Mass Spec Aimbot version 1.0

Basic functionality

Mass Spec Aimbot is a utility designed to help visualize ions and their isotopologues across the contents of one or more MS file. With it you can specify a base mass, possible labels, and possible adducts and the program will automatically calculate what m/z values to search for, even for MS2 data. You can also use the application to deep dive into the data, visualizing spectra at specific scans or even composite spectra over a RT window.

Loading files

Supported formats

- netCDF
- mzML
- mzXML

Process

From the initial screen look at the 'loaded files' dialog in the top-left corner and click "New". Select the file you wish to visualize, or multiple files at once by using ctrl or shift, and click "Open" to begin loading the data in the background. The files will initially be displayed in the dialog with grey text, but once they have been loaded into memory the text will change to black. Click on any loaded file to display its TIC data in the top-right dialog, or if it hasn't loaded yet the program will swap priority to that file and display as soon as it loads.

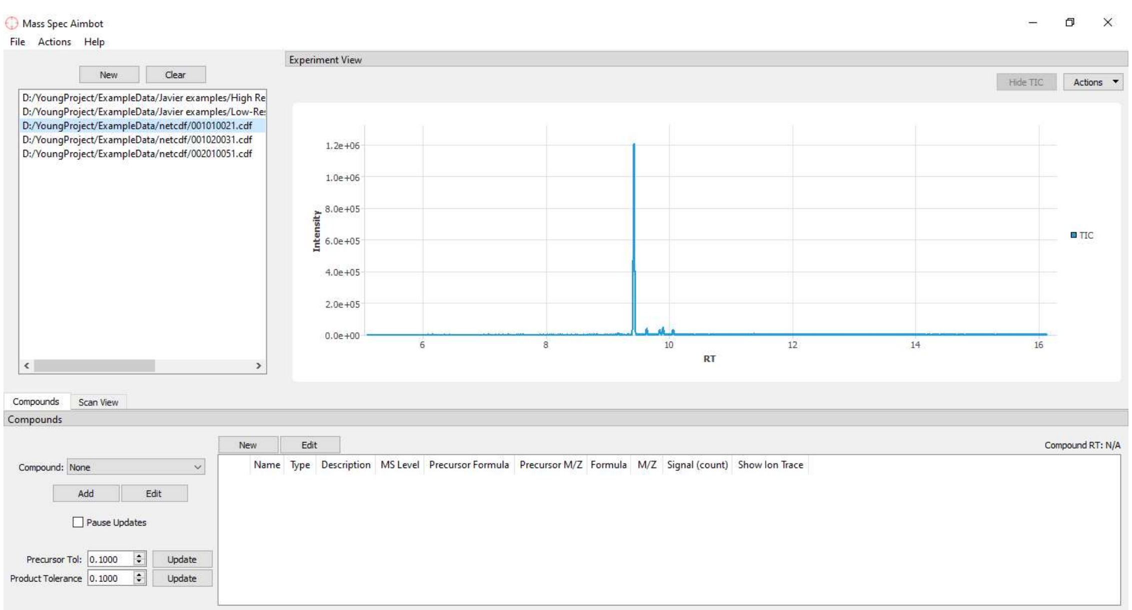

Figure 1: Mass Spec Aimbot with various files loaded

Targeted analysis

Manual targeting

Usually when looking at a file there is a particular RT window and ion of interest. You can track this in Mass Spec Aimbot by specifying a Compound, which the program understands as an ion or group of ions that can all be found around the same time point. Look at the bottom dialog and ensure that the "Compound" tab is selected. On the left side click "Add" to begin the process of defining your compound of interest.

A pop up window will appear asking for what name to use and approximately where in the RT range to search. You can also load from an external file at this screen, which will be covered in the next section.

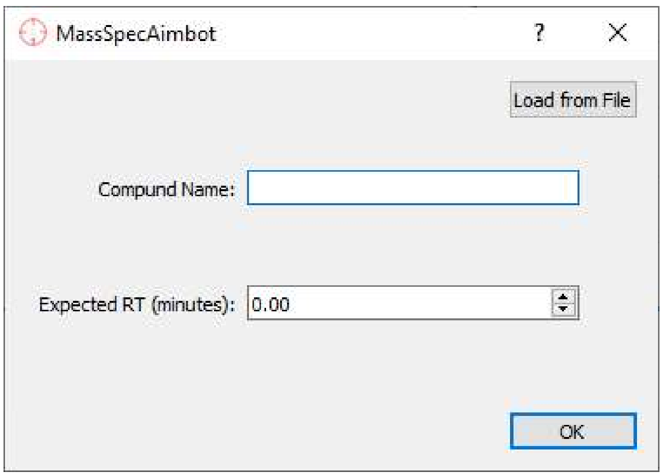

Figure 2: Defining a Compound name and RT

Since the compound will initially have no ions the program will go ahead and show the "Add/Edit Ion" window. From here specify what MS level you are using, which will in turn toggle whether the dialog requests information about the parent ion as well. Enter the ion formulae in the proper boxes, or if you don't know the formula off hand (as might happen if you are investigating an unknown peak) you can also use the m/z.

The "Isotope Labels" box is pre-filled with entries for 13C, 15N, and 2H, but each row can be edited to fit whatever chemical element and substitution mass you need. When working with MS2 data the program will automatically ensure that the maximum possible substitutions of the product ion is not greater than the parent ion, and when producing those permutations in the next step will ensure that the resulting isotopologues can exist.

On the right side there is a list of adducts, which can be changed from positive mode adducts to negative mode adducts by clicking the radio button above the list. Check all rows with adducts you would like to keep an eye out for, and check the “In Product” as well if you are using MS2 and expect to see the adduct on the product ion.

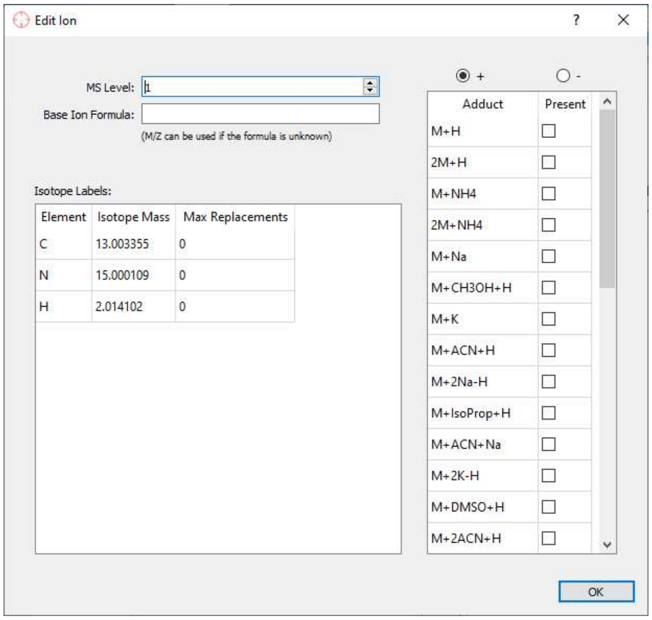

Figure 3: The Add/Edit Ion window

Loading from file

When adding a compound you can also load settings from outside sources. The first, and simplest, version of this is to start typing in the name of a compound you have defined in Mass Spec Aimbot in the past. The program will offer suggestions of what names it has saved in cache, and if you select one and tab out of the input box or click OK it will ask if you want to load the compound. Click “Yes” to skip the ion dialog and load the compound into the program or click “No” to ignore the old cache and create a new compound with that name to replace it.

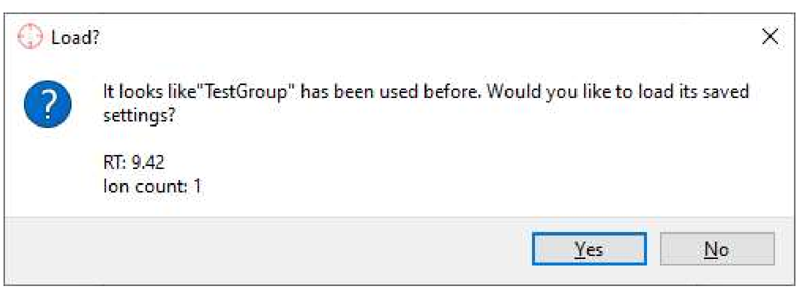

Figure 4: Loading from cache

Alternatively you can use the “Load from File” button to load a compound from a MatLab methods file, like you would use for int_GCMS or int_LCMS. Mass Spec Aimbot also has its own file format (.msabCGF) which can be imported in the same way. Once a file has been selected from the load dialog the prompt will close and the compounds will immediately be added to the list.

Navigating ‘Compound’ panel

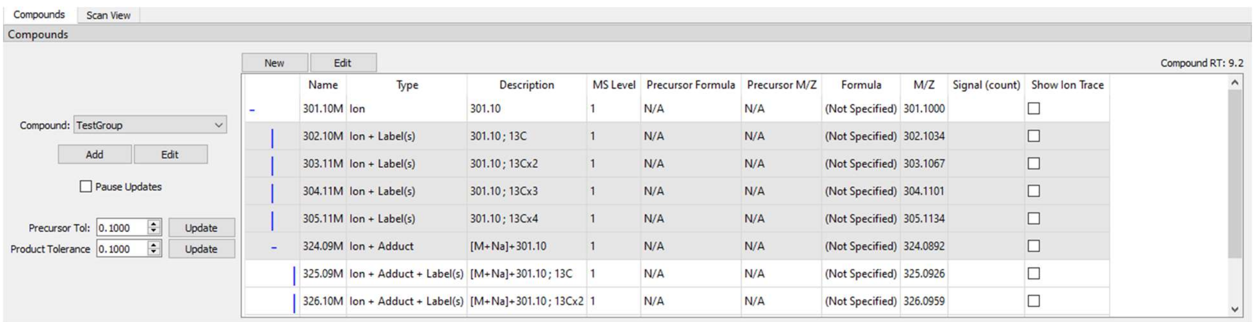

Figure 5: The Compound panel

Now that we’ve gone over the basic functions on how to add new compounds it’s time to look at the bottom dialog in detail. The top left corner that we’ve used so far contains a drop down list that allow you to select which compound you wish to work with, with the retention time displayed as a label in the top right corner.

The bottom left corner has two spin boxes for m/z tolerance, though when working with MS1 data the precursor tolerance box is visible but serves no function. The up/down buttons will increment the number along the most common tolerances- so from 0.5 -> 0.1 -> 0.05 -> 0.01 -> etc. At this time PPM is not supported, but that may change in the future. Once the desired tolerance is reached click the “Update” button, which will be shown in green if there are any changes to be made, to update the TIC panel and recalculate the integration export information.

The large table taking up most of the dialog is devoted to the ions and permutations on those ions. The buttons on top allow you to edit the list of base ions, with the ability to delete secondary base ions located under the edit dialog.

The leftmost column in the table allows isotopologues to be navigated in tree form. If a ‘+’ is visible on an ion that means you can click on the ‘+’ to expand the possible permutations, such as adding on a labeled atom or an adduct. If a ‘-‘ is visible you can click on that to close that tree branch. A ‘|’ indicates that no more permutations are possible from that branch given the constraints of the base ion. Whitespace is added before the symbols to indicate how deep into the tree the row is to help with organization.

The rightmost column contains a checkbox to indicate if you want to try to display the row as an ion trace in the TIC panel. This usage ignores the suggested RT and instead displays any points where the ion can be found along with its intensity.

Integration export

Once one or more compounds have been established Mass Spec Aimbot will begin compiling an integration export in the background. This takes into account the peak area around the RT point specified by the compound and shows the relative intensities of the isotopologues within that peak. To export the integration click on “Actions->Integrate…” in the menu bar and specify a location to output to.

File	Compound	Row Names	Row Descriptions	Expected RT	Base Ion	Peak RT Range	Iso custom name	Parent MZ Range	MZ Range	Absolute Intensity	Ion Fraction
001010021.cdf	TestGroup	301.10M	301.1	9.4	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.8417
001010021.cdf	TestGroup	302.10M	301.10 ; 13C	9.4	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.1336
001010021.cdf	TestGroup	303.11M	301.10 ; 13Cx2	9.4	?	9.386 - 9.448	303.11M	N/A	303 - 303.2	1.05E+06	0.02218
001010021.cdf	TestGroup	304.11M	301.10 ; 13Cx3	9.4	?	9.386 - 9.448	304.11M	N/A	304 - 304.2	1.13E+05	0.002392
001010021.cdf	TestGroup	305.11M	301.10 ; 13Cx4	9.4	?	9.386 - 9.448	305.11M	N/A	305 - 305.2	5159	0.0001094
001010021.cdf	TestGroup	306.12M	301.10 ; 13Cx5	9.4	?	9.386 - 9.448	306.12M	N/A	306 - 306.2	0	0
001010021.cdf	TestGroup	324.09M	[M+Na]+301.10	9.4	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
001010021.cdf	TestGroup	325.09M	[M+Na]+301.10 ; 13C	9.4	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
001010021.cdf	TestGroup	326.10M	[M+Na]+301.10 ; 13Cx2	9.4	?	9.386 - 9.448	326.10M	N/A	326 - 326.2	0	0
001010021.cdf	TestGroup	327.10M	[M+Na]+301.10 ; 13Cx3	9.4	?	9.386 - 9.448	327.10M	N/A	327 - 327.2	0	0
001010021.cdf	TestGroup	328.10M	[M+Na]+301.10 ; 13Cx4	9.4	?	9.386 - 9.448	328.10M	N/A	328 - 328.2	0	0
001010021.cdf	TestGroup	329.11M	[M+Na]+301.10 ; 13Cx5	9.4	?	9.386 - 9.448	329.11M	N/A	329 - 329.2	0	0
001010021.cdf	TestGroup2	301.10M	301.1	9.4	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.8416
001010021.cdf	TestGroup2	302.10M	301.10 ; 15N /// 301.10 ; 13	9.4	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.1336
001010021.cdf	TestGroup2	303.10M /// 303	301.10 ; 15N,13C /// 301.1	9.4	?	9.386 - 9.448	303.10M /// 303.09	N/A	303 - 303.2	1.05E+06	0.02222
001010021.cdf	TestGroup2	304.10M	301.10 ; 15N,13Cx2 /// 301	9.4	?	9.386 - 9.448	304.10M	N/A	304 - 304.2	1.15E+05	0.00243
001010021.cdf	TestGroup2	305.10M	301.10 ; 15Nx2,13Cx2	9.4	?	9.386 - 9.448	305.10M	N/A	305 - 305.2	5159	0.0001094
001010021.cdf	TestGroup2	324.09M	[M+Na]+301.10	9.4	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
001010021.cdf	TestGroup2	325.09M	[M+Na]+301.10 ; 15N /// [	9.4	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
001010021.cdf	TestGroup2	326.09M /// 326	[M+Na]+301.10 ; 15N,13C	9.4	?	9.386 - 9.448	326.09M /// 326.08	N/A	326 - 326.2	0	0
001010021.cdf	TestGroup2	327.09M	[M+Na]+301.10 ; 15N,13Cx	9.4	?	9.386 - 9.448	327.09M	N/A	327 - 327.2	0	0

Figure 6: Integration export from a single file with multiple test compounds

The export function will create a new folder with a summary file and a detail csv for each loaded experiment. If any experiments were mzMLs with chromatograms which matched your compounds they will be analyzed in a separate file in the export.

Manual inspection

In addition to automated integration it is possible to view the targeted compounds across the loaded files visually. To do this make sure that the compound of interest is defined and all files are finished loading then from the Actions menu select “Compare across files”.

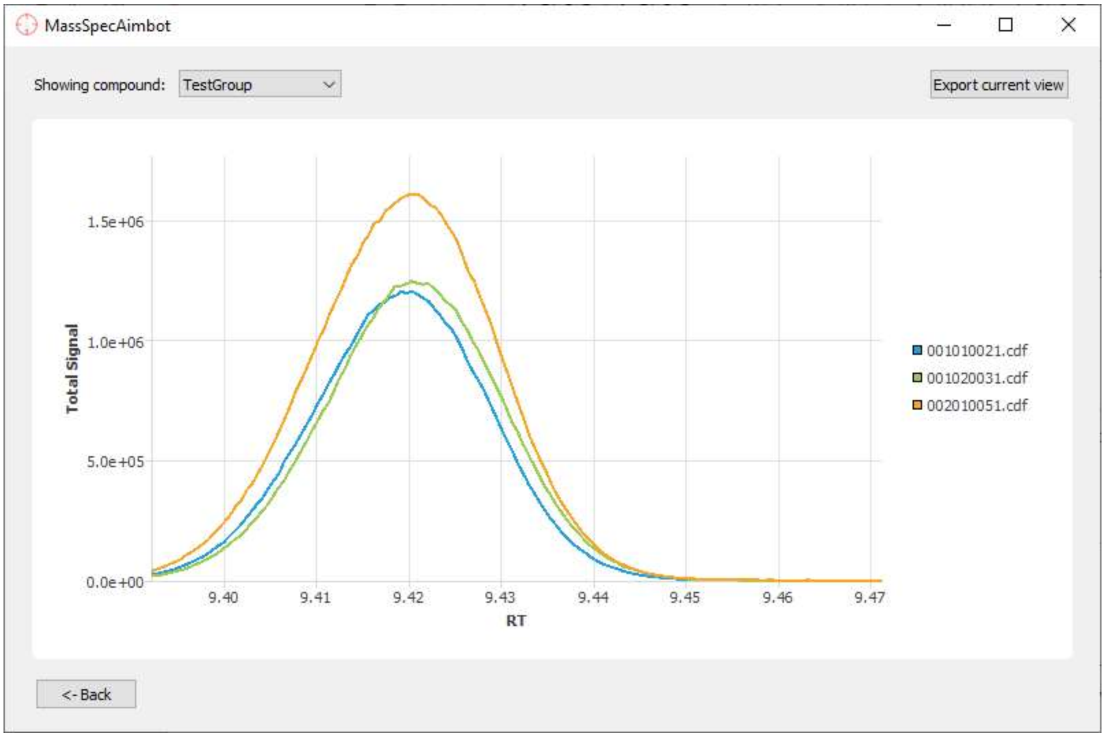

Figure 7: Comparing a compound's signal across files

Viewing spectrum

TIC panel

When a file is selected the top-right panel shows information about the spectrum contained within. By default this only shows the TIC, but if one or more ion trace is shown from the Compound panel it will also track individual ions across the file. Additionally if a compound is loaded it give the option to highlight the area of the selected peak as determined by the integration algorithm.

To navigate the TIC panel click and drag a portion of the graph to zoom in, and right click to reset zoom. When you are zoomed in you can pan left and right by clicking and dragging with the middle mouse button. Whenever the mouse is hovering over a valid time point a red bar will appear along with text indicating the RT of the cursor's location.

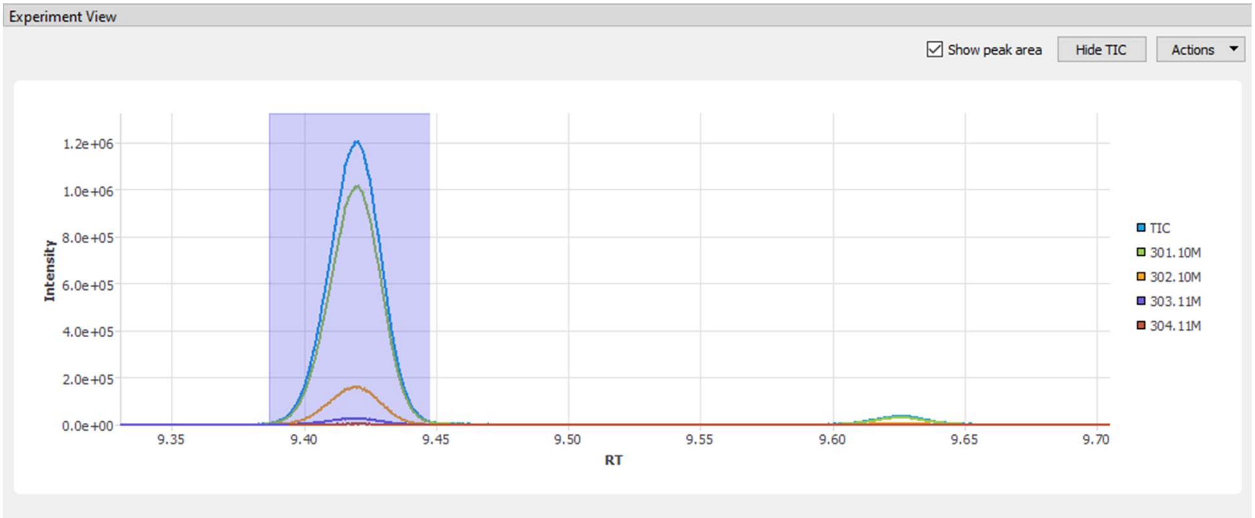

Figure 8: The TIC panel zoomed in on two peaks, with the integrated peak highlighted and three selected ion traces found.

Viewing a RT window

Once you have zoomed in on an area you can quickly view the contents of the visible window by clicking the "Export" button and selecting "…composite of current view". This will bring up a dialog showing the composition of the m/z and intensity values along the selected RT window (summed). This can be a quick way of determining what an unknown peak is made up of without having to look at individual spectra.

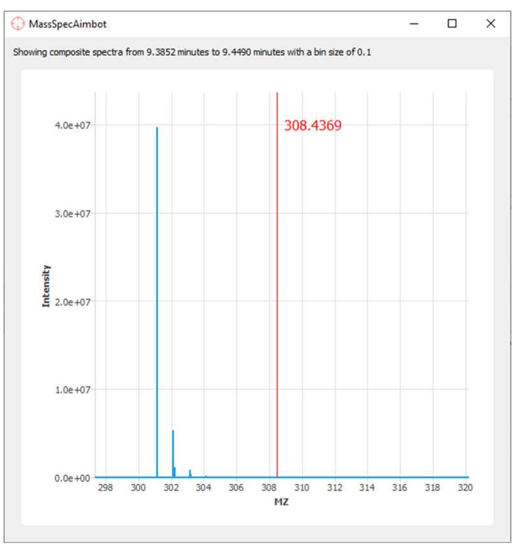

Figure 9: A composite view of a zoomed in peak within the test data, with a red bar indicating the m/z of the mouse's location

Viewing individual scans

Any time you click on a point in the TIC window Mass Spec Aimbot updates the scan panel with information about that time point. The scan panel is located in the same place as the Compound dialog, except in a different tab. It can be zoomed in and navigated the same way as the TIC panel except it follows m/z rather than RT as the bottom axis and tracks the individual scan time in the panel title.

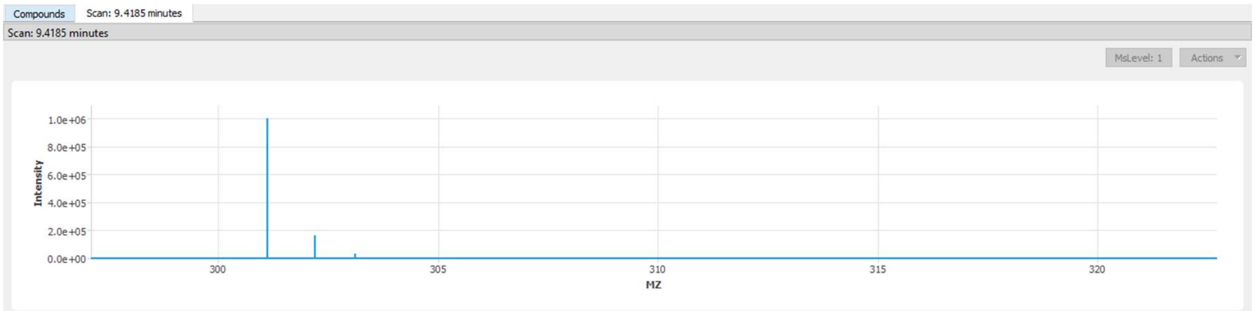

Figure 10: The scan panel activated and showing a single scan in the test data

Working with Chromatograms

Viewing

By default Mass Spec Aimbot only considers the spectra entries of an input file. When an mzML which contains chromatograms is loaded, however, it does have the capability of working with them in a limited capacity. The simplest interaction the program supports is simply viewing them from a filterable drop-down menu. To access this dialogue click “Actions” in the experiment view and select “Show mzML Chromatograms”

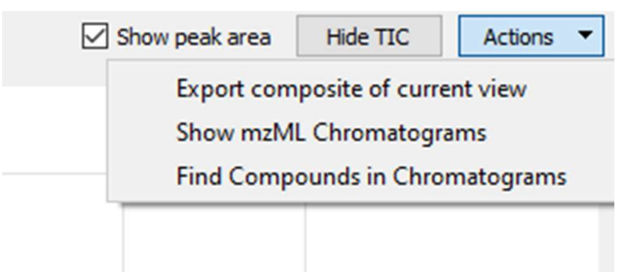

Figure 11: Location of Chromatogram functions

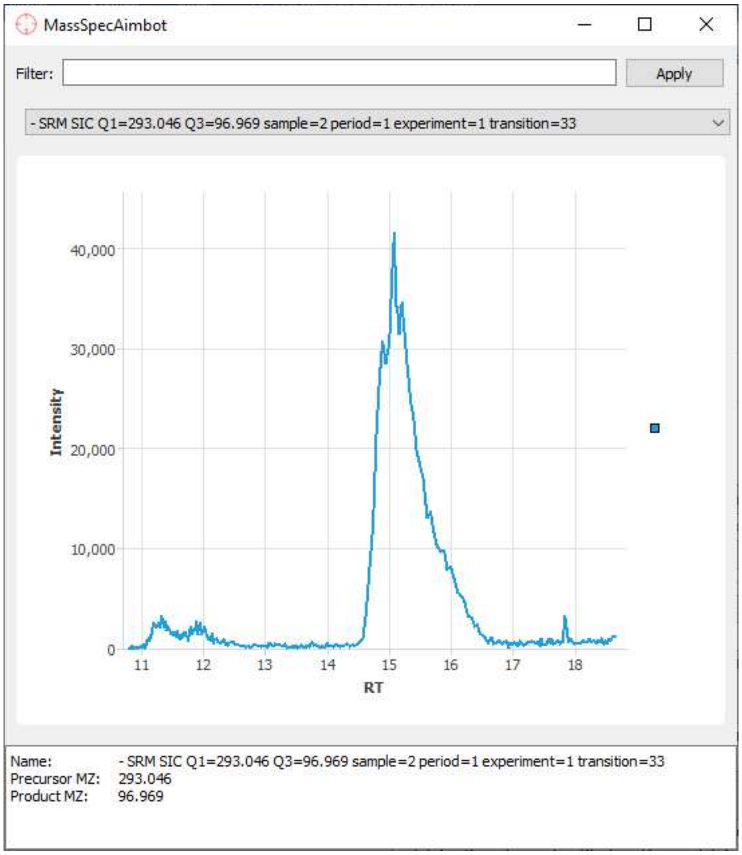

Figure 12: Example chromatogram

The drop-down box in the resulting dialog will contain every chromatogram present in the currently selected file. The filter box will allow you to display only chromatograms with the specified text in its name, which often already contains information such as the Q1 and Q3 MZ values.

Extracting groups

Loaded chromatograms can also be used for specifying compounds to search for in the spectra or integration export. If your experiment involved known heavy ions or expected adducts you can quickly look for them by clicking the "Actions" button in the experiment view as before and selecting "Find Compounds in Chromatograms".

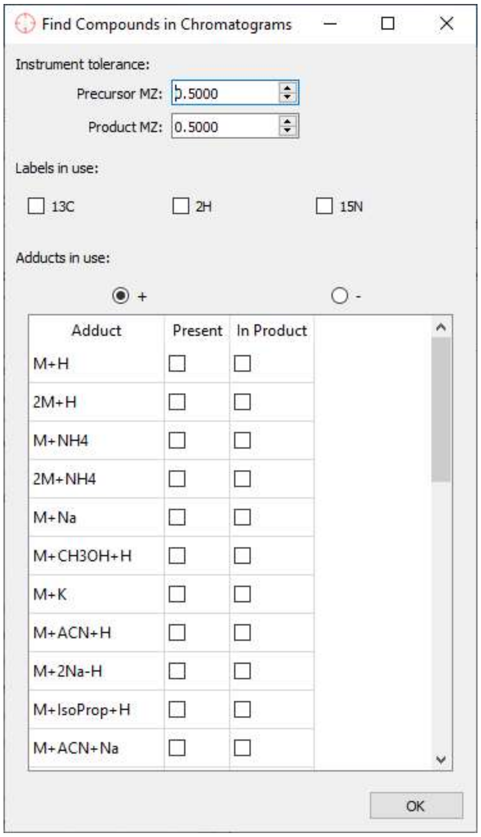

Figure 13: Chromatogram to Compound tool configuration

From the resulting dialog specify your instrument resolution, what heavy ions you expect to see, and any adducts that may have been previously taken into consideration. Once everything has been specified the program will go through the file's chromatogram masses and calculate any groups that look like they may belong to a compound with the given modifications.

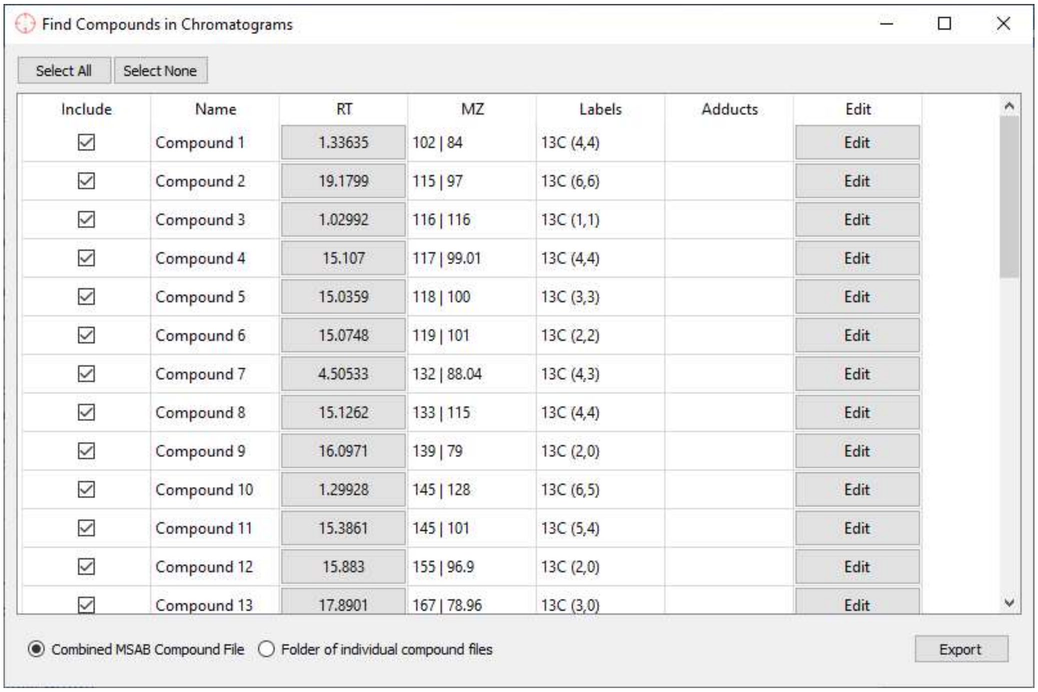

Figure 14: Chromatogram to Compound results page

Each compound in this list represents one potential match to the given parameters. For example, “Compound 1” represents a group of at least five chromatograms; the base of which has precursor mass of 102 and a product mass of 84, and the remaining ones having masses that match that base plus up to 4x13C modifications on both the precursors and products. The suggested RT is based on the highest point in the chromatogram, but can be edited by clicking on the button and using the resulting dialog to try to find a better point.

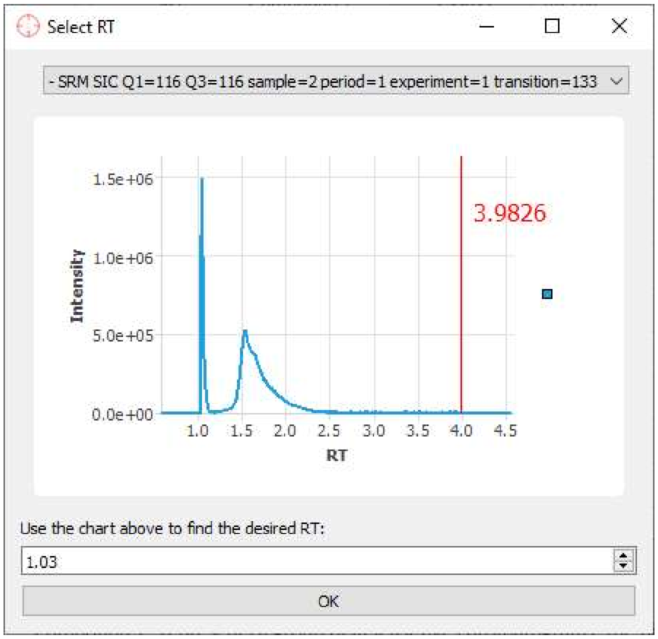

Figure 15: Chromatogram to Compound RT selection. Mouse over the graph to display exactly where the cursor is and enter the desired RT in the box at the bottom before clicking OK.

If any compounds look close but not quite what is expected they can be edited at any time with the edit button on the right side of the row. This works similarly to the Edit Ion window but contains extra information about the chromatograms in range.

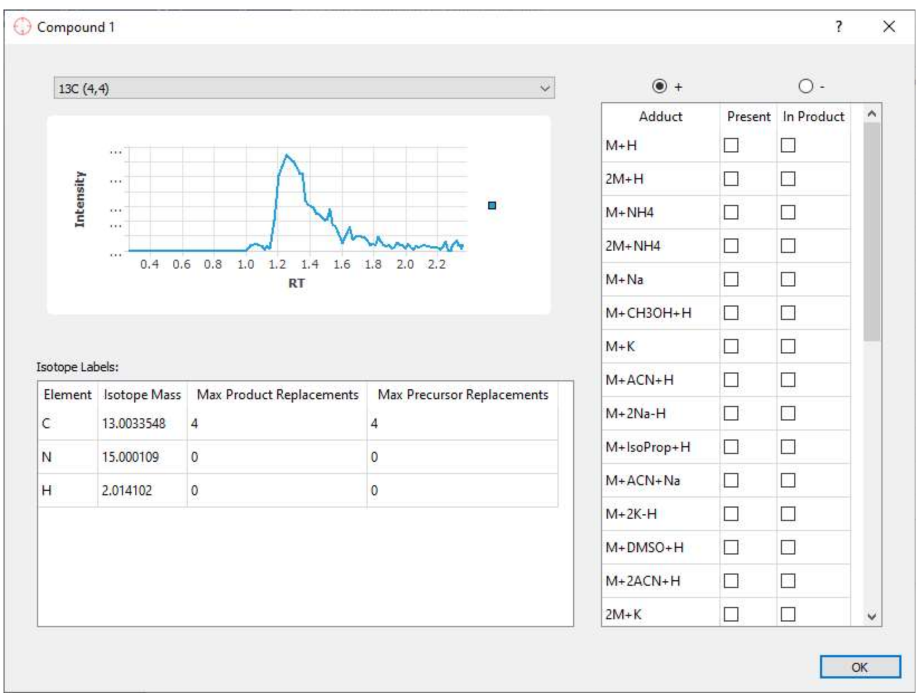

Figure 16: Chromatogram to Compound RT edit row dialog with 13C (4 precursor, 4 Product) chromatogram shown

Once all compounds of interest are selected they can be exported to either a single file or a group of files that can then in turn be imported from the “Add Compound” dialog.

Headless mode

To activate headless mode start the program with the `--headless` argument. Other arguments are as follow:

Create msabCGF file	[--MSABcgf] <(string)Output file name>
Merge with old msabCGF	[--append/--combine/--merge] <(string)Old file location>
Set CG name	[--name/-n] <(string)New name or name to append to>
Set CG rt	[--rt] <(double)New or replacement RT>
Set new ion formula	[--formula/-f] <(string or double) Formula or MZ>
Set precursor formula if MS2	[--precursor/-p] <(string or double) Precursor formula or MZ>
Set new ion expected intensity	[--intensity/-i] <(int) Intensity value>
Add label	[--label/-l] <(string)ElementSymbol> <(double)replacement mass> <(int)Product replacements> <(int, optional)PrecursorReplacements>
Add adduct	[--adduct/-a] <(string)AdductName> <(0 or 1,optional)can be on product>
Create compound split file	[--cgTree] <(string or 0)inputMSABcgf location> <(string) output csv>
Create integration file	[--integrate] <(string or 0)inputMSABcgf location> <(string)input spectra file location> <(string) output csv>