nanoEase M/Z Columns

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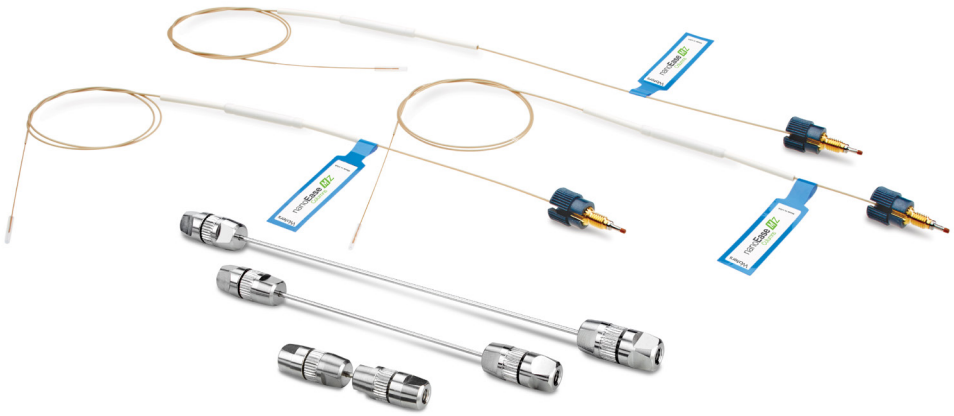
I. INTRODUCTION

Waters™ nanoEase M/Z Columns and Trap Columns are manufactured to exact specifications and are designed for use at a pressure up to 15,000 psi (1000 bar). nanoEase M/Z Columns are compatible with ACQUITY™ UPLC™ M-Class Systems (please see section II, part c for details).

Each nanoEase M/Z Column is individually tested to ensure that it passes stringent quality control.

nanoEase M/Z Columns use ZenFit™ Connection Technology which greatly simplifies creating nano- and micro-flow UPLC fluid connections. ZenFit Connectors are designed to improve the reproducibility of connections and to minimize dead volume in the flow path and thus peak tailing and poor efficiency. This document provides several essential recommendations for the successful use of nanoEase M/Z Columns.

nanoEase M/Z Columns



II. GETTING STARTED

a. Column Construction

Analytical Columns

nanoEase M/Z Columns are available in two hardware formats based on inner diameter (I.D.).

75 µm to 150 µm I.D. columns are packed inside a fused silica capillary, which is connected to the transfer tubing also made of fused silica. The entire column is sheathed with protective polymer tubing. On the inlet side, the column uses ZenFit Connectors. ZenFit Connectors are 10-32 threaded connectors for 1/16" O.D. tubing (see part c for column installation details). The column outlet is a 360 µm O.D. fused silica capillary which interfaces directly with a mass spectrometry source. The column is shipped with a protective piece of Teflon® tubing present over the outlet capillary.

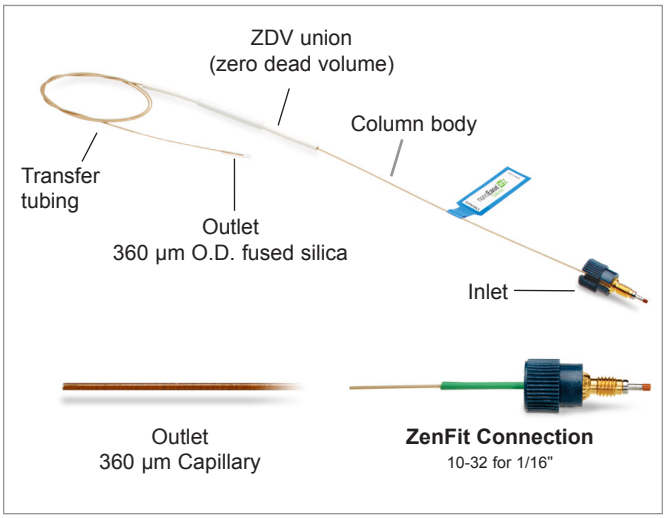


Figure 1. 75–150 µm I.D. column construction.

Columns with an I.D. of 300 µm are made of stainless steel. The inlet and outlet female ports are V-detail female connection ports used in analytical ACQUITY UPLC Columns and work with ZenFit Connectors. The size of a V-detail fitting is a 10–32 thread for 1/16" O.D. tubing.

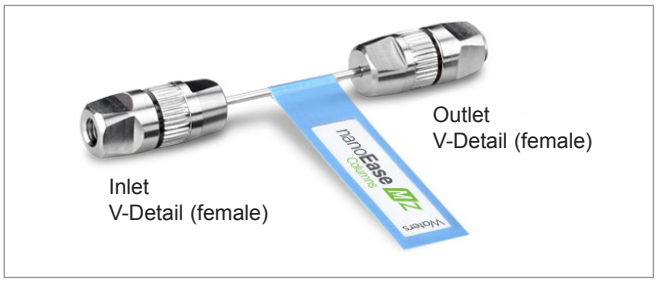


Figure 2. 300 µm I.D. column construction.

Trap Columns

The 180 µm I.D. trap columns are packed inside a fused, silica capillary, which connects to the transfer tubing also made of fused silica. The column body and transfer are sheathed with protective polymer tubing. The 180 µm I.D. trap columns are equipped with ZenFit Connectors on the inlet and outet side. These are 10-32 threaded connectors for 1/16" O.D. tubing (see part c for column installation details).

Trap columns with a 300 µm I.D. are constructed the same way as an analytical column with the same inner diameter (see above).

Trap columns are suitable for all applications, including 2D with dilution. They are also suitable for forward and reversed flow.

b. Preparation

A small piece of Teflon tubing is present on the outlet end of the column for those who want to connect the device to a UV or PDA detector rather than a mass spectrometer.

This tubing is removed when the column is being connected to an emitter, carefully remove the protective Teflon® tubing.

Note: The column is attached to a transfer tube with the use of a zero dead volume union. The union is hidden beneath the protective white polymer cover. Care must be taken not to apply excessive force on the ends of the nano column (e.g., when removing Teflon tubing at column outlet) that could cause this joint to separate.

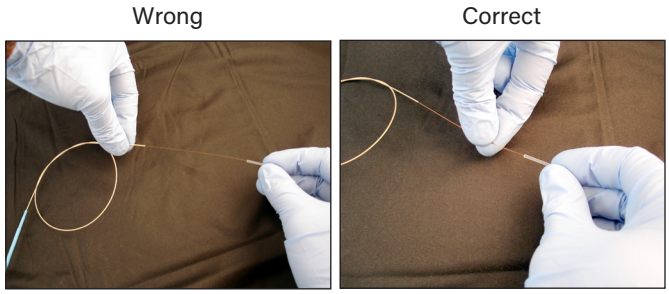


Figure 3. Correct removal of protective Teflon tubing.

Carefully hold the Teflon tubing with one hand and the bare-fused silica capillary, not the PEEK™ tubing, with the other hand to remove the Teflon sleeve (see Figure 4). Again, be careful NOT to apply unnecessary force when removing the Teflon tubing which could cause the joint between transfer tube and packed capillary to separate.

c. Column Installation

nanoEase M/Z Columns are equipped with ZenFit Connectors. To install the columns or trap columns simply follow these instructions.

What is ZenFit?

Waters ZenFit Connectors greatly simplifies creating nano- and micro-flow UPLC fluid connections. They are easy-to-use, re-usable, and made for tool-free use. The fittings eliminate the complexity and common variability of regular, conical compression fittings. The 15,000 psi liquid connection warrants reliable chromatographic results with each re-tightening cycle.

Where does ZenFit fit?

ZenFit Connectors are designed to be used in ports called out specifically for ZenFit or in V-detail style connection ports. The V-Detail connection port is a 10-32 threaded port for 1/16" O.D. tubing used in Waters UPLC equipment. ZenFit can be used on the injection valve and the Trap Valve Manager (TVM) of the ACQUITY UPLC M-Class and nanoACQUITY™ Systems. Please note that the following nanoTee and nano union as well as appropriate ZenFit tubing must be used when using nanoEase M/Z Columns and Trap Columns.

Note: It is NOT recommended to use ZenFit Connectors in non-Waters ports. Slight variations in port diameters may cause the ZenFit Connector to seal inadequately or interfere with the receiving port.

Description	P/N
nanoTee	700011518
nano Union	700011519

How does it work?

ZenFit Connectors create a fluid seal at the flat bottom face of the connection port. This greatly simplifies the installation process helping repeatedly creating consistent fluid connections.

ZenFit Connectors are designed for multiple tightening cycles.

Note: Ports specifically designed for ZenFit Connectors will not work with compression type fittings, and are only compatible with ZenFit Connectors.

How is it installed?

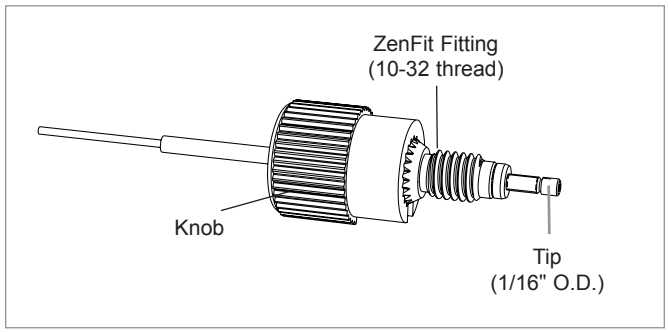


Figure 4. Schematic of a ZenFit Connector.

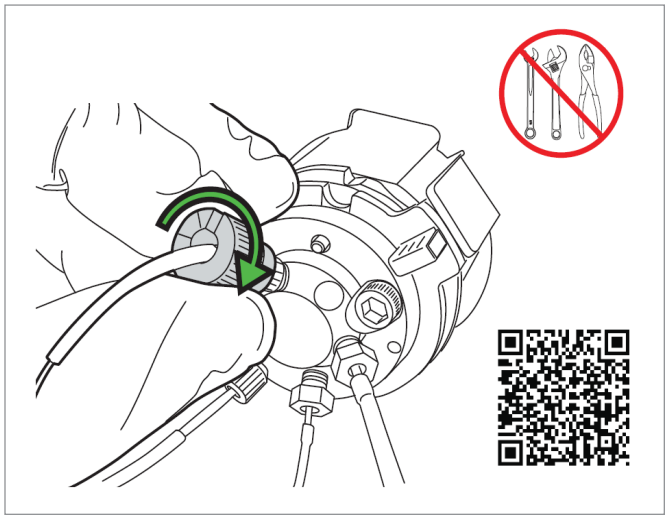


Figure 5. Tool-free installation of a ZenFit Connector.

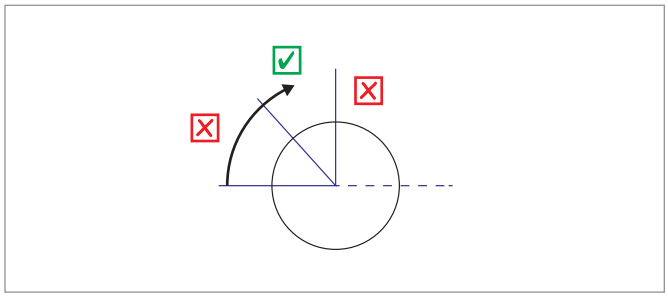


Figure 6. Finger tighten 1/8 to a 1/4 turn maximum.

1/8" to 1/4" turn

Thread the ZenFit Connector into the port until the tip touches the bottom of the port. Using the knob, hand tighten the fitting with your fingers as described above in Figure. This will create a seal between the tip seal and the bottom of the port. If a slow leak should be present, hand tighten the fitting slightly more.

Don't use excessive force as this may damage the fitting or the receiving port.

III. COLUMN USE

a. pH and temperature range

Each solid phase packed inside the columns has different characteristics and can be used with mobile phases in a different pH range which need to be considered when operating a column.

Also to be considered is that due to the differences in wetted built materials, 75 µm to 150 µm I.D. columns have a more limited pH range than columns with 300 µm I.D. Please see table below for details.

Table 3. pH and temperature range

Solid phase	pH range and max. temperature			
	300 µm I.D.		150 µm, 100 µm, 75 µm, and 180 µm I.D.	
BEH C_{18}, 130Å	pH 1–12	80 °C (low pH) 60 °C (high pH)	pH 2–10	80 °C (low pH) 60 °C (high pH)
BEH C_{18}, 130Å	pH 1–12	80 °C (low pH) 60 °C (high pH)	pH 2–10	80 °C (low pH) 60 °C (high pH)
BEH C_4, 130Å	pH 1–10	80 °C (low pH) 50 °C (high pH)	pH 2–10	80 °C (low pH) 50 °C (high pH)
CSH C_{18}, 130Å	pH 1–11	80 °C (low pH) 45 °C (high pH)	pH 2–10	80 °C (low pH) 45 °C (high pH)
HSS T3, 100Å	pH 2–8	45 °C (low pH) 45 °C (high pH)	pH 2–8	45 °C (low pH) 45 °C (high pH)
Symmetry C_{18}, 100Å	pH 2–8	45 °C (low pH) 45 °C (high pH)	pH 2–8	45 °C (low pH) 45 °C (high pH)

b. Mobile phases and buffers

- To maintain and ensure maximum column performance and lifetime, only use MS-grade solvents.
- Filter all aqueous buffers prior to use through a 0.2 µm filter. Solvents containing suspended particulate materials may clog the inlet of the columns. This may result in higher backpressure or distorted peak shape. Recommended buffers are listed in Table 4.
- Only prepare small quantities of mobile phase into fresh, clean bottles. Do not top up mobile phases. Exchange bottles and mobile phases after an extended period of time.

Eluents:

- A solvent: 100% water with 0.1% formic acid
- B solvent: 100% acetonitrile or methanol with 0.1% formic acid
- Seal wash solvent: water, may contain small amount of organic (no acid)
- Weak needle wash for peptides: 3% acetonitrile with 0.1% formic acid

See below for a list of recommended buffers.

Table 4. Recommended buffers and modifiers

Additive/buffer	pKa	Buffer range	Volatility (± pH unit)	Comments
Acetic acid	4.76	n/a	Volatile	Maximum buffering obtained when used with ammonium acetate salt. Used in 0.1–1.0% range.
Formic acid	3.75	n/a	Volatile	Maximum buffering obtained when used with ammonium formate salt. Used in 0.1–1.0% range.
Ammonium acetate (NH_4CH_2COOH)	4.76	3.76–5.76	Volatile	Used in the 1–10 mM range. Note that sodium or potassium salts are not volatile.
Ammonium formate (NH_4COOH)	3.75	2.75–4.75	Volatile	Used in the 1–10 mM range. Note that sodium or potassium salts are not volatile.

c. Sample preparation considerations

- Samples may contain buffers and residual reagents from approved digestion procedure.
- The sample must not contain other reagents, denaturants, detergents, lipids, and must be free of particulates.

Samples containing a significant amount of undigested protein per injection have been shown to limit or shorten column lifetimes, depending on the injected quantity and the column diameter.

Therefore, it is essential that proper sample preparation protocols are followed. Below are examples of recommended protocols for preparing plasma.

Table 5. Sample preparation

Sample type	Sample preparation
Protein precipitation >2:1 acetonitrile:water	This method effectively removes the majority of protein from solution, pay attention to final organic content.
Liquid/liquid extraction	This method effectively extracts the analyte leaving the protein behind, pay attention to final organic content.
Digestion	Proteins are broken into smaller pieces that no longer present an issue.
Affinity	Small amounts of specific proteins are extracted from the sample for analysis. This can be an effective way of preparing samples by removing residual protein.
Solid-phase extraction	This is an issue when the analyte has similar properties to proteins (e.g., the methods used to isolate larger peptides often leave significant protein content in the final extract). If solid-phase extraction is the sole sample preparation technique, multidimensional chromatography (i.e., trap and back-flush elution) is required.
Filter	Filters will not remove protein that is in solution.
Centrifugation	Centrifugation will not remove protein that's in solution, unless it's been crashed with protein precipitation (PPT).
Dilution	It depends on the dilution ratio, and the diluent. In general, small aqueous dilutions will not remove protein.
Urine	Typically low protein content and does not require organic additive to crash proteins. Dilution and centrifugation is recommended.
Bile	Typically low protein content and does not require organic additive to crash proteins. Dilution and centrifugation is recommended.
Microsomes	Microsomal incubations contain proteins. Pay close attention to sample preparation to ensure that minimal protein remains in the final sample extract.
Plasma	Plasma typically contains high concentrations of protein. Pay close attention to sample preparation to ensure that minimal protein remains in the final sample extract. Injecting a large amount of undigested proteins onto the column will result in compromised performance and shorter lifetimes.
Organic extracts	High amounts of organic affect the performance of any column, this effect is greater with small dimensions.

The use of multidimensional chromatography, specifically a trap and elution strategy, can provide further sample cleanup and facilitate injections of higher organic strength injection solvents without experiencing analyte breakthrough. This multidimensional chromatographic strategy is highly recommended when solid-phase extraction is the sole sample preparation technique (i.e., no additional sample preparation technique is used to remove large amounts of undigested protein).

It is preferable to prepare the sample in the initial mobile phase conditions or a weaker solvent for the best peak shape and sensitivity.

If the sample is not prepared in the mobile phase, ensure that the sample, solvent, and mobile phases are miscible in order to avoid sample and/or buffer precipitation.

Consider filtering samples with a 0.2 µm membrane to remove particulates. If the sample is dissolved in a solvent that contains an organic modifier (e.g., acetonitrile, methanol, etc.) ensure that the membrane/filter material is compatible with the solvents in use. Alternatively, centrifuge the sample for 20 minutes at 8000 RPM, followed by the transfer of the supernatant to an appropriate Waters TruView™ LCMS Certified Vial could be considered.

Below are two examples of commonly used sample preparation protocols:

Protein precipitation (PPT)*

1. Add acetonitrile to plasma at a ratio of 2:1 (acetonitrile:plasma).
2. Vortex (mix) for one minute.
3. Centrifuge at 5000 relative centrifugal force (RCF) for five minutes.
4. Remove supernatant.
5. Perform solid-phase extraction (SPE) cleanup (optional).*
6. Pipette into Waters TruView LCMS Certified Vial (e.g., p/n: 186005663CV).

Liquid/liquid extraction (LLE)*

1. Add hexane to plasma at a ratio of 10:1 (hexane:plasma).
2. Vortex (mix) for one minute.
3. Centrifuge at 5000 relative centrifugal force (RCF) for five minutes.
4. Remove supernatant and evaporate to dryness.
5. Reconstitute (e.g., initial gradient conditions solvents).
6. Centrifuge at 5000 relative centrifugal force (RCF) for five minutes (optional).
7. Perform solid-phase extraction (SPE) cleanup (optional).*
8. Remove supernatant and pipette into Waters TruView LCMS Certified Vial (e.g., p/n: 186005663CV).

**Additional sample cleanup steps using Oasis™ or Sep-Pak™ SPE Devices can provide cleaner samples, improved selectivity, higher sensitivity, and reduced matrix effects.*

d. Operating pressure, starting, and stopping the flow

Do not start or stop the flow to your nanoEase M/Z Column abruptly. It is critical that the flow be slowly increased or lowered via the ACQUITY UPLC M-Class System console to prevent column damage.

The inlet, or head, of a chromatography column experiences the largest amount of pressure in a LC system. The nanoEase M/Z Column Inlet is designed to operate at pressures up to 15,000 psi (1000 bar).

The fluidic pressure drops across the length of the column and the transfer tubing. The outlet should only experience atmospheric pressure. Do not operate the column in reverse flow direction.

e. Cleaning and storage

Cleaning

Changes in peak shape, peak splitting, shouldering peaks, shifts in retention, change in resolution or increasing backpressure may indicate column contamination. Flush with organic solvent to remove the non-polar contaminant(s), taking care not to precipitate any buffered mobile phase components. If this flushing procedure does not solve the problem, purge the column with the following cleaning and regeneration procedures.

- Use a cleaning routine that matches the properties of the samples and stationary phase type and will solubilize the suspected contaminate. Flush with 20 column volumes of solvent at an intermediate temperature of 40 °C. Return to the initial mobile-phase conditions by reversing the sequence.
- Purge the column with a sequence of progressively more non-polar solvents (e.g., water, methanol, acetonitrile, isopropanol, etc.) and reverse the purging order to end the procedure at initial mobile phase conditions.
- 25% water, 25% acetonitrile, 25% methanol, 25% IPA, and 0.1% formic acid is a good all-round cleaning solvent.
- If PEG contamination is found, flush system excessively with IPA.

Storage

When not in use, it is recommended to maintain flow through the nanoEase M/Z Column using 100% eluent B.

When the column needs to be disconnected from the system for long term storage, the column should be stored in 100% acetonitrile. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of MS-grade water followed by 10 column volumes of acetonitrile. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced.

Note: If the column has been run with a formate-containing mobile phase (e.g., ammonium formate, formic acid, etc.) and is purged with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and re-wetted with that same formate-containing mobile phase.

f. Method Example: Trap Elute Peptide Separation

This protocol is based on evaluating a 75 µm x 250 mm nanocolumn using a trap-and-elute method. The method should be modified accordingly in order to test columns of other dimensions.

1. Prepare the following solutions and other mixtures that are necessary for your LC-MS system. Make sure that all the solvents and reagents are of highest quality available (e.g., LC-MS grade), and are prepared fresh. It is recommended to discard the mobile phases and aqueous solutions after one week from the preparation date and prepare a new batch in order to avoid contamination.
   - Mobile phase A: water + 0.1% formic acid
   - Mobile phase B: acetonitrile + 0.1% formic acid
   - Weak needle wash: water + 0.1% trifluoroacetic acid
   - Strong needle wash: 25:25:25:25 water/acetonitrile/methanol/2-propanol + 0.2% formic acid
   - Diluent: water + 0.1% trifluoroacetic acid
   - Stock diluent: 50:50 water/acetonitrile + 0.1% trifluoroacetic acid
2. Prepare the injection samples and the stock.
   - Add 1 mL of stock diluent (50:50 water/ACN + TFA) into a fresh vial of MassPREP™ Enolase Digest with Phosphopeptides (p/n: 186003286). Close the cap and vortex for 1 minute. Label it as "Stock" and store in a refrigerator (1 pmol/µL).
   - Add 2 µL of stock to 998 µL diluent (water + TFA) in a LC-MS grade injection vial. Close the cap and vortex for 1 minute. Label it as "test sample" (2 fmol/µL).

     ** You may adjust the concentration of the injection sample depending on the sensitivity of the mass spectrometer. If so, please make sure the acetonitrile concentration in the sample matrix is below 0.5%.*
   - Add 25 µL of stock to 475 µL diluent in a LC-MS grade injection vial. Close the cap and vortex for 1 minute. Label it as "conditioning sample" (50 fmol/µL).

     ** You may use your typical conditioning samples.*
3. Set up an LC method following the below parameters.

Trapping condition	99.5:0.5 A/B, 15 µL/min x 2 min			
Analysis gradient		Flow rate	A	B
	–	300 nL/min	95	5
	55 min	300 nL/min	60	40
	57 min	300 nL/min	15	85
	62 min	300 nL/min	15	85
	65 min	300 nL/min	95	5
	110 min	300 nL/min	95	5
Injection	3 µL			
Column temp.	35 °C			
Sample temp.	10 °C			

4. Set up an ES+ MS method. An example MRM method for a triple quad MS is shown below. For a ToF or Orbitrap instrument, scan from 100 to 1200 amu. Set the dwell time/cycle time so that there are at least 20 points across a 10-sec peak (i.e., 0.5 seconds cycle time).

Capillary voltage	~2.5 kV, fine-tuned				
Source temp.	100 °C				
Peptide SRM conditions		Sequence	Transition	Cone V	Characteristics
	T3	SVYDSR	363.67 > 540.24	12 eV	Hydrophilic
	T19	HLADLSK	392.22 > 646.38	13 eV	Histidine-containing
	T18	NVPLYK	367.22 > 520.31	13 eV	Basic
	T22	TFAEALR	404.22 > 559.32	14 eV	Neutral
	T43p	VNQIGpTLSESIK	684.84 > 914.40	14 eV	Phosphorylated
	T42	AADALLLK	407.76 > 672.43	14 eV	Hydrophobic
	T32	YDLDFK	400.69 > 637.32	23 eV	Acidic

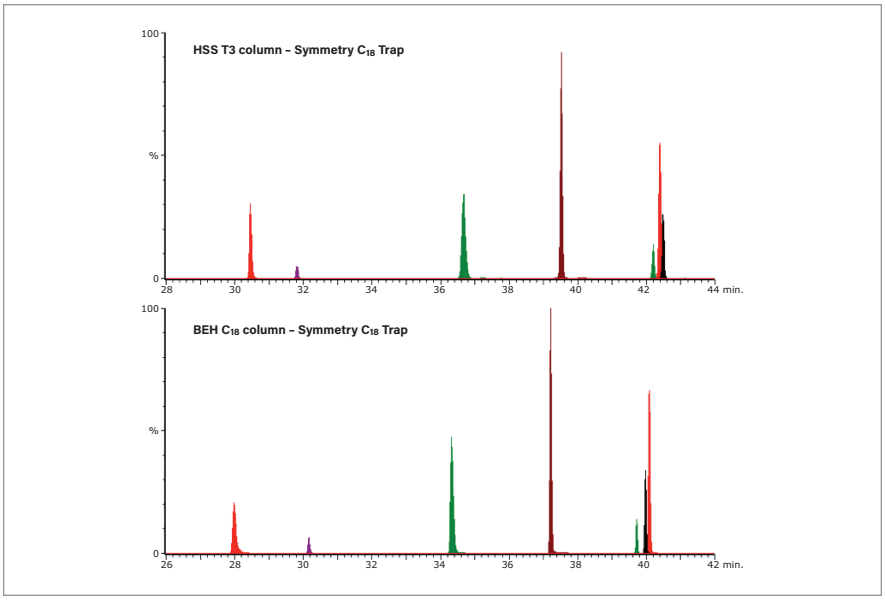


Figure 7. An example chromatogram separating the peptides using a nanoEase M/Z HSS T3 Column (top) and a nanoEase M/Z BEH C_{18} Column (bottom) with a nanoEase M/Z Symmetry™ C_{18} Trap. Both separations were performed using 75 µm x 250 mm columns at the flow rate of 0.3 µL/min. Note the 2-minute offset on the time axis (x-axis).

5. Qualify the LC-MS system per the instrument manufacturer's recommendation, such as tuning the nanoelectrospray source, calibrating the mass spectrometer, priming the mobile phases and needle wash solutions, etc.
6. Connect all fluidic tubing except for the trap and the column according to the manufacturer's recommended flow diagram.
7. Flush the lines with 50:50 A:B to remove any debris or dust particles in the fluidic lines and the connection port. Change the valve position to ensure that all fluidic lines are cleaned. For example, for an ACQUITY UPLC M-Class System, flush it with 50:50 A:B for 15 µL/min on both valve positions.
8. Connect the trap. Flush it with 50:50 A:B at 5 µL/min for 10 minutes. Perform the leak test up to 14,500 psi (if the system has an option).
9. Connect the 75 µm x 250 mm column inlet, but leave the end of the column open.
10. Flush the column and trap with 10:90 A:B at 0.3 µL/min and 5 µL/min, respectively for 30+ minutes.
11. Connect the end of the column to the emitter. Check the spray performance and its consistency.
12. Equilibrate with the initial condition for 30 minutes.
13. Inject the "conditioning sample" for 3–5 times.
14. Inject the "test sample" for 6 times. Take the results from the last four injections.

IV. TROUBLESHOOTING

a. Low pressure

If the backpressure of the column is unusually low and small droplets can be seen at the inlet, ZenFit Fitting may not be tightened enough. Tighten the fitting with your fingers with just a fraction of a turn.

b. High pressure

For the following system diagnosis, a basic single pump trap-elute scenario is assumed, as shown below:

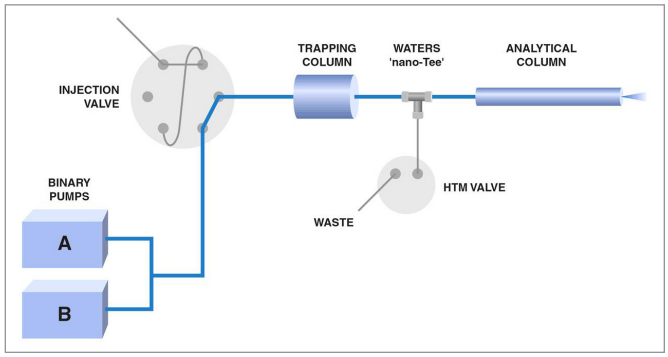


Figure 8. Basic single pump trap-elute scenario.

High system pressure can have two basic causes: a blockage at the emitter/outlet end of the column or a blockage at the inlet side of the column.

In case of an emitter blockage the column and transfer tubing are under high pressure conditions. In case of a blockage on the inlet side, the column and transfer tubing are not under high pressure conditions.

Diagnosis: Post-column blockage

The most common source of post-column clogs is a blocked nanospray emitter. A clogged emitter may still produce an ion signal; therefore this problem may only be diagnosed using the pressure trace in the console window:

Step 1: The pressure traces in the console window show an increase in pressure during the analytical gradient (red arrows) between two injections (highlighted by the red squares).

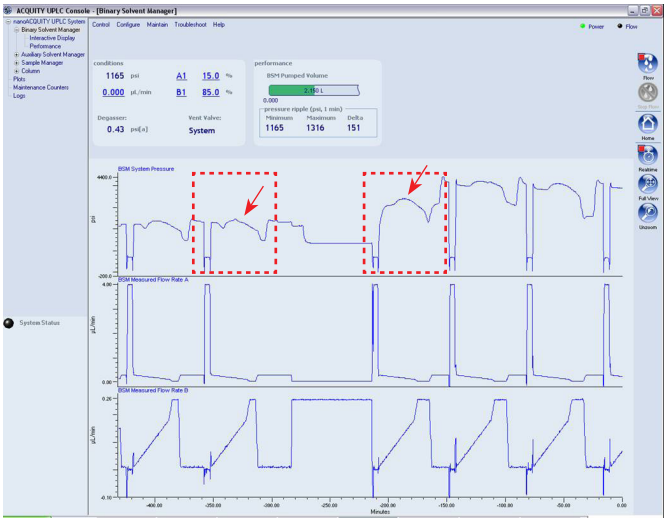


Figure 9. Post-column blockage—Step 1.

Step 2: Notice the pressure during the trapping portion of the analysis did not increase between injections (red arrows). This shows the increase in system pressure must originate after the trap column.

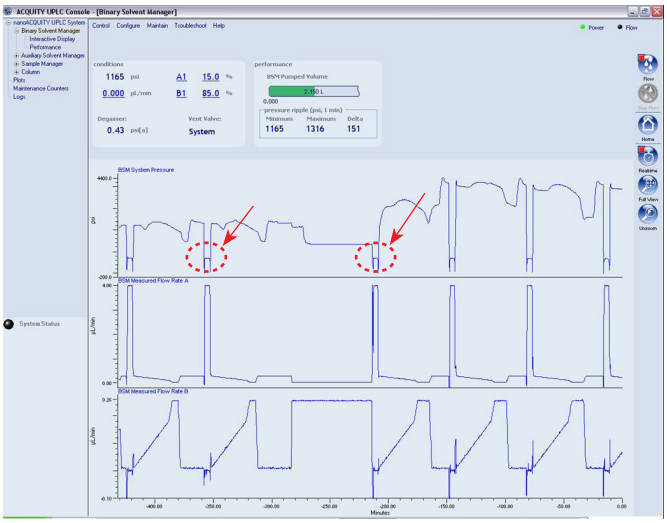


Figure 10. Post-column blockage – Step 2.

Step 3: To test for a clogged emitter, stop the pump flow, wait for the pressure to be released, shut off high voltage and remove the column transfer tubing from the Universal Sprayer (or other nanospray source).

If the system pressure drops more than 200 psi when disconnecting the transfer tubing, the emitter is clogged.

Please Note: In normal use, it is best to stop the flow and wait until the pressure drops before disconnecting the outlet tubing. However, some situations may require disconnecting the column outlet under higher pressure conditions to prevent permanent damage to the column.

If the column outlet or emitter is blocked and the column outlet pressure rises above ≥8000 psi (550 bar) for an extended period of time the joint between the column and transfer tubing in the ZDV union may become disconnected. This is permanent damage and will render the column unusable.

V. DOWNSTREAM MAINTENANCE

a. Checking the emitter tip

Examining a clogged nanospray emitter under a microscope may allow the user to determine the source of the clog.

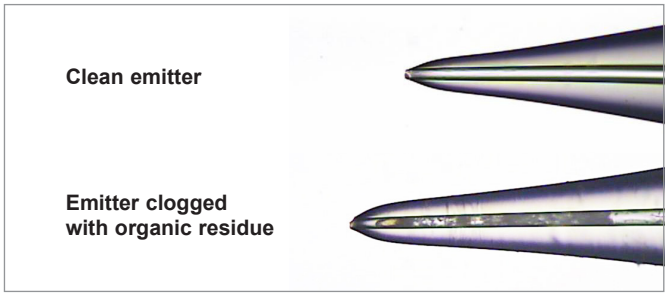


Figure 11. Clean and clogged emitters.

Organic material: The material will appear brown in color. Organic material can derive from a dirty sample or solvents and have accumulated over time in the emitter tip.

Fused silica particles: These particles will be transparent and have sharp, fractured edges. Fused silica particles can be created when a column and an emitter are joined with too much force, e.g., by an over tightened union or when the transfer tubing has been cut manually by the user.

b. Cleaning the union

It may be beneficial replacing the micro-union whenever a new emitter tip is being used.

- To order the Union – p/n: 700011518
- To order PEEK Fitting – p/n: 700011519

However, there are two cleaning methods recommended by Waters, as described below.

- Remove the clogged emitter and the column from the union on the universal sprayer.
- Remove the PEEK nuts on either end.

Loosen the screw on the side of the sliding portion of the sprayer and remove the union.

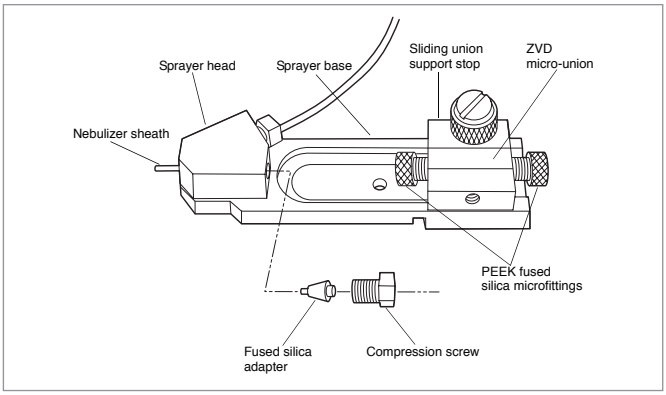


Figure 12. Universal NanoFlow Sprayer.

Clearing an obstruction from the micro-union

Required materials:

- Piece of 360 µm O.D. fused silica
- Stainless steel wire plug

Remove any tubing and microfittings from the union. Place the stainless steel wire plug on one of the union ends and screw it in place to dislodge the obstruction. Use a spare piece of fused silica capillary to push the obstruction out of the union. Follow one of the suggested cleaning methods below after removing the obstruction.

If the union cannot be cleared from the obstruction, replace the micro-union.

Cleaning the union — Method #1

As the union is 100% stainless steel, 20% nitric acid in water can be used to clean it. This aggressive procedure helps ensure that the union becomes free of even the strongest contaminations. In addition, it provides excellent electric contact for electrospray ionization. Wash the outside of the outlet capillary and fitting with 50:50 water:acetronitrile before reconnecting to union.

Note: Do not mix nitric acid with organic acids.

Cleaning the union — Method #2

The union can be cleaned by sonication in a solution of IPA/water for at least 15 minutes.

After sonication, remove any residual solvent by flushing the union with air or nitrogen. After cleaning, examine the union with a microscope and ensure that any particulate matter has been removed. While looking at the union be sure the internal threads and opening have not been scored or damaged in any way. If the obstruction within the union cannot be removed or it appears damaged please replace the union.

c. Replacing the emitter tip

Required materials:

- 1/4" open-end wrench
- Nanospray emitter
- Universal NanoFlow Sprayer (p/n: 289001685)
- Powder-free, sterile gloves

Place the wire pin plug at the back side of the union. This will mark the center of the union.

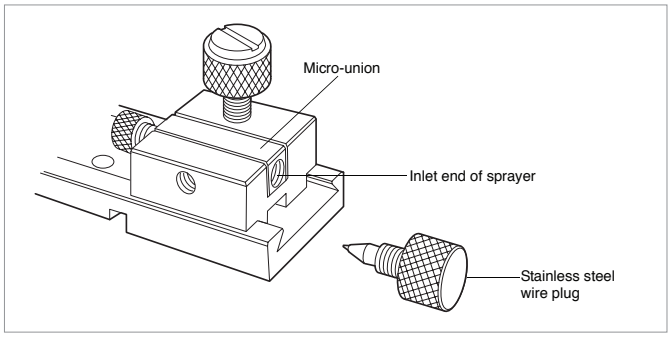


Figure 13. Replacing the emitter tip – Step 1.

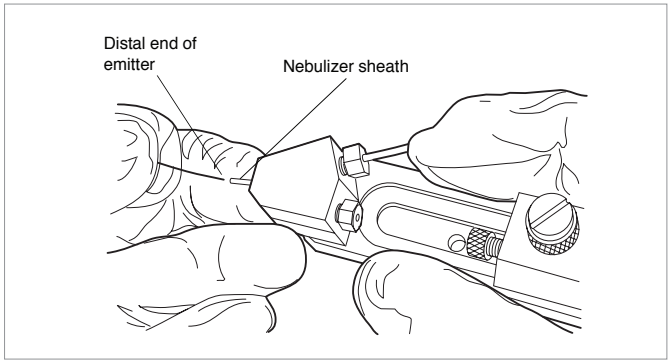


Figure 14. Replacing the emitter tip – Step 2.

Continue to push the emitter through the sprayer head and compression screw. When it emerges, grasp it, and pull it through. Guide the emitter into the PEEK microfitting until it rests against the wire plug. The emitter should pass freely all the way through the microfitting. Fingertighten the PEEK microfitting, securing the emitter in the micro-union.

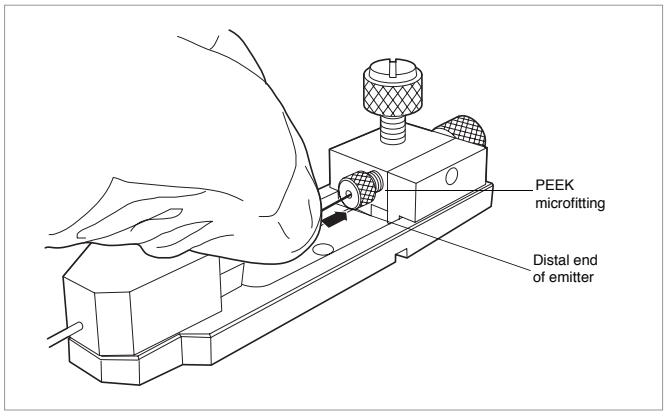


Figure 15. Replacing the emitter tip – Step 3.

Note: Check the microfitting if the emitter does not pass though freely, as the emitter stuck in the fitting will cause significant dead volume which could adversely affect the nanoLC performance.

Adjust the position of the micro-union so the emitter tip extends the appropriate amount (approx. 1 mm) beyond the nebulizer sheath.

- Thread the replacement emitter (back end first) through the front of the sprayer and into the union.
- Tighten the PEEK nut around the union carefully (do not over-tighten).
- Remove the pin plug and replace it with the transfer line from the nanoEase M/Z Column (again, do not over-tighten the fitting).

Note: Do not cut or modify the emitter or column tubing. All tubing and columns supplied by Waters are pre-cut and polished.

Improperly cut tubing can lead to "shedding" and introduction of fused silica particles or polyimide downstream of nanoEase M/Z 75, 100, or 150 µm I.D. Columns. This consequently can cause undesired system backpressure increases.

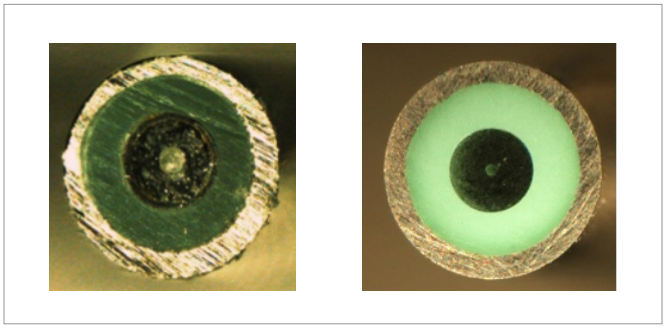


Figure 16. Improperly cut vs. properly cut and polished tubing.

VI. CAUTIONARY NOTE

Some products may be hazardous during and after use and are to be used by professional laboratory personnel trained in the competent handling of such materials. The responsibility for the safe use of products rests entirely with the purchaser and user. The safety data sheets (SDS) for these products are available at www.waters.com/sds.

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