ionopticks

GENERATION 4

Aurora Series® User Guide

UHPLC packed emitter columns

IonOpticks accessories featured in these guidelines:

IonOpticks XT Nanospray Flex Adapter (XTFLXAD01)

IonOpticks XS Installation Toolkit (XSITK01)

IonOpticks Heater Controller (IOHEATCON01)

HeatSync™ Controller (HSHEATCON02)

HeatSync™ Column Heater (HSCOLHTR01)

HeatSync™ Rapid 5 cm Column Housing (HS5CMH01)

HeatSync™ Regulator (HS5CMREG01)

Aurora Rapid® 5x75

HeatSync™ Column Heater Extension Cable (EXTCABLE03)

IonOpticks 30 mm Grounding Arm (GNDARM01)

IonOpticks 55 mm Grounding Arm (GNDARM02)

IonOpticks 50 cm High-voltage Cable (HVCABLE01)

IonOpticks 30 cm Earth Cable (HVCABLE02)

Recommended guidelines for optimal setup and operation of Aurora Series columns:

75 µm nano flow range 150 µm capillary flow range Aurora Frontier® 60x75 Aurora Ultimate® 25x150 AUR4-250150C18 AUR4-60075C18 AUR4-60075C18-CSI AUR4-250150C18-CSI AUR4-60075C18-XT AUR4-250150C18-XT AUR4-250150C18-XS Aurora Ultimate® 25x75 AUR4-25075C18 AUR4-25075C18-CSI AUR4-150150C18 Aurora Flite® 15x150 AUR4-25075C18-XT AUR4-150150C18-CSI AUR4-25075C18-XS AUR4-150150C18-XT AUR4-150150C18-XS Aurora Elite® 15x75 AUR4-15075C18 AUR4-15075C18-CSI Aurora Rapid® 8x150 AUR4-80150C18 AUR4-80150C18-CSI AUR4-15075C18-XT AUR4-15075C18-XS AUR4-8075C18-XT Aurora Rapid® 8x75 AUR4-8075C18 Aurora Rapid® 5x150 AUR4-50150C18 AUR4-8075C18-CSI AUR4-50150C18-CSI AUR4-8075C18-XT

AUR4-5075C18 AUR4-5075C18-CSI



Disclaimer

The use of "we" "us" or "our" in this User Guide are references to IonOpticks Pty Ltd ACN 621 674 459. The information in this User Guide including (without limitation) the recommendations, safety guidelines and product warnings with respect to the use of our products are to be read strictly subject to our terms and conditions which can be located at www.ionopticks.com and the limitations and exclusions of our liability found within those terms. Products should not be used if they appear damaged. We accept no liability for any loss or damage howsoever or wherever arising (including death and/or personal injury) which results from or is connected with the failure by the customer to use our products strictly in accordance with the directions in this User Guide.

Regulatory Compliance

IonOpticks evaluates its products to ensure full compliance with applicable North American and European regulations. IonOpticks columns, used in conjunction with the HeatSync™ Column Heater (HSCOLHTR01), IonOpticks Heater Controller (IOHEATCON01), HeatSync™ Controller (HSHEATCON02) and other accessories, are intended for use in the basic electromagnetic environment as defined in IEC 61326-1. For detailed information on emission level and permissible performance under the electromagnetic immunity conditions, please contact IonOpticks.

IonOpticks products are compliant with the Restriction of Hazardous Substances (RoHS) directive. All heating hardware and supplementary equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at their own expense. Changes or modifications to these products not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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Innovative Design. Transforming Proteomics.

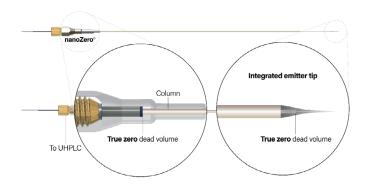
IonOpticks columns are differentiated by two key technological advances: a unique packed emitter design that enables maximum mobile phase velocity with no post-column dead volume; and our own nanoZero® technology that provides user friendly 'plug and play' connections with true zero pre-column dead volume. 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™ finger tight fitting (or equivalent) and the Aurora Series® column, the 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 lonOpticks 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



IonOpticks 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.

Mass Spectrometer Ion Sources

Thermo Scientific	Bruker	SCIEX	Newomics
EASY-Spray	CaptiveSpray	OptiFlow Turbo V	UniESI
Nanospray Flex	CaptiveSpray 2	OptiFlow Pro	DuoESI
	CaptiveSpray Ultra		
	CaptiveSpray Ultra 2		

UHPLC

Thermo Scientific	Bruker	Evosep	Waters
UtiMate series	nanoElute	Evosep One	M-Class
EASY-nLC 1000/1200	nanoElute 2	Evosep Eno	nanoAcquity
Vanquish Neo	proteoElute		

Column Heaters

IonOpticks	Bruker	Sonation
HeatSync™ Column Heater	Column Toaster Gen 1	Sonation Column Oven PRSO-V1
	Column Toaster Gen 2	Sonation Column Oven PRSO-V2

Fittings

- \bullet Thermo Scientific nanoViper $\mbox{^{TM}}$
- Waters ZenFit™

Product Specifications

Column format	Analytical column
Column type	Reversed-phase
For use with	UHPLC
Inner Diameter	75 μm / 150 μm
Pore size	120 Å
Pressure	>1700 bar
Temp. limits	60°C
Particle size	1.7 µm
pH stability	1–8
Stationary phase	C18



Preparing column heating apparatus

4.0 | HSCOLHTR01 IOHEATCON1 HSHEATCON02 HS5CMH01 HS5CMREG01 EXTCABLE03

Setting up the HeatSync™ Controller with HeatSync™ Column Heater



HSHEATCON02 & HSCOLHTR01



Turn on HeatSync™ Controller via the power switch on the back.



Plug in the HeatSync™ Column Heater into Channel 1 or 2.



If using a HeatSync™ Regulator, plug the column heater into the Regulator and then the Regulator into the Controller. A visual confirmation should temporarily display to confirm successful connection. The channel 1 icon should now appear on screen. The same will apply if using channel 2.



If using an extension cable, plug the column heater into the extension cable and then the extension cable into the HeatSync $^{\text{\tiny{TM}}}$ Controller.



Note: Only a single extension cable can be used with the HeatSvnc™ Column Heater. The use of multiple extension cables may cause a failure in the operation of the heater and could damage the Heater and Controller.



Once the cable is plugged in, the screen should illuminate with the current heater temperature.



Note: The actual column temperature may take 10-15 minutes to stabilise after the device shows it has reached the set temperature.



If desired, plug in a second column heater into the free channel and wait for the display to update.



Press the channel button to enter temperature selection mode, indicated by flashing white lights.



Use the rotary dial to select the desired temperature from 30 to 60 degrees C.



Press the channel button again to confirm temperature, or allow the selection mode to time out (30 seconds), indicated by a change to coloured lights showing the status of the controller.



Repeat steps 7-9 as needed to change and set the temperature on the desired channel



Wait for the HeatSync™ Controller to stabilise and turn teal. The HeatSync™ Controller is now setup and ready for use.

For a more in-depth guide including troubleshooting, please see the user manual for the HeatSync™ Controller.



Our product is compliant across multiple territories, as signified by the compliance markings on the product label.

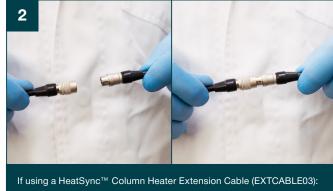


Setting up the IonOpticks Heater Controller with HeatSync™ Column Heater

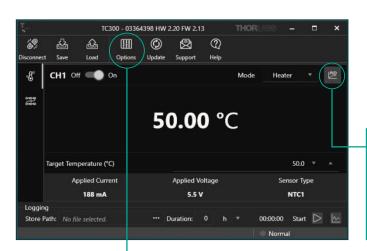


IOHEATCON1 & HSCOLHTR01





Using the USB cable and software supplied on unit, ensure settings on the lonOpticks Heater Controller match these found here:



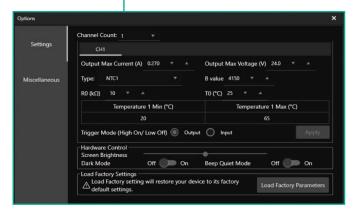
Plug extension cable into Channel 1 of the IonOpticks Heater Controller. Plug cable of the HeatSync™ Column Heater into he receiving port of the extension cable.



Note: Only a single extension cable can be used with the HeatSync[™] Column Heater. The use of multiple extension cables may cause a failure in the operation of the heater and could damage the Heater and Controller.



Ensure PID settings matches the shown configuration.



Use the 'Adjust' dial to set the desired temperature. We recommend using a temperature between 40-60°C. The maximum recommended temperature is 60°C.

The IonOpticks Heater Controller is now set up and ready for use.



Our product is compliant across multiple territories, as signified by the compliance markings on the product label.



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Fitting Aurora Series® columns to Bruker CaptiveSpray ion source

5.1 | Using Aurora Series CSI range with:

Bruker Column Toaster 1st Generation
Sonation Column Oven (PRSO-V1 and PRSO-V2)

Installing Aurora Series® CSI Housing on Bruker CaptiveSpray ion source

1 Unscrew the CSI probe from the CSI housing.

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









Ensuring rubber seal is in place between the metal housing and the Aurora Series CSI fitting.





The CSI housing is now ready to connect to the CaptiveSpray ion source.

Installing Aurora Series® CSI columns on Bruker CaptiveSpray ion source



Using Bruker Column Toaster 1st Generation



Retract the Column Toaster from the CaptiveSpray source.



Insert the CSI housing into the source, pressing with a small amount of pressure, and tighten the screw until finger-tight.



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

(If not sealed, try refitting before replacing rubber seals)

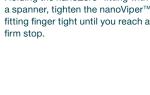


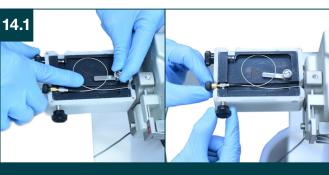
Move the Column Toaster in front of the CaptiveSpray source.





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





For Aurora Frontier, Aurora Ultimate and Aurora Elite 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



For Aurora Rapid 5 cm columns, use 30 mm Grounding Arm (GNDARM01) For Aurora Rapid 8 cm columns use 55 mm Grounding Arm (GNDARM02)

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



Place lid on the Column Toaster.



Install filter in the CaptiveSpray source air inlet.

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

Removal of CSI columns from your system

- Remove the lid of the Column Toaster and remove the column from underneath the hold arm.
- While holding the column gently unscrew the earth screw to release the column.
- Retract the heater back and to the side to access the CSI Housing
- Unscrew the housing and remove from the source.
- Repeat CSI housing installation steps in reverse and carefully place the protective cap back onto the CSI fitting. Press firmly to ensure that the cap is held securely.
- Screw the shipping plug into the nanoZero® union. For removal of the column for extended periods of time we recommend placing 30 ul of methanol in the nanoZero® before installing the shipping plug.

Installing Aurora Series® CSI columns on Bruker CaptiveSpray ion source



Using Sonation Column Oven (PRSO-V1 & PRSO-V2)



Retract the Column Toaster from the CaptiveSpray source.



Insert the CSI housing into the source, pressing with a small amount of pressure, and tighten the screw until finger-tight.



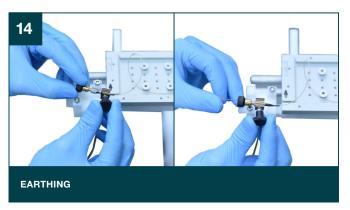
Using a gloved finger, block the air inlet and monitor the fore pressure. The fore vacuum needs to drop below 3e-01 mbar within 10 seconds to be considered usable. (If not sealed, try refitting before replacing rubber seals)



Move the Column Toaster in front of the CaptiveSpray source.



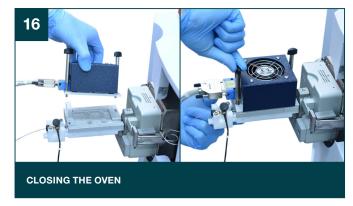
Holding the nanoZero® fitting with a spanner, tighten the nanoViper™ fitting finger tight until you reach a firm stop.



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.



Curl the column inside the column recess, and place the nanoZero® union and earth clip into the plastic holder



Place the heating plate onto the column recess and secure in position using the press pins. **Be careful to void pinching the column during this process.**



Install filter in the CaptiveSpray source air inlet.

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

Removal of CSI columns from your system

- Release the press pins and remove the heating plate from the column recess.
- Carefully uncoil the column from the heating recess and remove the earth cable clip.
- 3. Retract the heater back and to the side to access the CSI Housing
- 4. Unscrew the housing and remove from the source
- Repeat CSI housing installation steps in reverse and carefully place the protective cap back onto the CSI fitting. Press firmly to ensure that the cap is held securely.
- Screw the shipping plug into the nanoZero® union. For removal of the column for extended periods of time we recommend placing 30 ul of methanol in the nanoZero® before installing the shipping plug.

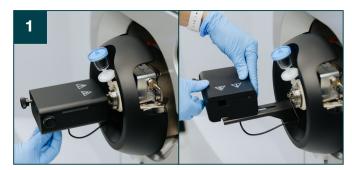
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Fitting Aurora Series® columns to CaptiveSpray 2, CaptiveSpray Ultra, CaptiveSpray Ultra 2 ion sources

5.2 | **Using Aurora Series CSI range with:**Bruker Column Toaster 2nd Generation

Installing Aurora Series® CSI columns on CaptiveSpray 2, CaptiveSpray Ultra and CaptiveSpray Ultra 2 ion sources

Using Bruker Column Toaster 2nd Generation



Loosen the thumb screw underneath the Column Toaster and slide it back to access the emitter lock.



Remove the filter from the CaptiveSpray source air inlet.



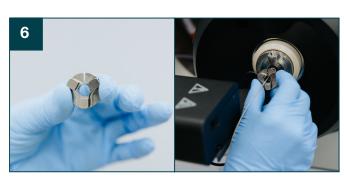
Unscrew the emitter lock anticlockwise.



Carefully remove the CSI Protective cap.



Carefully insert the CSI fitting into the CaptiveSpray source in a single motion (do not twist)



Using the emitter lock, insert the column through the gap and tighten the emitter lock clockwise to a stop.



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.



Install filter into the CaptiveSpray source air inlet.

Installing Aurora Series® CSI columns on CaptiveSpray 2, CaptiveSpray Ultra and CaptiveSpray Ultra 2 ion sources



Using Bruker Column Toaster 2nd Generation



Remove the lid of the Column Toaster and slide it in front of the CaptiveSpray source while placing the column through the recess of the heater



Remove the shipping plug from the nanoZero® union



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



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



For Aurora Rapid 5 cm columns, use 30 mm Grounding Arm (GNDARM01) For Aurora Rapid 8 cm columns use 55 mm Grounding Arm (GNDARM02)

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



Place the lid on Column Toaster.

The column is now installed and

ready for operation.

Removal of CSI columns from your system

- Remove the lid of the Column Toaster and remove the column from underneath the hold arm.
- 2. While holding the column gently unscrew the earth screw to release the column.
- 3. Retract the heater back and to the side to access the emitter lock.
- 4. Unscrew the emitter lock until completely loose and remove it.
- Hold the CSI fitting firmly and pull it towards you in a straight motion.
- Carefully place the protective cap back onto the CSI fitting and press firmly to ensure that the cap is held securely.
- Screw the shipping plug into the nanozero. For removal of the column for extended periods of time we recommend placing 30 ul of methanol in the nanozero before installing the shipping plug.

Note: 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.

Note: 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.



Fitting Aurora Series® columns to Thermo Scientific Nanospray Flex ion source

5.3 | Using Aurora Series range with:

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

Using Aurora Series XT range with:

IonOpticks XT Nanospray Flex Adapter (XTFLXAD01)

HeatSync™ Controller (HSHEATCON02)

HeatSync™ Rapid Housing Kit (HS5RHK01)

IonOpticks Heater Controller (IOHEATCON01)

Fitting Aurora Series® columns to Thermo Scientific Nanospray Flex ion source



Using Sonation Column Oven (PRSO-V1 & PRSO-V2)



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



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.



Curl the column inside the heating plate.

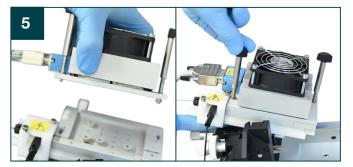


You will need:

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



Connect the high-voltage cable (HVCABLE01) to the nanoZero as shown.



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



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



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.

Installing the XT Nanospray Flex Adapter onto the Nanospray Flex source stage

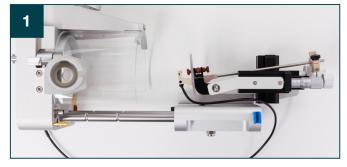




Tools Required

- 1.5 mm hex key
- 2. 2 mm hex key
- 3. 3 mm hex key
- 4. 2 x Nanospray Flex Adapter (NSFA) screw
- 5. Nanospray Flex Adapter

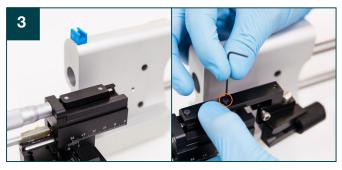




Place Nanospray Flex souce on its side, and slide the stage out.



Using a 2 mm hex key, unscrew the two screws holding the mounting arm in place.



Using gravity to hold the screws in place, drop one NSFA screw into the central hole on the Nanospray Flex Adapter mounting arm. Align the screw with the corresponding position on the Nanospray Flex source and use a 1.5 mm hex key to tighten screw into place.



Secure the remaining NSFA screw in the second hole.



Ensure that the high-voltage cable is positioned at the rear of the source, between the two stage rails.

Note: The high-voltage cable should not pass between the source mount and the stage.



Connect the high-voltage cable to the source



Adjust the angle of the column as desired using the angle adjustment screw, located beside the column mount, with a 3 mm hex key.



Slide the stage into the source mount, and attach the source to your mass spectrometer.



The adapter is now installed and ready for operation.

Installing Aurora Series® XT columns on Thermo Scientific Nanospray Flex ion source

Scan to watch video on our Help Centre



using the XT Nanospray Flex Adapter



Place the mass spectrometer in standby before sliding the stage out of the safety hood.



Retract the Z-axis control knob to prevent the emitter contacting the ion transfer tube when the column is inserted and the source stage slides into the operating position



Remove the shipping plug from the XT column cassette.

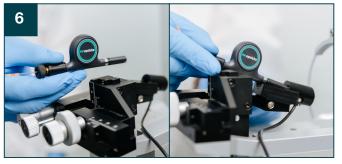


Holding the column cassette firmly, screw a nanoViper™ fitting finger tight into the nanoZero® until you reach a firm stop.



Remove protective cap.

Note: The emitter is not protected by a sheath and protrudes from the end of the cassette. To avoid damaging the emitter, do not twist the cap and ensure that the cap is removed in line with the cassette using a swift motion to completely separate the cap and the cassette.



Slide column cassette into the pre-installed Nanospray Flex Adapter along the column holder until it clicks into place.



Place the column heater onto the fin of the column cassette and press down firmly to ensure the heater is held in position.

8 Impetes

Slide the stage into position and adjust the x,y, and z axis to ensure a good emitter position. The emitter should be around 3 mm from the ion transfer tube.

The column is now installed and ready for operation.

Removal of XT columns from your system

- If possible, do not remove the column from a UHPLC system before the column performance falls and the columns requires replacement, however, removal for instrument servicing and maintenance is sometimes required.
- 2. For removal, run 80% B for 5min at operating flow rates.
- 3. Reduce flow to $0.002\,\mu l/min$ for 10 min or until the back pressure has stabilised below 10 bar.
- 4. Set MS system into standby mode.
- Slide Nanospray Flex stage out of the safety hood before gently removing the column cassette in a backwards motion from the Nanospray Flex Adapter along the column holder.
- The nanoViper can now be removed from the nanoZero®.
- 7. Carefully place the protective cap back onto the cassette and press firmly to ensure that the cap is held securely.
- Screw the shipping plug back into the nanoZero®. For removal of the column for extended periods of time we recommend placing 30µl of methanol in the nanoZero® before installing the shipping plug.

Note: 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.

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

Note: If using FAIMS, the emitter distance to the orifice is normally between 0.5 mm and 3 mm, with the most common distance being 0.5 mm. This is to ensure stable spray of the system.



Fitting Aurora Series® columns Thermo Scientific EASY-Spray ion source

5.4 Using Aurora Series XT range with:

EASY-Spray ion source ES081 and ES082

IonOpticks Heater Controller (IOHEATCON01)

HeatSync™ Controller (HSHEATCON02)

HeatSync™ Rapid Housing Kit (HS5RHK01)

Installing Aurora Series® XT columns on Thermo Scientific EASY-Spray ion source



ES081 and ES082



Remove the shipping plug from the column cassette.



Holding the column cassette firmly, screw a nanoViper fitting finger tight into the nanoZero until you reach a firm stop.



Remove protective cap.

Note: The emitter is not protected by a sheath and protrudes from the end of the cassette. To avoid damaging the emitter, do not twist the cap and ensure that the cap is removed in line with the cassette using a swift motion to completely separate the cap and the cassette.



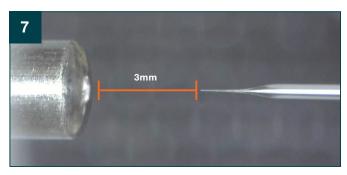
Ensure the Z-axis control knob has been set in the correct position using the Thermo Emitter Positioning Tool. If no tool is available, ensure that the control knob has been retracted to prevent the emitter contacting the ion transfer tube.



Slide column cassette into EASY-Spray source along the column holder until it clicks into place.



Place the column heater onto the fin of the column cassette and press down firmly to ensure that the heater is held in position.



Using the video feed from the source mounted camera, make fine adjustments to the z-axis emitter position as required. The emitter should be around 3 mm from the ion transfer tube. Please follow the instructions from the EASY-Spray Source.

The column is now installed and ready for operation.

Note: If using FAIMS, the emitter distance to the orifice is normally between 0.5 mm and 3 mm, with the most common distance being 0.5 mm. This is to ensure stable spray of the system.

Removal of XT columns from your system

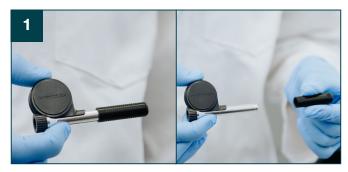
- If possible, do not remove the column from a UHPLC system before the column performance falls and the columns requires replacement, however, removal for instrument servicing and maintenance is sometimes required.
- 2. For removal, run 80% B for 5min at operating flow rates.
- 3. Reduce flow to 0.002 μ l/min for 10 min or until the back pressure has stabilised below 10 bar.
- 4. Set MS system into standby mode.
- 5. Gently remove the XT cassette from the source. The nanoViper can now be removed from the nanoZero®.
- 6. Carefully place the protective cap back onto the cassette and press firmly to ensure that the cap is held securely.
- Screw the shipping plug back into the nanoZero®. For removal of the column for extended periods of time we recommend placing 30µl of methanol in the nanoZero® before installing the shipping plug.

Important: 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.

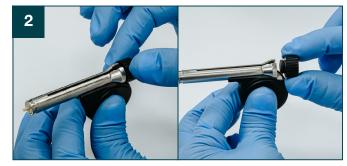
Note: The removal procedure can lead to fouling of the emitter tip and poor column performance. The lonOpticks replacement guarantee is not valid if a column has been removed from a UHPLC once in operation.

Installing an Aurora Rapid® 5 cm column into the HeatSync™ Rapid 5 cm Housing

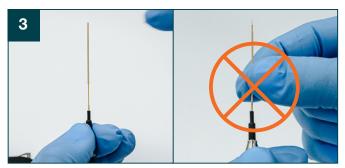
Using the HeatSync™ Rapid 5 cm Column Housing with Thermo Scientific ion sources



Remove cap from the HeatSync™ Rapid 5 cm Column Housing.



Unscrew the restraining fitting at the end of the adapter.



Take the Aurora 5 cm Rapid column and ensure that the beige protective sleeve is protecting the emitter.



Do not retract the beige protective sleeve. Insertion of the column into the housing will retract the sleeve automatically.



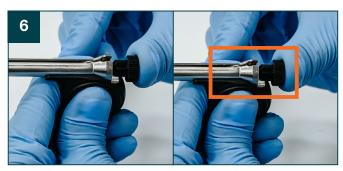
Hold the column with the shipping plug attached, as shown in the image.



Slowly lower the column through the opening and slot. Once the column is in the slot and the end of the protective sleeve is aligned with the small hole at the end of the slot, push it forward. Ensure the NanoZero is positioned correctly — the horizontal edge should be centered within the opening, as shown in the image.

Installing an Aurora Rapid® 5 cm column into the HeatSync™ Rapid 5 cm Housing (cont.)

Using the HeatSync™ Rapid 5 cm Column Housing with Thermo Scientific ion sources



Push the column further forward while maintaining the correct position of the $nanoZero^{\circledast}.$ The emitter will protrude through the adapter.



Remove the shipping plug from the



Screw the restraining fitting into the HeatSync $^{\text{\tiny{TM}}}$ Rapid 5 cm Column Housing to secure the column.



Holding the HeatSync[™] Rapid 5 cm Column Housing firmly, screw a nanoViper $^{\text{\tiny TM}}$ fitting finger tight into the nanoZero $^{\text{\tiny ©}}$ until you reach a firm stop.

The column is now installed and ready for operation.

Using the HeatSync™ Rapid 5 cm Column Housing with Thermo Scientific ion sources

If using the IonOpticks Heater Controller (IOHEATCON01)



Temperature displayed on Heater Controller	Actual column temperature inside housing
40	30
48	35
55	40
62	45
70	50

Set IonOpticks Heater to desired temperature. Refer to the below temperature ∆ table for applicable set points using the HeatSync™ Rapid 5 cm Column Housing



If using the HeatSync™ Controller (HSHEATCON02)



Connect the HeatSync™ Regulator, supplied in the HeatSync™ Rapid Housing Kit, into channel 1 of the HeatSync™ Controller.



4

Plug the HeatSync[™] Column Heater cable into the HeatSync[™] Regulator. '01' will be displayed temporarily to confirm the successful connection of the regulator, if using channel 1. If using channel 2, '02' will be displayed.

If a visual confirmation, or the Channel 1 or 2 icon does not display, this may indicate that the HeatSync $^{\text{TM}}$ Regulator is not correctly connected.

To change the target column temperature, press the desired LED Channel button.



Use the rotary touch dial in a clockwise/counter-clockwise motion to set desired column temperature.



The device has two major colour schemes, represented by an illuminated ring around the rotary touch dial and applicable channel button. Orange will be illuminated when the temperature target is in progress. Teal/Green will be illuminated when the device has reached its temperature target successfully.

Installing the HeatSync™ Rapid 5cm Column Housing into a Thermo Scientific EASY-Spray ion source

Using the IonOpticks Nanospray Flex Adapater

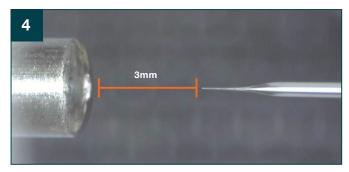
Ensure the Z-axis control knob has been set in the correct position using the Thermo Scientific Emitter Positioning Tool. If no tool is available, ensure that the control knob has been retracted to prevent the emitter contacting the ion transfer tube when the column is inserted.



Spray source along the column holder until it clicks into place.



Place the HeatSync™ Column Heater onto the fin of the housing and press down firmly to ensure that the heater is held in position.



Using the video feed from the source mounted camera, make fine adjustments to the z-axis emitter position as required. The emitter should be around 3 mm from the ion transfer tube. Please follow the instructions from the EASY-Spray Source.

The column is now installed and ready for operation.

Installing the HeatSync™ Rapid 5 cm Column Housing into the Nanospay Flex source





Using the IonOpticks Nanospray Flex Adapater



Place the mass spectrometer in standby before sliding the source stage out of the safety hood.



Retract the z-axis control knob to prevent the emitter contacting the ion transfer tube when the column is inserted and the source stage slides into the operating position.

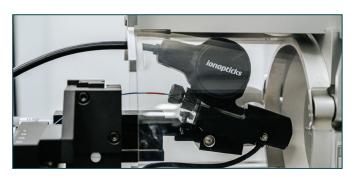


Reference the installation guide for the XT Nanospray Flex Adapter on page 18.





Slide the stage into position and adjust the x, y and z axis to ensure a good emitter position. The emitter should be around 3 mm from the ion transfer tube.



The column is now installed and ready for operation.

Removal of the column from HeatSync™ Rapid 5 cm Column Housing



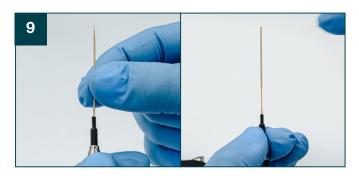
- If possible, do not remove the column from a UHPLC system before the column performance falls and the column requires replacement. However, removal for instrument servicing and maintenance is sometimes required.
- For the removal procedure, run 80% Buffer B for 5 minutes at operating flow rates.
- Reduce flow to 0.002 μl/min for 10 minutes, or until the back pressure has stabilised below 10 bar.
- 4 Set the mass spectrometer system into standby mode.
- Slide the HeatSync™ Rapid 5 cm Column Housing backwards from the source along the column holder (applicable for both EASY-Spray and Nanospray Flex ion sources).







Once the entire column is visible through the full length of the column opening, carefully lift the column up through the opening, ensuring close attention is paid to the emitter to avoid breakage.



Slide the protective sleeve back over the column to protect the emitter. For removal of the column for extended periods, we recommend placing 30 μl of methanol in the nanoZero $^{\circ}$ union before re-installing the shipping plug.



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



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

Fitting Aurora Series® columns to SCIEX OptiFlow Turbo V, OptiFlow Pro ion sources

5.5 | Using Aurora Series XS range with: lonOpticks XS Installation Toolkit (XSKIT01) SCIEX OptiFlow nanoflow probe

Installing Aurora Series® XS columns into SCIEX OptiFlow Turbo V ion source





Installing the XS Adapter





Insert the retraction end of the XS probe-adjustment tool into the nano probe, aligning the blade with the slot of the metal fitting inside. Turn the tool anticlockwise to reduce the depth of the metal fitting until 3 or 4 threads of PEEK are visible.



Flipping the XS probe-adjustment tool over, insert the depth-setting end of the tool into the nano probe, aligning the two projections with the slot of the metal fitting inside.



Rotate the probe-setting tool clockwise while pushing it gently towards the slotted metal fitting. Continue rotating the tool half a turn after the stepped feature of the tool makes full contact with the face of the probe.



Gently remove the tool from the probe



Wearing clean powder-free gloves, grip the main cassette body to







Turn the shipping plug counterclockwise to remove it.



Ensure the LC flow is set to zero then insert the solvent line fitting (ZenFit or nanoViper) into the nanoZero® union and rotate the finger grip on the solvent line fitting until a firm stop is reached.



Hold the column cassette fin in one hand and pinch the interface of the column and sleeve between a thumb and pointer finger to hold the sleeve in place (protecting the emitter tip).



Maintain grip on the interface between the sleeve and column, then guide the sleeve into the orifice of the nano probe installed on the source. Push the column with sleeve into the nano probe approximately 3 cm. Maintain the position of the column cassette, then retract the sleeve towards the cassette as far as possible.

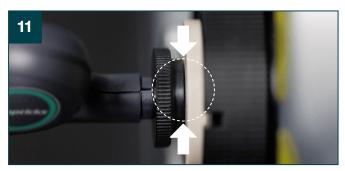


Push the column fully inside the probe until the thread of the black thumbwheel reaches the face of the nano probe. Hold the column cassette perpendicular to the probe, then rotate the black thumbwheel clockwise to screw it into the probe.

Installing Aurora Series® XS columns into SCIEX OptiFlow Turbo V ion source (cont.)



Installing the XS Adapter



Tighten the thumbwheel securely. When a firm stop is reached, the black O-ring is no longer visible when viewed from the side.



Grip the XS Adapter via the concave feature on each side. Install it by placing it underneath the column cassette with the prongs facing upwards and touching the beige probe face. Hold the adaptor level and then slide it upwards until it clicks in place under the column.



Holding the IonOpticks heater level, place it onto the fin of the column cassette, positioned so that the centre point of the heater is perpendicular with the finger notch on the XS Adapter. Use the finger notch to pinch and apply pressure to the heater until it makes contact with the adapter.

The column is now installed and ready for operation.

Removal of an XS column from your system

If possible, do not remove the column from a UHPLC system before the column performance falls and the columns requires replacement, however, removal for instrument servicing and maintenance is sometimes required.

- 1. For the removal procedure, run 80% B for 5min at operating flow rates.
- 2. Reduce flow to 0.002 ul/min for 10 min or until the back pressure has stabilised below 10 bar.
- 3. Set mass spectrometer system into standby mode.
- 4. Grip the XS adaptor via the concave features on each side.
- 5. Grip the projection at the rear of the heater where the cable attaches. Tilt the heater forwards to release.
- 6. Lift off the heater.
- 7. Grip the concave features on the adaptor.
- 8. Pull the adaptor straight down to release it from the XS column.
- 9. Rotate the thumbwheel counterclockwise to disengage it from the probe.
- 10. Pull the column straight out (perpendicularly) until the thumbwheel is approx. 3 cm from the front of the nano probe.
- 11. With the other hand, grip the sleeve and hold it close to the front of the nano probe.
- 12. Hold the sleeve in place and pull the column back until the thumbwheel is approx. 5 cm from the front of the nano probe.
- 13. Remove the column and sleeve from the probe.
- 14. Screw the shipping plug back into the nanoZero®. For removal of the column for extended periods of time we recommend placing 30µl of methanol in the nanoZero® before installing the shipping plug.
- 15. Place the column back in its box

Note: Removal of the solvent line from the nanoZero under high back pressure conditions can damage the stationary phase bed and lead to blockages and poor chromatographic performance.

Note: The removal procedure can lead to fouling of the emitter tip and poor column performance. The lonOpticks replacement guarantee is not valid if a column has been removed from a UHPLC once in 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 80% buffer B at a flow rate equivalent to the desired gradient flow rate 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.

Spray voltage

It is recommended to start with a spray voltage of 1500V and increase over time as required to maintain a stable spray. It is recommended that the spray voltage does not exceed 2500V. Exceeding a spray voltage of 2500V may result in damage to the emitter.



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

Operating environmental conditions

The laboratory room temperature must be maintained between 15 and 27 °C (59 and 81 °F). The relative humidity of the operating environment must be between 20 and 80%, with no condensation. The operating environment of the equipment should be free of vibrations.

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 μ l/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 lonOpticks 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

60 cm x 75 μm	2.65 μL
25 cm x 150 μm	4.42 µL
25 cm x 75 μm	1.1 µL
15 cm x 150 μm	2.66 μL
15 cm x 75 µm	0.66 μL
8 cm x 150 μm	1.4 µL
8 cm x 75 μm	0.35 μL
5 cm x 150 μm	0.88 μL
5 cm x 75 μm	0.22 μL

Column Temperature

The recommended operating temperature for Aurora columns is 40-60 °C. The maximum operating temperature is 60 °C.

Column equilibration

Before each run the column should be re-equilibrated 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.

Example data

Example data and raw data files for each column can be found in our help centre, or provided upon request.



Visit our help centre at: https://helpcentre.ionopticks.com/portal/en/kb/operation or contact: support@ionopticks.com for more information.

Application notes

Application and technical notes can be found in our Literature Room: https://ionopticks.com/resource-hub/





ionopticks

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