



NanoLC™ Systems

For TripleTOF® Systems

System Integration Test and Data Log



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Contents

Chapter 1 Introduction.....	5
About the Test.....	5
Time Required.....	6
Required Materials.....	6
Chapter 2 LC/MS System Integration Test.....	8
Create the Methods.....	8
Create the Autosampler Method.....	9
Create the LC Methods.....	9
Create the Acquisition Method.....	10
Prepare the System for Testing.....	15
Prepare the 1 pmol/μL Stock Solution of Beta-Galactosidase Digest	
Solution.....	15
Condition the System.....	15
Prepare the Sample for the System Integration Test.....	16
Verify System Readiness.....	16
Perform the System Integration Test.....	20
Create the LC/MS Acquisition Batch.....	20
Run the Batch.....	23
Assess the Results.....	24
Troubleshoot Peak Issues.....	28
Chapter 3 System Integration Test Data Log and Signoff.....	32
System Information.....	32
Peak Area and Resolution.....	33
Retention Time.....	34
Comments and Exceptions.....	36
Signoff.....	36
Appendix A Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation.....	37
Autosampler Method Parameters.....	37
Loading Pump Method Parameters.....	39
Gradient Pump Method Parameters.....	40
Appendix B Methods for SCIEX nanoLC 400 Systems—External Trap and Column	
Installation.....	42
Autosampler Method Parameters.....	42
Loading Pump Method Parameters.....	44
Gradient Pump Method Parameters.....	45
Appendix C Methods for NanoLC Ultra Systems— cHiPLC System Installation.....	47
Autosampler Method Parameters.....	47

Contents

Loading Pump Method Parameters.....	49
Gradient Pump Method Parameters.....	50
Appendix D Methods for NanoLC Ultra Systems—External Trap and Column	
Installation.....	52
Autosampler Method Parameters.....	52
Loading Pump Method Parameters.....	54
Gradient Pump Method Parameters.....	55
Appendix E Methods for NanoLC and Tempo Systems—cHiPLC System Installation.....	57
Autosampler Method Parameters.....	57
Loading Pump Method Parameters.....	59
Gradient Pump Method Parameters.....	60
Appendix F Methods for NanoLC and Tempo Systems—External Trap and Column.....	62
Autosampler Method Parameters.....	62
Loading Pump Method Parameters.....	64
Gradient Pump Method Parameters.....	65
Appendix G Connecting NanoLC and Tempo Systems with a cHiPLC System.....	67
Wire Connections.....	67
Appendix H Mass Spectrometer System Calibration.....	68
Prepare the [Glu1]-Fibrinopeptide B Dilution.....	68
Masses for [Glu1]-Fibrinopeptide B.....	69
Edit the Calibration Reference Table for [Glu1]-Fibrinopeptide B.....	70
Calibrate in TOF MS Mode.....	71
Calibrate in Product Ion Mode.....	73
Appendix I Plumbing Diagrams.....	76
Appendix J Best Practices.....	79
Sample Preparation Techniques.....	80
Appendix K Mass Assignments.....	81
Revision History.....	82

Introduction

1

This guide describes the steps for preparing for and performing an LC/MS/MS system integration test for a NanoLC™ system combined with the SCIEX TripleTOF® 4600, 5600/5600+, or 6600 mass spectrometer. The system might include the cHiPLC® system or an external column and one of the following NanoLC systems:

- SCIEX nanoLC 400 system
- NanoLC Ultra™ system
- NanoLC™ system
- Tempo™ system

Note: Read the safety instructions in the *Operator Guide* prior to running the system integration test.

Note: For information about assembly and operation of the NanoSpray® ion source, refer to the *NanoSpray® Ion Source Operator Guide*. For information about assembly and operation of the Digital PicoView® Nanospray ion source, refer to the *Digital PicoView Nanospray Source Hardware Manual*.

Note: Optimize the ion source before beginning this test.

About the Test

The test in this document determines the performance level of the LC/MS system. This test can be used as a measure of the system performance. The results from this test can provide a baseline measurement of system performance. The test can be performed regularly and used as a system quality control test in the future. Record the test results in the [System Integration Test Data Log and Signoff on page 32](#).

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.

If the LC system has been idle for two weeks or more, re-initialize transducers, then verify the flow rate and, if necessary, calibrate.

Before starting the test, make sure that the system is plumbed in a trap-and-elute configuration (with either the cHiPLC® system or an external trap and column). Refer to [Plumbing Diagrams on page 76](#).

Introduction

Note: The test in this document is written with Gradient 1 as the low-flow channel. If this is not true for the system being tested (for example, a 2D system), then make the appropriate changes throughout the test. Refer to [Table 1-1](#).

Table 1-1 Channel Flow Rate

Pump	Channel	Flow Rate
Gradient 1	Low flow	300 nL/min
Loading pump	High flow	5 µL/min

Time Required

Approximate time required:

1. Create the methods and batch: 45 minutes
2. Prepare the system for testing: 60 minutes (or 3 to 4 hours if the column needs conditioning)
3. Perform the test: 3 hours

Required Materials

Recommended solvents can be ordered from VWR:

- Burdick and Jackson acetonitrile with 0.1% formic acid (PN BJLC441-1.0)
- Burdick and Jackson water with 0.1% formic acid (PN BJLC452-1.0)

Note: If you are not using pre-mixed solvents, make sure that you use LC/MS-grade water, acetonitrile and formic acid.

Required materials for a cHiPLC[®] system installation:

- Reverse phase cHiPLC[®] column (75 µm × 15 cm ChromXP C18-CL 3 µm 120 Å) (PN 804-00001)
- cHiPLC[®] trap (200 µm × 0.5 mm ChromXP C18-CL 3 µm 120 Å) (PN 804-00006)
- Trap-and-elute jumper chip (PN 800-00389)
- LC/MS Peptide Calibration kit (PN 4465867)

Required materials for an external column installation:

- Reversed phase ChromXP nanoLC column (75 µm ID × 15 cm, ChromXP C18 3 µm 120 Å) (PN 805-00120)
- ChromXP nanoLC Trap column (350 µm ID × 0.5 mm, ChromXP C18 3 µm 120 Å) (PN 5016752)

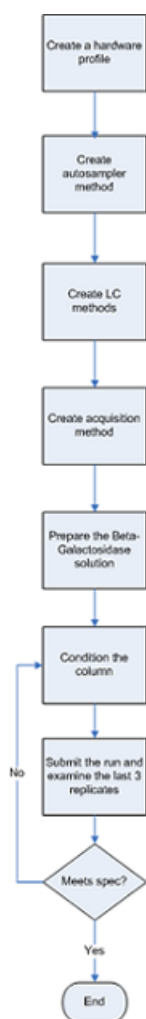
- LC/MS Peptide Calibration kit (PN 4465867)

LC/MS System Integration Test

2

Determine which LC system is installed and then complete the steps in the test as shown in [Figure 2-1](#).

Figure 2-1 System Integration Test Tasks



Create the Methods

Create the autosampler and LC methods in the Eksigent control software and the Acquisition method in the Analyst® TF software.

Create the Autosampler Method

1. Plumb the autosampler valve with a 10 μ L sample loop.
2. Open the Analyst[®] TF software.
3. In the **Autosampler** control window, click **Method Editor**.
4. Create the autosampler method for a trap-elute configuration. Refer to the appropriate appendix for the system.
 - [Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation on page 37](#)
 - [Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation on page 42](#)
 - [Methods for NanoLC Ultra Systems— cHiPLC System Installation on page 47](#)
 - [Methods for NanoLC Ultra Systems—External Trap and Column Installation on page 52](#)
 - [Methods for NanoLC and Tempo Systems—cHiPLC System Installation on page 57](#)
 - [Methods for NanoLC and Tempo Systems—External Trap and Column on page 62](#)
5. Type the name of the method as specified in the appendix, and then click **Save**.
6. Click **OK**.

Create the LC Methods

Fill the aqueous channel for each pump (side A) with buffer A. Fill the organic channel (side B) with buffer B. For the loading pump, buffer A is always used. Required buffers are shown in [Table 2-1](#).

Table 2-1 Mobile Phase Mixtures

	Binary Mixture A	Binary Mixture B	Modifier
Gradient 1	100% water	100% acetonitrile	0.1% formic acid
Gradient 2	100% water	100% acetonitrile	0.1% formic acid
Loading pump	100% water	N/A	0.1% formic acid

Create the Loading Pump Method

In this method, the loading pump is the pump with the microflow module.

1. Open the Analyst[®] TF software.
2. In the **Acquisition** window that opens (from the Eksigent control software), click the arrow in the top, right corner of the window to select the high-flow or loading pump.
3. Click **LC Methods**.

4. Create the loading pump method. Refer to the appropriate appendix for the system:
 - [Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation on page 37](#)
 - [Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation on page 42](#)
 - [Methods for NanoLC Ultra Systems— cHiPLC System Installation on page 47](#)
 - [Methods for NanoLC Ultra Systems—External Trap and Column Installation on page 52](#)
 - [Methods for NanoLC and Tempo Systems—cHiPLC System Installation on page 57](#)
 - [Methods for NanoLC and Tempo Systems—External Trap and Column on page 62](#)
5. Type the method name as specified in the appropriate appendix.
6. Click **Save**.
7. Click **OK**.

Create the Gradient Method

For the analytical gradient (typically on the gradient 1 pump with the nanoflow module), create the gradient method.

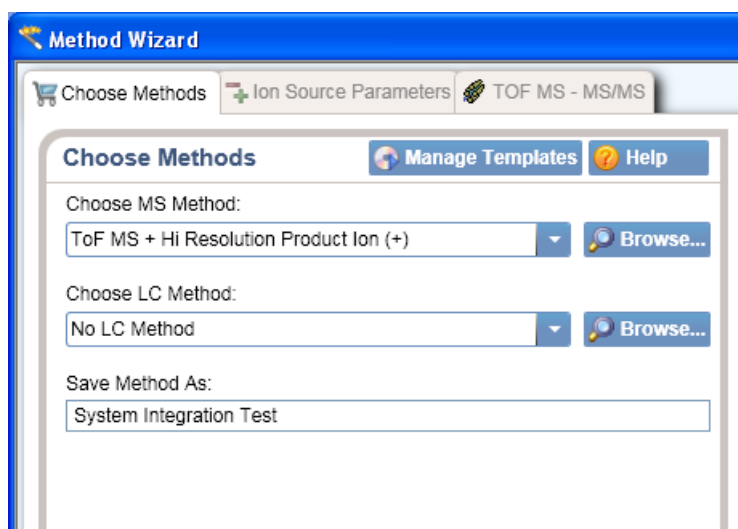
1. In the **Acquisition** window that opens (from the Eksigent control software), click the arrow in the top, right corner of the window to select the nanoflow or gradient pump.
2. Click **LC Methods**.
3. Create the gradient method. Refer to the appropriate appendix for the system:
 - [Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation on page 37](#)
 - [Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation on page 42](#)
 - [Methods for NanoLC Ultra Systems— cHiPLC System Installation on page 47](#)
 - [Methods for NanoLC Ultra Systems—External Trap and Column Installation on page 52](#)
 - [Methods for NanoLC and Tempo Systems—cHiPLC System Installation on page 57](#)
 - [Methods for NanoLC and Tempo Systems—External Trap and Column on page 62](#)
4. Type the method name as specified in the appropriate appendix.
5. Click **Save**.
6. Click **OK**.

Create the Acquisition Method

1. Close the Eksigent control software, if it is open.

2. Verify that the Analyst® TF software drivers are installed. Refer to the appropriate installation document for details.
3. Open the Analyst® TF software.
4. Create a hardware profile.
5. On the Navigation toolbar, click **Acquire > Method Wizard**.

Figure 2-2 Method Wizard Window - Choose Methods Tab



6. In the **Choose MS Method** list, select **ToF MS + Hi Resolution Product Ion (+)**.
7. Specify the LC method in one of the following ways:
 - If an autosampler and LC method have already been associated with an acquisition method, in the **Choose LC Method** list, select the existing acquisition method (.dam).
 - If an autosampler and LC method has not been used before, then select **No LC Method**.

Tip! Any method can be selected as it can be edited after creation of this acquisition method.

8. In the **Save Method As** field, type **System Integration Test** for the acquisition method, and then press **Enter**.
9. Click **Next**.
10. On the **Ion Source Parameters** tab, specify the source conditions that were determined during the ion source test. For tests for the NanoSpray® ion source, refer to the *Ion Source Tests, Specifications, and Data Log*. For tests for the Digital PicoView® Nanospray ion source, refer to the *NanoLC Systems Installation Checklist and Data Log*.

Figure 2-3 Method Wizard - Ion Source Parameters Tab

The screenshot shows the 'Ion Source Parameters' tab in the Method Wizard. The 'Ion Source' is set to 'Nanospray'. The parameters are as follows:

Parameter	Value
Curtain Gas (CUR):	20
Ion Source Gas 1 (GS1):	5
Ion Source Gas 2 (GS2):	0
Ion Spray Voltage Floating (ISVF):	2300
Interface Heater Temperature (IHT):	150

11. Click **Next**.
12. On the **TOF MS - MS/MS** tab, specify the acquisition parameters.

Note: The acquisition time should be more than 1 minute shorter than the LC run time.

Table 2-2 Acquisition Parameters

Parameter	Value
Start TOF Mass (Da)	400
Stop TOF Mass (Da)	1800
TOF Accumulation Time (s)	0.250
MS2 Start Mass (Da)	100
MS2 Stop Mass (Da)	1800
MS2 Accumulation Time (s)	0.5
Mass Spec Acquisition Duration (min)	42
Product of m/z (Da)	729.3652

Table 2-2 Acquisition Parameters (continued)

Parameter	Value
Collision Energy (V)	45
Collision Energy Spread (V)	3

Figure 2-4 Method Wizard - TOF MS - MS/MS Tab

Choose Methods Ion Source Parameters TOF MS - MS/MS

TOF MS - MS/MS Help

Start TOF Mass (Da): 400

Stop TOF Mass (Da): 1800

TOF Accumulation Time (s): 0.250015

MS2 Start Mass (Da): 100

MS2 Stop Mass (Da): 1800

MS2 Accumulation Time (s) (per precursor): 0.5

Cycle Time (s): 0.8

Mass Spec Acquisition Duration (min): 44.002

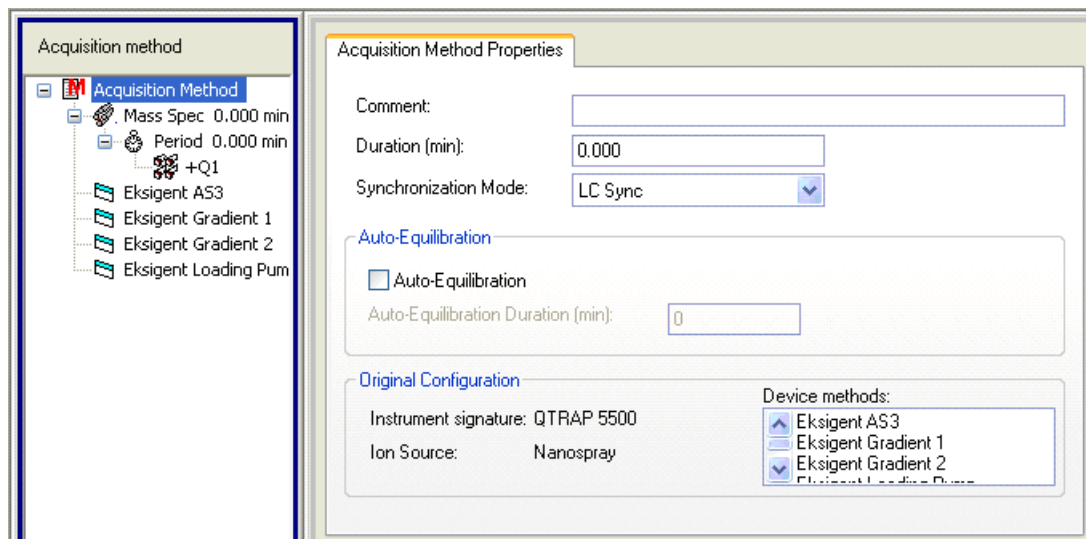
Product of m/z (Da)	Collision Energy (V)	Collision Energy Spread (V)
729.3652	45	3

13. Click **Finish**.

Add LC Information to the Acquisition Method

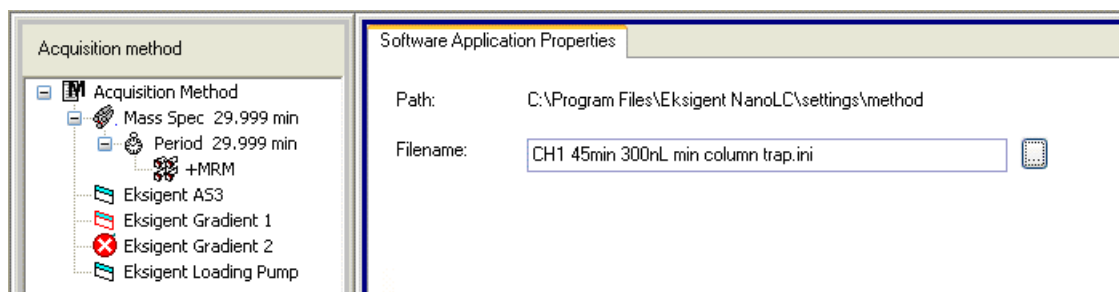
1. Click **Acquisition Method** in the left pane, and then set **Synchronization Mode** to **LC Sync**.

Figure 2-5 Acquisition Method Properties Tab—Synchronization Mode



2. Click the autosampler (for example, Eksigent AS2 or AS3) and then select the appropriate autosampler method. Refer to [Create the Autosampler Method on page 9](#).
3. If Eksigent Gradient 2 appears in the device list, right-click **Eksigent Gradient 2** and then clear the "Use" selection as it is not being used.
4. Click **Eksigent Gradient 1** and then select the appropriate gradient pump method. Refer to [Create the Gradient Method on page 10](#).

Figure 2-6 Software Application Properties Tab—Gradient 1 Method



5. Click **Eksigent Loading Pump** and then select the appropriate loading pump method. Refer to [Create the Loading Pump Method on page 9](#).
6. Save the acquisition method.

Prepare the System for Testing

Plumb the system in trap-elute configuration to perform the pre-column desalting workflow. Refer to [Plumbing Diagrams on page 76](#). For wiring information for a NanoLC™ or Tempo™ system with the cHiPLC®, refer to [Wire Connections on page 67](#).

Prepare the 1 pmol/μL Stock Solution of Beta-Galactosidase Digest 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*.

Required Materials

- Beta-galactosidase digest (PN 4465938)
- Buffer A

1. Add 625.0 μL of buffer A (100% water:0.1% formic acid) to the beta-galactosidase vial.
2. Vortex the vial for at least 30 seconds.
3. Using a centrifuge, spin the vial to bring the liquid down to the bottom of the vial before opening.
4. Repeat step 2 to step 3 to confirm dissolution.
5. Aliquot the stock solution into 50 μL volumes and then store them at –35 °C to –15 °C for up to one year.

Note: Aliquots of the beta-galactosidase stock solution can be stored at 4 °C for up to three days after thawing.

Condition the System

A trap and column typically require two to three runs with 200 fmol/μL of protein digest for conditioning. Prepare a 200 fmol/μL dilution and use it in two or three 1 μL injections at the beginning of the batch.

If the column is already conditioned, this procedure is not required. Proceed to [Prepare the Sample for the System Integration Test on page 16](#).

Prepare a 200 fmol/μL Dilution of the Beta-Galactosidase to Condition the System

Required Materials

- | |
|---|
| <ul style="list-style-type: none">Stock solution, prepared in Prepare the 1 pmol/μL Stock Solution of Beta-Galactosidase Digest Solution on page 15 |
|---|

- Combine 40 μL of buffer A (100% water:0.1% formic acid) with 10 μL of the stock solution in a clean vial.
- Vortex the vial for at least 30 seconds to properly mix the solution.
This step produces a 1:5 dilution, with a concentration of 200 fmol/μL.
- Transfer the solution to the autosampler vial and make sure there is no bubble on the bottom of the vial.

Prepare the Sample for the System Integration Test

Required Materials

- | |
|--|
| <ul style="list-style-type: none">Beta-galactosidase stock solution, prepared in Prepare the 1 pmol/μL Stock Solution of Beta-Galactosidase Digest Solution on page 15 |
|--|

Follow this procedure to prepare 400 μL of the working solution of beta-galactosidase. The system integration test uses a 1 μL injection of a 25 fmol/μL solution.

- Combine 390 μL of buffer A (100% water:0.1% formic acid) with 10 μL of the stock solution (1 pmol/μL) in a clean vial.
- Vortex the vial for at least 30 seconds to properly mix the solution.
This step produces a 1:40 dilution with a concentration of 25 fmol/μL.
- Transfer the solution to the autosampler vial and make sure that there is no bubble on the bottom of the vial.

Verify System Readiness

Make sure that the LC system meets performance specifications.

Note: For information about assembly and operation of the NanoSpray[®] ion source, refer to the *NanoSpray[®] Ion Source Operator Guide*. For information about assembly and operation of the Digital PicoView[®] Nanospray ion source, refer to the *Digital PicoView Nanospray Source Hardware Manual*.

Note: Optimize the ion source before beginning this test.

- Connect the flow from the LC system to the ion source and verify that the spray is stable by monitoring the background signal in the Analyst[®] TF software.

2. Equilibrate the LC/MS system with the starting conditions of the method outlined above.
3. On the Navigation bar, double-click **Manual Tuning**.
4. Type the key parameters from [Table 2-3](#) and then click **Start** to begin acquisition. Refer to the following figures as an example.

Figure 2-7 TOF MS Parameters

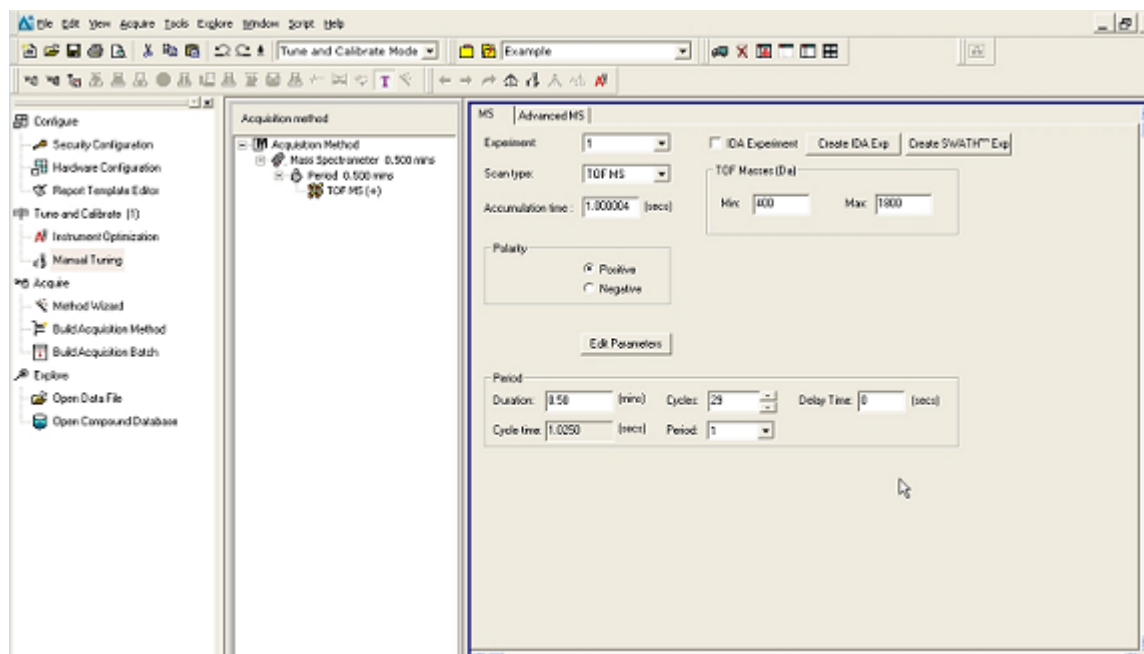


Figure 2-8 TOF Advanced MS Parameters

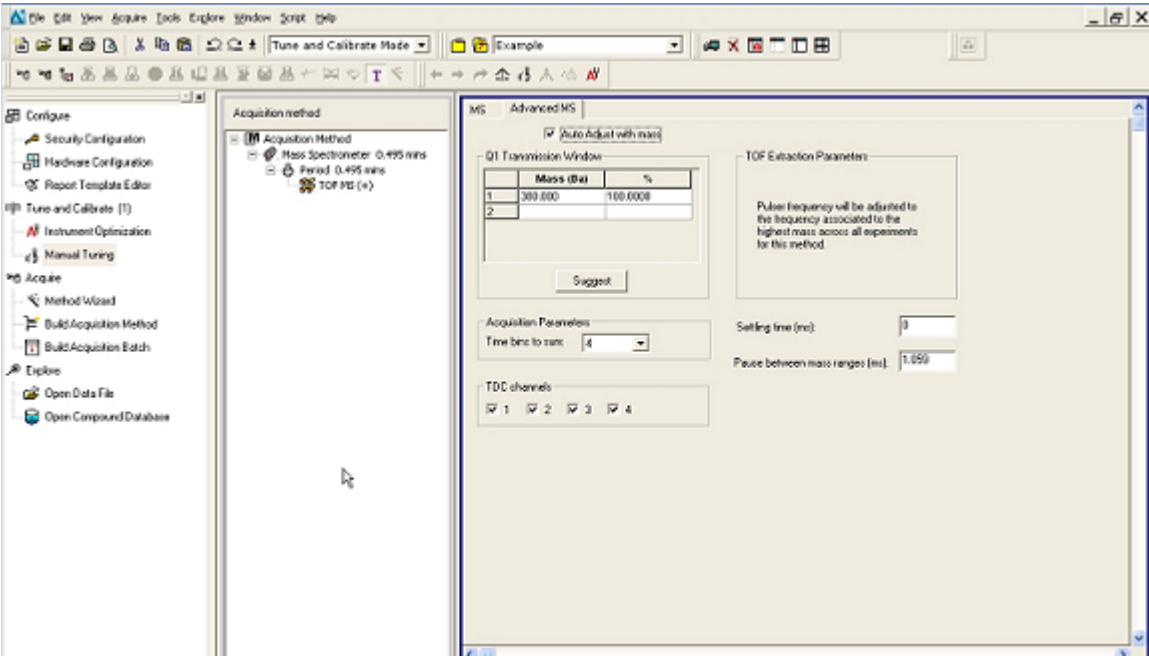


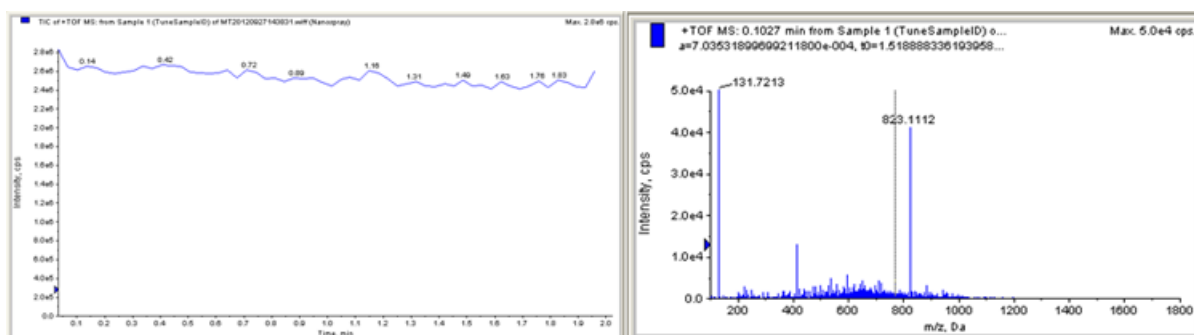
Table 2-3 Key Parameters

Parameter	Value
MS	
Scan type	TOF MS
Accumulation time (sec)	1.0
Polarity	Positive
TOF masses (Da)	400 to 1800
Duration (min)	0.5
Advanced MS	
MCA	On
Auto Adjust with mass	On
Q1 Transmission window	Default (with Auto-adjust)
Pulsar frequency	Default (with Auto-adjust)
Time bins to sum	4

Table 2-3 Key Parameters (continued)

Parameter	Value
Settling time	Default
Pause between mass ranges	Default
Source/Gas	
Ion Source Gas 1 (GS1)	5 (or as optimized)
Curtain Gas (CUR)	20 (or as optimized)
Temperature (TEM) (°C)	150 (or as optimized)
IonSpray Voltage Floating (ISVF) (V)	2300 (or as optimized)
Compound	
Declustering Potential (DP)	70 (or as optimized)

Figure 2-9 TOF MS Scan for Background Noise

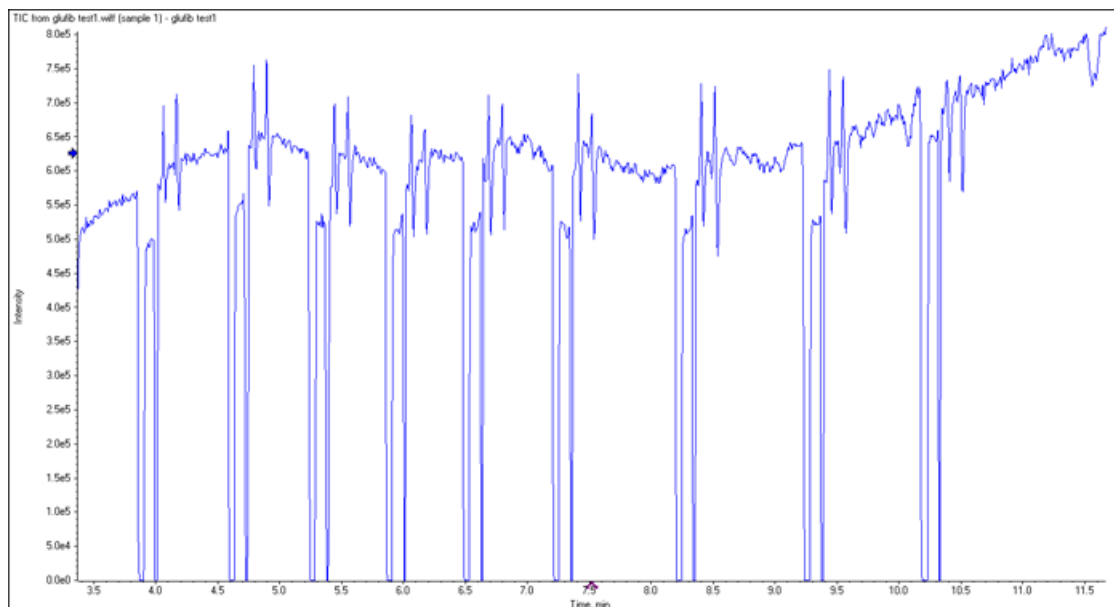


5. Make sure that the spray is still stable by monitoring the background signal with a TOF MS scan.

An example of stable spray is shown on the left side of [Figure 2-9](#) (spectrum on the right will vary system to system). Unstable spray appears as shown below (typical unstable spray induced by air bubbles).

- If air bubbles are present in the spray, verify that all post-column fittings and connections have been made properly.
- If the spray is not stable, retune the ion source by infusion. Refer to the *Operator Guide* for more information.

Figure 2-10 Unstable Spray Induced by Air Bubbles



Perform the System Integration Test

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 32](#).

Note: If the system includes a cHiPLC[®] system, make sure that the cHiPLC[®] system is in the Load position before beginning this test.

Create the LC/MS Acquisition Batch

1. Double-click **Build Acquisition Batch** on the **Navigation** bar.
2. On the **Sample** tab, in the **Acquisition** group, select the acquisition method created in [Create the Acquisition Method on page 10](#) from the list.

Figure 2-11 Sample Tab—Acquisition Group

Sample Locations Quantitation Submit

Select Method for Sample Set

Set: SET1

Add Set Remove Set

Add Samples Del Samples

Quantitation

none Quick Quant

Acquisition

☐ Use as Template ☐ Use Multiple Methods

System_Integration_Test_DATE Method Editor

3. Click **Add Set**.
4. Click **Add Samples**.

Figure 2-12 Add Sample Dialog

Add Sample

Sample name

Prefix: Sample Sample number: ☒ Number of digits: 3

Data file

Prefix: System Performance Tes Set name: ☒ Auto Increment: ☐

Sub Folder: Browse

New samples

Number: 9

OK Cancel Help

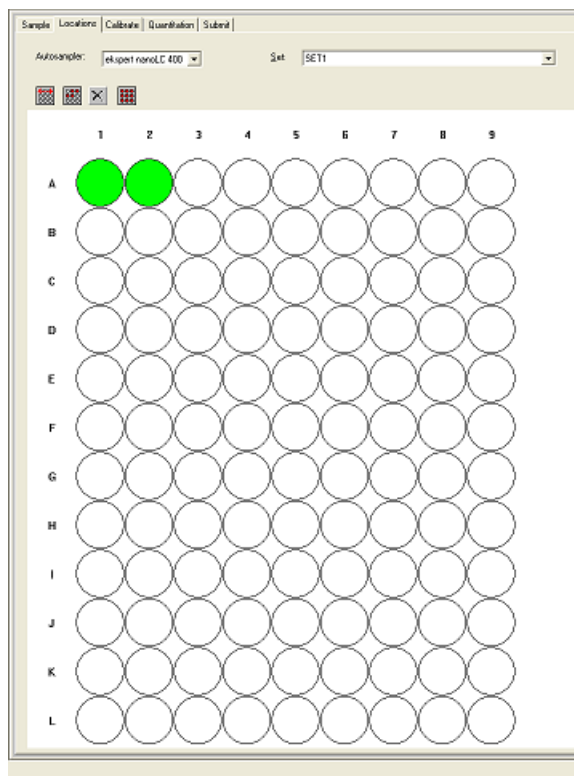
5. Specify the sample information as shown in [Figure 2-12](#).
6. In the **Number** field, type **10**.
7. Click **OK**.
8. Specify the rack and plate position for the samples as shown in [Figure 2-13](#).

Figure 2-13 Sample Tab—Method Creation

	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Acquisition Method	Data File	Inj. Volume (µl)
1	BGal 200 fmol/µL	By Row	1	48 vial by ro	2	1	Conditioning the system	Conditioning the system	-1.000
2	BGal 200 fmol/µL	By Row	1	48 vial by ro	2	1	Conditioning the system	Conditioning the system	-1.000
3	BGal 200 fmol/µL	By Row	1	48 vial by ro	2	1	Conditioning the system	Conditioning the system	-1.000
4	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
5	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
6	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
7	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
8	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
9	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
10	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
11	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
12	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000
13	BGal 10 fmol/µL	By Row	1	48 vial by ro	2	2	System Integration Test	System Integration Test	-1.000

9. Create a batch. Refer to [Figure 2-13](#).
10. Save the batch file as **YYYYMMDD LC TOFMS BGal Integration Test**.
11. On the **Location** tab, identify the position of the beta-galactosidase sample vial.

Figure 2-14 Locations Tab



Run the Batch

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

Figure 2-15 Submit Tab

Sample | Locations | Calibrate | Quantitation | **Submit**

Batch Owner name
Administrator

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/μL	1	1	1	Conditioning the system	none	Conditioning the system	SET1	Not
2	BGal 200fmol/μL	1	1	1	Conditioning the system	none	Conditioning the system	SET1	Not
3	BGal 200fmol/μL	1	1	1	Conditioning the system	none	Conditioning the system	SET1	Not
4	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
5	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
6	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
7	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
8	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
9	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
10	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
11	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
12	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not
13	BGal 10fmol/μL	1	1	2	System integration test	none	System integration test	SET1	Not

2. Click **View > Sample Queue**.
3. Click **Acquire > Start Sample**.

Assess the Results

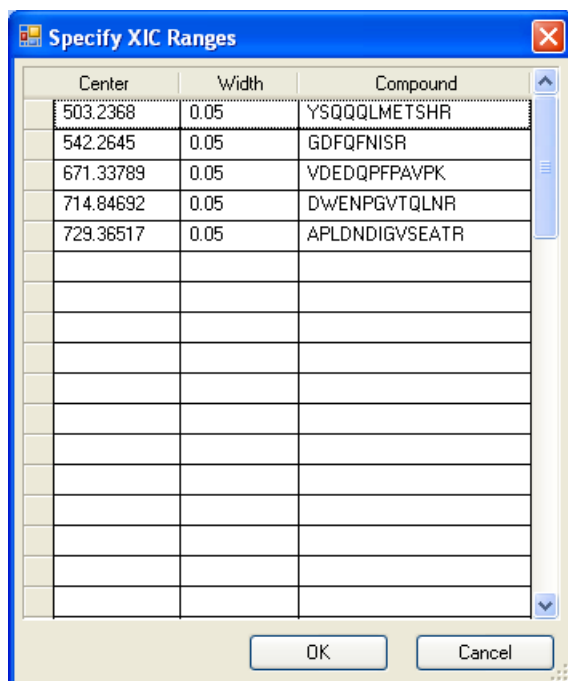
Assess the TOF MS Data in the PeakView® Software

1. Open the data file and double-click the arrow in the middle of the X-axis (Time, min) to display the individual TICs.
2. Right-click **TIC from Experiment 1, +TOF MS** in the top left corner of the graph.
3. Select **Remove all Traces Except Active** to display only the TOF MS TIC plot.
4. Extract the TOF MS peak XICs for the target peptides:
 - a. Click **Show > Extract Ions Using**.

- b. Specify the masses and extraction width for the peptides as shown in [Figure 2-16](#). Only the masses and widths are required.

Note: These peaks are the most stable and the most reproducible from batch-to-batch and from lot-to-lot.

Figure 2-16 Specify XIC Ranges Dialog



Center	Width	Compound
503.2368	0.05	YSQQQLMETSHR
542.2645	0.05	GDFQFNISR
671.33789	0.05	VDEDQPPFAVPK
714.84692	0.05	DWENPGVTQLNR
729.36517	0.05	APLDNDIGVSEATR

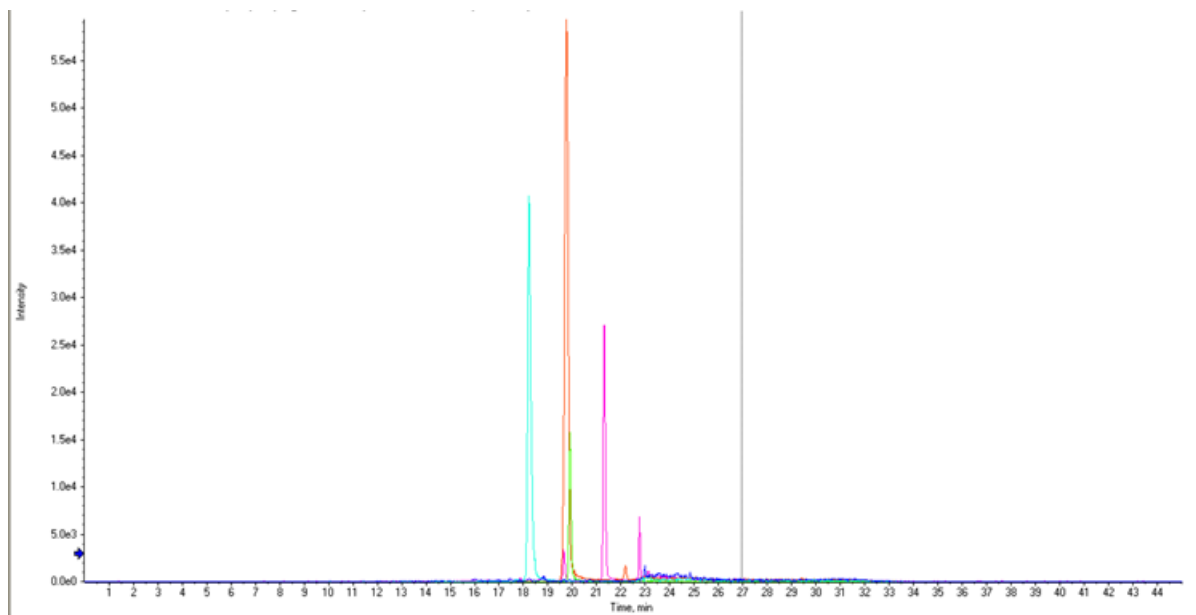
5. Click **OK**.

The XICs for each peptide are generated. The peak areas of the extracted peaks can be used to evaluate the MS sensitivity and the peak retention times can be used to evaluate the chromatography.

Note: The XIC can be used to evaluate the NanoLC LC/MS peak retention times and shapes. Peak widths, retention times, and XIC width vary from LC system to LC system depending on transfer line volumes, column type used, column age, and more.

6. In the **XIC** pane, click and drag across the range of the more intense peaks, as shown in [Figure 2-17](#).

Figure 2-17 TOF MS XICs of Peptides from Beta-galactosidase NanoLC Ultra Trap Elute Run (25 fmol on column)



7. Click **Window > Graph Selection Window**.
8. Record the peak area and resolution for each XIC.

Note: Only the peak area for the selected peptide XIC (in bold text in the heading in the top left corner) will be displayed in the **Graph Selection** window. Individually select each peptide XIC in the top left heading to display the corresponding peak area.

Note: This test is not a specification. Use this example to confirm injection and peak separation and shape only. Refer to the peak area specification in the *Installation Checklist and Data Log*. Refer to the "TOF MS Test" section in the *Ion Source Tests, Specifications and Data Log* document for resolution specifications for the NanoSpray[®] ion source.

9. Make sure that the peaks have good separation and shape using the PeakView[®] software:
 - a. Click **Window > Graph Selection Window**.

- b. Record the retention times of the chosen peaks.

Retention times will vary with each system. Time of elution of the first is about 14 minutes to 16 minutes. The longer elution time indicates that LC dead volume should be minimized.

Note: Most of the XICs obtained should have peak widths of approximately 0.2 minutes half height on average and have peak intensities similar to those shown in [Figure 2-17](#). Some peaks will be narrower and some will be broader.

Record the Results in the Data Log

1. Calculate the average peak area and average peak resolution for the last three injections for the following four peptides:
 - 729.4 m/z
 - 714.8 m/z
 - 671.3 m/z
 - 542.3 m/z
2. Record the values in [Table 3-8 on page 33](#) and [Table 3-9 on page 34](#).
3. Examine the last three injections to make sure that the average peak area for each of the four QC peaks meet the minimum requirements specified in the Data Log.
4. Record the retention time for the last three samples analyzed for the following three peptides:
 - 729.4 m/z
 - 671.3 m/z
 - 542.3 m/z

The absolute retention time will vary with each system.

Tip! Use a column oven to increase the retention time reproducibility. The temperature effect on retention time differs from peptide to peptide. In order to get the retention time reproducibility within specification, the temperature of the column should be stable within 1°C. Factors to consider include not only the column temperature itself, but the room temperature, especially during overnight runs as the room temperature can shift dramatically.

5. Calculate the difference between the latest and earliest elution of each peptide and record the difference in [Table 3-10 on page 34](#).
6. Make sure that the difference meets the minimum requirements specified in the Data Log.

Troubleshoot Peak Issues

This section provides information for troubleshooting peak related problems such as broad or tailing peak widths, lack of separation between peaks, and low peak area.

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.

CAUTION: Potential System Damage: If peak with problems persist and a cHiPLC[®] system is in use, do not attempt to troubleshoot the fittings connected to the chip.

No separation between the peaks

- Make sure that both pumps are delivering the correct amount of solvent.
- Make sure that the pressure drop upon injection is not too severe in the high-flow channel (change in pressure less than 300 psi).
- A large pressure drop upon injection suggests an air bubble has been introduced to the sample loop or is present in the trap column plumbing.

Note: The overall separation with trap and elute will often be less than with direct injection. Components that elute comparably on the trap and analytical column will not resolve on the analytical column and, as a result, spread out or bunch together.

Peak intensity or peak area is too low

- Verify that the correct amount of sample has been withdrawn from the autosampler vial by performing an aspiration test.
- Verify the performance of the mass spectrometer and the ion source spray. For the NanoSpray[®] ion source, refer to the infusion tests in the *Ion Source Tests, Specifications, and Data Log* document. For tests for the Digital PicoView[®] Nanospray ion source, refer to the *NanoLC Systems Installation Checklist and Data Log*.
- If there is a large pressure drop upon injection, inspect for air bubbles in the sample loop or trap column plumbing.

- Verify that the trap and analytical column are well conditioned with protein digest injections before performing this test. A trap typically requires two to three runs with 200 fmol of protein digest on the column for conditioning.
- Perform a direct injection with a protein digest on the analytical column to determine whether the problem is related to the trap, or 10-port switching valve, or the cHiPLC[®] system.
- If the first LC peak does not elute for a long time, inspect the system for dead volume.

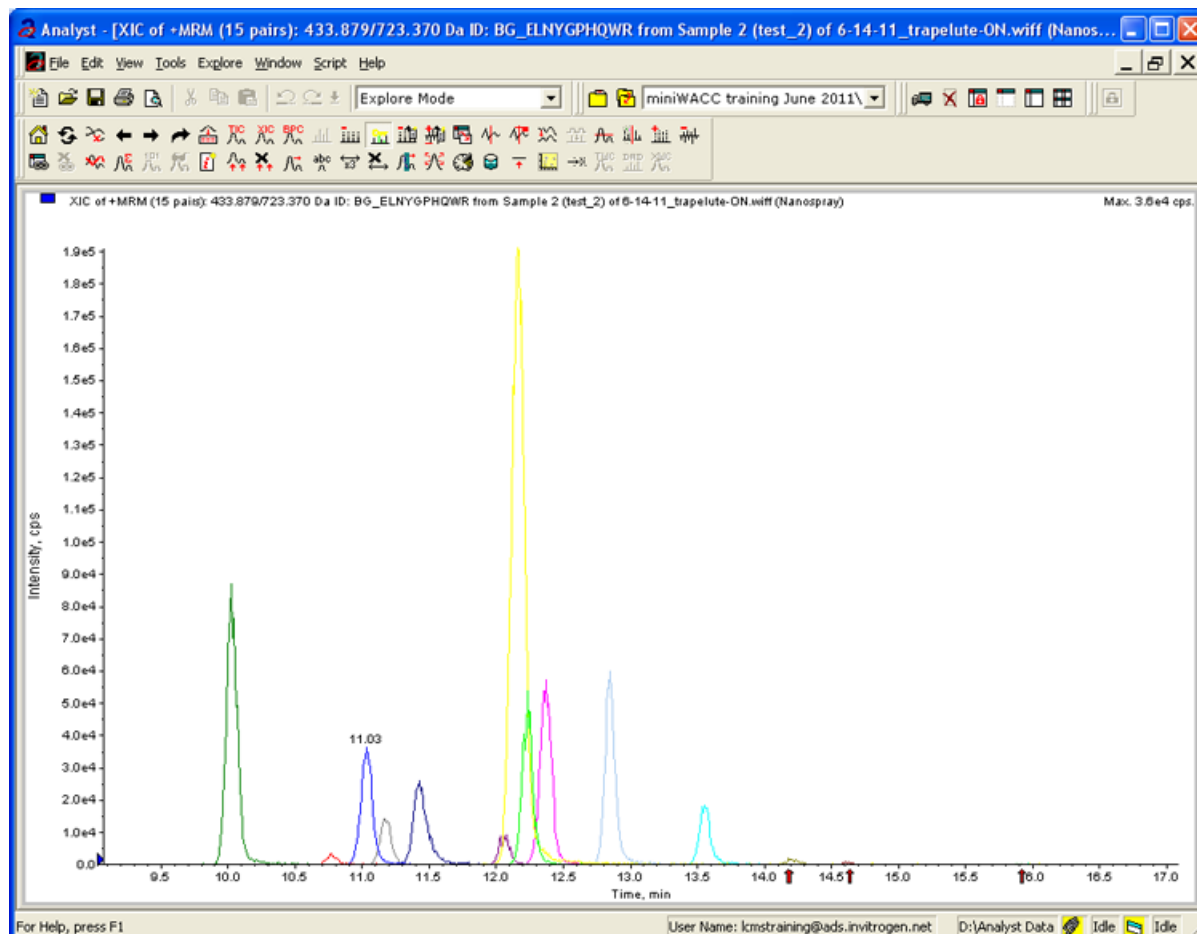
Tip! Minimize tubing length wherever possible and make sure that all the tubing for the flow path for the gradient channel has an inner diameter of 18 μm to 25 μm .

- If the early eluting peaks are not visible or are very low in intensity, replace the trap. Invisible or low-intensity peaks can result from trapping efficiency.

LC/MS System Integration Test

- If the late eluting peaks are not visible or are very low in intensity, the column might be getting old. In rare cases, the beta-galactosidase standard is degraded. See [Figure 2-18](#) for an example of a scan with an older column.

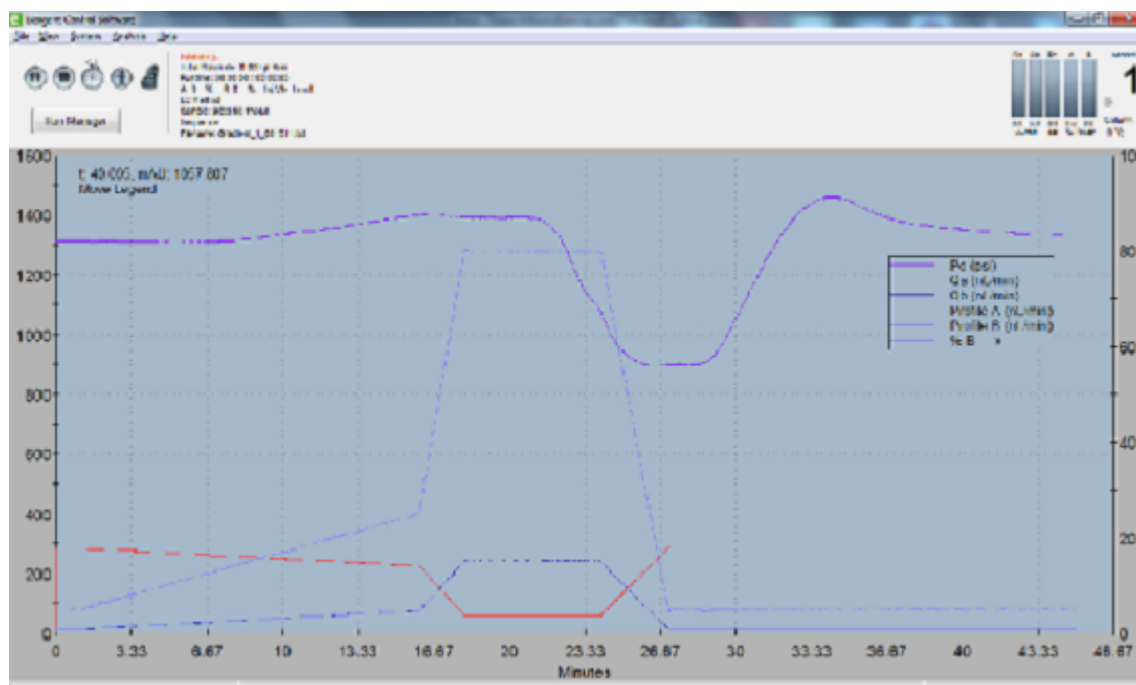
Figure 2-18 Extraction of All Peaks—Late Eluting Peaks Not Present



- Always monitor the column and trap pressure over time. Increasing pressure often indicates increasing blockage, probably at the ion source emitter tip. If the pressure drops quickly when the connection between the column and the ion source is unfastened, the tip is getting clogged and should be changed.
- For better long-term column lifetime, verify that the pressure drops at least 30% during the high organic flush of the column. Increase the duration of the high organic flush until a good pressure change is observed. This time might increase for the trap column configuration relative to the direct injection configuration.

- [Figure 2-19](#) shows a minimal pressure change upon injection (at time 0) and a 30% pressure decrease (at 23 minutes to 30 minutes) during the high organic flush.

Figure 2-19 Good Pressure Profile for a Trap-and-Elute Run



System Integration Test Data Log and Signoff

3

Beta-galactosidase lot number:	
--------------------------------	--

System Information

Table 3-1 Pump System Information

Model	
Location	
Serial number	

Table 3-2 Autosampler Information

Model	
Location	
Serial number	

Table 3-3 (Optional) cHiPLC[®] System Information

Model	
Location	
Serial number	

Table 3-4 (Optional) A/D Converter

Model	
Location	
Serial number	

Table 3-5 (Optional) Column Oven

Model	
Location	

Table 3-6 Ion Source Information

Model	
Serial number	

Table 3-7 Mass Spectrometer Information

Mass spectrometer model	
Mass spectrometer location	
Mass spectrometer serial number	

Peak Area and Resolution

Most of the XICs obtained should have peak widths of approximately 0.2 min half height on average. Some peaks will be narrower and some will be broader.

Additionally, verify that the peaks elute within five minutes of each other.

Table 3-8 TOF MS Data—Peak Area

TOF-MS	Peptide ID	Peak Area Spec	Avg. Peak Area
542.2645	GDFQFNISR	1.0×10^5	
671.3379	VDEDQFPAPVK	2.0×10^5	
714.8469	DWENPGVTQLNR	5.0×10^4	
729.3652	APLDNDIGVSEATR	2.0×10^5	
Specification Passed?			

System Integration Test Data Log and Signoff

Table 3-9 TOF MS Data—Average Peak Resolution

TOF-MS	Peptide ID	Average Resolution
542.2645	GDFQFNISR	
671.3379	VDEDQFPFAVPK	
714.8469	DWENPGVTQLNR	
729.3652	APLDNDIGVSEATR	
Resolution should be $\geq 25\,000$ for all peaks. Specification Passed?		

Retention Time

Record the retention time for the last three samples analyzed for the three peptides in [Table 3-10](#). The difference between the latest eluting time and the earliest eluting time should be less than 0.3 min for the SCIEX nanoLC 400 systems and 0.5 min for the NanoLC , Tempo and NanoLC Ultra systems.

Table 3-10 Retention Time

Peptide	Actual
Retention time—729.4 m/z	
Replicate A	
Replicate B	
Replicate C	
Difference Specification: SCIEX nanoLC 400 systems < 0.3 min NanoLC Ultra [®] , NanoLC [™] and Tempo [™] systems < 0.5 min	
Retention time—671.3 m/z	
Replicate A	
Replicate B	
Replicate C	

Table 3-10 Retention Time (continued)

Peptide	Actual
Difference	
Specification:	
SCIEX nanoLC 400 systems < 0.3 min	
NanoLC Ultra, NanoLC and Tempo systems < 0.5 min	
Retention time—542.3 m/z	
Replicate A	
Replicate B	
Replicate C	
Difference	
Specification:	
SCIEX nanoLC 400 systems < 0.3 min	
NanoLC Ultra, NanoLC and Tempo systems < 0.5 min	
Specification Passed?	

Tip! Use a column oven to increase retention time reproducibility. The temperature effect on retention time differs from peptide to peptide. To get the retention time reproducibility within specification, the temperature of the column should be stable within 1°C. Factors to consider include not only the column temperature itself, but the room environment, especially during overnight runs as the room temperature can shift dramatically.

Comments and Exceptions

Signoff

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

*Signature required on hard copy only.

Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation

A

This appendix includes method parameters for the SCIEX nanoLC 400 system with a cHiPLC[®] system. For an external trap and column installation, refer to [Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation on page 42](#).

Autosampler Method Parameters

Figure A-1 Method Editor Dialog

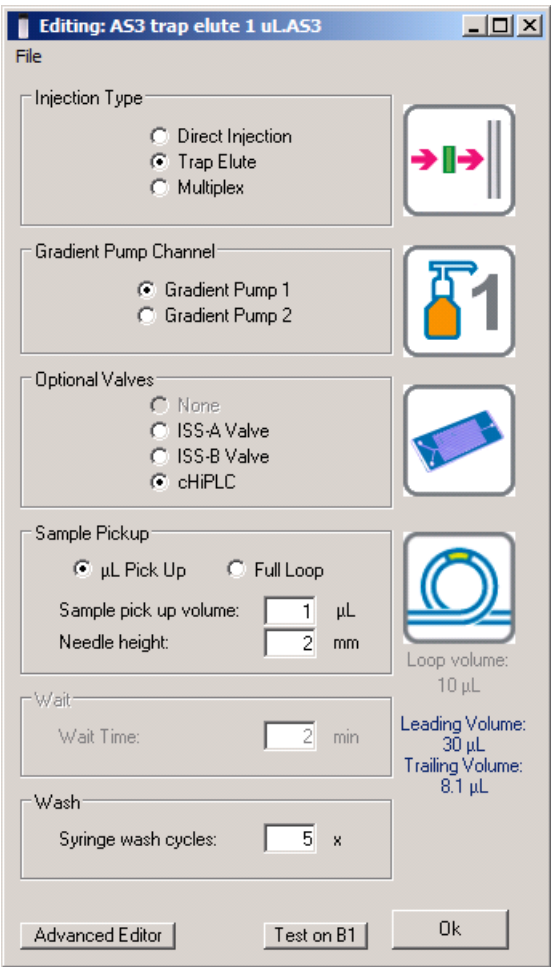


Table A-1 contains the autosampler method parameters as they are shown in the **Advanced Editor** dialog.

Table A-1 Advanced Editor Autosampler Method Parameters

Command	Description
Initialize	Autosampler Device
Wait	for 0:00:01
Needle Wash	Pre-wash—1 ×
Wait	for Gradient 1 ready to start
Wait	for Loading pump ready to start
Get Sample	µL Pickup—1 µL—0.5 µL/s—2 mm from bottom
Start	Loading Pump
Valve	Injector Inject
Wait	Wait for loading pump injection complete
Start	Gradient Pump 1
Valve	Injector Load
Needle Wash	Clean Up—5 ×

Loading Pump Method Parameters

Figure A-2 LC Method Settings Dialog—Run Conditions

The screenshot shows the 'LC Method Settings' dialog box with the 'Run Conditions' tab selected. The 'Selected Method' is 'Load Pump 10 min Trap Wash'. The 'Pre-Run' section has 'Flush column for 0.1 minutes using 100 % initial flowrate conditions.' checked. The 'Sample Injection' section has 'Standard: Sample valve opens prior to beginning Flow Profile and remains open.' selected. The 'Post-Run' section has 'Flush column for 1 minutes using 100 % ending flowrate conditions.' checked. Buttons for 'Save', 'Print', 'Delete', 'View Audit Trail', 'OK', and 'Cancel' are visible.

LC Method Settings

Selected Method

Name: Load Pump 10 min Trap Wash [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.
☒ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 2000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 2000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☒ Flush column for 1 minutes using 100 % ending flowrate conditions.

[Delete] [View Audit Trail] [OK] [Cancel]

Table A-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Figure A-3 LC Method Settings Dialog—Run Conditions Tab (cHiPLC System)

LC Method Settings

Selected Method

Name: CH1 45min 300nLmin cHiPLC trap [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.
☒ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 12000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 12000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

[Delete] [View Audit Trail] [OK] [Cancel]

Table A-3 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	95	5	
2	1	95	5	
3	16	75	25	
4	18	20	80	
5	24	20	80	
6	27	95	5	
7	45	95	5	

Note: Flow rate is set to 300 nL/min.

Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation

B

This appendix includes method parameters for the SCIEX NanoLC 400 system with an external trap and column. For a cHiPLC® installation, refer to [Methods for SCIEX nanoLC 400 Systems— cHiPLC System Installation on page 37](#).

Autosampler Method Parameters

Figure B-1 Method Editor Dialog

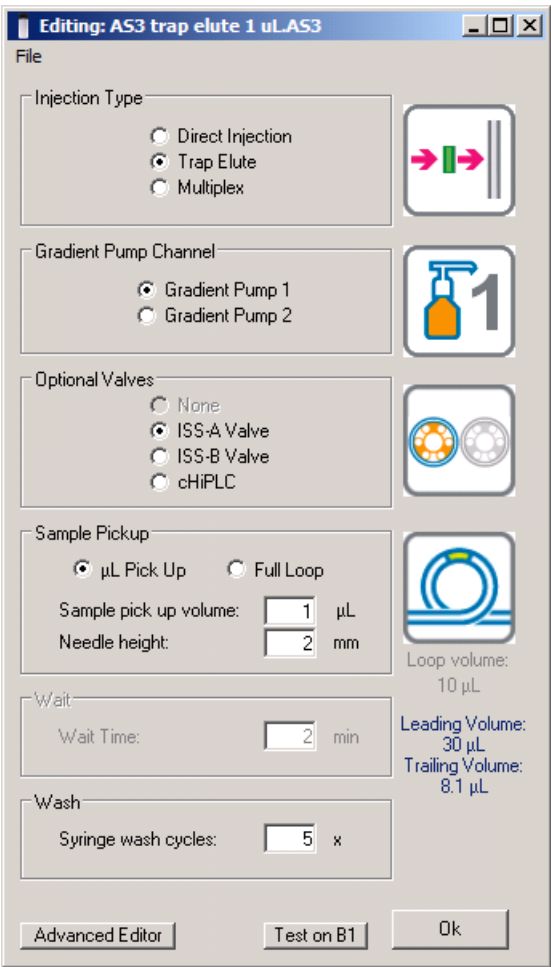


Table B-1 contains the autosampler method parameters as they are shown in the **Advanced Editor** dialog.

Table B-1 Advanced Editor Autosampler Method Parameters

Command	Description
Initialize	Autosampler Device
Valve	ISS-A Load
Needle Wash	Pre-wash—1 × using Wash Solvent 1
Wait	for Gradient 1 ready to start
Wait	for Loading Pump ready to start
Get Sample	μL Pickup—1 μL - 0.5 μL/s - 2 mm from bottom
Start	Loading Pump
Valve	Injector Inject
Wait	for Loading Pump injection complete
Valve	ISS-A Inject
Start	Gradient Pump 1
Valve	Injector Load
Needle Wash	Clean Up—5 × using Wash Solvent 1

Loading Pump Method Parameters

Figure B-2 LC Method Settings Dialog—Run Conditions

LC Method Settings

Selected Method

NameLoad Pump 10 min Trap Wash

Save

Print

Summary

Run Conditions

Gradient Profile

Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.

☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.

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

☐ Metered: Inject 2000 nL of sample at 100 % initial flowrate conditions.

☐ Rapid: Inject 2000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

Delete

View Audit Trail

OK

Cancel

Table B-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Figure B-3 LC Method Settings Dialog—Run Conditions Tab (External Column)

LC Method Settings

Selected Method

Name: CH1 45min 300nLmin column trap [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection

☒ None.
☐ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 12000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 12000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

[Delete] [View Audit Trail] [OK] [Cancel]

Table B-3 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	95	5	
2	1	95	5	
3	16	75	25	
4	18	20	80	
5	24	20	80	
6	27	95	5	
7	45	95	5	

Methods for SCIEX nanoLC 400 Systems—External Trap and Column Installation

Note: Flow rate is set to 300 nL/min.

Methods for NanoLC Ultra Systems— cHiPLC System Installation

C

This appendix includes method parameters for the NanoLC™ Ultra™ system with a cHiPLC® system. For an external trap and column installation, refer to [Methods for NanoLC Ultra Systems—External Trap and Column Installation on page 52](#).

Autosampler Method Parameters

This table contains the autosampler method parameters as they are shown in the **Method Editor** dialog.

Table C-1 Autosampler Method Parameters

Command	Value	Parameter	Values
Valve		Injector Load	
External Events		Wait for Gradient 1 Ready	
External Events		Wait for Loading Pump Ready	
Needle Wash	50 µL	Port 1	
Aspirate	19 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Aspirate	1 µL	Sample	Speed: 1 Height: 3
Wait	00:00:05		
Aspirate	0 µL	Sample	Speed: 1 Height: 3
Aspirate	5 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		

Table C-1 Autosampler Method Parameters (continued)

Command	Value	Parameter	Values
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
External Events		Start Loading Pump	
Valve		Injector Inject	
External Events		Wait for Loading Pump Inject	
Valve		Injector Load	
External Events		Start Gradient 1	
Dispense	25 µL	Waste	Speed: 5 Height: 0
Needle Wash	200 µL	Port 1	
END			

Loading Pump Method Parameters

Figure C-1 LC Method Settings Dialog—Run Conditions

The screenshot shows the 'LC Method Settings' dialog box with the 'Run Conditions' tab selected. The 'Selected Method' is 'Load Pump 10 min Trap Wash'. The 'Pre-Run' section has 'Flush column for 0.1 minutes using 100 % initial flowrate conditions.' checked. The 'Sample Injection' section has 'Standard: Sample valve opens prior to beginning Flow Profile and remains open.' selected. The 'Post-Run' section has 'Flush column for 1 minutes using 100 % ending flowrate conditions.' unchecked. Buttons for 'Save', 'Print', 'Delete', 'View Audit Trail', 'OK', and 'Cancel' are visible.

LC Method Settings

Selected Method

Name: Load Pump 10 min Trap Wash

Save Print

Summary Run Conditions Gradient Profile Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.

☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.

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

☐ Metered: Inject 2000 nL of sample at 100 % initial flowrate conditions.

☐ Rapid: Inject 2000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

Delete View Audit Trail OK Cancel

Table C-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Note: The events shown below verify the correct switching of the cHiPLC[®] valve. The signals at Time 0 will move the cHiPLC[®] valve to the Inject position and the signal at Time 45 will move the cHiPLC[®] valve back to the Load position.

Figure C-2 LC Method Settings Dialog—Run Conditions Tab (cHiPLC System)

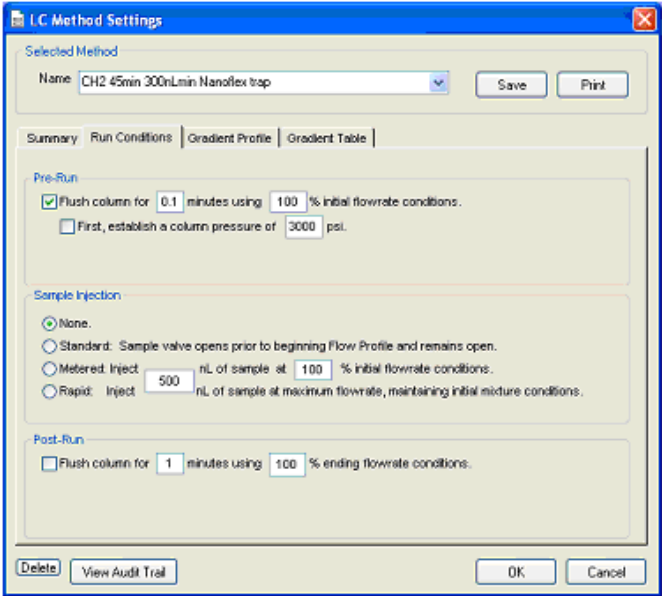


Table C-3 Gradient Method Parameters

Step	Time	% A	% B	Event
1	0	95	5	AUX3 TTL Low
2	0	95	5	AUX4 TTL High
3	0.1	95	5	AUX3 TTL High
4	16	75	25	
5	18	20	80	
6	24	20	80	
7	27	95	5	
8	45	96	5	AUX4 TTL Low

Note: Flow rate is set to 300 nL/min.

Methods for NanoLC Ultra Systems—External Trap and Column Installation

D

This appendix includes method parameters for the NanoLC Ultra™ system with an external trap and column. For methods for a cHiPLC® system installation, refer to [Methods for NanoLC Ultra Systems— cHiPLC System Installation on page 47](#).

Autosampler Method Parameters

This table contains the autosampler method parameters as they are shown in the **Method Editor** dialog.

Table D-1 Autosampler Method Parameters

Command	Value	Parameter	Values
Valve		Injector Load	
External Events		Wait for Gradient 1 Ready	
External Events		Wait for Loading Pump Ready	
Needle Wash	50 µL	Port 1	
Aspirate	19 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Aspirate	1 µL	Sample	Speed: 1 Height: 3
Wait	00:00:05		
Aspirate	0 µL	Sample	Speed: 1 Height: 3
Aspirate	5 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		

Table D-1 Autosampler Method Parameters (continued)

Command	Value	Parameter	Values
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
External Events		Start Loading Pump	
Valve		Injector Inject	
External Events		Wait for Loading Pump Inject	
Valve		Injector Load	
External Events		Start Gradient 1	
Dispense	25 µL	Waste	Speed: 5 Height: 0
Needle Wash	200 µL	Port 1	
END			

Loading Pump Method Parameters

Figure D-1 LC Method Settings Dialog—Run Conditions

LC Method Settings

Selected Method

NameLoad Pump 10 min Trap Wash

Save

Print

Summary

Run Conditions

Gradient Profile

Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.

☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.

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

☐ Metered: Inject 2000 nL of sample at 100 % initial flowrate conditions.

☐ Rapid: Inject 2000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

Delete

View Audit Trail

OK

Cancel

Table D-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Figure D-2 LC Method Settings Dialog—Run Conditions Tab (External Column)

LC Method Settings

Selected Method

Name: CH1 45min 300nLmin column trap [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.
☒ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 12000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 12000 nL of sample at maximum flowrate, maintaining initial mixture conditions.

Post-Run

☐ Flush column for 1 minutes using 100 % ending flowrate conditions.

[Delete] [View Audit Trail] [OK] [Cancel]

Table D-3 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	95	5	
2	1	95	5	
3	16	75	25	
4	18	20	80	
5	24	20	80	
6	27	95	5	
7	45	95	5	

Methods for NanoLC Ultra Systems—External Trap and Column Installation

Note: Flow rate is set to 300 nL/min.

Methods for NanoLC and Tempo Systems—cHiPLC System Installation

E

This appendix includes method parameters for the NanoLC™ and Tempo™ systems with a cHiPLC® system. Refer to [Connecting NanoLC and Tempo Systems with a cHiPLC System on page 67](#) for plumbing information. For an external trap and column installation, refer to [Methods for NanoLC and Tempo Systems—External Trap and Column on page 62](#).

Autosampler Method Parameters

This table contains the autosampler method parameters as they are shown in the **Method Editor** dialog.

Table E-1 Autosampler Method Parameters

Command	Value	Parameter	Values
Output		1-OFF	
Output		2-OFF	
Valve		Injector Load	
Aspirate	19 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Aspirate	1 µL	Sample	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Sample	Speed: 1 Height: 5
Aspirate	5 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		

Table E-1 Autosampler Method Parameters (continued)

Command	Value	Parameter	Values
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Output		1-ON	
Wait for input		1-LOW	
Valve		Injector Inject	
Wait for input		1-HIGH	
Valve		Injector Load	
Output		2-ON	
Dispense	25 µL	Waste	Speed:5 Height: 0
Needle wash	200 µL	Port 1	
END			

Loading Pump Method Parameters

Figure E-1 LC Method Settings Dialog—Run Conditions

The screenshot shows the 'LC Method Settings' dialog box with the 'Run Conditions' tab selected. The 'Selected Method' is 'Loading_Trap_Elute'. The 'Pre-Run' section has 'Flush column for 0.1 minutes using 100 % initial flowrate conditions.' checked. The 'Sample Injection' section has 'Standard: Sample valve opens prior to beginning Flow Profile and remains open.' selected. The 'Post-Run' section has 'Flush column for 0.5 minutes using 100 % ending flowrate conditions.' unchecked. Buttons for 'Save', 'Print', 'Delete', 'View Audit Trail', 'OK', and 'Cancel' are visible.

LC Method Settings

Selected Method

Name: Loading_Trap_Elute [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.
☒ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 5000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 5000 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]

Table E-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Figure E-2 LC Method Settings Dialog—Run Conditions Tab (cHiPLC System)

LC Method Settings

Selected Method

Name

Gradient_Trap_Elute

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.

Sample Injection

☐ None.

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

☐ Metered: Inject

5000

 nL of sample at

100

 % initial flowrate conditions.

☐ Rapid: Inject

5000

 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

Table E-3 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	95	5	
2	1	95	5	
3	16	75	25	
4	18	20	80	
5	24	20	80	
6	27	95	5	
7	45	95	5	

Methods for NanoLC and Tempo Systems—cHiPLC System Installation

Note: Flow rate is set to 300 nL/min.

Methods for NanoLC and Tempo Systems—External Trap and Column

F

This appendix includes method parameters for the classic NanoLC™ and Tempo™ systems with an external trap and column. For methods for a cHiPLC® system installation, refer to [Methods for NanoLC and Tempo Systems—cHiPLC System Installation on page 57](#).

Autosampler Method Parameters

This table contains the autosampler method parameters as they are shown in the **Method Editor** dialog.

Table F-1 Autosampler Method Parameters

Command	Value	Parameter	Values
Output		1-OFF	
Output		2-OFF	
Valve		Injector Load	
Aspirate	19 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Aspirate	1 µL	Sample	Speed: 1 Height: 5
Wait	00:00:05		
Aspirate	0 µL	Sample	Speed: 1 Height: 5
Aspirate	5 µL	Reagent-1	Speed: 1 Height: 5
Wait	00:00:05		

Table F-1 Autosampler Method Parameters (continued)

Command	Value	Parameter	Values
Aspirate	0 µL	Reagent-1	Speed: 1 Height: 5
Output		1-ON	
Wait for input		1-LOW	
Valve		Injector Inject	
Wait for input		1-HIGH	
Valve		Injector Load	
Output		2-ON	
Dispense	25 µL	Waste	Speed:5 Height: 0
Needle wash	200 µL	Port 1	
END			

Loading Pump Method Parameters

Figure F-1 LC Method Settings Dialog—Run Conditions

LC Method Settings

Selected Method

Name Loading_Trap_Elute

Save

Print

Summary

Run Conditions

Gradient Profile

Gradient Table

Pre-Run

☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.

☐ First, establish a column pressure of 3000 psi.

Sample Injection

☐ None.

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

☐ Metered: Inject 5000 nL of sample at 100 % initial flowrate conditions.

☐ Rapid: Inject 5000 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

Table F-2 Loading Pump Method Parameters (Gradient Table Tab)

Step	Time	Qa
1	0	2
2	10	2

Gradient Pump Method Parameters

Figure F-2 LC Method Settings Dialog—Run Conditions Tab (External Column)

LC Method Settings

Selected Method
Name: Ch2 45min 300nLpermin trap [Save] [Print]

Summary | **Run Conditions** | Gradient Profile | Gradient Table

Pre-Run
☒ Flush column for 0.1 minutes using 100 % initial flowrate conditions.
☐ First, establish a column pressure of 3000 psi.

Sample Injection
☐ None.
☒ Standard: Sample valve opens prior to beginning Flow Profile and remains open.
☐ Metered: Inject 5000 nL of sample at 100 % initial flowrate conditions.
☐ Rapid: Inject 5000 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]

Table F-3 Gradient Method Parameters (Gradient Table Tab)

Step	Time	% A	% B	Event
1	0	95	5	
2	1	95	5	
3	16	75	25	
4	18	20	80	
5	24	20	80	
6	27	95	5	
7	45	95	5	

Methods for NanoLC and Tempo Systems—External Trap and Column

Note: Flow rate is set to 300 nL/min.

Connecting NanoLC and Tempo Systems with a cHiPLC System

G

This appendix includes wiring information required when performing the system integration test with a NanoLC™ or Tempo™ system and the cHiPLC® system.

Wire Connections

[Table G-1](#) provides the wiring connections for a NanoLC™ system with the cHiPLC® system. The output on the NanoLC system (4-pin connector) is usually used for switching the external 10-port valve. However, the 10-port valve is not used when using the cHiPLC® system. Use the same communication ports, as shown in [Table G-1](#).

Table G-1 Pin Assignments

Output on NanoLC System (Valve I/O)	Input on cHiPLC® System
GND	Pin 1 (Ground) [1st on Left]
Vlv 1	Pin 3 (Position B)
Vlv 2	Pin 2 (Position A)
Pin 10 (Channel I/O)	Pin 6 (Interlock signal)

Mass Spectrometer System Calibration

H



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 [Glu¹]-Fibrinopeptide B, included in the LC/MS Peptide Calibration Kit (PN 4465867).

Note: Do not infuse the tuning solution and then the [Glu¹]-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 Digital PicoView[®] Nanospray ion source.

Prepare the [Glu¹]-Fibrinopeptide B Dilution

Required Material

- [Glu¹]-Fibrinopeptide B, included in the LC/MS Peptide Calibration Kit (PN 4465867)
- Standard diluent, included in the LC/MS Peptide Calibration Kit

For the list of masses for [Glu¹]-Fibrinopeptide B, refer to [Masses for \[Glu¹\]-Fibrinopeptide B on page 69](#).

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

Note: The [Glu¹]-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 [Glu¹]-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 [Glu¹]-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.

Masses for [Glu¹]-Fibrinopeptide B

Table H-1 [Glu1]-Fibrinopeptide B (Monoisotopic Molecular Weight, 1569.6696 Da)

Charge	(M+nH) ⁿ⁺ Monoisotopic m/z
+1	1570.6768
+2	785.8421 *
+3	524.2305*
+4	393.4247
+5	—
+6	—

* Indicates more commonly observed charged states.

[Table H-2](#) contains the exact monoisotopic masses for the theoretical cleavages of [Glu1]-Fibrinopeptide B, as calculated for positive ion mode.

Table H-2 Theoretical Fragment Ions of [Glu1]-Fibrinopeptide B

b ions		y ions	
<i>m/z</i>	Fragment	<i>m/z</i>	Fragment
—	—	1570.6768	EGVNDNEEGFFSAR
130.0499	E	1441.6342	GVNDNEEGFFSAR
187.0713	EG	1384.6128	VNDNEEGFFSAR
286.1397	EGV	1285.5444	NDNEEGFFSAR
400.1827	EGVN	1171.5014	DNEEGFFSAR
515.2096	EGVND	1056.4745	NEEGFFSAR
629.2525	EGVNDN	942.4316	EEGFFSAR
758.2951	EGVNDNE	813.3890	EGFFSAR
887.3377	EGVNDNEE	684.3464	GFFSAR
944.3592	EGVNDNEEG	627.3249	FFSAR
1091.4276	EGVNDNEEGF	480.2565	FSAR
1238.4960	EGVNDNEEGFF	333.1881	SAR
1325.5281	EGVNDNEEGFFS	246.1561	AR
1396.5652	EGVNDNEEGFFSA	175.1190	R
1552.6663	EGVNDNEEGFFSAR	—	—

Edit the Calibration Reference Table for [Glu¹]-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 H-1](#) to the **Reference Ions for TOF MS Calibration** table (on the left side of the dialog).

Figure H-1 Glu-Fibrinopeptide B Reference Table

Reference Table Editor

Name: ☒ Positive ☐ Negative Calibration Valve Position:

Reference Ions for TOF MS Calibration:

	Use	Compound Name	Precursor m/z (Da)	Use for MS/MS	CE for MS/MS	DP for MS/MS	Retention Time (min)
1	<input type="checkbox"/>	Diisooctyl phthalate,	391.28429	<input type="checkbox"/>	10.000	100.000	0.00
2	<input checked="" type="checkbox"/>	y4	480.25650	<input type="checkbox"/>	42.000	100.000	0.00
3	<input checked="" type="checkbox"/>	y6	684.34640	<input type="checkbox"/>	42.000	100.000	0.00
4	<input checked="" type="checkbox"/>	Glu-fibrinopeptide	785.84210	<input checked="" type="checkbox"/>	42.000	100.000	0.00
5	<input checked="" type="checkbox"/>	Y7	813.38900	<input type="checkbox"/>	42.000	100.000	0.00
6	<input checked="" type="checkbox"/>	Y8	942.43160	<input type="checkbox"/>	42.000	100.000	0.00
7	<input checked="" type="checkbox"/>	Y9	1056.47450	<input type="checkbox"/>	42.000	100.000	0.00
8	<input checked="" type="checkbox"/>	Y10	1171.50140	<input type="checkbox"/>	42.000	100.000	0.00
9	<input checked="" type="checkbox"/>	Y11	1285.54440	<input type="checkbox"/>	42.000	100.000	0.00
10	<input type="checkbox"/>			<input type="checkbox"/>			
11	<input type="checkbox"/>			<input type="checkbox"/>			
12	<input type="checkbox"/>			<input type="checkbox"/>			
13	<input type="checkbox"/>			<input type="checkbox"/>			
14	<input type="checkbox"/>			<input type="checkbox"/>			

Retention time is only used for non-CDS configuration. Retention Time Tolerance: +/- sec

Reference Ions for MS/MS Calibration:
(Product of 785.84210 Da)

	Use	Fragment Name	Fragment m/z (Da)
1	<input type="checkbox"/>		72.08080
2	<input type="checkbox"/>		120.08080
3	<input type="checkbox"/>		159.07640
4	<input checked="" type="checkbox"/>		175.11900
5	<input type="checkbox"/>		187.07130
6	<input type="checkbox"/>		246.15610
7	<input type="checkbox"/>		286.13970
8	<input checked="" type="checkbox"/>		333.18810
9	<input type="checkbox"/>		400.18270
10	<input checked="" type="checkbox"/>		480.25650
11	<input type="checkbox"/>		515.20960
12	<input type="checkbox"/>		627.32490
13	<input type="checkbox"/>		629.25250
14	<input checked="" type="checkbox"/>		684.34640

- Click **OK**.
- Click **OK** in the **Tuning Options** dialog.

Calibrate in TOF MS Mode

- Set the method parameters as shown in [Table H-3](#).

Table H-3 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 H-3 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)	3 (or as optimized)
Curtain Gas (CUR)	35 (or as optimized)
Interface Heater Temperature (IHT) (°C)	75 (or as optimized)
Ion Spray Voltage Floating (ISVF)	2300 (or as optimized)
Compound-dependent Parameters	
Collision energy (CE) (V)	40 (or as optimized)
Compound Parameters	
Declustering Potential	100 (or as optimized)

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 H-4](#).

Table H-4 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	Off
High resolution	On
Duration (min)	0.5
Advanced MS Parameters	
MCA	Off
Auto Adjust with mass	On
Q1 Transmission Window	Default (with Auto-adjust)

Table H-4 Method Parameters (continued)

Parameter	Value
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)	3 (or as optimized)
Curtain Gas (CUR)	35 (or as optimized)
Interface Heater Temperature (IHT) (°C)	75 (or as optimized)
Ion Spray 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 the calibration for the Product Ion mode.
15. Click **Close**.

Plumbing Diagrams



This appendix includes two plumbing diagrams for the trap-and-elute configuration, one for external trap-and-elute, the other for the cHiPLC[®] system.

Table I-1 Legend for the Plumbing Diagrams




Item	Description
1	Loading pump
2	Syringe dispenser
3	Sample needle
4	Sample loop
5	10-port valve
6	Waste
7	Column
8	To mass spectrometer
9	Nanoflow pump
10	Trap
11	cHiPLC [®] valve
12	Filter
13	cHiPLC [®] column chip
14	cHiPLC [®] trap column
15	cHiPLC [®] trap/elute jumper chip
16	Union
	Load
	Inject
	25 µM i.d., 360 µM o.d.

Table I-1 Legend for the Plumbing Diagrams (continued)



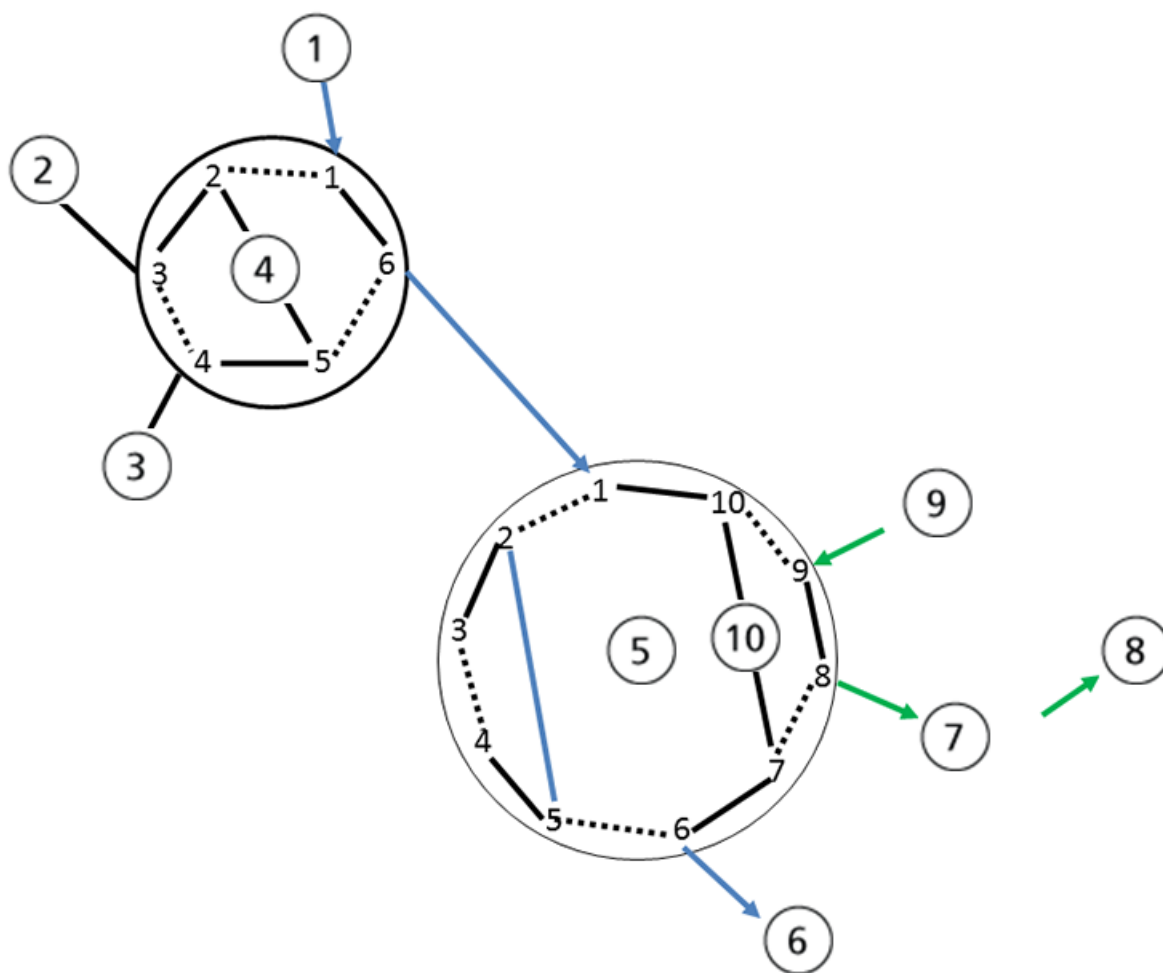
Item	Description
	30 μ M i.d., 180 μ M o.d.
	50 μ M i.d., 360 μ M o.d.

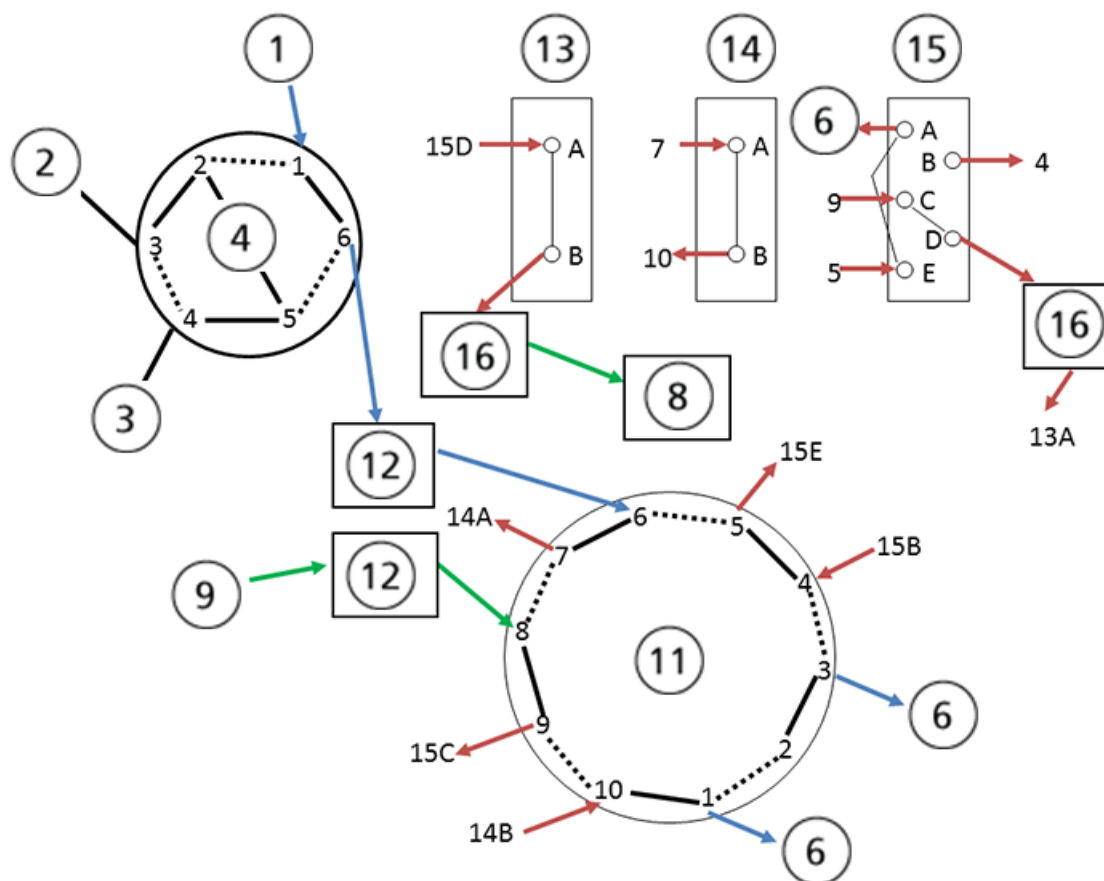
Figure I-1 Trap-and-elute Configuration with External Trap and Column



Plumbing Diagrams

In [Figure I-2](#), the nanoflow channel (usually gradient 2) is plumbed from pump to the filter attached to port 8 of the cHiPLC® system. The microflow channel (usually Channel 1) is plumbed from the pump to port 1 of the autosampler. Port 6 of the autosampler is plumbed to the filter attached to port 6 of the cHiPLC® system.

Figure I-2 Trap-and-elute Experiment with Optional cHiPLC System



Best Practices

J

This appendix describes best practices and sample preparation techniques for a NanoLC system.

- Keep the system turned on and fluid flowing at all times. If the system is shut off for more than a few days, then purge and possibly calibrate the system prior to use. Refer to the appropriate operator guide.
- Use LC/MS-grade pre-made solvents such as those from Burdick-Jackson (that is, HPLC-grade water with 0.1% formic acid and acetonitrile with 0.1% formic acid). These solvents can be ordered from VWR:
 - PN BJLC452-2.5 - Water with 0.1% formic acid
 - PN BJLC441-2.5 - Acetonitrile with 0.1% formic acid
- Verify that the gas flow rate and pressure are consistent. Do not disconnect the gas supply.
- Avoid biological growth.
 - Change solutions frequently.
 - Include 0.1% formic acid in all mobile phase bottles.
- Verify that the mobile phase solutions in the bottles match the mobile phase and composition values in the Eksigent control software (**System > Mobile Phases.**)

Table J-1 Recommended Mobile Phase Mixtures

	Binary Mixture A	Binary Mixture B	Modifier
Gradient 1	100% water	100% acetonitrile	0.1% formic acid
Gradient 2	100% water	100% acetonitrile	0.1% formic acid
Loading pump	100% water	N/A	0.1% formic acid

- Remove air from the mobile phase bottles weekly by purging the system a minimum of 10 times per channel.
- Keep the pump seal wash bottle filled with 5% methanol and change the solutions quarterly.
- Empty the waste bottle once a week (or more often if needed).
- Check the flow rate daily. Re-initialize the pressure transducers weekly.
- Pre-swage all fittings.
- When cutting silica, wash the end with methanol and flow solution through the cut end before connecting.
- Do not cut PEEK-lined fused silica tubing.
- Perform an autosampler Initial Wash or System Wash daily.

Sample Preparation Techniques



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

In general, the standard practices and procedures for reversed phase LC/MS/MS experiments using electrospray mass spectrometry also apply to the use of the NanoLC™ systems, including the cHiPLC® system.

- Use HPLC- or MS-grade solvents at all times.
- Avoid the use of non-volatile salts and buffers such as CHAPS, phosphate, TRIS, HEPES, and perchlorates. These additives will foul the electrospray source and mass spectrometer orifice.
- Centrifuge all samples at 10 000 rpm for 5 minutes to remove particulates from the sample solution.
- Dilute all samples to a maximum of 5% organic modifier to prevent sample precipitation in the chromatographic system and at the electrospray source. This also ensures binding to the stationary phase.
- Make sure that detergents used during sample preparation are reduced to a concentration less than 0.01% prior to analysis.

Mass Assignments

K

Table K-1 Beta-Galactosidase Mass Assignments

Peptide Fragment	Monoisotopic Molecular Weight (Da)	<i>m/z</i>	Charge State
ELNYGPHQWR	1298.62	433.88	3
FNDDFSR	899.38	450.7	2
YSQQQLMETSHR	1506.69	503.24	3
RDWENPGVTQLNR	1583.78	528.93	3
GDFQFNISR	1082.51	542.27	2
IDPNAWVER	1098.55	550.28	2
DVSLHKKPTTQISDFHVATR	2264.19	567.06	4
ITDSLAVVLQR	1213.70	607.86	2
VDEDQPFPAVPK	1340.66	671.34	2
LPSEFDLSAFLR	1393.72	697.87	2
DWENPGVTQLNR	1427.68	714.85	2
APLDNDIGVSEATR	1456.72	729.37	2
LSGQTIEVTSEYLFR	1741.89	871.95	2
VNWLGLGPQENYPDR	1756.85	879.43	2

Revision History

Revision	Reason for Change	Date
A	First release of document. Combined <i>NanoLC Systems System Integration Test and Data Log for TripleTOF® 4600 Systems</i> (PN D5033709 E), <i>NanoLC Systems System Integration Test and Data Log for TripleTOF® 5600/5600+ Systems</i> (PN D5033708 E), and <i>NanoLC Systems System Integration Test and Data Log for TripleTOF® 6600 Systems</i> (PN D5099640 A).	September 2016