SCIEX
The Power of Precision

Molecule Profiler Software

Software User Guide

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Get Started 1

Use the Molecule Profiler software to search for and report on molecules and their derivatives, including potential impurities and metabolites, in data acquired using the Analyst TF software and SCIEX OS.

The Molecule Profiler software supports the identification of small molecules, peptides, Antibody-drug conjugates and oligonucleotides under 10 kDa.

Select a Compound	Process a Batch	Review the Results	Refine the Results
Compound Library	Create a Batch	About the Results Workspace	Characterize MS/MS Data
Create a Biotransformation Set	Set Processing Parameters	About Results Filters	Correlate Results
Set Processing Parameters	Specify Batch Options	Edit Results	Create a Report in the Correlation Workspace
	OR	Add Multiple Spectra with the **Add MS/MS** Button	
	Import a Batch	Create a Report in the Results Workspace	

How Potential Molecules and Their Derivatives Are Found

The software has a series of peak finding strategies, or algorithms, that it uses to find potential molecules in a sample of interest. Refer to the section: About Peak Finding Strategies.

If a found peak is a predicted molecule, then the software assigns a specific name derived from the precursor or a combination of one or more transformations. Based on the workflow, the transformations could include a selected biotransformation set, potential cleavage metabolites or potential hydrolytic cleavages, or potential sequence fragments from an antibody.

For small molecule data analysis, the transformations include a selected biotransformation set and potential cleavage metabolites.

For peptide data analysis, the transformations include a selected biotransformation set and potential hydrolytic cleavages.

For antibody drug conjugate (ADC) data analysis, the transformations include a selected biotransformation set, potential cleavage metabolites, and potential sequence fragments from a digested antibody protein.

For oligonucleotide analysis, the transformations include a selection of biotransformation sets suitable for both metabolites and impurities, as well as potential cleavage metabolites and internal n–1 and terminus n+1 sequences.

If the Generic peak finding strategy is used, and if the peak is an unexpected molecule, then it is assigned a generic Loss of or Gain of name and the protonated adduct at the charge of the molecular ion.

If control files are selected with the sample, then the software performs a comparison between the sample and the control data. If analog files are also selected with the sample, then the software performs a comparison between the MS and analog data.

Users can change the parameters that control each algorithm. Refer to the section: Select Parameter Values.

Open the Molecule Profiler Workspace

The SCIEX OS software version 2.1.5 or later must be installed, and a valid Molecule Profiler software license must be activated.

1. Select the software from the Start menu: **Start > SCIEX OS > SCIEX OS**.

   If the software is configured for Integrated mode, then the home page opens.

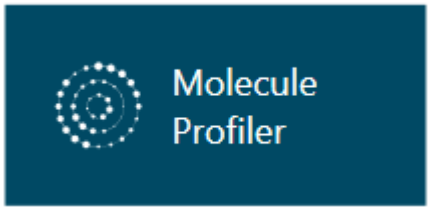
   If the software is configured for Mixed mode, then the Logon dialog opens. Continue with the following step.

2. If the Logon dialog opens, then type the user name and password of a user who is authorized to use the software, and then click **OK**.

   The Home page opens.

3. Click the Molecule Profiler tile.

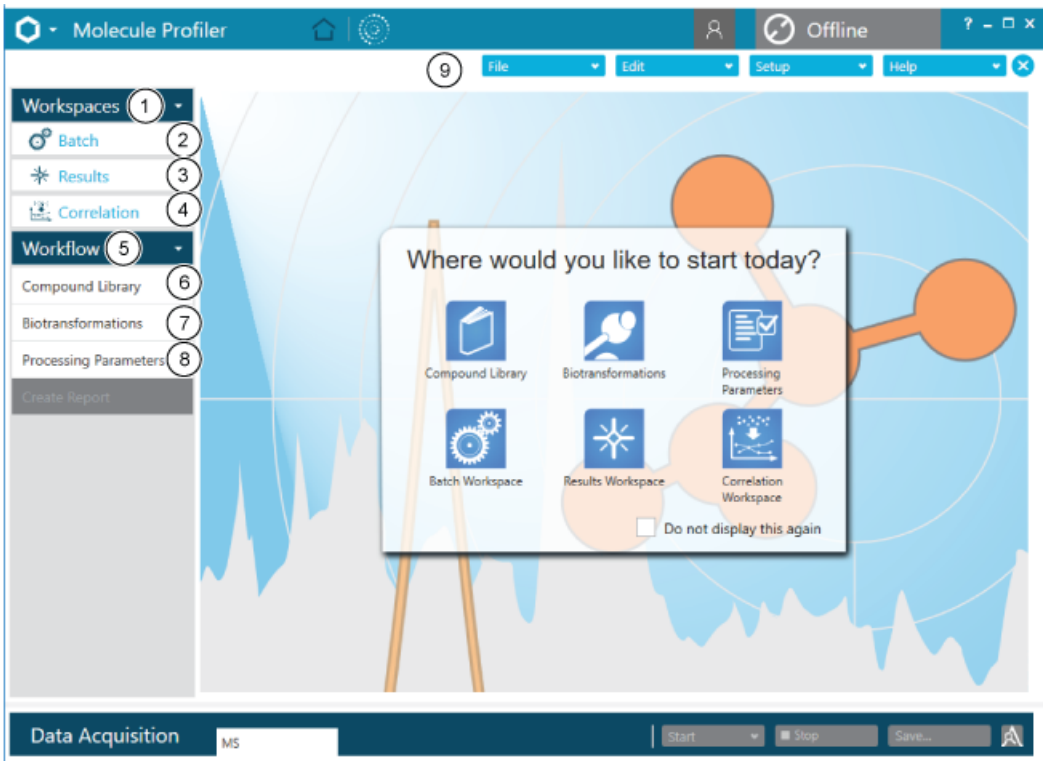
Figure 1-1 Molecule Profiler Tile



The Molecular Profiler workspace opens.

Molecule Profiler Window

Figure 1-2 Molecule Profiler Window



Item	Description
1	List of workspaces
2	Batch workspace. Use this workspace to search for potential metabolites. Refer to the section: About the Batch Workspace.
3	Results workspace. Use this workspace to view potential metabolites after processing. Refer to the section: About the Results Workspace.
4	Correlation workspace. Use this workspace to compare metabolites found in different Results files. Refer to the section: About the Correlation Workspace.
5	List of workflows
6	Compound library. Create and maintain a library of compounds. Refer to the section: Compound Library.
7	Biotransformations. Create and maintain lists of common transformations. Refer to the section: Biotransformation Sets.
8	Processing parameters. Create and maintain processing methods that can be used in the batch workspace. Refer to the section: Create Processing Methods.
9	Menu bar. Refer to the table: Table 1-1.

Table 1-1 Menu Commands

Item	Description
File Menu	
New	• **Batch**: Creates a new batch. Refer to the section: Create a Batch. • **Correlation**: Creates a new correlation. Refer to the section: Prepare for Correlation.
Open	• **Batch**: Opens a batch. • **Correlation**: Opens a correlation file. • **Results**: Opens a results file.
Save Batch	Saves the batch in the Batch workspace.
Save Batch As	Saves the batch in the Batch workspace with a different name.
Create Report	Creates a report. Refer to the section: Reports.
Recent reports	Opens a recent report.

Table 1-1 Menu Commands (continued)

Item	Description
Edit Menu	
Edit Name	Edits the name and formula for a compound.
Copy Selected Table	Copies the selected table.
Copy Selected Graph	Copies the selected graph.
Copy Batch Row	Copy the selected batch row.
Paste Batch Row	Paste the copied batch row in the selected location.
Clear Batch Row	Delete the contents of the selected batch row.
Delete Selected Row	Delete the selected row from the Results table. The software recalculates the results.
Undo Delete	Restores the last deleted row. The software recalculates the results.
Hide Unchecked Rows	Hides rows that are not selected.
Show Hidden Rows	Shows rows that are not selected.
Custom Elements	Opens the Custom Elements dialog. Use this dialog to define amino acids and oligonucleotide residues. Refer to the section: Custom Elements.
Setup Menu	
Compound Library	Opens the compound library. Refer to the section: Compound Library.
Biotransformations	Opes the list of biotransformation sets. Refer to the section: Biotransformation Sets.
Processing Parameters	Opens the processing methods window. Refer to the section: Create Processing Methods.

Table 1-1 Menu Commands (continued)

Item	Description
Filters	• **Results**: Set filters for the Results workspace. Refer to the section: About Results Filters. • **Correlation**: Set filters for the Correlation workspace. Refer to the section: About Correlation Filters. • **Interpretation**: Set filters for the interpretation workspace.
Create New Folder	Creates a folder. Refer to the section: Create Folders.

Create Folders

Folders store the files required by the software to find potential molecules in a sample of interest, as well as the Results files.

Custom folders can also be created to organize results.

1. Click **Setup > Create New Folder**.

   The Create New Folder dialog opens.

2. Type a **Name** for the folder.

   The **Location** field shows the installed location of the Data directory (C:\ProgramData\SCIEX\Molecule Profiler\Data). All folders that are created are stored in this directory.

3. Click **OK**.

   When a folder is created, two sub-folders are automatically created, the Processing Parameters folder and Results folder.

Custom Elements 2

The Custom Elements dialog contains these tabs:

- The AA List tab contains information for a list of standard amino acids. This information cannot be edited or deleted. Users can add custom amino acids to this list, and then modify or delete the added items, as required. The added amino acids are automatically added to the bottom of the list. However, the list can be sorted by clicking any one of the column headers.
- The AA Modifications tab contains the mass shift information for the various modifications that can be applied to peptide terminal groups and side-groups of amino acid residues. This information cannot be edited or deleted. Users can add custom amino acid modifications to the list, and then modify or delete the added items, as required. The added amino acid modifications are automatically added to the bottom of the list. However, the list can be sorted by clicking any one of the column headings.
- The Oligo List tab contains the predefined oligonucleotide residues and terminal groups. This information cannot be edited or deleted. Users can add new oligonucleotide residues and terminal groups to this list, and then modify or delete the added items, as required. The added residues are automatically added to the bottom of the list. However, the list can be sorted by clicking any one of the column headers.

Custom Amino Acids

Create a Custom Amino Acid

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.
2. Make sure that the AA List tab is selected.
3. Click **New**.

   The New Custom Amino Acid Residue dialog opens.
4. Complete the fields described in the following table and then click **OK**.

Table 2-1 New Custom Amino Acid Residue Dialog Fields

Field	Description	Acceptable Value
Name	Name for the amino acid	Alphanumeric
Symbol	Symbol for the amino acid	• Alphanumeric • First letter should be uppercase
Residue Formula	Formula for the amino acid	Empirical formula, using periodic elements. An enriched isotope can also be used as part of the formula. For example, 13C, where 13C indicates 13-carbon isotope.

The custom amino acid is added to the bottom of the amino acid table and shows the name, symbol, and mass.

Edit a Custom Amino Acid

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the AA List tab is selected.

3. Select the amino acid to be edited.

   Note: Only the custom amino acids that have been added by the user can be edited. Amino acids distributed with the software cannot be edited.

4. Click **Edit**.

   The Edit Custom Amino Acid Residue dialog opens.

5. Edit the fields described in the following table.

Table 2-2 Edit Custom Amino Acid Residue Dialog Fields

Field	Description	Acceptable Value
Name	Name for the amino acid	Alphanumeric
Symbol	Symbol for the amino acid	• Alphanumeric • First letter should be uppercase
Residue Formula	Formula for the amino acid	Empirical formula, using periodic elements

6. Click **OK**.

   The name, symbol, and mass of the selected custom amino acid are updated, if applicable, in the amino acid table.

Delete a Custom Amino Acid

Note: Deleting a custom amino acid that is used in a processing method or result can result in unexpected behavior.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the AA List tab is selected.
3. Select the amino acid to be deleted.

   Note: Only the custom amino acids that have been added by the user can be deleted. Amino acids distributed with the software cannot be deleted.

4. Click **Delete**.

   The custom amino acid is removed from the amino acid table.

Custom Amino Acid Modifications

Create a Custom Amino Acid Modification

Note: Custom amino acid modifications can only be applied to standard amino acids.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the AA Modifications tab is selected.

3. Click **New**.

   The New Custom Modification dialog opens.

4. Complete the fields described in the following table and then click **OK**.

Table 2-3 New Custom Modification Dialog Fields

Field	Description	Acceptable Value
Name	Name for the residue	Alphanumeric
Symbol	Symbol for the residue	• Must begin with _ • Alphanumeric • First letter should be uppercase
Formula Gain	Formula gained by the residue	Empirical formula, using periodic elements
Formula Lost	Formula lost by the residue	Empirical formula, using periodic elements
Mod Type	Position of the modification	Amino Acid, N-Terminus, C-Terminus, Protein N-Terminus, and Protein C-Terminus
Applies to AA	Name of the related amino acid	A single-letter representation of the standard amino acid to which the custom modification will be applied, for example P for proline. Leave this field empty to apply the custom modification to all standard amino acids.

The custom amino acid modification is added to the bottom of the amino acid modifications table and shows the symbol, mass shift, and name.

Edit a Custom Amino Acid Modification

1. Click **Edit > Custom Elements**.

The Custom Elements dialog opens.

2. Make sure that the AA Modifications tab is selected.
3. Select the modification to be edited.

Note: Only the modifications that have been added by the user can be edited. Modifications distributed with the software cannot be edited.

4. Click **Edit**.

   The Edit Custom Modification dialog opens.

5. Edit the appropriate fields described in the following table.

Table 2-4 Edit Custom Modification Dialog Fields

Field	Description	Acceptable Value
Name	Name for the residue	Alphanumeric
Symbol	Symbol for the residue	• Must begin with _ • Alphanumeric • First letter should be uppercase
Formula Gain	Formula gained by the residue	Empirical formula, using periodic elements
Formula Lost	Formula lost by the residue	Empirical formula, using periodic elements
Mod Type	Position of the modification	Amino Acid, N-Terminus, C-Terminus, Protein N-Terminus, and Protein C-Terminus
Applies to AA	Name of the related amino acid	A single-letter representation of the standard amino acid to which the custom modification will be applied, for example P for proline. Leave this field empty to apply the custom modification to all standard amino acids.

6. Click **OK**.

   The name, symbol, and mass shift of the selected custom modification are updated, if applicable, in the modifications table.

Delete a Custom Amino Acid Modification

Note: Deleting a custom amino acid modification that is used in a processing method or result can result in unexpected behavior.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the AA Modifications tab is selected.

3. Select the modification to be deleted.

   Note: Only the custom modifications that have been added by the user can be deleted. Modifications distributed with the software cannot be deleted.

4. Click **Delete**.

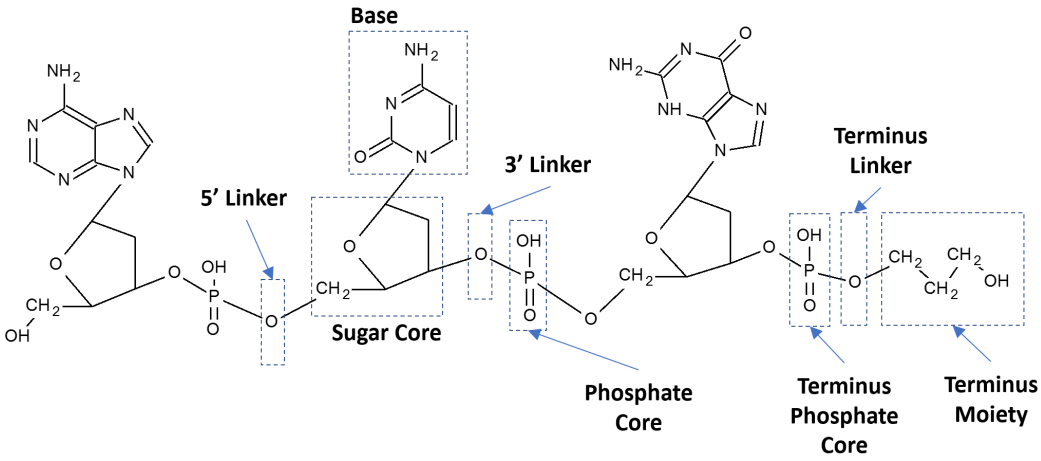
   The custom modification is removed from the modifications table.

Custom Oligonucleotide Residues or Terminal Groups

Use custom elements to create sequences that contain custom functional groups that can be added to the core structure of an oligonucleotide. These modifications can be entered in a sequence and then searched and identified by the Molecule Profiler software.

An oligonucleotide can be broken down into several substructures.

Figure 2-1 Oligonucleotide Substructures



Users can change the core substructures of an oligonucleotide or define a new core, terminus, and phosphate backbone. When creating a custom modified sequence, use this generalized structure:

5'-(Terminus Moiety)-(Terminus Linker)-(Terminus Phosphate Core)-(Residue Type)$_1$-…-(Residue Type)$_n$-(Terminus Phosphate Core)-(Terminus Linker)-(Terminus Moiety)-3'

In the New Oligo Residue or Terminus dialog, the **Type** field contains several predefined types of residues or terminus. These predefined types restrict editing to certain substructures of the oligonucleotide, to simplify the creation of modifications that are specific to the type itself. To understand how each type fits into the general structure outlined above, refer to the following table.

Table 2-5 Types

Type	Category	Editable Substructure
DNA	Residue Type	Base
DNA*	Residue Type	Base
RNA	Residue Type	Base
RNA*	Residue Type	Base
2'-O-Methyl RNA	Residue Type	Base
2'-O-Methyl RNA*	Residue Type	Base
Locked (LNA)	Residue Type	Base
Locked (LNA)*	Residue Type	Base

Table 2-5 Types (continued)

Type	Category	Editable Substructure
Other Residue	Residue Type	Base 5' Linker Sugar Core 3' Linker Phosphate Core
Phospho Terminus*	Terminus Moiety	Terminus Moiety
Phospho Terminus	Terminus Moiety	Terminus Moiety
Other Terminus	Terminus Moiety Terminus Linker Terminus Phosphate Core	Terminus Moiety Terminus Linker Phosphate Core

* Phosphorothioated backbone

The most flexible type for adding and editing chemical formulas is "Other Residue". It can be changed to accommodate multiple different custom substructures, allowing the user to define highly customized oligonucleotides. Similarly the Other Terminus type allows the user to define a custom 5' or 3'-terminus, linker, and core.

For an example, Refer to the section: Example Custom Oligonucleotide.

Create a Custom Oligonucleotide Residue or Terminal Group

Tip! To create an oligonucleotide residue or terminal group by copying an existing one, select the existing item on the Oligo List tab, and then click **New From**.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the Oligo List tab is selected.

   The list contains all of the predefined oligonucleotide residues and terminal groups.

3. Click **New**.

   The New Oligo Residue or Terminus dialog opens.

4. Complete the fields on the dialog. For examples, refer to the section: Example Custom Oligonucleotide.

5. Click **OK**.

   The custom residue or terminal group is added to the bottom of the table.

Edit a Custom Oligonucleotide Residue or Terminal Group

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the Oligo List tab is selected.
3. Select the residue or terminal group to be edited.

   Note: Only the residues and terminal groups that have been added by the user can be edited. Residues and terminal groups distributed with the software cannot be edited.

4. Click **Edit**.

   The Edit Custom Amino Acid Residue dialog opens.

5. Edit the properties of the residue or terminal group.
6. Click **OK**.

Delete a Custom Oligonucleotide Residue or Terminal Group

Note: Deleting a custom oligonucleotide residue or terminal group that is used in a processing method or result can result in unexpected behavior.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the Oligo List tab is selected.
3. Select the residue or terminal group to be deleted.

   Note: Only the residues and terminal groups that have been added by the user can be deleted. Residues and terminal groups distributed with the software cannot be deleted.

4. Click **Delete**.

   The custom residue or terminal group is removed from the table.

Import Oligonucleotide Residues and Terminal Groups

Oligonucleotide residues and terminal groups can be imported from a text file.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the Oligo List tab is selected.

   The list contains all of the predefined oligonucleotide residues and terminal groups.

3. Click **Import**.

   The Import Text File dialog opens.

4. Browse to the text file, select it, and then click **Open**.

Export Oligonucleotide Residues and Terminal Groups

Oligonucleotide residues and terminal groups can be exported to a text file.

1. Click **Edit > Custom Elements**.

   The Custom Elements dialog opens.

2. Make sure that the Oligo List tab is selected.

   The list contains all of the predefined oligonucleotide residues and terminal groups.

3. Select the oligonucleotide residues and terminal groups to be exported.

   Tip! Press **Ctrl+A** to select all of the residues and terminal groups in the list.

4. Click **Export**.

   The Save As dialog opens.

5. Type the name of the text file in which the exported oligonucleotide residues and terminal groups will be saved.

Compound Library 3

The Compound Library stores information including chemical formula, structure or sequence, isotope pattern, and MS/MS spectra for compounds. Users can also specify the reference spectrum for each compound. Each entry in the library can be used to create processing parameters.

The software is installed with a basic library of compounds but users can customize the library by adding, editing, and deleting entries.

Note: Each entry requires a chemical formula and at least one MS/MS spectrum.

Compound Library Options

Add a Structure | Add a Peptide Sequence | Add an Oligonucleotide Sequence

How Structures and Sequences are Used

Chemical structures and peptide and oligonucleotide sequences are used by the software to generate compound-specific parameter values, such as potential cleavage metabolites.

Note: The software automatically generates a chemical formula from the structure or sequence.

The software accepts both v2000 and v3000 mol files, including those with Markush or multiple structures.

Add a Structure

Use wiff files and txt files to add a reference spectrum to individual entries in the Compound Library.

1. In the Workflow panel, click **Compound Library**.

   The Compound Library dialog opens.

2. Do one of the following:

   - Create a new compound.
     a. Click **New** and then select **Structure** from the option list.
        The New Entry dialog opens.
     b. Type a **Name** for the compound and then click **OK**.
        The software automatically populates the **Compound name** field on the Compound Library dialog with the name provided

   - Select a compound from the list provided in the **Compound name** field.
     The Compound Library dialog is updated with the information corresponding to the selected compound.

3. Click **Open Structure**.

   The Open Structure File dialog opens.

4. Browse to and then select a valid mol file.

5. Click **Open**.

   The software populates the following fields in the Compound Library dialog:

   - Structure
   - Chemical formula
   - Polarity
   - Isotope Pattern

   By default, the **Adduct** field is populated with a singly-charged protonated adduct [M+H]+ or [M-H]–. The software also updates the **m/z** field with the appropriate information.

6. Select the **Polarity** of the acquisition.

   The **Isotope Pattern** and the **m/z** and **Adduct** values on the Compound Details tab are updated, based on the polarity selected.

7. Complete the following fields with the appropriate information:

   - Compound class
   - CAS number
   - Comments (For example, information about metabolite classes can be added to this field.)

8. Open the Experimental Data tab.

9. Do one of the following:

   - To add a reference MS/MS spectrum from a wiff file, continue with the section: Add a Reference MS/MS Spectrum from a wiff File.

- To add a reference MS/MS spectrum from a txt file, continue with the section: Add a Reference MS/MS Spectrum from a txt File.

Add a Peptide Sequence

Use wiff files and txt files to add a reference spectrum to individual entries in the Compound Library.

1. In the Workflow panel, click **Compound Library**.

   The Compound Library dialog opens.

2. Click **New** and then select **Sequence** from the option list.

   The New Entry dialog opens.

3. Type a **Name** for the compound and then click **OK**.

   The software automatically populates the **Compound name** field on the Compound Library dialog with the name provided.

4. Type the appropriate peptide sequence in the **Sequence** field.

   Note: The sequence can contain custom elements. Refer to the section: Custom Elements.

5. Click in the **Chemical formula** field.

   The software populates the following fields on the Compound Library dialog:

   - Chemical formula
   - Polarity
   - Isotope Pattern

   By default, the **Adduct** field is populated with a doubly-charged protonated adduct [M+2H]2+ or [M-2H]2–. The software also updates the **m/z** field with the appropriate information.

6. Select the **Polarity** of the acquisition.

   The **Isotope Pattern** and the **m/z** and **Adduct** values on the Compound Details tab are updated, based on the polarity selected.

7. Click the Experimental Data tab.

8. Do one of the following:

   - To add a reference MS/MS spectrum from a wiff file, continue with the section: Add a Reference MS/MS Spectrum from a wiff File.

- To add a reference MS/MS spectrum from a txt file, continue with the section: Add a Reference MS/MS Spectrum from a txt File.

Peptide Sequence Naming Conventions

Table 3-1 Peptide Sequences

Characteristic	Input Convention	Example
Multiple Chains	/	LIGHTCHAIN / HEAVYCHAIN
Modification on amino acid: Side group	[Symbol]	M[Oxi]
Modification on amino acid: C-Terminal	-[Symbol]	Y-[Ami]
Modification on amino acid: N-Terminal	[Symbol]-	[1Me]-Y
Linkages	• [*#] on each bonded residue • The number on each linked residue	S-S Bridge: MYC[*1]PEPC[*1]TIDE

Table 3-2 Linkages

Type of Linkage	Convention	Example
S-S bridge	Add [*#] to both residues in the bridge	Single Chain: MYC[*1]PEPC[*1]TIDE Multi-chain: LIGHTC[*1]HAIN / MC[*2]HEAVYC[*1]HAINC[*2]AD
Ester/Amide Bridge	Add '[O-1]' to one of the bonding residues	MYR[*1]PEPD[*1][O-1]TIDE
Cyclic	Add '[H]' on the C-Term	M[*1]YCPEPCTIDE[*1]-[H]
Loops: Linked residue at first or last index and the terminal groups are not part of the bridging bond	Explicitly add the terminal groups	[H]-C[*1]YCPEPCTIDC[*1]-[OH]

Add an Oligonucleotide Sequence

Optionally, add oligonucleotide compound information to the compound library. Compounds in the library have MS/MS spectra, which will be used during processing.

Note: If a compound is not in the library, then the user can add it to a processing method manually.

Sequences are added in text format. To capture the diverse set of therapeutic oligonucleotide modifications and custom elements, obey the rules for inputting sequences. Refer to the section: Oligonucleotide Sequence Naming Conventions. For a more detailed list of modifications and custom elements, refer to the section: Custom Elements.

1. In the Workflow panel, click **Compound Library**.
2. Click **New > Oligonucleoide Sequence**.

   The New Entry dialog opens.
3. Type the **Name** of the oligonucleotide sequence and then click **OK**.
4. Type the sequence in the **Sequence** table.

   Note: The sequence can contain custom elements. Refer to the section: Custom Elements.
5. Click the **Chemical formula** field to automatically update the chemical formula.
6. (Optional) Type information into the fields on the Compound Details tab.
7. Click the Experimental Data tab.
8. Do one of the following:
   - To add a reference MS/MS spectrum from a wiff file, continue with the section: Add a Reference MS/MS Spectrum from a wiff File.
   - To add a reference MS/MS spectrum from a txt file, continue with the section: Add a Reference MS/MS Spectrum from a txt File.

Oligonucleotide Sequence Naming Conventions

Oligonucleotide sequences can be specified using characteristic single-letter identifiers for the bases:

- Adenine (A)
- Cytosine (C)
- Thymine (T)
- Guanine (G)

- Uracil (U)

Oligonucleotide types such as Deoxyribonucleic acid (DNA, d), Ribonucleic acid (RNA, r), can be identified with the single-letter identifier added at the beginning of the sequence or, for mixed oligonucleotide types, interspersed between bases.

For oligonucleotides that contain synthetic nucleotides, such as locked nucleic acid (LNA), use the full symbol for each residue when defining the sequence. For example lA for LNA-A or moA for 2'-Mehtoxymethyl-A.

Backbone modifications, such as phosphorothioate (HPSO, *), are added at the end of each base.

Heavy atoms, such as Carbon-13(/13Cn/), are added after the specific oligonucleotide residue, where n denotes the number of heavy atoms.

Note: In the preceding example, the notation "/13Cn/" adds heavy atoms to the existing formula. It does not replace atoms in the nucleobase with a heavy label. To define an isotopically labeled nucleobase, a custom modification is required.

Use a slash (/) as the first and last character to identify a user-defined custom modification. To add custom modifications, and for examples of additional use cases for modifications and associated naming conventions, refer to the section: Custom Elements.

Table 3-3 Oligonucleotide Conventions

Characteristic	Input Convention	Example
DNA	d	dACG T
RNA	r	rACG U
Mixed DNA and LNA	d, l	dACG lT
Phosphorothioate backbone	*	dA*C*G* T*
2'Methoxymethyl (2'MOE) sugar modification	mo	moAmoCmoG moT
Carbon-13	/13Cn/	dACG T/13C2/
Custom residue	//	dACG /Other Residue/

Add a Reference MS/MS Spectrum from a wiff File

1. Click **Open wiff File**.

   The Select Data dialog opens.

2. Browse to the appropriate location, select a wiff file that contains a spectrum for the compound being added, and then click **OK**.

Note: The wiff file must contain the compound as a precursor ion.

Table 3-4 Add a Reference Spectrum

File contains multiple precursors	File contains one precursor
If there are multiple precursors in the selected wiff file, then the Select a Spectrum dialog opens with the following information shown in the Precursors table for each available precursor: • m/z • Time (min) • Quality • Charge	If the wiff file only contains one precursor, then the MS/MS Spectrum window is updated with the spectrum.
Select the check box for the filters to be applied. Select one or both filter options, as applicable. The **Precursors** table updates to show only the rows that meet the criteria specified.	The software uses the **m/z** and the **Charge** of the selected precursor, and the collision energy of the experiment to create a unique line of information in the **Spectra** field on the Compound Library dialog. For example, Prec(m/z), CE(collision energy from the experiment), Charge(Charge) is shown in the field. The title of the spectrum contains the **Polarity** and the **Compound name** from the Compound Information group, followed by the information from the **Spectra** field. The **Spectrum Details** contain the Instrument type, Retention time, Charge, and Collision energy corresponding to the selected MS/MS spectrum. This information is read-only.

Table 3-4 Add a Reference Spectrum (continued)

File contains multiple precursors	File contains one precursor
Select a row in the Precursors table. The MS/MS Spectrum window is updated with the spectrum for the selected precursor. **Tip!** Use **Ctrl**+click to select multiple rows. If multiple rows are selected, then the **MS/MS Spectrum** for the first selected precursor is shown.	—
If the **Charge state from** check box is selected, then select the **from** and **to** values from the options provided. The **from** value is equivalent to the minimum available charge state in the Precursors table. The **to** value is equivalent to the maximum available charge state shown in the Precursors table.	—
If the **Quality above** check box is selected, then type the appropriate value in the field provided.	—

Table 3-4 Add a Reference Spectrum (continued)

File contains multiple precursors	File contains one precursor
Click **OK**.	—
For each row selected in the Precursors table, the software uses the **m/z** and the **Charge** of the selected precursor, and the collision energy of the experiment to create a unique line of information in the **Spectra** field on the Compound Library dialog. For example, Prec(m/z), CE(collision energy from the experiment), Charge(Charge) is shown in the field. The information shown in the **Spectra** field and the spectrum shown in the **MS/MS Spectrum** field corresponds to the first row selected in the Precursors table. The title of the spectrum contains the **Polarity** and the **Compound name** from the Compound Information group, followed by the information from the **Spectra** field. The **Spectrum Details** contain the Instrument type, Retention time, Charge, and Collision energy corresponding to the selected MS/MS spectrum. This information is read-only.	—

3. (Optional) Select a different **Spectra** from the list provided.

   The **MS/MS Spectrum** and the **Spectrum Details** are updated to show the information related to the selection.

4. To save a spectrum as the predefined spectrum for the compound, select the appropriate **Spectra** from the list provided and then click **Set as Reference**.

   - Reference is added to the information shown in the **Spectra** field. For example, Prec (xx.xx), CE(xx), Charge(xx) - Reference is shown in the field.

5. Click **Save**.

6. Click **OK**.

   The new compound is saved in the library and the Compound Library dialog closes.

Add a Reference MS/MS Spectrum from a txt File

1. Click **Open txt File**.

   The Open txt File dialog opens.

2. Browse to the appropriate location, select an MS/MS txt file, and then click **OK**.

   The Spectrum Details dialog opens.

3. Type the appropriate information for the selected spectrum and then click **OK**.

   The software uses the information in the **Precursor mass (m/z)**, **Collision energy**, and **Charge** fields to generate the information in the **Spectra** field on the Compound Library dialog. For example, Prec (Precursor mass (m/z)), CE(Collision energy), Charge(Charge) is shown in the field.

   The information shown in the **Spectra** field and the spectrum shown in the **MS/MS Spectrum** field corresponds to the selected txt file.

   The title of the spectrum contains the **Polarity** and the **Compound name** from the Compound Information group, followed by the information from the **Spectra** field.

   The **Spectrum Details** contain the Instrument type, Retention time, Charge, and Collision energy corresponding to the selected MS/MS spectrum. This information is read-only.

4. (Optional) Select a different **Spectra** from the list provided.

   The **MS/MS Spectrum** and the **Spectrum Details** are updated to show the information related to the selection.

5. To save a spectrum as the predefined spectrum for the compound, select the appropriate **Spectra** from the list provided and then click **Set as Reference**.

   - Reference is added to the information shown in the **Spectra** field. For example, Prec (xx.xx), CE(xx), Charge(xx) - Reference is shown in the field.

6. Click **Save**.

7. Click **OK**.

   The new compound is saved in the library and the Compound Library dialog closes.

Add Information to the Compound Library from a Results Table

Note: This functionality is only available for small molecule and peptide Results files. The functionality is not available for ADC and oligonucleotide Results files.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. Select a row in the Potential Metabolites table, right click, and then select **Add to Compound Library**.

   Note: If the selected row does not contain an MS/MS spectrum, the **Add to Compound Library** option is not available.

6. Click **OK** in response to the confirmation message.

7. In the Workflow panel, click **Compound Library**.

   The Compound Library dialog opens. The added metabolite is added to the **Compound name** list.

Biotransformation Sets 4

Biotransformation sets are lists of common transformations.

About Biotransformations

Users can search for predicted metabolites using predefined biotransformation sets that are installed with the software, or they can create new biotransformation sets. For example, users might want to create a different set for each compound being analyzed. The installed biotransformations contain embedded information that is used during automated sequence or structure proposal.

Method-specific biotransformation sets are used as defaults for each method type. For example, the peptides method uses the biologics biotransformation set as its default. This biotransformation set contains the most relevant biotransformations for in-vivo metabolic reactions for peptides.

For oligonucleotides, select one of three predefined transformation sets:

- **Oligonucleotide Basic**: Provides a concise list of modifications and is restricted to those impacting the base or the backbone only.
- **Oligonucleotide Comprehensive**: Provides extensive coverage of all transformations that can occur during synthesis, in-vivo metabolism, and storage.
- **Oligonucleotide Metabolites**: Contains a subset of the comprehensive set that focuses on in-vivo transformations only.

Consider the origin of the sample carefully and then choose the most representative set. Users can build their own custom biotransformation set from default entries, or by adding new entries. Refer to the sections: Create a Biotransformation Set and Edit a Biotransformation Set.

Create custom biotransformations by identifying a change in chemical formula or by combining two existing biotransformations.

Custom biotransformations or biotransformations from existing sets can be included in any new biotransformation set that is created.

Tip! When assessing biologics data, select highly probable biotransformations to create a small set for faster analysis of the data.

Create a Biotransformation Set

1. In the Workflow panel, click **Biotransformations**.

   The Biotransformations dialog opens.

2. Click **New**.

   The New Biotransformation Set dialog opens.

3. Type a name for the set in the **Working biotransformation set** field.

4. Click **New Biotransformation**.

   The New Biotransformation dialog opens.

5. Type a name for the biotransformation in the **Name** field.

6. (Optional) Type the appropriate details related to the biotransformation in the **Description** and **Comments** fields.

7. Do one of the following:

Table 4-1 Create Biotransformation Sets

To create a single biotransformation	To create a combined biotransformation
Click **Single biotransformation**.	Click **Combined biotransformation**.
Identify the portion of the structure being lost in the **Formula from** field.	Select a biotransformation from each of the **Biotransformation 1** and **Biotransformation 2** fields.
Type the formula of the biotransformation in the **Formula to** field.	—

Note: The available biotransformations are those that exist in the working set.

Note: The software automatically calculates the change resulting from the biotransformation and populates the **Mass shift** field with this value.

8. Click **OK**.

   The new biotransformation is shown in both the working biotransformation set and the source biotransformation set tables.

9. Click **OK** to save the new biotransformation set.

   The New Biotransformation Set dialog closes.

10. Click **OK**.

    The Biotransformations dialog closes.

Edit a Biotransformation Set

1. In the Workflow panel, click **Biotransformations**.

   The Biotransformations dialog opens.
2. Select the appropriate **Set** from the list provided.
3. Click **Edit**.

   The Edit Biotransformation Set dialog opens showing the name of the selected set in the **Working biotransformation set** field.
4. Type a name for the appropriate set in the **Working biotransformation set** field.
5. Select a row in the working biotransformation set table.
6. Click **Edit Biotransformation**.

   The Edit Biotransformation dialog opens.
7. (Optional) Make any required changes in the **Name**, **Description**, and **Comments** fields.
8. (Optional) Do one of the following:

Table 4-2 Edit Biotransformation Sets

To create a single biotransformation	To create a combined biotransformation
Click **Single biotransformation**.	Click **Combined biotransformation**.
Identify the portion of the structure being lost in the **Formula from** field.	Select a biotransformation from each of the **Biotransformation 1** and **Biotransformation 2** fields.
Type the formula of the biotransformation in the **Formula to** field.	—

Note: The available biotransformations are those that exist in the working set.

9. Click **OK**.

   The updated biotransformation is shown in both the working biotransformation set and the source biotransformation set.
10. Click **OK** to save the changes.

The Edit Biotransformation Set dialog closes.

11. Click **OK**.

    The Biotransformations dialog closes.

Delete a Biotransformation Set

1. In the Workflow panel, click **Biotransformations**.

   The Biotransformations dialog opens.

2. Select the appropriate **Set** from the list provided.
3. Click **Delete**.

   A confirmation message opens.

4. Click **Yes**.
5. Click **OK**.

   The Biotransformations dialog closes.

Create Processing Methods 5

The software supports four workflows: small molecule, peptide, oligonucleotide, and ADC.

A method containing processing parameters that are specific to the sample file being studied must be created to find potential metabolites in a sample of interest.

Select the Method Type

↓

Select Parameter Values

↓

Set Generic Processing Parameters

↓

Set Compound-Specific Processing Parameters

Processing Parameters

The processing parameters in the Molecule Profiler software contain all of the attributes and values that allow wiff files to be processed. The processing function is used to find and characterize metabolites. The processing function also assigns confidence scores to metabolites.

These processing parameter templates are used:

- Small molecule
- Peptides
- Oligonucleotides
- ADC

The templates represent the compound and workflow types that are considered for the different types of analysis.

Note: When adding compound sequences, make sure that the sequence names are formatted correctly. Refer to the sections: Peptide Sequence Naming Conventions or Oligonucleotide Sequence Naming Conventions.

Select the Method Type

1. In the Workflow panel, click **Processing Parameters**.

   The processing parameters workspace opens.

2. Click **New** and then select the method type from the list provided.

3. Continue with step 2 of Select Parameter Values.

Select Parameter Values

1. In the Workflow panel, click **Processing Parameters**.

   The Processing Parameters workspace opens.

2. Type the Compound Information in the Processing Parameters workspace.

   - For small molecule and ADC workflows, click **Open Structure** in the Structure group, select the targeted mol file, and then import the structure.
   - For peptide and oligonucleotide workflows, type the appropriate sequence in the Sequence group.

   Tip! Alternatively, click **Select From Library** to select an entry from the compound library to fill in the structure or sequence. Only entries that match the workflow are available in the list. Refer to the section: Select a Compound from a Library.

3. Make sure that the **Polarity**, **Charge state**, and **Adduct** or **Ion type** are appropriate for the data set.

   Oligonucleotides are typically acquired in negative polarity or negative ion mode. The recommended charge range for oligonucleotides with masses of 10,000 Da or less is –2 to –20. Processing of oligonucleotides with masses greater than 10,000 Da is not recommended.

4. Select the peak finding strategies to use when finding potential metabolites. Refer to the section: About Peak Finding Strategies.

5. Configure the parameters that are independent of the compound being processed. Refer to the section: Generic Processing Parameters.

6. Configure the parameters that are dependent on the compound. Refer to the section: Compound-Specific Processing Parameters.

7. Click **Save and Close**.

8. Select the method storage location in the **Folder** field of the Save Processing Parameters As dialog.
9. Type a name for the method in the **Name** field and then click **OK**.

   The method is saved and the Processing Parameters workspace closes.

Select a Compound from a Library

1. In the Workflow panel, click **Processing Parameters**.

   The processing parameters workspace opens.
2. Click **Select From Library**.

   The Select From Library dialog opens.
3. Select a compound from the list in the **Compound name** field.

   Note: For small molecule and ADC processing parameters, only the entries identified as structures in the compound library are shown in the list. For peptide and oligonucleotide processing parameters, only the entries identified as sequences in the compound library are shown in the list.
4. Click **OK**.

   The processing parameters workspace is updated with the information from the selected compound.
5. To review or edit the reference MS/MS spectrum, click **Compound-Specific Parameters > Product Ions and Neutral Losses**.

   Note: The Reference MS/MS Spectrum pane is populated with the MS/MS spectrum from the selected compound.
6. (Optional) If multiple reference spectra are available, then navigate through the list and select a different spectrum, if appropriate.

   Note: When a different reference spectrum is selected, the Reference MS/MS Spectrum pane is refreshed and the information is cleared from the product ions and neutral losses table.
7. To configure the fragments table, click **Assign Fragments**.
8. Continue with step 5 of the section: Select Parameter Values.

About Peak Finding Strategies

Peak finding strategies refer to the algorithms the software uses to find potential metabolites in the sample of interest. Users can select specific algorithms in the Peak Finding Strategy group to process the data.

Algorithm	Description
TOF MS	
Predicted metabolites	• **Small molecule**: With this algorithm, the software searches for metabolites based on the selected biotransformation set, the predicted cleavage metabolites, and a combination of the two. • **Peptides**: With this algorithm, the software searches for metabolites based on the biotransformation set, the predicted catabolites, and a combination of the two. • **Oligonucleotides**: With this algorithm, the software searches for metabolites based on the biotransformation set, the predicted catabolites (including hydrolytic cleavage, terminus n+1 and internal n–1 products), and a combination of the two. • **ADC**: With this algorithm, the software searches for metabolites based on biotransformations, cleavages, antibody fragments, and a combination of the three. Refer to the section: Generic Processing Parameters. For each method, the **Available Adducts** selected in the MS Parameters tab are also included when using the combinations. **Note:** The **Predicted metabolites** option is recommended for processing of oligonucleotide data.
Generic peak finding	With this algorithm, the software searches for unexpected metabolites. The search can be refined further by selecting the **Apply mass defect filter** or the **Apply charge state filter**. The parameters that control this algorithm are found on the **Chromatographic Data** and **MS Parameters** tabs. Refer to the section: Generic Processing Parameters. **Note:** This option, in conjunction with the **Predicted metabolites** option, is recommended for processing of oligonucleotide data.

Algorithm	Description
Apply mass defect filter	This filter restricts the search to peaks that exhibit the filters selected on the **Mass Defect** range specified in the Compound-Specific Parameters. When this filter is selected, only those metabolites found by the generic peak finder that meet the criteria specified are included in the results.
Apply charge state filter	This filter restricts the search to peaks with a charge that is within the Charge state tab in the Compound Information group. When this filter is selected, only those metabolites found by the generic peak finder that meet the criteria specified are included in the results. **Note:** This option is not recommended for processing oligonucleotide data.
Mass defect	This algorithm is only applicable to small molecule methods. This algorithm uses fractional mass to filter the data. The compound, selected biotransformations, and potential cleavage metabolites all contribute to the available filters that allow users to search for specific metabolites within a range of masses. The parameters that control this algorithm are found on the Mass Defect tab. Refer to the section: Compound-Specific Processing Parameters.
Isotope pattern	This algorithm searches for metabolites that have an isotope pattern similar to the parent compound. **Tip!** If the compound is radio-labeled, then users can define the isotopic enrichment on the Processing Parameters dialog, by selecting **Compound-Specific Parameters > Isotope Pattern**. The parameters that control this algorithm are found on the Isotope Pattern tab. Refer to the section: Compound-Specific Processing Parameters.
TOF MSMS	
Note: This algorithm works only if the processing parameter method contains a reference MS/MS spectrum. The reference MS/MS spectrum can be from the entry in compound library, or it can be added manually on the Product Ions and Neutral Losses tab. Refer to the section: Compound-Specific Processing Parameters.	

Algorithm	Description
Find characteristic product ions	The software uses this algorithm to search the IDA data and the SWATH acquisition data for metabolites that have characteristic product ions to the parent compound. With this algorithm, users can search for all or a limited number of the identified ions. The parameters that controls this algorithm are found on the Product Ions and Neutral Losses tab. Refer to the section: Compound-Specific Processing Parameters.
All specified ions	When this option is selected, all of the identified ions are searched. For example, if four product ions are identified and then a search for peaks that have all of these ions is conducted, then only exact matches are identified as potential metabolites.
At least __ ions	When this option is selected, only the ions selected on the Product Ions and Neutral Losses tab are searched. For example, if the search is for peaks with at least two ions, then at least two ions out of the selected ions must be present in the MS/MS spectrum of the metabolite before a peak can be considered a metabolite.
Find characteristic neutral losses	The software uses this algorithm to search the IDA data and the SWATH acquisition data for metabolites that have neutral losses to the parent compound. The algorithm is not applicable to peptide and oligonucleotide workflows. With this algorithm, users can search for all or a limited number of losses. For example, if four neutral losses are identified and then a search for peaks that have all of these losses is conducted, then only exact matches are identified as potential metabolites. If the search is for peaks with at least two losses, then at least two of the selected losses must be present in the MS/MS spectrum of the metabolite before a peak can be considered a metabolite. The parameters that control this algorithm are found on the Product Ions and Neutral Losses tab. Refer to the section: Compound-Specific Processing Parameters.
All specified losses	When this option is selected, all metabolites are searched and all neutral losses are reported.

Algorithm	Description
At least __ losses	When this option is selected, only the losses selected on the Product Ions and Neutral Losses tab are searched. For example, if four neutral losses are identified and a search for peaks that have all of these losses is conducted, then only exact matches are identified as potential metabolites. If the search is for peaks with at least two losses, then at least two of the selected losses must be present in the MS/MS spectrum of the metabolite before a peak can be considered a metabolite.
Consider internal neutral losses	This algorithm is specific to SWATH acquisition data. This strategy will only work if at least two neutral losses are selected. The internal neutral loss is the delta between the two neutral losses formulas. Note that one neutral loss formula needs to be a subset of the other neutral loss formula in order for the "Find by Internal Neutral Loss" to take effect
Isotope pattern (SWATH Only)	This algorithm is specific to SWATH acquisition data. Precursors with a fragment isotope pattern that matches the fragment isotope pattern selected in the table on the Product Ions and Neutral Losses tab in the Compound-Specific Parameters are flagged as metabolites. The user must select one or more of the fragment isotope formula check boxes in the **Isotope Pattern** column. The experimental fragment isotope pattern must match the theoretical fragment isotope pattern within the MS/MS *m/z* tolerance and Intensity Tolerance specified on the MS/MS Parameters tab, in order for the peak to be considered a metabolite.

Generic Processing Parameters

Generic parameters are settings that are independent of the compound being processed. Each of the following tabs manage generic parameters:

Generic Parameters

↙	↙	↘	↘
Small Molecules	**Peptides**	**Oligonucleotides**	**ADC**
Biotransformations Tab	Biotransformations Tab	Biotransformations Tab	Biotransformations Tab
Chromatographic Data Tab	Chromatographic Data Tab	Chromatographic Data Tab	Chromatographic Data Tab
MS Parameters Tab	MS Parameters Tab	MS Parameters Tab	MS Parameters Tab

MS/MS Parameters Tab
Formula Prediction Tab (Small Molecule and ADC Methods)
Confirmation Scoring Tab
MS/MS Parameters Tab
Confirmation Scoring Tab
MS/MS Parameters Tab
Confirmation Scoring Tab
MS/MS Parameters Tab
Formula Prediction Tab (Small Molecule and ADC Methods)
Confirmation Scoring Tab

Biotransformations Tab

Identifies the biotransformation set that contains the expected biotransformations. The software includes predefined biotransformation sets. To create a custom biotransformation set, refer to the section: Create a Biotransformation Set.

Parameter	Description
Select Set	Selects a different biotransformation set, to be used for processing, from the database. When this option is selected, the software might show the following warning: "The selected biotransformation set might no longer exist in the biostransformations database." This occurs because the selected biotransformation set has been saved to the processing parameters file. Subsequent changes made to the biotransformations set in the Biotransformations workspace are not to saved to the processing parameters file. To reprocess using the saved biotransformation set, click **OK**, and then click **Cancel** on the Biotransformations dialog. To update the processing parameters file with a new biotransformation set, do this: a. Click **OK**. b. Select a biotransformations set. A message is shown: "If you select this new biotransformation set, you might not be able to re-select the existing set. Do you want to continue? c. Click **OK**.

Chromatographic Data Tab

Parameter	Description
Chromatographic Peak	
Retention time window	Specifies the range of retention times to search for potential metabolites. The size of the retention time (RT) window is directly proportional to processing time. Specify a value larger than 0.00 min to exclude the void volume of the column. The **to** value must be greater than the **from** value. We recommend that an RT window be set for all workflows, because a wide RT range can increase processing times very much. Ranges are highly dependent on the experiment being analyzed. Examine the RT window for each experiment. We recommend that the start time be slightly greater than 0.00 min, and that the end time be slightly after the peak of interest, or when the method enters the high elution or wash phase of the gradient.
MS data	Specifies the method for setting the XIC width. • **XIC width**: Specifies the width of the extracted ion chromatogram to be considered for processing. • **Automatic**: The software calculates the best width based on the selected data. The **Automatic** setting is recommended for oligonucleotide workflows. **Note:** If this option is selected when SWATH acquisition data is being processed, then the **XIC width** option is applied.
LC peak separation	Determines how closely eluting peaks are integrated. This parameter also handles chromatographic peaks with significant tailing. Set this parameter lower if there are closely eluting peaks. The lower setting allows peaks to be considered separately instead of as one peak.
TOF MS	
Minimum peak width	Excludes chromatographic peaks that have a width below this value. Set the value lower to include narrow peaks.

Parameter	Description
Minimum peak intensity	Removes chromatographic peaks that are below a given TOF MS intensity level from consideration. Use when there is noisy chromatographic data. By setting a threshold just above the level of noise, peaks that are likely the results of noise can be rejected. Examine peaks widths before processing data in the Molecule Profiler software or in viewer software, such as the Explorer workspace in SCIEX OS. Use a general average of all peaks examined to calculate the minimum peak width. For oligonucleotide methods that contain TOF MS or IDA experiments, a setting of 50 cps is recommended.
Use smoothing	Distinguishes peaks from noise by eliminating the variation in intensity within noise. Select when there is noisy chromatographic data. This option is recommended for oligonucleotide workflows.
Sample-control offset	Aligns the MS sample and control chromatograms. During processing, the software shifts all controls before comparing them to the sample.
Sample/control ratio	Specifies how many times larger a sample peak must be when compared to the control in order to be considered a metabolite.
TOF MS/MS	
Minimum peak intensity	This parameter is only used when processing SWATH acquisition data, with the MS/MS peak finding algorithms. This parameter is not used when processing IDA data. Removes chromatographic peaks that are below a given TOF MS/MS intensity level from consideration. Use when there is noisy chromatographic data. By setting a threshold just above the level of noise, peaks that are likely the results of noise can be rejected.
Analog data	
Wavelength (UV only)	Selects the wavelength to use when confirming potential metabolites.

Parameter	Description
Time offset from MS	Aligns the MS and analog chromatographic data. During processing, the software shifts the analog data before comparing it with the MS data. **Note:** The MS and analog chromatographic data can also be aligned, post-processing, in the Analog Interpretation workspace. Refer to the section: Change R.T. Offset.
LC peak separation	Determines how closely eluting peaks are integrated. This parameter also handles chromatographic peaks with significant tailing. Set this parameter lower if there are closely eluting peaks. The lower setting allows peaks to be considered separately instead of as one peak.
Minimum peak width	Excludes chromatographic peaks that have a width below this value. Set the value lower to include narrow peaks.
Minimum peak intensity	Removes chromatographic peaks that are below a given intensity level from consideration. Use when there is noisy chromatographic data. By setting a threshold just above the level of noise, peaks that are likely the results of noise can be rejected.
Use smoothing	Distinguishes peaks from noise by eliminating the variation in intensity within noise. Select when there is noisy chromatographic data.
Sample-control offset	Aligns the MS sample and control chromatograms. During processing, the software shifts all controls before comparing them to the sample.

MS Parameters Tab

Parameter	Description
m/z Tolerance	
MS m/z tolerance	Specifies a range for determining peaks in the MS spectrum. All masses within this range will be considered one unique peak. For a peak with an assigned experimental formula to be considered as a potential metabolite, the mass accuracy of the peak must be within the tolerance specified. This parameter is highly dependent on the calibration state of the instrument. For instruments calibrated within ±3 ppm, a value of 10 ppm is recommended for oligonucleotide methods that contain TOF MS or IDA experiments.

Parameter	Description
Minimum MS peak intensity	Specifies the minimum spectral threshold for MS peak intensity. Removes MS peaks that have an intensity below the specified spectral threshold from consideration. Set the value based on the level of noise in the spectra.
Isotope Pattern Tolerances	
MS m/z tolerance	Specifies the tolerance that is applied to the isotope pattern of metabolites. Only peaks with isotope *m/z* offset values that are within this tolerance are considered a match. For oligonucleotide methods that contain TOF MS or IDA experiments, a value of 10 mDa is recommended.
Intensity tolerance	Specifies the relative tolerance for the isotopic intensities as defined on the Isotope Pattern tab in the Compound-Specific Parameters. To be considered a match, the intensity ratio of two peaks must equal the expected ratio within this tolerance. For oligonucleotide methods that contain TOF MS experiments, a value of 20% is recommended.
Minimum Score	(Oligonucleotide methods) Specifies the minimum matching tolerance (in percent) for the observed isotope pattern for a metabolite, when compared to its expected isotope pattern. We recommend starting with a value of 0%, and then increasing the value as required to remove confirmed false positive identifications.
Limits	
Maximum number of unexpected metabolites	Select a maximum number of unexpected peaks that can be identified as potential metabolites. This setting affects the maximum number of peaks that can be identified by the generic peak finder. The generic peak finder interacts with the predicted metabolites peak finder. For example, if a smaller biotransformation set is selected for a complex sample, then the maximum number of unexpected metabolites will be high, and thus this setting will need to be increased. Typically, for process impurity oligonucleotide samples, a setting of 100 is recommended. For more complex samples this number should be increased.
Mass range window (m/z)	Limits the mass range in which to find potential metabolites.
Generic LC/MS Peak Finding	

Parameter	Description
Perform background subtraction	Specifies if background subtraction is to be performed. Select this option to remove the background ions if the background level is high in the LC/MS chromatogram. For oligonucleotide methods that contain TOF MS and TOF MS/MS experiments, this option is not recommended.
Available Adducts (Small molecule methods) A list of all of the supported adducts, based on the charge range defined in the Compound Information group.	
Use	Indicates whether the adducts are to be included in the search.
__ adduct(s) selected	(Read-only) Indicates the number of adducts that have been selected in the **Use** column of the Available Adducts table.
Advanced Ion Types (ADC, peptide, and oligonucleotide methods)	
Use	Indicates if the ions are to be included in the search.
__ adduct(s) selected	(Read-only) Indicates the number of ions that have been selected in the **Use** column of the Advanced Ion Types table.

MS/MS Parameters Tab

Parameter	Description
MS/MS Finding	
MS/MS m/z tolerance	Specifies a range for determining peaks in the MS/MS spectrum. The MS/MS *m/z* tolerance is the tolerance within which found fragment peaks in the MS/MS spectrum must match the selected fragments or neutral losses values specified on the Product Ions and Neutral Losses tab in the Compound-Specific Parameters so that the corresponding precursor peak can be considered a potential metabolite. For oligonucleotide methods that contain TOF MS/MS or IDA experiments, a value of 10 mDa is recommended.
Minimum MS/MS peak intensity	Removes from consideration MS/MS peaks that have an intensity below the specified spectral threshold. Set the value based on the level of noise in the spectra.
MS/MS Isotope Finding	

Parameter	Description
MS/MS m/z tolerance	Specifies a range for determining peaks in the MS/MS spectrum. For peaks in the MS/MS spectrum to be considered a match, the mass difference between two isotopic peaks must equal the expected difference within this tolerance. The MS/MS *m/z* tolerance is used when processing SWATH acquisition data with the Isotope pattern (SWATH only) peak finding strategy selected. For oligonucleotide methods that contain TOF MS/MS or IDA experiments, a value of 10 mDa is recommended.
Intensity tolerance	Specifies the relative tolerance around the isotopic intensities of the selected fragment formulas as defined in the selected **IP** cell on the Product Ions and Neutral Losses tab in the Compound-Specific Parameters. To be considered a match, the intensity ratio of two peaks must equal the expected ratio within this tolerance. This parameter also defines the smallest isotope that is considered part of the pattern. For example, if the intensity tolerance is 10%, then the smallest isotope that can contribute to the mass pattern must be 10% or higher of the 100%-defined peak. The **Intensity tolerance** is used when processing SWATH acquisition data with the Isotope pattern (SWATH only) peak finding strategy selected. For oligonucleotide methods that contain TOF MS/MS or IDA experiments, a value of 20% is recommended.
Source of Reference MS/MS Spectrum	
Control	Select a reference spectrum for the compound of interest. The spectrum can be selected from various locations. Selected reference spectrum is selected by default. We recommend that the **Selected reference spectrum** option is chosen when using the automatic structure or sequence generation function.
Sample	
Selected reference spectrum	
MS/MS Spectrum	
Use advanced MS/MS filter	This filter is used exclusively for SWATH acquisition data. The algorithms used by this filter include PCVG which is used to assign fragments from an MS/MS spectrum to a particular precursor for SWATH acquisition data, Only those fragments that can confidently be assigned to the precursor are shown in the MS/MS spectrum, depending on the position of the slider (**Comprehensive** or **Confident**).
Similarity and Fragment Interpretation	

Parameter	Description
MS/MS m/z tolerance	Select a mass accuracy tolerance to compare the reference MS/MS spectrum to the MS/MS spectrum of the metabolite. This parameter is also used when fragments are assigned in the Interpretation table. The mass accuracy of the assigned fragments must be within the MS/MS *m/z* tolerance provided. For oligonucleotide methods that contain TOF MS/MS or IDA experiments, a value of 10 ppm is recommended.
Minimum signal-to-noise ratio	Select a minimum ratio of signal to unwanted noise to compare the reference MS/MS spectrum to the MS/MS spectrum of the metabolite. This parameter is also used when fragments are assigned in the Interpretation table. The signal to noise ratio of the assigned fragments must be above the minimum signal to noise ratio provided.
Fragment Interpretation Options (Small Molecule and Peptide Methods)	
Number of fragment peaks selected for assignment	(Small molecule methods) Specifies number of MS/MS fragments that will be selected for assignment. The peaks are selected based on their intensities (the more intense peaks are selected first).
Break aromatic rings	(Small molecule methods) Breaks bonds that form part of an aromatic ring.
Maximum number of bonds to break	(Small molecule methods) Specifies the maximum number of bonds to break when assigning MS/MS fragments for interpretation.
Maximum number of C-C bonds to break	(Small molecule methods) Specifies the maximum number of C-C bonds to break when assigning MS/MS fragments for interpretation.
Fragment Types	(Peptide methods) Identifies the fragment type. Multiple types can be selected.
Maximum bonds to break	(Peptide methods) Specifies the maximum number of bonds to break.
Break linkages	(Peptide methods) Breaks linkages in the peptide or oligonucleotide sequence.

Formula Prediction Tab (Small Molecule and ADC Methods)

Parameter	Description
Search Constraints	
Elements from **Elements to**	Specifies the starting element that the software will use to propose formulas for potential metabolites.

Parameter	Description
Isotope Pattern Tolerances	
MS m/z tolerance	After the software identifies a theoretical predicted isotope pattern for a proposed formula, this parameter limits the allowed mass difference between isotopes when compared to the isotope pattern of the metabolite.
Intensity tolerance	After the software identifies a theoretical predicted isotope pattern for a proposed formula, this value limits the allowed difference in isotopic peak intensity when compared to the isotope pattern of the metabolite.
Ranking	
Contribution	Specifies whether formulas based on the MS spectrum or based on the MS/MS spectrum should be provided in the results.
Automatically weight MS/MS	Select to apply a logarithmic scale to the MS/MS weighting.
Rings and Double Bonds	
RDB from **RDB to**	Identifies a range of rings and double bonds in the proposed formulas of potential metabolites. If the number of rings and double bonds of a proposed formula does not fall within the specified range, then that formula will not be considered for the metabolite. The minimum value must be less than the maximum value.
Element Ratios	
Oxygen/ phosphorus count	Specifies the range of oxygen-to-phosphorus molecules that must be present in the proposed formulas. This parameter applies to both MS and MS/MS formulas.
Oxygen/sulphur count	Specifies the range of oxygen-to-sulphur molecules that must be present in the proposed formulas. This parameter applies to both MS and MS/MS formulas.

Confirmation Scoring Tab

When a potential metabolite is found in the sample of interest, the software assigns a confirmation score that indicates the likelihood that the peak found is a metabolite. The score is independent of the algorithms used to find metabolites and is based on various properties.

Note: For oligonucleotide methods, a value of 100 is recommended for **Isotope pattern**, and 0 for all other parameters.

Parameter	Description
Mass defect	(Small molecule methods) Indicates how closely the mass defect of the metabolite matches the mass defect of the parent compound, potential cleavage metabolites, or Phase II metabolites. **Note:** This attribute is not used for the calculation of the total confirmation score for ADC, peptide, and oligonucleotide data.
Isotope pattern	(Small molecule and ADC methods) Indicates whether the metabolite has an isotope pattern similar to the parent compound. This property has a score from 0 to 100. (Oligonucleotide methods) Indicates whether the metabolite has an isotope pattern similar to the expected isotope pattern. This parameter is very useful for filtering false positives. A value of 100 is recommended.
MS/MS	Indicates how close the MS/MS spectrum is to the reference spectrum. This property applies only if a reference spectrum is available. The MS/MS score has two components: • Quality: A measure of the ability to distinguish spectral peaks from background noise. • Similarity: The software calculates how close the MS/MS spectrum is to the reference spectrum, including product ions that were shifted based on known biotransformations. **Note:** If only TOF MS data is being processed, then set this parameter to **0**.
Mass accuracy	Indicates how close the found *m/z* value is to the expected *m/z* value. This property applies for predicted metabolites only.
Total confirmation score	(Read-only) Total of the four property values.

Tip! Type **0** in the Scoring table to ignore a specific property when scoring.

Compound-Specific Processing Parameters

Compound-specific processing parameters are settings that are dependent on the compound being processed. Each of the following tabs manage compound-specific parameters.

Compound-Specific Parameters

Small Molecules	Peptides	Oligonucleotides	ADC
Cleavage Metabolites Tab (Small Molecule and ADC Methods)	Catabolites Tab (Peptide Methods)	Catabolites Tab (Oligonucleotide Methods)	Cleavage Metabolites Tab (Small Molecule and ADC Methods)
Mass Defect Tab (Small Molecule Methods)	Isotope Pattern Tab	Isotope Pattern Tab	Isotope Pattern Tab
Isotope Pattern Tab	Product Ions and Neutral Losses Tab	Product Ions and Neutral Losses Tab	Product Ions and Neutral Losses Tab
Product Ions and Neutral Losses Tab			Antibody Details

Cleavage Metabolites Tab (Small Molecule and ADC Methods)

Identifies the potential cleavage metabolites of the parent compound. The method must contain a structure before the software can generate a list of potential cleavage metabolites.

Parameter	Description
Potential Compound Cleavages	
Maximum bonds to break	Specifies the maximum number of bonds to break.
Break ring bonds	Breaks bonds that form part of a ring.
Only break C-N bonds	Breaks only C-N bonds.
Cleavages selected	Indicates the number of cleavages that have been selected in the potential compound cleavages table. Automatically generated by the software.

Catabolites Tab (Peptide Methods)

Identifies the potential hydrolytic cleavages of the parent compound. The method must contain a peptide sequence before the software can generate a list of potential hydrolytic catabolites.

Parameter	Description
Potential Hydrolytic Cleavages	
Max. peptide bonds to break	Specifies the maximum number of peptide bonds to break.
Max. cross-links to break	Specifies the maximum number of cross-links to break.
Min. AA count	Specifies the minimum number of amino acids in the catabolite.
Catabolites selected	(Read-only) Indicates the number of catabolites that have been selected in the potential hydrolytic cleavages table.

Catabolites Tab (Oligonucleotide Methods)

Identifies the potential hydrolytic cleavages of the parent compound. The method must contain a peptide sequence before the software can generate a list of potential hydrolytic catabolites.

Parameter	Description
Potential Hydrolytic Cleavages	
Max. bonds to break	Specifies the maximum number of bonds that can break along the oligonucleotide backbone only, including loss of H_2PO_3. For nucleobase and sugar losses, refer to the section: Biotransformations Tab.
Min. Nucleotides	Specifies the minimum number of nucleotides used to generate potential catabolites and hydrolytic cleavages.
Include terminus n+1 sequences	Specifies whether to search for terminus n+1 impurities.
Include internal n-1 sequences	Specifies whether to search for internal n–1 impurities.
Catabolites selected	(Read-only) Indicates the number of catabolites that have been selected in the potential hydrolytic cleavages table.

Mass Defect Tab (Small Molecule Methods)

When complex biological samples are analyzed, these filters can help to remove background interference.

Parameter	Description
Mass Defect Filters	
Filters selected	Indicates the number of mass defect filters that have been selected in the mass defect filters table. Automatically generated by the software.
Filters	
Parent	Selected by default.
Glucuronidation	Selected by default.
Bis-Glucuronidation	Selected by default.
Glutathione	Selected by default.
Sulphate	Selected by default.

Isotope Pattern Tab

Parameter	Description
Isotope Pattern	Shows a graphical representation of the information listed in the Isotopes table. (Oligonucleotide methods) Shows a graphical representation of the isotopic distribution for the oligonucleotide, at a specified charge state. To change the charge state, select a different **Ion type** in the Compound Information.
Isotopic Enrichment	Specifies the isotopic enrichment of an atom that will be used in the formula of the parent compound. **Note:** To add an isotope element for ADC or small molecule methods, import the mol file that contains the isotope. **Note:** To change the isotopic enrichment for peptide and oligonucleotide formulas with enriched atoms, refer to the section: Edit the Isotopic Enrichment for Peptide and Oligonucleotide Formulas.
Isotopes	Shows the most intense isotopes, based on the formula and isotopic enrichment, if applicable, of the parent compound.
Isotope Pattern Intensity Cufoff for Metabolite XICs (%)	(Oligonucleotide methods) Specifies the cutoff value, in percent intensity, that is applied during peak area calculation to individual isotopes that are considered for XIC extraction. Isotopes with intensities below the cutoff are shown in red in the table.

Product Ions and Neutral Losses Tab

Parameter	Description
Reference MS/MS Spectrum	Identifies a spectrum to use when selecting product ions and neutral losses to match against the MS/MS of potential metabolites. The best source is a data file for a sample acquired under similar conditions as the sample of interest. The spectrum can be selected from one of two locations: • wiff file • Compound Library
Filters	
m/z From __ to __	Defines the mass range that is considered when populating the product ions and neutral losses table. Only the fragments that are within the selected range are shown in the product ions and neutral losses table.
Charge state From __ to __	Defines the charge state range that is considered when populating the product ions and neutral losses table. Only fragments with charges that are within the selected range are shown in the product ions and neutral losses table.
Only show product ions above (%)	Defines the minimum threshold for product ions to be included in the product ions and neutral losses table. Removes product ions that are below the given intensity from consideration.
Mass accuracy within (mDa)	Only fragments with mass accuracies that are within the specified value are shown in the product ions and neutral losses table.
Add product ions, neutral losses from Phase II metabolites	(Small molecule and ADC methods) Includes product ions and neutral losses from phase II metabolites in the product ions and neutral losses table.

Note: After all of the required changes have been made to the filters, click **Assign Fragments** to update the product ions and neutral losses table.

Antibody Details

Note: These compound-specific parameters are only applicable to ADC methods.

Parameter	Description
Protein Sequence	The protein sequence of the antibody.
Enzyme	Enzyme to be used to digest the protein.
Break disulfide bonds	Disulfide bonds are broken when this check box is selected.
Site of conjugation	Amino acid in the antibody that the drug molecule can be conjugated to.
Type of conjugation	The chemistry involved in conjugation of the drug molecule to the antibody.
Max. AA count	The maximum number of amino acids to be considered as potential fragments, after digestion.
Selected fragments	Automatically generated by the software. Indicates the number of fragments that have been selected in the table.

Edit the Isotopic Enrichment for Peptide and Oligonucleotide Formulas

Prerequisite
A custom amino acid, with or without a custom amino acid modification, must be created. Refer to the sections: Create a Custom Amino Acid and Create a Custom Amino Acid Modification. The custom amino acid or the custom amino acid modification must contain at least one enriched isotope.

1. In the Workflow panel, click **Processing Parameters.**

   The processing parameters workspace opens.

2. Click **New > Peptides** or **New > Oligonucleotide**

   Note: Alternatively, select an entry from the compound library to fill in the sequence.

3. Type the **Compound name** for the custom amino acid or oligonucleotide in the field provided.
4. Type the **Sequence** information for the custom amino acid or oligonucleotide. The sequence must include at least one enriched isotope.
5. Click in the **Chemical formula** field.

The **Chemical formula** field and the **m/z** value are populated with the information related to the custom amino acid.

Tip! An icon is shown above the sequence. Hover over the icon with the cursor to view the **Symbol** and **Residue Formula** of the custom amino acid used.

6. Click the **Compound-Specific Parameters > Isotope Pattern**.

   In the Isotopic Enrichment table, the residue formula of the custom amino acid is shown in the **Element** column and **100** is shown in the **Enrichment %** column.

7. Modify the **Enrichment %** value, as required.

8. Click **Save and Close**.

Search for Potential Molecules 6

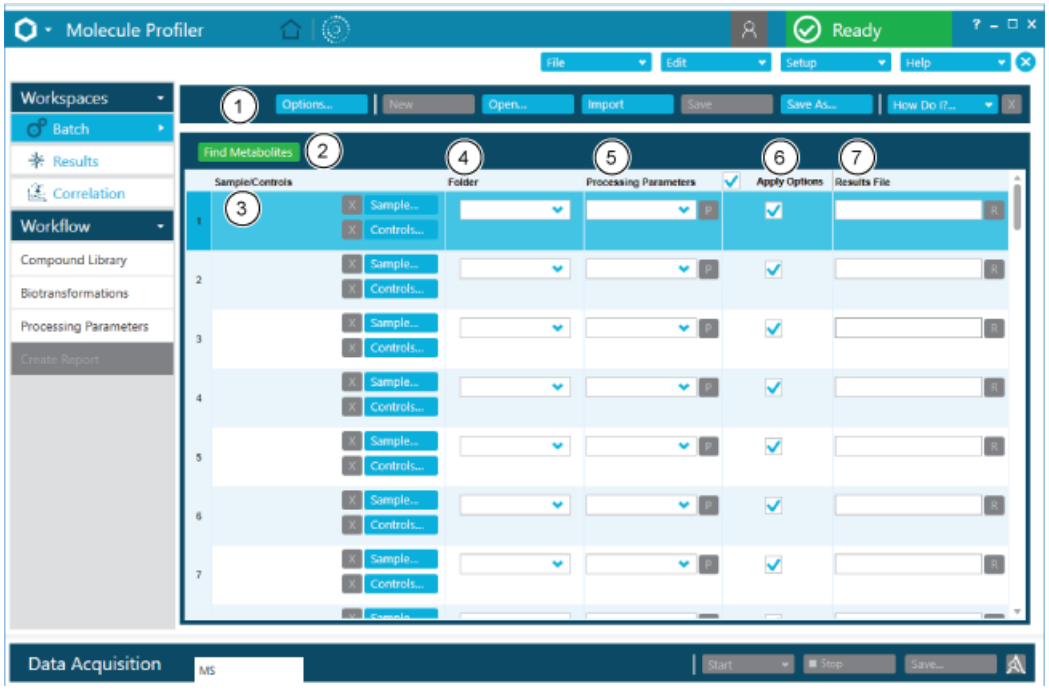
The Batch workspace is used to process multiple sample files at the same time. The batch table can be completed manually, or an existing batch can be imported to populate the table.

1. To manually prepare a batch, continue with the section: Create a Batch.
2. To open an existing batch, continue with the section: Open a Batch.
3. To import an existing batch, continue with the section: Import a Batch.

About the Batch Workspace

Use the Batch workspace to create batches of sample for processing.

Figure 6-1 Batch Workspace



Item	Description
1	Menu bar. Contains these buttons: • **Options**: Opens the Batch Processing Options dialog where the user can specify the options that are relevant to the batch. Refer to the section: Batch Processing Options. • **New**: Click to save the batch. Only available after a sample has been added to the **Sample/Controls** field. • **Open**: Opens the Open Processing Batch dialog where the user can select an existing batch to open. Refer to the section: Open a Batch. • **Import**: Opens the Batch Importer dialog where the user can select an Excel file to import. Refer to the section: Import a Batch. • **Save**: Saves the currently open batch file. Automatically replaces the existing version. Only available after the batch information has been modified. • **Save As**: Saves the currently open batch file. Users can assign a new name to the batch file.
2	**Find Metabolites** button. Starts processing of the batch.
3	**Sample/Controls** column. The **Sample** button opens the Select Data dialog where the user can select the sample of interest. The **Controls** button opens the Select Data dialog where the user can select the corresponding control sample. A maximum of five controls can be selected per sample.
4	**Folder** column. Provides a list of folder locations where the processing parameters and results are stored.
5	**Processing Parameters** column. Provides a list of the processing parameters that can be used to process the sample of interest. Only processing parameters that are stored in the selected **Folder** are available for selection.

Item	Description
6	**Apply Options** column. Selected by default. When selected, applies all of the Auto Assign and Report options chosen in the Batch Processing Options dialog to the samples and control samples in the batch. Options include: • Clear the **Apply Options** check box to clear all of the selected check boxes. • Clear specific sample check boxes. • Select specific sample check boxes. Refer to the section: Batch Processing Options. **Note:** Auto Assign options are not applicable for the oligonucleotide workflow.
7	**Results File** column. User-specified name for the Results file.

Specify Batch Options

Refer to the section: Batch Processing Options.

1. In the Workspace panel, click **Batch**.

   The Batch workspace opens.
2. Click **Options**.

   The Batch Processing Options dialog opens.
3. (Small molecule, peptide, and ADC workflows) On the Auto Assign tab:
   - Select the check box for each applicable option.
   - Type the appropriate value for each selected option.
4. On the Report tab:
   - Select the check box for each applicable option.
   - Type the appropriate value for each selected option.
5. Click **OK**.

   The batch options will be saved with the batch.

Batch Processing Options

Option	Description
Auto Assign **Note:** The auto assign options are independent of one another. They are considered "or" conditions. One or more of the selected options must meet the criteria specified. **Note:** The auto assign options are not applied to oligonucleotide samples.	
Assign Structures or Sequences	Proposes potential structures or sequences for metabolites that meet the criteria of the selected option. Dependent on the data type and processing parameters used (that is, whether it is a small molecule or a peptide). **Note:** Multiple options can be selected.
Metabolites with peak areas above (%)	Proposes potential structures or sequences for metabolites with the XIC peak area above the specified value.
Metabolites with analog peak areas above (%)	Proposes potential structures or sequences for metabolites with the analog peak area above the specified value.
Metabolites with MS/MS quality above	Proposes potential structures or sequences for metabolites that have an MS/MS quality above the specified value. This option is not applicable to oligonucleotide workflows.
Report **Note:** The report options are dependent on one another. They are considered "and" conditions. All of the selected options must meet the criteria specified.	
Report metabolites with assigned structures or sequences	Shows a check mark in the **Report** column of the Potential Metabolites table for any metabolite that has an assigned structure or sequence.
Report metabolites with peak areas above (%)	Shows a check mark in the **Report** column of the Potential Metabolites table for any metabolite that has a peak area above the specified value.

Option	Description
Report metabolites with analog peak areas above (%)	Shows a check mark in the **Report** column of the Potential Metabolites table for any metabolite that has an analog peak area above the specified value.
Report metabolites with scores above (%)	Shows a check mark in the **Report** column of the Potential Metabolites table for any metabolite that has a score above the specified value.

Create a Batch

Note: Only one sample in each row can be processed. A maximum of five controls can be selected for each sample. However, controls are not required for processing.

1. In the Workspace panel, click **Batch**.

   The Batch workspace opens.

   Tip! Select the **Open** option to retrieve a previously saved batch file. Refer to the section: Open a Batch.

2. Add an MS sample by doing this:

   a. In the first available row of the batch table, click **Sample**.

      The Select Data dialog opens.

   b. Click **Browse** and then navigate to the appropriate source folder.

   c. Select the wiff file and injection containing the sample of interest in the **Available** field and then click **>>**.

      The sample information is shown in the **Selected** field.

3. (Optional) Add an analog sample by doing this:

   Tip! If analog data was acquired, then select the **Use analog data** check box to automatically add analog data when adding the MS sample.

   a. In the first available row of the batch table, click **Use analog data**.

   b. Open the Analog Sample tab.

   c. Click **Browse** and then navigate to the appropriate source folder.

d. Select the analog sample in the **Available** field and then click **>>**.

The sample information is shown in the **Selected** field.

4. Click **OK**.

The **Sample/Controls** field of the selected row in the batch table is populated with the sample information.

5. (Optional) Add an MS control by doing this:

a. In the first available row of the batch table, click **Control**.

The Select Data dialog opens.

b. Click **Browse** and then navigate to the appropriate source folder.

c. Select the wiff file and injection containing the control in the **Available** field and then click **>>**.

The sample information is shown in the **Selected** field.

d. Continue with the following step to add an anolog control, or click **OK** to close this dialog.

6. (Optional) Add an analog control by doing this:

a. In the first available row of the batch table, click **Use analog data**.

b. Open the Analog Sample tab.

c. Click **Browse** and then navigate to the appropriate source folder.

d. Select the analog sample in the **Available** field and then click **>>**.

The sample information is shown in the **Selected** field.

e. Click **OK**.

The **Sample/Controls** field of the selected row in the batch table is populated with the sample information.

7. In the **Folder** column, select the folder where the processing parameters and Results files will be saved.

8. In the **Processing Parameters** column, select a processing parameters file.

Tip! To view the processing parameters file, click **P**. Change the parameters as required, and then click **Save and Close** to save them.

9. In the **Results File** column, type the name of the file in which the results will be stored.

10. Repeat step 2 to step 9 for each row in the batch.

Note: The maximum number of rows that can be processed in one batch is 200.

Tip! To facilitate the creation of the batch, rows can be copied and pasted or deleted. Refer to the sections: Copy and Paste a Batch Row or Clear a Batch Row.

Copy and Paste a Batch Row

Use the copy and paste options to edit a batch.

1. In the batch table, select the row to be copied.
2. Right-click and then select **Copy Batch Row**.
3. Select the destination row of the pasted information.
4. Right-click and then select **Paste Batch Row**.

Note: Any existing information in the destination row is overwritten.

Clear a Batch Row

Rows of sample information can be cleared when creating a batch.

1. In the batch table, select the row to be cleared.
2. Right-click and then select **Clear Batch Row**.

   All of the data is removed from the selected row.

Open a Batch

1. In the Workspace panel, click **Batch**.

   The batch workspace opens.
2. Click **Open**.

   The Open Processing Batch dialog opens.
3. Select the batch file and then click **OK**.
4. Do one of the following:
   - If the batch is complete, then continue with the section: Submit a Batch.

- If the batch is incomplete, then continue with the section: Create a Batch.

Import a Batch

1. In the Workspace panel, click **Batch**.
2. Click **Import**.

   The Batch Importer dialog opens.
3. Click **Browse**.

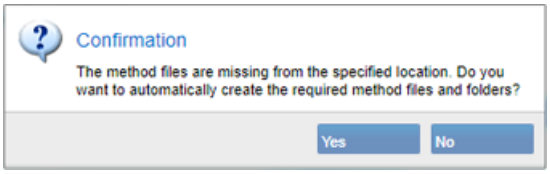
   The Open excel file dialog opens.
4. Navigate to and then select the appropriate Excel file.

   Note: The Excel file should be created using the example template (BatchImportTemplate.xlsx) that is distributed with the software. During installation, the template is installed in the following location: C:\ProgramData\SCIEX\Molecule Profiler\Batch Import Templates folder.
5. Click **Open**.

   The **Target batch file** field is populated with the name of the imported Excel file. This information can be modified, if required.
6. Do one of the following:
   - To convert the selected Excel file to a Molecule Profiler software batch and open batch in the Batch workspace, click **Convert and Open** and continue with step 7.
   - To convert the selected Excel file to a Molecule Profiler software batch that can be opened in the Batch workspace at a later time, click **Convert** and continue with step 7.
   - To cancel the import, click **Close**.
7. Do one of the following:
   - If the **Convert and Open** option was selected and all of the processing parameters (method files) and folders referenced in the Excel file are stored in the correct locations, then continue with the section: Submit a Batch.
   - If the **Convert** option was selected and all of the processing parameters (method files) and folders referenced in the Excel file are stored in the correct locations, then continue with the section: Save a Batch.

- If the **Convert and Open** option or **Convert** option was selected and a confirmation dialog is shown, then continue with step 8.

Figure 6-2 Confirmation Dialog



8. Do one of the following:

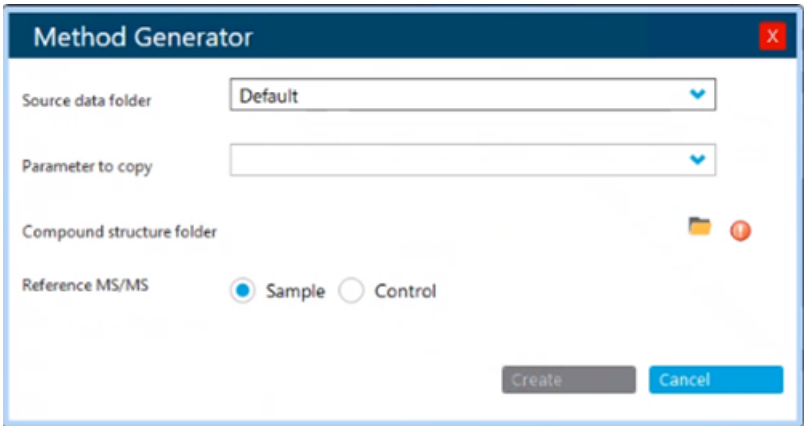
- (Small molecule workflow) To automatically create the required method files, continue with step 9.

Note: The Method Generator cannot be used with peptide, oligonucleotide, or ADC workflows.

- To manually create the required method files, click **No** and then cancel the import. Continue with the section: Create Processing Methods.

9. Click **Yes**.

Figure 6-3 Method Generator Dialog



10. Select the appropriate folder from the **Source data folder** list.
11. Select the appropriate method file from the **Parameters to copy** list.
12. Click the folder icon to the right of the **Compound structure folder** field and then browse to and select the folder that contains the precursor structures of the processing parameters.

13. Do one of the following:
    - Click **Sample** if the sample wiff file contains the reference MS/MS.
    - Click **Control** if the control wiff file contains the reference MS/MS.
14. Click **Create**.

    The processing parameters and any missing folders are created and stored in the location specified in the Excel spreadsheet.
15. Do one of the following:
    - If the **Convert and Open** option was selected, then continue with the section: Submit a Batch.
    - If the **Convert** option was selected, then continue with the section: Save a Batch.

Save a Batch

Information that has been added to the table in the Batch workspace can be saved for later use.

1. To save a batch using the same file name, click **Save**.
2. To create a batch using a different name, click **Save As**.

   The Save Processing Batch As dialog opens.
3. Type a unique **Name** and then click **OK**.

Submit a Batch

After the batch is prepared and the batch options have been specified, the batch is submitted for data processing.

Note: If required, the processing parameters can be modified for a sample of interest before the batch is submitted.

1. (Optional) To view the processing parameters for a sample, do this:
   a. Select the row containing the sample of interest and then click the **P** located to the right of the **Processing Parameters** field.

      The processing parameters associated with the selected sample are shown.
   b. Make any necessary modifications and then click **Save and Close**.

      The Batch workspace is shown.
2. Click **Save As**.

The Save Processing Batch As dialog opens.

3. Type a unique **Name** and then click **OK**.
4. Click **Find Metabolites**.

   Processing of the batch begins. A progress bar shows the status of the processing. During processing, the **P** is disabled to prevent modification of the parameters. When the processing for the row is complete, the **P** located to the right of the **Processing Parameters** field and the **R** located to the right of the **Results File** field are available.
5. Click the **R** to open the Results file in the Results workspace.

   Note: Any Auto Assign and Report options that were selected in the Batch Processing Options dialog are completed by the software.
6. (Optional) Complete step 1 to save the updated the processing parameters.
7. Click **Save**.

   Tip! Click **Save As** to save the batch with a different name.

View Results 7

Use the Results workspace to view the results of searching for potential metabolites in a sample of interest.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

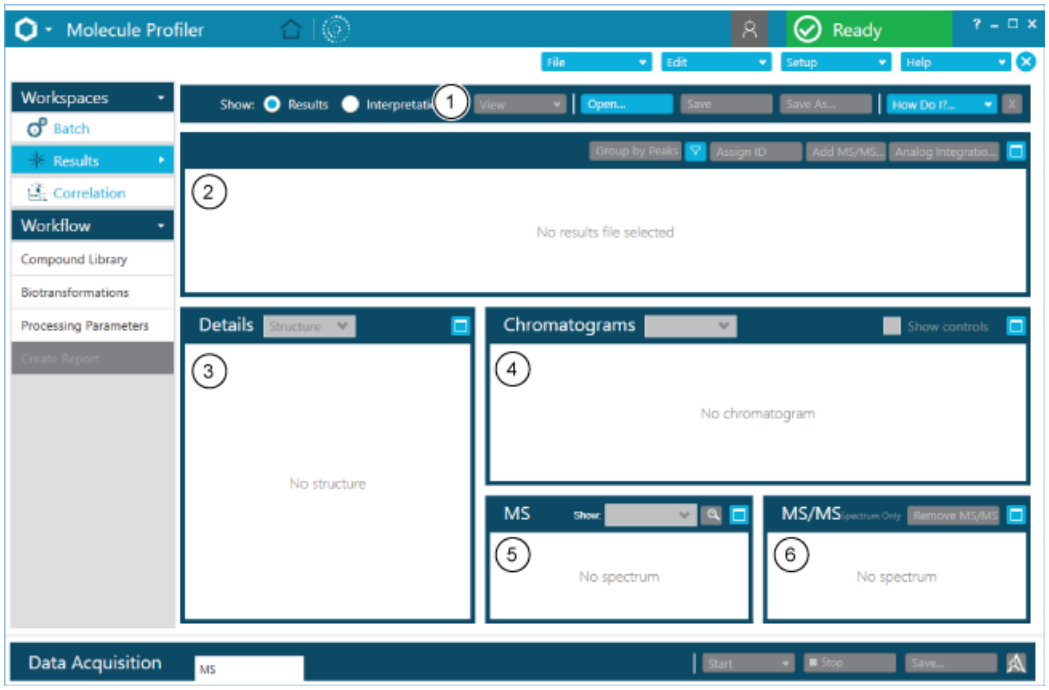
4. Click **OK**.

   The Results view is shown.

About the Results Workspace

After the software processes the data, use the Results workspace to view the list of potential metabolites.

Figure 7-1 Results Workspace



Item	Description

1	Menu bar. Contains these buttons:
	• **View**
	  • **Processing Parameters**: Shows the processing parameters for the results.
	  • **Batch Options**: Shows the batch processing options for the results.
	  • **Sample Details**: Shows detailed information about the sample.
	• **Open**: Opens the Open Results dialog where users can browse to the appropriate Results file.
	• **Save**: Saves the currently open Results file. Automatically replaces the existing version.
	• **Save As**: Saves the currently open Results file. Users can select the destination folder and assign a new name to the Results file.

2	Potential Metabolites pane. Lists all of the peaks that were found by the selected algorithms in the sample of interest. Refer to the section: About Peak Finding Strategies. Edit the results by deleting rows that do not contain potential metabolites, changing the name and formula, adding MS/MS spectra, and assigning peak IDs. Refer to the section: Edit Results. For a description of the columns in the Potential Metabolites table, refer to the section: Table 7-1. **Note:** Predefined filters might affect the potential metabolites shown in the list. Refer to the table: About Results Filters.

Item	Description
3	Details pane. Provides information about how the potential metabolite was scored. For each property in the processing method, **Scoring** list shows the score for the individual metabolite, as well as the total score for all metabolites, are shown. Refer to the section: Confirmation Scoring Tab. Scores are shown for **Mass Defect** (disabled for oligonucleotides), **Isotope Pattern**, **MS/MS**, **Mass Accuracy**, and **Total confirmation score**. For small molecule results, the **Structure** list shows the available structure. For ADC results, the **Structure** list shows both the available structure and the available sequence. For peptide results, the **Sequence** list shows the available sequence. For oligonucleotide results, the **Sequence** list shows the oligonucleotide sequence for the selected metabolite, as determined by the MS/MS assignment in the Interpretation view. If no assignment was made, then this field is blank.

Item	Description
4	Chromatograms pane. Enables the user to show different chromatograms for the potential metabolite that was found: • **Metabolites**: Shows the sum of all of the metabolite peaks currently found. All major chromatographic peaks are shown, with their RTs and peak IDs. The selected metabolite peak and any peaks eluting at the same retention time are yellow. • **XIC**: Shows an extracted ion chromatogram (XIC) for the selected metabolite. Isotopes selected for XIC extraction are labeled at the top of the graph. • **Mass Defect**: Shows the mass defect chromatograms used to identify the selected metabolite. This is specific to small molecules data only. • **Isotope Pattern**: Shows a chromatogram of all of the peaks with isotope patterns that match the parent compound. • **Product Ions**: Shows a chromatogram of all of the peaks with fragment ions that match the selected fragments on the Product Ion and Neutral Losses tab. • **Neutral Losses**: Shows a chromatogram of all of the peaks with neutral losses that match the selected neutral losses on the Product Ion and Neutral Losses tab. • **Isotope Pattern** (SWATH acquisition data): Shows a chromatogram of all of the peaks with fragment isotope patterns that match the selected fragment isotope patterns on the Product Ion and Neutral Losses tab. • **Analog Data**: Shows an analog chromatogram of all of the peaks. A peak label indicates matching peak IDs for both the analog peak and the corresponding MS peak. **Note:** The **Mass Defect**, **Isotope Pattern**, **Product Ions**, **Neutral Losses**, and the **Isotope Pattern** (SWATH acquisition data), chromatograms are available only if these algorithms were selected for processing. In addition, if analog data was processed, as defined in the processing method, then the **Analog Data** chromatogram is available in the **Chromatograms** list. **Note:** In oligonucleotide workflows, only the **Metabolites**, **XIC**, and **Analog Data** views are available. **Note:** If the **Show Controls** check box is selected, then both the **XIC** and the **Analog Data** chromatogram, if applicable, show the control traces.

Item	Description
5	MS pane. Shows the MS spectrum. The options in the **Show** list select the peaks to be highlighted: • **Default**: Shows a portion of the sample MS, centered around the *m/z* value of the selected metabolite. • **Mass Defect**: Highlights all of the *m/z* values that match any mass defect filter selected in the processing method. This is specific to small molecules data only. • **Isotope Pattern**: Highlights all of the *m/z* values that have the same isotope pattern as the parent compound. For oligonucleotide workflows, **Predicted Isotope Pattern** and **Charge Series** traces are overlaid by default. The default view shows the centroided TOF MS spectrum for the selected metabolites. The monoisotopic peak is labeled with the predicted charge, and a blue arrow indicates its position. Red arrows on the *m/z* axis indicate individual isotopes that were selected for XIC extraction and area determination. A theoretical isotopic envelope overlays centroid peaks to provide a goodness-of-fit assessment for observed data. To set the spectrum to full range, double-click below the *m/z* axis. To view the predicted location of additional charge states for the selected metabolite, pan and zoom by left-clicking and dragging across the *m/z* axis.
6	MS/MS pane. Shows an MS/MS spectrum for the selected metabolite. The source of this data is one of the following: • The sample IDA wiff file. • The sample SWATH acquisition wiff file. • The dedicated MS/MS wiff file that was added to the Results file. Refer to the section: Add Multiple Spectra with the **Add MS/MS** Button. For oligonucleotides, common product ion peaks that were matched to the reference MS/MS spectrum selected in the processing parameters are yellow. Unmatched product ions peaks are blue.

Table 7-1 Potential Metabolites Table Columns

Column	Description
Report	When selected, includes the metabolite in the final report.
Peak ID	Shows the Peak ID of the metabolite. The ID is based on the retention time and the mass of the metabolite. For all Parent metabolites, the **Peak ID** is blank. A -# is assigned to peaks with the same mass and retention time but different a charge. For example: M1-1, M1-2, M1-3, and so on.
Name	Shows the name of the metabolite. For ADC results, the names are preceded by the word Parent. Parent indicates that the small molecule drug (payload) and linker components are combined. For oligonucleotide results, the main component names are indicated by the words Parent and Ion charge. Biotransformation and cleavage products identified by the predicted peak finder have the characteristic 5' or 3' (n-#) notation. Generic peak finder results are indicated by either a Gain or Loss prefix.
Formula	Shows the neutral formula of the metabolite.
Assigned	When selected, indicates that information exists in the Interpretation workspace. For example, a structure or sequence might be present or the Fragments table might be populated.
Neutral Mass	Shows the neutral mass of the metabolite.
m/z	Shows the monoisotopic mass to charge ratio for the metabolite. For oligonucleotides results, if the monoisotope is not observed, then the software calculates its position and flags the *m/z* value with an (n), where n denotes the number of peaks that the monoisotope is away from the first observed peak.
Charge	Shows the charge of the metabolite.

Table 7-1 Potential Metabolites Table Columns (continued)

Column	Description
Peak Index	Reflects the isotope of the XIC peak area shown for the metabolite. • **Blank cell**: monoisotope • **1**: the first isotope after the monoisotopic peak • **2**: the second isotope after the monoisotopic peak, and so on If the column is not included in the table, then the XIC peak area is associated with the monoisotope of the metabolites. For metabolites identified by the predicted metabolite finding strategy, an estimated hypothetical base peak index is shown for a metabolite. For metabolites identified by other peak finding strategies, the experimental isotopic peak is shown. Usually, the experimental isotopic peak is the base peak. However, for IDA data, the experimental isotopic peak could be the index of the precursor ion.
ppm	Shows the mass accuracy (in ppm) of the metabolite.
R.T. (min)	Shows the retention time of the metabolite.
Peak Area	Shows the XIC peak area of the isotope whose peak index is shown in the **Peak Index** column.
% Area	Shows the % area of the XIC, based on the total number of metabolites in the table.
% Score	Shows the % score of the metabolite.
Analog - Peak Area	Shows the peak area of the analog peak. Available only when analog data has been processed.
Analog - % Area	Shows the % area of the analog peak. Available only when analog data has been processed.
Analog - R.T. (min)	Shows the retention time of the analog peak. Available only when analog data has been processed.

Show Filtered Spectrum Only

Note: This feature is not applicable for oligonucleotide workflows.

If the **Use advanced MS/MS filter** parameter was selected to process a SWATH acquisition data file on the MS/MS Parameters tab in the Generic Parameters group in the Processing Parameters workspace, then select this check box to show only an MS/MS spectrum filtered with the advanced filter. Clear the check box to show the background-subtracted spectrum.

Note: If the **Use advanced MS/MS filter** parameter was selected but only the background-subtracted spectrum is shown in the MS/MS spectrum, then this might be a result of:

- Blank filtered spectrum.
- Ineffective advanced filter caused by interference from a co-eluting peak that was approximately 10-fold or higher in intensity.
- Ineffective advanced filter caused by less than five data points across the precursor peak.

About Results Filters

Filters can be applied to refine the results shown in the Potential Metabolites table.

Tip! Click the filter icon to open the Results Filters dialog.

Table 7-2 Filters

Select this filter	To show this
Metabolites	
Top __ metabolites by peak area	Only the specified number of peaks that are the most abundant based on % peak area.
Reported metabolites	Only the metabolites that have been selected in the **Report** column.
Metabolites by adduct	Only the metabolites that were found by a primary adduct. A primary adduct is defined as the adduct which is the first visible selection in the **Advanced Ion Types** table of the **Generic Parameters > MS Parameters** tab. Options include: • Primary • Most intense
Assigned Metabolites	
Metabolites with structures or sequences assigned	Only the metabolites that have assigned structures (small molecules) or assigned sequences (peptides and oligonucleotides), as indicated by a check mark in the **Assigned** column in the Potential Metabolites table.
Retention Time Window	

Table 7-2 Filters (continued)

Select this filter	To show this
Retention time from __ to __	Only peaks within the specified range.
Peak Area	
Peak area from __ % to __ %	Only peaks with a % Area that is within the specified percentage range.
Analog peak area from __ % to __ %	Only peaks with a % Analog Area that is within the specified percentage range. If no analog files were provided for processing, then this filter has no effect.
Charge	
Charge from __ to __	Only the metabolites with a charge value that are within the specified range.
Score	
Overall score above __ %	Only peaks with an overall score that is above the specified value. Refer to the section: Confirmation Scoring Tab. For oligonucleotide workflows, we recommend that the isotope envelope be used as the sole parameter in confirmation scoring. We also recommend that the user set **Overall Score** above 20% to remove metabolites with low theoretical isotope overlap scores.
Mass Accuracy	
Accuracy within __ ppm	Only peaks with a mass accuracy that is within the specified range.
Mass Range	
m/z from __ to __	Only *m/z* values that are within the range specified.
Product Ions and Neutral Losses	
MS/MS similarity above __	Only peaks with an MS/MS similarity score that is above the specified value. If a reference spectrum was not provided, then this filter has no effect.
Minimum number of common product ions __	Only peaks that have at least the specified number of product ions in common with the parent compound. If a reference spectrum was not provided, then this filter has no effect.
Minimum number of common neutral losses __	Only peaks that have at least the specified number of neutral losses in common with the parent compound. If a reference spectrum was not provided, then this filter has no effect.

Note: Deleting and adding rows to the table will automatically update the **% Area** and **% Analog Area** of each metabolite, affecting how the peak area, analog peak area, and top metabolites by peak area filters are applied to the remaining rows.

Edit Results

Entries in the Potential Metabolites table can be edited or deleted to further refine the results.

Users can:

- Delete Rows
- Edit the Name and Formula of a Potential Metabolite
- Assign Peak IDs

Delete Rows

1. In the Workspace panel, click **Results**.

   The Results workspace opens.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select the appropriate file.
4. Click **OK**.

   The Results view is shown.
5. Select a row in the Potential Metabolites table.

   Tip! Click and press **Ctrl** or **Shift** to select multiple rows.
6. Click **Edit > Delete Selected Rows.**

   Tip! To revert the most recent deletion, click **Edit > Undo Delete**.
7. Click **Save**.

Edit the Name and Formula of a Potential Metabolite

Refer to the section: How metabolites are named by the software.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. Right-click a row in the Potential Metabolites table and select **Edit Name and Formula**.

   The Edit Name and Formula dialog opens.

6. Do one of the following to change the **Name**:

   - If applicable, select a name from the list of options provided.
   - Type a new name.

7. If applicable, select an adduct from the list of options provided.

   Note: If the adduct is changed, then the **Mass accuracy** of the metabolite is automatically updated.

8. Do one of the following to change the **Formula**:

   - If there is insufficent information available to determine a formula, then select **Unknown**.
   - To manually add a formula to the potential metabolite, select **Use** and then type a formula in the field provided.
   - If potential formulas were predicted by the software, then select **Automatic** and select an entry from the list.

     Note: If potential formulas were not predicted by the software, then **Automatic** is not available for selection.

   Note: The values in the **Mass accuracy** and **RDB** fields are automatically updated by the software when the new formula is added.

9. To identify the metabolite from the selected row as the parent compound, click **Assign as Parent**.

10. Click **OK**.

11. Click **Save**.

Note: For peptides, the order of the names is based on the mass accuracy of the proposed name and the number of manipulations required, for example, the number of bonds broken. That is, the proposed name for the peptide with a higher mass accuracy and fewer manipulations is shown at the top of the list.

Group by Peaks

Use the **Group by Peaks** button to group peaks that have the same neutral mass, such as the multiple charge states of a molecule, to show a summary table of the identified molecules, with **Peak Area**, **%Area**, and so on, totalled for all identified charge states. The peaks are grouped based on neutral mass and retention time tolerance.

Note: The group function is applicable to the oligonucleotide workflow only.

Assign Peak IDs

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. Review the current peak IDs in the Potential Metabolites table.

6. Make any changes to the table, including deleting rows and renaming metabolites.

7. Click **Assign ID**.

   Rows related to the same neutral formula and retention time are grouped together. One row is assigned the primary peak ID and the remaining rows in the group are assigned a sequential ID, one level below the primary peak ID. For example, if the assigned primary peak ID is M2, each remaining row is assigned a sequential ID beginning with M2. For example, M2-1, M2-2, and so on.

   Non-primary peak IDs are assigned to peaks with non-primary adducts. Non-primary adducts are those which have been selected in the Advanced Ion Types table but are not shown in the Ion type list. Only the primary peak IDs are shown in the Ion type list.

MS/MS Spectra

MS/MS spectra can be added, removed, or replaced for any metabolite. An MS/MS spectrum can be added manually by copying a single centroided MS/MS spectrum in the Explorer workspace, and then pasting it in the Results workspace, or automatically with the **Add MS/MS** button in the Results workspace.

Add Spectra by Pasting

Note: This is a beta feature.

Note: This feature is available only in the oligonucleotide workflow, for TOF-MS/MS and IDA data. Pasting MS/MS spectra from the IDA Explorer is currently not supported. Data files must be opened as a standard TIC and centroided in the Explorer workspace before being pasted into Molecule Profiler.

Note: The existing MS/MS spectrum in the Results file is not overwritten until the Results file is saved. To revert to the original spectrum for the selected metabolite, as stored in the Results file, click **Remove MS/MS** before saving the Results file.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and select the appropriate file, and then click **OK**.

   The Results view is shown.
4. Select a row in the Potential Metabolites table.
5. From the SCIEX OS Home page, open the Explorer workspace.
6. Select **File > Open Sample**.

   The Select Sample dialog opens.
7. Browse to the data file that contains the sample, click **+** to expand it, select the sample to be opened, and then click **OK**.

   The data file must be a wiff or wiff2 data file that contains TOF-MS/MS or IDA data.
8. If the data file contains IDA data, select **As a standard TIC** in the Open IDA Sample dialog, and then click **OK**.
9. Open an MS and MS/MS spectrum.

Tip! To open the spectra, set a selection window, or double-click a retention time in the TIC pane.

10. Right-click the header of the MS/MS spectrum, and then select **Remove All Traces Except Active**.
11. Select **Process > Centroid Spectrum**.

    The Centroid dialog opens.
12. Select whether to centroid on **Intensity**, **Height**, **Area**, or **Intensity sum above 50%**.
13. Select **Edit > Copy**.
14. Go to the Molecule Profiler workspace.
15. Click **Paste MS/MS**.

    The MS/MS spectrum is added.
16. Click **Save**.

Add Multiple Spectra with the Add MS/MS Button

Note: The existing MS/MS spectrum in the Results file is not overwritten until the Results file is saved. To revert to the original spectrum for the selected metabolite, as stored in the Results file, click **Remove MS/MS** before saving the Results file.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select the appropriate file, and then click **OK**.

   The Results view is shown.
4. Select a row in the Potential Metabolites table.
5. Click **Add MS/MS**.

   The Add MS/MS dialog opens.
6. Click **Select MS/MS**.

   The Select Data dialog opens.
7. Browse to and select the appropriate **Source** folder.
8. Click **OK**.

9. In the Available pane of the Select Data dialog, select the wiff file and injection containing an MS/MS spectrum and then click the icon (>>) to move the file to the Selected pane.

Tip! Up to 10 injections can be selected.

10. When all of the required files are shown on the Add MS/MS dialog, click **OK**.

    The software tries to find a matching spectrum for each metabolite.

11. Review the score of each metabolite.

    If the MS/MS spectrum was changed, then the software might recalculate the **% Score**.

12. Click **Save**.

    To edit specific fragment types within a single MS/MS spectrum, refer to the section: Oligonucleotide Workflows.

Remove Spectra

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. Select a row in the Potential Metabolites table.

6. Click **Remove MS/MS** in the MS/MS pane.

Note: If an IDA spectrum exists for the potential metabolite, then it is shown in the MS/MS pane.

7. Click **Save**.

Remove MS/MS Spectra Files

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.
4. Click **OK**.

   The Results view is shown.
5. Click **Add MS/MS**.
6. Select the appropriate wiff file in the **MS/MS Samples** field.
7. Click **Remove** and then click **OK**.

   All dedicated MS/MS spectra in the wiff file are removed from the results.
8. Click **Save**.

Characterize MS/MS Data 8

After identifying the peaks in a sample of interest, use fragment interpretation to help identify the structure of each potential metabolite.

About the Interpretation View

The Interpretation view in the Results workspace shows the data and tools required to help elucidate a potential structure for each metabolite in a Results file.

Interpretation Views

Small Molecule Interpretation View

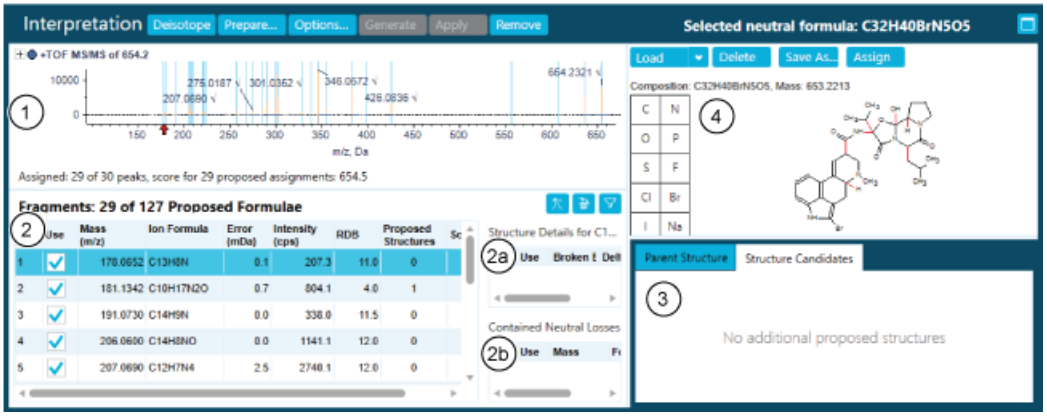
Peptides Interpretation View

Oligonucleotides Interpretation View

ADC Interpretation View

Small Molecule Interpretation View

Figure 8-1 Small Molecule Interpretation View



Item	Interface Component	Description
1	MS/MS pane	Shows the MS/MS spectrum of the selected metabolite. The mirror image of the reference MS/MS spectrum is also shown, if available. Asterisks identify peaks that have been selected for assignment. The source of this spectrum is the sample IDA wiff file, the sample SWATH acquisition wiff file, or a dedicated MS/MS wiff file that was added to the Results file. For a description of the functionality provided by the buttons, refer to the table: Table 8-1.
2	Fragments table	Lists all of the assigned fragments for the selected metabolite including their *m/z* value, the number of proposed structures, and the score. If a particular *m/z* value could be assigned different formulas, the table contains a row for each formula. By default, the row containing the highest score for each combination of formula and *m/z* value has the **Use** check box selected. **Tip!** Rows that do not have the **Use** check box selected can be hidden. To show all rows, right-click the table and then click **Show Hidden Rows**. For a description of the functionality provided by the icons, refer to the table: Table 8-3.
2a	Structure Details table	Lists the portions of the structure that could yield the selected fragment including the number of broken bonds, the delta H value, and the score. Selecting a row in this table highlights the related portion of the structure. By default, the fragment structure with the highest score has the **Use** check box selected.
2b	Contained Neutral Losses table	Contains the neutral losses from the two fragment masses.

Item	Interface Component	Description
3	Structure charts pane	Contains the following two tabs: • The Parent Structure tab, which contains the parent structure of the selected metabolite. • The Structure Candidates tab, which is an interactive histogram that contains a table showing the complete list of proposed structures, sorted in descending score order. Selecting a row in this table changes the structure shown in the Structure pane (item 2). To include a specific structure in the results, click the **Apply to Results** check box for that structure. Refer to the section: About the Structure Candidates Tab.
4	Structure pane	Enables the user to load a candidate structure for potential metabolites and provides basic drawing tools that enable users to edit the structure. For a description of the functionality provided by the buttons, refer to the table: Table 8-2.

Table 8-1 MS/MS Pane Buttons

Button	Description
Deisotope	Hides all of the isotopes in the MS/MS pane. Click again to show the isotopes.
Prepare	Opens the Interpret Data dialog where users can edit the details needed to interpret the selected metabolite (formula, active peaks, MS/MS spectrum recalibration).
Options	Opens the Options dialog where users can assign MS/MS fragments. Refer to the table: Table 8-15 in the section: Set Options.
Generate	Populates the Structure Candidate tab with automatically generated potential candidates for the selected metabolite. Refer to the section: About the Structure Candidates Tab.
Apply	Applies the interpretation changes to the selected peak.
Remove	Removes the assigned fragments and the metabolite structure from the selected peak.

Table 8-2 Structure Pane Buttons

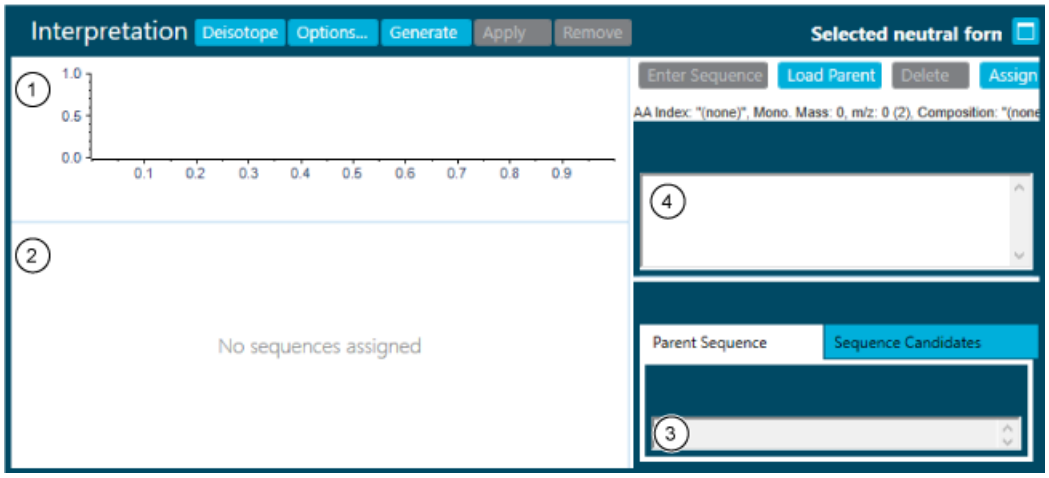
Button	Description
Load	• Load Parent: Opens the parent structure of the selected metabolite. • Load Structure: Opens a structure file for the selected peak.
Delete	Removes the visible structure from the Structure pane.
Save As	Enables the user to save the visible structure with a different file name.
Assign	Calculates the fragments and neutral losses for the potential structure and then assigns the ions to the MS/MS spectra.

Table 8-3 Fragments Table Icons

Icon	Description
	Adds a label for the selected fragment.
	Deletes all of the labels from the MS/MS spectrum.
	Opens the Interpretation Filters dialog. Refer to the section: About Interpretation Filters for Small Molecules.

Peptides Interpretation View

Figure 8-2 Peptides Interpretation View



Item	Interface Component	Description
1	MS/MS pane	Shows the MS/MS spectrum of the selected metabolite. The mirror image of the reference MS/MS spectrum is also shown, if available. The source of this spectrum is the sample IDA wiff file, the sample SWATH acquisition wiff file, or a dedicated MS/MS wiff file that was added to the Results file. For a description of the functionality provided by the buttons, refer to the table: Table 8-4.
2	Sequence pane	Enables the user to input a sequence. For a description of the functionality provided by the buttons, refer to the table: Table 8-5.
3	Fragments table	Contains a list of proposed formulas for the selected potential metabolites. The list includes the *m/z* values, sequences, fragment ion types (for example, y or b), charge, errors, and intensities. For a description of the functionality provided by the icons, refer to the table: Table 8-6.
4	Sequence charts pane	Contains the following two tabs: • The Parent Sequence tab, which contains the sequence of the parent drug. • The Sequence Candidates tab, which is an interactive histogram that contains a table showing a list of sequences that are being proposed by the software. A score, based on the percentage peak area assigned, is assigned to each proposed sequence. To apply a specific sequence to the results, select the **Apply to Results** check box for that sequence. The applied sequence shows as the default sequence after the Results file is closed and then opened again. Refer to the section: About the Sequence Candidates Tab.

Table 8-4 MS/MS Pane Buttons

Button	Description
Deisotope	Removes all of the isotopes from the MS/MS spectrum.
Options	Opens the Options dialog. Refer to the table: Table 8-16 in the section: Set Options.

Table 8-4 MS/MS Pane Buttons (continued)

Button	Description
Generate	Populates the Structure Candidate tab with automatically generated potential candidates for the selected metabolite. Refer to the section: About the Structure Candidates Tab.
Apply	Applies the interpretation changes to the selected peak.
Remove	Removes the assigned fragments and metabolite structure from the selected peak.

Table 8-5 Sequence Pane Buttons

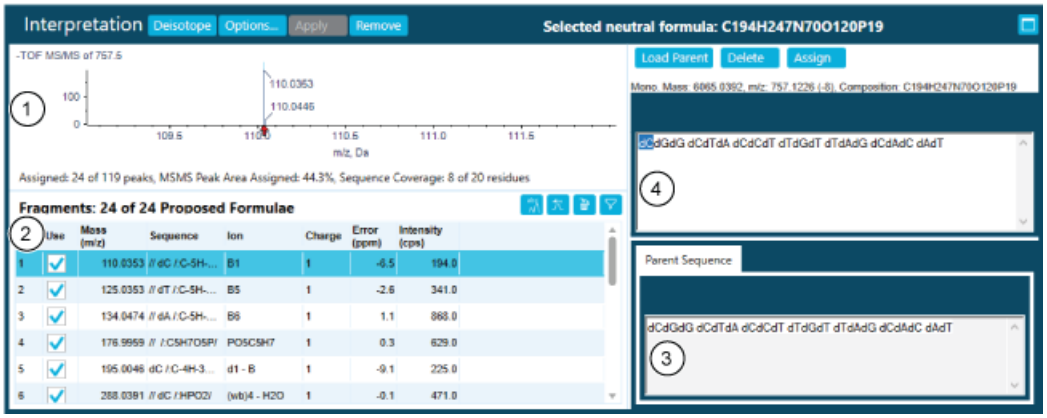
Button	Description
Enter Sequence	Enables the user to type a new sequence in the Sequence pane. Refer to the section: Peptide Sequence Naming Conventions.
Load Parent	Opens the parent sequence of the selected metabolite.
Delete	Removes the visible sequence from the Sequence pane.
Assign	Calculates the fragments and neutral losses for the potential structure and then assigns the ions to the MS/MS spectra.

Table 8-6 Fragments Table Icons

Icon	Description
	Adds labels for all of the peaks.
	Adds a label for the selected fragment.
	Deletes all labels from the MS/MS spectrum.
	Opens the Interpretation Filters dialog. Refer to the section: About Interpretation Filters for Peptides.

Oligonucleotides Interpretation View

Figure 8-3 Oligonucleotide Interpretation View



Item	Interface Component	Description
1	MS/MS pane	Shows the MS/MS spectrum of the selected metabolite. The mirror image of the reference MS/MS spectrum is also shown, if available. The source of this spectrum is the sample IDA wiff file, the sample SWATH acquisition wiff file, or a dedicated MS/MS wiff file that was added to the Results file. For a description of the functionality provided by the buttons, refer to the table: Table 8-7.
2	Fragments table	Contains a list of proposed formulas for the selected potential metabolites. The list includes the *m/z* values, sequences, fragment ion types (for example, y or b), charge, errors, and intensities. For a description of the functionality provided by the icons, refer to the table: Table 8-6.
3	Sequence charts pane	Contains the sequence of the parent drug.
4	Sequence pane	Enables the user to input a sequence. For a description of the functionality provided by the buttons, refer to the table: Table 8-5.

Table 8-7 MS/MS Pane Buttons

Button	Description
Deisotope	Removes all of the isotopes from the MS/MS spectrum.
Options	Opens the Options dialog. Refer to the table: Table 8-18 in the section: Set Options.
Apply	Applies the interpretation changes to the selected peak.
Remove	Removes the assigned fragments and metabolite structure from the selected peak.

Table 8-8 Sequence Pane Buttons

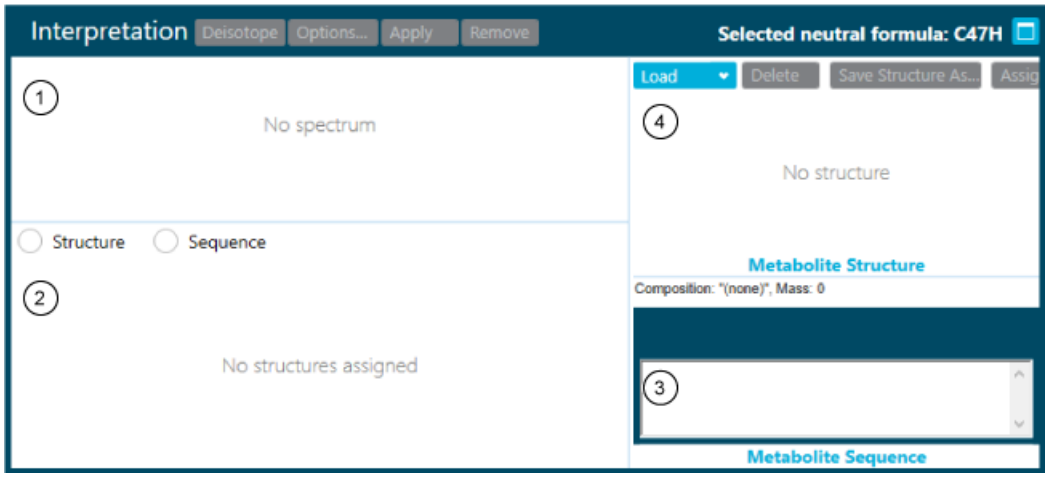
Button	Description
Load Parent	Opens the parent sequence of the selected metabolite.
Delete	Removes the visible sequence from the Sequence pane.
Assign	Calculates the fragments and neutral losses for the potential structure and then assigns the ions to the MS/MS spectra.

Table 8-9 Fragments Table Icons

Icon	Description
	Adds labels for all of the peaks.
	Adds a label for the selected fragment.
	Deletes all labels from the MS/MS spectrum.
	Opens the Interpretation Filters dialog. Refer to the section: About Interpretation Filters for Oligonucleotides.

ADC Interpretation View

Figure 8-4 ADC Interpretation View



Item	Interface Component	Description
1	MS/MS pane	Shows the MS/MS spectrum of the selected metabolite. The mirror image of the reference MS/MS spectrum is also shown, if available. The source of this spectrum is the sample IDA wiff file, the sample SWATH acquisition wiff file, or a dedicated MS/MS wiff file that was added to the Results file. For a description of the functionality provided by the buttons, refer to the table: Table 8-10.
2	Fragments table	Contains the following tabs: • The Structure tab, which lists all of the assigned fragments for the selected metabolite including their *m/z* value, the number of proposed structures, and the score. If a particular *m/z* value could be assigned different formulas, then the table contains a row for each formula. By default, the row containing the highest score for each combination of formulas and *m/z* value has the **Use** check box selected. • The Sequence tab, which lists all of the proposed formulas for the selected potential metabolites. The list includes the *m/z* values, sequences, fragment ion types (for example, y or b), charge, errors, and intensities. For a description of the functionality provided by the icons, refer to the table: Table 8-13.
3	Sequence pane	Shows the part of the sequence that is conjugated to the payload or linker moiety. To indicate the residue that is conjugated to the payload or linker moiety, select the residue, right-click, and then select **Mark Residue to Conjugate**.
4	Structure pane	Enables the user to load a candidate structure for potential metabolites and provides basic drawing tools that allow users to edit the structure. For a description of the functionality provided by the buttons, refer to the table: Table 8-11.

Table 8-10 MS/MS Pane Buttons

Button	Description
Deisotope	Removes all of the isotopes from the MS/MS spectrum.
Options	Opens the Options dialog. Refer to the table: Table 8-21 in the section: Set Options.
Apply	Applies the interpretation changes to the selected peak.
Remove	Removes the assigned fragments and metabolite structure from the selected peak.

Table 8-11 Structure Pane Buttons

Button	Description
Load	• **Load Parent Structure**: Opens the parent structure of the selected metabolite. • **Load Sequence**: Opens the sequence of the selected metabolite.
Delete	Removes the loaded structure from the Structure pane, the loaded sequence information from the Sequence pane, and the assigned structure and sequence information from the Fragments table.
Save Structure As	Enables the user to save the structure with a different file name.
Assign	Calculates the fragments and neutral losses for the potential structure and then assigns the ions to the MS/MS spectra.

Table 8-12 Fragments Table Buttons

Button	Description
Structure	Lists all of the assigned fragments for the selected metabolite including their *m/z* value, the number of proposed structures, and the score. If a particular *m/z* value could be assigned different formulas, then the table contains a row for each formula. By default, the row containing the highest score for each combination of formulas and *m/z* value has the **Use** check box selected.
Sequence	Lists all of the proposed formulas for the selected potential metabolites. The list includes the *m/z* values, sequences, fragment ion types (for example, y or b), charge, errors, and intensities.

Table 8-13 Fragments Table Icons

Icon	Description
	Adds labels for all of the peaks.
	Adds a label for the selected fragment.
	Deletes all labels from the MS/MS spectrum.
	Open the Interpretation Filters dialog. Refer to the section: About Interpretation Filters for ADC.

Manual Interpretation

Manual Interpretation

Small Molecule Workflow	Peptide Workflows	Oligonucleotide Workflows	ADC Workflow

Small Molecule Workflow

Load a Structure

Edit a Structure

Prepare for Structural Assignment → Edit the Name and Formula of a Potential Metabolite

Recalibrate the MS/MS Spectrum

Deisotope the MS/MS Spectrum

Select Active Peaks

Select Fragment Peaks for Assignment

Set Options

Assign Fragment Structures → Assign Proposed Formulae and Structures

Select a Formula Structure for Each Fragment

Attach Markush Structures

About Peak Labels → Add a Peak Label to the MS/MS Spectrum

About Interpretation Filters for Small Molecules

Load a Structure

Before starting structural elucidation of a metabolite, load the structure files that enable the software to determine potential fragment structures.

Note: If a structure is not loaded, then potential formulas can still be assigned to fragments.

1. In the Workspace panel, click **Results**.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select a Results file.
4. Click **OK**.
5. In the **Show** field, select **Interpretation**.
6. Select a row in the Potential Metabolites table.
7. In the Structure pane, click **Load** and then select the **Load Structure** option.

   The Open Structure File dialog opens.
8. Browse to and then select a structure file.

   Note: The software accepts structure files in either sdf or mol format.
9. If minor changes are needed, then edit the structure. Refer to the section: Edit a Structure.

Edit a Structure

After loading a structure for a specific metabolite, use the editing tools to make minor changes.

Tip! Use the editing tools to make minor changes to a structure, such as different attachment positions for a metabolic transformation. The editing tools should not be used to create new structures or make major changes to existing structures.

Table 8-14 Edit a Structure

To do this	Do this
Add an atom to a structure	Drag a specific symbol on the palette to the new position. The added atom forms a single bond with the closest existing atom.
Create new atoms on the palette	Click a blank square, type the symbol in the Specify Symbol dialog, and then click **OK**. **Tip!** Click the added square and type a new symbol to create different atoms.
Highlight a portion of the structure	Drag a circle around the required atoms and bonds.
Move one or more atoms	Drag a highlighted portion of the structure to the new position. If the portion is bonded to one other atom, then the bond moves to the new position. If the portion is bonded to two or more atoms, then the portion moves but the existing bonds remain the same.
Insert a structure into an existing structure	Right-click the structure and click one of the following: • **Insert .mol File** to add another structure. • **Insert Conjugate** to add a specific conjugate structure.
Delete one or more atoms	Right-click a highlighted portion of the structure and then click **Remove Selected Atoms**.
Create a bond	Select two non-bonded atoms, right-click the selection, click **New Bond**, and then select the type of bond.
Edit a bond	Right-click a bond, click **Set Bond Type**, and then select the type of bond.
Delete a bond	Right-click a bond and then click **Remove Bond**.
Change the charge state of an existing atom	Right-click the atom, click **Atom Charge State**, and then select the state.

Tip! To save the edited structure as a separate file, click **Save As**.

Tip! Structures can be saved as either mol or sdf files. Type the appropriate extension in the Save As dialog.

Prepare for Structural Assignment

There are four tasks that can be completed when a user is preparing for structural assignment:

- Edit the name or formula of the potential metabolite.
- Recalibrate the MS/MS spectrum.
- Select specific peaks in the MS/MS spectrum.
- Select fragment peaks for interpretation.

Note: If none of these tasks are required, then users can disregard these procedures and immediately assign fragment structures.

Edit the Name and Formula of a Potential Metabolite

Refer to the section: How metabolites are named by the software.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select the appropriate file.
4. Click **OK**.

   The Results view is shown.
5. Right-click a row in the Potential Metabolites table and select **Edit Name and Formula**.

   The Edit Name and Formula dialog opens.
6. Do one of the following to change the **Name**:
   - If applicable, select a name from the list of options provided.
   - Type a new name.
7. If applicable, select an adduct from the list of options provided.

   Note: If the adduct is changed, then the **Mass accuracy** of the metabolite is automatically updated.
8. Do one of the following to change the **Formula**:
   - If there is insufficent information available to determine a formula, then select **Unknown**.
   - To manually add a formula to the potential metabolite, select **Use** and then type a formula in the field provided.

- If potential formulas were predicted by the software, then select **Automatic** and select an entry from the list.

  Note: If potential formulas were not predicted by the software, then **Automatic** is not available for selection.

Note: The values in the **Mass accuracy** and **RDB** fields are automatically updated by the software when the new formula is added.

9. To identify the metabolite from the selected row as the parent compound, click **Assign as Parent**.
10. Click **OK**.
11. Click **Save**.

Note: For peptides, the order of the names is based on the mass accuracy of the proposed name and the number of manipulations required, for example, the number of bonds broken. That is, the proposed name for the peptide with a higher mass accuracy and fewer manipulations is shown at the top of the list.

Recalibrate the MS/MS Spectrum

1. In the Interpretation view, click **Prepare**.

   The Interpret Data dialog opens.
2. Click the MS/MS Details tab.
3. Select a fragment to use as a calibration point.
4. Right-click the selected fragment and then click **Set calibration points**.

   The color of the fragment circle changes to blue.
5. Repeat steps 3 and 4 to select additional calibration points.
6. To remove set calibration points, select the appropriate calibration points, right-click, and then select **Clear calibration points**.

   The color of the fragment circle reverts to green.
7. To view the details of a fragment, select a calibration point, right-click, and then select **Composition details**.

   The Fragment dialog opens, providing the *m/z* value, the mass error in both ppm and mDa, an indication of whether or not the proposed formula points to an even electron, and the RDB value (rings and double bonds) of the proposed formula.

8. To select a calibration point as the composition of the fragment or as a potential calibration point, select a calibration point, right-click, and then select **Select composition**.
9. Right-click the MS/MS spectrum and then click **Recalibrate**.

Note: To discard the recalibrated spectrum, right-click the spectrum and then click **Revert Calibration**.

Deisotope the MS/MS Spectrum

In the Interpretation view, when Deisotope is clicked, all of the isotopes are removed from the MS/MS spectrum. This provides a quick view of the monoisotopic peaks, which is useful when viewing SWATH acquisition data.

Note: Only the monoisotopes are shown in the Results Table regardless of whether or not this option is selected.

Select Active Peaks

Active peaks are the only peaks in the MS/MS spectrum that are available for fragment interpretation.

1. In the Interpretation view, click **Prepare**.

   The Interpret Data dialog opens.
2. Review the MS/MS spectrum.

   Blue arrows identify the current active peaks.
3. To select a peak, drag a square across the peak.
4. Double-click the selected peak.

   A blue arrow is shown below the selected peak.
5. To remove single peaks, drag the blue arrow below the border of the Interpret Data dialog.

   The blue arrow is removed from below the selected peak.

   Tip! To clear all active peaks, right-click the spectrum and then click **Clear All Markers**.
6. After selecting all active peaks, click **Find**.
7. Select the row with the formula that best matches the MS and MS/MS spectra.
8. Click **Select**.

Select Fragment Peaks for Assignment

Although several peaks might be identified as active, users can select to work with only those peaks that have the highest intensities.

1. In the Interpretation view, click **Options**.

   The Options dialog opens.

2. In the **Number of fragment peaks selected for assignment** field, type the appropriate number.

3. Click **OK**.

   Asterisks in the MS/MS spectrum identify the peaks selected for assignment.

Set Options

1. In the Interpretation view, click **Options**.

   The Options dialog opens.

2. Modify the fragmentation and labeling parameters as described in the table: Table 8-15.

Table 8-15 Options Dialog

Option	Description
Number of fragment peaks selected for assignment	Use this field to specify the number of fragment peaks that will be assigned. This number can be a subset of the total number of peaks that have been selected in the Prepare dialog. If it is a subset of the total number of peaks, then peaks are chosen in order of intensity.
Minimum signal-to-noise ratio	Use this field to specify the threshold used to assign fragment peaks. Peaks below this threshold will not be assigned. Noise is defined as the peak with the smallest intensity in the MS/MS spectrum.
MS/MS m/z tolerance (ppm or mDa)	For a fragment peak to be assigned a formula and potentially a structure, its mass accuracy must fall within the MS/MS *m/z* tolerance specified.
Fragmentation Settings	
Break aromatic rings	Select this check box to break the aromatic ring.

Table 8-15 Options Dialog (continued)

Option	Description
Maximum number of bonds to break	Use this field to specify the maximum number of bonds to break. Options include: • **1** • **2** • **3** • **4**
Maximum number of C-C bonds to break	Use this field to specify the maximum number of C-C bonds to break. Options include: • **0** • **1** • **2** • **3** • **4**
Label Settings	
Label peaks with	Use this field to specify the information that should be shown in the peak labels. Options include: • **Ion** • **Ion with ppm Error** • **Ion with mDa Error**
Apply options to all potential metabolites	Select this check box to apply the current options to all unassigned metabolites.

Assign Fragment Structures

To assign structures, the software links the fragment peaks in the MS/MS spectrum to potential portions of the candidate structure. Users can then select a formula and structure that best matches the *m/z* value of each fragment. After assignment takes place, the asterisks that identified peaks selected for assignment are replaced with either a check mark to show that assignment took place or an x to show that no assignment was possible.

Note: The fragmentation rules are embedded in the software and cannot be edited.

Assign Proposed Formulae and Structures

Each metabolite must have an MS/MS spectrum before fragment structures can be assigned. To add a spectrum, refer to the section: Add Multiple Spectra with the **Add MS/MS** Button.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. In the **Show** field, select **Interpretation**.

6. Load and edit a candidate structure. Refer to the sections: Load a Structure and Edit a Structure.

7. If required, prepare for structural assignment. Refer to the section: Prepare for Structural Assignment.

8. In the Structure pane of the Interpretation view, click **Assign**.

   Three tables are shown below the MS/MS pane: the Fragments table, showing the identified fragments, the Structure Details table, showing potential structures, and the Contained Neutral Losses table, showing the contained neutral losses.

   Note: If a structure is not loaded, then the software assigns only potential formulas to fragments.

Select a Formula Structure for Each Fragment

1. If appropriate, in the Interpretation view, right-click each of the Fragments, Structure Details, and Contained Neutral Losses tables and then click **Show Hidden Rows**.

   Note: In the Fragments table, the row containing the highest score for the *m/z* value has the **Use** check box selected. In the Structure Details table, the row with the highest score has the **Use** check box selected. In the Contained Neutral Losses table, all rows have the **Use** check box selected.

2. In the Fragments table, select the **Use** check box to identify the row that contains the most accurate formula for each *m/z* value.

   Tip! In the Fragments table, select the **Use** check box in more than one row to select multiple potential formulas for each fragment.

3. In the Structure Details table, select the **Use** check box to identify the portions of the structure that most accurately match the selected formula.
4. In the Contained Neutral Losses table, select the **Use** check box to identify the row that most accurately reflects the contained neutral losses.

   Tip! In the Structure Details and Contained Neutral Losses tables, select the **Use** check box in more than one row for a particular fragment.

5. Click **Apply**.

   The interpretation data is saved for the selected metabolite.
6. When all changes are made, click **Save**.

   Tip! To delete all interpretation data for a specific metabolite, click **Remove**.

About the Structure Candidates Tab

When automatic structure generation is used, the Structure Candidates tab in the Structure charts pane is populated with a list of structures for the selected metabolite that satisfy the conditions set in the Options dialog. Refer to the section: Batch Processing Options. The software generates structures for the following types of metabolites:

- Metabolites with one or two cleavages
- Single biotransformation metabolites
- Metabolites with one cleavage and single biotransformation

In the case of more complex metabolism, the user can provide or edit a custom metabolite structure and assess such structure proposal.

The list of structures (referred to as the histogram) contains the following columns of information:

Column	Description
Rank	Indicates the position or ranking of the structures.
Relative Evidence	The ranking or scoring is based on a comparison between the MS/MS spectrum of the parent structure and the MS/MS spectrum of the metabolites. Metabolite fragments are then compared against those of the parent to identify shifted and non-shifted fragments. Other attributes, such as the fragment intensity and the uniqueness of a proposal, are also considered in the overall ranking strategy. The final ranking indicates the probability that a biotransformation or a cleavage will take place at a particular atom index. This column also enables the user to toggle between structures. Refer to the section: Toggle between Structures.
Apply to Results	A selected check box indicates that the corresponding structure will be saved for the Results file.

The total number of candidates is shown above the histogram table, directly above the **Apply to Results** column.

Auto-generated structures cannot be edited. Users can load a structure, make any necessary modifications, and then select the **Apply to Results** check box to include the structure in the Results file. Refer to steps 7 and 8 of the sections: Load a Structure and Edit a Structure.

Toggle between Structures

- Click a blue bar in the histogram.

  The corresponding structure is shown in the Structure pane.

Select an Empty Pane

- Click the first line in the histogram.

  The first line in the histogram contains the words No structure. The Structure pane refreshes, with the phrase No structure shown.

Add a Structure

Note: Only one structure can be added to the list of auto-generated structures. If an additional structure is added, then the previous user-added structure is overwritten.

1. In the Structure pane, click **Load** and then select the **Load Structure** option.

   The Open Structure File dialog opens.

2. Browse to and then select a mol or sdf file.

3. Click **Open**.

   The selected structure is shown in the Structure pane and a line is added to the histogram table, immediately above the first auto-generated structure. The shade of blue for the loaded structure row is slightly different than the blue for the lines containing the auto-generated structures. The ranking is set a 0.

   The user-added structure can be edited. Any changes made to the structure will be held in memory when the user tabs out of the Structure pane.

Select a Structure to View

1. Click a blue bar in the histogram.

   The corresponding structure is shown in the Structure pane. By default only the first structure in the histogram has the Fragments table assigned.

2. To assign the Fragments table for a different structure, click the blue bar in the histogram and then click **Assign**.

Delete a Structure

1. Click a blue bar in the histogram.

   The corresponding structure is shown in the Structure pane.

2. In the Structure pane, click **Delete**.

   The structure is removed from the Structure pane, the selected blue line is removed from the histogram, and the Fragments table is removed. The structure of the next line in the histogram is shown in the Structure pane.

Attach Markush Structures

After assigning fragment structures, use Markush structures to show approximate positions for chemical modifications.

Note: Fragment structures cannot be assigned to a metabolite that contains a Markush structure.

1. Highlight a portion of the structure.
2. Right-click above or below the structure and then click **Attach Markush**.
3. Select either **Single Bond** or **Double Bond**.
4. In the Select Symbol dialog, type the required symbol or formula.
5. Click **OK**.

   The Markush structure is shown with a dashed line connecting it to the selected portion of the structure.

Note: Changes to the structure can be made after assigning interpretation data, if a Markush structure is attached. If the Markush structure is removed, then any changes will delete all interpretation data for the metabolite.

About Peak Labels

A peak can be labeled with:

- an ion formula or ion type (for a peptide)
- an ion formula or ion type (for a peptide) and ppm error
- an ion formula or ion type (for a peptide) and mDa error

Add a Peak Label to the MS/MS Spectrum

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. In the **Label peaks with** field, select the label type.
3. Click **OK**.
4. In the Fragments table, select the row containing the peak to be labeled.
5. Click  .

Tip! To remove all labels from the MS/MS spectrum, click  .

About Interpretation Filters for Small Molecules

Apply filters to refine the data shown in the Fragments table. To access the Interpretation Filters dialog, click the  icon in the Fragments table.

Filter	Description
Rings and Double Bonds	
RDB	• **Integer value (even-electron)**: Shows only fragments that have an integer value for rings and double bonds. • **Non-integer value (odd-electron)**: Shows only fragments that have a non-integer value for rings and double bonds.
Mass Range	

Filter	Description
m/z from __ to __	Shows only fragments with an *m/z* value that is within the specified range.
Mass Accuracy	
Accuracy within	Shows only fragments with a mass accuracy that is within the specified range. **Note:** Whether the mass accuracy is measured in mDa or ppm depends on the selection in the Options dialog.
Intensity	
Intensity above __ cps	Shows only fragments with an intensity value that is above the specified value.
Score	
Score above	Shows only fragments with a score that is above the specified value.
Structures	
Fragments with assigned structures	Shows only fragments that are associated with structures.

Peptide Workflows

Load a Sequence

Edit a Sequence

Set Options

Assign Fragment Sequences

About Peak Labels → Add a Peak Label to the MS/MS Spectrum

About Interpretation Filters for Peptides

Load a Sequence

1. In the Workspace panel, click **Results**.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select a Results file.

4. Click **OK**.
5. In the **Show** field, select **Interpretation**.
6. Select a row in the Potential Metabolites table.
7. Do one of the following:
   - If the Sequence pane is blank, then click **Load Parent**.
   - If a sequence already exists in the Sequence pane, and a new sequence needs to be added, then click **Enter Sequence** to clear the pane and then click **Load Parent**.

   The parent sequence is shown in the Sequence pane. The following label is added above the pane: **AA Index: [ ], Mono. Mass: [ ], m/z: [ ], Composition: [ ]**, where:

   - **AA Index**: (Amino Acid Index) The amino acid indices indicate the position of the first and last residue of the sequence in the parent sequence. If the catabolite sequence is not a subset of the parent sequence, then the AA Index is not shown.
   - **Mono. Mass**: The monoisotopic mass of the neutral component.
   - **m/z**: The mass-to-charge value. The charge is shown in brackets.
   - **Composition**: The uncharged element composition of the sequence.
8. If changes are required, then edit the sequence. Refer to the section: Edit a Sequence.

Edit a Sequence

After a sequence for a specific metabolite is created or loaded, it can be edited.

1. Click in the sequence where the changes are required.
2. Make the required changes. Refer to the section: Peptide Sequence Naming Conventions.

Set Options

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. Modify the fragmentation and labeling parameters. Refer to the table: Table 8-16.

Table 8-16 Options Dialog

Option	Description
Minimum signal-to-noise ratio	Use this field to specify the threshold used to assign fragment peaks. Peaks below this threshold will not be assigned. Noise is defined as the peak with the smallest intensity in the MS/MS spectrum.
MS/MS m/z tolerance (ppm or mDa)	Use this field to specify the MS/MS *m/z* tolerance. For a fragment peak to be assigned an ion type and a sequence, the mass accuracy must fall within the MS/MS *m/z* tolerance specified.
Fragmentation Settings	
Fragment Types	Select the appropriate fragment type. Multiple types can be selected. Options include: • **a** • **b** • **y**
Maximum number of bonds to break	Use this field to specify the maximum number of bonds to break. Options include: • **1** • **2** • **3** **Tip!** For more complex peptides, selecting 3 as the maximum number of bonds to break results in an increase in the amount of processing time required.
Break linkages	If there are linkages in the peptide sequence, then select this check box to allow the linkages between individual amino acids to be broken.
Label Settings	

Table 8-16 Options Dialog (continued)

Option	Description
Label peaks with	Use this field to specify the information that should be shown in the peak labels. Options include: • **Ion** • **Ion with ppm Error** • **Ion with mDa Error** • **Ion with Charge**
Apply options to all potential metabolites	Select this check box to apply the current options to all unassigned metabolites.

Assign Fragment Sequences

Note: The fragmentation rules are embedded in the software and cannot be edited.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. In the **Show** field, select **Interpretation**.

6. Load a sequence. Refer to the section: Load a Sequence.

7. In the Sequence pane, click **Assign**.

   The Fragments table is populated with the interpretation results for the loaded sequence, using the options selected. Refer to the section: Set Options. Green vertical lines, identifying the ions that are matched in the Fragments table, are added to the MS/MS pane. The label above the table is updated to indicate:

   - **Assigned: x of y peaks**. Indicates the number of peaks that have been assigned.
   - **MSMS Peak Area Assigned: x%**. Indicates the percentage of the MS/MS peak area that has been assigned.

- **Sequence Coverage: x of y consecutive amino acids**. Indicates the number of consecutive amino acids that are covered by the sequence.

About the Sequence Candidates Tab

When automatic sequence generation is used, the Sequence Candidates tab in the Sequence charts pane is populated with a list of sequences for the selected catabolite or metabolite that satisfy the conditions set in the Options dialog. Refer to the section: Batch Processing Options. The software generates sequences for the following types of metabolites or catabolites:

- n cleavages: up to four modifications on the cleavages
- parent: where n refers to any number of cleavages

The list of sequences (referred to as the histogram) contains the following columns of information:

Column	Description
Rank	Indicates the relative ranking of all the isomer sequences found for the specified metabolite. The rank is based on the MS/MS peak area assigned.
View sequence fragments	The percentage values indicate the scoring of the proposed sequence. This column also enables the user to toggle between sequences. Refer to the section: Toggle Between Sequences.
AA Index	Indicates the amino acid start and end of the sequence.
Apply to Results	A selected check box indicates that the corresponding sequence will be saved for the Results file.

The total number of candidates is shown above the histogram table, directly above the **Apply to Results** column.

Auto-generated sequences cannot be edited. Users can load a sequence, make any necessary modifications, and then select the **Apply to Results** check box to include the sequences in the Results file. Refer to step 7 of the sections: Load a Sequence and Edit a Sequence.

About Peak Labels

A peak can be labeled with:

- An ion formula or ion type (for a peptide)
- An ion formula or ion type (for a peptide) and ppm error
- An ion formula or ion type (for a peptide) and mDa error
- An ion formula or ion type (for a peptide) with charge

Add a Peak Label to the MS/MS Spectrum

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. In the **Label peaks with** field, select the label type.
3. Click **OK**.
4. Do one of the following:

Table 8-17 Add Peak Labels

To label one peak	To label all peaks
In the Fragments table, select the row containing the peak to be labeled.	Click 
Click  .	—

Tip! To remove all labels from the MS/MS spectrum, click  .

About Interpretation Filters for Peptides

Apply filters to refine the data shown in the Fragments table. To access the Interpretation Filters dialog, click the  icon in the Fragments table.

Filter	Description
Mass Range	
m/z from __ to __	Shows only fragments with an *m/z* value that is within the specified range.
Charge Range	
Charge from __ to __	Shows only fragments with a charge that is within the selected range. Options include: • **From range**: 1 to 10, inclusive • **To range**: 1 to 10, inclusive **Note:** The to range value must be greater than or equal to the from range value.

Filter	Description
Ion Type	
Fragment type	Select the appropriate fragment type. Multiple types can be selected. These options are available: • **a** • **b** • **y**
Mass Accuracy	
Accuracy within	Shows only fragments with a mass accuracy that is within the specified range. **Note:** Whether the mass accuracy is measured in mDa or ppm depends on the selection in the Options dialog.
Intensity	
Intensity above __ cps	Shows only fragments with an intensity value that is above the specified value.

Oligonucleotide Workflows

Load a Sequence

Edit a Sequence

Set Options

Assign Fragment Sequences

About Peak Labels → Add a Peak Label to the MS/MS Spectrum

About Interpretation Filters for Oligonucleotides

Load a Sequence

1. In the Workspace panel, click **Results**.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select a Results file.

4. Click **OK**.
5. In the **Show** field, select **Interpretation**.
6. Select a row in the Potential Metabolites table.
7. If the Sequence pane is blank, then do one of the following:
   - Click **Load Parent**.
   - Type or paste a sequence in the pane.

   The following label is added above the pane: **Mono. Mass: [ ], m/z: [ ], Composition: [ ]**, where:

   - **Mono. Mass**: The monoisotopic mass of the neutral component.
   - **m/z**: The mass-to-charge value. The charge is shown in brackets.
   - **Composition**: The uncharged element composition of the sequence.
8. If changes are required, then edit the sequence. Refer to the section: Edit a Sequence.

Edit a Sequence

After a sequence for a specific metabolite is created or loaded, it can be edited.

1. Click in the sequence where the changes are required.
2. Make the required changes. Refer to the section: Oligonucleotide Sequence Naming Conventions.

Set Options

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. Modify the fragmentation and labeling parameters. Refer to the table: Table 8-18.

Table 8-18 Options Dialog

Option	Description
Minimum signal-to-noise ratio	Use this field to specify the threshold used to assign fragment peaks. Peaks below this threshold are not assigned. Noise is defined as the peak with the smallest intensity in the MS/MS spectrum.
MS/MS m/z tolerance (ppm or mDa)	Use this field to specify the MS/MS *m/z* tolerance. For a fragment peak to be assigned an ion type and a sequence, the mass accuracy must fall within the MS/MS *m/z* tolerance specified.

Table 8-18 Options Dialog (continued)

Option	Description
Fragmentation Settings	
Fragment Types	Select the appropriate fragment type. Multiple types can be selected. Options include: • **a** • **b** • **c** • **d** • **y** • **Other** • **wb-H20** • **x** • **y** Refer to the section: Example Custom Oligonucleotide.
Maximum number of bonds to break	Use this field to specify the maximum number of bonds to break. Options include: • **1** • **2** A value of 2 is recommended. **Tip!** For more complex oligonucleotides, selecting 3 as the maximum number of bonds to break results in an increase in the amount of processing time required.
Maximum water and Base losses	Specifies the maximum water losses that can occur during fragmentation. A value of 1 is recommended.
Label Settings	

Table 8-18 Options Dialog (continued)

Option	Description
Label peaks with	Use this field to specify the information that should be shown in the peak labels. Options include: • **Ion** • **Ion with ppm Error** • **Ion with mDa Error** • **Ion with Charge**
Apply options to all potential metabolites	Select this check box to apply the current options to all unassigned metabolites.

Assign Fragment Sequences

Note: The fragmentation rules are embedded in the software and cannot be edited.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. In the **Show** field, select **Interpretation**.

6. Load a sequence. Refer to the section: Load a Sequence.

7. In the Sequence pane, click **Assign**.

   The Fragments table is populated with the interpretation results for the loaded sequence, using the options selected. Refer to the section: Set Options. Cyan vertical lines, identifying the ions that are matched in the Fragments table, are added to the MS/MS pane. The label above the table is updated to indicate:

   - **Assigned: x of y peaks**. Indicates the number of peaks that have been assigned.
   - **MSMS Peak Area Assigned: x%**. Indicates the percentage of the MS/MS peak area that has been assigned.

- **Sequence Coverage: x of y consecutive nucleotides**. Indicates the number of consecutive nucleotides that are covered by the sequence.

About Peak Labels

A peak can be labeled with:

- An ion formula or ion type (for a oligonucleotide)
- An ion formula or ion type (for a oligonucleotide) and ppm error
- An ion formula or ion type (for a oligonucleotide) and mDa error
- An ion formula or ion type (for a oligonucleotide) with charge

Add a Peak Label to the MS/MS Spectrum

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. In the **Label peaks with** field, select the label type.
3. Click **OK**.
4. Do one of the following:

Table 8-19 Add Peak Labels

To label one peak	To label all peaks
In the Fragments table, select the row containing the peak to be labeled.	Click 
Click  .	—

Tip! To remove all labels from the MS/MS spectrum, click  .

About Interpretation Filters for Oligonucleotides

Apply filters to refine the data shown in the Fragments table. To access the Interpretation Filters dialog, click the  icon in the Fragments table.

Filter	Description
Mass Range	
m/z from __ to __	Shows only fragments with an *m/z* value that is within the specified range.
Charge Range	
Charge from __ to __	Shows only fragments with a charge that is above the selected value. Valid values are from 1 to 10.
Ion Type	
Fragment type	Select the appropriate fragment type. Multiple types can be selected. These options are available: • **a** • **b** • **c** • **d** • **w** • **wb-H20** • **x** • **y** • **Other** • **Base loss** • **Water loss** • **Internals**
Mass Accuracy	
Accuracy within	Shows only fragments with a mass accuracy that is within the specified range. **Note:** Whether the mass accuracy is measured in mDa or ppm depends on the selection in the Options dialog.
Intensity	
Intensity above __ cps	Shows only fragments with an intensity value that is above the specified value.

ADC Workflow

Load a Structure

Edit a Structure

Load a Sequence

Edit a Sequence

Set Options

Assign Fragment Ions for Both Structure and Sequence

About Peak Labels → Add a Peak Label to the MS/MS Spectrum

About Interpretation Filters for ADC

Load a Structure

Before starting structural elucidation of a metabolite, loading a structure enables the software to determine potential fragment structures.

Note: If a structure is not loaded, then potential formulas can still be assigned to fragments.

1. In the Workspace panel, click **Results**.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select a Results file.
4. Click **OK**.
5. In the **Show** field, select **Interpretation**.
6. Select a row In the Potential Metabolites table.
7. In the Structure pane, click **Load** and then select the **Load Parent Structure** option.

   The pane is populated with the parent structure of the selected metabolite. The marked site of the attachment (as seen in the embedded processing parameters file) or the anchor atoms are shown in purple.
8. If minor changes are needed, then edit the structure. Refer to the section: Edit a Structure.

Edit a Structure

After loading a structure for a specific metabolite, use the editing tools to make minor changes.

Tip! Use the editing tools to make minor changes to a structure, such as different attachment positions for a metabolic transformation. The editing tools should not be used to create new structures or make major changes to existing structures.

Table 8-20 Edit a Structure

To do this	Do this
Add an atom to a structure	Drag a specific symbol on the palette to the new position. The added atom forms a single bond with the closest existing atom.
Create new atoms on the palette	Click a blank square, type the symbol in the Specify Symbol dialog, and then click **OK**. **Tip!** Click the added square and type a new symbol to create different atoms.
Highlight a portion of the structure	Drag a circle around the required atoms and bonds.
Move one or more atoms	Drag a highlighted portion of the structure to the new position. If the portion is bonded to one other atom, then the bond moves to the new position. If the portion is bonded to two or more atoms, then the portion moves but the existing bonds remain the same.
Insert a structure into an existing structure	Right-click the structure and click one of the following: • **Insert .mol File** to add another structure. • **Insert Conjugate** to add a specific conjugate structure.
Delete one or more atoms	Right-click a highlighted portion of the structure and then click **Remove Selected Atoms**.
Create a bond	Select two non-bonded atoms, right-click the selection, click **New Bond**, and then select the type of bond.
Edit a bond	Right-click a bond, click **Set Bond Type**, and then select the type of bond.
Delete a bond	Right-click a bond and then click **Remove Bond**.
Change the charge state of an existing atom	Right-click the atom, click **Atom Charge State**, and then select the state.

Tip! To save the edited structure as a separate file, click **Save As**.

Tip! Structures can be saved as either mol or sdf files. Type the appropriate extension in the Save As dialog.

Load a Sequence

1. In the Workspace panel, click **Results**.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select a peptide Results file.
4. Click **OK**.
5. In the **Show** field, select **Interpretation**.
6. Select a row in the Potential Metabolites table.
7. In the Structure pane, click **Load** and then select the **Load Sequence** option.

   The Sequence pane is populated with the parent sequence of the selected metabolite.
8. Select the residue to be conjugated on, right-click and then select **Mark Residue to Conjugate**.

   The selected residue is shown in purple.
9. If changes are required, then edit the sequence. Refer to the section: Edit a Sequence.

Edit a Sequence

After a sequence for a specific metabolite is created or loaded, it can be edited.

1. Click in the sequence where the changes are required.
2. Make the required changes. Refer to the section: Peptide Sequence Naming Conventions.

Set Options

1. In the Interpretation view, click **Options**.

   The Options dialog opens.
2. Modify the fragmentation and labeling parameters as described in the table: Table 8-21.

Table 8-21 Options Dialog

Option	Description
Number of fragment peaks selected for structure assignment	Use this field to specify the number of fragment peaks that will be assigned for the structure portion of the ADC. If it is a subset of the total number of peaks, then peaks are chosen in order of their intensities.
Minimum signal-to-noise ratio	Use this field to specify the threshold used to assign fragment peaks. Peaks below this threshold will not be assigned.
MS/MS m/z tolerance (ppm or mDa)	Use this field to specify the MS/MS *m/z* tolerance, in ppm or mDa. For a fragment peak to be assigned a formula and potentially a structure, its mass accuracy must fall within the MS/MS *m/z* tolerance specified.
Structure Fragmentation Settings	
Break aromatic rings	Select this check box to break the aromatic ring.
Maximum number of bonds to break	Use this field to specify the maximum number of bonds to break. Options include: • **1** • **2** • **3** • **4**
Maximum number of C-C bonds to break	Use this field to specify the maximum number of C-C bonds to break. Options include: • **0** • **1** • **2** • **3** • **4**
Sequence Fragmentation Settings	

Table 8-21 Options Dialog (continued)

Option	Description
Fragment Types	Select the appropriate fragment type. Multiple types can be selected. Options include: • **a** • **b** • **y**
Maximum number of bonds to break	Use this field to specify the maximum number of bonds to break. Options include: • **1** • **2** • **3** **Note:** For more complex peptides, selecting 3 as the maximum number of bonds to break results in an increase in the amount of processing time required.
Break linkages	If there are linkages in the peptide sequence, then select this check box to allow the linkages between individual amino acids to be broken.
Label Settings	
Label peaks with	Use this field to specify the information that should be shown in the peak labels. Options include: • **Ion** • **Ion with ppm Error** • **Ion with mDa Error** • **Ion with Charge**
Apply options to all potential metabolites	Select this check box to apply the current options to all unassigned metabolites.

Assign Fragment Ions for Both Structure and Sequence

Note: The fragmentation rules are embedded in the software and cannot be edited.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

4. Click **OK**.

   The Results view is shown.

5. In the **Show** field, select **Interpretation**.

6. Load a structure and a sequence. Refer to the section: Load a Structure and Load a Sequence.

   Note: Only one of a structure or a sequence must be loaded. This procedure is written based on the assumption that both have been loaded.

7. In the Structure pane, click **Assign**.

   Both the Structure view and the Sequence view under the TOF MS/MS spectra are populated, with the Structure view shown, by default.

   Note: If only the parent structure was loaded, then the Structures view of the Fragments table is shown. If only the parent sequence was loaded, then the Sequences view of the Fragments table is shown.

   In the Structure view, the Fragments table is populated with the identified fragments, the Structure Details table is populated with the potential structures, and the Contained Neutral Losses table is populated with the contained neutral losses. The results are based on the options selected. Refer to the section: Set Options. Light blue vertical lines, identifying the ions that are matched in the Fragments table, are added to the MS/MS pane.

   Note: If the structure has no interpretation results, then No structures assigned is shown in the Fragments table.

   The label above the Fragments table indicates:

- **Assigned:** a of b peaks (Structure: x, Sequence: y), where a is the sum of x and y and indicates the number of peaks that have been assigned, b indicates the total number of peaks, x indicates the number of rows in the Structures view and y indicates the number of rows in the Sequences view.
- **MSMS Peak Area Assigned: w%**, where w indicates the percentage area of the assigned peaks from the spectral data.

The Fragments table contains a **Use as Conjugate** column. This column contains a check for each row in the table. If the check box is available for selection, then a site of attachment is present on the proposed structure for the fragment. If the check box is not available for selection, then a site of attachment is not present. If the check box is selected, then the fragment is used for conjugation with the sequence. If the check box is not selected, then the fragment is not used. By default, a maximum of three fragments that contain a site of attachment are selected, based on accuracy and abundance. The first row in the table is selected by default.

8. Make sure that the Structures view is selected.
9. If appropriate, right-click each of the Fragments, Structure Details, and Contained Neutral Losses tables and then click **Show Hidden Rows**.

Note: In the Fragments table, the row containing the highest score for the *m/z* value has the **Use** check box selected. In the Structure Details table, the row with the highest score has the **Use** check box selected. In the Contained Neutral Losses table, all rows have the **Use** check box selected.

10. In the Fragments table, select the **Use** check box to identify the row that contains the most accurate formula for each *m/z* value.

Tip! Select the **Use** check box in more than one row to select multiple potential formulas for each fragment.

The assigned *m/z* value is shown in bold, italicized font.

11. In the Structure Details table, select the **Use** check box to identify the portions of the structure that most accurately match the selected formula.
12. In the Contained Neutral Losses table, select the **Use** check box to identify the row that most accurately reflects the contained neutral losses.

Tip! In the Structure Details and Contained Neutral Losses tables, select the **Use** check box in more than one row for a particular fragment.

13. Select the Sequences view.

In the Sequence view, the Fragments table is populated with the results of the interpretation, based on the options selected (refer to the section: Set Options), the conjugates selected in the Structures view, the selections made on the Product Ions and Neutral Losses tab of the Compound-Specific Parameters (refer to the section: Product Ions and Neutral Losses Tab), and the sequence. Green vertical lines, identifying the ions that are matched in the Fragments table, are added to the MS/MS pane.

Note: If the sequence has no interpretation results, then No sequences assigned is shown in the Fragments table.

The label above the table indicates:

- Assigned: a of b peaks (Structure: x, Sequence: y), where a is the sum of x and y and indicates the number of peaks that have been assigned, b indicates the total number of peaks, x indicates the number of rows in the **Structures** tab and y indicates the number of rows in the **Sequences** tab.
- **MSMS Peak Area Assigned: w%**, where w indicates the percentage area of the assigned peaks from the spectral data.

14. If appropriate, right-click the Fragments table and then click **Show Hidden Rows**.

Note: In the Fragments table, the row containing the highest score for the *m/z* value has the **Use** check box selected.

15. In the Fragments table, select the **Use** check box to identify the row that contains the most accurate formula for each *m/z* value.

Tip! Select the **Use** check box in more than one row to select multiple potential formulas for each fragment.

The assigned *m/z* value is shown in bold, italicized font.

16. After all changes have been made, click **Apply**.

The interpretation data is saved for the selected metabolite.

17. Click **Save**.

Tip! To delete all interpretation data for a specific metabolite, click **Remove**.

About Peak Labels

A peak can be labeled with:

- An ion formula or ion type (for a peptide)

- An ion formula or ion type (for a peptide) and ppm error
- An ion formula or ion type (for a peptide) and mDa error
- An ion formula or ion type (for a peptide) with charge

Add a Peak Label to the MS/MS Spectrum

1. In the Interpretation view, click **Options**.
   The Options dialog opens.
2. In the **Label peaks with** field, select the label type.
3. Click **OK**.
4. Do one of the following:

Table 8-22 Add Peak Labels

To label one peak	To label all peaks
In the Fragments table, select the row containing the peak to be labeled.	Click 
Click  .	—

Tip! To remove all labels from the MS/MS spectrum, click  .

About Interpretation Filters for ADC

Apply filters to refine the data shown in the Fragments table. To access the Interpretation Filters dialog, click the  icon in the Fragments table.

Filter	Description
Rings and Double Bonds	
RDB	• **Integer value (even-electron)**: Shows only fragments that have an integer value for rings and double bonds. • **Non-integer value (odd-electron)**: hows only fragments that have a non-integer value for rings and double bonds.
Mass Range	
m/z from __ to __	Shows only fragments with an *m/z* value that is within the specified range.

Filter	Description
Charge Range	
Charge from __ to __	Shows only fragments with a charge that is within the selected range. Options include: • **From range**: 1 to 10, inclusive • **To range**: 1 to 10, inclusive **Note:** The to range value must be greater than or equal to the from range value.
Ion Type	
Fragment type	Select the appropriate fragment type. Multiple types can be selected. Options include: • **a** • **b** • **y**
Mass Accuracy	
Accuracy within	Shows only fragments with a mass accuracy that is within the specified range. **Note:** Whether the mass accuracy is measured in mDa or ppm depends on the selection in the Options dialog.
Intensity	
Intensity above __ cps	Shows only fragments with an intensity value that is above the specified value.
Score	
Score above	Shows only fragments with a score that is above the specified value.
Structures	
Fragments with assigned structures	Shows only fragments that are associated with structures.

Automatic Interpretation

Automatic Interpretation

Small Molecule Workflow

Peptide Workflows

Small Molecule Workflow

Automatic generation of structures can be started in the following ways:

- In the Batch workspace, select the **Apply Options** check box to apply all of the Auto Assign options chosen in the Batch Processing Options dialog to the samples and control samples in the batch. At a minimum, the Assign Structures or Sequences option must be selected. Refer to the section: Batch Processing Options.
- In the Interpretation view of the Results workspace, click **Generate** in the MS/MS pane.

Peptide Workflows

Automatic generation of sequences can be started in the following ways:

- In the Batch workspace, select the **Options** check box to apply all of the **Auto Assign** options chosen in the Batch Processing Options dialog to the samples and control samples in the batch. At a minimum, the **Assign Structures or Sequences** option must be selected. Refer to the section: Batch Processing Options.
- In the Interpretation view of the Results workspace, click **Generate** in the MS/MS pane.

Toggle Between Sequences

- Click a blue bar in the histogram.

  The corresponding sequence is shown in the Sequence pane and the Fragments table is updated with the information related to the selected sequence. The label above the Sequence pane is updated to indicate the sequence number and the assigned rank. For example, Sequence x of y, rank = z.

Select an Empty Pane

- Click the first line in the histogram.

The first line in the histogram contains the words No sequence. The Sequence pane is cleared and the Fragments table refreshes, with the words No sequences assigned shown.

Add a Sequence

Note: Only one sequence can be added to the list of auto-generated sequences. If an additional sequence is added, then the previous user-added sequence is overwritten.

1. In the Sequence pane, click **Enter Sequence**.

   The Sequence pane is cleared and the Fragments table refreshes with the words No sequence assigned shown.

2. Click **Load Parent**.

   The parent sequence is shown in the Sequence pane and on the **Parent Sequence** tab of the Sequence charts pane.

3. Press **Tab** to validate the parent sequence.

   An underline is added to the sequence to indicate that it is valid. A new histogram is created on the Sequence Candidates tab, showing the user-added sequence in the line immediately above the first auto-generated sequence. The ranking for the user-added sequence will be 0. The blue bar will span the entire width of the table. However, the percentage will not be shown in the bar. The shade of blue for the loaded sequence row will be slightly different than the blue for the lines containing the auto-generated sequences. The number of sequences proposed will increase by one.

   The user-added sequence can be edited. Any changes made to the sequence will be held in memory when the user tabs out of the Sequence pane.

Select a Sequence to View

- Click a blue bar in the histogram.

  The corresponding sequence is shown in the Sequence pane. The Fragments table is updated with the information related to the selected sequence. The label above the Sequence pane is updated to indicate the sequence number and the assigned rank. For example, Sequence x of y, rank = z.

Delete a Sequence

1. Click a blue bar in the histogram.

   The corresponding sequence is shown in the Sequence pane.

2. In the Sequence pane, click **Delete**.

The sequence is removed from the Sequence pane and the row is removed from the histogram. The sequence of the next line in the histogram is shown in the Sequence pane and the Fragments table is updated with the information related to the selected sequence.

Correlate Results 9

When potential metabolites are found in several samples of interest, the results from each sample can be compared. This allows the user to see the differences and the similarities between the potential metabolites generated by several Results files. Metabolites from the different Results files are considered to be identical if they match the mass-to-charge ratio and retention time tolerances set in the Correlate Results dialog.

For oligonucleotide workflows, the software can group multiply charged metabolites with the same neutral mass and within the retention time tolerance in a single entry in the Correlation workspace. This feature is called Grouping. To enable the feature, select **Group results by analyte** when correlating the results. When the feature is enabled, multiply charged species are merged, facilitating comparison across Results files.

Note: Enable the group feature before correlating the Results files.

Prepare for Correlation

1. Click **File > New > Correlation**.

   The Correlate Results dialog opens.

2. Click **Add Results**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate files.

   Note: The selected files can contain different compounds. Analog data is not required for correlation.

4. Complete the **X-axis title** and the **X-axis units** fields.

   This assigns a label for the X-axis of the graphs in the Correlation workspace.

5. Type a unique value beside each Results file in the field corresponding to the X-axis label. For example, if the label assigned in step 4 is **Time**, then type the time for each Results file in the **Time** field.

6. Select **Include RRF in % area determination**, if appropriate.

Note: Do not select both **Include RRF in % area determination** and **Group results by analyte**.

If this option is selected, then the MS Area will be multiplied by the relative response factor. The change in area is shown in the Linear Graph, Bar Graph, and Table views of the Correlations Details pane. The change is not shown in the Potential Metabolites table.

7. (Oligonucleotide workflow) If required, select the **Group results by analyte** to group peaks based on neutral mass.
8. Customize the correlation. Refer to the section: Customize the Correlation.
9. In the **Correlation file name** field, type a name for the file.

Note: Do not include spaces in the file name.

10. To select a specific location to save the correlation file, click **Browse** and then select the appropriate folder.
11. Click **OK**.

    The software compares the metabolites found in the selected files and shows the results in the Correlation workspace.

Tip! The same correlation can be processing using different settings. In the Correlation workspace, click **Correlate Results**.

Customize the Correlation

After selecting the files to correlate, edit the parameters values in the Correlation Results dialog to improve the results.

Improve Peak Alignment

The retention times of individual Results files can be shifted to better correlate the selected files.

1. Before correlating results, do this:
   a. Open each of the appropriate Results file in the Results workspace.
   b. Review the retention time of a specific metabolite that is shown in all of the files.
2. Based on the shift that is shown in the Results file, in the Correlate Results dialog, type a value in the **R.T. Shift (min)** field beside the specific file.

Note: The **R.T. Shift (min)** field accepts values from –2.00 minutes to 2.00 minutes.

Define Peak Merging

Specific tolerances allow peaks with similar values to be considered the same peak.

1. Open each of the Results files in the Results workspace.
2. Identify the retention time and mass tolerance of a specific metabolite that is shown in all of the files.
3. In the Correlate Results dialog, in the Tolerances group, type a value in the **Retention time** field.

   Note: The **Retention time** field accepts values between 0.01 minutes and 0.25 minutes.

4. Type a value in the **MS m/z** field and then select either **ppm** or **mDa** as the unit of measure.

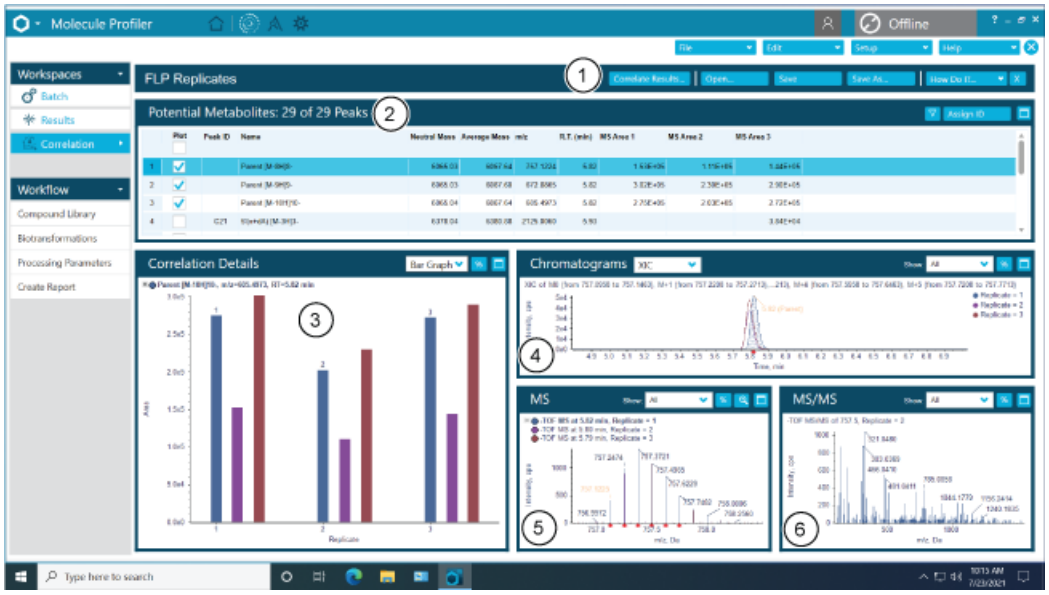
   Note: In oligonucleotide workflows, if the **Group results by analyte** option is selected, then only **ppm** is available.

   Note: The **MS m/z** field accepts values between 0.1 and 250.0.

About the Correlation Workspace

The Correlation workspace shows the comparison of potential metabolites that were found in the selected Results files.

Figure 9-1 Correlation Workspace



Item	Description
1	Menu bar. The menu bar contains these buttons: • **Correlate Results**: Opens the Correlate Results dialog. Refer to the section: Prepare for Correlation. • **Open**: Opens the Open Correlation dialog where users can browse to the appropriate correlation files. • **Save**: Saves the currently open correlation file. Automatically replaces the existing version. • **Save As** : Saves the currently open correlation file. Optionally, select the destination folder and assign a new name to the correlation file.
2	Potential Metabolites pane. Lists all of the correlated peaks based on the set tolerances. Each row lists a correlated potential metabolite, the **MS Area**, and the **Analog Area**, if applicable, from the Results files. An empty **MS Area** cell indicates that the metabolite was not found in the specific Results file. An empty **Analog Area** cell indicates that either the metabolite was not found in the Results file or the analog response was zero. This pane contains these buttons: • **Select values to filter peaks from the results.** (): Opens the Correlation Filters dialog where users can set values that will filter information that does not meet the criteria from the results. Refer to the section: About Correlation Filters. • **Assign ID**: Assigns a unique identifier to each peak in the Potential Metabolites table, based on the retention time and the *m/z* value.
3	Correlation Details pane. Allows users to compare correlated metabolites. Refer to the section: Compare Correlated Metabolites. Different metabolites and Results files can be selected. MS and analog data can be presented in the following formats: • **Linear Graph** or **Bar Graph**: Compares the intensity of each metabolite in each of the Results files in which it was found. • **Table**: Identifies the Results files in which each metabolite was found. Users can also select to show the occurrence, peak ID, or peak area in the table. **Note:** If a relative response factor was applied when preparing for correlation, then quantitative MS data is shown in the linear graphs and the bar graphs.

Item	Description
4	Chromatograms pane: Shows either an extracted ion chromatogram (XIC) or an analog chromatogram for the selected metabolite. The chromatograms can include data from one or from all of the Results files containing the metabolite.
5	MS pane: Shows the MS spectrum of the sample of interest from one or from all of the Results files containing the selected metabolite.
6	MS/MS pane: Shows the MS/MS spectrum of the selected metabolite from one or from all of the Results files containing the metabolite.

Note: If correlation results are grouped, then chromatographic, MS and MS/MS spectra are not shown.

Edit the Name of a Correlated Metabolite

1. In the Workspace panel, click **Correlation**.

   The Correlation workspace opens.
2. Click **Open**.

   The Open Correlation dialog opens.
3. Browse to and then select the appropriate correlation file.
4. Select a row in the Potential Metabolites table.
5. Click **Edit > Edit Name**.

   The Edit Name dialog opens.
6. Type a new metabolite name.
7. Click **OK**.

   The metabolite name changes to the new value.

Compare Correlated Metabolites

After correlating the metabolites contained in multiple Results files, users can compare selected specific metabolites in more detail.

1. In the Workspace panel, click **Correlation**.

   The Correlation workspace opens.

2. Click **Open**.

   The Open Correlation dialog opens.

3. Browse to and then select the appropriate file.

4. In the Potential Metabolites table, select the **Plot** check box beside the potential metabolites to be compared.

   The metabolites are shown in the Correlation Details pane.

5. To change the relative response factor of a specific metabolite, type a value in the **RRF** field.

   In the Linear graph and Bar graph, the MS area and the Analog area, if applicable, are multiplied by the RRF value.

   Note: This field is shown only if a relative response factor was used when preparing for correlation.

6. To show analog data in the Correlation Details pane, click **Analog data**.

7. To identify the files that contain the selected metabolites, select **Table** in the Correlation Details pane.

8. To show normalized data, click  .

   Tip! Normalized data can be shown in the Linear graph, the Bar graph, the XIC, the analog chromatogram, the MS spectrum, and the MS/MS spectrum.

9. To reassign the peak IDs of the Potential Metabolites in the correlated files, based on the retention time and the *m/z* value, click **Assign ID**.

About Correlation Filters

Apply filters to further refine the data shown in the Correlation table. Click the  icon to access the Correlation Filters dialog or click **Setup > Filters > Correlation**.

Filter	Description
Mass Range	
m/z from __ to __	Shows only peaks with an *m/z* value that is within the specified range.
Retention Time	

Filter	Description
R.T. from __ to __	Shows only peaks with a retention time that is within the specified range.
Occurrence	
Peaks in __ or more results files	Shows only peaks that are shown in the specified number of Results files. **Note:** The maximum value depends on the number of files selected for correlation. For example, if five Results files are selected to correlate, then a maximum of five occurrences of a peak can be selected.

Reports 10

To generate reports with the software, Microsoft Word 2010 or higher must be installed on the computer.

Users can create reports in Adobe PDF, Microsoft Word, and HTML. A report can also be sent directly to a printer.

The following report templates are installed with the software in the C:\ProgramData\SCIEX\Molecule Profiler\Report Templates folder:

- Correlation folder
  - Correlation Detailed Report
  - Correlation Summary Report
  - Correlation Group Report
- ResultsAndInterpret folder
  - Interpretation Detailed Report
  - Interpretation Summary Report
  - Results Detailed Report
  - Results Summary Report
- ResultsAndInterpret_ADC folder
  - Interpretation Detailed Report
  - Interpretation Summary Report
  - Results Detailed Report
  - Results Summary Report
- ResultsAndInterpret_Peptides folder
  - Interpretation Detailed Report
  - Interpretation Summary Report
  - Results Detailed Report
  - Results Summary Report

- ResultsAndInterpret_Oligo folder
  - Interpretation Detailed Report
  - Interpretation Summary Report
  - Results Detailed Report
  - Results Summary Report

Although each report can contain many pieces of information, the report only shows the contents of the Results file being reported at the time that the report is generated. If the Results file does not contain a specific piece of information, for example, isotopic enrichment, then the generated report will not contain that content and, in most cases, will not contain a field label or heading for that content. Any filters that have been applied to the Potential Metabolites table or the Fragments table are reflected in the report. For example, if the Potential Metabolites table is filtered to show only the top 5 of 23 peaks, only those 5 peaks are included in the report.

All graphs or spectra included in the report are shown at the default zoom level, regardless of the zoom level that has been selected in the user interface. All correlation graphs are reported with non-normalized data.

Note: When creating custom correlation report templates for use with grouped data, make sure to include "grouped" in the file name.

Create a Report in the Results Workspace

A report can be created for each of the small molecule, peptides, and ADC results.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.
2. Click **Open**.

   The Open Results dialog opens.
3. Browse to and then select the appropriate file.
4. Click **OK**.

   The Results view is shown.
5. In the **Report** column, select the corresponding check box for each metabolite to be included in the report.

   Metabolites that are not selected are not included in the generated report.
6. In the Workflow panel, click **Create Report**.

   The Create Report dialog opens.

7. Select a template for the report from the **Available templates** field.

   For a list of templates, refer to the section: Reports.

8. Select the appropriate **Formats** check boxes to create the required versions of the report files or to print the report.

   Note: Multiple formats can be selected.

9. For each format version selected, click **Browse** and then, on the Browse For Folder dialog, browse to and select a specific storage location for the report file.
10. Click **OK**.

    The Browse For Folder dialog closes.

11. For each format version selected, type a name for the report in the field provided.
12. (Oligonucleotide workflow) Select or clear the **Report grouping table for Results** check box, as required.
13. Click **Generate Report**.
14. If the **Print report** option was selected, then select the required print options on the Print dialog and click **OK**.

    The software generates the report.

Create a Report in the Correlation Workspace

A correlation report can be created for each of the small molecule, peptides, and ADC results.

1. In the Workspace panel, click **Correlation**.

   The Correlation workspace opens.

2. Click **Open**.

   The Open Correlation dialog opens.

3. Browse to and then select the appropriate file.
4. Click **Open**.

   The Correlation Results view is shown.

5. To include the correlation details for the metabolite of interest in the report, select the **Plot** check box.
6. In the Workflow panel, click **Create Report**.

   The Create Report dialog opens.

7. Select a template for the report from the **Available templates** field.

   For a list of templates, refer to the section: Reports.

   Note: If the correlation file does not contain grouped data, then only ungrouped report templates are available. If the correlation file contains grouped data, then only report templates with "grouped" in the file name are shown.

8. Select the appropriate **Formats** check boxes to create the required versions of the report files or to print the report.

   Note: Multiple formats can be selected.

9. For each format version selected, click **Browse** and then, on the Browse For Folder dialog, navigate to and select a specific storage location for the report file.
10. Click **OK**.

    The Browse For Folder dialog closes.
11. For each format version selected, type a name for the report in the field provided.
12. Click **Generate Report**.
13. If the **Print report** option is selected, then select the required print options on the Print dialog and click **OK**.

    The software generates the report.

Copy and Paste a Graph

Graphs can be copied from the Results workspace as well as the Compound Library and Processing Parameters dialogs.

1. Right-click the graph to copy and then click **Copy Selected Graph**.

   The graph is copied to the clipboard.
2. Paste the graph in another application, such as Microsoft Word.

Copy and Paste the Potential Metabolites Table

1. Right-click the table and then click **Copy Table** in the Results workspace.

   The table is copied to the clipboard.
2. Paste the table in Excel.

Analog Integration 11

Analog data is used to confirm that metabolites found using the mass spectrometer are actual metabolites and not false positives. Users who use analog in-line with the mass spectrometer will use this feature to optimize analog area integration and better visualize the association of MS peaks with analog peaks.

If a Results file that contains analog data is opened, then the **Analog Integration** button in the Potential Metabolites table is enabled.

When **Analog Integration** is clicked, the Analog Integration dialog opens.

The original Potential Metabolites table from the Results workspace is shown, with the following exceptions:

- Any analog peaks that do not have associated mass peaks are shown, but the MS-related columns are empty.
- An additional column, **Analog Signal in Control**, is shown immediately after the **Analog - R.T. (min)** column if analog control data exists. If there is no analog control data, this column is not shown.

The **Analog Signal in Control** column provides the following information:

- If the sample-to-control ratio of the analog peak is greater than the value specified in the Processing Parameters, then an x is shown in the column.
- If the sample-to-control ratio of the analog peak is less than the value specified in the Processing Parameters, then a check mark is shown in the column.

Manually Integrate Analog Data

Prerequisites:
• Results have been processed with analog data.

1. In the Workspace panel, click **Results**.

   The Results workspace opens.

2. Click **Open**.

   The Open Results dialog opens.

3. Browse to and then select the appropriate file.

   Note: The Results file must contain an analog chromatogram.

4. Click **OK**.

   The Results view is shown. If the Results file contains analog data, then the **Analog Integration** button in the Potential Metabolites table is enabled. If the Results file does not contain analog data, the button is not available.

5. Click **Analog Integration**.

   The Analog Integration dialog opens.

   In addition to the Potential Metabolites table, two chromatograms are shown. The first chromatogram, the Analog Sample chromatogram, shows all of the analog peaks within the retention time range specified in the Chromatographic Data tab of the Generic Processing Parameters. Refer to the section: Chromatographic Data Tab. The second chromatogram, the Extracted Ion Chromatogram (XIC) of the MS Sample, shows all of the peaks for the selected row. The XIC is updated each time a different row is selected in the Potential Metabolites table.

6. Select the Analog Sample chromatogram and then complete the following tasks, if required, to integrate the data:

   - manually integrate peaks
   - modify the existing integration of peaks
   - remove peaks

   As the changes are made, the software automatically updates the Analog Sample chromatogram.

7. (Optional) Select the **Show Controls** check box.

   A maximum of five control samples are shown below the Analog Sample chromatogram title. Refer to the section: Show Controls.

8. (Optional) Click **Baseline Subtract**.

   Baseline subtract is applied to both the Analog Sample and to any controls. The phrase "baseline subtracted" is appended to the title of the Analog Sample chromatogram and to any controls. Refer to the section: Perform a Baseline Subtract.

9. (Optional) Change the **R.T. Offset**. Refer to the section: Change R.T. Offset.

   The R.T. Offset applies to both the Analog Sample and the control traces.

10. (Optional) Apply the analog integration **Options**. Refer to the section: Set Analog Integration Options.

11. Do one of the following:
    - Click **Update Table**. Refer to the section: Update Table.
    - Click **Update Results and Close**. Refer to the section: Update Results and Close.
12. Do one of the following:
    - Click **Save** to save the currently open Results file and overwrite the existing version.
    - Click **Save As** to save the currently open Results file with a new name. The existing Results file is not updated.

Show Controls

1. In the Chromatograms pane of the Analog Integration dialog, select the **Show controls** check box.

   If applicable, a maximum of five controls are shown below the Analog Sample title in the Chromatograms pane. If applicable, a maximum of five controls are shown below the MS Sample title in the XIC pane.
2. Click the ⊞ icon to expand the list and show both the analog sample and the analog control or the MS Sample and the MS Control.
3. Click the ⊟ icon to collapse the list and show only the analog Sample or the MS Sample.
4. Select the **Show controls** check box again to remove the controls from the view.

Perform a Baseline Subtract

1. In the Chromatograms pane of the Analog Integration dialog, click **Baseline Subtract**.

   The Analog Sample chromatogram is baseline subtracted. Baseline subtraction is applied to both the analog sample and to any control traces. The phrase "baseline subtracted" is appended to the name of the Analog Sample chromatogram.
2. Click **Baseline Subtract** again to remove the baseline subtraction.

   The phrase "baseline subtracted" is removed from the name of the Analog Sample chromatogram.

Change R.T. Offset

- In the Chromatograms pane of the Analog Integration dialog, use the up and down arrows in the **R.T. Offset** field to change the retention time offset.

The peaks in the Analog Sample chromatogram are shifted by the specified retention time offset. When the Potential Metabolites table is updated or the results are updated, the values in the **Analog R.T. (min)** column are updated to reflect the shift in the specified retention time offset. The offset applies to both the analog sample and the control samples.

Set Analog Integration Options

1. In the Chromatograms pane of the Analog Integration dialog, click **Options**.

   The Analog Integration Options dialog opens.

2. Select the check box for each option to be applied.

Option	Description
Overlay XIC for peaks at the same analog retention time	Overlays MS Sample XICs for these traces with identical analog retention times.
Link x-axis	Links the X-axis of the Analog Sample chromatogram and the XIC chromatogram.

3. Click **OK**.

Update Table

When changes are made in the Analog Integration dialog, the **Update Table** option is enabled.

- Click **Update Table**.

  The information in the following columns of the Potential Metabolites table is updated to reflect any changes made to the analog peak integration, the analog retention time, and baseline subtraction:

  - The assigned **Peak ID** for the Analog Sample chromatogram might be updated to reflect any manual integration. The analog peak is considered a match with an MS peak if the retention time of the analog peak matches the retention of the MS peak, within a specified tolerance.
  - The **Analog - Peak Area** is updated to reflect any new integrated areas.

- The **Analog - % Area** is updated to reflect any changes in the algorithm. The **Analog - % Area** is calculated based on all of the analog peaks, both those associated with MS peaks and those not associated with MS peaks, within the time range specified in the Processing Parameters. If an analog peak is associated with more than one MS peak, then the analog peak area listed for a specific M# is calculated proportionally based on the XIC MS area of that M#, using the peak areas of all the associated MS peaks as the total.
- The **Analog - R.T. (min)** is updated to reflect any changes made to the retention time offset.

Note: These changes are not saved in the Results file and can be reverted by clicking **Cancel**.

Update Results and Close

When changes are made in the Analog Integration dialog, the **Update Results and Close** option is enabled.

1. Click **Update Results and Close**.

   A message opens, asking the user to confirm that the analog information should be updated based on the changes made.

2. Click **Yes**.

   The Analog Integration dialog closes. The information in the following columns of the Potential Metabolites table is updated to reflect any changes made to the analog peak integration, the analog retention time, and baseline subtraction:

   - The assigned **Peak ID** for the Analog Sample chromatogram might be updated to reflect any manual integration. The analog peak is considered a match with an MS peak if the retention time of the analog peak matches the retention of the MS peak, within a specified tolerance.
   - The **Analog - Peak Area** is updated to reflect any new integrated areas.
   - The **Analog - % Area** is updated to reflect any changes in the algorithm. The **Analog - % Area** is calculated based on all of the analog peaks, both those associated with MS peaks and those not associated with MS peaks, within the time range specified in the Processing Parameters. If an analog peak is associated with more than one MS peak, then the analog peak area listed for a specific M# is calculated proportionally based on the XIC MS area of that M# (using the peak areas of all the associated MS peaks as the total).

- The **Analog - R.T. (min)** is updated to reflect any changes made to the retention time offset.

Troubleshooting 12

For help with a particular issue, select the appropriate link:

Cannot Open a Structure File

Make sure that the structure file follows these conventions:

- Format: mol
- Version: v2000 or v3000
- Content: Does not contain text

Change User Permissions

When the Molecule Profiler software is installed, all users are given permission to read, write, and delete files in the installed user data folder. If the permissions are changed, then the software might not function correctly.

Note: The default location of the installed user folder is C:\ProgramData\SCIEX\Molecule Profiler Data.

No Potential Metabolites Found

To find more metabolites in the sample of interest:

- Select a different peak finding strategy. Refer to the section: About Peak Finding Strategies.
- Decrease the minimum chromatographic intensity on the Chromatographic Data tab. Refer to the section: Chromatographic Data Tab.
- Increase the **MS m/z tolerance** in the m/z Tolerance group on the MS Parameters tab. Refer to the section: MS Parameters Tab.
- Decrease the **Minimum MS peak intensity** in the m/z Tolerance group on the MS Parameters tab. Refer to the section: MS Parameters Tab.
- (Oligonucleotide workflow) Increase the **Intensity tolerance** in the Isotope Pattern Tolerances group on the MS Parameters tab.

Too Many Potential Metabolites Found

To reduce the number of potential metabolites found:

- Select a different peak finding strategy. Refer to the section: About Peak Finding Strategies.
- Increase the minimum chromatographic intensity on the Chromatographic Data tab. Refer to the section: Chromatographic Data Tab.
- Reduce the retention time window on the Chromatographic Data tab. Refer to the section: Chromatographic Data Tab.
- Reduce the mass range window on the MS Parameters tab. Refer to the section: MS Parameters Tab.
- Increase the **Minimum MS peak intensity** in the Isotope Pattern Tolerances group on the MS Parameters tab. Refer to the section: MS Parameters Tab.

Long Processing Times

Processing time is affected by many factors, including data complexity, processing parameters, workstation, and operating system.

To reduce the time needed for processing:

1. Close any other applications that are running on the workstation.
2. Change the processing parameter values. For example:
    - Reduce the number of algorithms selected.
    - Increase the minimum chromatographic intensity on the Chromatographic Data tab.
    - Reduce the retention time window on the Chromatographic Data tab.
    - Increase the minimum MS peak intensity on the MS Parameters tab.

- Reduce the mass range window on the MS Parameters tab.
- Reduce the number of mass defect filters selected (for small molecules only).
- Reduce the number of biotransformations.
- (Peptide, oligonucleotide, and ADC workflows) Reduce the number of catabolites generated by adjusting the compound-specific parameters.

Show the ProgramData Folder

The Microsoft Windows operating system might hide the C:\ProgramData folder. After installing the Molecule Profiler software, make sure that all users can view the C:\ProgramData\SCIEX\Molecule Profiler Data folder. If the folder is not visible, then complete the following procedure:

1. In File Explorer, click **View > Options**.

   The Folder Options dialog opens.
2. Select the View tab.
3. Click **Hidden files and folder > Show hidden files, folders, or drives**.
4. Click **Apply**.
5. Click **OK**.

Known Issues and Limitations

Results Data

- When determining the MS peak area, a time conversion factor of 60 is now being applied to the calculations.

Interpretation

- When preparing for structural assignment, always click **Find** after making changes to the Interpret Data dialog. The software recalculates the list of available formulas based on the selected settings.

Correlation

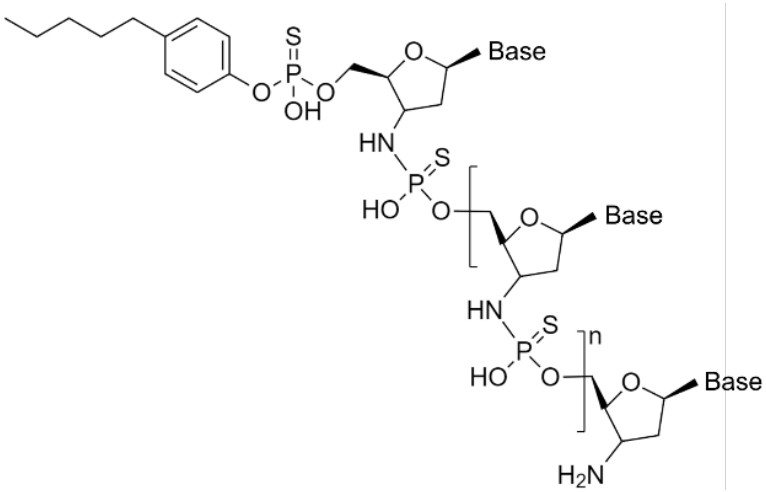
- When the relative response factor (RRF) of a specific metabolite is changed, the MS area is multiplied by the RRF value. The updated MS area of the selected metabolite is shown in the Correlation details pane, in each of the Linear graphs, Bar graphs, and Tables.

Reporting

- If conflicts with the Microsoft Word report templates occur when reports are created, then make sure that all of the Microsoft Office applications are closed and try again.

Example Custom Oligonucleotide A

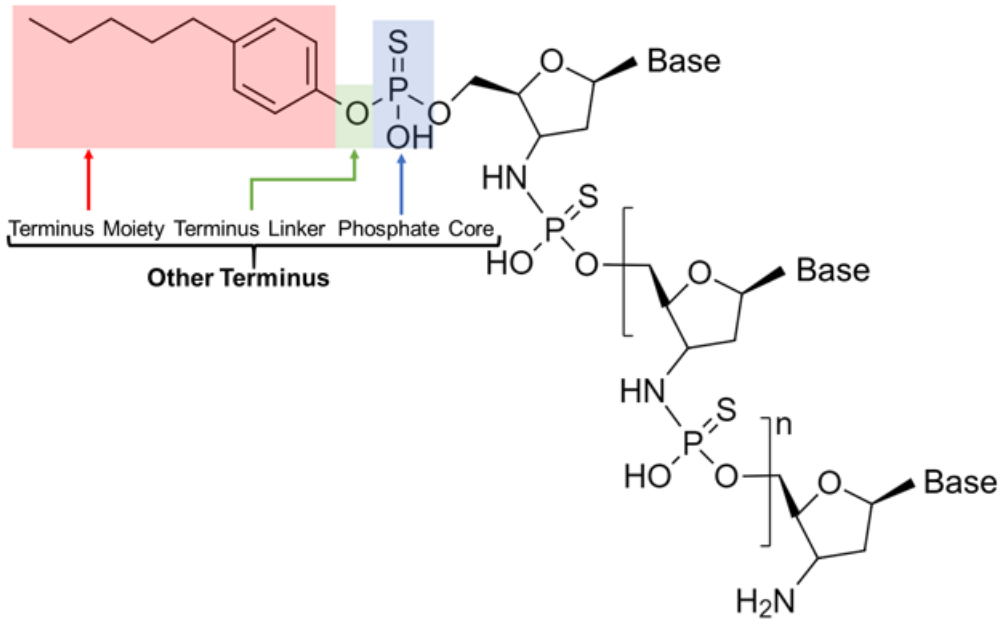
A thio-phosphoramidate oligonucleotide conjugated with a benzyl pentane linker tethered to the 5′-thio-phosphate end.

Sequence	Chemical Structure, Formula, and Monoisotopic Mass
5′-ATCGATCGTTTAAA-3′	**Figure A-1 $C_{149}H_{203}N_{65}O_{57}P_{14}S_{14}$ (4695.7400)** 

Create the Other Terminus

Follow the general scheme for identifying the substructures that make up the 5' linker moiety.

Figure A-2 Other Terminus



1. Click **Edit > Custom Elements**.
2. On the Oligo List tab, click **New**.

   The New Oligo Residue or Terminus dialog opens.
3. In the **Name** field, type a name, for example **5' benzyl-pentane terminus**.
4. In the **Symbol** field, type a symbol, for example **/CustomBP/**.
5. In the **Composition Type** field, select **Other Terminus**.
6. Complete the fields for the other terminus.

   Table A-1 Other Terminus Fields

Field	Value
Terminus Moiety	**C11H15**
Terminus Linker	**O**
Phosphate Core	**HOPS**

7. Click **OK**.

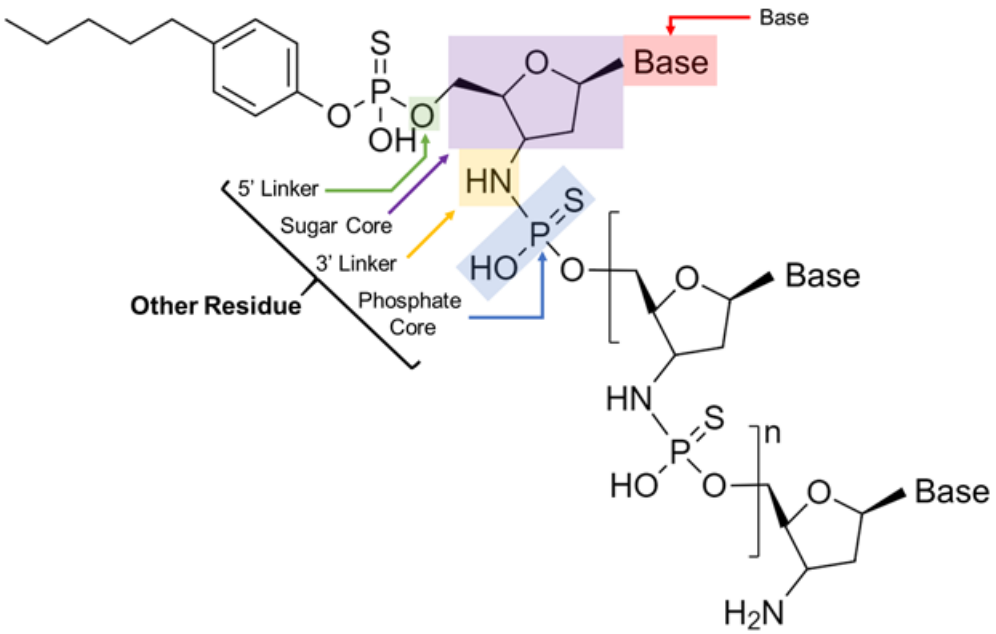
   A Warning dialog is shown, with the message, The "Terminus Moiety" field is usually odd electron. Do you want to continue?

8. Click **OK**.

Create the Internal Residues as Other Residues

Follow the general scheme for identifying the substructures that make up the custom bases.

Figure A-3 Other Residue



1. Click **Edit > Custom Elements**.
2. On the Oligo List tab, click **New**.

> **Tip!** When creating custom residues, define all four nucleotides. It is recommended to create a single nucleotide, and then click **New From** to create the three remaining nucleotides.

The New Oligo Residue or Terminus dialog opens.

3. For the Adenine nucleotide, follow these steps:
   a. In the **Name** field, type **Custom dA**.
   b. In the **Symbol** field, type **/CustomdA/**.
   c. In the **Composition Type** field, select **Other Residue**.

d. Complete the fields for the other residue.

Table A-2 Other Residue Fields

Field	Value
Base	**C5H4N5**
5' Linker	**O**
Sugar Core	**C5H70**
3' Linker	**NH**
Phosphate Core	**HPOS**

e. Click **OK**.

A Warning dialog is shown, with the message, The "Sugar Core" field is usually odd electron. Do you want to continue?

f. Click **OK**.

4. For each of the remaining three nucleotides, do this:

a. Select **/CustomdA/** and then click **New From**.

The New Oligo Residue or Terminus dialog opens.

b. Type the **Name**, **Symbol**, and **Base**. For base formulas, refer to the following table.

Table A-3 Base Formulas

Nucleotide	Name	Symbol	Base
Thymine	Custom dT	/CustomdT/	C5H5N2O2
Guanine	Custom dG	/CustomdG/	C5H4N5O
Cytosine	Custom dC	/CustomdC/	C4H4N3O

c. Click **OK**.

Write a Custom Sequence

1. Click **New > Oligonucleotide**.
2. In the Sequence pane, type:

/CustomBP/ /CustomdA//CustomdT//CustomdC/ /CustomdG//CustomdA//CustomdT/ /CustomdC//CustomdG//CustomdT/ /CustomdT//CustomdT//CustomdA/ /CustomdA//CustomdA/

3. Click in the **Chemical formula** field.

   C149H203N65O57P14S14 is shown in the field.

Glossary B

How metabolites are named by the software

Names are assigned to potential metabolites in two ways. If the peak is a predicted metabolite, then the name is based on the matching biotransformation, cleavage metabolite, or a combination of the two. If the peak is an unexpected metabolite, then it is named Loss of or Gain of.

The software also assigns a potential formula to each metabolite. Users can change the formula by either selecting a different formula from a list of formulas suggested by the software or by typing a formula manually.

IDA

An IDA method finds ions in full scan spectra during acquisition and then decides in real-time which ions to analyze by MS/MS.

peak IDs

The software labels potential metabolites using M1, M2, M3, and so on, based on retention time and *m/z* value.

relative response factor

The relative response factor (RRF) is a value by which the peak area is multiplied, to artificially increase or decrease the peak area. It can change the plotting of that peak area in the correlation details graph.

reference spectrum

The MS/MS spectrum of a specific compound that is used when identifying potential metabolites.

Contact Us

Customer Training

- In North America: NA.CustomerTraining@sciex.com
- In Europe: Europe.CustomerTraining@sciex.com
- Outside the EU and North America, visit sciex.com/education for contact information.

Online Learning Center

- SCIEX Now Learning Hub

SCIEX Support

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- sciex.com/request-support

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