PharmaFluidics
The micro-Chip Chromatography Company

Instruction Sheet

PharmaFluidics µPAC™-compatible EASY-Spray™ Emitter

Accessories

General description

For compatibility with a 50 or 200 cm µPAC™ column, the Thermo Scientific™ EASY-Spray™ emitter has been modified by PharmaFluidics. The inlet of the µPAC™-compatible EASY-Spray™ Emitter is equipped with a stainless-steel union (50 µm through-bore). For applications in nanoLC-MS, the combination of a µPAC™ column with a modified EASY-Spray™ emitter facilitates high-resolution separation of peptides/metabolites. The stainless-steel union enables virtually zero-dead-volume (ZDV) installation on any nanoLC system. To achieve the highest performance for the modified EASY-Spray™ emitter, the following guidelines must be adhered to. The EASY-Spray™ ion source user guide from Thermo Scientific describes in detail how to install an EASY-Spray™ emitter on an EASY-Spray™ ion source.

Connecting a modified EASY-Spray™ emitter to a µPAC™ column

- With a $^{3}/_{16}$" spanner, unscrew and remove the union attached to the outlet of the µPAC™ column.
- Connect the outlet of the µPAC™ column to the 50 µm through-bore union located on the inlet side of the modified EASY-Spray™ emitter.
- Ensure that the fitting is finger tight and then further tighten for a maximum ¼ turn with a $^{3}/_{16}$" spanner. **Do not overtighten!**

Grounding a µPAC™ column connected to a modified EASY-Spray™ emitter

- The µPAC™ 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 modified EASY-Spray™ emitter, ground the µPAC™ column using the blue grounding cable (swallow tail or crocodile clip options; P/N: 201901TLK).
- Connect the inlet of the modified emitter (*i.e.*, the stainless steel 50 µm through-bore union) to a grounded point on the mass spectrometer or the chassis of the HPLC. A schematic overview is shown in Figure 1.

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).

Initial conditioning of the µPAC™ column connected to a modified EASY-Spray™ emitter

- At an initial flow rate of 300 nL/min and a solvent composition of 1% B, the backpressure should be <150 bar.
- Once the pressure has stabilised, the flow rate can be increased, as required, until the maximum backpressure of 350 bar has been reached.
- After approximately 30 min, the column should be equilibrated and ready for use.

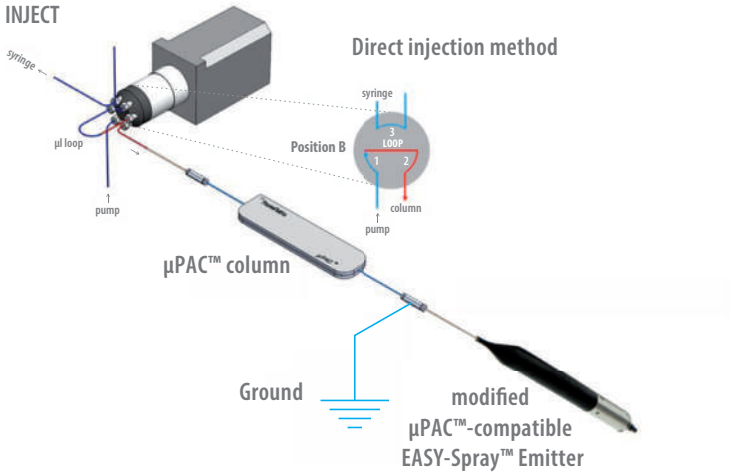

Figure 1: Installation and grounding of a µPAC™ column connected to a modified EASY-Spray™ emitter

Routine operation of the modified EASY-Spray™ emitter

- Start the nanoLC flow through the µPAC™ column and modified EASY-Spray™ emitter by slowly ramping the flow rate to 75% of the value indicated in the Quality Assurance report.
- Once the pressure stabilises, apply the flow rate used for analysing samples, *e.g.*, 300 nL/min.
- When the pressure has again stabilised, apply 1.5 kV to the emitter and wait until the spray stabilises. If the spray does not stabilise, slowly increase the voltage to a maximum of 2.5 kV.
- Using the initial solvent conditions, the nanoLC method must contain a column re-equilibration step equivalent to a minimum of 1× the µPAC™ column bed volume (*i.e.*, 3 and 10 µL for the 50 and 200 cm columns, respectively).

Disconnecting the EASY-Spray™ emitter

- Turn off the voltage to the modified EASY-Spray™ emitter.
- Stop the flow from the nanoLC and wait until the backpressure of the µPAC™ column has decreased and stabilised.

Stand-by/idle conditions

- After completion of a sample sequence, continue the flow from the nanoLC through the modified EASY-Spray™ emitter. **Ensure that voltage is applied to the modified emitter**; else irreparable damage to the emitter can occur.
- For the Thermo Scientific™ EASY-nLC™ system, an idle flow rate of 300 nL/min is recommended.
- For the Thermo Scientific™ UltiMate™ 3000 RSLCnano and other nanoLC systems, use 70% solvent B and set the idle flow rate to that used during the gradient.

Column storage with a modified EASY-Spray™ emitter

- µPAC™ columns can be stored for short periods in most mobile phases.
- For prolonged storage, flush the column with 5 - 10 column volumes of at least 70% acetonitrile or methanol in water.
- Run a solution of 70% acetonitrile through the modified EASY-Spray™ emitter at the flow rate used to analyse samples (*e.g.*, 300 nL/min).
- If the µPAC™ 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.

The voltage on the modified emitter should remain ON during these steps.

- Cap the modified EASY-Spray™ emitter, and cap both ends of the µPAC™ column with the provided PEEK blind union plugs.
- Store the column and the modified EASY-Spray™ emitter in the supplied protective boxes.

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/