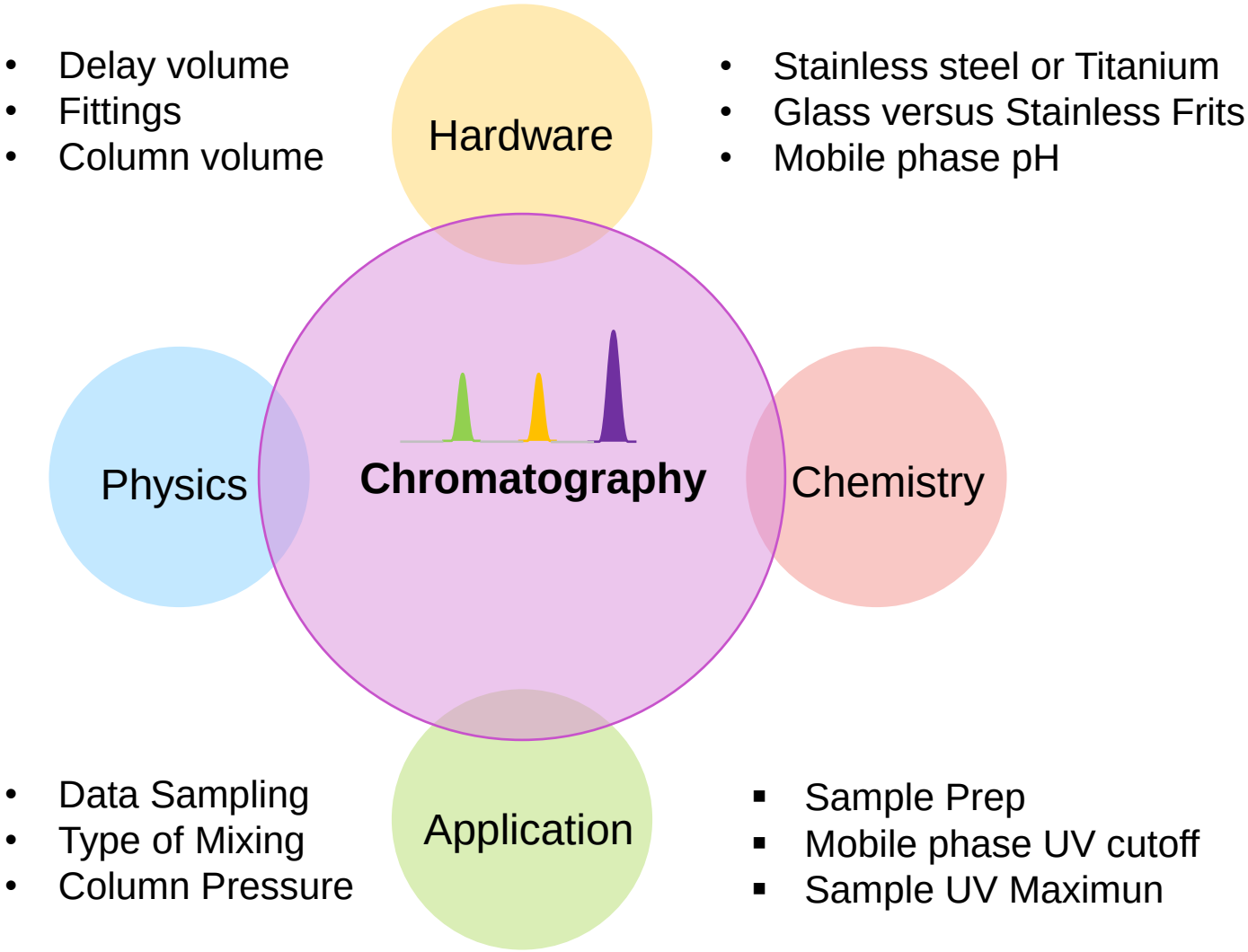
HPLC User Maintenance & Troubleshooting

Lori Sandford

Applications Chemist

Agilent Technologies

Schaumburg, IL



- Delay volume
- Fittings
- Column volume

- Stainless steel or Titanium
- Glass versus Stainless Frits
- Mobile phase pH

- Data Sampling
- Type of Mixing
- Column Pressure

- Sample Prep
- Mobile phase UV cutoff
- Sample UV Maximun

What are Chromatographers Looking for?

Best performance

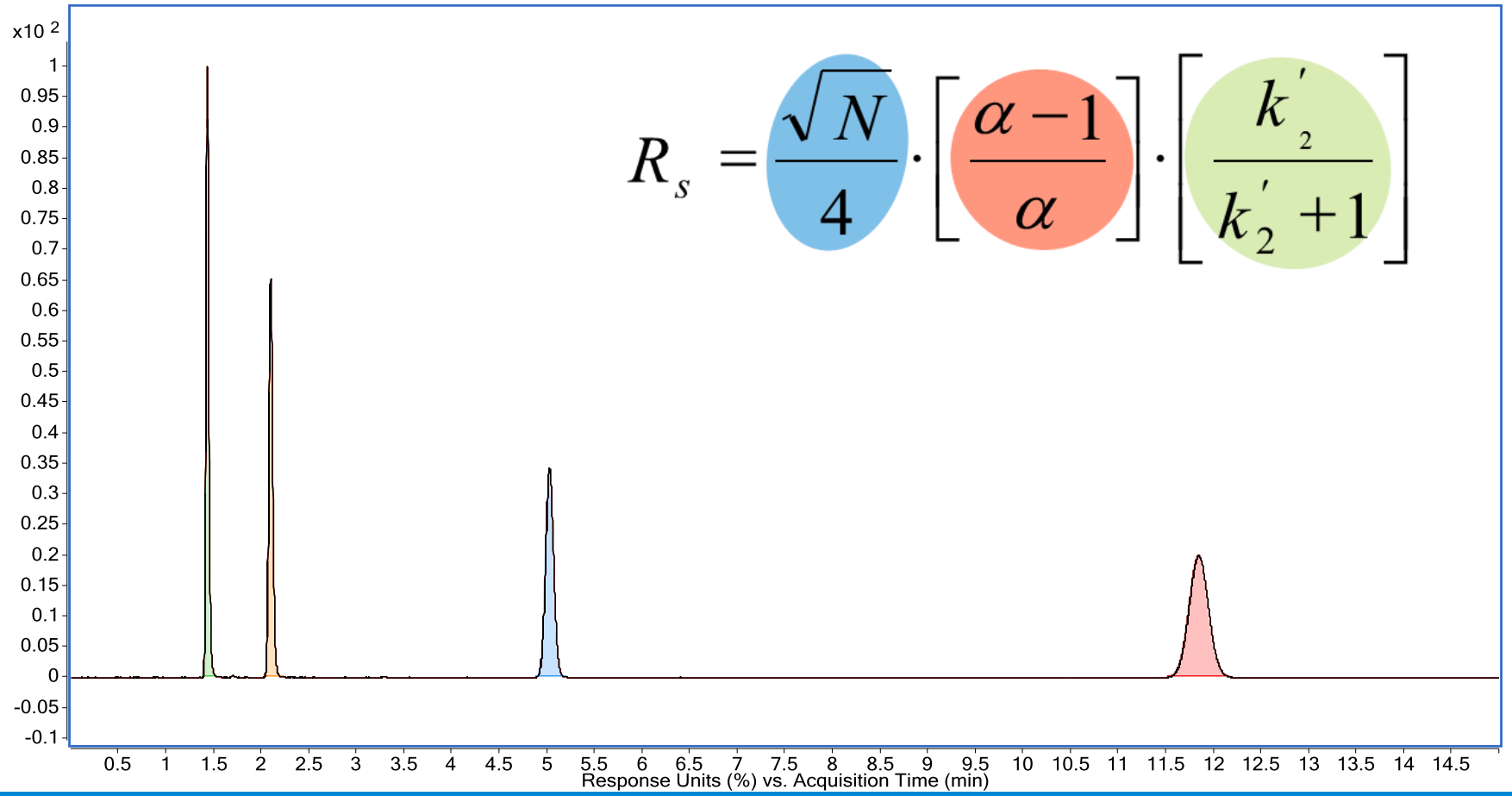
- Resolution
- Highest throughput
- Reproducibility
- Accuracy of results
- Sensitivity
- Standard, narrow bore and capillary column capability

Resolution

N = Number of theoretical. plates — Column length, particle size

α = Selectivity — Stationary and mobile phase, temperature

k = Retention — Stationary and mobile phase



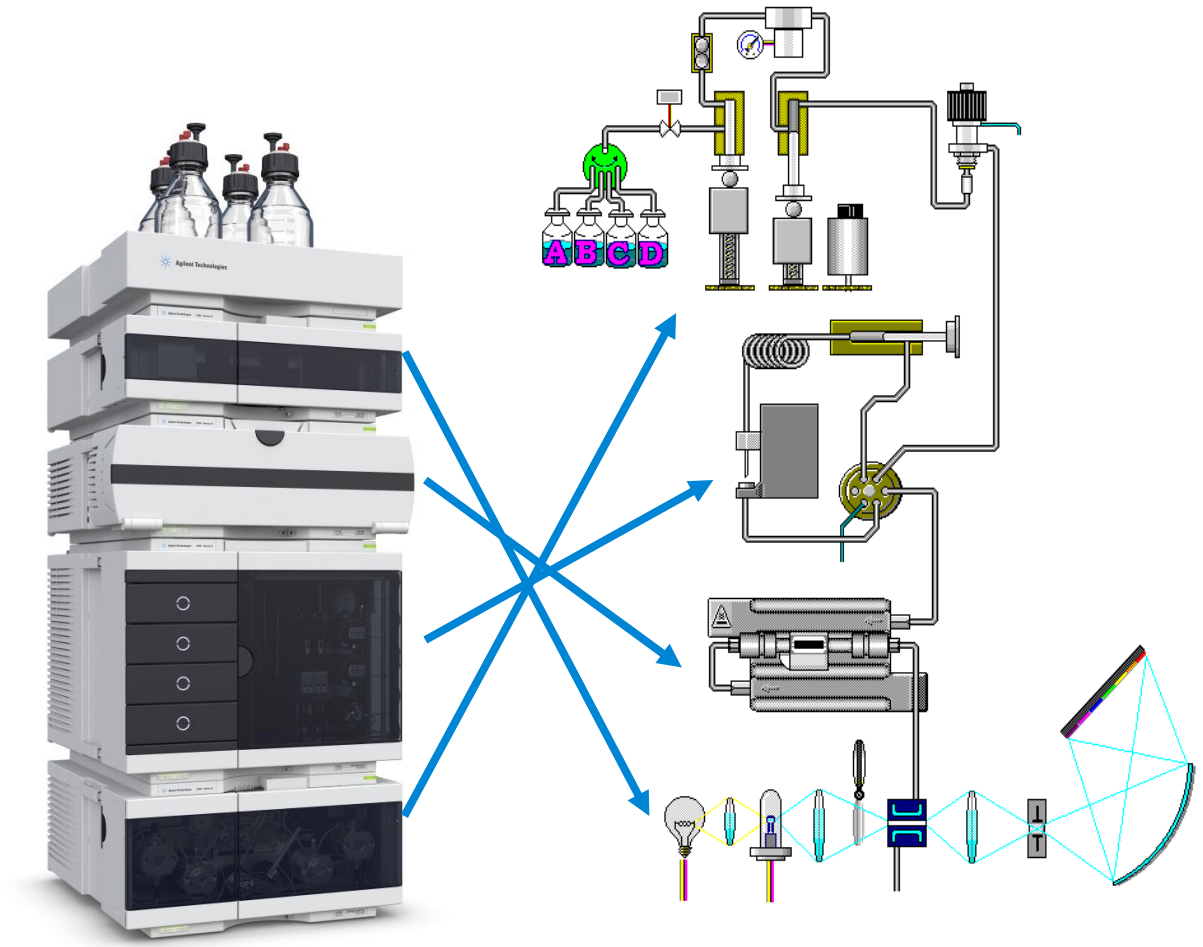
Know your HPLC System:

Detector

Column Comp.

Autosampler

Pump



Vacuum Degasser (integrated in Infinity II and Quat systems)

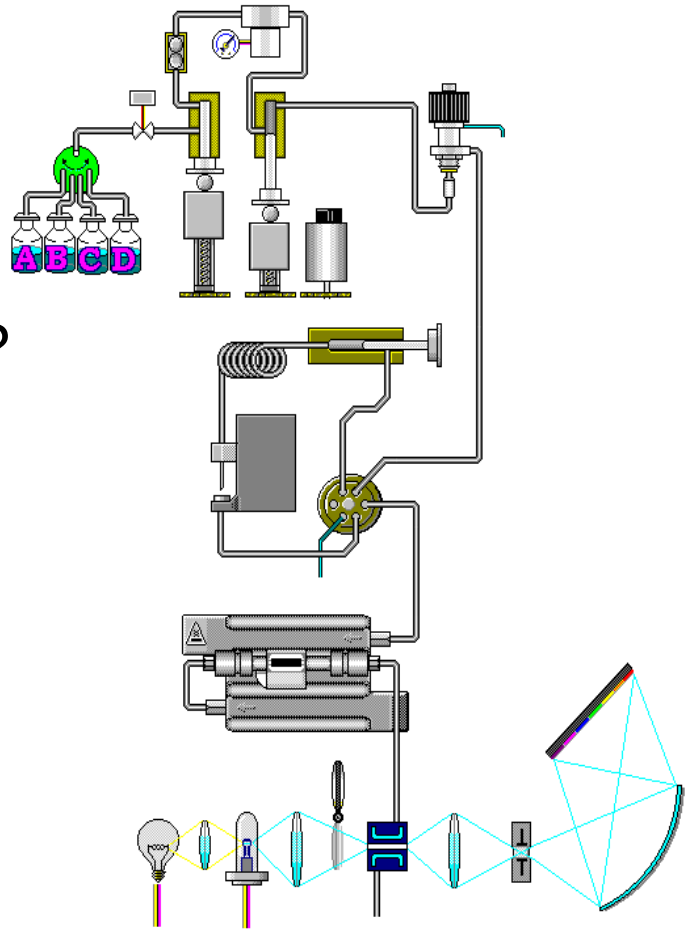
Know Your HPLC Flow Path:

Where are the moving parts?

Where can blockages to flow occur?

Where are the consumables that need to be replaced on a regular basis (PM)?

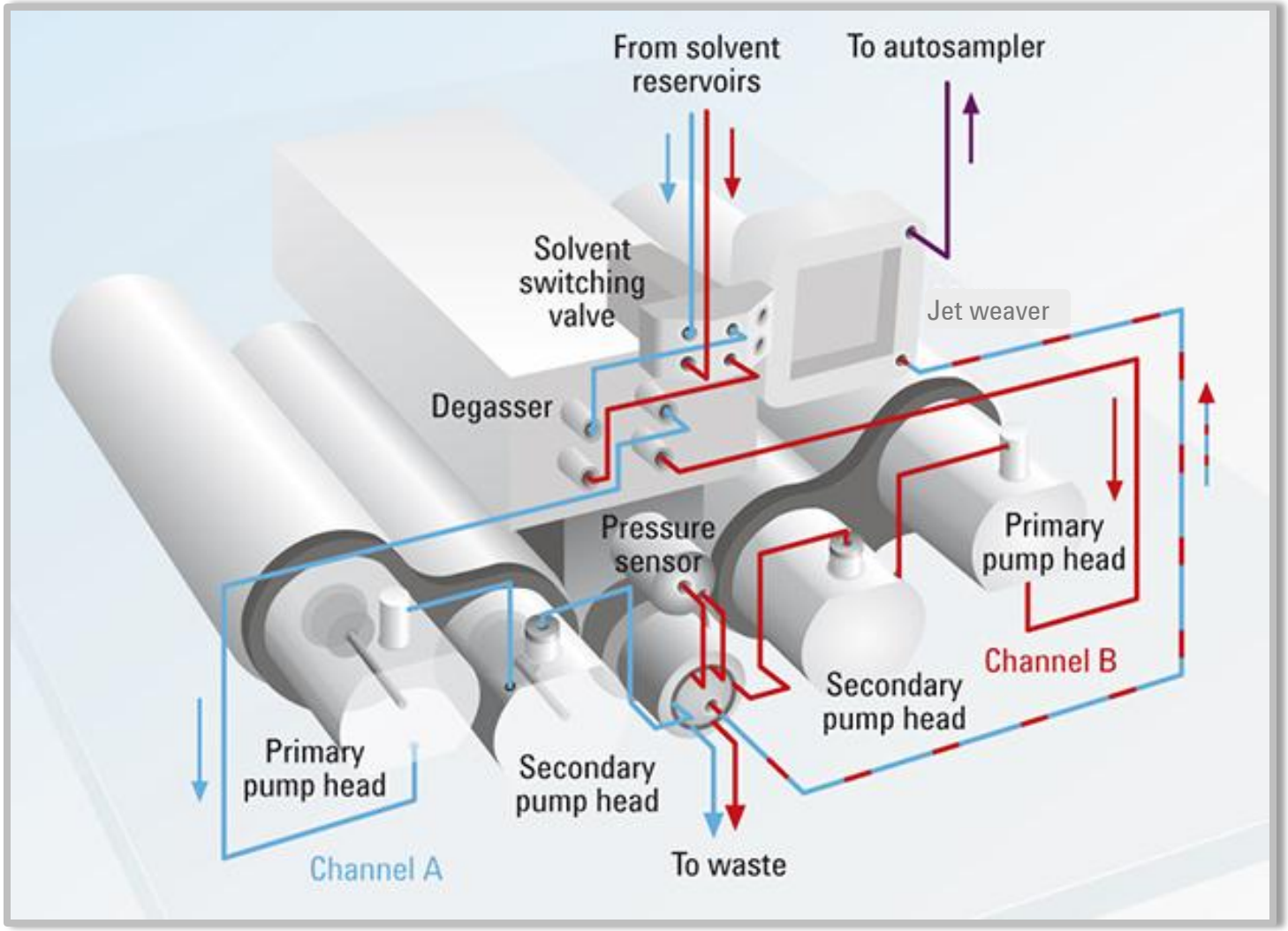
Where can leaks occur ?



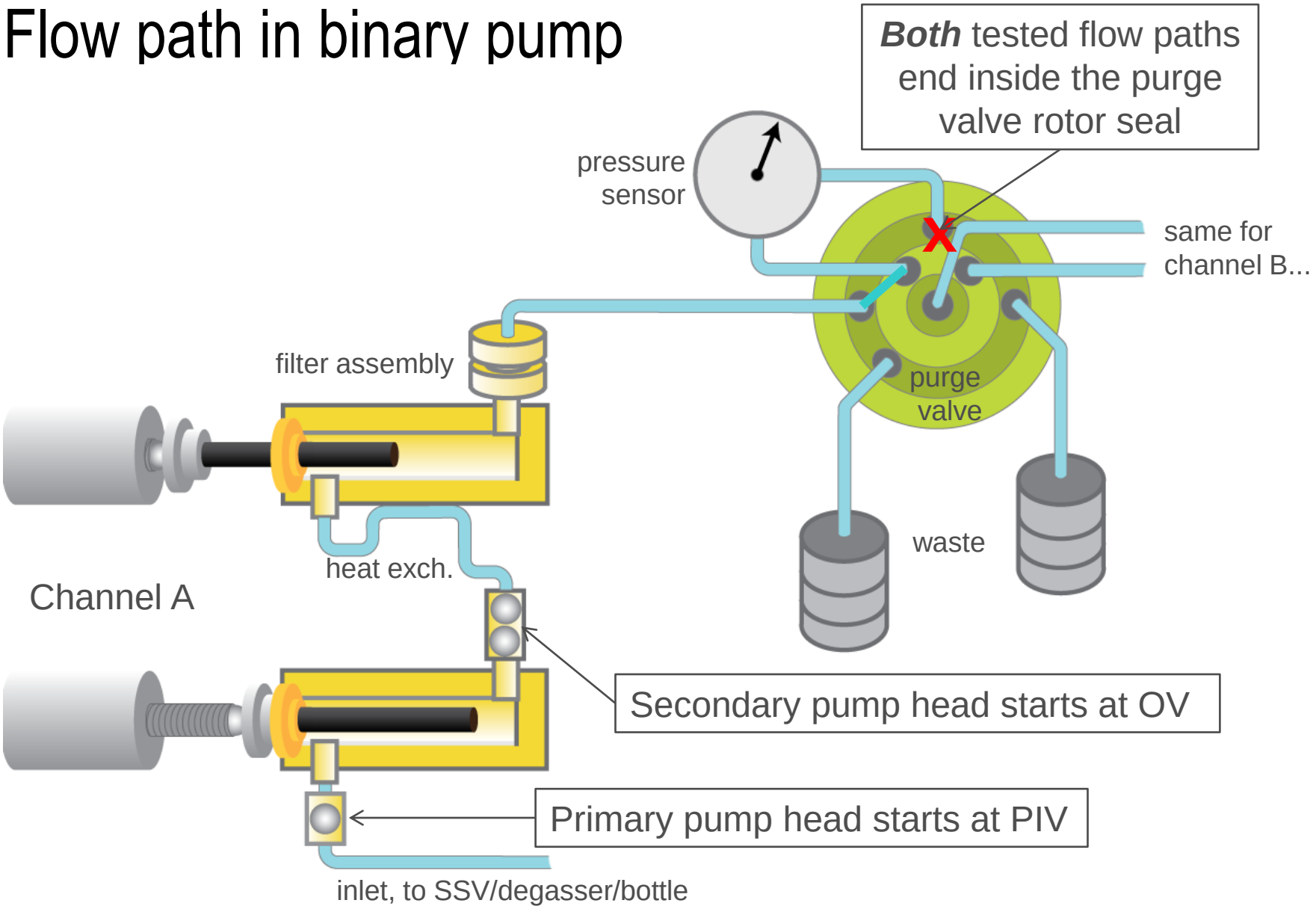
What can I do to eliminate, reduce or anticipate potential problems with the LC ?

1290 Infinity Binary Pump

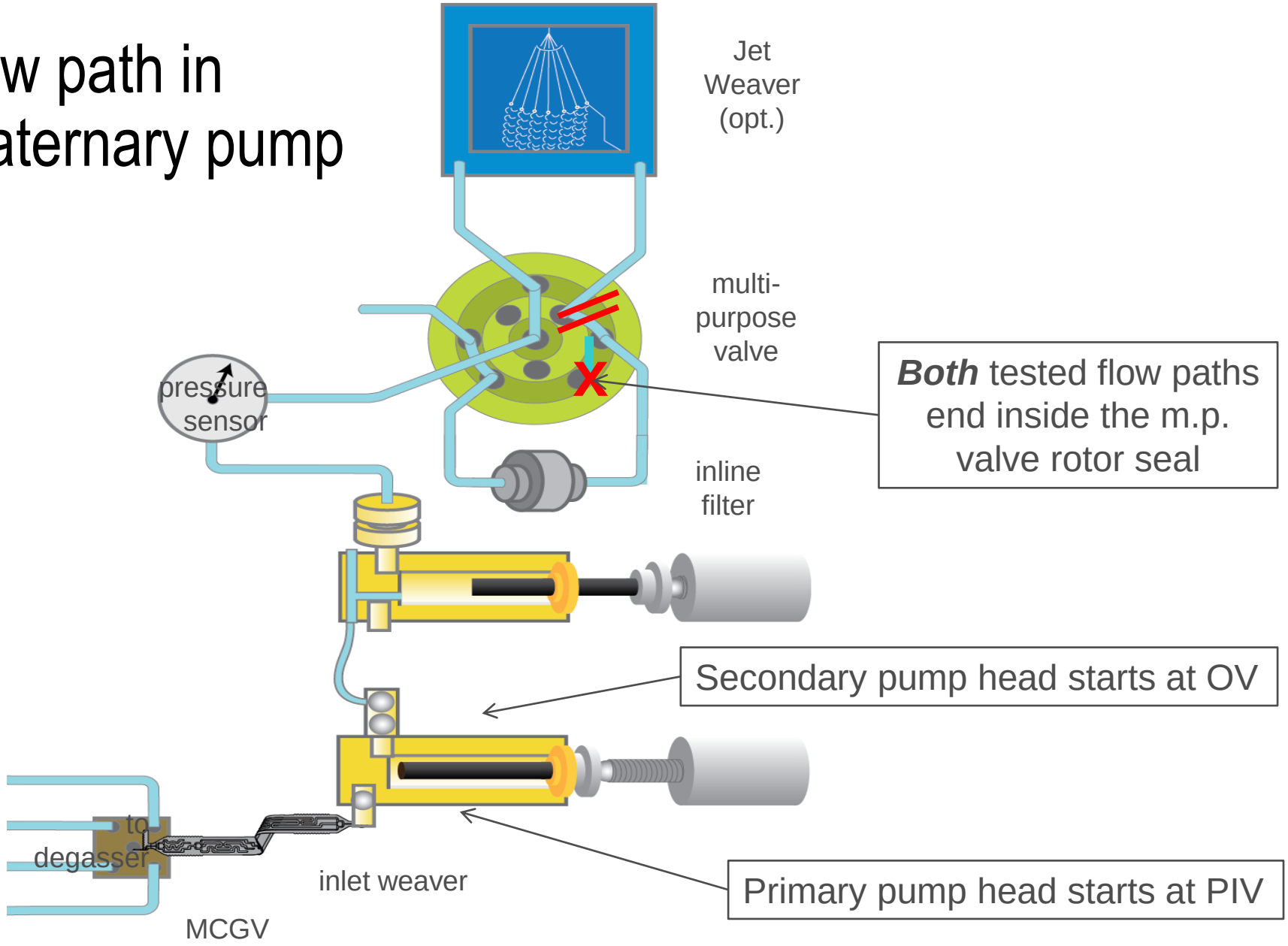
– the flow path



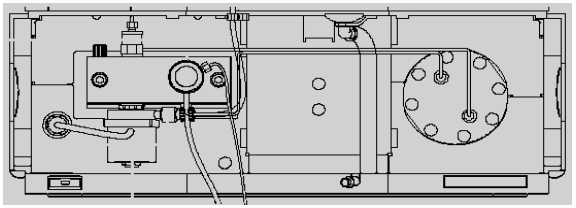
Flow path in binary pump



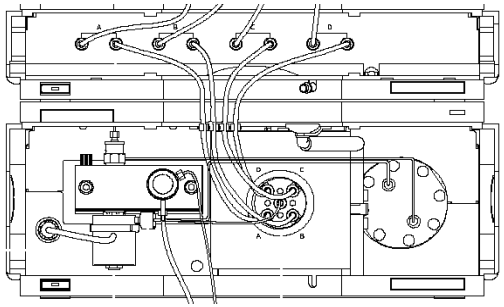
Flow path in quaternary pump



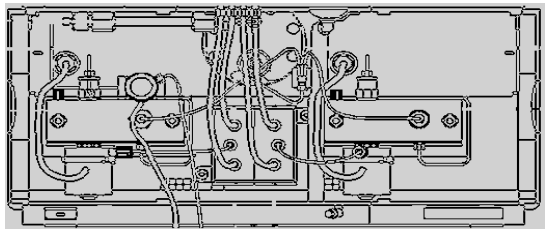
Agilent 1200 Series Pump Models - Analytical



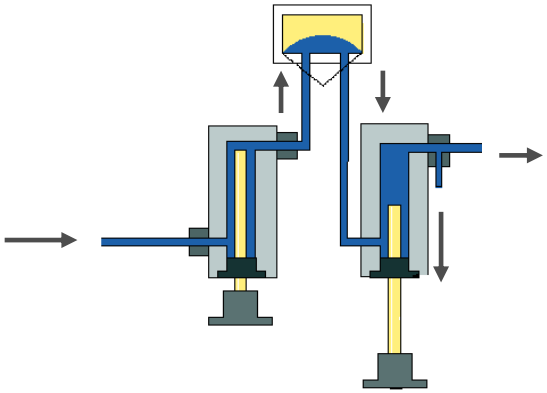
Isocratic Pump

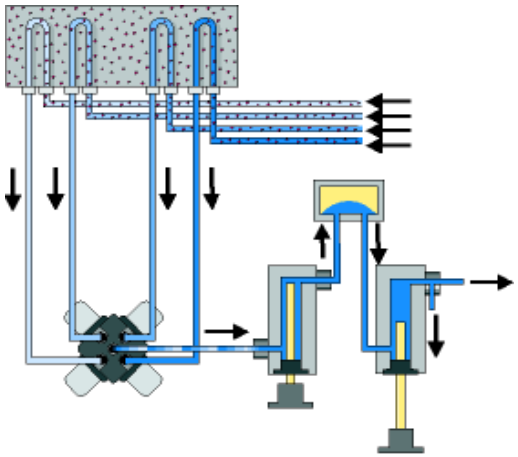


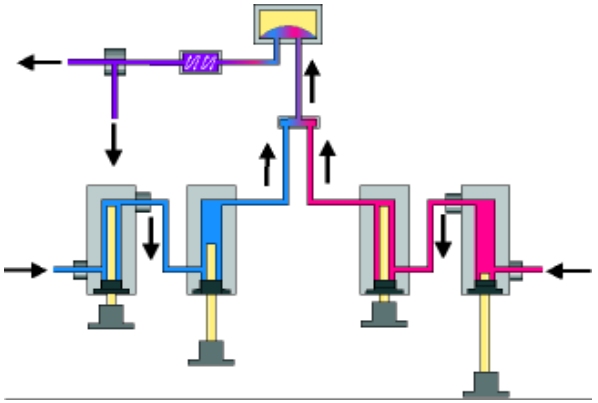
Quaternary Pump



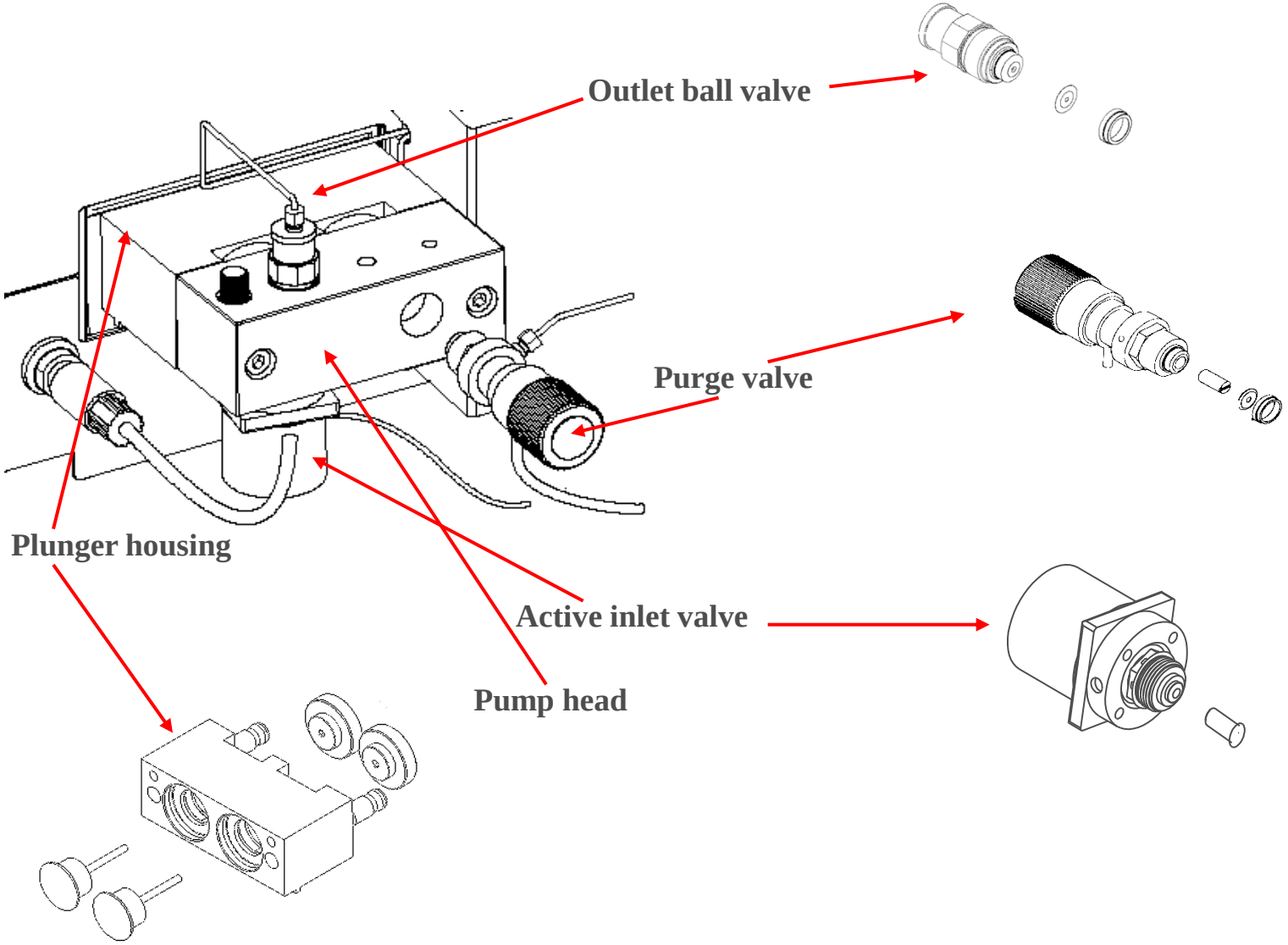
Std Binary Pump







Pump Head – Main Components



Pump and Degasser Maintenance

- Clean the degasser lines by flushing with isopropanol.
- When using buffers, flush with water, then with isopropanol.
- Check for air bubbles in outlet lines.
- Be aware of the possibility of microbial growth in aqueous phases.
- Check for solvent compatibility.
- Unused channels should be left in isopropanol.
- May have need to exchange the vacuum pump, sensor, solenoid valve, or vacuum chamber.

Overall Routine Pump Maintenance

1. Remove and disassemble the pump head.
2. Remove and clean pistons.
3. Replace piston seals.
4. If seal wash option is installed, replace wash seals and gaskets.
5. Inspect the springs.
6. Reassemble pump head and reinstall.
7. Perform seal wear-in procedure.
8. Replace PTFE frit in the purge valve.
9. Clean or replace the outlet ball valve.
10. Replace the AIV cartridge.
11. Flush with isopropanol.
12. Clean or replace solvent inlet filters.
13. Clean the leak sensor.
14. Make certain the waste tube is in place.
15. Test the pump (Pressure and Leak Test).

- Covered by an annual Agilent LC PM contract

When to use purge, prime, condition ?

Purge
Change solvents
When pump is refilled with new/different mobile phase the purge valves allows both pump heads (binary pump) to be connected to waste at the same time

Prime
When the pump is dry
When Purge and Condition still show exhausted pressure ripple

Condition
When first starting up for the day or after changing solvents
When pump pressure ripple or composition ripple is too high (mixing noise) air bubble is hidden in pump head (listen)
best once a day to condition for smooth operation

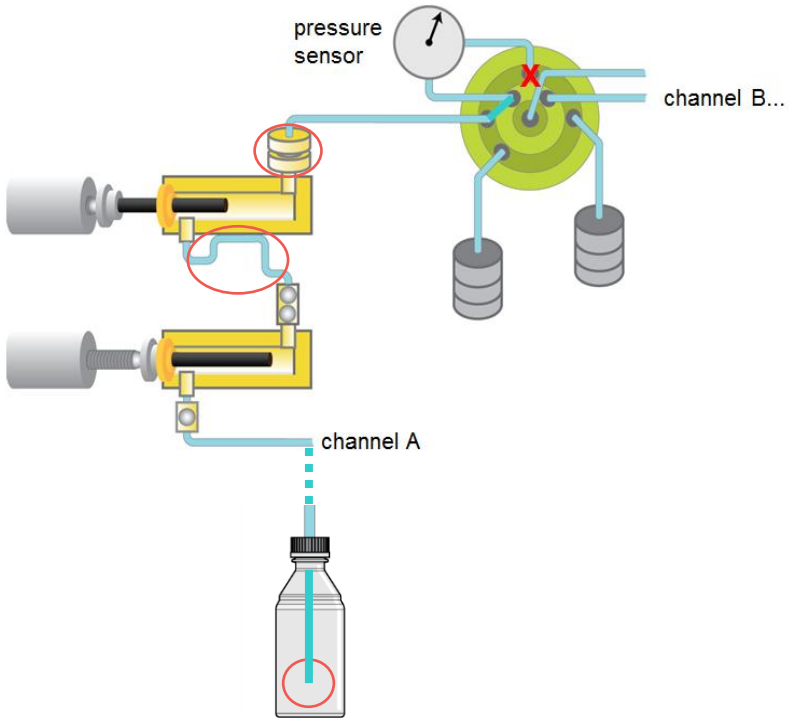
Filters and Bottle necks for blockages

- **Solvent inlet** filters in solvent bottles
  glass: 20um – replace if needed!
  SST: 12-14um – replace, opt sonicate
- **Inlet weaver** (mixer) between MCGV & prim head (quat only)
- **Heat exchanger** (bent? Connection?)

- **High pressure filter** assemblies at outlet of secondary pump heads: 5um – replace (see cleaning procedure)
- Quaternary pump: **inline filter** at multiple purpose valve MPV: 0.3um – replace
- **Jet weaver** (optional with 35um, 100ul, 380ul)

  Troubleshoot: Disconnect other modules behind pump

➔ **Flow path before pressure sensor is uncontrolled !**



Examples: used / unused Filters

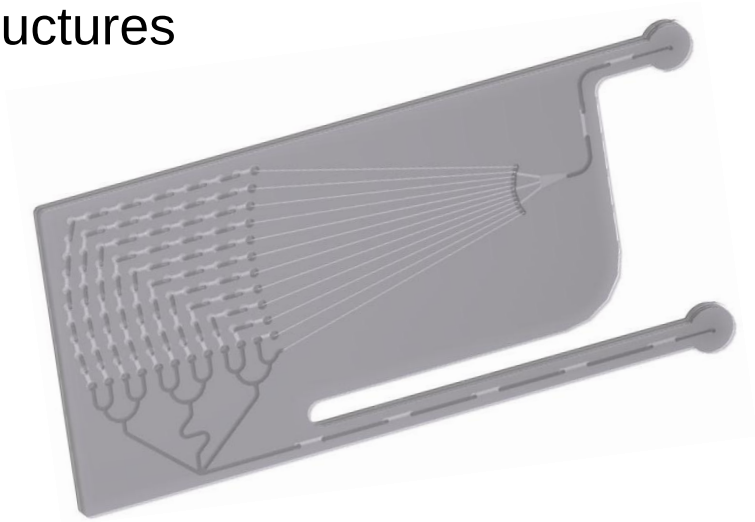
Glass filters: 3150 - 0944

Stainless Steel Filters: 01018 – 60025

(less volume, no Na+ ions)

Example: Jet Weaver & Inlet Weaver

- **Multi-layer technology**
  Diffusion bonded stainless steel, etched structures

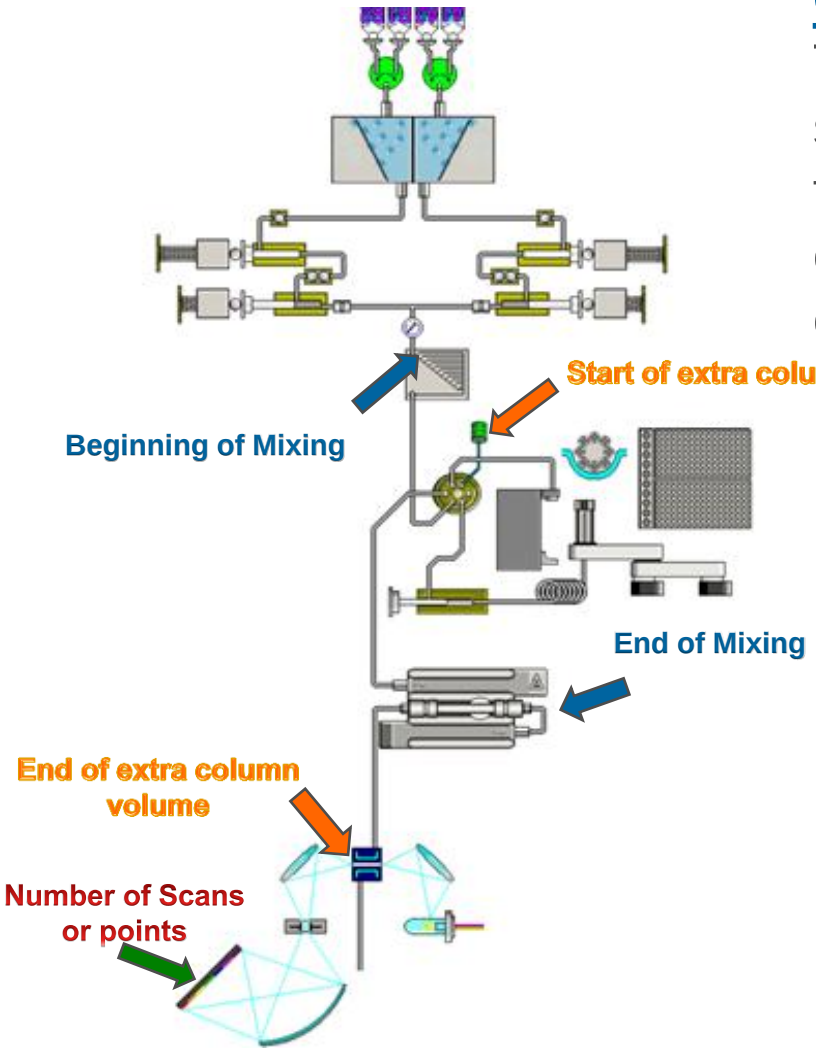


- **Two mixers in one cartridge**
  Standard mixer with 35 µl volume & 100ul adds delay volume of 45ul and 75ul

- **Optional mixer in quat pump with V380**
  which adds 150ul delay volume

- **Mixer for TFA applications with**
  adds 380 µl volume delay volume

Considerations for HPLC systems



Gradient Delay or Dwell Volume

The volume between the point of mixing of solvents (usually in the mixing chamber or at the proportioning valves in the liquid chromatograph) and the head of an LC column.

Extracolumn Volume

The volume between the effective injection point and the effective detection point, excluding the part of the column containing the stationary phase. It comprises the volumes of the injector, connecting lines and frits, and the detector. It determines the ***extracolumn effects.***

System – Signal height

System dispersion

- *"Dispersion is the sample bandspreading or dilution which occurs in connecting tubing, sample valves, flow cells and in column end-fittings."*

Peak height: Loss of sensitivity

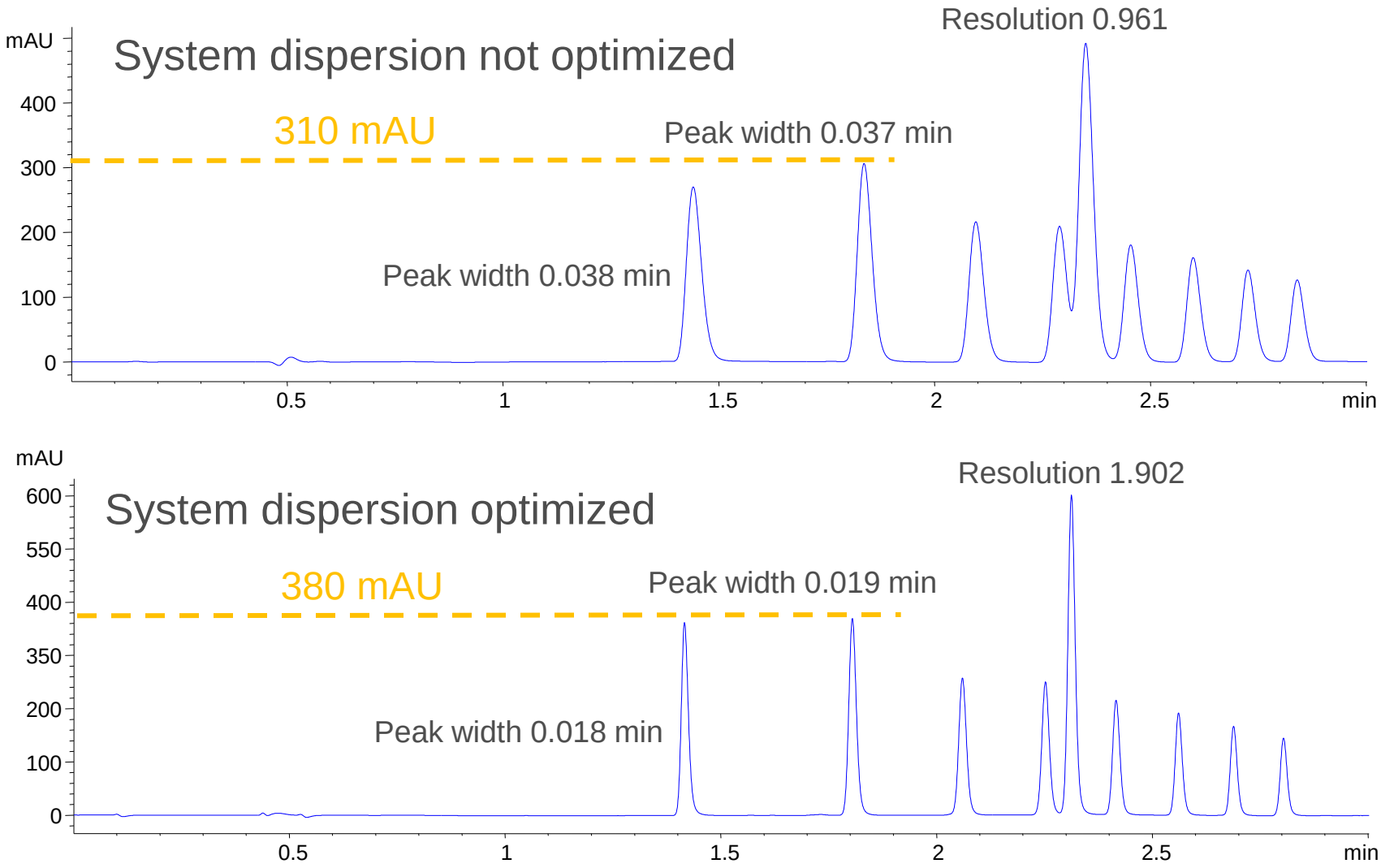
Peak width: Loss of resolution

- Capillaries (Inner diameter, length)

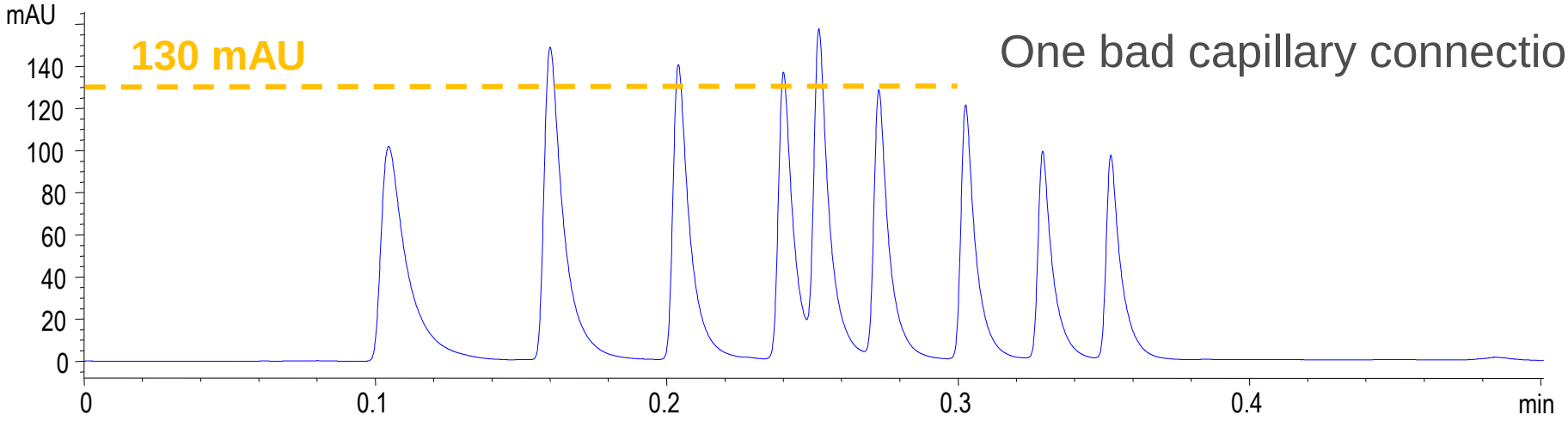
$$\sigma^2 = \frac{\pi \cdot r^4 \cdot F \cdot L}{24 \cdot D_m}$$

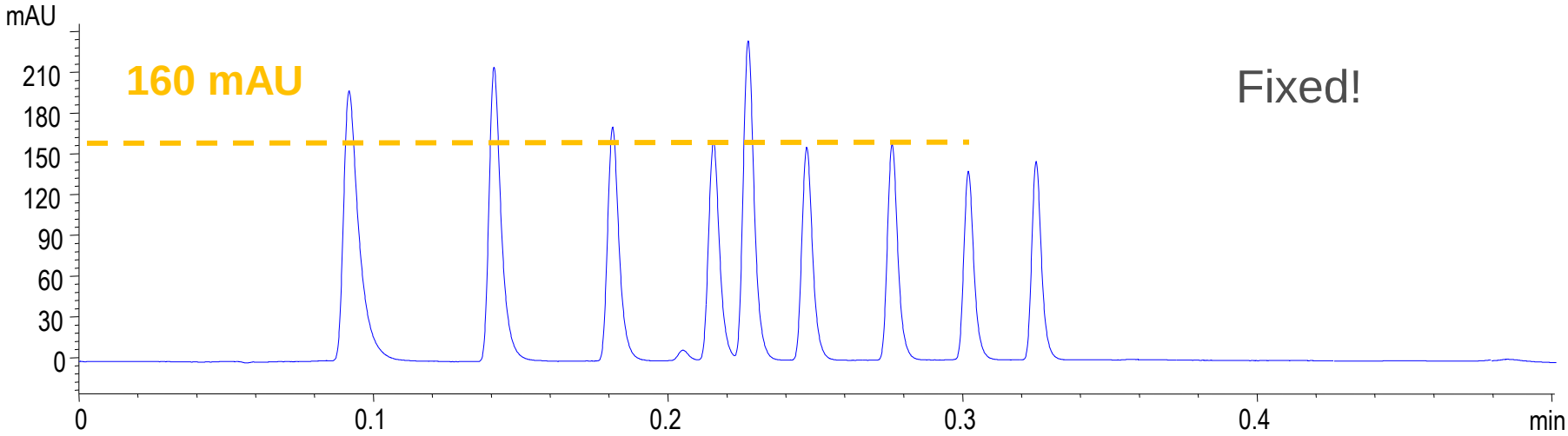
Aris-Taylor Equation

System – Signal Height



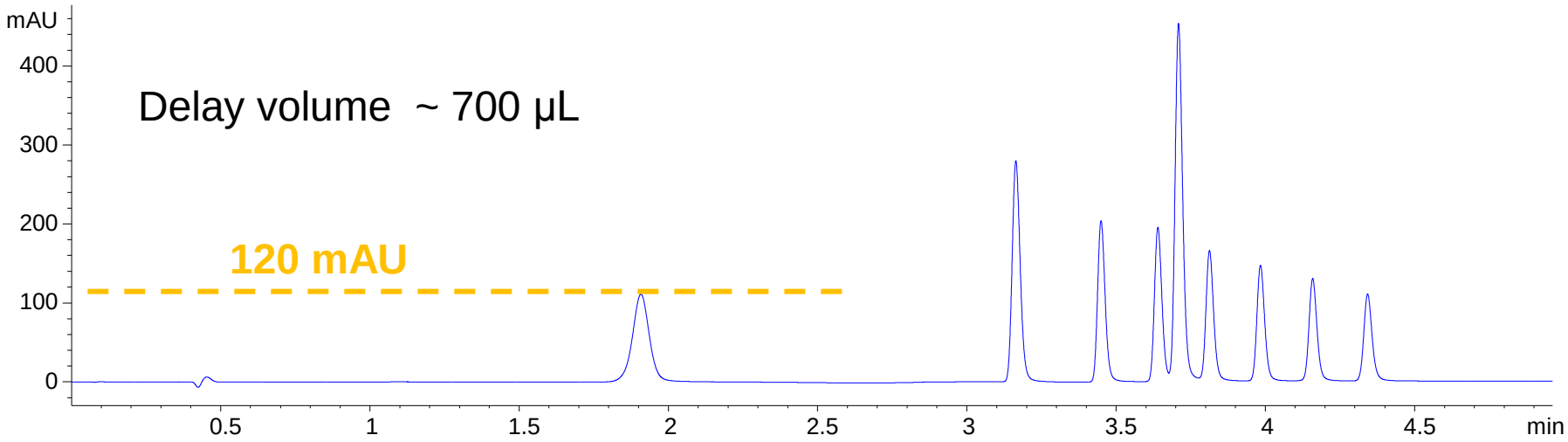
Influence post-column capillary connections

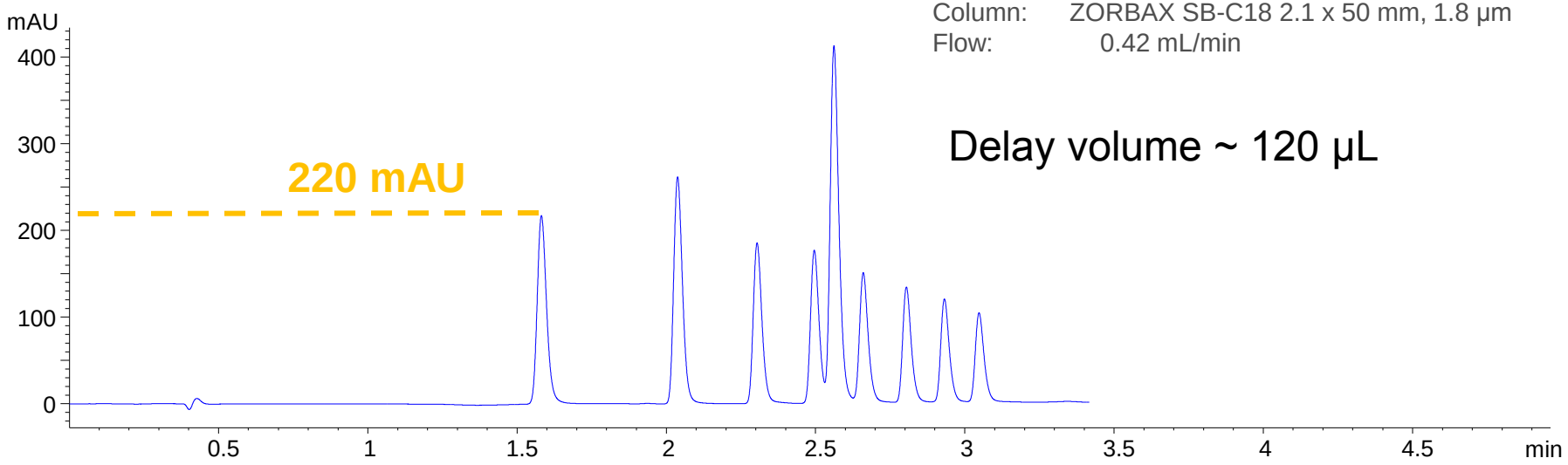




System – Signal height

System volumes – Delay volume

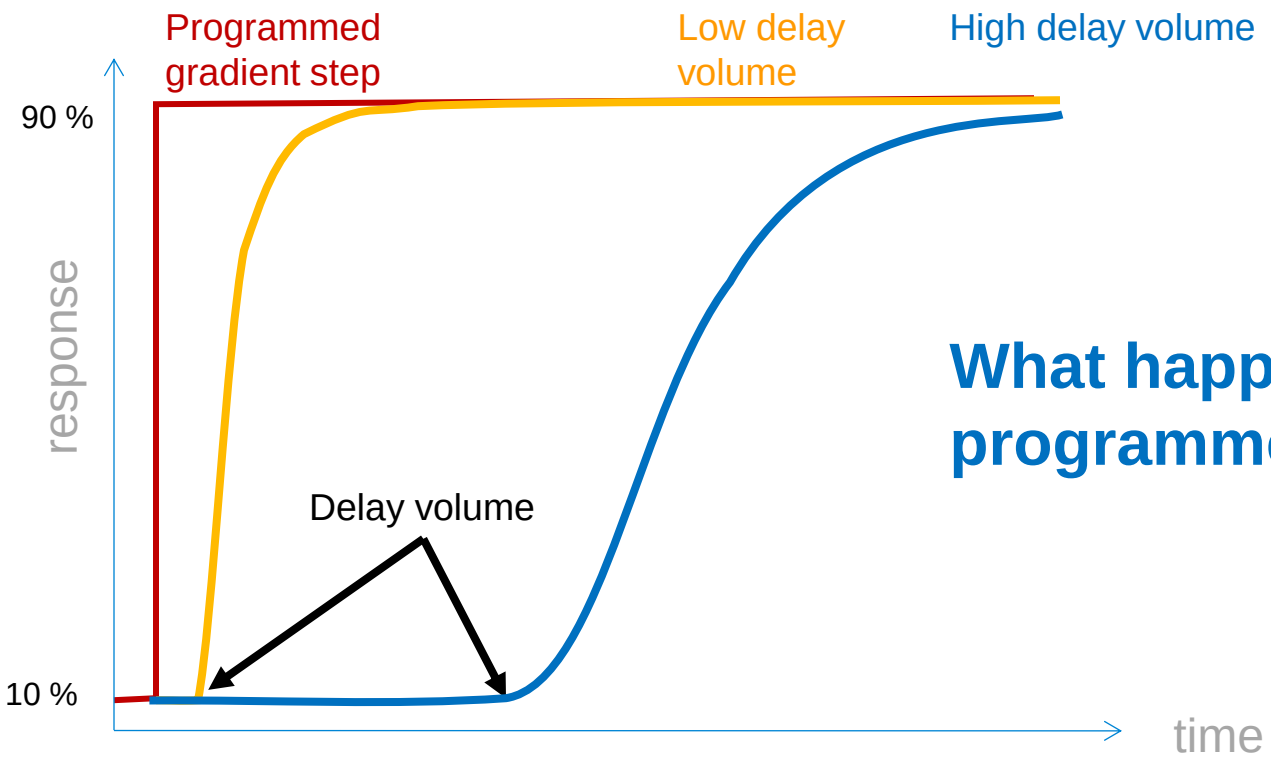




Column: ZORBAX SB-C18 2.1 x 50 mm, 1.8 µm
Flow: 0.42 mL/min

Delay volume

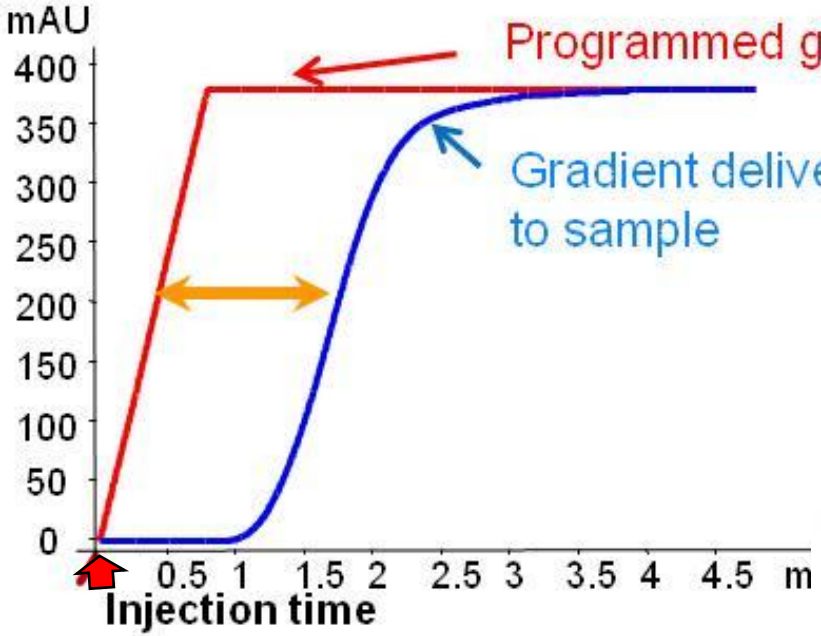
Impact of low delay volume



What happens with a programmed gradient?

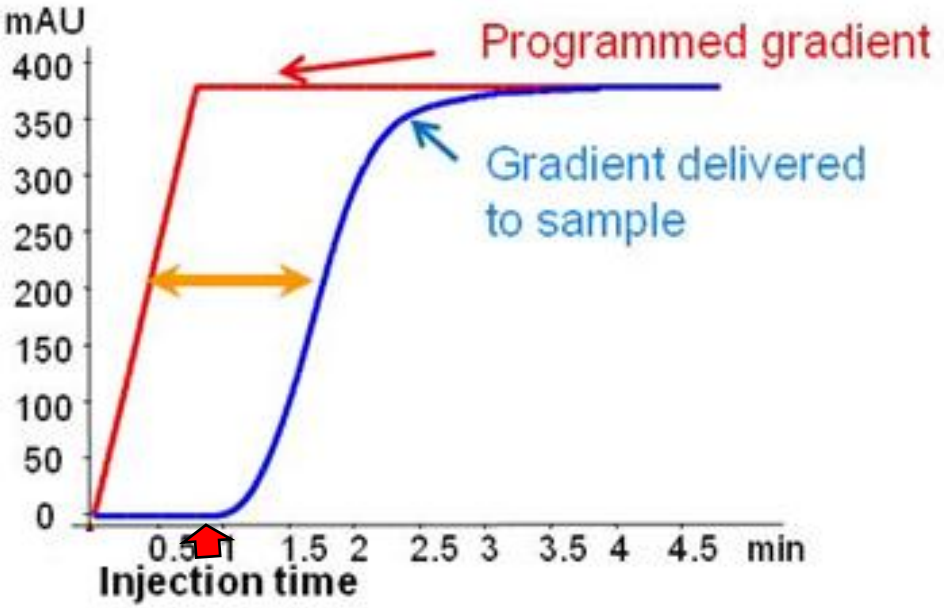
Total delay volume of the system (sum of capillaries, mixer, cells, valves..)

Effects of Delay Injection Program



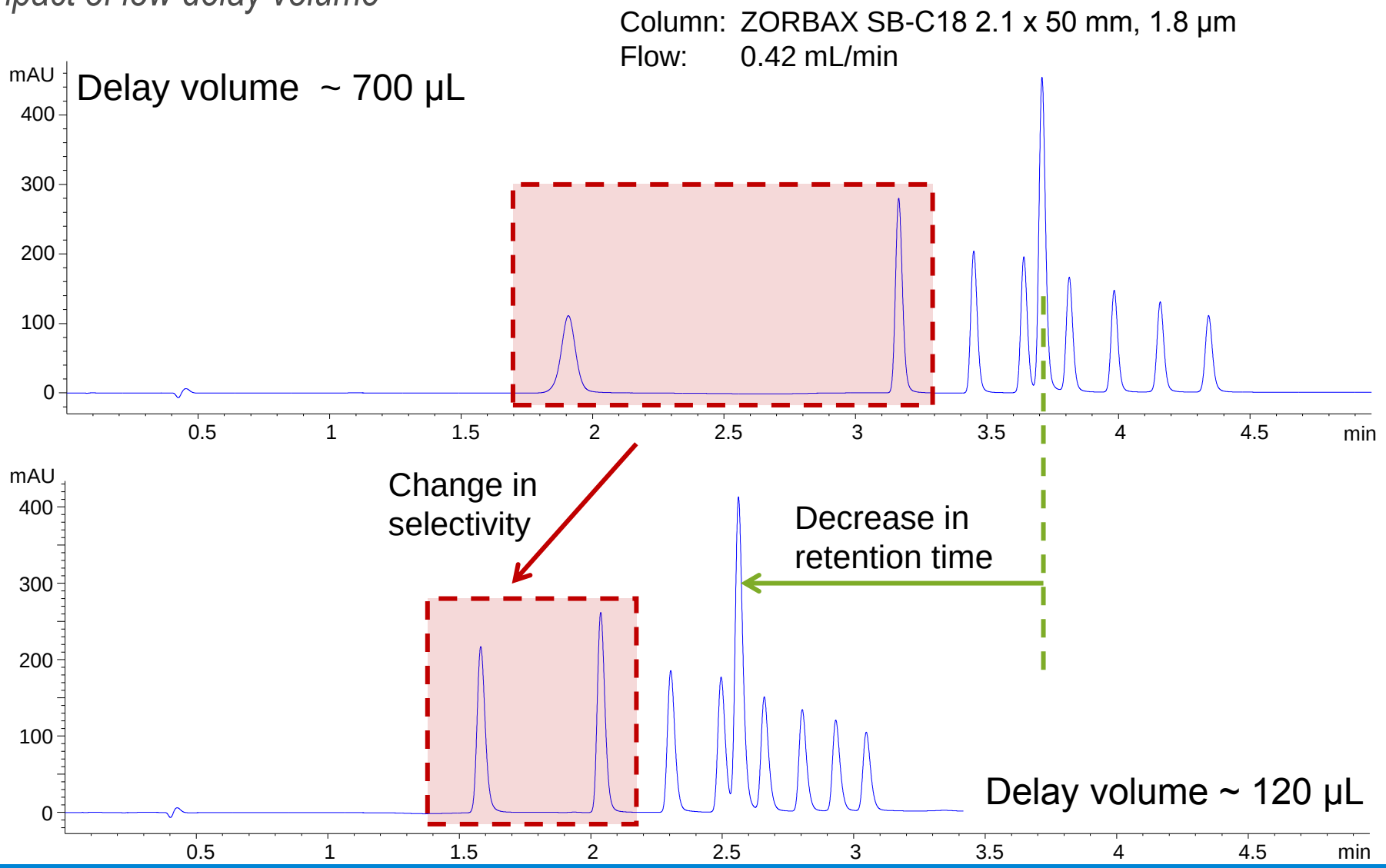
Standard Injection

Delayed Injection



Delay volume

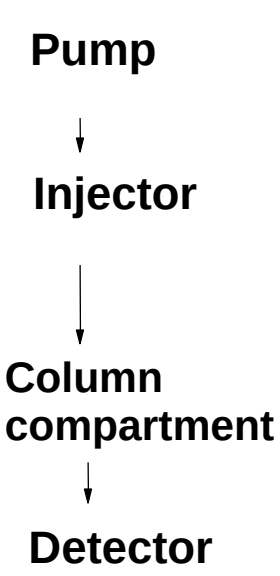
Impact of low delay volume



Performance Characteristics of an HPLC System

Influenced by one module...

Influenced by several modules...



Flow: accuracy, precision
Composition: accuracy, precision

Repeatability of retention times
Delay volume

Injection volume precision
Linearity, dynamic range
Carry over

Repeatability of peak areas
Dead volume

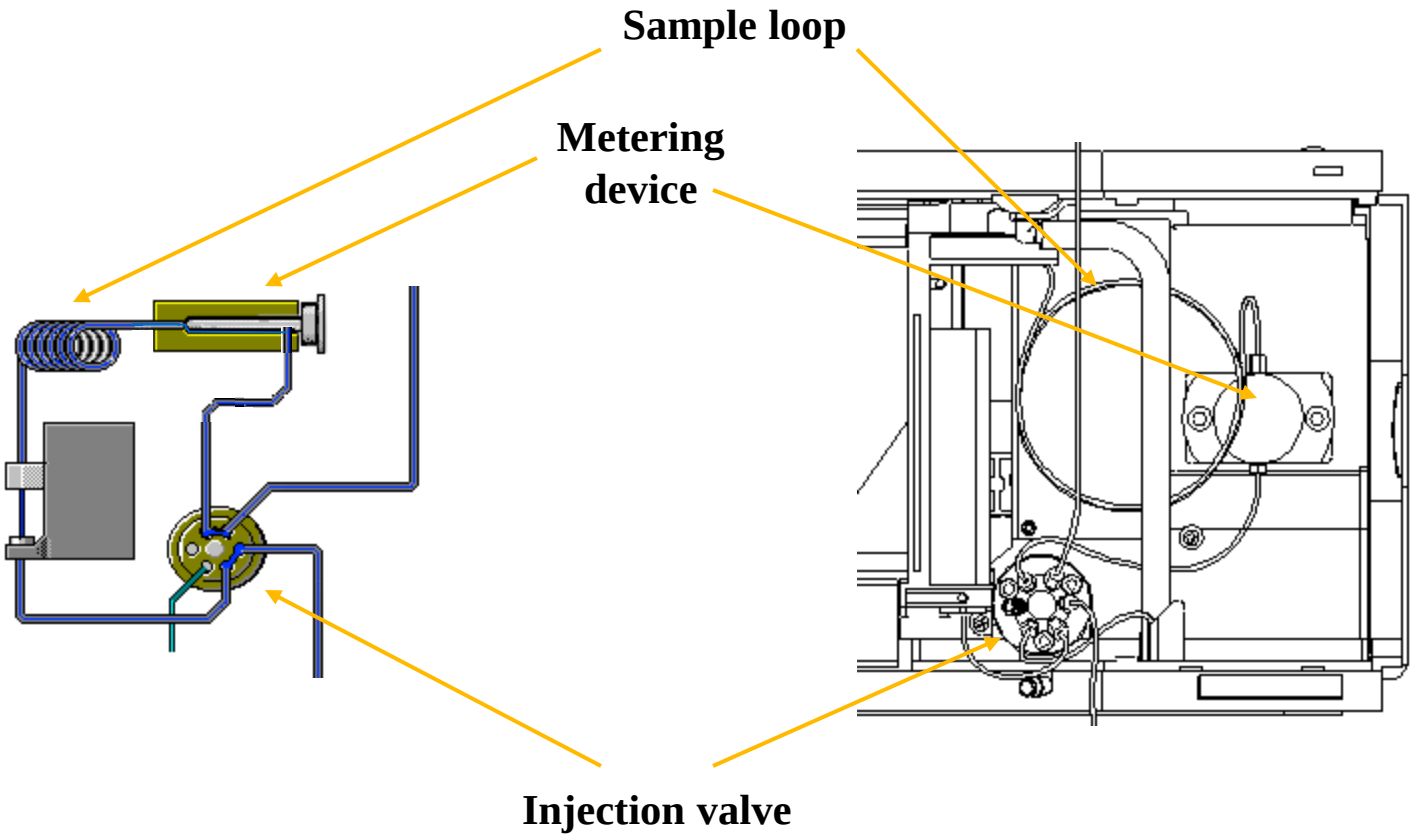
Column temperature accuracy
Column temperature precision

Peak elution order

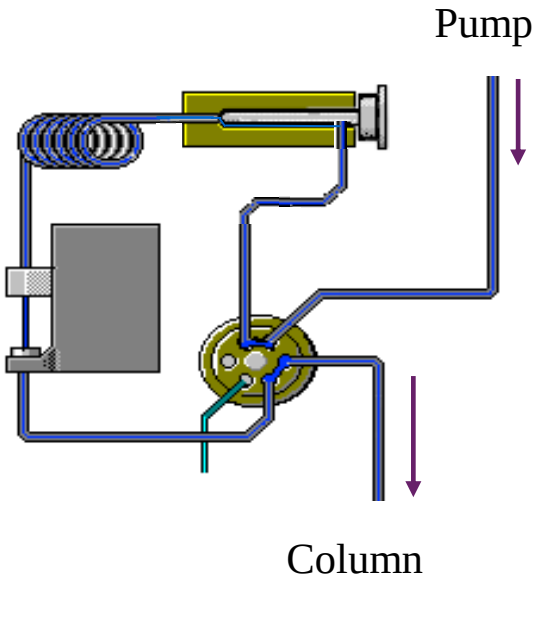
Wavelength: accuracy, precision
Signal linearity
Spectral resolution (DAD only)

Baseline: noise, drift and wander

Schematic of Injection System

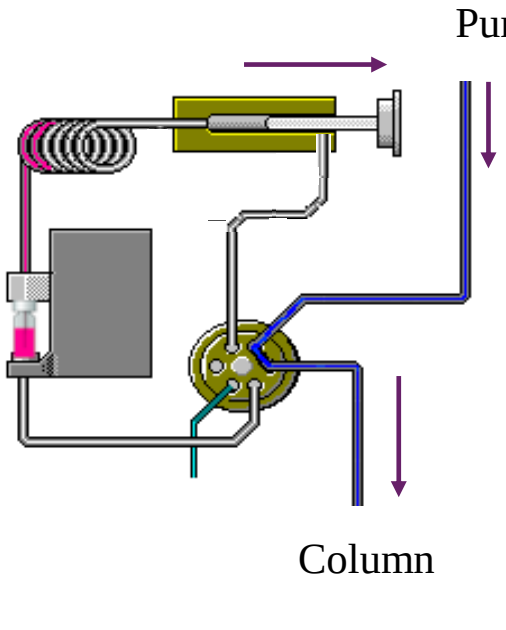


Principle of Operation



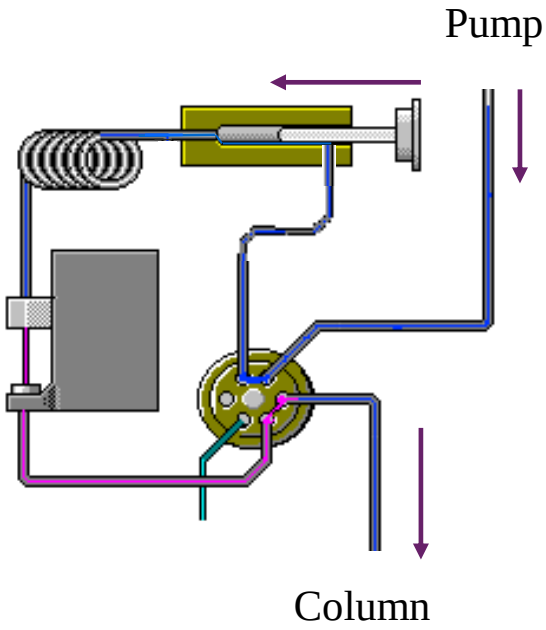
Prior to Injection

Valve in Mainpass Position



Draw Sample

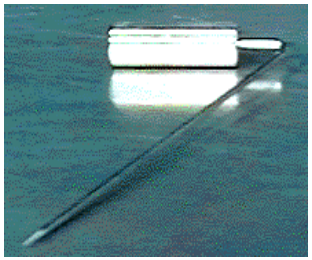
Valve in Bypass Position

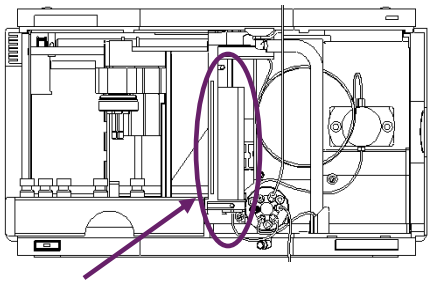


Injection and Run

Valve in Mainpass Position

Exchanging the Needle/Needle Seat – Standard Autosampler G1329A.





Needle/Needle seat

Parts:

Needle **G1313-87201**

Needle seat **G1313-87101 (0.17 mm i.d.)**

or **G1313-87103 (0.12 mm i.d.)**

Tools:

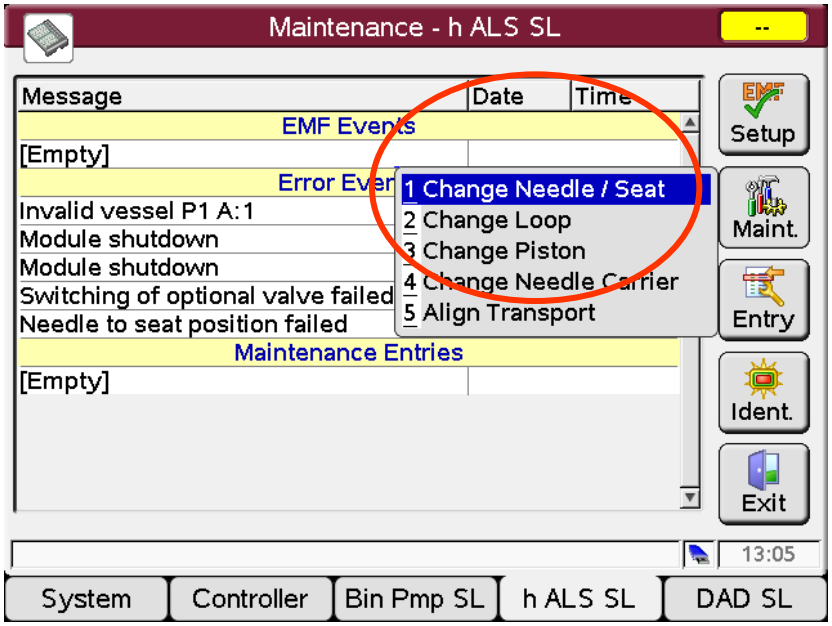
Wrench ¼ inch

Hexagonal key 2.5 mm

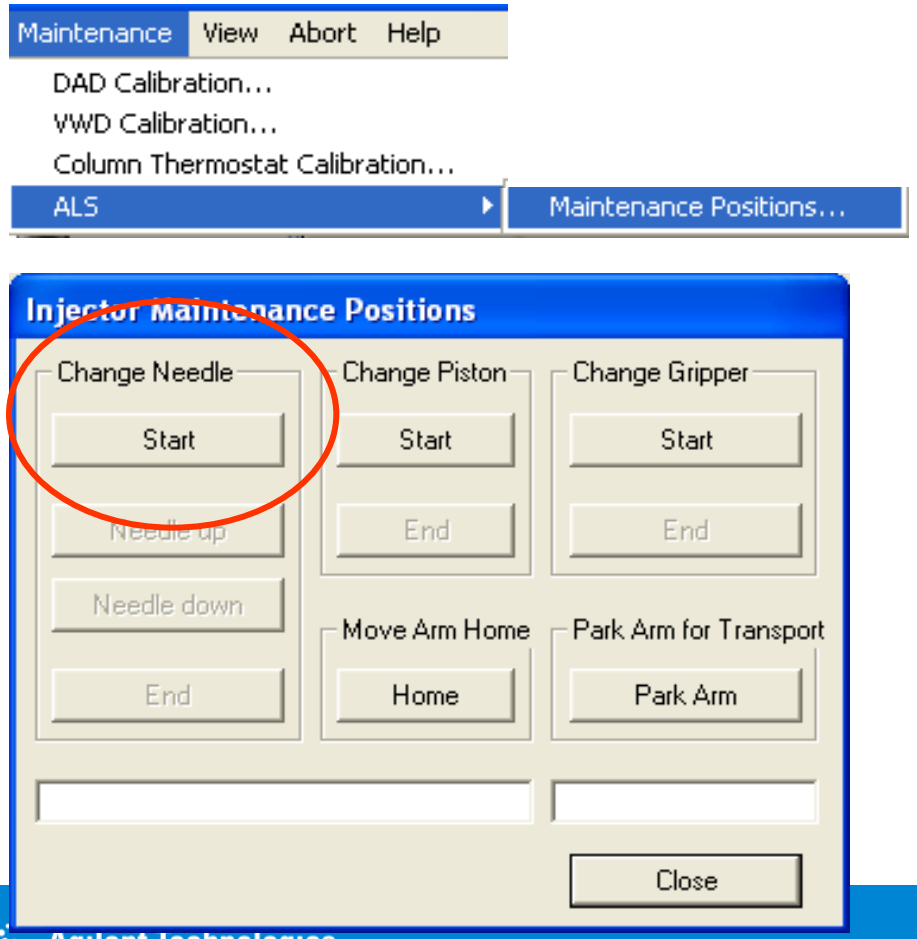
Autosampler Maintenance Functions

<u>Before beginning needle or needle seat replacement:</u>
Select "Change Needle" in the autosampler maintenance function.

Instant Pilot:



ChemStation:



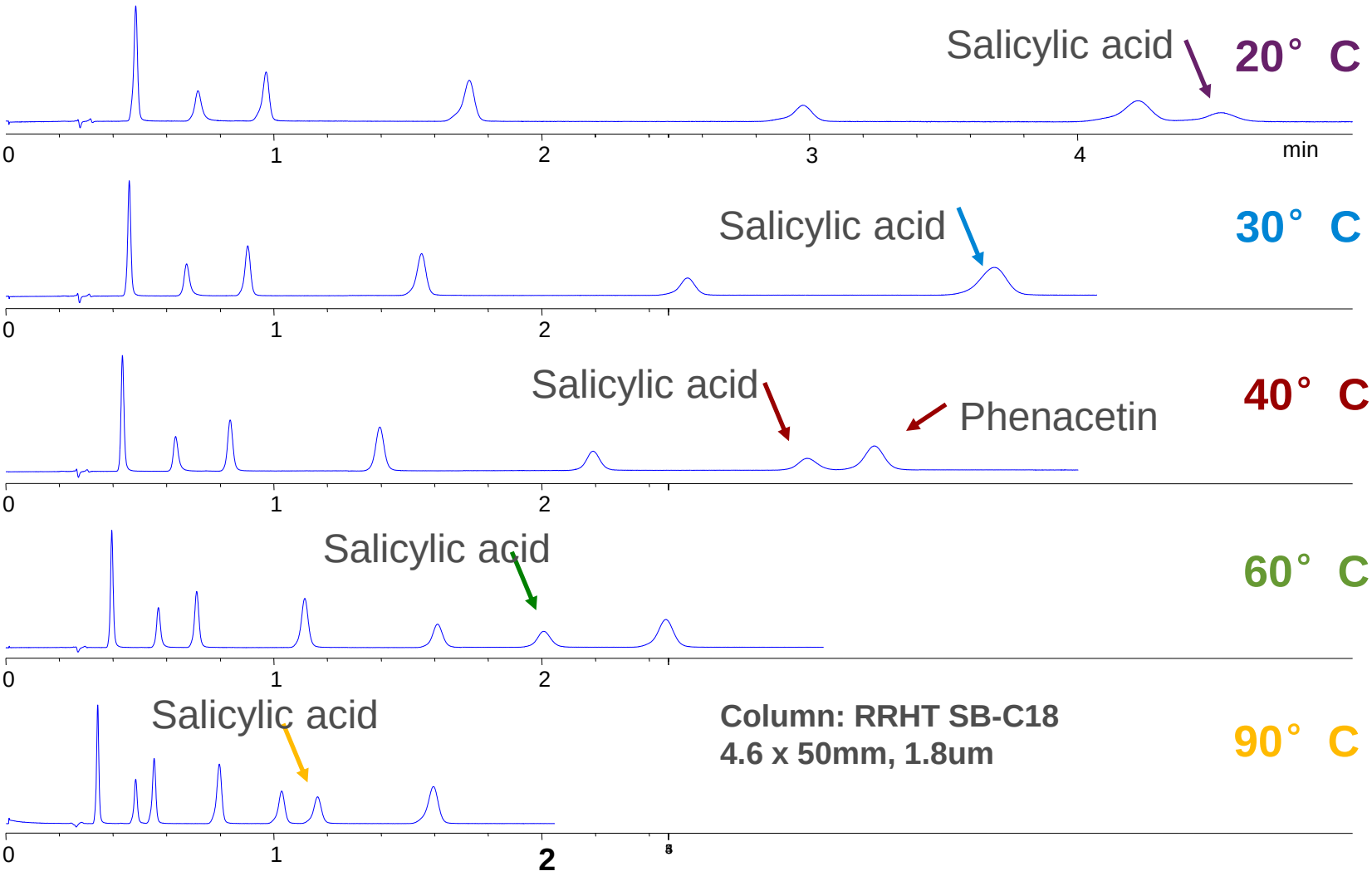
Thermostatted Column Compartment

- **Important performance characteristics**

- **Influence on...**

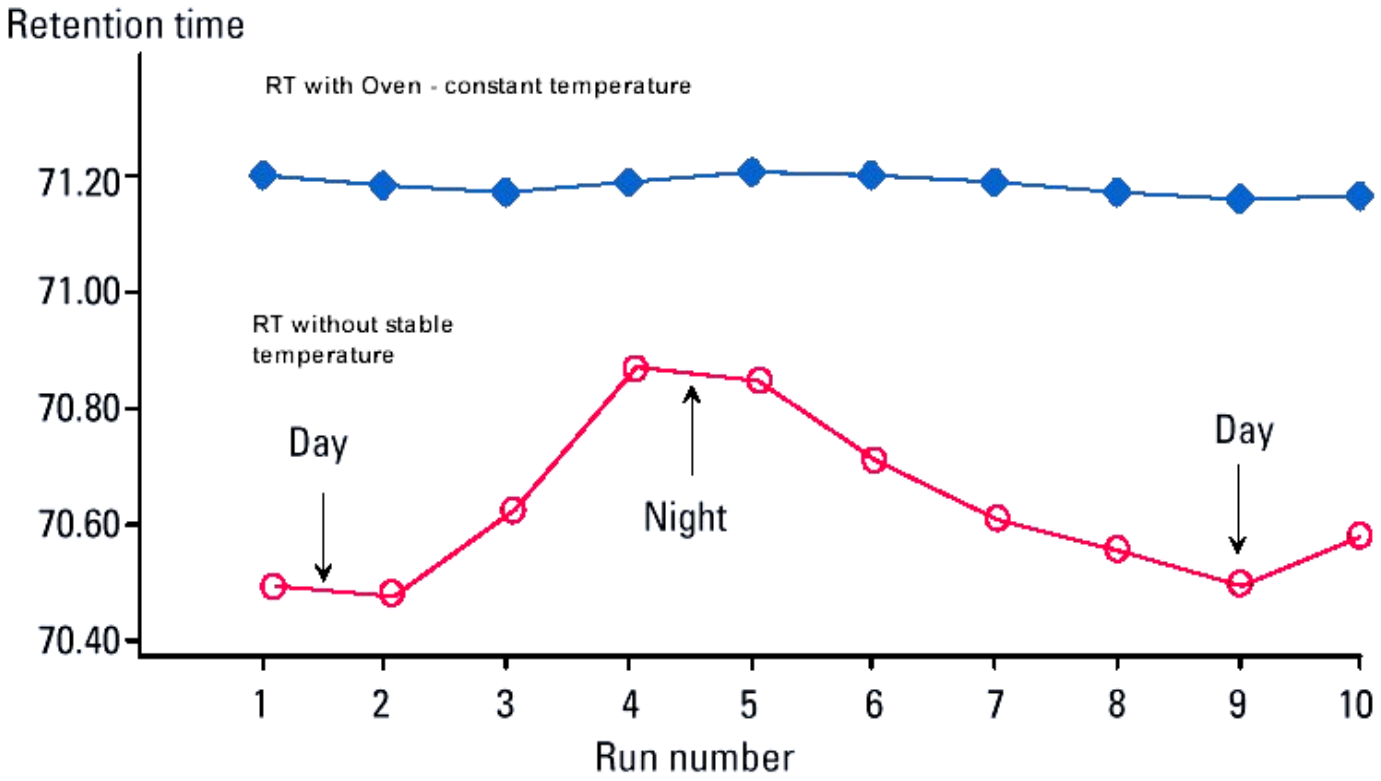
- Excellent temperature accuracy
  - Elution order
  - Peak identification
- Excellent temperature precision
  - Elution order
  - Retention time precision
  - Peak identification

Effect of Temperature on Separation



Conditions: A: 0.1% formic acid B: ACN w/ 0.1% formic acid (85:15) Detection: UV 254 nm

Column Oven

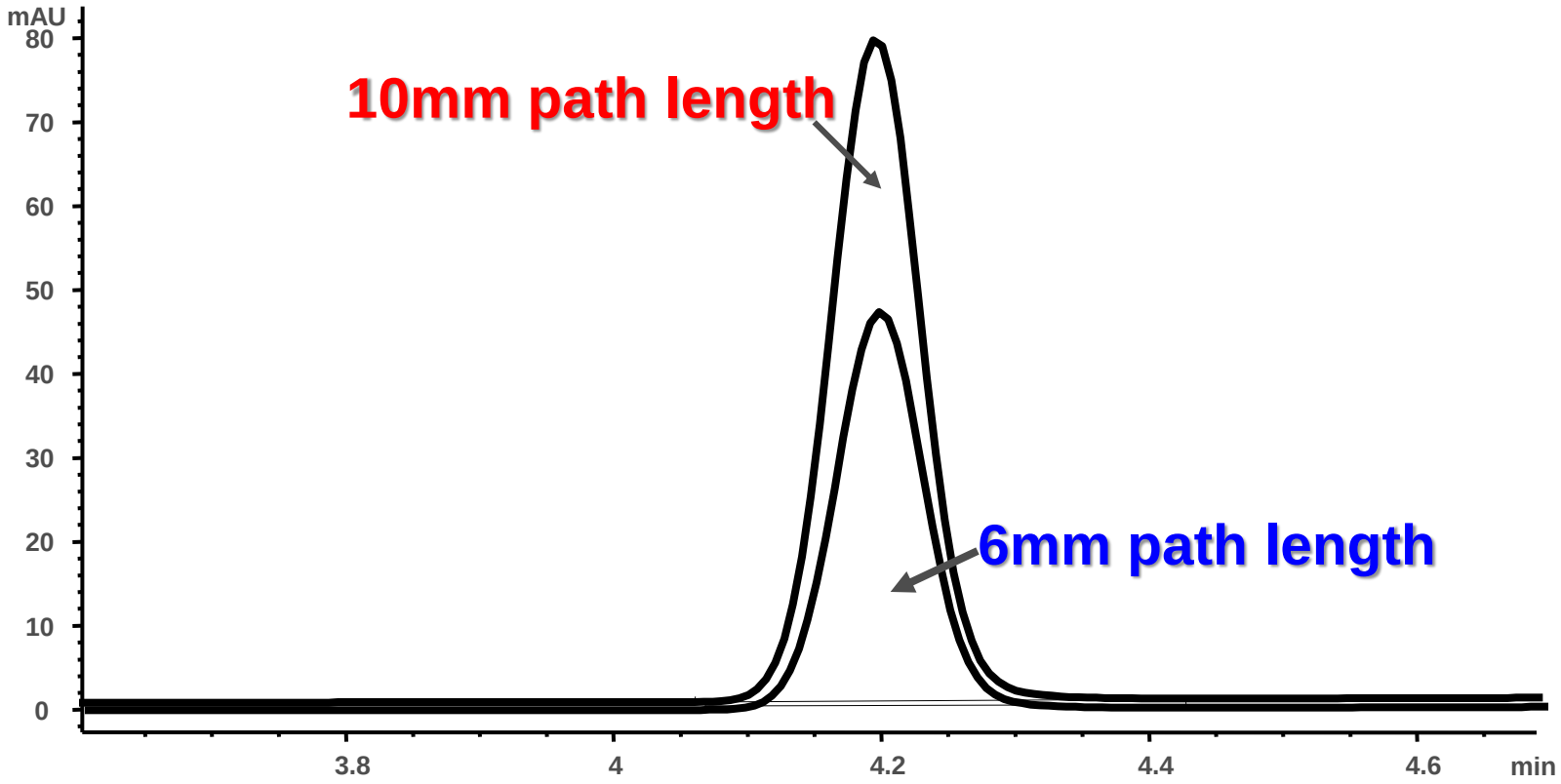


Constant temperature for solvent and column is required to perform reproducible results.

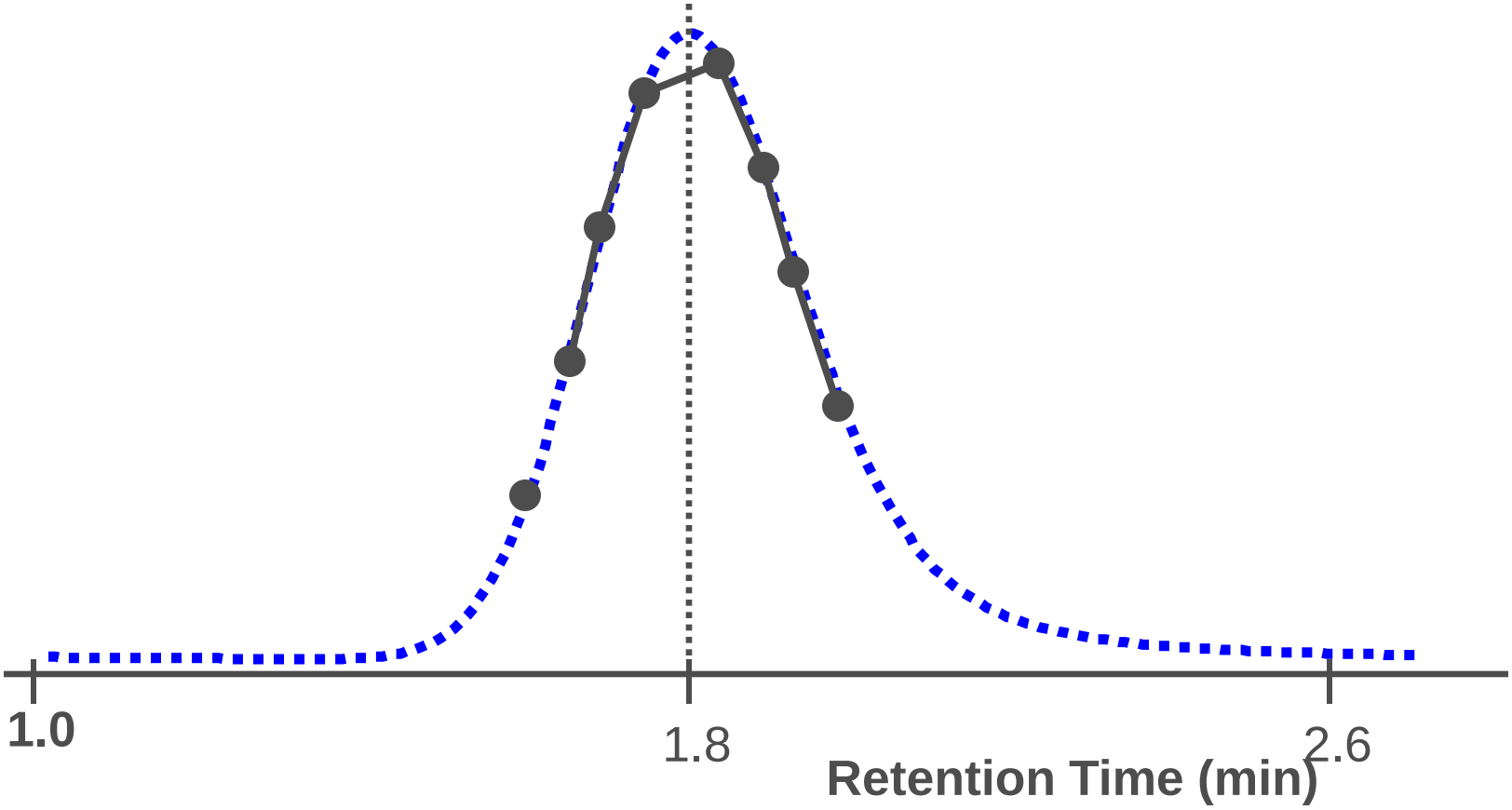
HPLC UV/Vis Detectors

Important performance characteristics	**Influence on...**
Variable Wavelength and Diode Array Detector	Variable Wavelength and Diode Array Detector
Low noise, wander and drift Wide linear range	Detection limit, quantitation limit Confidence in quantitation at high and low concentrations Accuracy of peak areas/heights Precisons of peak areas/heights
Very good wavel. accuracy Excellent wavel. precision	Diode Array Detector only
Diode Array Detector only	Accuracy of spectra, peak identification by spectra
High spectral resolution Excellent spectral sensitivity	Accuracy of spectra, peak identification by spectra at low concentrations

Influence of Pathlength on Signal Sensitivity

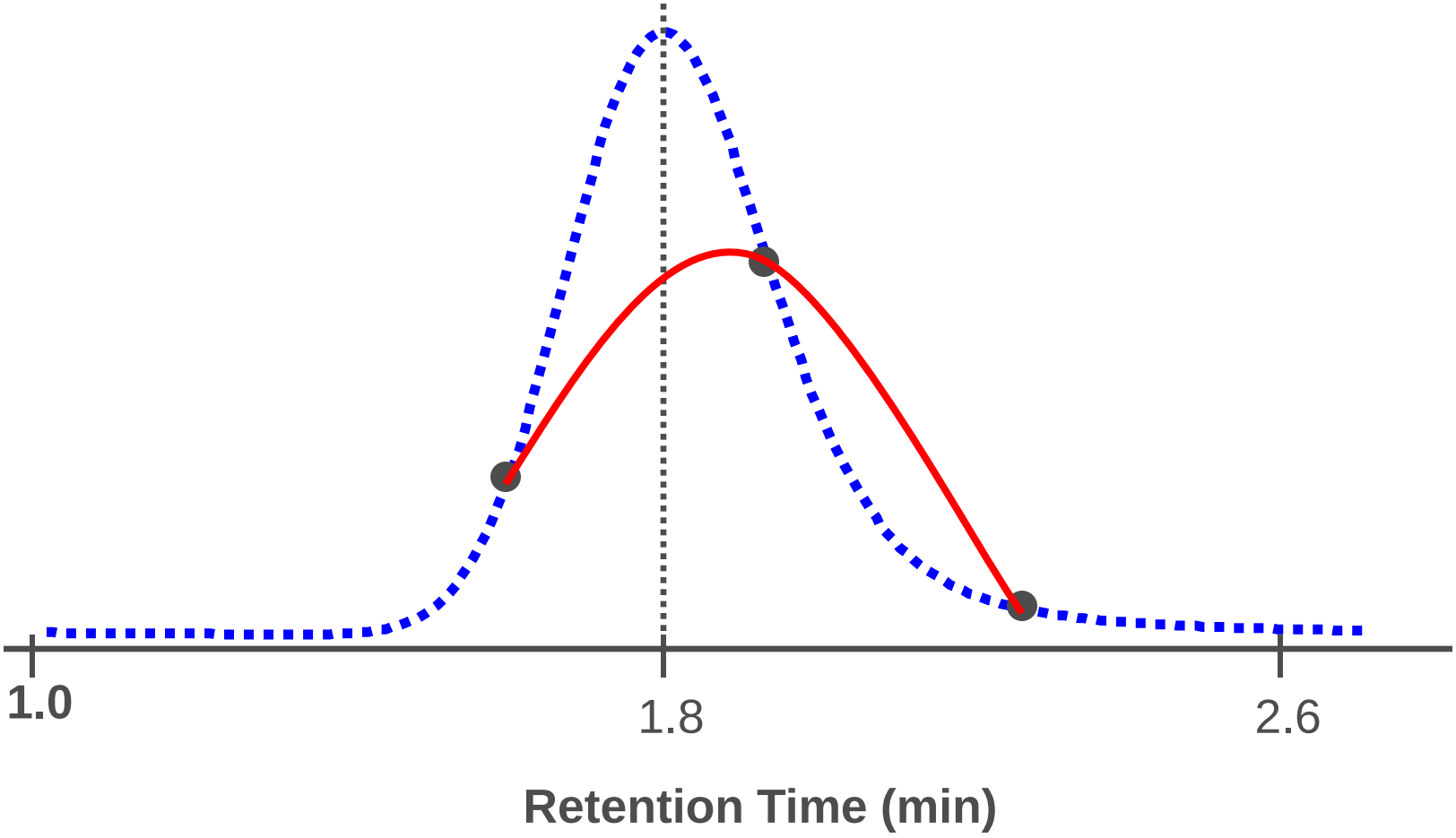


Determining Peak Apex



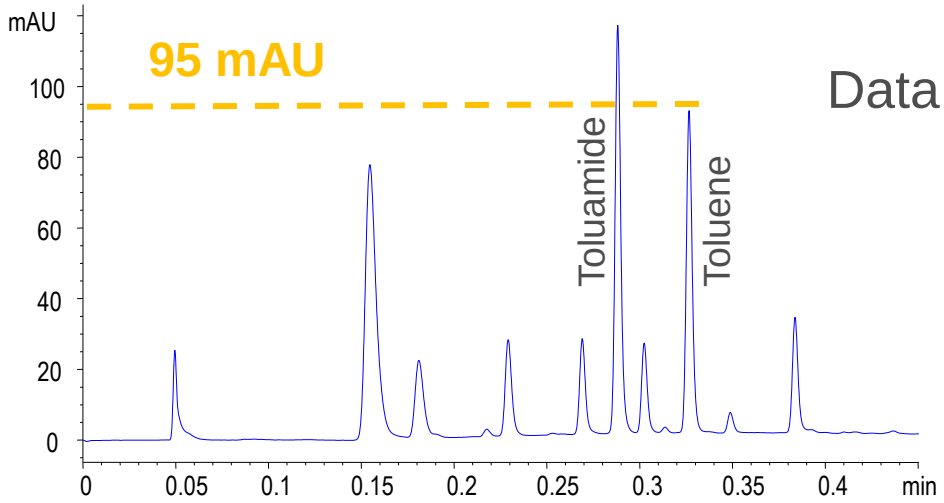
Fit highest data point and those on each side
to a quadratic equation, solve for highest point

Determining Peak Apex



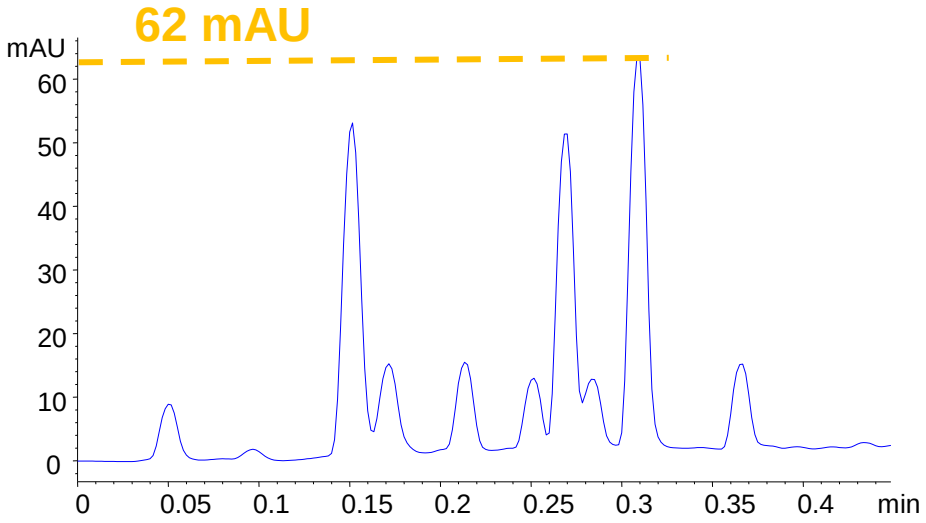
Sensitivity

Data rate – Peak height



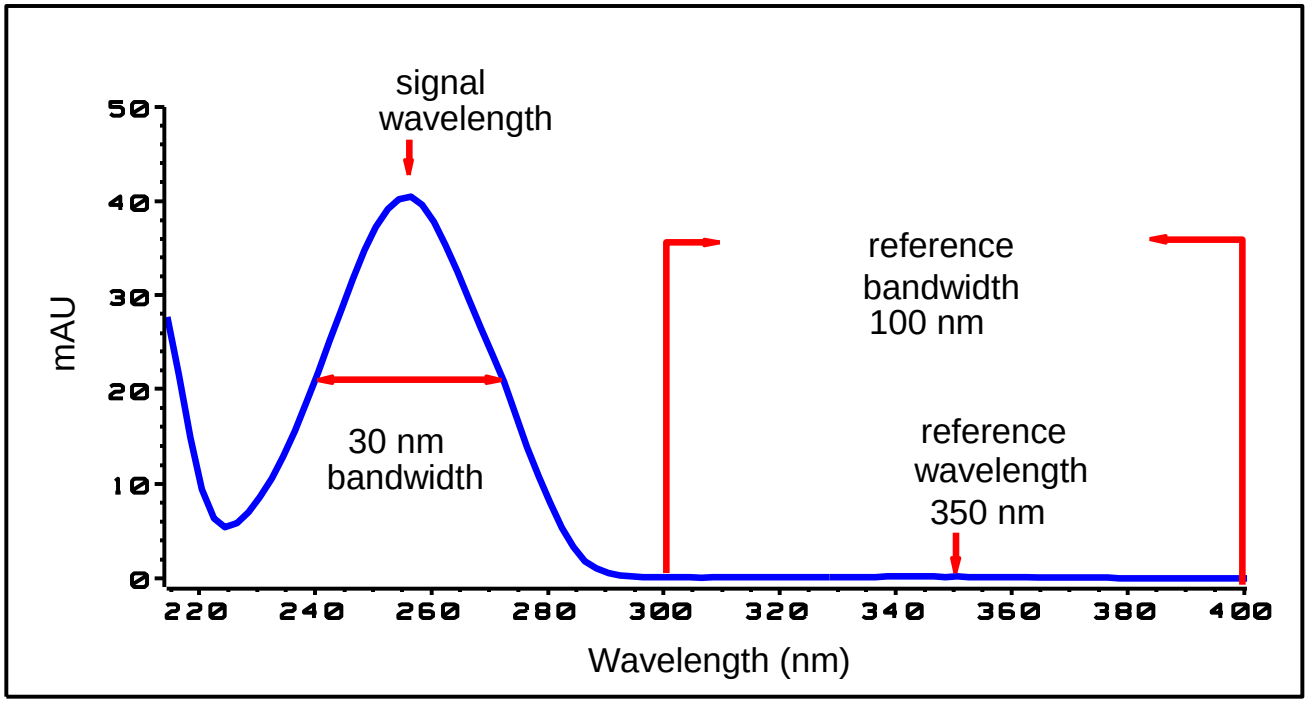
Data rate 80 Hz

Data rate 10 Hz

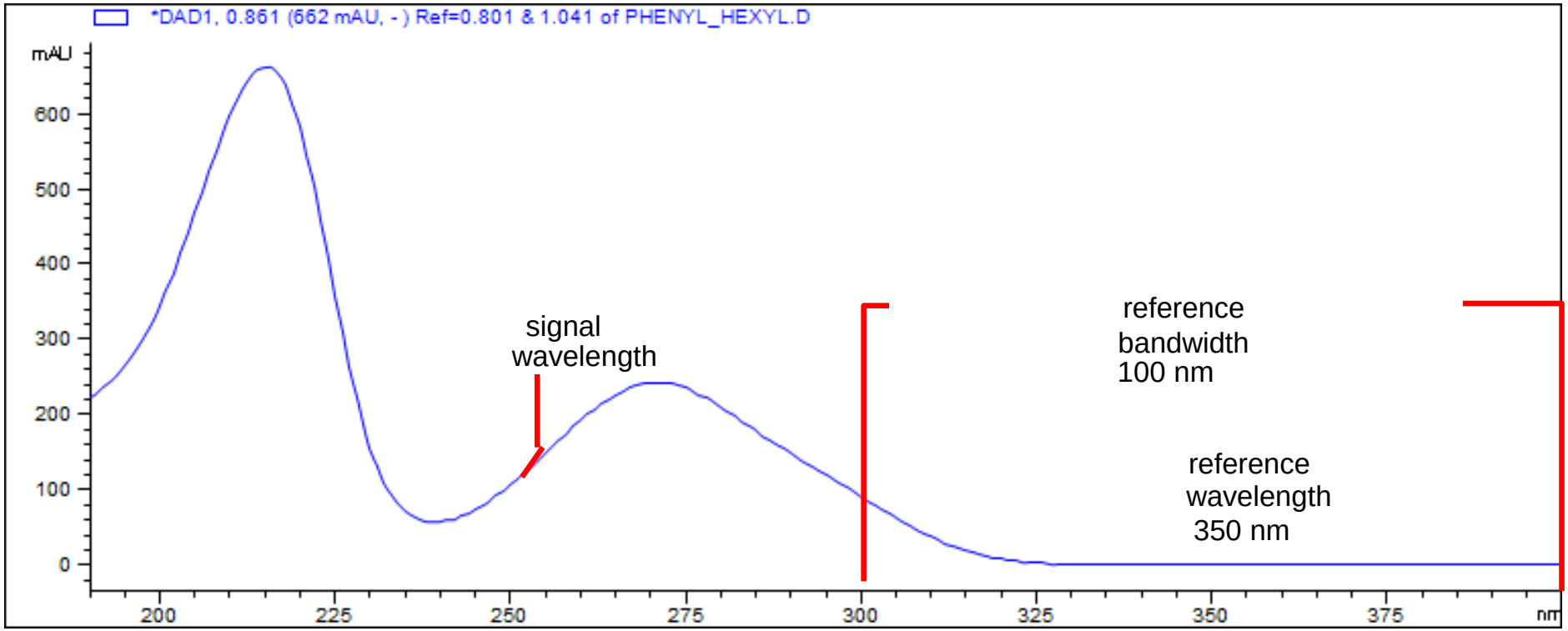


Peak height increases with increasing data rate!

Sample Signal and Reference Optimization

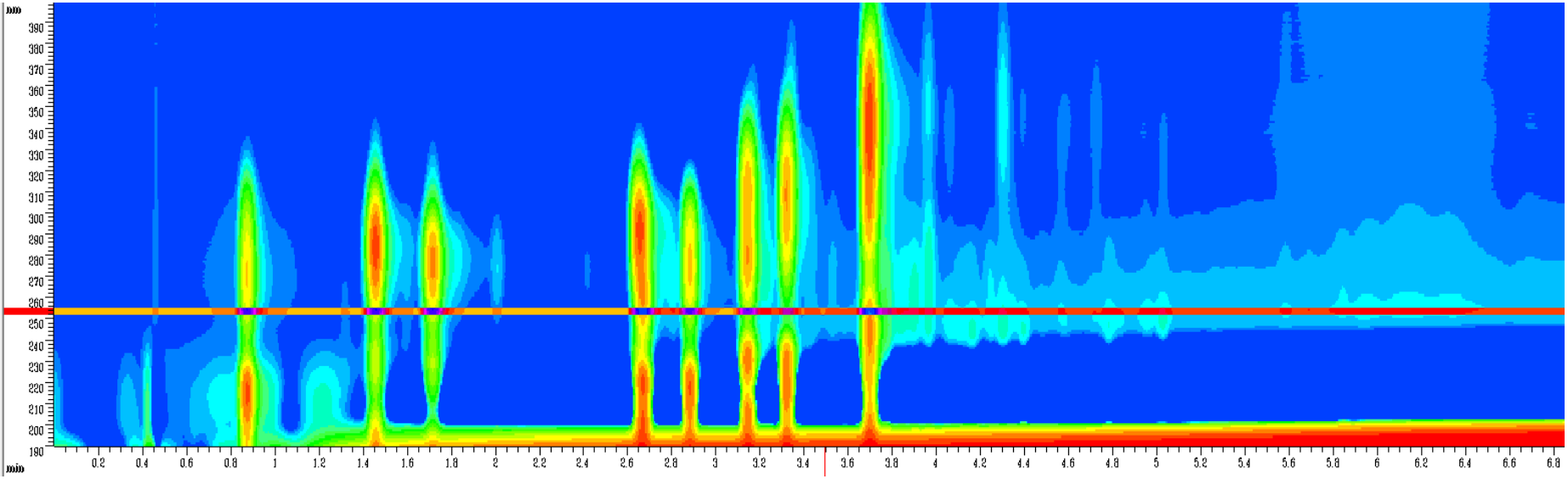


Total Signal with Diode Array Detection

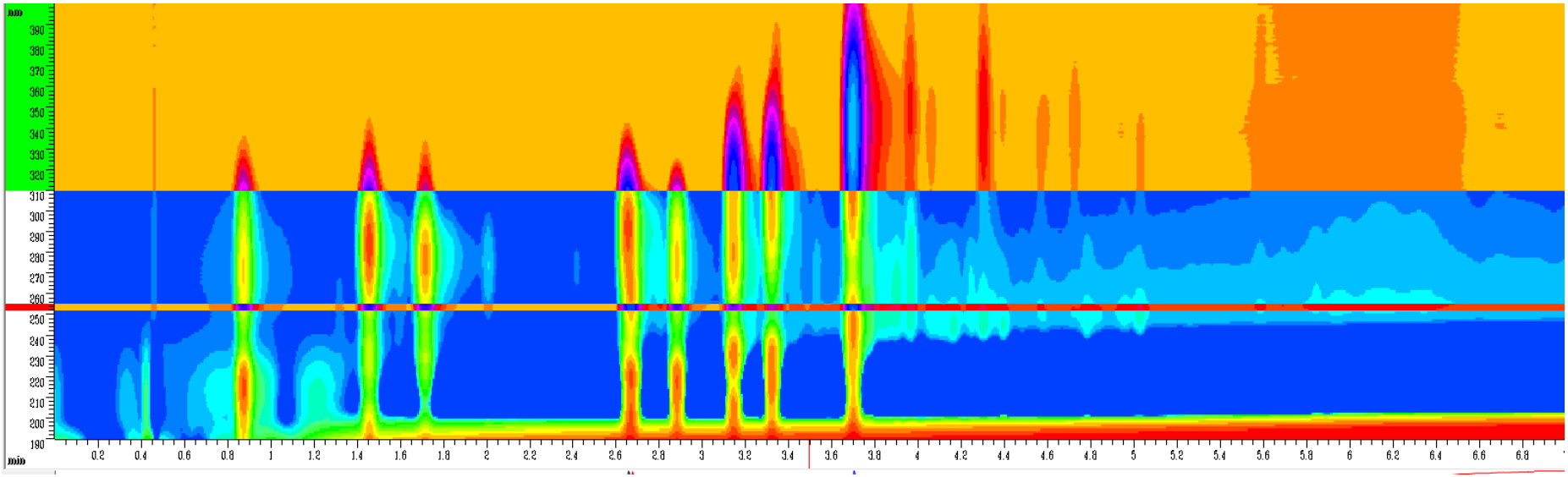


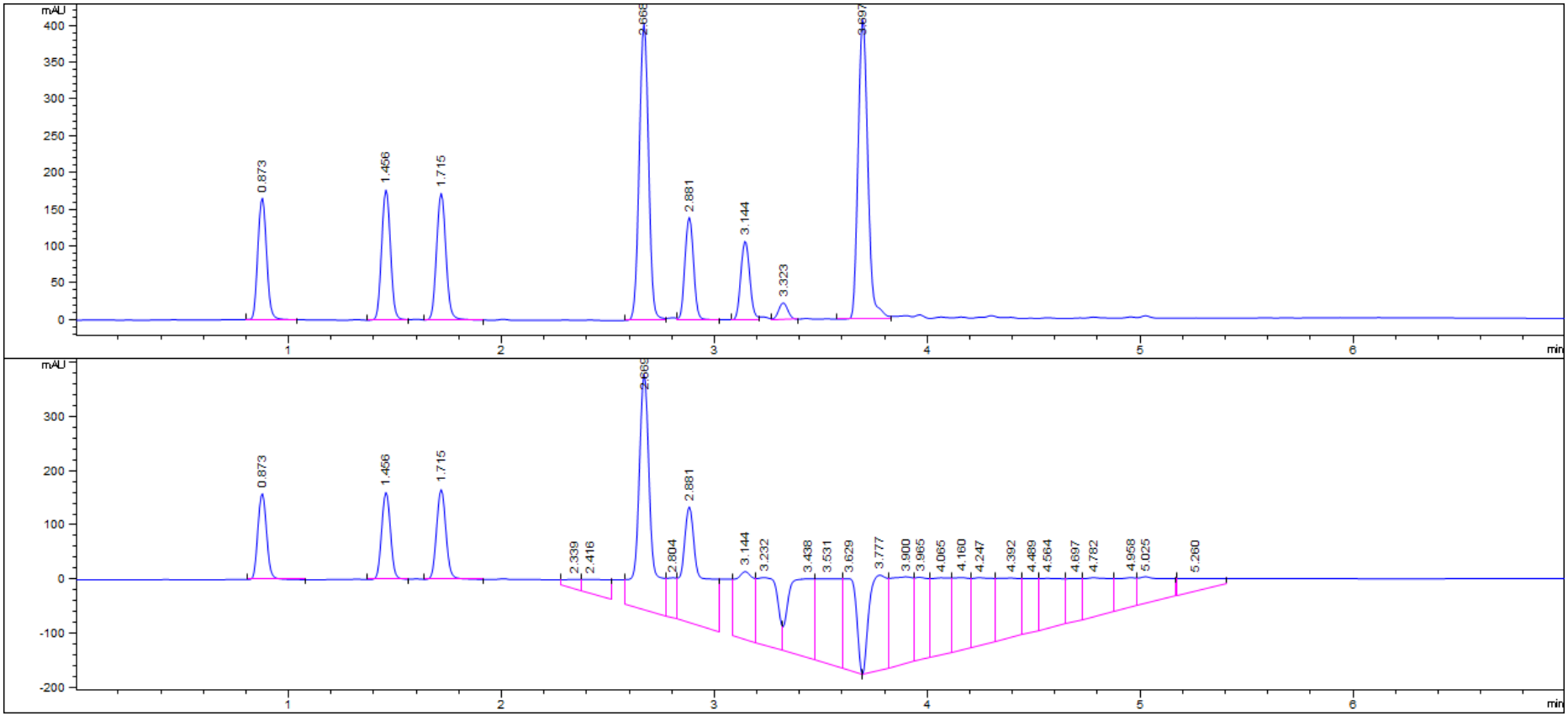
$$\frac{\text{Absorbance}_{\text{Sample wavelength}} + \text{Absorbance}_{\text{averaged over Bandwidth}}}{\text{\# of wavelengths used}} - \frac{\text{Absorbance ref}_{\text{wavelength}} + \text{Absorbance}_{\text{averaged over Bandwidth}}}{\text{\# of wavelengths used}} = \textbf{Total Absorbance}$$

Isoabsorbance plot without reference

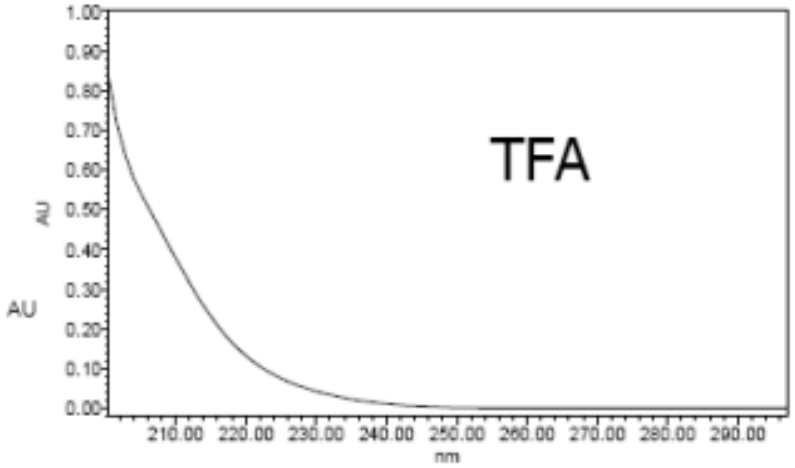


Isoabsorbance plot with reference

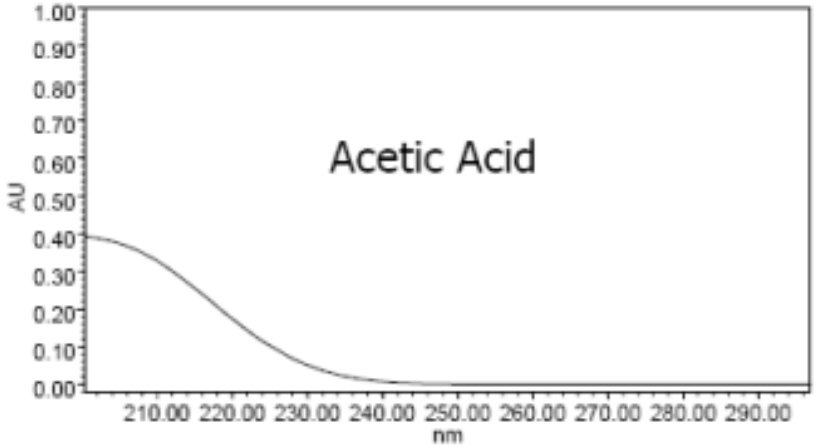



mAU
0.873
1.456
1.715
2.668
2.881
3.144
3.323
3.697
min
mAU
0.873
1.456
1.715
2.339
2.416
2.668
2.804
2.881
3.144
3.232
3.438
3.531
3.629
3.777
3.900
3.965
4.065
4.160
4.247
4.392
4.489
4.564
4.697
4.782
4.958
5.025
5.260
min

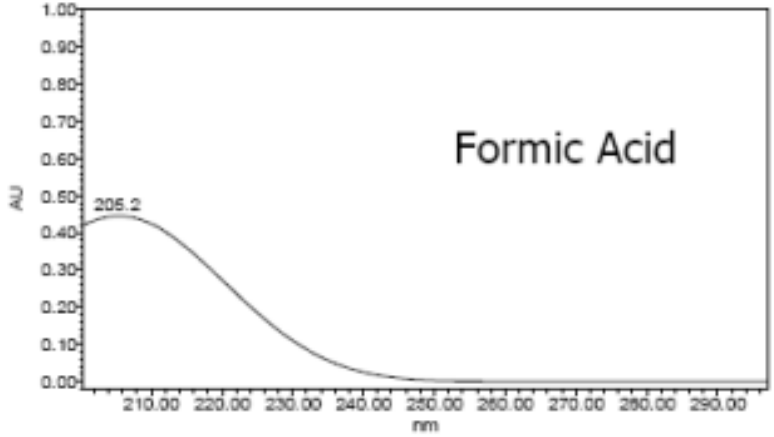
UV spectrum of 10 nM mobile phase



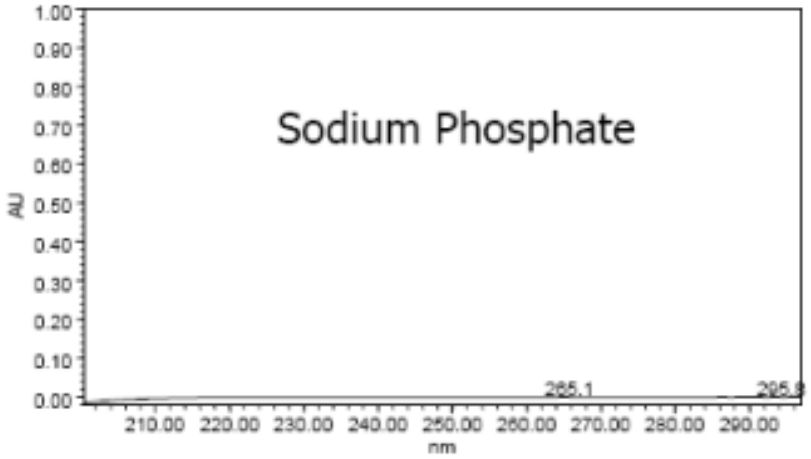
pH 0.94



pH 2.78



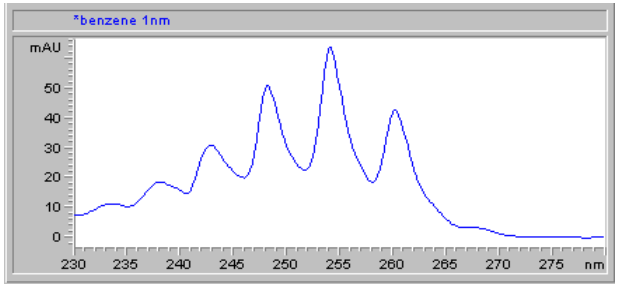
pH 2.26



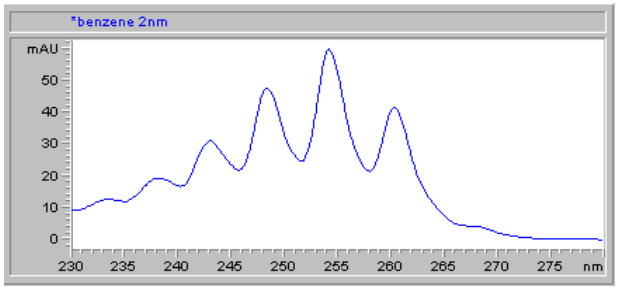
pH 2.0

Optimizing Slit

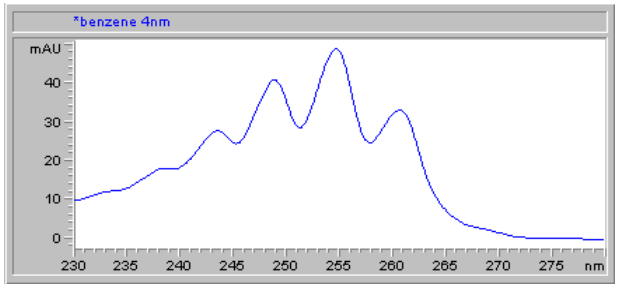
1 nm

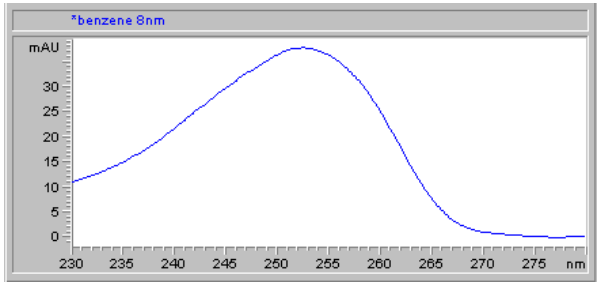


2 nm

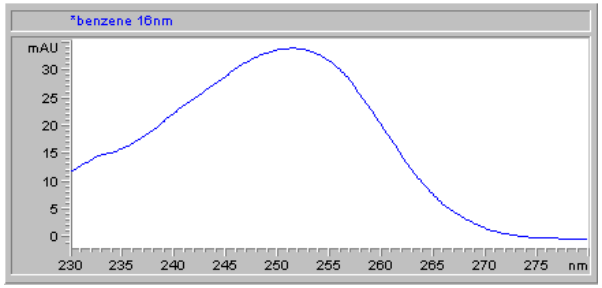


4 nm





8 nm



16 nm

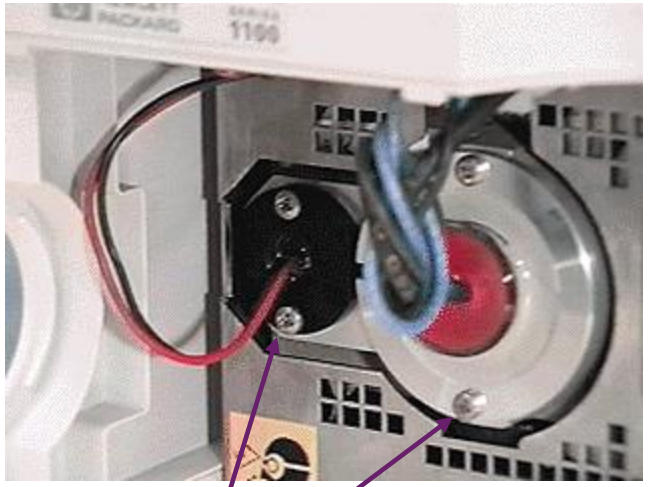
Tip #8 - Routine Maintenance Procedure – DAD/MWD Uv Detectors

- Clean the leak sensors
- Check the waste tube
- Exchange the lamp (if necessary)
- Clean the flow cell (if necessary)
- Wavelength calibration
- Holmium Oxide Test
- Intensity test
- Cell test
- Dark current test
- Filter test

Available Diagnostic Tests

- Covered by an annual Agilent LC PM contract -

Replacing the DAD/MWD Lamps



Step 1: Remove the lamp by unscrewing both screws and unplugging it.

Step 2: Install the lamp into the housing (auto-aligning) and tighten screws.

Step 3: Check wavelength calibration

Step 4: Check lamp intensity (for future reference)

Deuterium Lamp (1000 hours): 2140-0590

Deuterium Lamp (2000 hours): 2140-0820*

***includes RFID tag read by SL modules**

Tungsten lamp G1103-60001

Tip # 9: Care of Detector Flow Cells

✓Avoid the use of alkaline solutions with pH > 9.5 which can attack quartz and impair optical performance.

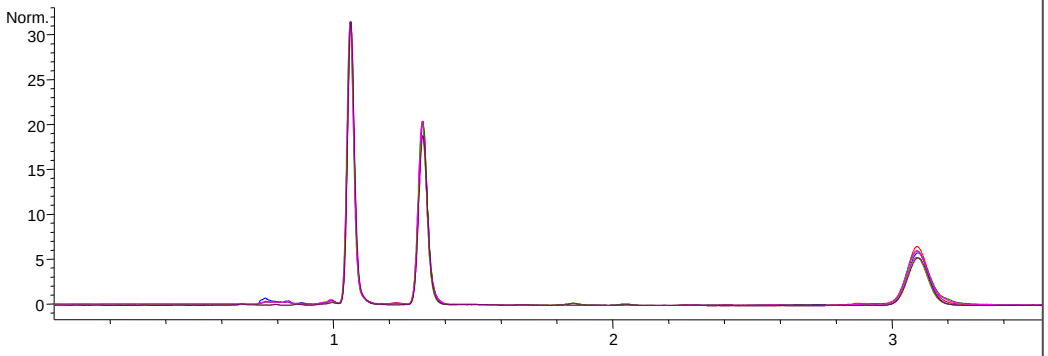
✓Prevent crystallization of buffers or salts which will lead to blockage and damage.

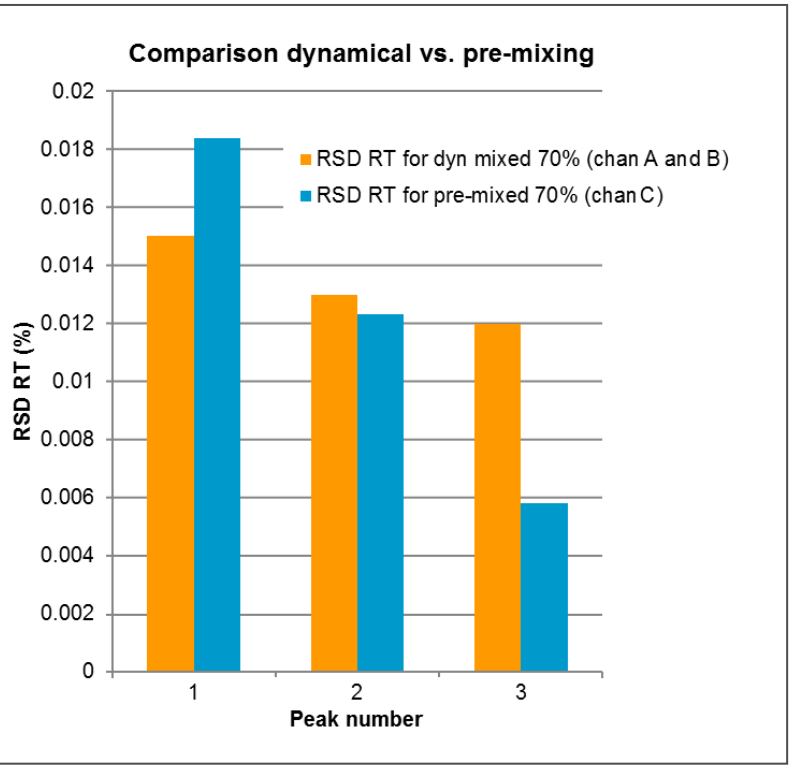
✓Aqueous solvents can allow algae growth. Don't leave 100% water standing in the flow cell. When leaving LC idle, pump a mobile phase with at least 5-10% of organic solvent.

✓Observe the pressure limits of flow cells. Be careful when using detectors in series or fraction collectors.

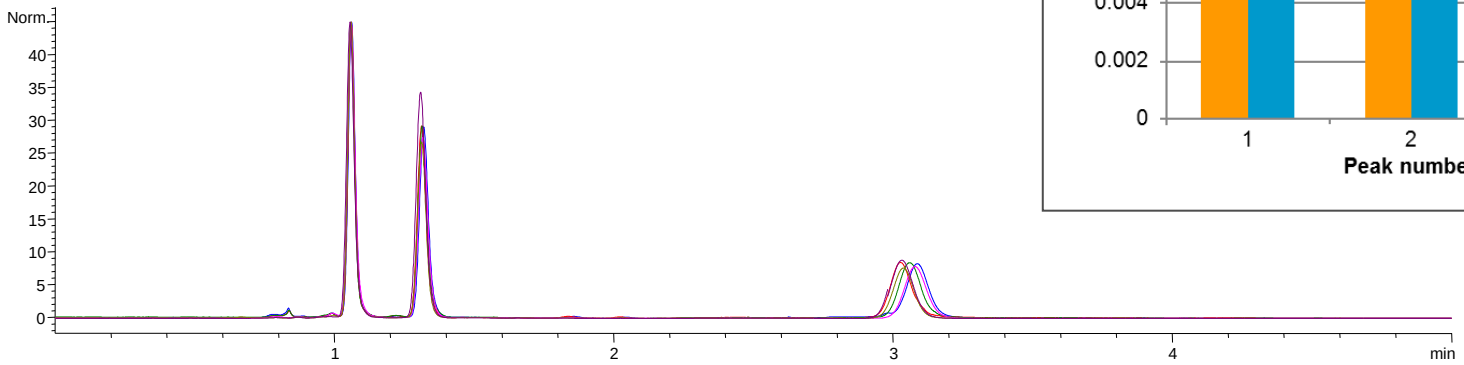
Dynamically Mixed vs. Premixed Mobile Phases (prepared by one user)

6 dynamically mixed mobile phases

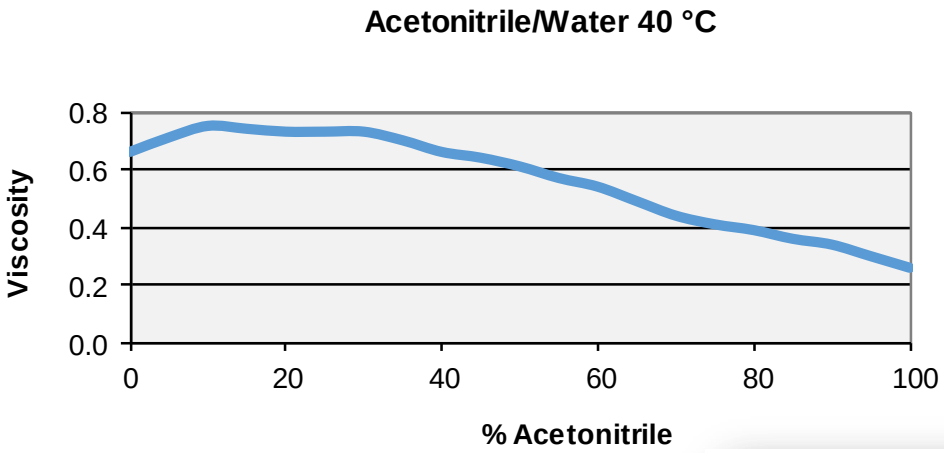




6 premixed mobile phases



Non-ideal mixtures



Maximum viscosity of acetonitrile/water mixtures at approx. 10 % acetonitrile.

Maximum viscosity of methanol/water mixtures at approx. 50 % methanol.