DataApex
Clarity™
CHROMATOGRAPHY SOFTWARE

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.
Chromatogram (blue underlined) marks clickable links referring to related chapters.

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 Spectrometry

The **Clarity MS Extension** is an optional part of the **Clarity Chromatography Station**. 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).

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 activated by entering an appropriate user code during the installation of **Clarity** or later using the *Help - User Code…* command from the ***Clarity*** main window.

To enable the MS Extension on an ***Instrument***, you have to set the corresponding instrument type. To set the instrument type, click the ... button in the ***System Configuration*** dialog.

In the invoked *Instrument Type Setting* dialog, select the GC-MS or LC-MS option.

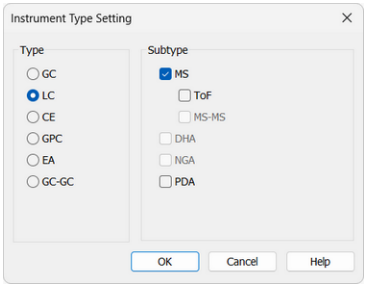


*Fig. 1: Setting up the **LC-MS** Instrument*

4 Key Features

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

- **MS Chromatogram window** - Standard **Clarity** Chromatogram window was modified to additionally display the spectral information. 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** – The MS method defines which extracted ion signals are used for compound identification and quantification. Each compound can have its own reference signals, enabling the detection of multiple compounds within the same retention time.
- **Library search** - **Clarity MS Extension** allows the user to use spectral libraries for peak identification and/or compound confirmation.
- **NIST Libraries** – **Clarity** uses the search mechanism of the *NIST MS Search* program (version 3.0.0.2, year 2023) and by default installs the NIST17 DEMO library for demonstration. It supports the NIST Library format for storing spectra and works seamlessly with commercial NIST Libraries (2023 or older) as well as with user-created libraries in the same format.
- **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 Columns** - 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 it is fragmented.
- **m/z** means *Mass to Charge* ratio. It is calculated by dividing the mass of an ion by its charge. In most cases, ions have a charge of +1 or –1, which means the m/z value is numerically equal to the ion's mass. However, for ions with higher charges, the m/z value is lower than the actual mass.
- **MS Spectrometer** is an analytical instrument used in Mass Spectrometry. It typically consists of several key components: Sample Inlet; Ion Source, where analyte molecules are ionized into charged particles; Mass Analyzer, which separates these ions according to their *m/z* values; and Detector, which measures the number and intensity of the ions. In chromatographic applications, this entire setup is often referred to as an **MS detector**, as it serves as the detection module connected to a chromatograph.
- **MS Spectrum** is the basic information coming out of the MS Detector - it is a set of data describing the relative intensities of ions with particular *m/z* values 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) 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* value, 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 representation of a mass spectrum, showing discrete peaks as vertical lines ("sticks") at their *m/z* positions with heights proportional to intensity. Unlike a raw spectrum, it does not display the full continuous profile of the signal.

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

The *MS Chromatogram* window is used on GC-MS and LC-MS instruments instead of the standard ***Chromatogram*** window. It offers similar functionality but includes specific features related to mass spectrometry. This section focuses only on the differences and additional features specific to the *MS Chromatogram* window.

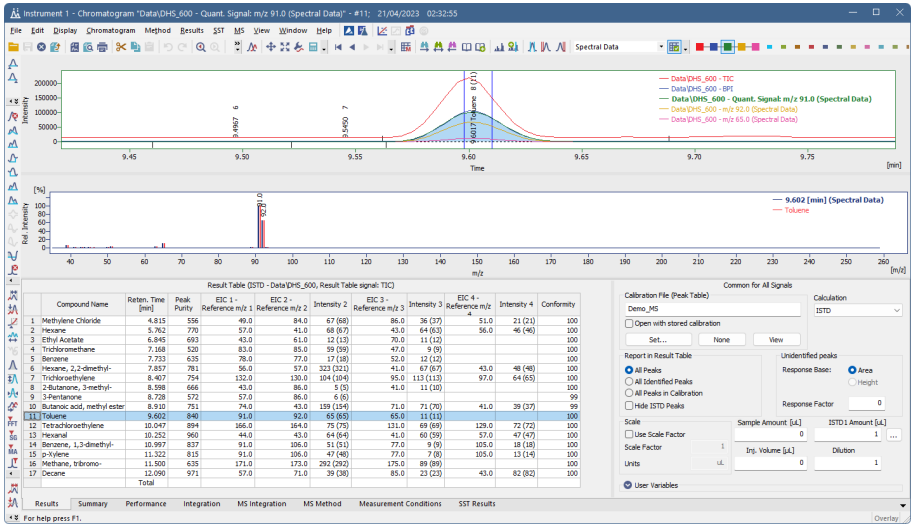


Fig. 2: MS Chromatogram window

Spectral graph

The graph area in the MS Chromatogram window is divided into two parts:

- Chromatogram (top graph) – displays selected signals over time, such as TIC (Total Ion Current), BPI (Base Peak Intensity), quantification m/z and reference m/z signals.
- Spectrum (bottom graph) – shows the mass spectrum corresponding to the currently selected peak or time range.

When a compound is selected, its spectrum is displayed in the spectral graph. A compound can be selected by clicking on it in the chromatogram, or by selecting a row in the Result Table or in the MS Method Table. Selecting a compound in the chromatogram automatically highlights it in the tables, and vice versa. If a library spectrum is available, the spectral graph also displays the matching library spectrum in an overlay.

To view the spectrum for any chromatogram section, use the *MS - Show Spectrum* command.

By default, the spectrum shows relative intensity vs. m/z. You can change this to absolute intensity or adjust label settings in the Graph Properties - MS Spectra dialog (via the Properties… local menu command).

The *Select Spectral Data* drop-down can be used to select displayed spectral data when multiple of them are stored in a chromatogram.

MS Offsetting

A special display mode called *MS Offsetting* lets you visually align signals to the same baseline while keeping their relative intensities. This is useful for comparing signals (e.g. TIC vs. reference ions) and minimizing background noise. You can enable it in the *Graph Properties - Signals* dialog.

Signals in MS Extension

The type and number of displayed signals depend on the selected compound. Different peaks can have different quantification and reference signals and the visible signals may update dynamically based on the current selection.

Signals appear in the following order:

- *Standard signals* defined in *System Configuration* or *Method Setup*.
- *Quantification signal* of the selected peak – this can be one of the reference m/z signals, TIC, their sum, or even an external signal.
- *Reference ion signals* of the selected peak. If a reference ion is also used as a quantification signal, it is shown only once.
- *User-created temporary signals* – additional signals generated on-the-fly from raw data.

A maximum of 32 signals can be displayed for one MS chromatogram. This includes standard, reference, TIC, BPI, external and temporary signals. It is not possible to display temporary signal that would exceed this number.

Temporary Signals

Temporary signals are created on-the-fly from raw data and are not part of the chromatogram file itself. They are visible only on MS instruments and are regenerated whenever needed.

They include:

- All EIC (Extracted Ion Chromatogram) signals used for reference or quantification
- Any summed signals derived from multiple m/z values (e.g. sum of references)
- User-defined signals created by Add Temporary Signal command. These signals disappear after you close the *Chromatogram* window and have to be set again if needed.

View Menu

Maximize Spectrum

The command maximizes the spectrum to cover the entire window (hides the bottom tabs). Double click near the splitter (the cursor will change to ) to achieve the same

effect.

Show All

The command restores default view showing chromatogram, spectrum and the bottom tabs.

MS Measurement Conditions

On the *Measurement Conditions* tab of the *MS Chromatogram window*, *Show MS Conditions…* button is available. This opens the *MS Measurement Conditions* dialog, which displays information provided by the MS detector control module.

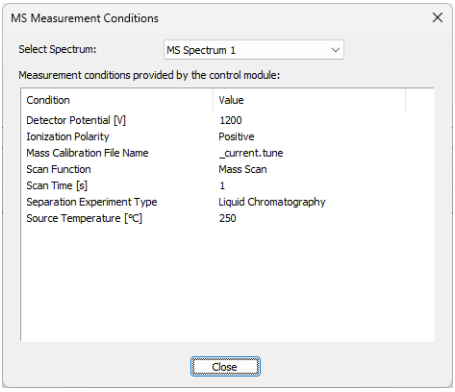


Fig. 3: MS Measurement Conditions dialog

More MS features in Chromatogram window

Changes and new features specific to the MS Chromatogram window that require additional explanation are described in these dedicated sections:

- MS menu
- Result Table
- Integration Table
- MS Integration Table
- MS Method tab
- Import Chromatogram
- Graph Properties - MS Spectra

5.1.1 MS Menu

Menu containing all commands available in the MS Extension.

Icon	Command	Description
	MS Integration	Switches the display of the lower part of the MS Chromatogram window to the *MS Integration* tab, showing *Integration Table* for compound's quantification signals.
	MS Method	Switches the display of the lower part of the MS Chromatogram window to the *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 basic NIST demo libraries stored in the \DATAFILES\NIST\ subdirectory of the **Clarity** working directory (C:\CLARITY\ by default), which can be used instead. It is necessary to select the folder that contains the libraries, with each library represented as a subdirectory. This is required for **Clarity** to function correctly.
	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. It defines whether, for compounds already present in the *MS Method*, the displayed spectrum is the one selected in the method. By default this option is checked, meaning that while the focus changes to different compound defined in the *MS Method*, "active" spectral data chosen in the *Select Spectral Data* dropdown will change.
	Single Compound Search…	Changes the cursor so you can select a retention time in the chromatogram. After you mark the region, the corresponding spectrum is taken and the *MS Search* dialog opens on the *Single Compound Search* tab.
	Automatic Compound Search…	Opens the MS Search dialog on the *Automatic Compound Search* tab.

Icon	Command	Description
	Target Compound Search…	Opens the MS Search dialog on the *Target Compound Search* tab.
	**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 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.
	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 as Temporary	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 Temporary m/z Signal - Manually…	Using this function invokes the *Add Temporary m/z Signal Manually* dialog described in the section **Add Temporary m/z Signal - Manually… on pg. 12** allowing to extract the signal on any chosen m/z from the raw data. It is possible to get a signal for the range of several defined m/z values. Manual Temporary signal is created only if there is free signal slot available in the chromatogram.

Icon	Command	Description
	Remove All Temporary m/z Signals	Serves for removing all user-added temporary signals from the display in the MS Chromatogram window.
	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 to set the defaults for mean calculation interval and background intervals.
	Create Spectrum Label	Creates labels in the MS spectral graph. Two options enable to create either Text labels or Line labels.
	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

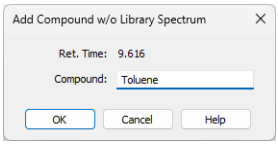


Fig. 4: Add Compound w/o Library Spectrum dialog

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…

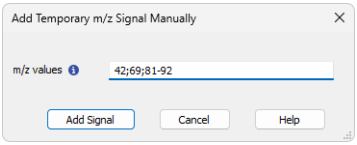


Fig. 5: Add Temporary m/z Signal - Manually…

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.: 42;69;81-92.

5.1.1.1 MS Search

MS Search dialog is used to set the parameters for search in spectral libraries. The search is performed by *NIST MS Search* program mechanism on libraries linked to **Clarity** - either *NIST DEMO17* library installed by default, or commercial libraries purchased by the user. The search results (Compound Name (identity), Match, R. Match) returned by *NIST MS Search* are the same as if the search was performed directly in *MS Search* with the same parameters. The only exception is the *Probability* value, which may differ slightly. It is only an approximate, indicative value derived from the current set of fixed-result values.

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.

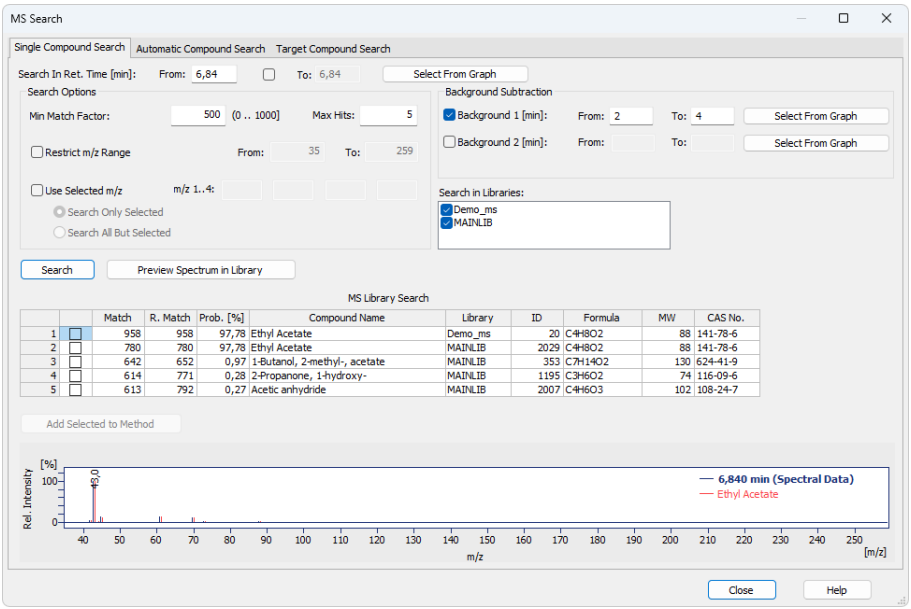


Fig. 6: MS Search - Single Compound Search dialog

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. A single click selects an exact retention time. Holding the CTRL key, you can select a retention time range by clicking once for the beginning and once for the end of the interval.

Search Options

Section allowing to set several parameters for the spectral search.

Min Match Factor

Restricts the hits returned to Clarity from the *MS Search* program to just those with match factor higher than or equals to the value set in the field. Changing the value in the field only applies when the search is invoked again. Valid numbers are whole numbers from 0 to 1000.

Max Hits

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

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. 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 spectral search. It is possible to select multiple libraries.

Background Subtraction

Up to two background intervals can be selected to be subtracted from the spectrum before the search. Such background intervals 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. A single click selects an exact time for background subtraction. Holding the **CTRL** key, you can select a time range by clicking once for the beginning and once for the end of the interval.

Search

When invoked, the search in *NIST Libraries* is performed using the external *MS Search* program.

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

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 in the search options in the *MS Search* program, manual search performed there may thus yield different results. To change parameters used for the search in MS Search program, *Options - Library Search Options* command must be used and changes must be done in the *Library Search Options* dialog (on various tabs).

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 defined in the *Search In Ret. Time* field 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.

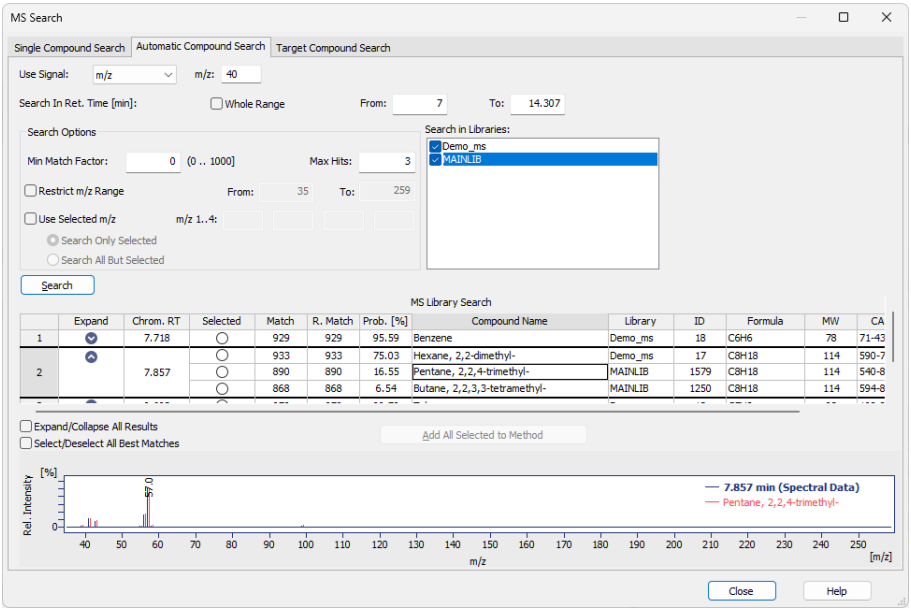


Fig. 7: MS Search - Automatic Compound Search dialog

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 covers the entire range of the chromatogram where the spectral data are available - typically, this means the whole chromatogram. If needed, search interval can be specified in the *from* and *to* edit boxes, enabled after unchecking the check box *Whole Range*. 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

Limits the number of hits returned to Clarity from *MS Search* program for each compound. 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. 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 shows the search results in the following columns:

- *Expand* - Allows you to view additional results for each compound. Click the chevron in this column or use the *Expand/Collapse All Results* checkbox to show or hide all results at once.
- *Chrom. RT* - Retention time of the compound in the chromatogram.
- *Selected* - Marks the library match to be added to the *MS Method*. You can select the best hit directly or expand the list to view and change other matches.
- *Match*, *Reverse Match*, *Probability %* - Numerical indicators of the similarity between the acquired and library spectra.

- *Compound Name*, *Library*, *ID*, *Formula*, *MW* and *CAS No.* - Identification and source information for the matched compound.

By default, the table lists the number of best matches for each peak up to the value specified in *Max Hits*. If fewer results that otherwise match the search criteria (e.g. have *Min Match Factor* higher than set value) are available, only those are displayed.

Only one search result per peak can be selected.

Add All Selected to Method

Transfers the selected compounds from the *MS Library Search table* to the *MS Method table*, including their compound names and retention times. These values can be modified later 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 is used to locate the position or presence of the desired compound in the chromatogram. It is possible to quickly browse the spectra across the whole chromatogram and find the one that best matches 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.

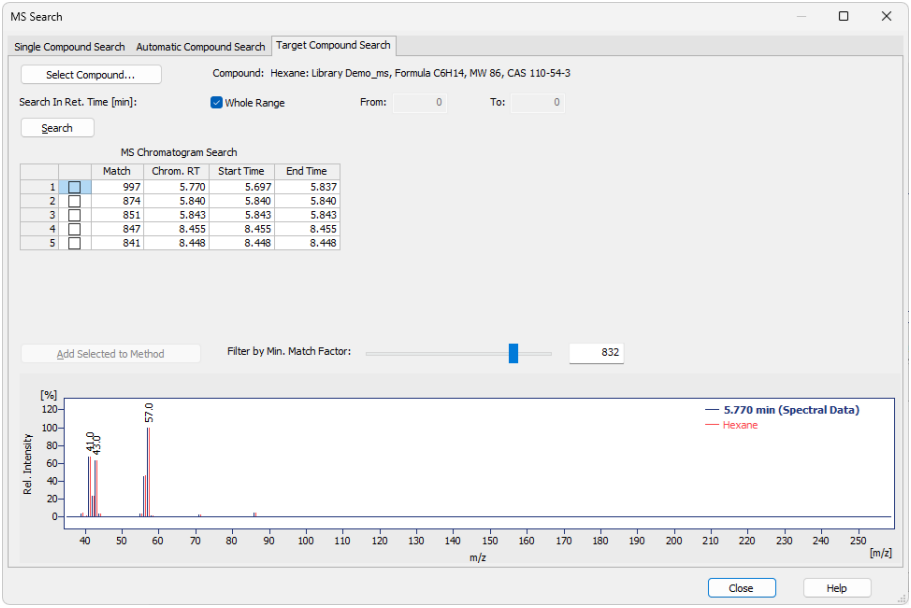


Fig. 8: MS Search - Target Compound Search dialog

Select Compound…

Opens the *Select NIST Compound* dialog for selecting the desired compound.

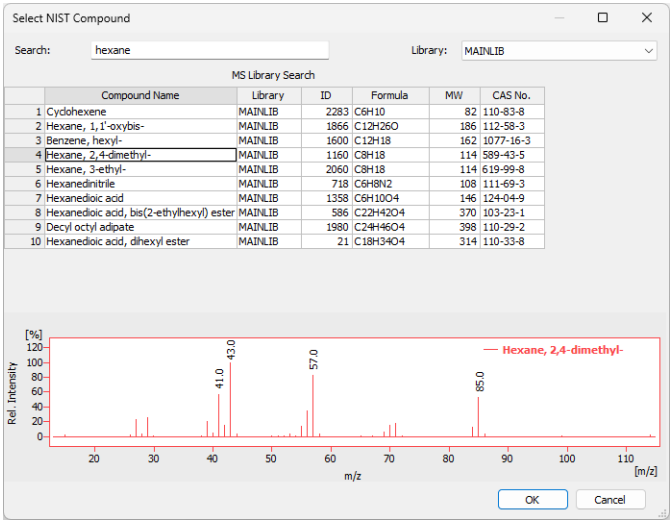


Fig. 9: Select NIST Compound dialog

Select the library from the *Library* drop-down menu and enter the compound name in 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* to return to *MS Search - Target Compound Search* dialog, where the selected compound is displayed in the *Compound* field.

Search In Ret. Time

By default, the search interval is the whole range where spectral data are defined. If needed, such search interval can be specified in the *from* and *to* edit boxes, enabled after unchecking the check box *Whole Range*.

Search

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

MS Chromatogram Search table

This table shows the search results in the following columns: *Chrom. RT*, *Match*, *Start Time* and *End Time*. The result can be filtered using the *Filter by Min. Match Factor* slider.

Add Selected to Method

Adds the checked rows from *MS Library Search* in 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

Use the slider (or enter the value in the box next to it) to set the minimum *Match factor* for intervals displayed in the *MS Chromatogram Search* table.

- Move right: increases the minimum required *Match Factor*. This may split or narrow intervals and eventually hide them if the value exceeds the best match.
- Move left: decreases the minimum required *Match Factor*, which widens existing intervals or adds new ones with lower-quality matches.

The slider should be set so that the displayed interval is representative for the compound before pressing *Add Selected to Method*.

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, serves for saving the spectrum into the user library.

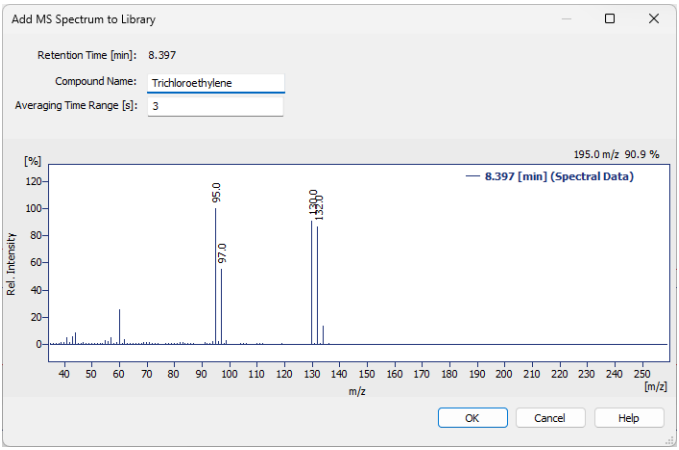


Fig. 10: Add MS Spectrum to Library dialog

The dialog lets the user to set several parameters before sending the spectrum to the library. 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.

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

The *Mean and Background* dialog serves for setting default values for mean and background interval operations. You can open it from the *MS - Mean and Backgrounds - Configure Mean and Backgrounds…* menu command.

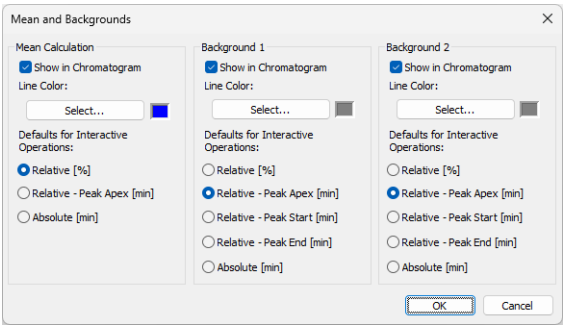


Fig. 11: Mean and Backgrounds dialog

Mean Calculation

Mean Calculation section is used to define how the mean calculation interval is displayed in the chromatogram graph and to set the default values 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 color of vertical marker lines representing 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. **26**.

Background 1..2

Background 1 and *Background 2* section is used to define how the *Background 1* and *Background 2* intervals are shown in the chromatogram graph and to set the default values 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 color of the vertical marker lines representing 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. **26**.

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.

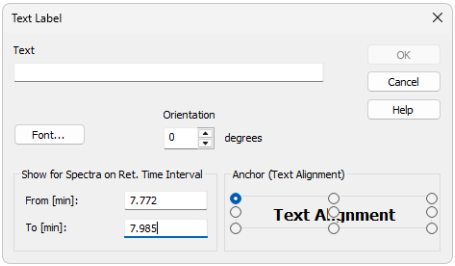


Fig. 12: Text Label

Text

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

Font…

Selects the font, size and color of characters. Invoking this 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. Appears only when the dialog is opened by double-clicking on the existing label.

Show for Spectra on Ret. Time Interval

Defines the retention time interval in which the label will be visible. Set the retention time span using the *From [min]* and *To [min]* fields. The label will be shown if the spectrum is within the defined retention time interval, or if its retention time range overlaps the interval.

Anchor (Text alignment)

Defines the method of label anchoring. One of nine 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.

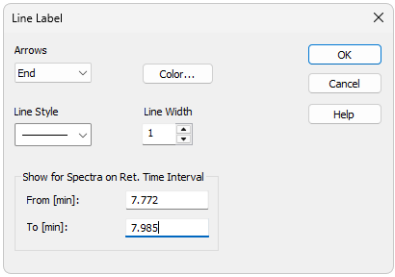


Fig. 13: Line Label

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.

Delete

Deletes the selected label. Appears only when the dialog is opened by double-clicking on the existing label.

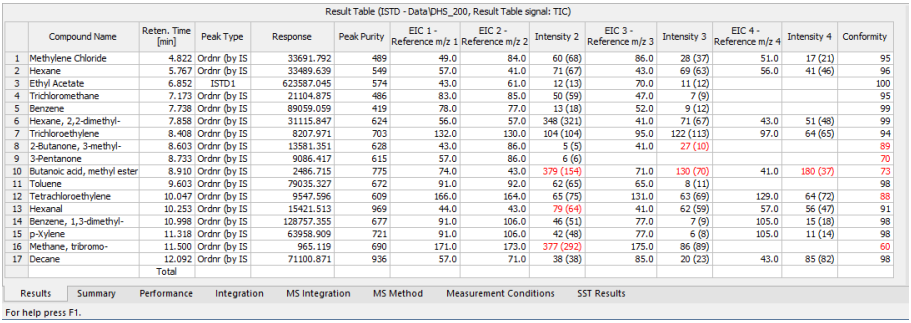
Show for Spectra on Ret. Time Interval

Defines the retention time interval in which the label will be visible. Set the retention time span using the *From [min]* and *To [min]* fields. The label will be shown if the spectrum is within the defined retention time interval, or if its retention time range overlaps the interval.

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\DHS_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	Ordnr (by IS	33691.792	489	49.0	84.0	60 (68)	86.0	28 (37)	51.0	17 (21)	95
2	Hexane	5.767	Ordnr (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	ISTD1	623587.045	574	43.0	61.0	12 (13)	70.0	11 (12)			100
4	Trichloromethane	7.173	Ordnr (by IS	21104.875	486	83.0	85.0	50 (59)	47.0	7 (9)			95
5	Benzene	7.738	Ordnr (by IS	89059.059	419	78.0	77.0	13 (18)	52.0	9 (12)			99
6	Hexane, 2,2-dimethyl-	7.858	Ordnr (by IS	31115.847	624	56.0	57.0	348 (321)	41.0	71 (67)	43.0	51 (48)	99
7	Trichloroethylene	8.408	Ordnr (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	Ordnr (by IS	13581.351	628	43.0	86.0	5 (5)	41.0	27 (10)			89
9	3-Pentanone	8.733	Ordnr (by IS	9086.417	615	57.0	86.0	6 (6)					70
10	Butanoic acid, methyl ester	8.910	Ordnr (by IS	2486.715	775	74.0	43.0	379 (154)	71.0	130 (70)	41.0	180 (37)	73
11	Toluene	9.603	Ordnr (by IS	79035.327	672	91.0	92.0	62 (65)	65.0	8 (11)			98
12	Tetrachloroethylene	10.047	Ordnr (by IS	9547.596	609	166.0	164.0	65 (75)	131.0	63 (69)	129.0	64 (72)	88
13	Hexanal	10.253	Ordnr (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	Ordnr (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	Ordnr (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	Ordnr (by IS	965.119	690	171.0	173.0	377 (292)	175.0	86 (89)			60
17	Decane	12.092	Ordnr (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.

Fig. 14: Result Table - MS Chromatogram

The compounds and values in the *Result Table* are taken from various sources:

- from the MS Calibration
- from the MS Method for compounds not present in the MS Calibration (these appear without amounts)
- from detector signal peaks that match neither MS Calibration nor MS Method, in which case most fields remain empty

Peak Purity

Shows the spectral similarity of a particular spectra contained in the peak, as defined by parameters set in the MS Method - Peak Purity dialog accessible from the MS Method tab. If *Peak Purity* is calculated using the *Five* points method and the *Intensity Threshold* is set too high (above the peak inflection points), an error message is shown together with a tooltip suggesting a solution.

If the chromatogram does not contain spectral information, the spectral information are not selected for the compound in the *MS Method Table* or the peak quantified on

the external signal is outside of the spectrum range, the *Peak Purity* value will not be calculated.

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 different" is set by the *Max. Conformity Error* parameter on the MS Method tab. If the expected relative intensity is below *100*, the value is considered an absolute limit. If 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 score showing how similar a compound's spectrum is to library spectrum assigned in the *MS Method Table*, on the scale of *0* (completely different) to *100* (identical). If the *Conformity* value is too low, the value is displayed in red. The limit for "too different" 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*) or 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 is used to set up the method parameters.

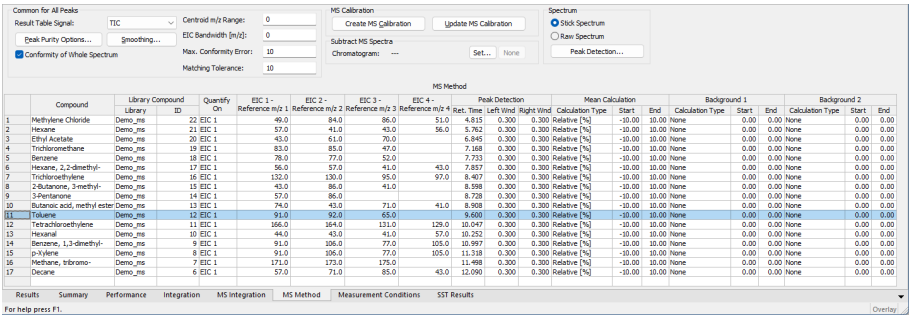


Fig. 15: MS Method tab

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 (displayed in the Result Table).

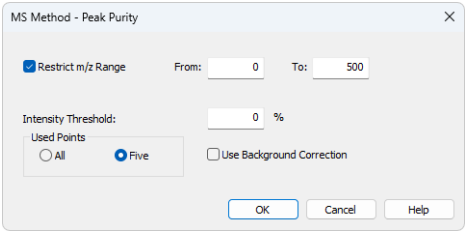


Fig. 16: MS Method - Peak Purity dialog

Restrict m/z Range

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

Intensity Threshold

This parameter specifies the section of a peak, expressed as a percentage of the detected peak height, used for peak purity evaluation. It defines the interval where spectra are included in calculations.

Used Points

Sets whether the calculations of peak purity value will be carried out based on spectra in *All* peak data points or only *Five* significant points, which are:

- the *peak purity start* (located in one-third of the distance between the start point of the interval set by *Intensity Threshold* and peak apex)
- the *peak purity inflexion point* (in two-thirds of the distance between the start point of the interval set by *Intensity Threshold* and peak apex)
- the *peak apex*
- the other *peak purity inflexion point* (in one-third of the distance between the peak apex and end point of the interval set by *Intensity Threshold*)
- the *peak purity end* (in two-thirds of the distance between the peak apex end point of the interval set by *Intensity Threshold*)

Use Background Correction

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

Smoothing…

Clicking the button opens the *MS Method - Spectrum Smoothing* dialog. It is used to define 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 the *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.

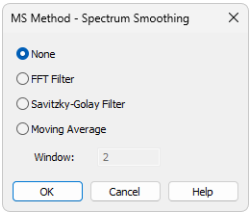


Fig. 17: MS Method - Spectrum Smoothing dialog

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: If 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 relative *Intensity* of reference m/z ions.

Matching Tolerance

Sets the tolerance for the peak maximum position to match peaks in 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 information 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 the MS Calibration file based on the data in the *MS Method* are *Compound Name*, *Quantified On*, *Retention Time*, *Left Window*, *Right Window* and *Peak Selection*.

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.

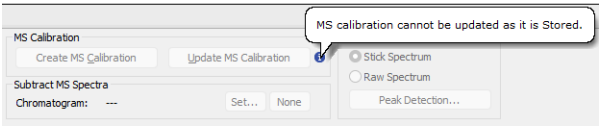


Fig. 18: Displayed tooltip for Stored MS Calibration

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. If multiple spectral data sets are present in the chromatograms, subtraction is performed pair by pair: the first spectrum of the subtraction chromatogram is subtracted from the first spectrum of the opened chromatogram, the second from the second, and so on. If the spectral data do not have the same spectral parameters (Start m/z, End m/z, m/z Step, data frequency), a bi-linear interpolation is performed on the chromatograms prior to the subtraction.

Spectrum

Section defining the way how spectra are displayed in the spectral graph. The user may want to display the spectra in:

- *Stick Spectrum* mode
- *Raw Spectrum* mode, where the library spectra are ignored

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 - deciding on placing representative m/z sticks into the raw spectra while in *Raw Spectrum* display mode.

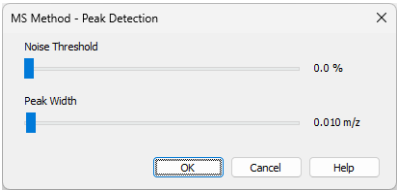


Fig. 19: MS Method - Peak Detection

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. 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* , *Automatic Compound Search*  and *Target Compound Search*  functions from the MS Menu.

Compound

Shows the name of the compound. The name is by default taken from the *MS Library* if the user uses the library search, but can be changed. Each row in the *MS Method Table* must have a unique *Compound* name.

Spectral Data

Selects which spectral data are used to work with the given compound. The column is hidden by default and is filled in with currently active spectral data at the moment of compound's creation, but can be changed later. Even for compounds quantified on external signals, the column is used to provide the source for reference EIC signals.

Note: Within the MS extension, it is possible to work with a chromatogram that contains no spectral data - this may happen for example when the MS detector is switched off in the method but a different detector is left active. Compounds found on such external signal can still be quantified even without the presence of spectral information if their *Quantify on* is set to external signal as well.

Library Compound

Sets the link to the MS library used in the compound evaluation. The link is defined by the *Library* name and compound *ID*. If no spectral libraries are used, the *Library* column will be empty and the *ID* will be set to *0* for the compound.

Note: The columns are colored yellow when Raw spectra are displayed (*Raw Spectrum* mode, no library spectra used). Cells in a row may be highlighted in red to warn that either: the compound ID specified in *Library Compound* column is not available in the defined library, or that the library itself is not at the expected location.

Quantify on

Defines on which signal will the particular compound be quantified. The options are 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, which usually includes *TIC*, 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. **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* and *Peak Selection* is the same as described in the *Calibration Table* of the *Calibration* window.

Retention time and windows define the expected peak retention time and a span to lower and higher retention times, respectively, to search for the peak.

Peak Selection column (hidden by default) defines which peak will be taken if more than one is found in the given time interval on the specific 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 shortened 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* is used to define how the peak spectrum - 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 [%]** - the spectrum shown and compared is calculated as the average spectrum over the interval from *Start* to *End*. The values entered into these fields are expressed as percentages of the peak length, with *–100* representing the peak start, *0* the peak apex, and *100* the peak end.
- **Relative - Peak Apex [min]** - the spectrum shown and compared is calculated as the average spectrum over 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]** - the spectrum shown and compared iis calculated as the average spectrum over the interval from *Start* to *End* The values entered are absolute retention times.

The default settings are *Relative* with *Start* of *–10*, *End* of *10*. There are restrictions on possible values entered into the fields - 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 Type*s 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.

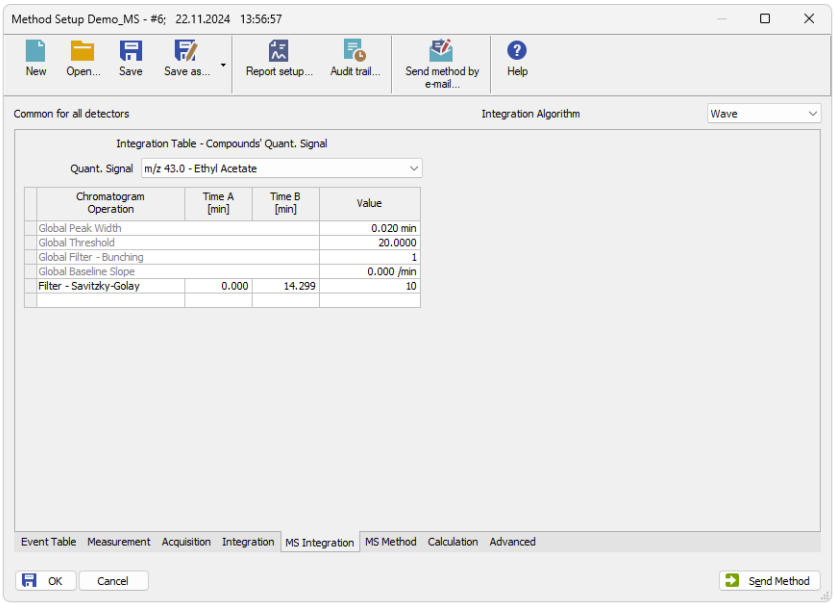


Fig. 20: MS Integration tab

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 Filter - 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].

5.1.5 Import MS Chromatogram

The import dialog opened after selecting files to import in *Select files to import MS chromatograms* dialog depends on the format of the imported MS data.

For the *.CDF format, the *MS Import AIA File* dialog opens (modified from the standard *Import AIA File* dialog). For the *.MZDATA, *.MZML and *.MZXML formats, the *Import*

MS Data File dialog opens. The two dialogs have some features different so they are described separately.

5.1.5.1 MS Import AIA File

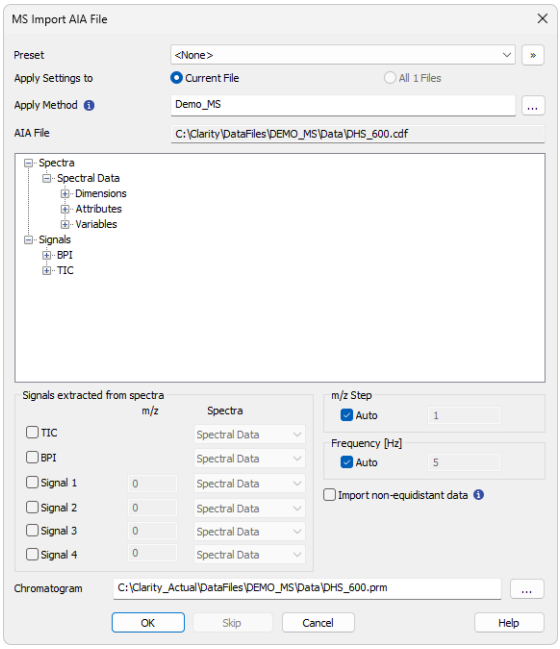


Fig. 21: MS Import AIA File dialog

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). The setting for multiple files is applied to all files imported using the same import dialog (as technically user can select multiple files to import that are exported to different format).

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

Name of the imported file including directory path.

AIA file content preview

Pane that displays the content detected inside the active *.CDF file to import. The file must contain one or more spectral data and may also contain one or more signals, stored in separate structures. **Clarity** displays the data divided into *Spectra* and *Signals* sections, and offers the preview on parameters of the data and/or few first data points.

Note: The *.CDF file which does not contain at least one spectral data structure will be imported through *Import AIA File* dialog instead.

Signals extracted from spectra

Defines which signals should be extracted from the spectral data and stored to the chromatogram as standard signals. A chromatogram in **Clarity** must contain at least one standard signal (on top of possible spectral data), so this section allows to create such signal(s) in case the *.CDF file does not contain any. Alternatively, even when the *.CDF file contains such signals, additional signals can be created through this section. If there are multiple spectral data in the *.CDF file, the section also allows to define from which spectral data the particular signal will be created.

The *TIC* (Total Ion Current) and/or *BPI* (Base Peak Intensity) signals can be created only from particular spectral data selected or from every spectral data available in the *.CDF file. Up to four additional signals are possible, with the m/z precision being limited by the *m/z Step* value of the chromatogram. If the specified m/z value is outside the measured spectrum in the imported chromatogram, the import will not be possible.

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 try to read the m/z step value defined in the data (if it is defined there) or 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, while setting it too high will cause a loss of 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 distorted peak shapes. Setting it too high will create artificial points where the real ones are missing. These artificial points are generated as a linear interpolation between existing points.

Import non-equidistant data

Clarity only works with chromatograms that use equidistant data point spacing. This option allows to import the non-equidistant data as well. The data will be interpolated to equidistant during the import. Please note that MS chromatograms exported to *.CDF files from **Clarity** before version 10.2 did not contain the equidistant data flag so will need to use this option.

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.

5.1.5.2 Import MS Data File

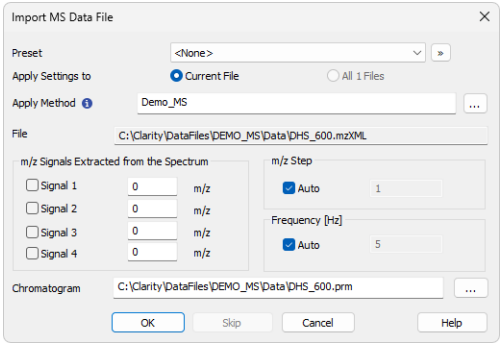


Fig. 22: Import MS Data File dialog

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). The setting for multiple files is applied to all files imported using the same import dialog (as technically user can select multiple files to import that are exported to different format).

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.

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. 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, while setting it too high will cause a loss of 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 distorted peak shapes. Setting it too high will create artificial points where the real ones are missing. These artificial points are generated 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

Clarity provides two different ways to export data acquired on MS instrument. The appropriate export method depends on whether you need to preserve the full MS data suitable for further processing in external MS software or need to only export selected MS signals as standard chromatograms, similar to chromatogram export on non-MS instruments.

5.1.6.1 Exporting full MS data

To export the complete MS chromatogram in an MS-specific data format, use the menu command *File - Export - Export MS Chromatogram…*. This command opens the *Export MS Chromatogram* dialog.

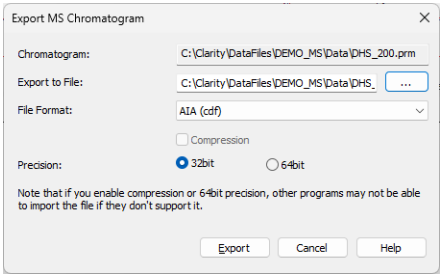


Fig. 23: Export MS Chromatogram dialog

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. Different formats may support different features being exported - namely, in case of a chromatogram with more than one spectral information, the *AIA (cdf)* will export all spectra and also all standard signals to the *.CDF file, while other export formats will only export first spectral data and no standard signals.

Compression

Sets whether the data will be compressed during the export to *mzML* and *mzXML* formats. The target software must support import of compressed data to import it succesfully.

Precision

Sets the data precision during the export. Select the precision according to the target software into which the data will be imported, as some applications support only 32-bit data. **Clarity** supports import with 32-bit precision set.

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.

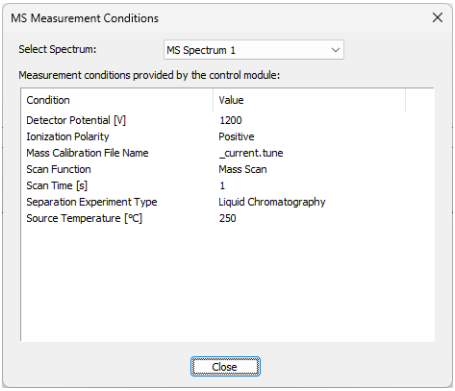


Fig. 24: MS Measurement Conditions dialog

5.1.6.2 Exporting MS data as non-MS chromatogram

To export MS data as a standard chromatogram, use the menu command *File - Export - Export Chromatogram…*.

This command opens the *Export Chromatogram* dialog, which has the same layout and behavior as the standard *Export Chromatogram* dialog used for non-MS instruments. For a detailed description of the dialog controls, see the chapter Export Chromatogram.

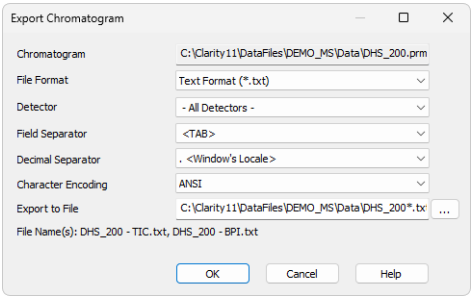


Fig. 25: Export Chromatogram dialog

Using *Export Chromatogram…* allows you to export only predefined MS signals that were configured before the measurement (depending on the specific MS detector setup).

The number of available signals and their m/z definitions are set prior to acquisition, typically in the detector setup in *System Configuration* and in the MS tab of the method.

By default, the available signals include the *Total Ion Chromatogram* (TIC) and the *Base Peak Intensity* (BPI). *Extracted Ion Chromatograms* (EIC) can also be exported, provided they were defined before the measurement, therefore signals created temporarily after the run are not available for export.

The exact configuration workflow and available options are specific to the MS detector control module. Please refer to the documentation of the corresponding MS detector for details.

The export behavior depends on the selected file format: for *.CHR and *.ASC, all selected signals are exported into a single file, whereas for *.TXT and *.CDF, a separate file is created for each signal.

5.1.7 Graph Properties - MS Spectra tab

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

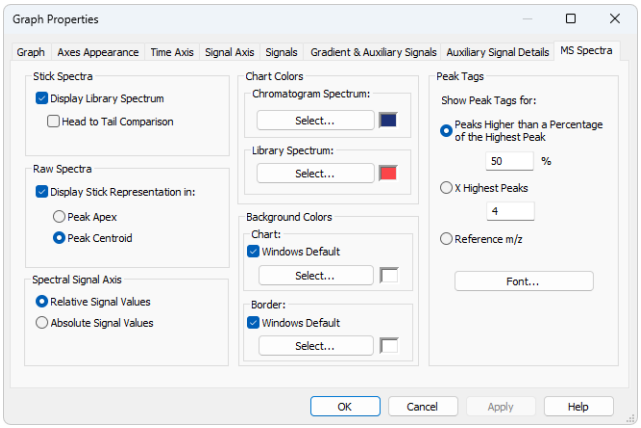


Fig. 26: Graph Properties - MS Spectra

Stick Spectra

Section for 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

The measured spectrum is shown upwards and the library spectrum downwards on the same m/z axis, so matching peaks form mirror images. If not selected, both spectra will be displayed in overlay.

Raw Spectra

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

Spectral Signal Axis

Section for changing the signal axis display.

- *Relative signal values* (default), where library spectra are matched with the compound spectrum using the first reference ion as the 100% reference.
- *Absolute signal values*, where the highest ion in the spectrum is taken as the 100% reference for library spectra.

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 MS Calibration

The *MS Calibration* window resembles the standard *Calibration* window but adds several MS-specific functions.

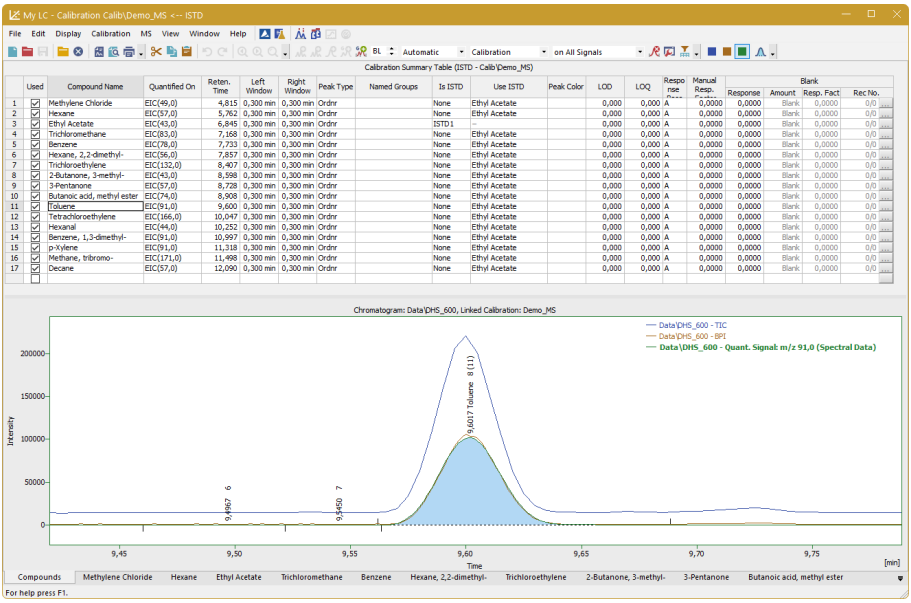


Fig. 27: MS Calibration window

Like the MS Chromatogram window, it contains only one **Calibration Table** even if multiple signals are present. In standard **Clarity**, compounds are usually entered manually. Here, the **Calibration Table** can be filled directly from the MS Method using the *Create MS Calibration* or *Update MS Calibration* commands.

The following information is transferred automatically: *Compound Name*, *Quantify On*, *Retention Time*, *Left Window*, *Right Window* and *Peak Selection* (hidden by default). If *Best Match* or *Weighted EIC Sum* are used, spectral data are also updated.

Calculations are based on the defined quantification signal. The new *MS* menu provides a command to fill calibration responses from these signals. The signal used for each compound is shown in the *Quantified On* column.

Caution: *Left Window* and *Right Window* define the search interval on the quantification signal. If multiple compounds are quantified on the same signal and their windows overlap, Clarity shortens them to avoid overlap. Windows on different signals do not affect each other.

Quantified On

Displays the MS signal used for calibration of each compound. Normally filled when calibration is created via *Create MS Calibration*. If left empty, it is updated when *Fill All Responses from Quant. Signals* is used and the chromatogram contains the required MS information. If settings differ between the MS Method and MS Calibration, the values in the calibration take precedence.

If a peak cannot be found on the specified signal (e.g., it is missing in the chromatogram), its response is not updated and a message is recorded in the calibration and station audit trail.

Two compounds cannot share the same *Retention Time* and *Quantified On*; at least one of these parameters must differ.

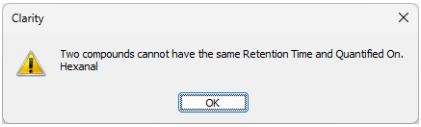


Fig. 28: Warning when Retention Time and Quantified On are identical for two compounds.

Fill All Responses from Quant. Signals

This command (or icon) fills the selected calibration level with responses of all peaks. Each response is taken from the compound's quantification signal and integrated according to the calibration standard chromatogram. This option is disabled when opening a stored MS Calibration.

Caution: This function cannot be used if compound settings differ between the *MS Method* and the *MS Calibration*. In such case, responses are not updated and a warning message is displayed.

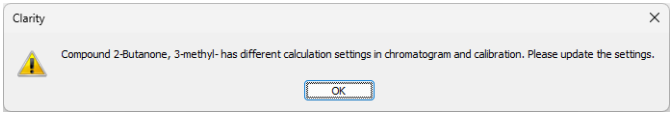


Fig. 29: Warning displayed when compound settings differ in MS Method and MS Calibration.

5.3 Method Setup

Chapters about Method Setup.

5.3.1 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. **26**.

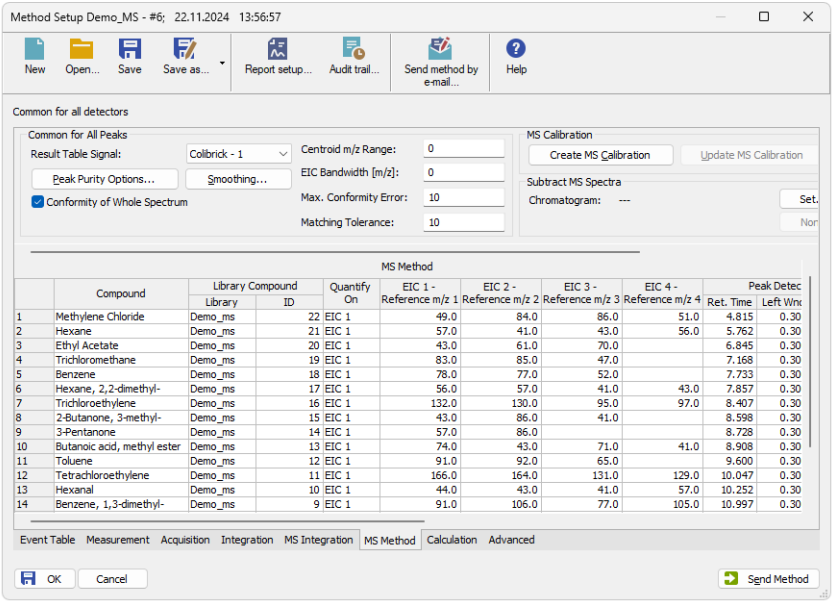


Fig. 30: Method Setup - MS Method tab

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 method file, and measure rest of the chromatograms with it. Modifications in the *Method Setup* dialog may cause side effects which will not be, without the feedback from chromatogram graphs, clearly visible.

5.3.2 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.

For more information see MS Integration.

5.4 Data Acquisition

The *Data Acquisition* window behaves the same way as on a standard instrument.

When using an MS Instrument, two additional commands are available in the View menu:

Show Signal(s)

Switches the display of the *Data Acquisition* window to showing the detector signals.

Show MS Spectrum

Switches the display to the real-time spectrum acquired by the MS detector during a running analysis.

These commands are available at any time. However, Clarity displays spectra only during an active acquisition.



Fig. 31: 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.

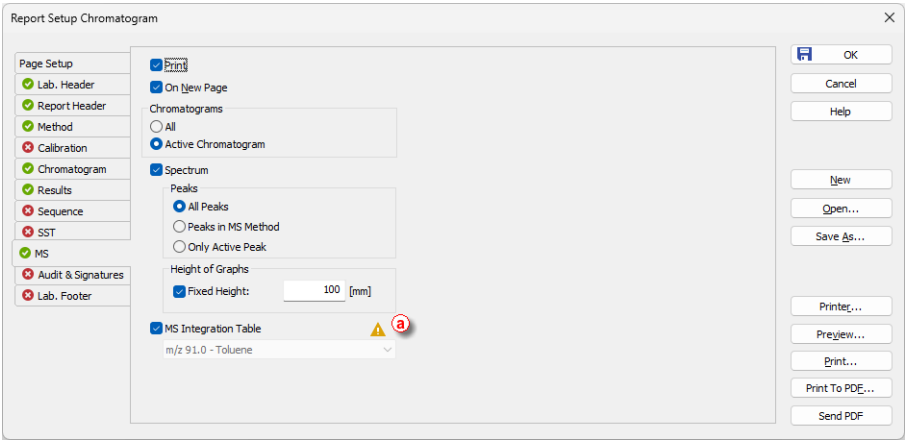


Fig. 32: Report Setup - MS

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 working with Clarity instruments configured for the MS Extension are described in this chapter. Only topics specific to MS mode are covered; for details on standard operation, refer to the **Clarity User Guide**.

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

Preparation of the MS Method

Setting MS Instrument described on pg. **51**.
Opening the Demo_MS project described on pg. **51**.
Setting MS Search position described on pg. **52**.
Creating MS Method described on pg. **54**.
Editing MS Method described on pg. **60**.
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. **63**., Creating and filling your own library described on pg. **53**. and Managing MS Library described on pg. **54**.

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 Integration of signals in MS

In MS Extension, two types of signals can be integrated: Quantification Signals and Result Table Signal.

Result Table Signal

Result Table Signal serves for filling the *Result Table* with peaks other than those mentioned in *MS Method*. Any signal from the detectors configured on the particular Instrument can be selected as Result Table Signal.

- Switch to Integration tab in Chromatogram window.
- In the drop-down list select Result Table Signal (as defined on MS Method tab). If the Result Table Signal is not selected, integration table will not be shown at all.
- Apply integration operations as desired.

Quantification Signal

Quantification Signal serves for quantification of compounds mentioned in MS Method. Integration table is unique for each quantification signal. When multiple compounds are quantified on the same quantification signal they will share the same table.

- Switch to *MS Integration* tab in *Chromatogram* window.
- In the drop-down list select desired compound (defined by m/z ion and the name). Chromatogram will be focused on the compound's peak.
- Apply integration operations as desired.

Note: Even if you select Result Table Signal, e.g. TIC also as your Quantification Signal for any of your compounds, the integration table from Result Table Signal will not be used for such Quantification Signal. Each quantification signal has its own integration table.

6.4 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, conformity 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*, either in a commercial one or a user-created library. **Clarity** works with NIST Libraries and saves the files in NIST-compatible format. A small Demo_MS library is installed by default in C:\CLARITY\DATAFILES\NIST directory. If you already have NIST Libraries installed, we recommend using them, as they usually contain more spectra. 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).

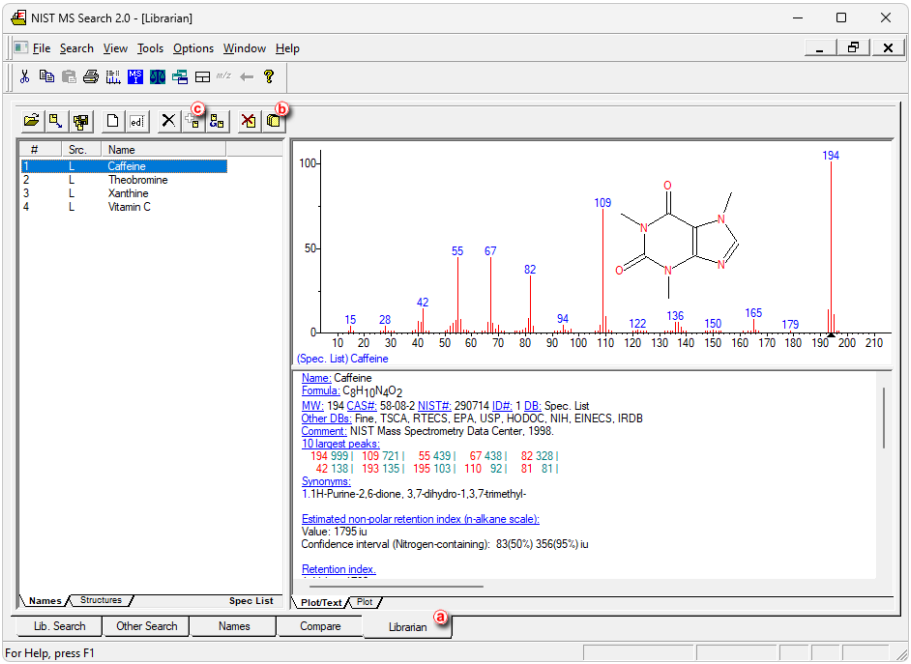
Caution: Make sure that the Demo_MS library is on the spot to ensure the demo data function properly.

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

6.4.1 Creating and filling your own MS 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 to this library.



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**..

6.4.2 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.

6.5 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 in TIC

When a spectral library is available (for example, the Demo_MS library included with the Demo_MS project), you can create an MS method. Clarity offers three search options:

- Single Compound Search: Locks the mouse to the graph so you can select a peak to search for in the library.
- Automatic Compound Search: Automatically searches all peaks identified in the TIC.
- Target Compound Search: Searches the chromatogram for a compound whose spectrum exists in the library.

After selecting a search function, the corresponding dialog or tab will appear. For details, see the instructions for that specific dialog.

In either case, one or more compounds are added to the MS method using the names found in the library.

Using Spectral Libraries - compounds not visible in TIC

You can also add peaks that are not visible or identified in the *TIC* to the *MS Method*. To do this, you will usually need to useTarget Compound Search or locate the peak position for a known m/z value.

To extract a temporary signal at a given m/z, use the *MS – Add Selected m/z Signal* or *MS – Add Selected m/z Signal – Manual…* command. This lets you choose the m/z in the spectra graph or enter it directly in a dialog. Then zoom in on the found peak in the graph and runSingle Compound Search .

If you have selected too many temporary signals, you can hide them using *MS – Remove Selected m/z Signals*.

Not using Spectral Libraries

When spectral libraries are not used, adding compounds to the MS Method is slightly different. Instead of Single Compound Search or Automatic Compound Search, use the*MS - Add Compound w/o Library Spectrum…* command.

This opens the Add Compound w/o Library Spectrum, where you can:

- Select the retention time of the desired compound in the chromatogram (interactively in the graph).
- Enter the compound name.

The compound is then added to the *MS Method Table*, with its *Library* column left blank.

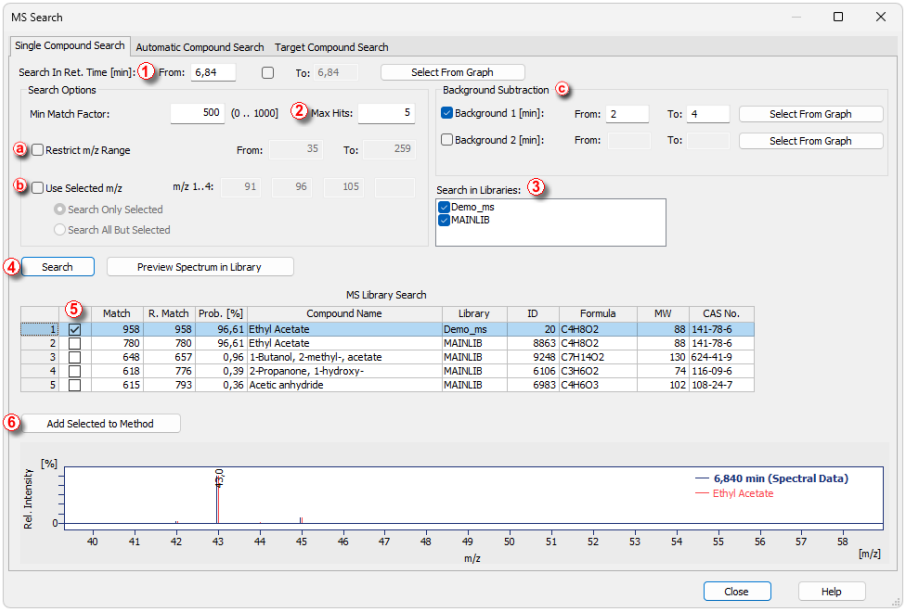
Note: Some of the functions in the *Result Table* will not work for compounds that do not have a compound from spectral library linked. These include the comparison of expected and actual relative intensities for reference ions, as well as compound *Conformity*.

Using non-MS signals (e.g. PDA)

If you want to add a compound quantified using a non-MS signal measured within an MS configuration (for example, a PDA signal), follow the procedure described in this Working with non-MS Signals (e.g. PDA) on MS Instrument chapter.

6.5.1 Using Single Compound Search

When you run a Single Compound Search, the Single Compound Search tab of the MS Search dialog opens.



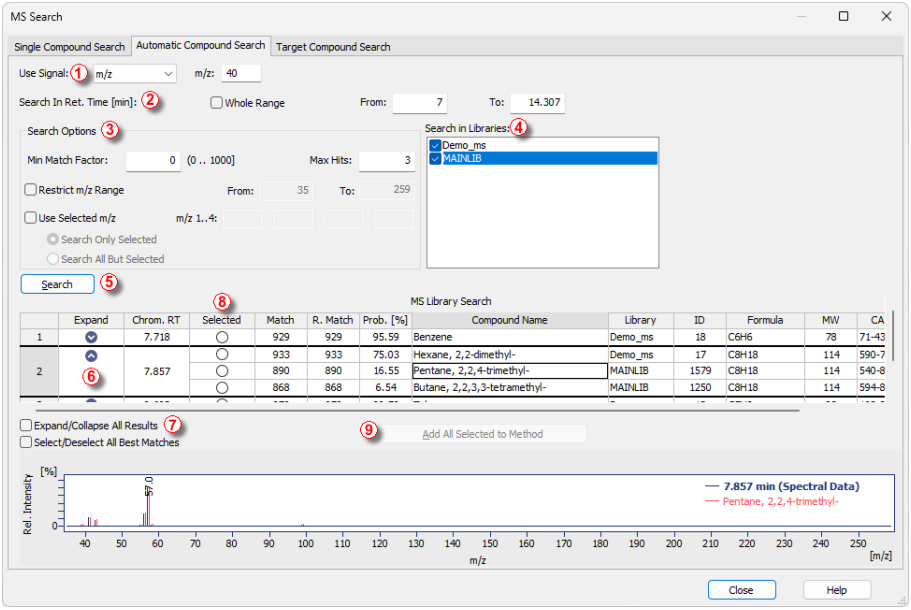
1. In *Search in Ret. Time* ① , you can specify a single retention time or a retention time range in which to search.
2. Set *Max Hits* ② – this defines how many results will be returned to Clarity. This setting applies only if the *MS Search* application is closed when you press *Search*.
3. Optionally, limit the search by selecting *Restrict m/z Range* ⓐ and/or *Use Selected m/z* ⓑ . This can make the search faster but the search may be more inaccurate. Additionally, *Background Subtraction* ⓒ option may be applied.
4. Select the library/libraries to search in using *Search in Libraries* ③ section. Here, both the Demo_ms and MAINLIB libraries are chosen.
5. Click the *Search* ④ button to start the search.

Note: Search parameters for the MS Search program are pre-set to *Spectrum Search Type* - *Identity*, *Quick*; *Presearch*: *Off*. The values may be changed, please see Appendix for more details.

6. Review the results, which are sorted by *Match Factor* . Click a row to display the matching library spectrum overlaid with the actual spectrum in the graph for visual comparison.
7. When you are satisfied with the match (or multiple matches), use the *check box* ⑤ for the chosen compound and click *Add Selected to Method* ⑥ to add the compound to the *MS Method*.

6.5.2 Using Automatic Compound Search

When you run an Automatic Compound Search, the Automatic Compound Search tab of the MS Search dialog opens.



1. In *Use Signal* ① , choose the signal on which peaks will be detected and matched with the spectral libraries. You can select any standard signal (including TIC), an external signal, or a signal extracted from raw data (in which case select the desired m/z in the provided field).
2. Optionally adjust the search parameters as needed, i.e. *Search In Ret. Time* ② , *Search Options* ③ or *Search in Libraries* ④ . These options are the same as in Single Compound Search.
3. Click the *Search* ⑤ button. The search will automatically run for all peaks identified in the TIC, BPI, or on the selected m/z in the chromatogram, using the selected libraries. By default, only the best match for each peak is displayed in the table.
4. To view additional matches for a peak, click the *Expand icon* ⑥ in that row. To expand or collapse results for all peaks, use the *Expand/Collapse All Results* checkbox ⑦ .
5. After reviewing the results, select the desired matches in the *Selected* column using the check box ⑧ (maximum one search result per peak can be selected).
6. Click *Add All Selected to Method* ⑨ to add the chosen compounds to the *MS Method*.

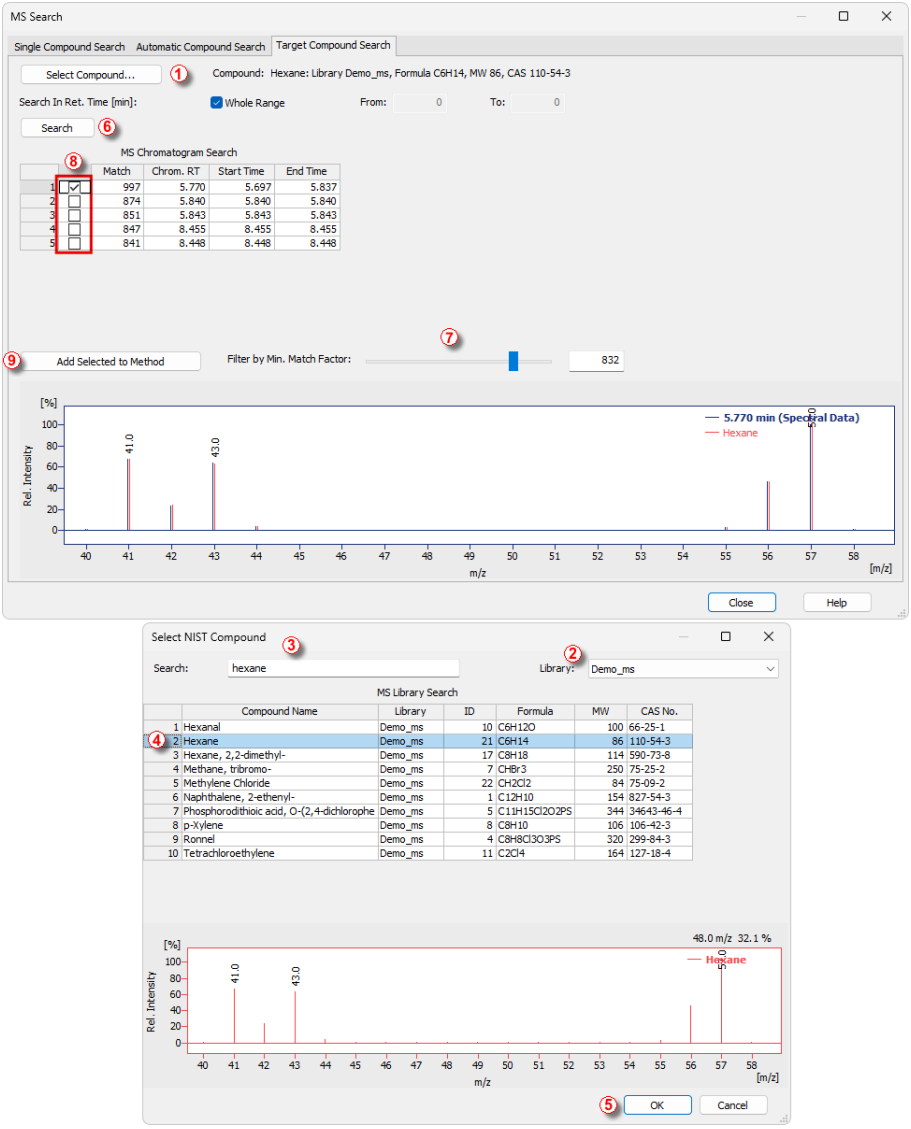
6.5.3 Using Target Compound Search

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. When you run an Target Compound Search, the Target Compound Search tab of the MS Search dialog opens.

1. Click *Select Compound* ① to open the *Select NIST Compound* dialog.
2. In the *Select NIST Compound dialog*, choose the correct spectral library to search in by using the *Library* ② drop-down menu.
3. Type in the name of the compound into the *Search* ③ field. The table lists library compounds with names that best match the typed 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.

4. In the *MS Library Search* ④ table, select the desired compound.
5. Click the *OK* ⑤ button to return to the *MS Search - Target Compound Search* dialog.
6. Click the *Search* ⑥ button. The *MS Chromatogram Search* table will list chromatogram intervals where the spectra match the target compound.
7. You can adjust the *Filter by Min. Match Factor* ⑦ slider or enter a value in the box to filter the matches.
8. When you are satisfied with the match (or multiple matches), use the check box ⑧ for the chosen compound and click *Add Selected to Method* ⑨ to add the compound to the *MS Method*.



6.5.4 Improving Match probability

This topic describes how to improve Match probability with library compounds.

Restrict m/z Range

Allows to limit the m/z range used in the search. If you know the approximate m/z values of interest, you can exclude the rest and consequently increase the Match

probability. If you enter an incorrect range, a dialog will inform you of the valid m/z range for the current chromatogram.

Use Selected m/z

Defines which m/z values should or should not be included in the search.

- *Search Only Selected* – searches only the defined m/z values. Suitable when you know the typical m/z for the compounds of interest.
- *Search All But Selected* – excludes the selected m/z values from the search. Useful, for example, when you want to avoid interference from a solvent signal.

Background subtraction

Specifies exact retention time points or intervals to be excluded from the search. These values can be entered manually or selected directly in the graph.

Preview Spectrum in Library

Shows the search results, based on the above-mentioned options, directly in the NIST library. You can check here, whether your setting were right, or even find out the typical m/z for selected compounds.

6.5.5 Editing MS Method

MS method is defined on the MS Method tab of the MS Chromatogram window. You can quickly get there using the *MS Method* icon from the *MS toolbar*. The *MS Method* tab can be edited.

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.

6.6 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 valuesaccording to the needs of your analysis.

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

- enter m/z values for the peaks directlyinto the cell, using either the values you already know, those listed in a table, or those visible in the graph.
- 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.

Note: You can choose which spectral lines will have peak tags displayed 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.

6.7 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. Since most calibration data are already entered in the *MS Method*, they are automatically transferred to 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. 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 method file to be able to measure according to it, and connect the calibration file with the chromatogram to see the calibrated results.

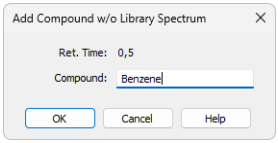
6.8 Working with non-MS Signals (e.g. PDA) on MS Instrument

This chapter describes the workflow for compound identification, and possible quantification of compounds on non-MS signals using an MS instrument. Although the signal is not acquired by MS detector, all processing steps must be performed within the *MS Method*.

It explains how to configure *MS Method* for compounds on a selected non-MS signal and perform integration using the *MS Integration* tab.

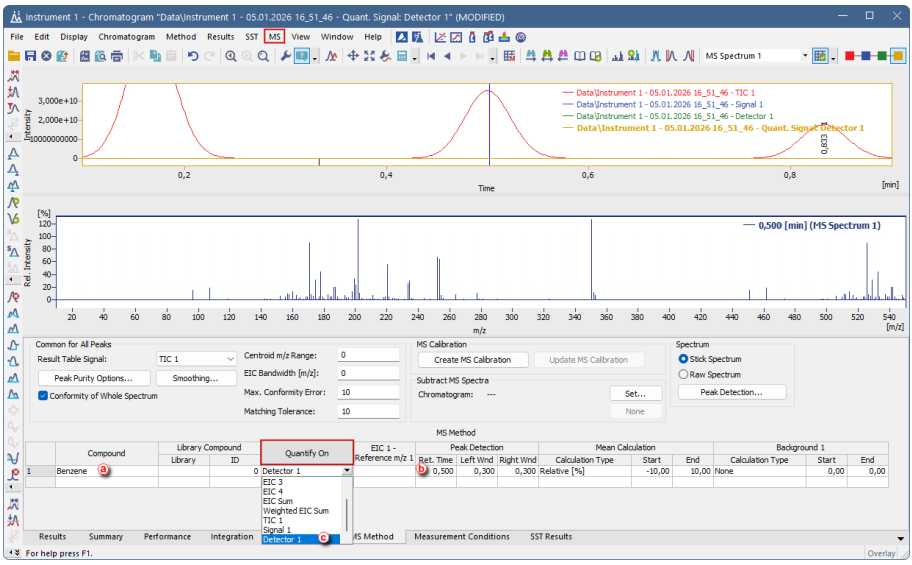
Caution: Ensure that both detectors are configured on the active instrument, providing MS and non-MS signals.

1. Open measured chromatogram and navigate to the *MS Method* tab. Even when working with non-MS signals, such as PDA, all quantification must be configured and processed within the *MS Method*.
2. From the *MS* menu, select *Add Compound w/o Library Spectrum...*. In the chromatogram, position the vertical cursor at the apex of the compound peak. Then, in the opened dialog, enter the compound name as shown in the following image.



Note: Alternatively, you can add another compound in the *MS Method* tab by filling in the compound table, entering the compound name in the *Compound* column ⓐ , and specifying the retention time corresponding to the approximate compound's peak maximum in the *Ret. Time* column. ⓑ

3. For each compound quantified using non-MS signal, select the required non-MS detector signal in the *MS Method* and change *Quantify On* from default EIC to your desired signal ⓒ . In this case configuration of an *EIC Reference* is not required.



4. Define the peak integration parameters for each signal as needed. Integration of non-MS signals must be performed using the *MS Integration* tab. For more information about *MS Integration*, please refer to: Integration of signals in MS.

5. Then proceed using the standard MS workflow for creating an *MS calibration*, as described in the Clarity MS Operation chapter.

Note: All results are reported in a single *MS result* table, as MS results are not separated by signal type.

Note: Integration parameters in Clarity can be defined either as global, affecting all quantification signals, or as local, applied only to a specific signal (see chapter MS Integration). This distinction is particularly important in MS data processing, where different signals may have vastly different signal scales. For example, TIC or extracted ion signals can reach values in the order of millions, while normalized spectral signals are typically scaled to 1 (A.U.). In such cases, global parameters such as *Global Threshold* cannot be set to a single value suitable for all signals. Local parameters (e.g., *Local Threshold*, *Local Peak Width*) should therefore be used to ensure correct integration of each signal independently.

6.9 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 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.

7 Appendix - setting different search parameters

Search performed in the *MS Search* program may follow dozens of different settings. Clarity software does not directly offer all of these settings, but some may be forced to **Clarity** searches using configuration file.

In *MS Search*, these search parameters are available through *Options - Library Search Options* command. Using the configuration file, only two settings can be changed from **Clarity** software - *Spectrum Search Type* and *Presearch*. When nothing is entered in the configuration file, the used parameters in **Clarity** are :

- *Spectrum Search Type - Identity*, *Quick*
- *Presearch - Off*

To change the values, locate the OTHERS.INI file in /CFG/ subdirectory of **Clarity** installation directory (C:/CLARITY by default) and open it in a text editor (e.g. Notepad). Create a new section for MS parameters looking as below:

```
[MS]
SearchMode=Q
Presearch=Default
```

The available values for parameters are:

- *Spectrum Search Type* - key *SearchMode* , available values *I* (meaning *Identity*, *Normal*) or *Q* (meaning *Identity*, *Quick*)
- *Presearch* - key *Presearch*, available values *Default* or *Off*

In future versions, new configurable values or parameters may be added.