



Eksigent MicroLC 200/200 Plus Systems

Triple Quad™ and QTRAP® Systems

System Integration Test and Data Log



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Contents

Chapter 1 Introduction	4
About the Test.....	4
Time Required.....	4
Required Materials.....	5
Chapter 2 Create the LC and Autosampler Methods	6
Create the LC Method.....	6
Create the Autosampler Method.....	10
Create the Acquisition Method.....	12
Chapter 3 Prepare the System for Testing	17
Verify System Readiness.....	17
Prepare the 1 µg/mL Stock Solution.....	17
Prepare the Sample to Be Tested.....	18
Equilibrate the System.....	19
Chapter 4 Perform the System Integration Test	22
Create the LC/MS Acquisition Batch and Quantitation Method	22
Run the Batch.....	25
Verify the Results.....	26
Verify the Results and Verify the Integration.....	26
Verify the Mean Area and % CV.....	29
Verify Peak Widths at Half Height and Retention Times.....	29
Chapter 5 Troubleshooting	32
Troubleshooting Peak Related Problems and System Crashes.....	32
Troubleshooting Using Pressure and Flow Data.....	36
Troubleshooting Using the Chromatogram.....	38
Chapter 6 System Integration Test Data Log and Signoff	41
Test Results.....	41
Specifications.....	41
Signoff.....	42
Revision History	43

This document describes the steps for preparing and performing an LC/MS system integration test for the Eksigent MicroLC 200/200 Plus system configured with the HALO 0.5 x 50 mm 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, SCIEX QTRAP® 4500 system
- API 5000™ system
- SCIEX Triple Quad™ 5500 system, SCIEX QTRAP® 5500 system
- SCIEX Triple Quad™ 6500 system, SCIEX QTRAP® 6500 system

CAUTION: Potential System Damage. Prior to operating the system, refer to “Safety Instructions” in the *Operator Guide* for detailed information on the safe use and operation of the system.

About the Test

Use this test as a measure of the Eksigent MicroLC 200/200 Plus system performance in isolation of the performance of the other components. Results from the test can become the baseline performance for the system and 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 you have consistent peak shape and peak intensity.

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

- HALO 0.5 x 50 mm column (PN 805-10100)
- Triazine System Suitability Solution (PN 4376887)
- 2 µL PEEKsil sample loop (PN 5017798)
- One of the following electrode assembly kits for the Turbo V™ or IonDrive™ ion source:
 - 65 µm ID electrode (PN 5029342)—preferred
 - 50 µm ID electrode (PN 5028466)
 - 25 µm ID electrode (PN 5028467)

Create the LC and Autosampler Methods

2

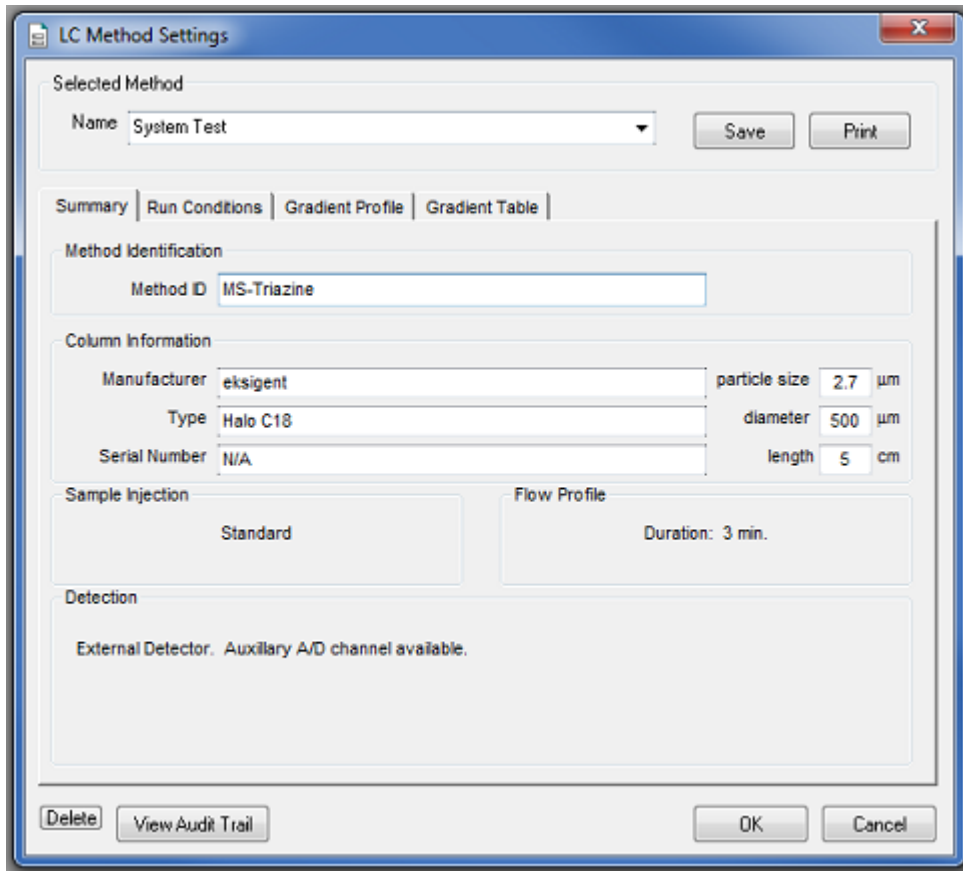
Note: The active hardware profile must include the CTC autosampler and the Eksigent control software to perform a run with the Analyst[®] software. The hardware profile is configured at installation. However, if you are using a different computer or have uninstalled the Analyst[®] software, then the hardware profile may not be correct. Verify that the correct hardware profile is present or create a profile before you begin. Refer to the *Operator Guide* for information on verifying and creating a hardware profile.

Create the LC method in the Eksigent control software and the autosampler method in the Analyst[®] software.

Create the LC Method

1. Click **LC Methods**.
2. In the **Name** box, type a name for the method, and then click **Save**.
3. On the **Summary** tab, specify the values as shown.

Figure 2-1 LC Method Settings Dialog—Summary Tab



4. On the **Run Conditions** tab, specify the **Pre-Run** and **Pre-Injection** values as shown.

Create the LC and Autosampler Methods

Figure 2-2 LC Method Settings Dialog—Run Conditions Tab

The screenshot shows the 'LC Method Settings' dialog box with the 'Run Conditions' tab selected. The 'Selected Method' section shows 'System Test' in the Name dropdown, with 'Save' and 'Print' buttons. The 'Pre-Run' section has three options: 'Flush column for 1.0 minutes using 100 % initial flowrate conditions.' (checked), 'First, establish a column pressure of 3000 psi.' (unchecked), and 'Stabilize column temperature at 35 °C prior to injecting sample and beginning Flow Profile.' (checked). 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 2000 nL of sample at 100 % initial flowrate conditions.', and 'Rapid: Inject 2000 nL of sample at maximum flowrate, maintaining initial mixture conditions.'. The 'Post-Run' section has one option: 'Flush column for 1 minutes using 100 % ending flowrate conditions.' (unchecked). At the bottom are 'Delete', 'View Audit Trail', 'OK', and 'Cancel' buttons.

5. On the **Gradient Table** tab, type the values for the method as shown.

Figure 2-3 LC Method Settings Dialog—Gradient Table Tab

LC Method Settings

Selected Method

Name: System Test

Save Print

Summary | Run Conditions | Gradient Profile | Gradient Table

	Time (min)	% A	% B	Event
1	0	80	20	
2	1	10	90	
3	2	10	90	
4	2.1	80	20	
5	3	80	20	
6				
7				
8				
9				
10				
11				
12				
13				

Flow Mode

Conserved flow
 Independent flow

Profile Editor

Total flowrate: 40 µL/min

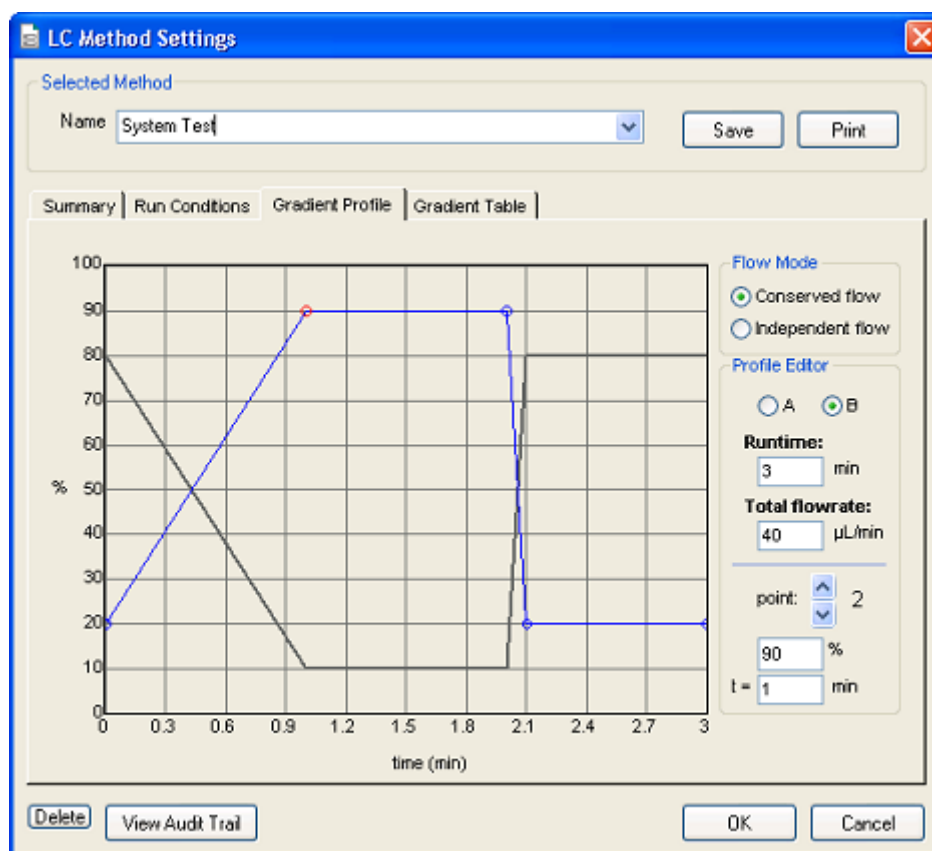
Delete View Audit Trail OK Cancel

The final lines in the gradient are used to equilibrate the column and sample loop with the initial conditions for the run.

6. On the **Gradient Profile** tab, review the profile shown.

Create the LC and Autosampler Methods

Figure 2-4 LC Method Settings Dialog—Gradient Profile Tab



7. Click **Save**.
8. Click **OK**.

Create the Autosampler Method

1. On the Navigation bar in the Analyst[®] 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 **microLC200-Injection-RevB.cyx**.

Note: Field Service Employees should download the latest .CYX file from the SharePoint site and leave a copy of the file with the customer.

4. In the **Injection Volume** field, type **10** (the volume to be aspirated into the sample loop).
5. Make the following changes to the parameters in the **Cycle Arguments** table.
 - a. Type **1** for both **Front Volume** and **Rear Volume**.
 - b. Select **Wash2** for **Second Wash Solvent**.

Figure 2-5 CTC Autosampler Basic Properties Tab

CTC PAL Autosampler Basic Properties

Loop Volume1 (µl): 10 Actual Syringe (µl): 100
 Loop Volume2 (µl): 10 Injection Volume (µl): 10.000

Available Cycles: microLC200 Inject Rev

Syringe: 100µDLW

Description:

Parameter	Value
Airgap Volume (µl)	1
Front Volume (µl)	1
Rear Volume (µl)	1
Sample Aspirate Speed (µl/s)	2
Pulup Delay (ms)	500
Num of Wash1 PreDips	1
Num of Wash2 PreDips	0
Inject to	LC Vlv1
Injection Speed (µl/s)	1
Needle Gap for Vlv Cleans (mm)	0
First Wash Solvent	Wash1
Valve Clean Time 1 (s)	5
Needle Clean Time 1 (s)	2
Second Wash Solvent	Wash2
Needle Clean Time 2 (s)	2
Valve Clean Time 2 (s)	5
Replicate Count	1
Final Wash Solvent	Wash1
0 or 1 Final Cleans	0
Final Needle Clean Time (s)	2
Final Valve Clean Time (s)	5

Default All

6. Verify that the parameters in the **Cycle Arguments** table are as shown in [Table 2-1](#).
 If the values are not the same, edit them as needed.

Table 2-1 Cycle Arguments Parameters

Parameter	Value	Parameter	Value
Airgap Volume (µL)	1	Valve Clean Time 1 (s)	5
Front Volume (µL)	1	Needle Clean Time 1 (s)	2
Rear Volume (µL)	1	Second Wash Solvent	Wash2
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
Num of Wash2 PreDips	0	Final Wash Solvent	Wash1
Inject to	LCVlv1	0 or 1 Final Cleans	0
Injection Speed (µL/s)	1	Final Needle Clean Time (s)	2
Needle Gap for Vlv Cleans (µm)	0	Final Valve Clean Time 2 (s)	5
First Wash Solvent	Wash1		

Create the Acquisition Method

1. Close the Eksigent control software, if it is open.
2. Open the Analyst[®] software.
3. On the **Navigation** bar under **Acquire**, double-click **Build Acquisition Method**.
The **Acquisition Method Editor** opens.
4. In the **Acquisition Method Browser** pane, click the autosampler, and then select the autosampler method created previously.
Refer to [Create the Autosampler Method](#).
5. Select the LC method.
 - a. In the **Acquisition Method Browser** pane, click **Eksigent 1**.
 - b. Click ... (Browse) to view the available LC methods.
 - c. Click the name of the method created previously, and then click **Open**.
6. In the **Acquisition Method Browser** pane, click **Mass Spec** to create the mass spectrometer acquisition method.
7. Specify the acquisition method parameters as shown in [Table 2-2](#) and [Table 2-3](#).

Note: Source/Gas parameters may 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 Acquisition Method Parameters

Parameter	Value
MS	
Scan Type	MRM (MRM)
Duration (min)	3
Polarity	Positive
Q1/Q3 Masses and CE	Refer to tables below.
Advanced MS	
Q1 Resolution	Unit
Q3 Resolution	Unit
Pause (ms)	5.00
Source/Gas**	
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)
Compound	
Declustering Potential (DP)	70
Collision Exit Potential (CXP)	30

Note: The values for Entrance Potential (EP) and Collision Cell Exit Potential (CXP) differ by mass spectrometer. Enter the appropriate values from [Table 2-3](#).

Create the LC and Autosampler Methods

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

System	EP	CXP
API 3200 system 3200 QTRAP system	10	4
API 4000 system 4000 QTRAP system SCIEX Triple Quad 4500 system SCIEX QTRAP 4500 system	10	10
API 5000 system SCIEX Triple Quad 5500 system SCEX QTRAP 5500 system	10	10
SCIEX 6500 system	10	13

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

Note: Field Service Employees may download a .CSV file from the SharePoint site, import it and save it locally for customer use.

Note: Add the CE (collision energy) column to the table view by right-clicking the table and selecting **CE** on the menu.

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
226.2	142.3	10	Prometon 1	51	29
226.2	184.2	10	Prometon 2	51	23
242.2	158.1	10	Prometryn 1	41	27
242.2	200.0	10	Prometryn 2	41	23

Table 2-4 MRM Transitions for API 3200 and 3200 QTRAP Systems (continued)

Q1	Q3	Dwell	ID	DP	CE
230.2	146.0	10	Propazine 1	51	31
230.2	188.3	10	Propazine 2	51	23
242.2	186.0	10	Terbutryn 1	41	23
242.2	68.2	10	Terbutryn 2	41	55

Table 2-5 MRM Transitions for 4000 and 4500 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
226.2	142.3	10	Prometon 1	71	29
226.2	184.2	10	Prometon 2	71	23
242.2	158.1	10	Prometryn 1	61	27
242.2	200.0	10	Prometryn 2	61	23
230.2	146.0	10	Propazine 1	71	31
230.2	188.3	10	Propazine 2	71	23
242.2	186.0	10	Terbutryn 1	61	23
242.2	68.2	10	Terbutryn 2	61	55

Table 2-6 MRM Transitions for Triple Quad 5500/6500 Systems and QTRAP 5500/6500 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

Create the LC and Autosampler Methods

Table 2-6 MRM Transitions for Triple Quad 5500/6500 Systems and QTRAP 5500/6500 Systems (continued)

Q1	Q3	Dwell	ID	DP	CE
226.2	142.3	10	Prometon 1	91	29
226.2	184.2	10	Prometon 2	91	23
242.2	158.1	10	Prometryn 1	81	27
242.2	200.0	10	Prometryn 2	81	23
230.2	146.0	10	Propazine 1	91	31
230.2	188.3	10	Propazine 2	91	23
242.2	186.0	10	Terbutryn 1	81	23
242.2	68.2	10	Terbutryn 2	41	55

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

Prepare the System for Testing

3

To prepare the system to run the system integration test, complete the following procedures:

1. [Verify system readiness.](#)
2. [Prepare the stock sample solution.](#)
3. [Prepare the sample to be tested.](#)
4. [Equilibrate the system.](#)

Verify System Readiness

Prior to running this test, make sure that the system has been calibrated. To calibrate the system, perform the procedures, "Reinitialize the Pressure Transducers" and "Calibrate Flowmeters" in the *Operator Guide*.

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
<ul style="list-style-type: none">• Methanol• 100 µg/mL Triazine System Suitability Solution (PN 4376887)

1. Create a 10 µg/mL solution using the volumes from the first row of [Table 3-1](#).
2. Create a 1 µg/mL solution using the volumes from the second row of [Table 3-1](#).

Prepare the System for Testing

Table 3-1 Solution Dilution

Stock Solution Volume	Dilution Solvent Volume	Final Concentration
100 μ L of 100 μ g/mL triazine test mixture	900 μ L MeOH	10 μ g/mL
100 μ L of 10 μ g/mL sample solution	900 μ L MeOH	1 μ g/mL

Prepare the Sample to Be Tested

Required Materials

- | |
|--|
| <ul style="list-style-type: none">• Stock solution, prepared in Prepare the 1 μg/mL Stock Solution on page 17 |
|--|

1. Combine the specified amount of the 1 μ g/mL triazine stock solution with 50:50 MeOH:H₂O in a clean vial. Refer to [Table 3-2](#).
2. Vortex the vial for at least 30 seconds to properly mix the solution.
3. Transfer the solution to the autosampler vial and make 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 and make sure that there is no bubble on the bottom of the vial. Refer to [Equilibrate the System on page 19](#).

Table 3-2 Sample Dilutions by Mass Spectrometer

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

Equilibrate the System

Make sure that the LC column is not connected.

1. Verify the following mobile phases are loaded on the system.

Prepare the System for Testing

Table 3-3 Mobile Phases

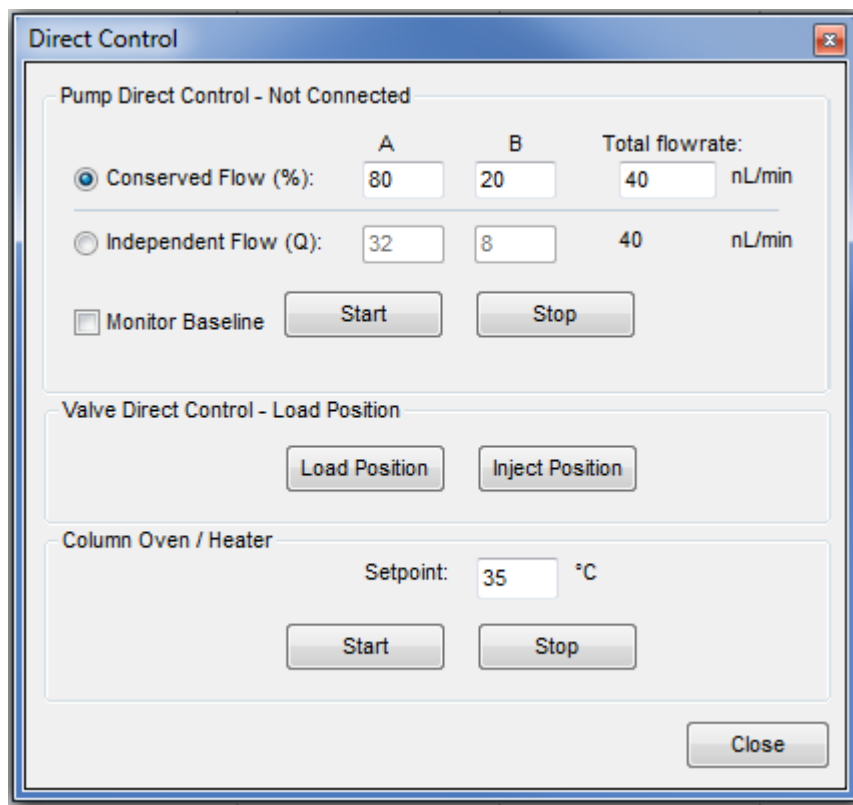
Buffer	Mixture	Channel
Buffer A	100% water:0.1% formic acid	Channel A
Buffer B	100% acetonitrile:0.1% formic acid	Channel B

2. In the Analyst[®] software, equilibrate the mass spectrometer.
 - a. On the **Navigation** bar, click **Tune and calibrate**.
 - b. Click **Manual Tuning**.
 - c. In the tuning method, type the source and gas parameters from [Table 2-2](#). Adjust the source and gas parameters as needed.
 - d. Type **10** in the **Time [Min.]** field, and then click **OK** to manually start the equilibration.
3. Start the pump.
 - a. In the Eksigent control software, click **System > Direct Control**.
 - b. Select the **Conserved Flow** option and set **A (%)** to **80** and **B (%)** to **20**.

This is the mobile phase composition used for equilibration.
 - c. Type the **Total flowrate** of **40** $\mu\text{L}/\text{min}$.

- d. Click **Start** to start the pump.

Figure 3-1 Direct Control Dialog



4. In the **Column Oven/Heater** section, type the **Setpoint** of **35**, and then click **Start**.

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

5. Flush the injection valve:
 - a. In the **Valve Direct Control** section, alternately click **Load Position** and **Inject Position**.
 - 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.
6. Click **Start** in the **Pump Direct Control** section to start the pump again.
7. After approximately three minutes, click **Stop** in the **Pump Direct Control** section to halt the pump.

Perform the System Integration Test

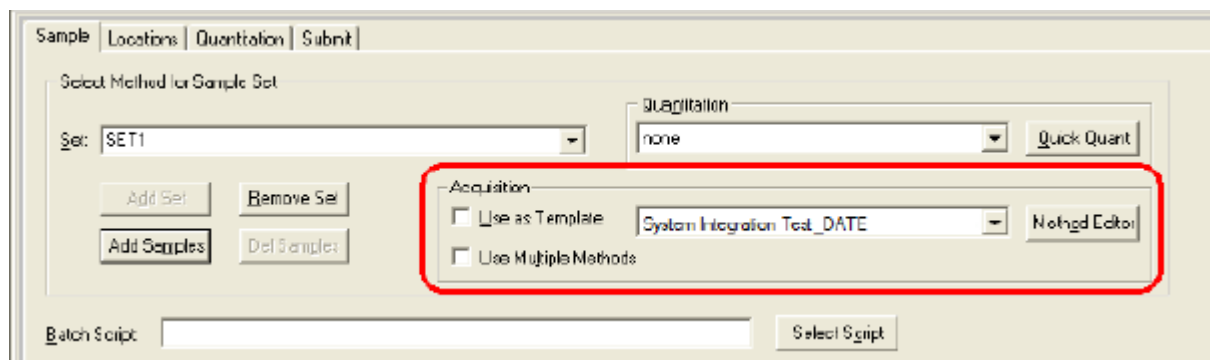
4

Create the LC/MS acquisition batch, run the batch and then verify the results. Record the test results in the [System Integration Test Data Log and Signoff on page 41](#).

Create the LC/MS Acquisition Batch and Quantitation Method

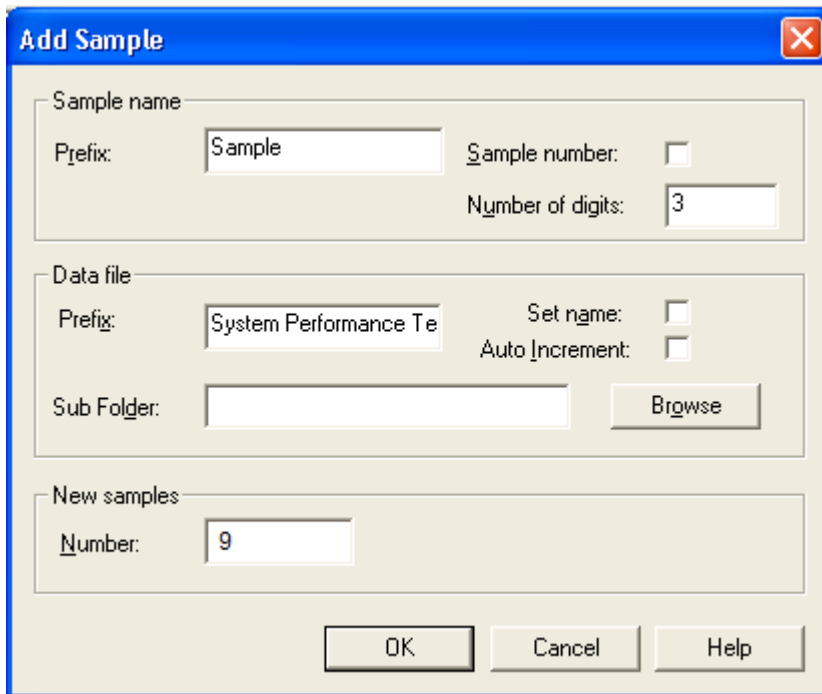
1. Open the Analyst® software.
2. Double-click **Build Acquisition Batch** on the **Navigation** bar.
3. On the **Sample** tab, in the **Acquisition** group, select the acquisition method created in [Create the Acquisition Method on page 12](#) from the list.

Figure 4-1 Sample Tab—Acquisition Group



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

Figure 4-2 Add Sample Dialog



6. Specify the sample information as shown in [Figure 4-2](#).
7. In the **Number** field, type **9**.
8. Click **OK**.
9. Specify the rack and plate position for the samples as shown in [Figure 4-3](#).

Perform the System Integration Test

Figure 4-3 Batch Editor—Sample Tab

Batch Editor: [API Instrument - New Batch]

Sample | Locations | Calibrate | Quantitation | Submit

Select Method for Sample Set

Set: SET1

Quantitation: none

Acquisition: System Integration Test

	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Data File	Inj Volume (µl)
1	blank001	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000
2	blank002	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000
3	blank003	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000
4	10 ng/ml triazine mix_1	1	1	VT54	1	2	System Integration Test 2013-02-07	10.000
5	10 ng/ml triazine mix_2	1	1	VT54	1	2	System Integration Test 2013-02-07	10.000
6	10 ng/ml triazine mix_3	1	1	VT54	1	2	System Integration Test 2013-02-07	10.000
7	blank007	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000
8	blank008	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000
9	blank009	1	1	VT54	1	1	System Integration Test 2013-02-07	10.000

10. In the **Vial Position** column, type the position of the vial containing the test mixture or blank, as required.
11. For 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.
12. Make sure that the **Injection Volume** on the **Sample** tab is 10 µL.
13. Click **Quick Quant** to open the **Create Semi-Automatic Quantitation Method** dialog.

Figure 4-4 Create Semi-Automatic Quantitation Method Dialog

Data Source: Period 1 / Expt. 1 Smoothing Width: 3 points.

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	Prometon 1		226.200 / 142.300
6	Prometon 2		226.200 / 184.200

OK Cancel Help

14. Select **3** from the **Smoothing Width** list.
15. Click **OK**, and when prompted, type **triazine test** for the method name.

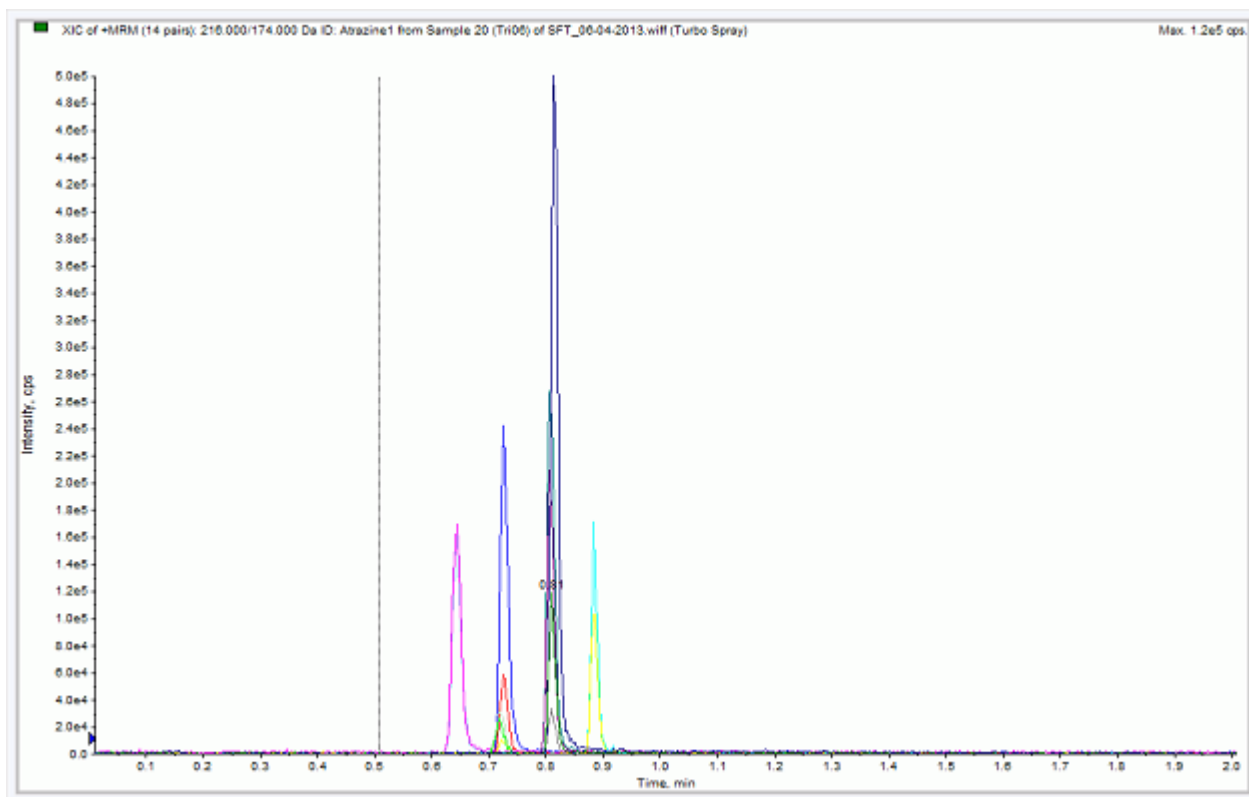
Run the Batch

If this is the first time the column has been used, 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 click  (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) should be < 4000 PSI.
6. If the first run was to condition the column, then re-run the batch to acquire data for analysis.
Refer to [Figure 4-5](#) for representative data.

Perform the System Integration Test

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

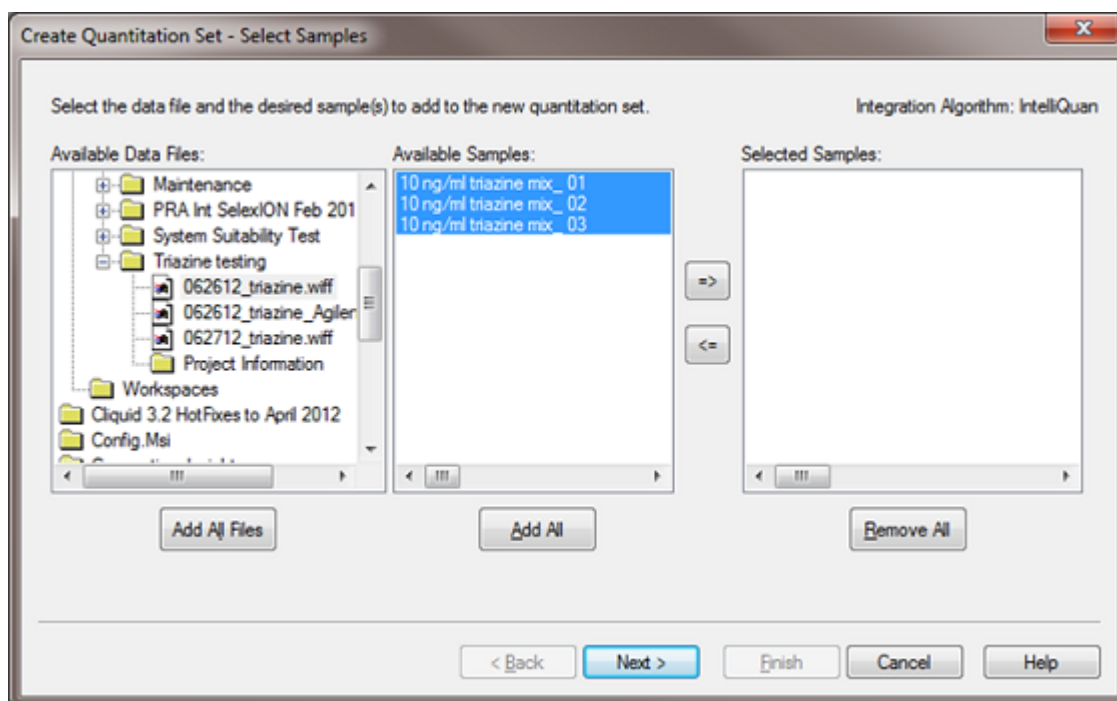


Verify the Results

Verify the Results and Verify the Integration

1. When the run is finished, double-click **Quantitation wizard** in the **Quantitation** menu.
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, make sure that the injections selected are from the test run.

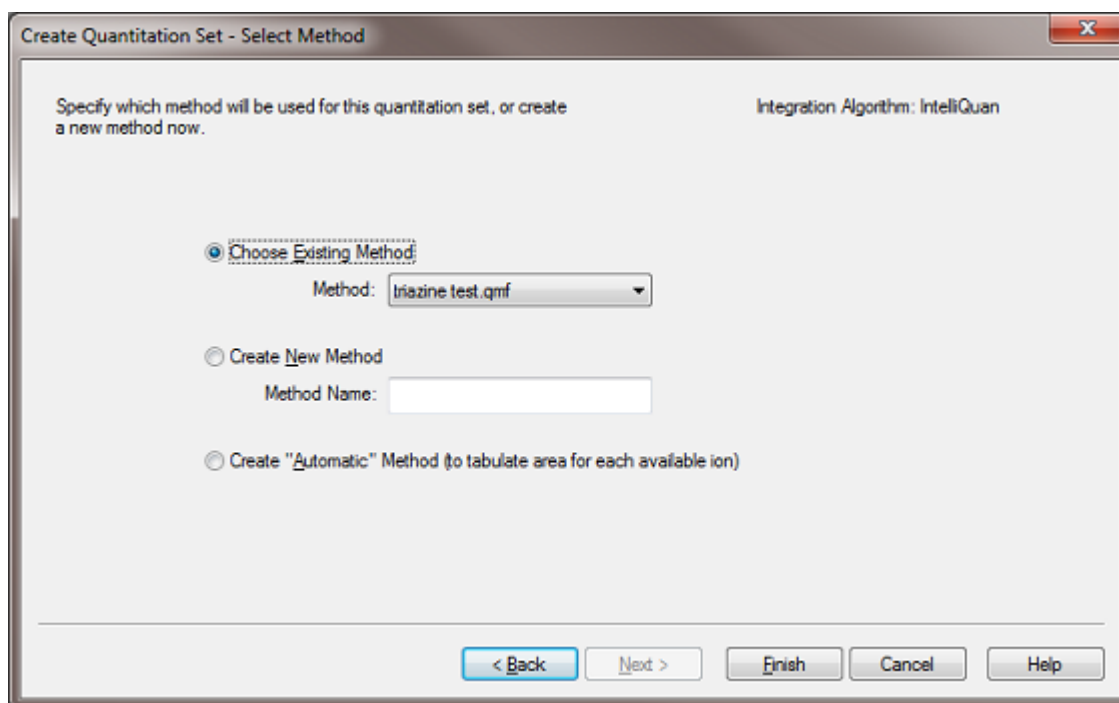
Figure 4-6 Quantitation Wizard—Select Samples Page



4. Click **Next** and **Next** on the following screen.
5. In the **Select Method** page, select **Choose Existing Method** and **triazine test** in the **Method** list, and then click **Finish**.

Perform the System Integration Test

Figure 4-7 Quantitation Wizard—Select Methods Page



The results table opens.

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

Figure 4-8 Results Table—Changing Sample Type

	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

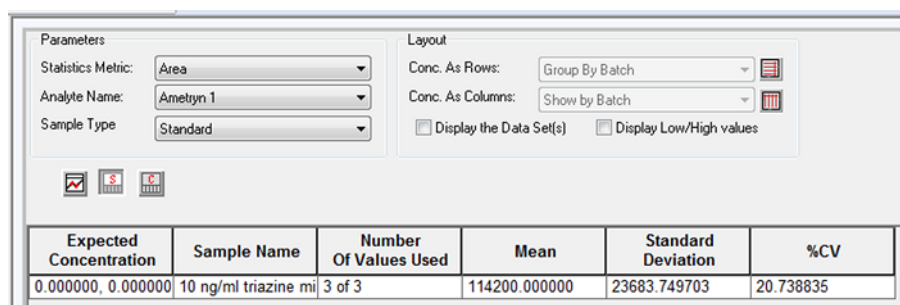
- a. On the first line, change the **Sample Type** to **Standard**.
- b. Right-click **Sample Type** and select **Fill Down**.

7. Right-click in the margin above the results table, and then select **Analyte > Ametryn 1**.
Only the results for Ametryn 1 are displayed.
8. Select **Tools > Peak Review > Pane**.
The chromatograms for the MRM transition are displayed below the results table.
9. Click the forward and back arrows in the chromatogram pane to view the integration for each chromatogram.
10. After the data has been reviewed, repeat step 7 through step 9 for the Atrazine 1 and Terbutryn 1 transitions.

Verify the Mean Area and % CV

1. After reviewing the integration, on the **Tools** menu, select **Statistics**.
2. In the **Statistics Metric** list, select **Area**.
3. For each of the four MRM transitions:
 - a. Compare the Mean and % CV values to the specifications in the [System Integration Test Data Log and Signoff on page 41](#).
 - b. Record the values in [Table 6-1 on page 41](#).
4. Delete the **Statistics** pane.

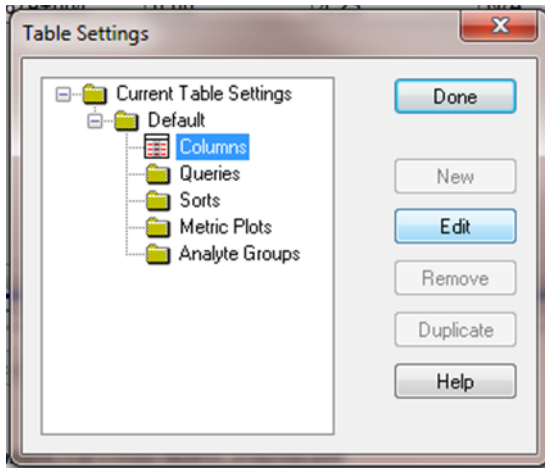
Figure 4-9 Statistics Summary Pane



Verify Peak Widths at Half Height and Retention Times

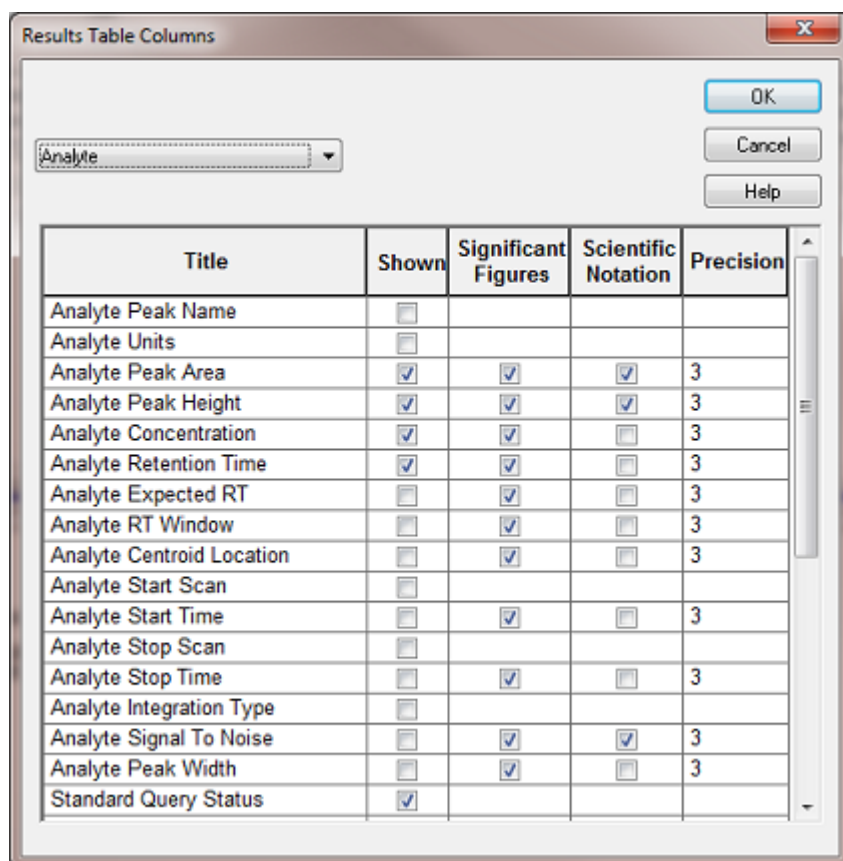
1. Open the **Results Table Columns** dialog.
2. Right-click on the area above the results table and select **Table Settings > Edit**.
3. In the **Table settings** dialog, click **Columns**, and then click **Edit**.

Figure 4-10 Table Settings Dialog



4. In the **Results Table Columns** dialog, select the columns for the table.

Figure 4-11 Results Table Columns Dialog



- a. Select **Analyte** in the list.
 - b. In the table, select **Analyte Retention Time** and **Analyte Peak Width at 50% Height**.
 - c. Make sure that **Analyte Peak Area**, **Analyte Peak Height**, and **Analyte concentration** are also selected.
 - d. Click **OK** to close the **Results Table** dialog.
5. Click **Done** to close the **Table Settings** dialog.
- The results table updates to display 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 41](#).
 - b. Record the values in [Table 6-1 on page 41](#).

This section provides information for troubleshooting issues with the MicroLC 200/200 Plus system.

1. Repeat the system integration test. Refer to [Perform the System Integration Test on page 22](#).
2. Examine the chromatograms for the blank injections.
 - a. Are there peaks similar to those in the sample? If so, there is carryover. Follow the steps in [Identify and Resolve Carryover Issues](#), then repeat the system integration test.
3. Compare the chromatograms for the sample to those from the original system integration test. [Figure 5-5](#) shows a chromatogram with good results. After each troubleshooting step, repeat the system test.
 - a. [Resolve General Issues](#).
 - b. Do the retention times change from injection to injection? Refer to [Resolve Drifting Retention Times](#).
 - c. Are the peaks broader than or tailing more than the original chromatogram? Refer to [Peak widths are too broad or are tailing](#).
 - d. Is the background level high? Refer to [Front, pre-eluting or low intensity peaks](#).
 - e. Are peaks missing? Refer to [Missing or low intensity peaks](#).
4. Examine the pressure traces in the Eksigent control software. Refer to [Table 5-1 on page 36](#) for anomalies in the pressure traces and their possible causes.

Troubleshooting Peak Related Problems and System Crashes

This section provides information for troubleshooting peak related problems and system crashes.

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.

Front, 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 HTC-xt PAL 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 HTC-xt PAL autosampler method has been configured properly:
 - There is a large enough volume loaded into injection loop
 - The **Inject to:** value is set to **LC Vlv1** (not Waste)
 - The **Post Inject Delay** value is set to **>5000** ms
- Make sure that the injection alignment is correct (position and needle penetration) and that the injection port fitting seals on the syringe needle.

LC system crashes or loses communication

- Make sure that the proper grounding is in place between the ion source and the LC system.
- Make sure the pump is connected to a USB 2.0 port on the computer (connections to a USB 3.0 port do not work correctly).

Low signal

- A pressure drop-out at the start of a trace is indicative of air in the sample loop. Refer to "Sample Position in the Sample Loop" in the *Operator Guide*.
 - Examine the injection valve fittings and autosampler for leaks. Consider performing a pressure test. Refer to "Perform a Pump Leak Check" in the *System Calibration* document.
 - Make sure the values for **Front Sample** and **Rear Sample** in the **Cycle Parameters** table in the **Acquisition** window are 1 μ L.

Figure 5-1 Acquisition Window Showing Pc, With Air Bubbles in Sample Loop

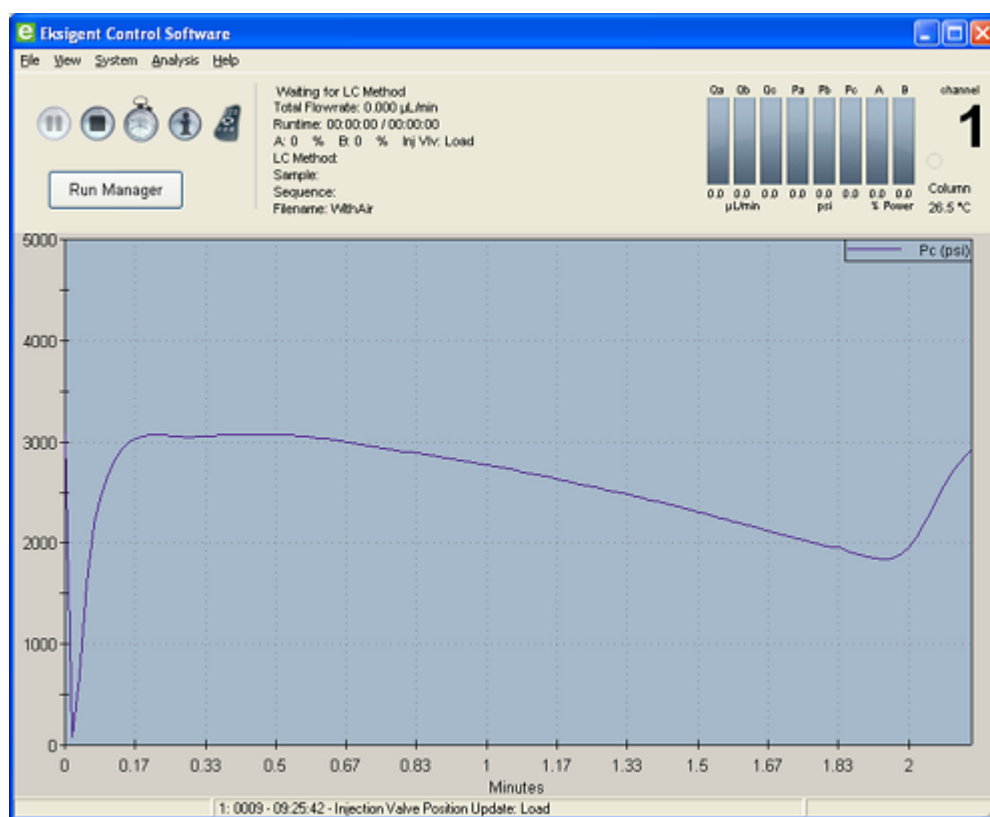
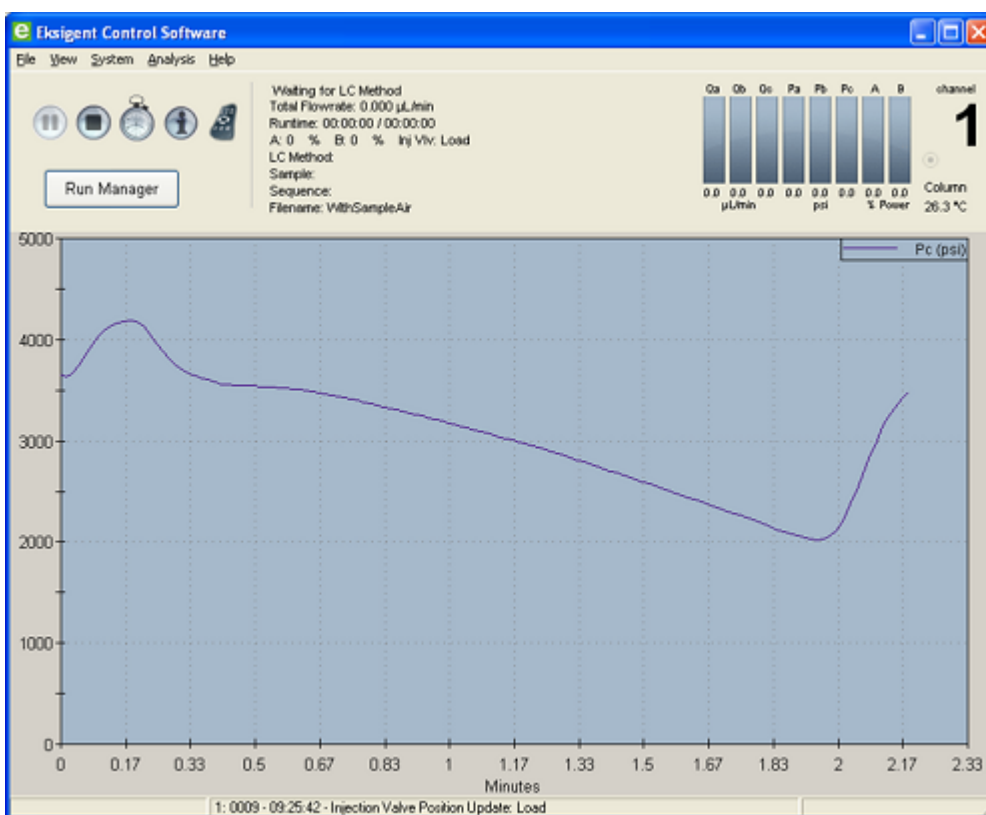


Figure 5-2 Acquisition Window Showing P_c, Without Air Bubbles in Sample Loop

Resolve Drifting Retention Times

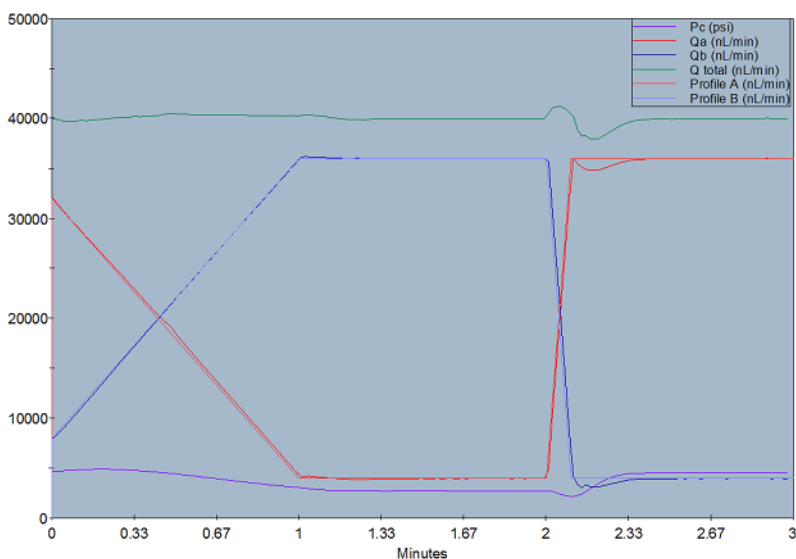
If the column temperature varies over the course of the test, retention times may drift.

- Make sure 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.
- Re-initialize the pressure transducers (refer to "Re-initialize the Pressure Transducers" in the *Operator Guide*).

Troubleshooting 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. [Figure 5-3](#) is an example of pressure and flow data for the system test

Figure 5-3 Example Pressure and Flow Data for the System Integration Test



Use [Table 5-1](#) to troubleshoot some issues that appear in the pressure and flow data.

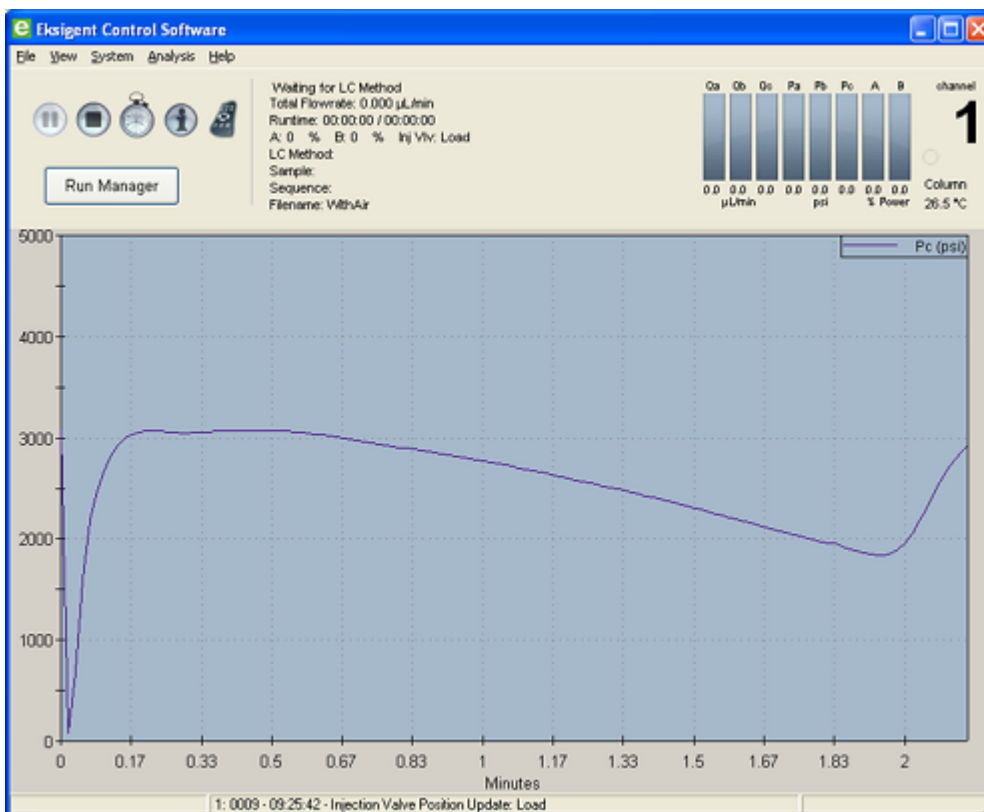
Table 5-1 Common Anomalies in MicroLC 200/200 Plus System Pressure Data

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-out at the start of a trace ([Figure 5-4](#)) can indicate that there is air in the sample loop (refer to "Sample Position in the Sample Loop" in the *Operator Guide*).

Figure 5-4 Air Bubbles in the 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[®] software, make sure the values for **Front Sample** and **Rear Sample** in the Cycle Parameters table in the **Acquisition Method** window are 1 µL and enter 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 FSE for assistance.

Troubleshooting Using the Chromatogram

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

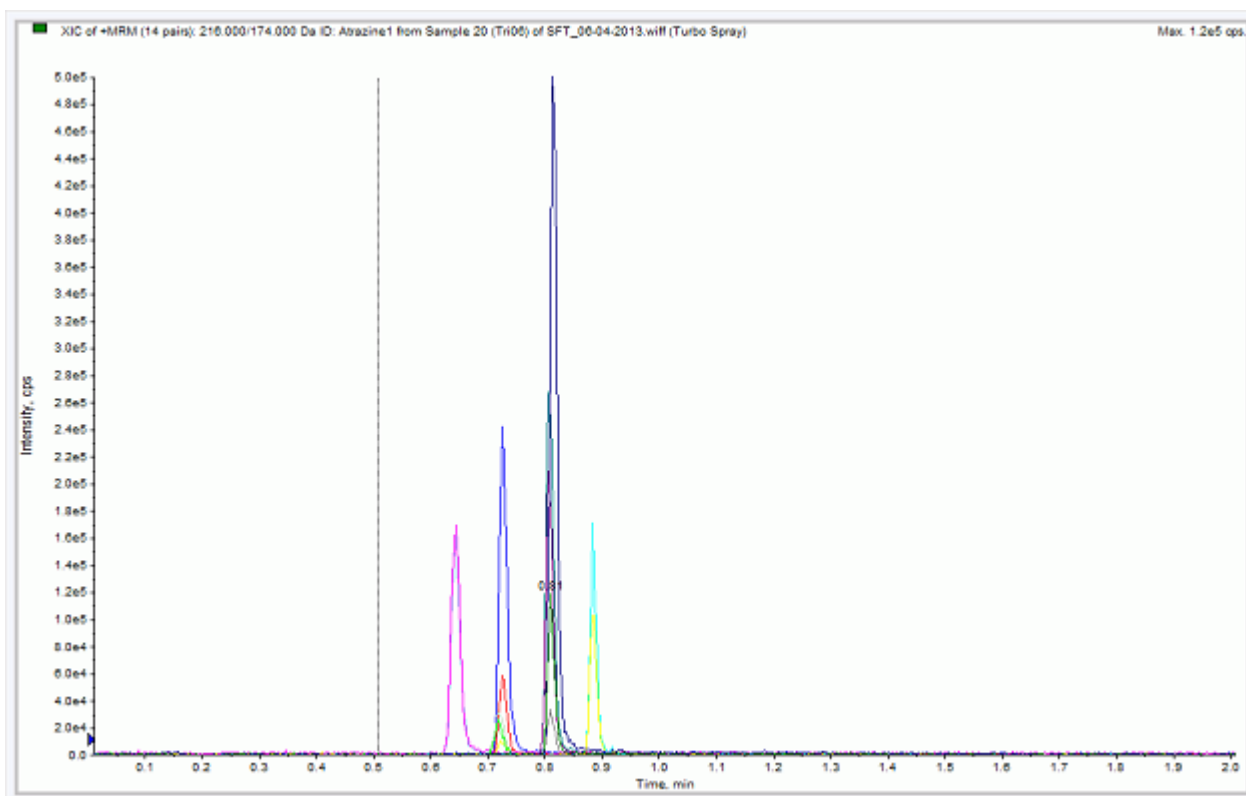
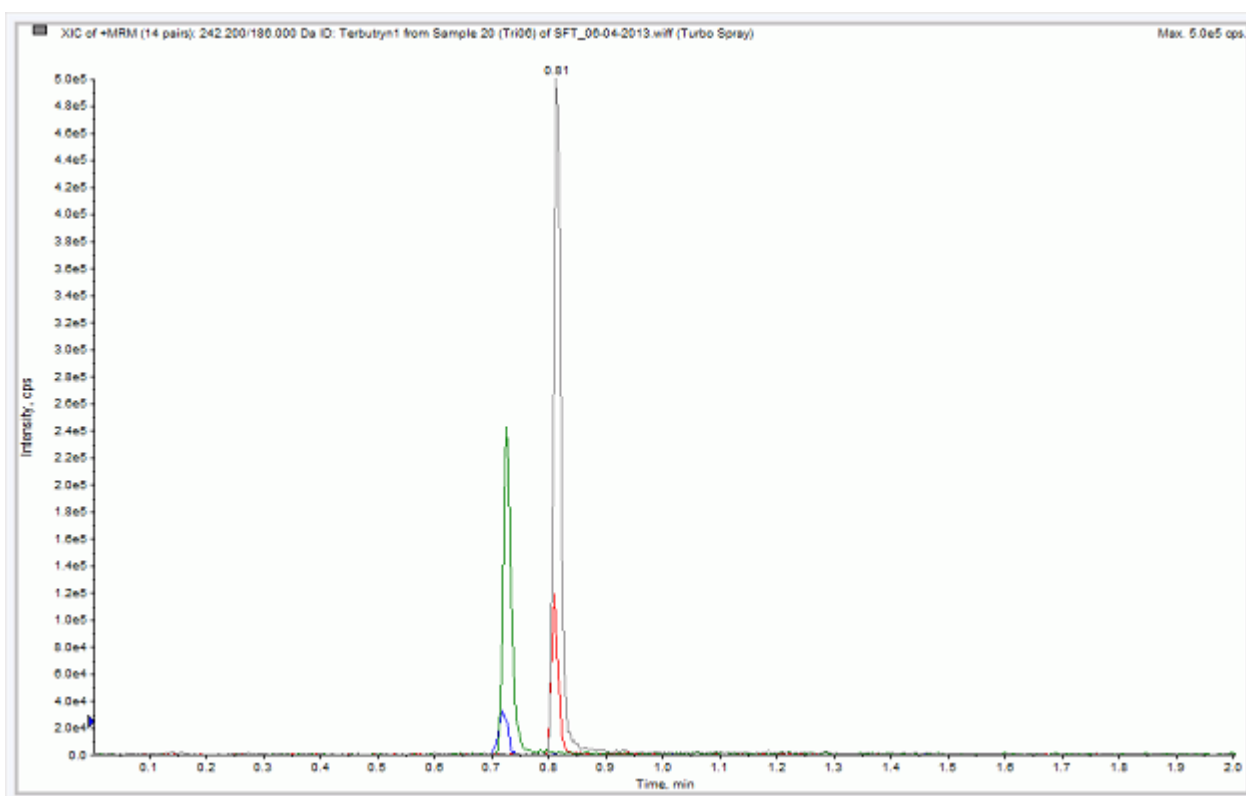


Figure 5-6 Example Chromatograms for the System Integration Test—XICs for QC Peaks



Identify and Resolve Carryover Issues

For a well-tuned MicroLC 200/200 Plus 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 it should be resolved first.

Carryover is most often due to problems with the injection port or the DLW.

Test the Injection Port

- Inspect the injection port and make sure:
 - The port is plumbed correctly.
 - No fluid is leaking from the injection port. The presence of fluid at the injection port suggests a problem with the needle alignment at the port position and needle penetration or the tightness of the fitting and the syringe needle.

Test the Waste Line

Run a test sample and make sure that the fluid is flowing out the waste line. During the flow, watch for bubbles. The presence of bubbles may indicate a plug in the line.

Troubleshooting

- Fluid should emerge during wash cycles.
- Fluid should emerge during injection. To test, create a run with a 50 μ L injection that will overflow the loop.

Test the DLW

Make a mark on each pump and verify the marks move when the pumps should be flowing. Refer to "Autosampler Method" in the *Operator Guide* for more information about when the DLW pumps operate.

Resolve General Issues

- Make sure the correct solvents are being used and the solvents in the bottle match the settings in the Eksigent control software

System Integration Test Data Log and Signoff

6

Test Results

Complete this table with the results for the five analytes from the triazine solution. Refer to [Specifications on page 41](#) for the mean area specification. Select **Guideline Met** if the value is within 10%.

Mass Spectrometer:	
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Table 6-1 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 or Propazine 1					
Guideline Met?					

Table 6-2 Notes

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Specifications

Mean Area Specification

System Integration Test Data Log and Signoff

Table 6-3 Instrument Response (CPS)

Analyte	3200 10 ng/mL	4000/4500 1 ng/mL	5000/5500/6500 0.1 ng/mL
Ametryn 1	8.0×10^4	7.5×10^4	3.5×10^4
Atrazine 1	1.5×10^4	6.5×10^4	3.0×10^4
Propazine 1	n/a	n/a	n/a
Terbutryn 1	1.0×10^4	1.0×10^4	5.0×10^4

% CV Specification

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

Retention Time Specification

Chromatographic peaks for the four analytes should be <0.030 minutes in width when measured at half maximum (peak width at half maximum).

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

Signoff

Contact name		Date (yyyy-mm-dd)	
Contact signature*			
FSE name		Date (yyyy-mm-dd)	
FSE signature			

*Signature required on hard copy only.

Revision History

Document Number	Reason for Change	Date
D5032053 A	First release of document.	June 2012
D5032053 B	Updated screen captures to reflect changes to software. Removed support for TripleTOF systems. Updated the "Run the Test" procedure to include creating quantitation method. Added procedure to verify the mean area and %CV.	December 2012
RUO-IDV-05-1295-A D5032053 C	Updated document template. Updated part numbers for electrodes, locations of files. Reordered steps in "Prepare the System for Testing" and updated "Equilibrate the System" procedure. Generalized references to ion sources.	March 2014
RUO-IDV-05-1295-B D5032053 D	Applied new template and incorporated Eksigent MicroLC 200 Plus. Combined document with customer-facing version to create a single <i>System Integration Test</i> document. Replaces D5077470. Added "System Integration Test Data Log and Signoff" section.	September 2014
RUO-IDV-05-1295-C D5032053 E	Updated company name. Updated steps for equilibrating the mass spectrometer. Added additional item for troubleshooting system crashes. Removed simazine as one of the analytes to be analyzed.	April 2015