

nanoACQUITY UPLC System with HDX Technology System Guide

Revision A

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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Contacting Waters

Contact Waters[®] with enhancement requests or technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us via the Internet, telephone, or conventional mail.

Waters contact information:

Contacting medium	Information
Internet	The Waters Web site includes contact information for Waters locations worldwide. Visit www.waters.com .
Telephone and fax	From the USA or Canada, phone 800 252-HPLC, or fax 508 872 1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site.
Conventional mail	Waters Corporation 34 Maple Street Milford, MA 01757 USA

Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

Considerations specific to the HD Exchange system

High voltage hazard



Warning:

- To avoid electric shock, do not remove instruments' protective panels. The components they cover are not user-serviceable.
- To avoid nonlethal electric shock when the instrument is in Operate mode, avoid touching the areas marked with the high voltage warning symbol. To touch those areas, first put the instrument in Standby mode.

Safety advisories

Consult [Appendix A](#) for a comprehensive list of warning and caution advisories.

Operating the HD Exchange system

When operating the nanoACQUITY UPLC Hydrogen Deuterium Exchange system, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

Symbol	Definition
 <p>Waters Corporation 34 Maple Street Milford, MA 01757 U.S.A.</p>	Manufacturer
 <p>Waters Corporation Floats Road Wythenshawe Manchester M23 9LZ United Kingdom</p>	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
 <p>ABN 49 065 444 751</p>	Australia C-Tick EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use

Audience and purpose

The HD Exchange with nanoACQUITY UPLC System Guide *can address experienced, qualified users; systems administrators; installation engineers and field service engineers.*

A system guide explains in detail procedures associated with configuring and installing a product.

Intended use of the HD Exchange system

Waters designed the HD Exchange system as a research tool to perform analyses of protein dynamics and changes to protein conformation. The HD Exchange system is for research use only and is not intended for use in diagnostic applications.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for

standards should include the entire range of QC samples, typical specimens, and atypical specimens.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

ISM classification

ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with IEC CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

EC authorized representative



Waters Corporation
Floats Road
Wythenshawe
Manchester M23 9LZ
United Kingdom

Waters Corporation (Micromass UK Ltd.)
Floats Road
Wythenshawe
Manchester M23 9LZ
United Kingdom

Telephone: +44-161-946-2400
Fax: +44-161-946-2480
Contact: Quality manager

Table of Contents

Copyright notice	ii
Trademarks	ii
Customer comments	ii
Contacting Waters	iii
Safety considerations	iii
Considerations specific to the HD Exchange system.....	iv
Safety advisories	iv
Operating the HD Exchange system	iv
Applicable symbols.....	v
Audience and purpose.....	v
Intended use of the HD Exchange system.....	v
Calibrating	v
Quality-control	vi
ISM classification	vi
ISM Classification: ISM Group 1 Class B	vi
EC authorized representative	vii
1 nanoACQUITY UPLC System with HDX Technology	1-1
System components	1-2
Hydrogen deuterium exchange	1-2
nanoACQUITY UPLC HDX Manager	1-3
2 Setting up the system	2-1
Configuring the system	2-1
Configuration using a nanoACQUITY UPLC System with HDX Technology.....	2-3
Fitting installation recommendations	2-5

Making plumbing and gas connections with a nanoACQUITY UPLC System with HDX Technology	2-6
Configuration using a nanoACQUITY UPLC System with HDX Automation.....	2-11
Assembling the LEAP Autosampler	2-12
Making the electrical connections in a nanoACQUITY UPLC System with HDX Automation.....	2-13
Restoring backup files	2-17
Making the plumbing and gas connections in a nanoACQUITY UPLC System with HDX Automation.....	2-17
Making the waste connections in a nanoACQUITY UPLC System with HDX Automation.....	2-19
Checking/setting HDX Automation Manager needle penetration values ..	2-22
Installing columns	2-23
3 Maintenance Procedures	3-1
Inspecting and replacing the HDX Automation Manager injection unit syringe	3-1
Cleaning and lubricating the HDX Automation Manager sample manager	3-2
Maintaining your injection port components	3-4
Spare parts	3-6
Safety and handling	3-6
Defrost	3-7
A Safety Advisories	A-1
Warning symbols	A-2
Task-specific hazard warnings.....	A-2
Specific warnings	A-3
Caution advisory	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-11
Electrical symbols.....	A-11

Handling symbols	A-12
B Materials of Construction and Compatible Solvents	B-1
Preventing contamination	B-1
Items exposed to solvent	B-1
Wetted materials.....	B-1
Solvents used to prepare mobile phases	B-2
C Specifications of the HDX Manager	C-1
Electrical power specifications	C-1
Communications specifications	C-2
Physical specifications	C-2
Environmental specifications	C-3
Miscellaneous specifications	C-3
D Start up kit components	D-1
Index	Index-1

1 nanoACQUITY UPLC System with HDX Technology

The nanoACQUITY UPLC[®] System with HDX Technology, combined with high resolution mass spectrometry, is used to analyze changes in higher order protein structure, including protein conformation and protein interactions. The three-dimensional shape in solution, as driven by secondary, tertiary, and quaternary interactions, can provide in-depth information about:

- Drug binding to a protein target molecule
- Stability for biopharmaceutical product development
- Protein-protein interactions

Contents:

Topic	Page
System components	1-2
Hydrogen deuterium exchange	1-2
nanoACQUITY UPLC HDX Manager	1-3

Ensure that these prerequisites are met:

- The site is properly prepared.
- If a Xevo G2 Q-ToF is part of the system, it is installed and set up according to the instructions in the *Waters Xevo G2 Q-ToF Installation Manual*, P/N 715002401. See Recommended System Configurations in the ACQUITY console help.
- If a Synapt G2 is part of the system, it is installed and set up according to the instructions in the *Waters Synapt G2 MS/HDMS Installation Manual*, P/N 715002112. See Recommended System Configurations in ACQUITY console help.
- MassLynx 4.1 with appropriate SCNs for Xevo G2 Q-ToF or Xevo ToF or Synapt G2 are installed.

System components

The nanoACQUITY UPLC System with HDX Technology is available in two configurations.

- nanoACQUITY UPLC System with HDX Technology - standalone system, with manual injection of sample.
- nanoACQUITY UPLC System with HDX Automation- includes a robotic autosampler providing sample preparation and injection capabilities.

The nanoACQUITY UPLC System with HDX Technology includes the following components:

- nanoACQUITY UPLC HDX Manager
- nanoACQUITY binary solvent manager (nanoBSM)
- nanoACQUITY auxiliary solvent manager (nanoASM)
- nanoACQUITY cart
- Mass spectrometer, such as: Xevo[®] G2 ToF, Xevo G2 QToF, or SYNAPT[®] G2
- DynamX[™]
- MassLynx[™] 4.1
- PLGS 2.5

The nanoACQUITY UPLC HDX System with Automation combines the standalone configuration, minus the cart, with the following components:

- LEAP HDX Automation Manager
- Automation Shelf

Hydrogen deuterium exchange

Relatively pure, aqueous solutions of proteins are exposed to deuterium oxide (D₂O) or “heavy water.” The deuterium (D) exchanges with amide hydrogen on the peptide backbone. The rate of exchange is a function of the solvent accessibility and hydrogen bonding for each amide hydrogen. Sites with the greatest uptake are generally more solvent accessible and the free amide hydrogen is available to exchange.

Once the deuterium interacts with the target protein, the protein may be analyzed while intact, or may be subjected to proteolysis with pepsin or other acidic protease to localize conformational information.

Utmost care must be taken to preserve deuterium incorporation. All operations must be performed rapidly within a strict acidic pH range. The chromatographic separation must be done at 0 °C.

The goal is to quantify small changes in mass caused by the uptake of deuterium in biologically significant proteins using high-resolution, accurate mass spectrometry. The Automated system combined with LEAP is useful for high-throughput analysis required for the drug candidate screening.

A typical HD exchange method includes the following procedural steps:

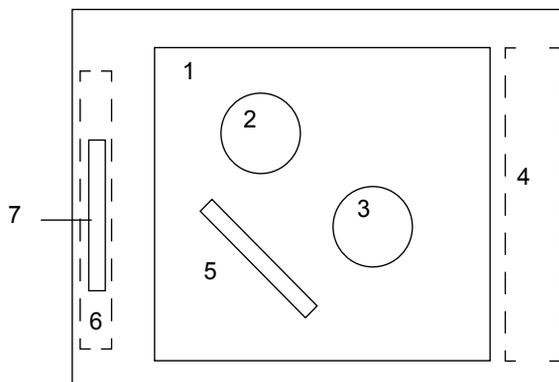
1. A protein is incubated with D₂O.
2. At either a set endpoint or at timed intervals the D₂O incubation is quenched and the sample is placed into the injection loop and diverted to the digestion column for digestion. The ASM supplies eluent.
3. Following peptic digestion, peptide fragments are eluted from the digestion column to the Vanguard™ trapping column at 0 °C. The trap valve is in the waste position to allow the ASM to wash unwanted solutes away, retaining the peptide fragments on the trapping column.
4. The trap valve changes so that the trapping column is in-line with the analytical column, and gradient elution proceeds from the trap to the analytical and into the mass spectrometer.

MS data are acquired in MS^E mode using MassLynx™ software. Once collected, data are analyzed using Protein Lynx Global SERVER 2.5 (PLGS) and DynamX Software.

nanoACQUITY UPLC HDX Manager

The HDX manager offers online pepsin digestion, online desalting, and UPLC® separations at 0 °C. System LEDs indicate the power (green/off) and status (red/green) of the HDX manager.

HDX manager components:



HDX manager components:

Number	Component
1	Sample chamber
2	Injection valve
3	Trap valve
4	Fluidic Block
5	Analytical column
6	Digestion chamber
7	Digestion column

Sample chamber

- Thermally-controlled chamber, ambient to 0 °C
- One trap valve
- One injection valve
- Two heater-cooler engines
- An analytical-column holder
- A sample injection port

Digestion chamber

- Thermally-controlled column heater, 10 °C to 45°C
- Plumbing manifold

Other components

- eCord™ support for analytical columns
- Internal and external plumbing connections
- Front panel with LEDs and mechanical door latch
- Control boards, power supply, and cable
- Back panel outputs and inputs
- Condensation management tray
- Pressure regulator
- USB to RS-232

The sample chamber contains an injection valve, trapping valve, analytical column and a Vanguard trap column. The sample chamber can be maintained between 0 °C and room temperature.

The digestion chamber, a small chamber on the left-hand side of the manager, contains the digestion column for the digestion phase. The digestion column sits in-line between the injection valve and the trap valve. The digestion column in the digestion chamber can be accessed independently of the sample chamber.

2 Setting up the system

A Waters field service engineer installs the system. Prior to installation, refer to the nanoACQUITY UPLC™ with HDX Automation / Technology System Site Preparation Guide for detailed information on the plumbing and electrical connections. Also note that your mass spectrometer must be operational and proven prior to configuration of the system.

Contents:

Topic	Page
Configuring the system	2-1
Installing columns	2-23

Configuring the system

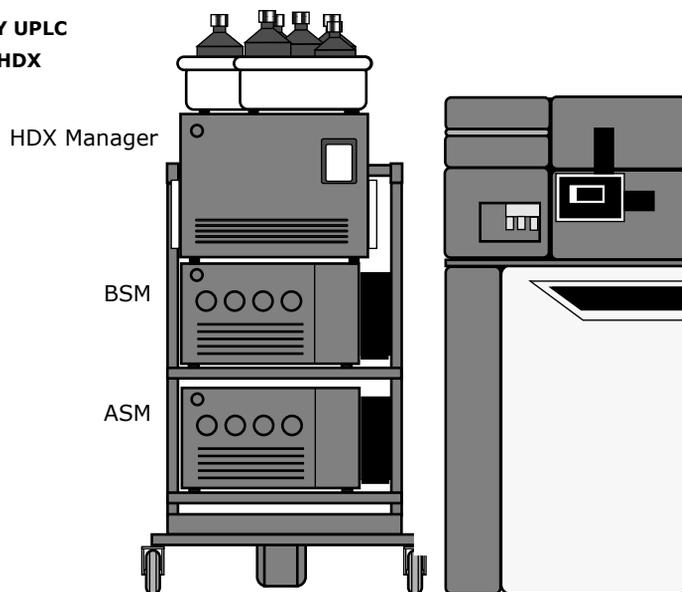
The HD exchange can be performed using either of two system configurations; nanoACQUITY UPLC System with HDX Technology or nanoACQUITY UPLC System with HDX Automation.

nanoACQUITY UPLC Systems with HDX Technology, installed on a nanoACQUITY cart, have an HDX manager, two solvent managers, and a mass spectrometer. An operator performs sample preparation and injection manually.

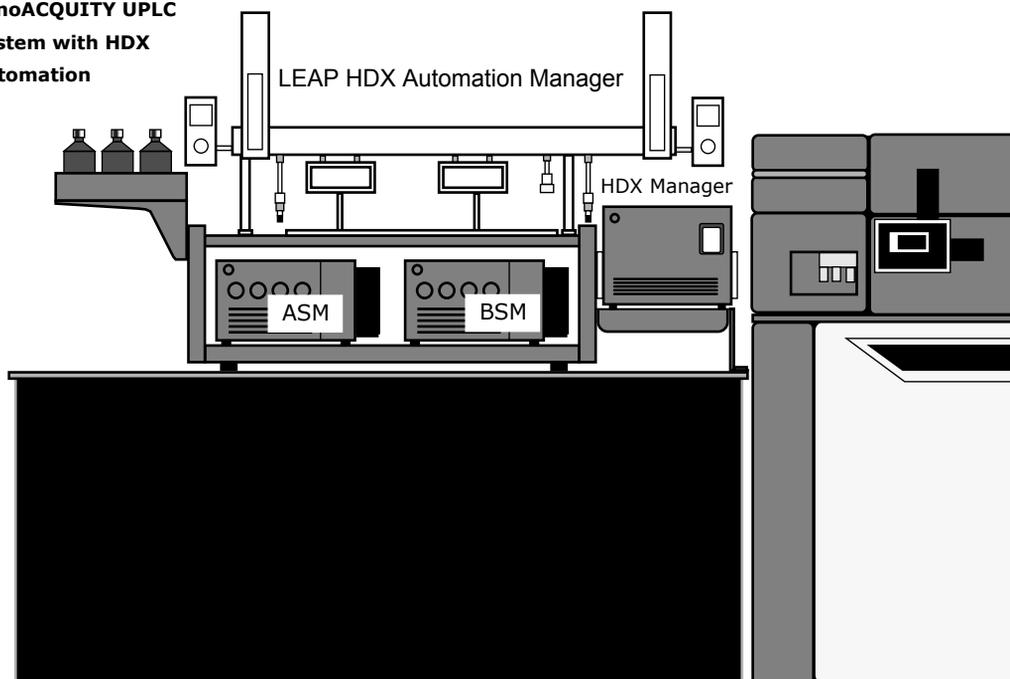
nanoACQUITY UPLC Systems with HDX Automation, installed on a bench-top accessory, include the same components, minus the cart, as a standalone system, plus an optional LEAP HDX Automation Manager (an online robotic autosampler 120-cm system) for sample preparation and injection.

Examples of nanoACQUITY UPLC HDX systems:

nanoACQUITY UPLC System with HDX Technology



nanoACQUITY UPLC System with HDX Automation



Configuration using a nanoACQUITY UPLC System with HDX Technology

Positioning the components with a nanoACQUITY UPLC system with HDX technology

The standalone system uses the nanoACQUITY System Cart.

To position the components:

1. Position the nanoACQUITY HDX System components on the nanoACQUITY System Cart as follows:
 - nanoACQUITY Auxiliary Solvent Manager - bottom cart position
 - nanoACQUITY Binary Solvent Manager - above the Auxiliary Solvent Manager
 - nanoACQUITY UPLC HDX Manager - on top of the Binary Solvent Manager
 - Solvent trays - on the top-rear and top-right of the HDX Manager, making sure the injection port is accessible



Warning: Ensure the Bottle Trays Chassis have been installed.

2. Position the nanoACQUITY System Cart to the left of the mass spectrometer.

Making electrical connections in a nanoACQUITY UPLC system with HDX technology

Follow the steps in this section to make power and communications connections for a nanoACQUITY UPLC System with HDX Technology.

To make electrical connections:

1. Connect the BSM and ASM Ethernet cables to the Ethernet switch on the HDX manager.
2. Connect the AC power cords from the BSM, ASM and HDX manager to the nanoACQUITY System Cart.

3. Connect the AC power cords from the mass spectrometer, rotary/scroll pump, system PC, and nanoACQUITY System Cart to a dedicated, grounded AC power source.
4. For systems with a Synapt G2 mass spectrometer, make the following Ethernet connections:
 - Synapt G2 Ethernet port to the Ethernet switch on the HDX manager.
 - System PC Ethernet port to the Ethernet switch on the HDX manager.
5. For systems with a Xevo G2 QTOF mass spectrometer, make the following connections:
 - EPC upper Ethernet port to the Xevo G2 QTOF Ethernet port.
 - EPC lower Ethernet port to the System PC Ethernet port.
 - EPC Com port to the System PC Com port.
 - EPC AC cord to a dedicated, grounded AC power source.
6. Power on HDX Manager.
7. Power on all other system components.

Electrical connections for a nanoACQUITY UPLC system with HDX technology:

Component	To
HDX Manager (AC power)	nanoACQUITY System Cart power strip
Binary Solvent Manager (Ethernet port)	Ethernet switch on the HDX Manager
Binary Solvent Manager (AC power)	nanoACQUITY System Cart power strip
Auxiliary Solvent Manager (Ethernet port)	Ethernet switch on the HDX Manager
Auxiliary Solvent Manager (AC power)	nanoACQUITY System Cart power strip
Mass spectrometer (AC power)	Dedicated, grounded power source
Rotary/Scroll pump (AC power)	Dedicated, grounded power source

Electrical connections for a nanoACQUITY UPLC system with HDX technology: (Continued)

Component	To
System workstation (AC power)	Dedicated, grounded power source
nanoACQUITY System Cart (AC power)	Dedicated, grounded power source
System workstation (Ethernet port)	Ethernet switch on the HDX Manager
Systems with a Synapt G2 Mass Spectrometers	
Synapt G2 mass spectrometer (Ethernet port)	Ethernet switch on the HDX Manager
Systems with a Xevo G2 QToF Mass Spectrometers	
EPC (AC power)	Dedicated, grounded power source
EPC (upper Ethernet port)	Ethernet port on the Xevo G2 QToF
EPC (lower Ethernet port)	Ethernet switch on the HDX Manager
EPC (EPC COM cable)	COM port on the System workstation

Fitting installation recommendations

Tightening tubing assemblies:

1. Start the fitting into the port.
2. Grasp the fused silica tubing and gently push it into the fitting as you continue to tighten.
3. Bottoming the fused silica tubing into the port minimizes system dead volume.
4. Tighten the fitting according to the recommendations in the following table.

Installation recommendations for fittings:

Fitting	Recommended Tightening	
6-40 nano (M-detail)	Finger tight plus 1/2 turn	Snug plus 1/6-turn (60°)
10-32 (V-detail)	Finger tight plus full turn	Snug plus 1/6-turn (60°)



Caution: Do not cross-thread the M-detail fitting during installation. The M-detail fitting is considered snug when the fitting begins to grab the capillary tubing.

Making plumbing and gas connections with a nanoACQUITY UPLC System with HDX Technology

To make plumbing and gas connections with an HDX Technology System, open or remove the nanoACQUITY UPLC HDX Manager front door, use the tubing and parts from the various instrument and system startup kits to make the plumbing connections between system components.

Note: The external tubing connections to the HDX fluidic block should be made first.

To reconfigure plumbing and gas connections:

1. Insert the pod holder into its bracket near the front of the HDX manager. The pod holder allows you to easily plumb the two valves.

Pod holder:



2. Use the M2 hex driver from the kit to remove the injection valve from the HDX manager. Place the valve into the pod holder.
3. Referring to the plumbing connection diagram and the plumbing and gas connection details table, make the connections to the injection valve.
4. After completing all connections, reinstall the injection valve, then repeat the steps for the trapping valve.
5. Follow these steps to assemble and install the injection port components, or replace the filter within the precolumn microfilter assembly:
 - a. Slide a ferrule onto the inject port tube.
 - b. Lightly insert the tube with ferrule onto the union
 - c. Insert the 250 μ L syringe into the injection port at the top of the HDX manager until it just bottoms out in the union.
 - d. With the tubing end facing up, finger tighten the union/tubing assembly in the injection port within the HDX manager.
 - e. Cut a 4-inch section of the 0.005-inch PEEK tubing, then insert it into a 2 μ m precolumn microfilter.
 - f. Finger-tighten the lower fitting collar to secure the tubing.
 - g. Finger-tighten the precolumn microfilter assembly into the bottom of the injection union.
 - h. Use a finger-tight fitting to secure the open end of the precolumn microfilter assembly to port 2 of the injection valve.

6. Feed the column outlet line from center port of the fluid block to the mass spectrometer inlet.



Caution: Turn off gas.

7. Use the “tee” and 6-mm teflon tubing to connect the nitrogen source to both the mass spectrometer and the HDX manager.
8. **Note:** Refer to the nanoACQUITY System Guide for installation instructions for the BSM and ASM.

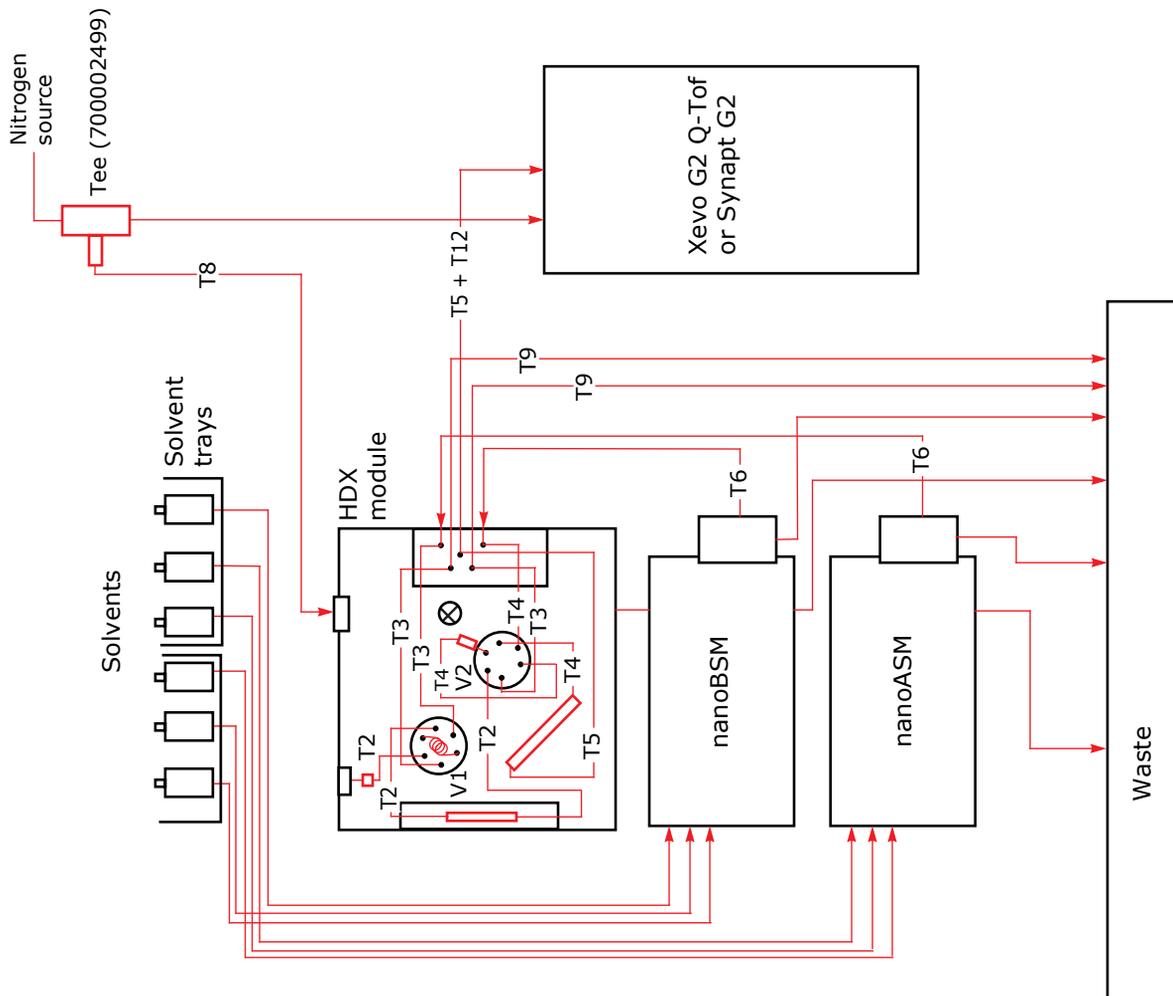
Plumbing and gas connection details in a standalone system:

Waters P/N	Description	Diagram identifier	From:	To:
430001325	50 μ L loop	N/A	V1 Port 1	V1 Port 4
WAT022995	PEEK tubing hand cut, 0.005 in. ID	T2	V1 Port 2	Inj Filter
WAT022995	PEEK tubing hand cut, 0.005 in. ID	T2	V1 Port 6	Digestion column inlet
WAT022996	PEEK tubing (hand cut, 0.010 in. ID)	T3	V1 Port 3	HDX fluid block (inner)
WAT022996	PEEK tubing (hand cut, 0.010 in. ID)	T3	V1 Port 5	HDX fluid block (inner)
430001837	Capillary tube with fitting 75 μ m x 10 in and Van Guard trap column (401003946)	T4	V2 Port 1	V2 Port 4
WAT022995	PEEK tubing hand cut, 0.005 in. ID	T2	V2 Port 2	Digestion column outlet

Plumbing and gas connection details in a standalone system: (Continued)

Waters P/N	Description	Diagram identifier	From:	To:
WAT022996	PEEK tubing (hand cut, 0.010 in. ID)	T3	V2 Port 3	HDX fluid block (inner)
430001837	Capillary tube with fitting 75 µm x 10 in.	T4	V2 Port 5	HDX fluid block (inner)
430001837	Capillary tube with fitting 75 µm x 10 in.	T4	V2 Port 6	Analytical column inlet
430000252	PEEK tubing 0.010 in. ID	T9	HDX fluid block (outer)	Waste
430000252	PEEK tubing 0.010 in. ID	T9	HDX fluid block (outer)	Waste
430002409	V-M tubing (75 µm)	T6	HDX fluid block (outer)	ASM outlet
430002409	V-M tubing (75 µm)	T6	HDX fluid block (outer)	BSM outlet
430000229	PEEK tubing (0.0025 in. ID)	T5	Analytical column outlet	MS inlet (through the HDX fluid block)
WAT271055	Pharmed tubing (hand cut, 1/4 in. OD)	T12	HDX fluid block (outer)	MS inlet
430003111	FEP Tubing, 6mm OD	T8	HDX nitrogen inlet	“Tee” (700002499)

Plumbing connection diagram:



Configuration using a nanoACQUITY UPLC System with HDX Automation

Assembling the bench-top accessory, including the bottle holder and shelf

1. Attach the two bottle holder supports to the left side of the rack using four M5x25 Torx screws, four M5 kep nuts, and eight washers.
2. Attach the bottle holder tray to the bottle holder shelf using four M5 kep nuts and flat washers.
3. Attach the bottle holder tray/shelf assembly to the supports using four M4x10 screws.
4. Attach the HDX manager shelf to the rack using two M5x16 Torx screws, flat washers, lock washers and nuts.
5. Attach the triangular HDX manager lock plate to the shelf using two M5x10 Torx screws.

Positioning the components in a nanoACQUITY UPLC System with HDX Automation

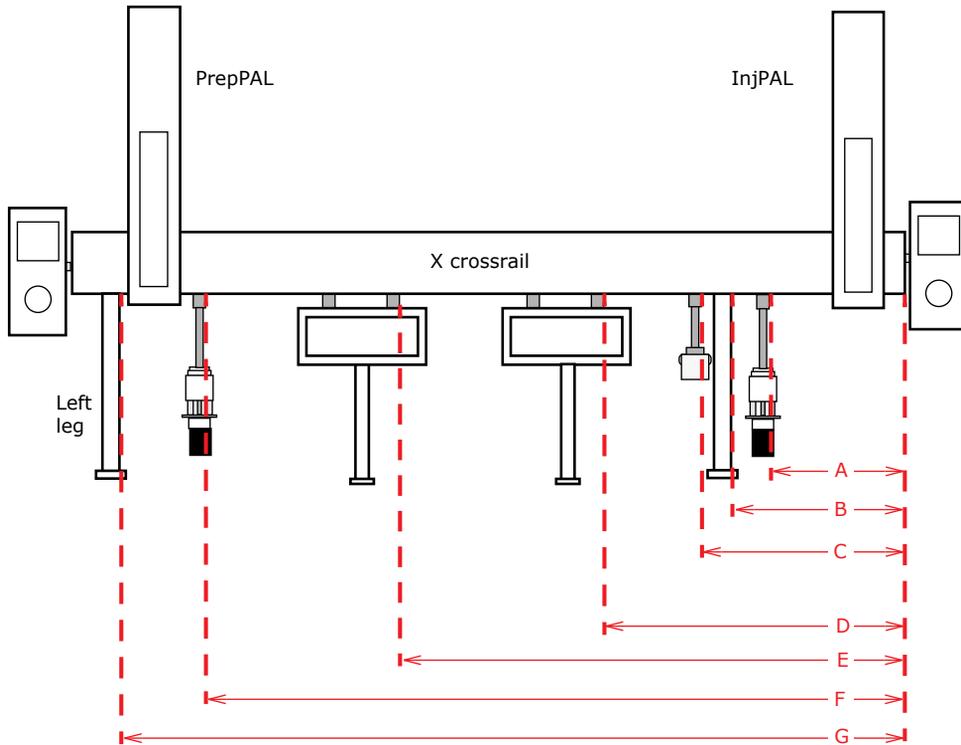
Follow these steps to position the system components.

1. Position the bench-top accessory on a suitable cart or bench to the left of the mass spectrometer.
2. Place the nanoACQUITY Auxiliary Solvent Manager on the lower-left side of the bench-top accessory rack.
3. Place the nanoACQUITY Binary Solvent Manager on the lower-right side of the bench-top accessory rack.
4. Place the stainless steel drip tray on top of the bench-top accessory rack, aligning the front edge of the drip tray with the front edge of the bench-top accessory rack, making sure that the drain hole and two waste tube holes in the drip tray align with the three holes on the bench-top accessory rack.
5. Place the HDX manager on the shelf, then secure it in place via the HDX manager lock plate using the M4 Torx screw.

Assembling the LEAP Autosampler

Use the following diagram and table to determine the exact mounting location of each LEAP Autosampler leg and object.

LEAP autosampler leg and object:



Dimensions from right edge of X crossrail:

Dimension identifier and component	Dimension
A — Fast Wash Station (with bottle holder)	8-1/2 in. (216 mm)
B — Right leg	10-5/8 in. (270 mm)
C — 10 mL vial holder	12-1/2 in. (318 mm)
D — Stack Cooler2 (right)	18-1/2 in. (470 mm)
E — Stack Cooler1 (left)	30-7/8 in. (784 mm)

Dimensions from right edge of X crossrail: (Continued)

Dimension identifier and component	Dimension
F — Fast Wash Station (with bottle holder)	42 in. (1067 mm)
G — Left leg	45- ¹ / ₁₆ in. (1145 mm)

To assemble the LEAP autosampler:

1. Attach the two legs onto the X crossrail using a T20 Torx driver, then place the autosampler into approximate position on the bench-top accessory rack.



Warning: Stack cooler must have their support feet installed before assembly to rail.

2. Install the stack coolers with their support feet.
3. Assemble each injection unit and mount each to a Y crossrail.
4. Install a plunger holder and syringe adapter into each injection unit.
5. Attach each injection unit/Y crossrail assembly to the X crossrail (one on each end) using a T20 Torx driver.
6. Assemble the 10 mL vial holder, wash pump, stack coolers, and wash station, then attach each object to X crossrail.
7. Attach the two hand-held keypads to the ends of the X crossrail, and attach the safety shield (not shown in diagram).

Making the electrical connections in a nanoACQUITY UPLC System with HDX Automation

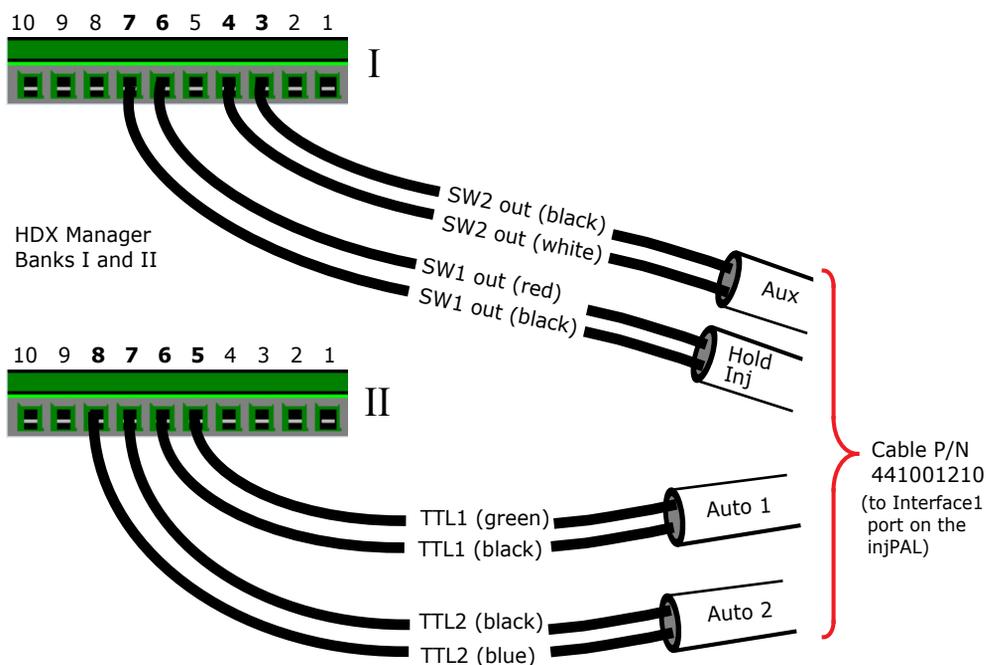
Follow the steps in this section to make power and communications connections for an Automated system. The following table contains a summary of these connections.

Electrical connections in a nanoACQUITY UPLC HDX automated system:

Connect the LEAP rear panel port marked:	To:
TERMINAL (injPAL port from rear of autosampler)	Keypad (inject side)
SER1 (injPAL port from rear of autosampler)	USB Converter
WASH STATION (injPAL port from rear of autosampler)	Wash station object
POWER (injPAL port from rear of autosampler)	PAL port on the LEAP power supply
TERMINAL (prepPAL port from rear of autosampler)	Keypad (prep side)
INTERFACE1 (injPAL port from rear of autosampler)	HDX Manager Event terminal banks I and II via cable P/N 441001210.
SER1 (prepPAL port from rear of autosampler)	USB Converter
WASH STATION (prepPAL port from rear of autosampler)	Wash station object
POWER (prepPAL port from rear of autosampler)	PAL port on the LEAP power supply
Other system components	
HDX Manager (AC power)	Dedicated, grounded power source
Binary Solvent Manager (Ethernet port)	Ethernet switch on the HDX Manager
Binary Solvent Manager (AC power)	Dedicated, grounded power source
Auxiliary Solvent Manager (Ethernet port)	Ethernet switch on the HDX Manager
Auxiliary Solvent Manager (AC power)	Dedicated, grounded power source
System workstation (AC power)	Dedicated, grounded power source
Systems with Synapt G2 mass spectrometers	
Synapt G2 mass spectrometer (Ethernet port)	Ethernet switch on the HDX Manager

Electrical connections in a nanoACQUITY UPLC HDX automated system:

Connect the LEAP rear panel port marked:	To:
System workstation (Ethernet port)	Ethernet switch on the HDX Manager
Systems with a Xevo G2 QTof mass spectrometers	
EPC (AC power)	Dedicated, grounded power source
EPC (upper Ethernet port)	Ethernet port on the Xevo G2 QTof
EPC (lower Ethernet port)	Ethernet switch on the HDX Manager
EPC (EPC COM cable)	COM port on the System workstation



To make the electrical connections:

1. Connect the nearest keypad to the left “Terminal” port on the rear of the X crossrail.
2. Connect the USB converter to a USB port on the PC.

3. Connect an RS9B-6000 cable from the prepPAL “SER1” port on the X crossrail to a serial port on the USB converter.
4. Connect the Fast Wash Station to the prepPAL “Wash Station” port on the back of the X crossrail.
5. Connect the event cable-end of the Interface 1 cable from the right “Interface1” port on the injPAL side of the crossrail to terminal ports 6 and 7 (Hold Inject) on the HDX manager. Connect the left “Power” port on the X crossrail to one “PAL” port on the LEAP power supply.
6. Connect the second keypad to the right “Terminal” port on the X crossrail.
7. Connect an RS9B-6000 cable from the right “SER1” port on the X crossrail to a serial port on the USB converter.
8. Connect the Fast Wash Station cable to the injPAL “Wash Station” port on the back of the X crossrail.
9. Connect the right “Power” port on the X crossrail to the second “PAL” port on the LEAP power supply.
10. Connect the two Stack Coolers to their Peltier power supplies.
11. Connect the two Peltier power supplies and the LEAP power supply to the dedicated C power source.
12. Make all LEAP PC power and electronic connections:
 - Both Serial 1 connections on the inj and prep side of the LEAP to the USB Hub
 - AC power to the dedicated power source
13. Make all system PC power and electronic connections:
 - PC network card to a port on the HDX manager Ethernet switch
 - Any USB port to the USB RS-232 converter using provided USB cable
 - AC power to the dedicated power source
14. Power on all system components.

Restoring backup files

To restore backup files:

1. Copy the HDX_prep.sss and HDX_inj.sss backup files to the Desktop.
2. Open the PAL Loader, then click Setup.
3. Select the appropriate COM port for the prepPAL arm, then click OK. You should hear a tone on the hand-held keypad when communications is achieved.
4. From the PAL Loader, click Update. The Update Flash memory dialog box opens.
5. Browse to the Desktop, select the HDX_prep.sss backup file, then click Open.
6. Click Start Update to load the new file. The process takes several minutes.
7. When the Update of target memory succeeded message appears, click Close.
8. Click Start PAL, then Exit in the PAL loader dialog box to terminate control by the Loader program.
9. Repeat [step 2](#) through [step 8](#) for communication to the injPAL arm, choosing appropriate COM port. You should hear a tone on the hand-held keypad when communications is achieved.

Making the plumbing and gas connections in a nanoACQUITY UPLC System with HDX Automation

To make plumbing and gas connections with an Automated system, open or remove the nanoACQUITY UPLC HDX Manager front door, use the tubing and parts from the various instrument and system startup kits to make the plumbing connections between system components. To reconfigure these connections, use the following steps.

Note: Refer to the plumbing diagrams and tubing table during installation.

To make the plumbing and gas connections:

1. Connect external tubing to the HDX fluidic block.
2. Open or remove the HDX manager front door.

3. Insert the pod holder into its bracket near the front of the HDX manager. The pod holder allows you to easily plumb the two valves.
4. Use the M2 hex driver from the kit to remove the injection valve from the HDX manager. Place the valve into the pod holder.
5. Refer to the plumbing connection diagram to make the connections to the injection valve.
6. After completing all connections, reinstall the injection valve, then repeat the steps for the trapping valve.
7. Follow these steps to assemble and install the injection port components:
 - a. Slide a ferrule onto the inject port tube as shown.

Pod holder:



- b. Lightly insert the tube with ferrule into the union.
- c. With the tubing end facing up, finger tighten the union/tubing assembly in the injection port within the HDX manager.
- d. Insert the 250 uL syringe into the injection port at the top of the HDX manager until it just bottoms out in the union.
- e. Secure the union an additional 1/4-turn using the 5/16-in wrench.
- f. Cut a 4-inch section of the 0.005-inch PEEK tubing, then insert it into a 2 um precolumn microfilter.
- g. Finger-tighten the lower fitting collar to secure the tubing.
- h. Finger-tighten the precolumn microfilter assembly into the bottom of the injection union.

- i. Use a finger-tight fitting to secure the open end of the precolumn microfilter assembly to port 2 of the injection valve.
8. Feed the column outlet line from center port of the fluid block to the mass spectrometer inlet.
9. Connect the nitrogen outlet on the HDX manager to the chiller stacks using tubing.
10. Connect the LEAP wash stations to a suitable container.
11. Connect the LEAP solvent bottles to the wash stations.

Making the waste connections in a nanoACQUITY UPLC System with HDX Automation

Follow the steps below to make the waste connections in an Automated system.



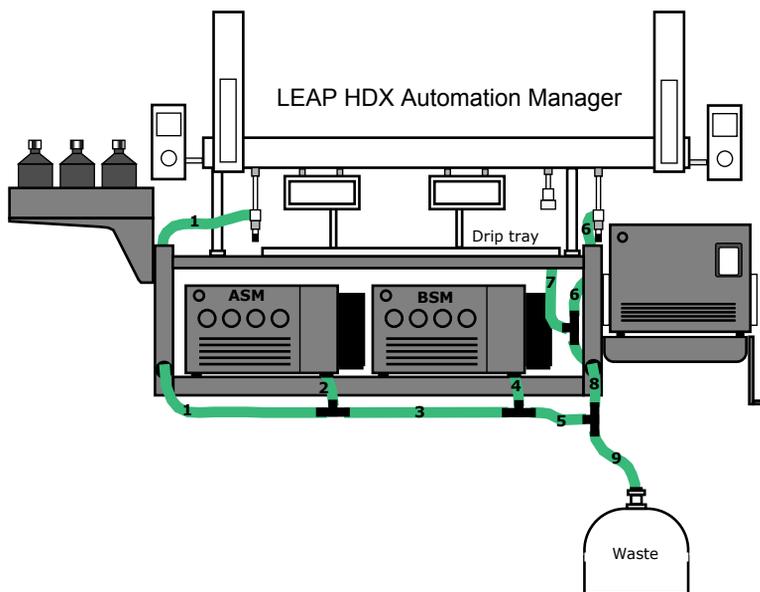
Warning: To avoid fluid backup, ensure that the waste container is below the stack, ensure there are no crimps or bends in the waste lines and ensure waste and degasser line ends are not immersed in waste fluid.

To make the waste connections:

1. For each step that follows, use Figure 2 as a guide to determine the length and location of clear waste tubing that you need to cut to make the connection. You also need four “tees” (P/N 700003907) to complete the connections.
2. Route a section of waste from the waste port of the prepPAL Fast Wash Station, through the prepPAL side of the Benchtop Accessory, then on to

one side of a “tee” that is positioned below the ASM. See tubing section “1” in Waste connection in an automated system.

Waste connection in an automated system:



3. Connect a section of waste tubing from the waste port of the ASM to the “tee” that is positioned below the ASM. See tubing section “2”.
4. Connect a section of waste tubing from the “tee” that is positioned below the ASM to a “tee” that is positioned below the BSM. See tubing section “3”.
5. Connect a section of waste tubing from the waste port of the BSM to the “tee” that is positioned below the BSM. See tubing section “4”.
6. Connect a section of waste tubing from the “tee” that is positioned below the BSM to a “tee” that is positioned below the right side of the Benchtop Accessory. See tubing section “5”.
7. Route a section of waste tubing from the waste port of the *right* Fast Wash Station, then into the right side of the Benchtop Accessory to one side of a “tee” (positioned to the right of the BSM). See tubing section “6”.

8. Connect a section of waste tubing from the waste port on the right-front underside of the Benchtop Accessory drip tray to the “tee” that is positioned to the right of the BSM. See tubing section “7”.
9. Connect a section of waste tubing from the “tee” that is positioned to the right of the BSM to the “tee” that is positioned below the right side of the Benchtop Accessory. See tubing section “8”.
10. Connect a section of waste tubing from the “tee” that is positioned below the right side of the Benchtop Accessory to a suitable waste container. See tubing section “9”.
11. Route the BSM and ASM degasser vent tubes to a suitable container.



Warning: To avoid releasing solvent vapors into the room, route the degasser vent tube into a fume hood or other suitable exhaust system, or to a suitable waste container, ensuring the end of the tubing is above the fluid level.

Checking/setting HDX Automation Manager needle penetration values

Refer to this section to check, and if necessary, set the needle penetration values for injectors, trays, wash stations, and vials. The procedure describes the steps for an injector valve. Use the steps as a guide for checking/setting the needle penetration values for other system components.

To check/set needle penetration values:

1. From the Job Queue screen, select F1 Menu > Setup > F3 > Enter > Objects > F3 > Enter > Injectors > LC Vlv1. The Injector: LC Vlv1 screen appears.

Injector: LC Vlv1 screen example:

Injector: LC Vlv1			
Position X		246.9mm	
Position Y		2.6mm	
Position Z		86.0mm	
Needle Penetr		20.0mm	
Valve		DC6-1-C	
2002/10/26		11:15:36	
Check Pos	Clear Pos	Movto Zero	Home
F1	F2	F3	F4

2. Scroll to Needle Penetr, then press the Enter button.
3. Press F1 Check Pos and listen carefully as the needle moves down into the injector port.
4. Follow these steps:
 - a. Rotate the outer knob *slowly clockwise* until the needle reaches the bottom of the inject port and you see the inj arm raise up.
 - b. Press the **Enter** button to set the value.
5. Select F4 Home. The syringe moves to the home position and the Job Queue screen displays.

Note: After adjusting the needle penetration values, back up the current configuration.

Installing columns

Chromatography part number from Waters:

	Mode	Description	
Peptide Separation	Trap	VanGuard BEH Pre-column 2.1x5 mm P/N 186003977	
	Analytical	BEH C18 1.7 µm, 1.0 x 100 mm P/N 186002346	
Protein Separation	Desalt	MassPREP Micro desalting column 2.1x5 mm (P/N 186004032)	
	Analytical	BEH C4 1.7 µm 2.1 x 50 mm if protein separation is necessary	
Applied Biosystems	Online pepsin digestion	Poroszyme® immobilized Pepsin cartridge 2.0x30 mm (P/N 186004495)	

Note: For peptide separations, a digestion column is required, such as Poroszyme from Life Technologies.

To install the digestion column:

1. On the inlet side of the column, cut piece of tubing from supplied T2 and connect using finger-tight fitting.
2. Feed the tubing through the column heater into the HDX Manager.
3. Connect the other end of the inlet-side tubing to V1, port 6, on the Plumbing Connections diagram using finger-tight fitting.
4. On the outlet side of the column, cut piece of tubing from supplied T2 and connect using finger-tight fitting.
5. Feed the tubing through the column heater into the HDX Manager.
6. Connect the other end of the outlet side tubing to V2, port 2, using finger-tight fitting.

7. Place the column inside the column heater.

To install the analytical column:

1. On the inlet side of the column, connect tubing T4. Use a 1/4-inch wrench to tighten.
2. Connect the other end of the inlet side tubing to V2, port 6. Use a 1/4-inch wrench to tighten.
3. On the outlet side of the column, connect tubing T5 using a 1/4-inch wrench and a 5/16-inch wrench to tighten.
4. Connect the other end of the outlet side tubing to the mass spectrometer inlet through the HDX fluid block, using finger-tight fitting.

To install the VanGuard trap column:

1. Connect the inlet side of the column directly to V2, port 1, using a 5/16-inch wrench.
2. On the outlet side of the column, connect tubing T2.
3. Connect the other end of the tubing to V2, port 4, using a 1/4-inch wrench and a 5/16-inch wrench to tighten.

To prepare the system for injections:

1. In the MassLynx inlet editor window, click the Additional Status tab.
2. Enter an appropriate flow setting for the BSM.
3. Enter a digestion/trapping flow rate in the A side of the ASM.
4. Enter a lock mass flow rate in the B side of the ASM.
5. Enter the sample chamber temperature for the HDX Manager.
6. Enter the digestion temperature for the HDX Manager.

7. Wait 30 to 60 minutes for the HDX Manager temperatures to equilibrate.

Inlet Editor

The screenshot displays the 'Inlet Editor' software window. The title bar reads 'Untitled.bgm, Untitled.hdx - Inlet Method'. The menu bar includes 'File', 'View', 'Tools', 'LC', 'Autosampler', and 'Help'. A toolbar with various icons is located below the menu bar. On the left side, there is a vertical navigation pane with icons and labels for 'Status', 'Inlet', 'Autosampler', and 'ACQ-HDX'. The main area is titled 'Status' and contains a sub-panel with tabs for 'Status', 'Additional Status', and 'Solvent Levels'. The 'Status' tab is active, showing data for 'BSM' and 'ASM'. The 'BSM' section displays '6521 psi', '40.000 uL/min', and '50.0 %' for both 'A1' and 'B1' channels. The 'ASM' section displays '106 psi', '25.00 uL/min', and '692 psi', '10.00 uL/min' for 'A1' and 'B1' channels. Below this, the 'HDX Manager' section shows 'Position: Analytical', 'Chamber: 0.0 °C', and 'Digestion: 20.0 °C'. A 'Run' button is visible. At the bottom left, it says 'For Help, press F1'.

 **Caution:** For solvent pumped through the digestion column, pH should not exceed 4.0 and acetonitrile should not exceed 5% by volume.

3

Maintenance Procedures

This chapter provides the maintenance guidelines and procedures necessary to maintain the HDX Manager's performance.

Keep to a maintenance schedule, and perform maintenance as required and described in this chapter.

Contents:

Topic	Page
Inspecting and replacing the HDX Automation Manager injection unit syringe	3-1
Cleaning and lubricating the HDX Automation Manager sample manager	3-2
Maintaining your injection port components	3-4
Spare parts	3-6
Safety and handling	3-6
Defrost	3-7

Inspecting and replacing the HDX Automation Manager injection unit syringe

Inspect the syringe for wear and replace it if necessary.

To inspect the injection unit syringe:

1. From the Job Queue screen, select F1 Menu > F1 Chang Syr. The injection unit moves to a location that facilitates removal of the syringe.
2. Loosen the plunger retaining screw, then move the plunger slightly out of the plunger holder.
3. Pull the syringe adapter out, then carefully up to remove the syringe adapter with the syringe from the injection unit.

4. Replace the syringe, then check the needle penetration position for each injection valve.
5. Prime the syringe. If necessary, manually prime the syringe to completely remove air bubbles.

Cleaning and lubricating the HDX Automation Manager sample manager

Follow these steps to clean and lubricate the guide rollers, tracks, and toothed rack within the X and Y axis units. Use the lubricant and brush that come in the Performance Maintenance kit.

Notes:

- The sample manager must be powered off when performing these steps.
- If observed, clean any black material that might have flaked off the X and Y axes.
- Refer to Cleaning and lubricating the X-axis as required.

To clean and lubricate the sample manager:

1. Manually move the sample manager components back and forth across each axis to check for smoothness. Note any areas of resistance.
2. Use the brush from the kit to carefully clean the guide rollers, tracks, and toothed rack for the X and Y axis units.
3. Apply only a *small* amount of lubricant to the components.

Note: Do not apply lubricant to any Teflon parts.

4. Manually move the sample manager components back and forth across each axis to evenly distribute the lubricant and check for smoothness. Ensure that there are no areas of resistance.

Cleaning and lubricating the X axis:



Cleaning the toothed rack



X axis roller

Cleaning the Y axis toothed rack:



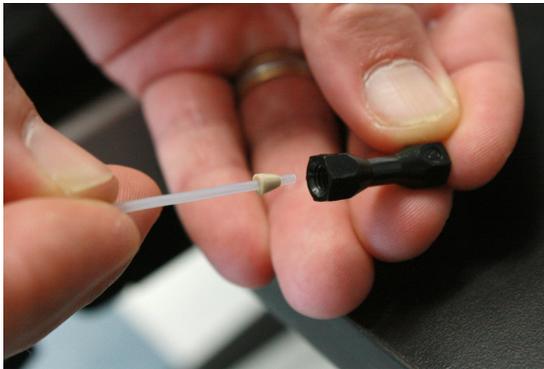
Maintaining your injection port components

To unclog your injection port flow path, follow these procedures:

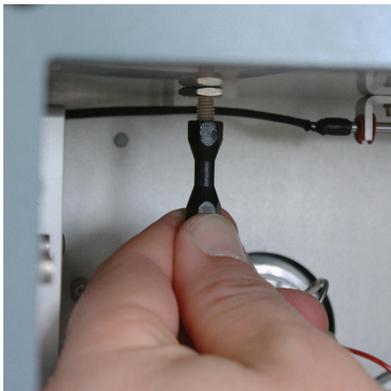
1. Slide a ferrule (WAT021817) onto the inject port tube (4300003053) as shown.



2. Lightly insert the tube with ferrule into the union (WAT02-04).



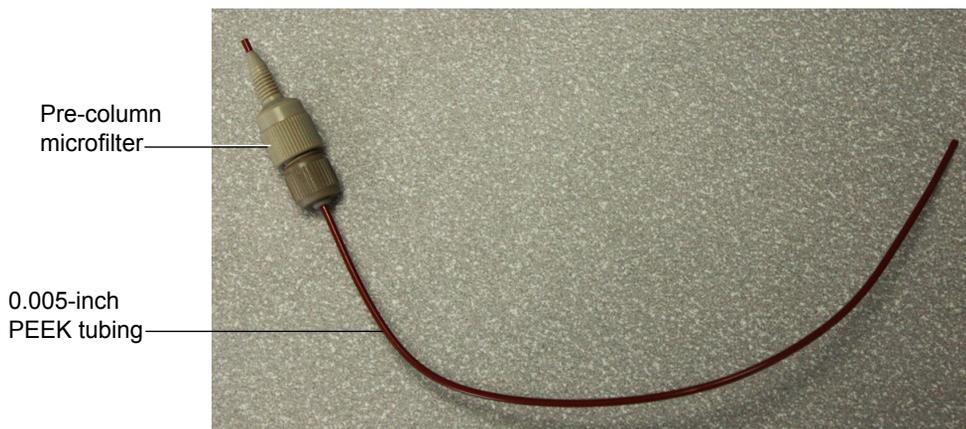
3. With the tubing end facing up, finger tighten the union/tubing assembly in the injection port within the module.



4. Insert the 250 μL syringe into the injection port at the top of the module until it just bottoms out in the union.



5. Secure the union an additional 1/4-turn using the 5/16-in wrench.
6. Cut a 4-inch section of the 0.005-inch PEEK tubing (WAT027-010), then insert it into a 2 μL pre-column microfilter (401003946).
7. Finger-tighten the lower fitting collar to secure the tubing.



8. Pre-column microfilter assembly
9. Finger-tighten the pre-column microfilter assembly into the bottom of the injection union.

10. Use a finger-tight fitting (410000760) to secure the open end of the pre-column microfilter assembly to port 2 of valve 1.

Spare parts

To ensure that your system operates as designed, use only Waters Quality Parts®. Visit www.waters.com/wqp for information about Waters Quality Parts, including how to order them.

Safety and handling

Bear in mind the following safety considerations when performing maintenance procedures:



Warning: The instrument components can be contaminated with biologically hazardous materials. Always wear chemical-resistant, powder-free gloves while handling the components.



Warning: To prevent injury, always observe Good Laboratory Practice when handling solvents, changing tubing, or operating the instrument. Know the physical and chemical properties of the solvents used (see the Material Safety Data Sheets for the solvents in use).



Warning: To avoid electric shock,

- do not remove the instrument's panels. There are no user-serviceable items inside the instrument.
- ensure that the instrument is in Standby mode before commencing any maintenance.



Warning: The probe and source can be hot. To avoid burn injuries, take great care while working with these components.



Caution: When performing maintenance inside the source enclosure, ensure that the following criteria are met:

- Instrument is in Standby mode.
- LC flow is diverted to waste or set to off.
- Desolvation gas is turned off.

See [Appendix A](#) for safety advisory information.

For more information regarding contamination control, see “Controlling Contamination in UltraPerformance LC[®]/MS and HPLC/MS Systems” (part number 715001307).

Defrost

Purge nitrogen should be used during defrost. Issues with icing are somewhat more noticeable if the nitrogen purge gas is turned off (not a recommended mode of operation), due to moisture entering through the cracks and frosting near them. The units are easily defrosted by turning off the power for 30 minutes or raising the chamber temperature to 10°C for 10 minutes. As the ice begins to melt, remove ice pieces and dry the water with a towel.

Note: Ice tends to form where the door is leaking if the nitrogen purge gas is turned off. This tends to help seal up the crack but could result in the door being frozen shut if there is no purge gas and the door is not opened for more than a week. The 10°C for 10 minutes defrost cycle would allow a frozen door to be opened.

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

Contents:

Topic	Page
Warning symbols	A-2
Caution advisory	A-5
Warnings that apply to all Waters instruments	A-5
Electrical and handling symbols	A-11

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of sharp-point puncture injury.)



Warning: (Risk of hand crush injury.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



Warning: (Risk of exposure to a toxic substance.)



Warning: (Risk of personal exposure to laser radiation.)



Warning: (Risk of exposure to biological agents that can pose a serious health threat.)



Warning: (Risk of tipping.)



Warning: (Risk of explosion.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



Warning: Pressurized nonmetallic, or polymer, tubing can burst.

Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.



Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid nonlethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that can contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.



Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practice, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.



Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practice (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*.



Warning: To avoid the harmful effects of personal contact with solvents, including inhalation, observe Good Laboratory Practice when you handle them. See the Material Safety Data Sheets for the solvents you use.

Caution advisory

Caution advisories appear where an instrument or device can be subject to use or misuse capable of damaging it or compromising a sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.



Caution: To avoid damaging the instrument's case, do not clean it with abrasives or solvents.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.



Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意: 未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意: 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。

주의: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意: 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。



Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.



Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告: 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。



警告: 当有压力的情况下使用管线时, 小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀, 大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸설폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があります、その場合、チューブは極めて低い圧力で破裂します。



Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告: 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

警告: 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告: ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

Electrical and handling symbols

Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

B Materials of Construction and Compatible Solvents



Warning: To confirm the integrity of the source exhaust system, you must address any safety issues raised by the contents of this Appendix.

Contents:

Topic	Page
Preventing contamination	B-1
Items exposed to solvent	B-1
Solvents used to prepare mobile phases	B-2

Preventing contamination

For information on preventing contamination, refer to *Controlling Contamination in LC/MS Systems*. Visit www.waters.com.

Items exposed to solvent

The items that appear in the following table can be exposed to solvent. You must evaluate the safety issues if the solvents used in your application differ from the solvents normally used with these items. See [page B-2](#) for details about the most common ingredients used to prepare mobile phases.

Wetted materials

316 SST, Fused Silica, Carbon filled PEEK (High Pressure Valves), PEEK (low pressure plumbing lines) PTFE, TFE.

Solvents used to prepare mobile phases

Frequently used solvents

- Methanol
- Acetonitrile with 0.1% formic acid
- Water with 0.05 to 0.1% formic acid

Not compatible

- THF
- Toluene
- Methylene chloride

The following lists the most common ingredients used to prepare mobile phases for reverse-phase LC/MS (API):

- Water
- Methanol
- Acetonitrile
- Formic acid (<0.1%)
- Acetic acid (<0.1%)
- Trifluoroacetic acid (<0.1%)
- Ammonium acetate (<10 mM)
- Ammonium formate (<10 mM)

These solvents are not expected to cause any problems with the materials identified on [page B-1](#).

C Specifications of the HDX Manager

Contents:

Topic	Page
Electrical power specifications	C-1
Communications specifications	C-2
Physical specifications	C-2
Environmental specifications	C-3
Miscellaneous specifications	C-3

Electrical power specifications

Electrical specifications:

Attribute	Specification
Protection class ^a	Class I
Oversvoltage category ^b	II
Pollution degree ^c	2
Moisture protection ^d	Normal (IPXO)
 Line voltages, nominal	Grounded AC
Voltage range	100 to 240 Vac
Frequency	50/60 Hz
Maximum VA input	400 VA

- a. **Protection Class I** – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. **Overvoltage Category II** – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. **Pollution Degree 2** – A measure of pollution on electrical circuits that can produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.
- d. **Moisture Protection** – Normal (IPX0) – IPX0 means that no Ingress Protection against any type of dripping or sprayed water exists. The “X” is a placeholder that identifies protection against dust, if applicable.

Communications specifications

Communications Class I specifications:

Attribute	Specification
Contact closures (3)	One to the MS (if required), one to the injection trigger, and one to/from LEAP/CTC
RS-232	
Ethernet	

Physical specifications

Physical specifications:

Attribute	Specification
Height	29.9 cm (11.5 inches)
Width	34.3 cm (13.5 inches)
Depth	67.3 cm (26.5 inches)
Weight	20.41 kg (45.0 pounds)

Environmental specifications

Environmental specifications:

Attribute	Specification
Operating temperature	0 to 28 °C (32 to 82.4°F)
Operating humidity	20 to 80%, non-condensing
Shipping and storage temperatures	-35 to 65°C (-31 to 149°F)
Shipping and storage humidity	20 to 80%, non-condensing

Miscellaneous specifications

Performance specifications:

Item	Specification
Maximum operating pressure	10,000 psi for ASM, BSM only
Flow rate range	200 nL/min to 100 µL/min (BSM)
	200 nL/min to 1000 µL/min (ASM)
Temperature control ranges:	<ul style="list-style-type: none">• Ambient down to 0 °C for sample chamber.• Column chamber is 10°C above sample chamber ambient up to a maximum of 45 °C.

D Start up kit components

The following spreadsheet details the parts included in the start up kits for the nanoACQUITY UPLC with HDX Technology, and the nanoACQUITY UPLC with HDX Automation.

System startup kits:

Number	Systems Startup Kits
200000304	Startup Kit, nanoACQ, UPLC Sys HDX Tech.
200000311	Startup Kit, nanoACQ UPLC Sys HDX Auto
200000303	Startup Kit, nanoACQUITY UPLC HDX Mgr

Index

A

ACQUITY UPLC H-Class
specifications
 electrical C-1
 environmental C-3
audience and purpose v

B

biohazard warning A-4
burst warning A-3

C

caution symbol A-5
chemical hazard warning A-5
compatible solvents B-1
construction materials B-1
contamination, preventing B-1

D

defining
 valve needle penetration depth
 2-22

E

EC authorized representative vii
electrical specifications
 ACQUITY UPLC H-Class C-1
electrical symbols A-11
environmental specifications
 ACQUITY UPLC H-Class C-3
equipment guidelines iv, A-5

F

flammable solvents A-3

H

handling symbols A-12

I

intended use v
ISM classification vi, vii

M

maintenance
 safety 3-6
 schedule 3-1
mass spectrometer shock hazard A-4
materials of construction B-1
mobile phases, solvents used in
 preparation of B-2

P

performance specifications
 quaternary solvent manager C-3
preventing contamination B-1
purpose and audience v

Q

quaternary solvent manager
specifications
 performance C-3

R

replacement parts 3-6

S

safety advisories A-1
safety procedures, maintenance 3-6
solvents
 compatible B-1
 exposure of SQ detector
 components to B-1
 use in mobile phases B-2
spare parts 3-6
symbols
 caution A-5

electrical [A-11](#)

handling [A-12](#)

warning [A-2](#)

V

valve needle penetration depth,
defining [2-22](#)

W

warning symbols [A-2](#), [A-5](#)