PharmaFluidics
The micro-Chip Chromatography Company

Instruction Sheet

PharmaFluidics 50 cm µPAC™ C18 nano-LC column

Analytical Column

General description

PharmaFluidics µPAC™ C18 analytical columns are micro-fabricated nano-HPLC columns specifically designed 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 50 cm µPAC™ analytical column bed are:

- pillar diameter 5 µm
- inter-pillar distance 2.5 µm
- pillar length/bed depth 18 µm
- external porosity ($V_{interstitial}/V_{total}$) 59%
- bed channel width 315 µm
- bed length 50 cm
- column volume 3 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™ analytical column is encased in an aluminium jacket. Attached to both ends of the µPAC™ analytical column are fused silica capillaries protected by PEEK tubing (blue). Each end is fitted with stainless steel unions that are closed with blind PEEK union plugs. To guarantee column performance, the PEEK tubing and fittings must never be removed.

Operating guidelines

- Upon receipt, inspect the column. If there are any signs of damage, immediately notify your local PharmaFluidics representative.
- 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 when diagnosing possible chromatographic problems.

To prevent damage, handle the µPAC™ analytical column, capillaries and accessories with care. Compared to conventional nano-LC columns, no special requirements are needed.

Preparation for use: The µPAC™ analytical 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 300 nL/min at a backpressure of 30 to 60 bar. Under these conditions, the void volume time is approximately 10 minutes.

The 50 cm µPAC™ analytical columns can be operated at flow rates between 0.1 and 2.0 µL/min (do not exceed the maximum column pressure of 350 bar).

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 for direct injection, do not use a sample loop > 5 µL. Loading the sample at a higher flow rate (max. 2.0 µL/min or 350 bar) substantially reduces sample loading time.

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 0.5 µg total protein can be injected without overloading the column. 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.

Connection to a switching/injection HPLC valve

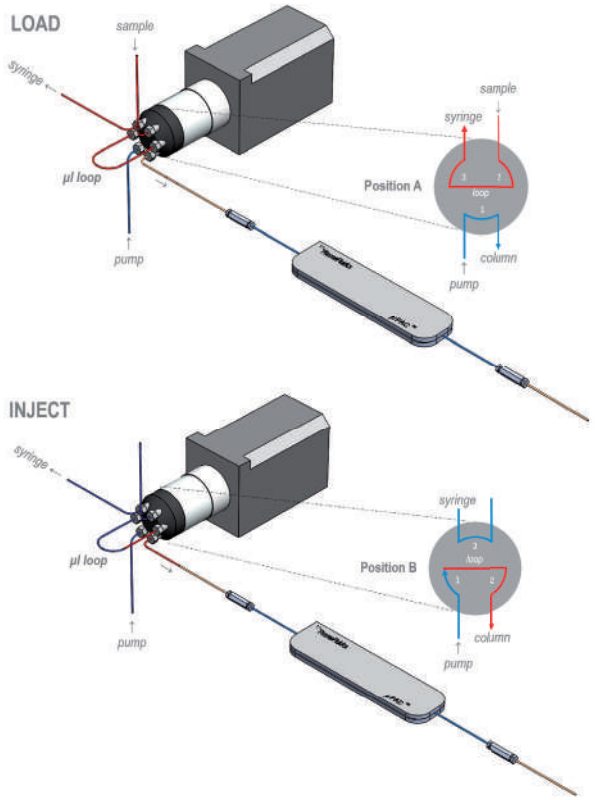

Figure 1: Connection scheme for µL volume injection

- Secure the µPAC™ analytical column into the column heater/compartment of the HPLC system with the **Click it!** µPATCH (P/N: 201902TLK).
- The µPAC™ analytical column is connected to a standard HPLC 6-port switching/injection valve with the blue **Connect it!** 25 µm I.D. × 25 cm PEEKsil™ capillary (P/N: 201904TLK) (Figure 1).
- Screw the black PEEK 1/16" finger tight fitting (labelled: to valve) of the PEEKsil™ capillary into the appropriate port on a standard HPLC switching/injection valve. **Do not overtighten!**
- To assess that the connection to the HPLC valve is leak-free, start the HPLC pump (@ 1% solvent B) and gradually step the flow rate up to 1.5 µL/min. The backpressure should be between 20 and 30 bar.
- Stop the pump flow and after the backpressure drops, screw the beige PEEK 1/32" finger tight fitting (labelled: to µPAC™) into the zero-dead-volume (ZDV) union on the inlet side of the µPAC™ analytical column. **Do not overtighten!**
- To assess that the connection of the PEEKsil™ capillary to the inlet of the µPAC™ analytical column is leak-free, apply the desired flow rate, *e.g.*, 300 nL/min (to a maximum backpressure of 320-350 bar).
- If a leak occurs at the interface between the PEEKsil™ capillary and the inlet capillary of the µPAC™ analytical column, disconnect the beige PEEK 1/32" finger tight nut and reassemble.
- At 500 nL/min, the column back pressure should be between 50 and 110 bar and solvent droplets should appear from the outlet of the µPAC™ analytical column.

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

Connection to MS/UV detector

Once the µPAC™ analytical column has been connected to the HPLC switching/injection valve, connect the outlet of the µPAC™ analytical column to a UV detector or a mass spectrometer.

- Stop the pump flow and wait until the back pressure has decreased and stabilised.
- A stainless-steel low-dead-volume (LDV) reducing union is attached to the outlet tubing of the µPAC™ analytical column. Connect the reducing union to a 280 µm O.D. × 20 µm I.D. fused silica transfer capillary (UV) or directly to a nanoESI emitter (MS) with the PEEK fitting (VICI P/N: C360NFPKG) included in the accessory bag.
- Ensure that the fused silica capillary/emitter is cleanly cut and assemble the connection with care, finger tight should be sufficient. **Do not overtighten!**
- Gradually increase the pump flow to the desired rate, *e.g.* 300 nL/min. Small droplets should appear at the tip of the capillary or emitter.
- A rapid increase in back pressure is indicative of overtightening the connector or clogging of the capillary/emitter.
- If a drastic increase in backpressure is observed during routine operation, assess the connection to the capillary/emitter and replace the PEEK fitting as required.

Grounding

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

- The µPAC™ analytical 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 nanoESI emitter, ground the µPAC™ analytical column using the **Ground it!** blue coiled grounding cable (swallow tail and crocodile clip options; P/N: 201901TLK).
- Connect the outlet (*i.e.*, the stainless-steel reducing union) to a grounded point on the mass spectrometer or the chassis of the HPLC. Grounding for three different electrospray ionisation configurations is shown in Figure 2.

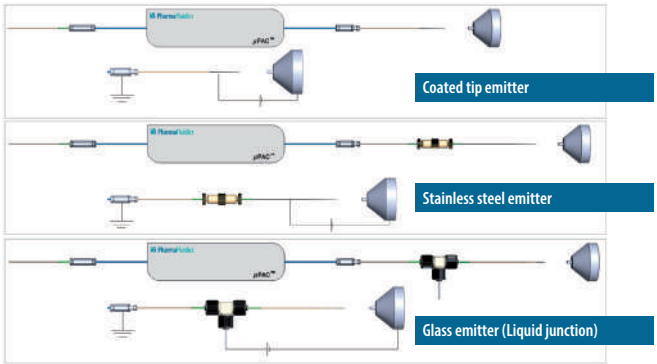

Figure 2: Overview of grounded electrospray ionisation interfaces

Column operation

- When developing a chromatographic method, remember that the 50 cm µPAC™ analytical column has an internal volume of 3 µL.
- Following elution of the analytes, the µPAC™ analytical column requires re-equilibration to starting conditions with at least 3 µL of initial mobile phase.

Column maintenance

- The µPAC™ analytical 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™ analytical 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™ analytical column that the solvent flow is not obstructed.
- If the increase in backpressure is due to the µPAC™ analytical column, reverse the flow direction and flush with 5 - 10 column volumes of mobile phase. This should return the µPAC™ analytical column to the original backpressure.
- The µPAC™ analytical column is symmetrical and column performance is identical in both directions.

Column storage

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

Part numbers for spare parts and consumables

VICI 4-20 nL valve	C4N-4004-.004EUHA
VICI PEEK fitting for 360 µm O.D. tubing	C360NFPKG
VICI SS internal ZDV union (inlet union)	ZU.5T
VICI SS internal reducing union (outlet union)	C360RU.5FS2
VICI 360 µm end plug	C360PPK
VICI ZDV union end plug	ZU.5FPK
µPAC™ 1/16" Connection PEEKsil™ Capillary	201904TLK
µPAC™ Grounding Cable	201901TLK
µPATCH	201902TLK
µPAC™ Spanner	201903TLK

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/