

A Gram of Prevention

Simple Tips for Maintaining
LC Instrument Performance

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Applications Engineer

January 21, 2014

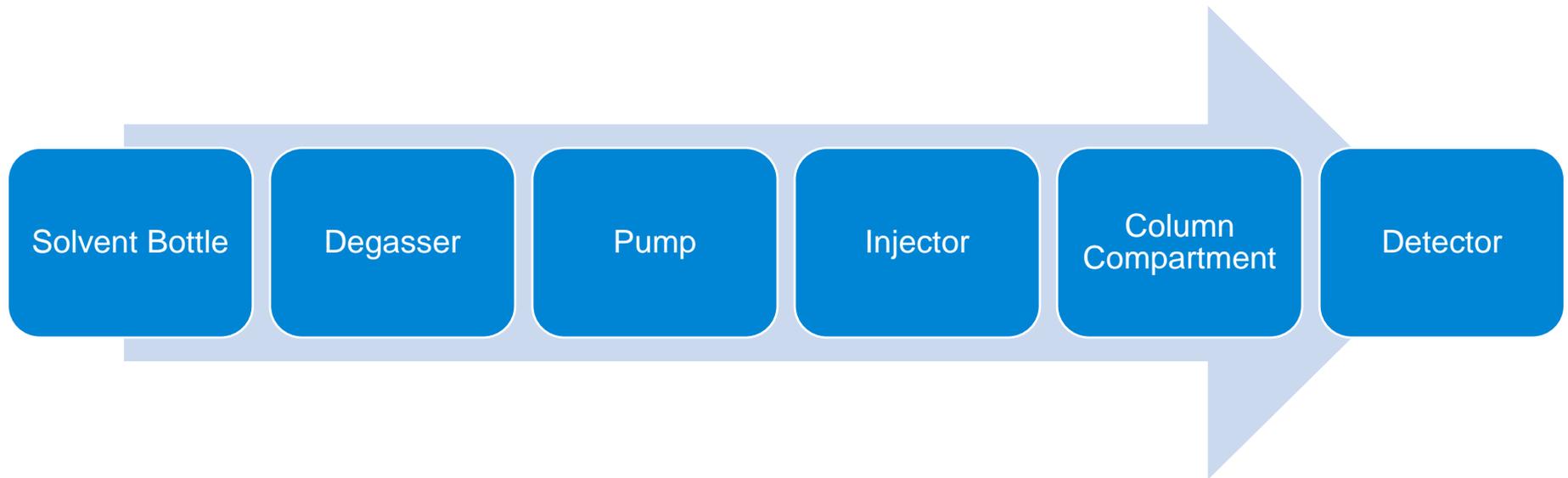
Overview

- Know the flow path of your instrument (1260/1200/1100)
- What is in your solvent bottle?
- Capillaries and fittings
- Pump care
- Injector care
- Filters and filtration
- Detector care
 - UV
 - MS
- Consider an Agilent service contract



Your Instrument's Flow Path

- Where can blockages to flow occur?
- Where are the consumables that need to be replaced on a regular basis?
- Where can leaks occur?
- What can I do to reduce or anticipate potential problems with the LC?



Your Instrument's Flow Path

Solvent cabinet →

Degasser →

Pump →

Autosampler →

Column compartment →

Detector →

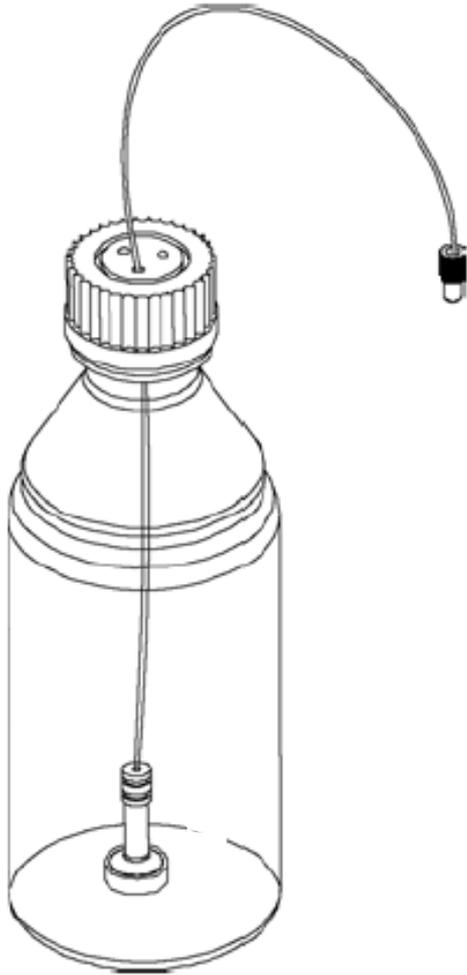


What is in Your Solvent Bottle?

- Use only quality HPLC or MS grade solvents
- These do not need to be filtered
- Buffer and salt solutions do need to be filtered
- Filter porosity: 0.45 or 0.2 μm
- Make sure the filter material is compatible
- Avoid algae/microbial growth
 - Frequently replace the mobile phase with a clean bottle
 - Adding some organic to aqueous mobile phases can inhibit growth
 - Consider avoiding light exposure
- Can cause degasser problems
- Can be a source of ghost peaks



What is in Your Solvent Bottle?



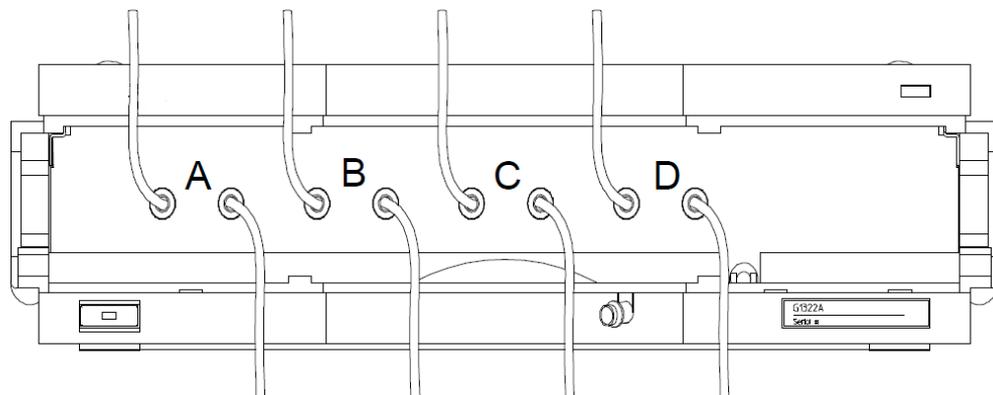
- Solvent inlet filter
- Not a replacement for good mobile phase hygiene
- Glass solvent inlet filter (20 μm), 5041-2168
- Stainless Steel solvent inlet filter, 01018-60028



Degasser Care



- Check for bubbles in outlet lines
- Avoid blockages by flushing out buffer salts when changing mobile phases
- When switching solvents, make sure they are miscible
- Do not leave the degasser for an extended period of time with aqueous mobile phase to avoid microbial growth
- Unused channels should be left in isopropanol



Pump Care – Flow Path



Proportioning Valve

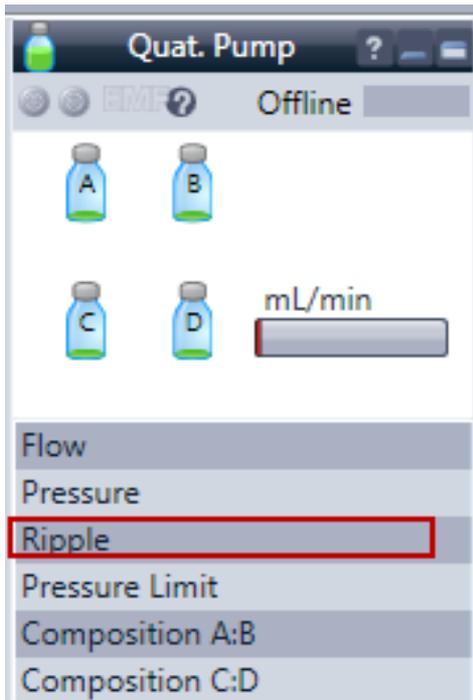
Inlet Valve

Pump Head

Outlet Valve

Purge Valve

Pump Care

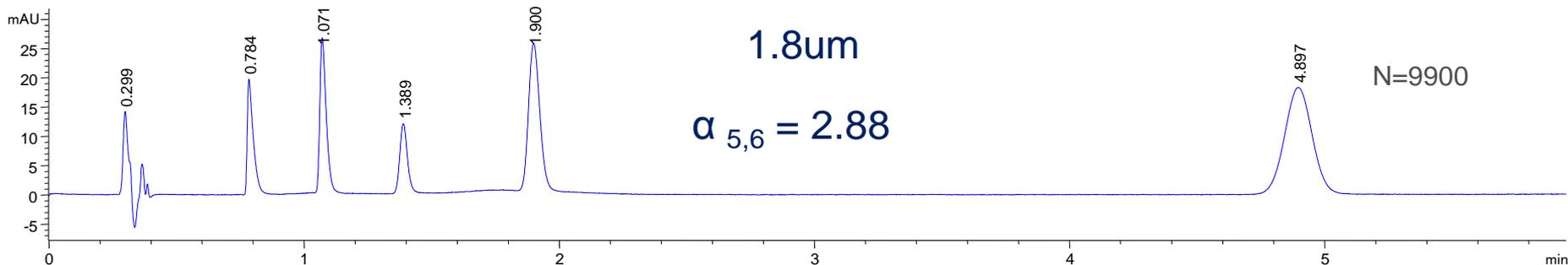
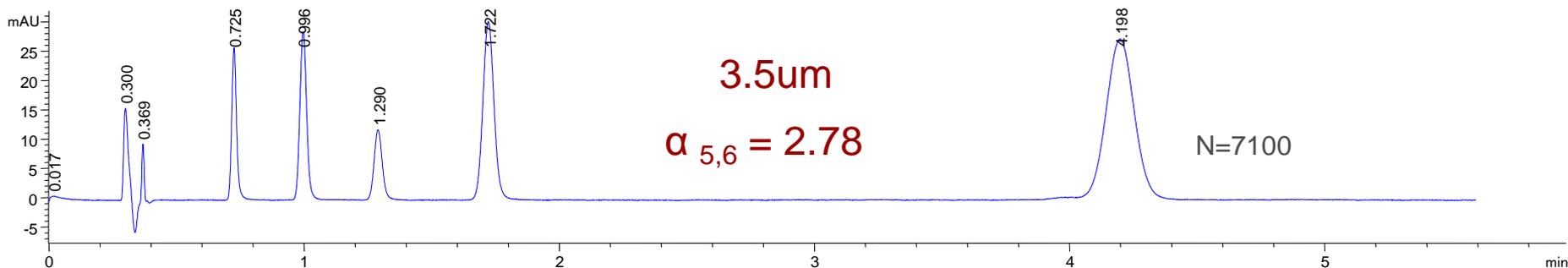
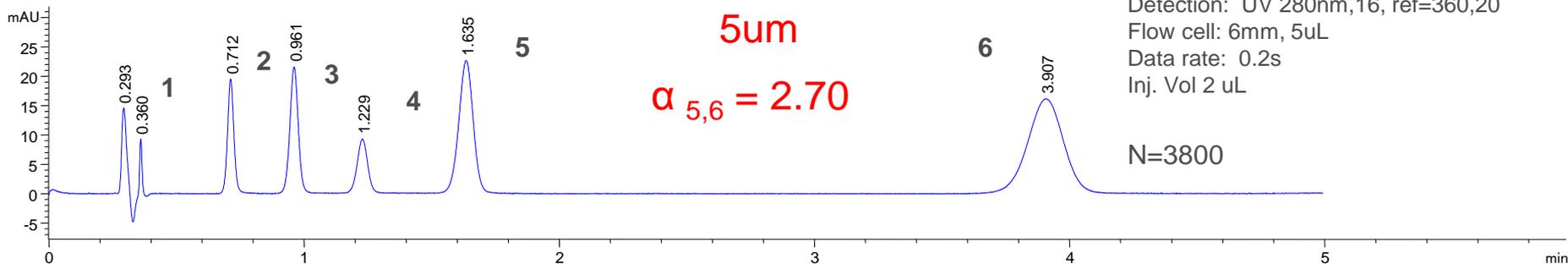


- Pressure ripple is a measure of pump performance
- Convenient way to assess condition of your pump
- Value should be stable

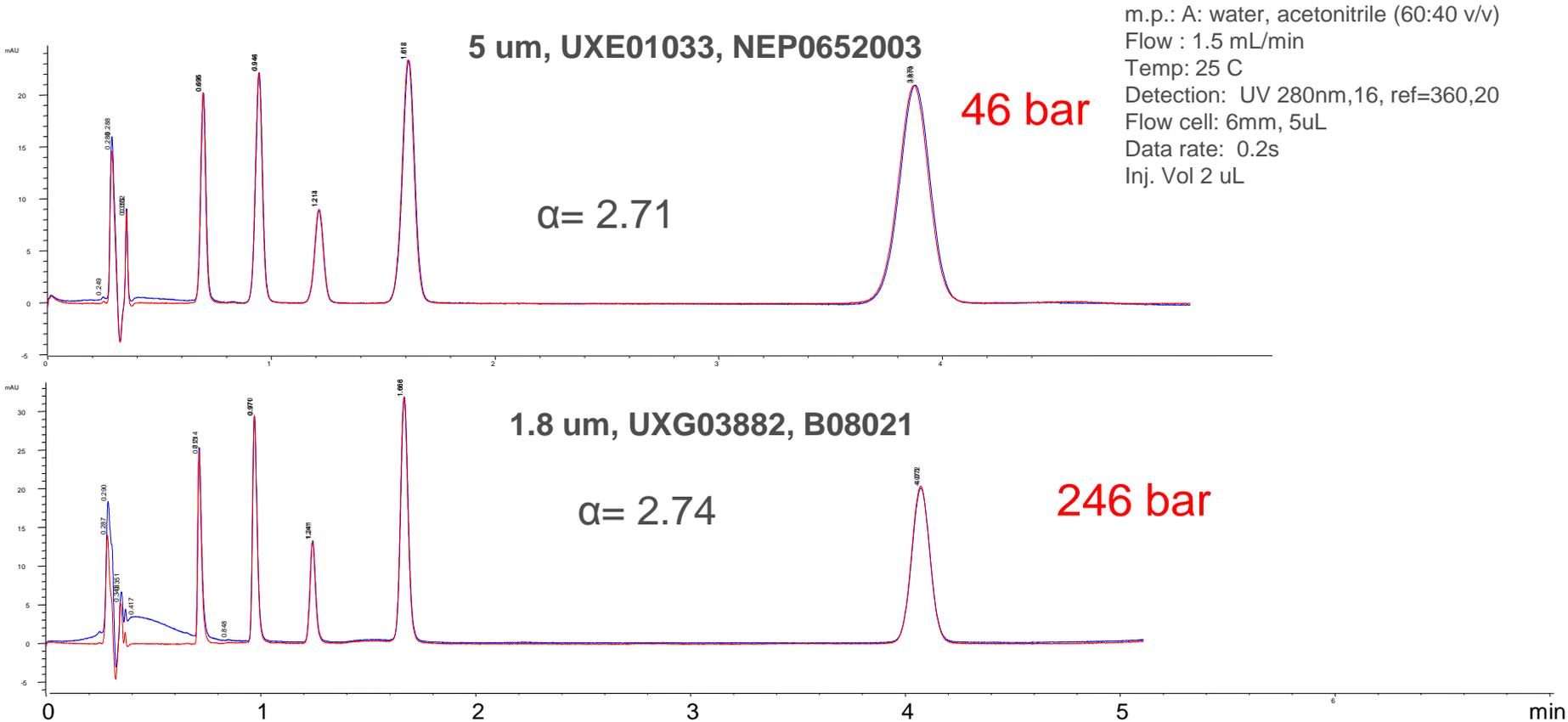
Pump Care

Eclipse Plus C18, 4.6 x 50 mm

m.p.: A: water, B: acetonitrile (60:40 A:B)
Flow : 1.5 mL/min
Temp: 25 C
Detection: UV 280nm,16, ref=360,20
Flow cell: 6mm, 5uL
Data rate: 0.2s
Inj. Vol 2 uL



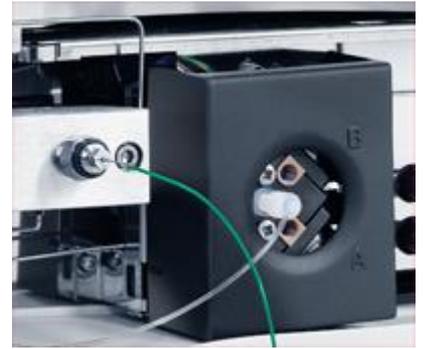
Pump Care Solvents Premixed



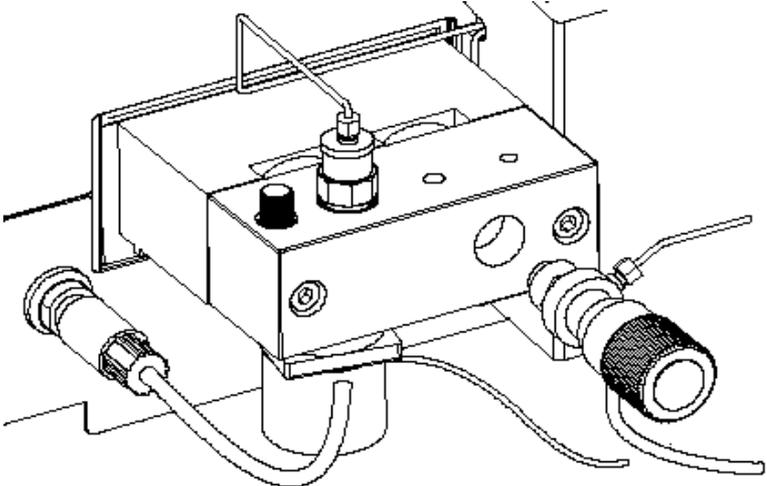
Pump Care

Proportioning Valve

- Solvent selection valve on binary pump
- Gradient valve on quaternary pump
- Allows the pump to vary solvent mixtures and form gradients
- Not much routine maintenance required here
- Avoid blockages and leaks by flushing out buffer salts when changing mobile phases
- Issues can lead baseline problems and poor retention-time precision



Pump Care Inlet Valve



Active inlet valve



Active inlet valve



Active inlet valve cartridge



14 mm wrench
8710-1924



Passive inlet valve

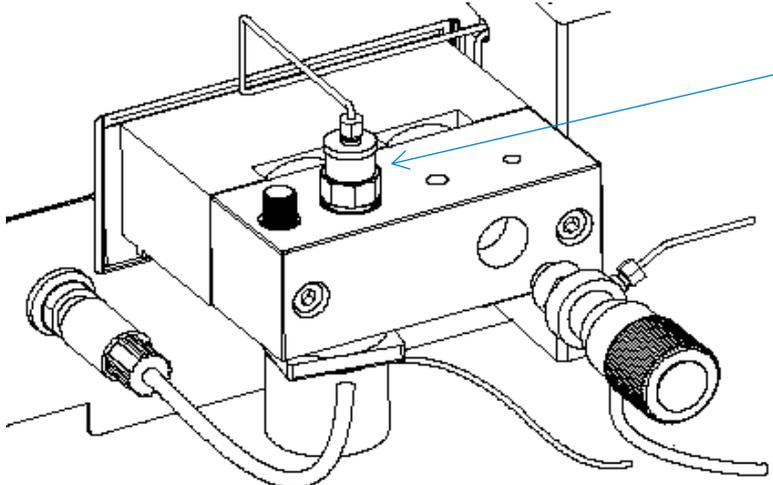
Pump Care

Inlet Valve

- If pressure ripple is unstable, the active inlet valve cartridge may be dirty
- Inlet valve issues can lead to
 - poor pump performance
 - detector baseline noise
 - unstable system pressure
 - poor retention-time precision

Pump Care

Outlet Ball Valve



14 mm wrench
8710-1924

Pump Care

Outlet Ball Valve

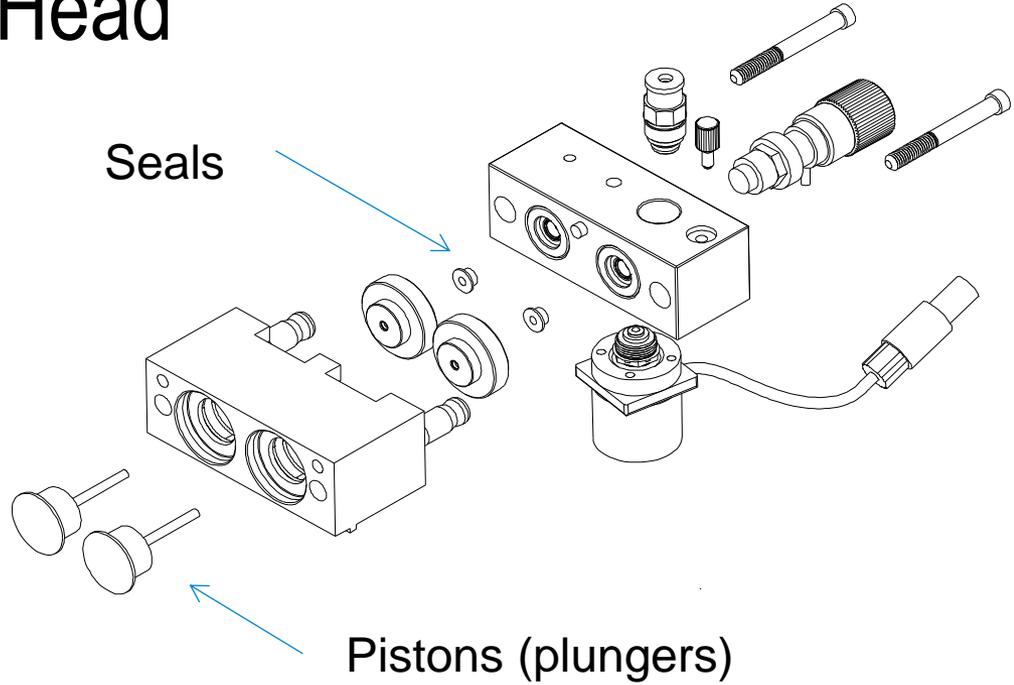
- A failing outlet ball valve causes backflow of solvent
 - poor pump performance
 - detector baseline noise
 - unstable system pressure (pressure ripple),
 - poor retention-time precision
- Outlet valves on older Agilent LC's have a separate gold seal cap, which can still be replaced
- However, the current valve design has an integrated gold seal



5067-4728

Pump Care

Pump Head



5064-8211

Pump Care

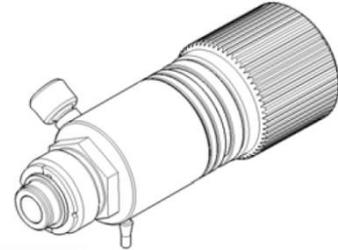
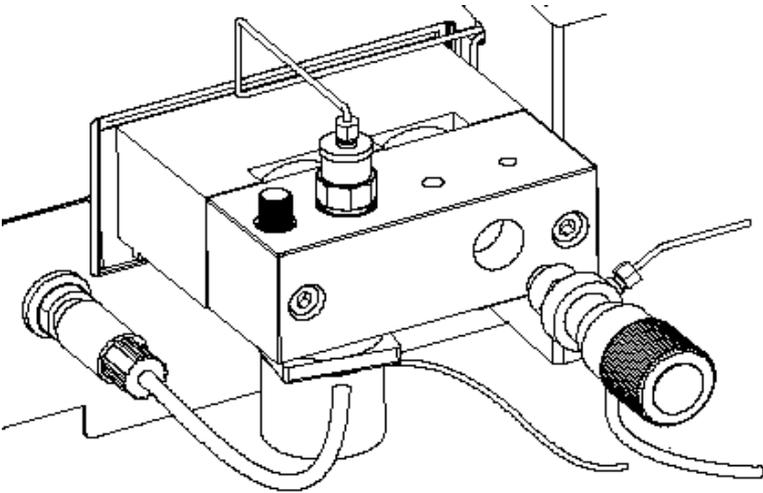
Pump Head



- Perform seal wear-in procedure after installation of black reversed-phase seals
- Replace on a regular basis, before there is a problem
- Set up a replacement schedule for your instrument based on usage and mobile phase composition
- Leaking pump seals can lead to
 - poor pump performance
 - unstable system pressure (pressure ripple)
 - Detector baseline noise
 - poor retention-time precision

Pump Care

Purge Valve



14 mm wrench

Seal cap
5067-4728

PTFE frits
01018-22707

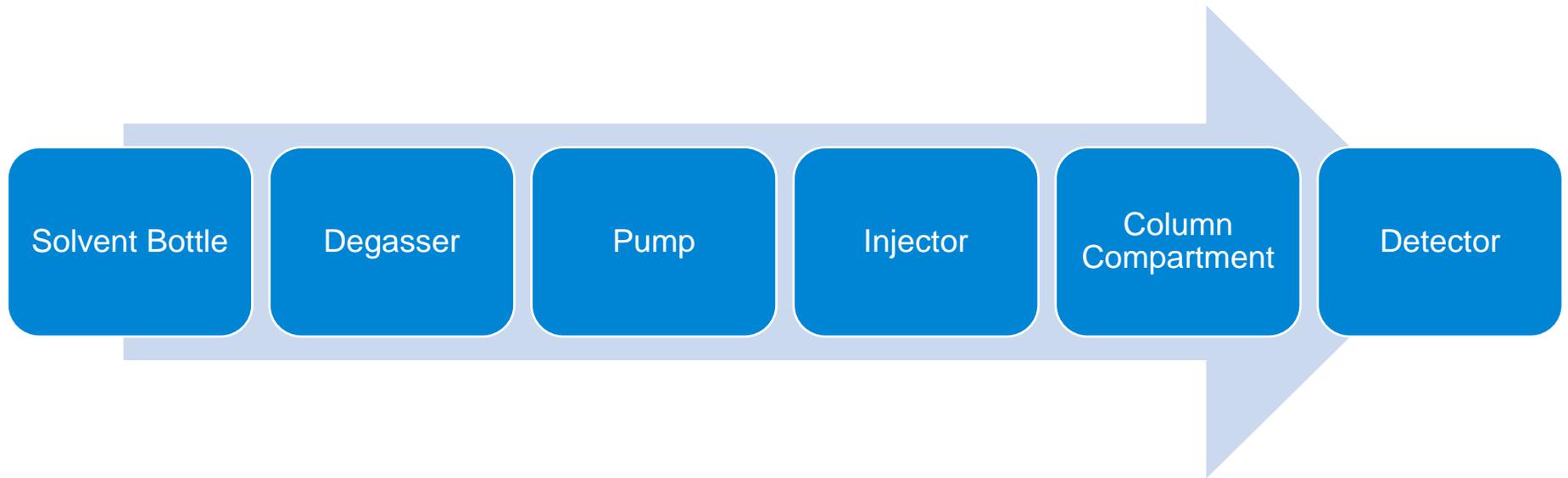
Pump Care

Purge Valve

- Dirty frit in the purge valve often a source of high pressure
- A pressure drop of >10 bar across the frit (5 mL/min water with purge valve open) could indicate a blockage
- Change at the same time and after changing pump seals



Your Instrument's Flow Path



Autosampler Care

- Two main types
 - Standard: gripper arm moves vial to needle
 - Well Plate (HiP): needle arm moves to the sample
- Five main maintenance points:
 - Needle
 - Loop capillary
 - Needle seat
 - Injection valve rotor seal
 - Metering device seal



Autosampler Care Standard ALS



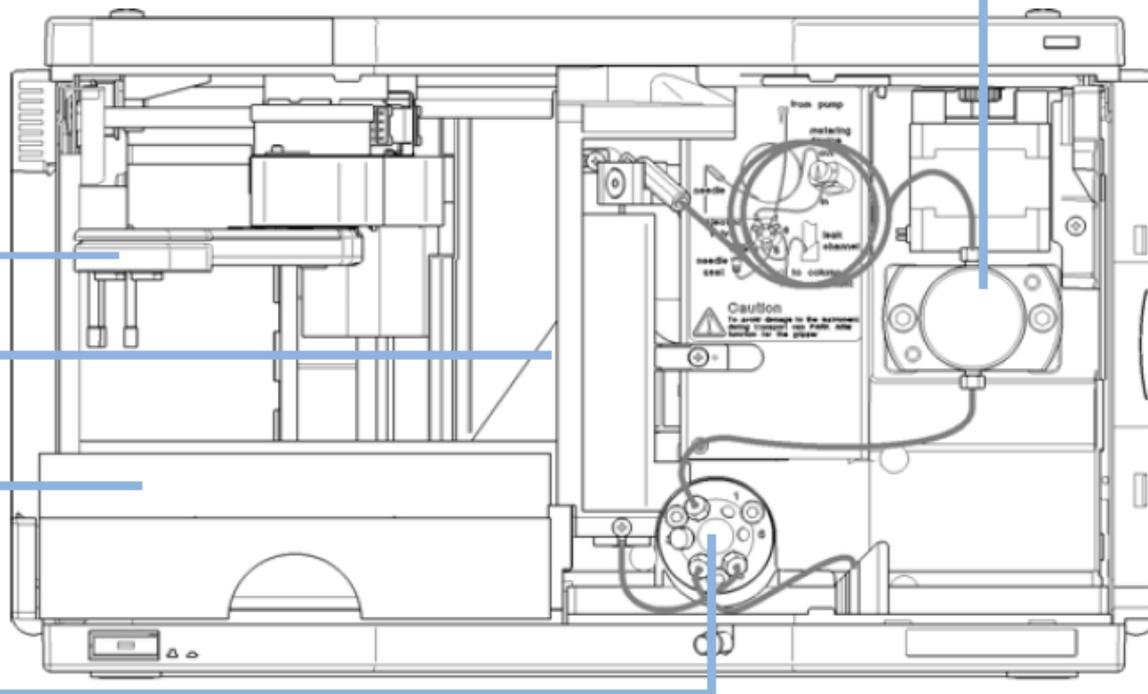
Analytical head

Gripper arm

Needle seat

Vial tray

Switching valve



Autosampler Care

Well Plate ALS



Metering device

Loop capillary

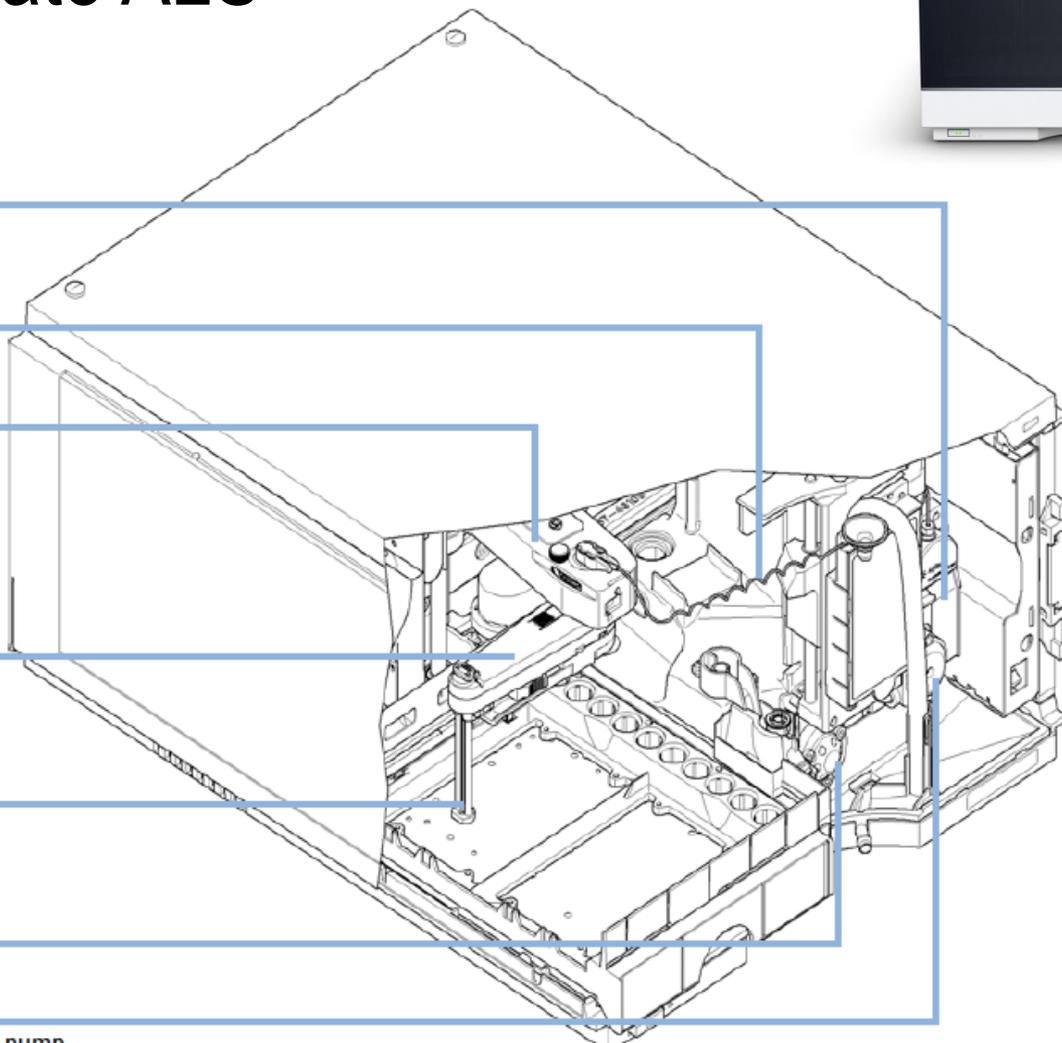
Transport assembly

Needle carrier

Needle

Injection valve

Needle wash peristaltic pump



Autosampler Care

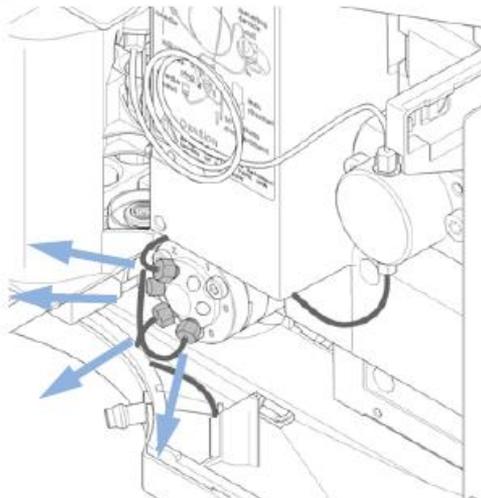


- Exchange the rotor seal after approximately 30000 to 40000 injections
- or when injection reproducibility or leakage indicates wear
- Exchange the metering seal when autosampler reproducibility indicates seal wear

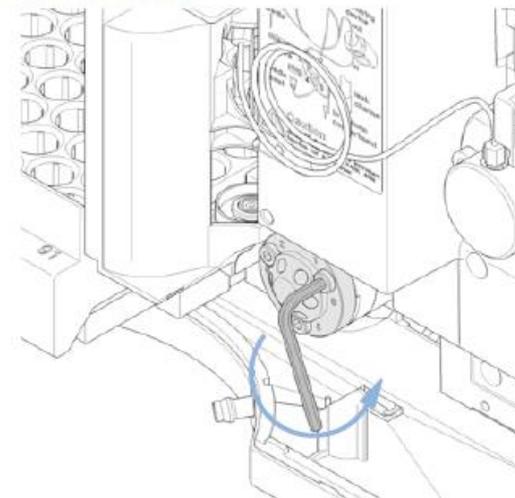
Autosampler Care



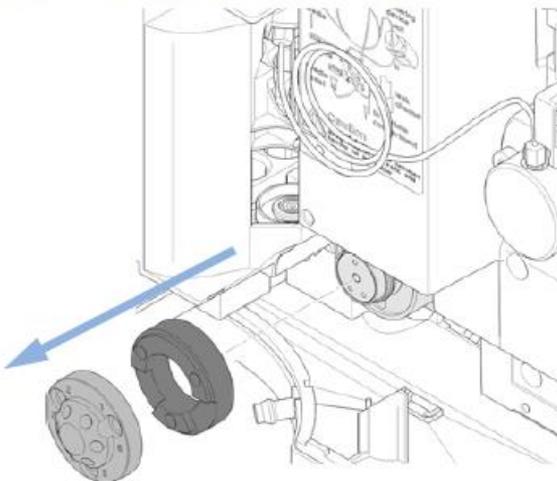
1 Remove all capillary fittings from the injection-valve ports.



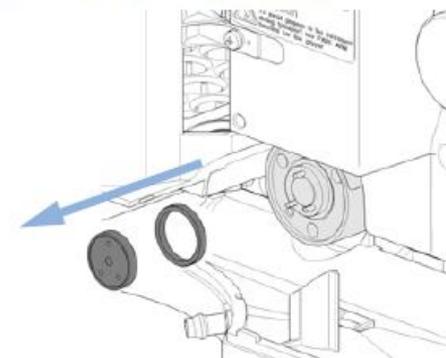
2 Loosen each fixing bolt two turns at a time. Remove the bolts from the head.



3 Remove the stator head and stator ring.



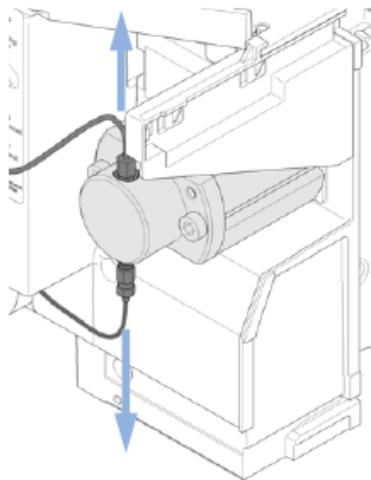
4 Remove the rotor seal and isolation seal.



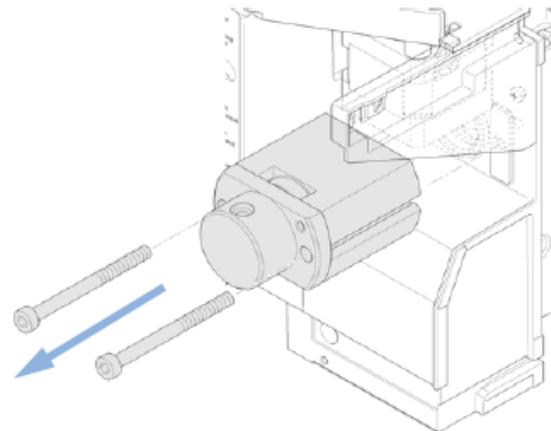
Autosampler Care



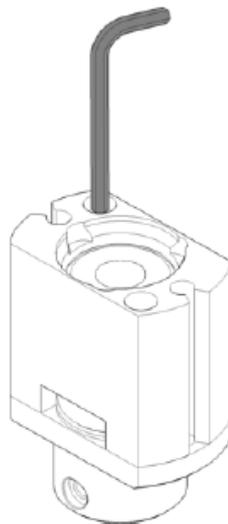
1 Remove the two capillaries from the metering-head assembly.



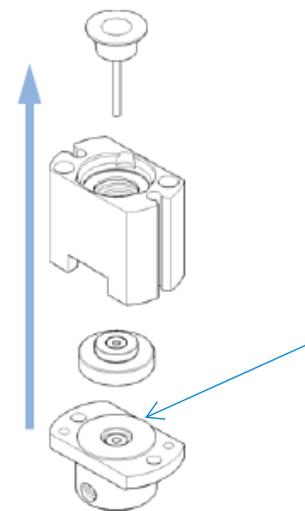
2 Remove the two fixing bolts, and pull the head assembly away from the sampler. Notice that the closed side of the metering head faces upwards.



3 Remove the two fixing bolts from the base of the metering head assembly.



4 Disassemble the metering head assembly.

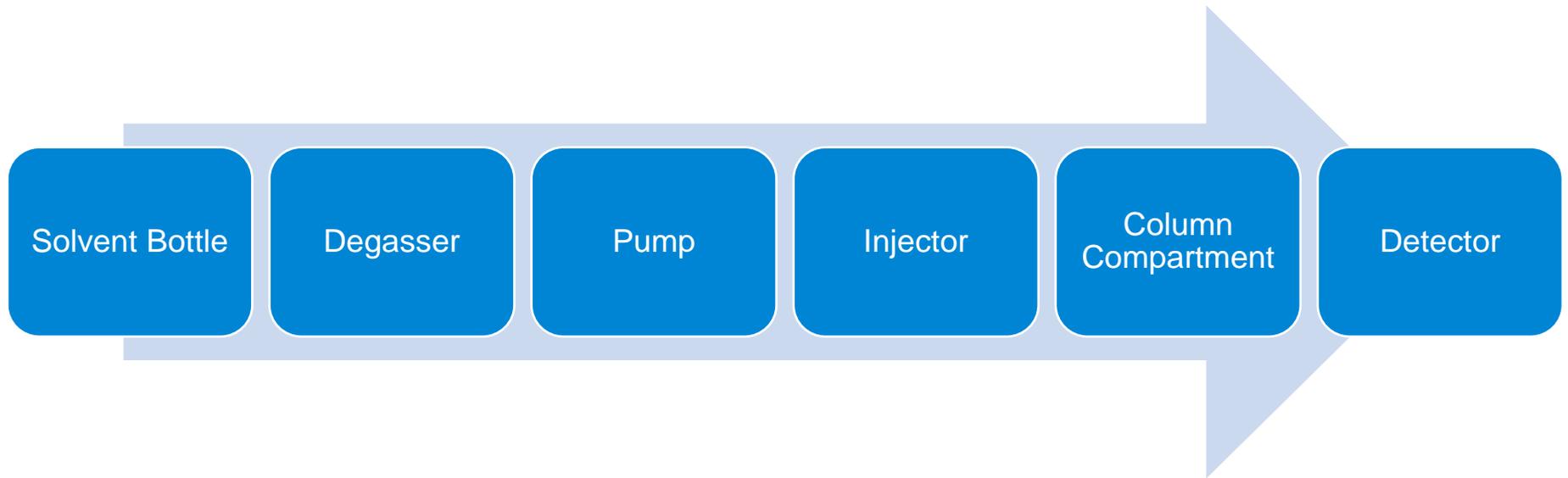


Autosampler Care

- Agilent autosamplers use wide opening (9 mm) vials
- Choose Agilent Certified vials
 - Tested for full compatibility
 - Vial neck and shoulder are proper height
 - Competitors do not meet our exact specifications



Your Instrument's Flow Path



Filtration

What causes high pressure?

- Particulates in mobile phase
 - Improperly filtered buffer solutions
 - Buffer precipitation
 - Microbial growth
 - Seal debris
- Particulates in the sample
 - Precipitated sample (sample solvent-mobile phase mismatch)
 - Insoluble matrix components

Common blockage points

- Purge valve frit
- Autosampler needle/needle seat
- Column frit

Filtration

Filter Selection

- Consider the particle size of your column
- Inlet frit on columns with particle size 2.7 or larger is nominally 2 μm
- Smaller on sub-2 μm columns
- Choose 0.2 μm filters when using sub-2 μm columns
- 0.45 μm acceptable for larger particle size columns
- Check the compatibility of the filter with your mobile phase/sample solvent



Filtration

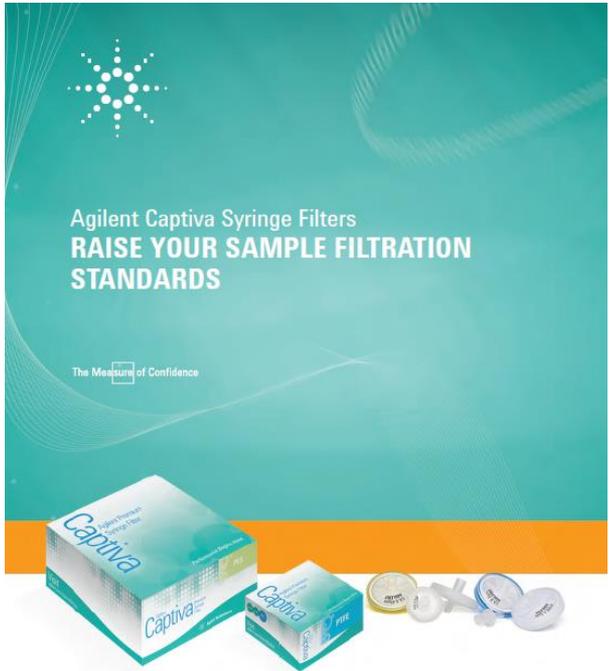
Legend	Polypropylene membrane	Polyethersulfone membrane	Cellulose Acetate membrane*	Polytetrafluorethylene membrane	Regenerated Cellulose membrane	Nylon membrane	Glass Fiber membrane*	Housing Methacrylate Butadiene Styrene	Housing Polypropylene
Filter	PP	PES	CA	PTFE	RC	Nylon	GF	MBS	PP
Housing									
Solvents									
Acetone	**	—	—	**	**	**	**	—	**
Acetonitrile	*	—	—	**	**	N/A	**	—	**
Benzene	—	—	*	**	**	**	**	—	**
Benzyl alcohol	**	—	—	**	**	**	**	—	*
n-Butyl acetate	N/A	—	—	**	**	**	**	—	**
n-Butanol	**	*	*	**	**	**	**	**	**
Carbon tetrachloride	*	—	—	**	**	**	**	—	—
Chloroform	*	—	—	**	**	**	**	—	**
Cyclohexane	**	—	*	**	**	**	**	*	*
Diethylacetamide	**	—	—	**	**	**	**	—	**
Diethyl ether	*	—	*	**	**	**	**	—	**
Dimethyl formamide	**	—	—	**	*	*	**	—	*
Dimethylsulfoxide	**	—	—	**	**	**	**	—	**
Dioxane	*	—	—	**	**	**	**	—	**
Ethanol, 98%	**	**	*	**	**	**	**	—	*
Ethyl acetate	*	—	—	**	**	**	**	—	*
Ethylene glycol	**	**	*	**	**	**	**	**	**
Formamide	N/A	**	—	**	*	**	**	**	**
Gasoline	*	*	*	**	**	**	**	**	**
Glycerin	**	**	*	**	**	**	**	*	*
n-Heptane	—	**	*	**	**	**	**	*	**
n-Hexane	—	**	*	**	**	**	**	*	*
Isopropanol	**	**	*	**	**	**	**	—	**
Isopropyl acetate	N/A	—	—	**	**	**	**	—	**
Methanol, 30%	**	**	N/A	**	**	**	**	**	**
Methanol, 98%	**	*	—	**	**	**	**	**	*
Methyl acetate	*	—	—	**	**	**	**	—	*
Methylene chloride	*	—	—	**	**	**	**	—	**

*CA and GF membranes in MBS housing for 28 mm size

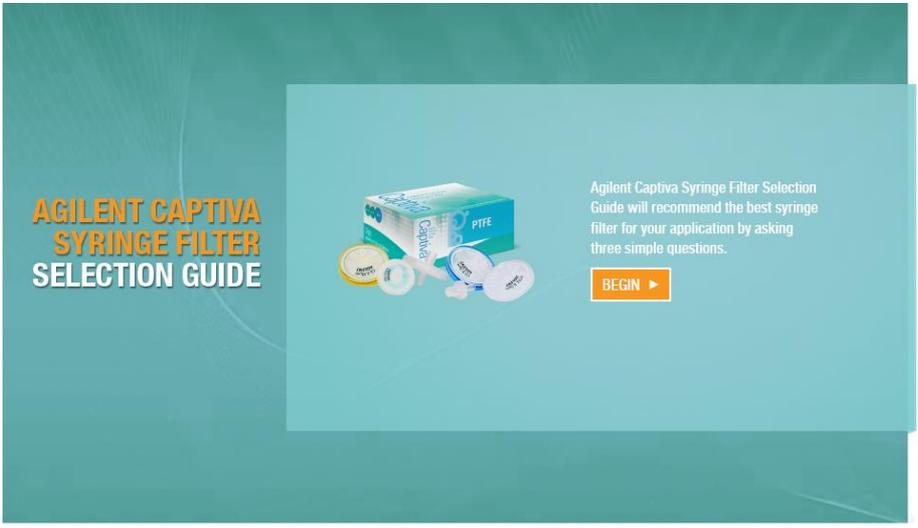
Contact time:24 hours at 20 °C

Chemical compatibilities can be influenced by various factors. Therefore, we recommend that you confirm compatibility with the fluid you want to filter before performing a pilot filtration run before you start your actual filtration.

Filtration – Agilent Captiva



5991-1230EN



filtrationselectiontool.chem.agilent.com

Filtration

In-Line Filters



RRLC in-line filter
0.2 µm pore size filter, max 600 bar
4.6 mm ID, 5067-1553
2.1 mm ID, 5067-1551

1290 Infinity LC in-line filter, 0.3 µm,
1200 bar, 5067-4638

- In-line filters can help extend the life of your column
- Not intended to be a replacement for good sample cleanup

Capillaries

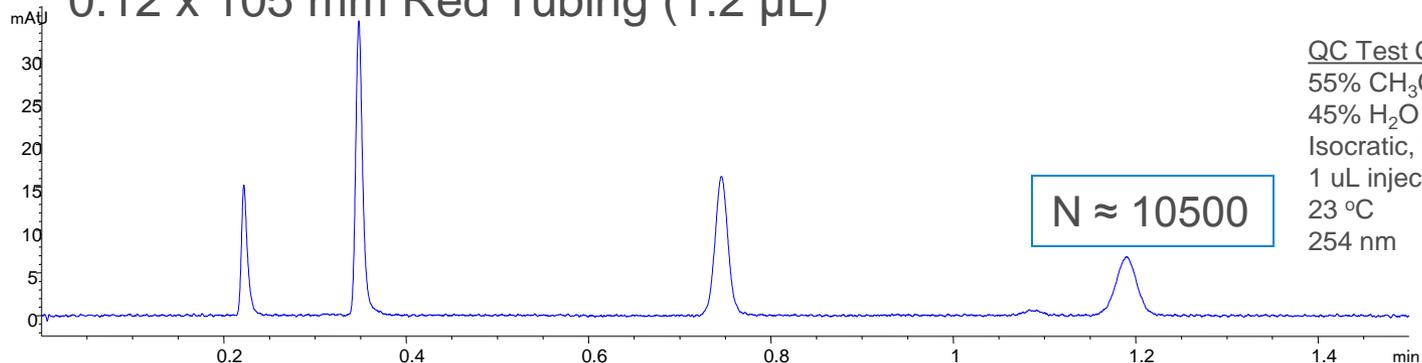


- Capillary internal diameters
 - 0.17 mm ID
 - 0.12 mm ID
 - Color coded
- Know what is on your instrument
- Replace with the same ID and length
- Tubing volume and system volume
- Changes can affect peak shape and retention times

Internal diameter (mm)	Color
0.015	Orange
0.025	Yellow
0.05	Natural PEEK
0.075	Black
0.1	Purple
0.12	Red
0.17	Green
0.20/0.25	Blue
0.3	Grey
0.50 to 0.53	Bone white

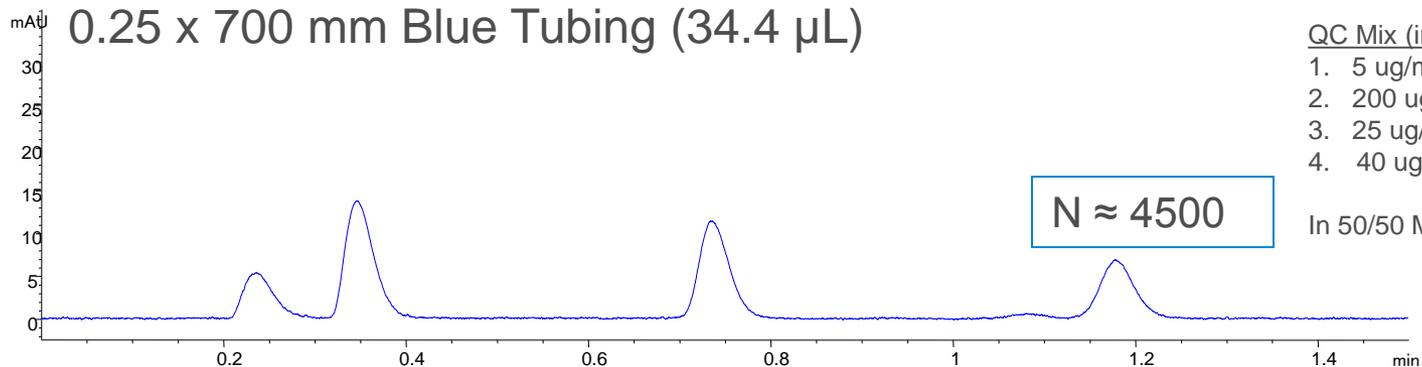
Capillaries

0.12 x 105 mm Red Tubing (1.2 μ L)



QC Test Conditions:
55% CH₃CN
45% H₂O
Isocratic, 0.6 mL/min
1 μ L injection of QC Mix
23 °C
254 nm

0.25 x 700 mm Blue Tubing (34.4 μ L)



QC Mix (in elution order):
1. 5 μ g/mL uracil
2. 200 μ g/mL phenol
3. 25 μ g/mL 4-chloro-nitrobenzene
4. 40 μ g/mL naphthalene

In 50/50 MeCN/Water

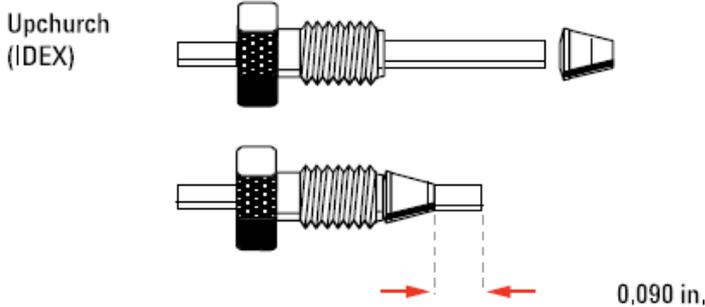
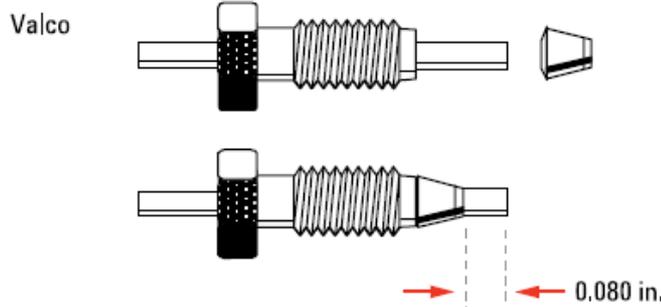
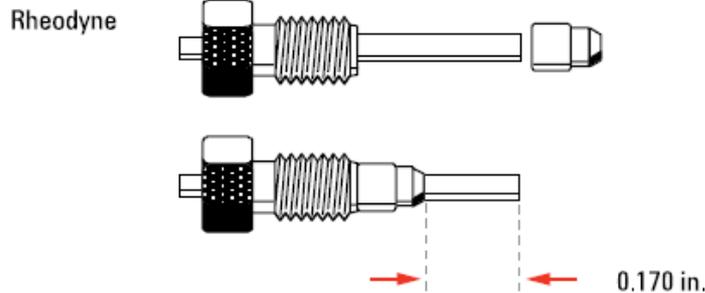
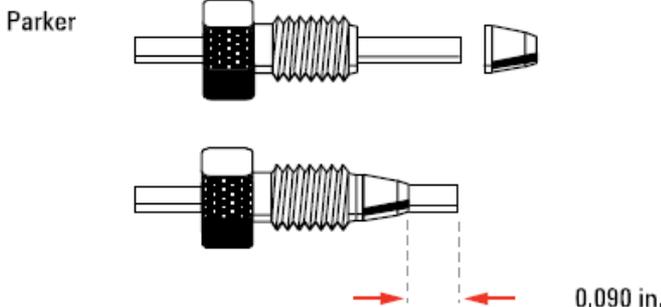
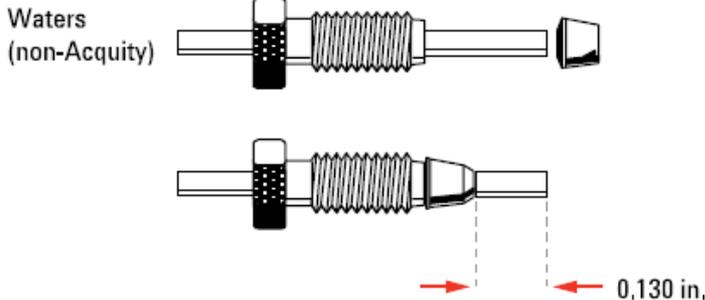
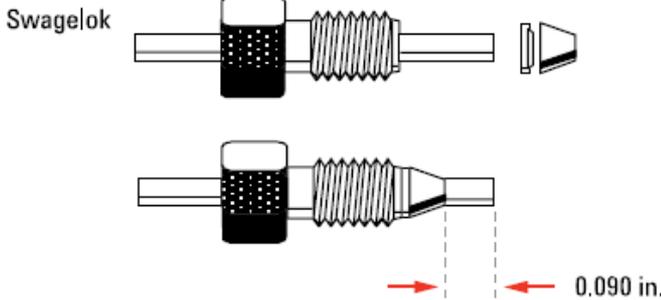
- 2.1 x 50 mm, 1.8- μ m Eclipse Plus C18
- Peak broadening when larger volume tubing installed between autosampler & column
- 43% of the efficiency is lost with too much extra column volume

Fittings

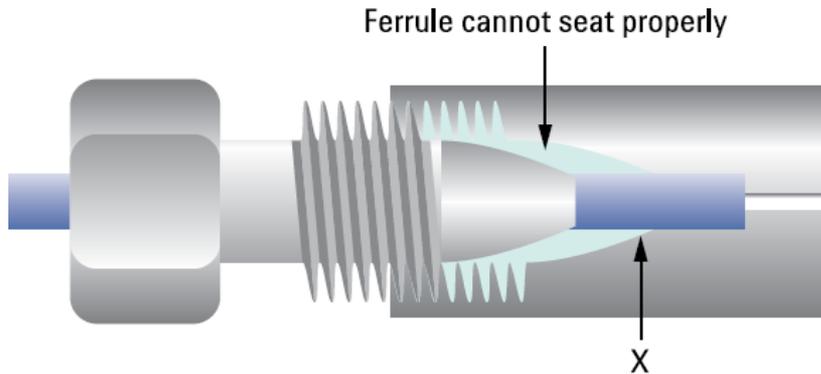
- Problems with improper stainless steel tubing connections
 - Source of leaks
 - Mistaken for chromatography issues
- Different manufacturers supply different types of fittings
- Use the fittings recommended for your system
- Agilent LC systems use Swagelok-type fittings for most connections
- Polymeric finger tight fittings can be a convenient alternative for column connections
 - PEEK (< 400 bar)
 - Polyketone (up to 600 bar)



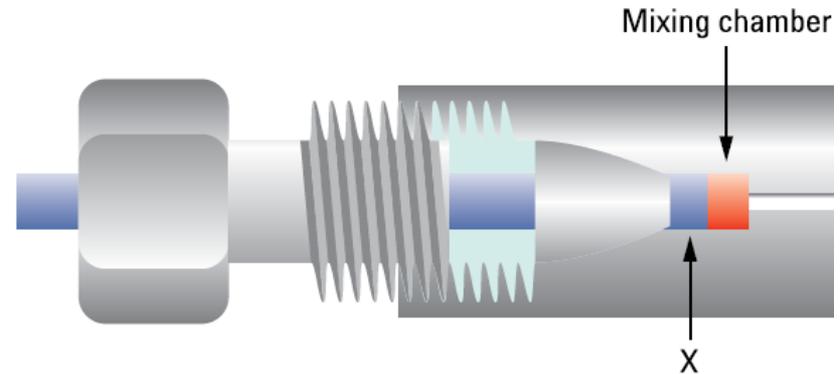
Fittings



Fittings



If dimension X is too long, leaks will occur



If dimension X is too short, a dead-volume or mixing chamber, will occur

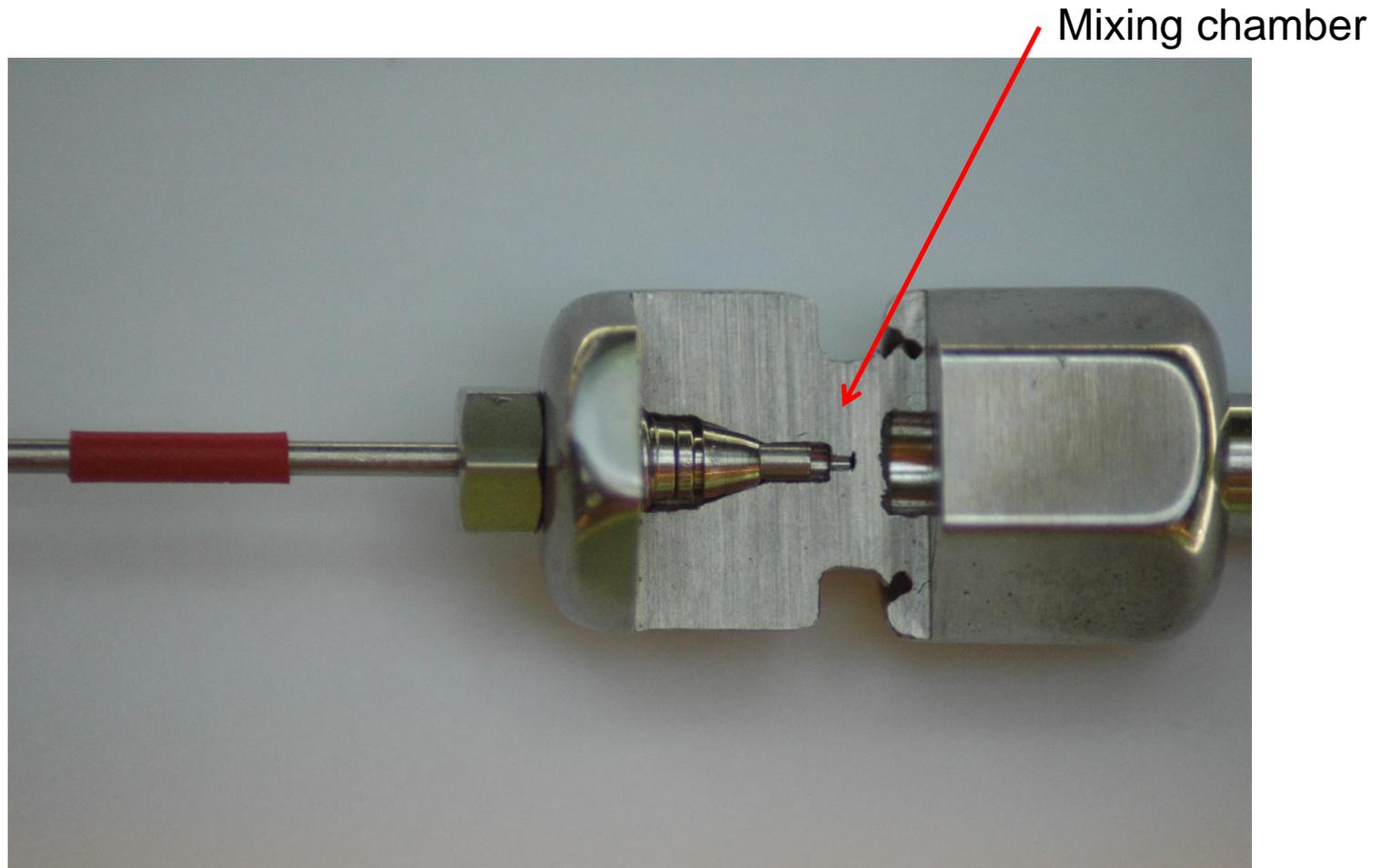
- **This will broaden or split peaks or cause tailing**
- **It will typically affect all peaks, but especially early eluting peaks**

Fittings



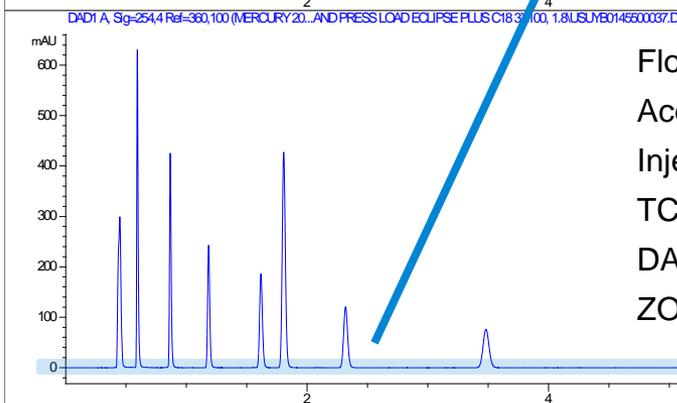
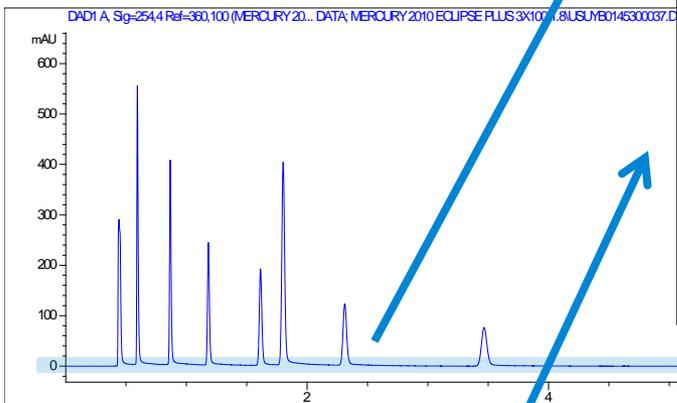
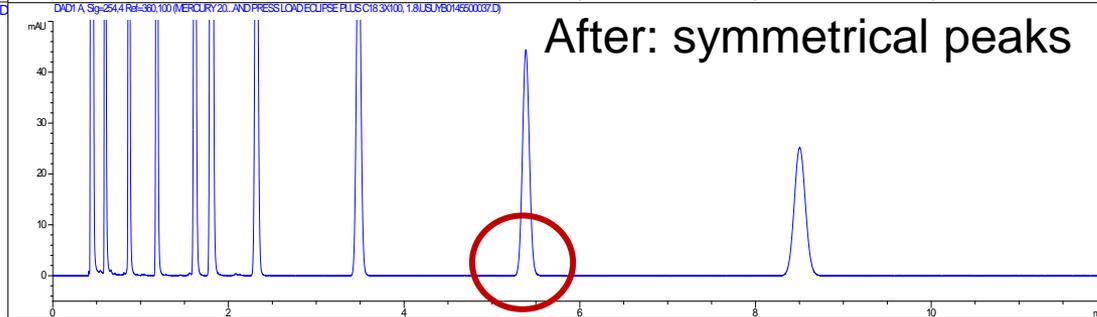
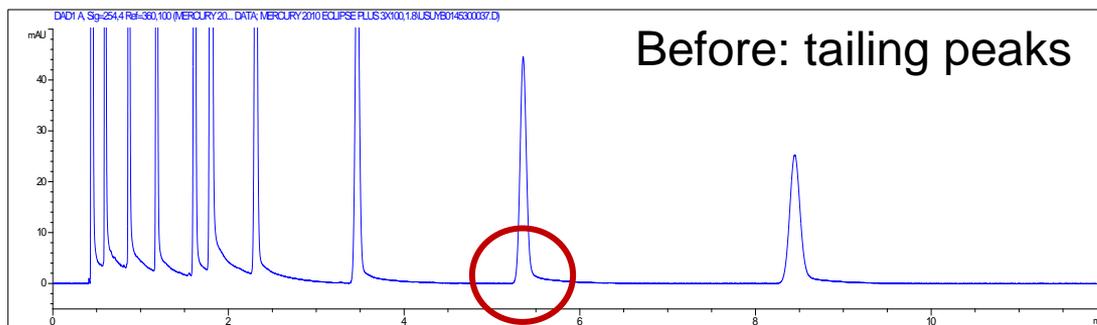
Proper Fit

Fittings



Fitting Mismatch

Fittings



Flow Rate: 0.833 mL/min
Acetonitrile/Water (60:40)
Injection Volume: 2.00 μ L
TCC: 26 $^{\circ}$ C
DAD: Sig=254,4nm Ref=360,100nm
ZORBAX Eclipse Plus C18, 3.0 mm x 100 mm, 1.8 μ m

Swaging Your Fittings

Step 1



Select a nut that is the right length for the fitting.

Step 2



Slide the nut over the end of the tubing.

Step 3



Carefully slide the ferrule components on after the nut. Finger-tighten the assembly while making sure the tubing is completely seated in the bottom of the end fitting.

Step 4



Use a wrench to gently tighten the fitting by 1/4 to 1/2 turn where you want to connect it; this will force the ferrule to seat onto the tubing. Do not over-tighten!

Step 5

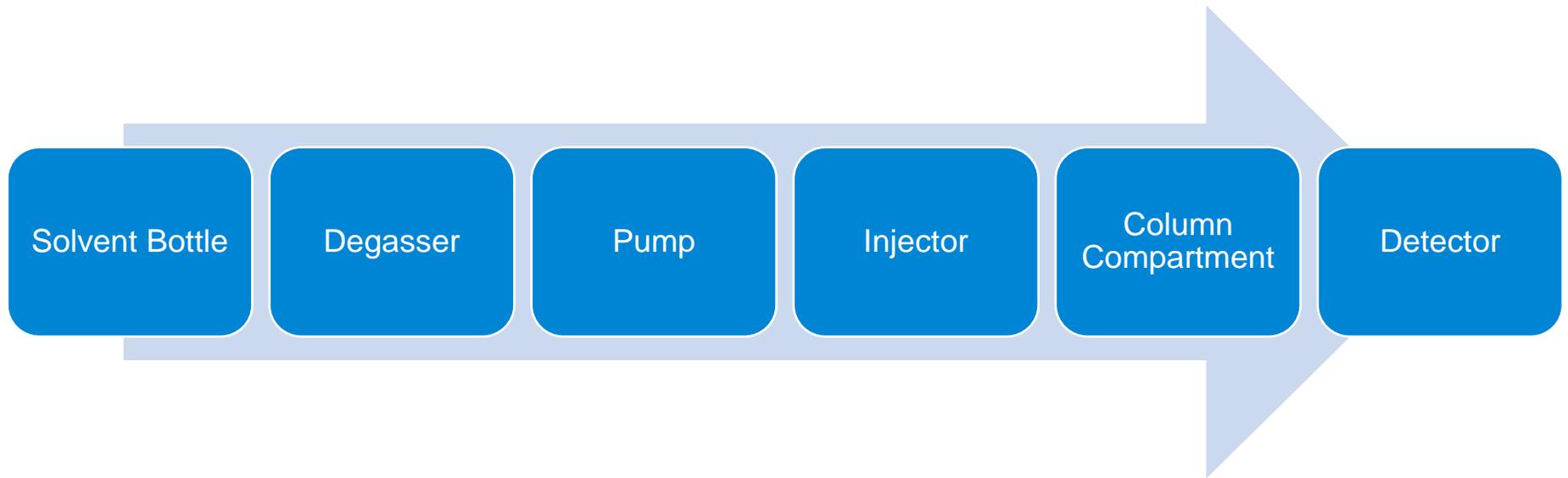


Once you are sure your fitting is complete, loosen the nut and inspect the ferrule for correct position on the tubing.



1/4 in wrench

Your Instrument's Flow Path



Detector Care

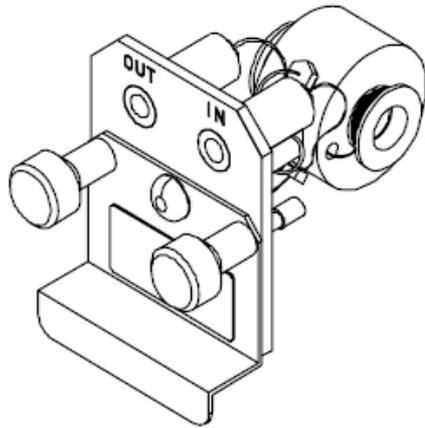
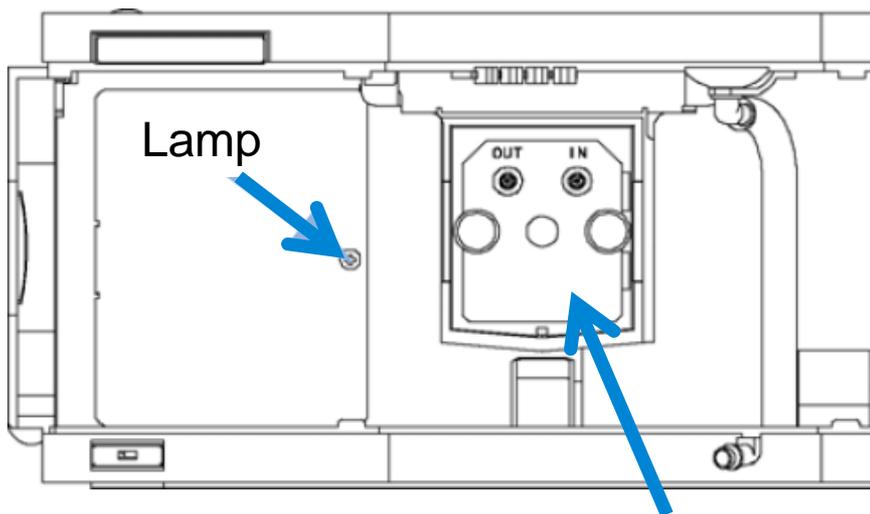
UV Detectors

- Two types
 - VWD
 - DAD/MWD
- Simple Maintenance
 - Lamp replacement
 - Flow cell cleaning or replacement
- Keep in mind the pressure rating of your flow cell – another detector fraction collector in the flow path will increase the backpressure on the flow cell
- Avoid using flow cells with quartz windows at pH 9.5 or greater
- Make sure the flow cell contains 5 or 10% organic to prevent microbial growth when not in use
- Avoid leaving buffer solutions in the flow cell which can crystallize

Detector Care

UV Detectors

VWD



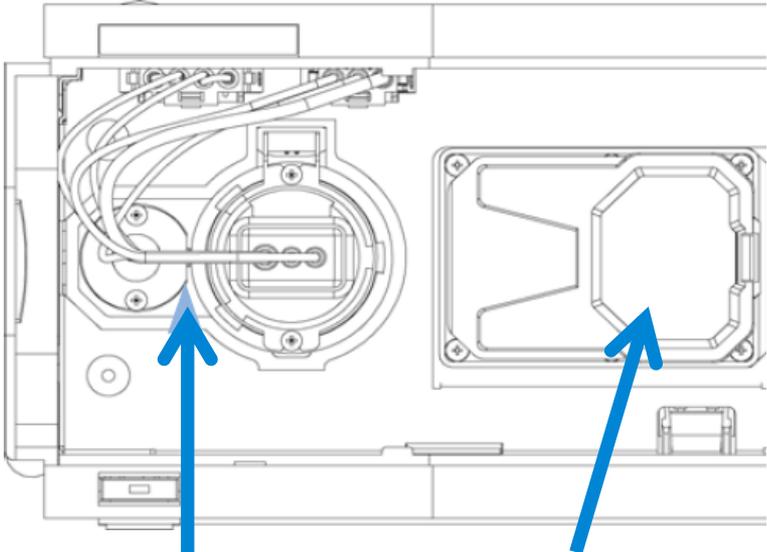
Flow Cell

Detector Care

UV Detectors



DAD

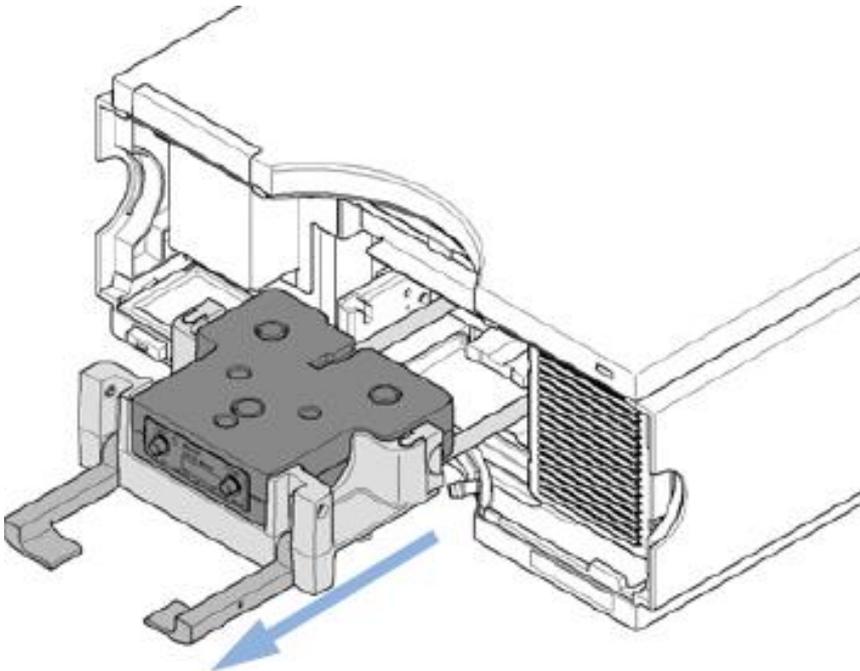


Lamps

Flow Cell

Detector Care

UV Detectors



DAD on 1290/some 1260 systems



Max-Light cartridge
(G4212A/B)

Detector Care Lamps

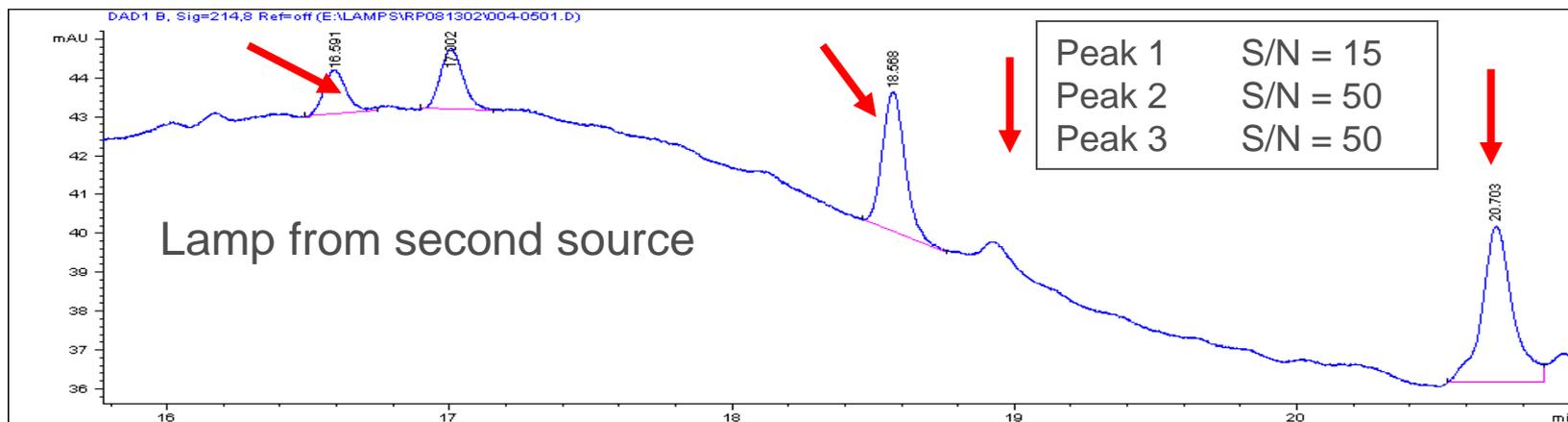
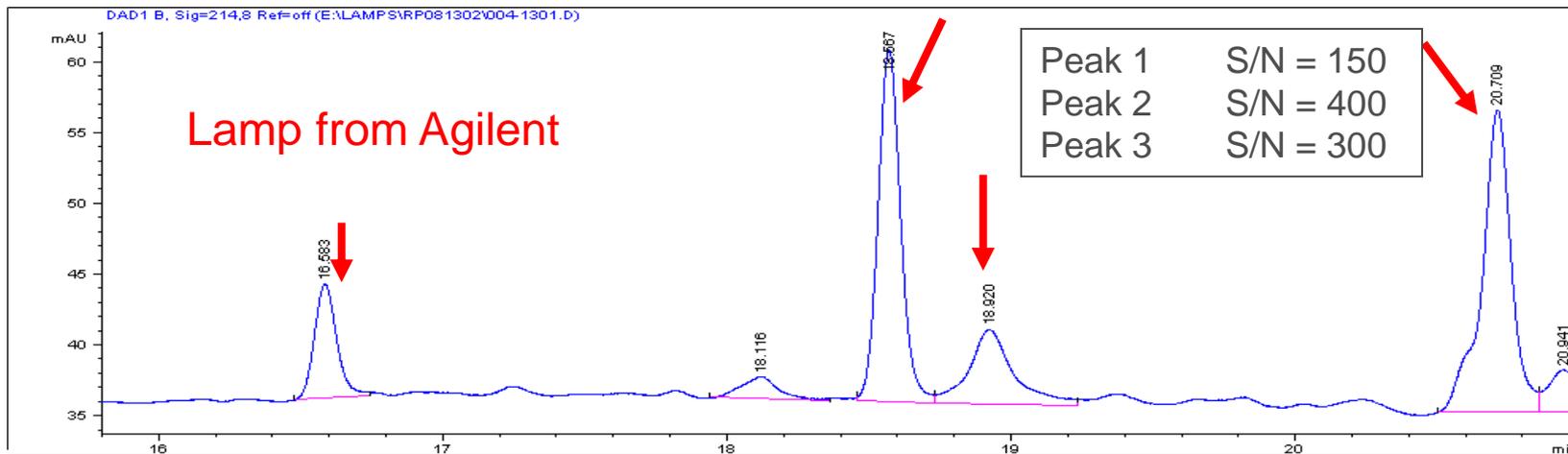
Agilent Lamps

- designed and certified for Agilent detectors
 - much narrower aperture providing increased light intensity and decreased noise
 - higher signal-to-noise ratio
-
- VWD detectors use a single deuterium lamp
 - DAD uses deuterium and tungsten lamp
 - Long life lamps last up to 2000 hours



DAD tungsten lamp

Detector Care Lamps



Detector Care

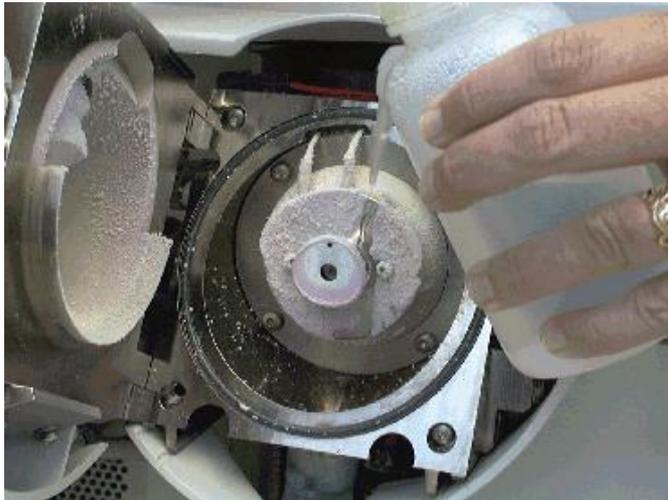
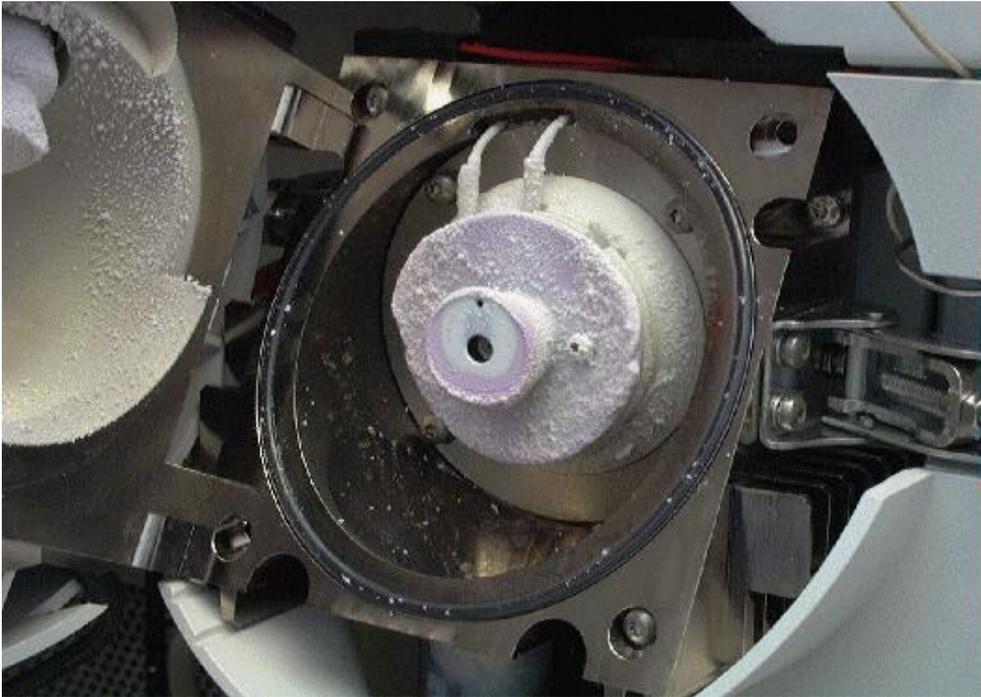
MS Detectors

Flush the nebulizer	Daily after use to flush the tubing, valves, and nebulizer
Replace the nebulizer needle	When plugged
Clean the spray chamber	Daily or when carryover is suspected
Check the rough pump fluid level	Check weekly for color and level; replace every six months



Detector Care

MS Detectors



Cleaning the spray chamber

Detector Care

MS Detectors



Clean Source



After SPE



After PPT

Summary

- Please refer to the manual for the maintenance details on the specific modules in your system
- Some parts you will want to replace on a regular basis, before there is a problem
- Some should be kept on hand in case there is a problem, but do not necessarily need to be replaced frequently
- Develop a maintenance routine that works for you
- Contact: LC-column-support@agilent.com