ionopticks

Aurora 3™
GENERATION

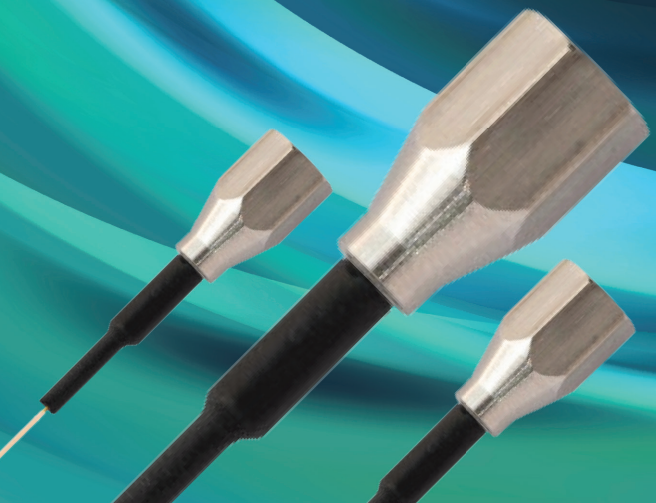
User Guide

Aurora Series™

UHPLC packed emitter columns

Recommended guidelines for optimal setup and operation of Aurora Series columns:
Part No. AUR3-25075C18 and AUR3-25075C18-CSI
Part No. AUR3-15075C18 and AUR3-15075C18-CSI
Part No. AUR3-50150C18 and AUR3-50150C18-CSI
Part No. 5CMNFSH1 - 5cm AUR Nanospray Flex housing

For more information, visit www.ionopticks.com



Index.

ionopticks

Innovative Design. Transforming Proteomics.

Our columns are differentiated by two key technological advances: a unique packed emitter design that enables maximum mobile phase velocity with no post-column dead volume; and our own nanoZero® technology that provides user friendly 'plug and play' connections with true zero pre-column dead volume. Together, these features combine to maximise chromatographic efficiency and dramatically enhance performance, providing a best in class solution for peptide and metabolite LC-MS separations.

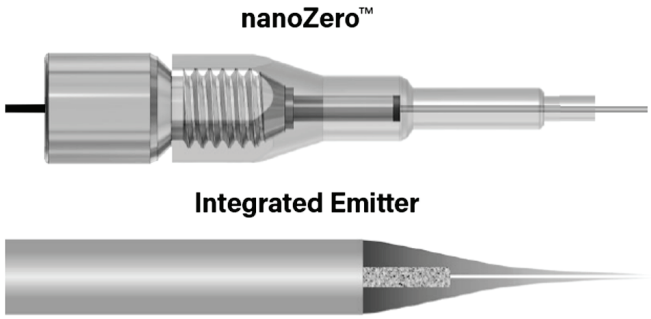
Product Features

- **Integrated emitter with zero post-column dead volume.**
- **Pre-fitted with nanoZero® to provide a zero-dead-volume female union between a nanoViper™ Fingertight fitting (or equivalent) and the Aurora series column.**
- **nanoZero® fitting incorporates a 10-32 internal thread for easy installation.**
- **Designed to withstand ultra-high-performance LC (UHPLC) backpressures of >1700 bar.**
- **nanoZero® is electrically conductive to allow a voltage connection to the entrance of the column.**

Performance guarantee

Our columns are subjected to rigorous quality control procedures under the direct eye of our Senior Scientists. All IonOpticks products are covered by our 100% performance guarantee. Any item not meeting our high-performance expectations due to manufacturing defects will be replaced without charge to the customer.

Please contact us at support@ionopticks.com if you have any concerns relating to your column. Our terms and conditions are provided with every quote, but we want to make sure you are receiving a quality product every time so please do not hesitate to get in touch with any feedback or concerns.



Compatibility.

Aurora columns are compatible with a wide range of LC-MS systems. Compatibility is not limited to equipment presented in this guide. Please contact support@ionopticks.com for enquiries regarding instrument compatibility.

UHPLC	BRUKER	THERMO SCIENTIFIC	WATERS
	nanoElute	Dionex UHPLC systems	nanoAcquity
		Easy-nLC 1000/1200	M-Class
		Vanquish Neo	

Mass Spectrometers	BRUKER	THERMO SCIENTIFIC
	timsTOF Pro	Fusion Eclipse (+/- FAIMS)
	timsTOF Pro 2	Exploris 480 / 240 / 120 (+/- FAIMS)
FITTINGS	timsTOF SCP	Orbitrap Fusion
nanoViper	timsTOF fleX	Orbitrap Fusion Lumos
	maXis II ETD	Orbitrap Elite
COLUMN OVENS	Impact II	LTQ Orbitrap XL
Sonation	Compact	Q-Exactive
Bruker Column Toaster	Amazon	Q-Exactive HF

Product Specifications.

Column format:	Analytical column	**Pore size**	120Å	**Particle size**	1.7µm
Column type:	Reversed-phase	**Max pressure**	>1700 bar	**pH stability**	1-8
For use with:	UHPLC	**Temp limits**	60°C (low pH)	**Stationary phase**	C18

Installation of Aurora Series columns.

Setup of UHPLC systems

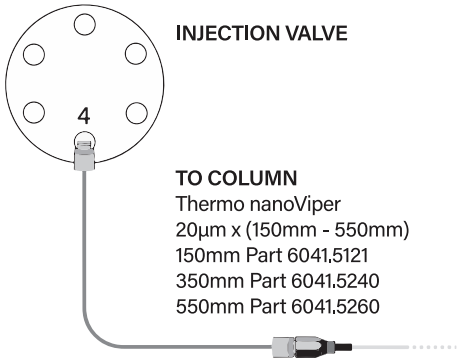
We recommend operation of Aurora series columns in a one column mode (direct injection). Please refer to manufacturers guidelines for operating your UHPLC in a one column mode.

Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety goggles meeting AS/NZS 1336 requirements or equivalent. Puncture and chemical-resistant gloves should also be worn at all times.

Fitting the column to your UHPLC system.

THERMO SCIENTIFIC

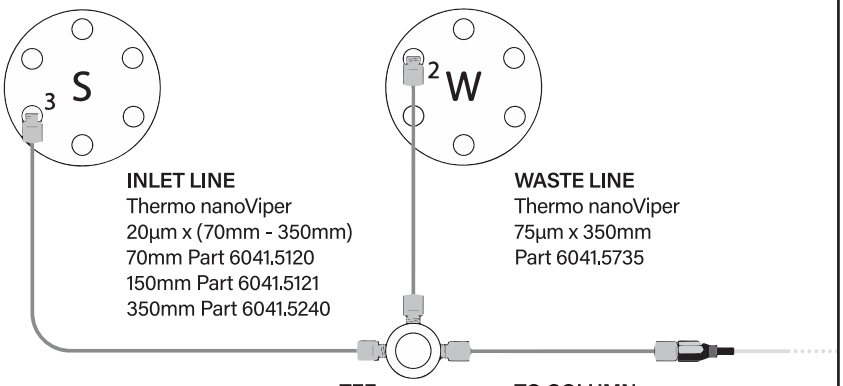
Dionex UltiMate 3000 RSLC



INJECTION VALVE

TO COLUMN
Thermo nanoViper
20µm x (150mm - 550mm)
150mm Part 6041.5121
350mm Part 6041.5240
550mm Part 6041.5260

EASY-nLC 1000/1200



INLET LINE
Thermo nanoViper
20µm x (70mm - 350mm)
70mm Part 6041.5120
150mm Part 6041.5121
350mm Part 6041.5240

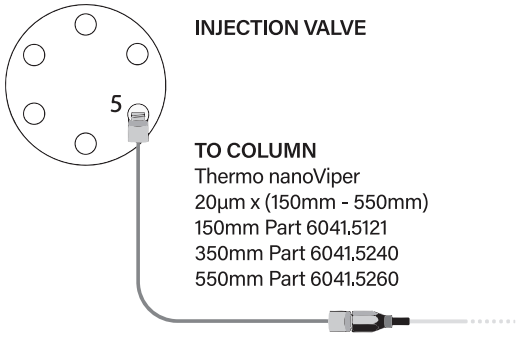
WASTE LINE
Thermo nanoViper
75µm x 350mm
Part 6041.5735

TEE
Thermo
Part SC901

TO COLUMN
Thermo nanoViper
20µm x (150mm - 550mm)
150mm Part 6041.5121
350mm Part 6041.5240
550mm Part 6041.5260

BRUKER

nanoElute

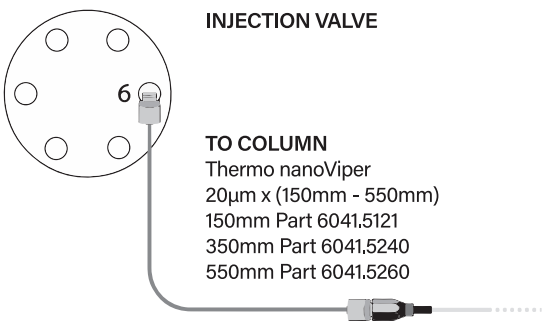


INJECTION VALVE

TO COLUMN
Thermo nanoViper
20µm x (150mm - 550mm)
150mm Part 6041.5121
350mm Part 6041.5240
550mm Part 6041.5260

WATERS

nanoACQUITY and M-Class



INJECTION VALVE

TO COLUMN
Thermo nanoViper
20µm x (150mm - 550mm)
150mm Part 6041.5121
350mm Part 6041.5240
550mm Part 6041.5260

For Bruker CaptiveSpray source.

For optimal usage, please adhere to the following instructions:

CAUTION Handling of fused-silica or glass tubing and tips can result in serious personal injury, including eye and skin injury. Use safety goggles meeting AS/NZS 1336 requirements or equivalent. Puncture and chemical-resistant gloves should also be worn at all times.

1 **Unscrew CSI probe from CSI housing**

2 **Disassemble rear metal housing of CSI probe insert (2 screws)**

3

Carefully remove Aurora CSI protective cap.

4

Insert Aurora CSI fitting into CSI probe. Insert using a twisting motion to prevent pinching of rubber seal.

5

Remove the protective plug from the nanoZero® union.

6

6.1

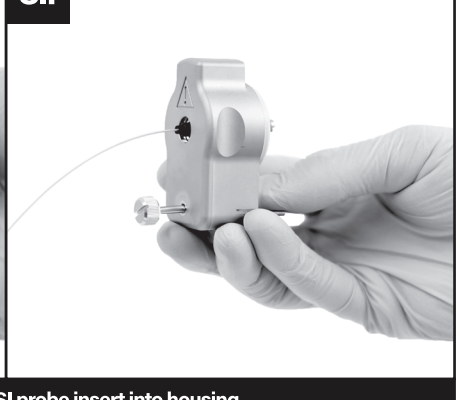
Feed Aurora column through the rear metal housing and reassemble CSI probe (2 screws), ensuring rubber seal is in place between metal housing and Aurora CSI fitting.

7

Feed Aurora column with nanoZero® fitting through CSI probe housing.

8

8.1



Feed column through CSI housing and screw CSI probe insert into housing.

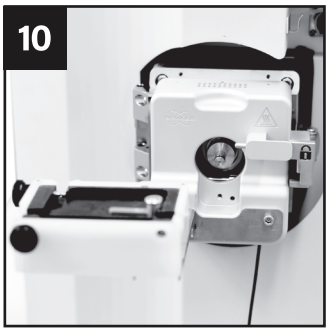
9

The CSI housing is now ready to connect to your LC-MS system.

For Bruker CaptiveSpray source

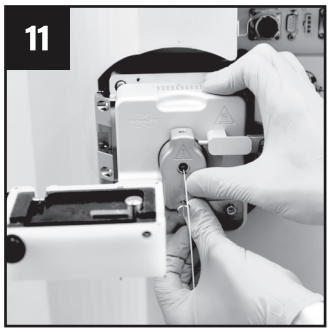
if using Bruker Column Toaster

10



Retract the column heater from the CaptiveSpray source

11



Insert the CSI Housing into the source, press with a small amount of pressure and tighten the screw until finger tight.

12

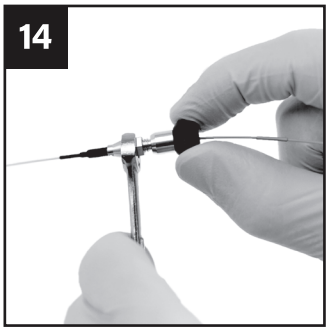
Using a gloved finger, block the air inlet and monitor the Fore pressure. The Fore vacuum needs to drop to below 3e-01 mbar within 10 seconds to be considered usable.

If not sealed, try refitting before replacing rubber seals (Refer Bruker CSI troubleshooting guide).

13

Move the column heater in front of the CaptiveSpray source.

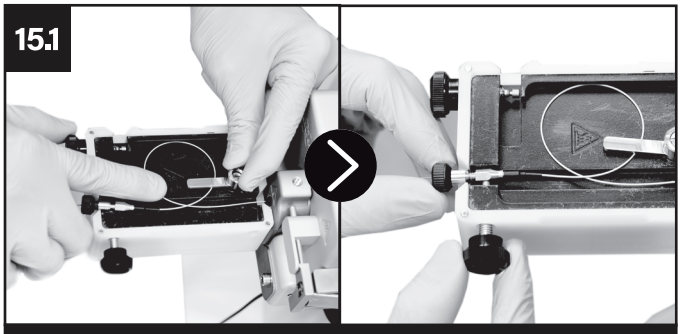
14



Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop.

Operation of the column with an under tight fitting can result in a fall in performance that can not be recovered with additional tightening.

15.1

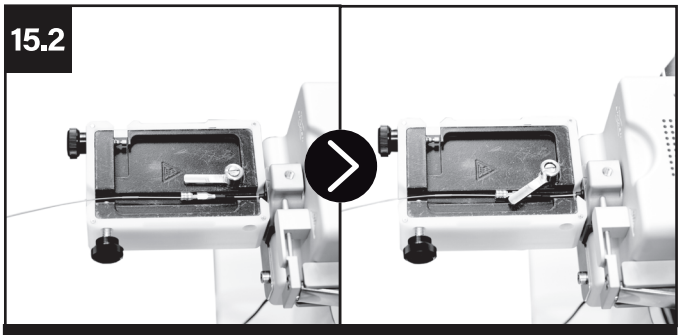


For 15cm and 25cm Aurora columns

Curl the column inside the heating plate and hold in position by placing under the holding arm.

Tighten the earth screw onto the nanoZero® to ensure that the nanoZero® is grounded and held in position.

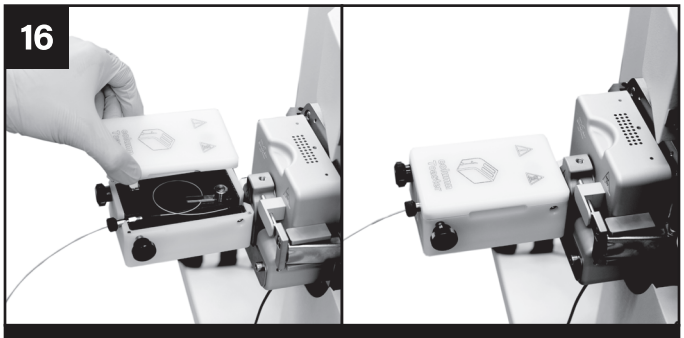
15.2



For 5cm RAPID Aurora columns

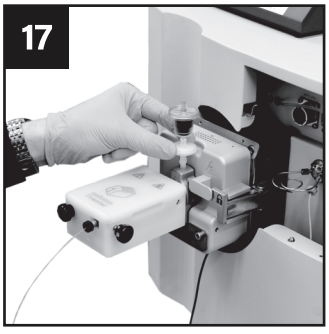
Place column directly into the heater recess. Hold in position by placing the holding arm onto the nanoZero®. This requires a replacement metal holding arm (supplied by IonOpticks) to allow an earth connection with the nanoZero®.

16



Place lid on heater

17



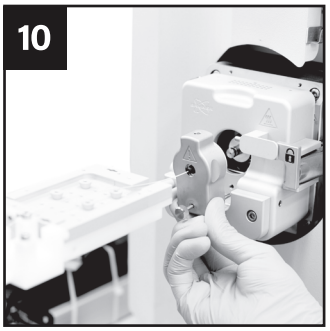
Install filter into the CSI housing air inlet.

The column is now installed in the heater and ready for operation.

For Bruker CaptiveSpray source

if using Sonation Column Oven (PRSO-V1 and PRSO-V2)

10

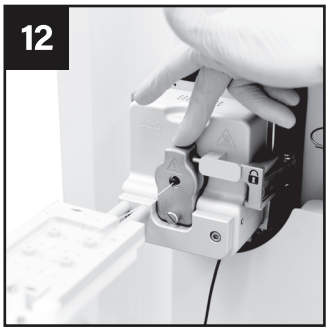


Retract the column heater from the CaptiveSpray source

11

Press the CSI Housing with a small amount of pressure and tighten the screw until finger tight.

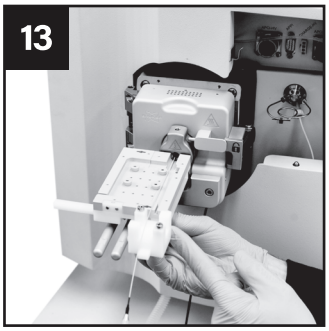
12



Using a gloved finger, block the air inlet and monitor the Fore pressure. The Fore vacuum needs to drop to below 3e-01 mbar within 10 seconds to be considered usable.

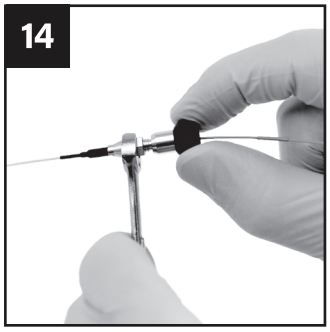
If not sealed, try refitting before replacing rubber seals (Refer Bruker CSI troubleshooting guide).

13



Move the column heater in front of the CaptiveSpray source.

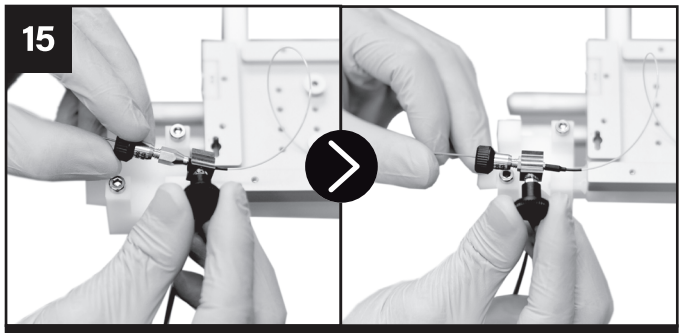
14



Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop.

Operation of the column with an under tight fitting can result in a fall in performance that can not be recovered with additional tightening.

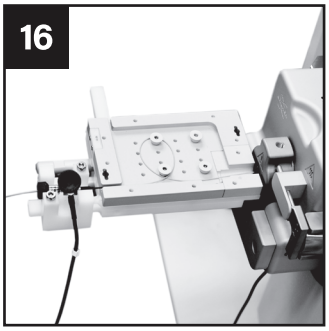
15



Earthing

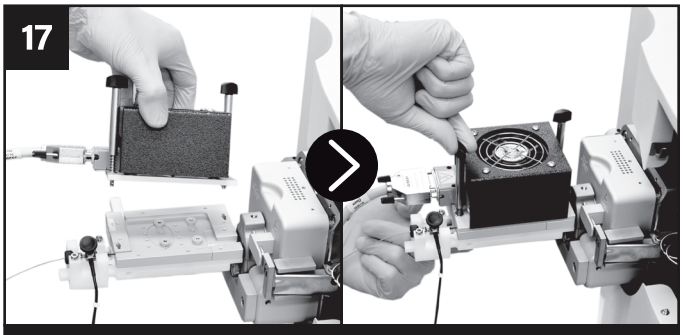
Place the earth cable clip over the thin section of the nanoZero® and slide over the hexagonal section to make a firm connection. If the clip feels loose, remove the clip, pinch in the clip arms and repeat the process.

16



Curl the column inside the heating plate and place the nanoZero® and earth clip into the plastic holder.

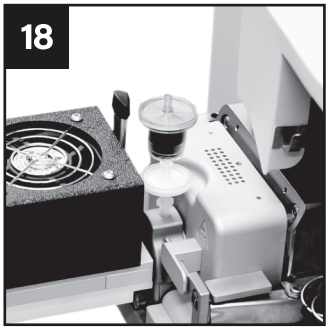
17



Closing the heater

Place top plate on heater and secure in position using the press pins.
Be careful to avoid pinching the column during this process.

18



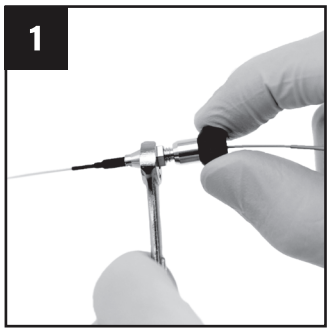
Install filter into the CSI housing air inlet.

The column is now installed in the heater and ready for operation.

For Thermo Scientific Nanospray Flex

with Sonation Column Oven (PRSO-V1 and PRSO-V2)

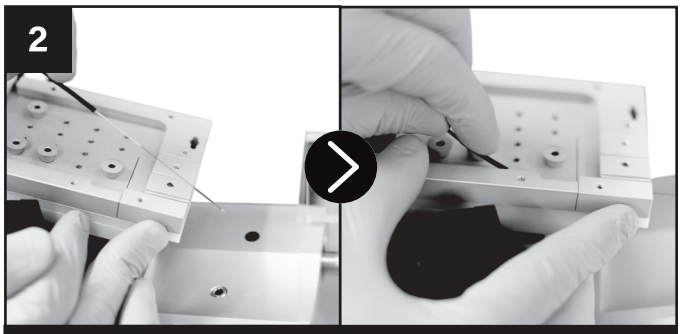
1



Holding the nanoZero® with a spanner, tighten the nanoViper fitting finger tight until you reach a firm stop.

Operation of the column with an under tight fitting can result in a fall in performance that can not be recovered with additional tightening.

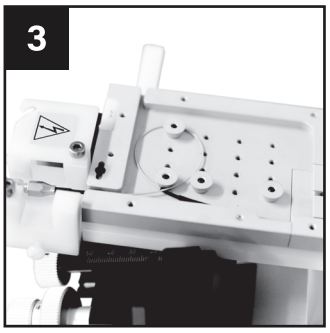
2



Preparing the column

Carefully slide protective sheath backwards towards the nanoZero® fitting to expose the emitter tip. Press the holding clamp on the heater open and place the column into the heater. The column emitter should extend 15-20mm beyond the heating plate.

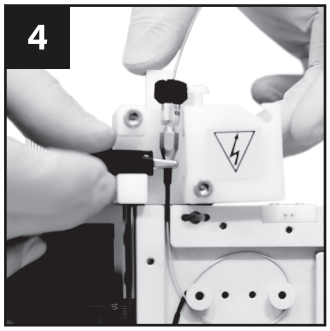
3



Curl the column inside the heating plate.

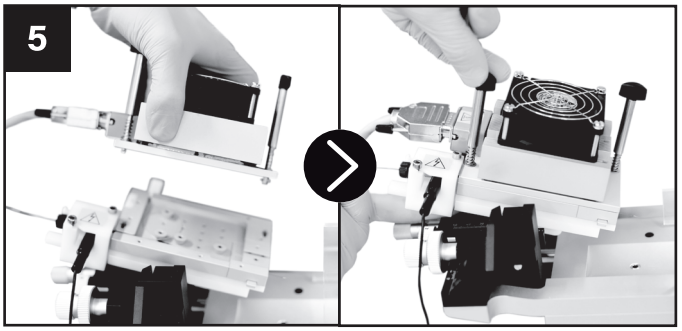
You will need:
1 x HVCABLE01
for connection of the source high-voltage to the nanoZero® fitting.

4



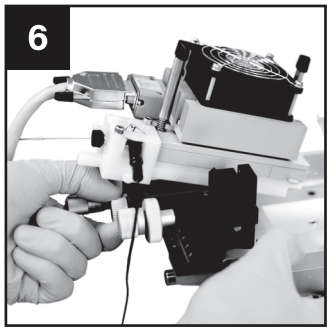
Connect the HVCABLE01 to the nanoZero as shown.

5



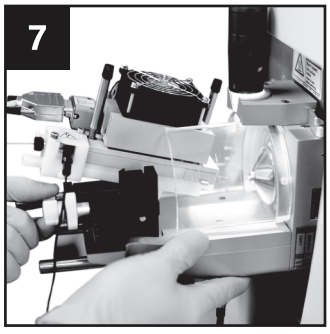
Place top plate on heater and secure in position using the press pins. Be careful to avoid pinching the column during this process.

6

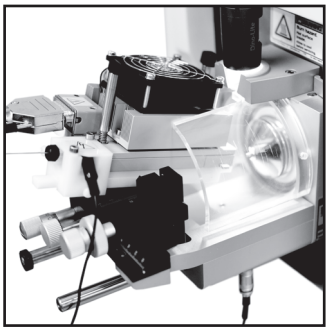


Ensure that the stage is fully retracted in the z-axis before moving the source towards the mass spectrometer.

7



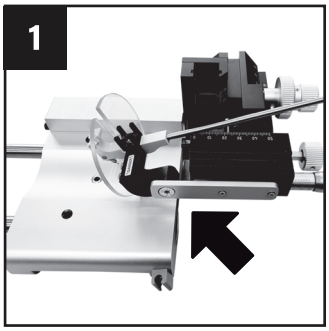
Slide the source into position and adjust the x, y and z axis on the stage to ensure a good emitter position. The emitter should be 3-5mm from the ion transfer capillary.



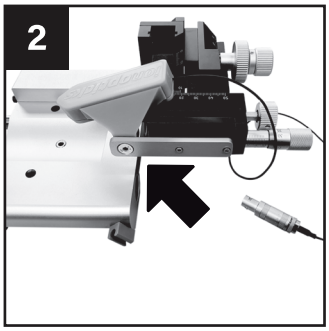
The column is now installed in the heater and ready for operation.

For Thermo Scientific Nanospray Flex

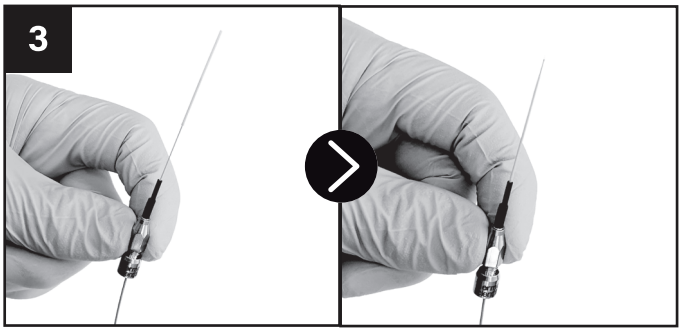
with 5cm RAPID column housing (Part No. 5CMNFSH1)



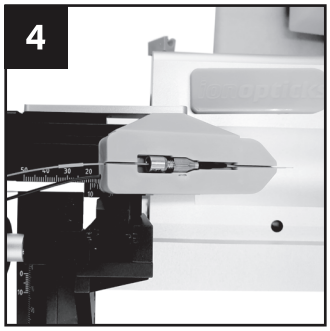
Unscrew and remove the existing fastening bolt, marked by the arrow, from the source.



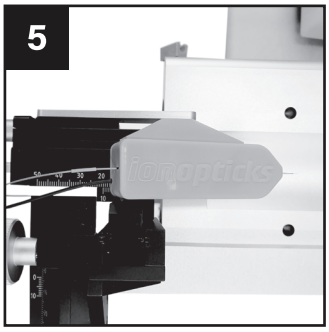
Attach the housing using the supplied bolt and nut. Attach the source to the mass spectrometer and plug in voltage connection into the source.



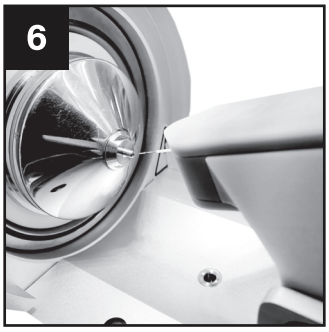
Fit the nanoViper into the Aurora column nanoZero® fitting. Expose the emitter tip by sliding the protective sheath towards the nanoZero®.



Install Aurora column and nanoViper line into the housing as shown.



Place lid on housing.



Manoeuvre emitter tip into the desired position using the source stage controls. The emitter should be 3-5mm from the ion transfer capillary.

The column is now installed and ready for operation.

Operation of Aurora Series columns.

Aurora series columns initial operation

Once the column is connected to your UHPLC system and is placed inside the source heater or housing, begin operation using 70% buffer B at a flow rate of 400nl/min for around 10 minutes or until the pressure is stable for several minutes. Voltage should be applied once the mobile phase reaches the emitter tip. It is recommended that at least one gradient is run without sample injection before assessment of column performance using standards.

Standby and Idle conditions

To optimise column lifetime and performance, it is recommended that the instrument continues to run at the desired operating pressure and ideally continues to run blank samples using mobile phase gradients typical of normal operation. Spray voltages should be maintained during these operations.

Extended periods of time at isocratic flow will reduce column performance. Column performance can be recovered by running a blank gradient without sample injection.

Removal from a UHPLC

If possible, do not remove the column from a UHPLC system, however, removal is sometimes required. For the removal procedure, run 80% B for 5min at operating flow rates before reducing flow to 0.002 µl/min for 10min or until the back pressure has stabilised below 10 bar. Set MS system into standby mode. The nanoViper can now be removed from the nanoZero®. For removal of the column for extended periods of time we recommend placing 30µl of methanol in the nanoZero® and screwing in the plug supplied during transport.

Removal of the nanoViper from the nanoZero® under high back pressure conditions can damage the stationary phase bed and lead to blockages and poor chromatographic performance.

The removal procedure can lead to fouling of the emitter tip and poor column performance. The IonOpticks replacement guarantee is not valid if a column has been removed from a UHPLC once in operation.

Recommended Buffer compositions:

Buffer A 99.9% MilliQ Water, 0.1% formic acid
Buffer B 99.9% Acetonitrile, 0.1% formic acid

Column Volumes

25cm X 75µm	1.1µl
15cm X 75µm	0.66µl
5cm X 150µm	0.88µl

Column Temperature

The recommended operating temperature for Aurora columns is 50°C. The maximum operating temperature is 60°C.

Column equilibration

Before each run the column should be requilibrated using a minimum of 4 column volumes of 100% buffer A.

Sample Loading

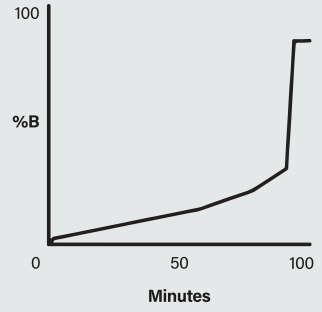
Samples should be loaded onto the column in 100% Buffer A. Samples loaded on to the column should be de-salted and should not contain any contaminants (salts, detergents, solid particles, etc). Loading contaminated samples onto the column may disrupt solvent flow or foul the emitter tip leading to a loss of performance.

Aurora™ ULTIMATE

25cm Column
90min gradient

Time (min)	Composition (% Buffer B)	Flow Rate (µl/min)
0	0	0.400
1	2	0.400
2	5	0.400
57	17	0.400
78	25	0.400
91	35	0.400
94	85	0.400
101	85	0.400

Example gradient:

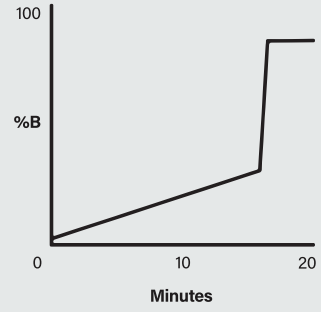


Aurora™ ELITE

15cm Column
17min gradient

Time (min)	Composition (% Buffer B)	Flow Rate (µl/min)
0	5	0.400
17	30	0.400
17.5	85	0.400
20	85	0.400

Example gradient:

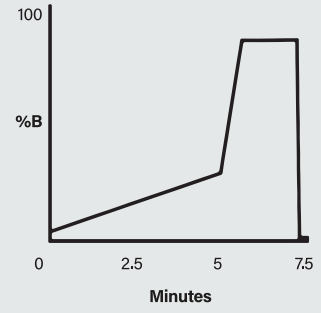


Aurora™ RAPID

5cm Column
5min gradient

Time (min)	Composition (% Buffer B)	Flow Rate (µl/min)
0	5	2
5	34	2
5.5	85	2
6.5	85	2
6.8	0	2
7.5	0	2

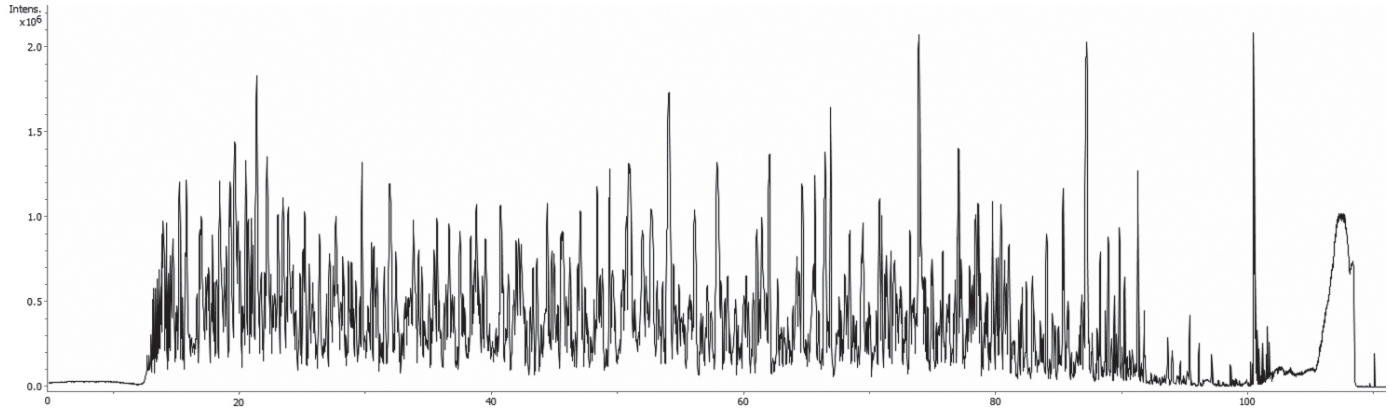
Example gradient:



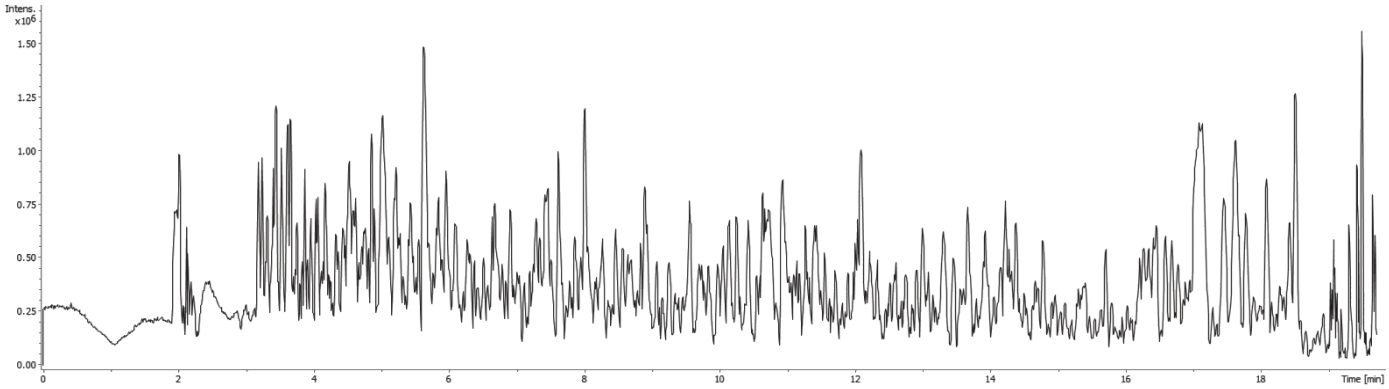
Example data.

The BPC plots shown represent typical results (shown for Hela tryptic digest).

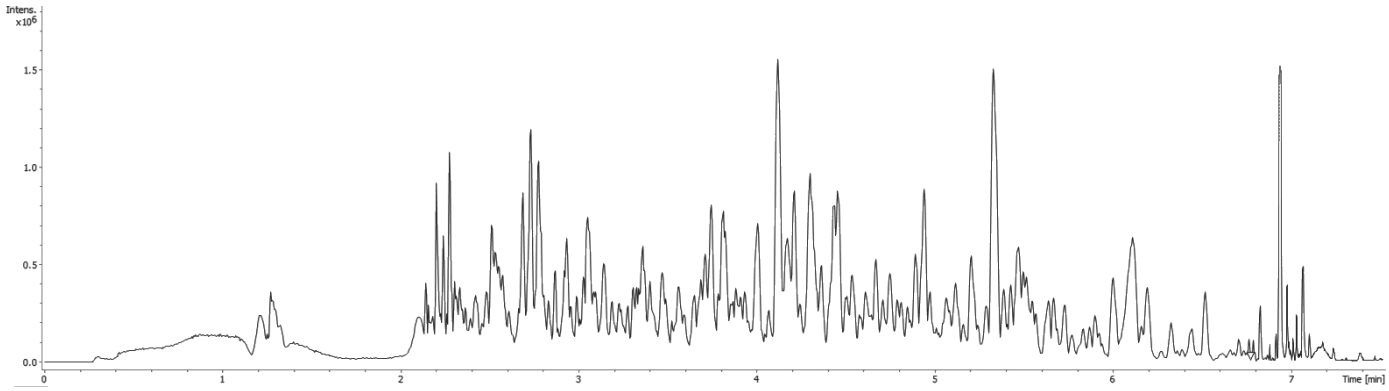
25cm Aurora Ultimate™ Column, 90min Gradient (200ng injection)



15cm Aurora Elite™ Column, 17min Gradient (200ng injection)



5cm Aurora Rapid™ Column, 5min Gradient (100ng injection)



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