

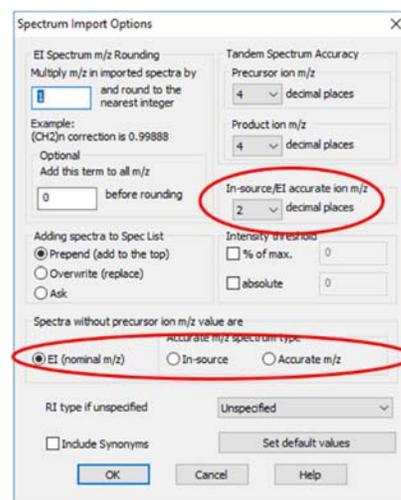
# Exporting Spectra with Accurate Mass from the Thermo Orbitrap to the NIST Mass Spectral Search Program

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The **NIST Mass Spectral Search Program (MS Search)** works with accurate mass as well as integer mass spectra. Several mass spectrometry manufacturers will export spectra from their data analysis software to MS Search with either accurate  $m/z$  values or integer values. The Thermo **Xcalibur** mass spectrometry software has always been very closely tied to **MS Search**. **Xcalibur** is a suit of programs: **Qual Browser**, **Quan Browser**, **Library Browser**, etc. The **Library Browser** selection from the **Xcalibur** dashboard brings **MS Search** into focus. **Xcalibur** users build, edit, and manage user libraries with **MS Search**. It has never been possible to send anything but integer  $m/z$ -value data from **Qual Browser** when exporting spectra to the **Library Browser (MS Search)**. Thermo data was primarily produced by transmission quadrupole mass spectrometers (both in GC/MS and LC/MS) and NIST did not have searches that used accurate  $m/z$ -value data. Today both LC/MS and GC/MS are produced with accurate  $m/z$ -value data using the Orbit trap. NIST has searches that are specific for product-ion MS/MS data and accurate  $m/z$ -value data in spectra in the NIST Tandem Library as well as user libraries. Due to the availability of an accurate  $m/z$ -value search routine in **MS Search** for EI data, users are wanting to build accurate  $m/z$ -data libraries for use with the GC Orbitrap. **Qual Browser's** limitation of exporting only integer  $m/z$ -value data made all this not possible.

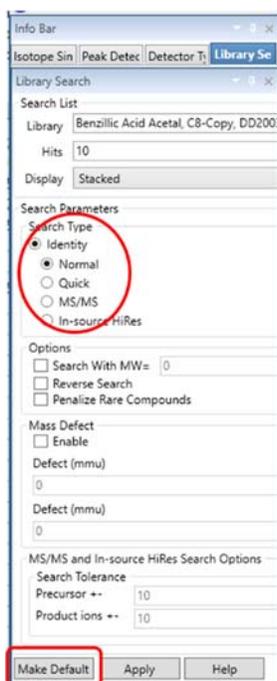
In the latest release of **Xcalibur** there is a new data analysis program in addition to **Qual Browser**, **FreeStyle**. **Freestyle** does allow for direct export of accurate  $m/z$ -value spectra from Thermo data files. Unfortunately, the initiation of this feature is not the default for **FreeStyle** and is not intuitive, even to the most experienced of users. This document is a guide as how to imitate the export of accurate  $m/z$ -value data from **FreeStyle** to **MS Search**.

The first step is to set **MS Search** to accept other than integer  $m/z$ -value data. From the **Xcalibur** Dashboard select the **Library Browser** icon. When **MS Search** opens, select **Options** from the **Main Menu** and select **Spectrum Import Options**. This results in the display of the **Spectrum Import Options** dialog box. The **EI** radio button in the **Spectra without precursor ion  $m/z$  value:** will be selected by default, unless it has already been changed. Select **In-source** or **Accurate  $m/z$** . In the **In-source/EI accurate ion  $m/z$**  area of the dialog box, enter the number of decimal place the imported value is to have. Once these two settings are made, put the Mouse pointer on the **OK** button to close the dialog box and save the changes.



Return to the **Xcalibur** dashboard. Unfortunately, Thermo did not properly implement the calling of **MS Search** from the **Xcalibur** dashboard, thus the return button in the button bar of **MS Search** remains grayed. The only way to return to the **Xcalibur** dashboard is to used the **Alt-Tab** keystroke command are select the **Xcalibur** icon from Windows Taskbar. The **Switch to Caller** button does work when you send spectra from **FreeStyle** to **MS Search**.

Select the **FreeStyle** icon on the **Xcalibur** dashboard. **FreeStyle** opens with the **Workspace Options** tab full-screen view. A dialog box in the center shows the version of **FreeStyle** along with the names of the last files opened and a series of selections. Either select a previously-used file from the dialog box or select the red **File** tab and select a file name. The initial display is of a reconstructed total ion current chromatogram in the top horizontal panel and a spectrum in a second panel just below the RTIC chromatogram panel. These two panels occupy about 80% of the display. There is a vertical command panel, from the top to the bottom of the display, on the left. There are a series of tabs across the top of the program's total display. Select the third tab (**Workspace Processing**). The display changes to have five icons on the left of center with only the **Library Manger** icon not grayed. Put the Mouse pointer on the title bar of the spectrum (lower horizontal panel) and click the left Mouse button once. The tile bar changes to a darker blue and the four icons on the left of the **Library Manager** icon are no longer grayed. Put the Mouse pointer on the **NIST Search** icon and click the left



Mouse button. This causes the left panel display to change. Were as only three tabs were visible at the top of this panel, there are now four. The view is of the **Library Search** tab. In the area labeled **Search Parameters**, under **Search Type**, under the radio button labeled **Identity** are four more radio buttons. The radio button labeled **Normal** is selected. Select the radio button next to the **MS/MS** or **In-source HiRes** label. Next, put the Mouse pointer **Apply** button and click the left Mouse button.

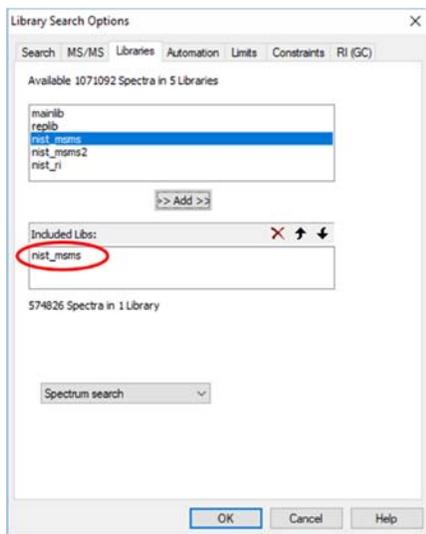
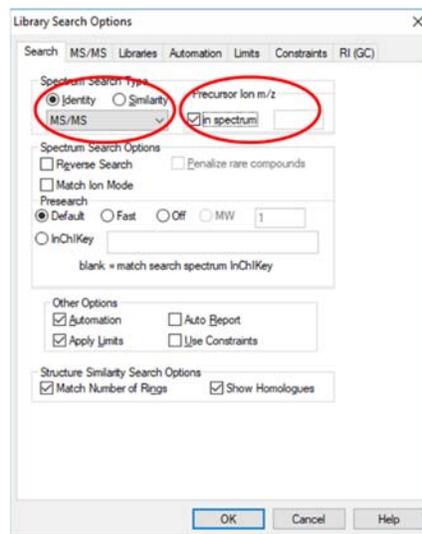
All spectra from now on will be exported to **MS Search** with accurate mass. It is important that the desired search type and libraries are selected in **MS Search**. From this point forward, any search done in **FreeStyle** will be an **Identity/In-source HiRes** or **MS/MS** search rather than the **Identity/Normal** search, that one would want to do on an EI spectrum. To allow search a search will require that the selections be set to **Normal** or **Quick EI Identity** search and the **Apply** button be selected. Now, spectra will only be exported as integer  $m/z$ -values The above process of selecting either the **In-source HiRes** or **MS/MS** radio button followed by clicking on the **Apply** button will have to be repeated to export an accurate  $m/z$ -value spectrum to **MS Search**. If **FreeStyle** is closed and reopened, all the changes will be lost and to export accurate  $m/z$ -value spectra, the process will have to be repeated.

The export of accurate  $m/z$ -value spectra to **MS Search** can be retained when the program is exited by putting the Mouse pointer on the **Make Default** button and clicking the left Mouse button after setting the **Search Parameters** type to **Identity/MS/MS** or **In-source Hi Res**. This will cause export of accurate  $m/z$  spectra even if **FreeStyle** is exited and restarted. The sequence will need to be repeated, including clicking on the **Apply** button to return to a normal EI Search. You, cannot have your cake and eat it too.

There is another confusing point about **FreeStyle** and the **MS Search** Libraries. At the top of the left-hand panel's **Library-Search** tab view is a list box that contains the names of all the libraries in the **NIST MS Search Program's \MSSEARCH** folder separated by commas. It is not clear if these are the libraries that are searched when a search is done internal to **FreeStyle**. A library can be deleted using the **Thermo Library Manage** dialog box displayed by selecting the **Library Manger** icon in **FreeStyle**. Unfortunately, this process permanently deletes the library from the **\MSSEARCH** folder of the **NIST MS Search Program**. Thermo should be asked about this.

## Export of Spectra to MS Search for use with the NIST Tandem Library

The **NIST Mass Spectral Search Program** now as an **MS/MS Identity Search**. This search should be used with the **nist\_msms** product ion library and/or user product-ion libraries. To use the **MS/MS Identity Search**, select **Library Search Options** from the **Options** menu displayed on the **Main Menu** bar or displayed when the **Library Search Options** button on the button bar just above the **Spec List** in the **Lib Search** tab view is chosen. This will display the **Library Search Options** dialog box. Next, select **MS/MS** from the dropdown list box under the **Identity** radio button. This causes the **Precursor ion m/z** area just to the right of the **Identity** and **Similarly** radio buttons to be ungrayed. If the mass spectrum sent to **MS Search** by the spectrometry data analysis program has the line header **Precursor m/z**: above the peak list, select the **in spectrum** check box in the **Library Search Options**' dialog box. If the spectrum does not have this, it will be necessary to enter a value of the precursor ion for the spectrum to be searched. At present, the Thermo **FreeStyle** software does not export product-ion mass spectra with a precursor ion specified.



After specifying the **Spectrum Search Type**, select the **Libraries** tab of the **Library Search Options** dialog box. The two libraries in the **Include Libs:** window of this dialog box will probably be **mainlib** and **replib**. Highlight them and click on the X just above the window to remove them. Next, highlight the **nist\_msms** library in the **Available Libraries** window and then click on the **Add** button, which is no longer grayed. This will copy the **nist\_msms** library to the **Include Libs:** window.

You may want to adjust the settings in the **MS/MS** tab view of this dialog box. Before making any changes, you should consult the **Help File** or the **MS Search Program's** manual. If the exporting software does not send the **Precursor m/z**: notation and you have entered the appropriate value for the precursor, you can have **MS Search** perform an automatic search when the spectrum is imported. Otherwise you should disable the **Automation** check box in the **Search** tab view of the **Library Search Options**' dialog box.

This allows you to import the spectrum to the **Spec List**, view and select the **m/z** value for the precursor-ion just before specifying it in the **Library Search Options**' dialog box.