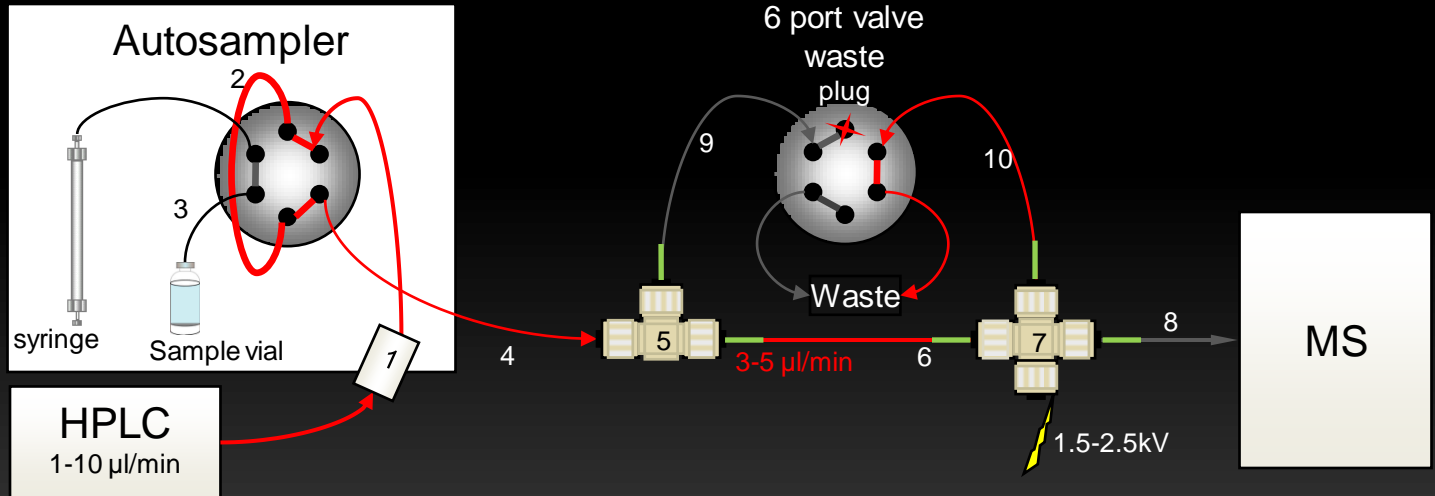
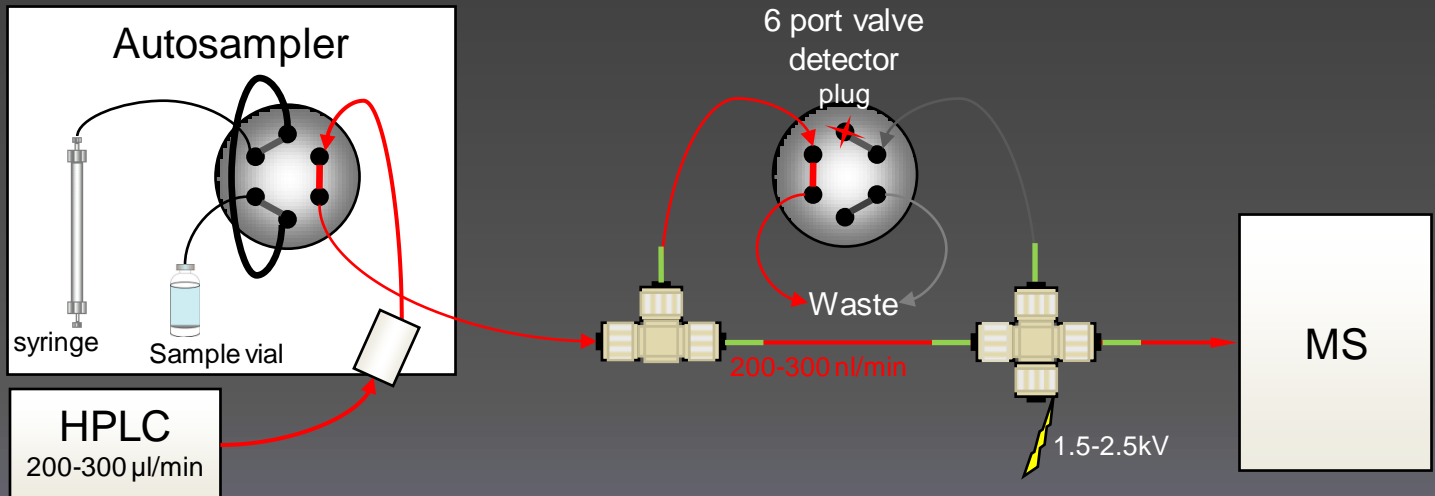


# High flow pump, two split system

Injection/  
Trapping



Analysis



## 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.



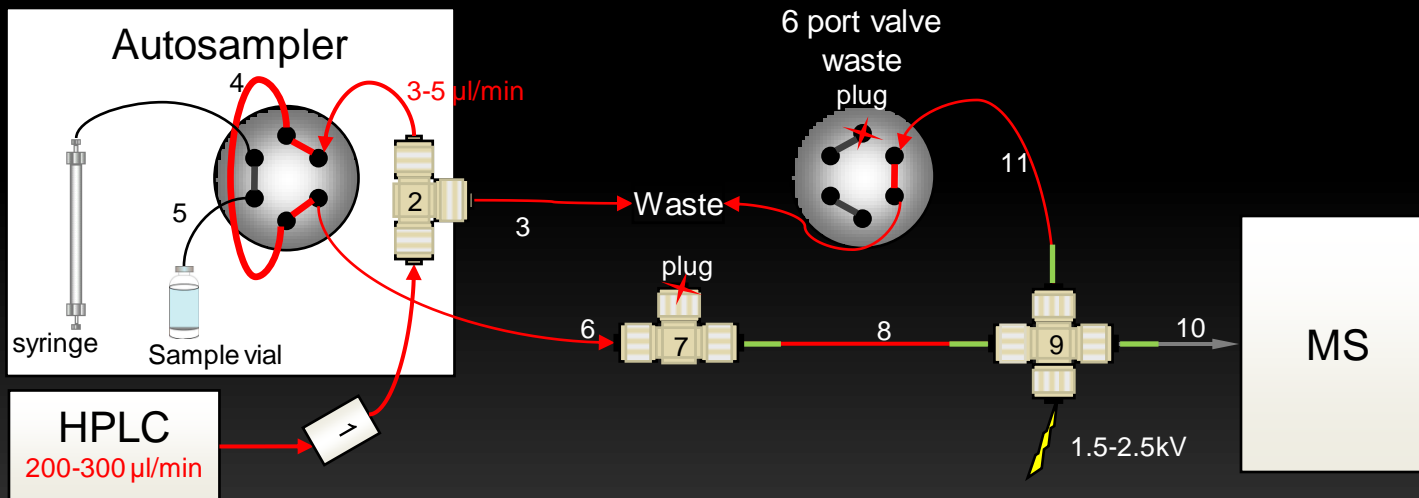
### Cons:

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

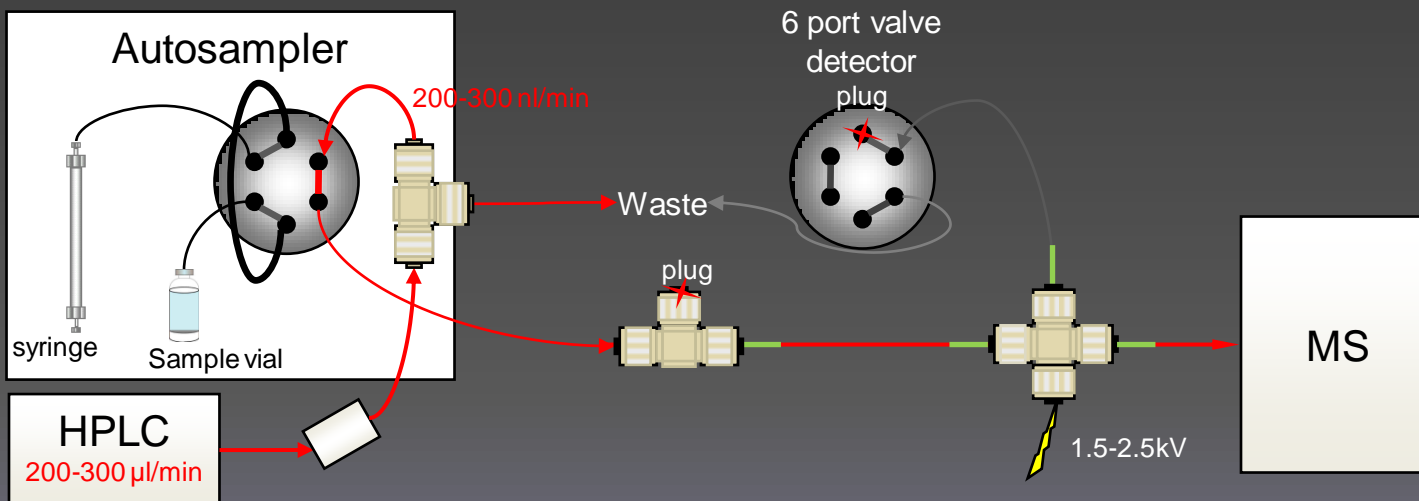
1. Optional in-line solvent filter (Upchurch A314 with 2  $\mu\text{m}$  peek frit A702) connected via peek tubing (127  $\mu\text{m}$  ID) to reduce risk of clogging downstream lines/columns
2. Sample loop; e.g. PEEKsil tubing 15 cm x 1/16" x 0.3 mm ID: 10.603  $\mu\text{l}$  (Upchurch part#630015)
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  $\text{nl}/\text{min}$
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

Injection/  
Trapping



Analysis



# 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

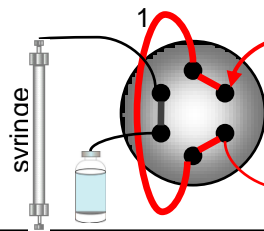
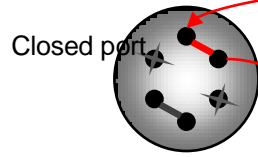
1. Optional in-line solvent filter (Upchurch A314 with 2  $\mu\text{m}$  peek frit A702) connected via peek tubing (127  $\mu\text{m}$  ID) to reduce risk of clogging downstream lines/columns
2. Peek MicroTee (Upchurch P-890), NOTE: mount as close to the AS valve as possible to minimize void volume, 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 : 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 200Å 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 100Å 5 $\mu\text{m}$  10 cm long
11. Flow split : fused silica 100  $\mu\text{m}$  ID x 15 cm open in waste position

# Nano-flow system

Injection/  
Trapping

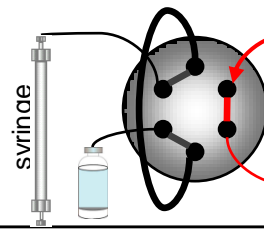
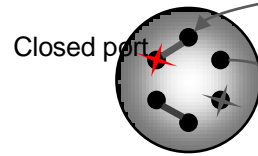


e.g. nanoAcquity

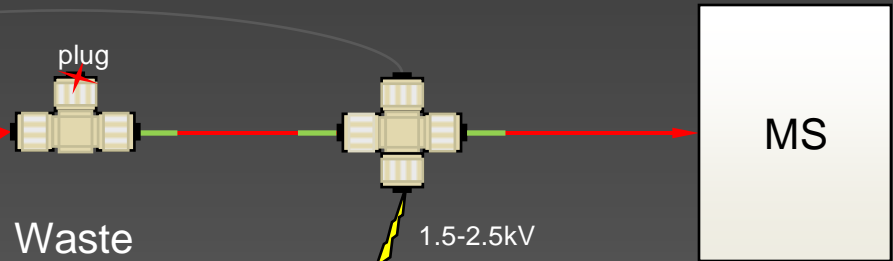
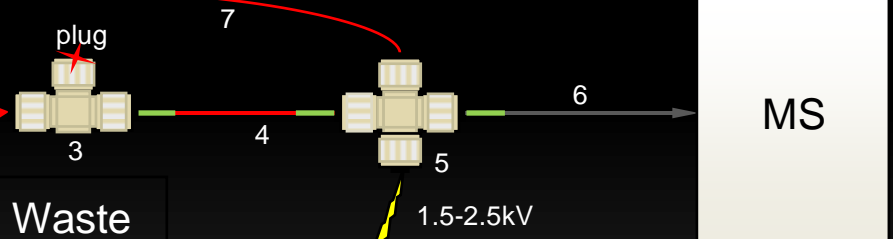


pump  
1-4  $\mu\text{l}/\text{min}$

e.g. nanoAcquity



pump  
200-300 nL/min



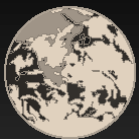
Analysis

# Nano-flow system



## 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 ss loop: 5 or 10  $\mu$ l
2. Transfer line fused silica 25-40  $\mu$ m ID x 25 cm: 0.1-0.3  $\mu$ l
3. Peek MicroTee (Upchurch P-775) closed with one plug
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 200A 5 $\mu$  c.a. 2-4 cm long (NOTE we reuse the Integrafrit by flushing the beads out using the HPLC)
5. Peek MicroCross (Upchurch P-777), high voltage applied through platinum or gold wire
6. Empty tip or separation column: e.g. fused silica 75  $\mu$ m ID x 10-60 cm, tip pulled manually with microflame, packing MagicC18AQ 100A 5 $\mu$  10 cm long
7. Flow split : fused silica 50-100  $\mu$ m ID x 20-30 cm open during loading