

Universal NanoFlow Sprayer

Installation and Maintenance Guide

71500110107/Revision C

Waters

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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. 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.

When you develop methods, follow the “Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory,” *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Considerations specific to the Universal NanoFlow Sprayer

High voltage hazard



Warning: High voltage danger.

- To avoid electric shock, never operate the NanoLockSpray or NanoFlow Z-spray source without the protective sprayer cover in place. Do not remove the mass spectrometer's 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.

Operating the Universal NanoFlow Sprayer

When operating the Universal NanoFlow Sprayer, follow standard quality-control (QC) procedures and the guidelines presented in this section.

See also: *NanoLockSpray User's Guide*, the relevant instrument user's guide, and the MassLynx online Help.

Audience and purpose

The audience for these instructions is experienced mass spectrometer users and field service engineers.

The purpose of these instructions is to explain how to install and maintain the Universal NanoFlow Sprayer.

Intended use of the Universal NanoFlow Sprayer

Waters designed the Universal NanoFlow Sprayer to work with fused silica emitters on Micromass NanoFlow and NanoLockSpray™ MS sources.

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.

To calibrate mass spectrometers, consult the calibration section for the operator's guide of the instrument you are calibrating.

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.

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1 Installing the Universal NanoFlow Sprayer

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Overview

The Waters® Universal NanoFlow™ Sprayer is specially designed to work with fused silica emitters on Micromass NanoFlow and NanoLockSpray™ MS sources. The sprayer head's compact profile accommodates 360-µm OD fused silica emitters of 5- to 7-cm lengths. For use with PicoTip® and TaperTip™ emitters, or other 360-µm OD fused silica emitters, the sprayer features a zero-dead-volume union held in place by an adjustable stop. The zero-dead-volume union allows a direct end-to-end connection between the emitter and a 360-µm OD nanocolumn outlet end, which minimizes post-column bandspreading for nanoLC applications.

Attaching the nebulizer tube and fittings

If tubing and fittings are already attached to the sprayer, proceed to “[Installing or replacing a nanospray emitter](#)”, on [page 1-6](#).

Required materials

- 1/16-inch PTFE tubing
- 1/16-inch stainless steel compression screw
- 1/16-inch Swagelok[®] nut
- 1/4-inch open-end wrench
- PEEK[™] ferrule (see [Appendix A](#) for the part number)
- Powder-free, sterile gloves
- Swagelok back ferrule
- Swagelok front ferrule
- Universal NanoFlow Sprayer (see [Appendix A](#) for the part number)



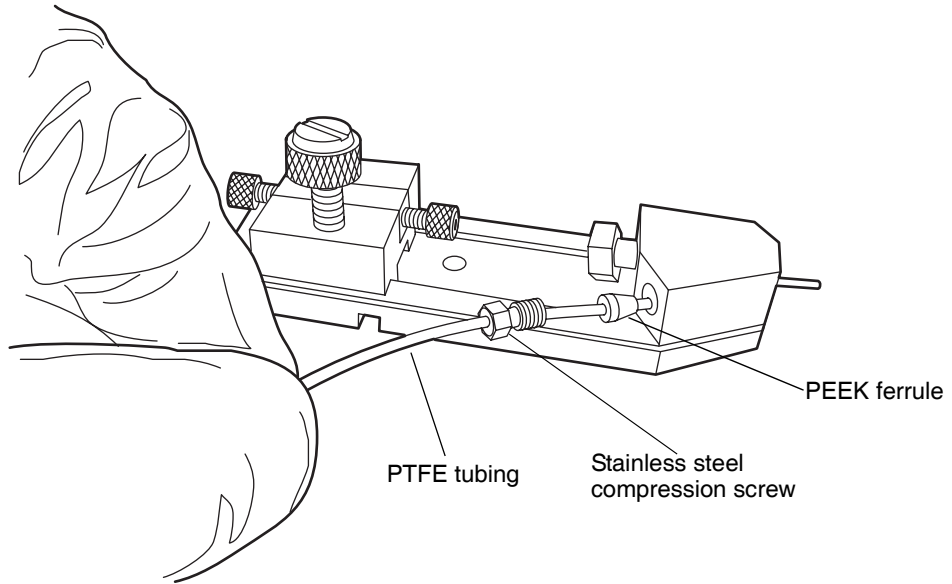
Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

See also: *Controlling Contamination in UltraPerformance LC[®]/MS and HPLC/MS Systems.*

To attach the nebulizer tube and fittings:

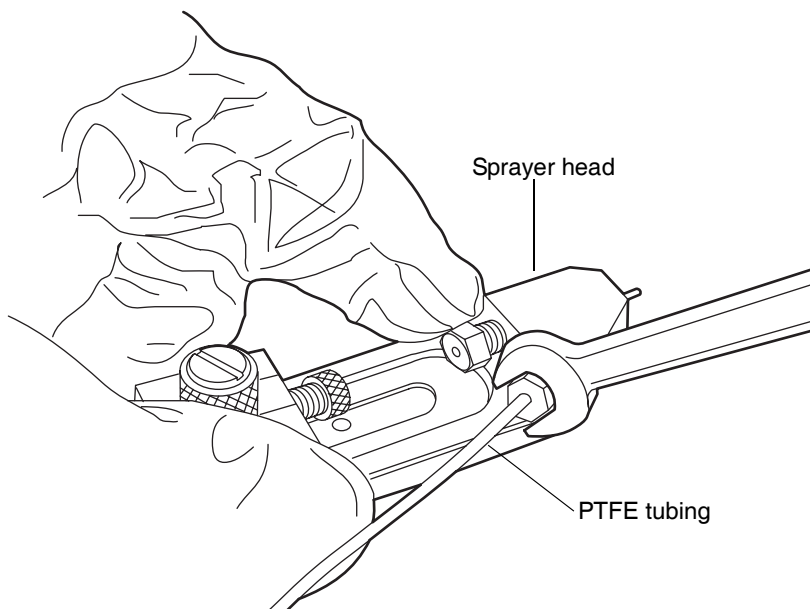
1. Place a 1/16-inch stainless steel compression screw and a PEEK ferrule on the end of the 1/16-inch PTFE tubing.

1/16-inch compression screw and PEEK ferrule on PTFE tubing



2. Secure the tubing to the sprayer head, tightening the compression screw with the 1/4-inch wrench.

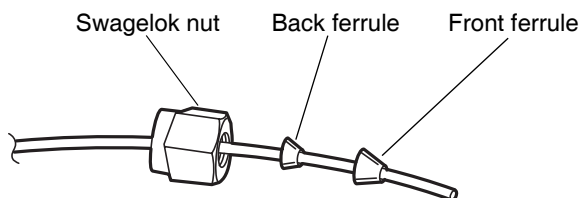
Securing 1/16-inch PTFE tubing to the sprayer head



Tighten only enough to swage the PEEK ferrule onto the tubing (three-quarters of a turn beyond hand-tight).

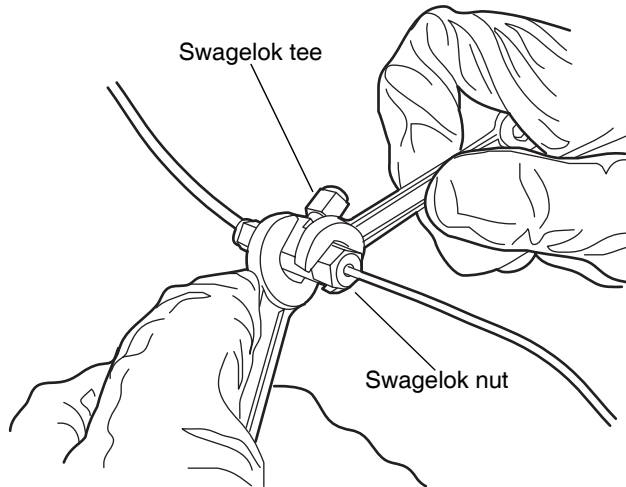
3. Place a 1/16-inch Swagelok nut, back ferrule, and front ferrule on the other end of the 1/16-inch PTFE tubing.

Swagelok nut, back ferrule, and front ferrule on 1/16-inch PTFE tubing



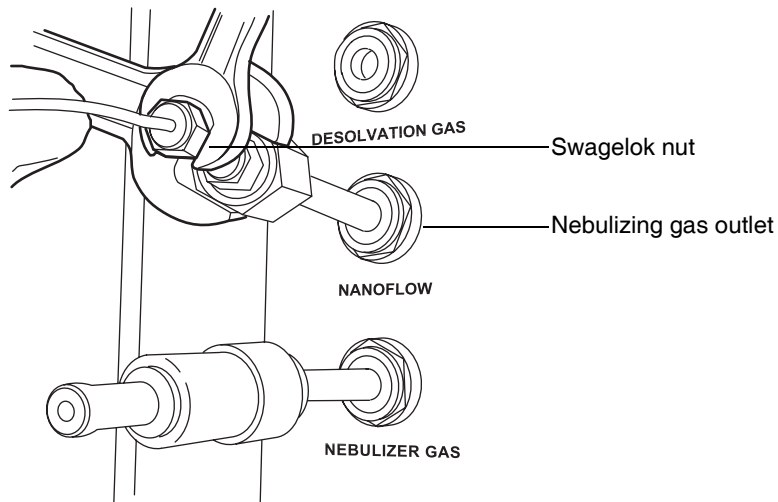
4. Secure the Swagelok nut to the 1/16-inch Swagelok tee of the NanoLockSpray's nebulizing gas supply.

Securing the nut into the NanoLockSpray's nebulizing gas supply



5. If you are using a standard Nanoflow Z-spray source, secure the Swagelok nut to the nebulizing gas outlet.

Securing the nut to the nebulizing gas outlet



6. Finger tighten the nut to secure the Swagelok fitting to the tubing. Then, while securely holding the mating fitting with a wrench, tighten the nut three-quarters of a turn using the 1/4-inch wrench.
7. After the stainless steel ferrule is swaged onto the tubing, finger-tighten the Swagelok nut on the nebulizing gas outlet.

Installing or replacing a nanospray emitter

Preparing the sprayer

Required materials

- Powder-free, sterile gloves
- Universal NanoFlow Sprayer

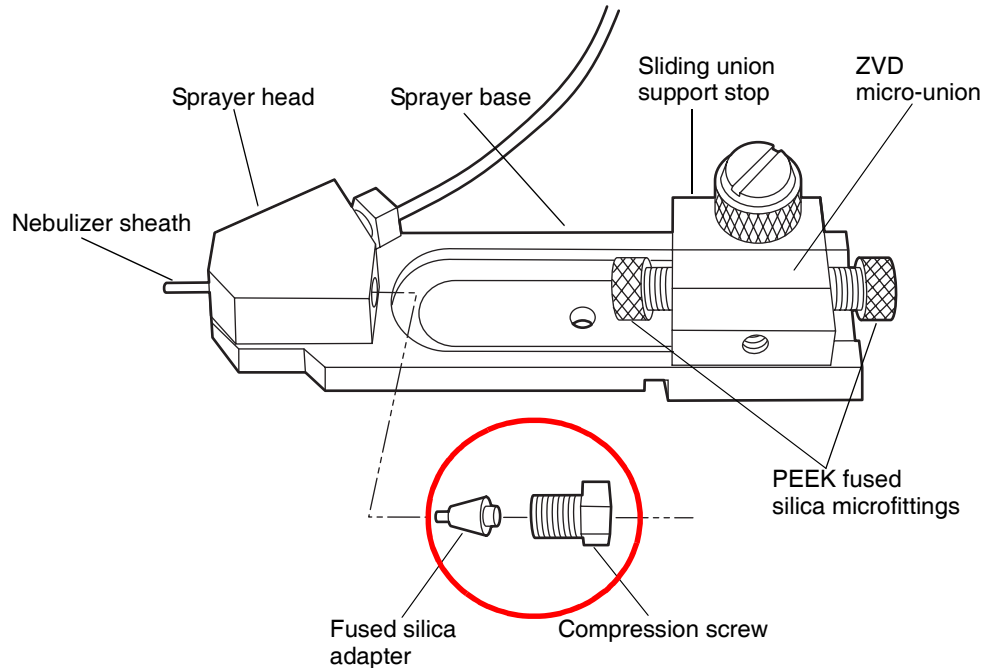


Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To prepare the sprayer:

1. Ensure the polyimide fused silica adapter is in place in the sprayer head.

Fused silica adapter



2. Place the 1/16-inch, stainless steel, compression screw over the fused silica adapter. Screw it into the head, leaving it loose.
3. Ensure that the sliding union support stop is attached to the sprayer base by the dove-tail fitting. The sliding union support stop has a ZVD micro-union secured to it with an Allen-head set screw and PEEK fused silica microfittings on each end.

Preparing the micro-union

Required materials

- Powder-free, sterile gloves
- Stainless steel wire plug
- Universal NanoFlow Sprayer (see [Appendix A](#) for the part number)

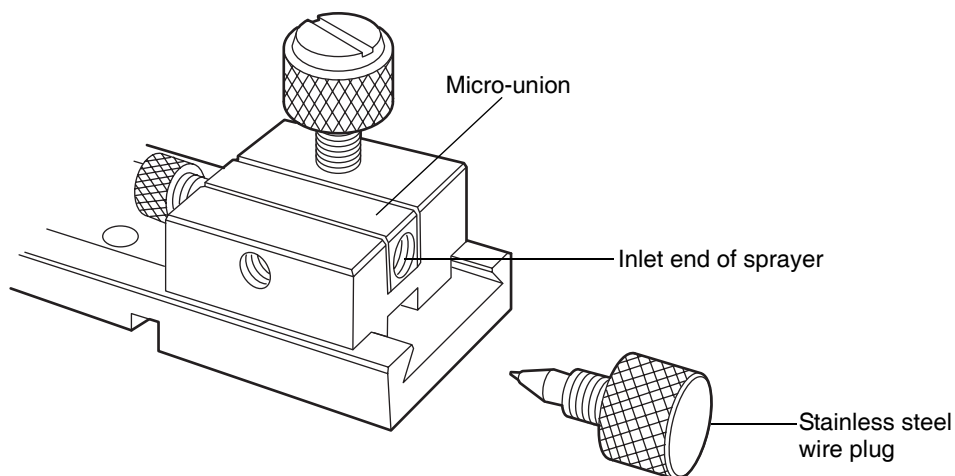


Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To prepare the micro-union:

1. A stainless steel wire plug ensures the distal end of the nanospray emitter corresponds to the exact mid-point of the micro-union.

Inlet end of sprayer and stainless steel wire plug



2. Remove the PEEK microfitting on the inlet end of the sprayer, and replace it with the stainless steel wire plug.

Placing the nanospray emitter in the sprayer

Required materials

- 1/4-inch open-end wrench
- Nanospray emitter
- Powder-free, sterile gloves

- Universal NanoFlow Sprayer (see [Appendix A](#) for the part number)



Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To place the nanospray emitter in the sprayer:

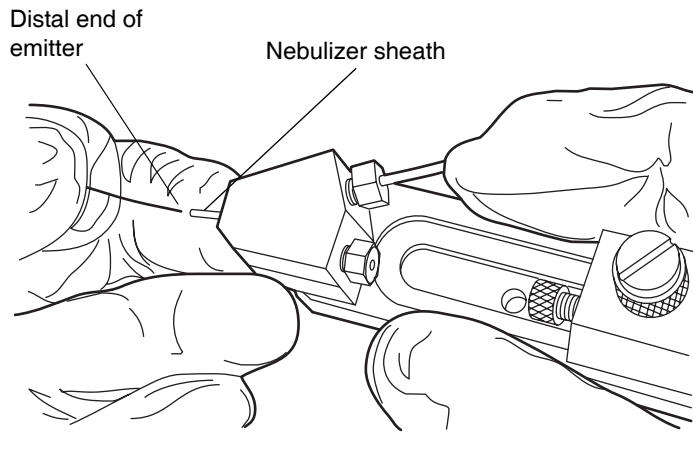
1. Ensure the compression screw, in the sprayer head, and the PEEK microfitting, in the micro-union, are loose.
2. Remove a nanospray emitter from its packaging, and carefully thread the distal end through the nebulizer sheath.



Caution:

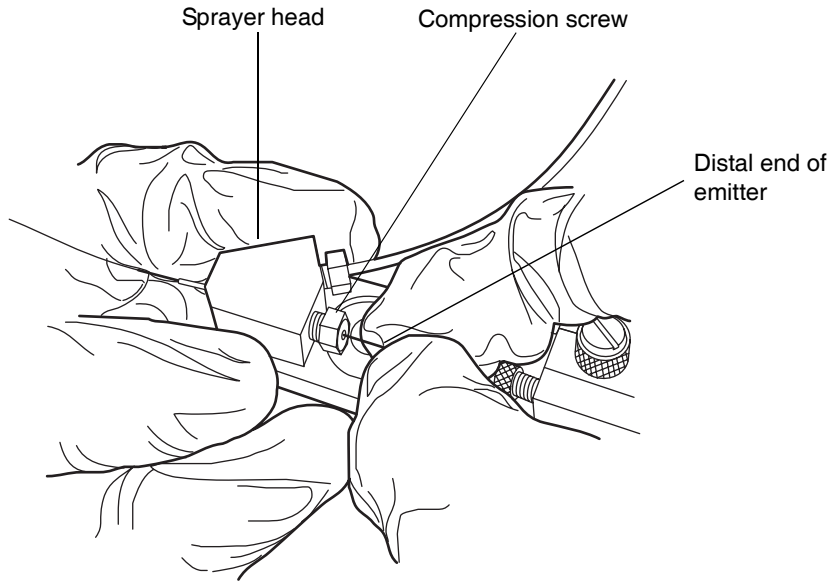
- To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.
- To avoid damaging the emitter, do not grasp or hold it by its proximal (spray) end. Always grasp the emitter by its larger, 360- μ m, outer body.
- Do not cut the distal end of the emitter. It has been precut and polished.

Threading the emitter into the nebulizer sheath



3. Continue to push the emitter through the sprayer head and compression screw. When it emerges, grasp it, and pull it through.

Pulling the emitter through the sprayer head and compression screw

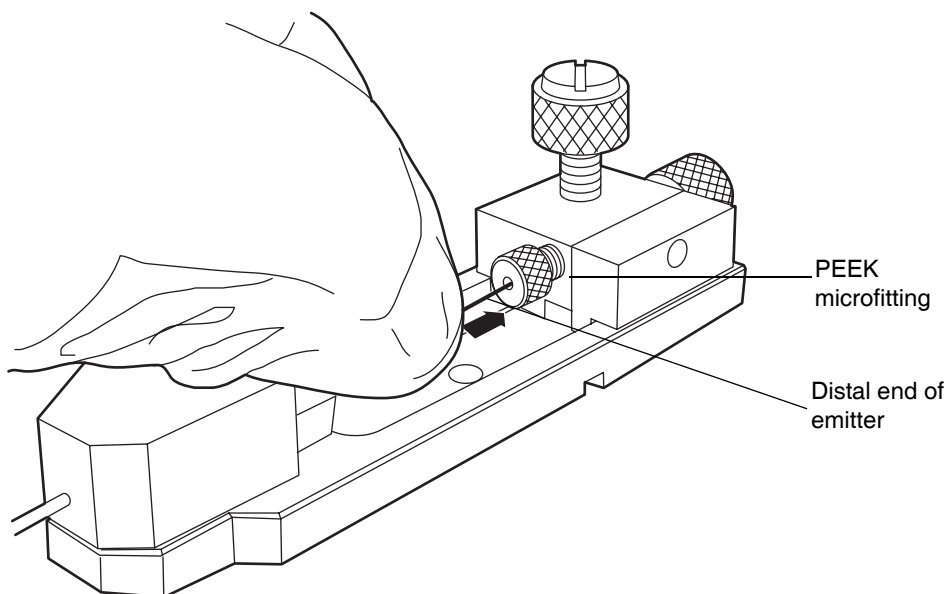


4. As the emitter emerges from the compression screw, carefully guide it into the PEEK microfitting and in the ZVD union.

5. Continue to push the emitter forward, into the micro-union, so that it finally rests against the wire plug.

Tip: The emitter should pass freely, all the way through the microfitting. If it binds or does not pass fully through, significant dead volume, which could adversely affect nanoLC performance can result. If necessary, remove the microfitting and ensure that the emitter can freely pass through it. See [“Reusing microfittings”](#) on [page 2-2](#).

Guiding the end of the emitter into the PEEK microfitting



6. Finger-tighten the PEEK microfitting to secure the emitter in the micro-union.



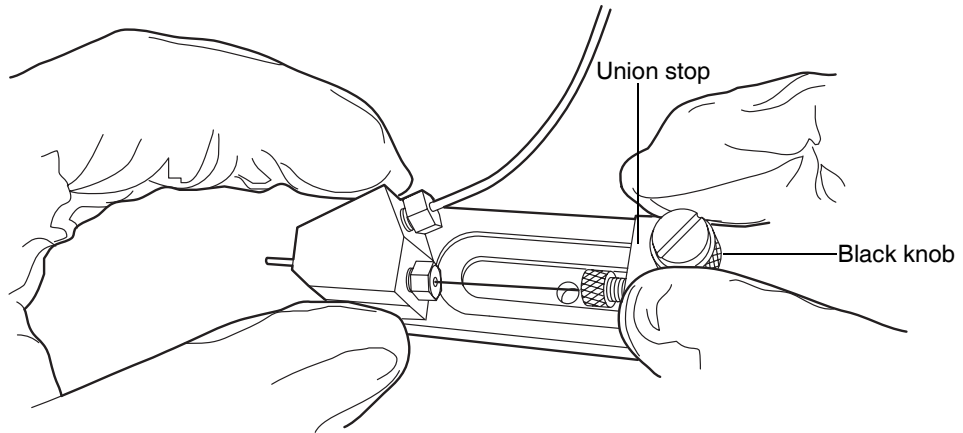
Caution: To avoid crushing the emitter end, do not overtighten the PEEK microfitting.

Tip: You can avoid crushing the distal end of the emitter by carefully turning the fitting to the point that the emitter just seats in the union but is not pushed too far against the wire plug. Then remove the wire plug from the other end, and complete turning the microfitting until it is finger-tight.

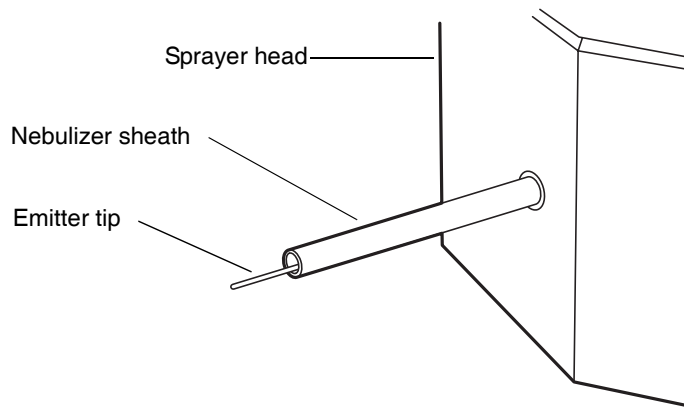
7. Loosen the black knob of the union stop by turning it counterclockwise.

8. Grasp the black knob and slide the union stop either backward or forward so that approximately 1 mm of the tip extends beyond the nebulizer sheath. Then tighten the black knob.

Sliding the union backward or forward

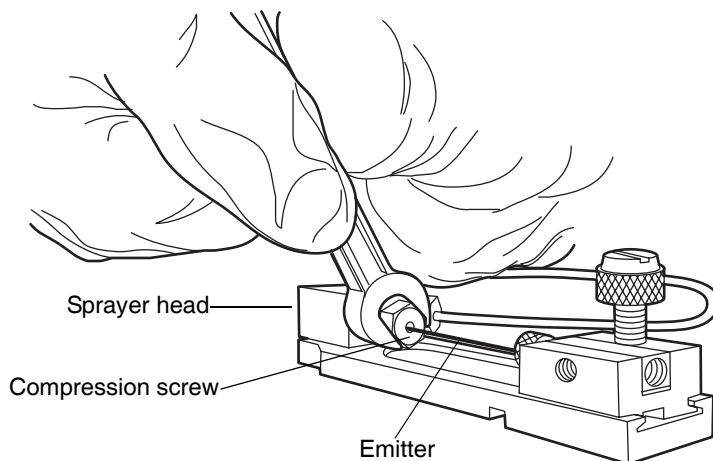


Close-up view of the emitter tip extended beyond the nebulizer sheath



- Secure the emitter to the sprayer head by tightening the 1/16-inch compression screw. Be careful not to overtighten the screw.

Securing the emitter to the sprayer head



Placing the sprayer on the stage platform



Warning: High voltage danger. To avoid electrical shock, never operate the NanoLockSpray or NanoFlow Z-spray source without the protective sprayer cover in place.

Required materials

- Powder-free, sterile gloves
- Universal NanoFlow Sprayer (see [Appendix A](#) for the part number)



Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To place the sprayer on the stage platform:

- Slide the NanoLockSpray source stage back from the source region. If you are attaching the sprayer to the stage platform of a NanoFlow

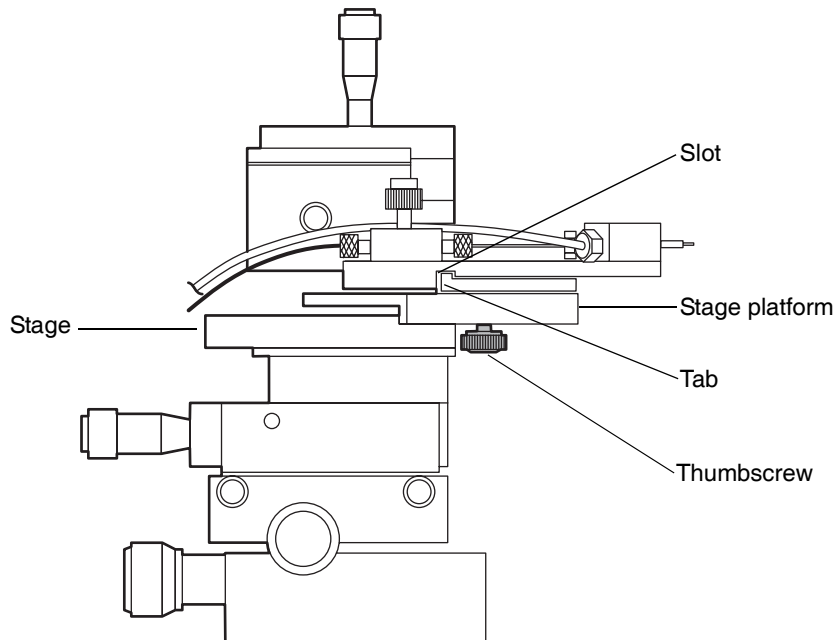
Z-spray source, loosen the stop screw, grasp the handle, and rotate the stage clockwise, 90 degrees from the source housing.

2. Remove the protective sprayer cover.
3. Align the slot in the base of the sprayer with the tab on the platform.
4. Thread the captive thumbscrew, located underneath the stage platform, into the hole in the sprayer base.

Tip: When installing a new sprayer, ensure the thumbscrew is in the hole at the rear of the stage platform.

5. Tighten the thumbscrew to secure the sprayer on the stage platform.

Attaching the sprayer to the stage platform



Attaching a syringe pump or nanocolumn to the sprayer inlet

Recommendations:

- Waters recommends a pre-cut, polished, fused silica piece as the best union between the nanocolumn and emitter. Waters NanoEase™ and nanoACQUITY UPLC® columns are fitted with a column outlet pigtail, which has a pre-cut polished end. An improperly cut fused-silica line can cause poor chromatographic performance when used with a nanocolumn at flow rates under 500 nL/min.
- When infusing a calibrant or reference solution into the sprayer, you should place some type of in-line microfilter (for example, Upchurch part M-500, M-520, M-538, or equivalent) between the solvent delivery system and the sprayer inlet to avoid clogging the micro-union or emitter.

Required materials

- PEEK microfitting (see [Appendix A](#) for the part number)
- Powder-free, sterile gloves
- Universal NanoFlow Sprayer (see [Appendix A](#) for the part number)

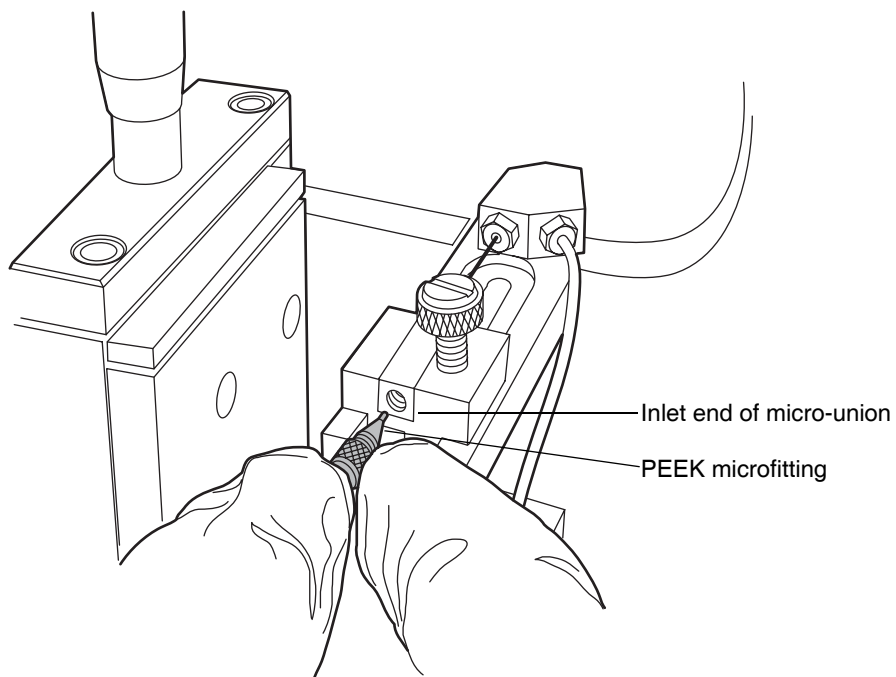


Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To attach a syringe pump or nanocolumn to the sprayer:

1. Attach a PEEK microfitting to the inlet end of the sprayer micro-union.

Installing the PEEK microfitting

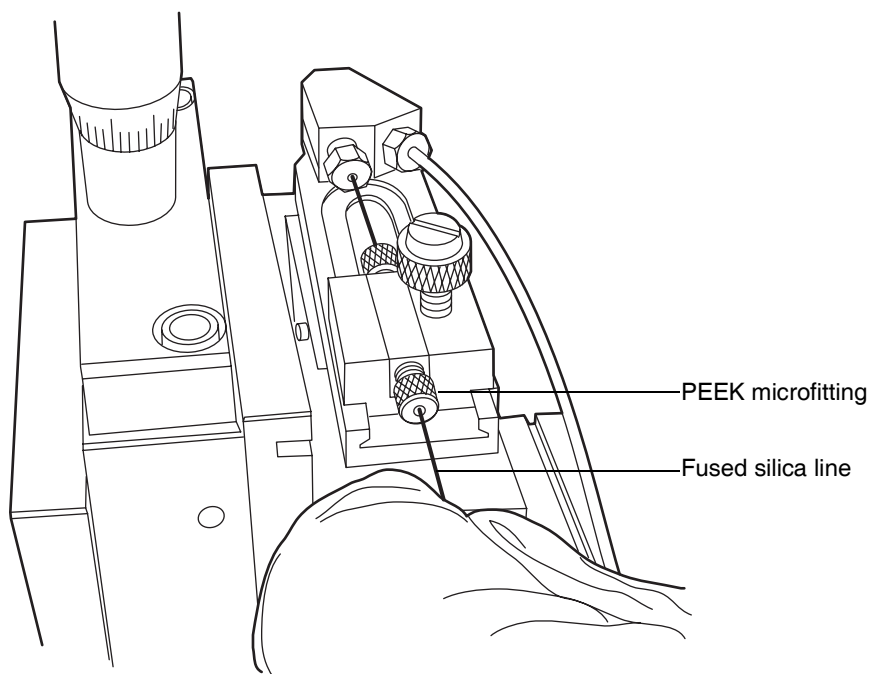


2. Carefully guide a length of 360- μ m OD fused silica tubing from a syringe pump, or nanocolumn, through the PEEK microfitting and, ultimately, into the micro-union.

Requirement: Inside the micro-union, the end of the tubing must butt against the emitter.

3. Finger tighten the PEEK microfitting to secure the tubing in the micro-union.

Guiding the fused silica line into the PEEK microfitting



Replacing the protective sprayer cover and gas lines

Required materials

Powder-free, sterile gloves



Warning: High voltage danger. To avoid electrical shock, never operate the NanoLockSpray or NanoFlow Z-spray source without the protective sprayer cover in place.



Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

To refit the protective sprayer cover and gas lines:

1. If you are using a Q-ToF Premier™ or Synapt™ NanoLockSpray source, refit the clear protective sprayer cover by sliding it forward into the stage slots.
2. If you are using a NanoLockSpray or NanoFlow Z-Spray source, replace the protective sprayer cover, and fasten it to the stage platform.

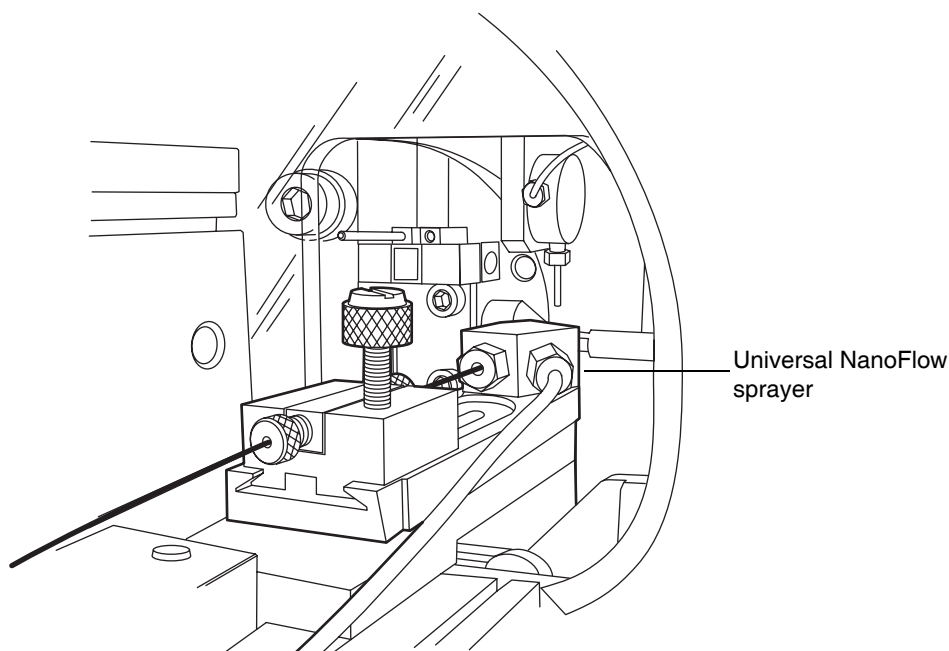
3. Move the stage back into the source housing, and secure it in place.



Caution:

- To avoid damaging the sprayer, determine the positioning of the sprayer before moving the stage into the source housing. Ensure the sprayer does not touch the sample cone or NanoLockSpray baffle.
- To prevent the sprayer from electrically discharging inside the source, carefully position the sprayer so that it is within approximately 5 mm of the NanoLockSpray baffle or sample cone.

Universal NanoFlow Sprayer in the Q-ToF Premier or Synapt NanoLockSpray source housing (protective cover removed for illustrative purposes)



4. Reattach the sprayer's nebulizer gas line to the NanoSpray or NanoFlow gas supply, if necessary. Refer to [“Attaching the nebulizer tube and fittings”](#) on [page 1-6](#).

Optimizing the nanospray signal

Optimizing the signal with the Waters Universal NanoFlow Sprayer and nanospray emitter installed involves infusing a test solution like glufibrionpeptide at a specified flow rate. While infusing the standard solution, you must adjust the capillary high voltage, X-Y-Z stage positioning, cone gas, and nebulizer gas for maximum signal intensity and stability. At typical nanoLC flow rates of 200 to 500 nL/min, a capillary voltage of 2.0 to 3.8 kV serves as a good starting point. For the NanoLockSpray source, extending the emitter tip beyond the nebulizer sheath from 1 mm to between 2 and 3 mm can sometimes yield better stability.

2

Maintaining the Universal NanoFlow Sprayer

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Reusing microfittings	2-2
Replacing the fused silica adapter	2-3



Warning: To prevent injury, always observe Good Laboratory Practices when you handle solvents, change tubing, or operate the system. Know the physical and chemical properties of the solvents you use. See the Material Safety Data Sheets for the solvents in use.

Cleaning the micro-union and microfittings



Caution: To avoid contaminating the emitter or sprayer with skin oils, powder, or other contaminants, always wear powder-free, sterile gloves when handling the emitter or sprayer.

Required materials

- 1.5-mm Allen wrench
- HPLC-grade methanol or isopropyl alcohol
- Pure, dry nitrogen

To clean the micro-union and microfittings:

1. Remove the micro-union from the stop by loosening the Allen-head screw with the 1.5-mm Allen wrench.
2. Sonicate the micro-union and microfittings in a beaker of HPLC-grade (or better) methanol or isopropyl alcohol.
3. Direct a stream of pure, dry nitrogen over the micro-union and microfittings to dry them.

Tip: A spare micro-union and microfittings are provided in the Universal NanoFlow Sprayer startup kit.

Clearing an obstruction from the micro-union

Required materials

- Piece of 360- μ m OD fused silica
- Stainless steel wire plug

To clear an obstruction from the micro-union:

1. Remove any tubing and microfittings from the union.
2. Place the stainless steel wire plug on one of the union ends, and screw it in place to dislodge the obstruction.
3. Use a spare piece of fused silica to push the obstruction out of the union.

Requirement: If you cannot clear the obstruction, replace the micro-union.

Reusing microfittings

You can reuse microfittings several times before they need replacing.

Required materials

- Piece of 360- μ m OD fused silica
- Stainless steel wire plug

To reuse microfittings:

1. Ensure that a piece of fused silica can pass freely and all the way through the fitting without binding.
2. If necessary, carefully insert the stainless steel wire plug into the microfitting to reopen it.

Tip: Replace the microfitting if a piece of 360- μ m OD fused silica does not pass freely through it, or if it leaks.

Replacing the fused silica adapter

Replace the fused silica adapter when

- the emitter does not pass freely through the sprayer head
- the emitter no longer stays in place in the sprayer head when you tighten the compression screw 1 to 3/4 turn with a 1/4-inch wrench.

Required materials

- 1/4-inch open-end wrench
- Ferrule removal tool (see [Appendix A](#) for the part number)
- Fused silica adapter

To replace the fused silica adapter:

1. Remove the compression screw.
2. Extract the fused silica adapter from the sprayer head. It can be helpful to insert a piece of 360- μ m OD fused silica into the adapter and wiggle it back and forth to remove the fused silica adapter from the sprayer head. If necessary, use the ferrule removal tool supplied in the startup kit to remove the fused silica adapter from the sprayer head.

A Universal NanoFlow Sprayer Kit

This appendix lists the parts included in the Universal NanoFlow Sprayer Kit (part number 205000320).

Universal NanoFlow Sprayer Kit contents

Item	Part number
1.5 mm hex key	700002112
Capillary union	405004196
Cleaving tool	605000118
Ferrule removal tool kit	605000117
Ferrule set, .062, two-piece	410001349
Pre-cut TaperTip™ emitter (5-pack)	186003932
NanoFlow sprayer kit list	716001653
PEEK ferrules, 1/16-inch (3)	WAT021817
PEEK fitting, one-piece, 6-40, short (5)	405004199
Polyimide reducing ferrules (3)	410001525
Read Me First document	716001779
Rheodyne fitting	7010-009
Swagelok nut, 1/16-inch tube, stainless steel	41000247
Tube, nat., .062 × .020 × 27 inches	430000617
Universal NanoFlow sprayer	289001685
Universal NanoFlow Sprayer Installation and Maintenance Guide	71500110107

