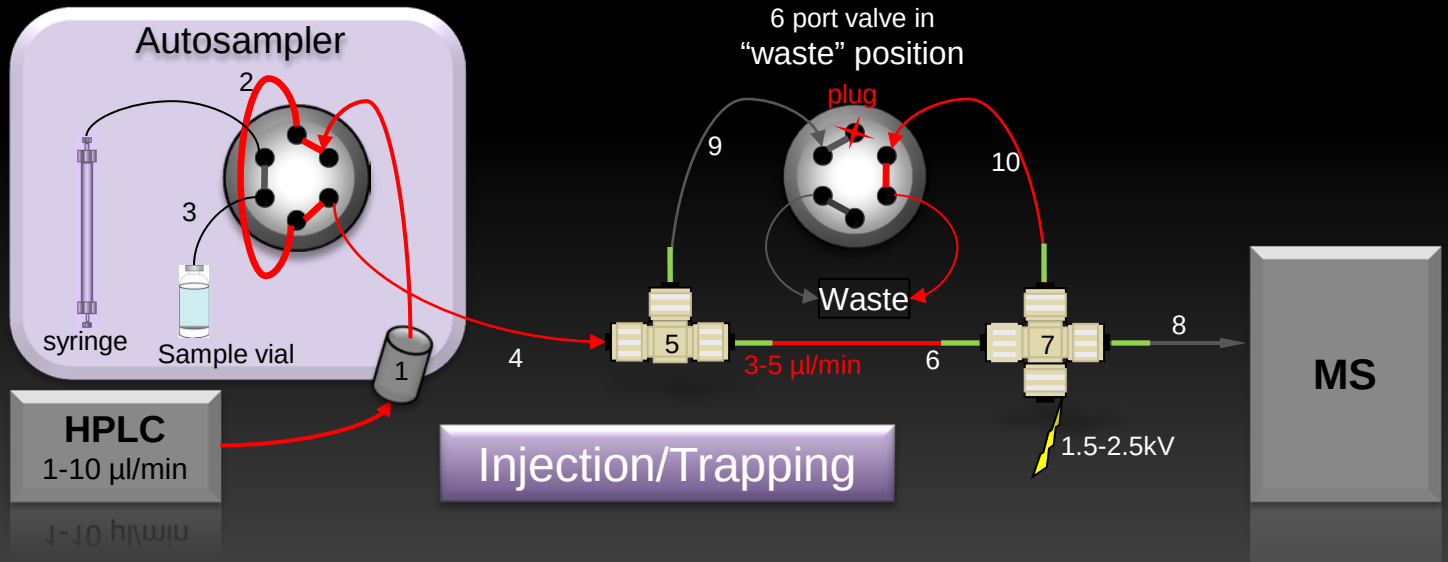
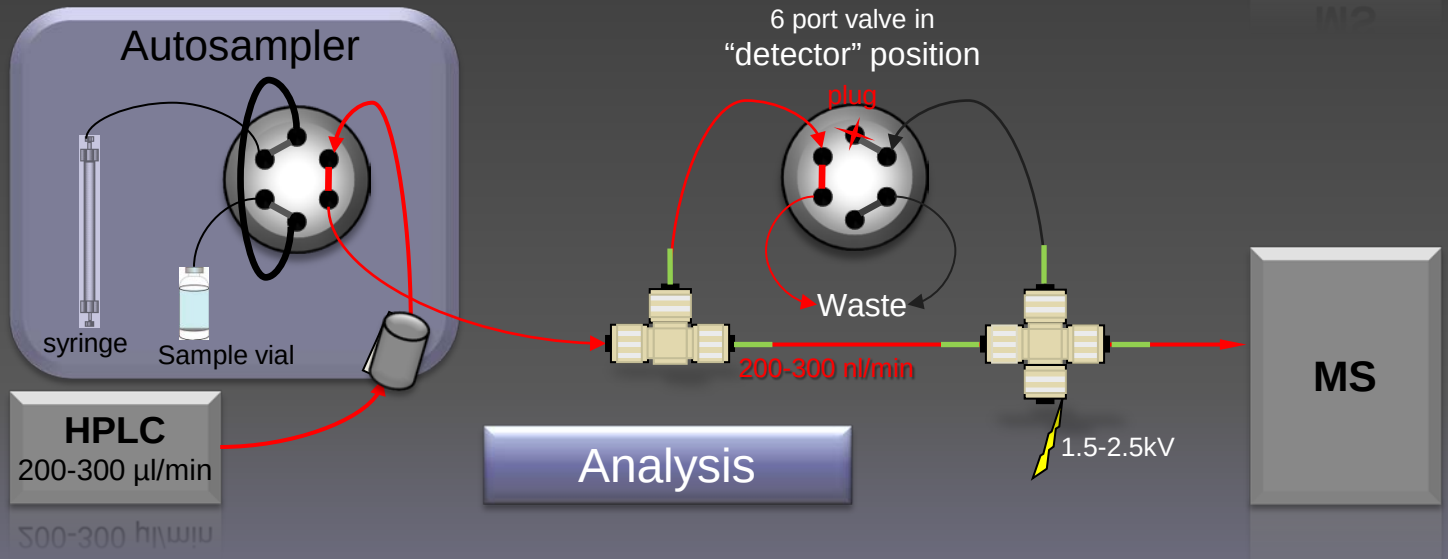
High flow pump, two split system





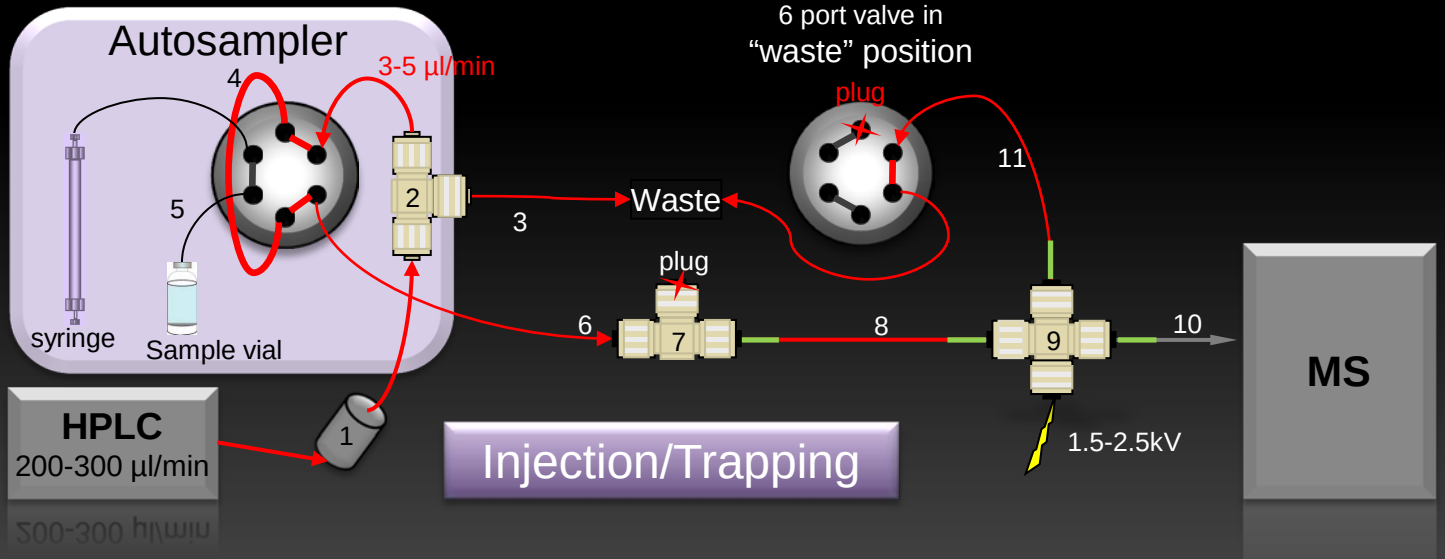
High flow pump, two split system

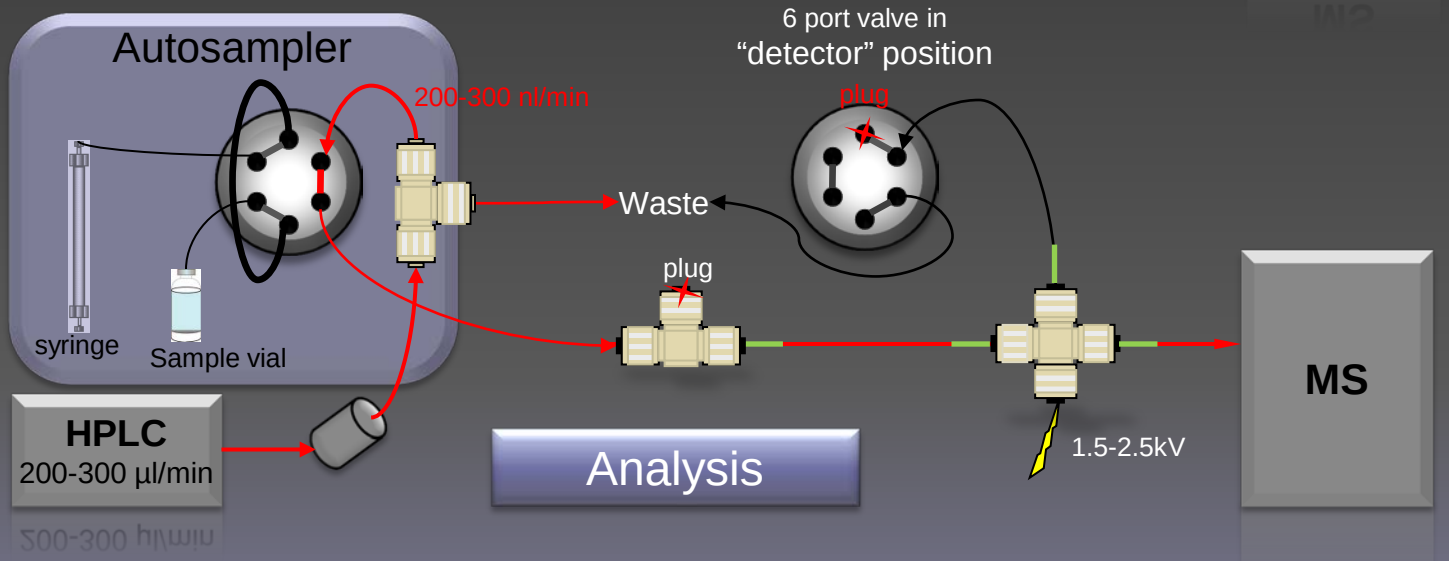
Pros:
Flow split close to the trap ensures fast gradient transitions as the flow rate is 200-300 µl/min up to the first split.

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 loose sample

1. Optional in-line solvent filter (Upchurch A314 with 2 µm peek frit A702) connected via peek tubing (127 µm 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 µl (Upchurch part#630015)
3. Injection needle (home made for Spark Holland Endurance AS 100 µm ID x 37 cm: 3 µl)
4. Transfer line fused silica 50-100 µm ID x 25 cm: 0.5-2 µl
5. Peek MicroTee (Upchurch P-775 or P-875 w/ mounting whole)
6. Trap column: e.g. fused silica 100 µm ID x 20 cm = 1.6 µl (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200Å 5µm 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 µm ID x 10-60 cm tip pulled manually with microflame torch, packing MagicC18AQ 100A 5µ 10 cm long
9. Flow split : fused silica 25-50 µm 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 µm ID x 15 cm open in waste position

High flow pump, single constant open split system





High flow pump, single constant open split system

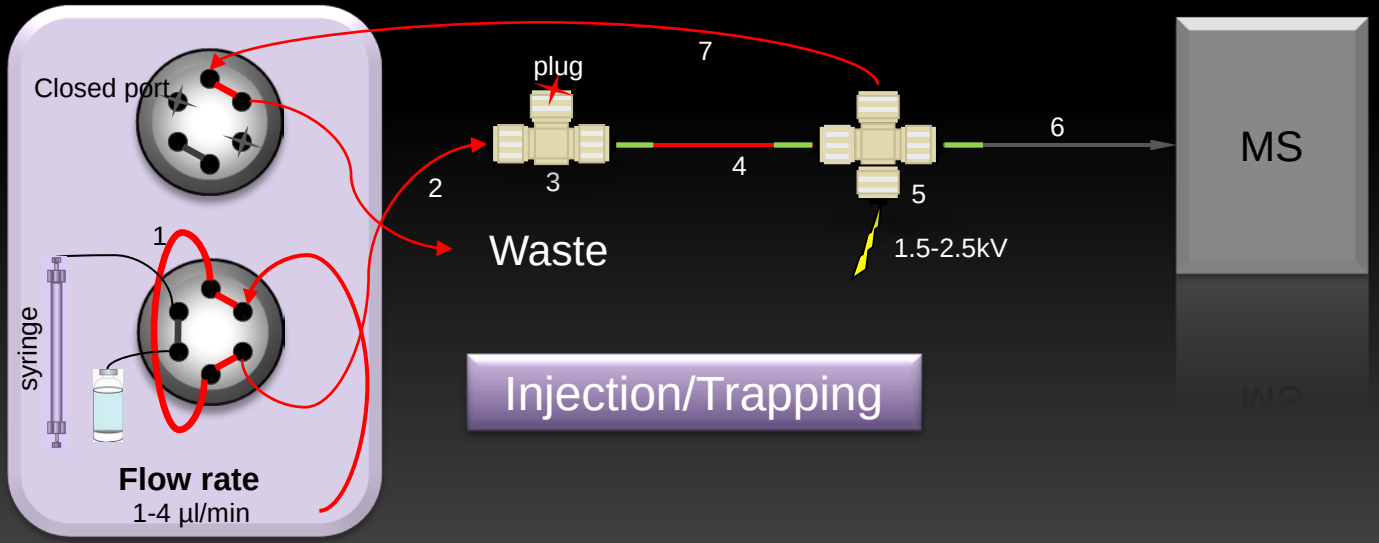
Pros:
- Constant flow rate at pump (200-300 µl/min)
- Reduced the risk of pressure fluctuations
- Self regulated flow rate through trap and column
- Reduced risk of sample loss during loading

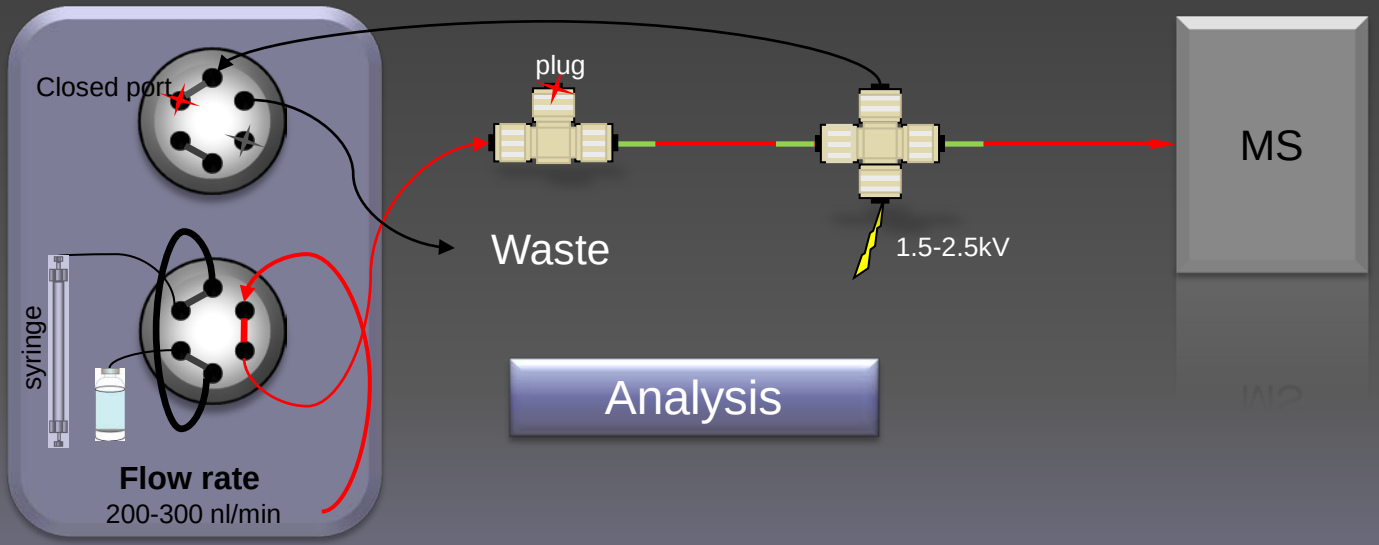
Cons:
- Increased void volume leads to increased delay time

1. Optional in-line solvent filter (Upchurch A314 with 2 µm peek frit A702) connected via peek tubing (127 µm 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 x127 µm ID = 630 nl, 5cm x 50µm ID = 98 nl)
3. Flow split : PEEK or fused silica 25-50 µm 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 µl (Upchurch part#630015)
5. Injection needle (home made for Spark Holland Endurance AS 100 µm ID x 37 cm: 3 µl)
6. Transfer line fused silica 50-100 µm ID x 25 cm: 0.5-2 µl
7. Peek MicroTee (Upchurch P-775 or P-875 w/ mounting whole)
8. Trap column: e.g. fused silica 100 µm ID x 20 cm = 1.6 µl (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200Å 5µm 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 µm ID x 10-60 cm tip pulled manually with microflame torch, packing MagicC18AQ 100A 5µ 10 cm long
11. Flow split : fused silica 100 µm ID x 15 cm open in waste position

Nano-flow system

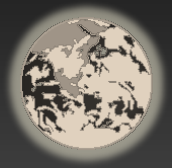
e.g. nanoAcquitiy





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 loop (ss): 5 or 10 µl
2. Transfer line fused silica 25-40 µm ID x 25 cm: 0.1-0.3 µl
3. Peek MicroTee (Upchurch P-775) closed with one plug
4. Trap column: e.g. fused silica 100 µm ID x 15 cm = 1.18 µl (PicoTip Integrafrit # IF360-100-50-N-5) packed with MagicC18AQ 200Å 5µ 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 µm ID x 10-60 cm, tip pulled manually with microflame or laser puller, packed with MagicC18AQ 100Å 5µ 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)