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part of AB SCIEX

# Eksigent MicroLC 200/200 Plus Systems

## For TripleTOF® Systems

### System Integration Test and Data Log

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AB Sciex Pte. Ltd.
Blk 33, #04-06
Marsiling Ind Estate Road 3
Woodlands Central Indus. Estate.
SINGAPORE 739256

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# Introduction 1

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 AB SCIEX mass spectrometers:

- TripleTOF® 4600 system
- TripleTOF® 5600 system
- TripleTOF® 6600 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 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.

Refer to *Mass Spectrometer System Calibration on page 42*. If the Eksigent system has been idle for two weeks or more, re-initialize transducers, then verify the flow rate and, if necessary, calibrate.

## 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™ 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

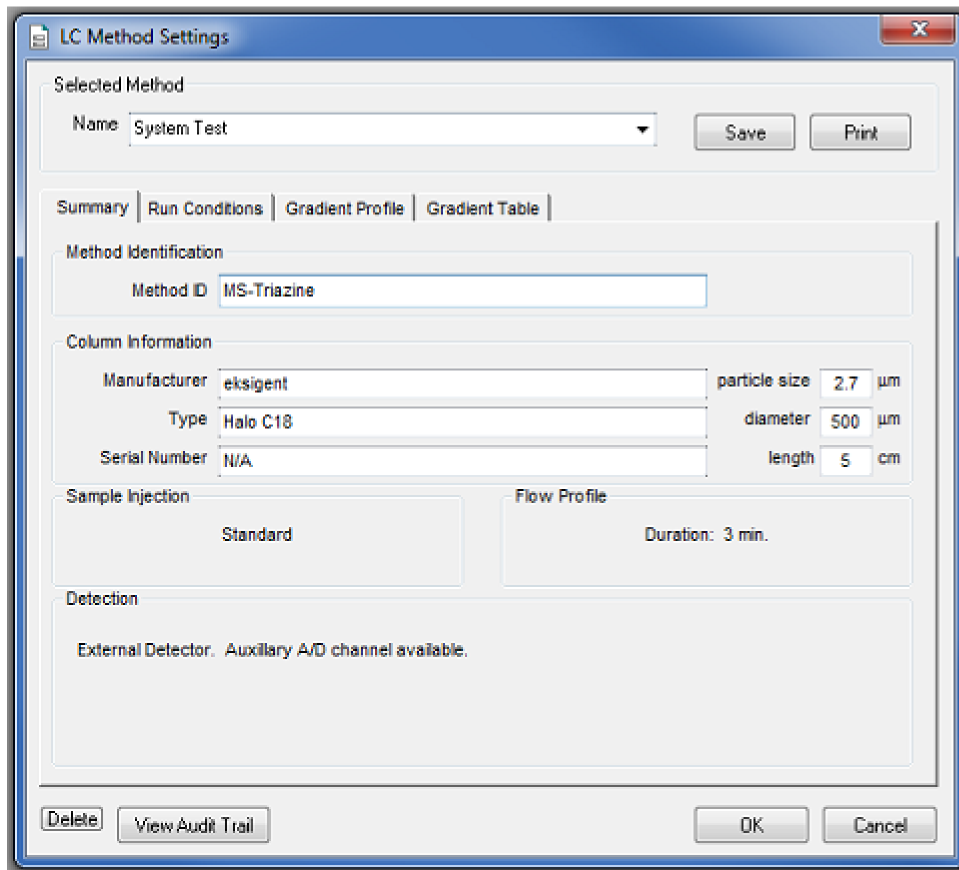
**Note:** The active hardware profile must include the autosampler and the Eksigent control software to perform a run with the Analyst® TF software. The hardware profile is configured at installation. However, if you are using a different computer or have uninstalled the Analyst® TF software, then the 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® TF 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.

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

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

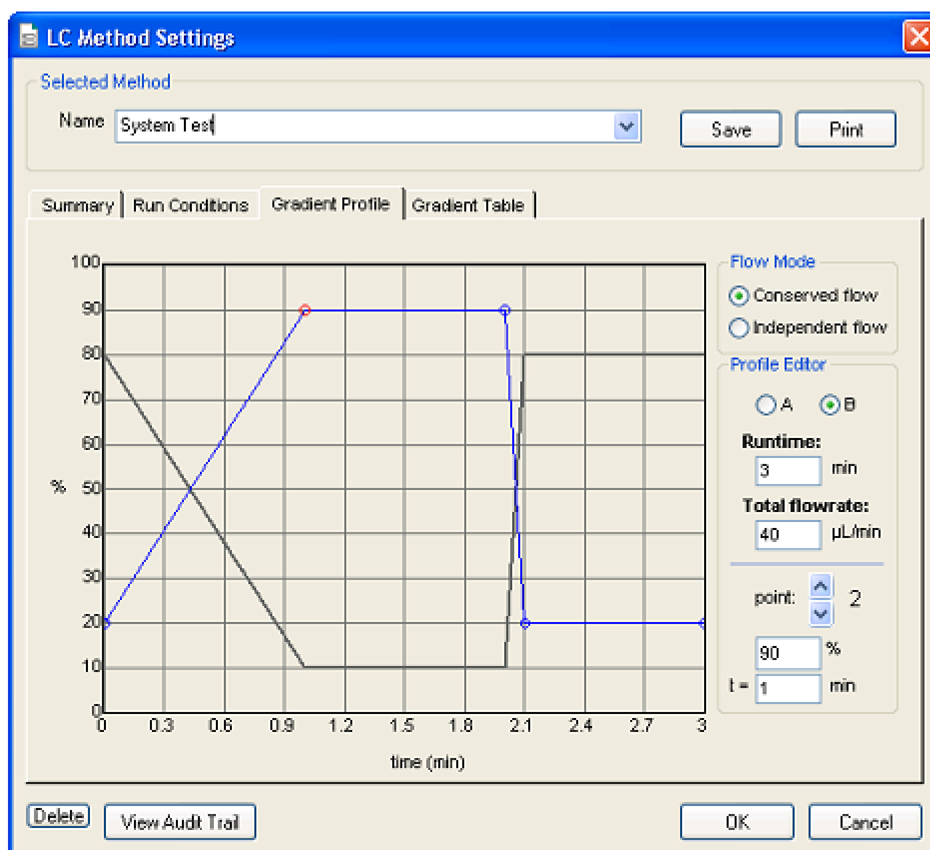
Profile Editor: Total flowrate: 40 µL/min

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.

**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® 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 **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. In the **Cycle Arguments** table, type **1** for both **Front Volume** and **Rear Volume**.
6. In the **Cycle Arguments** table, select **Wash2** for **Second Wash Solvent**.

**Figure 2-5 CTC Autosampler Basic Properties Tab**



| Parameter | Value |
|---|---|
| Airgap Volume (µl) | 1 |
| Front Volume (µl) | 1 |
| Rear Volume (µl) | 1 |
| Sample Aspirate Speed (µl/s) | 2 |
| Pullup 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 |



7. Verify that the parameters in the **Cycle Arguments** table are as shown in Table 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

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

1. Close the Eksigent control software, if it is open.
2. Open the Analyst® TF software.
3. On the **Navigation** bar under **Acquire**, double-click **Build Acquisition Method**.

   The Acquisition Method Editor opens.
4. Set the autosampler and LC method parameters as follows:
   a. In the **Acquisition Method Browser** pane, click **CTC PAL Autosampler**.

b. In the **Available Cycles** list, select **microLC200-Injection-RevB.cyx** or the latest .CYX file.

5. Verify the parameters in the Cycle Arguments table are as shown. If the values are not the same, edit them as needed.

**Table 2-2 Autosampler Method Parameters**

| Parameter | Value |
| --- | --- |
| Airgap Volume (µL) | 1 |
| Front Volume (µL) | 1 |
| Rear Volume (µL) | 1 |
| Sample Aspirate Speed (µL/s) | 2 |
| Pullup Delay (ms) | 500 |
| Num of Wash1 PreDips | 1 |
| Num of Wash2 PreDips | 0 |
| Inject to | LCVlv1 |
| 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 2 (s) | 5 |

6. 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**.
7. In the **Acquisition Method Browser** pane, click **Mass Spec** to create the mass spectrometer acquisition method.
8. Enter 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.
9. Click **Edit Parameters** to display the **Parameters Settings** dialog.
   a. Set the Source/Gas Parameters as shown.

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

   b. Click the **Compound** tab and set the Compound parameters as shown.

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

   c. Click **OK**.
10. 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 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 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*.

**Table 3-1 Solution Dilution**

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

# Prepare the Sample to Be Tested

1. Combine the specified amount of the 1 µg/mL triazine stock solution with 50:50 MeOH:$H_2O$ in a clean vial.
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 17*.

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

| System | Target Concentration | Dilution (Stock Solution + 50:50 MeOH:$H_20$) |
|---|---|---|
| AB SCIEX TripleTOF 4600 system<br>AB SCIEX TripleTOF 5600/5600+ system<br>AB SCIEX TripleTOF 6600 system | 10 ng/mL | Two serial dilutions:<br>a. 100 µL stock solution + 900 µL MeOH:$H_20$ (to make 100 ng/mL)<br>b. 100 µL of 100 ng/mL + 900 µL MeOH:$H_20$ |

# Equilibrate the System

Make sure that the LC column is connected.

| Required Materials |
|---|
| • Stock solution, prepared in *Prepare the 1 µg/mL Stock Solution on page 15* |

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

**Table 3-3 Mobile Phases**

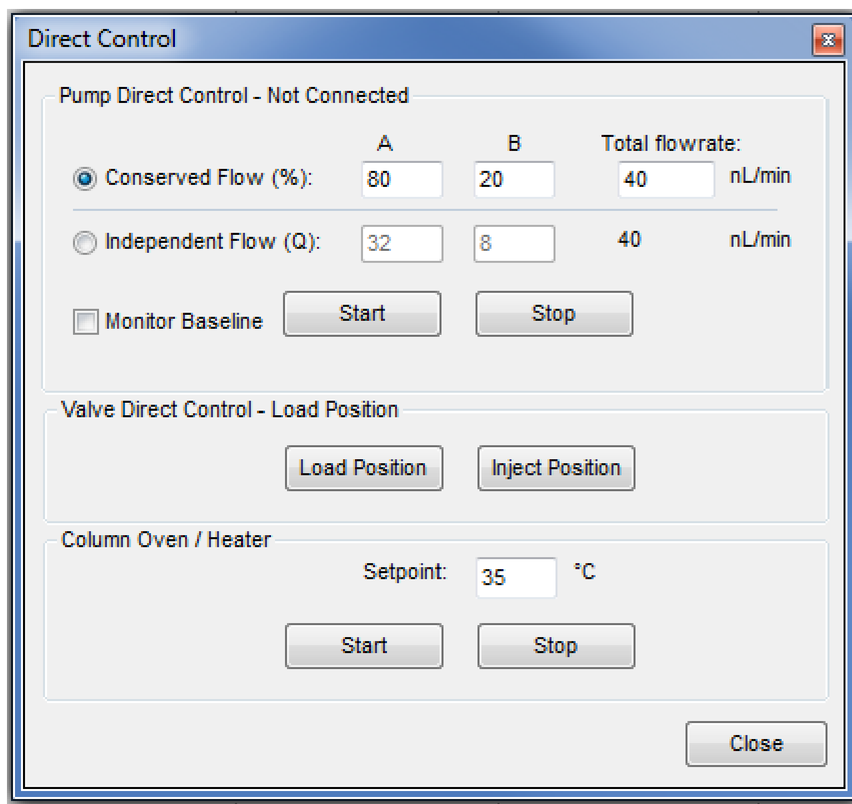
| Buffer | Mixture | Channel |
|---|---|---|
| Buffer A | 100% water:0.1% formic acid | Channel A |
| Buffer B | 100% acetonitrile:0.1% formic acid | Channel B |

2. In the Analyst® TF software, equilibrate the mass spectrometer.
   a. On the **Navigation** bar, click **Acquire**.
   b. Click **View Sample Queue**.
   c. Click **Acquire > Equilibrate**. The **Equilibrate** dialog opens.
   d. Select the acquisition method created previously.
   e. To equilibrate, type **10** in the **Time [Min.]** field, and then click **OK**.

3. In the Eksigent control software, click **System > Direct Control**.
4. Select the **Conserved Flow** option and set **A** (%) to **80** and **B** (%) to **20**.

   This is the mobile phase composition used for equilibration.
5. Type the **Total flowrate** of **40** µL/min.

**Figure 3-1 Direct Control Dialog**




6. 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.

7. In the **Valve Direct Control** section, flush the injection valve by alternately clicking **Load Position** and **Inject Position**.
8. Make sure that the final position of the valve is at **Load**.
9. 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 39*.

## Create the LC/MS Acquisition Batch and Quantitation Method

1. Open the Analyst® TF 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.

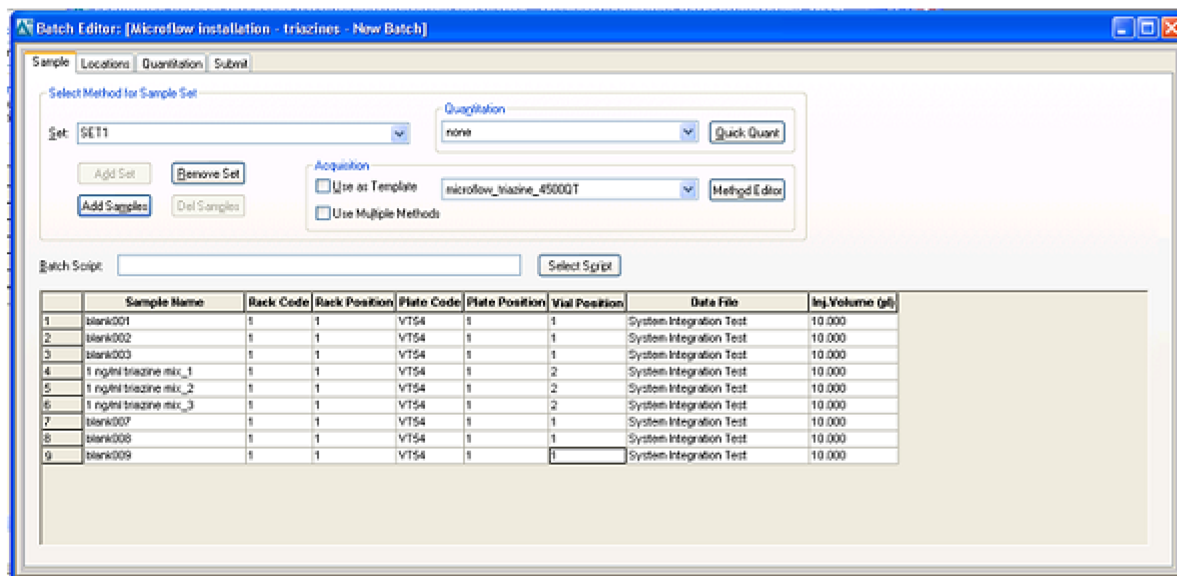
**Figure 4-1 Sample Tab—Acquisition Group**

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*.

**Figure 4-3 Sample Tab—Method Creation**



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**



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 to condition the column and then run it a second time for the test.

1. Click **Submit** on the **Submit** tab.

**Figure 4-5 Submit Tab**

Sample | Locations | Calibrate | Quantitation | Submit

Batch Owner name: Administrator

Submit

Submit Status
Number of samples in the Batch: 13. Number of DataFiles: 2.

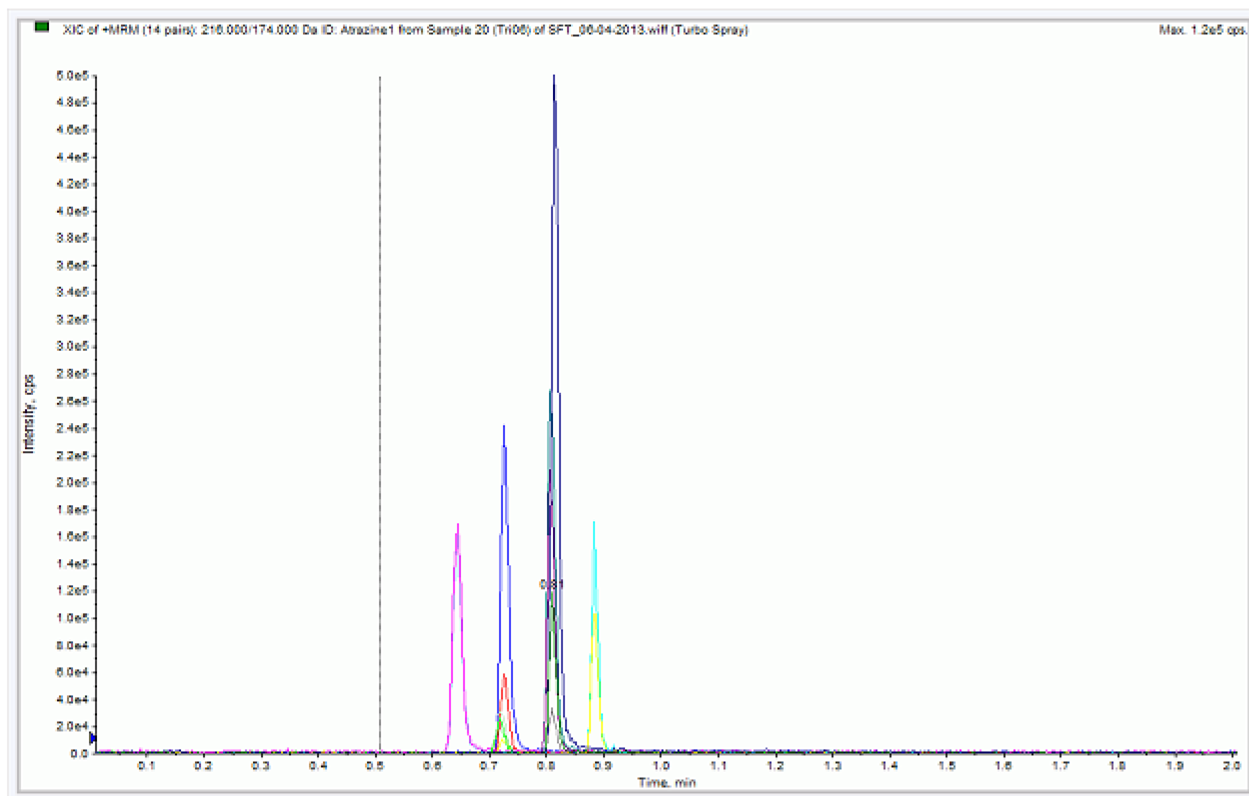
| | Sample Name | Rack Position | Plate Position | Vial Position | Acquisition Method | Quantitation | Data File | Set Name | Submit Status |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BGal 200fmol/uL | 1 | 1 | 1 | Conditioning the system | none | Conditioning the system | SET1 | Not |
| 2 | BGal 200fmol/uL | 1 | 1 | 1 | Conditioning the system | none | Conditioning the system | SET1 | Not |
| 3 | BGal 200fmol/uL | 1 | 1 | 1 | Conditioning the system | none | Conditioning the system | SET1 | Not |
| 4 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 5 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 6 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 7 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 8 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 9 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 10 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 11 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 12 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |
| 13 | BGal 10fmol/uL | 1 | 1 | 2 | System integration test | none | System integration test | SET1 | Not |

2. Click **View > Sample Queue.**
3. Verify that the system is not in Tuning mode and is set to "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 4-6* for representative data.

**Figure 4-6 Example Chromatograms for the System Integration Test—All XICs**

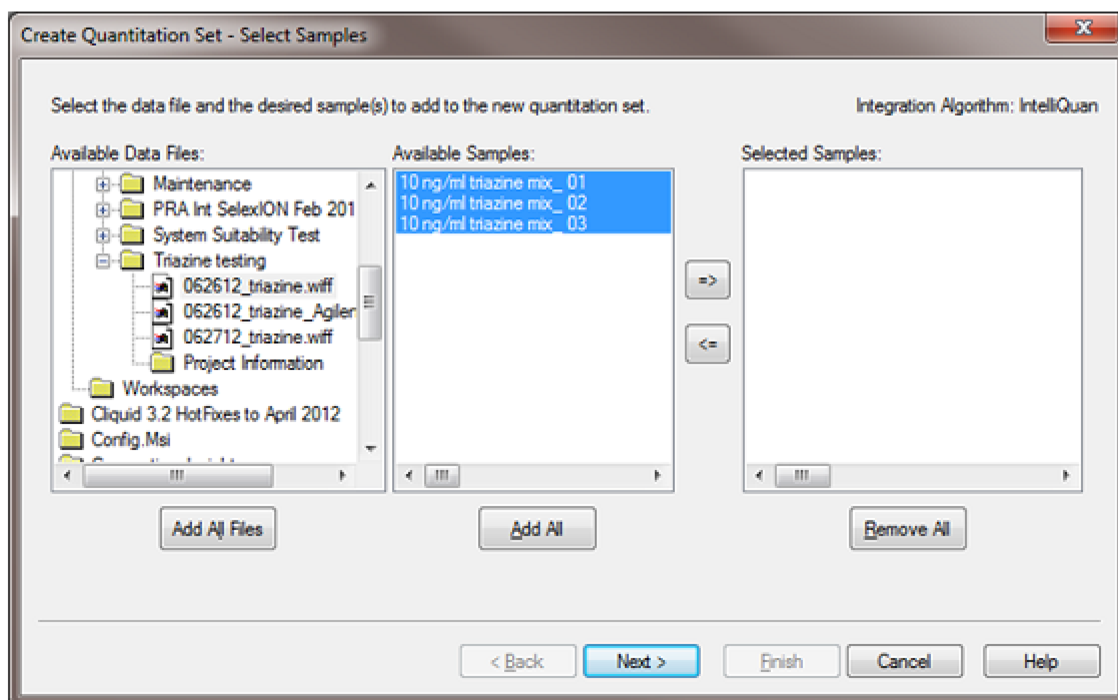



# Verify the Results

## Verify the Results and Verify the Integration

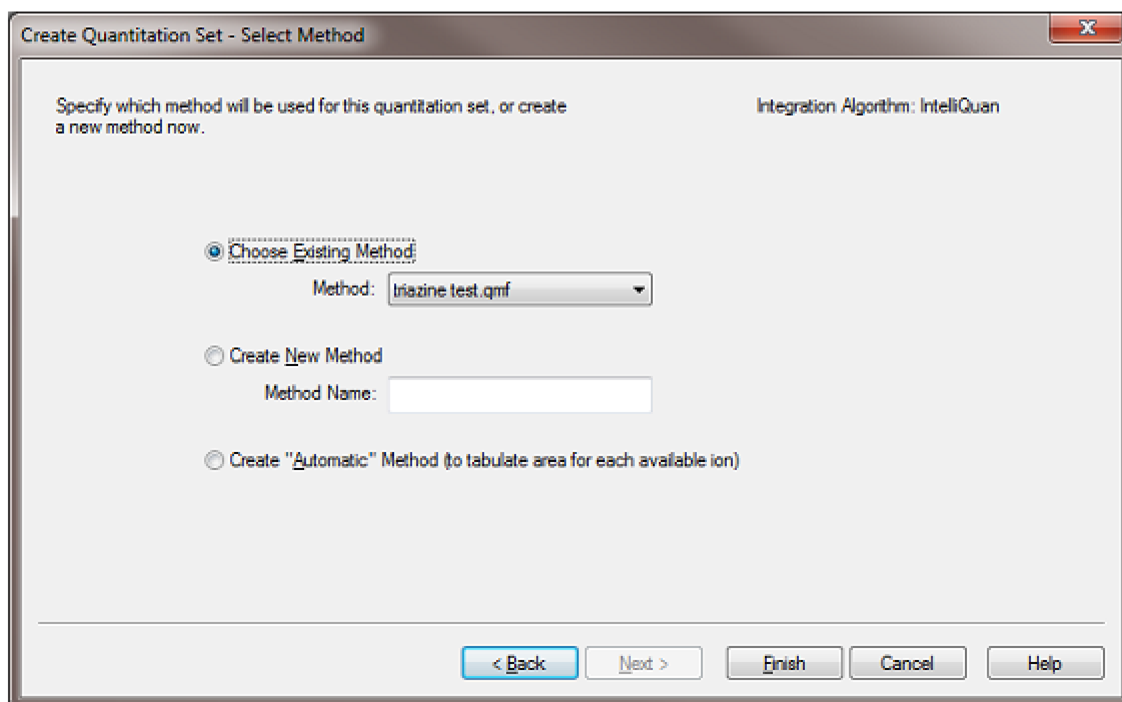
1. When the run is finished, double-click **Quantitation wizard** in the **Quantitation** menu.
2. Select the data file **System Integration Test <date>**.
3. Click **Add All** to move the three injections to the **Selected Samples** list. If a conditioning run was submitted, make sure that the injections selected are from the test run.

**Figure 4-7 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**.

**Figure 4-8 Quantitation Wizard—Select Methods Page**




The results table opens.

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

**Figure 4-9 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 Unknown | 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 **Analyst > 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.

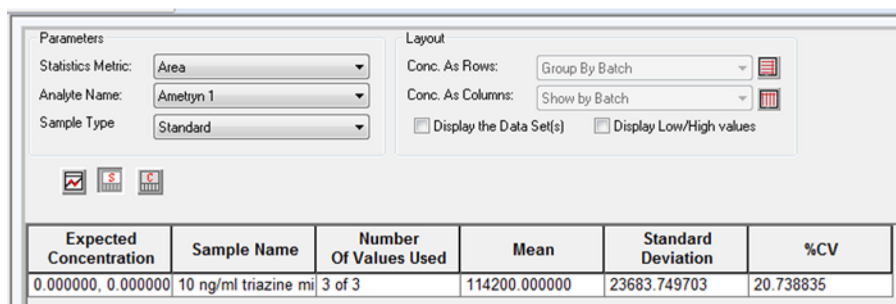
9. Click the forward and back arrows in the chromatogram pane to view the integration for each chromatogram.

10. After the data has been reviewed, repeat step *7* through step *9* for the following transitions: Atrazine 1, Simazine 1, and Terbutryn 1.

## 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 39*.
   b. Record the values in *Table 6-1 on page 39*.
4. Delete the **Statistics** pane.

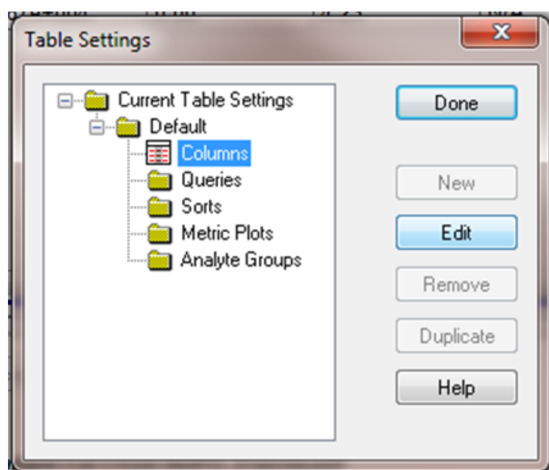
**Figure 4-10 Statistics Summary Pane**




## Verify Peak Widths at Half Height and Retention Times

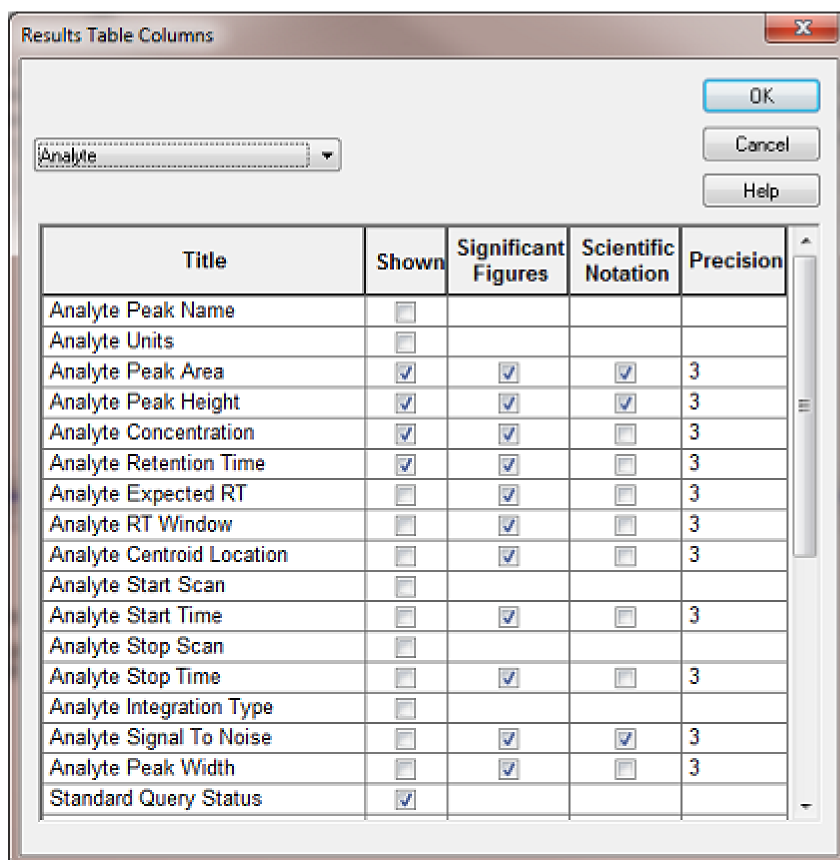
1. Open the **Results Table Columns** dialog.
2. Right-click on the area above the results table and select **Table Settings > Edit**.
3. In the **Table settings** dialog, click **Columns**, and then click **Edit**.

**Figure 4-11 Table Settings Dialog**




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

**Figure 4-12 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 39*.

   b. Record the values in *Table 6-1 on page 39*.

# Troubleshooting 5

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 19*.
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 34* 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.

## 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 µL.

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

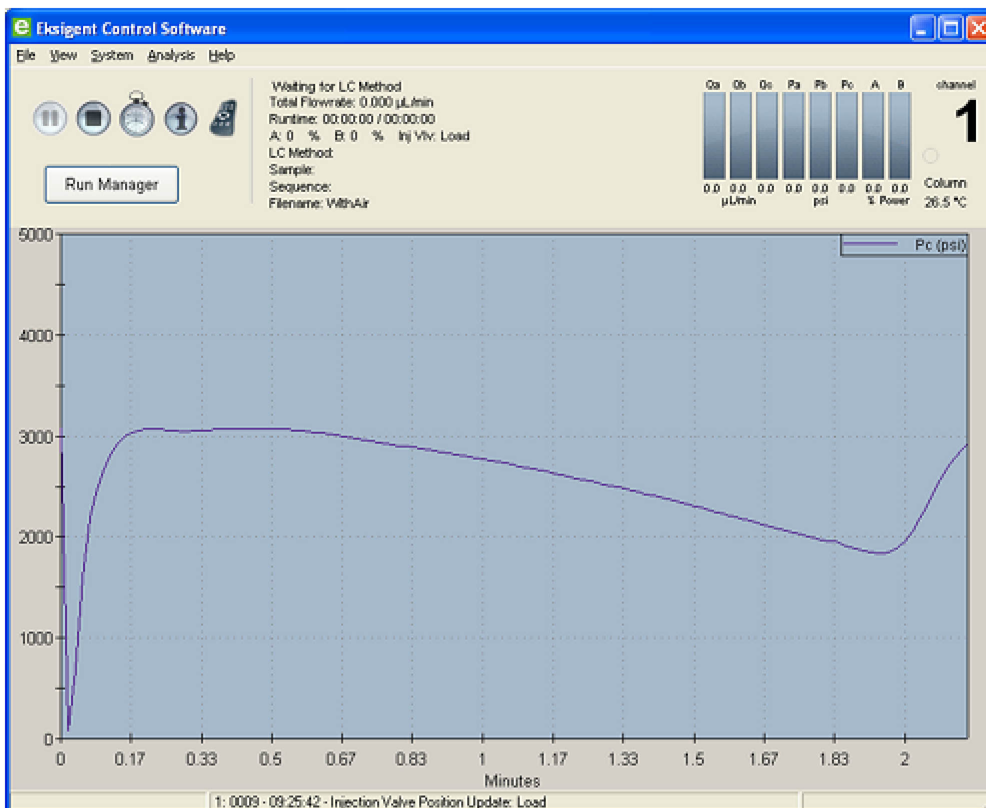


**Figure 5-2 Acquisition Window Showing Pc, Without Air Bubbles in Sample Loop**

## Resolve Drifting Retention Times

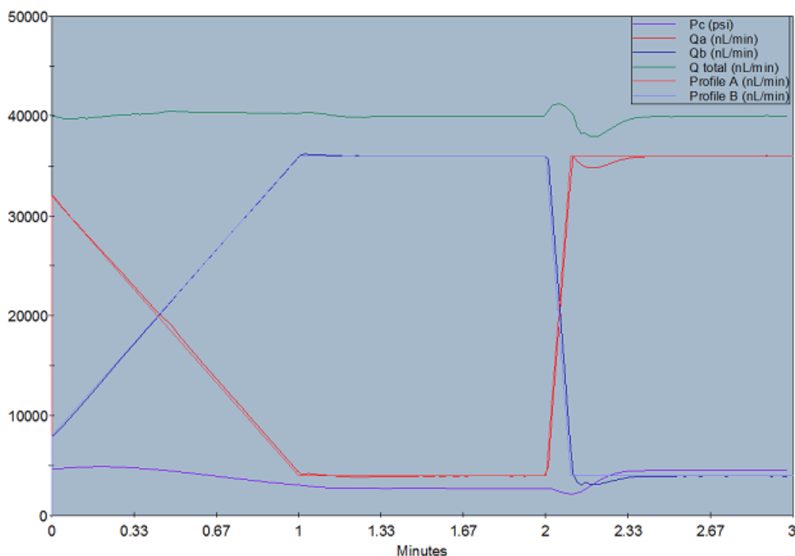
If the column temperature varies over the course of the test, retention times may drift.

- 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.

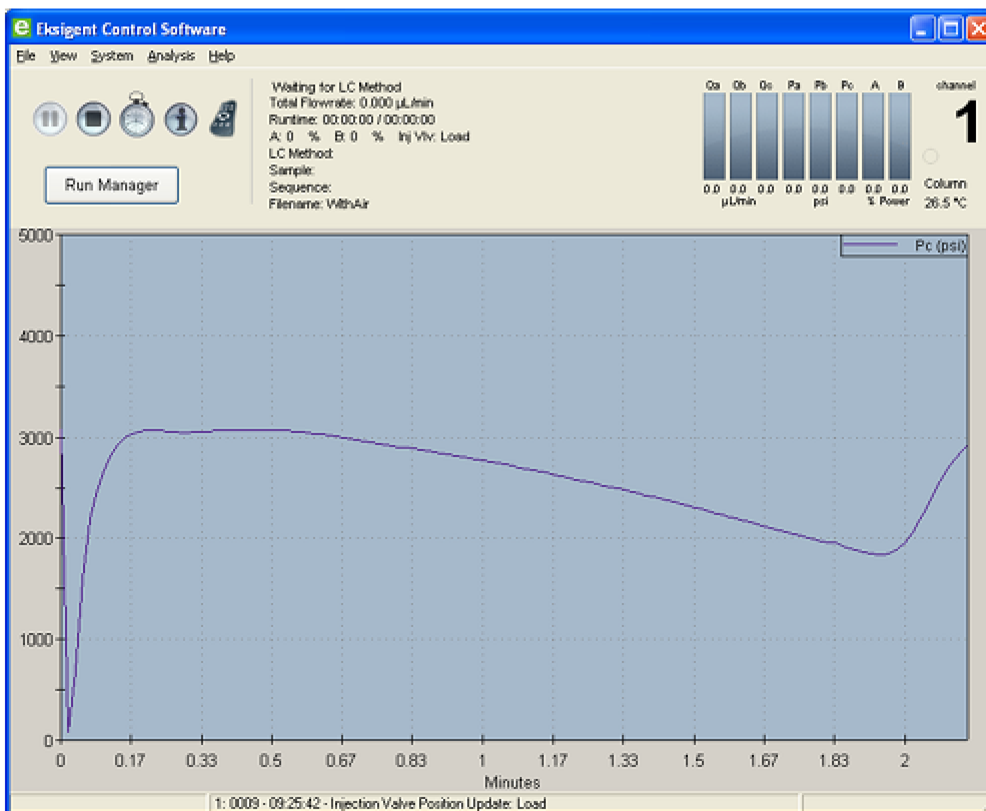
**Table 5-1 Common Anomalies in MicroLC 200/200 Plus System Pressure Data**

| 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 an FSE for assistance.

# Troubleshooting Using the Chromatogram

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

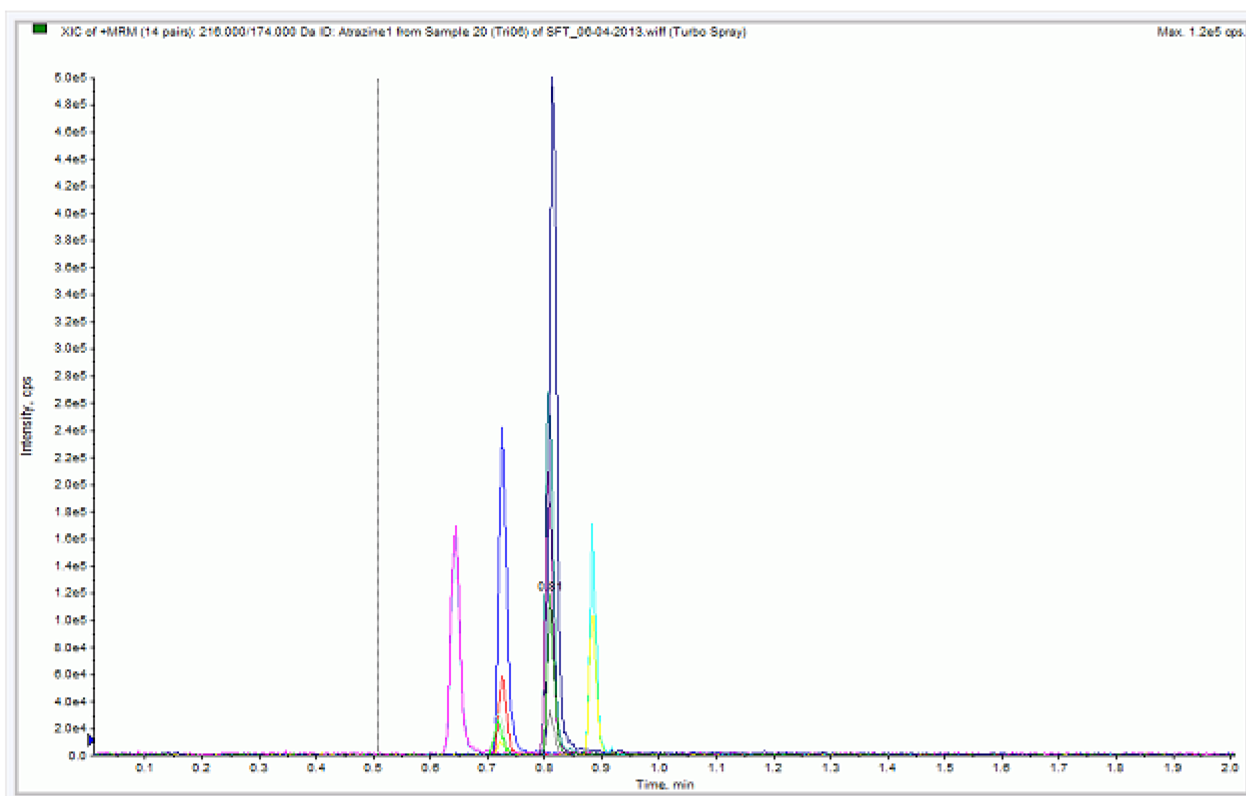
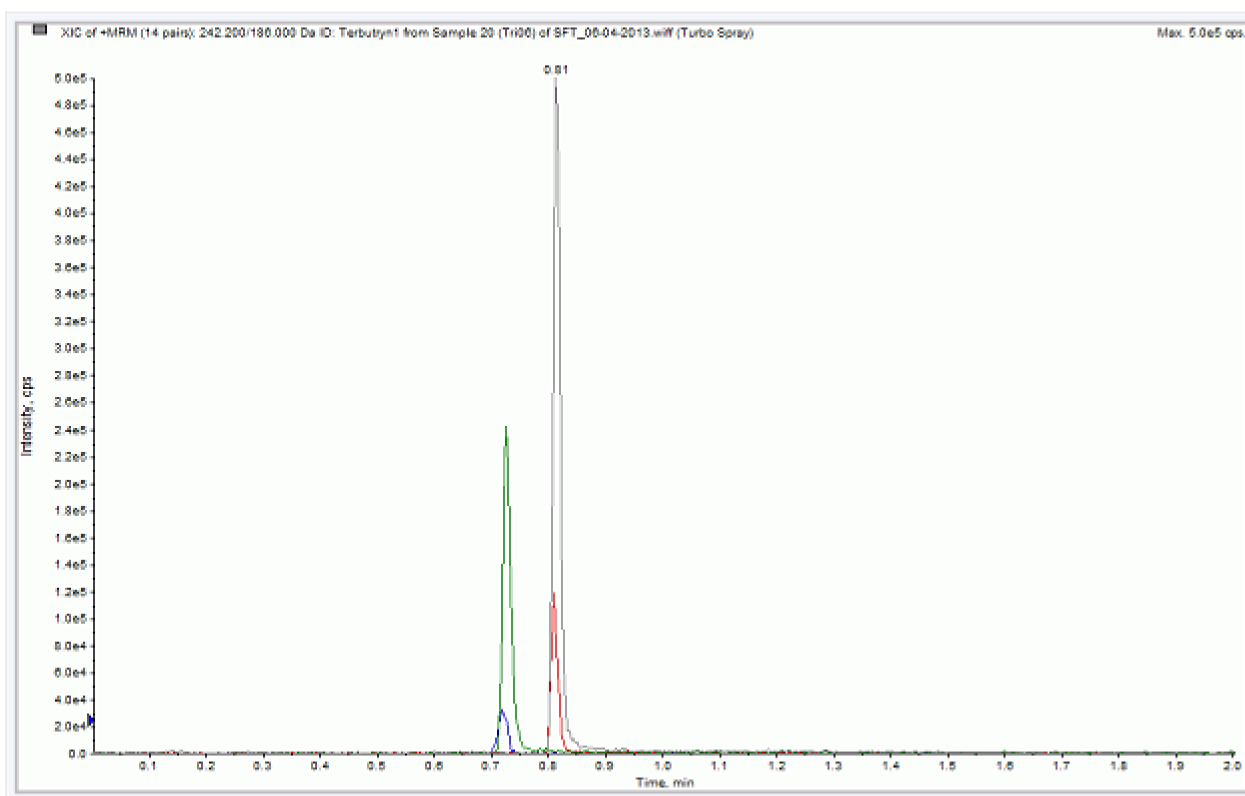


**Figure 5-6 Example Chromatograms for the System Integration Test—XICs for QC Peaks**




# Identify and Resolve Carryover Issues

For a well-tuned MicroLC 200/200 Plus system, carryover should be nonexistent (for a blank, there should be no peaks with the 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.

- Fluid should emerge during wash cycles.
- Fluid should emerge during injection. To test, create a run with a 50 µL injection that will overfill 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 *Table 6-1 on page 39* for the mean area specification. Select **Guideline Met** if the value is with 10%.

| Mass Spectrometer: | |
|---|---|

**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 | | | | | |
| Simazine 1 | | | | | |
| Terbutryn or Propazine 1 | | | | | |
| | | | | Guideline Met? | |

**Table 6-2 Notes**

# Specifications

## Mean Area Specification

**Table 6-3 Instrument Response (CPS)**

| Analyte | 4600/5600/6600 10 ng/mL |
| --- | --- |
| Ametryn 1 | $7.5 \times 10^4$ |
| Atrazine 1 | $6.5 \times 10^4$ |
| Simazine 1 | $4.0 \times 10^4$ |
| Propazine 1 | $7.5 \times 10^4$ |
| Terbutryn 1 | n/a |

## % 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

<table>
<tr><td>Contact name</td><td></td><td rowspan="2">Date (yyyy-mm-dd)</td><td rowspan="2"></td></tr>
<tr><td>Contact signature*</td><td></td></tr>
<tr><td>FSE name</td><td></td><td rowspan="2">Date (yyyy-mm-dd)</td><td rowspan="2"></td></tr>
<tr><td>FSE signature</td><td></td></tr>
</table>

*Signature required on hard copy only.

# Mass Spectrometer System Calibration

# A

**WARNING! Radiation Hazard, Biohazard, or Toxic Chemical Hazard. Follow all safety guidelines and applicable local regulations when handling, storing, and disposing of system components. Components might have been exposed to hazardous materials.**

If the mass spectrometer has not been calibrated recently, then calibrate the system using the [Glu$^1$]-Fibrinopeptide B, included in the LC/MS Peptide Calibration Kit (PN 4465867).

**Note:** Do not infuse the tuning solution and then the [Glu$^1$]-Fibrinopeptide B solution. Use mobile phase A to thoroughly flush the lines between running tests to avoid clogging the emitter tips on the NanoSpray® ion source or DPV-450 Digital PicoView® Nanospray ion source.

## Prepare the [Glu$^1$]-Fibrinopeptide B Dilution

| Required Material |
| --- |
| • [Glu1]-Fibrinopeptide B, included in the LC/MS Peptide Calibration Kit (PN 4465867)<br>• Standard diluent, included in the LC/MS Peptide Calibration Kit |

For the list of masses for [Glu$^1$]-Fibrinopeptide B, refer to *Masses for [Glu1]-Fibrinopeptide B*.

**Note:** Always prepare the dilution just before running the test.

**Note:** The [Glu$^1$]-Fibrinopeptide B might become lodged in the rubber septum of the vial. Gently tap or shake it down before opening the vial. Then, partially remove the septum to reveal a slot. Add the dilution solvent through the slot. Then push the septum back into place and mix well to dissolve.

1. Add 900 µL of Standard diluent (0.1% formic acid, 10% acetonitrile) to the glass amber vial containing 0.1 mg [Glu$^1$]-Fibrinopeptide B.
2. Cover the vial tightly, shake it, and then vortex it for at least 2 minutes, to make sure that the peptide is fully dissolved.

**Note:** Peptide concentration may vary depending on the total peptide content and peptide purity of the standard solution. Refer to the Certificate of Analysis provided by the vendor. At 100% purity, 0.1 mg [$Glu^1$]-Fibrinopeptide B dissolved as described in the preceding steps produces a stock solution with a concentration of approximately 66.67 pmol/µL.

3. Aliquot the stock solution in 50 µL volumes into clean tubes. Freeze unused aliquots at –20°C for future use.
4. Put 50 µL of the stock solution into a clean tube and then add 450 µL of standard diluent.
5. Vortex the tube for 30 seconds.

   This is a 1:10 dilution, providing 500 µL of a 6.7 pmol/µL solution.
6. Put 50 µL of the 6.7 pmol/µL solution into another clean tube.
7. Add 450 µL of standard diluent.
8. Vortex the tube for 30 seconds.

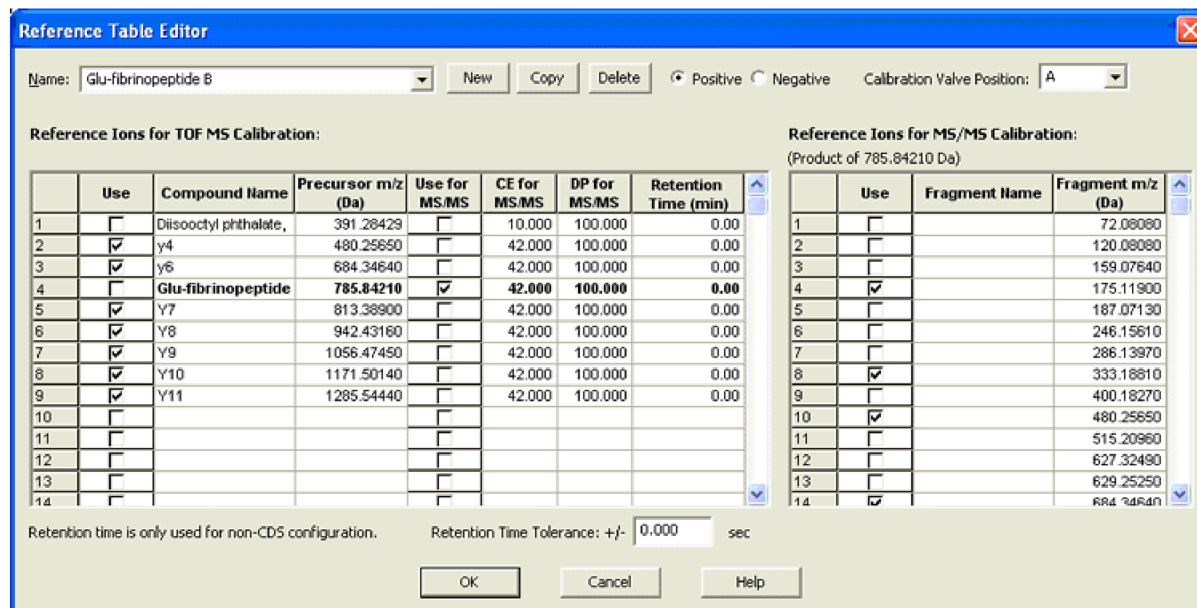
   This is a 1:10 dilution, providing 500 µL of the 667 fmol/µL solution.
9. Put 50 µL of the 667 fmol/µL solution into another clean tube.
10. Add 450 µL of standard diluent.
11. Vortex the tube for 30 seconds.

    This is a 1:10 dilution, providing 500 µL of the final 66.7 fmol/µL solution, to be used for the infusion test.

# Edit the Calibration Reference Table for [$Glu^1$]-Fibrinopeptide B

1. In the Navigation bar, click **Tune and Calibrate** sidebar.
2. Click **Tools** > **Settings** > **Tuning Options** .
3. On the **Calibration** tab, click **Reference**.
4. In the **Reference Table Editor**, select **Glu-Fibrinopeptide B** in the **Name** list.
5. Add the masses in *Figure A-1* to the **Reference Ions for TOF MS Calibration** table (on the left side of the dialog).

**Figure A-1 Glu-Fibrinopeptide B Reference Table**




6. Click **OK**.
7. Click **OK** in the **Tuning Options** dialog.

# Calibrate in TOF MS Mode

1. Set the method parameters as shown in *Table A-1*.

**Table A-1 Method Parameters**

| Parameter | Value |
|---|---|
| **MS Parameters** | |
| Scan type | TOF MS |
| Accumulation time (sec) | 1 |
| Polarity | Positive |
| TOF masses (Da) | 400 to 1800 |
| Duration (min) | 0.5 |
| **Advanced MS Parameters** | |

**Table A-1 Method Parameters (continued)**

| Parameter | Value |
|---|---|
| MCA | Off |
| Auto Adjust with mass | On |
| Q1 Transmission window | Default (with Auto-adjust) |
| Pulsar frequency | Default (with Auto-adjust) |
| Time bins to sum | 4 |
| Settling time | Default |
| Pause between mass ranges | Default |
| **Syringe Pump Method Parameters** | |
| Flow rate (µL/min) | 0.5 |
| Syringe Size (µL) | 100 Gastight (1.46 mm) |
| **Source/Gas Parameters** | |
| Ion Source Gas 1 (GS1) | 2 (or as optimized) |
| Curtain Gas (CUR) | 25 (or as optimized) |
| Interface Heater Temperature (IHT) (°C) | 150 (or as optimized) |
| IonSpray Voltage Floating (ISVF) | 2300 (or as optimized) |
| **Compound-dependent Parameters** | |
| Collision energy (CE) (V) | 40 (or as optimized) |
| Compound Parameters | |
| Declustering Potential | 100 |

2. Make sure that spray is stable.
3. Click **Acquire** and acquire at least 30 seconds of scan data.
4. In the **TIC** window (lower left) highlight 30 seconds of TIC signal to average.
5. Double-click the highlighted area.

6. In the window that appears (at the bottom of the screen), right-click and select **Re-Calibrate TOF** from the menu that appears.
7. In the **TOF Calibration** window, select **[Glu1]-Fibrinopeptide B** in the **Reference Table** list.
8. Make sure that proper experimental masses have been identified from the infusion spectrum and match up with the reference table theoretical masses.
9. Review the **Average Error** value displayed to the right of the **Calculate New Calibrations** button.
10. Click **Calculate New Calibrations**.
11. Verify that the **Average Error** value has dropped to less than 2 ppm.
12. In the **Calibration Values** area, click **Calibrate Spectrum**.
13. In the **Save Current Calibration** area, select the **Set as Instrument Default and Overwrite Current File** check boxes.
14. Click **Entire File** to save new calibration for the TOF MS mode.
15. Click **Close**.

# Calibrate in Product Ion Mode

1. Set the method parameters as shown in *Table A-2*.

**Table A-2 Method Parameters**

| Parameter | Value |
|---|---|
| **MS Parameters** | |
| Scan type | Product Ion |
| Product of | 785.8 |
| Accumulation time (sec) | 1 |
| Polarity | Positive |
| TOF masses (Da) | 100 to 1800 |
| High sensitivity | On |
| Duration (min) | 0.5 |
| **Advanced MS Parameters** | |
| MCA | Off |
| Auto Adjust with mass | On |

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

| Parameter | Value |
|---|---|
| Q1 Transmission Window | Default (with Auto-adjust) |
| Pulsar Frequency | Default (with Auto-adjust) |
| Time bins to sum | 4 |
| Settling time | Default |
| Pause between mass ranges | Default |
| **Source/Gas Parameters** | |
| Ion Source Gas 1 (GS1) | 2 (or as optimized ) |
| Curtain Gas (CUR) | 25 (or as optimized) |
| Interface Heater Temperature (IHT) (°C) | 150 |
| IonSpray Voltage Floating (ISVF) | 2300 (or as optimized ) |
| **Compound-dependent Parameters** | |
| Collision energy (CE) (V) | 45 (or as optimized) |
| **Resolution Parameters** | |
| Q1 resolution | Unit |

2. Make sure that spray is stable.
3. Click **Acquire** and acquire at least 30 seconds of scan data.
4. In the **TIC** window (lower left) highlight 30 seconds of TIC signal to average.
5. Double-click the highlighted area.
6. In the window that appears (at the bottom of the screen), right-click and select **Re-Calibrate TOF** from the menu that appears.
7. In the **TOF Calibration** window, select **[Glu1]-Fibrinopeptide B** in the **Reference Table** list.
8. Make sure that proper experimental masses have been identified from the infusion spectrum and match up with the reference table theoretical masses.
9. Review the **Average Error** value displayed to the right of the **Calculate New Calibrations** button.
10. Click **Calculate New Calibrations**.

11. Verify that the **Average Error** value has dropped to less than 2 ppm.
12. In the **Calibration Values** area, click **Calibrate Spectrum**.
13. In the **Save Current Calibration** area, select the **Set as Instrument Default and Overwrite Current File** check boxes.
14. Click **Entire File** to save new calibration for the Product Ion High Sensitivity mode.
15. Click **Close**.
16. In the method parameters, select **High Resolution**.
17. Repeat step *5* to *29* to calibrate in Product Ion, High Resolution mode.

# Revision History

| Document Number | Reason for Change | Date |
|---|---|---|
| D5042727 A | First release of document. | March 2013 |
| D5042727 B \| RUO-IDV-05-1294-A | 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 |
| D5042727 C \| RUO-IDV-05-1294-B | Applied new template and incorporated Eksigent MicroLC 200 Plus. Combined document with customer-facing version to create a single *System Integration Test* document. Replaces D5077470. Added "System Integration Test Data Log and Signoff" section. | October 2014 |