

Thermo

# EASY-nLC 1200

## Getting Started Guide

Touch-screen application version 4.2

60053-97272 Revision C 2019



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scientific

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Software version: Touch-screen software version 4.2

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## Contents

# Preface

This guide describes how to operate and maintain the Thermo Scientific™ EASY-nLC™ nanoflow liquid chromatography (LC) instrument by using the integrated touch-screen application.

Before you turn on the instrument for the first time, read the first three chapters in this guide. Before you run your first sample, review the chapters that describe how to configure the software, prepare the system for operation, and submit your first sample set (batch).

## Contents

- [Related Documentation](#)
- [Cautions and Special Notices](#)
- [Contacting Us](#)

### ❖ To suggest changes to the documentation or to the Help

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.



## Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for the EASY-nLC instrument as PDF files:

- *EASY-nLC 1200 User Guide for the Xcalibur Data System (version 2.1 or later)*
- *EASY-nLC 1200 Preinstallation Requirements Guide*
- *EASY-nLC 1200 Troubleshooting and Maintenance Guide*
- *Safety and Regulatory Guide*

The EASY-nLC 1200 also ships with a printed copy of the *Safety and Regulatory Guide*. This guide contains important safety information about Thermo Scientific liquid chromatography (LC) and mass spectrometry (MS) systems. Make sure that all lab personnel have read and have access to this document.

You can find the EASY-nLC Series manuals in the following locations:

- The USB flash drive provided in the EASY-nLC accessory kit
- The data system computer

❖ **To view the product manuals on the data system computer**

Choose **Start > All Programs > Thermo Instruments > Manuals > LC Devices > Thermo**.

❖ **To view the EASY-nLC Help**

- From the EASY-nLC view of the Instrument Setup window, choose **Help > Thermo EASY-nLC Help** to open the Help to the Welcome topic.
- To view the Help topic for the current view, page, or dialog box, press the F1 key for information about setting parameters. Or, from the EASY-nLC view of the Instrument Setup window, choose **Help > Help On Current Item**.

For more information, visit [www.thermoscientific.com](http://www.thermoscientific.com).

## Cautions and Special Notices

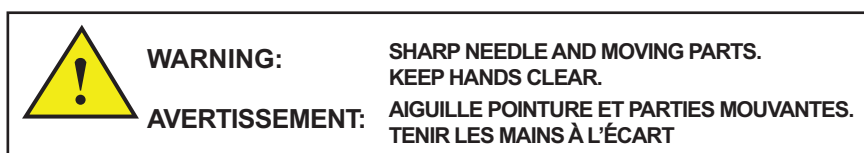
Make sure to follow the safety practices presented in this guide, and observe the safety and special notices that appear in boxes.

Observe all written safety precautions during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument and might result in damage to the instrument, personal injury, or loss of life.

### Special Notices

The following two stickers appear on the EASY-nLC instrument:

This sticker warns you that the instrument includes a sharp needle and moving parts that are accessible to the operator. To prevent personal injury or damage to parts of the EASY-nLC instrument, take care when loading samples into the instrument's tray compartment.



This sticker alerts you to consult this manual for instructions on how to operate the instrument.



The safety and special notices in the documentation include the following:



**CAUTION** Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.



**CAUTION** Highlights electrical hazards to humans or property.



**CAUTION** Highlights a sharp object hazard to humans.



**CAUTION** Highlights an eye hazard to humans.



**CAUTION** Highlights a chemical hazard to humans, property, or the environment.



**CAUTION** Highlights lifting hazards.

**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

**IMPORTANT** General Residual Hazards






**WARNING—Biohazard** Human-sourced material, for example microorganisms, cell cultures, tissues, body fluids, and other biological agents can transmit infectious diseases. To avoid infections with these agents:

- Assume that all biological substances are at least potentially infectious.
- Wear personal protective equipment as required by the hazard and follow good laboratory practice.
- Dispose of bio-hazardous waste in an environmentally safe manner that is consistent with local regulations. Follow a regulated, approved waste disposal program.

**IMPORTANT** Operate the instrument with closed side panels only.

## Contacting Us

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Contact us	Customer Service and Sales	Technical Support
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	(U.S.) 1 (561) 688-8731	(U.S.) 1 (561) 688-8736
	<a href="mailto:us.customer-support.analyze@thermofisher.com">us.customer-support.analyze@thermofisher.com</a> 	<a href="mailto:us.techsupport.analyze@thermofisher.com">us.techsupport.analyze@thermofisher.com</a> 



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### ❖ To order consumable and spare parts for the EASY-nLC instrument

For the EASY-nLC 1200 instrument, go to

<https://www.thermofisher.com/order/catalog/product/LC140>





# Introduction

The EASY-nLC instrument consists of a compact binary liquid chromatography instrument that provides split-free flows down to the low nanoLiter/min range, an autosampler that can hold standard sample vials and microtiter plates, and a built-in instrument control application with a touch-screen interface.

The EASY-nLC 1200 instrument operates in the ultra-high-performance pressure range of 0 to 1200 bar (see [Figure 1](#)).

**Figure 1.** EASY-nLC 1200



To familiarize yourself with the EASY-nLC instrument, read this chapter.

## Contents

- [Instrument Hardware and Software Components and Connections](#)
- [Autosampler Tray Compartment](#)
- [External Solvent Lines](#)
- [Back Panel](#)
- [Column Setup Options](#)
- [Solvent System Components](#)
- [Predefined Steps for Sample Runs](#)
- [Pump Flow Control](#)

# Instrument Hardware and Software Components and Connections

The EASY-nLC instrument consists of the following hardware and software components (see [Table 1](#)).

**Table 1.** EASY-nLC instrument components

Hardware	
Operating pressure range	1 to 1200 bar
Pumps	3 high-pressure pumps
Pressure sensors	3 pressure sensors, one for each pump
Valves	4 high-pressure rotary valves 4 check valves
Flow sensors	2 nano-flow sensors
Autosampler:	<ul style="list-style-type: none"> <li>• Holds one microtiter plate (96 or 384 wells) plus an additional 6 vials or one 6 × 8 vial adapter plus an additional 6 vials.</li> <li>• Holds four liquid containers for solvents, rinse, and waste.</li> <li>• Can be cooled to a maximum of 20 °C [68 °F] below ambient temperature.</li> </ul>
Computer	Embedded computer
Monitor	Pressure sensitive screen that you can use with gloves Screen size: 12.1 in. (30.7 cm) Resolution: 800 × 600 pixels
Mains power	120/230 Vac, 50/60 Hz, 250 W
Software	
EASY-nLC stand-alone, touch-screen application (version 4.2)	

Installing the EASY-nLC instrument as part of an LC/MS system requires only four connections:

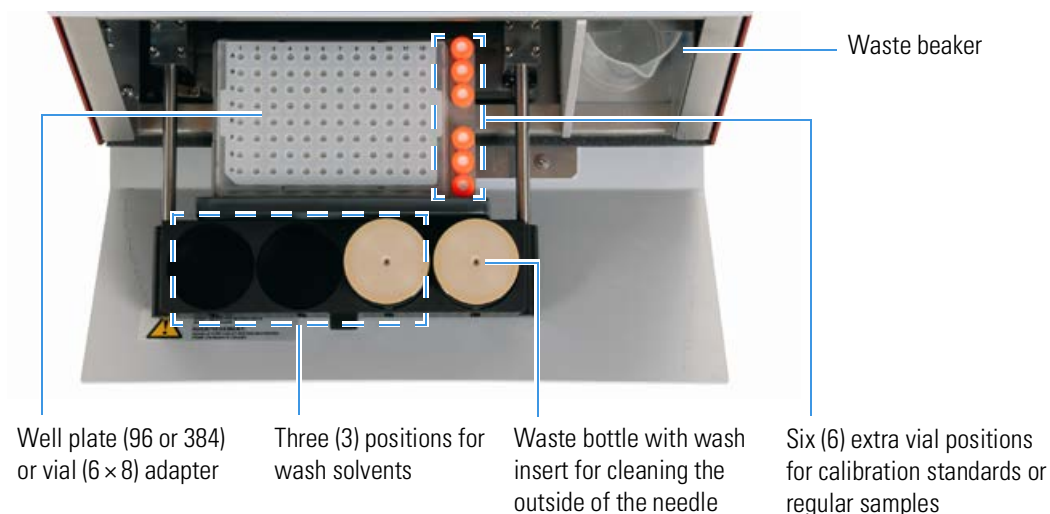
- Solvent outlet connection to the mass spectrometer’s ion source
- Power cable connection to a grounded line power source
- Contact closure connection between the EASY-nLC instrument and the mass spectrometer (see “[Connecting to the Mass Spectrometer through Contact Closure](#)” on page 42)
- Ethernet connection between the EASY-nLC instrument’s built-in computer and the MS system (see “[Setting Up the Ethernet Connection to the Support Server](#)” on page 47)

To control the EASY-nLC instrument with the Thermo Xcalibur™ data system or Thermo Scientific application, you must also connect the EASY-nLC instrument to the data system computer.

## Autosampler Tray Compartment

Figure 2 shows the tray compartment and the waste beaker for the ASC autosampler model. You can open the tray compartment door manually or by using the touch-screen controls.

**Figure 2.** Tray compartment for the current ASC model (viewed from above)



The tray compartment holds the sample tray and the wash bottles. The tray compartment for the ASC model holds an additional six vials. The compartment to the right of the tray compartment holds the waste beaker.

The autosampler draws solvent from wash bottle W3 to perform a standard wash and ejects the solvent into wash bottle W4, which contains the needle wash insert. The autosampler can also perform a custom wash using the solvents from wash bottles W1–W3.

## External Solvent Lines

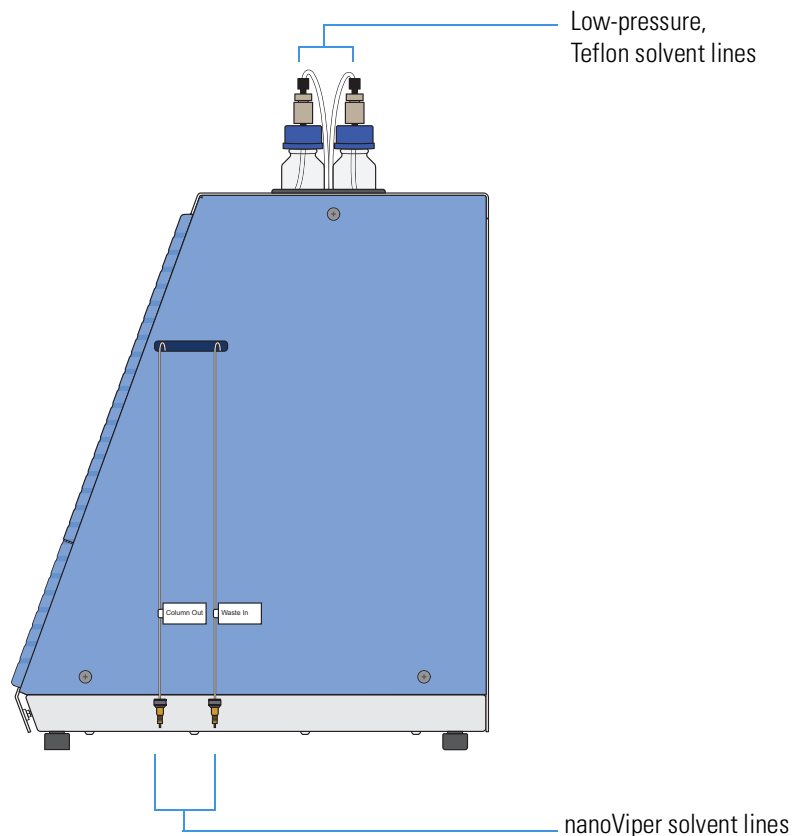
Two PEEKsil™ or nanoViper™ solvent lines exit from the slot in the right side instrument panel, and two low-pressure Teflon™ solvent lines exit through the solvent bottle holder on the top of the instrument. The Column Out line connects to the column assembly's inlet, and the Waste In line connects to the column assembly's venting Tee. The low-pressure solvent lines connect to the solvent A and solvent B reservoir bottles.

To use LC columns that have bare fused-silica ends with the EASY-nLC 1200 instrument, order the UHPLC Liquid Junction Kit. This kit contains modified Column Out and Waste In lines as well as the appropriate fittings and unions for 360 µm OD tubing. For information about installing the modified Column Out and Waste In lines, refer to the *EASY-nLC Series Troubleshooting and Maintenance Guide*. For information about the column setups for these types of LC columns, see “Setting Up a Column Assembly with Fused-Silica Columns” on page 103.

A complete Nano LC Connections Guide is available here:  
<https://tools.thermofisher.com/content/sfs/manuals/PP-64583-LC-MS-NanoLC-Complete-Easy-Guide-PP64583-EN.pdf>

Figure 3 shows the right side panel of the EASY-nLC 1200 instrument.

**Figure 3.** Column Out and Waste In solvent lines and low-pressure, Teflon solvent lines



## Back Panel

The connections for system communication (as part of an LC/MS system), contact closure, and line power are on the instrument's back panel (see [Figure 4](#)).

**Figure 4.** Back panel of the EASY-nLC 1200 instrument shipped from the factory in San Jose, California, U.S.A.

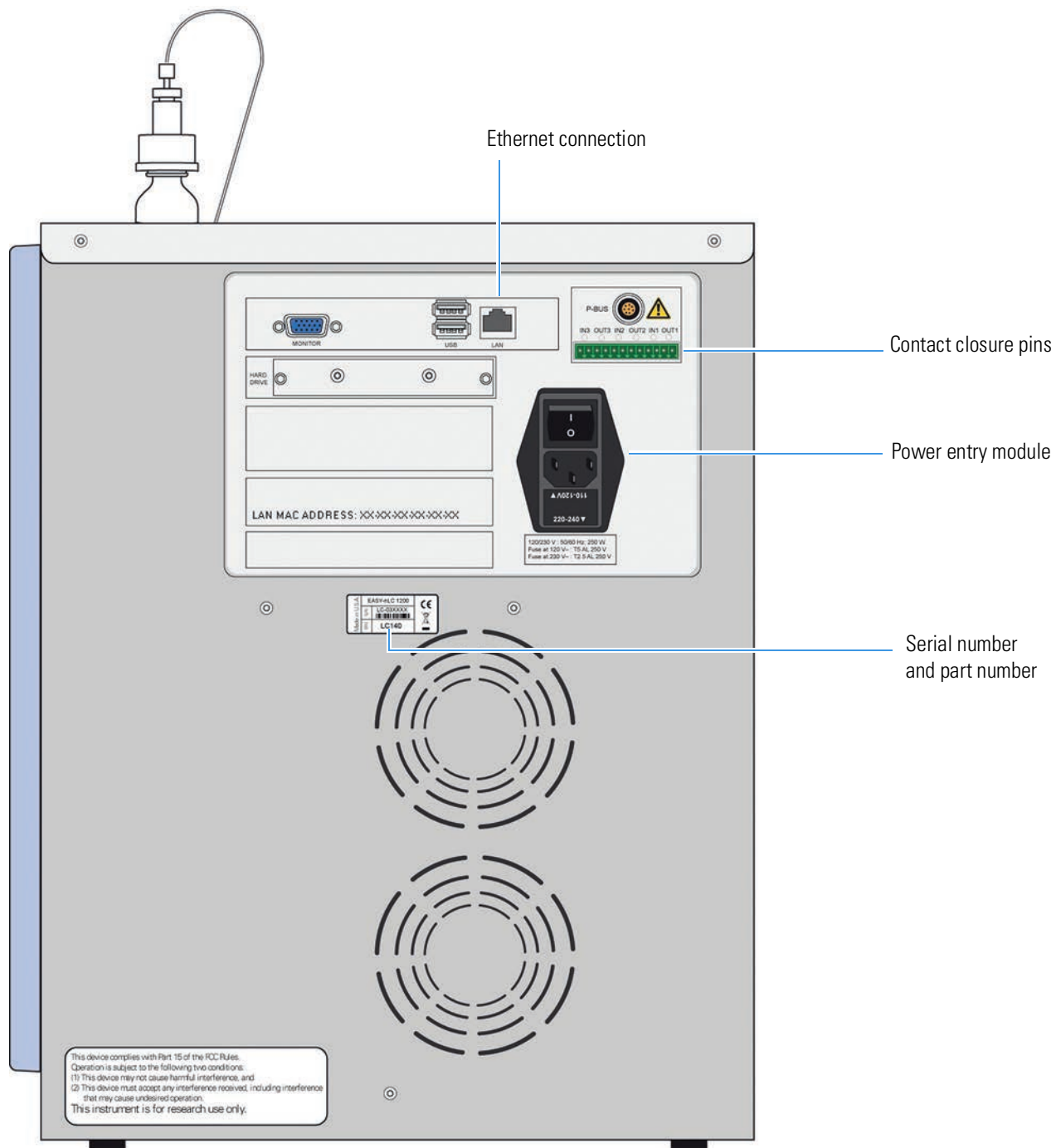


Table 2 lists the function of each back panel element.

**Table 2.** Back panel elements from top to bottom and left to right

<b>Element</b>	<b>Description</b>
Monitor	Output for connection of external display
USB	Two ports for keyboard or mouse or removable USB storage device
LAN	For 10/100/1000 MB/sec Ethernet connection
P-BUS	For communication with add-on devices
IN/OUT pins	Contact closure (primarily for communication with the mass spectrometer)
LAN MAC Address	MAC address of the embedded computer (Gives the EASY-nLC instrument a unique network identifier on your local network.)
I O	Power switch
Fuse holder below I O switch	Replaceable fuse ratings: <ul style="list-style-type: none"><li>• For 120 V, T 5 AL, 250 V</li><li>• For 230 V, T 2.5 AL, 250 V</li></ul>
S/N	Instrument serial number
P/N	Product order code

## Column Setup Options

Two solvent lines exit from the right side of the instrument. Behind the instrument's right panel, the solvent line labeled Column Out connects to port 4 of valve S, and the solvent line labeled Waste In connects to port 2 of valve W.

The fittings and columns shipped with the EASY-nLC instruments are suitable for the Nanospray Flex™ ion source and EASY-Spray™ ion source. For information about setting up the column assemblies for the Nanospray Flex™ and EASY-Spray™ ion sources, see “[Setting Up the Column Assembly](#)” on page 99.

A complete Nano LC Connections Guide is available here:

<https://tools.thermofisher.com/content/sfs/manuals/PP-64583-LC-MS-NanoLC-Complete-Easy-Guide-PP64583-EN.pdf>

You can connect the EASY-nLC instrument and enable the EASY-nLC software for these column configurations:

- “[Two-Column Setup](#),” next section
- “[One-Column Setup](#)” on page 8

### Two-Column Setup

A two-column setup consists of a precolumn and an analytical column.

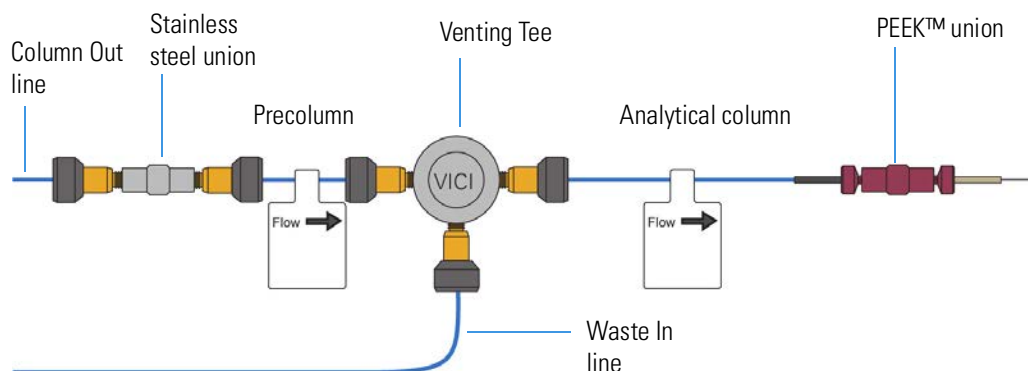
**Tip** When you install the two-column assembly, make sure that the **One Column Setup** check box is not ticked in the EASY-nLC device section on the Devices page of the Maintenance menu (see “[Setting Up the Properties for the HPLC Device](#)” on page 83). Confirm on the Home > Overview page that the configuration is correct.

In a two-column setup (**One Column Setup** check box is not ticked), valve W is in position 1–6 during the sample loading step. The flow through solvent A passes through the venting Tee and exits valve W into the waste beaker.

[Figure 5](#) shows the two-column assemblies for the Nanospray Flex ion source and the EASY-nLC 1200 instrument, respectively.

**Note** The EASY-nLC 1200 instrument ships with nanoflow LC columns for both ion sources. When installing the EASY-nLC instrument, a Thermo Fisher Scientific field service engineer uses the appropriate column to test the instrument performance.

**Figure 5.** Two-column assembly for the EASY-nLC 1200 instrument with a Nanospray Flex ion source



## One-Column Setup

A one-column setup consists of an analytical column, the venting Tee, and a union and sleeve connection to an emitter. The Column Out solvent line and the analytical column connect to parallel legs of the venting Tee. The Waste In solvent line connects to the perpendicular leg of the Tee.

**Tip** When you install a one-column setup, select the **One Column Setup** check box in the EASY-nLC device section on the Devices page of the Maintenance menu (see “[Setting Up the Properties for the HPLC Device](#)” on page 83).

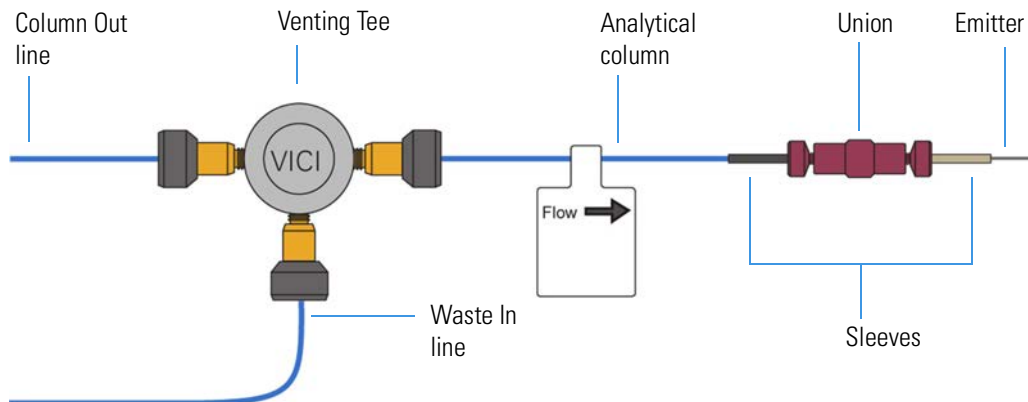
In a one-column setup (**One Column Setup** check box is selected), valve W is in position 1–2 during the sample loading step. The flow through solvent A passes through the venting Tee and the analytical column.

**IMPORTANT** Make sure that you use the venting Tee for the one-column setup as well as for the two-column setup. Inside the instrument housing, the other end of the Waste In line connects to port 2 of valve W. During the Prepare Gradient step of a sample run, valve W provides pressurized venting to the solvent system through the venting Tee. When the Waste In line is not connected to the venting Tee, the system pressure can only vent through the analytical column. Because venting through the column takes longer than venting through the Tee, the total run time is significantly longer than if you used the venting Tee for a one-column setup.



Figure 6 shows the one-column assembly for the Nanospray Flex source and the EASY-nLC 1200 instrument, respectively.

**Figure 6.** One-column setup for the EASY-nLC 1200 instrument and Nanospray Flex source



## Solvent System Components

The EASY-nLC solvent system has three syringe pumps (A, B, and S); three pressure sensors, one for each pump; two flow sensors, one for pump A and one for pump B; four rotary valves (A, B, S, and W); two check valve assemblies, one for solvent A and one for solvent B; and a mixing Tee where solvents A and B are mixed under high pressure.

For more information about the solvent system's hardware components, see the following descriptions:

- [Syringe Pumps](#) (next section)
- [Pressure Sensors](#)
- [Flow Sensors](#)
- [“Six-Port Rotary Valves”](#) on page 12
- [“Check Valve Assemblies”](#) on page 14
- [“Mixing Tee”](#) on page 14

## Syringe Pumps

The syringe pumps perform these functions (see [Table 3](#)):

**Table 3.** Syringe pump functions

Component	Function
Pumps A and B	Draw solvent from the solvent bottles on top of the instrument, empty solvent to the waste beaker in the compartment to the right of the autosampler tray compartment, and deliver solvent to the mixing Tee.
Pump S	Draws solvent from the W3 bottle, draws sample from the specified sample location into the needle, and empties solvent to the W4 bottle in the autosampler tray compartment. It is also used for aspirating solvent from W1 and W2, if a custom wash is activated.

**CAUTION** Solvent B acetonitrile concentration >95% can damage the instrument!

For maintenance purposes, the built-in instrument control software tracks the volume pumped by each pump. For information about recording maintenance for the pumps, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

## Pressure Sensors

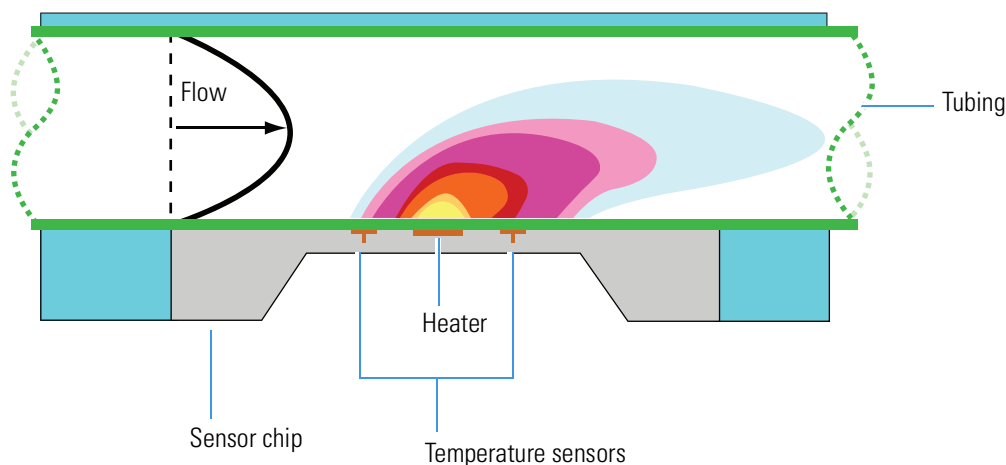
The three pressure sensors are connected to the solvent path between the syringe pump outlets and the six-port, three-position valves.

Using the strain gauge principle to measure pressure accurately, the pressure sensors are flow-through, zero-dead-volume (ZDV) devices with a wetted path that contains 1  $\mu\text{L}$  of swept volume. The pressure sensors for the EASY-nLC 1200 instrument have an operating range of 0 to 20 000 psi (1379 bar).

## Flow Sensors

Two flow sensors provide feedback control for pumps A and B. The two flow sensors are liquid mass flow meters with a patented technology. Each flow meter consists of a length of fused silica capillary, a sensor chip, a heating resistor, and two temperature sensors (see Figure 7).

**Figure 7.** Flow sensor schematic



Based on CMOSens™ Technology  
Principle patented: US Pat. 6 813 944

The flow sensors determine the flow rate of solvents passing through the solvent system by measuring the heat transfer through the fused silica capillary. Outside the capillary, a heating resistor on a thermally optimized membrane is maintained above ambient temperature. When liquid flows through the capillary, the temperature distribution upstream and downstream of the heating resistor is disturbed. The two temperature sensors measure this temperature asymmetry.

Because the heat transfer depends on both the solvent flow rate and the solvent mixture, you must recalibrate the flow sensors whenever you change the solvent types for solvents A and B. For example, if you use methanol instead of acetonitrile for the solvent B mixture, recalibrate the flow sensors as described in the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

**CAUTION** Solvent B acetonitrile concentration >95% can damage the instrument!

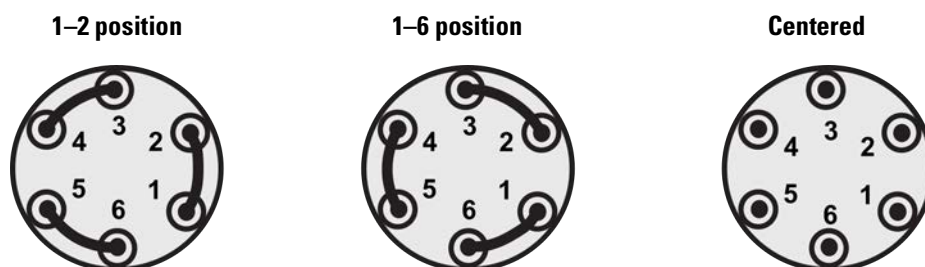
The EASY-nLC 1200 instrument uses two flow sensors with a maximum measuring range of 4.5  $\mu\text{L}/\text{min}$ .

**Tip** When the flow rate produced by pump A or B exceeds the maximum measuring range of the respective flow sensor, use the pump position readback ( $x.x/140 \mu\text{L}$ ) above the pump graphic to monitor the flow rate.

## Six-Port Rotary Valves

The six-port rotary valves can be in three different positions: 1–2 position, 1–6 position, and centered (see [Figure 8](#)).

**Figure 8.** Valve positions



For maintenance purposes, the built-in instrument control software tracks the number of valve shifts for each valve. For information about checking the device's usage counters, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

**Note** The port configuration of the actual valve is rotated (port 1 is at 4 o'clock).

Depending on the valve position, the rotary valves perform these functions (see [Table 4](#)):

**Note** The valve ports are only compatible with nanoViper fittings. Use of other connections may damage the valve.

**Table 4.** Rotary valve functions

Valve	Function
Valves A and B	Direct the solvent flow from syringe pump A and B, respectively.
	1–2 position Directs the solvent flow toward the check valve. When the valve is in the 1–2 position, the pump draws solvent from the solvent bottle on top of the instrument or empties solvent to the waste beaker.
	1–6 position Directs the solvent flow toward the mixing Tee.
	Centered <ul style="list-style-type: none"> <li>Prevents backflow into the solvent line upstream of the mixing Tee.</li> <li>Allows the backpressure for the solvent A and B lines to equalize before the gradient step of a sample run.</li> <li>Enables pump B to pre-pressurise in the Prepare gradient step.</li> <li>Provides backpressure for several maintenance scripts.</li> </ul>
Valve S	Directs the solvent flow from syringe pump S and the solvent flow from the mixing Tee.
	1–2 position The solvent flow bypasses the sample loop.
	1–6 position The solvent flow passes through the sample loop.
	Centered <ul style="list-style-type: none"> <li>Provides backpressure for the Flush Air script for pump S.</li> <li>Provides backpressure for the system leak test.</li> <li>Shuts off the flow to the Column Out line and provides backpressure during the Flow Sensor Calibration script.</li> </ul>
Valve W	Directs the solvent flow through the venting Tee to the waste beaker or provides pressure venting to the system.
	1–2 position Provides backpressure to direct flow towards the analytical column (blind nut in port 2).
	1–6 position Directs the solvent flow from the venting Tee to the waste beaker.
	Centered Blocks the solvent flow through the valve to the waste beaker.

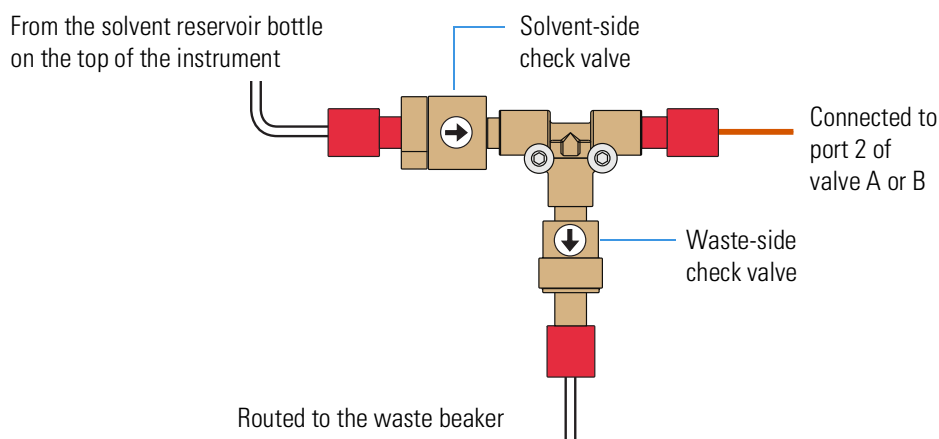
## Check Valve Assemblies

When the corresponding high-pressure (6-port rotary) valves for pumps A or B are in position 1–2, the check valves perform these functions:

- The solvent-side check valve prevents backflow into the associated solvent inlet line when the associated pump is emptying solvent to the waste beaker.
- The waste-side check valve prevents backflow from the waste beaker when the associated pump is filling and drawing solvent from the associated solvent bottle.

Figure 9 shows the check valve connections.

**Figure 9.** Check valve connections



## Mixing Tee

The mixing Tee mixes solvents A and B before the solvent stream reaches valve S. When connecting the solvent lines to the mixing Tee, connect the solvent A and solvent B lines to perpendicular ports of the mixing Tee.

## Predefined Steps for Sample Runs

During sample runs, the EASY-nLC instrument goes through a series of predefined steps.

- “[Overview of a Sample Run](#),” next section, provides a brief overview of the sample steps.
- “[Sequence of Events for Each Step of a Sample Run](#)” on [page 18](#) provides a more illustrative explanation of each step.

### Overview of a Sample Run

During sample runs, the active steps are highlighted in bold on the Home > Overview page (see [Figure 10](#)).

**Figure 10.** Current step area of the Home > Overview page

Pickup sample	Initialize system	Refill AB
Load sample	Equilibrate precolumn	
Prepare gradient	Equilibrate analytical column	
Gradient	Autosampler wash + refill S	

To minimize the cycle time, the instrument runs these steps in parallel:

- Refill pumps A, B, and S
- Column equilibration steps and Pickup Sample step
- Gradient step and Autosampler Wash + Refill S step

Table 5 summarizes the steps of a sample run.

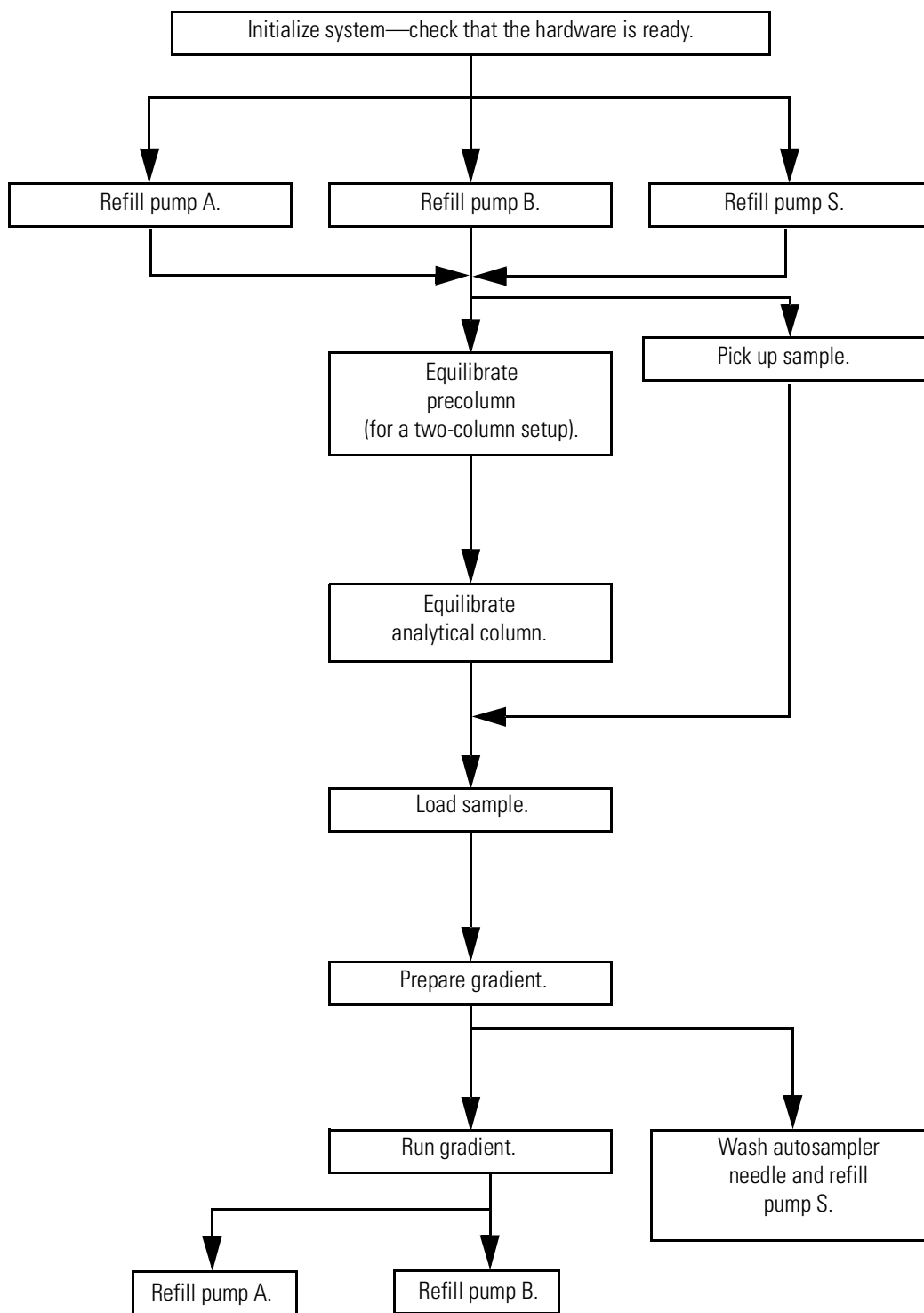
**Table 5.** Sample run steps

Step	Action
Refill A B	Pumps A and B draw solvent from the solvent bottles on top of the instrument. The instrument then zeros the pressure sensors. At beginning of a sample run, the pumps are checked for drawn air after refilling.
Precolumn and analytical column equilibration	Pump A delivers solvent to the system. For a two-column system, solvent A passes through the precolumn and then out to the waste beaker. After the precolumn equilibration finishes, valve W switches to position 1–2 to direct the solvent flow through the analytical column.
Pickup sample	The autosampler moves the needle to the sample position specified in the batch table (injection sequence). Then pump S aspirates sample from the specified vial or well position into the sample loop attached to valve S.
Load sample	With valve S in position 1–6, pump A delivers solvent to the system, flushing sample out of the sample loop and onto the precolumn or the analytical column. Valve W is in position 1–6 for a two-column system and position 1–2 for a one-column system.
Prepare gradient	The system vents through the Waste In line or builds up pressure to a precalculated pressure. Pump B builds up pressure to match the precalculated pressure of pump A. Pump B pumps a fixed volume of solvent B into the loop to ensure the alignment of solvents at the mixing Tee. Then valves S and W switch to position 1–2. With valves S and W in position 1–2, the solvent flow bypasses the sample loop and is directed through the column.
Gradient	Pumps A and B deliver solvent to the system. As the percent composition of solvent B increases, analytes elute off the column or columns.
Autosampler wash + refill S	Pump S draws solvent from one or more wash bottles, washes the autosampler needle and the sample loop, and then refills. At beginning of a sample run, the pump is checked for drawn air after refilling.



Figure 11 shows a flow chart of the sample run steps.

Figure 11. Flow chart of the sample run steps



### Sequence of Events for Each Step of a Sample Run

To understand what happens during a sample run, see the following step descriptions:

- “Refill Pumps A, B, and S Step,” next section
- “Pickup Sample Step” on page 20
- “Column Equilibration Steps” on page 22
- “Load Sample Step” on page 24
- “Prepare Gradient Step” on page 26
- “Gradient Step” on page 29
- “Autosampler Wash Step” on page 30

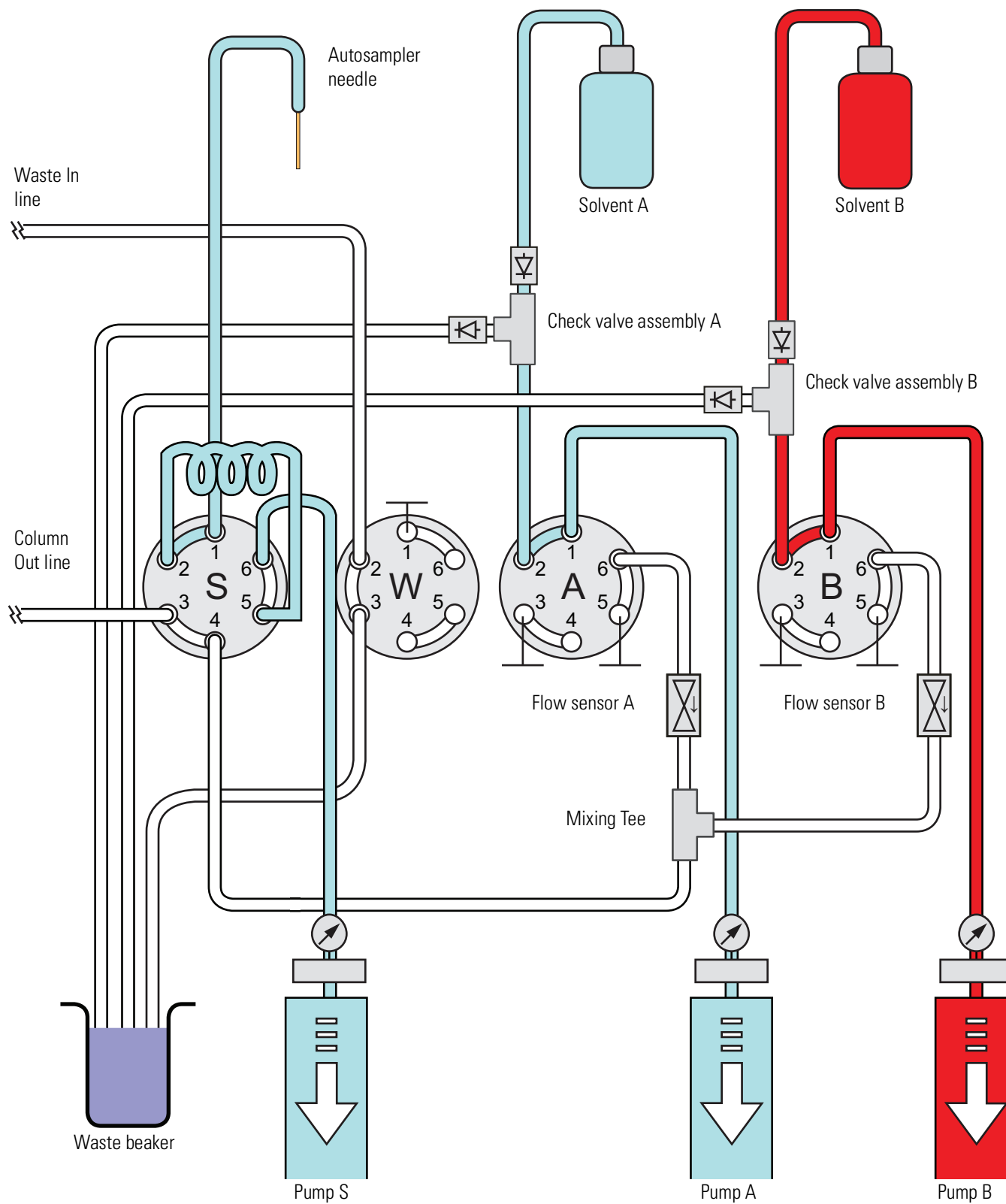
#### Refill Pumps A, B, and S Step

The instrument refills pumps A and B. With valves A and B in the 1–2 position, the corresponding check valves control the direction of the solvent flow (see [Figure 12](#)). When the pump begins to draw solvent from the solvent bottle, the corresponding check valve assembly opens to the solvent bottle and closes to the waste beaker. After a brief waiting period, the instrument zeros pressure sensors A and B.

As pumps A and B are refilling, the autosampler needle moves to the refill location (w3) and pump S draws solvent, if not already full. Once a pump is refilled, the pressure sensor of this pump is zeroed and a check for trapped air inside this pump is performed. During this check the respective valve moves to CENTER position.

During the air check of pump S, the autosampler needle moves to the W4 location.

**Figure 12.** Valves A and B in position 1–2 and pumps A and B in fully retracted position



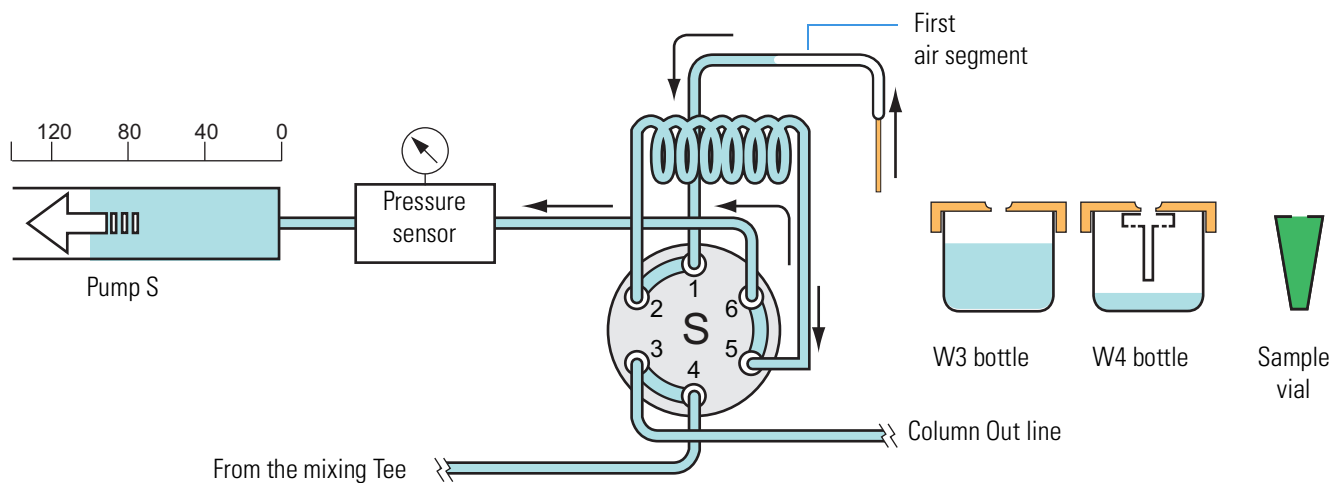
## Pickup Sample Step

The Pickup Sample step includes this sequence of actions.

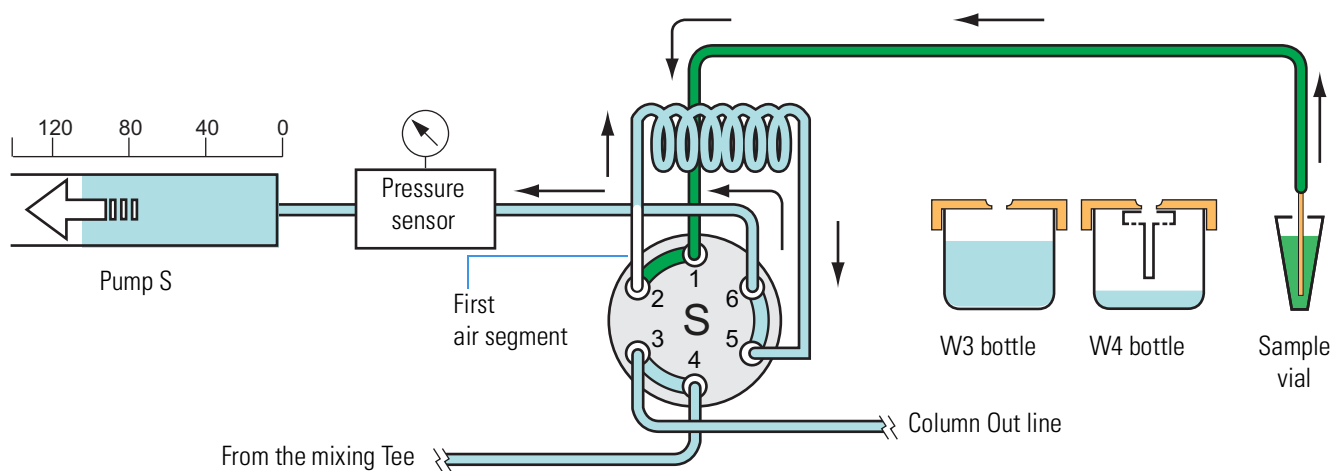
Action	
1	Pump S moves to a fixed position (filled with approximately 100 $\mu\text{L}$ of solvent for an instrument with a 20 $\mu\text{L}$ sample loop) by either dispensing excess solvent to the W4 bottle or drawing solvent from the W3 bottle.
2	Pump S draws 1 $\mu\text{L}$ of air (the first air segment) into the needle.
3	The XYZ robot moves the needle holder to the specified sample vial or microplate well location.
4	The needle descends into the sample solution.
5	Pump S draws the requested sample pickup volume into the needle.  <b>Note</b> The EASY-nLC instrument performs only partial loop injections. To avoid drawing sample into pump S, the application prevents you from entering a sample pickup volume larger than the sample loop size minus two microliters. The extra two microliters are required for the two air segments that bracket the sample.
6	The XYZ robot raises the needle out of the sample solution.
7	To transfer the sample into the loop, pump S draws a fixed volume of air into the needle. This fixed volume of air ( $\sim 10.25 \mu\text{L}$ ) is equal to the needle volume plus the valve port-to-port volume plus an additional 1 $\mu\text{L}$ of air (the second air segment) to bracket the sample solution inside the loop.

Figure 13 shows pump S drawing the first air segment into the needle. Figure 14 shows pump S drawing the first air segment into the sample loop, while drawing the requested sample volume into the needle. Figure 15 shows pump S drawing the second air segment through the needle and into the sample loop. At the end of the Pickup Sample step, the sample loop holds the requested sample volume of 10  $\mu\text{L}$ , two 1  $\mu\text{L}$  air segments, and approximately 8  $\mu\text{L}$  of solvent A.

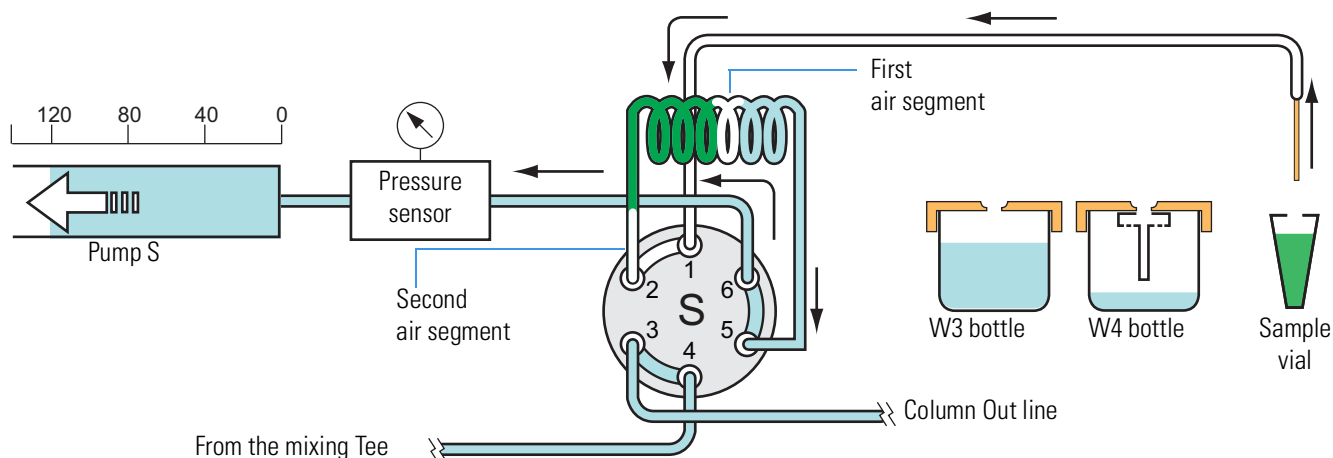
**Figure 13.** Pickup Sample step—with pump S drawing the first air segment into the needle



**Figure 14.** Pickup Sample step—with pump S drawing 10  $\mu\text{L}$  of sample from a sample vial



**Figure 15.** Pickup Sample step—with the requested sample volume loaded into the loop and bracketed by two air segments



## Column Equilibration Steps

For a one-column setup, the sample run includes the analytical column equilibration step. For a two-column setup, the sample run includes both a precolumn equilibration step and an analytical column equilibration step.

The Equilibrate Precolumn step (see [Figure 16](#)) includes this sequence of actions.

**Action**

- |   |   |
|---|---|
| 1 | Valve A and valve W switch to position 1–6. Valve B switches to the centered position. Valve S switches to position 1–2.  |
| 2 | Pump A pushes the specified solvent volume through the precolumn at the specified pressure or flow rate. Excess solvent A exits the venting Tee and passes through valve W to the waste beaker. |

The analytical column equilibration step includes this sequence of actions.

**Action**

- |   |  |
|---|--|
| 1 | <p>For a two-column setup, valve A remains in position 1–6, valve B remains in the centered position, and valve S remains in position 1–2. Valve W switches to position 1–2.</p> <p>For a one-column setup, valve A switches to position 1–6. Valve B switches to the centered position. Valve S switches to position 1–2. Valve W switches to position 1–2.</p> |
| 2 | Pump A pushes the specified solvent volume through the analytical column at the specified pressure or specified flow rate.   |



## Load Sample Step

After pump S draws the sample into the sample loop (see “Pickup Sample Step” on page 20), valve S switches to position 1–6 and pump A pushes solvent A through the sample loop, flushing the sample out of the sample loop into the Column Out line and onto the precolumn for a two-column setup or the analytical column for a one-column setup.

The Load Sample step includes this sequence of actions.

Action	
1	Valve S switches to position 1–6.  Valve W does one of the following: <ul style="list-style-type: none"><li>• Switches to position 1–6 for a two-column setup allowing excess solvent A to flow to the waste beaker.</li><li>• Remains in the 1–2 position for a one-column setup, directing the solvent flow through the analytical column.</li></ul>
2	Pump A pushes the specified solvent volume through the sample loop at a specified pressure or the specified flow rate. In a two-column system, solvent A vents to the waste beaker through valve W.

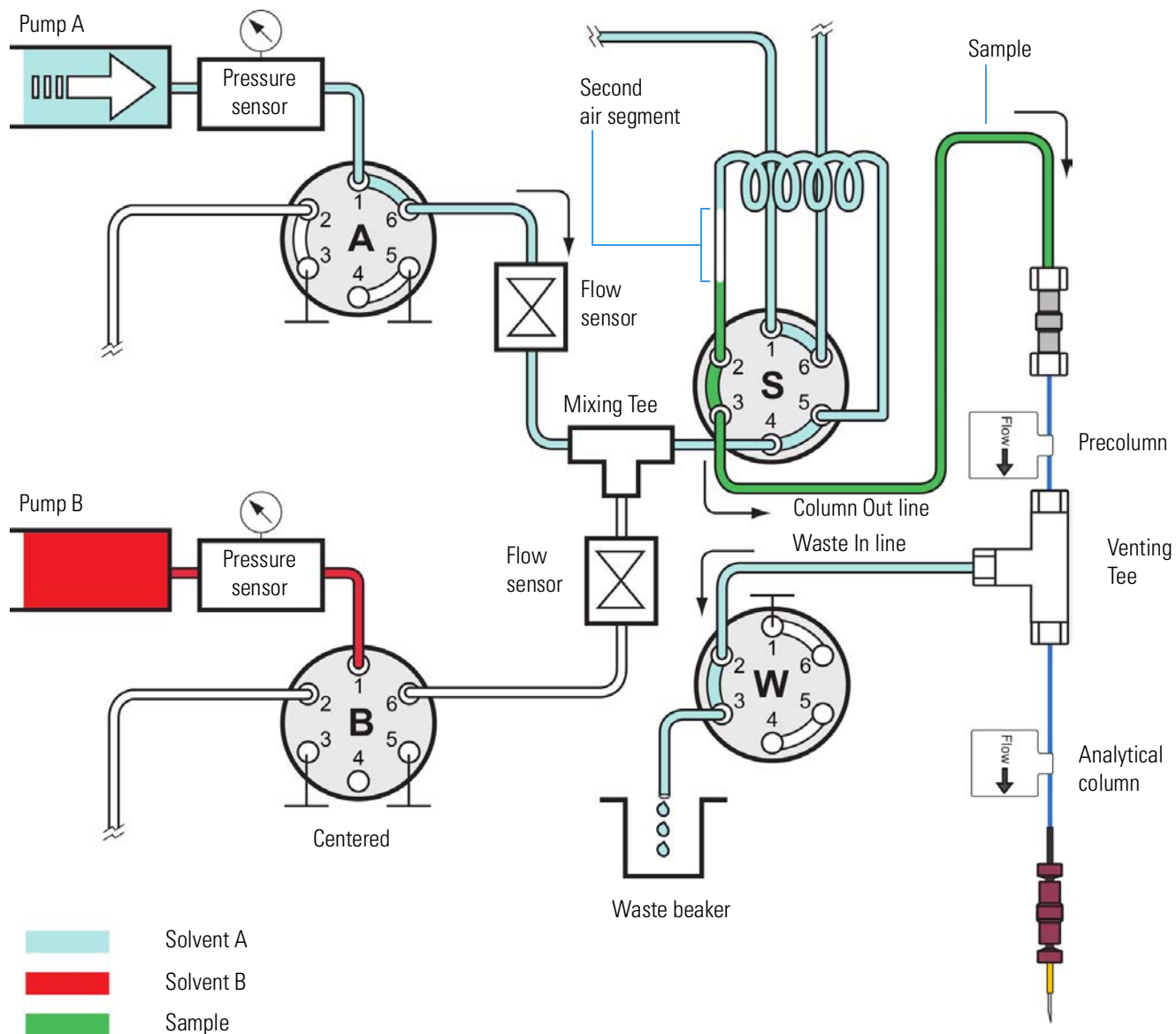





Figure 17 shows the Load Sample step of a sample run for a two-column setup. Valves A, S, and W are in the 1–6 position. Valve B is in the centered position.



**Figure 17.** Load Sample step (two-column setup)



## Prepare Gradient Step

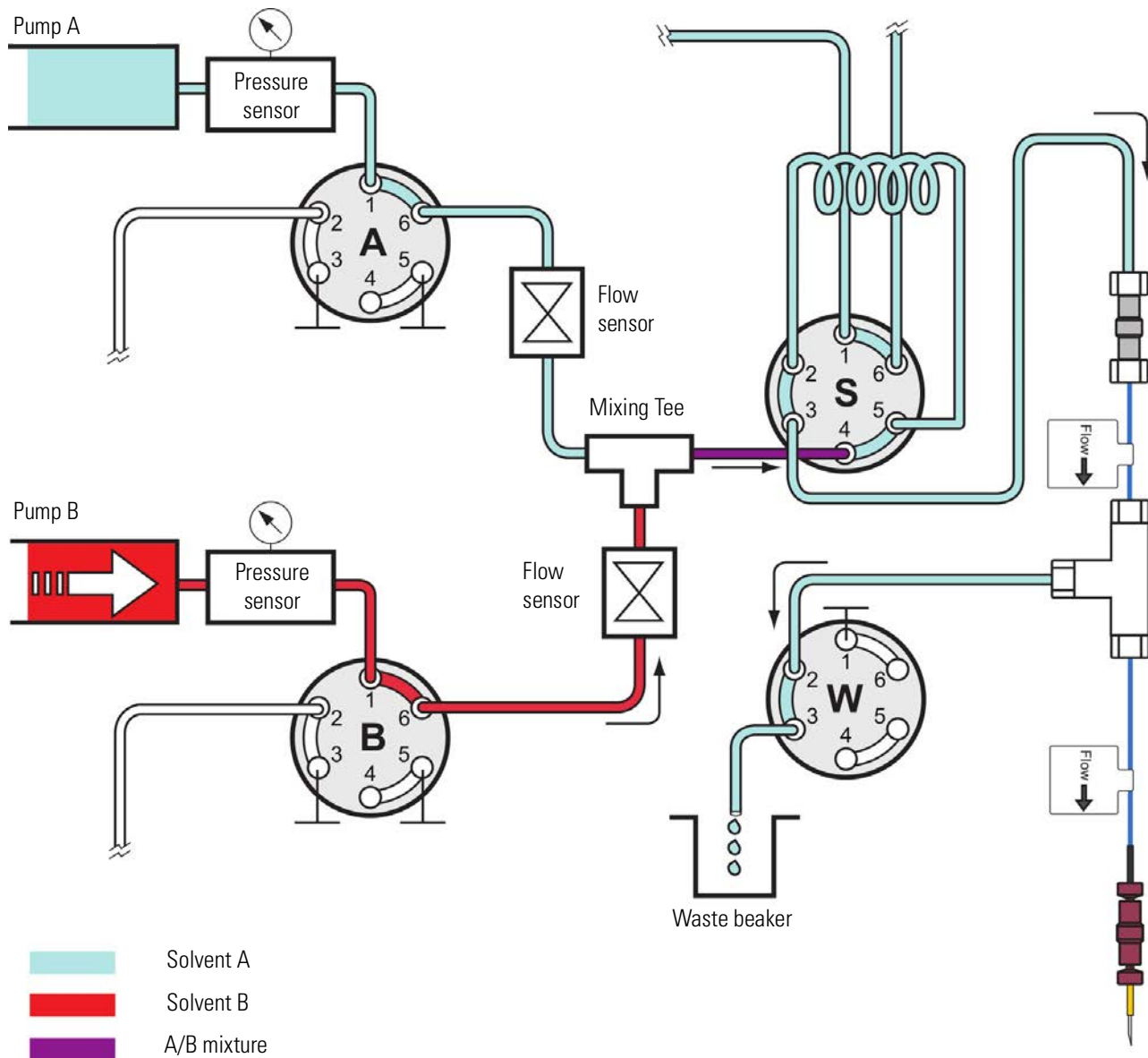
Before the gradient begins, this sequence of actions occurs.

Action	
1	The built-in instrument control software calculates the volume of solvent A required to complete the gradient program. Pump A refills if it does not contain a sufficient volume of solvent to complete the gradient program.
2	With valves A, W, and S in position 1–6 and valve B in the centered position, the system vents through the Waste In line to a precalculated pressure, or increases the pressure to the precalculated pressure with valve W in position 1–2.  Pump B builds up pressure to match the precalculated pressure of pump A.
3	Valve B switches to position 1–6. If valve W was in position 1–2 in the previous step, it also switches to position 1–6. The Intelligent Flow Control (IFC™) system now controls pumps A and B at the precalculated pressure.  With all of the valves in position 1–6, pump B pumps a fixed volume of 0.5 µL of solvent B toward valve S to ensure correct alignment of solvents A and B up to valve S. See <a href="#">Figure 18</a> .
4	Valve W switches to position 1–2. The Automatic Flow Control (AFC™) system now controls pumps A and B.  To align solvents A and B up to valve S, the system delivers a gradient consisting of a minimum of 2% solvent B or the gradient start solvent B%, whichever is higher, until it pumps 0.4 µL of solvent A into the sample loop (see <a href="#">Figure 19</a> ).
5	Valve S moves to position 1–2, which allows the mobile phase to bypass the sample loop, and the instrument sends the closure signal to the mass spectrometer.

**Note** During the Prepare Gradient step, a slight amount of solvent B enters the sample loop. This does not affect the next run because the Autosampler Wash step flushes the sample loop before the next run starts.

[Figure 18](#) shows 0.5 µL of solvent B being pumped toward valve S during the Prepare Gradient step. [Figure 19](#) on [page 28](#) shows 0.4 µL of solvent A being pumped toward valve S during the Prepare Gradient step.

**Figure 18.** Excess 0.5  $\mu\text{L}$  of solvent B pumped toward valve S during the Prealign Gradient step





## Gradient Step

The gradient step includes this sequence of actions.

### Action

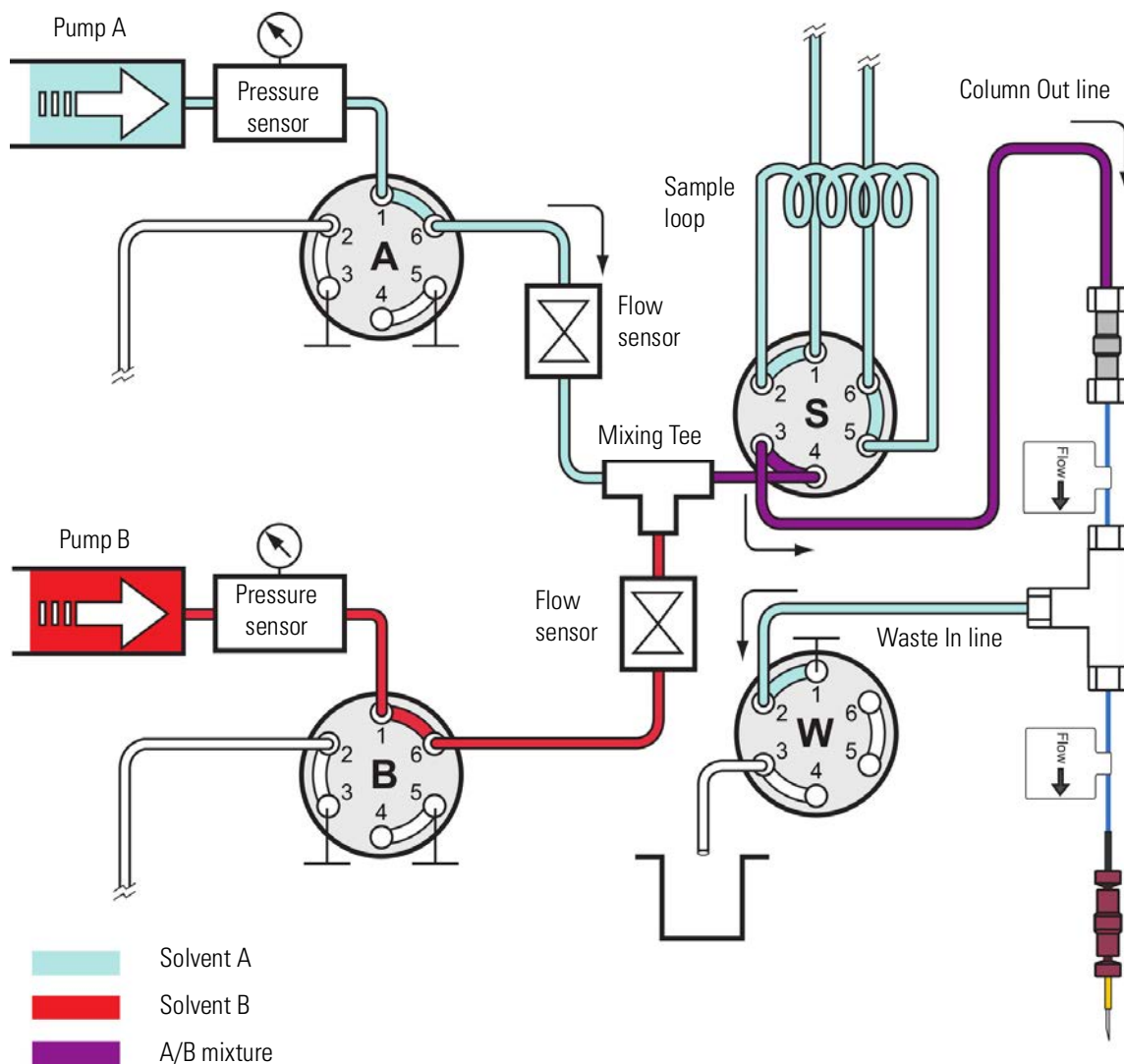
- 1 The instrument runs the gradient program.

**Note** With valve S in position 1–2, the mobile phase bypasses the sample loop during the gradient program. Therefore, the sample loop does not add any gradient delay volume to the system.

- 2 If either pump runs out of solvent during the gradient run, both pumps refill. The refilling process takes approximately 2 minutes.

Figure 20 shows the gradient step of a sample run. Mobile phase from the mixing Tee bypasses the sample loop as it enters and exits valve S through ports 4 and 3, respectively.

**Figure 20.** Gradient step with valve A and valve B in position 1–6 and autosampler wash step with valve S in position 1–2



## 1 Introduction

Predefined Steps for Sample Runs

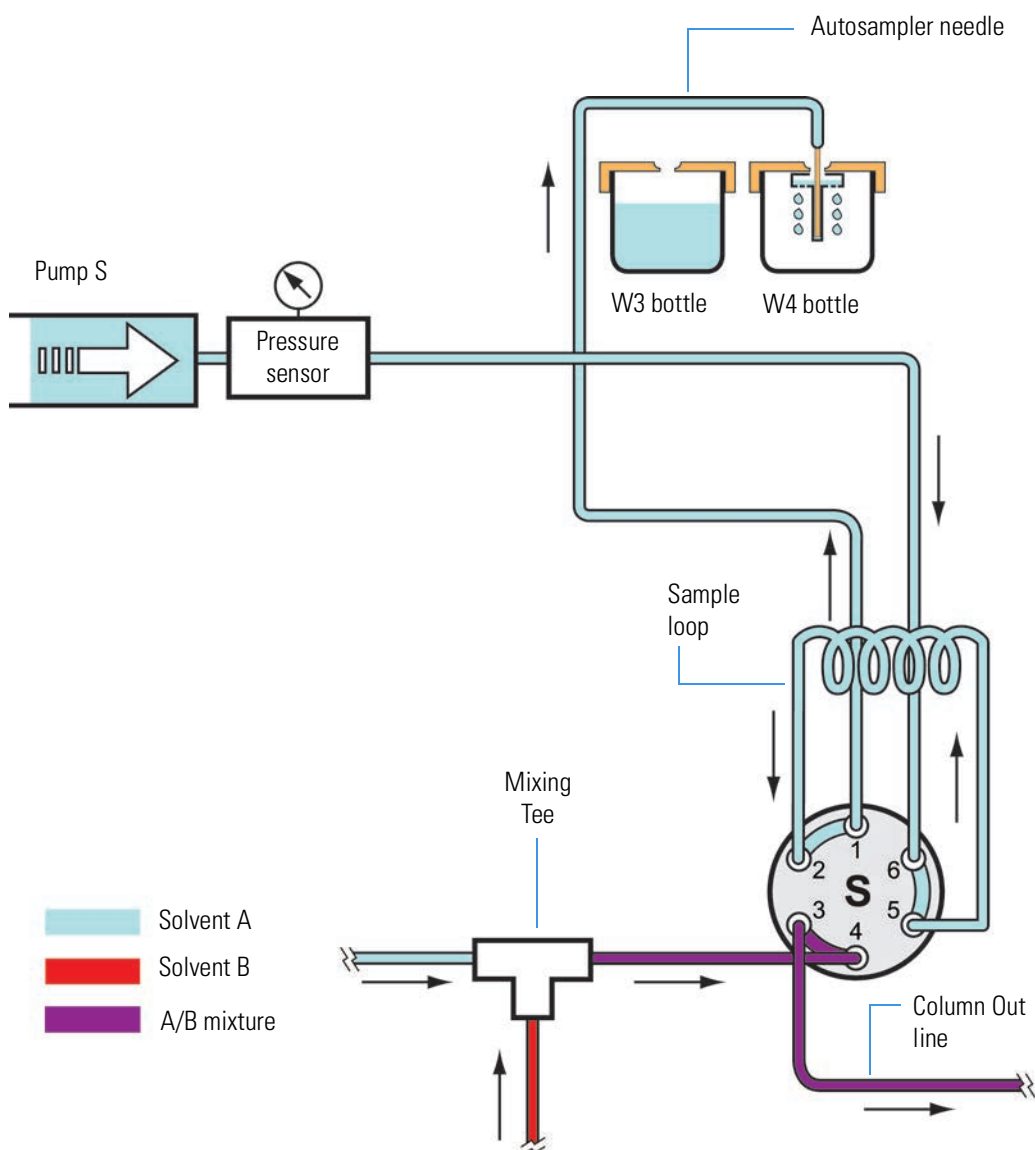
### Autosampler Wash Step

The standard autosampler wash step includes this sequence of actions.

Action	
1	The autosampler needle moves to the W4 bottle.
2	Pump S pushes the specified volume of solvent through the sample loop and needle. As the wash solvent exits the needle, it overfills the needle insert and washes the exterior surface of the needle. See <a href="#">Figure 21</a> .
3	Pump S refills with solvent A from the W3 bottle.

The Autosampler Wash and Refill S step occurs in parallel with the Gradient step.

**Figure 21.** Autosampler wash step with valve S in position 1–2



## Pump Flow Control

The EASY-nLC instrument contains split-free, high-pressure syringe pumps capable of delivering flows from 1 nL/min to 300  $\mu$ L/min.

These flow control systems optimize instrument performance:

- “Automatic Flow Control System,” next section
- “Intelligent Flow Control System” on page 32
- “Load Speed Protection System” on page 33

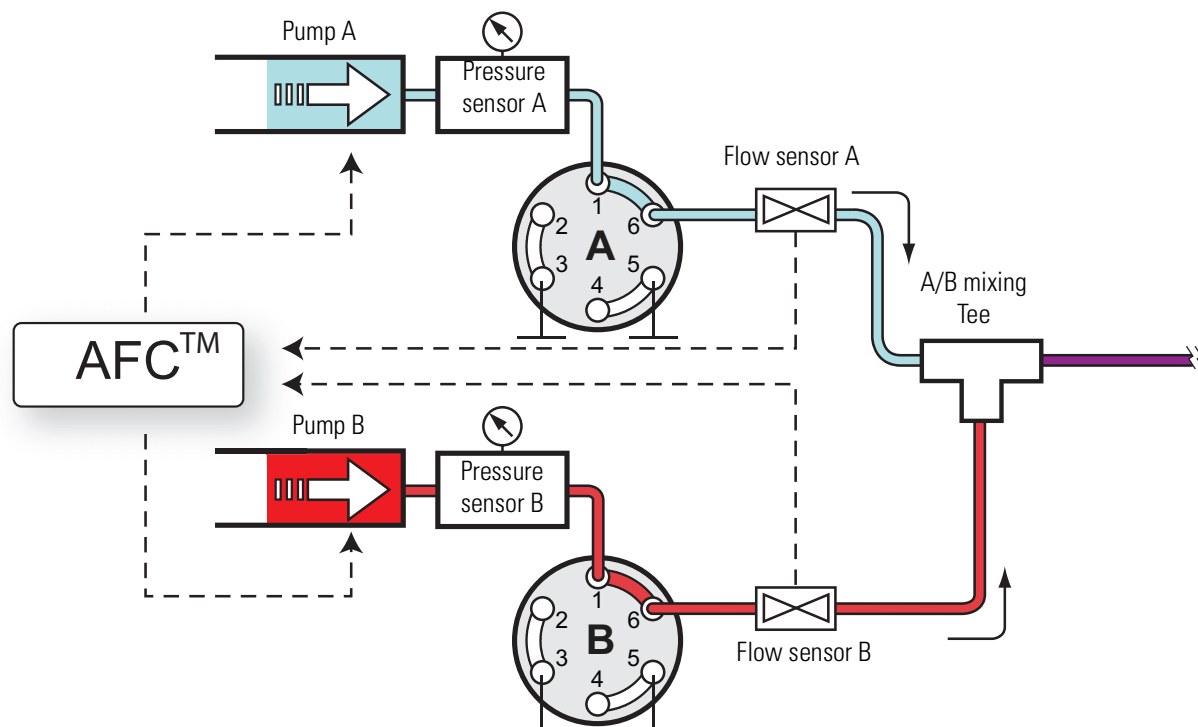
### Automatic Flow Control System

The AFC system (see Figure 22) is active during these processes:

- The gradient step of a sample run
- The second part of the prepare gradient step
- The execution of the isocratic flow maintenance script

By using the flow sensor feedback to regulate pumps A and B, the AFC system accurately maintains the programmed flow rate and solvent composition, even during sudden pressure changes caused by solvent composition changes. For accuracy, the AFC system requires a minimum pressure of 20 bar.

**Figure 22.** Schematic diagram of the AFC system



## Intelligent Flow Control System

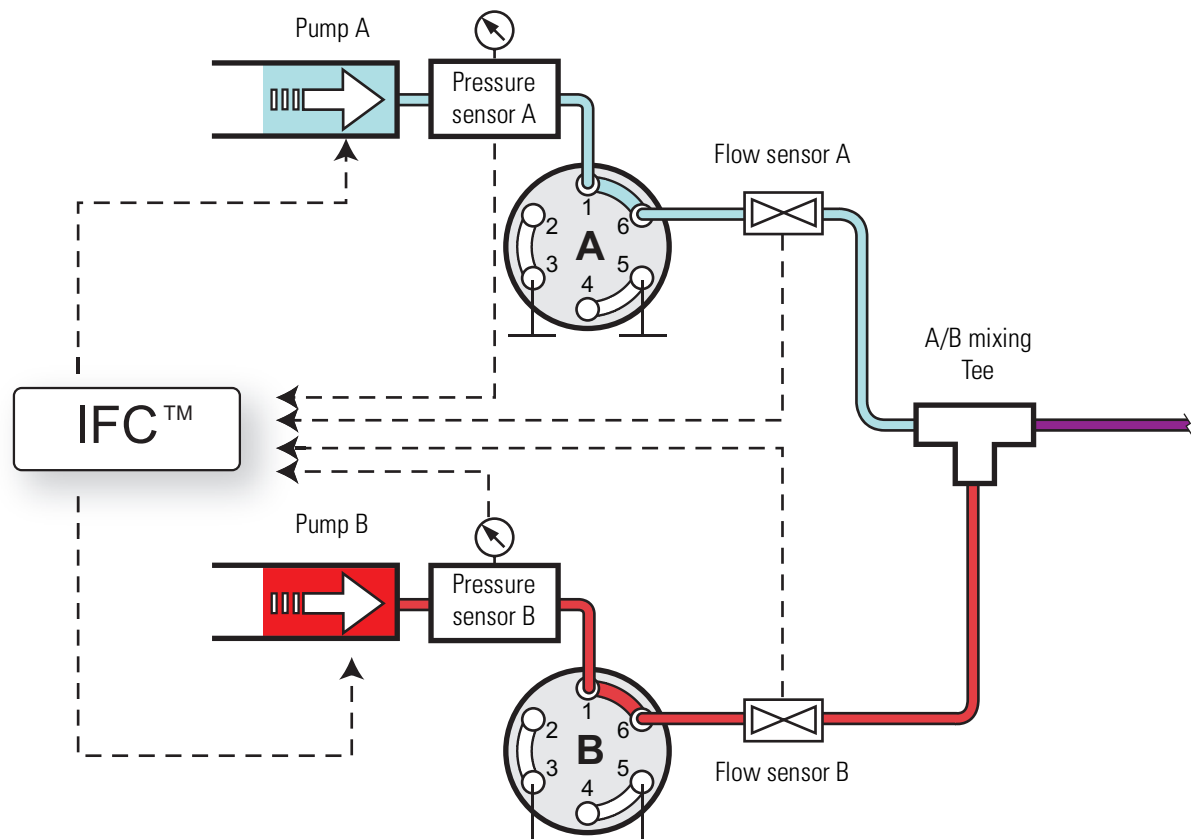
The IFC system (see [Figure 23](#)) is active during these processes:

- The column equilibration and sample loading steps of a sample run
- The first part of the prepare gradient step for pumps A and B
- The execution of the precolumn equilibration, analytical column equilibration, leak, and backpressure scripts

By using both the pressure sensor and flow sensor feedback, the IFC system continually regulates pumps A and B. By regulating the pumps A and B with both a flow and pressure feedback system, the IFC system enhances system performance as follows:

- Reduces the loss of productivity caused by instrument stoppage related to overpressure (for instance, stoppage caused by viscous samples during the sample loading step).
- Uses the pressure range of the instrument more effectively. For example, when you set a maximum pressure for the sample loading and column equilibration steps instead of a flow rate, the system automatically adjusts the flow rate to the maximum acceptable value.
- Builds the pressure rapidly during the prepare gradient step.

**Figure 23.** Schematic diagram of the IFC system





You can program the column equilibration and sample loading steps as follows:

- Specify both a set flow and a maximum pressure. The setting reached first (which becomes the limiting parameter) depends on the dimensions of the attached column or columns. If the system reaches the set pressure before it reaches the set flow rate, the IFC system reduces the flow rate to maintain the pressure. If the system reaches the set flow rate before the pressure limit, the pump maintains the set flow rate.
- Specify only a set flow. The IFC system maintains the set flow rate unless the pressure exceeds the maximum system pressure.
- Specify only a maximum pressure. The IFC system adjusts the flow rate to maintain the set pressure.

The duration of both the equilibration and loading steps depends on the total volume of solvent specified and the actual flow rate. When the set pressure is the limiting factor, the system adjusts the actual flow rate, which varies the duration of these steps.

**Tip** If the duration of the equilibration and loading steps must be fixed, limit these steps by flow rather than by pressure. Use the appropriate precolumn and analytical column maintenance scripts to determine the appropriate flow rates for your LC columns.

## Load Speed Protection System

When the Load Speed Protection system (LSP) is on, the batch run stops if the system reaches the set max pressure before it reaches the set flow during the sample loading step. Stopping the batch run when the system pressure rises above an acceptable level prevents the system from running the remaining samples when the column or tubing has become clogged.

For instructions about how to turn on the Load Speed Protection system, see [“Setting Up the Properties for the HPLC Device”](#) on page 83.

## **1 Introduction**

Pump Flow Control

## Installing the EASY-nLC Instrument

To install the EASY-nLC instrument or move the instrument from one laboratory benchtop to another, follow the installation instructions in this chapter after reviewing the “[Cautions and Special Notices](#)” on [page xiii](#).

**Note** This chapter describes the laboratory requirements and the back panel connections for the EASY-nLC instrument as a stand-alone LC system or as an inlet to a mass spectrometer.

- For information about installing the LC columns, the sample plates, the solvent bottles, and the wash bottles, see [Chapter 5, “Preparing the EASY-nLC Instrument for Use.”](#)
- For information about connecting the column assembly to the mass spectrometer’s ion source, refer to the manual provided with the ion source.
- For information about the internal solvent lines, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

### Contents

- [Lifting Instructions](#)
- [Laboratory Requirements](#)
- [Back Panel Connections](#)

## Lifting Instructions

The EASY-nLC 1200 instrument weighs 35 kg (77.2 lb). For safety reasons, use two people to move the instrument to a table cart for transport and wear gloves.



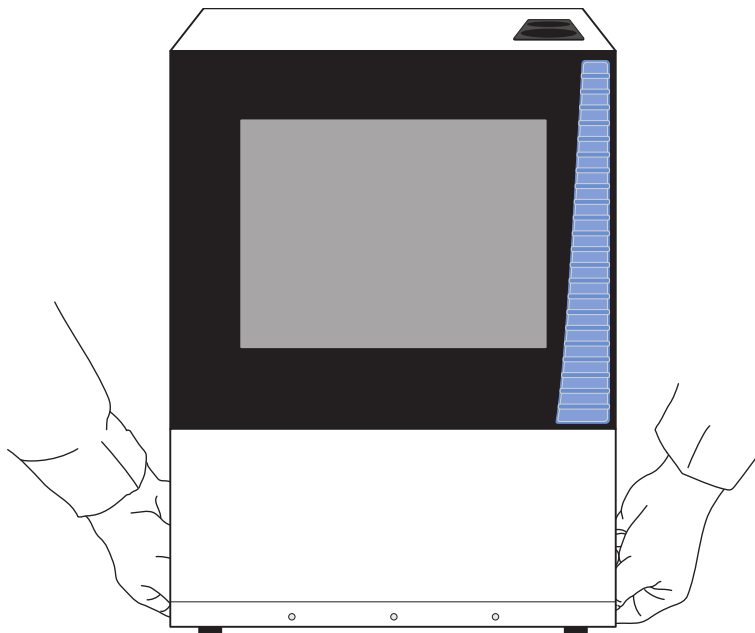
**CAUTION** At more than 30 kg (66 lbs), the EASY-nLC instrument is too heavy for one person alone to lift. When moving the instrument, use two people to place it on a table cart for transport.



**CAUTION** Before you move the EASY-nLC instrument from one benchtop to another, close down the EASY-nLC instrument (see “[Closing Down the EASY-nLC Instrument](#)” on [page 69](#)), disconnect the EASY-nLC instrument from line power, the detector, and the data system computer (if applicable). For information about installing or removing the column assembly from the mass spectrometer’s ion source, refer to the manual provided with the ion source.

[Figure 24](#) shows the lift points for the EASY-nLC instrument. With one person on each side of the instrument, lift the instrument from the bottom with one hand while stabilizing the top portion of the instrument with the other hand.

**Figure 24.** Lifting the EASY-nLC 1200 instrument by hand



## Laboratory Requirements

This section describes the laboratory requirements for the EASY-nLC instrument. For more information about the requirements for the EASY-nLC instrument, refer to the *EASY-nLC Series Preinstallation Requirements Guide*. For information about the preinstallation requirements for a Thermo Scientific mass spectrometer, refer to its preinstallation requirements guide.

Requirements:

- “[Benchtop Dimensions and Weight Capacity](#),” next section
- “[Internet Access](#)” on [page 39](#)
- “[Power and Fuses](#)” on [page 39](#)
- “[Temperature and Humidity](#)” on [page 39](#)

## Benchtop Dimensions and Weight Capacity

Place the EASY-nLC instrument on a benchtop or movable table that can support a minimum of two times the instrument weight. For the EASY-nLC instrument, make sure that the table can support a minimum of 70 kg (154 lb).

[Table 6](#) lists the dimensions of the EASY-nLC 1200 instruments. Allow for at least 15 cm (6 in.) of free space at the back of the instrument for proper air circulation.

**Table 6.** EASY-nLC instrument dimensions

Dimension	EASY-nLC 1200
Width	36 cm (14.2 in.)
Depth	38 cm (15.1 in.)
Height	45 cm (17.7 in.)

To minimize extra column volume between the LC column outlet and the detector inlet, place the EASY-nLC instrument as close as possible to the high-performance liquid chromatography (HPLC) detector, mass spectrometer, or both. However, to avoid exposure to direct heat, place the EASY-nLC instrument away from any detector or mass spectrometer vents.

[Figure 25](#) shows a typical benchtop setup for an EASY-nLC instrument connected to a Thermo Scientific mass spectrometer. Connecting the data system computer to your local area network (intranet) requires an additional network card.

Using the setup shown in [Figure 25](#), you can control the EASY-nLC instrument from the local data system computer or from another data system computer on the intranet; however, the EASY-nLC instrument might not be able to communicate with the remote support server. For information about connecting the EASY-nLC instrument to the remote support server, see “[Setting Up the Ethernet Connection to the Support Server](#)” on [page 47](#).

## 2 Installing the EASY-nLC Instrument

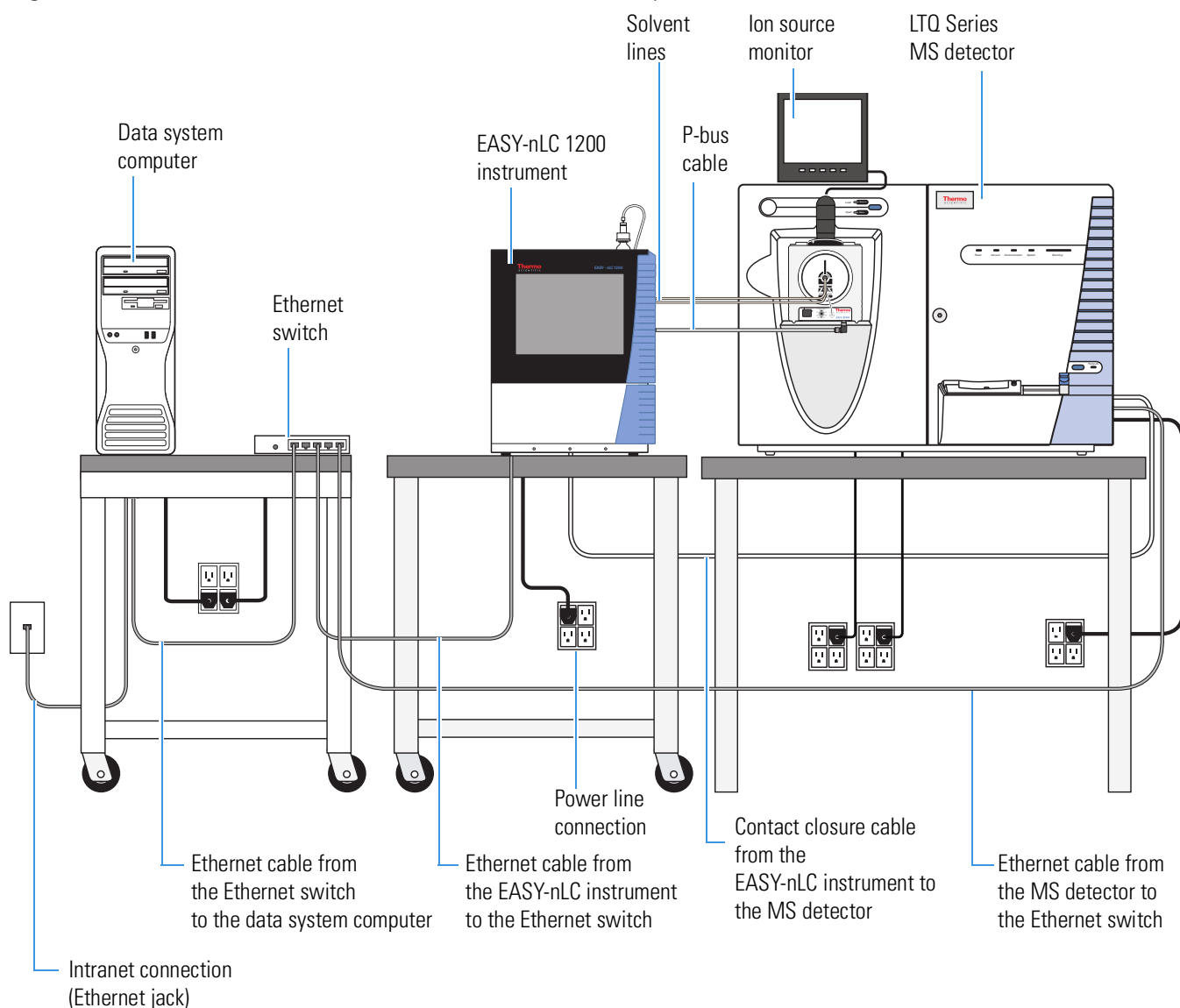
### Laboratory Requirements



**CAUTION** Before you move the EASY-nLC instrument from one benchtop to another, close down the EASY-nLC instrument (see “Closing Down the EASY-nLC Instrument” on page 69), disconnect the EASY-nLC instrument from line power, the detector, and the data system computer (if applicable). For information about installing or removing the column assembly from the mass spectrometer’s ion source, refer to the manual provided with the ion source.

**Note** Figure 25 shows the EASY-Spray source, which gets its power through the P-bus cable connection to the EASY-nLC instrument.

**Figure 25.** EASY-nLC instrument and LTQ™ Series MS detector setup



## Internet Access

For online support and monitoring (remote support), provide Internet access for the EASY-nLC instrument within reach of the laboratory benchtop.

**IMPORTANT** The EASY-nLC instrument uses Secure Shell (SSH™), a network protocol for secure data communication, to connect to the support server, which uses the Linux™ operating system. Port 22 is the standard TCP port for SSH programs.

To use the remote support feature, the firewalls for your local network must allow outgoing TCP/IP traffic from the EASY-nLC instrument to the support server at IP address 46.226.219.10, port 22.

## Power and Fuses

Use the EASY-nLC instrument only with properly grounded appliances and power sources.

You can plug the EASY-nLC instrument into these grounded line power sources:

- 120 Vac, 50/60 Hz, 250 W
- 230 Vac, 50/60 Hz, 250 W

**Note** To determine the appropriate rating for an uninterruptible power supply (UPS), assume it is 250 W.

The fuse requirements for the EASY-nLC instrument are as follows:

- For 120 Vac, one T 5 AL 250 V fuse (5 × 20 mm, IEC 60127)
- For 230 Vac, one T 2.5 AL 250 V fuse

All fuses supplied with the instrument are UL Listed and CSA certified.

**Note** Depending on the nanospray ion source, your nanospray system might need an additional power receptacle. The EASY-Spray ion source requires an additional power receptacle, whereas the Nanospray Flex ion source draws its line power from the mass spectrometer.

## Temperature and Humidity

Avoid locations with high air humidity or extreme changes in temperature (such as direct sunlight, drafts, directly below an air conditioning or heating vent, or directly next to the mass spectrometer or HPLC detector vent).

For optimal autosampler plate cooling, place the unit in an area where the working temperature is within 5 to 30 °C (41 to 86 °F).

The optimal humidity range is between 20 to 80% RH. Avoid condensing humidity.

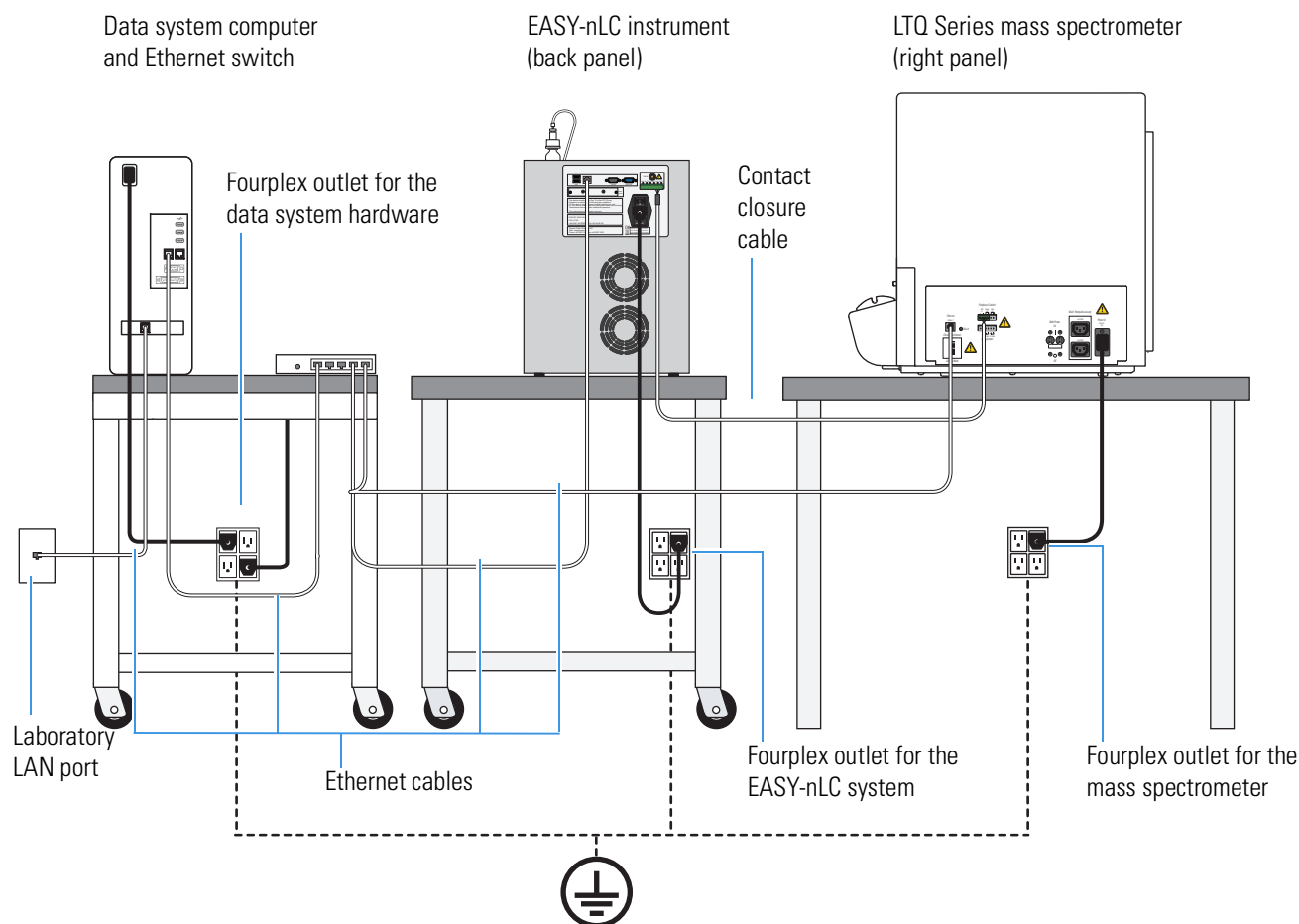
## Back Panel Connections

To connect the EASY-nLC instrument to a mass spectrometer, the intranet within your facility, the Internet for remote support, and other peripheral hardware, follow these procedures:

- “Connecting to Line Power” on page 41
- “Connecting to the Mass Spectrometer through Contact Closure” on page 42
- “Connecting the Ethernet Communication Cables” on page 44
- “Attaching a Mouse and Keyboard to the USB Connections” on page 48
- “Attaching Add-on Devices through the P-Bus and RS-232 Ports” on page 48

Figure 26 shows an LC/MS system with an EASY-nLC instrument, an LTQ Series mass spectrometer, and a data system computer. The LC system, mass spectrometer, and data system hardware are connected to three separate fourplex outlets that are hardwired to a common protective earth ground. This figure does not show the monitoring system for the nanoflow ion source or the external rotary pumps for the mass spectrometer.

**Figure 26.** LC/MS system with an EASY-nLC instrument, an LTQ Series mass spectrometer, and a data system computer





## Connecting to Line Power

Each EASY-nLC instrument comes with a 2.5 m (8.2 ft) long power cord.



**CAUTION** Power cords can become frayed and damaged with use. If the power cord is frayed or damaged, replace it with an equivalent power cord.

To meet compliance and safety requirements, the replacement power cord must be no longer than 2.5 m (8.2 ft) and be certified by recognized organizations for your country (for example, UL, CSA, SEMKO, VDE, or TÜV).

Installing an EASY-nLC instrument requires a minimum of one outlet. The electrical outlet must be earth ground hard-wired to the main panel.

If you are using the EASY-nLC instrument as an inlet to an MS detector, the LC/MS system might require two or more fourplex outlets. The interconnected electrical outlets for the LC/MS system and the data system computer must have a common point to one ground connector (see [Figure 26](#) on [page 40](#)).



**CAUTION** When using the EASY-nLC instrument as an inlet to an LC/MS system, the EASY-nLC instrument, the mass spectrometer, the nanospray ion source (if it connects to line power) and the (optional) data system hardware must have a common ground.

Connecting the hardware to external grounds at different potentials can do the following:

- Create a ground loop that causes noise and interference.
- Damage the EASY-nLC instrument's built-in computer.



**CAUTION** Improper grounding of the instrument creates an electrical safety hazard.

### ❖ To connect the EASY-nLC instrument to line power

1. If you are moving an existing instrument from one benchtop to another, make sure that the fuse is installed correctly.

For information about installing the fuse, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

2. Connect the supplied power cord to the power entry module on the back panel of the EASY-nLC instrument and to a properly grounded electrical outlet.

## Connecting to the Mass Spectrometer through Contact Closure

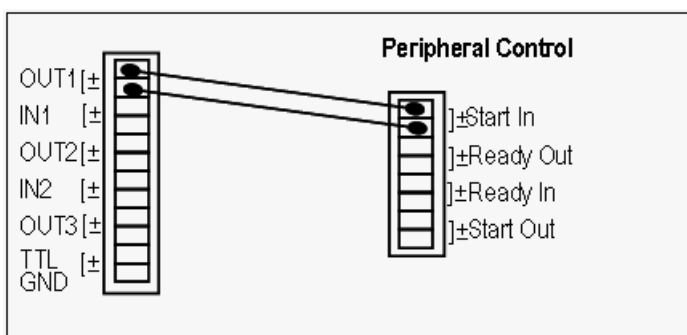
The EASY-nLC touch-screen application contains preconfigured values for most of the standard mass spectrometers used in proteomics. For instructions about setting up the software configuration for the mass spectrometer connection, see “[Setting Up Communication with the Mass Spectrometer](#)” on page 71.

**IMPORTANT** Occasionally, Thermo Fisher Scientific releases new software for the EASY-nLC instrument. After downloading and installing new software on the EASY-nLC, you must reselect the MS in the Configuration menu.

Figure 27 and Figure 28 show the hardwire connections between the EASY-nLC instrument and an LTQ Series mass spectrometer or Orbitrap Fusion™ mass spectrometer, respectively.

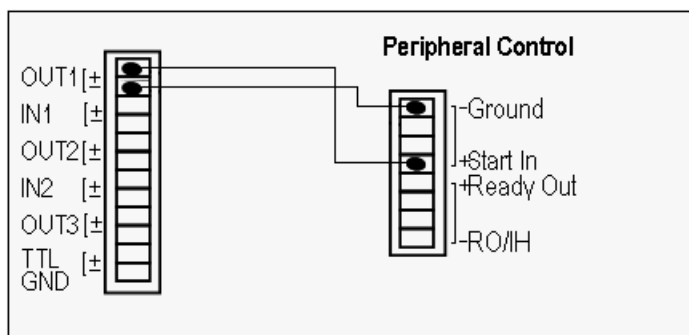
**Figure 27.** Two-way contact closure connection for an LTQ Series mass spectrometer

EASY-nLC 12-pin connector      Mass spectrometer  
input and output pins



**Figure 28.** Two-way contact closure connection for an Orbitrap Fusion mass spectrometer

EASY-nLC 12-pin connector      Mass spectrometer  
input and output pins



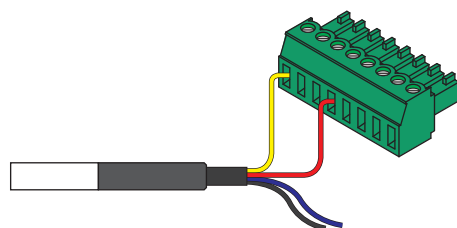
To make contact closure between the EASY-nLC instrument and the Orbitrap Fusion mass spectrometer, you must modify the Thermo Fisher contact closure cable by replacing one of the two-pin terminal blocks with the 8-pin terminal block provided in the MS Setup Kit.

❖ **To modify the contact closure cable for the Orbitrap Fusion mass spectrometer**

1. Using a small flat-blade screwdriver, loosen the screws for pins 1 and 2. Then pull the wires out of the terminals.
2. Using a small flat-blade screwdriver, connect the cable wires to the 8-pin terminal block provided in the Orbitrap Fusion MS Setup Kit as follows:
  - Connect the yellow wire to terminal 1.
  - Connect the red wire to terminal 4.

Figure 29 shows the pin outs for the modified MS connector.

**Figure 29.** 8-pin terminal block with wires connected



❖ **To connect the contact closure cable**

Using a contact closure cable provided by Thermo Fisher Scientific (see [Table 7](#)), connect the cable to the mass spectrometer and the EASY-nLC instrument.

For more information, refer to the graphic for the selected mass spectrometer in the Contact Closure area of the Configuration > Connections page.

**Table 7.** Contact closure cables

Description	Part number
Thermo Fisher contact closure cable	LC160
ABI/MDS Sciex™ contact closure cable	LC161
Bruker™/Agilent™ contact closure cable	LC162
Waters™/Micromass™ contact closure cable	LC163
Varian™ contact closure cable (Varian is now a part of Agilent Technologies Inc.)	LC164

## Connecting the Ethernet Communication Cables

How you set up the Ethernet communication cables depends on the type of communication you are trying to establish.

Follow the appropriate procedure to set up communication between the EASY-nLC computer and the data system computer, the intranet for data exchange, or the remote support server.

- [Setting Up the Ethernet Connections to the Data System Computer](#)
- [Setting Up the Ethernet Connection to a Local Network for Data Exchange](#)
- [Setting Up the Ethernet Connection to the Support Server](#)

### Setting Up the Ethernet Connections to the Data System Computer

For an LC/MS system with an EASY-nLC instrument and a Thermo Scientific mass spectrometer, you operate the system from a data system computer with the Xcalibur data system and the device drivers for the EASY-nLC instrument and a Thermo Scientific mass spectrometer.

**Note** You can create instrument methods and injection sequences, submit and process injection sequences, and monitor the instrument status and injection progress with the external data system. The external data system does not include maintenance scripts or direct controls for the EASY-nLC instrument. To perform tasks such as flushing air from the LC system, you must use the touch-screen application.

[Figure 25 on page 38](#) and [Figure 26 on page 40](#) show a typical benchtop setup for an LC/MS system with an EASY-nLC instrument, a Thermo Scientific mass spectrometer, a data system computer, and an Ethernet switch.

The EASY-nLC computer and the Thermo Scientific mass spectrometer communicate with the data system computer through an Ethernet connection routed through an Ethernet switch connected to the data system computer. To make the Ethernet connections, use the shielded Ethernet cables supplied in the instrument accessory kits.

When you order the EASY-nLC instrument with a Thermo Scientific mass spectrometer, the Ethernet card for the LC/MS system is preset at the factory to this IP address: 172.16.0.101. While installing the LC or LC/MS system, your local Thermo Fisher Scientific field service engineer checks the IP address setting.

If the data system computer has a second network card, you can connect the data system computer to the intranet. After you connect the data system computer to the intranet, you can view the files on the EASY-nLC instrument's computer hard drive from other computers on the intranet. When you also set up a remote desktop connection, you can monitor and control the LC/MS system from other computers on the intranet.

For information about setting up a remote desktop connection, refer to the Microsoft™ Windows™ operating system Help.

❖ **To connect the Ethernet cables for an LC/MS system controlled from a data system computer**

1. Connect the EASY-nLC instrument and the Thermo Scientific mass spectrometer to the Ethernet switch as follows:
  - a. Plug a shielded Ethernet cable into the LAN port on the instrument's back panel.
  - b. Plug the other end of the cable into the Ethernet switch.
2. Connect the Ethernet switch to the data system computer as follows:
  - a. Verify that the IP address for the dedicated Ethernet card is set to 172.16.0.101.
  - b. Plug a shielded Ethernet cable into the appropriate Ethernet port in the data system computer.
  - c. Plug the other end of the cable into the Ethernet switch.
3. If the Ethernet switch has a power save button, make sure that the button is in the Off position.

The current model of the Ethernet switch that ships with Thermo Scientific mass spectrometers has a power save button labeled ECO friendly.

**IMPORTANT** When the power-save feature is on, the instrument can lose communication with the data system computer.

4. To connect the data system computer to the intranet, go to the next procedure, "[To connect the data system computer to the intranet.](#)"

❖ **To connect the data system computer to the intranet**

1. If the data system computer does not already have a second network card, install one.
2. Connect the network card's LAN port to a laboratory LAN port with intranet access using a shielded Ethernet cable.

When the data system computer is connected to a laboratory LAN port, you can access the admin folder on the EASY-nLC instrument's computer hard drive.

❖ **To control the LC/MS system remotely from another computer on the intranet**

1. Connect the hardware as described in these procedures:
  - [To connect the Ethernet cables for an LC/MS system controlled from a data system computer](#)
  - [To connect the data system computer to the intranet](#)
2. Set up a remote desktop connection between the data system computer and the remote local area computer.

For information about setting up a remote desktop connection, refer to the Windows operating system Help.

## Setting Up the Ethernet Connection to a Local Network for Data Exchange

You can access the chromatographic methods and injection batches stored on the EASY-nLC instrument's computer hard drive after you make the following connections:

- When you connect a stand-alone EASY-nLC instrument to a laboratory LAN port with intranet access, you can access these files from other computers on the intranet.
- When you connect the EASY-nLC instrument to a data system computer through an Ethernet switch, you can access these files from the data system computer.
- When you connect the EASY-nLC instrument to a data system computer through an Ethernet switch and connect the data system computer to a laboratory LAN port with intranet access, you can access these files from other computers on the intranet.

**Note** When you control the EASY-nLC instrument in the stand-alone mode, the chromatographic methods and batches reside on the integrated computer hard drive.

When you control the EASY-nLC instrument from a data system computer, your instrument methods and sequences created with the data system reside on the data system computer.

### ❖ To access the files on the EASY-nLC instrument's computer hard drive from another computer on the intranet

1. Do one of the following:
  - For a stand-alone EASY-nLC instrument, use a shielded Ethernet cable to connect the LAN port on the back panel of the EASY-nLC instrument to a laboratory LAN port with intranet access.
  - For an EASY-nLC instrument that is part of a Thermo Scientific LC/MS system, connect the Ethernet cables as described in [“Setting Up the Ethernet Connections to the Data System Computer”](#) on page 44.
2. Set up the software connection for the EASY-nLC instrument as described in [“Setting Up the Local Area Network Connection”](#) on page 74.

With a Thermo Scientific LC/MS system, you can access the files on the EASY-nLC system from the data system computer.

To access the files on the EASY-nLC instrument's computer hard drive from a computer on the intranet (other than the local data system computer for an LC/MS system), you need the IP address, the user login and password for the EASY-nLC instrument.

## Setting Up the Ethernet Connection to the Support Server

You can have Thermo Fisher Scientific Technical Support remotely diagnose problems with your EASY-nLC instrument.

Thermo Fisher Scientific recommends the following hardware setup when you want to establish a communication link between the EASY-nLC instrument and the remote support server.

For information about initiating the communication link with the support server, refer to the Remote Support chapter in the *EASY-nLC Series Troubleshooting and Maintenance Guide*.

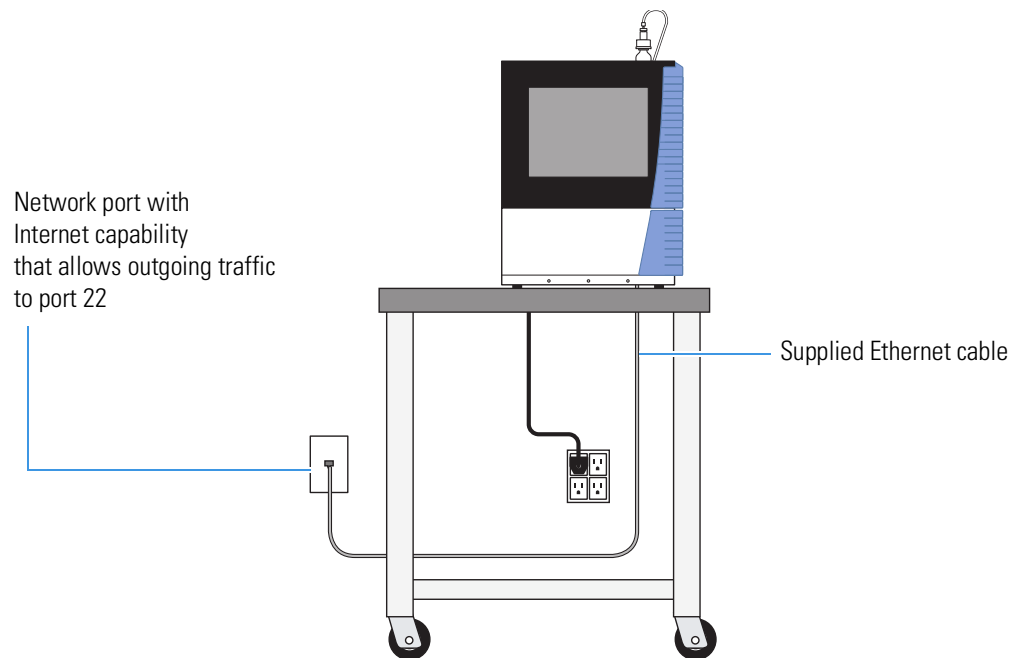
### ❖ To connect the EASY-nLC to the local network that has Internet capability

1. If the EASY-nLC instrument is connected to a data system computer or an Ethernet switch as shown in [Figure 25](#) on [page 38](#), disconnect the Ethernet cable from the LAN port of the EASY-nLC instrument.
2. Using a Category 6 network cable, connect the EASY-nLC instrument directly to the local network that has Internet capability to access the support server (see [Figure 30](#)).

**IMPORTANT** For remote diagnostics, connect the EASY-nLC instrument directly to the Internet access port using the supplied Category 6 network cable.

To use the remote support feature, the firewalls for your local network must allow outgoing TCP/IP traffic from the EASY-nLC instrument's computer to the support server at IP address 46.226.219.10, port 22.

**Figure 30.** EASY-nLC instrument connected to a local network port with Internet capability



## Attaching a Mouse and Keyboard to the USB Connections

You can use a mouse and keyboard instead of the built-in touch screen, but Thermo Fisher Scientific recommends using the touch-screen interface. For more information, see “Using the Touch Screen” on page 53.

Connect the USB mouse or keyboard by using the USB connectors on the back panel (see Figure 4 on page 5) of the instrument. The EASY-nLC application detects the mouse or keyboard within 30 seconds. The application does not recognize all USB-based keyboards or mice.

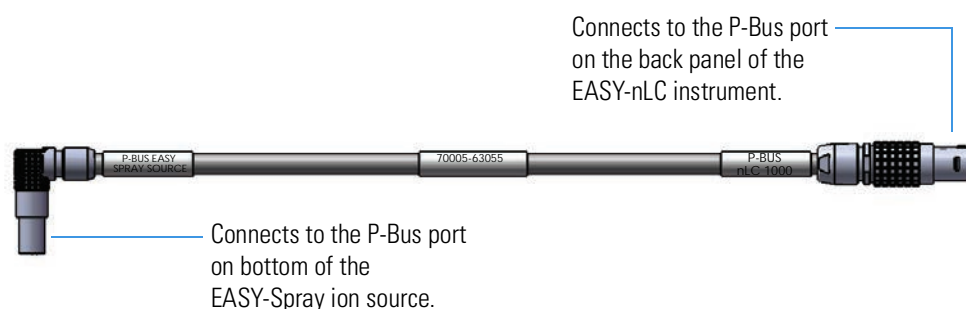
## Attaching Add-on Devices through the P-Bus and RS-232 Ports

The EASY-nLC instrument can control and power add-on devices through the P-bus port, the RS-232 interface, or both. These ports are on the back panel of the instrument (see Figure 4 on page 5).

### ❖ To control the temperature of the EASY-Spray ion source’s column heater

1. Using the P-bus cable shown in Figure 31, connect the EASY-nLC instrument to the EASY-Spray ion source as follows:
  - Connect the P-BUS NLC end of the cable to the P-Bus port on the back panel of the EASY-nLC 1200 instrument.
  - Connect the P-BUS EASY-SPRAY SOURCE end of the cable to the P-Bus port on the bottom of the EASY-Spray ion source.

**Figure 31.** EASY-Spray source to EASY-nLC 1200 cable (P/N LC167)



The EASY-nLC instrument provides power to the EASY-Spray ion source through the P-Bus cable.



2. Add the EASY-Spray ion source to the Devices list on the Maintenance > Devices page as described in [“Adding Devices to the EASY-nLC Devices List”](#) on page 162.

**Tip** If the temperature readout on the EASY-Spray ion source is blank, make sure that the P-Bus cable is properly connected and that the EASY-nLC instrument is turned on.

If the temperature readout in the EASY-Spray dialog box (available from the Home > Overview page of the touch-screen application) displays a question mark and the temperature readout on the EASY-Spray ion source displays two dashes “--”, make sure that the EASY-Spray column is plugged into the EASY-Spray ion source’s Heater port.

If the temperature can not be set on the touch screen, close the window and remove the device from the configuration while keeping the P-Bus cable connected on both ends and add it back in to the configuration. At this point you may see a temperature reading or you may see the letters „bL“.

Set the desired temperature on the touch screen, wherein the set and actual temperatures should match within a few seconds. (Note: If you manually turn the temperature control knob on the source, it will disable the feature. Go back and set the temperature on the touch screen again.)

For more information about the EASY-Spray ion source, refer to the *EASY-Spray Series Ion Source User Guide*.

## **2** Installing the EASY-nLC Instrument

### Back Panel Connections

# Integrated Instrument Control Software

The EASY-nLC instrument is controlled by software that runs on an integrated computer; this means you can operate the instrument without the aid of a data system computer.

**Note** When you are using the EASY-nLC instrument as part of a Thermo Scientific LC/MS system, you can use the Xcalibur data system to create chromatographic methods and batches and to acquire and process mass spectral data from the mass spectrometer.

For information about controlling the EASY-nLC instrument from the Xcalibur data system, refer to the data system Help. You can access Help from most of the user-interface pages by pressing the F1 key on the keyboard connected to the data system computer.

You can use the built-in instrument control application for the EASY-nLC instrument to set up instrument methods (sample processing protocols), schedule batches (injection sequences), view the run progress and instrument status, manage users, and carry out maintenance and repair work.

To familiarize yourself with the touch-screen application, review this chapter.

## Contents

- [Turning On the EASY-nLC Instrument](#)
- [Using the Touch Screen](#)
- [Viewing the System Status](#)
- [User Interface Layout and Application Menu Structure](#)
- [Logging into the EASY-nLC Instrument](#)
- [Closing Down the EASY-nLC Instrument](#)

## Turning On the EASY-nLC Instrument

### ❖ To turn on the instrument

Press the On/Off (I/O) switch on the back panel of the instrument (Figure 32).

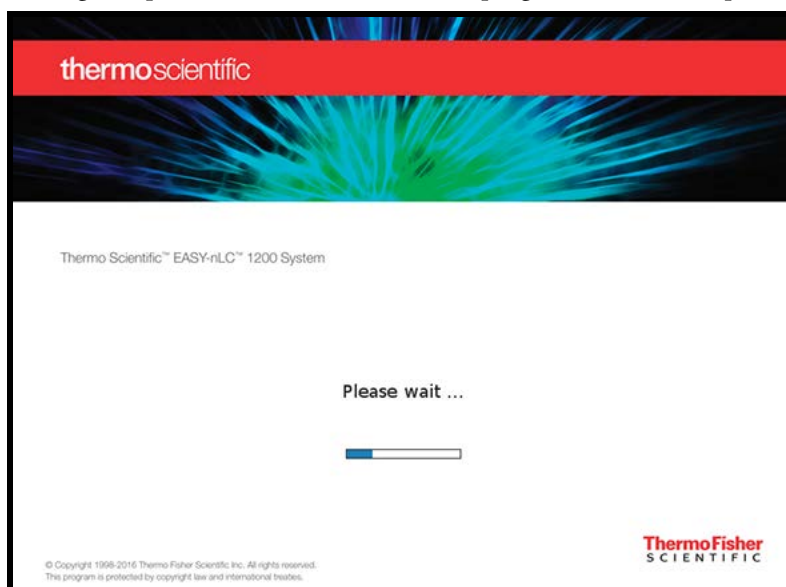
**Figure 32.** On/Off switch on the back panel



The startup procedure takes approximately 5 to 10 minutes while the instrument does the following:

1. Starts the internal computer.
2. Initializes the software components.
3. Checks the hardware components (pumps, valves, autosampler, plate-cooler, flow sensor, and so on).
4. Starts the Linux application.

During this process, a status bar indicates progress on the startup screen.



## Using the Touch Screen

You control the EASY-nLC instrument directly from the touch screen.

**Note** You can attach a USB-based mouse and keyboard to the instrument's back panel (see “Attaching a Mouse and Keyboard to the USB Connections” on page 48), but the user interface has been optimized for touch-screen interaction.

The instrument monitor is a transparent layer of glass (Figure 33) that detects finger pressure (even when the operator is wearing gloves) and sends the corresponding commands to the computer.

**Figure 33.** Touch-screen control for the EASY-nLC instrument



The touch-screen controls consist of buttons, tables, input fields, and interactive graphical instrument components. To use the touch-screen controls, see these procedures:

- [Using the Buttons](#)
- [Entering Alphanumeric Text](#)
- [Using Tables and Input Fields](#)
- [Using the Interactive Graphical Controls](#)

## Using the Buttons

Press buttons only one time for a specific action. If the action is irreversible or could cause a system error if the instrument is not prepared for the action, the EASY-nLC application displays a confirmation box where you can cancel or accept the action. For example, when you press Eject/Insert Tray on the Home > Overview page, a confirmation box appears to remind you to remove obstacles from the area in front of the tray compartment before opening the tray compartment door. To continue the action, you must press Accept.

## Entering Alphanumeric Text

In most cases, pressing a button on the touch screen causes the instrument to carry out a certain operation. However, when the application requires text or numeric input, the application displays a keyboard or numeric keypad (Figure 34) in front of the main window so that you can enter values by pressing the appropriate keys. When you press a key, it is highlighted in blue until you press another key.

**Figure 34.** Touch-screen keyboard and numeric keypad



## Using Tables and Input Fields

You can edit many tables (usually when you have entered the data yourself). You can also access single cells by touching the cell twice to open a keyboard/keypad display for changing the entry.

Long tables have vertical scroll bars that you move either by pressing the up or down arrow symbols, or by dragging the scroll bar and moving it explicitly.

In some tables you can select multiple rows by pressing check boxes on several rows or complete columns by pressing the table headings.

If a screen has blank input fields, press inside the field to enter data. The application displays a keyboard/keypad.

## Using the Interactive Graphical Controls


The four valves, three pumps, autosampler, and optional EASY-Spray ion source appear as interactive graphical components on the Home > Overview page (see [Figure 37](#) on [page 57](#)). When you press a graphical component, a dialog box with direct controls for that component opens.

For information about using the manual controls for the valves, pumps, and autosampler, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

To control the temperature of the EASY-Spray ion source when the instrument is not running a method, follow these procedures:

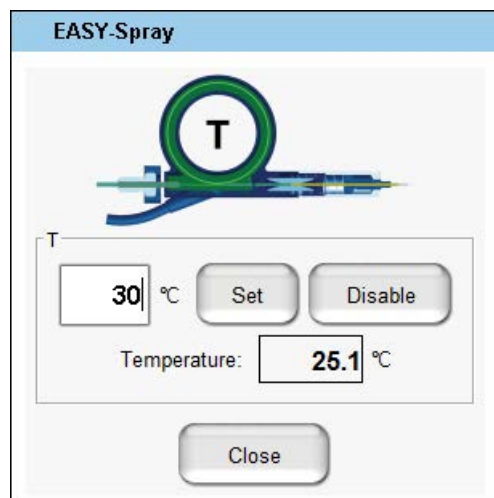
- To open the EASY-Spray (direct control) dialog box
- To modify the temperature setting of EASY-Spray ion source
- To turn off temperature control and return the ion source to ambient room temperature

### ❖ To open the EASY-Spray (direct control) dialog box

On the Home > Overview page, press the **EASY-Spray** icon, .

The EASY-Spray dialog box opens ([Figure 35](#)).

**Figure 35.** EASY-Spray dialog box

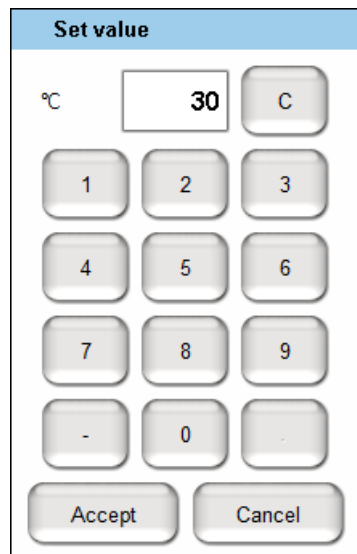


### ❖ To modify the temperature setting of EASY-Spray ion source

1. Open the EASY-Spray dialog box as described in the previous procedure.
2. Press the temperature box.

The Set Value dialog box opens ([Figure 36](#)).

**Figure 36.** Set Value dialog box



The image shows a 'Set value' dialog box. At the top, there is a blue header with the text 'Set value'. Below the header, on the left, is a '°C' symbol. To its right is a white input field containing the number '30'. Further right is a 'C' button. Below these elements is a numeric keypad with buttons for digits 1 through 9, a '-' button, and a '0' button. At the bottom of the dialog box are two buttons: 'Accept' on the left and 'Cancel' on the right.

3. Press the number pad to enter a temperature from **30** to **60** °C.

The application does not accept temperatures below 30 °C or above 60 °C. The application displays temperatures outside this range in red.

4. After you enter an appropriate temperature, press **Accept**.

The Set Value dialog box closes and the application downloads the new temperature setting to the EASY-Spray ion source.

5. To close the EASY-Spray dialog box, press **Close** (see [Figure 35](#) on [page 55](#)).

#### ❖ **To turn off temperature control and return the ion source to ambient room temperature**

1. Open the EASY-Spray dialog box as described in “[To open the EASY-Spray \(direct control\) dialog box](#)” on [page 55](#).
2. Press **Disable** (see [Figure 35](#) on [page 55](#)).

The application turns off the EASY-Spray ion source’s temperature control and the ion source’s temperature returns to the ambient laboratory temperature.

3. To close the EASY-Spray dialog box, press **Close** (see [Figure 35](#) on [page 55](#)).



## Viewing the System Status

You can view the system status of the system hardware components and the run status on the Home > Overview page (Figure 37).

**Figure 37.** Overview page of the Home menu

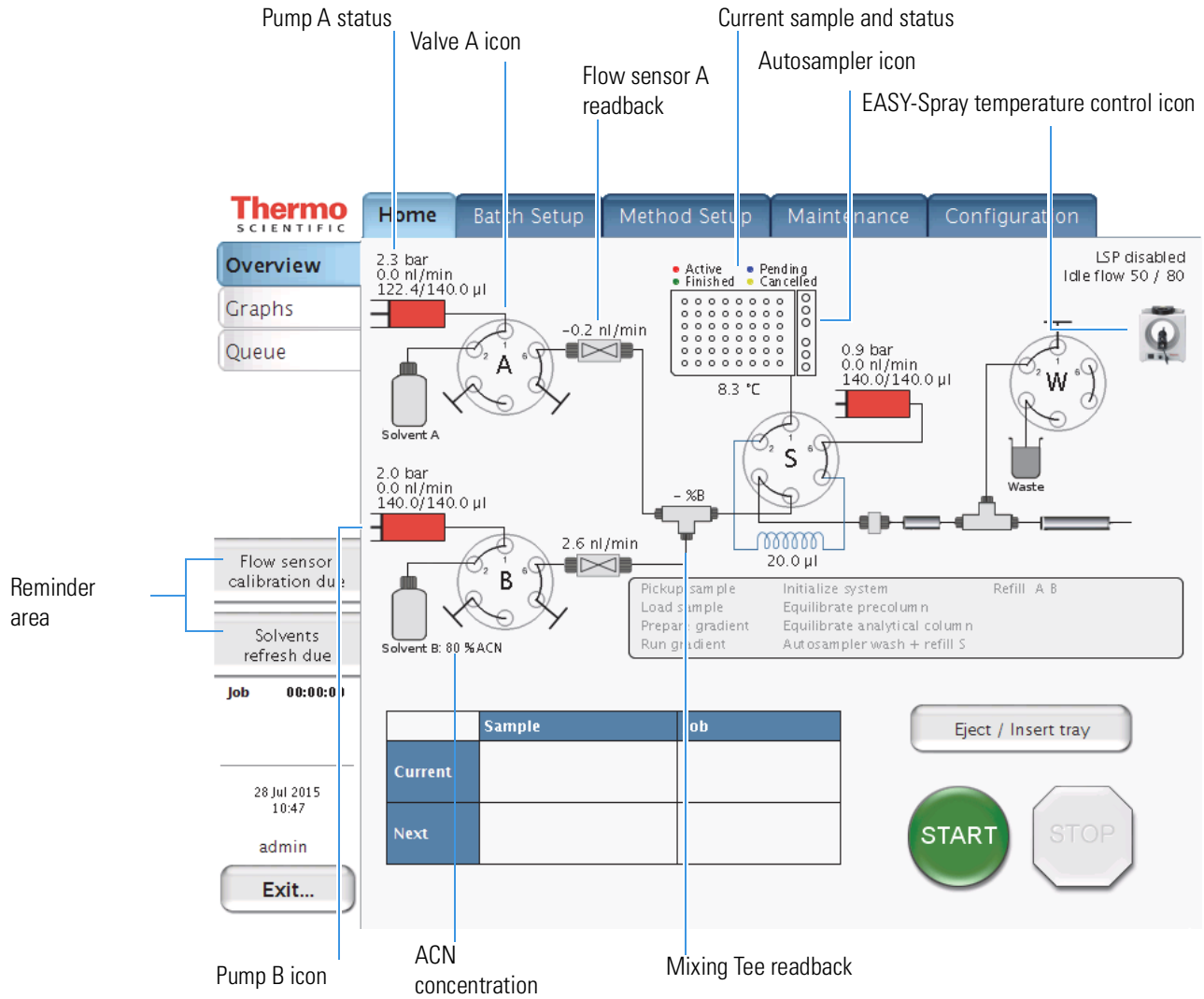


Table 8 describes the system readbacks.

**Table 8.** System readbacks

Readback	Description
Pump	Displays the backpressure, flow rate, and position for the associated pump. The flow rate readback is negative when the pump is refilling.
Flow sensor	Displays the measured flow rate from the associated pump (see <a href="#">“Flow Sensors”</a> on page 11).
Mixing Tee	Displays the %B solvent composition.
Autosampler	Displays the active, pending, and finished samples for a batch run and the temperature of the cooling unit.
ACN concentration	Displays the current ACN concentration

The valve icon shows the position of the associated valve (see [“Six-Port Rotary Valves”](#) on page 12).

For more information about viewing the system status, see [“Monitoring the Run”](#) on page 153.

In the reminder area dues are displayed:

“Flow sensor calibration due”	Shows if the last successful calibration was more than 6 months ago.
“Solvent refresh due”	Shows that the last acknowledged solvent refresh was more than 14 days ago.
“Solvent refreshed” acknowledge button	Shows that the last acknowledged solvent refresh was less than 14 days ago.

Click on the reminder for further actions:

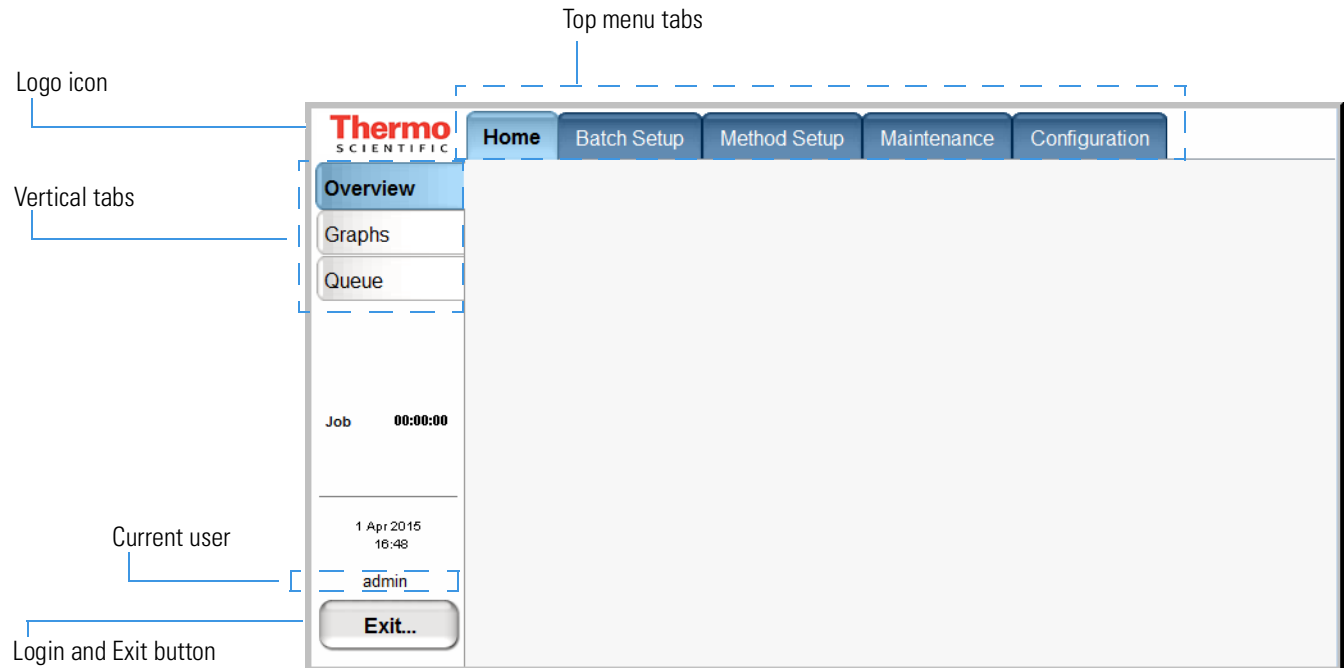
The “Flow sensor calibration due” button is linked to the flow sensor calibration script.

Clicking on “Solvent refresh due” and “Solvents refreshed” shows a solvent refresh confirmation.

## User Interface Layout and Application Menu Structure

The application has five menu tabs at the top of the touch screen (see [Figure 38](#)) and each menu has two or more pages (vertical tabs at the left).

**Figure 38.** EASY-nLC user interface layout with menus



In this guide, the term *menus* refers to the tabs at the top of the touch screen and the term *page* refers to the screens opened by pressing the vertical tabs.

During a session, the application tracks which page (vertical tab) you selected inside each menu (top tab) and shows that page when you next choose that menu.

The Thermo Scientific logo icon in the upper left corner and the Login button in the lower left corner are always available.

To log in or log off, press **Login** (see “[Logging into the EASY-nLC Instrument](#)” on [page 66](#) and “[Closing Down the EASY-nLC Instrument](#)” on [page 69](#)).

To view version information for the application, press the **Logo** icon.

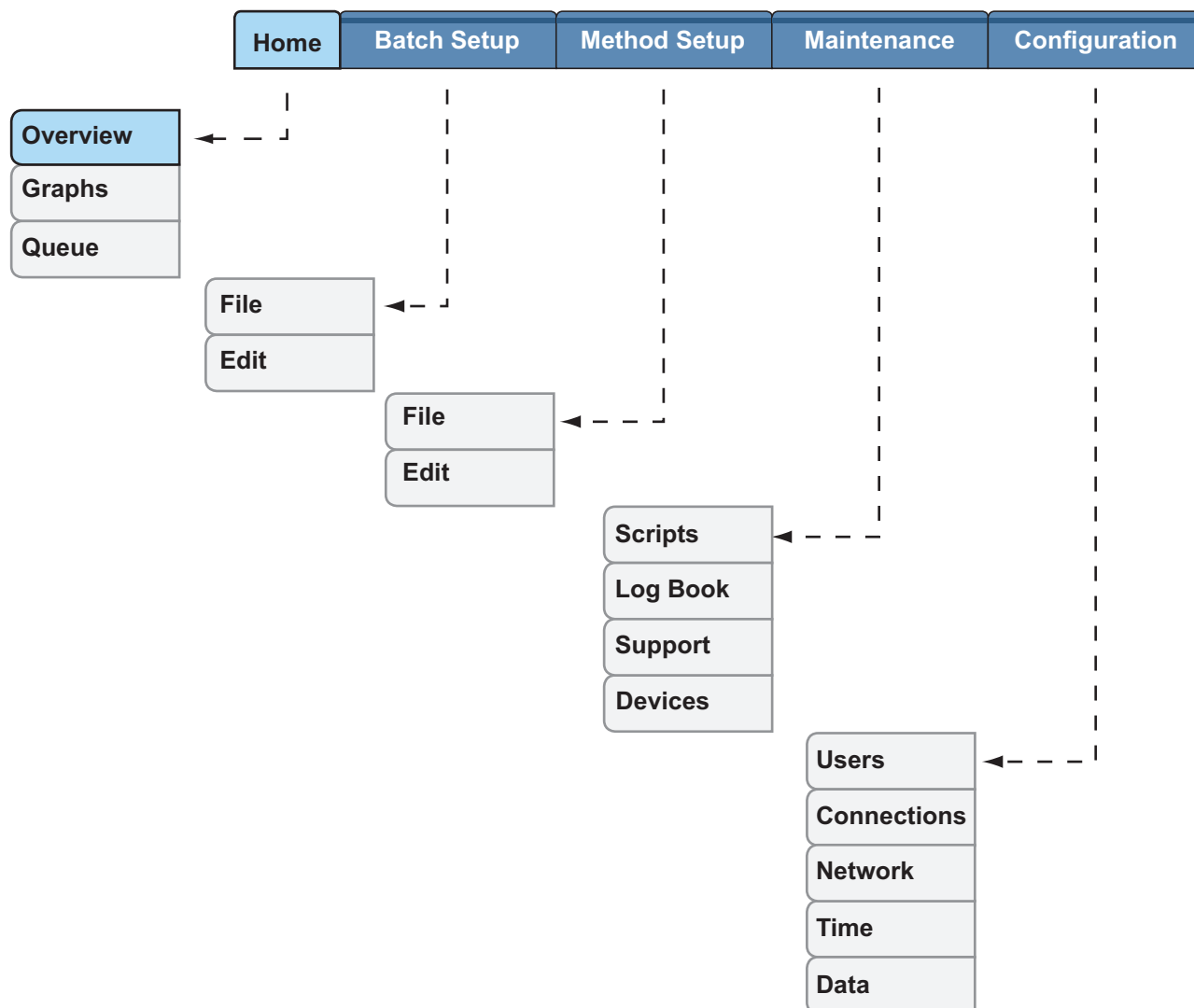
To access a specific page of the application, first press the appropriate menu tab and then press the appropriate page tab. In this guide, the following nomenclature describes the navigation to a specific page of the user interface: Menu > Page.

Some of the pages have two or more views. For example, the Batch Setup > Edit page has two views: View Rack and View List. You access these views by pressing View Rack or View List on the page.

The Method Setup > Edit page contains a five-page wizard. You access the wizard pages by pressing the left and right arrows at the bottom of the touch screen.

Figure 39 shows a schematic of the user-interface structure. When you navigate to a *Menu > Page*, the menu and page tabs are highlighted in light blue.

**Figure 39.** Menu and page structure



See the following menu descriptions (on pages 61–65):

- [Home Menu](#)
- [Batch Setup Menu](#)
- [Method Setup Menu](#)
- [Maintenance Menu](#)
- [Configuration Menu](#)

## Home Menu

Use the three pages of the Home menu to view the status of your sample runs and the instrument components; edit batch jobs in the queue; and take direct control of the valves, the pumps, the autosampler’s XYZ robot, and the cooler temperature.

Page	Function	Reference
Overview	Manually control the valves, the pumps, and the autosampler components.	Refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i> .  <a href="#">“Preparing and Loading Samples”</a> on page 129
	Monitor the system status.	<a href="#">“Viewing the System Status”</a> on page 57 and <a href="#">“Monitoring the Run”</a> on page 153
Graphs	Display up to four graphs, each selectable from a list, with time-dependent data for the active run. The selection list includes Pump A, Pump B, Pump S, Valve A, Valve B, Valve S, Valve W, Sample Cooling, and Gradient.	<a href="#">“Using the Graphs Page in the Home Menu”</a> on page 155
Queue	View a list of the batch jobs, currently running or waiting.	<a href="#">“Editing the Running Batch”</a> on page 157

## Batch Setup Menu

Use the two pages of the Batch Setup menu to set up sample processing jobs. For information about setting up and submitting a batch run, see these procedures: “[Creating a Batch](#)” on [page 146](#) and “[Starting Sample Acquisition](#)” on [page 152](#).

Page	Function	Reference
File	View or change a directory tree of saved batch jobs. You can create or import new jobs and create or delete directories on this page.	“ <a href="#">Viewing the Batch List and Setting Up a Batch Record</a> ” on <a href="#">page 146</a>
Edit	Set up new batch jobs.	
View Racks view	Select the vial or well positions for the batch job from a graphical view of the configured plate format.	“ <a href="#">Specifying the Method, Sample Positions, and Number of Injections</a> ” on <a href="#">page 147</a>
View List view	View the batch job as a list of samples in the order of processing. Each row in the table holds information about the position, name, and method for that position.	“ <a href="#">Editing the Running Batch</a> ” on <a href="#">page 117</a>

## Method Setup Menu

Use the pages of the Method Setup menu to set up the chromatographic methods for your samples (see “[Creating a Method](#)” on page 130).

**Note** If the method does not control an external device, the Method Setup wizard contains five pages. If the method controls external devices, such as the Advion™ RePlay™ device or the EASY-Spray ion source, the Method Setup wizard includes additional pages. For information about setting up a method that includes the RePlay device, see [Chapter 7, “Installing and Controlling External Devices.”](#)

Page	Function	Reference
File	View a directory tree of saved methods. You can create or import new methods or create or delete directories from this page.	“ <a href="#">Browsing for a Stored Method or Creating a New Method File</a> ” on page 130
Edit	Method Setup wizard with six pages—	
1/6	Select the method that you want to edit and enter a description of the method. If you installed external devices, select the external devices that you want the method to control.	“ <a href="#">Starting the Method Setup Wizard</a> ” on page 132
2/6	Set up the sample pickup and sample loading steps of the method.	“ <a href="#">Setting Up the Sample Pickup and Loading Steps</a> ” on page 133
3/6	Control the liquid chromatography gradient.	“ <a href="#">Building the Gradient</a> ” on page 136
4/6	Control the equilibration of the precolumn, the analytical column, or both.	“ <a href="#">Setting Up the Column Equilibration Steps</a> ” on page 138
5/6	Control the autosampler wash step.	“ <a href="#">Setting Up the Autosampler Wash Step</a> ” on page 140
6/6	Control the column temperature of the EASY-Spray ion source.	“ <a href="#">Specifying the Temperature of the EASY-Spray Column Heater</a> ” on page 143

## Maintenance Menu

Use the pages of the Maintenance menu to perform a number of functions designed to provide and improve system status.

Page	Function	Reference
Scripts	View a collection of useful maintenance procedures for keeping the instrument functioning properly and to help diagnose and resolve possible problems.	<p>For information about purging and flushing the pump, see <a href="#">“Purging and Flushing the Pumps”</a> on page 95.</p> <p>For information about equilibrating the columns and determining the appropriate flow rate for your chromatographic method, see <a href="#">“Equilibrating Columns”</a> on page 124.</p> <p>For information about other maintenance scripts, refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i>.</p>
Log Book	Electronically enter all the service actions that you perform on the instrument.	For information about the log book, refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i> .
Support	Set up support from Thermo Fisher Scientific.	Refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i> .
Devices	Add an external device to the system.	<a href="#">Chapter 7, “Installing and Controlling External Devices.”</a>
	Check and reset the device usage counters.	For information about the device usage counters, refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i> .
	Set up the global configuration for the HPLC.	<a href="#">“Setting Up the Properties for the HPLC Device”</a> on page 83
	Calibrate the autosampler.	Refer to the <i>EASY-nLC 1200 Troubleshooting and Maintenance Guide</i> .



## Configuration Menu

Use the pages of the Configuration menu to control the instrument setup and to manage the system configuration.

Page	Function	Reference
Users	Manage user accounts (create, edit, and delete).	<a href="#">“Creating New User Accounts” on page 79</a>
Connections	Select the mass spectrometer that is connected to the EASY-nLC instrument, set up the communications protocol, and view a contact closure wiring diagram for the selected mass spectrometer.	<a href="#">“Setting Up Communication with the Mass Spectrometer” on page 71</a>
Network	Set up or view how the EASY-nLC instrument’s computer is identified in a connected computer network. Consult your IT administrator before making changes to the system.	<a href="#">“Setting Up the Local Area Network Connection” on page 74</a>
Time	Set the internal clock in the EASY-nLC instrument and the display format used throughout the application to print date and time information.	<a href="#">“Modifying the Time Settings” on page 81</a>
Data	Purges excess data on the hard drive of the EASY-nLC instrument’s computer. Removing unnecessary data improves the computer’s performance.	<a href="#">“Purging the Computer Hard Drive” on page 82</a>

## Logging into the EASY-nLC Instrument

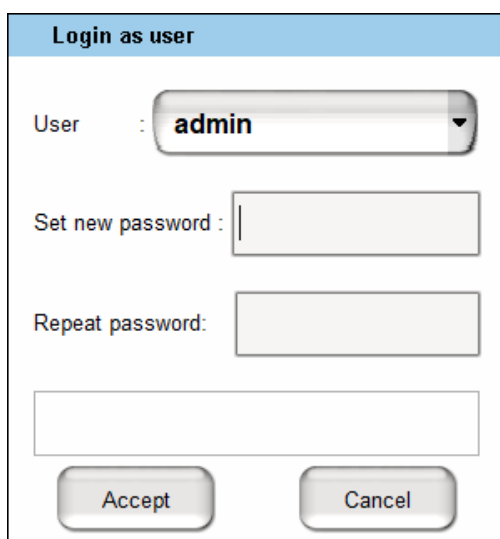
The EASY-nLC instrument's built-in software has a user management system that assigns specific access rights to each user. By default, it has two predefined users: "guest" and "admin". The default user account is "guest" (and does not require a password). With this account, you can monitor the system but you cannot enter data or issue instrument commands.

When the application is started, it automatically logs you in as "guest". **Guest** appears above the Login button.

#### ❖ To start using or configuring the EASY-nLC instrument's built-in software for the first time

1. In the User list, select **admin** (see [Figure 40](#)).

**Figure 40.** EASY-nLC first login dialog box

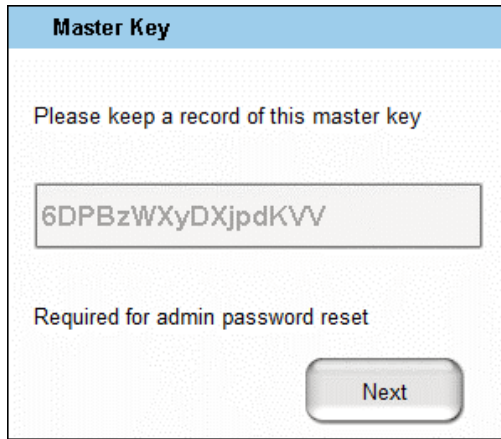


The screenshot shows a dialog box titled "Login as user". It features a "User" dropdown menu with "admin" selected. Below this are two text input fields: "Set new password" and "Repeat password". There is also an empty text box below the "Repeat password" field. At the bottom of the dialog are two buttons: "Accept" and "Cancel".

2. Set a new password for the user **admin** with a maximum of 8 characters.
3. Repeat the password in the box below.
4. Press **Accept**.
5. A dialog box appears showing the master key. This key is required to reset the administrator password.

**IMPORTANT** Keep a record of the master key and keep it in a safe place.

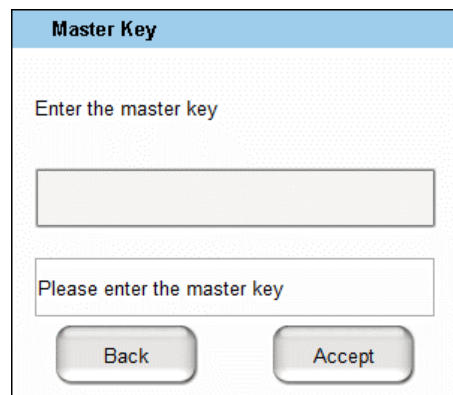
**Figure 41.** Master key dialog box



The dialog box has a blue header with the text "Master Key". Below the header, the text "Please keep a record of this master key" is displayed. A text box contains the alphanumeric string "6DPBzWXyDXjpdKVV". Below the text box, the text "Required for admin password reset" is shown. At the bottom right, there is a "Next" button.

6. Reenter the master key for confirmation.

**Figure 42.** Master key verification



The dialog box has a blue header with the text "Master Key". Below the header, the text "Enter the master key" is displayed above an empty text input field. Below this field, the text "Please enter the master key" is displayed above another empty text input field. At the bottom, there are two buttons: "Back" on the left and "Accept" on the right.

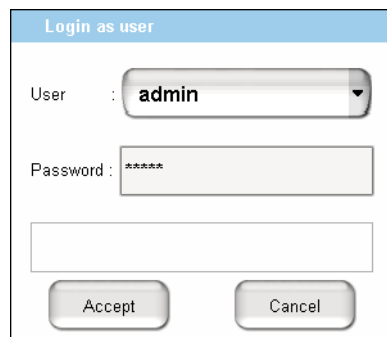
### 3 Integrated Instrument Control Software

Logging into the EASY-nLC Instrument

#### ❖ To start using or configuring the EASY-nLC instrument's built-in software

1. In the User list, select **admin** (see [Figure 43](#)).
2. In the Password box, enter the correct password.
3. Press **Accept**.

**Figure 43.** EASY-nLC login dialog box

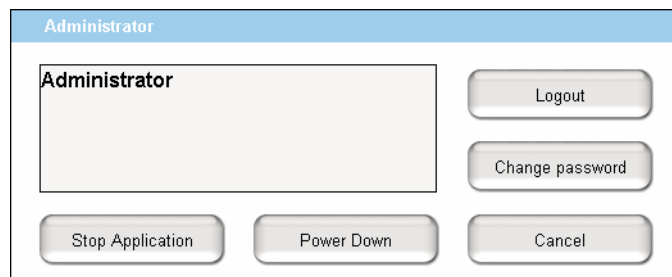


#### ❖ To log out of the EASY-nLC instrument

1. Press **Exit**.

A confirmation box opens (see [Figure 44](#)).

**Figure 44.** Confirmation box from pressing Exit in the lower left corner of the touch screen



2. Press **Logout**.

An administrator can start a controlled power-down or exit the application from this dialog box. If you are not an administrator, you can only log out or change your password.

## Closing Down the EASY-nLC Instrument

With the exception of an emergency shutdown, closing down the instrument in a controlled manner is important to allow all the components to shut down in an orderly sequence. Using this controlled method saves important data so that the instrument starts with the correct information the next time you use it.



**CAUTION** With the exception of an emergency shutdown, closing down the instrument in a controlled manner is important to allow all the components to shut down in an orderly sequence. If you turn off the power switch during normal operation, you risk damaging essential system components. Follow the procedure outlined here whenever possible.

### ❖ To turn off the EASY-nLC instrument

1. Press **Exit** (lower left corner of the touch screen).

A confirmation box opens (see [Figure 44](#) on [page 68](#)).

2. Press **Power Down**.

The EASY-nLC application displays a white screen with a small progress bar. When the progress bar fills completely and the message prompts you to turn off the instrument, go to the next step.



**The instrument can now be turned off safely**

Thermo Scientific™ EASY-nLC™ 1200 System

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This program is protected by copyright law and international treaties.

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SCIENTIFIC

3. After receiving the message that you can safely turn off the instrument, turn off the power switch on the back panel of the instrument.

### **3 Integrated Instrument Control Software**

Closing Down the EASY-nLC Instrument

## Configuring the EASY-nLC System

After turning on the instrument and logging in as a system administrator, you must configure the instrument's integrated software application for use and define the network connection for the instrument.

To set up the application configuration, follow the configuration instructions in this chapter.

### Contents

- [Setting Up Communication with the Mass Spectrometer](#)
- [Setting Up the Local Area Network Connection](#)
- [Modifying User Permissions](#)
- [Modifying the Time Settings](#)
- [Purging the Computer Hard Drive](#)
- [Setting Up the Properties for the HPLC Device](#)

## Setting Up Communication with the Mass Spectrometer

After the EASY-nLC injects sample onto the analytical column, it signals the mass spectrometer to start data acquisition.

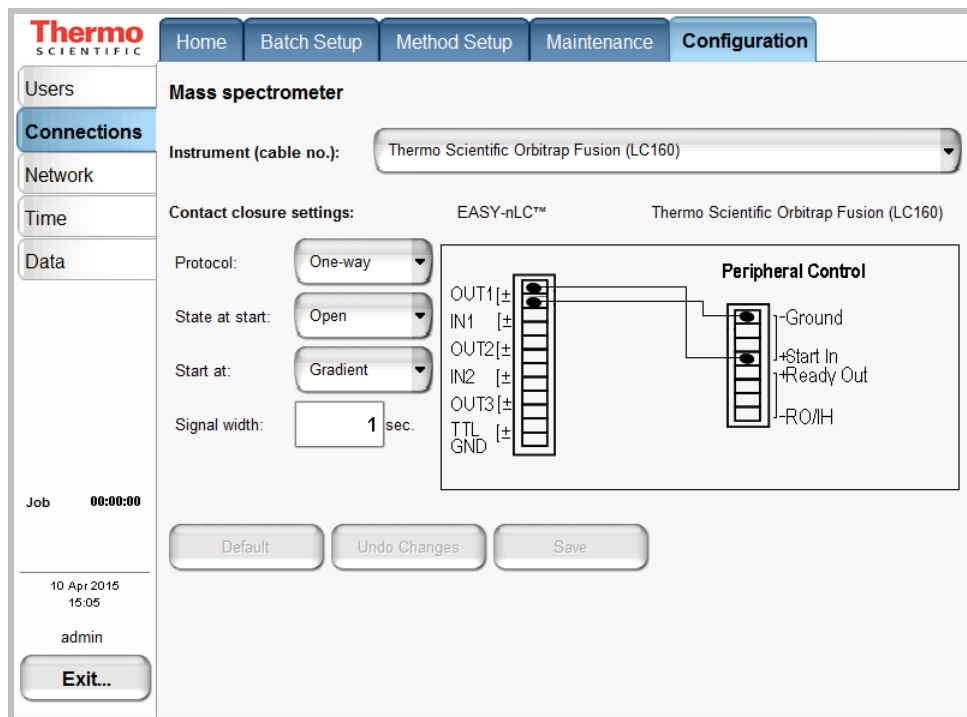
**IMPORTANT** Before you connect the EASY-nLC instrument to a mass spectrometer, make sure that the protective grounding is shared between the instruments.

### ❖ To set up the mass spectrometer connection

1. Press **Configuration > Connections**.

The Configuration > Connections page opens. [Figure 45](#) shows the contact closure graphic for an Orbitrap Fusion mass spectrometer. For information about connecting the contact closure cable for a Thermo Scientific mass spectrometer, see [“Connecting to the Mass Spectrometer through Contact Closure”](#) on [page 42](#).

Figure 45. Connections page of the Configuration menu



2. From the Instrument (cable no.) list, select the instrument by manufacturer.

**Tip** The Instrument (cable no.) list contains the most common mass spectrometers (by manufacturer) used in proteomics, but if you cannot find your particular mass spectrometer, select **Generic**.

If your mass spectrometer does not appear on the list and you would like to see it supported in a future release of the EASY-nLC application, provide this information to Thermo Fisher Scientific Customer Service (see “[Contacting Us](#)” on page xv).

When you select the instrument, a graphic of the contact closure connections appears in the Contact Closure Settings area, but the default selections remain the same.

3. In the Contact Closure Settings area, do the following:
  - a. Check the connections on the graphic for the selected mass spectrometer.
  - b. From the Protocol list select **One-way** or **Two-way** synchronization (see [Figure 45](#)).
    - If the graphic for the selected mass spectrometer (contact closure cable) shows only Start In signals, select **One-way** synchronization. With a one-way connection, the EASY-nLC instrument sends a start signal to the mass spectrometer.



- If the graphic for the selected mass spectrometer shows two-way communication between the mass spectrometer and the EASY-nLC instrument, select **Two-way** synchronization. With a two-way connection, the EASY-nLC instrument waits for a ready signal from the mass spectrometer.
- c. In the State at Start list, select **Open** or **Closed**.
- For a Thermo Scientific mass spectrometer, select **Open**. For other mass spectrometers, refer to the documentation provided with the mass spectrometer or contact Thermo Fisher Scientific Customer Service.
- d. In the Start At list, select **Gradient** or **Sample Loading**.
- If you want data acquisition to start when the mobile phase gradient starts, select **Gradient**.
  - If you are running the instrument in the one-column mode and you want data acquisition to start when the instrument loads the sample onto the analytical column, select **Sample Loading**.

**Note** By setting the Start At value to be the sample loading start, you might be able to acquire data for early-eluting peptides that would not ordinarily bind to the column material and elute in the solvent front.

Moving the Start At point to the start of the sample loading step can lead to small retention time variations, due to possible differences in pressure buildup before the A/B mixing starts in the actual gradient.

- e. In the Signal Width box, enter an appropriate signal width.
- The default signal width is 1 second, which is sufficient for a Thermo Scientific mass spectrometer. For other mass spectrometers, refer to the documentation provided with the mass spectrometer or contact Thermo Fisher Scientific Customer Service.

## Setting Up the Local Area Network Connection

If you connect the EASY-nLC instrument to the local network (see “[Setting Up the Ethernet Connection to a Local Network for Data Exchange](#)” on page 46) and set up the network configuration for the application, you can access the EASY-nLC instrument’s computer from other computers on the network.

**IMPORTANT** Ask your local IT administrator to help you configure the EASY-nLC instrument on the local network. The following information will help you through the process.

### ❖ To connect the EASY-nLC instrument to a local network

1. Connect the Ethernet cables as described in (see “[Setting Up the Ethernet Connection to a Local Network for Data Exchange](#)” on page 46).
2. Press **Configuration > Network**.

The Network page (Figure 46) contains the configuration address information.

**Figure 46.** Network page of the Configuration menu

The screenshot shows the Thermo Scientific EASY-nLC Configuration menu. The 'Configuration' tab is selected, and the 'Network' sub-tab is active. The 'LAN' section is visible, showing the following configuration:

- Profile: Internet
- Configuration:  Dynamic  Fixed
- IP: 172 . 22 . 50 . 18
- Subnet: 255 . 255 . 255 . 0
- Gateway: 10 . 128 . 12 . 1
- DNS: 10 . 128 . 28 . 26
- Name: LC-030024
- Domain: [Empty]

An 'Apply' button is located below the LAN configuration. Below the LAN section, the 'Support server' section is visible, showing:

- IP: 46 . 226 . 219 . 10
- Default IP button

An 'Apply' button is located below the Support server configuration. The left sidebar contains navigation options: Users, Connections, Network (selected), Time, and Data. At the bottom of the sidebar, there is an 'Exit...' button. The main content area also shows 'Solvents Refreshed' and 'Job 00:00:00'.

3. In the Profile list, select one of the following:
  - To connect to a local area network, select **MS LAN**.
  - To connect to the remote support server, select **Internet**.

4. For Configuration, select the **Dynamic** (addressing DHCP) option or the **Fixed** (IP address) option and press **Apply** to activate your selection.

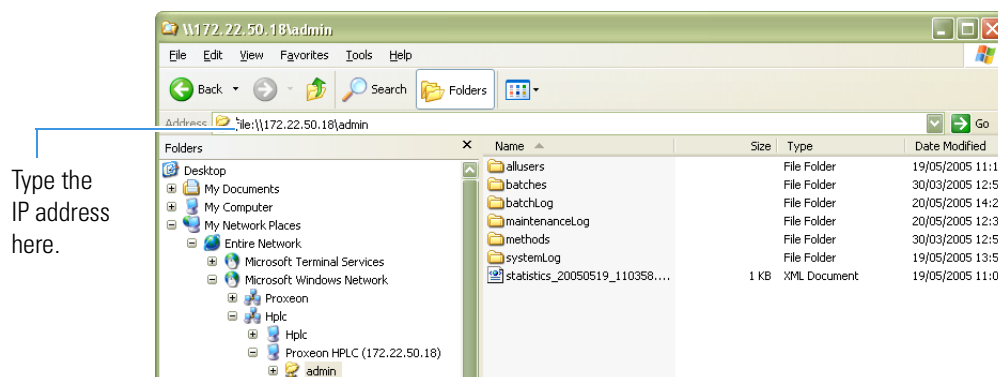
Your IT administrator knows which option to select.

If IT asks about a MAC address for the instrument—that is, the instrument’s unique network identifier—it is labeled on the connector panel on the back of the instrument. The identifier is a series of numbers and letters and might look like this:

MAC address: 00:E0:4B:07:89:65

External users can now access the file server in the EASY-nLC instrument’s computer to look at log files, for example. Microsoft Windows Explorer might display the file server as shown in Figure 47.

**Figure 47.** File server access through Windows Explorer



❖ **To access the admin folder on the EASY-nLC instrument’s computer hard drive from another computer on the network**

1. Type the IP address of the instrument (in the Address list for Windows Explorer), for example, **file:\\172.22.50.18**, and then press ENTER.

A login dialog box appears.

You need a valid user name and password to enter the file space. The user name and password are the same as those on the EASY-nLC instrument.

2. Enter the same user name and password that you use to log in locally to the EASY-nLC instrument.

## Modifying User Permissions

The four types of users are Guest (default), Normal, Super User, and Administrator.

Each user type has access to different levels of functionality in the EASY-nLC application (Table 9).

**Table 9.** User types and permission levels for each menu

Menu	User type			
	Guest	Normal	Super User	Administrator
Home	Read only <sup>a</sup>	Read/Write <sup>b</sup>	Read/Write	Read/ Write
Batch Setup	Read only	Read/Write	Read/Write	Read/Write
Method Setup	Read only	Read/Write	Read/Write	Read/Write
Maintenance	N/A	N/A	Read/Write	Read/Write
Configuration	N/A	N/A	N/A	Read/Write

<sup>a</sup>User has read-only access.

<sup>b</sup>User has read-write access.

Having a Guest user account means you can access the top menu but not enter data or issue instrument commands.

If a feature is not available, the application grays (blanks) it out, so the user cannot access that function.

Follow these procedures to change passwords and create new user accounts:

- “Changing the Administrator Password,” next section
- “Repeat the password.” on page 78
- “Changing Your Password from the Exit Menu” on page 80

## Changing the Administrator Password

### ❖ To change the administrator password

1. Log in as **admin** (otherwise, see “Logging into the EASY-nLC Instrument” on page 66).
2. Press **Configuration > Users**.

The Configuration > Users page opens (Figure 48).

**Figure 48.** Users page of the Configuration menu

The screenshot shows the 'Users' configuration page in the Thermo Scientific software. The interface includes a top navigation menu with 'Home', 'Batch Setup', 'Method Setup', 'Maintenance', and 'Configuration'. The 'Configuration' menu is selected. On the left, a sidebar lists 'Users' (selected), 'Connections', 'Network', 'Time', 'Data', 'Job', and 'Exit...'. The main content area has a 'User login' field, 'Name', 'Password', and 'Re enter' fields. Below these are radio buttons for 'Level' (Normal, Super, Admin). There are four 'Graphs' buttons, each set to 'Don't care'. At the bottom, there are 'Open', 'Close', 'Save', 'Delete', and 'New' buttons.

3. Press **Open**.

The Select User dialog box opens.

4. In the User list, select **Admin**.
5. Enter the new password as follows:
  - a. Press the **Password** box.  
The Enter Password dialog box opens.
  - b. Type a new password, and then press **OK**.
  - c. Press the **Re enter** box.  
The Enter Password dialog box opens.
  - d. Type the password a second time to confirm it, and then press **OK**.
6. Press **Save** to save the user account information.

### Resetting the Administrator Password

❖ **To use the master key to reset the administrator password**

1. Type the master key in the field below the password field. For details about the master key, see [“Logging into the EASY-nLC Instrument”](#) on page 66.

**Figure 49.** EASY-nLC login dialog box (master key)

The screenshot shows a dialog box titled "Login as user". It contains a "User" dropdown menu with "admin" selected. Below it is a "Password" field. Underneath the password field is a text input field with the placeholder text "Enter master key to reset password". At the bottom of the dialog are two buttons: "Accept" and "Cancel".

2. Press **Accept** to continue.
3. Set the new password.

**Figure 50.** EASY-nLC login dialog box (password)

The screenshot shows a dialog box titled "Login as user". It contains a "User" dropdown menu with "admin" selected. Below it are two text input fields: "Set new password" and "Repeat password:". Underneath these fields is a text input field with the placeholder text "Password reset for 'admin'". At the bottom of the dialog are two buttons: "Accept" and "Cancel".

4. Repeat the password.
5. Press **Accept** to continue.

## Creating New User Accounts

Use the Users page to create new user accounts for all the people or groups that will use the instrument. Each user has a private file space for batch jobs and methods that other users (apart from system administrators) cannot see.

In addition, there is a public file space for methods and batch jobs that all users can see and use. Only Super Users and Administrators can copy files into the public spaces and only Administrators can delete files and directories from the public space.

### ❖ To create a new user account

1. Log in as **admin** (otherwise, see “[Logging into the EASY-nLC Instrument](#)” on page 66).
2. Press **Configuration > Users**.

The Configuration > Users page opens (see [Figure 48](#) on page 77).

3. Press **New**.

The User Login, Name, Password, and Re enter boxes also become available for data input.

4. Make entries in the User Login and Name boxes.
5. Select the privileges for the user by level: **Normal**, **Super**, or **Admin**. For user privileges by user type, see [Table 9](#) on page 76.

If you want the user to carry out instrument maintenance, you must select Super User as the user type. If you want the user to restore data to the instrument’s computer hard drive, select Admin as the user type.

6. Create an initial password for the defined user by using the Password and Re enter boxes.

Users can change their passwords from the Login/Exit menu.

7. Press **Save**.

## Changing Your Password from the Exit Menu

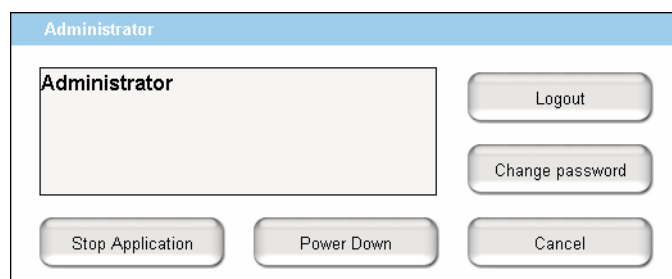
After logging in, all users can change their password from the Exit menu.

### ❖ To change your password

1. Press **Exit** in the lower right corner of the touch screen.

The exit confirmation box opens for the current user (see [Figure 51](#)).

**Figure 51.** Exit confirmation box for the current user



2. Press **Change Password**.

The Change password for: *current user* dialog box opens.

3. Make the appropriate entries.
4. Press **Accept** to accept the new password and close the dialog box.



## Modifying the Time Settings

Use the Configuration > Time page (Figure 52) to set both the internal clock in the EASY-nLC instrument and the display format used throughout the application for date and time information.

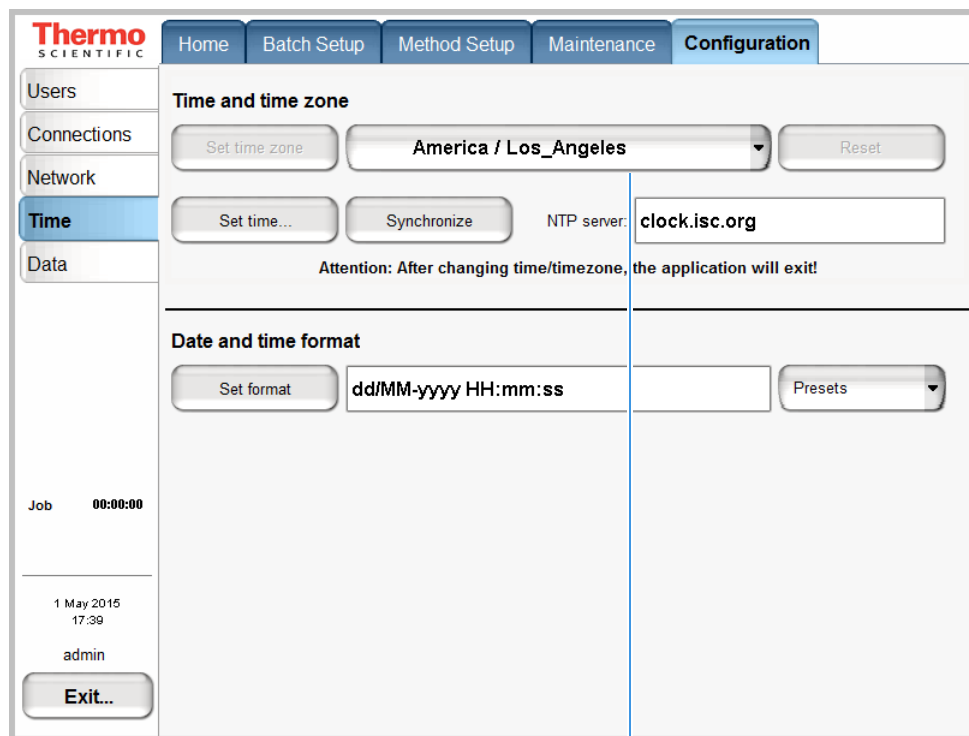
**Note** You cannot modify the time settings when the system is active.

### ❖ To set the time zone

1. Press **Configuration > Time**.

The Configuration > Time page opens (see Figure 52).

**Figure 52.** Time page of the Configuration menu



Continent/city list

2. Select a time zone from the continent/city list.

The Set Time Zone button becomes available.

3. Press **Set Time Zone**.

### ❖ To change the display format for the time and date

1. Select a format from the Presets list.

2. Press **Set Format**.

The time and date format changes immediately on the Maintenance > Log Book page.

## Purging the Computer Hard Drive

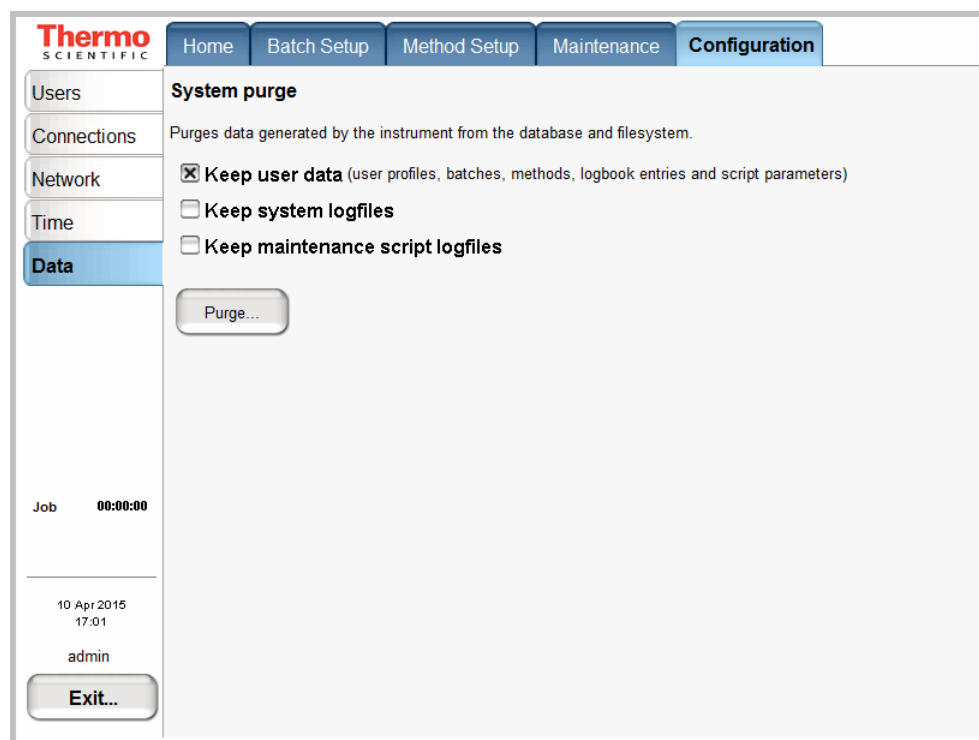
Use the Configuration > Data page to remove excess data from the computer's hard drive. Purge the hard drive when the instrument becomes less responsive or sluggish, as removing excess data improves system performance.

### ❖ To remove unnecessary files from the instrument's computer

1. Press **Configuration > Data**.

The Configuration > Data page opens (Figure 53).

**Figure 53.** Data page of the Configuration menu



2. Select the check boxes for the data that you want to keep.
3. Press **Purge**.

The application deletes the excess data. When the purge is complete, the Linux application restarts.

**Note** Perform the System Purge only when necessary. In the case of errors the system data facilitates troubleshooting.

## Setting Up the Properties for the HPLC Device

Use the Properties page for the EASY-nLC instrument on the Devices page of the Maintenance menu to set the global instrument configuration settings such as the one- or two-column setup, loop size, idle flow settings, and load speed protection.

### ❖ To set up the global instrument configuration for the HPLC device

1. Press **Maintenance > Devices**.
2. In the Devices list, select **EASY-nLC (HPLC)**.
3. Press the **Properties** tab.

The Properties view for the HPLC device on the Maintenance > Devices page opens (Figure 54).

**Figure 54.** Devices page of the Maintenance menu, showing the HPLC Properties settings

The screenshot shows the Thermo Scientific software interface. At the top, there are navigation tabs: Home, Batch Setup, Method Setup, Maintenance (selected), and Configuration. On the left, a vertical menu includes Scripts, Log Book, Support, and Devices (selected). Below this menu, there are status indicators for 'Solvents Refreshed', 'Job 00:00:00', and a timestamp '16 Jul 2015 15:36' with the user 'admin'. An 'Exit...' button is at the bottom left. The main area is titled 'Devices' and contains a table with the following data:

Name	Type
Cooled Autosampler (ASC) [Autosampler]	Autosampler
EASY-Spray	Column Heater
EASY-nLC™ [HPLC]	HPLC

To the right of the table are buttons for 'Add Device', 'Remove Device', and 'Assign Slave'. Below the table, there are tabs for 'About', 'Summary', and 'Properties' (selected). The 'Properties' section is titled 'EASY-nLC™ [HPLC]' and contains the following settings:

- Loop volume: 20.0 µl
- Idle flow rate: 50 nl/min
- Idle mixture: 80 %B
- One column setup
- Load speed protection (LSP)
- Automatic pre-run maintenance

At the bottom of the properties section are buttons for 'Factory', 'Reset', and 'Apply'.

4. Press the corresponding box to open the keypad and enter the appropriate numeric values for these parameters: Loop Volume, Idle Flow Rate, and Idle Mixture.
5. Make the appropriate selections for these check boxes: **One Column Setup**, **Load Speed Protection (LSP)**, and **Automatic Pre-run Maintenance**.

Table 10 describes the parameters in the Properties view for the EASY-nLC instrument on the Devices page of the Maintenance menu.

**Table 10.** HPLC parameter descriptions (Sheet 1 of 2)

Parameter	Description
Loop Volume	<p>Specifies the nominal size of the installed sample loop.</p> <p>Ensure that the correct loop size is selected. If the loop selection is incorrect this will affect the sample aspiration and positioning in the loop and will have a negative affect on data quality.</p>
Idle Flow Rate	<p>Specifies the solvent flow rate when the instrument is idle. This feature activates when the last sample in a batch is done.</p> <p>Maintaining long-term emitter stability (with glass emitters) might require constant flow through the system.</p>
Idle Mixture	<p>Specifies the solvent mixture as a percentage of solvent B when the instrument is idle. The default percentage of solvent B is 75% B.</p> <p>For best results, use a minimum of 70% B to help prevent deposition on the emitter tip, both metal and borosilicate.</p>
One Column Setup	<p>Specifies the one-column setup.</p> <p>The diagram on the Overview page of the Home menu displays the column setup.</p>

**Table 10.** HPLC parameter descriptions (Sheet 2 of 2)

Parameter	Description
Load Speed Protection	<p>When this check box is selected, the instrument uses the flow rate entered in the Sample Loading area on page 2 of the Method Setup wizard (see “<a href="#">Setting Up the Sample Pickup and Loading Steps</a>” on <a href="#">page 133</a>) to load the sample onto the precolumn (or the analytical column for a one-column setup). During a batch run, the sequence stops immediately if the system reaches the maximum pressure limit entered in the Sample Loading area.</p> <p>The LSP feature ensures that the solvent delivery system loads each sample onto the column at the same flow rate. Because the flow rate used to load the sample onto the column can affect sample-to-sample reproducibility for quantitation experiments, use the LSP feature to maximize sample-to-sample reproducibility for quantitation experiments.</p> <p>LSP is only active during the sample loading step of a run.</p> <p>To use the LSP feature correctly, you must enter both a flow and a maximum pressure limit for sample loading in the method file. To determine an appropriate flow rate for your LC columns, use the precolumn and analytical column equilibration scripts.</p>
Automatic Pre-run Maintenance	<p>Selecting this check box turns on the automatic Flush Air script. When you start a new batch from the touch-screen application or a new Xcalibur sequence from a data system computer, the instrument runs the automatic Flush Air script if the previous batch (or sequence) started more than 8 hours ago.</p> <p>This check box is selected by default. Thermo Fisher Scientific recommends leaving the check box selected.</p>

## **4 Configuring the EASY-nLC System**

Setting Up the Properties for the HPLC Device

# Preparing the EASY-nLC Instrument for Use

To prepare the EASY-nLC instrument for use, log in to the instrument with either Super User or Administrator privileges (see “Logging into the EASY-nLC Instrument” on page 66), and follow these procedures:

## Contents

- [Daily and Weekly Maintenance Tasks](#)
- [Preparing the Solvent Bottles and Waste Containers](#)
- [Executing Maintenance Scripts](#)
- [Purging and Flushing the Pumps](#)
- [Setting Up the Column Assembly](#)
- [Using nanoViper Fittings](#)
- [Using the Viper Union](#)
- [Equilibrating Columns](#)

## Daily and Weekly Maintenance Tasks

For optimal system performance, check the solvent levels, and run the Purge and Flush Air script on a daily basis. However, you do not need to stop a sequence that is already running to perform these actions.

Because the vapor pressures of formic acid, water, and acetonitrile differ, the solvent composition changes over time. Refill the solvent bottles with fresh solvent to maintain a consistent solvent composition on a weekly basis. Discard the old solvents and fill the bottles with new, do not top up.

### ❖ To check the solvent levels

1. Visually inspect solvent bottle A, solvent bottle B, and the autosampler bottle in position W3 (and the bottles in position W1 and W2 if used). Refill the bottles with fresh solvent if necessary.

**Tip** You can also inspect the purity of the solvent to ensure no visible precipitates have formed that might lead to blockages in the pump line.

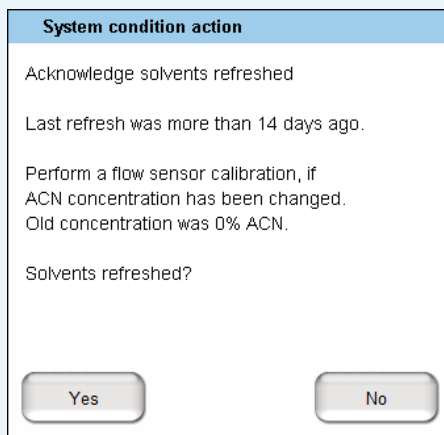
2. Visually inspect the autosampler wash bottle in position W4 and the waste container in the pump compartment, and empty them if necessary.

**Note** A solvent refresh reminder (“Solvent refresh due”) is shown on the left menu bar, if the last acknowledged solvent refresh is more than 2 weeks ago.

If the solvent refresh was acknowledged within the last 2 weeks, a “Solvents refreshed” button is shown.



To acknowledge that both solvents have been refilled, press either the “Solvent refresh due” or the “Solvents refreshed” button and choose “Yes”.



**System condition action**

Acknowledge solvents refreshed

Last refresh was more than 14 days ago.

Perform a flow sensor calibration, if ACN concentration has been changed.  
Old concentration was 0% ACN.

Solvents refreshed?

Yes No

❖ **To draw fresh solvent into the system and to flush air out of the system**

Run the **Purge Solvent** with two purge iterations. Then run the **Flush Air** script with a flush threshold of 12  $\mu$ L.

## Preparing the Solvent Bottles and Waste Containers

As you prepare the solvent bottles and empty the waste containers, you work with hazardous solvents and chemicals such as methanol, acetonitrile, formic acid, and so on. Before working with hazardous solvents and chemicals, review the specific hazards for each substance by reading the Material Safety Data Sheets (MSDSs) provided by the manufacturer. When working with solvents and chemicals, wear the appropriate safety gear.





**CAUTION** When working with solvents, wear safety glasses and safety gloves. Make sure that the gloves are compatible with the solvents you are using.

When working with volatile hazardous chemicals, use an appropriate fume hood.

Before shipment, the EASY-nLC instrument’s solvent system is flushed with methanol.



**CAUTION** Methanol (CAS number: 67-56-1) is highly flammable and toxic by inhalation, ingestion, or skin absorption. Take appropriate measures to protect yourself and your equipment. Make sure that the mobile phases are miscible with methanol or flush the A and B solvent lines with an intermediate solvent.

To set up the two bottles that contain the mobile phase solvents, the four autosampler bottles, and the waste beaker, follow these procedures:

- “Preparing the Solvents,” next section
- “Preparing the Solvent A and B Bottles” on page 90
- “Installing the Autosampler Wash Bottles and Waste Beaker” on page 92

## Preparing the Solvents

The installation solvents for the EASY-nLC 1200 instrument are as follows:

- Solvent A: 0.1% formic acid in water  
 Solvent B: 0.1% formic acid in a mixture of 80% acetonitrile in water  
 Wash solvent 3: 0.1% formic acid in water

**Note** Acetonitrile concentrations greater than 95% in water can shorten the lifetime of components.

To prepare these solutions, order the appropriate UHPLC-MS or LC/MS-grade solvents or solvent blends. Table 11 lists the solvents and solvent blends that you can order from Thermo Fisher Scientific.

Thermo Fisher Scientific recommends using pre-mixed solvents to enhance reproducibility.

**Table 11.** Solvents

Solvent/reagent	Grade	Size	Catalog number
Water	Optima™ UHPLC-MS	1 L	W8-1
Acetonitrile	Optima™ UHPLC-MS	1 L	A956-1
Formic acid (99.5%)	Optima™ LC/MS	10×1mL	A117-10X1AMP

**Table 11.** Solvents

Solvent/reagent	Grade	Size	Catalog number
Water with 0.1% formic acid	Optima™ LC/MS	1 L	LS118-1
80% acetonitrile, 20% water with 0.1% formic acid	Optima™ LC/MS	500mL	LS122-500
0.1% formic acid in acetonitrile	Optima™ LC/MS	1 L	LS120-1



**CAUTION** Solvent contaminants can cause system blockages and poor spray stability. To minimize instrument problems caused by solvent contaminants, follow these guidelines for the EASY-nLC instrument:

- Use only UHPLC-MS or LC/MS-grade solvents.
- Use only commercially manufactured LC/MS-grade solvent blends, as mixing solvents in a typical laboratory environment can introduce contaminants as well as solvent concentration errors.
- Do **not** filter UHPLC-MS-grade solvents, as filtering solvents can introduce contaminants.
- Do **not** use HPLC-grade solvents, as HPLC-grade solvents contain more contaminants than LC/MS-grade solvents.
- Do **not** use water from laboratory purification systems, as laboratory purified water contains more contaminants than UHPLC-MS-grade water.



**CAUTION** Store and handle all chemicals in accordance with standard safety procedures.

The Optima LC/MS mobile phases can be ordered through [www.fishersci.com](http://www.fishersci.com) (search for Optima LC/MS)

## Preparing the Solvent A and B Bottles

Follow these recommended steps to prepare the two solvent bottles:

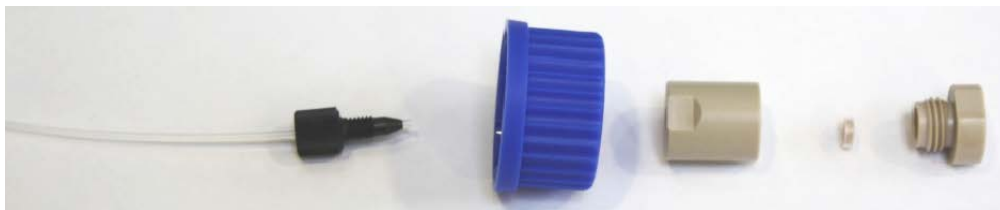
### ❖ To prepare the solvent A and B bottles

1. Fill the 25 ml Schott Duran™ blue cap bottles with the mobile phase solvents.
2. Degas the solvents by sonicating the solvent bottles in a sonication bath or by sparging the solvents with helium gas.

**Note** Sparging can lead to variation in solvent concentrations.

3. Place the bottles in the holder on top of the instrument: solvent A bottle in the front and solvent B bottle in the back position.
4. Mount the inline filter assembly on the blue cap lids as shown in [Figure 55](#) and [Figure 56](#).

**Figure 55.** Inline filter assembly (exploded view)



**Figure 56.** Assembled inline filter



**IMPORTANT** To establish proper surface wetting, prime the filters with methanol or acetonitrile.

## Installing the Autosampler Wash Bottles and Waste Beaker

The autosampler compartment holds the sample trays, up to four wash bottles, and a waste beaker (see Figure 57). You can operate the instrument after installing the wash bottle that the autosampler uses for needle cleaning (position W4), the wash bottle that contains solvent A for pump S (position W3), and the waste beaker that collects the waste from pumps A and B and from the venting Tee through valve W.

To access the autosampler compartment, press the Eject/Insert Tray button on the Home > Overview page.

**Figure 57.** Autosampler compartment



### ❖ To install the waste beaker and prepare and install the wash bottles

1. Fill one of the wash bottles with solvent A and degas it.
2. Turn on the instrument and make sure that the area in front of the tray compartment door is free from obstruction.
3. Press **Home > Overview**. Then press **Eject/Insert Tray**.

A confirmation box appears to remind you to remove obstacles from the area in front of the tray compartment before commanding the instrument to open the tray compartment door.

4. Press **Accept**.

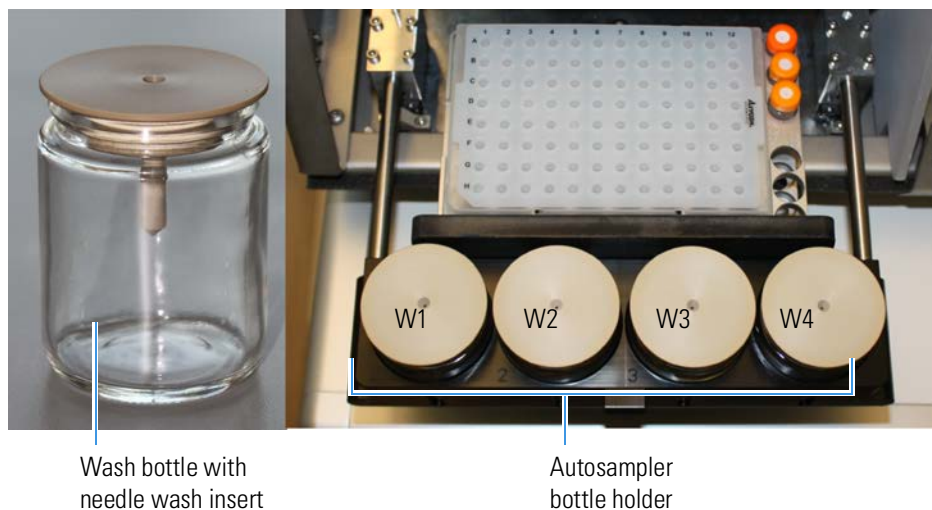
The tray compartment door opens.

5. Put the bottle with the needle wash insert into position W4 (see [Figure 58](#)).



**CAUTION** You must place the wash bottle with the needle wash insert in position W4. Placing this bottle in any other position will cause irreparable damage to the autosampler needle during an injection cycle.

**Figure 58.** Wash bottle with needle wash insert and view of the four positions on the autosampler bottle holder



6. Put a wash bottle that contains solvent A into position W3.

Pump S draws solvent from this position. You can place wash solvents for customized washing procedures in positions W1 and W2.

7. Make sure all the bottles are installed with the lids fitting tightly.
8. Place the waste container (a 250 mL plastic beaker) into the compartment on the right side of the autosampler compartment.
9. Press **Eject/Insert Tray** to move the plate back into the instrument.

## Executing Maintenance Scripts

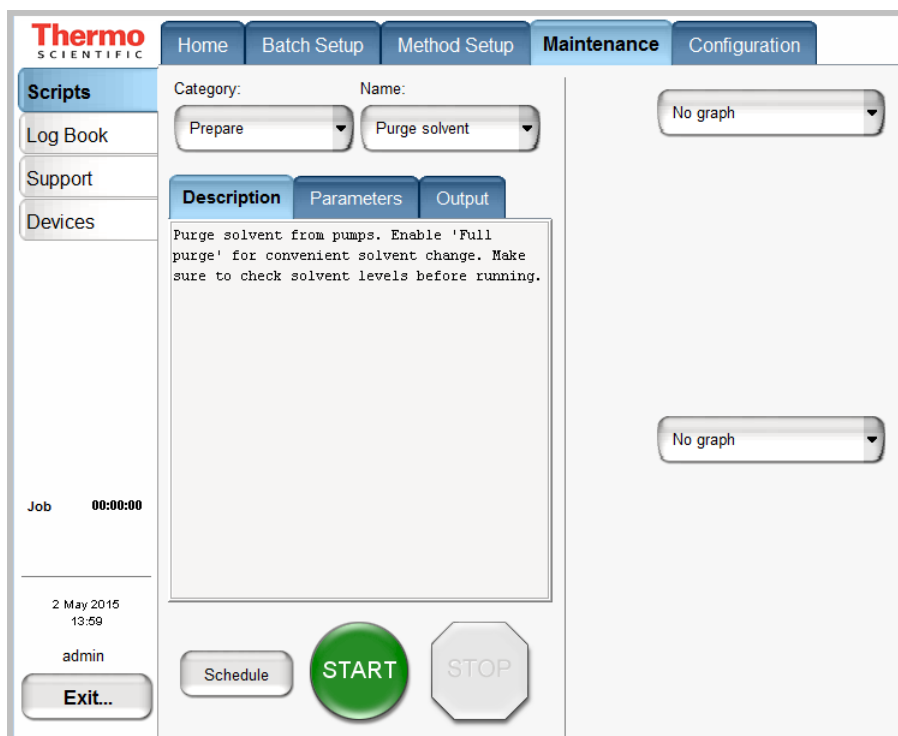
The EASY-nLC application has a set of maintenance scripts that you can access from the Scripts page (Figure 59) of the Maintenance menu.

Use the scripts in the Prepare category for common tasks such as drawing fresh solvent through the solvent system, flushing air out of the system, and equilibrating the columns.

Before you start any of the maintenance scripts, prepare the instrument as described in “Preparing the Solvent Bottles and Waste Containers” on page 88.

**Note** You cannot execute maintenance scripts when the EASY-nLC instrument is running batches.

**Figure 59.** Scripts page of the Maintenance menu



The green START button is active when a job or script is ready to execute. After you press START, the STOP button replaces the START button and remains active until the job or script ends. During execution you can terminate a job or script by pressing the red STOP button.

When the Schedule button is available, you can automate the start of the Flow Sensor calibration script and all of the scripts in the Prepare category. You cannot schedule the remaining scripts because they require supervision or hardware changes to the instrument.

## Purging and Flushing the Pumps

Because the EASY-nLC pumps are purged with methanol before delivery, thoroughly purge the pumps with the mobile phase solvents before doing any analytical runs. In addition, purge the pumps when switching to a new solvent.

The purge pump job fills the selected pump or pumps with solvent and then ejects the solvent into the waste container. You can specify how many iterations of this job you want performed.

After purging the pumps, flush them to remove any air that might be trapped inside.

### ❖ To purge and flush pumps A and B

1. Before you purge the pumps with fresh solvent, make sure that the solvent bottles A and B on the top of the instrument are filled with solvent.
2. Press **Maintenance > Scripts**.

The Scripts page of the Maintenance menu opens.

3. Set up the purge solvents job as follows (see [Figure 60](#)):
  - a. In the Category list, select **Prepare**.
  - b. In the Name list, select **Purge Solvent**.
  - c. Press the **Parameters** tab.
  - d. In the Purge Iterations box, enter **10**.

This is the number of times that the pumps will completely refill and eject the solvent.

**Tip** To enter the number of purge iterations, press the empty cell in the Value column. The Set Value dialog box appears. Use the numeric keypad to type the number of purge iterations that you want. Then press **Accept**. The new value appears in the Value column.

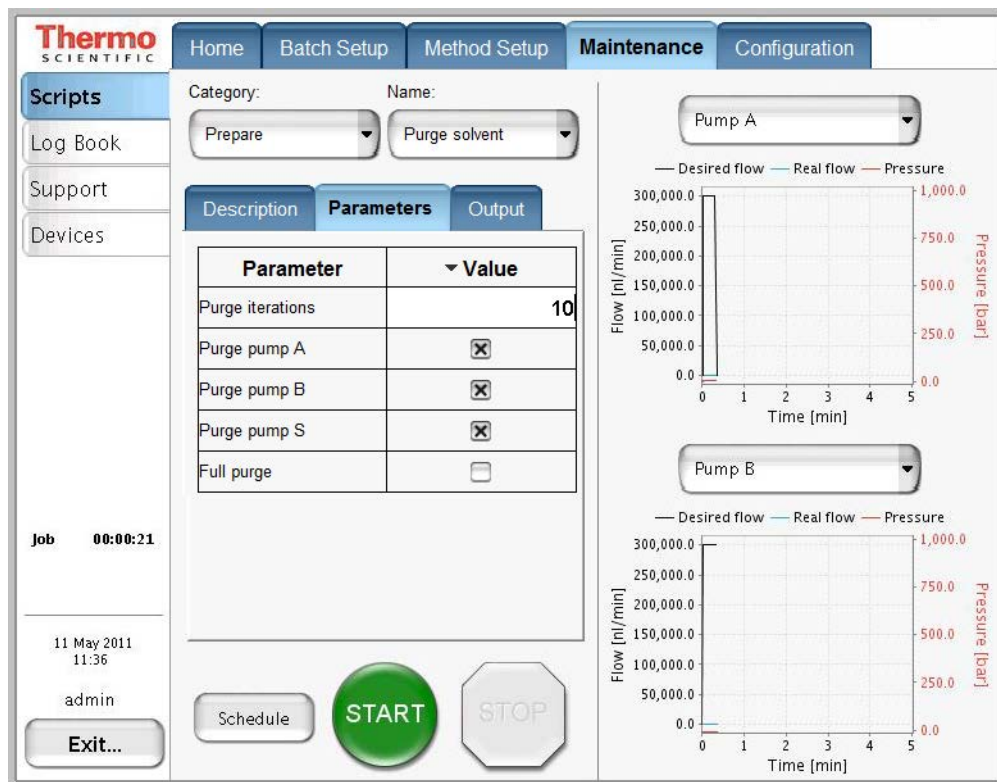
- e. Select the **Purge Pump A**, **Purge Pump B**, and **Purge Pump S** check boxes.

**Note** The pumps draw and eject solvent as follows:

- Pump S draws solvent from bottle W3 in the autosampler bottle holder and ejects solvent into bottle W4.
- Pump A and pump B draw solvent from the solvent A and B bottles on top of the instrument and eject solvent to the waste cup.

- f. The option “full purge” sets the purge iterations for pumps A & B to 10 iterations and for pump S to 5 iterations. The “full purge” option is recommended for refreshment of solvents in all pumps.

Figure 60. Purge solvent job parameters



4. To save these settings and continue, press **Schedule**.

The Queue page of the Home menu appears.

5. Set up the flush air job as follows (see Figure 61):
  - a. Press **Maintenance > Scripts**.
  - b. In the Category list, select **Prepare**.
  - c. In the Name list, select **Flush Air**.
  - d. Press the **Parameters** tab.
  - e. In the Flush Vol. Threshold [ $\mu$ L] box, enter 12 $\mu$ l.

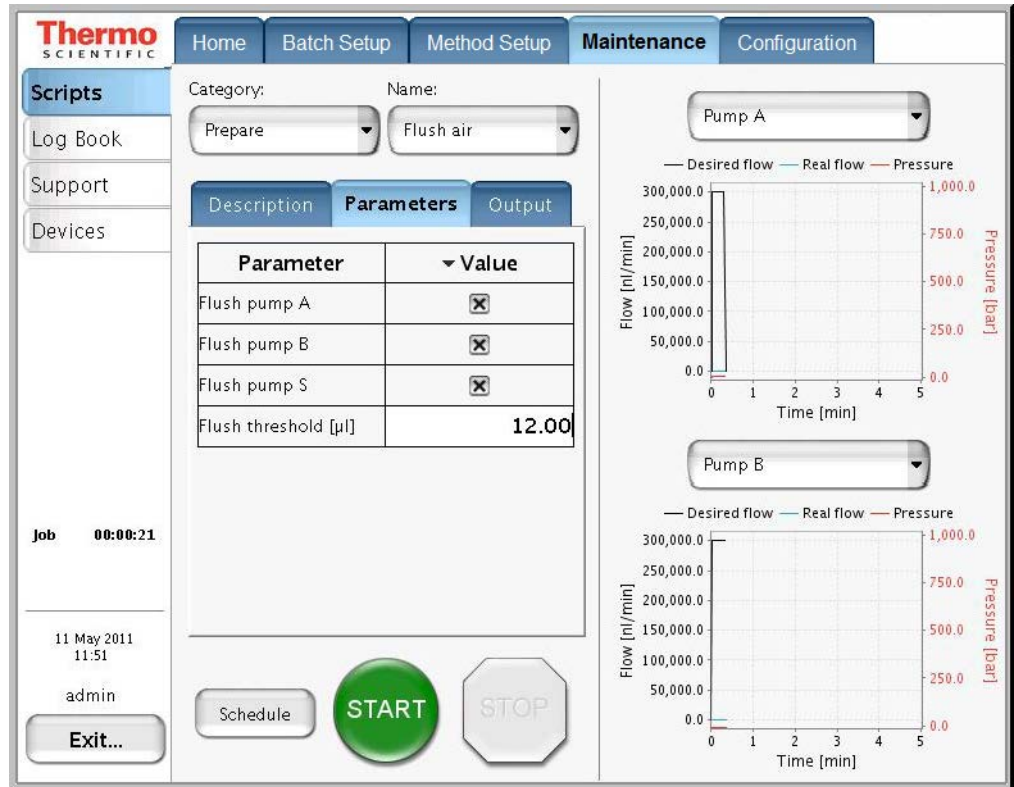
This values helps to ensure an acceptable amount of solvent compressibility.

**Tip** To enter the flush volume, press the empty cell in the Value column. The Set Value dialog box appears. Use the numeric keypad to type the volume that you want. Then press **Accept**. The new value appears in the Value column.

- f. Select the **Flush Pump A**, **Flush Pump B**, and **Flush Pump S** check boxes.



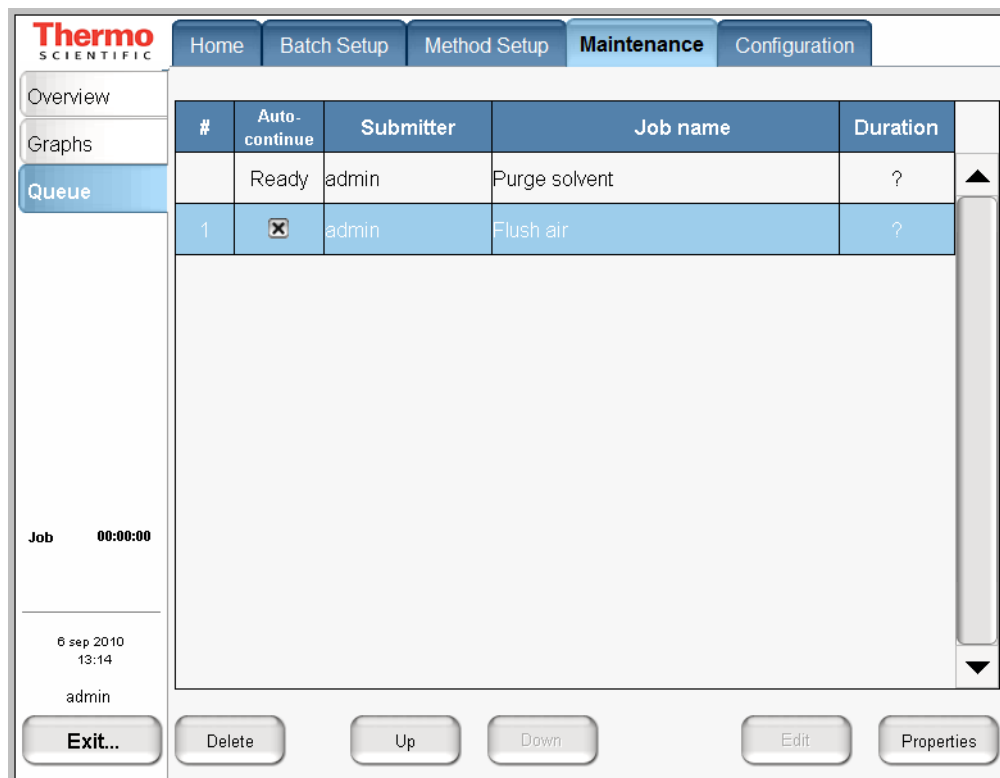
Figure 61. Flush air job parameters



6. Press **Schedule**.

The Queue page (Figure 62) of the Home menu appears.

Figure 62. Queue page of the Home menu, showing the queued jobs



7. In the Jobs list, make sure the check box in the Auto-Continue column is selected for **Flush Air**.
8. Press **Overview**.  
The Overview page of the Home menu opens.
9. Press **START**.  
The default selections appear in the two graph windows on the Scripts page of the Maintenance menu, but you can change the selection for a window from its corresponding list. These graphs help you to monitor the job.
10. Wait for the jobs to end (the flush air job continues until the flush air volume values are below the specified threshold).

**Note** While a maintenance job or a script is running, you cannot start other jobs or scripts.

## Setting Up the Column Assembly

To integrate the EASY-nLC instrument with a Thermo Scientific mass spectrometer, Thermo Fisher Scientific provides two nanospray ion sources: the Nanospray Flex source and the EASY-Spray source.

You use different columns for these two sources. The Nanospray Flex source uses standard nanoflow columns for both the one- or two-column setup. The EASY-Spray source uses an EASY-Spray column as the analytical column in both the one- or two-column setup. To create a two-column setup for the EASY-Spray source, use a standard nanoflow column with nanoViper fittings as the precolumn.

A complete Nano LC Connections Guide is available here:

<https://tools.thermofisher.com/content/sfs/manuals/PP-64583-LC-MS-NanoLC-Complete-Easy-Guide-PP64583-EN.pdf>

To set up the column assembly for your nanospray source, follow the appropriate procedure:

- “Setting Up the Column Assembly for the Nanospray Flex Source,” next section
- “Setting Up the Column Assembly for the EASY-Spray Source” on page 116

### Setting Up the Column Assembly for the Nanospray Flex Source

If your LC/MS system has a Nanospray Flex ion source, follow the appropriate procedures to set up the column assembly.

- “Setting Up the Column Assembly for an EASY-nLC 1200 Instrument,” next section
- “Setting Up a Column Assembly with Fused-Silica Columns” on page 103
- “Connecting a Sleeved Emitter to the Analytical Column” on page 115



**CAUTION** Wear protective gloves and safety glasses when handling the solvent lines. To prevent contamination, use lint-free and powder-free gloves.

### Setting Up the Column Assembly for an EASY-nLC 1200 Instrument

For an EASY-nLC Instrument with a Nanospray Flex ion source, you can use standard columns with nanoViper fittings or fused-silica columns.

Depending on the columns you are installing, follow the appropriate procedure:

- “Setting Up a Standard Column Assembly,” next section
- “Setting Up a Column Assembly with Fused-Silica Columns” on page 103

### Setting Up a Standard Column Assembly

Use this procedure for an EASY-nLC 1200 instrument with a Nanospray Flex ion source and standard columns that have nanoViper fittings.

Table 12 and Table 13 list the columns, fittings, and unions that make up the two-column assembly.

**Note** The EASY-nLC 1200 instrument ships with two analytical columns: an EASY-Spray column and a standard analytical column with nanoViper fittings. For set up a two-column assembly, you must order the precolumn.

**Table 12.** Column fittings and precolumn (trap column)

Item	Description	Part number
Precolumn (trap column) with nanoViper fittings	Acclaim™ PepMap 100 C18, 3 μm, Separation Bed 75 μm i.d. x 2 cm, Total Length 70 mm with nanoViper connections	164946
PEEK ZDV union with fittings	0.76 in. length, 0.035 in. thru-port, for 1/32 in. OD tubing	G10-0035 (IDEX PN P-771)
Stainless steel union, ZDV (Viper™ union)	For 1/16 in. OD tubing (HPLC-to-Column connector)	SC900
Venting Tee	Stainless steel, 0.15 mm bore, for 1/16 in. OD tubing (nanoliter-dead-volume-tee)	SC901
PEEK sleeves (black) for 280 μm fused-silica	330 μm ID, 1/32 in. OD	SC903

**Table 13.** Acclaim™ PepMap™ C18, 2 μm nanoViper columns with 45 mm fused silica at end

Column length (cm)	Column OD (μm)	Column ID (μm)	Part number
15	280	50	164943
15	280	75	164940
25	280	50	164944
25	280	75	164941
50	280	50	164945
50	280	75	164942
75	360	75	164939

**Table 14.** EASY-Spray C18 columns

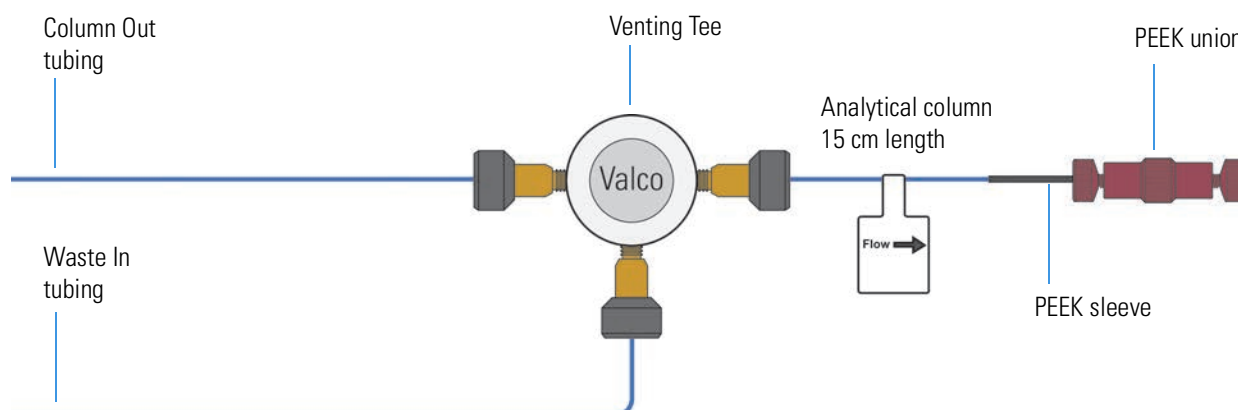
Column length (cm)	Column OD ( $\mu\text{m}$ )	Column ID ( $\mu\text{m}$ )	Part number
15	75	3	ES800A
15	50	2	ES801A
25	75	2	ES802A
50	75	2	ES803A
15	75	2	ES804A
75	75	2	ES805A

Make sure that you connect the venting Tee to the Waste In line for both the one-column and two-column setups.

❖ **To connect the column assembly for the Nanospray Flex source to the EASY-nLC 1200 instrument**

1. Do one of the following:
  - For a one-column setup, go to [step 2](#).
  - For a two-column setup, go to [step a](#).
  - a. For a one-column setup, connect the Column Out line directly to the venting Tee ([Figure 63](#)). Then go to [step 3](#).

**Figure 63.** One-column assembly connected to the EASY-nLC 1200 instrument's Column Out and Waste In tubing



2. For a two-column setup, do the following:
  - a. Connect one end of the precolumn to the receiving port of the venting Tee that is parallel to the analytical column. Slowly tighten the fitting until you feel resistance.
  - b. Connect the Viper union (stainless steel ZDV) to the free end of the precolumn. Slowly tighten the fitting until you feel resistance.

## 5 Preparing the EASY-nLC Instrument for Use

### Setting Up the Column Assembly

- c. Insert the nanoViper fitting on the Column Out line into the free end of the Viper union. Slowly tighten the fitting until you feel resistance.
- d. Tighten the nanoViper fittings by an additional 45 degrees (1/8-turn). Do not tighten the fittings by more than a 1/4-turn (see “Using nanoViper Fittings” on page 119 and “Using the Viper Union” on page 121).

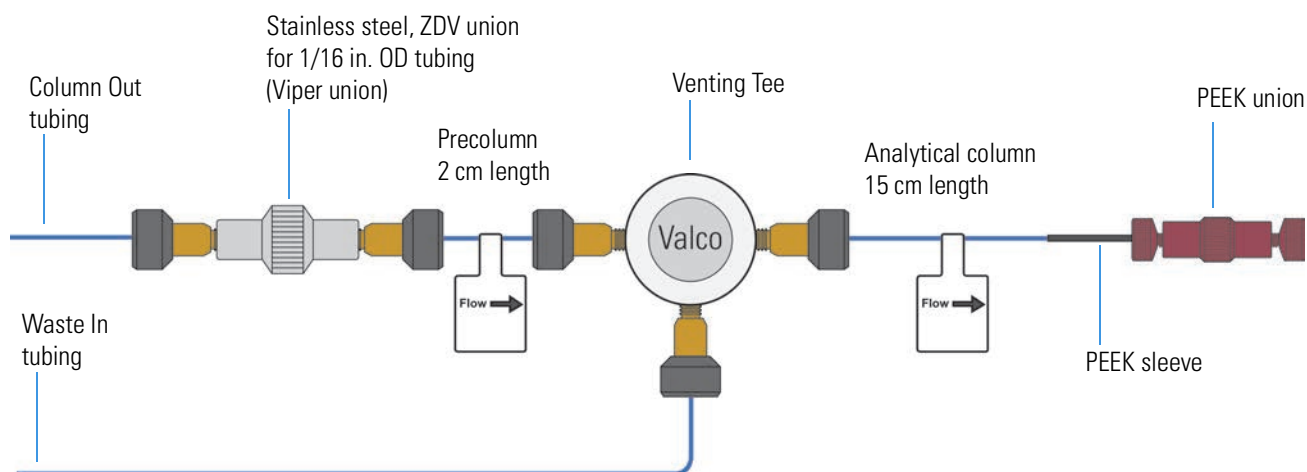


**CAUTION** Because the fittings seal against each other inside the union, do not tighten them by more than 90 degrees (1/4-turn). Overtightening the fittings can irreparably damage their sealing surfaces.

3. Connect the Waste In line to the venting Tee as follows:
  - a. Remove the plug from the third port of the venting Tee.
  - b. Insert the nanoViper fitting at the end of the Waste In line into the free port of the Tee (see Figure 63 and Figure 64). Slowly tighten the fitting until you feel resistance. Then tighten the fitting by an additional 1/8- to 1/4-turn. Do not tighten the fitting by more than a 1/4-turn (see “Using nanoViper Fittings” on page 119).

Figure 64 shows the two-column setup. Both the one-column and two-column setups use the venting Tee.

**Figure 64.** Two-column assembly connected to the EASY-nLC 1200 instrument's Column Out and Waste In tubing



## Setting Up a Column Assembly with Fused-Silica Columns


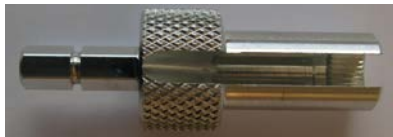
Use this procedure for an EASY-nLC 1200 instrument with a Nanospray Flex ion source and columns that have exposed fused-silica ends.

When you are using a Nanospray Flex ion source and you want to use a pulled fused-silica analytical column, you must modify the EASY-nLC 1200 instrument and install the column using the components in the EASY-nLC UHPLC Liquid Junction Kit.

For information about modifying the EASY-nLC instrument by installing the Column Out and Waste In lines that are supplied in the EASY-nLC UHPLC Liquid Junction Kit, refer to the *EASY-nLC 1200 Troubleshooting and Maintenance Guide*.

Table 15 lists the items in the UHPLC Liquid Junction Kit (ES269).

**Table 15.** UHPLC Liquid Junction Kit contents (Sheet 1 of 2)

Description	Part number
Column Out line with a nanoViper fitting at one end and bare fused-silica tubing at the other end	6041.5290
Waste In line with a nanoViper fitting on one end and bare fused-silica tubing at the other end	6041.5289
UHPLC liquid junction cross with a platinum electrode and two-piece protective cover	N/A
 <p>The image shows the UHPLC liquid junction cross, which is a black cylindrical component with a central fitting. Below it are two protective covers: a bottom cover and a top cover. The top cover features a yellow lightning bolt warning symbol. Two screws are also shown next to the covers.</p>	
Tightening tool used to torque internally-threaded knurled nut fittings (quantity: 2)	(IDEX) P-278
	 <p>The image shows a tightening tool, which is a long, thin metal rod with a knurled section in the middle and a hexagonal end.</p>

**Table 15.** UHPLC Liquid Junction Kit contents (Sheet 2 of 2)




Description		Part number
Microferrules (quantity: 3)		(IDEX) PK-152
Blind plug microferrule		(IDEX) P-116
UHPLC fused-silica union, which consists of the following: <ul style="list-style-type: none"> <li>• PEEK holder</li> <li>• Two internally-threaded knurled nuts</li> <li>• Two microferrules</li> <li>• Stainless steel cartridge with coned ports and a 280 µm thru-hole</li> </ul>		ES272

Figure 67 shows an internal view of this union.

**Note** The UHPLC fused-silica union has two functions:

- For a two-column setup, it connects the Column Out line to the inlet of a 360 µm OD precolumn.
- For system leak testing and backpressure testing, it connects the Column Out line to the Waste In line. For information about leak testing, refer to the *EASY-nLC Series Troubleshooting and Maintenance Guide*.

Figure 65 shows a one-column setup with the modified Column Out and Waste In lines and a fused-silica column with an integrated emitter.

**Figure 65.** One-column setup with the modified Column Out and Waste In lines

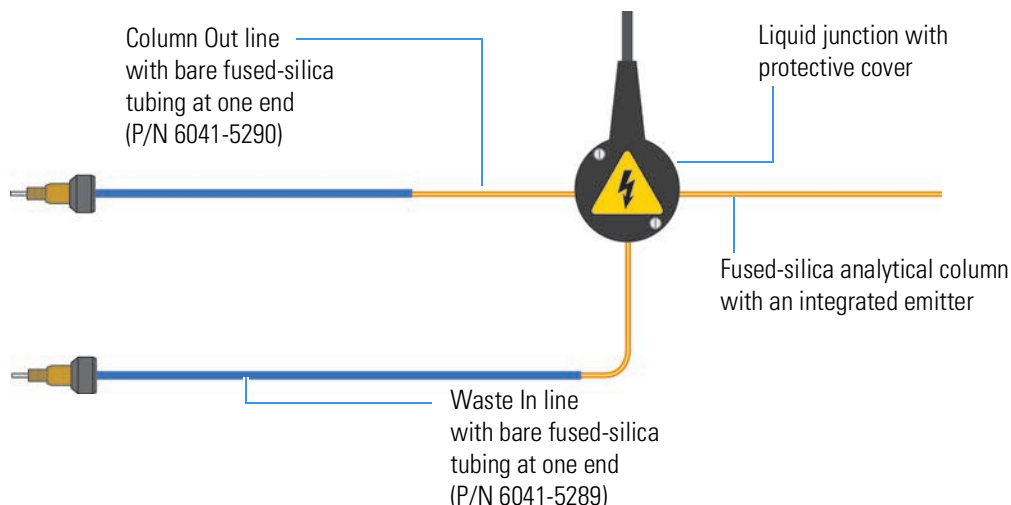
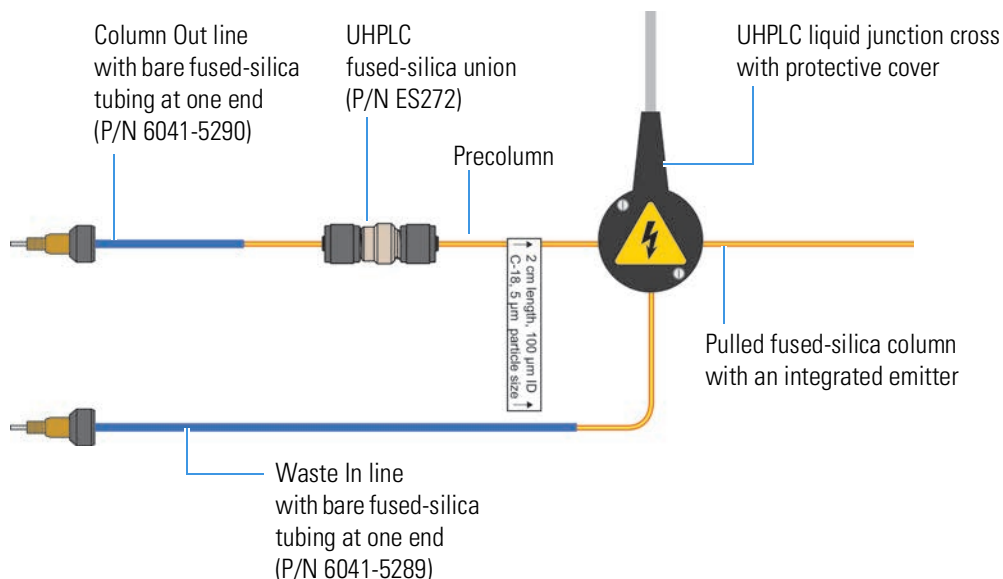




Figure 66 shows a two-column setup with the modified Column Out and Waste In lines and an analytical column with an integrated emitter. Both the precolumn and analytical column have bare fused-silica ends. The UHPLC fused-silica union connects the Column Out line to the precolumn's inlet.

**Figure 66.** Two-column setup with the modified Column Out and Waste In lines and an analytical column with an integrated emitter



The UHPLC fused-silica union is designed for a two-column assembly that includes nanoflow columns with fused-silica ends. Figure 67 shows an enlarged cross section of this union. The ends of the externally-threaded PEEK holder have different internal depths. The stainless steel cartridge, which has coned ports and a 280 µm thru-hole, fits within the deeper end of the PEEK holder. The microferrules are designed for sleeveless use with 360 µm OD fused-silica tubing. The two internally-threaded knurled nuts secure the tubing to the PEEK holder.

**Figure 67.** UHPLC fused-silica union (enlarged cross section)

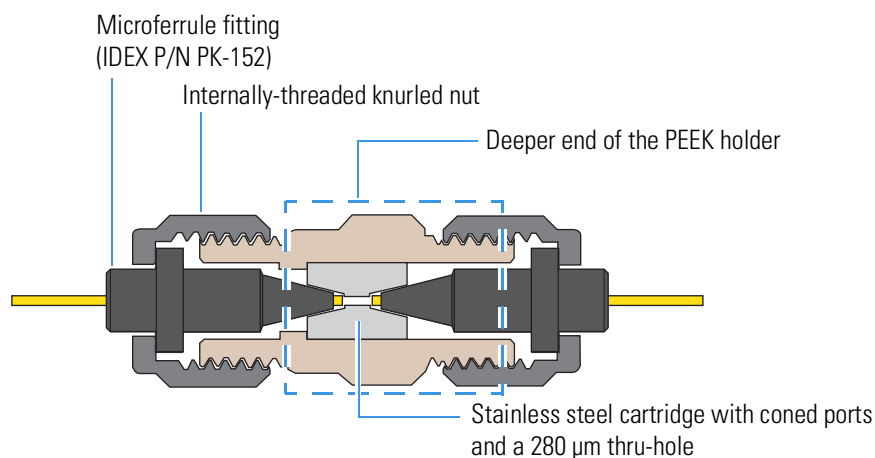
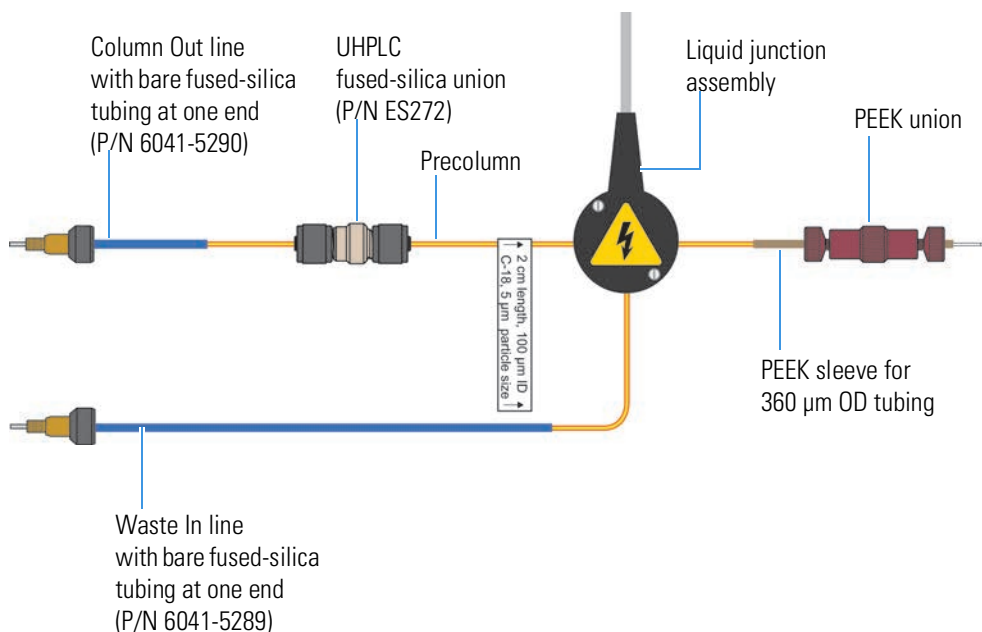


Figure 68 shows a two-column setup with the modified Column Out and Waste In lines and an analytical column with an external emitter.

**Figure 68.** Two-column setup with the modified Column Out and Waste In lines and an analytical column with an external emitter



Use the following tools and materials to set up the column assembly.

Tools	Parts and materials
<ul style="list-style-type: none"> <li>• Small flat-blade screwdriver</li> <li>• Tightening tool (in kit)</li> </ul>	<ul style="list-style-type: none"> <li>• UHPLC Liquid Junction Kit (P/N ES269)</li> <li>• Powder-free safety gloves</li> </ul>

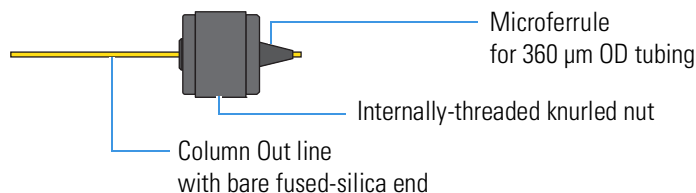
To install the column assembly and prepare the system for operation, follow these procedures:

- To connect the column assembly to the UHPLC liquid junction cross, do one of the following:
  - “To connect a two-column assembly to the UHPLC liquid junction cross,” next procedure
  - or–
  - “To connect a one-column assembly to the UHPLC liquid junction cross” on page 109
- “To mount the UHPLC liquid junction cross onto the DirectJunction” on page 110
- “To prepare a system with the UHPLC liquid junction cross for operation and optimize its performance” on page 112

❖ **To connect a two-column assembly to the UHPLC liquid junction cross**

1. Slip an internally-threaded nut and a microferrule (components of the UHPLC fused-silica union) onto the Column Out line (Figure 69).

**Figure 69.** Column Out line with nut and ferrule



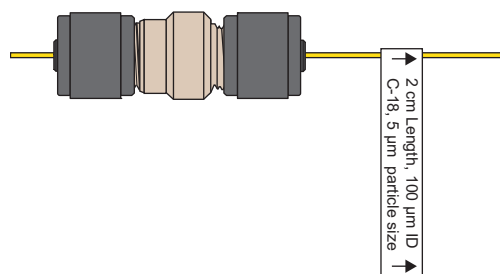
2. Insert the stainless steel cartridge into the deeper end of the externally-threaded PEEK holder (see Figure 67 on page 105). Then insert the Column Out line into the deeper end of the PEEK holder and hand-tighten the nut (Figure 70).

**Figure 70.** Column Out line connected to the deeper end of the PEEK holder



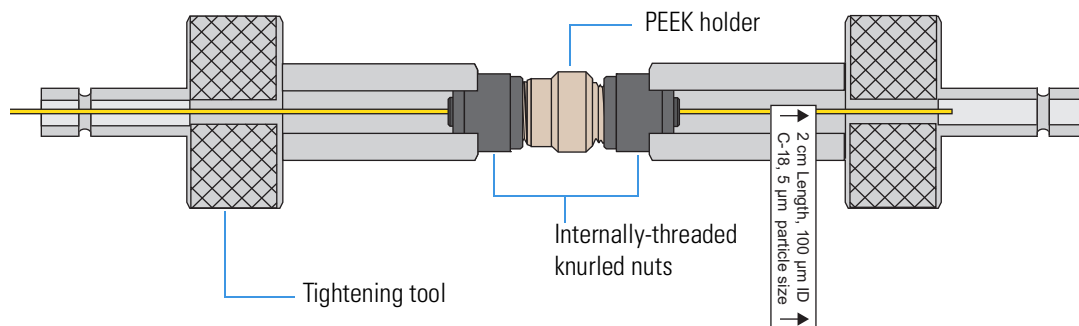
3. Slip an internally-threaded knurled nut and a ferrule onto the inlet end of a precolumn. Then insert the inlet end of the precolumn into the PEEK holder and hand-tighten the nut (Figure 71).

**Figure 71.** Column Out line and precolumn connected to the PEEK holder



4. Using the tightening tool provided in the UHPLC Liquid Junction Kit, tighten the internally-threaded knurled nuts an additional one-quarter turn (Figure 72).

**Figure 72.** Using the tightening tools to tighten the nuts an additional one-quarter turn





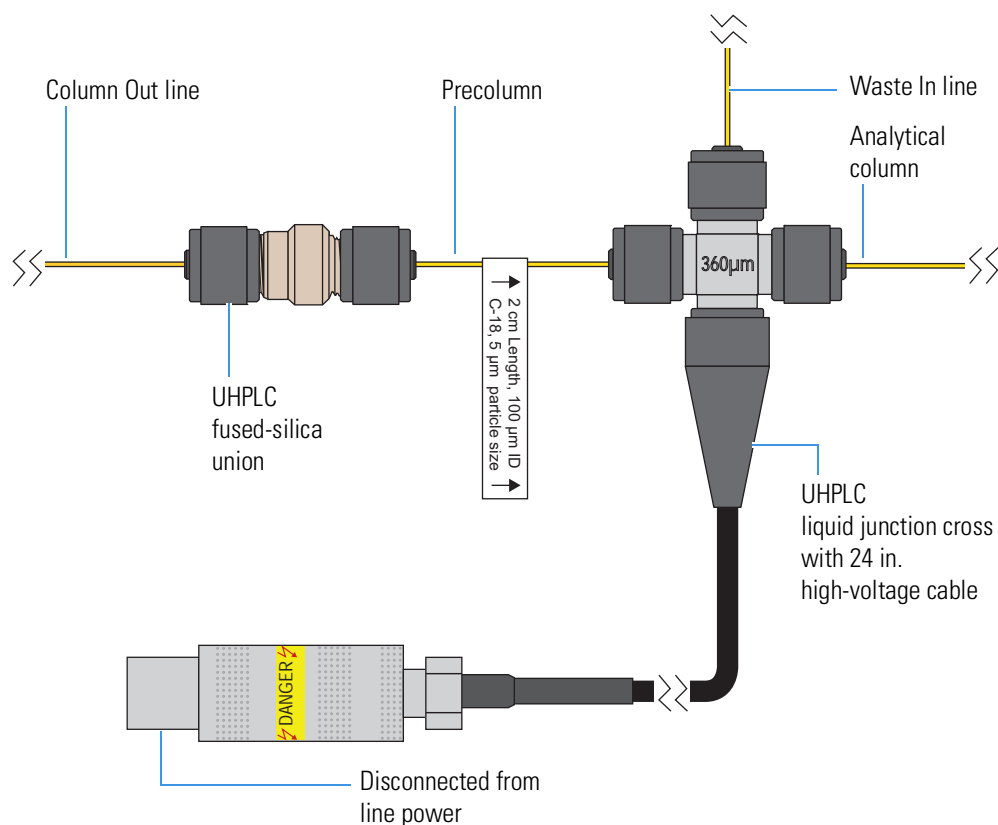
**CAUTION Danger Electric Shock Hazard!**

Before handling the UHPLC liquid junction cross, make sure that it is disconnected from line power.

- Remove the internally-threaded knurled nuts and the microferrules from the UHPLC liquid junction cross. Using these nuts and ferrules, connect the precolumn, the analytical column, and the Waste In lines to the UHPLC liquid junction cross as follows:
  - Connect the outlet of the precolumn and the inlet of the analytical column to the parallel ends of the cross.
  - Connect the Waste In line to the perpendicular end of the cross.

Figure 73 shows the plumbing connections for the UHPLC liquid junction cross. Make sure that the columns connect to the parallel ends of the cross.

**Figure 73.** UHPLC liquid junction cross connections for a two-column assembly



- Tighten the three internally-threaded knurled nuts an additional one-quarter turn using the tightening tool.

❖ To connect a one-column assembly to the UHPLC liquid junction cross

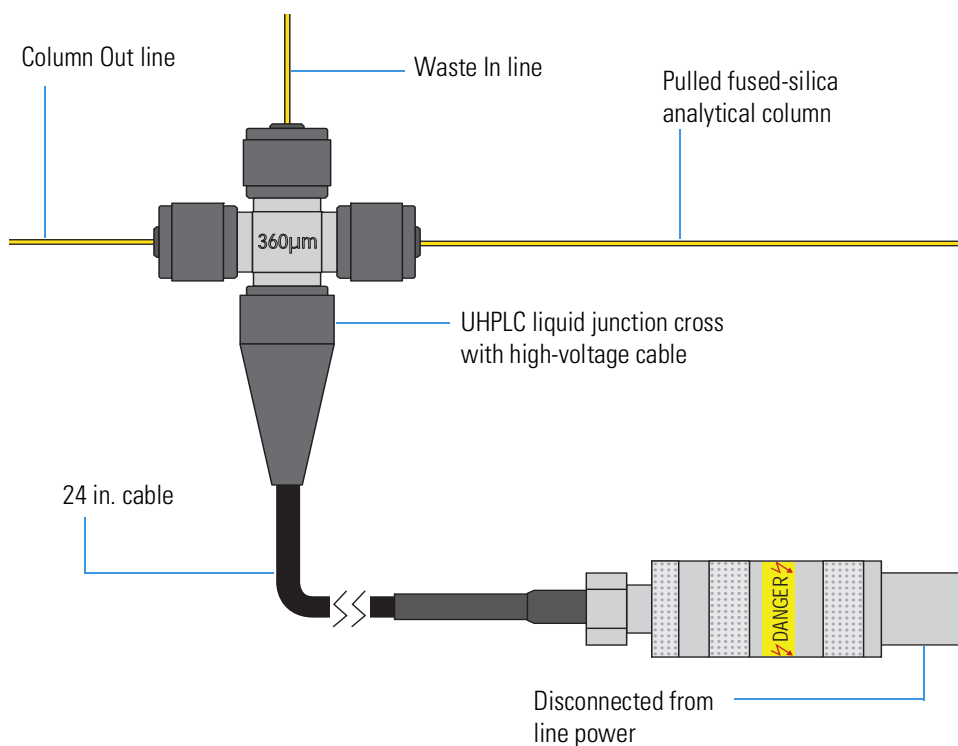


**CAUTION** Before handling the UHPLC liquid junction cross, make sure that it is disconnected from line power.

1. Remove the externally-threaded knurled nuts and the microferrules from the UHPLC liquid junction cross. Then, using these nuts and ferrules, connect the Column Out line, the analytical column, and the Waste In lines to the UHPLC liquid junction cross as follows:
  - Connect the Column Out line and the inlet of the analytical column to the parallel ends of the cross.
  - Connect the Waste In line to the perpendicular end of the cross.

Figure 74 shows the plumbing connections for the UHPLC liquid junction cross. Make sure that the Column Out line and the analytical column connect to the parallel ends of the cross.

**Figure 74.** UHPLC liquid junction cross connections for a one-column setup



2. Tighten the three internally-threaded knurled nuts an additional one-quarter turn using the tightening extender tool.

❖ To mount the UHPLC liquid junction cross onto the DirectJunction

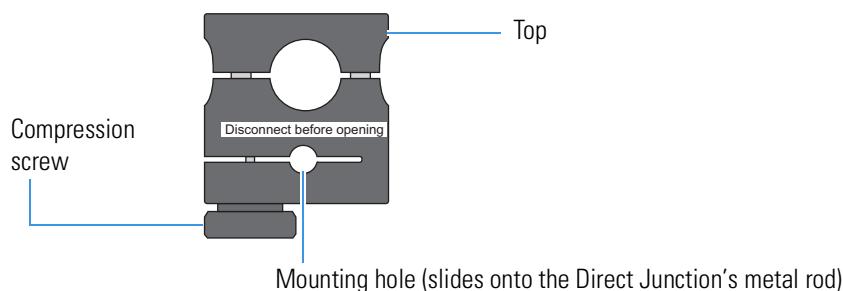


**CAUTION** During operation, the UHPLC liquid junction cross is at high voltage. To avoid personal injury, make sure that the UHPLC liquid junction cross is securely mounted inside its two-piece protective cover.

1. Using a flat-blade screwdriver, remove the two screws that secure the top of the protective cover to its body.

Figure 75 shows a profile view of the two-piece protective cover.

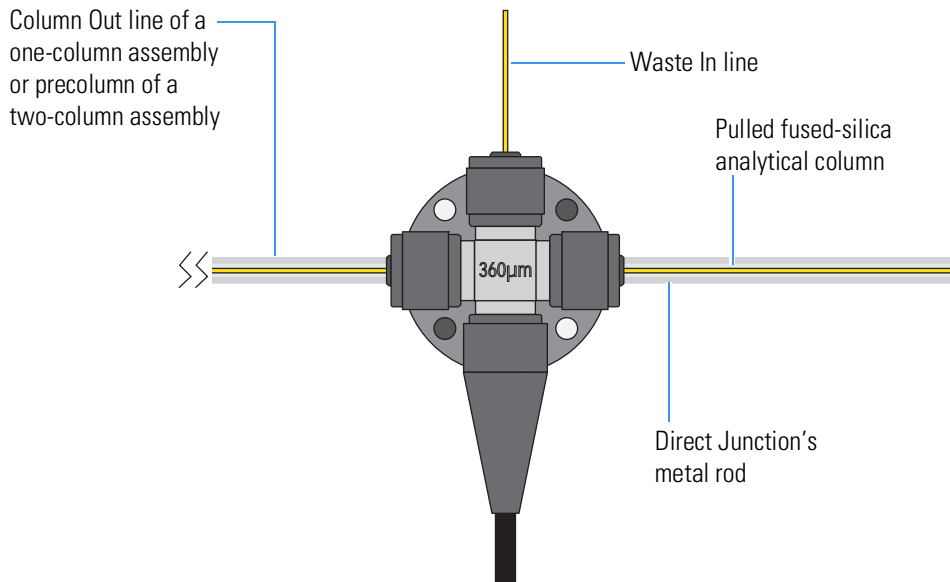
**Figure 75.** Protective cover (profile view)



2. Slide the protective cover's body onto the DirectJunction's metal rod. If necessary, loosen the compression screw in the body until the hole in the body fits over the rod, and tighten the screw.
3. Mount the UHPLC liquid junction cross onto the body of the protective cover (Figure 76). Then, place the top of the protective cover over the liquid junction cross.

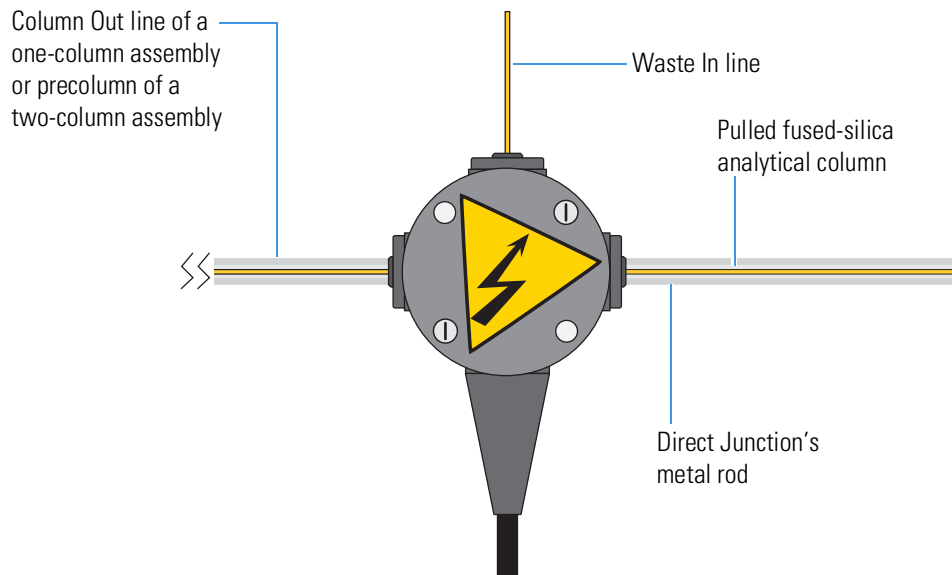
Both the top and the body of the cover contain two magnets. When you place the top onto the body, the magnetic attraction holds the two pieces together.

**Figure 76.** UHPLC liquid junction cross mounted onto the body of the protective cover



- Using the two screws that you removed in [step 1](#) on [page 110](#), secure the top of the protective cover to its body ([Figure 77](#)).

**Figure 77.** UHPLC liquid junction cross secured within the protective cover



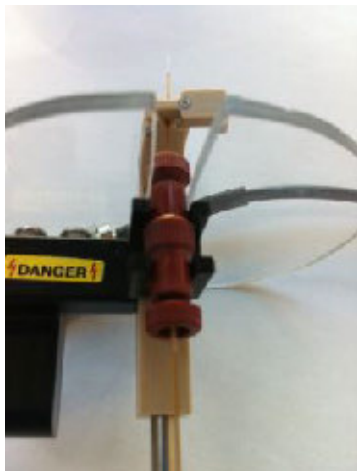
- If necessary, connect an emitter to the outlet of the analytical column as described in [“Connecting a Sleeved Emitter to the Analytical Column”](#) on [page 115](#).
- Mount the emitter on the DirectJunction and secure it with the clamp ([Figure 78](#)).

**Figure 78.** Column assembly mounted on DirectJunction



7. If the column is longer than the DirectJunction, use a 1/32 in. PEEK connector G10-0035 (IDEX PN P-771) to secure the emitter tip (Figure 79).

**Figure 79.** PEEK connector used to secure a long analytical column



8. Connect the high voltage cable from the UHPLC liquid junction cross to the socket underneath the ion source (Figure 80).

**Figure 80.** High-voltage cable connected to the ion source



❖ **To prepare a system with the UHPLC liquid junction cross for operation and optimize its performance**

1. Turn on the EASY-nLC instrument and log in (see “[Logging into the EASY-nLC Instrument](#)” on page 66).
2. Remove air from the Waste In and Column Out lines by running the Precolumn Equilibration script. Do the following:
  - a. Open the Precolumn Equilibration script as follows:
    - i. On the touch screen, press **Maintenance > Scripts**.
    - ii. In the Category list, select **Prepare**.
    - iii. In the Name list, select **Precolumn Equil**.



- b. Set up the script parameters as follows:
  - i. Press the **Parameters** tab.
  - ii. Enter the following settings:
    - Volume: **30**  $\mu\text{L}$
    - Flow: (Leave Empty)
    - Max Press: Set to the maximum pressure rating of the installed columns.
- c. Press **Start**.

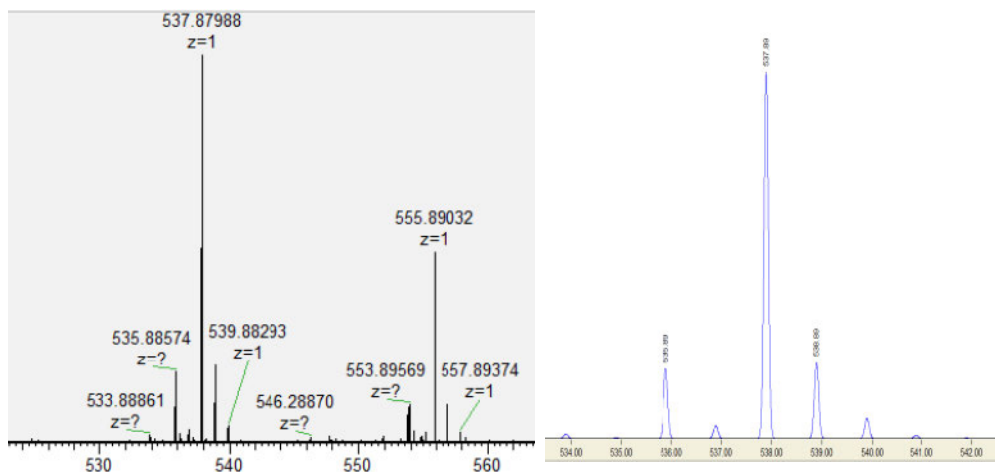
**IMPORTANT** To maintain optimal system performance, be aware of the following:

- Repeatedly connecting and disconnecting the same fused-silica column to the UHPLC fused-silica union or UHPLC liquid junction cross can damage the polyimide protection layer of the column and cause column blockage.
- The Liquid Junction Kit is designed for 360  $\mu\text{m}$  OD columns. Using the kit with columns of other dimensions can cause leakage, delayed elution, and non-reproducibility of chromatographic performance.
- Use only IDEX brand Very High Pressure (VHP) ferrules for 360  $\mu\text{m}$  OD tubing to connect tubing to the UHPLC fused-silica union and the UHPLC liquid junction cross. Using ferrules that are not specifically designed for ultra-high pressure, 360  $\mu\text{m}$  OD, fused-silica tubing can cause leaks and add swept volume.
- A poor connection between the analytical column and the emitter can cause broad chromatographic peaks.

For routine applications with the UHPLC liquid junction cross, follow these guidelines:

- Apply a high voltage potential from 2.0 to 2.5 kV. The actual voltage applied once the spray has been optimized depends on the length and ID of the analytical column.
- Use formic acid rather than acetic acid as a mobile phase modifier to avoid the formation of peptide-Fe cluster ions with acetic acid  $[(C_2H_4O_2)_n-nH + 3Fe + O]^+$  as well as the water clusters of these ions  $[(C_2H_4O_2)_n-nH H_2O + 3Fe + O]^+$ . You can distinguish ions that contain iron by their isotope distribution (Figure 81).

**Figure 81.** Spectrum of peptide-Fe cluster ions and the theoretical isotope distribution of these ions



When you use the UHPLC liquid junction cross, Thermo Fisher Scientific recommends that you maintain a supply of spare parts (see Table 16).

**Table 16.** Spare parts for a column assembly with a pulled fused-silica column

Description	Part number
VHP sleeveless cross for 360 um OD tubing	UH-752 (IDEX)
VHP microferrule for 360 μm OD tubing	PK-152 (IDEX)
UHPLC spare blind plug	P-116 (IDEX)
Tightening tool to tighten internally-threaded knurled polymer fittings	P-278 (IDEX)
Column Out tubing (modified)	6041.5290
Waste In tubing (modified)	6041.5289
PEEK connector (red) for 1/32 in. OD tubing	G10-0035 (IDEX PN P-771)

## Connecting a Sleeved Emitter to the Analytical Column

Before you mount the column assembly onto the ion source, connect an emitter to the analytical column. Borosilicate emitters require sleeves. Stainless steel emitters come pre-inserted into microsleeves.

For information about ordering stainless steel emitters sleeves, visit <https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-60053-97127-Nanospray-Flex-User-Man6005397127-EN.pdf>

Table 17 lists the sleeves for 1/32 in. fittings that you can order to install a borosilicate emitter.

**Table 17.** Thermo Fisher Scientific sleeves for 1/32 in. fittings and borosilicate emitters

Color	Use	Part number
Natural	For 360 µm OD tubing	SC603
Black	For 280 µm OD tubing	SC903

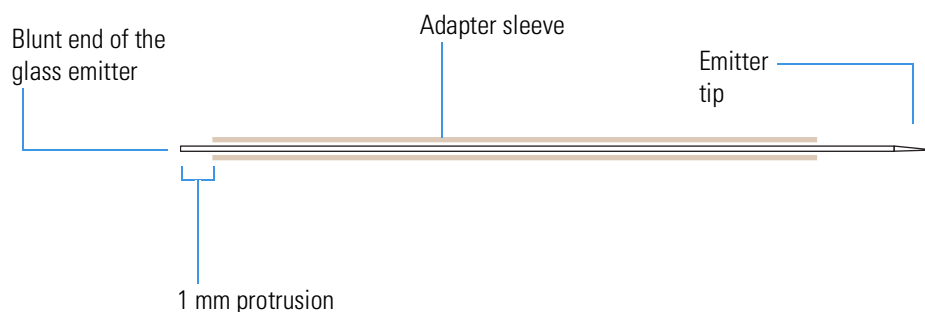


**CAUTION** Because the emitter tip is sharp enough to puncture skin and easily damaged, do not touch it.

### ❖ To connect an emitter to the PEEK union

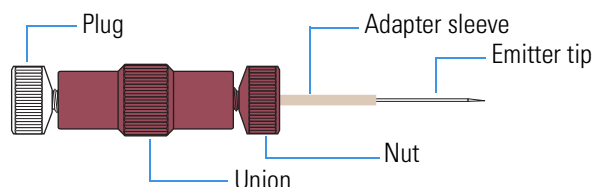
1. If you are using the preassembled column assembly, disconnect the analytical column from the PEEK union G10-0035 (IDEX PN P-771).
2. Connect the white distance-gauge plug to one end of the PEEK union.
3. Connect the emitter to the other end of the PEEK union as follows:
  - a. For a glass emitter, thread the blunt end of the emitter through the adapter sleeve. Ensure that the blunt end of the emitter does not protrude from the (20 mm length) sleeve by more than 1 mm (Figure 82).

**Figure 82.** Sleeved emitter (4x scale)



- b. To ensure proper seating, slide the blunt end of the sleeved emitter through the PEEK nut until it protrudes slightly (approximately 1 to 2 mm) from the front of the fitting.
- c. As you gently press the sleeved emitter against the union port, screw the nut into the port until fingertight (Figure 83). For a stainless steel emitter, turn the nut an additional half-turn (180 degree) once you feel resistance.
- d. Verify that the connection is secure.

**Figure 83.** Sleeved emitter connected to the PEEK union



4. Remove the gauge from the PEEK union and reconnect the analytical column to the union.

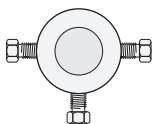


## Setting Up the Column Assembly for the EASY-Spray Source

If your LC/MS has an EASY-Spray source, follow the appropriate procedure to connect either a one- or two-column setup.

- “Connecting an EASY-Spray Column to an EASY-nLC 1200 Instrument” on page 117

The EASY-Spray Ion Source Kit contains the fittings and sleeves for the plumbing connections (see Table 18).

**Table 18.** Fittings and sleeves in the EASY-Spray Ion Source Kit

Image	Description	Part number
	A/B mixing/venting Tee, stainless steel, with three attached ferrules and nuts	SC901
	Sleeves, PEEK, 1/16 in. OD	IDEX™, F-233
	Viper union	SC900



**CAUTION** Wear protective gloves and safety glasses when handling the solvent lines. To prevent contamination, use lint-free and powder-free gloves.

## Connecting an EASY-Spray Column to an EASY-nLC 1200 Instrument

Follow the appropriate procedure for a one- or two-column setup. The two-column setup includes a precolumn in the column-out flow path to the venting Tee.

- “To connect an EASY-Spray column for a one-column setup to the EASY-nLC 1200 instrument,” next procedure
- “To connect a two-column setup with a precolumn and an EASY-Spray column to an EASY-nLC 1200 instrument” on page 118

**IMPORTANT** The venting Tee (P/N SC901) supplied in the EASY-Spray Ion Source Kit has three ports with attached fittings (stainless steel nut and ferrule). The attached fittings must not be used with the EASY-nLC 1200 instrument; however, to keep the internal components of the union aligned, remove only one of the three fittings at a time.

Use the two parallel ports to connect the sample transfer line. Use the perpendicular port to connect the waste line.

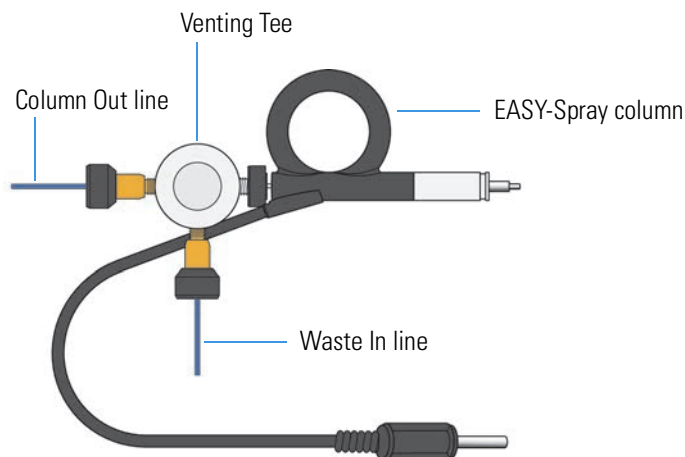
### ❖ To connect an EASY-Spray column for a one-column setup to the EASY-nLC 1200 instrument

Connect the nanoViper fittings at the ends of the Column Out and Waste In solvent lines and the nanoViper fitting at the inlet end of the EASY-Spray column to the venting Tee as follows:

- Remove the fitting (stainless steel nut and ferrule) from one of the venting Tee’s parallel ports. Insert the Column Out solvent line’s nanoViper fitting into the free port. Slowly tighten the fitting until you feel resistance. Then tighten the fitting by an additional 1/8- to 1/4-turn. Do not tighten the fitting by more than a 1/4-turn (see “Using nanoViper Fittings” on page 119).
- Remove the fitting from the venting Tee’s perpendicular port. Insert the Waste In solvent line’s nanoViper fitting into the free port, and carefully tighten the fitting.
- Remove the fitting from the remaining parallel port. Insert the nanoViper fitting at the inlet end of the EASY-Spray column into the venting Tee’s free parallel port, and carefully tighten the fitting.

Figure 84 shows the one-column setup for an EASY-Spray column and an EASY-nLC 1200 instrument.

**Figure 84.** One-column setup with an EASY-Spray column and an EASY-nLC 1200 instruments



**Tip** When you install the one-column assembly, make sure that the **One Column Setup** check box in the EASY-nLC device section on the Devices page of the Maintenance menu is selected (see “[Setting Up the Properties for the HPLC Device](#)” on page 83).

❖ **To connect a two-column setup with a precolumn and an EASY-Spray column to an EASY-nLC 1200 instrument**

1. Connect the Column Out line to the precolumn inlet with a Viper union as follows:
  - a. Insert the nanoViper fittings on the ends of the tubing into the Viper union.

**Note** Follow the recommended flow direction stated on the precolumn label.

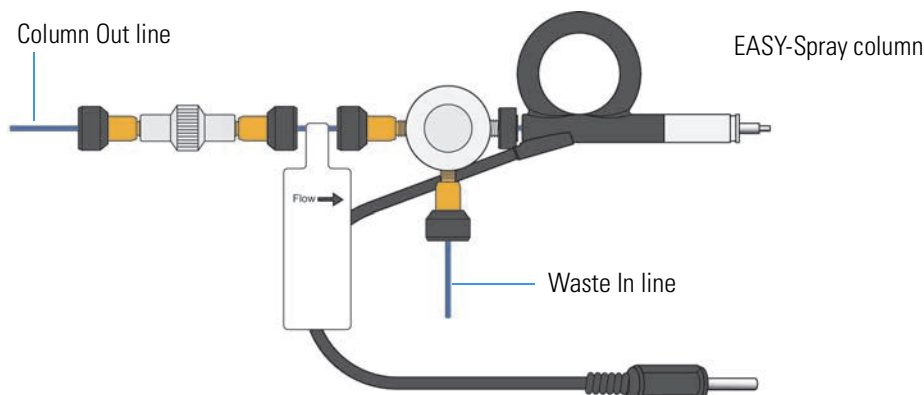
- b. Because the nanoViper fittings seal against each other inside the ZDV union, alternate tightening each fitting in increments until you feel resistance (see “[Using the Viper Union](#)” on page 121).
- c. Tighten both fittings by an additional 45 degrees (1/8-turn). Do not tighten the fittings by more than a 1/4-turn (see “[Using nanoViper Fittings](#)” on page 119).

**Note** The venting Tee has three ports. Use the two parallel ports to connect the sample transfer line. Use the perpendicular port to connect the waste line.

2. Connect the outlet end of the precolumn, the inlet end of the EASY-Spray column, and the Waste In line to the venting Tee as follows:
  - Insert the nanoViper fitting on the outlet end of the precolumn into one of the venting Tee’s parallel ports, and carefully tighten the fitting.
  - Connect nanoViper fitting on the inlet end of the EASY-Spray column to the venting Tee’s free parallel port, and carefully tighten the fitting.
  - Insert the fitting on the end of the Waste In line into the venting Tee’s perpendicular port, and carefully tighten the fitting.

Figure 85 shows a two-column setup for an EASY-nLC 1200 instrument and an EASY-Spray source.

**Figure 85.** Two-column setup for an EASY-nLC 1200 instrument and an EASY-Spray source

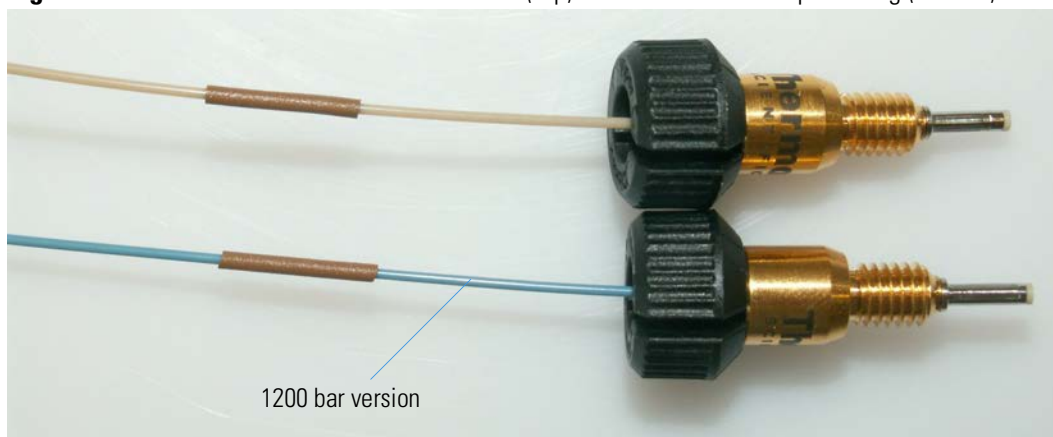


**Tip** When you install the two-column assembly, clear the **One Column Setup** check box in the EASY-nLC device section on the Devices page of the Maintenance menu (see “Setting Up the Properties for the HPLC Device” on page 83).

## Using nanoViper Fittings

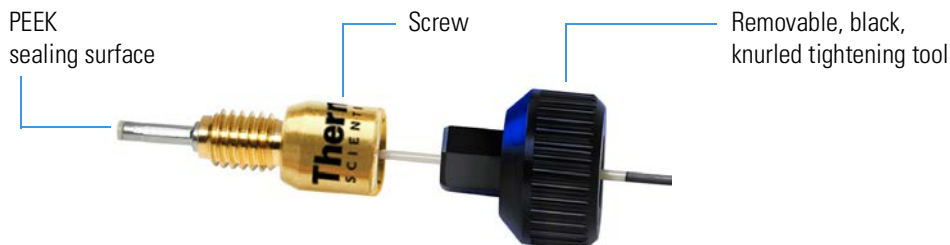
**IMPORTANT** The EASY-nLC 1200 requires the current version of nanoViper fitting systems with a specified pressure range up to 1200 bar. Older versions are only specified up to 1000 bar. The new 1200 bar nanoViper fitting system consists of blue PEEK tubing, which distinguishes it from the beige 1000 bar version, see Figure 86.

**Figure 86.** Visual difference between 1000 bar (top) and 1200 bar nanoViper fitting (bottom)



For the EASY-nLC 1200 instrument, most of the plumbing connections are made with nanoViper fittings (see [Figure 87](#)).

**Figure 87.** nanoViper fitting



Even though nanoViper fittings can withstand UHPLC backpressures of up to 1200 bar (-17 404 psi), they are fingertight fittings, which require only very small torques to seal. To avoid damage by overtightening, you must follow this procedure.

❖ **To connect a nanoViper fitting to a receiving port**

1. Insert the nanoViper fitting into the target port and slowly rotate the screw clockwise until you feel resistance.
2. Turn the screw clockwise to an angle between 0 and 45 degrees (1/8-turn) using the black, knurled tightening tool.

**IMPORTANT** To prevent damage to the sealing surface of the nanoViper fitting, take care not to overtighten the nanoViper fitting.

3. Run the Leaks script for the system as described in the *EASY-nLC Series Troubleshooting and Maintenance Guide*.

When the leak test ends, the system is at atmospheric pressure.

**IMPORTANT** To extend the lifetime of the nanoViper fittings, open and close connections at atmospheric system pressures only. Opening and closing connections at high system pressures can reduce the lifetime of the fitting system.

4. If the Leaks script fails because the new connection is not leak tight, use the black knurled nut to turn the screw up to an additional 45 degrees. Do not turn the screw beyond an angle of 90 degrees from where you felt the initial resistance.



## Using the Viper Union

For an EASY-nLC instrument with the standard Column Out and Waste In lines, use a Viper union to connect the Column Out line to the Waste In line during a system leak test or to connect the Column Out line to the precolumn of a two-column assembly. The Viper union is a true zero-dead-volume (ZDV) union, which means that the fittings connected to its receiving ports seal against each other inside the union.

### ❖ To connect tubing with nanoViper fittings to the Viper union

1. Insert the nanoViper fittings on the ends of the tubing into the Viper union.
2. Alternate tightening each fitting in increments until you feel resistance.
3. Tighten both fittings by an additional 45 degrees (1/8-turn). Do not tighten the fittings by more than a 1/4-turn (see “Using nanoViper Fittings” on page 119).



**CAUTION** Because the nanoViper fittings seal against each other inside the union, do not tighten them by more than 90 degrees (1/4-turn). Overtightening the fittings can irreparably damage their sealing surfaces.

Figure 88 shows two solvent lines with nanoViper fittings connected to the Viper union. The Viper union makes a zero-dead-volume connection between the two nanoViper fittings.

**Figure 88.** Viper union connecting tubing with nanoViper fittings

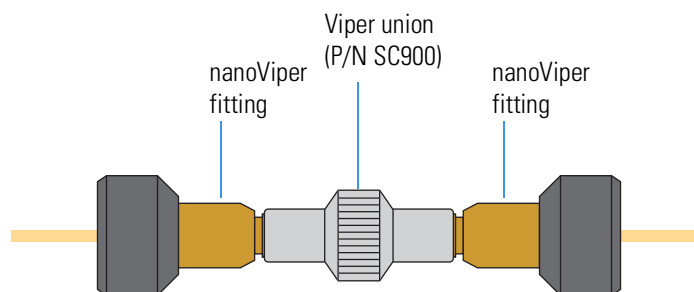
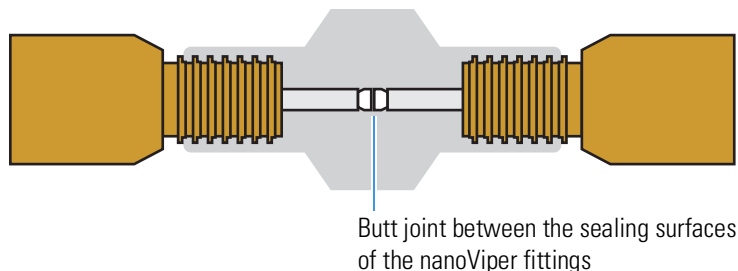


Figure 89 shows an internal view of the Viper union with nanoViper fittings connected to both ports. The two nanoViper fittings seal against each other in the center of the union, and a portion of the last thread on each nanoViper fitting is visible.

**Figure 89.** Viper union with nanoViper fittings connected to both ports (internal view)



## Conditioning the Analytical Column (for initial operation)

### ❖ To condition the analytical column

1. Open the Parameters view of the Column Conditioning script as follows:
  - a. Press **Maintenance > Scripts**.  
The Maintenance > Scripts page opens.
  - b. In the Category list, select **Prepare**.
  - c. In the Name list, select **Column Conditioning**.
  - d. Press the **Parameters** tab.

The Parameters view of the Column Conditioning script opens (see [Figure 90](#)).

**Figure 90.** Column Conditioning parameters

The screenshot shows the Thermo Scientific software interface. The top navigation bar includes tabs for Home, Batch Setup, Method Setup, Maintenance, and Configuration. The 'Maintenance' tab is active. On the left, there is a 'Scripts' menu with options like Log Book, Support, and Devices. The main area displays the 'Column Conditioning' script parameters. The 'Parameters' tab is selected, showing a table with the following data:

Parameter	Value
Total Duration [min]	40
Max Pressure [bar]	800
No. of Steps	10

Below the table are buttons for 'Schedule', 'START', and 'STOP'. The 'START' button is highlighted in green. There are also 'No graph' dropdown menus on the right side of the interface.

2. Set up the script parameters as follows:
  - a. In the Total Duration [min] box, enter the duration for conditioning the analytical column.  
A minimum time of 40 minutes is recommended.
  - b. In the Max Pressure [bar] box, enter the specified maximum pressure value of your analytical column.  
If the Max Pressure field is left empty, the pump operates at the maximum system pressure.

- c. In the No. of Steps box, enter the number of steps until the specified maximum pressure value is reached.

As a typical value, 10 steps are recommended.

Please see also the respective column documentation for the best parameters, particular column or columns.

## Equilibrating Columns

After you initially install the column assembly, equilibrate the column or columns and determine an appropriate flow rate for the sample loading step (see “[Setting Up the Sample Pickup and Loading Steps](#)” on [page 133](#)) of the chromatographic method.

Follow these procedures to equilibrate the columns and to determine the appropriate flow rate for your chromatographic method:

- “[Equilibrating the Precolumn](#),” next section
- “[Equilibrating the Analytical Column](#)” on [page 126](#)

### Equilibrating the Precolumn

❖ **To equilibrate the precolumn and determine the flow rate for sample loading**

1. Open the Parameters view of the Precolumn Equilibration script as follows:
    - a. Press **Maintenance > Scripts**.
- The Maintenance > Scripts page opens.
- b. In the Category list, select **Prepare**.
  - c. In the Name list, select **Precolumn Equil.**
  - d. Press the **Parameters** tab.

The Parameters view opens (see [Figure 91](#)).

**Figure 91.** Precolumn equilibration parameters

The screenshot shows the Thermo Scientific software interface. At the top, there are tabs for Home, Batch Setup, Method Setup, Maintenance, and Configuration. The 'Maintenance' tab is selected. On the left, there is a navigation menu with options: Scripts, Log Book, Support, and Devices. The 'Scripts' section is active, showing a 'Category' dropdown set to 'Prepare' and a 'Name' dropdown set to 'Precolumn equili...'. Below this, there are three tabs: Description, Parameters, and Output. The 'Parameters' tab is selected, displaying a table with the following data:

Parameter	Value
Volume [µl]	12.00
Flow [µl/min]	
Max pressure [bar]	800.00

At the bottom of the interface, there are several buttons: 'Exit..', 'Schedule', a large green 'START' button, and a grey 'STOP' button. On the right side, there are two 'No graph' dropdown menus.

2. Set up the script parameters as follows:
  - a. In the Volume [μL] box, enter the volume of solvent A to be used to equilibrate the precolumn.

For best results, use at least 10 column volumes to equilibrate the precolumn.

- b. In the Flow [μL/min] box, enter the flow rate for the precolumn equilibration step.  
If the flow field is left empty, the pump operates at the set pressure.
  - c. In the Max Pressure [bar] box, enter the maximum allowed pressure for the equilibration step.

If the pressure field is left empty, the pump operates at the set flow (as long as it is below the instrument maximum pressure of 1180 bar). If both a flow and a max pressure are specified, the pump flow is limited by whichever parameter is reached first.



**CAUTION** The maximum pressure rating for the columns is as follows:

- The maximum pressure rating is 1200 bar for the PepMap columns supplied with the EASY-nLC 1200 instrument.

Running the instrument at pressures higher than the column's maximum pressure rating reduces the column lifespan.

Thermo Fisher Scientific recommends running the equilibration at a set pressure rather than a set flow, to make sure the column pressure limit and the instrument pressure limit are not exceeded (see [Figure 91](#) on [page 124](#)). If the flow field is left empty, the pump will run at the specified pressure. For best results, use an equilibration volume of 10 column volumes.

Item	Maximum pressure (in bar)
EASY-nLC 1200 instrument	1180
PepMap column	1200
EASY-Spray column	Refer to the specifications provided with the column.

3. Press **START**.

The default graphs appear in the two graph windows, but you can change the selections from their corresponding lists. These graphs help you to monitor the progress.

4. Monitor the actual flow rate to become familiar with the flow/pressure relationship on your particular column.

## Equilibrating the Analytical Column

### ❖ To equilibrate the analytical column

1. Open the Parameters view of the Analytical Column Equilibration script as follows:
  - a. Press **Maintenance > Scripts**.  
The Maintenance > Scripts page opens.
  - b. In the Category list, select **Prepare**.
  - c. In the Name list, select **Analytical Col equil**.
  - d. Press the **Parameters** tab.

The Parameters view of the Analytical Column Equilibration script opens (see [Figure 92](#)).

**Figure 92.** Analytical column equilibration parameters

Parameter	Value
Volume [µl]	6.00
Flow [µl/min]	
Max pressure [bar]	800.00

2. Set up the script parameters as follows:
  - a. In the Volume [µL] box, enter the volume of solvent A to be used to equilibrate the analytical column.  
For best results, use at least 10 column volumes to equilibrate the analytical column.
  - b. In the Flow [µL/min] box, enter the flow rate for the analytical column equilibration step.  
If the flow field is left empty, the pump operates at the set pressure.

- c. In the Max Pressure [bar] box, enter the maximum allowed pressure for the equilibration step.

If the pressure field is left empty, the pump operates at the set flow (as long as it is below the instrument maximum pressure of 1180 bar).

If both a flow and a maximum pressure are specified, the pump flow is limited by whichever parameter is reached first.



**CAUTION** Running the instrument at pressures higher than the column's maximum pressure rating reduces the column lifespan.

Thermo Fisher Scientific recommends running the equilibration at a set pressure rather than a set flow, to make sure the column pressure limit and the instrument pressure limit are not exceeded. If the flow field is left empty, the pump will run at the specified pressure. For best results, use an equilibration volume of 10 column volumes.

**Table 19.** Summarized recommended equilibration volumes for typical column configurations

Length (mm)	ID (µm)	Recommended equilibration volumes (µL) - equals approximately 10 column volumes
150	75	6
150	50	6
250	75	10
500	75	20
750	75	30

3. Press **START**.

The default graphs appear in the two graph windows, but you may change the selections from their corresponding lists. These graphs help you to monitor the progress.

4. Monitor the actual flow rate to become familiar with the flow/pressure relationship on your particular column or columns.

## 5 Preparing the EASY-nLC Instrument for Use

### Equilibrating Columns



# Running Samples Using the Integrated Software

To create a method and run your first sample batch by using the integrated instrument control software, follow these procedures:

## Contents

- [Preparing and Loading Samples](#)
- [Creating a Method](#)
- [Creating a Batch](#)
- [Starting Sample Acquisition](#)
- [Monitoring the Run](#)
- [Stopping Sample Acquisition](#)
- [Editing the Running Batch](#)
- [Troubleshooting a Sample Run](#)

## Preparing and Loading Samples

The EASY-nLC instrument ships with adapters for vials (54 vials in a 6 × 8+6 configuration), microplates (96- and 384-well), and PCR strips.

If you are using a new plate format, you must add the plate format to the list of configured plate formats and calibrate the plate as described in the *EASY-nLC Series Troubleshooting and Maintenance Guide*.

### ❖ To prepare the sample vials or microplates

1. Fill some vials or microplate wells with your sample, preferably a known standard.
2. Make sure that no air is trapped in the sample vials or the microplate wells and that the sample is in the bottom of the vials or wells. To do this, tap the vial or microplate gently against a hard surface to have the sample move to the bottom.
3. Put on the vial cap or plate mat.

❖ **To load a microwell plate or vials into the autosampler tray compartment**

1. Use the touch-screen controls to open the tray compartment door as follows:
  - i. On the Home > Overview page, press **Eject/Insert Tray**.  
A confirmation box appears to remind you to remove obstacles from the area in front of the tray compartment before opening the tray compartment door.
  - ii. Make sure that the area in front of the tray compartment is clear, and press **Accept**.

The tray compartment door opens and the plate holder moves forward.

2. Load your microwell plate or sample vials.
3. On the Home > Overview page, press **Eject/Insert Tray**.

## Creating a Method

To create a method, follow these procedures:

1. [“Browsing for a Stored Method or Creating a New Method File,”](#) next section
2. [“Starting the Method Setup Wizard”](#) on page 132
3. [“Setting Up the Sample Pickup and Loading Steps”](#) on page 133
4. [“Building the Gradient”](#) on page 136
5. [“Setting Up the Column Equilibration Steps”](#) on page 138
6. [“Setting Up the Autosampler Wash Step”](#) on page 140
7. (Optional) [“Specifying the Temperature of the EASY-Spray Column Heater”](#) on page 143
8. [“Saving the Method Settings”](#) on page 144

## Browsing for a Stored Method or Creating a New Method File

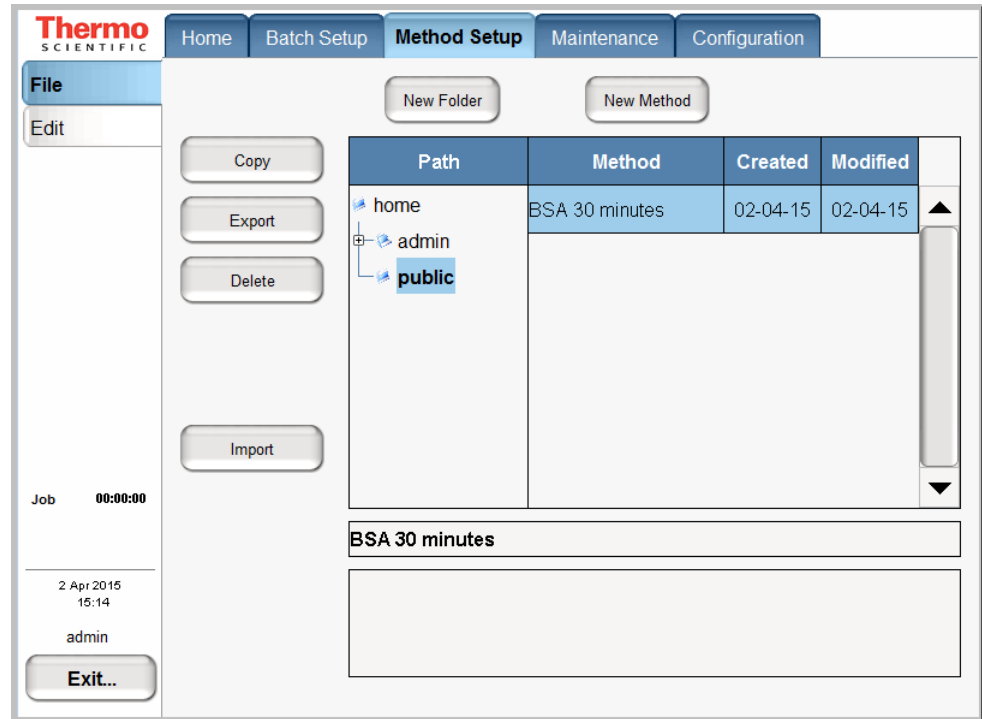
Use the Method Setup > File page to browse for stored methods or to select the file folder and enter a name for the new method.

❖ **To browse for a stored method**

1. Press **Method Setup > File**.

The Method Setup > File page opens (see [Figure 93](#)).

**Figure 93.** File page of the Method Setup



2. To view the methods in the folder, press the file folder name in the Path column.
3. To view a description of the method (if available), press the method name.

❖ **To create a new method**

1. Open the Method Setup > File page (see [Figure 93](#)).
2. Press **New Method**.

The Create New File dialog box opens.

3. In the Folder box, browse to the directory where you want to store the new method.
4. In the New Name box, enter a method name.
5. (Optional) In the Description box, enter a description of the method.
6. Press **Save**.

## Starting the Method Setup Wizard

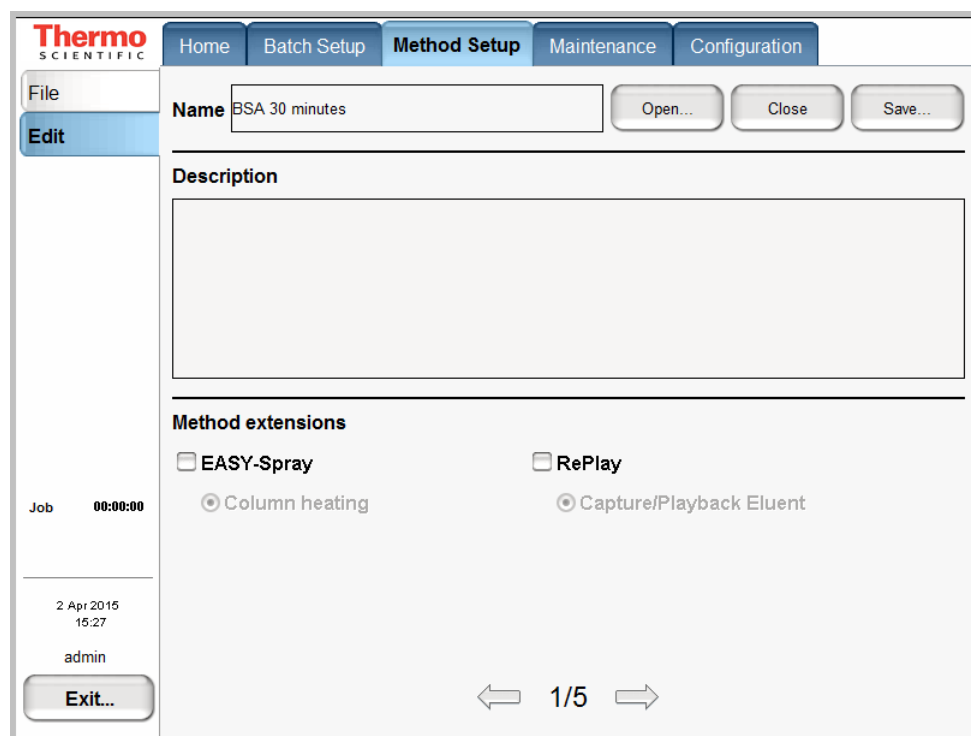
The Method Setup wizard consists of five or more pages that guide you through all the parameters required for a working LC method.

Use first page of the Method Setup wizard to select the method that you want to edit and the external devices that want to control.

### ❖ To start the Method Setup wizard

1. Press **Method Setup > Edit**.
2. If you are not already on the page 1 (see [Figure 94](#)), go to page 1 by pressing the appropriate arrow near the bottom of the touch screen.

**Figure 94.** Method Setup > Edit page 1



3. Press **Open**.

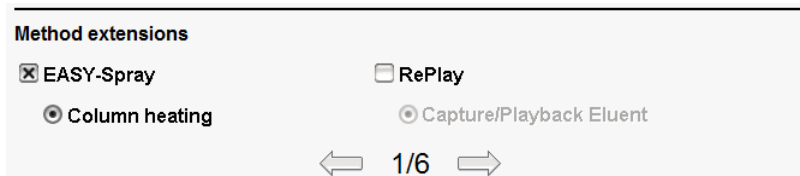
The Select Method to Open dialog box opens.

4. Select the method that you want to edit.
5. Press **Accept**.

❖ **To control an external device**

In the Method extensions area, select the check box for the device.

- To control the column temperature of the EASY-Spray ion source, select the **EASY-Spray** check box.



- To control the Replay device, select the **RePlay** check box.

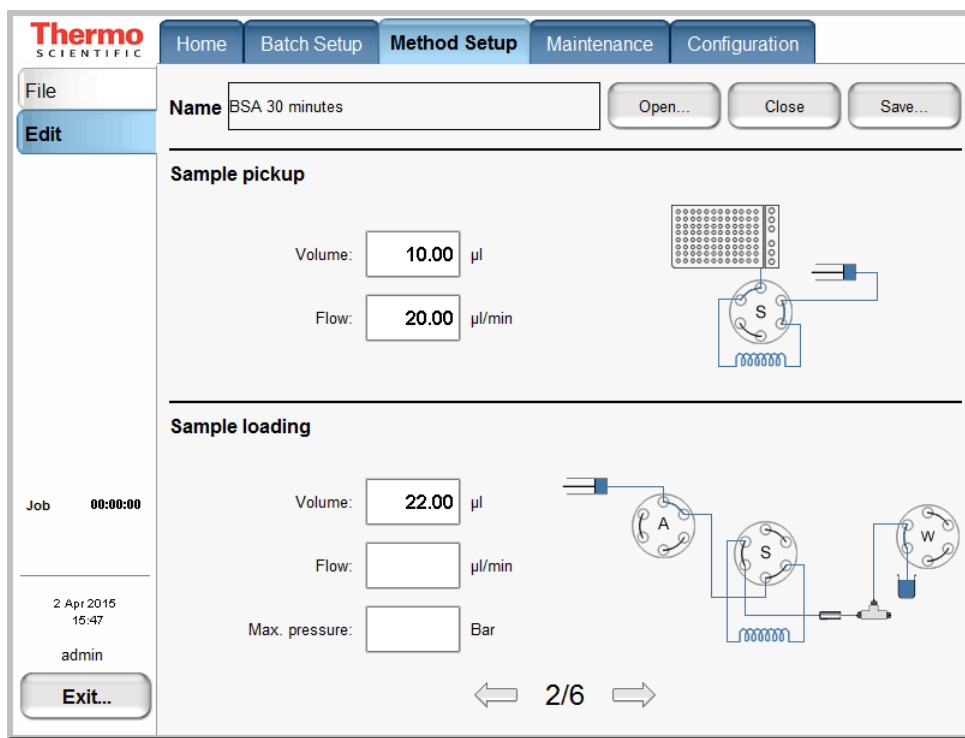
By default, the Method Setup wizard contains five pages. For each external device that you add, the wizard increases by one page.

To continue setting up the method, press the right arrow to go to the next page of the Method Setup wizard (page 2/x, where x equals the total number of pages).

## Setting Up the Sample Pickup and Loading Steps

Use the Method Setup > Edit 2/x page (see [Figure 95](#)) to set the parameters for picking up the sample from the well plate and loading it onto the precolumn.

**Figure 95.** Edit 2/x page of the Method Setup wizard



❖ **To set up the sample pickup parameters**

1. In the Sample Pickup area, press the Volume box and enter the default amount of sample to be picked up.

The autosampler withdraws the requested sample volume; it does not pick up excess sample.

To prevent contamination of pump S, the maximum volume is set to the configured sample loop size minus 2  $\mu\text{L}$ . For example, the maximum sample pickup for an instrument with a 20  $\mu\text{L}$  sample loop is 18  $\mu\text{L}$ .

You can overwrite this setting when you set up the injection sequence from an external Thermo Scientific data system such as the Xcalibur data system.

2. Press the Flow box to set the flow rate at which the autosampler picks up sample.

Range: 0 to 40  $\mu\text{L}/\text{min}$

Set the speed according to the sample viscosity and accuracy needed. For typical samples, 20  $\mu\text{L}/\text{min}$  is optimal. Setting the flow rate too high can draw air bubbles into the sample plug.

❖ **To set up the sample loading parameters**

1. In the Sample Loading area, press the Volume box and enter the volume of solvent A to be used to load the sample from the sample loop onto the precolumn (or analytical column in a one-column setup).

Range: 0 to 137

Normally it is sufficient to load the precolumn with  $2 \times \text{sample pickup volume} + 2 \mu\text{L}$  or a minimum of 6  $\mu\text{L}$ , whichever is larger.

2. Press the Flow box and enter the flow rate for sample loading onto the precolumn (or analytical column in a one-column setup).

If you leave the Flow box empty, the pump operates at the set pressure.

Range: 0 to 100  $\mu\text{L}/\text{min}$

**Tip** The application accepts values from 0 to 100  $\mu\text{L}/\text{min}$ , but the IFC algorithm (see “[Intelligent Flow Control System](#)” on [page 32](#)) prevents the flow rate for the sample loading step from exceeding 25  $\mu\text{L}/\text{min}$  based on the backpressure produced by the system tubing alone without the columns attached.

Use the column equilibration scripts to determine the appropriate flow rate for your application.

3. Press the Max. Pressure box and set the maximum allowed pressure.

If you leave the Max. Pressure box empty, the pump operates at the set flow (as long as it is below the maximum instrument pressure of 1180 bar).

**Tip** If you specify both a flow rate and a maximum pressure, the pump flow is limited by the parameter that is reached first. If you leave the flow and max pressure boxes empty, the IFC algorithm allows pump A to run at the maximum system pressure.

**IMPORTANT** If you enable the LSP feature and specify a maximum pressure, the batch run stops if the system reaches the maximum pressure setting before it reaches the specified flow rate during sample loading.

To continue setting up the method, go to the Edit 3/x page by pressing the right arrow.

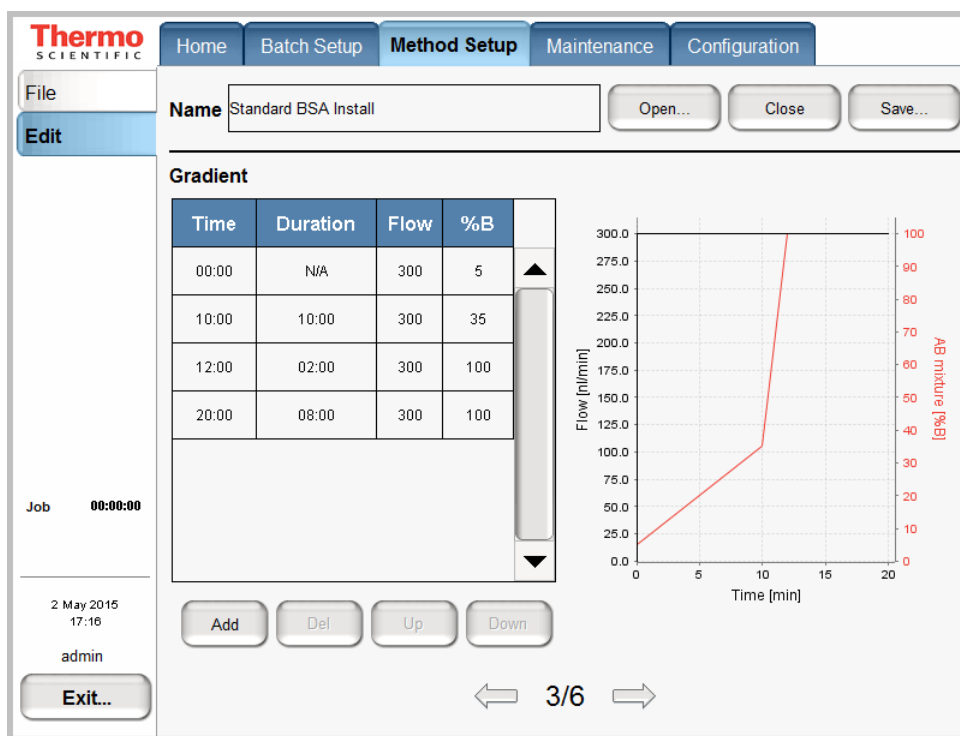
## Building the Gradient

Use the Method Setup > Edit 3/x page to build the gradient. This page consists of a schematic view for setting parameters and a graph view showing the gradient as a function of time (see Figure 96).

Use the four buttons at the bottom of the page to add lines to the gradient, move lines up and down, and delete lines. To move or delete lines, first select the line by pressing it.

The rest of this tutorial guides you through building a gradient for analyzing a BSA digest on a C18 column. Because this tutorial is intended to help you set up a method, make sure to set the parameters appropriately for your analyte and column characteristics.

**Figure 96.** Edit 3/x page of the Method Setup wizard



**Note** When you press a cell in the gradient table, the keypad dialog box opens.

### ❖ To build a gradient

1. In the first row of the gradient table, do the following:
  - a. Press the Flow column cell and enter **300** nL/min. Then press **ACCEPT**.
  - b. Press the % B column cell and enter **5** (%). Then press **ACCEPT**.The gradient is now set to start at 300 nL/min and 5 %B.
2. Press **ADD** to add another row.



3. In the second row of the gradient table, do the following:
  - a. Press the Duration column cell and enter **10** minutes. Then press **ACCEPT**.
  - b. Press the Flow column cell and enter **300** nL/min. Then press **ACCEPT**.
  - c. Press the % B column cell and enter **35** (%). Then press **ACCEPT**.
4. Press **ADD** to add another row.
5. In this new row, set duration to **2** min, flow to **300** nL/min, and % B to **100**.
6. Press **ADD** to add another row.
7. In this new row, set the duration to **8** min, flow to **300** nL/min, and % B to **100**.

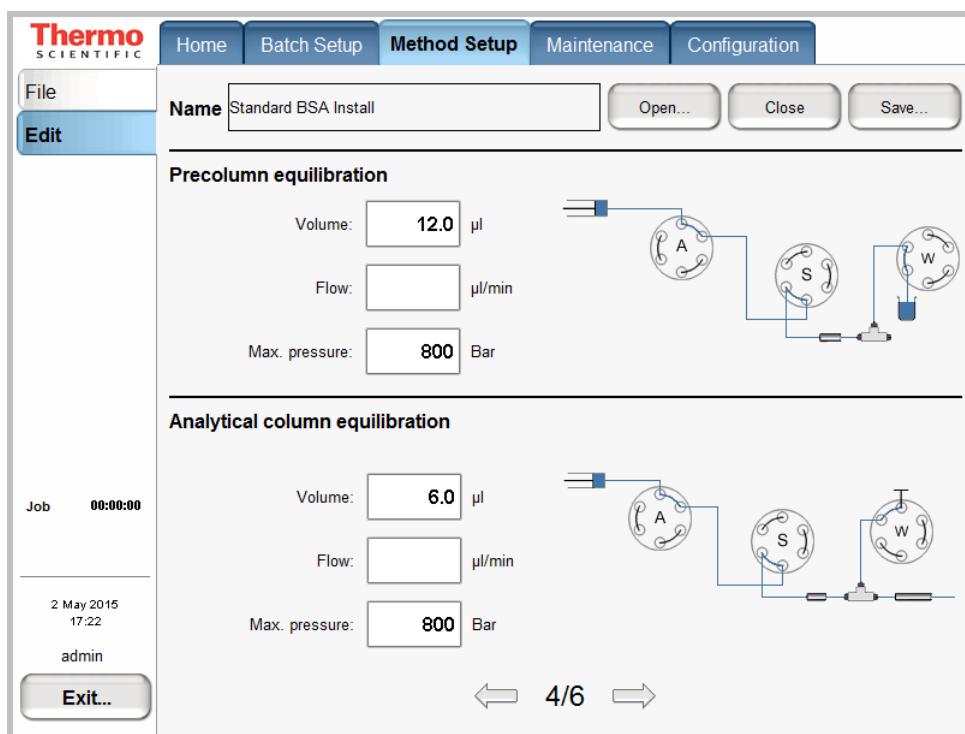
**Note** The Method Setup wizard includes a separate page for setting up the column equilibration parameters, so you do not need to add a column equilibration step to the end of the gradient program. The column equilibration steps run parallel with the sample pickup step at the beginning of a sample run.

To continue setting up the method, go to the Edit 4/*x* page by pressing the right arrow.

## Setting Up the Column Equilibration Steps

Use the Method Setup > Edit 4/x page to set up the parameters for the column equilibrations (see [Figure 97](#)).

**Figure 97.** Edit 4/x page of the Method Setup wizard



### ❖ To set up the precolumn equilibration

1. In the Precolumn Equilibration area, press the Volume box to set the amount of solvent A to use for equilibration of the precolumn.

For best results, use at least 10 column volumes to equilibrate the precolumn.

2. Press the Flow box to set the flow rate for performing the equilibration of the precolumn.

If you leave the Flow box empty, the pump operates at the set pressure during a sample run.

3. Press the Max. Pressure box to set the maximum allowed pressure.

If you leave the Max. Pressure box empty, the pump operates at the set flow (as long as it is below the instrument pressure of 1180 bar).

**Note** If you specify both a flow rate and a maximum pressure, the pump flow is limited by the parameter that is reached first.

❖ **To equilibrate the analytical column**

1. In the Analytical Column Equilibration area, press the Volume box to set the amount of solvent A to use for equilibration of the analytical column.

For best results, use at least 10 column volumes to equilibrate the analytical column.

Range: 0 to 137

2. Press the Flow box to set the flow rate for performing the equilibration of the analytical column.

If you leave the Flow box empty, the pump operates at the set pressure during a sample run.

3. Press the Max. Pressure box to set the maximum allowed pressure.

If you leave the Max. Pressure box empty, the pump operates at the set flow (as long as it is below the instrument pressure of 1180 bar).

---

**Note** If you specify both a flow rate and a maximum pressure, the pump flow is limited by the parameter that is reached first.

---

To continue setting up the method, go to the Edit 5/*x* page by pressing the right arrow.

## Setting Up the Autosampler Wash Step

Use the Method Setup > Edit 5/x page for setting up the autosampler washing procedures (see Figure 98). The EASY-nLC instrument conducts the wash while the gradient is running.

Use the standard wash step or set up a custom wash cycle as described in these procedures:

- [Setting Up a Standard Wash Step](#)
- [Setting Up a Custom Wash Program](#)

### Setting Up a Standard Wash Step

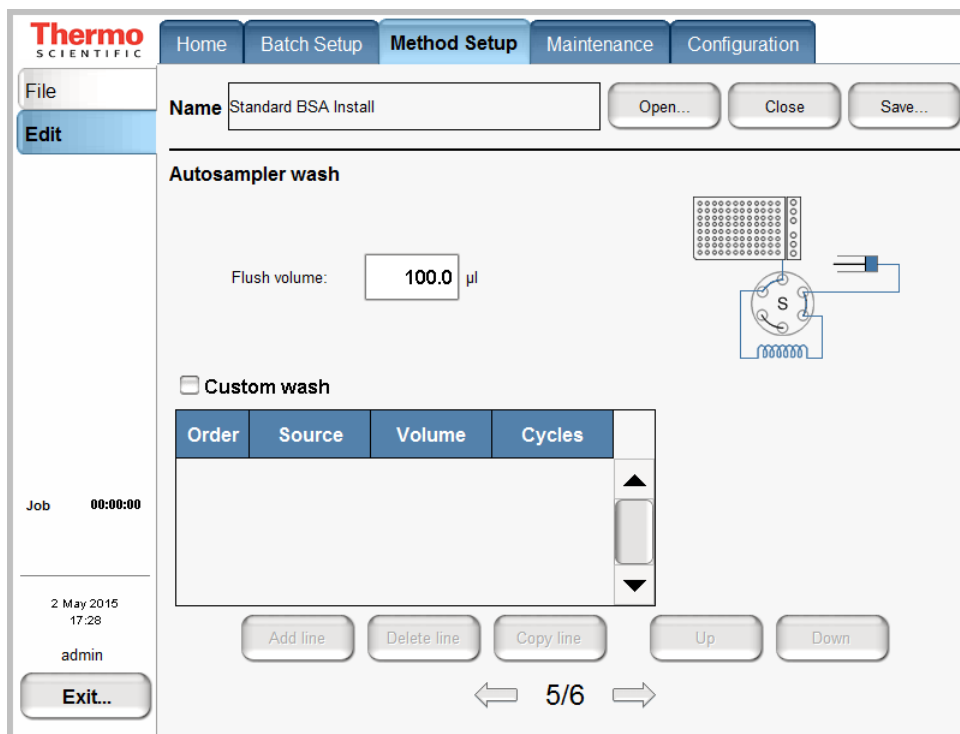
For most samples in low concentrations, flushing the needle and loop with solvent A is sufficient. You can set the amount of solvent for this action in the Flush Volume box.

Use 100 µL as the default flush volume.

**Note** This equation defines the minimum flush volume that you can set:

$$\text{loop volume} + \text{needle volume} + 1 \mu\text{L}.$$

Figure 98. Edit 5/x page of the Method Setup wizard



❖ **To set up a standard wash step**

1. Do not select the **Custom Wash** check box.
2. In the Flush Volume box, type the volume of solvent A (from wash bottle 3) that the autosampler uses to flush the needle and the sample loop.

The range depends on the sample loop size. For the 20 µL sample loop, the range is 30.25 to 100.75 µL.

**Setting Up a Custom Wash Program**

For a more thorough wash of the needle and loop, select the **Custom Wash** check box. Use this option to set up a user-definable procedure for washing the loop and needle.

**Tip** The custom wash program automatically ends with a preset volume of solvent A from bottle W3.

The Custom Wash table consists of three user-editable parameters (see [Table 20](#)). The Order column lists the order in which the autosampler performs the steps. To change the order, use the Up and Down buttons.

**Table 20.** Custom wash parameters

Parameter	Description
Order	Defines the order of the wash steps. To change the order of the wash steps, use the Up and Down buttons.
Source	Specifies the wash solvent source for the current wash step.  Selections: Bottle 1, Bottle 2, or Bottle 3  Bottles 1 and 2 contain custom wash solvents. Bottle 3 contains solvent A.
Volume	Specifies the volume that pump S draws from the source location for each wash cycle.  To avoid drawing solvent into pump S, for a 20 µL sample loop, the maximum volume is 28 µL.  $\text{needle volume} + \text{loop volume} - 2 \mu\text{L}$
Cycles	Specifies the number of wash cycles for the wash step.  For each cycle, the autosampler draws the specified volume of wash solvent from the specified source and ejects the wash solvent into wash bottle 4.

When the custom wash has ended, the autosampler automatically empties the pump and refills with solvent from bottle 3 to be ready for the next injection.

❖ **To set up a custom wash program**

1. Select the **Custom Wash** check box.

The custom wash table becomes available.

2. For each row that you want to add to the program, press **Add Line**.

You can select a different wash solvent for each row.

3. Edit each row as follows:

- In the Source column, select the wash solvent source.

The selections are Bottle 1, Bottle 2, or Bottle 3.

**Note** The EASY-nLC autosampler holds up to four bottles: three for washing solvents and the fourth for ejecting waste and cleaning the outside of the injection needle.

- Bottles 1 and 2 in positions W1 and W2 contain custom wash solvents.
- Bottle 3 in position W3 contains solvent A.
- Bottle 4 in position W4 contains the needle wash insert.

- In the Volume column, type a volume from **0** to the *sample loop size* plus 8  $\mu\text{L}$ .

- In the Cycle column, type a value from **0** to **10**.

4. To edit an existing wash program, do the following:

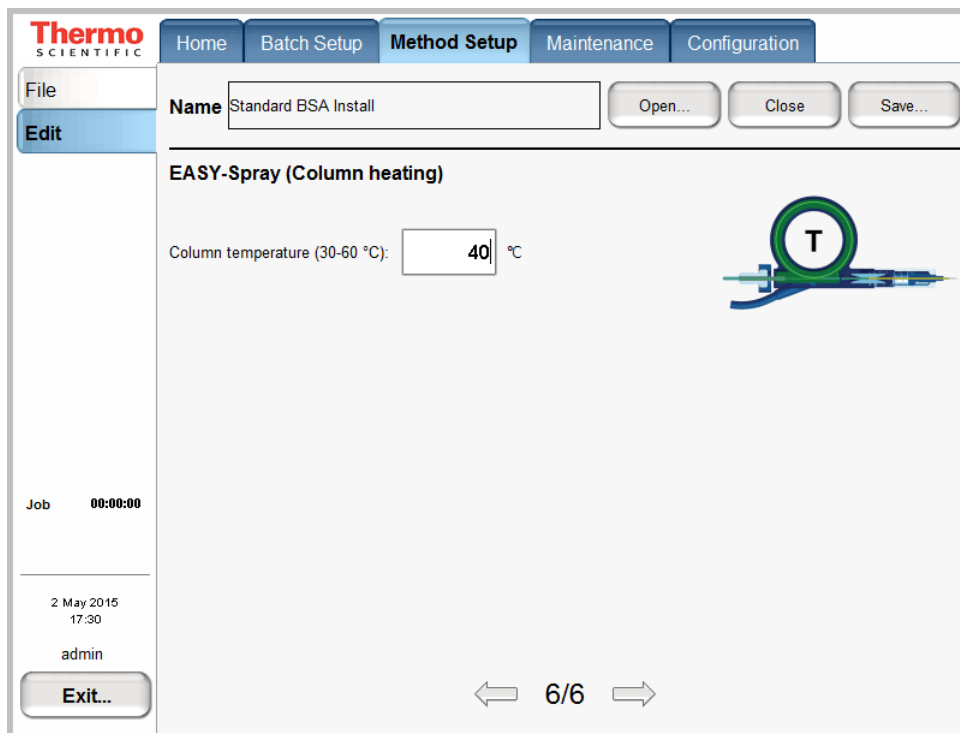
- To add a row to the program, press **Add Line**.
- To remove a row from the program, press **Delete Line**.
- To copy a selected line, press **Copy Line**.
- To move a selected row up or down, press **Up** or **Down**, respectively.

When you finish setting up the method parameters, save the method as described “[Saving the Method Settings](#)” on [page 144](#).

## Specifying the Temperature of the EASY-Spray Column Heater

If your LC/MS system includes the EASY-Spray ion source, use the Method > Edit 6/x page to specify the temperature of the EASY-Spray column heater.

**Figure 99.** Edit page 6/x of the Method Setup wizard



### ❖ To specify the column temperature

1. Press the temperature box.  
The Set Value dialog box opens.
2. Press the number pad to enter a temperature from **30** to **60** °C.  
The application does not accept temperatures below 30 °C or above 60 °C.
3. After you enter an appropriate temperature, press **Accept**.

## Saving the Method Settings

After you enter the method settings, save the method.

### ❖ To save the method

1. Press **Save** in the top right corner of the Method Setup wizard.

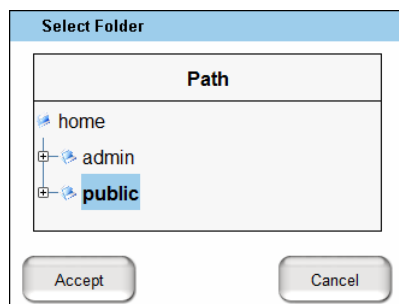
The Save File dialog box opens.

2. Select the folder where you want to store the method as follows:

- a. Press the Folder box.

The Select Folder dialog box opens (Figure 100).

**Figure 100.** Select Folder dialog box



- b. Select the appropriate folder.

- c. Press **Accept**.

The Select Folder dialog box closes and the folder location appears in the Save As dialog box.

3. Enter the method name as follows:

- a. Press the Name box.

The virtual keyboard opens.

- b. Use the virtual keyboard to type the method name.

- c. Click **OK**.

The virtual keyboard closes and the new method name appears in the Save File dialog box (Figure 101).



**Figure 101.** Save File dialog box

The image shows a 'Save file' dialog box with a light blue title bar. Below the title bar, there are three input fields. The first is labeled 'Folder:' and contains the text '/home/public/'. The second is labeled 'Name:' and contains the text 'Caffeine assay'. The third is labeled 'Description:' and is currently empty. At the bottom of the dialog, there are two buttons: 'Save' on the left and 'Cancel' on the right.

4. (Optional) In the Save As dialog box, enter a description for the method in the Description box.
5. Click **Save** to save the method.

The Save File dialog box closes. If the method already exists, the Overwrite Method dialog box opens.

6. If the Overwrite Method dialog box opens, press **Yes** to overwrite the existing method or press **No** to close the Save File dialog box without saving the method.

## Creating a Batch

Use the Batch Setup > Edit page to set up and schedule batches for sample acquisition on the EASY-nLC instrument. (See also “Preparing and Loading Samples” on page 129 and “Creating a Method” on page 130.)

To create a batch, follow these procedures:

- “Viewing the Batch List and Setting Up a Batch Record,” next section
- “Specifying the Method, Sample Positions, and Number of Injections” on page 147

## Viewing the Batch List and Setting Up a Batch Record

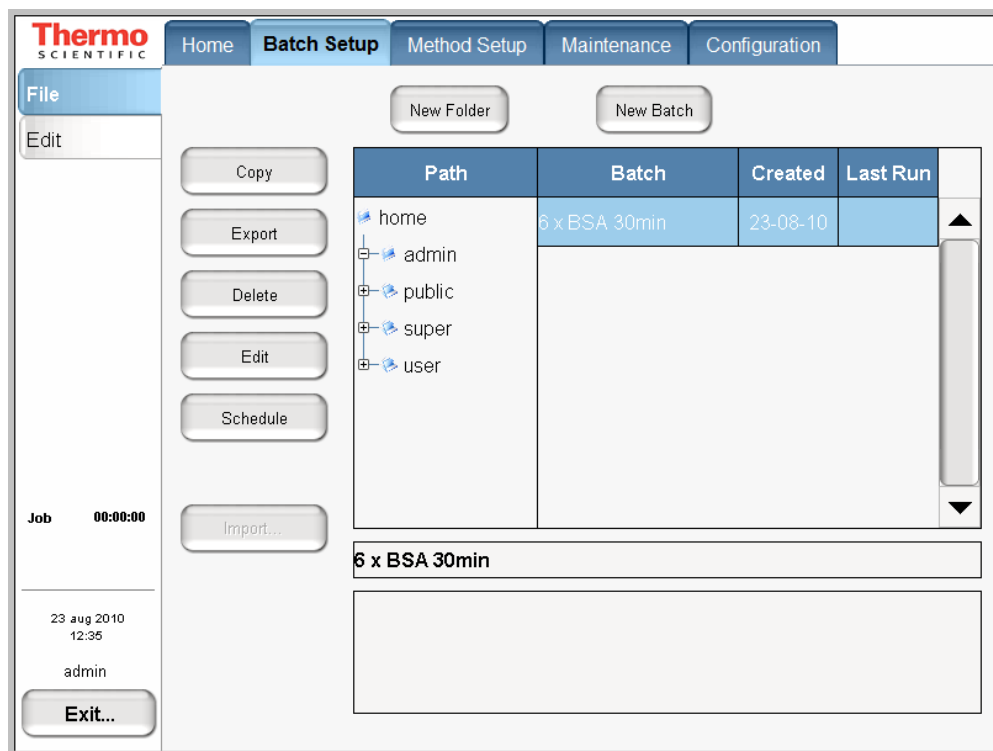
Use the Batch Setup > File page to view a list of stored batches, import a batch from another EASY-nLC instrument, and create an empty batch record (that is, name a new batch and select its folder location).

### ❖ To view a list of batches

1. Press **Batch Setup > File**.

The Batch Setup > File page opens (see Figure 102).

**Figure 102.** File page of the Batch Setup menu



2. To view your batches, in the Path column, press the folder where you store your batches.

**❖ To specify the batch name and folder location**

1. On the Batch Setup > File page, press **New Batch**.

The Create New File dialog box opens.

2. In the Folder box, browse to the folder where you want to store the batch.
3. In the New Name box, enter a name for the batch.
4. (Optional) In the Description box, enter a description of the batch.
5. Press **Save**.

The batch name appears under the selected folder.

To set up the batch, go to the next section, [“Specifying the Method, Sample Positions, and Number of Injections.”](#)

## Specifying the Method, Sample Positions, and Number of Injections

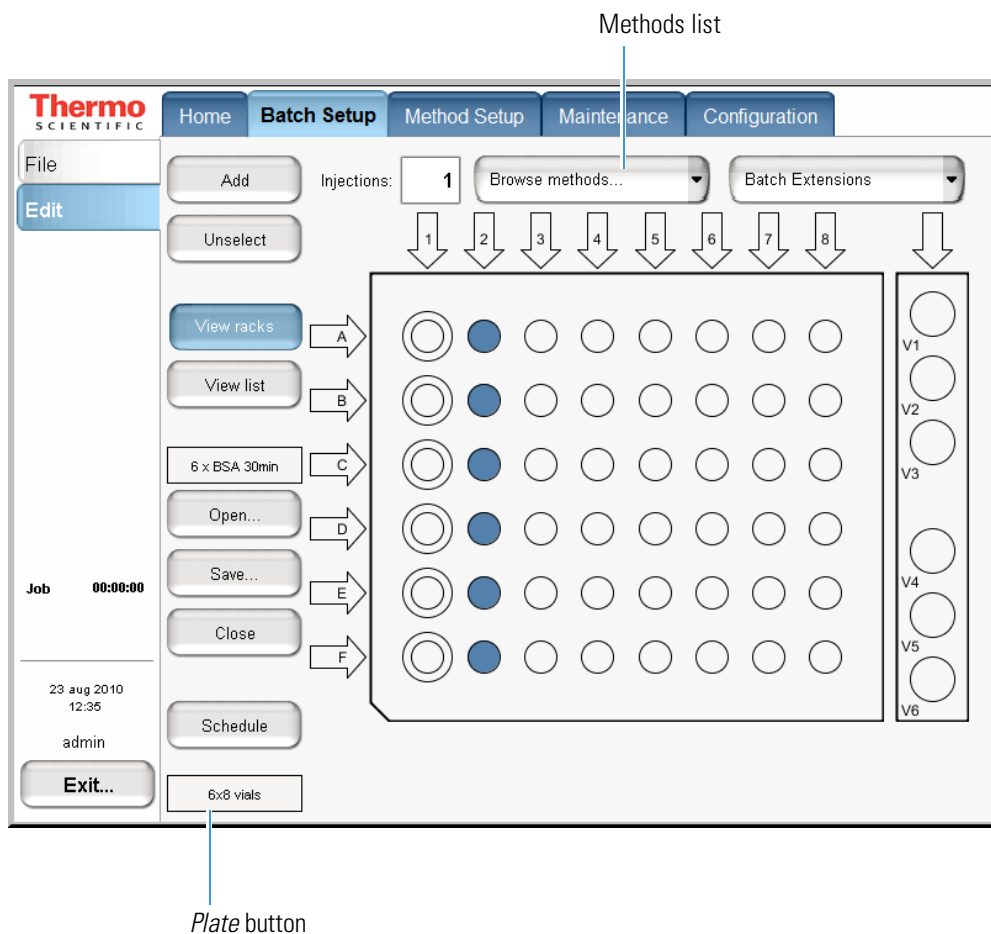
A batch lists the sample injection order, the vial or well positions where the samples are located, the number of injections per sample, and the method used to run the sample.

**❖ To create a batch by using the View Racks view**

1. Press **Batch Setup > Edit**.
2. If you do not see a plate overview, press **View Racks**.

A plate view for the selected plate format appears. [Figure 103](#) shows the plate view for the 6 × 8 vial plate format.

Figure 103. View Racks view on the Edit page of the Batch Setup menu



3. If the plate format is not set to your installed plate, do the following:
  - a. Press the *Plate* button.

**Note** The *Plate* button is the rectangular button imprinted with the plate name on the lower left side. The default plate is 6 × 8 vials.

The Autosampler Rack Configuration dialog box opens.

- b. Select a plate format from the list.
- c. Press **Accept**.

The new plate format appears on the rectangular *Plate* button.

4. If you have already created a name and selected a folder location for the batch, do the following:
  - a. Press **Open**.
  - b. Select the batch.
  - c. Press **Accept**.

The batch name appears in the batch name box.





5. In the Injections box, enter the number of injections per sample.

Default: 1

6. Browse the methods list next to the Injections box, and select the method that you want to use for the entire batch or for a subset of sample runs in the batch.

If you have not already created a method, create one by following the instructions in “Creating a Method” on page 130.

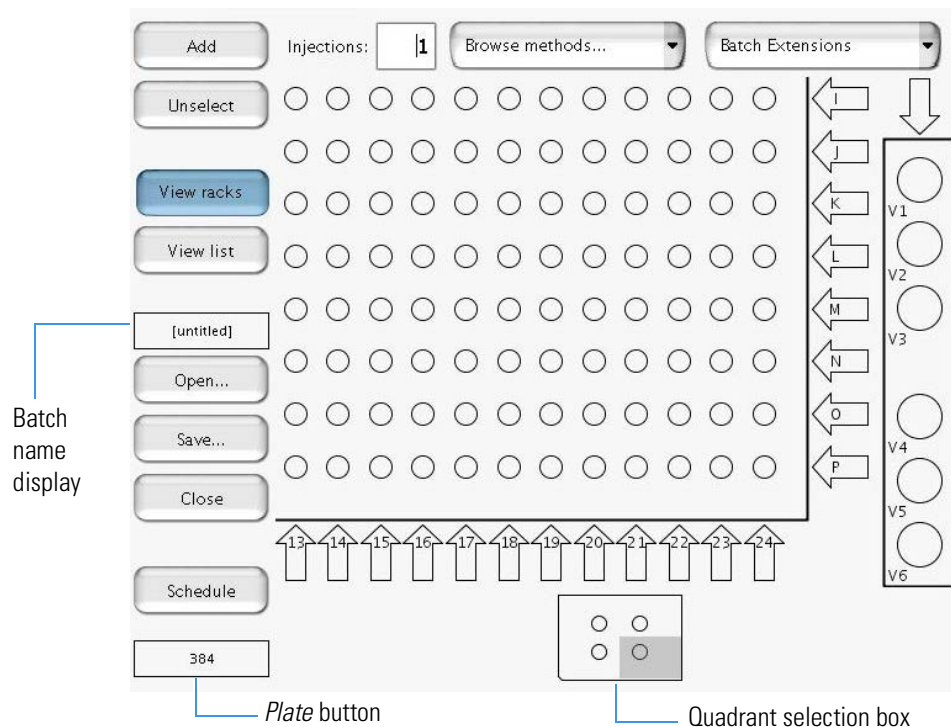
**Tip** To use different methods in the same batch, select the method, select the vial or well positions that you want to run with this method (see [step 7](#)), and then press **Add**.

7. Select the vials or wells that contain the samples that you want to run in the order that you want the instrument to process them.
  - To select an individual vial or well, press the vial or well position in the graphic.
  - To select an entire column, press the DOWN ARROW above the column. For example, press the arrow labeled “1” to select all the vials (A1 to F1) or wells (A1 to H1) from the first column (and first quadrant of a 384-well plate). For the third or fourth quadrant of a 384-well plate, press the UP ARROW below the column.
  - To select the well positions on a 384-well plate, use the quadrant selection box to move between quadrants (see [Figure 104](#)).
  - To select wells A1 to P1, do the following:
    - a. In the quadrant selection box, press the first quadrant, .
    - b. Press the DOWN ARROW, , above the first column.
    - c. In the quadrant selection box, press the third quadrant, .
    - d. Press the UP ARROW, , below the first column.

**Tip** You can clear the vial or well selections by pressing them again.

From left to right and top to bottom, the four quadrants of a 384-well plate are A1 to H12, A13 to H24, I1 to P12, I13 to P24.

**Figure 104.** View Rack view for a 384-well plate

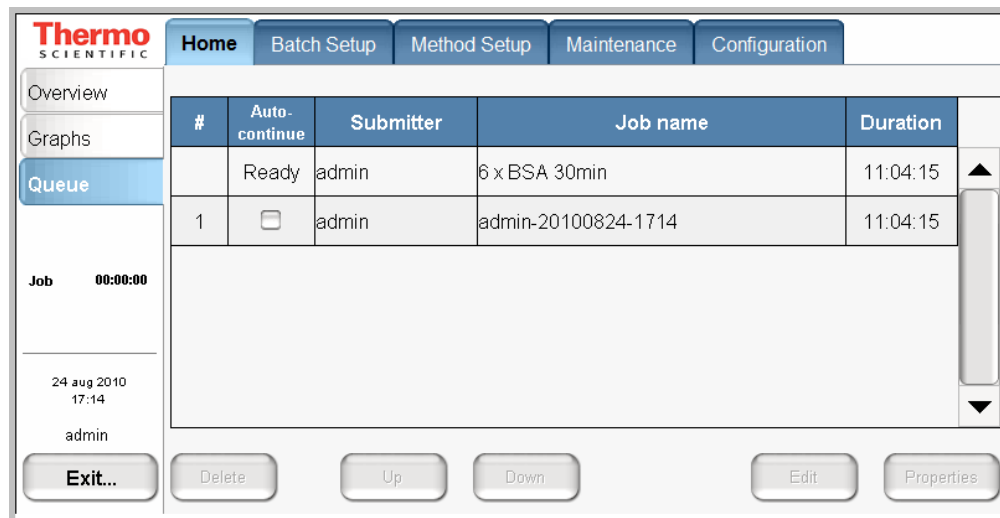


8. Press **Add** to link the selected vials or wells with the selected method.
9. To save the batch, do the following:
  - a. Press **Save**.  
The Save File dialog box opens.
  - b. If you have not already done so, select the appropriate folder and enter a name for the batch.
  - c. Press **Save**.

10. Press **Schedule** (see [Figure 103](#) on [page 148](#)) to submit the batch to the queue.

The application switches to the Queue page of the Home menu (see [Figure 105](#)).

**Figure 105.** Queue page of the Home menu, showing the queued jobs



11. If necessary, move any scheduled batch in the job queue by selecting it and pressing **Up** or **Down**.

You can also delete batches from the Queue by selecting the batches and pressing Delete.

For a batch run to start automatically after the previous batch is finished, you must select its corresponding check box in the Auto-Continue column. In the case shown in [Figure 105](#), the admin-20100824-1714 job does not auto-continue when the 6 x BSA 30 min job is done.

## Starting Sample Acquisition

Before starting the sample acquisition, make sure that the LC/MS system is properly set up. This includes having contact closure (see [“Connecting to the Mass Spectrometer through Contact Closure”](#) on page 42). Also make sure that you have set up the mass spectrometer or any other detector to acquire data during the sample run.

### ❖ To start a batch run

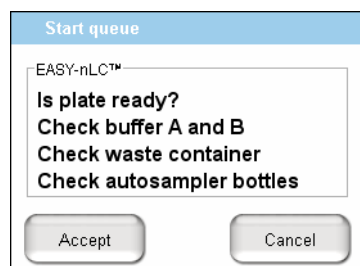
1. Create a batch and submit it to the queue as described in the previous procedure.
2. Press **Home > Overview**.

The Overview page of the Home menu opens.

3. Press **START** to start running the batch.

A message box opens, prompting you to check that you have installed the sample plate, filled solvent bottles A and B, installed the W3 bottle with solvent A, installed the W4 bottle with the needle insert, and emptied the waste beaker (see [Figure 106](#)).

**Figure 106.** Start Queue message box



4. Press **Accept**.

If the Automatic Pre-run Maintenance feature (touch-screen application version 3.2 or later) is turned on and more than 8 hours have elapsed since the last sequence began, the instrument automatically runs the Flush Air script. After the Flush Air script finishes, the instrument starts processing the batch. For information about turning on the Automatic Pre-run Maintenance feature, see [“Setting Up the Properties for the HPLC Device”](#) on page 83.



## Monitoring the Run

You can monitor the run and the system status during analysis from two different pages of the Home menu: Overview or Graphs.

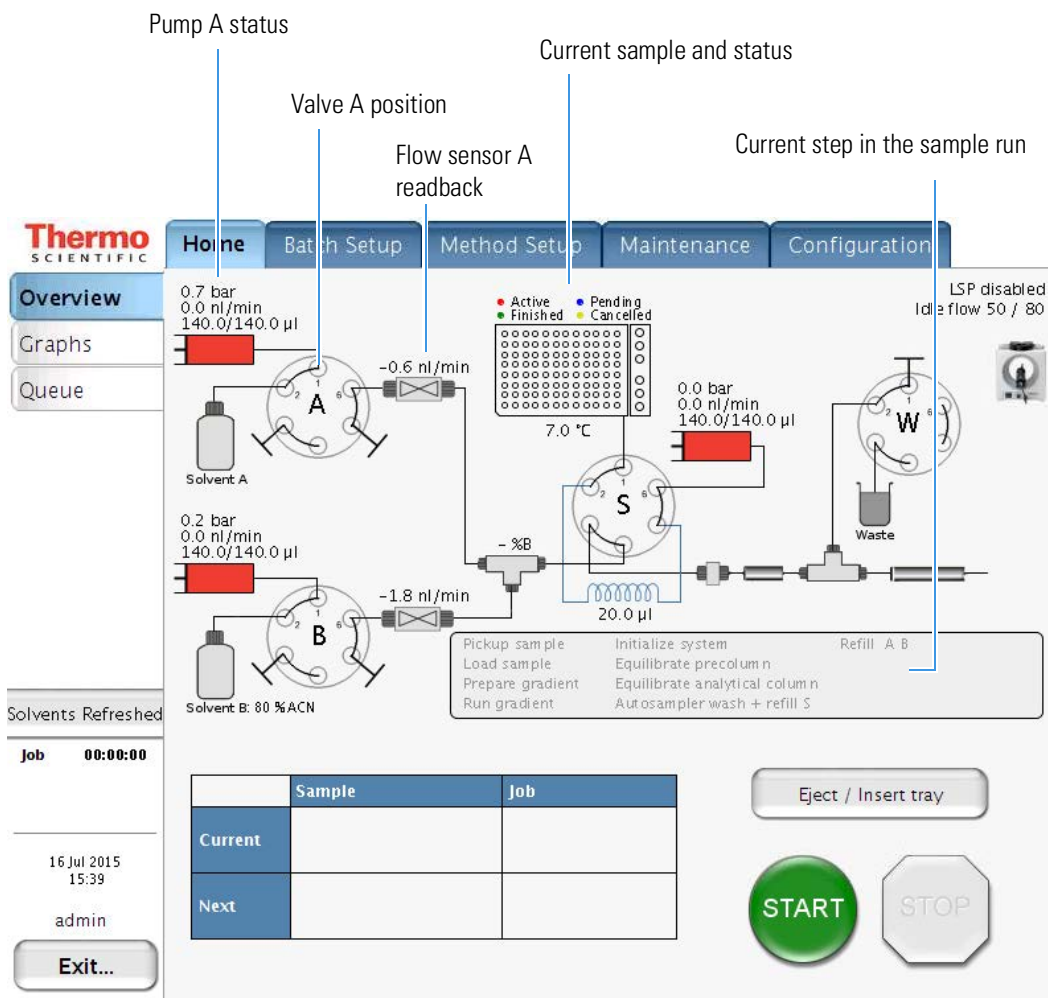
Follow these procedures to monitor the run and view the system status:

- “Using the Overview Page in the Home Menu,” next section
- “Using the Graphs Page in the Home Menu” on page 155

### Using the Overview Page in the Home Menu

The Overview page provides a full schematic overview of the system, complete with real-time updating of all critical component data (Figure 107).

**Figure 107.** Overview page of the Home menu



The system schematic provides information on these items:

- Hardware parts in the system:
  - Position, pressure, and flow for pumps A, B, and S
  - Valve positions (1–2, 1–6, or centered)
  - Percentage of solvent B being delivered
  - Actual flow being measured by the flow sensors

**Note** When pumps A and B are delivering solvent to the mixing Tee, the flow sensors measure the actual flow rate. During the refill A B step, pumps A and B are drawing solvent from the solvent bottles.

- Samples to be analyzed, plus their current status
- Current sample and job, plus the next sample and job
- Current step in the sample run
- If the EASY-nLC instrument controls the temperature of the EASY-Spray ion source's column heater, the column temperature setting appears (in Centigrade) in the third column of the run step area.

Pickup sample	Initialize system	Refill AB
Load sample	Equilibrate precolumn	
<b>Prepare gradient</b>	Equilibrate analytical column	<b>Column:T:35</b>
Gradient	Autosampler wash + refill S	

Column temperature readback

When the batch starts, the green START button is grayed out and the red STOP button becomes active. When the instrument is running, three counters measure the time for job, sample, and gradient.

## Using the Graphs Page in the Home Menu

The Graphs page displays up to four graphs with time-dependent data for the currently active sample (see Figure 108).

**Figure 108.** Graphs page of the Home menu

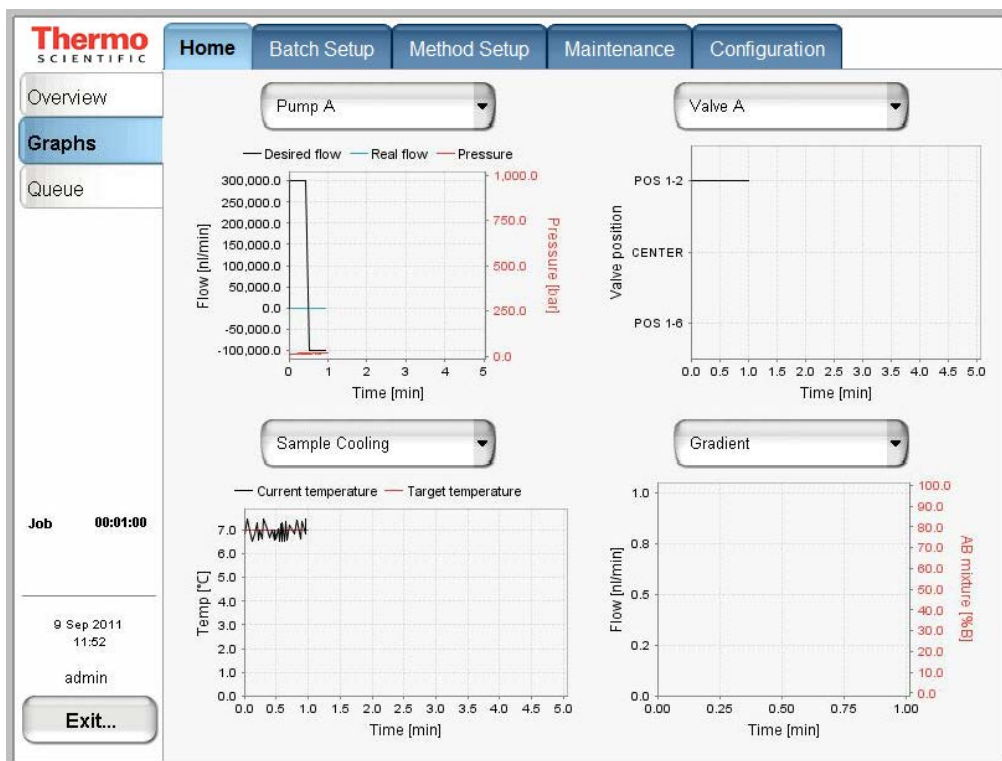


Table 21 describes the different types of graphs that are available. To change graphs, select from their corresponding lists.

**Table 21.** Graphical data types

Graph	Description
Pump A, pump B, and pump S	Shows the desired flow (black line), real flow (blue line), and pressure (red line) over time for each of the three pumps.
Valves A, B, S, and W	Shows the valve position over time for each of the four valves.
Gradient	Shows the theoretical gradient (dotted red line) and the actual gradient dynamically calculated on the feedback values from the pump flow sensors (full red line), plus the actual flow calculated on the feedback values from the pump flow sensors.
Temperature	Shows the measured temperature on the plate cooler over time.

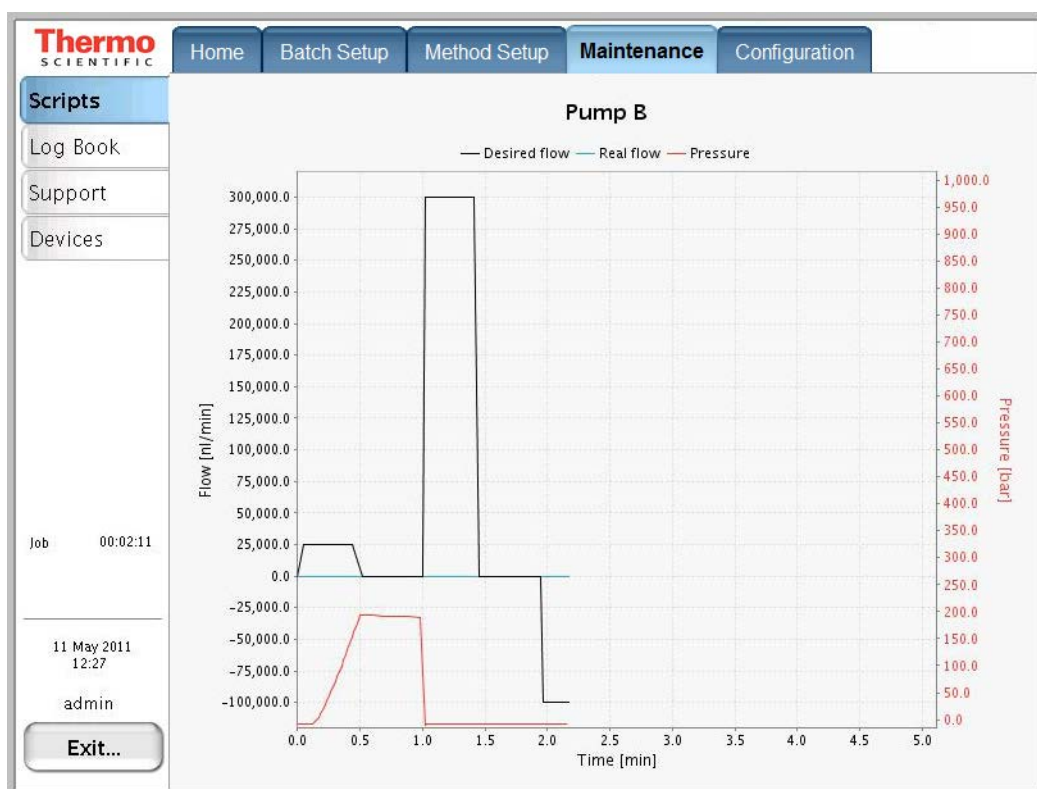
You can change the graph size as follows:

- Touch the graphs to enlarge them and then touch them again to minimize the view.

Figure 109 shows the enlarged flow graph for pump B.

- Touch the y axis of each graph to cycle the Flow axis through several preset scales.

Figure 109. Pump B graph enlarged



## Stopping Sample Acquisition

You can stop the sample acquisition during the run by pressing the red STOP button on the Home > Overview page. This halts all actions on the HPLC system and the following buttons become active:

- **Restart Sample:** Runs the method from its beginning again. Only press this button if you are sure you have enough sample in the vial or microtiter plate.
- **Cancel Sample:** Cancels the current sample and skips to the next sample in the batch job.
- **Cancel Batch:** Cancels the entire current batch job.
- **Eject Plate:** Ejects the plate for your inspection to help you determine how to proceed.

## Editing the Running Batch

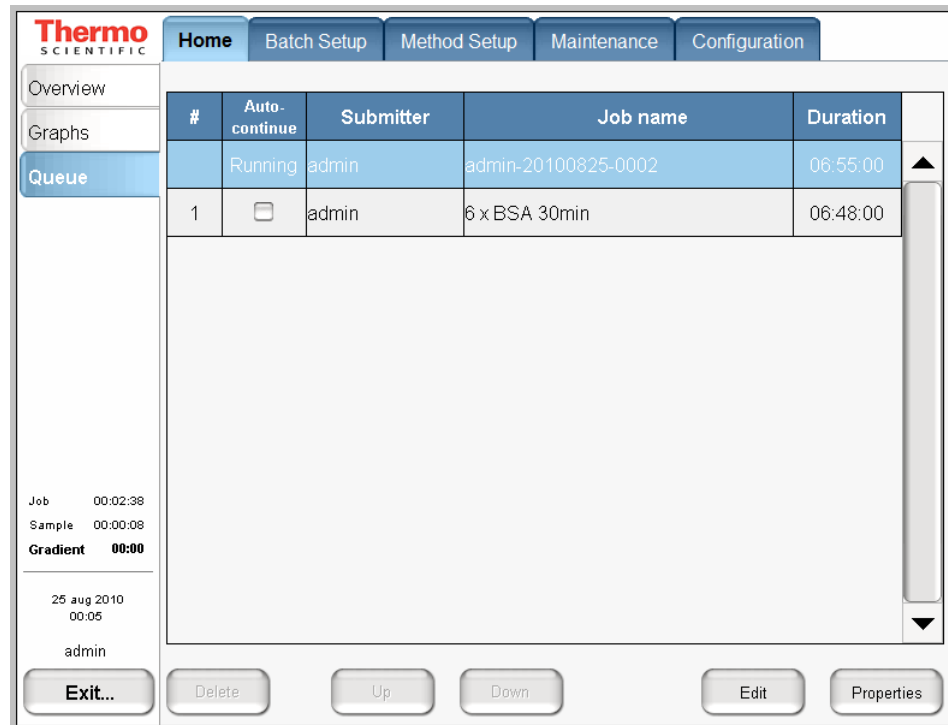
You can edit the running batch if you want to add samples or remove samples that the instrument has not already run.

### ❖ To edit the running batch

1. Press **Home > Queue**.

The Queue page of the Home menu opens (see [Figure 110](#)).

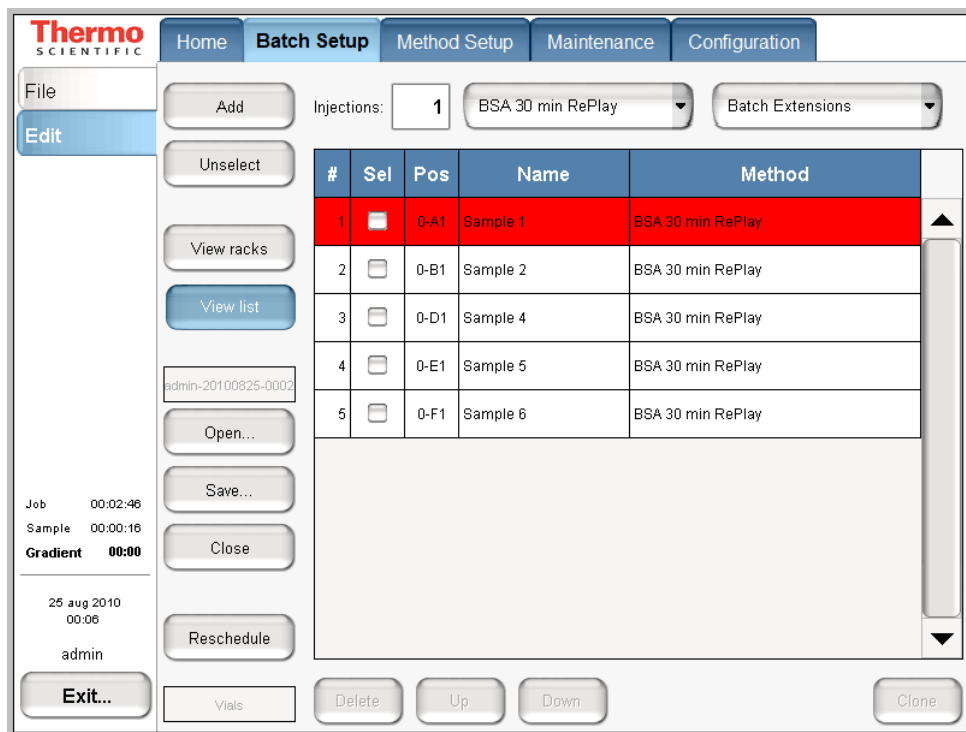
**Figure 110.** Queue page of the Home menu



2. Select the batch job from the list and press **Edit**.

The Batch Setup > Edit page opens (see [Figure 111](#)).

Figure 111. Edit page of the Batch Setup menu



3. Edit the batch—that is, add, change, or remove samples that are not yet tested.
4. Press **Reschedule**.

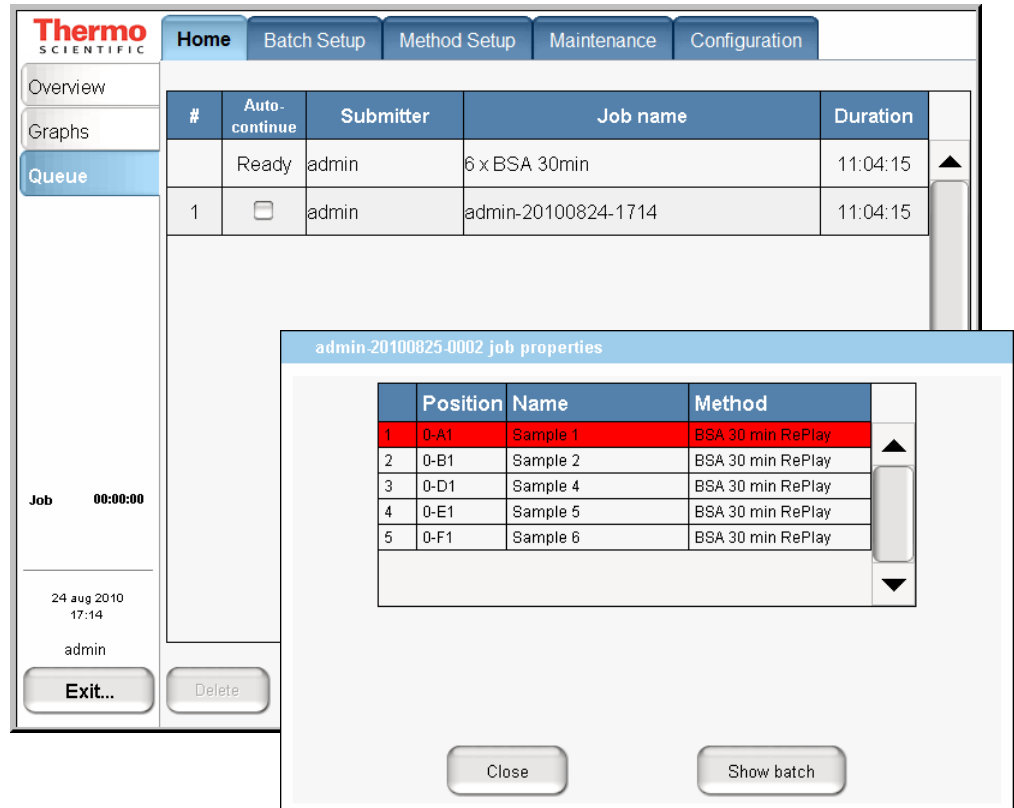
The batch continues running.

**IMPORTANT** If you do not reschedule the batch after completing the batch edit, the instrument stops after the current sample run.

5. (Optional) Check the sample list as follows:
  - a. Press **Home > Queue**.
  - b. Press **Properties** (see [Figure 110](#) on page 157).
  - c. Press **Show Samples**.

The Job Properties box opens (see [Figure 112](#)) and the Show Batch button appears in place of the Show Samples button.

Figure 112. Sample list job properties on the Queue page



d. Click **Close**.

# Troubleshooting a Sample Run

If a problem occurs during a sample analysis, the EASY-nLC instrument displays the error in a pop-up box and stops the sample processing.

#### ❖ To troubleshoot an error condition that stops the sample processing

1. Press **Cancel Batch**.
2. Refer to the troubleshooting tips or follow the troubleshooting procedures in the *EASY-nLC Series Troubleshooting and Maintenance Guide*.

After you fix the problem, reschedule the batch (possibly changing the number of samples, their well positions, or both).



## Installing and Controlling External Devices

The EASY-nLC instrument (with the 3.2 version of the touch-screen software) can control the Advion Replay device and the EASY-Spray ion source's column temperature.

To add the EASY-Spray device to the EASY-nLC device list or to install and use the optional Advion RePlay device, follow the instructions in this appendix.

The RePlay device was specifically developed for nanoflow LC/MS analyses. It splits the sample from one injection, so a second analysis is provided with no additional sample required. The RePlay device consists of a primary column, a six-port valve, a flow sensor, tubing, a control panel, and a secondary column/emitter. It performs a second analysis of one sample without a reinjection, which allows for twice the analytical possibilities from every nanoLC injection. For more information, visit the Advion website:

[www.advion.com/biosystems/replay/index.php](http://www.advion.com/biosystems/replay/index.php)

Thermo Fisher Scientific does not offer support for—or is in any way responsible for—the functionality of the RePlay device itself and the associated tubing and columns that it requires.

For information about connecting the EASY-Spray ion source to the EASY-nLC instrument, refer to the *EASY-Spray Series Ion Source User Guide*.

### Contents

- [Adding Devices to the EASY-nLC Devices List](#)
- [Connecting the RePlay Device to the EASY-nLC Instrument](#)
- [Setting Up a Method with the RePlay Device](#)
- [Monitoring the Run of a RePlay Method](#)
- [Effect of Additional Run Time for a RePlay Experiment](#)

## Adding Devices to the EASY-nLC Devices List

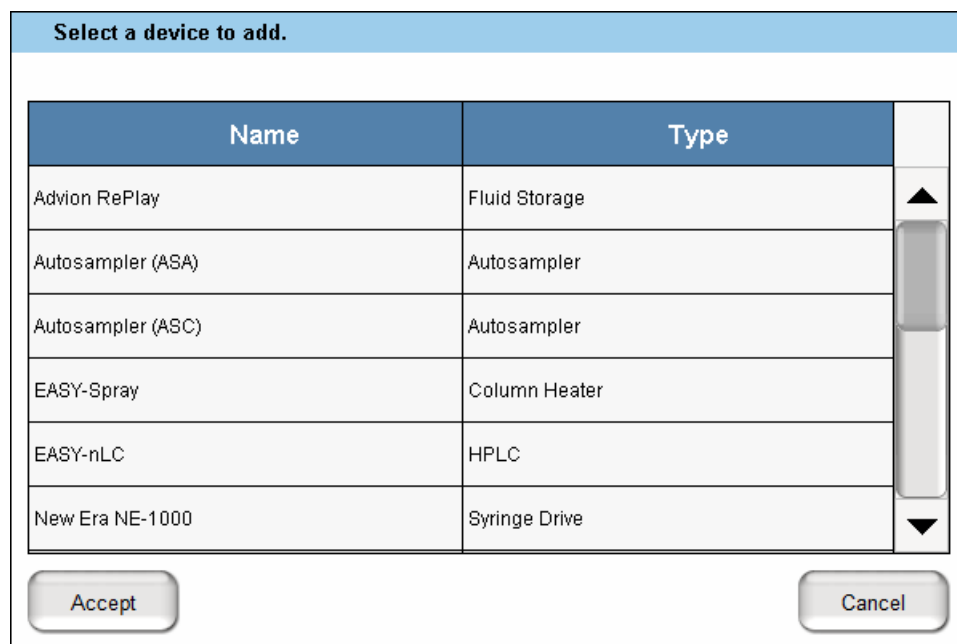
The EASY-nLC instrument (with the 3.2 version of the touch-screen software) can control the Replay device and the EASY-Spray's column heater.

### v To add a device to the device list in the EASY-nLC application

1. Press **Maintenance > Devices** and check that the device is not already present in the Devices list. If not, add the device as described in the following steps.
2. For each device that you want to add, do the following:
  - a. Press **Add Device**.

The Select a Device to Add dialog box opens.

**Figure 113.** Select a Device to Add dialog box



- b. Do one of the following:
      - To add the Replay device, select **Advion RePlay** and press **Accept**.
      - To add the EASY-Spray device, select **EASY-Spray** and press **Accept**.
3. Verify that the Devices list includes the added device.

Selecting the device displays information about the device driver on the About page (see [Figure 114](#) and [Figure 115](#)).

**Figure 114.** About information for the RePlay device

**Devices**

	Name	Type	
	Piston Pump [Pump S]	Piston Pump	▲
	RePlay	Fluid Storage	▼
	Rotary Valve [Valve A]	Rotary Valve	▼

Add Device  
 Remove Device  
 Assign Slave

**About**

**RePlay**

**Manufacturer:** Advion BioSciences, Inc.  
**Product:** RePlay  
**Type:** Fluid Storage  
**Driver info:** 0.1.0  
**Firmware info:** <N/A>  
**Serial Number:** <N/A>  
**Usages:** Capture/Playback Eluent  
**Slaves:** <N/A>

**Figure 115.** About information for the EASY-Spray device

**Devices**

	Name	Type	
	EASY-Spray	Column Heater	▲
	EASY-nLC™ [HPLC]	HPLC	▼
	Piston Pump (flow feedback) [Pump A]	Piston Pump w/ flow fee...	▼

Add Device  
 Remove Device  
 Assign Slave

**About**

**EASY-Spray**

**Manufacturer:** Thermo Scientific  
**Product:** EASY-Spray  
**Type:** Column Heater  
**Driver info:** 0.1.1  
**Firmware info:** devType:3; fwVersion:N/A; serialNumber:1  
**Serial Number:** serialNumber:1  
**Usages:** Column heating  
**Slaves:** <N/A>

## Connecting the RePlay Device to the EASY-nLC Instrument

Before you can use the RePlay device with the EASY-nLC instrument, you must connect the contact closure cables and the solvent lines.

The RePlay device has a remote port on its back panel and a six-port valve (see [Figure 116](#)) on its front panel.

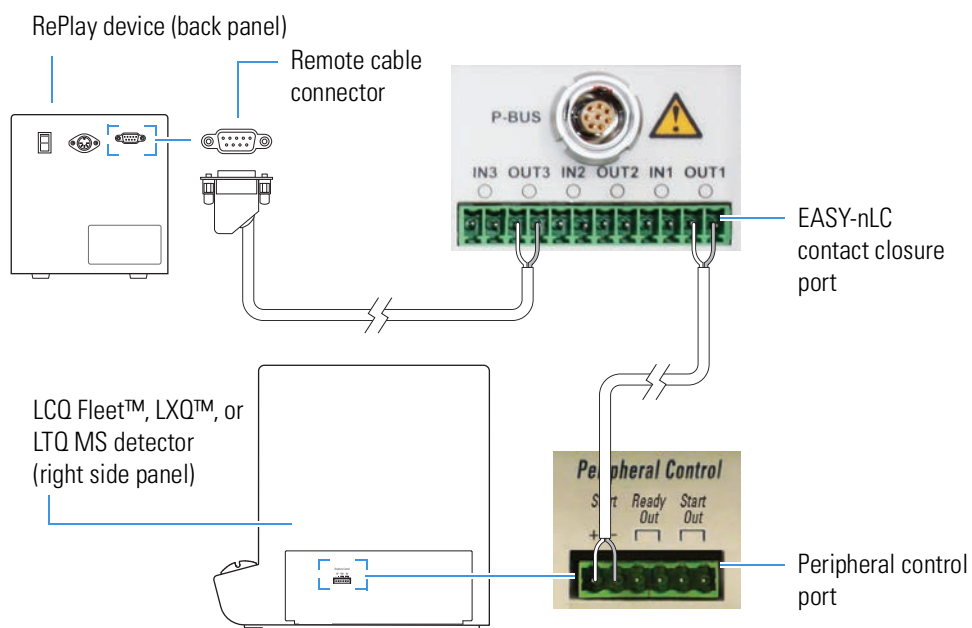
**Figure 116.** Front panel of the RePlay device (courtesy of the Advion Inc. website)



### v To connect the RePlay device to the EASY-nLC instrument

1. Turn off the EASY-nLC instrument.
2. Connect the Remote cable port on the back panel of the RePlay device to the Out3 pins on the back panel of the EASY-nLC instrument (see [Figure 117](#)).

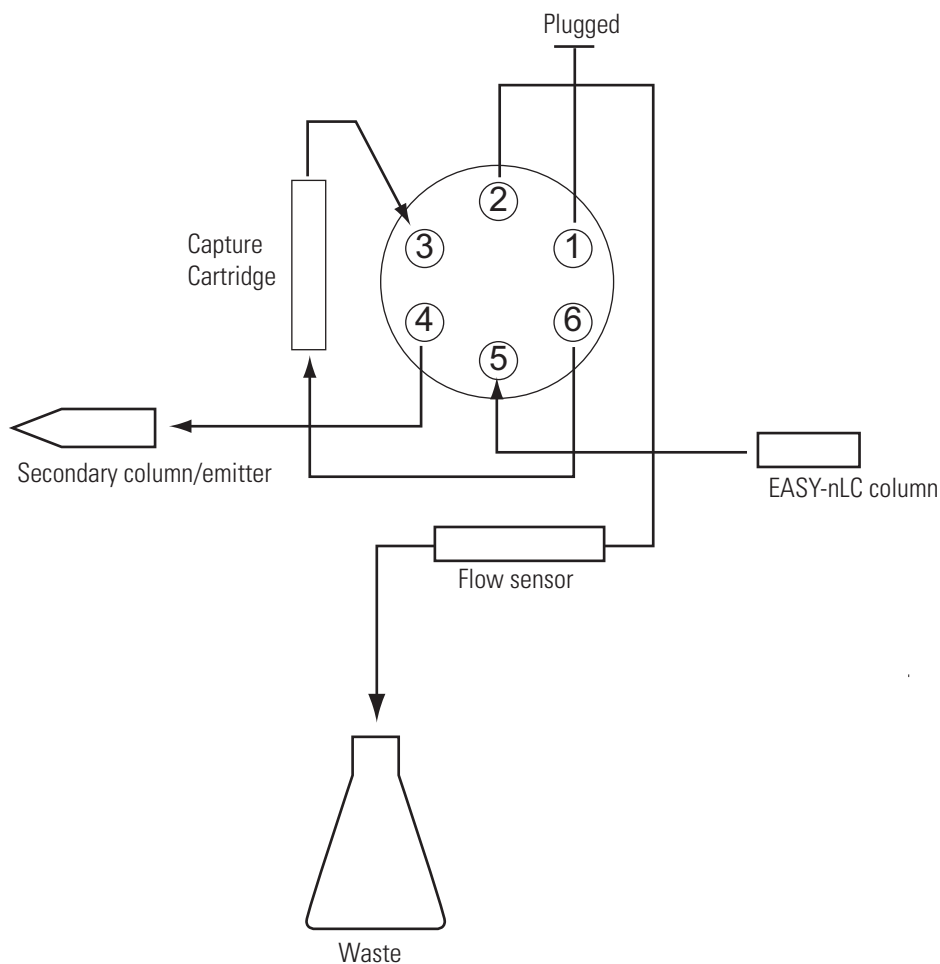
**Figure 117.** RePlay device connection to the EASY-nLC contact closure port



3. Connect the solvent lines to the valve on the front panel of the RePlay device (see [Figure 118](#)) as follows:
  - Connect the output end of the EASY-nLC analytical column to position 5 of the RePlay valve.
  - Connect position 4 of the RePlay valve to the secondary column/emitter.

**Note** For more information about connecting and using the RePlay device, refer to the *Advion RePlay User's Manual* supplied by Advion Biosystems, Inc.

**Figure 118.** Valve connections for the standard plumbing configuration



4. Turn on the EASY-nLC instrument.

## Setting Up a Method with the RePlay Device

### ▼ To set up a method with the RePlay device

1. To use the RePlay device, enable the method extension on page 1 of the Method Setup wizard (you can also open an existing method and enable its extension).

An extra RePlay Method Setup page is now available (see [Figure 119](#)).

**Figure 119.** Page 1/7 of the Method Setup wizard

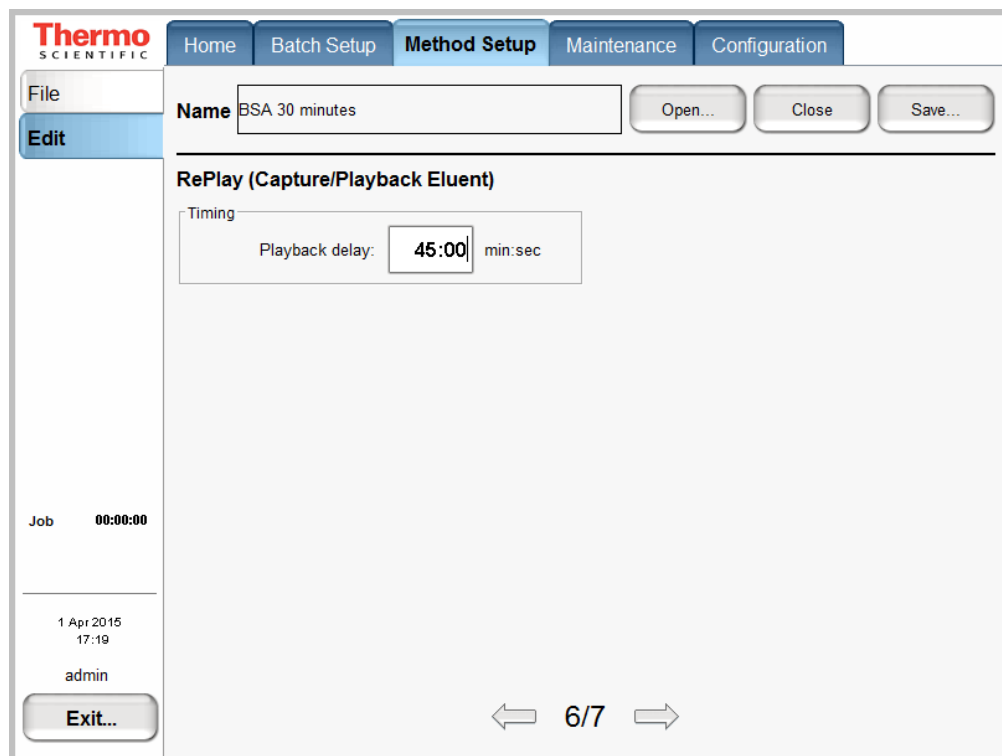
2. On page 3 of the Method Setup wizard, properly set up the gradient. For more information, see [“To build a gradient”](#) on [page 136](#).

The RePlay device uses a nanoflow splitter and a valve to split the EASY-nLC eluent to a direct online analysis and to a Capture Cartridge in the device. The RePlay valve switches to direct the captured chromatogram to the mass spectrometer for a second analysis of the same injection. To acquire the second analysis, the gradient has to be prolonged.

3. On page 6, enter the playback delay relative to the MS START signal (which can be the start of either sample loading onto the columns or the start of the gradient, and set from the Configuration > Connections page).

This value, as shown in Figure 120, indicates how long into the gradient to switch back to playback mode. At the end of the gradient, the contact closure control reverts to capture mode.

Figure 120. RePlay Playback Delay setting



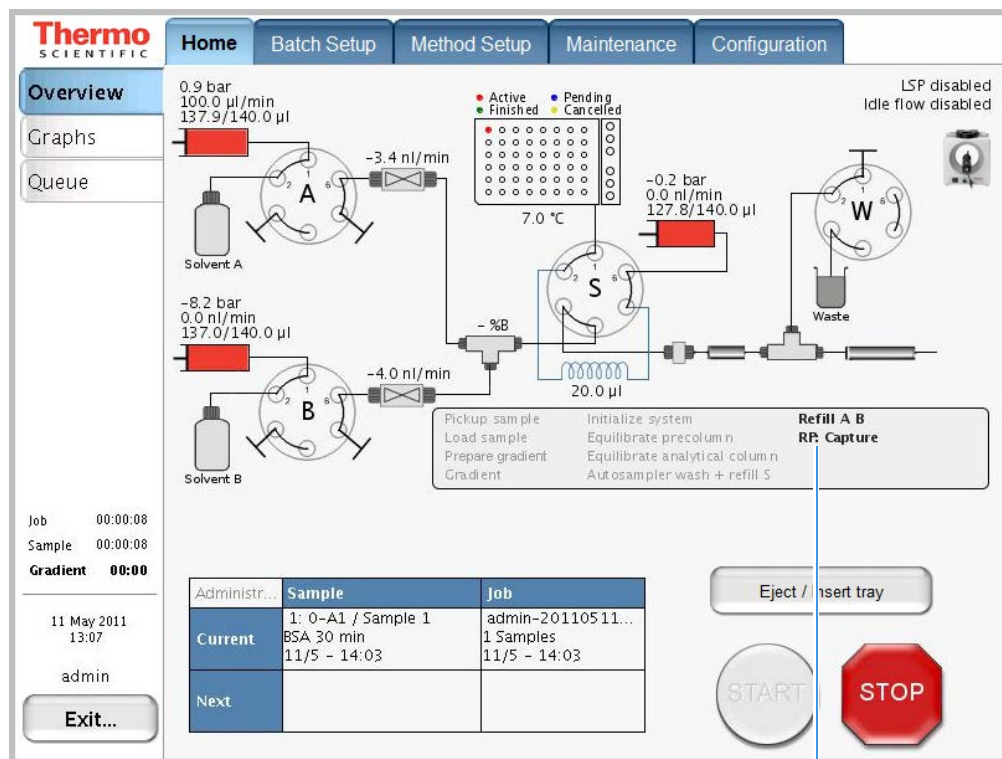
## Monitoring the Run of a RePlay Method

The readback for a RePlay method displays Capture or Replay.

▼ **To monitor the run of a RePlay method**

1. When running a sample using a method with the RePlay extension, press **Home > Overview**.
2. View the RePlay (“RP”) mode readback in the center status box (see [Figure 121](#)).

**Figure 121.** RePlay method readback



RePlay method readback



## Effect of Additional Run Time for a RePlay Experiment

For a typical RePlay application—injecting and loading 5  $\mu\text{L}$  of sample with a gradient of 60 minutes + 30 minutes RePlay—the full timing is as follows:

- 2 minutes – Refilling pumps at 100  $\mu\text{L}/\text{min}$
- 2 minutes – Injecting 5  $\mu\text{L}$  at 20  $\mu\text{L}/\text{min}$  and waiting for pressures to settle
- 12 minutes – Loading the sample onto the analytical column with 15  $\mu\text{L}$  at 1.25  $\mu\text{L}/\text{min}$
- 60 minutes – Gradient + autosampler wash
- 30 minutes – RePlay while also reequilibrating the analytical column

In total, you can achieve an MS utilization rate of close to 85 percent (90/106), despite the fact that the EASY-nLC/MS system cannot overlap (parts of) two runs in parallel.

## **7 Installing and Controlling External Devices**

Effect of Additional Run Time for a RePlay Experiment



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