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# M3 MicroLC System

## For SCIEX Triple Quad™ and QTRAP® Systems

### System Integration Test and Data Log



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

- API 3200™ system, 3200 QTRAP® system
- API 4000™ system, 4000 QTRAP® system
- SCIEX Triple Quad™ 4500 system, QTRAP® 4500 system
- API 5000™ system
- SCIEX Triple Quad™ 5500 system, QTRAP® 5500 system
- SCIEX Triple Quad™ 6500 system, QTRAP® 6500 system

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

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## About the Test

Use this test as a measure of the M3 MicroLC 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)
- Triazine System Suitability Solution (PN 4376887)
- 5 µL PEEKsil sample loop (PN 5017799)
- 65 µm i.d. electrode (PN 5029342)

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**Note:** The active hardware profile must include the CTC autosampler and the Eksigent control software to perform a run with the Analyst<sup>®</sup> software. The hardware profile is configured at installation. However, if a different computer is being used or the Analyst<sup>®</sup> 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.

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Create the LC method in the Eksigent control software and the autosampler and acquisition methods in the Analyst<sup>®</sup> software.

## Create the Gradient LC Method

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

1. Click **LC Methods** to open the LC Method Settings dialog.
2. In the **Name** field, type **Gradient 1 Method**, and then click **Save**.
3. On the Summary tab, type the values shown in [Figure 2-1](#).

Figure 2-1 Gradient 1 Method—Summary Tab

The screenshot shows the 'LC Method Settings' dialog box with the 'Summary' tab selected. The 'Selected Method' section shows 'Gradient 1 Method' in a dropdown menu, with 'Save' and 'Print' buttons to its right. Below this are four tabs: 'Summary', 'Run Conditions', 'Gradient Profile', and 'Gradient Table'. The 'Summary' tab contains the following sections:

- Method Identification:** A text box for 'Method ID'.
- Column Information:** A table of column details:

|               |              |               |                   |
|---------------|--------------|---------------|-------------------|
| Manufacturer  | SCIEX        | particle size | 2.7 $\mu\text{m}$ |
| Type          | HALO Peptide | diameter      | 500 $\mu\text{m}$ |
| Serial Number | N/A          | length        | 5 cm              |
- Sample Injection:** A text box containing 'Standard'.
- Flow Profile:** A text box containing 'Duration: 2 min.'.
- Detection:** A text box containing 'External Detector. Auxillary A/D channel available.'

At the bottom of the dialog are buttons for 'Delete', 'View Audit Trail', 'OK', and 'Cancel'.

4. Click the Run Conditions tab and type the values shown in [Figure 2-2](#).

Figure 2-2 Gradient 1 Method—Run Conditions Tab

The screenshot shows the 'LC Method Settings' dialog box with the 'Run Conditions' tab selected. The 'Selected Method' is 'Gradient 1 Method'. The 'Pre-Run' section has three checked options: 'Flush column for 1 minutes using 100 % initial flowrate conditions.', 'Stabilize column temperature at 35 °C prior to injecting sample and beginning Flow Profile.', and 'First, establish a column pressure of 3000 psi.' The 'Sample Injection' section has four radio button options: 'None.', 'Standard: Sample valve opens prior to beginning Flow Profile and remains open.' (selected), 'Metered: Inject 10000 nL of sample at 100 % initial flowrate conditions.', and 'Rapid: Inject 10000 nL of sample at maximum flowrate, maintaining initial mixture conditions.' The 'Post-Run' section has one unchecked option: 'Flush column for 0.5 minutes using 100 % ending flowrate conditions.' Buttons for 'Delete', 'View Audit Trail', 'OK', and 'Cancel' are at the bottom.

LC Method Settings

Selected Method

Name Gradient 1 Method Save Print

Summary Run Conditions Gradient Profile Gradient Table

Pre-Run

Flush column for 1 minutes using 100 % initial flowrate conditions.

First, establish a column pressure of 3000 psi.

Stabilize column temperature at 35 °C prior to injecting sample and beginning Flow Profile.

Sample Injection

None.

Standard: Sample valve opens prior to beginning Flow Profile and remains open.

Metered: Inject 10000 nL of sample at 100 % initial flowrate conditions.

Rapid: Inject 10000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

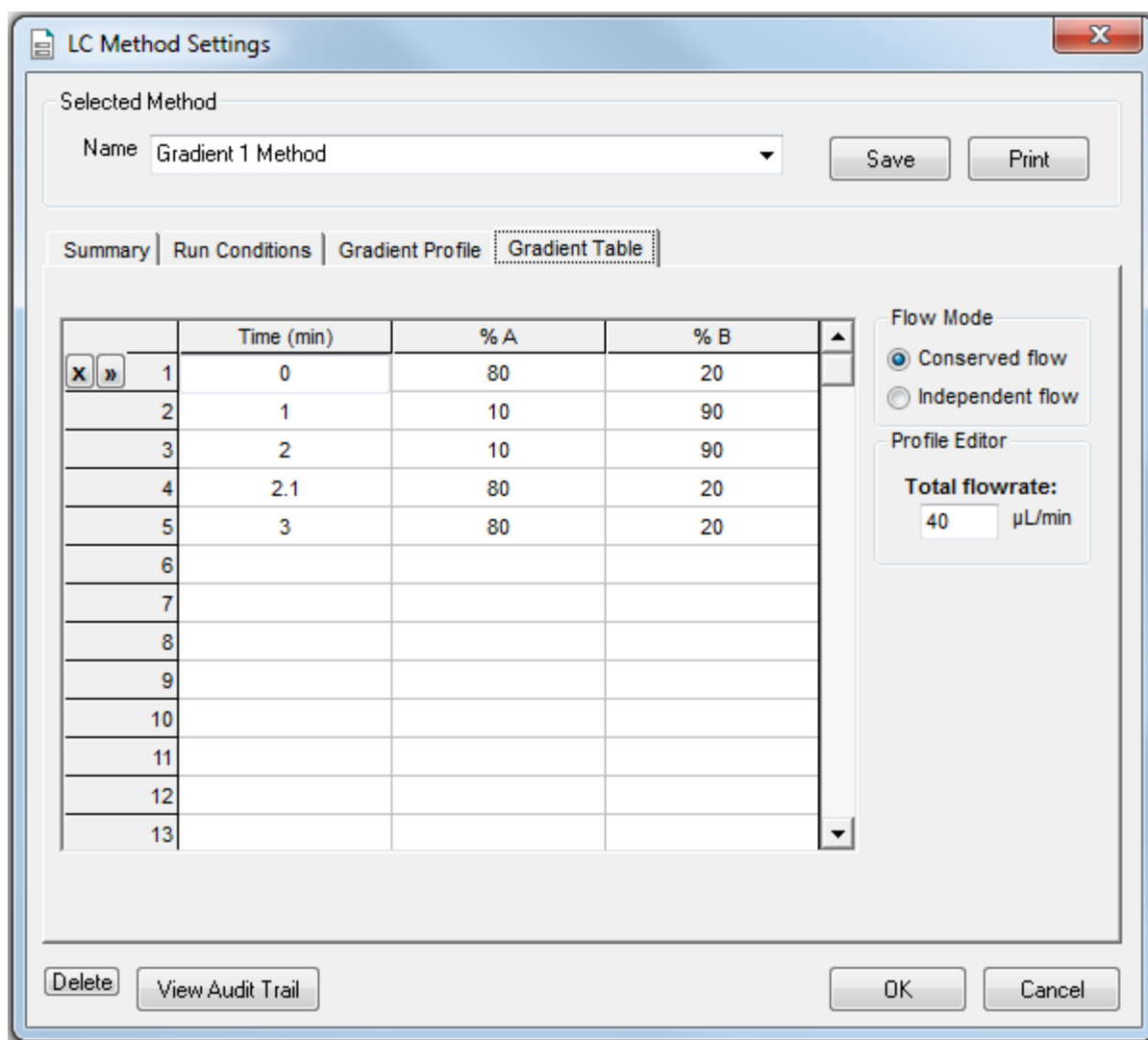
Flush column for 0.5 minutes using 100 % ending flowrate conditions.

Delete View Audit Trail OK Cancel

5. Click the **Gradient Table** tab and set the flow mode, the gradient parameters, and the flow rate. Refer to [Figure 2-3](#).



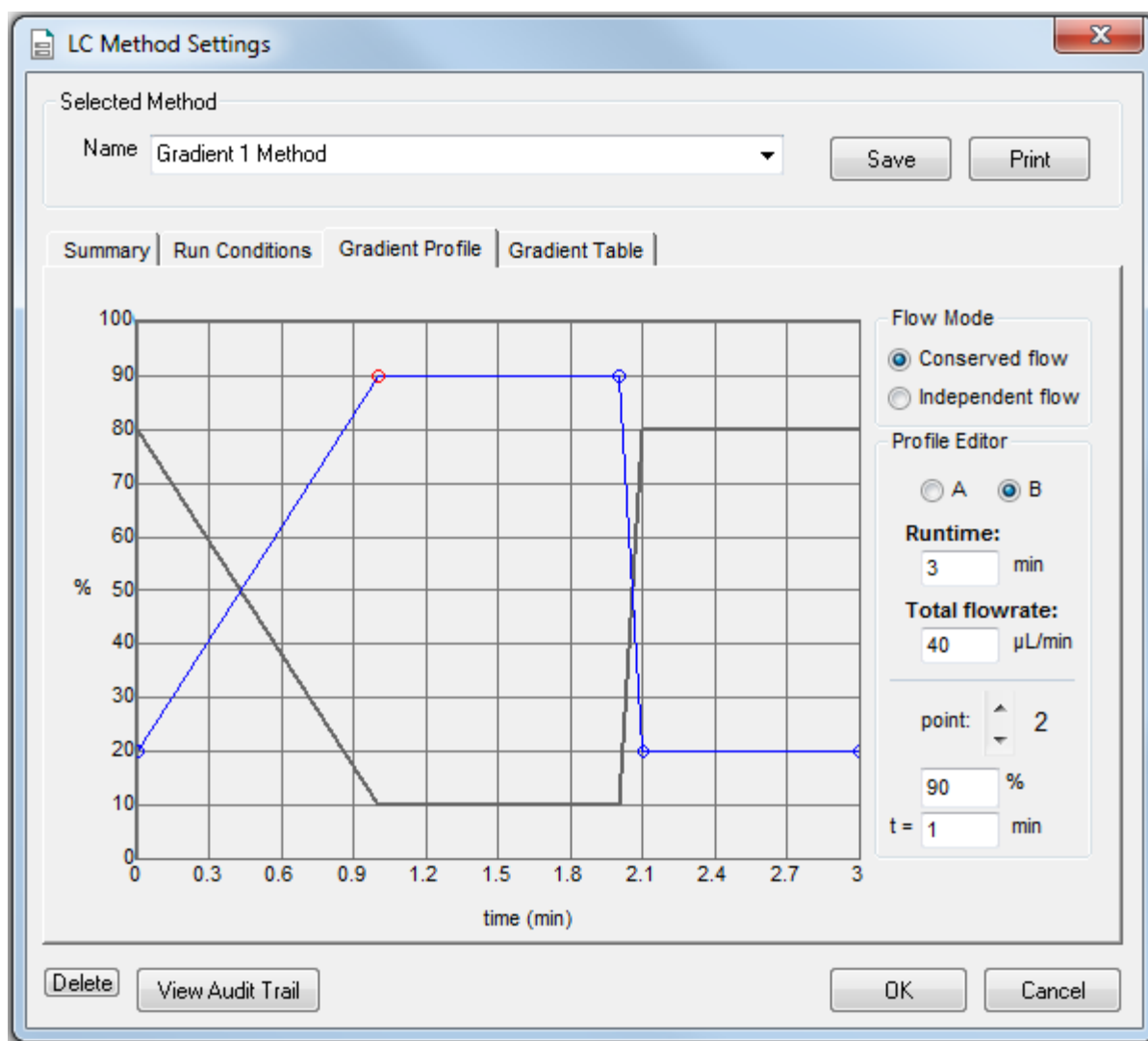
Figure 2-3 Gradient 1 Method—Gradient Table Tab



6. Click the Gradient Profile tab to view a graphical representation of the gradient. Refer to [Figure 2-4](#).

## Create the Methods

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.

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

## Create the Autosampler Method for the System Integration Test

1. On the Navigation bar in the Analyst<sup>®</sup> 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 Direct Injection RevA**.

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**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.

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4. In the **Injection Volume** field, type **6** (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.

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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—Direct Injection 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     |

## Create the Methods

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**Table 2-1 Cycle Arguments Table—Direct Injection 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

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. In the Acquisition Method Browser pane, click **Mass Spec** to create the mass spectrometer acquisition method.
3. Type the acquisition method parameters shown in [Table 2-2](#).

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**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.

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**Table 2-2 Acquisition Method Parameters**

| Parameter           | Value  |
|---------------------|--|
| <b>MS</b>           |  |
| Scan Type           | MRM (MRM)  |
| Duration (min)      | 3  |
| Polarity            | Positive   |
| Q1/Q3 Masses and CE | Refer to <a href="#">Table 2-4</a> , <a href="#">Table 2-5</a> , and <a href="#">Table 2-6</a> . |
| <b>Advanced MS</b>  |  |
| Q1 Resolution       | Unit   |
| Q3 Resolution       | Unit   |

**Table 2-2 Acquisition Method Parameters (continued)**

| Parameter                      | Value                  |
|--------------------------------|------------------------|
| Pause (ms)                     | 5.00                   |
| <b>Source/Gas</b>              |                        |
| Curtain Gas (CUR)              | 20 (or as optimized)   |
| CAD Gas                        | Medium                 |
| IonSpray Voltage (IS) (V)      | 5000 (or as optimized) |
| Ion Source Gas 1 (GS1)         | 20 (or as optimized)   |
| Ion Source Gas 2 (GS2)         | 20 (or as optimized)   |
| Temperature (TEM)              | 350 (or as optimized)  |
| <b>Compound</b>                |                        |
| Declustering Potential (DP)    | 70                     |
| Collision Exit Potential (CXP) | 30                     |

4. Type the parameters shown in [Table 2-3](#)

The values for Entrance Potential (EP) and Collision Cell Exit Potential (CXP) differ by mass spectrometer. Be sure to type the appropriate values.

**Table 2-3 Values for Entrance Potential and Collision Cell Exit Potential**

| System   | EP | CXP |
|--|----|-----|
| API 3200™ system<br>3200 QTRAP® system   | 10 | 4   |
| API 4000™ system<br>4000 QTRAP® system<br>SCIEX Triple Quad™ 4500 system<br>QTRAP® 4500 system | 10 | 10  |

## Create the Methods

**Table 2-3 Values for Entrance Potential and Collision Cell Exit Potential (continued)**

| System   | EP | CXP |
|--|----|-----|
| API 5000™ system<br>SCIEX Triple Quad™ 5500 system<br>QTRAP® 5500 system | 10 | 10  |
| SCIEX Triple Quad™ 6500 system<br>QTRAP® 6500 system                     | 10 | 13  |

- Right-click the **Mass Ranges Table** and select **DP** from the menu to add the DP (declustering potential) column to the table. Add the CE (collision energy) as well.
- Type the MRM transitions in the **Mass Ranges Table**.

The transitions are shown in [Table 2-4](#), [Table 2-5](#), and [Table 2-6](#).

**Tip!** Field Service Employees can download a .CSV file from the SharePoint site, import it, and save it locally for customer use. To use the file, click **Import List** and select the file.

**Table 2-4 MRM Transitions for API 3200™ and 3200 QTRAP® Systems**

| Q1    | Q3    | Dwell | ID          | DP | CE |
|-------|-------|-------|-------------|----|----|
| 228.2 | 186.2 | 10    | Ametryn 1   | 46 | 23 |
| 228.2 | 96.1  | 10    | Ametryn 2   | 46 | 33 |
| 216.0 | 174.0 | 10    | Atrazine 1  | 46 | 23 |
| 216.0 | 104.1 | 10    | Atrazine 2  | 46 | 39 |
| 242.2 | 186.0 | 10    | Terbutryn 1 | 41 | 23 |
| 242.2 | 68.2  | 10    | Terbutryn 2 | 41 | 55 |

**Table 2-5 MRM Transitions for the 4000 and 4500 Series of Systems**

| Q1    | Q3    | Dwell | ID         | DP | CE |
|-------|-------|-------|------------|----|----|
| 228.2 | 186.2 | 10    | Ametryn 1  | 66 | 23 |
| 228.2 | 96.1  | 10    | Ametryn 2  | 66 | 33 |
| 216.0 | 174.0 | 10    | Atrazine 1 | 66 | 23 |
| 216.0 | 104.1 | 10    | Atrazine 2 | 66 | 39 |

Table 2-5 MRM Transitions for the 4000 and 4500 Series of Systems (continued)

| Q1    | Q3    | Dwell | ID          | DP | CE |
|-------|-------|-------|-------------|----|----|
| 242.2 | 186.0 | 10    | Terbutryn 1 | 61 | 23 |
| 242.2 | 68.2  | 10    | Terbutryn 2 | 61 | 55 |

Table 2-6 MRM Transitions for SCIEX Triple Quad™ 5500 and 6500 Systems and QTRAP® 5500 and 6500 Systems

| Q1    | Q3    | Dwell | ID          | DP | CE |
|-------|-------|-------|-------------|----|----|
| 228.2 | 186.2 | 10    | Ametryn 1   | 86 | 23 |
| 228.2 | 96.1  | 10    | Ametryn 2   | 86 | 33 |
| 216.0 | 174.0 | 10    | Atrazine 1  | 86 | 23 |
| 216.0 | 104.1 | 10    | Atrazine 2  | 86 | 39 |
| 242.2 | 186.0 | 10    | Terbutryn 1 | 81 | 23 |
| 242.2 | 68.2  | 10    | Terbutryn 2 | 81 | 55 |

7. 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 health and safety precautions, refer to the mass spectrometer *System User Guide* or *Hardware Guide*.

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

### Required Materials

- Methanol
- 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  |
|---|
| <ul style="list-style-type: none"><li>• Stock solution, prepared in <a href="#">Prepare the 1 µg/mL Stock Solution on page 16</a>.</li><li>• 50:50 mixture of MeOH:H<sub>2</sub>O</li><li>• Water with 0.1% formic acid</li></ul> |



1. Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:H<sub>2</sub>O and, for the final dilution, H<sub>2</sub>O with 0.1% formic acid, in a clean vial. Refer to [Table 3-2](#).

For the final dilution, be sure to use H<sub>2</sub>O 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 19](#).

## Prepare the System for Testing

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

| System   | Target Concentration | Dilution (Stock Solution + 50:50 MeOH:H <sub>2</sub> O)  |
|--|----------------------|--|
| API 3200™ system<br>3200 QTRAP® system   | 10 ng/mL             | Two serial dilutions:<br>a. 100 µL of stock solution + 900 µL of MeOH:H <sub>2</sub> O (to make 100 ng/mL)<br>b. 100 µL of 100 ng/mL + 900 µL of H <sub>2</sub> O with 0.1% formic acid  |
| API 4000™ system<br>4000 QTRAP® system<br>SCIEX Triple Quad™ 4500 system<br>QTRAP® 4500 system                                   | 1.0 ng/mL            | Three serial dilutions:<br>a. 100 µL of stock solution + 900 µL of MeOH:H <sub>2</sub> O (to make 100 ng/mL)<br>b. 100 µL of 100 ng/mL + 900 µL of MeOH:H <sub>2</sub> O (to make 10 ng/mL)<br>c. 100 µL of 10 ng/mL + 900 µL of H <sub>2</sub> O with 0.1% formic acid  |
| API 5000™ system<br>SCIEX Triple Quad™ 5500 system<br>QTRAP® 5500 system<br>SCIEX Triple Quad® 6500 system<br>QTRAP® 6500 system | 0.1 ng/mL            | Four serial dilutions:<br>a. 100 µL of stock solution + 900 µL of MeOH:H <sub>2</sub> O (to make 100 ng/mL)<br>b. 100 µL of 100 ng/mL + 900 µL of MeOH:H <sub>2</sub> O (to make 10 ng/mL)<br>c. 100 µL of 10 ng/mL + 900 µL of MeOH:H <sub>2</sub> O (to make 1 ng/mL)<br>d. 100 µL of 1 ng/mL + 900 µL of H <sub>2</sub> O 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**

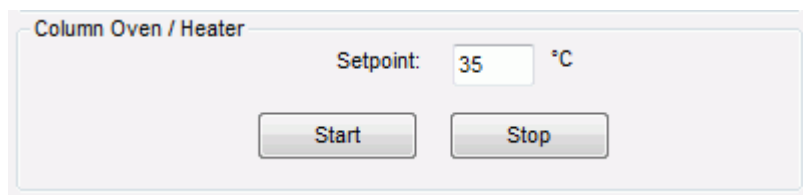
| Buffer                 | Mixture                            | Channel   |
|------------------------|------------------------------------|-----------|
| <b>Gradient 1 Pump</b> |                                    |           |
| Buffer A               | 100% water:0.1% formic acid        | Channel A |
| 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. 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**



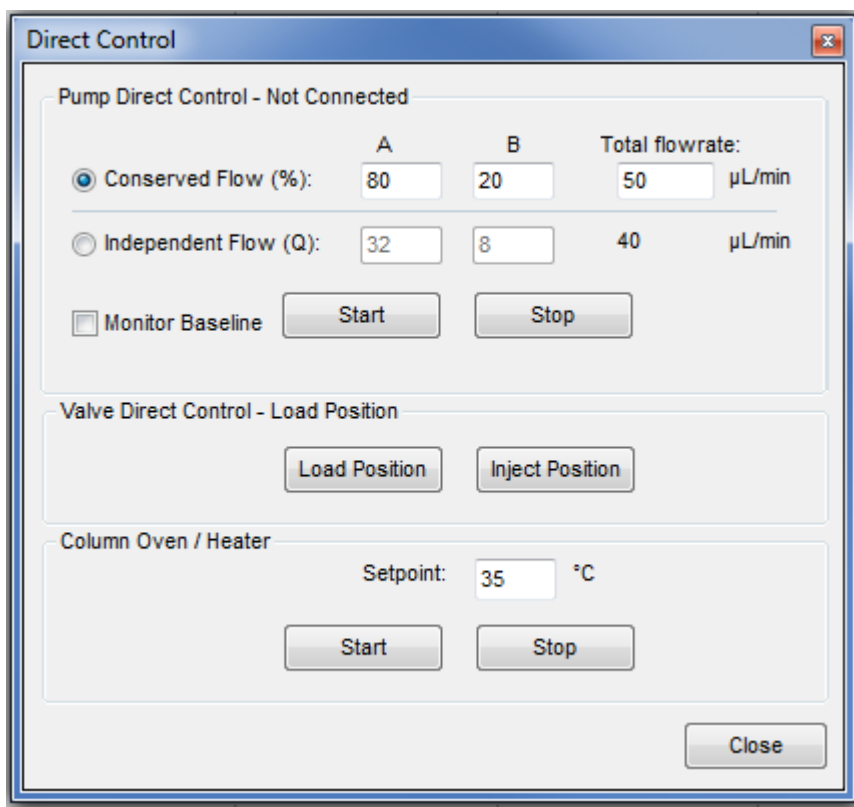
- c. Close the compartment so that the oven can reach the specified temperature.
4. Start the pump.
    - a. Select the **Conserved Flow (%)** option and set **A** to **80** and **B** to **20**.  
This is the mobile phase composition used for equilibration.
    - b. Type a **Total flowrate** of **50** µL/min.
    - c. Click **Start**.
  5. Flush the injection valve.
    - a. In the **Valve Direct Control** section, alternate clicking **Load Position** and **Inject Position**.

## Prepare the System for Testing

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- b. Make sure that the final position of the valve is at **Load**.
- c. In the **Pump Direct Control** section, click **Stop** and then connect the LC column.

**Figure 3-2 Direct Control Dialog**



6. In the Analyst<sup>®</sup> 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**.
7. Click **Start** in the **Pump Direct Control** section.
8. Adjust the source and gas parameters as required until a stable spray is achieved and then click **Stop** to end tuning.
9. Click **Stop** in the **Pump Direct Control** section and then close the Direct Control dialog.

# Perform the System Integration Test

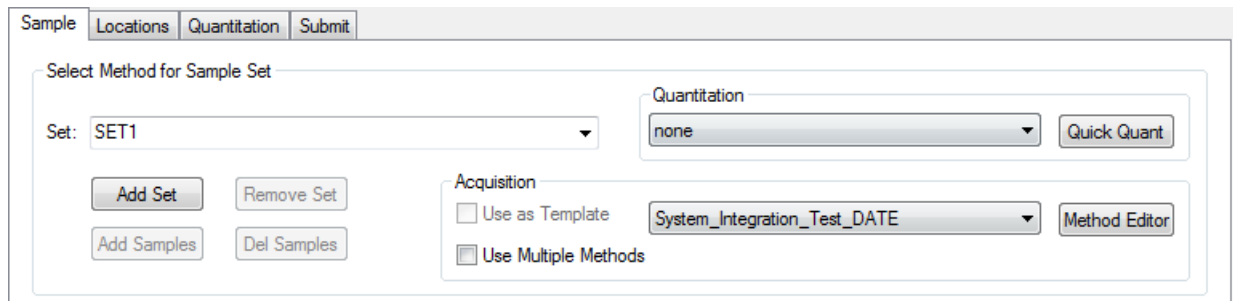
# 4

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 38](#).

## Create the Acquisition Batch and Quantitation Method

1. Open the Analyst<sup>®</sup> 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 12](#) from the list.

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



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

**Figure 4-2 Add Sample Dialog**

The 'Add Sample' dialog box contains the following fields and controls:

- Sample name:**
  - Prefix:
  - Sample number:
  - Number of digits:
- Data file:**
  - Prefix:
  - Set name:
  - Auto Increment:
  - Sub Folder:
- New samples:**
  - Number:

Buttons:

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 "x ng/mL triazine mix\_y", where "x" is the target concentration and "y" is 1, 2, or 3.
10. Make sure that the **Injection Volume** (in the table on the **Sample** tab) is 6  $\mu$ L.
11. Click **Quick Quant** to open the Create Semi-Automatic Quantitation Method dialog.

**Figure 4-4 Create Semi-Automatic Quantitation Method Dialog**

Internal Standards

|   | Name | Q1 / Q3 |
|---|------|---------|
| 1 |      |         |
| 2 |      |         |
| 3 |      |         |

Analytes

|   | Name        | Internal Standard | Q1 / Q3           |
|---|-------------|-------------------|-------------------|
| 1 | Ametryn 1   |                   | 228.200 / 186.200 |
| 2 | Ametryn 2   |                   | 228.200 / 96.100  |
| 3 | Atrazine 1  |                   | 216.000 / 174.000 |
| 4 | Atrazine 2  |                   | 216.000 / 104.100 |
| 5 | Terbutryn 1 |                   | 242.200 / 186.000 |
| 6 | Terbutryn 2 |                   | 242.200 / 68.200  |

12. Select **3** from the **Smoothing Width** list.
13. Click **OK**, and when prompted, 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.

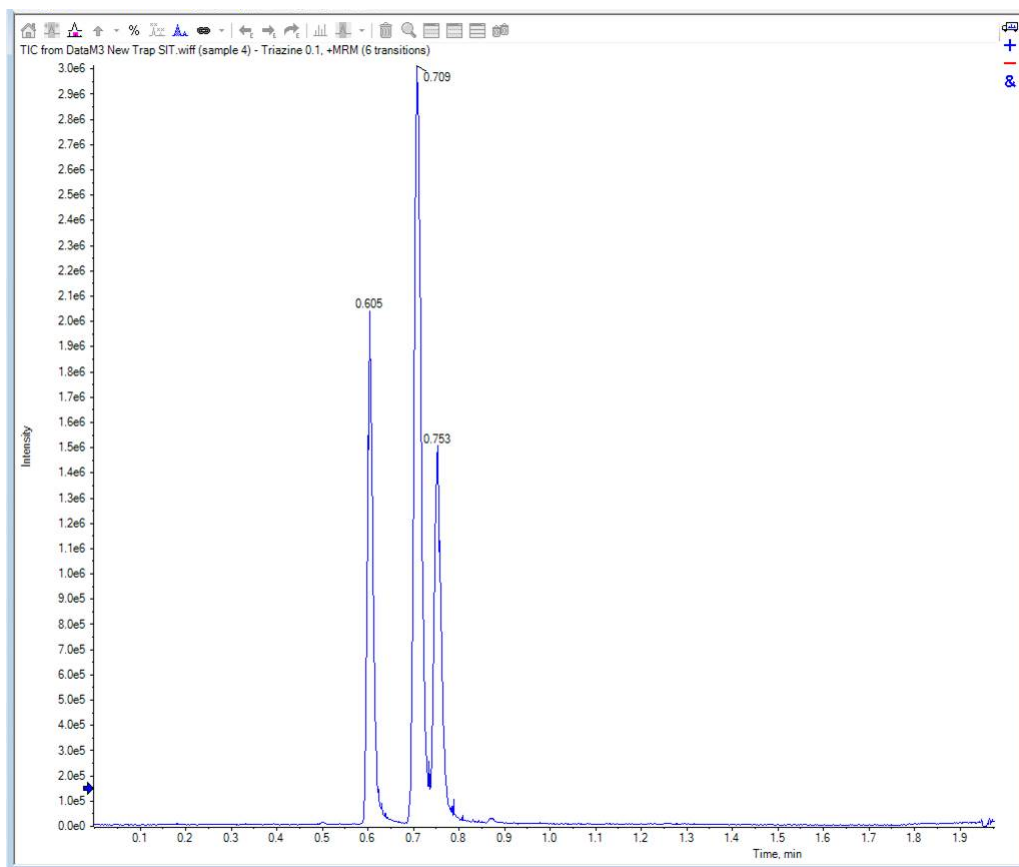
1. Click **Submit** on the **Submit** tab.
2. Click **View > Sample Queue**.
3. Verify that the system is not in Tuning mode and then click **Acquire > Ready**.
4. Click **Acquire > Start Sample**.
5. Monitor the system pressure ( $P_c$ ) in the upper right corner of the Acquisition window of the Eksigent control software.

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

6. (Optional) If the first run was to condition the column, then run the batch again to acquire data for analysis. Refer to [Figure 4-5](#) for representative data.



Figure 4-5 Example Chromatogram for the System Integration Test—All XICs



## View the Results and Verify the Integration

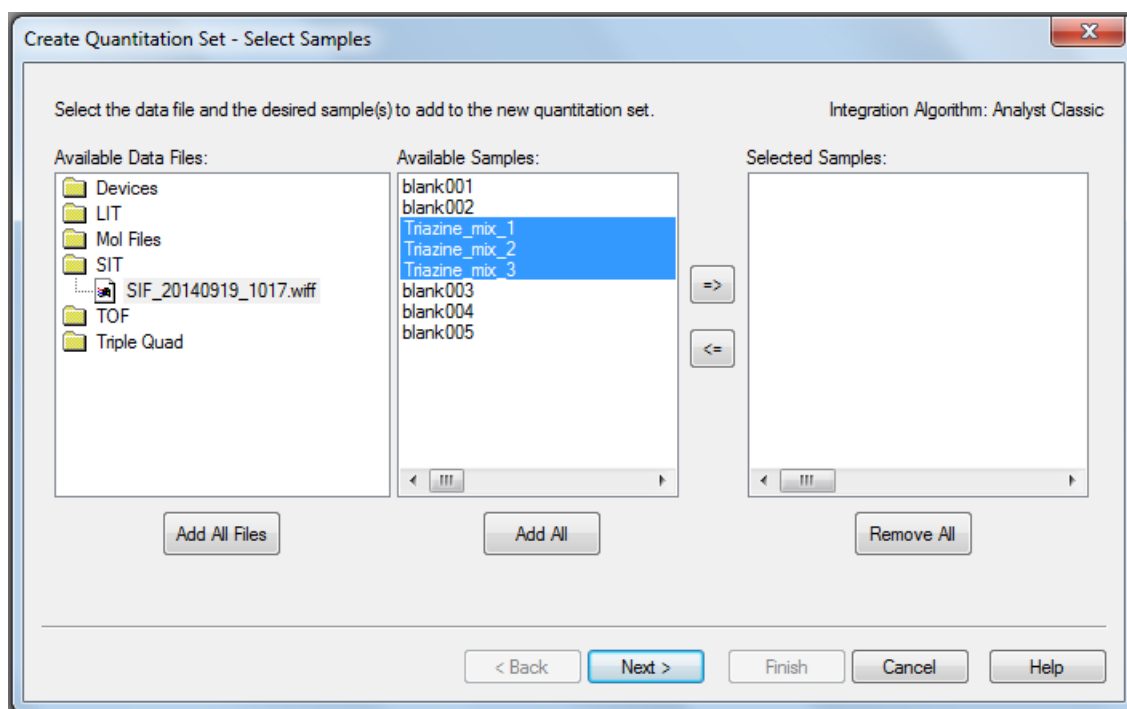
1. When the run is finished, in the Navigation bar, under **Quantitation**, double-click **Quantitation Wizard**.
2. Select the data file **System Integration Test <date>**.
3. Click **Add All** to move the three injections to the **Selected Samples** list.

If a conditioning run was submitted, then make sure that the injections selected are from the test run.

## Perform the System Integration Test

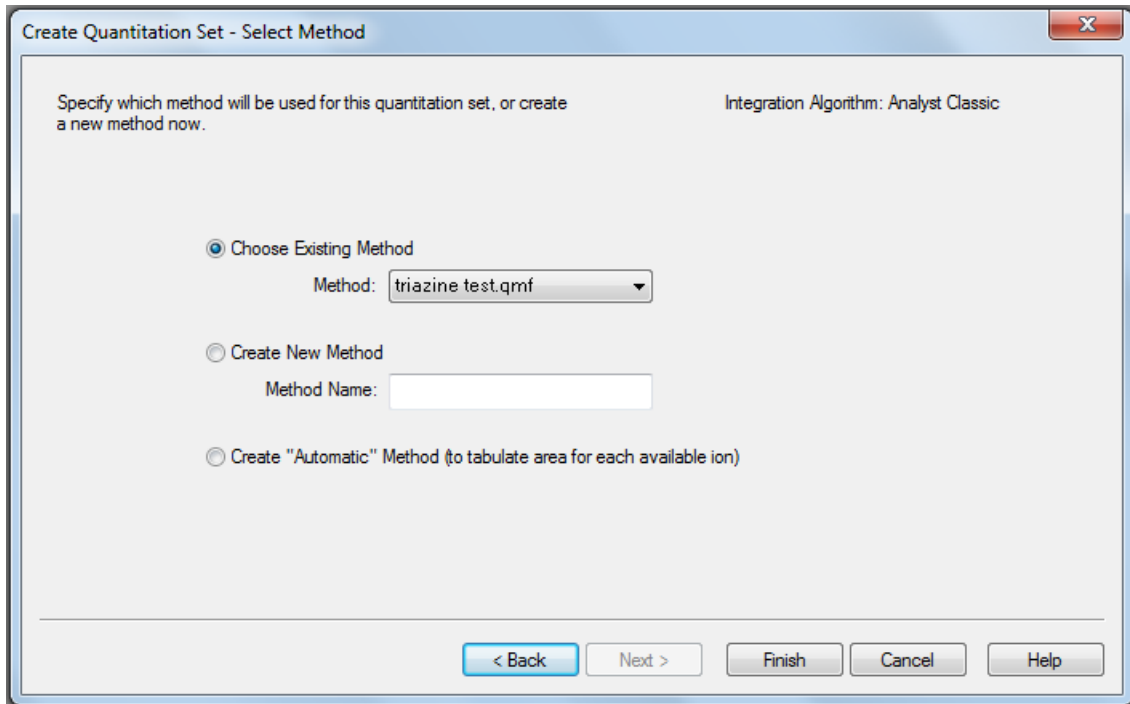
---

**Figure 4-6 Create Quantitation Set—Select Samples Dialog**



4. On the Create Quantitation Set - Select Settings & Query dialog, click **Next**.
5. Click **Next**.
6. On the Create Quantitation Set - Select Method dialog, select **Choose Existing Method** and **triazine test** in the **Method** list, and then click **Finish**.

Figure 4-7 Create Quantitation Set—Select Method Dialog



The Results Table opens.

7. Change the **Sample Type** to **Standard**.

Figure 4-8 Results Table—Changing Sample Type

Full Layout  
Query: None  
Idle  
Sort: Unsorted

|   | Sample Name         | Sample ID | Sample Type     | File Name          | Analyte Peak Area (counts) | Analyte Peak Height (cps) |
|---|---------------------|-----------|-----------------|--------------------|----------------------------|---------------------------|
| 1 | 10 ng/ml triazine m |           | Unknown         | Analyst Data\Proje | 8.76e+004                  | 1.69e+004                 |
| 2 | 10 ng/ml triazine m |           | Unknown         | Analyst Data\Proje | 3.16e+004                  | 5.99e+003                 |
| 3 | 10 ng/ml triazine m |           | Standard        | Analyst Data\Proje | 5.14e+004                  | 8.23e+003                 |
| 4 | 10 ng/ml triazine m |           | Quality Control | Analyst Data\Proje | 1.64e+004                  | 3.02e+003                 |
| 5 | 10 ng/ml triazine m |           | Blank           | Analyst Data\Proje | 6.85e+004                  | 1.23e+004                 |
| 6 | 10 ng/ml triazine m |           | Double Blank    | Analyst Data\Proje | 9.97e+004                  | 1.72e+004                 |
| 7 | 10 ng/ml triazine m |           | Solvent         | Analyst Data\Proje | 9.41e+004                  | 1.85e+004                 |
| 8 | 10 ng/ml triazine m |           | Unknown         | Analyst Data\Proje | 6.00e+004                  | 1.23e+004                 |

- a. On the first line, change the **Sample Type** to **Standard**.
  - b. Right-click **Sample Type** and then select **Fill Down**.
8. Right-click inside the margin above the Results Table, and then select **Analyte > Ametryn 1**.

## Perform the System Integration Test

---

Only the results for Ametryn 1 are shown.

9. Click **Tools > Peak Review > Pane**.

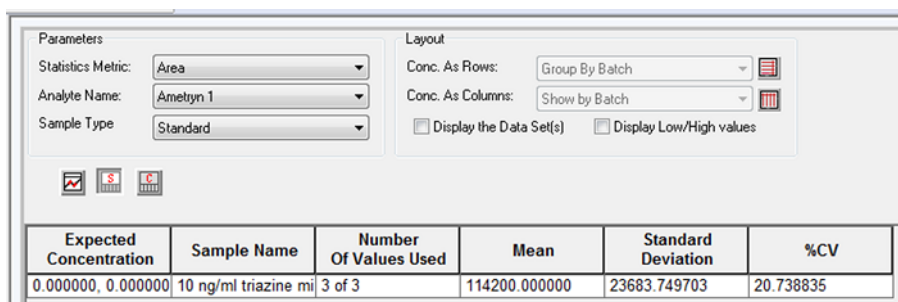
The chromatograms for the MRM transition are shown below the Results Table.

10. Click the forward and back arrows in the chromatogram pane to view the integration for each chromatogram.
11. After the data has been reviewed, repeat step 8 through step 10 for the Atrazine 1 and Terbutryn 1 transitions.

## Verify the Mean Area and %CV

1. After reviewing the integration, click **Tools > Statistics**.
2. In the **Statistics Metric** list, select **Area**.
3. For each of the MRM transitions:
  - a. Compare the Mean and %CV values to the specifications in the [System Integration Test Data Log and Signoff on page 38](#).
  - b. Type the values in [Table 6-4 on page 39](#).
4. Delete the Statistics Summary pane.

Figure 4-9 Statistics Summary Pane



The screenshot shows the Statistics Summary Pane with the following settings:

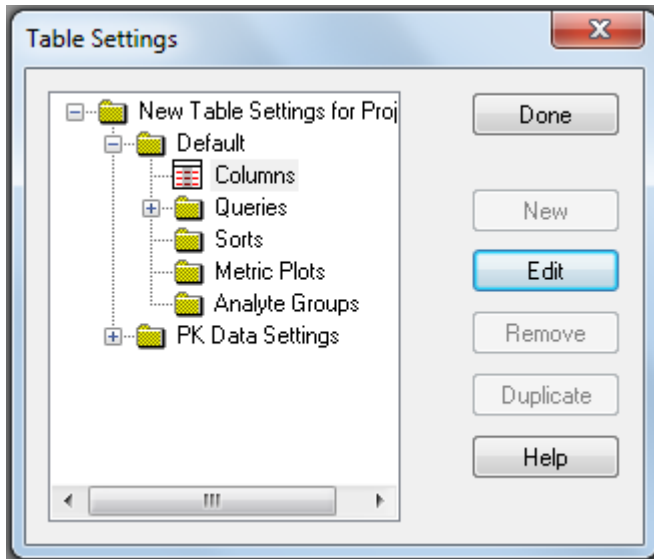
- Parameters: Statistics Metric: Area, Analyte Name: Ametryn 1, Sample Type: Standard
- Layout: Conc. As Rows: Group By Batch, Conc. As Columns: Show by Batch,  Display the Data Set(s),  Display Low/High values

| Expected Concentration | Sample Name          | Number Of Values Used | Mean          | Standard Deviation | %CV       |
|------------------------|----------------------|-----------------------|---------------|--------------------|-----------|
| 0.000000, 0.000000     | 10 ng/ml triazine ml | 3 of 3                | 114200.000000 | 23683.749703       | 20.738835 |

## Verify Peak Widths at Half Height and Retention Times

1. Open the Results Table Columns dialog.
2. Right-click on the area above the Results Table and then click **Table Settings > Edit**.
3. In the Table Settings dialog, click **Columns > Edit**.

Figure 4-10 Table Settings Dialog

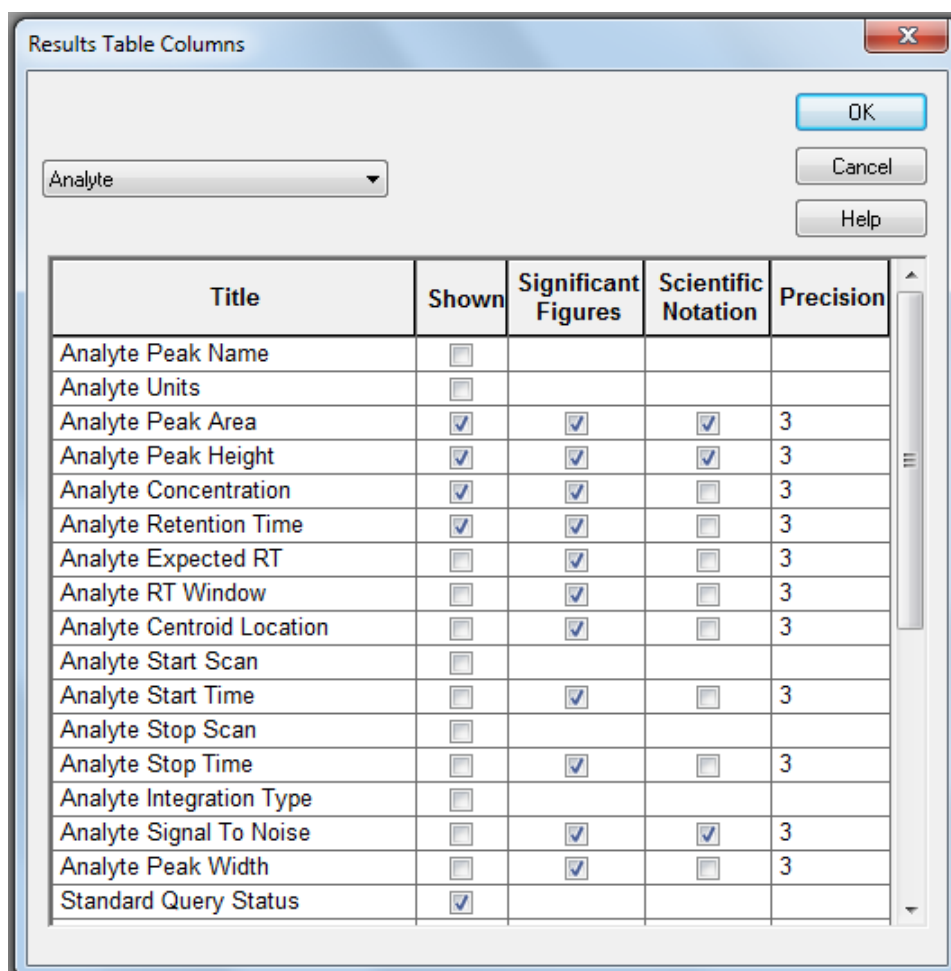


4. In the Results Table Columns dialog, select the columns to be shown in the table.

## Perform the System Integration Test

---

Figure 4-11 Results Table Columns Dialog



- a. Select **Analyte** in the list.
  - b. In the **Shown** column of the table, select the **Analyte Retention Time** and **Analyte Peak Width at 50% Height** check boxes.
  - c. Make sure that the **Analyte Peak Area**, **Analyte Peak Height**, and **Analyte Concentration** check boxes are also selected.
  - d. Click **OK**.
5. Click **Done**.
- The Results Table updates to show the selected columns.
6. For each MRM transition:
- a. Compare the experimental values with the specifications in the [System Integration Test Data Log and Signoff on page 38](#).

- b. Type the values in [Table 6-4 on page 39](#).

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

1. Repeat the system integration test. Refer to [Perform the System Integration Test on page 21](#).
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 33](#).
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 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 50 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.
- 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:

## Troubleshooting

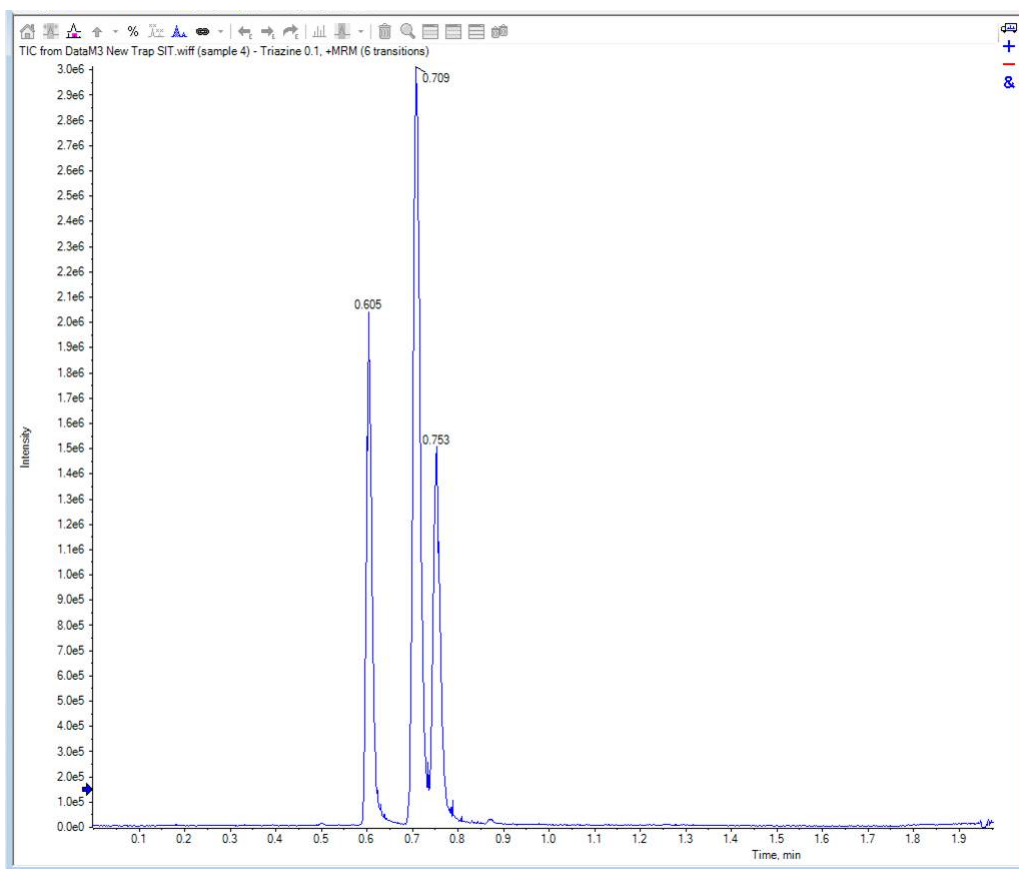
---

- 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

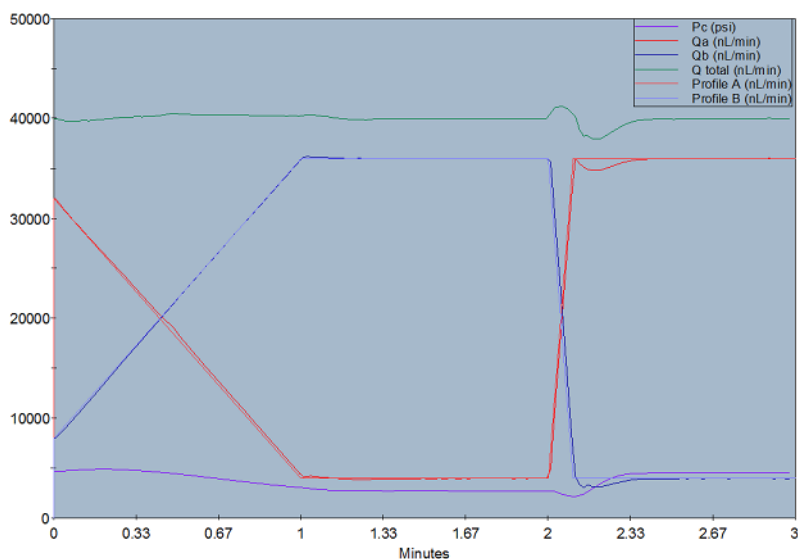
Figure 5-1 Example Chromatogram for the System Integration Test



## 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. |

## Pc Drops at the Beginning of a Run

A pressure drop at the start of a trace (Figure 5-3) can indicate that there is air in the sample loop. Refer to “Sample Position in the Sample Loop” in the *Operator Guide*.

Figure 5-3 Acquisition Window Showing Pc, with Air Bubbles in Sample Loop

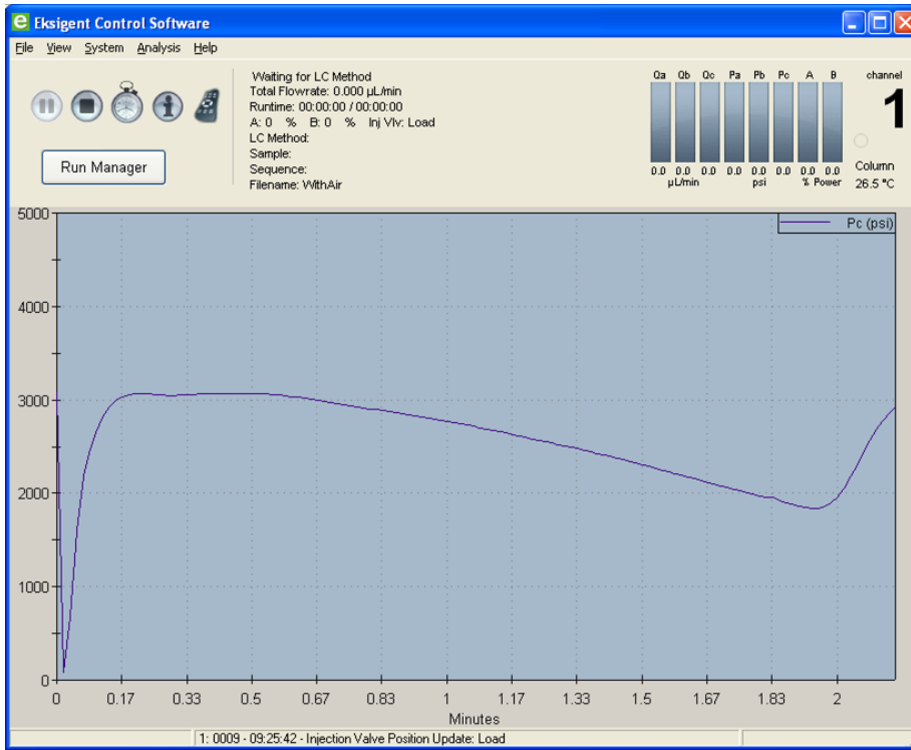


Figure 5-4 Acquisition Window Showing Pc, without Air Bubbles in Sample Loop



## Troubleshooting Steps

- Examine the injection valve fittings and autosampler for leaks. Check that the injection port fitting is snug and the needle penetration depth is correct. Refer to "Configure the Autosampler" in the *Operator Guide*.
- In the Analyst<sup>®</sup> software, make sure that the values for Front Sample and Rear Sample in the Cycle Parameters table in the Acquisition Method window are 1  $\mu$ L and use an injection volume large enough to overfill the loop. The recommended volume is 2 to 5 times the volume of the sample loop installed on the system.
- Air bubbles in the sample loop can also indicate an internal leak in the pump. Contact a SCIEX Field Service Employee (FSE) for assistance.

# 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 40](#).

**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          |                    |              |                     |                      |                                |
| Terbutryn 1         |                    |              |                     |                      |                                |
| Specifications Met? |                    |              |                     |                      |                                |

**Table 6-5 Notes**

|  |
|--|
|  |
|--|

## Specifications

### Mean Peak Area Specification

For each analyte, the mean peak area should be  $\geq$  the value in the table below.

**Table 6-6 Minimum Mean Peak Area (CPS)**

| Analyte     | 3200 10 ng/mL     | 4000/4500 1 ng/mL | 5000/5500/6500<br>0.1 ng/mL |
|-------------|-------------------|-------------------|-----------------------------|
| Ametryn 1   | $8.0 \times 10^4$ | $7.5 \times 10^4$ | $3.5 \times 10^4$           |
| Atrazine 1  | $1.5 \times 10^4$ | $6.5 \times 10^4$ | $3.0 \times 10^4$           |
| Terbutryn 1 | $1.0 \times 10^4$ | $1.0 \times 10^4$ | $5.0 \times 10^4$           |

### 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

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| Revision | Reason for Change   | Date          |
|----------|---|---------------|
| A        | First release of document.  | October 2015  |
| B        | Correction to peak area specification for ametryn 1. Correction to MRM transition in acquisition method.  | February 2016 |
| C        | Correction to peak area specifications. 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   |
| D        | Updated autosampler method parameters for new injection port.   | December 2016 |