PharmaFluidics
The micro-Chip Chromatography Company

Instruction Sheet

PharmaFluidics µPAC™ capLC C18 column

Analytical Column

General description

PharmaFluidics µPAC™ C18 capLC columns are micro-fabricated capillary flow HPLC columns specifically designed for reversed-phase separation of small molecules and peptides. The separation beds of the µPAC™ capLC 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™ capLC bed are:

- pillar diameter 5 µm
- inter-pillar distance 2.5 µm
- pillar length/bed depth 28 µm
- external porosity ($V^{interstitial}/V_{total}$) 59%
- bed channel width 1 mm
- bed length 50 cm
- column volume 10 µL

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™ capLC column is encased in an aluminium jacket. Integrated on both ends of the µPAC™ capLC column are 1/16" stainless steel unions. Via 1/16" connection tubing, this design expedites connection of the µPAC™ capLC column to any third-party LC vendor.

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™ capLC column, capillaries and accessories with care. Compared to conventional capillary-flow HPLC columns, no special requirements are needed.**

Preparation for use: The µPAC™ capLC columns are filled with 70% acetonitrile. Before use, flush the column with, *e.g.*, 100% acetonitrile or methanol and then with at least 10 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 for short gradients (≤ 30 min), 5 µL/min for intermediate gradients (60 min), and 2 µL/min for longer gradients (≥ 90 min). At a flow rate of 10 µL/min, the backpressure is between 180 and 220 bar. Void volume time is approximately 1 minute.

Depending on separation requirements, the µPAC™ capLC column can be utilised over a flow rate range of 1 to 15 µL/min (as long as the maximum column pressure of 350 bar is not exceeded).

Sample solvent: To maximise column efficiency, dissolve samples in the initial HPLC solvent (or in a solvent with a weaker elution strength than the initial solvent).

Injection volume: If the initial binding capacity of the solutes in the sample solvent is higher than in the starting solvent (typically the case for hydrophobic samples in ~99% water), it is possible to inject several microlitres (1 - 5 µL) of sample and concentrate on the column.

To minimise sample loading time, do not use a sample loop with a capacity > 20 µL.

Optimal column efficiency can be achieved for separating polar or small molecules by nanolitre injection volumes. This can be performed by user-defined partial loop injection programs (25-100 nL) or by an external nanolitre injection valve (4-20 nL) (VICI P/N: C4N-4004-.004EUHA).

Sample capacity: To analyse tryptically-digested samples, the equivalent of 2.0 µg total protein can be injected without overloading the column. When analysing small molecules or metabolites, the recommended sample capacity is 15 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.

Connection to a switching/injection HPLC valve

- Secure the µPAC™ capLC column into the column heater/compartment of the HPLC system with the µPATCH (P/N: 201902TLK).
- The µPAC™ capLC column is connected to a standard HPLC 6-port switching/injection valve with a 360 µm O.D. 50 µm I.D. fused silica capillary with a 1/16" connection (Figure 1).
- Remove the blind union plugs from the inlet and outlet of the µPAC™ capLC column. Connect the fused silica capillary extending from the HPLC 6-port switching/injection valve to the inlet union of the column. Ensure that the fitting is finger tight and then tighten further with a spanner for a maximum ¼ turn. **Do not overtighten!**

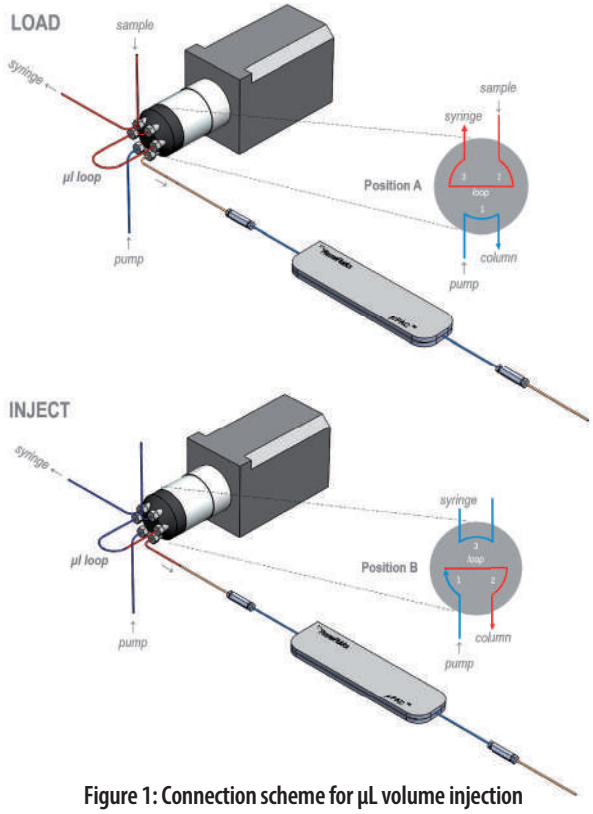

Figure 1: Connection scheme for µL volume injection

Connection to MS/UV detector

Once the µPAC™ capLC column is connected to the HPLC switching/injection valve, the outlet of the µPAC™ capLC column can be connected to a UV detector or mass spectrometer.

- Stop the pump flow and wait until the back pressure has decreased and stabilised.
- A 1/16" stainless steel low-dead-volume union (minimising column dispersion) is connected to the outlet tubing of the µPAC™ capLC column. Connect the union to a 360 µm O.D. 50 µm I.D. fused silica transfer capillary (UV) or directly to an ESI emitter (MS).
- Gradually step up the flow rate and droplets should appear at the tip of the capillary or emitter.
- A rapid increase in back pressure is indicative of either overtightening of the connector or clogging of the capillary/emitter.

Grounding

If the µPAC™ capLC column is connected to the ESI source of a mass spectrometer, the column must be correctly grounded.

- The µPAC™ capLC column is primarily composed of the semi-conductor silicon. If no precautions are taken to shunt the high voltage to ground, there is a dramatic chromatographic peak broadening effect.
- Before applying high voltage to the ESI emitter, ground the µPAC™ capLC column using the blue coiled grounding cable (swallow tail and crocodile clip options; P/N: 201901TLK).
- Attach the clip end of the cable to the stainless-steel union grounding point located near the outlet of the µPAC™ capLC column, as indicated in Figure 2.
- Attach the other end of the cable to a grounded point on the mass spectrometer or the chassis of the HPLC.

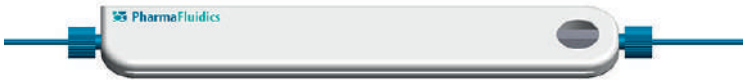

Figure 2: Image of the µPAC™ capLC column

Column operation

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

Column maintenance

- The µPAC™ capLC 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™ capLC column from potentially clogging.
- If the backpressure increases above 150% of the original value, systematically evaluate from the HPLC pump to the inlet of the µPAC™ capLC column that the solvent flow is not obstructed.
- If the increase in backpressure is due to the µPAC™ capLC column, reverse the flow direction and flush with 5 - 10 column volumes of mobile phase. This should return the µPAC™ capLC column to the original backpressure.

Column storage

- µPAC™ capLC columns can be stored for short periods in most mobile phases. For prolonged storage, it is recommended that the µPAC™ capLC column is
- flushed with 5 - 10 column volumes of a mobile phase with at least 70% acetonitrile or methanol in water.
  If the µPAC™ capLC 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™ capLC column with the provided PEEK blind union plugs.
- Store the µPAC™ capLC column in the supplied protective box.

Part numbers for spare parts and consumables

VICI 4-20 nL valve	C4N-4004-.004EUHA
VICI zero dead volume $^{1}/_{16}$" plug	ZP1*
µPAC™ Grounding Cable	201901TLK

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/