ThermoFisher SCIENTIFIC

HPLC columns

µPAC HPLC columns connection guide

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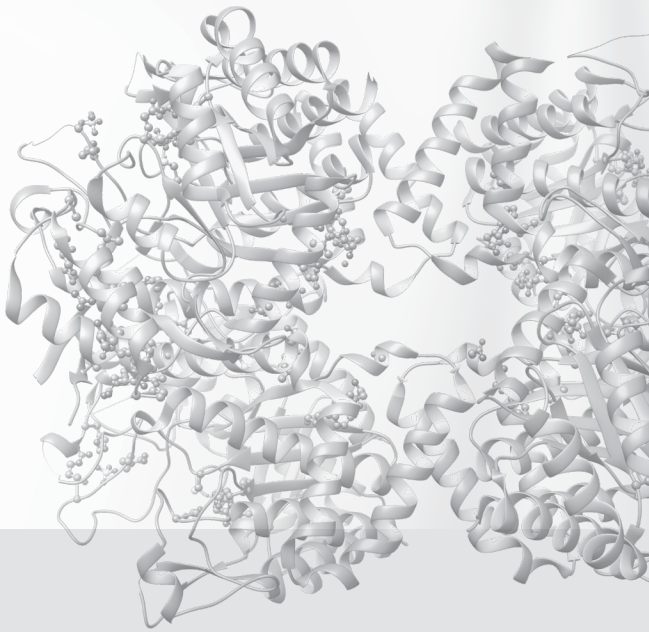
This connection guide describes how to connect Thermo Scientific™ µPAC™ HPLC Columns to either a Thermo Scientific™ EASY-Spray™ or a Thermo Scientific™ Nanospray Flex™ Ion Source. Various system set-ups are included.



Introducing nanoViper column connections

nanoViper connections overview

nanoViper is a fingertight UHPLC fitting system with virtually zero-dead-volume. You'll experience successful nanoLC connections the first time, giving you peace of mind.

- Carefully tighten the nut fingertight using the black knurled nut
- Do not over tighten—maximum of 1/8 turn after fingertight
- Remove the black knurled nut when tightened, after installation

Recommendation: Use the torque tool. One is provided with the Thermo Scientific™ Vanquish™ Neo UHPLC System, and is available to purchase separately.

Learn more about tool-free LC connections for HPLC, UHPLC, and low-flow UHPLC systems

nanoViper fitting

Double nanoViper (DNV) column

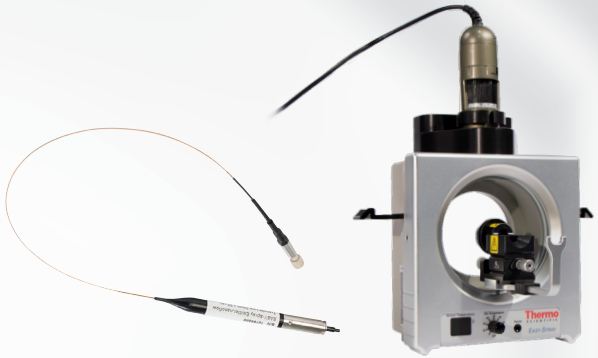
Connect µPAC HPLC column, nanoLC to EASY-Spray ion source

Connecting the µPAC HPLC column nano to Vanquish Neo UHPLC system and EASY-Spray ion source

Vanquish Neo UHPLC system

Steps required to make the connections

- Connect the transfer line coming from the Vanquish Neo UHPLC System autosampler valve to the pre-fitted HPLC column inlet union (side A), tighten by hand
- Remove the outlet union from the µPAC column (side B) using two spanners (3/16" opening)
- Connect the 1/32" outlet fitting of the µPAC column to the inlet union of the µPAC compatible EASY-Spray emitter first by hand and finally using a spanner with 3/16" opening, turning clockwise 1/8th of turn
- **Ground the column*** on the outlet union using the grounding cable



EASY-Spray ion source

Part name	Part number
20 µm I.D. × 550 mm nanoViper capillary	6250.5260
µPAC HPLC column, nanoLC—includes inlet reducing union, outlet union, grounding cable	All µPAC HPLC columns, nanoLC
µPAC compatible EASY-Spray emitter	EMI-easysprayB

** For suitable grounding points, see **Column pressure and grounding***

Connect µPAC HPLC column, nanoLC to Nanospray Flex ion source

µPAC Flex iON Connect ESI-MS interface

Connecting the µPAC HPLC column, nanoLC to Vanquish Neo UHPLC system and Nanospray Flex ion source using the µPAC Flex iON Connect ESI-MS interface

Vanquish Neo UHPLC system

Steps required to make the connections

- Connect the transfer line coming from the Vanquish Neo UHPLC system autosampler valve to the pre-fitted µPAC HPLC column inlet union (side A), tighten by hand
- Remove the outlet union from the µPAC column (side B) using two spanners (3/16” opening)
- The µPAC Flex iON Connect **ESI-MS interface** consists of a docking unit (lower part) and spray unit (upper part)
- Docking unit: slide over the rod and connect HV and **grounding cables***
- Spray unit: connect the outlet fitting of the µPAC column to the inlet union of the µPAC Flex iON Connect **ESI-MS interface** by hand and finally using a spanner with 3/16" opening, turning clockwise 1/8th of turn. Connect emitter with PEEK fitting to outlet of Flex iON Connect **ESI-MS interface**
- Connect the spray unit to the docking unit

Nanospray Flex ion source

Part name	Part number
20 µm I.D. × 550 mm nanoViper capillary	6250.5260
µPAC HPLC column, column, nanoLC—includes inlet reducing union, outlet union, grounding cable	All µPAC HPLC columns, nanoLC
µPAC Flex iON Connect ESI-MS interface	EMI-flexionB

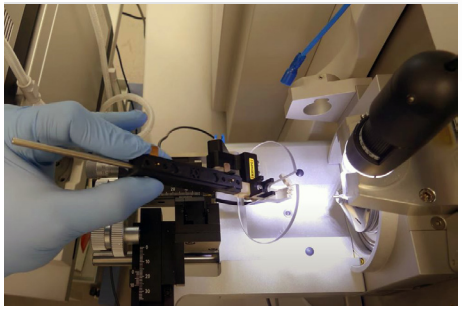
** For suitable grounding points, see **Column pressure and grounding***

Position µPAC Flex iON Connect Emitter in the Nanospray Flex ion source

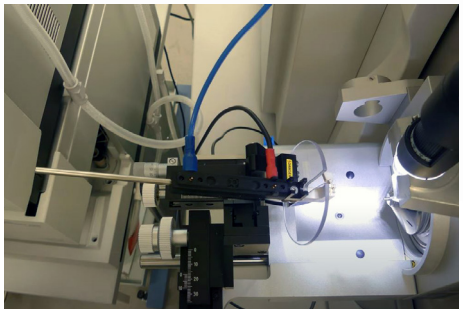
Steps required to optimize the emitter position in the Nanospray Flex ion source

- Place the mass spectrometer in standby
- Retract the DirectJunction™ adaptor on the sliding rails (A)
- Slide the docking unit (lower part) of the **µPAC** Flex iON Connect **ESI-MS interface** onto the rod (B)
- Connect the **grounding cable*** to the widest contact point and to the grounding point on the NanoSpray Flex ion source (C)
- Attach the red high voltage cable to the electrical socket at the bottom of the NanoSpray Flex ion source and the other end to the smallest contact point of the docking unit
- Stop the LC flow
- Connect the µPAC column outlet fitting to the inlet of the spray unit (upper part) of the µPAC Flex iON Connect **ESI-MS interface** by hand and finally using a spanner with 3/16" opening, turning clockwise 1/8th of turn
- Connect a 360 µm O.D. emitter using the one-piece PEEK fitting to the mass spectrometer facing side of the spray unit
- Connect the spray unit to the docking unit (magnets)
- Set flow and observe a liquid drop on the emitter
- Place the mass spectrometer in operate
- Adjust the position of the emitter using the XYZ-manipulator (D)

A

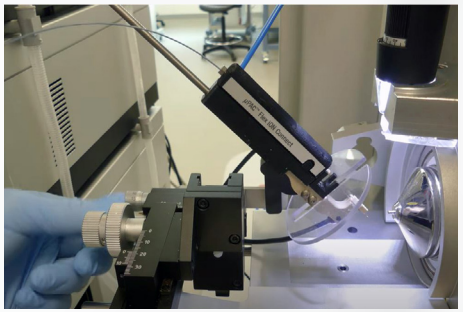


B



C

D



** For suitable grounding points, see **Column pressure and grounding***

Connect µPAC HPLC column nano to Nanospray Flex ion source

Liquid junction using a µPAC™ Flex iON Connect Emitter

Connecting the µPAC HPLC column nano to Vanquish Neo UHPLC system and NanoSpray Flex ion source using a metal emitter

Vanquish Neo UHPLC system

Steps required to make the connections

- Connect the transfer line coming from the Vanquish Neo UHPLC system autosampler valve to the pre-fitted µPAC HPLC column inlet union (side A), tighten by hand
- Connect a fused silica tubing to the outlet union of the µPAC column (side B) using a one-piece PEEK fitting
- Connect the other end of the fused silica tubing to the red PEEK union and using a sleeve connect the stainless-steel emitter on the other side of the PEEK union
- **Ground*** this outlet union using the grounding cable

Nanospray Flex ion source

Part name	Part number
20 µm I.D. × 550 mm nanoViper capillary	6250.5260
µPAC HPLC column, nanoLC—includes inlet reducing union, outlet union, grounding cable and one-piece PEEK fitting	All µPAC HPLC columns, nanoLC
Fused silica capillary 360 µm O.D. 20 µm I.D.	(IDEX) FS-120
Red PEEK union of 1/32" O.D.	(IDEX) P-771
Sleeves for connecting OD 280 µm capillary to unions	SC903
Metal emitter	ES542

** For suitable grounding points, see **Column pressure and grounding***

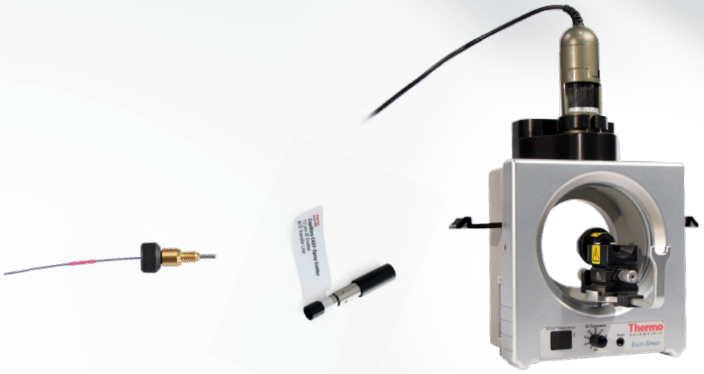
Connect µPAC HPLC column CapLC to EASY-Spray ion source

Connecting the µPAC HPLC column CapLC to Vanquish Neo UHPLC system and EASY-Spray ion source

Vanquish Neo UHPLC system

Steps required to make the connections

- Connect a nanoViper transfer line coming from the Vanquish Neo UHPLC system autosampler valve to the µPAC HPLC column inlet union (side A), tighten by hand
- Connect a nanoViper transfer line from the µPAC column outlet (side B) to a EASY-Spray bullet emitter
- **Ground*** the µPAC HPLC column using the grounding cable on the outlet union of the µPAC column
- Align the EASY-Spray emitter in the EASY-Spray ion source

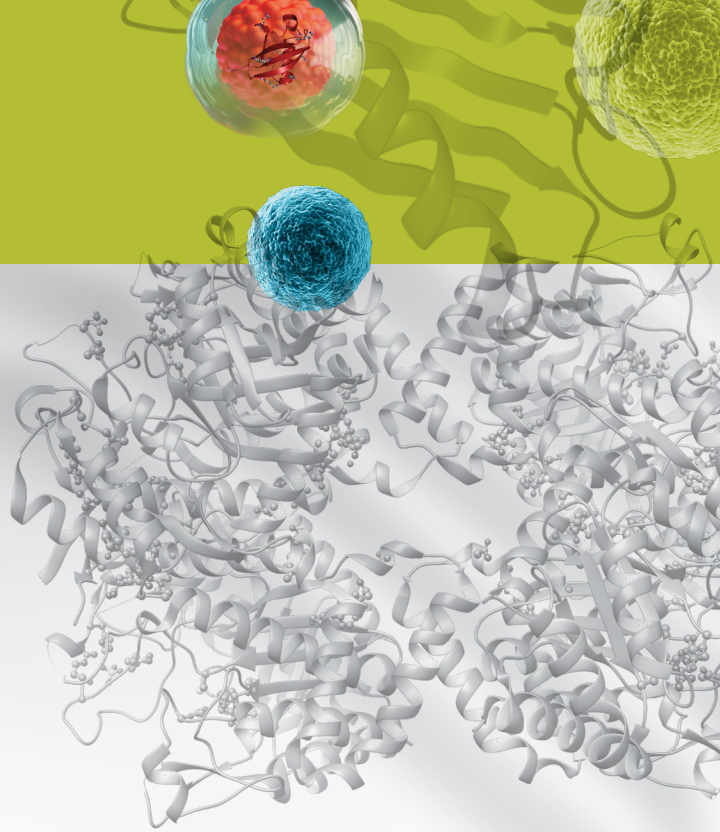


EASY-Spray ion source

Part name	Part number
2 × 50 µm I.D. × 550 mm nanoViper capillary	6250.5560
µPAC HPLC column, CapLC—includes grounding cable	All µPAC HPLC columns, CapLC
Thermo Scientific™ EASY-Spray™ Capillary Emitters	ES992

** For suitable grounding points, see **Column pressure and grounding***

Column equilibration



System and column equilibration

- 1.5 column volumes are recommended for good equilibration of the column. Equilibration can be performed at higher flow rates to be more time efficient.
- Tip: Equilibration protocols are available on the Vanquish Neo UHPLC instrument software

Product	Column void volume (µL)	Equilibration volume (µL)	Max. flowrate (µL/min)
µPAC HPLC Column, 50 cm NanoLC	3	4.5	1.5
µPAC HPLC Column, 200 cm NanoLC	9	13.5	1.0
µPAC HPLC Column, 50 cm CapLC	10	15	10.0

Column pressure and grounding

Column pressure

- Maximum pressure of the µPAC HPLC columns is 350 bar. Exceeding this value will cause irreversible damage to column.
- Ensure this pressure limit is set in all configuration and method files on the LC instrument.

350 Bar
5000 psi

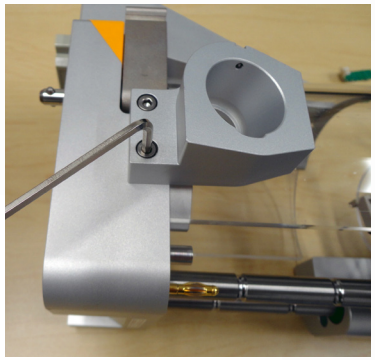
Column grounding

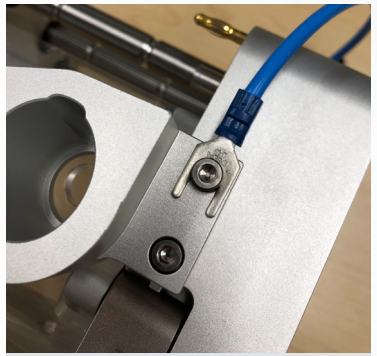
- A µPAC column is mainly comprised of silicon which is a semi-conductor. The high voltage applied by the mass spectrometer can have a dramatic impact on the chromatographic behavior if this voltage is not shunted to ground.
- Ground the µPAC column at the outlet union using the including grounding cable (A).
- Suitable grounding points include for example the LC chassis (B), or designated grounding point on the mass spectrometer source as displayed on the pictures (C and D).

A

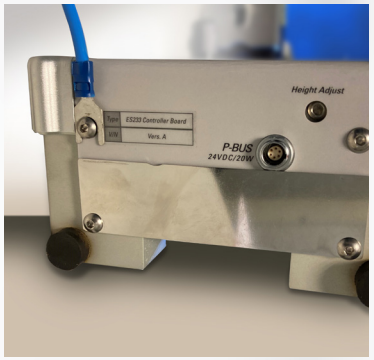
B LC chassis

C Nanospray Flex ion source





D EASY-Spray ion source



Learn more at **thermofisher.com/lowflowHPLCcolumns**