# M3 MicroLC-TE System

## For TripleTOF® Systems

### System Integration Test and Data Log

RUO-IDV-05-1537-C | D5088500 C

December 2016

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# Introduction 1

This document provides the procedures for preparing and performing an LC-MS/MS system integration test for the SCIEX M3 MicroLC-TE system configured with the C18 HALO Peptide-ES 0.5 mm i.d. × 5 cm and the Chrom XP C18 trap columns and one of the following SCIEX mass spectrometers:

- TripleTOF® 4600 system
- TripleTOF® 5600/5600+ system
- TripleTOF® 6600 system

**CAUTION: Potential System Damage. Prior to operating the system, refer to "Operational Precautions and Limitations" in the *Operator Guide*.**

# About the Test

Use this test as a measure of the M3 MicroLC-TE system performance in isolation of the performance of the other components. Results from the test can become the baseline performance for the system. The test can be performed regularly and used as a system quality control test in the future.

Perform this test when the mass spectrometer is known to be operating well and meeting performance specifications. Refer to the *Ion Source Tests, Specifications, and Data Log* document.

Repeat the test until consistent peak shape and peak intensity are achieved.

## Time Required

Approximate time required:

1. Sample preparation: 15 minutes
2. Create the methods and batch: 10 to 15 minutes
3. Equilibrate the system: 3 to 5 minutes
4. Perform the test: 27 minutes
5. Verify the test results: 15 minutes

## Required Materials

- C18 HALO Peptide-ES 0.5 mm i.d. × 5 cm column (PN 5039577)

- Chrom XP C18 trap column, cartridge holder (PN 5027467), and cartridge (PN 5028897)
- Triazine System Suitability Solution (PN 4376887)
- 50 µL stainless steel sample loop (PN 5040770)
- 65 µm i.d. electrode (PN 5029342)

# Create the Methods 2

**Note:** The active hardware profile must include the CTC autosampler and the Eksigent control software to perform a run with the Analyst® TF software. The hardware profile is configured at installation. However, if a different computer is being used or the Analyst® TF software has been reinstalled, then the hardware profile might not be correct. Before creating the methods, verify that the correct hardware profile is present or create a hardware profile. Refer to the *Operator Guide* for information on creating and verifying a hardware profile.

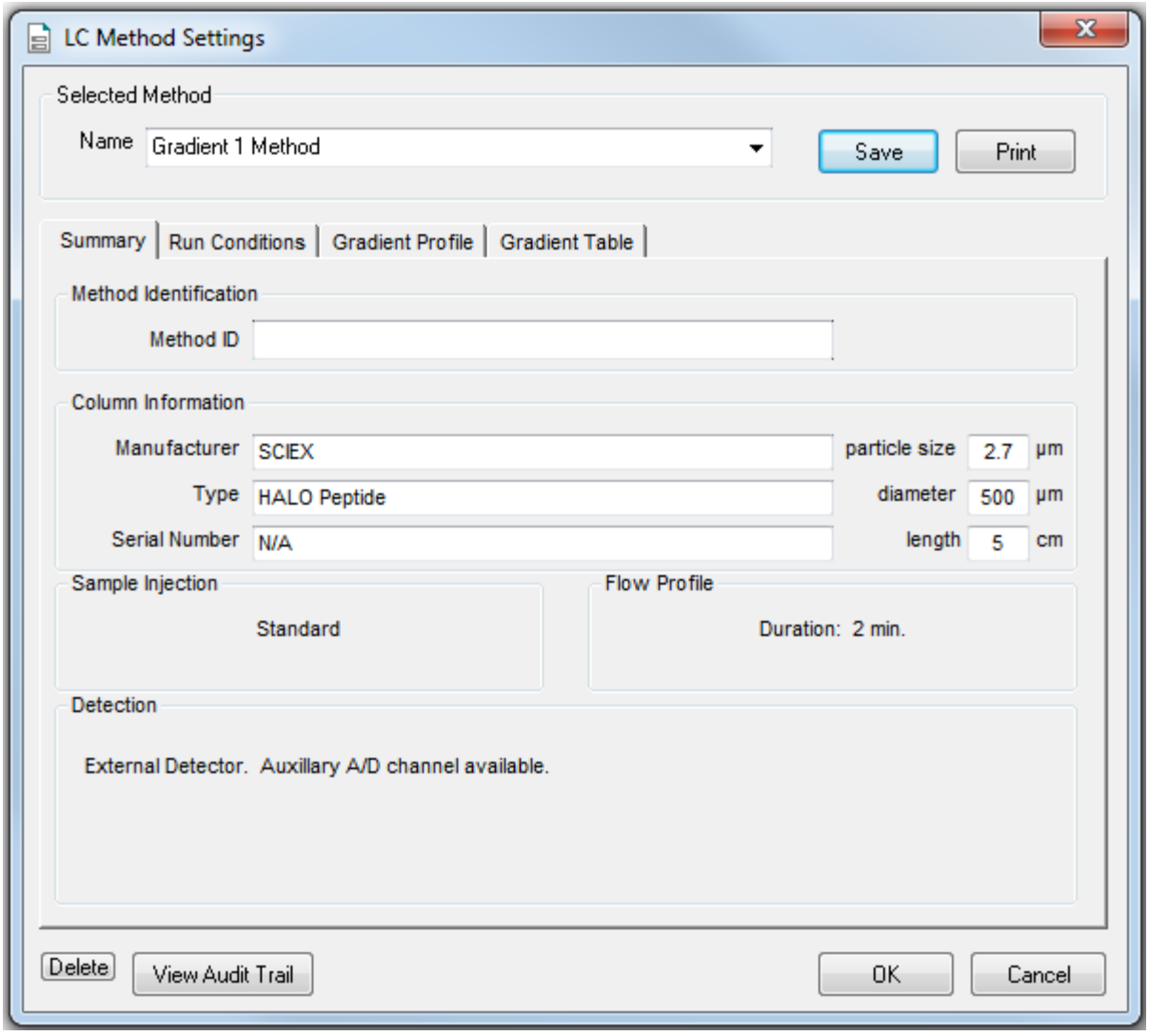
Create the LC methods (one for the Gradient 1 pump and one for the Gradient 2 pump) in the Eksigent control software and the autosampler and acquisition methods in the Analyst® TF software.

## Create the Gradient LC Method

This method is used to separate the samples on the analytical column.

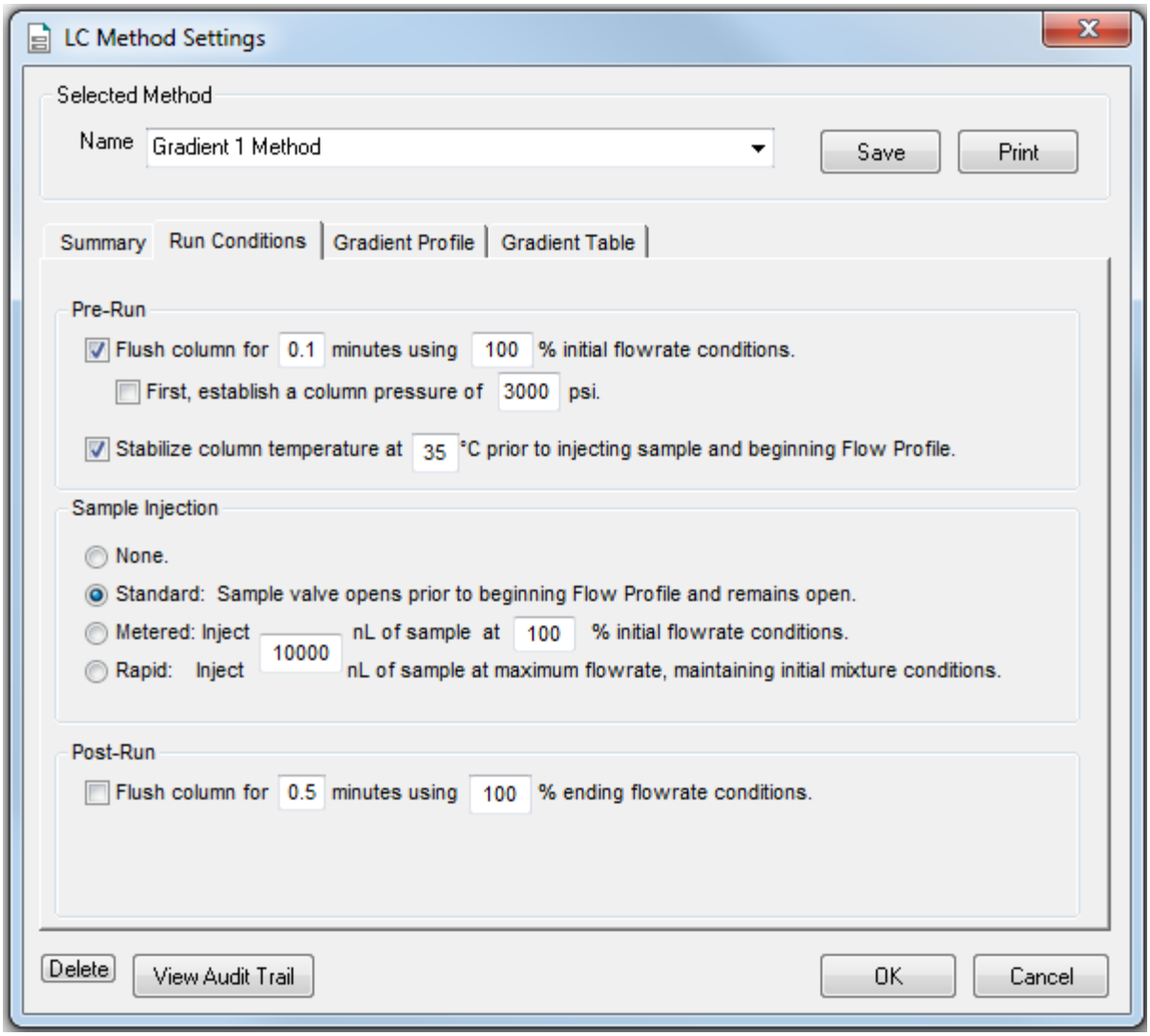
1. In the Eksigent control software Acquisition window, click the arrows to select **Gradient 1** in the **Channel** area.
2. Click **LC Methods** to open the LC Method Settings dialog.
3. In the **Name** field, type **Gradient 1 Method**, and then click **Save**.
4. On the Summary tab, type the values shown in Figure 2-1.

**Figure 2-1 Gradient 1 Method—Summary Tab**



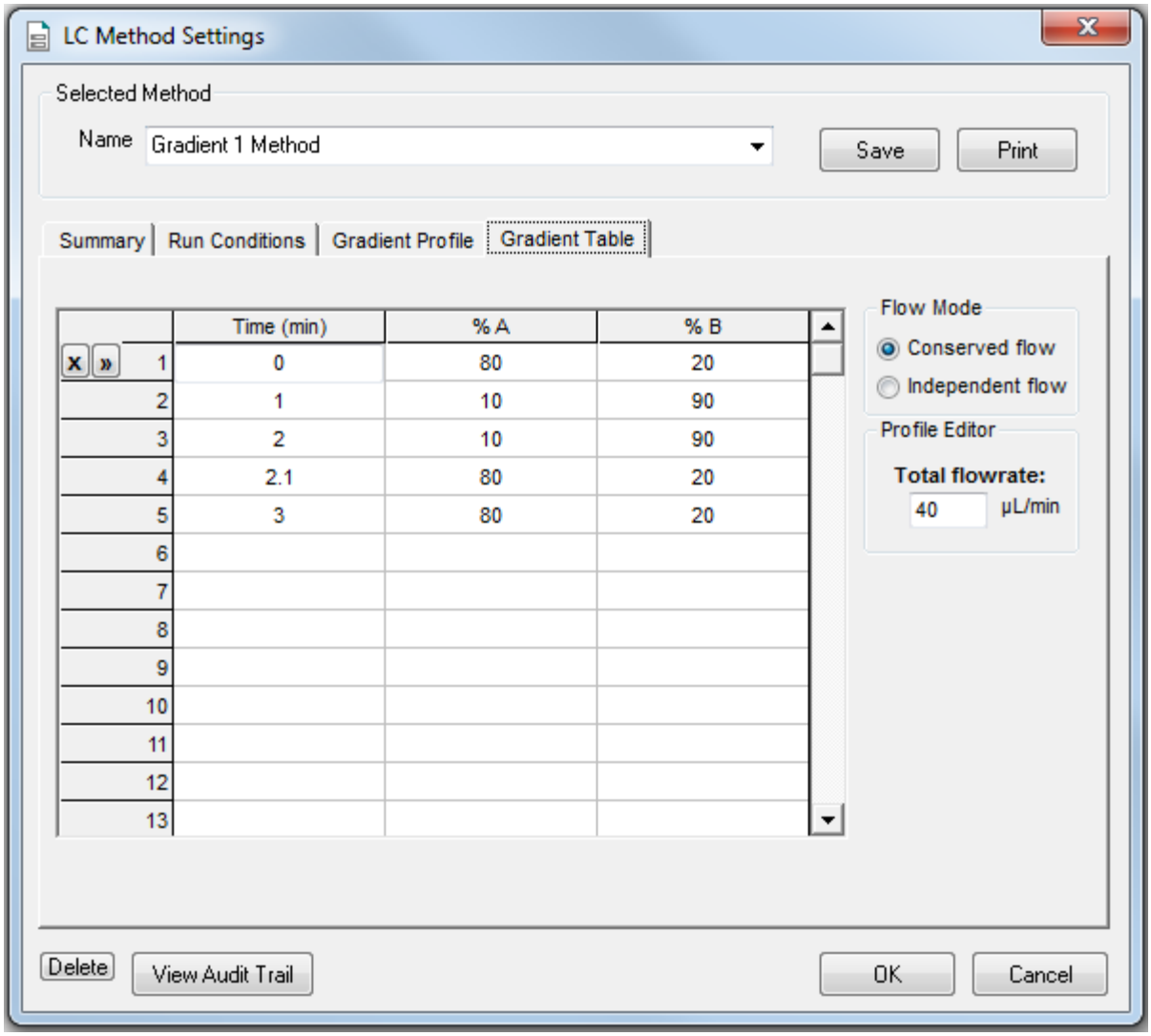

5. Click the Run Conditions tab and type the values shown in Figure 2-2.

**Figure 2-2 Gradient 1 Method—Run Conditions Tab**



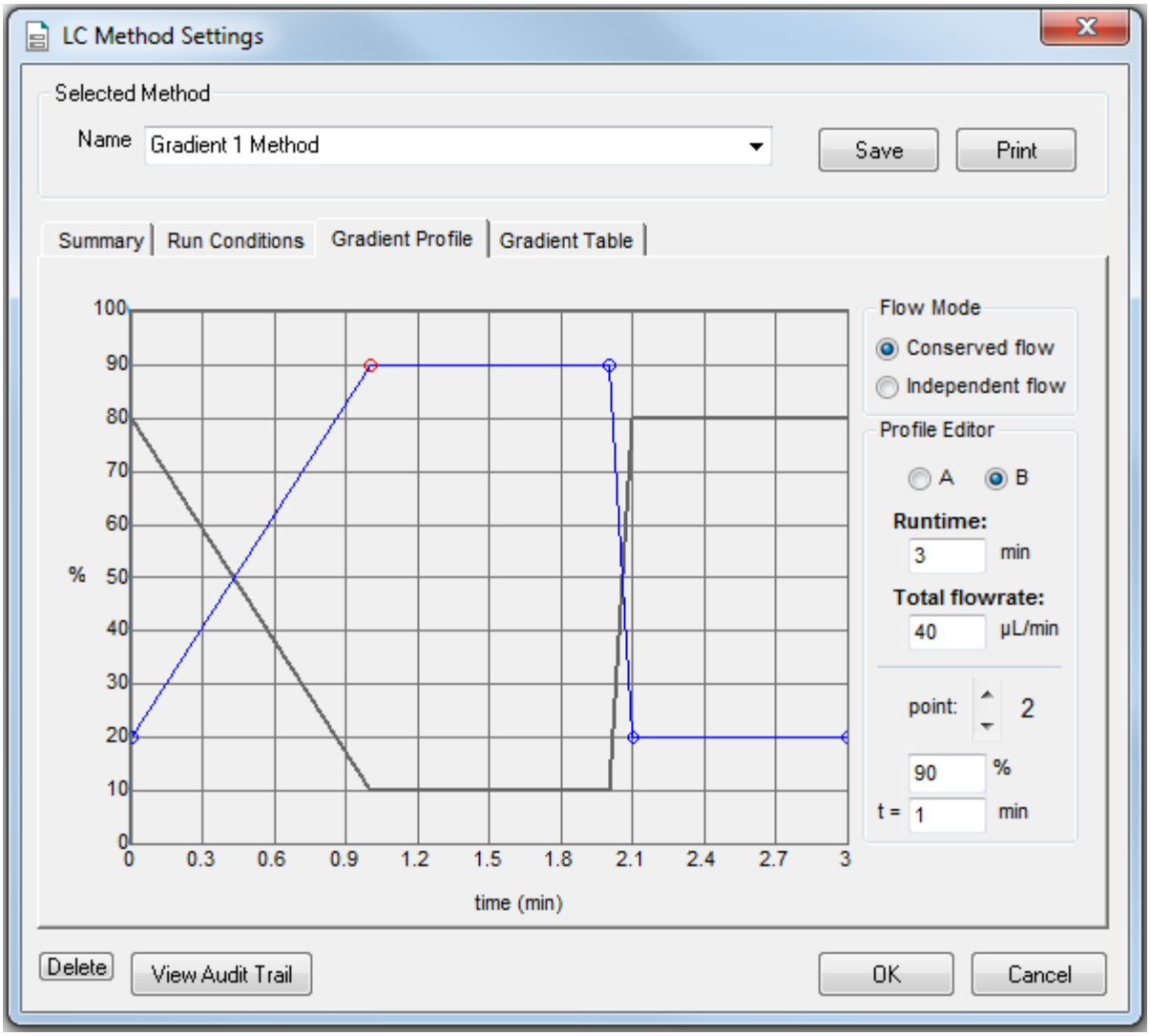

6. Click the **Gradient Table** tab and set the flow mode, the gradient parameters, and the flow rate. Refer to Figure 2-3.

**Figure 2-3 Gradient 1 Method—Gradient Table Tab**




7. Click the Gradient Profile tab to view a graphical representation of the gradient. Refer to Figure 2-4.

**Figure 2-4 Gradient 1 Method—Gradient Profile Tab**




The last two steps in the method allow for the aqueous solvent to flow through the sample loop before the next sample is injected.

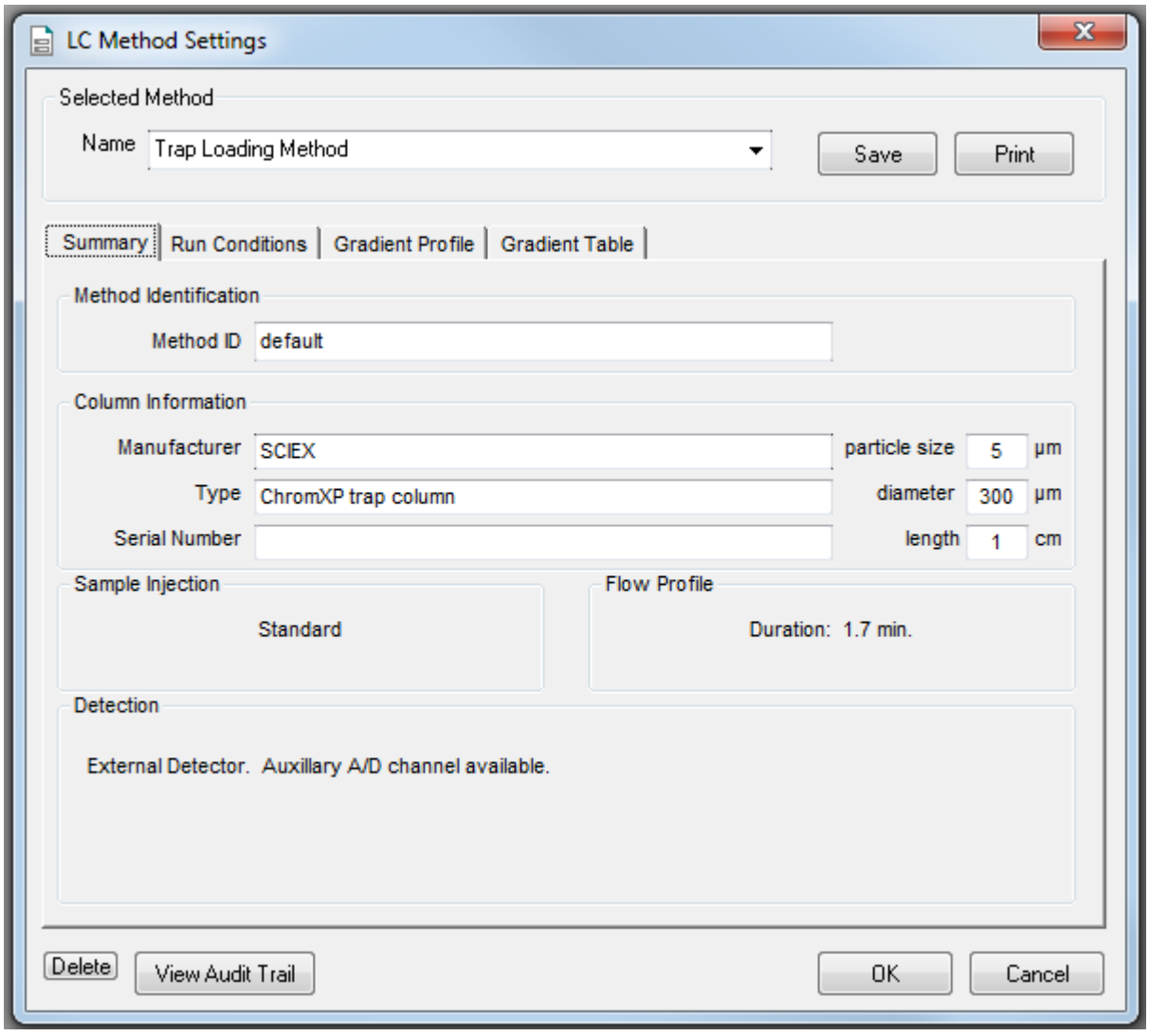
8. Click **Save** and then click **OK**.

# Create the LC Method to Load the Trap Column

This method is used to load the sample onto the trap column.

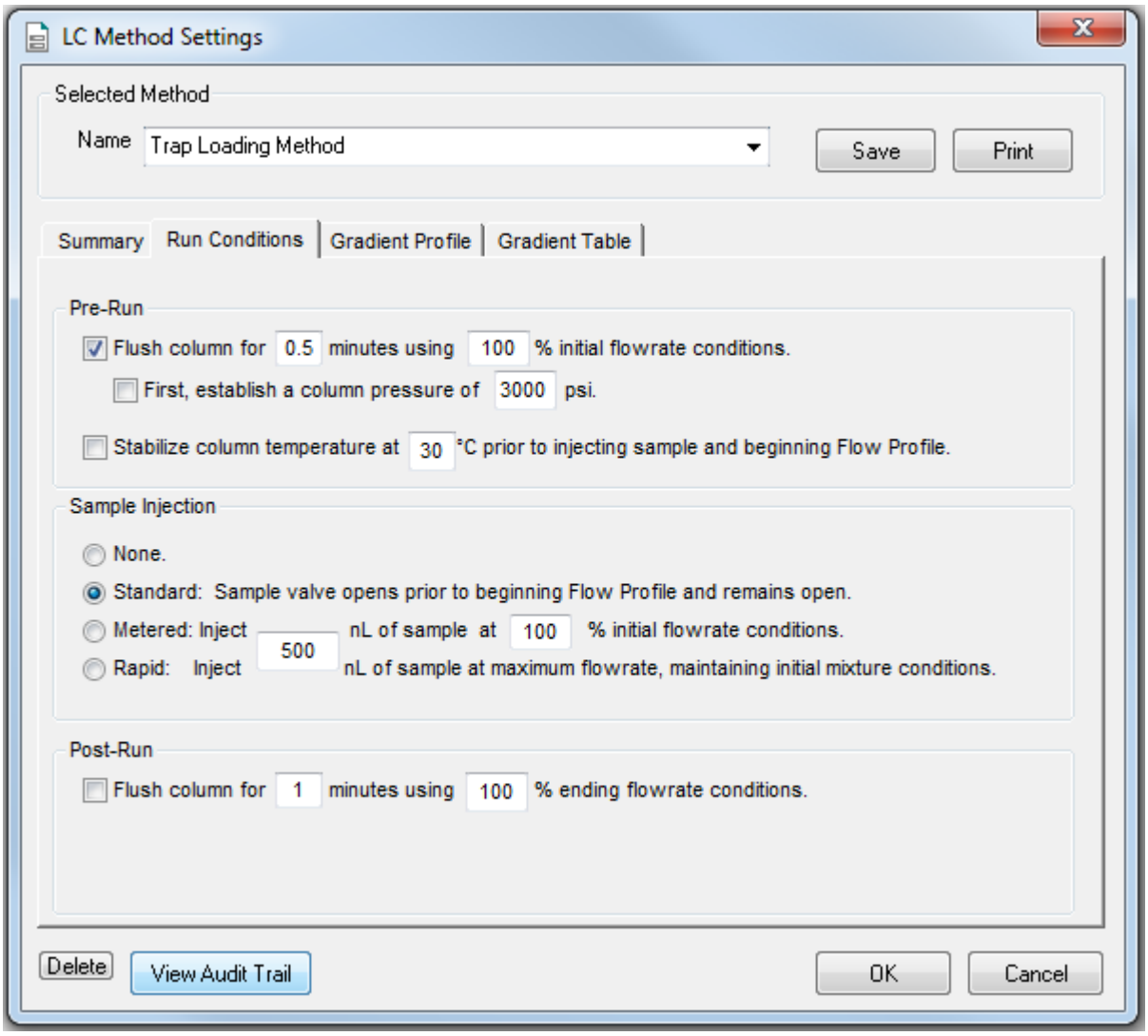
1. In the Eksigent control software Acquisition window, click the arrows to select **Gradient 2** in the **Channel** area.
2. Click **LC Methods** to open the LC Method Settings dialog.
3. In the **Name** field, type **Trap Loading Method** and then click **Save**.
4. In the Column Information section, type the values shown in Figure 2-5.

**Figure 2-5 Trap Loading Method—Summary Tab**



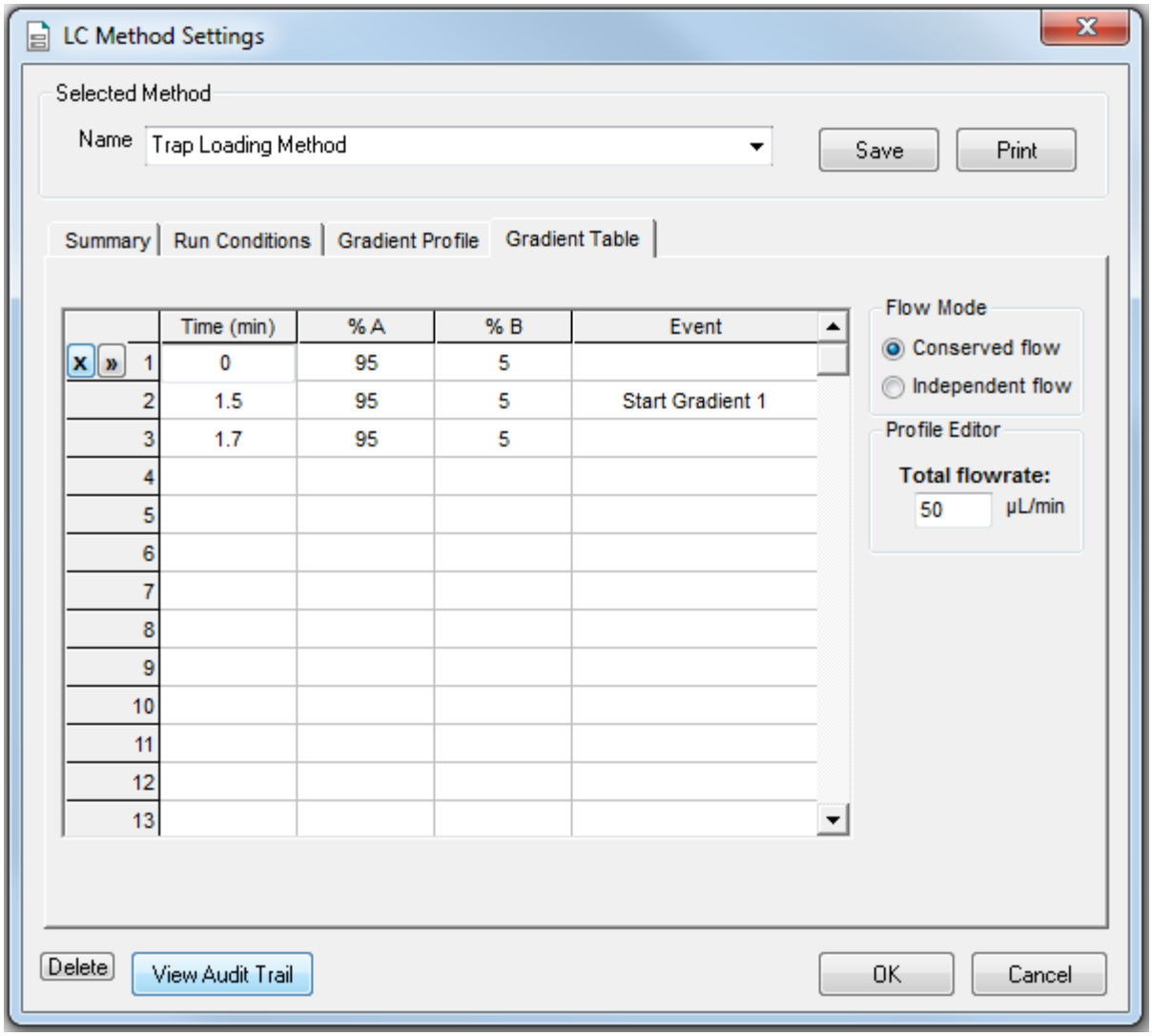
5. Click the **Run Conditions** tab and then type the values shown in Figure 2-6.

**Figure 2-6 Trap Loading Method—Run Conditions Tab**




6. Click the **Gradient Table** tab and then set the flow mode, the gradient parameters, and the flow rate. Refer to Figure 2-7.

**Figure 2-7 Trap Loading Method—Gradient Table Tab**



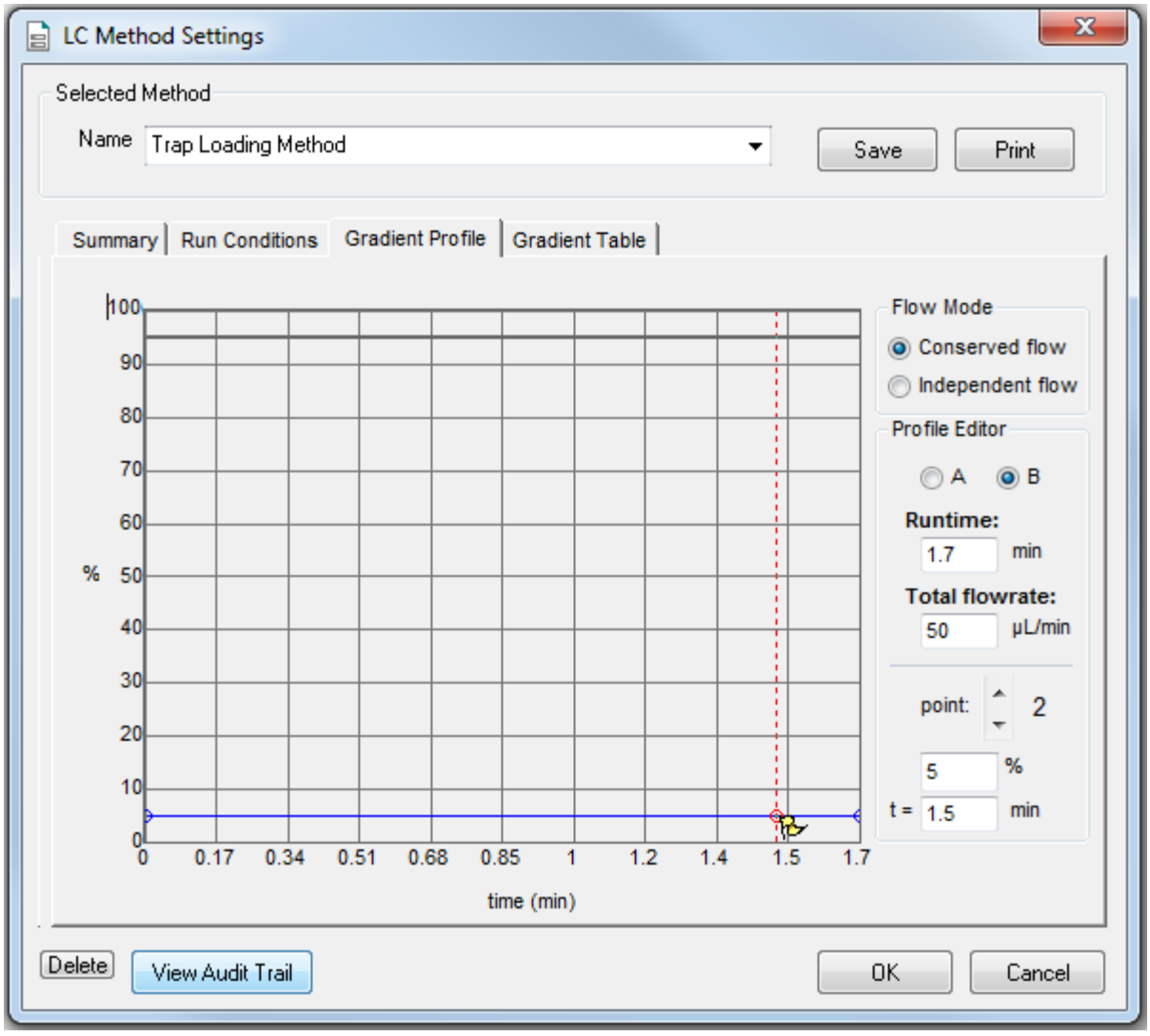

**Note:** If there is carryover from one sample to the next, add a step at the end of the method to wash the sample loop with the organic mobile phase.

7. At 1.5 min, click the **Event** cell and then select **Start Gradient 1**.

   This event starts the Gradient 1 pump, which switches the trap column in-line with the analytical column. The sample will be eluted from the trap column onto the analytical column.

8. Click the **Gradient Profile** tab to view a graphical representation of the gradient. Refer to Figure 2-8.

**Figure 2-8 Trap Loading Method—Gradient Profile Tab**




9. Click **Save** and then click **OK**.

# Create the Autosampler Method for the System Integration Test

1. On the Navigation bar in the Analyst® TF software, under **Acquire**, double-click **Build Acquisition Method**.

   The Acquisition Method Editor opens.
2. In the Acquisition Method Browser pane, click **CTC PAL Autosampler**.
3. In the **Available Cycles** list, select **M3 MicroLC Trap Elute RevA.**

   **Note:** The autosampler method installed with the system may have a different name than listed above. Use the most recent autosampler method supplied by SCIEX.
4. In the **Injection Volume** field, type **60** (the volume to be aspirated into the sample loop).
5. Edit parameters in the Cycle Arguments table.
   a. Type **1** in the **Front Volume (µL)** field.
   b. Type **1** in the **Front Airgap Volume (µL)** field.
   c. Type **0** in the **Needle Gap for 2nd and Final Vlv Clean** field.
   d. Select **Wash2** for **Final Wash Solvent.**

   **Note:** The **Rear Airgap Volume (µL)** field requires a value of at least 0.01.
6. Verify that the parameters in the **Cycle Arguments** table are as shown in the following table.

   If the values are not the same, then edit them as needed.

**Table 2-1 Cycle Arguments Table—Trap-and-Elute Parameters**

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Rear Airgap Volume (µL) | 1 | First Wash Solvent | Wash1 |
| Rear Volume (µL) | 1 | Valve Clean Time 1 (s) | 5 |
| Front Volume (µL) | 1 | Needle Clean Time 1 (s) | 2 |
| Front Airgap Volume (µL) | 1 | Second Wash Solvent | Wash1 |
| Sample Aspirate Speed (µL/s) | 2 | Needle Clean Time 2 (s) | 2 |
| Pullup Delay (ms) | 500 | Valve Clean Time 2 (s) | 5 |
| Num of Wash1 PreDips | 1 | Replicate Count | 1 |

**Table 2-1 Cycle Arguments Table—Trap-and-Elute Parameters (continued)**

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| Num of Wash2 PreDips | 0 | Final Wash Solvent | Wash2 |
| Inject to | LCVlv1 | 0 or 1 Final Cleans | 1 |
| Injection Speed (µL/s) | 1 | Final Needle Clean Time (s) | 2 |
| Needle Gap for 2nd and Final Vlv Clean (mm) | 0 | Final Valve Clean Time 2 (s) | 5 |

# Create the Acquisition Method

This method has two experiments, a TOF MS scan and a product ion scan.

1. Select the LC method for the Gradient 1 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 1**.
   b. Click **...** (browse) to view the available LC methods.
   c. Click **Gradient 1 Method** and then click **Open**.
2. Select the LC method for the Gradient 2 pump.
   a. In the Acquisition Method Browser pane, click **Gradient 2**.
   b. Click **...** (browse) to view the available LC methods.
   c. Click **Trap Loading Method** and then click **Open**.
3. In the Acquisition Method Browser pane, click **Mass Spec** to create the mass spectrometer acquisition method.
4. Type the appropriate information in the **MS** tab to create the TOF MS scan.
   a. In the **Scan type** list, select **TOF MS**.
   b. Type **0.150010** in the **Accumulation time** field.
   c. In the **TOF Masses (Da)** section, type **100** and **1000** for the **Min:** and **Max:** masses.
   d. In the **Duration** field, type **3.001** minutes.
5. Click **Edit Parameters** to open the Parameters Settings dialog.

a. Set the Source/Gas Parameters as shown in Table 2-2.

**Note:** Source/Gas parameters can vary between systems and electrodes. Determine the best value for the system. Make sure that the electrode position is optimized before creating the acquisition method.

**Table 2-2 Source/Gas Parameters**

| Parameter | Value |
|---|---|
| Ion Source Gas 1 (GS1) | 60 (or as optimized) |
| Ion Source Gas 2 (GS2) | 30 (or as optimized) |
| Curtain Gas (CUR) | 30 (or as optimized) |
| Temperature (TEM) | 350 (or as optimized) |
| IonSpray Voltage Floating (ISVF) | 5000 (or as optimized) |

b. Click the **Compound** tab and set the Compound parameters as shown in Table 2-3.

**Table 2-3 Compound Parameters**

| Parameter | Value |
|---|---|
| Declustering Potential (DP) | 80 |
| Collision Energy (CE) | 10 |

c. Click **OK**.

6. Save the method as **System Integration Test_DATE**, where *DATE* is today's date.

# Prepare the System for Testing 3

## Verify System Readiness

Prior to running this test, make sure that the system is calibrated. To calibrate the system, perform the following procedures described in the *Operator Guide*:

- Reinitialize the Pressure Transducers
- Measure the Flow Rate
- Calibrate the Flowmeters, if necessary

## Prepare the 1 µg/mL Stock Solution

**WARNING! Toxic Chemical Hazard. Follow all safety guidelines when handling, storing, and disposing of chemicals. For heath and safety precautions, refer to the mass spectrometer *System User Guide*.**

This procedure generates 1 mL of a 1 µg/mL stock solution.

| Required Materials |
|---|
| • Methanol<br>• 100 µg/mL Triazine System Suitability Solution (PN 4376887) |

1. Create a 10 µg/mL solution using the volumes shown in the first row of Table 3-1.
2. Create a 1 µg/mL solution using the volumes shown in the second row of Table 3-1.

**Table 3-1 Solution Dilution**

| Stock Solution Volume | Dilution Solvent Volume | Final Concentration |
|---|---|---|
| 100 µL of 100 µg/mL triazine test mixture | 900 µL of MeOH | 10 µg/mL |
| 100 µL of 10 µg/mL sample solution | 900 µL of MeOH | 1 µg/mL |

# Prepare the Test Sample

| Required Materials |
|---|
| • Stock solution, prepared in Prepare the 1 µg/mL Stock Solution on page 18.<br>• 50:50 mixture of MeOH:$H_2O$<br>• Water with 0.1% formic acid |

1. Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:$H_2O$ and, for the final dilution, $H_2O$ with 0.1% formic acid, in a clean vial. Refer to Table 3-2.

   For the final dilution, be sure to use $H_2O$ with 0.1% formic acid.

2. Mix the solution for at least 30 seconds using a vortex mixer.
3. Transfer the solution to the autosampler vial, making sure that there is no bubble on the bottom of the vial.
4. Prepare the blank by filling an autosampler vial with Mobile Phase A, making sure that there is no bubble on the bottom of the vial. Refer to Equilibrate the System on page 20.

**Table 3-2 Sample Dilutions by Mass Spectrometer**

| System | Target Concentration | Dilution |
|---|---|---|
| TripleTOF® 4600 system<br>TripleTOF® 5600/5600+ system<br>TripleTOF® 6600 system | 1 ng/mL | Three serial dilutions:<br>a. 100 µL of stock solution + 900 µL of MeOH:$H_2O$ (to make 100 ng/mL)<br>b. 100 µL of 100 ng/mL + 900 µL of MeOH:$H_2O$ (to make 10 ng/mL)<br>c. 100 µL of 10 ng/mL + 900 µL of $H_2O$ with 0.1% formic acid |

# Equilibrate the System

1. Disconnect the tubing to the LC column inlet.
2. Verify that the mobile phases in Table 3-3 are loaded on the system.

**Table 3-3 Mobile Phases**

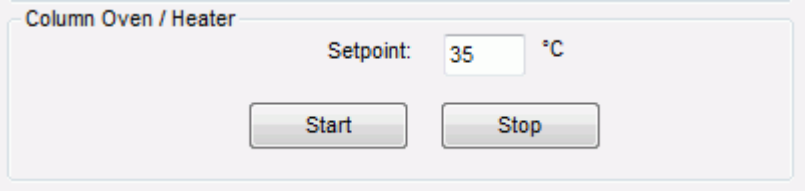
| Buffer | Mixture | Channel |
|---|---|---|
| **Gradient 1 Pump** | | |
| Buffer A | 100% water:0.1% formic acid | Channel A |
| Buffer B | 100% acetonitrile:0.1% formic acid | Channel B |
| **Gradient 2 Pump** | | |
| Buffer A | 100% water:0.1% formic acid | Channel A |
| Buffer B | 100% acetonitrile:0.1% formic acid | Channel B |

3. Allow the column oven to pre-heat.

**Note:** The column oven reaches the correct temperature quickly, but the column can take as long as 30 minutes to fully equilibrate.

   a. In the Eksigent control software, click **System > Direct Control**.
   b. Click the **Channel** buttons to select **Gradient 1**.
   c. In the **Column Oven/Heater** section, type **35** in the **Setpoint** field and then click **Start**.

**Figure 3-1 Direct Control Dialog–Column Oven/Heater Section**




   d. Close the compartment so that the oven can reach the specified temperature.
4. Make sure that the **Trap-Elute Mode** checkbox in the **Injection Valve Direct Control** section is checked.
5. Start the pump.

a. Select the **Conserved Flow (%)** option and set **A** to **80** and **B** to **20**.

   This is the mobile phase composition used for equilibration.

b. Type a **Total flowrate** of **50** µL/min.

c. Click **Start**.

6. Flush the injection valve.

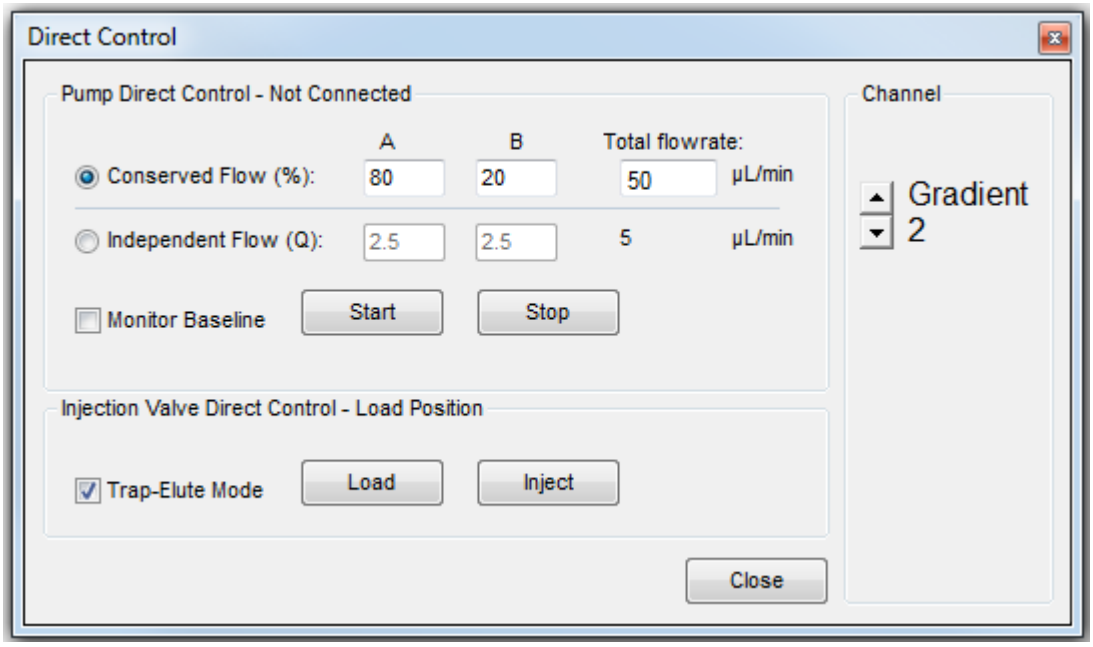
   a. Click the **Channel** buttons to select **Gradient 2**.

   b. In the **Valve Direct Control** section, alternate clicking **Load Position** and **Inject Position**.

   c. Make sure that the final position of the valve is at **Load**.

   d. In the **Pump Direct Control** section, click **Stop** and then connect the LC column.

**Figure 3-2 Direct Control Dialog**




7. In the Analyst® TF software, equilibrate the mass spectrometer.

   a. On the Navigation bar, under **Tune and Calibrate**, double-click **Manual Tuning.**

   b. In the tuning method, type the source and gas parameters from Table 2-2.

   c. Type **10** in the **Time [Min.]** field and then click **OK**.

8. In the Direct Control dialog, click the **Channel** buttons to select **Gradient 1**.

9. Click **Start** in the **Pump Direct Control** section.

10. Adjust the source and gas parameters as required until a stable spray is achieved and then click **Stop** to end tuning.

11. Click **Stop** in the **Pump Direct Control** section and then close the Direct Control dialog.

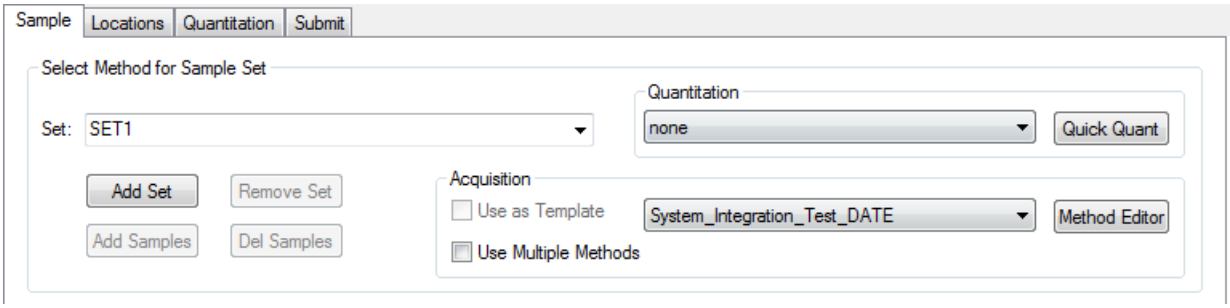
# Perform the System Integration Test 4

Create the acquisition batch, run the batch and then verify the results. Type the test results in the System Integration Test Data Log and Signoff on page 36.

## Create the Acquisition Batch

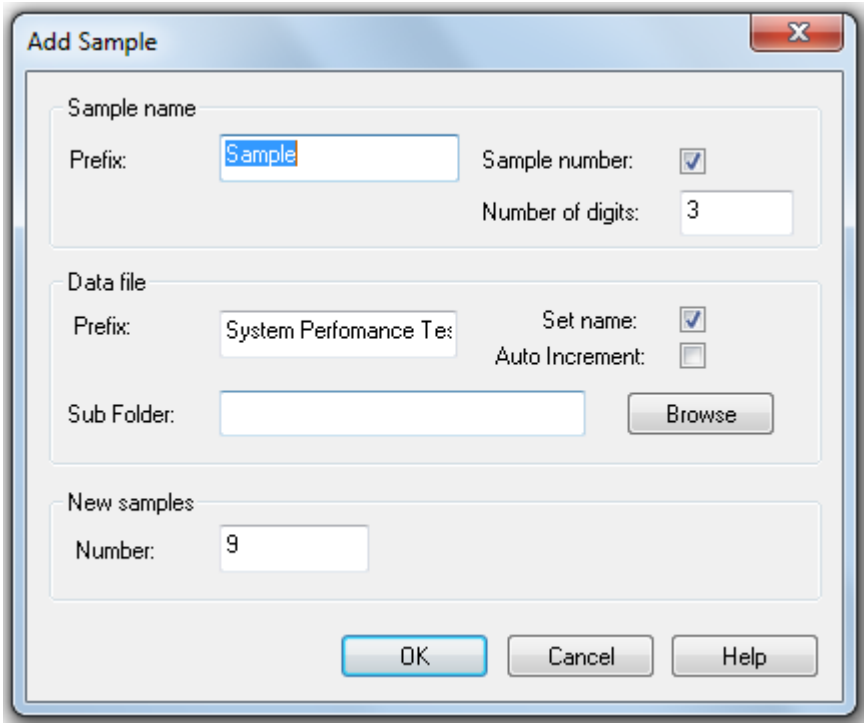
1. Open the Analyst® TF software.
2. On the Navigation bar, under **Acquire**, double-click **Build Acquisition Batch.**
3. On the **Sample** tab, in the **Acquisition** group, select the acquisition method created in Create the Acquisition Method on page 16 from the list.

**Figure 4-1 Sample Tab—Acquisition Group**



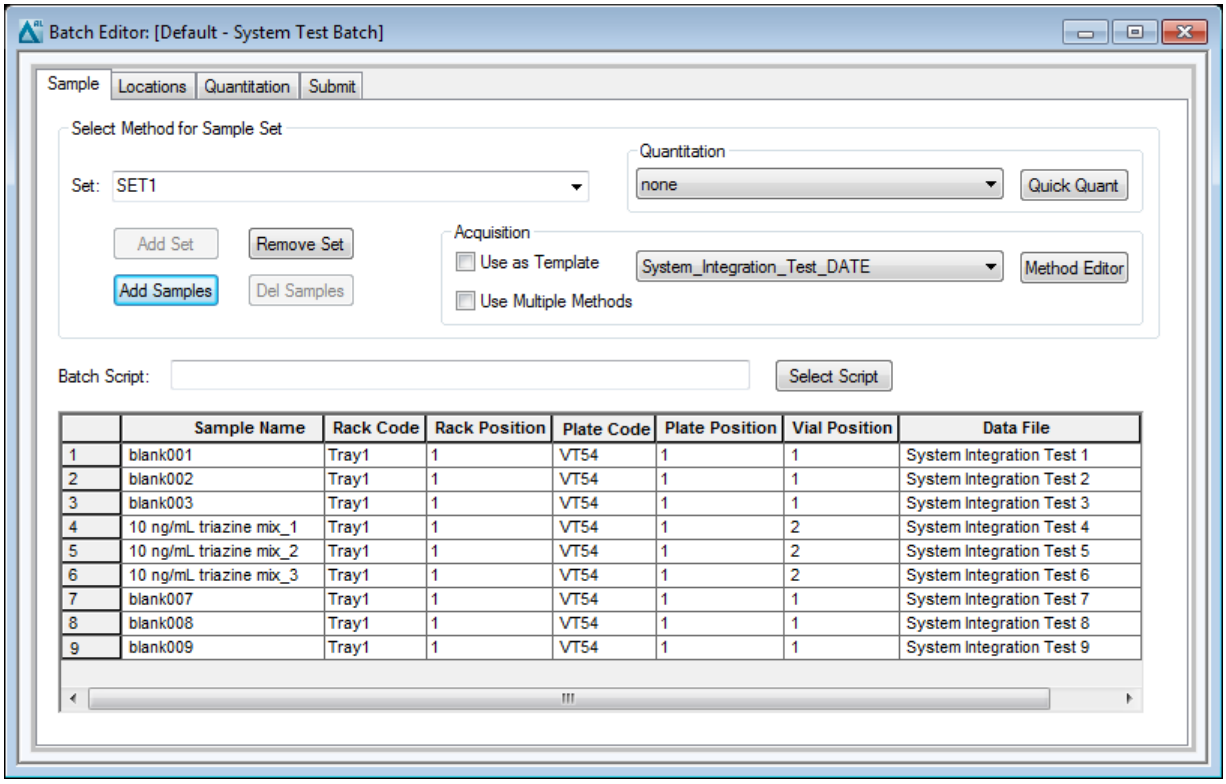

4. Click **Add Set**.
5. Click **Add Samples**.

**Figure 4-2 Add Sample Dialog**




6. Type the sample information shown in Figure 4-2 and then click **OK**.
7. Type the rack and plate position for the samples shown in Figure 4-3.

**Figure 4-3 Batch Editor—Sample Tab**




| | Sample Name | Rack Code | Rack Position | Plate Code | Plate Position | Vial Position | Data File |
|---|---|---|---|---|---|---|---|
| 1 | blank001 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 1 |
| 2 | blank002 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 2 |
| 3 | blank003 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 3 |
| 4 | 10 ng/mL triazine mix_1 | Tray1 | 1 | VT54 | 1 | 2 | System Integration Test 4 |
| 5 | 10 ng/mL triazine mix_2 | Tray1 | 1 | VT54 | 1 | 2 | System Integration Test 5 |
| 6 | 10 ng/mL triazine mix_3 | Tray1 | 1 | VT54 | 1 | 2 | System Integration Test 6 |
| 7 | blank007 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 7 |
| 8 | blank008 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 8 |
| 9 | blank009 | Tray1 | 1 | VT54 | 1 | 1 | System Integration Test 9 |

8. In the **Vial Position** column, type the position of the vial containing the test mixture or blank, as required.
9. In rows 4 through 6, edit the **Sample Name** field to read "1 ng/mL triazine mix_y", where "y" is 1, 2, or 3.
10. Make sure that the **Injection Volume** (in the table on the **Sample** tab) is 60 µL.
11. Select **File > Save** and type **triazine test** for the method name.

# Run the Batch

If this is the first time the column has been used, then run the batch once to condition the column and then run it a second time for the test.

1. Click **Submit** on the **Submit** tab.
2. Click **View > Sample Queue.**
3. Verify that the system is not in Tuning mode and then click **Acquire > Ready.**
4. Click **Acquire > Start Sample.**

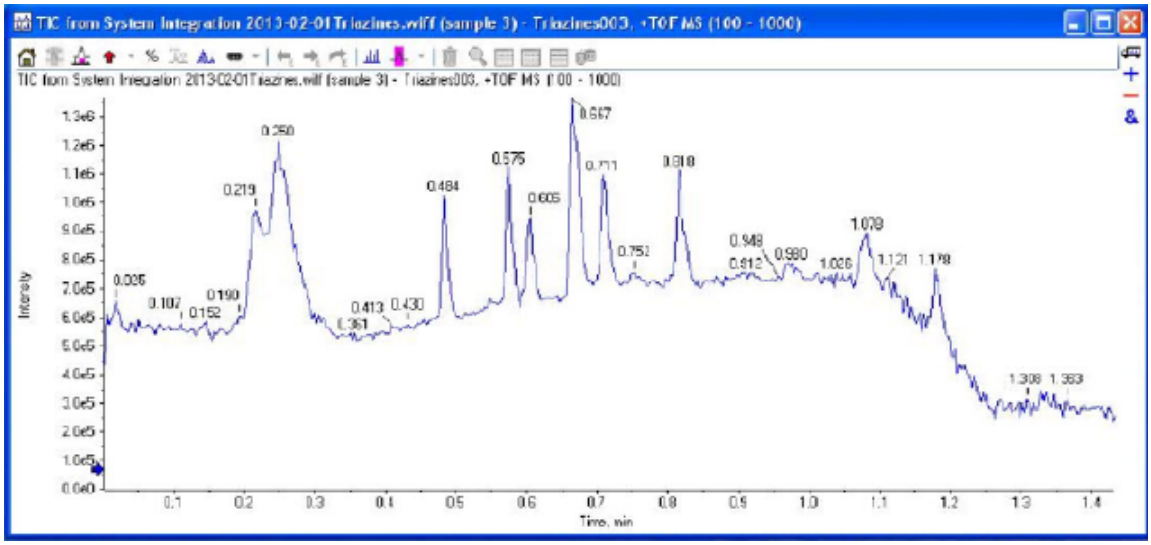
5. Monitor the system pressure ($P_C$) in the upper right corner of the Acquisition window of the Eksigent control software.

   During this test, the expected system pressure ($P_C$) for Gradient 1 should be <4000 psi.

6. If the first run was to condition the column, then run the batch again to acquire data for analysis.

   Refer to Figure 4-4 for representative data.

**Figure 4-4 Example Data for the System Integration Test—TIC in PeakView® Software**

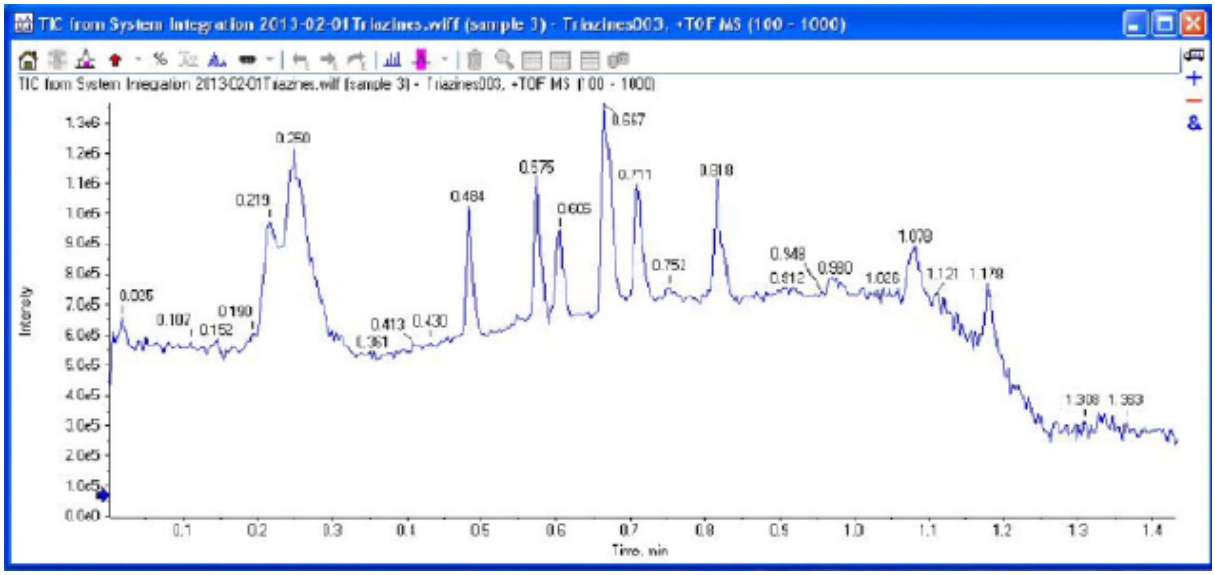



# Verify the Results Using the PeakView® Software

1. When the run is finished, open the file and view the TIC for the experiment.
   a. Double-click **PeakView** to open the **PeakView®** software.
   b. Click **File > Open Wiff Sample** to open the Select Sample dialog.
   c. Select the file and click **OK**.

      The TIC is shown.

**Figure 4-5 TIC for the TOF MS Scan of Triazines**




2. Create the XICs.
   a. Click **Show > Show Extract Ions Using Dialog.**
   b. In the Specify XIC Ranges dialog, type in the information from Table 4-1 and then click **OK**.

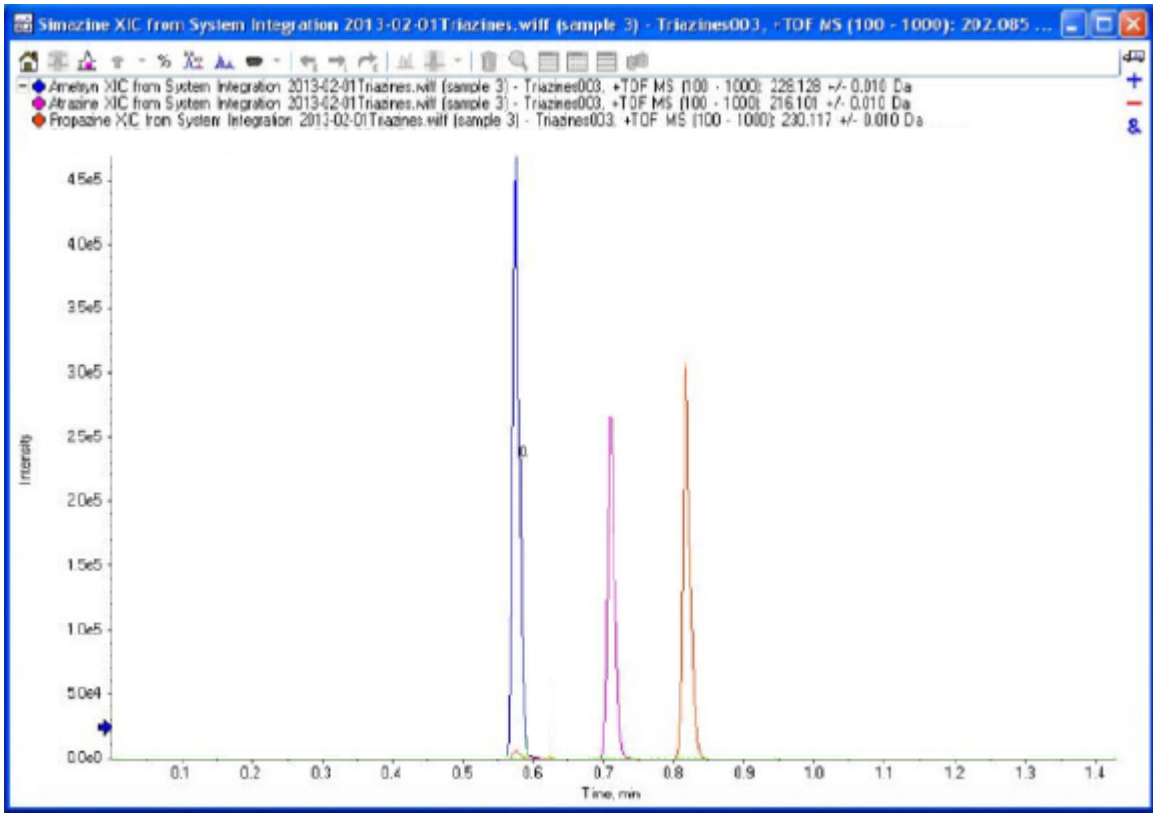
      The XICs are shown.

**Tip!** Field Service Employees can download a text file from the SharePoint site and save it locally for customer use. To use the file, in the Specify XIC Ranges dialog, right-click and select **Import From Text File**, and then select the file.

**Table 4-1 XICs to Extract**

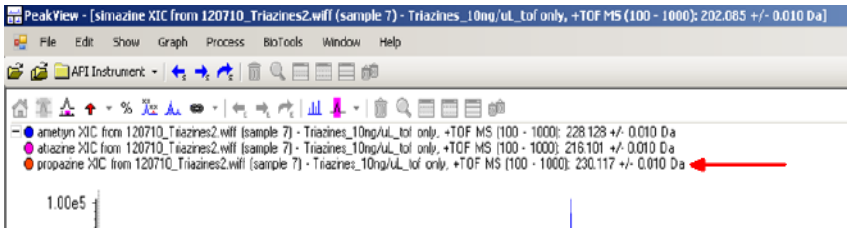
| Center | Width | Compound |
|---|---|---|
| C9H17N5S | 0.02 | Ametryn |
| C8H14ClN5 | 0.02 | Atrazine |
| C9H16ClN5 | 0.02 | Propazine |

**Figure 4-6 XICs for the Triazines in TOF MS Mode**



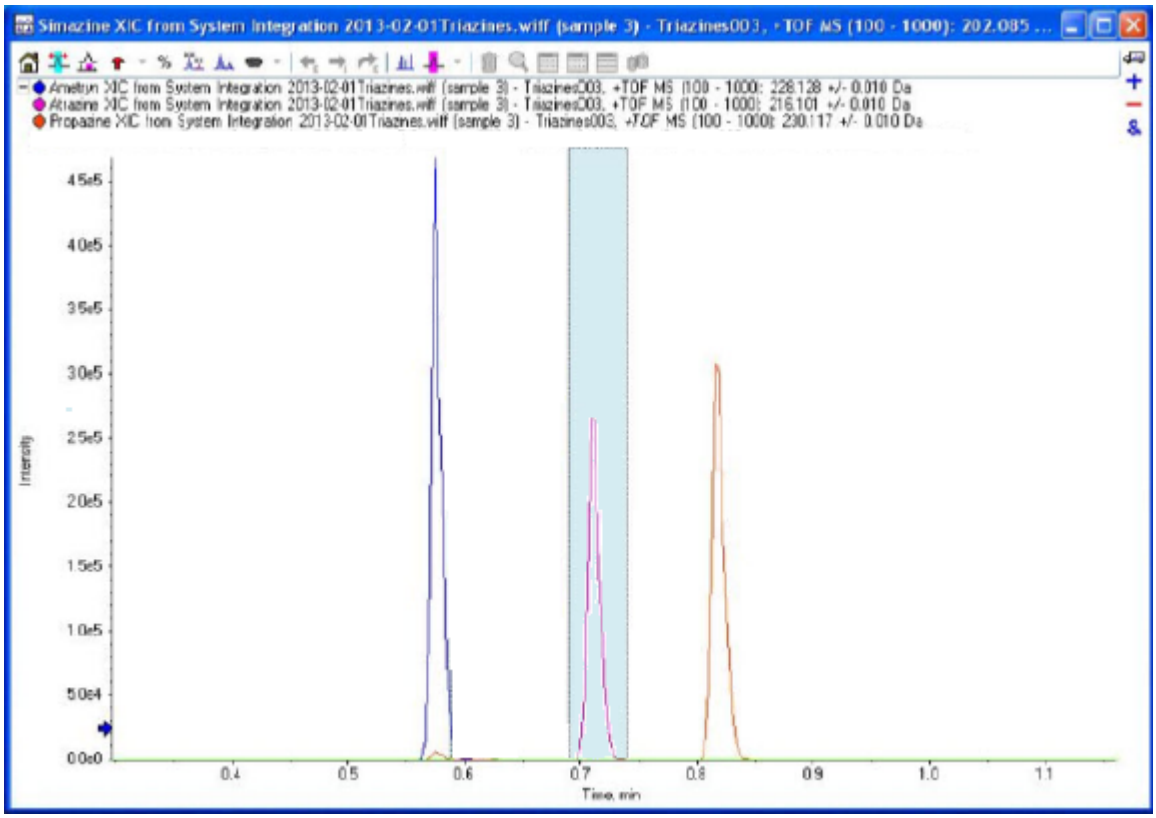

3. For each analyte in the plot, show the peak area and peak width at half-height.
   a. Select the analyte of interest by clicking the appropriate line.

**Figure 4-7 Select the Analyte**



b. Drag to select the peak in the plot.

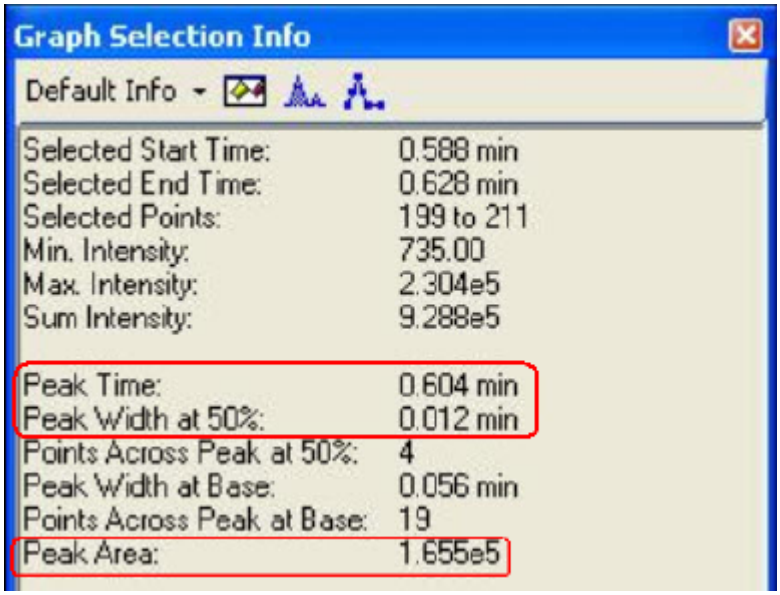
**Figure 4-8 Selecting the Peak**




c. Click **Window > Graph Selection Window.**

d. Type the **Peak Time**, **Peak Width at 50%**, and **Peak Area** into a text editor so the values can be copied for later use.

**Figure 4-9 Graph Selection Info Window Showing Peak Information**




4. Repeat step 3 for the other two samples.
5. Calculate averages and %CV for each analyte.

**Tip!** Field Service Employees can download a file from the SharePoint site and save it locally for customer use. To use the file, copy the peak areas, retention times, and peak widths at half-height recorded in 3 into the spreadsheet. Refer to Figure 4-10.

**Figure 4-10 Spreadsheet for Calculating Averages and %CV**

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **Analyte** | | **area 1** | **area 2** | **area 3** | | **mean** | **stdev** | **%CV** |
| 2 | | | | | | | | | |
| 3 | **ametryn** | | 2.69E+05 | 2.71E+05 | 2.83E+05 | | 2.74E+05 | 7057.148 | **2.571534** |
| 4 | **atrazine** | | 1.67E+05 | 1.83E+05 | 1.94E+05 | | 1.82E+05 | 13454.74 | **7.413079** |
| 5 | **propazine** | | 1.91E+05 | 2.22E+05 | 2.31E+05 | | 2.15E+05 | 20960.2 | **9.762553** |
| 6 | | | | | | | | | |
| 7 | | | | | | | | | |
| 8 | | | | | | | | | |
| 9 | **Analyte** | | **RT 1** | **RT 2** | **RT 3** | | **mean** | **stdev** | **%CV** |
| 10 | | | | | | | | | |
| 11 | **ametryn** | | 1.12E+00 | 1.21E+00 | 1.31E+00 | | 1.21E+00 | 0.095044 | **7.833284** |
| 12 | **atrazine** | | 1.12E+00 | 1.21E+00 | 1.31E+00 | | 1.21E+00 | 0.095044 | **7.833284** |
| 13 | **propazine** | | 1.12E+00 | 1.21E+00 | 1.31E+00 | | 1.21E+00 | 0.095044 | **7.833284** |
| 14 | | | | | | | | | |
| 15 | | | | | | | | | |
| 16 | **Analyte** | | **width 1** | **width 2** | **width 3** | | **mean** | **stdev** | |
| 17 | | | | | | | | | |
| 18 | **ametryn** | | 1.12E+00 | 1.21E+00 | 1.31E+00 | | 1.21E+00 | 0.095044 | |
| 19 | **atrazine** | | 1.12E+00 | 1.21E+00 | 1.32E+00 | | 1.22E+00 | 0.100167 | |
| 20 | **propazine** | | 1.13E+00 | 1.20E+00 | 1.31E+00 | | 1.21E+00 | 0.090738 | |
| 21 | | | | | | | | | |

6. For each analyte, type the average area and %CV, the average retention time and %CV, and the average peak width at half-height in the System Integration Test Data Log and Signoff on page 36.

# Troubleshooting 5

This section provides information for troubleshooting issues with the M3 MicroLC-TE system.

1. Repeat the system integration test. Refer to Perform the System Integration Test on page 23.
2. Examine the chromatograms for the blank injections.
   a. Are there peaks similar to those in the sample? If so, then there is carryover. Complete Identify and Resolve Carryover Issues on page 32 and then repeat the system integration test.
3. Compare the chromatograms for the sample to those from the original system integration test. Figure 5-1 on page 34 shows a chromatogram with good results.
   After each troubleshooting step, repeat the system integration test.
   a. Continue with Resolve General Issues on page 33.
   b. Do the retention times change from injection to injection? Refer to Resolve Drifting Retention Times on page 33.
   c. Are the peaks broader than or tailing more than the original chromatogram? Refer to Peak Widths are Too Broad or are Tailing on page 33.
   d. Is the background level high? Refer to Fronting, Pre-eluting or Low Intensity Peaks on page 33.
   e. Are peaks missing? Refer to Missing or Low Intensity Peaks on page 34.
4. Examine the pressure traces in the Eksigent control software. Refer to Table 5-1 on page 35 for anomalies in the pressure traces and their possible causes.

# Identify and Resolve Carryover Issues

For a well-tuned M3 MicroLC-TE system, carryover should be minimal. For a blank, there should be no peaks with retention times matching the sample. When carryover is present, it can cause several other issues, so carryover should be resolved first.

Carryover is most often caused by issues with the injection port or the dynamic load and wash (DLW) system.

## Test the Injection Port and the DLW System

- Inspect the injection port and make sure that there is no fluid leaking from the injection port. The presence of fluid at the injection port suggests an issue with the needle alignment at the port position and needle penetration, or the tightness of the fitting and the syringe needle.

- Run a test injection and make sure that the fluid is flowing out the waste line from the valve. To test, create a run with a 100 µL injection that will overfill the loop. During the flow, watch for bubbles exiting the waste line. The presence of bubbles can indicate a poor connection or issue with the operation or priming of the syringe or DLW system.

# Resolve General Issues

- Make sure that the correct solvents are being used and that the solvents in the bottle match the settings in the Eksigent control software.
- Make sure that the proper grounding is in place between the ion source and the LC system.

# Troubleshoot Peak-Related Issues

## Resolve Drifting Retention Times

If the column temperature varies over the course of the test, then retention times might drift.

- Make sure that the column heater is on during the test. The column can take up to 30 minutes to stabilize to the temperature of the column heater.
- Initialize the pressure transducers. Refer to "Re-initialize the Pressure Transducers" in the *Operator Guide*.

## Peak Widths are Too Broad or are Tailing

- Inspect all connections in the flow path to verify that there are no dead volumes.
- Look at connections post-column and around the trap column. A small increase in peak width is often seen when a trap column is used.
- Confirm that a microflow electrode is present in the ion source.

## Fronting, Pre-eluting or Low Intensity Peaks

- Make sure that Wash1 and Wash2 on the wash station are configured properly and have the proper solvents. The Wash1 solvent should be organic and the Wash2 solvent should be aqueous.
- Verify that the DLW pumps are operating properly and flowing through the injection valve/port.
- Make sure that the sample loaded by the autosampler is fully flushing the injection loop prior to injection.
- Make sure that the sample has been diluted in the proper solvent (that is, low organic in sample solution).

## Missing or Low Intensity Peaks

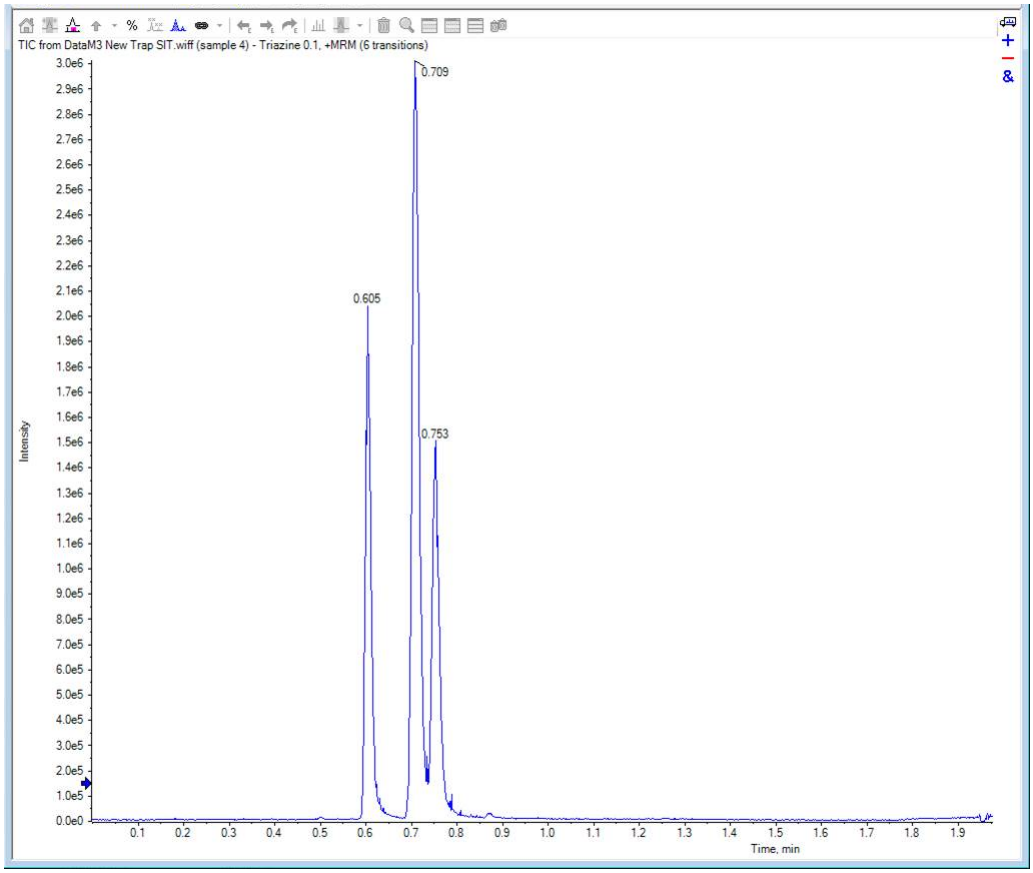
Make sure that the autosampler method has been configured as follows:

- There is a large enough volume loaded into injection loop.
- The **Inject to:** value is set to **LC Vlv1**, not **Waste.**

Make sure that the injection alignment is correct (position and needle penetration) and that the injection port fitting seals on the syringe needle.

# Troubleshoot Using the Chromatogram

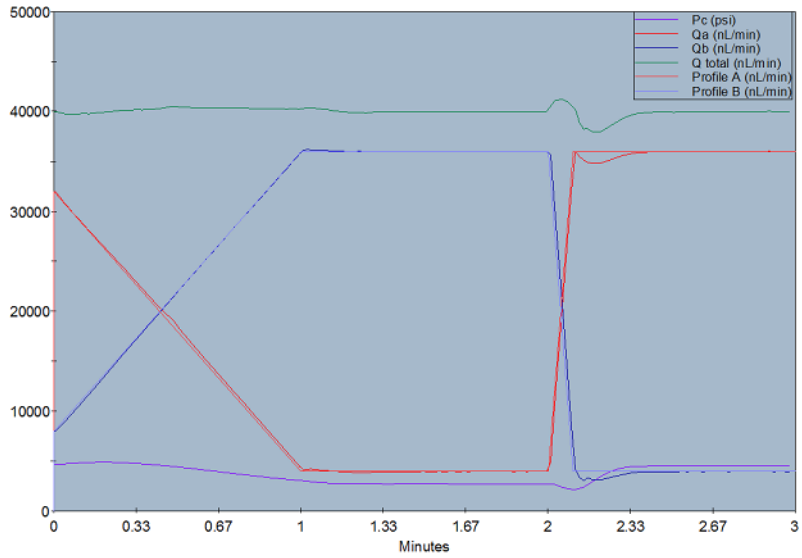
**Figure 5-1 Example Chromatogram for the System Integration Test**



# Troubleshoot Using Pressure and Flow Data

The pressure and flow data files that are automatically saved by the Eksigent control software can be helpful in diagnosing LC issues. Refer to Figure 5-2.

**Figure 5-2 Example Pressure and Flow Data for the System Integration Test**




Use Table 5-1 to troubleshoot some issues that might occur in the pressure and flow data.

**Table 5-1 Common Anomalies in System Pressure Data**

| Symptom | Possible Cause |
|---|---|
| Pressure drops at the beginning of the run and then recovers. | Air bubble in the sample loop. |
| Pressure in both pumps initially increases and then decreases. | Column, probe, or tubing is partially clogged. |
| Sudden increase in pressure followed by a drop after injection. | Sample solvent is different than the mobile phase. |

# System Integration Test Data Log and Signoff 6

## System Information

**Table 6-1 System Information**

| | |
|---|---|
| Model | |
| Location | |
| Serial number | |

**Table 6-2 Ion Source Information**

| | |
|---|---|
| Model | |
| Serial number | |

**Table 6-3 Mass Spectrometer Information**

| | |
|---|---|
| Mass spectrometer model | |
| Mass spectrometer location | |
| Mass spectrometer serial number | |

## Test Results

Complete this table with the results for the three analytes from the triazine solution. Refer to Specifications on page 38.

**Table 6-4 Test Results**

| Analyte | Mean Area (Counts) | %CV (Counts) | Mean Retention Time | %CV (Retention Time) | Mean Peak Width at Half-Height |
|---|---|---|---|---|---|
| Ametryn 1 | | | | | |
| Atrazine 1 | | | | | |
| Propazine 1 | | | | | |
| | | | | Specifications Met? | |

**Table 6-5 Notes**

| |
|---|
| |

# Specifications

### Mean Peak Area Specification

For each analyte, the mean peak area should be ≥ the value in the table below.

**Table 6-6 Minimum Mean Peak Area**

| Analyte | 4600/5600/5600+/6600 1 ng/mL |
|---|---|
| Ametryn 1 | $7.5 \times 10^4$ |
| Atrazine 1 | $6.5 \times 10^4$ |
| Propazine 1 | $7.5 \times 10^4$ |

### Area %CV Specification

All analyte areas should have a %CV of <15% based on replicate injections.

### Peak Width Specification

Chromatographic peaks for each analyte should be <0.030 minutes in width when measured at half maximum (peak width at half maximum).

### Retention Time Specification

For each analyte, the retention times of consecutive runs (n=3) should have a %CV of <3%.

# Signoff

| | | | |
|---|---|---|---|
| Organization | | | |
| Customer contact name | | Date (yyyy-mm-dd) | |
| Customer contact signature* | | | |
| FSE name | | Date (yyyy-mm-dd) | |
| FSE signature | | | |

* Signature required on hard copy only.

# Revision History

| Revision | Reason for Change | Date |
|---|---|---|
| A | First release of document. | October 2015 |
| B | Modified final sample dilution step to use water instead of methanol and water. Added fields for system information. Updated images for test results. | August 2016 |
| C | Updated autosampler method parameters for new injection port. | December 2016 |