REF

1811107; 1811112; 1893470-74; 1893476-77;
1893479-80; 1893482-84; 1893625-27;
1895617; 1895619; 1895798-99; 1895802-04;
1895806; 1895808-10; 1895813; 1895817;
1895828; 1895838; 1895846; 1895833;
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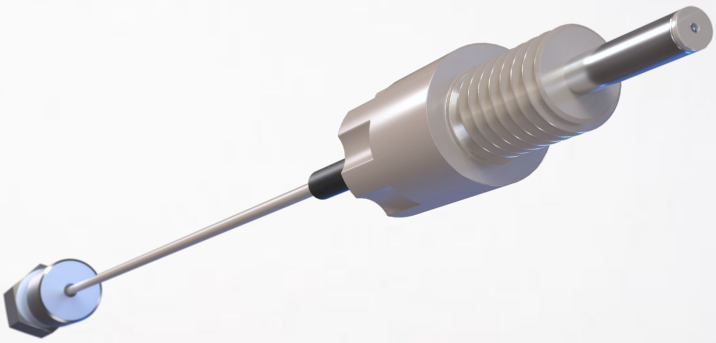

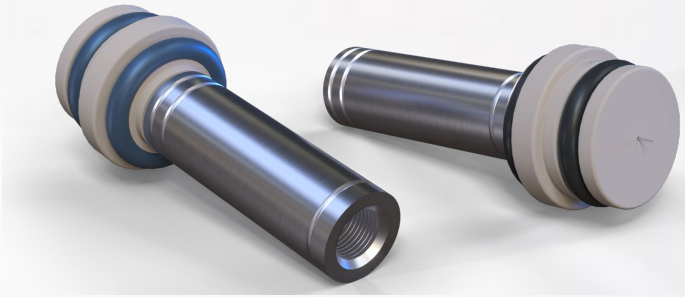

RUO

Instructions for Use

PepSep Columns & Emitters

HPLC & LC-MS Consumables

Revision B

Innovation with Integrity

Document Number: 5048686

Table of contents

1 Legal and regulatory notices 4
1.1 All rights reserved 4
1.2 Warranty 4
1.3 Use of trademarks 4
1.4 Limitations on use 4
1.5 Document History 4
1.6 General information 5

2 Product description 6
2.1 PepSep columns 6
2.2 CaptiveSpray 2 emitter 6

3 Compatibility 8
3.1 PepSep columns 8
3.2 CaptiveSpray 2 emitter 8

4 Video installation guides 9

5 Specifying the separation column in Hystar Software 10

6 Column & emitter installation 13

7 Detaching column & emitter 16

8 Operating conditions 18

9 Column care for storage and cleaning 20
9.1 Cleaning 20
9.2 Storage 20

10 Troubleshooting 21
10.1 Backpressure is low 21
10.2 Backpressure is high 22
10.3 Loading time too long 22

11 Symbols 23

12 Manufacturer 24
12.1 Contact 24
12.2 Webstore 24

13 Equipment Disposal 25

1 Legal and regulatory notices

Read this section before proceeding to the rest of the sections.

1.1 All rights reserved

1.2 Warranty

The information contained in this document is subject to change without notice.

Bruker Daltonics GmbH & Co. KG makes no warranty of any kind with regard to this material, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

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Bruker Daltonics GmbH & Co. KG assumes no responsibility for the use or reliability of its software on equipment that is not furnished by Bruker.

Avoid mechanical shock

Mechanical shock can have an impact on column performance.

1.3 Use of trademarks

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

1.4 Limitations on use

For Research Use Only (RUO)

This product has no declared clinical intended purpose and is not for clinical diagnostic use. Any clinical diagnostic use is at the user's own risk and responsibility.

Hyperlink disclaimer

Bruker Daltonics GmbH & Co. KG makes no express warranty, neither written nor oral, and is neither responsible nor liable for data or content from the linked Internet resources presented in this document.

1.5 Document History

Title:	Instructions for Use PepSep columns & emitters
Revision	Revision B (November 2024)
First revision	June 2022

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

Section	Changes
First edition	No changes
Rev_B	Major changes
1	CaptiveSpray 2 Emitter added
2	New chapter added (Compatibility)
3	New chapter added (Video installation guides)
4	Minor text changes (Specifying the separation column in Hystar Software)
4.1	New screenshots (How to add a column)
5	New screenshots (Column preparation)
6	CaptiveSpray 2 Emitter added (Column & Emitter installation)
7	New chapter added (Detaching Column & Emitter)
8	Minor text changes (Operating conditions)
9	Minor text changes (Column care for storage and cleaning)
10	CaptiveSpray 2 Emitter added (Troubleshooting)
11	Text changes (Warranty)
14	Contact information added (Manufacturer)

Table 1.1: Document changes

1.6 General information

Please read this information carefully before using this column. All PepSep columns & emitters are individually manufactured and tested to meet stringent specification criteria. The following measures will enhance its performance and lifetime.

2 Product description

2.1 PepSep columns

PepSep columns are packed with C18 beads and are available in various lengths, inner diameters, and particle sizes. High Performance PepSep UHPLC nanoFlow columns are pre-mounted for safe and easy connections. PepSep columns are tested to be used for up to 1000 bar (14,500 psi). Columns are connected without tools.

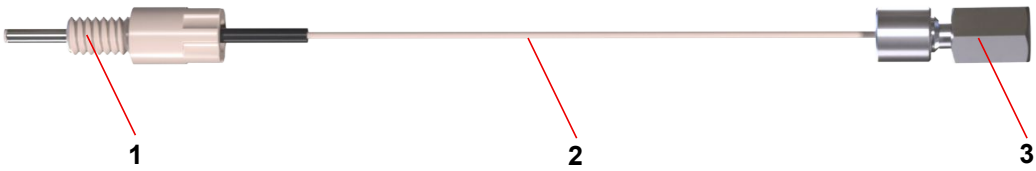

Figure 2.1: PepSep column with nanoConnect (Outlet) and UHP union (Inlet)

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

2.2 CaptiveSpray 2 emitter

The CaptiveSpray 2 emitter provides a seamless plug-and-play-setup, ensuring effortless column connections via a pre-assembled zero-dead-volume union. The emitters are available with 10 µm or 20 µm internal diameter.

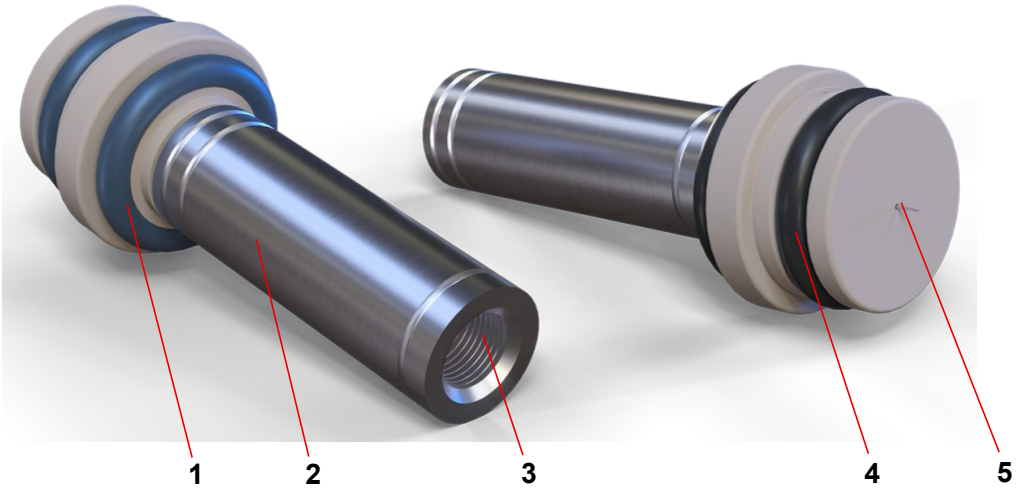

Figure 2.2: Captive Spray 2 emitter with pre-mounted union

1	Blue O-ring (unique for 10 µm emitter)
2	Pre-mounted zero-dead-volume union

3	Thread of 10-32 UNF receiving union (one ring on this side of the union = 10 µm ID emitter, two rings on this side of the union = 20 µm ID emitter)
4	Black O-ring (unique for 20 µm emitter)
5	Emitter tip

3 Compatibility

3.1 PepSep columns

Column inlet (high-pressure end of the column, UHP union):

- Connects to Bruker nanoLC series instruments and other LC systems via transfer line.

Column outlet (low-pressure end of the column, nanoConnect):

- nanoConnect system ensures a zero-dead-volume connection to the union of the CaptiveSpray 2 emitter.

3.2 CaptiveSpray 2 emitter

The CaptiveSpray 2 emitter is compatible with all Bruker CaptiveSpray ion sources. It has a pre-assembled zero-dead-volume union. The 10-32 UNF receiving thread connects to any 10-32 UNF thread male nut. A bottom sealing connection is required. Ferrule and sleeve sealing is not supported.

Recommended flow rates*:

- CaptiveSpray 2 Emitter 10 µm: 50 - 450 nL/min
- CaptiveSpray 2 Emitter 20 µm: 300 - 5000 nL/min

*__Note:__ 10 µm emitters often provide a more stable spray at lower flow rates.

4 Video installation guides

We are excited to present a comprehensive series of instructional videos, including the installation of columns, through the Bruker Training Academy website. Additionally, you can find instructional videos around instruments, ion sources, software, data acquisition, data processing and workflows.

For column and emitter installation please watch **Installing a Column CSI 2**.

Please visit *Videos Customer Training Academy - Mass Spectrometry | Bruker**

*registration needed

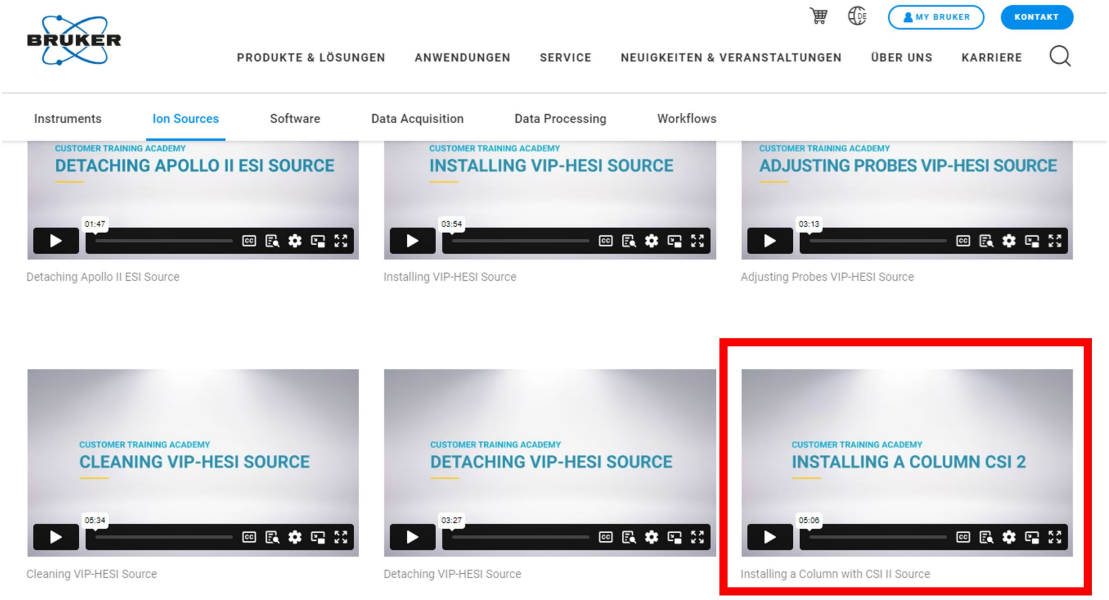

Figure 4.1: Screenshot of Video Customer Training Acadamy website

5 Specifying the separation column in Hystar Software

The following software-related steps describing column setup in Hystar are specific for Bruker nanoLC users.

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

- Define the separation columns in the **Columns** tab of the configuration editor (see section below).
  - Changing the columns definition does not affect any defined instrument configuration.
- The plug-in supports user defined LC columns.

How to add a new column

1. Open the **Configuration Editor**.
2. Click the **Columns** tab.

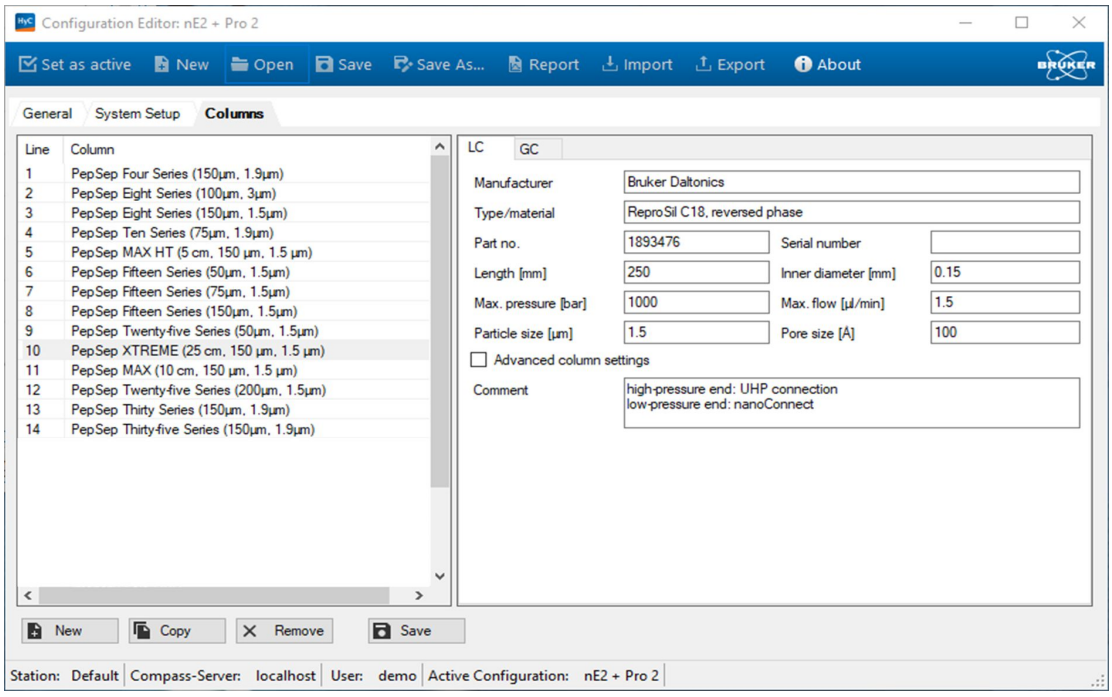

Figure 5.1: Columns tab with the list of predefined columns

3. Click **New** to add a new column.
4. Enter a name for the new columns in the **Column Name** dialog.

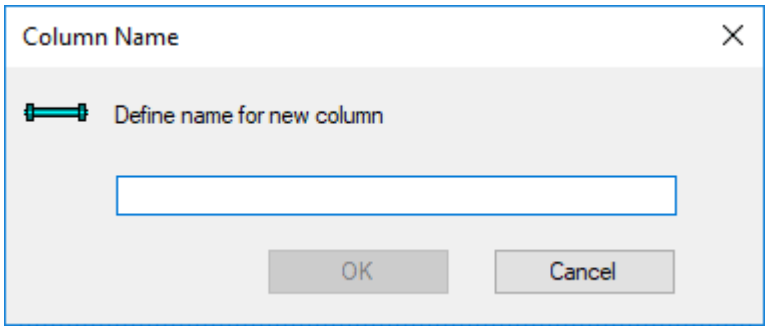

Figure 5.2: Column name dialog

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

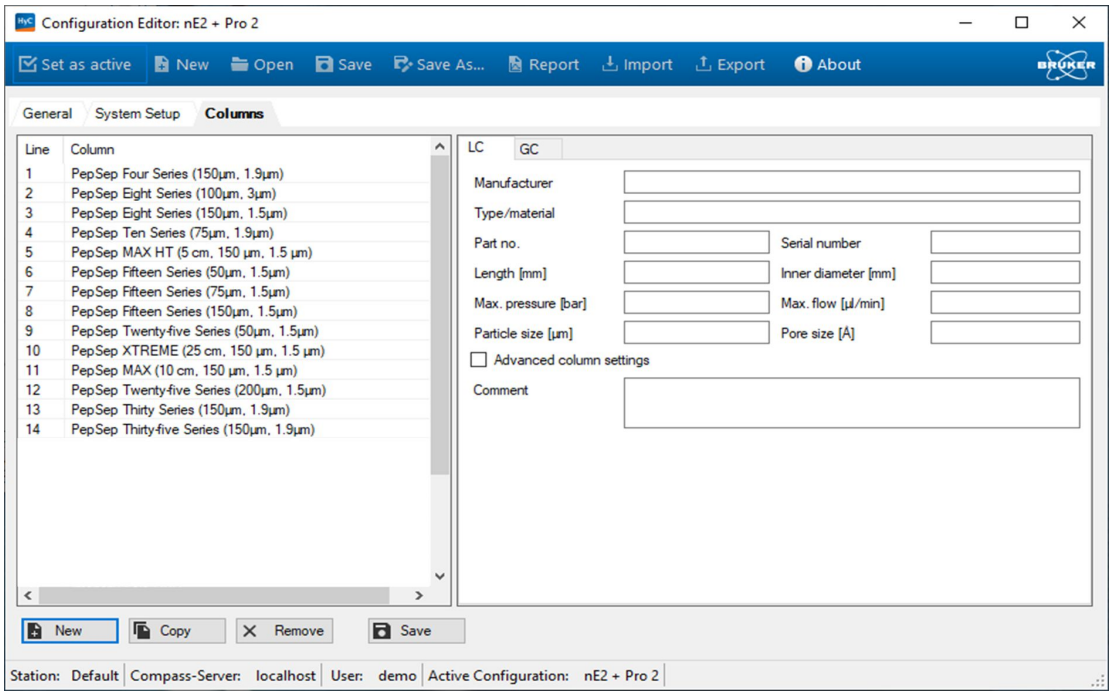

Figure 5.3: New column added to column list

6. Enter specifications of the new column (mandatory input: Length [mm], Inner diameter [mm], particle size [µm], or pore size) and/or a comment.

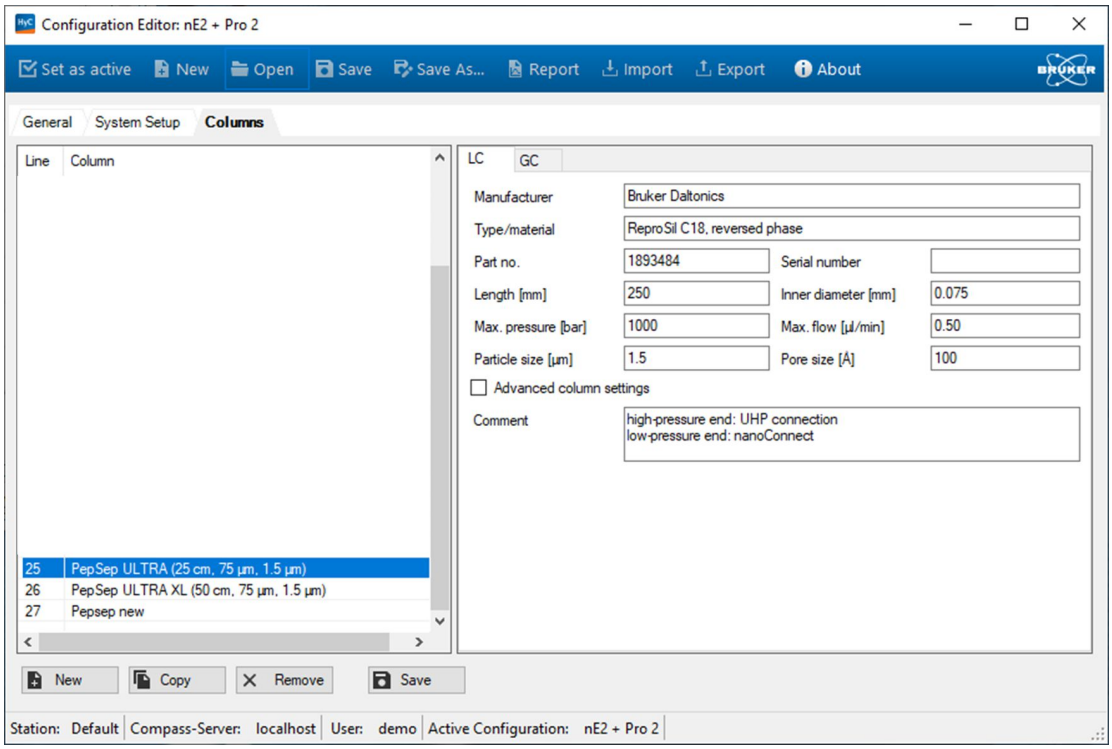

Figure 5.4: Columns tab with new column and corresponding specifications

7. Click **Save** to save the new specifications.
   ⇨ If the **Save** button is greyed out, try selecting a different column on the left side, then return to the previous column and click **Save**.
8. Click **X** in the upper right-hand corner to close the **Configuration Editor.**

6 Column & emitter installation

For any further assistance regarding the CaptiveSpray 2 Emitter, CaptiveSpray Source and Column Toaster please refer to the CaptiveSpray user manual and use the installation videos linked in *Video installation guides [9]*.

Connect the separation columns carefully to the CaptiveSpray 2 Emitter to ensure stable electrospray and good chromatographic performance.

The following software-related steps describing column setup in Hystar are specific for Bruker nanoLC users.

Use LC-MS grade solvents to prepare 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.

1. Open the emitter lock of the CaptiveSpray source.
2. Remove the protecting caps from the CaptiveSpray 2 Emitter and carefully insert the emitter.
3. Close the emitter lock.
4. Select **Separation column** in Hystar.

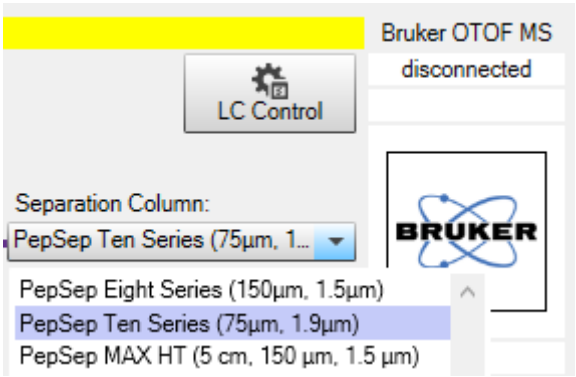

Figure 6.1: Column selection

5. Click the **Direct Flow** gearwheel in LC Control in Hystar.
6. Choose appropriate flow rate.
7. Click **Run.**

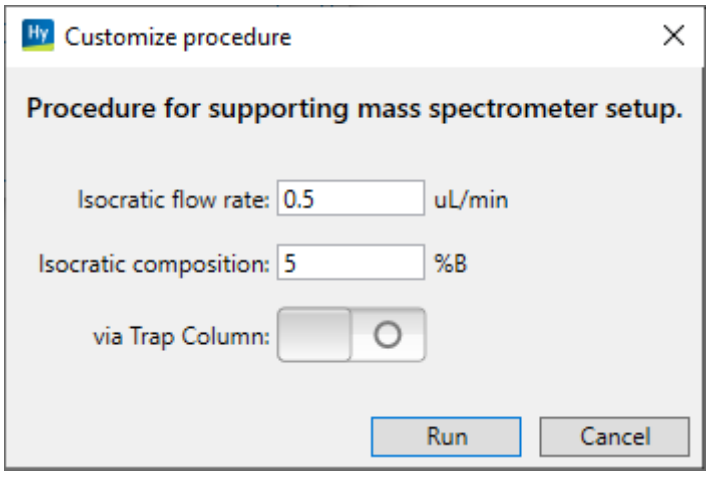

Figure 6.2: ***Direct flow dialog in the LC Control***

8. Attach the transfer line to the column when a droplet emerges from the transfer line.
9. Make sure the column flow direction is correct (as depicted on column label).
10. Optically inspect the column outlet integrity when a droplet emerges from the column.
11. Mount the column finger tight into the union and loosen this connection by a quarter and retighten after ~20 seconds.
    ⇨ Do not use any tools for tightening and do not overtighten!

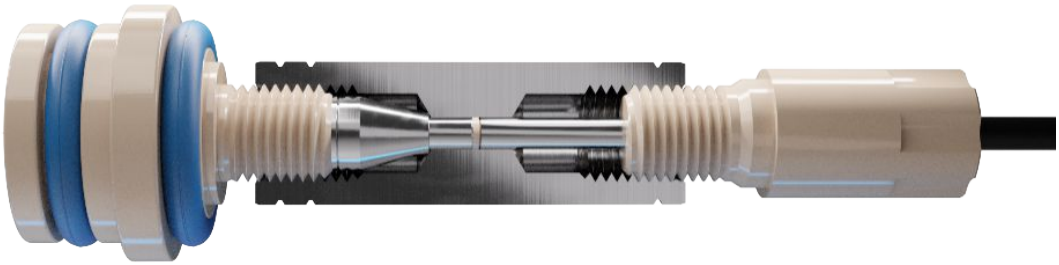

Figure 6.3: CaptiveSpray 2 emitter and PepSep column zero-dead-volume connection in union

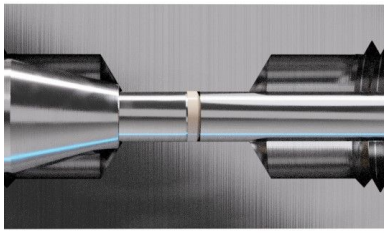

Figure 6.4: Magnification of the direct connection (zero-dead-volume bottom seal)

12. Carefully rotate column toaster under the column and secure the column in place using the oven fixation screw and the grounding screw.
13. Tighten the thumbscrew at the bottom of the column toaster to fix the position.
14. Close the column oven's lid.
15. In Hystar, click on the **Preparation** gearwheel in LC Control.
16. Activate **Include column preparation**.
17. Click **Run**.

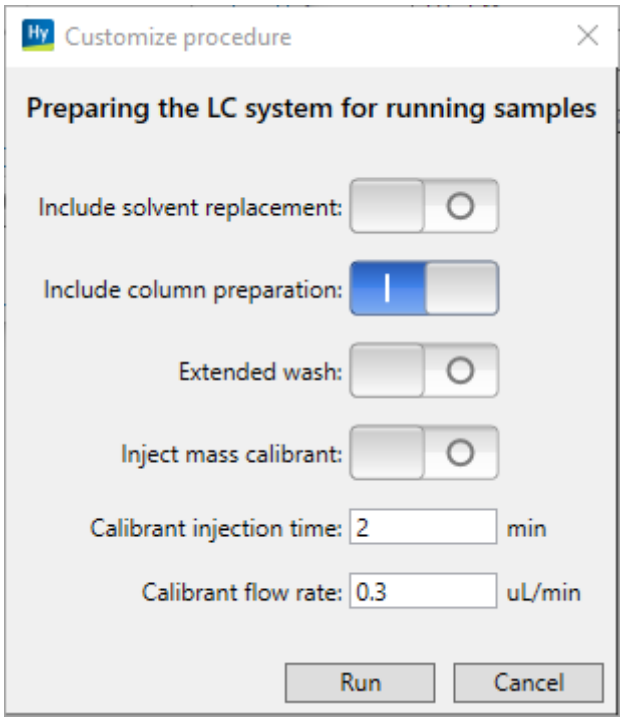

Figure 6.5: Preparation dialog in LC Control (Hystar)

18. When columns are not in use, use idle flow as stated in chapter *Operating conditions [▷ 18]* to avoid damaging the emitter tip by high voltage.

⚠ WARNING

Be aware of rapid pressure changes when starting or stopping the flow!

Pump acceleration and deceleration can cause significant pressure increases.

7 Detaching column & emitter

For any further assistance regarding the CaptiveSpray 2 Emitter, CaptiveSpray Source and Column Toaster please refer to the CaptiveSpray user manual and use the installation videos linked in *Video installation guides [9]*.

The following software-related steps describing column setup in Hystar are specific for Bruker nanoLC users.

Use LC-MS grade solvents to prepare 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.

▶ Deactivate the source in timsControl by deselecting the checkbox in the **Source** tab.

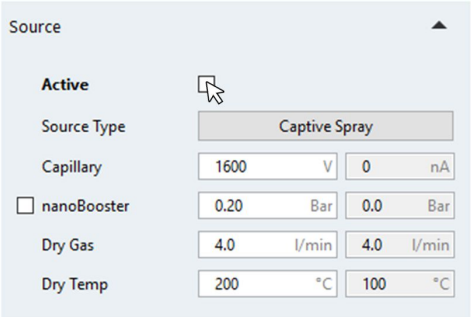

Figure 7.1: Source settings in timsControl software

1. In LC Control click on **Column** gearwheel, activate **Detach separation column.**
2. Click **Run.**

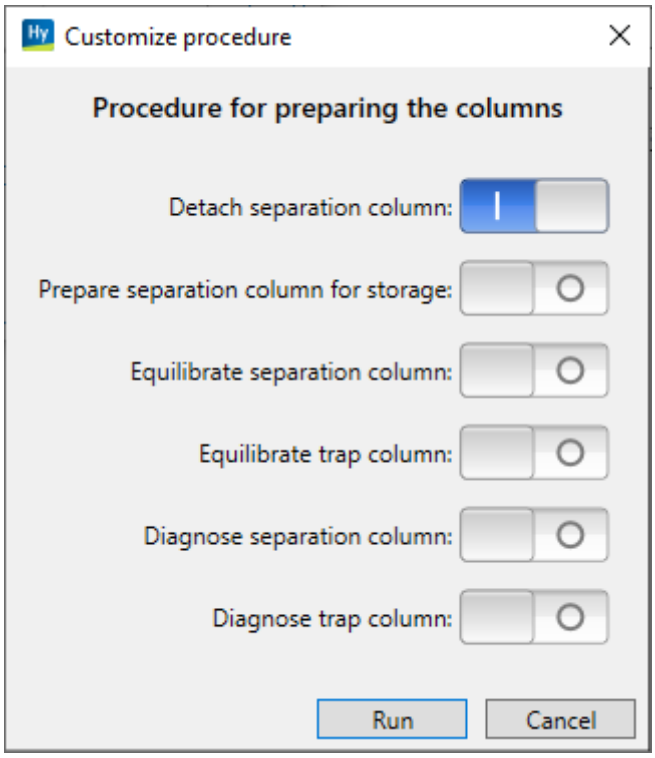

Figure 7.2: Column dialog in LC Control (Hystar)

1. Open the column toaster lid.
2. Release the grounding screw and the oven fixation screw.
3. Loosen the thumbscrew at the bottom of the column toaster and slide it back.
4. Detach the column from the emitter union.
5. Detach the transfer line from the column.
6. Open the emitter lock and remove the emitter.
7. Carefully put on the protective caps for column & emitter to ensure safe storage.

8 Operating conditions

⚠ WARNING

Delicate components.

Handle all components with care.

- ▶ Avoid overflexing the columns and damaging the emitter tip.
- ▶ Ensure the operating area remains clean to prevent any damage or contamination to the parts in use.

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

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.

NOTICE

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.
11. Enable LC idle flow when the system is inactive to prevent any damage or blockage of the column and emitter. The idle flow typically 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 LC status window in HyStar and select **Preferences**.
- Set the **Idle Flow** rate and the composition of the mobile phase.
- If the application required use of a trap column, enable idle flow via the trap column.

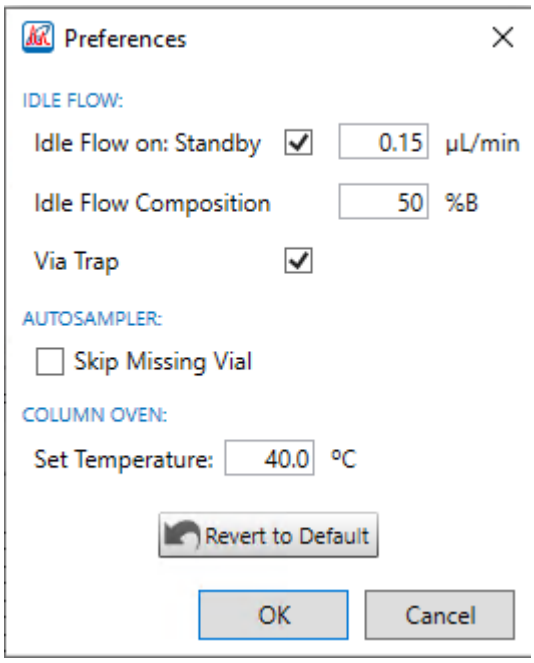

Figure 8.1: Preferences dialog

9 Column care for storage and cleaning

9.1 Cleaning

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

⚠ WARNING

Severe 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 2 µL 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

Table 9.1: Gradient for column cleaning procedure

9.2 Storage

The following steps are necessary to store the PepSep column for a period of time.

1. Clean the column as stated in *Table 9.1 [▶ 20]*.
2. Place a vial with a storage liquid (e.g. 100% acetonitrile) in position 1 (rear position) of the autosampler's wash module.
3. Use the **Prepare separation column for storage** procedure (Hystar).
   ⇨ The separation column is flushed with ten column volumes.
4. Start **Detach Separation Column** procedure (Hystar).
5. Cap the ends of the column.

10 Troubleshooting

The following software-related steps describing column setup in Hystar are specific for Bruker nanoLC users.

For general troubleshooting:

1. Click on **Smart diagnostics** in LC Control (Hystar).
2. Activate **Flow restriction test** and **Leak test**.
3. Follow instructions.

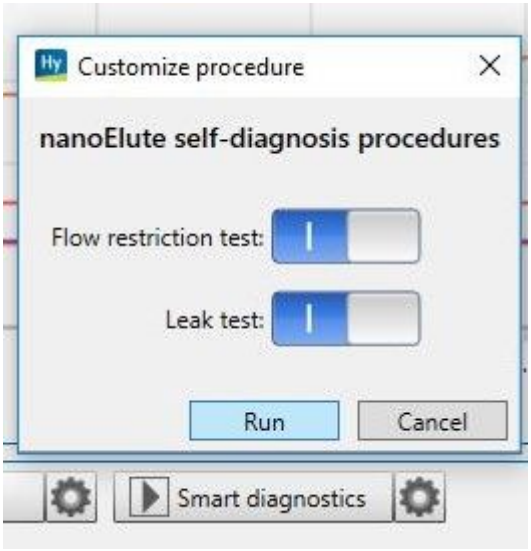

Figure 10.1: Smart diagnostics dialog

For diagnosing column issues:

1. Click **Column** gearwheel in LC Control.

   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.

10.1 Backpressure is low

Reasons for low backpressure:

- Connection between the transfer line and the column is not tight:
  - Look for small liquid droplets
  - Retighten the connection
  - Try new/other transfer line

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

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

Should none of these steps help to resolve the issue, change the column.

10.2 Backpressure is high

Reasons for high backpressure:

- Blockage to the emitter:
  - Disconnect the emitter and check the backpressure again.
  - Check the emitter using the CSI Transfer Line Kit (#1886191)
    For 10 µm ID Emitter use 50-500 nL/min
    For 20 µm ID Emitter use 50-5000 nL/min
    Wait for at least 10 minutes and check emitter tip for small liquid droplet
  - If no droplet is observed, exchange the emitter.
- Blockage on the column:
  - A column exchange may be necessary.

10.3 Loading time too long

Run the test routine **Diagnose separation column** in Hystar.

11 Symbols

The following symbols appear on the product labeling.

	Manufacturer
	Catalogue number
	Research use only
	Consult instructions for use

12 Manufacturer

Bruker Daltonics GmbH & Co. KG
Fahrenheitstrasse 4
28359 Bremen
Germany

12.1 Contact

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Application support:
Email: esi.appl.support@bruker.com
Tel.: +49 421 2205-493
Sales
Web: *https://store.bruker.com/*

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Bruker Scientific LLC
40 Manning Road
Billerica, MA 01821
USA
Service support:
Email: ms.support.us@bruker.com
Tel.: +1 978 6633660-1445

12.2 Webstore

PepSep Consumables as well as other components, can be ordered directly from the *https://store.bruker.com/en-DE/* or via email to your local Bruker office or representative.

13 Equipment Disposal

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

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