

Clarity Extensions

MS Extension


Extensions

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:

Instrument (blue text) marks the name of the window, to which the text refers.

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 (when you already are in the topic describing the window).

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, there are text sections written in format other than normal text. These sections are formatted as follows:

Note: Notifies the reader of possibly interesting information.

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

■ Marks the problem statement or trouble question.

Description: Presents any closer 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** evaluation extension is an optional **Extension** for the **Clarity Chromatography Station** (from version 5.0). 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).

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, choose the **LC-MS** or **GC-MS** (as appropriate) from the *Instrument Type* field in the **System Configuration** dialog.

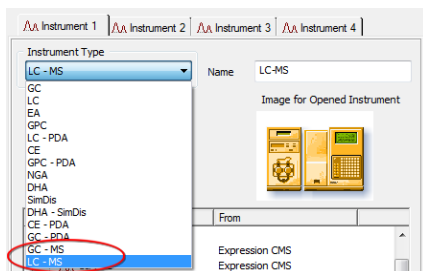


Fig 1: Setting up the MS Instrument

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.

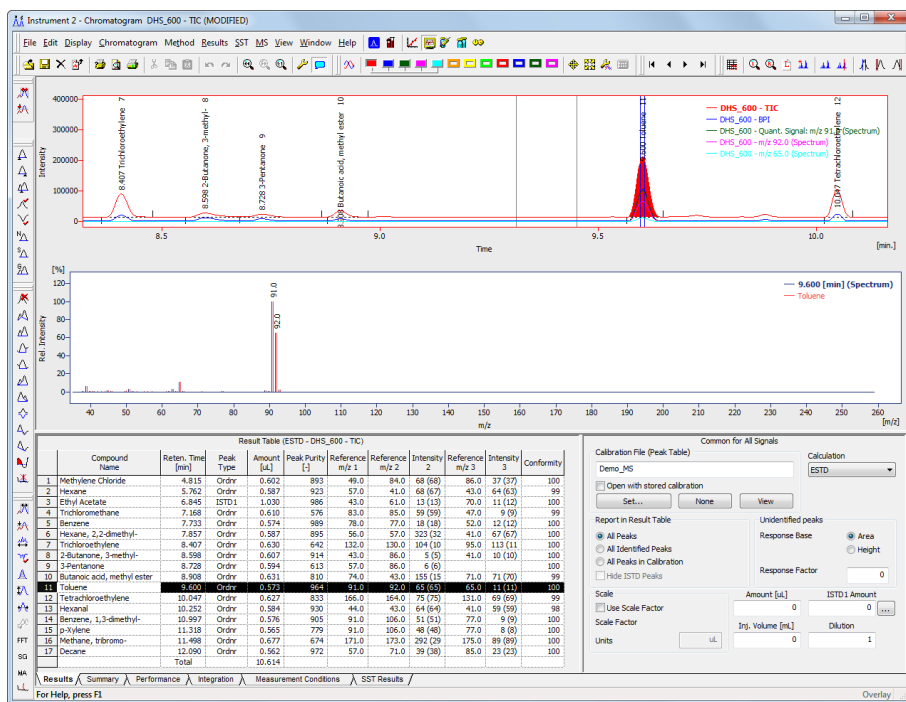


Fig 2: MS Chromatogram window

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 in the **Result Table**. Each peak in the **MS Extension** is defined by up to four *Reference m/z* 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 reference m/z for them will differ too.

The 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. The 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. 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 [Measurement Conditions - 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](#), [Measurement Conditions - MS Method tab](#), [Integration Table](#), [Import Chromatogram](#) and the [Graph Properties - MS Spectra](#) tab.

5.1.1 MS Menu

Menu containing all commands available in the MS Extension.

	MS Method	Switches the display of the lower part of the MS Chromatogram window to the Measurement Conditions - MS Method tab.
Set NIST Libraries Directory...	Allows to set the correct location of the NIST Libraries, if they are installed on the computer. Clarity installation includes also basic NIST demo libraries which are stored in the /UTILS/NIST/MS SEARCH subdirectory of the Clarity working directory (C:/CLARITY by default), which can be used instead.	
<i>Note:</i>		It is necessary to select the folder where the library names are displayed as subdirectories for Clarity to function.
Select Spectral Data	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.	
Prefer Spectral Data from Method	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.	
	Single Compound Search...	Opens the MS Libraries 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 " MS Libraries " on pg 13.
	Automatic Compound Search...	Opens the MS Libraries 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 " MS Libraries " on pg 15.
	Target Compound Search...	Opens the MS Libraries 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 " MS Libraries " on pg 17.

 Manage Libraries...	Opens the MS Search external program which allows to create user libraries, manage them and copy compound spectra to them.
 Add Spectrum to Library...	Sends the selected spectrum to the MS Search external program via Add MS Spectrum to Library dialog described on pg 20 .
 Show Spectrum	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 Ctrl and clicking twice allows to select the time interval through which the spectrum will be averaged.
 Add Reference m/z	When you are in a MS Method Table row, using this command locks the cursor in the spectrum graph. Clicking on any time adds the given m/z ion clicked on as a reference m/z for the compound. When all four reference m/z ions are filled in, clicking on a new ion in this manner will replace the fourth reference m/z.
Add Compound w/o Library Spectrum...	When invoked, locks the cursor in the chromatogram graph pane. Clicking on any time will invoke the Add Compound w/o Library Spectrum dialog described in the section Add Compound w/o Library Spectrum on pg 12
Add Selected m/z Signal	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.
Add Selected m/z Signal - Manual...	Using this function invokes the Add Selected m/z Signal dialog described in the section Add Selected m/z Signal on pg 12 allowing to extract the signal on any chosen m/z from the raw data if maximum signals in the chromatogram is not achieved yet. The signal is extracted using the <i>EIC Bandwidth</i> value from the Measurement Conditions - MS Method tab, so it is possible to get a signal for the range of m/z's by setting the central m/z and appropriate bandwidth.

<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</i> or <i>Add Selected m/z Signal - Manual...</i> function, but not the Quantification signal.
Remove Selected m/z Signals	Serves for removing the no-longer necessary extracted signals from the display in the MS Chromatogram window. The currently active extracted signal will be closed upon invoking this command.
Mean and Backgrounds	Command opening sub-menu with commands used for setting the mean and background evaluation intervals.
 Set Mean Calculation Interval	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 Mean and Backgrounds dialog.
 Set Background 1 Interval	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 Mean and Backgrounds dialog.
 Set Background 2 Interval	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 Mean and Backgrounds dialog.
Configure Mean and Backgrounds...	Opens the Mean and Backgrounds 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 23).

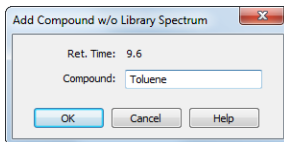
Add Compound w/o Library Spectrum

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.

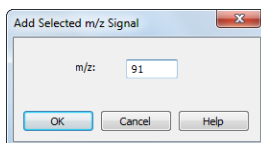
Add Selected m/z Signal


Fig 4: Add Selected m/z Signal dialog

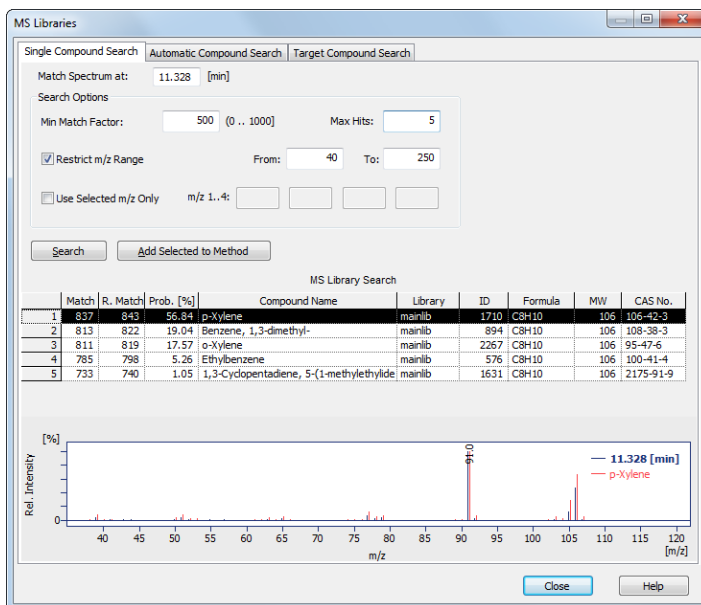
The dialog invoked after using the *Add Selected m/z Signal - Manual...* command allows to extract the selected m/z signal from the raw data. Any values complying with spectral precision are allowed to be entered into the dialog.

5.1.1.1 MS Libraries

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



The screenshot shows the 'MS Libraries' dialog box with the 'Single Compound Search' tab selected. The 'Match Spectrum at' field is set to '11.328 [min]'. Under 'Search Options', 'Min Match Factor' is 500, 'Max Hits' is 5, and 'Restrict m/z Range' is checked with 'From' 40 and 'To' 250. Below the search options is a table of search results.

Match	R. Match	Prob. [%]	Compound Name	Library	ID	Formula	MW	CAS No.
1	837	845	56.84	p-Xylene	mainlib	1710	C8H10	106-106-42-3
2	813	822	19.04	Benzene, 1,3-dimethyl-	mainlib	694	C8H10	106-108-50-3
3	811	819	17.57	o-Xylene	mainlib	2267	C8H10	106-95-47-6
4	785	798	5.26	Ethylbenzene	mainlib	576	C8H10	106-100-41-4
5	733	740	1.05	1,3-Cyclopentadiene, 5-(1-methylethylidene)	mainlib	1631	C8H10	2175-91-9

Below the table is a mass spectrum plot showing 'Rel. Intensity [%]' on the y-axis and 'm/z' on the x-axis. A blue line represents the spectrum at 11.328 min, and a red line represents the reference spectrum for p-Xylene. The x-axis ranges from 40 to 120 m/z.

Fig 5: MS Libraries - Single Compound Search dialog

Match Spectrum at

Defines the retention time in the chromatogram. The spectrum from that retention time will be compared with the spectral libraries.

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 the value set in the field.

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 **MS**

Search program is closed.

Restrict m/z Range

Sets the range of m/z values that will be compared with the library spectra.

Use Selected m/z Only

Performs the search only using 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.

Search

When invoked, the search in **NIST Libraries** is performed using the external **MS Search** program. The MS Libraries being searched in can be set in the **MS Search** program (menu options *Options - Library Search Options - Libraries* tab), along with other search parameters. To get the search results back to **Clarity**, it is necessary to use the *File - Switch to Caller* ← command in the **MS Search** program.

Note: Searched parameters changed in the [MS Libraries - Single Compound Search](#) dialog will not be applied to the search routine if the **MS Search** program is not closed at the time of pressing the *Search* button. This is the restriction of the **MS Search** program.

Add Selected to Method

Adds the selected compound into the **MS Method Table**. The *Retention Time* of the compound in the table will be set to the time shown in the *Match Spectrum at* field, the name of the compound will be set to *Compound Name* value from the **MS Library Search** table and can be later changed in 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.

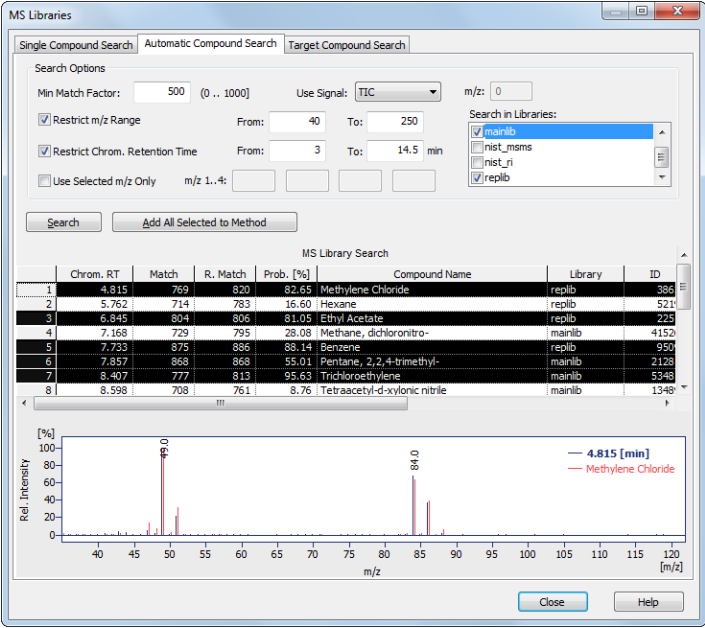
Spectrum graph

The graph displays the overlay of the chromatogram signal as set in the *Match Spectrum at* 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, its 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 local menu on 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.

Note: *Automatic Compound Search* gives just a single "best" hit for each peak found in the selected interval of retention times. It is necessary to check each peak search results carefully before adding all compounds to the **MS Method** table.



MS Library Search

	Chrom.	RT	Match	R. Match	Prob. [%]	Compound Name	Library	ID
1	4.815	769	820	82.65	Methylene Chloride	replib	386	
2	5.762	714	783	16.60	Hexane	replib	521	
3	6.843	804	866	81.00	Ethyl Acetate	replib	223	
4	7.168	729	795	29.08	Methane, dichloroortho-	mainlib	4152	
5	7.733	875	886	88.14	Benzene	replib	950	
6	7.857	868	868	55.01	Pentane, 2,2,4-trimethyl-	mainlib	2128	
7	8.407	777	813	95.63	Trichloroethylene	mainlib	5348	
8	8.598	708	761	8.76	Tetraacetyl-d-xylic nitrile	mainlib	1348	

Chromatogram Plot: Rel. Intensity vs m/z. Peaks at 4.815 [min] (Methylene Chloride) and 86.0 [min].

Fig 6: MS Libraries - Single Compound Search dialog

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 the value set in the field.

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).

Restrict m/z Range

Sets the range of m/z values that will be compared with the library spectra.

Restrict Chrom. Retention Time

Sets the range of retention times in which the automatic search will be performed.

Use Selected m/z Only

Performs the search only using up to four selected m/z values.

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. To apply the settings to the search, the **MS Search** external program must be closed when the *Search* button is pressed.

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.

Add All Selected to Method

Adds the selected compounds into the **MS Method Table**. The *Retention Time* of the compounds in the table will be set to the times shown in the *Chrom. RT* field, 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**.

Note: The MS Library Search table allows multi-selection using the Ctrl and Shift keys, so that the addition of more rows to the table at the same time is possible.


MS Library Search table

This table contains the results of the search. The table contains the *Chrom. RT*, *Match*, *Reverse Match*, *Probability*, *Compound Name*, *Library*, *ID*, *Formula*, *MW* and *CAS No.* columns and gives just the best result for each peak found on a signal defined in the *Use Signal* field.

Spectrum graph

The graph displays the overlay of the chromatogram signal as set in the *Chrom. RT* column and the library spectrum of a compound with best match, as displayed 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](#).

Target Compound Search

Target Compound Search tab serves for looking up the position or presence of the known 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.

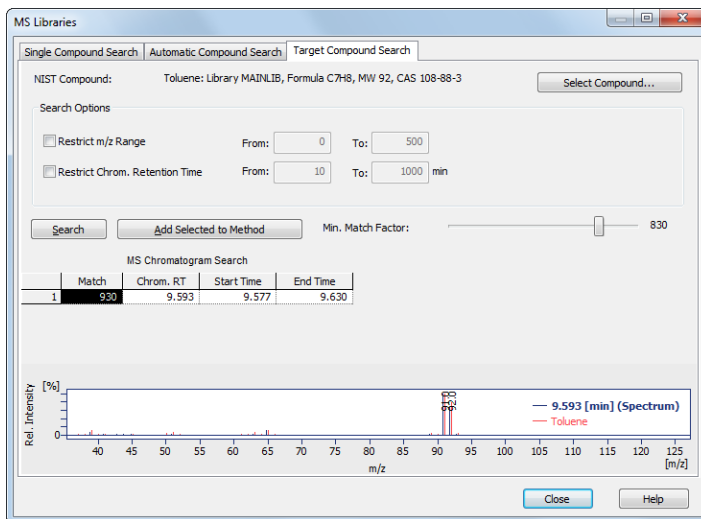


Fig 7: MS Libraries - Target Compound Search dialog

NIST Compound

Shows the NIST compound for whose spectrum you will search. The informational field bears the information on the library in which the spectrum was found, formula, molecular weight and CAS number of the compound.

Select Compound...

Opens the Select NIST Compound dialog for selecting the desired compound:

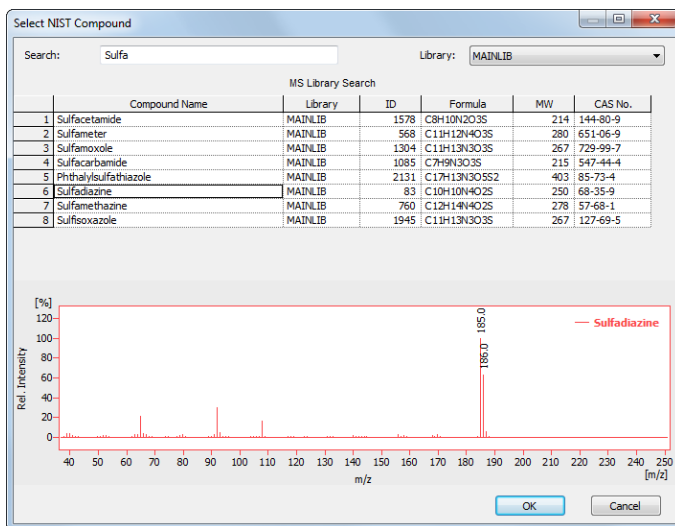


Fig 8: Select NIST Compound dialog

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 compound you wish in the table and press the *OK* button to return to [MS Libraries - Target Compound Search](#) dialog.

Search Options

Restrict m/z Range

Sets the range of m/z values that will be compared with the library spectra.

Restrict Chrom. Retention Time

Sets the range of retention times in which the target search will be performed.

Search

When invoked, the search is started. To apply the settings to the search, the **MS Search** external program must be closed when the *Search* button is pressed.

Add Selected to Method

Adds the selected compound into the **MS Method Table**. The *Retention Time* of the compounds in the table will be set to the times shown in the *Chrom. RT* field, 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**.

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 follows the settings of the *Min. Match Factor* slider.


Min. Match Factor

Move the slider to the right or left to change the intervals displayed in the **MS Chromatogram Search** table. 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 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 pressing the *Add Selected to Method* button.

Spectrum graph

The graph displays the overlay of the chromatogram signal as set in the *Chrom. RT* column and the library spectrum of the spectrum of the compound being searched for. 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](#).

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.

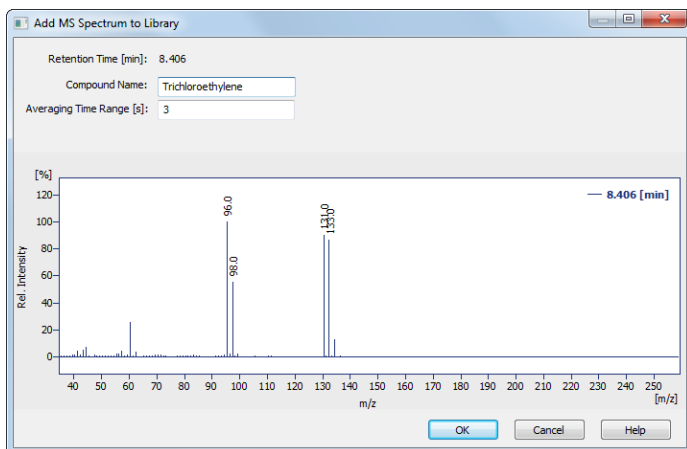


Fig 9: Add MS Spectrum to Library dialog

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.

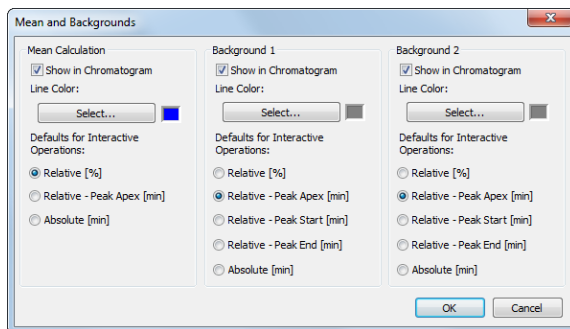


Fig 10: Mean and Backgrounds dialog

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 its 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 "**Measurement Conditions - MS Method**" on pg 28.

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 "**Measurement Conditions - MS Method**" on pg 28.

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.

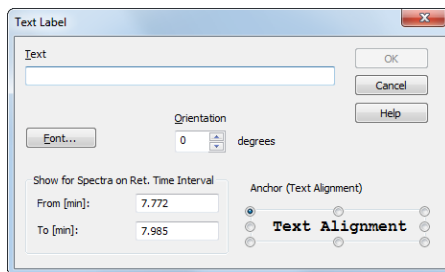


Fig 11: Text Label

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.

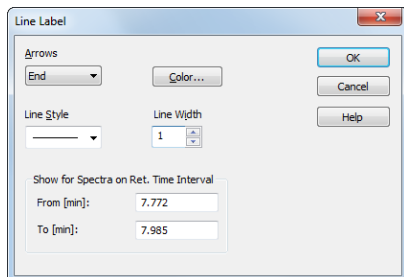


Fig 12: Line Label

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 (ESTD - DHS_200 - TIC)													
	Compound Name	Reten. Time (min)	Peak Type	Amount [uL]	Peak Purity [%]	Reference m/z 1	Reference m/z 2	Intensity 2	Reference m/z 3	Intensity 3	Reference m/z 4	Intensity 4	Conformity
1	Methylene Chloride	4.822	Ordnr	0.324	791	49.0	84.0	60 (68)	86.0	28 (37)	51.0	17 (21)	96
2	Hexane	5.767	Ordnr	0.576	843	57.0	41.0	71 (67)	43.0	66 (63)	56.0	41 (46)	97
3	Ethyl Acetate	6.852	ISTD1	1.188	986	43.0	61.0	13 (13)	70.0	12 (12)			100
4	Trichloromethane	7.173	Ordnr	0.318	513	83.0	85.0	51 (59)	47.0	7 (9)			96
5	Benzene	7.738	Ordnr	0.311	929	78.0	77.0	14 (18)	52.0	9 (12)			99
6	Hexane, 2,2-dimethyl-	7.858	Ordnr	1.301	800	56.0	57.0	345 (321)	41.0	71 (67)	43.0	51 (48)	100
7	Trichloroethylene	8.408	Ordnr	0.655	560	132.0	130.0	104 (104)	95.0	122 (113)	97.0	64 (65)	95
8	2-Butanone, 3-methyl-	8.603	Ordnr	0.230	790	43.0	86.0	5 (5)	41.0	24 (10)			92
9	3-Pentanone	8.733	Ordnr	0.187	681	57.0	86.0	6 (6)					91
10	Butanoic acid, methyl ester	8.910	Ordnr	0.711	766	74.0	43.0	299 (154)	71.0	110 (70)	41.0	119 (37)	93
11	Toluene	9.603	Ordnr	0.416	861	91.0	92.0	63 (65)	65.0	9 (11)			99
12	Tetrachloroethylene	10.047	Ordnr	0.561	686	166.0	164.0	67 (75)	131.0	62 (69)	129.0	65 (72)	92
13	Hexanal	10.253	Ordnr	0.397	949	44.0	43.0	79 (64)	41.0	62 (59)	57.0	55 (47)	88
14	Benzene, 1,3-dimethyl-	10.998	Ordnr	0.445	944	91.0	106.0	48 (51)	77.0	7 (9)	105.0	16 (18)	99
15	p-Xylene	11.318	Ordnr	0.387	750	91.0	106.0	42 (48)	77.0	6 (8)	105.0	12 (14)	98
16	Methane, tribromo-	11.500	Ordnr	0.852	617	171.0	173.0	383 (292)	175.0	85 (89)			60
17	Decane	12.092	Ordnr	0.751	946	57.0	71.0	38 (38)	85.0	20 (23)	43.0	85 (82)	98
	Total			9.609									

Fig 13: Result Table - MS Chromatogram

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 **Measurement Conditions - MS Method** tab.

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 **Measurement Conditions - 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 **Measurement Conditions - MS Method** tab.

Note: The result is also influenced by the setting of the *Conformity of Whole Spectrum* checkbox on the **Measurement Conditions - 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.

5.1.3 Measurement Conditions - MS Method

Measurement Conditions - MS Method tab in the **MS Chromatogram** window serves for setting up the **MS Method**. This tab is different to other tabs in the **Measurement Conditions** section in the fact that it is editable.

Fig 14: Measurement Conditions - 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.

Purity Threshold

Restricts the spectra with *Peak Purity* average match factor below the value specified.

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). The *Purity Threshold* is not used in *Five* point calculations.

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.

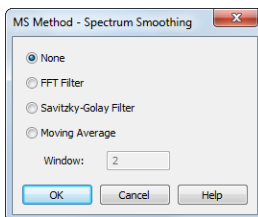


Fig 16: MS Method - Spectrum Smoothing dialog

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.

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, but compounds already present in the [MS Calibration](#) file are not influenced in any way.

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:

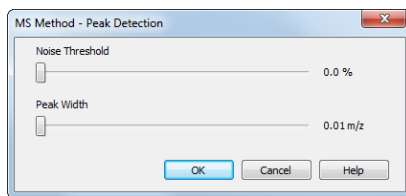


Fig 17: MS Method - Peak Detection

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*.

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 9..

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 and the peak with the *Best Match* of its spectrum to the expected library spectrum that is in the interval. *Nearest* is the default option.

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 Integration Table

The **Integration Table** in the **MS Extension** works little differently than in standard **Clarity**. The table is the same for standard signals and also behaves the same for these, but works differently for quantification signal which is by far the most important signal for evaluating the result in the **MS Extension**.

The **Integration Table** for the quantification signal looks the same, but is in fact composed of multiple parts. The quantification signal meaning change with each peak selected, but for the software it behaves like a simple signal. Some **Integration Table** items are common for the whole signal (and all peaks represented on it), but some are specific for each particular peak and some are even missing on the quantification signal. The items common to whole quantification signal, those specific for each peak and items missing are listed in following table:

Tab 1: Integration Table items

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

This information is important in understanding which parameters can be changed just for one peak and which will influence all peaks detected on the quantification signal.

Note: Take care to the active signal when editing the **Integration Table** for the quantification signal - it is necessary to select the signal before editing the table, otherwise the integration for the first standard signal (most often TIC) will be edited instead. After the signal is selected, it is maintained even after changes of active peak.

5.1.5 Import MS Chromatogram

The dialog for importing the data to **MS Chromatogram** window invoked using the *File - Import Chromatogram...* menu command is also slightly modified from the standard **Import AIA File** dialog. Only different functions are described in this topic.

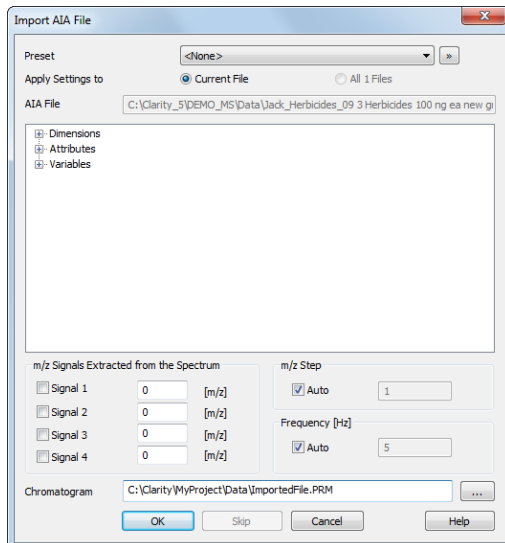


Fig 18: Import AIA File dialog

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.

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.

5.1.6 Export MS Chromatogram

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

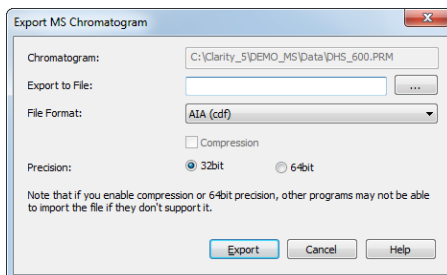


Fig 19: Export MS Chromatogram dialog

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.

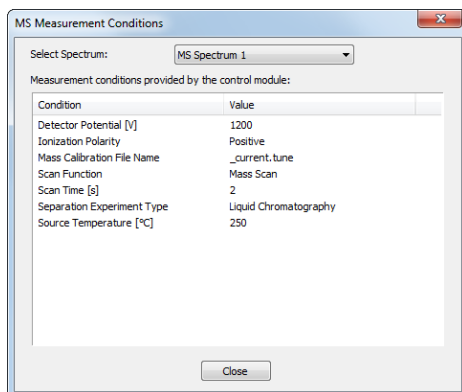


Fig 20: MS Measurement Conditions dialog

5.1.7 Graph Properties - MS Spectra tab

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

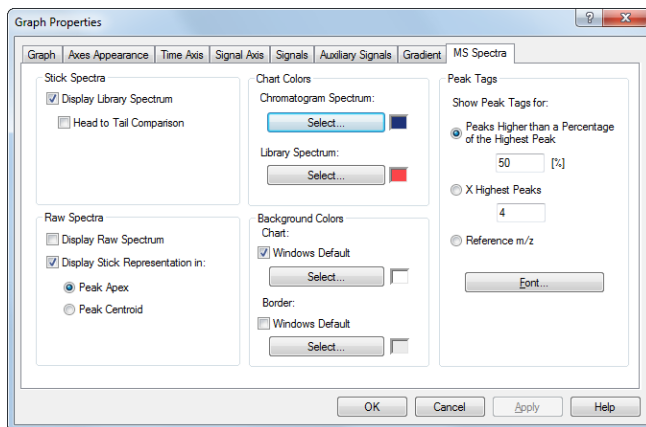


Fig 21: Graph Properties - MS Spectra

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 Raw Spectrum

Sets the presence/visibility of the chromatogram spectrum in the form of raw spectra.

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

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 **Measurement Conditions - MS Method** tab in measured chromatogram and which is described in the chapter "**Measurement Conditions - MS Method**" on pg 28.

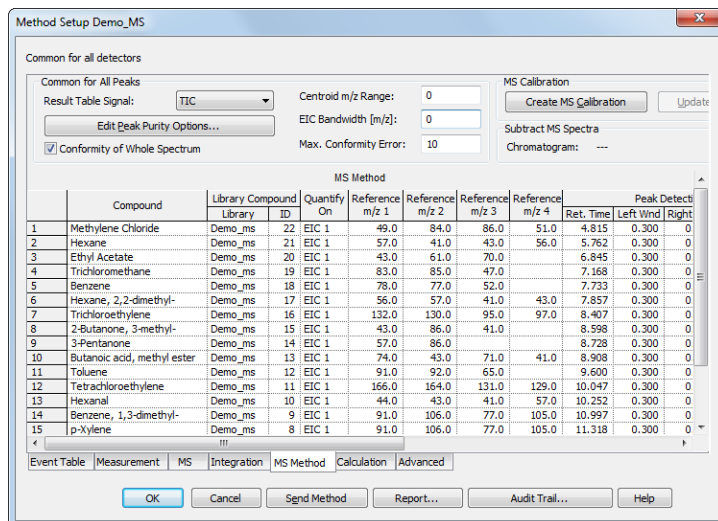


Fig 22: Method Setup - MS Method tab

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 - Integration

The [Method Setup - Integration](#) tab is exactly the same as in standard **Clarity** and also functions the same. This, however, means that the **Integration Table** for the quantification signal is not visible on the tab - the quantification signal is temporary, not present in the configuration/on the Instrument, and thus the user cannot switch to its **Integration Table** by using the *Select Detector* function. The table can thus be only edited on the measured chromatogram, but when the chromatogram method is saved as a template method, the **Integration Tables** for any temporary signals are saved too and applied to any new chromatograms measured according to such method.

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.

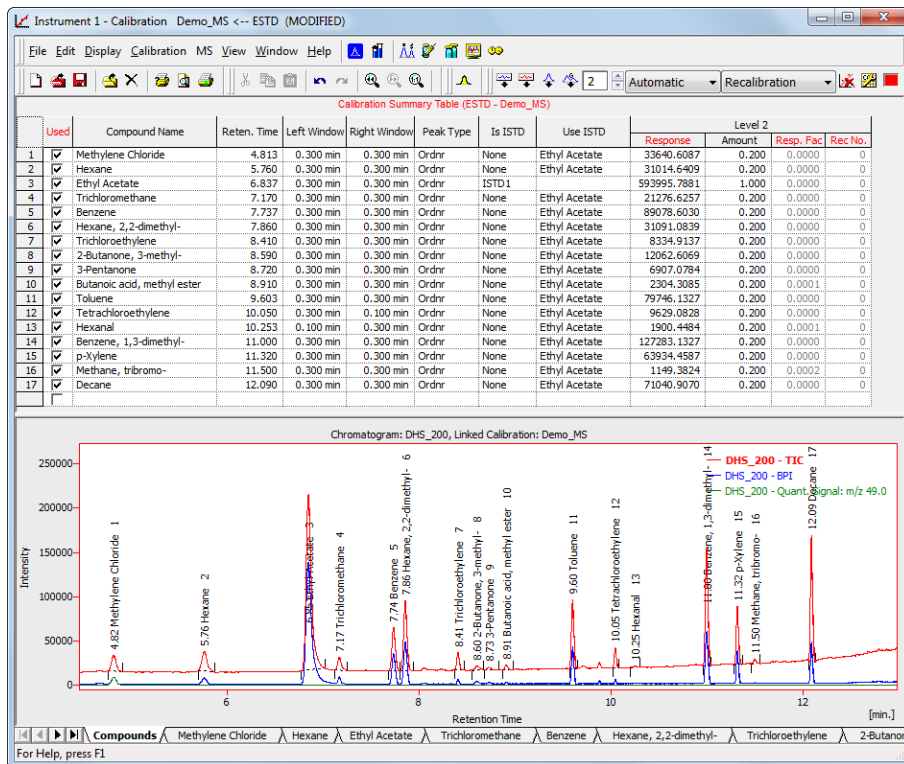



Fig 23: MS Calibration window

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 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**, **Reten. Time**, **Left Window**, **Right Window** and **Peak Selection** (hidden by default). The **Best Match** option, if set in the **MS Method Table**, is changed to **Biggest** in the **Calibration Table**.

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.

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.

5.4 Data Acquisition

The [Data Acquisition](#) window works the same on the **MS Instrument** as on standard **Clarity Instrument** - all of the functions are the same. Moreover, the **MS Extension** adds the *Show Spectrum* command to the *View* menu, which allows to display the current spectrum as acquired by the detector at that moment. The function works only during the acquisition, the displayed spectrum is renewed as soon as the new one is gathered.

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.

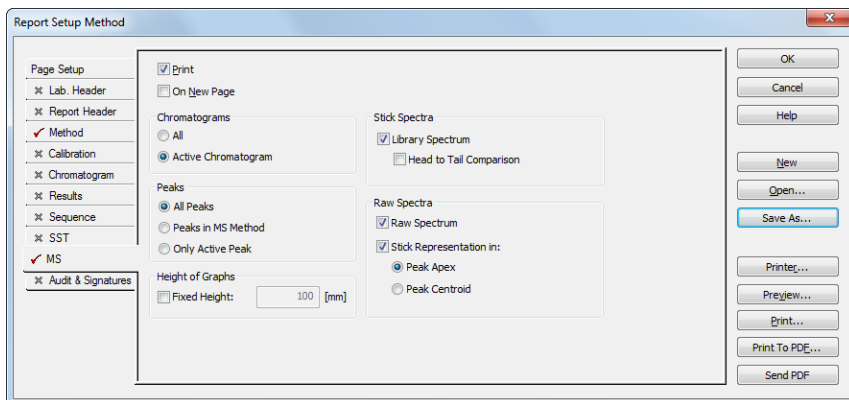


Fig 24: Report Setup - MS

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*).

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*), 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.

Stick Spectra

Defines the format of the stick spectra to be printed. In case the stick spectra are selected in the MS method of the chromatogram, this section defines whether the *Library Spectrum* will be printed too, possibly even in *Head to Tail Comparison*.

Raw Spectra

Defines the format of the raw spectra to be printed. In case the raw spectra are selected in the MS method of the chromatogram, this section defines whether the *Raw Spectrum* will be printed and/or whether just the *Stick Representation* of the *m/z* peaks will be printed, either placed in the *Peak Apex* or in the *Peak Centroid*.

Note: The spectra are printed in WYSIWYG form - what is displayed in the [MS Chromatogram](#) window will be printed. Be careful with cuts in the spectra as these cuts will be applied to all spectra being printed, possibly hiding some spectral lines.

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 50.

Opening the Demo MS project described on pg 50.

Setting MS Libraries position described on pg 50.

Creating MS Method described on pg 52.

Editing MS Method described on pg 54.

Defining Reference m/z values described on pg 55.

Creating the MS Calibration

Creating of the MS Calibration described on pg 56.

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 Evaluation** extension might want to perform other actions like **Importing MS Chromatogram** described on pg 57., **Creating and filling your own library** described on pg 51. and **Managing MS Library** described on pg 51.

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 DEMO_MS 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 Libraries 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 MS Extension**, saved in the folder in C:/CLARITY/UTILS/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*, a dialog will prompt you to select the location of the **MS Library** on the computer. You can go with the default **Clarity** Library folder (/CLARITY/UTILS/NIST folder) or set your custom one.


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


The creation of the **MS Library** is incorporated into the **NIST Search** program used externally, accessed from the *MS toolbar* in the **MS Chromatogram** window. All of the commands are also available in the MS menu:

- 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.

The selected compounds (or spectra) can be shown in the **MS Search** application by performing the *Single Compound Search* function (see below) or using the *Add Spectrum to Library*  icon or command – the view will lock in the graph and will let you select the spectrum, then 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.

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 Libraries](#) 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 Libraries](#) 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 Libraries](#) 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.

- Press the *Search* button. The spectrum of the selected compound will show in the graph below the table.
- Press the *OK* button to return to the [MS Libraries - Target Compound Search](#) dialog.
- Decide whether you want to restrict the search parameters using the *Restrict m/z Range* and/or *Restrict Chrom. Retention Time* functions.
- 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 *Min. Match Factor* slider to the right or left to improve the found intervals.
- Click on the interval in the **MS Chromatogram Search** table to display the comparison of the library spectrum with the spectrum in the interval.
- Once content 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** (*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 *Reference m/z* field on that row.

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.

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 **Import AIA File** dialog will open.
- 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.