From Instrument to Column

Tracking Down the Problem

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Troubleshooting Topics

System pressure

- Increased pressure
- Low pressure
- Pressure fluctuations

Separation

- Changing retention time
- Loss of resolution

Peak shape

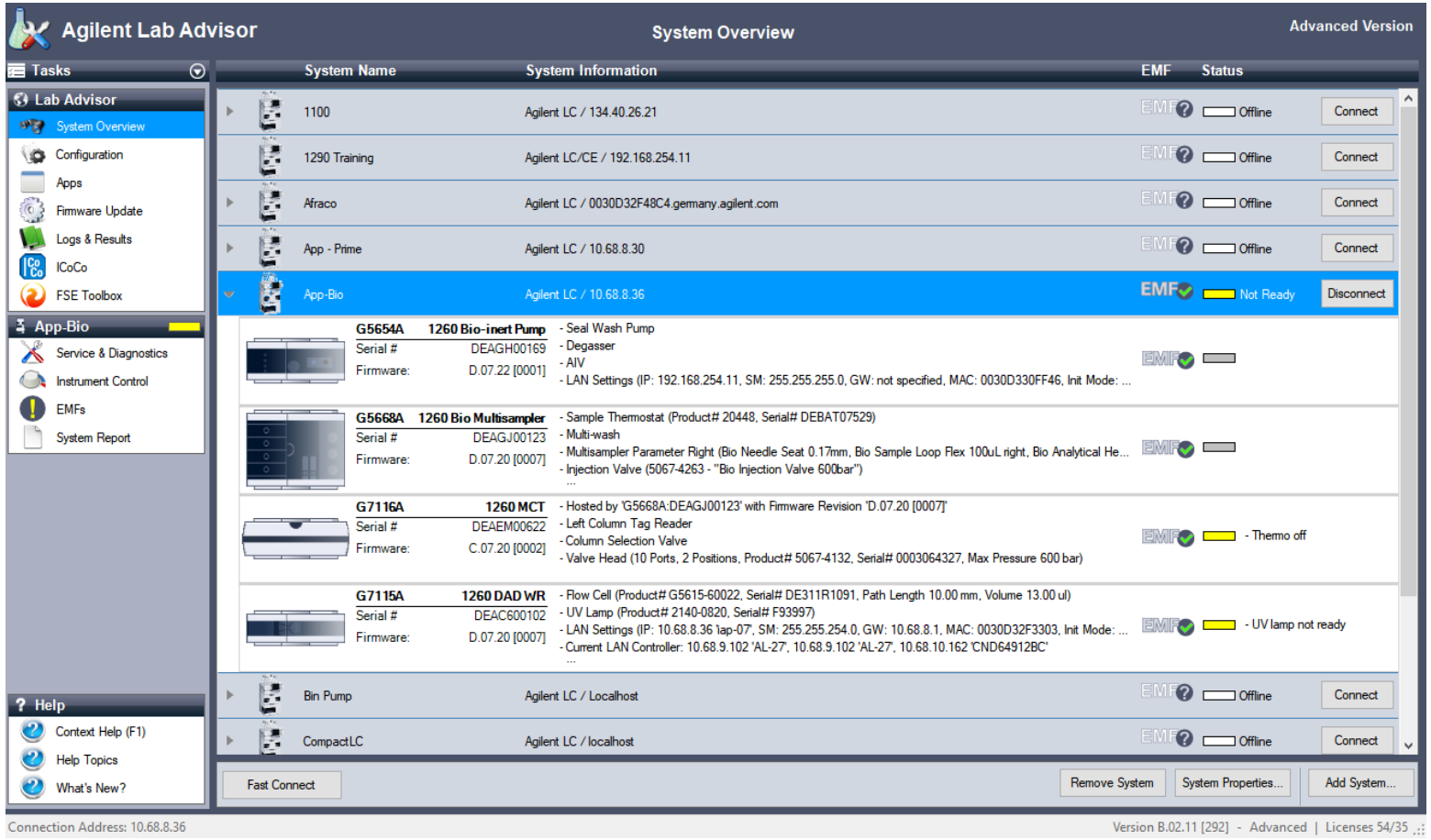
- Tailing
- Broadening
- Fronting
- Peak splitting and doubling

Detection

- Noisy baseline
- Reduced intensity or sensitivity
- Drifting baseline

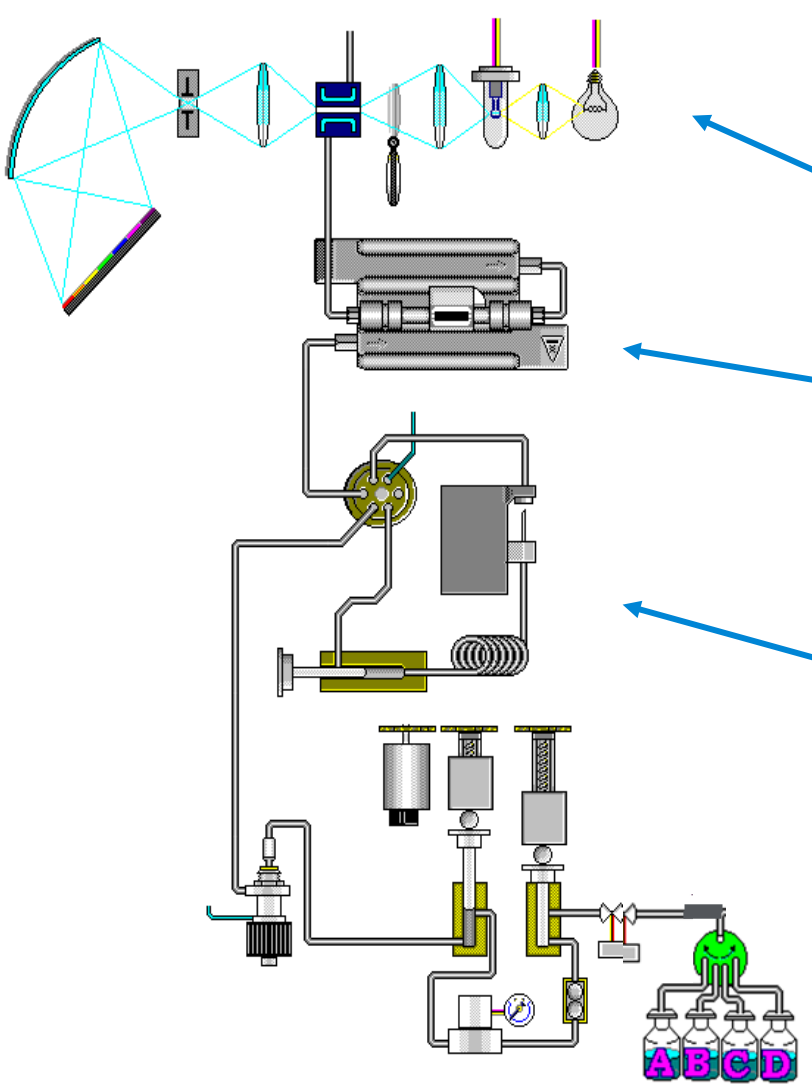
Agilent Lab Advisor

- 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



System Pressure

Understand Your LC System and Follow the Flow Path



Detector

Column compartment

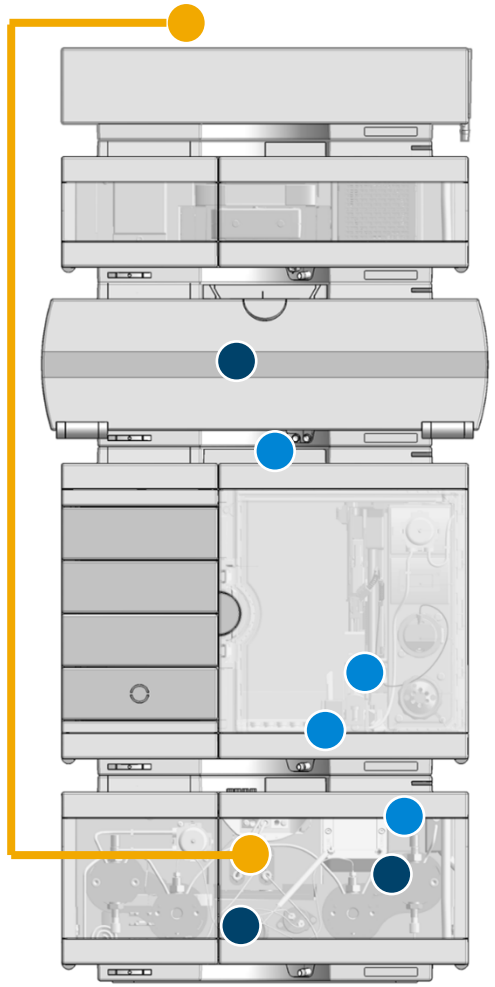
Autosampler

Pump

Changes in System Pressure

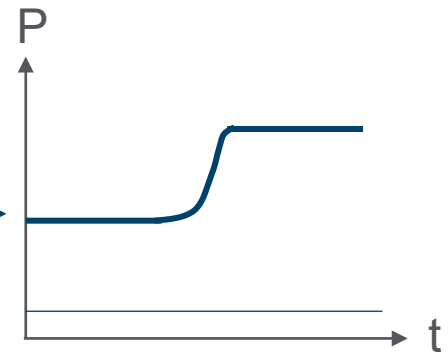
Increased Pressure/Overpressure and Blockages

	Potential cause	Recommended action
●	Clogging of filter frits in the high-pressure flow path	• Identify the culprit by logical elimination process and replace affected part. • Use clean, prefiltered solvent • Prevent algae growth in water 
●	Plugging of capillaries, needles, and needle seats	
●	Wrong solvent	• Check for correct mobile phase • Check solvent reservoir and tube connections

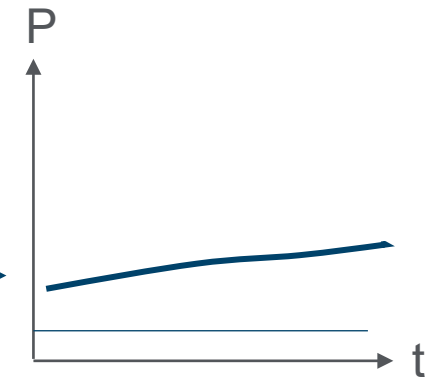


Blockages and Clogging

Characteristics	
Parts affected	Blockages: • Capillaries, needle, and needle seat • Detector flow cells Clogging: • Filter frits (inline filter, column filter)
Characteristic	
Identification	• Check easily accessible points: needle seat, purge valve, column • Disconnect capillaries one-by-one, starting at detector and moving back toward the pump
Possible root cause	• Debris from mechanically worn parts (needle seat material, rotor seal at injection valve) • Coring of vial septa material
Instant action/First aid	• Replace part • Backflush affected part
Preventive measures	• Proper preventive maintenance schedules, replace worn parts regularly • Use high quality septa • Install inline filters

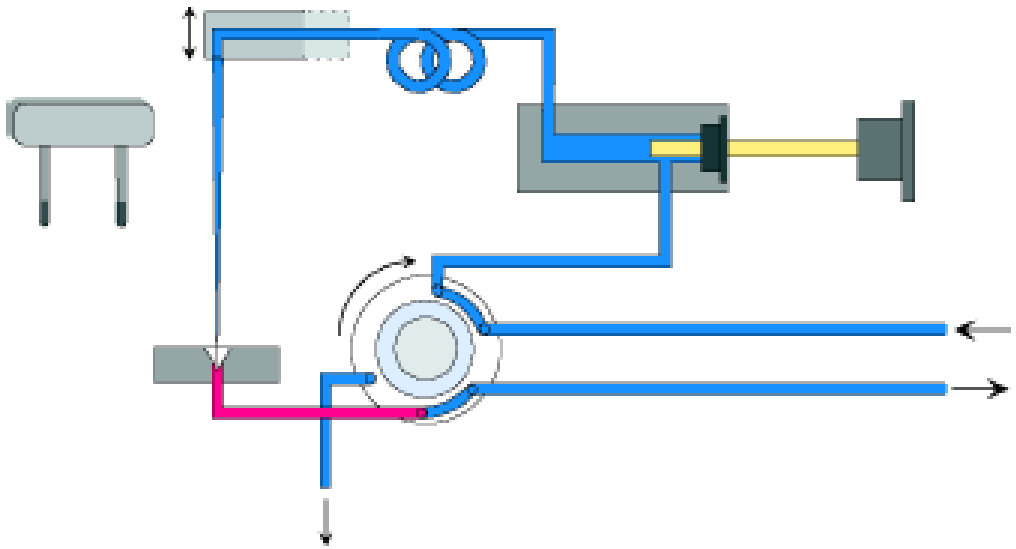


Blockages: Instant pressure increase step

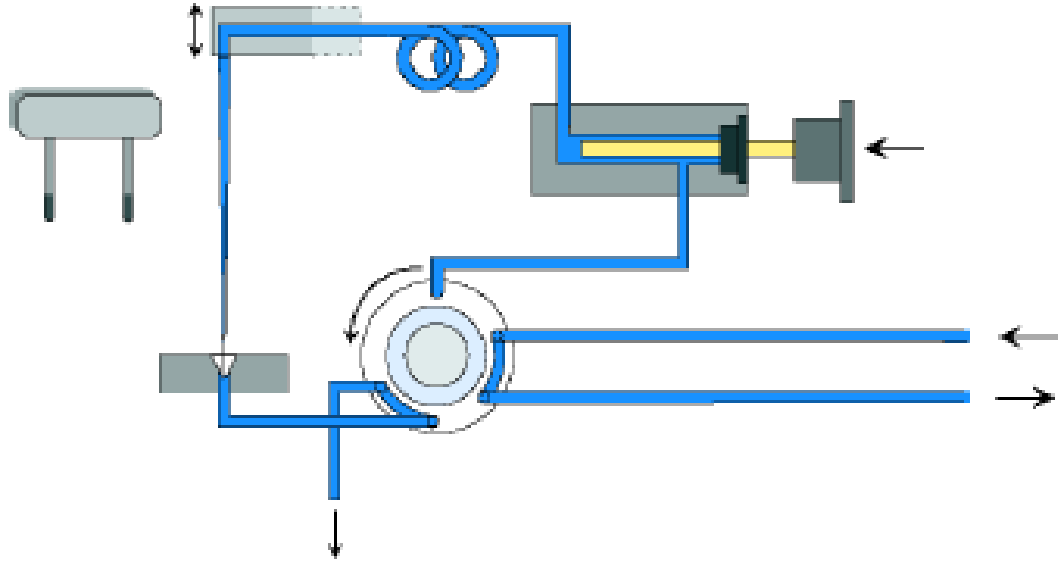


Clogging: Constant pressure increase over time

Checking for Blocked Needle Seat



Mainpass Position



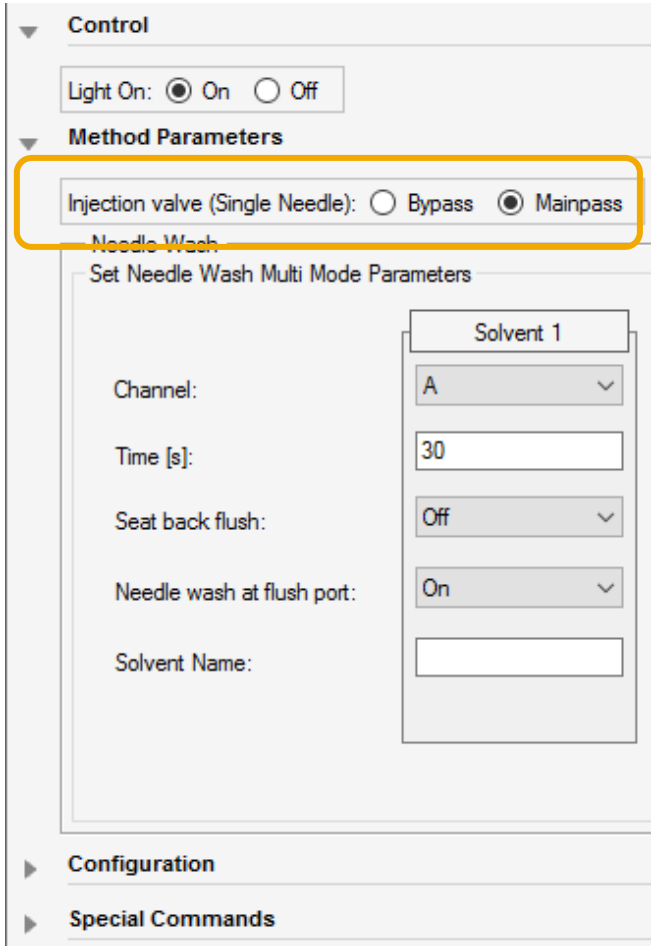
Bypass Position

Checking for Blocked Needle Seat

Track the typical operating pressure for a given application

To troubleshoot:

- Create high pressure on the system by turning on flow
- While the pressure is climbing, move the sampler to the “Bypass” position
- Watch the pressure when the valve switches to “Bypass”
- If the pressure drops immediately, then the source of the high pressure is in the portion of the flow path specific to the bypass position
  - Needle Seat
    - Where the sample first meets the mobile phase
    - **Most commonly clogged piece of tubing in an LC**
  - Needle
    - Less commonly clogged
    - Watch for issues with septa

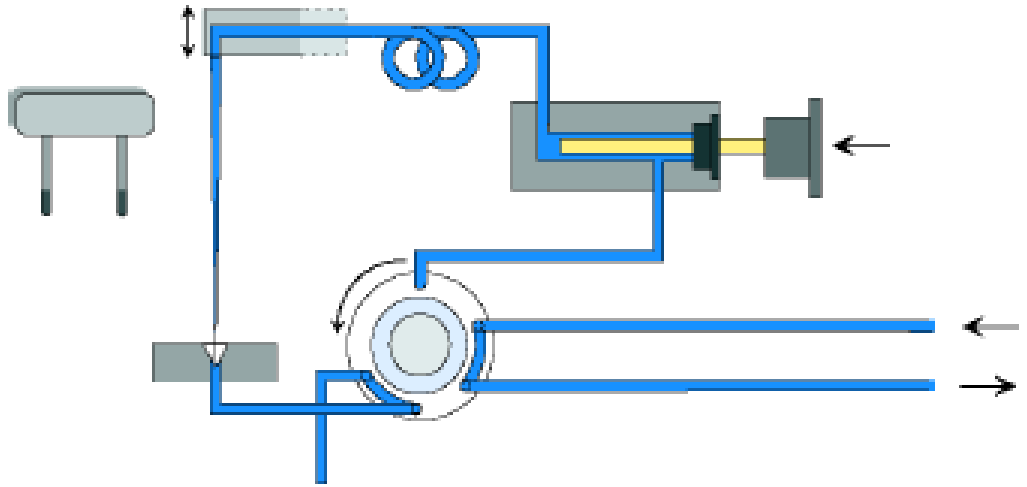


Checking for Blocked Needle Seat

Loop
- Not commonly clogged
- Watch for issues with sample

Metering head
- Never exposed to sample
- Consider solvent issues

Injection valve
- Most common issue is with rotor seal
- Look for scratches on stator face

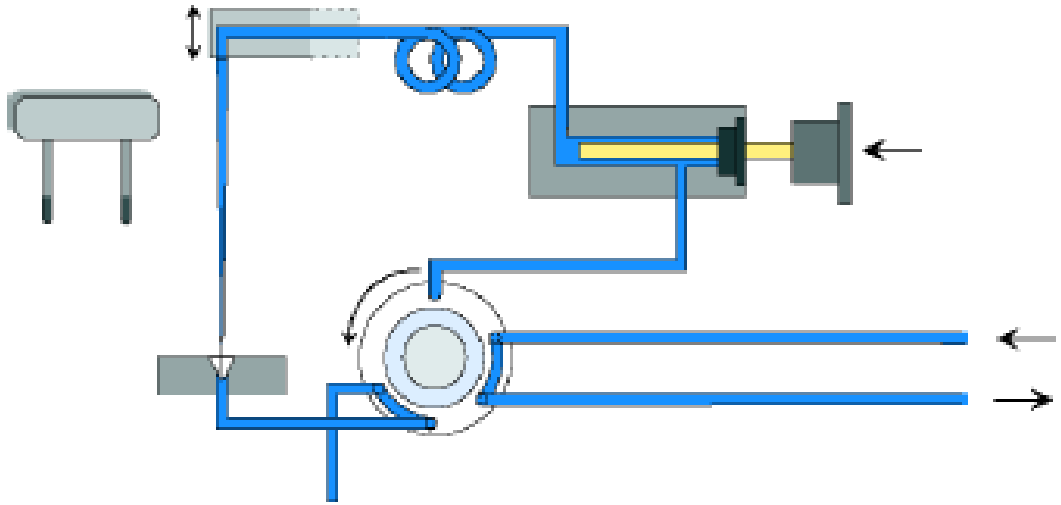


Checking for Blocked Needle Seat

Driving the needle into debris may result in a clogged needle or seat

- In well plate samplers, bottom sensing gives the most consistent position
- But this isn't recommended if there's debris in the bottom of the sample vial
- For vial samplers, a zero offset is approximately 2 mm from the bottom of a 2 mL vial

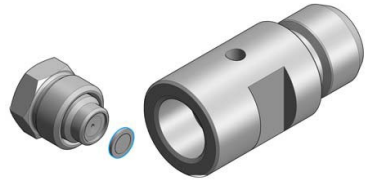
Needle height position without bottom sensing:
G1367E/G4226A 54 vial tray = 4 mm
G1367E/G4226A 100 vial tray = 2.5 mm
G7167X 54 vial tray = 5 mm

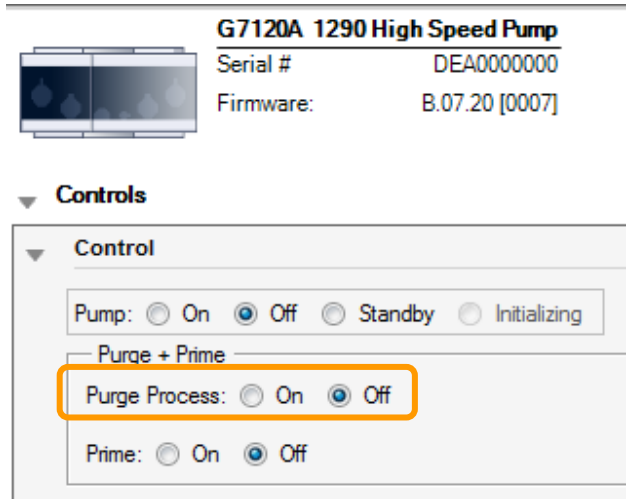


Locating a Clog

If the pressure doesn't drop when the valve switches to "Bypass", the issue is likely outside the sampler.

- Purge valves:
  - With 1260 Infinity II model pumps, open the manual purge valve. The pressure should drop to between 0 and 5 bar**. If the pressure is higher than this, the PTFE filter may be clogged.**
  - With 1290 Infinity II model pumps, purging is done through an automated valve, activated using software. 1290 binary pumps have the same PTFE filter, 1290 quaternary pumps have a 5 µm filter frit.





Locating a Clog

PTFE replacement on a 1260 pump:

1. Remove pump outlet and purge waste tubing
2. Unscrew the purge valve using a 14 mm wrench
3. Remove the gold seal cap
4. Remove the frit
5. Install the new frit, slot side up
6. Replace the gold seal cap
7. Reinstall the valve

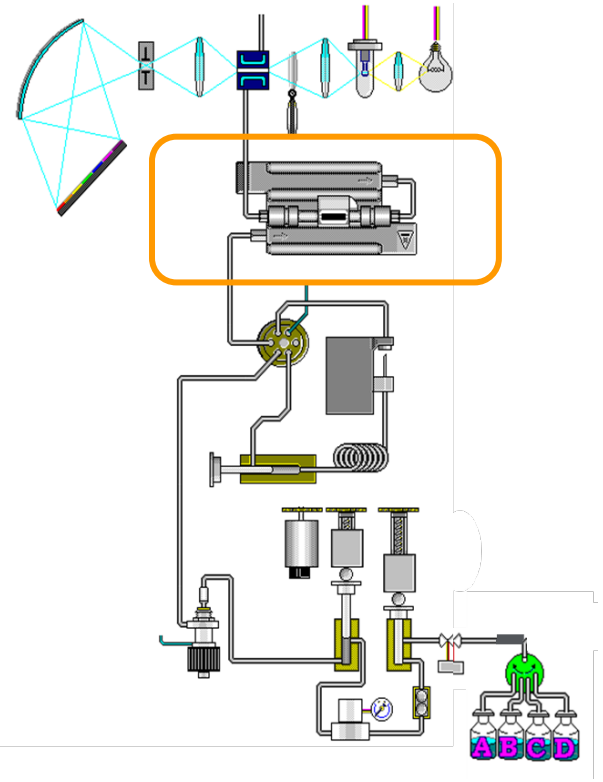
Re-align the waste tubing in the correct orientation during installation.

Locating a Clog

If the pressure doesn't drop when the valve switches to "Bypass", the issue is likely outside the sampler.

Column:

- Open the fitting at the inlet of the column.
- Pumping 1 mL/min of water through an Agilent LC with 0.17 mm id tubing typically shows a pressure of 40 bar.
- If the pressure is much higher than this, a capillary may be clogged. If the pressure appears "normal" the issue may be with the column.

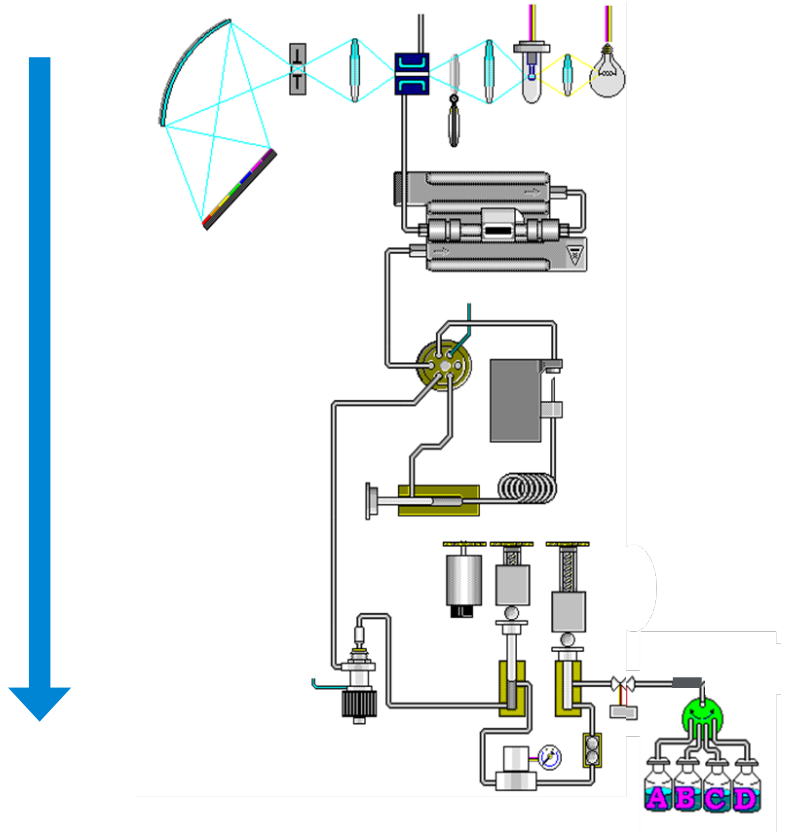


Locating a Clog

If the pressure doesn't drop when the valve switches to "Bypass", the issue is likely outside the sampler.

Working backwards from detectors:

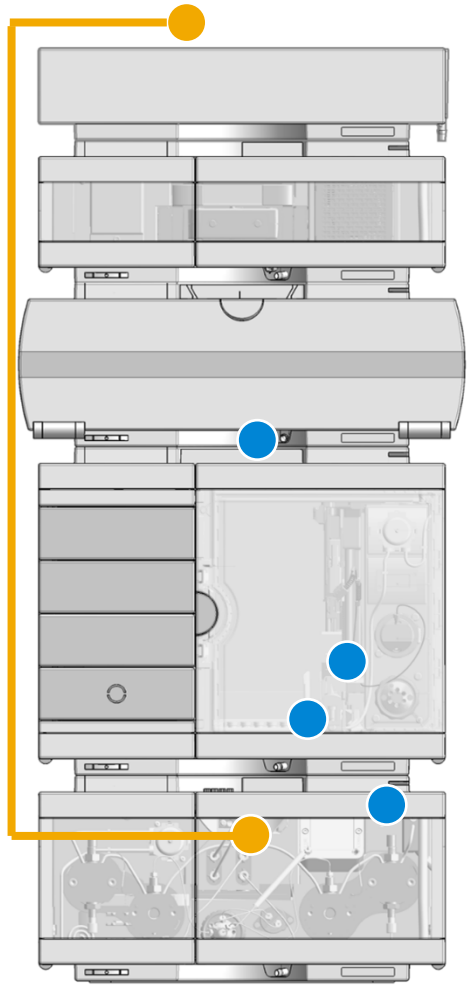
- Clogs are located by opening a fitting, typically at most a half turn.
- If the pressure drops, the clog is downstream from the fitting or towards the detector. If pressure remains high, the clog is upstream or towards the pump.



Changes in System Pressure

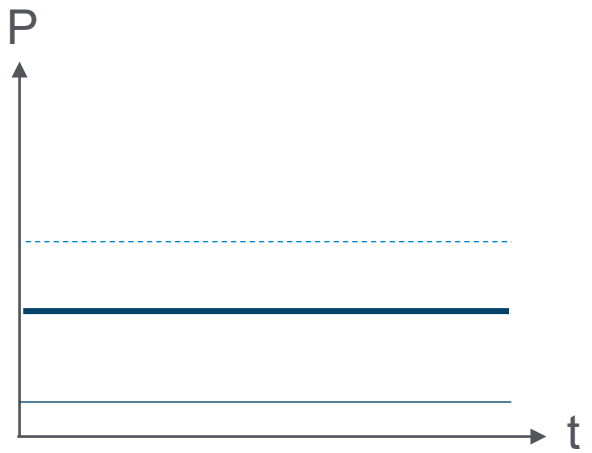
Low pressure

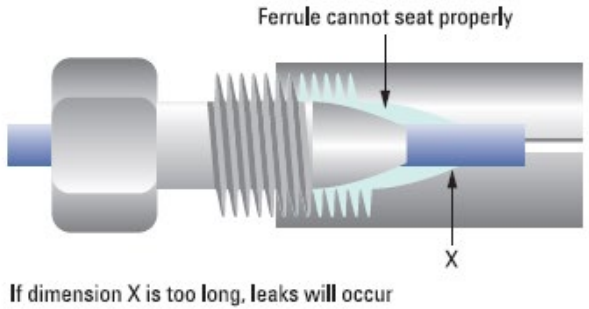
	Potential cause	Recommended action
●	Leak in high-pressure flow path	• Visual inspection of flow path • Instrument diagnostic tests
●	Wrong mobile phase	• Check for correct mobile phase • Check solvent reservoir and tube connections



Leaks

Characteristics	
Parts affected	• Potentially all parts in the flow path • High potential at frequently operated fitting connections (such as the column inlet) and parts with high mechanical stress (rotor seal, needle, and needle seat)
Characteristic	• Lower pressure • Potentially impacting retention times and peak shape
Identification	• Drops of solvent or residues of salt • System diagnostic tests
Possible root cause	• Loose or bad fitting connections • Cracked capillaries • Worn needle and needle seat
Instant action/first aid	• Replace affected parts • Renew or redo fitting connection
Preventive measures	• Use proper fitting connections • Replace fittings and wear parts in time

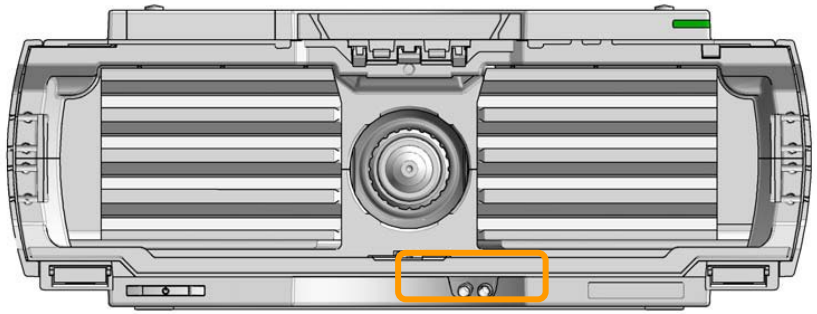


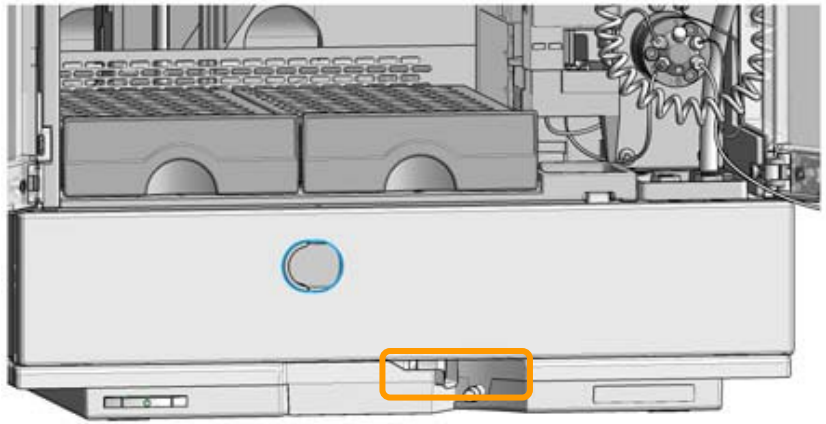


If dimension X is too long, leaks will occur

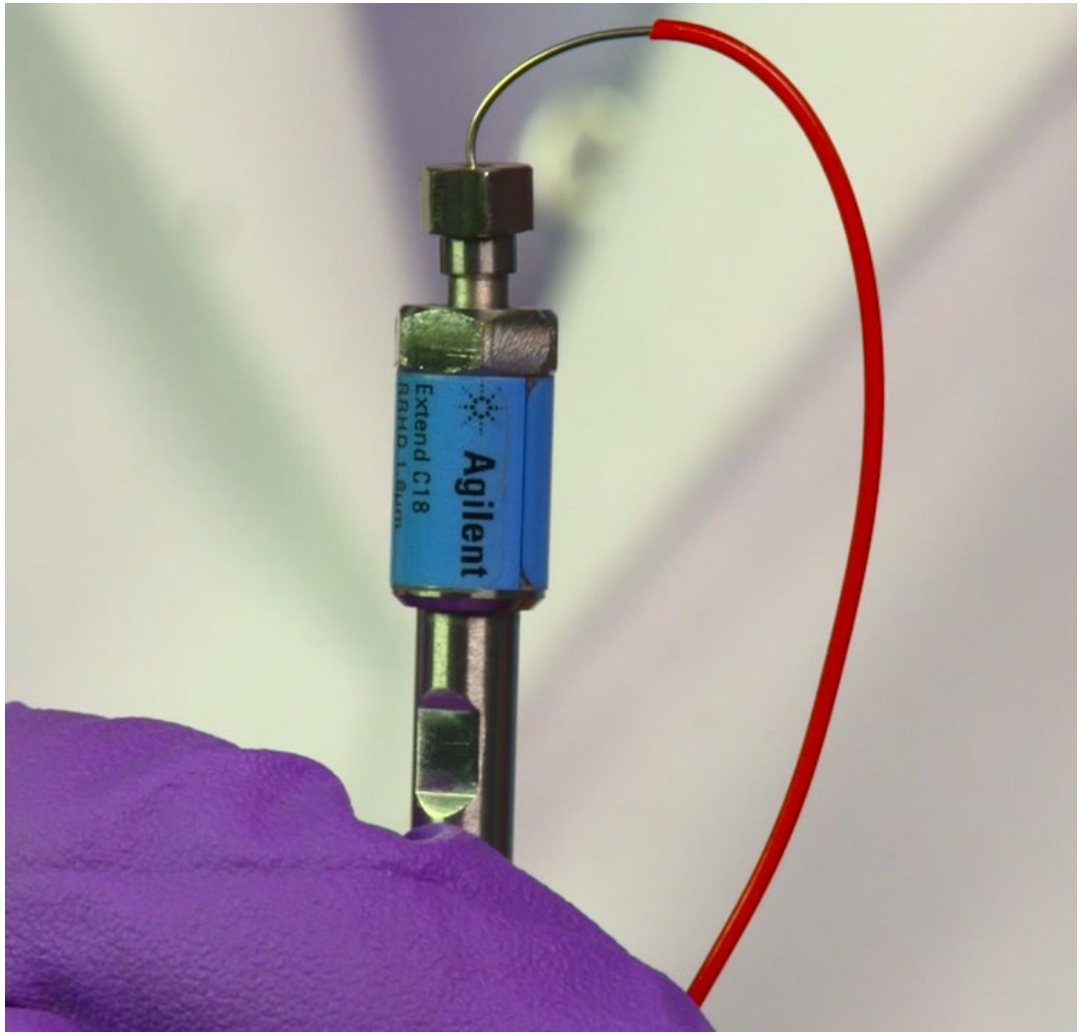
How Do I Locate a Leak?

- Each Agilent LC module is equipped with a leak sensor
- If liquid is detected, the entire LC stack will shut down
- The LC will not start up again until the sensor has been dried and returned to temperature





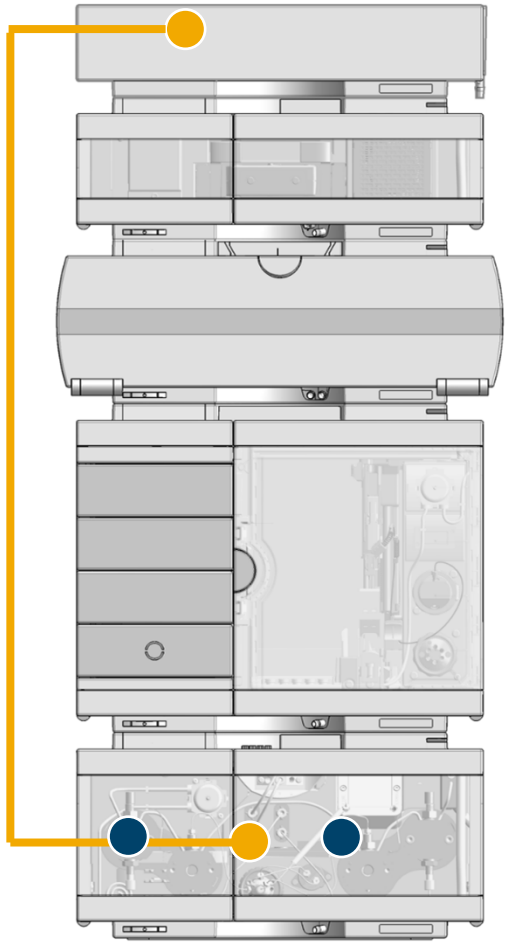
Overtightened Fittings



Changes in System Pressure

Pressure fluctuations

Potential Cause	Recommended Action
Air in the system	• Prime and flush instrument • Check for sufficient solvent supply • Check for correct plumbing (SSV/MCGV) • Check for correct degassing
Malfunctions at pump head	• Perform pump head diagnostic tests LA • Replace defective parts • Implement proper maintenance schedule
Cavitation effects	• Check for flow restrictions (solvent bottle to pump head inlet) • Clean or replace parts • Verify that solvent supply is positioned above pump inlet



Important to know

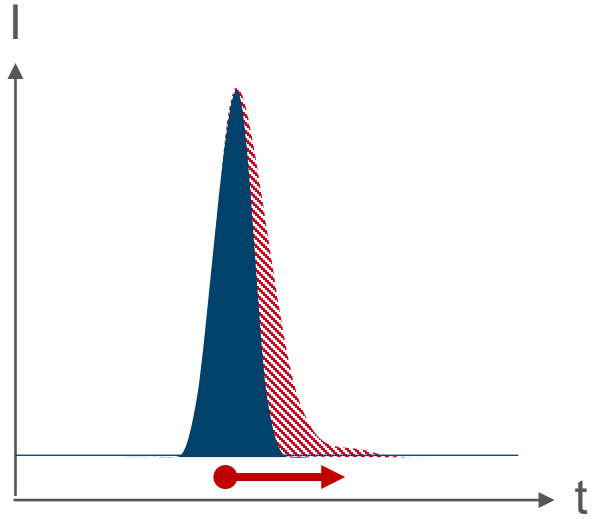
Pressure fluctuations typically also impact the UV signal due to refractive index effects.

Peak Shape

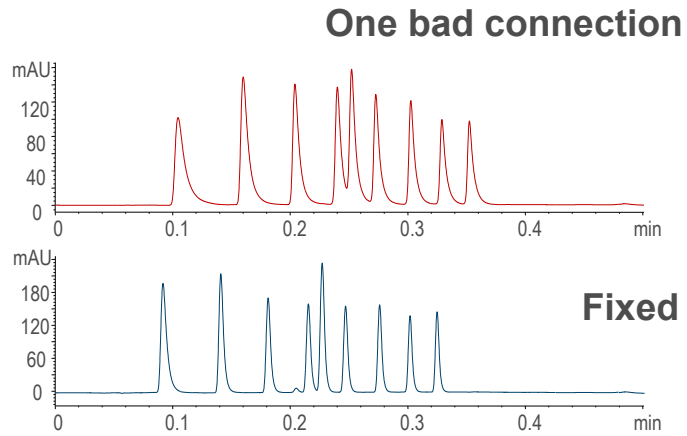
Changes in Peak Shape

Peak tailing

If applicable to some peaks	Recommended Action
Secondary interactions	• Check pH of mobile phase (most likely) • Pump malfuntion
Small peak eluting on tail of larger peak	

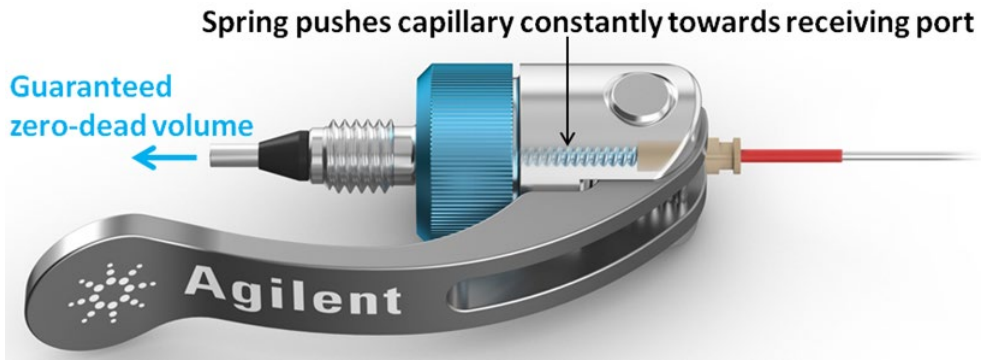


If applicable to all peaks	Recommended Action
Poor tubing connections; high dispersion volume	• Minimize number of connections • Check connections/fitting condition and proper seat of fittings • Use fittings with spring-loaded function
Column damage	• Use specialty, polymeric or sterically protected column • Column cleaning



InfinityLab Quick Connect and Quick Turn Fittings

- Spring-loaded design
- Easy to use
- Works for all column types
- Reusable
- Consistent ZDV connection



Quick Connect Fitting
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

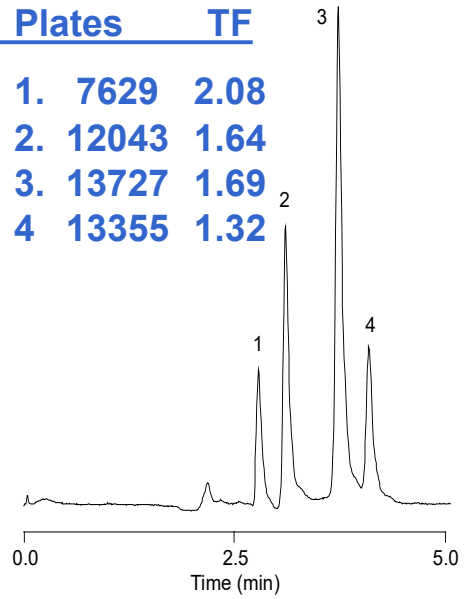
Quick Turn Fitting
- Finger tight up to 400 bar
- Up to 1300 bar with a wrench
- Compact design

Peak Tailing: Column Contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5 mm Mobile phase: 20% H2O : 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

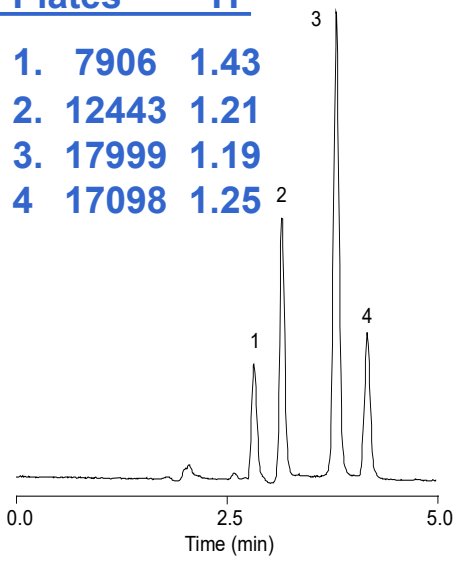
QC test forward direction

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



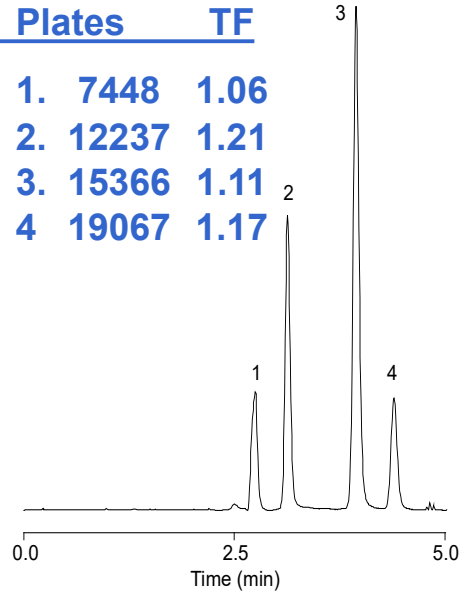
QC test reverse direction

	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

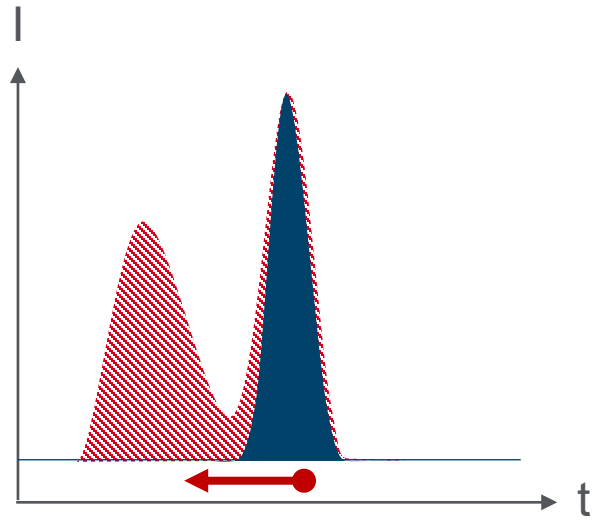
	Plates	TF
1.	7448	1.06
2.	12237	1.21
3.	15366	1.11
4	19067	1.17



Changes in Peak Shape

Peak splitting/doubling

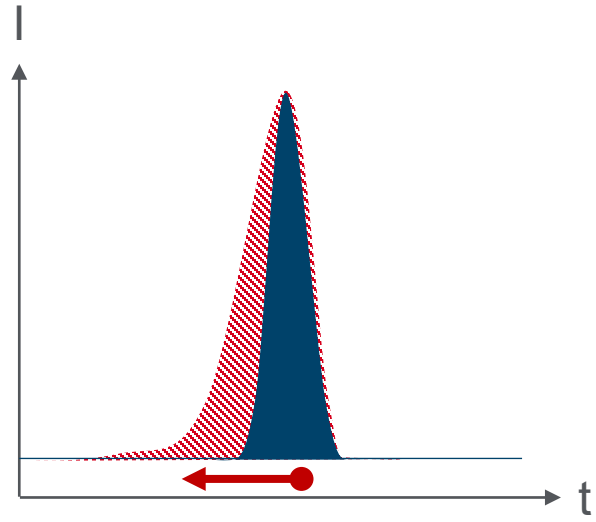
	Potential Cause	Recommended Action
	Partially plugged column frit	• Backflush column (if applicable) • Use inline filter • Use guard column
	Column void	• Replace column • Use guard column • Use less aggressive mobile phase conditions
	Sample volume overload	• Use smaller injection volume
	Sample solvent incompatibility with mobile phase	• Use mobile phase or weaker miscible solvent as injection solvent
	Issues with injection valve	• Check injector valve parts • Replace worn parts

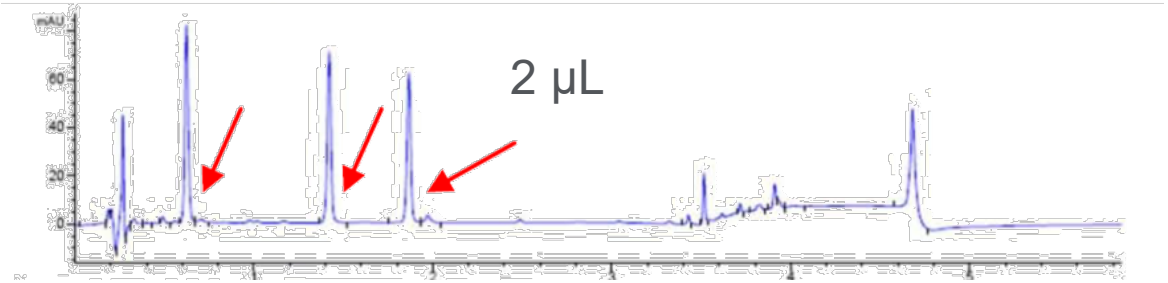


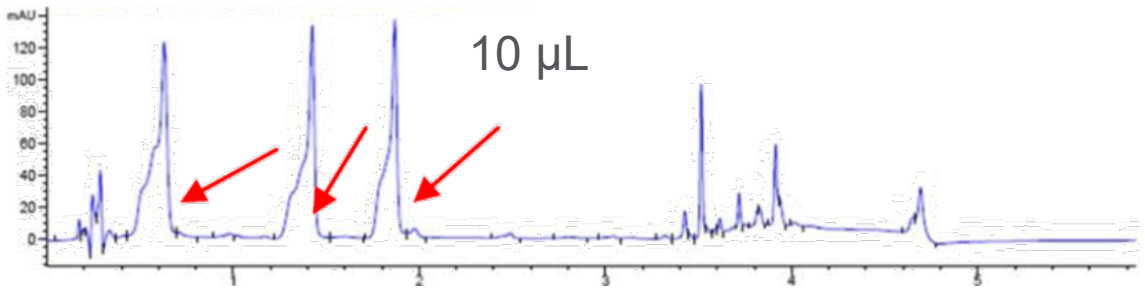
Changes in Peak Shape

Fronting

	Potential Cause	Recommended Action
	Channeling in column	• Replace column • Use guard columns
	Column overload	• Use higher capacity column (increase length, diameter, or change to high-capacity material) • Decrease sample amount



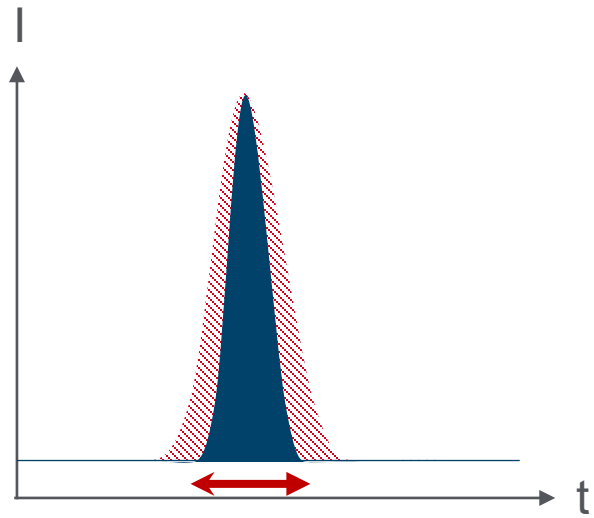




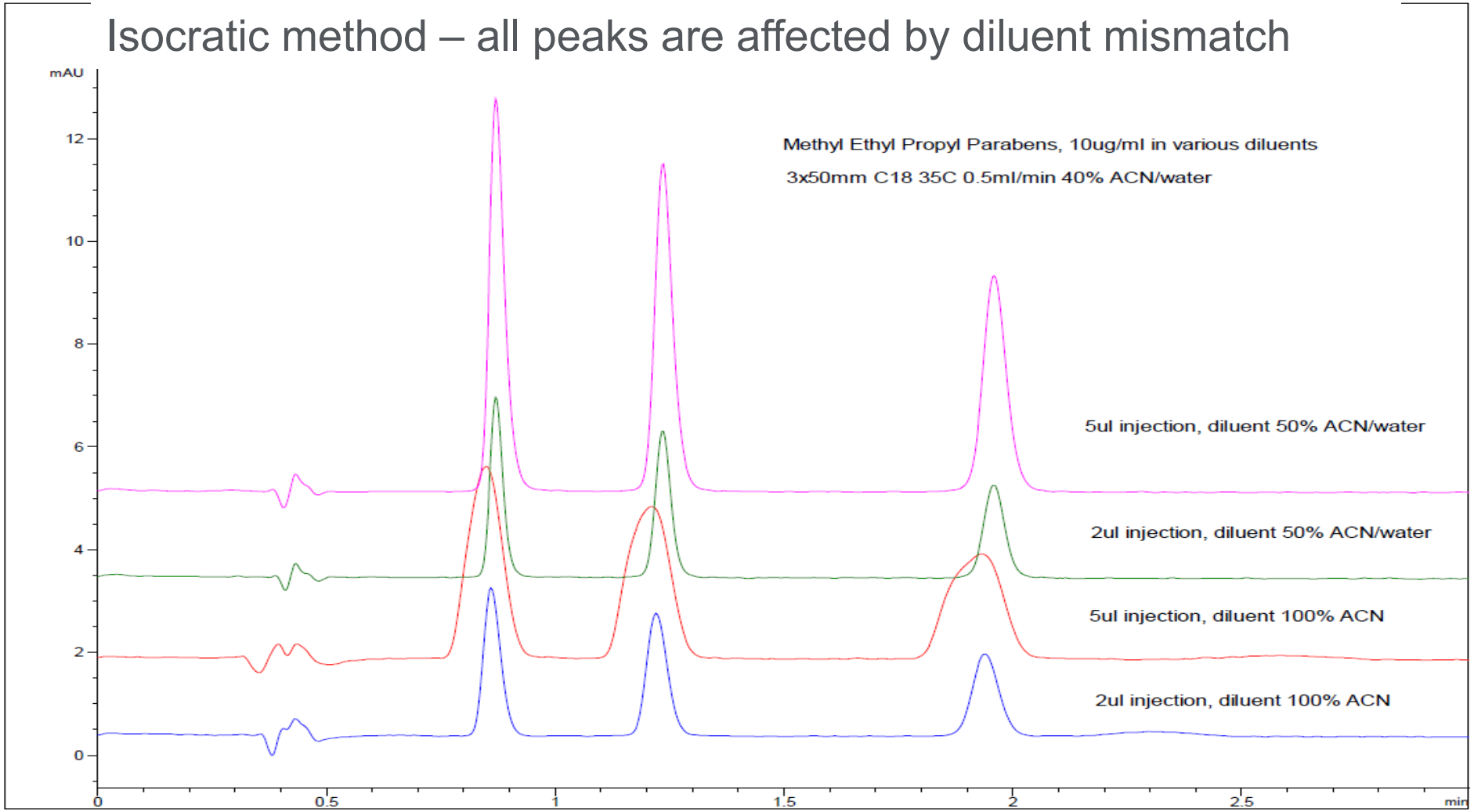
Changes in Peak Shape

Peak broadening

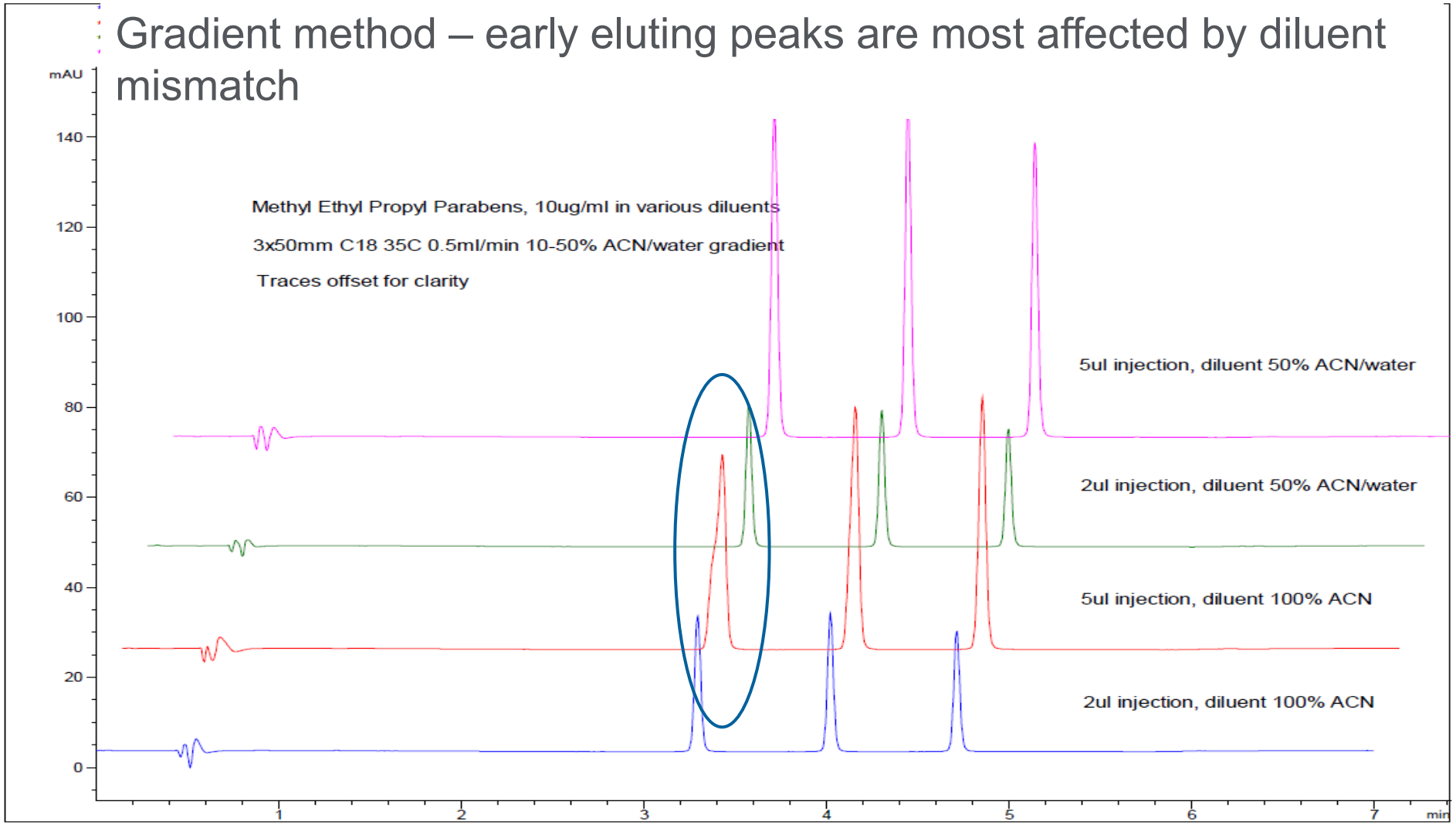
	Potential Cause	Recommended Action
	Injection volume too large	• Decrease injection volume
	Long retention times	• Use gradient elution or stronger mobile phase
	System settings	• Check data collection rate • Adjust the detector setting or time constant to the fastest possible value without compromising signal-to-noise.
	Viscosity of mobile phase too high	• Increase column temperature
	Detector cell volume too large	• Use smallest possible cell volume
	Improper fittings and connections	• Ensure that your fitting connections are correct
	Extra tubing volume on system	• Ensure that the tubing is narrow and as short as possible to avoid extra volume
	Sample diluent too strong	• Reduce diluent strength



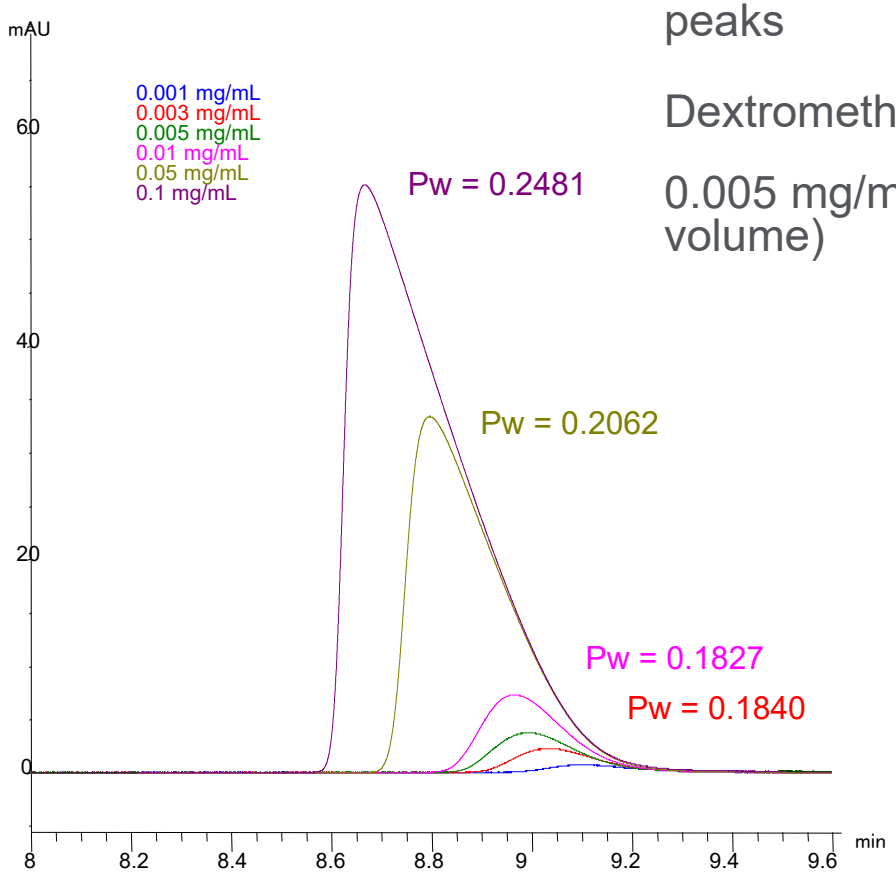
Strong Diluents Can Disrupt Equilibration – Isocratic Method



Strong Diluents Can Disrupt Equilibration – Gradient Analysis



Comparison of Peak Shape at Low and High Loads Broadening and Tailing

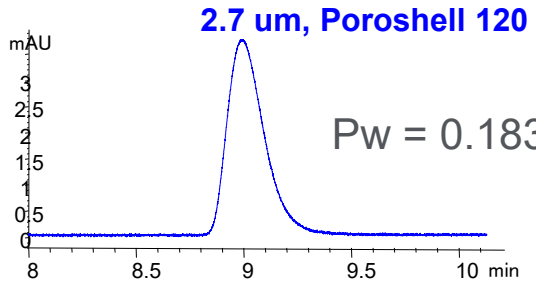


High sample loads give broad or broad and tailing peaks

Dextromethorphan is 35% broader at high load

0.005 mg/mL dextromethorphan (4.1 uL injection volume)

Low sample loads provide symmetrical, nontailing peaks with narrow peak widths

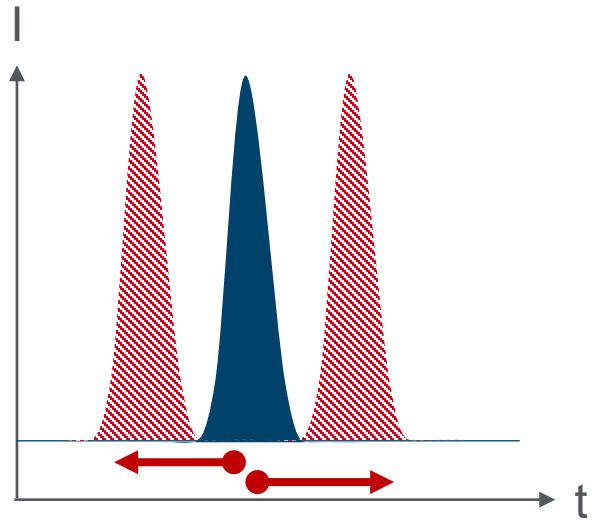


Changes in Separation

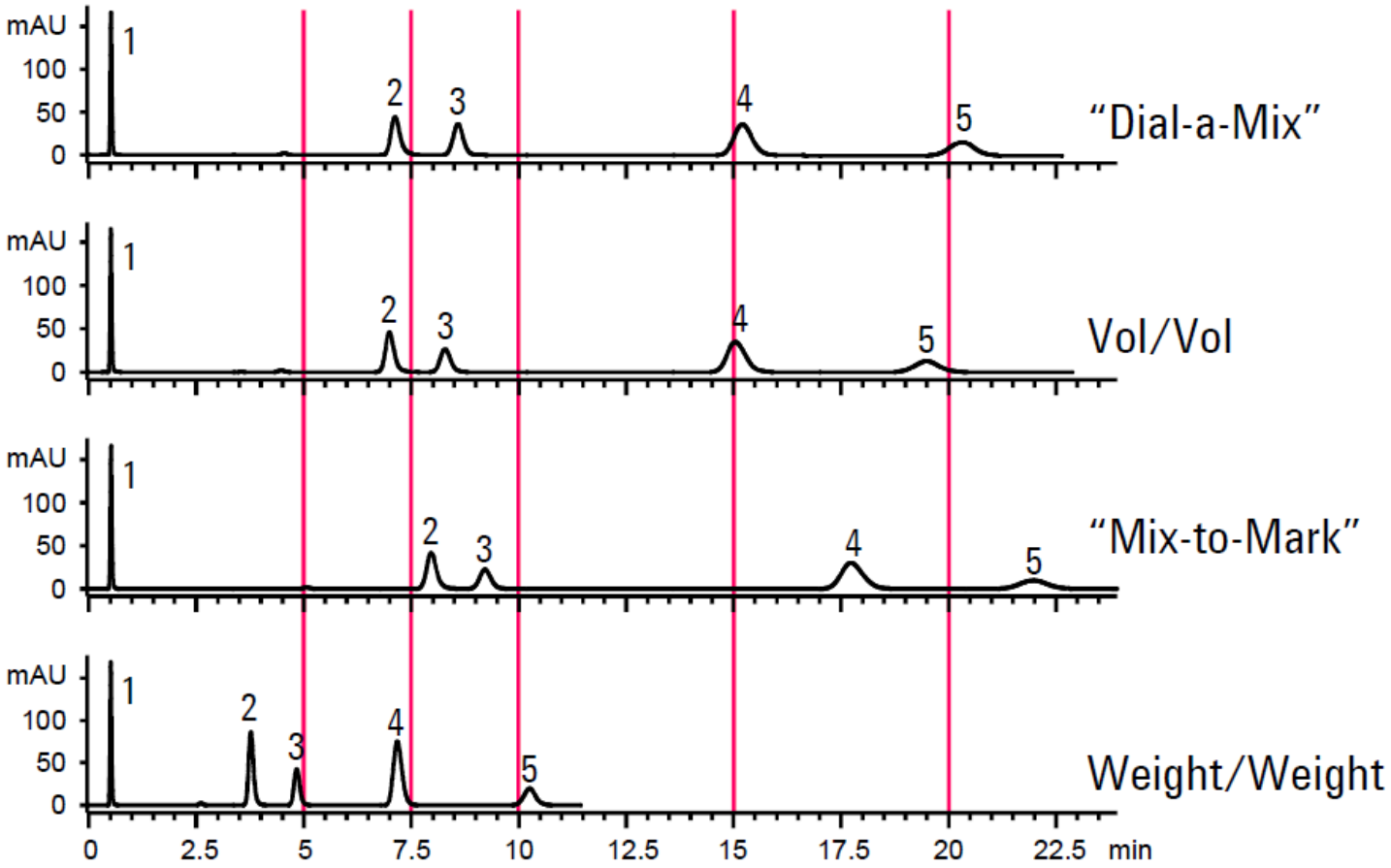
Changes in Separation

Retention time changing

	Potential Cause	Recommended Action
	Inconsistent online mobile phase mixing	Ensure gradient system is delivering constant composition check vs. manual preparation of mobile phase
	Flow rate changing	Check ‘pressure fluctuation'
	Column temperature varying	Thermostat column and ensure constant lab temperature
	Equilibration time insufficient with gradient run or change in isocratic mobile phase	Flush with at least 10 column volumes after solvent change or gradient conclusion
	Selective evaporation of mobile phase component	Keep solvent reservoirs covered prepare fresh mobile phase
	Buffer capacity insufficient	Use > 20 mM concentration of buffer
	Contamination buildup	Occasionally flush column with strong solvent to remove contaminants
	First few injections – adsorption on active sites	Condition column by initial injection of concentrated sample
	Column overloaded with sample	Decrease injection volume or concentration
	Mobile phase composition changing	Follow ‘best practices'



Mobile Phase Preparation

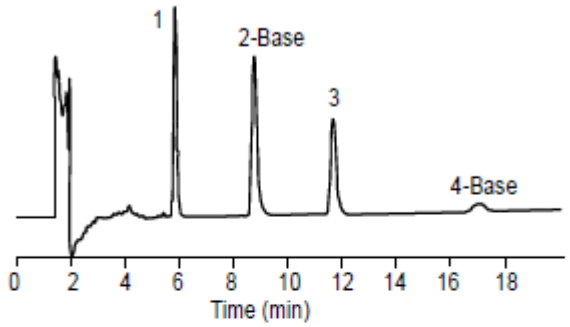


Agilent 1100 with quaternary pump
ZORBAX Eclipse XDB-C8 Rapid-Resolution (3.5µm), 4.6 x 50 mm
Agilent Part No. 935967-906
Dial-a-Mix= A: water B: MeOH, pump 50% B
Vol/Vol=250 mL water + 250 mL MeOH, pump 100%
Mix-to-Mark = 250 mL MeOH, fill to 500 mL with water, pump 100%
Premixed (w/w) = 200 g MeOH + 200 g water, pump 100%
UV 254 nm
1 mL/ min.

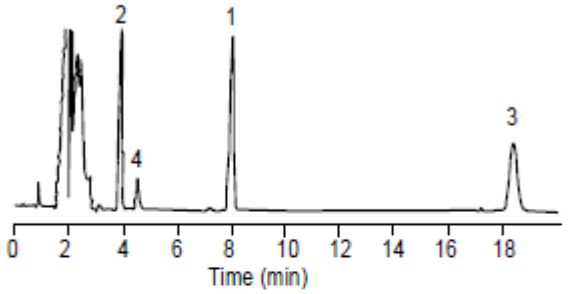
Retention Time Shift – Selectivity Differences Due to Incorrect pH

pH 4.5 shows selectivity change from lot-to-lot for basic compounds

pH 4.5 - Lot 1

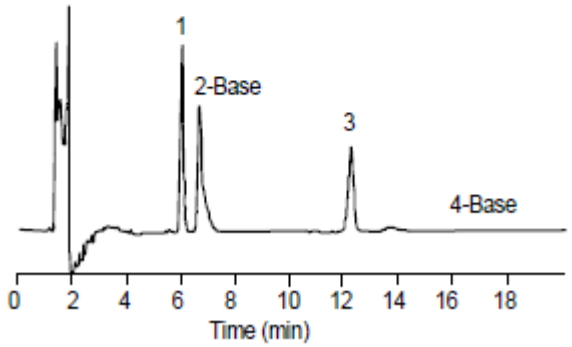


pH 3.0 - Lot 1

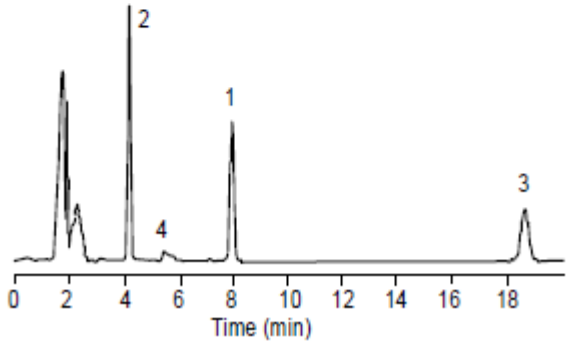


pH 3.0 shows no selectivity change from lot-to-lot

pH 4.5 - Lot 2



pH 3.0 - Lot 2



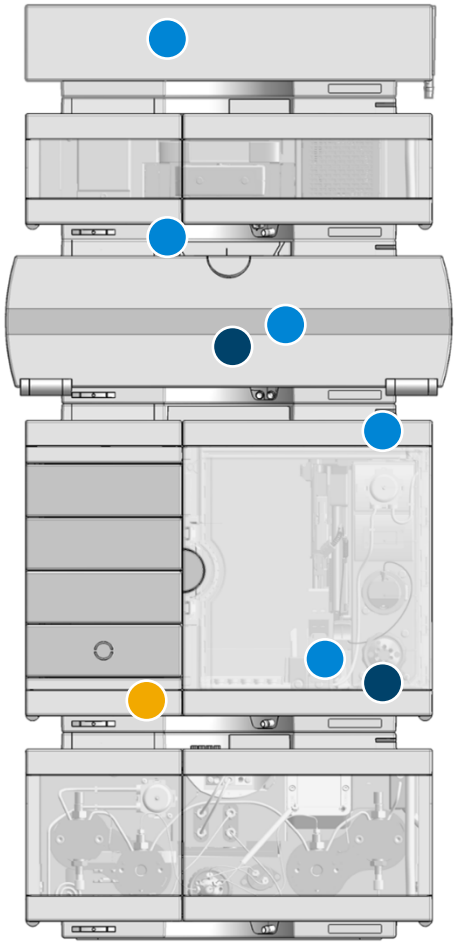
- For method ruggedness
  - Test three different column lots
  - Compare R_s for the three lots
    - If ΔR_s is too large, modify method

Changes in Separation

Ghost peaks, carry over

	Potential Cause	Recommended Action
●	Peaks from previous injection	• Flush column to remove contaminants • Check with blank injection
●	Specific interaction with metal surfaces	• Passivate instrument • Use InfinityLab Deactivator Additive • Use bio-inert LC equipment
●	Contamination or unknown interferences in samples	• Proper sample cleanup

BIO INERT



Mobile Phase Hygiene

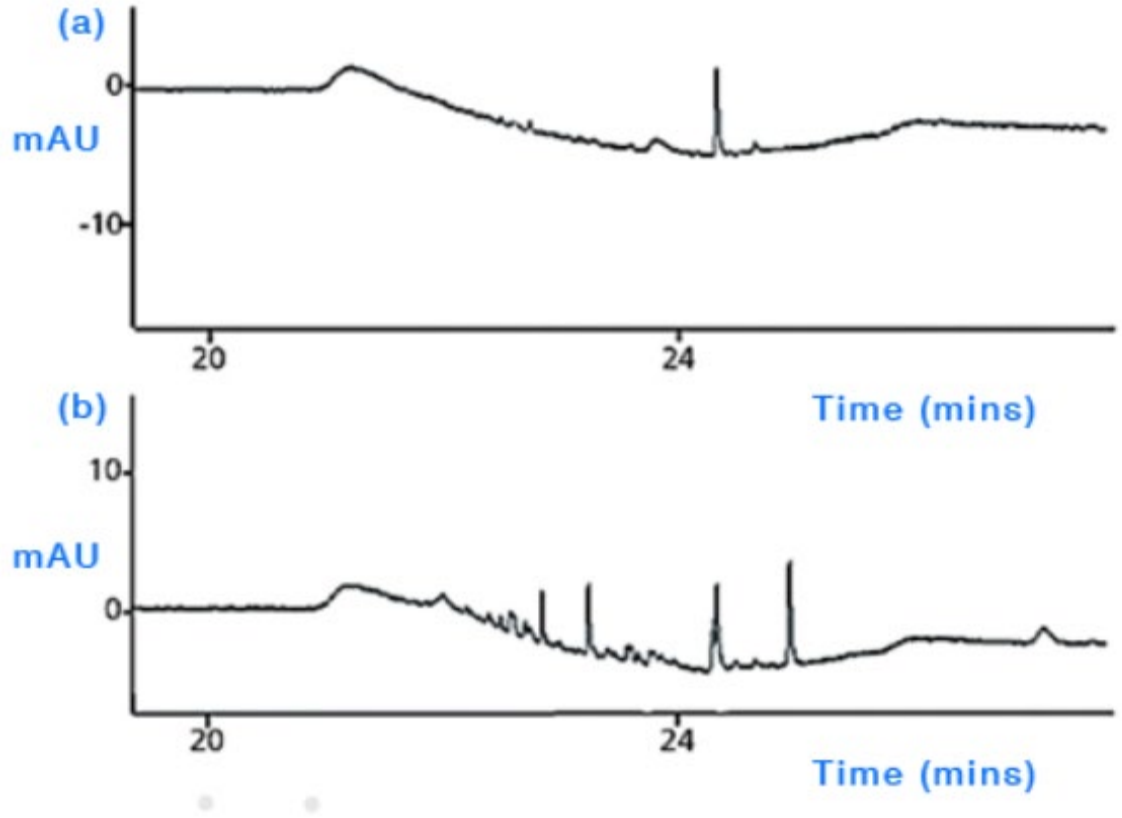
Contaminated mobile phases can cause:

- Lower sensitivity
- Rising/drifting baselines
- Higher noise
- Ghost peaks with gradient separations

Often the issue is confused with autosampler carryover.

It can be identified by repeating the gradient run without sample injection or increasing the pre-run equilibration.

Always run multiple blanks before standards or samples to distinguish gradient artifacts from possible carryover.

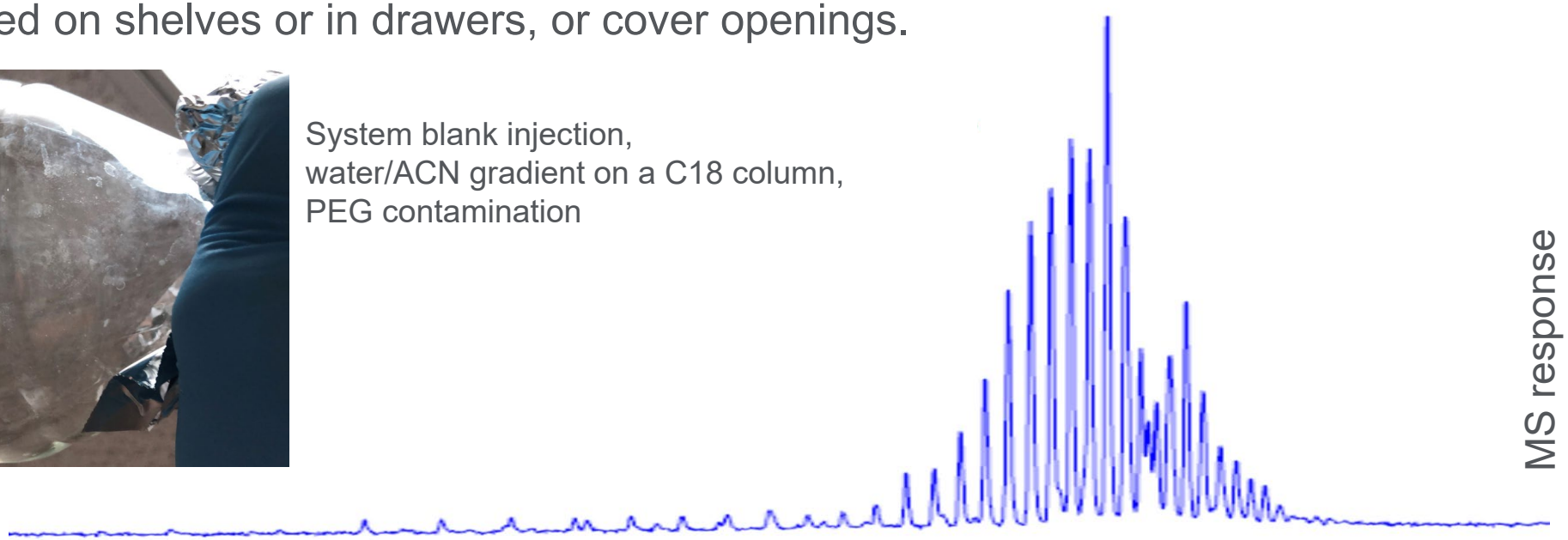


Mobile Phase Hygiene: Glassware

Improper cleaning of solvent bottles can cause contamination of mobile phases and result in gradient artifacts

- Wash solvent bottles with hot water, deionized water, and organic solvent (IPA or acetonitrile).
- Leave glassware inverted on paper towels on a bench or clean pegboard dowels to dry.
- Avoid using detergents. If it is necessary to use detergents to get glassware clean, rewash with plenty of hot and cold water so that all detergent residues are removed. Follow with deionized water and organic (IPA or acetonitrile) rinses.
- Store glassware inverted on shelves or in drawers, or cover openings.

System blank injection,
water/ACN gradient on a C18 column,
PEG contamination



Mobile Phase Hygiene: Solvent Purity and Buffer Preparation

- Use HPLC grade organic mobile phases
- Use HPLC grade water or Milli-Q DI water
- Use HPLC grade reagents, including salts, ion pair reagents, and base and acid modifiers
- Always rinse pH electrodes thoroughly when measuring/adjusting the pH of the mobile phase
- Prepare fresh buffers to avoid contaminants from the growth of bacteria or algae
- Filter your mobile phase buffer with 0.45 µm filter before use
- Solvent filters installed at the end of solvent lines should be replaced periodically

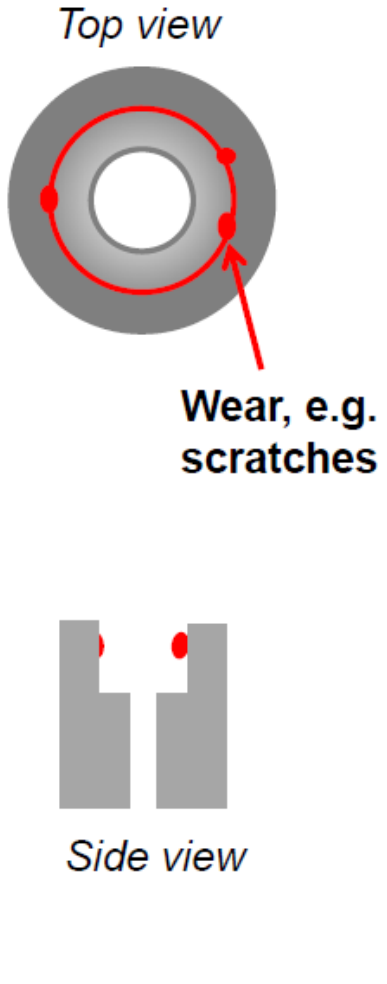
100% ACN

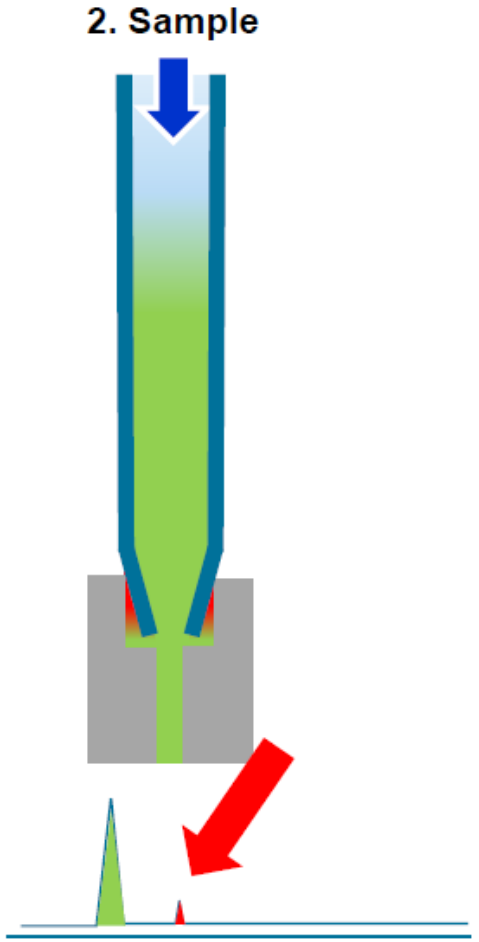
90%ACN+10% buffer (10 mM phosphate)

Autosampler Carryover

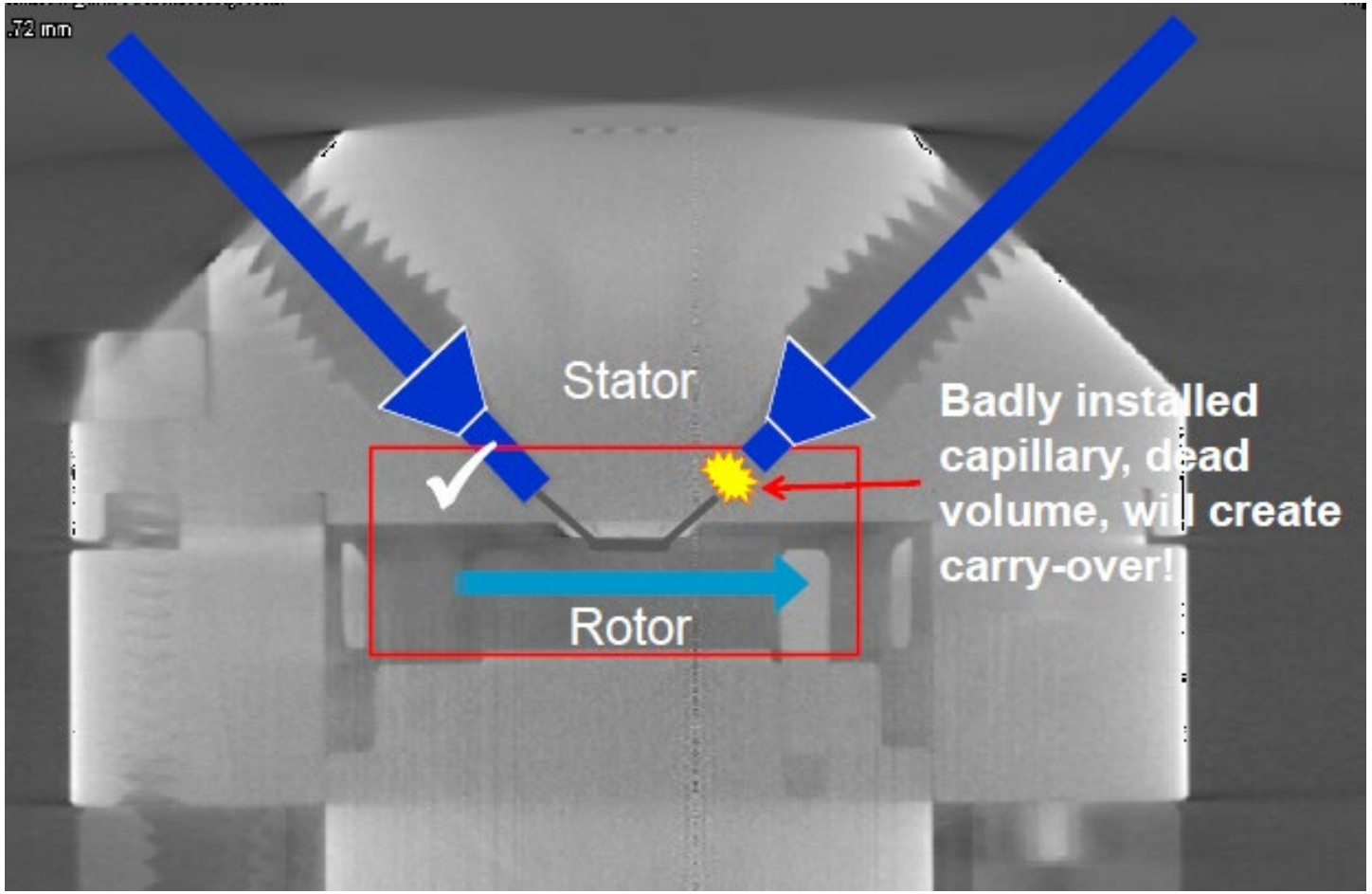
Common sources:

- Exterior of needle (use needle wash)
- Worn needle seat
- Worn rotor seal
- Poorly made fitting





Autosampler Carryover

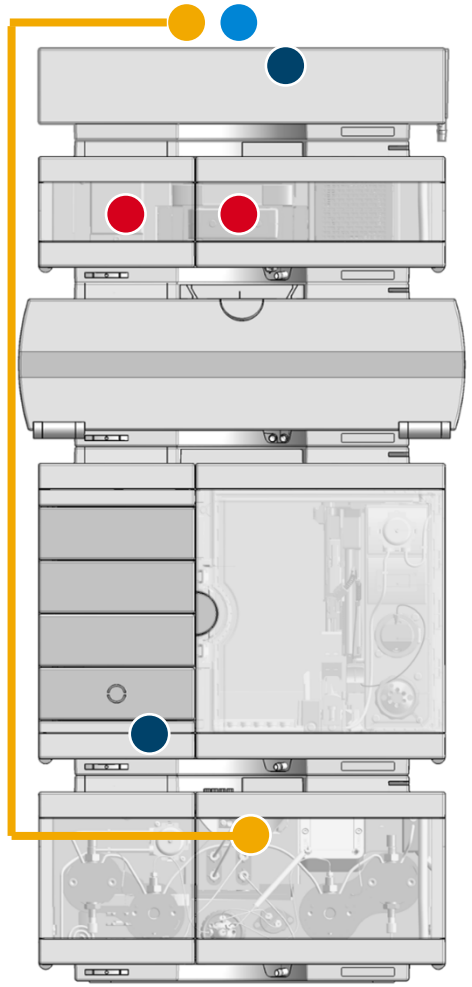


Changes in Detection

Changes in Detection

Noisy baseline

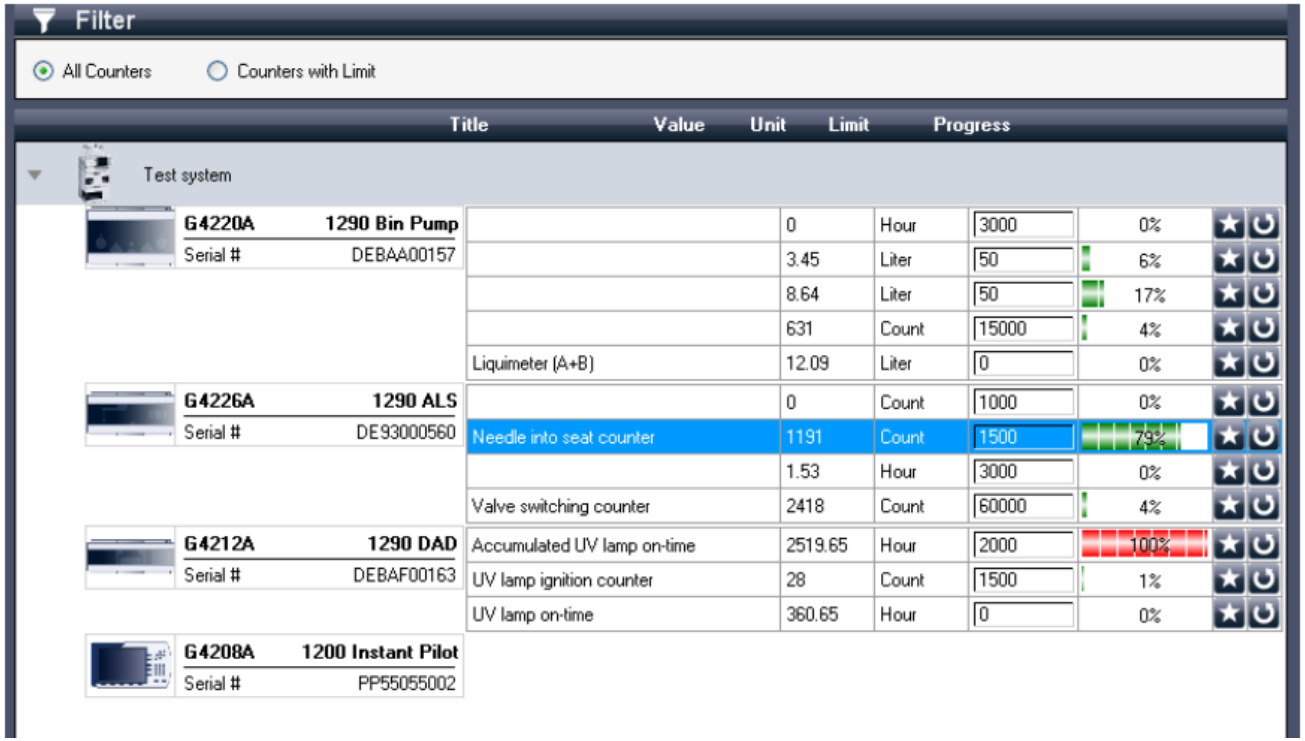
	Potential Cause	Recommended Action
●	Gas bubbles in mobile phase	• Apply degassing • Check degasser performance
●	Low difference between sample and mobile phase absorbance	• Check absorbance values of sample vs. mobile phase
●	Contamination	• Use degassed HPLC-grade solvents • Flush system • Clean up the sample
●	Detector optics	• Perform intensity test • Check signal with flow cell removed if possible • Replace lamp
	Pressure instability	• Check ‘pressure fluctuation'



UV Lamp Tests

How do I know if my UV lamp is good?

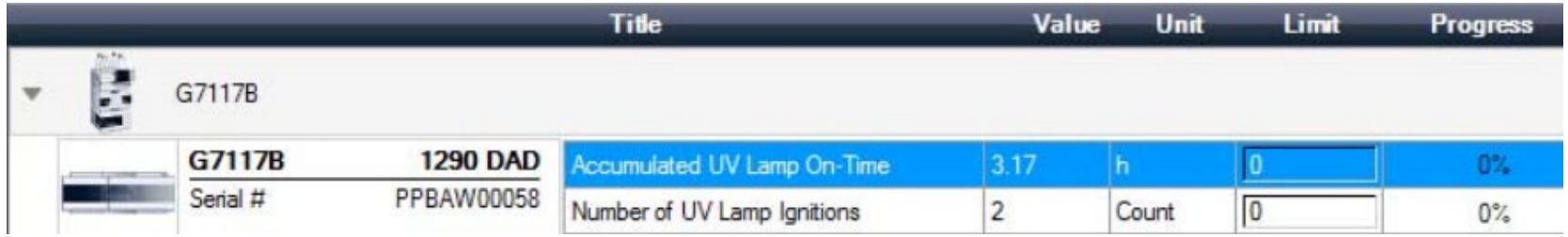
- Visual inspection of an equilibrated baseline
- Accumulated UV lamp on-time from RFI tag or Lab Advisor
- Lab Advisor intensity test
- Lab Advisor ASTM drift and noise test
- Lab Advisor cell test



UV Lamp Tests

Diode array and multiple wavelength

Counters and hours



The useable lifetime of a deuterium lamp will depend on it's use:

- How many hours has it been on?
- How many times has it been ignited?
- What wavelength is being used?

UV Lamp Tests

Diode array and multiple wavelength

Intensity test

Intensity Test — 1260 HPLC » 1260 HPLC » G7115A:DEAC600377

General | Limits | Signals

Test Name	Intensity Test	**Description**	The test scans the Intensity spectrum generated by the UV and VIS Lamp.
Module	G7115A:DEAC600377 (1260 DAD WR)		
Status	Passed		
Start Time	2/21/2019 3:21:31 PM		
Stop Time	2/21/2019 3:22:15 PM		

Test Procedure

1. Check Prerequisites...
2. Remove Flow Cell.
3. Scan Intensity Spectrum...
4. Evaluate Data...

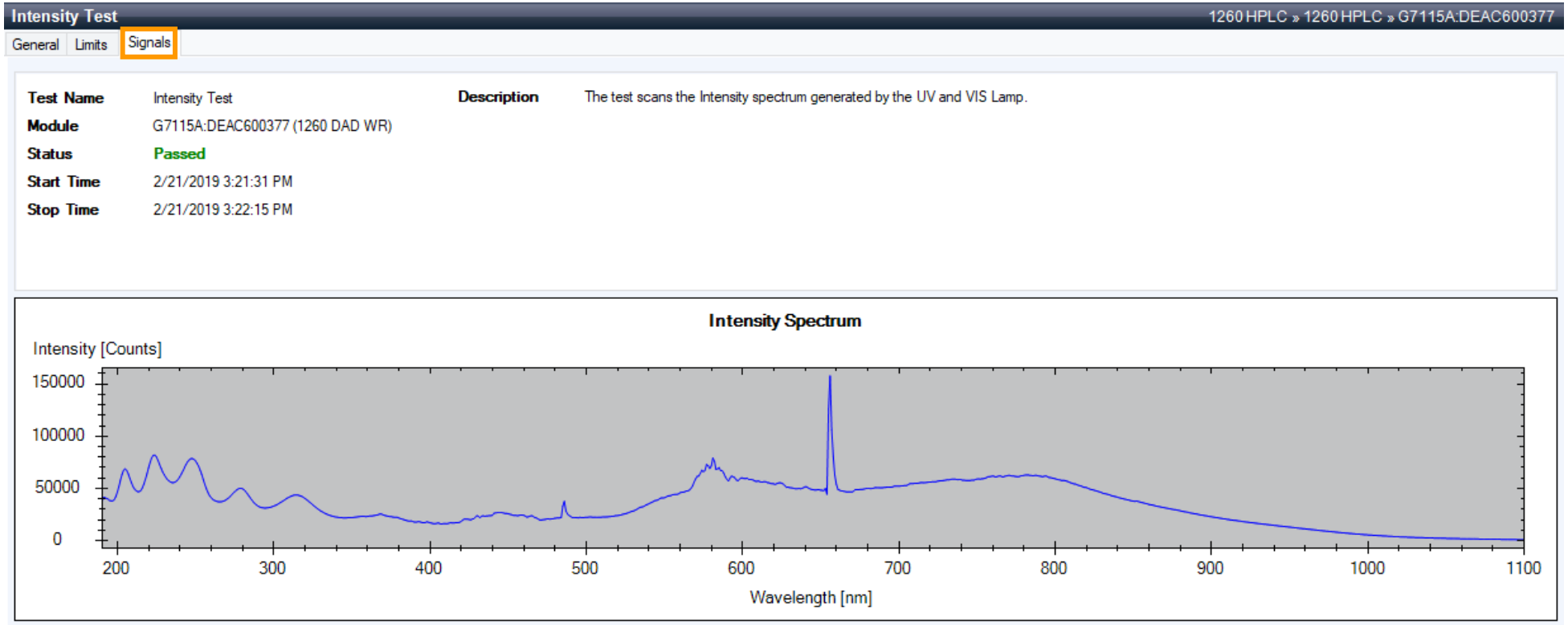
Result

Name	Value
Accumulated UV Lamp Burn Time	126.35 h
UV Lamp On-Time	0.02 h
Accumulated Vis Lamp Burn Time	262.45 h
Vis Lamp On-Time	0.02 h
Lowest Intensity in Range 190 - 220 nm	36289 Counts
Lowest Intensity in Range 190 - 220 nm	2000 Counts
Lowest Intensity in Range 221 - 350 nm	21963 Counts
Lowest Intensity in Range 221 - 350 nm	5000 Counts
Lowest Intensity in Range 351 - 500 nm	16150 Counts
Lowest Intensity in Range 351 - 500 nm	2000 Counts
Lowest Intensity in Range 501 - 950 nm	13102 Counts
Lowest Intensity in Range 501 - 950 nm	2000 Counts
Highest Intensity in Range 190 - 350 nm	81934 Counts
Highest Intensity in Range 190 - 350 nm	2000 Counts
Highest Intensity in Range 700 - 950 nm	62919 Counts
Highest Intensity in Range 700 - 950 nm	2000 Counts
Highest Intensity for D2 Alpha Line (600 - 700 nm)	157676 Counts
Highest Intensity for D2 Alpha Line	2000 Counts
Spectrum Integral	31863112
UV Integral (190 - 349 nm)	7384053

UV Lamp Tests

Diode array and multiple wavelength

Intensity test



The profile of the intensity scan changes as a lamp ages

UV Lamp Tests

Diode array and multiple wavelength

ASTM drift and noise

ASTM Drift and Noise Test — 1260 HPLC » 1260 HPLC » G7115A:DEAC600377

General | Limits | Signals

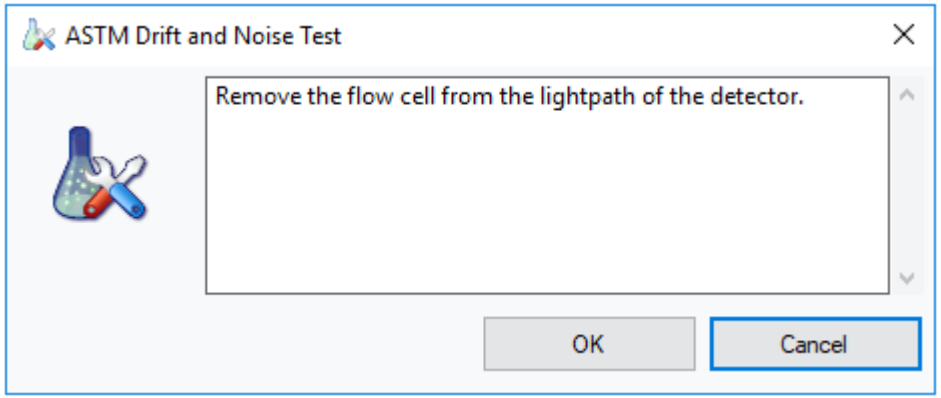
Test Name	ASTM Drift and Noise Test	**Description**	The test performs ASTM drift and noise evaluation without reference.
Module	G7115A:DEAC600377 (1260 DAD WR)		
Status	Passed		
Start Time	2/21/2019 4:56:26 PM		
Stop Time	2/21/2019 5:16:47 PM		

Test Procedure

1. Check Prerequisites...
2. Remove Flow Cell.
3. Measure Noise...
4. Evaluate Data...

Result

Name	Value
Accumulated UV Lamp Burn Time	127.94 h
UV Lamp On-Time	1.60 h
Minimum Lamp On-Time	1 h
Accumulated Vis Lamp Burn Time	264.03 h
Vis Lamp On-Time	1.61 h
Minimum Lamp On-Time	1 h
Signal Drift value at 254 nm (UV)	-0.138 mAU/h
Maximum Allowed Drift	-1 ... 1 mAU/h
Signal Noise value at 254 nm (UV)	0.008 mAU
Maximum Allowed Noise	0 ... 0.02 mAU
Signal Drift value at 750 nm (Vis)	-0.415 mAU/h
Maximum Allowed Drift	-1 ... 1 mAU/h
Signal Noise value at 750 nm (Vis)	0.013 mAU
Maximum Allowed Noise	0 ... 0.02 mAU



UV Lamp Tests

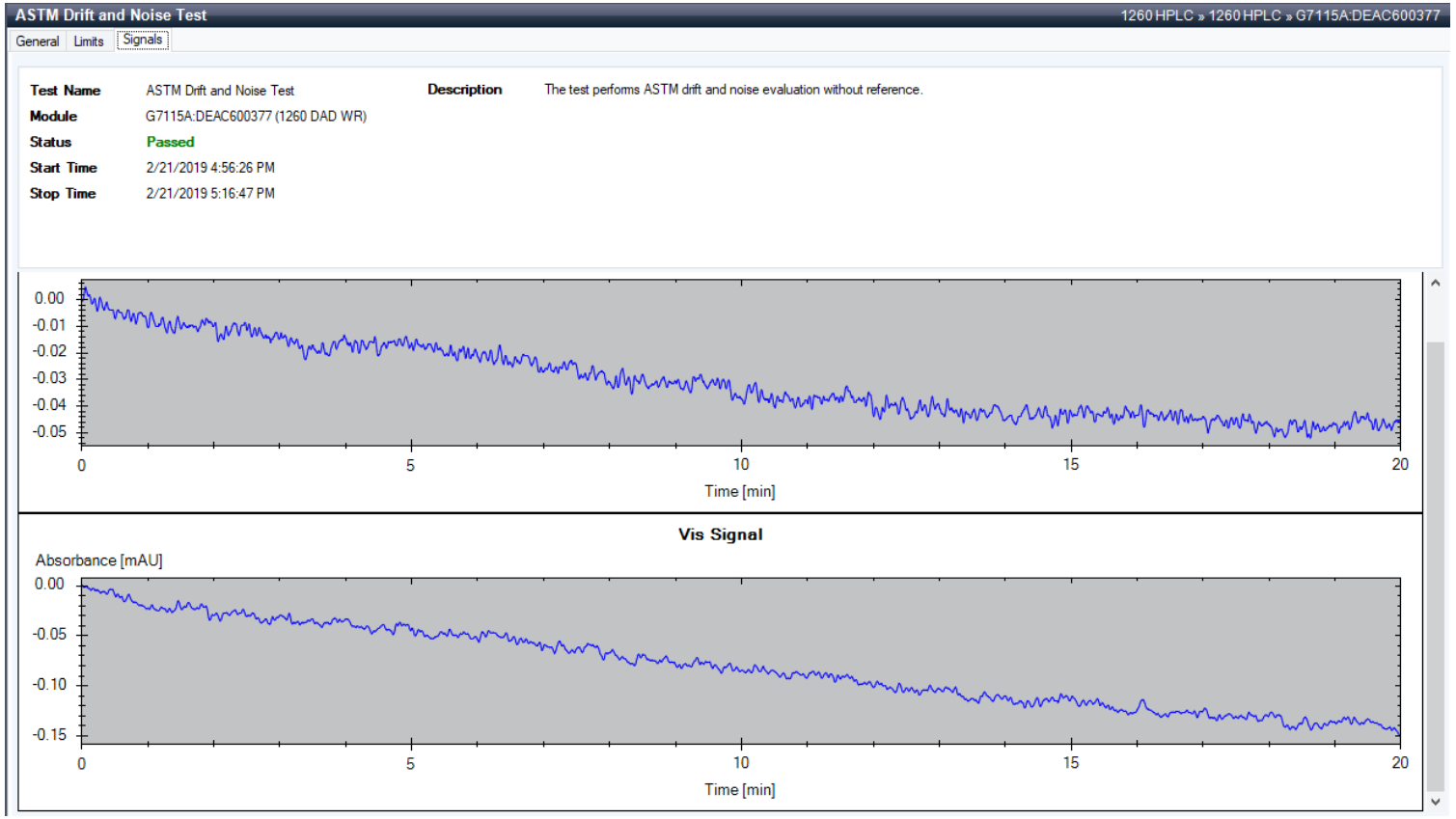
Diode array and multiple wavelength

Name	Value	Description
Minimum Lamp On-Time	1 h	The minimum lamp on-time to perform a noise check.

Name	Lower limit	Upper limit	Description
Maximum Allowed Noise	0 mAU	0.02 mAU	The maximum allowed Signal noise in mAU.
Maximum Allowed Drift	-1 mAU/h	1 mAU/h	The maximum allowed Signal drift in mAU.

ASTM drift and noise

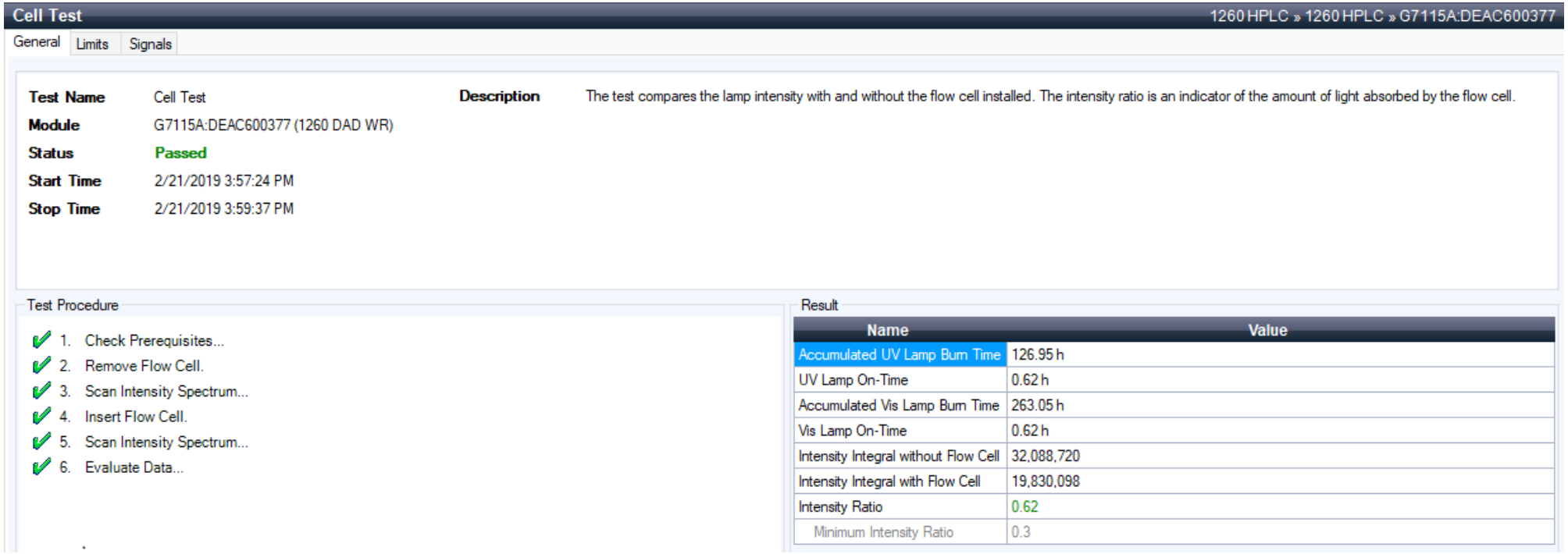
Run on a monthly basis, this test can help track the natural decline of the lamp and perhaps raise awareness of a dirty cell.



UV Lamp Tests

Diode array and multiple wavelength

Cell test

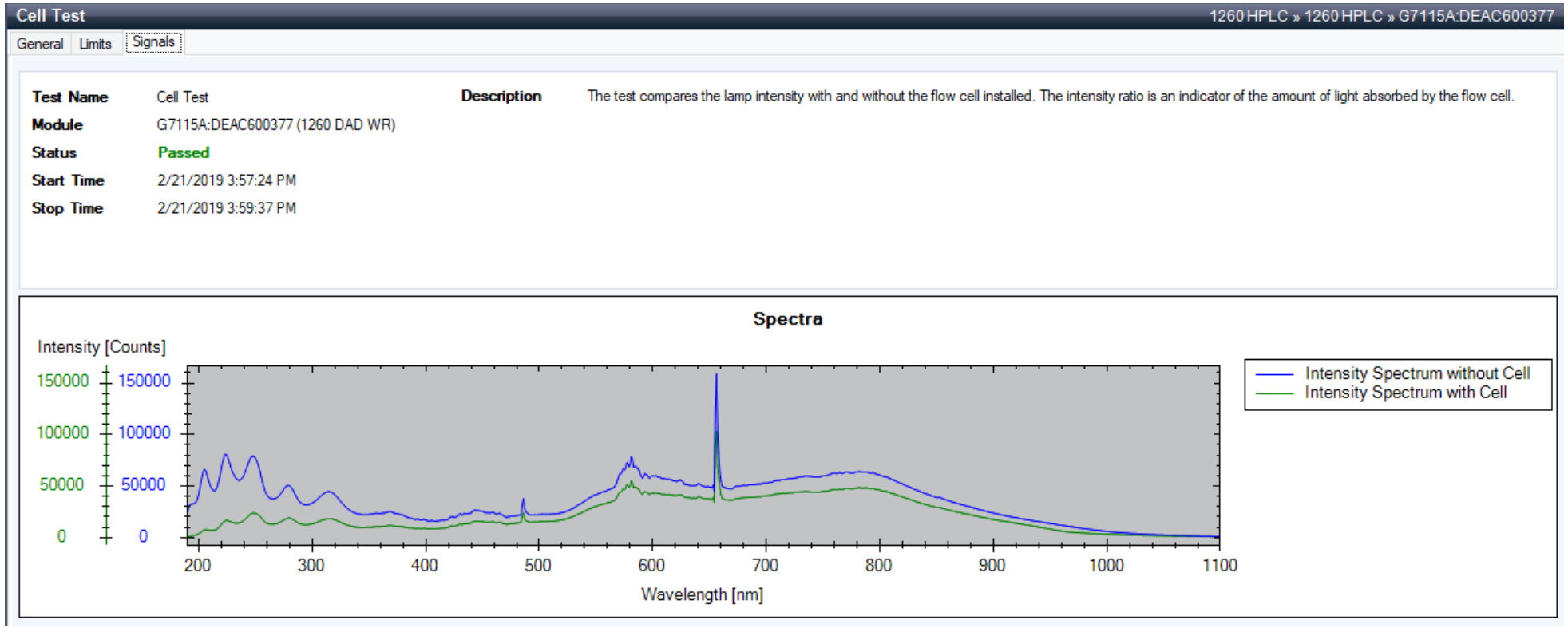


Diode array detectors with the fiber optic style flow cell require a Max Light test cell for this test (part number G4212-60011).

UV Lamp Tests

Diode array and multiple wavelength

Cell test



Example of scans with and without the cell installed.

How Do I Know if My UV Lamp is Good?

Diode array and multiple wavelength

Baseline inspection

Various factors contribute to the specific amplitude and pattern of baseline noise and drift, including the specific wavelength, mobile phase, room temperature, and data rate.

How Do I Know if My UV Lamp Is Good?

Diode array and multiple wavelength

Baseline inspection

Long cycle wave
This is a rhythmic change in the baseline where the periodicity may be hours.
- Environmental influences

Short cycle wave
This is a rhythmic change in the baseline where the periodicity may be seconds or minutes.
- Solvent mixing noise
- Mechanical issue in pump

If the cycle of the wave does not appear to be mixing noise, evaluate the health of the lamps through Lab Advisor intensity, noise and drift tests. Also, the cleanliness of the flow cell can be evaluated through the cell test.

Excessive drift
In a UV baseline, light scattering shows up as drift. If the baseline is drifting more than expected, empty and rinse the solvent bottles, refilling with fresh solvent. Perform a cell test to check the cleanliness of the flow cell.

Useful Parts

Parts that address potential issues and help to ease your daily tasks

Part Description	Information	Part number
InfinityLab Stay Safe caps	Prevents solvent evaporation; changes in mobile phase concentration	Various www.agilent.com/chem/staysafecaps
InfinityLab Quick Connect and Quick Turn fittings	With spring-loaded function for optimized dead volume reduction	Various www.agilent.com/chem/infinitylabfittings
Blank nut, long, 10-32	Blank nut, PEEK with steel core; for system diagnostic tests; finger tight up to 1300 bar, easy to use and gentle on receiving port	5043-0277
Agilent Captiva syringe filters	Solve issues like inlet clogging, increased backpressure, and retention time shift by filtering your samples	Various www.agilent.com/chem/filtration
InfinityLab Poroshell 120 columns	High efficiency and high resolution; available in 18 chemistries	Various www.aglient.com/chem/discoverporoshell

InfinityLab Poroshell 120 columns

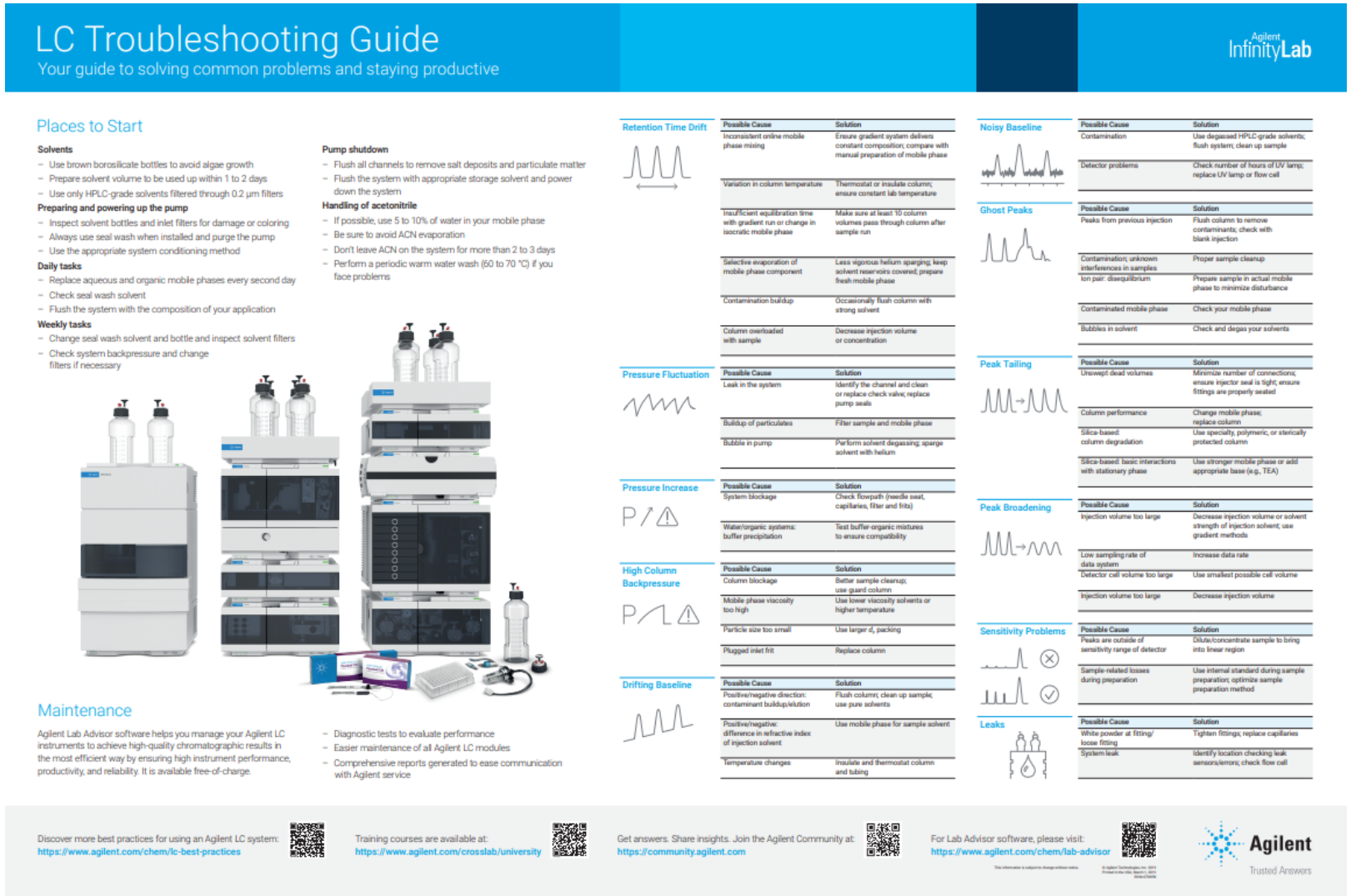

InfinityLab Stay Safe cap on solvent bottle

InfinityLab Quick Connect fitting

InfinityLab Quick Turn fitting


Blank nut, 5043-0277

LC Troubleshooting Poster Available



LC Troubleshooting Guide

Your guide to solving common problems and staying productive

Agilent InfinityLab

Places to Start

Solvents
- Use brown borosilicate bottles to avoid algae growth
- Prepare solvent volume to be used up within 1 to 2 days
- Use only HPLC-grade solvents filtered through 0.2 µm filters

Preparing and powering up the pump
- Inspect solvent bottles and inlet filters for damage or coloring
- Always use seal wash when installed and purge the pump
- Use the appropriate system conditioning method

Daily tasks
- Replace aqueous and organic mobile phases every second day
- Check seal wash solvent
- Flush the system with the composition of your application

Weekly tasks
- Change seal wash solvent and bottle and inspect solvent filters
- Check system backpressure and change filters if necessary

Pump shutdown
- Flush all channels to remove salt deposits and particulate matter
- Flush the system with appropriate storage solvent and power down the system

Handling of acetonitrile
- If possible, use 5 to 10% of water in your mobile phase
- Be sure to avoid ACN evaporation
- Don't leave ACN on the system for more than 2 to 3 days
- Perform a periodic warm water wash (60 to 70 °C) if you face problems

Maintenance

Agilent Lab Advisor software helps you manage your Agilent LC instruments to achieve high-quality chromatographic results in the most efficient way by ensuring high instrument performance, productivity, and reliability. It is available free-of-charge.

- Diagnostic tests to evaluate performance
- Easier maintenance of all Agilent LC modules
- Comprehensive reports generated to ease communication with Agilent service

Retention Time Drift

Possible Cause	Solution
Inconsistent online mobile phase mixing	Ensure gradient system delivers constant composition; compare with manual preparation of mobile phase
Variation in column temperature	Thermostat or insulate column; ensure constant lab temperature
Insufficient equilibration time with gradient run or change in isocratic mobile phase	Make sure at least 10 column volumes pass through column after sample run
Selective evaporation of mobile phase component	Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase
Contamination buildup	Occasionally flush column with strong solvent
Column overloaded with sample	Decrease injection volume or concentration

Pressure Fluctuation

Possible Cause	Solution
Leak in the system	Identify the channel and clean or replace check valve; replace pump seals
Buildup of particulates	Filter sample and mobile phase
Bubble in pump	Perform solvent degassing; sparge solvent with helium

Pressure Increase

Possible Cause	Solution
System blockage	Check flowpath (needle seat, capillaries, filter and frits)
Water/organic systems: buffer precipitation	Test buffer-organic mixtures to ensure compatibility

High Column Backpressure

Possible Cause	Solution
Column blockage	Better sample cleanup; use guard column
Mobile phase viscosity too high	Use lower viscosity solvents or higher temperature
Particle size too small	Use larger d_p packing
Plugged inlet frit	Replace column

Drifting Baseline

Possible Cause	Solution
Positive/negative direction: contaminant buildup/elution	Flush column; clean up sample; use pure solvents
Positive/negative: difference in refractive index of injection solvent	Use mobile phase for sample solvent
Temperature changes	Insulate and thermostat column and tubing

Noisy Baseline

Possible Cause	Solution
Contamination	Use degassed HPLC-grade solvents; flush system; clean up sample
Detector problems	Check number of hours of UV lamp; replace UV lamp or flow cell

Ghost Peaks

Possible Cause	Solution
Peaks from previous injection	Flush column to remove contaminants; check with blank injection
Contamination; unknown interferences in samples	Proper sample cleanup
Ion pair: disequilibrium	Prepare sample in actual mobile phase to minimize disturbance
Contaminated mobile phase	Check your mobile phase
Bubbles in solvent	Check and degas your solvents

Peak Tailing

Possible Cause	Solution
Unswept dead volumes	Minimize number of connections; ensure injector seal is tight; ensure fittings are properly seated
Column performance	Change mobile phase; replace column
Silica-based: column degradation	Use specialty, polymeric, or sterically protected column
Silica-based: basic interactions with stationary phase	Use stronger mobile phase or add appropriate base (e.g., TEA)

Peak Broadening

Possible Cause	Solution
Injection volume too large	Decrease injection volume or solvent strength of injection solvent; use gradient methods
Low sampling rate of data system	Increase data rate
Detector cell volume too large	Use smallest possible cell volume
Injection volume too large	Decrease injection volume

Sensitivity Problems

Possible Cause	Solution
Peaks are outside of sensitivity range of detector	Dilute/concentrate sample to bring into linear region
Sample-related losses during preparation	Use internal standard during sample preparation; optimize sample preparation method

Leaks

Possible Cause	Solution
White powder at fitting/ loose fitting	Tighten fittings; replace capillaries
System leak	Identify location checking leak sensors/errors; check flow cell

Discover more best practices for using an Agilent LC system: https://www.agilent.com/chem/lc-best-practices

Training courses are available at: https://www.agilent.com/crosslab/university

Get answers. Share insights. Join the Agilent Community at: https://community.agilent.com

For Lab Advisor software, please visit: https://www.agilent.com/chem/lab-advisor

Agilent Trusted Answers

Request yours today at www.agilent.com/chem/troubleshootLC

Resources for Support

- *New!* HPLC Advisor App: HPLC Advisor app | Agilent
- LC Troubleshooting poster: 5994-0709EN
- Resource page: http://www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
- InfinityLab Supplies catalog: 5991-8031EN
- LC handbook: 5990-7595EN
- YouTube – Agilent channel (maintenance videos)
- Agilent service contracts

Contact Agilent Chemistries and Supplies Technical Support

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

Available in the U.S. and Canada 8-5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com