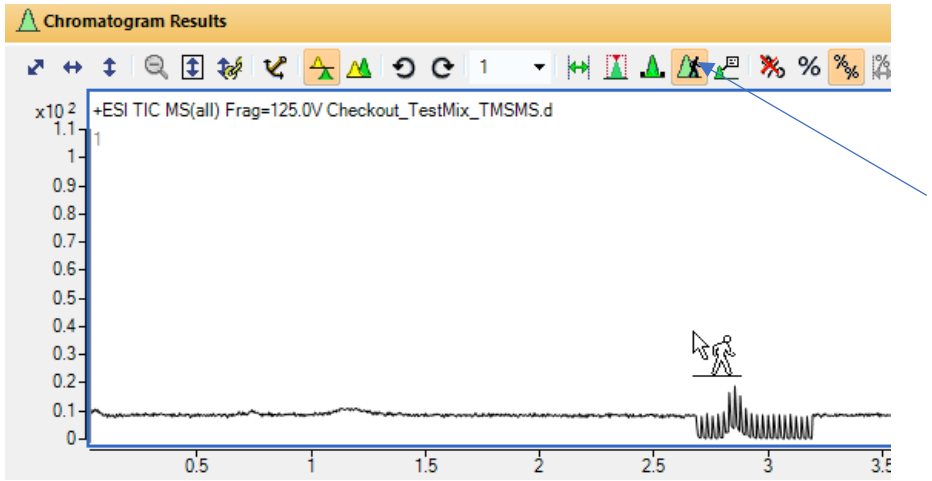


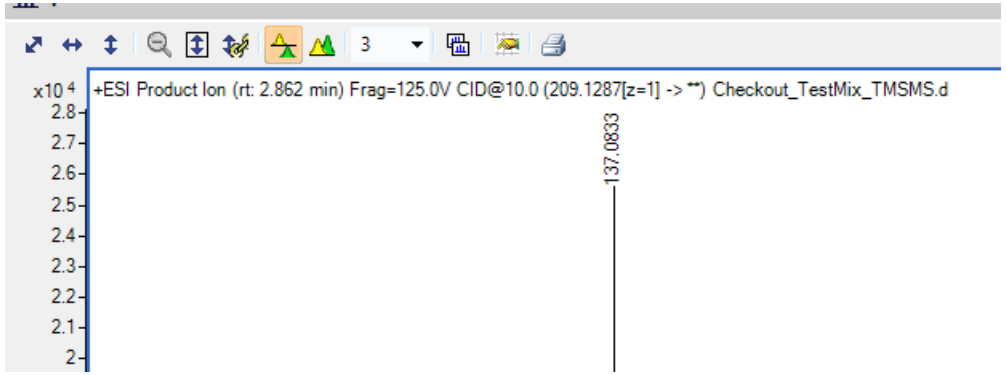


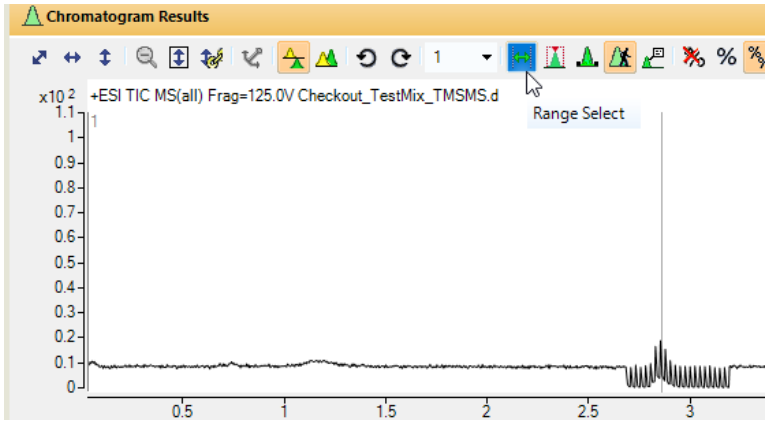


Walking Chromatogram or What Some Call the “Walking Man”

- Left click on the Walking Chromatogram Icon
- Walking icon will appear
- Left click on wherever you want to see spectrum
- Use left and right arrows on keyboard to step through the spectra one by one
- Header on displayed spectrum will tell you the details in a separate spectrum window
- To get out of this mode, hit “range select” icon







Expanding Chromatograms or Spectra

- Just Right click and drag to form a box
- Then autoscale to return to full scale plot