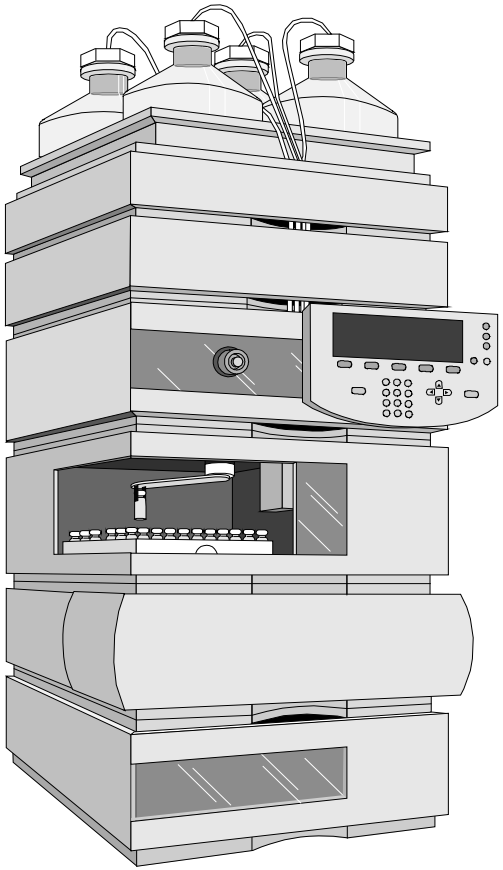
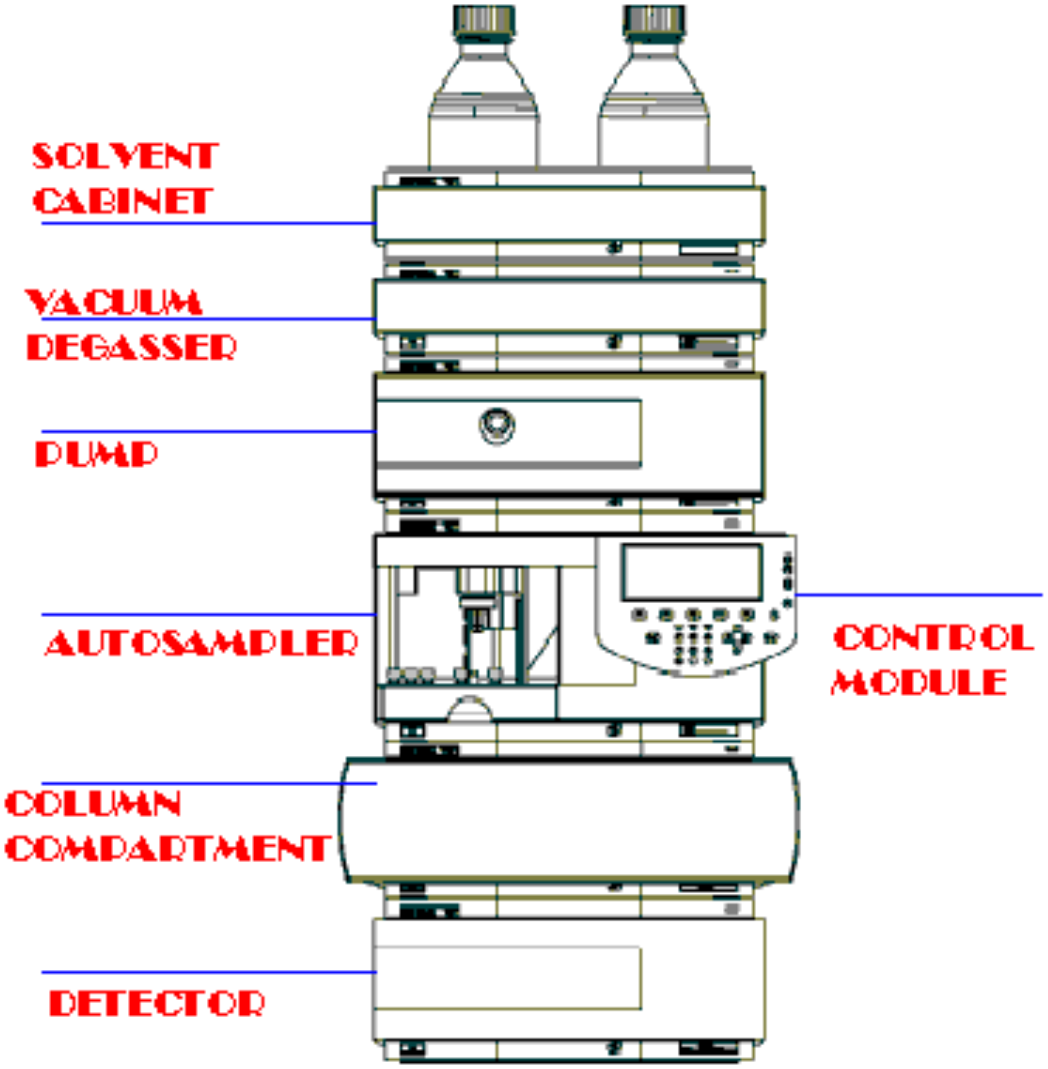
Agilent Technologies
1200/1100 Series HPLC
Troubleshooting and Maintenance Seminar

Patrick Cronan & Sue D'Antonio
LC Applications Scientists

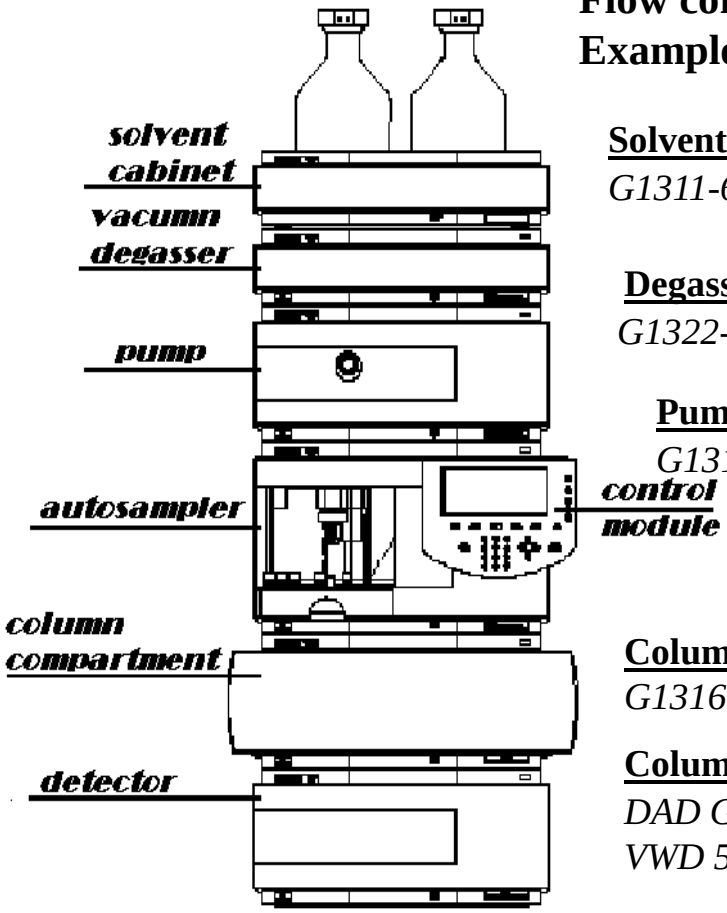
Agilent Technologies HPLC Systems



- All stackable modules for small bench space
- Single system control module
- Front access for customer maintenance
- Ergonomic tubing organization for lowest delay volume and bandspreading
- Single CAN cable connection
- All modules have RS 232, HP IB, Start/Stop, CAN


SOLVENT CABINET
VACUUM DEGASSER
PUMP
AUTOSAMPLER
CONTROL MODULE
COLUMN COMPARTMENT
DETECTOR

THE STACK



Flow connections in the stack:
Example setup with 0.17mm ID green capillaries

Solvent bottles - degasser:
G1311-60003 (bottle-head assembly, PTFE-tubings)

Degasser - pump:
G1322-67300 (PTFE-tubings)

Pump - autosampler:
G1312-67305 (SST, green)

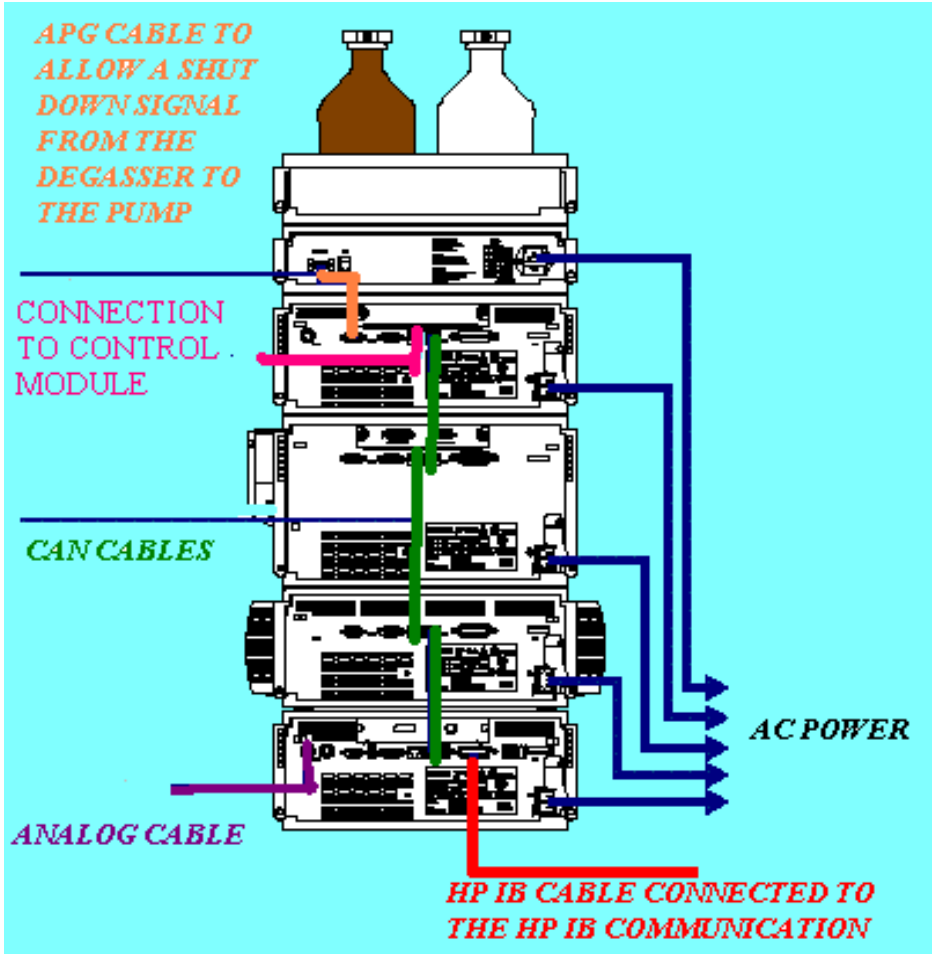
Autosampler - column compartment:
G1313-87305 (SST, green)

Column compartment - column:
G1316-87300 (SST, green)

Column - detector:
DAD G1315-87311 (SST, coated)
VWD 5062-8522 (PEEK)

Detector - waste:
DAD 0890-1713 (PTFE, wide bore)
VWD 5062-8535 (PEEK)
5062-2463 (corrugated waste tubing, reorder pack)

REAR CONNECTIONS



CAN CABLES BETWEEN MODULES

CAN CONNECTION TO THE CONTROLLER

ANALOG OUT PUT FOR DATA COMMUNICATION TO A NON HP DATA SYSTEM

HP IB CABLE TO COMPUTER

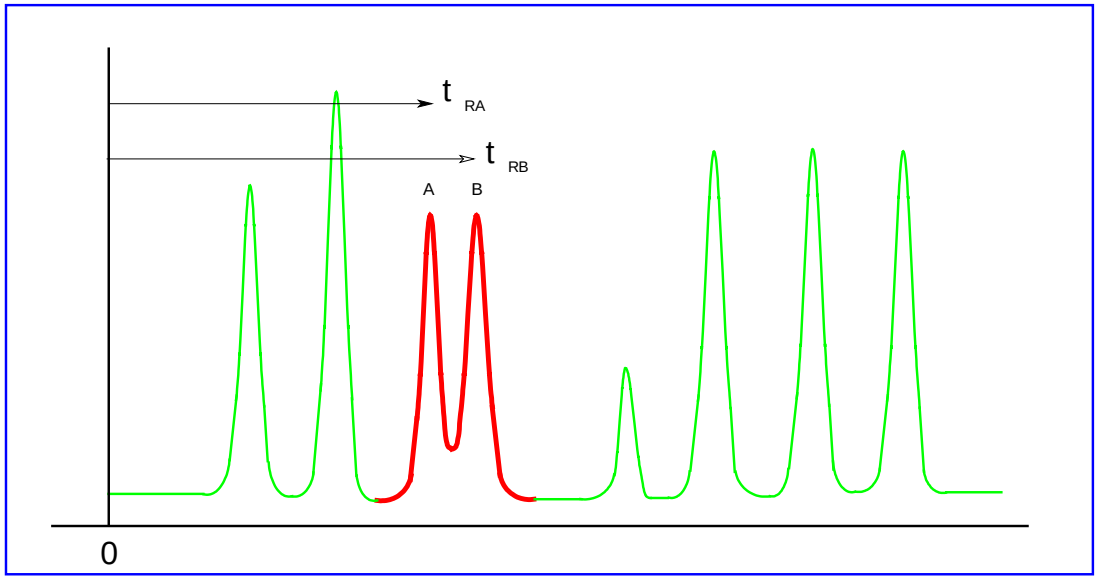
LAN Connections on Newer Systems

What are Chromatographers Looking for?

Better performance

- Baseline separation in shortest time
- Repeatability of results
- Accuracy of results
- Sensitive detection
- Standard, narrow bore and capillary column capability

The Goal of Separation
-Resolution Between Sample Components



$$R = 2\left(\frac{t_{RB} - t_{RA}}{W_A + W_B}\right)$$

$$R = 1.176\left(\frac{t_{RB} - t_{RA}}{W_{1/2A} + W_{1/2B}}\right)$$

R - resolution

t - retention time of component B

t - retention time of component A

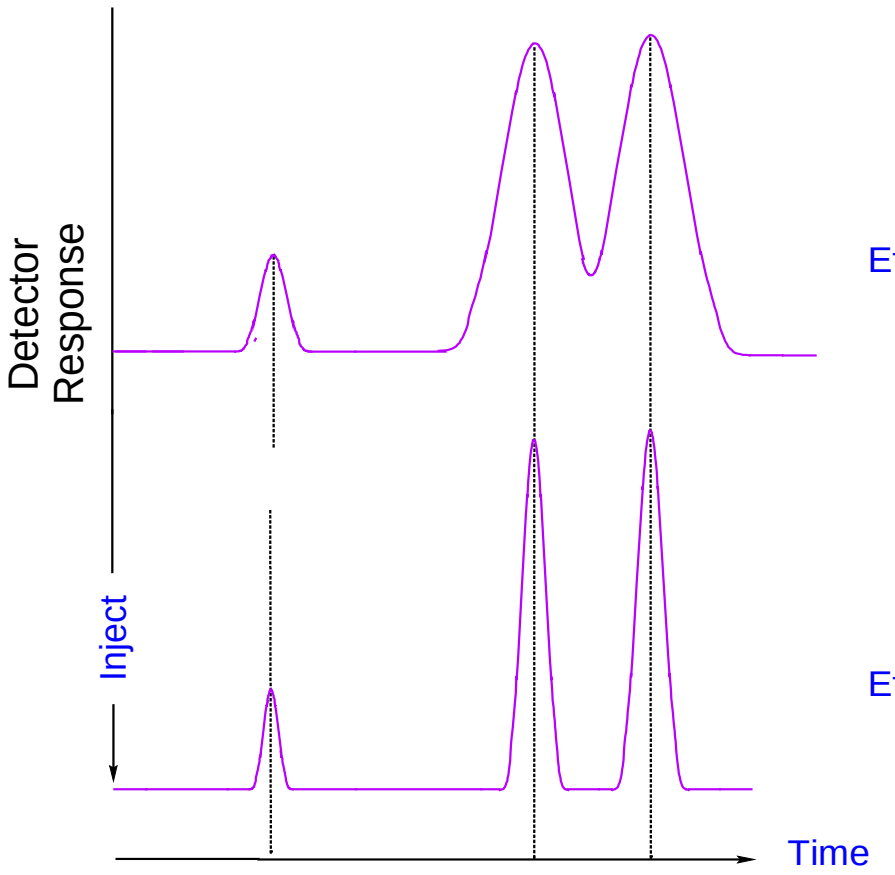
w - width at base of peak

w - width at half-height

The Goal of Separation
-Resolution Between Sample Components

$$R = \frac{1}{4}\sqrt{N} \times \frac{\alpha - 1}{\alpha} \times \frac{k'}{1 + k'}$$

Efficiency *Selectivity* *Capacity*



Low Efficiency

High Efficiency

Resolution

R_s, defined as the amount of separation between two adjacent peaks, is given by:

$$R_s = (1/4)\,(\alpha - 1)\,(N)^{1/2}\,[k/(1 + k)]$$

where **k** is the average value for the two peaks.

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

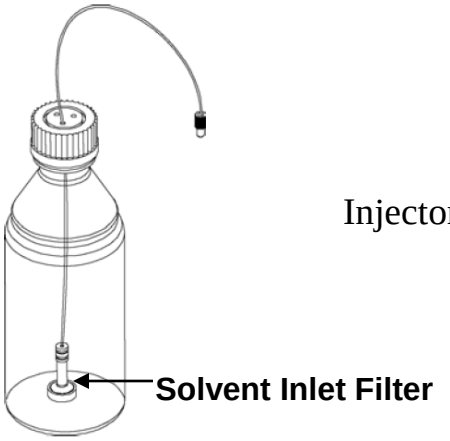
Baseline: noise, drift and wander

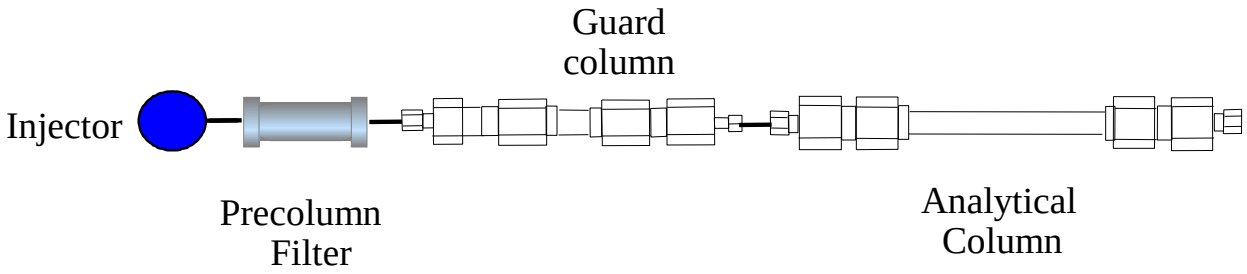
Instrument Status Indicator

The instrument status indicator indicates one of four possible instrument conditions:

- **When the status indicator is OFF** (and power switch light is on), the quaternary pump is in a prerun condition, and is ready to begin an analysis.
- **A green status indicator**, indicates the quaternary pump is performing an analysis (run mode).
- **A yellow indicator** indicates a not-ready condition. The quaternary pump is in a not-ready state when it is waiting for a specific condition to be reached or completed (for example, immediately after changing a setpoint), or while a self-test procedure is running.
- **An error condition is indicated when the status indicator is red.** An error condition indicates the quaternary pump has detected an internal problem which affects correct operation of the quaternary pump. Usually, an error condition requires attention (for example, leak, defective internal components). An error condition always interrupts the analysis.

Solvent Filters





Solvent Inlet Filer

Stainless Steel or glass with 10 micron porosity.

Removes particulates from solvent.

Precolumn Filter

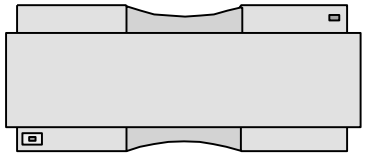
Used between the injector and guard column.

2 to 0.5 micron

Removes particulates from sample and autosampler wear debris.

Must be well designed to prevent dispersion.

Agilent 1200 Series Vacuum Degasser



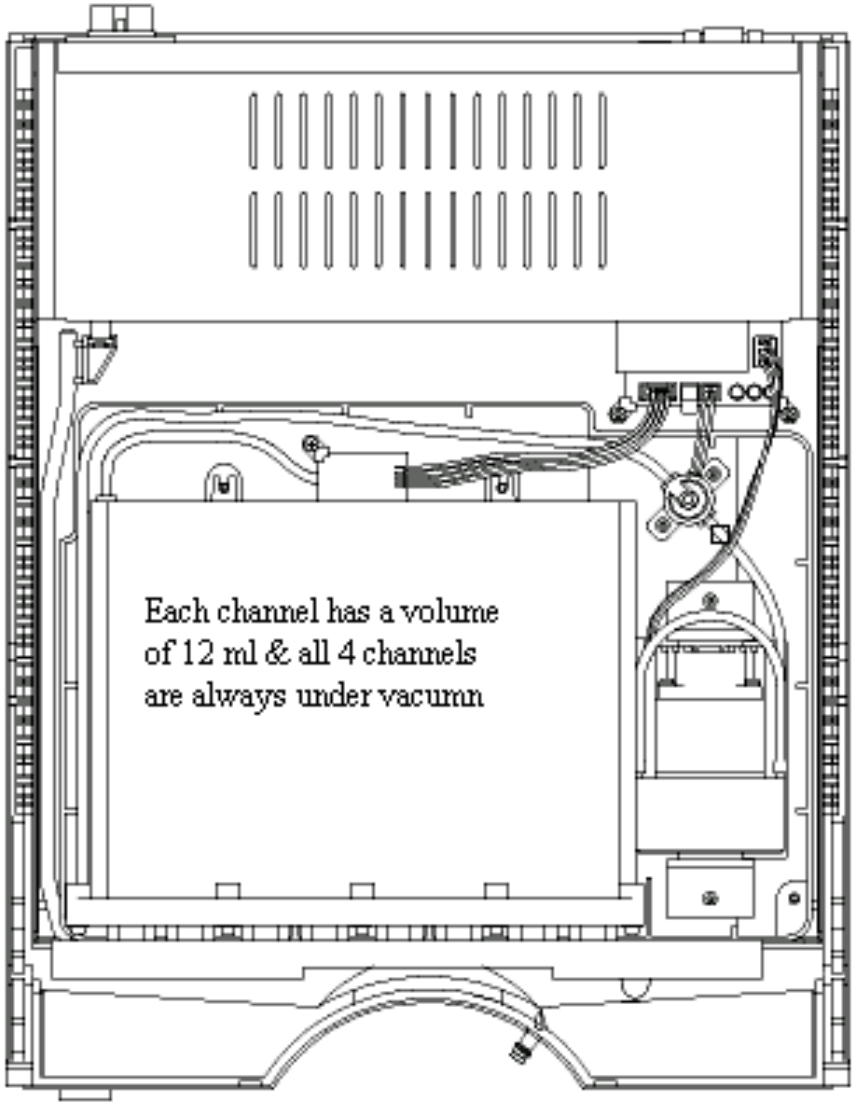
Recommended for ...

- reliable performance with gradient pumps using low pressure mixing
- improved performance with all pump designs at low flowrates
- use with detectors requiring oxygen-free mobile phase

Features/Benefits

- Convenient and cost effective alternative to helium sparging
- High degassing efficiency for trouble-free system operation (<1.5 ppm oxygen at 10 ml/min)
- Low internal volume (12 ml) for fast solvent changeover
- Up to 4 channels for highest versatility
- Stackable with other Agilent 1200 Series modules

The Vacuum Degasser

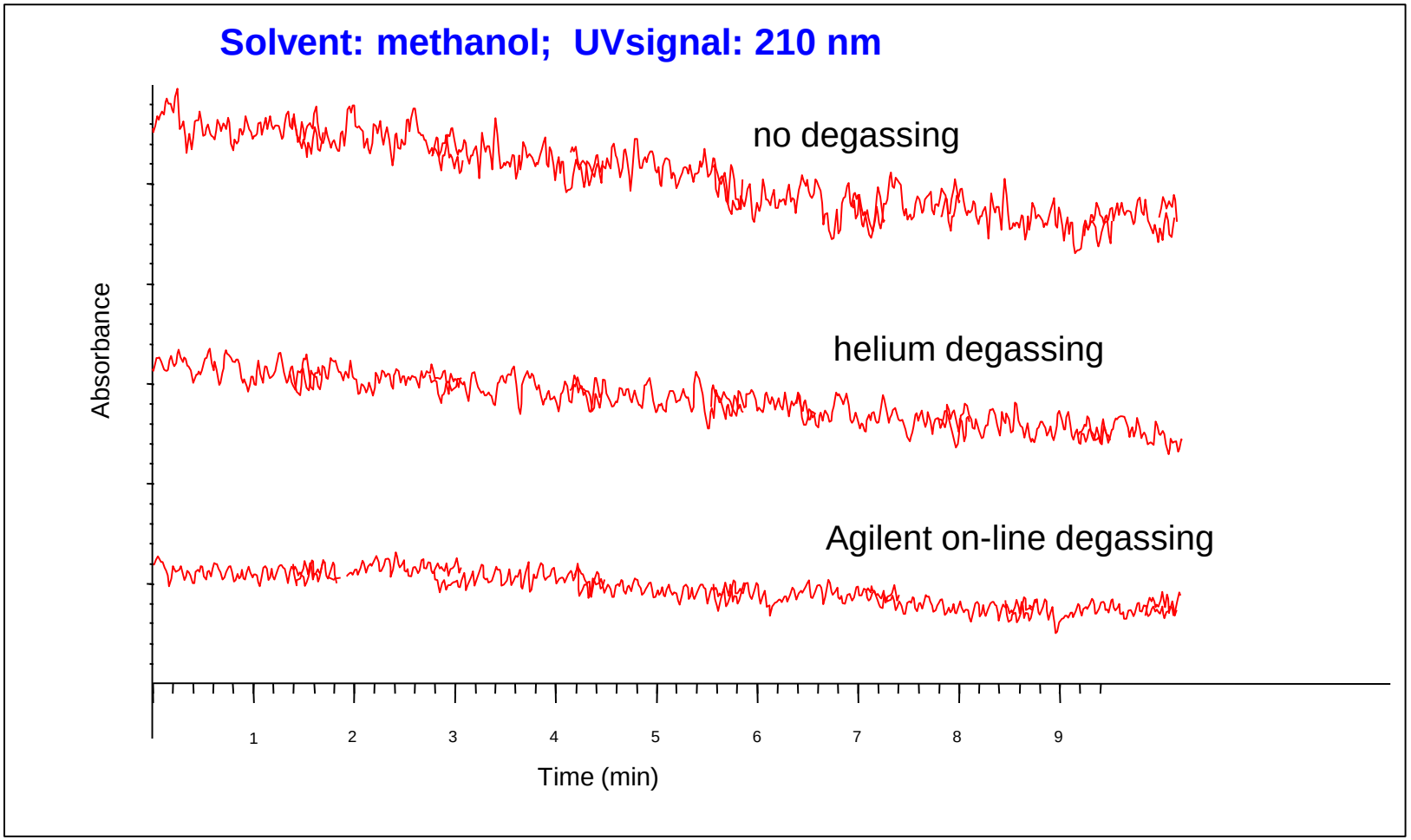


Priming and Purging the System

- The system can be primed either by drawing solvent through the degasser with a syringe or by pumping with the pump.
- Priming the system with a syringe is recommended, when:
  - vacuum degasser or connected tubings are used for the first time or vacuum tubes are empty or
  - changing to solvents that are immiscible with the solvent currently in the vacuum tubes.
- Priming the system by using the pump at high flow rate (3–5 ml/min) is recommended, when:
  - pumping system was turned off for a length of time (for example, overnight) and if volatile solvent mixtures are used, or
  - solvents have been changed.

Agilent 1200 Series On-line Degasser

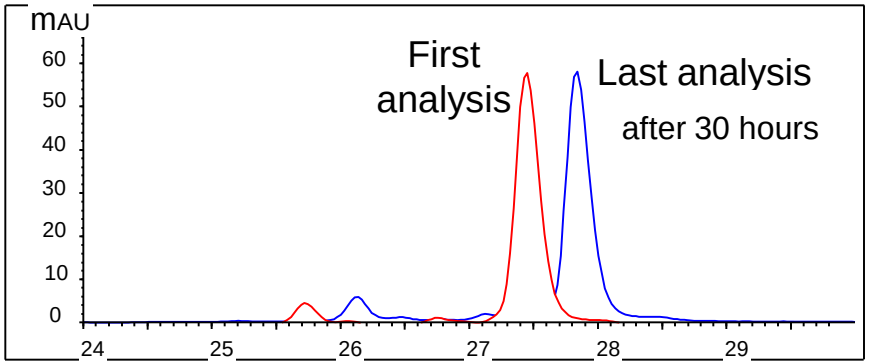
-Influence on Detector Baseline



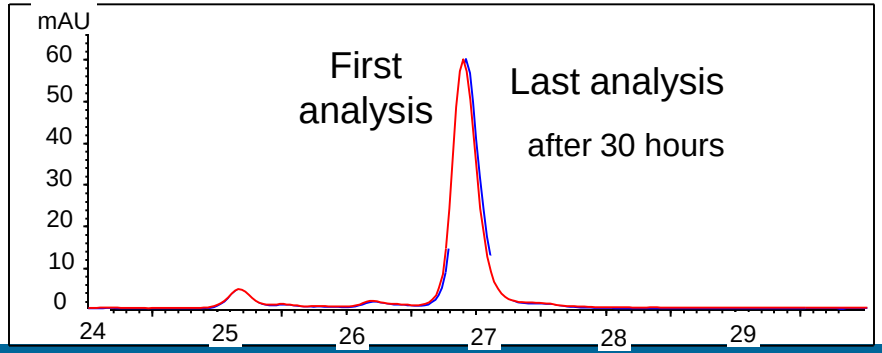
Agilent 1200 Series On-line Degasser

-Influence on Reproducibility

Lysozyme Analysis



Helium Degassing



On-line Vacuum Degassing

Pump

- Important characteristics
- Influence on...

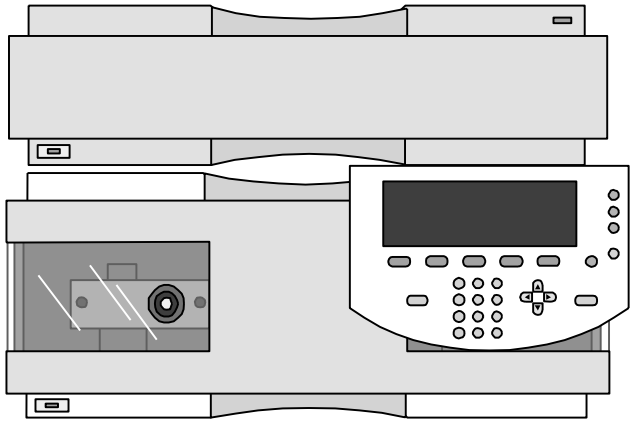
Common to isocratic and gradient pumps

Flow accuracy	Retention time and peak area precision (system to system)
Flow precision	Retention time and peak area precision (within one system)
Pressure pulsation	Baseline noise

Gradient pumps only

Delay volume in low and high pressure mixing	Gradient shape and precision
Composition accuracy	Retention time and peak area precision (system to system)
Composition precision	Retention time and peak area precision (within one system)

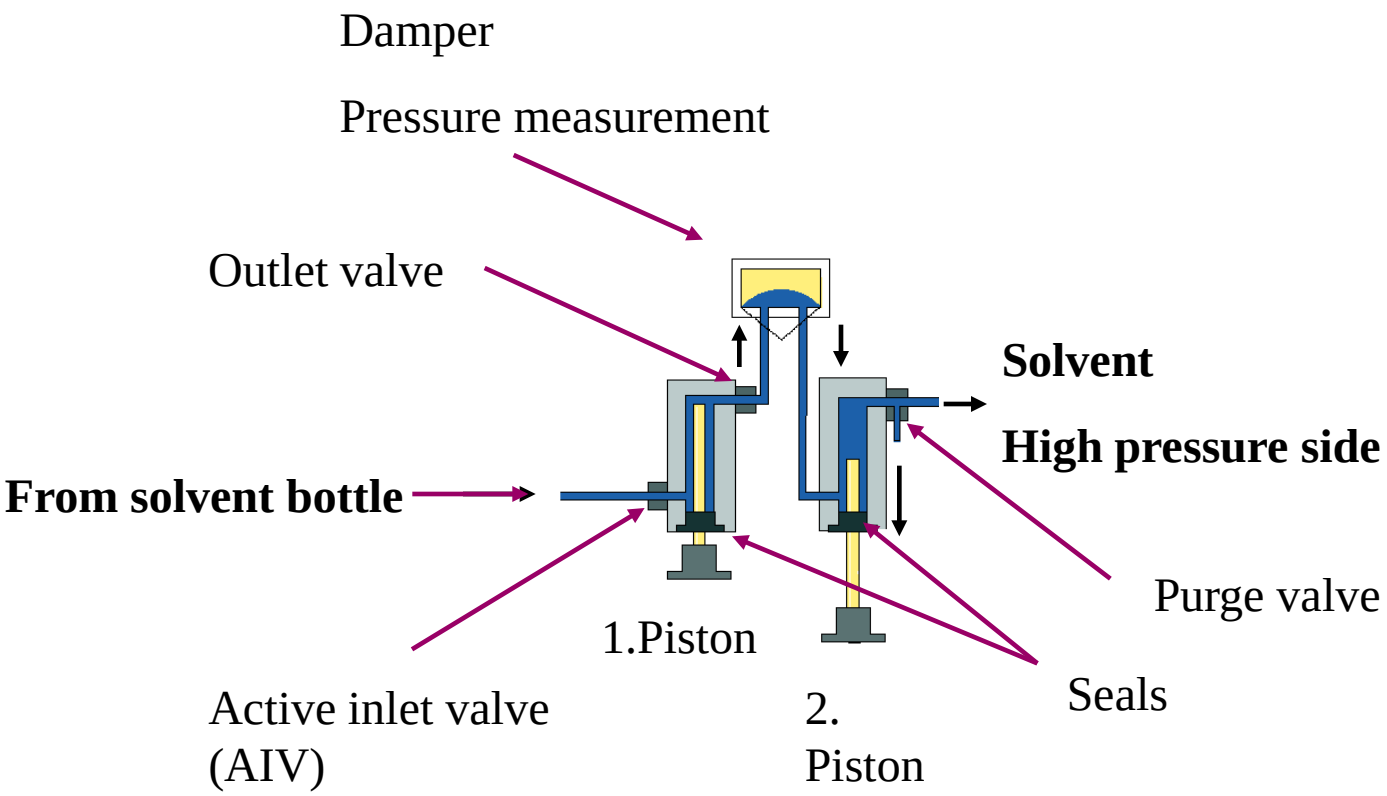
The Agilent 1260/1200/1100 Series Pumps



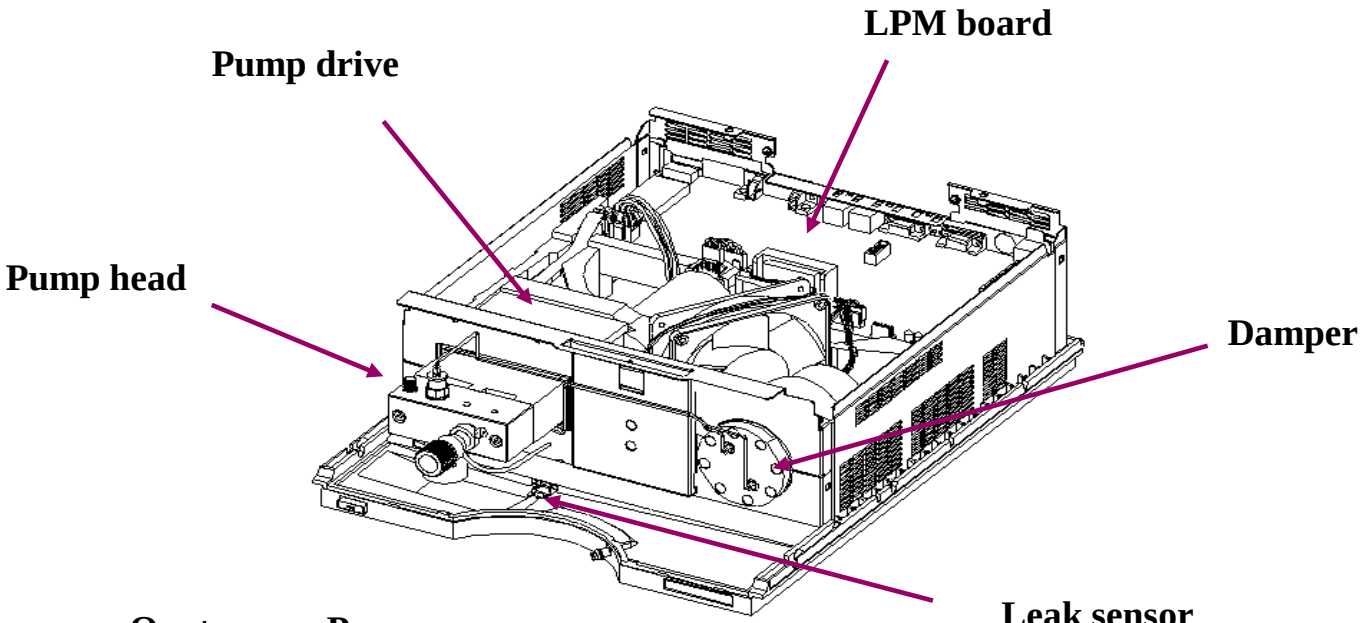
Common Features

- Complete with solvent bottle, filter, cabinet, purge valve and tubing's for fast start up
- Dual-piston, variable stroke volume, and pulse dampener for pulseless flow
- Automatic stroke volume adjustment for excellent mixing and noise-free baselines
- Improved valves for longer life and lower replacement cost
- Optional seal wash for trouble-free operation with high salt mobile phases
- Easy to maintain and repair

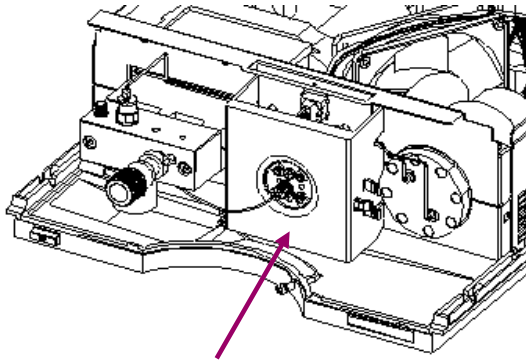
Operating Principle of the Dual Piston Pump



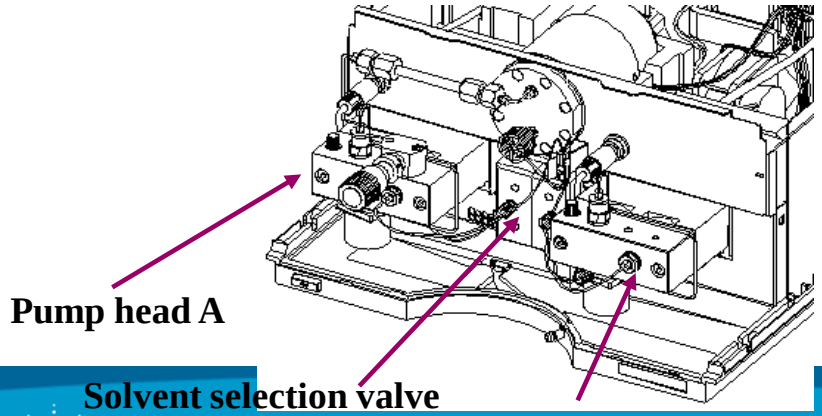
Pump - Main Components



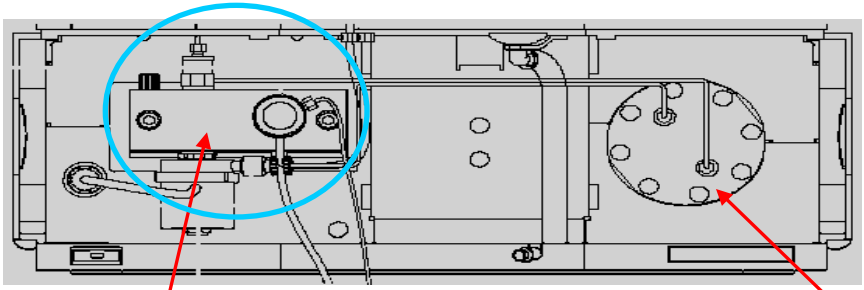
Quaternary Pump

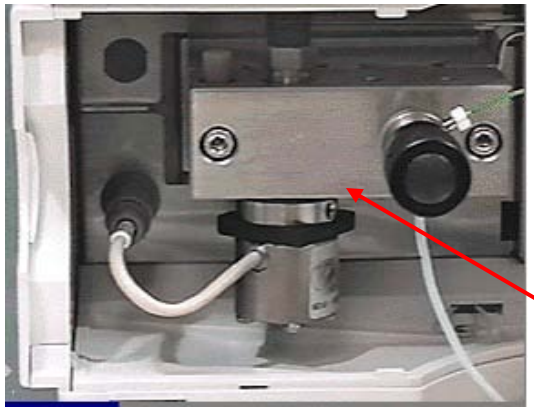


Binary Pump



Isocratic Pump



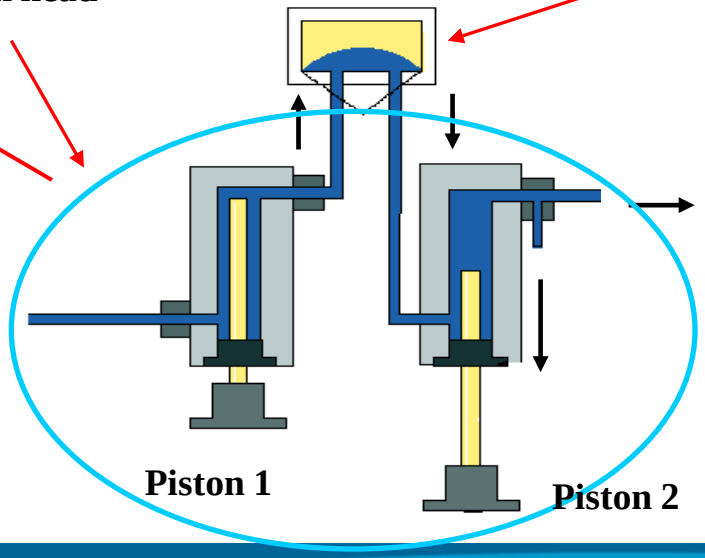


Piston head

Damper

(Pressure measurement)

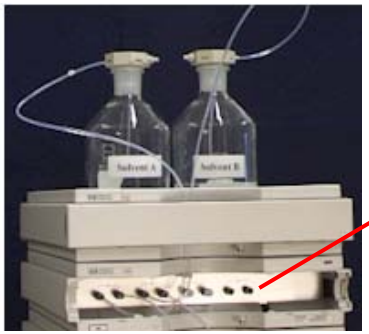
Working Principle



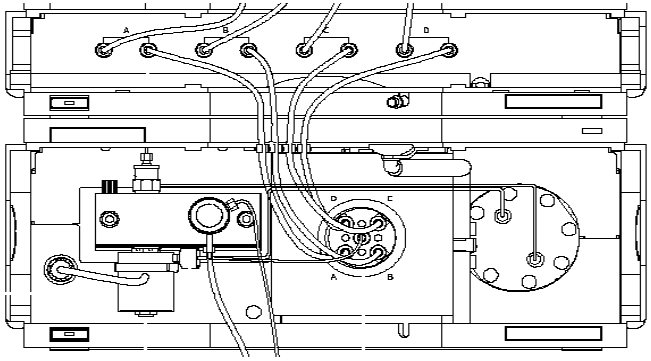
Maintenance areas:

- Seals,
- AIV,
- Outlet valve,
- Purge valve,
- Pistons.

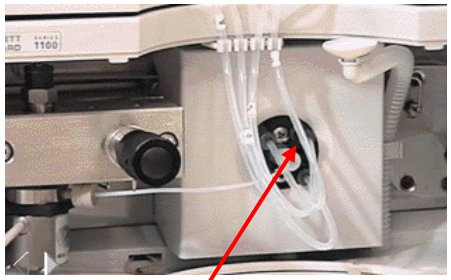
Quaternary Pump with Vacuum Degasser



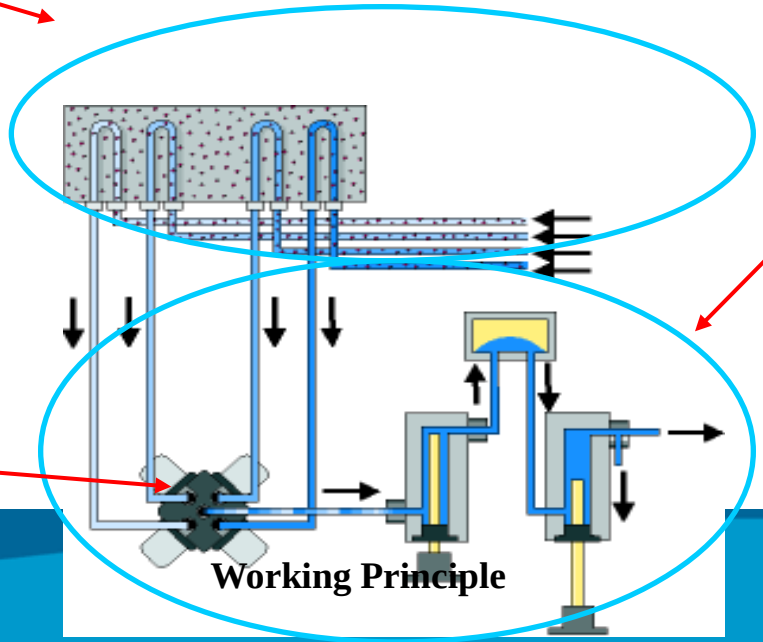
Vacuum degasser



Quaternary Pump



MCGV



Working Principle

Maintenance areas:

Seals,

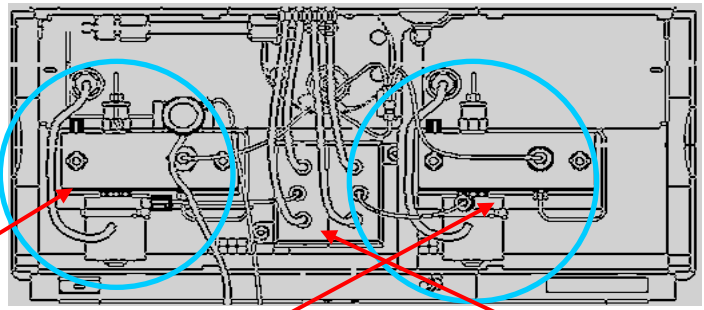
AIV,

Outlet valve,

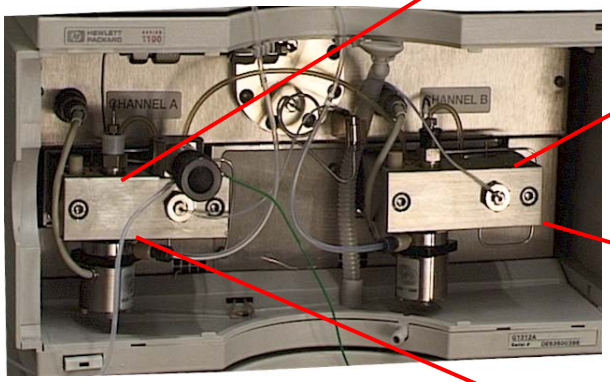
Purge valve,

Piston.

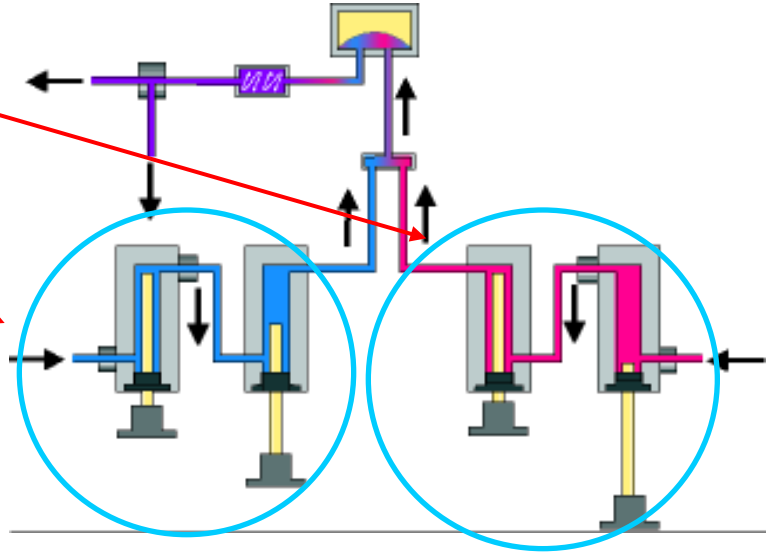
Binary Pump



Solvent Selection Valve (Option)



Pumpheads

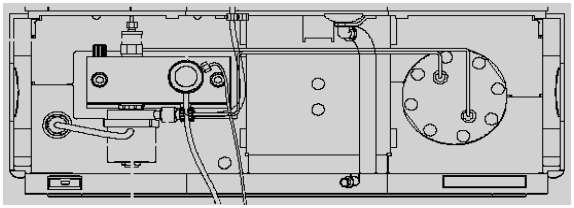


Maintenance areas:

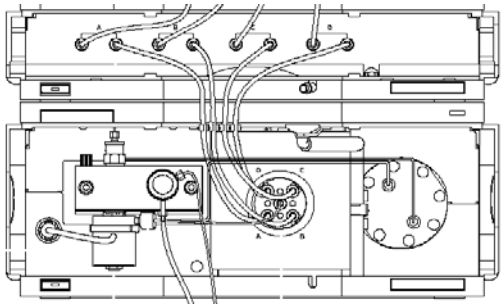
- Seals,
- AIV,
- Outlet valve,
- Purge valve,
- Piston.

Working Principle

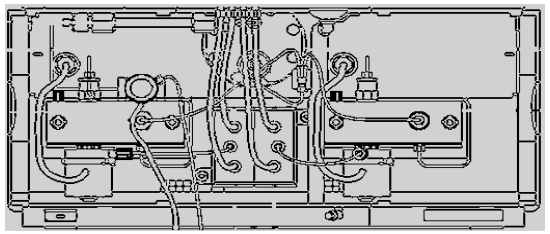
Pump Models for 1100 & 1200



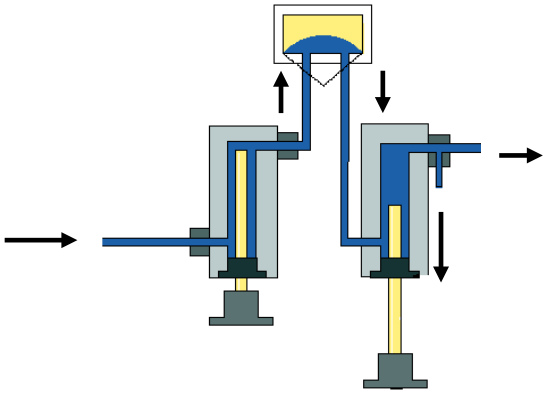
Isocratic Pump

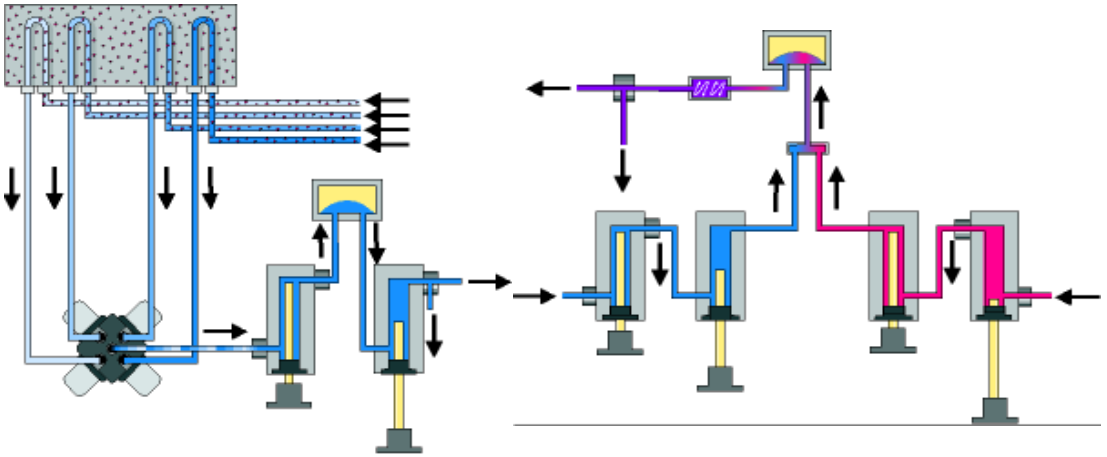


Quaternary Pump

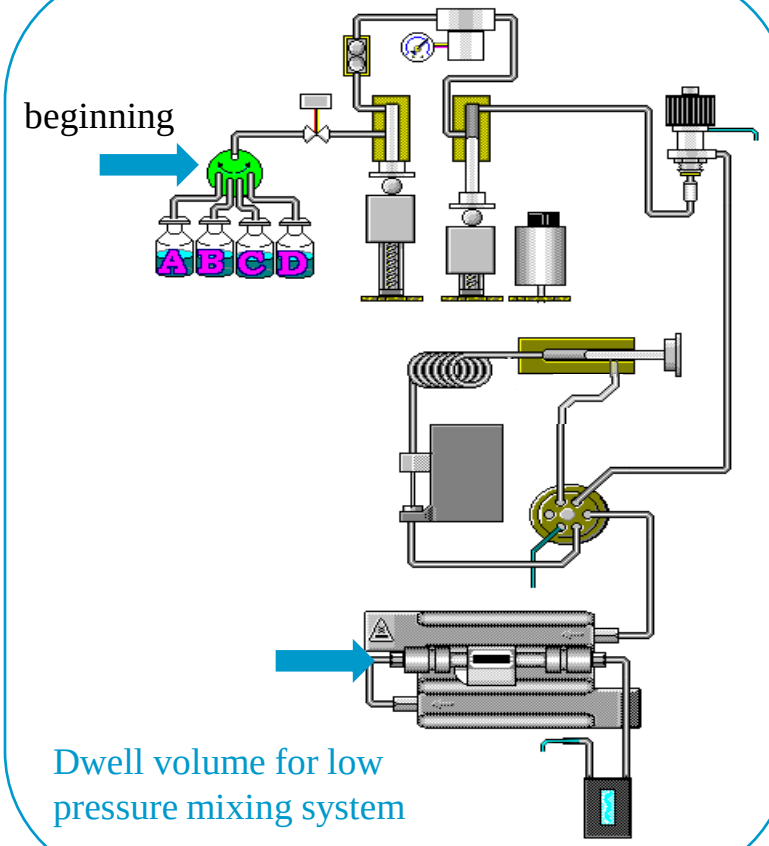


Binary Pump

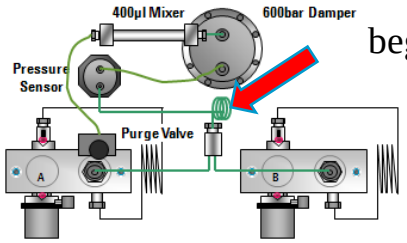




UHPLC Volume Effects

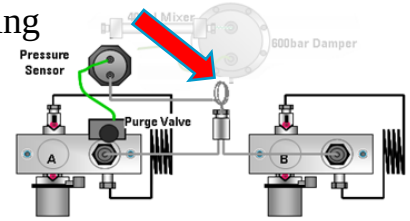


Dwell volume for low pressure mixing system

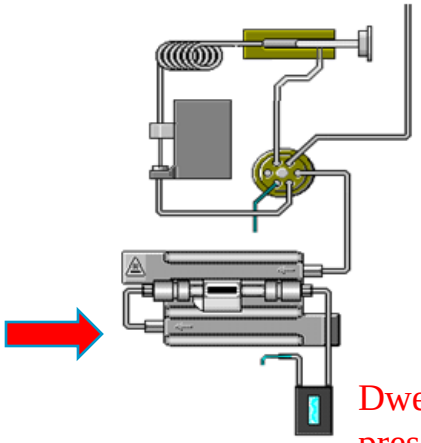


Standard delay mode

beginning



Low delay mode



Dwell volume for high pressure mixing system

- Dwell volume = volume from formation of gradient to the column
- Behaves as isocratic hold at the beginning of gradient.

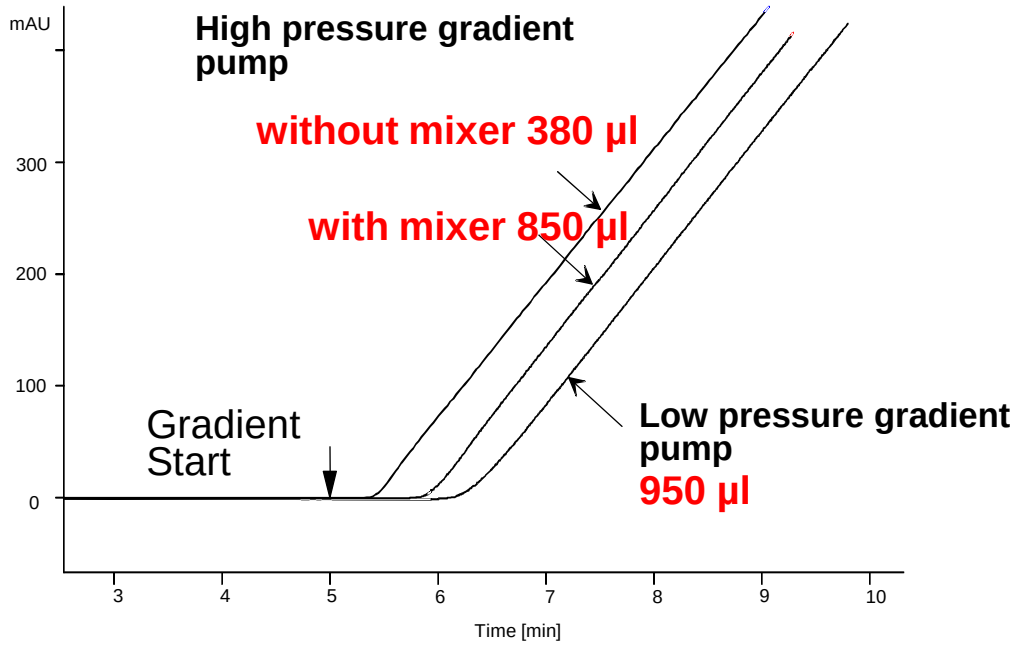
Comparison of System Delay Volumes

		1090	1050	1200 Quat.	1200 Bin.
Pump	w/o mixer	300-500	800-1200	800-1200	180-480
	w/ mixer	1050-1250	n/a	n/a	600-900
Mixer		750	n/a	n/a	420
		V (loop)	327 + V (inj)	300 + V (inj)	300 + V (inj)
Autosampler		N/A	8	6.2	6.2
Standard					
	Bypass	4.1 or 8.2	15 ul	3 or 6	3 or 6
		0	0	0	0
Column compartment standard					
	Bypass				
	min Range	304-504	1242-1442	1203-1406	189-489
	Max Range	1058-1258			906-1206

System Delay Volume with Low Pressure and High Pressure Gradient Pump

Agilent 1100 / 1200 Pumps

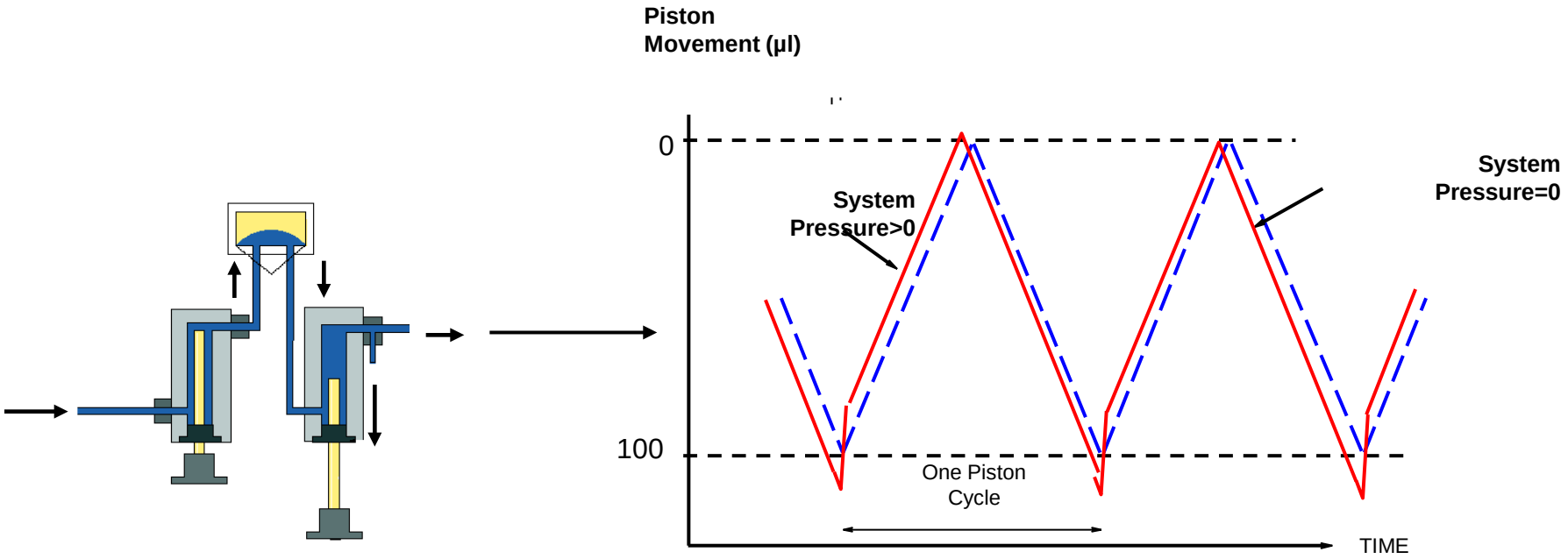
Performance



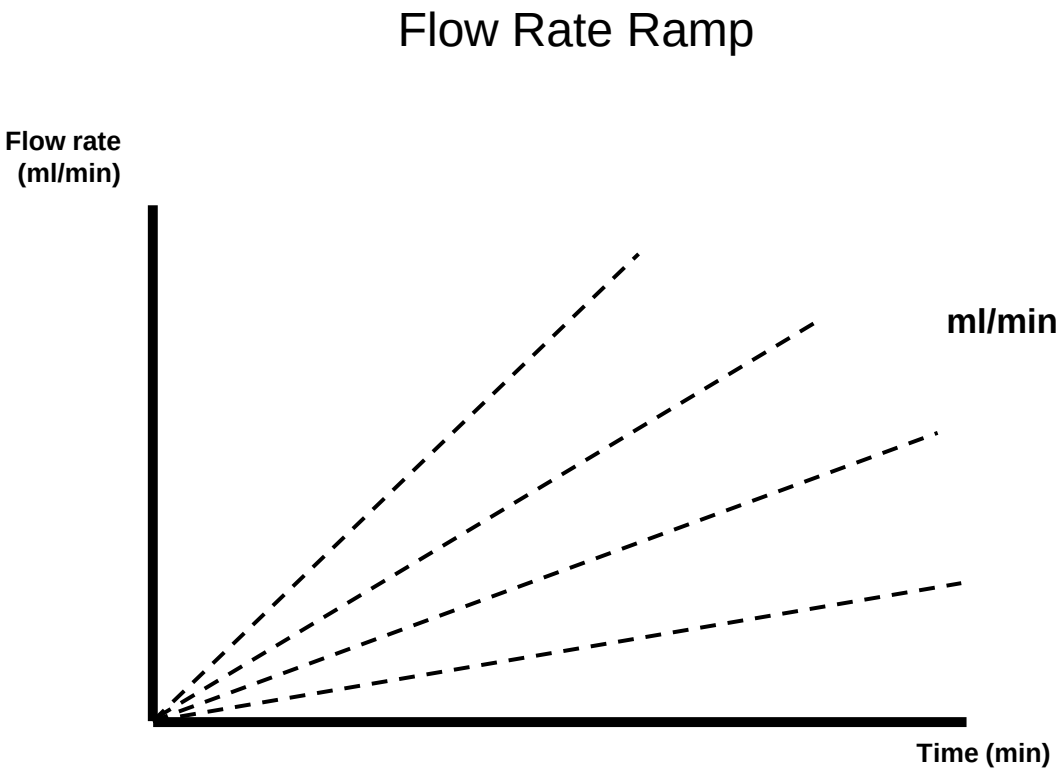
Solvent A: Water
Solvent B: Water+0.5% Acetone

Flow rate: 1.0 ml/min
Pressure: 130 bar

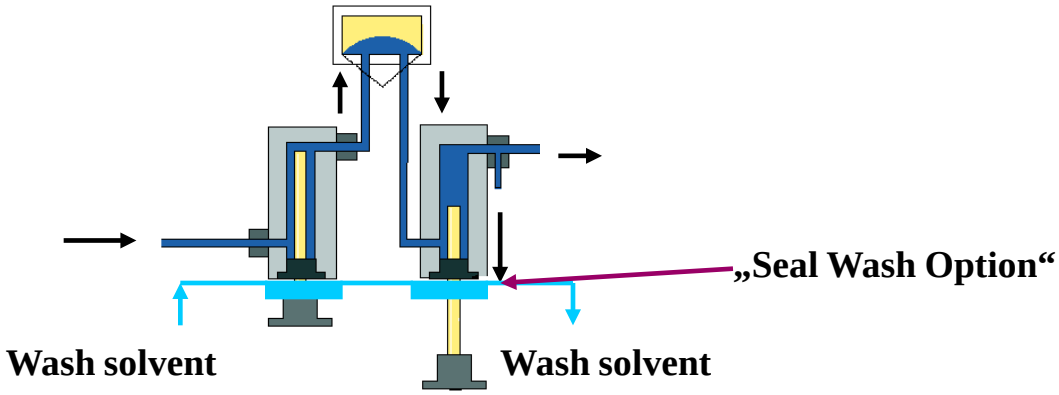
Solvent Compressibility Corrections



Column Protection: Soft Start Function

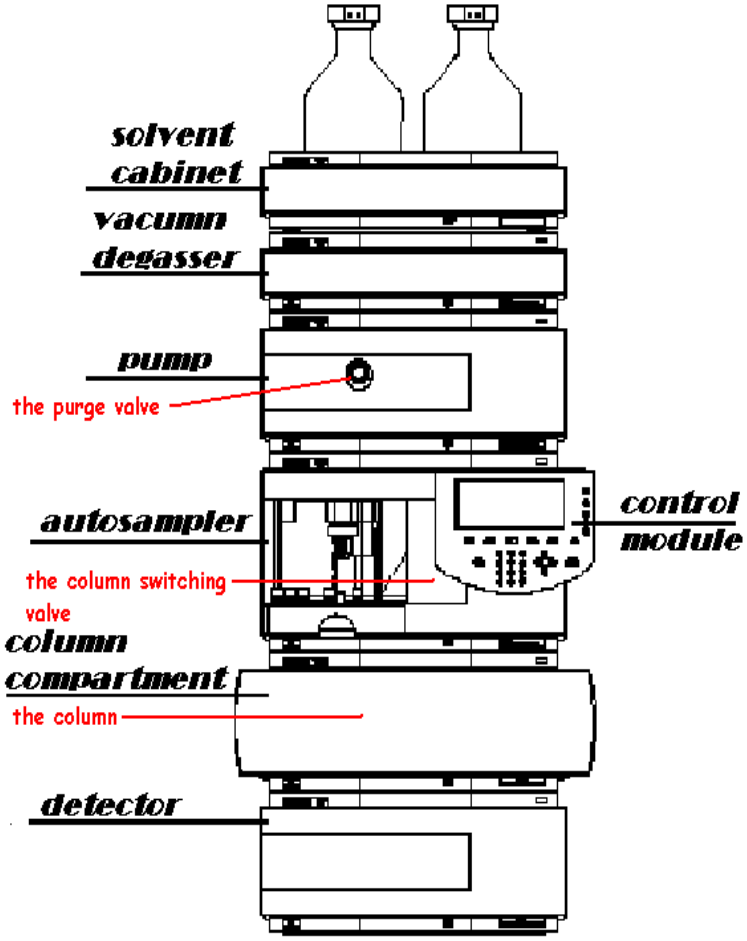


Seal Wash Option

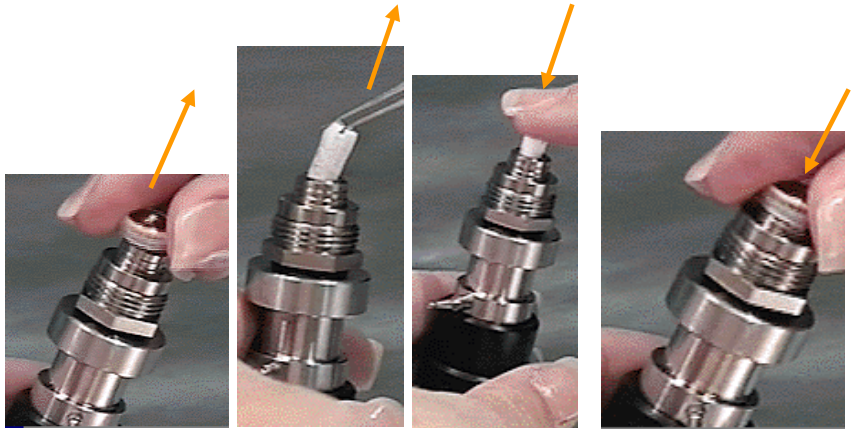


The *Seal Wash Option* can be installed to protect your pistons and seals from the routine use of highly concentrated aqueous buffer solutions (>0.01M)

The 3 point break

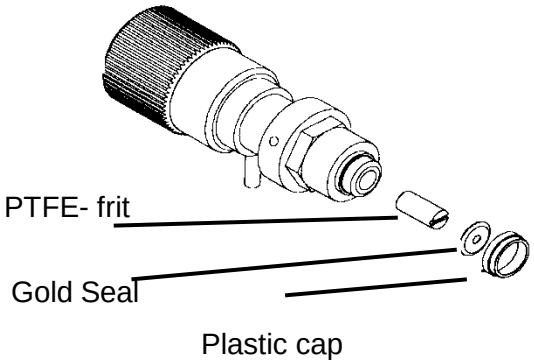


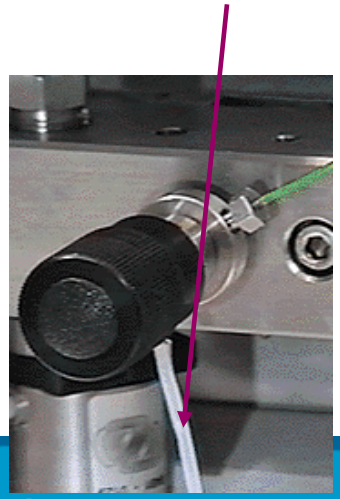
Purge Valve – Exchanging the PTFE Frit

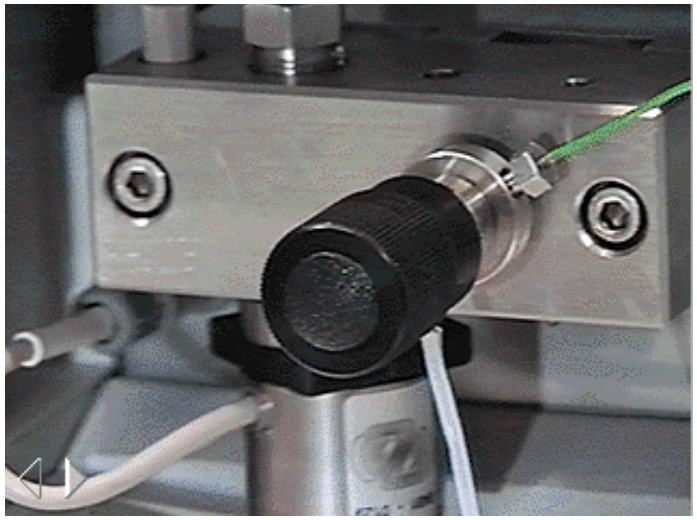


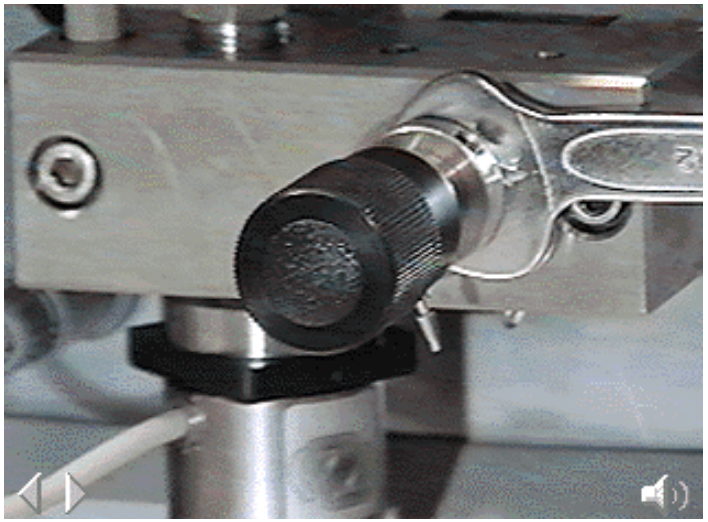
1. **Unscrew the valve using a 14 mm wrench**
2. **Remove the plastic cap and the gold seal**
3. **Take out the frit (tweezers)**
4. **Install a new frit**
5. **Replace the gold seal and the plastic cap**
6. **Install the valve**

Note: Realign the waste tube in the correct orientation during installation.







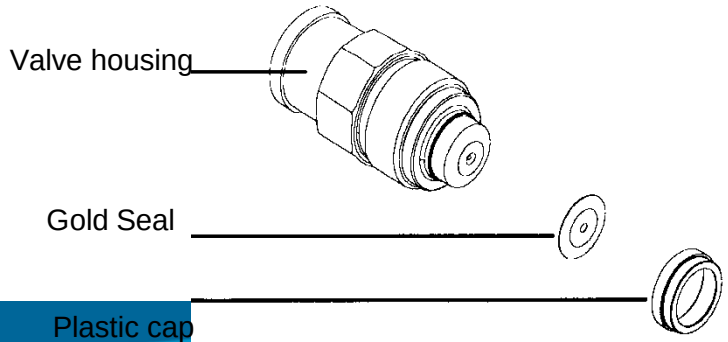


The Outlet Ball Valve

Outlet Valve – Cleaning or Exchanging the Outlet Ball Valve

1. **Remove the capillary from the valve**
2. **Remove the valve using a wrench**
3. **Clean the ball valve in the ultrasonic bath or replace the ball valve**
   a. **Take off the plastic cap and gold seal**
   b. **Replace the ball valve**
   c. **Replace the gold seal and cap**
4. **Reinstall**

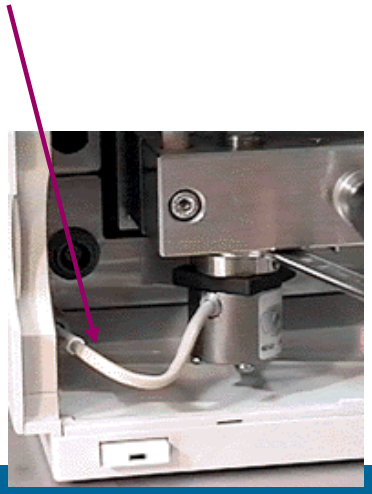
Note: The outlet ball valve of the binary pump has an additional sieve (**5063-6505**)



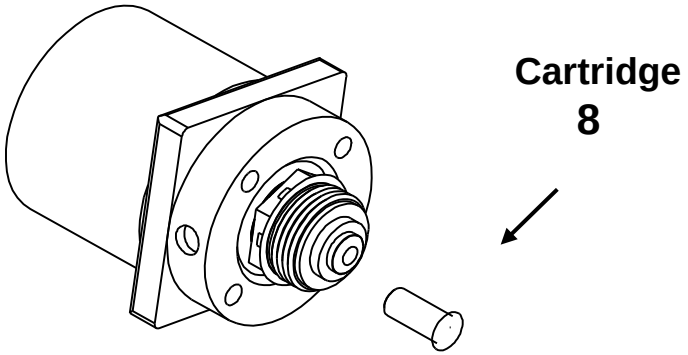
Active Inlet Valve (AIV) – Old/New

1. **Remove the AIV using a 14 mm wrench**
2. **Change the cartridge (new design)**
3. **Change the gold seals when necessary**
4. **Reinstall the AIV**

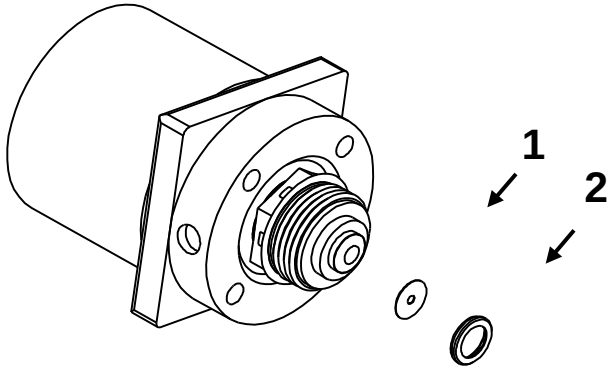
Note: Properly position the AIV cable when you reinstall the valve



New design

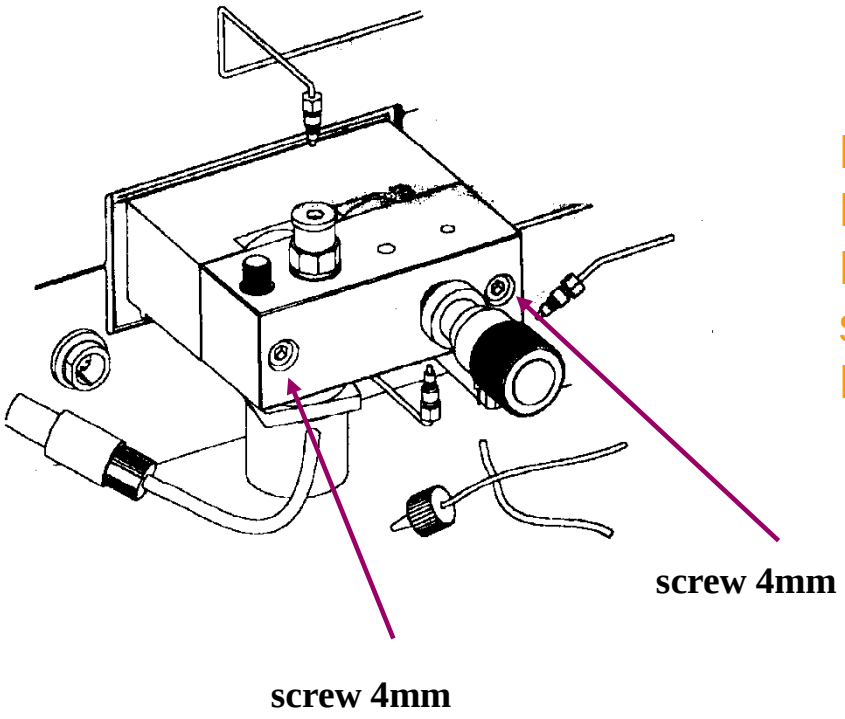


Old design



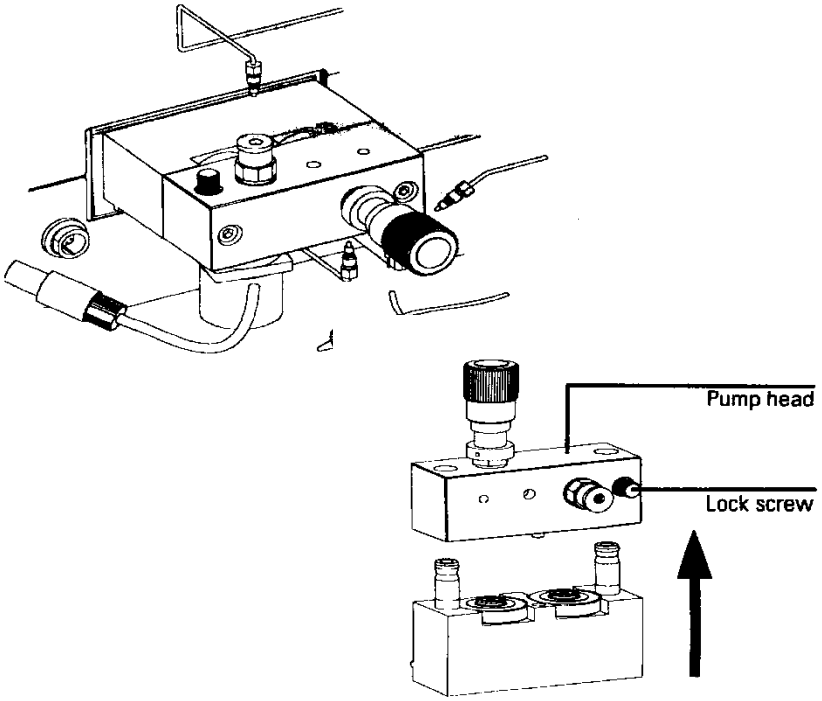
Active Inlet Valve (AIV)

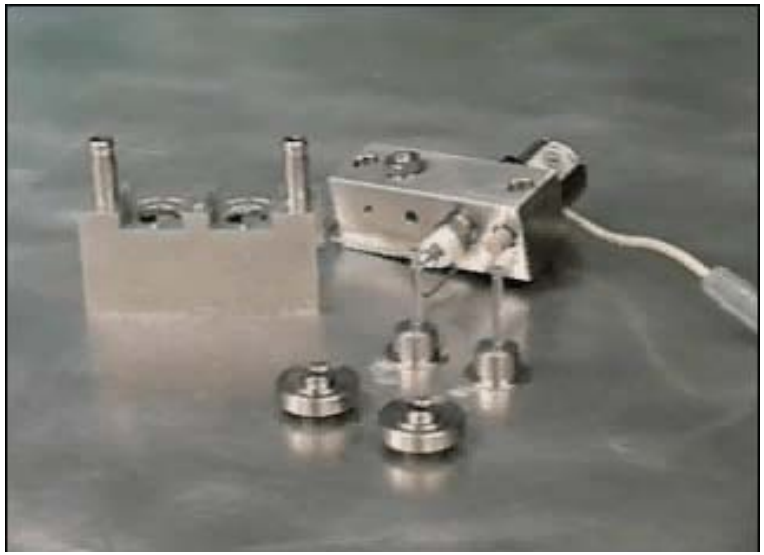
Removing the Pump Head



Remove all capillaries
Disconnect the AIV supply cable
Remove both pump head hexagonal screws
Remove the pump

Disassembling the Pump Head

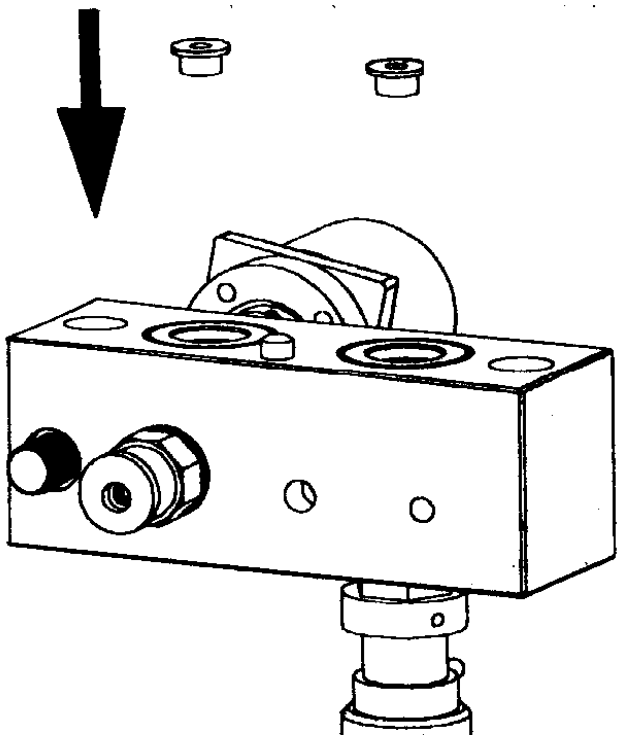




The two parts of the pump head are disconnected by releasing the lock screw.

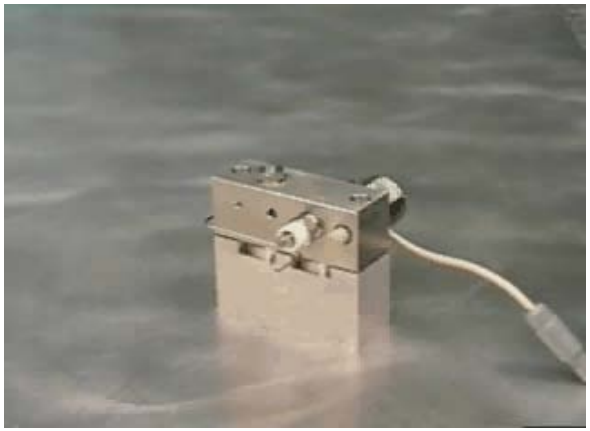
Changing the Pump Seals

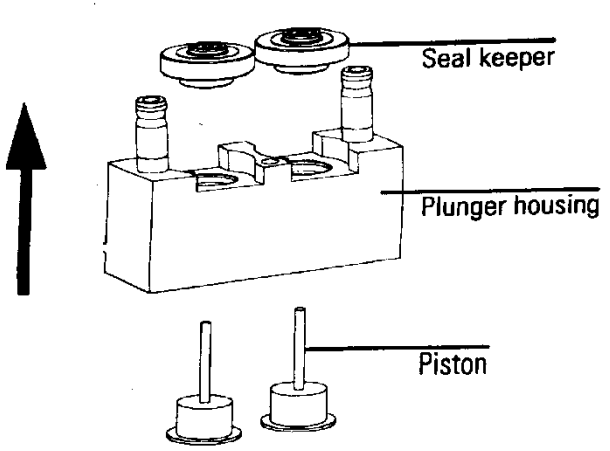
1. Remove the old seals.
2. Remove the wear retainers, if present.
3. Clean the pump chambers.
4. Insert new seals.
5. Reassemble the pump head.
6. Perform seal wear-in procedure for standard seals



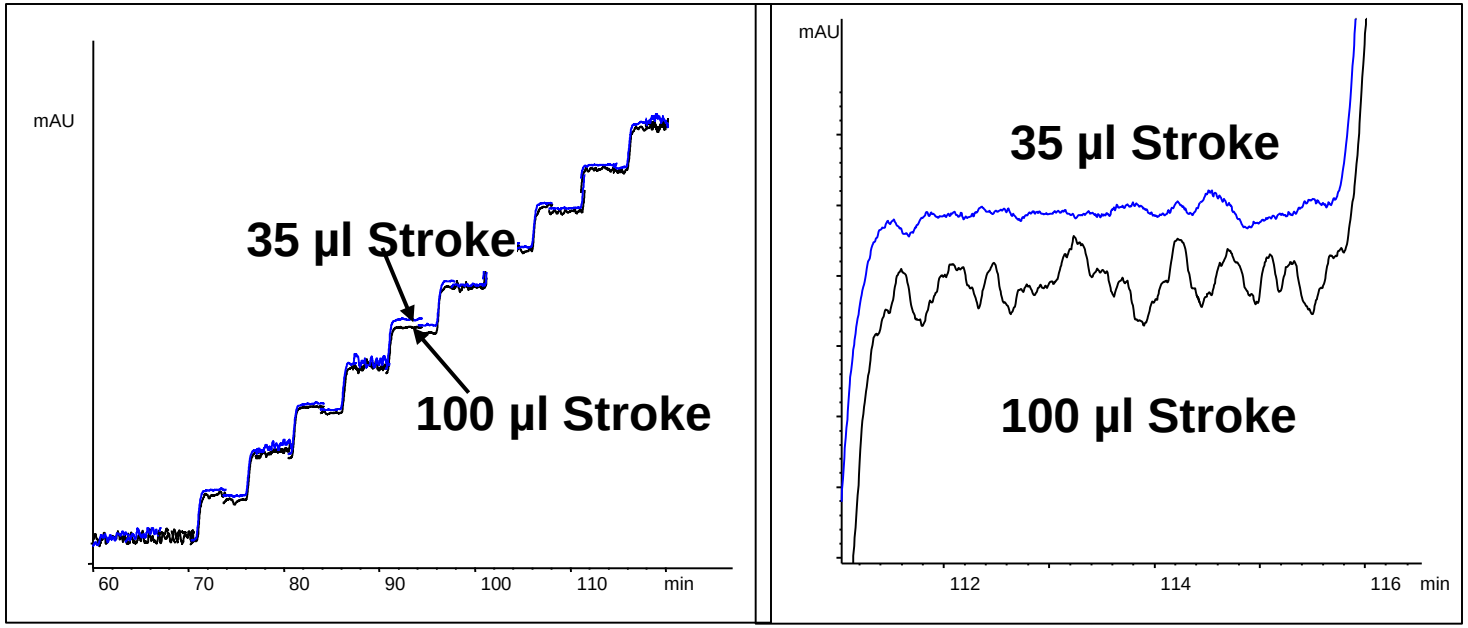
Changing and Inspecting the Sapphire Pistons

1. **Disassemble the pump head assembly**
2. **Check the plunger surface and remove any deposits or layers with alcohol or tooth paste**
3. **Replace the pistons if scratched**
4. **Reassemble the pump head**
5. **Check to make certain there are not any fractures in the springs**
6. **Put in the pistons**





Agilent 1200 Series Quaternary Pump: Variable Stroke Influence on Composition Ripple

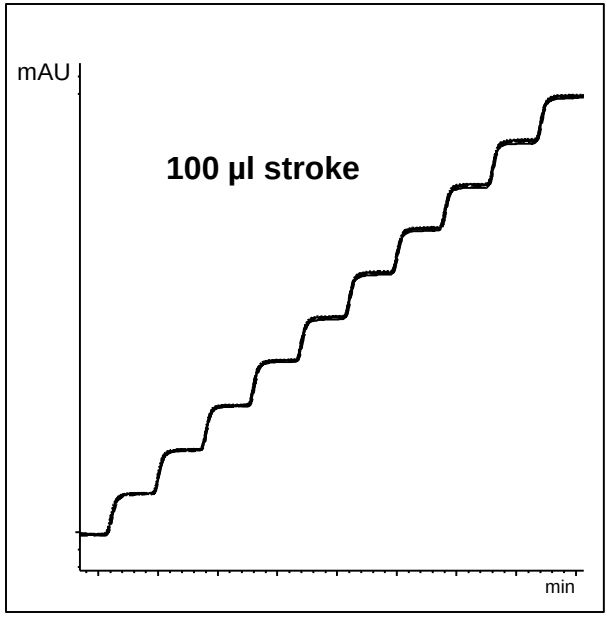


Tracer	**Water/Methanol +0.5% Acetone**
Flow rate	**1 ml/min**
Gradient	**55-65 %**

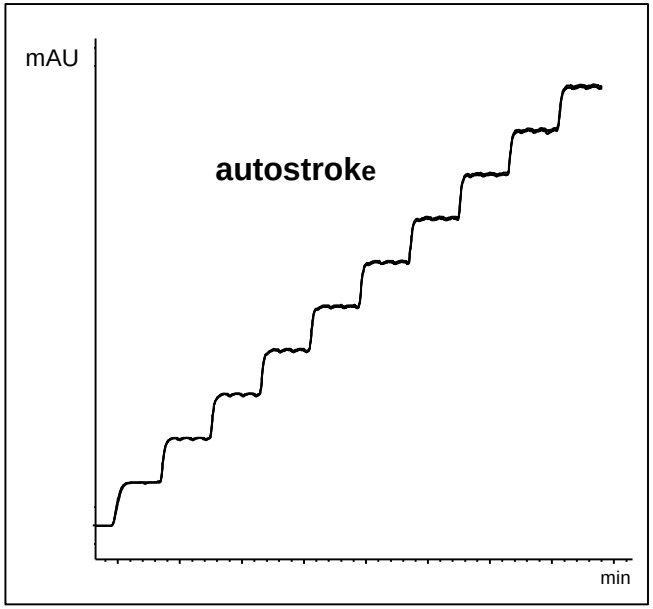
Agilent 1200 Series Pumps:

Gradient Performance 0-10%B

Quaternary Pump



Binary Pump



Tracer Water/Water
+0.5% Acetone

Flow rate 1 ml/min

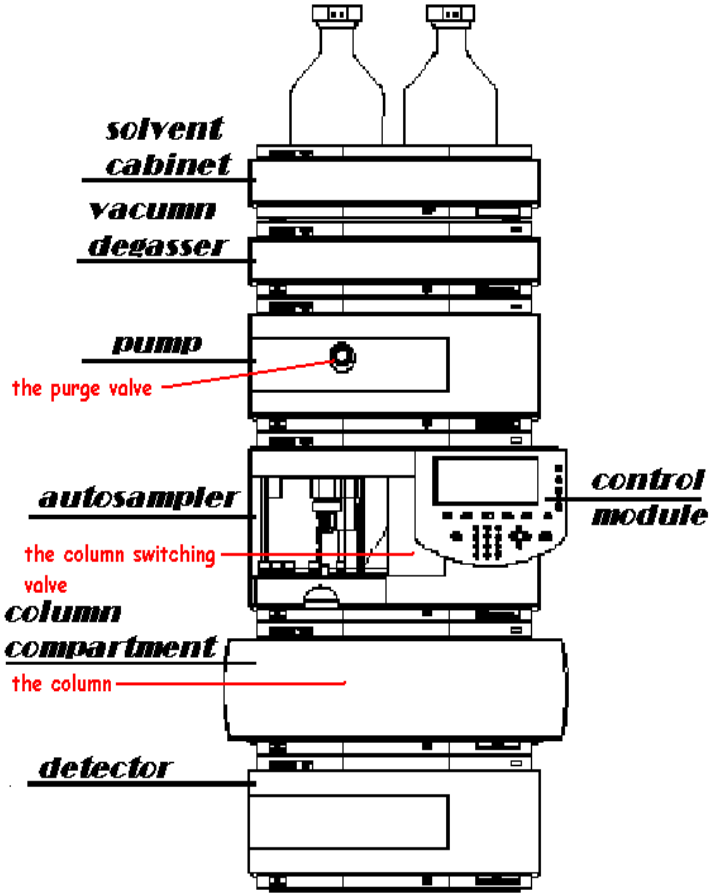
Gradient 0-10 %

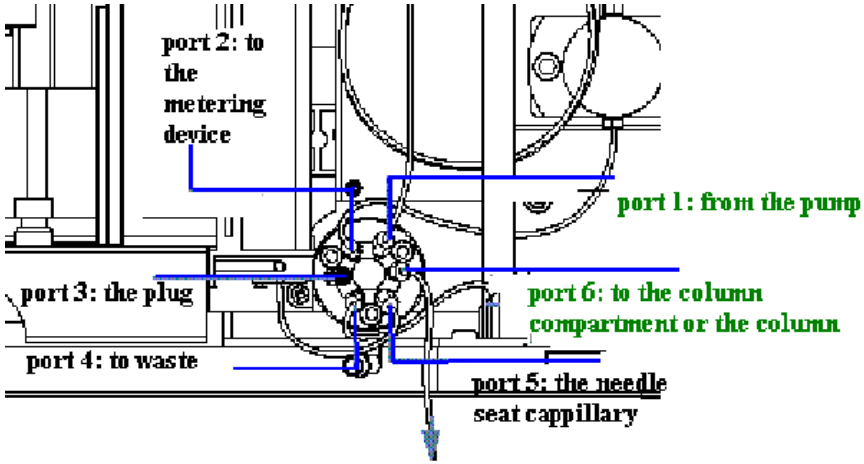
Injector

- **Important performance characteristics**
  - Injection volume precision
  - Wide linearity
  - Minimum carry over
  - Wide dynamic injection volume

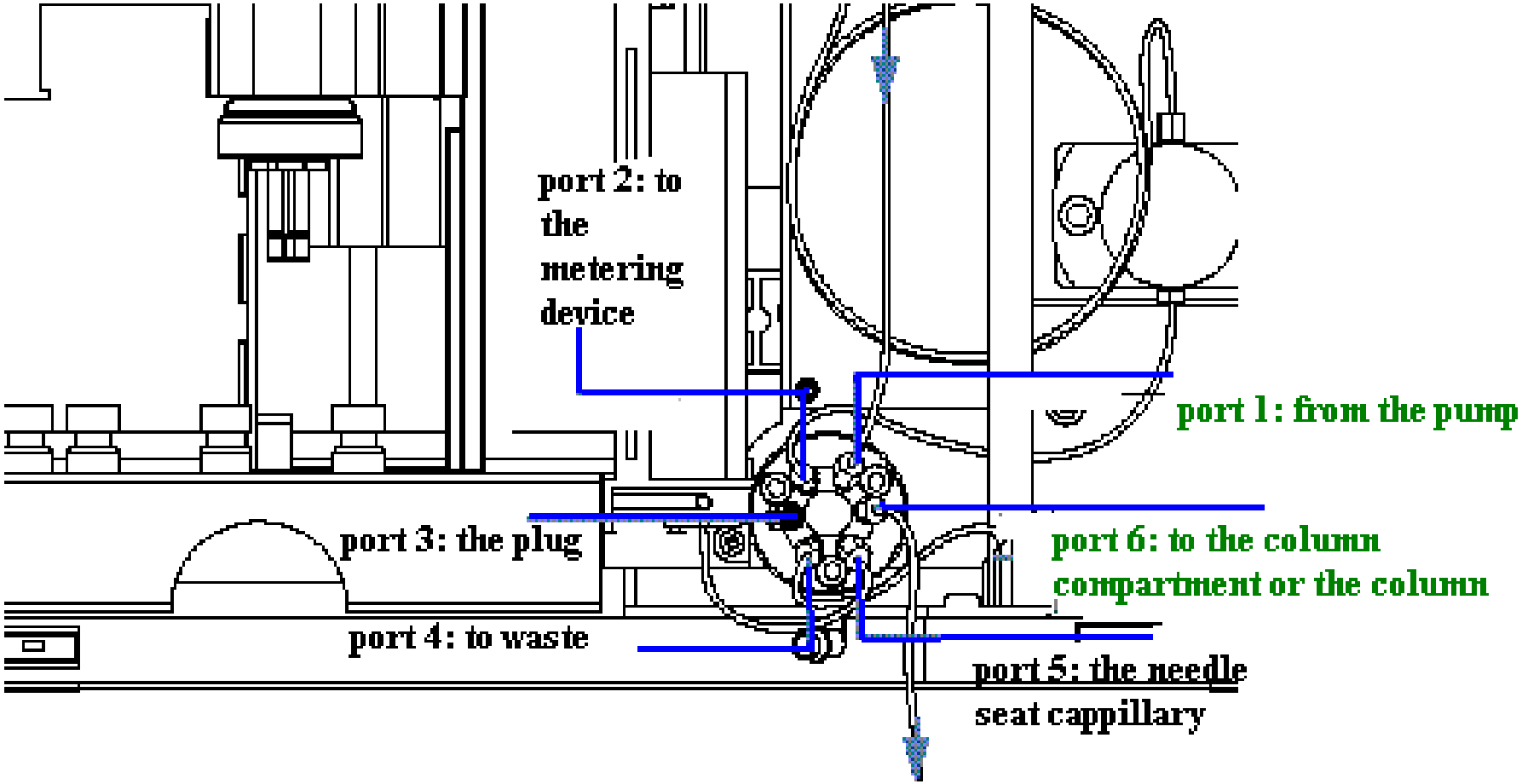
- **Influence on...**
  - Precision of peak area/height
  - Accuracy of peak area/height (when using different injection volumes)
  - Precision of peak area/height
  - Versatility, application range

The 3 point break



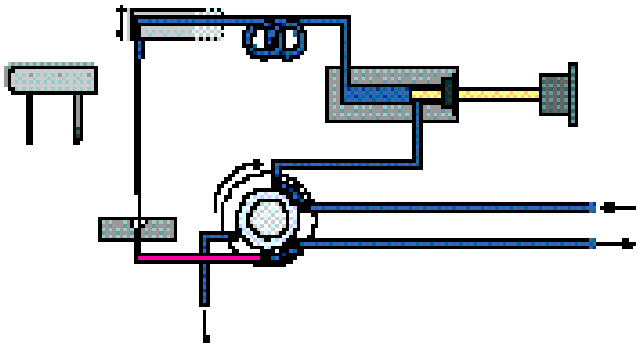


The autosampler connections

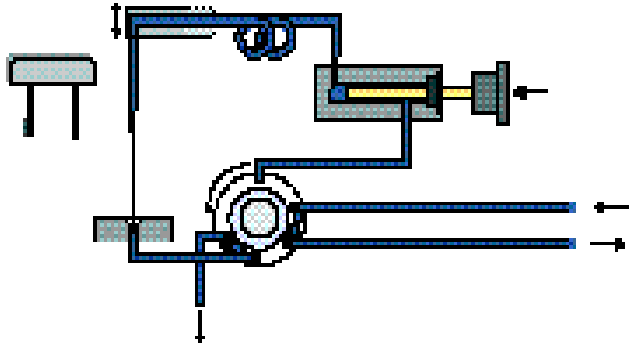


The injection sequence

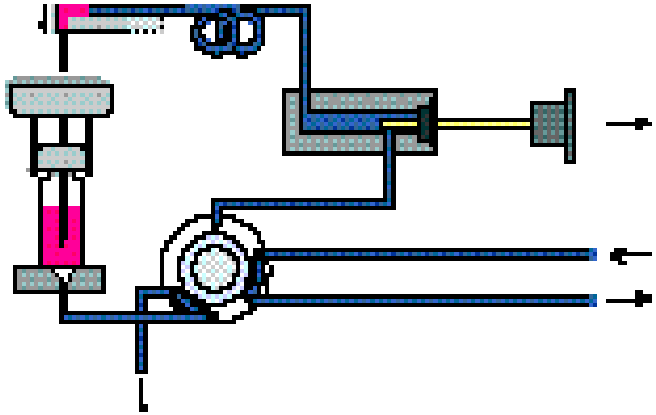
Mainpass Position



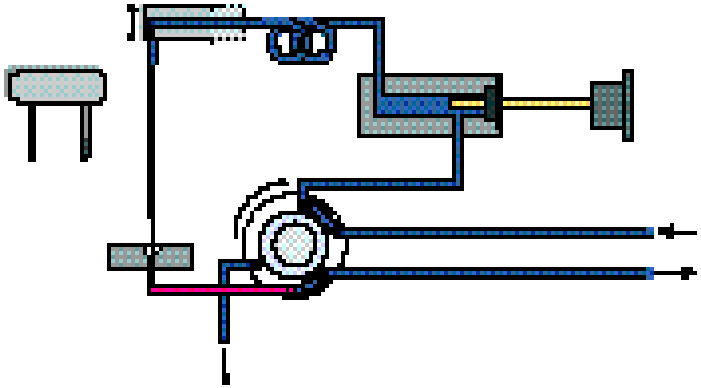
Bypass Position

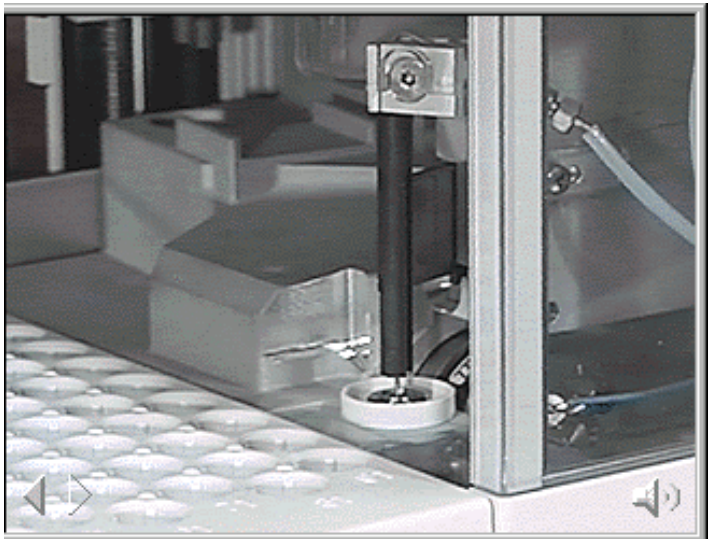


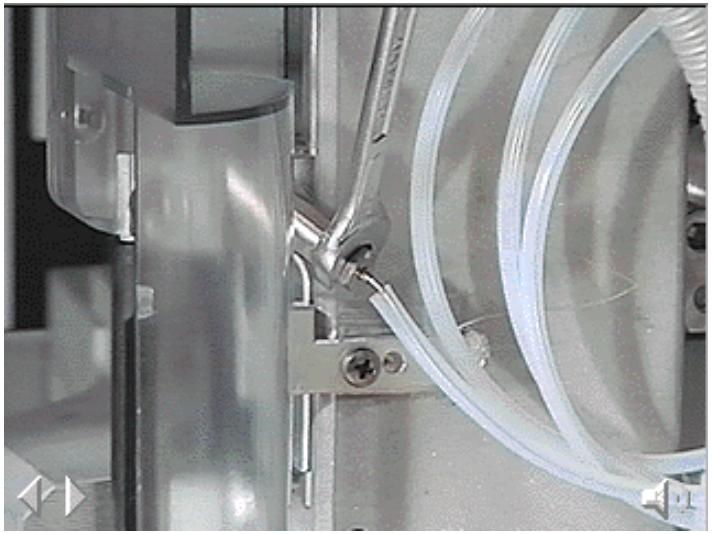
Drawing the Sample

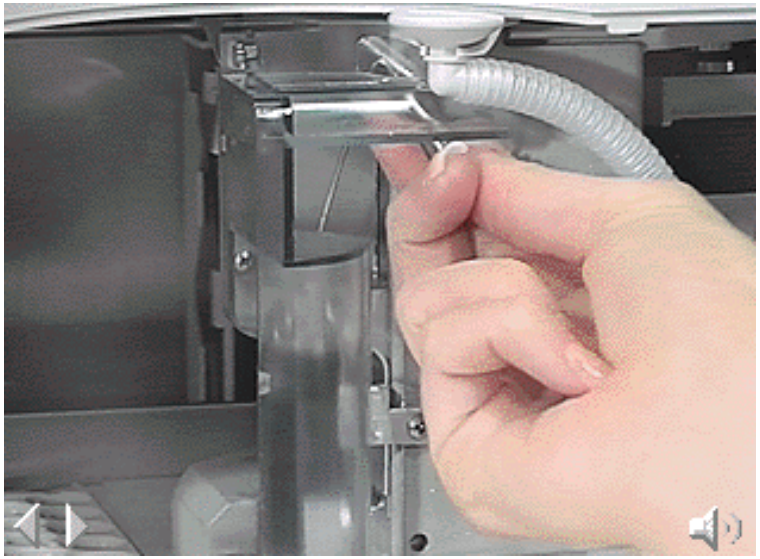


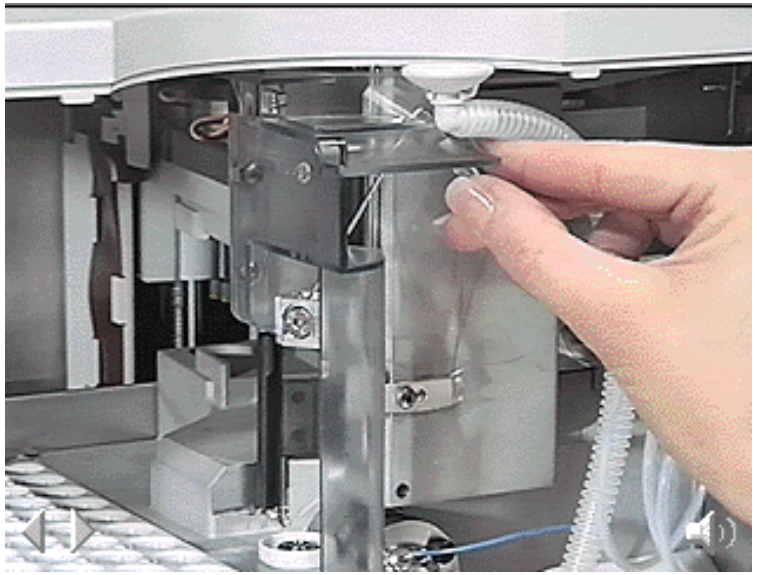
Mainpass Position (Sample Injection)

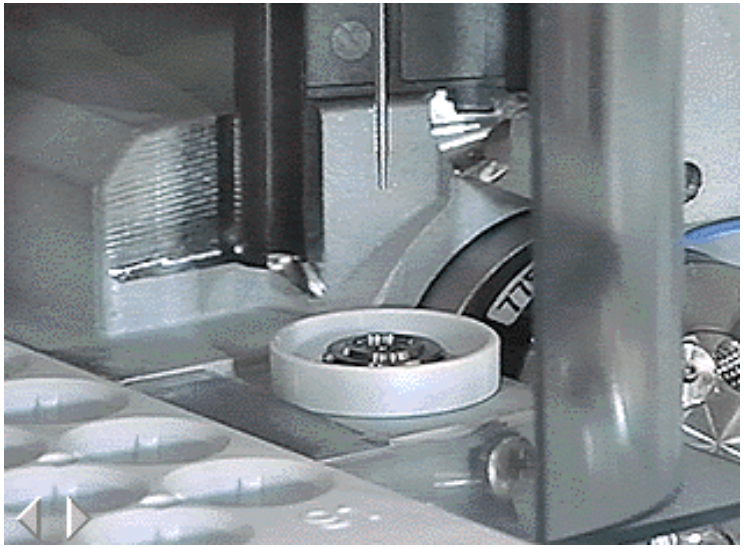


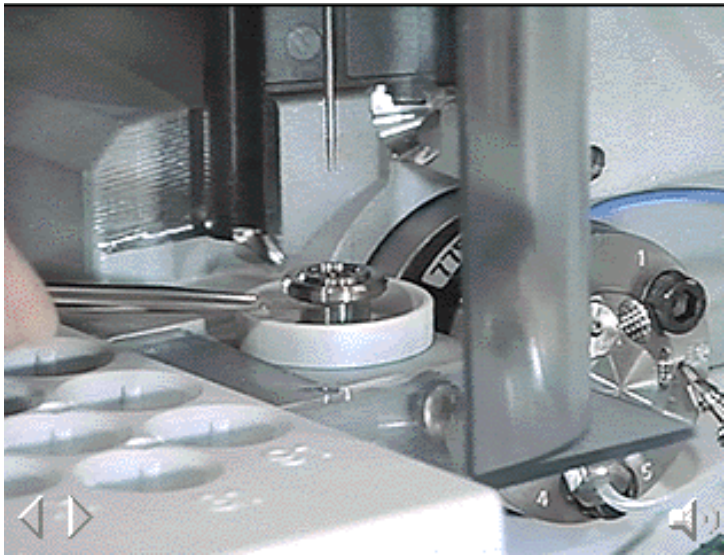






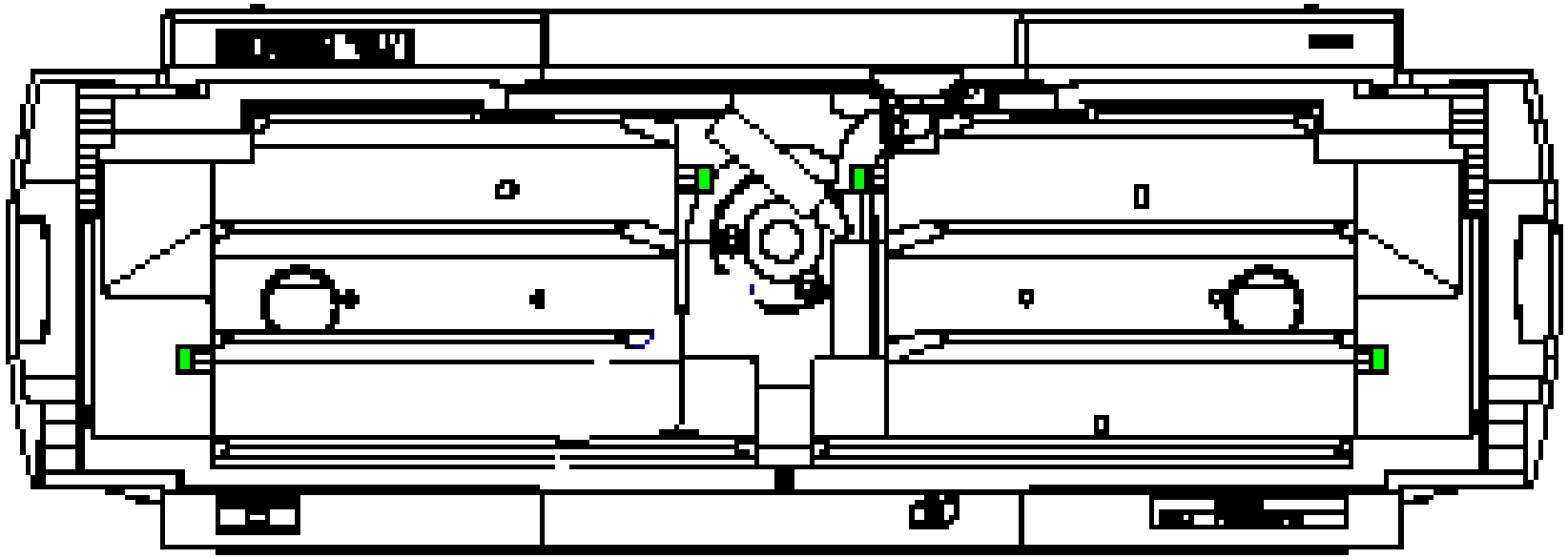


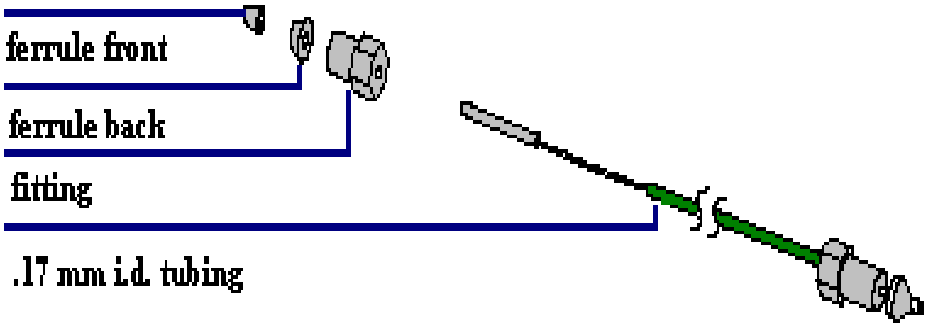




Thermostatted Column Compartment

Important performance characteristics	Influence on...
Excellent temperature accuracy	Elution order Peak identification
Excellent temperature precision	Elution order Retention time precision Peak identification



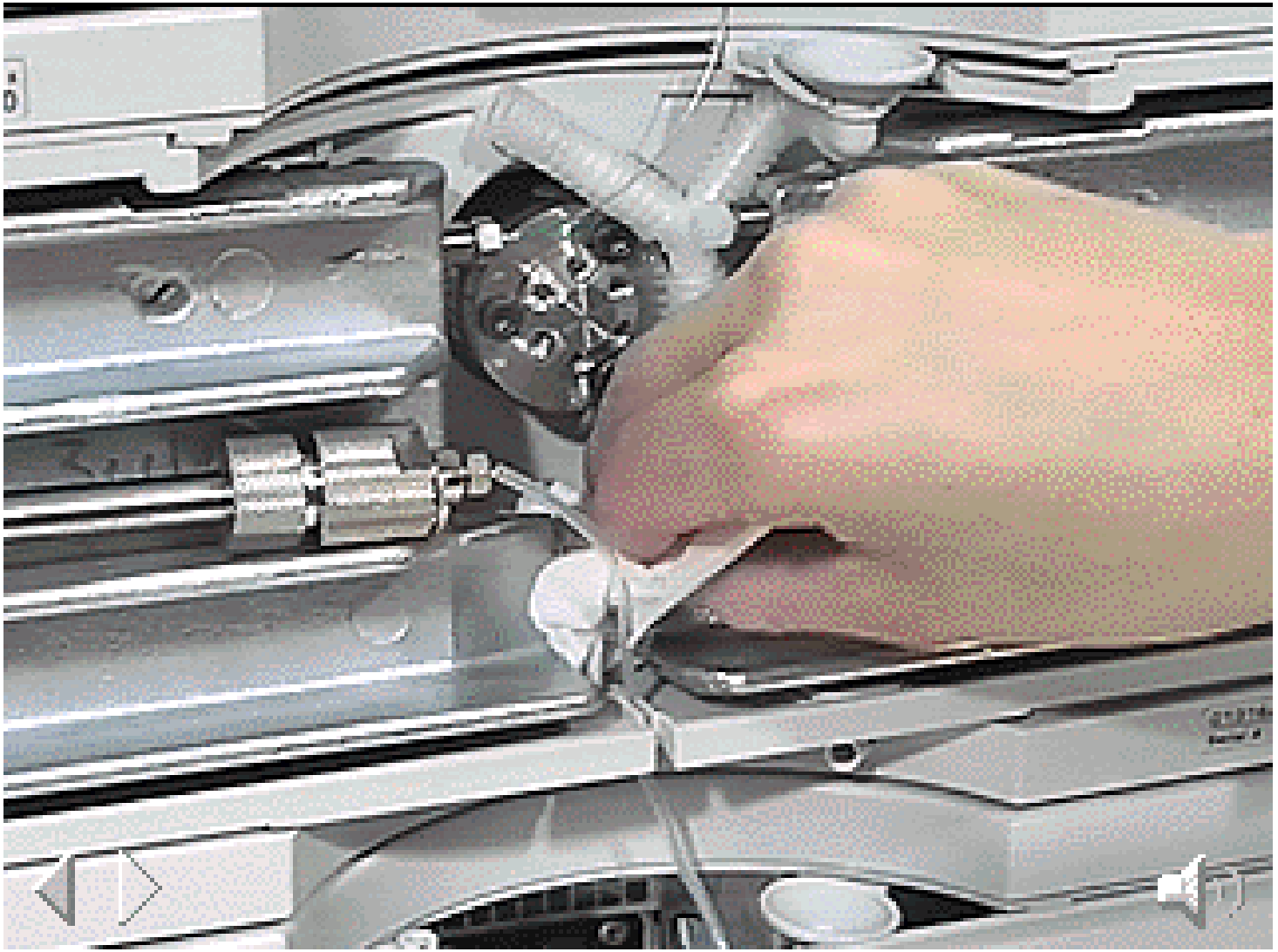


The column compartment is an optional module the output of the autosampler (port 6) is attached to the right side, the left side or the column switching valve.

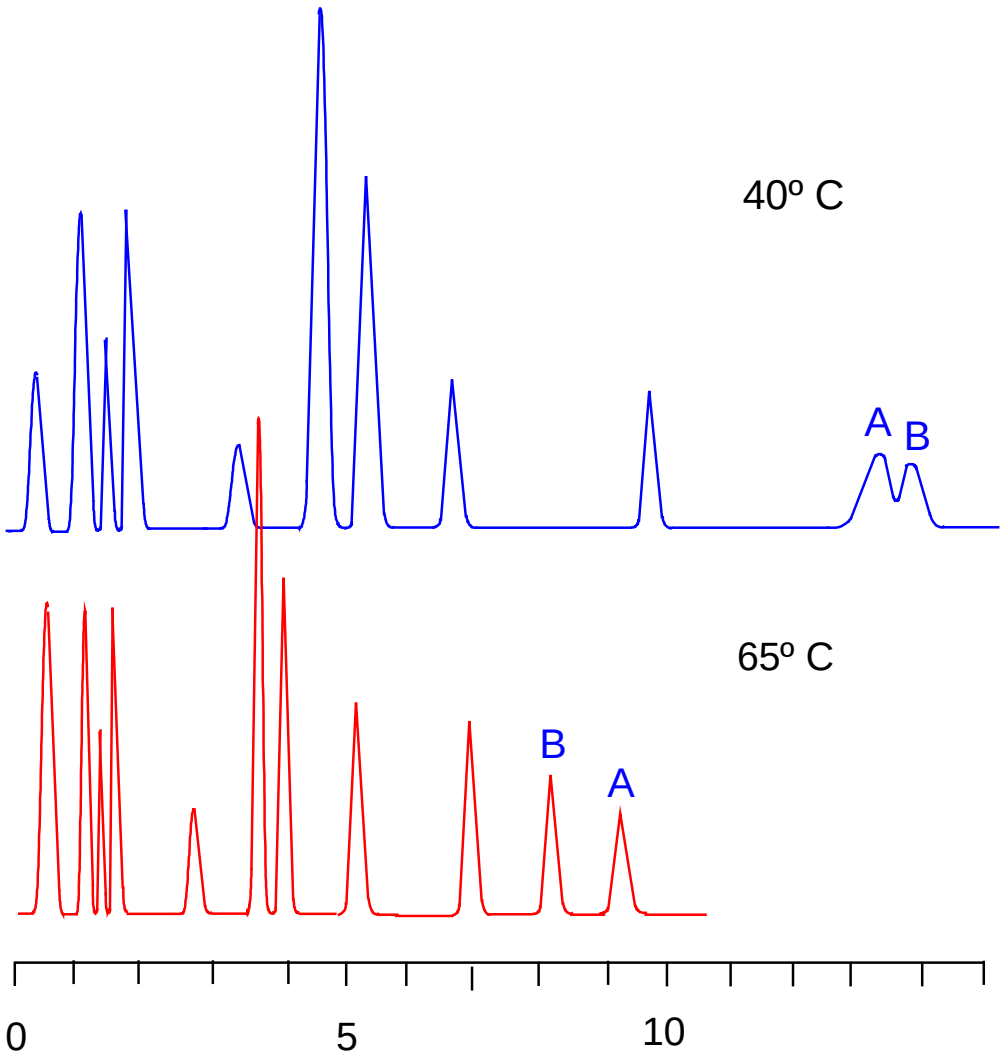
1200 Series TCC SL
Pre-column Heater and Post-column Cooler

- L-shaped pre-column heater
- Volume: 1.6 µl
- Mounted on carrier

- U-shaped Post-column cooler
- Volume: 1.5 ul
- Mounted on Carrier



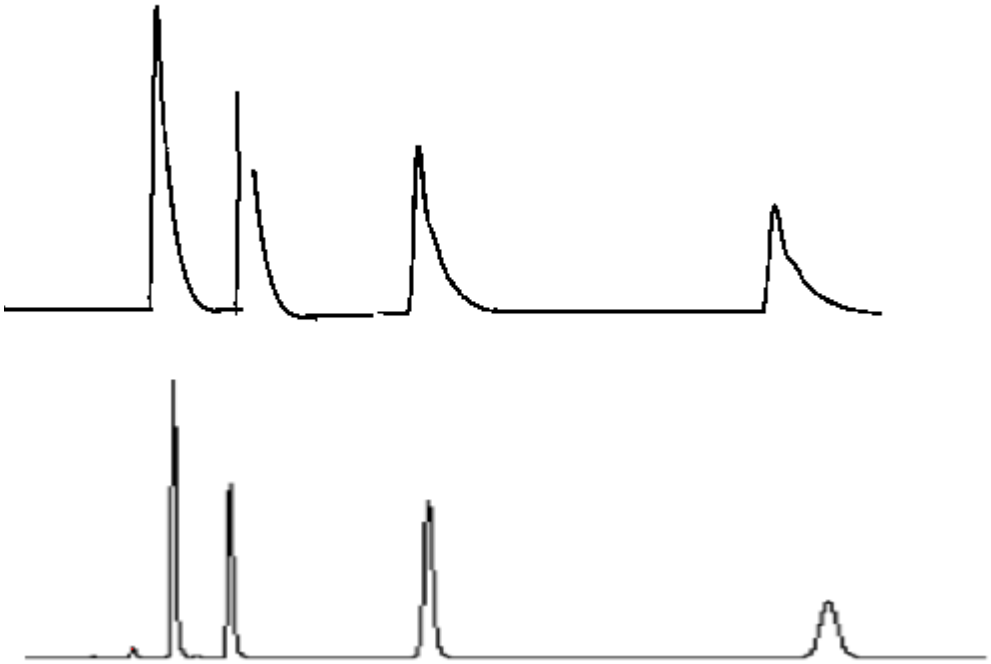
Effect of Temperature on Separation



Split Peaks

Can be caused by:

- Column contamination
- Partially plugged frit
- Column void
- Injection solvent effects



Peak Tailing, Broadening and Loss of Efficiency

May be caused by:

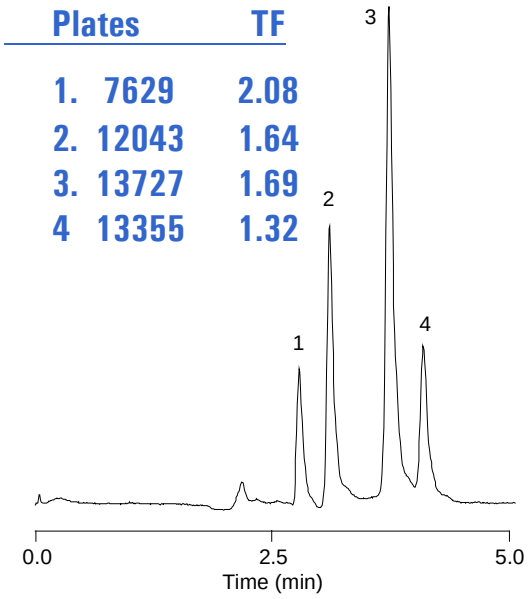
- Column “secondary interactions”
- Column void
- Column contamination
- Column aging
- Column loading
- Extra-column effects

Peak Tailing
Column Contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5µm Mobile Phase: 20% H_2O : 80% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 nm Sample: 1. Uracil 2. Phenol 3. 4-Chloronitrobenzene 4. Toluene

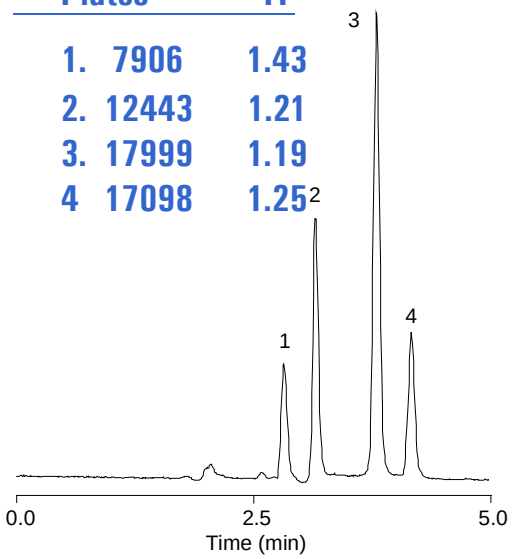
QC test forward direction

	Plates	TF
1.	7629	2.08
2.	12043	1.64
3.	13727	1.69
4	13355	1.32



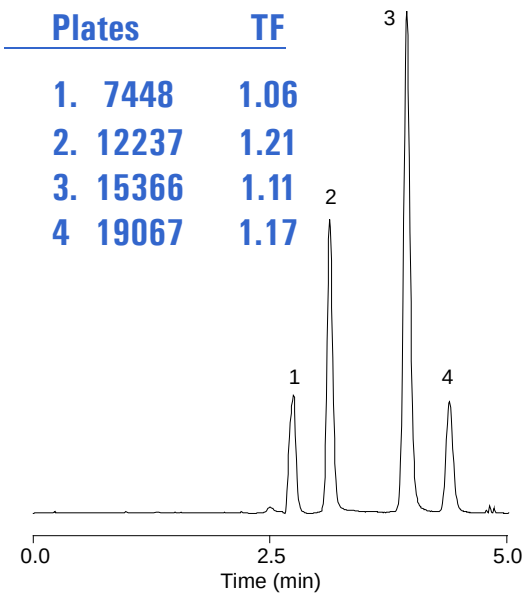
QC test reverse direction

	Plates	TF
1.	7906	1.43
2.	12443	1.21
3.	17999	1.19
4	17098	1.25



QC test after cleaning 100% IPA, 35°C

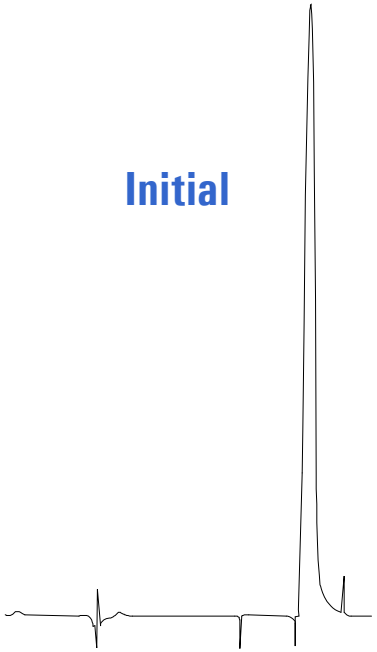
	Plates	TF
1.	7448	1.06
2.	12237	1.21
3.	15366	1.11
4	19067	1.17



Peak Broadening & Splitting

Mobile Phase: 50%ACN: 50% Water : 0.2% TEA
(~ pH 11)

Initial



After 30 injections

- Multiple peak shape changes can be caused by the same column problem. In this case a void resulted from silica dissolved at high pH.

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
Very good wavel. accuracy Excellent wavel. precision	Accuracy of peak areas/heights Precisons of peak areas/heights
Diode Array Detector only	Diode Array Detector only
High spectral resolution	Accuracy of spectra, peak identification by spectra
Excellent spectral sensitivity	Accuracy of spectra, peak identification by spectra at low concentrations

Flow Cells with longer path lengths yield higher signals

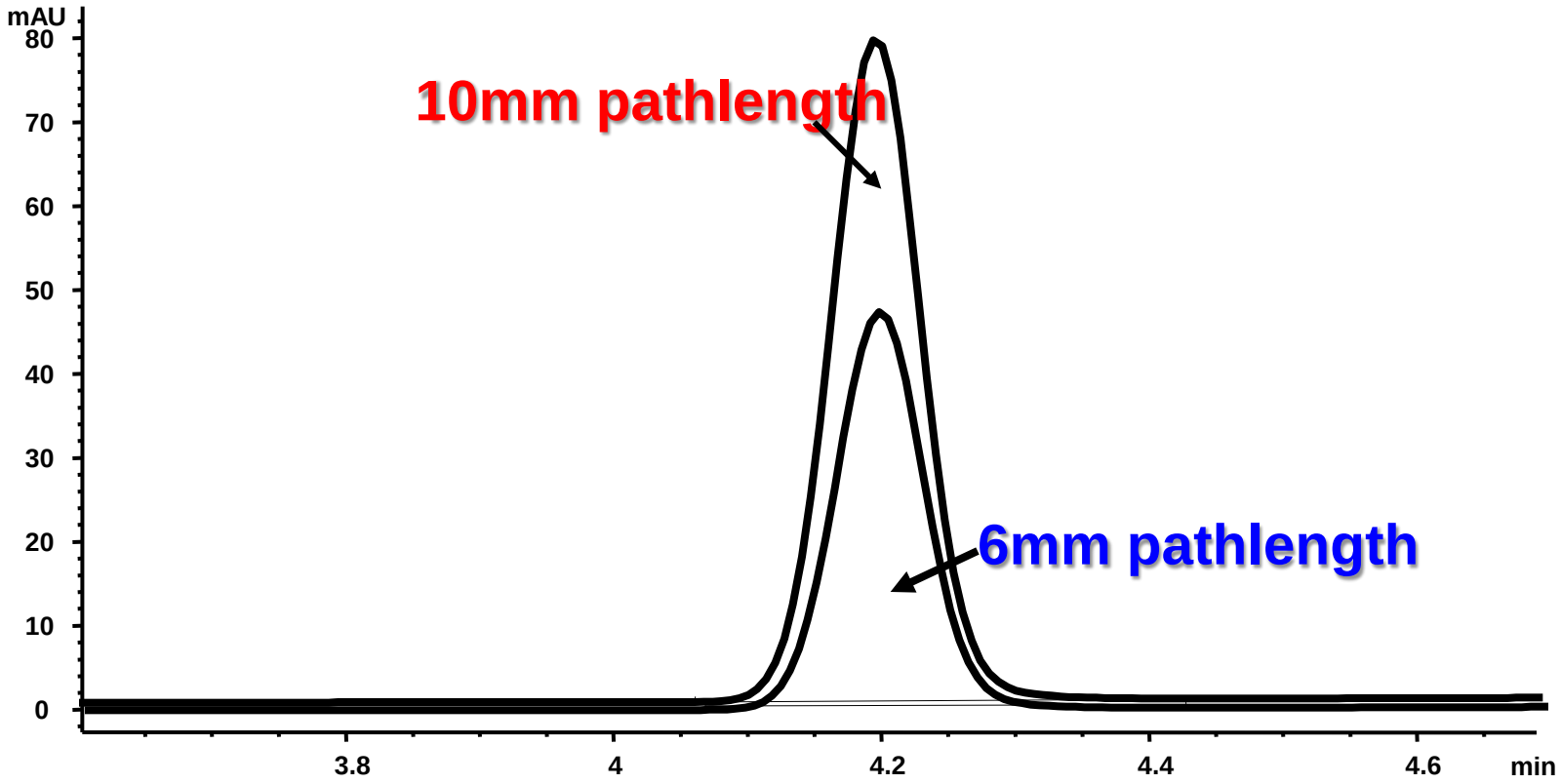
VWD STANDARD CELL

Standard: 14-µl volume, 10-mm cell path length and 40 bar

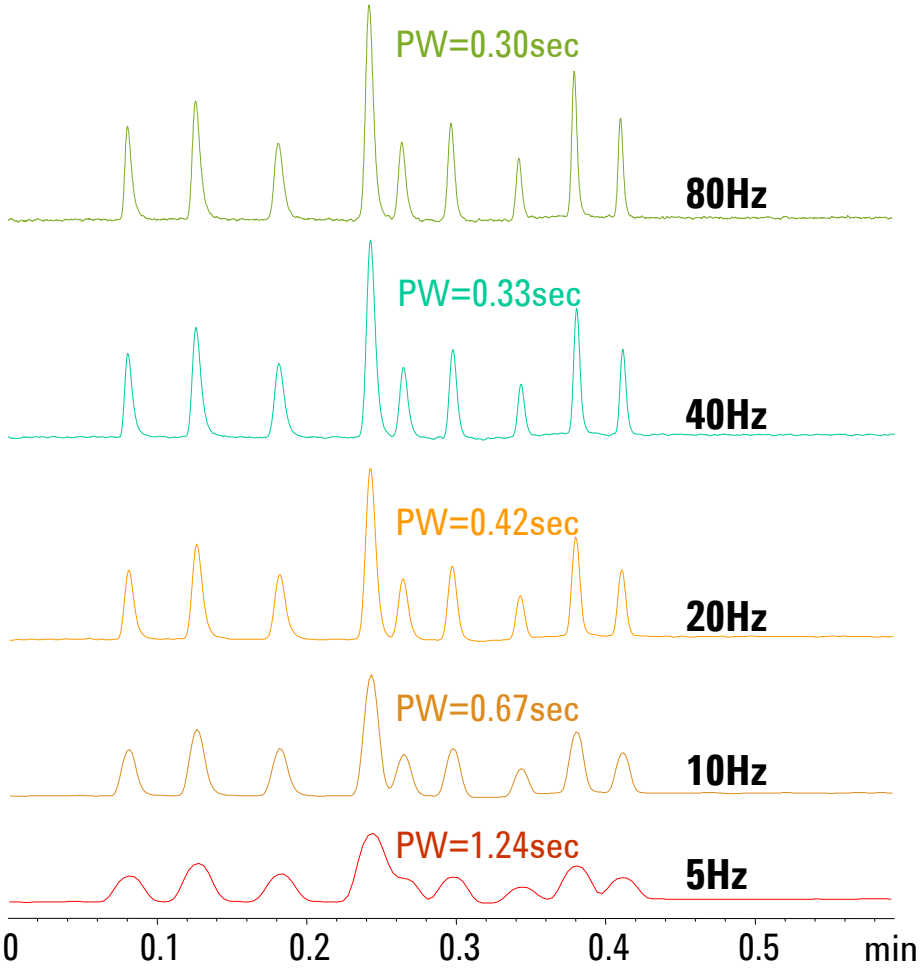
DAD STANDARD CELL

Standard: 13 µl volume, 10 mm cell path length and 120 bar

Influence of Pathlength on Signal Sensitivity



Benefit of 80Hz Data Acquisition Rate
Peak Width, Resolution and Peak Capacity in Ultra-Fast LC



20Hz versus 80Hz

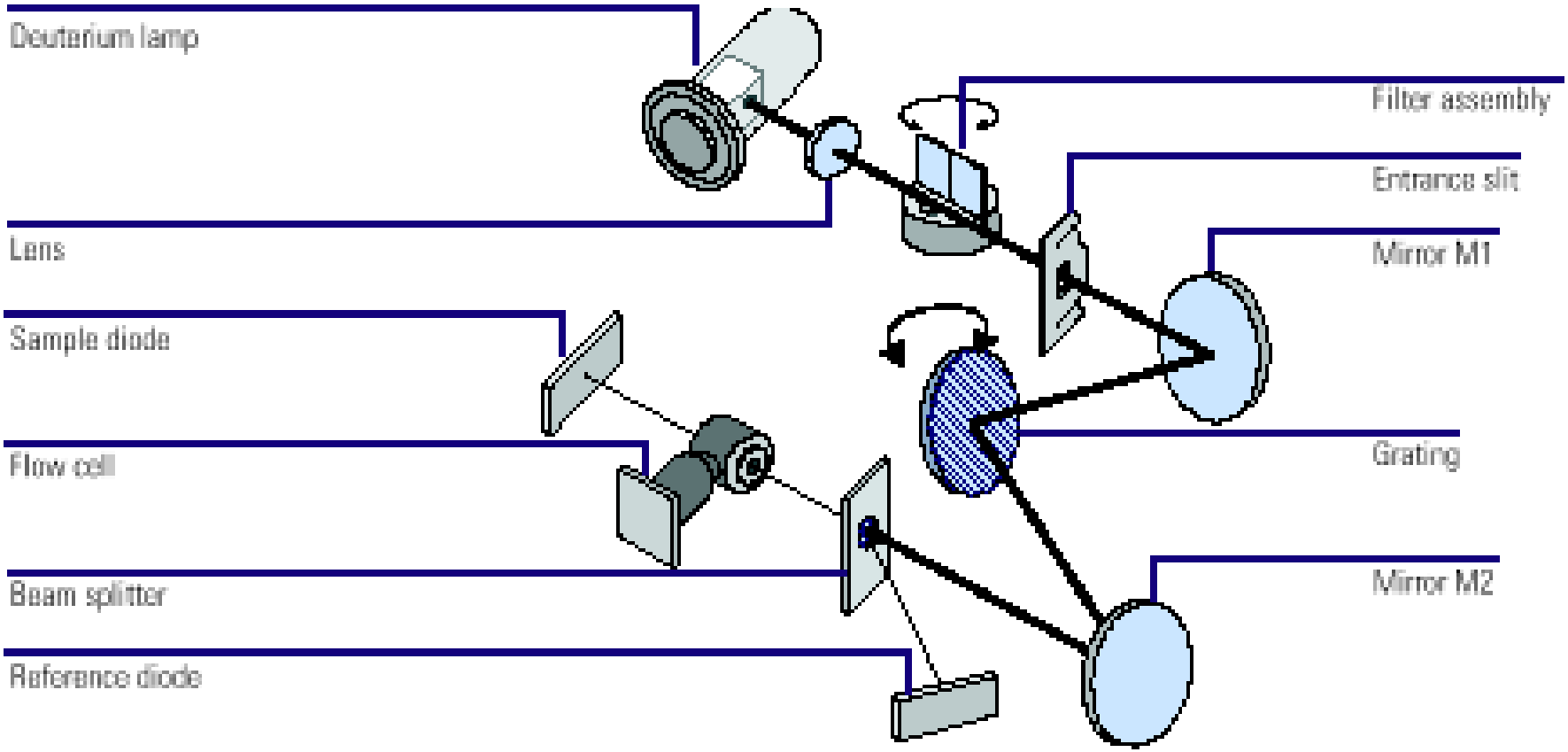
+ 40% Peak Width
– 40% Peak Capacity
– 30% Resolution
– 70% Apparent Column Efficiency

10Hz versus 80Hz

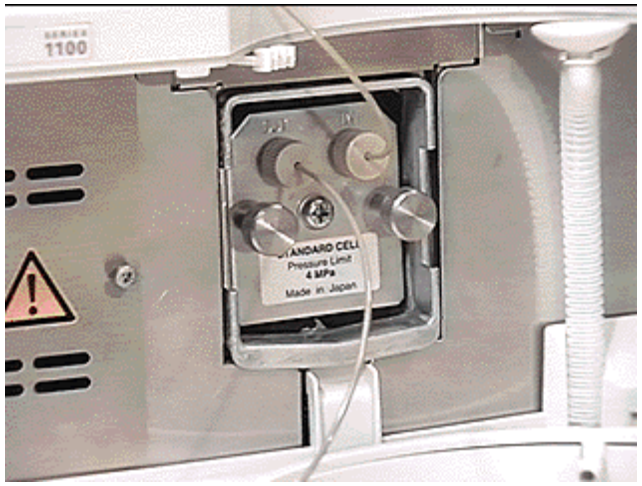
+ 120% Peak Width
– 120% Peak Capacity
– 90% Resolution
– 260% Apparent Column Efficiency

- Sample: Phenone Test Mix
- Column: Zorbax SB-C18, 4.6x30, 1.8um
- Gradient: 50-100% ACN in 0.3min
- Flow cell: 5ul

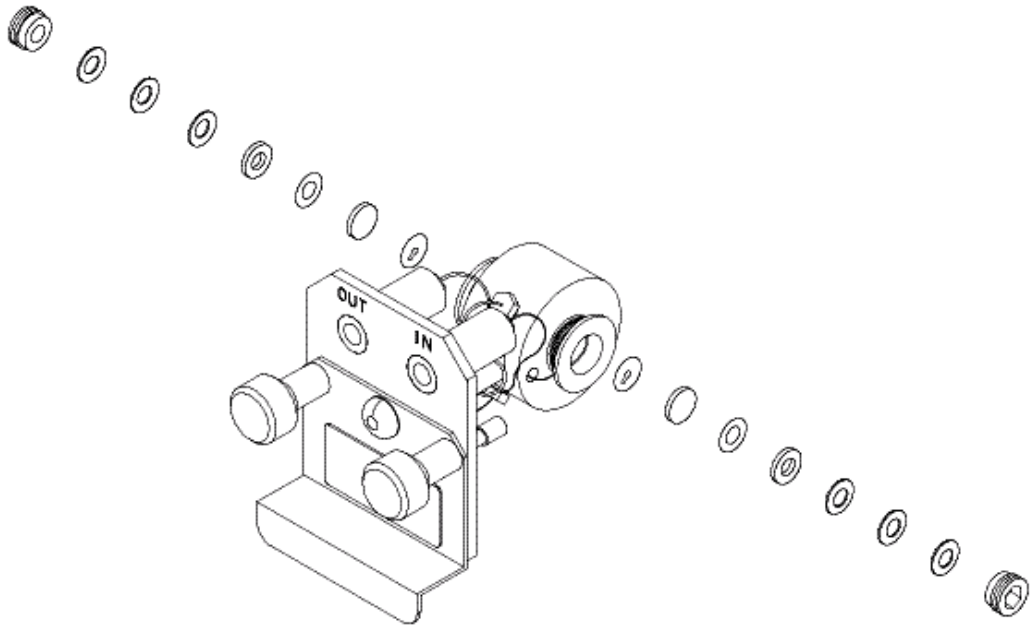
Figure 6 **Optical Path of the Variable Wavelength Detector**



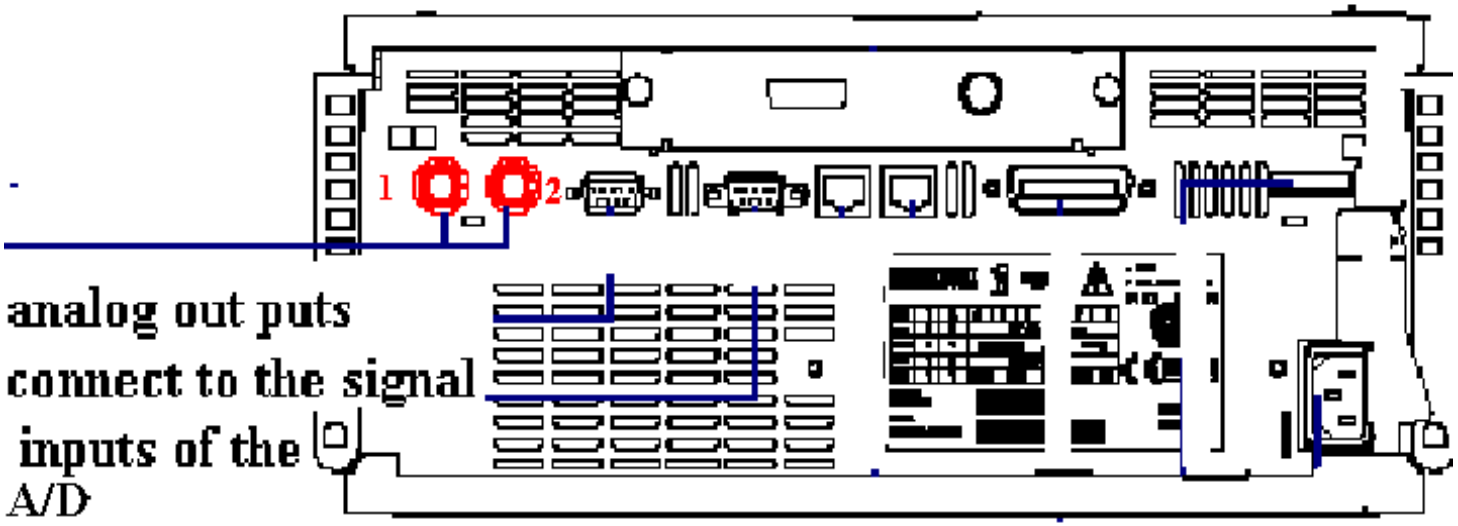
Changing the VWD Lamp


1100
OUT
IN
Pressure Limit
4 MPa
Made in Japan


OUT
IN
4 MPa
Made in Japan


OUT
IN

Connections to an A/D box



Analog connections

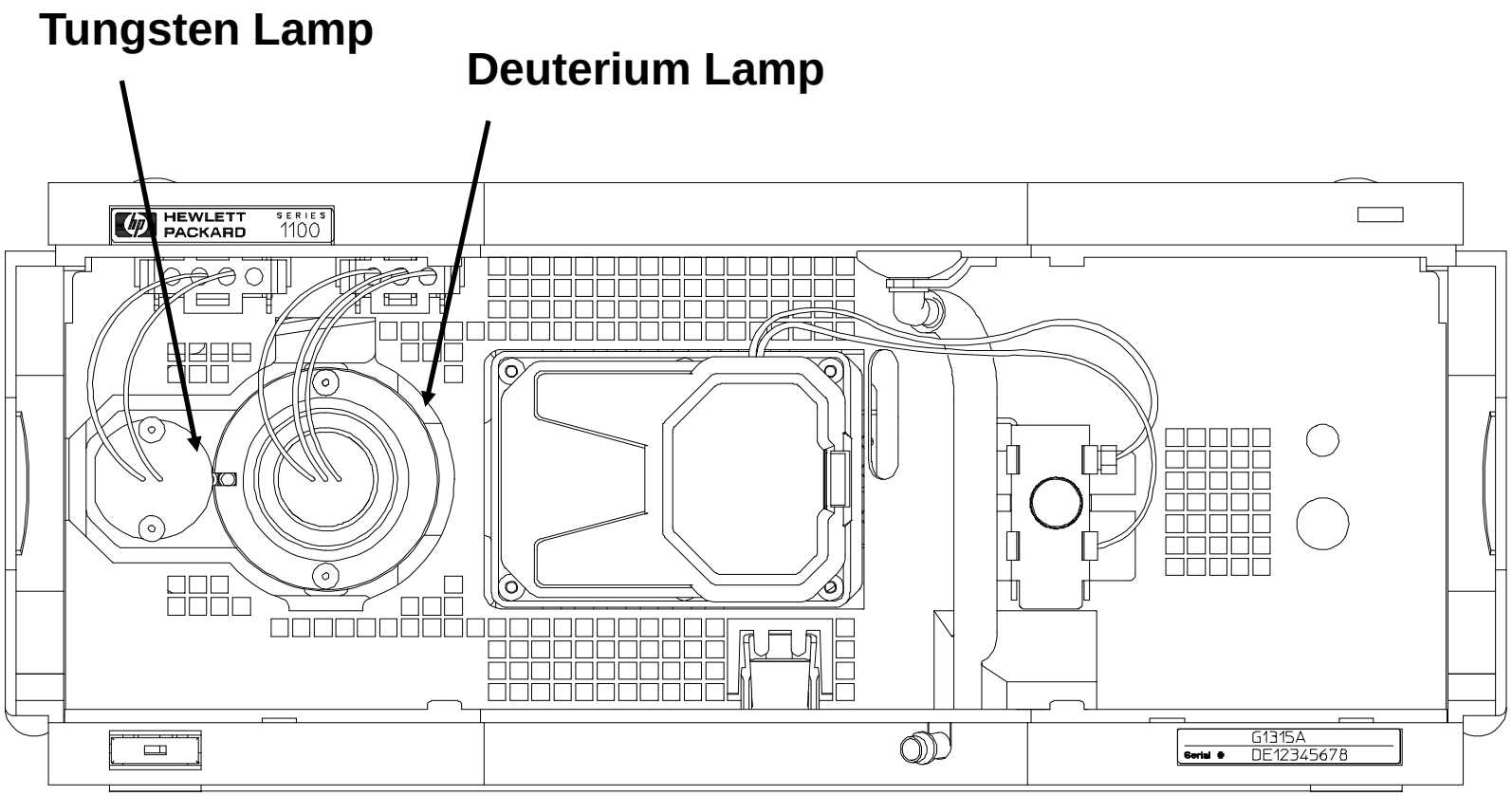
General purpose (spade lugs) *01046-60105*

Please note the analog cable and connections are the same for all 1200 detector outputs to a non HP data system

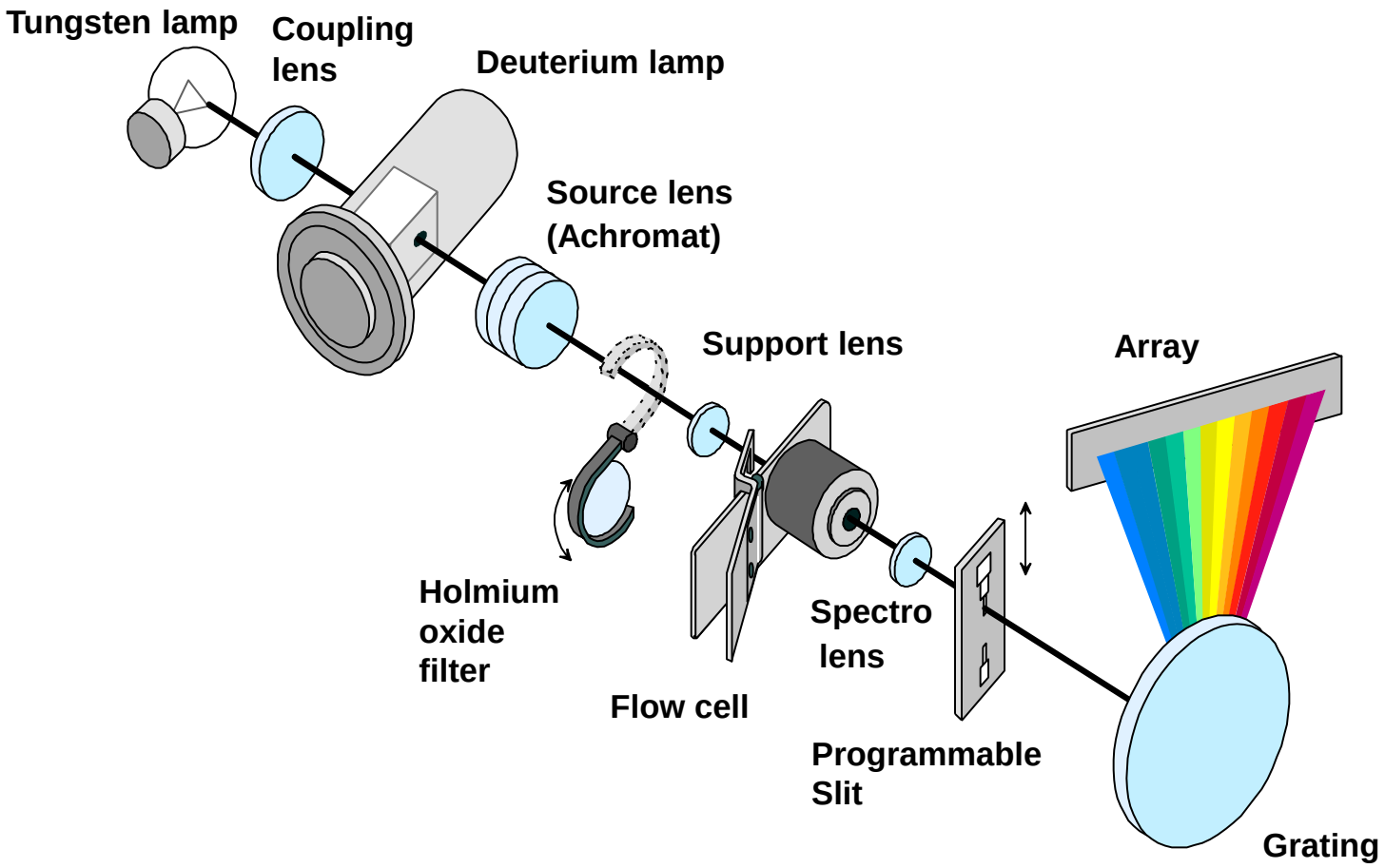
HP 1200 Diode Array

- **Wavelength Range:** 190 - 950 nm
- **Lamps:** Shine-through deuterium lamp (uv-range)
  Tungsten lamp (vis-range)
- **Slits:** Programmable electromechanical; 1, 2, 4, 8 and 16 nm
- **Noise:** 2x10-5 AU at 254 nm and 750 nm
- **Flow Cells:** STD 10 mm 13 ul, Semi-micro 6 mm 5 ul, Micro/HP 10 mm 1.7 ul, 500 nl flow cell

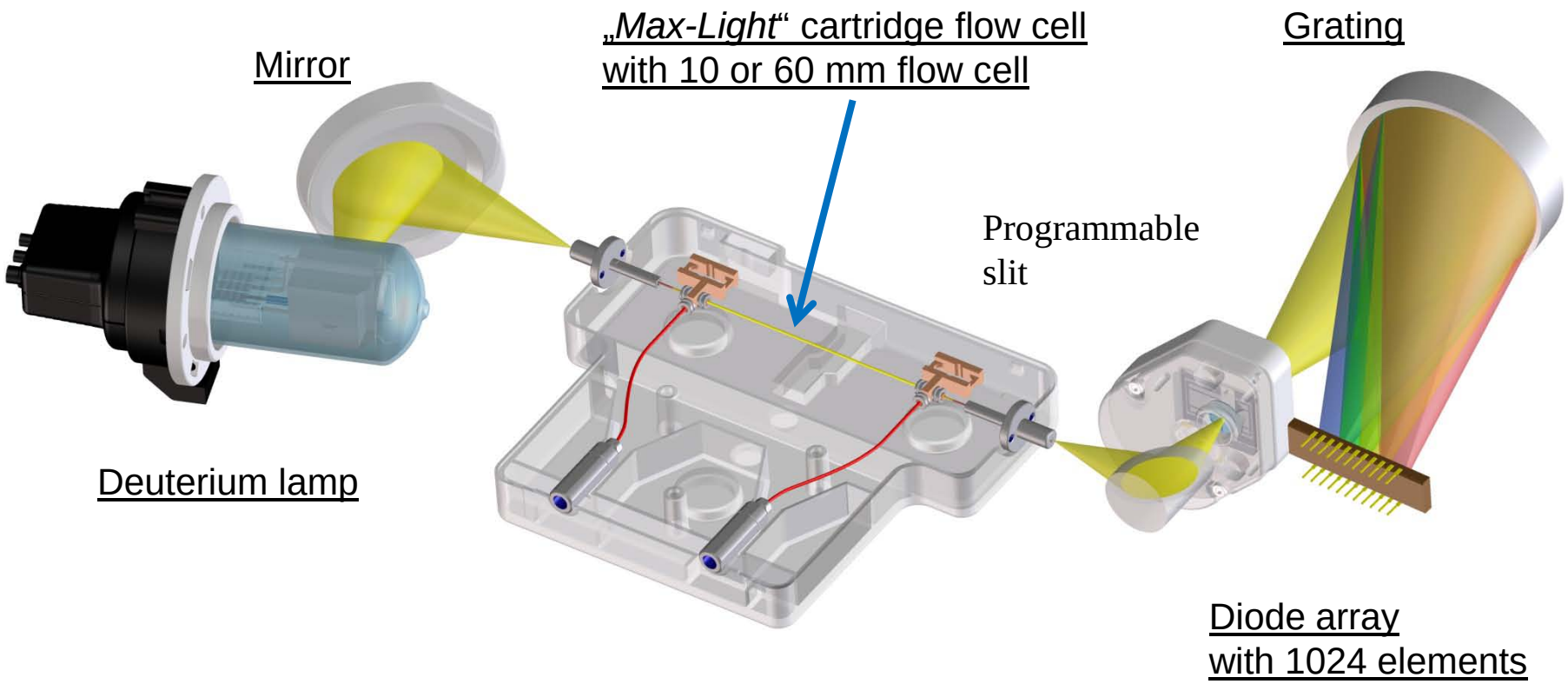
Frontal View – DAD/MWD



Optical Path - HP 1200



1260 &1290 Infinity Diode Array Detectors



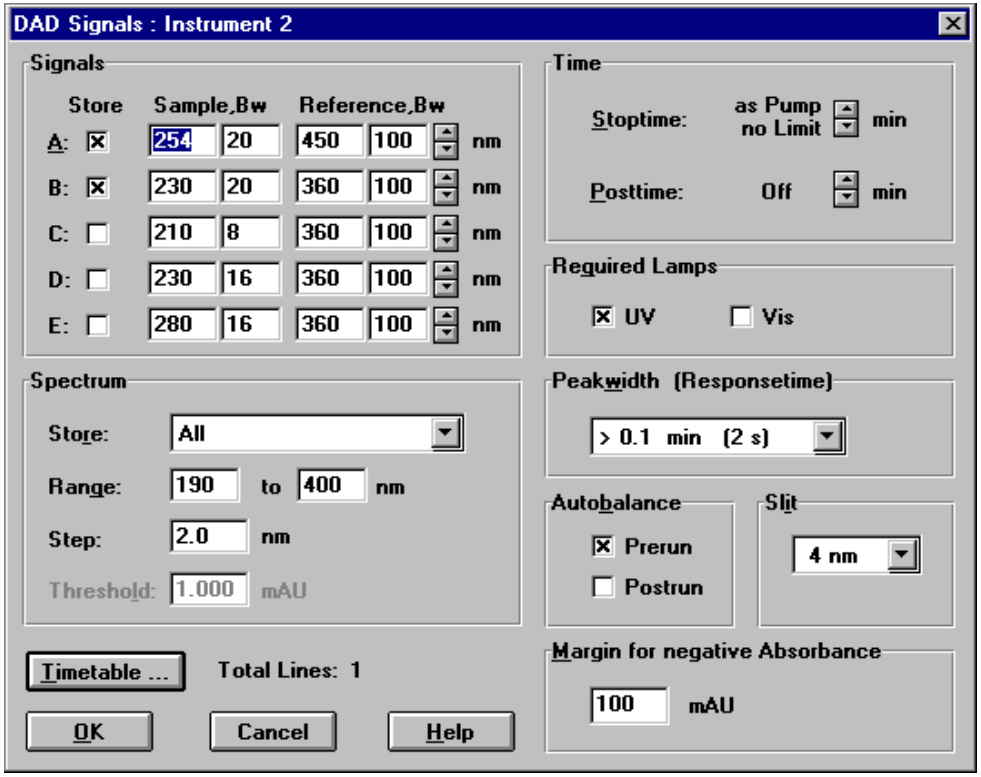
Optofluidic Waveguides: Max-Light Flow Cells

Total-internal reflection in a non-coated fused silica fiber

Diode Array Parameters - Review

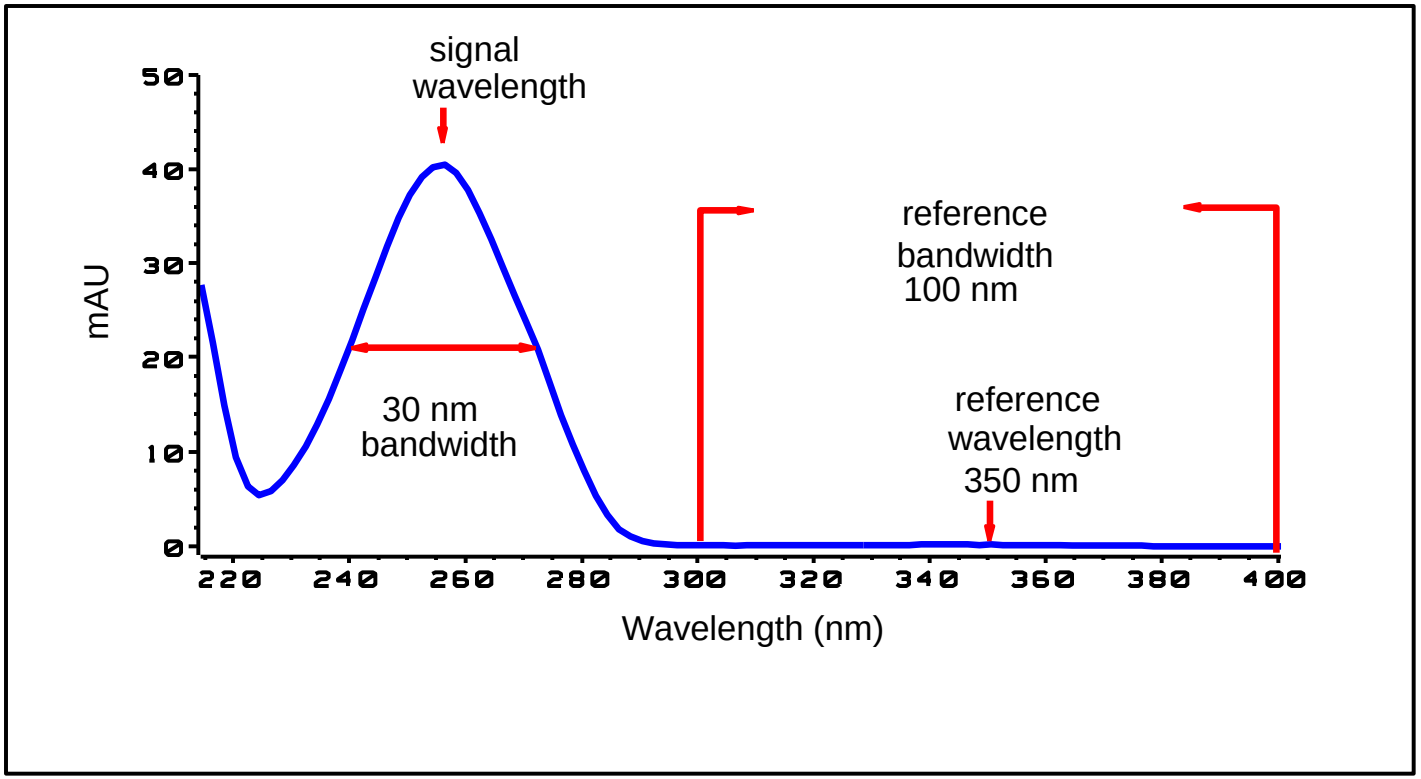
Signal Storage

Spectral Storage Parameters

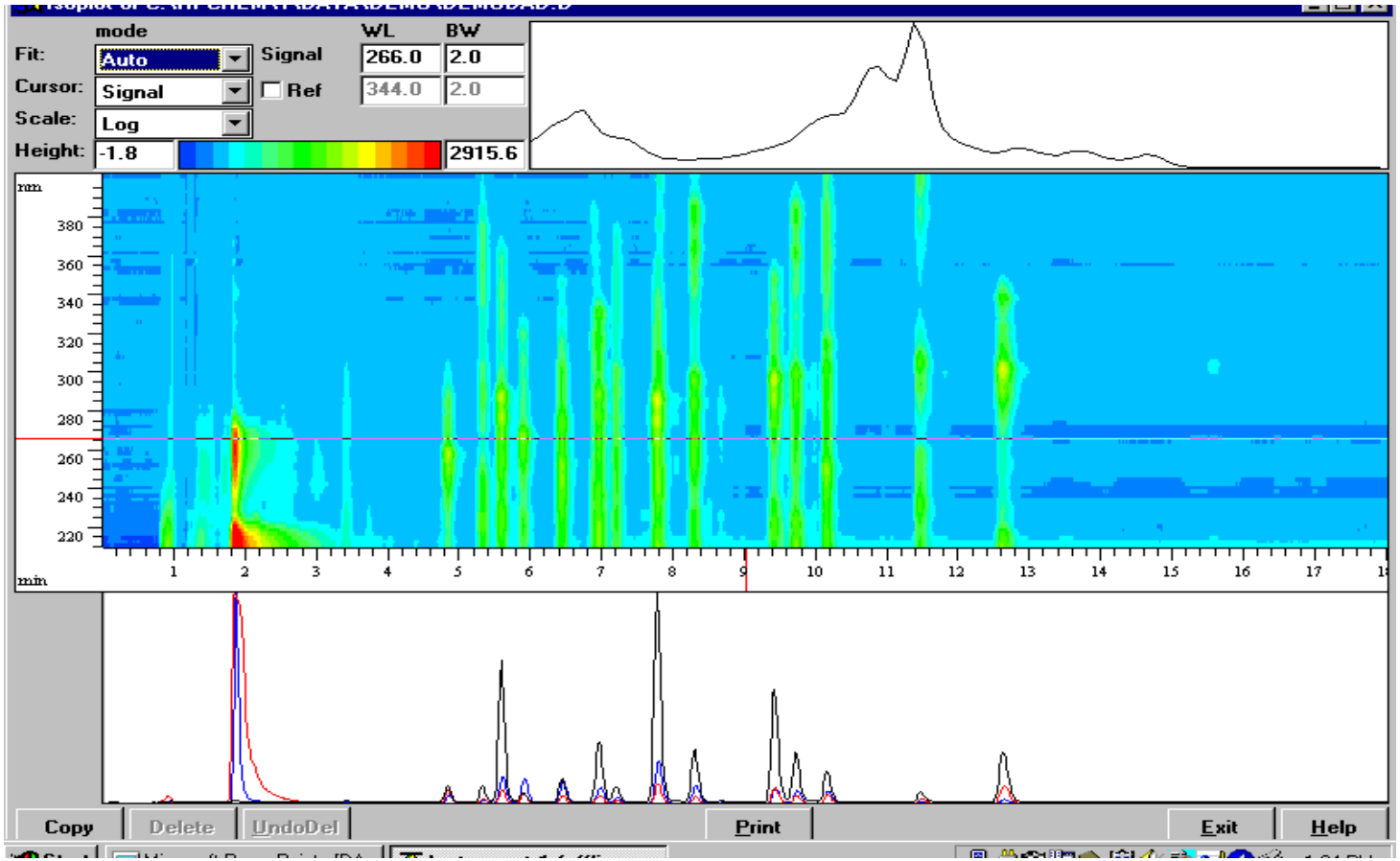


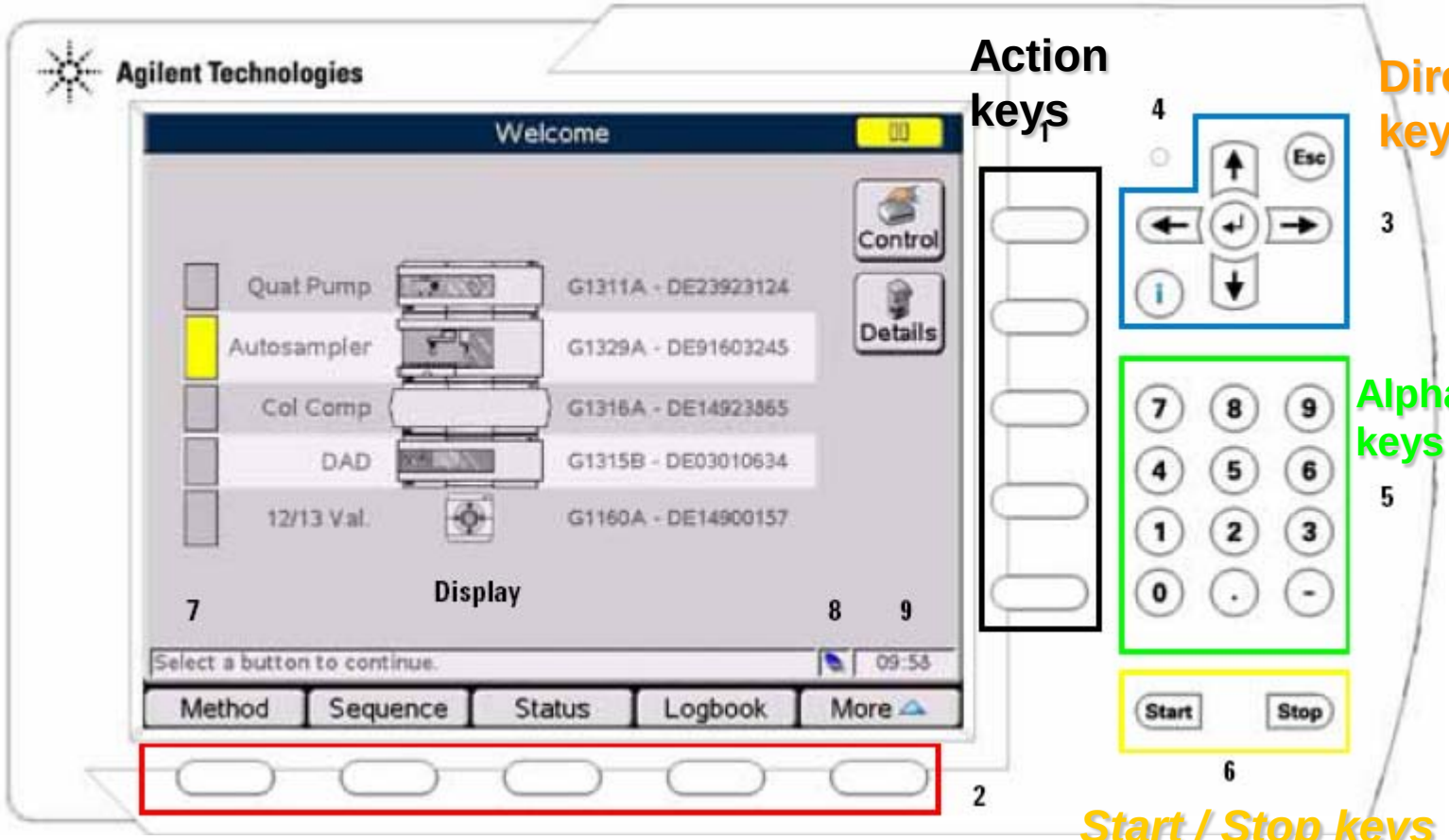
Response Time

Sample Signal and Reference Optimization



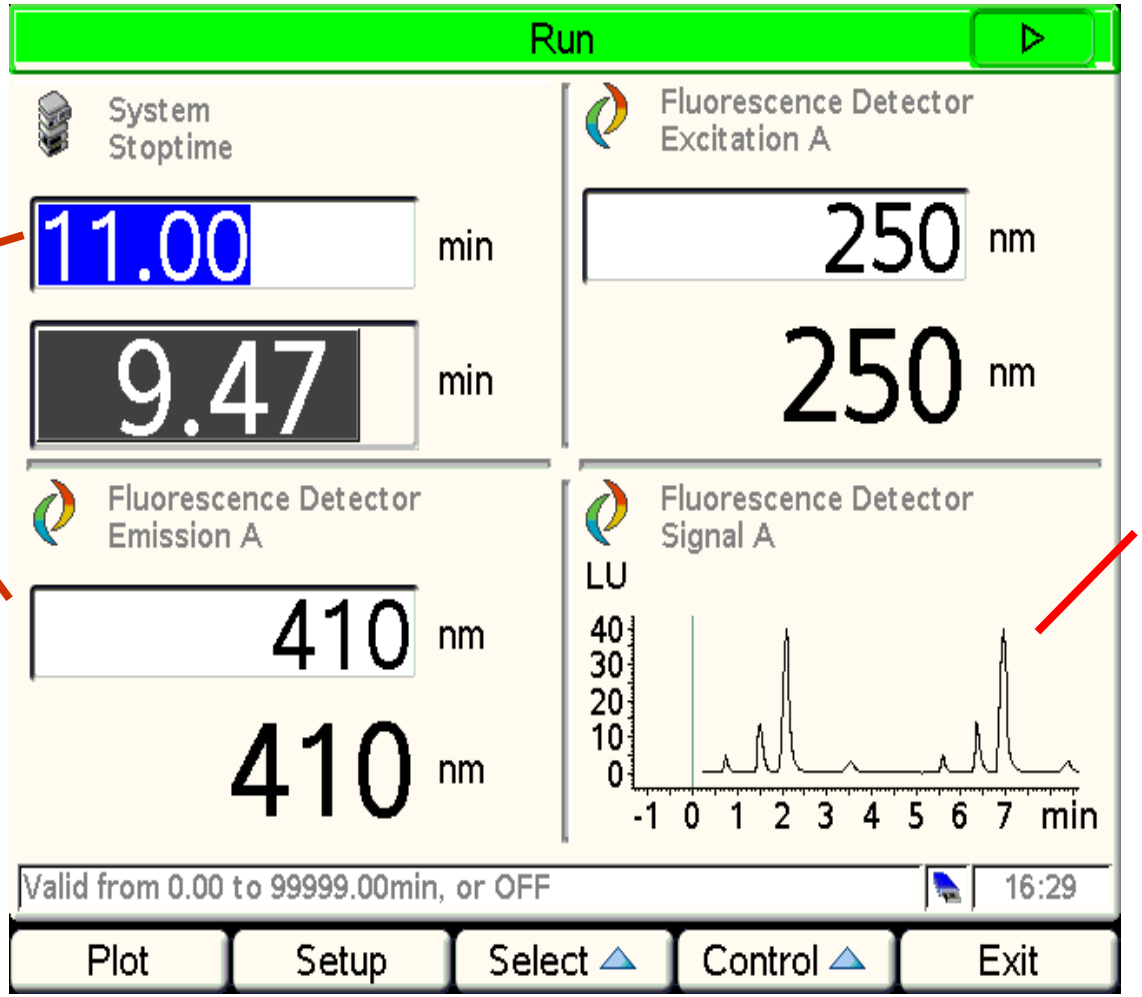
Diode Array: Signal Extraction with Isoplot




Agilent Technologies
Welcome
Control
Details
Quat Pump
G1311A - DE23923124
Autosampler
G1329A - DE91603245
Col Comp
G1316A - DE14923865
DAD
G1315B - DE03010634
12/13 Val.
G1160A - DE14900157
Display
7
8
9
Select a button to continue.
09:58
Method
Sequence
Status
Logbook
More
2
Action keys
4
Esc
3
7
8
9
4
5
6
1
2
3
0
.
-
5
Start
Stop
6
Direction keys
Alphanumeric keys
Start / Stop keys
Navigation keys

Customizable Status Display

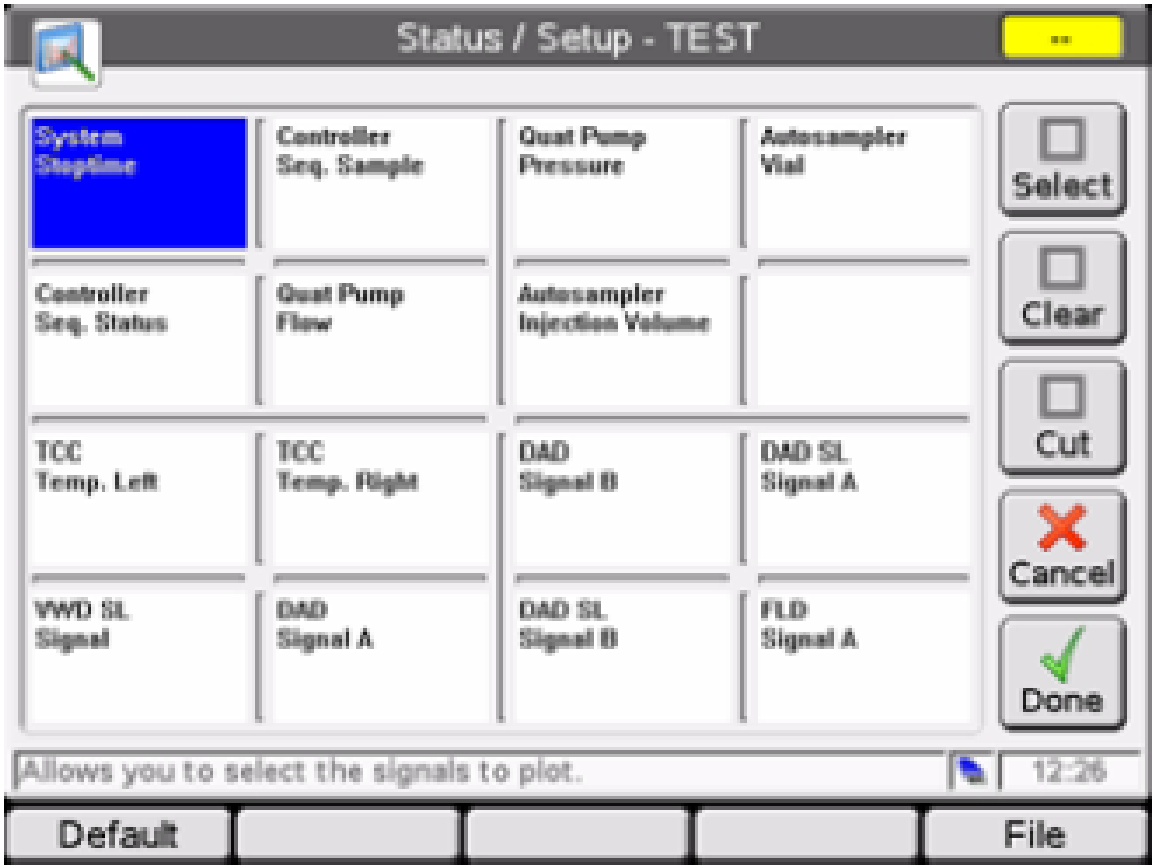
Direct parameter change



Signal Plot

Tiles customizable by user

Press the **Setup** button.



allows the selection of a signal/parameter.

clears a selected field.

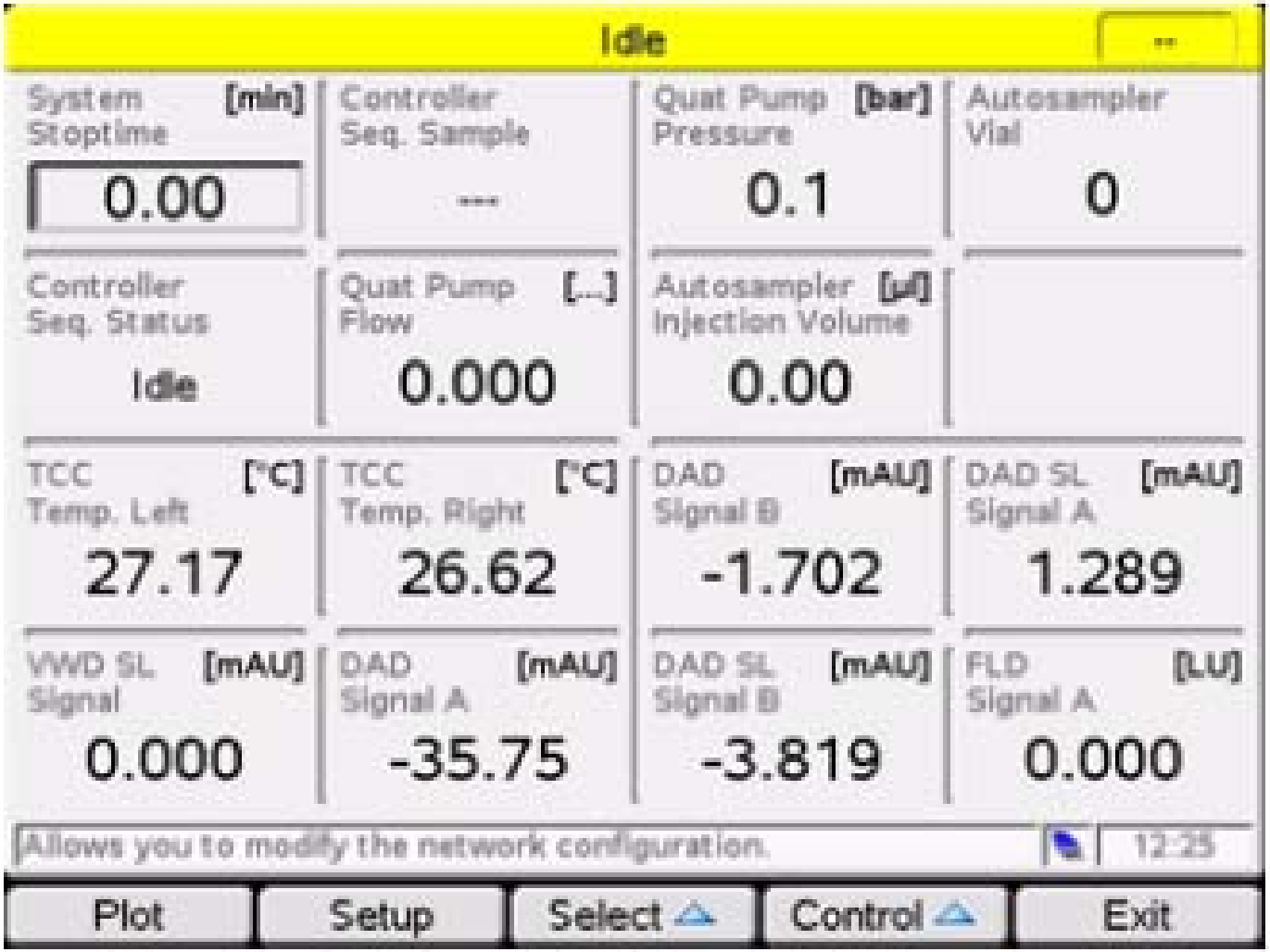
cuts a selected field to be pasted to another position.

leaves this screen without changes.

leaves this screen with all changes.

Default: takes signals from all modules
File: load/save a setup.

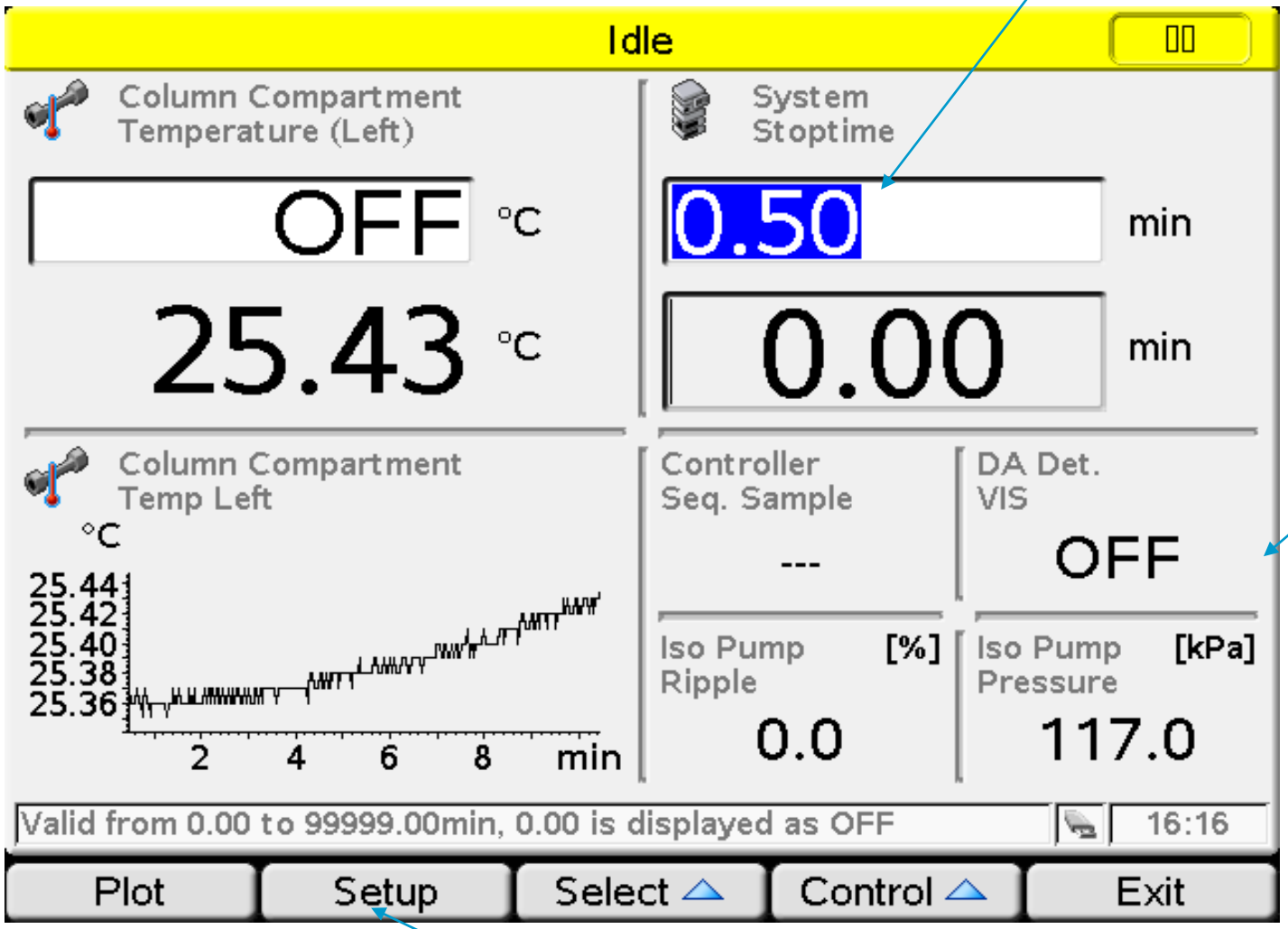
Setup of a Status Information Screen



When the Status Information screen has not been setup before, it will show from each module in the system one or more signals/parameters

Customizable Status Display

Direct parameter change



Tile with 4 subtiles

Screen customizable

Agilent 1200 Series
Instant Pilot

Unsupported Feature – Space Invaders

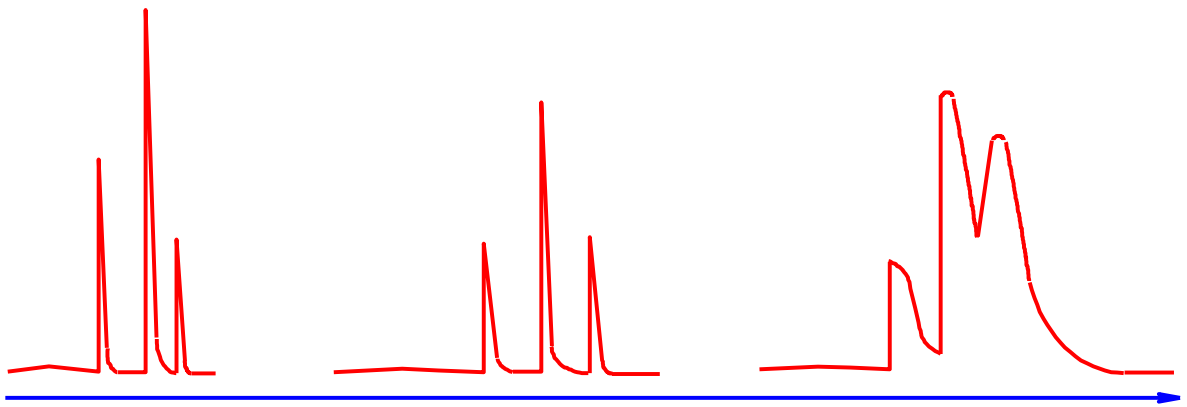
Welcome screen

Enter

3 5 1 1 3 7

H O B B I T

Extra-Column Dispersion



- Use short, small internal diameter tubing between the injector and the column and between the column and the detector.
- Make certain all tubing connections are made with matched fittings.
- Use a low-volume detector cell.
- Inject small sample volumes.

Dispersion is effected by

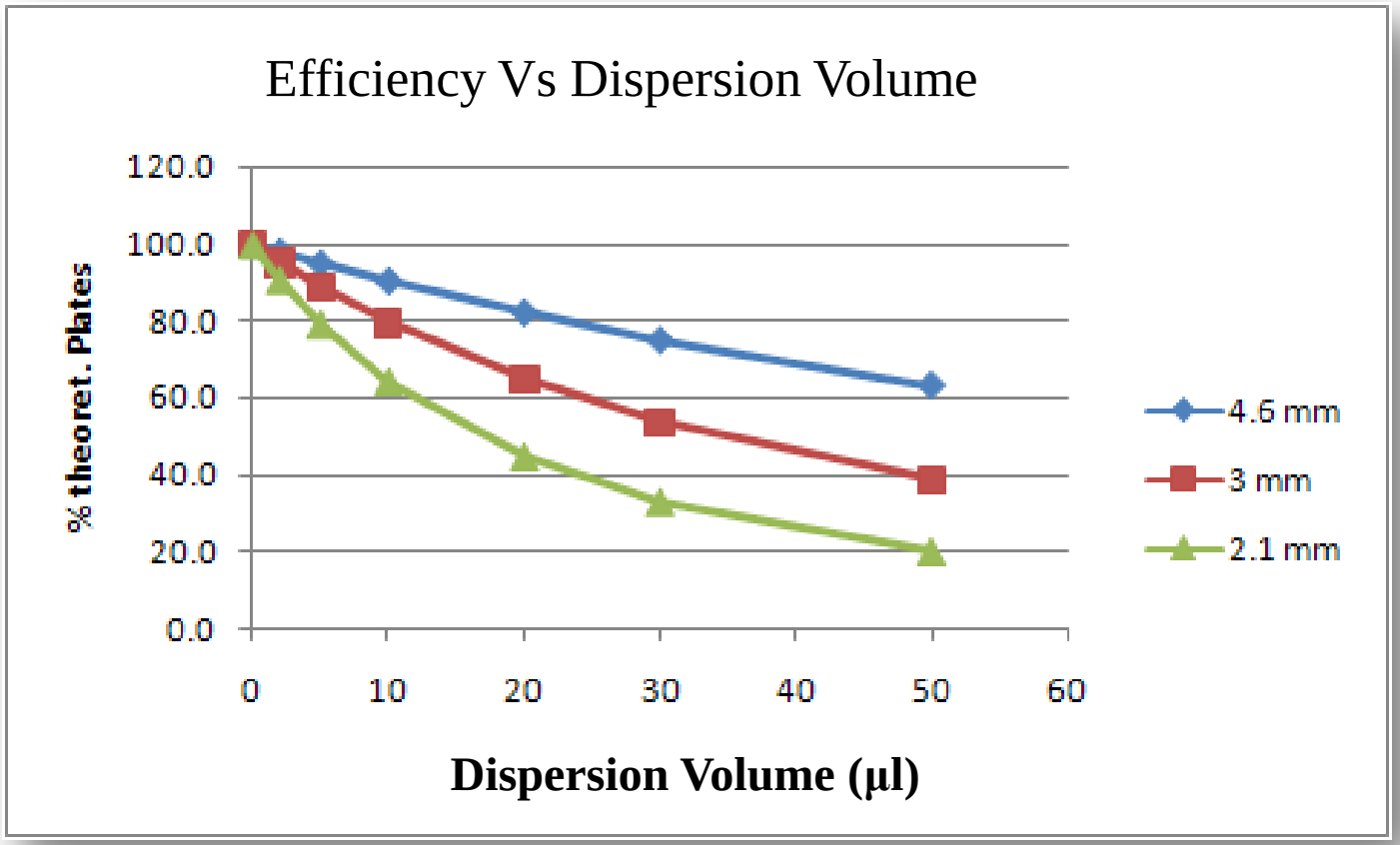
Tubing (amount and id)

Connections (number of)

Flow cell volume (larger >)

The goal is to have the smallest id tubing, least number of connections, and the lowest flow cell volume. This will give the best number for dispersion.

Dispersion

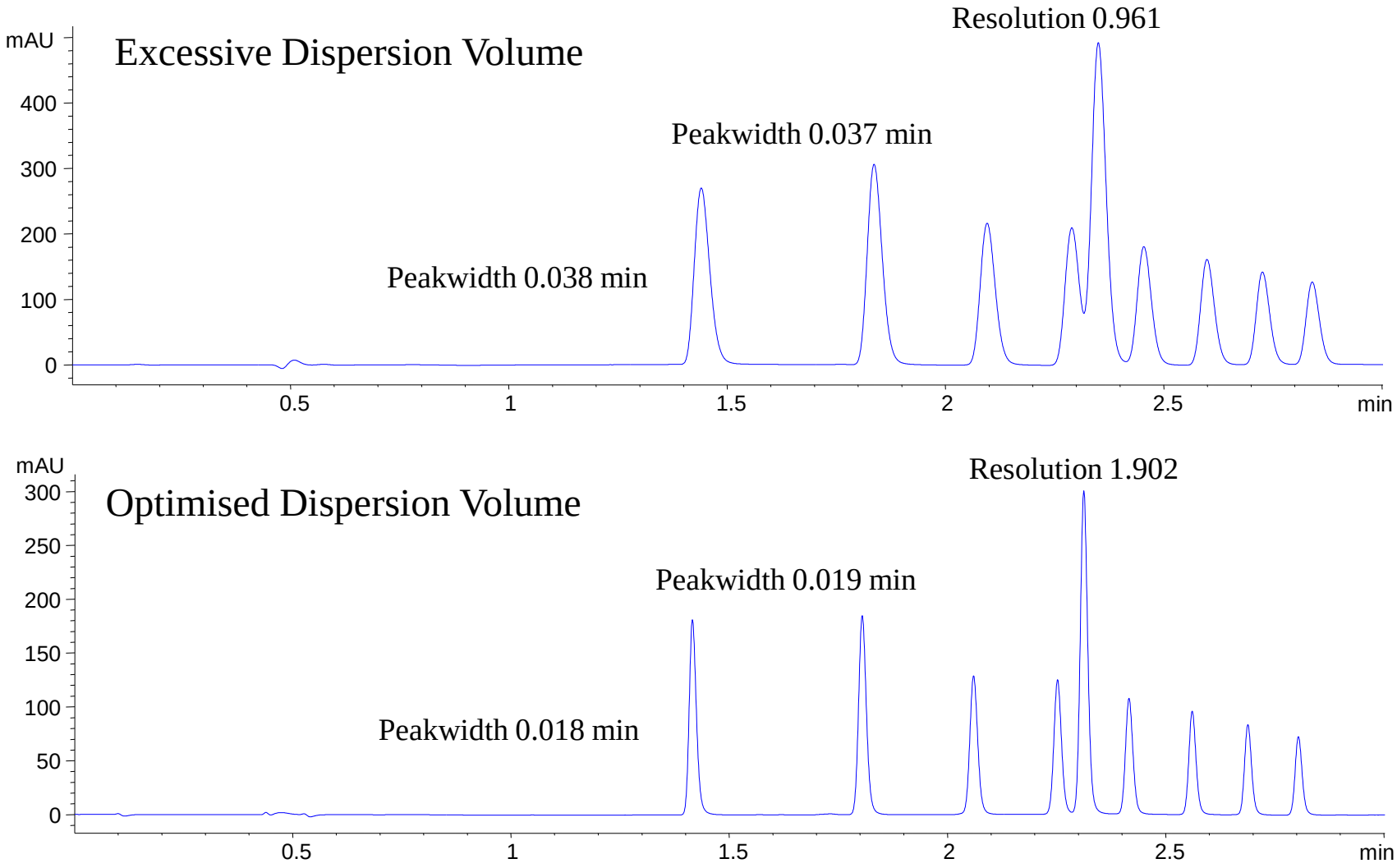


Flow rates converted for Column i.d.

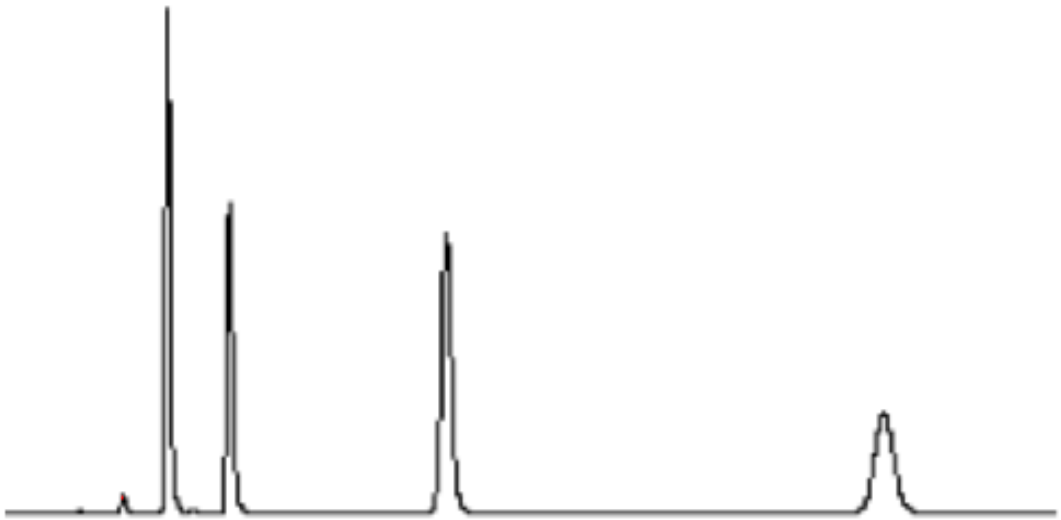
4.6 mm Column	1.00 mL/min
3.0 mm Column	0.43 mL/min
2.1 mm Column	0.21 mL/min

Column Dimension
Length = 50 mm
Particle size = 1.8 µm

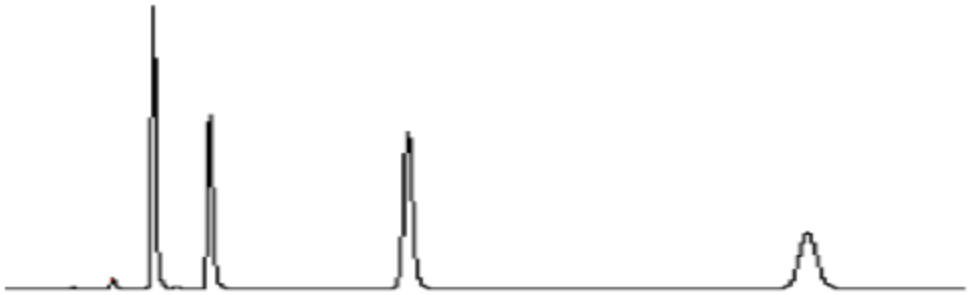
Dispersion

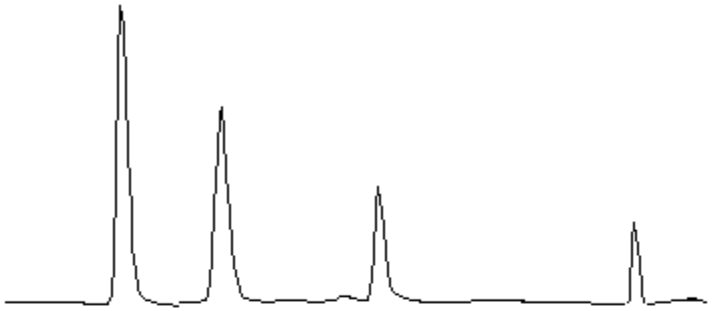

Excessive Dispersion Volume
Resolution 0.961
Peakwidth 0.037 min
Peakwidth 0.038 min
mAU
400
300
200
100
0
0.5
1
1.5
2
2.5
min
Optimised Dispersion Volume
Resolution 1.902
Peakwidth 0.019 min
Peakwidth 0.018 min
mAU
300
250
200
150
100
50
0
0.5
1
1.5
2
2.5
min

A typical chromatogram



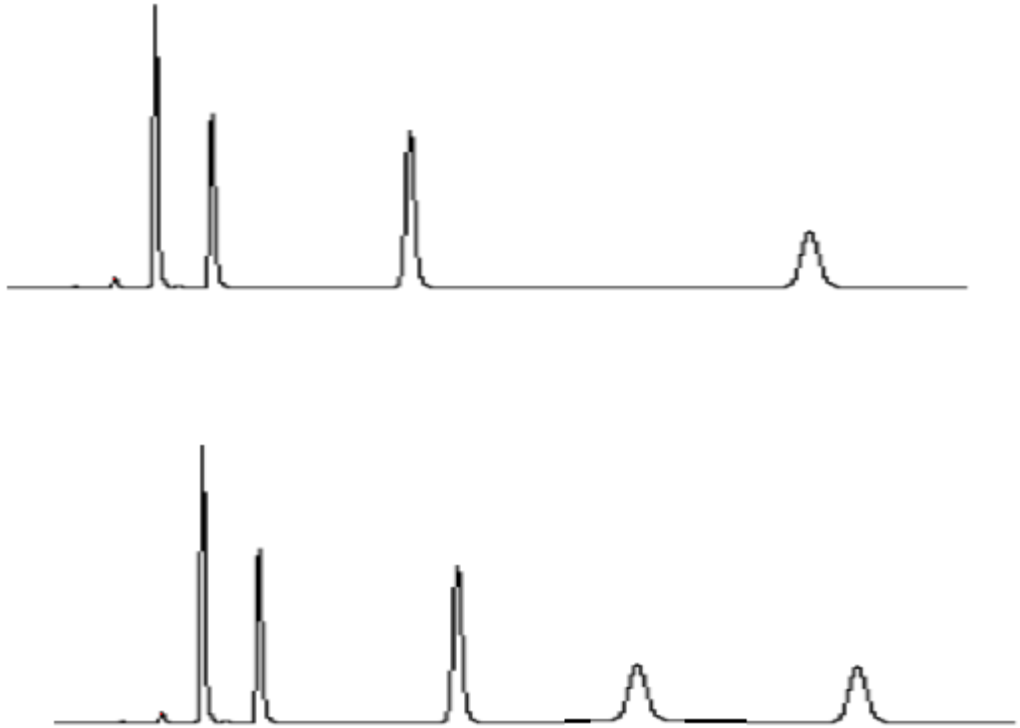
Peak Tailing
Injector Seal Failure





- Overdue instrument maintenance can cause peak shape problems.

Broad Peaks
Unknown “Phantom” Peaks



- The extremely low plates are an indication of a very late eluting peak from the preceding run.

Determining the Cause of Peak Tailing

- Evaluate mobile phase effects - alter mobile phase pH and additives to eliminate secondary interactions
- Evaluate column choice - try column with high purity silica or different bonding technology
- Reduce sample load
- Eliminate extra-column effects
- Flush column and check for aging/void

Questions?