Agilent G6825AA METLIN Personal Metabolite Database for MassHunter Workstation

Quick Start Guide

This guide describes how to install and use METLIN Personal Metabolite Database for MassHunter Workstation.

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Where to find more information

Use the online help for in-depth information not given in this *Quick Start Guide*. Display online help in either of the following ways:

- Select either **Contents**, **Index**, or **Search** from the METLIN Personal Metabolite Database software **Help** menu.
- Press the **F1** key to get more information about a window, tab, or dialog box.

Agilent Technologies

What is METLIN Personal Metabolite Database software?

METLIN Personal Metabolite Database for MassHunter Workstation provides tools to access and use the METLIN Personal metabolite database for metabolite searches based on LC/MS data. The METLIN database is probably the best-known and most-comprehensive metabolite database in the world today. It currently includes annotated lists of over 23,000 endogenous and exogenous metabolites, as well as di- and tripeptides; each entry includes mass, chemical formula, and structure information.

METLIN Personal Metabolite Database software allows you to:

- Do automated searches on multiple compounds, which saves time.
- Do private, on-site searches, which keeps intellectual property safe.
- Customize the database by adding proprietary compounds.
- Customize the database by adding retention times to compounds in the database.
- Search a database using mass only, mass and optional retention time, or mass and retention time required. The use of retention time increases the compound identification specificity.
- Search on text.
- Search on empirical formula.
- Search on retention time.
- Do automated or manual searches from Agilent MassHunter Qualitative Analysis software.
- Link to websites such as PubChem, Kyoto Encyclopedia of Genes and Genomes (KEGG), Human Metabolome Database (HMP), LIPID Metabolites And Pathways Strategy (LMP), and public METLIN (Scripps Center for Mass Spectrometry) for more information on many compounds.

METLIN Personal Metabolite Database software supports mass lists output from Agilent MassHunter Mass Profiler and GeneSpring MS software.

Installation

If the METLIN Personal Metabolite Database for MassHunter Workstation is not already installed on your system, install it as follows.

Before you start

Uninstall previous version of METLIN Personal Metabolite Database for MassHunter Workstation that exist on your system before installing the new version.

Installation

1. Insert the CD-ROM labeled Agilent G6825AA into the CDROM drive.
2. Click the Windows **Start** button and select **Run**.
3. Type **x:\setup**, where **x** is the letter of the CDROM drive, and click **OK**.
4. Follow the instructions on the screen to install the software.

To uninstall the software

Use the **Add/Remove** window of the Control Panel.

Startup

Select **Programs > Agilent > MassHunter Workstation > METLIN Personal Metabolite Database** from the Windows Start menu, as shown below, to start the METLIN Personal Metabolite Database for MassHunter Workstation software.



NOTE Databases created with a previous version can be opened and searched with the current software, but they *cannot be edited*, unless they are converted as described on page 26.

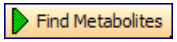
Main Window

The METLIN Personal Metabolite Database for MassHunter Workstation main window has the following areas and controls.

- "Toolbar"(below)
- "Menus" (below)
- "Tabs" on page 7
- "Search Results Table" on page 8
- "Structures" on page 9

Toolbar

The following items are available on the METLIN Personal Metabolite Database for MassHunter Workstation toolbar:

Button	Equivalent Menu Item
	Database > Find Metabolites
	File > Print Results
	File > Export Results
	File > New Database
	File > Open Database
	Help > Contents

Menus

The following menu options are available in METLIN Personal Metabolite Database for MassHunter Workstation software:

- "File menu" on page 5
- "Database menu" on page 5
- "Edit Metabolites menu" on page 6
- "View menu" on page 6
- "Metabolomics Links menu" on page 6
- "Help menu" on page 7

File menu

New Database Allows you to create a subset of the master METLIN database or to create a custom database that can be edited and personalized for your lab. This is done by creating a copy of an existing database with a new name.

Open Database Allows you to open a different database.

Backup Database Allows you to make a copy of the database for archive purposes.

Import Metabolites Allows you to select a file that contains metabolites to add to a custom database if editing is enabled. If you start with an empty database, then you are essentially creating a custom database from the imported metabolites.

Export Results Allows you to export batch summary results for use with other programs, such as spreadsheets and word processors.

Print Results Allows you to print search results and structures.

Exit Closes the METLIN Personal Metabolite Database software window.

Database menu

Find Metabolites Searches the database for metabolites that match criteria specified on the Single Search tab or Batch Search tab. Metabolites that match the search criteria are displayed in the Search Results table at the bottom of the window.

Allow Editing A toggle that enables or disables editing operations to a custom database. *A master METLIN database cannot be edited.*

Database Information Allows you to view information about the current database and edit the description for custom databases with editing enabled.

Run METLIN Database Update Utility Opens the METLIN Database Update Utility program, which allows you to select custom databases that were created with a previous release of METLIN Personal Metabolite Database and convert them to the current format. See “To update a database from a previous version” on page 26.

Edit Metabolites menu

These menu items are for use with custom databases with editing enabled only and are only available when the Edit Metabolites tab is displayed.

Add New Creates a new metabolite in the database with a mass of 0 named "New Compound". The new compound is displayed at the end of the Results table. Enter information such as RT, Formula, Name, and Structure for the new metabolite in the Edit Metabolites tab.

Save As New Creates a new metabolite in the database using the current values of the edit fields on the Edit Metabolites tab. The new compound is displayed at the end of the Results table.

Update Selected Updates the information for the current metabolite in the database. The updated information is reflected in the Results table.

Delete Selected Removes the selected lines of the Results table, which removes the selected metabolites from the database. Use **Ctrl+click** to select multiple lines.

View menu

Add/Remove Columns Opens the Add/Remove Columns dialog box, which allows you to specify what information is displayed in the Search Results table.

Restore to Default Columns Displays the columns in the Search Results table as they were originally set in the software.

Structure Details Increases the size of the current structure for easier viewing.

Metabolomics Links menu

Allows you to access the following websites for information on metabolomics:

- Agilent Metabolomics
- Agilent Metabolomics Lab
- METLIN (Scripps Center for Mass Spectrometry)
- PubChem (National Center for Biotechnology Information)
- KEGG (Kyoto Encyclopedia of Genes and Genomes)
- HMP (Human Metabolome Database)
- Lipid MAPS (LIPID Metabolites And Pathways Strategy)

Help menu

Contents, Index, and Search Opens the online help window for METLIN Personal Metabolite Database software, with the appropriate tab displayed.

View End User License Agreement Displays the license agreement for METLIN Personal Metabolite Database software in a Notepad window.

About Displays the version and build number of the METLIN Personal Metabolite Database software.

Tabs

The following tabs are available in METLIN Personal Metabolite Database for MassHunter Workstation software:

Single Search tab

Allows you to search for metabolites in the database. The search is started by clicking the **Find Metabolites** button on the toolbar, selecting **Find Metabolites** from the Database menu, or pressing the **F5** key. Search results are displayed in the Results table in the lower part of the window. See "To find metabolites" on page 11.

Batch Search tab

Allows you to search for metabolites in the database by specifying a mass list and optional retention times. The search is started by clicking the **Find Metabolites** button on the toolbar, selecting **Find Metabolites** from the Database menu, or pressing the **F5** key. Search results are displayed in the Results table in the lower part of the window. See "To find metabolites from a mass list" on page 15.

Batch Summary tab

Shows a summary view of all Best fit hits from a Batch Search. Results can be exported for use with other applications. You can also add comments and update retention times for custom databases with editing enabled. See "To view batch summary results" on page 17

Edit Metabolites tab

For custom databases that allow editing, you can use this tab to edit information for metabolites and to add new metabolites. Before editing existing metabolites, do a Single or Batch search, so that the metabolites to be modified are present in the Search Results table.

Search Results Table

This area in the lower part of the METLIN Personal Metabolite Database software window allows you to view search results. It also allows you to select metabolites for editing on the Edit Metabolites tab (*custom databases with editing enabled only*).

Viewing search results

Clicking on a row of the table displays structure information about that metabolite in the Structures area, if the structure is available in the database.

- To change which columns of information are displayed in the table, right-click in the table and select **Add/Remove Columns** from the shortcut menu.
- To sort the table by the entries in a column, click the heading for the column of interest.
- To change the order of the columns of information in the table, drag a column heading to a different position. Repeat this until the desired order is achieved.
- To resize a column to fit the information contained in it, double-click on the right column divider in the heading of the column of interest.
- To resize a column to a specific width, drag the separator between the column headings to the right or left until the column is the desired width.

Editing metabolite information in the database

You can edit information in the database for the metabolites shown in the Search Results table as follows, if you are using a custom database with editing enabled.

- Select the row (metabolite) of interest in the table. Make changes to the data for that row that appears in the Edit Metabolites tab. When you click the **Update Selected** button, the database is updated and the changes are reflected in the results table.
- Add a metabolite to a custom database in either of the following ways:

- **Add New** - Click the **Add New** button to create a new metabolite in the database with a mass of 0 named “New Compound”. The new compound is displayed at the end of the Results table. Enter information such as Mass, RT, Formula, Name, and Structure for the new metabolite in the Edit Metabolites tab. The information for the new metabolite is updated in the database and reflected in the results table when you click the **Update Selected** button.
- **Save As New** - Click the **Save As New** button to create a new metabolite in the database using the current values of the edit fields on the Edit Metabolites tab. The new compound is displayed at the end of the Results table.

- To remove a metabolite from the database, select the row (metabolite) of interest in the table, then click the **Delete Selected** button. Use **Ctrl+click** to select multiple lines for deletion.

Structures

Structure tab

This area on the right side of the METLIN Personal Metabolite Database software window displays structure information for the metabolite currently selected in the Results table, if it is available in the database. Clicking the  button opens the Structure Details dialog box and displays a larger image of the structure.

MOL Text tab

Displays the text version of the structure. If the current database is a custom database with editing enabled, you can copy text from a molecular drawing tool that is capable of generating MOL file text and paste it into this area to update the structure of the selected metabolite. Or you can use the  button in the Structure area to load a structure from a **.MOL** file. The Structure  button is available on the Edit Metabolites tab only.

Getting Started

The following topics will help you get started with Agilent METLIN Personal Metabolite Database for MassHunter Workstation. This guide and the online help explain these activities.

- "Doing a database search" on page 11
- "Doing a batch search" on page 15
- "Database operations" on page 20
- "Custom database operations" on page 22

Doing a database search

Searching a database for metabolites matching certain criteria can be broken into the following activities, which are described in the following pages:

- "To find metabolites" (below)
- "To view search results" on page 12
- "To change how data is displayed in the Results table" on page 13
- "To print search results" on page 13

To find metabolites

1. If the database you want to search is not already open, open it in either of the following ways:
   - Select **Open Database** from the File menu, *or*
   - Click the  button on the toolbar.
2. Click on the **Single Search** tab.
3. To return the contents of the entire database, leave the search fields blank and skip to Step 7. Otherwise, set the desired mass search criteria:
   a. **Mass** and **Mass tolerance** in **ppm** or **mDa**
   b. **Type of mass** (**[M+H]+**, **Neutral**, or **[M-H]-**)
   - Select **[M+H]+** mass type if you are entering an observed positive ion into the database. The neutral mass value will be automatically calculated, assuming the ion was a positive protonated ion. If **Include cations** is selected in Step 3c, the neutral mass of a radical cation is calculated. Only those compounds that have been defined as cations in the database will be compared to the calculated neutral mass of a radical cation.
   - Select **Neutral** if the mass is the calculated neutral mass value, usually a value that was calculated by Molecular Feature Extraction (MFE) software. *Do not select this value for a mass observed in a spectrum.* Ions observed in a spectrum have a charge and need to be converted to neutral to work with the database. For observed ions use either the **[M+H]+** or **[M-H]-** selection. If **Neutral** is selected, you can choose **Include anions** or **Include cations** for the **Radical ion search mode** setting in Step 3c.

- Select **[M-H]-** mass type if you are entering an observed negative ion into the database. The neutral mass value will be automatically calculated, assuming the ion was a negative deprotonated ion. If **Include anions** is selected in Step 3c, the neutral mass of a radical anion is calculated. Only those compounds that have been defined as anions in the database will be compared to the calculated neutral mass of a radical anion.

c **Radical ion search mode**:

- **Include neutrals** is normally always selected. Clear it if you want to search radical ions only.
- If you selected **Neutral** for the mass type in Step 3, then select **Include anions** *or* **Include cations** if you also want to consider those chemicals that don't pick up or lose a proton to ionize. Searching *both* modes will return false matches.
- Choose **Include anions** if the data was acquired in *negative* ion mode.
- Choose **Include cations** if the data was acquired in *positive* ion mode.

4 When searching the master METLIN metabolite database or a custom database without retention time data, be sure to *clear* the **Require** checkbox for **Retention time**. When searching a database that contains retention time information for metabolites, enter a retention time and RT tolerance if you want to consider retention times in the search.

5 If desired, enter a **Formula**, **Name**, or an ID number from METLIN, CAS, KEGG, HMP, or LMP databases to find metabolites based on these fields. You can also enter text in the **Notes** field to search for a text string, such as “drugs”.

6 Initiate the search in any of the following ways:

- Click the **Find Metabolites** button on the toolbar,
- Select **Find Metabolites** from the Database menu, *or*
- Press the **F5** key.

7 View search results in the Search Results table at the bottom of the window. For more information, see “To view search results” on page 12 and “To change how data is displayed in the Results table” on page 13.

To view search results

1 Do a search on the Single Search tab as described in “To find metabolites” on page 11.

2 View search results in the Search Results table at the bottom of the window. You can modify how search results are displayed as described in “To change how data is displayed in the Results table” on page 13.

3 Click on a metabolite in the Results table to view its structure information in the Structures area on the right side of the window, if the structure is available in the database.

4 Click on the **Edit Metabolites** tab to edit information for custom databases that allow editing, as described in "To edit a custom database" on page 22.

5 To print results, see "To print search results" on page 13.

To change how data is displayed in the Results table

This topic relates to search results displayed on the Single Search, Batch Search, Batch Summary, and Edit Metabolites tabs.

1 To sort the table by the entries in a column, click the heading for the column of interest.

2 To change the order of the columns of information in the table, drag a column heading to a different position. Repeat this until the desired order is achieved.

3 To resize a column to fit the information contained in it, double-click on the right column divider in the heading of the column of interest.

4 To resize a column to a specific width, drag the separator between the column headings to the right or left until the column is the desired width.

5 To change which columns of information are displayed in the table:

   a Right-click in the table and select **Add/Remove Columns** from the shortcut menu to open the Add/Remove Columns dialog box.

   b Move the items of information that you want to display into the **Show these columns** list.

   c Click **OK**.

   The Results table will be updated to reflect the selected columns of information.

To print search results

Results can be printed from the Single Search, Batch Search, Batch Summary, and Edit Metabolites tabs.

1 If you want to print only certain metabolites, highlight those rows in the Results table using **Ctrl**+click.

2 Open the Print Results dialog box in *any* of the following ways:

   - Select **Print Results** from the File menu.
   - Click the  button on the toolbar.
   - Right-click in the results table and select **Print Results** from the shortcut menu.

3 Select desired report options from the following:

- **Printer** (destination)
- **Print Preview** if you want to view the report on the screen first.
- **Fit to page** to size the report to fit the width of the page. This may be important if you are displaying more than the standard number of columns.
- **Orientation** (**Landscape** or **Portrait**). The default is Landscape, as it allows more columns of information.

4 Select the desired report content options from the following:

- **All rows** or **Only highlighted rows** of the results table
- **Include search parameters**
- **Include unmatched masses**
- **Print structures** and the size (**240 x 160**, **360 x 240**, or **540 x 360**)

5 Click **OK** to generate the report.

If you selected **Print Preview** in Step 3, review the report in the Print preview pane. Click the  button on the toolbar to print the report.

NOTE For Single Search, only the first 30 metabolites results will be printed.

Doing a batch search

Searching a database for metabolites based on a mass list can be broken into the following activities, which are described in the following pages:

- "To find metabolites from a mass list" (below)
- "To view batch search results" on page 16
- "To view batch summary results" on page 17
- "To export batch summary results" on page 18

To find metabolites from a mass list

1. If the database you want to search is not already open, open it in *either* of the following ways:
   - Select **Open Database** from the File menu.
   - Click the  button on the toolbar.
2. Click on the **Batch Search** tab.
3. Enter a mass list in *one* of the following ways:
   - Click the **File** button to the left of the mass list table to open a mass list file output by MassHunter Qualitative Analysis (**.csv** file), Mass Profiler (**.xls** file), or GeneSpring MS (**.txt** file)
   - Copy and paste a compound list from MassHunter Qualitative Analysis
   - Type in masses and retention times directly into the table
4. Select or enter the **Type of mass** (normally **Neutral** for Batch Search) and **Mass tolerance**, in **ppm** or **mDa**.
5. Select **Radical ion search mode** options as follows:
   - **Include neutrals** is normally always selected.
   - Select **Include anions** and/or **Include cations** if you also want to consider those few chemicals that don't pick up or lose a proton to ionize.
6. Select one of the following options for **Retention times**:

Option	Meaning	Application
Ignore	Don't consider retention time even if it is present in the list	When searching the master METLIN metabolite database or a custom database *without* retention time data.

Optional	Use retention time information to rank a metabolite higher if its retention time matches with the observed retention time.	Any. Select this option if you plan to update retention times in a custom database from the mass list as described in "To update retention time data" on page 24.
Required	Consider only metabolites that match the specified retention time range.	When searching a database that contains retention time information for metabolites.

7 Initiate the search in *any* of the following ways:

- Click the **Find Metabolites** button on the toolbar.
- Select **Find Metabolites** from the Database menu.
- Press the **F5** key.

8 When the search is complete, the number of hits for each mass appears in the **Hits** column of the mass list, and the list is automatically sorted to show the masses with the most hits at the top of the list. To sort the list by a different criterion, click the heading for the column of interest (**Mass** or **RT**).

Masses with conflicting hits are shown in red text in the Mass List table.

9 Click on a row in the Mass List table to display the metabolites found for that mass in the Search Results table in the lower part of the window. For more information on viewing batch search results, see "To view batch search results" on page 16.

10 Select and view the best metabolite hit for each mass as described in "To view batch summary results" on page 17.

To view batch search results

1 Do a search on the Batch Search tab as described in "To find metabolites from a mass list" on page 15.

2 When the search is complete, the number of hits for each mass appears in the **Hits** column of the mass list, and the list is automatically sorted to show the masses with the most hits at the top of the list. To sort the list by a different criterion, click the heading for the column of interest (**Mass** or **RT**). Masses with conflicting hits are shown in red text in the Mass List table.

3 Click on a row in the Mass List table to display the metabolites found for that mass in the Search Results table in the lower part of the window.

Tip For ease of reviewing results, use the **F4** key to scroll down the Mass List and the **Shift+F4** keys to scroll back up. The information in the Results table will be updated as you scroll through the mass list.

4 (*Optional*) Modify how the batch search results are displayed as described in "To change how data is displayed in the Results table" on page 13.

5 See "To view batch summary results" on page 17 for information on selecting and viewing the best metabolite hit for each mass.

6 Click on a compound in the Results table to view its structure information in the Structures area on the right side of the window, if the structure is available in the database.

7 Click on the **Edit Metabolites** tab to edit information for custom databases that allow editing, as described in "To edit a custom database" on page 22.

8 To print batch results, see "To print search results" on page 13.

To view batch summary results

1 Do a search on the Batch Search tab as described in "To find metabolites from a mass list" on page 15.

2 Click on a row in the Mass List table to display the metabolites found for that mass in the Search Results table in the lower part of the window. Masses with conflicting hits are shown in red text in the Mass List table.

3 Review the entries marked in the **Best** column for each mass and if necessary, edit the Batch Search Results table to select the best metabolite hit by marking the desired row (metabolite). You can also *clear* the box for the selected hit to ignore all the results for that metabolite, as another way of resolving the conflict.

4 Click the **Batch Summary** tab to view only the best hits from the Batch Search Results table. Compounds with conflicting mass hits are shown in red text in the results table.

5 (*Optional*) Modify how the batch summary results are displayed as described in "To change how data is displayed in the Results table" on page 13.

6 Click on a compound in the Results table to view its structure information in the Structures area on the right side of the window, if the structure is available in the database.

7 Click on the **Edit Metabolites** tab to edit information for custom databases that allow editing, as described in "To edit a custom database" on page 22.

8 To export batch summary results, see "To export batch summary results" on page 18.

9 To print batch summary results, see "To print search results" on page 13.

10 (*Optional*) For a custom database with editing enabled, you can update retention times in the database for the metabolites shown in the Batch Summary Results table. See "To update retention time data" on page 24.

To export batch summary results

1. Do a batch search as described in "To find metabolites from a mass list" on page 15.
2. Select and view the best metabolite hit for each mass as described in "To view batch summary results" on page 17, or clear the box for the selected hit to ignore all the results for that metabolite.
3. (*Optional*) Modify the display of data in the Batch Summary tab as described in "To change how data is displayed in the Results table" on page 13.
4. Open the Export Results dialog box in *any* of the following ways:
   - Select **Export Results** from the File menu.
   - Click the  button on the toolbar.
   - Right-click in the results table and select **Export Results** from the shortcut menu in the Batch Summary results table.
5. (*Optional*) Click the **Select** button to specify a different file name, location, or file type for the export file.

NOTE A default file name, location, and format are provided for the export file as follows:

- If the mass list was opened from a file, the export file name is: **<massListFileName>-<timestampInSecs>.txt**
- If you entered the mass list manually, the export file name is: **MassList-<timestampInSecs>.txt**
- The default location for the export file is **MassHunter\Metlin\Results**.
- The default output file format is **.TXT** (tab-separated values), but you can select **.CSV** (comma-separated values) instead.

6. Select one or more of the following types of information to include in the report:
   - **Search parameters**
   - **Results**
   - **Unmatched masses** (available only if the **Results** checkbox is marked)
7. Mark the **Open results file after exporting** option to open the batch summary results file automatically in the associated application.
8. Click **OK** to output the results.
9. The results will open in the associated application such as Excel if you selected the **Open results file** option in Step 7. Otherwise open the exported results file in your application of choice at a later time.

NOTE

When a **.csv** file is opened in Excel, the Text Import Wizard may be displayed automatically. If so, follow the prompts to specify the proper delimiter for your file, e.g. comma or tab.

If the wizard is *not* displayed automatically, all the values for each row appear in the first column. To split the text into columns, select the first column (A), then start the wizard by selecting **Text to Columns** on the Data tab, in the Data Tools group.

Database operations

The following operations apply to both Master and custom databases and are described in the following pages:

- "To open a database" (below)
- "To back up a database" (below)
- "To view database information" on page 21

To open a database

1 Open the Select Database File dialog box in *either* of the following ways:

- Select **Open Database** from the File menu.
- Click the  button on the toolbar.

2 Select the database of interest (**.mtl** file extension) and click the **Open** button.

The following database files are supplied with the software:

- **Metlin.mtl** - the master METLIN database. This database *cannot* be edited, but can be copied to create a custom database that can be edited. See "To create a custom database" on page 22.
- **Metlin_Empty.mtl** - an empty master database, which can be used to create a custom database for importing metabolites. To create a database and import metabolites, see "To create a custom database" on page 22 and "To import metabolites" on page 25.

To back up a database

1 Select **Backup Database** from the File menu.

2 Select a folder for the backup file or accept the default folder, which is:

MassHunter\databases\Archive

3 Type in a file name for the backup file or accept the default file name, which is in the form:

<dbname>.<yyyy.mm.dd>.mtl

where **dbname** is the name of the current database, **yyyy** is the current year, **mm** is the current month, and **dd** is the current day of the month.

4 Click the **Save** button. A backup copy of the database is created using the specified file name and location.

To view database information

1. Select **Database Information** from the Database menu.
2. View the following information about the database:
   - Database file name
   - The number of metabolites (entries) in the database
   - Type (**Master** or **User**)
   - Edit allowed (**Yes** or **No**)
   - Version number
   - Description - the description can be edited if the current database is a custom database with editing enabled.
3. Click **OK** to close the Database Information dialog box.

Custom database operations

The following operations apply only to custom databases and are described in the following pages:

- "To create a custom database" (below)
- "To edit a custom database" (below). This includes adding and removing compounds.
- "To update retention time data" on page 24
- "To import metabolites" on page 25
- "To update a database from a previous version" on page 26

To create a custom database

1 Select **New Database** from the File menu or click the  button on the toolbar.

2 Do the following steps on the Create a New METLIN Database dialog box:

a Select a starting database for the new database. Use **Metlin_Empty.mtl** if you want to create an empty database for importing metabolites.

b Type in a name for the new database.

c Enter a description for the new database.

d Click the **Create** button to create the new database.

The new database becomes the current working database and is automatically enabled for editing.

To edit a custom database

Use this procedure to edit information for metabolites, to add a new metabolite, or to remove a metabolite.

1 Open the custom database of interest.

2 Enable editing by marking **Allow Editing** on the Database menu.

3 Do a search using *either* of the following:

- "To find metabolites" on page 11.
- "To find metabolites from a mass list" on page 15.

If you want to have the entire database available for editing, then use Single Search with no criteria specified. This search returns all entries in the database.

4 When the search is complete, click the **Edit Metabolites** tab.

5 To update information for a particular metabolite:

a Select the metabolite of interest by clicking on it in the Search Results table. Information for the selected metabolite is displayed in the Edit Metabolites tab.

b Update information on the left side of the Edit Metabolites tab, such as Name, Mass, RT, Formula, CAS, KEGG, HMP, LMP, and Radical ion type.

c Update structure information if desired in either of the following ways:

- Click on the MOL Text tab and paste in MOL file text that you have copied from a molecular drawing tool (**Ctrl+V**), *or*
- Click the  button in the Molecule area to open a **.MOL** file.

d Click the **Update Selected** button. The information for the metabolite is updated in the database and the information you entered in Step 5b is reflected in the Results table.

6 To add a new metabolite to the database:

a Click the **Add New** button. A new metabolite is created in the database with a mass of 0 named "New Compound" and the new compound is displayed at the end of the Results table.

b Enter or modify information for the new metabolite as described in Step 5 above.

c Click the **Update Selected** button. The information you entered for the new metabolite is updated in the database and the information is reflected in the Results table.

Alternate method to add a new metabolite

Enter information for a new metabolite on the left side of the Edit Metabolites tab, such as Name, Mass, RT, Formula, CAS, KEGG, HMP, LMP, and Radical ion type, then click the **Save As New** button. A new metabolite is created in the database using the specified values. The new compound is displayed at the end of the Results table.

7 To remove a metabolite from the database:

a Select the metabolite of interest by clicking on it in the Search Results table.

b Click the **Delete Selected** button.

To update retention time data

1. Open the custom database of interest. Be sure that editing is enabled by marking **Allow Editing** on the Database menu.
2. Click on the **Batch Search** tab.
3. Enter a mass list in *one* of the following ways:
   - Click the **File** button to the left of the mass list table to open a mass list file output by MassHunter Qualitative Analysis (**.csv** file), Mass Profiler (**.xls** file), or GeneSpring MS (**.txt** file).
   - Copy and paste a compound list from MassHunter WorkStation Software - Qualitative Analysis.
   - Type in masses and retention times directly into the table.

NOTE Every mass in the mass list must have a retention time value for this procedure.

4. Select *one* of the following options for **Retention times**:
   - **Optional** - Use this option to search a database that either has no retention time information or has retention times missing for some metabolites that you now want to update.
   - **Required** - Use this option if you only want to identify and update those metabolites that already have retention time information in the database. Specify an **RT tolerance** value in minutes as well.
5. Set other search criteria if desired as described in "To find metabolites from a mass list" on page 15.
6. Initiate the search in *any* of the following ways:
   - Click the **Find Metabolites** button on the toolbar.
   - Select **Find Metabolites** from the Database menu.
   - Press the **F5** key.
7. When the search is complete, the number of hits for each mass appears in the **Hits** column of the mass list, and the list is automatically sorted to show the masses with the most hits at the top of the list. To sort the list by a different criterion, click the heading for the column of interest (**Mass** or **RT**).

   Masses with conflicting hits are shown in red text in the Mass List table.
8. Click on a row in the Mass List table to display the metabolites found for that mass in the Search Results table in the lower part of the window. For more information on viewing batch search results, see "To view batch search results" on page 16.

9 Select and view the best metabolite hit for each mass as described in "To view batch summary results" on page 17 or clear the box for the selected hit to ignore that metabolite.

NOTE All conflicting results must be resolved before retention time data can be updated.

10 Click the **Apply Retention Times** button to apply retention times from the Mass List in the Batch Search tab for each of the metabolites listed in the Batch Summary table.

To import metabolites

Use the following procedure to import metabolites into a custom database or to create a custom database from a metabolite list created by another application. If you start with an empty database, then you are essentially creating a custom database from the imported metabolites.

1 Prepare the import file. The format required for the import file is described in the online help. The **.CSV** file can be created in *any* of the following ways:
   - Exported from METLIN Personal Metabolite Database software as described in "To export batch summary results" on page 18.
   - Exported from another application such as Agilent Mass Profiler or GeneSpring MS software.
   - Manually in Excel.
2 Open the custom database of interest or create a new database.

NOTE You can create an new database for importing metabolites from **Metlin_Empty.mtl** as described in "To create a custom database" on page 22.

3 Enable editing on this database by marking **Allow Editing** on the Database menu.
4 Select **Import Metabolites** from the File menu.
5 Select the **.CSV** or **.TXT** file prepared in Step 1 that contains the metabolites of interest, then click the **Open** button.
6 To view the new metabolites in the database, do a search on the Single Search tab with no criteria or criteria that you know will find the metabolites of interest. The metabolites will be listed in the Search Results table in the lower part of the window.

NOTE The mass is calculated from the formula, unless the difference is > 0.0001 Da. In that case, the Mass and Formula values will be as read from the input file, and those metabolites will be added to the file: **<ImportFileName>-MassErrorsLog.csv**, where **<ImportFileName>** is the name of the import file selected in Step 5. The information in the log file can be used to determine the correct Formula or Mass. Typically errors will occur when 1) the mass value was the mass from the spectrum and not a neutral mass, or 2) the formula contained the adduct (such as Cl).

7 Metabolites that were not imported due to problems with the input file are saved in the file **<ImportFileName>-ImportLog.csv**, where **<ImportFileName>** is the name of the import file selected in Step 5. Edit this log file to fix the problems, then import the corrected file.

8 (*Optional*) See "To edit a custom database" on page 22 if you want to modify information for the imported metabolites.

To update a database from a previous version

Use the following procedure to convert a custom database from a previous version to the format used in the current METLIN Personal Metabolite Database software. You can also update the content at the same time, if entries are from the METLIN metabolite database.

NOTE Databases created with a previous version can be opened and searched with the current software, but they *cannot be edited*, unless they are converted as described here.

1 Close any program that is using a METLIN database that you want to convert, such as MassHunter Qualitative Analysis or Mass Profiler. If the database of interest is open in METLIN Personal Metabolite Database, either exit the software or open a different database. Otherwise you will get a warning message when you start the utility in Step 2.

NOTE Databases that are currently in use by another application *cannot* be updated.

2 Open the Database Update Utility in *either* of the following ways:

- Select **Run METLIN Database Update Utility** from the Database menu in METLIN Personal Metabolite Database software window.
- Select **Programs > Agilent > MassHunter Workstation > METLIN Personal Metabolite Database Update Utility** from the Windows Start menu.

3 In the section labeled **Step 1. Select the databases to update**:

a Click the **Select** button to open a file select dialog box.

b Select one or more databases to update (***.mtl** files).

c Click the **Open** button. The selected databases appear in the Databases list.

4 In the section labeled **Step 2. Select the type of update**, select the appropriate option from the following choices:

- If the database contains entries with METLIN ID#s that you want to update from the current METLIN metabolite database, then select **Update all content and convert to new format** to update. Entries that don't have a METLIN ID# are unchanged.
- If the database does *not* contain information from METLIN metabolite database or you do *not* want to update the entries, then select **Just convert to new format**.

5 In the section labeled **Step 3. Convert the selected databases**, click the **Convert** button to start converting the selected databases.

Progress is shown in the box, as well as any errors that occur. If a selected database is already in the new format, it will be skipped. (**Metlin.mtl** is always skipped, because it is assumed to be the new version.)

Backup copies of the databases in their original format are copied to the **Archive** folder (a subdirectory of the **Databases** folder).

6 When finished, click the **Close** button to exit the Database Update Utility.

Familiarization Tutorial

Exercise 1. Search for metabolites in a database

Steps	Detailed Instructions	Comments
1 Open the master METLIN database.	**a** Select **Open Database** from the File menu, *or* click the  button on the toolbar. **b** Select the **Metlin.mtl** file, and click the **Open** button.	Skip this step if the master METLIN database is already open.
2 Click on the **Single Search** tab.		
3 Leave the search fields blank.	Be sure that the **Require** checkbox for Retention time is *cleared*, since the master METLIN database does *not* contain retention time information.	This will return the contents of the entire database. See "To find metabolites" on page 11 for a discussion of search parameters to narrow your search.
4 Click the **Find Metabolites** button on the toolbar to begin searching.		You can also start the search in either of the following ways: • Select **Find Metabolites** from the Database menu. • Press the **F5** key.
5 Review the search results in the table in the lower half of the window.		The results are sorted from low mass to high mass.
6 View structure information for a selected metabolite.	**a** Click on a metabolite in the Results table. Its structure is displayed in the Structures area on the right side of the window. **b** Click on the **MOL Text** tab to view the text version of the structure. **c** Click the  button to view a larger image of the structure.	The structure for the selected metabolite is displayed if it is available in the database.
7 Sort the table by name.	Click the heading of the **Name** column. Click it again to sort by name in the opposite order.	Clicking on any column heading will sort the table according to the values in that column.

Steps	Detailed Instructions	Comments
8 Reorder the columns in the table.	**a** Drag a column heading to a different position. **b** Repeat until the desired order is achieved.	
9 Resize a column.	You can resize columns in either of the following ways: • Double-click on the right column divider in the heading of the column of interest to resize the column to fit the information contained in it. • Drag the separator between column headings to the right or left until the column of interest is the desired width.	
10 Change which columns of information are displayed in the table.	**a** Right-click in the table and select **Add/Remove Columns** from the shortcut menu. **b** Move the items of information that you want to display from the **Available columns** list into the **Show these columns** list using the **Add->** button. **c** Move the items of information that *you don't want to display* out of the **Show these columns** list into the **Available columns** list using the **<-Remove** button. **d** Click **OK**.	Alternate Methods: • You can also open the Add/Remove Columns dialog box by selecting **Add/Remove Columns** from the View menu. • You can move individual items between the two lists by double-clicking them. Note that the original column layout can be restored at anytime by selecting **Restore to Default Columns** from the View menu.
11 Print results.	**a** To print only selected results, highlight those rows in the Results table using **Ctrl+click**., then select **Only highlighted rows** as one of the print options in Step 11c below. **b** Click the  button on the toolbar to open the Print Results dialog box. **c** Select print options. For a discussion of print options, see "To print search results" on page 13. **d** Click **OK**. If you selected the Print Preview option, review the report in the Print Preview window, then click the  button to print it.	You can also open the Print Results dialog box in either of the following ways: • Select **Print Results** from the File menu • Right-click in the results table and select **Print Results** from the shortcut menu.

Exercise 2. Batch search from a mass list

Steps	Detailed Instructions	Comments
1 Open the master METLIN database.	**a** Select **Open Database** from the File menu, *or* click the  button on the toolbar. **b** Select the **Metlin.mtl** file, and click the **Open** button.	Skip this step if the master METLIN database is already open, as it will be after Exercise 1.
2 Click on the **Batch Search** tab.		
3 Open a mass list.	**a** Click the **File** button to the left of the mass list table. **b** Select the **RBC_Metabolites_Neg.txt** mass list file in the **MassHunter\Metlin\Mass List Files** folder. **c** Click the **Open** button.	• Mass lists can also be copy-and-pasted into METLIN Personal Metabolite Database software from Qualitative Analysis. • See online help in MassHunter Qualitative Analysis for instructions on how to output a mass list.
4 Set the search parameters.	**a** Select the **Neutral Masses** option and set the **Mass tolerance** value to 10 ppm (the default). **b** Mark **Ignore** for **Retention times**. **c** Mark only the **Include neutrals** option for **Radical ion search mode**.	The master METLIN database does *not* include retention times.
5 Click the **Find Metabolites** button on the toolbar to begin searching.		You can also start the search in either of the following ways: • Select **Find Metabolites** from the Database menu, *or* • Press the **F5** key.
6 Review search results.	When the search is complete, the number of hits for each mass appears in the **Hits** column of the mass list, and the list is automatically sorted to show the masses with the most hits at the top of the list. Masses with conflicting hits are shown in red text in the Mass List table. See Figure 1 on page 32.	To sort the mass list by a different criterion, click the heading for the column of interest (**Mass** or **RT**).

Steps	Detailed Instructions	Comments
7 View hits for selected masses.	Click on a row in the Mass List table to display the metabolites found for that mass in the Batch Search Results table in the lower part of the window.	For ease of reviewing results, use the **F4** key to scroll down the Mass List and the **Shift**+**F4** keys to scroll back up. The information in the Results table will be updated as you scroll through the mass list. The batch search results table can be resorted as described in "To change how data is displayed in the Results table" on page 13.
8 Review the entries marked in the Best column for each mass and edit the Batch Search Results table to select the best metabolite hit for a particular mass.	Mark the **Best** column for the row (metabolite) you believe is the best metabolite hit for each mass. In many cases, this will be the one already selected by the software.	You can also clear the box for the selected hit to ignore all the results for that metabolite, as another way of resolving the conflict.
9 View the best hit results.	Click the **Batch Summary tab**. The Best hit for each mass is shown in the Batch Summary table in the lower part of the window. See Figure 2 on page 33.	Compounds with conflicting mass hits are shown in red text in the results table. The batch summary results table can be resorted as described in "To change how data is displayed in the Results table" on page 13.
10 Export batch summary results.	**a** Click the  button on the toolbar to open the Export Results dialog box. **b** Select the options to include **Search parameters**, **Results**, and **Unmatched masses**. **c** Mark the **Open results file after exporting** option. **d** Click **OK** to output the results. The results will open in the associated application such as Excel. The following export file is created in the **MassHunter\Metlin\Results** folder: **RBC_Metabolites_Neg-<timestamp>.txt**	You can also open the Export Results dialog box in either of the following ways: • Select **Export Results** from the File menu. • Right-click in the Batch Summary results table and select **Export Results** from the shortcut menu. You can select a different file name, location or type as described in "To export batch summary results" on page 18.

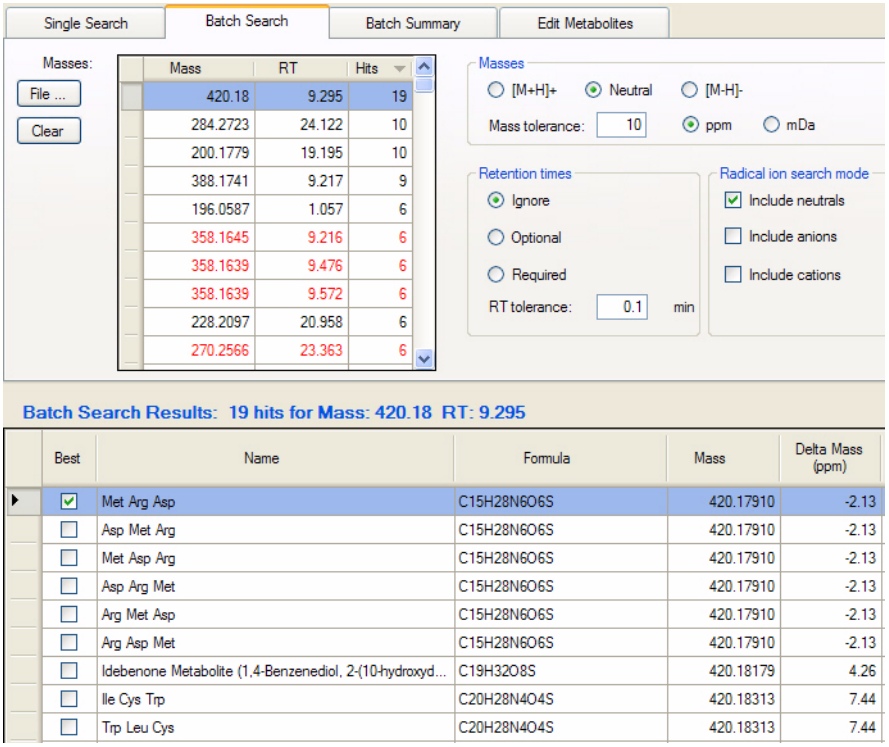
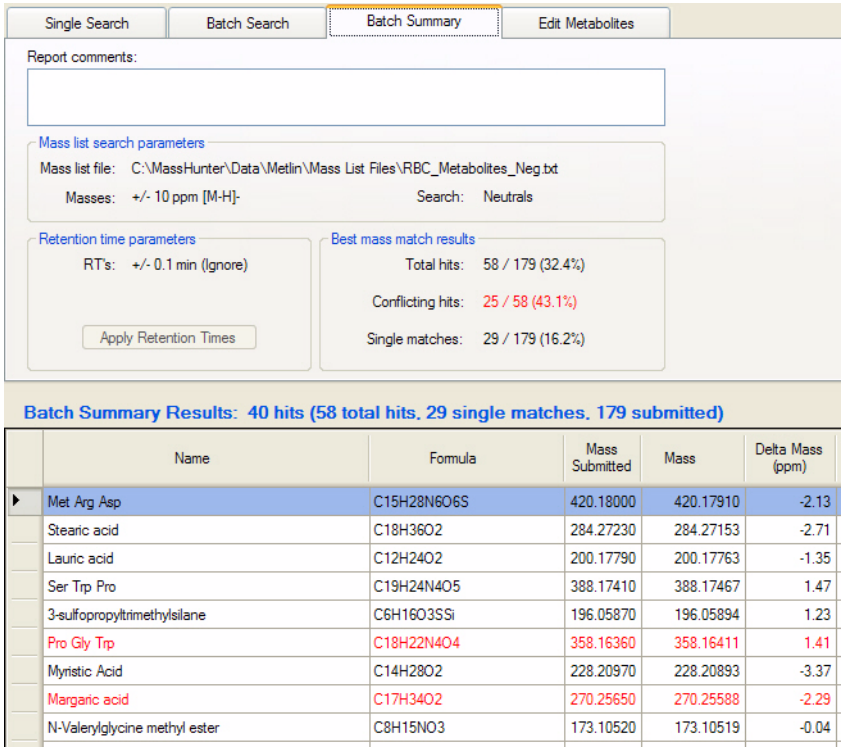


Figure 1 Results of Batch Search



Name	Formula	Mass Submitted	Mass	Delta Mass (ppm)
Met Arg Asp	C15H28N6O6S	420.18000	420.17910	-2.13
Stearic acid	C18H36O2	284.27230	284.27153	-2.71
Lauric acid	C12H24O2	200.17790	200.17763	-1.35
Ser Trp Pro	C19H24N4O5	388.17410	388.17467	1.47
3-sulfopropyltrimethylsilane	C6H16O3SSi	196.05870	196.05894	1.23
Pro Gly Trp	C18H22N4O4	358.16360	358.16411	1.41
Myristic Acid	C14H28O2	228.20970	228.20893	-3.37
Margaric acid	C17H34O2	270.25650	270.25588	-2.29
N-Valerylglycine methyl ester	C8H15NO3	173.10520	173.10519	-0.04

Figure 2 Batch Summary Results

Exercise 3. Create a custom database

In this exercise, you will create a custom database that is a subset of the master METLIN database.

Steps	Detailed Instructions	Comments
1 Open the Create a New METLIN Database dialog box.	Select **New Database** from the File menu, or click the  button on the toolbar.	
2 Create the new database.	**a** Select **Metlin.mtl** as the starting database for the new database. **b** Type in a name for the new database, e.g. **Metlin_Subset**. **c** Enter a description for the new database, such as "My database". **d** Click the **Create** button to create the new database.	The new database becomes the current working database and is automatically enabled for editing.
3 Edit the database.	See "Exercise 4. Edit a custom database".	

Exercise 4. Edit a custom database

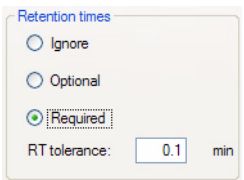
In this exercise, you will edit the custom database that you created in Exercise 3.

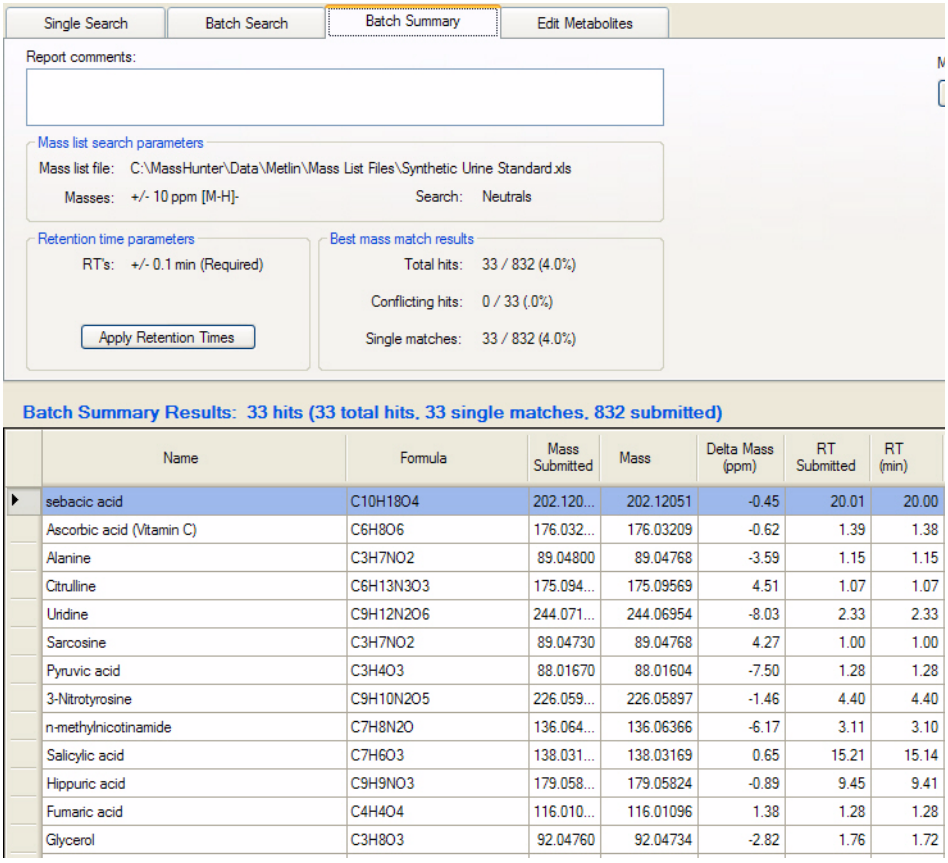
Steps	Detailed Instructions	Comments
1 Open the custom database you created in Exercise 3, if it is not already open.	**a** Select **Open Database** from the File menu, *or* click the  button on the toolbar. **b** Select the **Metlin_Subset.mtl** file, and click the **Open** button.	Skip this step if the custom database is already open, as it will be if you just completed Exercise 3.
2 Verify that editing is allowed for this database.	Open the **Database** menu and verify that there is a check mark next to **Allow Editing**.	
3 Do a search that returns the entire contents of the database.	**a** Click the **Single Search** tab. **b** Leave the search fields blank. **c** Click the **Find Metabolites** button on the toolbar to start the search. The search results appear in the table in the lower half of the window.	See "Exercise 1. Search for metabolites in a database" on page 28 for more information.
4 Click the **Edit Metabolites** tab.		
5 Update information for a particular metabolite.	**a** Select the metabolite of interest by clicking on it in the Search Results table. **b** Update information on the left side of the Edit Metabolites tab, such as Mass, RT, Formula, Name, Radical ion type, KEGG ID, CAS ID, HMP ID, and LMP ID. **c** Click the **Update Selected** button to apply the changes to the database.	Information for the selected metabolite is displayed in the Edit Metabolites tab. The updated information will be changed in the database and reflected in the Results table.
6 Update structure information for a particular metabolite.	**a** Select the metabolite of interest by clicking on it in the Search Results table. **b** Click the  button in the Molecule area to open a **.MOL** file. **c** Click the **Update Selected** button. The information you entered will be changed in the database.	You can also update structure information for a selected metabolite by clicking on the **MOL Text** tab and pasting in MOL file text that you copied from a molecular drawing tool (**Ctrl**+**V**).

Steps	Detailed Instructions	Comments
7 Add a new metabolite to the database.	**a** Click the **Add New** button. A new metabolite is created in the database with a mass of 0 named "New Compound", and the new compound is displayed at the end of the Results table. **b** Enter or modify information for the new metabolite as described in Step 5 above. **c** Click the **Update Selected** button. The information you entered for the new metabolite is updated in the database and the information is reflected in the Results table.	**Alternate method:** **a** Enter information for a new metabolite on the left side of the Edit Metabolites tab, such as Mass, RT, Formula, Name, Radical ion type, KEGG ID, CAS ID, HMP ID. and LMP ID. **b** Click the **Save As New** button. A new metabolite is created in the database using the specified values. The new compound is displayed at the end of the Results table.
8 Remove a metabolite from the database.	**a** Select the metabolite of interest by clicking on it in the Search Results table. **b** Click the **Delete Selected** button.	The selected metabolite is removed from the database.

Exercise 5. Add retention times to a database

In this exercise, you will add retention times to a custom database.

Steps	Detailed Instructions	Comments
1 Open the custom database you created in Exercise 3, if it is not already open.	**a** Select **Open Database** from the File menu, *or* click the  button on the toolbar. **b** Select the **METLIN-Urine.mtl** file, and click the **Open** button.	This sample database has ~70 urinary metabolites with retention times.
2 Enable editing on this database.	Open the **Database** menu and mark **Allow Editing**.	
3 Click the **Batch Search** tab.		
4 Open a mass list file that contains retention time information for the masses.	**a** Click the **File** button to the left of the mass list table. **b** Select **Synthetic Urine Standard.xls** in the **MassHunter\Metlin\Mass List Files** folder. **c** Click the **Open** button.	• This mass list was output from Mass Profiler. • Each mass in the mass list must have an associated retention time value.
5 Set the search parameters.	**a** Mark **Retention times Required** as shown below: 	
6 Start the search.	Click the **Find Metabolites** button on the toolbar.	
7 Click the **Batch Summary** tab.	See Figure 3 below.	There are no conflicting hits in this example.
8 Update retention times in the database from the current mass list.	Click the **Apply Retention Times** button.	Retention time information is updated in the database for each of the metabolites listed in the Batch Summary table.



Name	Formula	Mass Submitted	Mass	Delta Mass (ppm)	RT Submitted	RT (min)
sebacic acid	C10H18O4	202.120...	202.12051	-0.45	20.01	20.00
Ascorbic acid (Vitamin C)	C6H8O6	176.032...	176.03209	-0.62	1.39	1.38
Alanine	C3H7NO2	89.04800	89.04768	-3.59	1.15	1.15
Citrulline	C6H13N3O3	175.094...	175.09569	4.51	1.07	1.07
Uridine	C9H12N2O6	244.071...	244.06954	-8.03	2.33	2.33
Sarcosine	C3H7NO2	89.04730	89.04768	4.27	1.00	1.00
Pyruvic acid	C3H4O3	88.01670	88.01604	-7.50	1.28	1.28
3-Nitrotyrosine	C9H10N2O5	226.059...	226.05897	-1.46	4.40	4.40
n-methylnicotinamide	C7H8N2O	136.064...	136.06366	-6.17	3.11	3.10
Salicylic acid	C7H6O3	138.031...	138.03169	0.65	15.21	15.14
Hippuric acid	C9H9NO3	179.058...	179.05824	-0.89	9.45	9.41
Fumaric acid	C4H4O4	116.010...	116.01096	1.38	1.28	1.28
Glycerol	C3H8O3	92.04760	92.04734	-2.82	1.76	1.72

Figure 3 Batch Summary Results with Retention Time Required

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In this Book

This *Quick Start Guide* presents first steps to install and use the METLIN Personal Metabolite Database for MassHunter Workstation.

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