Preventative and Routine Maintenance for Your HPLC System

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CSD Applications Engineer
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Agilent
Trusted Answers

Preventative and Routine Maintenance for Your HPLC System

Agenda

- Tools and Resources
- Best Practices
- Daily Maintenance
- Weekly Maintenance
- Monthly Maintenance
- Quarterly and Semi-Annual Maintenance
- Annual Maintenance



Preventative and Routine Maintenance for Your HPLC System

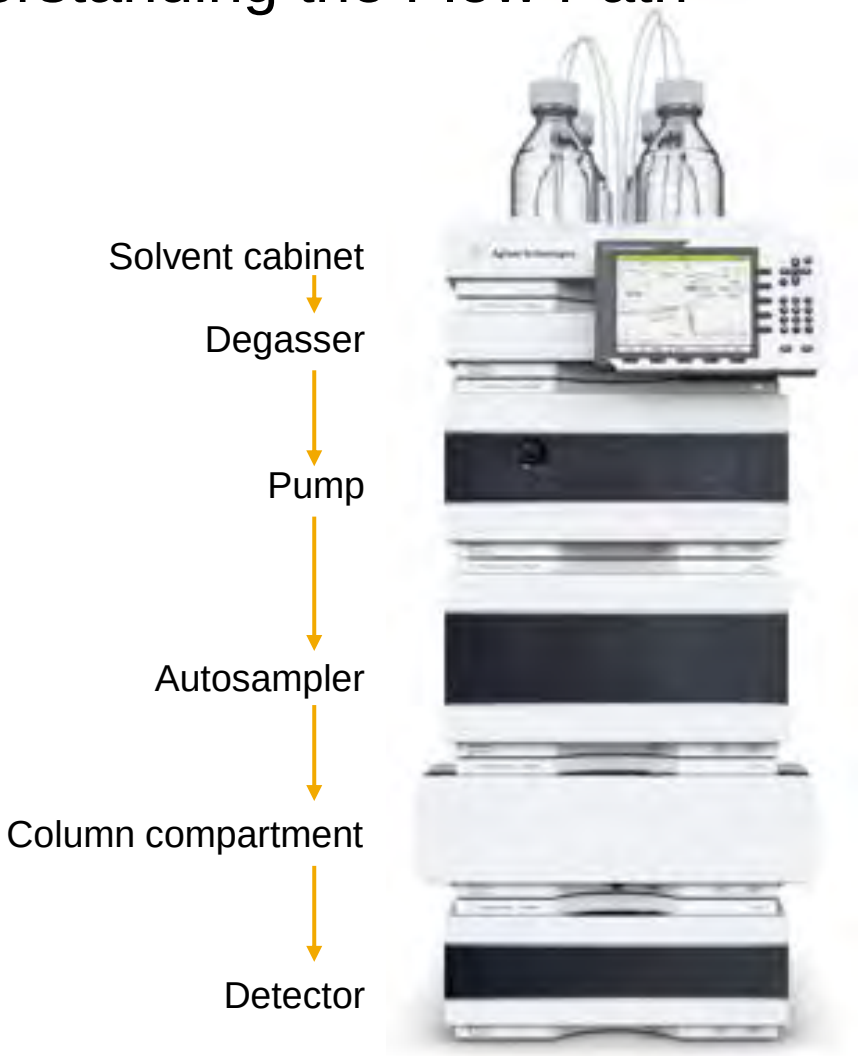
Tools and Resources

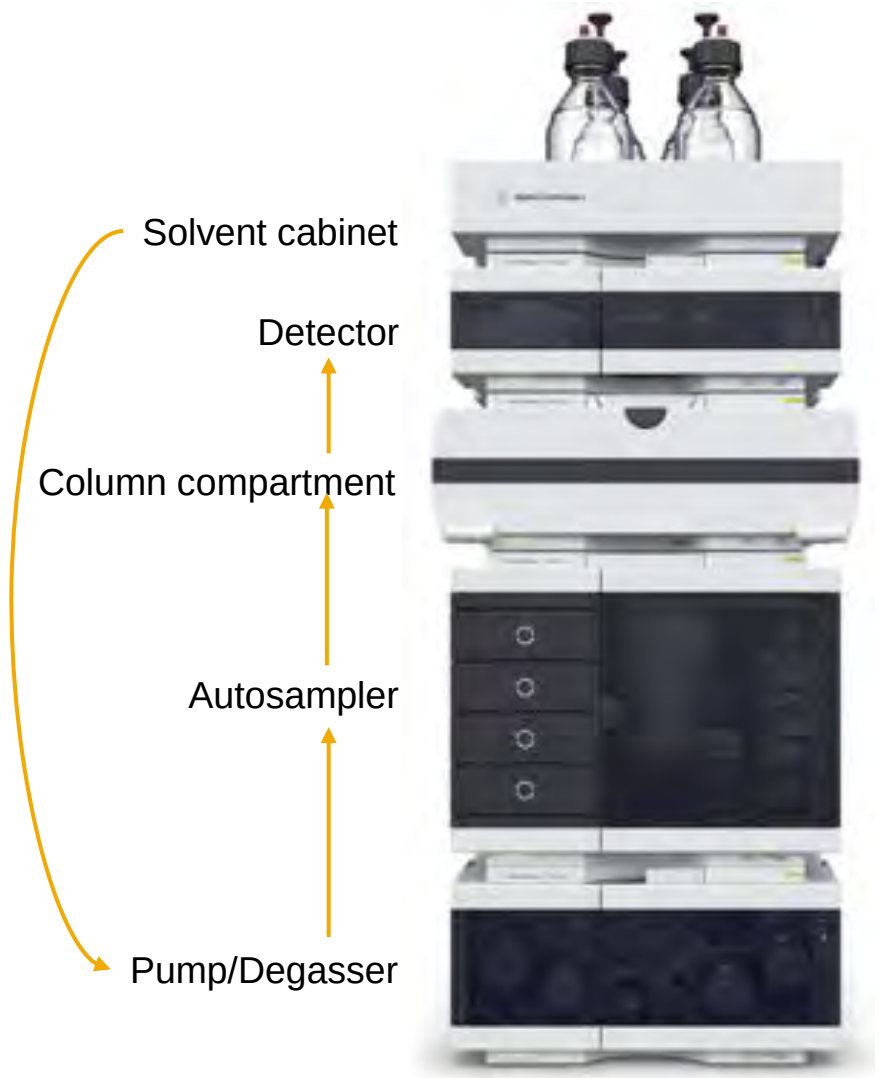


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Tools and Resources

Understanding the Flow Path





Tools and Resources

Instrument Model Numbers



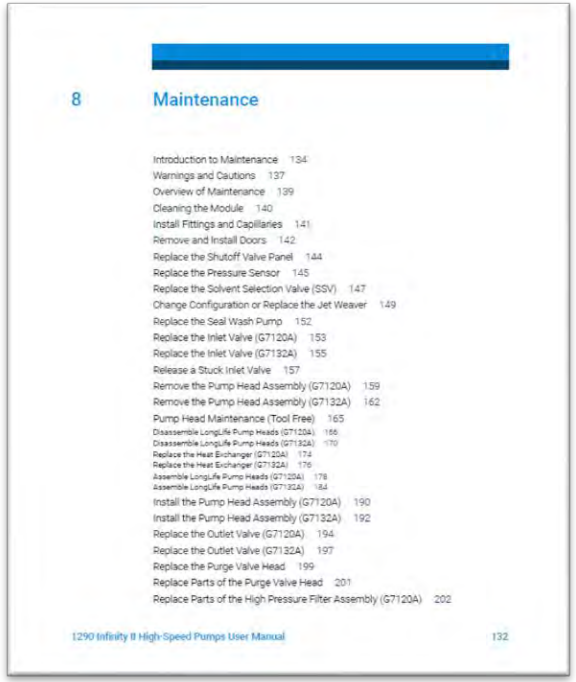


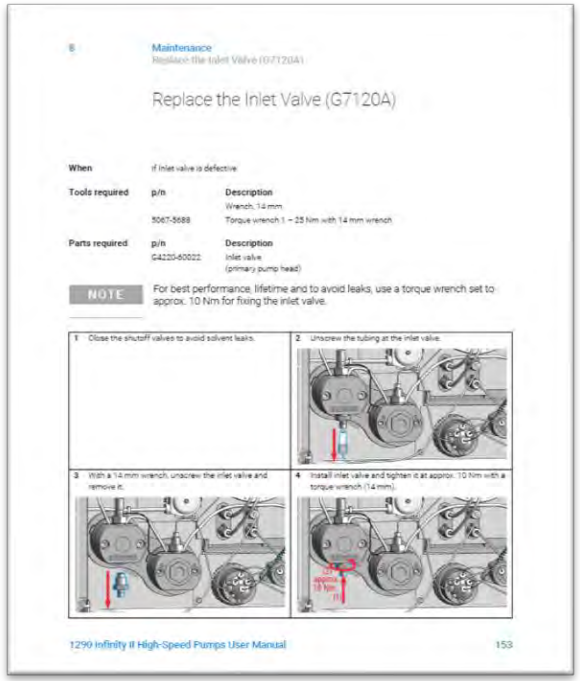
G#####A/B/C

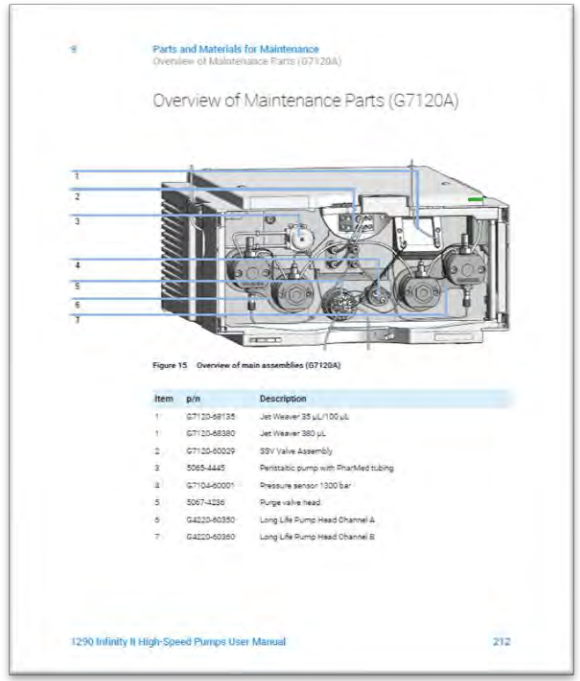
Tools and Resources

Module User Guides









User guides include information on:

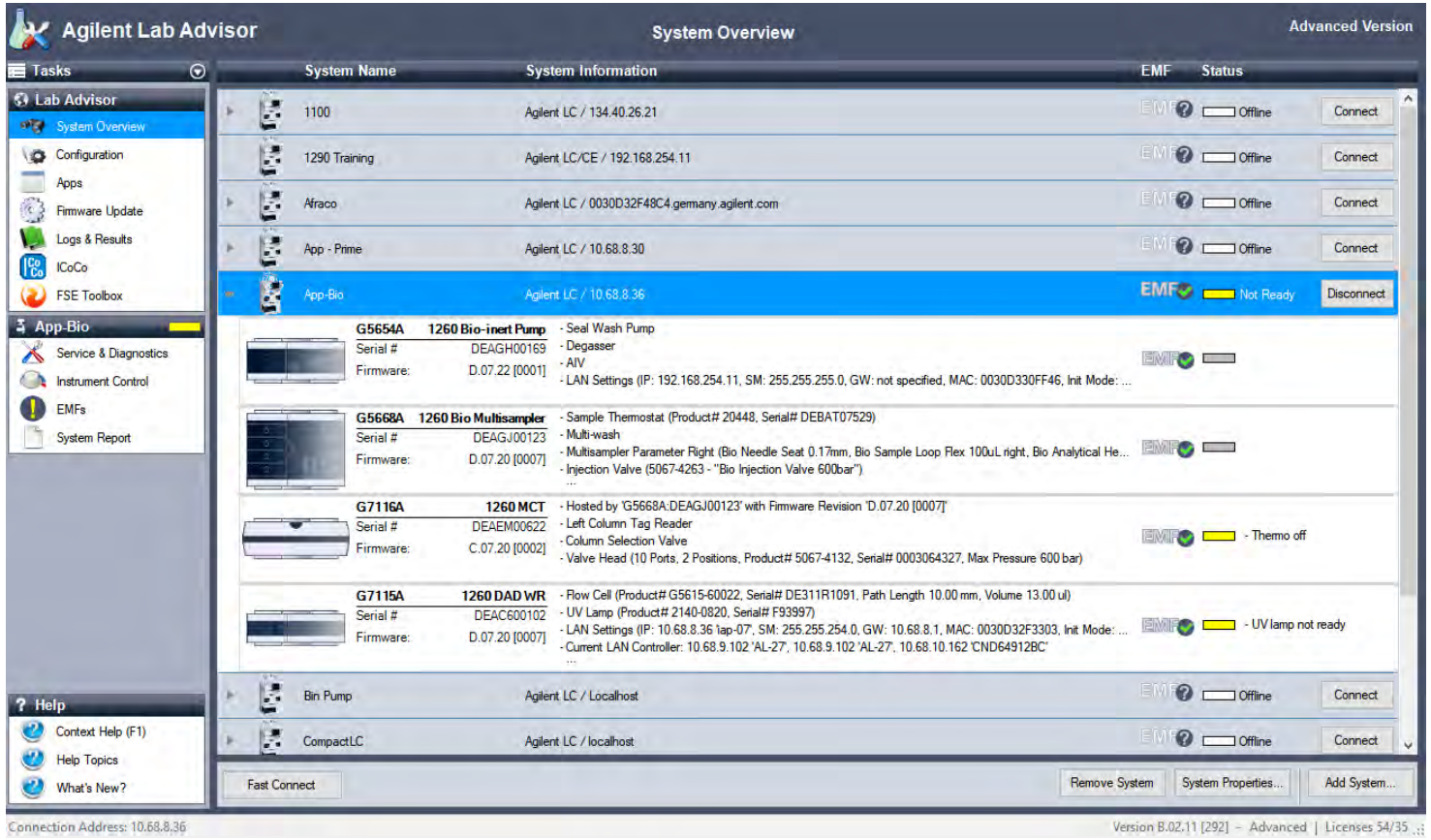
- Module specifications
- Optimization guidelines
- Detailed maintenance procedures
- List of recommended consumables for the module

HPLC User Guides

Tools and Resources

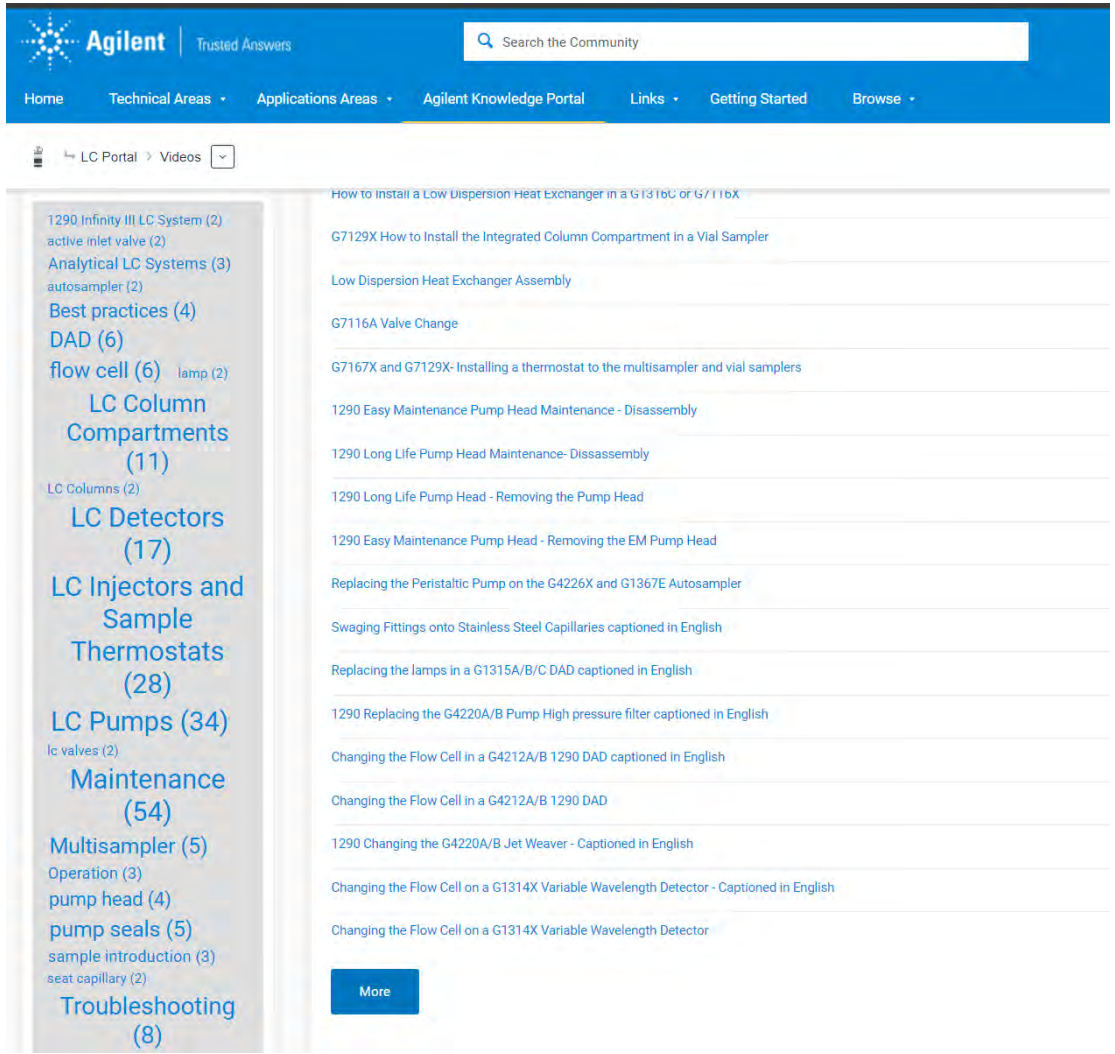
Agilent Lab Advisor

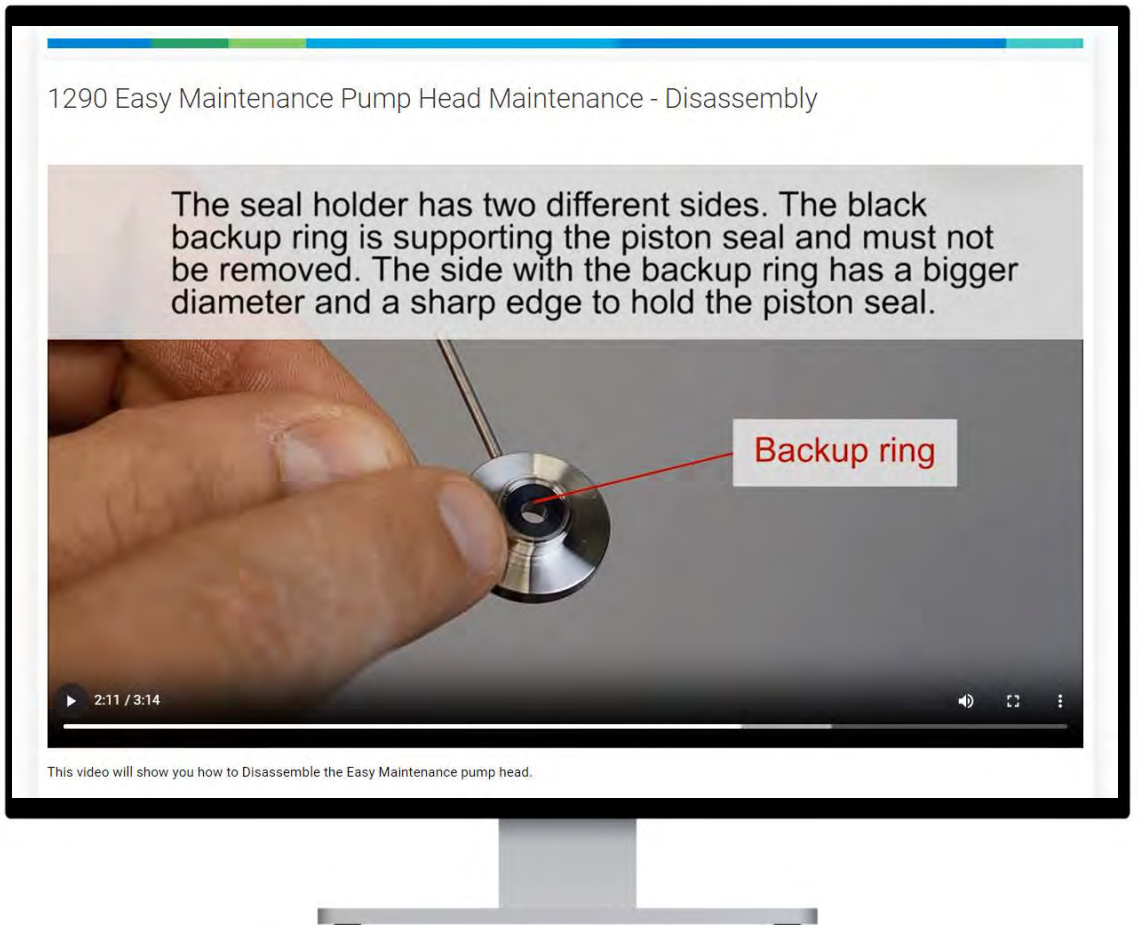
- Tools for calibration, diagnosis, and maintenance
- Daily instrument tests
- General calibration and maintenance procedures
- Advanced version also available for expert level troubleshooting
- EMF (Early Maintenance Feedback) shows the number of valve switches or pumped solvent



Tools and Resources

Agilent Community

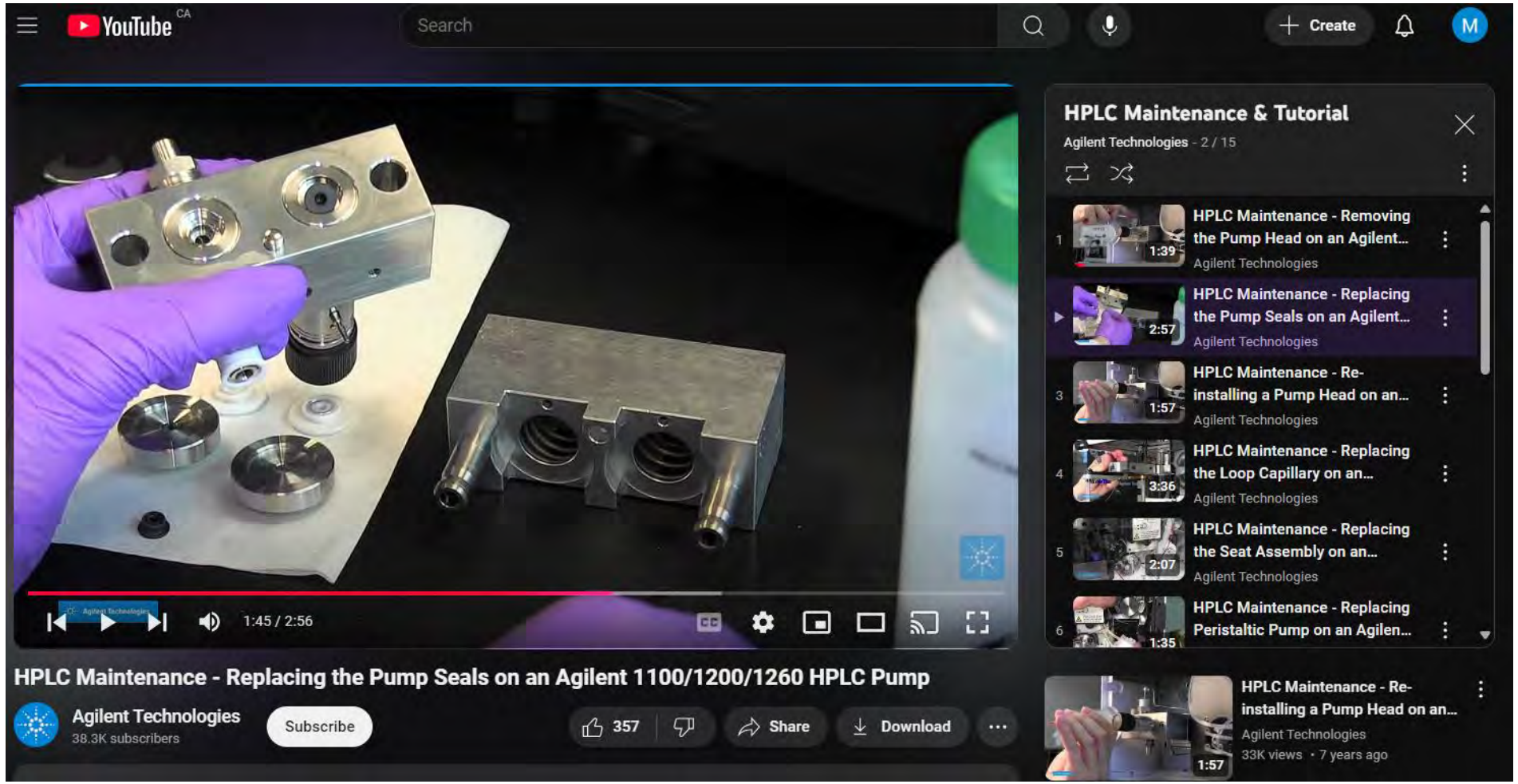




Agilent Community

Tools and Resources

Agilent YouTube Channel



[Agilent Technologies - YouTube]

Tools and Resources

Tools for Preventative Maintenance



InfintyLab system tool kit
G7120-68708



Restriction capillary, 0.12 x 2000 mm capillary for testing system pressure without a column
5022-2159



Zero-dead-volume union, for maintenance that requires taking the column offline
5022-2184



14 mm wrench, required for many maintenance procedures
8710-1924

5022-2159 | Agilent

8710-1924 | Agilent

5022-2184 | Agilent

G7120-68708 | Agilent

Preventative and Routine Maintenance for Your HPLC System

Best Practices



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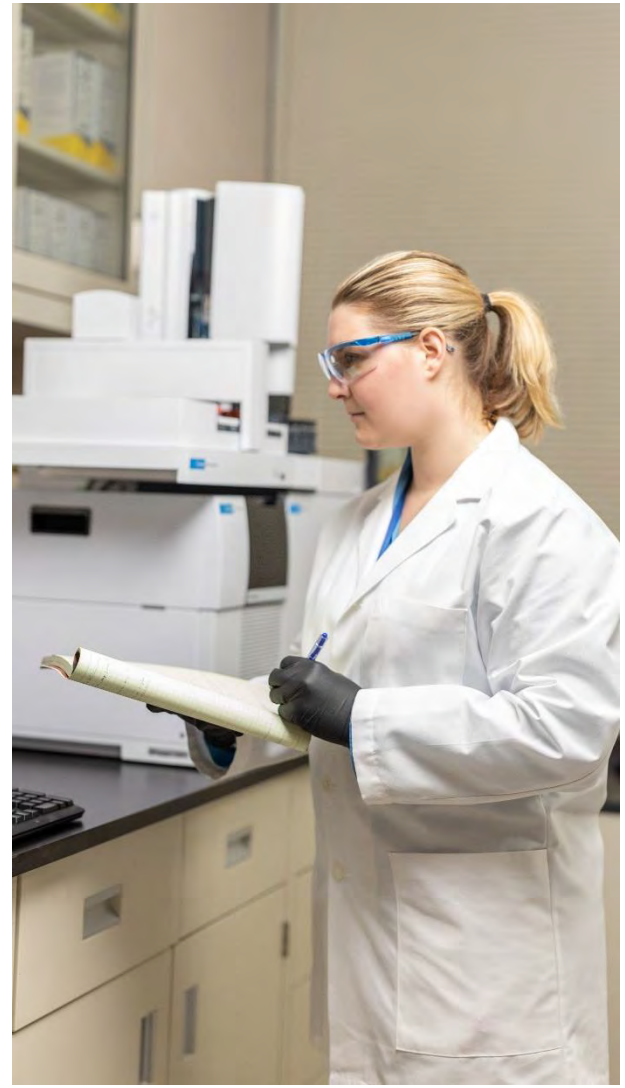
Best Practices

Documentation

Documentation is crucial for compliance and can serve as a reference for troubleshooting and future operations.

What documentation should be included:

- Methods being run on the instrument
  - Track method pressures
- Parts numbers of consumables replaced on the instrument
- Preventative maintenance
- Any abnormal performance



Best Practices

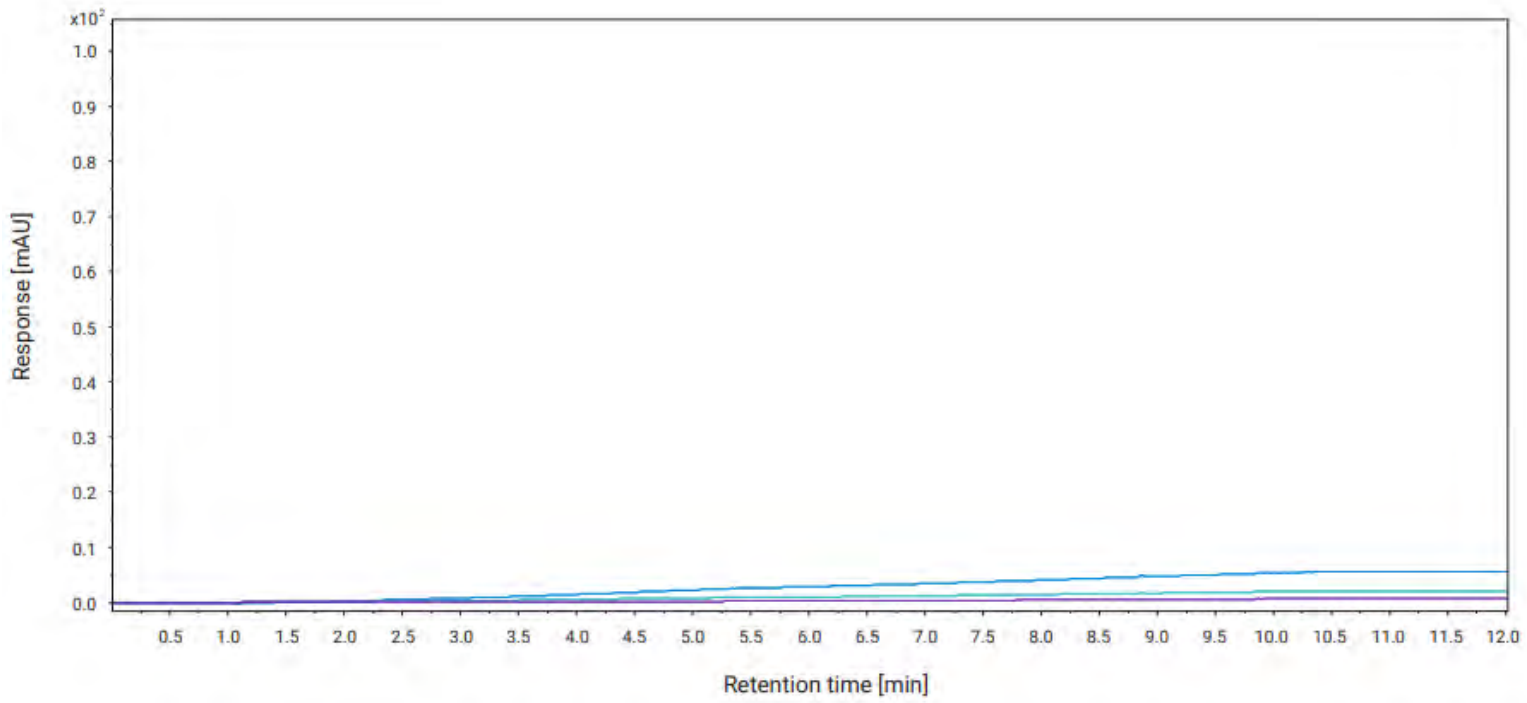
Use Quality Solvents

HPLC or LCMS grade solvents

- Lowest impurity levels, reducing ghost peaks in gradient runs
- 0.2 µm pre-filtering safeguards system from contaminants and clogging
- Highest lot-to-lot reproducibility



Water/Methanol Gradient Overlay at 210 nm, 225 nm, and 254 nm



Gradient from 5-95% ACN. Detection wavelengths 210 nm (blue), 225 nm (turquoise), and 254 nm (purple); Range: 0-100 mAU

Maximize Your Efficiency With Precision Solvents (agilent.com)

Best Practices

Solvent Safety

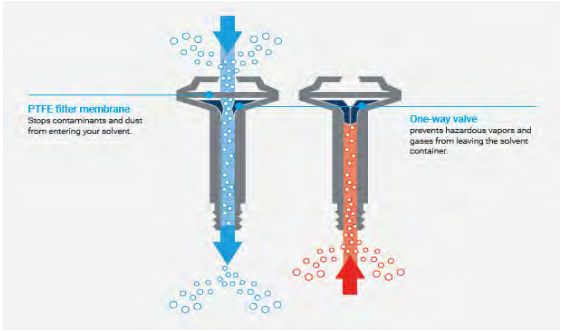
- Minimize harmful vapors and maintain solvent consistency
- Prevent solvents from leaching into the air
- Increase long-term mobile phase consistency



InfinityLab solvent bottles
Ergonomic, safer handling, easier to grab



Time strip tells when to replace venting valve
Venting valve only allows air in one direction



InfinityLab Stay Safe caps
Keep solvent concentration constant and stops solvent vapor from leaching into the air



Waste container with Stay Safe cap for waste
Keeps harmful solvent vapors in the container

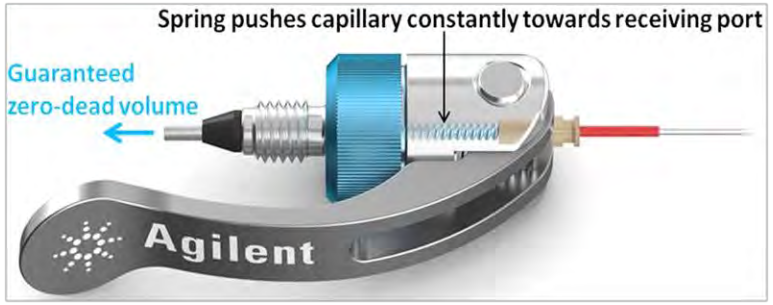
Agilent InfinityLab Stay Safe Caps and Accessories

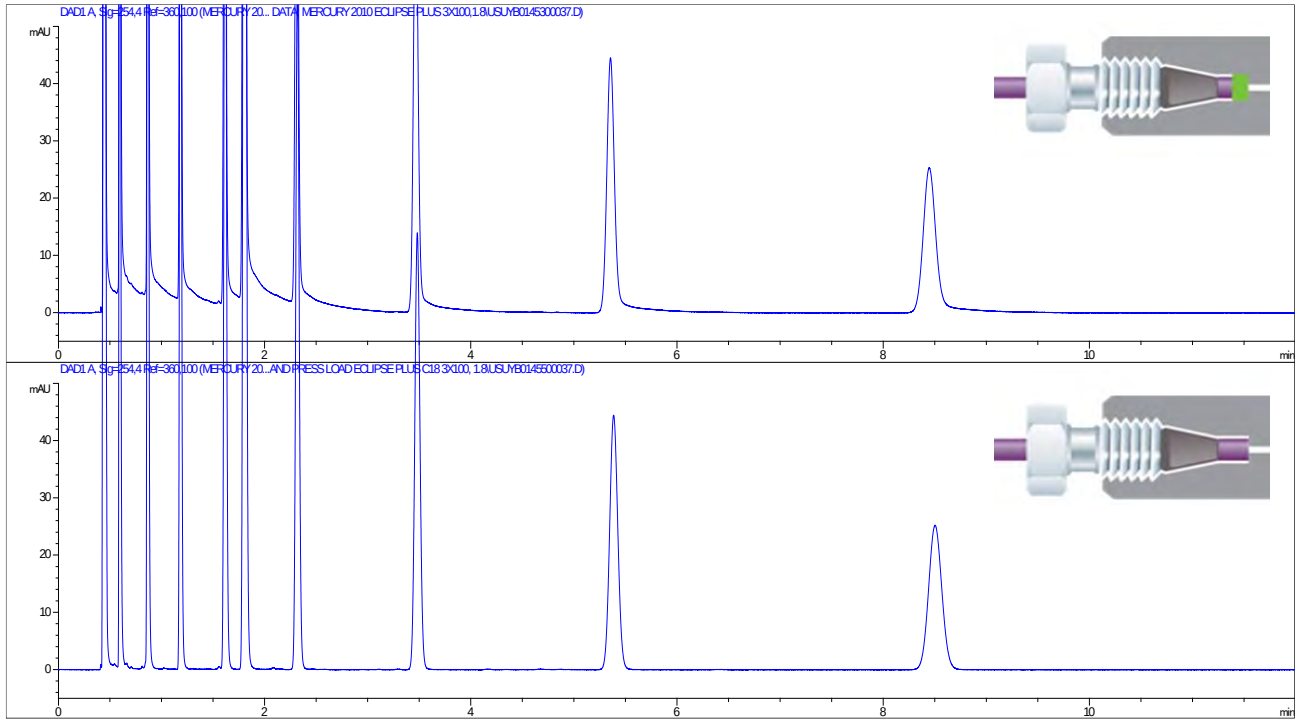
Best Practices

Capillary Connections

- Poor connections can be a source of peak broadening, especially if dead volume is created.
- In the chromatograms to the right, we see that with a poor connection all peaks are tailing.
- Fittings such as the InfinityLab Quick Connect or Quick Turn can ensure perfect fitting every time.







[Quick Connect Fittings]

Preventative and Routine Maintenance for Your HPLC System

Daily Maintenance



Daily Maintenance

- Mobile phase maintenance
  - Clean solvent bottles
  - Aqueous mobile phase
  - Organic mobile phase
- Check seal wash solvent level
- Purge each channel with fresh solvent at 2.5-3.0 mL/min for 5 minutes before operation
- Equilibrate the system with composition and flow rate of subsequent method



Daily Maintenance

Mobile phase maintenance

Mobile phases should be replaced in a clean glass solvent bottle every day.

- Rinse bottle with desired solvent before refilling it
- Avoid using surfactant-based detergents when cleaning mobile phase bottles

Never “top off” existing mobile phase with freshly prepared mobile phase.



Daily Maintenance

Mobile phase maintenance

Aqueous mobile phases are prone to microbial growth and precipitations.

- After all buffer salts have been dissolved and pH has been adjusted the mobile phase should be filtered through 0.22 µm filter
- Label solvent bottle with bottle content and filling date
- Consider adding 5-10% organic to the aqueous mobile phase to inhibit microbial growth



5191-6776 | Agilent

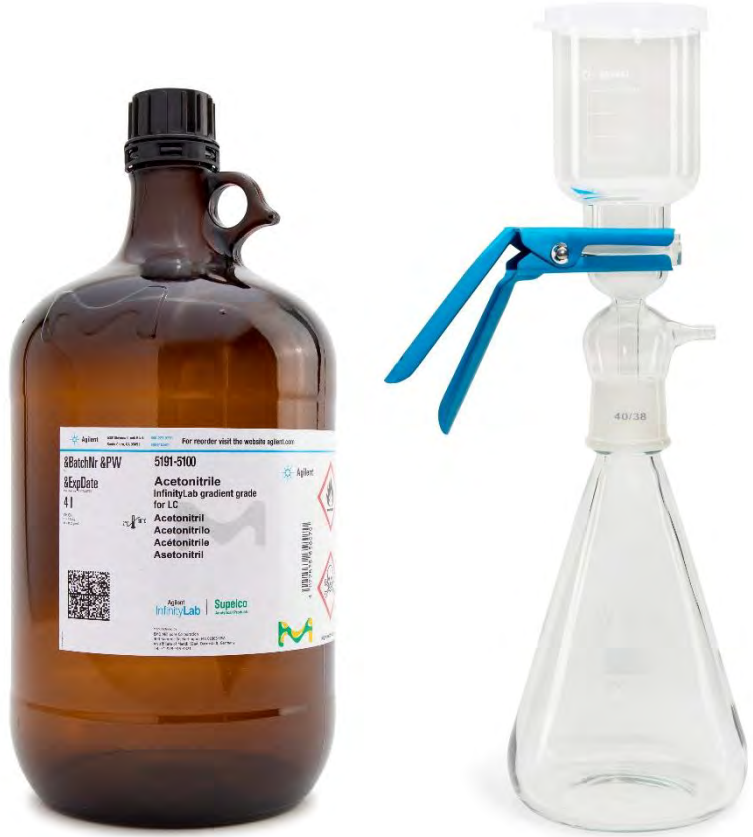
Daily Maintenance

Mobile phase maintenance

Organic mobile phases should be replaced every second day at the latest

- Acetonitrile and other organic solvents can be filtered using 0.22 µm PTFE filter
- Acetonitrile should be stored in an amber bottle to prevent photochemical reactions and oxidation
- Label solvent bottle with bottle content and filling date

Filtering through nylon filters is not recommended for high sensitivity LCMS



5191-5100 | Agilent

5191-6776 | Agilent

technical-overview-vacuum-filtration-system-infinitylab-5994-1504en-agilent.pdf

Preventative and Routine Maintenance for Your HPLC System

Weekly Maintenance



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Weekly Maintenance

- ❑ Change seal wash solvent and bottle (10% isopropanol in water)
- ❑ If applications with salts were used, flush all channels with water and remove possible salt deposits manually
- ❑ Inspect solvent inlet filters for dirt or blockages



Weekly Maintenance

Flushing procedure

This procedure should be used to remove salt deposits from the flow path and surfaces in contact with salt-containing mobile phases.

1. Flush the column with the recommended storage solvent.
2. Replace the column with a union, replace the salt-containing solvent bottle with a new bottle of HPLC-grade water at room temperature.
3. Clean the bottle head assembly using lint-free wipes to minimize carryover of remaining salt solution into the new water bottle.
4. Autosampler: perform at least a 15-minute purge with water to remove salt residues from all lines. If necessary, manually clean needle/seat/washport.
5. Purge each pump channel that has pumped buffer separately, for at least 10 minutes at 5 mL/min.
6. Flush the entire system flow path with water for at least 10 minutes at 2 mL/min. During this step switch the injection valve and column selection valve (if installed) position every 1 minute. Repeat this until every position has been selected for at least 5 times.
7. Replace water with fresh solvent bottles to minimize salt carryover.

This procedure should be used when switching from salt-containing mobile phases to methods running high organics

Best Practices for Using an Agilent LC System Technical Note

Preventative and Routine Maintenance for Your HPLC System

Monthly Maintenance



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Monthly Maintenance

- ❑ Use performance standard to verify instrument performance
- ❑ Check expiration dates on stored chemical stocks
- ❑ Check certification dates on lab devices such as balances, pipettes and thermometers
- ❑ Backup data
- ❑ Routine PC maintenance

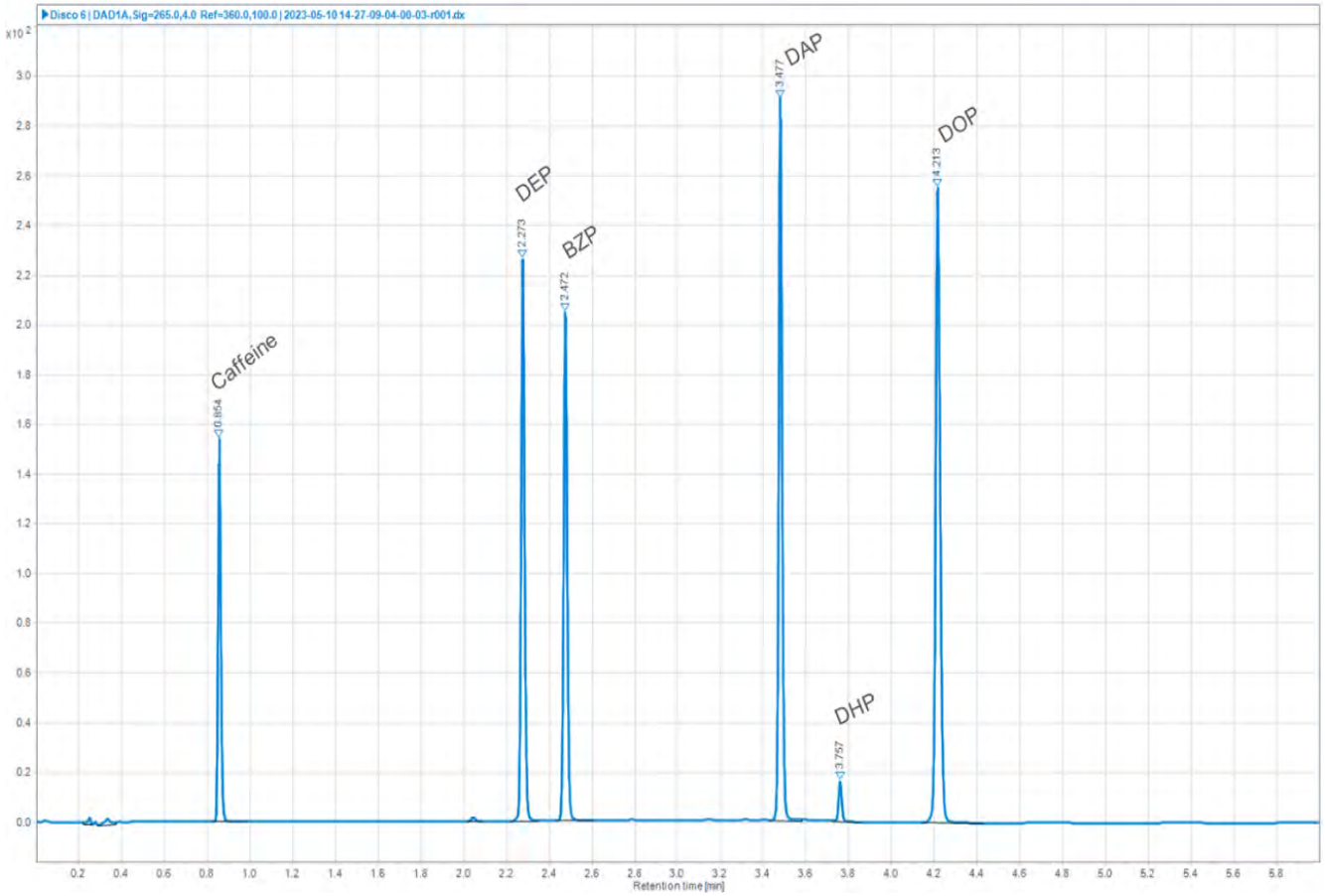


Monthly Maintenance

InfinityLab LC performance standard

This standard can be used in conjunction with internal standards and qualification standards to verify instrument performance.

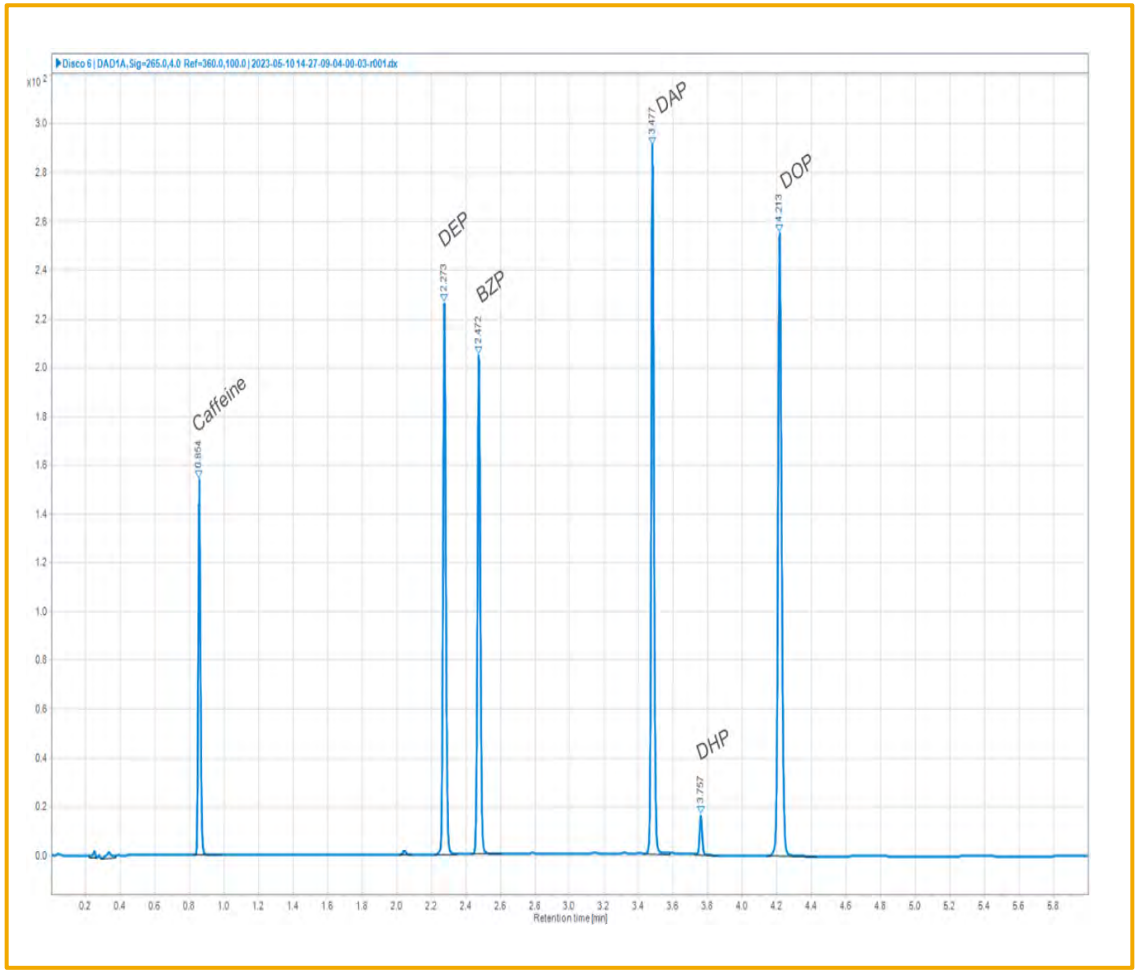
1290 Infinity II LC Method with DAD detector	
Parameter	**Value**
Flow Rate	1 mL/min
Column Temp.	40 °C
Mobile Phase A	LC grade Water with 0.1% Formic Acid
Mobile Phase B1	LC grade acetonitrile with 0.1% Formic Acid
%B Mobile Gradient	5% at 0 min
	95% at 3 min
	95% at 4.5 min
	5% at 5 min
Post Time	2 min
Inj. Volume	3 µL
DAD Wavelength	265nm



[Introducing the Agilent InfinityLab LC Performance Standard]
[Introducing the Agilent InfinityLab LC Performance Workflow]

Monthly Maintenance

InfinityLab LC performance standard



Analyte	Note/System Indicators	Affected System Component
Caffeine	A compound that elutes very early in the method will probe the LC pump for gradient delivery.	Pump
Diethyl Phthalate (DEP)	Half of the elution pair that evaluates the resolving power of the chromatographic system.	Column
Benzophenone (BZP)	A critical pair with DEP.	Pump
Diamyl Phthalate (DAP)	Paired with DEP, the resolution ratio between the two yields the gradient peak capacity. This peak area ratio (PAR) can also be used to evaluate loss at the injector.	Autosampler
Dihexyl Phthalate (DHP)	Spiked at low concentrations to monitor the ability to detect compounds at trace level (~ 1-2% of the total chromatogram).	Detector
Dioctyl Phthalate (DOP)	A late eluting compound used to ensure the gradient is appropriate for hydrophobic analytes, eluting at the end of the gradient.	Solvent composition

Preventative and Routine Maintenance for Your HPLC System

Quarterly and Semi-Annual Maintenance



Quarterly and Semi-Annual Maintenance

Quarterly Maintenance

- ❑ Run module diagnostic tests through Lab Advisor
- ❑ Replace frit in purge valve
- ❑ Flush aqueous channels with isopropanol
- ❑ Wipe down/clean the system
- ❑ Check consumables inventory and restock

Semi-Annual Maintenance

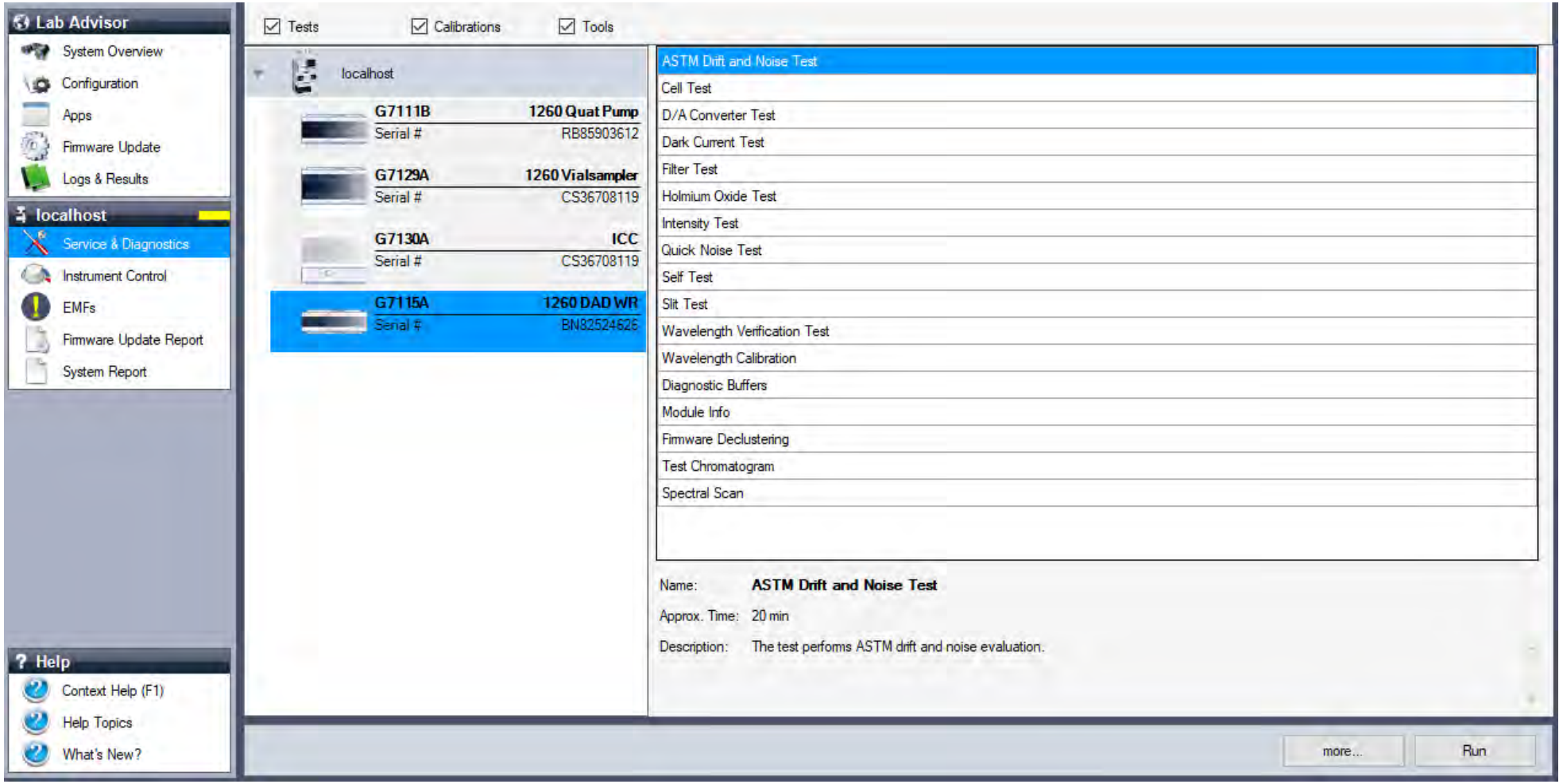
- ❑ Replace or clean solvent inlet filters
- ❑ Check Stay Safe solvent vent and replace as needed



Quarterly and Semi-Annual Maintenance

Lab Advisor Diagnostic Tests

Diagnostic tests are procedures for individual modules that will result in a passed/failed statement.



Quarterly and Semi-Annual Maintenance

Lab Advisor Diagnostic Tests

Pump

- Leak rate test
- Pressure test

Autosampler

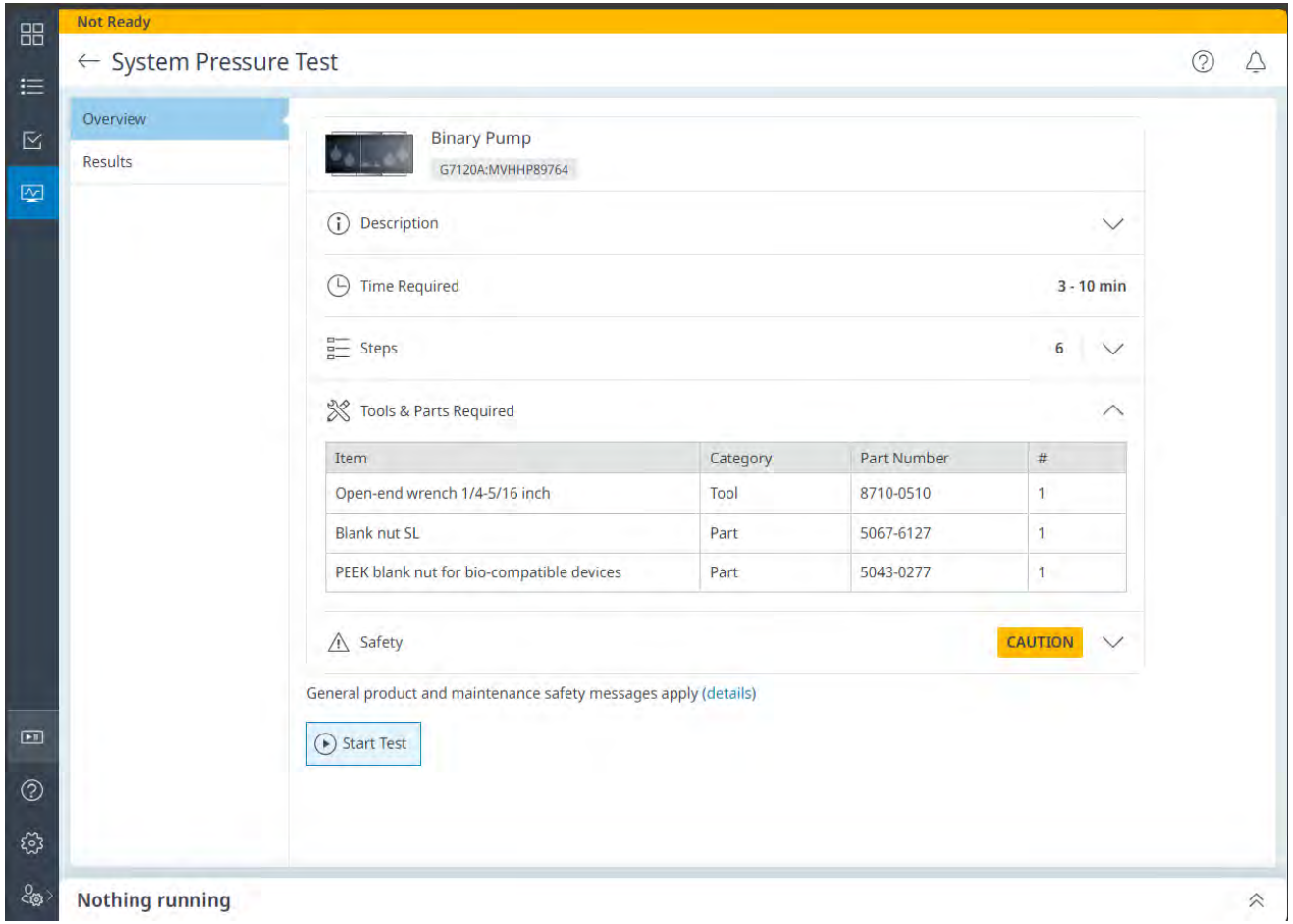
- Pressure test
- Accuracy by mass test

Column compartment

- Heating and cooling speed and accuracy

UV detector

- Intensity test
- Wavelength verification
- Dark current test
- Filter test
- ASTM drift and noise test



Quarterly and Semi-Annual Maintenance

Replace Frit in Purge Valve

- A dirty frit is a source of high pressure
- A pressure drop of >10 bar across the frit when running at 5 mL/min with purge valve open could indicate a blockage
- Always change frit after changing pump seals





PTFE frits

01018-22707

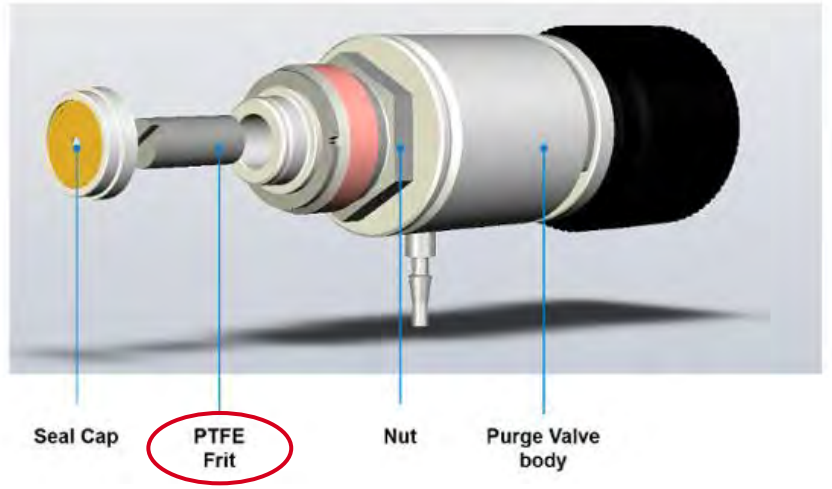


Seal cap

5067-4728

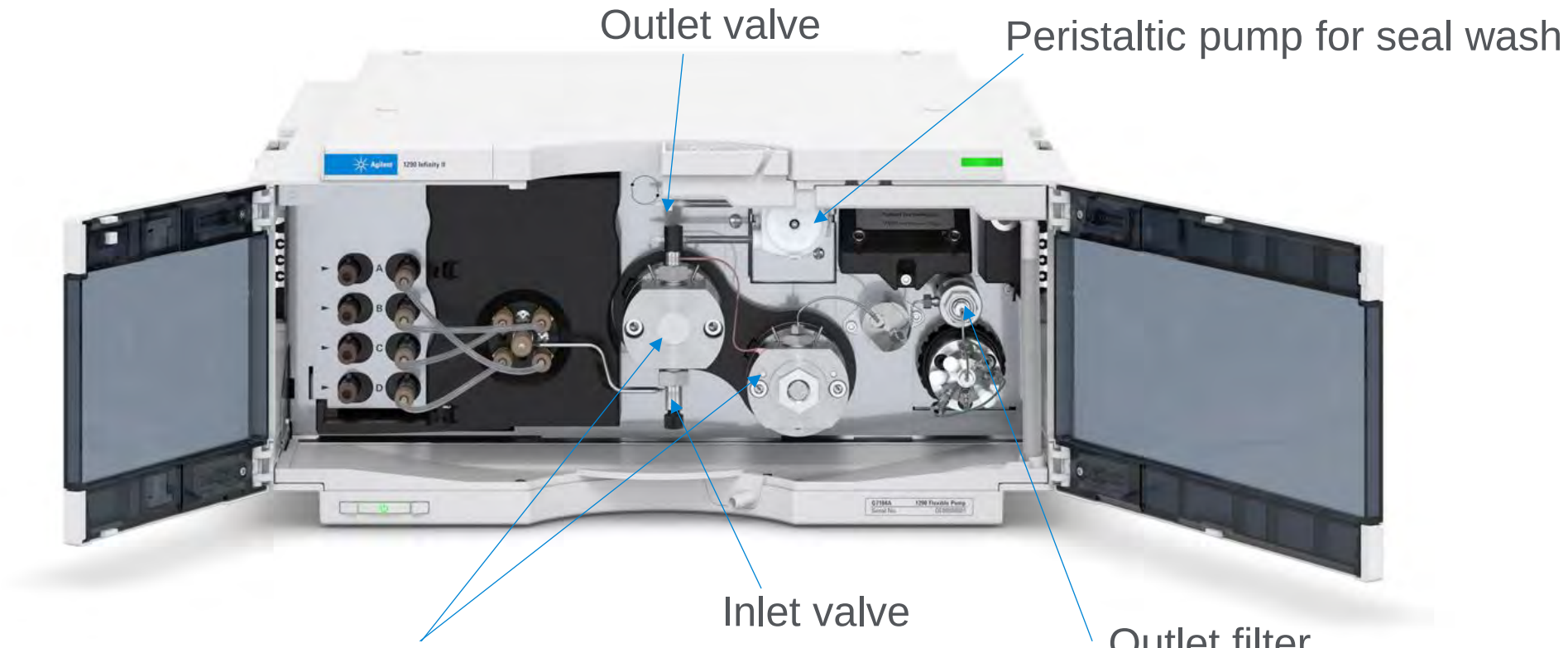


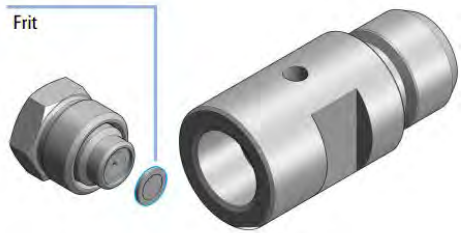
14 mm wrench



Quarterly and Semi-Annual Maintenance

Replace Frit in Purge Valve





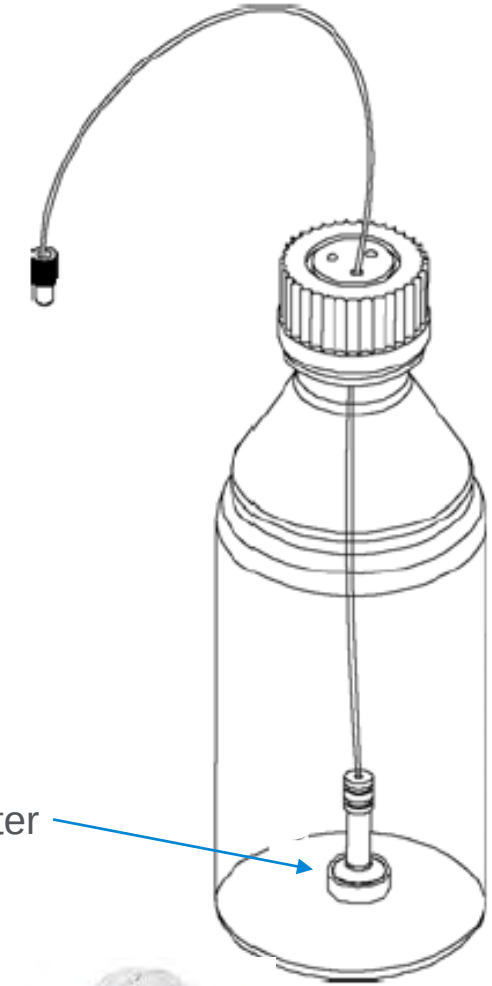
Quarterly and Semi-Annual Maintenance

Solvent Inlet Filters

A dirty solvent inlet frit can cause cavitation. The “bubbles” formed can lead to pump issues. Inlet filters are not a replacement for good mobile phase hygiene.

Replace or clean glass solvent inlet filters:

1. Remove the solvent inlet filters
2. Compare filter to a clean, unused solvent filter
3. Replace the glass filter or soak them in concentrated nitric acid (35%) for one hour.
4. Flush well with water or column damage may occur
5. Reinstall the solvent inlet filter





Stainless steel solvent inlet filter (recommended for LC-MS), 01018-60025



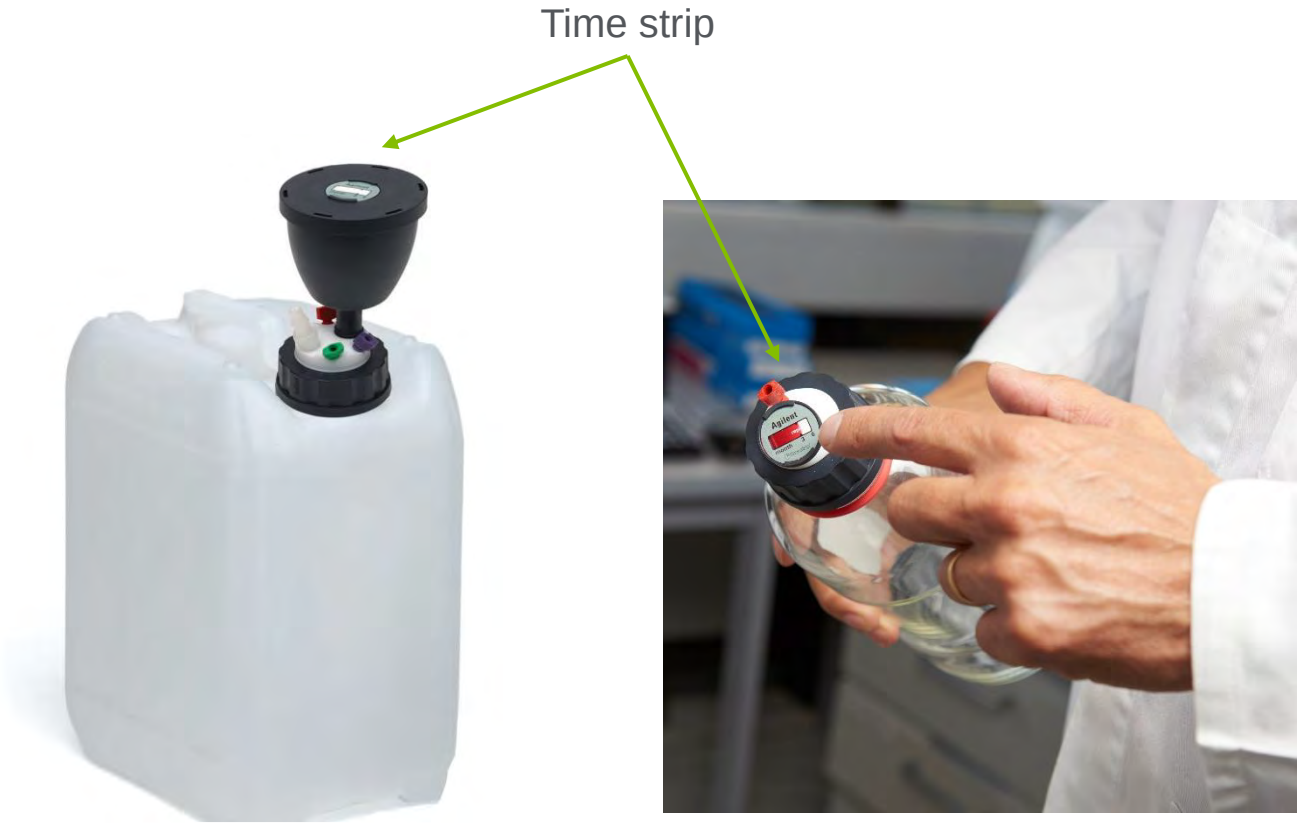
Glass solvent inlet filter (20 µm), 5041-2168

Quarterly and Semi-Annual Maintenance

Stay Safe Solvent Vent

The venting valve on the solvent bottle and the charcoal filter on the waste container have time strips that let you know when to change the filter.





Preventative and Routine Maintenance for Your HPLC System

Annual Maintenance



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Annual Maintenance

Major Preventative Maintenance

Pump

- ❑ Replace piston seals
- ❑ Replace frit in purge valve
- ❑ Run module diagnostic tests through Lab Advisor

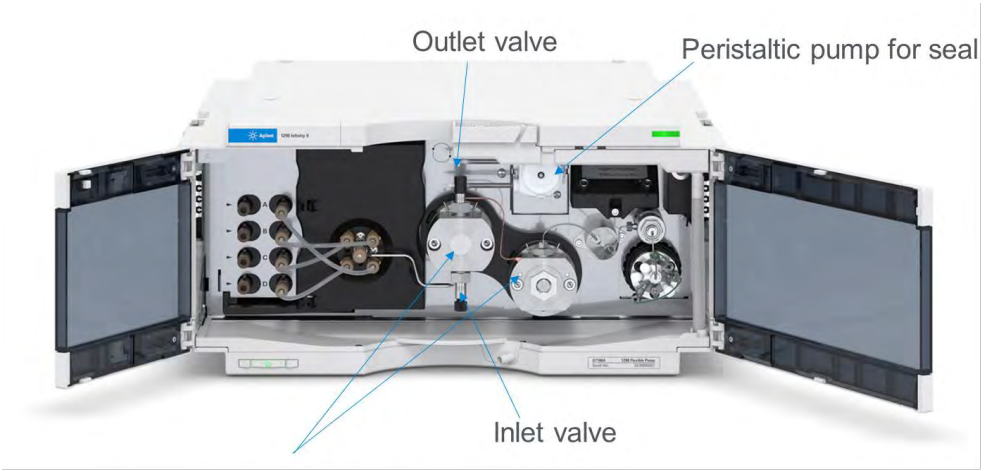


Annual Maintenance

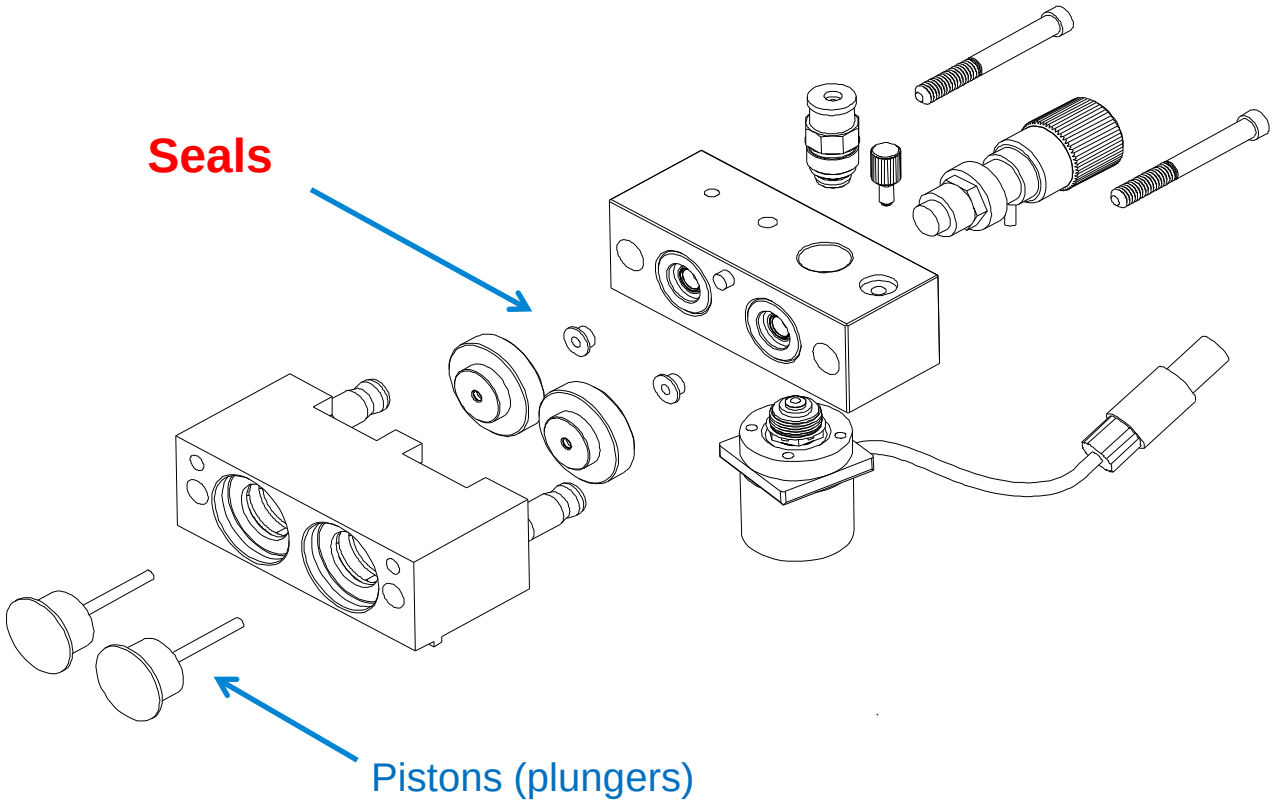
Major Preventative Maintenance

Pump

Replace piston seals



Pump head (pump seals/wash seals)





5063-6589, PTFE (reversed-phase)



0905-1420, polyethylene (normal phase)

0905-1719, 1290/high speed/flexible pump

Replace piston seals

Annual Maintenance

Major Preventative Maintenance

Autosampler/Multisampler

- ❑ Replace needle and needle seat
- ❑ Replace rotor seal
- ❑ Run module diagnostic tests through Lab Advisor



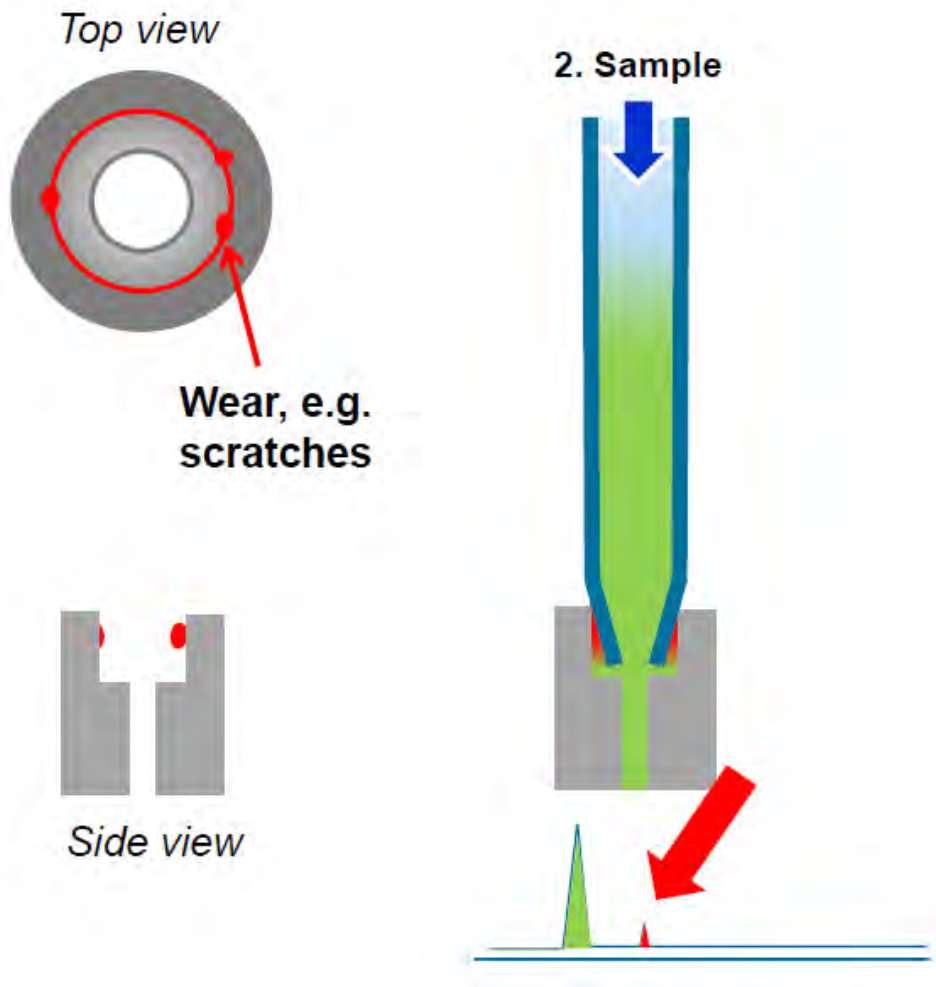
Annual Maintenance

Major Preventative Maintenance

Autosampler/Multisampler

Replace needle and needle seat

- Wear can affect sample delivery into the system
- May see carryover, broader peaks or reproducibility issues
- Always replace the needle seat and the needle together



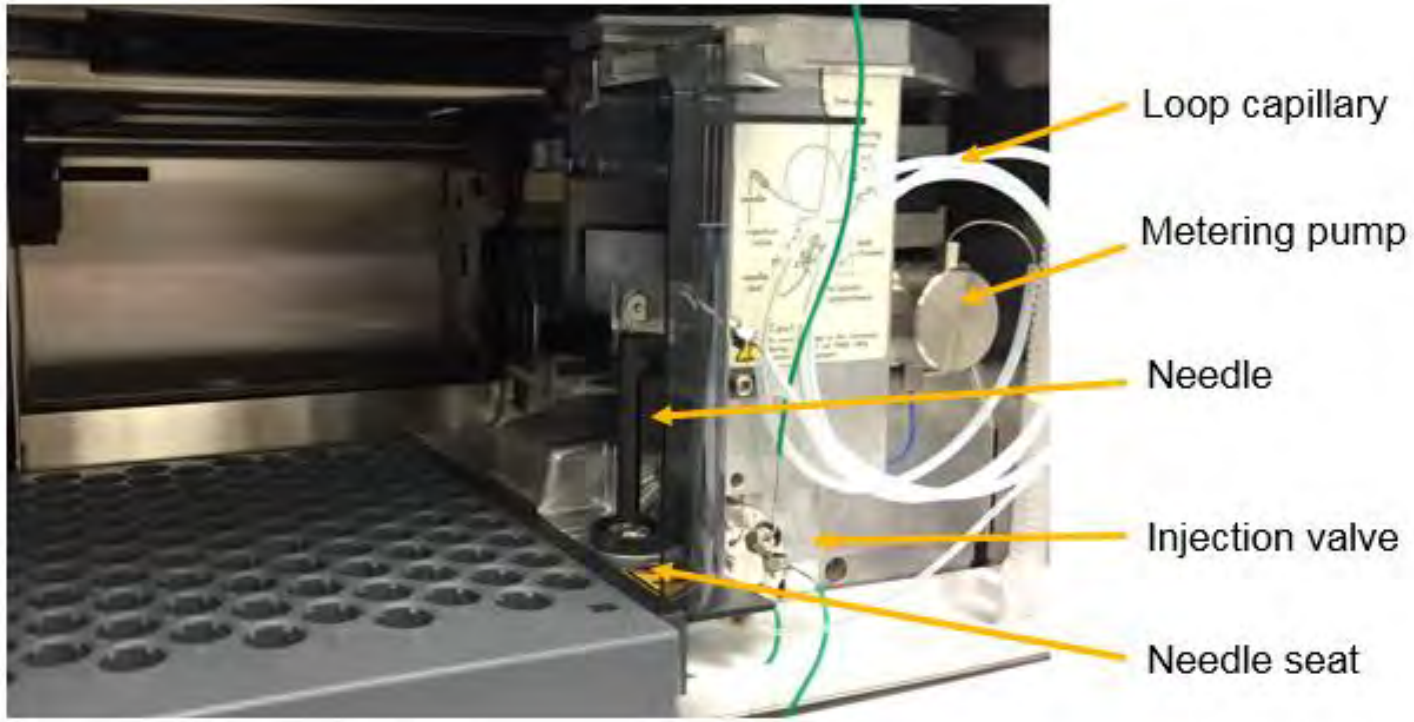
Change Needle and Needle Seat: Agilent YouTube

Annual Maintenance

Major Preventative Maintenance

Autosampler/Multisampler

Replace needle and needle seat





Autosampler needle



Multisampler needle



Well plate sampler needle



Needle seat

[Change Needle and Needle Seat: Agilent YouTube]

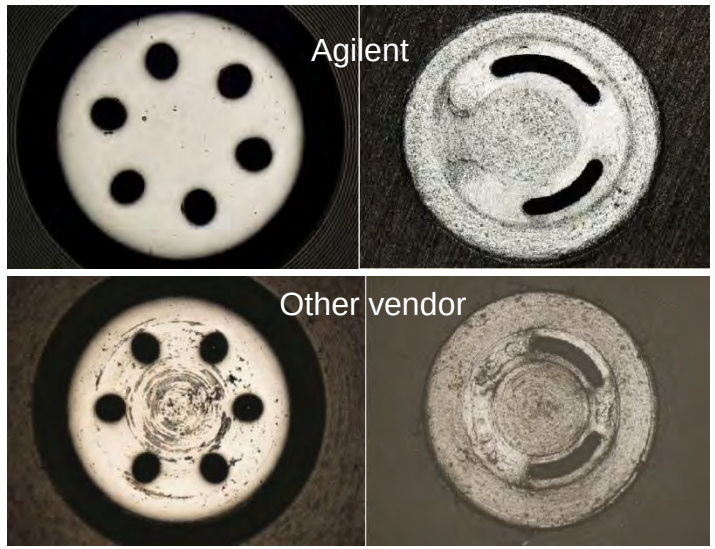
Annual Maintenance

Major Preventative Maintenance

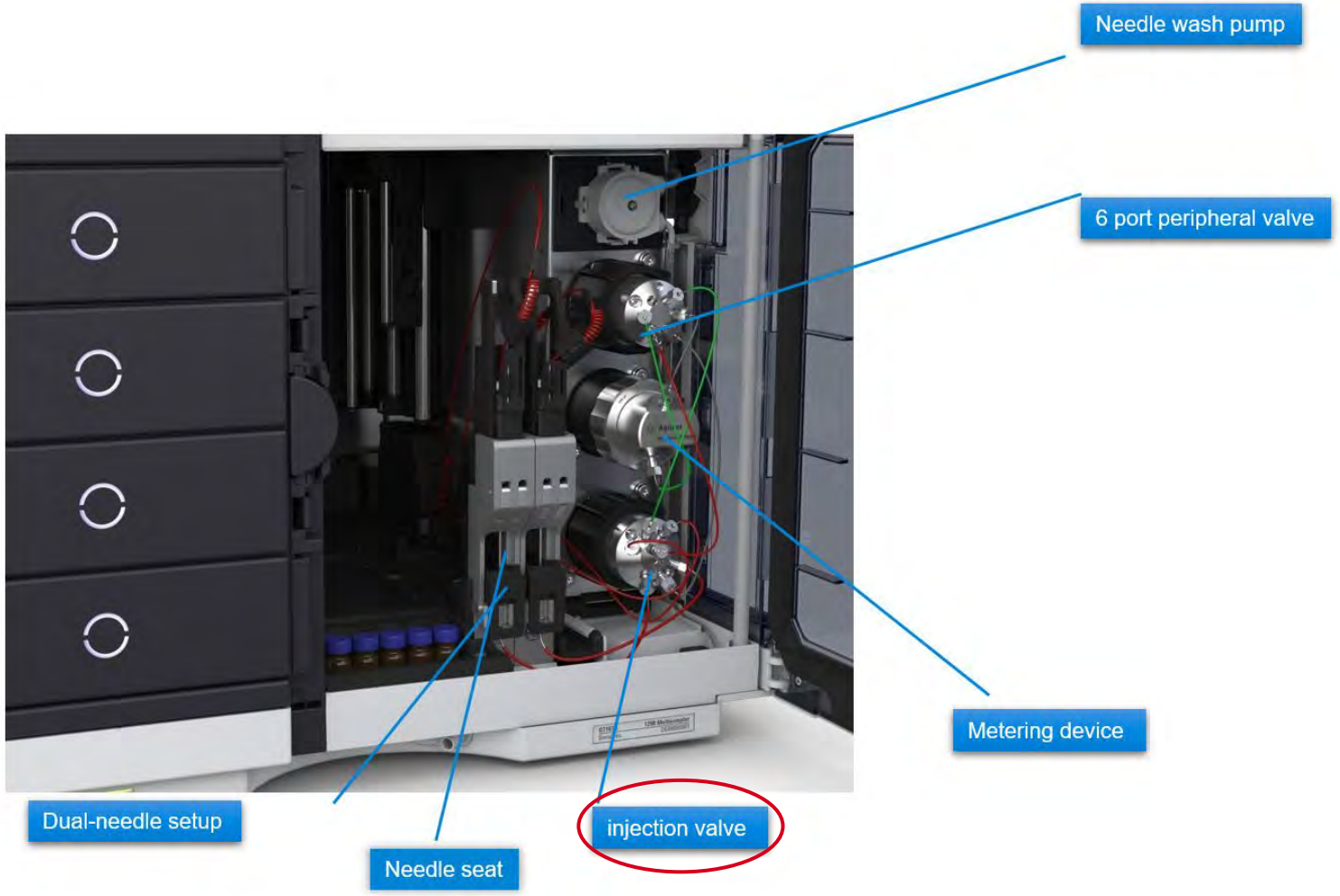
Autosampler/Multisampler

Replace rotor seal

- Rotor seal wear is often a cause of sample carryover



Stator and rotor surface of 30,000 (Agilent) or 26,000 (other vendor) switch cycles



Replace Rotor Seal: Agilent YouTube

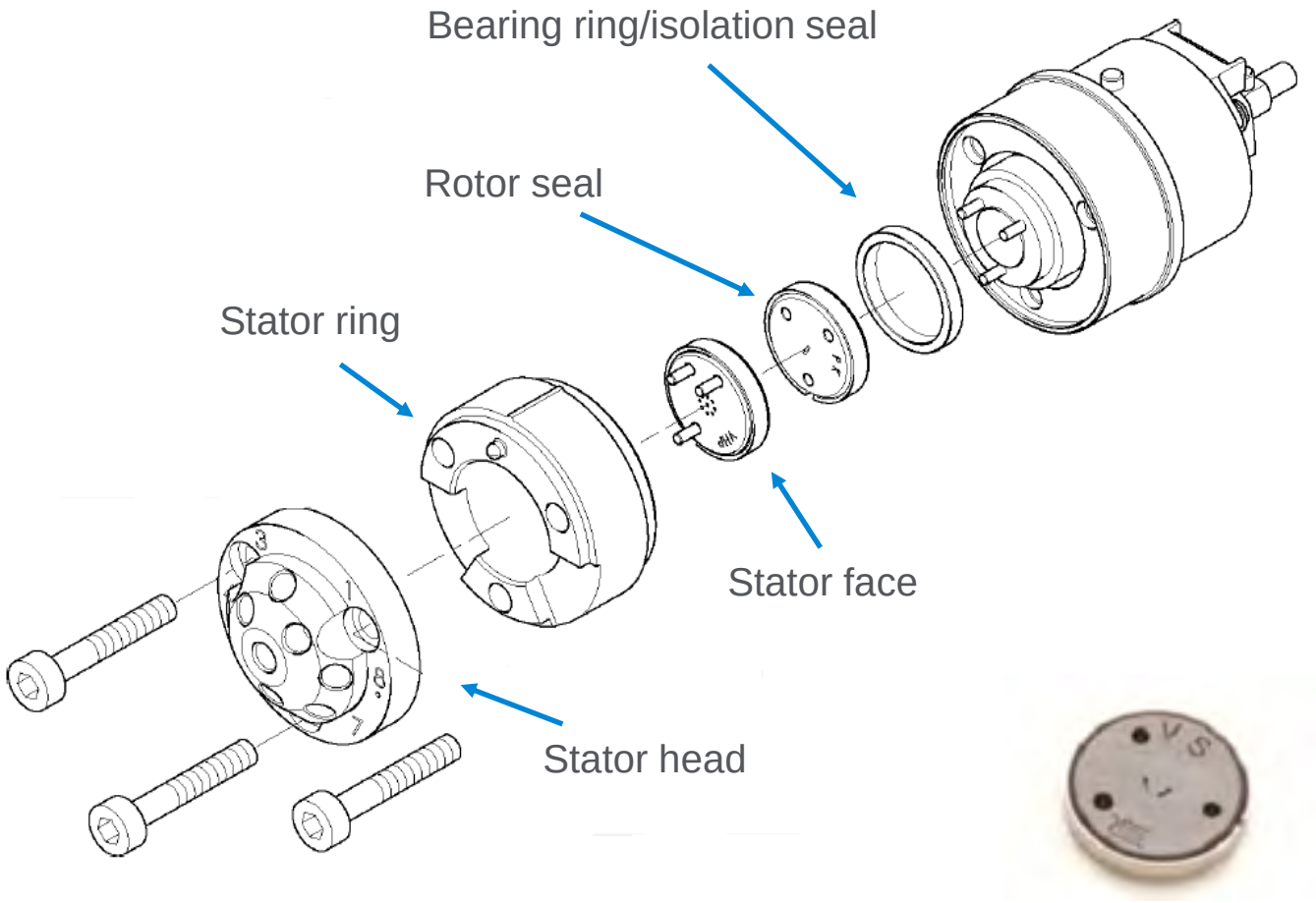
Annual Maintenance

Major Preventative Maintenance

Autosampler/Multisampler

Replace rotor seal

- Polyimide rotor seal is suitable for mobile phases in the pH range of 2.3 to 9.5
- PEEK rotor seal has high chemical resistance and is suitable for mobile phases in the pH range of 1 to 14



[Replace Rotor Seal: Agilent YouTube]

Annual Maintenance

Major Preventative Maintenance

Column compartment

- ❑ Replace rotor seal
- ❑ Run module diagnostic tests through Lab Advisor



Annual Maintenance

Major Preventative Maintenance

Column compartment

Replace rotor seal

- The correct rotor seal depends on the type of valve head installed.





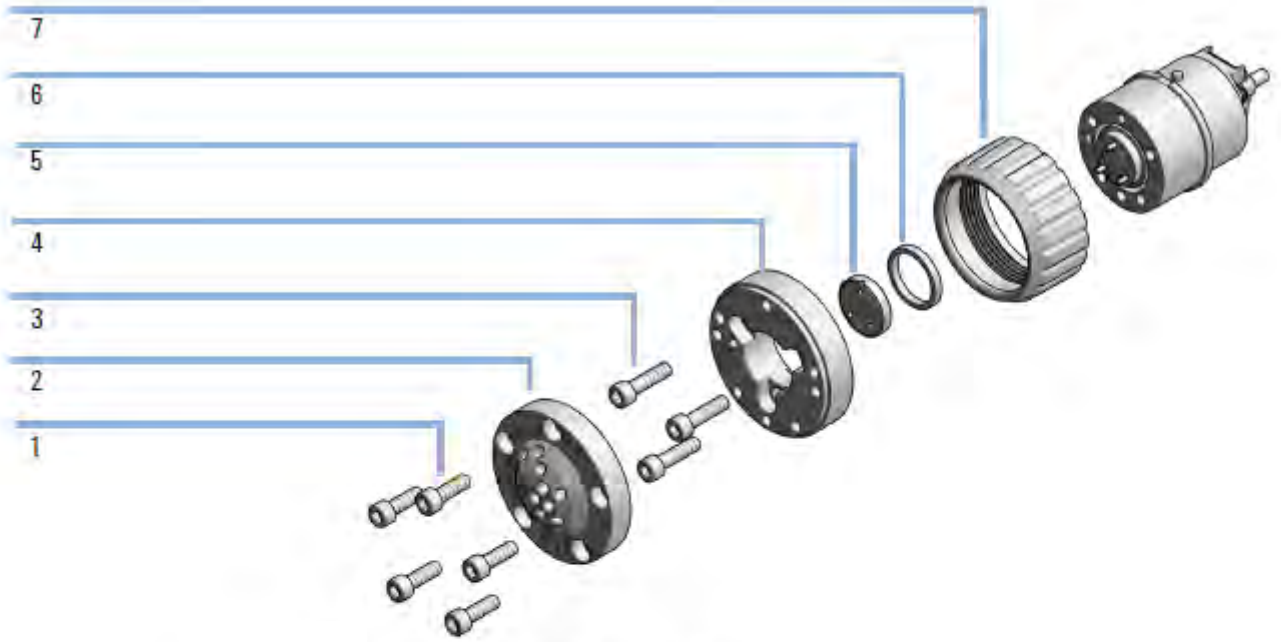
Annual Maintenance

Major Preventative Maintenance

Column compartment

Replace rotor seal

- The correct rotor seal depends on the type of valve head installed.



Valve Head Parts (example)

1	Stator screws
2	Stator head assembly
3	Stator ring screws (not available)
4	Stator ring (available for service only)
5	Rotor seal
6	Bearing ring
7	Spanner nut (available for service only)

Annual Maintenance

Major Preventative Maintenance

UV Detector

- ❑ Inspect flow cell for leaks
- ❑ Replace lamp if intensity test fails
- ❑ Run module diagnostic tests through Lab Advisor



Annual Maintenance

Major Preventative Maintenance

UV Detector

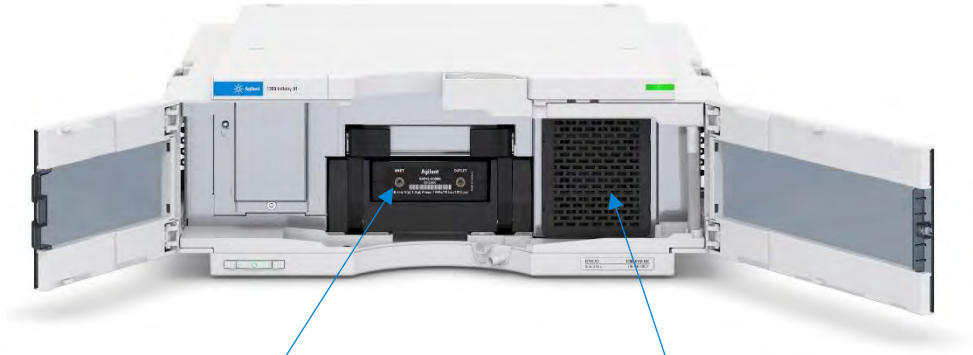
Inspect flow cell for leaks

- UV detector will have either a flow cell or a Max-Light cartridge
- Visually inspect the flow cell or Max-Light cartridge for leaks









[Replacing the Flow Cell: Agilent YouTube]

Annual Maintenance

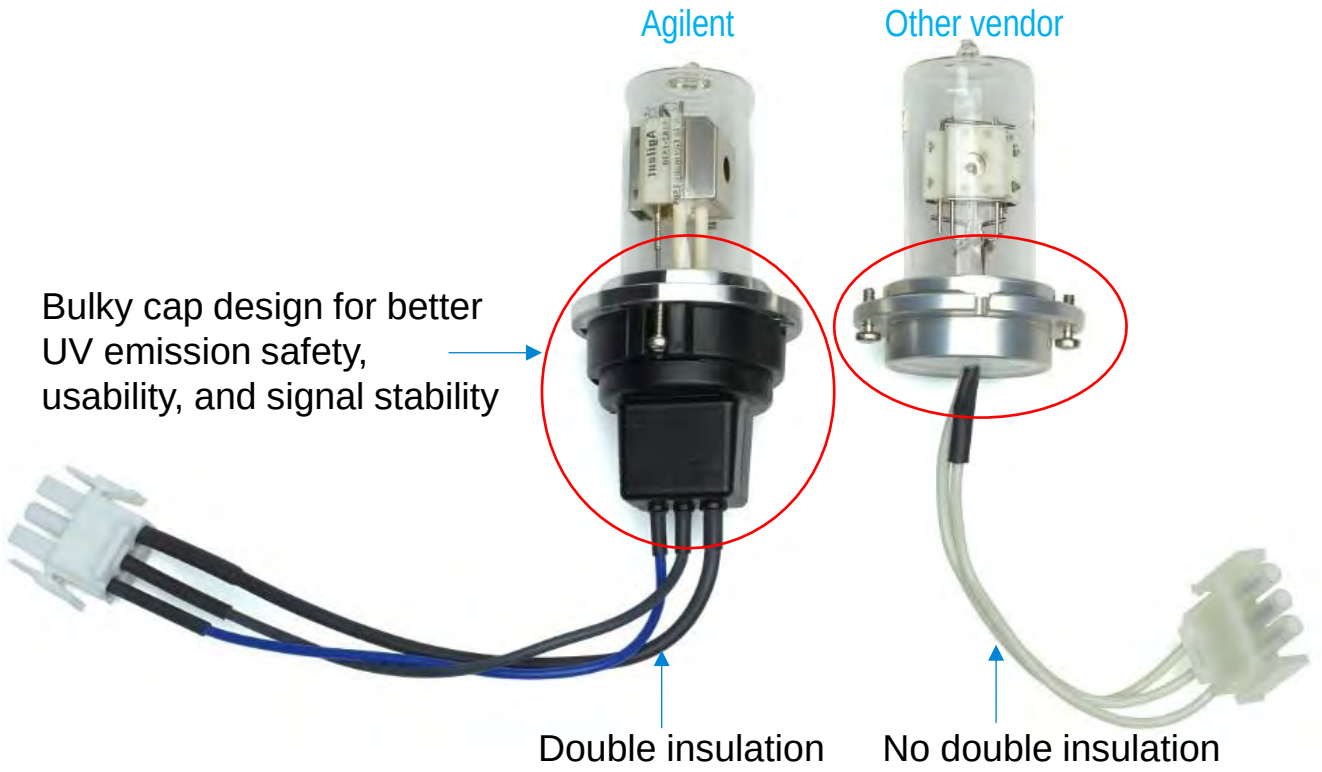
Major Preventative Maintenance

UV Detector

Replace lamp if intensity test fails

Agilent lamps:

- Designed and certified for Agilent detectors
- Narrower aperture provides increased light intensity and decreased noise
- Higher signal-to-noise (S/N) ratio
- Typical long-life lamp life is 2000 hours



Replacing the Lamp: Agilent YouTube

Annual Maintenance

Major Preventative Maintenance

UV Detector

Replace lamp if intensity test fails

When changing the lamp:

- Do not touch the glass bulb
- Any deposit in the light path can affect performance
- Wait 10 minutes for warmup



[Replacing the Lamp: Agilent YouTube]

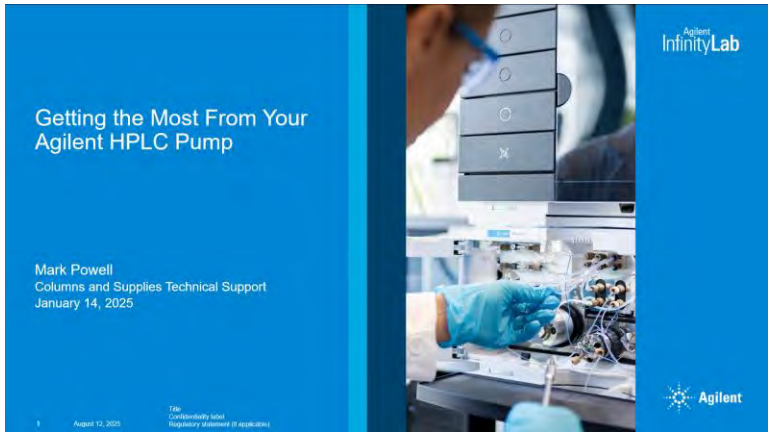
Preventative and Routine Maintenance for Your HPLC System

Additional Resources

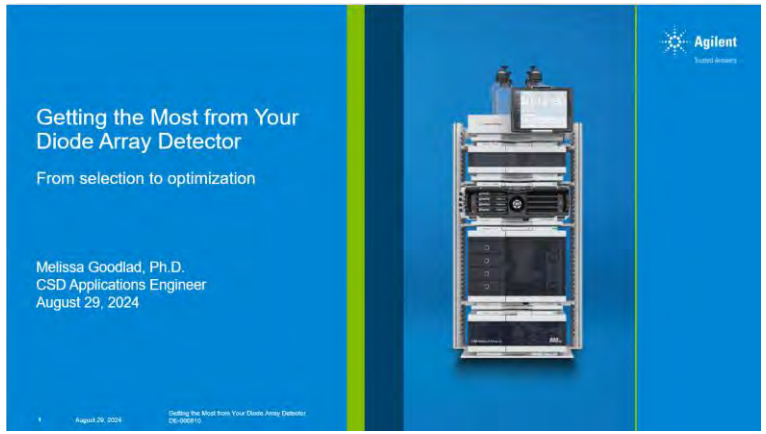


Agilent

On Demand Webinars









[Getting the Most from Your Agilent HPLC Pump]

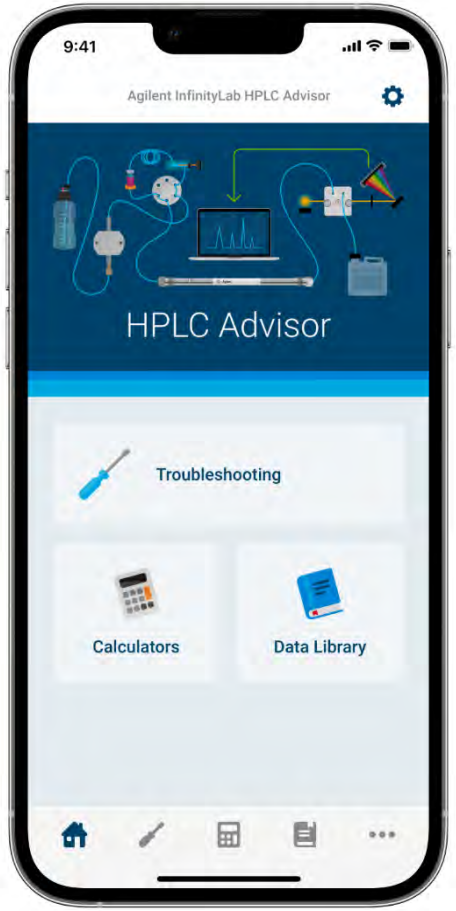
[Getting the Most from Your Agilent HPLC Autosampler or Manual Injector]

[Getting the Most from Your Diode Array Detector: From Selection to Optimization]

[LC & LC/MS Column Webinars | Agilent]

Agilent Resources for Support

- Resource page http://www.agilent.com/chem/agilentresources
  - Online selection tools, “How-to” videos
  - Column user guides - https://www.agilent.com/en-us/support/liquid-chromatography/kb005965
- HPLC Advisor app for troubleshooting and method calculators: HPLC Advisor app | Agilent
- Application Note Finder: Application Finder | Agilent
- Tech support: http://www.agilent.com/chem/techsupport
- Agilent University http://www.agilent.com/crosslab/university
- YouTube – Agilent Channel
- Your local product specialists





Agilent Resources for Support

Quick Reference Guides

- 1260 Infinity II LC with Multisampler
- 1260 Infinity II LC with Vialsampler
- 1260 Infinity II Prime System
- 1290 Infinity II with Multisampler
- 1290 Infinity II with Vialsampler
- 1260 Infinity II Bio-Inert LC
- 1260 Infinity II SFC System
- 1260 Infinity II Preparative LC System
- 1220 Infinity II
- Infinity II Bio LC



Quick Reference Guide

Agilent
Trusted Answers

Agilent InfinityLab Supplies for the Agilent 1260 Infinity II LC with Multisampler

Agilent Technologies is committed to optimizing your laboratory's productivity, so we have produced this list of the most commonly ordered supplies and parts for the 1260 Infinity II LC with Multisampler.

Pump supplies	PM*	Part number
Bottle head assembly		G7120-60007
Glass filter, solvent inlet, 20 µm pore size		5041-2168
Stainless steel filter, solvent inlet, 10 µm pore size		01018-60025
Piston seal PTFE, 2/Pk (reversed phase)	1 each	5063-6589
Piston seal PE, 2/Pk (normal phase)	1 each optional	0905-1420
Sapphire piston		5063-6586
Passive inlet valve		G1312-60066
Passive inlet valve (normal phase)		G1312-60166
Cartridge for active inlet valve, 600 bar		G1312-60020
Outlet valve		G1312-60067
Outlet valve (normal phase)		G1312-60167
Purge valve		G1312-60071
PTFE frits, for purge valve (pack of 5)	1 each	01018-22707
Seal cap for purge valve	1 each	5067-4728
Wash seal PTFE (reversed phase)	2 each	0905-1175
Wash seal PE (normal phase)	2 each optinonal	0905-1718
Gasket seal wash (pack of 6)	1 each	5062-2484
Peristaltic pump for seal wash (pharmed tubing)	1 each	5065-4445
PM kit for isocratic / quaternary pump	kit	G1310-68741
PM kit for binary pump	kit	G1312-68741
Seal wash preventive maintenance kit for quaternary and binary pumps		G1310-68742

* PM (preventive maintenance) relevant part information:
- x each: amount of part needed per PM
- kit: preventive maintenance kit that includes all standard parts for PM
- optional: part required for PM only if referring option is installed

Contact Agilent Chemistries and Supplies Technical Support

Available in the USA and Canada 8-5 all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com



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Delaware
HPLC/dissolution



Ryan Birney
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Delaware
GC/ spectroscopy



Mark Sinnott
Application Engineer
Idaho
GC



Alex Ucci
Application Engineer
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GC/sample preparation



Questions?





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