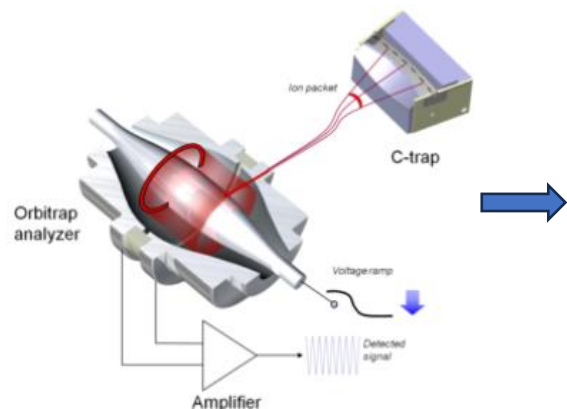


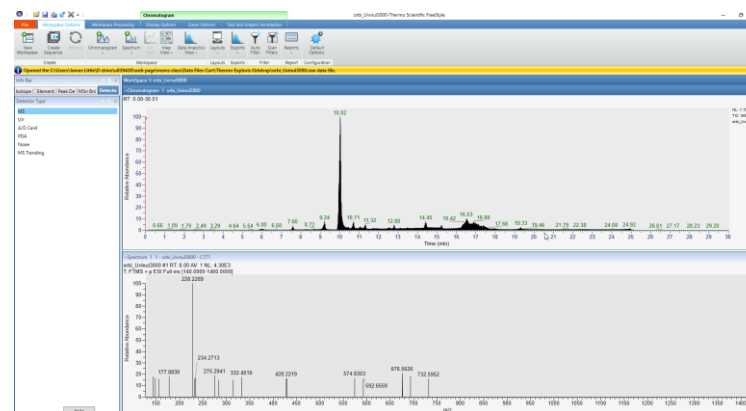
Data Processing Using Thermo Freestyle Software with NIST MSMS Search for Compound Identifications (Alternative Less Efficient Approaches)

James Little
Mass Spec Interpretation Services
<https://littlesandsailing.com/>
11/06/2025

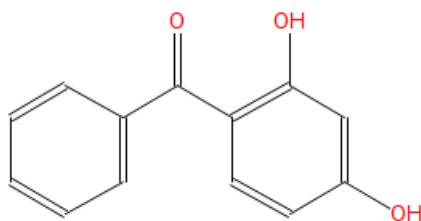
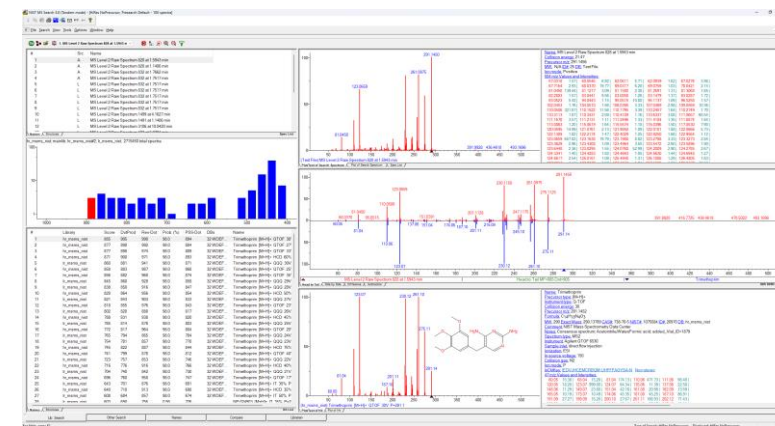
Thermo Orbitrap MSMS



Thermo Freestyle



NIST MSMS Search



Uvinul 3000
MW 214
Thermo Orbitrap
LC-MS and Non-Targeted MSMS Analyses (Positive and Negative Modes)
with
Photo Array Detector (PDA) and UV-VIS Analyses

In My Opinion, Preferred Way of Processing Data *(shared with me by colleague)*

- However, several other ways for processing data in Freestyle
- Some might think simpler, but after initially saving the layout in the “preferred approach,” really no advantage in my opinion to these simpler approaches

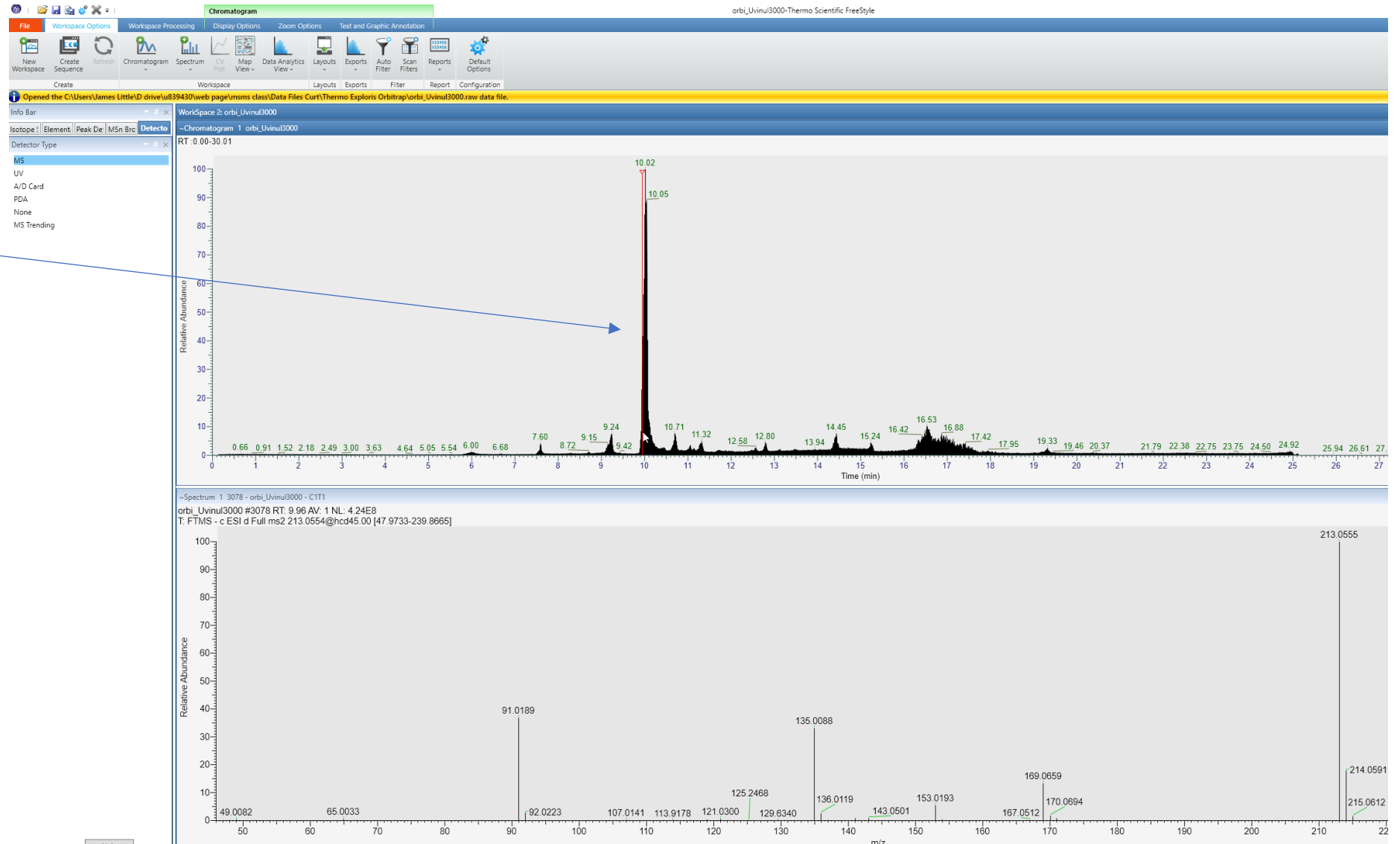
Windows Arrow Key Approach Using Total Ion Chromatogram, **NOT** Parsed into Separate Ones

After opening the file, the total ion chromatogram is displayed, just left click on the front of the peak, note red line showing the location of selected peak

Then just “walk” through the peak using the left and right arrows on keyboard

The Header on the spectrum in bottom box will tell you the polarity and if a MS1 or MS2 peak is being displayed

The MS2 spectrum of interest can be sent to NIST MS search



Manually Opening Windows

Opened the C:\Users\James Little\D drive\u8:

Info Bar

Isotope| Elemer| Peak D| MSn B| Detect| Scan F

Scan Filters

Filter Parameters

Raw File Name : orbi_Uvinul3000

Time range (min): * ☐ Track

FTMS + c ESI d Full ms2 371.3158@hcd45.0C
FTMS + c ESI d Full ms2 376.2595@hcd45.0C
FTMS + c ESI d Full ms2 377.3264@hcd45.0C
FTMS + c ESI d Full ms2 388.2539@hcd45.0C
FTMS + c ESI d Full ms2 390.2982@hcd45.0C
FTMS + c ESI d Full ms2 391.2846@hcd45.0C
FTMS + c ESI d Full ms2 392.3135@hcd45.0C
FTMS + c ESI d Full ms2 393.2975@hcd45.0C
FTMS + c ESI d Full ms2 394.3295@hcd45.0C
FTMS + c ESI d Full ms2 399.2505@hcd45.0C
FTMS + c ESI d Full ms2 414.3582@hcd45.0C
FTMS + c ESI d Full ms2 420.3322@hcd45.0C
FTMS + c ESI d Full ms2 424.3634@hcd45.0C
FTMS + c ESI d Full ms2 425.1021@hcd45.0C
FTMS + c ESI d Full ms2 425.3049@hcd45.0C
FTMS + c ESI d Full ms2 427.1176@hcd45.0C
FTMS + c ESI d Full ms2 432.2805@hcd45.0C
FTMS + c ESI d Full ms2 437.1936@hcd45.0C
FTMS + c ESI d Full ms2 438.3789@hcd45.0C
FTMS + c ESI d Full ms2 447.0840@hcd45.0C
FTMS + c ESI d Full ms2 449.0999@hcd45.0C
FTMS + c ESI d Full ms2 453.1734@hcd45.0C
FTMS + c ESI d Full ms2 468.3898@hcd45.0C
FTMS + c ESI d Full ms2 473.2647@hcd45.0C
FTMS + c ESI d Full ms2 476.3064@hcd45.0C
FTMS + c ESI d Full ms2 482.4052@hcd45.0C
FTMS + c ESI d Full ms2 493.3480@hcd45.0C
FTMS + c ESI d Full ms2 512.4155@hcd45.0C
FTMS + c ESI d Full ms2 520.3325@hcd45.0C
FTMS + c ESI d Full ms2 556.4418@hcd45.0C
FTMS + c ESI d Full ms2 570.4573@hcd45.0C
FTMS + c ESI d Full ms2 600.4683@hcd45.0C
FTMS + c ESI d Full ms2 608.3858@hcd45.0C
FTMS + c ESI d Full ms2 614.4838@hcd45.0C
FTMS + c ESI d Full ms2 628.1943@hcd45.0C
FTMS + c ESI d Full ms2 639.1651@hcd45.0C
FTMS + c ESI d Full ms2 644.4946@hcd45.0C
FTMS + c ESI d Full ms2 654.3314@hcd45.0C
FTMS + c ESI d Full ms2 658.5099@hcd45.0C
FTMS + c ESI d Full ms2 659.1310@hcd45.0C
FTMS + c ESI d Full ms2 663.4533@hcd45.0C
FTMS + c ESI d Full ms2 702.2131@hcd45.0C
FTMS + c ESI d Full ms2 702.5370@hcd45.0C
FTMS + c ESI d Full ms2 771.6456@hcd45.0C
FTMS + c ESI d Full ms2 776.2321@hcd45.0C
FTMS + c ESI d Full ms2 902.3948@hcd45.0C
FTMS - p ESI Full ms [140.0000-1400.0000]
FTMS + p ESI Full ms [140.0000-1400.0000]

Export Print Help

Workspace Options | Workspace Processing | Display Options | Zoom Options | Text and Graphic Annotation

Create Sequence Refresh Chromatogram Spectrum CV Plot Map View Data Analytics View Layouts Exports Auto Filter Scan Filters Reports Default Options

Create Workspace Layouts Exports Filter Report Configuration

Opened the C:\Users\James Little\D drive\u839430\web page\msms class\Data Files Curt\Thermo Exploris Orbit

Workspace 2: orbi_Uvinul3000

~Chromatogram 1 orbi_Uvinul3000

RT : 0.00-30.01

Scan Filters

Open the Scan Filters page in the Info Bar to display filtered chromatograms.

1) Click on the Scan Filters Icon in Workspace Processing Tab

2) That will open the list of ions, the positive and negative ion MS1 choices are at the **bottom** of the list

3) If you want to get another trace, for example, the PDA, click on Insert trace

4) The PDA or UV trace is found in the "Detector" Tab

Chromatogram

File | Workspace Options | Workspace Processing | Display Options

New Workspace Create Sequence Refresh Chromatogram Spectrum CV Plot Map View

Create

Opened the C:\Users\James Little\D drive\u839430\web page\msms class\Data Files Curt\Thermo Exploris Orbit

Info Bar

Isotope| Elemer| Peak D| MSn B| Detect| Scan F

Scan Filters

Filter Parameters

Raw File Name : orbi_Uvinul3000

Ranges

Insert Trace

Insert View

Insert Chromatogram Trace

Add another Chromatogram trace within the selected Chromatogram view.

Opened the C:\Users\James Little\D drive\u8:

Info Bar

Isotope| Elemer| Peak D| MSn B| Detect| Scan F

Detector Type

MS
UV
A/D Card
PDA
None
MS Trending