

## MS EXTENSION

Clarity Extension

ENG

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To facilitate the orientation in the **MS Extension** manual and **Clarity** chromatography station, different fonts are used throughout the manual. Meanings of these fonts are:

*Open File* (italics) describes the commands and names of fields in **Clarity**, parameters that can be entered into them or a window or dialog name.

WORK1 (capitals) indicates the name of the file and/or directory.

ACTIVE (capital italics) marks the state of the station or its part.

The bold text is sometimes also used for important parts of the text and the name of the **Clarity** station. Moreover, some sections are written in format other than normal text. These sections are formatted as follows:

---

**Note:** Notifies the reader of relevant information.

---

**Caution:** Warns the user of possibly dangerous or very important information.

---

**Marks the problem statement or trouble question.**

**Description:** Presents more detailed information on the problem, describes its causes, etc.

**Solution:** Marks the response to the question, presents a procedure how to remove it.

# 1 MS - Mass Spectroscopy

The **Clarity MS Extension** is an optional part of the **Clarity Chromatography Station** (from version 5.0, it received major update in version 8.7, refer to **Clarity 8.7 vs 8.6** manual for more information). It is intended for data acquisition and evaluation of data measured on **MS Detectors**.

**MS Extension** is also compatible with **Clarity Offline** software.

## 2 Specification

The **MS Extension** is an optional, fully integrated part of **Clarity** software. It can be ordered as a part of new datastation or as an Extension to existing datastation (p/n A38).


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*Note:* MS Extension has higher demands on your PC configuration. We recommend PC with SSD drive installed. Please check recommended PC configuration listed in the D016 Datasheet - Compatibility Table.

## 3 Installation

The **MS Extension** is enabled by appropriate user code entered during installation or later by using the *Help - User Code* command from the *Clarity* main window.

To switch an Instrument to a mode where it can collect and evaluate MS data, select the **LC-MS** or **GC-MS** option from the *Instrument Type Setting* dialog.

*Instrument Type Setting* dialog is invoked by clicking on the  button in the *System Configuration* dialog.

Options that are technically possible and have been purchased are enabled by default. Otherwise they are automatically disabled.

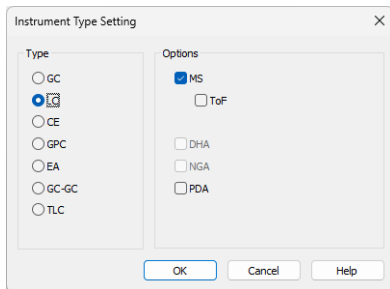


Fig 1: Setting up the **LC-MS** Instrument

## 4 Key Features

The **MS Extension** brings the following features to the **Clarity** station:

- **MS Chromatogram window** - standard **Clarity** Chromatogram window was modified to display the spectral information too. The graph pane now holds one more graph showing the spectrum at any given time or at a time span, which is noted in the top-right corner in the legend area.
- **MS Method** - MS method allows the user to select the extracted signals on which the compounds will be identified and quantified. Each compound may use different reference signals, so that more than one compound may be identified in any part of the chromatogram.
- **Library search** - **Clarity MS Extension** allows the user to use spectral libraries for peak identification and/or compound confirmation.
- **NIST Libraries** - **Clarity** uses the **NIST Library** format for storing spectra, and works flawlessly with commercial **NIST Libraries** or those created by the user.
- **Automatic Compound Search** - **Clarity** allows to search any selected signal automatically for the presence of spectra that are defined in the spectral library.

The following standard **Clarity** features will also help the user to make the best of the **MS** analysis in **Clarity**:

- **User calculations** - the user can define custom calculations in the **Result Table** and **Summary Table**. With the use of the integrated editor you can create your own columns from the original ones and use individual mathematical functions.
- **Reports** - user selectable report sections, WYSIWYG formatting of Graphs and Tables, and the possibility to report the spectral information for any peak in the chromatogram.
- **Batch** - automatic batch processing, display, export or print of any number of chromatograms.
- **Summary Result Table** - displays and prints selected results from all simultaneously displayed chromatograms.

## 4.1 Basic principles and terms

- **Mass Spectrometry (MS)** is an analytical detection method that determines the structure of compounds from the ions to which the compound is fragmented.
- **m/z** means **Mass to Charge** ratio. It is the value particular ions are divided by. Most common usage is the usage of ions with the charge of +1 or -1, making the m/z effectively just the mass of the ion.
- **MS Detector** is the device serving for the **Mass Spectrometry**. It generally composes of several parts - **Sample Inlet**, **Ion Source** where the compounds entering the detector are fragmented to ions, **Mass Analyzer** in which the particular ions are separated based on their **m/z** ratio and **Detector**, where the ions are detected.
- **MS Spectrum** is the basic information coming out of the **MS Detector** - it is a set of data describing the relative intensities of ions of particular **m/z** fragmented from the compound.
- **Raw Data** is a matrix of the spectra and the retention time.
- **Total Ion Current (TIC)** represents the total signal coming from the **MS Detector**, in fact summing all the individual ion intensity values from each spectra to single value.
- **Base Peak Intensity (BPI)** is another interesting signal in the MS practice - it represents the signal showing the intensity of the highest intensity ion from each spectra.
- **Extracted Ion Current (EIC)** is the signal over time of one single ion of selected **m/z**, as gained from the **Raw Data**.
- **Raw Spectrum** is a continuous spectrum (similar to the UV spectrum) of the compound - individual data points are connected by a curve.
- **Stick Spectrum** is a simplified version of the **Raw Spectrum**, where "Peaks" made by small imprecision of the **MS Detector** are instead represented by a single **m/z** value, so called **Stick**.

# 5 MS Extension Description

After installation, new functions of the **MS Extension** will be available. Only features changed or added to the **Clarity** standard mode are listed and described here.

## 5.1 MS Chromatogram window

On the **GC-MS** and **LC-MS** Instrument the standard *Chromatogram* window was replaced by *MS Chromatogram* window. The functionality of *MS Chromatogram* window differs slightly from the functionality of the *Chromatogram* window, but most of the functions are common between both windows.

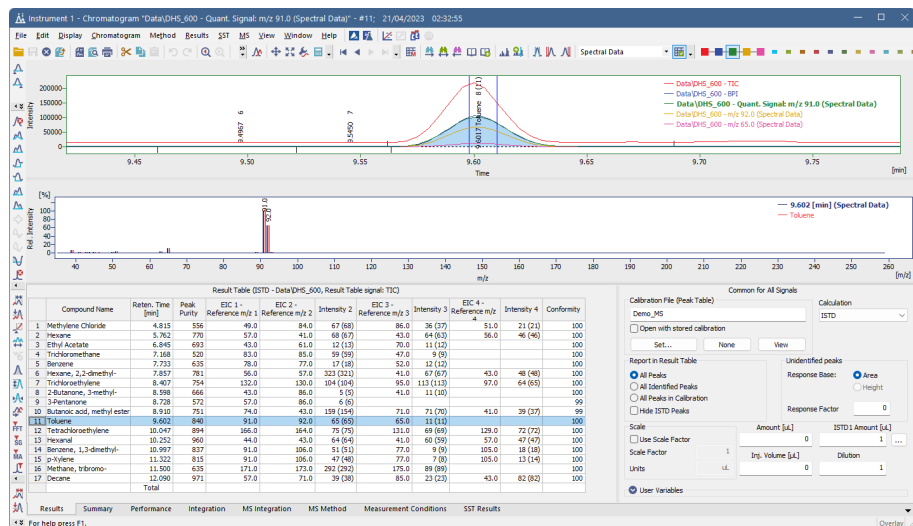


Fig 2: MS Chromatogram window

Graph pane is divided into two parts in the *MS Chromatogram* window - original graph as in standard *Chromatogram* window, and the spectrum part displaying the spectrum at any given time or time interval. There is a special mode called *MS Offsetting* for displaying the signals in the *MS Chromatogram* window, allowing to offset different signals to the same baseline level while keeping the ratios of the signals, which can be set in the *Graph Properties - Signals* dialog invoked by using the *Properties...* command from the local menu. This setting helps to eliminate the background noise on some signals (notably TIC) for optical evaluation of correct ion ratios. The settings for the behavior of the spectral graph can be selected using the *Graph Properties - MS Spectra* dialog invoked by using the *Properties...* command from the local menu.

## Signals in MS Extension

The number of signals displayed in the graph will change contextually as the user changes focus in the graph itself or select any signal or compound in the **Result Table** or on *MS Method*, *MS Integration* and *Integration* tabs. Each peak in the **MS Extension** is defined by up to four *EIC X - Reference m/z X Reference* signals and a *Quantification* signal, which may be TIC, any of the Reference signals, sum of those or even an external signal. Different peaks may (and indeed will) differ in number of such defined signals and in the signals themselves - the EIC - reference m/z for them will differ too.

Order under which the signals are displayed is defined by the signals set in the *System Configuration* (and later *Method Setup*) that were used during the acquisition of the chromatogram. Standard signals defined in the *System Configuration* and *Method Setup* dialogs will be displayed first. Next, if a peak is selected, will be it's quantification signal, followed by the signals of reference ions. If one of the reference ions is at the same time used as a quantification signal, it will not be displayed for the second time. Next in order will be any signals displayed by the user from the raw data.

The standard signals behave exactly as the users are used to in **Clarity**, and they are saved when the chromatogram is saved. The rest of the signals are so called "Temporary Signals" created according to the information set in the **MS Method** and created always fresh from the raw data when they are needed. If an MS chromatogram is opened on non-MS **Clarity** Instrument, only the standard signals will be available for inspection and work, but the temporary signals will not.

## Result Table

Another major change in the *MS Chromatogram* window touches the **Result Table**. On non-MS Instruments each signal has it's own **Result Table**, whereas on MS Instrument, the **Result Table** is common through all signals. The table header which normally changes color to match the color of the active signal is black on MS Instrument regardless of the signal selected.





The **Result Table** is a combination of two separate result tables - one created according to the *MS Method Table*, second one created according to the selected standard signal (Result Table Signal). The information on the signal used for this purpose is stored in the header of the table and can be changed by using the *Result Table Signal* option on the upper part of the *MS Method* tab.





## Other changes in MS Chromatogram window




All changes in the *MS Chromatogram* window are described on particular places - MS menu, *Result Table*, *Integration Table*, *MS Integration Table*, *MS Method tab*, *Import Chromatogram* and the *Graph Properties - MS Spectra* tab.

### 5.1.1 MS Menu

Menu containing all commands available in the MS Extension.

<b>MS Integration</b>	Switches the display of the lower part of the <i>MS Chromatogram</i> window to the <i>MS Integration</i> tab, showing <i>Integration Table</i> for compound's quantification signals.
 <b>MS Method</b>	Switches the display of the lower part of the <i>MS Chromatogram</i> window to the <i>MS Method</i> tab.
<b>Set NIST Libraries Directory...</b>	Allows to set the correct location of the NIST Libraries, if they are installed on the computer. <b>Clarity</b> installation includes also basic <b>NIST</b> demo libraries which are stored in the \DATAFILES\NIST\ subdirectory of the <b>Clarity</b> working directory (C:\CLARITY\ by default), which can be used instead.
<p><i>Note:</i> It is necessary to select the folder where the library names are displayed as subdirectories for <b>Clarity</b> to function.</p>	
<b>Select Spectral Data</b>	Command for working with multiple spectra in a single chromatogram. Opens a sub-menu allowing to switch to different spectrum. The same action may be also performed from the select-box in the MS toolbar.
<b>Prefer Spectral Data from Method</b>	Command for working with multiple spectra in a single chromatogram. Sets whether for compounds already present in the MS Method the spectrum displayed will be the spectrum selected in the method.
 <b>Single Compound Search...</b>	Opens the <i>MS Search</i> dialog on the <i>Single Compound Search</i> tab. More information on the <i>Single Compound Search</i> tab can be found in the chapter " <b>MS Search</b> " on pg 12.
 <b>Automatic Compound Search...</b>	Opens the <i>MS Search</i> dialog on the <i>Automatic Compound Search</i> tab. More information on the <i>Automatic Compound Search</i> tab can be found in the chapter " <b>MS Search</b> " on pg 15.
 <b>Target Compound Search...</b>	Opens the <i>MS Search</i> dialog on the <i>Target Compound Search</i> tab. More information on the <i>Target Compound Search</i> tab can be found in the chapter " <b>MS Search</b> " on pg 18.

	<b>Manage Libraries...</b>	Opens the <b>MS Search</b> external program which allows to create user libraries, manage them and copy compound spectra to them.
	<b>Add Spectrum to Library...</b>	Sends the selected spectrum to the <b>MS Search</b> external program via <i>Add MS Spectrum to Library</i> dialog described on pg 21.
	<b>Show Spectrum</b>	Allows to display a spectrum at any given time or any time interval. Invoking this command locks the cursor in the graph pane - just clicking on the time shows the spectrum at that point, pressing <b>Ctrl</b> and clicking twice allows to select the time interval through which the spectrum will be averaged.
	<b>Add Reference m/z</b>	When you are in a <b>MS Method Table</b> row, using this command locks the cursor in the spectrum graph. Clicking on any m/z stick adds the given m/z ion as a reference m/z for the compound. When all four reference m/z ions are filled in, you need to delete one of the four reference m/z ions before adding a new one.
	<b>Add Compound w/o Library Spectrum...</b>	When invoked, locks the cursor in the chromatogram graph pane. Clicking on any time will invoke the <i>Add Compound w/o Library Spectrum</i> dialog described in the section <b>Add Compound w/o Library Spectrum on pg 11</b>
	<b>Add Selected m/z Signal as Temporary</b>	Using this function locks the cursor in the spectrum graph. Clicking on any m/z will add the extracted signal of that m/z to the chromatogram if maximum signals in the chromatogram is not achieved yet.
	<b>Add Temporary m/z Signal - Manually...</b>	Using this function invokes the <i>Add Temporary m/z Signal Manually</i> dialog described in the section <b>Add Temporary m/z Signal - Manually... on pg 11</b> allowing to extract the signal on any chosen m/z from the raw data if maximum signals in the chromatogram is not achieved yet. It is possible to get a signal for the range of several defined m/z values.

<i>Note:</i>	Maximum of 24 signals is allowed in any MS chromatogram. This includes TIC, BPI, external signals, reference signals and any extracted signals using <i>Add Selected m/z Signal as Temporary</i> or <i>Add Temporary m/z Signal - Manually...</i> function, but not the Quantification signal.
<b>Remove All Temporary m/z Signals</b>	Serves for removing the no-longer necessary temporary added signals from the display in the <i>MS Chromatogram</i> window. All temporary added signals will be closed upon invoking this command.
<b>Mean and Backgrounds</b>	Command opening sub-menu with commands used for setting the mean and background evaluation intervals.
	<b>Set Mean Calculation Interval</b>
Using this function locks the cursor in the MS chromatogram graph. Clicking on any time point sets the start of the mean calculation interval, clicking for the second time selects the end of the mean calculation interval. Both values are inserted into the MS method of the chromatogram for the given peak in the format specified in <i>Mean and Backgrounds</i> dialog.	
	<b>Set Background 1 Interval</b>
Using this function locks the cursor in the MS chromatogram graph. Clicking on any time point sets the start of the background 1 interval, clicking for the second time selects the end of the background 1 interval. Both values are inserted into the MS method of the chromatogram for the given peak in the format specified in <i>Mean and Backgrounds</i> dialog.	
	<b>Set Background 2 Interval</b>
Using this function locks the cursor in the MS chromatogram graph. Clicking on any time point sets the start of the background 2 interval, clicking for the second time selects the end of the background 2 interval. Both values are inserted into the MS method of the chromatogram for the given peak in the format specified in <i>Mean and Backgrounds</i> dialog.	
<b>Configure Mean and Backgrounds...</b>	
Opens the <i>Mean and Backgrounds</i> dialog used for defining the defaults for mean calculation interval and background intervals.	

**Create Spectrum Label**

Command serving for creating labels in MS spectral graph. Two options enable to create either **Text** labels or **Line** labels (for more details see the chapter **Create Spectrum Label** on pg 24).

**Switch MS Warnings Off**

Using this option hides **MS Extension** related warnings above the **Result Table** in the **MS Chromatogram** window.

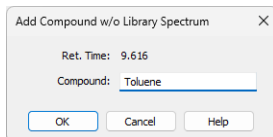
**Add Compound w/o Library Spectrum**

Fig 3: Add Compound w/o Library Spectrum dialog

The dialog allows to enter a new row into the **MS Method Table**. Selecting a unique *Compound* name is necessary.

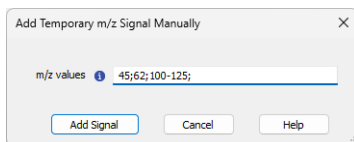
**Add Temporary m/z Signal - Manually...**


Fig 4: Add Temporary m/z Signal - Manually...

The dialog invoked after using the *Add Temporary m/z Signal - Manually...* command allows to extract the desired m/z signal from the raw data. Any values complying with spectral precision are allowed to be entered into the dialog, the values can be input as single values separated by semicolon (;) or as a range using dash (-). E.g.: 45;62;100-125.

### 5.1.1.1 MS Search

*MS Search* dialog serves for setting the parameters for the search in spectral libraries. **Clarity** can perform three types of search - *Single Compound Search*, *Automatic Compound Search* and *Target Compound Search*.

#### Single Compound Search

*Single Compound Search* tab serves for performing a simple search of a single spectrum in one or more spectral libraries. It is invoked by using the *Single Compound Search*  command from the *MS* menu of the *MS Chromatogram* window.

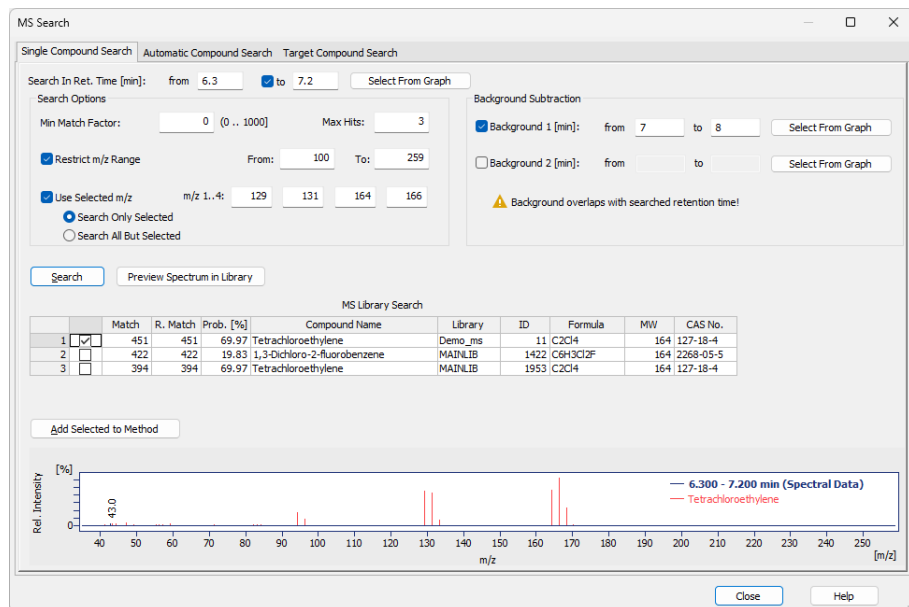


Fig 5: MS Search - Single Compound Search dialog

#### Search In Ret. Time

Either an exact retention time or an interval can be selected to search in. The values can be input manually or by selecting in the graph. The spectrum from the selected retention time will be compared with the spectral libraries and results will be returned based on further setting, i.e. *Search Options* and *Background Subtraction*.

#### Select From Graph

When this button is clicked, a mouse cursor will be focused into the graph, where either an exact retention time can be selected by one click into the graph, or a range of retention time can be selected: while holding the **CTRL**

key, first click into the graph to select the beginning of the desired interval, and second click into the graph to select the end of the desired interval.

### Search Options

Section allowing to set several parameters for the spectral search.

#### Min Match Factor

Restricts the hits returned to **Clarity** from MS Search program to just those with match factor higher than or equals to the value set in the field. Valid numbers are whole numbers from 0 to 1000.

#### Max Hits

Restricts the number of hits returned from the MS Search program to **Clarity**. Changing the value in the field only applies when the search is invoked again. Valid values are whole numbers from 1 to 100.

#### Restrict m/z Range

Sets the range of m/z values that will be searched in the library spectra.

#### Use Selected m/z

- **Search Only Selected** performs the search using only up to four selected m/z values. Using this option searches the libraries only for spectra containing the selected m/z ions with non-zero intensities, giving best matches to compounds that have the relative intensities of such sticks closest to ones in the chromatogram.

*Note* When the *Restrict m/z Range* together with *Search Only Selected* are set, only those m/z ions belonging to this restricted range are searched.

- **Search All But Selected** excludes from the search up to four ions selected in the *m/z 1..4* fields.

### Background Subtraction

Up to two backgrounds can be selected to be subtracted from the spectrum before the search. Such backgrounds can be input either as an exact retention time or as an interval.

### Select From Graph

When this button is clicked, a mouse cursor will be focused into the graph, where either an exact time for background subtraction can be selected by one click into the graph, or a range of time for background subtraction can be selected: while holding the **CTRL** key, first click into the graph to select the beginning of the desired interval, and second click into the graph to select the end of the desired interval.

### Search

When invoked, the search in **NIST Libraries** is performed using the external **MS Search** program. The MS Search being searched in can be set in the

**MS Search** program (menu options *Options - Library Search Options - Libraries* tab), along with other search parameters.

### Preview Spectrum in Library

When invoked, the selected spectrum will be shown in **NIST Libraries** using the external **MS Search** program. More parameters can be set in the **MS Search** program (menu *Options - Library Search Options - Libraries* tab).

**Caution:** Parameters changes in the *MS Search - Single Compound Search* dialog will not be applied until the *Search* or *Preview Spectrum in Library* button is pressed once again.

### Add Selected to Method

Adds the checked rows from MS Library Search into the **MS Method table**.

### MS Library Search table

This table contains the results of the search from the **MS Search** program. The table contains the *Match*, *Reverse Match*, *Probability*, *Compound Name*, *Library*, *ID*, *Formula*, *MW* and *CAS No.* columns, as used in the **MS Search** program.

### Spectrum graph

The graph displays the overlay of the chromatogram signal as set in the *Search In Ret. Time* field used for the library search and the library spectrum of a compound selected in the *MS Library Search* table. Standard operations (zooming, etc.) are allowed in the graph, it's format (mainly peak tag format, overlay/head-to-tail mode, spectra colors) is dependent on the settings on the *Graph Properties - MS Spectra tab*. It is possible to open the tab using the context menu invoked by right-click in the graph area.

## Automatic Compound Search

*Automatic Compound Search* tab serves for performing an initial search on a chromatogram where the user knows what he can expect. It is possible to quickly fill the whole **MS Method** table by running an *Automatic Compound Search*. The function is invoked by using the *Automatic Compound Search* command from the *MS* menu of the *MS Chromatogram* window.

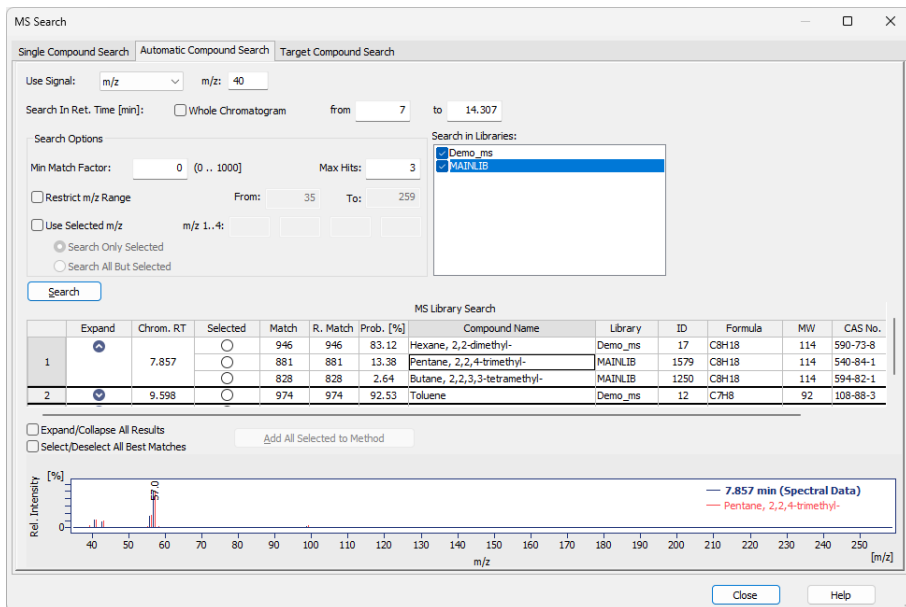


Fig 6: MS Search - Automatic Compound Search dialog

### Use Signal

Sets the signal on which the peaks will be detected and their spectra will be matched with spectral libraries. It is possible to select any standard signal including *TIC*, as well as external signals and signals extracted from the raw data (in such case you have to select the desired *m/z* in the appropriate field). While adding found compounds to *MS Method*, the signal set in this combo box will be propagated to *Quantify On* column in **MS Method** table.

### Search In Ret. Time

By default the search interval is in the whole chromatogram. If needed, such search interval can be specified in the *from* and *to* edit boxes, enabled after unchecking the check box *Whole Chromatogram*. The spectrum from the selected retention time will be compared with the spectral libraries and results will be returned based on further setting, i.e. *Search Options*.

### Search Options

Section allowing to set several parameters for the spectral search.

**Min Match Factor**

Restricts the hits returned to **Clarity** from MS Search program to just those with match factor higher than or equals to the value set in the field. Valid numbers are whole numbers from 0 to 1000.

**Max Hits**

Enables to show up to 100 hits for each peak found in the selected interval of retention times (or in a whole chromatogram). Only the best match is shown, remaining results for each peak can be shown by clicking on the chevron in *Expand* column or by clicking on *Expand/Collapse All Results* check box. Only one compound per each peak can be selected .

**Restrict m/z Range**

Sets the range of m/z values that will be searched in the library spectra.

**Use Selected m/z**

- **Search Only Selected** performs the search using only up to four selected m/z values. Using this option searches the libraries only for spectra containing the selected m/z ions with non-zero intensities, giving best matches to compounds that have the relative intensities of such sticks closest to ones in the chromatogram.

*Note:* When the *Restrict m/z Range* together with *Search Only Selected* are set, only those m/z ions belonging to this restricted range are searched.

- **Search All But Selected** excludes from the search up to four ions selected in the *m/z 1..4* fields.

**Search in Libraries**

Allows to select the libraries which should be used for the automatic search. It is possible to select multiple libraries.

**Search**

When invoked, the search is started and results are displayed in the *MS Library Search* table.

*Note:* In larger libraries and on slower computers, the search may take several minutes to complete. A progress dialog is displayed during it's course.

**MS Library Search table**

This table contains the results of the search. The table contains the *Expand*, *Chrom. RT*, *Selected*, *Match*, *Reverse Match*, *Probability %*, *Compound Name*, *Library*, *ID*, *Formula*, *MW* and *CAS No.* columns and gives a number of best results for each peak found on a signal defined in the *Use Signal* field according to the *Max Hits* field. In case the search produces less results then the *Max Hits* value, only the found results are provided. By default, no peak

has any library match preselected. It is possible to directly set the best match for the peak by using the *Selected* column checkbox on a given row or to display other matches and change the selection for each particular compound by the *Expand* column icon. It is also possible to use the *Expand/Collapse All Results* and *Select/Deselect All Best Matches* checkboxes modify the whole table instead just the single row.


#### **Add All Selected to Method**

Adds the checked rows from MS Library Search into the **MS Method table**. The *Retention Time* of the compounds in the *MS Method* table will be set to the times shown in the *Chrom. RT* field in the **MS Library Search** table, the name of the compounds will be set to *Compound Name* value from the **MS Library Search** table and can be later changed in the **MS Method table**.

#### **Spectrum graph**

The graph displays the overlay of the chromatogram signal as set in the *Search In Ret. Time* field used for the library search and the library spectrum of a compound selected in the *MS Library Search* table. Standard operations (zooming, etc.) are allowed in the graph, it's format (mainly peak tag format, overlay/head-to-tail mode, spectra colors) is dependent on the settings on the *Graph Properties - MS Spectra tab*. It is possible to open the tab using the context menu invoked by right-click in the graph area.

## Target Compound Search

*Target Compound Search* tab serves for looking up the position or presence of the desired compound in the chromatogram. It is possible to quickly browse the spectra in the whole chromatogram and find the one with the best match with the spectrum of the target compound. The function is invoked by using the *Target Compound Search*  command from the *MS* menu of the *MS Chromatogram* window.

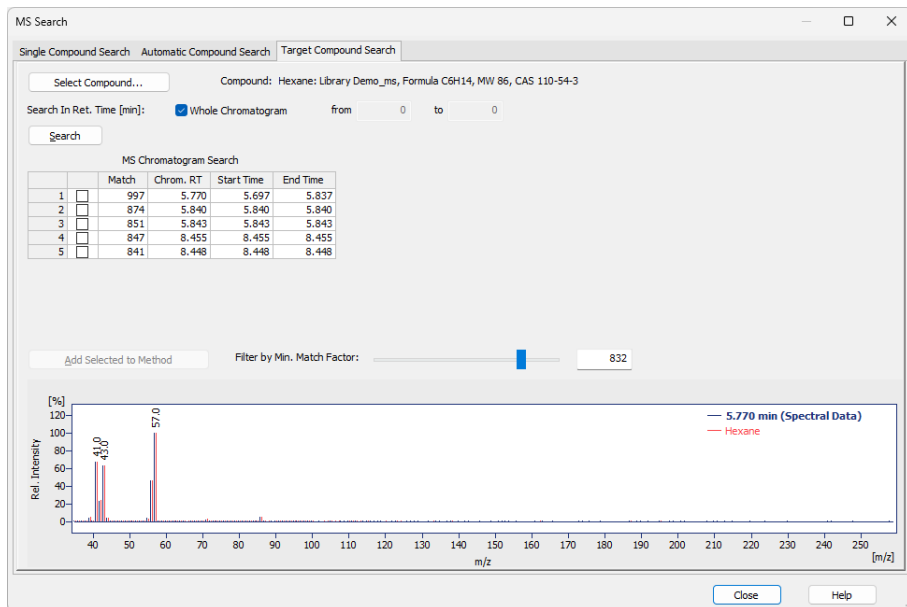


Fig 7: MS Search - Target Compound Search dialog

### Select Compound...

Opens the Select NIST Compound dialog for selecting the desired compound. The selected compound is then displayed in the *Compound:* field.

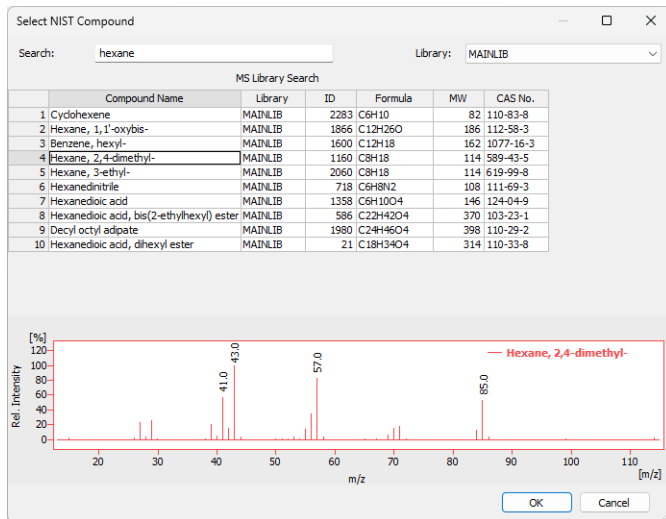


Fig 8: Select NIST Compound dialog

Select the library to search in in the *Library* drop-down menu and type the name of the compound to search for into the *Search* field. The table below the field will restrict the available compounds in real time. Select the desired compound in the table and press the OK button to return to *MS Search - Target Compound Search* dialog.

### Search In Ret. Time

By default the search interval is in the whole chromatogram. If needed, such search interval can be specified in the *from* and *to* edit boxes, enabled after unchecking the check box *Whole Chromatogram*.

### Search

When invoked, the search is started and the result are shown in the *MS Chromatogram Search*.

### MS Chromatogram Search table

This table contains the results of the search. The table contains the *Chrom. RT*, *Match*, *Start Time* and *End Time* columns and is filtered by *Filter by Min. Match Factor* slider.

### Add Selected to Method

Adds the checked rows from MS Library Search into the **MS Method table**. The *Retention Time* of the compounds in the *MS Method* table will be set to the times shown in the *Chrom. RT* field in the **MS Chromatogram Search** table.

### Filter by Min. Match Factor


Move the slider to the right or left to change the intervals displayed in the **MS Chromatogram Search** table or input the desire value directly to the edit

box next to the slider. The intervals are those in which all spectra have the Match of at least the value set on the slider. Moving the slider to the right (but still below the best match) will result in raising the number of intervals shown (in **MS Chromatogram Search**) as some particular spectra will not fall into the minimal match interval (they will split the interval to more than one), raising it even more will produce narrowing interval for the best match and later no interval displayed at all (as the *Min. Match Factor* will be higher than best match in the search. Moving the slider to the left will widen the interval and possibly add more intervals as similar spectra with worse matches will be available in different parts of the chromatogram too. It is important to set the slider for your search to a position where the interval shown is representative enough for the compound before selecting the desired one and pressing the *Add Selected to Method* button.

### **Spectrum graph**

The graph displays the overlay of the chromatogram signal as set in the *Search In Ret. Time* field used for the library search and the library spectrum of a compound selected in the *MS Library Search* table. Standard operations (zooming, etc.) are allowed in the graph, it's format (mainly peak tag format, overlay/head-to-tail mode, spectra colors) is dependent on the settings on the *Graph Properties - MS Spectra tab*. It is possible to open the tab using the context menu invoked by right-click in the graph area.

### 5.1.1.2 Add MS Spectrum to Library

Dialog invoked by using the *Add Spectrum to Library*  menu command, serving for saving the spectrum into the user library.

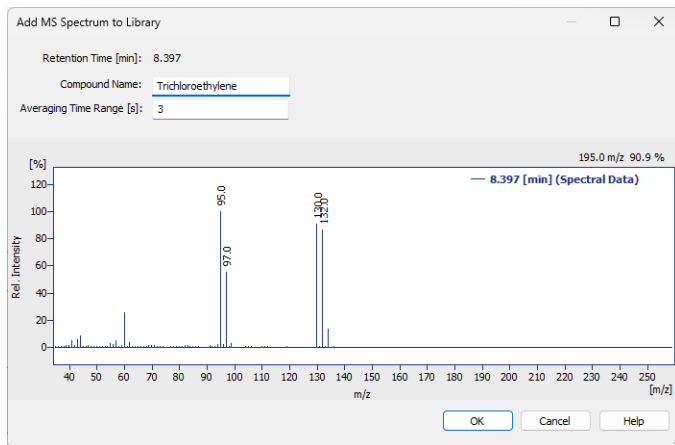


Fig 9: Add MS Spectrum to Library dialog

The dialog allows the user, prior to sending the spectrum into the library, to set several parameters. The spectrum is sent to the **MS Search** external program after pressing the **OK** button.

#### Retention Time [min]

Shows the retention time of the spectrum taken in minutes.

#### Compound Name

Serves for assigning a name to the spectrum when it is sent to the MS library. If the field is left empty, the retention time will be used instead.

#### Averaging Time Range [s]

Serves for setting the time interval in which the spectrum will be calculated as an average spectrum. The value entered into the field is the whole width of the interval.

### 5.1.1.3 Mean and Backgrounds

Dialog invoked by using the *Configure Mean and Background Intervals...* menu command, serving for setting the defaults for mean and background interval operations.

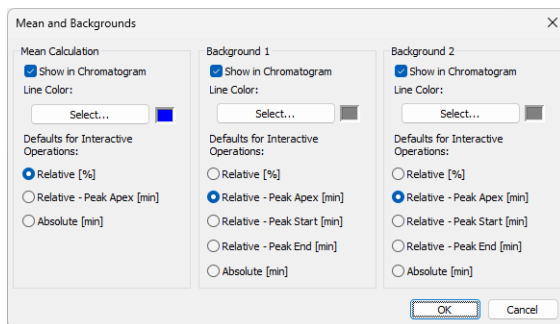


Fig 10: Mean and Backgrounds dialog

#### Mean Calculation

*Mean Calculation* section serves for defining how the mean calculation interval is shown in the chromatogram graph and what are the defaults for the interactive *Set Mean Calculation Interval* function.

##### Show in Chromatogram

Sets whether the vertical lines showing the mean spectra calculation interval will be displayed in the chromatogram graph. For each interval, two vertical lines may be shown, first indicating the start of the mean spectra calculation interval, second marking it's end.

##### Line Color

Sets the line color for the vertical marker lines which represent the mean spectra calculation interval.

##### Defaults for Interactive Operations

Sets how will the mean spectra calculation interval be inserted in the **MS Method** table when the *Set Mean Calculation Interval* interactive command is used. The meaning of particular options is described in the chapter "**MS Method**" on pg 29.

#### Background 1..2

*Background 1* and *Background 2* section serves for defining how the *Background 1* and *Background 2* intervals are shown in the chromatogram graph and what are the defaults for the interactive *Set Background 1 Interval* and *Set Background 2 Interval* functions.

##### Show in Chromatogram

Sets whether the vertical lines showing background 1(2) spectra interval will be displayed in the chromatogram graph. For each interval, two

vertical lines may be shown, first indicating the start of the interval, second marking it's end.

**Line Color**

Sets the line color for the vertical marker lines which represent the background 1(2) spectra calculation interval.

**Defaults for Interactive Operations**

Sets how will the background 1(2) interval be inserted in the **MS Method** table when the *Set Background 1 Interval* and *Set Background 2 Interval* interactive commands are used. The meaning of particular options is described in the chapter "**MS Method**" on pg 29.

### 5.1.1.4 Create Spectrum Label

Adds labels and lines to the spectrum graph in the **MS Extension**.

#### Text

Appends a text label. Select the command and click on the desired location to open the *Text Label* dialog. The *Text Label* dialog may also be invoked by double-clicking on the actual text label to modify it.

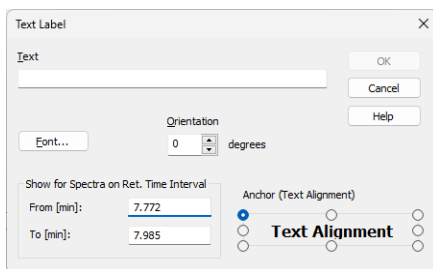


Fig 11: Text Label

#### Text

The text of the user's label is entered/edited on this line.

#### Font...

Selects the font, size and color of characters. Invoking the command opens the standard *Font* dialog.

#### Orientation

Select the orientation of the label text in degrees counted counterclockwise (e.g. enter 0 for horizontal or 90 for vertical orientation). Allowed values are 0 - 359.

**Note:** You can use *Orientation* field only when using **TrueType** fonts.

#### Delete

Deletes the selected label.

#### Show for Spectra on Ret. Time Interval

Defines the retention times in which the label will be visible. Set the retention time span using the *From [min]* and *To [min]* fields. If the spectrum displayed is taken in the retention time falling into such interval or in case the range of retention times of the displayed spectrum at least overlaps the set retention time range, the label will be displayed.

#### Anchor (Text alignment)

Defines the method of label anchoring. One of eight points on the label border or the label center may be selected as the anchor point. The label is then firmly "bound" by that point to the nearest spectrum point (in the m/z, relative intensity coordinates).

A label can be shifted and/or scaled in the same way as in usual drawing applications. Left clicking on a label displays the controlling frame and handles for sizing it. The font size will change with the frame size. To move the frame containing the label, click inside the frame and drag.

## Line

Adds connecting lines. To create a line label, invoke the command and then left click and hold the button on the desired starting point of the line, drag to the desired end point and release. The *Line Label* dialog will open to determine the properties of the line. The dialog can also be called by double-clicking on the actual line label to change its appearance.

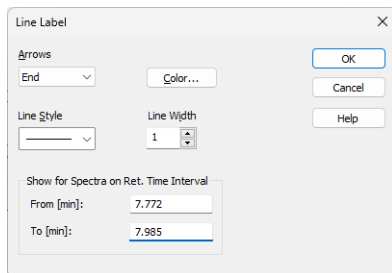


Fig 12: Line Label

### Arrows

Selects optional line end:

*None* - No arrows will be placed on the line.

*Beginning* - An arrow will be added to the line beginning.

*End* - An arrow will be added to the line end.

*Both* - Both line ends will be provided with arrows.

### Color...

Selects the line color.

### Line Style

Defines the line style. If the *Line Width* parameter is greater than 1, the line will be solid. Possible variants for the *Line Style* are solid line, broken line, dotted line, dashed and dotted line or dashed and double dotted line.

### Line Width

Defines the line width in pixels (1 to 6). Lines with the width greater than 1 have to be solid.

### Show for Spectra on Ret. Time Interval

Defines the retention times in which the label will be visible. Set the retention time span using the *From [min]* and *To [min]* fields. If the spectrum displayed is taken in the retention time falling into such interval or in case the range of retention times of the displayed spectrum at least overlaps the set retention time range, the label will be displayed.

A line can be shifted or sized in the same way as in drawing applications. Left clicking on a line will display the controlling frame and handles the sizing of the line. To shift the line, left click and hold anywhere on the line except the end points, drag to the desired location and release.

## 5.1.2 Result Table

**Result Table** in the **MS Extension** contains several columns that are not present in standard **Clarity**. These columns are described below the picture. Also, some columns from standard **Result Table** are not available in the **MS Extension**.

Result Table (ISTD - Data\PHS_200, Result Table signal: TIC)												
Compound Name	Reten. Time [min]	Peak Type	Response	Peak Purity	EIC 1 - Reference m/z 1	EIC 2 - Reference m/z 2	Intensity 2	EIC 3 - Reference m/z 3	Intensity 3	EIC 4 - Reference m/z 4	Intensity 4	Conformity
1 Methylene Chloride	4.822	Ordrr (by IS	33691.792	489	49.0	84.0	60 (68)	86.0	28 (37)	51.0	17 (21)	95
2 Hexane	5.767	Ordrr (by IS	33489.639	549	57.0	41.0	71 (67)	43.0	69 (63)	56.0	41 (46)	96
3 Ethyl Acetate	6.852	ISTD 1	623587.045	574	43.0	61.0	12 (13)	70.0	11 (12)			100
4 Trichloromethane	7.173	Ordrr (by IS	21104.875	486	83.0	85.0	50 (59)	47.0	7 (9)			95
5 Benzene	7.738	Ordrr (by IS	89059.059	419	78.0	77.0	13 (18)	52.0	9 (12)			99
6 Hexane, 2,2-dimethyl-	7.858	Ordrr (by IS	31115.847	624	56.0	57.0	348 (321)	41.0	71 (67)	43.0	51 (48)	99
7 Trichloroethylene	8.408	Ordrr (by IS	8207.971	703	132.0	130.0	104 (104)	95.0	122 (113)	97.0	64 (65)	94
8 2-Butanone, 3-methyl-	8.603	Ordrr (by IS	13581.351	638	43.0	86.0	5 (5)	41.0	27 (10)			89
9 3-Pentanone	8.733	Ordrr (by IS	9086.417	615	57.0	86.0	6 (6)					70
10 Butanoic acid, methyl ester	8.910	Ordrr (by IS	2486.715	775	74.0	43.0	379 (154)	71.0	130 (70)	41.0	180 (37)	73
11 Toluene	9.603	Ordrr (by IS	79035.327	672	91.0	92.0	62 (65)	65.0	8 (11)			98
12 Tetrachloroethylene	10.047	Ordrr (by IS	9547.596	609	166.0	164.0	65 (75)	131.0	63 (69)	129.0	64 (72)	88
13 Hexanal	10.253	Ordrr (by IS	15421.513	969	44.0	43.0	79 (64)	41.0	62 (59)	57.0	56 (47)	91
14 Benzene, 1,3-dimethyl-	10.998	Ordrr (by IS	128757.355	677	91.0	106.0	46 (51)	77.0	7 (9)	105.0	15 (18)	98
15 p-Xylene	11.318	Ordrr (by IS	63958.909	721	91.0	106.0	42 (48)	77.0	6 (8)	105.0	11 (14)	98
16 Methane, tribromo-	11.500	Ordrr (by IS	965.119	690	171.0	173.0	377 (292)	175.0	86 (89)			60
17 Decane	12.092	Ordrr (by IS	71100.871	936	57.0	71.0	38 (38)	85.0	20 (23)	43.0	85 (82)	98
Total												
Results	Summary	Performance	Integration	MS Integration	MS Method	Measurement Conditions	SST Results					
For help press F1.												

For help press F1.

Fig 13: Result Table - MS Chromatogram

**Note:** The compounds and values in the **Result Table** are taken from several places. Firstly, they are taken from **MS Calibration**. Secondly, compounds that are present in the **MS Method** but not in the **MS Calibration** are added (such compounds are missing amounts). Lastly, detector signal peaks that match compounds neither from **MS Calibration** nor **MS Method** are added with most of the fields empty.

### Peak Purity

Shows the spectral similarity of particular spectra contained in the peak, as defined by parameters set in the **MS Method - Peak Purity** dialog accessible from the **MS Method** tab. In case the **Peak Purity** is calculated using the **Five points method** and the **Intensity Threshold** is set too high to be over peak inflexion points, **Error** result is displayed with a tooltip hinting for solution.

### Reference m/z 1..4

Displays the m/z of the particular reference ion as set in the **MS Method Table**.

### Intensity 2..4

Displays the relative intensity of the given reference m/z ion stick to the first reference m/z ion stick. The value in the parentheses is displaying the expected relative intensity calculated from the library spectrum of the compound. If the value of the relative intensity is too different from the expected value, the cell contents are displayed in red. The limit for "too low" is set by the **Max. Conformity Error** parameter on the **MS Method** tab so that when the expected relative intensity is below 100, the value is considered an absolute limit, when the expected value is above 100, the value entered in the **Max. Conformity Error** field is considered a relative limit in %.

### Conformity

Displays the numerical representation of compound's spectrum similarity to spectrum from spectral library set for the compound in the **MS Method Table** on the scale of 0 (totally different) to 100 (completely the same). If the *Conformity* value is too low, the value is displayed in red. The limit for "too low" is again set by the *Max. Conformity Error* parameter on the **MS Method** tab.

*Note:* The result is also influenced by the setting of the *Conformity of Whole Spectrum* checkbox on the **MS Method** tab - while it is checked, the conformity of the whole spectra is compared, while when the checkbox is unchecked, the conformity is only calculated from the reference m/z ions.

There are several **MS Extension** related **Warnings** that can be displayed above the result table. For example warnings are displayed when settings for a compound differs in **MS Method** and **MS Calibration**, when spectrum is missing but is required (using options *Best Match* in *Peak Selection* or *Weighted EIC Sum* in *Quantify On*), and when a compound is present in **MS Calibration** but missing in **MS Method**. These can be turned off by using option *MS - Switch MS Warnings Off*.

5.1.3 MS Method

*MS Method* tab in the *MS Chromatogram* window serves for setting up the **MS Method**.

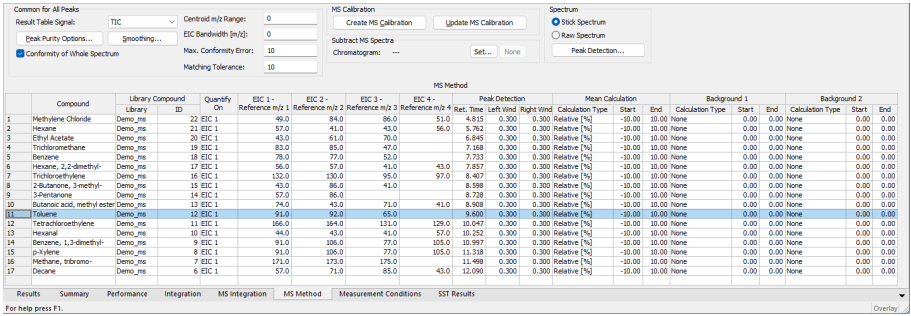


Fig 14: MS Method tab

Common for All Peaks

Items in this section are common properties of all peaks defined in the **MS Method** and shown in the *Result Table*.

Result Table Signal

Sets the signal for the display in the *Result Table*. **MS Result Table** is the superposition of a standard signal (set in this field) and peaks defined in the **MS Method Table**. The default value is *TIC* signal.

Peak Purity Options...

Using the button invokes the *MS Method - Peak Purity* dialog used for defining the calculation method of *Peak Purity* value (as displayed in the *Result Table*).

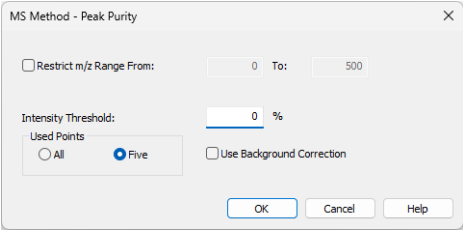


Fig 15: MS Method - Peak Purity dialog

Restrict m/z Range

Restricts the comparison of the spectra only to the m/z range specified in the *From* and *To* fields to the left.

Intensity Threshold

Defines the start and end spectrum for peak purity evaluation in % of the detected peak height. Available values range includes integer numbers in the range <0 - 100>.

**Used Points**

Sets whether the calculations of peak purity value will be carried out based on *All* spectra or only the *Five* significant points (peak start, peak apex, peak end, both peak inflexion points).

**Use Background Correction**

The baseline (background) for individual  $m/z$  values is interpolated between peak start and peak end points. With the *Use Background Correction* checked, the spectra are measured against this surface.

**Smoothing...**

Using the button invokes the *MS Method - Spectrum Smoothing* dialog used for defining the way how MS spectra are displayed - the available options are *None*, *FFT Filter*, *Savitzky-Golay Filter* and *Moving Average* filter. The last two allow to set the *Window* on which the smoothing will be applied - the higher *Window* value, the smoother the data will be. Side effect of such operation is increased noise and in some cases, shift of the spectral lines on the  $m/z$  axis.

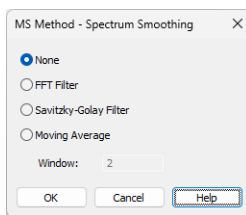


Fig 16: *MS Method - Spectrum Smoothing* dialog

**Conformity of Whole Spectrum**

Sets whether the *Conformity* values in the *Result Table* will be calculated just from defined *Reference  $m/z$*  values or from the whole spectra. Calculating with the whole spectra is more precise, but also slower.

**Centroid  $m/z$  Range**

Allows to artificially lower the precision of calculating the  $m/z$  stick intensities and values. Default value is 0 (as in the data).

**EIC Bandwidth [ $m/z$ ]**

Allows to define the allowed imprecision of  $m/z$  values to be still considered in the same  $m/z$  stick.

*Note:* In case the *Centroid  $m/z$  Range* is set to 1 ( $m/z$  sticks shown in the **Spectrum Graph** are with the e.g. 40, 41, 42, ...) and *EIC Bandwidth [ $m/z$ ]* is set to 0.2, any spectrum containing data with 0.1 imprecision to the expected value will be considered as having that ion in. For example, any spectrum containing  $m/z$  line of 159.9 - 160.1 will be considered to have a stick of  $m/z$  160.

**Max. Conformity Error**

Sets the value of maximal error in spectra conformity calculations. This value is used in the *Result Table* for the calculations of *Conformity* and also for relative *Intensity* of reference m/z ions.

**Matching Tolerance**

Sets the tolerance of the peak maximum position so that the peak found on the *Result Table Signal* is matched to a peak as defined in **MS Method Table**. The value is defined as a percentage of the *Left Wnd* and *Right Wnd* compound property, as defined in the **MS Method Table**. Default value is 10 %.

**MS Calibration**

This section serves for creating and maintaining calibration files from already filled in **MS Method**.

**Create MS Calibration**

Using the button creates a new calibration file and fills it with information already entered into the **MS Method** table. Columns being filled in in the MS Calibration file

**Update MS Calibration**

The button is only active when a calibration file is already connected to the **MS Chromatogram**. Pressing it updates the calibration file with compounds which are present in the **MS Method** but not in the calibration. Compounds already present in the **MS Calibration** file get the information in the *Quantified On*, *Reten. Time*, *Right Window*, *Left Window* and *Peak Selection* updated. In certain situation (when using *Best Match* in *Peak Selection* or *Weighted EIC Sum* in *Quantify On*) spectral information is required and it is updated as well. The responses and amounts of those compounds are not influenced in any way. Compounds present in the calibration, but not in the **MS Method** table, are not influenced in any way.

The button is disabled when **MS Chromatogram** is opened with **Stored MS Calibration** and *tooltip* is displayed next to it.

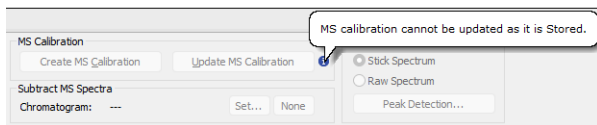


Fig 17: Displayed tooltip for Stored MS Calibration

**Subtract MS Spectra**

Serves for setting the subtraction chromatogram (most probably the blank) for signal subtraction. No scaling or moving of any data points is performed, just simple subtraction.

## Spectrum

Section defining the way how spectra are displayed in the spectral graph. The user may want to display the spectra in the *Stick Spectrum* mode or in the *Raw Spectrum* mode. The display options for both modes are set in the *Graph Properties - MS Spectra* dialog, accessible through local menu of the spectrum graph.

## Peak Detection

Using the button invokes the *MS Method - Peak Detection* dialog used for defining of the MS Spectra peak detection mechanism:

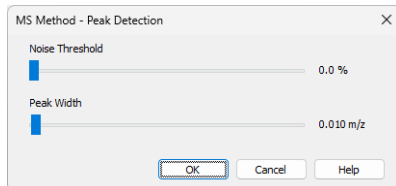


Fig 18: MS Method - Peak Detection

The mechanism is simplified peak detection mechanism only using two parameters for the whole spectra - *Noise Threshold* and *Peak Width*. Both parameters are set using sliders.

### Noise Threshold



Sets the noise threshold for the detection of the peaks - the higher the value, the less peaks will be detected in the spectrum.

### Peak Width

Sets the peak width parameter for the detection of the peak. This value is dependent on the spectrum precision of the MS detector - the denser the spectra are, the better peak detection can be achieved. Higher values of *Peak Width* will cause in fewer peaks detected.

**Note:** While setting the peak detection parameters, it is necessary to be careful. Wrong settings may invalidate any stick representations of the peaks, among other things (sticks representing the peaks in spectrum will be clearly off if wrong parameters are set).

## MS Method Table

The table defining the **MS Method** parameters. Rows in the table correspond to compounds of interest, columns hold the information for particular compound. It is possible to add the compounds into the table either manually (by editing the last empty row) or by using the *Single Compound Search*  and *Automatic Compound Search*  functions from the *MS Menu*.

### Compound

Shows the name of the compound in the **MS Method**. The name is by default taken from the **MS Library** if the user uses the library search, but can be changed. No two rows of the **MS Method Table** can have the same *Compound* name.

### Library Compound

Defines the link to the library if the MS libraries are used in the Extension. The link is defined by the *Library* name and compound *ID*. In case no spectral libraries are used, the *Library* column will be empty and the *ID* will be set to 0 for particular compound.

*Note:* The columns are colored yellow in case Raw spectra are displayed (no library spectra are worked with in the *Raw Spectrum* mode), or the cells on a row may be colored red to warn the user that the compound with the *ID* as set in *Library Compound* column is not available in the library defined in the *Compound* column, or that the library itself is not at the expected place.

### Quantify on

Defines on which signal will the particular compound be quantified. The options are *TIC*, any of the four reference extracted ions (*EIC1..4*), *EIC Sum*, *Weighted EIC Sum* or any standard signals defined in the method (either from the MS detector or from external detector). The default option is *EIC1*.

*Note:* Spectrum and spectrum related information are necessary for using *Weighted EIC Sum*.

### EIC 1..4 - Reference m/z 1..4

Columns for setting up the reference m/z ions for compounds in the **MS Method Table**. The values for m/z ions may be entered either manually or interactively using the *Add Reference m/z* function from the **MS Menu** in the chapter "**MS Menu**" on pg 8.

### Peak Detection

This section of the **MS Method Table** serves for setting the areas of the chromatogram where the given peak will be searched for. The meaning of the *Ret. Time*, *Left Wnd* and *Right Wnd* is the same as described in the **Calibration Table** of the *Calibration* window - they define the expected peak retention time and a span to lower and higher retention times, respectively, to search for the peak. *Peak Sel.* column (hidden by default) means peak selection and defines which peak will be taken if more than one is found in the time interval on the quantification signal - the options are peak *Nearest* to the expected retention time, *First* peak in the interval, *Last* peak in the interval, *Biggest* peak in the interval (based on *Response Base* settings in the *MS Calibration*) and the peak with the

*Best Match* of it's spectrum to the expected library spectrum that is in the interval. *Nearest* is the default option.

**Caution:** *Left Wnd* and *Right Wnd* define the search interval on the quantification signal which is specified for compound. When two (or more) compounds are quantified on the same signal and their *Windows* are set in a way they would overlap, the *Windows* are shorten so no overlap happens. On the other hand, *Windows* of compounds quantified on different signals do not influence each other in any way.

### Mean Calculation


*Mean Calculation* section of the **MS Method Table** serves for defining how the spectrum of the peak which will be later used for spectral comparison with the library spectrum is calculated. The *Calculation Type* defines the means of the calculation, the *Start* and *End* columns the boundaries. The following *Calculation Types* are available:

**None** - a single spectrum from the peak apex is taken.

**Relative [%]** - spectrum being shown and compared is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered into these fields are in percent from the peak beginning to peak end, -100 representing peak start, 0 peak apex and 100 peak end.

**Relative - Peak Apex [min]** - spectrum being shown and compared is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered are times in minutes relative to peak apex - negative values mean retention time lower than peak apex, positive values retention time higher than peak apex.

**Absolute [min]** - spectrum being shown and compared is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered are absolute retention times.

The default settings are *Relative* with values of *Start* of -10, *End* of 10. There are restrictions on possible values entered into the fields - basically the time entered into the *Start* column must be lower than the time in the *End* column for the compound. The values and *Calculation Type* may be filled in either manually by entering the values into the cells, or using the  *Set Mean Calculation Interval* function.

### Background 1..2

*Background 1* and *Background 2* section of the **MS Method Table** serves for defining the background spectra used for subtraction from the spectrum of the peak. The subtracted spectrum is used for the comparisons with the spectral libraries and for the display. The *Calculation Type* defines the means of the calculation, the *Start* and *End* columns the boundaries. The following *Calculation Types* are available:

**None** - no spectrum is subtracted.



**Relative [%]** - spectrum being subtracted is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered into these fields are in percent from the peak beginning to peak end, -100 representing peak start, 0 peak apex and 100 peak end. Values lower than -100 and higher than 100 are allowed.

**Relative - Peak Apex [min]** - spectrum being subtracted is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered are times in minutes relative to peak apex - negative values mean retention time lower than peak apex, positive values retention time higher than peak apex.

**Relative - Peak Start [min]** - same calculation as for *Relative - Peak Apex* option, only the referenced time point is the peak start, not peak apex.

**Relative - Peak End [min]** - same calculation as for *Relative - Peak Apex* option, only the referenced time point is the peak end, not peak apex.

**Absolute [min]** - spectrum being subtracted is calculated as an average of the spectra in the interval from *Start* to *End*. The values entered are absolute retention times.

The default settings are *None*. In case both *Background* intervals are defined, the calculated spectrum is an average of the two background spectra, if just one interval is defined, the spectrum subtracted is fully defined by it. The values and *Calculation Type* may be filled in either manually by entering the values into the cells, or using the  *Set Background 1 Interval* and  *Set Background 2 Interval* functions.

---

**Note:** Using the *Background* intervals will make the processing of MS chromatograms and work with them slower.

5.1.4 MS Integration

The *Integration tab* shows **Integration Table** for detectors' signals, and is the same as in standard **Clarity**. This table is for integration of standard signals coming from detectors, serving as *Result Table Signal*. The *MS Integration tab* shows **Integration Table** for compound's quantification signals, the most important signals for evaluating the result in the **MS Extension**. The **Integration Table** is unique for each quantification signal. When more compounds use the same quantification signal, such signal is shared among them. For example, having compound A and compound B quantified on the same m/z value means, that changing **Integration Table** for one of them results in the same changes for the second one.

Integration Algorithm (Common for All Signals) Wave

Integration Table - Compounds' Quant. Signals  
Data\PHS\_600, Integration Algorithm: Wave rev. 1

Quant. Signal m/z 91.0 - Toluene

Chromatogram Operation	Time A [min]	Time B [min]	Value
Global Peak Width			0.020 min
Global Threshold			20.0000
Global Filter - Bunching			1
Global Baseline Slope			0.000 /min
Filter - Savitzky-Golay	0.000	14.299	10

ResultsSummaryPerformanceIntegrationMS IntegrationMS Method

Fig 19: MS Integration tab

Therefore, some **Integration Table** items are common for all signals, but some are specific for each particular quantification signal, and some are even missing on the quantification signal. The items common to all signals, those specific for each quantification signal and items missing are listed in following table:

Tab 1: Integration Table items

table items common to all signals	table items specific for each quantification signal	table items not available on quantification signal
Global Peak Width	Local Peak Width	Detect Negative
Global Threshold	Local Threshold	Baseline Clamp Negative
Global Bunching	Baseline Allow Crossing	Baseline Cut Negative
Integration Interval	Baseline Spike Removal	Baseline Reject Negative
Minimal Area	Baseline Lock	Peak Flow Marker
Minimal Height	Baseline Valley	Peak Force Peak Name

table items common to all signals	table items specific for each quantification signal	table items not available on quantification signal
Minimal Half Width	Baseline Together	Group Add Group
Valley To Valley Slope	Baseline Forward Horizontal	Group Remove Group
Tangent Area Ratio	Baseline Backward Horizontal	
Tangent Slope Ratio	Baseline Front Tangent	
Detector Delay	Baseline Tail Tangent	
Filter - FFT	Peak Start	
Filter - Savitzky-Golay	Peak End	
Filter - Spike	Peak Both	
Filter - Moving Average	Peak Add Positive	
Noise Evaluation	Peak Add Negative	
ASTM Noise Evaluation	Peak Solvent	
6-Sigma Noise Evaluation		
Drift Evaluation		

This information is important in understanding which parameters can be changed just for one quantification signal and which will influence all quantification signals.

*Note:* In **MS Extension** integration operations *Global Threshold*, *Local Threshold* or *Min. Height* are displayed in the **Integration Table** and logged in audit trail without unit, whereas integration operation *Min. Area* is displayed in the **Integration Table** and logged in audit trail with time unit only - [s].

## 5.1.5 Import MS Chromatogram

The shape of dialog for importing the data to *MS Chromatogram* window invoked using the *File - Import Chromatogram...* menu command depends on the format from which the MS data are imported. For import from \*.CDF format, the dialog opened is *MS Import AIA File* dialog (slightly modified from the standard *Import AIA File* dialog). For import from \*.MZDATA, \*.MZML and \*.MZXML formats, the *Import MS Data File* dialog is opened. Both dialogs share most of their features in common.

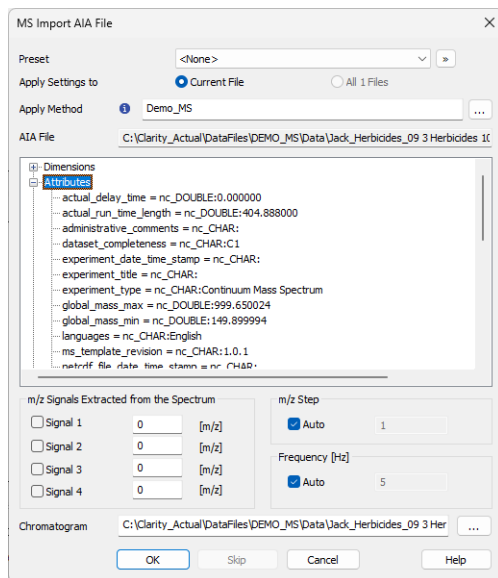


Fig 20: MS Import AIA File dialog

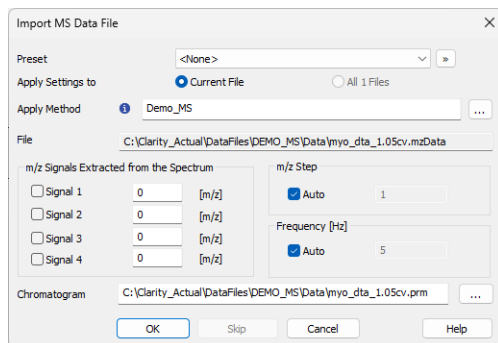



Fig 21: Import MS Data File dialog

**Preset**

Allows to select a preset from the list of stored import presets. A preset can be created / deleted / managed using the  button.

**Apply Setting to**

Radio-button deciding whether the settings for the import will be applied just to *Current File* selected (the one as defined by the *AIA File/File* field) or to *All n Files* selected for import (if multiple are selected).

**Apply Method**

Choose method whose parameters (integration table, attached calibration, etc.) will be applied into the imported chromatogram. By default, displayed method matches method in the *Single Analysis* dialog. Leaving this field blank applies default parameters.

**AIA File/File**

Name of the imported file including directory path.

**m/z Signals Extracted from the Spectrum**

Defines which signals should be extracted from the spectral data and stored to the chromatogram as standard signals. Up to four signals are allowed, with the m/z precision being limited by the *m/z Step* value of the chromatogram.

**Note:** If the specified m/z value is outside the measured spectrum in the imported chromatogram, the measured value closest to this value is used instead.

**m/z Step**

Defines the precision of the m/z values being imported, set in amu (atomic mass unit) or Da (Dalton). While the *Auto* checkbox is checked, the import mechanism will decide the optimal *m/z Step* itself, unchecking it allows the user to select the desired value. Setting the value manually too low will create unnecessary larger data, setting it manually too low will cause the loss off the m/z precision.

**Frequency [Hz]**


Defines the sampling frequency of the spectral data being imported. While the *Auto* checkbox is checked, the import mechanism will decide the optimal frequency itself, unchecking it allows the user to select the desired value.

**Note:** Setting the *Frequency* too low manually will create chromatograms with less-than-ideal data points and non-ideal peak shapes, setting it too high manually will cause artificial points to be created where the real ones are missing. The artificial points are created as a linear interpolation between existing points.

**Chromatogram**

Name and location of the resultant chromatogram created after importing the spectral file. By design, the program will store the original name of the

imported file along with the \*.PRM suffix and the path to the directory of the current project.

To select another name and location of the resultant chromatogram, open the *Save As...* dialog using the  button.

## 5.1.6 Export MS Chromatogram

The dialog for exporting the MS data is invoked using the *File - Export - Export Chromatogram...* menu command.

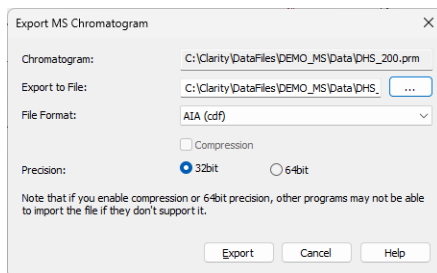


Fig 22: Export MS Chromatogram dialog

### Chromatogram

Displays the name of the chromatogram that will be exported.

### Export to File

Allows to define the file name of the chromatogram export, including its path. If no value is selected, the exported file will be named according to the original chromatogram.

### File Format

Defines the file format to which the chromatogram will be exported. **Clarity** supports the export to *AIA (cdf)*, *mzXML*, *MzML* and *mzData* formats.

### Compression

Sets whether the data will be compressed during the export to *mzML* and *mzXML* formats.

### Precision

Sets the data precision during the export. **Clarity** and a range of other software is *32bit* applications.

**Note:** If you enable the *Compression* or *64bit Precision*, other programs may not be able to import the file if they don't support it.

The header of the exported file will contain information provided by the control module - the type of the ionization, number of ion sources etc. To check the information before exporting, the user may use the *Show MS Conditions...* button on the *Measurement Conditions - Instrument* tab of the *MS Chromatogram* window, which will open the *MS Measurement Conditions* dialog.

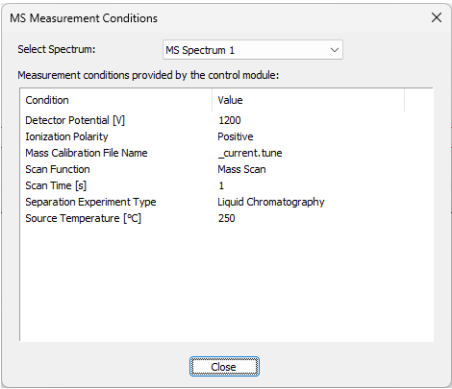


Fig 23: MS Measurement Conditions dialog

## 5.1.7 Graph Properties - MS Spectra tab

Allows to set the display form of the spectra graph in the **MS Extension**.

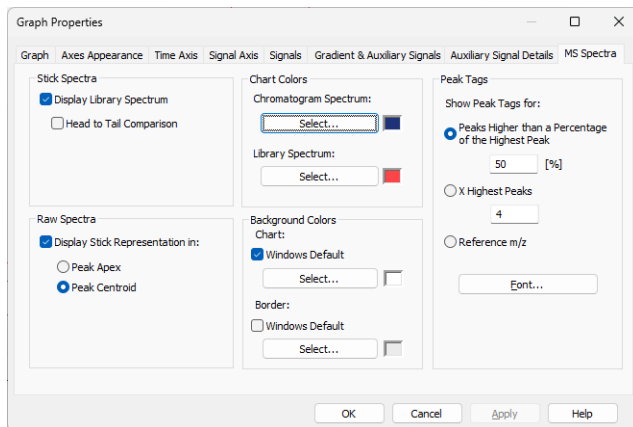


Fig 24: Graph Properties - MS Spectra

### Stick Spectra

Area setting the appearance of the spectrum graph when it is switched to the *Stick Spectrum* mode.

#### Display Library Spectrum

Sets the visibility of the library spectrum. This will be only visible for peaks defined in the **MS Method Table** with the *Library Compound* selected.

#### Head to Tail Comparison

Selects whether the spectrum graph and the Library graph will be displayed as a Head to Tail comparison. If not selected, both spectra will be displayed in overlay.

### Raw Spectra

Area setting the appearance of the spectrum graph when it is switched to the *Raw Spectrum* mode.

#### Display Stick Representation in

Sets the presence/visibility of the stick representations of peaks in the raw spectra. The stick representations may be placed either into *Peak Apex* of a detected peak or in a *Peak Centroid* of a detected peak. Which peaks are detected is influenced by settings in the **MS Method - Peak Detection** dialog.

### Chart Colors

Sets the coloring of the *Chromatogram Spectrum* and *Library Spectrum*. Each color may be defined independently using the appropriate *Select...*

button. The *Chromatogram Spectrum* coloring will be valid for both stick spectra and raw spectra.

### Background Colors

Selects the background color - *Chart* section - and border color - *Border* section - of a spectrum graph.

**Windows Default** - Color will automatically be adopted from the **MS Windows** appearance scheme. If deselected, the color will be set according to the *Select* button.

**Select...** - Color selection button.

### Peak Tags

Defines the placement and format of peak tags in the spectrum graph. The setting is common to both chromatogram spectrum and library spectrum.

#### Show Peak Tags for:

Sets which sticks in the spectrum graph will have peak tags.

**Peaks Higher than a Percentage of the Highest Peak** - peak tags will be displayed for any sticks with relative intensity higher than set amount (relative to the highest stick).

**X Highest Peaks** - peak tags will be displayed for set amount of highest sticks.

**Reference m/z** - peak tags will be displayed for the sticks marked as reference m/z in the **MS Method Table**.

#### Font

Allows to set the font for the peak tags in the spectral graph.

## 5.2 Method Setup

Chapters about Method Setup.

### 5.2.1 Method Setup - MS Method

*Method Setup - MS Method* is the method part holding information on the MS evaluation method. It contains the same fields and functions that are available on the *MS Method* tab in measured chromatogram and which is described in the chapter "**MS Method**" on pg 29.

Method Setup Demo\_MS - #3; 20/02/2023 15:14:23

Buttons: New, Open..., Save, Save as..., Report setup..., Audit trail..., Send method by e-mail..., Help

Common for all detectors

Common for All Peaks

Result Table Signal: TIC 1

Centroid m/z Range: 0

EIC Bandwidth [m/z]: 0

Max. Conformity Error: 10

Matching Tolerance: 10

MS Calibration

Create MS Calibration

Subtract MS Spectra

Chromatogram: ---

MS Method

	Compound	Library Compound		Quantify On	EIC 1 -	EIC 2 -	EIC 3 -	EIC 4 -
		Library	ID		Reference m/z 1	Reference m/z 2	Reference m/z 3	Reference m/z 4
1	Methylene Chloride	Demo_ms	22	EIC 1	49.0	84.0	86.0	5
2	Hexane	Demo_ms	21	EIC 1	57.0	41.0	43.0	5
3	Ethyl Acetate	Demo_ms	20	EIC 1	43.0	61.0	70.0	5
4	Trichloromethane	Demo_ms	19	EIC 1	83.0	85.0	47.0	5
5	Benzene	Demo_ms	18	EIC 1	78.0	77.0	52.0	5
6	Hexane, 2,2-dimethyl-	Demo_ms	17	EIC 1	56.0	57.0	41.0	4
7	Trichloroethylene	Demo_ms	16	EIC 1	132.0	130.0	95.0	9
8	2-Butanone, 3-methyl-	Demo_ms	15	EIC 1	43.0	86.0	41.0	5
9	3-Pentanone	Demo_ms	14	EIC 1	57.0	86.0	71.0	5
10	Butanoic acid, methyl ester	Demo_ms	13	EIC 1	74.0	43.0	71.0	4

Event Table | Measurement | MS | Integration | MS Integration | MS Method | Calculation | Advanced

Buttons: OK, Cancel, Send Method

Fig 25: Method Setup - MS Method tab

As the development and possible modifying of the MS Method pretty much needs an interaction with the measured data, it is advisable to develop the method on a measured chromatogram and then save it as a template method. Modifications in the *Method Setup* dialog may cause side effects which will not be, without the feedback from chromatogram graphs, clearly visible.

## 5.2.2 Method Setup - MS Integration

The *Method Setup - Integration* tab is exactly the same as in standard **Clarity** and also functions the same. The *Integration tab* serves for integration of standard signals coming from detectors, serving as *Result Table Signals*.

The *Method Setup - MS Integration* tab shows **Integration Table** for compound's quantification signals, the most important signals for evaluating the result in the **MS Extension**. The **Integration Table** is unique for each quantification signal. When more compounds use the same quantification signal, such signal is shared among them. For example, having compound A and compound B quantified on the same  $m/z$  value means, that changing **Integration Table** for one of them results in the same changes for the second one..

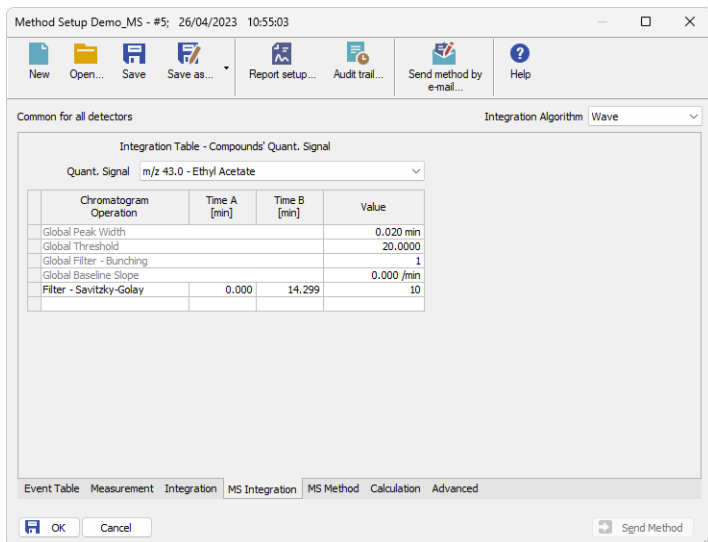


Fig 26: MS Integration tab

Therefore, some **Integration Table** items are common for all signals, but some are specific for each particular quantification signal, and some are even missing on the quantification signal. The items common to all signals, those specific for each quantification signal and items missing are listed in following table:

Tab 2: Integration Table items

table items common to all signals	table items specific for each quantification signal	table items not available on quantification signal
Global Peak Width	Local Peak Width	Detect Negative
Global Threshold	Local Threshold	Baseline Clamp Negative
Global Bunching	Baseline Allow Crossing	Baseline Cut Negative
Integration Interval	Baseline Spike Removal	Baseline Reject Negative
Minimal Area	Baseline Lock	Peak Flow Marker
Minimal Height	Baseline Valley	Peak Force Peak Name
Minimal Half Width	Baseline Together	Group Add Group
Valley To Valley Slope	Baseline Forward Horizontal	Group Remove Group
Tangent Area Ratio	Baseline Backward Horizontal	
Tangent Slope Ratio	Baseline Front Tangent	
Detector Delay	Baseline Tail Tangent	
Filter - FFT	Peak Start	
Filter - Savitzky-Golay	Peak End	
Filter - Spike	Peak Both	
Filter - Moving Average	Peak Add Positive	
Noise Evaluation	Peak Add Negative	
ASTM Noise Evaluation	Peak Solvent	
6-Sigma Noise Evaluation		
Drift Evaluation		

This information is important in understanding which parameters can be changed just for one quantification signal and which will influence all quantification signals.

**Note:** In **MS Extension** integration operations *Global Threshold*, *Local Threshold* or *Min. Height* are displayed in the **Integration Table** and logged in audit trail without unit, whereas integration operation *Min. Area* is displayed in the **Integration Table** and logged in audit trail with time unit only - [s].

## 5.3 MS Calibration

*MS Calibration* window resembles the standard *Calibration* window in most functions, but also brings several new functions or differences to the standard *Calibration* window.

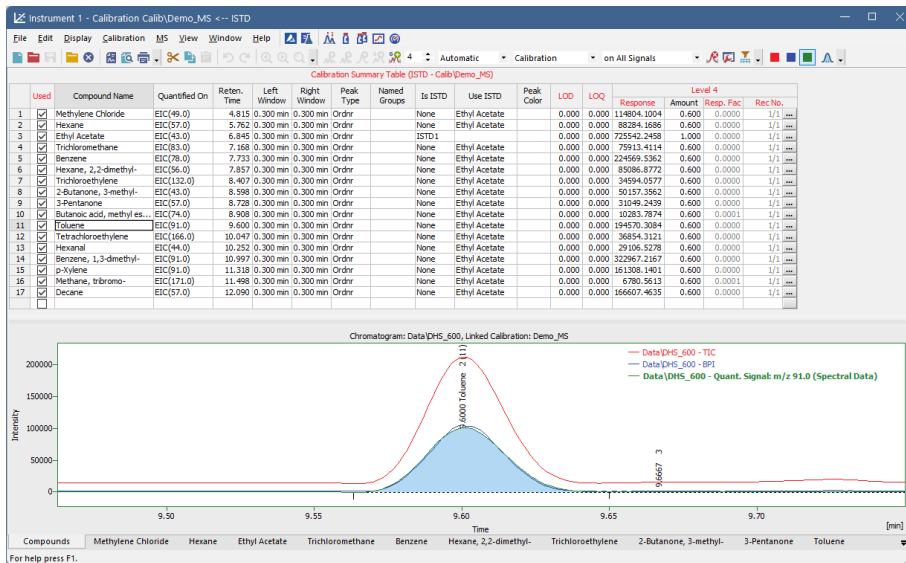


Fig 27: MS Calibration window

The same as *MS Chromatogram* window has just one **Result Table**, the *MS Calibration* window contains just a single **Calibration Table** even when it contains multiple signals. In standard *Clarity*, the user would be filling the compounds into the **Calibration Table**. However, as the compound table is already filled in the *MS Method*, it is possible to fill the **Calibration Table** directly from there by using the *Create MS Calibration* or *Update MS Calibration* functions. Several information from the **MS Method Table** are transferred directly into the calibration file, namely *Compound Name*, *Quantify On*, *Reten. Time*, *Left Window*, *Right Window* and *Peak Selection* (hidden by default). In certain situation (when using *Best Match* in *Peak Selection* or *Weighted EIC Sum* in *Quantify On*) spectral information is required and it is updated as well.

As the calculations for peaks in the **MS Extension** are carried out according to the quantification signal, the responses of the quantification signal should be used to fill in responses to the **Calibration Table**. A new *MS* menu item is added to the window, containing just a single command. Which quantification signal is used for particular compound is defined in the *Quantified On* column.

**Caution:** *Left Window* and *Right Window* define the search interval on the quantification signal which is specified for compound. When two (or more) compounds are quantified on the same signal and their *Windows* are set in a way they would overlap, the *Windows* are shortened so no overlap happens. On the other hand, *Windows* of compounds quantified on different signals do not influence each other.

### Quantified On

The column shows a notation of the MS signal on which the compound is calibrated. Under normal conditions the calibration is created from the MS chromatogram by using the *Create MS Calibration* button, the *Quantified On* column is in such case filled with values as present in the *MS Chromatogram* window. User can also manually change the values if he wants to. In case the calibration is created directly in the *Calibration* window, the column is left empty. On an attempt to use the *Fill All Responses from Quant. Signals* command / button with the empty *Quantified On* column the calibration standard chromatogram is checked for the presence of the MS calibration information and updates the *Quantified On* column accordingly. In case the peak has different quantification information filled in the calibration standard **MS Method Table** and in the *Calibration* window *Quantified On* column, the information from the *Quantified On* column takes precedence.

When for any reason the peak is not found on a signal (because the peak is not present on the signal, the signal does not exist in the calibration standard chromatogram etc.), the response on the given level is not updated and message is logged in the calibration and station audit trail.

**Note:** Two compounds cannot have the same *Retention Time* and *Quantified On* at the same time. At least one of the parameters must differ. This means that it is possible to evaluate multiple compounds with identical *Retention Time* if they are quantified on different quantification signals.

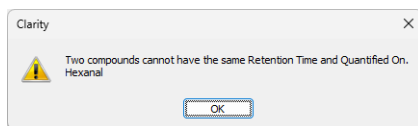



Fig 28: Warning when *Retention Time* and *Quantified On* are identical for two compounds.

### Fill All Responses from Quant. Signals

Using this command or the  icon fills the currently selected calibration level with the responses of all peaks, each response calculated from the respective quantification signal of the given peak. The attempt to get the response of the peak is performed by querying the chromatogram of the calibration standard for the response of the peak found on the signal as defined in the calibration, with the integration as defined in the calibration standard. This option is disabled, when opening *Stored MS Calibration*.

**Caution:** Fill All Responses from Quant. Signals cannot be used successfully when settings for a compound in **MS Method** and **MS Calibration** doesn't match. Following message is displayed and responses are not updated.

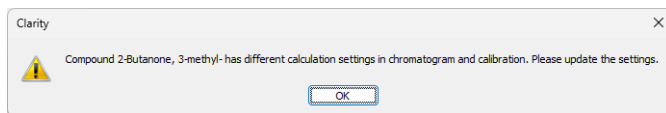


Fig 29: Warning displayed when compound settings differs in MS Method and MS Calibration.

**Note:** When a new compound is manually created in *Calibration Summary Table* there is not pre-filled any default value for *Quantified On* column. Default values for all other columns are described in **Clarity** Help in chapter **Calibration**.

## 5.4 Data Acquisition

The **Data Acquisition** window works the same on the **MS Instrument** as on standard **Clarity Instrument**.

Moreover, the **MS Extension** adds the *Show Spectrum* toggle command to the *View* menu, which displays the real time spectrum acquired by the detector during running analysis. This command can be selected only during running acquisition. When selected the spectrum is shown only during the analysis.

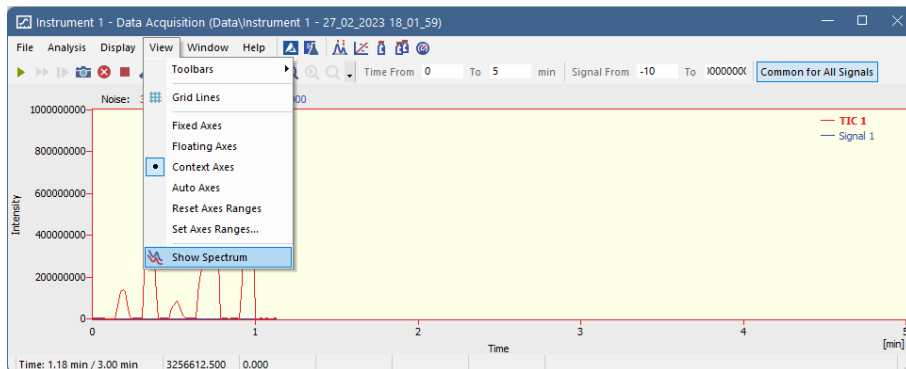


Fig 30: MS Data Acquisition window

## 5.5 Report Setup - MS

Determines the content and layout of the MS spectra printed from the chromatogram.

**Caution:** The *Report Setup - MS* section items will only be printed from the *MS Chromatogram* window.

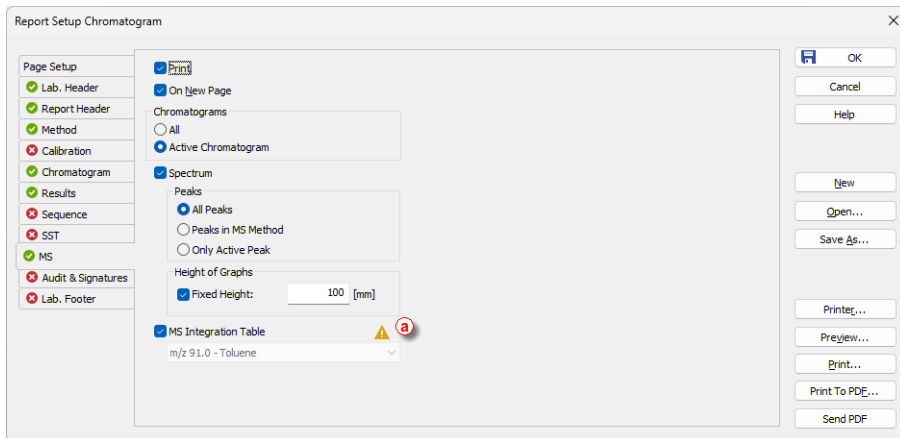



Fig 31: Report Setup - MS

### Print

Allows/disallows the printing of the relevant part. If checked, the symbol  will be shown before the tab name and other fields on the tab will become editable.

### On New Page

If checked, the relevant part will be printed on a new page.

### Chromatograms

Determines which chromatograms should be included in the printout in case more than one is opened. You can choose to include to the report the spectra from all opened chromatograms (*All*) or just the spectra from the active chromatogram (*Active Chromatogram*).

### Spectrum

Governs whether spectral graphs will be printed. Once enabled, lower level sections of *Peaks* and *Height of Graphs* get enabled as well to determine the number and size of spectral graphs. All spectra are printed as they are in the chromatogram, therefore if the chromatogram uses stick spectra, those will be printed.

### Peaks


Determines the peaks whose spectra will be printed in the report. The options are to print the spectra for all peaks present in the **Result Table**

*All Peaks*), all compounds found in the **MS Method** (*Peaks in MS Method*) or only the spectrum of the active peak (*Only Active Peak*). In case the last option is selected in combination with the selection of *All Chromatograms* from the *Chromatograms* section, the spectrum will be printed for each chromatogram if it contains the peak with the same name in the **Result Table**.

### Height of Graphs

If the *Fixed* checkbox is checked, the chromatogram will be printed with a fixed height instead of the fixed ratio 2:3. The permitted lower height range is *30 mm*, the upper height range is not set - the graph will be scaled to the page height if larger than the page itself.

### MS Integration Table

After checking the *Quant. Signal* checkbox, integration tables of quantification signals will be printed, based on selection in combobox. Either all quantification signals or selected (one) quantification signal can be printed. When selected quantification signal is not presented in MS Method Table, following error warning  will be shown. Tooltip telling what is wrong, when mouse cursor hovers over the icon, will be shown.

## 6 Clarity MS operation

Basic operating principles for the work on **Clarity Instruments** configured on use with **MS Extension** are described in this chapter. Only topics related to **MS** mode are discussed here, refer to the **Clarity User Guide** for detailed description of operations in standard mode.

The typical workflow for somebody working with routine analyses should consist of:

### Preparation of the MS Method

Setting MS Instrument described on pg **54**.

Opening the Demo\_MS project described on pg **54**.

Setting MS Search position described on pg **54**.

Creating MS Method described on pg **57**.

Editing MS Method described on pg **59**.

Defining Reference m/z values described on pg **60**.

### Creating the MS Calibration

Creating of the MS Calibration described on pg **61**.

### Routine measurements

Routine measurements will work the same as in standard **Clarity**, performing the integration and identification of peaks automatically, connecting the calibration and showing the results. No significant changes of the workflow are expected compared to non-MS chromatography.

Additionally, user of the **Clarity MS Extension** might want to perform other actions like Importing MS Chromatogram described on pg **62**., Creating and filling your own library described on pg **55**. and Managing MS Library described on pg **56**.

## 6.1 Setting MS Instrument

- Start **Clarity** and open the *System Configuration* dialog.
- Switch to the correct tab in the right part of the *System Configuration* dialog.
- Set the *Instrument Type* parameter to *GC - MS*, *LC - MS* or *GC-MS ToF*, depending on the MS detector you want to use.
- Save and close the *System Configuration* dialog using the *OK* button.

## 6.2 Opening MS demo project

There is a project containing DEMO data for MS Extension prepared in the software. It contains a set of chromatograms, prepared matching calibration and sample MS method. To open the DEMO\_MS project:

- In the opened *Instrument* window, select the *File - Project...* menu command. The *Project Setup* dialog will open.
- Use the *Open...* button to select the *DEMO\_MS* project in the *Open Project* dialog, then press the *OK* button.

Alternatively, the DEMO\_MS project may be opened directly when opening **Clarity** Instrument by selecting it in the *Login Dialog*.

## 6.3 Setting MS Search position

There are two modes of work available in **Clarity** - using libraries (typical to GC-MS applications) or not using them (typical to LC-MS applications). Some of the **MS Extension** features may not be available without the spectral libraries usage (spectra matches, conformities etc.), however the use of spectral libraries is not mandatory.







- If you want to use spectrum identification features, **Clarity** expects that any compound defined in the MS method is identified in the **MS Library**, be it a commercial one or a library self-made by the user. **Clarity** cooperates with the **NIST Libraries** and also saves the files in NIST-compatible format. A small **Demo\_MS** library is pre-created in the installation of the **Clarity**, saved in the folder in C:\CLARITY\DATAFILES\NIST directory. If you already have **NIST Libraries** installed, we would strongly recommend to use those libraries instead as they are probably more rich. Typical placement of the **NIST Libraries** is in the C:\NISTXX folder.
- During the first opening of the *Instrument* window set as *LC-MS* or *GC-MS*, **Clarity** will automatically select the default location of the **MS Library**, for default **Clarity** installation located in (\CLARITY\DATAFILES\NIST folder).

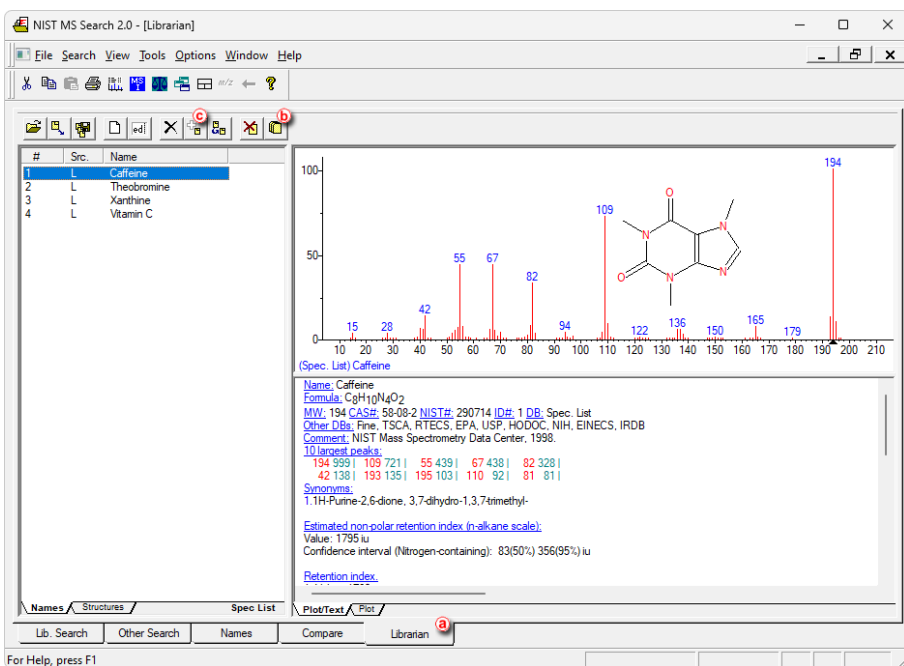
**Caution:** Make sure that the **Demo\_MS** library is on the spot to ensure the demo data function properly.


- You can later change the location of the libraries by using the *MS – Set NIST Libraries Directory...* command from the *MS Chromatogram* window.

### 6.3.1 Creating and filling your own library

Own **MS Library** can be created using external program **NIST MS Search** accessed either from the *MS toolbar* or from the *MS* menu in the *MS Chromatogram* window:


- Use the *Manage Libraries*  button to open the **MS Search**.
- Switch to the *Librarian* tab .
- Use the *Create Library*  button  to create your own library, and *Add to Library*  button  to add the selected compounds into it.




To be able to add selected compounds (or spectra) to own library, such compounds must be selected in the *Librarian* tab. To do so, you can either perform the *Single Compound Search* or use *Add Spectrum to Library*  icon or command from the *MS Chromatogram* window.

#### Add Spectrum to Library

After clicking the *Add Spectrum to Library* icon or command, the view will lock in the graph and will let you select the spectrum. After selecting the spectrum the *Add MS Spectrum to Library* dialog for inserting that particular **MS Spectra** appears:

- Set the *Averaging Time Range* field to perform averaging and smoothing of the spectra inserted into the library – if not selected, the actual spectra as clicked into the graph will be stored.
- Set a *Compound Name* under which you want to add the spectrum into the library.
- Press the OK button.
- The **MS Search** program will open, which allows you to add the spectrum into the library upon switching to the *Librarian* tab and using the *Add to Library*  button.


### Single Compound Search

After clicking the *Single Spectrum Search* icon or command, the view will lock in the graph and will let you select the spectrum. After setting the desired parameters and clicking the *Search* button the **MS Search** program will open. Switch to the *Librarian* tab and using the *Add to Library*  button add the spectrum into the library.

---

*Note:* For more details on *Single Search Compound* please see the chapter "MS Search" on pg 12..

## 6.3.2 Managing MS Library

To manage the **MS Library** (change the compound names or comments, save and rename libraries, ...), use the *Manage Libraries*  button on the toolbar or menu command, which will open the **MS Search** program on the *Librarian* tab. The operations performed there are standard **NIST** functions, so for help on them please revert to the **MS Search** help.

## 6.4 Creating MS Method

Creation of the MS method consists of adding compounds to the MS Method table. In processing the compounds, it strongly depends on the fact whether the compound is identified as a peak on TIC (or another selected "standard" signal) or not, and on the usage of spectral libraries.

### Using spectral libraries - compounds visible on TIC

When there is a library present (you can use the **Demo\_MS** library packed with the **Demo\_MS** project), it is possible to create the MS method. Use either *Single Compound Search* (locks the mouse to the graph and lets you select the peak for which you want to perform the search), *Automatic Compound Search* (performs the Automatic search on all peaks identified on the TIC) or *Target Compound Search* (looks the chromatogram for a compound which has spectrum in the library) function. Appropriate dialog/tab appears, please see the description of work with that particular dialog:

Single Compound Search

Automatic Compound Search

Target Compound Search

Either way, one or more compounds are inserted in the MS method, with the name found in the library.

### Using Spectral Libraries - compounds not visible on TIC

You can, of course, add other peaks which are not visible/identified in the **TIC** to the **MS Method** too. To do so, you will probably need to use the *Target Compound Search* or find the position of the peak on some particular  $m/z$  you know. To extract a temporary signal on the given  $m/z$ , use the *MS – Add Selected  $m/z$  Signal* or *MS – Add Selected  $m/z$  Signal – Manual...* command. This will allow you to select the  $m/z$  in the spectra graph or directly set it in the special dialog. Zoom to the found peak in the graph and use the *Single Compound Search* function. If too many temporary signals are selected, you can hide them by using *MS – Remove Selected  $m/z$  Signals* command.

### Not using Spectral Libraries

When you are not using Spectral Libraries, the procedure of adding compounds to the **MS Method** is slightly different. Instead of using the *Single Compound Search* or *Automatic Compound Search* functions, the *MS – Add Compound w/o Library Spectrum...* command should be used instead. It will invoke the *Add Compound w/o Library Spectrum* dialog which allows you to set the retention time where the desired compound is found in the chromatogram (selected interactively in the graph) and the name of the compound, after which it will be added to the **MS Method Table**. The *Library* column for such compound will be blank.


**Note:** Some of the functions in the **Result Table** will not work for compounds that do not have a compound from spectral library linked. These include the comparison of expected and actual relative intensities for reference ions, as well as compound *Conformity*.

### 6.4.1 Using Single Compound Search

In case of Single Compound Search, the *Single Compound Search* tab of the *MS Search* dialog opens.

- Set the *Max Hits* field – this governs the number of hits later returned to **Clarity**, but only applies if the **MS Search** application is closed when you press the *Search* button.
- Decide whether you want to restrict the search parameters using the *Restrict m/z Range* and/or *Use Selected m/z Only* functions - the search is quicker, but may be imprecise.
- Press the *Search* button.

**Note:** When you do this for the first time, the libraries to search in might not be properly selected – you will have to do so on your own in the **MS Search** software, by using the *Options – Library Search Options* command, switch to the *Libraries* tab and move the desired libraries to the right side of the dialog.

- For the first try, when the libraries were not successfully set, you might need to redo the search by pressing the *Search* button.
- Once the search is successfully performed, return to **Clarity** using the *Switch to Caller*  button.
- The results will show, sorted by the *Match Factor*.
- By clicking on the row in the table you can switch between the results found, which will change the library spectrum in the graph part overlaid with the real spectrum for visual confirmation. The graph allows standard operations like zooming etc.
- Once content with the match, select the given row and press the *Add Selected to Method* button.

### 6.4.2 Using Automatic Compound Search

In case of Automatic Compound Search, the *Automatic Compound Search* tab of the *MS Search* dialog opens. Operation is very similar to the *Single Compound Search*, just after pressing the *Search* button, the automatic search of all peaks identified in **TIC/BPI/on selected m/z** in the chromatogram is performed through selected libraries, and only the best match for each peak is shown in the table. You can then select any number of rows (by using **Shift+click** and **Ctrl+click**) and press the *Add All Selected to Method* button.

### 6.4.3 Using Target Compound Search


Target Compound Search is a two-step process serving for finding the presence or position of given compound (for which the user knows the spectrum) in the chromatogram. The *Target Compound Search* tab of the *MS Search* dialog opens.

- Press the *Select Compound...* button to open the *Select NIST Compound* dialog.
- Select the correct spectral library to use in the *Library* drop-down menu.
- Type in the name of the compound into the *Search* field. The table below the field will start to populate with compounds from the library which have the name as similar as possible to the typed compound name.

*Note:* The compound name search ignores any apostrophes, commas, dashes etc. you may type. This is the restriction of the **MS Search** external program.

- Select the desired compound in the *MS Library Search* table.
- Press the *OK* button to return to the *MS Search - Target Compound Search* dialog.
- Press the *Search* button. The **MS Chromatogram Search** table will populate with chromatogram intervals where the spectra are similar to the spectrum of the compound searched for.
- Move the *Filter by Min. Match Factor* slider to the right or left to improve the found intervals, or input the desired value into the edit box next to this slider.
- Click on the interval in the **MS Chromatogram Search** table to display the comparison of the library spectrum with the spectrum in the interval.
- When satisfied with the match, select the given row and press the *Add Selected to Method* button.

### 6.4.4 Editing MS Method


MS method is defined on the *Measurement Conditions – MS* tab of the *MS Chromatogram* window. You can quickly get there using the *MS Method*  icon from the *MS toolbar*. Unlike other tabs in the *Measurement Conditions* group, the *MS Method* tab can be edited here.


You can perform most of the operations with the MS method here, including defining the Identification/Reference *m/z*, intervals for given peak detection, it's mean spectra calculation, background subtraction intervals etc. You can also rename the compound here, the newly defined name will override the Library name, but will not change it in the library itself.

## 6.5 Defining Reference m/z values

You can define **Quantification** (*Quantify On* column) and **Reference** (*EIC 1..4 - Reference m/z 1..4* columns) m/z according to the needs of your analysis.

When setting the Reference m/z values for the given peak, you can either:

- enter the m/z's for the peaks directly by entering the values you know, which are tabulated or which you see in the graph into the particular cell.
- enter the numbers interactively using the *Add Reference m/z*  icon or menu command while you have a row in the **MS Method Table** selected; while using the icon/command, the mouse will be locked in the **MS Spectra** graph and you will select the desired m/z stick. The m/z value of this stick will be filled in the first free *EIC - Reference m/z* field on that row.


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**Note:** You can define the peak tags being shown in the **MS Spectra** graph by using the *Properties...*  command from the graph local menu and going on the *MS Spectrum* tab.

Setting of the **Quantification** signal is straightforward - select one of the options in the *Quantify On* column for the given compound (table row). Each compound can be quantified on different signal.


## 6.6 Creating of the MS Calibration

To create the **MS Calibration** file, it is enough to click the *Create MS Calibration* button on the **MS Method** pane. Most of the things that users normally fill in in the **Calibration** window were already filled into the **MS Method**, so all of these information will be automatically copied into the **MS Calibration** window. Most of the operations are the same in the **MS Calibration** window as are in standard **Clarity** calibration.

Even though **MS Evaluation** uses a lot of signals, only one calibration compound table is present in the **MS Calibration** window. The quantification signal changes for each peak depending on what is set as a quantification signal in the **MS Method**. There is a new *Fill All Responses from Quant.*

*Signals*  button in the **MS Calibration** window, which will fill in the respective responses from all peaks present in the **MS Method** to the opened calibration level. The new button is there to cope with the switching quantification signals on different peaks.

To create the **MS Calibration**:

- On the **MS Method** tab in the **MS Chromatogram** window, press the *Create MS Calibration* button.
- The view will switch to the **MS Calibration** window.
- Open the calibration standard file as usual.
- Select the correct calibration level and ensure all calibration parameters are set correctly (calibration/recalibration, automatic/manual, ...).
- Fill in the particular compound amounts into the *Amount* column.
- Use the *Fill All Responses from Quant. Signals*  button.

Same as in standard **Clarity**, you have to save the created **MS Method** as a template to be able to measure according to it, and connect the calibration file with the chromatogram to see the calibrated results.

## 6.7 Importing MS Chromatogram

- Open the Chromatogram window on the *Instrument* where **MS Extension** is active.
- Use the *File – Import Chromatogram...* command to get the import selection. Select a file and press the *Open* button.

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**Note:**       **Clarity** currently supports \*.CDF, \*.MZDATA, \*.MZXML and \*.MZML files for the import, proprietary formats can be converted using the ProteoWizard MSConvert (<http://proteowizard.sourceforge.net>) software.

- The *MS Import AIA File* or *Import MS Data File* dialog will open (based on the format to import from).
- You can check particular import parameters, and select up to four *m/z Signals* to be automatically extracted from the imported file. The *TIC* and *BPI* signals will be created from the imported data automatically. Also, you may manually enter the *m/z Step* and *Frequency* of the detector, or let **Clarity** count the parameter from the imported data.
- Press the *OK* button to import the file.