Agilent 6100 Series Quadrupole LC/MS Systems

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

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This guide is valid for the C.01.06 or later revision of the Agilent ChemStation software for the Agilent 6100 Series Quadrupole LC/MS systems, until superseded.

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CAUTION

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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.

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.

Content

1

Prepare for the Analysis

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 µm, *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

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. LC OpenLAB	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 Binary Pump > Control** on the **Instrument** menu to open the Pump Control dialog box. **b** Select **Standby** and click **OK**.	Alternate method: • Select **Standby** from the Pump shortcut 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** Right-click the pump icon. **b** Select **Method**. **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 green button in the Binary Pump pane. ⊖ **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.

Steps	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 **Method**. **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.

Steps	Detailed Instructions	Comments
1 Disconnect the column from the detector and MS.	**a** Turn the pump off by clicking the red button in the Binary Pump pane. ⏻ **b** Disconnect the column from the detector and MS. **c** 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, *p/n 873700-902*	**a** Turn the pump on. **b** 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).

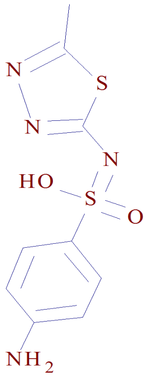
Steps	Detailed Instructions	Comments
3 Condition the column as follows, using the solvents made up in Task 2, step 6: • Flow rate – 0.4 mL/min • 100% B for 1/2 hour • 50% B for 1/2 hour	**a** Click **Set up Instrument Method** on the **Instrument** menu to open the Setup Method dialog box. **b** Click the **Binary 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 (*p/n 873700-902*). • If your column is *not* 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 **Column Comp.** 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.

Steps	Detailed Instructions	Comments
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 *do* need to reconnect prior to the exercises in Chapter 2, “Set Up and Run a Scan Method.”

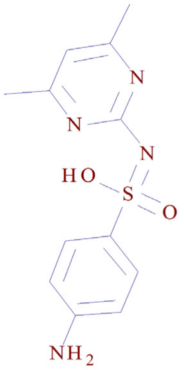
Exercise 2. Prepare the samples for the analyses

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:

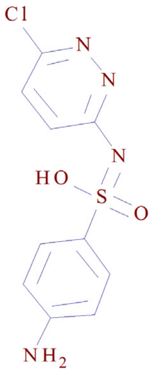
- sulfamethizole $(M+H)^+$ = 271
- sulfamethazine $(M+H)^+$ = 279
- sulfachloropyridazine $(M+H)^+$ = 285
- sulfadimethoxine $(M+H)^+$ = 311.



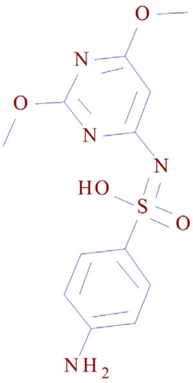
Sulfamethizole



Sulfamethazine



Sulfachloropyridazine



Sulfadimethoxine

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/µL. You will also prepare a solvent blank.

Steps	Detailed Instructions	Comments
1 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 (NH_4HCO_2). **c** Cap the vial.	• The original sulfa mixture is dissolved in a solvent mixture of 70% water and 30% acetonitrile.
2 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

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**. 	
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.

2

Set Up and Run a Scan Method

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

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 18
- "Task 2. Enter MS acquisition parameters" on page 20

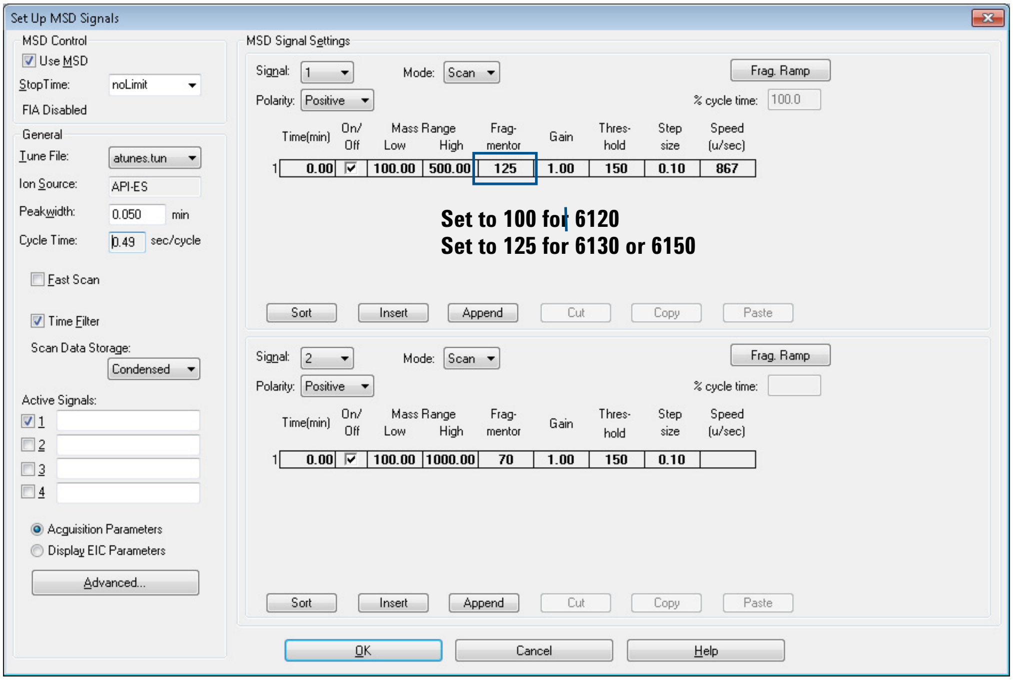
Task 1. Enter LC acquisition parameters

Steps	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**.  	
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 **HiP Sampler** tab. **c** In the **Injection volume** box, type `1` for a 1-µL injection.	

Steps	Detailed Instructions	Comments
5 Enter pump parameters.	**a** Click the **Binary 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=5mM ammonium formate in water Solvent B=5mM ammonium formate in methanol	
6 Set up the gradient timetable: 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	**a** Open the **Timetable** area in the lower part of the tab, click **Add**, and type the first line. **b** Click **Add** and type the second line. **c** Repeat step b for lines 3 and 4.	
7 Enter a column compartment temperature of 40 ^{o}C.	**a** Click the **Column Comp.** tab on the Setup Method dialog box. **b** Click the option button for **^{o}C**. **c** Type `40.0` for **^{o}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: > 0.10 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.
9 Select Data Acquisition only in the Run Time Checklist.	**a** Click **Run Time Checklist** on the **Method** 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

Steps	Detailed Instructions	Comments
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**.	• 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**).



Set Up MSD Signals

MSD Control
☑ Use MSD
StopTime: noLimit
FIA Disabled

General
Tune File: atunes.tun
Ion Source: API-ES
Peakwidth: 0.050 min
Cycle Time: 0.49 sec/cycle
☐ Fast Scan
☑ Time Filter
Scan Data Storage: Condensed
Active Signals:
☑ 1
☐ 2
☐ 3
☐ 4
● Acquisition Parameters
○ Display EIC Parameters

MSD Signal Settings

Signal: 1 Mode: Scan
Polarity: Positive
% cycle time: 100.0

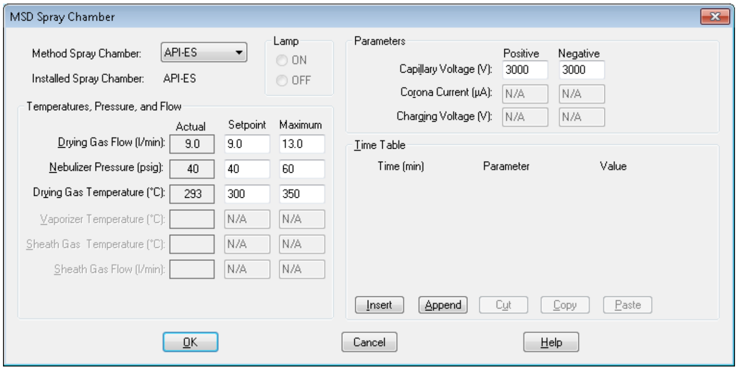
	Time(min)	On/ Off	Mass Range Low	Mass Range High	Frag-mentor	Gain	Thres-hold	Step size	Speed (u/sec)
1	0.00	☑	100.00	500.00	125	1.00	150	0.10	867

Set to 100 for 6120
Set to 125 for 6130 or 6150

Signal: 2 Mode: Scan
Polarity: Positive
% cycle time:

	Time(min)	On/ Off	Mass Range Low	Mass Range High	Frag-mentor	Gain	Thres-hold	Step size	Speed (u/sec)
1	0.00	☑	100.00	1000.00	70	1.00	150	0.10	

Steps	Detailed Instructions	Comments
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: • Drying gas flow: 7 L/min • Nebulizer pressure: 30 psi • Drying gas temperature: 350°C • Sheath gas temperature: 360 C° • Sheath gas flow: 12 L/min • Capillary voltage: 4000 V • Nozzle voltage: 2000 V	**a** Right-click the **MSD** icon on the system diagram and select **Spray Chamber**.  **b** Enter the parameters described in step 2 and shown in the figure below. **c** Click **OK**.	



MSD Spray Chamber

Method Spray Chamber: API-ES
Installed Spray Chamber: API-ES
Lamp: ○ ON ○ OFF

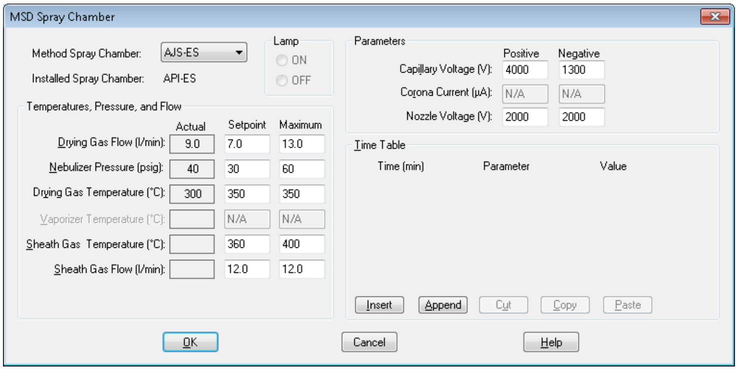
Temperatures, Pressure, and Flow

	Actual	Setpoint	Maximum
Drying Gas Flow (l/min):	9.0	9.0	13.0
Nebulizer Pressure (psig):	40	40	60
Drying Gas Temperature (°C):	293	300	350
Vaporizer Temperature (°C):		N/A	N/A
Sheath Gas Temperature (°C):		N/A	N/A
Sheath Gas Flow (l/min):		N/A	N/A

Parameters

	Positive	Negative
Capillary Voltage (V):	3000	3000
Corona Current (µA):	N/A	N/A
Charging Voltage (V):	N/A	N/A

For all models except 6150 with Agilent Jet Stream technology



MSD Spray Chamber

Method Spray Chamber: AJS-ES
Installed Spray Chamber: API-ES
Lamp: ○ ON ○ OFF

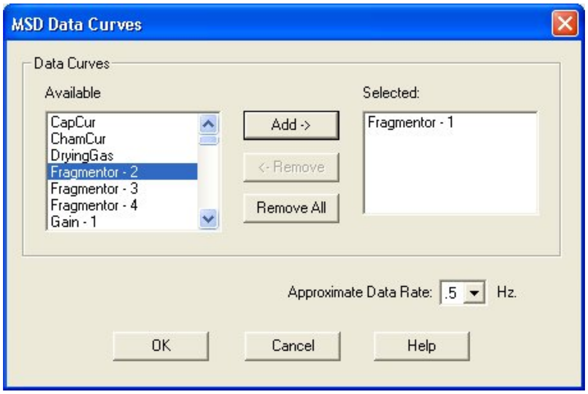
Temperatures, Pressure, and Flow

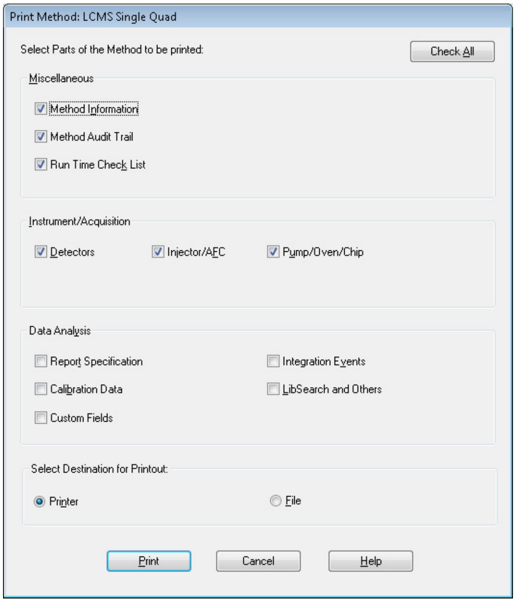
	Actual	Setpoint	Maximum
Drying Gas Flow (l/min):	9.0	7.0	13.0
Nebulizer Pressure (psig):	40	30	60
Drying Gas Temperature (°C):	300	350	350
Vaporizer Temperature (°C):		N/A	N/A
Sheath Gas Temperature (°C):		360	400
Sheath Gas Flow (l/min):		12.0	12.0

Parameters

	Positive	Negative
Capillary Voltage (V):	4000	1300
Corona Current (µA):	N/A	N/A
Nozzle Voltage (V):	2000	2000

For 6150 with Agilent Jet Stream technology

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**.  Selected: Fragmentor - 1 Approximate Data Rate: .5 Hz.	
4 Save the method.	**a** Select **Method > 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**.	

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: LCMS Single Quad

Select Parts of the Method to be printed:

Miscellaneous
☑ Method Information
☑ Method Audit Trail
☑ Run Time Check List

Instrument/Acquisition
☑ Detectors
☑ Injector/AFC
☑ Pump/Oven/Chip

Data Analysis
☐ Report Specification
☐ Integration Events
☐ Calibration Data
☐ LibSearch and Others
☐ Custom Fields

Select Destination for Printout:
● Printer
○ File

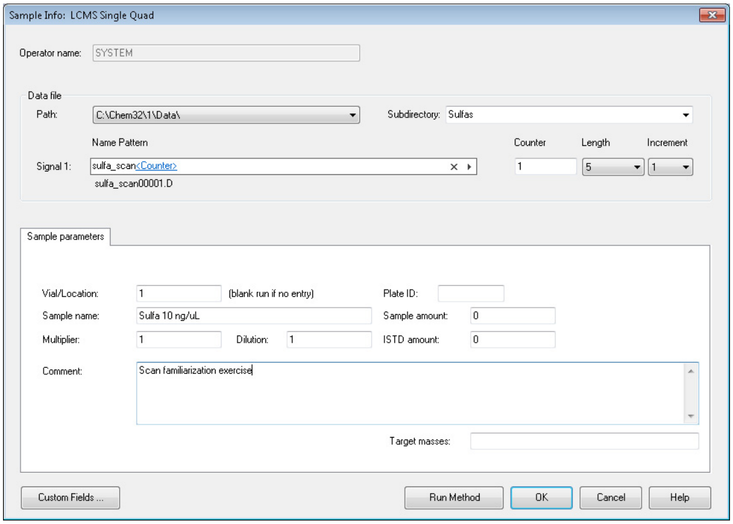
Exercise 2. Acquire data with the full-scan method

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

Steps	Detailed Instructions	Comments
1 Display the Single Sample toolbar.	• In the top toolbar, click the single sample icon. 	
2 Display the Sample Information dialog box.	**a** Click **Sample Info** on the RunControl menu.	

Steps	Detailed Instructions	Comments
3 Enter the sample information: • Operator name • Subdirectory: Sulfas • Prefix: Sulfa_scan • Location: Vial 1 • Sample Name: Sulfas 10 ng/µL • Comment: Scan familiarization exercise	**a** Enter the parameters described in step 3 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 Info: LCMS Single Quad

Operator name: SYSTEM

Data file

Path: C:\Chem32\1\Data\

Subdirectory: Sulfas

	Name Pattern	Counter	Length	Increment
Signal 1:	sulfa_scan<Counter> sulfa_scan00001.D	1	5	1

Sample parameters

Vial/Location: 1 (blank run if no entry)

Plate ID:

Sample name: Sulfa 10 ng/uL

Sample amount: 0

Multiplier: 1

Dilution: 1

ISTD amount: 0

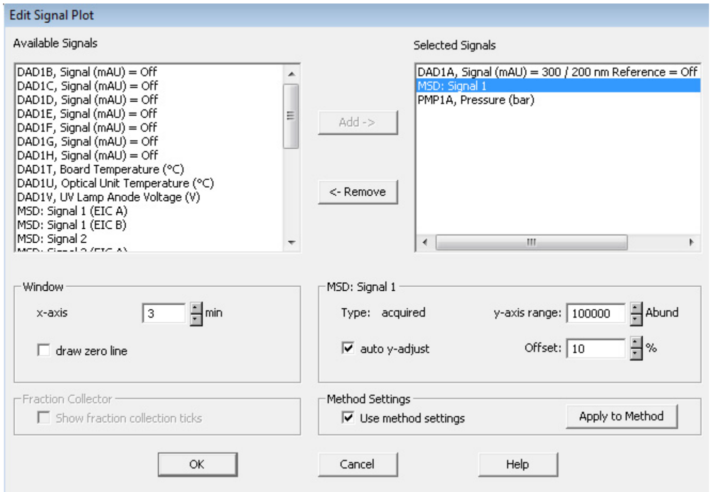
Comment: Scan familiarization exercise

Target masses:

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 13.
2 Inject the sulfa mix sample.	• Click the **Single Sample** button to start the run. Single Sample	This button is present only when you have selected Single Sample mode from the top toolbar.

Steps	Detailed Instructions	Comments
3 Monitor the total ion chromatogram and the UV chromatogram during data acquisition.	**a** From the Online Plot window, click the **Change** button. **b** In the list of **Available Signals**, select **DAD A: Signal=272,16 Reference=360,100** and click **Add**. **c** In the list of available signals, select **MSD: Signal 1** and click **Add**. **d** 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*.



Edit Signal Plot

Available Signals

DAD1B, Signal (mAU) = Off
DAD1C, Signal (mAU) = Off
DAD1D, Signal (mAU) = Off
DAD1E, Signal (mAU) = Off
DAD1F, Signal (mAU) = Off
DAD1G, Signal (mAU) = Off
DAD1H, Signal (mAU) = Off
DAD1T, Board Temperature (°C)
DAD1U, Optical Unit Temperature (°C)
DAD1V, UV Lamp Anode Voltage (V)
MSD: Signal 1 (EIC A)
MSD: Signal 1 (EIC B)
MSD: Signal 2

Selected Signals

DAD1A, Signal (mAU) = 300 / 200 nm Reference = Off
MSD: Signal 1
PMP1A, Pressure (bar)

Window
x-axis 3 min
☐ draw zero line

MSD: Signal 1
Type: acquired
y-axis range: 100000 Abund
☑ auto y-adjust
Offset: 10 %

Fraction Collector
☐ Show fraction collection ticks

Method Settings
☑ Use method settings

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.

3

Qualitative Data Analysis

This chapter shows you how to analyze data when you need to identify or confirm sample components.

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.

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.*
- 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.

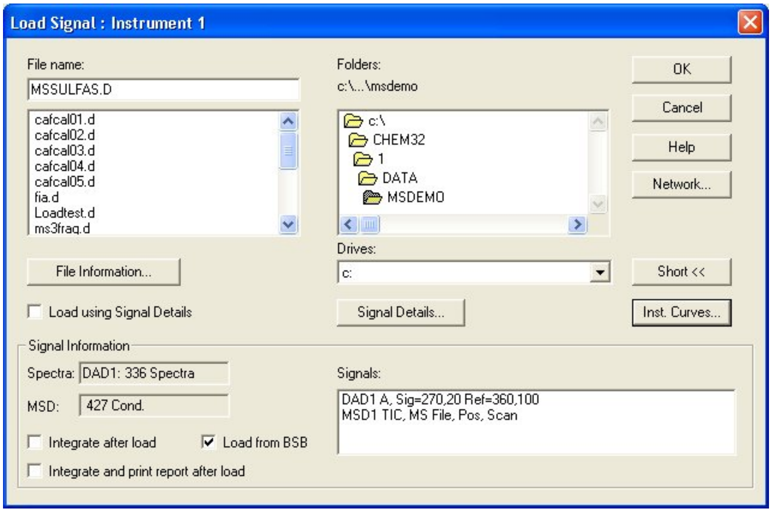
For the tasks on the following pages, perform the exercises in the order they appear. Try the steps on the left without the detailed instructions. If you need more help, follow the detailed instructions on the right.

Exercise 1. Display and manipulate chromatograms

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**.  	
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.  	

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 folder, either: • **C:\CHEM32\1\DATA\SULFAS**, or • **C:\CHEM32\1\DATA\MSDEMO**. **c** Select **the data file.** **d** **Set other parameters as shown below** and click **OK**.	• For other ways to load signals, see the Data Analysis chapter in the *Concepts Guide*. • If you wish to complete Chapter 4, “Set Up and Run a SIM Method”, then you need to process the data file you generated in Chapter 2. You need the report from that data file to set up your SIM groups.



File name: MSSULFAS.D

cafcal01.d
cafcal02.d
cafcal03.d
cafcal04.d
cafcal05.d
fia.d
Loadtest.d
ms3frag.d

Folders: c:\...\msdemo

c:\
CHEM32
1
DATA
MSDEMO

Drives: c:

☐ Load using Signal Details

Spectra: DAD1: 336 Spectra

MSD: 427 Cond.

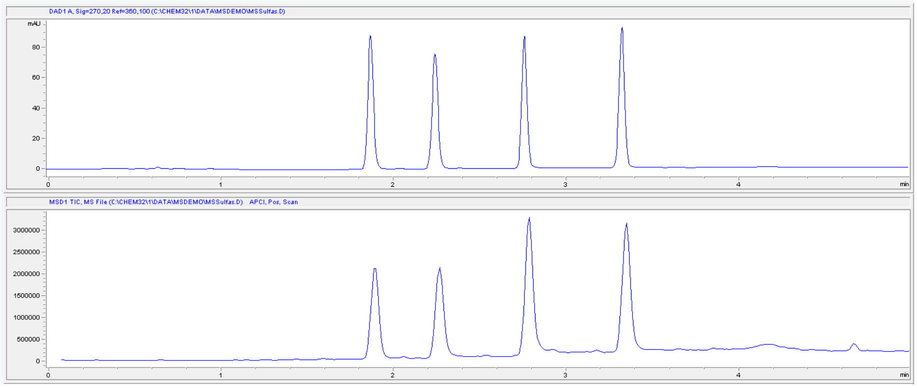
☐ Integrate after load ☑ Load from BSB

☐ Integrate and print report after load

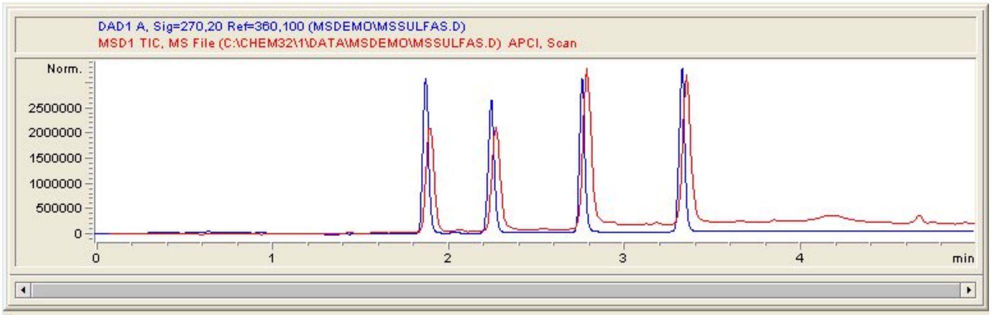
Signals:

DAD1 A, Sig=270,20 Ref=360,100
MSD1 TIC, MS File, Pos, Scan

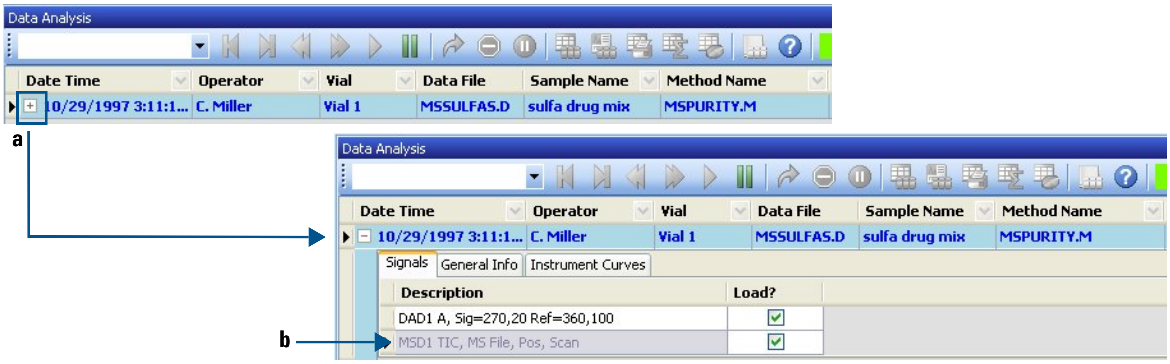
Steps	Detailed Instructions	Comments
5 Verify that you see the DAD and MS chromatograms.	**a** Check that you see a display that is similar to the one shown below. **b** Verify that you see the DAD signal in the top chromatogram. **c** Confirm that you see the MSD signal in the bottom chromatogram.	



6 Change the chromatogram view so that 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.



Steps	Detailed Instructions	Comments
7 Remove the DAD signal from the display.	**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 *Concepts Guide*.

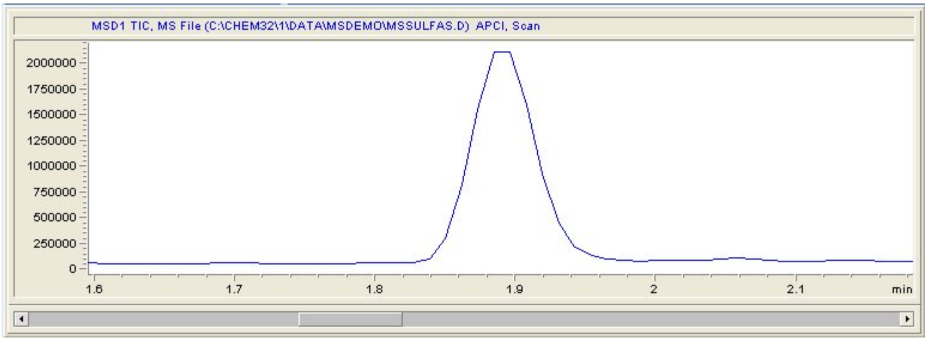


Date Time	Operator	Vial	Data File	Sample Name	Method Name
10/29/1997 3:11:1...	C. Miller	Vial 1	MSSULFAS.D	sulfa drug mix	MSPURITY.M

Description	Load?
DAD1 A, Sig=270,20 Ref=360,100	☑
MSD1 TIC, MS File, Pos, Scan	☑

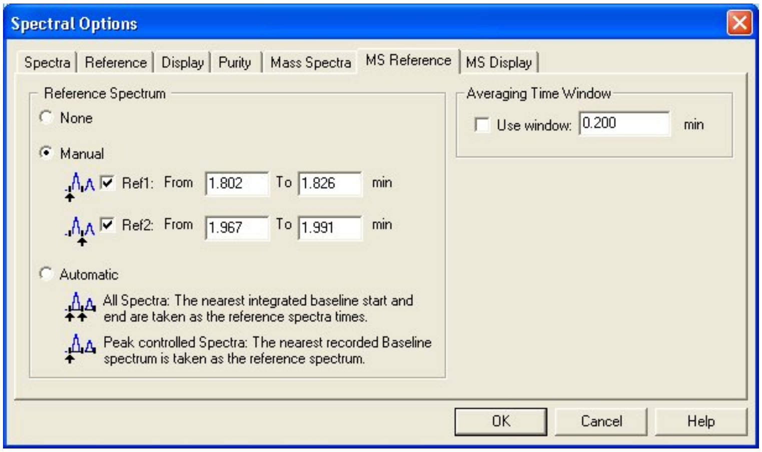
Exercise 2. Examine mass spectra

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
1 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.	

Steps	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, do one of the following at the chromatographic baseline just before the peak: • Click to select a single spectrum. • Click and drag to select an average spectrum.	
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, do one of the following at the chromatographic baseline just after the peak: • Click to select a single spectrum. • Click and drag to select an average spectrum.	
5 View your reference spectra.	**a** If you cannot see the spectra, adjust the size and location of the window labeled **Reference Mass Spectrum(a)**. **b** Note the two background spectra — one before the peak and one after.	

Steps	Detailed Instructions	Comments
6 Set the spectral options to do manual background subtraction.	**a** Click the icon to display the Spectral Options dialog box. **b** Click the **MS Reference** tab. **c** Under **Reference Spectrum**, click **Manual**. **d** Mark the check boxes for **Ref1** and **Ref2**. Note that the time ranges of the reference spectra that you just selected are specified there. **e** Click **OK**.	• The spectral options apply to all 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 subtraction. MSD, Manual Reference



Reference Spectrum

○ None

● Manual

☑ Ref1: From 1.802 To 1.826 min

☑ Ref2: From 1.967 To 1.991 min

○ Automatic

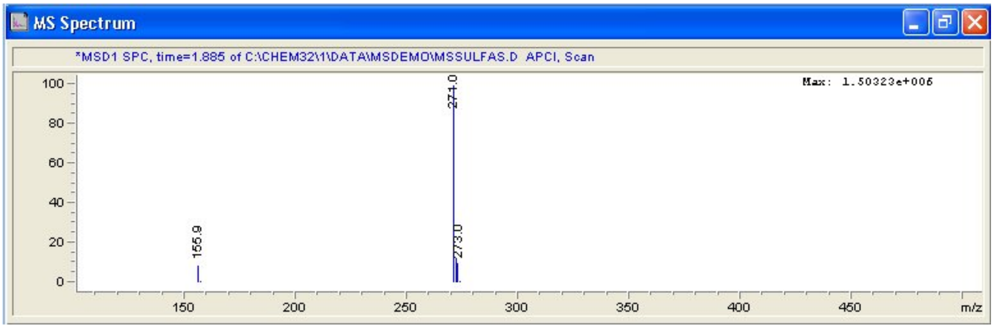
All Spectra: The nearest integrated baseline start and end are taken as the reference spectra times.

Peak controlled Spectra: The nearest recorded Baseline spectrum is taken as the reference spectrum.

Averaging Time Window

☐ Use window: 0.200 min

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 elution order for the 279 and 285 may change.



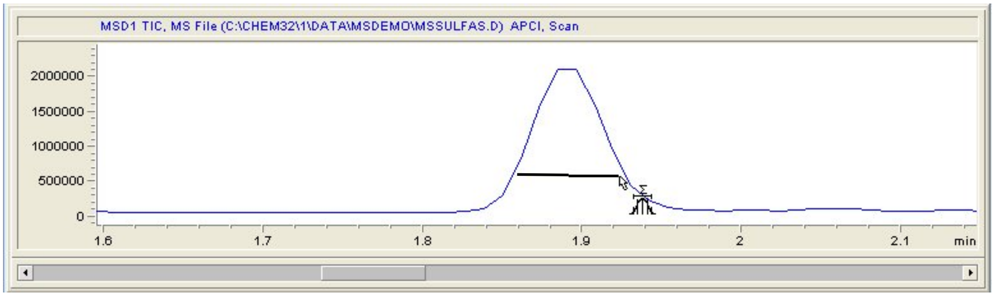
MS Spectrum

*MSD1 SPC, time=1.885 of C:\CHEM32\1\DATA\MSDEMO\MSSULFAS.D APCI, Scan

Max: 1.50323e+006

8 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 mouse across the peak, as shown below. **c** View the average spectrum in the window labeled **MS Spectrum**.	• When a chromatographic peak consists of a single compound, an average spectrum is usually more accurate.

Steps	Detailed Instructions	Comments



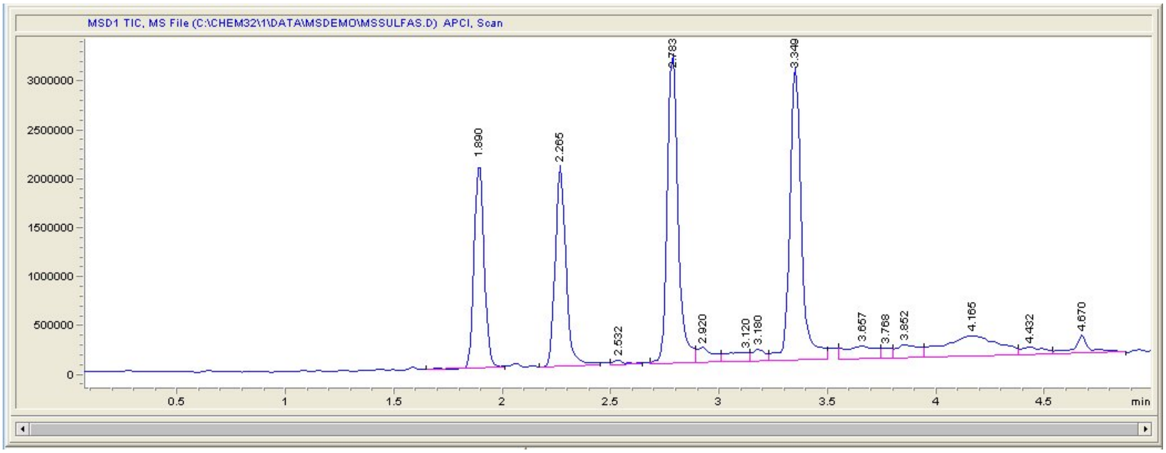
9 Be sure to see step 6 in "Exercise 3. Integrate the chromatogram" for an easier, faster way to display spectra.

Exercise 3. Integrate the chromatogram

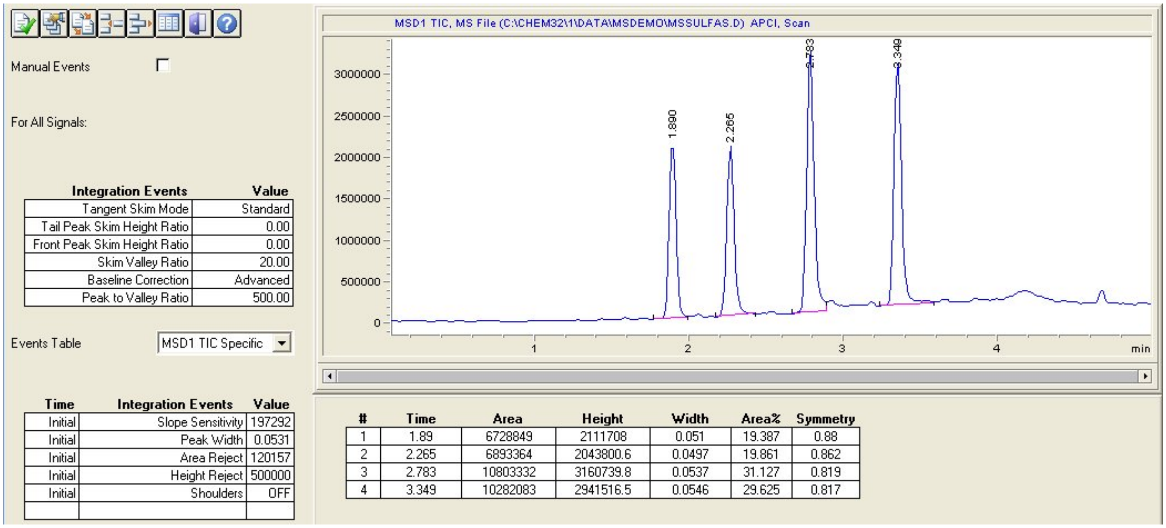
In this exercise, you learn to set integration events and integrate the chromatogram. Even if you do not care about quantitation, integration helps locate peaks for other purposes. For example, after integration, mass spectra of each peak can be printed with a report.

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

Steps	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.



Steps	Detailed Instructions	Comments
4 Adjust the integration parameters to get only four integrated peaks.	**a** Click the icon for **Edit/Set Integration Events Table**. **b** In the **Integration Events** table, for **Baseline Correction**, select **Advanced**. **c** For **Height Reject**, type `500000`. **d** Click the **Integrate current Chromatogram** icon. **e** Verify that your results are similar to those shown below.	- For detailed information about integration events, see *Agilent ChemStation: Understanding Your ChemStation*.



Manual Events ☐

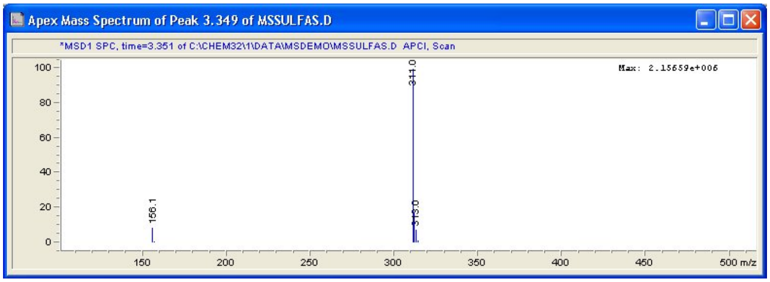
For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Tail Peak Skim Height Ratio	0.00
Front Peak Skim Height Ratio	0.00
Skim Valley Ratio	20.00
Baseline Correction	Advanced
Peak to Valley Ratio	500.00

Events Table: MSD1 TIC Specific

Time	Integration Events	Value
Initial	Slope Sensitivity	197292
Initial	Peak Width	0.0531
Initial	Area Reject	120157
Initial	Height Reject	500000
Initial	Shoulders	OFF

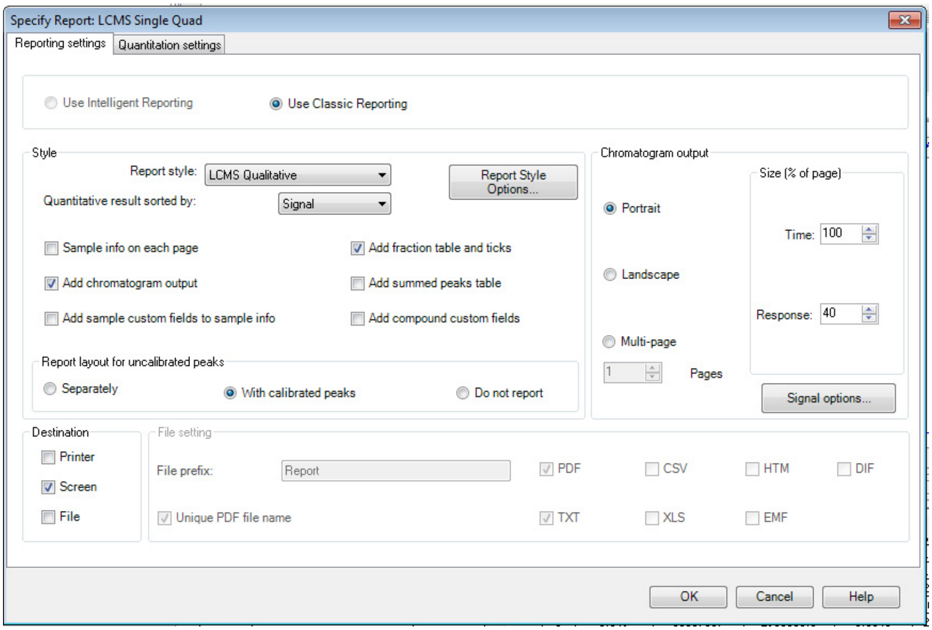
#	Time	Area	Height	Width	Area%	Symmetry
1	1.89	6728849	2111708	0.051	19.387	0.88
2	2.265	6893364	2043800.6	0.0497	19.861	0.862
3	2.783	10803332	3160739.8	0.0537	31.127	0.819
4	3.349	10282083	2941516.5	0.0546	29.625	0.817

Steps	Detailed Instructions	Comments
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 42.
6 Use the integrated chromatogram as the basis for a faster way to display background-subtracted spectra.	**a** Click the **Spectrum** 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.  Apex Mass Spectrum of Peak 3.349 of MSSULFAS.D *MSD1 SPC, time=3.351 of C:\CHEM32\1\DATA\MSDEMO\MSSULFAS.D APCI, Scan 	• 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.

Exercise 4. Print a report

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

Steps	Detailed Instructions	Comments
1 Specify the **LCMS Qualitative** report style, with the report printed to the screen.	**a** Select **Report > Specify Report**. **b** In the Specify Report dialog box, under **Destination**, mark the check box for **Screen**. **c** For **Report Style**, select **LCMS Qualitative**. **d** Check that other settings are as shown below. **e** Click **OK**.	



Specify Report: LCMS Single Quad

Reporting settings | Quantitation settings

○ Use Intelligent Reporting
● Use Classic Reporting

Style
Report style: LCMS Qualitative
Quantitative result sorted by: Signal
☐ Sample info on each page
☑ Add fraction table and ticks
☑ Add chromatogram output
☐ Add summed peaks table
☐ Add sample custom fields to sample info
☐ Add compound custom fields

Report layout for uncalibrated peaks
○ Separately
● With calibrated peaks
○ Do not report

Chromatogram output
● Portrait
○ Landscape
○ Multi-page
Pages: 1

Size (% of page)
Time: 100
Response: 40

Destination
☐ Printer
☑ Screen
☐ File

File setting
File prefix: Report
☑ PDF
☐ CSV
☐ HTM
☐ DIF
☑ Unique PDF file name
☑ TXT
☐ XLS
☐ EMF

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.

4

Set Up and Run a SIM Method

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

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

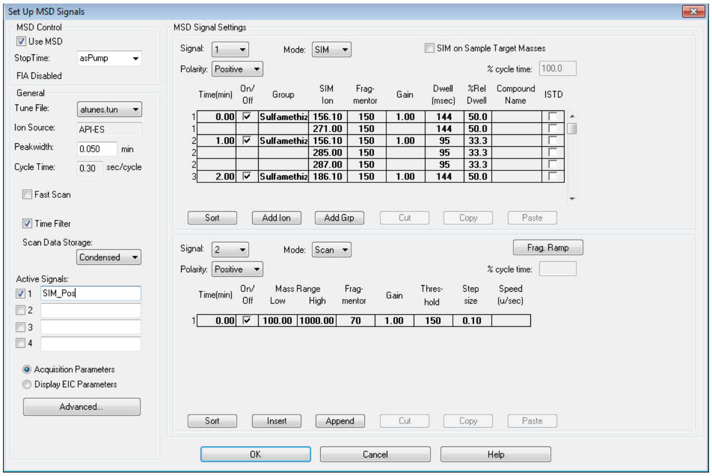
Task 1. Load the scan method you created previously

Steps	**Detailed Instructions**	**Comments**
1 Display Method and Run Control view.	• In the view selection area of the ChemStation window, click **Method and Run Control**.  	
2 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**.	
3 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

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**.	• 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.
2 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 Ions 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.

Steps	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 Ions 285, 287, and 156. • Sulfamethazine: Time 2.3, SIM Ions 279 and 186. • Sulfadimethoxine: Time 3.3, SIM Ions 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, *all* 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 *m/z* 287.



☑ Use MSD
StopTime: asPump
FIA Disabled
Tune File: atunes.tun
Ion Source: API-ES
Peakwidth: 0.050 min
Cycle Time: 0.30 sec/cycle
☐ Fast Scan
☑ Time Filter
Scan Data Storage: Condensed
Active Signals:
☑ 1 SIM_Pos
☐ 2
☐ 3
☐ 4
● Acquisition Parameters
○ Display EIC Parameters

Signal: 1 Mode: SIM ☐ SIM on Sample Target Masses
Polarity: Positive % cycle time: 100.0

	Time(min)	On/ Off	Group	SIM Ion	Frag-mentor	Gain	Dwell (msec)	%Rel Dwell	Compound Name	ISTD
1	0.00	☑	Sulfamethiz	156.10	150	1.00	144	50.0		☐
1				271.00	150		144	50.0		☐
2	1.00	☑	Sulfamethiz	156.10	150	1.00	95	33.3		☐
2				285.00	150		95	33.3		☐
2				287.00	150		95	33.3		☐
3	2.00	☑	Sulfamethiz	186.10	150	1.00	144	50.0		☐

Signal: 2 Mode: Scan
Polarity: Positive % cycle time:

	Time(min)	On/ Off	Mass Range Low	Mass Range High	Frag-mentor	Gain	Thres-hold	Step size	Speed (u/sec)
1	0.00	☑	100.00	1000.00	70	1.00	150	0.10	

Steps	Detailed Instructions	Comments
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

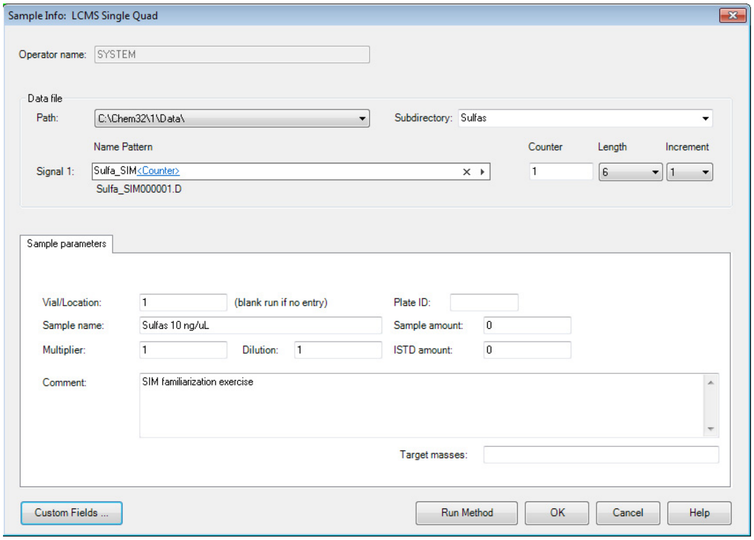
Now you are ready to acquire data for the sulfa mix with the method you just created. This exercise consists of the following tasks:

- "Task 1. Enter sample information" on page 48
- "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. 	
2 Display the Sample Information dialog box.	**a** Click **Sample Info** on the RunControl menu.	

Steps	Detailed Instructions	Comments
3 Enter the sample information: • Subdirectory: Sulfas • Prefix: Sulfa_SIM • Location: Vial 1 • Sample Name: Sulfas 10 ng/µL • Comment: SIM familiarization exercise	**a** Enter the parameters described in step 3 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.



Operator name: SYSTEM

Data file

Path: C:\Chem32\1\Data\

Subdirectory: Sulfas

	Name Pattern	Counter	Length	Increment
Signal 1:	Sulfa_SIM<Counter>	1	6	1
	Sulfa_SIM000001.D			

Sample parameters

Vial/Location: 1 (blank run if no entry)

Plate ID:

Sample name: Sulfas 10 ng/uL

Sample amount: 0

Multiplier: 1

Dilution: 1

ISTD amount: 0

Comment: SIM familiarization exercise

Target masses:

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 13.
2 Inject the sulfa mix sample.	• Click the **Single Sample** start button. 	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

Set Up and Run a Sequence

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.

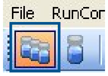
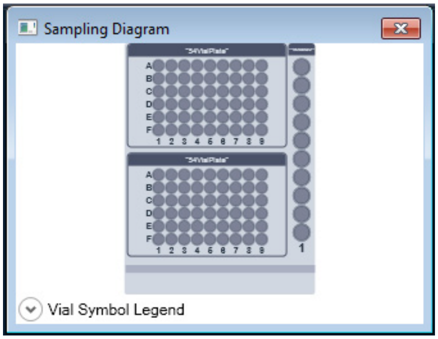
Before you start

- 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.

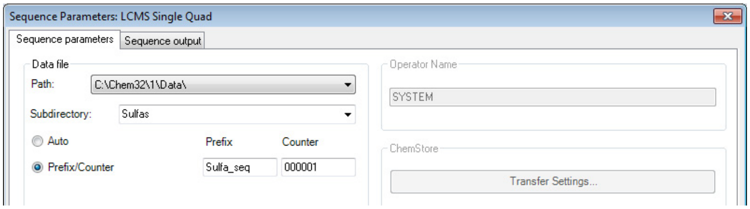
For details about sequences, see the automation chapter in *Agilent ChemStation: Understanding Your ChemStation*.

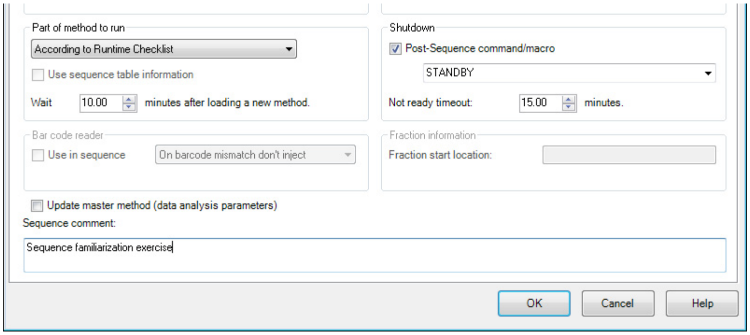
Exercise 1. Set up a sequence

Task 1. Prepare to create a new sequence

Steps	Detailed Instructions	Comments
1 Display Method and Run Control view.	• In the view selection area of the ChemStation window, click **Method and Run Control**.  Method and Run Control	
2 Display the **Sequence Toolset**.	• In the top toolbar, click the icon to display the **Sequence Toolset**.  	
3 Display the **Autosampler Tray** diagram.	• Click **Sampling Diagram** on the View menu.  	
4 Initiate setup of a new sequence.	• Select **Sequence> Sequence Table**.	
5 Save the sequence under a new name, **SULFA MS SIM 1.S**	**a** Select **Sequence > Save Sequence Template As**. **b** For **Name**, type `SULFA MS SIM 1.S`. **c** Click **OK**.	

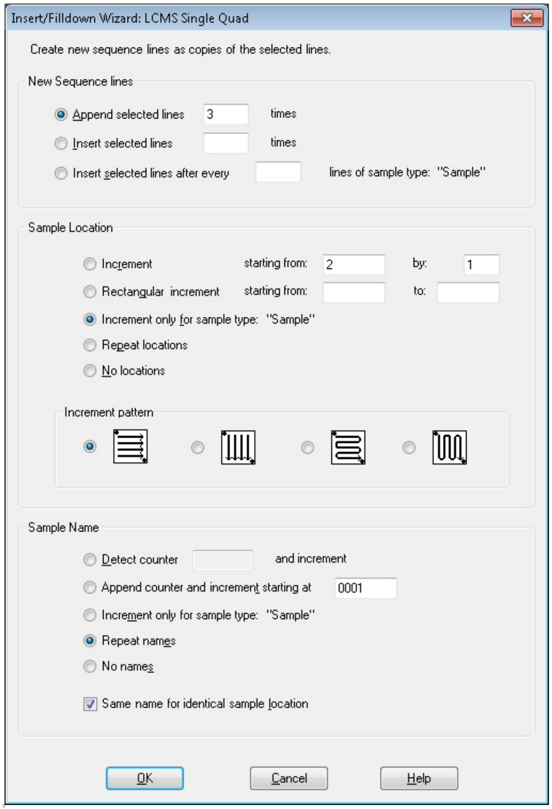
Task 2. Edit sequence parameters

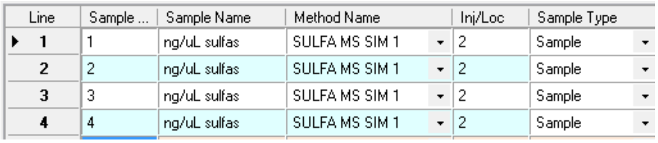
Steps	Detailed Instructions	Comments
1 Open Sequence Parameters dialog box.	• Click **Sequence > Sequence Parameters**.	• The sequence parameters are settings that are common to all the samples in the sequence.
2 Enter the sequence parameters for **Data File**.	a Enter the following parameters, shown in step 1. • 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.
	 Sequence Parameters: LCMS Single Quad Sequence parameters | Sequence output Data file Path: C:\Chem32\1\Data\ Subdirectory: Sulfas ○ Auto ● Prefix/Counter — Prefix: Sulfa_seq — Counter: 000001 Operator Name: SYSTEM ChemStore Transfer Settings...	

Steps	Detailed Instructions	Comments
3 Enter the rest of the sequence parameters:	**a** Enter the following parameters shown in the figure below. • Parts of methods to run: According to Runtime Checklist • Wait: 10 minutes after loading a new method • Shutdown: STANDBY • Not Ready Timeout: 15 minutes • Sequence Comment: Sequence familiarization exercise **b** Click **OK**.	• 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 method to run: According to Runtime Checklist ☐ Use sequence table information Wait 10.00 minutes after loading a new method. Bar code reader ☐ Use in sequence On barcode mismatch don't inject ☐ Update master method (data analysis parameters) Sequence comment: Sequence familiarization exercise Shutdown ☑ Post-Sequence command/macro STANDBY Not ready timeout: 15.00 minutes. Fraction information Fraction start location: Post-Sequence Command/Macros are 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.	Two examples of Post-Sequence Command/Macros are: • 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

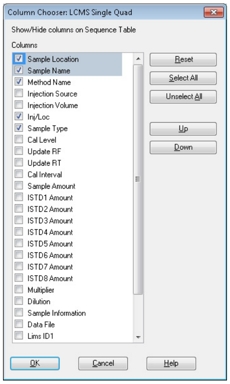
Steps	Detailed Instructions	Comments
1 Set up the sequence table to: • Run duplicate injections of a blank. • Run duplicate injections of the sulfa mix at three concentrations: 1, 5 and 10 ng/µL. • Use the method **SULFA MS SIM 1.M**, that you created in Chapter 4, “Set Up and Run a SIM Method”.	**a** Click **Sequence > Sequence Table**. **b** In the first line, type these values: • Sample Location = `1` • Sample Name = `ng/ul sulfas` • Method Name=`SULFA MS SIM 1` • Inj/Loc=`2` • Sample Type=`Sample` **c** Select **Line 1**. **d** Click the **Insert/Filldown Wizard** button. **e** Complete the Insert/Filldown Wizard as shown in the next figure.	• 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**.

Steps	Detailed Instructions	Comments
	 Insert/Filldown Wizard: LCMS Single Quad Create new sequence lines as copies of the selected lines. New Sequence lines ● Append selected lines 3 times ○ Insert selected lines times ○ Insert selected lines after every lines of sample type: "Sample" Sample Location ○ Increment starting from: 2 by: 1 ○ Rectangular increment starting from: to: ● Increment only for sample type: "Sample" ○ Repeat locations ○ No locations Increment pattern ● ○ ○ ○ Sample Name ○ Detect counter and increment ○ Append counter and increment starting at 0001 ○ Increment only for sample type: "Sample" ● Repeat names ○ No names ☑ Same name for identical sample location	
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 can recreate the table format below.



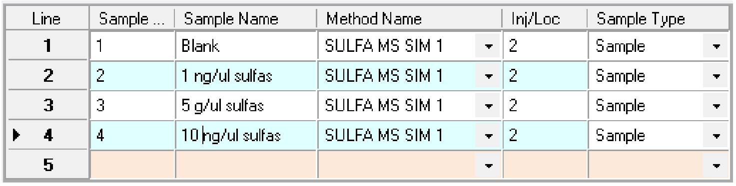
Line	Sample ...	Sample Name	Method Name	Inj/Loc	Sample Type
▸ 1	1	ng/uL sulfas	SULFA MS SIM 1	2	Sample
2	2	ng/uL sulfas	SULFA MS SIM 1	2	Sample
3	3	ng/uL sulfas	SULFA MS SIM 1	2	Sample
4	4	ng/uL sulfas	SULFA MS SIM 1	2	Sample

Steps	Detailed Instructions	Comments
3 (Optional) Customize the sequence table to match the format in step 2.	**a** Click the **Column Chooser** button. **b** Click **Unselect All** to clear all check boxes. **c** Click **OK**.	• For descriptions of any columns you removed, see the online Help.



☑ Sample Location
☑ Sample Name
☑ Method Name
☐ Injection Source
☐ Injection Volume
☑ Inj/Loc
☑ Sample Type
☐ Cal Level
☐ Update RF
☐ Update RT
☐ Cal Interval
☐ Sample Amount
☐ ISTD1 Amount
☐ ISTD2 Amount
☐ ISTD3 Amount
☐ ISTD4 Amount
☐ ISTD5 Amount
☐ ISTD6 Amount
☐ ISTD7 Amount
☐ ISTD8 Amount
☐ Multiplier
☐ Dilution
☐ Sample Information
☐ Data File
☐ Lims ID1

Steps	Detailed Instructions	Comments
4 Type the following sample names into the table: • Vial 1 – blank • Remaining vials – sulfa mix at 1, 5 and 10 ng/µL	**a** Modify the **Sample Name** for each sample, as shown below. **b** Click **OK**.	

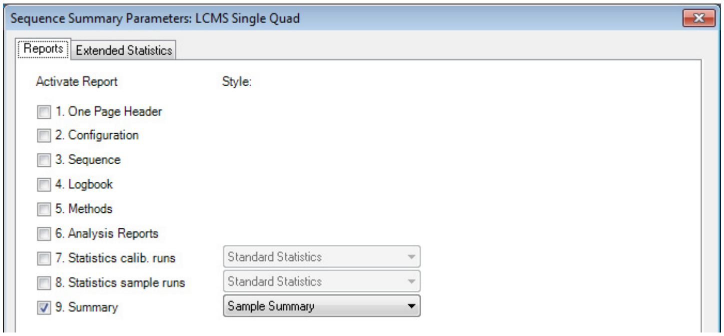


Line	Sample ...	Sample Name	Method Name	Inj/Loc	Sample Type
1	1	Blank	SULFA MS SIM 1	2	Sample
2	2	1 ng/ul sulfas	SULFA MS SIM 1	2	Sample
3	3	5 g/ul sulfas	SULFA MS SIM 1	2	Sample
4	4	10 ng/ul sulfas	SULFA MS SIM 1	2	Sample
5					

Steps	Detailed Instructions	Comments
5 Save the sequence.	• Click the **Save Sequence** button in the Sequence toolset.	

Task 4. Set up the sequence output

Steps	Detailed Instructions	Comments
1 Set up the sequence to print a short sequence summary to the printer.	**a** Click **Sequence > Sequence Parameters > 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 Parameters 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.

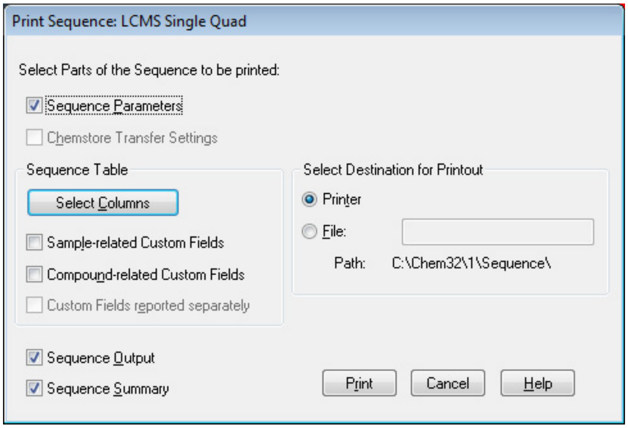


Sequence Summary Parameters: LCMS Single Quad

Reports | Extended Statistics

Activate Report | Style:

☐ 1. One Page Header
☐ 2. Configuration
☐ 3. Sequence
☐ 4. Logbook
☐ 5. Methods
☐ 6. Analysis Reports
☐ 7. Statistics calib. runs — Standard Statistics
☐ 8. Statistics sample runs — Standard Statistics
☑ 9. Summary — Sample Summary

Steps	Detailed Instructions	Comments
2 Save the sequence.	- Click the **Save Sequence** button in the Sequence toolset.	
3 Print the sequence.	**a** Select **Sequence > Print Sequence Template**. **b** Mark the check boxes as shown in the figure below. **c** Click the **Print** button.  Print Sequence: LCMS Single Quad Select Parts of the Sequence to be printed: ☑ Sequence Parameters ☐ Chemstore Transfer Settings Sequence Table Select Columns ☐ Sample-related Custom Fields ☐ Compound-related Custom Fields ☐ Custom Fields reported separately ☑ Sequence Output ☑ Sequence Summary Select Destination for Printout ● Printer ○ File: Path: C:\Chem32\1\Sequence\ Print Cancel Help	If you click the **Print All** button, you print all the parts of the sequence rather than the items you just specified.

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 13.
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 data acquisition.	**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: 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
Quantitative Data Analysis

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\MSDEMO**. The file names are **CAFCAL0X.D**, where **x** is a number from **1** to **5**.

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

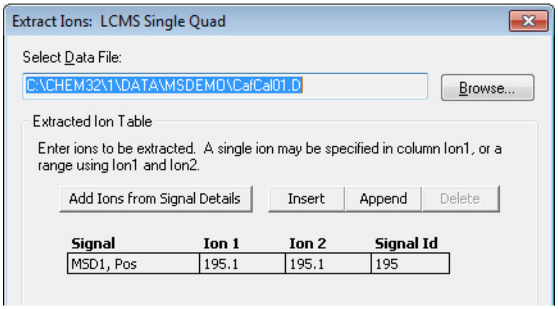
In this task, you load a default method and save it to a new name. You later modify the new method to create a calibrated method.

Steps	Detailed Instructions	Comments
1 Display Data Analysis view.	• In the view selection area in the lower left of the ChemStation window, click **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.

Steps	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\MSDEMO**. **c** Select **the data file CAFCAL01.D.** **d** If necessary, clear the check box for **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 *Concepts Guide*.
2 Extract the major ion of caffeine.	**a** Select **File > Extract Ions**. **b** For Ion 1, type `195.1`. **c** For Ion 2, type `195.1`. **d** Click **OK**.	• The 195 ion is the $(M+H)^+$ ion.



Extract Ions: LCMS Single Quad

Select Data File: C:\CHEM32\1\DATA\MSDEMO\CafCal01.D

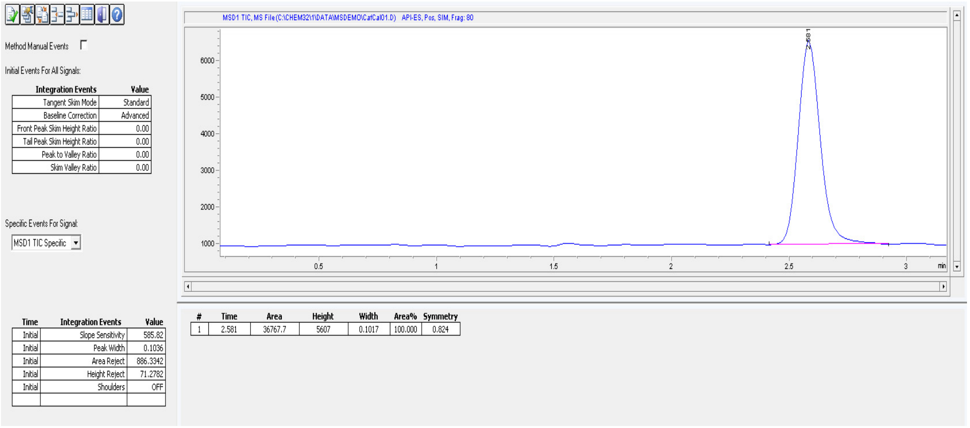
Extracted Ion Table

Enter ions to be extracted. A single ion may be specified in column Ion1, or a range using Ion1 and Ion2.

Signal	Ion 1	Ion 2	Signal Id
MSD1, Pos	195.1	195.1	195

Steps	Detailed Instructions	Comments
3 Display the Calibration Toolset.	• Click the **Calibration** icon, which is near the middle of the window. Calibration	

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.
5 (Optional) 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**.	• For these exercises, you save the method frequently, but you could wait instead until you had established all the method settings.



Method Manual Events ☐

Initial Events For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Baseline Correction	Advanced
Front Peak Skim Height Ratio	0.00
Tail Peak Skim Height Ratio	0.00
Peak to Valley Ratio	0.00
Skim Valley Ratio	0.00

Specific Events For Signal: MSD1 TIC Specific

Time	Integration Events	Value
Initial	Slope Sensitivity	585.82
Initial	Peak Width	0.1036
Initial	Area Reject	886.3342
Initial	Height Reject	71.2782
Initial	Shoulders	OFF

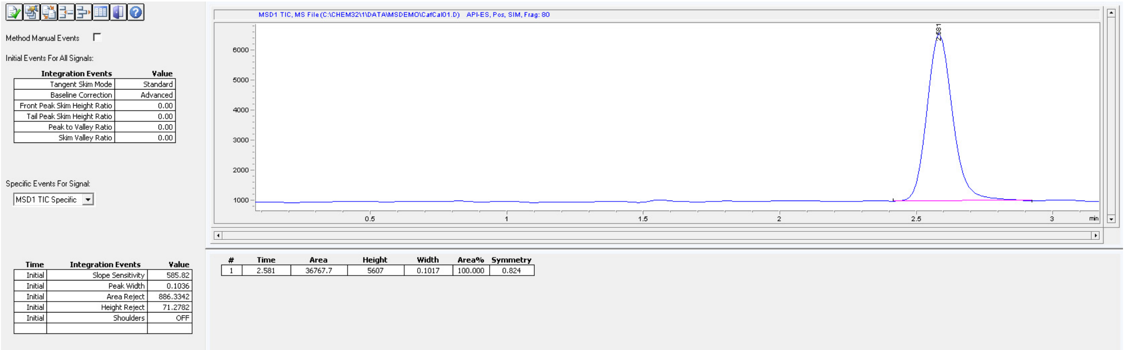
MSD1 TIC, MS File (C:\CHEM32\1\DATA\MSDEMO\CafCal01.D) API-ES, Pos, SIM, Frag: 80

#	Time	Area	Height	Width	Area%	Symmetry
1	2.581	36767.7	5607	0.1017	100.000	0.824

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.
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 Your ChemStation*.



Method Manual Events ☐

Initial Events For All Signals:

Integration Events	Value
Tangent Skim Mode	Standard
Baseline Correction	Advanced
Front Peak Skim Height Ratio	0.00
Tail Peak Skim Height Ratio	0.00
Peak to Valley Ratio	0.00
Skim Valley Ratio	0.00

Specific Events For Signal: MSD1 TIC Specific

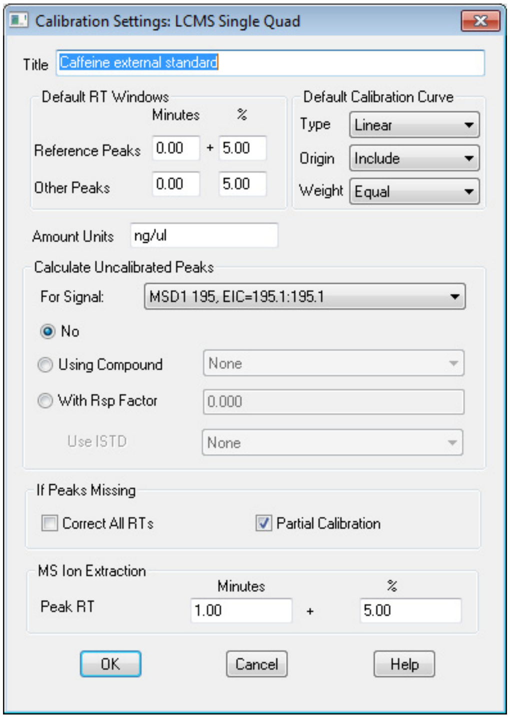
Time	Integration Events	Value
Initial	Slope Sensitivity	585.82
Initial	Peak Width	0.1036
Initial	Area Reject	886.3342
Initial	Height Reject	71.2782
Initial	Shoulders	OFF

MSD1 TIC, MS File (C:\CHEM32\1\DATA\MSDEMO\CafCal01.D) API-ES, Pos, SIM, Frag: 80

#	Time	Area	Height	Width	Area%	Symmetry
1	2.581	36767.7	5607	0.1017	100.000	0.824

Steps	Detailed Instructions	Comments
4 Save the integration events with the method in memory.	• Click the icon to exit and save the integration results. ✓	
5 (Optional) 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**.	

Task 4. Set general calibration parameters

Steps	Detailed Instructions	Comments
1 Establish calibration parameters.	**a** Select **Calibration** > **Calibration Settings**. **b** In the **Title** box, type a title, for example `Caffeine external standard`. **c** Leave the rest of the items at the default settings, shown below. **d** Click **OK**.  Calibration Settings: LCMS Single Quad Title: Caffeine external standard Default RT Windows (Minutes + %): Reference Peaks 0.00 + 5.00; Other Peaks 0.00 5.00 Default Calibration Curve: Type Linear; Origin Include; Weight Equal Amount Units: ng/ul Calculate Uncalibrated Peaks: For Signal: MSD1 195, EIC=195.1:195.1 ● No ○ Using Compound: None ○ With Rsp Factor: 0.000 Use ISTD: None If Peaks Missing: ☐ Correct All RTs ☑ Partial Calibration MS Ion Extraction: Peak RT (Minutes + %): 1.00 + 5.00 	
2 (Optional) 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**.	

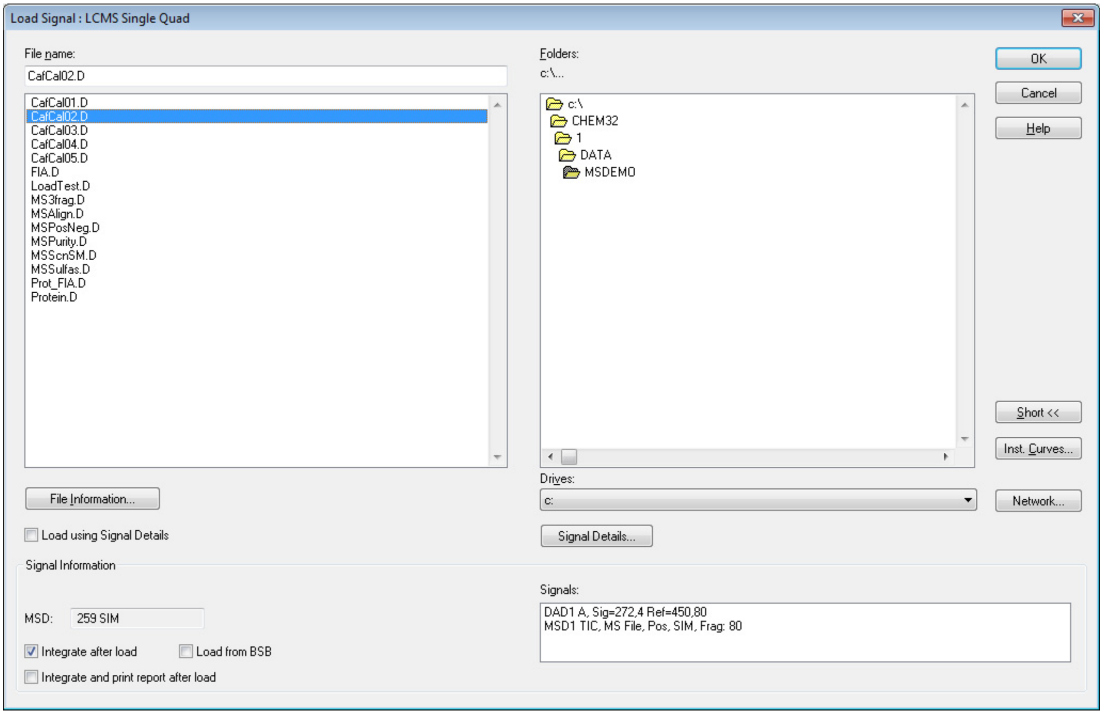
Task 5. Set up the calibration curve

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

Steps	Detailed Instructions	Comments
1 Display the Calibration Toolset.	- Click the **Calibration** icon, which is near the middle of the window.	
2 Add the low-level standard to the calibration curve.	**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.

#	RT	Signal	Compound	Lvl	Amt[ng/ul]	Area	Rsp.Factor	Ref	ISTD	#
1	2.580	MSD1 195	caffeine	1	0.500	36769.000	1.3598e-5	No	No	

Steps	Detailed Instructions	Comments
3 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 using Signal Details**. **d** Mark the check box for **Integrate after load**. **e** Check that your dialog box looks like the one below. **f** Click **OK**.	• These settings enable you in a single step to load the appropriate signal(s) and integrate them.


Load Signal : LCMS Single Quad

File name: CafCal02.D

CafCal01.D
CafCal02.D
CafCal03.D
CafCal04.D
CafCal05.D
FIA.D
LoadTest.D
MS3frag.D
MSAlign.D
MSPosNeg.D
MSPurity.D
MSScnSM.D
MSSulfas.D
Prot_FIA.D
Protein.D

Folders: c:\...

c:\
CHEM32
1
DATA
MSDEMO

Drives: c:

☐ Load using Signal Details

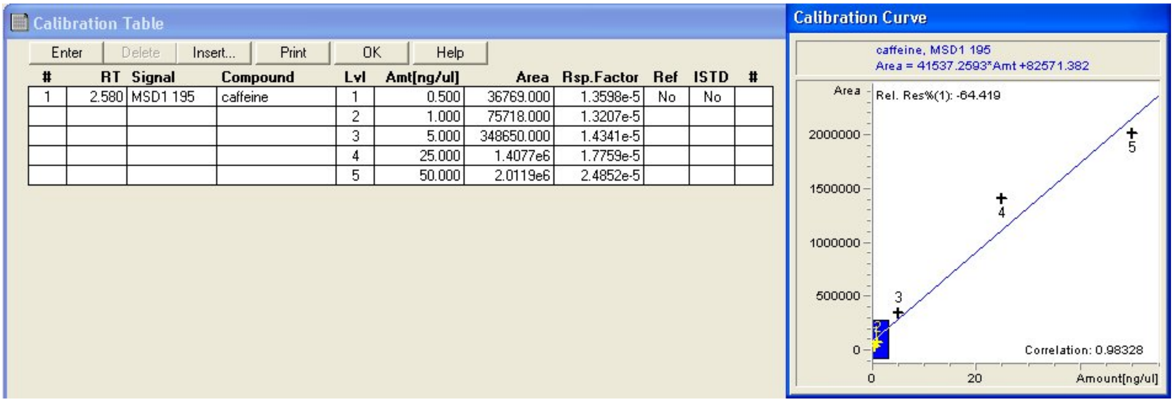
Signal Information

MSD: 259 SIM

☑ Integrate after load
☐ Load from BSB
☐ Integrate and print report after load

Signals:
DAD1 A, Sig=272,4 Ref=450,80
MSD1 TIC, MS File, Pos, SIM, Frag: 80

Steps	Detailed Instructions	Comments
4 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.	
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.

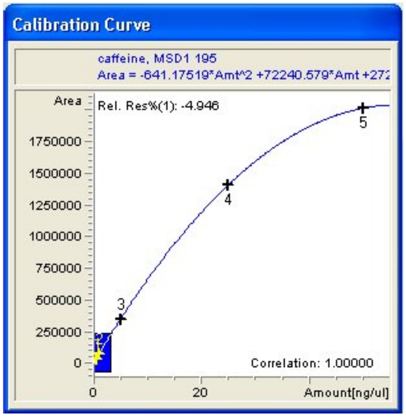


#	RT	Signal	Compound	Lvl	Amt[ng/ul]	Area	Rsp.Factor	Ref	ISTD	#
1	2.580	MSD1 195	caffeine	1	0.500	36769.000	1.3598e-5	No	No	
				2	1.000	75718.000	1.3207e-5			
				3	5.000	348650.000	1.4341e-5			
				4	25.000	1.4077e6	1.7759e-5			
				5	50.000	2.0119e6	2.4852e-5			

caffeine, MSD1 195
Area = 41537.2593*Amt +82571.382

Rel. Res%(1): -64.419

Correlation: 0.98328

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.  	
7 (Optional) 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**.	

Task 6. Explore options to refine the calibration

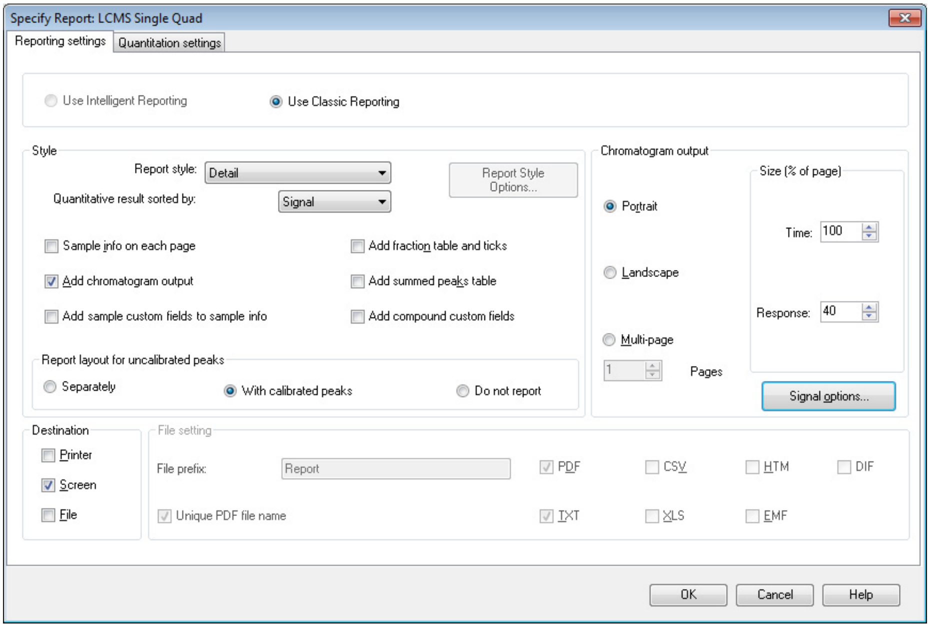
This exercise describes additional calibration table layouts that give you more calibration options. You do not need these options to process the caffeine demonstration data, but you may need them when you process your own samples.

Steps	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
1 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 > Specify Report**. • Click the **Specify Report** icon. **b** Enter parameters as described in step 1 and shown in the next two figures. **c** Click **Quantitation settings**. **d** Click **OK**.	



Specify Report: LCMS Single Quad

Reporting settings | Quantitation settings

○ Use Intelligent Reporting ● Use Classic Reporting

Style

Report style: Detail

Quantitative result sorted by: Signal

☐ Sample info on each page
☑ Add chromatogram output
☐ Add sample custom fields to sample info
☐ Add fraction table and ticks
☐ Add summed peaks table
☐ Add compound custom fields

Report layout for uncalibrated peaks

○ Separately ● With calibrated peaks ○ Do not report

Chromatogram output

● Portrait
○ Landscape
○ Multi-page
1 Pages

Size (% of page)

Time: 100

Response: 40

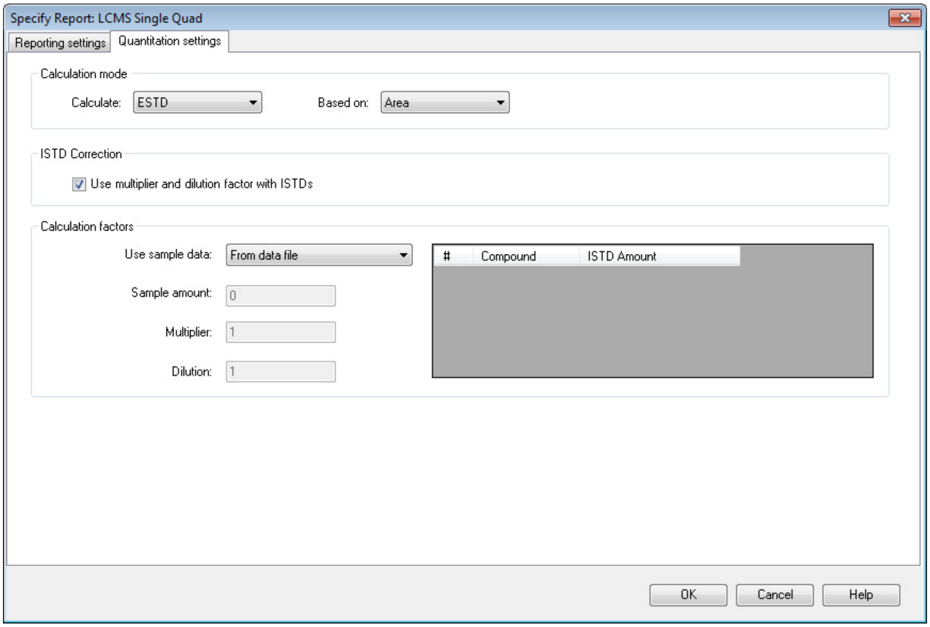
Destination

☐ Printer
☑ Screen
☐ File

File setting

File prefix: Report

☑ PDF ☐ CSV ☐ HTM ☐ DIF
☑ Unique PDF file name
☑ TXT ☐ XLS ☐ EMF



Calculation mode

Calculate: ESTD

Based on: Area

ISTD Correction

☑ Use multiplier and dilution factor with ISTDs

Calculation factors

Use sample data: From data file

Sample amount: 0

Multiplier: 1

Dilution: 1

#	Compound	ISTD Amount

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**.	

Steps	Detailed Instructions	Comments
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.

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