



A practical guide for connecting nano- and capillary-flow columns to an EASY-Spray MS source

## Introduction

This technical guide describes how to connect a Thermo Scientific™ MabPac™ capillary column with double nanoViper fittings to a capillary-flow emitter used with the Thermo Scientific™ EASY-Spray™ source. It also describes how to connect 10 and 45 mm useable silica nano and capillary columns to either nano-flow or capillary-flow emitters.

## Thermo Scientific™ MAbPac™ RP double nanoViper™ column

Table 1. Parts needed to connect a MAbPac RP double nanoViper column and a EASY-Spray source

Part name	Part no.
MAbPac RP double nanoViper capillary column (4 µm, 150 µm x 150 mm)	164947
Capillary flow emitter (ID 15 µm)	ES994
Thermo Scientific™ Viper™ transfer line (20 µm x 550 mm)	6041.5260
2 Viper unions	6040.2304

## Connecting the column to mass spectrometer

1. Connect the inlet of MAbPac RP capillary column (indicated by the arrow) to the line coming from the autosampler valve (delivering the mobile phase) using a viper union by carefully tightening the nut finger tight. Place the column in the column oven. (Operating temperature of MAbPac columns are typically ranging from 40 °C to 90 °C. Failure to connect in the direction of flow indicated by the arrow may cause damage to the column or the system).
2. Create a connection between the column outlet and the viper transfer line using the second viper union.



Connecting MAbPac RP double nanoViper column to EASY-Spray source

3. Connect the other end of the transfer line to the capillary flow emitter by carefully tightening the nut finger tight.
4. Plug the capillary flow emitter into EASY-Spray source.

Table 2. Parts needed to connect a MAbPac RP EASY-Spray column and an EASY-Spray source

Part name	Part no.
MAbPac RP capillary column (4 µm, 150 µm x 150 mm)	ES907
Viper transfer line (20 µm x 550 mm)	6041.5260
2 Viper unions	6040.2304

## Thermo Scientific™ MAbPac™ RP EASY-Spray™ capillary column

### Connecting the column to mass spectrometer

1. Use the Viper union to connect the transfer line to the line coming from autosampler valve (delivering the mobile phase). Carefully tighten the nut finger tight.
2. Install the second viper union to the inlet of MAbPac RP EASY-Spray column.
3. Connect the transfer line to the second viper union already attached to column.
4. Plug the MAbPac RP EASY-Spray column connected to HPLC into EASY-Spray source.
5. Plug the temperature control cable of the EASY-Spray column into the heater port of the EASY-Spray source and set the temperature by the integrated temperature controller knob.

## Thermo Scientific™ Acclaim™ PepMap™, PepMap™, and Accucore™ nanoViper columns with 10 mm usable silica outlet

Columns included in Appendices/Table 1 can be installed based on the instructions detailed below.

Table 3. Parts needed to connect Acclaim PepMap, PepMap, and Accucore nanoViper columns with 10 mm usable silica and an EASY-Spray source

Part name	Part no.
Columns with 10 mm usable silica	Listed in Appendices, Table 1
Nano flow emitter (ID 10 µm)	ES993
Viper transfer line (20 µm x 550 mm)	6041.5260
Viper union	6040.2304
Micro tight adapter	6720.0074
Red sleeve, 0.025" OD x 0.013" ID for 280 µm OD capillary	6720.0075
Nano connector with two sleeves	6720.039
Fused silica transfer line (20 µm x 300 mm)	164724

## Connecting column to mass spectrometer

1. Use the Viper union to connect the inlet of the column to the line coming from autosampler valve (delivering the mobile phase). Create a finger tight connection.
2. Slide one of the black nuts of the nano connector and the transparent union onto the fused silica transfer line. Thread the other black nut onto the column outlet.
3. Slide the end of the silica transfer line and the column outlet into the nano connector sleeve until they meet each other in the middle. It is important that the connection is free of dead volume.
4. Tighten both black nuts equally to ensure the transparent sleeve is centered in the middle of the union. The silica transfer line can be cut to any desired length.
5. Thread the red sleeve onto the other end of the silica transfer line and drive it through the nut of micro tight adapter. The sleeve tightly fits in the nut and its end is in-line with the end of the tip of nut. Position the capillary so that it reaches the end of the sleeve but does not stick out. Tighten the nut carefully into the adapter.
6. Connect the viper transfer line to the micro tight adapter by carefully tightening the nut finger (introducing a gap between column outlet results in peak broadening and deteriorated resolution, shown by Appendices/Figure 1).
7. Connect the other end of the Viper transfer line to the nano flow emitter by carefully tightening the nut finger tight.
8. Plug the emitter into the EASY-Spray source.



**Table 4. Parts needed to create connection between PepMap nanoViper FS columns with 45 mm usable silica and EASY-Spray source**

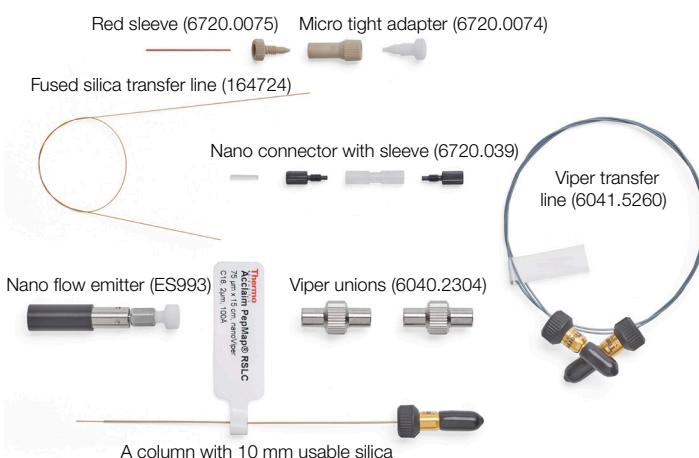
Part name	Part no.
Columns with 45 mm usable silica (FS)	Listed in Appendices, Table 2
Nano flow emitter (ID 10 µm)	ES993
Viper transfer line (20 µm x 550 mm)	6041.5260
Viper union	6040.2304
Micro tight adapter	6720.0074
Red sleeve, 0.025" OD x 0.013" ID for 280 µm OD capillary	6720.0075

## Thermo Scientific™ PepMap™ nanoViper FS columns with 45 mm usable silica outlet

Columns included in Appendices/Table 2 can be installed based on the instructions detailed below.

### Connecting column to mass spectrometer

1. Use the Viper union to connect the inlet of the column to the line coming from autosampler valve (delivering the mobile phase). Create a finger tight connection.
2. Thread the red sleeve onto the outlet of the column (onto the useable silica capillary) and drive it through the nut of micro tight adapter. The sleeve tightly fits in the nut and its end is in-line with the end of the tip of the nut. Position the capillary so that it reaches the end of sleeve but does not stick out. Tighten the nut carefully into the adapter.
3. Connect the Viper transfer line to the micro tight adapter by carefully tightening the nut finger tight.
4. Connect the other end of viper transfer line to the nano flow emitter by carefully tightening the nut finger tight.
5. Plug the emitter into the EASY-Spray source.



**Parts necessary to use for connecting columns with 10 mm usable silica capillary to EASY-Spray source**

The effect of the 30 mm silica transfer line (set up of the column is detailed by Appendices/Table 1) on the separation compared to the set up described by Appendices/Table 2 is shown by Appendices/Figures 2-3.



Connecting columns with 45 mm usable silica capillary to EASY-Spray source

## Appendices

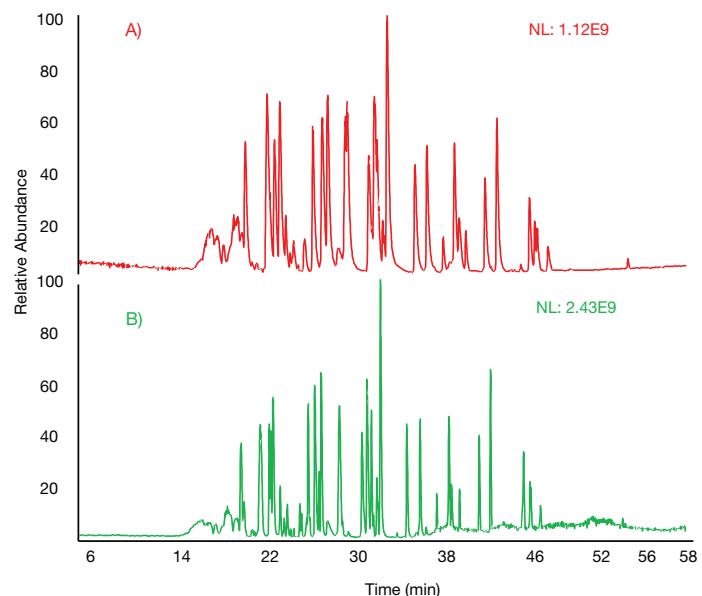


Figure 1. A shows a wrong connection between column outlet and silica transfer line using the micro tight adapter. B shows a chromatogram with zero dead volume connection between column outlet and transfer line.

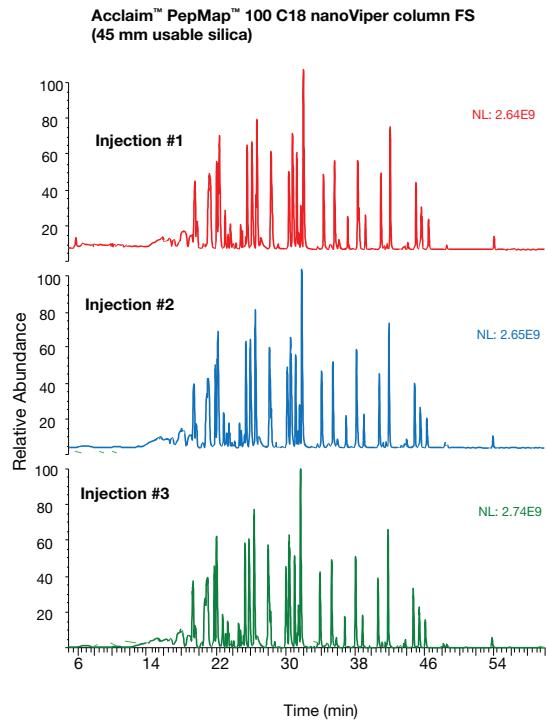
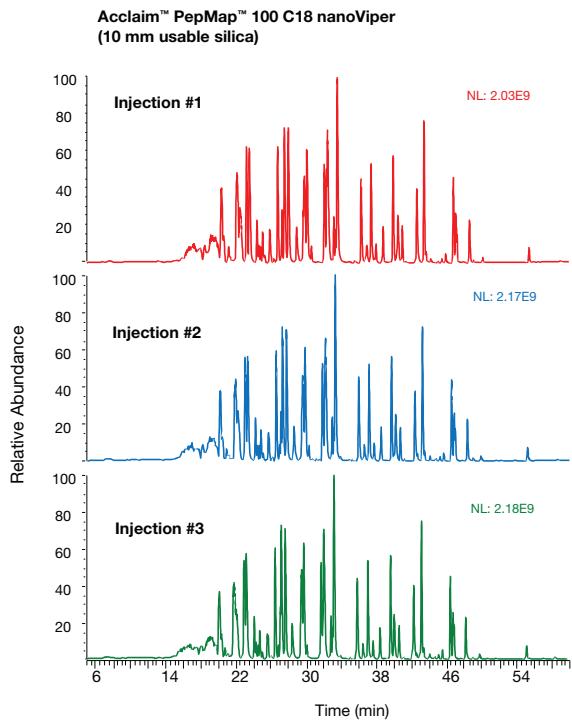


Figure 2. The panel on the left shows BPCs of 200 fmol BSA digest in triplicates using column with 10 mm usable silica (installation is detailed in Appendices/Table 1). Panel on the right depicts the BPCs of 200 fmol BSA digest using column with 45 mm usable silica (installation is described by Appendices/Table 2). Separations were performed with a 5-50% "B" gradient ("A": 0.1% formic acid in water, "B": 0.1% formic acid, 80% acetonitrile and 19.9% water) at 300 nL/min for 60 min and detected with a Thermo Scientific™ Q Exactive™ Plus Orbitrap™ Mass Spectrometer.

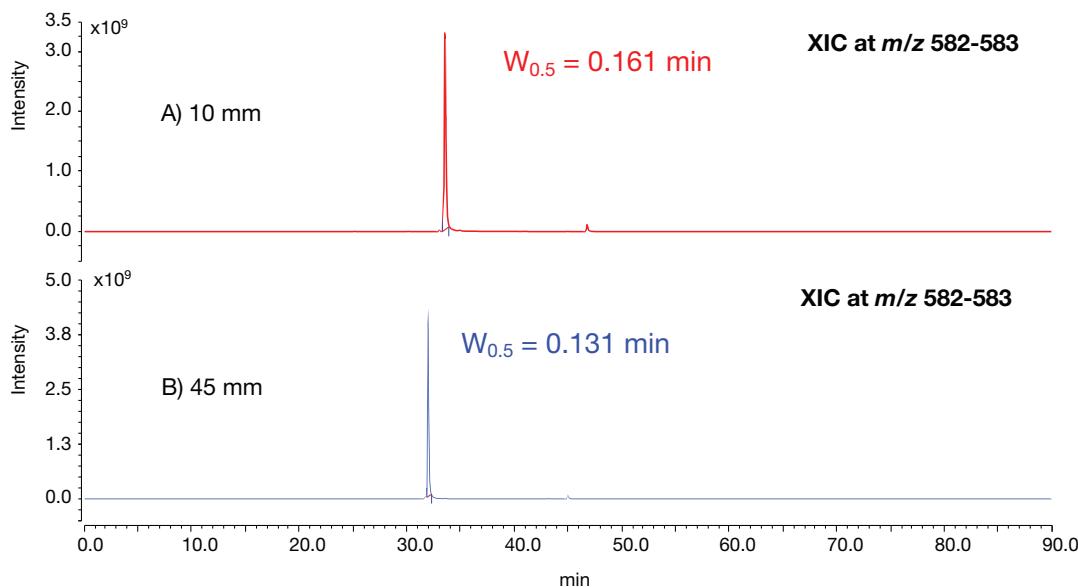


Figure 3. XICs of LVNELTAFAK peptide show the peak width at 50% of the peak half.

Table 1. Acclaim PepMap, PepMap, and Accucore HPLC nanoViper columns with 10 mm usable silica

Part name	Part no.
Acclaim PepMap 100 C18 nanoViper (2 $\mu$ m, 50 $\mu$ m x 50 mm)	164561
Acclaim PepMap 100 C18 nanoViper (2 $\mu$ m, 50 $\mu$ m x 150 mm)	164562
Acclaim PepMap 100 C18 nanoViper (2 $\mu$ m, 75 $\mu$ m x 50 mm)	164563
Acclaim PepMap 100 C18 nanoViper (2 $\mu$ m, 75 $\mu$ m x 150 mm)	164534
Acclaim PepMap 100 C18 nanoViper (3 $\mu$ m, 75 $\mu$ m x 50 mm)	164567
Acclaim PepMap 100 C18 nanoViper (3 $\mu$ m, 75 $\mu$ m x 150 mm)	164568
Acclaim PepMap 100 C18 nanoViper (3 $\mu$ m, 75 $\mu$ m x 250 mm)	164569
Acclaim PepMap 100 C18 nanoViper (3 $\mu$ m, 75 $\mu$ m x 500 mm)	164570
PepMap C8 nanoViper (3 $\mu$ m, 75 $\mu$ m x 150 mm)	164706
PepMap C18 nanoViper (3 $\mu$ m, 50 $\mu$ m x 50 mm)	164712
PepMap C18 nanoViper (3 $\mu$ m, 50 $\mu$ m x 150 mm)	164713
PepMap C18 nanoViper (3 $\mu$ m, 50 $\mu$ m x 250 mm)	164714
PepMap C18 nanoViper (3 $\mu$ m, 50 $\mu$ m x 500 mm)	164715
Accucore 150 C18 nanoViper (2.6 $\mu$ m, 75 $\mu$ m x 150 mm)	16126-157569
Accucore 150 C18 nanoViper (2.6 $\mu$ m, 75 $\mu$ m x 500 mm)	16126-507569
Accucore 150 C4 nanoViper (2.6 $\mu$ m, 75 $\mu$ m x 150 mm)	16526-157569
Accucore 150 C4 nanoViper (2.6 $\mu$ m, 75 $\mu$ m x 500 mm)	16526-507569

Table 2. PepMap nanoViper FS columns with 45 mm usable silica

Part name	Part no.
PepMap 100 C18 nanoViper FS (3 µm, 75 µm x 150 mm)	164738
PepMap 100 C18 nanoViper FS (3 µm, 75 µm x 500 mm)	164739
PepMap C18 nanoViper FS (2 µm, 75 µm x 750 mm)	164939
PepMap C18 nanoViper FS (2 µm, 75 µm x 150 mm) 1200 bar	164940
PepMap C18 nanoViper FS (2 µm, 75 µm x 250 mm) 1200 bar	164941
PepMap C18 nanoViper FS (2 µm, 75 µm x 500 mm) 1200 bar	164942
PepMap C18 nanoViper FS (2 µm, 50 µm x 150 mm) 1200 bar	164943
PepMap C18 nanoViper FS (2 µm, 50 µm x 250 mm) 1200 bar	164944
PepMap C18 nanoViper FS (2 µm, 50 µm x 500 mm) 1200 bar	164945

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