PharmaFluidics
The micro-Chip Chromatography Company

Instruction Sheet

PharmaFluidics µPAC™ C18 Trapping column

Trapping Column

General description

PharmaFluidics µPAC™ C18 Trapping columns are micro-fabricated HPLC columns specifically designed for use in conjunction with a µPAC™ analytical column for reversed-phase separation of small molecules and peptides. The separation beds of the µPAC™ columns are fabricated by etching interstitial volumes from a crystalline silicon substrate following lithographic definition of a pillar array. This creates a stationary-phase support that is organised into a reproducible, perfectly-ordered pattern. Concatenation of several of these channels enables the fabrication of long, yet compact, columns. The key characteristics of the µPAC™ Trapping column bed are:

- pillar diameter 5 µm
- inter-pillar distance 2.5 µm
- pillar length/bed depth 18 µm
- external porosity (Vinterstitial/Vtotal) 59%
- bed channel width 2 mm
- bed length 10 mm
- column volume 900 nL

To increase surface retention, the pillars are superficially porous and have a shell thickness of 300 nm and pore sizes of 100 to 200 Å. To create a hydrophobic stationary phase, the porous surface has been uniformly modified with octadecyl alkyl chains (C18).
For protection against mechanical damage, the silicon µPAC™ Trapping column is encased in an aluminium jacket. Attached to both ends of the µPAC™ Trapping column are 10 cm × 40 µm I.D. fused silica protected by $^{1}/_{16}$" PEEK tubing (blue). Each end is equipped with $^{1}/_{16}$" stainless steel fitting to enable direct connection to a HPLC valve or via a vented-tee configuration. To guarantee column performance, the PEEK tubing and fittings must never be removed.

Never remove the fittings on the PEEK tubing extending from the aluminium jacket, nor cut the PEEK tubing. This will damage the µPAC™ Trapping column and prevent further use.

Operating guidelines

- Upon receipt, inspect the column. If there are any signs of damage, notify your local PharmaFluidics representative immediately.
- Record the column type and serial number (located on the back of the aluminium jacket), purchase date and operating limits.
- Keep a record of column use with the provided test chromatogram. This record will be essential in diagnosing possible chromatographic problems.

To prevent damage, handle the µPAC™ Trapping column, capillaries and accessories with care. Compared to conventional HPLC trap columns, no special requirements are needed.

Preparation for use: The µPAC™ columns are filled with 70% acetonitrile. Before use, flush the column with, *e.g.*, 100% acetonitrile or methanol and then with at least 5 µL of the desired starting solvent.

Mobile phase: Only use filtered and/or degassed LCMS-grade mobile phases. To prevent crystallisation and/or precipitation of solutes, alternate between miscible mobile phases, *e.g.*, acetonitrile (ACN), methanol (MeOH), isopropanol (IPA), trifluoroacetic acid (TFA), formic acid (FA). A high-pressure in-line filter between the pump and injector is recommended.

Column pressure: Maximum operating pressure is 350 bar (5,000 psi).

Flow rate: The optimal flow rate for a water-acetonitrile mobile phase solvent system is 10 µL/min at a backpressure of 15 to 30 bar.

µPAC™ Trapping columns can be operated at flow rates between 0.1 and 20 µL/min (do not exceed the maximum column pressure of 350 bar).

Sample solvent: To maximise column efficiency, the recommendation is to dissolve samples in 0.1% TFA containing a low percentage of organic modifier (*e.g.*, 1% acetonitrile). For a double pump in-valve trapping configuration, a loading buffer comprised of 1% acetonitrile, 0.1% TFA is strongly advised.

Sample capacity: To analyse tryptically-digested samples, the equivalent of 1.0 µg total protein can be injected without overloading the column (note, however, that the recommended maximal protein load on the 50 cm µPAC™ analytical columns is 0.5 µg). When analysing small molecules or metabolites, the recommended sample capacity is 5 ng/molecule.

Column temperature: Maximum operating temperature is 60°C (140°F).

pH range: Avoid using mobile phases < pH 1.5 and > pH 7.0.

Recommended configurations

The µPAC™ Trapping column is completely symmetrical and can be used in both directions without risk of damage to the separation bed.

Depending on the HPLC system and personal preference, two configurations are possible:

- **In-valve:** the µPAC™ Trapping column is connected directly to the HPLC switching valve.
- **Vented-tee:** the µPAC™ Trapping column is configured in-line with the analytical column via an internal reducing connector T-piece.
- A 1/16" spanner is required to attach the µPAC™ Trapping column to a HPLC valve or vented-tee. Ensure that the fitting is finger tight and then further tighten for a maximum ¼ turn. **Do not overtighten!**

Column operation

- When developing a chromatographic method, remember that the µPAC™ Trapping column has an internal volume of 900 nL.
- Following elution of the analytes, the µPAC™ Trapping column requires re-equilibration to starting conditions with at least 10 µL of the initial mobile phase.

Column maintenance

- The µPAC™ Trapping column is relatively resistant to clogging. Nevertheless, injecting samples that contain particulate matter with a diameter > 0.5 µm is inadvisable.
- Filtering samples before injection with a 0.5 µm cut-off filter can protect the µPAC™ Trapping column from potentially clogging.
- If the backpressure increases above 300% of the original value, systematically evaluate from the HPLC pump to the inlet of the µPAC™ Trapping column that the solvent flow is not obstructed.
- If the increase in backpressure is due to the µPAC™ Trapping column, reverse the flow direction and flush with 5 - 10 column volumes of mobile phase. This should return the µPAC™ Trapping column to the original backpressure.
- The µPAC™ Trapping column is symmetrical and column performance is identical in both directions.

Column storage

- µPAC™ Trapping columns can be stored for short periods in most mobile phases.
- For prolonged storage, it is recommended that the µPAC™ Trapping column is flushed with 5 - 10 column volumes of a mobile phase with at least 70% acetonitrile or methanol in water.
- If the µPAC™ Trapping column has been used with buffered mobile phases, remove the buffer by flushing with 5 - 10 column volumes of a mobile phase with 50% acetonitrile or methanol in water and then flush with the storage mobile phase.
- Seal both ends of the µPAC™ Trapping column with the provided black plastic caps.
- Store the µPAC™ Trapping column in the supplied protective box.

Further information

For column specifications, pressure limits, pH range, tips and tricks including operational instructions, visit:
https://www.pharmafluidics.com/our-products/

For technical support visit:
https://www.pharmafluidics.com/contact-us/