

Agilent MassHunter LC/SQ ChemStation Integration Software

Quick Start Guide

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This guide describes how to use the Agilent MassHunter LC/SQ ChemStation Integration Software version B.04.00. This software has two main parts: an interactive translator and an automated translator that is run when you run a ChemStation method.

Interactive translator

You can use this program to manually translate one or more ChemStation LC/SQ data files to the MassHunter format. Data files are translated outside of the ChemStation program in a separate program, *LC/SQ ChemStation to MassHunter Translator*.



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Automated translator

The automated translator is run as part of a ChemStation method. After the data is acquired, the data can be automatically translated to the MassHunter data format. In addition, you can automatically run a MassHunter Qualitative Analysis method when you run a ChemStation method.



What is the Agilent MassHunter LC/SQ ChemStation Integration Software?

These programs let you translate LC/MS ChemStation data files to the MassHunter format so that they can be analyzed with the Agilent MassHunter Workstation Software - Qualitative Analysis and Quantitative Analysis programs. You can either translate the data file after it is acquired automatically as part of running the method or interactively translate the data file later.

LC/MS ChemStation Data and the Qualitative Analysis Program

With the Qualitative Analysis program, you can do Find Compounds by Formula on your translated LC/MS ChemStation data. The Qualitative Analysis program can also present large amounts of data for review in one central location. You can do these tasks for any type of LC/MS data that you open:

- Extract and display chromatograms.
- View and extract peak spectra.
- Subtract background.
- Integrate the chromatogram.
- Create basic reports from preconfigured templates.
- Customize report templates using the Report Designer Add-in that is run in Microsoft Excel.

You can also set up methods to automatically do the tasks in the list, as well as others, when you open the data files.

LC/MS ChemStation Data and the Quantitative Analysis Program

You can also quantitate your translated LC/MS ChemStation data with the Quantitative Analysis program.

You can quantitate very low amounts of material with the following unique features:

• Curve Fit Assistant to test all fits and statistics on curve quality.

- Automated, parameter-free integrator that uses a novel algorithm, which has been optimized for triple quad data, but can work well for single quad data also.
- Batch Table window to help you review and operate on an entire batch of data at once.
- Automatic detection and identification of outliers.
- Compounds-at-a-Glance.
- Rapid set up of Quantitative Analysis methods using information in the Acquisition method (compound names, SIM ions, ISTD designations, etc.).
- Easy export of the Batch Table to Excel spreadsheets or upload to a LIMS system.

Where to find more information

- Use the online Help for in-depth information not given in this *Quick Start Guide*. Two different online Help files are available for the ChemStation Integration program. Press **F1** in the interactive LC/SQ ChemStation to MassHunter Translator program to open the online Help for that program. Click the **Help** button in the dialog boxes in the ChemStation program to open the online Help for the automated ChemStation Integration program.
- Use the online Help for the Qualitative Analysis program and the Quantitative Analysis program to learn how to use these programs. Click the Help > Contents command or press F1 in either program to open the online Help for that program.
- Refer to these guides to learn how to use the Agilent MassHunter Workstation Software data analysis programs. They are included with the MassHunter Workstation software:
 - Qualitative Analysis Familiarization Guide
 - Quantitative Analysis Familiarization Guide

Installation

- 1 Check that no other program is running on your system, including Windows Update.
- **2** If a previous version of the Agilent MassHunter LC/SQ ChemStation Integration Software is present, use the Windows Add or Remove Programs in the Control Panel to remove any previous versions.
- **3** If you have an installation disc:
 - **a** Put the installation disc into the disc drive.
 - **b** Browse to the folder MassHunter LC & SQ ChemStation Integration Software.
- 4 If you do not have an installation disc, copy the file CSIntegrationSetup_xxx.exe to your local hard drive.
- 5 For Windows 7 systems, right-click CSIntegrationSetup_xxx.exe and then click Run as Administrator. For Windows XP systems, double-click CSIntegrationSetup_xxx.exe.
- 6 In the Welcome page, click Next.
- 7 In the License Agreement page, click I accept the terms of the license agreement, then click Next. If you want to print the License Agreement, click Print and the License Agreement is printed to the default printer.
- 8 In the Choose Destination Location page, click Next to accept the default destination location.

The default is C:\Program Files\Agilent\MassHunter\Workstation\ ChemStationIntegration.

9 In the **Choose location for customer data files** page, click **Next** to accept the default destination location.

The default is **D:\MassHunter**.

- 10 In the Start Copying Files page, review the settings and then click Next.
- 11 When the InstallShield Wizard Complete message is displayed, click Finish.
- **12** Open the **readme.txt** file on the installation media.

Getting Started

This section contains the tasks you need to know to operate the LC/SQ Chemstation to MassHunter Translator program.

These tasks are used in the interactive MassHunter Translator program:

- "To select one or more data files to translate" on page 6
- "To select the folder to use to place the translated data files" on page 7
- "To manually translate data files" on page 7

These tasks are used in the automated MassHunter Translator program:

- "To enable ChemStation Integration to MassHunter Qualitative Analysis" on page 8
- "To disable integration with MassHunter Qualitative Analysis" on page 9
- "To link Qualitative Analysis Method" on page 9
- "To remove link to Qualitative Analysis Method" on page 10

To select one or more data files to translate

This task is part of the interactive ChemStation Integration program.

- **1** Start the ChemStation Integration program. Do one of the following:
 - Click Start > All Programs > Agilent > MassHunter Workstation > LC-SQ ChemStation Translator
 - Click Start > Programs > Agilent > MassHunter Workstation > LC-SQ ChemStation Translator
- **2** Select the LC/MS ChemStation data file (s) that you wish to translate from the File Browser list.

To choose multiple files that are listed next to each other, press the **Shift** key while selecting the data files with the left mouse button.

To choose multiple files that are not listed next to each other, press the **Ctrl** key while selecting the data files with the left mouse button.

3 Click **1** in the File Browser toolbar.

You can also right-click the File Browser pane and then click **Add File(s)** from the shortcut menu.

To select the folder to use to place the translated data files

You can either place the translated data files into the same folder as the input data file or you can select a new folder.

This task is part of the interactive ChemStation Integration program.

Same folder as data files

1 Click Same location as input data file under Data file creation.

Selected folder

- 1 Click At location under Data file creation.
- **2** Click under **Data file creation**. The **Browse For Folder** dialog box is opened.
- **3** Select the folder where you want to place the translated files. You click **Make New Folder** to create a new folder.

If the MassHunter Workstation Software - Qualitative Analysis program or Quantitative Analysis program is installed on the same computer, you may select the default MassHunter data folder that is usually **D:\MassHunter\ Data**. If the folder that you specify does not exist when you start the translation, the translation fails.

4 Click OK.

To manually translate data files

This task is part of the interactive ChemStation Integration program.

- **1** Do the steps in "To select one or more data files to translate" on page 6.
- **2** Do the steps in "To select the folder to use to place the translated data files" on page 7.

- **3** Click **()** in the Translation File List toolbar.
- **4** You can also right-click the Translation File List pane and click **Translate** in the shortcut menu to start translating the selected data files.
- **5** Check the **Status** column to verify that each translation completed successfully.

To enable ChemStation Integration to MassHunter Qualitative Analysis

This task is part of the automated ChemStation Integration that is part of the ChemStation program.

- Click MassHunter > Configuration in the LC/MS ChemStation's Method and Run Control view. The MassHunter LC/SQ Integration Configuration dialog box is opened.
- **2** Mark the **Translate to MassHunter Data** check box. To use the ChemStation data file in the MassHunter Qualitative Analysis program, the ChemStation data file needs to be translated to the MassHunter format.
- **3** Click either the **Same location as input data file** button or the **At location** button.
- **4** If you click **At location**, then click the **Browse** button. The **Select a destination folder** dialog box is opened. Then, select the folder where you want to place the translated data file and click **OK**.
- **5** Mark the **Run MassHunter Qualitative Analysis Method** check box. When this check box is marked, the MassHunter Qualitative Analysis Method is automatically run as part of the ChemStation method.

6 Click OK.

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OK		Cancel	Help			

To disable integration with MassHunter Qualitative Analysis

This task is part of the automated ChemStation Integration that is part of the ChemStation program.

- 1 Click MassHunter > Configuration in the LC/MS ChemStation's Method and Run Control view. The MassHunter LC/SQ Integration Configuration dialog box is opened.
- **2** Clear the **Translate to MassHunter Data** check box. If this check box is cleared, then the ChemStation data file is not automatically translated.
- **3** Clear the **Run MassHunter Qualitative Analysis Method** check box. When this check box is cleared, the MassHunter Qualitative Analysis Method is not run as part of the ChemStation method.
- 4 Click OK.

To link Qualitative Analysis Method

This task is part of the automated ChemStation Integration that is part of the ChemStation program.

1 Click **MassHunter > Edit Link DA Method** in the main menu.

- **2** Select the correct folder in the **Folders** list box. The name of the selected folder is shown directly above the list box. If necessary, select a different drive in the Drives box.
- **3** In the list box under the **Name** field, select the MassHunter Qualitative Analysis method name.
- **4** Click the **OK** button. You can also double click the name of the method in the list box to close this dialog box.

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Types:	Drives:	

5 In ChemStation, click the Method > Run Time Checklist command. In the Run Time Checklist dialog box, mark the Standard Data Analysis check box.

To remove link to Qualitative Analysis Method

This task is part of the automated ChemStation Integration that is part of the ChemStation program.

- 1 Click the **MassHunter > Remove linked DA Method** command.
- **2** Verify that you want to remove the link to the MassHunter Qualitative Analysis method that is shown in the box.
- **3** Click the **OK** button.

Interactive LC/SQ Chemstation To MassHunter Translator Panes

Main Window

This section contains information on the user interface for the LC/SQ Chemstation to MassHunter Translator program. The user interface contains four different panes:

- "File Browser Pane" on page 11
- "Translation Parameters Pane" on page 12
- "Translation File List Pane" on page 12
- "Translation Status Details Pane" on page 14

This program is started from the Agilent MassHunter Workstation group. You click the **LC/SQ Chemstation Translator** command.

File Browser Pane

You can browse through the files on the system and select LC/MS ChemStation data files that you wish to translate. If you double-click on the name of an LC/MS ChemStation data file, that data file name is added to the Translation File List table. You can also right-click this pane and click **Add File(s)** from the shortcut menu to add one or more files to the Translation File List table.

Toolbar



ø

Adds the currently selected Chemstation data file to the Translation File List table

Refreshes the File Browser list

Shortcut Menu

Add File(s) Adds the currently selected files to the Translation File List table.

Translate Adds the currently selected files to the Translation File List table and starts the translation process for the files marked for translation.

Translation Parameters Pane

Data file creation

Same location as input data file The translated data files are placed in the same folder as the LC/MS ChemStation data files. If you click this option, then the MassHunter version of the data will be added to the ChemStation .D folder, and the same data file can be processed in ChemStation and MassHunter.

At location The translated LC/MS ChemStation data files are placed in the folder specified. You click — to select a different folder. If the specified folder does not exist when you translate the data files, the translation fails.

Overwrite files

Prompt before overwriting When the translation program is running, a message box is displayed if a data file would be overwritten. This message box asks whether or not you want to overwrite the existing data file.

Always overwrite When the translation program is running, data files are overwritten if the same name as an existing data file is used. No message box is displayed.

Translation Parameters Toolbar



Restores the default parameters in the Translation Parameters pane

Translation File List Pane

Translation File List Table

Check Box Allows you to select which files to translate. When a data file is first added to the **Translation File List** table, the check box in that row is marked. When the data file is translated, the check box is cleared. When new data files are added to this table, they are appended to the end of the table and the check boxes are marked automatically.

Data File(s) The name of the data file to translate.

Status The status of the translation process. When you first add a data file to the Translation File List table, the **Status** column is set to **Not Started**. When a file has been translated, the **Status** column is set to **Completed**. If an error occurs during the translation, the **Status** column is set to **Failed**.

An icon is placed next to the **Status** if a problem occurred.



An error occurred while the file was being translated. The problem is described in the **Message (if any)** column.



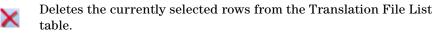
A warning occurred while the file was being translated. The warning is described in the **Message (if any)** column.

Message (if any) If an error or warning occurred while translating the ChemStation data file, the error or warning message is listed in this column.

Toolbar

Starts translating the files that are listed in the Translation File List table.

Stops translating the files. This button is only available when you are translating files.



Clears all lines in the Translation File List table.



Toggles whether or not to show the Translation Status Details pane.

Shortcut Menu

Translate Starts translating the files listed in the Translation File List table.

Stop Stops translating the files listed in the Translation File List table.

Delete Rows Deletes the currently selected row or rows from the Translation File List table.

Clear Table Deletes all rows in the Translation File List table.

Set check mark on highlighted files Marks the check box in the Check Box column for each row that is highlighted.

Remove check mark on highlighted files Clears the check box in the Check Box column for each row that is highlighted.

Translation Status Details Pane

File The name of the file that is selected in the Translation File List table.

Translation Status The status of the translation process. Possible values include:

- Not Started
- Completed
- Completed with warning
- Failed

Translation Time The length of time it took to translate the ChemStation file.

Message (if any) If an error or warning occurred during translation, it will be displayed here.

Error Details (if any) If an error or warning occurred during translation, details from the program are shown here. This information is for internal use only.

Shortcut menu

Copy Copies the information in this pane to the Clipboard.

Select All Selects all information in this pane.

Automated LC/SQ Chemstation To MassHunter Translator Menu

This section contains information on the user interface for the Automated LC/SQ Chemstation to MassHunter Translator program. This user interface is part of the LC/MSD ChemStation user interface.

- "MassHunter Menu" on page 15
- "Translation Parameters Pane" on page 12
- "Translation File List Pane" on page 12
- "Translation Status Details Pane" on page 14

MassHunter Menu

Configuration Opens the "MassHunter LC/SQ Integration Configuration Dialog Box" on page 15.

Edit Link DA Method Opens the "Link to Masshunter DA Method Dialog Box" on page 16.

Remove Link DA Method Opens the "Remove linked DA Method Dialog Box" on page 17.

About Opens the **About** dialog box.

MassHunter LC/SQ Integration Configuration Dialog Box

You select whether or not to translate the ChemStation data file automatically here, and you also select whether or not to run the MassHunter Qualitative Analysis method as part of running a method in ChemStation.

PostRun Options

Translate to MassHunter Data Whether or not to automatically translate the ChemStation data format to the MassHunter data format.

If this check box is marked, then any ChemStation method that is run needs to have a MassHunter Qualitative Analysis method linked to it. When you save a ChemStation method, the software checks if a MassHunter Qualitative Analysis method is linked to the current ChemStation method. If no Qualitative Analysis method is linked, you are automatically asked to select a MassHunter Qualitative Analysis method to link to the ChemStation method that you are saving.

Data file creation

Same location as input data file The MassHunter data file that is created is placed in the same folder as the ChemStation data file.

At location The Masshunter data file that is created is placed in the specified folder. You can directly type the folder name in the text box.

Browse button Opens the **Select a destination folder** dialog box. After selecting the location to place the data file, you click **OK** to close the dialog box.

Run MassHunter Qualitative Analysis Method Whether or not to automatically run the Qualitative Analysis method when the ChemStation method is run. If this check box is marked but the data file was not translated, the MassHunter Qualitative Analysis method is not run.

Link to Masshunter DA Method Dialog Box

You select the method to run in the MassHunter Qualitative Analysis program in this dialog box. This method is linked to the current ChemStation method.

Name The name of the MassHunter Qualitative Analysis method to link to. This MassHunter method is run when you mark the option to run the MassHunter Method in the **MassHunter LC/SQ Integration Configuration** dialog box.

Type Either Method(*.M) or All Types (*.*). This value limits the type of files and folders that are shown in the list box below the Name box. You select the MassHunter method in the list box below the Name box. The value Method(*.M) is the proper value in this dialog box.

Folders The folder that is currently shown in the list box below the Name box. This is the path to the MassHunter method. You can change this path by selecting a different folder in the list box below this.

Drives The currently selected drive. You can switch to a different drive if the MassHunter method is stored on a different drive.

Remove linked DA Method Dialog Box

You can remove the link to a MassHunter Qualitative Analysis method by using this dialog box. You are only removing the link. The MassHunter Qualitative Analysis method is not removed or changed.

DA Method to be removed The name of the MassHunter Qualitative Analysis method that is currently linked to this ChemStation method. If you click OK, the link to this MassHunter method is removed.

The MassHunter Qualitative Analysis Method is not removed.

NOTE

Familiarization Exercises for Automated Workflows

This section shows you the different workflows that you can do when you combine the LC/MSD ChemStation Data Acquisition software with the MassHunter Qualitative Analysis program and the MassHunter Quantitative Analysis program.

- "Exercise 1. Synthetic compound confirmation and purity assessment" on page 19
- "Exercise 2. Protein Confirmation" on page 29
- "Exercise 3. High Throughput Screening" on page 30
- "Exercise 4. Quantification (Target Compound Analysis)" on page 33

Exercise 1. Synthetic compound confirmation and purity assessment

The MassHunter Qualitative Analysis program can confirm the molecular weight that you enter in the Sequence table.

The following three tasks will lead you through this workflow.

- "Task 1. Set up Molecular Weight Confirmation method in MassHunter Qualitative Analysis" on page 20
- "Task 2. Set up ChemStation Method and ChemStation" on page 25
- "Task 3. Run a sequence in ChemStation" on page 27

Task 1. Set up Molecular Weight Confirmation method in MassHunter Qualitative Analysis

In this task, you use the Qualitative Analysis program to update the worklist parameters to run a Molecular Weight Confirmation algorithm. The worklist actions are run when the Qualitative Analysis method is run.

Steps		Detailed Instructions	Comments	
1	In MassHunter Qualitative Analysis, open your most up-to-date method for this analysis.	 a Double-click the Qualitative Analysis icon. b Open the method default.m. c Save the method to a new name (for example, <i>iii_MolecularWeight</i> <i>Confirmation.m</i>, where <i>iii</i> are your initials. 	 Make sure that you are opening a MassHunter Qualitative Analysis method. 	
2	Set up the parameters for the Find Compounds by Formula algorithm.	 a Click Find by Formula - Options in the Find Compounds by Formula section in the Method Explorer. b Click the Formula Source tab. c Click the Worklist button. d Review the other parameters in this section. 	 You can use any of the demo files that are supplied with the software to test the worklist actions. You can get help on any tab in the Method Editor window by pressing the F1 key. 	

× Method Editor: Find Compounds by Formula - Options				
🚮 🔄 - 🍽 - 💽 - Method Items- 😕 🎼				
A Formula Source Formula Matching Positive lons Negative lons Scoring Ret				
(type a comma-separated list of form	tulas, e.g., "C6H6, CH4")			
Compound exchange file (.CEF):				
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O Mass and retention time (retention time optional)				
Mass and retention time (retention tim	ne required)			
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Figure 1 Find Compounds by Formula - Options section in the Method Editor in Qualitative Analysis

Steps	Detailed Instructions	Comments
 3 (optional) Set up the parameters for the Sample Purity options of the Find Compounds by Formula algorithm. 	 a Click Find by Formula - Sample Purity in the Find Compounds by Formula section in the Method Explorer. b Click the Options tab. c Mark the Compute sample purity check box. d Review the other parameters in this section. 	

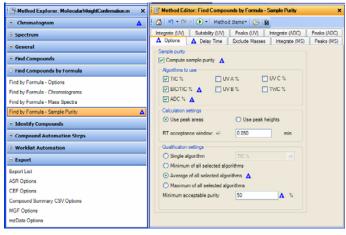


Figure 2 Find Compounds by Formula - Sample Purity section in the Method Editor in Qualitative Analysis

4 Set up the parameters for generating a compound report	 a Click Compound Report in the General section in the Method Explorer. b Mark the sections that you want to include in the report in this tab. c Click Common Reporting Options in the General section in the Method Explorer. d (optional) Change the template for the Compound report template. e Click the Options tab. f Review the parameters. 	• If you want to customize a report, you can learn how by using the Agilent MassHunter Workstation Software Reporting Familiarization Guide.
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Familiarization Exercises for Automated Workflows

Steps	Detailed Instructions	Ca
170	Method Editor: Compound Report	
Method Explorer: Default.m		^
+ Chromatogram	👬 🤟 - 🕅 - Method Items - 😕 🗿	
Spectrum	Compounds I Show compound table	
🖃 General	Sort by: Retention time	
Analysis Report		
Compound Report	Sort order: Increasing Exclude details for unidentified compounds	
Common Reporting Options		
File Open Actions	Chromatograms	
Extraction Data Format	Show user chromatogram(s)	
Find Compounds	Show compound chromatogram(s)	
Find Compounds by Formula	Compound spectrum (MS)	
Find by Formula - Options		
Find by Formula - Chromatograms	Show predicted isotope match table	
Find by Formula - Mass Spectra	Show MS spectrum (zoomed in on special peaks)	
Find by Formula - Sample Punity	Zoom padding: - 30.0 + 30.0 m/z	
Identify Compounds	Overlay predicted isotope distribution	
Compound Automation Steps	Compound spectrum (MS/MS) Show MS/MS peak table	
Worklist Automation		
Export	Ubray search results	
	Show library spectrum Show difference spectrum	

Figure 3 Compound Report section in the Method Editor in the Qualitative Analysis program

- **5** Set up the parameters for the destination for the report.
- a Click **Reporting Options** in the Worklist Automation section in the Method Explorer.
- **b** Mark whether or not to print the report.
- c (optional) Select the printer to use.
- d (optional) Mark whether or not to save the report as an Excel file and/or as a PDF file.
- The settings in this tab are used when the method is run automatically.

Agilent MassHunter LC/SQ ChemStation Integration Quick Start Guide

Steps		Detailed Instructions	Comments
Method Explorer: Default.m	×	Kethod Editor: Reparing Options	
Compound Report Common Reporting Options File Open Actions Exacelon Data Format Find Compounds by Formula Find by Formula - Options Find by Formula - Options Find by Formula - Mars Spectra Find by Formula - Sample Purity Hother Spectra Worklist Actions Selected Ranges Export Export Export Export Export Export Export Export Export Find Spectra CEF Options		Model - Color - Color - Method Items - Color - Co	
Figure 4 Reporting Set up the parameters Worklist Actions.		 e a Click Worklist Actions in the Worklist Automation section on the Method Explorer. b Click the button to clear the items in the Actions to be run list. c Double-click the Find Compounds by Formula action in the Available actions list. d Click the Generate Compound Report action in the Available actions list and then click the • button to copy the action to the Available actions list. e (optional) Click the Export action in the Available actions list and then click the 	 The actions in the Actions to be run list are executed in order. Make sure the actions are in the order shown in "Worklist Actions in Qualitative Analysis" on page 24. If you want to use the Analytical Studio Reviewer program, you need to export an ASR file.

Familiarization Exercises for Automated Workflows

teps	Detailed Instructions	Comments
Method Explorer: Default.m	x Rahod Editor: Assign Actions to Run from Worklist ×	
Compound Report		
	▲ 🚰 🗠 • 🔍 • Method Items• 😕 🕅	
Common Reporting Options	Available actions	
File Open Actions	Generate Formulas from Compound	
Extraction Data Format	Compound Automation with Report Compound Automation without Report	
Find Compounds	Chromatogram Peak Survey with Apalysis Report	
= Find Compounds by Formula	Chromatogram Peak Survey without Analysis Report Delete All Previous Results Delete Find Compounds Results	
Find by Formula - Options	Export Export AI Chromatograms to CSV Files	
Find by Formula - Chromatograms	Export All Deconvoluted Spectra to CSV Files	
Find by Formula - Mass Spectra	Correlate TIC with MFE Compounds	
Find by Formula - Sample Purity	▼	
Identify Compounds	Actions to be run Find Compounds by Formula	
+ Compound Automation Steps	Generate Compound Report Excert	
Worklist Automation		
Reporting Options		
Worklist Actions		
Selected Ranges	• X	
B Export		
Export List		
ASR Options		
CEF Options	v l	

7 Save the method.
Click Method > Save to save the method to the current method.
Or click Method > Save As to give the method a new name.

Task 2. Set up ChemStation Method and ChemStation

This task gives you the basic steps to set up an LC/MS method with the ChemStation Data Acquisition software.

Before you start

• Read the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide that came with your system.

For more information on setting up methods in the LC/MS ChemStation software, see the *Agilent 6100 Series Single Quad LC/MS System Familiarization Guide* that came with your system.

S	teps	Detailed Instructions	Comments	
1	Edit the entire method.	 a Display the Method and Run Control view. b Open the method DEF_LC.M. c Save the method under a new name. d Edit all LC parts of the method. e Select Data Acquisition and Data Analysis in the Run Time checklist. f Save the method again. 	 If you need help, see Task 1 in the "Set up a full-scan acquisition method" section of the Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide. 	
2	Enter MS acquisition parameters.	 a Enter parameters for the quadrupole mass spectrometer (MS). b Enter parameters for the spray chamber of the ion source. c Save the method. 	 You can use the Method > Edit Entire Method command to review and edit every section of the method, including the Data Analysis portion. 	
3	Configure ChemStation to translate the ChemStation data file to the MassHunter data file format and to run a MassHunter Qualitative Analysis method.	 a Click MassHunter > Configuration. b Mark the Translate to MassHunter Data check box. c Click the Save location as input data file button. d Mark the Run MassHunter Qualitative Analysis Method check box. e Click OK. 	 These parameters are not part of a method. You only need to do this step once, and then these parameters are set until you change them. 	

Familiarization Exercises for Automated Workflows

Detailed Instructions	Comments	
Browse		
		Browse

Figure 6 MassHunter LC/SQ Integration Configuration dialog box in LC/MSD ChemStation

- **4** Link the current ChemStation method to the MassHunter method.
- a Click MassHunter > Edit Link DA Method.
 - **b** Select the method to link to.
 - c Click OK.

 You need to do this step for any method that you are going to run.
 Each ChemStation method contains a link to a MassHunter Qualitative Analysis method. Multiple ChemStation methods can link to the same Qualitative Analysis method.

Name: MOLECULARWEIGHTCONFIRMATION.M	Folders: c:\\methods	ОК
COPY OF DEFAULT.M DEFAULT.M METABOLOMICS-DEFAULT.M MOLECULARWEIGHTCONFIRMATION M SCREENING-DEFAULT.M SCREENING-DEFAULT.M	c:\ masafunter methods B04.00 B0ConfirmIntactProtein-Default.m	Cancel
Types: Method(*.M)	Drives:	Network

Figure 7 Link to MassHunter DA Method in ChemStation

- **5** Save the ChemStation method.
- a Click Method > Save Method As.
- **b** Give the method a name and click
 - OK.

Task 3. Run a sequence in ChemStation

After you set up the ChemStation method to automatically link to the MassHunter Qualitative Analysis method, you run a sequence to do molecular weight confirmation.

Steps	Detailed Instructions	Comments
1 Review parameters in the Sequence Parameters dialog box.	 a Click the Sequence > Sequence Parameters command. b Review the parameters and make any necessary changes. c Click OK. 	

Steps	Detailed Instructions	Comments
Add a sample to the Sequence Table. Add the target mass to the table in the Targeted Mass column.	 a Click the Sequence > Sequence Table command. b Enter the parameters for the sample. c Enter the target masses in the Target Masses column. d Click Run Sequence. 	 You enter the ChemStation method in this table. You also enter the Target Masses in this table.
equence Table: INSTRUMENT 1		
Currently Running Line: Method	Viat Inc	
Sample Into for 1.	1	
6		
Inset Cu Ccey Parin AggendLine InsetFillDow Waad Unat Waad Custon Fields	Undo All Plun Sequence OK Cancel Halp	

Figure 8 Sequence Table dialog box in ChemStation

Configure Table

Exercise 2. Protein Confirmation

The MassHunter Qualitative Analysis program can do Protein Confirmation when you use the additional product Agilent MassHunter BioConfirm software.

Task 1. Set up the Qualitative Analysis method to do Protein Confirmation

The *Agilent MassHunter BioPharma Workflow Guide* describes the process for setting up a method to automate Sequence matching.

Task 2. Set up ChemStation method to run the MassHunter Protein Confirmation method

Follow the steps in "Task 2. Set up ChemStation Method and ChemStation" on page 25 to set up the ChemStation method to link to the method created in the previous task.

Task 3. Run a sequence in ChemStation to do Protein Confirmation

Follow the steps in "Task 3. Run a sequence in ChemStation" on page 27 to run the ChemStation method developed in the previous task. When this ChemStation method is run, the MassHunter Qualitative Analysis method that is linked to the ChemStation method is also run.

Exercise 3. High Throughput Screening

The MassHunter Quantitative Analysis program can screen for compounds.

The following three tasks will lead you through this workflow.

- "Task 1. Set up ChemStation method to translate the ChemStation data files to the MassHunter Data File Format" on page 30
- "Task 2. Run a sequence in ChemStation to get the data files to do High-Throughput Screening" on page 32
- "Task 3. Run a batch in the MassHunter Quantitative Analysis Program" on page 32

Task 1. Set up ChemStation method to translate the ChemStation data files to the MassHunter Data File Format

This task gives you the basic steps to set up an LC/MS method with the ChemStation Data Acquisition software.

Steps	Detailed Instructions	Comments	
1 Edit the entire method.	 a Display the Method and Run Control view. b Open the method DEF_LC.M. c Save the method under a new name. d Edit all LC parts of the method. e Select Data Acquisition and Data Analysis in the Run Time checklist. f Save the method again. 	 If you need help, see Task 1 in the "Set up a full-scan acquisition method" section of the Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide. 	
2 Enter MS acquisition parameters.	 a Enter parameters for the quadrupole mass spectrometer (MS). b Enter parameters for the spray chamber of the ion source. c Save the method. 	 You can use the Method > Edit Entire Method command to review and edit every section of the method, including the Data Analysis portion which is described in the next task. 	

Steps	Detailed Instructions	Comments
3 Configure ChemStation to translate the ChemStation data file to the MassHunter data file format but do not run a MassHunter Qualitative Analysis method.	 a Click MassHunter > Configuration. b Mark the Translate to MassHunter Data check box. c Click the Save location as input data file button. d Clear the Run MassHunter Qualitative Analysis Method check box. e Click OK. 	 You clear the Run MassHunter Qualitative Analysis Method check box because this workflow uses the MassHunter Quantitative Analysis program, so you do not want to run the MassHunter Qualitative Analysis program.

Translate to Ma	ssHunter Data.			
Data file cr	ealion			
⊙ Sa	me location as input data	ile.		
⊖ At	location.		Browse	
🔲 Run MassHunt	er Qualitative Analysis Me	hod.		

Figure 9 MassHunter LC/SQ Integration Configuration dialog box in LC/MSD ChemStation

4 Save the method.

a Click Method > Save Method As.

- ${\boldsymbol b}\ \ \, \mbox{Give the method a name and click}$
 - OK.

Task 2. Run a sequence in ChemStation to get the data files to do High-Throughput Screening

Follow the steps in "Task 3. Run a sequence in ChemStation" on page 27 to run the ChemStation method developed in the previous task. This method does translate the ChemStation data file into the MassHunter data format, but it does not run a MassHunter Qualitative Analysis method.

Task 3. Run a batch in the MassHunter Quantitative Analysis Program

Follow the directions in the *Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide*, except use the data files you generated in "Task 2. Run a sequence in ChemStation to get the data files to do High-Throughput Screening" on page 32.

Exercise 4. Quantification (Target Compound Analysis)

Task 1. Set up ChemStation method to translate the ChemStation data files to the MassHunter Data File Format for Target Compound Analysis

Follow the steps in "Task 1. Set up ChemStation method to translate the ChemStation data files to the MassHunter Data File Format" on page 30 to run the ChemStation method developed in the previous task. This method does translate the ChemStation data file into the MassHunter data format, but it does not run a MassHunter Qualitative Analysis method.

Task 2. Modify a method in ChemStation to set up Compound Names and ISTDs

In the **Set Up MSD Signals** dialog box, you enter the Compound Names and the ISTDs. To use this feature, you need to install the patch on B.04.03 or install C.01.xx or greater. This patch is available on the Agilent MassHunter LC/SQ ChemStation Integration Software media.

ISD Control	MSD Signal Settings
V Use MSD	Signal: 1 Mode: SIM SIM SIM SIM SIM SIM SIM SIM
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IA Disabled	
ieneral	Time(min) On/ SIM Frag- Time(min) Off Group Ion mentor Gain (msec) Dwell Name ISTD
une File: 🛛 🛛 🕶	1 0.00 V Group 1 279.20 70 1.00 590 100.0 Sulfametha
on Source: API-ES	2 3.50 ▼ Group 2 271.10 70 1.00 590 100.0 Sulfamethiz
eakwidth: 0.100 min	3 5.50 🔽 Group 3 285.10 70 1.00 590 100.0 Sulfachloro
vcle Time: 0.60 sec/cycle	4 6.50 V Group 4 311.20 70 1.00 590 100.0 Sulfametho 5 6.80 V Group 5 281.20 70 1.00 590 100.0 ISTD-Sulfa V
✓ Time <u>Filter</u> Scan Data Storage:	Signat: 2 Mode: Scan Frag. Ramp Polarity: Positive % cycle time:
Condensed	Time(min) On/ Mass Range Frag- Off Low High mentor Gain hold size
Active Signals: 2 2 3	Time(min) Off Low High mentor Gain hold size

Figure 10 Set Up MSD Signals dialog box

Task 3. Run a sequence in ChemStation to get the data files to do Target Compound Analysis

Follow the steps in "Task 3. Run a sequence in ChemStation" on page 27 to run the ChemStation method developed in the previous task. This method does translate the ChemStation data file into the MassHunter data format, but it does not run a MassHunter Qualitative Analysis method.

Task 4. Run a batch in the MassHunter Quantitative Analysis Program

Follow the directions in the Agilent MassHunter Workstation Software *Quantitative Analysis Familiarization Guide*, except use the data files you generated in "Task 2. Run a sequence in ChemStation to get the data files to do High-Throughput Screening" on page 32.

Familiarization Exercises for Automated Workflows

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In this book

This guide has instructions for installing and using the Agilent MassHunter LC/SQ ChemStation Integration Software.

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