We take care of our customers

BRUKER

REF

1842619	1893470-485	1895797-852
1842621	1893625-627	
1866154	1895616-619	

Instructions for Use

PepSep Columns

For Research Use Only. Not for use in clinical diagnostic procedures.

CARE products are designed to support our worldwide customers with high-quality consumables, accessories and dedicated kits.

RUO

www.bruker.com/care

Language: en

Doc. No. 5048686 Revision A (June 2022)

Table of Contents

Document history

Title:	Instructions for Use PepSep Columns
Revision:	Revision A (June 2022)
Document number:	5048686
First revision:	June 2022

The following table describes important changes from the previous revision of this document.

List of changes
First Edition

Use of trademarks

The names of actual companies and products mentioned herein may be the trademarks of their respective owners.

1 Product description

PepSep Columns are packed with Dr. Maisch Reprosil C18 beads and are available in different lengths, inner diameters, and particle sizes.

High Performance PepSep UHPLC nanoFlow Columns are pre-mounted for safe and easy connections. The high-pressure end of the column connects to the Bruker nanoElute UHPLC series instruments using a 10/32” thread female union. Connection to other LC systems can be performed using a 1/16” union.

The column’s low-pressure end uses a nanoConnect connector with 10/32” thread male union allowing connection to any 10/32” thread receiving union. The nanoConnect system ensures a zero dead volume connection to the Captive Spray emitter as well as to any other emitter connected to a 10/32” thread union.

PepSep Columns are tested to be used for up to 1000 bar (14,500 psi). Columns are connected using finger-tight connections.

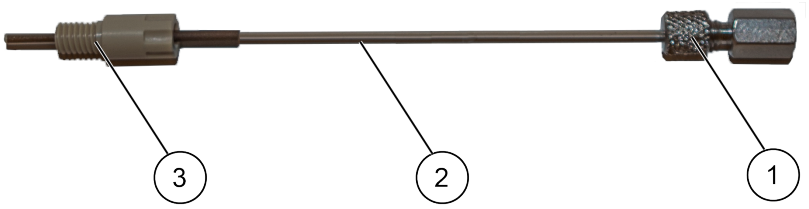


Figure 1 Bruker nano column with UHP union and the nanoConnect screw

1	UHP-union
2	Packed fused silica with peek tubing protection
3	nanoConnect

Table 1 Legend Bruker nano column

2 Specifying the separation column in HyStar Software

PepSep Columns are often predefined in HyStar. In case the PepSep Columns are not predefined in the HyStar release, please follow the below instructions.

(1) Define the separation columns in the “Columns” tab of the configuration Editor (see section 3).

(2) The columns’ definition is not part of the instrument configuration saved on the Compass Server, but is saved to a separate file (*.xmc) on the hard disk in

`C:\BDALSystemData\HyStar\LcPlugin\HardwareSetup.`

(3) Changing the columns definition does not affect any defined instrument configuration.

(4) The nanoElute plug-in supports user defined LC columns.

3 How to add a new column

(1) Open the Configuration Editor.

(2) Click the **Columns** tab (see Figure 2).

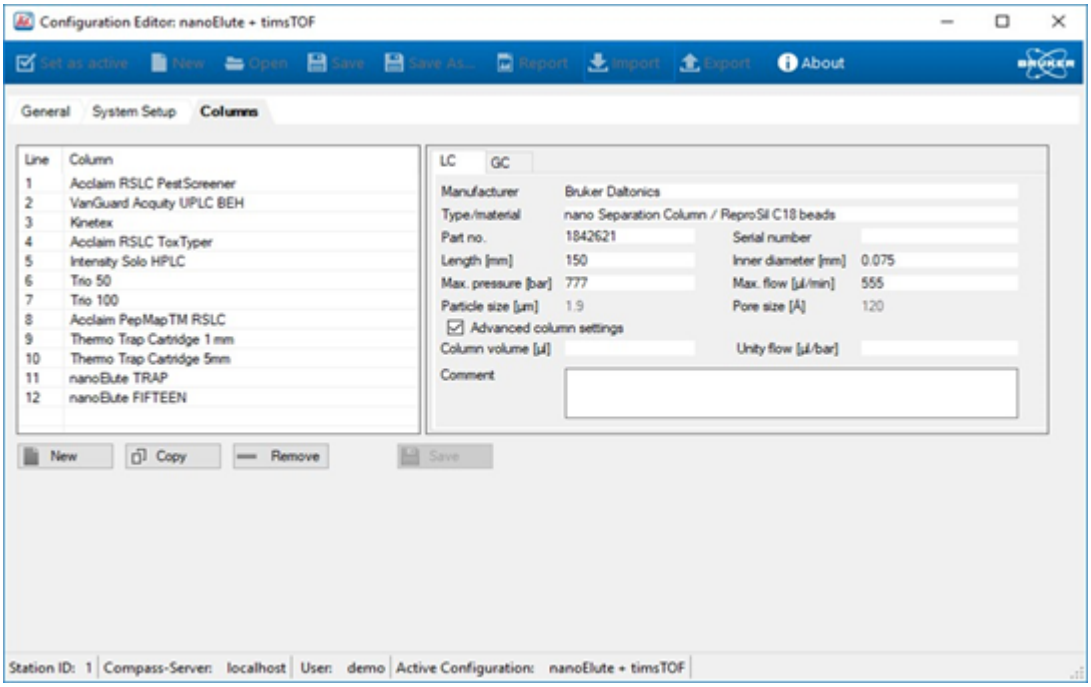


Figure 2 Columns tab with the list of predefined columns

(3) Click **New** to add a new column.

(4) Enter a name for the new column in the **Column Name** dialog (see Figure 3)

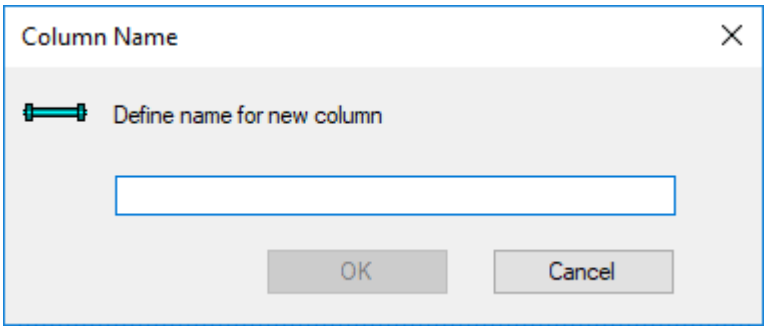


Figure 3 Column name dialog

(5) Click **OK** to add the new column without specifications to the columns lists.

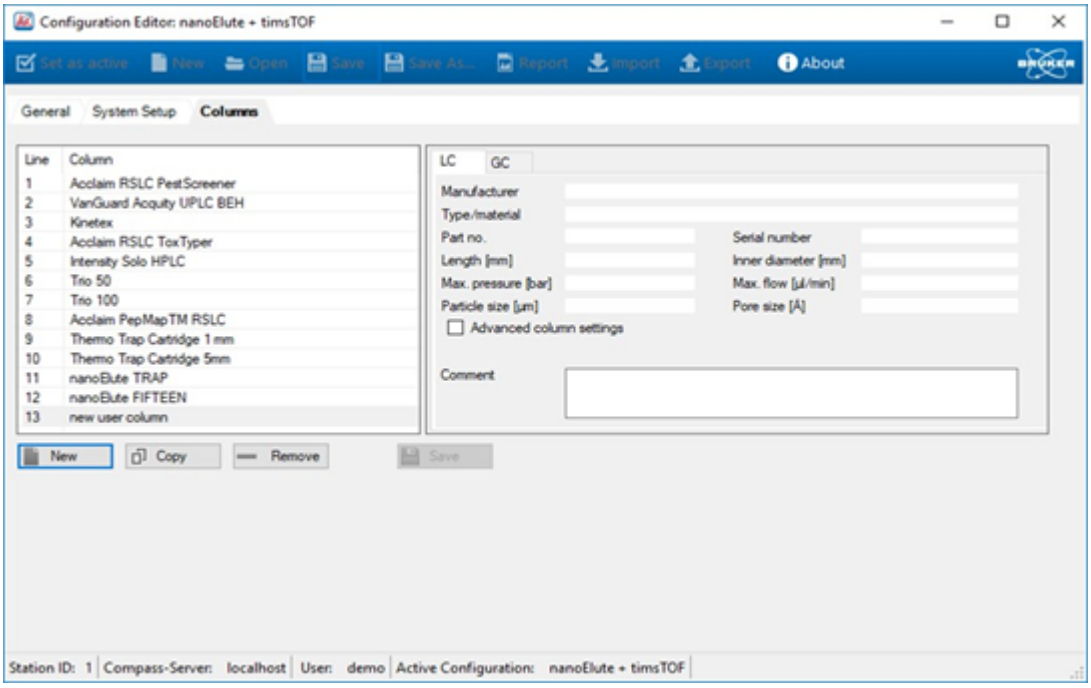


Figure 4 New column added to column list

(6) Enter specifications of the new column (e.g. manufacturer, type/material, part number, length, particle size, or pore size) and/or a comment (see Figure 5)

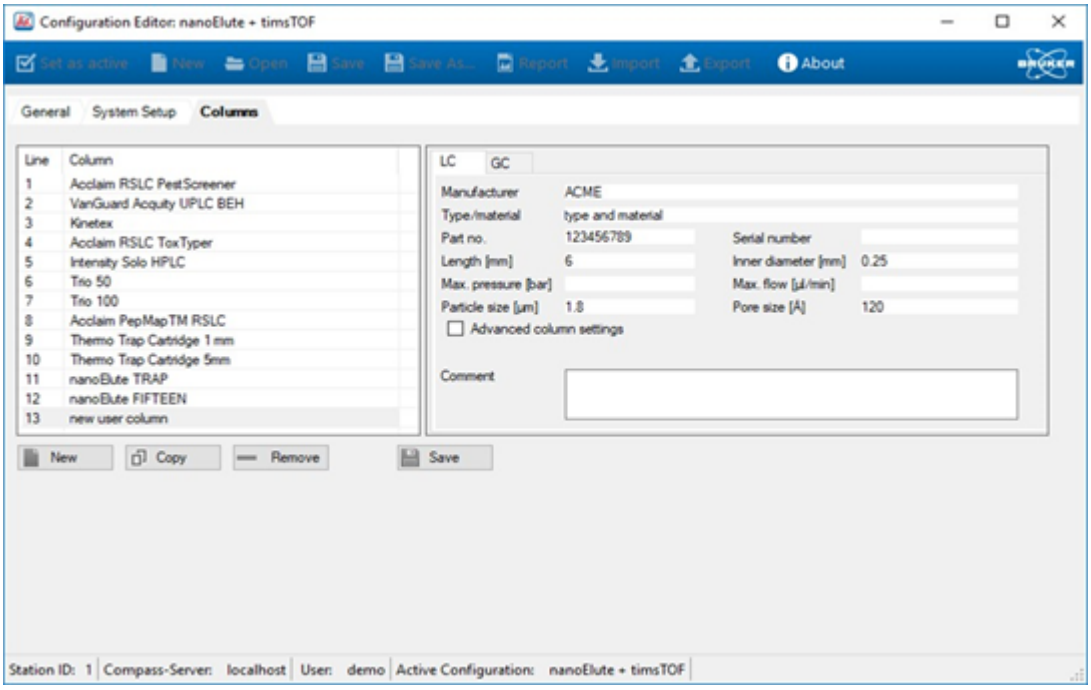


Figure 5 Columns Tab with new column and corresponding specifications

(7) Click **Save** to save the new specifications.

(8) Click **X** in the upper right-hand corner to close the Configuration Editor.

4 Column preparation

Use LC-MS grade solvents for preparing mobile phases.

The following common mobile phases are used for peptide separation with C18 columns:

- Mobile phase A: 99.9 % water, 0.1 % formic acid
- Mobile phase B: 99.9 % acetonitrile, 0.1 % formic acid

Specific recommended mobile phases can be found on the column leaflet in the column box.

(1) Select the defined **Separation Column** from the drop-down box of the HyStar nanoElute status window.

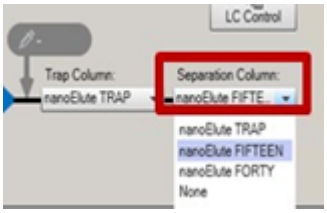


Figure 6 Column selection

(2) Open the **LC Control** window

(3) Click the **Preparation** gearwheel in the **LC Control** window, activate **Include column preparation** and click **Run**.

(4) Allow the procedure to complete (approx. 25 min.).

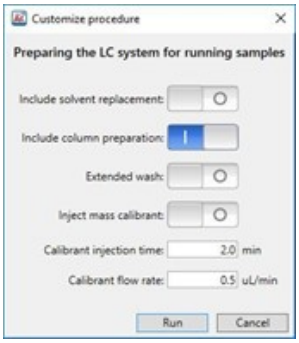


Figure 7 Preparation dialog in the LC Control nanoElute PlugIn

CAUTION Be vigilant of rapid pressure changes when starting or stopping flow!
Pump acceleration and deceleration can cause significant pressure increases, which can induce column decompression and inflict damage to the separation column. The nanoElute Plug-in in HyStar monitors and controls the values according to the preset values.
For other LC systems, check the values and adjust accordingly.

(5) Flush the separation column with at least five column volumes organic mobile phase and afterwards five times with the aqueous mobile phase.

(6) This will equilibrate the stationary phase and flush possible debris from the column frit.

CAUTION Do not connect the column to the ESI emitter during this procedure!

5 Column installation

It is strongly recommended to carefully connect the separation columns to the ESI emitter to ensure / enable stable electrospray and good chromatographic performance.

CAUTION Spray instability may be observed during the gradient or the LC flow may be obstructed if instructions are not followed.

(1) Mount the union onto the emitter, before the column is attached.

- The emitter has a ferrule which determines and ensures optimal position in the union.

(2) Tighten the emitter firmly, but do not overtighten!

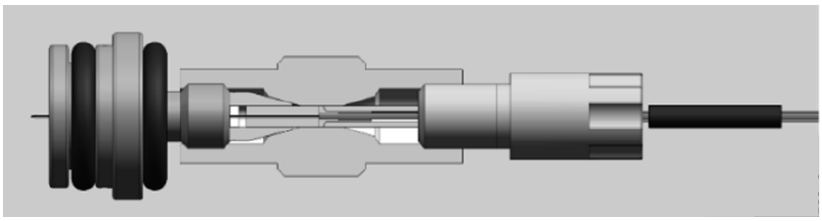


Figure 8 ZDV-Sprayer with thru hole union and analytical column

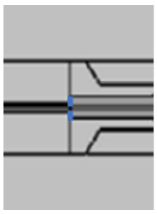


Figure 9 Magnification of the direct connection

(3) Make sure the column flow direction is correct.

(4) Attach the column to the transfer line of the nanoElute UHPLC, the capillary coming from the injection valve.

(5) Flush the column using the column preparation function in the user manual Compass HyStar (chapter "Definitions of columns").

(6) Mount the column finger tight into the union and place it into the column oven.

(7) Tighten the oven fixation screw until contacting the union between column and transfer capillary.

- This fixation point also acts as an electrical grounding.

(8) Close the column oven’s lid.

(9) Activate idle flow at 5% B with a flow rate of 500 nl/min to avoid column and ZDV sprayer damage from overheating.

(10) Use idle flow if no measurement is active.

(11) Observe stability of the spray signal.

Note To remove residual air bubbles from the connections and column it is recommended using a higher flow rate of 1000 nL/min for 10 – 20 minutes

6 Operating conditions

The following conditions are necessary to ensure sure long lasting columns.

Note Unique column information can be found on the column leaflet inside the column box.

(1) Use high purity LC-MS grade solvents for eluent and sample preparation.

(2) Filter aqueous mobile phases (0.2 µm filter) before use.

(3) Do not use nylon-based filters since they can introduce a contaminant that is seen in the mass spectrometer

(4) Ensure mobile phase pH is in the range of 2 to 8.

Note The pH can only be measured in an aqueous mobile phase, and it always refers to the aqueous part of the mobile phase.

(5) Select column operating temperatures between 40 °C and 60 °C.

(6) The best compromise is to use 50°C for operating columns packed with the C18 stationary phase.

(7) Utilize recommended column specific flow rates.

(8) Inject, whenever possible, freshly prepared and filtered, or thoroughly centrifuged samples.

(9) Resuspend samples in acidic aqueous solvents such as diluted formic acid or trifluoroacetic acid (0.1%).

(10) Store samples in vials with low protein binding and deactivated surfaces, eg., QuanRecovery MaxPeak HPS vials w. cap (can be ordered via Bruker representative, catalogue # 1892198).

(11) Enable LC idle flow when the system is inactive. With the nanoElute, the idle flow starts a few minutes after the last acquisition run or LC inactivity.

- **Idle Mode** is enabled by default but can be disabled in the **Preferences** dialog.
- Right-click under the nanoElute status window In HyStar and select **Preferences**.
- Set the **Idle Flow** rate and the composition of the mobile phase.
- If the application requires use of a trap column, enable idle flow via the trap column (see Figure 10).

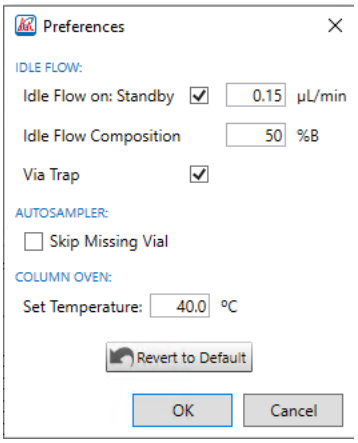


Figure 10 Preferences dialog

7 Column care for storage and cleaning

7.1 Cleaning

Column cleaning may be necessary if the non-ID-based metrics deteriorates for typical column criteria (RT shifts, broadening peak width, rising back pressure).

WARNING

Servere injuries possible when using Trifluoroethanol (TFE)

TFE is a flammable liquid, not easily biodegradable, and can cause severe eye and fertility damage.

- All solvents used must be of LC-MS grade.
- Carefully read the manufacturer's information before working with Trifluoroethanol and always observe the corresponding instructions.

(1) Inject 2µl of 2,2,2-Trifluoroethanol (TFE) to remove peptide and protein remains from the separation column.

(2) Run the gradient depicted in the table below.

(3) Select a flow which is 50 -100 % lower than the flow used for sample separations.

(4) Repeat this method twice.

Time [min]	Mobile phase A [%] (aqueous)	Mobile phase B [%] (organic)
0	80	20
20	0	100
25	0	100
30	80	20

7.2 Storage

The following steps are necessary to store the Bruker nanoFlow Column for a period of time.

(1) Clean the column (see section 7.1).

(2) Use the **Prepare separation column for storage** procedure.

(3) Place a vial with a storage liquid (e.g., water/acetonitrile: 30/70 (V/V), 0.1% formic acid) in position 1 (rear position) of the autosampler's wash module.

- The separation column is flushed with ten column volumes.

(4) Start **Detach Separation Column** procedure.

(5) Cap the ends of the column.

8 Troubleshooting

The nanoElute plug-in offers further options for checking the column quality.

- **Equilibrate separation column:** The separation column is flushed with five column volumes organic mobile phase and afterwards five times with aqueous mobile phase to ensure the correct mobile phase.
- **Diagnose separation column**: The separation column is checked for blockage and leakage.

(1) Run the test routine **Diagnose separation column**:

- The separation column is checked for a blockage and leakage.

8.1 Backpressure is low

Reasons for low backpressure:

- Connection between the transfer line and the nanoFlow Column is not tight,
    - Look for small liquid droplet,
    - Retighten the connection.

This can be seen only when the separation column is operated at ambient temperature.

Note The backpressure will be significantly lower at 50°C as compared to the ambient temperature.

8.2 Backpressure is high

Reasons for high backpressure:

- Blockage to the emitter:
    - Disconnect the emitter and check the backpressure again.
    - If needed exchange the emitter.
- Blockage on the column:
    - A column exchange may be necessary.

9 Warranty

Each column batch is tested on the specification stated in the column information leaflet.

Note Be aware that mechanical shock can have an impact on column performance.

Note The warranty does not apply if the UHP connection or nanoConnect is damaged or altered.

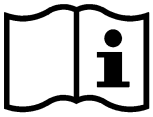
10 Disposal

At the end of the column lifetime, dispose of the column in the same way as the samples separated on it.

The common standards for the disposal of laboratory materials in the country of use apply.

11 Symbols

The following symbols are used in the labeling:

	Catalogue number		Consult instructions for use
	Research Use Only		Manufacturer

12 Manufacturer

Bruker Daltonics GmbH & Co. KG.
Fahrenheitstrasse 4
28359 Bremen
Germany
Phone: +49 421 2205-0

Application contact:
Email: esi.appl.support@bruker.com
Phone: +49 421 2205 493

Service contact:
Email: service.bdal.de@bruker.com
Phone: +49 421 2205 350

The Bruker nanoFlow Columns as well as other components can be ordered directly from the Bruker LabScape webstore or via email to your local Bruker office or representative.

Descriptions and specifications supersede all previous information and are subject to change without notice.