

# HPLC User Maintenance & Troubleshooting



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- Delay volume
- Fittings
- Column volume

## Hardware

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

## Physics

# Chromatography



## Chemistry

- Data Sampling
- Type of Mixing
- Column Pressure

## Application

- Sample Prep
- Mobile phase UV cutoff
- Sample UV Maximum

# 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

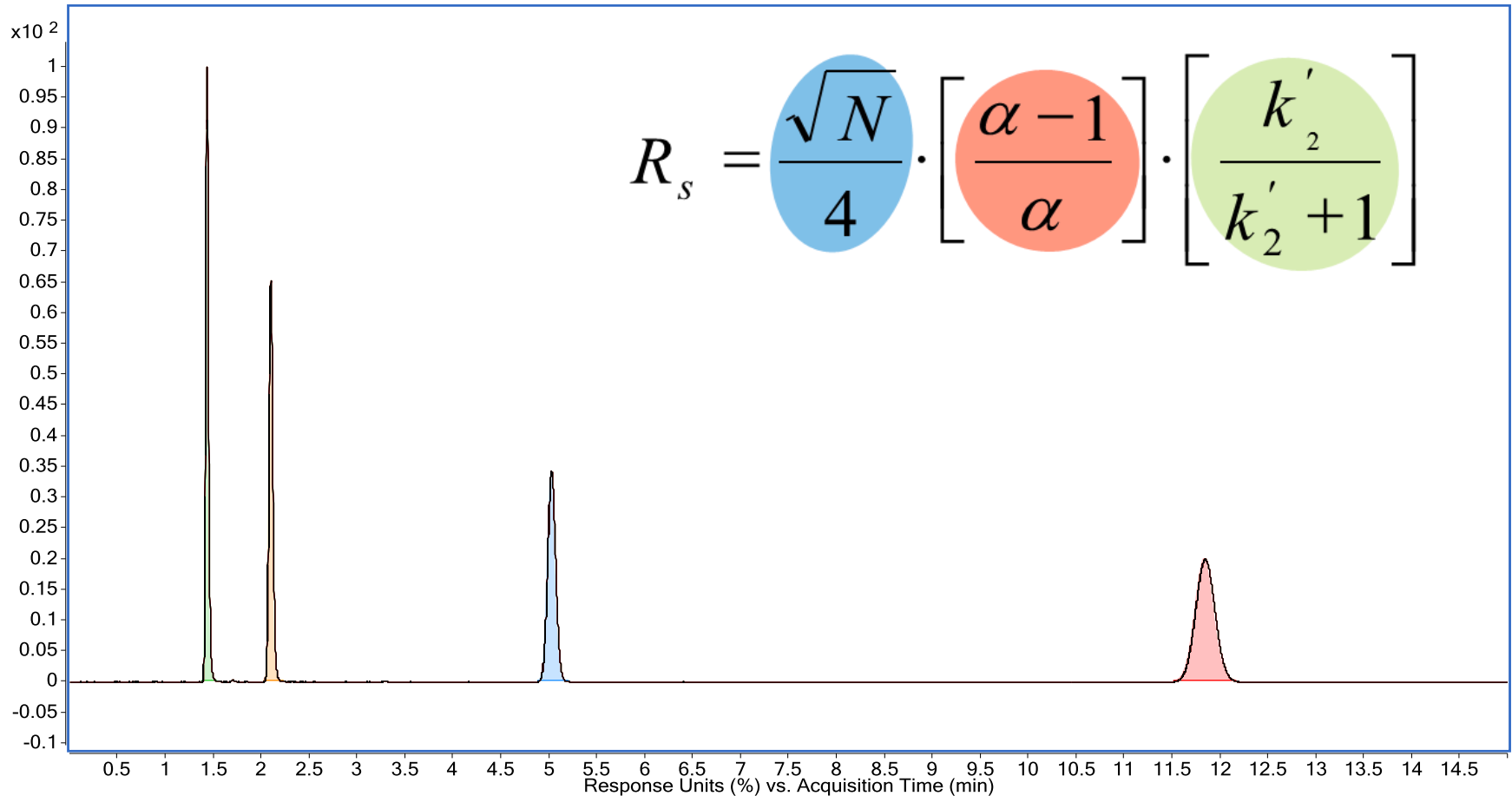
$\alpha$  = Selectivity

$k$  = Retention

Column length, particle size

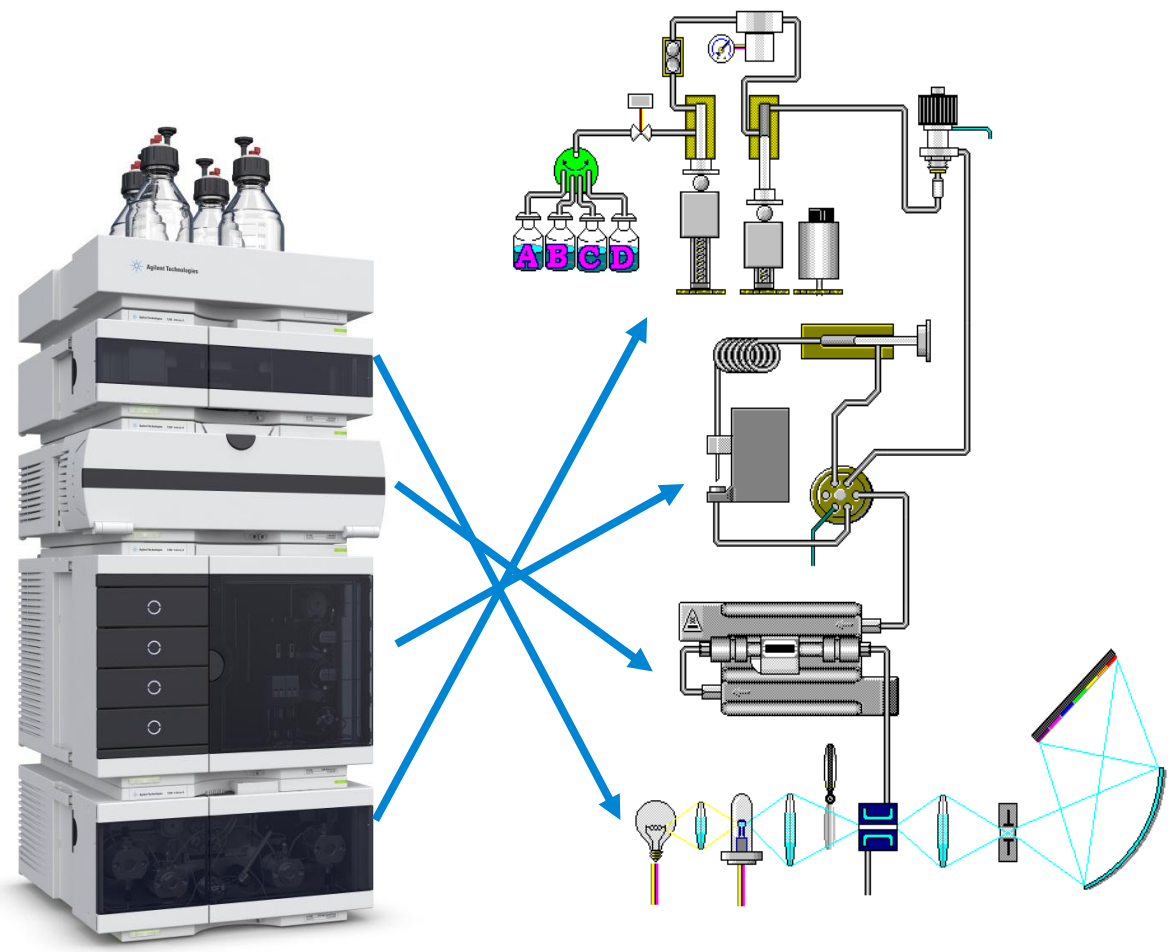
Stationary and mobile phase, temperature

Stationary and mobile phase



# Know your HPLC System:

Detector  
Column Comp.  
Autosampler  
Pump



Vacuum Degasser (integrated in Infinity II and Quat systems)

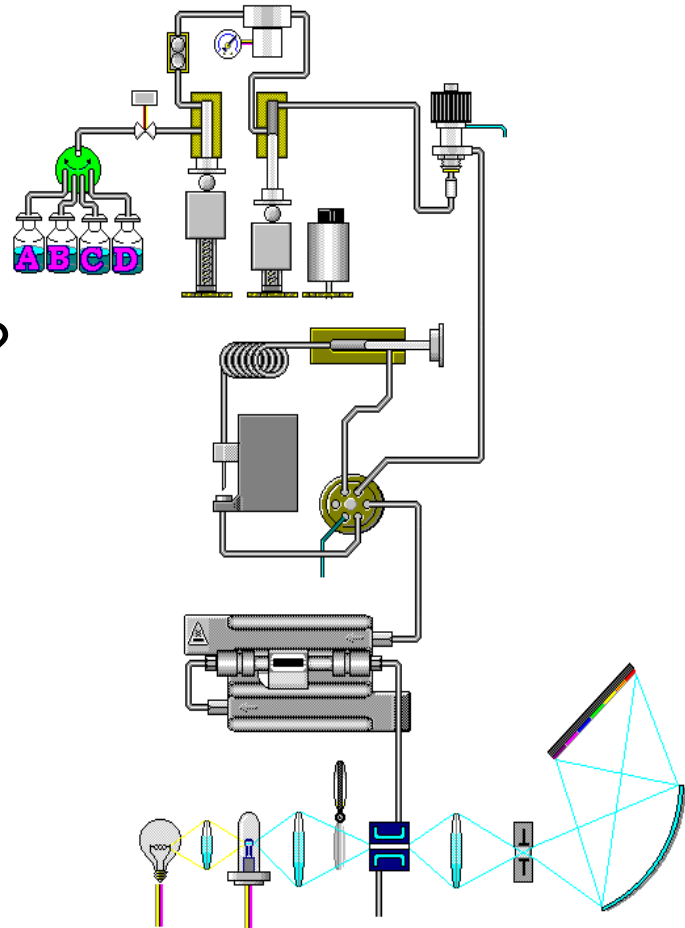
# Know Your HPLC Flow Path:

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)?

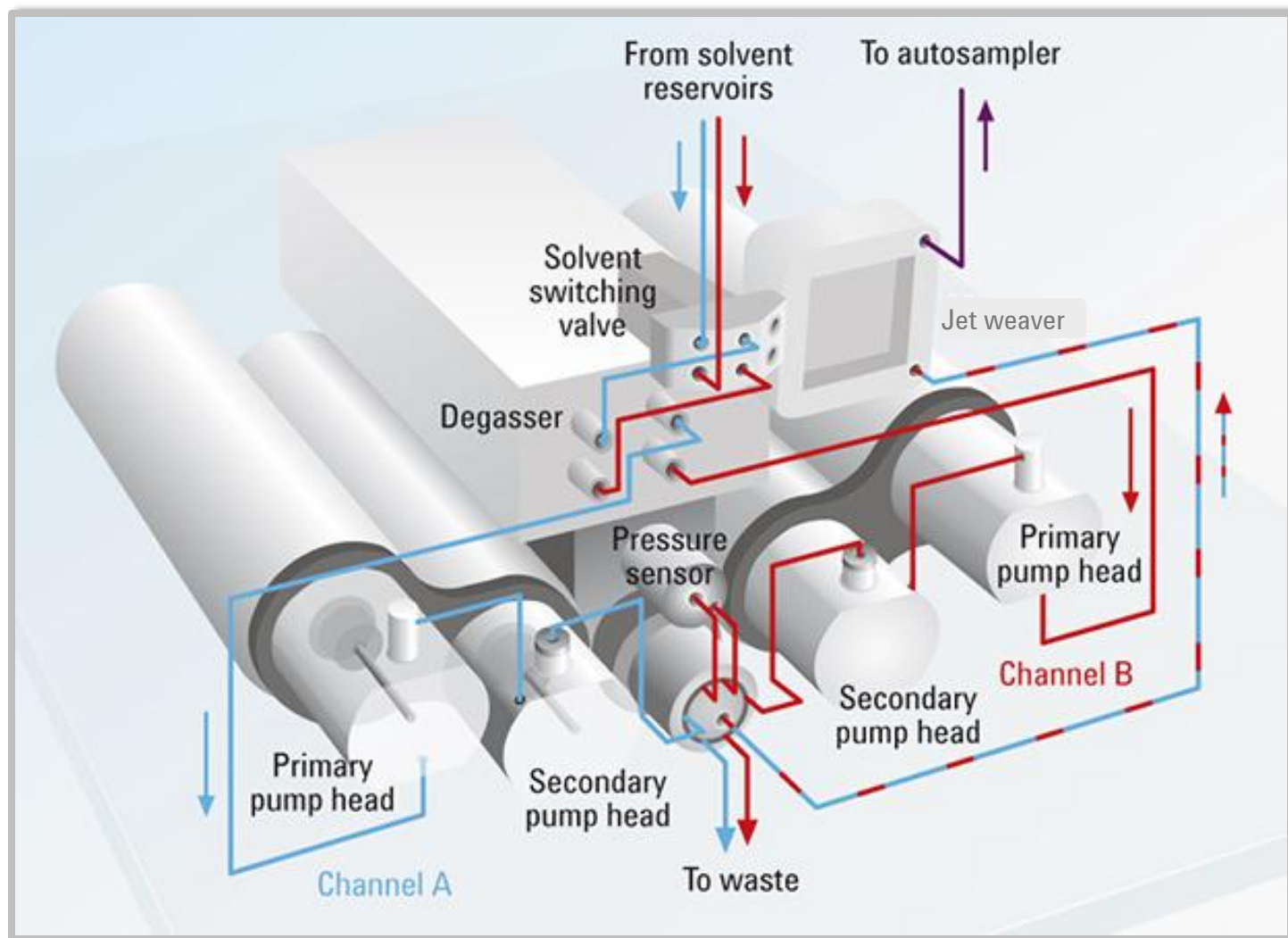
Where can leaks occur ?



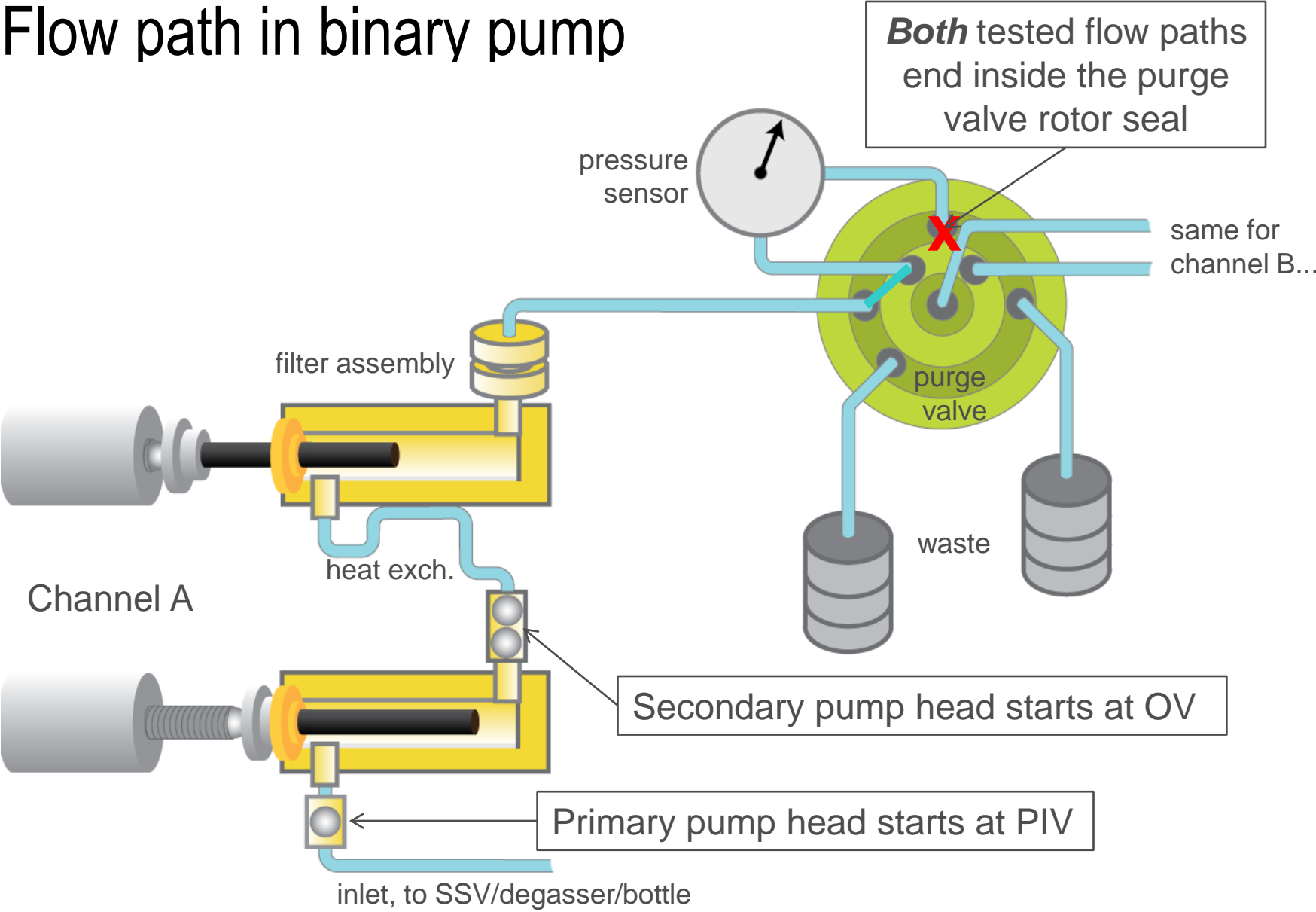
***What can I do to eliminate, reduce or anticipate potential problems with the LC ?***

# 1290 Infinity Binary Pump

– *the flow path*

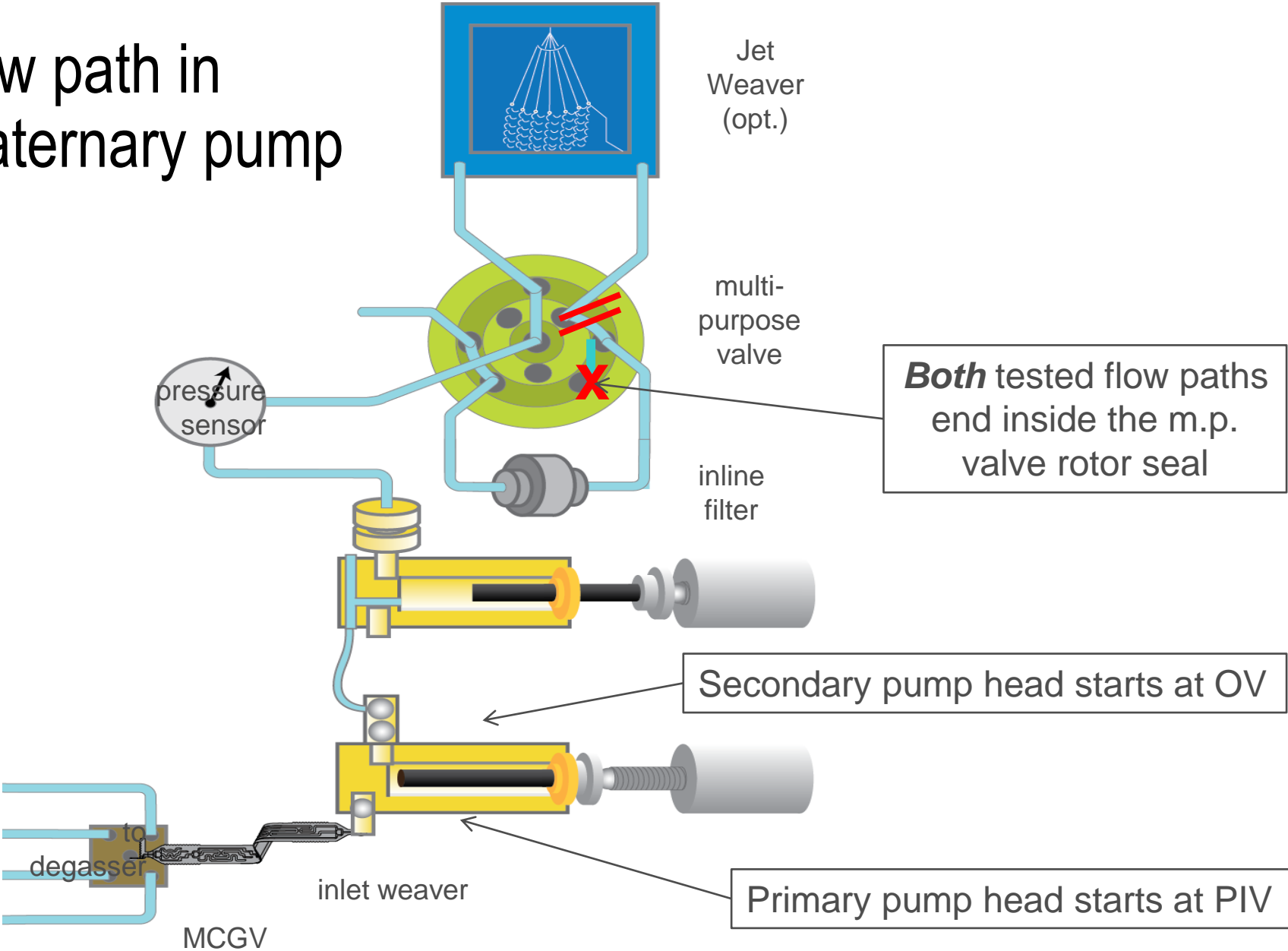


# Flow path in binary pump

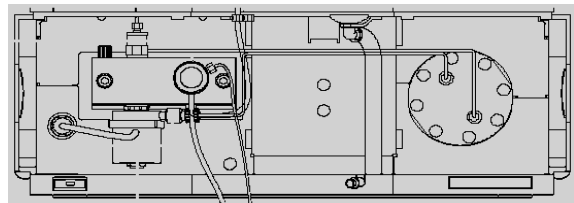




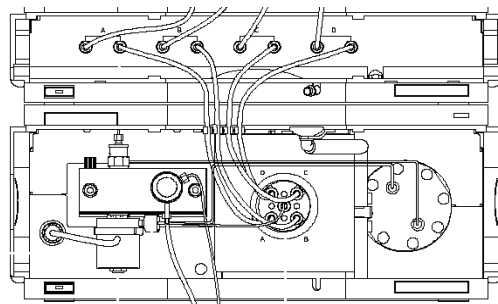
# Flow path in quaternary pump



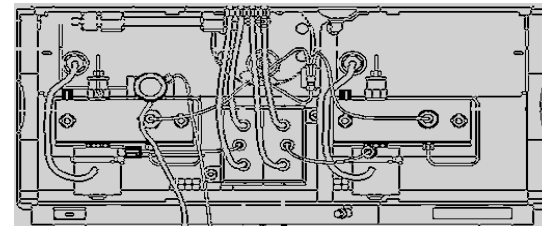
# Agilent 1200 Series Pump Models - Analytical



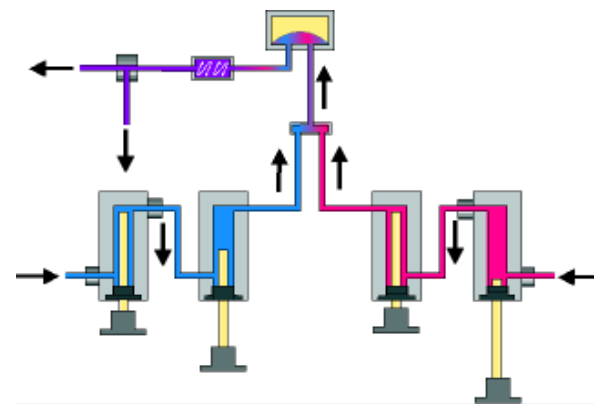
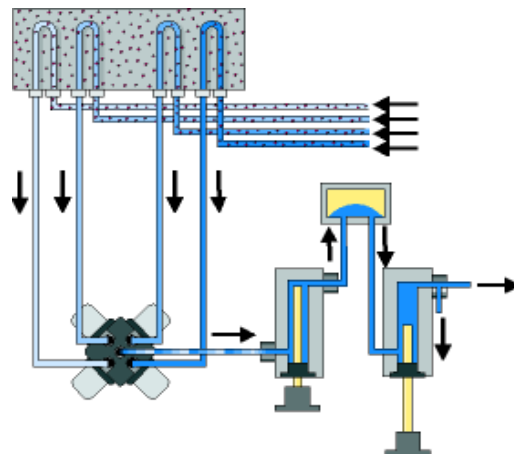
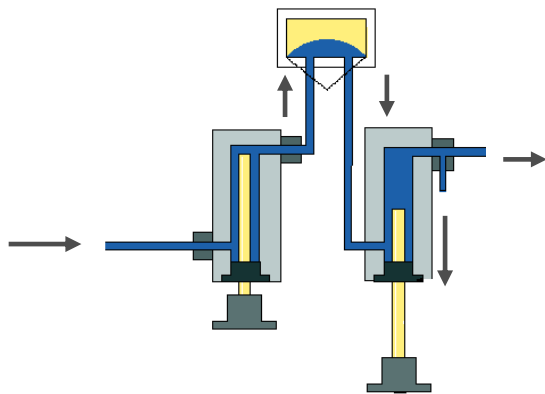
Isocratic Pump



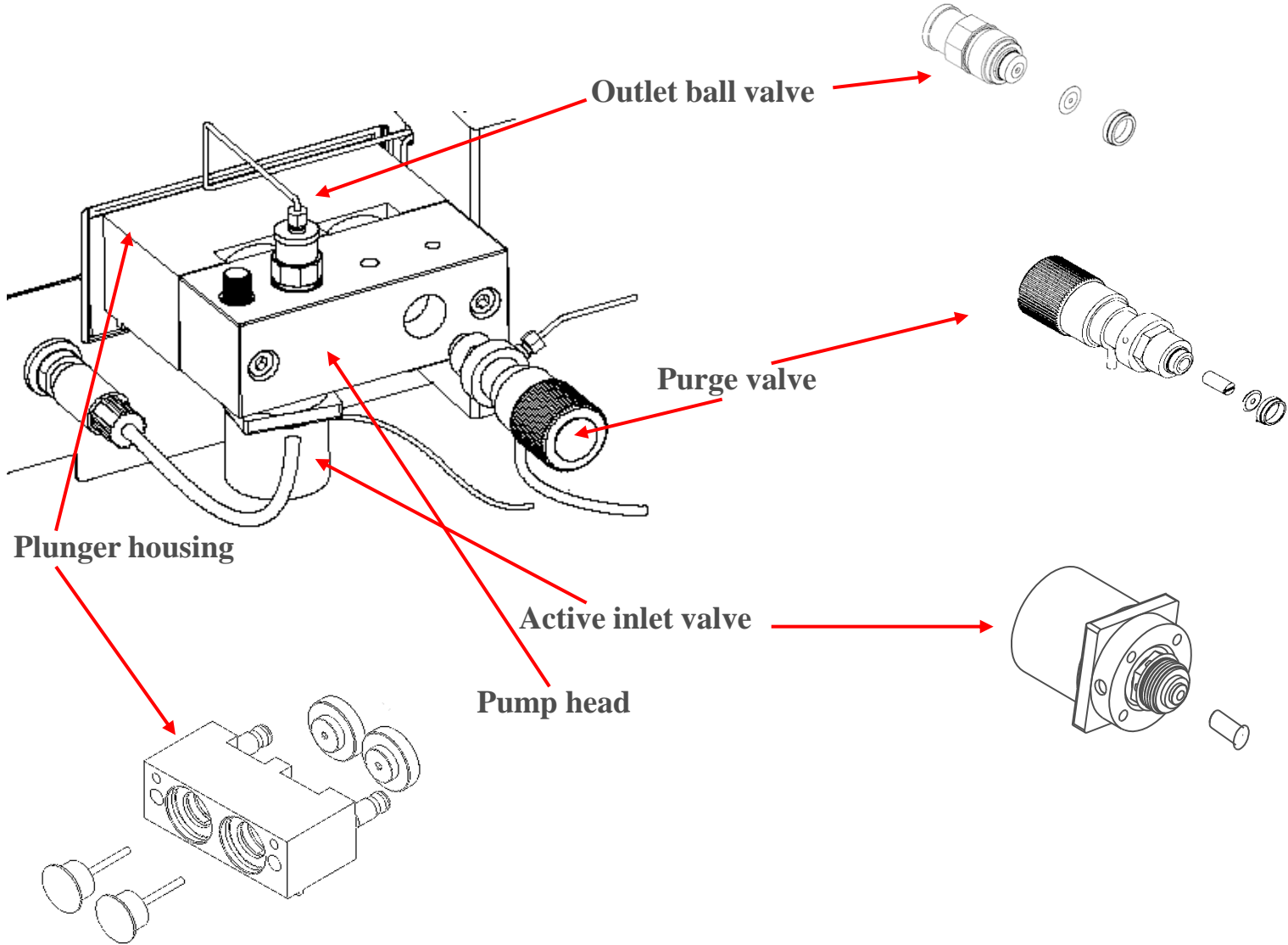
Quaternary Pump



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)

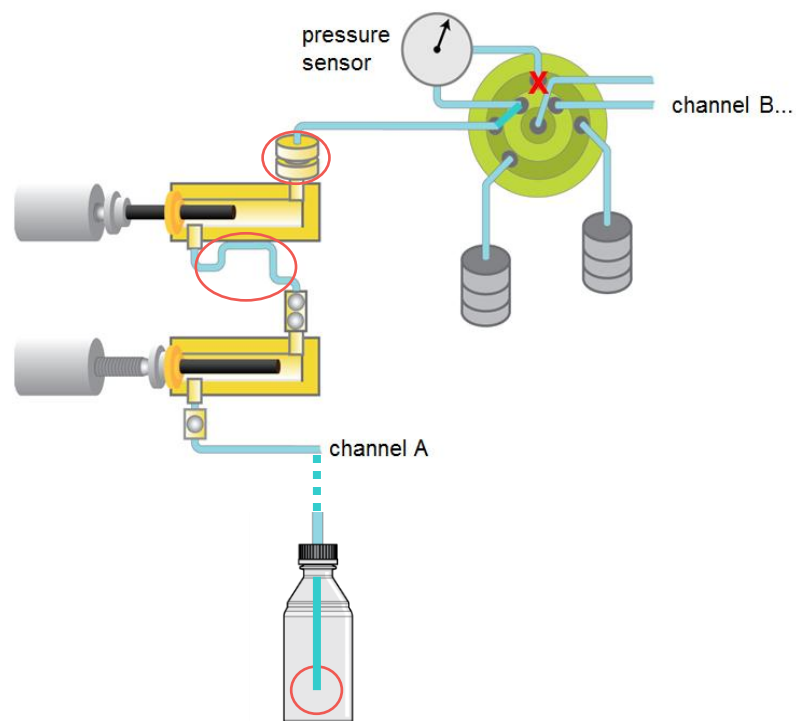
best once a day to condition for smooth operation

# 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<sup>+</sup> ions)





# Example: Jet Weaver & Inlet Weaver

- **Multi-layer technology**

Diffusion bonded stainless steel, etched structures

- **Two mixers in one cartridge**

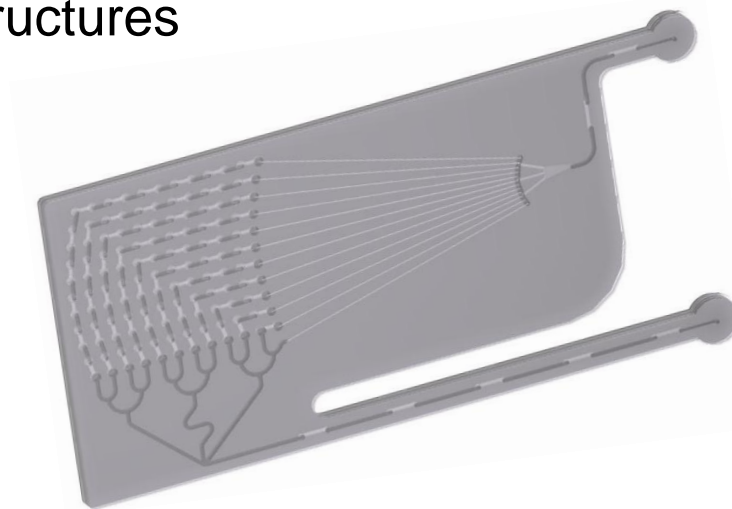
Standard mixer with 35  $\mu$ l volume & 100ul adds delay volume of 45ul and 75ul

- **Optional mixer in quat pump with V380**

which adds 150ul delay volume

- **Mixer for TFA applications with**

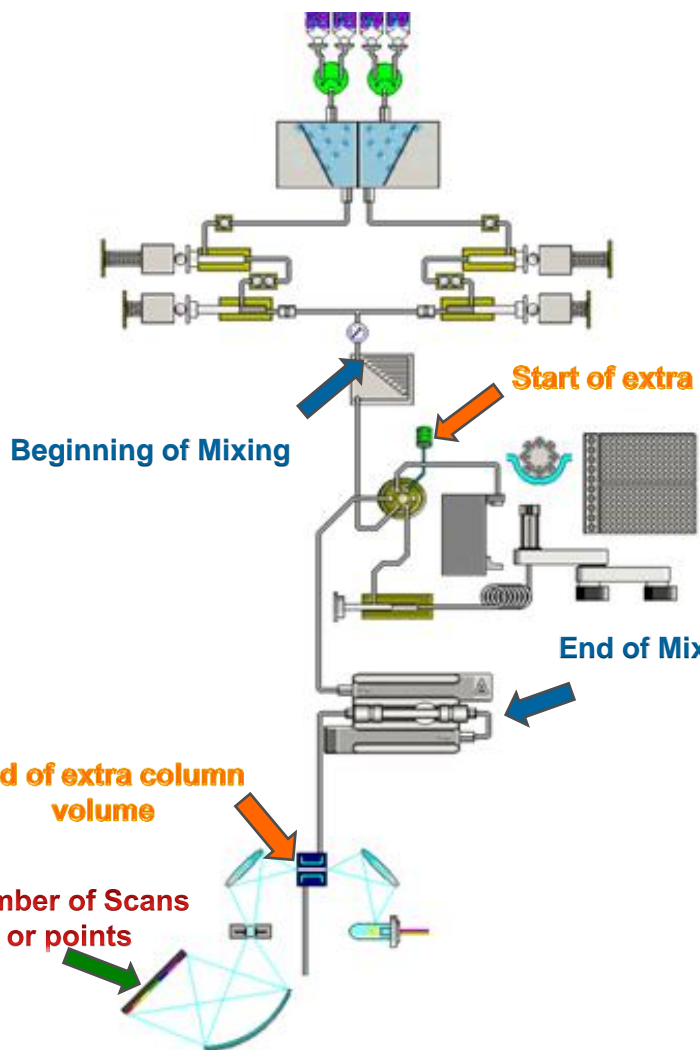
adds 380  $\mu$ l volume delay volume



# 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.



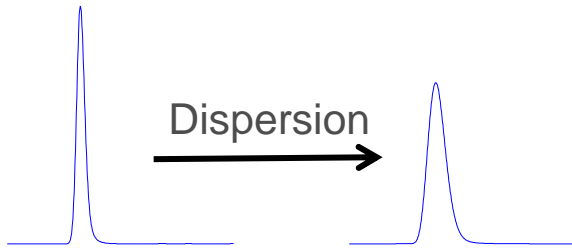
## Extracolumn Volume

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

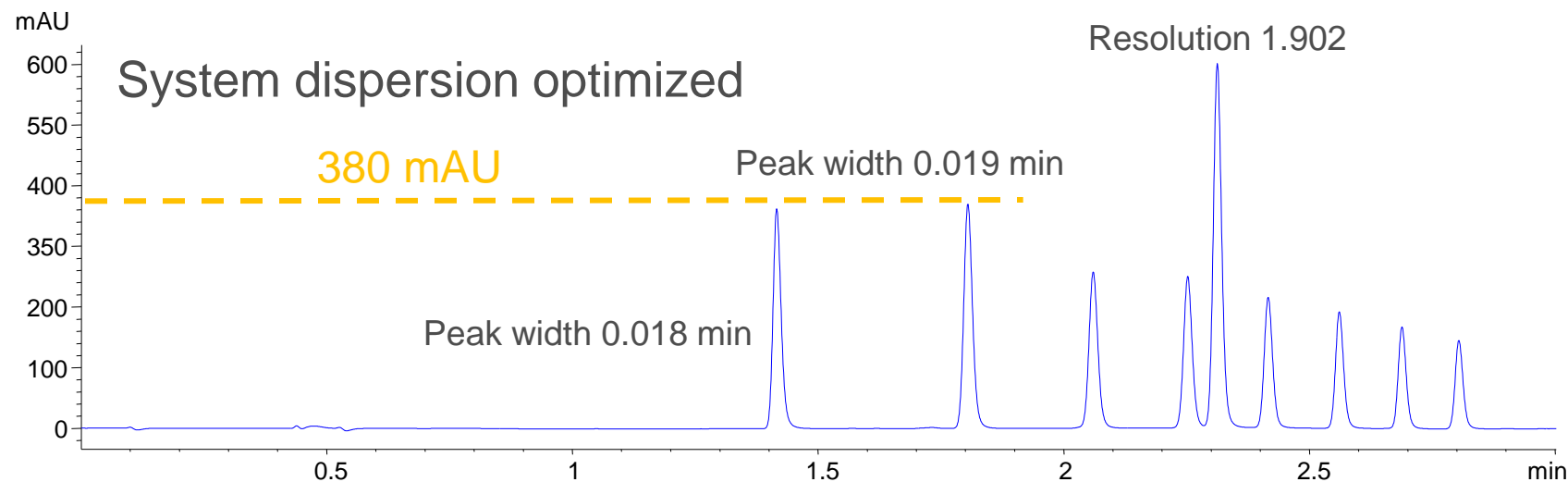
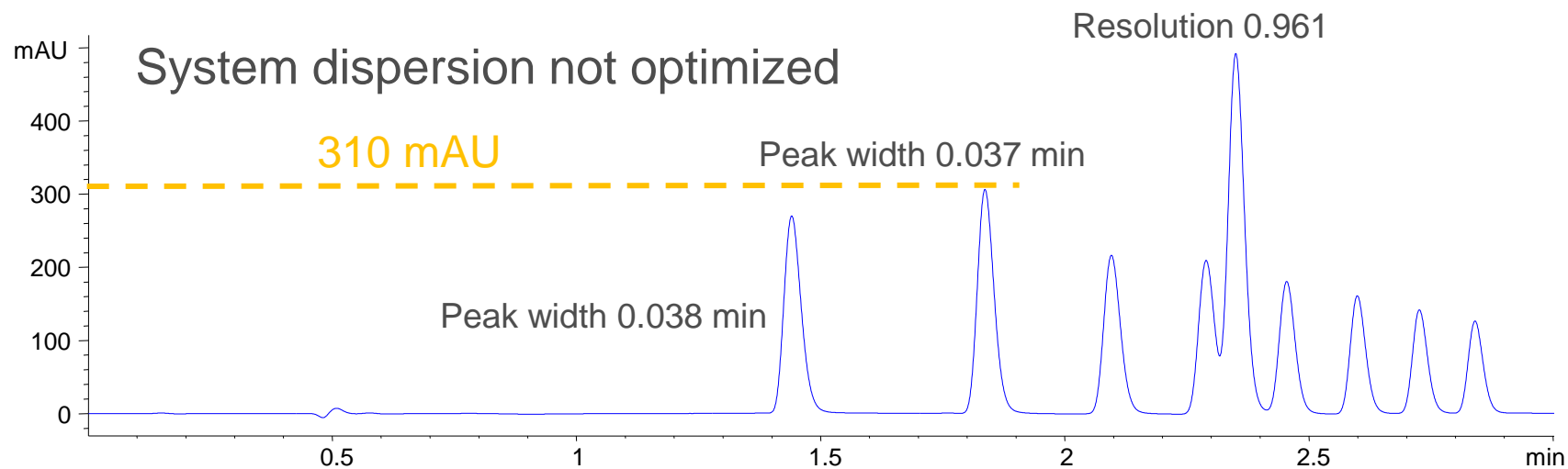
Peak width: Loss of resolution

- Capillaries (Inner diameter, length)

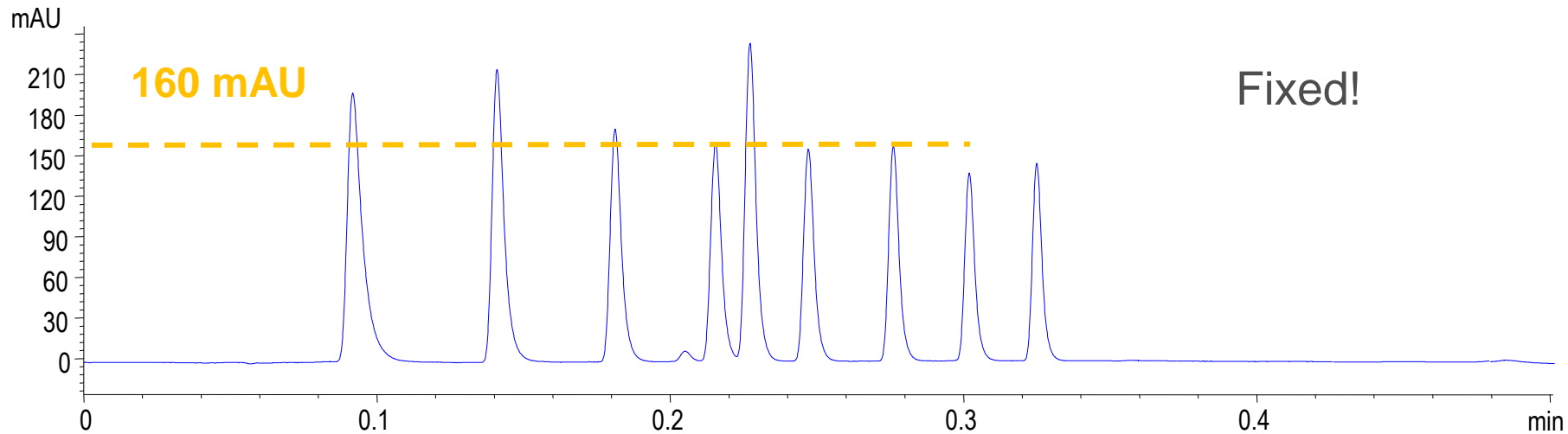
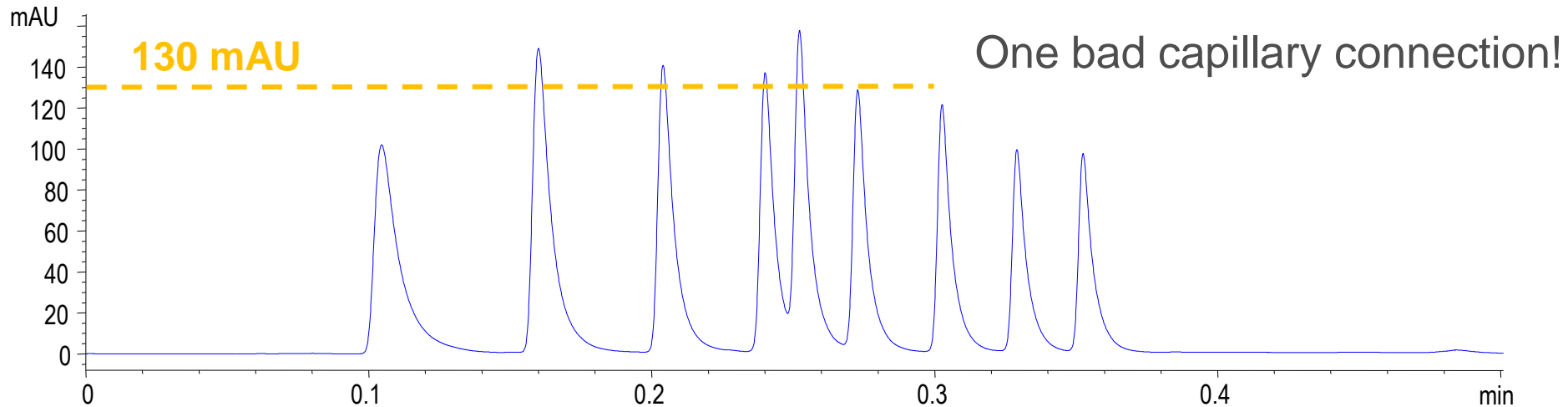
$$\sigma^2 = \frac{\pi \cdot r^4 \cdot F \cdot L}{24 \cdot D_m}$$

*Aris-Taylor Equation*

# System – Signal Height

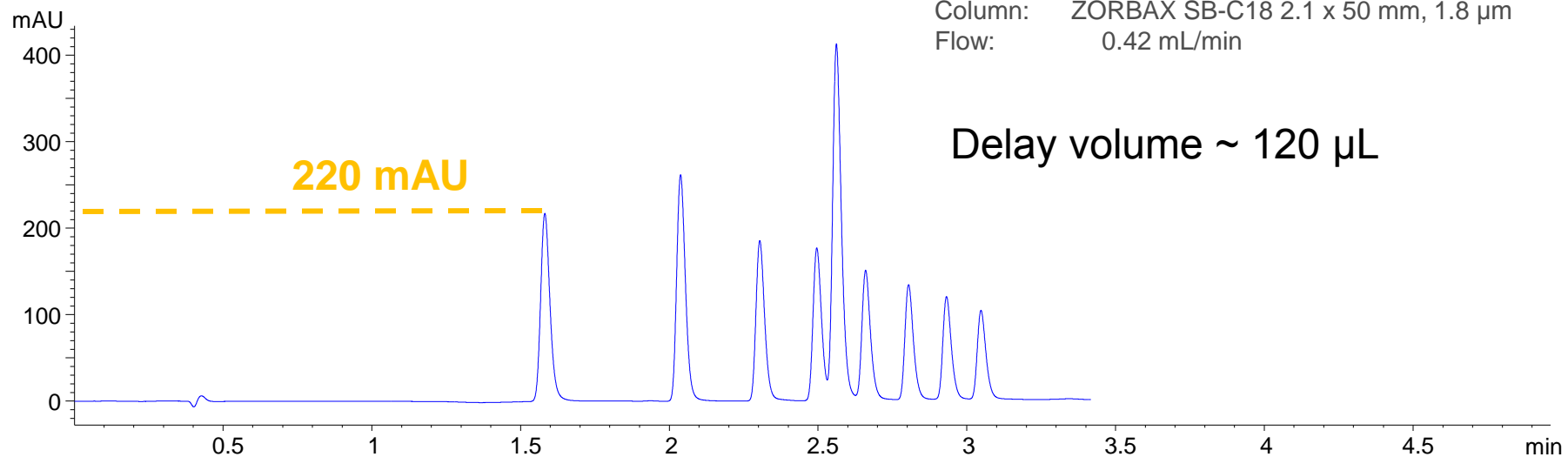
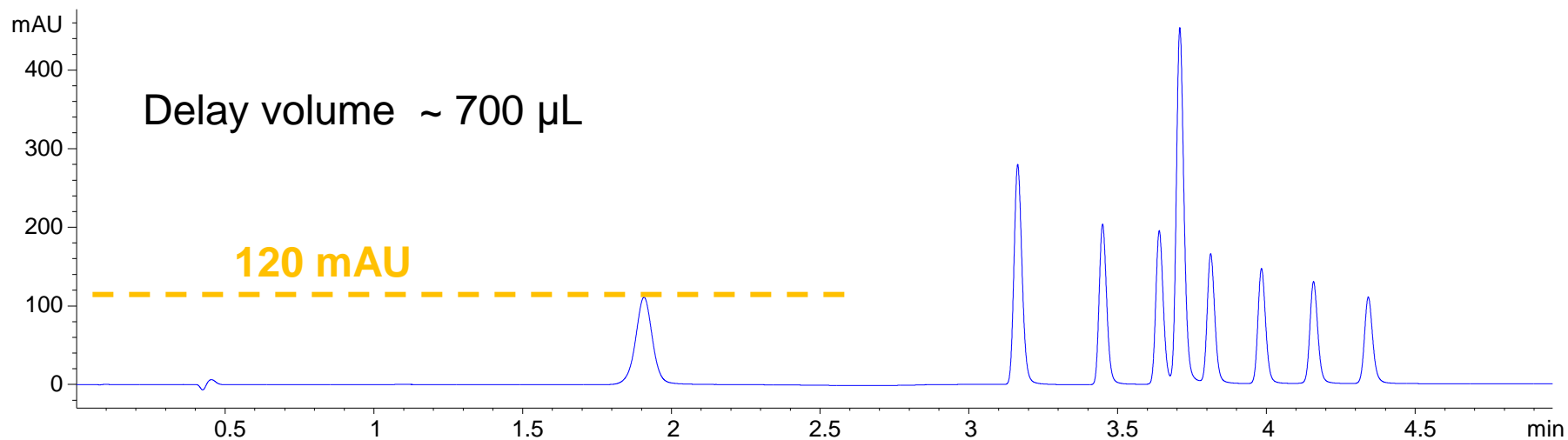


# Influence post-column capillary connections



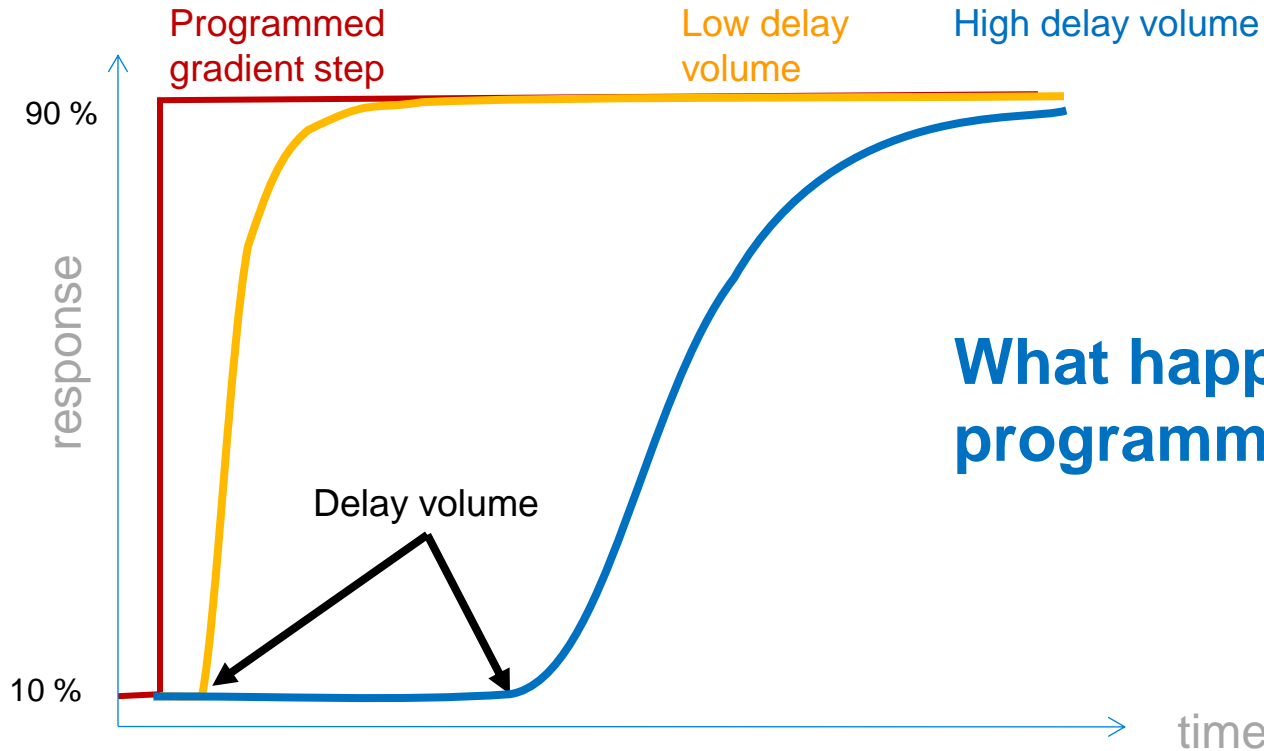
# System – Signal height

*System volumes – Delay volume*



# Delay volume

*Impact of low delay volume*



**What happens with a programmed gradient?**

High delay vol.

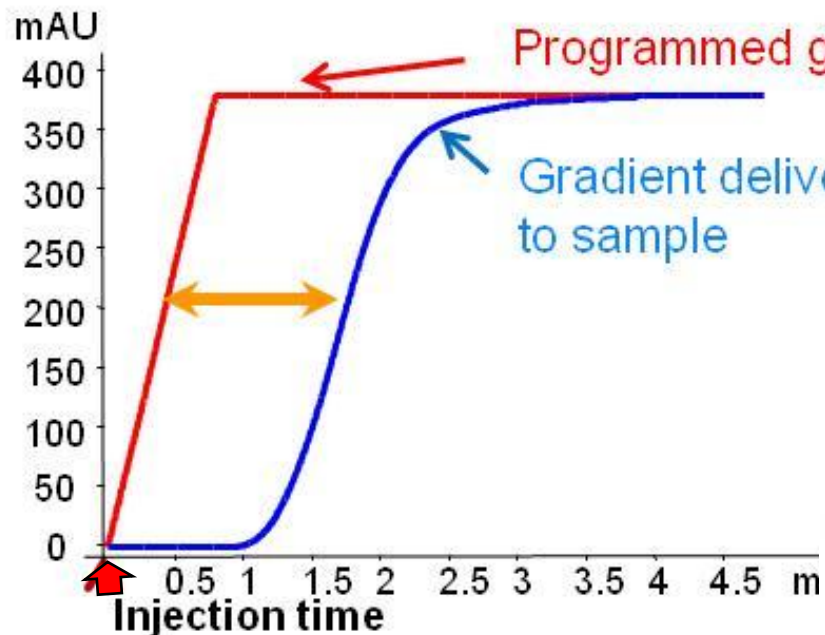


Low delay vol.



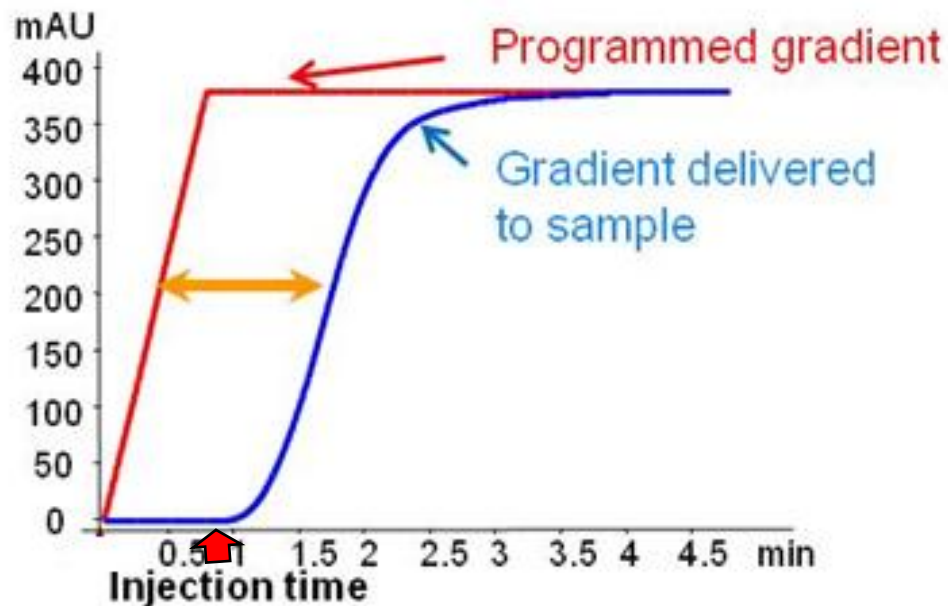
Total delay volume of the system (sum of capillaries, mixer, cells, valves..)

# Effects of Delay Injection Program



**Standard Injection**

**Delayed Injection**



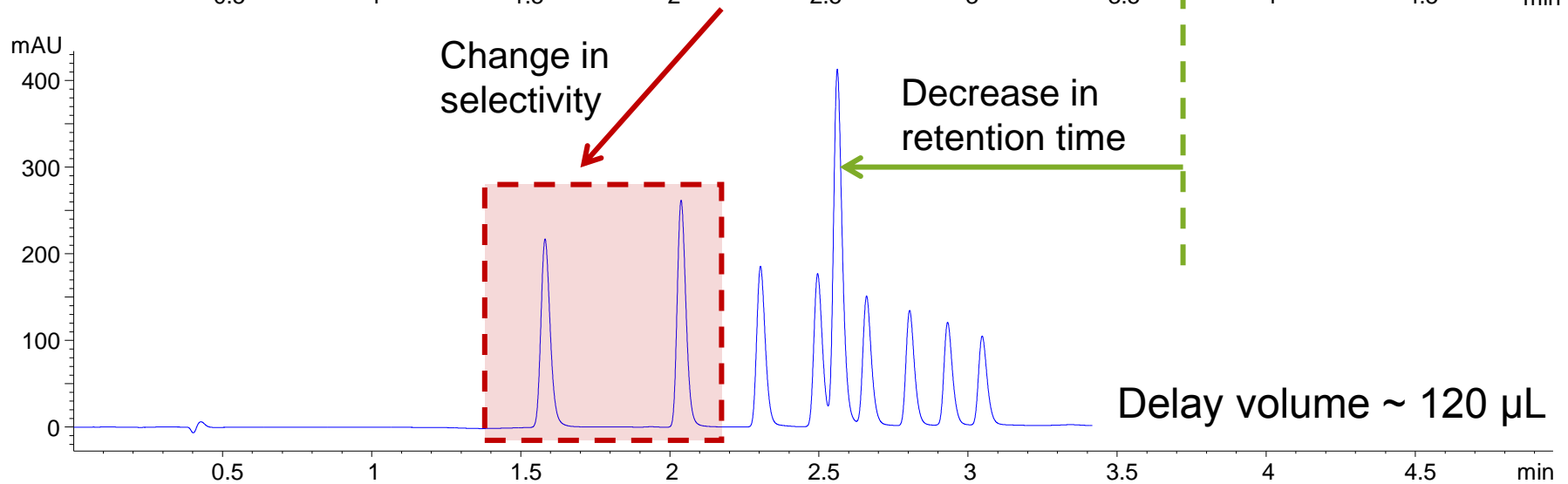
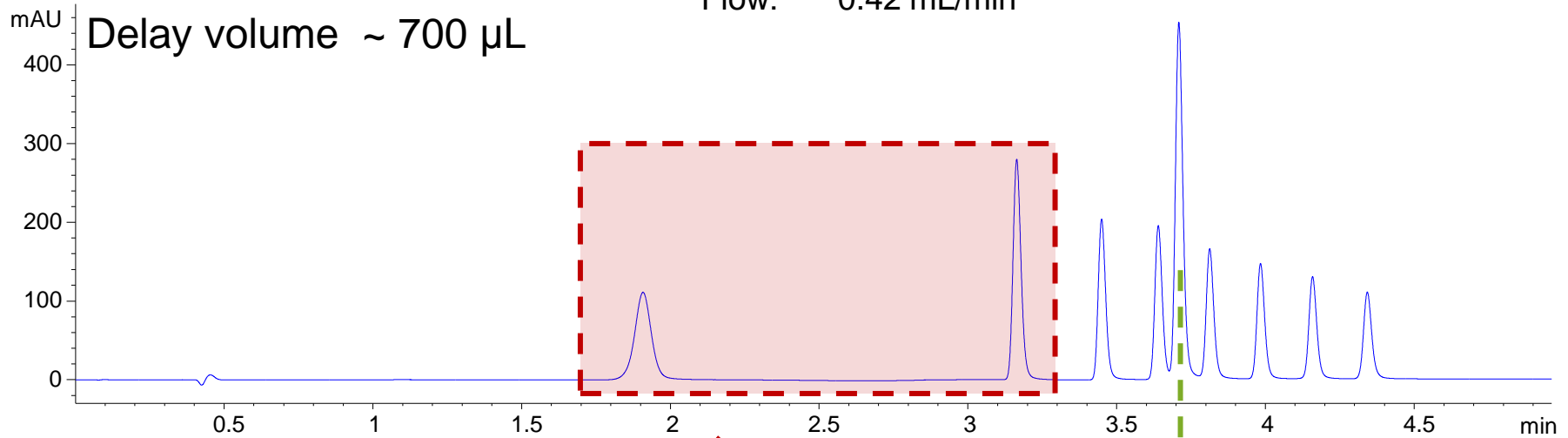


# Delay volume

*Impact of low delay volume*

Column: ZORBAX SB-C18 2.1 x 50 mm, 1.8  $\mu\text{m}$

Flow: 0.42 mL/min



# Performance Characteristics of an HPLC System

Influenced by one module...

**Flow: accuracy, precision**  
**Composition: accuracy, precision**

**Injection volume precision**  
**Linearity, dynamic range**  
**Carry over**

**Column temperature accuracy**  
**Column temperature precision**

**Wavelength: accuracy, precision**  
**Signal linearity**

**Spectral resolution (DAD only)**

**Pump**



**Injector**



**Column compartment**



**Detector**

Influenced by several modules...

**Repeatability of retention times**  
**Delay volume**

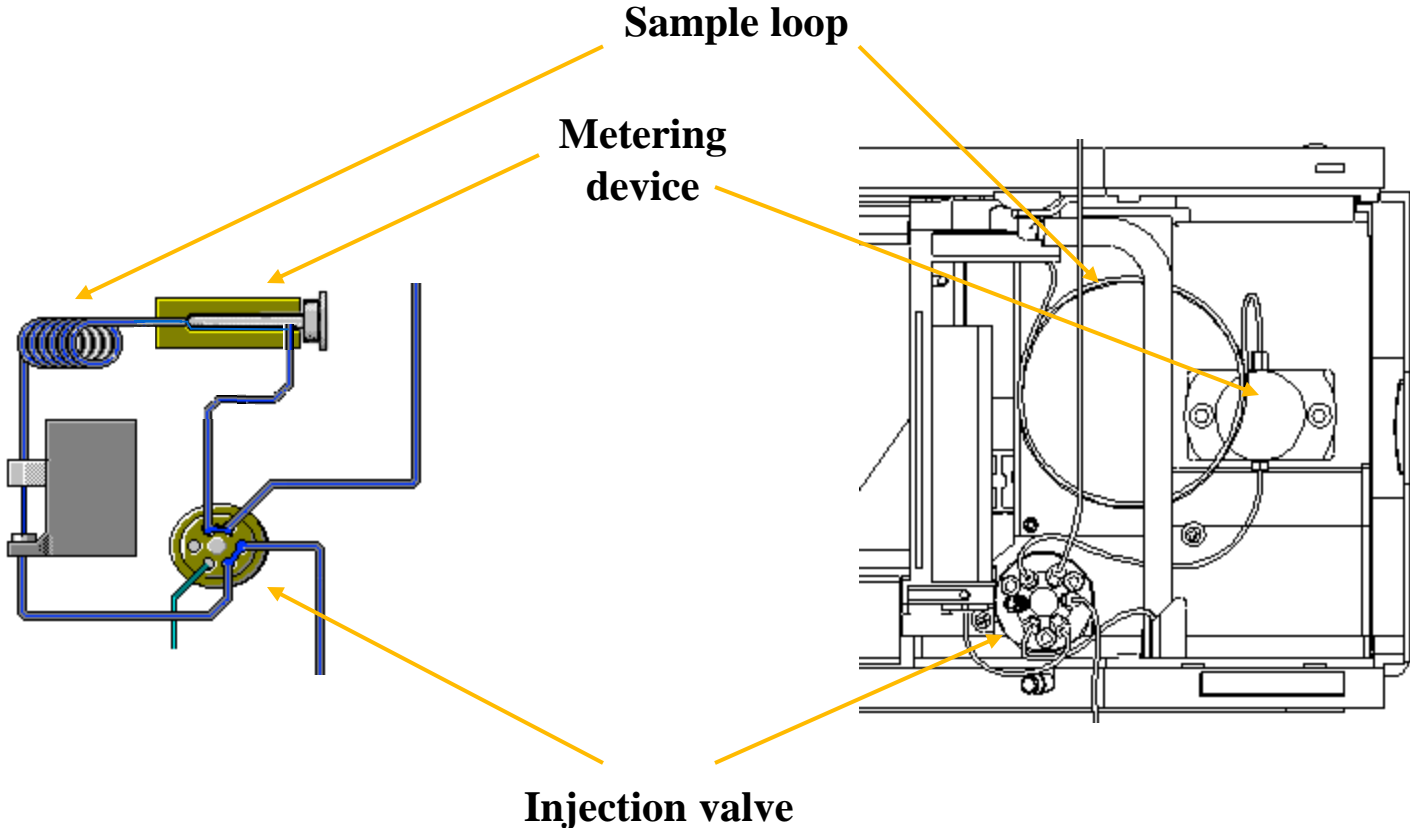
**Repeatability of peak areas**  
**Dead volume**

**Peak elution order**

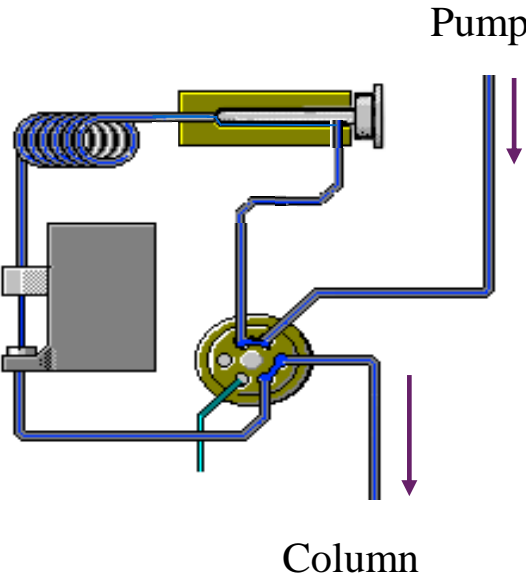
**Baseline: noise, drift and wander**



# Schematic of Injection System

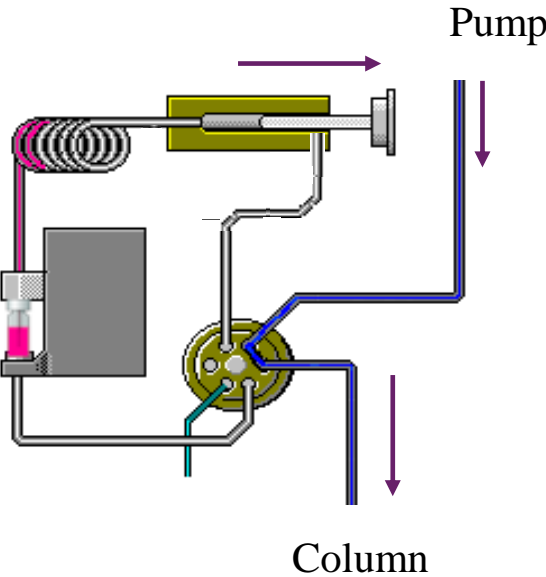


# Principle of Operation



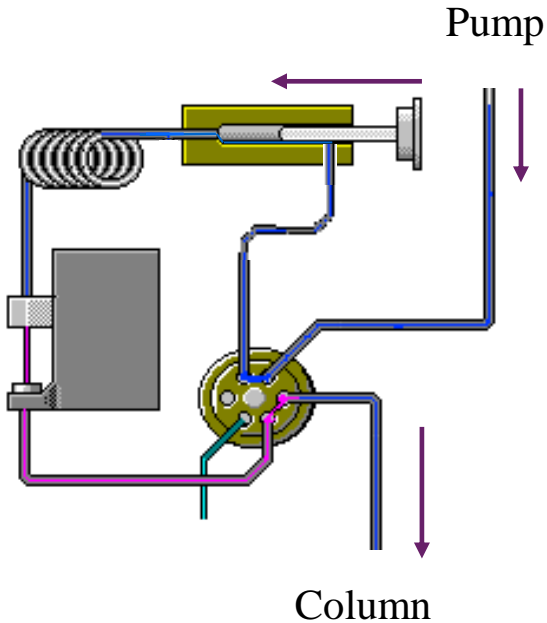
**Prior to Injection**

**Valve in Mainpass Position**



**Draw Sample**

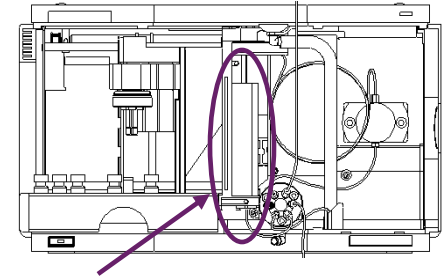
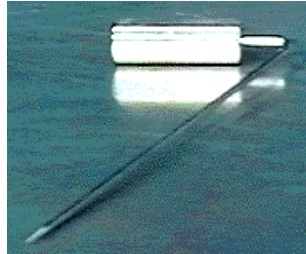
**Valve in Bypass Position**



**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

**Before beginning needle or needle seat replacement:**  
**Select "Change Needle" in the autosampler maintenance function.**

### Instant Pilot:

The screenshot shows the 'Maintenance - h ALS SL' window. A context menu is open over the 'Error Events' section, with '1 Change Needle / Seat' selected. The menu items are:

- 1 Change Needle / Seat
- 2 Change Loop
- 3 Change Piston
- 4 Change Needle Carrier
- 5 Align Transport

The window also displays sections for 'EMF Events', 'Error Events', and 'Maintenance Entries'. The status bar at the bottom shows 'System', 'Controller', 'Bin Pmp SL', 'h ALS SL', and 'DAD SL'.

### ChemStation:

The screenshot shows the 'Maintenance' menu in ChemStation. The 'ALS' option is selected, and the 'Maintenance Positions...' sub-menu is open.

The screenshot shows the 'Injector Maintenance Positions' dialog box. The 'Change Needle' section is selected, and the 'Start' button is highlighted. The dialog contains several sections with 'Start' and 'End' buttons:

- Change Needle: Start, Needle up, Needle down, End
- Change Piston: Start, End
- Change Gripper: Start, End
- Move Arm Home: Home
- Park Arm for Transport: Park Arm

A 'Close' button is located at the bottom right of the dialog.

# Thermostatted Column Compartment

- **Important performance characteristics**

- Excellent temperature accuracy
- Excellent temperature precision

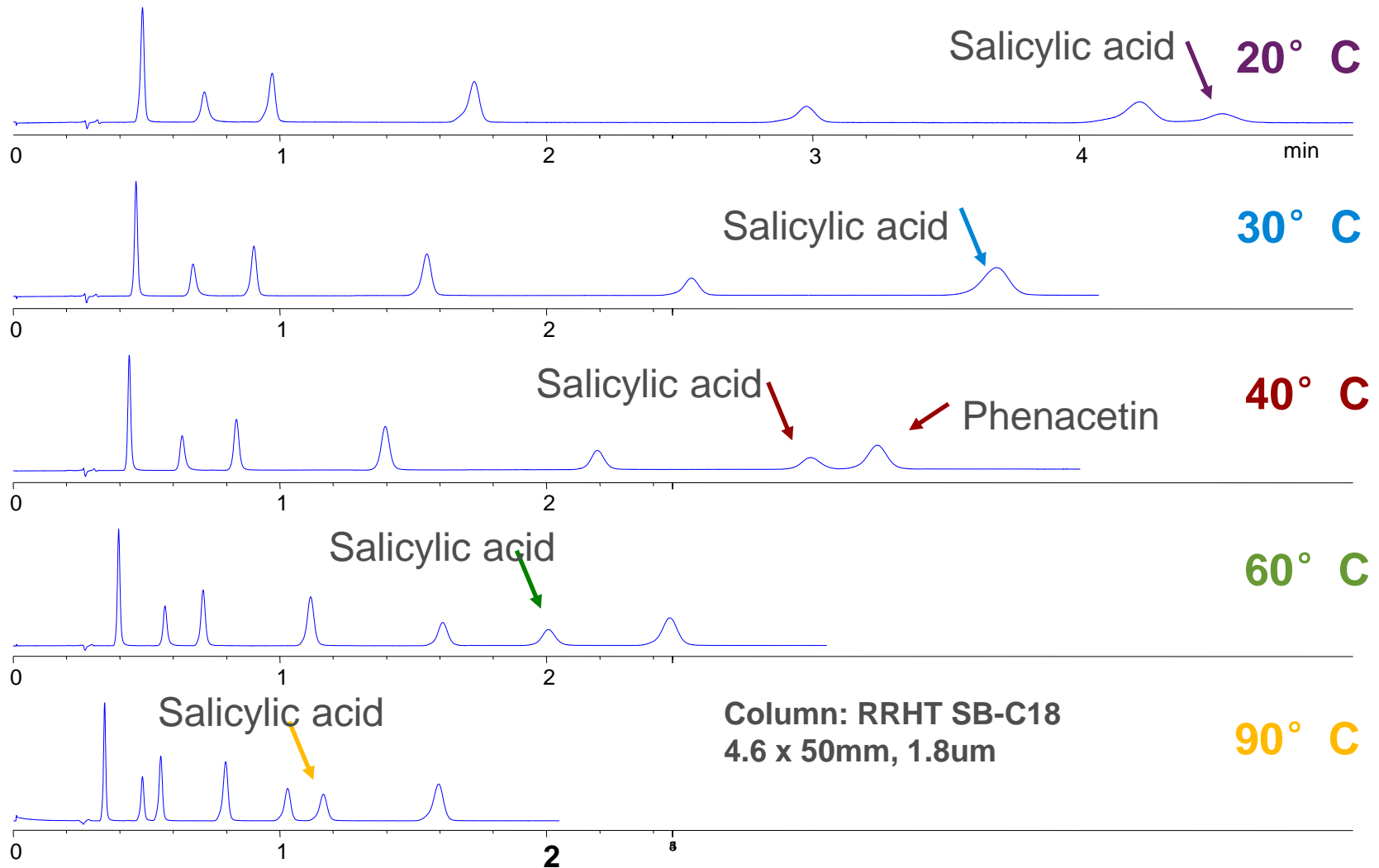


- **Influence on...**

- Elution order
- Peak identification
  
- Elution order
- Retention time precision
- Peak identification



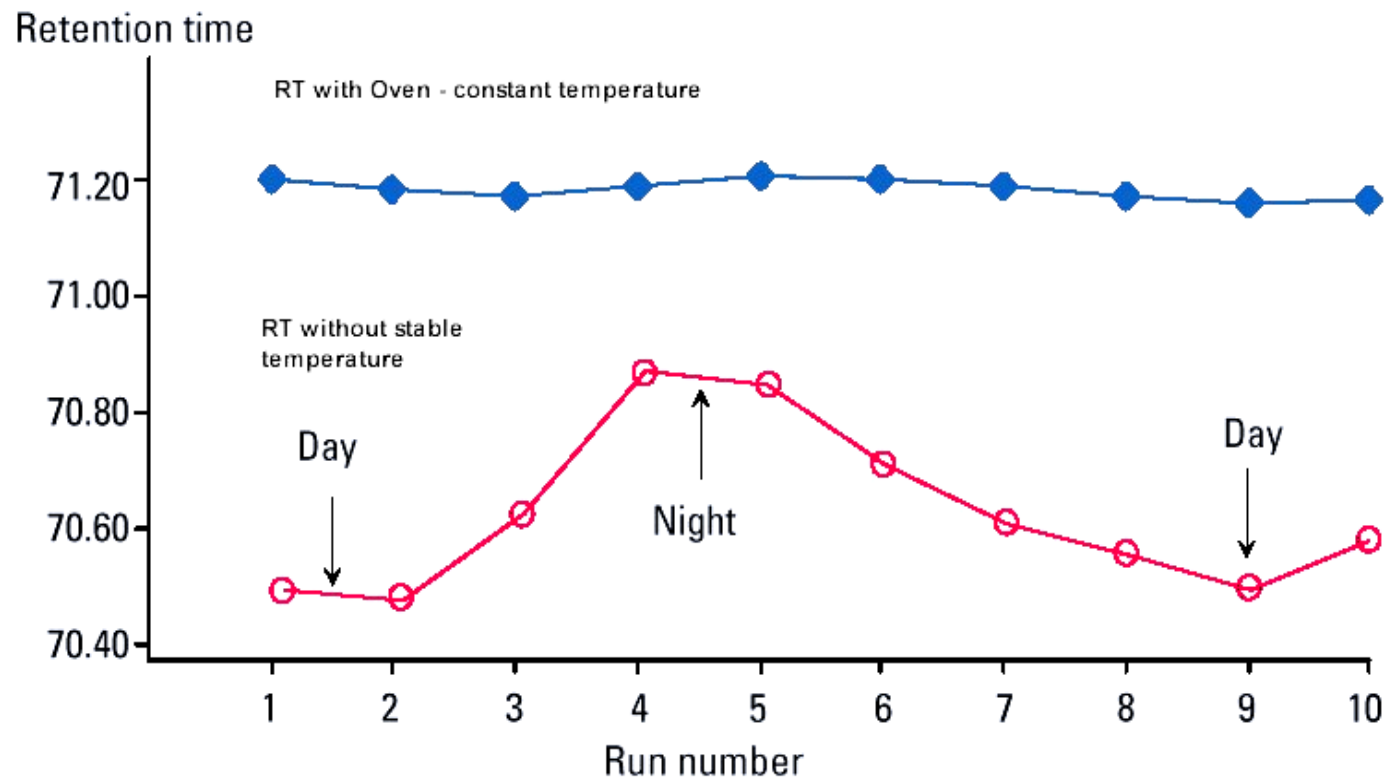
# Effect of Temperature on Separation



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

- Variable Wavelength and Diode Array Detector
- Low noise, wander and drift
- Wide linear range
- Very good wavel. accuracy
- Excellent wavel. precision
- Diode Array Detector only
- High spectral resolution
- Excellent spectral sensitivity

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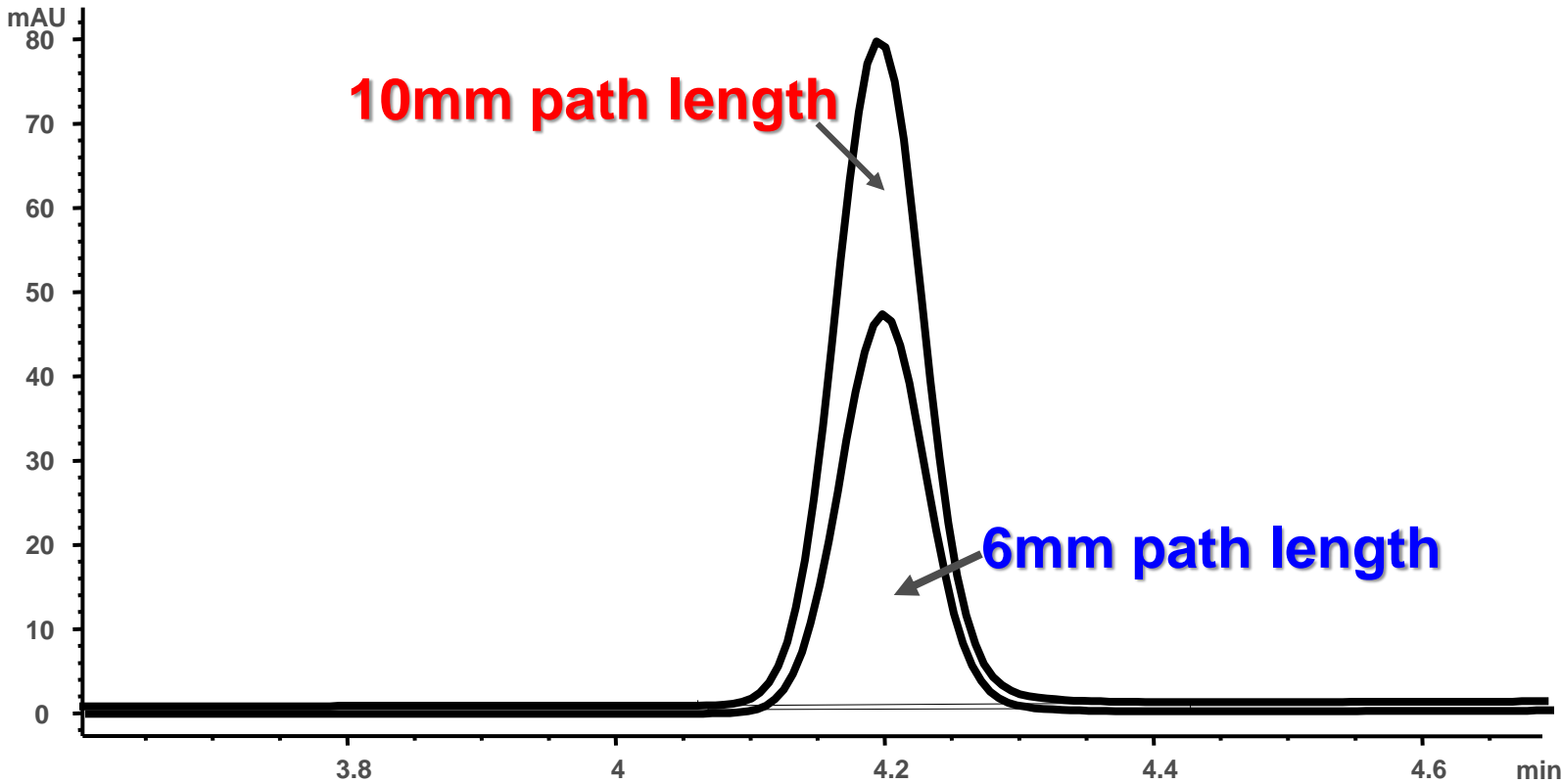
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## ● Influence on...

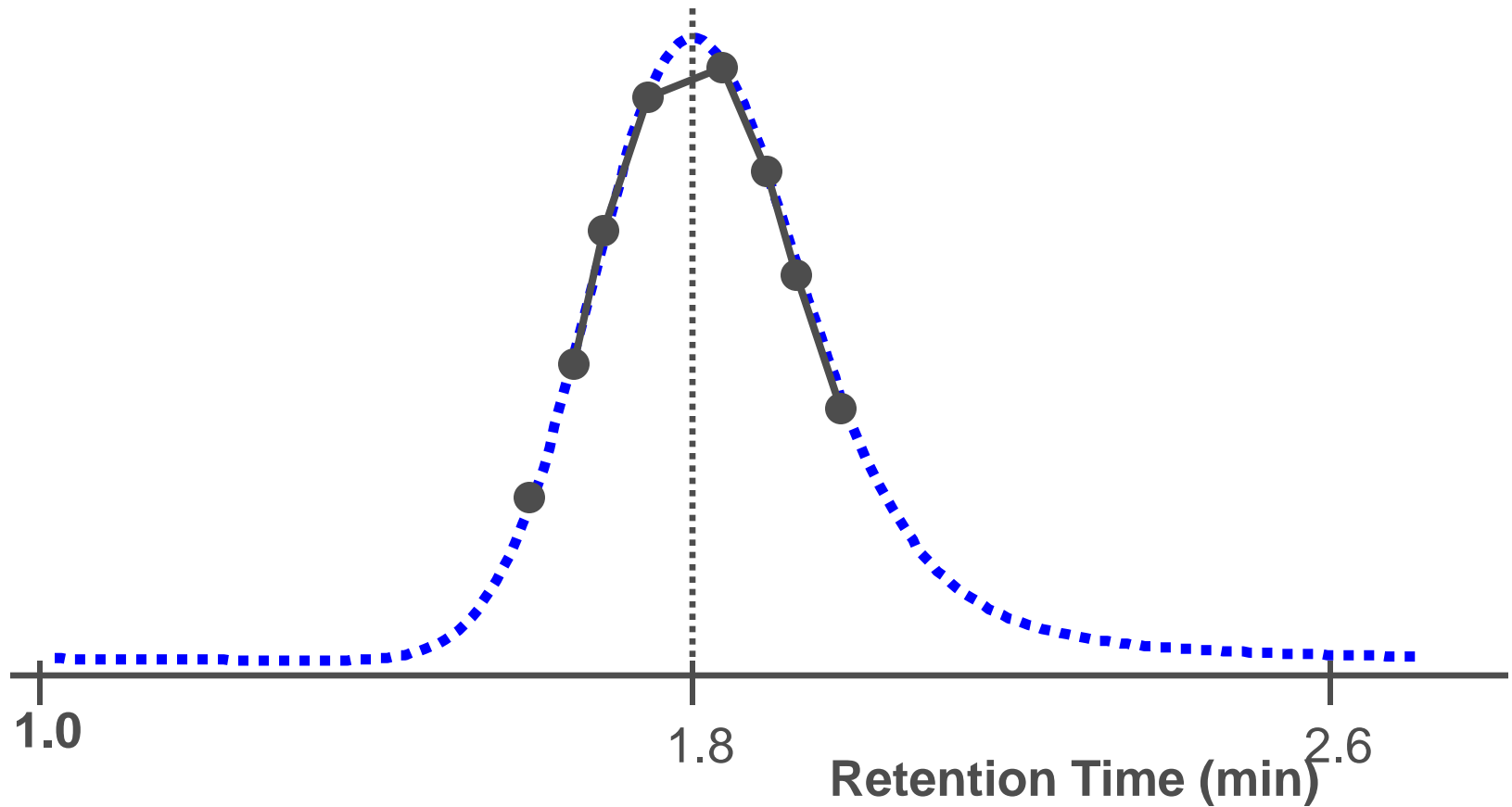
- Variable Wavelength and Diode Array Detector
- Detection limit, quantitation limit
- Confidence in quantitation at high and low concentrations
- Accuracy of peak areas/heights
- Precisions of peak areas/heights
- Diode Array Detector only
- Accuracy of spectra, peak identification by spectra
- Accuracy of spectra, peak identification by spectra at low concentrations



# Influence of Pathlength on Signal Sensitivity

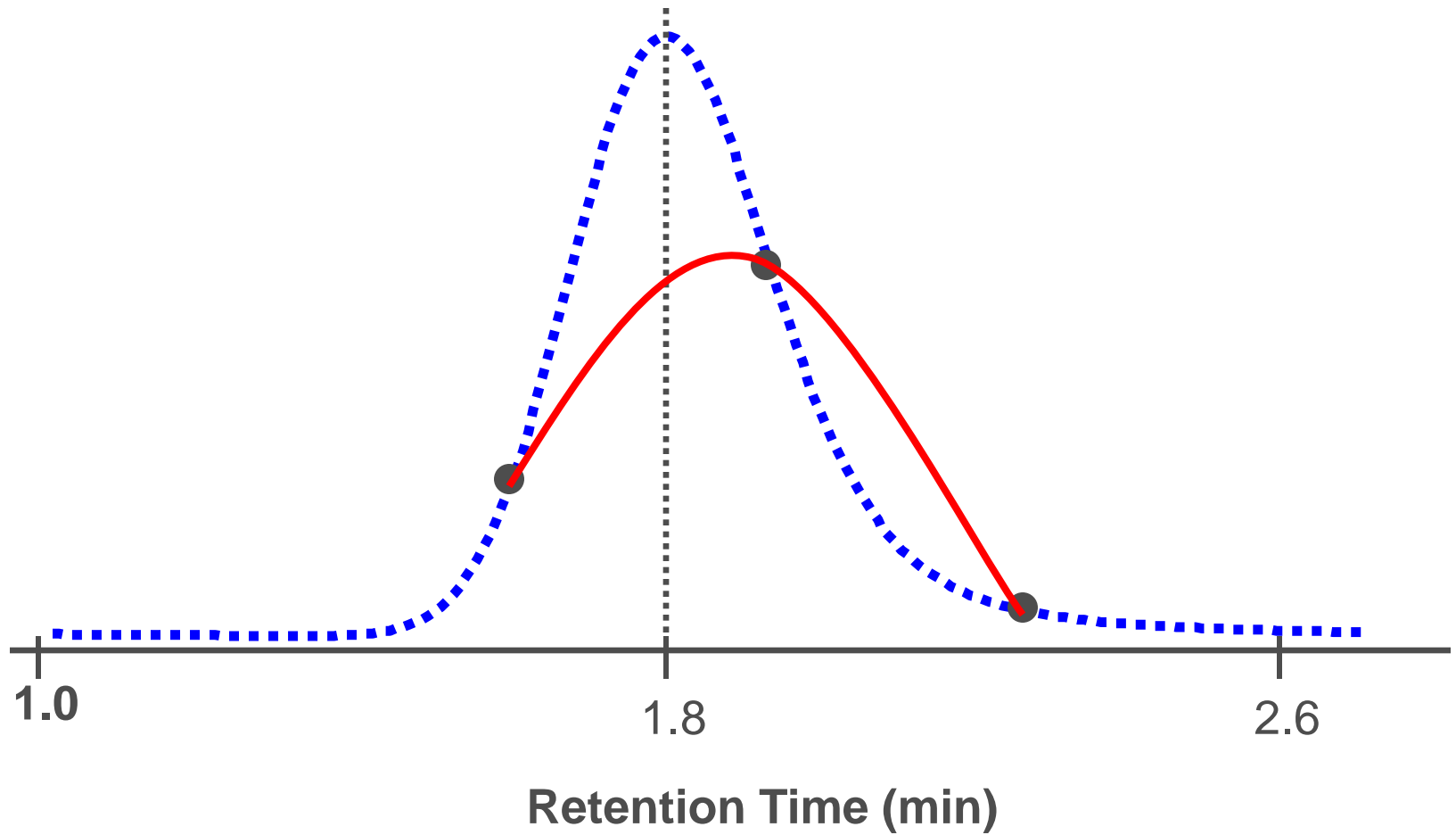


# Determining Peak Apex



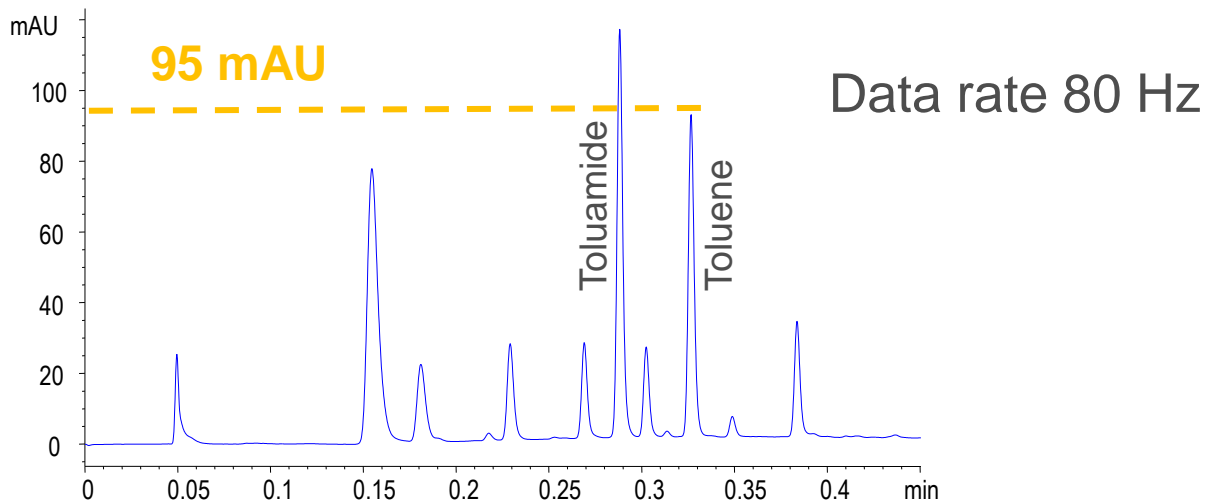
Fit highest data point and those on each side to a quadratic equation, solve for highest point

# Determining Peak Apex

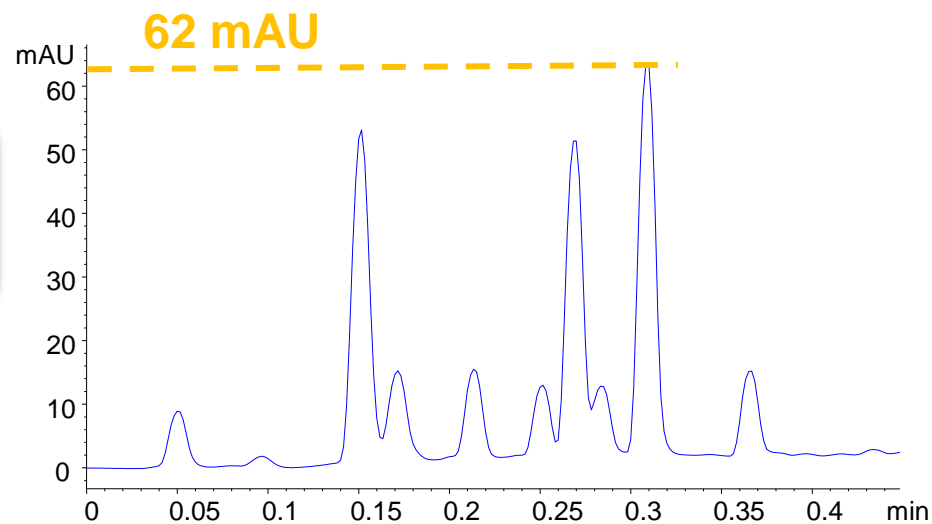


# Sensitivity

*Data rate – Peak height*



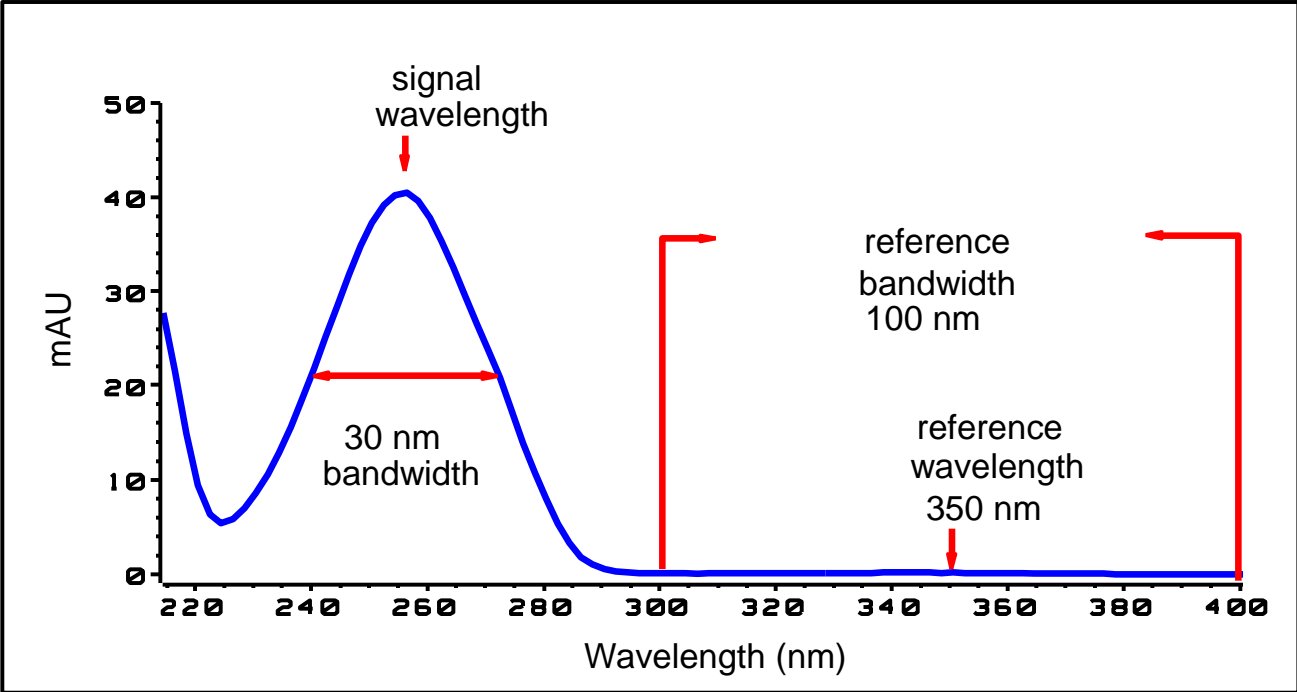
Data rate 10 Hz



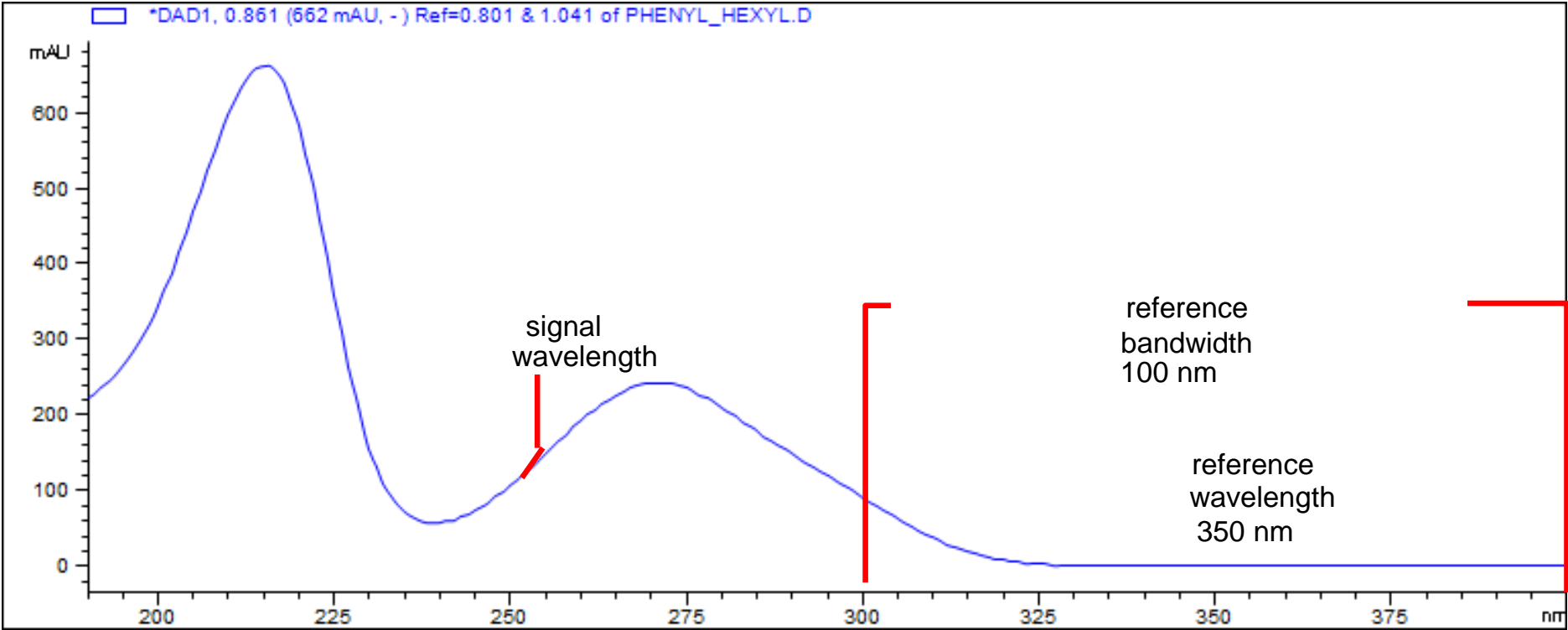
*Peak height increases with increasing data rate!*



# Sample Signal and Reference Optimization



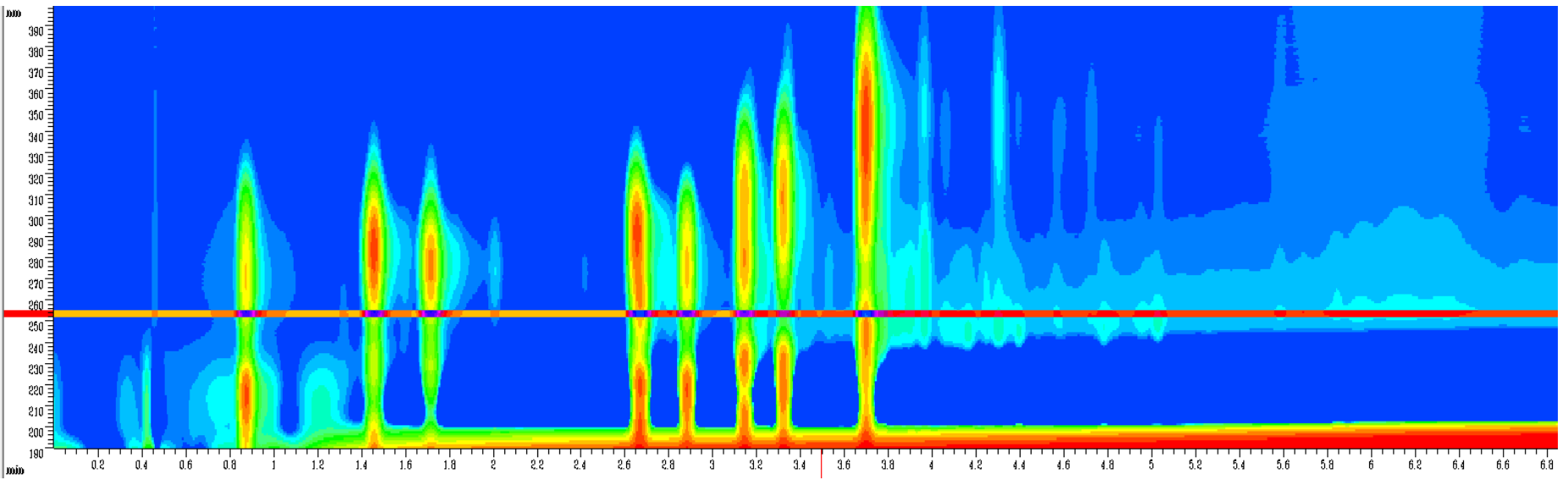
# Total Signal with Diode Array Detection



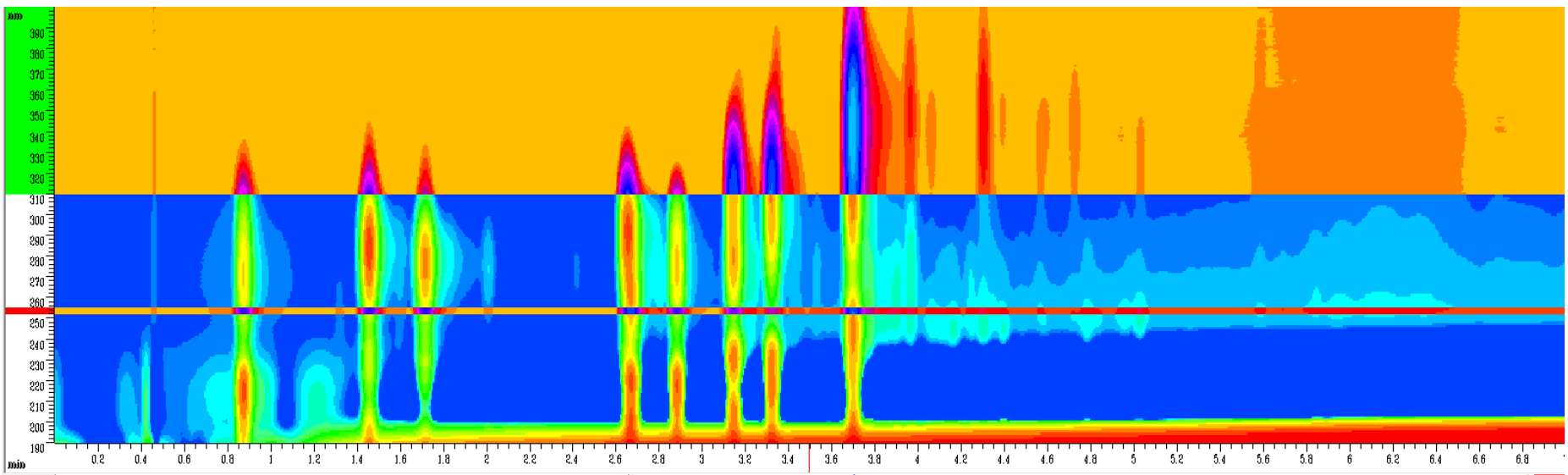
$$\frac{\text{Absorbance}_{\text{Sample wavelength}} + \text{Absorbance}_{\text{averaged over Bandwidth}}}{\# \text{ of wavelengths used}} + \frac{\text{Absorbance}_{\text{ref wavelength}} + \text{Absorbance}_{\text{averaged over Bandwidth}}}{\# \text{ of wavelengths used}} = \text{Total Absorbance}$$

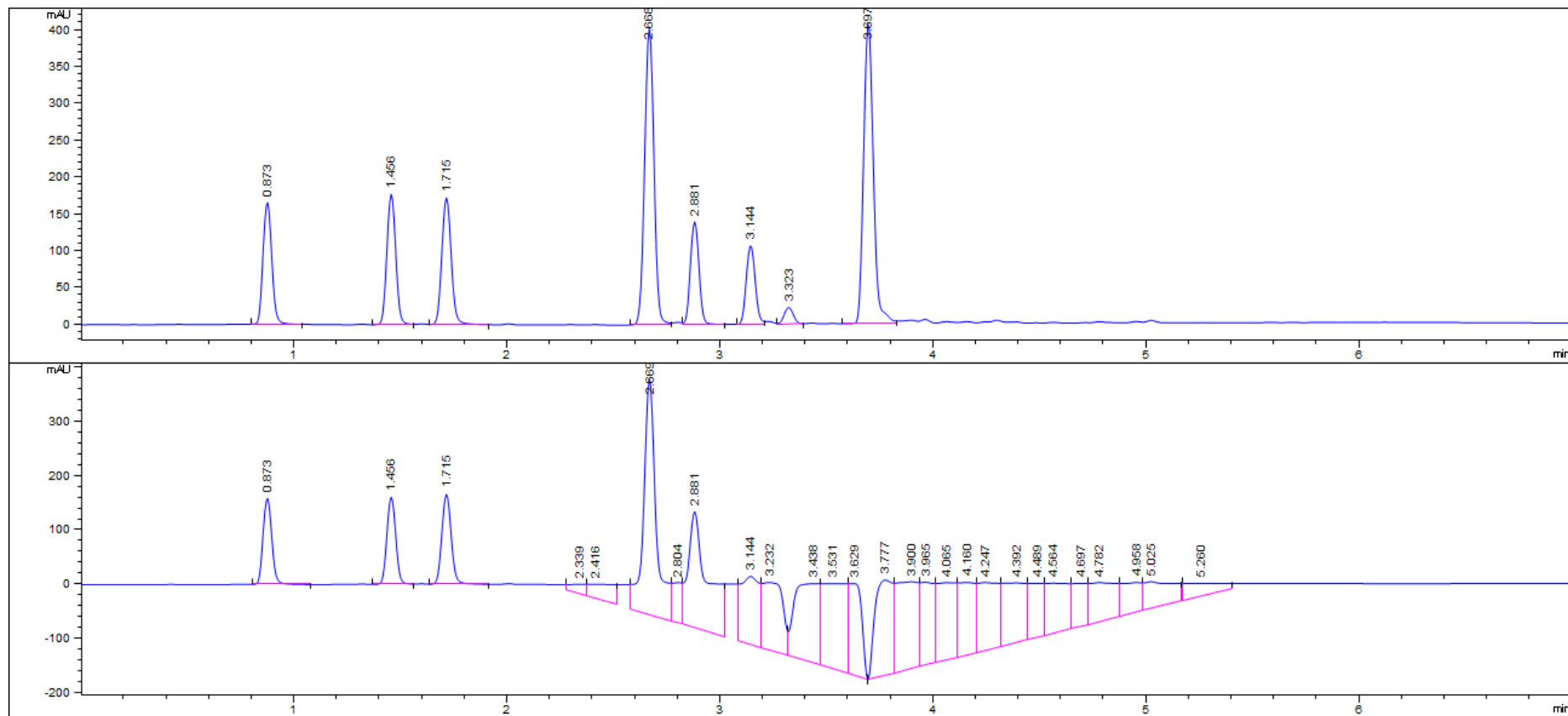


# Isoabsorbance plot without reference

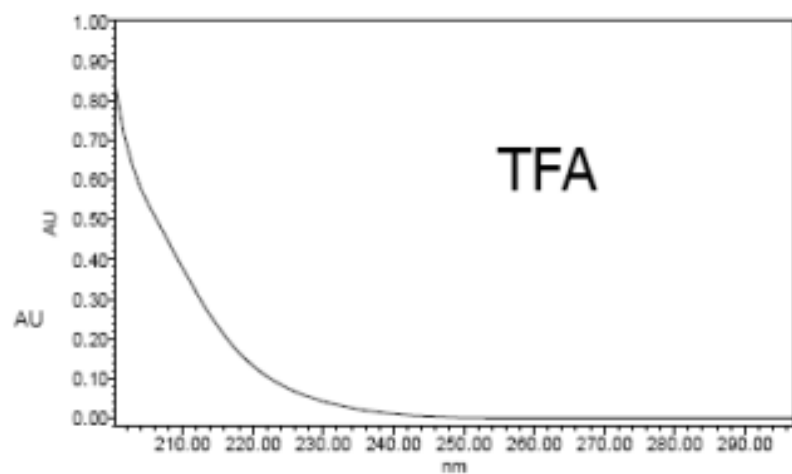


# Isoabsorbance plot with reference

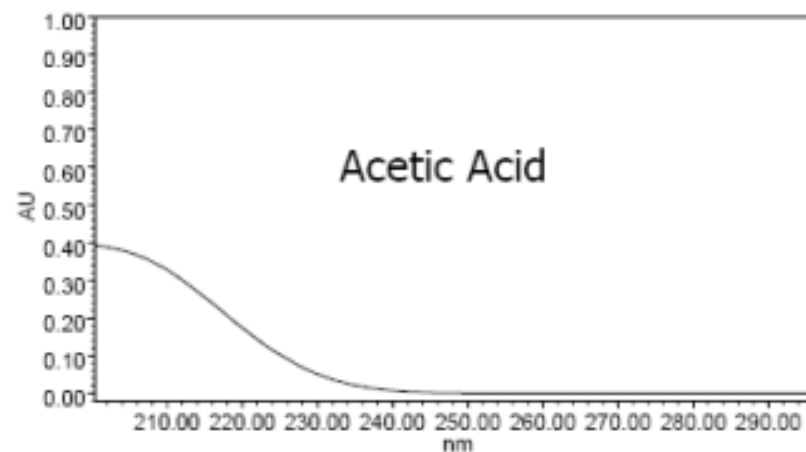




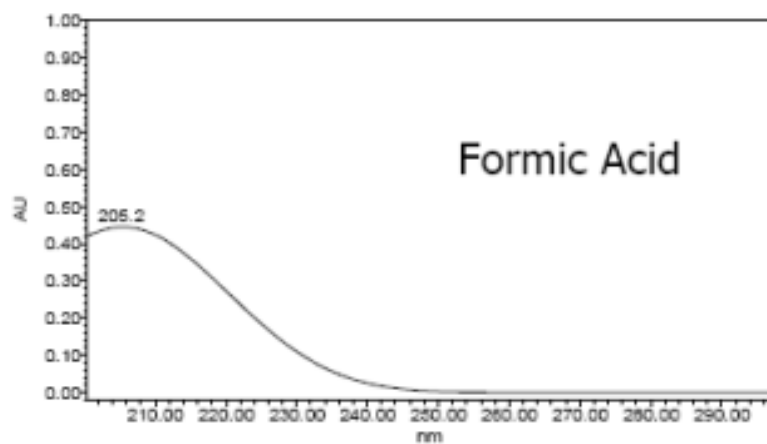
## UV spectrum of 10 nM mobile phase



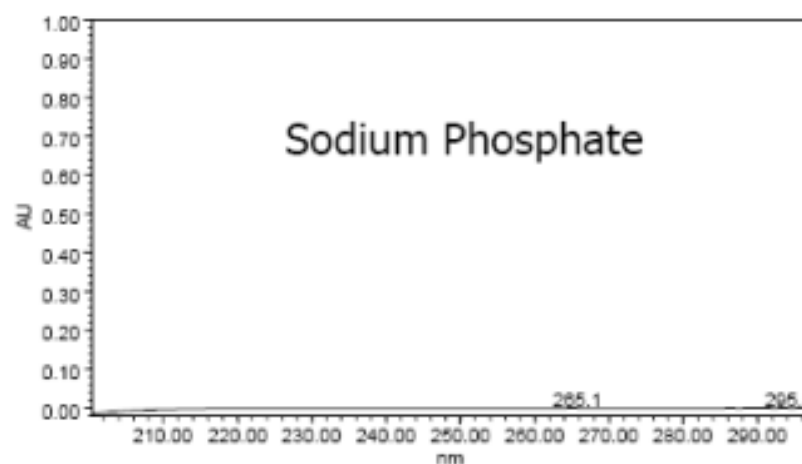
pH 0.94



pH 2.78



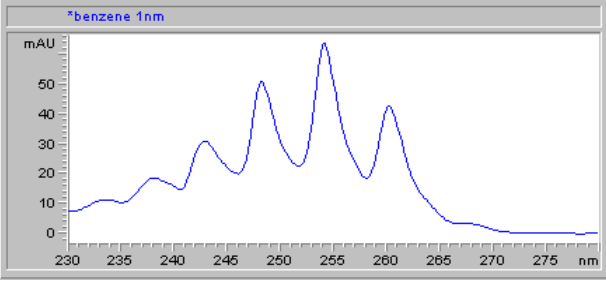
pH 2.26



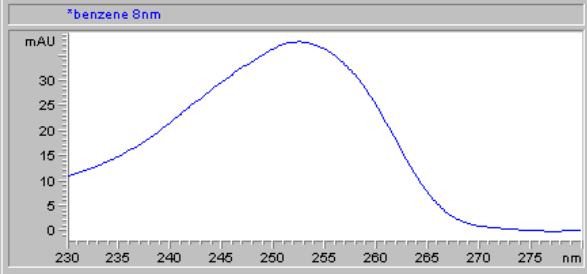
pH 2.0

# Optimizing Slit

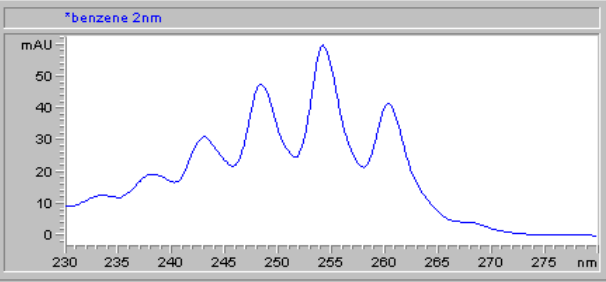
1 nm



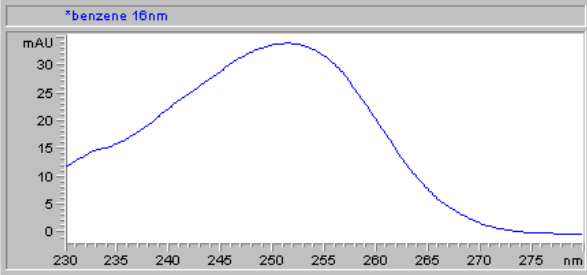
8 nm



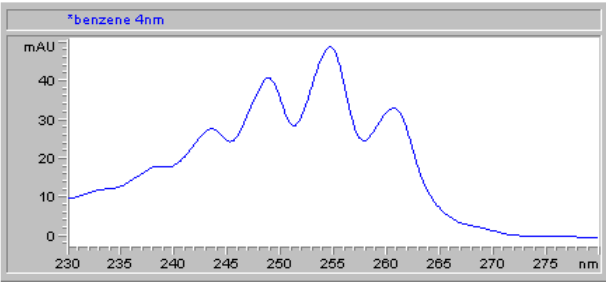
2 nm



16 nm



4 nm



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

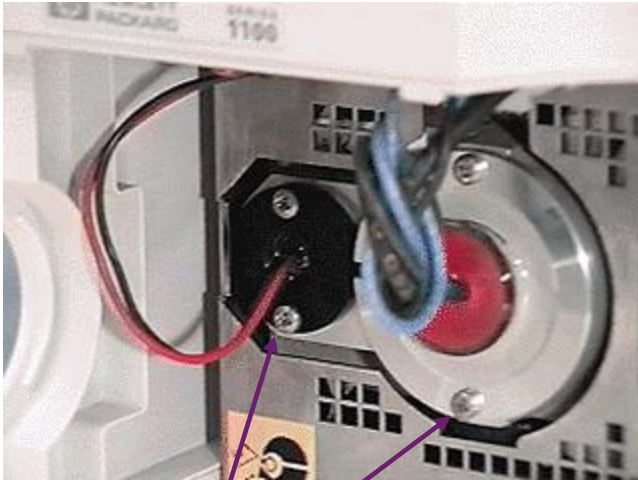
- Clean the leak sensors
- Check the waste tube
- Exchange the lamp (if necessary)
- Clean the flow cell (if necessary)
- Wavelength calibration
- Holmium Oxide Test
- Intensity test
- Cell test
- Dark current test
- Filter test

### 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

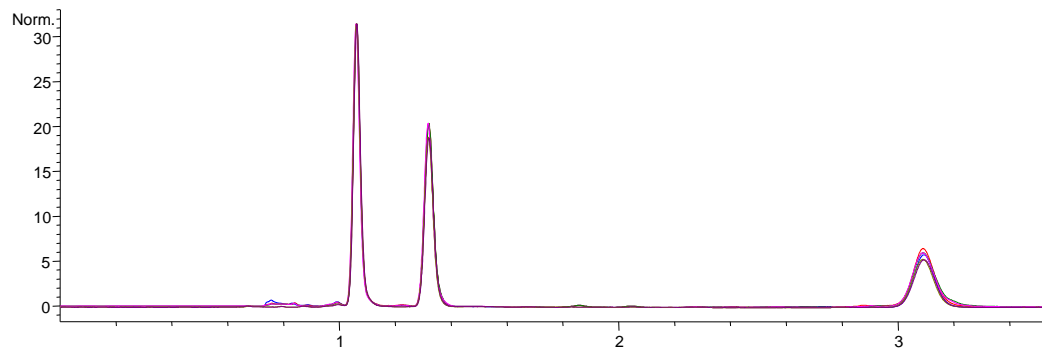
- ✓ Avoid the use of alkaline solutions with  $\text{pH} > 9.5$  which can attack quartz and impair optical performance.
- ✓ Prevent crystallization of buffers or salts which will lead to blockage and damage.
- ✓ Aqueous solvents can allow algae growth. Don't leave 100% water standing in the flow cell. 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.



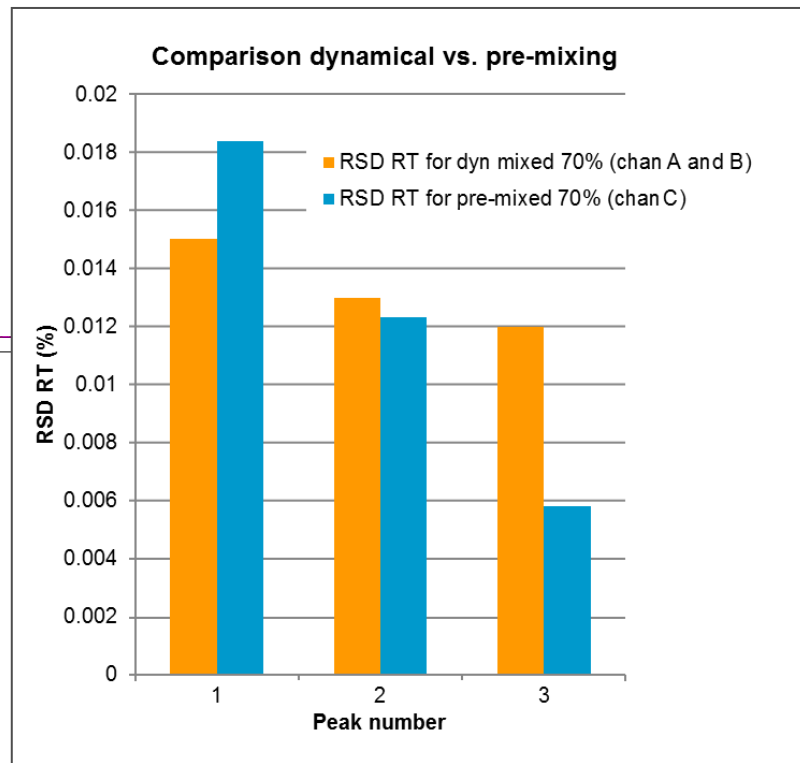
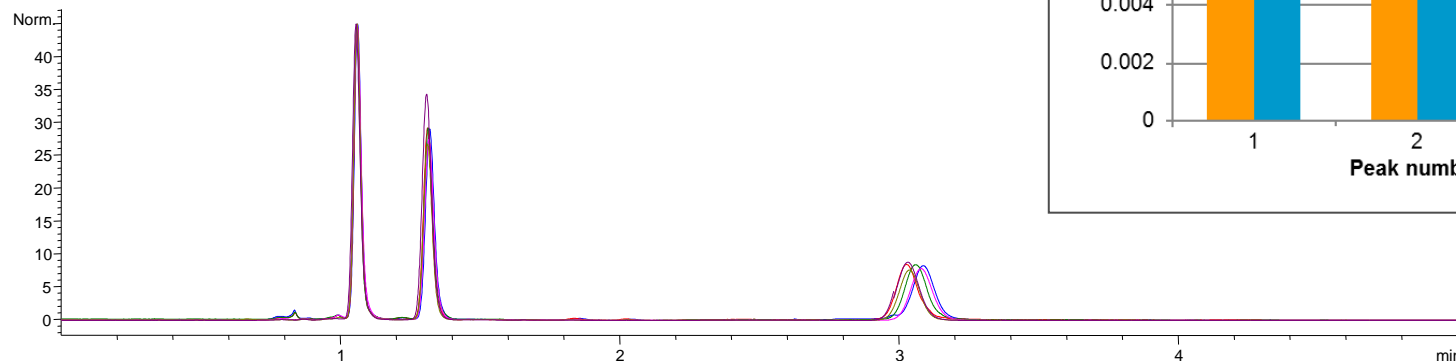
# Dynamically Mixed vs. Premixed Mobile Phases

## (prepared by one user)

6 dynamically mixed mobile phases



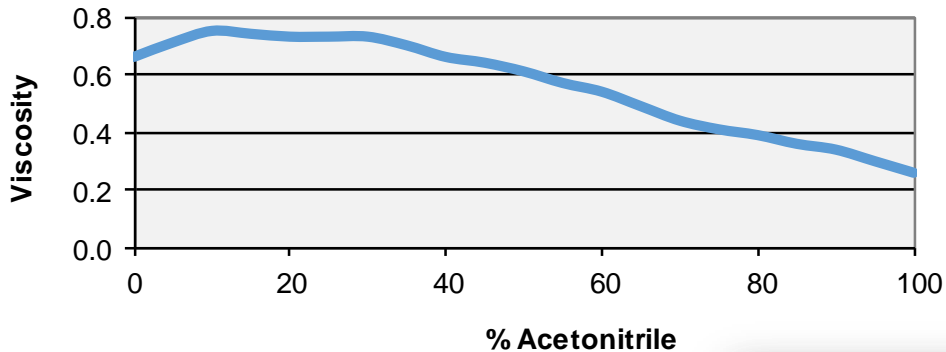
6 premixed mobile phases





# Non-ideal mixtures

Acetonitrile/Water 40 °C



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

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

Methanol/Water 40 °C

