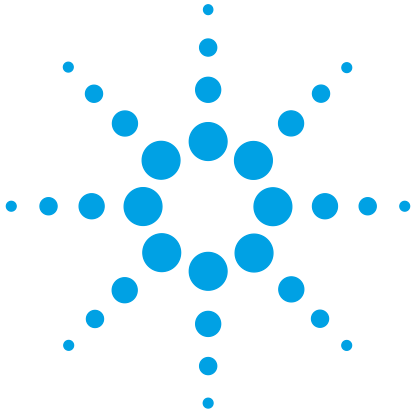

Agilent G1701EA MSD Productivity ChemStation

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

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In This Guide

This guide contains a step-by-step exercise to help you become familiar with your Agilent 7890A GC/5975 MSD with G1701EA MSD Productivity ChemStation software.

To successfully use this book, you need the following:

- GC Inlet: Split/Splitless Inlet with EPC (default inlet configuration)
- Column: HP-5ms 30 m x 250 µm x 0.25 µm
- Sample: 5975 MSD Sample (P/N 05970-60045) or (P/N 5074-3025 Japan only)
- MSD Tuning Calibrator: PFTBA (perfluorotributylamine)

Before operating your instruments, be sure to read all safety and regulatory information included with your instruments.

1 Start Up the System

Start up your system hardware and software for data acquisition.

2 Tune the MS

Determine whether the instrument is correctly tuned.

3 Create a Method for Qualitative Analysis

Create a new qualitative analysis scan method from the system default method.

4 Run the Scan Method

Run the method created in Chapter 3 to acquire sample data.

5 Qualitative Data Analysis

Use the Enhanced Data Analysis program to analyze the data generated in Chapter 4.

6 Create a SIM Quantitation Method

Create a SIM method from the scan method created in Chapter 3.

7 Run a Sequence

Create and run a sequence using the method created in Chapter 6.

8 Set Up a Quantitation Database

Set up a database with compounds and calibrators to identify unknown samples.

9 Generate a Report

Generate a report automatically after a run or at a later point from previously acquired data.

10 Recalibrate and Quantitate Unknowns

Modify a sequence for recalibration and then use it to quantitate an unknown sample.

11 Create a Cool Down Method

Create and store a maintenance method.

12 Shut Down the System

13 Frequently Asked Questions

Where to Find Information

Hardware

In addition to this document, Agilent provides several learning products that document how to install, operate, maintain, and troubleshoot the 7890A GC/5975 MSD. This information can be found on the Agilent Technologies GC and GC/MS Hardware User Information and Utilities DVDs that ship with your instrument.

The Agilent Technologies GC and GC/MS Hardware User Information and Utilities DVDs that ship with your instrument provides an extensive collection of online help, videos, and books for current Agilent gas chromatographs, mass selective detectors, ion traps, and GC samplers. Included are localized versions of the information you need most, such as:

- Getting Familiar documentation
- Safety and Regulatory guides
- Site Preparation checklists
- Installation information
- Operating guides
- Maintenance information
- Troubleshooting details

Software

For an introduction to, and where to find more information on, the G1701EA MSD Productivity ChemStation see the *Agilent G1701EA GC/MSD ChemStation Getting Started manual.*

Contents

1 Start Up the System

In this chapter, the startup checklist is reviewed for instrument readiness. If necessary, changes are made to the instrument hardware configuration to handle the data acquisition of the samples that are run in this manual. With the instruments off and the G1701EA MSD Productivity ChemStation not running, the instruments are started and the MSD is pumped down. Finally, a method is loaded in preparation for bringing all instrument parameters to settings required for data acquisition.

Start up the Hardware

1. Review the Agilent 7890A Gas Chromatograph Operating Guide (P/N G3430-90011) and the Agilent 5975 Series MSD Operation Manual (P/N G3170-90036) for important safety information and start up details before powering on your instruments.
2. Verify that the split/splitless (S/SL) inlet septum, liner, and O-ring are clean, properly installed, and in good condition.
3. Install a conditioned (HP-5ms 30 m x 250 µm x 0.25 µm) column in the GC. Attach the column inlet to the S/SL inlet and its outlet to the MSD transfer line. See the Agilent 5975 Series MSD Operation Manual for details.
4. Verify the EI ion source is installed.
5. Verify 99.9995% purity helium is attached to the carrier gas supply of the S/SL inlet.
6. Power on the 7890A GC.
7. From the GC keypad, turn off the oven, Aux 2 heated zone (GC/MSD transfer line), and inlet heater. If equipped, turn off any GC detectors.
8. Before you turn on or attempt to operate the MSD verify the following:
   - The vent valve must be closed (the knob turned all the way clockwise).
   - All other vacuum seals and fittings must be in place and fastened correctly.
   - The front side plate screw should not be tightened.
   - The MSD is connected to a grounded power source.
   - The GC/MSD interface extends into the GC oven.
   - A conditioned capillary column is installed in the GC inlet and in the GC/MSD interface.
   - The GC is on, but the heated zones for the GC/MSD interface, the GC inlet, and the oven are off.
   - Carrier gas of at least 99.9995% purity is plumbed to the GC with the recommended traps.
   - The foreline pump exhaust is properly vented.
9. Open the MSD analyzer top cover.
10. Close the MSD vent valve.
11. Press the **Power** button on the front of the MSD to power it on. The foreline pump will make a gurgling noise.

Press lightly on the metal box mounted on the MSD side board until the air noise stops to ensure a correct seal.

12 Close the MSD analyzer top cover.

13 On the MSD local control panel:

a Press **Menu** repeatedly until **Maintenance** appears.

b Press **Item** repeatedly until **Pumpdown** appears.

c Press **Yes/Select** to start the pumpdown.

The pumpdown is completely automatic and does not require operator actions.

After the turbo pump starts and the ion gauge value reaches 100 mTorr, allow the MSD to operate for a minimum of 2 hours before acquiring sample data.

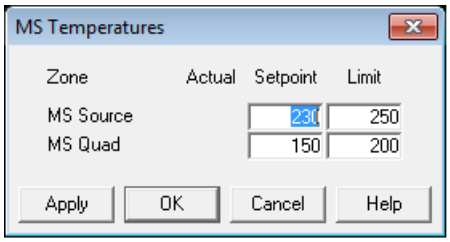
Run the ChemStation Software

The GC and MSD must both be running before starting an online session of the ChemStation product. If reports are to be printed, a printer must be installed on the computer.

1 Power on the PC.

2 From the PC desktop, select the ChemStation Instrument Control shortcut icon, to display the Enhanced ChemStation **Instrument Control** window.

3 If the Actual MS temperatures have not reached their Setpoints, the **MS Temperatures** dialog box will appear. Enter new setpoints if needed and click **OK**. The screen will appear repeatedly until the temperatures are reached.

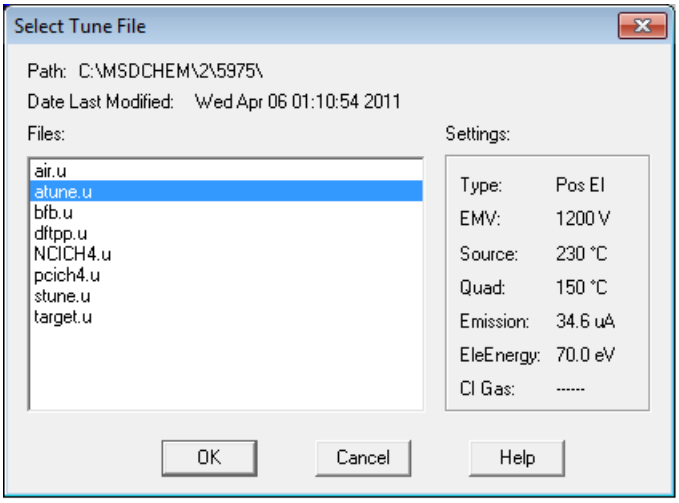


4 Set the default printer to PDF Printer, if a PDF writer like Adobe Acrobat is installed on the computer.

Select the Tune File

1 From the **Enhanced ChemStation main control** window, select **View > Tune and Vacuum Control...**. to display the **Tune and Vacuum Control** window.

2 Select **File > Load Tune Parameters**. The **Select Tune File** dialog box opens.



3 From the **Files** list, select **atune.u**. The **atune.u** file contains the optimal MSD parameter settings determined during the last autotune run.

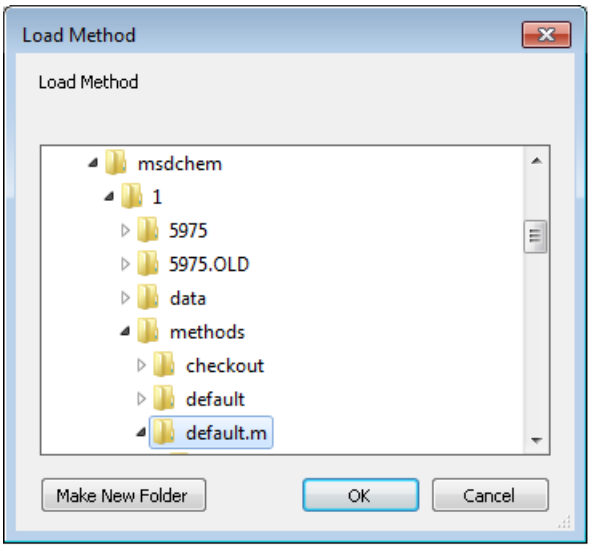
4 Select **OK**. The **atune.u** tune file is loaded and the dialog box closes.

Load the Method

1 Select **View > Instrument Control** to close the **Tune and Vacuum Control**.... and display the Enhanced ChemStation **Instrument Control** window.

2 Select the **Load Method** button,  . The **Load Method** dialog box opens.

3 Navigate to and select **default.m** in the msdchem/1/methods directory.



4 Select **OK**.

2
Tune the MS

This chapter provides a brief introduction to tuning and explains how to run an autotune on the instrument. An autotune report is generated as well as a report to evaluate the autotune results. This report is reviewed to see which items pass or fail the evaluation. Finally, we look at how we can graphically view the variation in tuned parameters that are plotted over a number of recent autotune runs.

Introduction

Tuning is the process that adjusts the MS for good performance over the entire mass range. Using a known compound as a calibrator, the tune parameters are set to achieve sensitivity, resolution, and mass assignments for the known calibration ions.

Tuning is performed using either the autotune or manual tune features.

Manual tune allows you to adjust an MS tune parameter while viewing the results easily in profile scans and spectra.

Manual tuning is used:

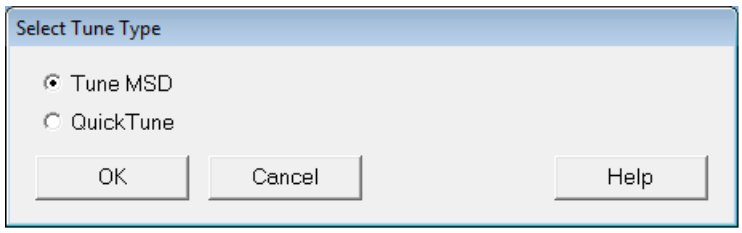
- To achieve maximum sensitivity by sacrificing some resolution
- To tune specifically for the very low end of the mass range (< 150 amu)
- To tune with a compound other than the standard calibrator

To access manual tune parameters select **Parameters > Manual Tune** from the **Tune and Vacuum Control** window or select **Instrument > Edit MS Tune Parameters** from the **Instrument Control** window. Please see the ChemStation online help for details on using manual tune.

The autotune program described in this section adjusts the MS for good performance over the entire mass range and is recommended for most applications.

Run Autotune

1 From the **Instrument Control** window select **Instrument > Tune MSD...** to display the **Select Tune Type** dialog box.



2 Select **Tune MSD** and click **OK** to close the dialog box and start the autotune procedure.

The system uses the PFTBA (perfluorotributylamine) calibrator to tune the instrument. When the tune is complete, the mass 69, 219, and 502 profile scans are displayed with abundance and peak widths noted. See Figure 1. The tune report is also generated as shown in Figure 2 on page 21.

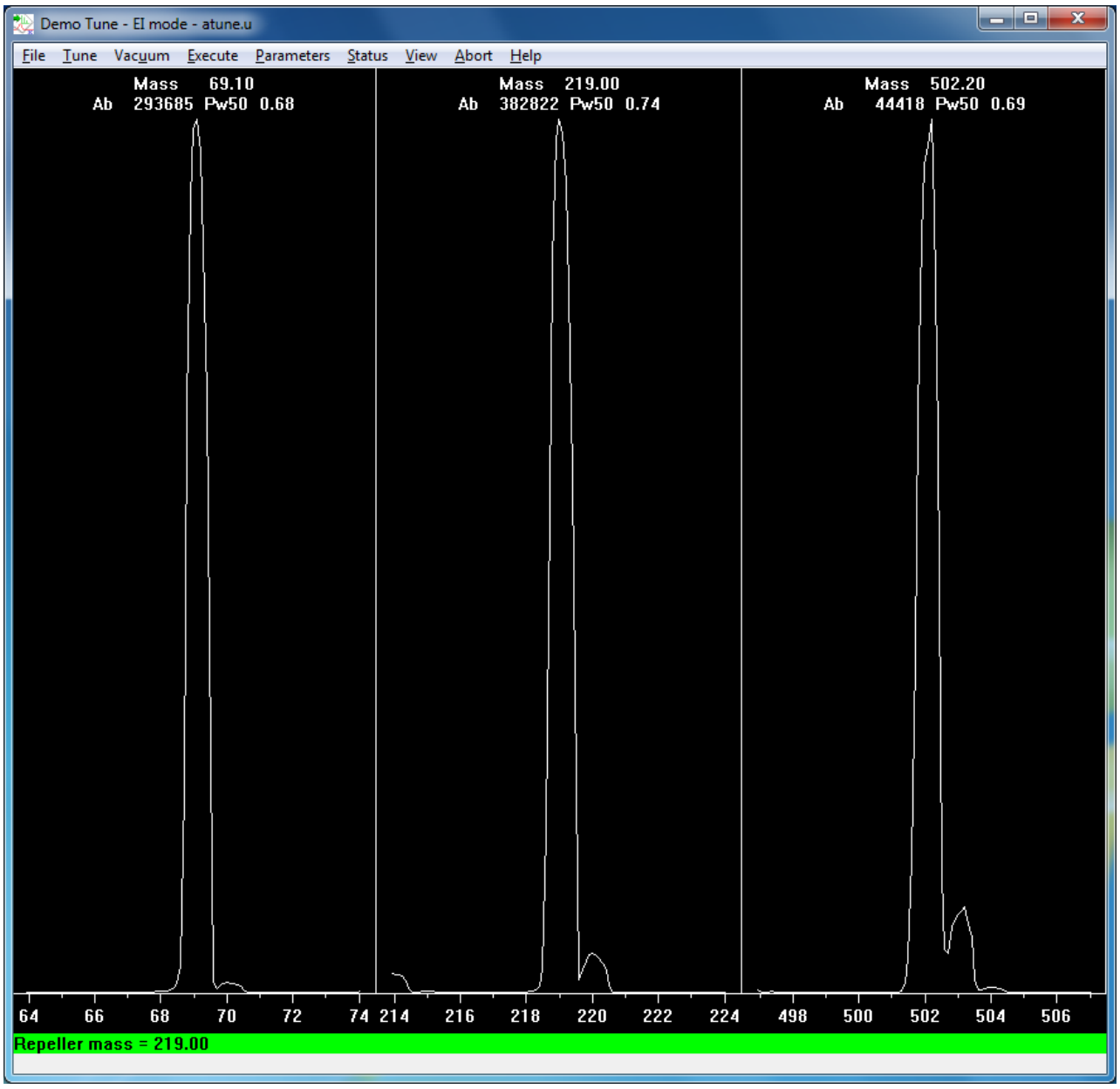


Figure 1 Profile scan results for mass 69, 219, and 502

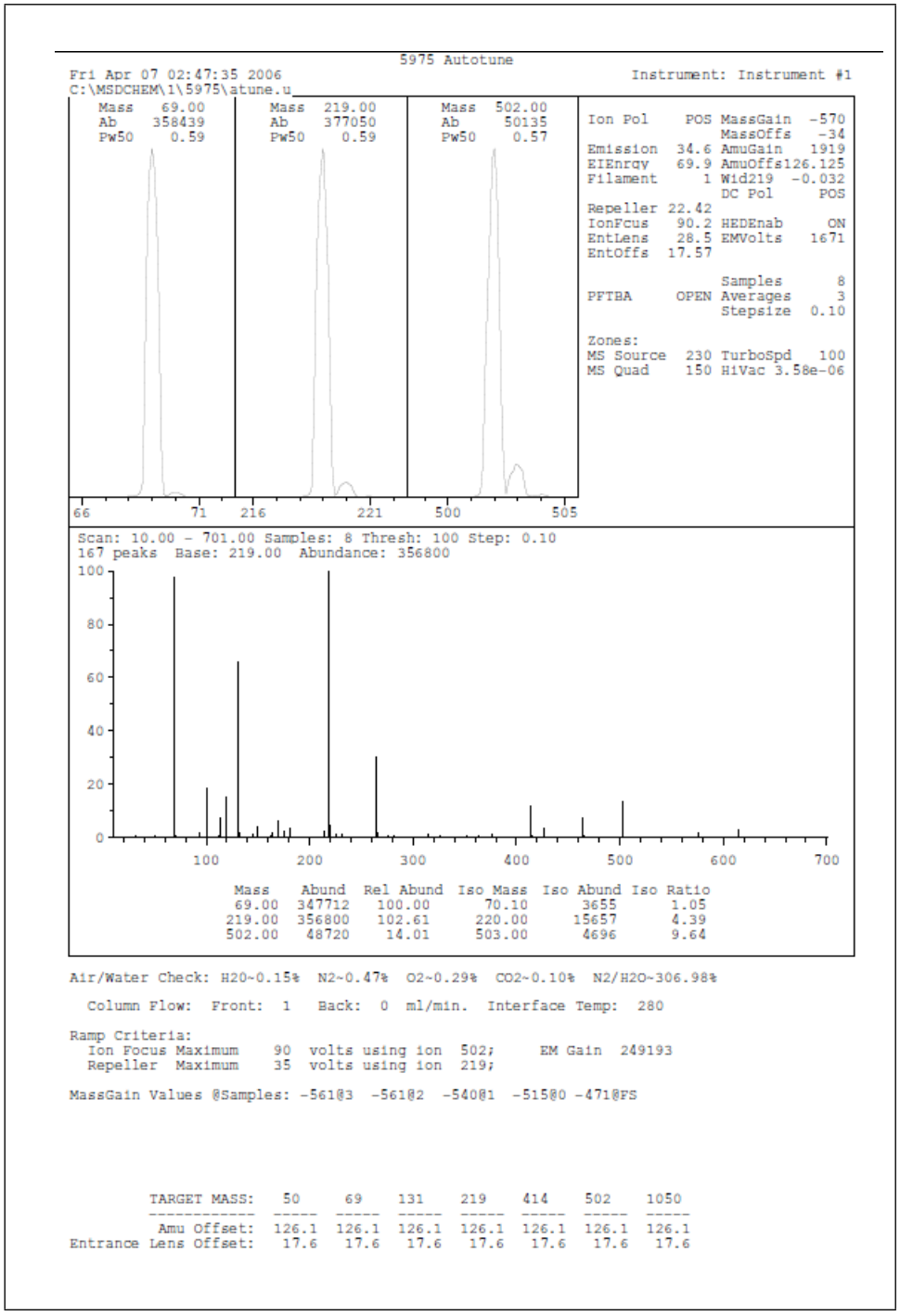


Figure 2 Autotune report

Evaluate the Autotune Results

1. Select **View > Instrument Control**.
2. Select **Checkout > Evaluate Tune**. The system compares your tune parameter results to preset acceptable results and displays the System Verification report. See Figure 3.
3. Review the report. Criteria marked as **OK** are functioning correctly. If all criteria are marked **OK**, **Tune portion of System Verification passed** is printed on the last line of the report. See Figure 3.

If one or more criteria do not pass verification, the incorrect behavior and suggested corrective actions are described. See Figure 4 on page 23 where the report shows a high ratio of mass 18 to 69. This report warns of a high amount of water in the system and a corrective action to be taken.

```
          System Verification - Tune (Detector Optimization) Portion

Instrument Name                 : Instrument #1
DC Polarity                     : Positive
Filament                        : 1
BasePeak should be 69 or 219                                    Ok
Position of mass 69                                  69.00      Ok
Position of mass 219                                219.00      Ok
Position of mass 502                                502.00      Ok
Position of isotope mass 70                          70.01      Ok
Position of isotope mass 220                        220.00      Ok
Position of isotope mass 503                        503.04      Ok
Ratio of mass 70 to mass 69(0.5 - 1.6%)              1.09       Ok
Ratio of mass 220 to mass 219(3.2 - 5.4%)            4.19       Ok
Ratio of mass 503 to mass 502(7.9 - 12.3%)           9.73       Ok
Ratio of 219 to 69 should be > 40% and is          103.07       Ok
Ratio of 502 to 69 should be > 2.4% and is          13.58       Ok

Mass 69 Precursor (<= 3%)                            0.10       Ok
Mass 219 Precursor (<= 6%)                           0.21       Ok
Mass 502 Precursor (<= 12%)                          0.26       Ok

    Testing for a leak in the system
Ratio of 18 to 69 (<20%)                             0.22       Ok
Ratio of 28 to 69 (<10%)                             0.43       Ok

Electron Multiplier Voltage                       1671          Ok

    Tune portion of System Verification passed.
```

Figure 3 Passing system verification tune report

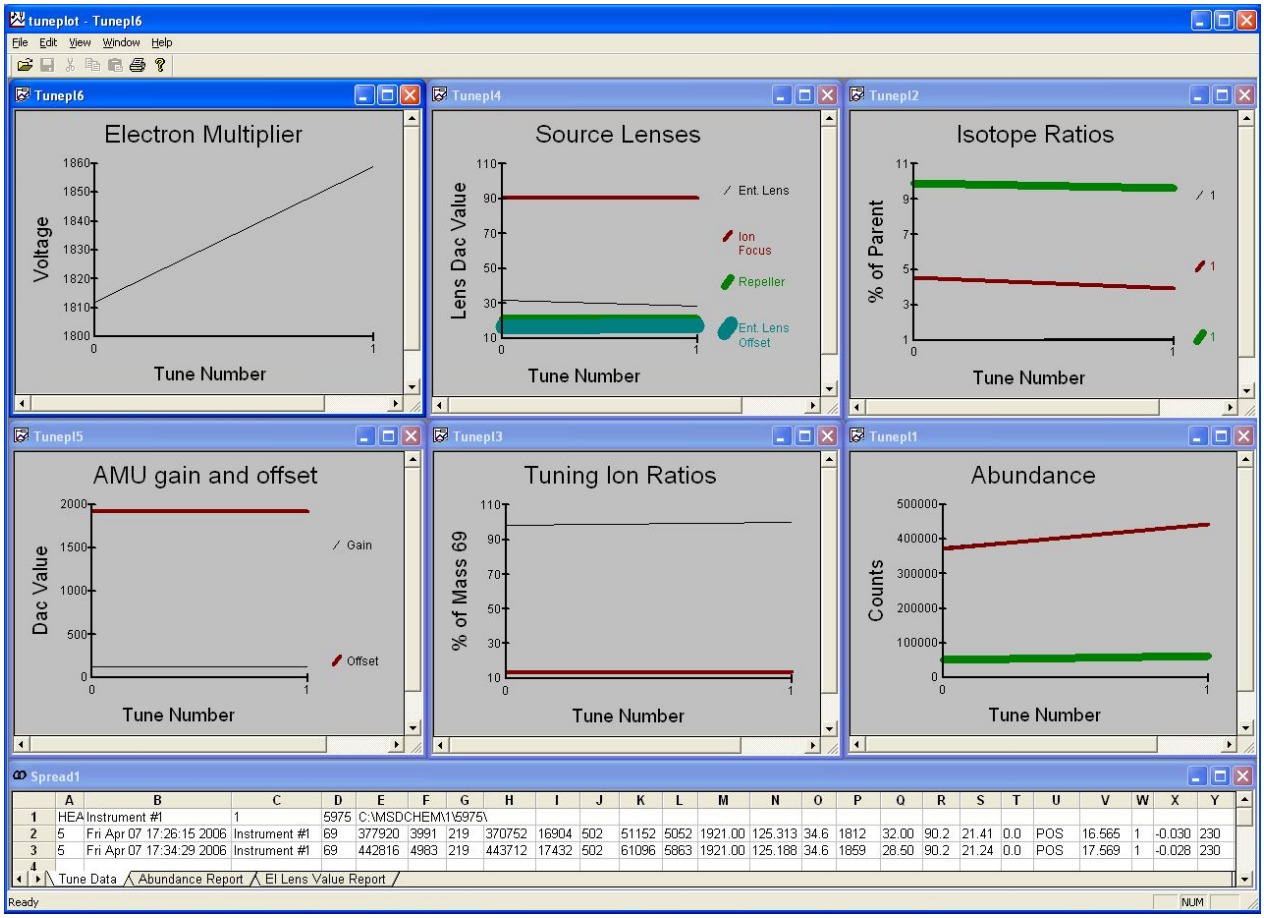
```
            System Verification - Tune (Detector Optimization) Portion

Instrument Name                 : Instrument #1
DC Polarity                     : Positive
Filament                        : 1
BasePeak should be 69 or 219                                    Ok
Position of mass 69                                  69.00      Ok
Position of mass 219                                218.98      Ok
Position of mass 502                                501.96      Ok
Position of isotope mass 70                          70.07      Ok
Position of isotope mass 220                        219.94      Ok
Position of isotope mass 503                        502.95      Ok
Ratio of mass 70 to mass 69(0.5 - 1.6%)              1.34       Ok
Ratio of mass 220 to mass 219(3.2 - 5.4%)            4.33       Ok
Ratio of mass 503 to mass 502(7.9 - 12.3%)          10.80       Ok
Ratio of 219 to 69 should be > 40% and is           96.83       Ok
Ratio of 502 to 69 should be > 2.4% and is          14.71       Ok

Mass 69 Precursor (<= 3%)                            0.42       Ok
Mass 219 Precursor (<= 6%)                           0.26       Ok
Mass 502 Precursor (<= 12%)                          0.45       Ok

    Testing for a leak in the system
Ratio of 18 to 69 (<20%)                            38.86       High
Ratio of 28 to 69 (<10%)                             6.50       Ok
    There is a high amount of water in your system.
    Wait 24 hours for the system to bake out and rerun system verification.

Electron Multiplier Voltage                    1671             Ok

    One or more specifications was out of range.
    Please correct before continuing.

    Failure of one or more tests may be caused by
    selecting the wrong DC Polarity.
    Please verify that the correct DC Polarity has been set
    by removing the detector cover and checking the label
    at the top of the EID.
```

Figure 4 Failing system verification tune report

Tune History Trends

1 Select **View > Instrument Control.**

2 Select **Checkout > View Previous Tunes**... to display the **Tuneplot** window plotting the results of recent tune parameters.



3 Create a Method for Qualitative Analysis

This chapter describes how to create an acquisition method that will be used later to identify all compounds in an Agilent standard sample. The method is created by editing the default method to include an MS scan that is set to identify all ions created by EI of each compound.

Introduction

The method we are creating will be used to find the known compounds in the Agilent sample P/N 05970-60045 (P/N 5074-3025 Japan only). The sample compounds are in isooctane solvent in 1 mL ampules of 10 ng/µL, 100 ng/µL, and 100 pg/µL concentrations and are shown in Table 1.

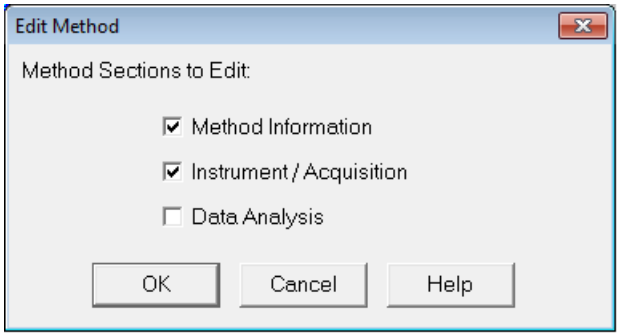
Table 1 Sample Compound list

Compound	MW	Formula
Dodecane	170	$C_{12}H_{26}$
Biphenyl	154	$C_{12}H_{10}$
4-Chlorobiphenyl (PN 05970-60045 only)	188	$C_{12}H_9Cl$
Methyl palmitate	270	$C_{17}H_{34}O_2$

The MS part of the method is required to scan for all ions contained in the range that includes all the molecular weights for these compounds. As seen in the table, the range of the molecular ions is from 0 to 270 so we will scan for ions from 0 to 300 in the method.

Edit the Entire Method

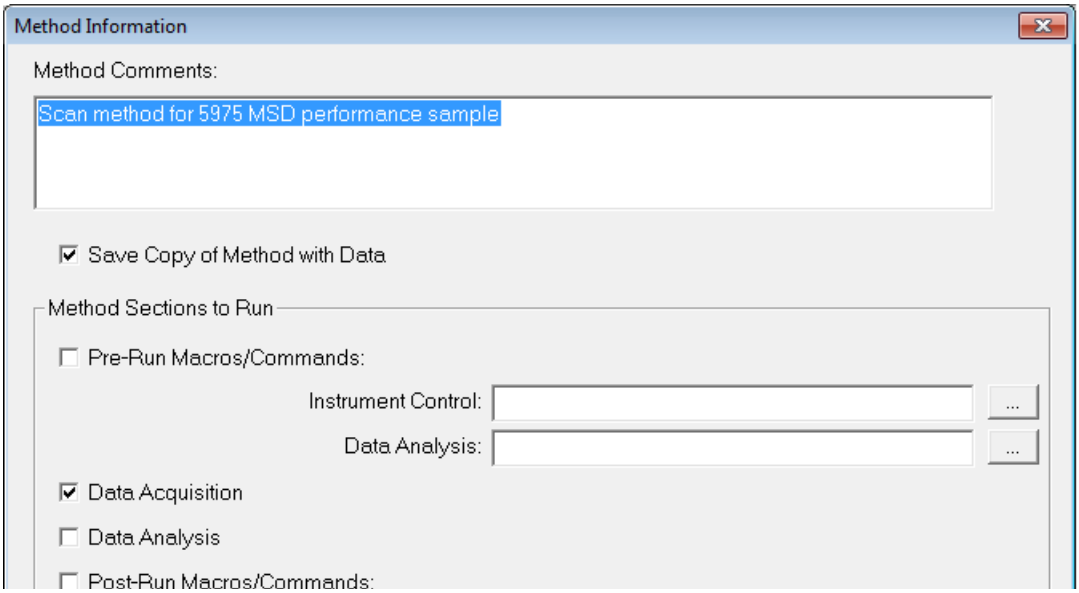
1 With the default method loaded, see "Load the Method" on page 16, select the **Edit Entire Method...** button,  to edit the currently loaded method. The **Edit Method** dialog box opens.



2 Mark the **Method Information** and **Instrument/Acquisition** checkboxes only. Clear the **Data Analysis** checkbox.

Selecting **Instrument/Acquisition** displays all the dialog boxes required to edit the acquisition parameters for both the GC and MS parts of the currently loaded method. We are not modifying the **Data Analysis** part of the method at this time.

3 Select **OK** to close the **Edit Method** dialog box. Because **Method Information** was selected, the **Method Information** dialog box opens.

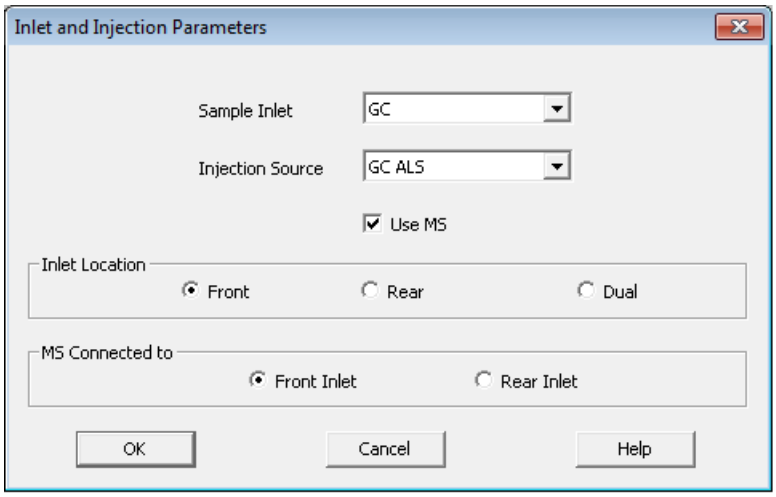


4 In the **Method Comments** field, enter a description of this method.

5 Mark the **Save Copy of Method With Data** checkbox. When the ChemStation acquires sample data using this method, it automatically saves a copy of the method along with the data.

6 In the **Method Sections To Run** area, mark the **Data Acquisition** checkbox only. The data analysis will not be run at this time.

7 Select **OK** to close the **Method Information** dialog box and display the **Inlet and Injection Parameters** dialog box.



8 From the **Sample Inlet** dropdown list, select **GC**.

9 From the **Injection Source** dropdown list, select your source.

- If you are injecting from the GC using the Automatic Liquid Sampler (**ALS**), select **GC ALS**.
- If you are manually injecting or using another injection source, select **Manual**.

10 Mark the **Use MS** checkbox to allow the ChemStation to turn on the MS analyzer and save the MS sample data acquired during the run. You would only uncheck this box when you have a GC (non-MS) detector and you were acquiring data for the GC detector only.

11 In the **Inlet Location** area, select the location where your S/SL inlet is attached to the MS through the column.

12 In the **MS Connected to** area, select the location where your S/SL inlet is attached to the MS through the column.

13 Select **OK** to close the **Inlet and Injection Parameters** dialog box and display the **GC Edit Parameters** window.

Check the GC configuration

1 Select the **Configuration** button,  . See the ChemStation Online Help for more information.

2 With the **Miscellaneous** tab selected, set the **Pressure Units** to **psi.** Under **Valve Configuration** set all **Valve Type** fields to **Not Installed**, and verify that the **MSD transfer line** is shown as a **Thermal Aux Type**.

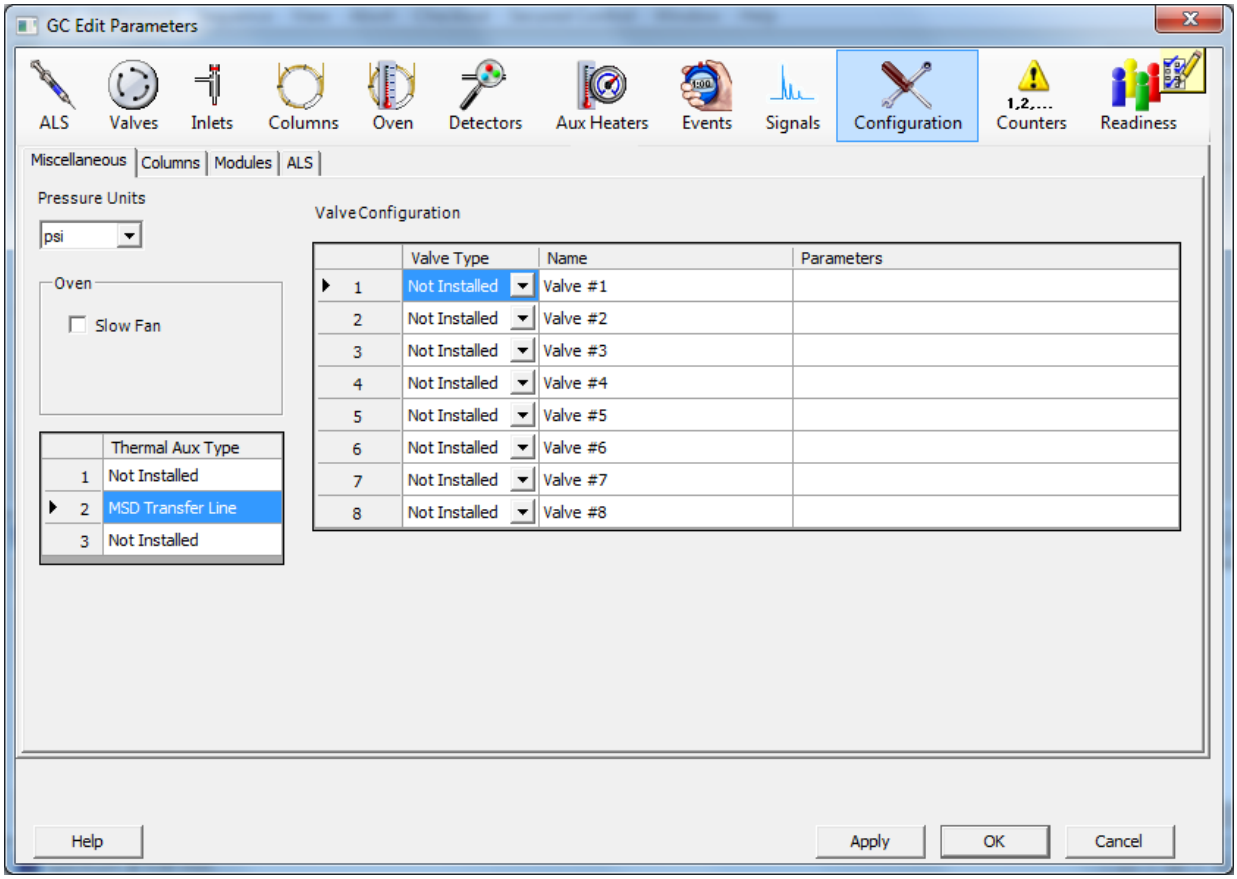


Figure 5 Miscellaneous configuration tab

3 Select the **Columns** tab to display the columns configuration parameters. The HP-5ms checkout column supplied with the MS should be listed under **Column**.

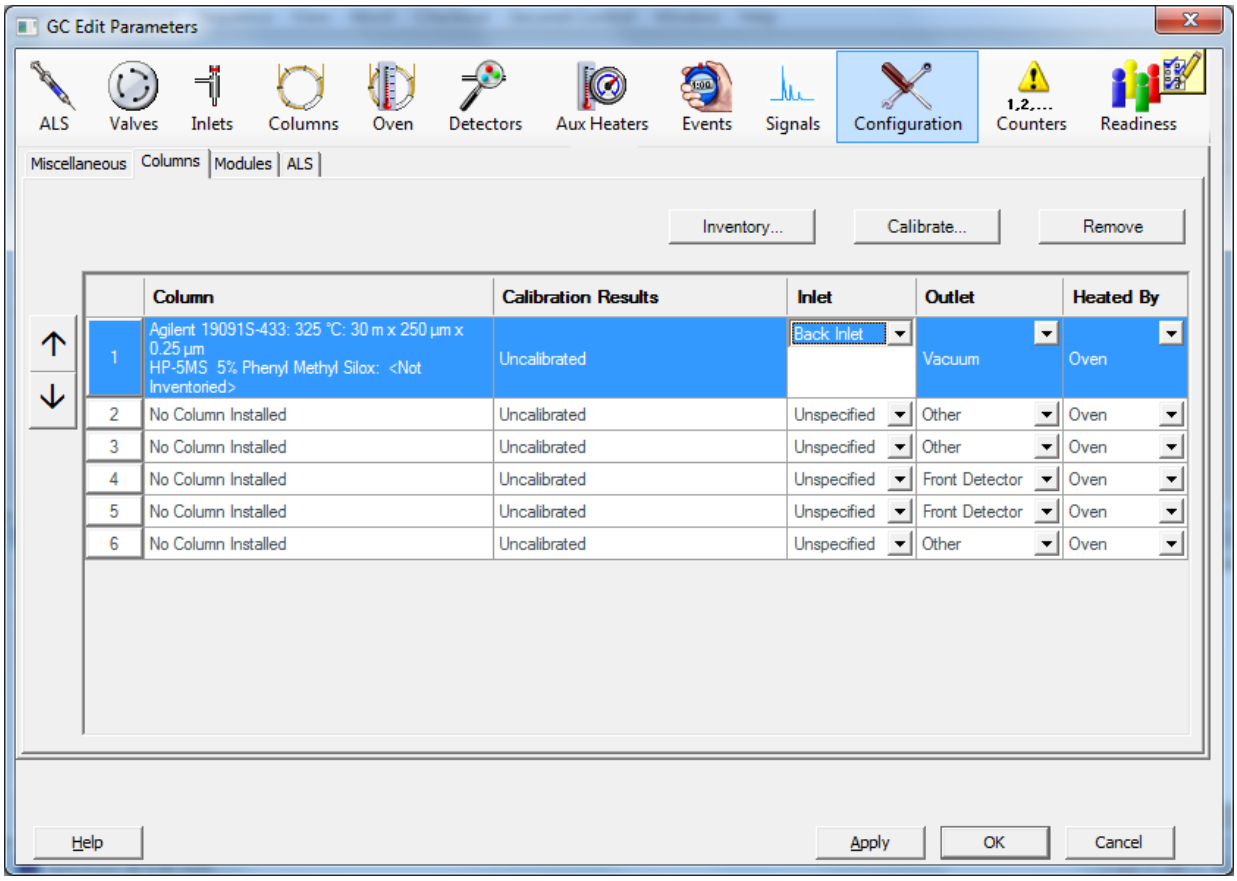


Figure 6 Column configuration tab

4 If a different **Column** is configured to the inlet location you are using or is attached to the MS, select it and click **Remove**.

5 If the HP-5ms is not listed under **Column**, click the **Inventory** button and add it to inventory before listing it here. See "Add a column to ChemStation local inventory" on page 48.

6 If required, use the up and down arrow keys to put the HP-5ms column in the **1** position.

7 For the **Inlet** pressure for this column, select the **Front** or **Back Inlet** from the dropdown.

8 For the column **Outlet** pressure select **Vacuum** for the MS

9 For the column **Heated By** select **Oven** from the dropdown.

10 Click the **Apply** button and then select the **Modules** tab.

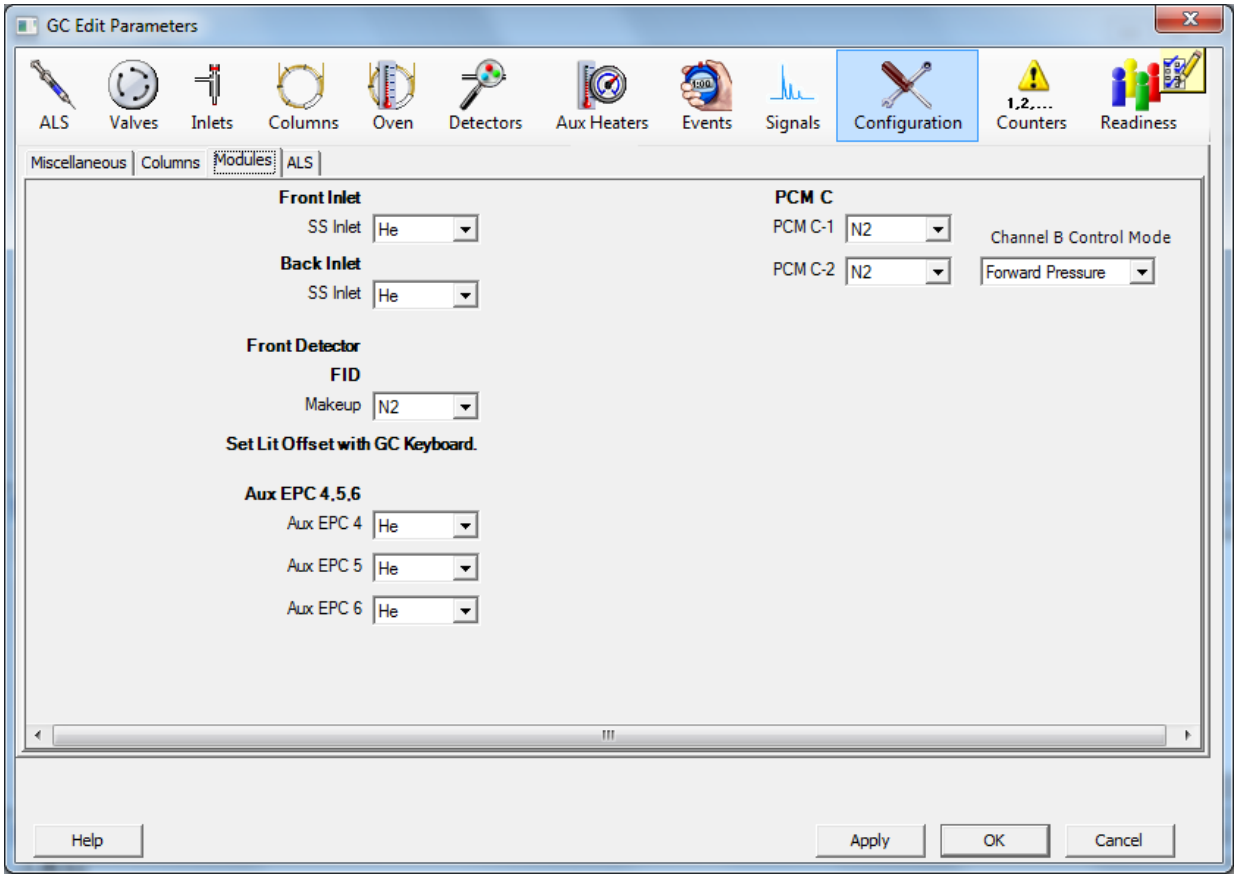


Figure 7 Modules configuration tab

11 Select **He** gas from the dropdown for the inlet connected to column 1. The system uses the properties of helium to obtain an accurate flow and pressure relationship for the column.

12 Click the **Apply** button to download any edits to the GC.

Set the GC readiness state

1 Select the **Readiness** button, 

. The **Readiness** parameters are displayed.

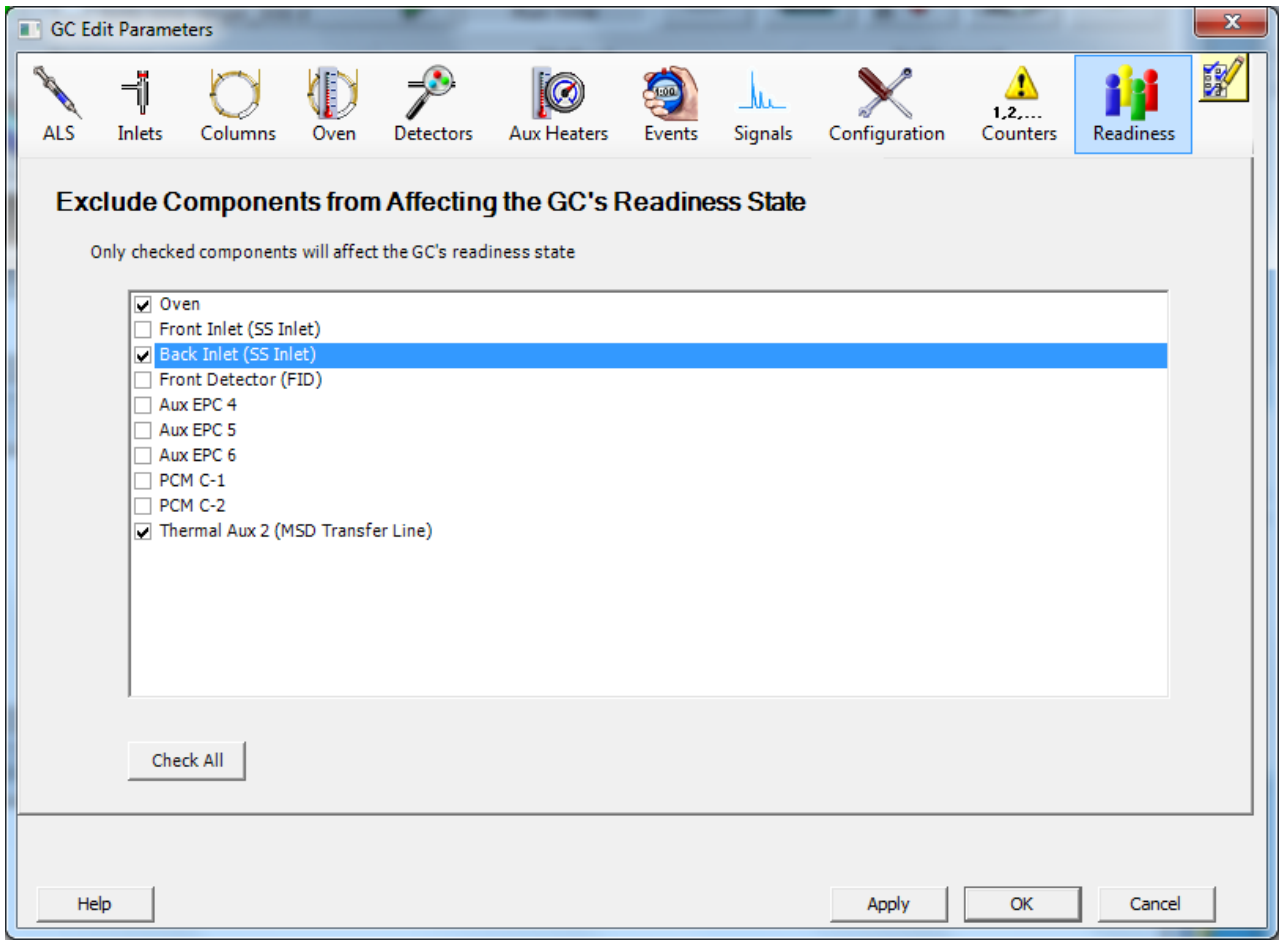


Figure 8 Readiness state component selection

2 Select the **Oven**, **SS Inlet** (attached to column 1), and **Thermal Aux 2** (MSD Transfer Line). These selections require the GC to wait until all setpoints related to the oven, inlet, and transfer line are held at a steady value before allowing a run to begin.

3 Click **Apply** to download these selections to the GC.

Set the GC oven parameters

1 Select the **Oven** button,  . The **Oven** parameters are displayed.

For this example we require an oven program that initially holds the column temperature at 50 °C. When the run starts, the column temperature is increased from this temperature to 300 °C at a rate of 35 °C/min. The column is then held at 300 °C for an additional 2 minutes. At this time the oven is cooled down to 50 °C to await the next data acquisition run.

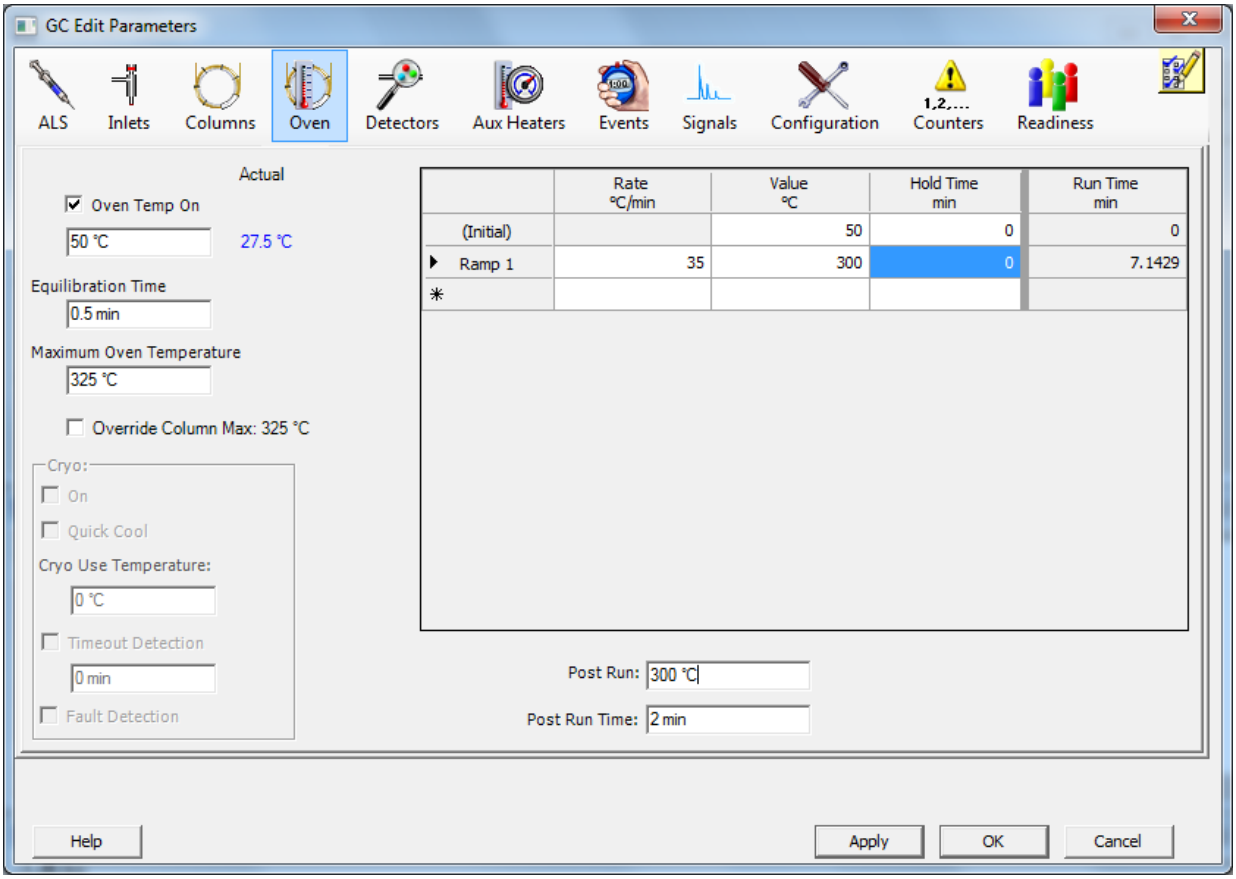


Figure 9 GC oven parameters

2 Mark the **Oven Temp On** checkbox and enter `50 °C` in the corresponding field.

3 In the **Equilibration Time** field, enter `0.5 min`.

4 In the **Maximum Oven Temperature** field, enter `325 °C`. This is the maximum temperature for the HP-5ms column.

5 Clear the **Override Column Max**. **325 °C** checkbox.

6 In the **Oven Ramp** table, enter the settings shown in Table 2.

Table 2 Oven ramp settings

Oven Ramp	Rate	Value	Hold Time
	°C/min	°C	min
(Initial)		50	0
Ramp 1	35.00	300	0

7 In the **Post Run** field, enter `300 °C`.

8 In the **Post Run Time** field, enter `2 min` to hold the 300 °C oven temperature for 2 minutes after the run is finished before cooling down to 50 °C for the start of the next run.

9 Select **Apply** to download these settings to the GC.

Set the GC column parameters

1 Select the **Columns** button,  . The **Column** parameters are displayed.

2 Check the Column information in the **Selection** list.

- Column: 19091S-433 (HP-5ms 30 m x 250 µm x 0.25 µm)
- In: front or back (split/splitless inlet position)
- Out: Vacuum

3 Mark the **Control Mode** checkbox.

4 In the **Flow Setpoint** field, enter **1.0 mL/min**. The **Pressure**, **Average Velocity**, and **Holdup Time Setpoints** will be calculated and displayed in the corresponding fields.

5 In the dropdown list, select **Constant Flow**.

6 In the **Post Run** field, enter **1.0 mL/min**.

7 Select **Apply** to download these settings to the GC.

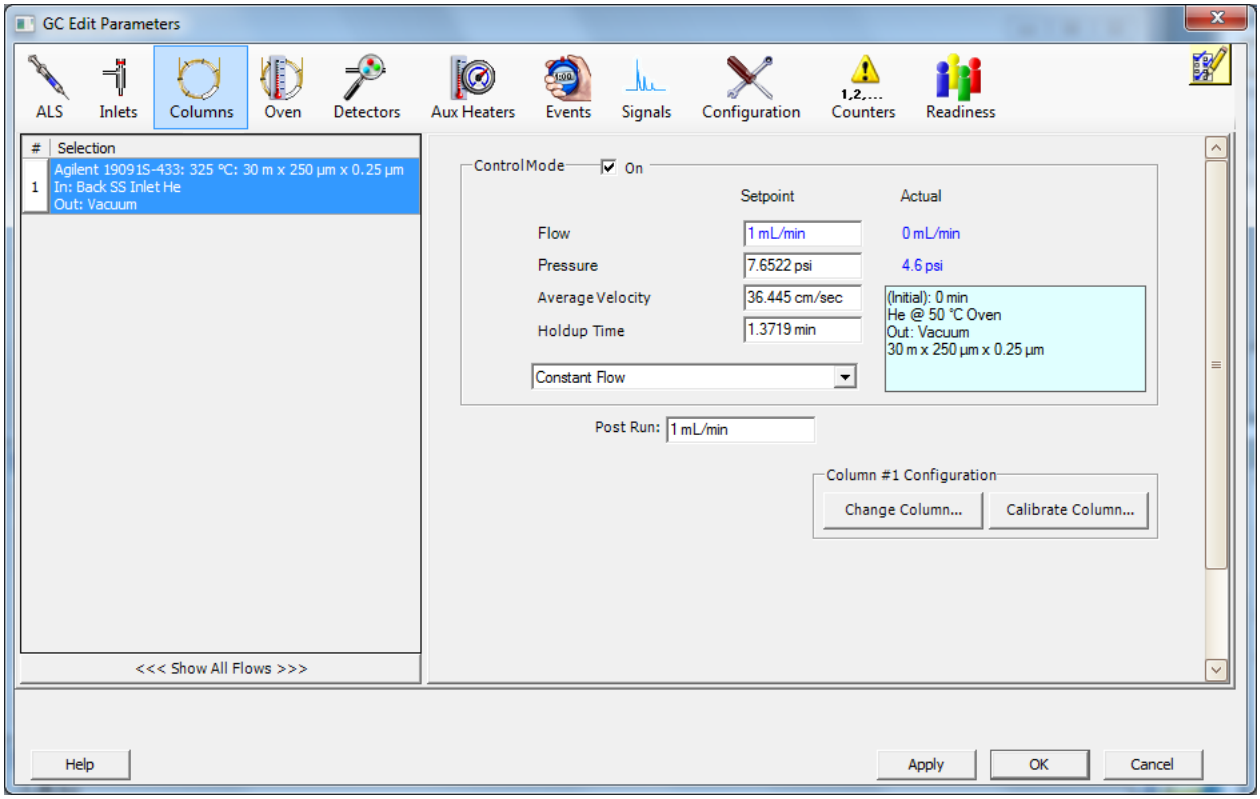


Figure 10 GC columns parameters

Set the GC inlet parameters

1 Select the **Inlets** button,  . The **Inlet** parameters are displayed.

2 Select the **Front** or **Back** tab, depending on your hardware configuration.

3 Mark the **Heater** checkbox and enter **250 °C** in the corresponding **Setpoint** field.

4 Mark the **Pressure** checkbox. The **psi** in the corresponding **Setpoint** field is automatically set when the column flow rate is set.

5 Mark the **Septum Purge Flow** checkbox and enter **3 mL/min** in the corresponding **Setpoint** field.

6 From the **Septum Purge Flow Mode** drop down list, select **Standard**.

7 In the **Gas Saver** area:

   a Mark the **On** checkbox.

   b In the field below, enter `20 mL/min`.

   c In the **After** field, enter `2 min`.

8 In the **Mode** area:

   a From the **Mode** drop down list, select **Splitless**.

9 In the **Purge Flow to Split vent** area:

   a In the field, enter `50 mL/min`.

   b In the **Start Time** field, enter `1`.

10 Select **Apply**.

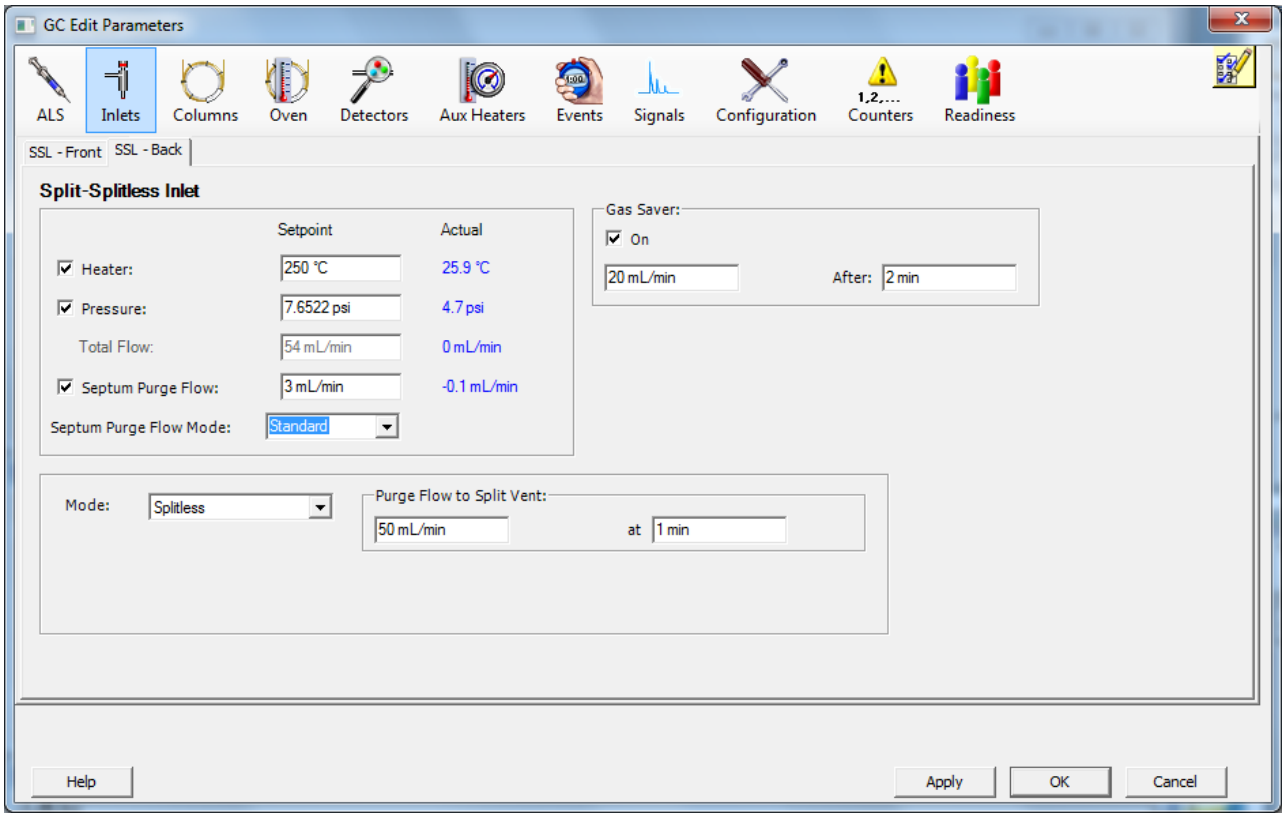


Figure 11 GC inlet parameters

Set the GC injector parameters

If you are not using the autosampler, skip this section.

1 Select the **ALS** button,  .

2 Select the **Front Injector** or **Back Injector** tab, depending on your hardware configuration.

3 In the **Injection** area:

   a Verify that the **Syringe Size** matches your hardware configuration.

   b In the **Injection Volume** field, enter `1`.

4 In the **Washes and Pumps** area:

   a For **Solvent A Washes**, enter `5` in the **PostInj** field.

   b For **Sample Washes**, enter `3` in the **PreInj** field.

   c For **Sample Pumps**, enter `5` in the **PreInj** field.

5 Select the **Advanced** button,  . Additional options are displayed in the window.

6 In the **Plunger Speed** area, select **Fast**.

7 In the **Sampling Depth** area,

   a Mark the **Enable** checkbox.

   b In the field, enter `3.6`.

8 Select **Apply**.

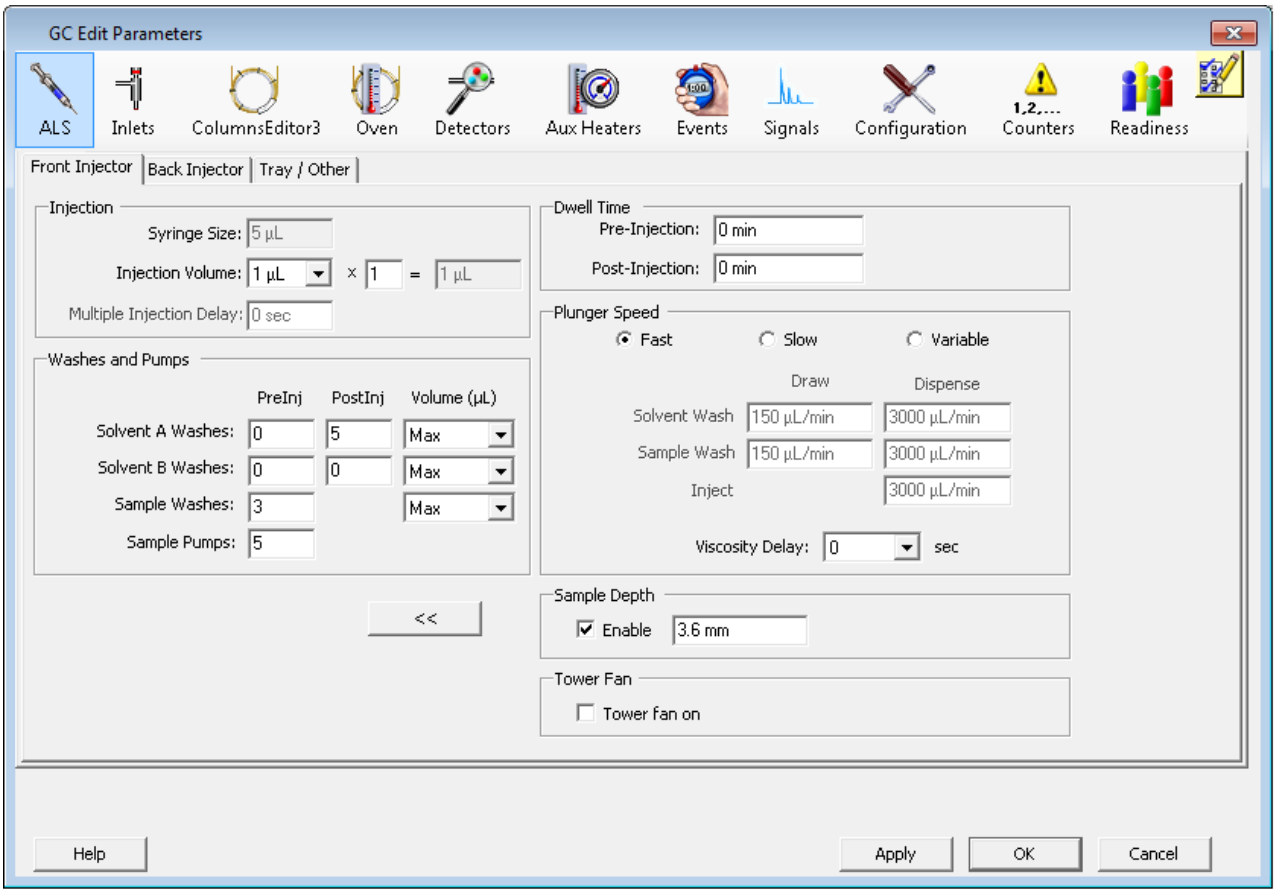


Figure 12 ALS parameters

Set the GC Aux heaters parameters

1 Select the **AUX Heaters** button,  .

2 For **Thermal Aux 2**, mark the **On** checkbox.

3 In the **Ramps** table, enter `280` in the **Value °C** field.

4 Select **Apply**.

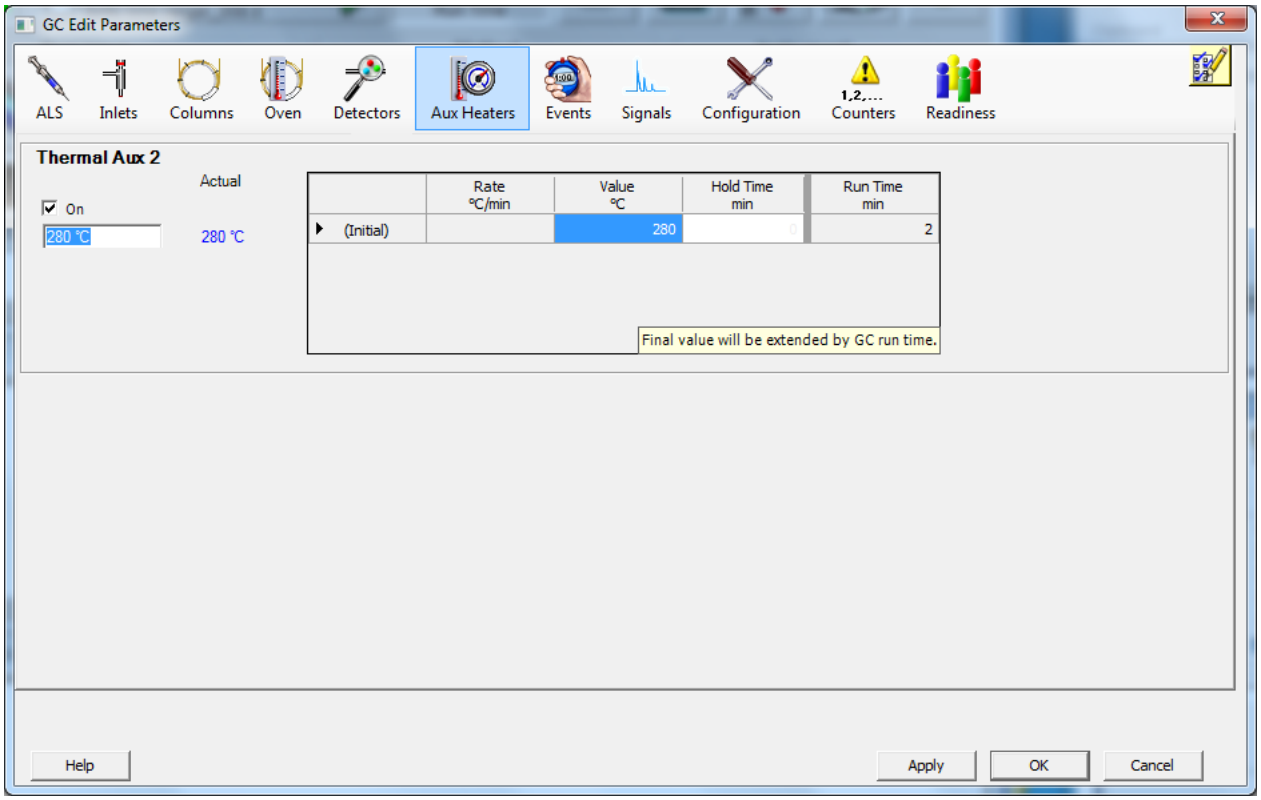


Figure 13 GC aux heaters parameters

Set the GC signals parameters

1 Select the **Signals** button, 

.

2 In the **Signal Source** dropdown list, select **None** for all the signal sources.

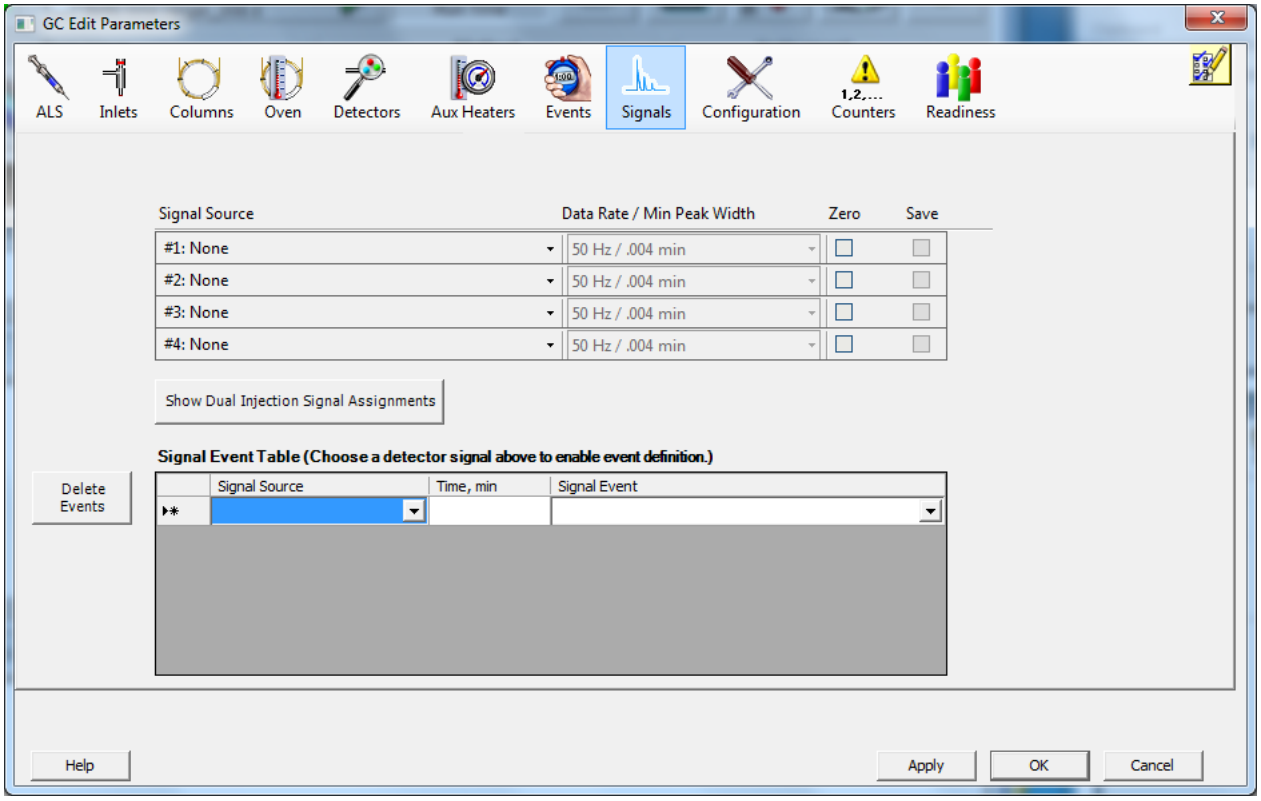


Figure 14 GC signals parameters

3 Select **OK** to download the selected parameters to the GC and close the **GC Edit parameters** window. The **GC Detector Data** dialog box opens. See Figure 15 on page 42.

Edit the GC real time plots to display

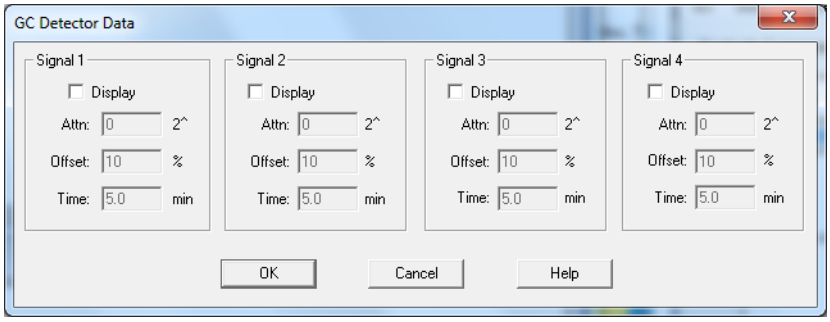


Figure 15 Selecting GC signals to plot in real time

4 From the **GC Detector Data** dialog box, clear the checkboxes for all signals. We will not be plotting GC signals.

5 Select **OK** to save the settings and close the dialog box. The **MS Tune File** dialog box opens. See Figure 16.

Edit the MS parameters

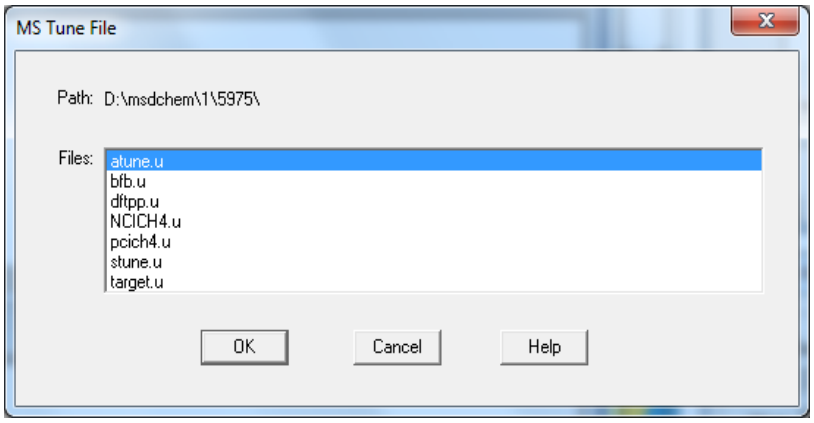


Figure 16 Selecting the method MS tune parameter file

1 Select **atune.u** from the **File** list.

2 Select **OK** to assign the tune file to the current method and close the **MS Tune File** dialog box. The **MS SIM/Scan Parameters** dialog box opens.

3 In the **MS Instrument** area enter:

   a In the **Solvent Delay** field, enter `3.00 min`.

   b In the **EMV mode** drop down list, select **Gain Factor.**

   c In the **Gain Factor** field, enter `1.00`.

d In the **Acq. mode** drop down list, select **Scan**.

e In the **Scan Speed** drop down list, select **Normal**.

f Clear the **Acquire both Scan and SIM data** checkbox.

4 In the **Real-Time Plot** area **Time Window** field, enter `10`.

5 In the **MS Window 1** area:

a From the **Plot Type** dropdown, select **Total**.

b In the **Y-Scale** fields, enter `0` to `2000000`.

6 In the **MS Window 2** area:

a From the **Plot Type** dropdown, select **Spectrum**.

b In the **Y-Scale** fields, enter `0` to `1000000`.

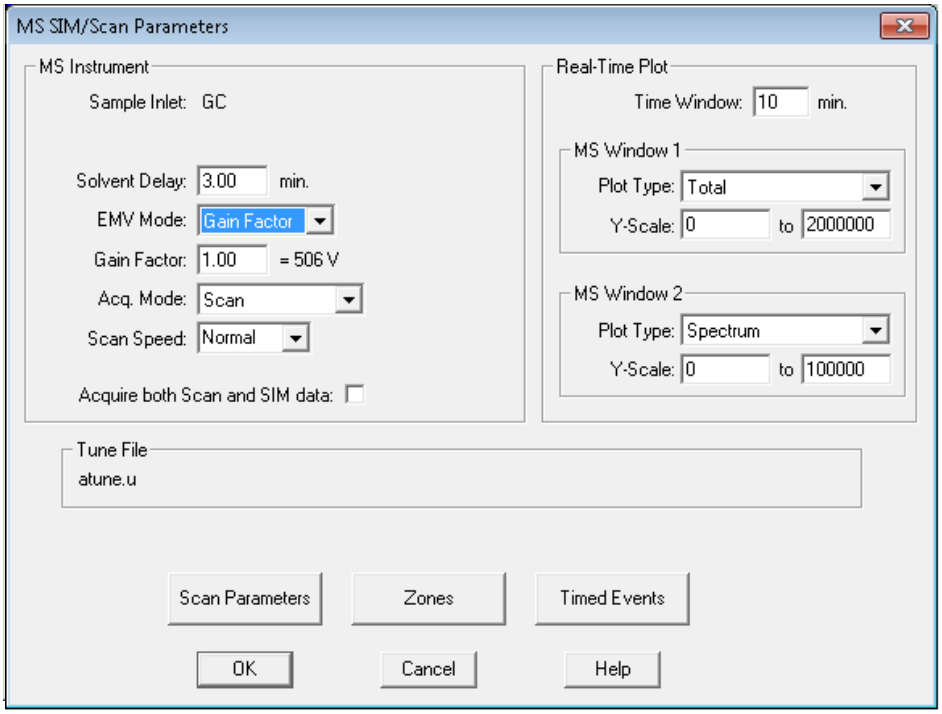


Figure 17 Setting the MS scan parameters

7 Select **Scan Parameters**. The **Edit Scan Parameters** dialog box opens.

8 Select the **Scanning Mass Range** tab:

a Mark the **Scan Group 1** checkbox.

b In the **Start at Mass** field, enter `50.00`.

c In the **End at Mass** field, enter `300.00`.

This scan range includes all the expected ions.

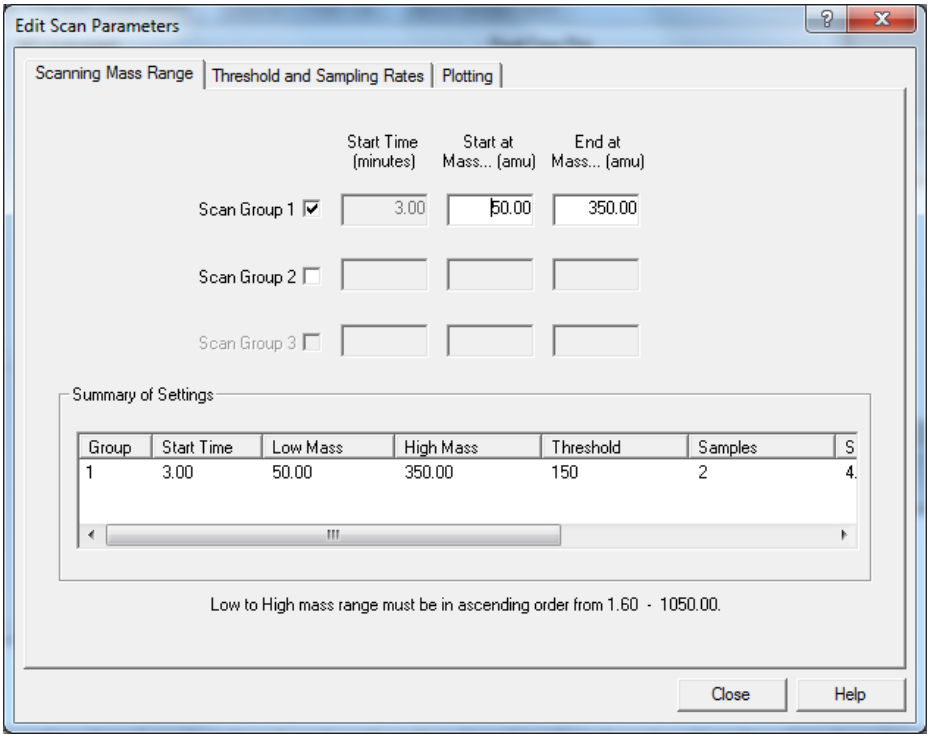


Figure 18 Specify the scan range

9 Select the **Threshold and Sampling Rates** tab:

a In the **Threshold** field, enter `40`.

b In the **Sampling Rate** field, enter `3`.

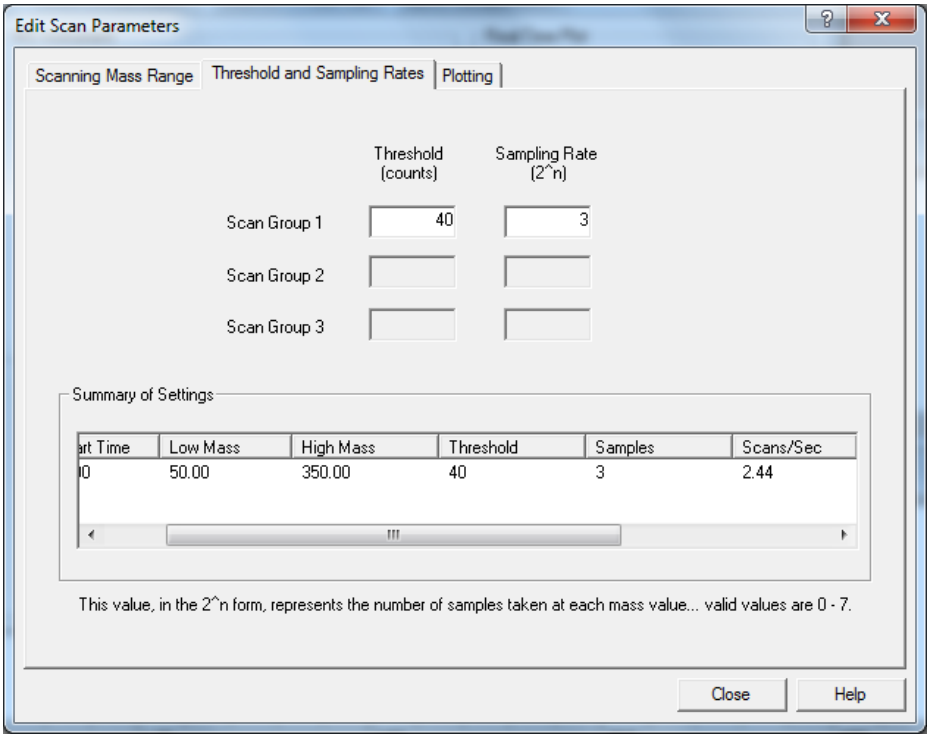


Figure 19 Setting the sampling rate and threshold filter

10 Select the **Plotting** tab, In the **Plot Window #2** area:

a Under **Low Mass**, enter `50`.

b Under **High Mass**, enter `350`.

Plot Window #1 was set to be a TIC so no plotting entry is required. **Plot Window #2** is a spectrum including all ions found between 50 and 350 *m/z*.

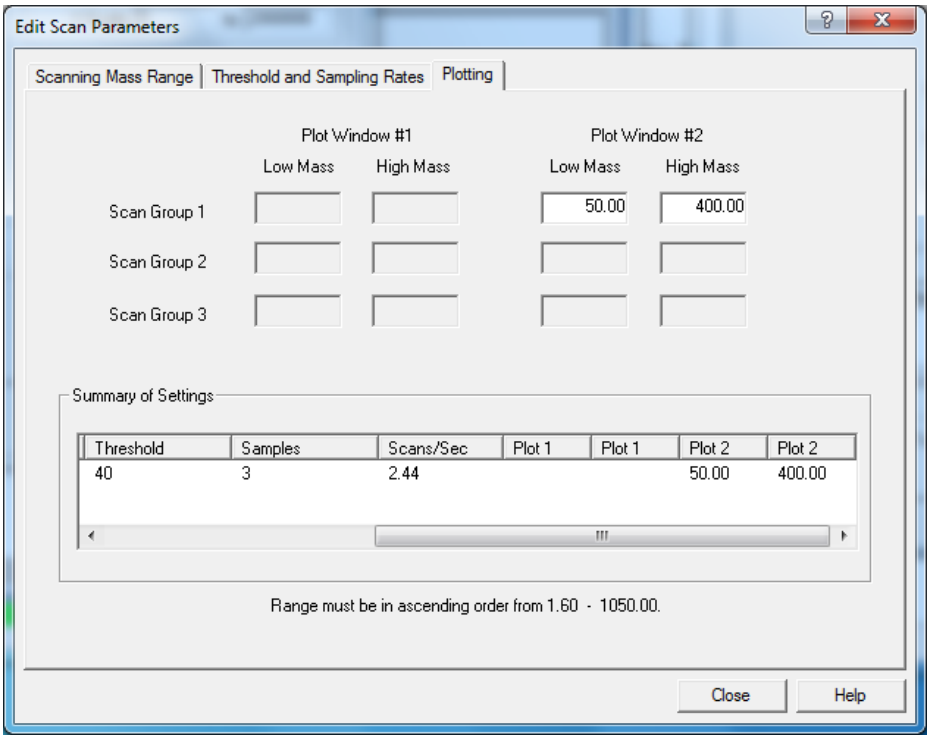


Figure 20 Specifying the real time plot scan range

11 Select **Close** to save the settings and return to the **MS SIM/Scan Parameters** dialog box.

12 Select **OK** to save the parameters and close the dialog box. The **Save Method As** dialog box opens. See Figure 21.

Save the method

1 Enter `demoscan.M` in the **Method File** field.

2 Select **OK** to save the current ChemStation method as **demoscan.m** method.

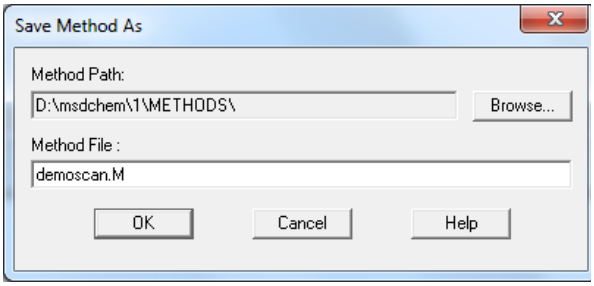


Figure 21 Saving the method

General Information for Editing the GC Parameters

Open the GC edit parameters window

1 From **Instrument Control** select the **GC Parameters** button to display the **GC Edit Parameters** window. See Figure 9 on page 33.

2 When a parameter button at the top of the screen is selected, the button is highlighted in blue and the settings for that parameter are displayed in the right panel. The GC instrument status is shown in the left panel.

Table 3 lists a description of the **GC Edit Parameters** window buttons.

Table 3 GC Edit Parameters window buttons

Button	Action
Apply	Downloads any settings that have been changed to the GC.
OK	Downloads any settings that have been changed to the GC and closes the **GC Edit Parameters** window.
Cancel	Discards any settings that have been changed and closes the **GC Edit Parameters** window.
Help	Displays help topics for the current parameter.

Add a column to ChemStation local inventory

Use the **Add Column to Local Inventory** dialog box to select a column from the **Column Catalog** and add it to your **Local Column Inventory.** This example adds the supplied checkout column to local inventory.

1 Select the **Configuration** icon to display the columns configured for the instrument.

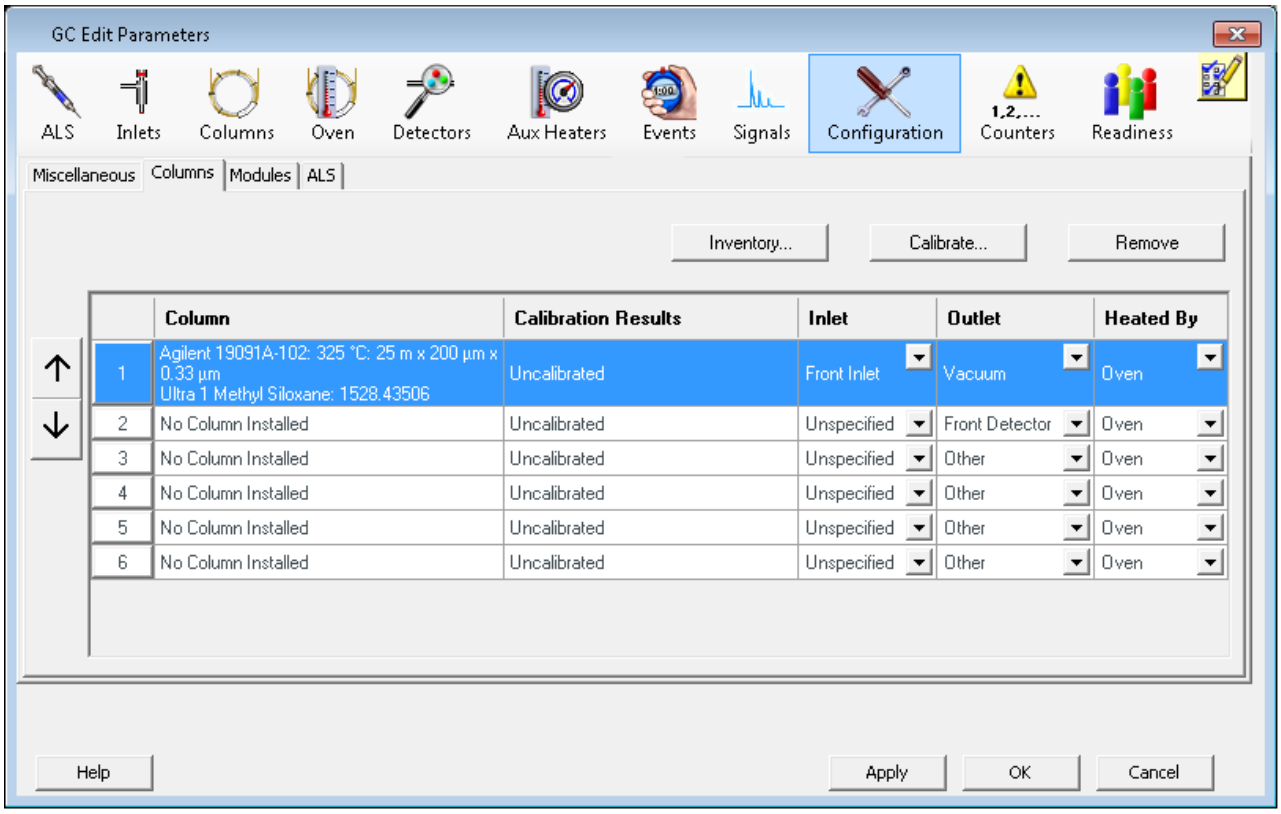


Figure 22 Columns configured for the instrument

2 Click **Inventory** to display the **Install Column 1** dialog box containing a list of columns in local inventory.

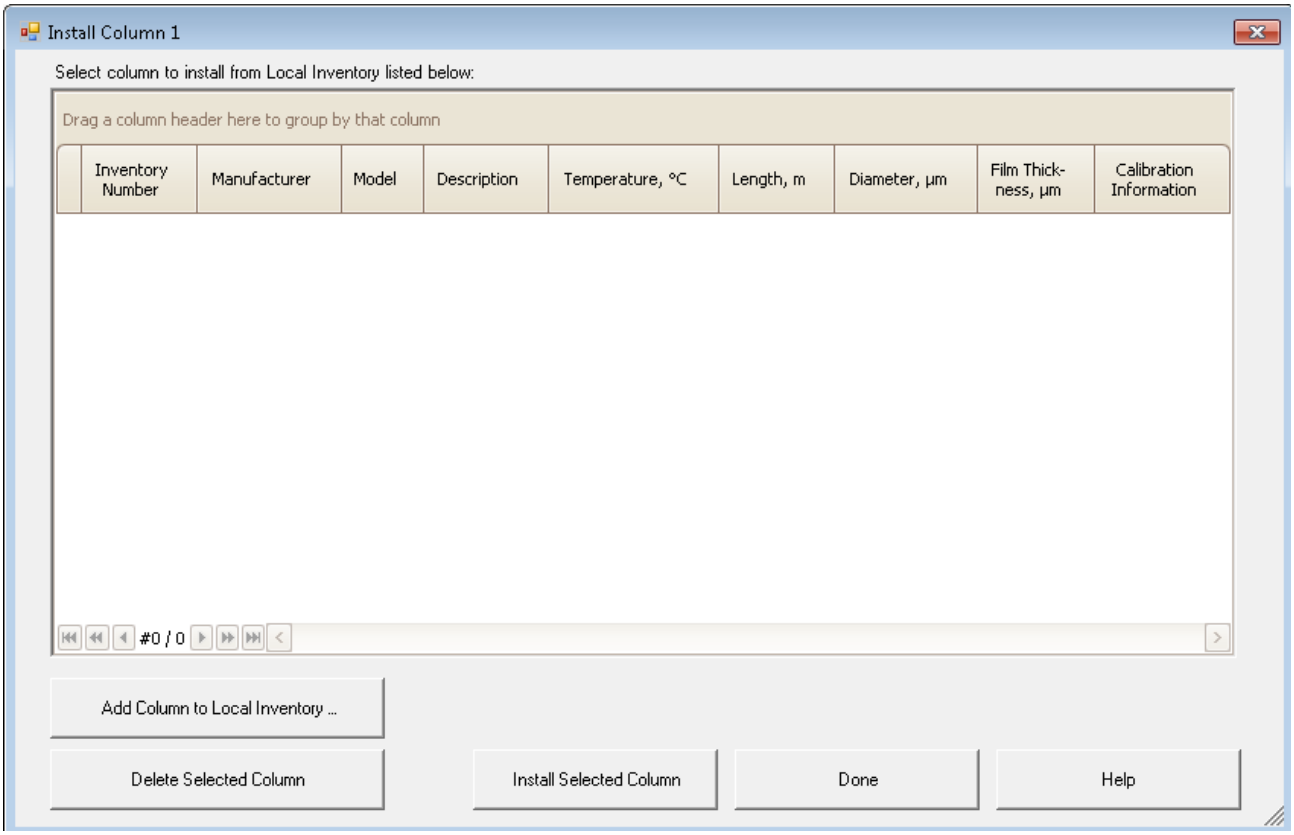


Figure 23 The local inventory of columns

3 Click **Add Column to Local Inventory** to display the **Add Column to Local Inventory** dialog box.

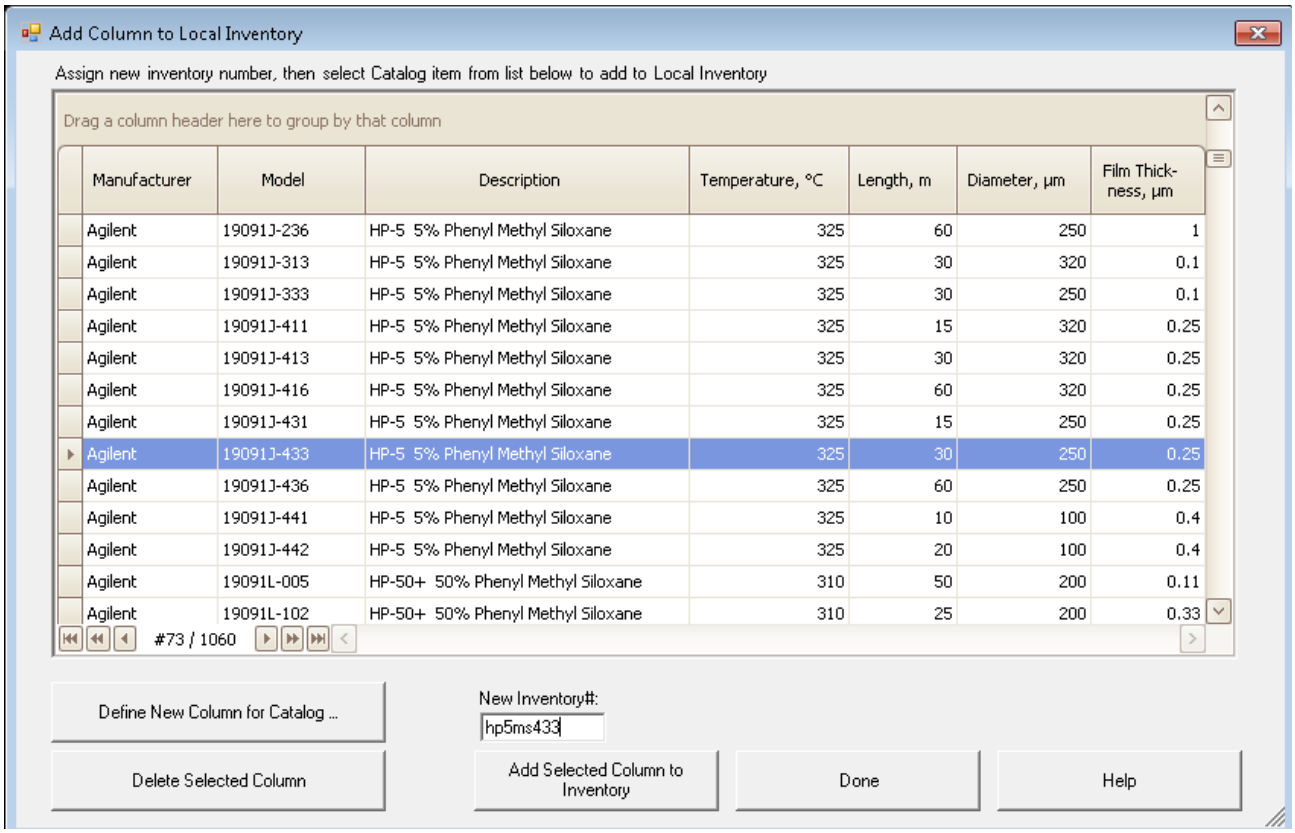


Figure 24 The catalog of columns

4 Scroll down the list of columns to model number **19091J-433** and enter `hp5ms433` as the **New Inventory#**.

5 Click **Add Selected Column to Inventory** to display the **Install Column 1** dialog box with the selected column now added to the local inventory list. .

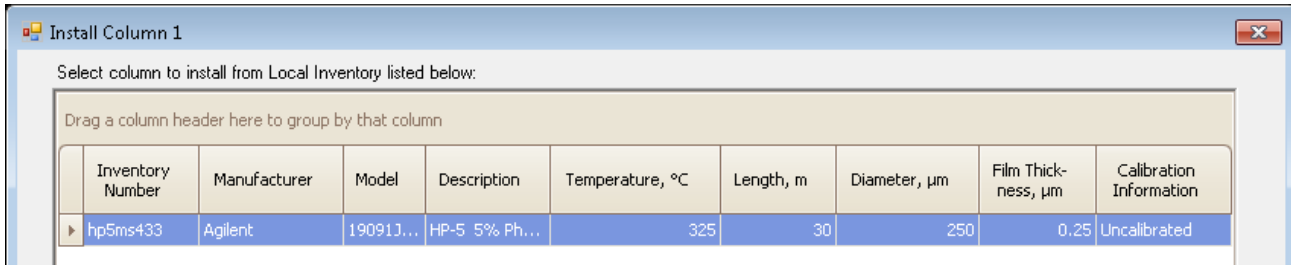


Figure 25 Local inventory with the added column

Columns added to local inventory can be quickly added and configured for the instrument. See "Select and configure a column" on page 51.

Select and configure a column

This example selects a column previously added to local column inventory and configures it as column number 1. See "Add a column to ChemStation local inventory" on page 48.

1 Select the **Configuration** icon and click on the **Column** description for column 1 to select it. The column number selected here will be replaced with the column we are adding.

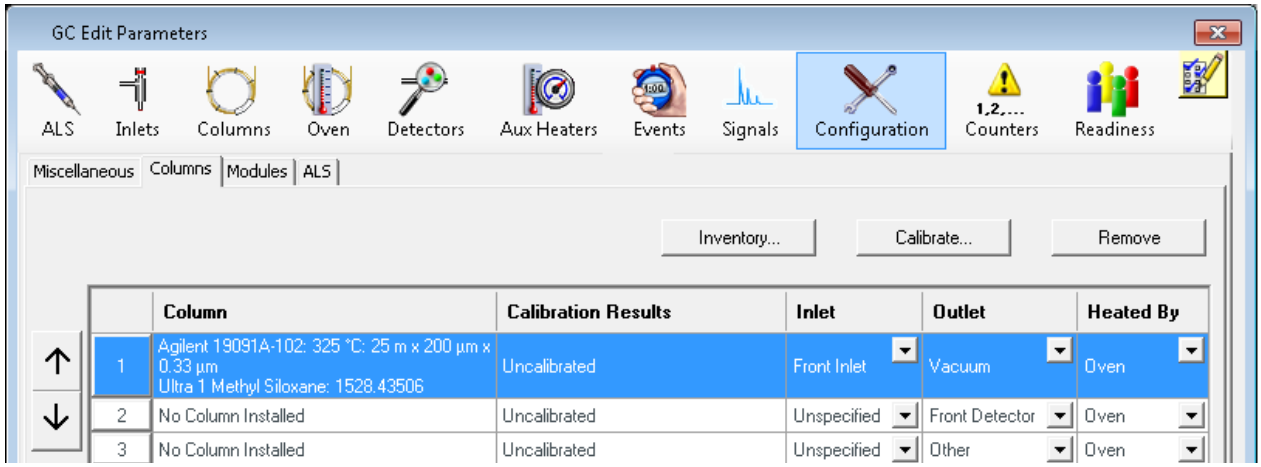


Figure 26 Columns configured for the instrument

2 Click **Inventory** to display the **Install Column 1** dialog box containing a list of columns in local inventory.

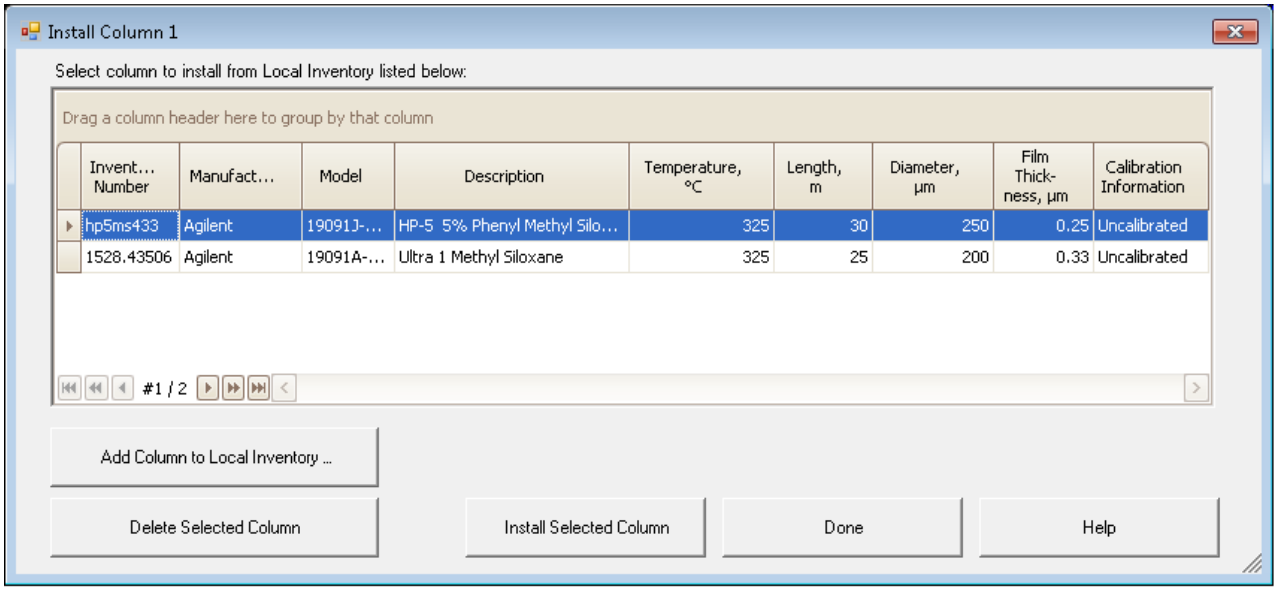


Figure 27 The local inventory of columns

3 Select a column from the local inventory list and click **Install Selected Column** to display the **Configuration** panel for **Edit GC Parameters** with the selected column replacing the previously configured column 1 for the instrument.

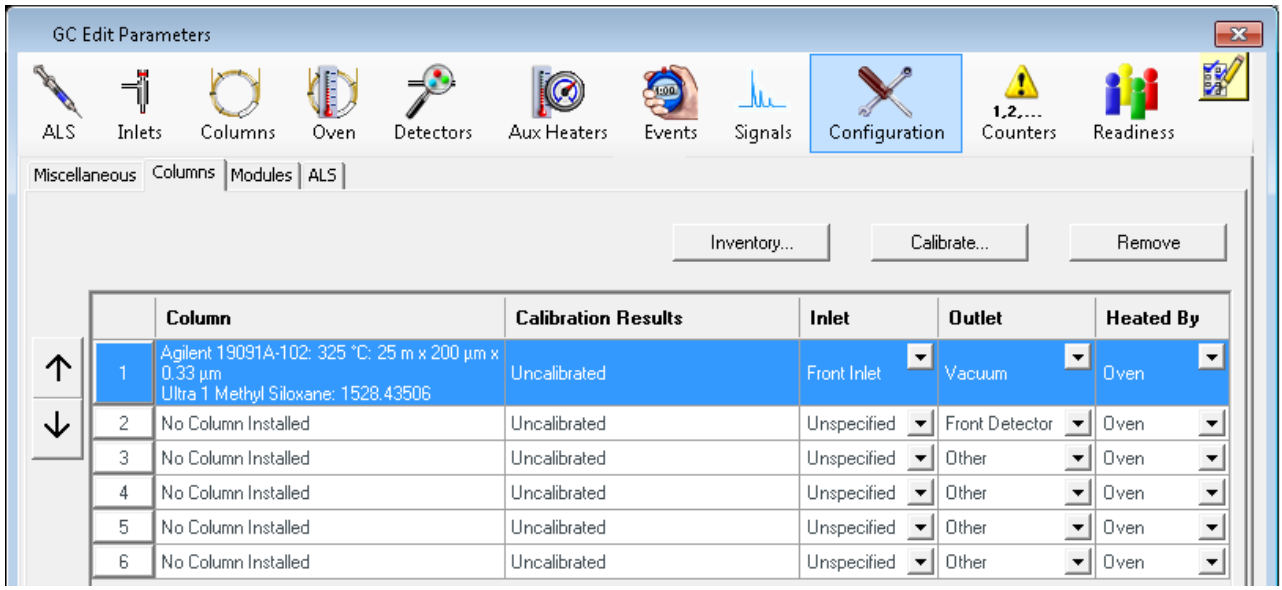


Figure 28 Columns configured for the instrument

4 Under the **Inlet** heading dropdown, select the item the column inlet is attached to.

5 Under the **Outlet** heading dropdown, select the item the column outlet is attached to. For an MS select **Vacuum**.

6 Under the **Heated By** heading dropdown, select the method for controlling the column temperature.

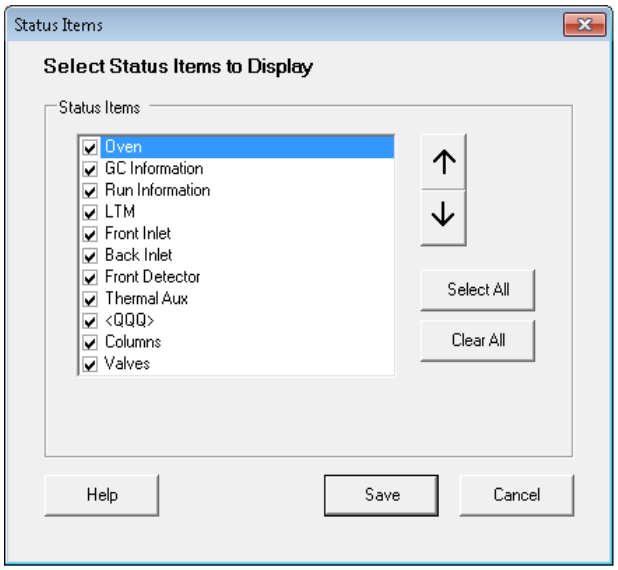
Upload parameters from the 7890A GC

1 On the **Instrument > GC Edit Parameters** screen, right-click in the blank area.

2 From the shortcut menu, select **Upload Method from GC**.

Customize the status panel view

1 In the status panel, select the **Setup Actuals** button, the **Status Items** dialog box opens.



2 Mark the checkboxes of the items in the **Status Item** list that you want to have displayed in the status panel.

3 To move an item up or down in the displayed list, select the item and then the up or down arrow buttons until it is in the desired position.

4 Select **Save** to save the settings and return to the **GC Edit Parameters** window.

4
Run the Scan Method

In this chapter, a sample is prepared for data acquisition and the ALS is loaded with the sample, the solvent wash vial, and a solvent waste vial. The single sample is run and during the data acquisition a snapshot is taken to demonstrate how it is possible to look at partial analysis results before a run is completed. Finally, the logbook showing actions taken during the run is reviewed.

Prepare the Sample

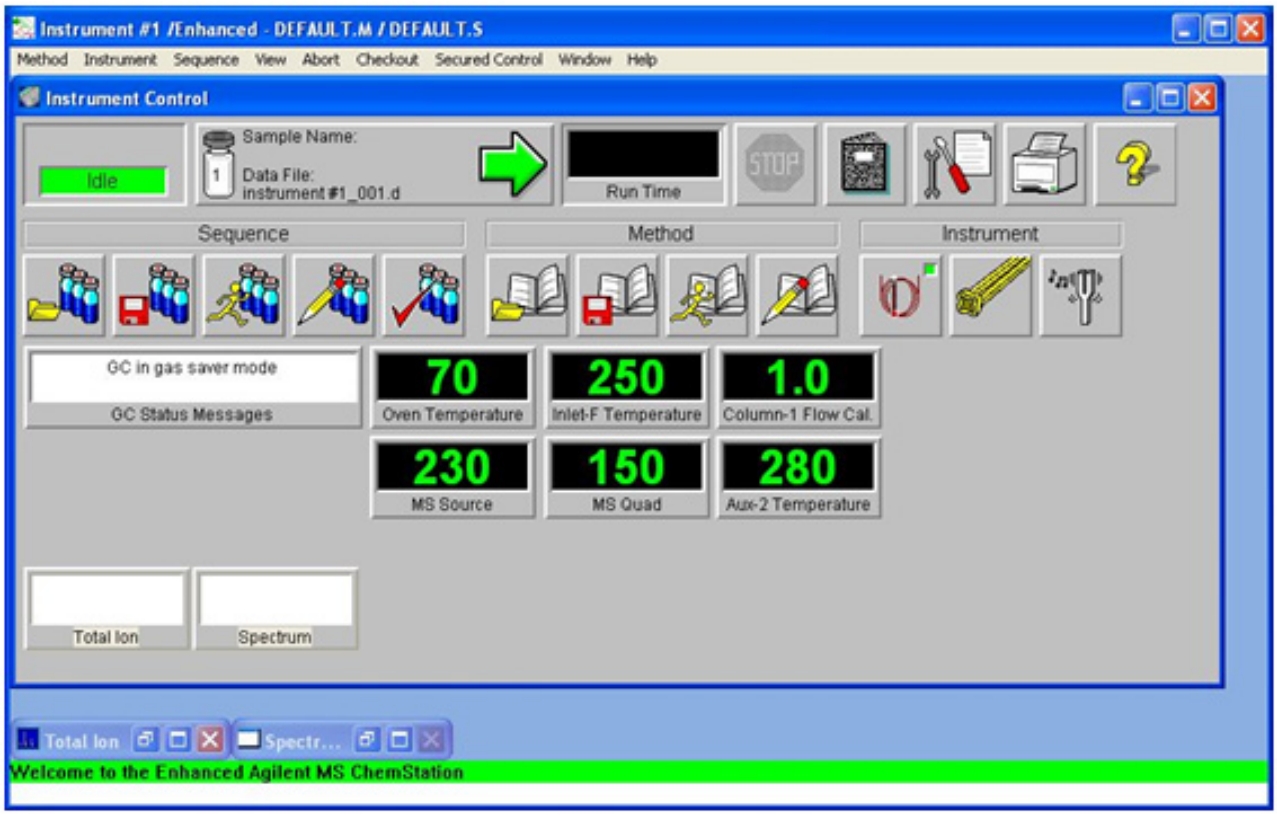
1. Fill a sample vial with the contents of the 10 ng/mL 5975 MSD Sample (P/N 05970-60045 or P/N 5074-3025 Japan only) and cap the vial.

   If you are not using an ALS skip the remaining steps.

2. Place the sample vial into position 1 of the GC sample tray.
3. Fill a solvent wash vial with isooctane and place it in injector turret location A for solvent wash mode A, B.
4. Place a waste vial in turret location B specified for solvent wash mode A, B.

Load the Method

1 From the PC desktop, select the **ChemStation** shortcut icon, the **Instrument Control** window opens.



2 Select the **Load Method** button,  to open the **Load Method** window. Navigate to and select **demoscan.M**.

3 Select **OK** to load the method and close the dialog box.

Run the Method

1 Select the **Run Method** button,  . The **Start Run** dialog box opens with the **GC ALS**, **Inlet Location**, and **MS Connected to** selections pre-populated.

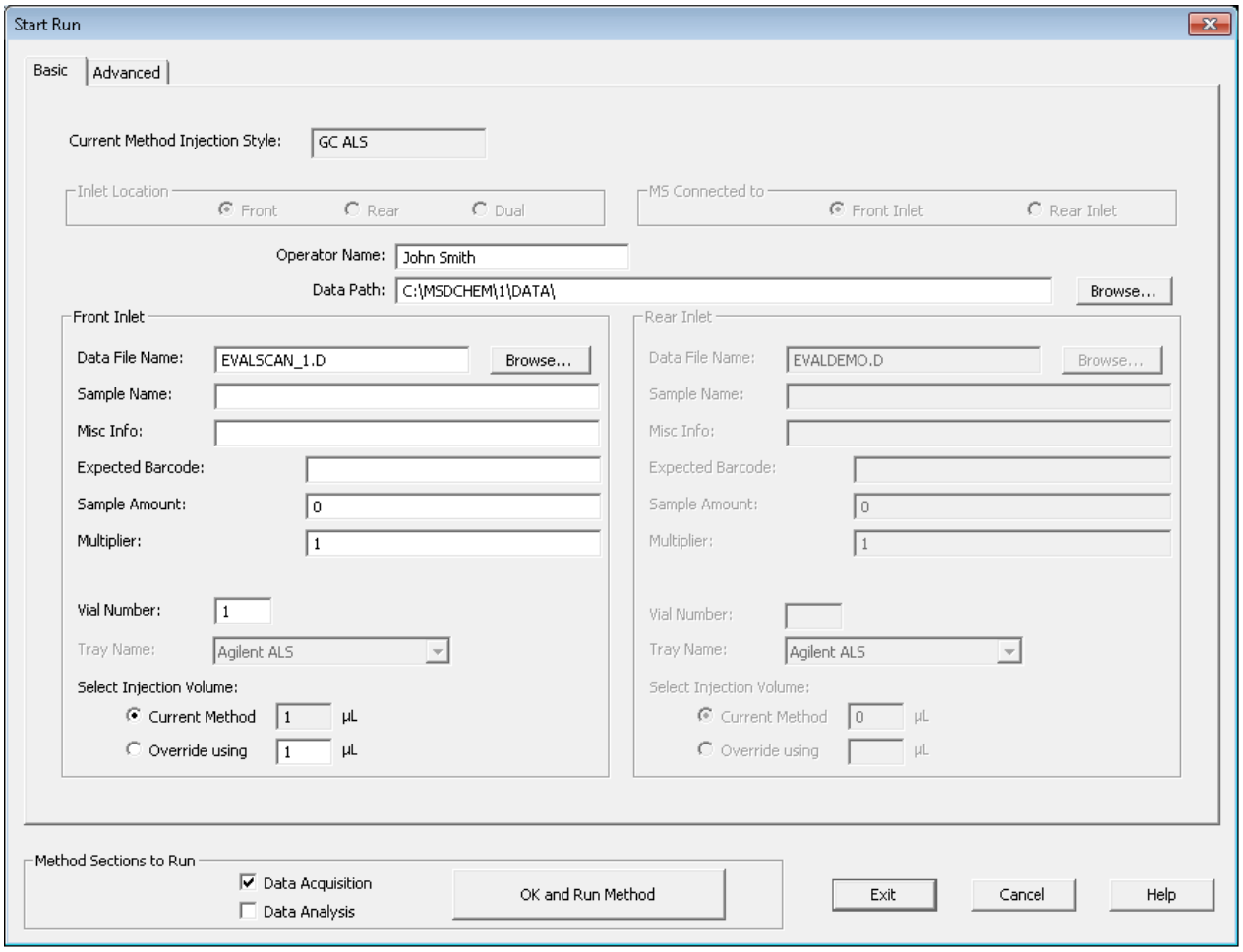


Figure 29 Start a single sample run

2 In the **Operator Name** field, enter your name.

3 In the **Front Inlet** area:

   a In the **Data File Name** field, enter `EVALSCAN_1`.

   b In the **Sample Name** field, enter a name for your sample (optional).

   c In the **Misc Info** field, enter a description of your scan (optional).

   d In the **Expected Barcode** field, enter a barcode (optional).

   e In the **Vial Number** field, enter `1`.

4 In the **Method Selections to Run** area:

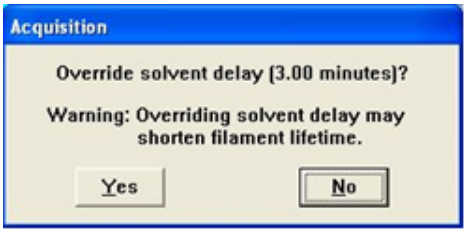
   a Mark the **Data Acquisition** check box.

   b Clear the **Data Analysis** check box.

5 When the instrument is in a ready state as shown by a green **Idle** indicator in the upper left hand corner, select **OK** and **Run Method** to close the dialog box and start the run. The ready state indicator changes to Run. See Figure 30 on page 60.

   If the instrument was not in a ready state, the system will prompt for you to override. When the status is Ready, the dialog box will close automatically.

   During the solvent delay the system will prompt for you to override. When the time is up, the dialog box will close automatically.



6 Observe the TIC real time plot and go to "Take a Snapshot" on page 61 after the second compound elutes.

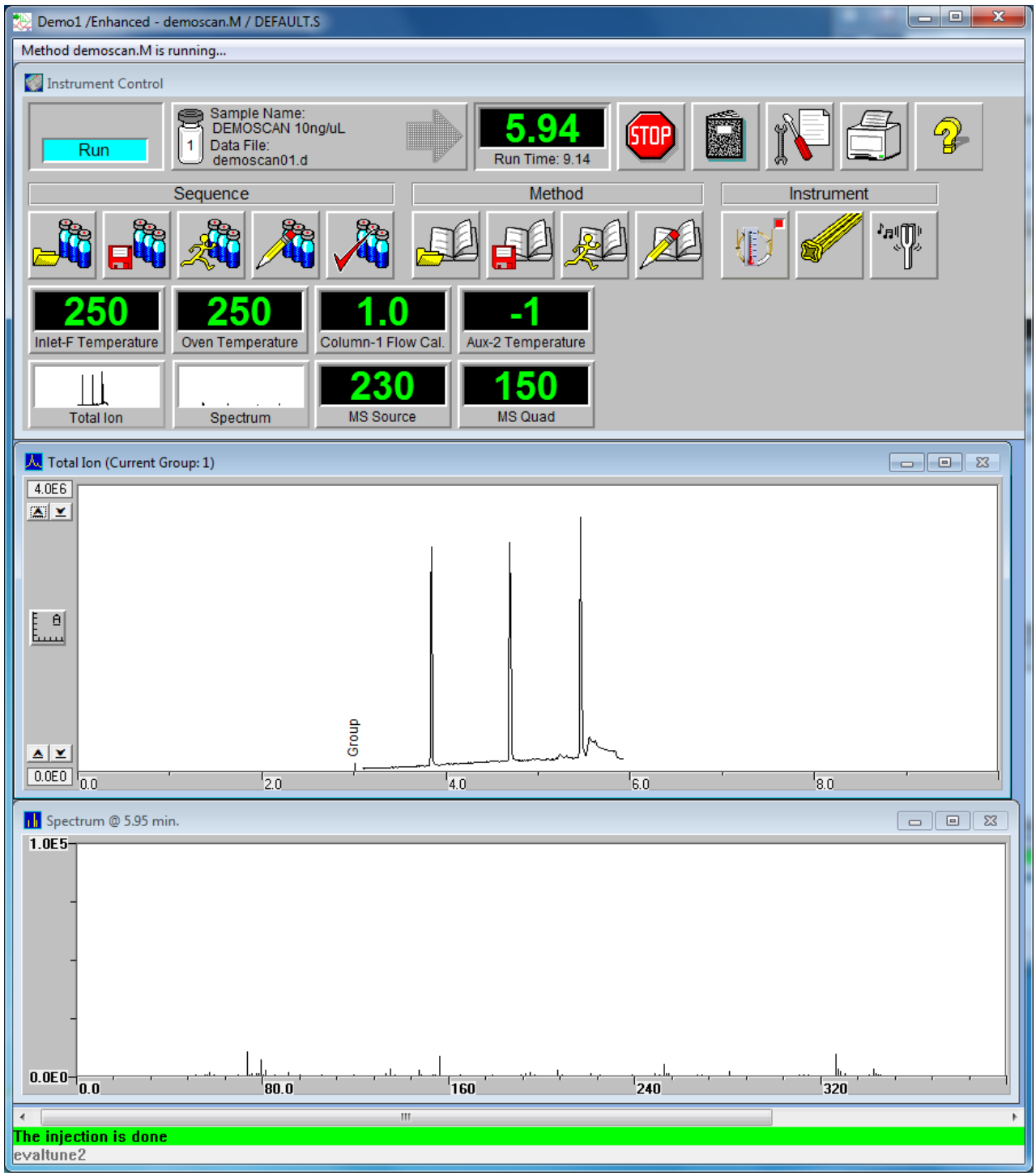


Figure 30 Instrument control window during single sample run

Take a Snapshot

Snapshot is useful when a compound of interest elutes early during a long run and you want to analyze that compound immediately. The system creates a snapshot data file with data that has been acquired up to the time the Snapshot is taken.

1 During the run select **View > Data Analysis** to open the data analysis view.

2 Select **File > Take Snapshot**. The data analysis windows opens displaying the TIC obtained for the run up to this point in time.

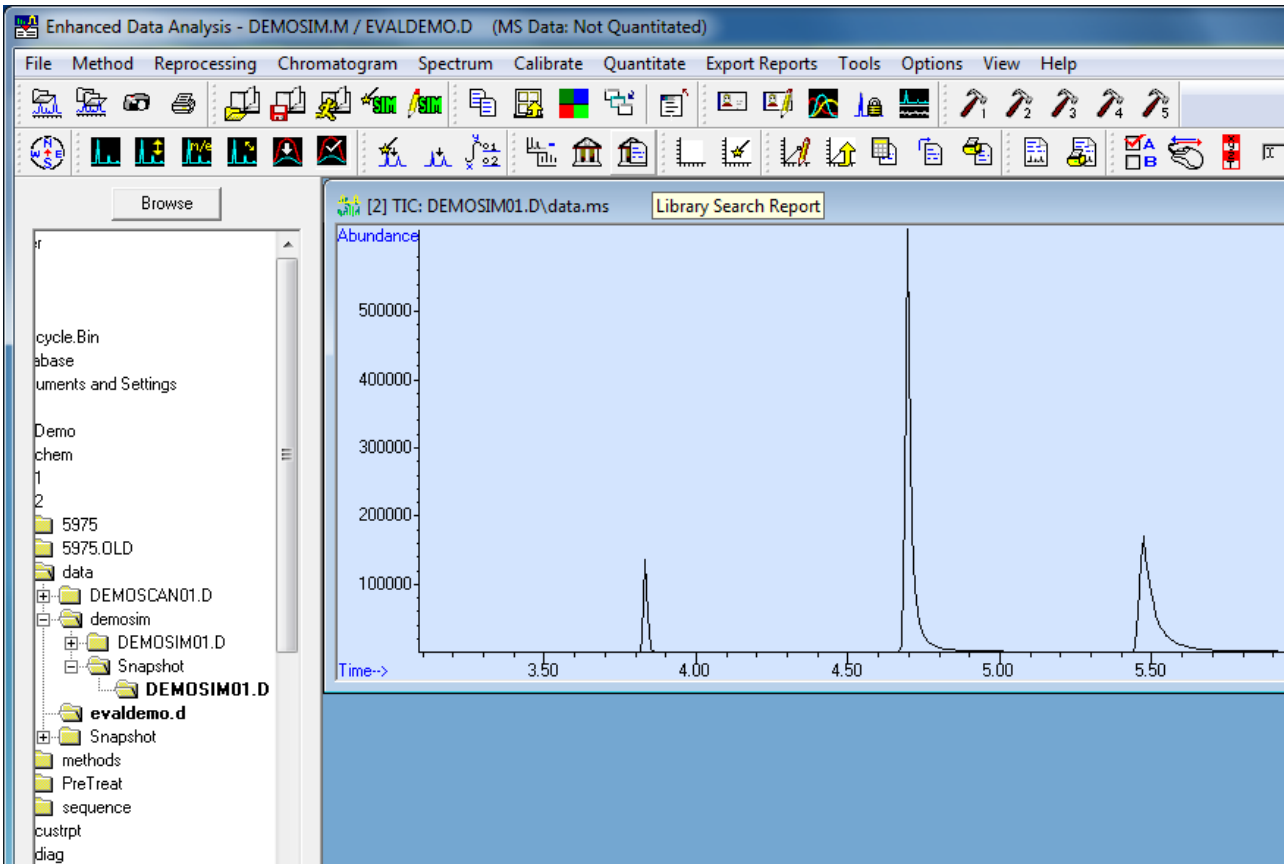


Figure 31 The TIC of the snapshot data file

Observe the location of the snapshot data file in the navigation pane. It is placed in the data directory specified for the run under the snapshot subdirectory and given the same name as the data file specified for the sample.

3 Analyze the compound of interest.

4 Exit data analysis and return to the **Instrument Control** view.

View the Logbook

The system keeps a logbook named MSLOGBK.LOG that records all instrument error and status messages prior to and during acquisition.

The Current Logbook lets you review instrument diagnostic information and any mass spec malfunctions recorded during the current and previous acquisitions. It is located in the instrument directory.

1 Select the **Logbook** button,  . The **Logbook** menu opens.

2 Select **Current Logbook** to display the active log.

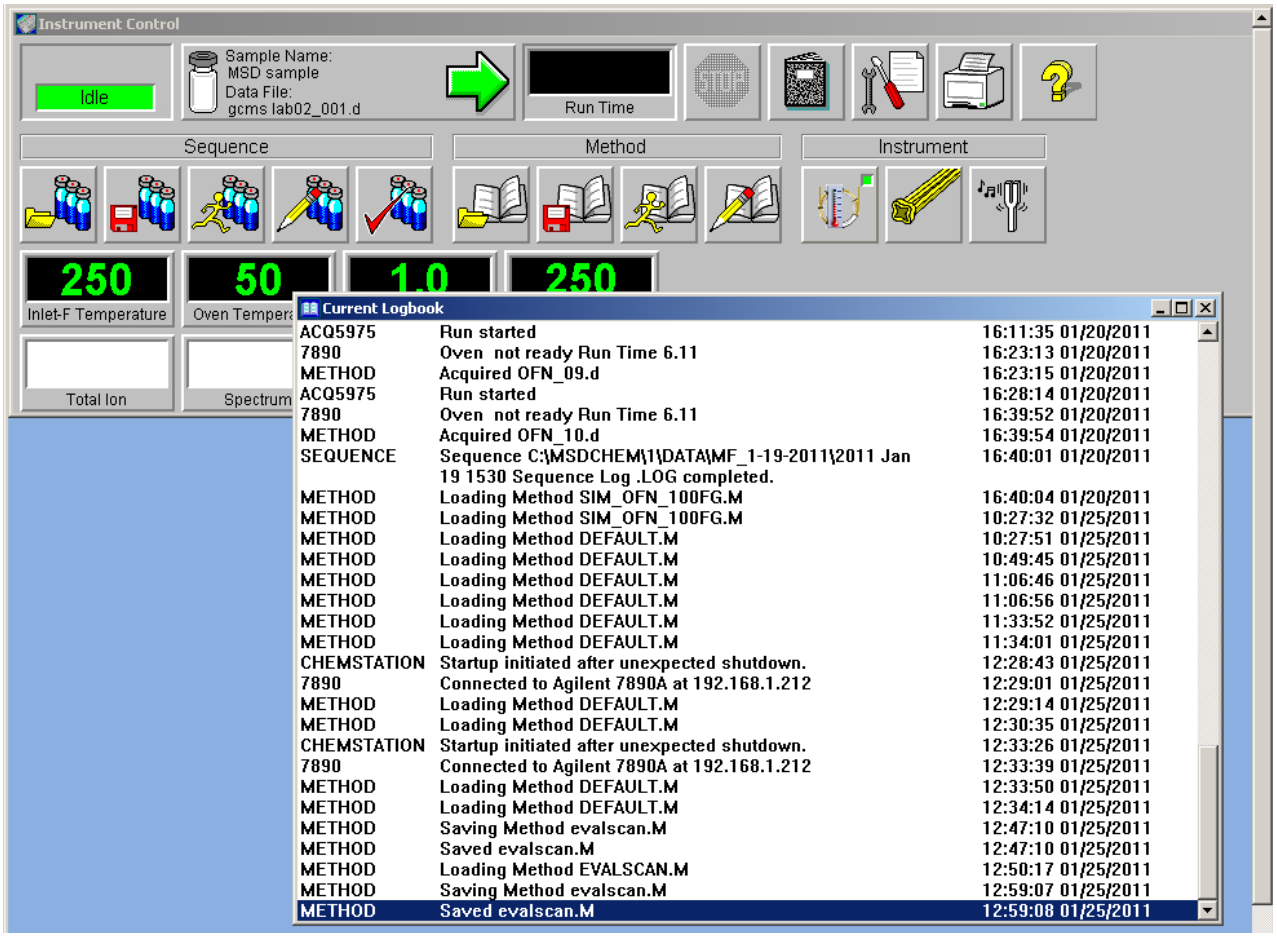


Figure 32 The current logbook is open

3 With the logbook open, select the **Logbook** button again and then from the menu select:

- **Open Logbook** to select a a logbook to open from a list of all logbooks in the instrument directory.
- **Clear Logbook** to delete the currently displayed logbook.
- **Save As Logbook** to save the displayed logbook into a new file.
- **Print Logbook** to print the displayed logbook.

4 Exit the Instrument Control program.

5
Qualitative Data Analysis

Qualitative data analysis identifies the compounds in your sample by:

- Integrating the peaks in your acquisition scan data
- Identifying the ions in the spectra from those peaks
- Comparing the ions from the peaks it found to ions in a library of known compounds, stored on your system
- Reporting the identity of the compound(s) found for each peak

This chapter reviews each of these processes.

Integrate Peaks

Integration is a tool for finding the peaks in a chromatogram and determining their size. In qualitative analysis integration is required for producing a percent report, doing a library search on integrated peaks, and producing a library search report.

1 Start the data analysis program using the desktop Data Analysis icon,  .

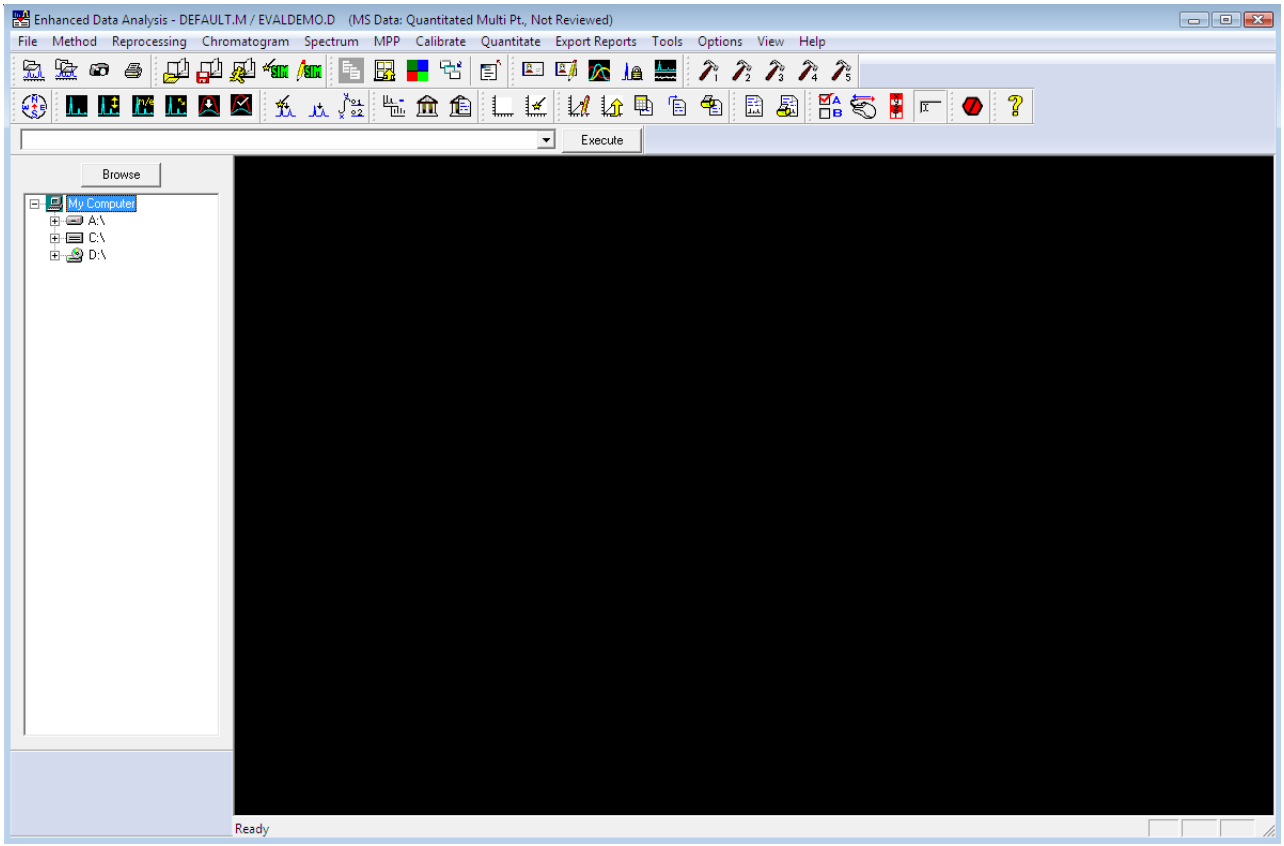
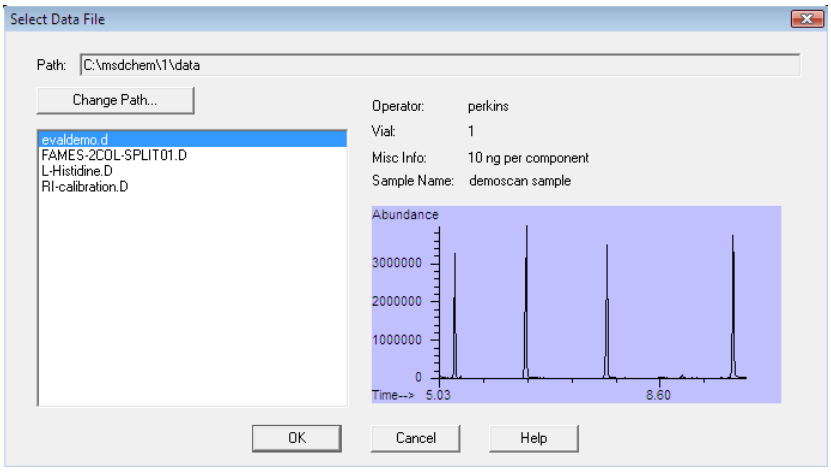
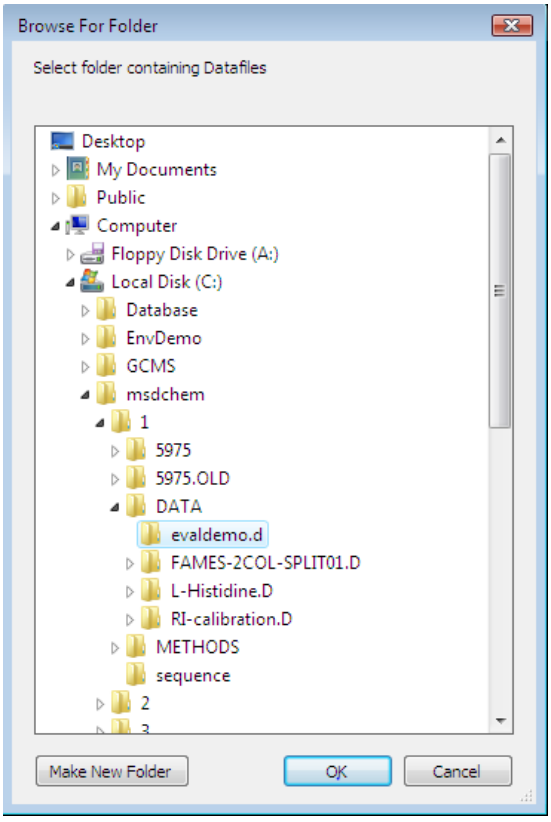


Figure 33 The initial data analysis window

2 Select the **Load Data File** button,  . The **Select Data File** dialog box is displayed.



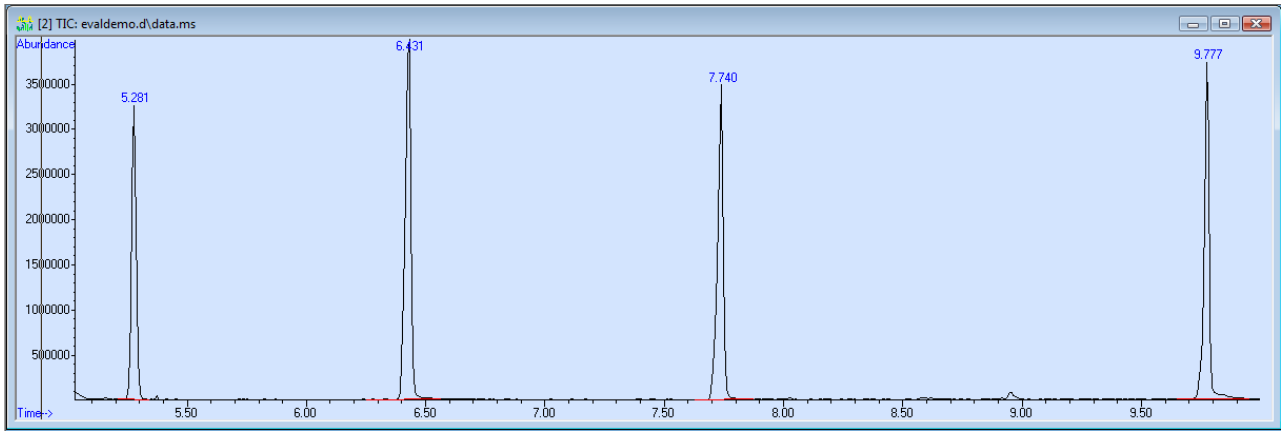
3 Select **Change Path**. The **Browse for Folder** dialog box opens.



4 Navigate to **evaldemo.d**. This is the data file from the scan analysis of our sample.

5 Select **OK**.

6 In the **Select Data File** dialog box, select **OK**. The data file is loaded and the total ion chromatogram (TIC) is displayed.

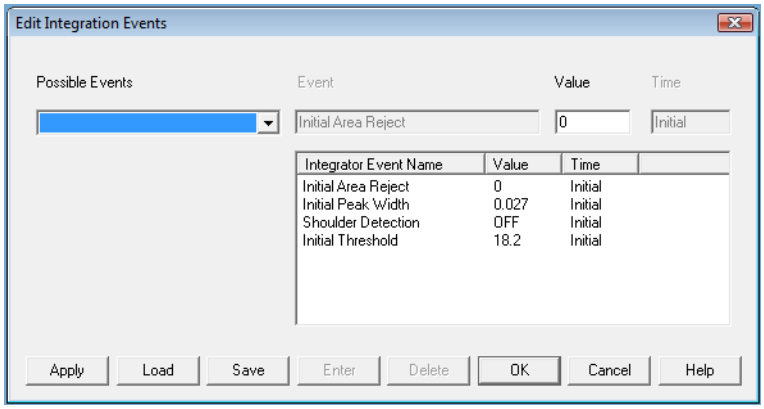


Edit the integration events

When the data analysis part of your method is run, the chromatogram is integrated using autointegrate. Most of the chromatogram can be successfully integrated by using the ChemStation default auto integration parameters. However, you can customize the auto integration parameters and add integration events for your specific chromatograms. These events are saved and used when your method is run.

1 Select the **Integration Parameters** button,  . The **Edit Integration Events** dialog box opens.

This example assumes the **ChemStation Integrator** is the specified integrator



2 To change **Initial Area Reject**, **Initial Peak Width**, or **Initial Threshold**:

- **a** Select the parameter you wish to change in the **Integrator Event Name** list. The parameter is displayed in the **Event** field and the current value is displayed in the **Value** field.
- **b** Enter the custom value in the **Value** field.
- **c** Select **Enter**. The custom value is now listed in the **Value** list.

3 To change **Shoulder Detection**:

- **a** Select **Shoulder Detection** in the **Integrator Event Name** list. The parameter is displayed in the **Event** field and the current setting is displayed in the **Value** field.
- **b** Select the **Value** field. An **Edit Integration Events** confirmation message appears.
- **c** Select **Yes** to change the setting.

4 To add integration events:

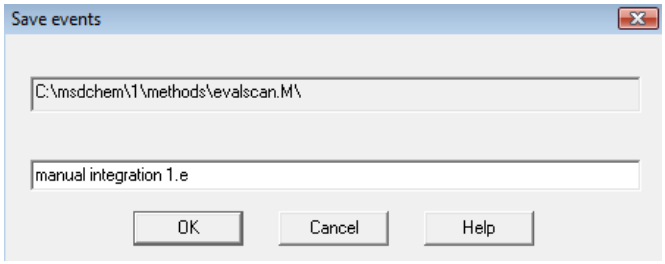
   a From the **Possible Events** drop down list, select the event to add to your integration.

   b Enter the required information in the **Value** or **Time** fields.

   c Select **Enter**. The event and value or time is now listed in the **Integrator Event Name**, **Value**, **Time** list.

5 Select **Apply** to view the results in the **TIC** window.

6 Select **Save** to save the auto integration parameters. The **Save Events** dialog box opens.

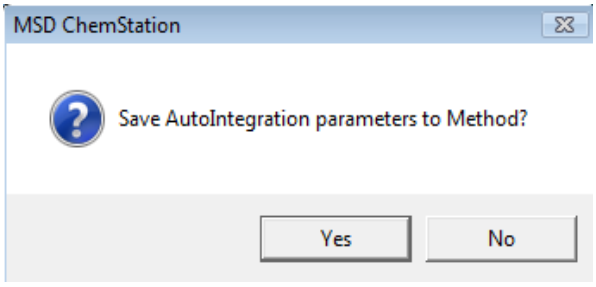


7 Enter a file name.

8 Select **OK** to close the **Edit Integration Event**s dialog box. The results are displayed in the **TIC** window.

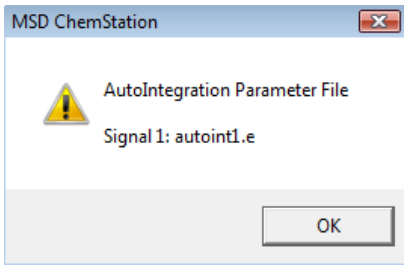
Save the integration events to the method

1 Select the **AutoIntegrate** button,  . The integration results appear in the **TIC** window (Figure 34) and a confirmation message appears.



2 Select **Yes** to save the integration or **No** to continue without saving this integration to the method.

If you selected **Yes**, a confirmation message appears displaying the saved auto integration parameter file name. Select **OK** to save the integration to the method.



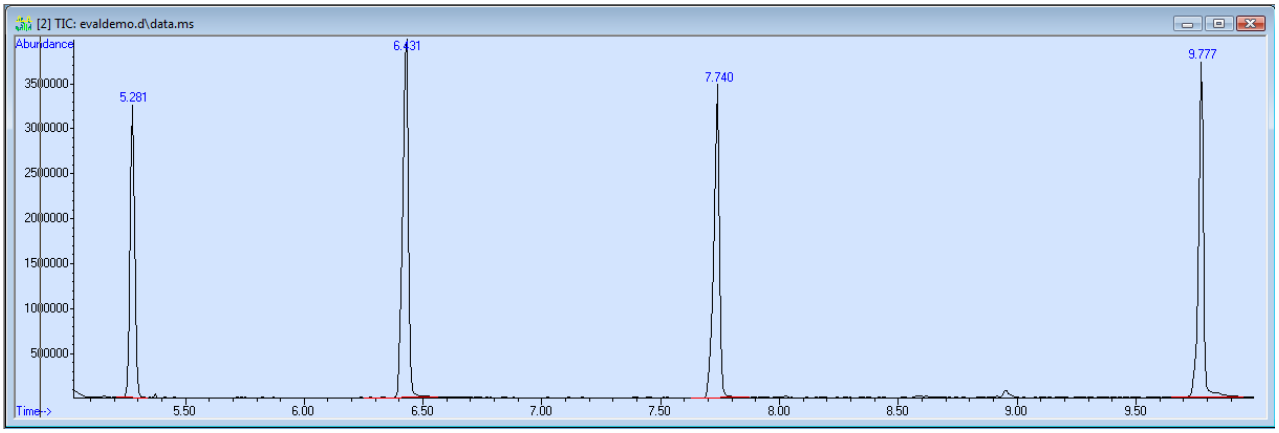
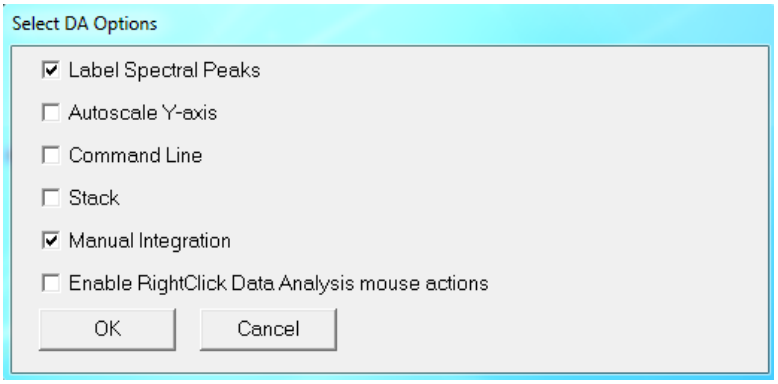


Figure 34 Integrated chromatogram

Manually integrate peaks

1 If required, "Edit the integration events" or load a saved integration events file.

2 Select **Tools > Options** to display the **Select DA Options** dialog box.



3 Select **Manual Integration** to turn it on and click **OK**. The mouse cursor changes to a crosshair in the **TIC** window.

4 Right-click the TIC to display a context menu. Select **Enable standard Data Analysis mouse actions** from the menu.

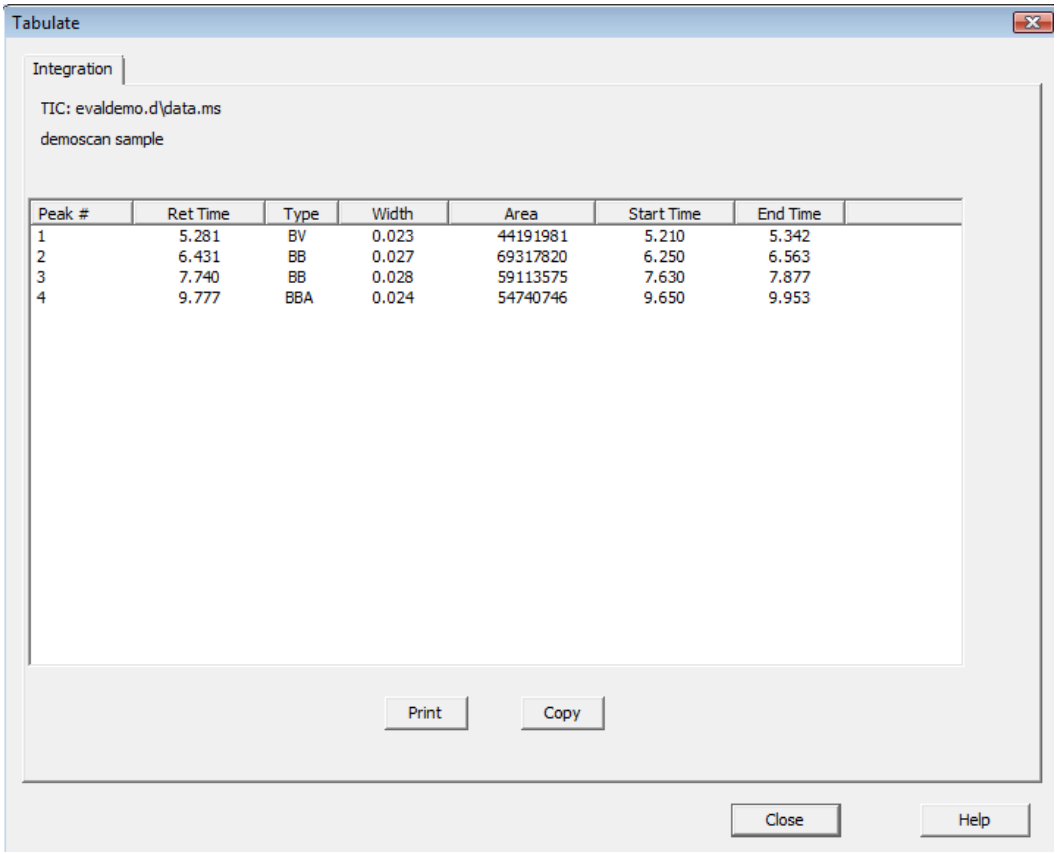
5 Click and drag the left mouse button to zoom in on the peak of interest in the chromatogram.

6 Click and drag the right mouse button to draw an integration baseline on the peak. When you release the button, the peak will be integrated, using the integrator you have selected.

If you want to delete an integrated peak, put the cursor on it and double-click the right mouse button.

View the integration results in a table

1 Select **Chromatogram > Integrate Results...**. The **Tabulate** window opens and lists the results.



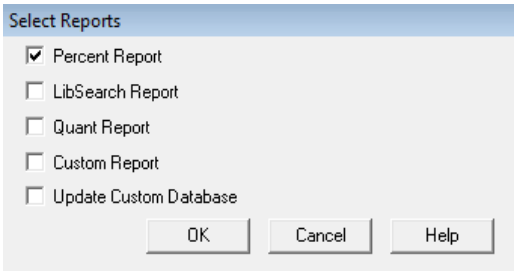
2 To print the integration table, select **Print** and navigate to your printer.

3 To copy the table to your clipboard for use in another application, such as MS Excel, select **Copy**.

4 Select **Close** to close the dialog box.

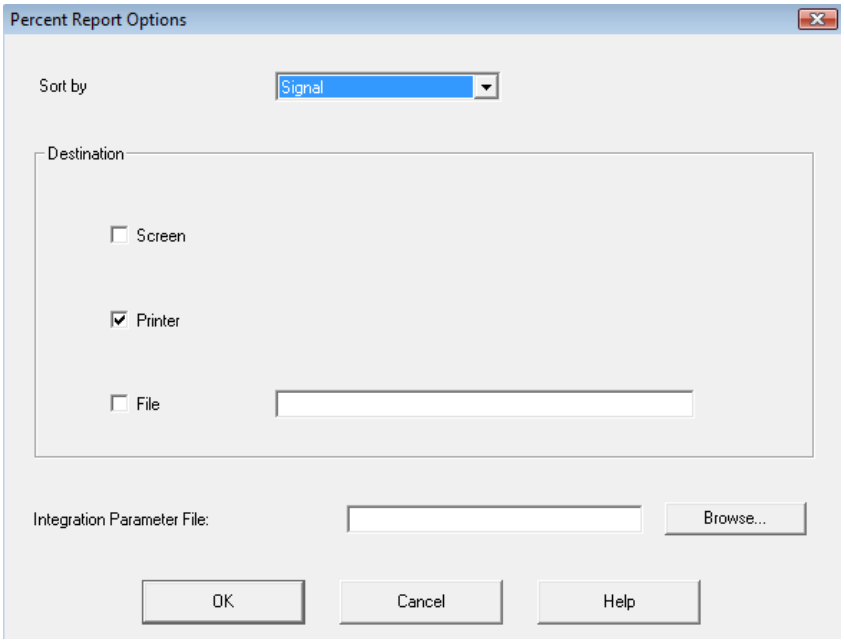
Edit the Method to Generate a Report

1 Select **Method > Edit Method**. The **Select Reports** dialog box opens.



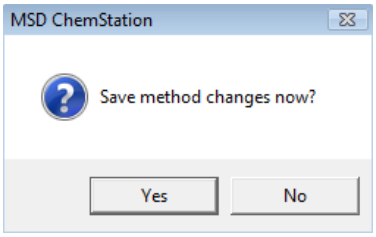
2 Check **Percent Report** and **OK.** Other report types can also be selected.

The **Percent Report Options** dialog box opens.

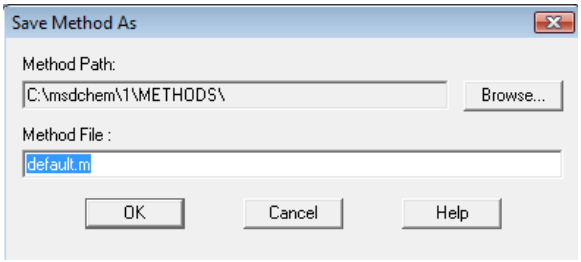


3 In the **Destination** pane, check where you want the report to be generated.

4 Select **OK**. A confirmation message appears.

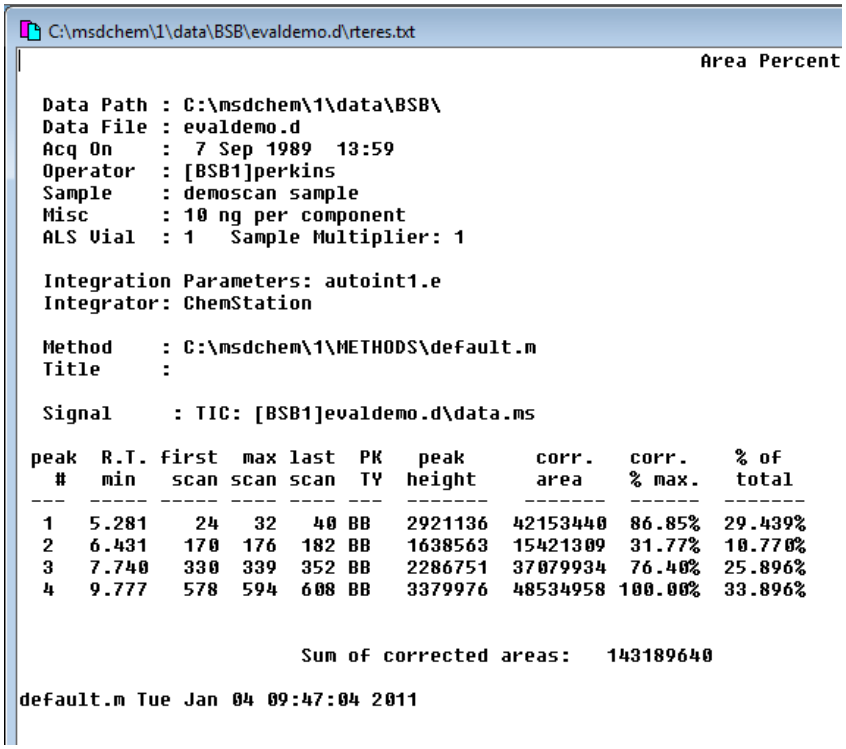


5 Select **Yes**. The **Save Method As** dialog box opens.



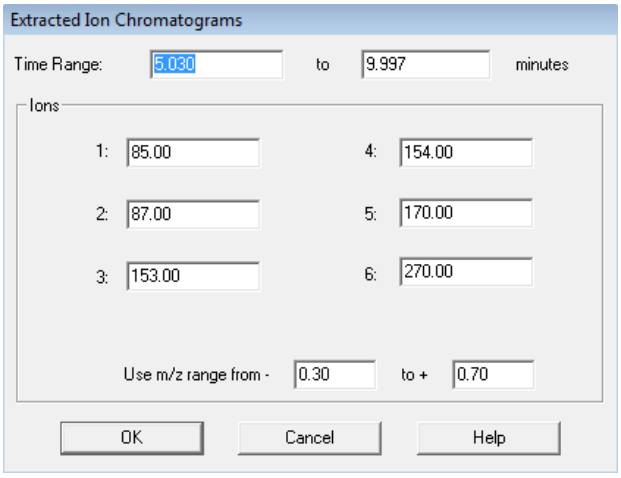
6 Select **OK** to save the setting to the current method.

7 To interactively generate a report, select **Chromatography > Percent Report**. The report is displayed in a new window.



Display Extracted Ion Chromatograms (EIC)s

1 Select the **Ion Chromatograms** button  . The **Extracted Ion Chromatograms** dialog box opens.

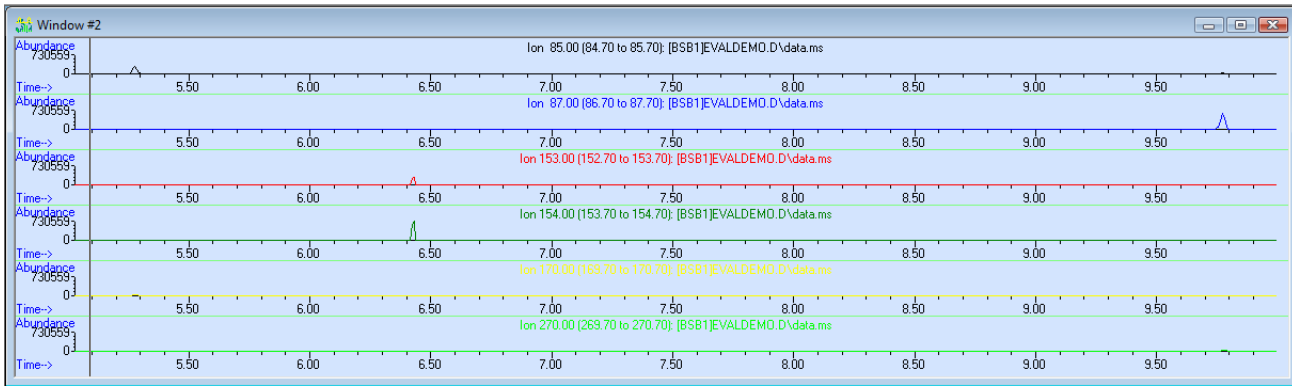


2 In the **Time Range** fields, enter the range you wish to extract. The complete time range of the data file is initially displayed. You can specify a shorter time range by entering the appropriate starting and ending values.

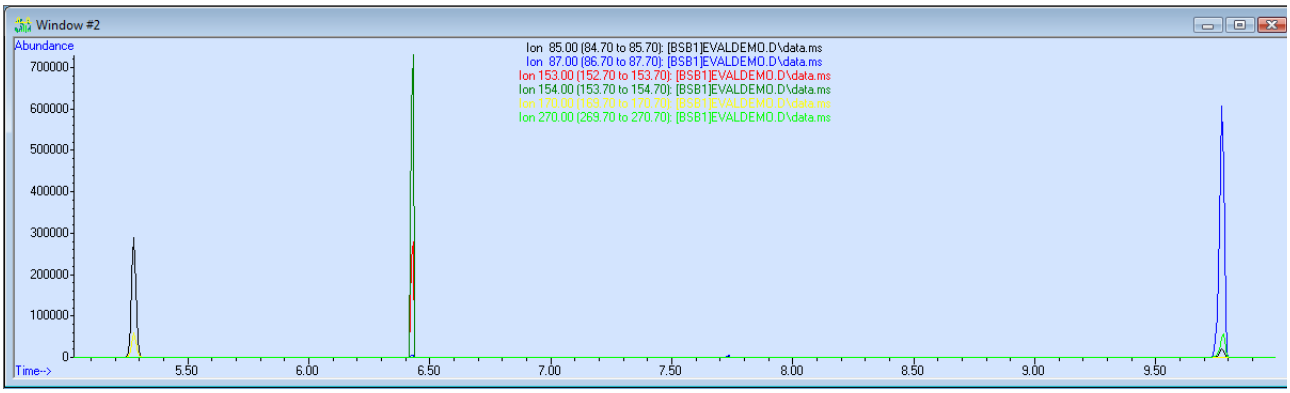
3 In the **Ions** area, enter the ion masses of interest. You can specify up to six ions.

4 In the **Use *m/z* range from** fields, enter the range of interest. The default *m/z* range for each ion is -0.3 to +0.7 of the ion mass specified. You can change the range by entering the appropriate starting and ending values.

5 Select **OK**. A window opens displaying a chromatogram for each ion.



6 Select the **Merged Format** button  to toggle from a chromatogram that displays the ions separately to one that displays the ions superimposed.



Enable or Disable the Right Mouse Click Context Menu

A right mouse click context menu can be enabled to allow you easy access to common data analysis tasks directly from a chromatogram or spectrum window rather than from using the main menu or toolbar buttons.

Selecting the **Switch Data Analysis Mouse Actions** button from the toolbar toggles between enabling and disabling the context menu. When the enhanced data analysis context menu is enabled, the standard right button mouse actions are disabled. The enabled context menu is shown in Figure 35.

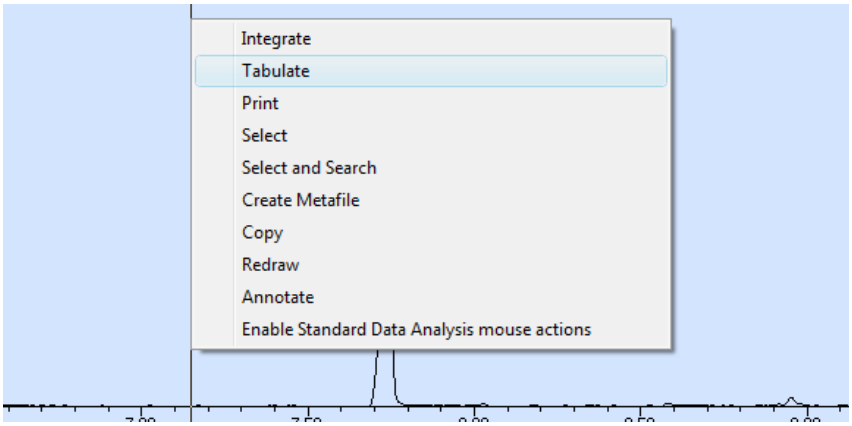


Figure 35 Right mouse click context menu

Certain mouse actions like averaging peak spectra and manually editing the baseline of a peak require the standard mouse actions.

Analyze Data

To perform these actions you must be using the standard mouse actions. See the preceding topic for details.

1 Enlarge the first peak using a left mouse click and drag to create a rectangle around the peak. The chromatogram is enlarged for the selected area. This is the peak for the compound Dodecane.

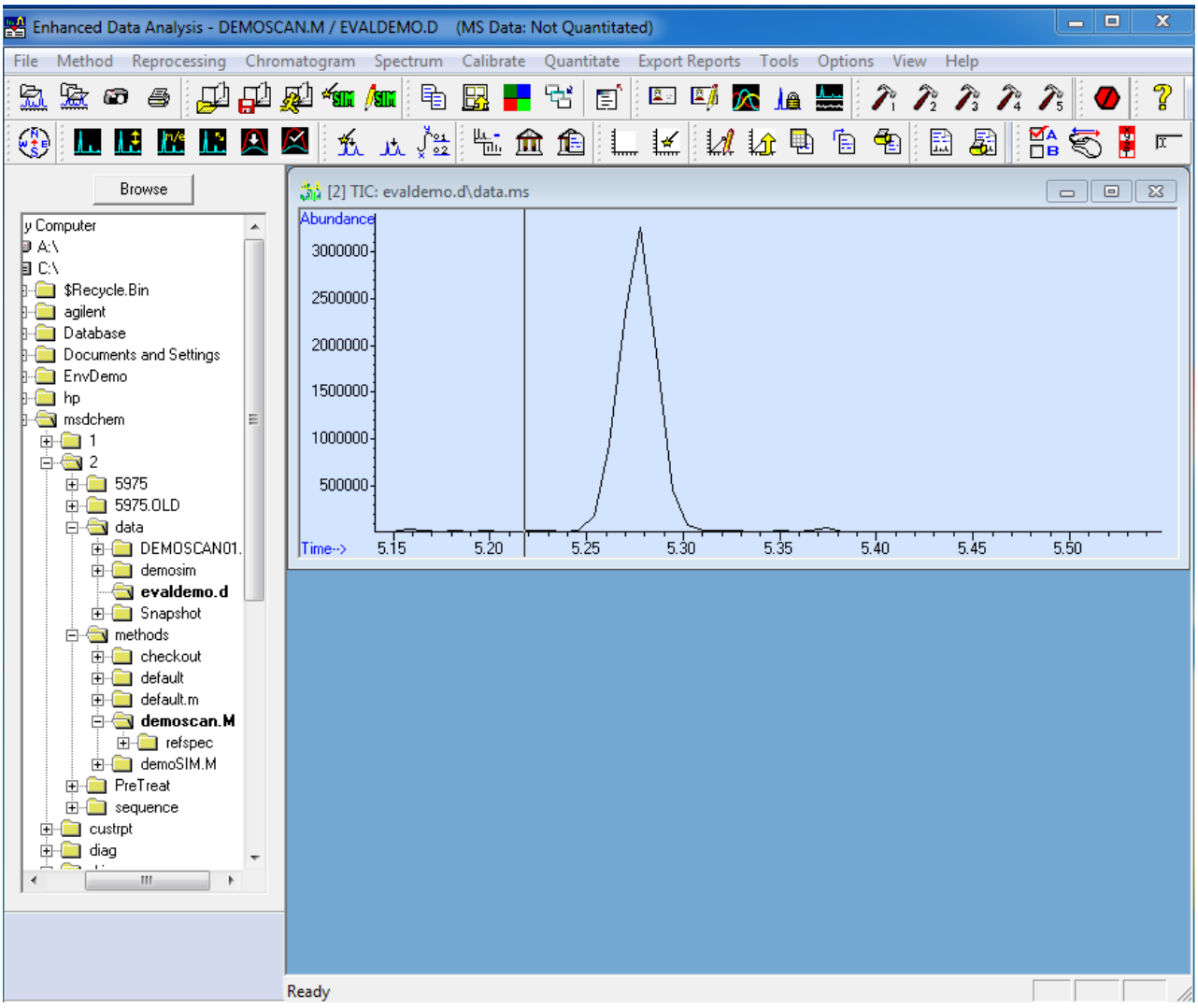
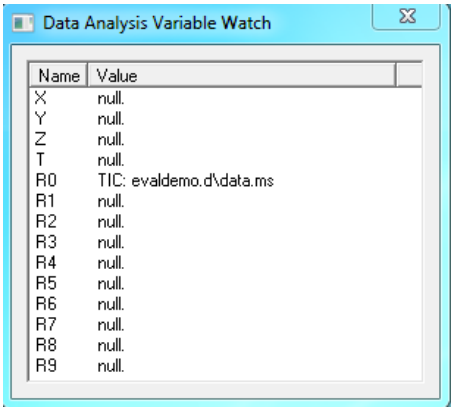


Figure 36 The enlarged peak

2 Enable the stack window:

a From the main menu, select **Tools > Options**.

b In the **Select DA Options** dialog box, check **Stack** and **OK**. The **Data Analysis Variable Watch** window opens.



3 Position the cursor at the highest point of the first peak and double right mouse click to display the spectrum.

You must be using the standard mouse actions.

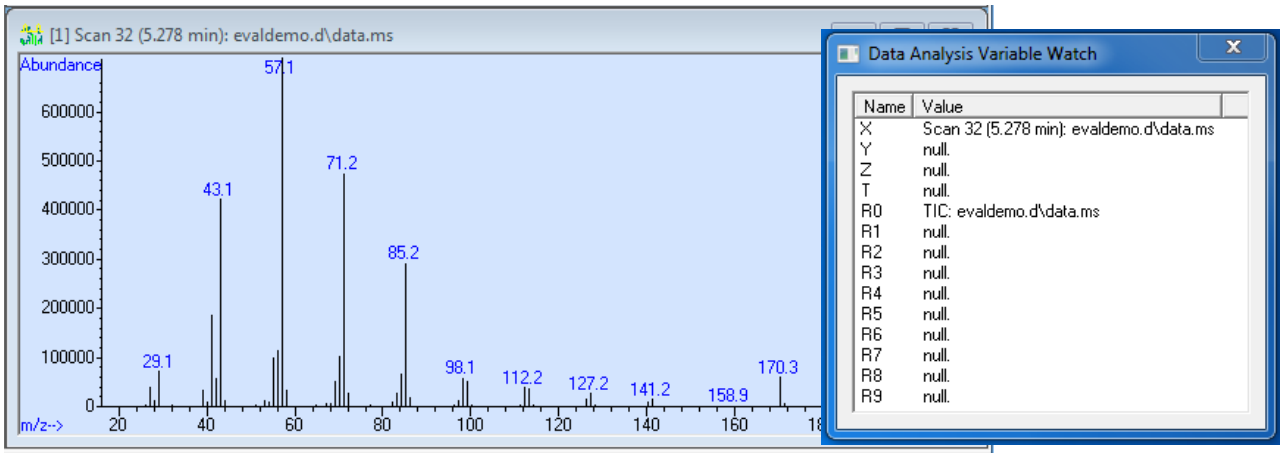


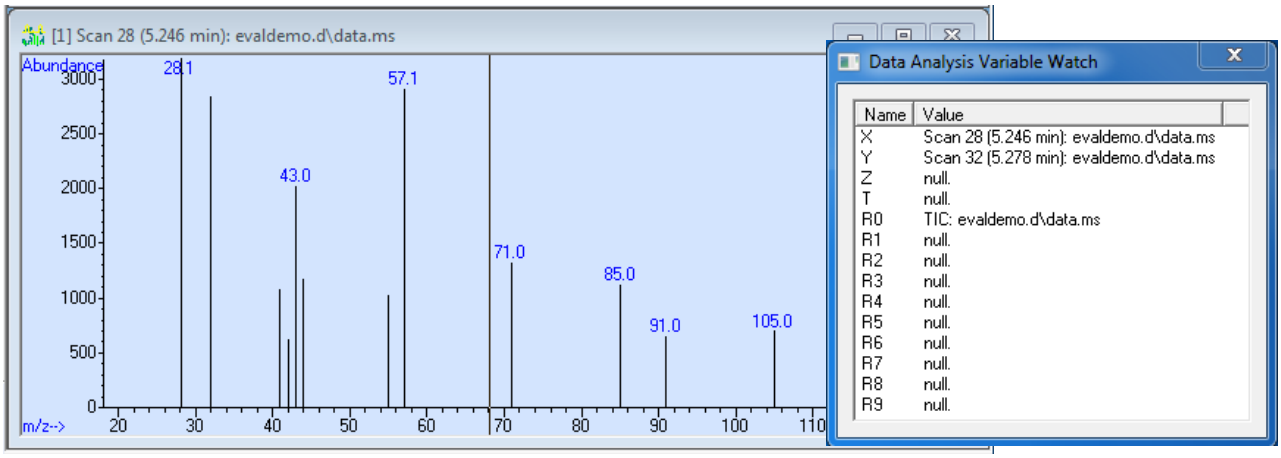
Figure 37 The spectrum at the peak apex

The **Data Analysis Variable Watch Window** now shows the peak spectrum in the **X** register.

Subtract the baseline noise from the spectra

Use spectral subtraction to improve the quality of your spectra by subtracting the baseline signal (noise) from peaks of interest.

1 With the peak apex stored in the Stack X register, position the cursor on the peak at its baseline and double right mouse click. The spectrum is displayed and placed in the **X** register in the **Data Analysis Variable Watch** window. The previous spectrum (peak apex) in the **X** register is moved to the **Y** register.



2 Select the **Subtract** button,  . The difference (Y - X) will be displayed as a spectrum labeled with a [-] following its title. See Figure 38.

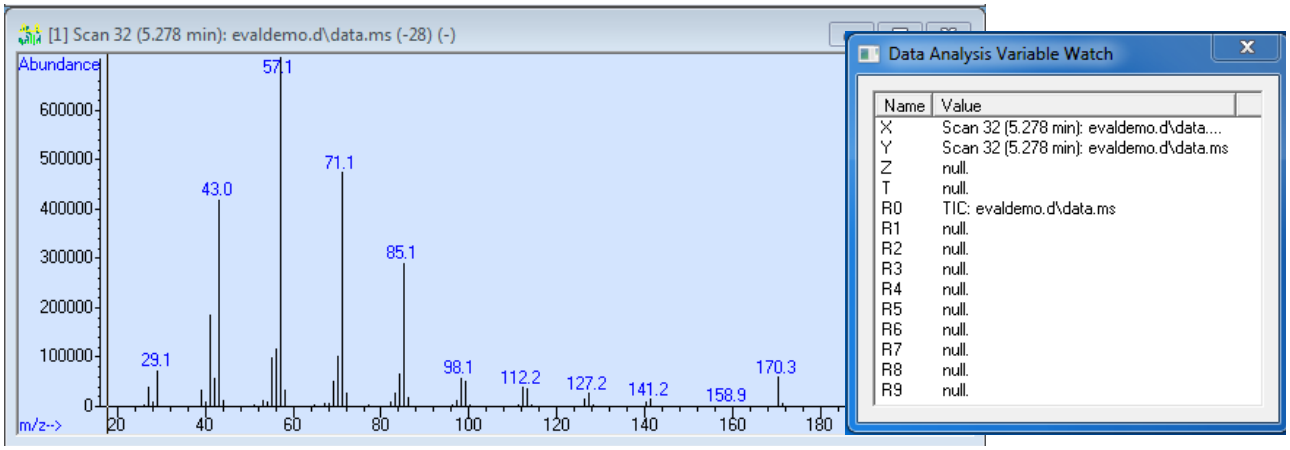


Figure 38 Subtracted spectrum for Dodecane

Select target and qualifier ions

Target ion

One target ion must be selected for each compound to be quantified (target compound). Ideally, the target ion is characteristic of the target compound and distinguishes it from other compounds with similar retention times.

Qualifier ions

Qualifier ions are secondary characteristic ions present in the mass spectrum of the target compound. The presence and correct amounts relative to the target ion of these ions support the identification of the correct target compound

Selection of peak and qualifier ions for Dodecane

Examination of the spectrum for Dodecane in Figure 38 on page 81 shows that Dodecane (mw = 170) molecular ion of 170 is present and will be used as the target ion. The 85 ion at half the mw of Dodecane is also significant and will be used as the qualifier ion.

Selection of peak and qualifier ions for the other compounds

Repeat the procedures under "Analyze Data" on page 79 selecting the other compound peaks in our sample and determining the target and qualifier ions for these compounds. Suggested selections are shown in Table 4 and will be used to set up a SIM acquisition and quantitative analysis later.

Table 4 Target and qualifier ion selections

Compound	Target Ion	Qualifier Ion	Dwell time
Biphenyl	154	153	60
Dodecane	170	85	60
Chlorobiphenyl	188	152	60
Methyl Palmitate	270	87	60

Search the Spectral Library

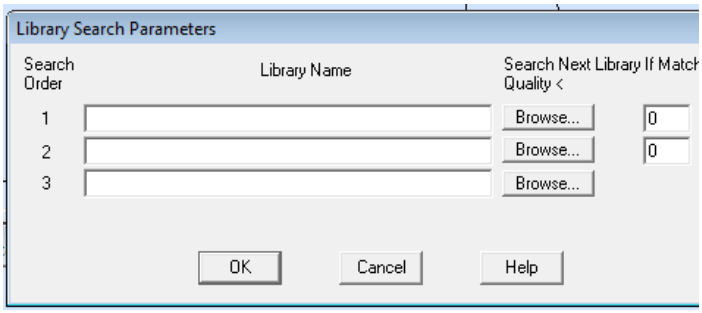
A library search compares the spectrum of an unknown compound against a library of reference spectra. The search identifies those spectra from the reference library that are most similar to the spectrum of the unknown compound.

You can do a search on an individual peak (spectrum) or on all integrated peaks in the TIC.

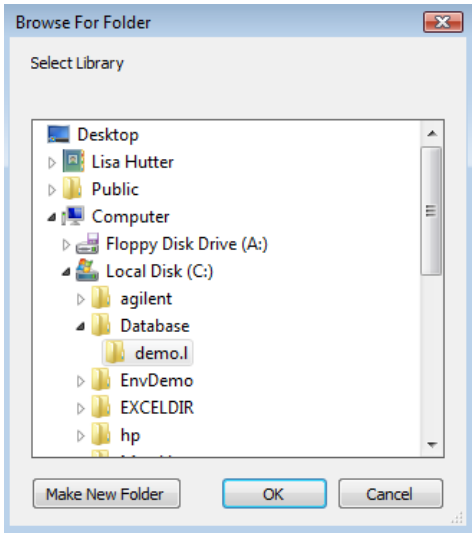
Search for an individual spectrum

1 Select a spectrum to search (**X** in **Data Analysis Variable Watch** window). See Figure 38 on page 81.

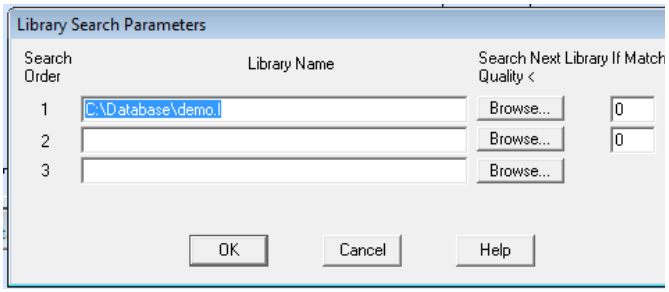
2 Select the **Select Library** button,  . The **Library Search Parameters** dialog box opens.



3 Select **Browse** to open the **Browse for Folder** window. Navigate to the demonstration library **demo.l** and select it.

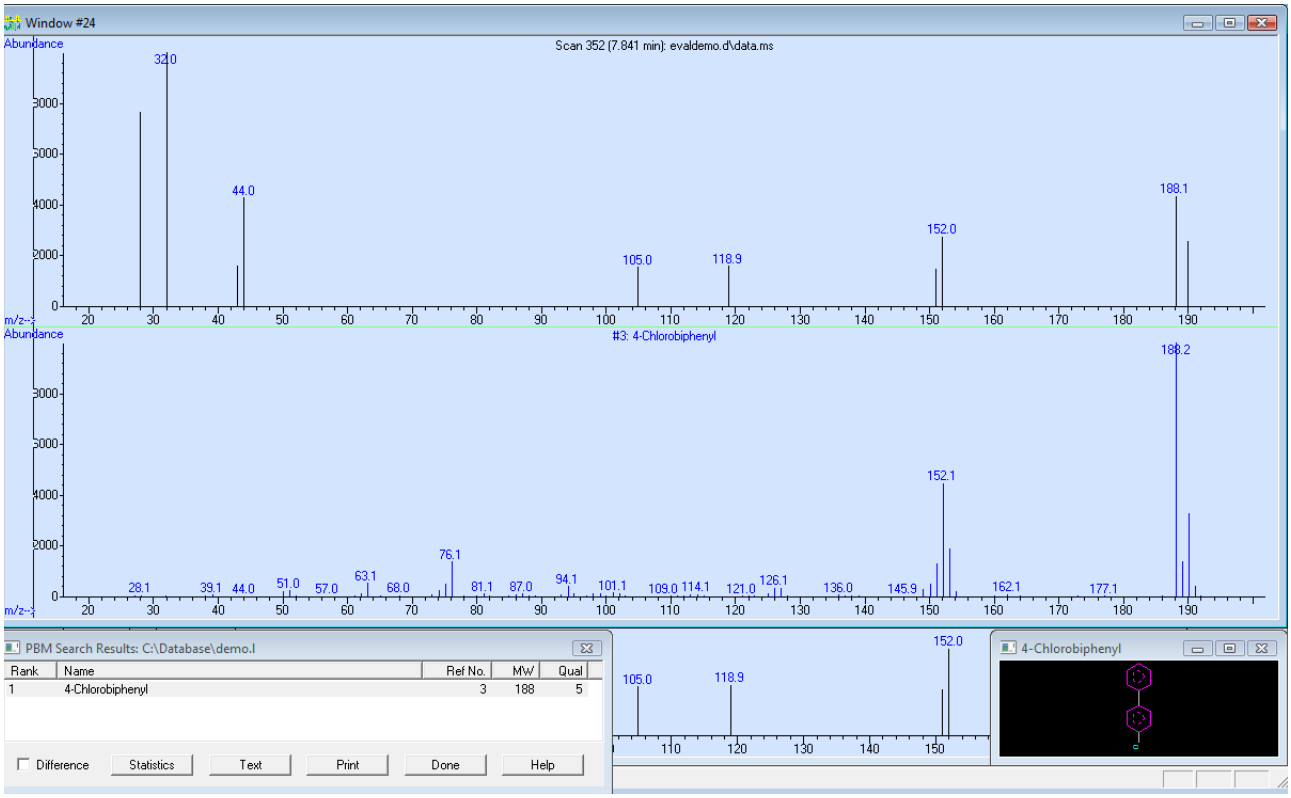


4 Select **OK**. The file path is entered and this library will be searched first. Use positions 2 and 3 to add any additional libraries you have purchased and installed.



5 Select **OK** to save selections.

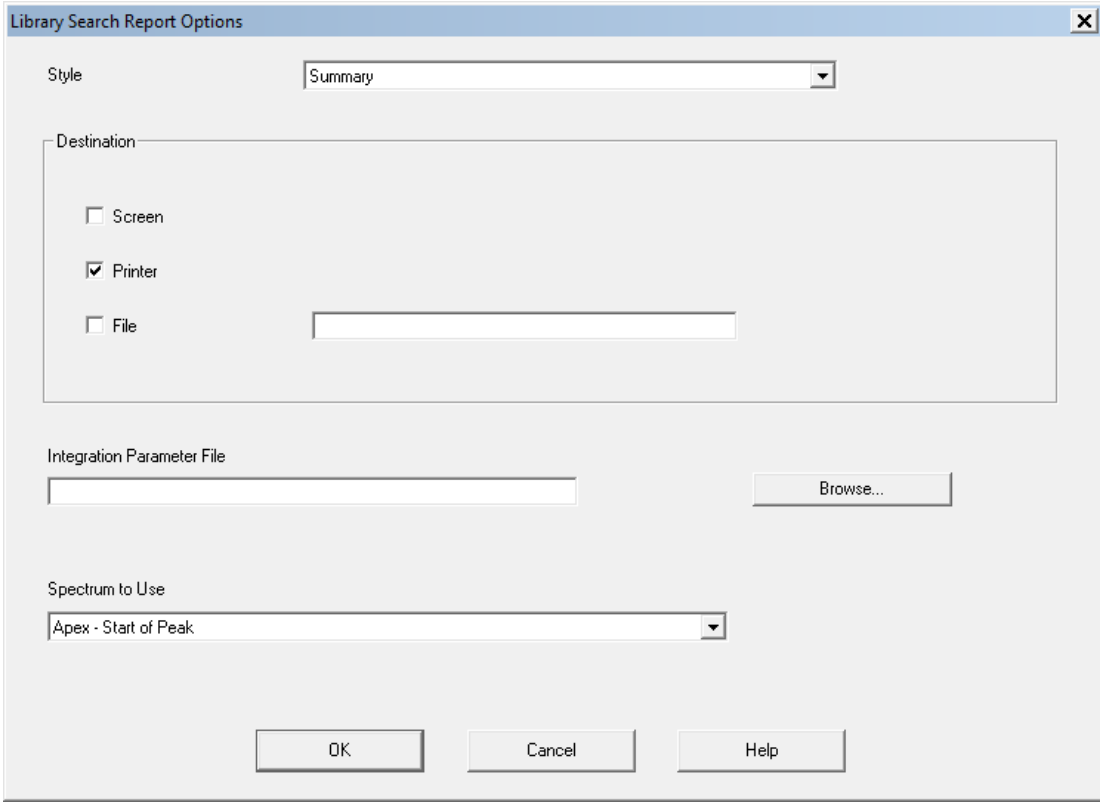
6 Double right-click on the spectrum. A search is performed and the results are displayed.



Generate an automated library search report

1 Open the data file.

2 Select the **Library Search Report** button,  . The **Library Search Report Options** dialog box opens.

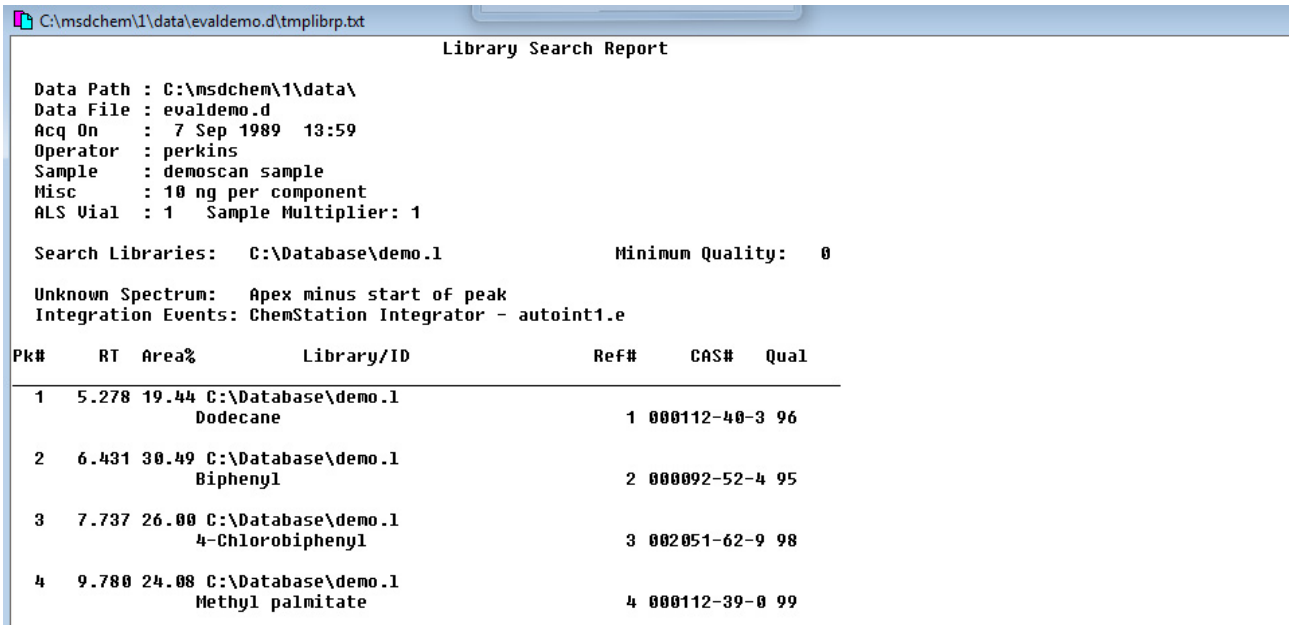


3 From the **Style** drop down list select **Summary**.

4 In the destination area, check **Printer**.

5 From the **Spectrum to Use** drop down menu, select **Apex- Start of Peak.** This selection automatically subtracts the spectrum at the start of the peak from the spectrum at the peak apex which you performed manually in the previous section **"Subtract the baseline noise from the spectra"** on page 81.

6 Select **OK** to generate the report.



C:\msdchem\1\data\evaldemo.d\tmplibrp.txt

Library Search Report

Data Path : C:\msdchem\1\data\
Data File : evaldemo.d
Acq On : 7 Sep 1989 13:59
Operator : perkins
Sample : demoscan sample
Misc : 10 ng per component
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: C:\Database\demo.l Minimum Quality: 0

Unknown Spectrum: Apex minus start of peak
Integration Events: ChemStation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.278	19.44	C:\Database\demo.l Dodecane	1	000112-40-3	96
2	6.431	30.49	C:\Database\demo.l Biphenyl	2	000092-52-4	95
3	7.737	26.00	C:\Database\demo.l 4-Chlorobiphenyl	3	002051-62-9	98
4	9.780	24.08	C:\Database\demo.l Methyl palmitate	4	000112-39-0	99

Figure 39 The library search report

Print a Window, TIC, Spectrum, or Method

Once you set your printer you can print a window, scan, spectrum, or method for the data file you are viewing on the screen.

Select a printer

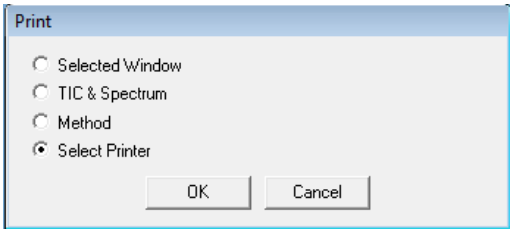
1 Select **File > Select Printer**.
2 Select printer from the list of printers on your system.
3 Select **OK**.

To change the page orientation

1 Select **File > Printer Setup**.
2 Select **Orientation**.
3 Select **OK**.

Select an item to print

1 Select **File > Print**. The Print dialog box is displayed.

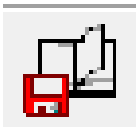


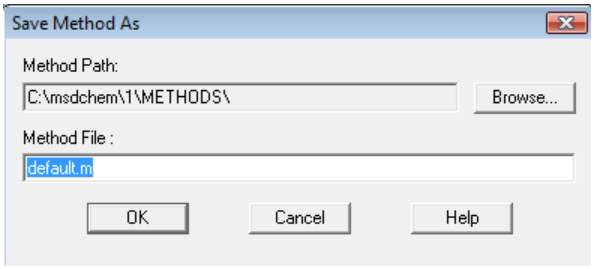
2 Select:

- **Selected Window** to print an open window and enter the window number from the window header in the Input dialog box.
- **TIC & Spectrum** to print these graphs.
- **Method** to print the method parameters.
- **Select Printer** to select a printer from the list of printers on your system.

3 Select **OK** to print your selection.

Save the Data Analysis Method

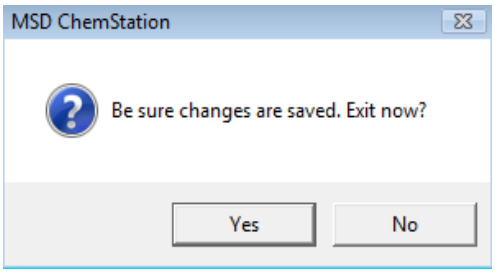
1 Select the **Save Method** button,  . The **Save Method As** dialog box opens.



2 Enter a name for the method and select **OK** to save the updated parameters to this method.

Exit the Data Analysis Program

1 Select **File > Exit**. A warning message appears.



2 Select **Yes** to close the program.

If you have not saved your method, you will lose changes if you click **Yes** to exit now.

6
Create a SIM Quantitation Method

This chapter describes how to create a SIM method for our standard sample using the target and qualifier ions found during qualitative analysis. We also examine how to set up a method that performs simultaneous SIM and scan data acquisition.

Introduction

Selected ion monitoring (SIM) mode is a data acquisition technique where only selected ion fragments are monitored in order to obtain maximum sensitivity.

To find appropriate conditions for the SIM data acquisition, analyze your scan data for:

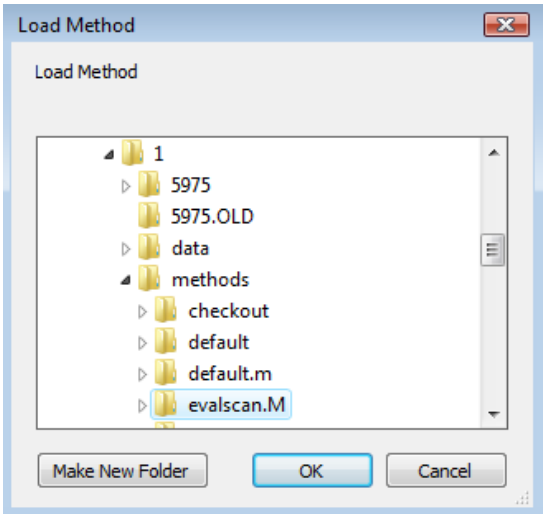
- **Ions (*m/z*) monitored for each peak** - MS SIM parameters allow you to define up to 100 groups of up to 60 ions each for selected ion monitoring, however, Agilent recommends you use as few ions as possible to maximize the signal to noise ratio.
- **The best time to switch groups** - Agilent recommends that you choose a time to switch groups where the peaks are well separated to avoid variations in retention time due to sample matrix effects.

Create a SIM Method

1 From the **Instrument View**, select the **Load Method** button, the **Load Method** dialog box opens.

2 Navigate to and select **evalscan.M**.

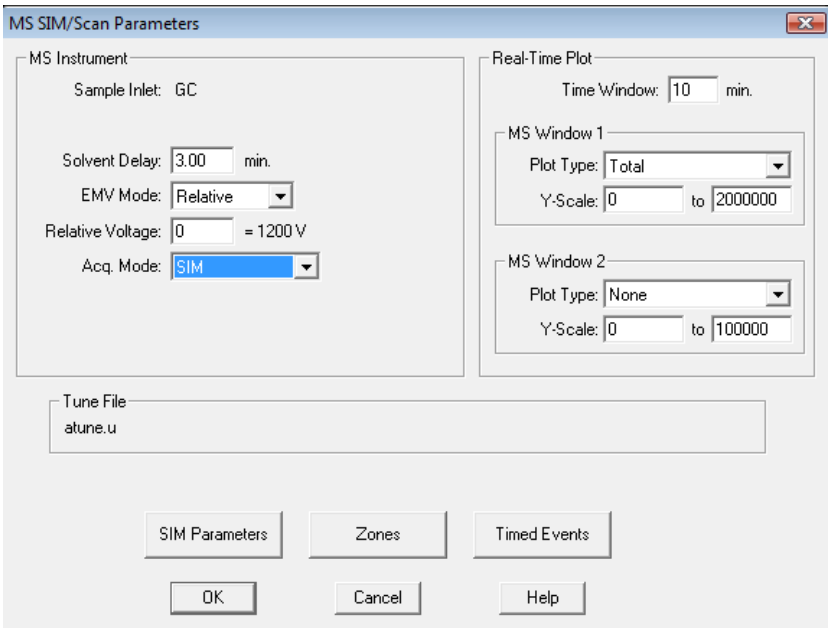
Since the GC acquisition parameters in this method were set for good chromatographic data resolution, use this method as a starting point and only change the MS parameters in the method.



3 Select **OK** to load the method and close the dialog box.

4 Select the **MS Parameters** button,  . The **MS SIM / Scan Parameters** dialog box opens.

5 From the **Acq. Mode** dropdown box, select **SIM**.



6 Select **SIM Parameters**. The **Edit SIM Parameters** dialog box opens. See Figure 40.

7 In the **Group** field, enter `1`. Group 1 appears in the right panel table.

8 For **Resolution**, select **High**.

9 In the **Edit Ion** area enter the values for all 4 ions in the group 1 ions time segment.

   a In the ***m/z*** and **Dwell** fields enter the ion values for these compounds from Table 5 on page 93.

   b After each ion addition, select **Add/Modify Ion**.

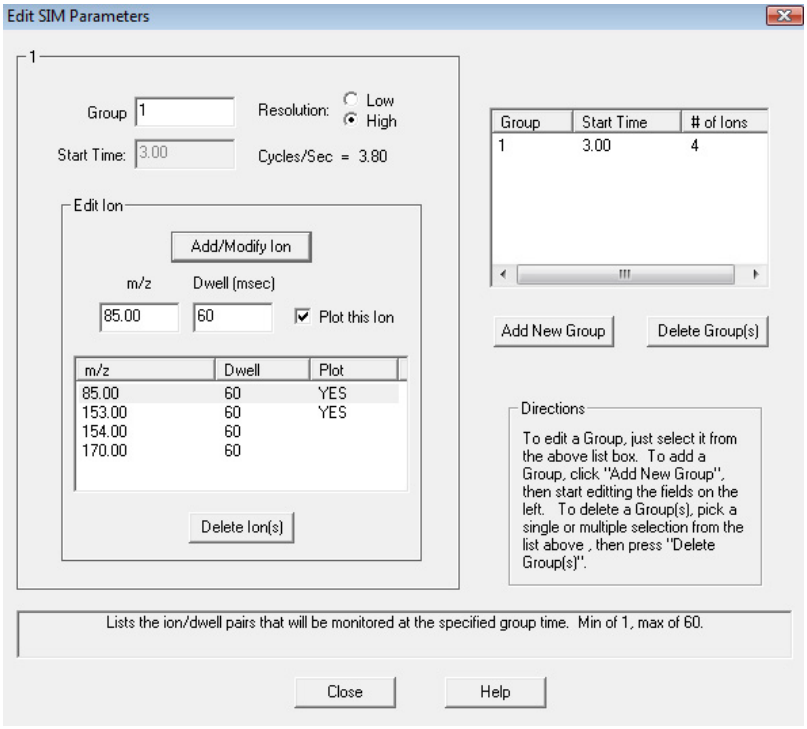


Figure 40 Entering Group 1 ions

Table 5 SIM ion selection

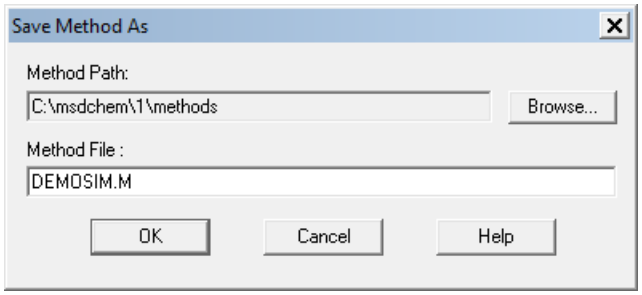
Compound	Target Ion	Qualifier Ion	Dwell time
Biphenyl	154	85	60
Dodecane	170	85	60
Chlorobiphenyl	188	152	60
Methyl pamitate	270	87	60

10 Select **Close** to save settings and return to the **MS SIM / Scan Parameters** dialog box.

11 Select **OK**.

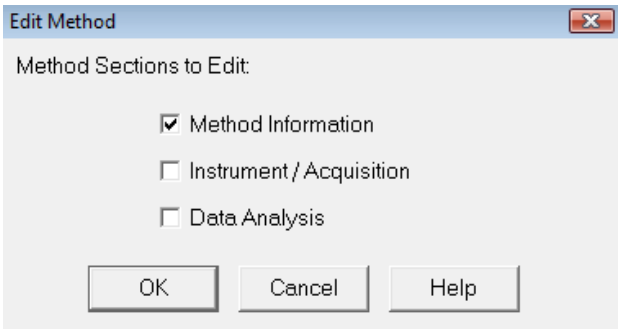
12 Select the **Save Method** button,  . The **Save Method As** dialog box opens.

13 In the **Method File** field, enter **demosim** and select **OK**.



14 Select the **Edit Entire Method**... button,  . The **Edit Method** dialog box opens.

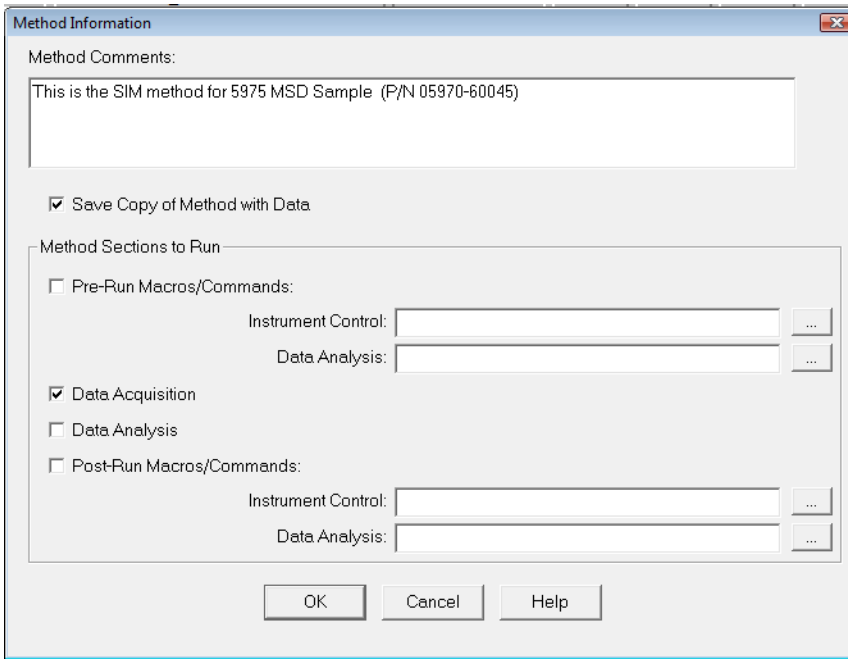
15 Mark the **Method Information** check box only. Clear the **Data Analysis** and **Instrument/Acquisition** check boxes.



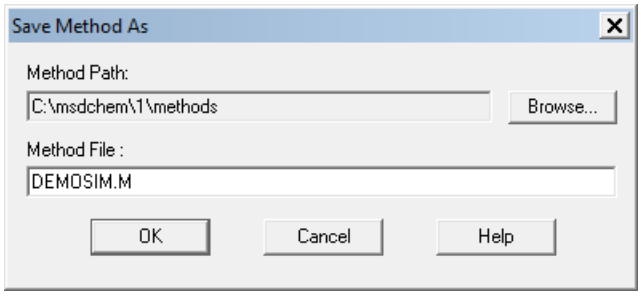
16 Select **OK**. The **Method Information** dialog box opens.

17 In the **Method Comments** field, enter a description of this method.

18 In the **Method Sections To Run** area, mark the **Data Acquisition** check box.



19 Select **OK**. The **Save Method As** dialog box opens.

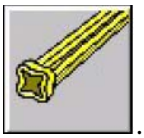


20 Confirm that **demosim** is entered in the **Method File** field and select **OK**.

Simultaneously Acquire Scan and SIM Data (SIM/Scan Mode)

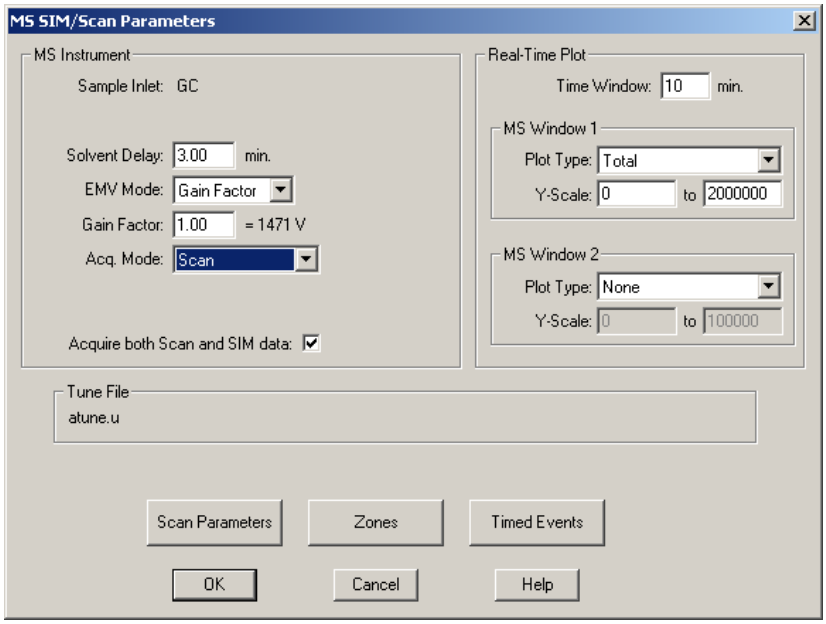
If we start with a method containing Scan parameters and then also enter SIM parameters like we did for the evalsim.m method, our method already contains all parameters required except one. We only need to check a box that specifies that we want to acquire both types of data simultaneously.

In SIM/Scan mode the number of data points taken in each mode is reduced and we will see how that impacts the total cycle frequency.

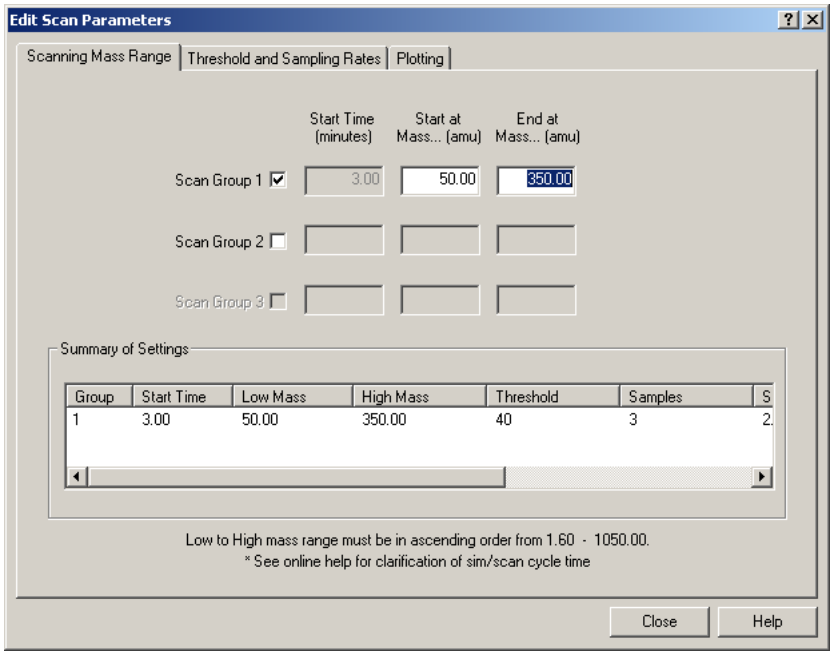
1 Select the **MS Parameters** button,  . The **MS SIM / Scan Parameters** dialog box opens.

2 Mark the **Acquire Scan and SIM data** check box.

3 From the **Acq. Mode** dropdown box, select **Scan**.



4 Select **Scan Parameters**. The **Edit Scan Parameters** dialog box opens and we can view our previous settings.



5 Select the **Mass Range** tab and note the asterisk.

   The asterisk in the **Summary Of Settings** table, **(Scans/Sec*)** denotes that the **Scans/Sec** displayed here does not represent the actual cycles. See "SIM/Scan Mode Cycle Frequency" on page 98, for more information.

6 Write down the cycle frequency for the scan mode.

7 Select **Close** to return to the **MS SIM/Scan Parameters** dialog box.

8 From the **Acq. Mode** dropdown box, select **SIM**.

9 Select **SIM Parameters**. The **Edit SIM Parameters** dialog box opens where we can view our previous settings.

10 Select the **Mass Range** tab and write down the cycle frequency for the SIM mode.

11 Select **Close** to return to the **MS SIM/Scan Parameters** dialog box.

12 Select **OK** to save the parameters and close the dialog box.

13 Save the method with the name **sim_scan.M**.

The individual cycle frequencies recorded here will be used to calculate the actual cycle frequency in the next section "SIM/Scan Mode Cycle Frequency" on page 98.

SIM/Scan Mode Cycle Frequency

In SIM/Scan mode, to complete one cycle the MSD acquires a single group of SIM data followed by a single group of Scan data. It may be necessary to increase the Scan speed or decrease the SIM dwell time to achieve the desired number of data points for effective chromatographic integration. See Figure 41.

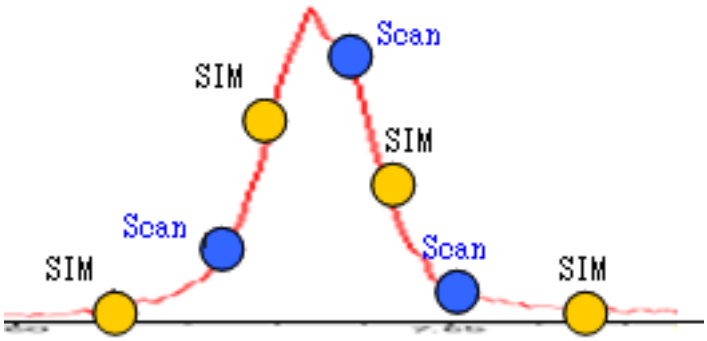


Figure 41 SIM/Scan mode

Actual cycle frequency is calculated with the equation in Figure 42.

$$\text{SIM/Scan Cycle Frequency} = \frac{1}{\left(\frac{1}{A}+\frac{1}{B}\right)\times 1.05}$$

Where A = Scan cycles per second
B = SIM cycles per second

Figure 42 SIM/Scan cycle

When switching from the SIM data acquisition mode to the Scan mode, about 5% of the available run time will be consumed.

For our example, Scan = 2.44 cycles/sec and SIM = 1.97 which results in an actual cycle time of 1.04 cycles/sec. To improve the number of data points, we could reduce the SIM dwell time, and increase the scan speed.

7
Run a Sequence

This chapter describes how to create and run a sequence.

A sequence is a list of samples to be analyzed and a designated method to be used for each analysis. Once defined, the sequence may run unattended, automatically processing the samples defined in the sequence.

When an ALS is installed, the entire analysis, from injection of the sample through reporting of results, can be automated to save you time.

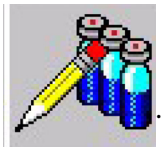
The data files generated when running this sequence will be used later for developing a quantitate analysis.

Prepare the Samples

1. Prepare 1:2 serial dilutions of the 100 ng/mL 5975 MSD Sample (P/N 05970-60045 or P/N 5074-3025 Japan only) in hexane to make a 50 ng/mL and a 25 ng/mL method calibration sample.
2. Prepare 1:2 serial dilutions of the 10 ng/mL 5975 MSD Sample (P/N 05970-60045 or P/N 5074-3025 Japan only) in hexane to make a 5 ng/mL and 2.5 ng/mL method calibration sample.
3. Fill the vials with approximately 500 µL of each standard (2.5, 5, 10, 25, and 50 ng/mL).

   If you are not using an ALS skip the remaining steps.
4. Place the sample vials in increasing order of concentration into positions 1 through 5 of the GC sample tray.
5. Fill a solvent wash vial with isooctane and place it in injector turret location A for solvent wash mode A, B.
6. Place an empty waste vial in turret location B specified for solvent wash mode A, B.

Create the Sequence

1 Select the **Edit Sequence** button,  . The **Sample Log Table** opens.

2 In sample row 1 under the **Type** column, click in the cell to activate the dropdown list, and select **Sample.**

3 Under the **Vial** column, enter `1` if you placed the lowest concentration sample in the ALS tray position 1.

4 Under the **Sample** column, enter `Standard 5 ng/mL`.

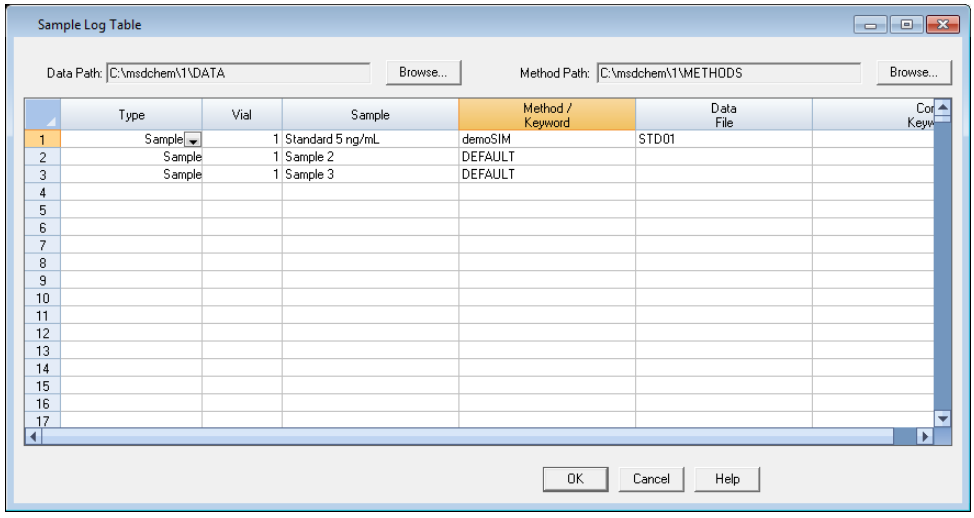
5 Under the **Method/Keyword** column:

   a Right mouse click and select **Browse for Method**. The **Browse for Folder** dialog box opens.

   b Navigate to and select **demoSIM**.

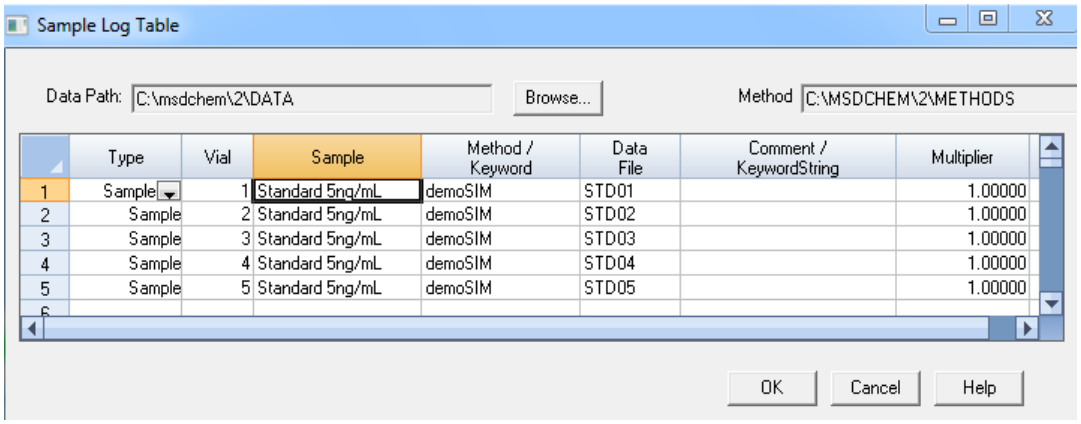
   c Select **OK**. The method name appears in the column.

6 In the **Data File** column, enter `STD01`.



7 Highlight rows 1 to 5.

8 Right mouse click and select **Repeat Row & increment**. Four lines are added to the table with incremented vial number and data file names.

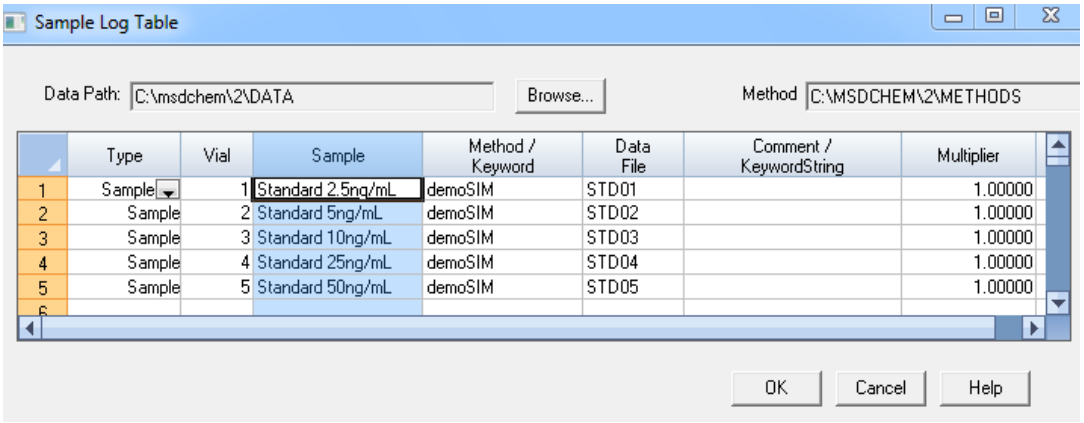


9 In row 1, under **Sample** column, change the value to **2.5 ng/mL**.

10 In row 3, under **Sample** column, change the value to **10 ng/mL**.

11 In row 4, under **Sample** column, change the value to **25 ng/mL**.

12 In row 5, under **Sample** column, change the value to **50 ng/mL**.

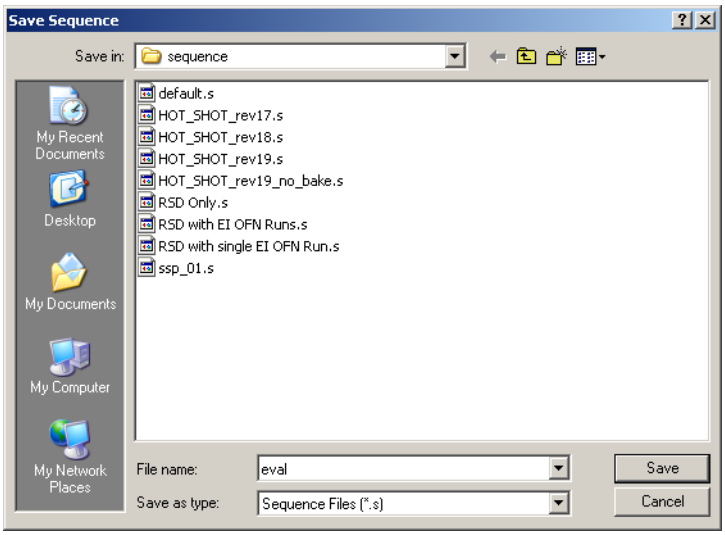


13 Select **OK** to close the **Sample Log Table**.

Save the Sequence

1 Select the **Save Sequence As...** button,  . The **Save Sequence** dialog box opens.

2 In the **File name** field, enter `eval`.

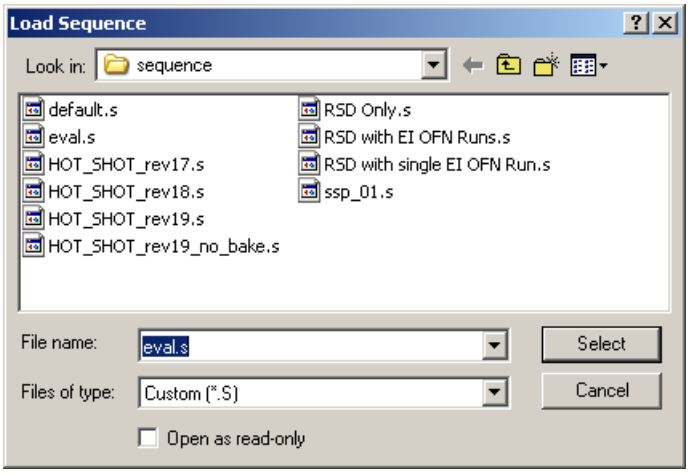


3 Select **Save**. The dialog box closes and the sequence is saved.

Load the Sequence

1 Select the **Load Sequence** button,  . The **Load Sequence** dialog box opens.

2 In the **File Name** field, enter `eval.s`.



3 Click **Select** to close the dialog box and load the sequence.

Run the Sequence

1 Select the **Run Sequence** button,  . The **Start Sequence** dialog box opens.

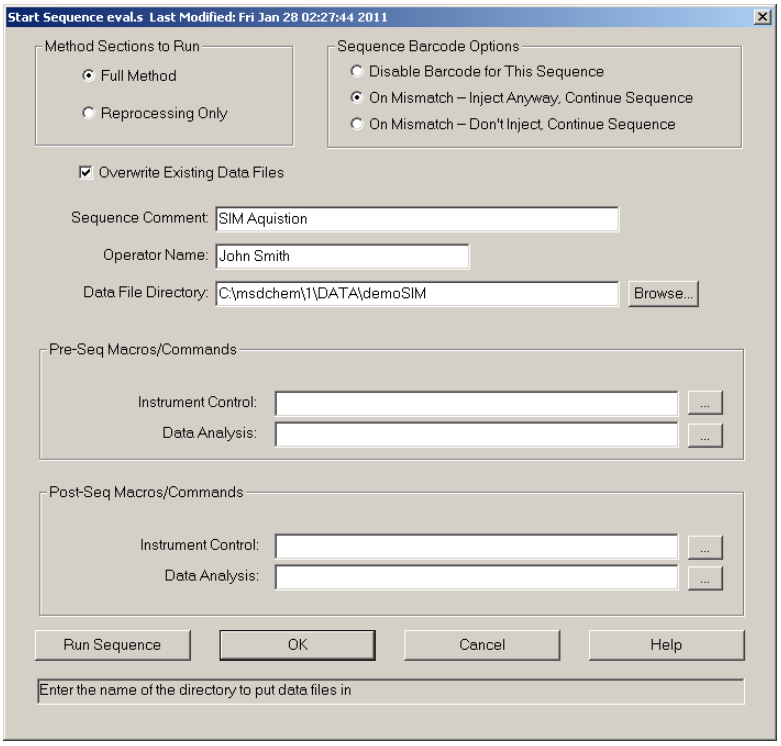
2 In the **Method Sections to Run** area, select **Full Method**.

3 In the **Sequence Comment** field, enter a description of the sequence.

4 In the **Operator Name** field, enter your name.

5 In the **Data File Directory** field, add `demosim` to the path.

6 Select **Run Sequence**.



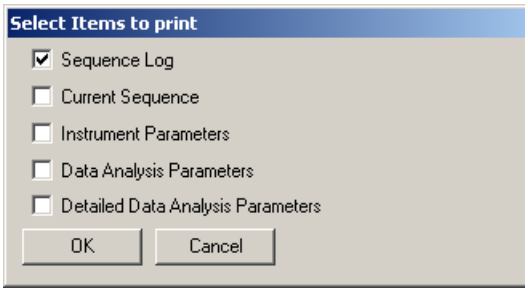
The **Sequence Status** bar is displayed. During the sequence run, you can monitor the number of the samples run, the number of samples remaining, and the current sample vial being processed. Use the controls on the bar to pause the sequence, access data analysis, or edit sequence sample entries that have not yet run.

Figure 43 The sequence status bar

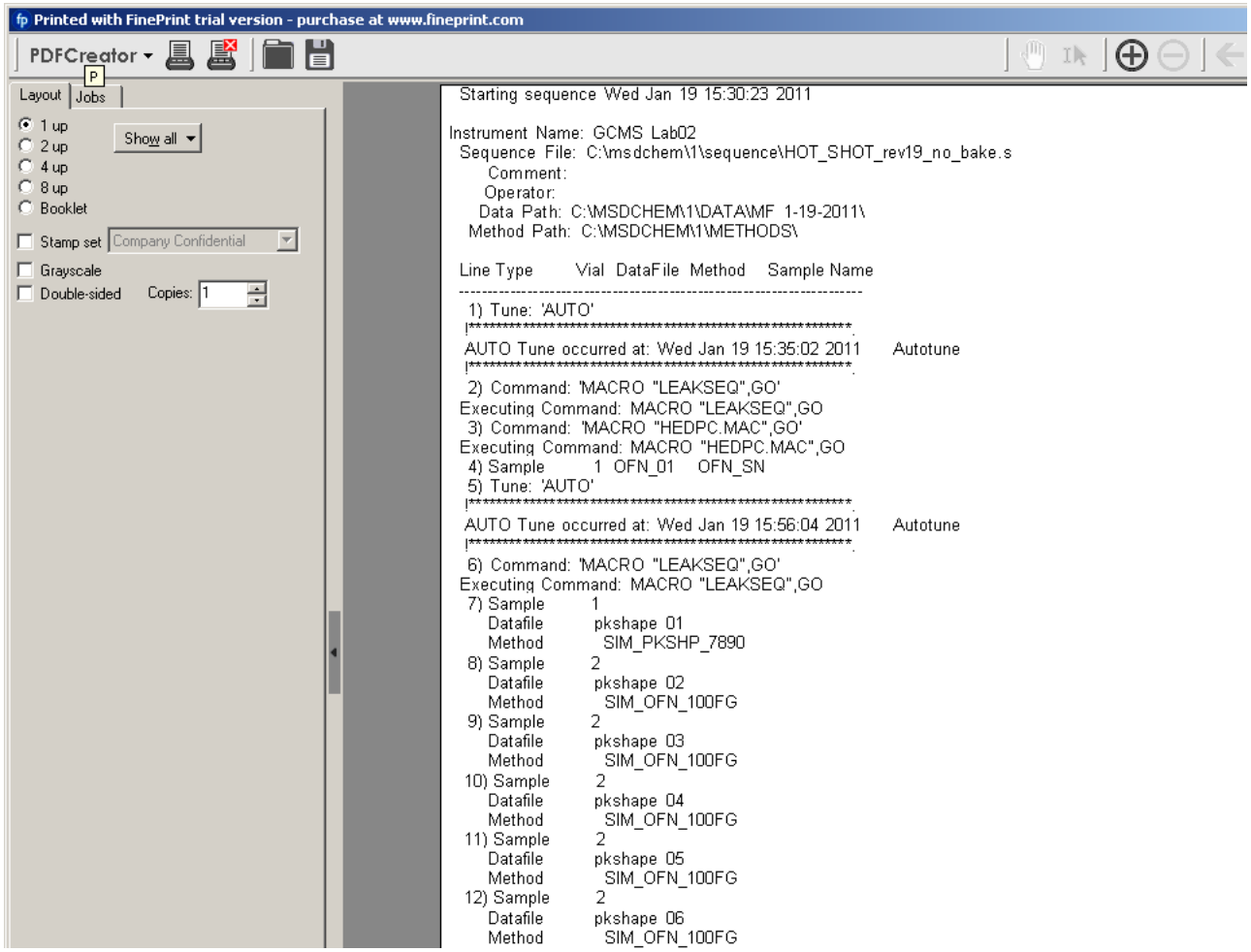
Print the Sequence Log

1 Select the **Print** button,  . The **Select Items to Print** dialog box opens.

2 Mark the **Sequence Log** checkbox.



3 Select **OK**. The **Sequence log** is displayed for printing.



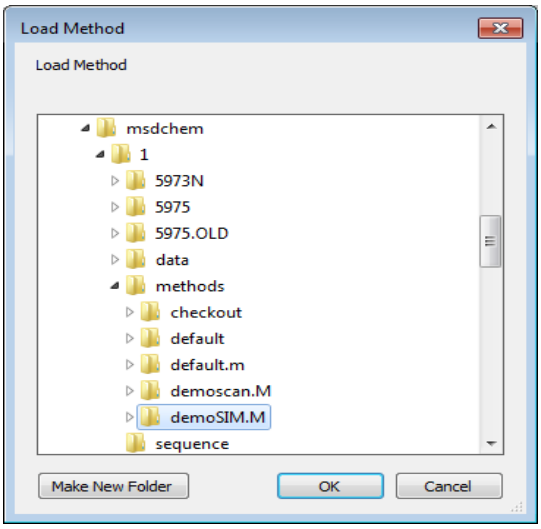
8
Set Up a Quantitation Database

This chapter describes how to add compounds to the database. After a compound is identified, quantitative data analysis determines the amount of the compound in your sample by comparing the response from an unknown amount of compound with the response from a known measured amount of the compound stored in the quantitation database.

Add Compound Entries for the Database

1 Start the Enhanced Data Analysis program.

2 Select the **Load Method** button,  . A confirmation message dialog box may open. If so, select **Yes**. The **Load Method** window opens.



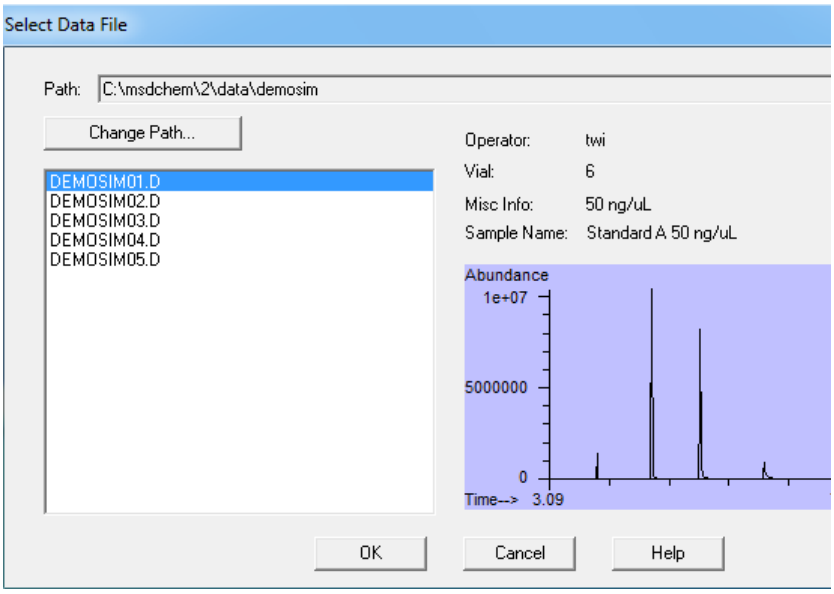
3 Select the demosim method and click **OK**.

4 Select the **Load Data File** button,  . The **Select Data File** dialog box opens.

5 Select **Change Path.** The **Browse for Folder** window opens.

6 Navigate to and select **C:\msdchem\1\data\demosim**.

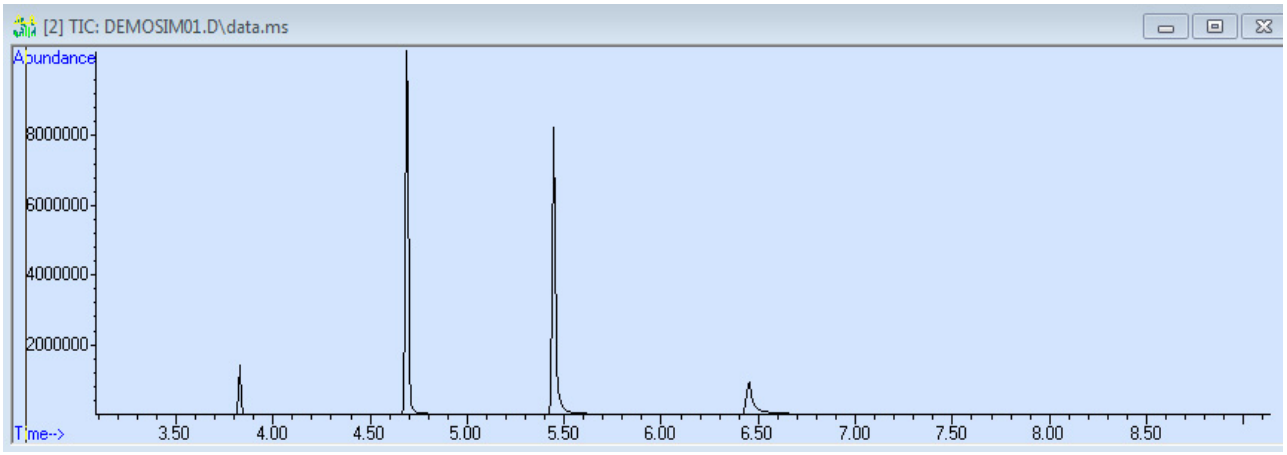
7 Select **OK**. The path is displayed in the **Path** field.



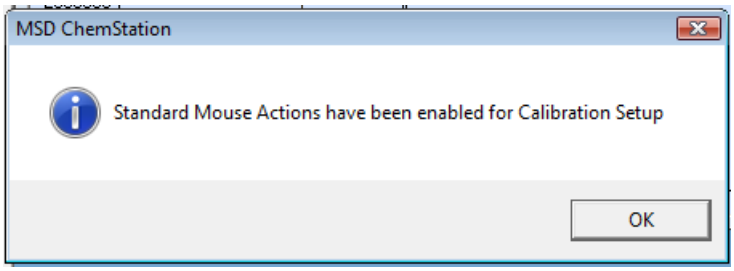
8 From the list of files, select **DEMOSIM01.D**.

Later we will use the load next file function. It remembers this data directory and the last file selected from it and automatically loads the next data file with the click of an icon.

9 Select **OK**. The **TIC** window opens.

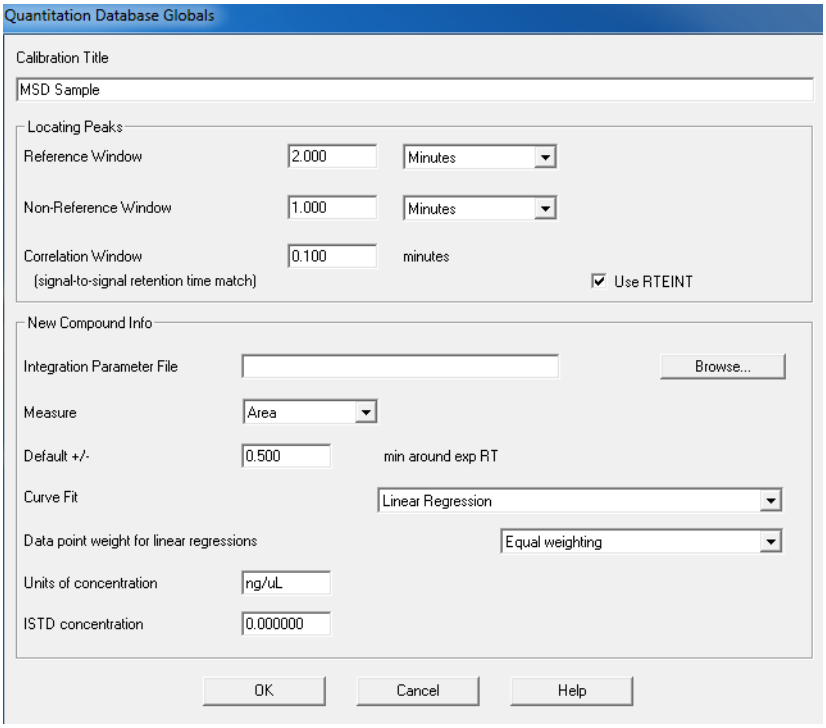


10 Select the **Setup Quant** button,  . A confirmation message may appear. Select **OK**.



11 Select **OK**. The standard right mouse buttons are enabled.

12 The **Quantitation Database Globals** dialog box opens.



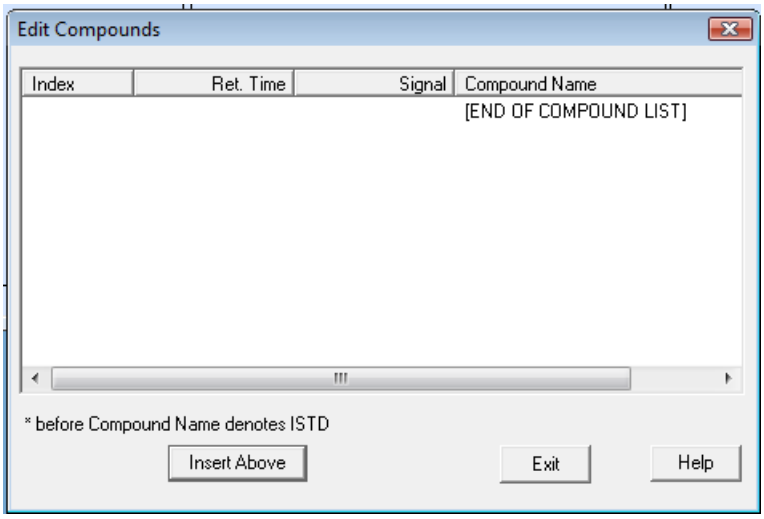
13 Enter the following information to set parameters that will initially be set for all compounds in this database. If some compounds need different parameters they can be changed later in the database.

a Calibration Title-`MSD Sample`.

b Units of Concentration - `ng/uL`

c Select **Use RTEINT**. The RTE integrator is recommended for MS data.

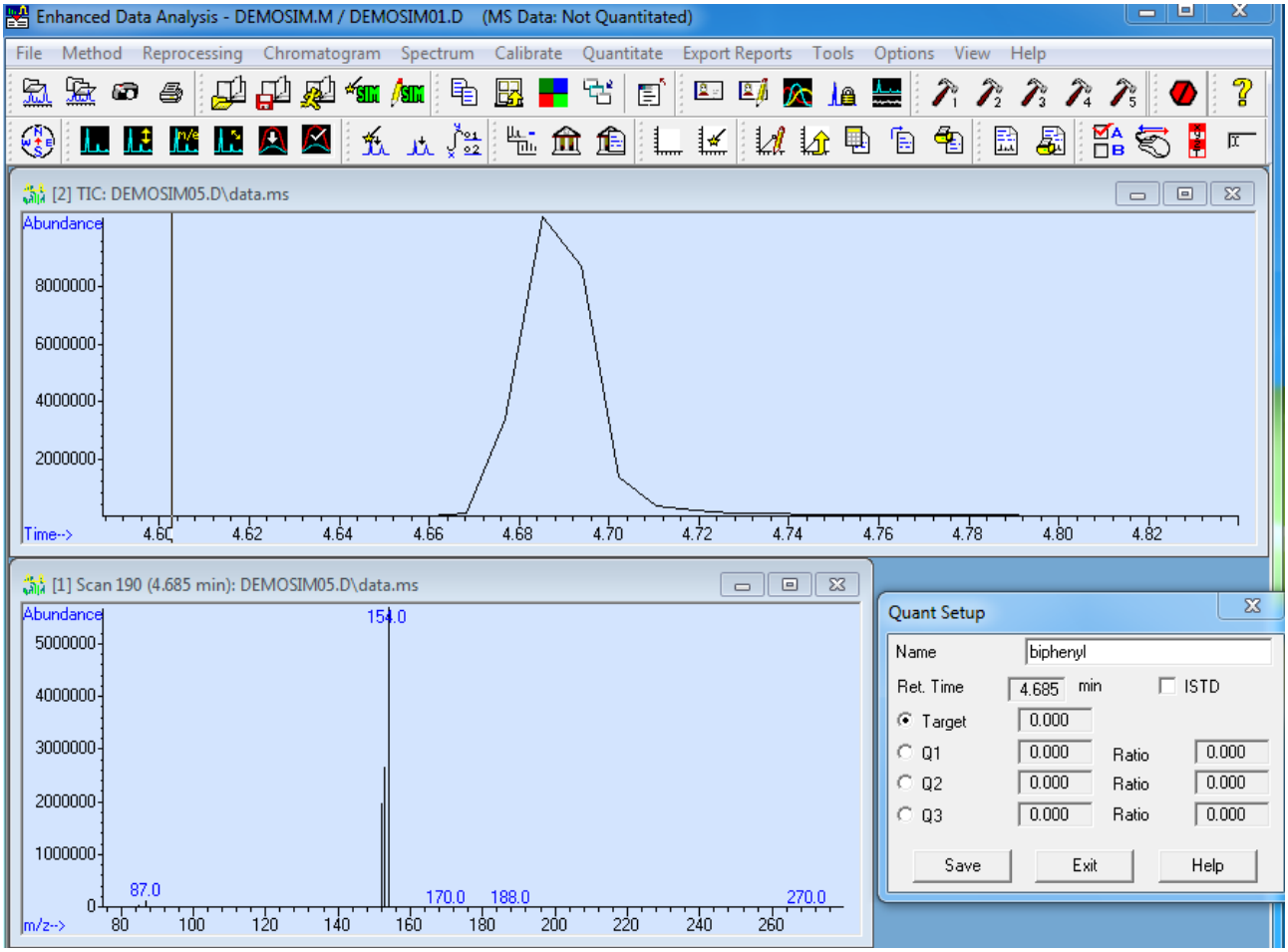
14 Select **OK** to save the settings and open the **Edit Compounds** dialog box.



Identify compounds

The first part of setting up a Quantitation database is identifying and naming the compounds by selecting target and qualifier ions from a known sample.

1 From the **Edit Compounds** dialog box, select **Insert Above**. The **Quant Setup** dialog box opens.



2 In the **name** field, enter the first compound name, `biphenyl`.

3 In the TIC window, enlarge the biphenyl peak (near RT 4.7).

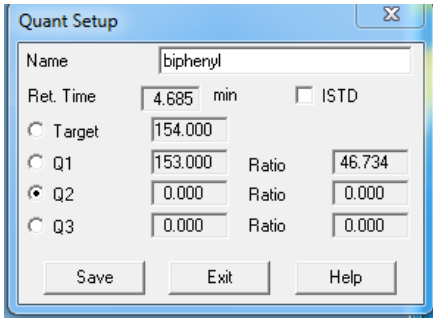
4 Position the cursor at the highest point of the peak and double right mouse click. The RT is added to the **Ret. Time** field. The **Scan** is displayed in the lower window and the RT is displayed for the **Ret. Time** in the **Quant Setup** dialog box.

Target is selected in the **Quant Setup** dialog box.

5 In the scan window, position the bulls eye cursor on the target ion (154) and click both mouse buttons simultaneously. The *m/z* is displayed for the **Target**.

Q1 is selected in the **Quant Setup** dialog box.

6 In the scan window, position the cursor on the first qualifier ion (153) and click both mouse buttons simultaneously. The *m/z* is added to the **Q1** field and the ratio is calculated and added to the **Ratio** field.



To clear an incorrect ion selection, select the radio button for that ion. Next, simultaneously click both mouse buttons with the cursor positioned on an area not containing an ion.

7 Select **Save** to add the biphenyl peak to the database and clear the **Quant Setup** dialog box.

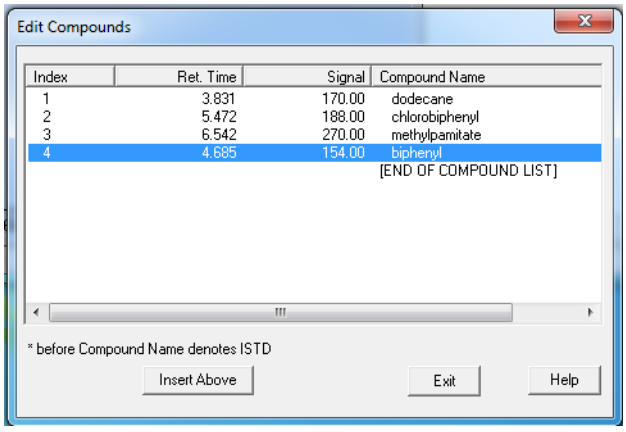
8 Add the remaining compounds using the target and qualifier ions identified in qualitative analysis.

Table 6 Target and qualifier ion selections

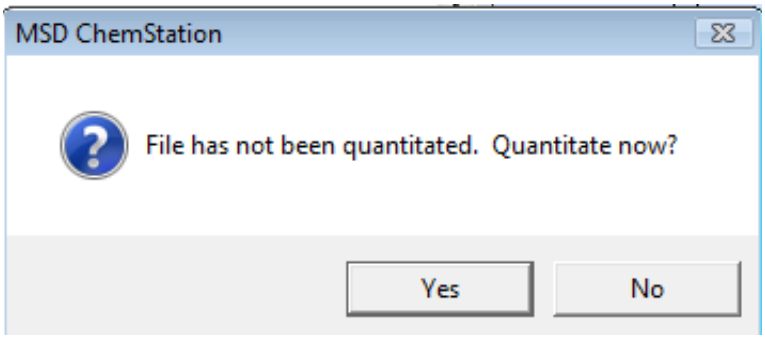
Compound	Target Ion	Qualifier Ion	Dwell time
Biphenyl	154	153	60
Dodecane	170	85	60
Chlorobiphenyl	188	152	60
Methyl pamitate	270	87	60

9 When all compounds are added, select **Exit** to return to the **Edit Compounds** dialog box.

10 Review the compound list. If any corrections need to be made, double-click on the compound and reenter the information in the **Quant Setup** dialog box.



11 Select **Exit**. A confirmation message appears.



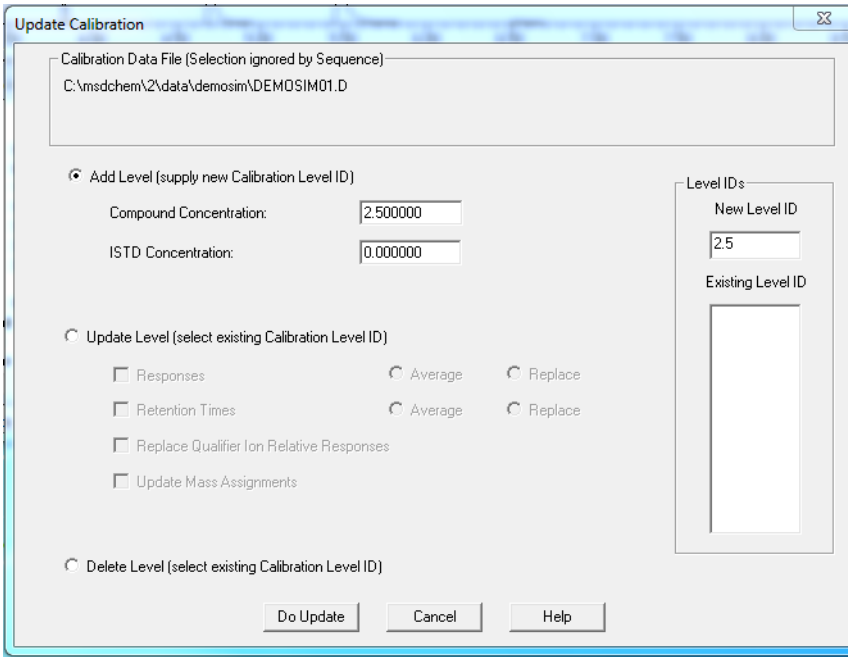
This procedure continues with the next section "Add the Calibration Curve" on page 115.

Add the Calibration Curve

The second part of setting up a Quantitation database is entering the compound concentrations from a group of samples. Each sample in the group contains a different compound concentration used to create the calibration curve.

Add calibrator level 1

1 Select **Yes** to the confirmation message that appears in step 11 of "Identify compounds" above. The **Update Calibration** dialog box opens.



2 For the first calibrator,

   a Select **Add Level**.

   b **Compound Concentration** enter `2.500000`.

   c In the **Level IDs** area, enter `2.5` in the **New Level ID** field.

3 Select **Do Update**. The **Edit Compounds** dialog box opens and displays the first calibration point.

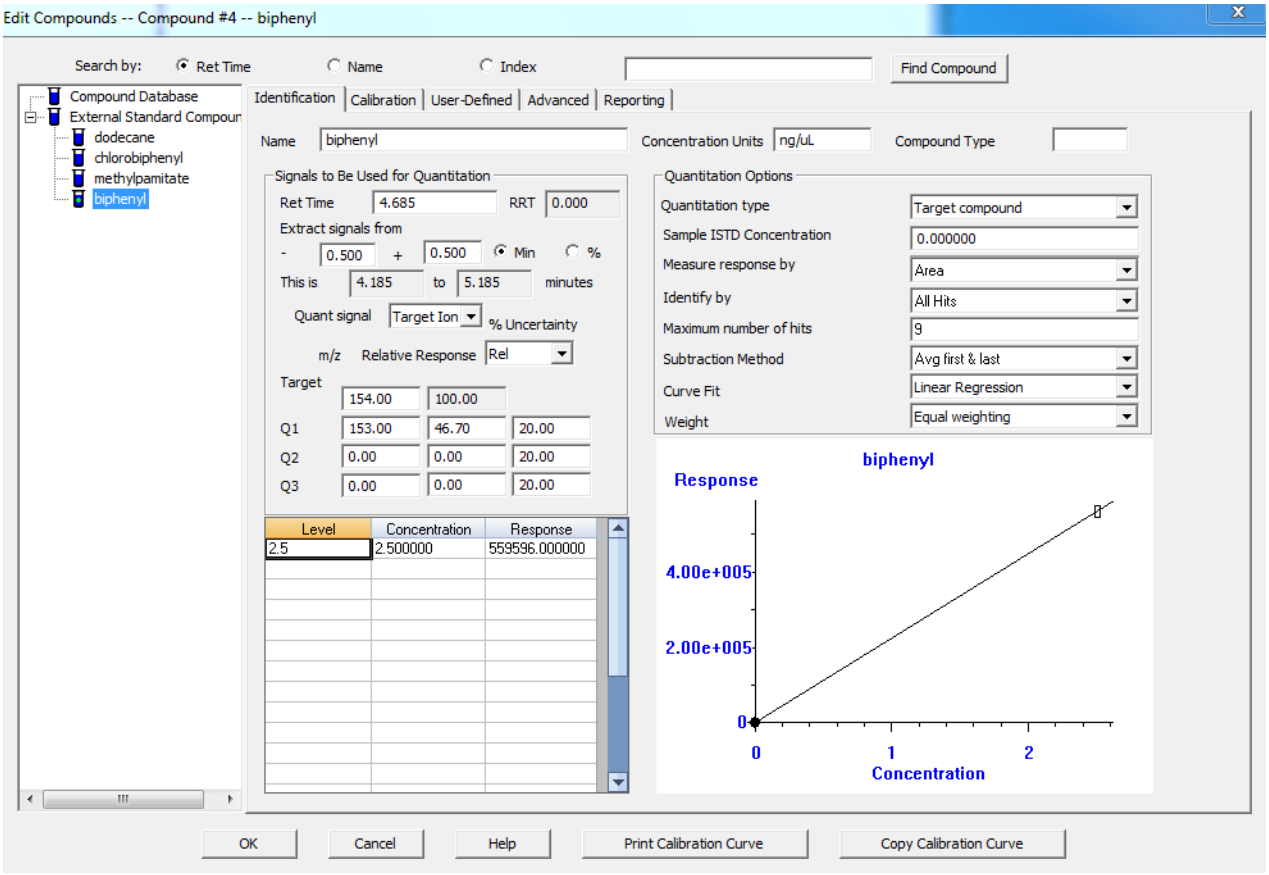


Figure 44 The first calibration point is added to the calibration curve

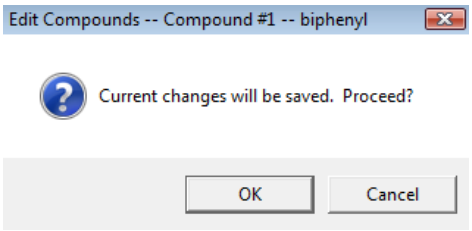
4 Select the **Identification** tab.

5 In the **Quantitation Options** area, select:

   a **Identify by - All Hits**

   b **Subtraction Method - Avg first & last**

6 Select **OK**. A confirmation message appears.



7 Select **OK** to save the changes.

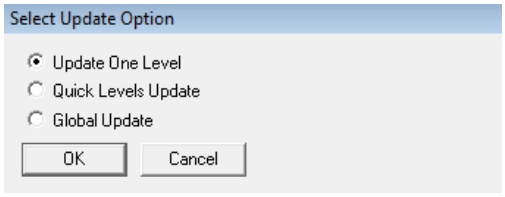
The Quantitation Report window opens. To add additional calibration levels, see the next section.

Add calibrator levels 5, 10, 25, and 50 to the calibration curve

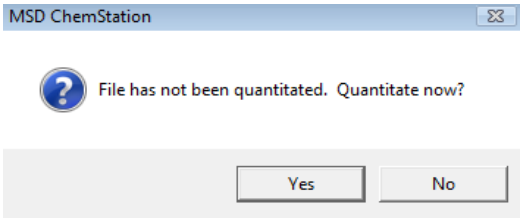
Repeat this procedure for DEMOSIM02 (level = 5), DEMOSIM03 (level = 10), DEMOSIM04 (level = 25), and DEMOSIM05 (level = 50).

1 Select the **Load Next Data File** button,  . The next data file is automatically loaded.

2 Select the **Update Calibration** button,  . The **Select Update Option** dialog box opens.



3 Select **Update One Level** and **OK**. A confirmation message appears.



4 Select **Yes**. The **Update Calibration** dialog box opens.

5 For this calibrator,

   a Select **Add Level**.

   b **Compound Concentration** for DEMOSIM02 (level = 5), DEMOSIM03 (level = 10), DEMOSIM04 (level = 25), and DEMOSIM05 (level = 50).

   c In the **Level IDs** area, enter for DEMOSIM02 (level = 5), DEMOSIM03 (level = 10), DEMOSIM04 (level = 25), and DEMOSIM05 (level = 50) the **New Level ID** field.

6 Select **Do Update**. The **Edit Compounds** dialog box opens and displays the new calibration point.

7 Select the **Identification** tab.

8 In the **Quantitation** area, select:

   a **Identify by - All Hits**

   b **Subtraction Method - Avg first & last**

9 Continue with the above steps under "Add calibrator levels 5, 10, 25, and 50 to the calibration curve" until all concentration levels are added. The completed calibration curve is shown in Figure 45.

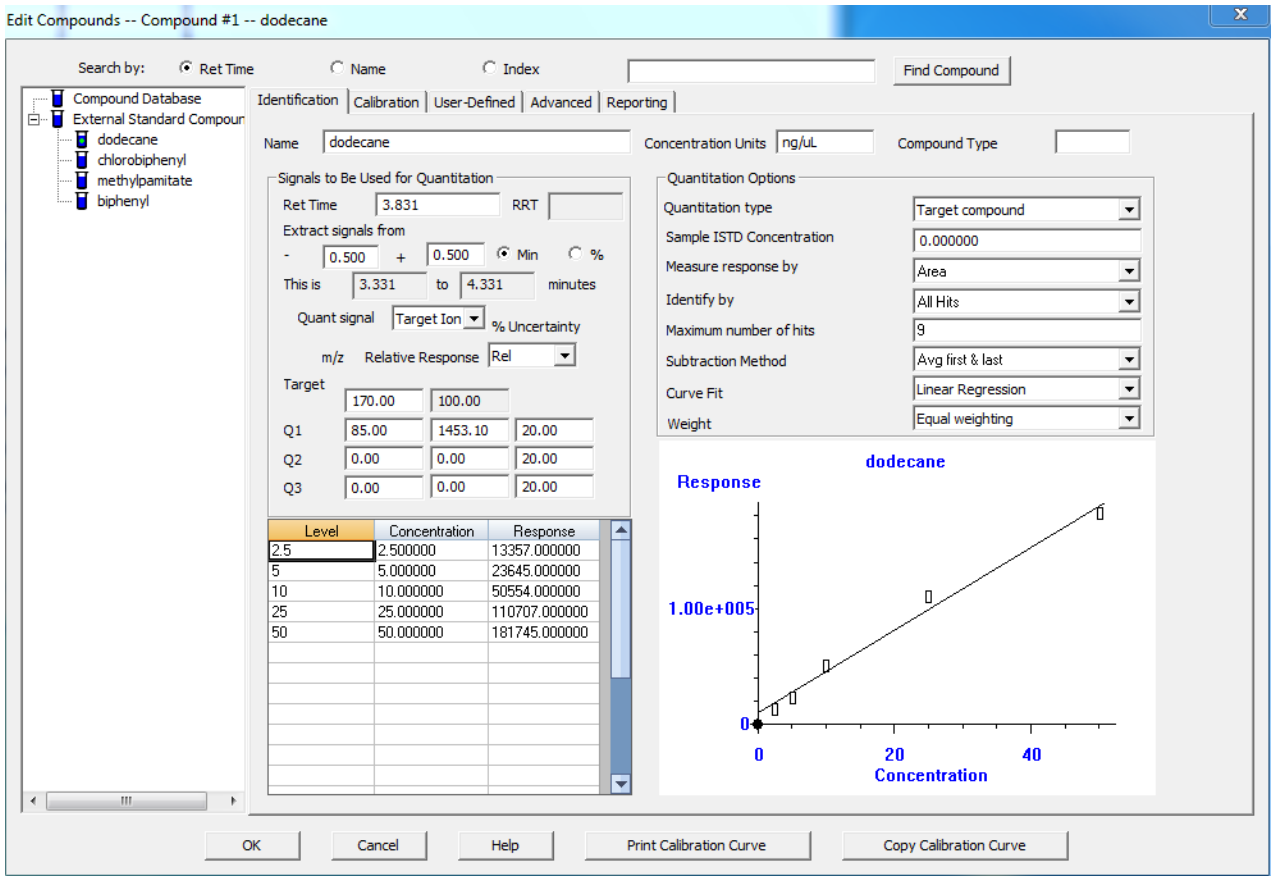


Figure 45 The completed quant database

10 Select **OK** to close the window.

Save the database

1. Select the **Save Method** button,  . The **Save Method As** dialog box opens with the name of the current method displayed in the **Method Path** and **Method File** fields.
2. Select **OK**.

View or Edit an Existing Database

1 Select the **Edit Compounds** button,  . The **Edit Compounds** dialog box opens.

2 Select a compound in the navigation tree. The corresponding information is displayed in each tab.

3 To copy the calibration curve to your clipboard for use in another application, select **Copy Calibration Curve**.

4 To print the calibration curve, select **Print Calibration Curve**.

Identification tab

- Name of the compound
- Concentration units
- Compound type
- Retention time information
- Signals to be used for quantitation
- Calibration information
- Quantitation parameters

Calibration tab

- Concentration units
- Response for each level ID

User-Defined tab

- A1 through A3 - alphanumeric items with a maximum of 19 characters
- N5 through N9 - numeric items

Advanced tab

- Area Correction Mass
- Correction Factor
- Integration parameter files for target and qualifier compound quantitation. The **Sum?** field allows you to add the response of the designated qualifier ion to the response of the target ion. This method is valid only in area quantitation using the extended area quantitation method.

Reporting tab

- CAS # - designed for a Chemical Abstract Service number. However, you may use this for any other number or information about the compound.
- Surrogate / Matrix Spike Amount
- Matrix A and B concentrations
- Signal level minimum and maximum
- MS database name
- Reference Spectrum number

9
Generate a Report

This chapter explains how to modify your method to generate a report at the end of each sample run and how to interactively generate a report from the **Data Analysis** view.

Generate a Report Automatically After the Run

Load the method

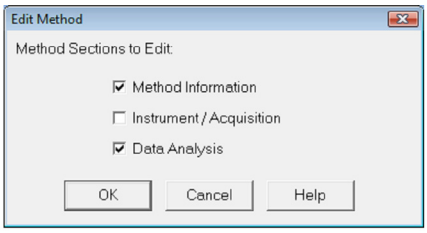
1 From the **Instrument View**, select the **Load Method** button,  . The **Load Method** window opens.

2 Navigate to and select **demosim.m**.

3 Select **OK** to close the dialog box and load the method.

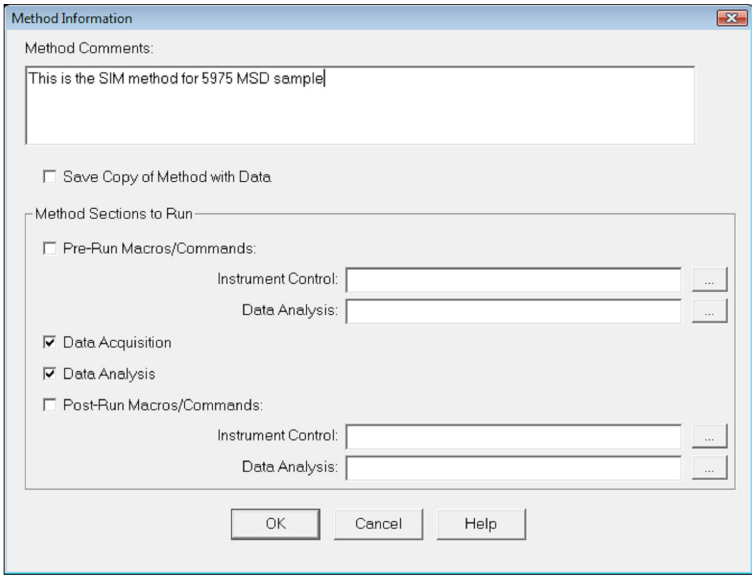
Edit the method to generate a report

1 From the **Instrument View**, select the **Edit Entire Method**... button,  . The **Edit Method** dialog box opens.



2 Mark the **Method Information** and **Data Analysis** check boxes only. Clear the **Instrument/Acquisition** check box.

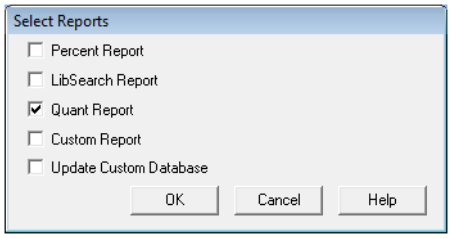
3 Select **OK**. The **Method Information** dialog box opens.



4 In the **Method Comments** field, enter a description of this method.

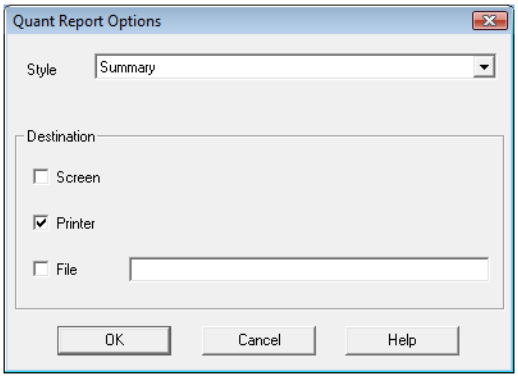
5 In the **Method Sections To Run** area, mark the **Data Acquisition** and **Data Analysis** check boxes, and clear the **Post-Run Macro/Commands** check box.

6 Select **OK**. The **Select Reports** dialog box opens.



7 Mark the **Quant Report** check box and clear all other check boxes.

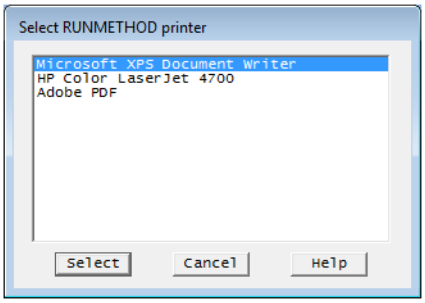
8 Select **OK.** The **Quant Report Options** dialog box opens.



9 From the **Style** drop down list, select **Summary**.

10 In the **Destination** area, mark the **Printer** check box and clear all the other check boxes.

11 Select **OK**. The **Select RUNMETHOD printer** dialog box opens.

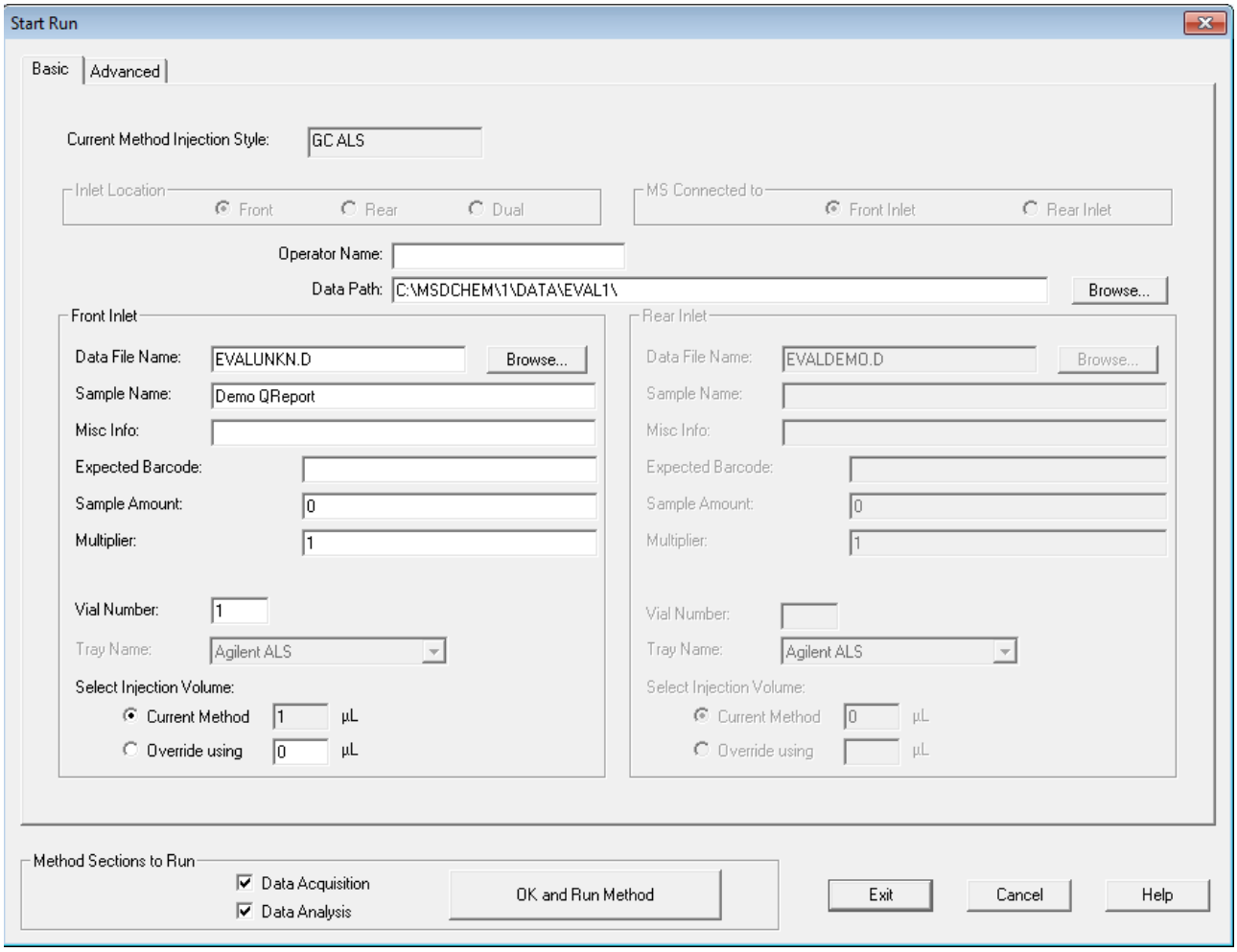


12 Select a printer and click **Select**. The **Save Method As** dialog box opens.

13 Select **OK** to save the setting to the current method or enter a new file name for the method.

Run the method and generate the report

1 With the method modified to print a quantitation summary report loaded, click on the green arrow to display the **Start Run** dialog box.



2 In the **Data Path** field, add `eval1` to the path.

3 In the **Data File Name** field, enter `evalunkn.d`.

4 In the **Operator Name** field, enter your name.

5 In the **Sample Name** field, enter a sample name.

6 Enter the **Vial** number for your sample location in the ALS.

7 In the **Method Sections to Run** area, select **Data Acquisition and Data Analysis.**

8 Select **OK and Run Method**. The method is run and the summary quantitation report is automatically generated after the run is completed.

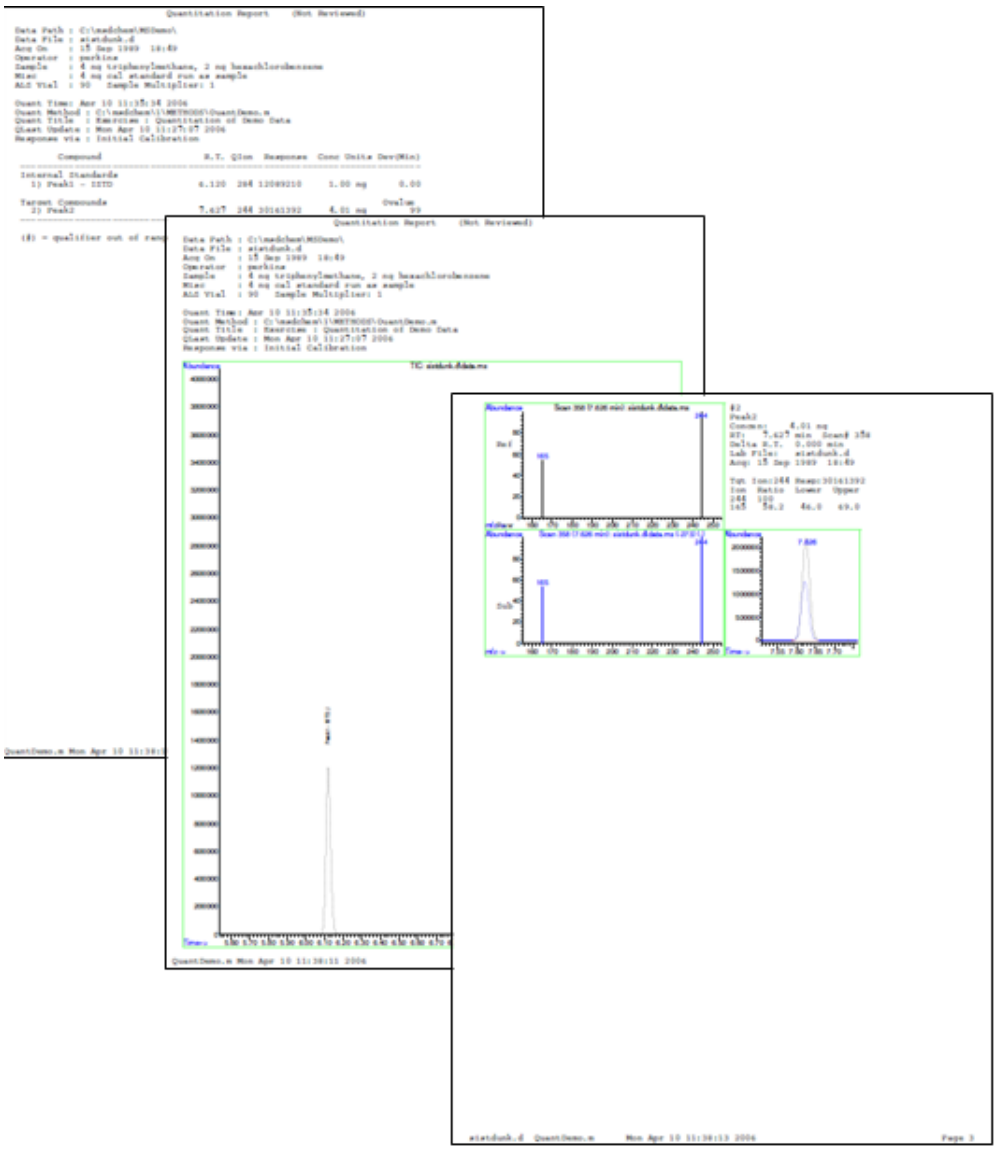


Figure 46 Summary quantitation report

Generate a Detailed Report for Previously Acquired Data

Load the method

1 Start the data analysis program by using the desktop icon,  .

2 From the **Instrument View**, select the **Load Method** button.  . The **Load Method** dialog box opens.

3 Navigate to and select **demosim.M** and then **OK**.

Load the data file

1 From the tool bar select the **Load Data File** button,  . The **Select Data File** dialog box.

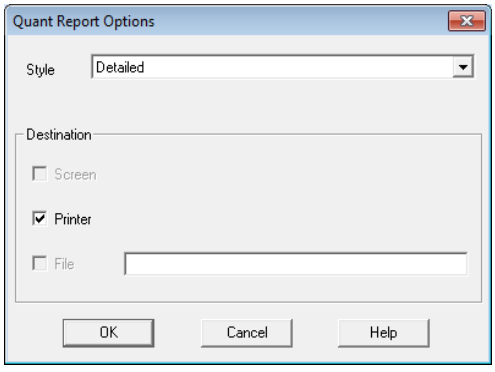
2 From the list, select **evalunkn.d**.

3 In the **Path** field, enter `C:\msdchem\1\DATA\eval1`.

4 Select **OK** to load the file and close the dialog box.

Generate a detailed quantitation report

1 Select the **Generate Reports** button,  . The **Quant Reports Options** dialog box opens.



2 From the **Style** drop down list, select **Detailed**.

3 In the **Destination** area, mark the **Printer** check box and clear all the other check boxes.

4 Select **OK**. The dialog box closes and the report is printed.

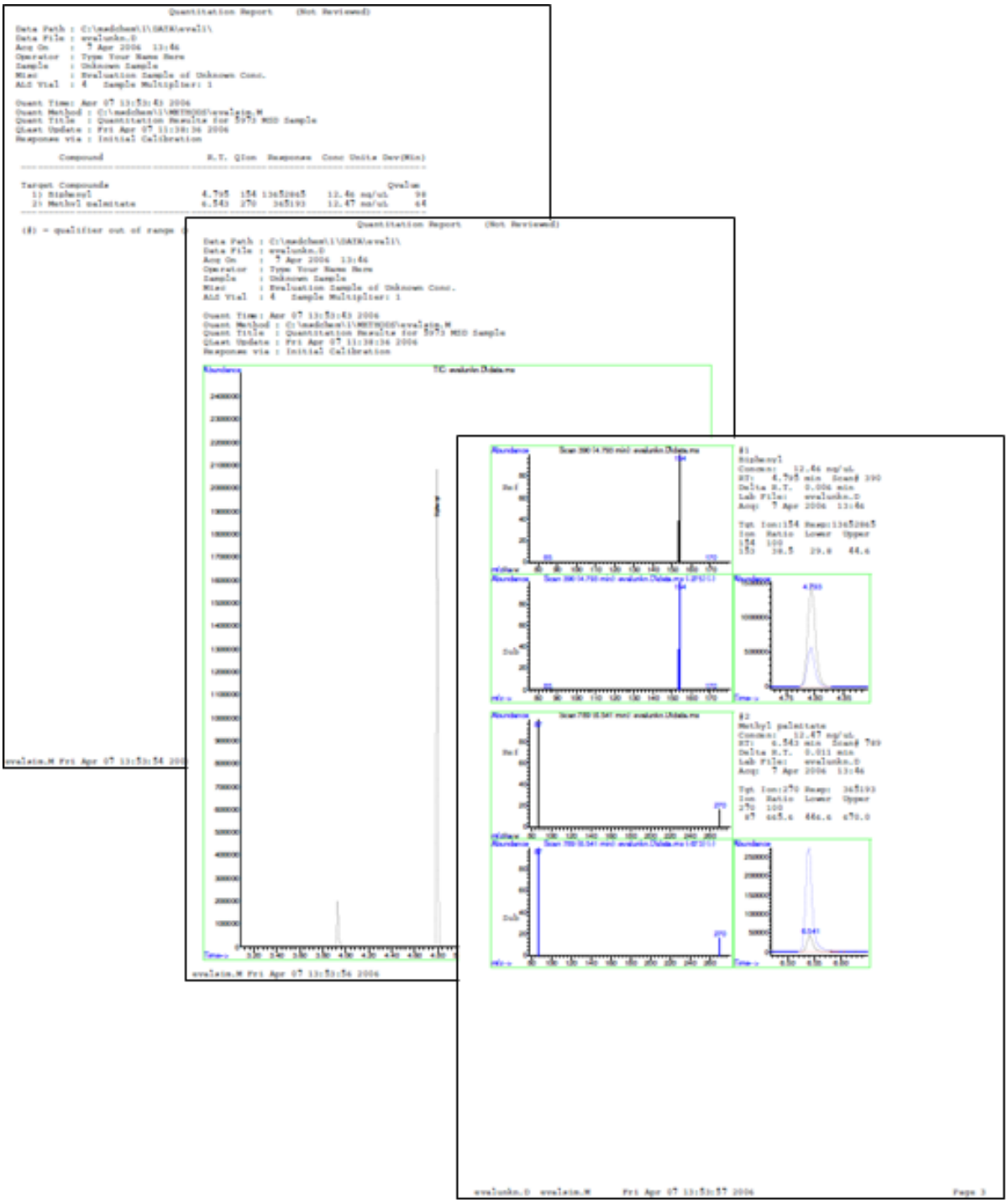
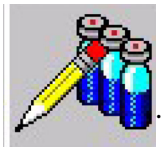


Figure 47 Detailed quantitation report

10
Recalibrate and Quantitate Unknowns

Regular recalibration is required to account for changes in your system. The ChemStation can perform this recalibration automatically using the recalibration sequence described here. This is normally done on a scheduled basis that precedes the running of samples.

Create a Recalibration Sequence

1 Select the **Edit Sequence** button,  . The **Sample Log Table** opens.

2 In sample row 1 under the **Type** column, click in the cell to activate the drop down list and select **Calibration.**

3 Under the **Vial** column, enter `1` if you place the lowest concentration sample in the ALS tray position 1.

4 Under the **Sample** column, enter `Std 2.5ng`.

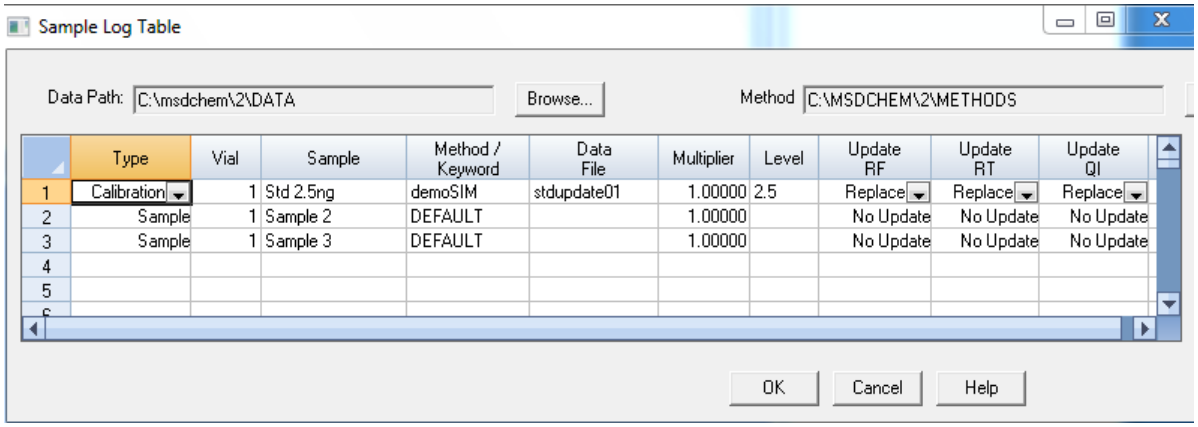
5 Under the **Method/Keyword** column:

   a Right mouse click and select **Browse for Method**. The **Browse for Folder** dialog box opens.

   b Navigate to and select **demosim.M**.

   c Select **OK**. The method name appears in the column.

6 Under the **Data File** column, enter `Stdupdate01`.



7 Under **Level** column, enter `2.5`.

8 Under **Update RF** column, click in the cell to activate the drop down list and select **Replace**.

9 Under **Update RT** column, click in the cell to activate the drop down list and select **Replace**.

10 Under **Update QI** column, click in the cell to activate the drop down list and select **Replace**.

11 Highlight rows 1 to 5.

12 Right mouse click and select **Repeat Row & increment**. Four lines are added to the table with incremented vial number and data file names.

13 In row 2, under **Sample** column, change the value to **Std 5 ng**.

14 In row 3, under **Sample** column, change the value to **Std 10 ng**.

15 In row 4, under **Sample** column, change the value to **Std 25 ng**.

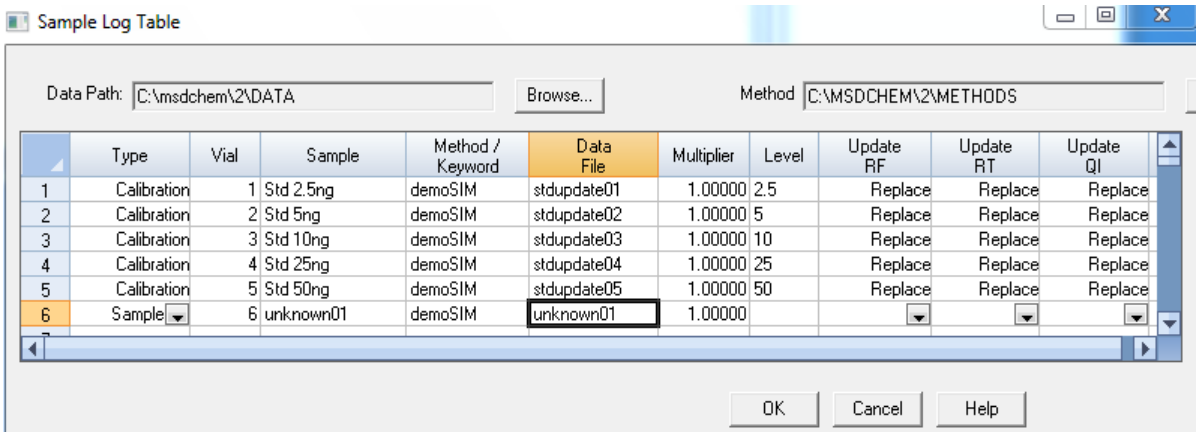
16 In row 5, under **Sample** column, change the value to **Std 50 ng**.

17 In row 2, under **Level** column, change the value to **5**.

18 In row 3, under **Level** column, change the value to **10**.

19 In row 4, under **Level** column, change the value to **25**.

20 In row 5, under **Level** column, change the value to **50**.



	Type	Vial	Sample	Method / Keyword	Data File	Multiplier	Level	Update RF	Update RT	Update QI
1	Calibration	1	Std 2.5ng	demoSIM	stdupdate01	1.00000	2.5	Replace	Replace	Replace
2	Calibration	2	Std 5ng	demoSIM	stdupdate02	1.00000	5	Replace	Replace	Replace
3	Calibration	3	Std 10ng	demoSIM	stdupdate03	1.00000	10	Replace	Replace	Replace
4	Calibration	4	Std 25ng	demoSIM	stdupdate04	1.00000	25	Replace	Replace	Replace
5	Calibration	5	Std 50ng	demoSIM	stdupdate05	1.00000	50	Replace	Replace	Replace
6	Sample	6	unknown01	demoSIM	unknown01	1.00000				

21 In row 6, enter an unknown sample for analysis as shown in the figure.

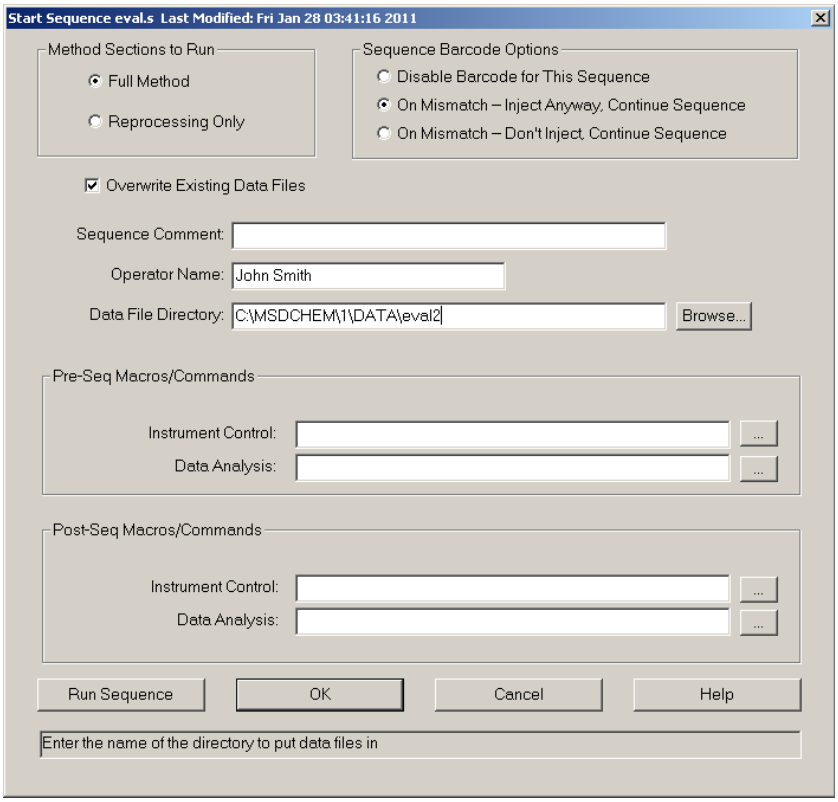
22 Select **OK** to close the **Sample Log Table**.

Save the Sequence

1. Select the **Save Sequence As...** button,  . The **Save Sequence** dialog box opens.
2. In the **File name** field, enter `updatequant`.
3. Select **Save**. The dialog box closes and the sequence is saved.

Run the Sequence

1 Select the **Run Sequence** button,  . The **Start Sequence** dialog box opens.



2 In the **Method Sections to Run** area, select **Full Method**.

3 In the **Sequence Comment** field, enter a description of the sequence.

4 In the **Operator Name** field, enter your name.

5 In the **Data File Directory** field, add `eval2` to the path.

6 Select **Run Sequence**. The calibration table of the demoSIM method is updated and the unknown sample results are calculated/reported with the recalibrated calibration curve.

11
Create a Cool Down Method

This chapter describes how to create and store a method to use for instrument maintenance tasks. Using this type of method helps prevent damage to the instrument electronics and columns and avoid injuries such as burns or shocks.

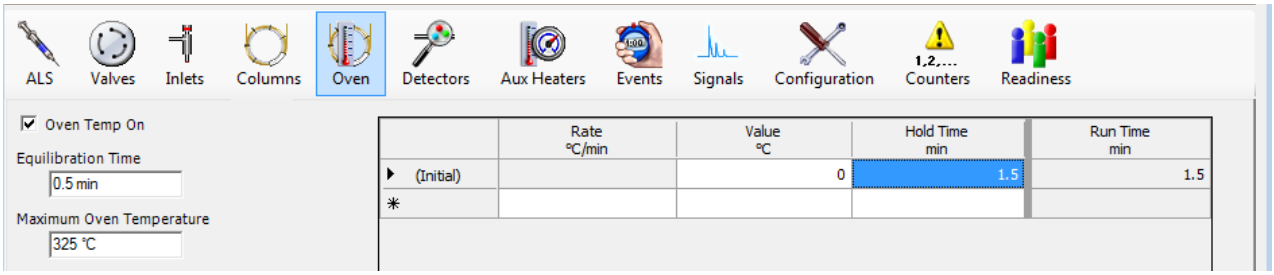
Create the Cool Down Method

1 Select **View > Instrument Control**.

2 Select the **GC Edit Parameters** button,  . The **GC Edit Parameters** window opens.

3 Select the **Oven** button,  . The oven parameters are displayed.

4 In the **Oven Ramp** table, clear the **Rate** and **Value** entries.



5 Select the **Inlets** button,  . The inlet parameters are displayed.

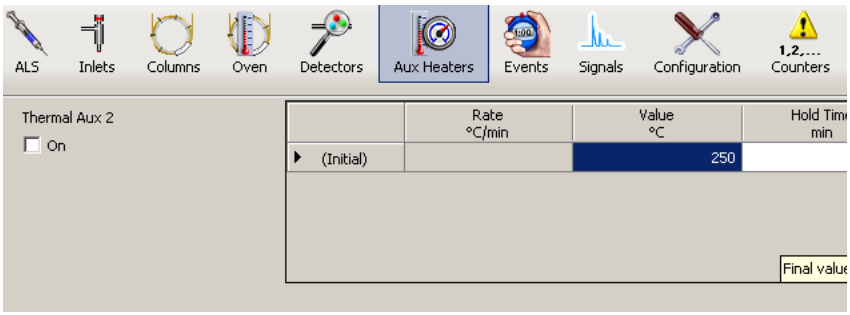
6 Select the **front** or **back** tab, depending on your hardware configuration.

7 Mark the **Heater** check box and enter `35°C` in the corresponding field.

8 Mark the **Pressure** check box. Column flow must be maintained to prevent damage to the column when hot.

9 Select the **AUX** button,  .

10 Clear the **On** check box for the **Aux 2 Heater**.



11 Select **OK.**

12 Select the **Save Method** button,  . The **Save Method As** dialog box opens.

13 In the **Method File** field, enter `cool down`.

14 Select **OK**.

Use the Cool Down Method

To use the cool down method, load the method, access the E**dit GC Parameters** window, and right mouse click in the right panel. Select **Download Method to GC** from the context menu. A confirmation message is displayed.

Select **OK** to close the message and return to the **GC Edit Parameters** window.

When the GC enters the Ready state, perform the maintenance.

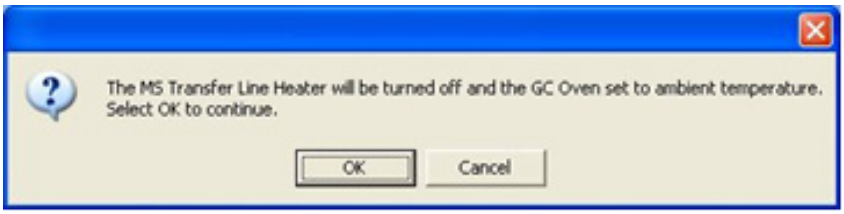
12
Shut Down the System

This chapter describes how to shut down the MS and GC.

Shut Down the MS

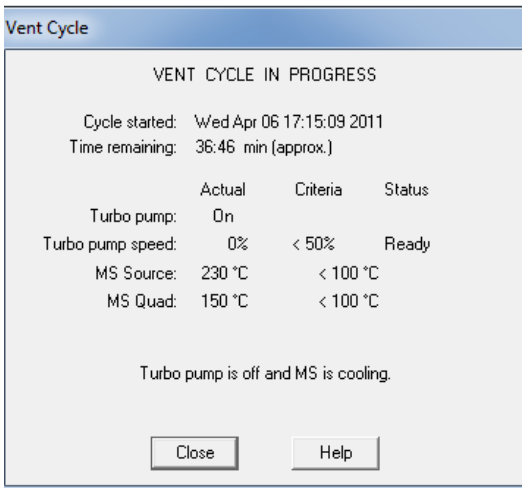
1 Select **View > Tune and Vacuum Control**....

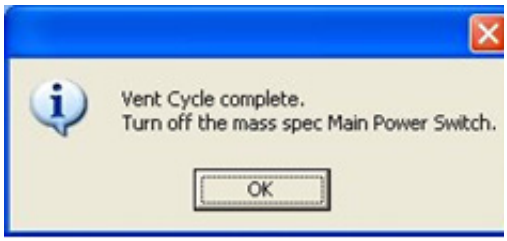
2 Select **Vacuum > Vent...** A confirmation message appears.



3 Select **OK**.

4 The **Vent Cycle** dialog status window opens and remains open until the vent is complete. You can close the dialog box by selecting **Exit**, however, the process continues. To reopen the **Vent Cycle** status window, select **View > Vacuum Status**.





5 Select **OK** to close the dialog box.

Do not turn off the MS at this time if you are first cooling down the instrument. The **Instrument Control** window will close when a configured instrument is powered off.

6 Select **Close**.

Shut Down the GC

1 In **Instrument Control,** load the GC cool down method.

2 Access the **Edit GC Parameters** window.

3 Right mouse click in the right panel and select **Download Method to GC** from the context menu. A confirmation message is displayed.

4 Select **OK** to close the message and return to the **GC Edit Parameters** window.

5 Close the **Edit GC Parameters** window and exit the ChemStation.

6 When the GC enters the Ready state turn off the power to the GC and the MS.

7 Turn off the carrier gas.

8 Power off the PC and all peripheral equipment.

13
Frequently Asked Questions

Q. How often should the MSD be tuned?

A. Perform an Autotune on a regular basis: weekly or monthly depending on use of the MSD. Perform a Check Tune daily to validate the performance of your instrument. If needed, perform a Quick Tune.

Q. There are two autotune options: Tune MSD and Quick Tune. What are the differences between them?

A. The Tune MSD maximizes the instrument sensitivity over the calibrant (PFTBA) mass range (69, 219, and 502). Quick Tune updates the peak width, mass assignment, and abundance.

Q. An analyte elutes before the solvent peak. How can data be acquired before the solvent peak as well as after?

A. Method parameters that control the MSD can be modified to update the method to capture data prior to the solvent peak. To update the method, use the timed events table to turn off the filament and data detection after the analyte elutes but before the solvent elutes. Set an event to turn the filament and the detector back on after the solvent peak has eluted.

Q. The sensitivity for some analytes has become reduced while some are not being detected at all. How can this be corrected?

A. Decreased sensitivity with the GC/MSD system may be caused by the following situations:

- Sample: Analytes have evaporated or deteriorated in the sample.
- Column: Column may be contaminated; column maintenance is recommended.
- GC Inlet: Inlet liner, split vent, or septum may be dirty, damaged or contaminated; inlet maintenance is required.
- Column Connection: Loose injection port ferrule or MSD transfer line ferrule, column installed incorrectly at the inlet or transfer line.
- Injector: The syringe is plugged with septum material or is using an incorrect sampling volume.

- Ion Source: The ion source has become contaminated or dirty; clean the source or replace the necessary parts.
- Method Parameters:
  - MSD Parameters: Incorrect mass assignments are being used with your method.
  - GC Parameters: Method uses incorrect split ratio or requires a longer purge time.

To improve your sensitivity:

- Perform autotune to verify MSD performance.
- Refer to the hardware manual for step by step troubleshooting procedures.
- Call Agilent Technologies Customer Support.

Q. When loading a data file, the error message **"No MS Data"** appears. What does this mean and what is the cause?

A. **"No MS Data"** means that the data file selected does not contain the data.ms within the datafile.d. Typically this occurs when the user forgets to save the MS data file within the method parameters, the remote start/stop cable is not connected, or the acquisition was aborted or terminated.

Q. When right-clicking on the TIS, the spectrum does not display, and the cursor is a (+) instead of a line. What causes this?

A. This is generally caused by the manual integration feature turned ON in Data Analysis. In this mode, to turn OFF manual integration, use the **Manual Integration** option in the data analysis option dialog box. The cursor in the chromatogram window should return to a vertical line.

Q. How does the Match Quality of library search results relate to the compound?

A. The Match Quality of the unknown is identified as the reference. Values greater than 90 are very good matches. Values less than 50 mean that substantial differences exist between the unknown and reference and the match should be regarded as uncertain. Differences in probability values of ±5 are generally not significant. An asterisk (*) before the probability value indicates that the molecular ion was used in the match. Because many factors affect the match quality and ordering of the compounds in the hit list, the list should be viewed as an interpretative guide to the unknown's identity. It is the chemist's responsibility to

determine whether the match identity is correct. For example, graphical comparison of the unknown's mass spectrum with that of an authentic sample, knowledge of the sample's history, and other pertinent information should be considered.

Q. Why does the library search list different spectra for the same compound?

A. Commercially available databases such as NIST or WILEY libraries contain MSD data for instruments from several manufacturers for one compound. This means search results may list duplicate compounds. To avoid this duplication, edit your Search Strategy to remove duplicate CAS numbers. See the online help for instructions on how to do this.

Q. Can a compound in a spectral library be viewed manually?

A. Yes it can. The Parametric Retrieval feature allows you to manually specify search criteria for your spectra. It retrieves a spectrum from the specified library based on those criteria and displays the results. The online help contains instructions for how to set up the criteria for your manual search.

Q. Can a chromatogram be redrawn to a different scale? How?

A. There are three ways to redraw the image of a chromatogram:

- Zoom in the area of interest in the existing chromatogram. Click within the area of interest and drag the cursor to define the area for the new chromatogram.
- Using the **Data Analysis** menu, click **Chromatogram > Chromatogram Scaling...** Select the chromatogram to be rescaled, and specify the scaling method to be used.
- Use the **DRAW** command to rescale the chromatogram and define the window location of the image. Refer to the online help for more detailed instructions on how to perform this action.

Q. After column maintenance or replacement, the chromatographic peak is missing. How can the peak be recovered?

A. The chromatographic peak is normally determined by the retention time window where the peak of interest would display. After a column change or maintenance, this retention time will shift. Perform a retention time update.

Q. Why would an Extracted Ion Chromatogram (EIC) be used instead of a Total Ion Chromatogram (TIC) for quantitation?

A. An EIC gives more stable results compared to the TIC.

Q. If autointegration does not work on a peak, can it still be integrated?

A. Yes, the peak can be integrated manually. For some cases, manual integration mode is the only method to use. Turn on manual integration under **Tools > Options** > **A/B.** Select the area of the peak that you would like to integrate. Refer to the online help for complete instructions on how to manually integrate.

Q. How can integration results be exported?

A. Click **Chromatogram > Integration Results**... Tabulation of the integration results associated with the current data file is displayed. Click the **Copy** button to save tabulated data to the clipboard. Now the results can be pasted into another application package.

Q. How can chromatogram graphics be exported?

A. To copy a selected Data Analysis window to the clipboard use the **Tools** > **Copy Window** menu. Answer the prompt for the number of the graphics window to be copied ('1' for spectrum, '2' for TIC). Click **OK** to copy the selected window to the clipboard. Now the graphics can be pasted into another application package. Alternatively, right-click **Data Analysis**, right-click in the window of interest, and copy and paste the image into another application.

Q. Why are the integration results on the quantitation report and my integration results different?

A. Integration results on the quantitation report are generated using the extracted ion chromatogram (EIC) of the target ion specified in the compound on the first page of the quantitation database while the integration results generated manually are based on the total ion chromatogram (TIC). Specific integration events can be used to integrate if the compound data file is specified on the third page of the quantitation results database. If the extracted ion chromatogram and the total ion chromatogram use the same integration event file, you may get the same integration results.

Q. Why would the chromatogram show a peak if the quantitation report shows a N.D?

A. There are two possible reasons. First, incorrect integration events may be used for quantitation. To check this, open the **Edit Compound** dialog with **Calibration >Edit Compound**....Select the compound of interest on the left panel. Click the **Advanced** tab to show the Integration Parameter File being used. The second cause may be that the concentration of the peak was lower than the quantitation limit. Integration parameters are set to integrate at least the lowest concentration standard sample peak. The area reject or other event may restrict small peaks from being integrated. In this case, the peak is lower than the quantitation limit; therefore, N.D. is appropriate. Please refer to the online help for additional information.

Q. Why does the quantitated data file show different results for the qualifier ion ratio?

A. The qualifier ion ratio on the first page of the quantitation database is calculated using the abundance of the qualifier ion relative to the abundance of the target ion when the compound was registered (=abundance ratio). The qualifier ion ratio can also be calculated using the integration of the spectrum (area of the curve) of the qualifier ion relative to the integration of the target ion spectrum (=area ratio). You can specify which way you would like the qualifier ion ratio to be calculated. See the online help for instructions.

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