Agilent G3300AA
LC/MSD TOF Software

Familiarization Guide

Agilent Technologies

Notices

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In this Guide...

The Familiarization Guide presents step-by-step exercises to help you learn to use the Agilent LC/MSD TOF. You do these exercises with the ES demo sample shipped with the system.

1 Prepare the instrument

These exercises help you prepare the LC and TOF for sample analyses.

2 Set up a method

In these exercises, you enter LC and TOF parameters and save and print the method.

3 Run an interactive single sample

These exercises help you enter sample and data file information for a single sample and run the sample.

4 Set up and run worklists

In these exercises you learn to do these tasks:

- Set up and run a worklist to produce an empirical formula confirmation report
- Modify the data analysis method used to confirm empirical formulas and regenerate the report.
- Set up and run a worklist to optimize TOF acquisition parameters.
- Set up and generate a report on the parameter optimization data

Before you start...

This guide assumes that the LC/MSD TOF system, both hardware and software, has been installed, the LC modules and TOF have been configured and the performance verified, and the system has been turned on. If this has not yet been done, see the *Agilent G3250AA LC/MSD TOF System Installation Guide*.

The exercises in this guide use this equipment and materials:

- Agilent 1100/1200 LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Electrospray (ESI) ion source
- ES demo sample (p/n 59987-20030), which ships with the LC/MSD TOF system
- Zorbax Eclipse XDB-C8 column, 4.6 X 150 mm, 5 microns (p/n 993967-906), which ships with the binary pump.
- Solvents–5MM ammonium acetate in water (A) and acetonitrile (B)

Although it is recommended that you use the ES demo sample and conditions described above and in the exercises, you can customize these steps for your particular samples, column and instrument configuration.

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This chapter presents familiarization exercises to help you learn how to prepare the instrument for sample analysis. This chapter provides the practical experience to accompany general and conceptual material:

- Background information, Chapter 2, Instrument Preparation, *Concepts Guide*
- General instructions, Step 2—Prepare the instrument, *Quick Start Guide*

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Make sure that you read "Before you start..." on page 4 to prepare for the exercises.

Exercise 1. Prepare the LC modules

In this exercise, you prepare the LC modules to equilibrate the LC column.

Steps	Detailed Instructions	Comments
1 Start the software.	See the *Quick Start Guide*, "Step 1—Start the software" on page 5.	
2 Make sure that the LC flow does not go into the TOF.	See the *Quick Start Guide*, "Switch LC stream to Waste" on page 11.	
3 Purge the pump.	See the *Quick Start Guide*, "Purge the binary pump" on page 12.	
4 Set the following LC conditions to prepare the column: • Pump: 0.5 mL/min. • Left column controller temperature: 30°C • Maximum controller temp: 35°C • UV lamp ON; Visible lamp OFF	**a** Click the Method pane icon, if necessary. **b** Click the **Bin Pump** tab, and enter `.5 mL/min`. **c** Click the **Column** tab, and enter `30` for the **Left** temperature. **d** Click **Apply**. **e** Right-click the **TCC** device panel in the Instrument Status pane. **f** Select **Control**, set the maximum temperature to `35` and click **OK**. **g** Right-click the DAD device panel in the Instrument Status pane. **h** Select **UV lamp** to turn the UV lamp on.	

Exercise 2. Set up real-time displays

In this exercise you set up to view real-time parameter values (actuals) and real-time plots of chromatograms and spectra.

You may want to view these as you equilibrate the column.

Steps	Detailed Instructions	Comments
1 Set up to view real-time parameter values (actuals).	See the *Quick Start Guide*, "Set up to view real-time parameter values (actuals)" on page 16.	
2 Set up to view the real-time chromatographic baseline for the DAD and the pump pressure.	See the *Quick Start Guide*, "Set up chromatographic display" on page 17.	
3 Set up to view the DAD spectra.	See the *Quick Start Guide*, "Set up spectral display" on page 18.	
4 Monitor the displays until the column is equilibrated.	See the *Quick Start Guide*, "Condition or equilibrate the column" on page 14.	

Exercise 3. Prepare the TOF

In these exercises you calibrate and tune the TOF and reconnect the flow to the TOF.

Steps	Detailed Instructions	Comments
1 Load the Agilent autotune method for the ESI ion source.	**a** Select **File > Open > Method**. **b** Select **ESIautotune.m** and click **OK**.	• Agilent recommends that you open this method to set default TOF acquisition parameters.
2 Check to make sure the calibration is OK.	**a** Click the Method pane icon if the pane is not visible. **b** Click the **MS TOF** tab, and select the positive polarity if not selected. **c** Click the **Calibration** tab. **d** Click **Check Calibration**.	• This step checks the calibration coefficients and prints a report that shows how the calibration curve fits the current data. This report can alert you to the need to calibrate your instrument. • Assume the calibration is not OK.

Steps	Detailed Instructions	Comments
3 Calibrate the TOF.	• Click **Calibrate** to start calibration. The valve for Bottle B automatically opens when you click Calibrate.	• This exercise assumes that all ten masses for the Agilent standard have been marked (recommended). If you want to use fewer masses, click Show Extended. • If you want to use a different calibration standard, click the Show Extended and click Load Mass List to select a different list of masses.
4 Check to make sure the tune is OK.	• Click the **Tune** tab, and click **Checktune**.	• Evaluate the checktune report. This exercise assumes the tune is not OK.
5 Do a Quick Tune.	• Click **Quicktune**.	• This tune takes only a few minutes. An autotune report automatically prints.
6 Do an Autotune.	• Click **Autotune**.	• This tune takes 15 to 20 minutes. Do this one for practice.
7 Do a manual tune on the accelerator focus. (Assume your run uses mass range of 100 to 1000.)	**a** Click **Show Extended**. **b** Select **118, 622,922** as the masses for which you intend to optimize the accelerator focus. **c** Select **Accelerator focus** as the **Parameter**. **d** Select **Height** and **Resolution** as the variable to optimize. **e** Click **Ramp**.	• Note that an optimization curve appears on screen.
8 After the column is equilibrated, switch the LC flow to the TOF.	See the *Quick Start Guide*, "Switch LC stream to MS" on page 22.	
9 Monitor the TOF baseline and spectral display.	See the *Quick Start Guide*, "Monitor TOF baseline and spectral displays" on page 22.	

Exercise 4. View and work with the system logbook

These exercises show you how to modify and save the logbook.

Steps	Detailed Instructions	Comments
1 Find two ways to open the system logbook.	• Select **Tools > System Logbook Viewer**, or click the Logbook icon in the toolbar.	
2 Archive the logbook entries.	• Select **File > Save Logbook As**	• You save the logbook as a .log file to any directory that you choose. • The system saves logbook entries for only a week. You may want to archive at least once a week. • You cannot print the logbook as a .log file.
3 Print the logbook.	**a** Right-click the Logbook Viewer and select **Export**. **b** Select either **HTML** or **Tabbed text** as the file format. **c** Click the **Browse** button to select a directory and a filename. **d** Click **OK**. **e** Open and print the file.	
4 Change the logbook to display error and warning messages only.	**a** Click the **Filter Events** icon. **b** Mark the **Apply filter** check box. **c** Select **Event Type** in the **Columns** list. **d** Mark **Error** and **Warning** in the **Show only** list.	• If no errors or warnings appear in your logbook, follow these instructions anyway to learn how to filter the events.
5 Find where to troubleshoot the error and warning messages.	• Select the error message and press **F1**.	If the error or warning occurs for the TOF hardware, you may see a troubleshooting message when you press F1. For all other events, errors and warnings, you see the first page of the Online Help. You can search in the Error Messages chapter for information on other error messages.

1 Prepare the instrument

2
Set up an acquisition method

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This chapter provides familiarization exercises to help you learn how to set up acquisition methods to perform sample analyses. This chapter provides the practical experience to accompany the general and conceptual information in these manuals:

- Background information, Chapter 3, Set Up Acquisition Methods, *Concepts Guide*.
- General instructions, Step 3—Set up acquisition methods, *Quick Start Guide*

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Exercise 1. Enter LC parameters

In this exercise, you enter the conditions for the analysis of the ES demo sample.

Steps	Detailed Instructions	Comments
1 Pump. • Flow: 0.5 mL/min • Stop Time: 3.5 minutes • Post Time: 1.5 minutes • Gradient: Initial: 30% 1.5 min: 30% 3.0 min: 50%	**a** Click the Method pane icon if necessary, and click the **Bin Pump** tab. **b** Enter the values for the **Flow, Stop Time** and **Post Time** as in Step 1. **c** Click the **Timetable** tab. **d** Enter `30% B` at **0.0 min.** and `50%` at **3 minutes**.	
2 Well-plate sampler • Make sure to configure your sampler correctly. • Volume: 1 microliter	**a** Click the Instrument Status pane icon if necessary. **b** Right-click the WPS device panel in the Instrument Status pane. **c** Select **Configuration**, and click the **Tray** tab. **d** If the tray configuration of your sampler does not match the one in this tab, select the type and orientation of your tray or trays. **e** Click the **WPS** tab in the Method pane, and enter the volume of 1 microliter.	
3 TCC • Temperature: 30°C	• Click the **Column** tab, and enter `30`.	
4 Diode array detector (DAD) • Signal A: 270 nm, BW 16, Reference On: 360, BW 50 • Signal B: 210 nm, BW 16, Reference On: 360, BW 50	**a** Click the **DAD** tab **b** Select **Signal Data** as the **Storage Mode**. **c** Mark **Store** for **Signals A and B**. **d** Enter the wavelengths as in Step 4.	

Exercise 2. Enter TOF data and acquisition parameters

In this exercise, you enter data and acquisition parameters that you set up to change during a run in the next exercise. The values entered here are the values used throughout the run time and for one scan unless otherwise specified in the next exercise.

Steps	Detailed Instructions	Comments
1 Set the polarity as positive, and enter these data parameters: • Storage: Profile • Mass range: 100 to 1000 • Transients/scan: 5000 • Cycles/sec: 1	**a** Click the **MS TOF** tab, and select the polarity as **Positive**. **b** Click the **Data** tab, and enter the values as in Step 1.	
2 Acquisition parameters: • Gas temp: 320 • Drying gas: 10 L/min • Nebulizer: 40 psi • Capillary: 4000V • Fragmentor: 150V • Skimmer: 60V	• Click the **Acquisition** tab and enter the values as in Step 2.	

Exercise 3. Set up to change TOF parameters during a run

In this exercise, you set up a time segment in addition to the initial one. The second segment has two scans.

Steps	Detailed Instructions	Comments
1 Add a time segment at 2.5 min with this value change for the first scan: • Mass Range: 300 to 1000	• Enter `2.5 min` in the **Time** field, and click **Add**. • With the **2.5 min** segment selected, change the **Mass Range** on the Data tab to 300-1000.	
2 Add another scan to the segment at 2.5 min with this value change for the second scan: • Fragmentor: 190	**c** With the **2.5 min** segment selected, click **Add** next to the **Scan** list. **d** With **Scan 2** selected, click the **Acquisition** tab and enter `190` for the **Fragmentor voltage**.	

Exercise 4. Set up reference mass correction

To obtain the mass accuracy specified with the TOF system, you enable a mass correction using a reference standard that passes through the TOF with the sample.

Steps	Detailed Instructions	Comments
1 Enable reference mass correction to use the reference standard in Bottle A.	**a** Click the **Ref. Masses** tab. **b** Mark the **Enable reference mass correction** check box. **c** Mark the **Bottle A** check box.	• If you do not use Bottle A, the system uses a standard that you infuse into the system or a background ion selected from an appropriate mass list.
2 Create a new mass list from the ESIpos_default mass list. • Call this list *iii*esdemo, where "*iii*" represents your initials. • Include masses 118, 622, 922 only.	**a** Click **Edit Mass List**. **b** Select **ESIpos_default** as the mass list used to create a new list. **c** Enter the **Name**, `iiiesdemo`. **d** Click **Save As New List**. **e** Delete all masses from the list except 118, 622, 922. **f** Click **Save List**, and click **OK**.	• Note that you must enter a name for the new mass list before you can do anything in this dialog box. • You can never edit the default mass lists. You can only use them as templates for new mass lists.
3 Select the *iii*esdemo mass list, then select 118 and 922 for the mass correction.	**a** Click **Select Mass List**. **b** Select the **iiiesdemo** list. **c** Mark **118** and **622**.	

Exercise 5. Set up TOF signals for the real-time plot display

In this exercise you select the TOF signals that you intend to view in the Real-time Plot.

Steps	Detailed Instructions	Comments
1 Set up to monitor the actual parameter value for the Chamber.	**a** Click the **Chromatogram** tab. **b** Select **Actuals** from the **Type** list. **c** Select **Chamber** from the **Actuals** list. **d** Enter a label. **e** Click **Add**.	
2 Set up to monitor an extracted ion chromatogram at 285.	**a** Select **EIC** from the **Type** list. **b** Enter 285 for the mass. **c** Enter a label. **d** Click **Add**.	
3 Set up to monitor scan 2 for the time segment at 2.5 min.	**a** Select **TIC** from the **Type** list. **b** Enter a label. **c** Select **2.5** from the **Segment** list. **d** Select **Scan 2** from the **Scan** list. **e** Click **Add**. **f** Click **Apply**.	
4 Make sure that the system added the new signals to the Edit Signal Plot list of signals for the real-time plot.	**a** Click the Real-time Plot icon, if necessary. **b** Right-click the chromatographic plot. **c** Select **Change**.	• The new TOF signals should appear in the Selected Signals list, not the Available Signals list.

Exercise 6. Save and print the acquisition method

This exercise shows you how to save and print the acquisition method that you have just set up.

Steps	Detailed Instructions	Comments
1 Save the method as iiiesdemo.	• Select **File > Save** to save the current method, or select **File > Save As** to save a new method.	• Before you save the method, you can add a pre-run or post-run script in the Properties tab. With these scripts you can prepare a column before injection or equilibrate a column after injection before the next run. Do not modify any files provided by Agilent since they may be overwritten the next time the system is upgraded.
2 Print the method	• Select **File > Print > Acquisition Method**.	

3
Set up and run single samples

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This chapter provides familiarization exercises to help you learn how to acquire and analyze the data for a single sample. This chapter provides the practical experience to general and conceptual material in the other guides:

- Background information, Chapter 4, Data Acquisition and Chapter 5, Data Analysis, *Concepts Guide*
- General instructions, "Step 4—Acquire data" on page 30, *Quick Start Guide*

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Exercise 1. Enter sample run information

TOF data is organized based on Analyst Projects. The data files and sample information are saved to a project. This exercise shows you how to enter sample and data file information for a single sample and then begin to acquire data on the sample.

Steps	Detailed Instructions	Comments
1 Open the *iii*esdemo method, then enter this sample information: • Name: *iii*esdemo1, where "*iii*" represents your initials • Position of sample in your sampler • Custom information	**a** Select **File > Open > Method**. **b** Select ***iii*esdemo.m**, and click **OK**. **c** Click the **Sample** tab in the Method pane. **d** Enter the information in Step 1.	• The system stores the custom information with the data file, where it can be viewed in the Quant Info section of the sample information in Analyst.
2 Create a new project called iiiesdemo, where "*iii*" represents your initials.	**a** Double-click the Analyst QS icon in the TOF Software group window. **b** Select **Tools > Projects > Create**. **c** Enter *iii*esdemo as the name of the project. **d** Minimize or close Analyst.	• The projects listed in the Sample tab have already been created. You may want to create one of your own.
3 Enter this data file information: • Select the project, *iii*esdemo. • iiiesdemo.wiff as the data file name	**a** Select the project, **iiiesdemo**. **b** Enter *iii*esdemo.wiff as the data file name	
4 Enter this run information: • Acquisition Only as the Part of method to run	• Select **Acquisition Only** from the **Part of Method to Run** drop-down list. • Click **Apply**.	

Exercise 2. Start data acquisition and print the real-time plot

In this exercise you start data acquisition and print the real-time plot during data acquisition.

Steps	Detailed Instructions	Comments
1 Start the sample.	• Click the Start icon for single samples.	•
2 Print the real-time plot.	**a** Select **File > Print > Real-time plot**. **b** Select to print the plot, send it to a file or view the plot on the screen. **c** Click **OK**.	•

Exercise 3. Review the data

This exercise shows you how to do basic Analyst review operations and adjust the spectrum peak find parameters.

Steps	Detailed Instructions	Comments
1 In Analyst, open the data file of the sample you just ran.	**a** Double-click **Analyst QS** in the TOF Software group window. **b** From the **Project** list on the top toolbar, select **iiiesdemo**. **c** Click **Open Data File** under the Explore folder. **d** Select **iiiesdemo1** and click **OK**.	
2 Zoom into the first two peaks, then zoom out again.	**a** Drag the cursor to produce a rectangle under the baseline of the first two peaks. **b** Draw another rectangle under the peaks. **c** Draw another rectangle on the y-axis next to the peaks. **d** Double-click the chromatogram to zoom out.	

Steps	Detailed Instructions	Comments
3 List peak areas, then change the following integration parameter and reintegrate: • Threshold to eliminate all peaks except four sulfa compounds	**a** Right-click the chromatogram to bring up the shortcut menu. **b** Select **List Data** to see peak areas. **c** Drag the arrow along the y-axis of the chromatogram to change the threshold. **d** Right-click the chromatogram and select **List Data** again to reintegrate??	
4 Extract ion 285 for each time segment and scan.	**a** Click the arrow along the x-axis for the first time-segment. **b** Select **Extract Ions**. **c** Enter 285 and click **OK**. **d** Repeat steps a-c for the second time segment.	
5 View sample information	• Click the sample information icon on the toolbar.	
6 View the TOF spectra.	**a** Right-click the chromatogram. **b** Select **Show spectrum**.	
7 Change the peak find parameter to 1000 to find more peaks.	**a** Select **Script > Agilent Peak Finder Parameters.dll.** **b** Enter 1000 for the **Maximum number of peaks**, and click **OK**.	
8 View TOF actuals.	**a** Right-click the spectrum and select **Actuals**.	

4
Set up and run worklists

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This chapter provides familiarization exercises to help you learn how to do these tasks:

- Create and run a worklist to confirm the empirical formulas for the compounds in your samples
- Customize the data analysis method used for this worklist and regenerate the empirical formula confirmation report.
- Set up and run a worklist to optimize TOF parameters.
- Set up and generate a report on the data from the worklist to optimize parameters

This chapter provides the practical experience to accompany the general and conceptual material in the other guides.

- Background information,Chapter 4, “Data Acquisition,” and Chapter 5, “Data Analysis” in the *Concepts Guide*.
- General instructions, “Step 4—Acquire data" on page 30, *Quick Start Guide*

Each exercise is presented in a table with three columns:

- Steps – Use these general instructions to proceed on your own to explore the software.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Exercise 1. Set up a worklist to confirm empirical formulas

The four data analysis methods that come with the software are default.anm, mfe.anm, efc.anm, and mass_list.anm. You will use the data analysis method efc.anm to generate an empirical formula confirmation report. When you use the new method and a list of formulas in the worklist, you can confirm the formulas in your samples.

Steps	Detailed Instructions	Comments
1 Add four samples to the worklist - Sulfa 1, Sulfa 2, Sulfa 3, Sulfa 4 - with the following information: • Sample position: any four positions convenient for your sampler • Data file: Sulfa 1.4.wiff to be saved to the project, iiiesdemo\Data. • Acquisition method: iiiesdemo.m • Data analysis method: efc.anm • Injection volume: 1	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Add Multiple Samples**. **c** Enter the sample name and data file name so that the numbering takes place automatically. **d** Change the directory path for the data files to **iiiesdemo\Data**. **e** Select the **iiiesdemo.m** acquisition method. **f** Make sure that the data analysis method is **efc.anm**. This method has the EFC report selected when it is shipped from the factory. **g** Enter an injection volume of `1`, and click **OK**.	• If another worklist already exists in the Worklist pane, click New to create this worklist. • The efc.anm method has the EFC report selected when it is shipped from the factory. If you have modified and saved this method in your lab, open the method in the Data Analysis Method Editor to verify that the method still has the EFC report selected. If it doesn't, then select the EFC report and save the method.
2 Hide the following columns: • Sample Type • Inj. Vol. • Comment	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Show/Hide Columns**. **c** Clear the check boxes for **Sample Type**, **Inj. Vol.** and **Comments**.	• You are hiding these columns, not deleting them. The software recognizes their values even though they do not appear in the worklist.
3 For all four samples, add columns for the formulas for the sulfa compounds: • Sulfamethazine: $C_{12}H_{14}N_4O_2S$ • Sulfachloropyridazine: $C_{10}H_9ClN_4O_2S$ • Sulfadimethoxine: $C_{12}H_{14}N_4O_4S$ • Sulfamethizole: $C_9H_{10}N_4O_2S_2$	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Add Column(s)**. **c** Select **EFC**. **d** Enter `Formula 1` for the column name and `C12H14N4O2S` for the first formula. **e** Click **OK**. **f** Repeat steps b-e for the other three compounds.	• The column name must always have the word Formula as the first part of the name to enable generation of the empirical formula confirmation report. • The EFC report will return an error if a formula is not entered in the worklist.

Steps	Detailed Instructions	Comments
4 Save the worklist as iiiesdemo.	**g** Select **File > Save As**, enter the name and click **Save**.	

Exercise 2. Run and edit the worklist

This exercise shows you how to start the worklist and monitor the samples that are running.

Steps	Detailed Instructions	Comments
1 Make sure that the worklist is set to run both data acquisition and analysis.	**a** Right-click the upper-left-hand cell of the worklist spreadsheet. **b** Select **Worklist Run Parameters**. **c** Select **Both Acquisition and DA** from the **Part of method to run** list. **d** Click **OK**.	
2 Start the worklist.	• Click the **Start** icon for the worklist.	• You do not have to save the worklist in order to start it. • The first sample row turns blue as the software begins to acquire data.
3 Change the sample name and the data file name of the last sample to Sulfa 5.	**a** Double-click the **Sample** cell in the last row and enter `Sulfa 5`. **b** Double-click the **Data** file cell in the last row and enter `Sulfa 5`.	• When you move to edit any part of the worklist, tracking is automatically turned off. You must turn it back on to see which sample is running.
4 Turn tracking back on.	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Track Worklist Run** from the menu.	• Worklist tracking is also automatically turned off when the Left Mouse clicks on the worklist or when any menu operation except Worklist Parameter and Worklist Report is used. Worklist tracking is turned off even if you cancel from the requested action.

Exercise 3. Customize and regenerate the report

You do not modify the default.anm or the efc.anm data analysis method in Analyst. You use a separate Data Analysis Method Editor created by Agilent. This exercise shows you how to use the editor and regenerate the empirical confirmation report.

Steps	Detailed Instructions	Comments
1 Make sure that the Data Analysis Method Editor contains the default values. Then change the following parameters in the efc.anm method: • Select Empirical Formula Confirmation report • Relative Peak Height Threshold: 10% • Use spectrum from apex of peak • Minimum abundance: 5000	**a** Double-click the Data Analysis Method Editor icon in the TOF software group window. **b** Select **Edit > Set to Defaults**. **c** Select **Select DA Operations** from the **Edit** menu in the Data Analysis Method Editor window. Select **Formula Confirmation** report. Remove **Mass List** report if it was selected. **d** In the Chromatogram tab, replace 1% with `10%` for the **Relative Peak Height Threshold**. **e** Click the **Spectrum** tab and select **Use spectrum from the apex of the peak**. **f** Click the **Report Options** tab and replace 1000 with `5000` for the **Minimum abundance to include with the table**.	• Every time you open the Data Analysis Method Editor, the default.anm is opened. If someone in the lab changed the defaults to other values, then this exercise may not work properly unless you reset the method to the default values.
2 Save the data analysis method as iiiesdemo.anm.	**a** Select **File > Save As**. **b** Enter *`iii`*`esdemo.anm` and click **Save**.	

Steps	Detailed Instructions	Comments
3 Regenerate the report with the new method.	**a** Click the first cell of the Data Analysis Method column and click the down arrow. **b** Select **iiiesdemo.anm**. **c** Right-click the first cell of the DA Method column and select **Fill > Column**. **d** Right-click the upper-left-hand corner of the worklist spreadsheet. **e** Select **Worklist Run Parameters**. **f** Select **DA Only** from the **Part of method to run** list. **g** Click **OK**. **h** Click the **Start** icon for the worklist.	• You must hide the down arrow before you can right-click to bring up the menu with the Fill item.

Exercise 4. Set up a worklist to optimize TOF parameters

You can also optimize TOF acquisition parameters with a worklist. To eventually generate a report on this optimization, you save the data on all samples to one data file. You can easily do this within a batch of samples. This exercise shows you how to set up a batch within a worklist to evaluate the signal as the fragmentor voltage changes.

Steps	Detailed Instructions	Comments
1 Create a batch and add four samples to the batch - Frag 1, Frag 2, Frag 3, Frag 4 - with the following sample information: • Sample position: any four positions convenient for your sampler • Data files: Frag 1- 4.wiff to be saved to the project, iiiesdemo\Data. • Acquisition method: iiiesdemo.m • Injection volume: 1	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Add Batch**. **c** Right-click the upper-left-hand corner of the batch spreadsheet. **d** Select **Add Multiple Samples**. **e** Enter the sample name and data file name so that the numbering takes place automatically. **f** Change the directory path for the data files to **iiiesdemo\Data**. **g** Select the **iiiesdemo.m** acquisition method. **h** Enter an injection volume of `1`, and click **OK**.	• This worklist does not require a data analysis method, but you can leave the default.anm method listed as the method. The software does not use this method when formula columns are not present.
2 Hide the following columns: • DA Method • Sample Type • Inj. Vol. • Comment	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Show/Hide Columns**. **c** Clear the check boxes for **DA Method**, **Sample Type**, **Inj. Vol.** and **Comments**, and click **OK**.	• You are hiding these columns, not deleting them. The software recognizes their values even though they do not appear in the worklist.
3 For all four samples, add a column for the fragmentor parameter, and enter these values: • Frag 1: 225 • Frag 2: 200 • Frag 3: 175 • Frag 4: 150	**a** Right-click the upper-left-hand corner of the worklist spreadsheet. **b** Select **Add Column(s)**. **c** Select **MS Parameters**. **d** Select **Fragmentor**, and click the **>** button. **e** Click **OK**. **f** Enter the values into the column.	
4 Save the worklist as Fragwklst.	**g** Click **File > Save As**. Enter `Fragwklst`; click **Save**.	

Exercise 5. Add a sample to the worklist during the run

As your batch is running, you can easily add single samples to the worklist whose purpose is different than than that for the samples in the batch. This exercise shows you how to add a single sample for empirical formula confirmation to a worklist whose batch for parameter optimization is running.

Steps	Detailed Instructions	Comments
1 Add a single sample to the worklist (not the batch) called Sulfa 6 during the run. Use the same information as in "Exercise 1. Set up a worklist to confirm empirical formulas" on page 27.	**a** Click **Back to Worklist**. **b** Right-click the upper-left-hand corner of the worklist spreadsheet. **c** Select **Worklist Run Parameters**. **d** Select **Both Acquisition and DA** from the **Part of the method to run** list. **e** Click **OK**. **f** Click the **Start** icon for the worklist. **g** Right-click the upper-left-hand corner of the worklist spreadsheet. **h** Click **Add Sample**. **i** Add the method and sample information.	• Even though you use only an acquisition method for the Batch, you select both acquisition and DA for the worklist because you are adding a sample that requires both. • The software completes the samples in the batch first, then runs the additional sample.
2 Save the worklist after data acquisition is complete.	• Select **File > Save**.	

Exercise 6. Set up and generate a parameter optimization report

An Agilent script to set up and generate a report on the data from the worklist for parameter optimization is available in Analyst. This report compares the data from each sample so that you can compare the signals and judge which value is best.

Steps	Detailed Instructions	Comments
1 Start the parameter optimization wizard in Analyst.	**a** Double-click the **Analyst QS** icon from the TOF Software group window. **b** Select the ***iii*esdemo** project. **c** Select **Script > Parameter Optimization.dll**.	•
2 Make the following selections in the wizard: • FragBatchdata.wiff • Use all samples in this file • Use all defaults.	• Select the **FragBatchdata.wiff** file in the **Available Files** list. • Mark all the samples, if necessary, and click **Next**. • Click **Next** again to accept defaults. • Click **Finish**.	• The Parameter Optimization report appears comparing the signals from each of the samples in the Batch.

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In This Book

The Familiarization Guide presents exercises to help you use the LC/MSD TOF system. In this guide you learn:

- How to prepare the instrument for sample analysis
- How to set up a method and run an interactive sample
- How to set up and run a worklist to confirm empirical formulas
- How to set up and run a worklist to optimize TOF parameters

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