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EASY-Spray Columns

Guidance for column set up and installation

Tips to maximize column lifetime

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EASY-Spray Column Tips and Tricks

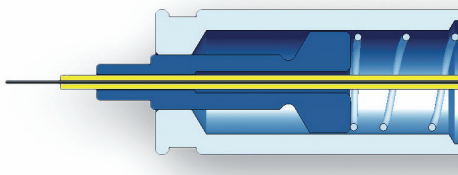
This document provides guidance for Thermo Scientific™ EASY-Spray™ column set up, installation and storage, as well as tips-and-tricks to extend column lifetime and best practice for nanoLC operation.

Definitions

Initial Operation: First time use or after a period of 8 hours without flow.

Standby/Idle: Period after a sequence where a low flow rate and voltage are applied.

Storage: Periods of over 1 day when the column will be stored. Columns can be stored either on the source, or returned to their box.



1

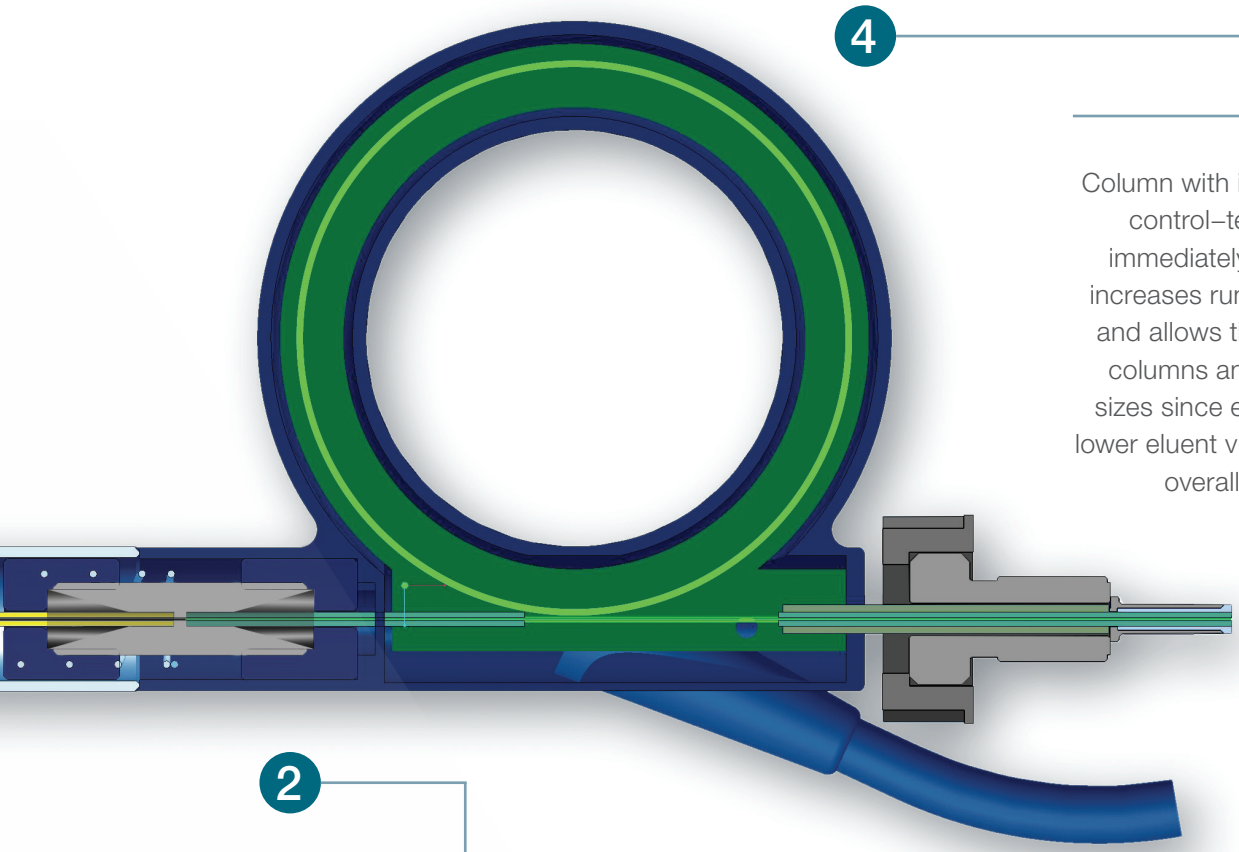
Precision positioned glass emitter – quality controlled and polished fused silica emitter with a uniform inner diameter of 7 µm delivers an exceptionally stable spray and extended emitter cap to protect tip from breakage.

The EASY-Spray Ion Source User Guide, (Literature Code: 60053-97260) is a valuable reference document for initial set up and troubleshooting of the source.

EASY-Spray column.

EASY-Spray source.

EASY-Spray column design features



4

Column with integrated temperature control–temperature control immediately before the MS inlet increases run to run reproducibility and allows the use of even longer columns and/or smaller particle sizes since elevated temperatures lower eluent viscosity and reduce the overall back pressure.

2

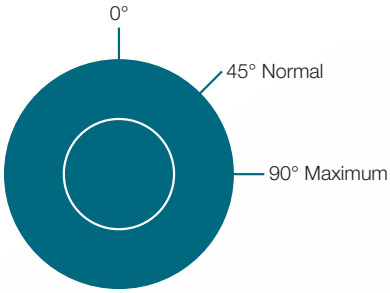
Integrated through-hole designed union ensures a high voltage connection is placed on the eluate between the column outlet and emitter outlet.

3

Thermo Scientific™ nanoViper™ fitting: Easy-to-use, stainless steel fingertight fitting to 1200 bar eliminates column damage due to over-tightening and experimental damage due to bad connections.

EASY-Spray column set up

Upon first use of a new EASY-Spray column, adjust the emitter position in the EASY-Spray source using the Emitter Positioning Tool (P/N ES232), as per the instructions in the EASY-Spray Ion Source user manual. After properly setting the emitter position, it is safe to proceed with mounting the EASY-Spray column.



Step 1: Connect the column by carefully tightening the nanoViper connection until the point of first resistance (also known as 0º mark).

Step 2: Continue to tighten the black knurl to a position between 0° and 45º past 0º mark.

NOTE: Do not overtighten the nanoViper fitting otherwise the connection might be damaged.

EASY-Spray column initial operation

Start with a flow rate of 50 nL/min and increase stepwise in 50 nL/min steps until the maximum column pressure is reached or system pressure is reached. Each step should last for 2 minutes.

Mobile phase: Use a mobile phase that reflects the starting conditions of the desired gradient. After this initial conditioning phase, reduce the flow rate to method starting conditions and follow the instructions for routine operation as detailed on page 5.

EASY-Spray column routine operation

Start the flow through the column by slowly ramping to 75% of the flow rate, indicated in the test conditions on the Quality Assurance Report provided with every EASY-Spray column.

Wait until the pressure stabilizes then apply the intended flow for the experiment.

As soon as the pressure has stabilized apply spray voltage, starting at 1.5 kV and wait for the spray to stabilize. This might take a few minutes. If a stable spray cannot be achieved slowly increase the spray voltage up to a maximum of 2.5 kV.

Once a stable spray is established make sure it is stable for 5 minutes at both high aqueous (95% water) and high organic conditions (70% acetonitrile). If the spray becomes unstable under these conditions increase the spray voltage to a maximum of 2.5 kV.

Remember

The LC method must contain a 'column equilibration' step which should be consistent with minimum of 5× the column volume at starting conditions.

Standby/idle conditions

After a sequence has finished it is recommended that the instrument continues to run to ensure that there is flow through the emitter while applying voltage

For Thermo Scientific™ EASY-nLC™ systems set an idle flow rate similar to the standard one for the given column.

For Thermo Scientific™ UltiMate™ 3000 RSLCnano or other nanoLC systems set an idle flow rate similar to the gradient flow rate with 70% B and ensure that the emitter voltage is switched on.

Remember

Voltage should be applied at all times when flow is set on the column.

Disconnecting EASY-Spray columns

Step 1: Turn off the voltage to the emitter.

Step 2: Stop the flow and wait until the EASY-Spray column is depressurized.

NOTE: Never remove the EASY-Spray column if the inlet pressure is above 5 bar (70 psi). This can damage the EASY-Spray column.

EASY-Spray column storage

Step 1: Before storing columns ensure that the sample is completely eluted from the column. This can be done by prolonged washing (30 min) with a high concentration of mobile phase B. Leave the emitter voltage on so that eluted peptides will not deposit on the outside of the emitter.

Tip: It is good practice to make the final injection in a sequence a blank injection.

Step 2: Run a solution of 70% v/v acetonitrile in water at the flow rate used to run samples through the EASY-Spray column.

Tip: Remember to keep the voltage on for this step

Remember
After storage, run the 'Column Initial Operation' protocol.

Increase the lifetime of your EASY-Spray column

During routine maintenance or troubleshooting of the HPLC system, ensure that the EASY-Spray column is removed from the flow path. To avoid pressure shocks do not disconnect the column until it is at, or below, 5 bar pressure.

Ensure sample cleanliness by removing excessive particulates and salts. This can be achieved by either using SPE, or use of a trap/pre-concentration column (see sample preparation considerations on page 10).

Ensure the column is conditioned correctly after it is installed (see initial operation section on page 4).

If unstable spray is observed, increase the voltage up to a maximum of 2.5 kV in incremental steps.

Increase the lifetime of your emitter

The biggest contributing factor to short emitter lifetimes is when a voltage is applied with no flow passing through the column and emitter. This can cause undue stress, which may compromise the emitter integrity.

Note: Avoid long periods where voltage is applied to the emitter without flow being delivered through the emitter. It is recommended to switch off the spray voltage before switching off the flow to the EASY-Spray column.

In a two-column set up on the EASY-nLC systems ensure sample loading onto the trap column is not excessively long (less than 5 minutes). There will be no flow through the emitter during this time.

Keep the emitter clean and free from debris, as contamination can affect the spray quality. Instructions for cleaning the emitter tip are given in the EASY-Spray Ion Source user guide.

Guidance for EASY-Spray users observing poor baseline stability (unstable spray) without "sputtering" at emitter tip

Step 1: Align the EASY-Spray source using the emitter positioning tool according the EASY-Spray Ion Source User Guide. This will set the X and Y axis positions.

Step 2: Insert the EASY-Spray column into the EASY-Spray source with 50% B at the flow rate being used in the method.

Step 3: Using the MS tune software, acquire a baseline. For instructions on acquiring a TIC, refer to the specific software guide supplied with your specific MS configuration.

Step 4: Adjust the position of the EASY-Spray column emitter using the Z-axis control knob on the source, while observing changes in the TIC. The goal is to minimize the instability in relation to the baseline intensity.

Remember

Detailed instructions for locating the set screws are given in the EASY-Spray Ion Source user guide.

Guidance for EASY-nLC users experiencing spray stability issues

Step 1: Run Purge scripts for A and B

Step 2: Run Flush Air scripts for A, B and S

Step 3: Run Pump A and B leak test scripts.

Step 4: Run system leak test. Ensure the column is removed and use a nanoViper union to connect the column-out and waste-in capillaries.

The system has to pass all leak tests.

Step 5: Clean the ion transfer tube on the mass spectrometer according to instructions.

Step 6: Flush the waste line with different percentages of solvent B (e.g. 10%, 50%, 90%, 25–50 µL each) to remove build up of salts and other matrix components.

Step 7: Replace the existing waste line with a new one.

Step 8: Only for EASY-nLC 1000 systems. If spray stability problems persist after all above mentioned steps you can exchange the waste line with a longer one which has a smaller inner dimension (e.g. 20 µm × 950 cm, P/N 6041.5122). This may improve spray stability in some cases.

Before implementing guidance steps 1–8 consider the following points:

1: It is not recommended to replace the venting tee with a nanoViper union in a direct injection (one-column) set up since this will result in impaired gradient reproducibility.

2: Please note that the addition of the 20 µm ID × 950 mm length waste line leads to an increased system backpressure when solvent is pumped through this line. This has several consequences which should be considered carefully before installing the longer waste line.

- During sample loading the flow is split at the venting tee. The split ratio depends on the respective backpressures of the column and the waste line. Hence, the increased backpressure on the waste line will result in an increased flow percentage directed through the column, most likely between 5–10%. Therefore, make sure that the samples do not contain high levels of salt and other contaminations which would foul the MS system.

- Sample loading in a pre-concentration (2-column) set up will take longer if Intelligent Flow Control (IFC) is used.

- The backpressure test is no longer usable since it relies on the backpressure values observed with the standard configuration.

Disclaimer
This guidance is intended to be part of the overall troubleshooting process and may not work in all circumstances. Before attempting this tip the EASY-nLC system has to be tested for fluidic leakages, which can also result in spray instability.

NOTE: These steps may require engineer assistance.

Best practice in nano LC–MS

Mobile phase preparation

Always use LC-MS grade solvents and additives. Lower grade reagents will result in premature column aging and emitter clogging, and a higher background signal in the MS.

Use only fresh, degassed solvents which are compatible with the column packing material. Degas the solvents for 30 minutes in an ultrasonic bath at 30–40 °C. Add volatile additives after sonication.

Switch only between mutually miscible mobile phases. Typical solvents and additives include acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), trifluoroacetic acid (TFA), formic acid (FA) and water.

Do not top up solvents but exchange solvents on a weekly basis. This minimizes the risk of large changes in solvent composition or bacterial growth.

Always use solvent bottles rinsed with water, acetonitrile and a mixture of both. This prevents the accumulation of contaminants in the bottles.

Do not externally filter solvents. Filtering using the in-line filters installed on the HPLC reservoirs is sufficient and it is recommended that the in-line filters on the solvent lines are always used.

Air bubbles in the mobile phase can cause spray instability.

Sample preparation considerations

Sample quality greatly affects column lifetime. To avoid premature clogging of columns and emitters, use samples that are free from particulates, salts, contaminants or undigested protein. If in doubt, consider an additional sample clean-up step and a pre-concentration (two-column) set up to protect the analytical column. Thermo Fisher Scientific provides a suitable range of Nano Trap columns and cartridges which can be chosen to match the column chemistry selected. Visit: **thermofisher.com**

C18 based SPE tips allow fast removal of particulates, salts and undigested protein.
Thermo Fisher Scientific offers a variety of suitable products (P/N 60109-201, P/N 60109-209, P/N 60109-401, P/N 60109-412). Visit: **thermofisher.com**

The Thermo Scientific™ SOLAμ™ SPE 96 well plates allow reproducible and robust clean up peptides, with the potential to pre-concentrate your sample 20 fold. Removing the blow down step, often associated with larger bed weight SPE products, helps maintain sample integrity (P/N 60209-001, P/N 60209-002, P/N 60209-003, P/N 60209-004, P/N 60209-005).

Instrument considerations

When operating an EASY-nLC 1000 or 1200 system run the purge (2 iterations) and flush air (12 µL) scripts daily or before starting a long sequence. This will minimize build-up of air in the system.

When operating an UltiMate 3000 RSLCnano system try to keep the High Pressure Gradient (HPG) pump running. After longer periods of no flow, purge both blocks and the flowmeter of the HPG pump in order to remove air bubbles from the system.

Regularly check for leaks in the nanoLC system since leaks can cause flow instability and retention time variability.

Column and equilibration volumes

The table below is a guide to the volume required at the start (or end) of a gradient to equilibrate the column ready for the next injection. As a guide use the stated 5× column volume, but more may be used as needed.

Column	Length (mm)	ID (µm)	Pressure (bar)	Volume (µL)	Column equilibration volume (µL)*
ES900	150	75	500	0.66	3.5
ES901	150	50	800	0.29	1.5
ES902	250	75	1000	1.10	5.5
ES903	500	75	1000	2.21	11.0
ES904	150	75	800	0.66	3.5
ES905	750	75	1200	3.31	16.5
ES911	150	75	800	0.66	3.5
ES912	150	75	500	0.66	3.5

* Figures are rounded to nearest 0.5 µL

For further information on Thermo Scientific™ low-flow columns, sample prep and accessories visit: **thermofisher.com/EasySpray**

For technical assistance visit: **thermofisher.com/chromexpert**

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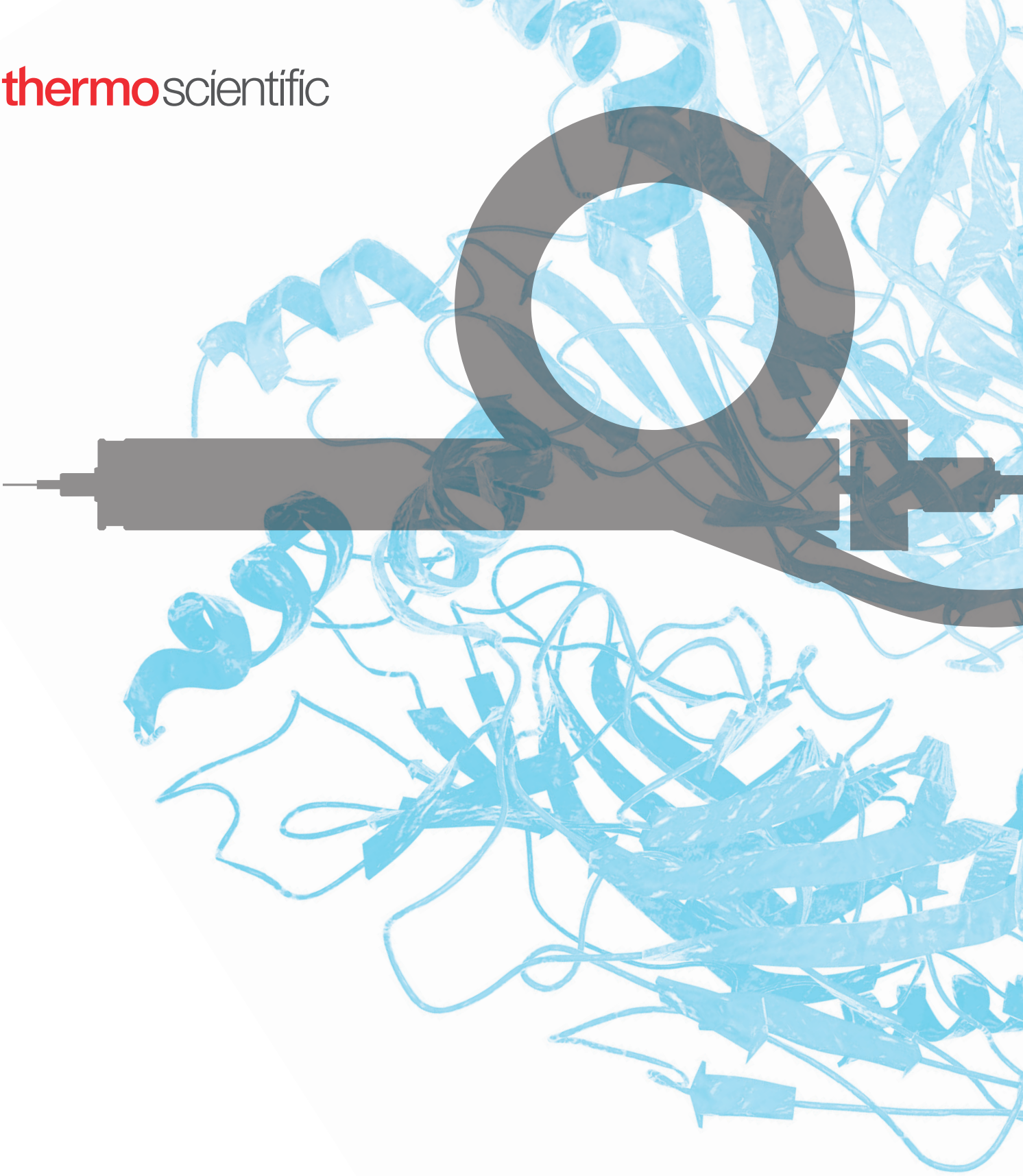

Find out more at **thermofisher.com/EasySpray**

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