
LAB
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Science Together
55 Years
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Troubleshooting

Tips and tricks for FPLC users

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Peak broadening & Delay

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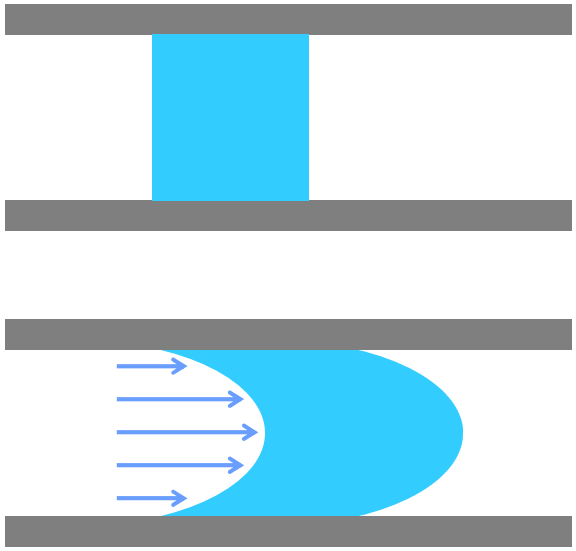
Peak broadening & resolution



Use tubing as short as possible

Use tubing with small ID

- higher resolution
- lower system volume
- but higher pressure

System Dead Volume

Volume in the system from injection to detection

Column Dead Volume/Void Volume

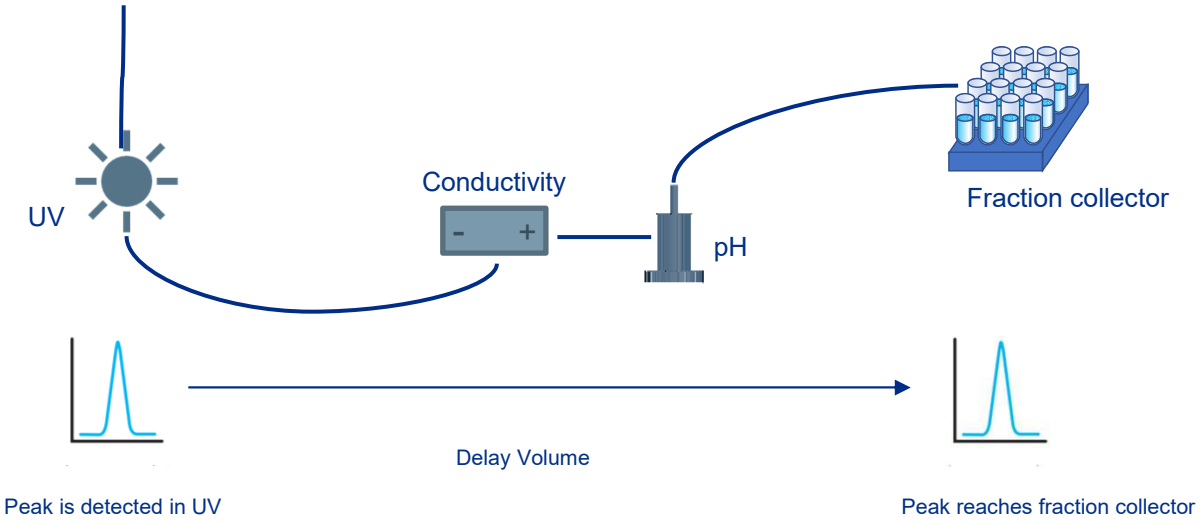
Volume of the fluid phase in the column

Delay/Dwell Volume

Volume from mixing chamber to column

Delay volume

Fractionation delay volume: UV detector flow cell to fraction collector...



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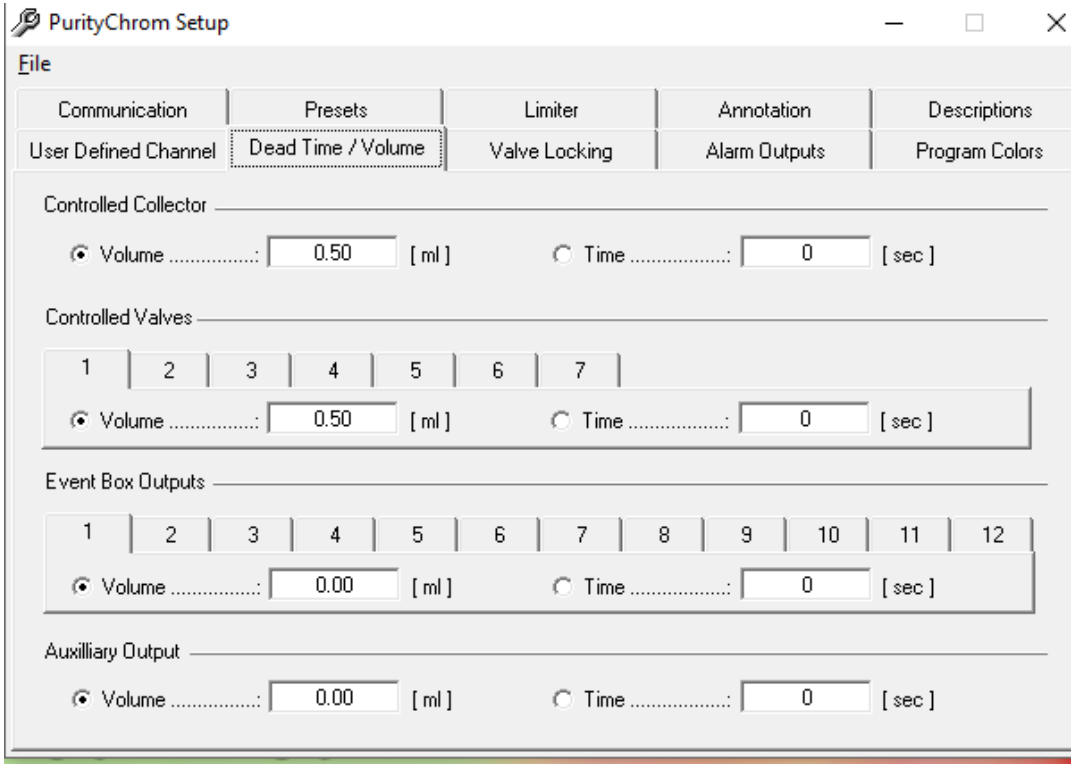
Delay volume in PurityChrom



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Injection

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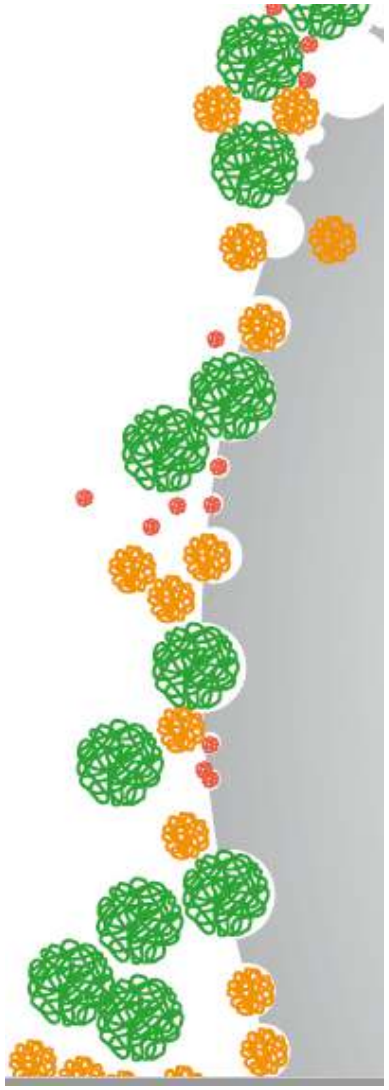
Sample volume

Sample volume affects resolution using SEC

No effect with capture method



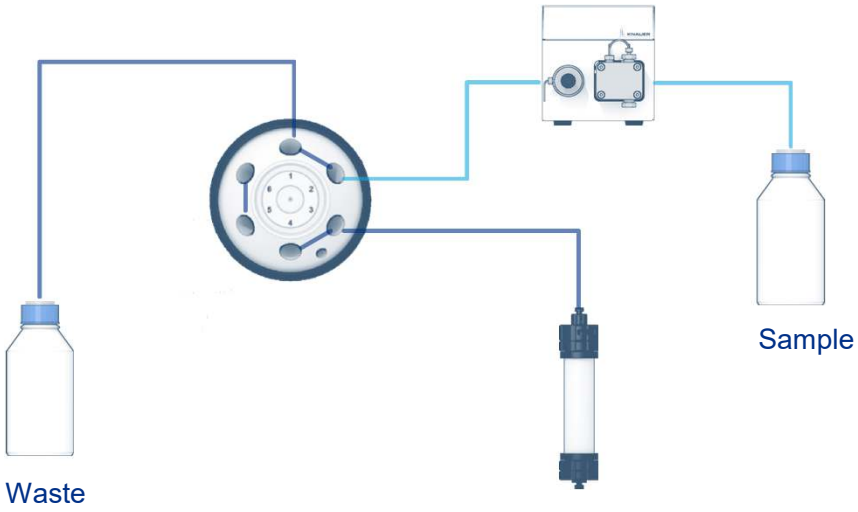
Injection via Sample loop

Full loop 3-5x volume overload

Partial loop fill

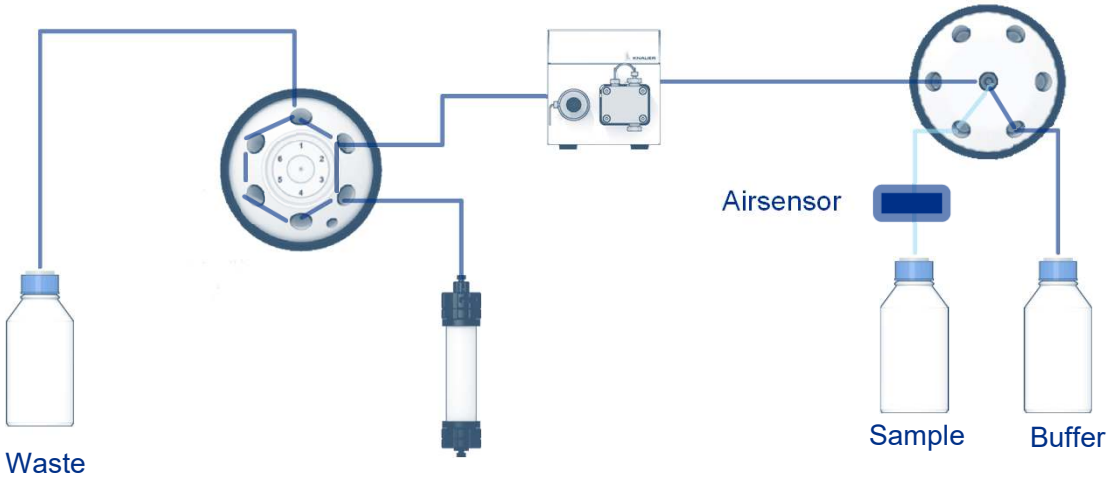
- Half loop volume
- Reproducibility ↓
- Sample loss ↓

Injection via Sample pump



Sample loss in tubing

Injection via Sample pump with air sensor



Less sample loss in tubing

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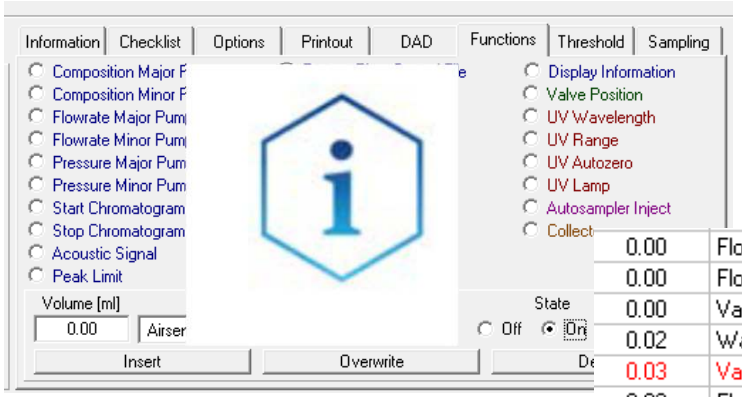
Program automatic sample application 1



Do not activate „Hold“ button

Sample application will not be recorded

0.00	Flowrate Major Pump	0.00 ml/min Constant Flow
0.00	Flowrate Minor Pump	5.00 ml/min Constant Flow
0.00	Valve Position	Injection = Inject
0.02	Wait for Input Signal	Airsensor = ON
0.03	Valve Position	Injection = Load
0.03	Flowrate Major Pump	1.00 ml/min Constant Flow
0.03	Flowrate Minor Pump	0.00 ml/min Constant Flow

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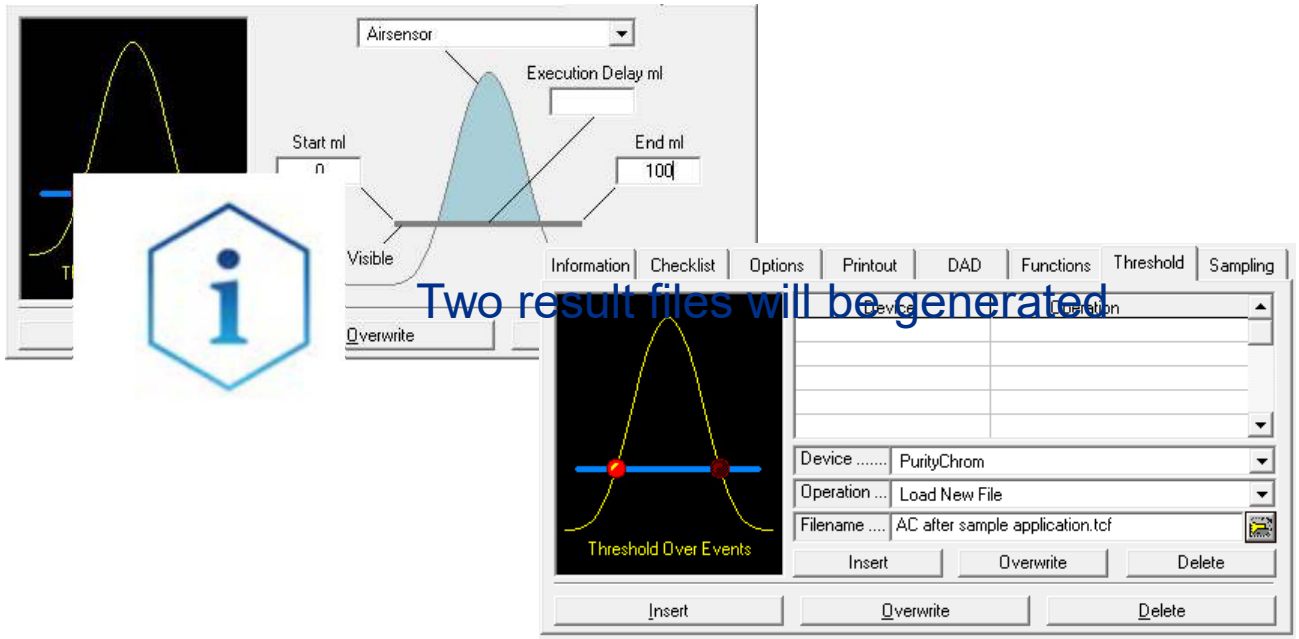
Program automatic sample application 2



Two result files will be generated

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Fluctuating pressure

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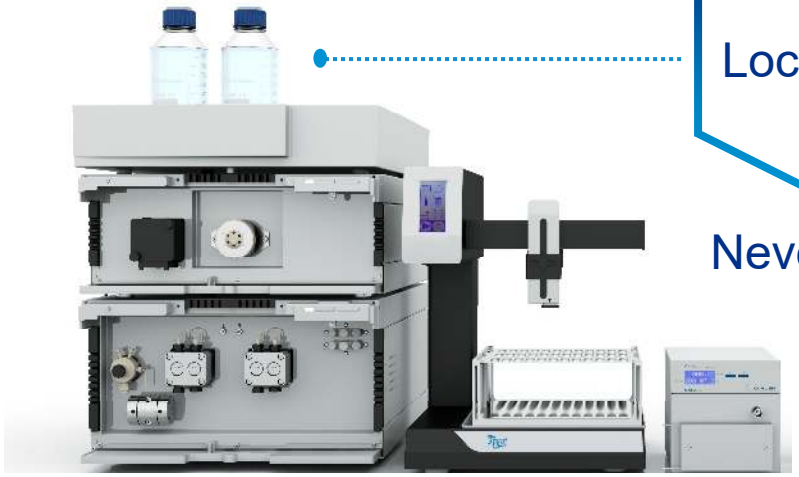
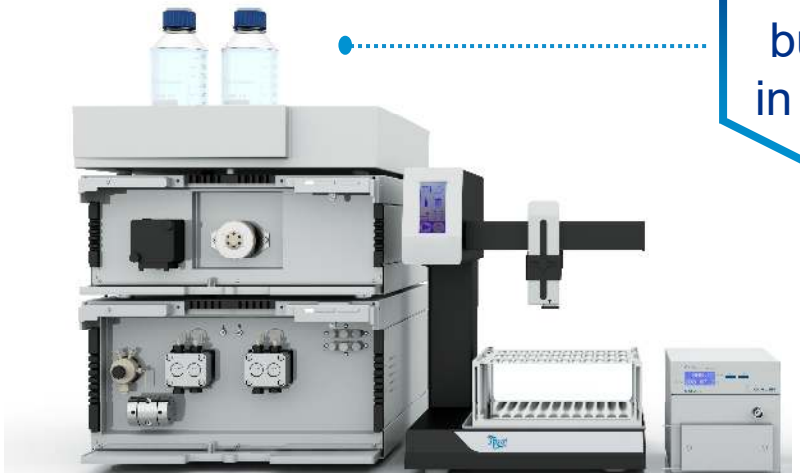
Origin of air bubbles or fluctuating pressure



Location

Never place bottles below the pump

Origin of air bubbles or fluctuating pressure



Air bubble in buffer

Degass buffers and adjust to used temperature

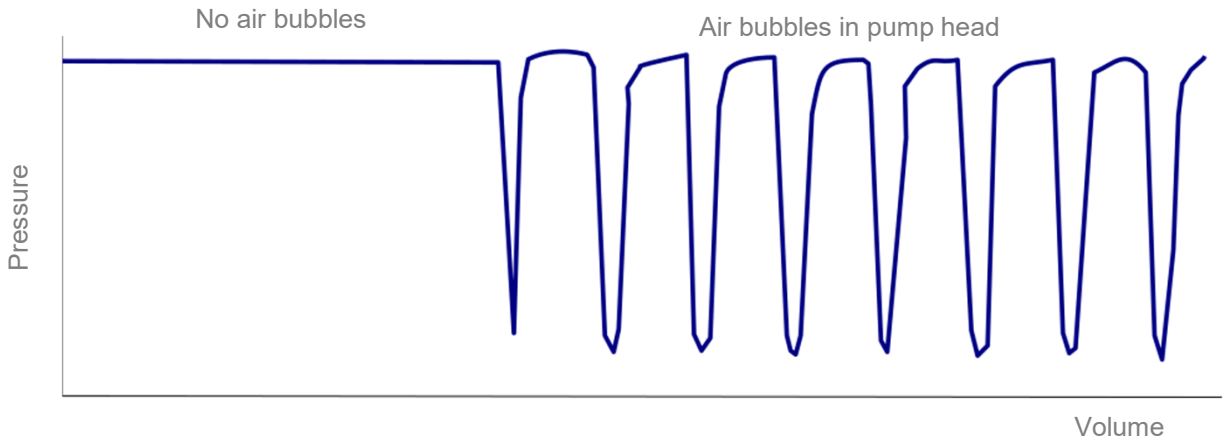
Origin of air bubbles or fluctuating pressure

Air in pump head

Check for prefilled inlets

Purge with 50 % of maximum flow rate

Detect air bubbles in pump head



Check pressure curve

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Use an Airsensor to protect the column

Airsensor stops system

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Fluctuating detector signal

Origin of fluctuating detector signal

Dirty flow cell

Clean flow cell according to manual

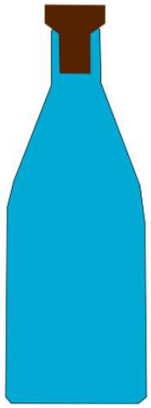
Origin of fluctuating detector signal

Air in flow cell

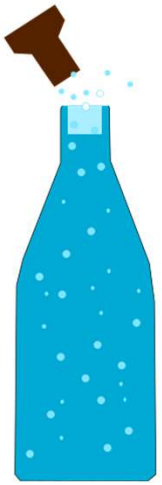
Flush flow cell

Use back pressure regulator

Why back-pressure regulator?

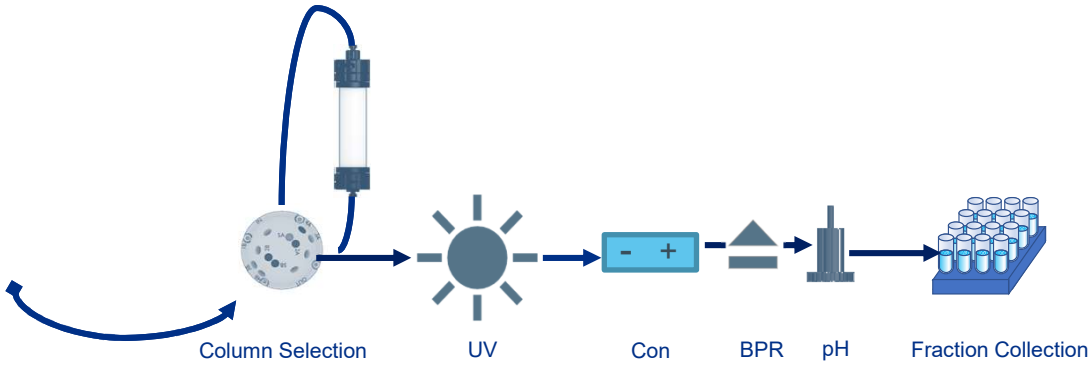


No air bubbles visible with corc on.



Air bubbles arise when corc is removed

Where do I connect the back-pressure regulator



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System pressure

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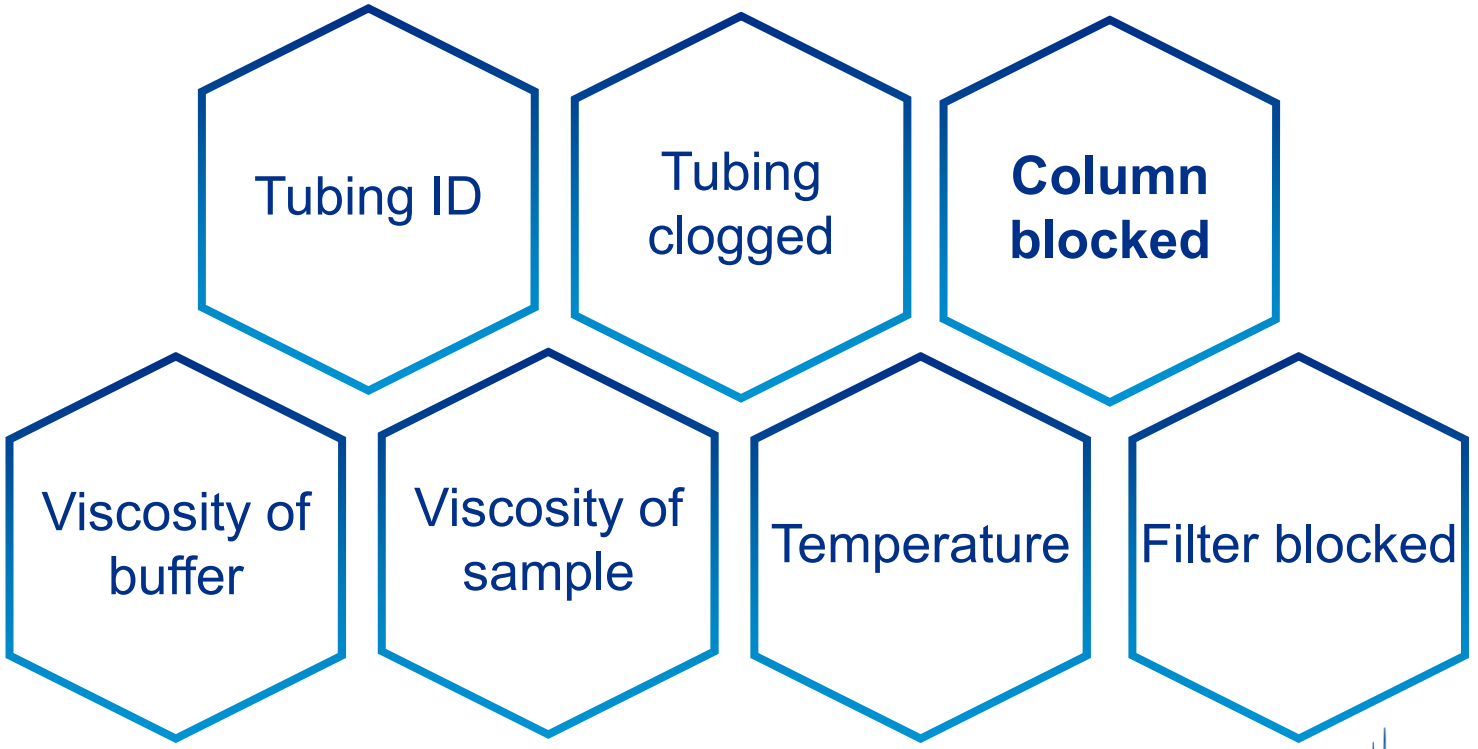
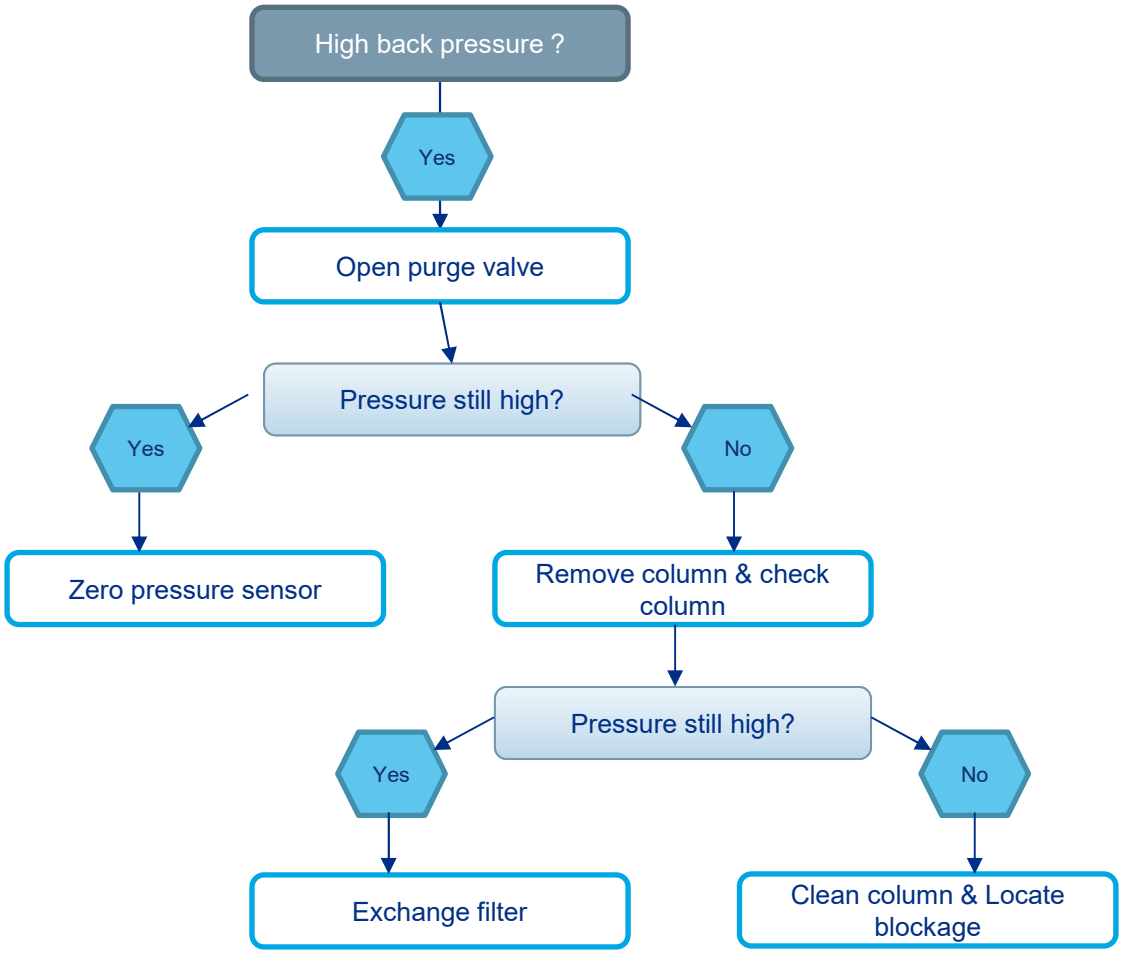
Backpressure origin





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Backpressure origin

Filter

Replace with empty cartridge

Use inline filter

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System maintenance

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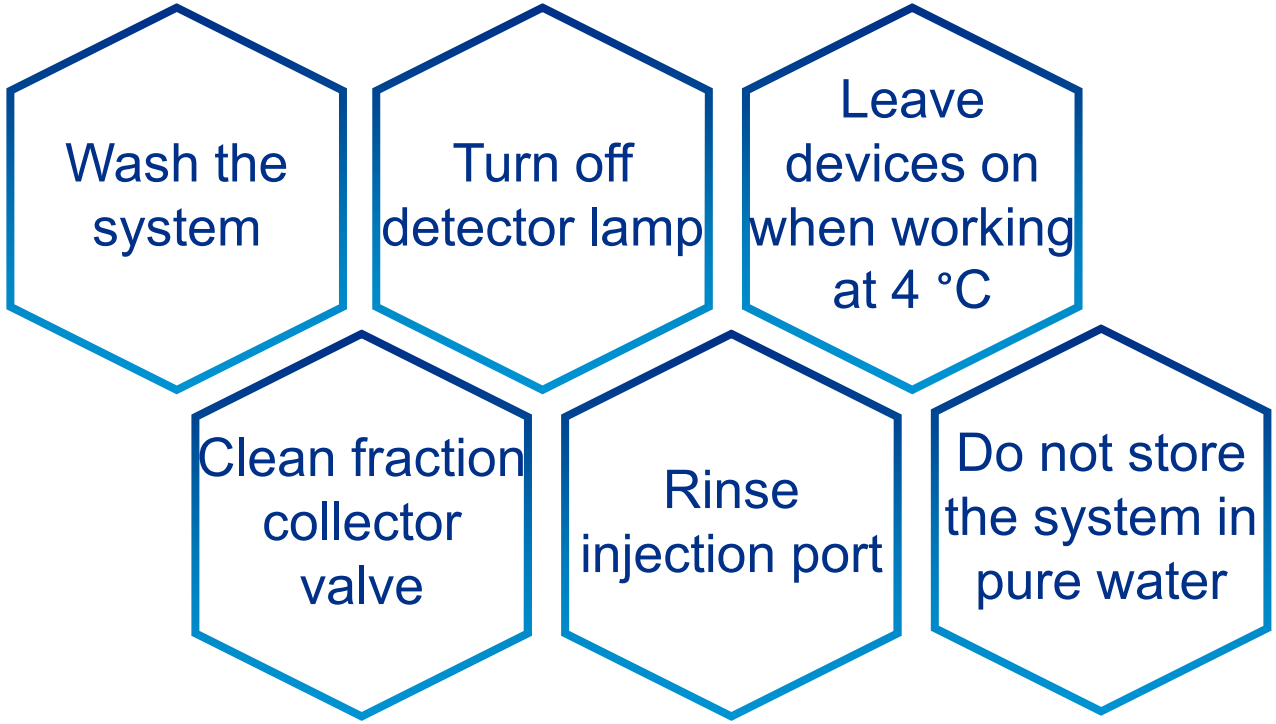
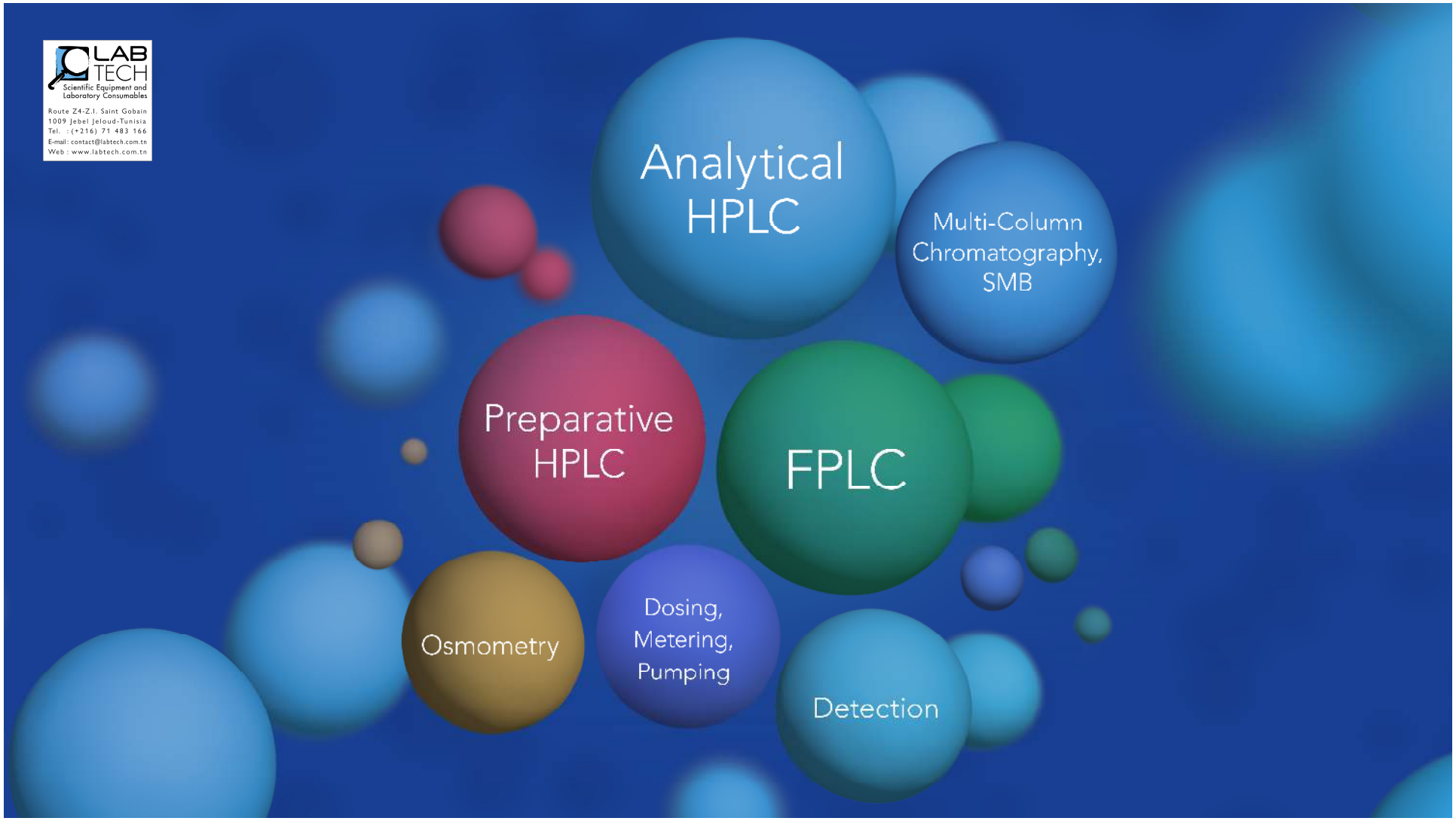
Before the run

- Centrifuge & filter your samples
- Degas & filter your buffers
- Temper your buffer
- Switch on detector
- Purge the pumps
- Check for tubes in fraction collector
- Wash the system
- Equilibrate the column

Check for leakages, stable pressure and UV signal

After the run & system storage

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