APPLICATION BRIEF 22013

Clean-up procedures for nanochromatography columns

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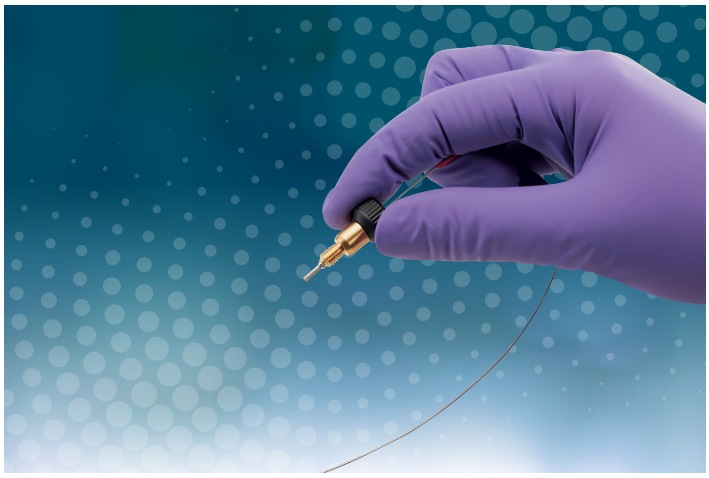
Introduction

Nanochromatography columns, just like analytical flow columns, can retain organic material over time leading to a loss of chromatographic performance (i.e., retention time inconsistency, poor resolution, or peak shape) and bleed either in the form of high organic eluting peaks or a consistent background. In nanoflow systems, cleaning a column may be more complex due to differences in plumbing configurations. In general, there are two ways to clean a system: plug injections of strong organic solvents and see-saw gradients of acetonitrile. If a trap is plumbed inline, then the autosampler may not be able to access the column with a plug type injection. In this case the user should combine the two techniques by using a plug injection to clean the trap and see-saw gradients to clean the column.

Important notes

- Clogging of the column can be attributed to two problems: particulate buildup from solvents, samples, or flow path, and organic buildup over time. The two phenomena can be distinguished by the rate of pressure increase. Particulate clogs produce a sudden increase in back pressure while organic buildup generally produces a slow and steady increase in pressure.
  - Particulate clogging issues can be mitigated by using a Thermo Scientific™ Viper™ inline filter inserted post autosampler.



  - Gradual organic buildup issues can be mitigated with preventive maintenance utilizing the see-saw and plug injection procedure described in this application brief.
- All nano systems show a small number of organic eluting contaminants from mobile phases and liquid chromatography (LC) system components. These naturally occurring levels can be determined when a new trap columns and LC column have been installed and cleaned with a few gradients. A water blank can provide the needed data.

Materials required

- Thermo Scientific™ ChromaCare™ LC-MS biologics flush solution (Fisher Scientific P/N MB1241)
- Thermo Scientific™ Viper™ inline filter, titanium, 0.5 µm frit pore size (P/N 6036.1045)

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Protocol

See-saw gradients

1. Build a short gradient using your standard elution system. To the end of the gradient, add a series of rapid increases and decreases in the amount of organic solvent steps creating the see-saw cleaning gradient. See example (Figure 1).

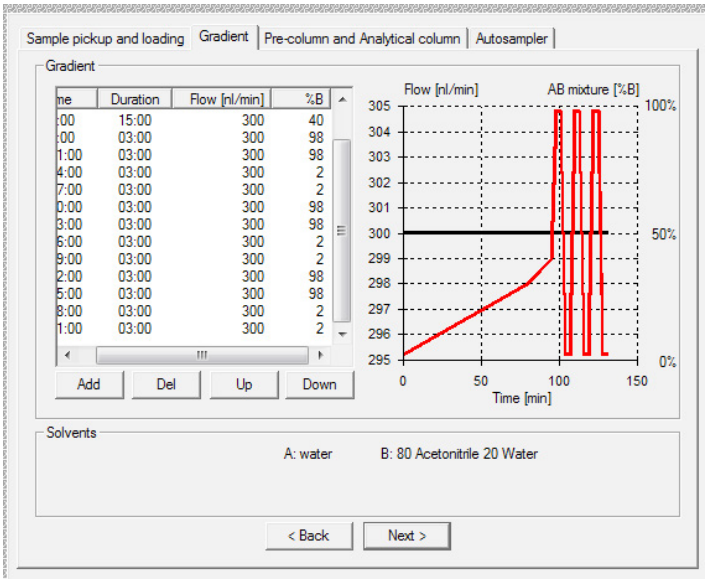

Figure 1. Cleaning gradient example

2. Run the gradients with the nano spray voltage on to eliminate buildup on the emitter tip which could cause spray problems.

3. Monitor the background data, repeating runs until the chromatogram meets your needs.

4. Run a standard to verify column performance (standard conditions are included on the QAR provided with the column).

Plug injections

1. Place one milliliter of ChromaCare biological flush solution into a glass vial, if your autosampler permits it.

2. Inject 10 µL of the solution using a short gradient to elute the organic contaminants form the column.

Note: Plug injections in combination with see-saw gradients are an excellent combination and are suggestion if the trap is plumbed inline, as the autosampler may not be able to access the column with a plug type injection.

Current versions of product instructions are available at **thermofisher.com/chromexpert**

Learn more about Bio LC columns and products at **thermofisher.com/biolc**

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