

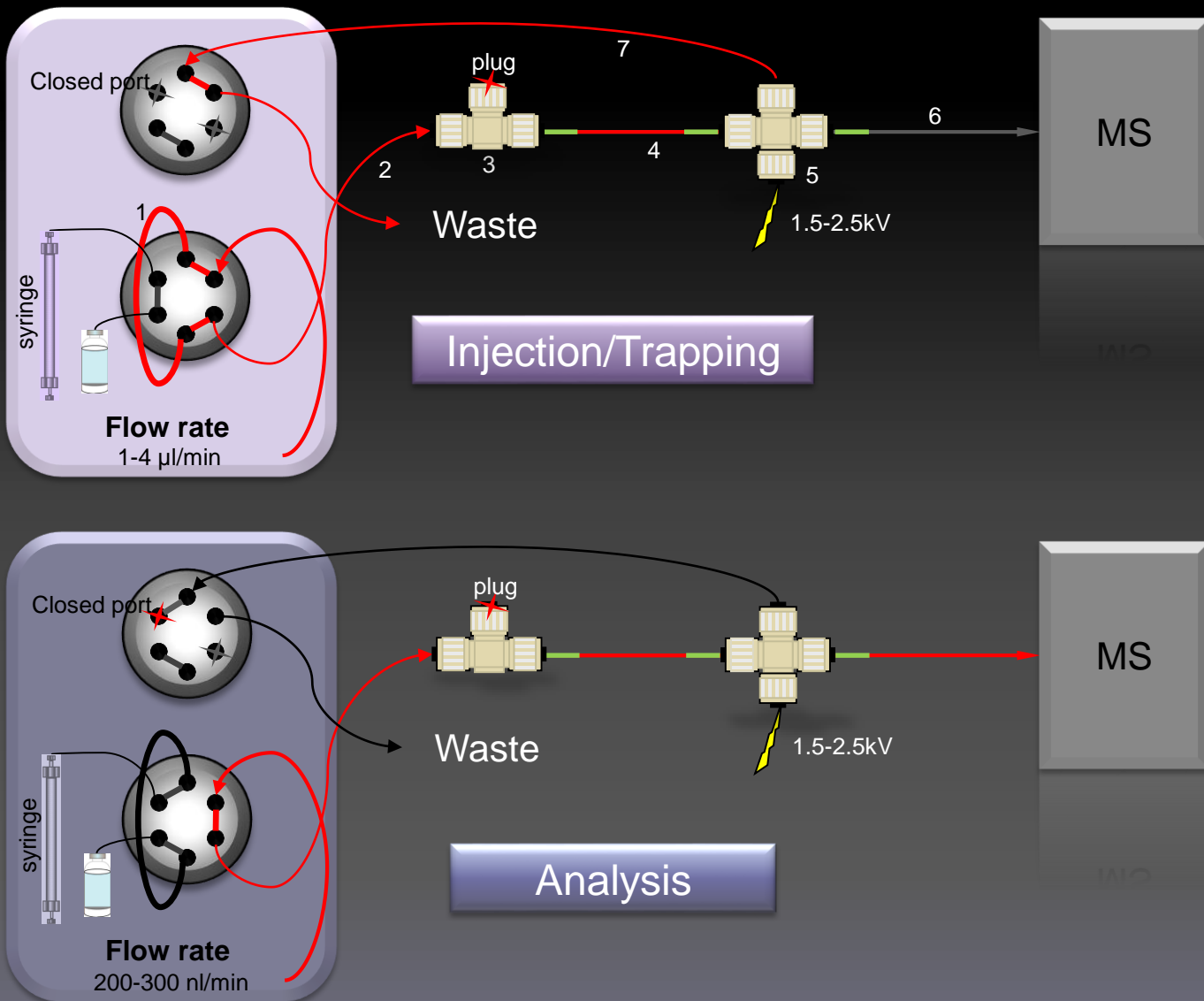
# Nano-flow system



EASY-nLC



nanoAcquity





## Pros:

- Low flow, less waste!
- Improved peak capacity and peak shape
- High chromatographic reproducibility

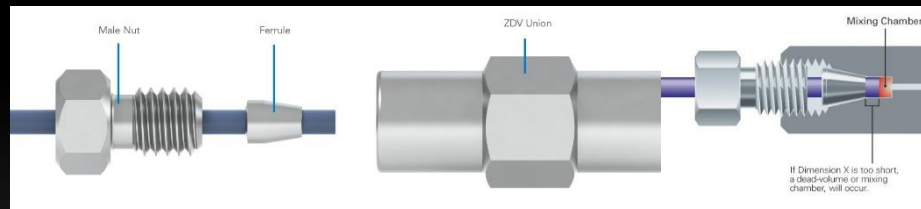


## Cons:

- Flow rate needs to be adjusted for every new column
- More sensitive to solvent impurities
- more difficult to find leaks

1. Sample loop (ss): 5 or 10  $\mu$ l
2. Transfer line fused silica 25-40  $\mu$ m ID x 25 cm: 0.1-0.3  $\mu$ l  
For the EASYnLC use Thermo Scientific [6041.5290] LQD JNCTN COL OUT,IDXODXL 20X3, Fisher [50-134-7912]
3. Peek MicroTee IDEX/Upchurch [P-775] closed with one plug [P-116] use with ferrule [F-172 ] and sleeves [F-185]
4. Trap column: e.g. fused silica 100  $\mu$ m ID x 15 cm = 1.18  $\mu$ l (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200Å 5 $\mu$  c.a. 2-4 cm long (NOTE we reuse the Integrafrit by flushing the beads out using the HPLC)
5. Peek MicroCross IDEX/Upchurch [P-777], high voltage applied through platinum or gold wire, use with ferrule [F-172 ] and sleeves [F-185]
6. Empty tip or separation column: e.g. fused silica 75  $\mu$ m ID x 10-60 cm, tip pulled manually with microflame or laser puller, packed with MagicC18AQ 100Å 5 $\mu$  10-60 cm long (avoid any void volume between trap and column, cut the column to desired length, such that beads are packed all the way to the end of the fused silica, most commonly used column length at UWPR is 20-30cm)
7. Transfer line fused silica 75  $\mu$ m ID x 40 cm  
For the EASYnLC use Thermo Scientific [6041.5289] UHPLC WASTE INIDXADXL 75UMX360, Fisher [50-134-7911]

## Connecting Fused silica to a six port valve

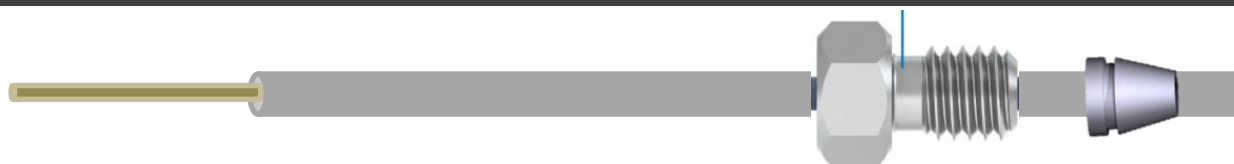


Check out IDEX's Fitting Primer:

<https://www.idex-hs.com/literature-tools/educational-materials/fitting-primer/>

**PLEASE NOTE:** Because the ferrule is permanently attached to the tubing and because of standard machining tolerances, we highly recommend that any pre-swaged stainless steel fitting only be used in the mating port into which it was initially swaged. Failure to do this may result in dead volume or solvent leakage.

**Additionally,** for proper tightening of a pre-swaged stainless steel fitting into its mating port, we recommend wrench tightening only an additional 1/4 to 1/2 of a turn past finger tight, followed by subsequent monitoring of the connection for any leaks. Should leaking occur, simply continue to tighten the fitting a little at a time until the leak stops. Also, should it become necessary to tighten the fitting more than one complete revolution past finger tight, IDEX Health & Science recommends that the fitting be replaced, as excessive tightening is typically indicative of a damaged product.

**Fused Silica:**

- 360  $\mu$ m OD

**Peek tubing sleeve:**

- Gray 1/16" OD x 0.015 " ID cut to length Waters [WAT022997]
- Orange 1/16" OD x 0.016 " ID x 1.3" IDEX [F-230]
- Orange 1/16" OD x 0.020 " ID cut to length IDEX [1532]

**Nut:**

- Compression screw Waters [700002635]
- Nut IDEX [U-400]
- Ferrule Valco style IDEX [U-320]

**Ferrule:**

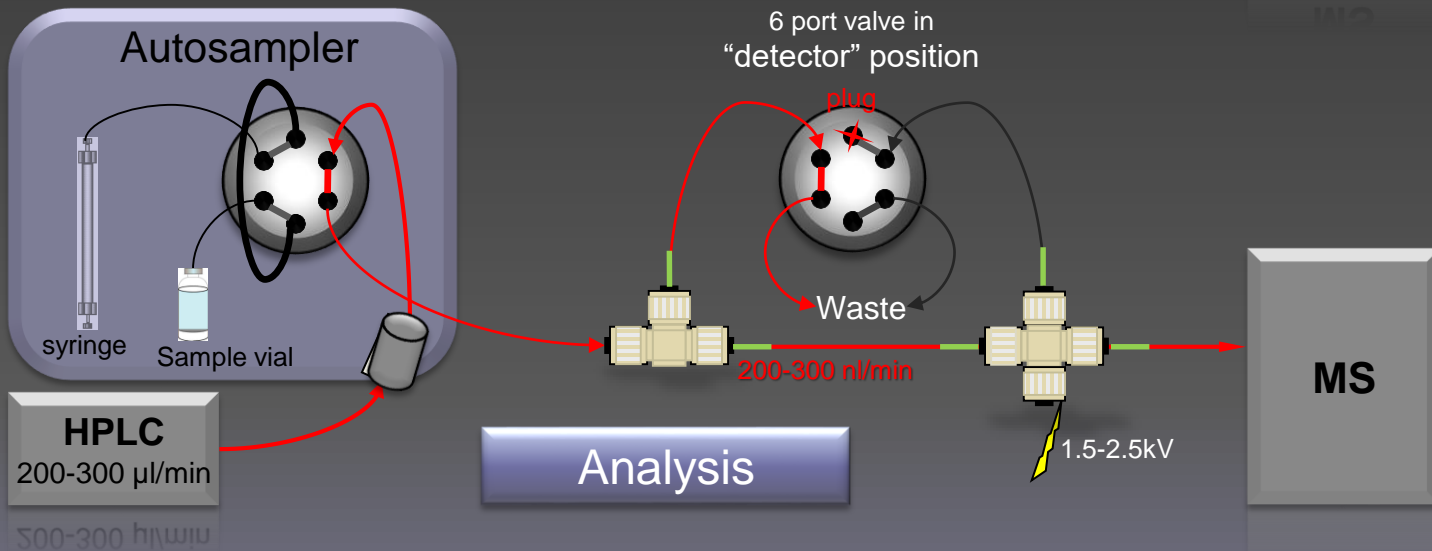
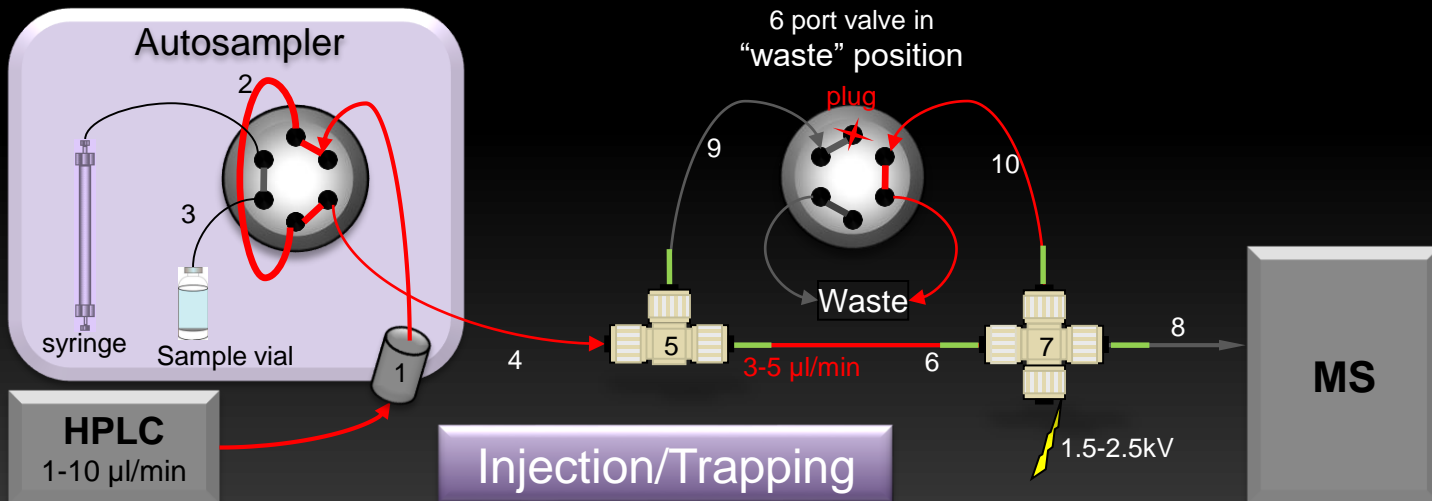
- Two piece ferrules Waters [700002635]
- Ferrule standard IDEX [U-401]
- Ferrule Valco style IDEX [U-321]

Alternatively nanoViper lines connect to the six port valve and terminate in fused silica:

20  $\mu$ m ID: Thermo Scientific [6041.5290] LQD JNCTN COL OUT, IDXODXL 20X3, Fisher [50-134-7912]

75  $\mu$ m ID: Thermo Scientific [6041.5289] UHPLC WASTE INIDXADXL 75UMX360, Fisher [50-134-7911]

# High flow pump, two split system



## High flow pump, two split system



## Pros:

Flow split close to the trap ensures fast gradient transitions as the flow rate is 200-300  $\mu\text{l}/\text{min}$  up to the first split.

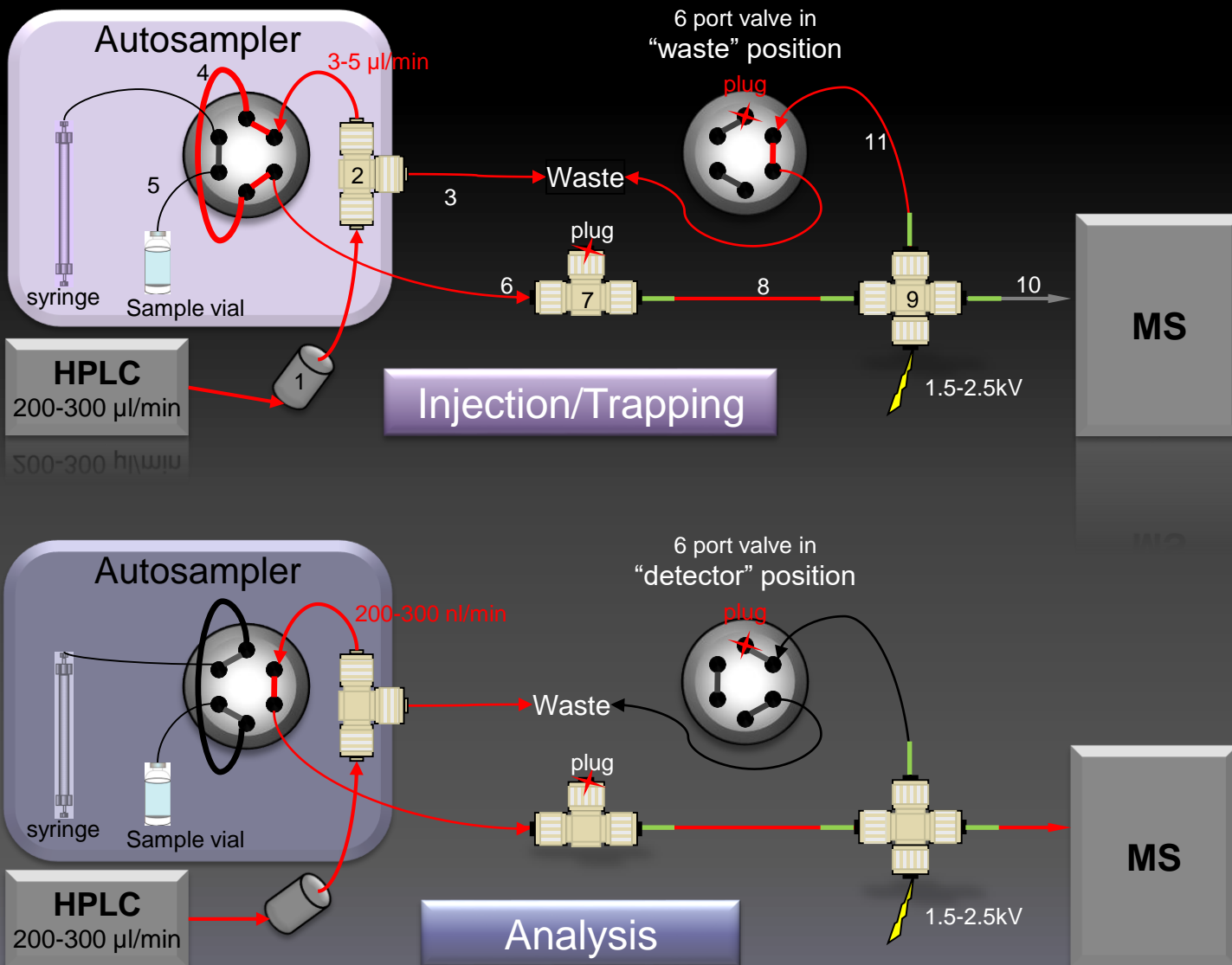


## Cons:

The flow rate changes from loading to analysis may cause pressure fluctuations affecting the packing of the trap/column or induce leaks  
If the first split leaks during loading you'll lose sample

1. Optional in-line solvent filter (Upchurch A314 with 2  $\mu\text{m}$  peek frit A702) connected via peek tubing (127  $\mu\text{m}$  ID) to reduce risk of clogging downstream lines/columns
2. Sample loop; e.g. PEEKsil tubing 15 cm x 1/16" x 0.3 mm ID: 10.603  $\mu\text{l}$  (Upchurch part#630015)
3. Injection needle (home made for Spark Holland Endurance AS 100  $\mu\text{m}$  ID x 37 cm: 3  $\mu\text{l}$ )
4. Transfer line fused silica 50-100  $\mu\text{m}$  ID x 25 cm: 0.5-2  $\mu\text{l}$
5. Peek MicroTee (Upchurch P-775 or P-875 w/ mounting whole)
6. Trap column: e.g. fused silica 100  $\mu\text{m}$  ID x 20 cm = 1.6  $\mu\text{l}$  (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200Å 5 $\mu\text{m}$  c.a. 2-4 cm long
7. Peek MicroCross (Upchurch P-777), high voltage applied through 0.5 platinum or gold wire
8. Empty tip or separation column: e.g. fused silica 75  $\mu\text{m}$  ID x 10-60 cm tip pulled manually with microflame torch, packing MagicC18AQ 100Å 5 $\mu\text{m}$  10 cm long
9. Flow split : fused silica 25-50  $\mu\text{m}$  ID x 15-30 cm open in detector position; adjust ID and length to regulate flow rate through column to 200-300 nl/min
10. Flow split : fused silica 100  $\mu\text{m}$  ID x 15 cm open in waste position

# High flow pump, single constant open split system



# High flow pump, single constant open split system



## Pros:

- Constant flow rate at pump (200-300  $\mu\text{l}/\text{min}$ )
- Reduced the risk of pressure fluctuations
- Self regulated flow rate through trap and column
- Reduced risk of sample loss during loading



## Cons:

- Increased void volume leads to increased delay time

1. Optional in-line solvent filter (Upchurch A314 with 2  $\mu\text{m}$  peek frit A702) connected via peek tubing (127  $\mu\text{m}$  ID) to reduce risk of clogging downstream lines/columns
2. Peek MicroTee (Upchurch P-890), NOTE: mount as close to the AS valve as possible to minimize void volume, use small ID line to connect to AS valve (e.g. 5cm x 127  $\mu\text{m}$  ID = 630 nl, 5cm x 50 $\mu\text{m}$  ID = 98 nl)
3. Flow split : PEEK or fused silica 25-50  $\mu\text{m}$  ID x 15-30 cm open in detector position; adjust ID and length to regulate flow rate through column to 200-300 nl/min
4. Sample loop; e.g. PEEKsil tubing 15 cm x 1/16" x 0.3 mm ID: 10.603  $\mu\text{l}$  (Upchurch part#630015)
5. Injection needle (home made for Spark Holland Endurance AS 100  $\mu\text{m}$  ID x 37 cm: 3  $\mu\text{l}$ )
6. Transfer line fused silica 50-100  $\mu\text{m}$  ID x 25 cm: 0.5-2  $\mu\text{l}$
7. Peek MicroTee (Upchurch P-775 or P-875 w/ mounting whole)
8. Trap column: e.g. fused silica 100  $\mu\text{m}$  ID x 20 cm = 1.6  $\mu\text{l}$  (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200 $\text{\AA}$  5 $\mu\text{m}$  c.a. 2-4 cm long
9. Peek MicroCross (Upchurch P-777), high voltage applied through 0.5 platinum or gold wire
10. Empty tip or separation column: e.g. fused silica 75  $\mu\text{m}$  ID x 10-60 cm tip pulled manually with microflame torch, packing MagicC18AQ 100A 5 $\mu$  10 cm long
11. Flow split : fused silica 100  $\mu\text{m}$  ID x 15 cm open in waste position