

Agilent 6100 Series Quadrupole LC/MS Systems

Familiarization Guide



Notices

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Manual Part Number

G1960-90080

Edition

Revision A, September 2011

Printed in USA

Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Santa Clara, CA 95051

This guide is valid for the B.03.01 or later revision of the Agilent ChemStation software for the Agilent 6100 Series Quadrupole LC/MS systems, until superseded.

If you have any comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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In this Guide...

This guide presents a series of exercises to help you learn the basic operation of your Agilent 6100 Series LC/MS system.

If you have any comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

1 Prepare for the Analysis

Use these exercises to prepare the LC, to dilute a sulfa demonstration sample, and to check the tune on the MS.

2 Set Up and Run a Scan Method

Learn how to set up a scan method and acquire data for the sulfa demonstration mix.

3 Qualitative Data Analysis

Learn how to examine chromatograms and spectra to identify sample components. In these exercises, you review data from the sulfa sample you analyzed in Chapter 2, or from a data file that you received with your ChemStation software.

4 Set Up and Run a SIM Method

Learn how to set up a selected ion monitoring (SIM) method and acquire data for the sulfa demonstration mix.

5 Set Up and Run a Sequence

Use these exercises to set up an automated sequence for SIM analyses of the sulfa mix at various concentrations, and to acquire data with that sequence.

6 Quantitative Data Analysis

Learn how to analyze data when you need to quantify sample components. These exercises use caffeine data files that you received with your ChemStation software.

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Prepare for the Analysis

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This chapter presents exercises to help you learn how to:

- Prepare the LC and column for an analysis
- Prepare the samples that you analyze in these exercises
- Check the tune settings of the MS and adjust if necessary.

Before you start

- Order the sample: Agilent Electrospray LC Demo Sample, p/n 59987-20033.
- Order the column: Agilent ZORBAX SB-C18, 2.1 mm x 30 mm, 3.5 μ m, *p/n 873700-902*.
 - You may use another similar column, but you may need to adjust the HPLC conditions to obtain good separation.
- Make sure that the electrospray source is installed.
- Read the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide and Chapter 2 of the Agilent 6100 Series Quadrupole LC/MS Systems Concepts Guide.



Exercise 1. Prepare the LC to run the sample

Exercise 1. Prepare the LC to run the sample

For the following tasks, try the steps in the first column. If you need more help, follow the detailed instructions in the middle column.

Task 1. Start up ChemStation

Steps	Detailed Instructions	Comments
1 Open the ChemStation window.	 Click the ChemStation icon on the desktop. Copentage 	 Alternate method: From the Start menu, select: All Programs > Agilent ChemStation > Instrument 1 online.

Task 2. Purge the pump

Use these instructions with the binary and quaternary pumps. See the ChemStation online Help for instructions for the capillary and nanoflow pumps.

Steps	Detailed Instructions	Comments
1 Display the Method and Run Control view.	 In the view selection area in the lower left, click Method and Run Control. Method and Run Control 	
2 Place the pump in standby mode.	 a Click More Pump > Control HPLC Pump on the Instrument menu to open the Pump Control dialog box. b Select Standby and click OK. 	Alternate method:Select Standby from the Pump context menu.
 3 Prepare solvents used in these familiarization exercises. A – 5 mM ammonium formate in water B – 5 mM ammonium formate in methanol 	 a Into a 1-liter reservoir of HPLC-grade water, add 1 mL of 5 M ammonium formate. b Into a 1-liter reservoir of HPLC-grade methanol, add 1 mL of 5 M ammonium formate. 	 The part number for ammonium formate is G1946-85021. Each ampoule contains 2.2 mL of ammonium formate solution.
4 Replace the solvent bottles with the ones you just prepared.	• Replace the bottles for channels A and B.	
5 Open the purge valve.	 a Turn the black purge valve on the front of the pump counter-clockwise two turns. b Place the tubing that exits the pump into a 250-mL or larger beaker. 	
6 Enter a flow of 5 mL/min and 50% B, using water in channel A and methanol in channel B.	 a Click the pump icon. b Select Set up Pump. c Enter the parameters in step 6 and click OK. 	• Be sure to use HPLC-grade solvents.
7 Turn the pump on and monitor the tubing for bubbles.	 a To turn the pump on, click the little button to the lower right of the solvent delivery (pump) icon. b Monitor for bubbles. 	 Purge for about 3 minutes to pass 3X the volume for the binary pump. If you wish, you may purge each channel individually first, to ensure that neither is air-locked.

Task 3. Prepare the column for the analyses

St	teps	Detailed Instructions	Comments
8	After the bubbles are gone and the purge is complete, enter a flow of 1 mL/min and 100% B.	 a Right-click the pump icon. b Select Set up pump. c Enter the new parameters in step 8, and click OK. 	
9	Purge a short while longer, and then close the purge valve.	a Continue to purge for a short while.b Close the black valve.	For more information on purging the pump, see the reference manual that you received with your pump.

Task 3. Prepare the column for the analyses

In the exercises in the next chapters, you analyze a mixture of four sulfonamide compounds. To perform the analyses in the following chapters, you must first condition and equilibrate your column.

S	iteps	D	etailed Instructions	(Comments
1	Disconnect the column from the detector and MS.	b	Turn the pump off by clicking the little button to the lower right of the solvent delivery (pump) icon. Disconnect the column from the detector and MS. Place the open end of the tubing that exits the column into the beaker.	•	 To prevent detector contamination, allow the column effluent to go directly to the waste beaker.
2	 Flush the column with 100% methanol at 1 mL/min (5 to 10 min). ZORBAX SB-C18, 2.1 mm × 30 mm, 3.5 μm, <i>p/n 873700-902</i> 	a b	Turn the pump on. Flow methanol through the column under the conditions used in Task 2, step 8.	•	 The data sheet shipped with the column cartridge recommends that you flush with 20 to 30 column- volumes of 100% methanol (approximately 5 to 7.5 mL).

Task 3. Prepare the column for the analyses

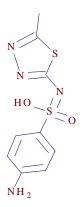
Steps	Detailed Instructions	Comments
 3 Condition the column as follows, using the solvents made up in Task 2, step 6: Flow rate – 0.4 mL/min 100% B for 1/2 hour 50% B for 1/2 hour 	 a Click Set up Instrument Method on the Instrument menu to open the Setup Method dialog box. b Click the Pump tab. c Enter the flow rate in step 3. d For Solvent B, type 100 and click Apply. e Wait 30 minutes. f For Solvent B, type 50 and click Apply. g Wait 30 minutes. 	 At a flow rate of 0.4 mL/min, the checkout column should produce about 70 to 80 bar pressure (measured without any fittings at the column exit). If, after you perform these steps, the pump pressure through the column is too high, order a replacement SB-C18 column (<i>p/n 873700-902</i>). If your column is <i>not</i> new, you can reduce the length of time that you condition the column.
 4 Equilibrate the column at the analysis conditions: 12% B for 1/2 hour at 40 °C 	 a For Solvent B, type 12 and click OK. b Click the TCC tab on the Setup Method dialog box. c For Temperature, type 40 and click OK. 	• While you condition and equilibrate the column, you may complete step 5 in this exercise and then work on the rest of the exercises in this chapter. Be sure to complete step 6 before you go on to the next chapter.
 5 While the column equilibrates, set parameters for the MS spray chamber so it can heat and equilibrate as well. Drying gas flow: 8 L/min Nebulizer pressure: 35 psig Drying gas temperature: 300 °C Capillary voltage: 3000 V For 6150 with Agilent Jet Stream technology: Sheath Gas Flow: 12 L/min Sheath Gas Temp: 360°C Nozzle Voltage: 0 V 	 a Right-click the MSD icon on the system diagram and select Spray Chamber. b Enter the parameters described in step 5. c Click OK. d Wait 10 minutes before you tune the MS. 	
6 Reconnect the column to the DAD and MS.		• You can complete "Exercise 3. Check the current MS tune values and adjust if necessary" either with or without the column connected to the DAD and MS, but you <i>do</i> need to reconnect prior to the exercises in Chapter 2, "Set Up and Run a Scan Method."

Exercise 2. Prepare the samples for the analyses

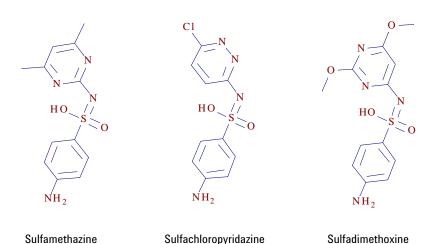
Exercise 2. Prepare the samples for the analyses

In the exercises in the next chapters, you analyze a mixture of four sulfonamide compounds. The Electrospray LC Demo Sample (p/n 59987-20033), contains five ampoules with 100 ng/µL each of these compounds:

- sulfamethizole $(M+H)^+ = 271$
- sulfamethazine $(M+H)^+ = 279$
- sulfachloropyridazine $(M+H)^+ = 285$
- sulfadimethoxine $(M+H)^+ = 311$.



Sulfamethizole



To perform the analyses in the following chapters, you must first prepare the sample at various dilutions. The final concentrations will be 1, 5 and 10 ng/ μ L. You will also prepare a solvent blank.

Exercise 2. Prepare the samples for the analyses

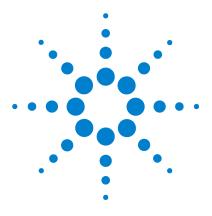
Steps	Detailed Instructions	Comments
 Prepare a 1:10 dilution of the original sample in a 1-mL autosampler vial. Final concentration is 10 ng/µL You will use this sample for the scan analysis in Chapter 2, and for the SIM analyses in Chapter 4 and Chapter 5. 	 a Transfer 100 μL of the sulfa mixture into the autosampler vial. b Add 900 μL of 90:10 water:methanol that contains 5 mM ammonium formate (NH₄HCO₂). c Cap the vial. 	 The original sulfa mixture is dissolved in a solvent mixture of 70% water and 30% acetonitrile.
 Prepare a 1:20 dilution of the original sample in a 1-mL autosampler vial. Final concentration is 5 ng/μL You will use this sample for the SIM analysis in Chapter 5. 	 a Transfer 50 μL of the sulfa mixture into the autosampler vial. b Add 950 μL of 90:10 water:methanol that contains 5 mM ammonium formate. c Cap the vial. 	
 3 Prepare a 1:100 dilution of the original sample in a 1-mL autosampler vial. Final concentration is 1 ng/μL You will use this sample for the SIM analysis in Chapter 5. 	 a Transfer 10 μL of the sulfa mixture into the autosampler vial. b Add 990 μL of 90:10 water:methanol that contains 5 mM ammonium formate. c Cap the vial. 	
 4 Prepare a solvent blank in a 1-mL autosampler vial. You will use this sample for the SIM analysis in Chapter 5. 	 a Into the autosampler vial, transfer 990 µL of 90:10 water:methanol that contains 5 mM ammonium formate. b Cap the vial. 	

Exercise 3. Check the current MS tune values and adjust if necessary

Exercise 3. Check the current MS tune values and adjust if necessary

The MS is very stable and does not need to be tuned very often. You can usually tune just once a month, or once a week at most. You can use the Check Tune program described in this exercise to confirm that the MS is in adjustment.

Steps	Detailed Instructions	Comments
1 Switch to the MSD Tune view.	 In the view selection area in the lower left, click MSD Tune. MSD Tune 	
2 Select the tune file.	 a In the Select Tune File dialog box, select ATUNES.TUN. b Keep the default of Positive Polarity (Standard). c Click OK. d In the status bar near the top of the MSD Tune view, verify that you see the following: Mode is API-ES Source is ESI (electrospray) 	 Make sure that you use an appropriate calibrant with an appropriate source.
3 Run a Check Tune.	 From the Tune menu, select Check Tune. Note that Check Tune requires values for comparison that are determined from a previous Autotune. Autotune is normally run during installation. 	 Check Tune is normally all that you need to do to confirm that the MS settings are correct. If Check Tune indicates a problem with your MS settings, then proceed to step 4 and/or step 5.
4 If Check Tune report suggests that you adjust peak widths or mass axis, then do that.	 a From the Tune menu, select Adjust Mass Peak Width. b From the Tune menu, select Calibrate Mass Axis. 	
5 If the Check Tune report shows poor sensitivity, which indicates that your MS settings are significantly out of adjustment, then run a full Autotune.	 From the Tune menu, select Autotune > Positive Polarity. 	 The exercises in this manual use only the positive ion mode and standard scan speeds, so it is not necessary to tune for negative polarity or fast scan.



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Set Up and Run a Scan Method

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Task 1. Enter sample information 24
Task 2. Acquire the data 25

These exercises show you how to set up a scan data acquisition method for the demonstration sample (sulfa mix) and to acquire data with that method.

The LC parameters that you enter in these exercises are appropriate for the standard Agilent 1100/1200/1260/1290 Series liquid chromatography (LC) systems. You must enter LC parameters that are appropriate for your LC model.

To view the results of these exercises, see Chapter 3, "Qualitative Data Analysis."

Before you start

- Review the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide and Chapter 3 of the Agilent 6100 Series Quadrupole LC/MS Systems Concepts Guide.
- Prepare the LC, column and sample as described in Chapter 1, "Prepare for the Analysis."

For the tasks on the following pages, try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.



Exercise 1. Set up a full-scan acquisition method

Exercise 1. Set up a full-scan acquisition method

This exercise changes the default method and saves it as a new method. This exercise consists of the following tasks:

- "Task 1. Enter LC acquisition parameters" on page 16
- "Task 2. Enter MS acquisition parameters" on page 19

Task 1. Enter LC acquisition parameters

S	teps	Detailed Instructions	Comments
1	Display the Method and Run Control view.	 In the view selection area in the lower left of the ChemStation window, click Method and Run Control. Method and Run Control 	
2	Open the method DEF_LC.M .	 a Select File > Load > Method. b If necessary, navigate to C:\CHEM32\1\METHODS. c Select DEF_LC.M and click OK. 	
3	Save the method under a new name, SULFA MS SCAN 1.M .	 a Select File > Save As > Method. b In the dialog box, for Name, type SULFA MS SCAN 1.M. c Click OK. d In the box for Comment for method history, type a comment. e Click OK. 	 You save the method now with a new name to avoid inadvertently overwriting the default method later.
4	Enter a volume of 1 μL for the injection.	 a Click Set up Instrument Method on the Instrument menu to open the Setup Method dialog box. b Click the ALS tab. c Click Standard injection. d In the Injection volume box, type 1 for a 1-μL injection. 	

Task 1. Enter LC acquisition parameters

Steps	Detailed Instructions	Comments
5 Enter pump parameters.	 a Click the Pump tab on the Setup Method dialog box. b Set the parameters as follows: Flow=0.400mL/min StopTime=7.00 min PostTime=3.00min Solvent A=Water 88% Solvent B=Methanol 12% 	
6 Set up the gradient timetable as it appears in the figure below.	 a Open the Timetable area in the lower part of the tab, click Insert, and type the first line. b Click Append and type the second line. c Repeat step b for lines 3 and 4. 	Set the timetable parameters as follows: Line 1 Time 1:00, %B=12, Flow=0.4 Line 2 Time 3:00, %B=100, Flow=0.4 Line 3 Time 6:00, %B=100, Flow=0.4 Line 4 Time 7:00, %B=12, Flow=0.4
7 Enter a column compartment temperature of 40 °C.	 a Click the TCC tab on the Setup Method dialog box. b Click the option button for °C. c Type 40.0 for °C. 	
8 Enter parameters for the diode-array detector (DAD).	 a Click the DAD tab on the Setup Method dialog box. b Enter the parameters shown below: Use Signal A: Wavelength 272nm, Bandwidth16 nm Reference Wavelength: 360 nm, Reference Bandwidth 100 nm Spectrum Store: All in peak Peakwidth: > 0.1 min c Click OK to close the Setup Method dialog box with the new setpoints. 	 The DAD is used in this example, but the variable wavelength detector (VWD) may be used analogously.

Task 1. Enter LC acquisition parameters

Steps	Detailed Instructions	Comments
9 Select Data Acquisition only in the Run Time Checklist.	 a Click Run Time Checklist on the RunControl menu. b Mark the Data Acquisition check box. c Click OK. 	 While it is common to include Data Analysis in the Run Time Checklist, for these exercises, you will view the results in Chapter 3, "Qualitative Data Analysis."
10 Save the new parameters to the method file, SULFA MS SCAN 1.M.	 a Select File > Save > Method. b In the box for Comment for method history, type a comment. c Click OK. 	

Task 2. Enter MS acquisition parameters

Task 2. Enter MS acquisition parameters

Steps

Detailed Instructions

- 1 Enter parameters for the quadrupole mass spectrometer (MS):
 - Signal 1, scan mode, positive polarity
 - Scan range: 100 to 500
 - Fragmentor: 100 V for the Agilent 6120; 125 V for the 6130 or 6150
 - · Gain: 1.00
 - Threshold: 150
 - Stepsize: 0.10
 - Peakwidth: 0.05 min
 - Scan data storage: Condensed
 - Active signals: 1 only

- a Right-click the MSD icon on the system diagram and select Set up MSD Signals.
- b Enter the parameters described in step 1 and shown in the figure below. Take care to enter the appropriate Fragmentor voltage for your MS model.
 c Click OK.
- Comments
- To save disk space you usually acquire line spectra (Scan Data Storage = Condensed). However, when you acquire spectra from intact proteins or protein digests/peptides, you must acquire and deconvolute profile spectra. (Scan Data Storage = Full).

MSD Control	MSD Signal Settings
✓ Use MSD StopTime: noLimit ▼ FIA Disabled	Signat: 1 Mode: Scan ▼ Polarity: Positive ✓
General Tune File:	Time(min) On / Mass Range Frag- Gain Thres-Step Off Low High mentor hold size
atunes.tun 👻	1 0.00 🔽 100.00 500.00 150 1.00 150 0.10
Source: API-ES Peakwidth 0.050 min	Set to 100 for 6120 Set to 125 for 6130 or 6150
0.48 sec/cycle	Sort Insert Append Cut Copy Paste
0.48 sec/cycle Ultra Fast Scan Time Filter	Sort Insert Append Cut Copy Paste Signal: 2
0.48 sec/cycle Ultra Fast Scan Time Filter Scan Data Storage Condensed 💌	Signat 2
0.48 sec/cycle Ultra Fast Scan Time Filter Scan Data Storage Condensed Active Signals:	Signat: 2 Frag. Ran Mode: Scan ▼ Polarity: Positive ▼ % cycle tin Time(min) 0n/ Mass Range Frag. Thres- Step
0.48 sec/cycle Ultra Fast Scan ✓ Time Filter Scan Data Storage Condensed ✓ Active Signals: ✓ 1	Signat: 2 Frag. Ran Mode: Scan Polanity: Positive ズ cycle tin Time(min) 0n/ Mass Range Frag. Frag. Time(min) 0ff Low High mentor Gain Thres- Step
 ✓ Ultra Fast Scan ✓ Time Filter Scan Data Storage ✓ Condensed ▼ Active Signals: ✓ 1 	Signal: 2 Frag. Rar Mode: Scan Polarity: Positive ✓ Time(min) On/ Off Low High mentor Gain hold size

Task 2. Enter MS acquisition parameters

Steps	Detailed Instructions	Commen
 2 Enter parameters for the spray chamber of the ion source: Drying gas flow: 9 L/min Nebulizer pressure: 40 psig Drying gas temperature: 300 °C Capillary voltage: 3000 V For 6150 with Agilent Jet Stream technology: Nebulizer pressure: 20 psi 	 a Right-click the MSD icon on the system diagram and select Spray Chamber. b Enter the parameters descril step 2 and shown in the figu below. c Click OK. 	
 Nebulizer pressure: 30 psi Drying gas flow: 7 L/min Drying gas temperature: 350°C Sheath gas flow: 12 L/min Sheath gas temperature: 360 C° Capillary voltage: 4000 V Nozzle voltage: 0 V Fragmentor: 200 V Multiplier gain: 3 	MSD Spray Chamber Method Spray Chamber: API-ES Installed Spray Chamber: Temperatures, Pressure, and Flow Actual Drying Gas Flow (I/min): Nebulizer Pressure (psig): Drying Gas Temperature (*C): Vaporizer Temperature (*C): Parameters Positive Capillary Voltage (V): N/A Charging Voltage (V): N/A	Setpoint Maximum 9.0 13.0 40 60 300 350 N/A N/A

Time (min)

For all models except 6150 with Agilent Jet Stream technology

Parameter

MSD Spray Chamber			×
Method Spray Chamber: AJS-ES Installed Spray Chamber: AJS-ES	C ON C OFF	Parameters Capillary Voltage (V): 13 Cojona Current (µA): N/	
Temperatures, Pressure, and Flow	Colorine Manimum	Nozzle Voltage (V): 20	00 2000
Actual Drying Gas Flow (I/min): 0.5	Setpoint Maximum 12.0 13.0	, 	
Nebulizer Pressure (psig): 1	35 60	Time (min) Parame	ter Value
Drying Gas Temperature (*C): 22	250 350		
⊻aporizer Temperature (*C):	N/A N/A		
Sheath Gas Temperature (*C): 28	150 150		
Sheath Gas Flow (I/min): 0.5	3.0 12.0	Insert Append Cut	<u>Copy</u> <u>Paste</u>
<u><u> </u></u>		ancel <u>E</u>	lelp

Value

For 6150 with Agilent Jet Stream technology

Task 2. Enter MS acquisition parameters

Steps	Detailed Instructions	Comments
3 Set up to store the fragmentor voltage throughout the run.	 a Right-click the MSD icon on the system diagram and select Data Curves. b Select Fragmentor - 1. c Click the Add button. d Click OK. 	- El
	MSD Data Curves	
	Fragmentor - 3	Selected: Add -> Fragmentor - 1 <- Remove All
	ОК	Approximate Data Rate: .5 💌 Hz. Cancel Help
4 Save the method.	 a Select Method > Save Moverwrite the method SULFA MS SCAN 1.M. b In the box for Comment history, type a comment c Click OK. 	for method

Task 2. Enter MS acquisition parameters

Steps	Detailed Instructions Comments	
5 Print the method.	 a Select Method > Print Method. b Mark the check boxes as shown in the figure below. c Click the Print button. 	
	Print Method: Instrument 1 Select Parts of the Method to be printed: Check All Miscellaneous ✓ Method Information ✓ Method Change History ✓ Run Time Check List Instrument/Acquisition ✓ Detectors ✓ Injector/AFC ✓ Pump/Oven/Chip ✓ Valves ✓ + Timetable ✓ + Timetable Ø ata Analysis ☐ Integration Events ☐ Calibration Data ☐ LibSearch and Others	
	Select Destination for Printout: Printer C File Print Cancel Help	

Exercise 2. Acquire data with the full-scan method

Now you are ready to acquire data for the sulfa mix with the method you just created. This exercise consists of the following tasks:

- "Task 1. Enter sample information" on page 24
- "Task 2. Acquire the data" on page 25

Task 1. Enter sample information

Task 1. Enter sample information

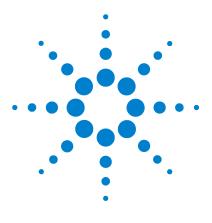
Steps	Detailed Instructions	Comments
1 Display the Single Sample toolbar.	• In the top toolbar, click the single sample icon.	
1 Display the Sample Information dialog box.	a Click Sample Info on the RunControl menu.	
 2 Enter the sample information: Operator name Subdirectory: Sulfas Prefix: Sulfa_scan Location: Vial 1 	 a Enter the parameters described in step 2 and shown in the figure below. b Click OK. 	 If you select Prefix/Counter, the file names increment automatically from one run to the next.
 Sample Name: Sulfas 10 ng/µL Comment: Scan familiarization exercise 	Sample Info: Instrument 1 Operator Name: Your name Data File Path: Path: C:\Chem32\1\DATA\ C Manual Prefix Sample Parameters Sulfa_scan Sample Name: Sulfas 10 ng/uL Sample Name: Sulfas 10 ng/uL Sample Amount: 0 Target Masses: Comment: Scan familiarization exercise 1	Subdirectory: SULFAS Counter: 00001 Vial 1 (blank run if no entry) Multiplier: 1 Dilution: 1
	Run Method OK	Cancel Help

Task 2. Acquire the data

S	teps	Detailed Instructions	Comments
1	Place the vial of sulfa sample you prepared at 10 ng/µL into position 1 in the autosampler.		• You prepared this sample in "Exercise 2. Prepare the samples for the analyses" on page 12.
2	Inject the sulfa mix sample.	Click the Single Sample buttor start the run. Single Sample	to This button is present only when you have selected Single Sample mode from the top toolbar.
3	Monitor the total ion chromatogram and the UV chromatogram during data acquisition.	 a From the Online Plot window, the Change button. b In the list of Available Signals select DAD A: Signal=272,16 Reference=360,100 and click A c In the list of available signals, s MSD: Signal 1 and click Add. d Monitor the MS signal to ensure stable baseline. 	MS signal is greater than 10%, the nebulizer and source chamber may require maintenance. See the Add. Agilent 6100 Series Single Quad select LC/MS System Maintenance Guide
		Edit Signal Plot Available Signals	
		Autosampler: Air Temperature Binary Pump: Pressure Binary Pump: Flow Binary Pump: %A Binary Pump: %B	Add -> Selected Signals DAD A: Signal=272,16 Reference=360 MSD: Signal 1
		x-axis 4 min T	D: Signal 1 ype: acquired y-axis range: 3000000 * Abund auto y-adjust Offset: 10 * %
		Fraction Collector	hod Settings Apply to Method
			1978

Task 2. Acquire the data

Steps	Detailed Instructions	Comments	
4 Save the signals for the Online Plot window.	 a In the Edit Signal Plot dialog box, click the Apply to Method button. b Save the method. 		
5 When the analysis is done, view the results.	• To view the results, go to the next exercise.	 The C18 column may require one or two injections of the sample to be fully conditioned. During these initial injections, everything may be eluted from the column in the void volume. Repeat the process and separation will occur. 	



Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide

Qualitative Data Analysis

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Exercise 3. Integrate the chromatogram 36
Exercise 4. Print a report 40

These exercises use the data file you generated in Chapter 2. Alternatively, you can use the sulfa demo data file that you received with the ChemStation software. This chapter shows you how to analyze data when you need to identify or confirm sample components.

Before you start

3

- Read the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide.
- Read the chapter on Data Analysis in the Agilent 6100 Series Quadrupole LC/MS Systems Concepts Guide.
- Set up and run the acquisition method in Chapter 2, "Set Up and Run a Scan Method" or that you have the **mssulfas.d** data file in the **MSDEMO** data folder on your system.

For the tasks on the following pages, perform the exercises in the order they appear. Try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.



Exercise 1. Display and manipulate chromatograms

Exercise 1. Display and manipulate chromatograms

In this exercise, you load chromatograms and change the chromatographic display.

Steps	Detailed Instructions	Comments	
1 Display Data Analysis view.	 In the view selection area of the ChemStation window, click Data Analysis. Data Analysis 		
2 Load the method SULFA MS SCAN 1.M.	 a Select File > Load > Method. b Navigate to the folder c:\CHEM32\1\METHODS. c Select the method file and click OK. 	 If you just completed the previous exercise, that method is already loaded. 	
3 Display the Signal Toolset.	 Click the Signal icon, which is near the middle of the window. 		

Exercise 1. Display and manipulate chromatograms

Steps	Detailed Instructions	Comments
 4 Do one of the following: Open the data file, SULFA_SCAN00001.D, which you acquired in Chapter 2. Open the data file mssulfas.d, located in the MSDEMO folder. 	 a Select File > Load Signal. b Navigate to the appropriate folds either: • C:\CHEM32\1\DATA\SULFA or • C:\CHEM32\1\DATA\MSDE c Select the data file. d Set other parameters as shown below and click OK. 	 Concepts Guide. If you wish to complete Chapter 4, "Set Up and Run a SIM Method",
	Load Signal : Instrument 1	
	File name: MSSULFAS.D cafcal01.d cafcal02.d cafcal02.d cafcal03.d cafcal04.d cafcal05.d file d Loadtest.d ms3traq.d File Information Load using Signal Details Signal Information Spectra MSD: 427 Cond. Integrate after load Integrate and print report after load	Folders: c:\\msdemo C:\ C CHEM32 DATA MSDEMO C: Signal Details Signals: DAD1 A, Sig=270,20 Ref=360,100 MSD1 TIC, MS File, Pos, Scan
5 Verify that you see the DAD and MS chromatograms.	 a Check that you see a display tha similar to the one shown below. b Verify that you see the DAD sign in the top chromatogram. c Confirm that you see the MSD signal in the bottom chromatogram. 	al

Exercise 1. Display and manipulate chromatograms

Step	DS	Detailed Instructions	Comments
t	Change the chromatogram view so hat the MS and UV chromatograms are overlaid in the display.	 a In the Signal Toolset near the middle of the window, click the icon to display overlaid signals. b Check that you see the overlaid chromatograms, as shown below. c Click the icon to display separate signals. 	• The icon in step a is also available in the Graphics Toolset, but in that toolset it toggles overlaid/separate. You click the icon shown above to turn on the display of the Graphics Toolset.
	DAD1 A, Sig=270,20 Ref=360,100 (MSDEMOW MSD1 TIC, MS File (C:\CHEM32\1\DATA\MSDE		
21 11 11			4 min
	Remove the DAD signal from the lisplay.	 a In the Navigation Table, click the + to display more information. b Under the Signals tab, double-click the signal labeled MSD1 TIC. c When you see the message about the method, click OK. d Verify that the DAD window is gone and only the TIC is displayed. 	 If you do not see the Navigation Table shown below, in the top toolbar, click the icon shown above. For other ways to remove signals, see the chapter on Data Analysis in the <i>Concepts Guide</i>.
Data :	Analysis		
D.	ate Time Operator Vial 0/29/1997 3:11:1 C. Miller Vial 1	✓ O O → → → → → → → → → → → → → → →	
a	0/29/199/ 3:11:1 C. Miller Vial 1		
	and the second se		ita File Sample Name 🗹 Method Name 🔗
-		0/29/1997 3:11:1 C. Miller Vial 1 MS	SULFAS.D sulfa drug mix MSPURITY.M

Signals General Info Instrument Curves

DAD1 A, Sig=270,20 Ref=360,100

MSD1 TIC, MS File, Pos, Scan

Description

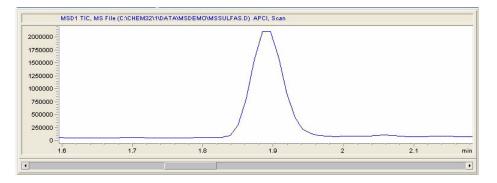
b -

Load?

Exercise 2. Examine mass spectra

In this exercise, you learn to display mass spectra. You choose background (reference) spectra that you can later subtract from the spectra of the peaks of interest. You learn how to display a single spectrum and an averaged spectrum for a peak.

Steps	Detailed Instructions	Comments	
 Zoom in on the first peak in the chromatogram. 	 a Click the icon to zoom in. b Use the mouse pointer to draw a rectangle around the peak. Take care to include the chromatographic baseline. c Check that your peak looks similar to the one below. d Note the width of the peak at half height. You will need this information to set up the SIM analysis in Chapter 4. 	 If you want to try again, you can zoom back out. Do one of the following: Double-click the chromatogram window. Click the icon to zoom out. 	



- 2 Display the Spectrum Toolset.
- a Click the Spectrum icon, which is near the middle of the window.
- b If there is not room under your chromatogram window to display spectra, use your mouse pointer to reduce the height of the chromatogram window.

Exercise 2. Examine mass spectra

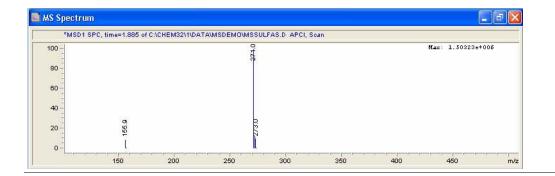
S	teps	Detailed Instructions	Comments
3	Get the first reference spectrum, to the left of the peak.	 a To select the first reference spectrum, click the icon that is highlighted here. b In the chromatogram window one of the following at the chromatographic baseline just before the peak: Click to select a single spe Click and drag to select an average spectrum. 	st ctrum.
4	Get a second reference spectrum, to the right of the peak.	 a To select the second reference spectrum, click the icon that is highlighted here. b In the chromatogram window one of the following at the chromatographic baseline just the peak: Click to select a single spe Click and drag to select an average spectrum. 	st after ctrum.
5	View your reference spectra.	 a If you cannot see the spectra the size and location of the w labeled Reference Mass Spectrum(a). b Note the two background sp. — one before the peak and c after. 	vindow

Exercise 2. Examine mass spectra

Manual.select new reference spectra thatdMark the check boxes for Ref1 andare close in time to each peak ofdRef2. Note that the time ranges of the reference spectra that you just selected are specified there.• Near the middle of the DataAnalysis window, you can view ar	Steps	Detailed Instructions	Comments
Spectral Options Spectral Reference Display Purity Mass Spectra MS Reference MS Display Reference Spectrum ^ None ^ Manual		 Spectral Options dialog box. Click the MS Reference tab. Under Reference Spectrum, click Manual. Mark the check boxes for Ref1 and Ref2. Note that the time ranges of the reference spectra that you just selected are specified there. 	 subsequent spectra until you change the options. If the chromatographic baseline changes over the course of the run select new reference spectra that are close in time to each peak of interest. Near the middle of the Data Analysis window, you can view and change the setting for background
Reference Spectrum Averaging Time Window ○ None □ Use window: □ 200 min ○ Manual ▲▲▲ From 1.802 To 1.826 min ▲▲▲ All Spectra: The nearest integrated baseline start and end are taken as the reference spectra times.			
		Reference Spectrum C None Manual ▲▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲	Averaging Time Window Use window: 0.200 min min min start and s.

Exercise 2. Examine mass spectra

Steps	Detailed Instructions	Comments	
7 Get a single background-subtracted spectrum for the first LC peak.	 a Click the icon to get a mass spectrum at any time point. b In the chromatogram window, click somewhere on the peak to get the spectrum. c If necessary for easier viewing, adjust the size and location of the window labeled MS Spectrum. d Verify that the spectrum is similar to the one shown below. 	 Under the conditions used to acquire the demo data file (mssulfas.d), the compounds elute in the following order: Sulfamethizole, m/z = 271 Sulfachloropyridazine, m/z = 285 Sulfamethazine, m/z = 279 Sulfadimethoxine, m/z = 311 Depending on the organic mobile phase and the modifiers, the elutior order for the 279 and 285 may change. 	



Exercise 2. Examine mass spectra

5	teps	Detailed Instructions	Comments
	Get a average background-subtracted spectrum for the first LC peak.	 a Click the icon to get an averaged mass spectrum. b In the chromatogram window, click and drag the mo across the peak, as shown below. c View the average spectrum in window labeled MS Spectrum 	ow. the
	MSD1 TIC, MS File (C:\CHEM32\1\DATA\MSDE	EMO\MSSULFAS.D) APCI, Scan	
	2000000 1500000 1000000		
	0	1.8 1.9	2 2.1 min

9 Be sure to see step 6 in "Exercise 3. Integrate the chromatogram" for an easier, faster way to display spectra.

Exercise 3. Integrate the chromatogram

Exercise 3. Integrate the chromatogram

In this exercise, you learn to set integration events and integrate the chromatogram. Even if you do not care about quantitation, integration helps locate peaks for other purposes. For example, after integration, mass spectra of each peak can be printed with a report.

Steps		Detailed Instructions	Comments
1	Display the total ion chromatogram in its entirety.	 a Minimize any spectral windows th hide the chromatogram window. b Click the icon to zoom out. 	at
2	Display the Integration Toolset.	 Click the Integration icon, which is near the middle of the window. 	n

Exercise 3. Integrate the chromatogram

Si	teps	Detailed Instructions	Comments		
3	Integrate the chromatogram.	 a Click the Auto Integrate icon, which is near the middle of the window. b Verify that the results are similar to those shown below. 	 Auto Integrate estimates initial integration parameters. If you do not see the retention times, in the graphics tools, click the icon to display retention times. If you do not see the pink integration baseline, in the graphics tools, click the icon to display baselines. 		
	MSD1 TIC, MS File (C:\CHEM32\1\DATA\MSDEM	IDIMSSULFAS.D) APCI, Scan	ę.		
	3000000		ri I		
	2500000-	565 1880	<u>}</u>		
	2000000 -				
	1500000 -				
	1000000 -				
	500000 -	2320	3.657 3.768 3.768 3.768 4.432 4.432		
		2.352			
1000	0.5 1	1.5 2 2.5 3	3.5 4 4.5 mi		
1 None of the	•				

Exercise 3. Integrate the chromatogram

St	eps		Detail	ed Instruc	tions			Comments
4	Adjust the integration paramet get only four integrated peaks.	ers to	b In t tab sel c For d Clic Chr e Ver	ck the icon egration E the Integra ile, for Bas ect Advan Height Re ck the Inte romatogra rify that yo hilar to tho	vents Tabl ation Event eline Corr ced. eject, type grate curr m icon. ur results :	e. [ts ection, 50000 ent are	0.	• For detailed information about integration events, see <i>Agilent</i> <i>ChemStation: Understanding Your</i> <i>ChemStation.</i>
			MSD1 TIC, N	MS File (C:\CHEM3	2111DATAMSDEM	MO\MSSULFAS	S.D) APCI, S	Scan
м	lanual Events	3000000						22 00 00 00 00 00 00 00 00 00 00 00 00 0
F	or All Signals:	2500000				890	2.265	
		2000000					1	
	Integration Events Value Tangent Skim Mode Standard Tail Peak Skim Height Ratio 0.00	1500000						
	Front Peak Skim Height Ratio 0.00 Skim Valley Ratio 20.00	1000000 -				10	1	
	Baseline Correction Advanced Peak to Valley Ratio 500.00	500000 -				\mathbb{I}	1	that
E	vents Table MSD1 TIC Specific 💌	0-				2		
		•						
	Time Integration Events Value		T:	.		5.2° Iu		•
	Initial Slope Sensitivity 197292 Initial Peak Width 0.0531	#	1.89	Area 6728849	Height 2111708	Width 0.051	Area% 19.387	Symmetry 0.88
	Initial Area Reject 120157	2	2.265	6893364	2043800.6	0.0497	19.861	0.862
	Initial Height Reject 500000	3	2.783	10803332	3160739.8	0.0537	31.127	0.819
	Initial Shoulders OFF	4	3.349	10282083	2941516.5	0.0546	29.625	0.817

500 m/z

Exercise 3. Integrate the chromatogram

Steps	Detailed Instructions	
5 Save the integration events to the method in memory.	• Click the icon to exit and save the integration results.	• To save the events to the method on disk, you also need to save the method to disk, as described in step 3 on page 41.
Use the integrated chromatogram as the basis for a faster way to display background-subtracted spectra.	 a Click the Spectrum Expectrum icon. b Click the icon to display the Spectral Options dialog box. c Click the MS Reference tab. d Under Reference Spectrum, click Automatic. e Click OK. f Click the icon to get a mass spectrum at the peak apex. g In the chromatogram window, click somewhere on the fourth peak to get the spectrum. h Verify that the spectrum is similar to the one shown below. 	 When you set Reference Spectrum, to Automatic, the software automatically takes the reference spectra for each peak, as described in the Spectral Options dialog box. The icon to get the mass spectrum at the peak apex is available only if you have integrated the chromatogram. No matter where you click on the peak, it gets the spectrum at the apex. With this tool, you may not need to zoom in on the chromatogram to achieve a precise location for the spectrum.
	Apex Mass Spectrum of Peak 3.349 of MSSULFAS.D "MSD1 SPC, time=3.361 of CACHEMO2YIDATAMSDEMOMSSULF 100 80 40 20 20 20 20 20 20 20 20 20 20 20 20 20	AS.D APCL Scan

Exercise 4. Print a report

Exercise 4. Print a report

O Separately

Destination

Printer

Report Layout For Uncalibrated Peaks

Screen

With Calibrated Peaks

Eile Eile

In this exercise, you print a report, which you will use in Chapter 4, "Set Up and Run a SIM Method."

S	teps	Detailed Instructions	Comments	
1	Specify the LCMS Qualitative report style, with the report printed to the screen.	 a Select Report > Specify b In the Specify Report dial under Destination, mark box for Screen. c For Report Style, select L Qualitative. d Check that other settings shown below. e Click OK. 	og box, the check CMS	
	Quantitative Results Calculate: Percent V Based On: ISTD Correction V Use Multiplier & Dilution Factor with		Calculation Factors Use Sample Data From Data Fi Amount 0.0000 If Multiplier 1.0000	
	Style Report Style: LCMS Qualitative	Report Style Options Add Fraction Table and Ticks	Dilution 1.0000 Ente	er Size

O Do Not Report

Unique pdf file name

File Settings

OK

1 Pages

Help

PDF

Cancel

O Multi-Page (Landscape)

EMF

MTH.

Response:

-

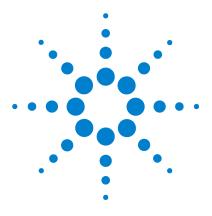
40

DIF

Signal Options...

Steps	Detailed Instructions	Comments
2 Print the report.	 a Select Report > Print Report. b After a short wait, view the Report window. c Verify that page 1 of the report contains header information and an integrated chromatogram. d At the bottom of the report window, click the Next button. e Verify that page 2 of the report shows extracted ion chromatograms and a mass spectrum of the first chromatographic peak. f Continue to click the Next button to view results for the three additional chromatographic peaks. g At the bottom of the report window, click the Print button. This prints a hard copy of the report. h At the bottom of the report window, click the Close button. 	 If you wish to complete Chapter 4, "Set Up and Run a SIM Method", then save the hard copy and refer to it when you set up your SIM groups. The extracted ion chromatograms are indicators of peak purity; if the retention times fail to coincide, the peak likely represents more than one compound.
3 Save the method.	 a Select File > Save > Method to overwrite the method SULFA MS SCAN 1.M. b In the box for Comment for method history, type a comment. c Click OK. 	 You save the method now so that your integration parameters, spectral display options, report settings, and other data analysis settings become part of the method.

Exercise 4. Print a report



4

Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide

Set Up and Run a SIM Method

Exercise 1. Set up a SIM acquisition method 44
Task 1. Load the scan method you created previously 44
Task 2. Enter MS acquisition parameters 45
Exercise 2. Acquire data with the SIM method 48
Task 1. Enter sample information 49
Task 2. Acquire the data 50

These exercises show you how to set up a data acquisition method that uses selected ion monitoring (SIM). You set up the method for the demonstration sample (sulfa mix) and then run the sample with that method.

To set up the SIM method, you modify the scan method that you created in Chapter 2. To set up the SIM acquisition, you need the following for each of the four sulfa compounds:

- The LC retention time
- The masses of ions in the spectrum

You get that information from the report you generated in Chapter 3.

Before you start

• Complete the previous exercises in this manual.



Exercise 1. Set up a SIM acquisition method

Exercise 1. Set up a SIM acquisition method

In this exercise, you start with your existing scan method and modify it for SIM analysis. You keep the same LC conditions and modify only the MS conditions. This exercise consists of the following tasks:

- "Task 1. Load the scan method you created previously" (below)
- "Task 2. Enter MS acquisition parameters" on page 45

Task 1. Load the scan method you created previously

Steps		Detailed Instructions	Comments	
4	Display Method and Run Control view.	 In the view selection area of the ChemStation window, click Method and Run Control. Method and Run Control 		
5	Open the method SULFA MS SCAN 1.M.	 a Select File > Load > Method. b If necessary, navigate to C:\CHEM32\1\METHODS. c Select SULFA MS SCAN 1.M and click OK. 		
6	Save the method under a new name, SULFA MS SIM 1.M .	 a Select File > Save As > Method. b In the dialog box, for Name, type SULFA MS SIM 1.M. c Click OK. d In the box for Comment for method history, type a comment. e Click OK. 	 You save the method now to avoid inadvertent overwrites of your scan method. 	

Task 2. Enter MS acquisition parameters

Task 2. Enter MS acquisition parameters

Steps	Detailed Instructions	Comments	
1 Enter the chromatographic peak width for the SIM analysis.	 a Right-click the MSD icon on the system diagram and select Set up MSD Signals. b When the Set Up MSD Signals dialog box is displayed, type 0.05 For Peakwidth. c Click OK. 	 The peak width is an important setting. It is used to calculate the appropriate SIM dwell times to deliver sufficient points across a chromatographic peak to give good quantitation. Peak width is defined as the full width at half maximum (FWHM), the width at 50% of the peak height. 	

Task 2. Enter MS acquisition parameters

Steps	Detailed Instructions	Comments	
 Set up the first SIM ions using the masses (to the nearest 0.1) that you observed in the spectra from your scan analysis: Sulfamethizole: Time 0, SIM lons 271 and 156. 	 a Under MSD Signal Settings, Signal 1, for Mode, select SIM. b In the table, for Fragmentor, type one of the following: 150 for the Agilent 6120 200 for the Agilent 6130 or 6150 c In the table, change Group 1 to Sulfamethizole, and for SIM Ion, refer to the spectrum on your printout and type the mass (to the nearest 0.1) for the 271 ion. d Click Add Ion, and type the mass for the sulfamethizole 156 ion. 	 In this example, each SIM group includes a pseudo-molecular ion and one fragment ion for confirmation. Note that the figure below does not show the fourth sulfa drug. 	
	StopTime: noLimit ▼ FIA Disabled Mode: SIM ▼ Polarity General Time(min) On/ Gro Tune File: atunes.tun ▼ Source: API-ES Peakwidth 0.050 min 2 1 Quele Time 0.30 sec/cycle Sott Addion IV Time Filter Scan Data Storage Signal: 2 ✓ Condensed ▼ Time(min) On/ Mode: Scan ▼ Polarity	sim Frag- Ion Gain Dwell %Rel (msec) Dwell methiz 156.10 150 1.00 139 50.0 - 2271.00 150 139 50.0 - - - 2271.00 150 1.00 92 33.3 - - - 2287.00 150 92 33.3 - - - - Add Grp Cut Copy Paste - - - - r Positive - % cycle time: - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - <td< td=""></td<>	

Task 2. Enter MS acquisition parameters

S	teps	Detailed Instructions	Comments	
3	 Set up the remaining SIM ions, using the masses (to the nearest 0.1) that you observed in the spectra from your scan analysis: Sulfachloropyridazine: Time 1.3, SIM lons 285, 287, and 156. Sulfamethazine: Time 2.3, SIM lons 279 and 186. Sulfadimethoxine: Time 3.3, SIM lons 311 and 156. 	 a Click Add Grp, and type the name, start time and mass (approximately 285) for sulfachloropyridazine. b Click Add Ion, and type the mass for the sulfachloropyridazine 156 ion. c Click Add Ion, and type the mass for the sulfachloropyridazine 287 ion. d Repeat these steps until you have entered two or three ions for each of the remaining compounds. e Click OK to close the Set Up MSD Signals dialog box. 	 Alternatively, instead of making separate groups for each compound as described here, <i>all</i> of the SIM ions could be entered into "Group 1", which could be re-named "Sulfonamides". The first SIM group can contain up to 100 ions. You may need to adjust the start time for each SIM group. Refer to your printout from Chapter 3 to determine a start time so that each group change occurs about midway between the chromatographic peaks. If the retention time difference between sulfachloropyridazine and sulfamethazine is less than 0.3 minutes, merge these ions into one group. The sulfachloropyridazine additionally includes the chlorine isotope at <i>m</i>/<i>z</i> 287. 	
4	Save the method.	 a Select Method > Save Method to overwrite the method SULFA MS SIM 1.M. b In the box for Comment for method history, type a comment. c Click OK. 		

Exercise 2. Acquire data with the SIM method

Exercise 2. Acquire data with the SIM method

Now you are ready to acquire data for the sulfa mix with the method you just created. This exercise consists of the following tasks:

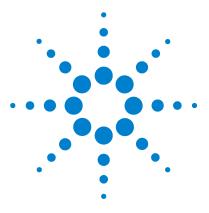
- "Task 1. Enter sample information" on page 49
- "Task 2. Acquire the data" on page 50

Task 1. Enter sample information

Steps	Detailed Instructions Comments
1 Display the Single Sample toolbar.	In the top toolbar, click the single sample icon.
1 Display the Sample Information dialog box.	a Click Sample Info on the RunControl menu.
 2 Enter the sample information: Operator name Subdirectory: Sulfas Prefix: Sulfa_SIM Location: Vial 1 	 a Enter the parameters described in step 2 and shown in the figure below. b Click OK. b If you select Prefix/Counter, the file names increment automatically from one run to the next.
 Sample Name: Sulfas 10 ng/µL Comment: SIM familiarization 	Sample Info: Instrument 1
exercise	Operator Name: Your name Data File Path: Path: C:\Chem32\1\DATA\ Image: Common Stress State Counter: Sample Parameters 000001 Sample Name: Sulfas 10 ng/uL Sample Name: Sulfas 10 ng/uL Sample Amount: 0 Multiplier: 1 ISTD Amount: 0 Date File Image: Sulfas 10 ng/uL Sample Amount: 0 Dilution: 1 Target Masses: Image: Silf familiarization exercise Run Method OK Cancel Help

Task 2. Acquire the data

Steps		Detailed Instructions	Comments	
1	Place the vial of sulfa sample you prepared at 10 ng/µL into position 1 in the autosampler.		• You prepared this sample in "Exercise 2. Prepare the samples for the analyses" on page 12.	
2	Inject the sulfa mix sample.	 Click the Single Sample start button. Single Sample 	This button is present only when you have selected Single Sample mode from the top toolbar.	
3	Monitor the total ion chromatogram and the UV chromatogram during data acquisition.	 a Activate the Online Plot window. b Monitor the MS signal to ensure a stable baseline. 	• If the baseline fluctuation for the MS signal is greater than 10%, the nebulizer and source chamber may require maintenance. See the Agilent 6100 Series Single Quad LC/MS System Maintenance Guide.	
4	When the analysis is done, view the results.	 a Display Data Analysis view. b Load the data file you just created. c Examine the DAD and MS chromatograms. 	• If you need help, follow the general procedure in "Exercise 1. Display and manipulate chromatograms" on page 28 in Chapter 3.	



5

Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide

Set Up and Run a Sequence

Exercise 1. Set up a sequence 52 Task 1. Prepare to create a new sequence 52 Task 2. Edit sequence parameters 53 Task 3. Set up the sequence table 55 Task 4. Set up the sequence output 58 Exercise 2. Run the sequence 60

These exercises show you how to set up a sequence for the SIM analysis of the demonstration sample (sulfa mix), and to acquire data with that sequence.

In the sequence, you run the sulfa mix at three concentrations: 1, 5 and 10 ng/ μL . You also run a solvent blank.

Before you start

- Read the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide and Chapter 3 of the Agilent 6100 Series Quadrupole LC/MS Systems Concepts Guide.
- Complete the previous exercises in this manual.

For details about sequences, see the automation chapter in *Agilent ChemStation: Understanding Your ChemStation.*



Exercise 1. Set up a sequence

Steps	Detailed Instructions	Comments
1 Display Method and Run Control view.	 In the view selection area of the ChemStation window, click Meth and Run Control. Method and Run Control 	hod
2 Display the Sequence Toolset .	In the top toolbar, click the icon to display the Sequence Toolset.	unCor
3 Display the Autosampler Tray diagram.	Vial Po Vial Symbol Legend	View menu.
4 Initiate setup of a new sequence.	Select Sequence> New sequence	ce . The empty default sequence file, DEF_LC.S , is loaded automatically.
5 Save the sequence under a new name, SULFA MS SIM 1.S	 a Select Sequence > Save Seque b For Name, type SULFA MS SI c Click OK. 	

Task 1. Prepare to create a new sequence

Task 2. Edit sequence parameters

S	teps	Detailed Instructions	Comments
1	Open Sequence Parameters dialog box.	 Click Sequence > Sequence Parameters. 	• The sequence parameters are settings that are common to all the samples in the sequence.
		Sequence Parameters; Instrument 1	X
		Operator Name: Your name Data File Path: C:\Chem32\1\DATA\ C Auto Prefix: Counter: Prefix/Counter sulfa_seq 000001	Subdirectory: SULFAS
2	Enter the sequence parameters for Operator Name and Data File .	 a Enter the following parameters, shown in step 1. Operator name: Your name Subdirectory: Sulfas Prefix: Sulfa_seq 	 To avoid overwrite of data files, type a new subdirectory for each sequence. The directory will be created if it doesn't already exist on your computer. Unique file names are automatically created for each data file within the subdirectory.

Task 2. Edit sequence parameters

ps	Detailed Instructions	mments			
inter the rest of the sequence arameters:	shown in the figure below. Parts of methods to run: According to Runtime Checklist	 If you wanted to run only reprocessing (data analysis), you would set that in Part of methods to run. The Wait allows the instrument to equilibrate when a new method is loaded. 			
	Part of methods to run	Shutdown			
	According to Runtime Checklist	Post-Sequence Command/Macro			
	Use Sequence Table Information	STANDBY			
	Wait 10 minutes after loading a new metho	od Not Ready Timeout: 15 minute:			
	Bar Code Reader	smatch C Inject anyway			
	Fraction Information	ChemStore			
	Fraction Start Location:	Transfer Settings			
	Sequence Comment: Sequence familiarization exercise				
	ОК	Cancel Help			

a convenient way to turn off lamps, pumps, etc. The command or macro is run at the end of the sequence or in the event of an error.

- MSSetState is a command that can change the MS state to standby. See the online Help for commands.
- SHUTDOWN.MAC is a macro that will shut down the system, but you must customize it before using it.

Task 3. Set up the sequence table

Steps	Detailed Instructions	Comments	
 Set up the sequence table to: Run duplicate injections of a blank. Run duplicate injections of the sulfa mix at three concentrations: 1,5 and 10 ng/μL. Use the method SULFA MS SIM 1.M, that you created in Chapter 4, "Set Up and Run a SIM Method". 	 a Click Sequence > Sequence Table. b Select the first line of the sequence table. In the sequence table, under Line, click the number 1. c Click the Cut button to delete the line. d Click the button for the Insert/Filldown Wizard, shown below. e Fill in the values and click OK. 	 In this step, you set up the parts of the sequence table that are common to all the samples. You will specify the sample names later in this exercise. There are a number of ways to add samples to the sequence table. This exercise illustrates just one of the ways — use of the Insert/Filldown Wizard. 	

Action Append Inset Fill down Every Inne(s)	 stected ranges:		Trom location	jular
Fields Clear all fields	Omit other sam Over <u>w</u> rite exist			
			ISTD Amount	
Clear all fields Method name	Over <u>w</u> rite exist	ing values		
Clear all fields Method name Samgle name Inj./Location	Over <u>w</u> rite exist Cal Le <u>v</u> el	ing values Average	ISTD Amount	

Task 3. Set up the sequence table

St	teps	Detail	ed Instructions			Comments			
2	View the sequence table that you have created so far.	 a Compare your table with the one below. b Note any differences, such as columns that are included and column widths. 				• Your results will likely differ, but in the next step you may recreate the table format below.			
		Line 1 2 3	Vial Sample Nam Vial 1 ng/ul sulfas Vial 2 ng/ul sulfas Vial 3 ng/ul sulfas	e		Method Name Inj/Vial Sample SULFA MS SIM 1 2 Sample SULFA MS SIM 1 2 Sample SULFA MS SIM 1 2 Sample			
		4	Vial 4 ng/ul sulfas			SULFA MS SIM 1 2 Sample			
3	(Optional) Customize the sequence table to match the format in step 2.	cor clic b Cle unr bel c Inc the d Dev for bel e Clic	he lower right-han ner of the dialog be sk the icon to custo sequence table. ar the check boxes necessary columns ow. rease the width of t sample name, as s crease the width of the method name, ow. ck OK .	ox, mize for any , as shov the colun shown be the colu as show	For descriptions of any columns you removed, see the online Help.				
			Column	Show?	Width				
			Vial		7				
			Sample Name	~	30				
			Method Name		15				
			Inj/Vial	V	7	4			
			Sample Type Cal Level		16 9				
			Update RF		13	4			
			Update RT	Г	12				
			Interval	Г	9				
			0 1 4 1		10				

Sample Amount ISTD Amount

Multiplier

Dilution

Datafile

Inj Volume

Lims ID Target Masses

AutoBalance

15

13 12

12

20

10

20

20

14

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Task 3. Set up the sequence table

e the following sample names into table:	а	Mod	lifv t						
iante. Vial 1 – blank Vemaining vials – sulfa mix at 1, 5	b		ple,	as	Sample Name for each shown below.				
nd 10 ng/µL		Line	,	Vial	Sample Name		Method Name	Inj/Vial	Sample Type
	Γ	1	V	'ial 1	Blank	9	SULFA MS SIM 1	2	Sample
		2	V	'ial 2	1 ng/ul sulfas	9	SULFA MS SIM 1	2	Sample
		3	V	ial 3	5 ng/ul sulfas	9	SULFA MS SIM 1	2	Sample
		4	V	ial 4	10 ng/ul sulfas	9	SULFA MS SIM 1	2	Sample
	emaining vials – sulfa mix at 1, 5	emaining vials – sulfa mix at 1, 5 nd 10 ng/μL	emaining vials — sulfa mix at 1, 5 nd 10 ng/µL	emaining vials – sulfa mix at 1, 5 nd 10 ng/μL Line 2 v 3 v 4 v	emaining vials – sulfa mix at 1, 5 nd 10 ng/μL <u>Line Vial</u> <u>1</u> Vial <u>2</u> Vial <u>3</u> Vial <u>4</u> Vial 4	emaining vials — sulfa mix at 1, 5 nd 10 ng/µL Line Vial Sample Name 1 Vial 1 Blank 2 Vial 2 1 ng/ul sulfas 3 Vial 3 5 ng/ul sulfas 4 Vial 4 10 ng/ul sulfas	emaining vials — sulfa mix at 1, 5 nd 10 ng/μL Line Vial Sample Name 1 Vial 1 Blank 2 Vial 2 1 ng/ul sulfas 3 Vial 3 5 ng/ul sulfas 4 Vial 4 10 ng/ul sulfas	emaining vials – sulfa mix at 1, 5 nd 10 ng/µL Line Vial Sample Name Method Name 1 Vial 1 Blank SULFA MS SIM 1 2 Vial 2 1 ng/ul sulfas SULFA MS SIM 1 3 Vial 3 5 ng/ul sulfas SULFA MS SIM 1 4 Vial 4 10 ng/ul sulfas SULFA MS SIM 1	emaining vials – sulfa mix at 1, 5 nd 10 ng/µL Line Vial Sample Name Method Name Inj/Vial 1 Vial 1 Blank SULFA MS SIM 1 2 2 Vial 2 1 ng/ul sulfas SULFA MS SIM 1 2 3 Vial 3 5 ng/ul sulfas SULFA MS SIM 1 2 4 Vial 4 10 ng/ul sulfas SULFA MS SIM 1 2

Task 4. Set up the sequence output

Task 4. Set up the sequence output

S	teps	Detailed Instructions	Comments			
1	Set up the sequence to print a short sequence summary to the printer.	 a Click Sequence > Sequence Output. b Mark the check box for Print Sequence Summary Report. c Mark the check box for Report to Printer. d Click the Setup button. e Fill in the dialog box as shown below. f Click OK in the Sequence Summary Parameters dialog box. g Click OK in the Sequence Output dialog box. 	 In addition to the sequence summary report, you can print individual sample reports, as specified in your method. (You do not print individual reports in this exercise.) For details about sequence reports, see the chapter on ChemStation reports in Agilent ChemStation: Understanding Your ChemStation. The setup shown in the dialog box below prints the simplest summary report. 			
		Sequence Summary Parameters: Instrum Activate report: Style: 1. One page header 2. Configuration 3. Sequence 4. Logbook 5. Methods 6. Analysis reports 7. Statistics calib. runs 8. Statistics sample runs Value V 9. Summary OK	ent 1			
2	Save the sequence.	Click the Save Sequence button in the Sequence toolset.				

Task 4. Set up the sequence output

Steps	Detailed Instructions	Comments
3 Print the sequence.	 a Select Sequence > Print Sequence. b Mark the check boxes as shown in the figure below. c Click the Print button. 	If you click the Print All button, you print all the parts of the sequence rather than the items you just specified.
	Sample Information Part Method and Injection Info Part File:	nation for Printout C:\CHEM32\1\SEQUENCE\

Exercise 2. Run the sequence

Exercise 2. Run the sequence

Now you are ready to acquire data with the sequence you just created.

Steps		Detailed Instructions	Comments
1	Confirm that your sequence includes four samples.	 Verify that the Autosampler Tray diagram shows four samples. 	
2	Place the samples you prepared in Chapter 1 into the appropriate positions in the autosampler.		You prepared the samples in "Exercise 2. Prepare the samples for the analyses" on page 12.
3	Inject the samples.	Click the Sequence start button on the Run Control Bar.	This button is only available if you have selected Sequence mode on the main toolbar.
4	(Optional) For the first blank analysis, monitor the total ion chromatogram and the UV chromatogram during	a Activate the Online Plot window.b Monitor the MS signal to ensure a stable baseline.	As the sequence progresses, the Autosampler Tray diagram is color-coded as follows:
	data acquisition.		Gray - samples that have been analyzed.
			White - samples not yet analyzed.
			Blue - the current sample.
5	When the sequence is done, view the Sequence Summary Report.	a Retrieve the report from the printer.b Examine the report to confirm that all the samples ran.	
6	When the sequence is finished, view the results.	 a Display Data Analysis view. b Load the first data file you just created. c Examine the DAD and MS chromatograms. d Repeat step b and step c for the other data files. 	 If you need help, follow the general procedure in "Exercise 1. Display and manipulate chromatograms" on page 28 in Chapter 3. When you analyze your own samples, you can set up your method to automatically generate a data analysis report for each sample in the sequence.



6

Agilent 6100 Series Quadrupole LC/MS System Familiarization Guide

Quantitative Data Analysis

Exercise 1. Create a method for quantification 62 Task 1. Create a new method 62 Task 2. Set up the signal for quantification 63 Task 3. Integrate the low-level standard 65 Task 4. Set general calibration parameters 67 Task 5. Set up the calibration curve 68 Task 6. Explore options to refine the calibration 72 Exercise 2. Process a sample and print a report 73

This chapter shows you how to use the ChemStation Data Analysis to perform quantification. The exercises in this chapter illustrate a simple calibration that uses data files that you received with your ChemStation software.

Before you start

- Read the Agilent 6100 Series Quadrupole LC/MS Systems Quick Start Guide.
- Read the chapter on Data Analysis in the Agilent 6100 Series Quadrupole LC/MS Systems Concepts Guide.
- Make sure that you have the caffeine data files on your ChemStation. Check for the files under
 C:\CHEM32\1\DATA\MSDEM0. The file names are CAFCAL0X.D, where x is a number from 1 to 5.



Exercise 1. Create a method for quantification

Exercise 1. Create a method for quantification

In this exercise, you create a calibrated method that you can use to quantify caffeine in the demo data.

Task 1. Create a new method

In this task, you load a default method and save it to a new name. You later modify the new method to create a calibrated method.

S	teps	Detailed Instructions	Comments
1	Display Data Analysis view.	 In the view selection area in the lower left of the ChemStation window, click Data Analysis. Data Analysis 	
2	Load the method DEF_LC.M .	 a Click File > Load > Method. b Navigate to the folder C:\CHEM32\1\METHODS. c Select the method file and click OK. 	
3	Save the method under the new name CAFFEINE CAL.M .	 a Select File > Save As > Method. b Navigate to the folder C:\CHEM32\1\METHODS. c In the dialog box, for Name, type CAFFEINE CAL.M. d Click OK. e In the box for Comment for method history, type a comment. f Click OK. 	

Task 2. Set up the signal for quantification

In this exercise, you add an extracted ion chromatogram (EIC) to the list of available signals for the method. Then you add this EIC to the Signal Details, so you can automatically load and integrate signals for the rest of the caffeine standards.

S	teps	Detailed Instructions	Comments
1	Open the data file CAFCAL01.D , located in the MSDEMO folder.	 a Select File > Load Signal. b Navigate to the folder: C:\CHEM32\1\DATA\MSDEM0. c Select the data file CAFCAL01.D. d If necessary, clear the check box fo Load using Signal Details. e In the Signals box, click the signal that begins with MSD1 TIC. f Click OK. 	 For other ways to load signals, see the chapter on Data Analysis in the <i>Concepts Guide</i>.
2	Extract the major ion of caffeine.	a Select File > Extract lons. b For lon 1, type 195.1. c For lon 2, type 195.1. d Click OK. Extract lons: Instrument 1 Select Data File: EXCHEM32X1XDATAXMSDEM0XCAFCAL01.0 Extracted lon Table Enter ions to be extracted. A single ion may be speci range using lon1 and lon2. Add lons from Signal Details Insert Signal Ion 1 lon 2 MSD1, Pos 195.1 195.1	• The 195 ion is the (M+H) ⁺ ion.
3	Display the Calibration Toolset.	• Click the Calibration icon, which is near the middle of the window.	

Task 2. Set up the signal for quantification

Steps	Detailed Instructions	Comments
4 Set up the signal for quantification.	 a Do one of the following: Click the icon to Edit current method signals. Select Calibration > Signal Details. b From the list of Available Signals, select MSD1 195, EIC=195.1:195.1. c Click Add to Method. d Click OK. 	 The EIC signal is available only because you loaded the 195 EIC in step 2.
	Signal Details: Instrument 1	
	Available Signals	Add to Method
	Insert Row Append Row Delete Row Signal Description	
	MSD1 195, EIC=195.1:195.1	
		→ → → → → → → → → → → → → → → → → → →
5 (Optional) Save the method under the same name (CAFFEINE CAL.M).	b In the box for Comment for method history, type a comment.	 For these exercises, you save the method frequently, but you could wait instead until you had
	c Click OK.	established all the method settings.

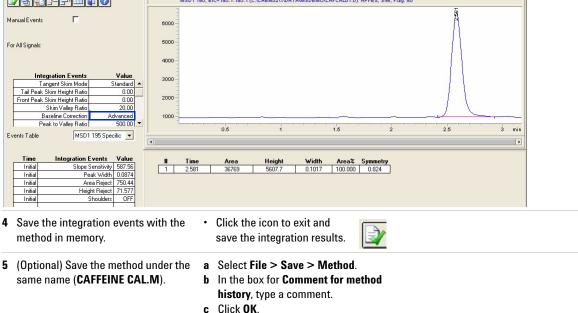
Task 3. Integrate the low-level standard

In this exercise, you establish integration parameters for your calibrated method. You use the low-level standard because it is usually the most difficult to integrate.

Steps	Detailed Instructions	Comments	
1 Display the Integration Toolset.	 Click the Integration icon, which is near the middle of the window. 		
2 Integrate the chromatogram.	 a Click the Auto Integrate icon, which is near the middle of the window. b Check that you have five integrated peaks with these initial settings. 	 Auto Integrate estimates initial integration parameters and then performs the integration. 	

Task 3. Integrate the low-level standard

Steps	Detailed Instructions	Comments	
3 Adjust the integration parameters to get one integrated peak.	 a Click the icon to Edit/Set Integration Events Table. b In the integration events for all signals, for Baseline Correction, select Advanced. c Click the Auto Integrate icon. d When you are prompted to save the events table, click Yes. e Verify that your results are the same or very similar to those shown below. 	• For detailed information about integration events, see Agilent ChemStation: Understanding You ChemStation.	



Task 4. Set general calibration parameters

- b In the box for Comment for method history, type a comment.
 - c Click OK.

2.580 MSD1 195

caffeine

1

Task 5. Set up the calibration curve

Task 5. Set up the calibration curve

In this exercise, you integrate the rest of the standards and add all standards to the calibration curve.

 Display the Calibration Toolset. Add the low-level standard to the calibration curve. 	• Click the Calibration icon, which is near the middle of the window.	
Calibration Table	 a Do one of the following: Click the New Calibration Table icon. Select Calibration > New Calibration Table. b Click Automatic Setup Level 1. c Click OK. d In the Calibration Table pane (shown below), under Compound, type caffeine and under Amt (amount), type 0.5. 	 Do not worry at this point if your calibration curve displays a message that says the curve is invalid.
Calibration Table		
Enter Delete Insert Print C # RT Signal Compound Lvl	DK Help	

36769.000

1 0.500

1.3598e-5 No No

Task 5. Set up the calibration curve

Steps	Detailed Instructions	Comments
Load and integrate the second standard.	 a Select File > Load Signal. b Under File name, select CAFCAL02.D. c Mark the check box for Load Signal Details. d Mark the check box for Integ after load. e Check that your dialog box loo the one below. f Click OK. 	rate
Load Signal : Instrument 1		
File name: cafcal02.d cafcal02.d cafcal03.d cafcal03.d cafcal05.d fia.d Loadtest.d ms3fraq.d File Information	Folders: c:\\msdemo CHEM32 CHEM32 The DATA MSDEMO Crives: C:	OK Cancel Help Network
C Load using Signal Details	Signals:	st. Curves
MSD: 259 SIM Integrate after load Load from BSB Integrate and print report after load	DAD1 A, Sig=272,4 Ref=450,80 MSD1 TIC, MS File, Pos, SIM, Frag. 80	
Add the second standard to the calibration curve.	a Click the icon to Add new level. b In the dialog box, for Default Amount, type 1 and	click

- OK.
- **c** Verify that the calibration table now has two entries, and the calibration curve contains two points.

Task 5. Set up the calibration curve

Steps	Detailed Instructions	
 5 Add the remaining three standards to the calibration table: CAFCAL03.D: 5 ng/μL CAFCAL04.D: 25 ng/μL CAFCAL05.D: 50 ng/μL 	 a Select File > Load Signal. b Under File name, select the next data file. c Verify that the chromatogram is properly integrated. d Click the icon to Add new level. e In the dialog box, for Default Amount, type the amount shown in step 5 and click OK. f Verify that the calibration table and the calibration curve contain the new entry. g Repeat step a through step f until you have added all the standards. h Confirm that your calibration table and calibration curve look like the ones below. 	 If multiple peaks are integrated in a chromatogram, retention time is used to find the correct peak for the calibration curve.

	Delete Inse	ert Print	OK	Help						caffeine, MSD1 195
E B	IT Signal	Compound	Lvi	Amt[ng/ul]	Area	Rsp.Factor	Ref	ISTD	#	Area = 41537.2593*Amt +82571.382
2.58	80 MSD1 195	caffeine	1	0.500	36769.000	1.3598e-5	No	No		Area - Rel. Res%(1): -64.419
			2	1.000	75718.000	1.3207e-5				
			3	5.000	348650.000	1.4341e-5			1	2000000 -
			4	25.000	1.4077e6	1.7759e-5				
			5	50.000	2.0119e6	2.4852e-5				1500000 -
										1000000 - 500000 - 3 + 0 - Correlation: 0.963

Task 5. Set up the calibration curve

Steps	Detailed Instructions	Comments
6 Refine the calibration curve	 a Select Calibration > Calibration Settings. b Under Default Calibration Curve for Type, select Quadratic. c Click OK. d Verify that your calibration curve now looks like the one below. 	re,
	Calibration Curve	
7 (Optional) Save the method same name (CAFFEINE CA		thod

Task 6. Explore options to refine the calibration

Task 6. Explore options to refine the calibration

This exercise describes additional calibration table layouts that give you more calibration options. You do not need these options to process the caffeine demonstration data, but you may need them when you process your own samples.

S	teps	Detailed Instructions	Comments	
1	Explore options to change the way calibration curves are constructed.	 a Select Calibration Table Options > Peak Details. b Verify that you see these columns in the calibration table: Curve Type Origin Weight 	 Note that this calibration table layout lets you change: Curve Type: The type of calibration curve (linear, quadratic, etc.) Origin: How the origin (zero point) is treated. Weight: The relative weights of the data points. 	
2	Explore options to add qualifier ions.	 a Select Calibration Table Options > Identification Details. b Verify that you see these columns in the calibration table: Resp % (response percent) +- (window for the response percent) Pk Usage (peak usage) 	 Note that this calibration table layout lets you define: Pk Usage: How the calibration uses the peak, for example, as a main calibration ion or a qualifier ion Resp %: The expected response of the qualifier ion, as a percentage of the main peak +-: A window for the expected percentage. 	
3	Display the original options for the calibration table.	 a Select Calibration Table Options > Overview. b Verify that the calibration table looks the same as in step 5 on page 70. 		

Exercise 2. Process a sample and print a report

In this exercise, you specify a report and test your calibration method by processing one of the standards as if it were a sample. You print a report of the results.

Steps	Detailed Instructions	Comments
 Specify a report with the following settings: Report destination: Screen External standard (ESTD) calculation, based on area Report style: Detail 	 a Do one of the following: Select Report > Specif Click the Specify Repoicon. b Enter parameters as described in step 1 and sl the figure below. c Click OK. 	rt 🕒
Specify Report: Instrument 1		
Quantitative Results Calculate: ESTD Based Or ISTD Correction Image: Construction Image: Construction Image: Use Multiplier & Dilution Factor with Multiplier & Dilution Factor with Style Report Style: Detail Image: Sample info on each page Image: Construction fields to sample info Sample Custom fields to Sample info Report Layout For Uncalibrated Peaks Separately Image: With Calibrated Peaks	h ISTDs Report Style Options Add Fraction Table and Ticks Add Summed Peaks Table Add Compound Custom fields	Calculation Factors Use Sample Data Amount D.0000 I# Compound ISTD.Amount Multiplier 1.0000 Enter Chromatogram Output O Poltrait O Landscape O Multi-Page (Landscape) I Pages Signal Options
Destination □ Printer ☑ Screen □ !	File Settings File Prefix Report	IZXT ESV EMF DF PDF ZLS HTM
	OK Cancel	Help

Exercise 2. Process a sample and print a report

Steps		Detailed Instructions	Comments	
2	Save the method under the same name (CAFFEINE CAL.M).	 a Select File > Save > Method. b In the box for Comment for method history, type a comment. c Click OK. 		
3	Load the standard of medium concentration.	 a Select File > Load Signal. b Under File name, select CAFCAL03.D. 		
4	Process the medium-level standard and print the report.	 a Do one of the following: Select Report > Print Report. Click the icon to preview results. b Verify that page 1 of the report contains header information, an integrated chromatogram, and an external standard report. c Check that the caffeine amount is about 5 ng/μL. d At the bottom of the report window, click the Next button. e Verify that page 2 of the report shows the calibration curve with the measured point identified with dotted lines. f (Optional) At the bottom of the report window, click the Print button so you get a hard copy. g At the bottom of the report window, click the Close button. 	 Another way to generate a hard copy is to click the Print Report icon. 	

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In This Book

When you do the exercises in this book, you learn how to:

- Prepare your LC/MS system for an analysis
- Set up methods for scan and selected ion monitoring analyses
- Acquire data
- Set up sequences for automated sample analyses
- Perform qualitative and quantitative analyses.

 ${}^{\textcircled{C}}$ Agilent Technologies, Inc. 2011

Printed in USA Revision A, September 2011



G1960-90080

