Waters™

LC-MS Toolkit

Online Help

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Table of contents

LC-MS Toolkit application overview....6

Configuring LC-MS Toolkit permissions, folder access, and policies....7

Creating a session....9

Creating a session using running samples....10

Browsing sessions....11

Deleting a session....12

Navigating a session....13

Viewing data in LC-MS Toolkit....16

Viewing injection information....16

Viewing chromatograms and spectra....16

Comparing data using the comparison view....22

Copying retention times for MRM data....24

Filtering channels....25

Adding and removing plot gridlines....26

Linking the *x* axes for displayed data....27

Viewing real-time data....27

Removing chromatograms and spectra from a session....29

Specifying the maximum number of MRMs to display....29

Lock mass correction....30

Viewing LC diagnostic channels....31

Viewing DDA data....32

Extracting spectra from DDA chromatograms on the view data page....33

Creating a DDA switch table....33
Filtering and sorting the rows in the DDA switch table....33
Extracting MS/MS spectra from the DDA switch table....34
Copying results from the DDA Switch Table....34

Processing chromatograms....35

Integrating a chromatogram....36
Viewing the peak list in an integrated chromatogram....39
Manual Integration....42

Extracting masses....44
Using Extract Masses....44
Using the Advanced Extract Tool....46

Smoothing a chromatogram....51

Signal-to-noise....52

Combining and subtracting mass spectra....53
Differences between Tof and Quad data when combining spectra....54

Combining mass spectra directly from a chromatogram....54

Extracting a single mass spectrum from a chromatogram....55

Combining and extracting mass spectra from XICs....55

Processing combined MS data....56

Smoothing a spectrum....58

The Mass Measure process....59

Centering a spectrum....60

Producing a deconvolved spectrum with BayesSpray....62

Producing a deconvolved spectrum with MaxEnt1....64

Producing a deconvolved spectrum with MaxEnt3....68

Creating an XIC from a mass spectrum....69

Creating a spectral peak table....69

Identifying candidate components....71

Elemental composition.....71
Saving and restoring elemental composition calculation settings.....80
Mass fragment 81

Locking LC-MS Toolkit..... 89

Unlocking LC-MS Toolkit.....90

Signing out of LC-MS Toolkit.....91

Processing options available by data type..... 92

About Help.....98

Additional resources.....98
Contacting Waters.....98
Customer comments..... 99
Copyright notice..... 99
LC-MS Toolkit Trademarks.....99

List of abbreviations.....101

AME 101
BPI 101
BSM 101
CM 101
DBE 101
DDA 101
LC-MS 101
MRM 102
MS 102

MS/MS 102

m/z 102

ppm 102

QSM 102

QTof 102

Quad 102

RMS 103

S:N 103

SM-FTN 103

SIR 103

SVG 103

TIC 103

Tof 103

TUV 103

UV 104

XC 104

XIC 104

LC-MS Toolkit application overview

The LC-MS Toolkit application is an application that launches from the waters_connect application Hub.

The LC-MS Toolkit application enables rapid investigation of LC-MS, LC-MS/MS, and optical (UV, PDA) data types outside the structured workflows of other waters_connect applications.

You can use the data processing tools to investigate chromatograms and spectra.

Configuring LC-MS Toolkit permissions, folder access, and policies

Before users can access and use LC-MS Toolkit, an administrator must ensure that users have the proper permissions assigned to their role and that they can access the required waters_connect folders.

By default, the Administrator role has all the required permissions assigned and can access all folders.

Permissions

The waters_connect Administration application (**Administration** > **Security** > **Roles**) allows the users with appropriate roles to view and manage permission settings.

Under Batches, assign the following permissions to LC-MS Toolkit users:

- Create LCMSToolkitSession
- Delete LCMSToolkitSession
- Modify LCMSToolkitSession

Users must have these permissions assigned to their role to create, delete, and modify sessions.

Folder access

The waters_connect Administration application (**Administration** > **Security** > **Access Management**) also allows a waters_connect user with appropriate permissions to assign folder access to waters_connect user accounts.

Users must have access to all projects and folders containing data that they will analyze in LC-MS Toolkit. To view and work with existing sessions, users must have access to the folder where the session resides.

Giving a user access to the Company folder also provides inherited access to all other folders. To prevent the user from accessing specific folders, remove the inherited user access from those folders.

Policies

Configurable policies are detailed below.

Table 1: LC-MS Toolkit policies

Settings	Description
Global policies – Timeout	The LC-MS Toolkit application locks a user's session based on the amount of minutes the user is inactive and the setting specified in the Timeout global policy. Users must supply their passwords when the application session is locked.

Table 1: LC-MS Toolkit policies (continued)

Settings	Description
	The Timeout policy can be configured using the waters_connect Administration application (**Administration** > **Security** > **Global Policies** > **Timeout**).
Data folder reason policies	You can apply a data folder reason policy when users delete a session in Explorer (**Administration** > **Security** > **Folder Policies**). You can require users to either supply a predefined reason or type their own reason for deleting the session. You can also require password authentication for this specific action. You cannot define custom reason and password requirements for modifying a session within LC-MS Toolkit. Instead, the data audit trail always displays the reason "Saving LC-MS Toolkit Session" when a session is modified. For more information, access the Help topic "Create and modify folder policies" in the waters_connect Administration application.
Data folder reasons	If the data folder reason policy requires a predefined reason, you must create at least one for the LCMSToolkitSession category (**Administration** > **Security** > **Predefined Reasons**). For more information, access the Help topic "Create and modify reasons for audit trail comments" in the waters_connect Administration application.

Event log

When you create, modify, or lock a session, an audit trail message is created and can be viewed in the event log (**Administration** > **Event Log**).

Creating a session

Organize and group your data within sessions in the LC-MS Toolkit application.

You must create a session before you select data to investigate. You can add data from multiple sample sets to the same session.

Each operation you perform in a session is automatically saved in the current draft version of the session.

To create a session:

1. On the LC-MS Toolkit homepage, in the CREATE A SESSION pane, type the name of the session.
2. In the Enter a description box, type a description, if needed.
3. In the Select a location box, select the location in which to save your session.

   Tips:

   - Sessions save to the Company folder by default.
   - If you change the location, the most recently used location becomes the default.
4. Click **Select data** and, in the Select Data dialog box, select injection data from the left-hand side, and then click  .

   Tips:

   - You can select or clear all injections in a sample set (or UNIFI analysis) by clicking the sample set name.
   - Click the arrow (>) next to the sample set name to expand it and select individual injections.
   - To clear an individual injection, click it again.
   - To select a range of injections in one action, press and hold `Shift` while clicking.
   - To view MassLynx data in LC-MS Toolkit, you must first import it into waters_connect using Explorer to create a sample set.

   Result: The selected data appears on the right-hand side in the Selected Data pane.
5. To remove injections from the list, click the injections in the Selected Data pane, and then click  .

   Tip: To quickly remove a range of injections, press and hold `Shift` while clicking.
6. With at least one injection selected, click **Add**.

   Result: Your session is created. See Navigating a session (Page 13).

Creating a session using running samples

There are two methods for creating a session from an acquisition that is in progress.

Launching a toolkit session from the Sample Submission application

On the Realtime Data page of the Sample Submission application, when the sample is running, click **Open with LC-MS Toolkit**. This creates a new toolkit session containing the sample that is in progress, indicated by the warning icon  .

Creating a new toolkit session in the LC-MS Toolkit application

Follow these steps to create a new toolkit session in the LC-MS Toolkit application:

1. Open the LC-MS Toolkit application.
2. Create a new session and browse to the folder containing the running samples.
3. Select the sample that displays a warning icon  .

   Note: If you point to the icon, a tooltip displays the message `Acquisition in progress or incomplete`.
4. Move the selected sample to the Selected Data list using the arrow.
5. Click **Add**. The new session opens.

   Note: You can also add the running samples to an existing session using the **Add Injections** option.

   Important: Before you add an injection, ensure that the chromatographic elution has begun and data is being acquired. If you add an injection to a session before this stage (for example, when the LC prepares to inject), no chromatogram appears. To view the acquiring datafile in this situation, do one of the following after data acquisition begins:

   - Remove and then re-add the injection.
   - Exit and return to the session.
   - Select a different injection, and then select the injection containing the acquired data again.
   - Create a new session including the injection.

Browsing sessions

When you create sessions, you can browse and open them from the LC-MS Toolkit home page.

Note: LC-MS Toolkit sessions also appear in the Explorer application, as follows:

- Sessions display "LCMSToolkitSession" for the item type.
- Inactive sessions (closed in LC-MS Toolkit) appear as published items.
- Active sessions (open in LC-MS Toolkit) appear with the status "In edit" indicated in the "Status Indicators" column.

To browse and open sessions from the LC-MS Toolkit home page:

1. If your session was recently created or opened, click its name within the Recent sessions list.
2. Otherwise, click **Browse**.
3. In the Browse Sessions dialog box, browse to the location of your session and select it.

   Tip: Sessions are represented by  and display the session name and the time they were last modified.
4. Click **Open**.

Deleting a session

You must open Explorer to delete a session, because you cannot delete sessions from within the LC-MS Toolkit application.

Deleting a session within Explorer cannot be undone.

Note: You cannot delete an active session with "In edit" status in Explorer. Close the session in LC-MS Toolkit by navigating to the home page from within the session.

To delete a session (in Explorer):

1. Open Explorer from the waters_connect application Hub.
2. In the Folders pane, browse to the folder containing your session.
3. In the Folder Contents pane, right-click the session and select **Delete**.
4. At the prompt, click **Yes**.

Navigating a session

LC-MS Toolkit sessions appear with the following items:

Session details side panel

The session details side panel (left-hand panel) displays the following information and options:

Item	Details
Session	The session name.
Version	The version of the session. When you reopen a published session, the version of the session is increased and a new draft is created. When you reopen a session that is in edit, you reopen the same version, and new changes are combined with previous changes in the same draft. See Closing a session (Page 15).
Location (click > to expand panel)	The folder in which the session resides.
Description (click > to expand panel)	The session description (if any).
Created by (click > to expand panel)	The user that created the session.
Created (click > to expand panel)	The date and time when the session was created.
Modified by (click > to expand panel)	The user that most recently modified the session. **Note:** Because opening the session modifies it, when you examine this field within a session, this is your username.
Modified (click > to expand panel)	The date and time when the session was last modified. **Note:** Opening a session is counted as a modification for this purpose.
ID (click > to expand panel)	The unique session identifier. Click  to copy this identifier to the clipboard. This identifier is unique to the session and the version.
Add injections	Click **Add Injections** to include additional injections in the session.

Item	Details
	In the Select Data dialog box, select the additional injections in the same way as when creating a session, and click **Add**. **Note:** You cannot add the same injection to a session twice.
View Injection Info	Opens the experimental record and post-run report for the selected injection, if available. See Viewing injection information (Page 16).
Injections	The list of currently included injections. The number of injections in the session is displayed. For each injection, the following information is displayed, where available: • Injection name • Sample description • Sample set (or UNIFI analysis) name • Date and time of acquisition You can drag injections in the list to reorder them, and you can click an injection to select its chromatograms and spectra in the main data view. To remove an injection from the session, click the cross (X). Injections may display warnings  , for example if there are acquisition or lock mass issues or if an injection is currently being acquired. Point to the icon for further information in a tooltip.
Collapse and expand	You can collapse  or expand  the side panel by clicking the appropriate icon.
Settings	Click the settings icon  to configure the truncation of injection names. In the Display Settings dialog box, use the Truncate Injection Information drop-down menu to select **Left** or **Right**, and then click **Close**. For injection names that are too long to display entirely in the side panel, this option determines whether the start (left) or end (right)

Item	Details
	of the name is displayed. By default, Left is selected. You can also point to the injection to display its full name in a tooltip.

Main data view

The main data view shows chromatograms and spectra for the selected injection. To examine chromatograms and spectra, see Viewing chromatograms and spectra (Page 16).

By default, all raw (acquired) chromatograms are initially shown for an injection, with the exception of lock mass reference channels. You can display reference channels by adding them in to the active filter (see Filtering channels (Page 25)).

Menu bar

The menu bar at the top of the page allows you to customize the main data view, toggle options, and access a comparison view. It also displays the current injection name (and description, if present).

The menu bar also displays controls for real-time updates to chromatograms as data is being acquired.

See the following topics:

- For the comparison view, see Comparing data using the comparison view (Page 22).
- For "X of Y Channels", see Filtering channels (Page 25).
- For the TIC and BPI options, see Processing chromatograms (Page 35).
- For Gridlines, see Adding and removing plot gridlines (Page 26).
- For "Link X-Axes", see Linking the x axes for displayed data (Page 27).
- For real-time data updates, see Viewing real-time data (Page 27).
- The Tools option is described below.

Tools menu

The tools menu, on the right-hand side of the session page, displays processing options for chromatograms and spectra. The options available depend on the item (or items) you select. For more information, see Processing chromatograms (Page 35) and Processing combined MS data (Page 56).

You can hide the tools menu and restore it by clicking the **Tools** toggle in the menu bar.

Closing a session

To close a session fully, navigate to the home page from the toolbar. This will publish the current draft (in edit) version of the session.

If you close the application instead, or sign out of waters_connect with the session open, the session remains in edit. You can see this in the Explorer application.

Viewing data in LC-MS Toolkit

The topics in this section describe data views, customizations, and factors affecting data display in LC-MS Toolkit.

Viewing injection information

You can view the instrument system and acquisition details for an injection in a session.

To view injection information:

1. In the session window, click **View Injection Info**.
2. In the View Injection Information dialog box, view the instrument system details and post run report on the Experiment Record and Post Run Report tabs.

   ### Notes:

   - On the Post Run Report tab, click ^ to collapse a section.
   - Click  to copy the information on the tabs to the clipboard, including sections that were collapsed on the Post Run Report tab.
   - If you want to view the method parameters used to acquire data, click **Open in Acquisition Method Editor**. Acquisition Method Editor opens in a separate browser window and displays the acquisition method that was used to run the injections. If your role contains the "Modify Acquisition Method" permission, you can adjust those settings as needed.
   - The experimental record and post-run report may not be available if the sample data was processed further before analysis in LC-MS Toolkit (for example, if you open a UNIFI analysis).
   - To display and open the acquisition method, the acquisition method must be present in the same database.

Viewing chromatograms and spectra

Chromatograms and spectra are displayed in individual panes in the main session data view, with chromatograms on the left and spectra on the right when both exist.

Data is displayed for one injection at a time, which you can change using the side panel. The top menu bar displays the injection name (and description, if present).

Each plot has a title containing channel information and other information where relevant (for example, the retention time range over which a spectrum was combined, or the analyte name for MRMs).

Color-coded lozenges indicate the nature of the data and any processing applied where relevant.

Notes:

- To drag plots to reorder them in the session window, position the pointer over the top of the plot.
- To reorder multiple plots, press **Ctrl** and click the check box on the top left-hand side of the plots, and then drag the plots.
- You can also select **Move To Top** or **Move To Bottom** in the Tools menu, to move a selected plot.

Tools

When investigating chromatograms and spectra, use the actions described in the following table:

Table 1: Chromatogram and spectrum actions

Description	Action
Maximize	Click to maximize a single chromatogram or spectrum.
Minimize	Click to minimize a maximized chromatogram or spectrum.
Collapse	Click to collapse the plot, showing only the title. **Tip:** You can collapse all chromatograms or spectra simultaneously using the Tools menu on the right-hand side of the session page. Click **Collapse All Chromatograms** or **Collapse All Spectra**.
Expand	Click to expand a collapsed plot. **Tip:** You can expand all chromatograms or spectra simultaneously using the Tools menu on the right-hand side of the session page. Click **Expand All Chromatograms** or **Expand All Spectra**.
Zoom in	Click and drag across the peaks of interest to zoom in on the *x* axis.

Table 1: Chromatogram and spectrum actions (continued)

Description	Action

	Tip: When you zoom in on a chromatogram or spectrum in the main session window or comparison view, you can zoom in on the *y* axis in two ways:
	• Click and drag horizontally to scale the *y* axis relative to the highest peak in the zoomed region.
	• Click and drag horizontally and vertically (a box) to scale the *y* axis to the height of the box.
	  • For chromatograms, the scaled *y* axis exactly matches the vertical start and end of the box.
	  • For spectra, the *y* axis begins at zero and ends at the top of the box.
	Zooming in does not prevent you from using processing methods that process the entire chromatogram or spectrum (for example, smoothing or integrating).

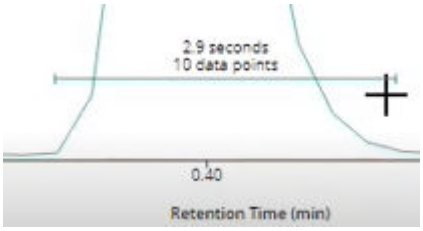
Zoom out	Click **Step back on zoom scale** .
Reset the zoom	Click **Reset zoom scale** .
Distance tool 	Press and hold **Shift**, and then click and drag to display the distance tool (+). • For a chromatogram, this tool displays the distance (minutes or seconds) and acquired data points within the dragged range. • For a mass spectrum, this tool displays the distance in *m/z* units. When you release the mouse button, the distance annotation persists on the chromatogram or spectrum. You can add multiple annotations. To clear the distance tool annotations, click **Remove user annotations** .

Table 1: Chromatogram and spectrum actions (continued)

Description	Action
	Tip: While dragging, you can move the mouse up or down. This moves the cross, helping you to measure distance accurately between peaks of different heights.
Download as an SVG file	Click **Download chart as SVG** . The SVG image is saved with a file name created from the injection and plot by default, and it matches your zoom.
Copy	Click **Copy data to clipboard** . You can copy the retention time and intensity data values from a chromatogram plot and the ion intensity and mass data values from a spectral plot, and then paste them into an external application such as Excel.

Plot data

Plots are displayed with *x* and *y* axes appropriate to the data type.

Peaks are annotated with *x* values (for example, retention time or *m/z*). Annotations update as you zoom.

Further annotations may be added after processing. For example:

- Peak area appears after integrating a chromatogram.
- For continuum MS spectra, pink lines mark the position of the peak centroid (the average of the *m/z* weighted by intensity), based on an initial peak detection.

  Note: This may not exactly match the results of your own centroid and mass measure operations in LC-MS Toolkit.
- Centered MS spectra also display the centered height or area, depending on your centering settings.

To view *x* and *y* axis values in a tooltip for the closest data point, press and hold **Ctrl**, and point to the desired data point. You may need to zoom in first to identify a specific point.

When you point to a chromatogram or spectrum, a cross (+) appears and moves to any location that you point to. The *x* and *y* axis values for the position of the cross appear in a box below the plot (when the plot is maximized, they appear above the plot).

At the top-right of the plot, the maximum *y* axis value (for example, intensity) is displayed.

Selecting and moving plots

To select a plot (for example, for processing), click the check box at the top-left of the pane. You can select multiple plots by pressing and holding **Ctrl** and then clicking the check box on each plot.

You can also move chromatograms and spectra within their own columns.

- To drag plots to reorder them in the session window, point to the top of the plot, and then click and drag.
- To reorder multiple plots, press **Ctrl** and click the check box on the top left-hand side of the plots, and then click and drag the plots.
- You can also select **Move To Top** or **Move To Bottom** in the Tools menu, to move a selected plot or multiple plots.

Customizing user preferences

General

To change the text size on chromatogram and spectrum plots, use the **Plot Text Size** and **Axis Label Size** drop-down options. You can select the small, medium or large based on your requirements.

Notes:

- The plot text size option applies to peak annotation, max intensity annotation, signal-to-noise annotation, legend text, and the distance tool.
- The axis label size option applies to the numerical values on the X and Y axis labels and tick labels.

The behavior when extracting spectra from MSe data can be modified using the **Automatic Extraction of High Energy Trace** toggle. Enabling this option generates both low and high energy spectra when extracting a single scan or combining with right mouse button from the low energy chromatogram.

Chromatogram

To set the annotation threshold as a percentage of the most intense peak in view, specify the "% of the most intense chromatographic peak" value in the **Chromatogram Annotation Threshold:** field.

To set the threshold relative to the most intense peak in the entire chromatogram, even when the plot is zoomed in, enable the **Threshold relative to most intense peak in entire chromatogram** toggle.

To view the scan number in the tooltip for datapoints on chromatogram plots, enable the **Display scan number in tooltip information** toggle. To visualize datapoints and their associated tooltips, hold `Ctrl` and hover the mouse over a shown datapoint.

Note: The scan number provides a reference for the user to determine if they are selecting exactly the same scans in a channel. It is more easily reproducible than using retention time values.

To customize the header of the XIC plot generated by the Advanced Extract Tool, enable the **Annotate non-summed XICs with user-defined compound identification** toggle. By default, this is disabled. Enabling it allows the user to add the required information in the header for improved plot identification.

By default, the **Height**, **Area**, and **Retention Time** check boxes are selected for integrated plot annotations. You can customize the annotations in the plot by selecting or clearing the required check boxes.

To view Base Peak Mass (BPM) annotations, enable the **Annotate Base Peak Mass** toggle. By default, this is disabled. Enabling it displays the annotation above the peaks in chromatograms for scanning data types. The 100 most intense peaks per channel are annotated. Where space is limited by other text on the plot, zooming in shows more BPM labels

Notes:

- BPM can be displayed for data acquired on Tof and Quad instruments.
- BPM annotations do not appear on processed plots, such as XIC, smoothed, or integrated plots.

To set the number of decimal places for the BPM annotation, specify the required value in the **BPM Decimal Places** field.

Note: The decimal places value can be set between 0 to 6.

When you generate an integrated or smoothed chromatogram plot, you can automatically replace the original plots with the respective processed plots by selecting the **After Integration**, **After Manual Integration**, or **After Smoothing** check box.

Spectrum

When you enable the **Annotate MS Resolution** toggle on the Spectrum tab in the User Preferences settings, the mass resolution of the most intense continuum ion in a spectrum plot is automatically calculated and appears as an annotation above the ion. The mass of the most intense ion (in Daltons) divided by the half height peak width gives the resolution value.

Notes:

- This allows you to calculate the resolution without resorting to manual measurements of peak widths. The resolution of the most intense ion appears on every plot displaying continuum data and updates automatically when you zoom into an area of the plot for the most intense ion.
- MS resolution can be automatically calculated on data without a lockmass channel.
- The resolution of ions in the lockmass reference channel can also be automatically labelled with mass resolution.

To set the annotation threshold as a percentage of the most intense ion in the view, specify the "% of the most intense ion" value in the **Spectra Annotation Threshold:** field.

To set the threshold relative to the most intense ion in the entire spectrum, even when the plot is zoomed in, enable the **Threshold relative to most intense ion in entire spectrum** toggle.

To customize the decimal places of the m/z values, specify the number of decimal places you require in the **m/z Decimal Places** field. By default, the **Use defaults** check box is selected. You can clear the check box to customize the number of decimal places.

Note: The decimal places value can be set between 0 to 5. The default decimal places value for data from Tof instruments is 5 and for Quad instruments is 2.

To view extracted scan number ranges in the headers of generated spectra, enable the **Display scan number in title** toggle.

Note: The scan number provides a reference for the user to determine if they are selecting exactly the same scans in a channel. It is more easily reproducible than using retention time values.

When you generate the centered, mass measured, or smoothed spectrum plot, you can automatically replace the original plots with the respective processed plots by selecting the **After Center**, **After Mass Measure**, or **After Smoothing** check box.

Comparing data using the comparison view

You can compare selected chromatograms and spectra in your session using the comparison view. This view offers further visualization options.

To compare data using the comparison view:

1. In the chromatogram or spectrum pane, for each of the plots you want to compare, click  **Add to comparison**.

   Notes:

   - To add all chromatograms in the main session window, click  **Add all visible chromatograms to Comparison View**.
   - You can include chromatograms and spectra from multiple injections.
2. In the top-left of the main session window, click **Comparison View**.

   Tip: If you point to the button, a tooltip displays the currently selected chromatograms and spectra.
3. In the Comparison View window, compare data using the settings described in the following table.

Table 2: Comparison view settings

Action	Setting
Select whether to show chromatograms or spectra.	Click **Chromatogram** or **Spectrum**.
Select the view mode for multiple chromatograms or spectra. You can overlay chromatograms or spectra in one plot, or stack them in separate plots.	Click **Stacked** or **Overlay**. **Notes:** • The overlay view legend orders the plots based on the highest *y* axis value (for most plots, this is intensity). • When you zoom in on a peak, the legend reorders based on the *y* axis values visible in the zoomed-in area. • When you overlay plots with differing *y* axis units, only one legend is shown, but all values remain in their original units.
View chromatograms grouped by injection.	Click **Injection**. **Note:** This is a third view mode for chromatograms only. It displays the selected chromatograms overlaid within their own injections, with different injections stacked.
Select the *y* axis values in Overlay or Injection mode (absolute or relative).	To specify relative values, select **Relative Y-axis**. **Notes:** • The default *y* axis view is absolute, which displays absolute values in original units. • The relative view displays *y* axis values as a percentage of their maximum value. • When you zoom in on a peak, the relative view updates the maximum value to consider only the zoomed-in area.
View tandem quadrupole MRM chromatograms grouped by transition.	Click **Transition** to display MRM chromatograms by transition. This option is not available for other data types.
Reorder plots in Stacked mode.	Point to the top of a plot, and then click and drag the plot to move it up or down.

Table 2: Comparison view settings (continued)

Action	Setting
Remove a plot from the comparison view.	In Stacked view mode, click **Remove from comparison**.
Return to the main session window.	Click **Go back to viewing all session data** .

Notes:

- Individual plots within the comparison view behave similarly to the main session window (see Viewing chromatograms and spectra (Page 16)). You can view information for a point, zoom in and out, measure distances, and download an SVG image. You can also maximize plots in injection and transition modes for MRM data.
- When you measure distances in Stacked mode, the distance tool displays the same information for each plot that it does in the main session window. In Overlay, Injection, or Transition mode, the distance tool only displays retention time distance (not data points) for chromatograms.
- Plots in the comparison view have linked *x* axes, so that plots sharing an *x* axis label zoom identically and display a common maximum axis value.
- Ions in spectra will only be labeled with *m/z* if their intensity is above 5% of the maximum peak in view.

4. In the main session window, you can adjust the contents of the comparison view as follows:

   - To include additional chromatograms or spectra in the comparison view, click **Add to comparison**.
   - To remove a chromatogram or spectrum from the comparison view, click **Remove from comparison**.
   - To clear all selected data for the comparison view, click **Remove all plots from Comparison View**.
   - You can also access the comparison view when filtering channels using the Compare Selected option (see Filtering channels (Page 25)).

Copying retention times for MRM data

For MRM data, you can copy the retention times of interest in the LC-MS Toolkit application to paste into the acquisition method in the waters_connect AME application and an external application such as Excel.

In AME, open the method used to acquire the data, or create a new method and add analytes that match the names of those in the data to import from LC-MS Toolkit (input values for precursor and product).

To copy retention times for MRM data:

1. In the Comparison View window, click **Injection** for the selected MRM chromatograms.
2. For the injection of interest, click **Toggle peak table** to display the peak table pane below the chromatogram panes. The peak table displays the following columns:
   - Channel Name
   - Retention Time (min)
   - Analyte Name
   - Group Name
   - Precursor (*m/z*)
   - Product (*m/z*)
3. In the peak table pane, click **Copy peak list to clipboard**. There are two copy options as follows:
   - **Copy Table**: Use this option to copy the results, along with the header, in a tab-delimited format. You can then paste it into Notepad, Excel, or other applications.
   - **Copy to AME**: Use this option to copy the results, without the header, in a tab-delimited format. You can then paste it into a waters_connect AME MRM method.
     1. In the peak table, click the method name to open AME in a separate window.
     2. In AME, click **Import analytes from the optimization tools** to import the copied peak list, and then select **Import retention times only**.

     Notes: The copied peak list is intended for use directly with AME on the same computer. It is not a copy of the text content of the table.
4. Click the cross to close the peak table.

Filtering channels

Use the channel filter to display selected raw (acquired) chromatogram plots in your session.

To filter channels:

1. In the top-left of the session window, click **x of y Channels**.

   Result: In the Filter Acquired Chromatograms dialog box, MRM and non-MRM channels are displayed in separate tables.

2. For MRM data, you can filter the table. In the Filters search bar, type the search filter, and then press **Enter**.

   Result: Each filter is displayed in a lozenge in the search bar. To remove a filter, click the cross (**x**) in the lozenge or press the backspace key. The result of each individual filter is combined with the other filters with an OR operation.

3. In the Include column, select the channels to display or use the options described in the following table.

   Table 3: Filter settings

Setting	Description
	Click to add all visible channels to the filter.
	Click to clear all visible channels from the filter.

   Notes:

   - Click the column headers to order-by when filtering.
   - The number of channels selected appears in parentheses on the **Apply** button.
   - When you create the session, all channels are selected by default, except for the following:
     - The lock mass reference channel for Tof data (select it to display the lock mass channel chromatogram)
     - LC diagnostic channels
   - Filtering the table for MRM data does not clear already-selected items that are excluded from the filter.

4. Click **Apply** to display the selected filtered data in the main session window, or click **Compare Selected** to replace the contents of the compare basket with the selected channels and go directly to the comparison view in Injection mode.

   Note: When you update the filter to add or remove a channel for one injection, the same change applies to channels with the same name in other injections.

Adding and removing plot gridlines

You can toggle plot gridlines on and off within the main session window and the comparison view. Your gridline selection persists within a session. When you create new sessions, gridlines are turned off by default.

To add or remove plot gridlines:

1. To add gridlines to plots, select the **Gridlines** toggle switch.
2. To remove gridlines from plots, clear the **Gridlines** toggle switch.

Linking the *x* axes for displayed data

You can link and unlink the *x* axes for chromatograms and spectra in the main session window. By default, the axes are linked.

When linked, plot actions such as zoom on one chromatogram or spectrum affect other plots with common *x* axis labels.

If you extract a chromatogram while linking is selected, the extracted chromatogram axis is automatically linked.

When unlinked, you can zoom plots independently.

If you link independently zoomed plots, the *x* axis is reset to its full extent.

To link or unlink *x* axes in the main session window:

Select or clear the **Link X-Axes** toggle in the top-right of the session window.

Viewing real-time data

You can view the following types of data in LC-MS Toolkit as the data is being acquired using Sample Submission:

- Tof data (RDa)
  - TIC/BPI
- QTof data (Xevo G2-XS, Xevo G3, and Xevo MRT)
  - TIC/BPI
- Quad data (excluding MRM and SIR modes)
- PDA and TUV data
- Diagnostic data (temperatures and pressures for LC modules)

While a sample injection acquisition is in progress, the **Refresh Chromatogram** icon appears. When you click , it updates the traces in view with all the available data. This causes all the chromatograms and time scale axes of all the plots to update.

Notes:

- You can process chromatograms during acquisition after refreshing.
- During acquisition, *m/z* values may differ from the final values when acquisition is complete, depending on your instrument and lock mass type. Because of this, the injection may display a warning that the data is not lock mass corrected, and when you process data before acquisition is complete you may obtain different results than when processing the same data after acquisition is complete.

To view real-time data:

1. Create a session from an acquisition that is in progress. For more information, see Creating a session using running samples (Page 10).

   Tip: When you open a toolkit session by either of the methods, it launches the application showing the data that is acquired up to that point in time. The running sample is indicated in the list in the left-hand pane by a warning icon  . If you point to the icon, a tooltip displays the message "Acquisition in progress or incomplete".

2. Click  to update all the visible chromatograms with the data acquired up to this point.

   Tip: You can click the refresh chromatogram icon multiple times. A refresh request takes longer if there are more plots or if a long time has elapsed since the last refresh was requested.

   Notes:

   - The refresh chromatograms icon is available only when data is being acquired.
   - All the processing tools appear after refreshing the chromatograms.
   - If you extract spectra from a Tof data chromatogram when a sample is running, the "Real Time" label is added to any spectrum that was extracted while the analysis was running to indicate that the lock mass correction was performed in real-time using only the available lock mass data at the time of extraction.
   - The "Real Time" label appears when you reopen a saved session. When a run finishes, it is recommended that you re-extract any plots showing the Real Time label. This finalizes the lock mass correction calculation, and the label will no longer appear on the new plot.
   - While chromatograms are being refreshed and the icon is gray, you cannot perform the following tasks until the action is completed:
     - Navigate to the home page (close the session).
     - Change your selection for TIC or BPI, linked *x* axes, and gridlines.
     - Access the comparison view.
     - Filter channels.
   - You can use the real-time extracted spectra with the mass fragment and elemental composition tools. However, centered spectra marked "real-time" have *m/z* values

corrected with a lockmass determined before the run was completed. Re-extracting the spectrum and using either tool after the run has completed may yield different results.

- If you defined the method with the lock mass setting disabled, the sample name displays a warning icon with the tooltip "The injection data is not lock mass corrected" while the sample is acquiring.
- If you extract any spectrum plot from an uncorrected sample, it displays a warning icon on the plot with the tooltip “Spectrum is not Lock Mass corrected”.

3. When the acquisition finishes, click for a final update of the chromatograms. The warning icon disappears from the sample name and any spectra now extracted no longer show the “Real Time” indicator. Data is fully lockmass corrected for any new processing operations performed.

Removing chromatograms and spectra from a session

You can remove plots from a session. The effect of removing the plot depends on whether it is a raw (acquired) chromatogram or an output of processing.

To remove a chromatogram or spectrum from a session:

1. In the chromatogram or spectrum pane, click the cross (X).

   Result: The chromatogram or spectrum is removed from the main session window.

   Notes:

   - For a raw (acquired) chromatogram, the cross displays the tooltip "Remove chromatogram from channel filter".
   - For chromatograms or spectra created by processing, the cross displays the tooltip "Delete Chromatogram" or "Delete Spectrum".

2. Whether the chromatogram or spectrum plot is removed (hidden) or deleted depends on the origin of the plot.

   - For a raw (acquired) chromatogram, the chromatogram is removed from view, but you can restore it (see Filtering channels (Page 25)).
   - For chromatograms or spectra created by processing, the plot is deleted, and you cannot restore it. However, any processing outputs created from it will persist.

Specifying the maximum number of MRMs to display

When viewing MRM data, you can limit the number of MRM chromatograms displayed in the main session window at one time.

To specify the maximum number of MRMs to display:

1. In the menu bar at the top of the main session window, type a number in the **Max MRMs** box, or use the arrows to adjust the value.

   Result: The maximum number of MRM chromatograms displayed for an injection is updated.

   Notes:

   - The default value is 10.
   - The Max MRMs box appears only when MRM data is present.

2. To identify whether the maximum limit is reached, navigate to the end of the chromatogram plots. If the limit affects the chromatograms displayed, the message `MAXIMUM PLOT LIMIT REACHED` appears.

Lock mass correction

Lock mass correction is normally applied during acquisition to data acquired with an appropriate lock mass reference channel (for example, Tof data). LC-MS Toolkit displays the corrected data.

Where lock mass correction is expected but is absent, you are warned in two places:

- When you select data to create a session, the injection displays a warning triangle ⚠ with the tooltip "The injection data is not lock mass corrected".
- In the session, the left-hand panel displays a warning triangle ⚠ next to the injection name, with the same tooltip, "The injection data is not lock mass corrected".

In a session, the lock mass reference channel chromatogram is not displayed by default. You can choose to display it (see Filtering channels (Page 25)).

Depending on your acquisition software, further considerations may apply, as follows:

Sample Submission data

Data acquired using Sample Submission in waters_connect is lock mass corrected (this includes standard, dynamic, and scheduled lock mass modes).

When you display real-time chromatogram updates for data that is being acquired (see Viewing real-time data (Page 27)), lock mass correction may not be applied to the data, depending on your instrument and lock mass correction method.

UNIFI data

Data acquired using UNIFI with dynamic and scheduled lock mass modes is lock mass corrected.

Data acquired using UNIFI with standard lock mass is not corrected unless it has also been processed to apply the correction. You can process the data in UNIFI to create an analysis that includes the correction.

MassLynx data

You can view MassLynx data in LC-MS Toolkit by importing it into waters_connect using the Explorer application.

MassLynx data acquired with **Acquire LockSpray - Do not apply correction** selected requires processing in UNIFI after import to apply the lock mass correction. You can then view the corrected data in LC-MS Toolkit by selecting it from the processed analysis.

MassLynx data acquired with **Acquire LockSpray - Apply correction** is not supported by LC-MS Toolkit. Although a correction has been applied, this correction is not detected and LC-MS Toolkit displays the lock mass warning.

Viewing LC diagnostic channels

Where you record diagnostic channels for your LC instruments, you can view their plots in LC-MS Toolkit by selecting them in the channel filter (see Filtering channels (Page 25)). You can also include these plots in the comparison view.

Notes:

- Plots from these channels display the "Diagnostic" lozenge.
- When you use the distance tool on these plots, it displays retention time distance.
- You cannot process data from these plots.

Examples of LC diagnostic channels include the following:

Table 4: LC diagnostic channel examples

Instrument	Data channel	Details
BSM (Binary Solvent Manager)	BSM Composition A or B	The composition of solvent A or B (%)
	BSM Flow Rate	System flow rate set point (mL/min)
	BSM System Pressure	Overall system pressure (in psi)
	BSM Primary A or B	Primary (left head) of pump A or pump B pressure (in psi)
	BSM Accumulator A or B	Accumulator (right head) of pump A or pump B pressure (in psi)
	BSM Degasser	Solvent degasser pressure (in psi)
QSM (Quaternary Solvent Manager)	QSM Composition A, B, C, or D	The composition of solvent A, B, C, or D (%)

Table 4: LC diagnostic channel examples (continued)

Instrument	Data channel	Details
	QSM Flow Rate	System flow rate set point (mL/min)
	QSM System Pressure	Overall system pressure (in psi)
	QSM Primary	Primary (left head) pressure (in psi)
	QSM Accumulator	Accumulator (right head) of pump A or pump B pressure (in psi)
	QSM Degasser	Solvent degasser pressure (in psi)
SM-FTN (Sample Manager - Flow Through Needle)	FTN Room Temp	Temperature at the air intake (°C)
	FTN Sample Temp	Temperature of the sample compartment (°C)
	FTN Column Temp	Temperature of the column compartment (°C)
	FTN Sample Pressure	Pressure of the sample loop (in psi)
	FTN Seal Force	Needle seal force (as a percentage)
	FTN Preheater Temp	Temperature at the active preheater (°C)
CM (Column Manager)	Temperature 1 or 2	Temperature of compartment 1 or 2 (°C)
	Preheater Temp	Temperature of the preheater (°C)

Viewing DDA data

If you load a data dependent acquisition (DDA) sample, you can view the DDA Tof MS Survey Scan chromatogram and a DDA Tof MSMS channel chromatogram that is a composite of all the Tof MSMS scans that are selected and switched on from the MS Survey channel.

Extracting spectra from DDA chromatograms on the view data page

Survey chromatogram channel

When you double-click a data point on the survey chromatogram, the following extracted spectra generate automatically:

- The spectrum from the selected survey MS scan data point
- One MSMS spectrum for each ion that was triggered by the survey scan

Note: When the MSMS channel contains multiple scans from the same switch mass, the spectrum displayed will be a combined spectrum of all scans.

MSMS chromatogram channel

When you double-click a data point on the MSMS chromatogram, the following extracted spectra generate automatically:

- The spectrum for the survey MS scan that triggered the MSMS scan
- The spectrum for the MSMS scan for the selected data point

Creating a DDA switch table

You can generate a DDA switch table using a toolbar option that tabulates all of the set masses that were triggered over the entire duration of the acquisition.

To create a DDA switch table:

1. Select the MS survey scan plot to see the DDA Switch Table option in the toolbar.
2. Select **DDA Switch Table**.

   The table appears with the following columns:

   - Switching Time
   - Set Mass
   - Function Name
   - Intensity

Filtering and sorting the rows in the DDA switch table

You can filter the rows in the DDA switch table as follows:

- To filter switching time, set mass, and intensity information, click  in the column heading. In the dialog box, specify the filter criteria, and then click **Apply**.

Note: To clear filtering for a single column, click  again, and then click **Clear** in the dialog box.

- To filter the rows for a single set mass, specify the desired set mass value in the **Filter by Set Mass** field.

  Note: To clear all filters, click  .

- To filter the rows using the retention time, click and drag across the peaks of interest to zoom in on the MS Survey chromatogram. The table is updated according to the plot range selection.

  Note: To clear all filters, click  or  .

In the DDA switch table, to sort information in ascending or descending order, click the column heading. To undo the sort, press and hold `Ctrl`, and then click the column heading again.

Extracting MS/MS spectra from the DDA switch table

To extract MS/MS spectra from the DDA switch table:

1. Select the required row or rows in the table.

   Note: You can use `Shift` or `Ctrl` to highlight multiple rows while making selections.

2. Click **Extract MS/MS Spectra**.

   A DDA Tof MSMS spectrum plot is generated for each selected row in the DDA switch table.

Copying results from the DDA Switch Table

Click  **Copy Exclusion/Inclusion list** to copy the results without the header, in a tab-delimited format. You can then paste it into a waters_connect AME method exclude/include table.

Processing chromatograms

You can perform the following processing operations on chromatograms from the Tools menu:

Table 1: Chromatogram processing options from the Tools menu

Operation	Details
Smooth	Smoothing reduces the high-frequency noise present in a chromatogram. You can choose between two smoothing methods. See Smoothing a spectrum (Page 58).
Integrate	Perform peak detection and quantitation on the chromatogram. See Integrating a chromatogram (Page 36).
Extract Masses	Specify masses for which an extracted ion chromatogram (XIC) will be created. See Using Extract Masses (Page 44).
Signal-to-noise	Calculate the signal-to-noise ratio of your chromatographic data based on signal and noise regions that you define. You can adjust the method of calculation. See Signal-to-noise (Page 52).
Combine spectra	Spectra are combined across a region you define. You can also define background regions to be subtracted from the result. Because of this, this operation includes both combining and subtracting spectra. See Combining and subtracting mass spectra (Page 53).

You can also perform two quick actions directly on the chromatogram, without using the tools menu:

Table 2: Processing options on the chromatogram

Operation	Details
Right-click and drag to combine spectra	You can quickly create a combined spectrum (without subtracting background regions) directly from the chromatogram. See Combining mass spectra directly from a chromatogram (Page 54).

Table 2: Processing options on the chromatogram (continued)

Operation	Details
Double-click to extract a spectrum	You can quickly extract a single spectrum directly from the chromatogram. See Extracting a single mass spectrum from a chromatogram (Page 55).

After any processing operation is complete, a lozenge appears on the output chromatogram or spectrum, named for (and color-coded by) the type of processing. Point to this lozenge for details of the processing settings used to create the output.

Notes:

- To display TIC or BPI data, click the **TIC** or **BPI** toggle in the top-right of the session window. This option does not apply to MRM data.
- To select additional chromatograms, press and hold `Ctrl`, and then select the check boxes on the additional chromatograms.
- To select contiguous chromatograms, press and hold `Shift`, and then select the check boxes on the start and end chromatograms.
- You can only perform operations appropriate to your starting chromatogram. See Processing options available by data type (Page 92).
- Some processing operations can be performed on multiple suitable chromatograms simultaneously, applying the same processing settings to each chromatogram. See Processing options available by data type (Page 92).
- When you process a chromatogram (except using Signal-to-noise), LC-MS Toolkit automatically selects the new plot and clears your previous selection.
- When you generate an integrated or smoothed chromatogram plot, you can automatically replace the original plots with the respective processed plots by selecting the **After Integration**, **After Manual Integration**, or **After Smoothing** check box in the User Preferences dialog.

Integrating a chromatogram

LC-MS Toolkit peak detection is based on detecting peaks in the second derivative chromatogram, and peak baselines are independently detected using liftoff and touchdown parameters.

This has several advantages over traditional integration, including:

- Peak detection using an apex in the second derivative chromatogram is more robust than detecting the rise at the start of a peak.
- Using the second derivative means that detection is not affected by a linear background gradient.
- Peak shoulders are readily detected using the second derivative.
- Independent baseline detection means that baseline placement does not affect which peaks are detected.

See also: Although the algorithmic implementation is not identical, many of the relevant concepts are described further in the ApexTrack section of the *Empower 3 Data Acquisition and Processing Theory Guide* (715005481), available from www.waters.com.

To integrate a chromatogram:

1. Select a chromatogram to process, using the top left-hand check box in the chromatogram pane.
2. Next to Integrate in the Tools menu, click  to specify integration settings as follows:

Table 3: Integration settings

Setting	Description
Apex Detection (Peak Width)	The peak width value affects several aspects of peak detection: • The widths of filters used by the software in smoothing for peak detection. • The minimum distance between peak apices. • Rejection of noise peaks that are notably wider or narrower than the width value. Select **Automatic** to allow the LC-MS Toolkit application to automatically determine the peak width. This sets the peak width to the width at 5% height of the peak with the highest magnitude in the second derivative. You can specify a value for the peak width by selecting **Manual**. Use the width of the largest peak measured at 5% of its height, expressed in minutes. **Recommendation:** Specify a peak width if the largest peak demonstrates one of the following properties: • It is saturated (the peak width value can be too great). • It coelutes with another peak (the peak width value can be too great). • It is noisy (the peak width value can be too small).

Table 3: Integration settings (continued)

Setting	Description
Apex Detection (Detection Threshold)	A threshold is applied to peak height in the second derivative chromatogram. Peak maxima that exceed this value are retained as peaks. Select **Automatic** to allow the LC-MS Toolkit application to automatically determine the detection threshold. The software calculates an automatic threshold from baseline noise within a peak-free area. This is detected and applied in the second derivative chromatogram. However, the threshold of the second derivative is proportional to the peak-to-peak noise in the original chromatogram, and the value is reported on the original chromatogram. You can specify a value for the detection threshold by selecting **Manual**. You specify a threshold on the chromatogram that the software automatically applies as a converted threshold in the second derivative chromatogram. **Tip:** You can point to the Integrated lozenge for previously processed data to see automatically applied thresholds, which may assist you in determining a suitable manual threshold.
Peak Integration (Liftoff%)	The start and end of a peak are determined by separate measurements to the initial peak detection. Liftoff determines the start point for peak integration. This is a gradient-based (first derivative) parameter. It is a percentage of the difference in gradient between the detector baseline and the point on the ascending peak profile where the second derivative is zero. Peak integration begins at the point with this gradient. The higher this value, the further up the peak integration begins. A value of zero means that the peak baseline is tangential to the detector baseline. A value of 100% means that the peak baseline is at the inflection point. The default value is 0%.
Peak Integration (Touchdown%)	The start and end of a peak are determined by separate measurements to the initial peak detection. Touchdown determines the end point for peak integration. This is a gradient-based (first derivative) parameter. It is a percentage of the difference in gradient between the detector baseline and the point on the descending peak profile where the second derivative is zero. Peak integration ends at the point with this gradient.

Table 3: Integration settings (continued)

Setting	Description
	The higher this value, the further up the peak integration ends. A value of zero means that the peak baseline is tangential to the detector baseline. A value of 100% means that the peak baseline is at the inflection point. The default value is 0.5%.
Peak Rejection (Minimum area)	Minimum peak area, after integration, required for the LC-MS Toolkit application to include a peak in the results. Peaks whose area values fall below the minimum area requirement are excluded from the results. **Tips:** • You can optimize this value by examining integrated peak lists for previously processed data (see Viewing the peak list in an integrated chromatogram (Page 39)). • You can use scientific notation to specify a value.
Peak Rejection (Minimum height)	Minimum height required for the LC-MS Toolkit application to include a peak in the results. Peaks with height values below the minimum height requirement are excluded from the results.
Enable Smoothing	Select to smooth the data. On the Smooth tab, specify the smooth settings. **See also:** Smoothing a chromatogram (Page 51), where the settings are described in detail.

3. Click **Integrate** or **Smooth & Integrate** in the dialog box to integrate the data, depending on your selections.

   Tip: To process the chromatogram directly from the Tools menu, without changing the settings from their default (or most recently used) values, click **Integrate** there instead.

Viewing the peak list in an integrated chromatogram

You can view the peak list for a single integrated chromatogram and for various types of data, such as MSe, TUV, MS, Product, Neutral Loss, SIR, MRM, and Precursor. You can view the retention time (RT), area (A), and height (H) annotations in the integrated peaks. You can also perform these tasks:

- Zoom in and out of selected areas in the chromatogram.
- Maintain zoom magnification while viewing the peak list.

- Customize the information that appears in the Peak List table by selecting and clearing the desired peak list columns.
- Sort information in ascending or descending order for each peak list column heading.
- Filter information based on a maximum of two applied filters for each peak list column heading.
- Copy information from the Peak List table to the clipboard, and then paste it into a spreadsheet.

To view the peak list in an integrated chromatogram:

1. Select a single integrated chromatogram, and then select **View Peak List** from the Tools menu.
2. In the Integrated Peak List dialog box, examine the chromatogram and the table.
   - On the chromatogram, click and drag to zoom in, and use the magnification tools to zoom out or reset the zoom scale.
   - The table displays the following information:

Table 4: Peak list table

Peak list column headings	Description
Retention time (min)	The retention time of the peak, which is the time (after injection) of the peak apex. Retention time is listed in minutes.
Area	The integrated peak area (intensity or absorbance integrated over time). **Notes:** • LC-MS Toolkit applies an integration scaling factor to peak height values to eliminate small values. • In the case of TUV absorbance, the value is shown as µAbsorbance (multiplied by 1×10^6). • In the case of TIC intensity, a scaling factor is applied to intensity (usually 1×10^3 or 1×10^6, depending on the type of detector). • When calculating the area, the time is converted to seconds, so the peak area after the scaling factor is applied is further multiplied by 60. • Peak area is also displayed as a peak annotation on the integrated chromatogram.
Percentage area (%)	Peak area, as a percentage of the summed area of all integrated peaks.

Table 4: Peak list table (continued)

Peak list column headings	Description
Height	The integrated peak height (intensity or absorbance). **Notes:** • LC-MS Toolkit applies an integration scaling factor to peak height values to eliminate small values. • In the case of TUV absorbance, the value is shown as µAbsorbance (multiplied by 1×10^6). • In the case of TIC intensity, a scaling factor is applied to intensity (usually 1×10^3 or 1×10^6, depending on the type of detector).
Percentage height (%)	Peak height as a percentage of the summed height of all peaks.
Width (sec)	Peak width (in seconds), which is the difference between the peak's start time, in minutes, and the peak's end time, in minutes, multiplied by 60 to convert the value to seconds.
Points across peak	The number of data points from peak start to peak end. **Note:** Optimum integration occurs when at least 15 data points span the peak.

Note: The table displays four headings by default:

- Retention time (min)
- Area
- Percentage area (%)
- Height

To add or remove columns, select the **Columns** drop-down menu on the right-hand side of the dialog box, and then select (or clear) the column headings that you want (or do not want) to appear in the table.

3. To sort information in ascending or descending order, click the peak list column heading.

   Tip: Hold **Ctrl** and click to reset the sorting for a column.

4. To filter information, click the filter icon in the column heading. In the filter dialog box, specify a maximum of two filter criteria, and then click **Apply**.

   Note: To clear filtering for a single column, click the filter icon again to open the filter dialog box, and then click **Clear**. To clear all filters, click **Clear all filters** .

5. To copy the entire contents of the peak list table, including any applied filters and all visible columns, click **Copy to Clipboard**. You can then paste the copied information into a spreadsheet.

   Note: Any customizations that you perform to the peak list table, such as filtering and sorting, persist every time you click **Close** to exit the Integrated Peak List dialog box, close the LC-MS Toolkit app, or reopen a session. However, if you click **X** or **Cancel** in the Integrated Peak List dialog box, customizations are not saved.

Manual Integration

Manual integration enables users to edit the assignments of peaks identified by the automatic integration process. Users can delete integrated peaks and select and edit the start and end points of an integrated peak. After making the necessary changes to the detected peak, the edited chromatogram can be added as a new plot to the View Data page.

Note: The manual integration option becomes available in the toolbar after the automatic integration process is performed.

To perform manual integration:

1. Select the integrated chromatogram.
2. In the tools section, select **Manual Integration**.

   The Manual Integration dialog appears.
3. On the chromatogram, click and drag to zoom in if required.
4. You can also perform the following functions on the manually integrated peaks:
   - Changing the start and end points of a peak (Page 43)
   - Splitting a peak (Page 43)
   - Deleting a peak (Page 44)
5. After making the necessary updates, select **Add** to apply all the changes.

   The Manual Integration dialog closes and a new plot appears with all the applied changes on the View Data page. The manually integrated chromatogram appears as a new plot with the "Manual Integration" lozenge.

   Notes:

   - If you click **Cancel**, the Manual Integration dialog closes without saving any of the applied changes. To make modifications, you must reopen the Manual Integration dialog.
   - To replace the previously integrated plot with the manually integrated plot, click the **Replace Chromatogram After Manual Integration** check box on the Chromatogram tab in the user preferences settings.

Changing the start and end points of a peak

To change the start and end points of a peak:

1. Right-click the peak for which you want to change the start and end points, and then click **Select Peak**.

   The current start and end points are shown in minutes as editable fields.

2. To set the start and end points of the peak, specify the required values in the **Start (min)** and **End (min)** fields in the Peak Settings option.

   Alternative: Click the **Start (min)** or **End (min)** field, and select the up or down arrow to increase or decrease the values by 0.0015 minute.

3. Click **Apply**.

   The data points move to the new positions.

   Note: The modified times set in the **Start (min)** and **End (min)** fields adjust to the retention times of the nearest acquired data points. Where a point was originally defined with a dropline to the baseline, the new position maintains a dropline. Where a point originally represented the first or last point in a group of detected peaks, the moved point causes the baseline to shift to the new start or end position. The revised baseline affects any other peaks defined above it.

Splitting a peak

To split a peak by adding a new dropline:

1. Right-click the peak you want to split, and then click **Select Peak**.

   The existing droplines to the baseline are listed in the dropline settings.

2. Select **Add new Dropline**.
3. Specify the required value in the **Dropline** field.

   Note: The new dropline must be specified at a time between the start and end points.

4. Click **Apply**.

   The new dropline is shown on the chromatogram by splitting the original peak into two.

   Notes:

   - Peak annotations for the new peak are displayed if space permits and the peak is above the threshold percentage defined in the User Preferences chromatogram settings.
   - When a new dropline is added to split a peak, or the start and end points of a peak are modified, the area "A" reported above peaks updates to reflect the new peak definitions. The calculated height "H" may also change due to an alternative curve being fitted to the peak based on the new start and end points.

Deleting a peak

To delete a peak in the integrated chromatogram:

1. Right-click the peak you want to delete, and then click **Delete Peak**.
2. Click **Apply**.

   The deleted peak is no longer shaded. The integration annotation and baseline for the deleted peak are removed.

Reverting Manual Integration changes

You can review and undo changes made during a manual integration session, such as removing droplines, reverting start and end positions, and reinstating deleted peaks.

To review and apply the changes:

1. Right-click anywhere on the manual integration plot.
2. Select **Review Edits** from the menu.

   A list of all changes made appears in the right-hand pane.
3. To revert a step and update the appearance of the chromatogram, highlight the item, and then click **Revert Last Change**.

   Note: Manual changes can be reverted one item at a time using the review edits feature. To discard all changes, click **Cancel** in the Manual Integration dialog box to exit without saving.
4. After reverting all the required steps, you can make further edits and apply them.
5. Click **Add** to finalize the changes.

   The Manual Integration dialog box closes, and a new plot is generated on the View Data page.

   Note: After the new plot is generated, changes cannot be reverted.

Extracting masses

You can extract masses to create an XIC (extracted ion chromatogram) for each of those masses, or a combined sum. You can also create an XIC for a mass range, summing signal over that range.

Using Extract Masses

To extract masses using the Extract Masses option:

1. Select an MS data chromatogram to process, using the top left-hand check box in the chromatogram pane.

Tips:

- You can select multiple chromatograms to extract the same masses from each chromatogram.
- You can also select an existing XIC. Masses are extracted from the original (raw) chromatogram data.
- You can extract masses from multiple XICs simultaneously. However, if the XICs share the same raw chromatogram, duplicate plots are not created.

2. Select **Extract Masses** and specify the following information:

Table 5: Extract masses settings

Setting	Description
Masses	Specify the *m/z* values you want to extract: • Use commas to separate several values (for example, "123.456, 789.123"). • Use a dash to specify a range (for example, "100.0-200.0"). • You can include both specific values and ranges.
Tolerance	Specify a tolerance to apply when you extract specific values. Data is extracted from a range created by adding the tolerance to, and subtracting it from, the specified *m/z*. Select from the following options, and specify a value: • **Da** (daltons). Tof and Quad data have different defaults (0.01 and 0.1, respectively). • **PPM** (parts per million). The default value is 5 ppm for both Tof and Quad data. **Notes:** • If you change the tolerance and extract masses, the settings you specify appear

Table 5: Extract masses settings (continued)

Setting	Description
	as the initial selection when you extract masses again. • The tolerance applies only to specific values, not ranges. • After processing, the tolerance appears in a tooltip when you point to the XIC lozenge.
Sum Individual XICs	Select this option to create a single summed XIC for a series of masses separated by commas. **Notes:** • The summed XIC is created from all comma-separated individual masses that you specify, even when separated by a range (for example "123.456, 100.0-200.0, 789.123" sums 123.456 and 789.123). • If you include ranges, each range creates a separate XIC and does not contribute to the sum. • The tolerance is applied to each mass before summation. • After processing, the summed XIC displays "Summed" before the individual masses in the plot title. Clear this option to create a separate XIC for every individual mass value and range.

3. Click **Extract**.

Using the Advanced Extract Tool

The Advanced Extract Tool allows the management of saved groups of ions for extraction that can be reused between samples. Compound ions of interest can be specified in groups and extracted simultaneously with a single click, without having to redefine each time.

Creating a new group in the Advanced Extract Tool

1. Select an MS data chromatogram to process, using the top left-hand check box in the chromatogram pane.
2. Select **Advanced Extract Tool**.
3. Click ⊕ to add a new group row.
4. Click in the **Name** and **Description** fields to specify the required information.

Adding compounds to a group

Compounds can be added to a group in any of the following ways.

Adding manually

In the Compounds section, click ⊕ to add a row and specify the details as follows:

- **Compounds where the molecular formula is known**
  Specify the compound name, formula, adduct, data type, and charge state. The monoisotopic and average masses will be automatically calculated.
- **Compounds where the monoisotopic mass is known**
  Specify the compound name, adduct, data type, monoisotopic mass, and charge state. Masses will not be automatically calculated. The defined monoisotopic mass value will be used when extracting.
- **Compounds where the average mass is known**
  Specify the compound name, adduct, data type, average mass, and charge state. Masses will not be automatically calculated. The defined average mass value will be used when extracting.

Notes:

- Data type is for information only and not used in any processing.
- Any duplications in compound or group names are highlighted in red in the table. You should correct these before extracting XICs or closing the dialog.
- Click the adduct field and select one or more adducts from the predefined list. The adduct mass is used in automatic *m/z* calculations if a formula has been defined.
- Click the charge field and specify one or more charge states. For multiple charge states, a comma-separated list or hyphenated range can be defined (for example, 1,2,3 or 1-3). The charge states will be used to calculate *m/z* values where a formula has been defined.
- To generate a table with the details of selected compounds in a group, select  .
- To select whether the calculated molecular mass should be based on monoisotopic or average mass values, select  .

Default compound groups

The application has predefined default groups for common classes of compounds and reference standards. For more information, see Table 6: Default compound group names and descriptions (Page 48).

Table 6: Default compound group names and descriptions

Group name	Description
LCMS QC Reference Standard	SKU: 186006963
Forensic Toxicology Installation Standard	SKU: 186007361-1
MassPrep Peptide Mix	SKU: 186002337
EquiSPLASH - Positive	Lipid Mix
EquiSPLASH - Negative	Lipid Mix
20 Pesticide Mix Standard	SKU: 18600634
Vitamins	Bioprocess
Organic Acids	Bioprocess
BioProcess Neg Ion	Bioprocess
Nucleobase Nucleoside	Bioprocess
Amino Acids	Bioprocess
Amino Acid Derivatives	Bioprocess
Choline	Bioprocess
Nucleotides	Bioprocess
CCM	Bioprocess

Notes:

- You can modify only the adduct and charge state fields in these default groups. This permits customization for different analytical conditions in your samples.
- If changes are made to adducts and charge states in a default group, click to revert to the default values, if required.
- You cannot add new compounds to the default groups. If additional compounds are required in a default group, click to copy the default group to clipboard, and then click to paste it in a new group. You can edit all the compounds in the new group and add new rows, if required.

Pasting from clipboard

You can paste compounds into the compound table from the clipboard. Click to copy these compounds from another group, and then click to paste it into the new group.

Alternatively, you can paste the compounds copied to the clipboard into Microsoft Excel and edit the seven column format to include new compounds or add missing information. Then, copy the Excel cells and click  to paste the compounds into a group.

Note: Pasted compounds will be appended below any compounds already defined in the table.

To delete a row, select the row using the check box and click  .

Note: You can also select a list of multiple rows to delete, using the check box in each row.

Using Advanced Extract Tool to extract XIC chromatograms

1. Select an MS data chromatogram to process, using the top left-hand check box in the chromatogram pane.
2. Select **Advanced Extract Tool**.
3. Select the group that contains the compounds that you want to extract as XIC chromatograms.
4. Select the check box of the compound rows that you want to generate XIC chromatograms.

   Note: If any selected compounds are out of the mass range of the selected data channel, the m/z cells are highlighted in orange, and you cannot extract the XIC chromatograms. The extract button remains unavailable until you deselect compounds with m/z values that are out of range.
5. On the Settings tab, specify the following information:

   Table 7: Extract masses settings

Setting	Description
Tolerance	Specify a mass tolerance to apply when you extract specific values. Data is extracted from a range created by adding the tolerance to, and subtracting it from, the specified *m/z*. Select from the following options, and specify a value: • **Da** (Daltons). Tof and Quad data have different defaults (0.01 and 0.1, respectively). • **PPM** (parts per million). The default value is 5 ppm for both Tof and Quad data. **Notes:**

Table 7: Extract masses settings (continued)

Setting	Description
	• If you change the tolerance and extract masses, the settings you specify appear as the initial selection when you extract masses again. • After processing, the tolerance appears in a tooltip when you point to the XIC lozenge.
Sum all XICs	Select this option to create a single summed XIC from all of the compounds selected for extraction in a group. **Notes:** • The same tolerance is applied to each mass before summation. • After processing, the XIC plot title displays "Summed" before a list of the summed masses.
Sum individual XICs per row	Select this option to sum XICs for each compound row. When selected, this generates one summed plot per selected row. **Notes:** • The generated XIC plot displays the sum of all charge states and adducts defined for the respective compound. • The header of the summed plot includes the compound name and a comma-separated list of the m/z values derived from the table row that have been summed.

6. Click **Extract**.

Note: You can add additional information derived from the Advanced Extract Tool compound definitions to the headers of generated XIC plots by enabling the **Annotate non-summed XICs with user-defined compound identification** toggle in the User Preferences settings.

Smoothing a chromatogram

Smoothing reduces the high-frequency noise present in a chromatogram.

Two methods are available for smoothing. Both move a window across the chromatogram, averaging the data points within the window to produce a point in the smoothed chromatogram.

- Mean. This calculates the arithmetic average of each set of data points within the window.
- Savitzky-Golay. This uses a least squares approach to optimally fit a set of data points within the window to a polynomial curve, and it uses the curve to weight the calculation of the average.

To smooth a chromatogram:

1. Select a chromatogram to process, using the top left-hand check box in the chromatogram pane.
2. Next to Smooth in the Tools menu, click  to specify smoothing settings as follows:

Table 8: Smooth settings

Setting	Description
Smoothing width	Set to the half width of the smoothing window (in data points). This is the number of data points before and after the center of the smoothing window. **Note:** If this value is too high, the smoothed chromatogram will be distorted.
Iterations	Smoothing is an iterative process. Set the number of times the smoothing is repeated. Increasing the number produces a heavier smoothing effect. **Note:** If this value is too high, the smoothed chromatogram will be distorted.
Smooth Type	Select the type of smoothing: • Mean - Calculates the arithmetic average of a set of data points. • Savitzky-Golay - Optimally fits a set of data points to a polynomial curve. **Tip:** Generally, mean smoothing provides the best smoothing for chromatograms, whereas Savitzky-Golay provides the best smoothing for spectra. Savitzky-Golay tends to enhance peak and valley shapes, as well

Table 8: Smooth settings (continued)

Setting	Description
	as preserve the height of the peaks better than mean smoothing. However, Savitzky-Golay can produce small artifacts on either side of the real peaks.

3. Click **Smooth** in the dialog box to center the data.

   Tip: To process the chromatogram directly from the Tools menu, without changing the settings from their default (or most recently used) values, click **Smooth** there instead.

Signal-to-noise

You can calculate the ratio of the peak heights to the level of noise in a chromatogram using one of two options:

- Root mean square (RMS). The greatest height of the signal above the mean noise is divided by the root mean square deviation of the noise.
- Peak-to-Peak. The greatest height of the signal range above the mean noise value is divided by the range of the noise.

To calculate signal-to-noise:

1. In the chromatogram pane, select the check box on the top, left-hand side to see the available processing options.
2. Select **Signal-to-noise**.
3. In the Signal-to-noise dialog box, click and drag to select the signal and noise regions, in order.

   Result: The calculated signal-to-noise value is displayed. You can now update the values of the selected signal and noise regions by typing in the **Adjust Signal Region** and **Adjust Noise Region** start and end fields.

   Notes:

   - To recalculate, click **Clear Selection**, and then select the new signal and noise regions.
   - To go into zoom mode, click  or  . The Marking Regions button changes to Mark Regions and you can click and drag to zoom instead of selecting regions.
   - To return to selecting the signal and noise regions, click **Mark Regions**.
   - Where the noise value is determined to be less than 1, a value of 1 is used for noise in the calculation, so that the signal-to-noise ratio is the signal value.
4. The default noise type is RMS. If necessary, select the noise type, **RMS** or **Peak-to-Peak**.

5. To add the processed data to the session window, click **Add**.

   Result: In the processed plot, the signal-to-noise operation is indicated by the S:N lozenge, and the signal-to-noise value appears above the signal region.

Combining and subtracting mass spectra

Combining and subtracting mass spectra produces a single spectrum by subtracting background spectra from the spectra combined across a TIC or BPI peak. The combination process depends on the data type. Peak intensities are averaged for Quad data but summed for Tof data. The process of combining also differs in other ways between those two data types (see Differences between Tof and Quad data when combining spectra (Page 54)).

A combined spectrum exhibits an enhanced signal-to-noise ratio and improved mass accuracy.

To combine and subtract mass spectra:

1. In the chromatogram pane, select the check box on the top, left-hand side to see the available processing options.
2. Select **Combine Spectra**.
3. In the Combine Spectra window, click and drag to mark up to three regions on the chromatogram, in order:
   a. The region over which to combine spectra (Combine Region)
   b. A first background region to subtract from the combined spectrum (Subtract Region 1)
   c. A second background region to also subtract from the combined spectrum (Subtract Region 2)

   Result: As you define each region, a combined spectrum displays above the chromatogram.

   Tip: After you define a region, its start and end retention times are displayed below the chromatogram. You can adjust the start and end retention time values by typing in the appropriate boxes.
4. Optionally, use the following controls to pause or adjust the process:
   a. Click **Stop Marking** to pause the marking process while defining regions, and zoom to examine the chromatogram. The Stop Marking button changes to Mark Regions. To resume marking after pausing, click **Mark Regions**.
   b. Adjust the Background Factor value to change the scaling factor applied to the background range selected, prior to subtraction. A higher value increases the effect of the background subtraction.
   c. When you combine and subtract from a low-energy MSe chromatogram, select the **Combine Spectra on High Energy Trace** option to automatically process the high-energy chromatogram with the same settings.

5. When you have marked all three regions, the Stop Marking button changes to Clear Selection. You can optionally click this button to restart the marking process.
6. After marking all three regions, click **Add** to create the combined and background-subtracted spectrum, denoted by the lozenge "Combined & Subtracted".

   Tip: You can click Add when only one or two regions are defined. If you click Add without defining either background region, no background subtraction is applied. The lozenge on the processed spectrum still indicates "Combined & Subtracted" but shows that no background regions were defined.

Differences between Tof and Quad data when combining spectra

Combining (or combining and subtracting) scans to produce a combined spectrum differs between Tof and Quad data because of the need for lock mass correction.

For Quad data, without a lock mass correction, ions within each scan strike the detector in order of their *m/z*, and the detector measures the intensity of ions over the duration of that scan. The analog-to-digital converter transforms the detector events into a set of time bins and intensity values for each scan. A mass calibration is then applied that converts the time bin to a *m/z* value, resulting in a set of *m/z* and intensity values for each scan.

The mass calibration is the same for each scan, meaning that further data transformations are not required, and intensities can be combined directly from common time (*m/z*) bins.

Tof data is normally lock mass corrected, which means that when time bins are converted to *m/z* values, a lock mass correction is applied in addition to the mass calibration. The lock mass correction factor at a given retention time offsets the *m/z* values to adjust for instrument drift. However, this means that the corrected position of the data points in a given scan may not align with the initial position of the bins—for example, the corrected values may fall between the original bins. Because the lock mass correction varies with retention time, corrected bin locations are also different at different retention times, so data points do not align across scans.

To allow intensities to be combined, each scan is resampled so that the data points across scans have common *m/z* values (in this context, resampling means creating new data points based on the existing ones). Intensity values at the offset *m/z* points are interpolated to provide an intensity value at shared, common *m/z* bin locations. The resampled intensities from each bin can now be summed together when combining the data.

Note: Limit the number of concurrent LC-MS Toolkit network users to five.

Combining mass spectra directly from a chromatogram

In addition to combining and subtracting spectra from the Tools menu, you can also combine mass spectra directly from a chromatogram.

When you combine spectra in this way, background subtraction is not applied.

To combine mass spectra directly from a chromatogram:

In the chromatogram pane, right-click and drag across the desired peaks.

Tip: Alternatively, you can manually specify ranges of the combine and subtract regions using the **Combine Spectra** option on the toolbar.

Result: The latest combined spectrum appears at the top on the right-hand side of the application window with the "Combined" lozenge.

Note: If you combine spectra from a low-energy MSe chromatogram, both the low collision energy and the high collision energy MSe chromatograms are processed and the generated spectral plots are automatically selected, creating two spectra over the same range.

Extracting a single mass spectrum from a chromatogram

You can quickly extract a single mass spectrum directly from a chromatogram.

To extract a single mass spectrum from a chromatogram:

In the chromatogram pane, double-click the desired point.

Result: The extracted spectrum is displayed at the top on the right-hand side of the application window with the "Extracted" lozenge.

Notes:

- The extracted spectrum is taken from the data point closest to your double-click location in the chromatogram.
- If you extract a spectrum from a low-energy MSe chromatogram, the closest spectrum in the high-energy MSe chromatogram is also extracted in a separate plot.

Combining and extracting mass spectra from XICs

You can create an XIC (extracted ion chromatogram) from a chromatogram or a spectrum. You can then combine, combine and subtract, or extract spectra from this XIC.

Importantly, when you create spectra from an XIC, the spectra are combined or extracted from the original source chromatogram. They are not limited to the *m/z* value or range included in the XIC.

This allows you to identify the retention time maximum for an *m/z* peak on the XIC, and then combine or extract full spectra accurately from that point to identify associated peaks in the original data.

Processing combined MS data

You can perform the following processing operations on a combined (or extracted) spectrum from the Tools menu:

- Smooth
- Mass measure
- Center
- BayesSpray deconvolution
- MaxEnt1 deconvolution
- MaxEnt3 deconvolution

You can also create an XIC directly from a spectrum without using the tools menu.

To process combined MS data:

1. In the combined spectrum pane, select the check box on the top left-hand side to see the available processing options in the Tools menu.

   #### Notes:

   - To select additional spectra, press and hold **Ctrl**, and then select the check boxes on the additional spectra.
   - To select contiguous spectra, press and hold **Shift**, and then select the check boxes on the start and end spectra.
   - Some processing operations can be performed on multiple suitable spectra simultaneously, applying the same processing settings to each spectrum.
   - Smooth and mass measure are available for spectra derived from Quad data.
   - All operations are available for most spectra derived from Tof data (you cannot deconvolve lock mass reference channels). See Processing options available by data type (Page 92).
   - You can only perform operations appropriate to your starting spectrum. Because of this, after you perform one of the operations, other options may not be available for the resulting spectrum.

2. Select a processing operation from the options available, and follow the appropriate guidance below.

Table 1: Spectrum processing options in the Tools menu

Operation	Details
Smooth	Smoothing reduces the high-frequency noise present in a chromatogram. Two methods are available for smoothing: • Mean • Savitzky-Golay Both methods slide a window along the chromatogram, averaging the data points in the window to produce a point in the smoothed spectrum. Savitzky-Golay is more commonly used for mass spectra. See Smoothing a spectrum (Page 58).
Mass Measure	Mass Measure performs peak detection on the spectrum and then reports the mass of the peak center as a single bar (centroid). You need not specify any parameters. Click **Mass Measure** in the Tools menu to perform this operation, and see The Mass Measure process (Page 59) for more information.
Center	Centering uses the points across a peak in a continuum trace to calculate the mass of the peak center. This produces a single bar from each peak in a continuum spectrum. You can apply this centering operation to Tof and Quad data and customize the parameters used. See Centering a spectrum (Page 60).
BayesSpray	BayesSpray is a nested sampling Bayesian inference algorithm for deconvolution of Electrospray mass spectral data, applicable to a wide range of molecules and molecular masses. See Producing a deconvolved spectrum with BayesSpray (Page 62).
MaxEnt1	The MaxEnt1 deconvolution algorithm uses the maximum entropy method to produce true molecular mass spectra from multiply charged Electrospray spectra.

Table 1: Spectrum processing options in the Tools menu (continued)

Operation	Details
	MaxEnt1 is suitable for deconvolution of larger molecules (for example, intact proteins). See Producing a deconvolved spectrum with MaxEnt1 (Page 64).
MaxEnt3	MaxEnt3 can be used for deconvolution of smaller molecules, such as peptides and smaller oligonucleotides. See Producing a deconvolved spectrum with MaxEnt3 (Page 68).

3. You can also create an XIC directly from the spectrum, without using the Tools menu (where this option does not appear). You can right-click and drag to extract a range, or double-click a single point. See Creating an XIC from a mass spectrum (Page 69).
4. After any processing operation is complete, a lozenge appears on the output spectrum (or spectra), named for (and color-coded by) the type of processing. Point to this lozenge for details of the processing settings used to create the output spectrum (except for Mass Measure, which requires no settings).

   Tip: When you process a spectrum, you can automatically replace the original plots with the respective processed plots by selecting the **After Integration** or **After Smoothing** check box in the User Preferences Settings dialog.

Smoothing a spectrum

Smoothing reduces high-frequency noise present in a spectrum.

Two methods are available for smoothing. Both move a window across the spectrum, averaging the data points within the window to produce a point in the smoothed spectrum.

- Mean. This calculates the arithmetic average of each set of data points within the window.
- Savitzky-Golay. This uses a least squares approach to optimally fit a set of data points within the window to a polynomial curve, and uses the curve to weight the calculation of the average.

To smooth a mass spectrum:

1. Select a combined continuum spectrum to process, using the top left-hand check box in the spectrum pane.
2. Next to Smooth in the Tools menu, click  to specify smoothing settings as follows:

Table 2: Smooth settings

Setting	Description
Smoothing width	Set to the half-width of the smoothing window (in data points). This is the number of data points before and after the center of the smoothing window. **Notes:** • For Quad data, set the Peak Width in Da. • If this value is too high, the smoothed spectrum is distorted.
Iterations	Smoothing is an iterative process. Specify the number of times the smoothing is repeated. Increasing the number produces a heavier smoothing effect. **Note:** If this value is too high, the smoothed spectrum is distorted.
Smooth Type	Select the type of smoothing: • Mean • Savitzky-Golay **Tip:** Generally, mean smoothing provides the best smoothing for chromatograms, whereas Savitzky-Golay provides the best smoothing for spectra. Savitzky-Golay tends to enhance peak and valley shapes, as well as preserve the height of the peaks better than mean smoothing. However, Savitzky-Golay can produce small artifacts on either side of the real peaks.

3. Click **Smooth** in the dialog box to center the data.

   Tip: To process the spectrum directly from the Tools menu without changing the settings from their default (or most recently used) values, click **Smooth** there instead.

The Mass Measure process

Mass Measure is designed to accurately detect Tof peaks and produce an accurate mass output value. It adjusts the *m/z* values of Tof spectra by resampling so that the peak width is constant across the mass range, allowing fixed width filters to be used in peak detection. Both first-

and second-derivative filters are then used to identify the start, end, and centroid (average *m/z* weighted by intensity) of the peaks. After peak detection, the data points are adjusted back to their original *m/z* locations (bins).

Mass Measure reports the centroid of each peak as a single bar, with the intensity of the centroided peak corresponding to the area of the original continuum peak. This means it quantitates by original peak area.

Mass Measure results are not identical to centering a peak using the Center operation on a combined spectrum, but they will be closest to Center results that are centered by area (not height).

You can use Mass Measure and Center outputs for operations requiring accurate mass values (for example, elemental composition calculations).

Centering a spectrum

The centering operation combines all the points across a peak in a continuum trace to calculate the mass of the peak center, taking the average of the *m/z* weighted by intensity. This produces a single bar from each peak in a continuum spectrum (a centroid).

You can adjust how much of the peak is included in the centroid calculation, and whether the centroided intensity is based on the height or area of the original peak.

You can center combined data that were not previously centered, mass measured, or created by deconvolution.

To center a mass spectrum:

1. Select a combined continuum spectrum to process, using the top left-hand check box in the spectrum pane.
2. Next to Center in the Tools menu, click  to specify centering settings as follows:

Table 3: Center settings

Setting	Description
Use top	Defines the amount of the peak to include in the centering calculation. The percentage refers to the height (intensity) of that point above the peak baseline, relative to the maximum height of the peak above the baseline. For example, the default value (include the top 80%) includes any points above 20% of the maximum peak height. A higher value includes more of the peak in the calculation, but also includes

Table 3: Center settings (continued)

Setting	Description
	more background noise from lower intensity points. **Recommended:** 60% to 95%
Minimum peak half width	Determines the minimum width of a peak at half-height to combine, in data point channels. **Tips:** • You can use the distance tool to estimate this value (see Viewing chromatograms and spectra (Page 16)). • For continuum data, Waters recommends that you use the default value (4) because there are sixteen data points collected per dalton, so this value is equivalent to 0.25 Da. • Too low a value allows peaks to be produced from narrow noise spikes. • Too high a value causes separate peaks to be combined into single centroid bars.
Report using	Select **Area** or **Height** to specify how the intensities of data points across the peak are reported in the centroided output. **Notes:** • Area reports the area of the continuum peak as the intensity of the centroided bar.

Table 3: Center settings (continued)

Setting	Description
	• For Tof data, the combined intensities of the included data points are summed. • For Quad data, the area is calculated using the trapezium rule. • Height reports the maximum intensity (height) of the continuum peak as the intensity of the centroided bar. • Height is typically used when processing Quad data and Area when processing Tof data.

3. Click **Center** in the dialog box to center the data.

   Tip: To process the spectrum directly from the Tools menu without changing the settings from their default (or most recently used) values, click **Center** there instead.

Producing a deconvolved spectrum with BayesSpray

BayesSpray is a nested sampling Bayesian inference algorithm for deconvolution of electrospray mass spectral data. It is applicable to a wide range of molecules and molecular masses.

It can process the same types of molecules as both MaxEnt1 and MaxEnt3, while producing cleaner deconvoluted spectral baselines.

BayesSpray requires a continuum input spectrum. BayesSpray in LC-MS Toolkit produces two output spectra:

- A deconvolved output spectrum ("BayesSpray") in centroid form. This is a neutral mass spectrum, including either monoisotopic or average masses.
- A mock spectrum in continuum form ("BayesSpray Mock"). This represents a recreation of the input data based on the final model, denoted by a "Simulated *m/z*" *x* axis. The mock spectrum should be a good match for the original input spectrum.

BayesSpray results are quantitative. The intensity of a centroided peak in the output spectrum is representative of the summed, multiply charged continuum peak areas in the input data that deconvolve to produce it.

Poster reference: For more information about BayesSpray, search www.waters.com for the reference "720003756en".

To produce a deconvolved spectrum with BayesSpray:

1. Select a combined continuum spectrum to process, using the top left-hand check box in the spectrum pane.
2. Next to BayesSpray in the Tools menu, click  to specify BayesSpray settings as follows:

Table 4: BayesSpray settings

Tab	Setting	Description
Basic Settings	Input Range	Specify the input *m/z* range to consider.
	Output Range	Specify the mass range of the output spectrum, in Da. Set the output range sufficiently wide to accommodate all target molecules that might be represented in the input data. **Note:** This is a single range. You cannot specify multiple separated output ranges.
	Output Average Masses	Select to display average masses in the output data.
	Charge Range	Select the range of charge states to consider during deconvolution.
	TOF Resolution	Specify the Tof resolution, *m/z* divided by the full-width half-maximum height (FWHM).
	Detailed Isotope Model	Select this option if your input data is at high enough resolution to resolve separate isotopic peaks (for example, the *m/z* isotope delta is greater than your peak width at half maximum).
Advanced Settings	Molecule Type	Select the molecule type so that the deconvolution process includes correct assumptions about elemental composition and isotopic profiles. • Protein • Oligonucleotide • Phosphorothioate Oligonucleotide • Glycan If you have an oligonucleotide with a mixture of phosphodiester and

Table 4: BayesSpray settings (continued)

Tab	Setting	Description
		phosphorothioate linkages, Waters recommends that you choose the model that represents the majority of the linkages.
	Maximum Species	BayesSpray models spectra containing up to this number of peaks. A higher number requires more processing time, but too low a number may cause masses to be missing from the final output.
	Iterations	BayesSpray deconvolution is an iterative process, with each iteration yielding a mass spectral output with improved fit to the data. A greater number of iterations may improve the fit, but it also causes a longer processing time.
	Charge Carrier	Specify the principal charge carrier by selecting an adduct from the list. **Tip:** From the charge carrier list, select Hydrogen unless you know that most of the charges result from other adducts.
	Number of Objects	This value is used to distribute the process over multiple processes. Although this improves the depth of exploration, it can increase the processing time. Specify the number of processes to use.

3. Click **Deconvolve** in the dialog box to begin processing.

   Tip: To process the spectrum directly from the Tools menu, without changing the settings from their default (or most recently used) values, you can click **BayesSpray** there instead.

Producing a deconvolved spectrum with MaxEnt1

The MaxEnt1 algorithm uses the maximum entropy method to produce true molecular mass spectra from multiply charged Electrospray spectra. The simplest spectrum (original peak distribution) that could account for the observed *m/z* data is generated, where simplest means the distribution with the highest entropy, or the least information beyond known constraints. This accounts for the observed data while making the fewest unnecessary assumptions.

MaxEnt1 is suitable for deconvolution of larger molecules (for example, intact proteins).

MaxEnt1 requires a continuum input spectrum and produces two output spectra:

- A deconvolved output spectrum ("MaxEnt1") in continuum form. This is a neutral average mass spectrum.
- A mock spectrum in continuum form ("MaxEnt1 Mock"). This represents a recreation of the input data based on the final model, denoted by a "Simulated *m/z*" *x* axis. The mock spectrum should be a good match for the original input spectrum.

MaxEnt1 is an iterative algorithm. It calculates an initial deconvolved spectrum, and then uses programmed knowledge of chemistry and mass spectrometer physics to synthesize a corresponding mock spectrum. It then compares the mock data to the observed data and uses the difference between the two to guide it to produce an improved deconvolved spectrum. The algorithm terminates when there is sufficiently little difference between mock and real data.

MaxEnt1 results are quantitative. Peak areas in the MaxEnt1 output spectrum are representative of the summed intensities of each component's multiply charged peaks in the input data.

Application note reference: For background details about MaxEnt1, search www.waters.com for the reference "720000553EN".

To produce a deconvolved spectrum with MaxEnt1:

1. Select a combined continuum spectrum to process, using the top left-hand check box in the spectrum pane.
2. Next to MaxEnt1 in the Tools menu, click  to specify MaxEnt1 settings as follows:

Table 5: MaxEnt1 settings

Tab	Setting	Description
Basic Settings	Input Range	Specify the input *m/z* range to consider.
	Output Range	Specify the mass range of the output spectrum, in Da. Set the output range sufficiently wide to accommodate all target molecules that might be represented in the input data. **Note:** This is a single range. You cannot specify multiple separated output ranges.
	TOF Resolution	Specify the Tof resolution value of your instrument.
	Include Isotope Width	Select this check box if isotopes are not resolved in your peaks (a single peak is produced, for example with charge states of large proteins). LC-MS Toolkit

Table 5: MaxEnt1 settings (continued)

Tab	Setting	Description
		calculates an isotopic contribution to the overall peak width. Peak widths are determined by combining the instrument peak width with the theoretical width of the isotopic envelope divided by the number of charges on the ion. Clear this check box if isotopes are (at least) partially resolved in the input data, and you require that isotopes appear resolved in the output data. In this situation, isotopes are deconvolved directly and a contribution to a single larger peak width is not required.
Advanced Settings	Iterations	Specify the maximum number of iterations performed. You can set this parameter only if you clear the "Iterate To Convergence" check box.
	Iterate To Convergence	Select this check box to continue deconvolution until convergence. The algorithm repeats its calculations until it finds a molecular mass spectrum that produces an *m/z* spectrum that matches the real data. This requires more processing time. If you clear the check box, specify a value for the maximum number of iterations to perform.
	Molecule Type	Select the molecule type, so that the deconvolution process includes correct assumptions about elemental composition and isotopic profiles. • Protein • Oligonucleotide • Phosphorothioate Oligonucleotide • Glycan If you have an oligonucleotide with a mixture of phosphodiester and phosphorothioate linkages, Waters

Table 5: MaxEnt1 settings (continued)

Tab	Setting	Description
		recommends that you choose the model that represents the majority of the linkages.
	Charge Carrier	Specify the principal charge carrier by selecting an adduct from the list. **Tip:** From the charge carrier list, select Hydrogen unless you know that most of the charges result from other adducts.
	Output Resolution	Specify the spacing, in Da, of data points on the output mass axis.
	Enable Noise Autoscaling	Select to update the initial noise estimate as the algorithm proceeds. Clear the check box to use the initial noise estimate. This may be more appropriate when the data contains many spikes.
	Minimum Intensities (Left)	This is an intensity fall-off criterion, based on the expectation that successive charge states of the ionized molecule follow an intensity distribution. Specify the minimum intensity of each consecutive charge state relative to the previous one, moving from the most intense charge state to lower *m/z* values (successively higher charge states). **Tip:** The default value 30% is suitable for most large molecules.
	Minimum Intensities (Right)	This is an intensity fall-off criterion, based on the expectation that successive charge states of the ionized molecule follow an intensity distribution. Specify the minimum intensity of each consecutive charge state relative to the previous one, moving from the most intense charge state to higher *m/z* values (successively lower charge states). **Tip:** The default value 30% is suitable for most large molecules.

3. Click **Deconvolve** in the dialog box to begin processing.

   Tip: To process the spectrum directly from the Tools menu, without changing the settings from their default (or most recently used) values, you can click **MaxEnt1** there instead.

Producing a deconvolved spectrum with MaxEnt3

MaxEnt3 can be used for deconvolution of smaller molecules, such as peptides and smaller oligonucleotides.

MaxEnt3 requires a continuum input spectrum and produces a centered output spectrum.

MaxEnt3 differs from BayesSpray and MaxEnt1 in several ways, including the following:

- MaxEnt3 deconvolves a multiply charged mass spectrum to a singly charged mass spectrum, instead of a neutral mass spectrum. This is denoted by a "Mass+H^+" or "Mass-H^+" *x* axis (depending on your data polarity).
- MaxEnt3 always assumes a proton charge carrier.
- In LC-MS Toolkit, MaxEnt3 produces only a deconvolved spectrum, not a mock spectrum.

To produce a deconvolved spectrum with MaxEnt3:

1. Select a combined continuum spectrum to process, using the top left-hand check box in the spectrum pane.
2. Next to MaxEnt3 in the Tools menu, click  to specify MaxEnt3 settings as follows:

   Table 6: MaxEnt3 settings

Setting	Description
Input Range	Specify the input *m/z* range to consider.
Output Range	Specify the mass range of the output spectrum, in Da. Set the output range sufficiently wide to accommodate all of the target molecules that might be represented in the input data. **Note:** This is a single range. You cannot specify multiple separated output ranges.
Tof Resolution	Specify the Tof resolution value of your instrument.
Maximum Charge	Select the maximum charge state to consider during the deconvolution.
Iterations	Specify the maximum number of iterations performed.

3. Click **Deconvolve** in the dialog box to begin processing.

   Tip: To process the spectrum directly from the Tools menu, without changing the settings from their default (or most recently used) values, you can click **MaxEnt3** there instead.

Creating an XIC from a mass spectrum

You can create an extracted ion chromatogram (XIC) directly from a mass spectrum without using the Tools menu. The XIC is created using your specified *m/z* value (or range) and the source chromatogram for the spectrum.

To create an XIC from a mass spectrum:

1. In the spectrum pane, perform one of the following actions directly on the spectrum:
   - Right-click and drag a range.
     - An XIC is created for the specified range.
   - Double-click a specific point.
     - For continuum data. an XIC is created for the specified *m/z* value.
     - For centroided (centered) or mass measured data, the XIC is created for the *m/z* value of the closest peak to your double-click location.

       Tip: Double-click the top of a peak to select it, because distance considers both *m/z* and intensity.
     - When you create an XIC by double-clicking, it includes a tolerance around the specified *m/z*.
2. To optionally change the tolerance applied when you double-click to create an XIC, use the Extract Masses option in the Tools menu to create an XIC directly from a chromatogram (see Using Extract Masses (Page 44)). The tolerance applied in this option also applies when you create an XIC by double-clicking a spectrum.

Creating a spectral peak table

You can create a spectral peak table for centroid or deconvoluted data and export it to use in other applications.

To create a spectral peak table:

1. Select a centroided or a MaxEnt1/MaxEnt3/BayesSpray processed plot to see the spectral peak table option in the toolbar.
2. Select the **Spectral Peak Table** option. The table appears with the following columns:

- Observed m/z
- Intensity
- % Intensity

3. To filter mass, intensity, and %intensity information, click  in the column heading. In the dialog box, specify the filter criteria, and then click **Apply**.

   Note: To clear filtering for a single column, click the filter icon again, and then click **Clear** in the dialog box.

4. To filter the top "n" rows, click  . In the **Display top:** field, specify the number of rows that you want to view, and then click **Apply**.

   Note: To clear all filters, click  .

5. To copy the results from the spectral peak table, click  . There are two copy options as follows:

   - **Copy Table**: Use this option to copy the results, along with the header, in a tab-delimited format. You can then paste it into Notepad, Excel, or other applications.
   - **Copy Exclusion/Inclusion list**: Use this option to copy the results, without the header, in a tab-delimited format. You can then paste it into a waters_connect AME method exclude/include table.

Identifying candidate components

LC-MS Toolkit includes a set of elucidation tools that you can use to investigate and potentially identify candidate components. The options available are as follows:

- Elemental composition (Page 71)
- Mass fragment (Page 81)

Elemental composition

LC-MS Toolkit includes an elemental composition calculator that calculates possible formulas that could account for an observed accurate mass. To restrict the number of possible formulas, you can filter by the numbers of different atoms, valence states, the DBE (double bond equivalent) value, and a series of chemical rules.

Elemental composition uses an algorithm, i-FIT, to score each remaining formula by the likelihood that the theoretical isotope pattern of the formula matches a cluster of peaks in the spectrum. This allows you to assess how well each theoretical elemental composition accounts for the observed data, and to identify which theoretical composition is most likely to be correct.

You can copy and paste the suggested formulas and composition results into an online resource for identification.

Including fragments

You can also analyze charged fragment profiles (with charge state 1) in the i-FIT calculation. To include fragments, you specify fragment-specific *m/z* values. You can include fragments from a high-energy spectrum to match with precursor data in the low-energy spectrum, or fragments from the same spectrum as the precursor. For each fragment, theoretical fragment ion compositions are calculated. Fragment compositions that match a subset of the elements in a precursor composition improve the evidence for that precursor composition. This is apparent in the i-FIT score for a precursor composition, which takes fragment data into account.

You can also disable the i-FiT filtering by clearing the **i-FiT** check box in the addition settings. This is useful in samples where suitable candidate formulas in noisy or low intensity data are rejected on the basis of their i-FiT scores. This helps the user to get the unfiltered results and make their own decision on the most likely match. By default, the **i-FiT** check box is selected.

Prerequisite: If you want to include high-energy charged fragments, you must use a low-energy and high-energy MSe spectrum from the same injection. These spectra must both be centered or mass measured, and they must be processed in exactly the same way with the same settings. This includes combining in the same way over the same retention time, and applying the same centering settings (if centering).

Tips:

- If you combine low-energy spectral data over the peak by right-clicking and dragging, the high-energy spectrum is automatically combined over the same retention time range.
- If you use the Combine Spectra option to combine and subtract spectra from the low-energy chromatogram, you can select the **Combine Spectra on High Energy Trace** option to automatically process the high-energy chromatogram in the same way.
- If you use the Combine Spectra option to combine and subtract spectra from each chromatogram separately, set the combine and subtract regions to have the same time values using the text fields.

To calculate elemental composition:

1. Select a mass measured or centered spectrum, and then click **Elemental Composition** from the Tools pane.

   #### Notes:

   - The Elemental Composition page appears with the spectrum on the left-hand side, elemental composition settings on the right, and results table beneath the settings.
   - If you are including a low-energy precursor and high-energy fragments in the calculation, select both low- and high-energy spectra before clicking **Elemental composition**. Both spectra appear on the left-hand side with the low-energy spectrum above the high-energy spectrum. You can link their *x* axis zoom by clicking **Link X-Axes** above the plots.
   - Individual plots behave like the main session window (see Viewing chromatograms and spectra (Page 16)). You can view information for a point, zoom in and out, measure distances, and download an SVG image. However, you cannot maximize the plots.

2. In the Settings tab, specify the **Basic Settings**, **Additional Settings**, and **Element Selection** settings according to the following table.

   Note: The Settings tab displays the name of loaded settings. This can be any of the four presets or a custom name. For example, Settings - Small Molecule Settings, Settings - Oligonucleotide Settings, and so on.

   Table 1: Setting descriptions

Setting	Action
Basic settings	
m/z	Type the mass-to-charge ratio you want to identify. **Tip:** Alternatively, you can select the *m/z* and calculate elemental composition immediately with the current settings. To do this, double-click the top of a peak in the spectrum plot (specifically, the low-energy spectrum, if you also include high-energy fragments).

Table 1: Setting descriptions (continued)

Setting	Action
	• When you double-click the spectrum, the mass-to-charge ratio value is taken from the nearest peak (the shortest distance to the top of a peak, considering *m/z* and intensity). • When you select a low intensity peak, zooming in helps you select the correct peak by increasing its plotted height and separating it from nearby peaks.
Fragment *m/z*	If you are including fragments, type the fragment mass-to-charge ratios you want to identify, with multiple fragments separated by commas (for example, "123.456, 789.123"). If you include both a low-energy and a high-energy spectrum, the fragments are matched from the high-energy spectrum. You cannot match fragments from both low-energy and high-energy spectra at the same time. If you only include one spectrum, the fragments are matched from that spectrum. **Tips:** • If you set this value to zero, no fragment ions are included in elemental composition identification. • When you include both a low-energy and high-energy spectrum, you can select fragments directly from the high-energy spectrum plot, as follows. This does not automatically start the calculation. 1. Double-click the top of a fragment peak in the high-energy spectrum to select it and populate the Fragment *m/z* box with its *m/z* value. 2. To add further fragments, press and hold **Ctrl** and then double-click their locations. 3. If you double-click another fragment without holding **Ctrl**, previous fragments are cleared and replaced by that fragment. • When you only include one spectrum, you can select fragments directly from the spectrum plot, as follows. This does not automatically start the calculation. 1. Click within the **Fragment m/z** box. 2. Double-click the top of a fragment peak in the spectrum to select it and populate the Fragment *m/z* box with its *m/z* value. 3. To add further fragments, press and hold **Ctrl** and then double-click their locations.

Table 1: Setting descriptions (continued)

Setting	Action
	4. If you double-click another fragment without holding **Ctrl**, previous fragments are cleared and replaced by that fragment. 5. To return to selecting the precursor *m/z*, click within the **m/z** box. • When you double-click a spectrum, the mass-to-charge ratio value is taken from the nearest peak (the shortest distance to the top of a peak, considering *m/z* and intensity). • When you select a low intensity peak, zooming in helps you select the correct peak by increasing its plotted height and separating it from other nearby peaks.
Number of results	Specify the maximum number of matching formula results to display.
Tolerance	Specify the tolerance for the error between the observed and theoretical *m/z* of the precursor in PPM (parts per million) or mDa (millidaltons). If you include fragments, the same tolerance applies to fragment matching as the precursor tolerance.
Adduct	The default adduct is protonation or deprotonation, depending on your data polarity. To specify an alternative adduct or select multiple adducts, click **Select**. In the Select Adduct dialog box, use the check boxes to choose the appropriate adduct or adducts for your ionization technique from the list, and then click **Select**.
Charge State	Specify the charge state of the ion. The results consider only this charge state. **Important:** This charge state specification applies only to the precursor, not to fragments (when included). For fragments, only charge state 1 is considered.
Fragment Ions Charge State	Specify the charge state of the fragment ion.
Electron State	Select one of three electron states to specify the valency of molecules to include in the results: **Even**, **Odd**, or **Both**.
Additional settings	
Minimum DBE	Specify a minimum for the Double Bond Equivalent value.[a]
Maximum DBE	Specify a maximum for the Double Bond Equivalent value.

Table 1: Setting descriptions (continued)

<table>
<tr><th>Setting</th><th>Action</th></tr>
<tr><td>i-FIT isotope number</td><td>Specify the number of isotopic peaks to include in the i-FIT score calculation.</td></tr>
<tr><td>Filters</td><td>Select a filter to restrict possible formulas to those that meet the conditions of each filter.</td></tr>
<tr><td>Senior Rule</td><td>• The sum of valences, or the total number of atoms with odd valences, is an even number.
• The sum of valences is greater than or equal to twice the maximum valence.
• The sum of valences is greater than or equal to twice the number of atoms minus 1.[a]</td></tr>
<tr><td>Hydrogen/ Carbon Ratio</td><td>The hydrogen/carbon ratio of the elemental composition formula must be between 0.1 and 6, based on data derived from a mass spectral database.[a]</td></tr>
<tr><td>Heteroatom/ Carbon Ratio</td><td>The ratios of elements other than hydrogen to carbon must be within the following common ranges, derived from a mass spectral database.[a]
<table>
<tr><th>Element ratio</th><th>Range</th></tr>
<tr><td>F/C</td><td>0-6</td></tr>
<tr><td>Cl/C</td><td>0-2</td></tr>
<tr><td>Br/C</td><td>0-2</td></tr>
<tr><td>N/C</td><td>0-4</td></tr>
<tr><td>O/C</td><td>0-3</td></tr>
<tr><td>P/C</td><td>0-2</td></tr>
<tr><td>S/C</td><td>0-3</td></tr>
<tr><td>Si/C</td><td>0-1</td></tr>
</table></td></tr>
<tr><td>Multi-Atom</td><td>The total number of elements other than H are within a common range derived from a mass spectral database.[a]
<table>
<tr><th>Element ratio</th><th>Range</th></tr>
<tr><td>N, O, P, S, all > 1</td><td>N < 10, O < 20, P < 4, S < 3</td></tr>
<tr><td>N, O, P, S, all > 3</td><td>N < 11, O < 22, P < 6</td></tr>
<tr><td>O, P, S, all > 1</td><td>O < 14, P < 3, S < 3</td></tr>
<tr><td>P, S, N, all > 1</td><td>P < 3, S < 3, N < 4</td></tr>
<tr><td>N, O, S, all > 6</td><td>N < 19, O < 14, S < 8</td></tr>
</table></td></tr>
</table>

Table 1: Setting descriptions (continued)

Setting	Action
i-FiT	This is useful in samples where suitable candidate formulas in noisy or low intensity data are rejected on the basis of their i-FiT scores. Clearing the i-FiT check box ensures the user views the unfiltered results and make their own decision on the most likely match. By default, the **i-FiT** check box is selected.
Element selection	Perform these steps: • Select whether to include elements and isotopes of the element as part of the elemental composition calculation from the list. • In the **Min** column, specify the minimum number of atoms of the element. In the **Max** column, specify the maximum number of atoms of the element. **Note:** When you perform fragment matching, the minimum number of atoms applies only to the precursor, not to fragments. • To include additional elements, click **Add Elements**, select the ones you want, and then click **Add**. **Tips:** • To filter the list of elements, type characters matching at least one element and press **Enter**. You can create several filters, which are additive. Filters are not case-sensitive. • After adding an element, click the arrow ▶ to show alternative (minor) isotopes. • Elements you add are automatically selected, but any minor isotopes of those elements are not. • To remove an element and its isotopes from the table, click **Remove element** 🗑. You can restore the element and its isotopes by clicking **Add Elements**. • Currently selected elements are also displayed on the Basic Settings tab, under **Elements Selected**. Alternative (minor) isotopes are displayed separately with their mass number, where present.

a. Kind, T.; Fiehn, O. Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. *BMC Bioinformatics* **2007**, *8*, 105.

3. To calculate possible elemental compositions matching your settings, click **Run Composition**.

Tips:

- You can run elemental composition again at any time by clicking **Run Composition**.
- To return all settings (apart from *m/z* values) to their defaults, click **Reset**.

4. Examine the possible formulas matching your settings in the results table.

Table 2: Results

Molecular Formula	m/z Formula	i-Fit Confidence (%)	Predicted m/z	Adduct	Neutral Mass	m/z error (PPM)	m/z error (mDa)
$C_{12}H_{29}FN_5OP$	$C_{12}H_{30}FN_5OP$	99.74	310.21665	$+H^+$	309.20938	0.38	0.12
$C_{10}H_{22}FN_4OP$	$C_{10}H_{23}FN_4OP$		265.15880			-0.01	0.00
$C_{21}H_{27}NO$	$C_{21}H_{28}NO$	0.26	310.21654	$+H^+$	309.20926	0.74	0.23

Table 3: Field description

Column	Details

Molecular Formula	List of possible formulas for the precursor, based on the elemental composition settings that can help you determine an accurate mass peak. By default, the results are ordered by the best i-FIT score.
	Notes:
	• The formula listed is the neutral formula. It does not contain charged adducts.
	• The number of each type of atom is shown in subscript following the atom.
	• Alternative (minor) isotopes are listed separately to the major isotope with their mass number in superscript form. For example:
	  • Carbon-12 is listed as C with no superscript mass number, but carbon-13 is listed as ^{13}C.
	  • A molecule containing six carbon atoms, of which four are carbon-12 and two are carbon-13, has its carbon component listed as $C_4{}^{13}C_2$.

m/z Formula	This formula shows the charged adduct detected by the mass spectrometer.
i-FIT Confidence % Percentage	The (posterior) probability of each elemental composition for the precursor, given the spectral data. A higher value indicates a greater probability that the elemental composition is correct.

Table 3: Field description (continued)

Column	Details
	Note: The total of the confidence scores for all matched elemental compositions always equals 100%. At least one matched composition must be present to calculate this value, and if only one elemental composition is matched, the value equals 100%.
Predicted *m/z*	Predicted mass-to-charge ratio of the precursor, including the charging adduct.
Adduct	The charging adduct for the precursor. **Note:** Where a precursor is matched with a specific adduct, fragment matching for that precursor formula considers the same adduct as the precursor.
Neutral Mass	Predicted neutral mass of the precursor (in daltons).
m/z error (PPM)	The error between the observed and expected mass-to-charge ratio for the precursor, expressed in parts per million.
m/z error (mDa)	The error between the observed and expected mass-to-charge ratio for the precursor, expressed in millidaltons.
DBE	Calculated Double Bond Equivalent value of the precursor.
i-FIT score	This score relates to the likelihood of observing the experimental spectrum given the theoretical isotope pattern. The lower the i-FIT score, the better the theoretical isotope pattern is at accounting for the observed data.
Normalized i-FIT	The negative of the natural logarithm of the posterior probability (i-FIT Confidence %), so a lower value indicates greater confidence that the elemental composition is correct. **Note:** Because this score is derived from the i-FIT Confidence %, at least one matched composition must be present to calculate this value. If only one elemental composition is matched, the value equals zero.

Notes:

- When you include fragments in the calculation, they contribute to the overall i-FIT results (score, confidence percentage, and normalized score).
- A subset of columns is displayed by default. To add or remove columns, select the **Columns** drop-down menu above the table, and then select (or clear) the column headings that you want (or do not want) to appear in the table.
- You can sort the results by clicking a column heading. To undo the sort, press and hold **Ctrl** and then click the column heading.

5. Click  ,  ,  , or  to resize the panes for an improved view of the results table and the spectrum plot.

Note: When you expand the pane:

- The result table shows more rows
- You can see additional ions and annotations on the spectrum plot once it is maximized.

6. If you included fragment *m/z* values, you can expand table rows to display details for matched fragments.

   a. Click ▶ to expand a row in the table.

   Note: You can only expand rows when fragments are matched.

   b. Matched fragments that contribute to the precursor results are displayed in the following columns:

Column	Details
Molecular Formula	The predicted formulas of any matched fragments.
Predicted *m/z*	Predicted mass-to-charge ratio of any matched fragments, including their charging adducts.
Neutral Mass	The predicted neutral mass of each matched fragment (in daltons).
m/z error (PPM)	The error between the observed and expected mass-to-charge ratio for each matched fragment, expressed in parts per million.
m/z error (mDa)	The error between the observed and expected mass-to-charge ratio for each matched fragment, expressed in millidaltons.
DBE	The calculated Double Bond Equivalent value for each matched fragment.

Note: Fragment data does not affect table sorting.

7. To copy and paste the results in a tab-delimited format, click **Copy elemental compositions to clipboard**, and then paste into another application such as Notepad or Excel. You can copy and paste the composition results from Excel or Notepad into a Web application for identification.

Notes:

- All columns are copied, whether selected on the page or not.
- Fragment data is also copied.

- In the exported data, subscript and superscript are not used in molecular formulas.
- Alternative (minor) isotopes are listed separately from the major isotope, with the mass number of the isotope and element in square brackets. For example:
  - Carbon-12 is listed as C with no mass number, but carbon-13 is listed as [13C].
  - A molecule containing six carbon atoms, of which four are carbon-12 and two are carbon-13, has its carbon component listed as C4[13C]2.

8. You can also save your elemental composition calculation settings for reuse (see Saving and restoring elemental composition calculation settings (Page 80)).
9. To return to the main session window, click **Go back to viewing all session data**  .

   Important:

   - Your settings persist when you return to the Elemental Composition page, except the precursor and fragment *m/z* values. If necessary, record your query *m/z* values before leaving the page.
   - Results of calculations do not persist. To keep results for a specific composition calculation, copy them to the clipboard.

Saving and restoring elemental composition calculation settings

For your convenience, you can save and restore elemental composition calculation settings as a profile. All settings are stored except for *m/z* values.

To save and restore elemental composition settings:

1. To save the current settings for the first time, click  in the Settings pane.
2. In the Save As dialog box, specify a name for the settings profile, and then click **Save**.
3. If you make further changes, you can save the updated settings directly from the Settings pane. To do this, click  .

   This replaces the settings in the most recently used profile.
4. To restore previously used settings, click  in the Settings pane.
5. Select a name from the list to load that settings profile, and then click **Apply Settings**.
6. To delete a settings profile, click  in the Settings pane, and then click the cross (x) next to the name of the settings profile that you want to delete.

Mass fragment

LC-MS Toolkit includes a fragment matching tool that you can use to generate fragments for a target component molecule using a series of chemically predictive algorithms. Fragment matching is based on systematic bond disconnection of the precursor structure. You assign each bond in the target component molecule a score to estimate how likely it is to break, with a low score indicating that a bond is likely to break. You can also specify unbreakable bonds.

LC-MS Toolkit generates theoretical fragments and compares their masses to peaks in the MS/MS for the identified component. The results table displays a list of peaks with matching theoretical fragment masses that you can use to assign structures to your observed fragment ions.

Only structures that meet the filters you specify are returned, and structures that require the breaking of too many bonds that are unlikely to break are excluded. This is achieved using a maximum score (penalty) parameter for the sum of bond scores. Because you assign a high score to bonds that are unlikely to break, the breaking of such bonds causes the total score to exceed the maximum and excludes the structure.

In addition to the V2000 .mol file format, the enhanced V3000 .mol file format is now supported for processing.

Hydrogen Difference

Fragment matches have a "Hydrogen Difference" (or "H-Difference"). This refers to the process by which LC-MS Toolkit calculates matches:

1. Elemental compositions matching the fragment *m/z* are calculated.
2. Potential structures are matched based on the non-hydrogen component of the elemental composition. Fragments containing the non-hydrogen component in a contiguous form are considered.
3. Where more than one match arises for a given *m/z*, the structures are ranked by score.

Because the structures are matched on the non-hydrogen component of the formula, they may show a difference in the number of hydrogens present (and their DBE value) from the elemental composition of the full formula. This is referred to as the Hydrogen Difference (or H-Difference) and is included in the penalty score and customizable limits.

Charge

Note that the calculated structures do not account for or assign charge positions.

Because of this and the H-Difference, you should propose your own fragmentation pathways for the creation of the matched fragment structures. However, the predicted fragment matches aid you considerably in predicting these pathways.

Requirements:

- You must obtain structure files for candidate molecules in .mol file format. These are available in online libraries.
- You must create a mass measured or centered spectrum containing the fragment peak data.

Reference: For background on the matching process, see: Hill AW, Mortishire-Smith RJ. Automated assignment of high-resolution collisionally activated dissociation mass spectra using a systematic bond disconnection approach. *Rapid Commun. Mass Spectrom.* 2005,*19 (21)*, 3111–8.

To identify candidate components using Mass Fragment:

1. Zoom in to the mass measured or centered spectrum you analyze.

   Tip: Fragments are only considered within the zoomed region.
2. Select the check box on the top, left-hand side of the mass measured or centered spectrum to see processing options in the Tools pane.
3. Click **Mass Fragment**.

   Result: The Mass Fragment page appears, with two tabs:

   - Import molecule files
   - Settings
4. Import your .mol file as follows:

   a. Click  .

   b. In the dialog box, browse to your .mol file and select it.

   Note: You can select a list of multiple candidate .mol files to load in the same session.

   c. Click **Open**.

   Note: You can select the .mol file to initiate an automatic reprocess against it with the same processing settings. This allows different candidates to be quickly assessed as potential matches.

   Tip: To change the .mol file, repeat the process selecting the new file.

   Note: When you import a .mol file for the first time, you do not need to click **Run**. Processing runs automatically using the default settings on the **Settings** tab.
5. If you need to customize settings and rerun the processing, select the **Settings** tab, and then specify the following settings:

   Table 4: Settings parameters description

Setting	Details
Bonds	The maximum number of bonds to break in the precursor molecule (up to 4).

Table 4: Settings parameters description (continued)

Setting	Details
Phenyl	The penalty score to apply when a phenyl bond is broken. The default is 8.
Aromatic	The penalty score to apply when an aromatic bond is broken. The default is 6.
Multiple	The penalty score to apply when a double or triple bond is broken. The default is 4.
Ring	The penalty score to apply when a ring bond is broken. The default is 2.
Other	The penalty score to apply when any other bond is broken. The default is 1.
Hetero	A multiplication factor that reduces the penalty for breaking of C-X hetero bonds (allows for easier breakage). The penalty score for the bond is multiplied by this value. The default is 0.5.
Alpha	The penalty score to apply when multiple bonds are broken on a single atom.
Hydrogen Difference	The maximum hydrogen difference to allow for a generated fragment.
Allow scores below	The maximum score to allow for a generated fragment. Potential fragments with scores higher than this value are excluded. **Notes:** • The score sums all bond-breaking penalties, alpha penalties, and hydrogen difference penalties. • If a bond falls into more than one category (for example, a single bond in a ring), then the higher score is applied to the bond. • The default is 8. This means that the default penalties permit a single aromatic bond breakage (with a score of 6), but they do not allow a phenyl

Table 4: Settings parameters description (continued)

Setting	Details
	bond breakage (with a score of 8) in the results. • If you increase the score value, it permits more of the fragmentation actions and associated penalties to occur before a fragment is rejected.
Delta (mDa)	The maximum mass error (difference between the observed and theoretical mass) to allow for a generated fragment, in mDa.
DBE Minimum	The minimum double bond equivalent (DBE) value to allow in a generated fragment.
DBE Maximum	The maximum double bond equivalent (DBE) value to allow in a generated fragment.
H Penalty	Penalty score (per hydrogen) to apply when a generated fragment has a hydrogen difference.
Mode	Specify the charge polarity of generated fragments. **Note:** The charge adduct is assumed to be H (protonation or deprotonation).
Use smart scores check box	When this box is selected, the Phenyl, Aromatic, Ring, Multiple, and Other selections are unavailable. LC-MS Toolkit uses a set of predetermined parameters to calculate the score. When **Use SmartScores** is cleared, the additional selections are enabled. LC-MS Toolkit uses the values of the additional selections to calculate the score given to a bond break.
Filter peaks by intensity	LC-MS Toolkit attempts to match fragments to all spectral peaks, unless you apply a filter. Select this option to filter matching so that it considers only the most intense peaks, and then specify the "Number of peaks" value to adjust the number of peaks included.

Table 4: Settings parameters description (continued)

Setting	Details
	Clear this option to consider all peaks in fragment matching.
Number of peaks	If you selected **Filter peaks by intensity**, specify an integer. Peaks are ordered by descending intensity (highest first), and this value defines the number of peaks included from the list for consideration in fragment matching. For example, if you specify `5`, the five most intense peaks are included. If you specify `0`, this has the same effect as clearing **Filter peaks by intensity**. All peaks are included in matching.

Note: Assigning lower scores to bonds or H-Differences, increasing the maximum score, allowing more bonds to break, and using wider tolerances all allow LC-MS Toolkit to consider more structures, but processing will take longer.

6. Click the **Run** button.

   Result: Matched fragments appear on the spectrum plot.

7. Examine the results in the Mass Fragment Results table.

   - Matched fragment structures are highlighted on the spectrum plot by the  icon.

     Note: Multiple fragments appear on the spectra when you position the pointer over the ion. When you position the pointer over an ion that is marked with  , the matched fragment will be displayed and the relevant row in the Mass Fragment Results table will be highlighted. You may need to zoom in on the plot to see the fragment icons corresponding to the low-intensity ions.

   - Below the spectrum plot, the Mass Fragment Results table displays matching formulas and the possible fragment structures for each experimental product ion.

     Note: The title of the table shows the number of matched assignments.

Table 5: Mass Fragment Results table

Measured m/z	Intensity	Substructure m/z	DBE	Mass Difference (mDa)	Mass Difference (PPM)	Formula	Formula Difference	No. of Substructures
71.07107	39	71.07295	1.0	-1.88	-26.49	C_4H_9O	$C_{17}H_{19}O$	3
74.09566	79	74.09643	-0.5	-0.77	-10.37	$C_4H_{12}N$	$C_{17}H_{16}O$	3
86.09715	129	86.09643	0.5	0.73	8.44	$C_5H_{12}N$	$C_{16}H_{16}O$	1
166.07643	287	166.07770	9.0	-1.27	-7.65	$C_{13}H_{10}$	$C_8H_{18}NO$	1
167.08390	1,436	167.08553	8.5	-1.63	-9.78	$C_{13}H_{11}$	$C_8H_{17}NO$	1

Table 6: Field description

Field	Details
Measured *m/z*	Observed mass (*m/z*) in the spectrum.
Intensity	A measure of abundance of ions.
Substructure *m/z*	Theoretical mass-to-charge ratio of the fragment.
DBE	The double bond equivalent.
Mass Difference (mDa)	The mass difference between the theoretical and observed masses, expressed in millidaltons.
Mass Difference (PPM)	The mass difference between the theoretical and observed masses, expressed in parts per million.
Formula	The formula of the neutral fragment.
Formula difference	The difference between the fragment formula and molecular formula.
No. of Substructures	Number of matched candidate fragments. The pane on the left-hand side displays the highest scoring fragment of the possible substructures, returned by the mass fragment processing algorithm.

Table 6: Field description (continued)

Field	Details
Structure	Displays graphical structure of fragment with the score, number of bonds broken, and number of hydrogen difference for each experimental product ion. These parameters are indicated by a string below the structure in the format "S:n B:n H:n". **Note:** This column is hidden by default.

Note: The result table displays compounds with superscripts to indicate where an isotope other than the monoisotopic mass is matched in a fragment.

- All columns except **Structure** are displayed by default. You can use the **Columns** drop-down menu above the table to select (or clear) the column headings that you want to be displayed in the table.
- The number of substructures column indicates when more than one possible structure matches a matched mass. The structure shown by default is the most likely structure based on score. If you want to reassign a less likely fragment structure, follow the instructions in step 10.

8. Select the **Import molecule files** tab to visualize the fragment on the molecule for the highlighted row on the results panel.
   - The red highlight shows the entire fragment that matches the formula on the highlighted row.

     Note: You can change the color of highlight using the color palette tool as required for better contrast with the structural atom colors.
   - The location of the bond break can be determined from the interface between the red highlighting and the non-highlighted portion of the molecule.
9. Click  ,  ,  , or  to resize the panes for an improved view of the results table and the spectrum plot.

   Note: When you expand the pane:
   - The result table shows more rows
   - You can see additional ions and annotations on the spectrum plot when it is maximized.
10. Reassign the alternative fragments in the Mass Fragments Results table where more than one substructure is available for a given mass as follows:

a. In the top-left of the mass fragment results panel, click  to view the alternative assignments.

b. In the dialog table that appears, select the alternative fragment to assign to each row where it is required.

c. Click **Apply**.

Note: When you select the row, the reassigned structure is updated in the table and displayed on the molecule pane and plot annotations.

11. Select the rows that you want to include in the PDF report using the check box in the first column, and then click  to generate the PDF report.

    The PDF report has the following information:

    - Sample Information (name, description, acquisition timestamp)
    - Combined spectrum and channel
    - Molecule structure
    - Processing settings
    - Mass Fragment Results table

    Note: The PDF report displays compounds with superscripts to indicate where an isotope other than the monoisotopic mass is matched in a fragment.

Locking LC-MS Toolkit

For security reasons, you can lock LC-MS Toolkit. Before you close your browser, lock or sign out of the application.

When the application locks, all waters_connect applications are locked and you must sign in again.

To lock LC-MS Toolkit:

Click the username menu and select **Lock**.

Note: The LC-MS Toolkit application is configured by default to lock after 30 minutes of inactivity. The default timeout is specified in Administration, within the Timeout global policy (**Security** > **Global Policies** > **Timeout**).

Unlocking LC-MS Toolkit

To unlock LC-MS Toolkit:

1. In the Application Locked dialog box, click **Unlock**.
2. In the Unlock screen dialog box, type your password and click **Unlock**.

Notes:

- Unlocking LC-MS Toolkit unlocks all open applications in the same waters_connect session.
- You can also unlock the LC-MS Toolkit application from the Hub window.

Signing out of LC-MS Toolkit

You can sign out of the LC-MS Toolkit application at any time. When you do so, you sign out of all waters_connect applications in the same session. For security reasons, lock or sign out of your applications before you close your browser.

To sign out of LC-MS Toolkit:

1. Click the username menu and select **Sign Out**.
2. In the Sign Out dialog box, click **Sign Out**.

Processing options available by data type

The processing options available for common plot data types (rows) are detailed here. Operations (columns) you can perform are indicated by "Y" (Yes). "N" (No) indicates that you cannot perform the operation on that data.

By default, the tables specify actions you can take for a single chromatogram or spectrum. Simultaneous processing options for multiple chromatograms or spectra are described in the notes below each table.

Raw data chromatograms

The options available for processing raw data chromatograms are as follows, listed by chromatogram type.

Chromatogram type	Smooth	Integrate	Extract masses (XIC)	Signal-to-noise	Combine spectra	Extract spectrum	View peak list	Extract wavelengths
MS	Y	Y	Y	Y	Y	Y	N	N
MS/MS	Y	Y	Y	Y	Y	Y	N	N
Low-energy MSe	Y	Y	Y	Y	Y	Y	N	N
High-energy MSe	Y	Y	Y	Y	Y	Y	N	N
UV	Y	Y	N	Y	Y	N	N	Y
MRM	Y	Y	N	Y	N	N	N	N
SIR	Y	Y	N	Y	N	N	N	N
Precursor	Y	Y	Y	Y	Y	Y	N	N
Neutral loss	Y	Y	Y	Y	Y	Y	N	N
Product	Y	Y	Y	Y	Y	Y	N	N
Tof lock mass reference channel	N	N	Y	N	Y	Y	N	N

Chromatogram type	Smooth	Integrate	Extract masses (XIC)	Signal-to-noise	Combine spectra	Extract spectrum	View peak list	Extract wavelengths
LC diagnostic channels	N	N	N	N	N	N	N	N

Notes:

- These options apply to chromatograms for which acquisition is complete, or in progress. For chromatograms that are being updated, see Viewing real-time data (Page 27).
- For chromatograms that allow you to select TIC or BPI, the processing options offered are not affected by your choice.
- "Combine spectra" includes both the Tools menu option, resulting in a combined and subtracted spectrum, and the quick combine option using right-click and drag on the chromatogram.
- "Extract spectrum" or "Extract Wavelength" refers to double-clicking a single point in the chromatogram to extract a spectrum.
- You can perform Smooth, Integrate, and Extract masses on multiple chromatograms simultaneously, where the operation is available for each individual chromatogram. Press `Ctrl` and then click the check box on each plot before processing. The same processing settings are applied to all chromatograms.

Chromatograms you create by processing

The options available for processing the chromatograms you create are as follows. Some options differ depending on the source of the original chromatogram:

- Tof or untargeted Quad MS data (MS, MS/MS, Low-energy MSe, High-energy MSe, Precursor, Neutral Loss, Product)
- Targeted quantification Quad MS data or non-MS data (PDA, TUV, MRM, SIR)

Chromatogram type	Smooth	Integrate	Extract wavelengths	Extract masses (XIC)	Signal-to-noise	Combine spectra	Extract spectrum	View peak list
XIC	Y	Y	N	Y	Y	Y	Y	N
XC	Y	Y	Y	N	Y	Y	Y	N
Smoothed (Tof or untargeted Quad)	N	Y	N	Y	Y	Y	Y	N
Integrated (Tof or	N	N	N	Y	Y	Y	Y	Y

Chromatogram type	Smooth	Integrate	Extract wavelengths	Extract masses (XIC)	Signal-to-noise	Combine spectra	Extract spectrum	View peak list
untargeted Quad)								
Smoothed and integrated (Tof or untargeted Quad)	N	N	N	Y	Y	Y	Y	Y
Smoothed (TUV, MRM, SIR)	N	Y	N	N	Y	N	N	N
Integrated (TUV, MRM, SIR)	N	N	N	N	Y	N	N	Y
Smoothed and integrated (TUV, MRM, SIR)	N	N	N	N	Y	N	N	Y
S:N	N	N	N	N	N	N	N	N
Smoothed (PDA)	N	Y	Y	N	Y	Y	N	N
Integrated (PDA)	N	N	Y	N	Y	Y	N	Y
Smoothed and integrated (PDA)	N	N	Y	N	Y	Y	N	Y

Notes:

- "Combine spectra" includes both the Tools menu option, resulting in a combined and subtracted spectrum, and the quick combine option using right-click and drag on the chromatogram.
- "Extract spectrum" refers to double-clicking a single point in the chromatogram to extract a spectrum.

- When you combine spectra, extract a spectrum, extract a wavelength, create a new XIC from an XIC or create a new XC from an XC, the spectral data is taken from the original chromatogram.
- You can perform Smooth, Integrate, and Extract masses on multiple chromatograms simultaneously, where the operation is available for each individual chromatogram. Press `Ctrl` and then click the check box on each plot before processing. The same processing settings are applied to all chromatograms.

Processing Tof mass spectra

The options available for Tof spectral processing (that create output plots) are as follows:

Spectrum type	Smooth	Mass measure	MaxEnt1	MaxEnt3	BayesSpray	Center	Extract XIC
Extracted	Y	Y	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y	Y
Smoothed	N	N	Y	Y	Y	Y	Y
Mass measured	N	N	N	N	N	N	Y
MaxEnt1	N	N	N	N	N	Y	N
MaxEnt1 mock	N	N	N	N	N	Y	N
MaxEnt3	N	N	N	N	N	N	N
BayesSpray	N	N	N	N	N	N	N
BayesSpray mock	N	N	N	N	N	N	N
Centered	N	N	N	N	N	N	Y
Combined and Subtracted	Y	N	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y	Y
Combined	Y	Y	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y (except for lock mass reference data)	Y	Y

Notes:

- The "Extracted" spectrum type refers to a spectrum created by double-clicking a specific point in a chromatogram.
- The "Combined" spectrum type refers to a spectrum created by right-clicking and dragging on a chromatogram.
- The "Combined and subtracted" spectrum type refers to a spectrum created using the "Combine Spectra" option in the Tools menu, which also applies background subtraction.
- The "Extract XIC" option refers to right-clicking and dragging a range, or double-clicking a point, to create an XIC. It is not available on the Tools menu.
- You can perform Smooth, Center, or Mass measure on multiple spectra simultaneously, where the operation is available for each individual spectrum. Press `Ctrl` and then click the check box on each plot before processing. The same processing settings are applied to all spectra.

Component identification from Tof mass spectra

The options available for component identification from Tof spectra are as follows:

Spectrum type	Elemental composition	Mass fragment
Extracted	N	N
Combined	N	N
Smoothed	N	N
Mass measured	Y	Y
MaxEnt1	N	N
MaxEnt1 mock	N	N
MaxEnt3	N	N
BayesSpray	N	N
BayesSpray mock	N	N
Centered	Y	Y
Combined and subtracted	N	N

Notes:

- The "Extracted" spectrum type refers to a spectrum created by double-clicking a specific point in a chromatogram.
- The "Combined" spectrum type refers to a spectrum created by right-clicking and dragging on a chromatogram.
- You can only perform component identification on one spectrum at a time, except in fragment identification within elemental composition elucidation, where you can select low-energy and high-energy data from the same injection.

Quad mass spectra

The options available for Quad spectral processing are as follows:

Spectrum type	Smooth	Center
Extracted	Y	Y
Combined	Y	Y
Smoothed	N	Y
Centered	N	N
Combined and subtracted	Y	Y

Notes:

- The "Extracted" spectrum type is created by double-clicking a specific point in a chromatogram.
- The "Combined" spectrum type refers to a spectrum created by right-clicking and dragging on a chromatogram.
- The "Combined and subtracted" spectrum type refers to a spectrum created using the Combine spectra option in the Tools menu, which also applies background subtraction.
- You can perform Smooth or Center on multiple spectra simultaneously, where the operation is available for each individual spectrum. Press `Ctrl` and then click the check box on each plot before processing. The same processing settings are applied to all spectra.

About Help

waters_connect LC-MS Toolkit Help

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Additional resources

Waters provides the following additional resources to ensure your continued success with its products.

Knowledge base: Obtain quick answers to your troubleshooting questions. Access support articles on Waters instrumentation, informatics, and chemistry.

eLearning courses: Learn anytime, anywhere, and at your own pace with eLearning courses.

Customer education: The Waters Educational Services team is the leading training organization empowering scientists to maximize their skills in UPLC, HPLC, LC-MS, and data management.

How-to video library: View/download the latest product how-to videos.

Contacting Waters

Contact Waters with technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us through the Internet, telephone, or conventional mail.

Contact method	Information
www.waters.com	The Waters website includes contact information for Waters locations worldwide.
iRequest	iRequest is a secure Web service form that allows you to request support and service for Waters instruments and software or to schedule a planned service activity. These types of support and services may be included as part of your maintenance plan or support plan. You may be charged for the requested service if you do not have appropriate plan coverage for your product.

Contact method	Information
	Note: In areas managed by authorized distributors, iRequest may not be available. Contact your local distributor for more information.
Local office contact information	For worldwide locations, telephone and conventional mail information is available at the Local Offices website.
Corporate contact information	Waters Corporation 34 Maple Street Milford, MA 01757 USA From the USA or Canada, phone 800-252-4752.

Customer comments

We seriously consider every customer comment we receive. Help us better understand what you expect from our documentation so that we can continuously improve its accuracy and usability. To report any errors that you encounter in this document or to suggest ideas for otherwise improving it, reach us at tech_comm@waters.com.

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List of abbreviations

AME

Acquisition Method Editor

BPI

Base Peak Intensity

BSM

Binary Solvent Manager

CM

Column Manager

DBE

Double bond equivalent

DDA

Data dependent acquisition

LC-MS

Liquid chromatography–mass spectrometry

MRM

Multiple Reaction Monitoring

MS

Mass spectrometry

MS/MS

Tandem mass spectrometry

m/z

Mass-to-charge ratio

ppm

Parts per million

QSM

Quaternary Solvent Manager

QTof

Quadrupole Time of Flight

Quad

Quadrupole

RMS

Root mean square

S:N

Signal-to-noise

SM-FTN

Sample Manager - Flow Through Needle

SIR

Selected Ion Recording

SVG

Scalable vector graphics

TIC

Total Ion Count

Tof

Time of flight

TUV

Tunable ultraviolet

UV

Ultraviolet

XC

Extracted Chromatogram

XIC

Extracted Ion Chromatogram