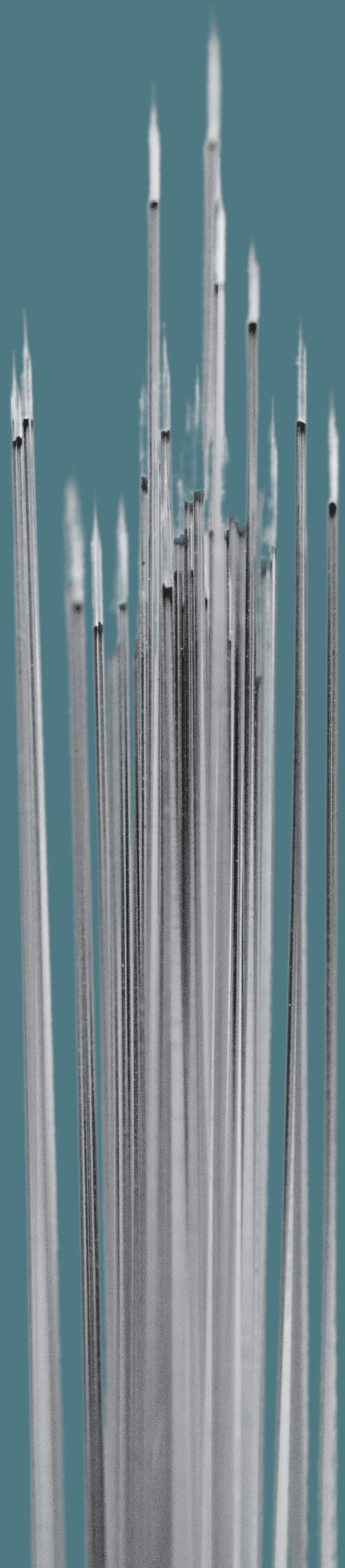
User Guide

Odyssey Series 3

UHPLC packed emitter column

Recommended guidelines for optimal setup and operation of Odyssey Series 3 columns:
ODY3-25075C18
ODY3-15075C18

For more information, visit www.ionopticks.com



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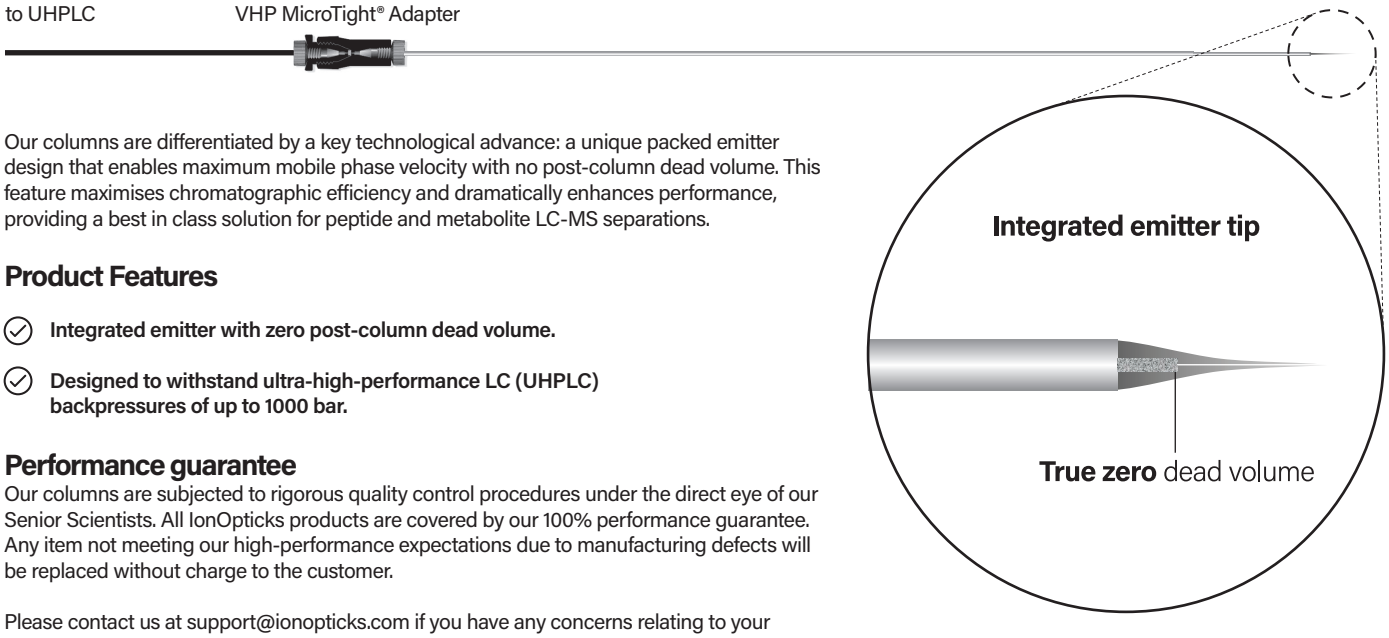
Innovative Design. Transforming Proteomics.



Our columns are differentiated by a key technological advance: a unique packed emitter design that enables maximum mobile phase velocity with no post-column dead volume. This feature maximises chromatographic efficiency and dramatically enhances performance, providing a best in class solution for peptide and metabolite LC-MS separations.

Product Features

- **Integrated emitter with zero post-column dead volume.**
- **Designed to withstand ultra-high-performance LC (UHPLC) backpressures of up to 1000 bar.**

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.

Odyssey Series 3 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
		Q-Exactive Plus

Product Specifications.

Column format:	Analytical column	**Pore size**	120 Å	**Particle size**	1.6 µm
Column type:	Reversed-phase	**Max pressure**	1000 bar	**pH stability**	1-8
For use with:	UHPLC	**Temp limits**	60 °C (low pH)	**Stationary phase**	C18

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Installation of Odyssey Series columns.

Setup of UHPLC systems

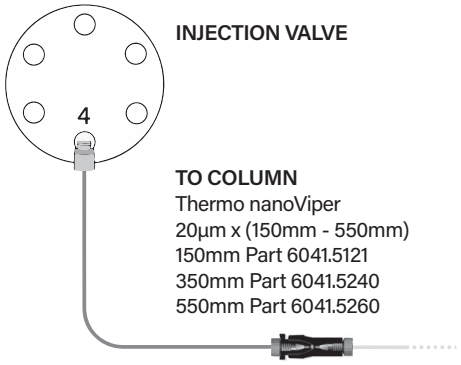
We recommend operation of Odyssey series columns in a one column mode (direct injection). Please refer to manufacturers guidelines for operating your UHPLC in a one column mode.

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

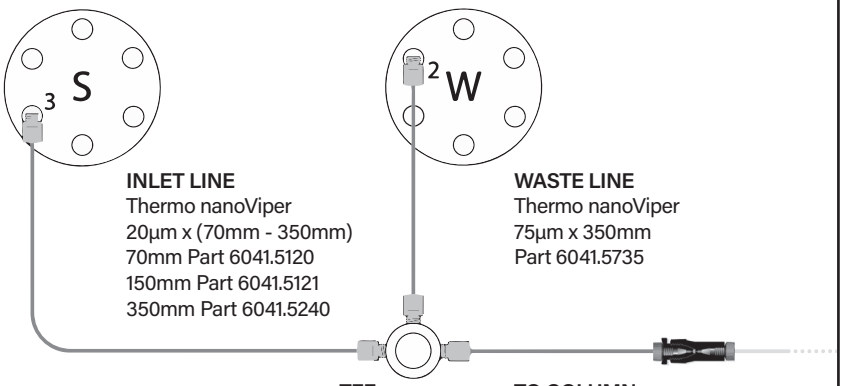
Dionex UltiMate 3000 RSLC



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

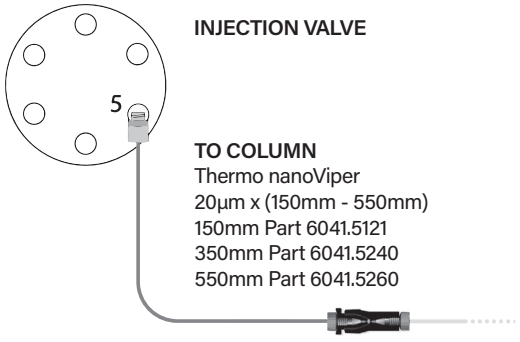
WASTE LINE
Thermo nanoViper
75µm x 350mm
Part 6041.5735

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)
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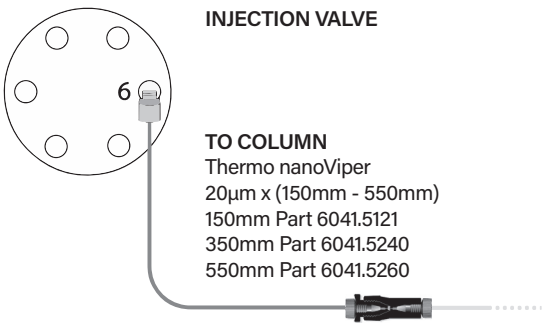
350mm Part 6041.5240
550mm Part 6041.5260

WATERS

nanoACQUITY and M-Class



INJECTION VALVE

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550mm Part 6041.5260

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Fitting Odyssey Series columns to your instrument

Recommended parts for optimal fitting:

Parts can be found at www.idex-hs.com

UH-750
VHP MicroTee® Assembly

OR

UH-634
VHP MicroTight® Adapter

Odyssey Series 3 columns are manufactured using 360µm outer diameter (OD) fused silica. Please ensure that any fittings used to connect the column are specifically designed for fused silica 360µm tubing. Note that the recommended parts included in this Guide have been have been proven to provide reproducible and reliable results.

WARNING Electrospray ionisation involves the use of potentially lethal high-voltage electrical current. Installation of equipment should be performed by qualified personnel in accordance with all applicable electrical codes or standards.

For optimal usage, please adhere to the following instructions:

1. Remove the nuts/ferrules from the tee assembly or adaptor union.
2. Slide a nut/ferrule onto the rear of the column, exposing the column beyond the ferrule by at least 1mm.
3. Screw the nut/ferrule/column assembly into the fitting until just finger tight (it's important not to overtighten at this point).
4. To determine that the ferrule has contacted the column, grip the column and gently slide it in and out and ensure that you feel a very slight resistance.

CAUTION Do not pull the column out beyond the ferrule nose during this procedure.

5. Push the column in until it butts firmly into the bottom of the fitting.
6. Carefully tighten the nut an additional amount. Use a tightening tool to achieve consistent results.

DO NOT OVER-TIGHTEN. The amount of tightening required is dependent on the type of fitting that you are using. Consult the fitting manufacturer for specific instructions relating to your set up.

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Operation of Odyssey Series columns.

Odyssey 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 400 nl/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 5 min 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 column can now be removed from the UHPLC. It is not recommended that the column is removed from the UHPLC for an extended period of time. It is recommended that a new cut with an appropriate tool is made to the entrance of the column before refitting to the UHPLC to ensure a reliable connection.

Removal of the column 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

25 cm X 75 µm 1.1 µl
15 cm X 75 µm 0.66 µl

Column Temperature

The recommended operating temperature for Odyssey Series columns is 40 °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. Column equilibration should be performed below 1000 bar.

Sample Loading

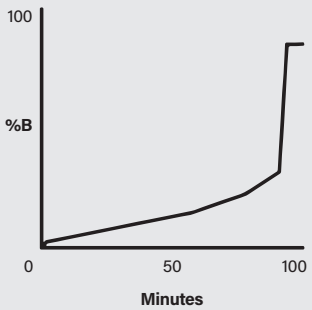
Samples should be loaded onto the column in 100% Buffer A. Samples loaded on to the column should be de-salted and should not contain any contaminants (salts, detergents, solid particles, etc). Loading contaminated samples onto the column may disrupt solvent flow or foul the emitter tip leading to a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

25 cm Column

90 min 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:

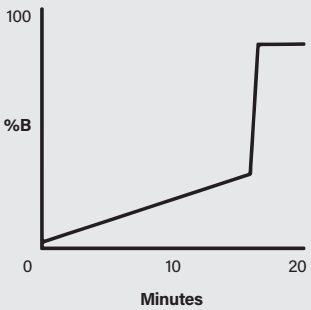


15 cm Column

17 min 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:



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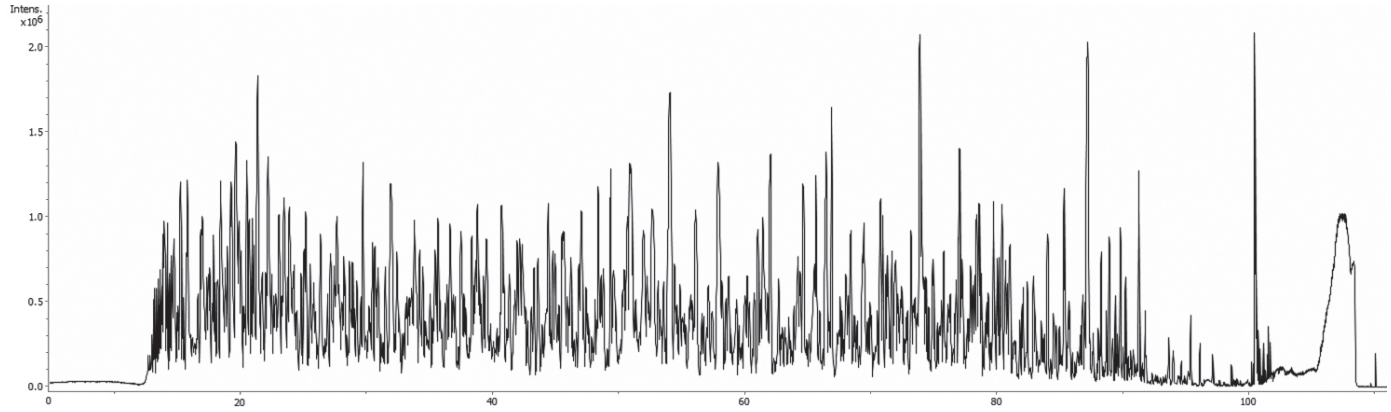
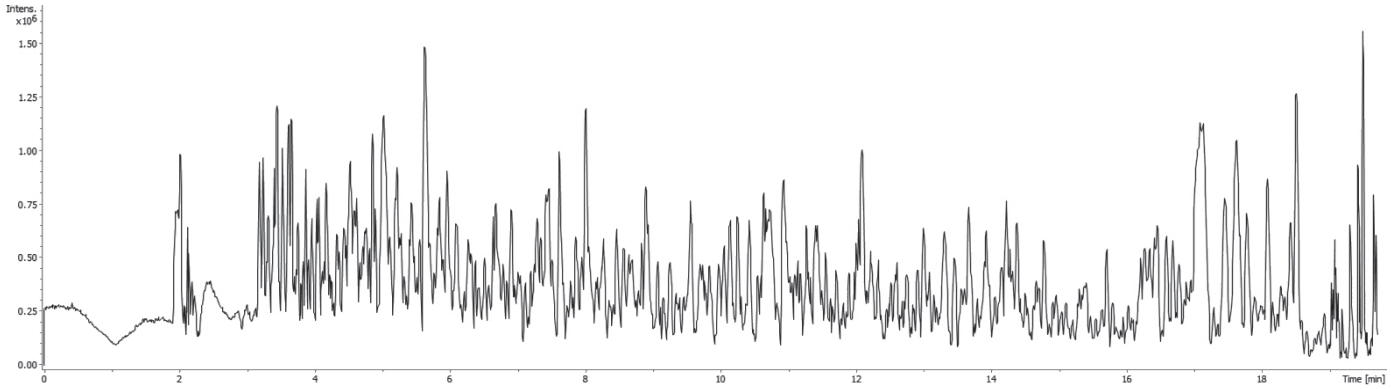
Example data.

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

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



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



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Odyssey series