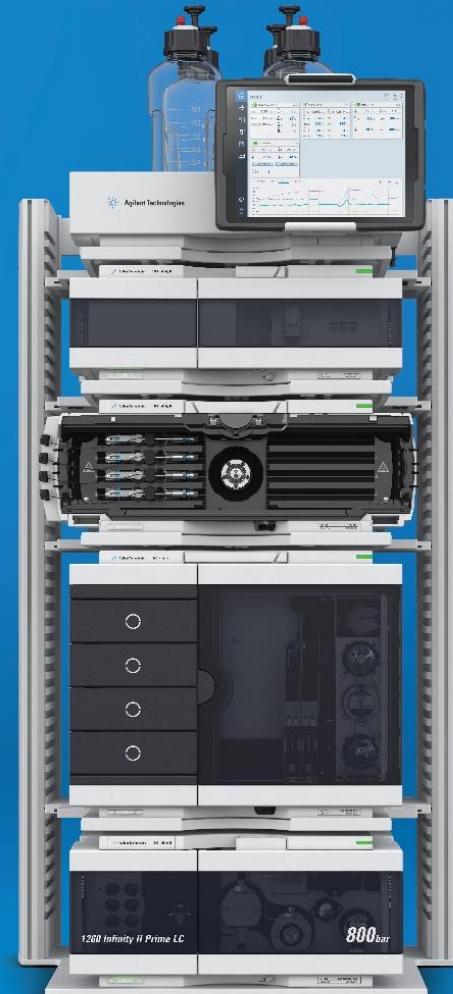


Getting the Most from Your Diode Array Detector

From selection to optimization

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August 29, 2024



Getting the Most from your Diode Array Detector (DAD)

Agenda

Introduction

Fundamentals

Maximizing Sensitivity

Spectral Acquisition

DAD Maintenance

Troubleshooting

Resources



Getting the Most from Your DAD

Introduction



Detector Type	Sensitivity	Selectivity	Response to Compounds	Advantages
Variable Wavelength Detector	ng–pg	—	80%	Low cost
Diode Array Detector	ng–pg	++	80%	Peak confirmation
Fluorescence	pg–fg	++	10%	High sensitivity
Electrochemical	pg–fg	+	>20%	High sensitivity
Conductivity	ng–pg	—	10%	Ion chromatography
Refractive Index	μg–ng	—	100%	Universal
Mass Spectrometer	ng–pg	++	<100%	Molecular weight and structural information

Getting the Most from Your DAD

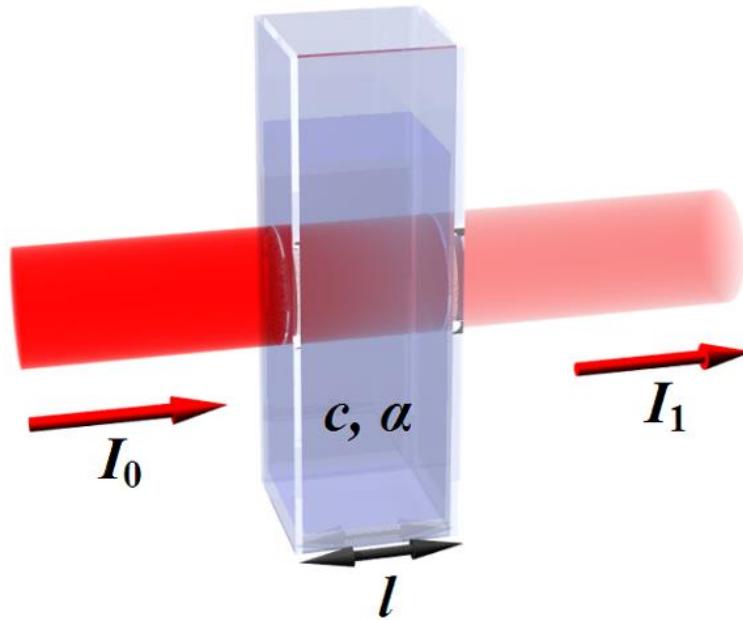
Fundamentals



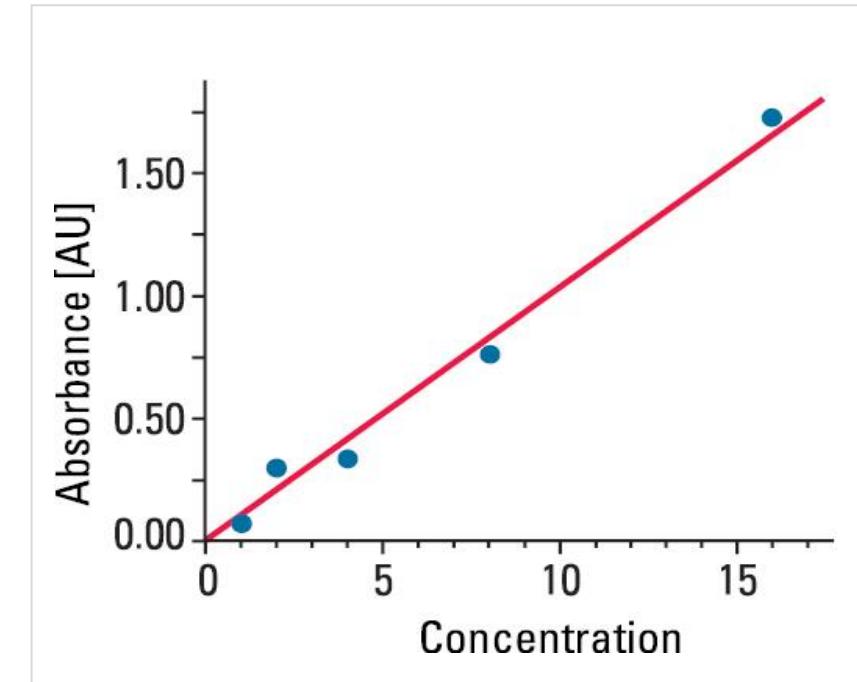
Fundamentals

Beer-Lambert law

$$A = -\log\left(\frac{I_1}{I_0}\right) = \alpha \cdot c \cdot l$$



- A: Absorption
- α : Extinction coefficient
- c: Concentration
- l: Path length

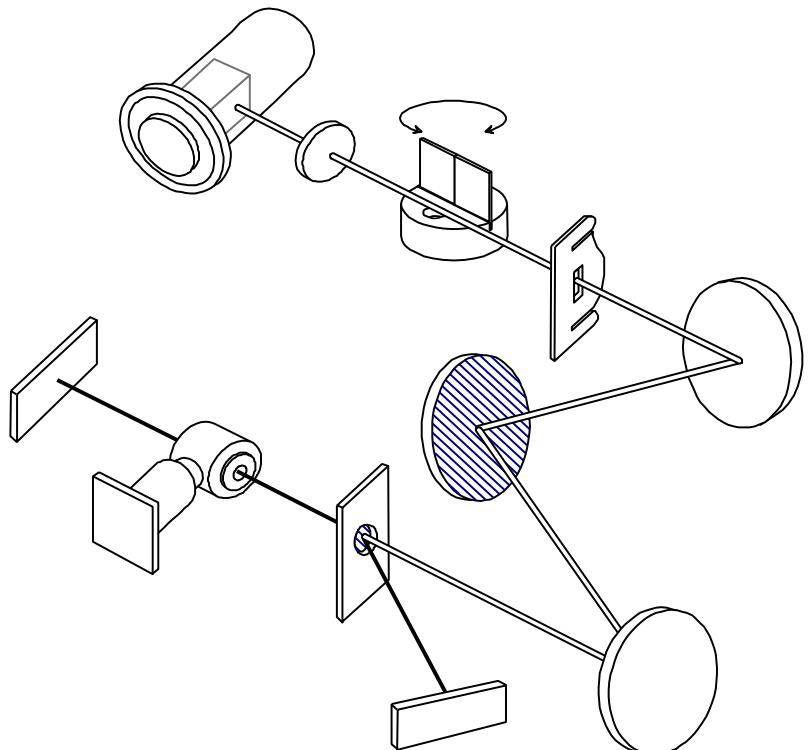


Example of calibration curve. Calibration is done by measuring A as c varies.

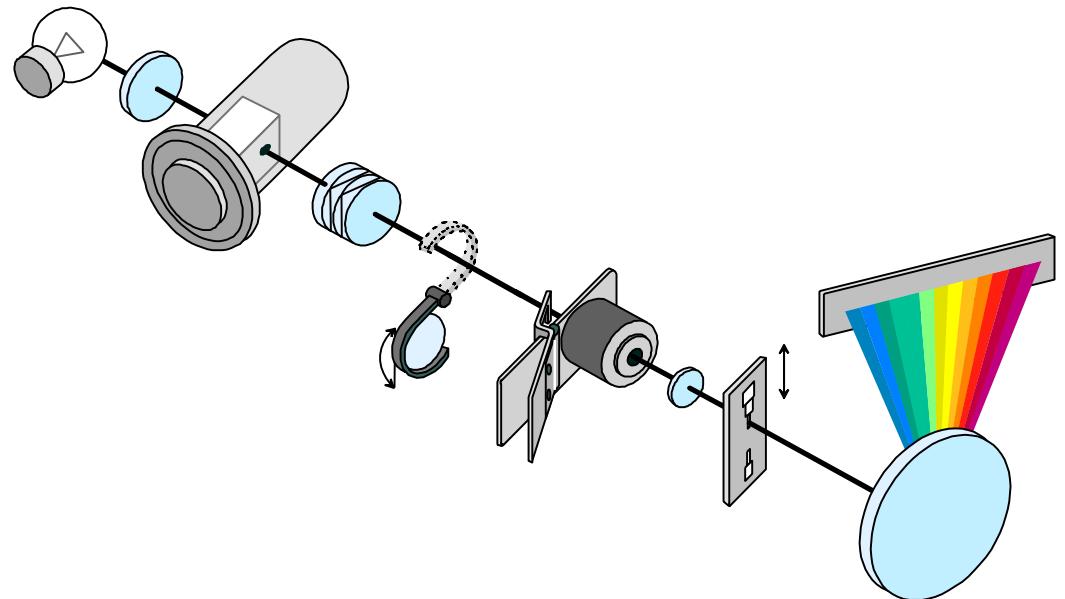
Fundamentals

UV-vis detection

Variable Wavelength Detector (VWD)

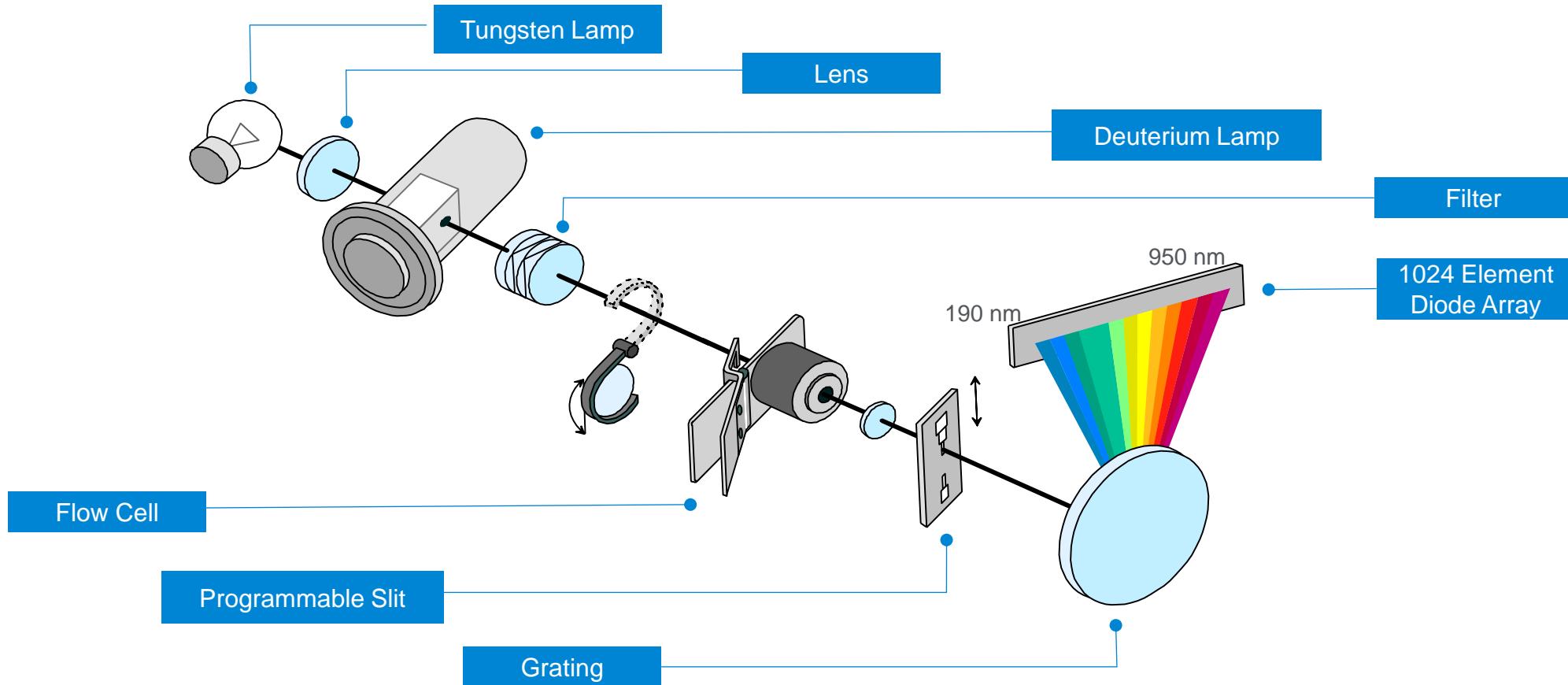


Multiple Wavelength Detector (MWD) & DAD



Fundamentals

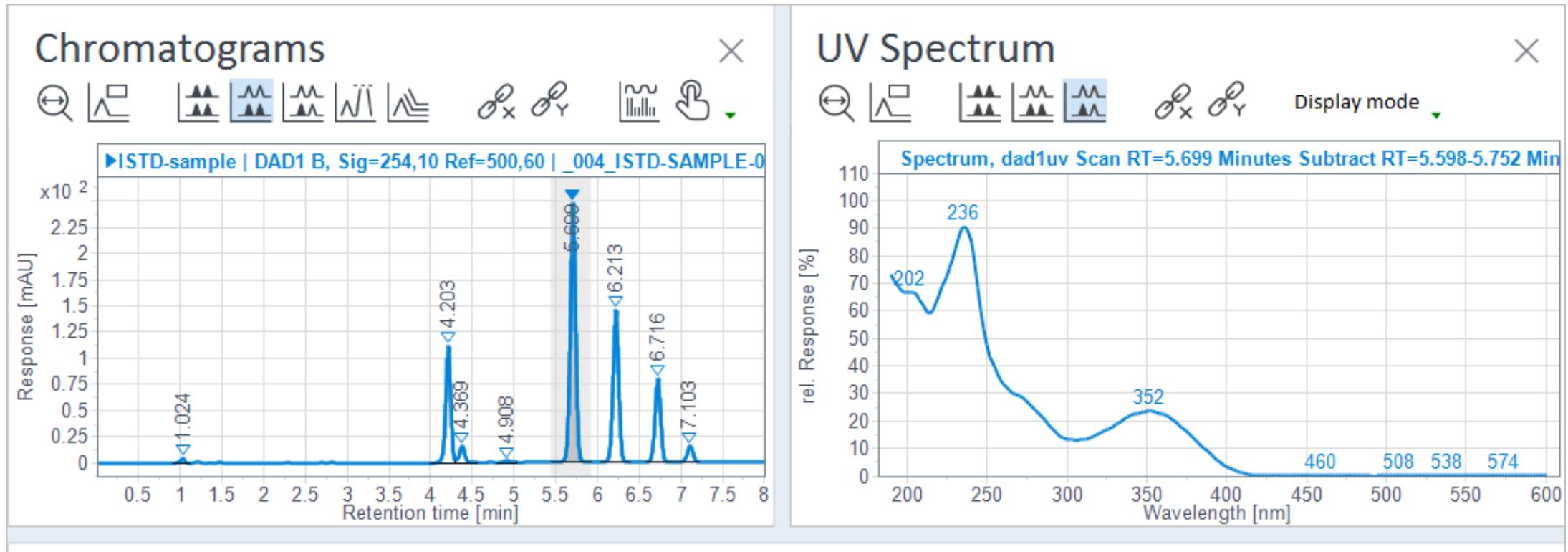
DAD and MWD



- Combination of deuterium-arc-discharge lamp for the ultraviolet (UV) wavelength range and a tungsten lamp for the visible (VIS) and short-wave near-infrared (SWNIR) wavelength range.
- A 1024-element array is used that gives high resolution over the whole wavelength range

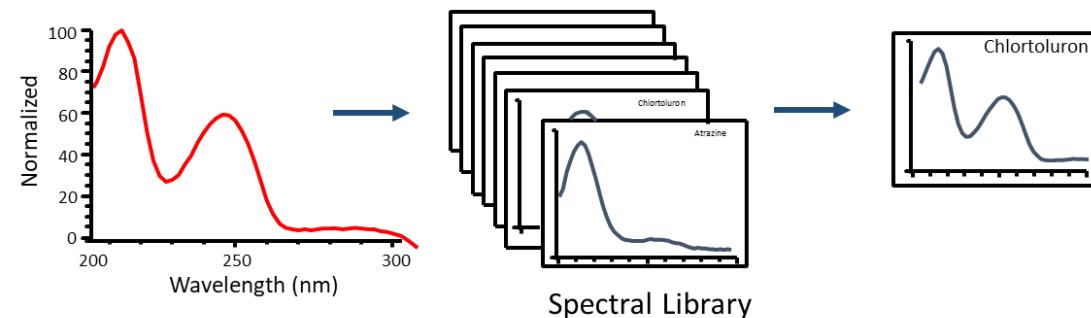
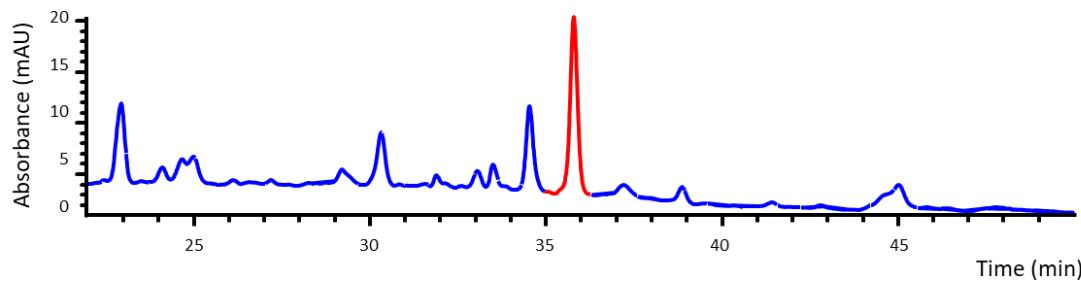
Fundamentals

DAD spectra collection



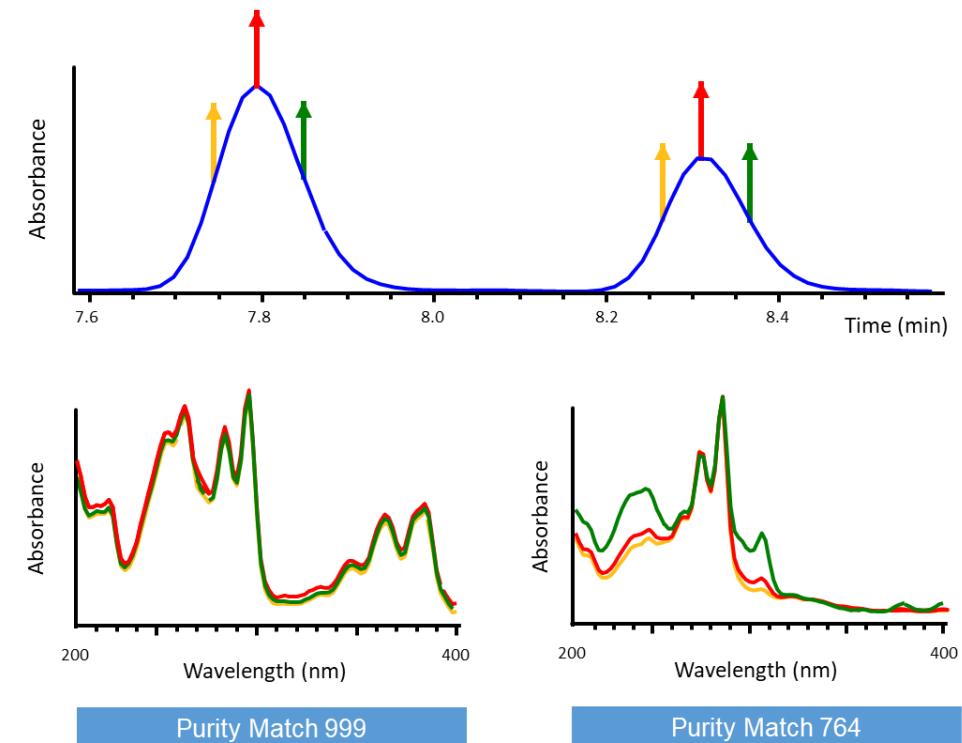
Fundamentals

UV spectrum confirmation



UV spectrum confirmation compares the apex spectrum with the reference spectrum, extracted from the apex of a reference (standard) peak.

Peak purity



Peak impurity is calculated by extracting all recorded spectra of a peak and comparing the remaining spectra with the spectrum of the peak apex.

Getting the Most from Your DAD

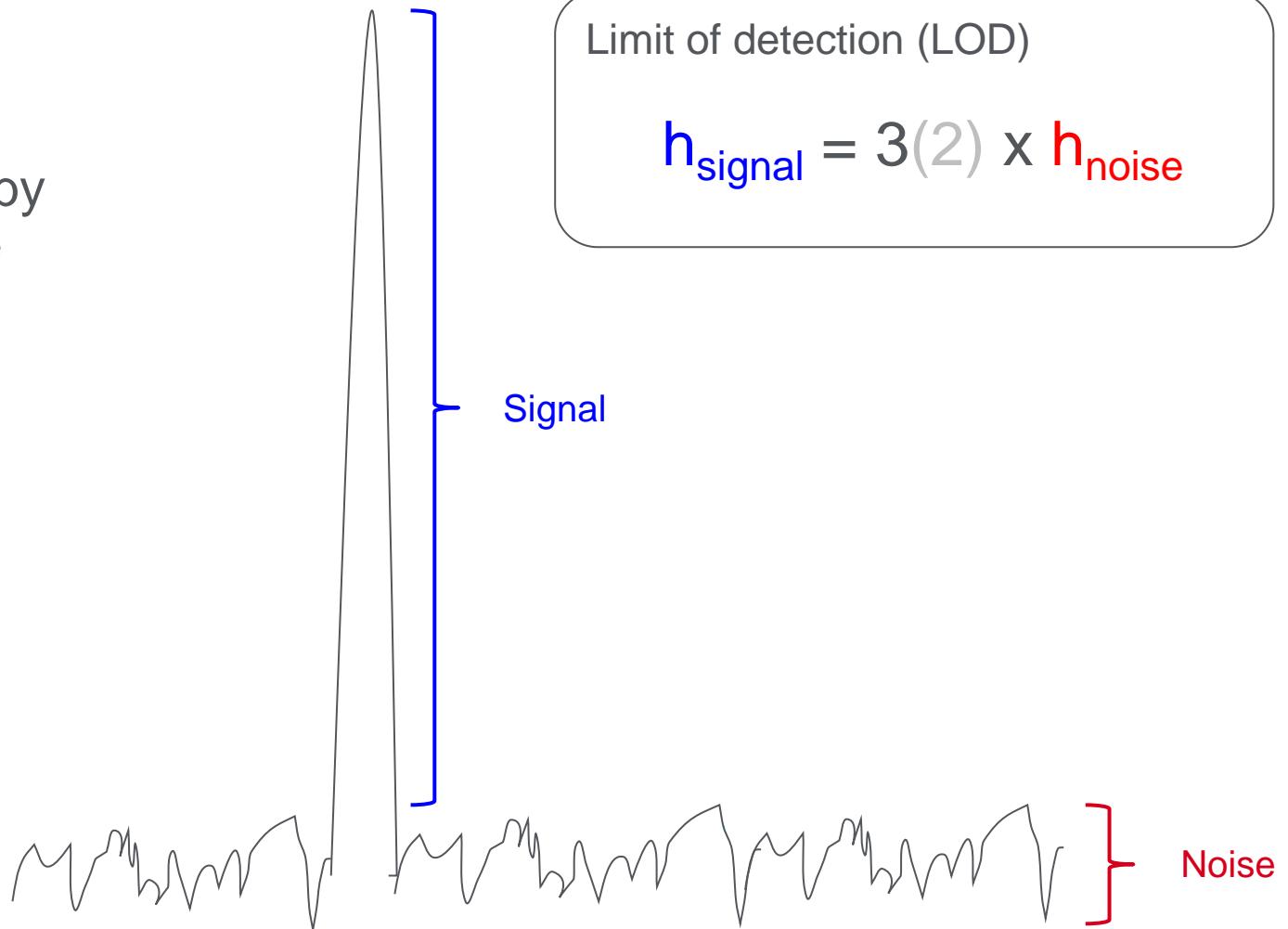
Maximizing sensitivity



Maximizing Sensitivity

What is sensitivity?

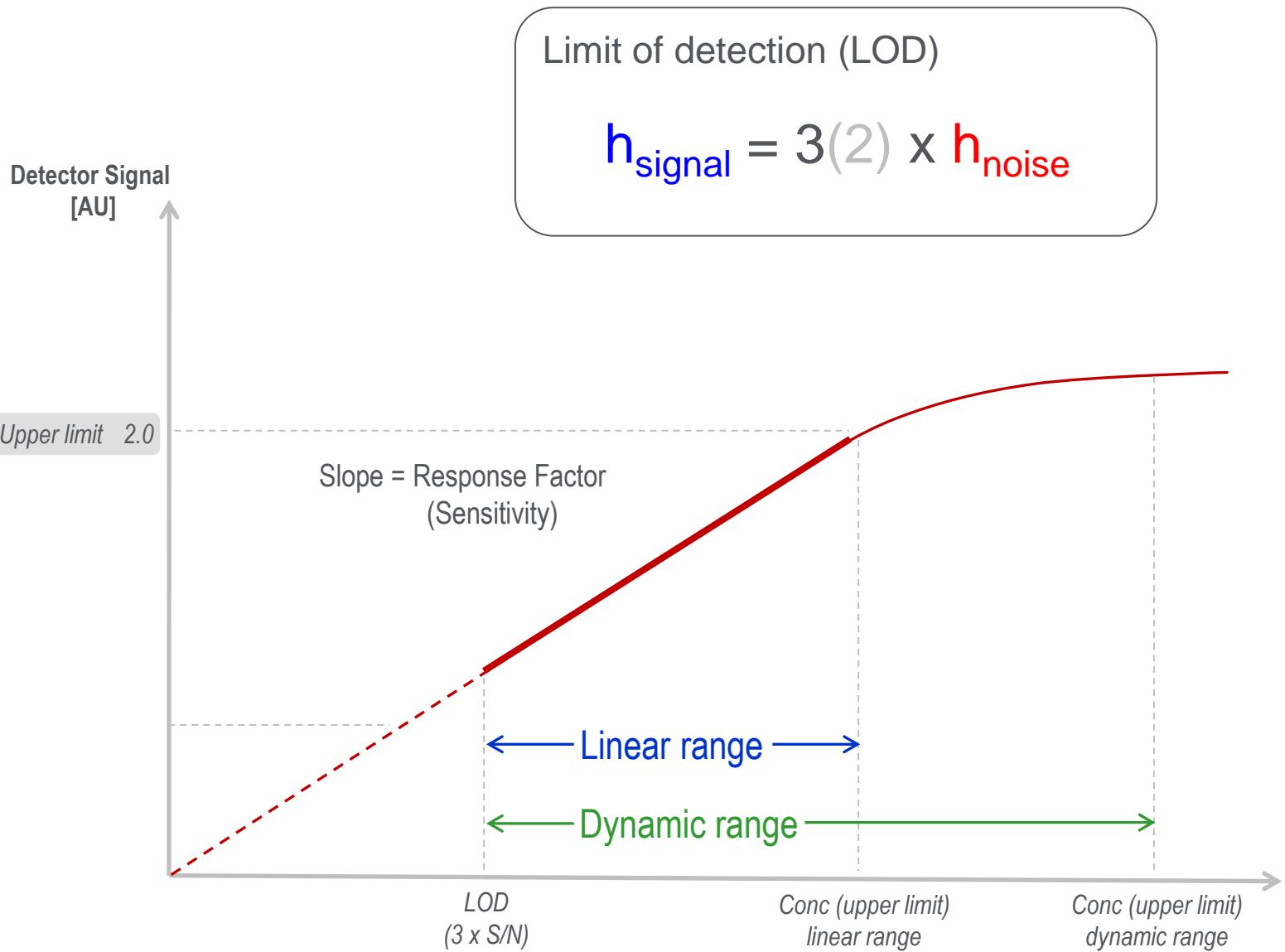
- Sensitivity is the ratio of peak height to baseline noise.
- Peak height must exceed noise by a certain factor to distinguish the peak from the noise
- The concentration where this is possible is the limit of detection



Maximizing Sensitivity

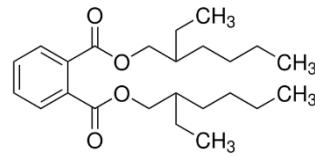
What is sensitivity?

- Maximizing sensitivity allows for lower LODs
- This is done be either increasing signal height, decreasing noise, or both



Maximizing Sensitivity

Factors affecting sensitivity



Analyte



System



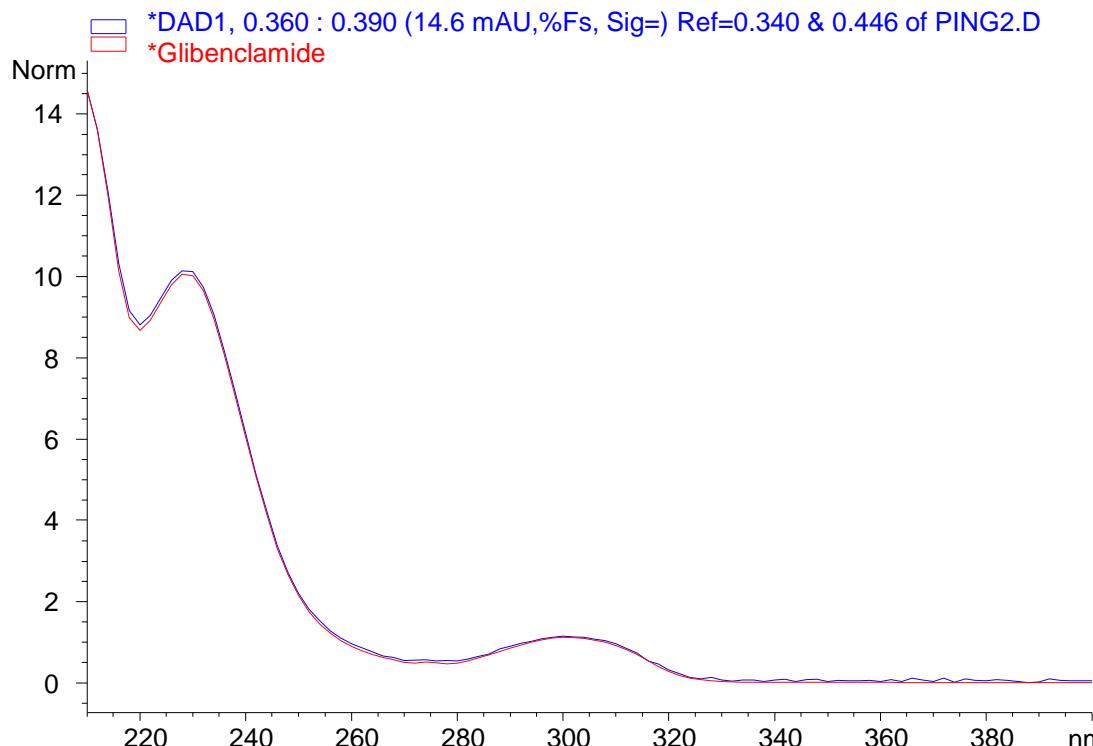
Detector

Increasing Peak Height	<ul style="list-style-type: none">• Chromophore• Increase sample concentration	<ul style="list-style-type: none">• Reduce extra column volume	<ul style="list-style-type: none">• Wavelength selection• Flow cell• Data rate• Programmable slit• Acquisition bandwidth
Decreasing Noise	<ul style="list-style-type: none">• Sample preparation	<ul style="list-style-type: none">• HPLC grade reagents	<ul style="list-style-type: none">• Reference wavelength• Reference bandwidth

Maximizing Sensitivity

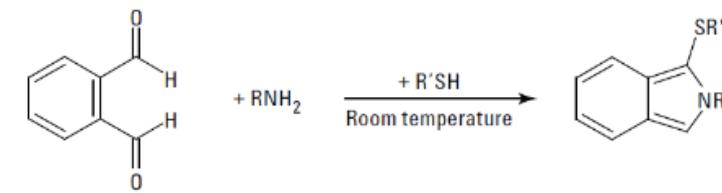
Analyte: Chromophores and derivatization

Chromophore

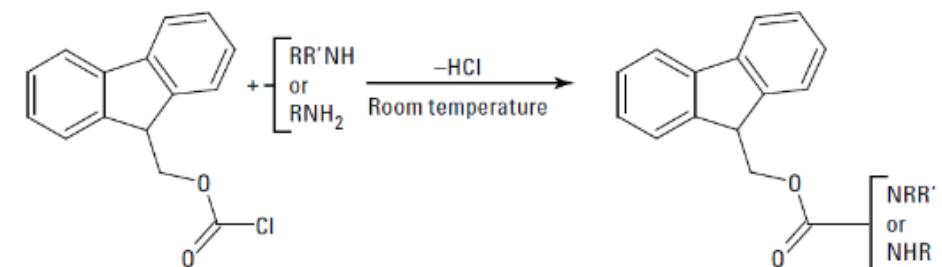


Derivatization

Ortho-phthalaldehyde (OPA)



9-fluorenylmethyl chloroformate (FMOC)



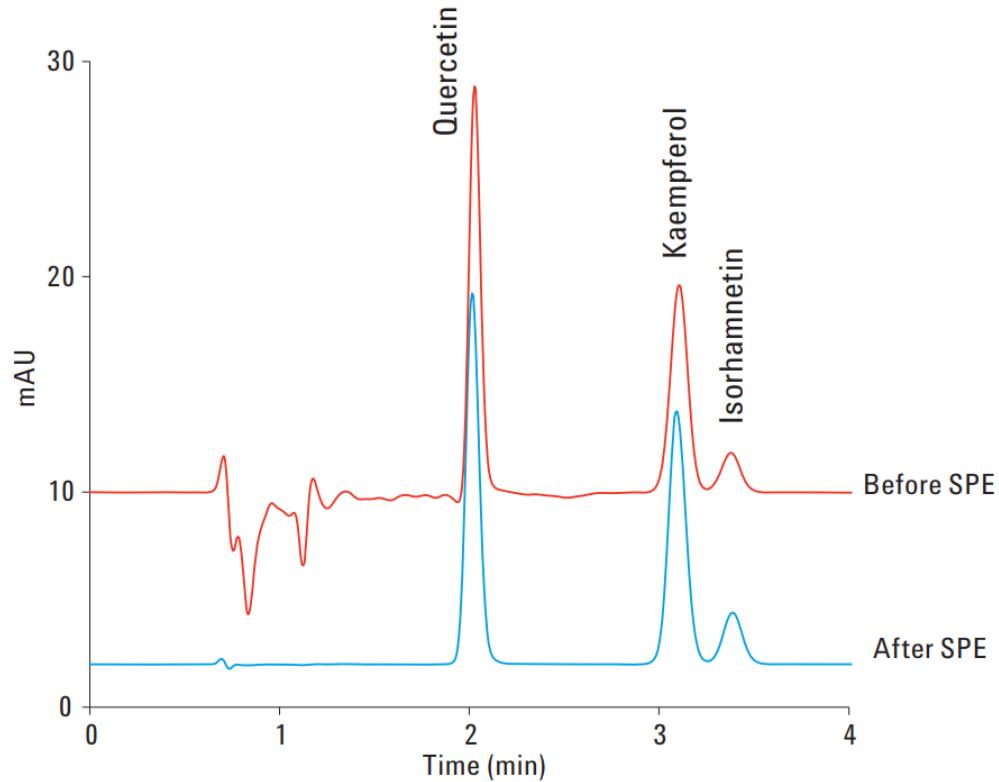
Maximizing Sensitivity

Analyte: Sample preparation

Sample preparation mitigate matrix effects by removing or reducing interferences.

Sample preparation can also concentrate samples that are present in low concentrations in original sample matrix.

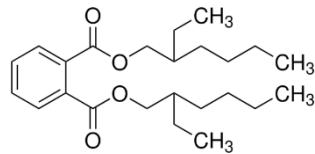
Column: 4.6 x 75 mm **Zorbax Eclipse Plus**, 3.5 μ m
Mobile phase: A: 0.5% phosphoric acid, B: Methanol
Elution: Isocratic 60% B
Injection volume: 5 μ L
Flow: 1 mL/min
DAD: 370 nm



[Determination of Flavonoids in Ginkgo Biloba Using Bond Elut Plexa Solid Phase Extraction](#)

Maximizing Sensitivity

Factors affecting sensitivity



Analyte



System



Detector

Increasing Peak Height

- Chromophore
- Increase sample concentration

- Reduce extra-column volume

- Wavelength selection
- Flow cell
- Data rate
- Programmable slit
- Acquisition bandwidth

Decreasing Noise

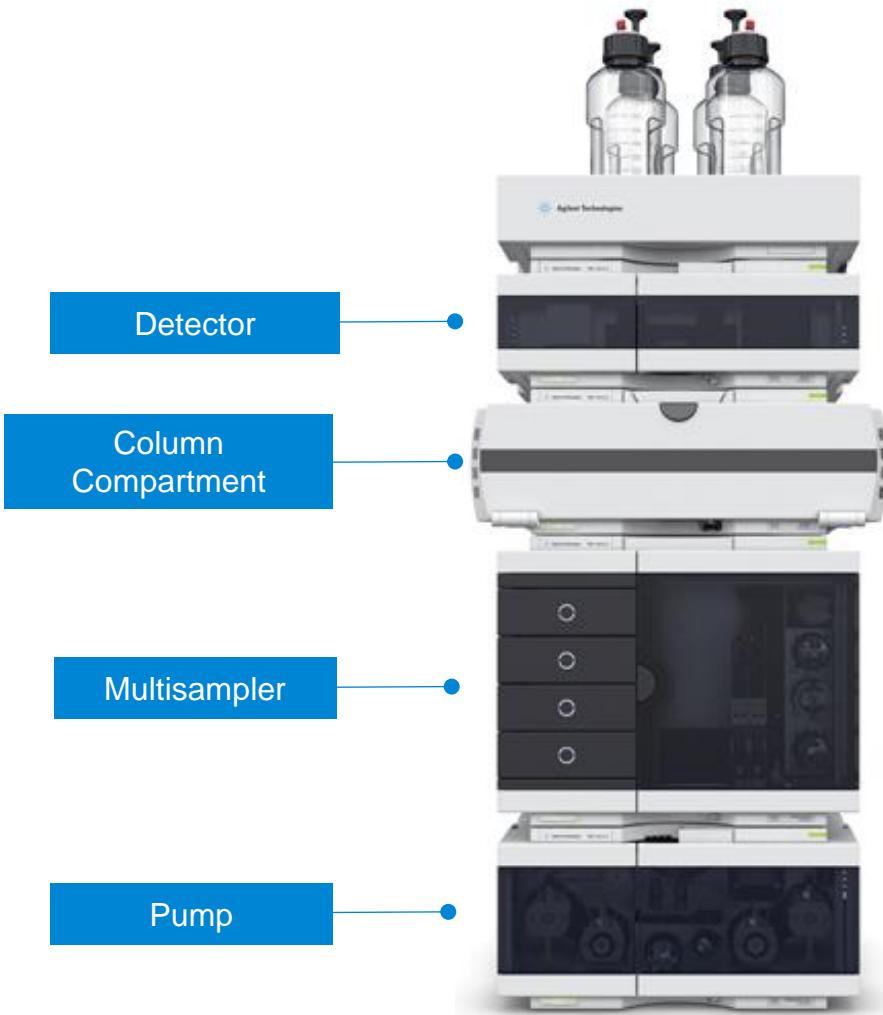
- Sample preparation

- HPLC grade reagents

- Reference wavelength
- Reference bandwidth

Maximizing Sensitivity

System: Extra-column volume

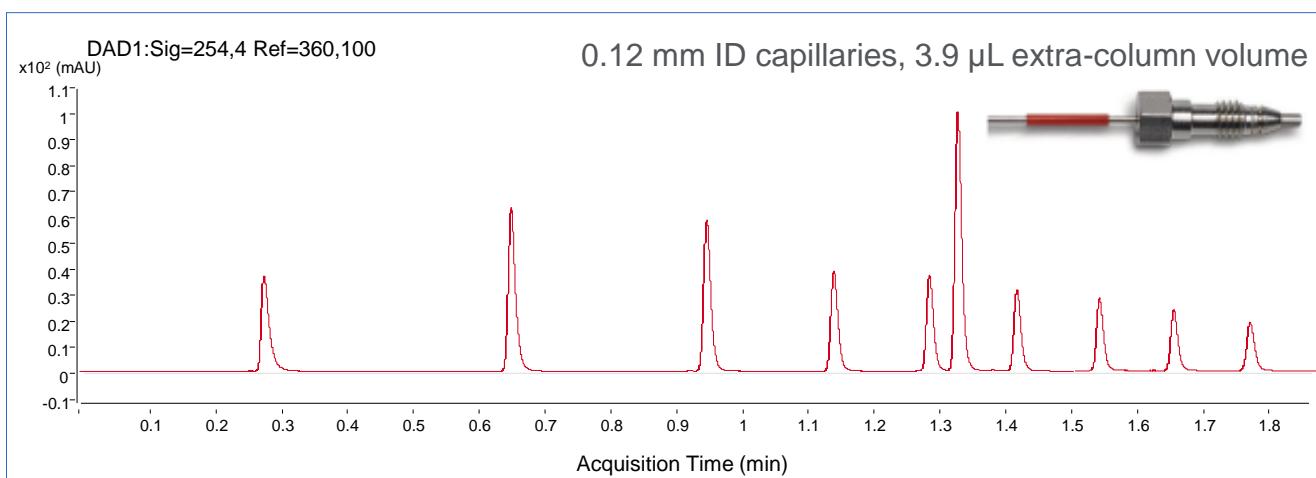
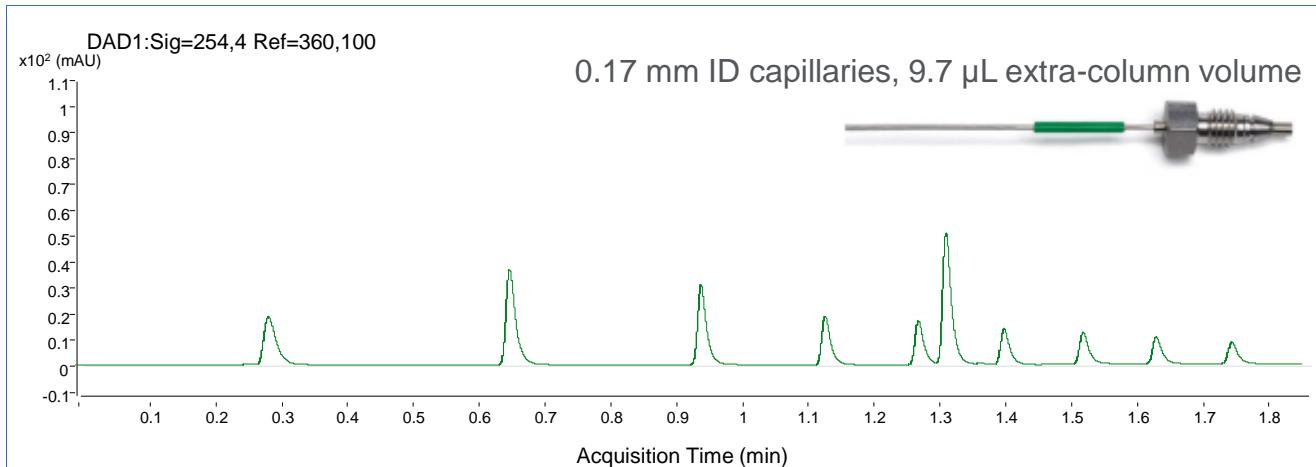


In liquid chromatography, the extra-column volume is the volume between the pump and detector that does not include the column. Extra-column volume is a source of peak dispersion. This volume depends on:

- Capillary lengths and diameters
- Capillary fittings
- Heat exchangers
- Flow cell volumes
- Injection volumes

Maximizing Sensitivity

System: Capillaries



- Reducing capillary diameters throughout the instrument reduces extra-column volume.
- Small dimension columns are most affected by extra-column volume.

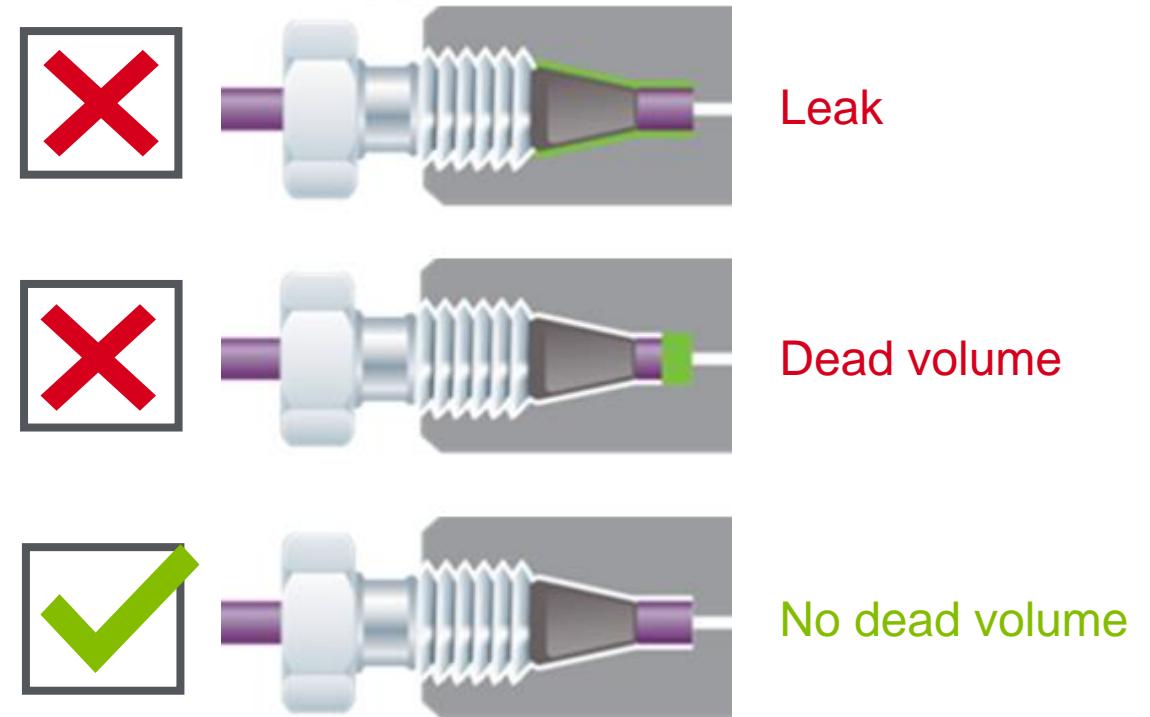
Capillary Length	10 mm	50 mm	100 mm	150 mm
Capillary ID	Volume	Volume	Volume	Volume
0.17 mm (green)	0.227 μ L	1.1 μ L	2.27 μ L	3.3 μ L
0.12 mm (red)	0.113 μ L	0.55 μ L	1.13 μ L	1.65 μ L

[Optimizing Performance of an Agilent Zorbax RRHD Eclipse Plus C18 Column by Enhancing and Agilent 1290 Infinity LC System for Ultra-Low Dispersion](#)

Maximizing Sensitivity

System: Capillary connections

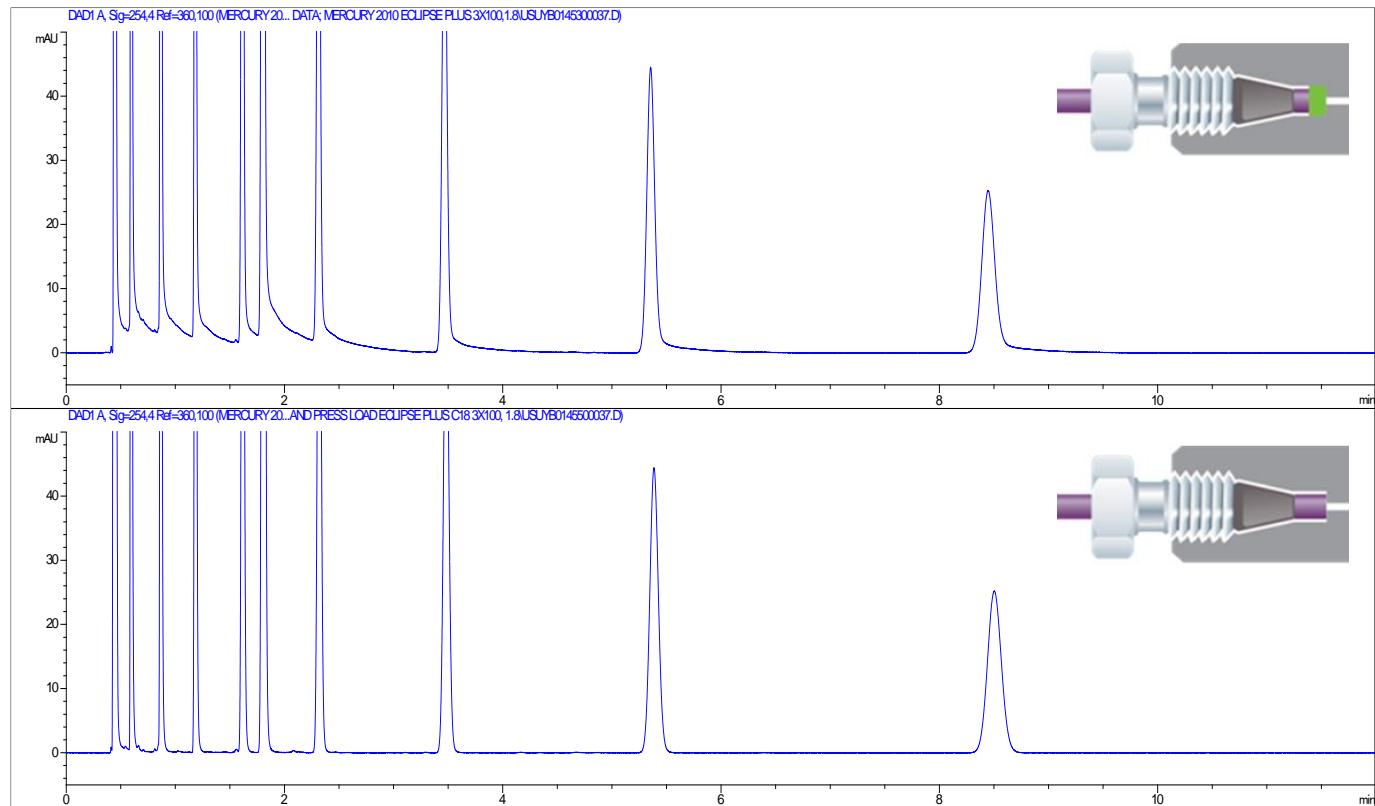
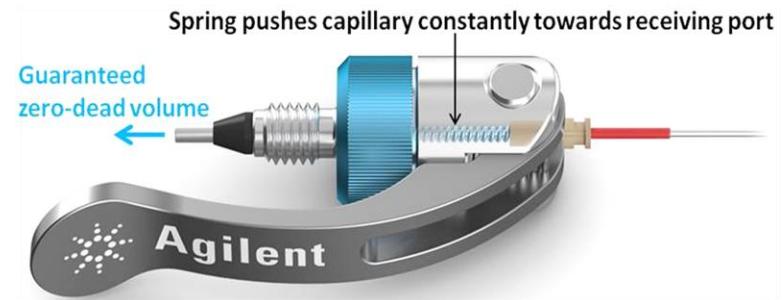
- Poor connections can be a source of peak broadening, especially if dead volume is created.



Maximizing Sensitivity

System: 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.



[flyer-quick-connect-fittings-infinitylab-5991-5164EN_agilent.pdf](#)

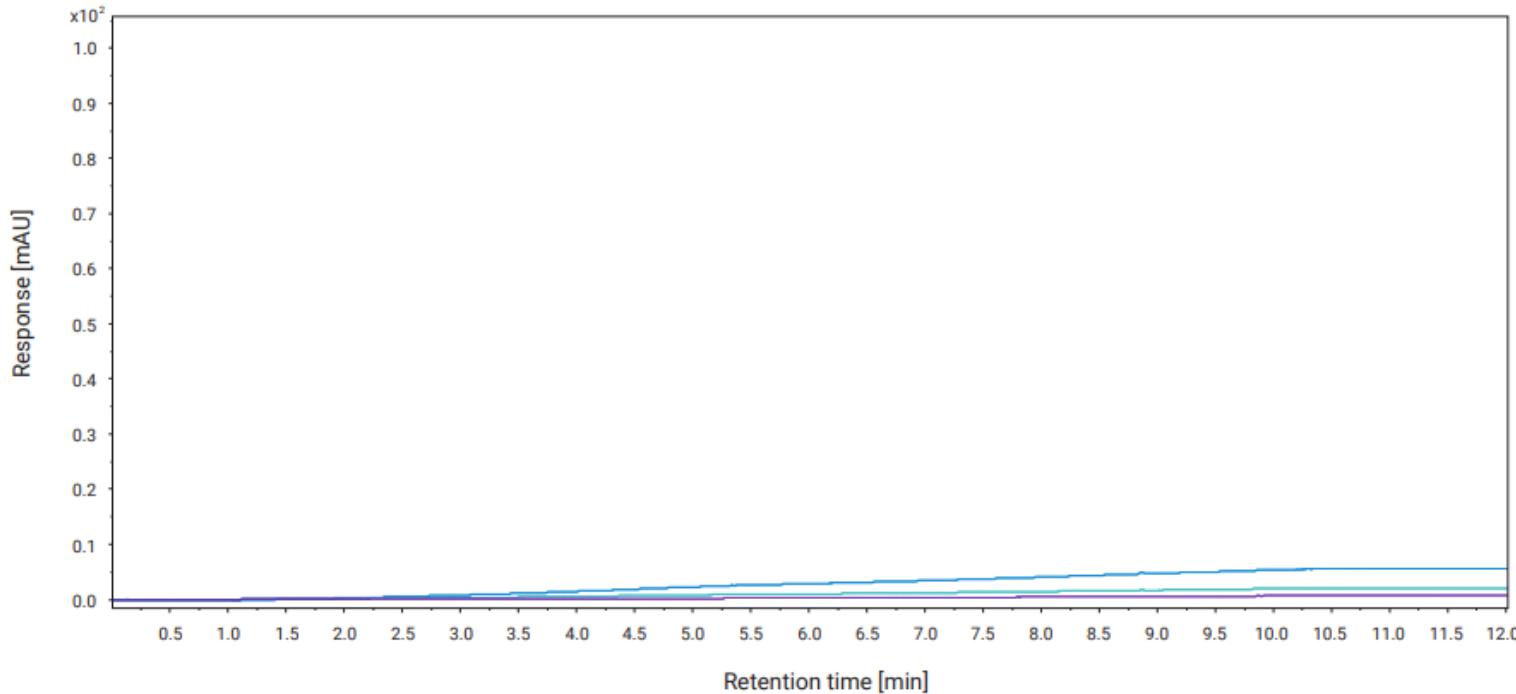
Maximizing Sensitivity

System: Quality reagents

HPLC or LCMS grade solvents

- Lowest impurity levels, reducing ghost peaks in gradient runs
- 0.2 μ m 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

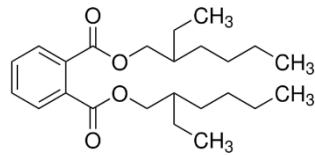


Gradient from 5-95% ACN. Detection wavelengths 210 nm (blue), 225 nm (turquoise), and 254 nm (purple); Range: 0-100 mAU

[Maximize Your Efficiency With Precision Solvents \(agilent.com\)](http://agilent.com)

Maximizing Sensitivity

Factors affecting sensitivity



Analyte



System



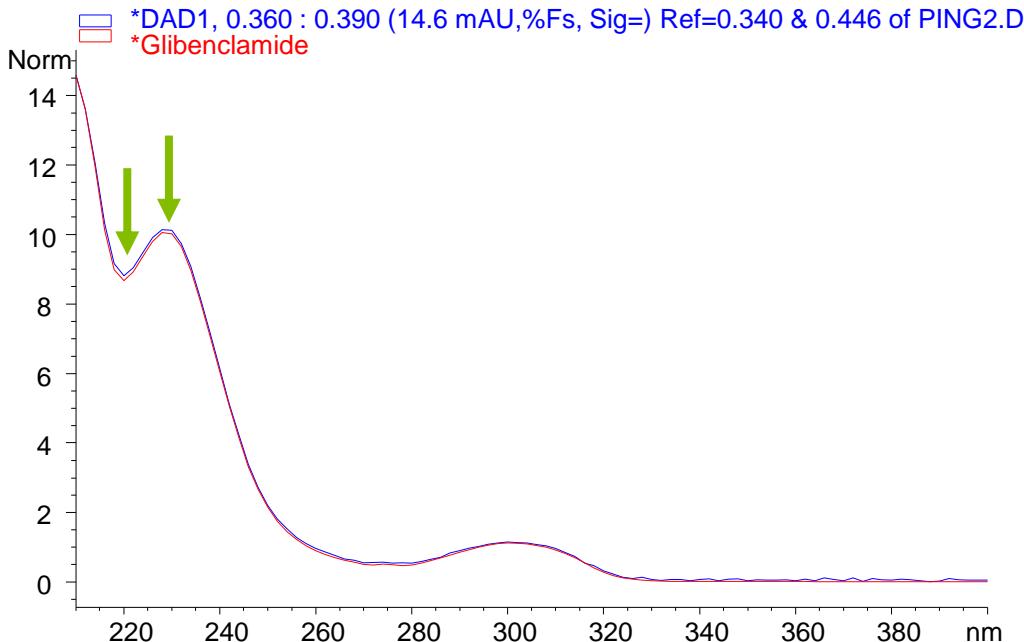
Detector

Increasing Peak Height	<ul style="list-style-type: none">• Chromophore• Increase sample concentration <ul style="list-style-type: none">• Reduce extra column volume	<ul style="list-style-type: none">• Wavelength selection• Flow cell• Data rate• Programmable slit• Acquisition bandwidth
Decreasing Noise	<ul style="list-style-type: none">• Sample preparation• HPLC grade reagents	<ul style="list-style-type: none">• Reference wavelength• Reference bandwidth

Maximizing Sensitivity

Detector: Wavelength selection

Maximum Absorbency



Choosing a wavelength where the analyte has either a peak or valley in the absorbance spectra will provide a more linear range of detection

UV-Transparency

Solvent	UV cutoff (nm)
N-Hexane	190
Toluene	285
Methylene chloride	233
Tetrahydrofuran	212
Acetonitrile	190
2-Propanol	205
Methanol	205
Water	<190

Mobile phase will have an absorbance of $A < 0.2$ AU at the wavelength used for detection of

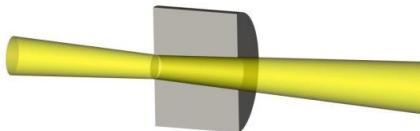
Maximizing Sensitivity

Detector: Conventional flow cells

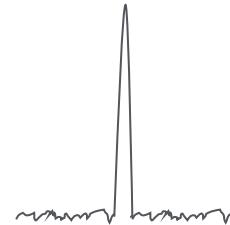
$$A = \alpha \cdot c \cdot l$$



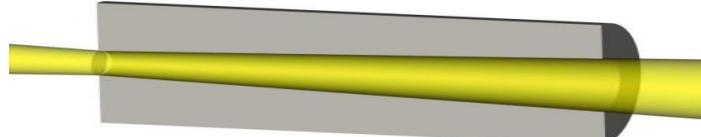
4.6 mm I.D. column



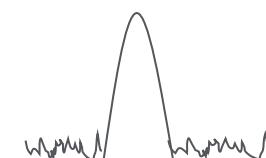
Pathlength: 10 mm
Cell volume: 13 μ L



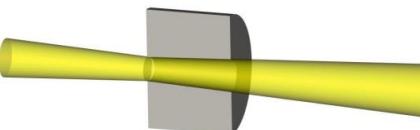
4.6 mm I.D. column



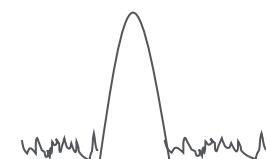
Pathlength: 60 mm
Cell volume: 78 μ L



2.1 mm I.D.
column



Pathlength: 10 mm
Cell volume: 13 μ L



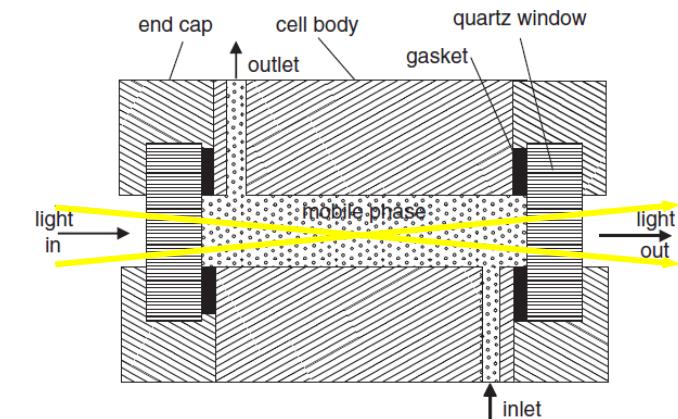
Maximizing Sensitivity

Detector: Max-Light cartridges

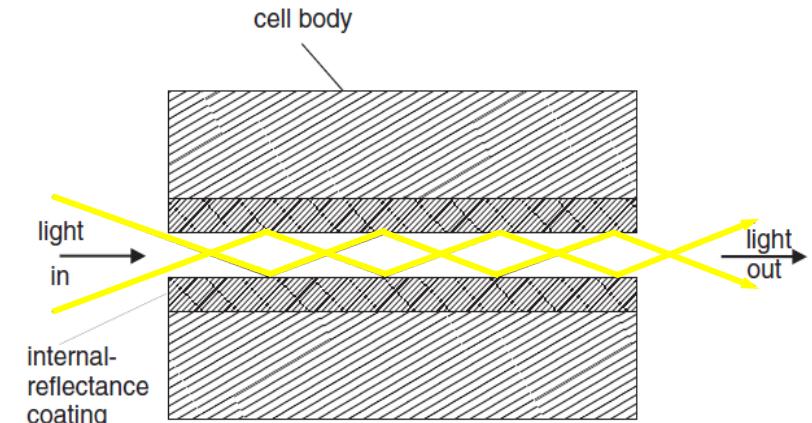
A non-coated fused-silica fiber is used which reflects the light coming from the lamp internally avoiding loss of light through the cell wall.



Conventional Flow Cell



Max-Light Cartridge



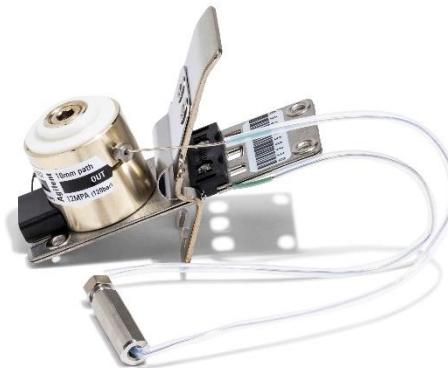
Total Internal Reflection

Maximizing Sensitivity

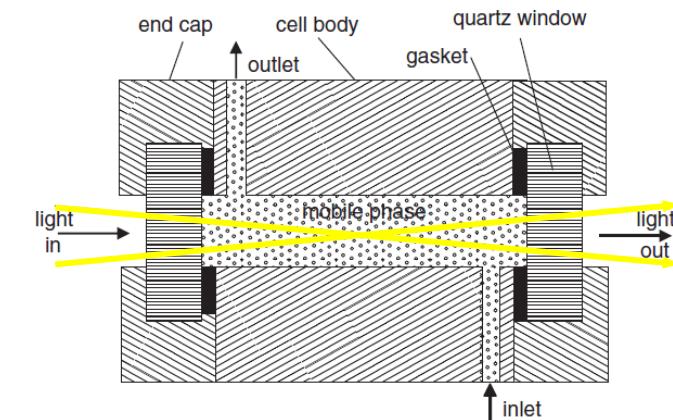
Detector: Max-Light cartridges

Advantages:

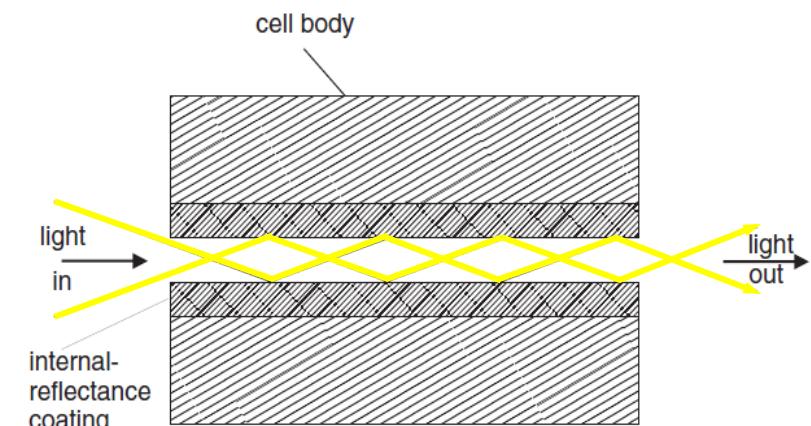
- High sensitivity but low cell volume
 - 60 mm cartridge: 4 μL
 - 10 mm cartridge: 1 μL
- Reduced RI (refractive index) and thermal effects (solvent temperature))
- No special care instructions for flow cell required
- Cartridge design for convenient handling



Conventional Flow Cell



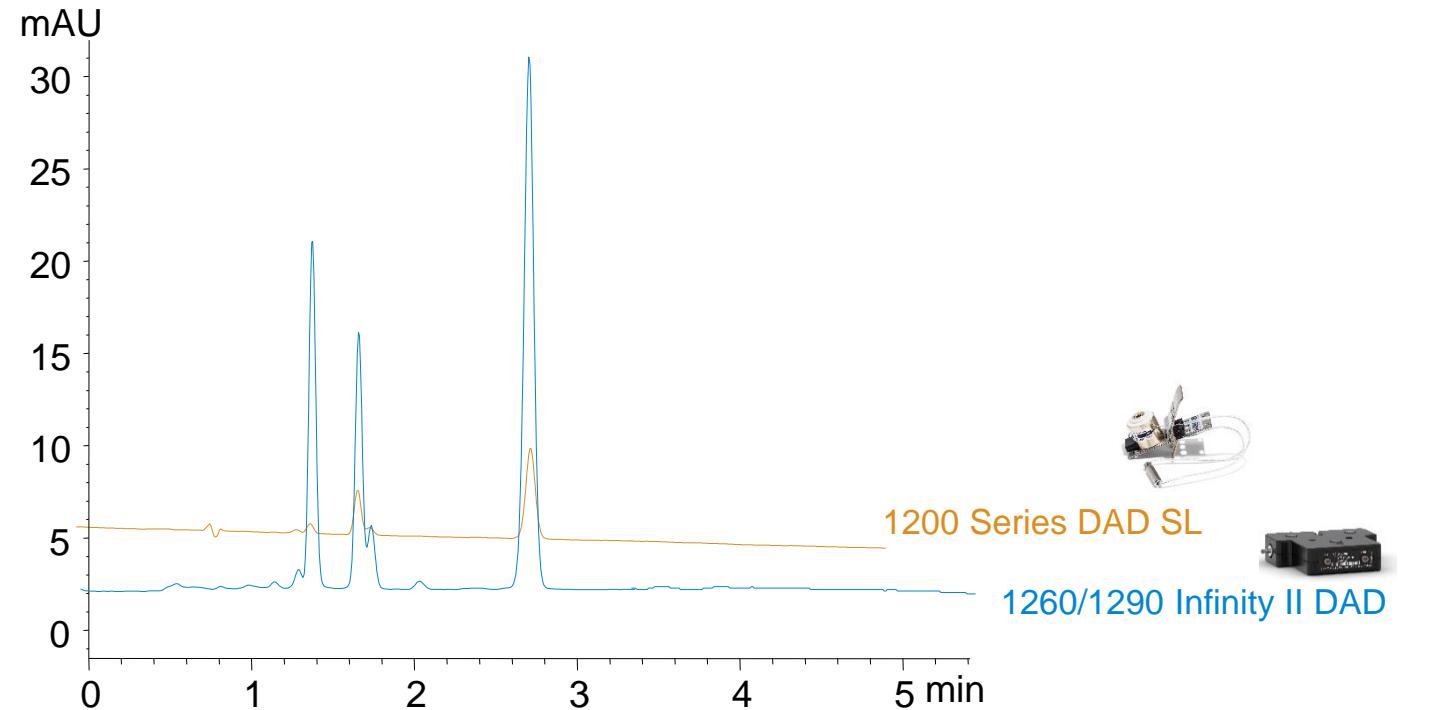
Max-Light Cartridge



Total Internal Reflection

Maximizing Sensitivity

Detector: Max-Light cartridges



Max-Light Cartridges are available for G7117A/B/C DADs

	1200 DAD SL 10 mm Flow Cell	1290 DAD 60 mm Max-Light
Height (mAU)	4.938	28.876
Noise (mAU)	0.0190	0.0098
Signal/Noise	259	2944
Sensitivity Increase		11.4X

Columns: 150 x 4.6mm **Zorbax** SB C18, 5 μ m
Sample: Anthracene: 835 pg/ μ L

Mobile phase: A: Water, B: Acetonitrile

Elution: isocratic 80 % B

Injection volume: 5 μ L

Flow: 1.5 mL/min

DAD: 251/4nm, Ref= 450/80nm, 2.5Hz, slit width 4nm

Maximizing Sensitivity

Detector: Data rate

Peakwidth

Stop time

As Peak

min

> 0.013 min (0.25 s response time) (20 Hz)

< 0.0008 min (0.008 s response time) (240 Hz)

> 0.0008 min (0.016 s response time) (240 Hz)

> 0.0016 min (0.031 s response time) (160 Hz)

> 0.0031 min (0.063 s response time) (80 Hz)

> 0.0063 min (0.13 s response time) (40 Hz)

> 0.013 min (0.25 s response time) (20 Hz)

> 0.025 min (0.5 s response time) (10 Hz)

> 0.05 min (1 s response time) (5 Hz)

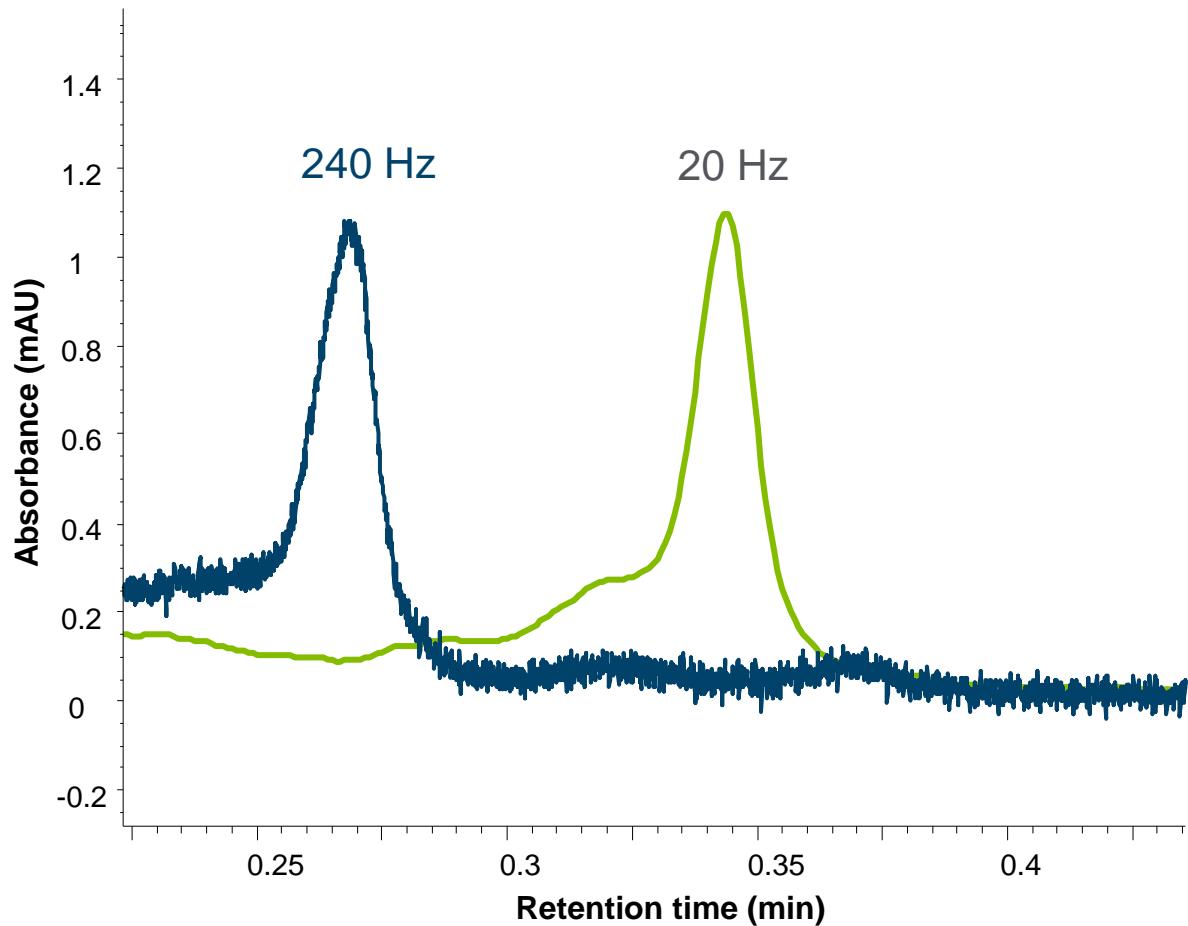
> 0.1 min (2 s response time) (2.5 Hz)

> 0.2 min (4 s response time) (1.25 Hz)

> 0.4 min (8 s response time) (0.62 Hz)

> 0.85 min (16 s response time) (0.31 Hz)

- For reliable determination, a minimum of 10 data points per peak is needed
- For quantitation 15 to 25 data points per peak is required
- If peaks co-elute or when there is low signal to noise 40 data points per peak should be used



Column: ZORBAX Eclipse Plus C18, 2.1x50 mm, 1.8 μ m
Column temperature: 35 °C; Flow rate: 1 mL/min
Gradient: 10-100% ACN in 3 min
Signal: 254 nm, Bandwidth: 4 nm
Reference: 360 nm, Bandwidth: 100 nm

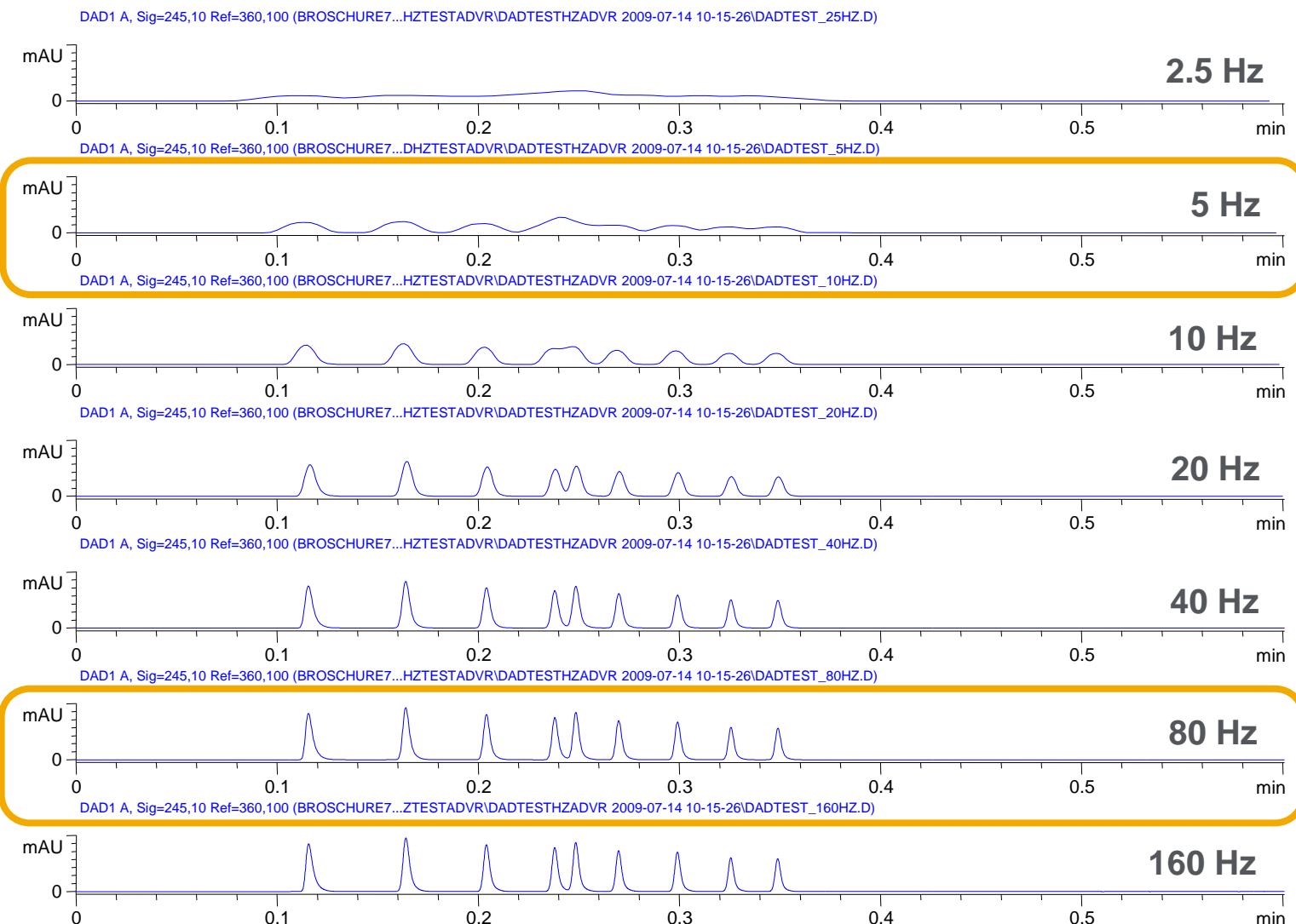
Maximizing Sensitivity

Detector: Data rate

When doing fast analysis on small volume columns with UV detection, it is important to optimize the detector response time.

The default setting in many instruments is 5 Hz which is insufficient to define the peaks, and a broad flattened peak is seen.

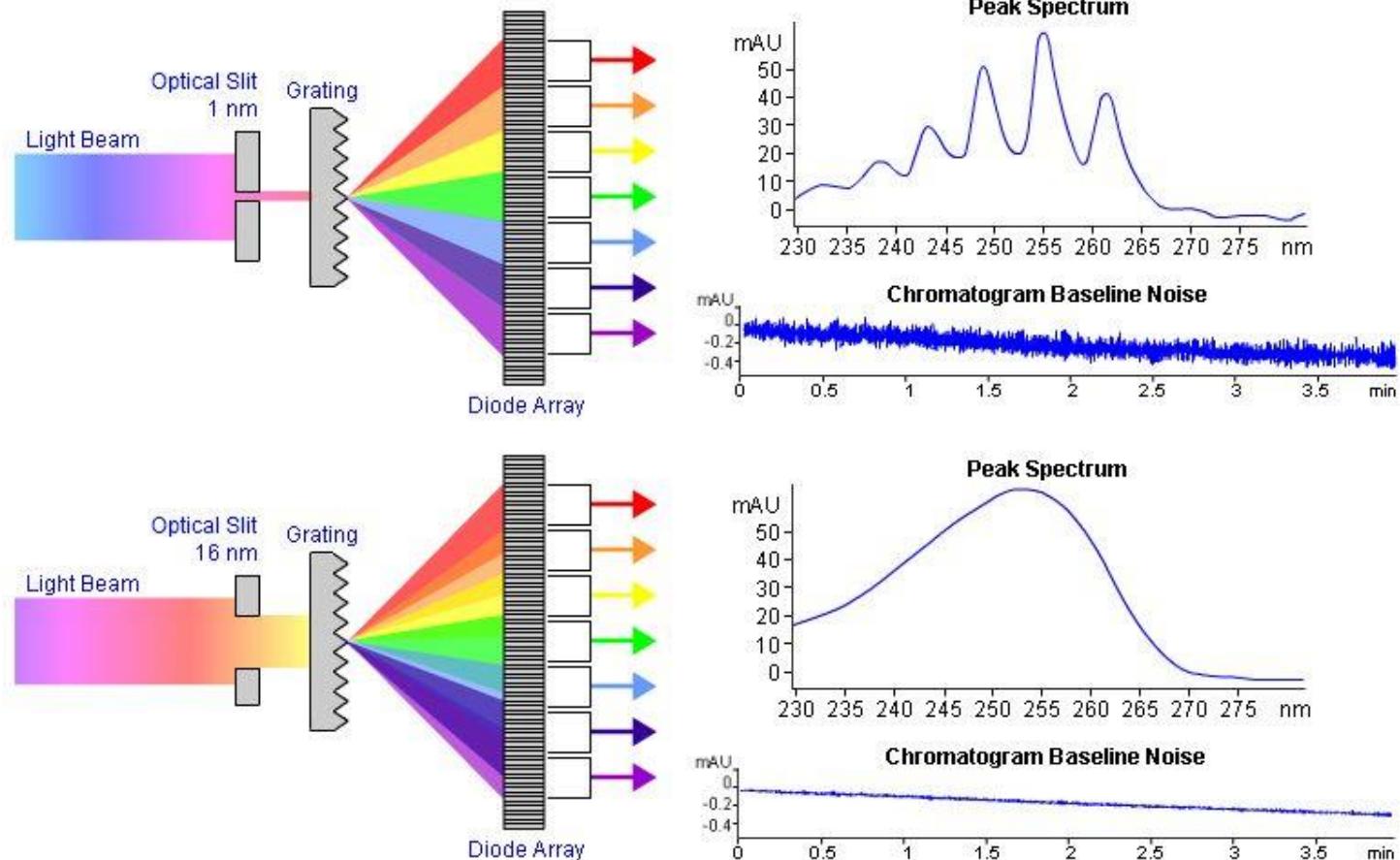
A calculated rate of at least 50 Hz is required to achieve the minimum of 10 data points per peak.



Maximizing Sensitivity

Detector: Programmable slit

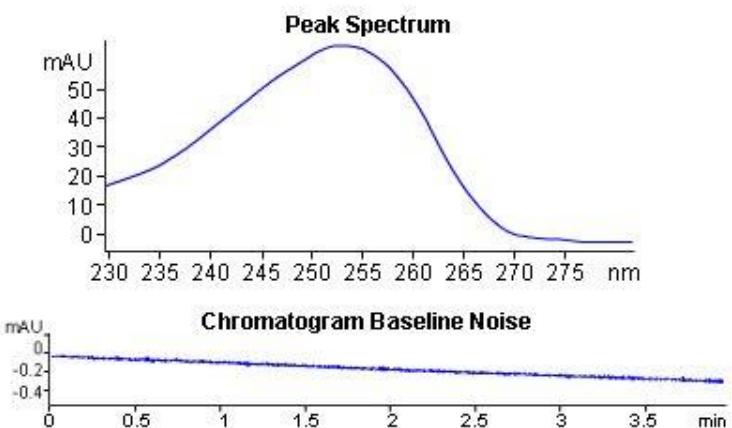
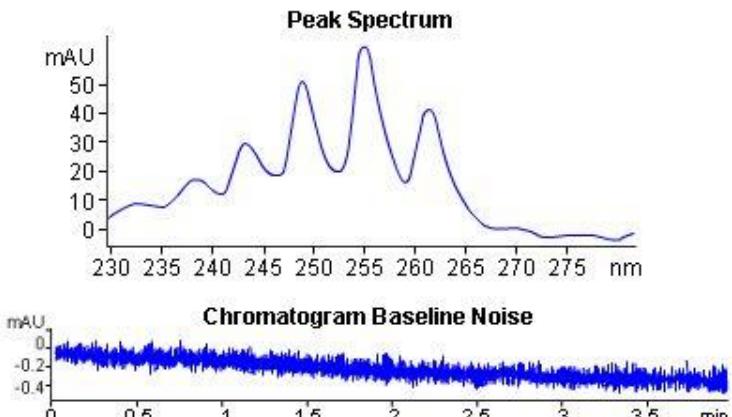
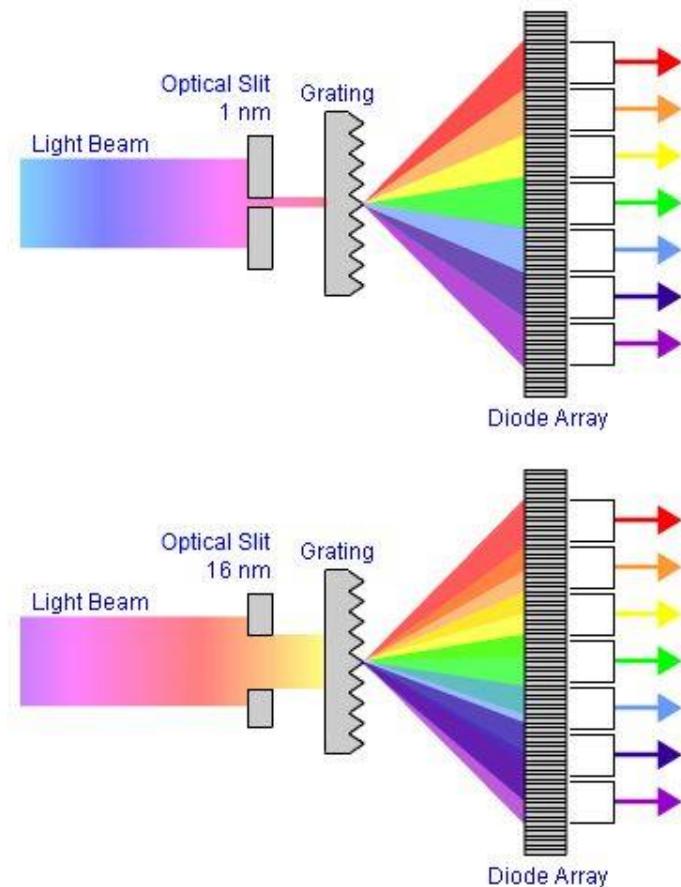
Varying the slit width allows control over how much light falls on the diode array



Maximizing Sensitivity

Detector: Programmable slit

- In most situations, a slit width of 4 nm will give the best results
- Use a narrow slit (1-2 nm) if you want to identify compounds with fine spectral structures, or if you want to quantify high concentrations
- Use a wide (8-16 nm) slit when your sample contains very small concentrations



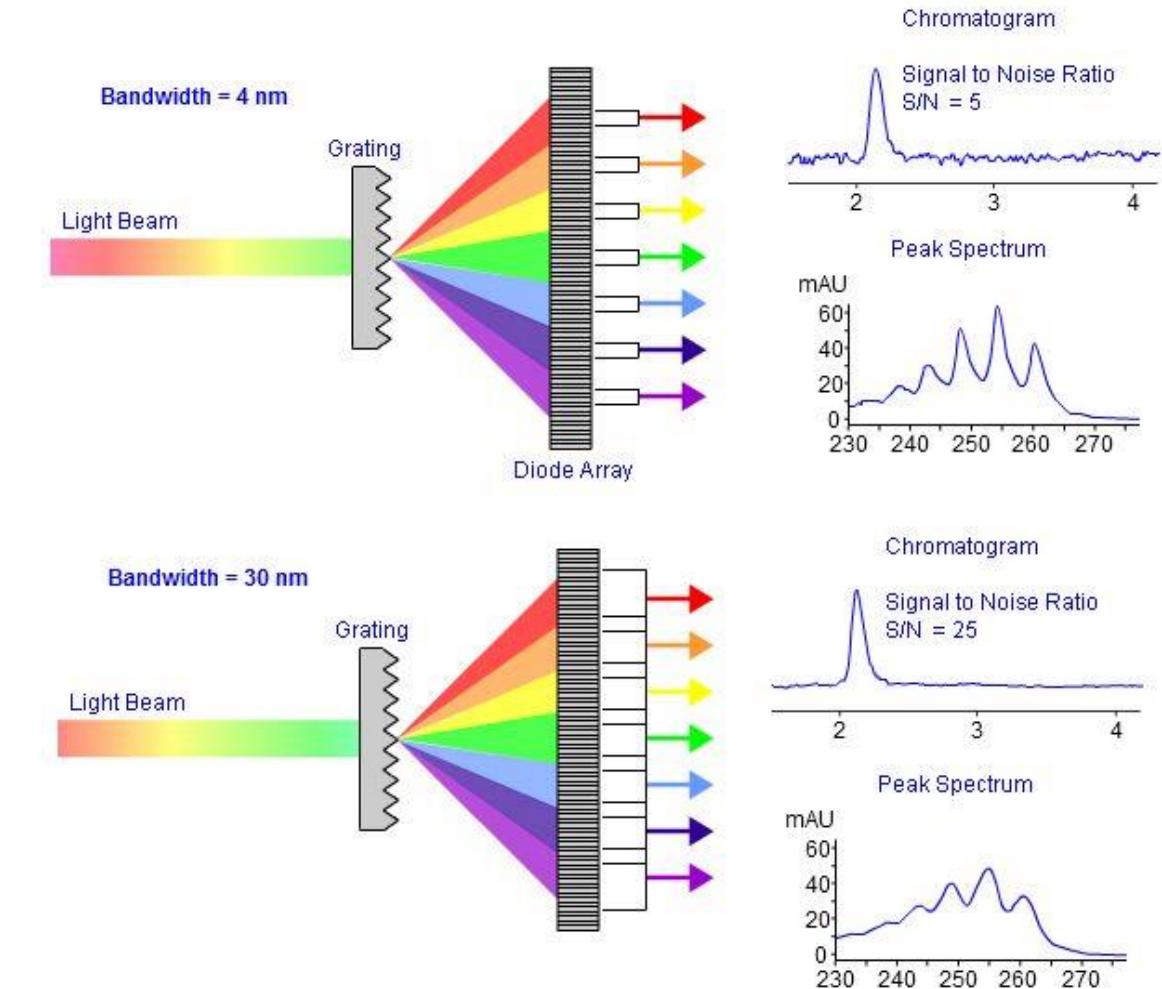
Detector	Programmable Slits
G1315A-D and G7115A	1, 2, 4, 8, 16 nm
G4212A and G7117B	1, 2, 4, 8 nm
G4212B and G7117A/C	4 nm fixed slit

Maximizing Sensitivity

Detector: Acquisition bandwidth

Bandwidth settings are related to the number of diode responses, which are averaged to obtain a signal at a particular wavelength.

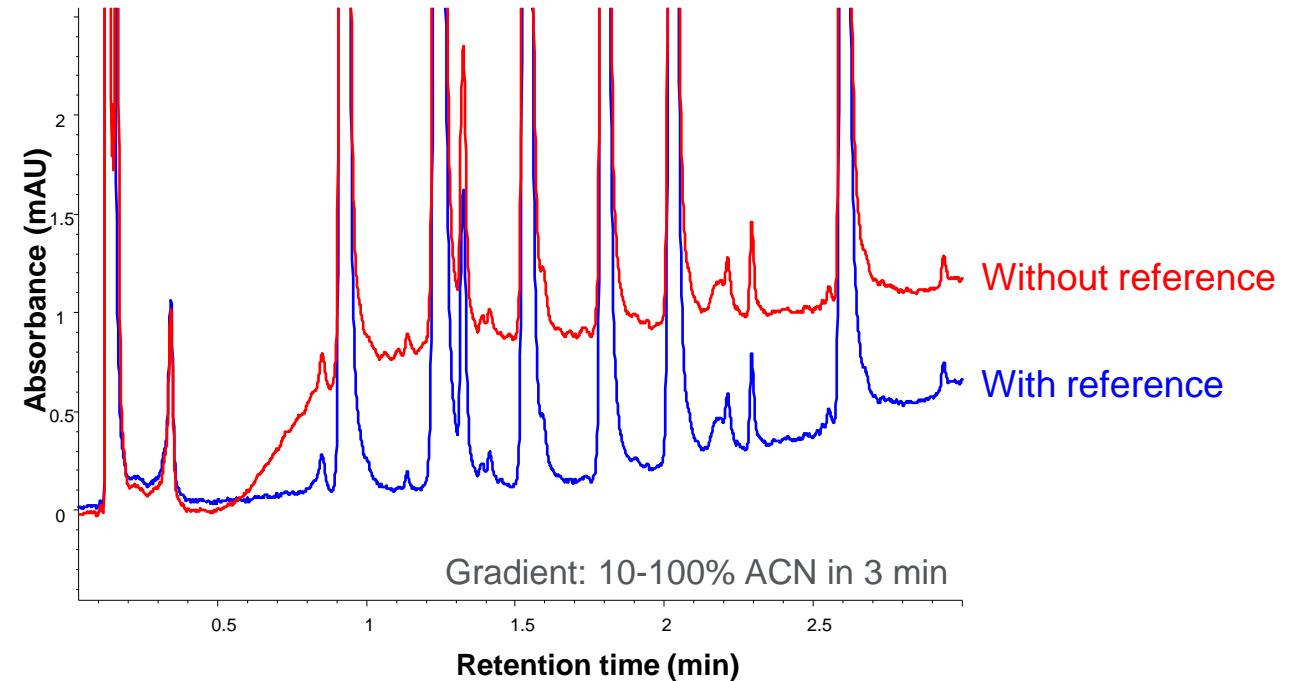
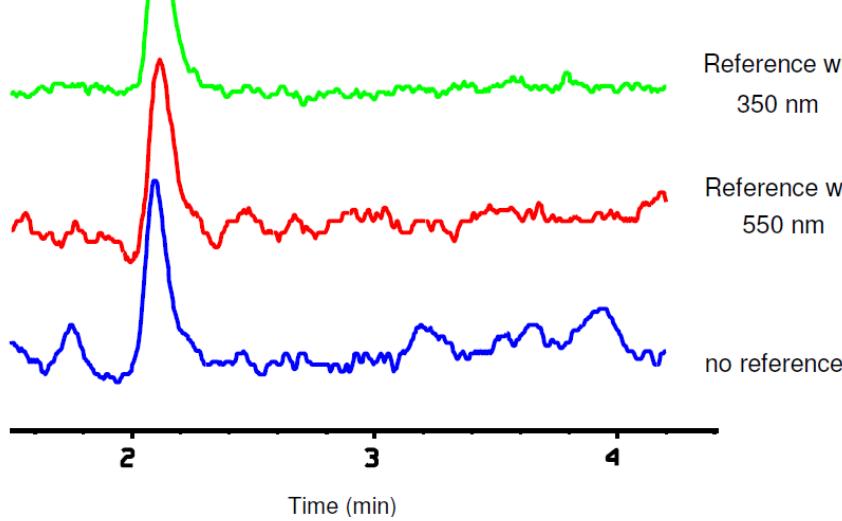
- Larger bandwidths average more signals and reduce noise but reduce selectivity and resolution.
- Smaller bandwidths average fewer signals, which increases selectivity and resolution but also increases noise.



Maximizing Sensitivity

Detector: Reference wavelength and bandwidth

The use of a reference wavelength is highly recommended to further reduce baseline drift and wander induced by room temperature fluctuations or refractive index changes during a gradient

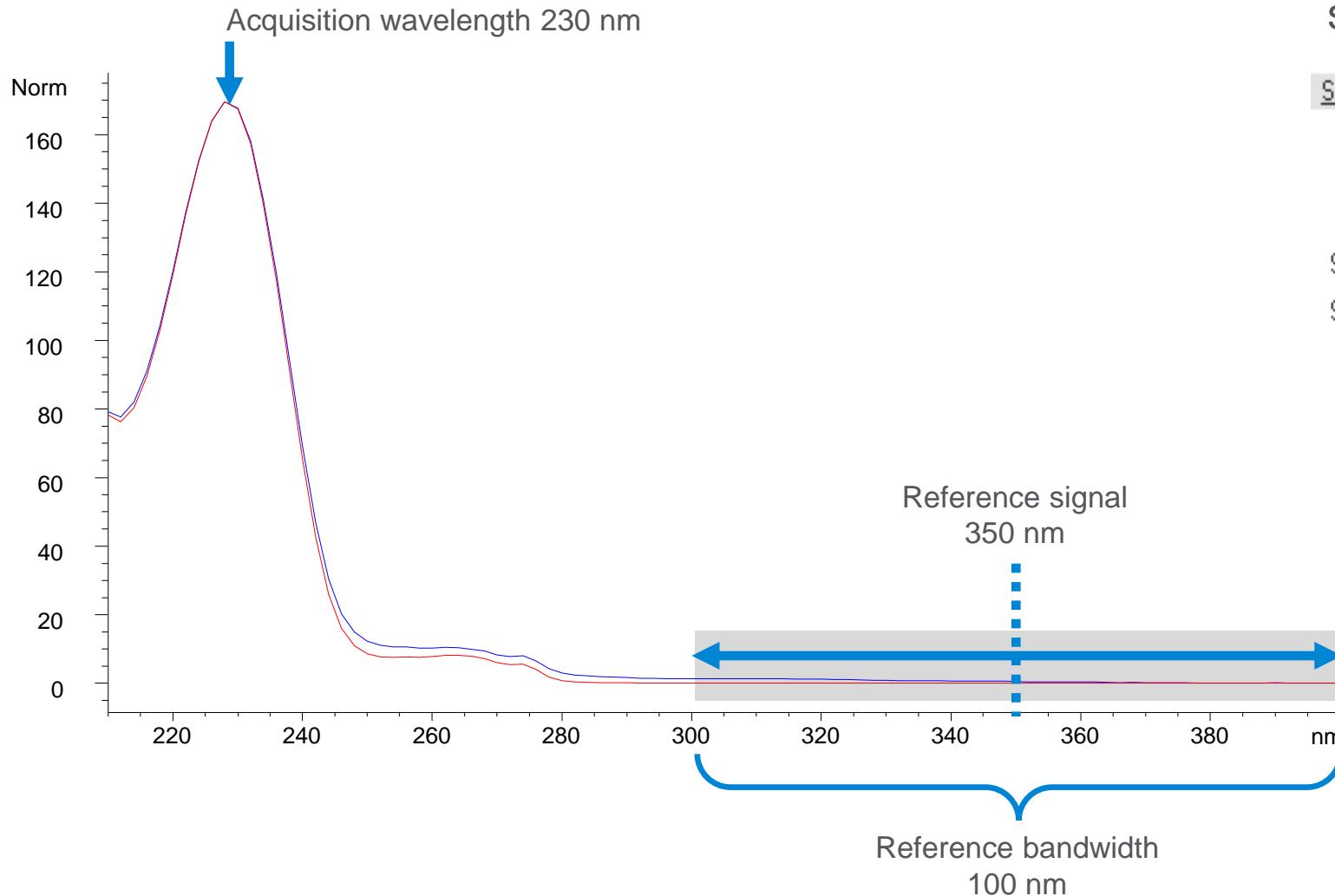


Reference wavelength can reduce noise due to temperature fluctuations and refractive index changes.

Reference wavelength can reduce the baseline drift observed during gradient methods .

Maximizing Sensitivity

Detector: Reference wavelength and bandwidth



The reference absorbance is subtracted from acquired absorbance.

Signals				
	Acquire Wavelength	Bandwidth	Reference Wavelength	
Signal A	<input checked="" type="checkbox"/> 254.0	4.0	<input checked="" type="checkbox"/> 360.0	100.0 nm
Signal B	<input checked="" type="checkbox"/> 254.0	4.0	<input type="checkbox"/> 360.0	100.0 nm

If the reference absorbance is higher than the acquired absorbance, it could result in a negative peak.

Getting the Most from Your DAD

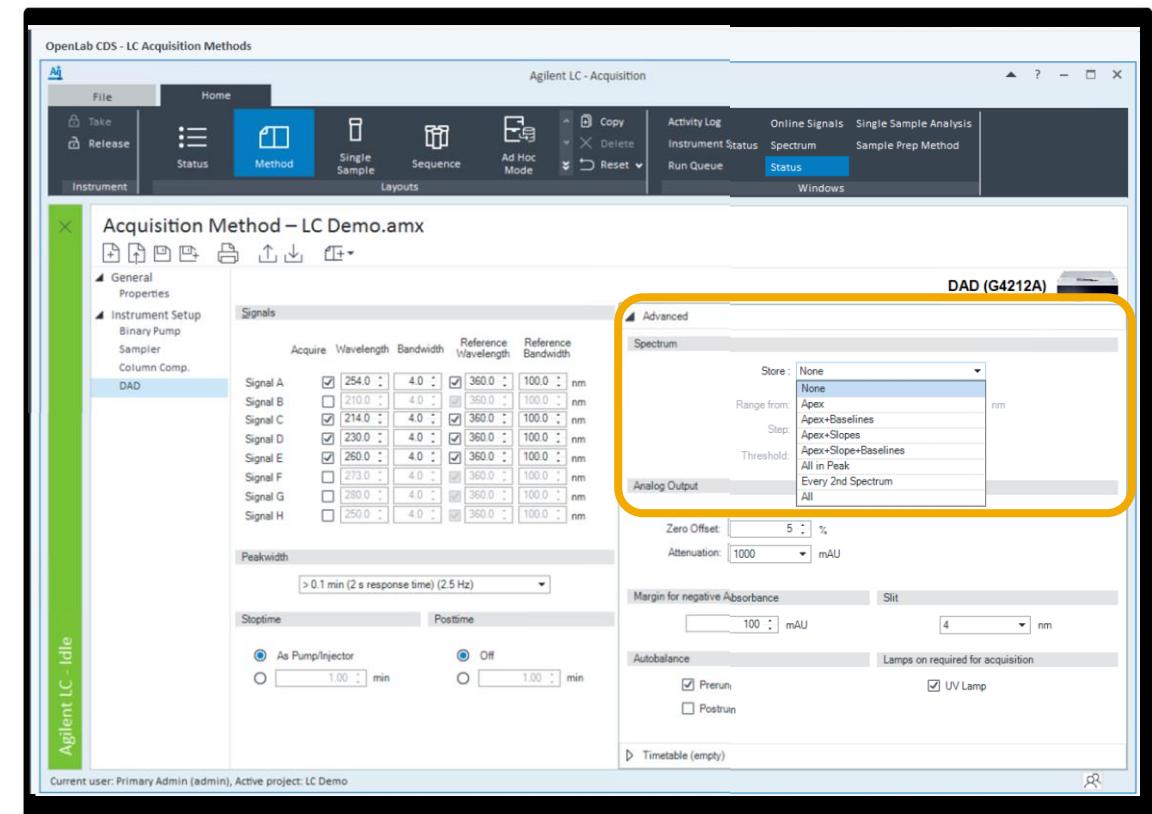
Spectral acquisition



Spectral Acquisition

Settings

- Spectrum storage
- Range
- Threshold



Spectral Acquisition

Spectrum storage

None	<ul style="list-style-type: none">• No spectra are taken.• No peak purity checks nor library search features can be used with this setting.
Apex	<ul style="list-style-type: none">• Only one spectrum per peak is stored• A peak must be higher than the value entered in Threshold setting.
Apex + Baselines	<ul style="list-style-type: none">• Spectra are taken at the apex, and baselines of the peak.• Recommended minimum to do library searches with baseline corrected spectra.
Apex + Slopes	<ul style="list-style-type: none">• Spectra are taken at the apex, upslope, and downslope of the peak.
Apex + Slopes + Baselines	<ul style="list-style-type: none">• Spectra are taken at the apex, baselines, upslope, and downslope of the peak.• Required to do a minimum peak purity check.
All in Peak	<ul style="list-style-type: none">• All spectra within the peak and above the threshold are taken.• Required for a full peak purity check.
Every 2nd spectrum	<ul style="list-style-type: none">• Spectra are taken continuously as for All, but only every second spectrum is stored; other spectra are discarded.• This reduces the amount of data storage necessary.
All	<ul style="list-style-type: none">• Spectra are taken continuously depending on the Peakwidth setting.

Spectral Acquisition

Range, step, and threshold

Range

- Range defines the wavelength range for spectral storage.
- Only the wavelength range where the compounds in your sample absorb contains information that is useful for purity checks and library searches. Reducing the spectrum storage range saves disk space.

Step

- Step defines the wavelength resolution for spectral storage. This resolution is dependent on the Peakwidth setting.
- Display of spectra, peak purity, and library search works best if a spectrum contains 5 to 10 data points per width of the absorbance bands.

Threshold

- Only spectra from peaks higher than threshold will be stored when a peak-controlled storage mode is selected.

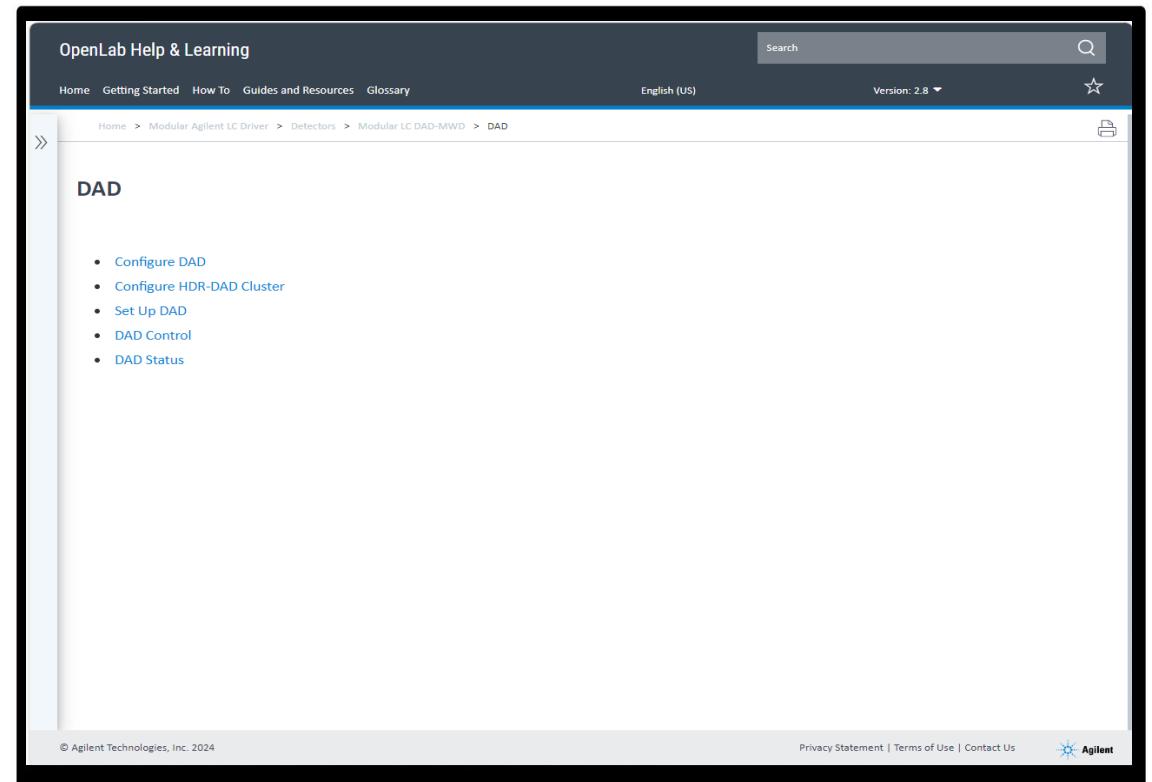
Spectral Acquisition

OpenLab Resources

[OpenLab CDS - LC Acquisition Methods
\(agilent.com\)](https://www.agilent.com)

[Optimizing DAD Acquisition Method Settings
- Articles - LC Portal - Agilent Community](https://www.agilent.com)

[OpenLab Help & Learning \(agilent.com\)](https://www.agilent.com)



Getting the Most from Your DAD

DAD maintenance



DAD Maintenance

Maintenance schedule

Daily	<ul style="list-style-type: none">• Inspect leak sensor• Inspect waste tubing
Weekly	<ul style="list-style-type: none">• Clean leak sensor• Run InfinityLab LC Performance Standard method
Monthly	<ul style="list-style-type: none">• Run Lab Advisor diagnostic tests• Inspect baseline
As Needed	<ul style="list-style-type: none">• Wavelength verification and calibration• Clean flow cell or Max-Light cartridge• Replace flow cell• Replace lamp

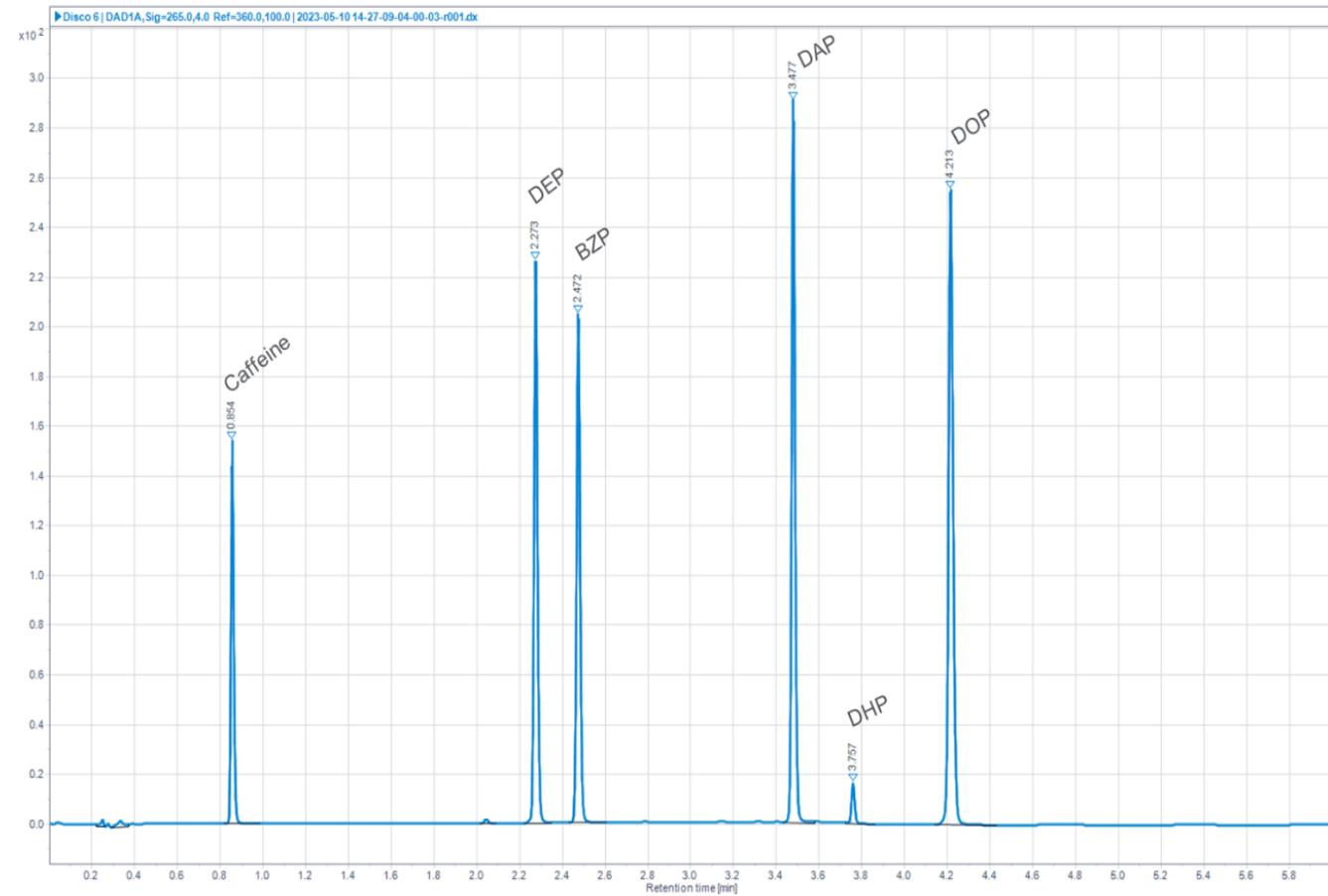


DAD Maintenance

InfinityLab LC performance standard

- This standard can be used in conjunction with internal standards and qualification standards to verify instrument performance on a day, week, month or annual basis.
- Customers can run this standard with acetonitrile and water **or** methanol and water with 0.1% formic acid buffer

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](#)

DAD Maintenance

InfinityLab LC performance standard

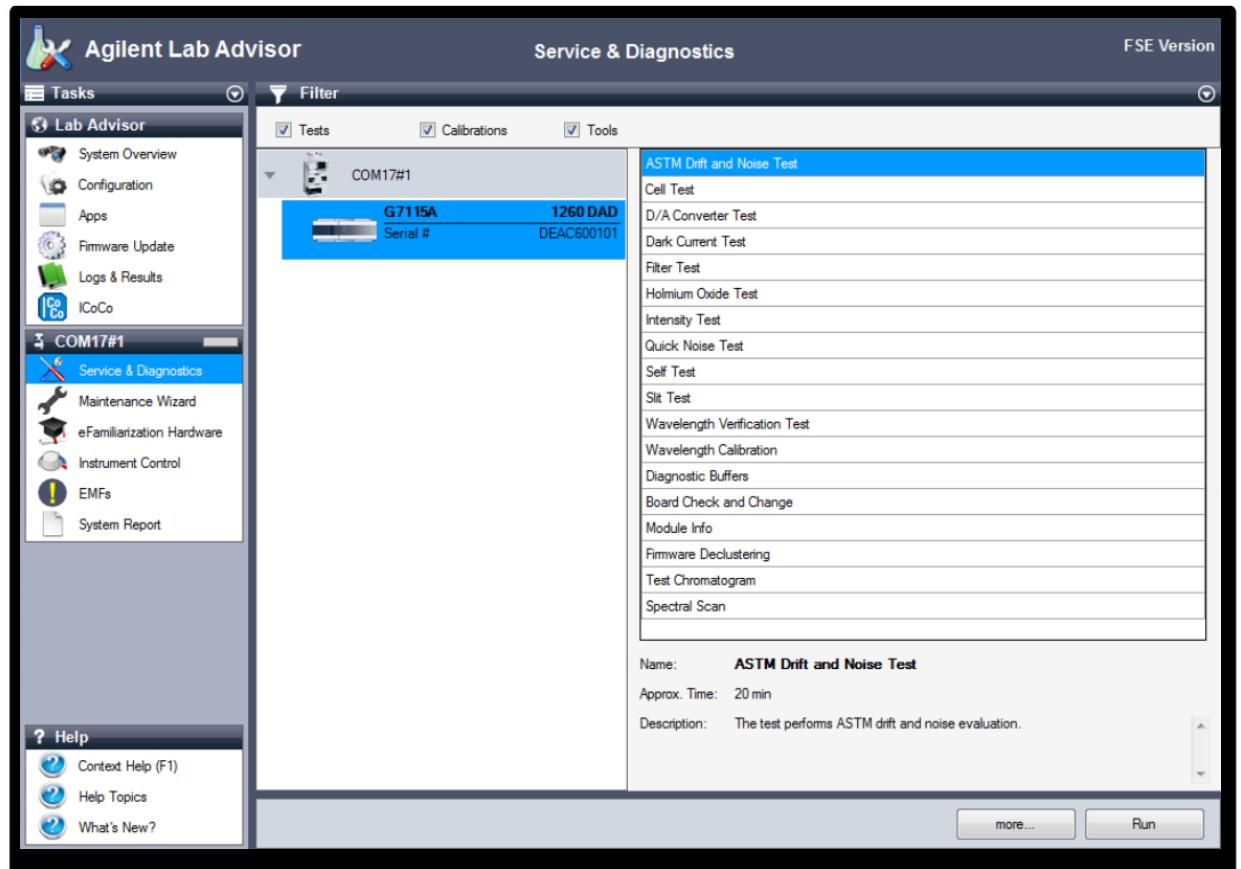


Analyte	Note/System Indicators	Affected System Component
Caffeine	A compound that elutes very early in the method will probe the LC pump for gradient delivery.	Pump
Diethyl Phthalate (DEP)	Half of the elution pair that evaluates the resolving power of the chromatographic system.	Column
Benzophenone (BZP)	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

DAD Maintenance

Lab advisor

Several maintenance and diagnostics tests available for UV detectors



DAD Maintenance

Lab advisor

Intensity test

- Lab Advisor will give a pass/fail result for different ranges across the visible and UV range.
- A lamp failing in the lower wavelengths may still be usable at the higher wavelengths.



Flow cell is removed for this test!

Intensity Test

1260HPLC > 1260HPLC > G7115A-DE

General	Limits	Signals
Test Name	Intensity Test	Description
Module	G7115A-DEAC600377 (1260 DAD WR)	The test scans the Intensity spectrum generated by the UV and VIS Lamp.
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...

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	3186312
UV Integral (190 - 349 nm)	7384053

DAD Maintenance

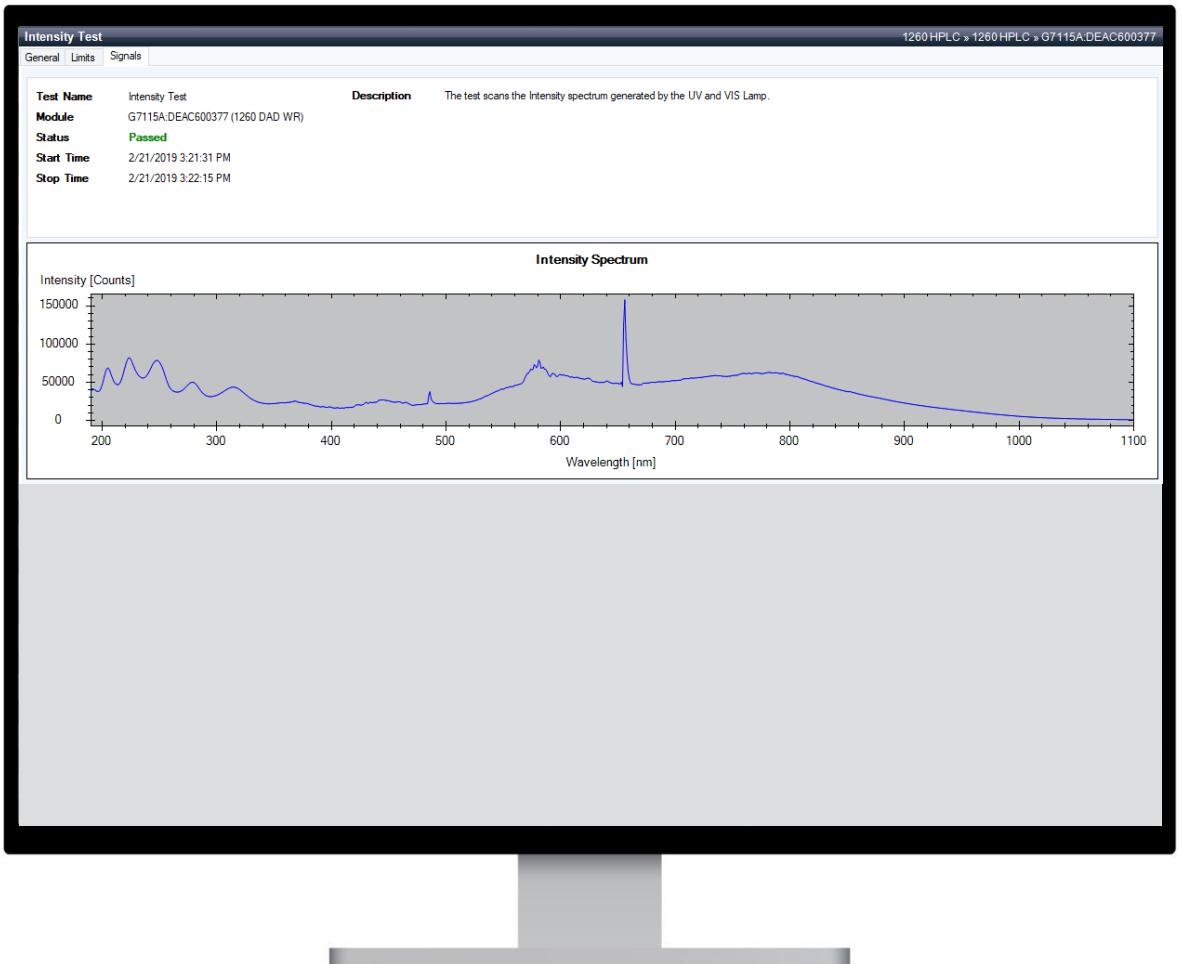
Lab advisor

Intensity test

- Lab Advisor will give a pass/fail result for different ranges across the visible and UV range.
- A lamp failing in the lower wavelengths may still be usable at the higher wavelengths.
- The profile of the intensity scan changes as a lamp ages



Flow cell is removed for this test!



DAD Maintenance

Lab advisor

ASTM drift and noise

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



Flow cell is removed for this test!

ASTM Drift and Noise Test

General Limits Signals

Test Name: ASTM Drift and Noise Test
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

Description: The test performs ASTM drift and noise evaluation without reference.

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
Noise value at 254 nm (UV)	0.008 mAU
Minimum Allowed Noise	0 ... 0.02 mAU
Drift value at 750 nm (Vis)	-0.415 mAU/h
Minimum Allowed Drift	-1 ... 1 mAU/h
Noise value at 750 nm (Vis)	0.013 mAU
Minimum Allowed Noise	0 ... 0.02 mAU

ASTM Drift and Noise Test

Remove the flow cell from the lightpath of the detector.

OK Cancel

DAD Maintenance

Lab advisor

Cell test

- To evaluate if a “dirty” flow cell is contributing to poor detection
- For G1315, G1365, G7115 or G7165 type detectors Lab Advisor will ask you to remove and then re-insert the flow cell, a UV scan is taken both with and without the cell and the ratio reported.
- For G4212 and G7117 type detectors the intensity with the flow cell is compared to that with the Test Cell.



Max Light test cell is part number
G4212-60011

Cell Test

General Limits Signals

Test Name: Cell Test
Module: G7115A:DEAC600377 (1260 DAD WR)
Status: Passed
Start Time: 2/21/2019 3:57:24 PM
Stop Time: 2/21/2019 3:59:37 PM

Description: The test compares the lamp intensity with and without the flow cell installed. The intensity ratio is an indicator of the amount of light absorbed by the flow cell.

Test Procedure:

- ✓ 1. Check Prerequisites...
- ✓ 2. Remove Flow Cell.
- ✓ 3. Scan Intensity Spectrum...
- ✓ 4. Insert Flow Cell.
- ✓ 5. Scan Intensity Spectrum...
- ✓ 6. Evaluate Data...

Result

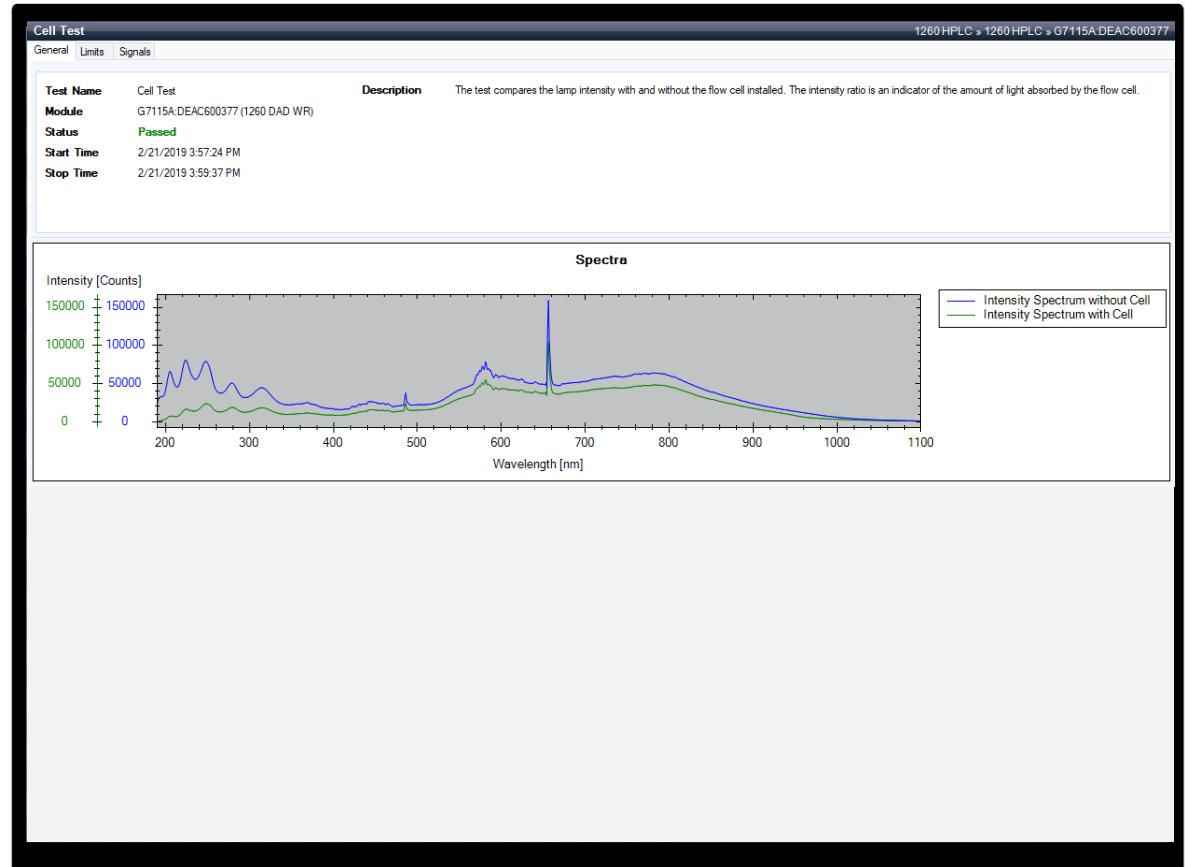
Name	Value
Accumulated UV Lamp Burn Time	126.95 h
UV Lamp On-Time	0.62 h
Accumulated Vis Lamp Burn Time	263.05 h
Vis Lamp On-Time	0.62 h
Intensity Integral without Flow Cell	32,088.720
Intensity Integral with Flow Cell	19,930.098
Intensity Ratio	0.62
Minimum Intensity Ratio	0.3

DAD Maintenance

Lab advisor

Cell test

- Example of scans with and without cell installed



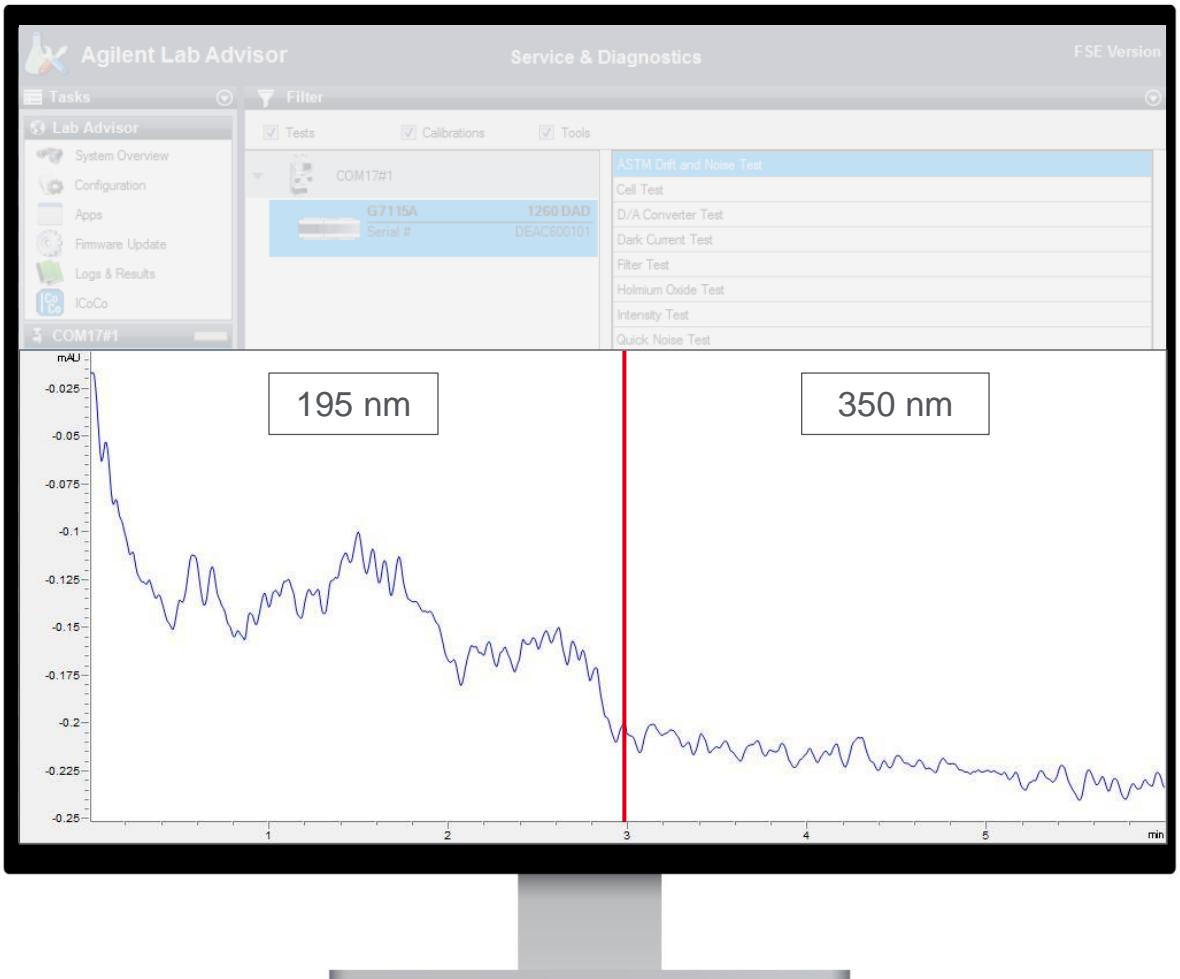
Flow cells must be thoroughly flushed with HPLC grade water

DAD Maintenance

Lab advisor

Baseline inspection

- Regular examinations of the baseline can provide helpful clues to the health of your lamp.
- There is a noticeable change in the drift and noise of the baseline when the wavelength is changed from 195nm to 350nm in chromatogram to the right.



DAD Maintenance

Care of flow cells

- Avoid the use of alkaline solutions with pH > 9.5 which can attack quartz and impair optical performance.
- Rinse flow cells with water after use to prevent crystallization of buffers or salts which will lead to blockage and damage.
- Aqueous solvents can allow algae growth. When leaving LC idle, pump a mobile phase with at least 5-10% of organic solvent.
- Ensure that the detector flow cell is bubble free by flushing with isopropanol or other organic solvent until a stable baseline is achieved.



DAD Maintenance

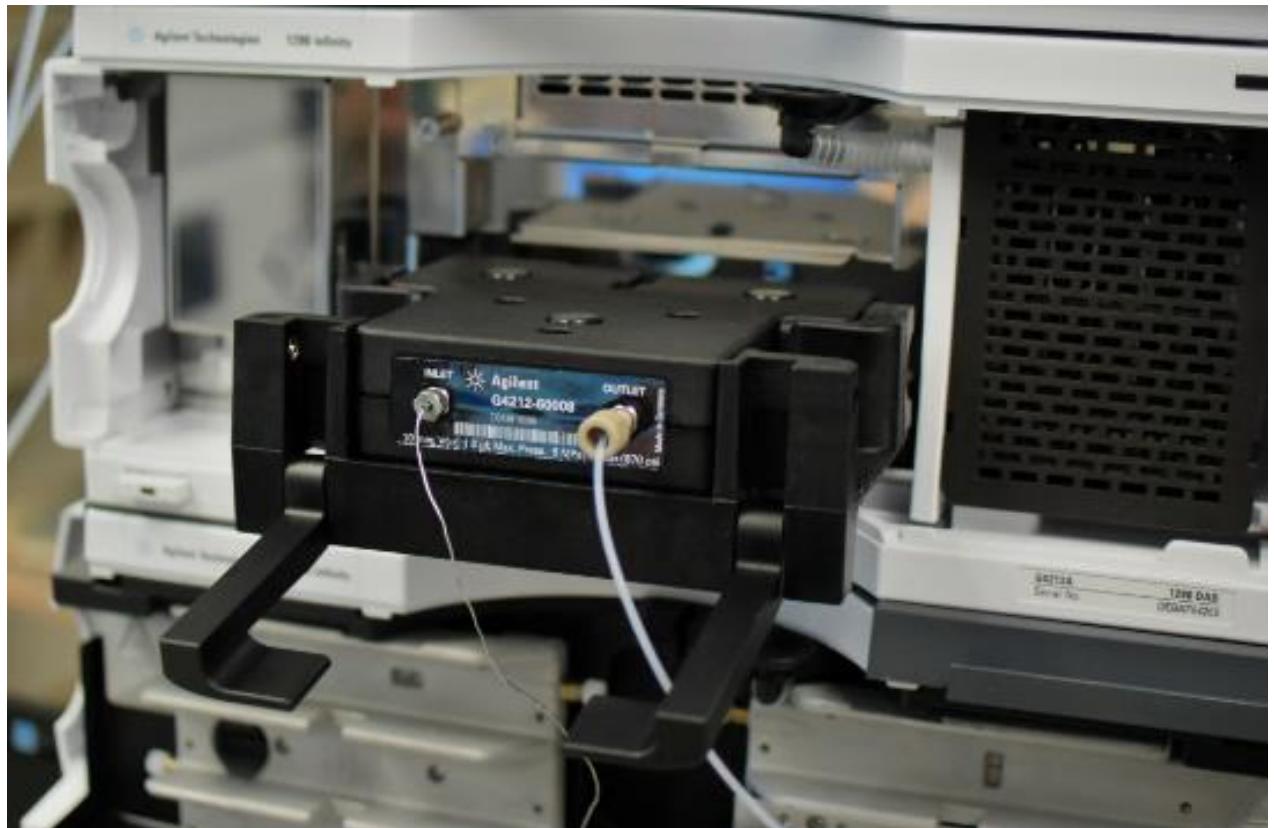
Flow cells

Max-Light Cartridge Cleaning

If there are low counts on the Intensity Test or the Cell Test

1. Flush the flow cell with isopropanol or ethanol for some time
2. Remove the cell from the cartridge holder
3. Carefully clean the light inlet and outlet using lens tissue or Q-tips dipped in alcohol

If alcohol cleaning fails, you can try the cell cleaning fluid (part number 5190-0530) or replace the cartridge.



[How to take care of your Agilent Max-Light Cartridge Cells Technical Note](#)

DAD Maintenance

Flow cells

Flow Cell Cleaning Guidance

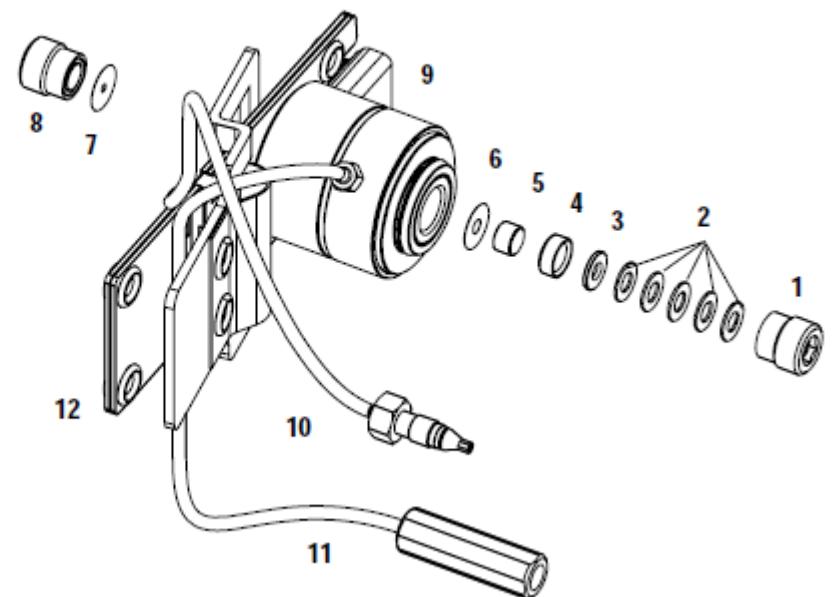
- Clean and reassemble one side of the flow cell before beginning the other side to prevent mixing the front and rear gaskets, which have different hole diameters (Number 6 and 7 in the diagram)
- While cleaning or replacing flow cell windows, if the washers fall out of the window assembly, they must be inserted in the correct order with a PTFE ring to prevent any leaks
- Clean the cell body with water or isopropanol
- After opening the cell, you should always use a new gasket

Orientation of Flow Cell Parts

NOTE

Gaskets # 6 and #7 have different hole diameters

- 1 - window screw
- 2 - spring washers
- 3 - compression washer
- 4 - window holder
- 5 - quartz window
- 6 - gasket (light in)
- 7 - gasket (light out)
- 8 - window screw (contains items 2, 3, 4 and 5)
- 9 - flow cell body
- 10 - inlet capillary
- 11 - outlet capillary
- 12 - holder

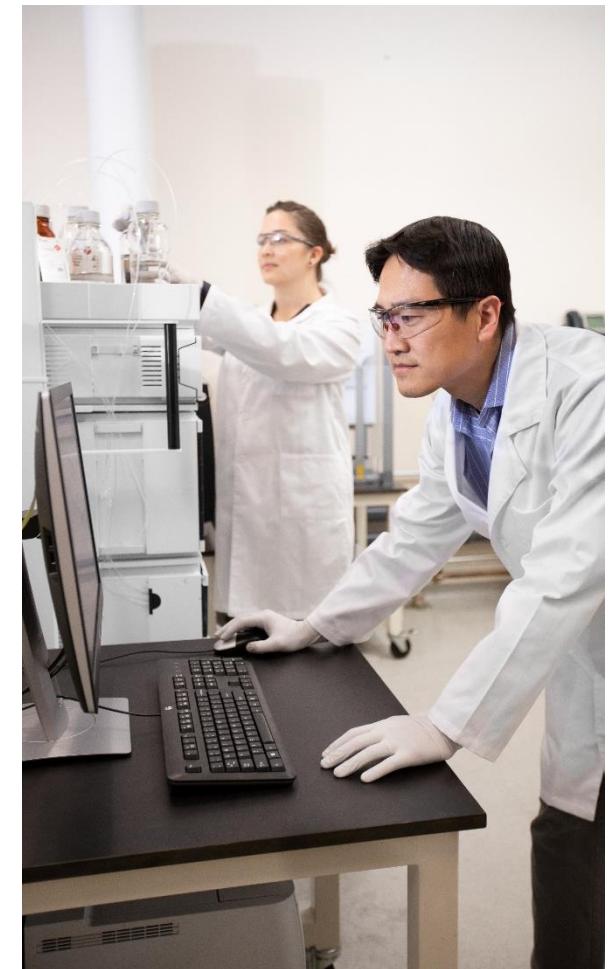


[HPLC Maintenance - Rebuilding the Flow Cell in an Agilent G1314X Variable Wavelength Detector \(VWD\) \(youtube.com\)](#)

DAD Maintenance

Best practices

- Frequently turning the lamp on and off will reduce lamp lifetimes.
- Warm up detector for at least one hour before first acquisition method.
- Install pressure relief valve (part number G4212-68001) when using DAD detectors are connected to a second detector.
- Only use the waste tubing designed for the DAD. Shorter lengths can cause bubbles in the flow cell.
- Keep environment temperature stable
 - Do not expose the detector to direct sun light
 - Do not expose the detector to direct air current from an HVAC



[Best Practices for Using an Agilent LC System Technical Note](#)

[Agilent Inline Pressure Relief Valve Kit Technical Note](#)

DAD Maintenance

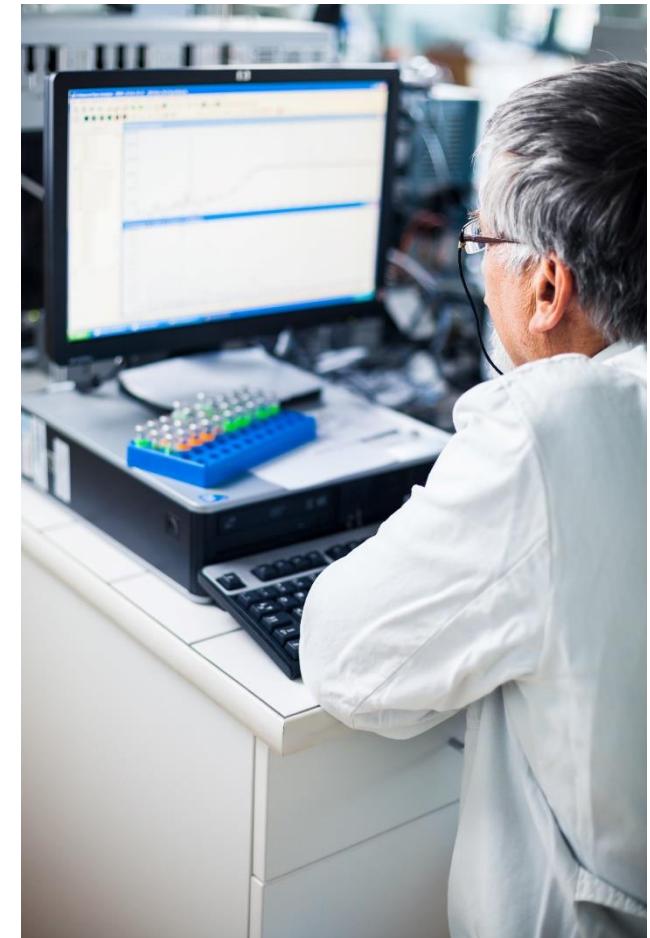
Maintenance resources

Manuals

- [Agilent 1200 Infinity Series Diode Array Detectors - User Manual](#)
- [Agilent 1260 Infinity Diode Array and Multiple Wavelength Detector User Manual](#)
- [Diode Array Detector WR and Multiple Wavelength Detector Agilent InfinityLab LC Series User Manual](#)
- [Diode Array Detectors Agilent InfinityLab LC Series User Manual](#)

Agilent YouTube Channel

- [HPLC Maintenance - Replacing the Lamps in an Agilent G1315X DAD or G1365X MWD \(youtube.com\)](#)
- [HPLC Maintenance - Rebuilding the Flow Cell in an Agilent G1314X Variable Wavelength Detector \(VWD\) \(youtube.com\)](#)
- [HPLC Maintenance - Replacing the Flow Cell in the Agilent G1315X DAD \(youtube.com\)](#)



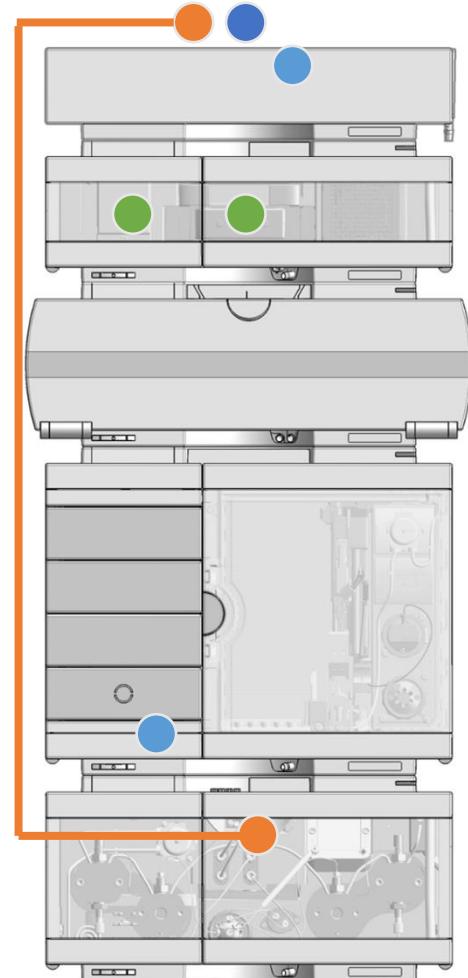
Getting the Most from Your DAD

Troubleshooting



Troubleshooting

Potential Cause	Recommended Action
Gas bubbles in mobile phase	<ul style="list-style-type: none">• Apply degassing• check degasser performance
Low difference between sample and mobile phase absorbance	<ul style="list-style-type: none">• Check absorbance values of sample vs. mobile phase
Contamination	<ul style="list-style-type: none">• Use degassed HPLC-grade solvents• flush system• Clean up the sample
Detector optics	<ul style="list-style-type: none">• Perform intensity test• Check signal with flow cell removed if possible• Replace lamp
Pressure instability	<ul style="list-style-type: none">• Check 'Pressure fluctuation'



Troubleshooting

Contacting Agilent instrument support

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

1-800-227-9770 option 3, option 2.

What to have ready when you call:

- Serial number of detector
- Most recent maintenance reports



Additional Resources

Resources



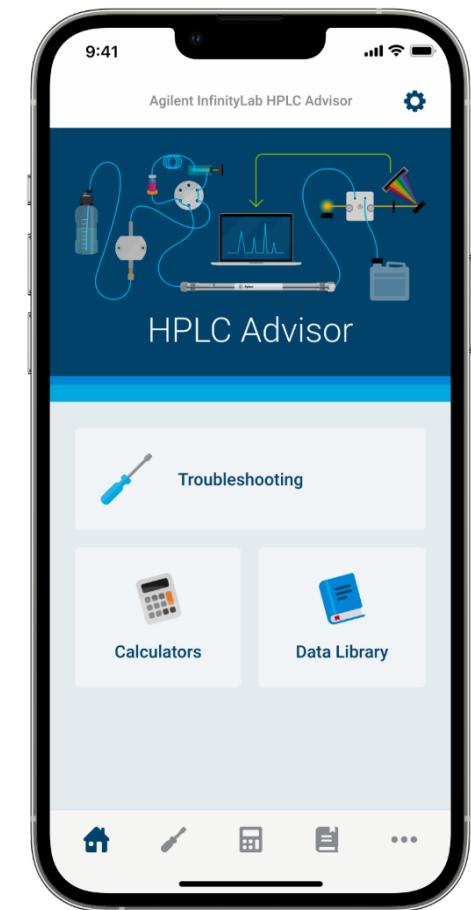
Agilent DAD Comparison

Product No	G1315D	G1315C	G7115A	G4212B	G7117C	G7117A	G7117B
Description short	1260 DAD VL	1260 DAD VL+	1260 Infinity II DAD WR	1260 DAD	1260 Infinity II DAD HS	1290 Infinity II DAD FS	1290 Infinity II DAD
	For high sensitivity in multi- λ -analysis and high-resolution spectral analysis	For high sensitivity in fast multi- λ -analysis and high-resolution spectral analysis	For high sensitivity in fast multi- λ -analysis and high-resolution spectral analysis	For ultra-sensitivity in fast multi- λ -analysis and spectral analysis	For ultra-sensitivity in fast multi- λ -analysis and spectral analysis	For ultra-sensitivity in fast multi- λ -analysis and spectral analysis	For ultra-sensitivity in ultra fast, multi- λ -analysis and high-resolution spectral analysis
Data rate	20 Hz	80 Hz	120 Hz	80 Hz	120 Hz	120 Hz	240 Hz
Signals	8 signals	8 signals	8 signals	8 signals	8 signals	8 signals	8 signals
Programmable Slit width	variable slit	variable slit	variable slit	fixed slit	fixed slit	fixed slit	variable slit
Wavelength range	190-950 nm	190-950 nm	190-950 nm	190-640 nm	190-640 nm	190-640 nm	190-640 nm
Flow cell type	Conventional	Conventional	Conventional	Max-Light Cartridge	Max-Light Cartridge	Max-Light Cartridge	Max-Light Cartridge
Flow cells	Nano, Analyt.Scale, Bio, Prep.Scale	Nano, Analyt.Scale, Bio, Prep.Scale	Nano, Analyt.Scale, Bio Prep.Scale	Analyt.Scale	Analyt.Scal Bio	Analyt.Scale Bio, HDR	Analyt.Scale Bio, HDR



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](#)



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



Contact Agilent Chemistries and Supplies Technical Support



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Mohit Patel
Application Engineer
Pennsylvania
HPLC



Mark Powell
Application Engineer
Delaware
HPLC/dissolution



Ryan Birney
Application Engineer
Delaware
GC/spectroscopy



Mark Sinnott
Application Engineer
Idaho
GC

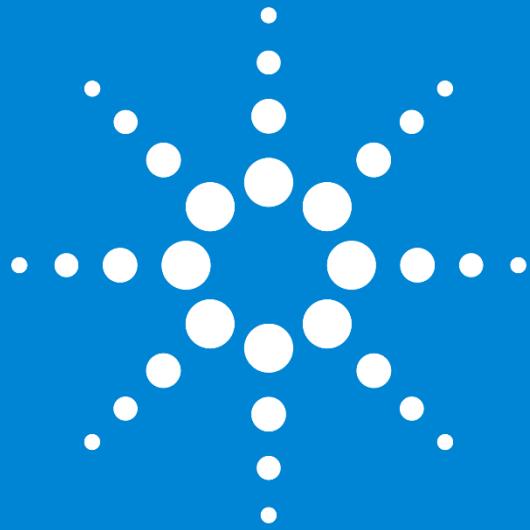


Alex Ucci
Application Engineer
Pennsylvania
GC/sample preparation



Questions?





Agilent

Trusted Answers