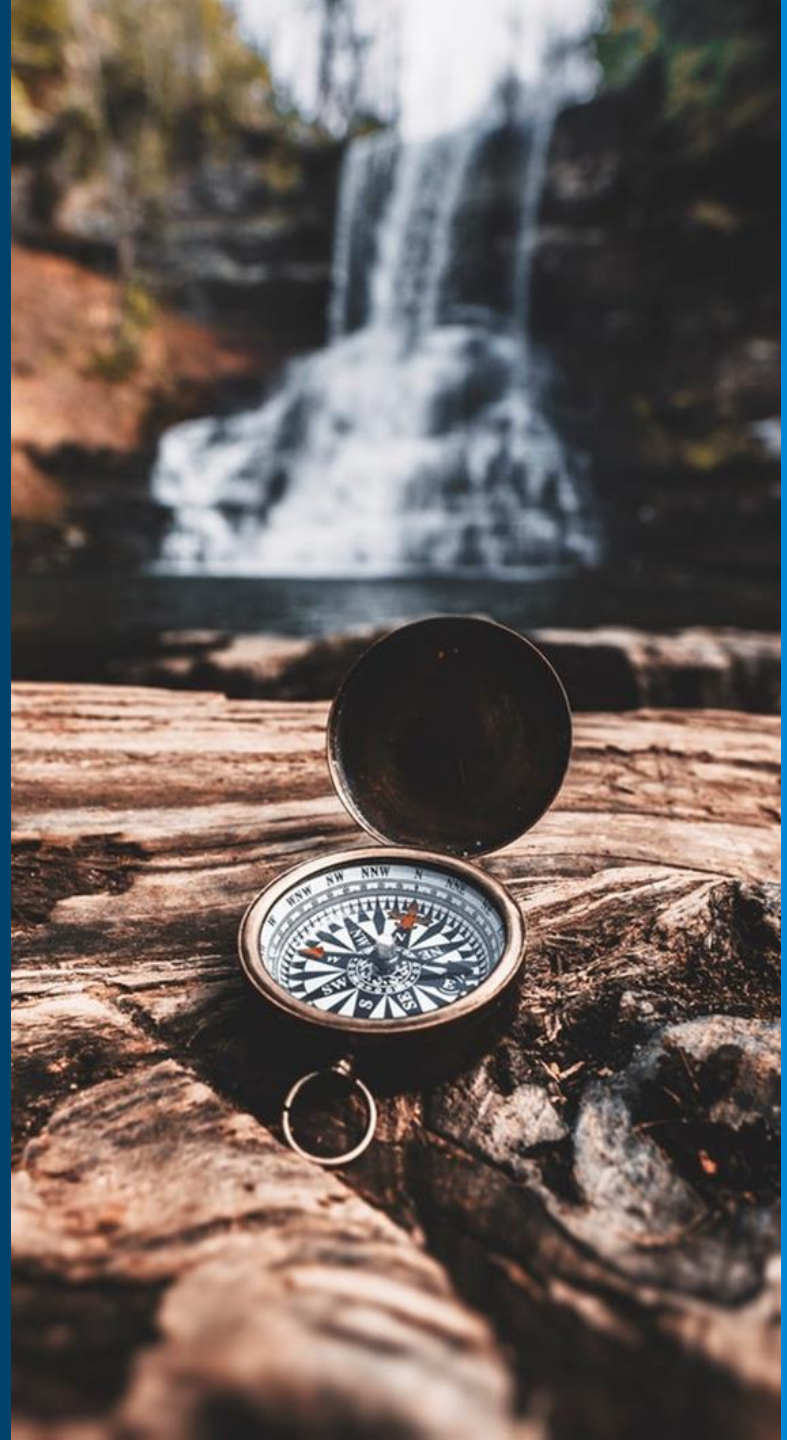
Surviving Chromatography

Part 1: Preventive Action

Mark Powell
Alexander Ucci



Agilent
Trusted Answers

Planning Ahead

- Planning and good habits can help prevent problems that can take you down the wrong path and cause you to lose your chromatography
- Care of your instrument:
  - supplies
  - sample prep
  - instrument configuration
- Careful attention to method development
- Save time and aggravation of troubleshooting

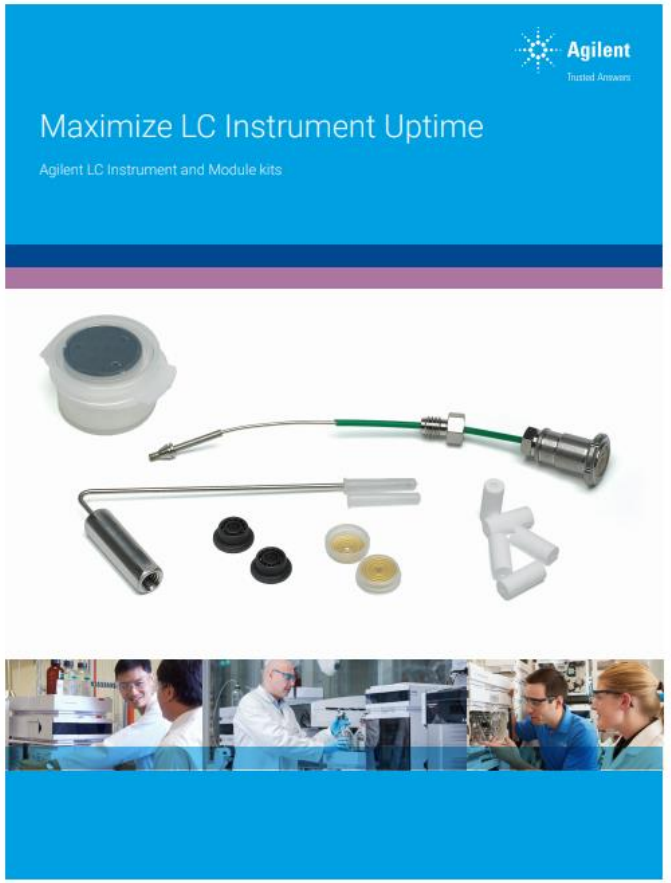
Instrument Supplies

- Critical points of instrument maintenance
- Supplies to have on hand

LC spare parts and lamps from Agilent compared to other vendors

Choosing 3rd party supplies could cause:

- System failure and increased downtime
- More frequent maintenance due to shorter life time of parts
- Contamination peaks
- Reduced signal-to-noise ratio and lower detection limit
- Inconsistent and inaccurate results
- In-efficiency and long-term higher cost-of-ownership



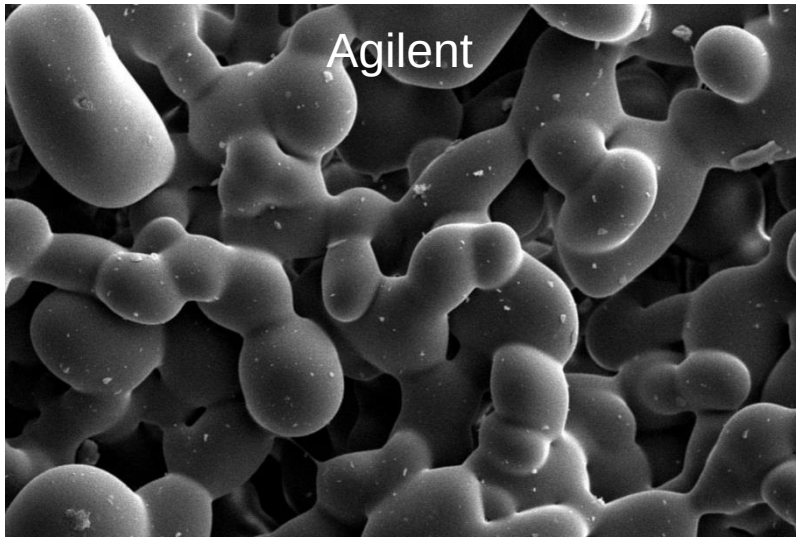
5994-0017EN

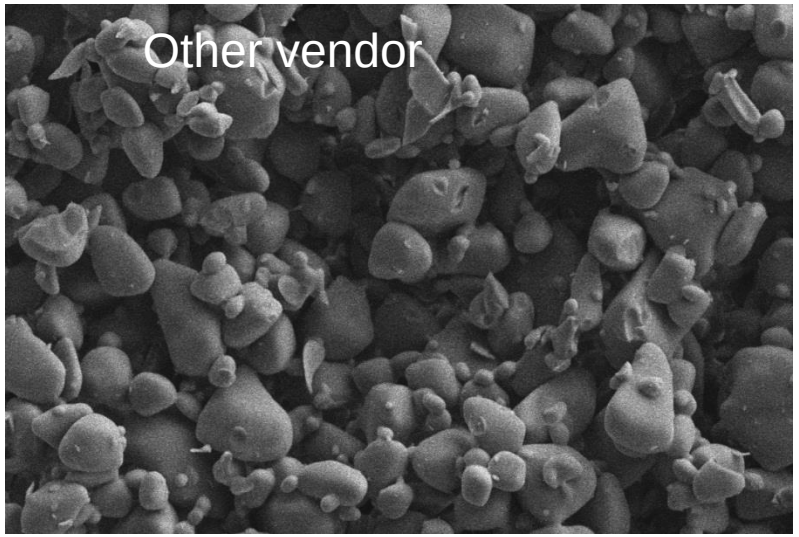
Solvent Bottles and Inlet Filters

- Inlet filters - Not a replacement for good mobile phase hygiene
  - Glass solvent inlet filter (20 µm), 5041-2168
  - Stainless steel solvent inlet filter (recommended for LC-MS), 01018-60028
- Use only high quality HPLC or MS grade solvents
  - Do not filter
- Filter buffer and salt solutions
  - Filter porosity: 0.45 or 0.2 µm
  - Make sure the filter material is compatible
- Avoid algae/microbial growth
  - Frequently replace the mobile phase with a clean bottle
  - Adding some organic to aqueous mobile phases can inhibit growth
  - Consider avoiding light exposure

Solvent Inlet Filters pore size

p/n: 5041-2168



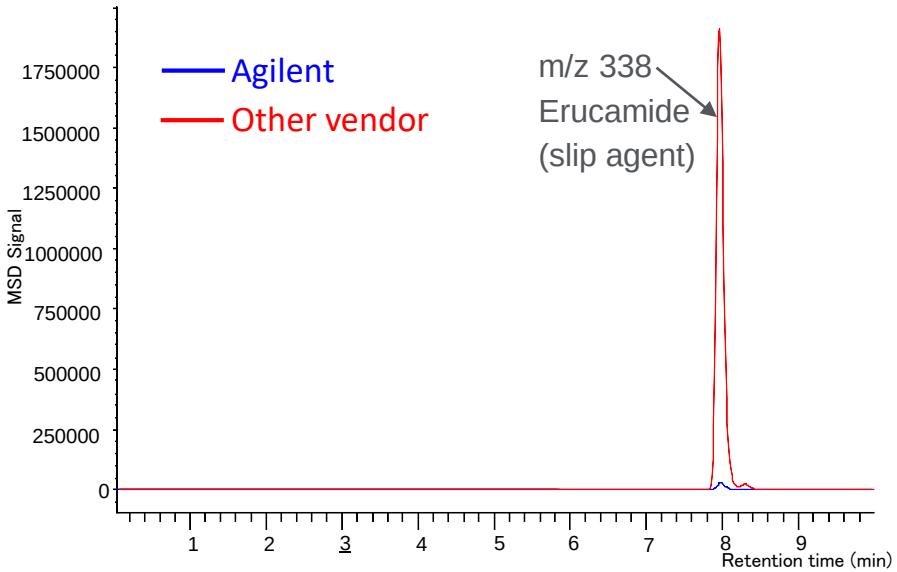


SEM images of solvent inlet glass filter from Agilent and other vendor

- Agilent solvent filter show uniform particle size and superior pore-size homogeneity, while inconsistent pore and particle size was evident on filter from other vendor.
- Too large pores lead to deficiency of filtration, while too small pores cause pressure increase.
- Too small particles can go into the flow path, blocking pump frit, capillaries or columns.

Solvent Inlet Filters cleanliness

p/n: 5041-2168



LC/MSD chromatograms of soaking solution from Agilent and other vendor solvent inlet filter

Packaging

- Agilent solvent inlet filters are packed in ultraclean antistatic bags with inner metallic coating, while other vendors use normal plastic bags.
- LC/MS analysis shows potential **contamination** through slip agent **when using 3rd party filters**.
- Specially shaped packaging of Agilent filters can avoid damage during transportation.

Pump supplies

Items to have on hand:

- PTFE frits
- Pump piston seals

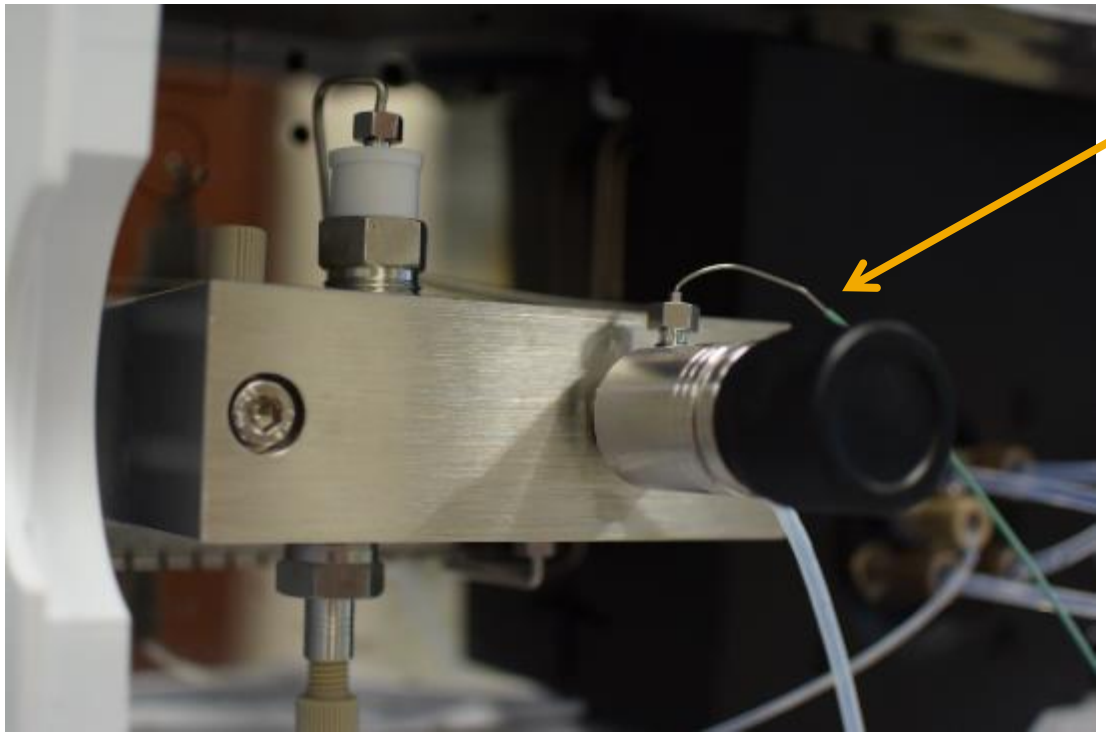
p/n: 01018-22707

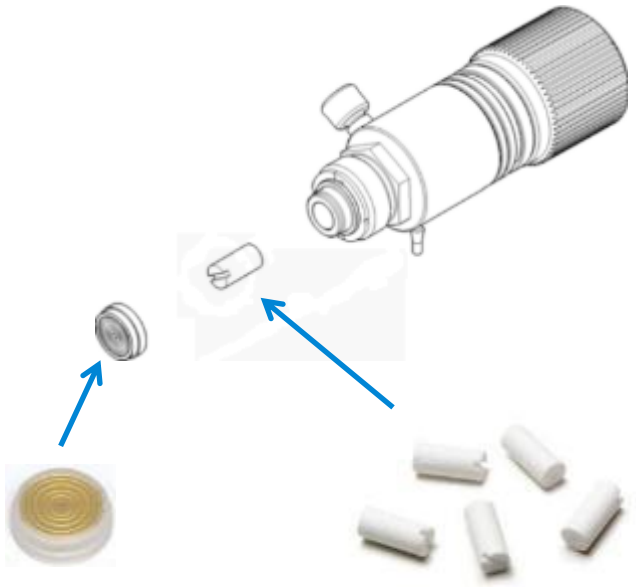
Typical Schedule for Pump

Item	Typical Schedule	Comments
Solvent inlet filter	Replace every 6 - 12 months	
PTFE frits in purge valve + gold seal	Every 12 months	
Piston seals	Every 12 months	When changing the seal, check the piston for scratches--replace if scratched
Inlet valve/cartridge, outlet ball valve	Every 24 months	

Adjust according to your samples, conditions, and performance goals

Pump Supplies
Purge Valve



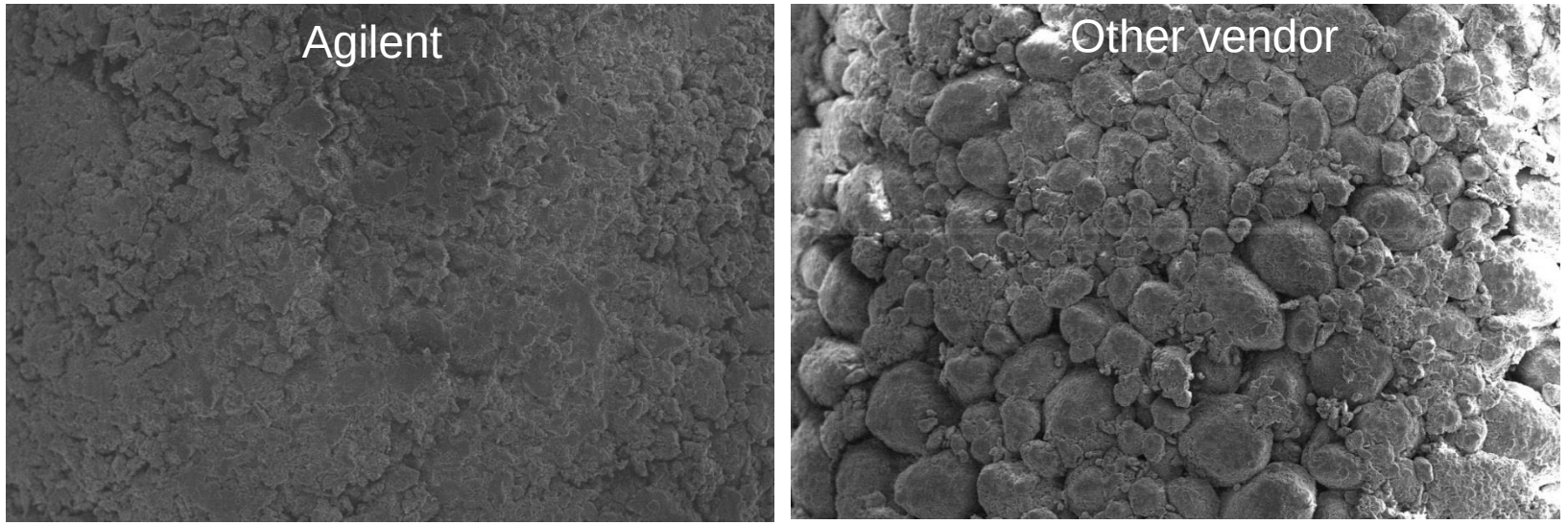


Seal cap
5067-4728

PTFE frits
01018-22707

PTFE Frits

p/n: 01018-22707

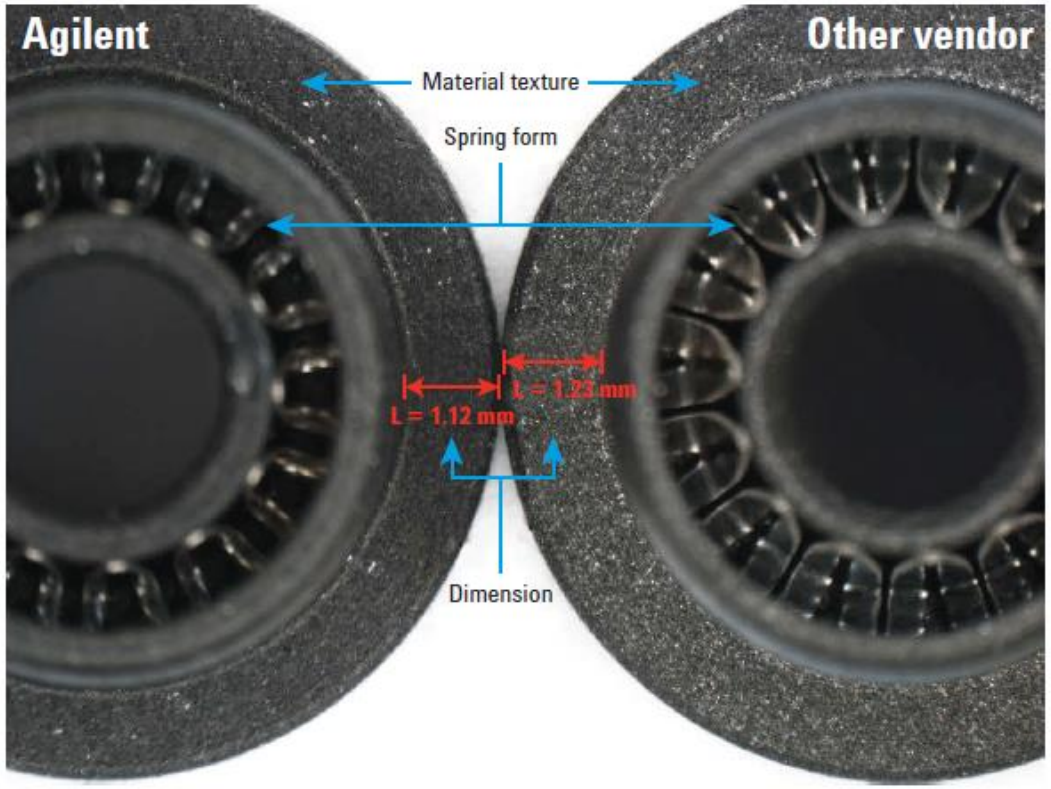


SEM images of PTFE frit from Agilent and other vendor

- Agilent frits show much more uniform particles than frits from other vendor.
- Agilent frits use defined particle size to avoid blockage or contamination.

Pump Piston Seals

p/n: 5063-6589



Photomicrographs of pump piston seal from Agilent and other vendor

- Piston seals from other vendor show different material texture, spring form and dimension than Agilent piston seals.
- Agilent has defined spring tightness for optimal strength:
  - too tight → more abrasion and shorter life time
  - too soft → air bubbles into pump head & pressure ripple
- Agilent uses proprietary polymer blend featuring:
  - optimal elasticity, firmness and hydrophobicity to reduce pressure ripple
  - wide temperature range 4-60°C
  - copper-free

Outlet Check Valves

p/n: G1312-60012

- Outlet check valves from other vendor still use Agilent´s old design.
- New design of Agilent check valves provides enhanced durability and reliability:
  - ✓Integrated gold-plated seal cap instead of gold seal cap to free users from maintenance
  - ✓Unique double-coned seat design provides higher resistance to high pressure and pressure changes, leading to lower pressure ripple, higher flow precision and longer life time.

Autosampler and Column Compartment

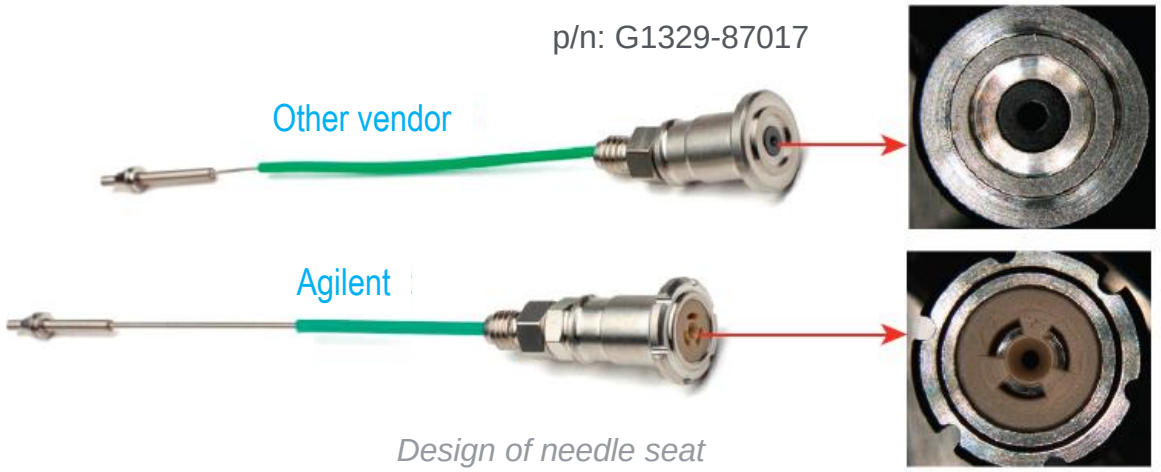
Maintenance points on Autosampler

- Needle
- Loop capillary
- Needle seat
- Injection valve rotor seal
- Metering device seal

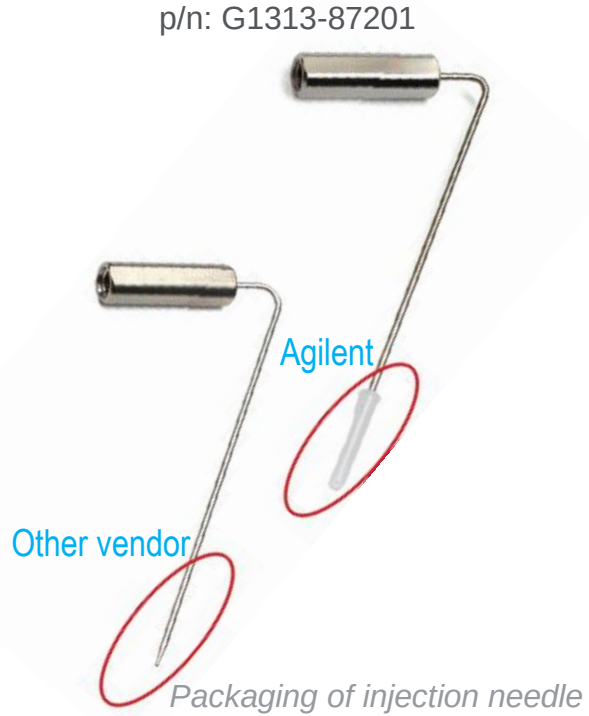
In-line Filters

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

Injection Needles and Needle Seats



Design of needle seat

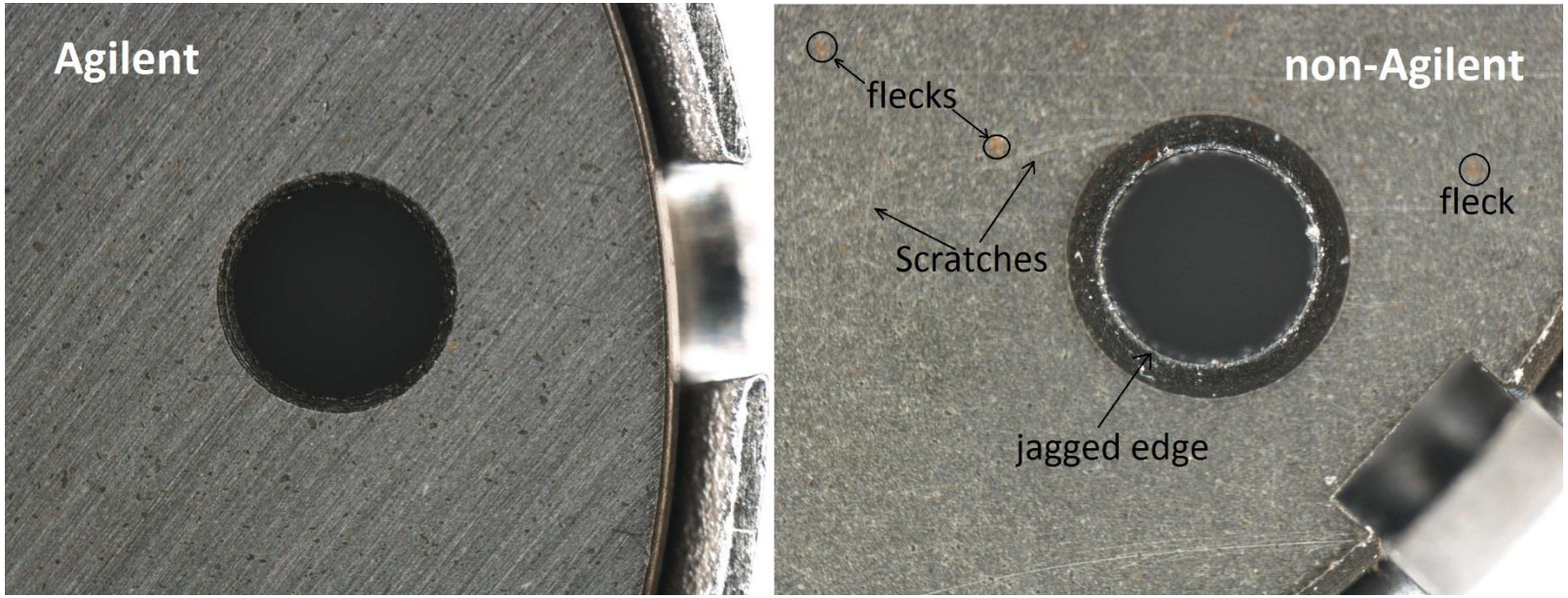


Packaging of injection needle

- Needle seat from other vendor still uses old design.
- Agilent developed new design with more robust material, improved performance, higher reliability and larger pH range (0-13).
- Needle tip from Agilent is protected by ultraclean plastic cap to avoid collision, abrasion, contamination and blockage from particulates.

Rotor Seals
surface smoothness

p/n: 0100-1853

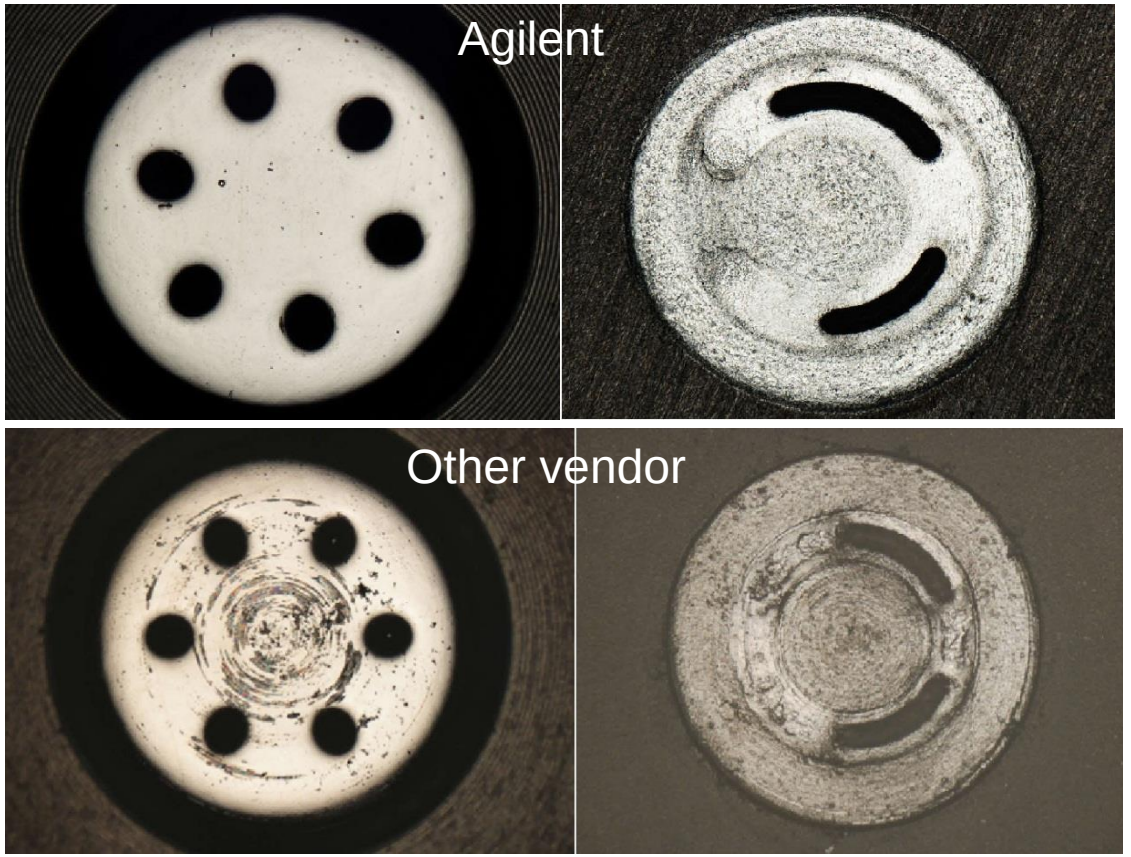


Photomicrographs of rotor seal from Agilent and other vendor

- Agilent rotor seal shows smooth flat surface while scratches, flecks and jagged hole edge were observed on third-party seal.
- Surface deficiencies can affect sealing ability resulting in leakage or sample carryover.

Rotor Seals
life time

p/n: 0100-1853



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

- After 30000 switches Agilent rotor surface still appears flat and consistent, and the contacting stator surface appears clean.
- Rotor seal from other vendor showed severe surface damage and contaminated stator surface after 26000 switches.

Rotor Seals
packaging

p/n: 0100-1853

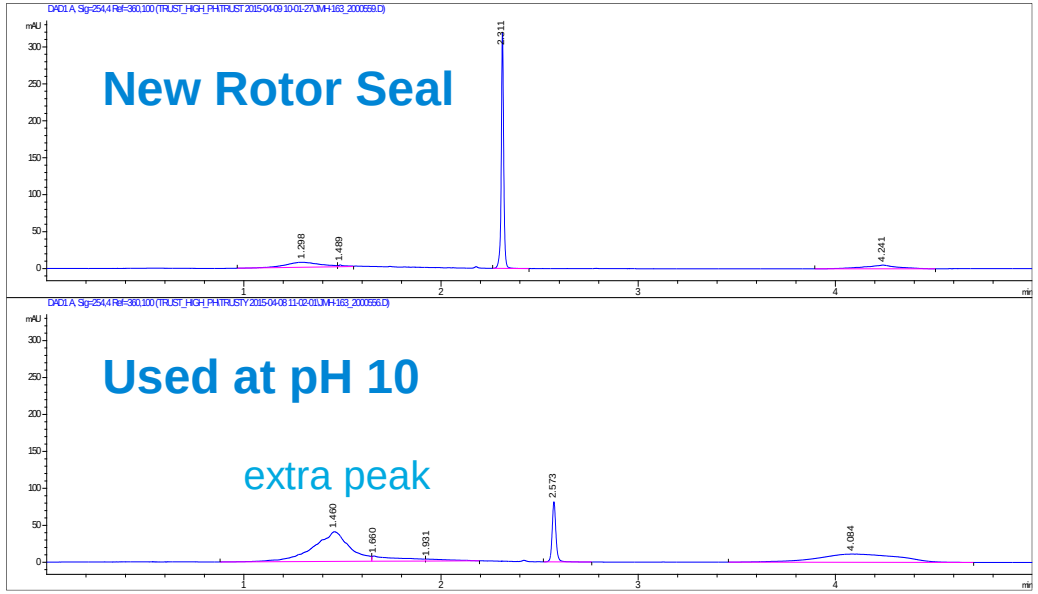
Agilent

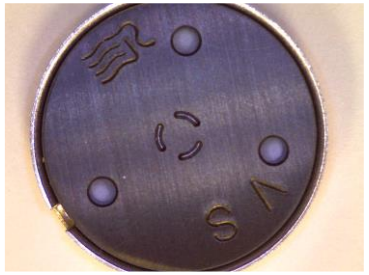
shape-stable plastic boxes to avoid surface damage and deformation during storage and transportation

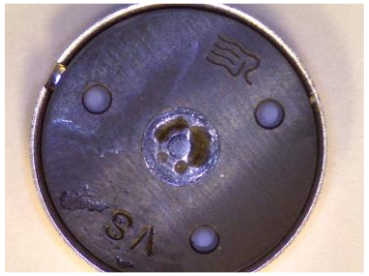
Other vendor

Normal plastic bags without special protection

Rotor Seal Choice







Vials

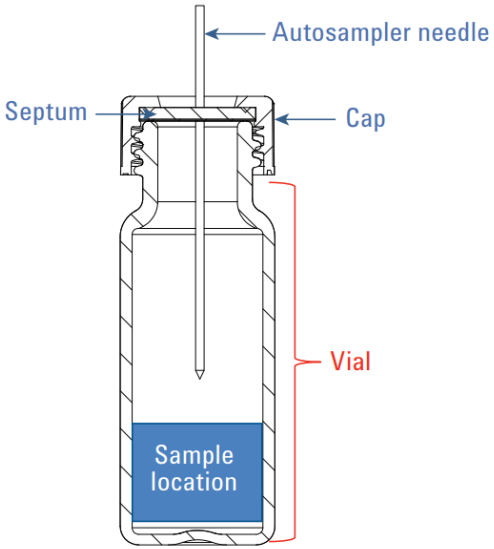
5991-6769EN
5990-9022EN

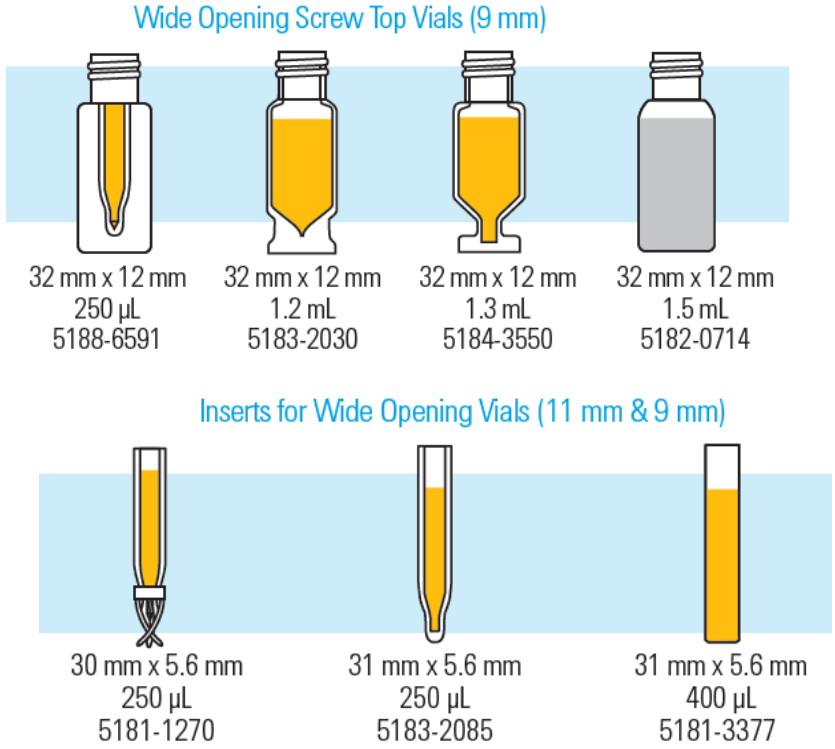
33/51 Best in glass: All vials are made of type 33-51 coefficient of expansion for top performance

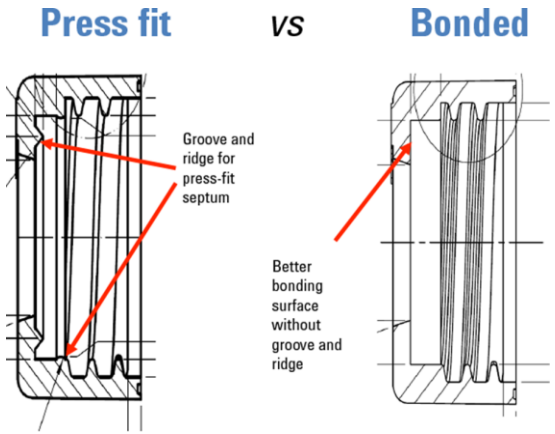
30+ Inspection points. So you get the tightest dimensional specifications, every time

Choose wide opening vials (9 mm) for Agilent autosamplers

- Choose Agilent Certified vials
  - Tested for full compatibility
  - Vial neck and shoulder are proper height
  - Competitors do not meet our exact specifications
  - Choose bonded caps to prevent septum push-through







Filtration

In-Line Filters

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

1290 Infinity II LC in-line filter, 0.3 µm -
- 1300 bar, *5067-6189*

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

Detector Care
UV Detectors

Two types

- VWD
- DAD/MWD

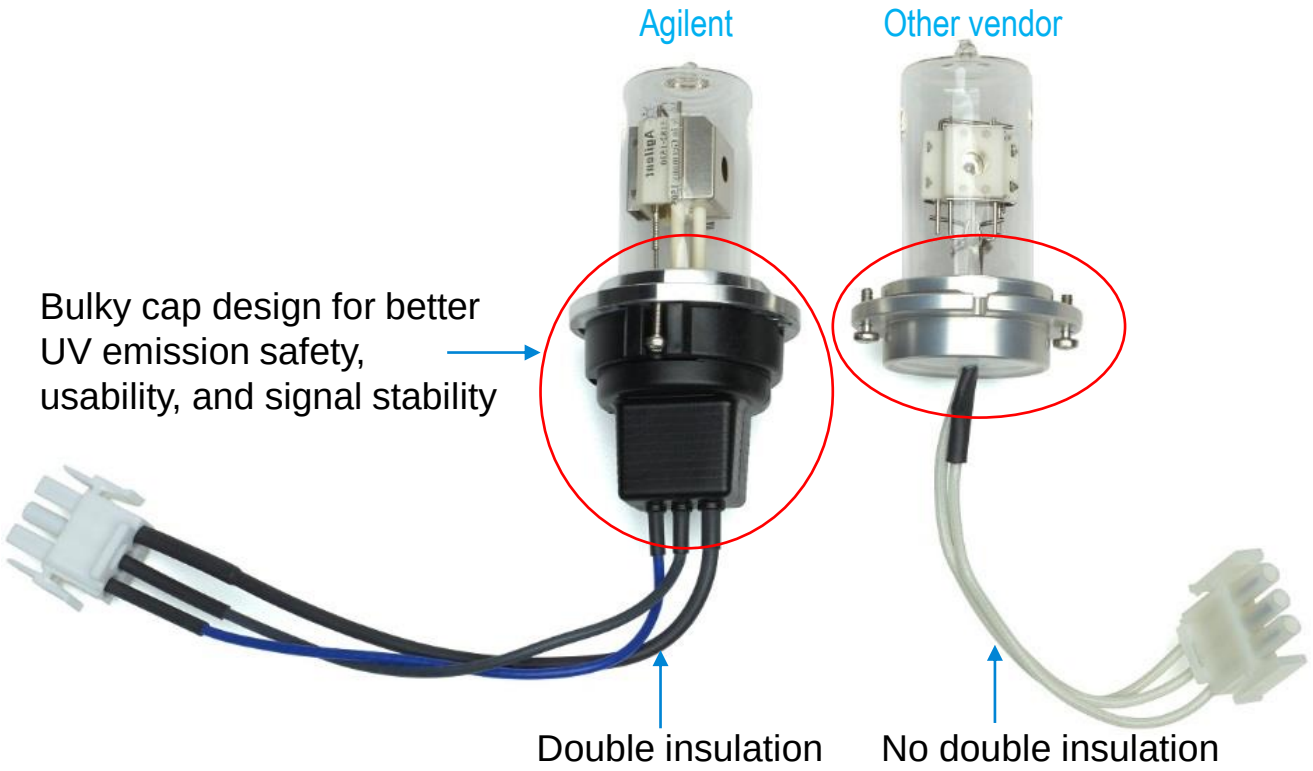
Simple Maintenance

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

Deuterium lamps inner design

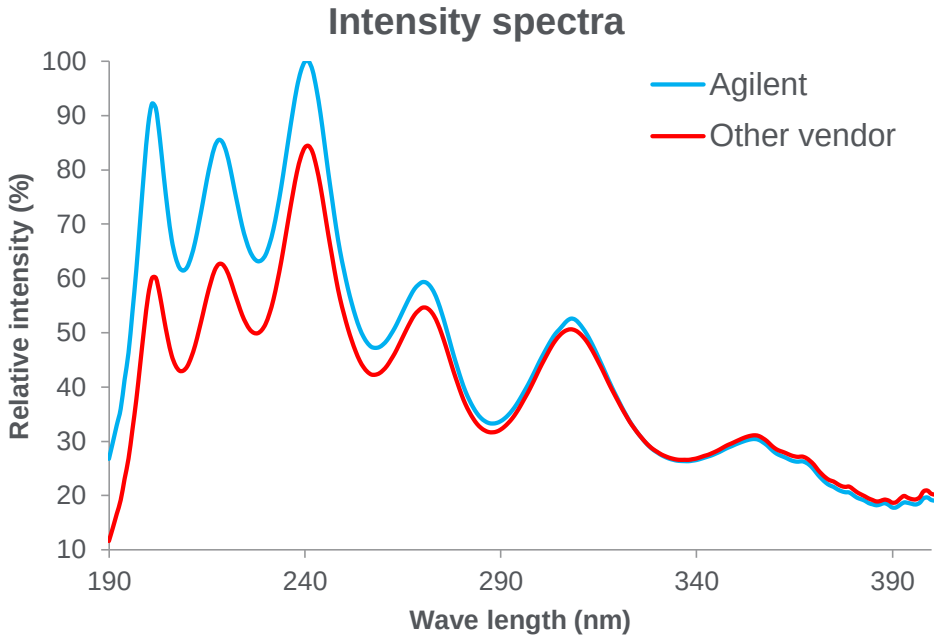
- Proprietary design of Agilent lamps provides higher S/N ratio and better stability against vibration or shaking.
- Agilent lamps are perfectly aligned to optical configuration of the detector.

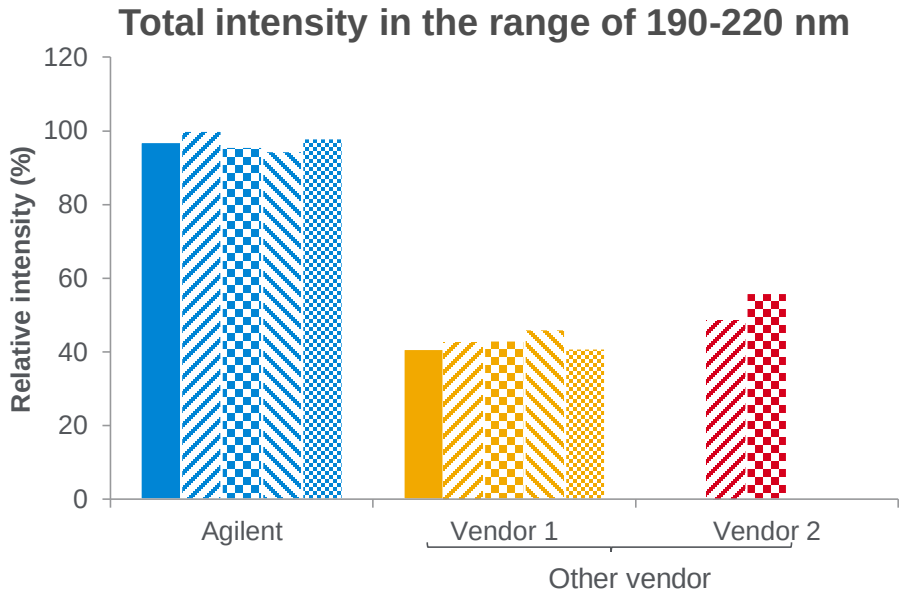
Deuterium lamps
cap and cable design



- Agilent´s bulky cap design blocks light emission and protects users from exposure to UV, leads to better temperature stability, and can be used as handle to simplify installation and deinstallation.
- Double-insulated cable meets stringent safety regulations, protecting users from potentical electrical shock through exposed wires.

Deuterium lamps
inital intensity





- Agilent lamps deliver higher intensity in the whole wavelength range, especially in the lower wavelength, up to 60% higher intensity from 190 to 220nm than lamps from other vendors.
- High initial intensity is crucial for long lamp life time and high S/N ratio.

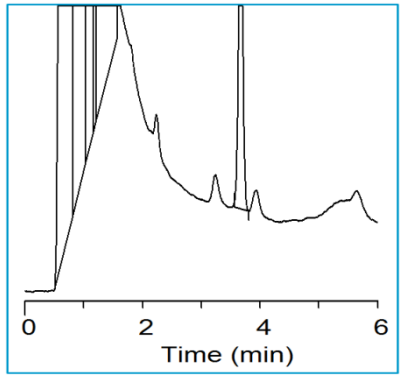
Sample Clean-Up

Filtration, Solid Phase Extraction, QUECHERS, and more!

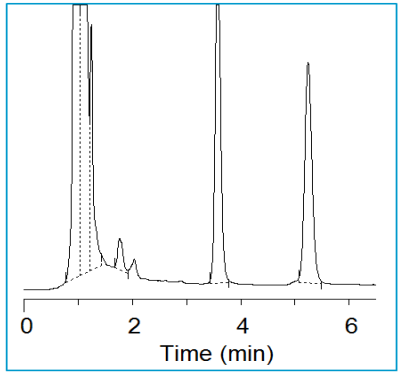
Why Perform Sample Clean-Up?

- To acquire desired sensitivity/selectivity
- To reduce contamination/carryover issues
- Use of sensitive and expensive instruments: *Protect your investment!!!*

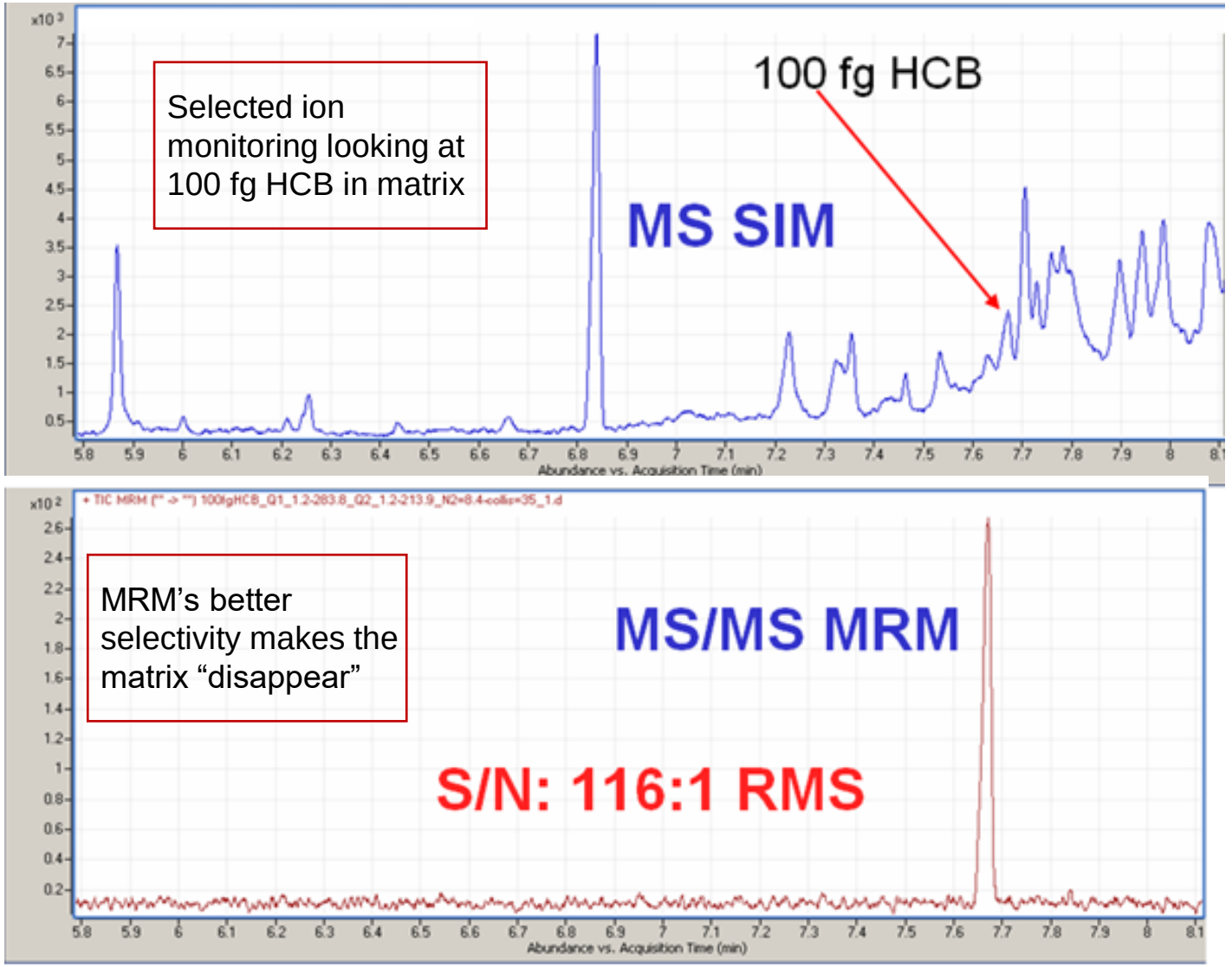
Pesticides in Avocado *without* sample clean-up



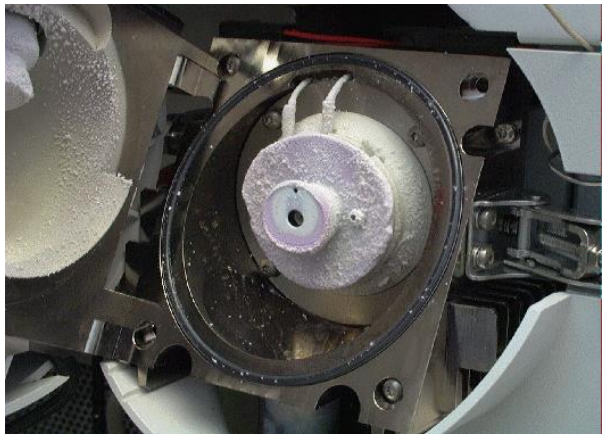
Pesticides in Avocado with sample clean-up



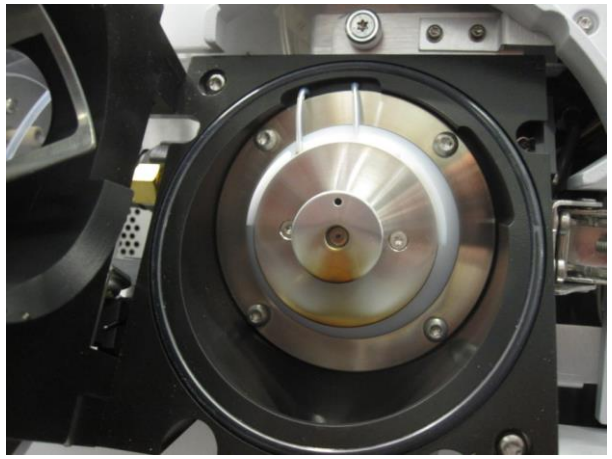
Tandem Mass Spectrometry and "The Case of the Disappearing Matrix"



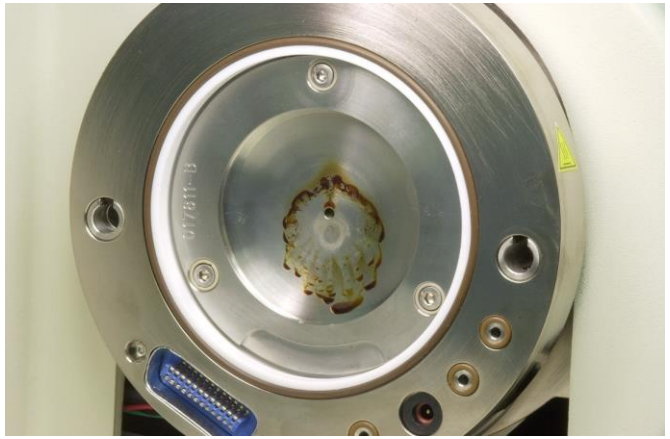
Instrument Contamination



Salt build-up in LC-MS ion source from unextracted salts



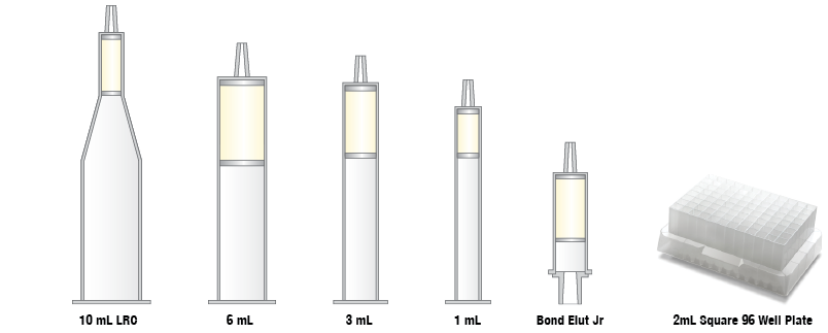
ESI Ion Source contamination after 3000x Urine Dilute/Shoot Injections



Curtain plate after injection of 25 samples with extractions from raisins without cleanup

Sample Clean-up Tools to Help you Survive

Solid Phase Extraction Cartridges and Plates



QUECHERS

Syringe Filters

Filtration Cartridges and Plates

Captiva EMR Lipid

Captiva Filtration and it's Benefit

Filtration is basic sample preparation method for all kinds of samples

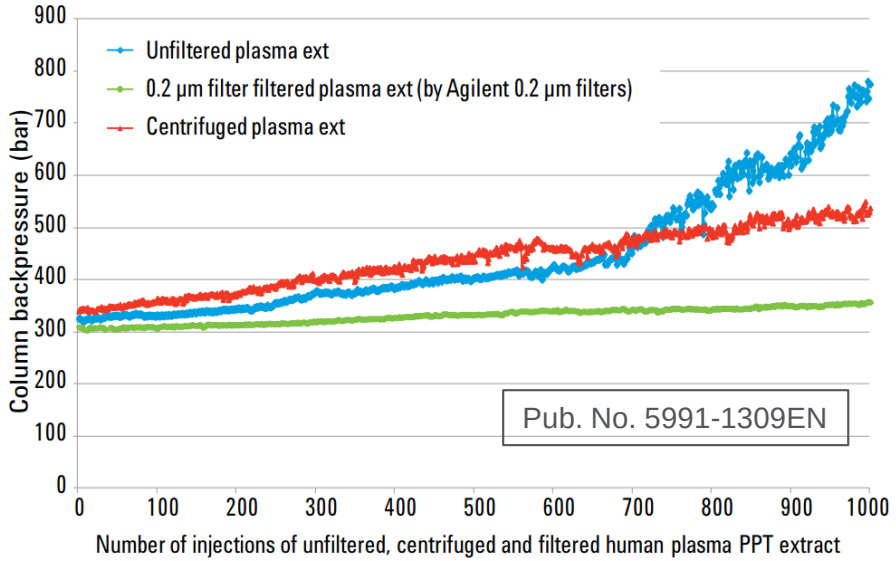
Physically removes particulates from the sample

Prevents blocking of capillaries, frits, and column inlet (especially for UHPLC)

Results in less downtime of the instrument for repairs

Results in less wear and tear on the critical moving parts of the injection valves

96-well plate formats available



Unfiltered, centrifuged, and filtered plasma extracts
Zorbax RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm column, PN 959757-902

Captiva Syringe Filters Guide 5991-1230EN

Syringe Filter Selection Tool

Captiva EMR-Lipid

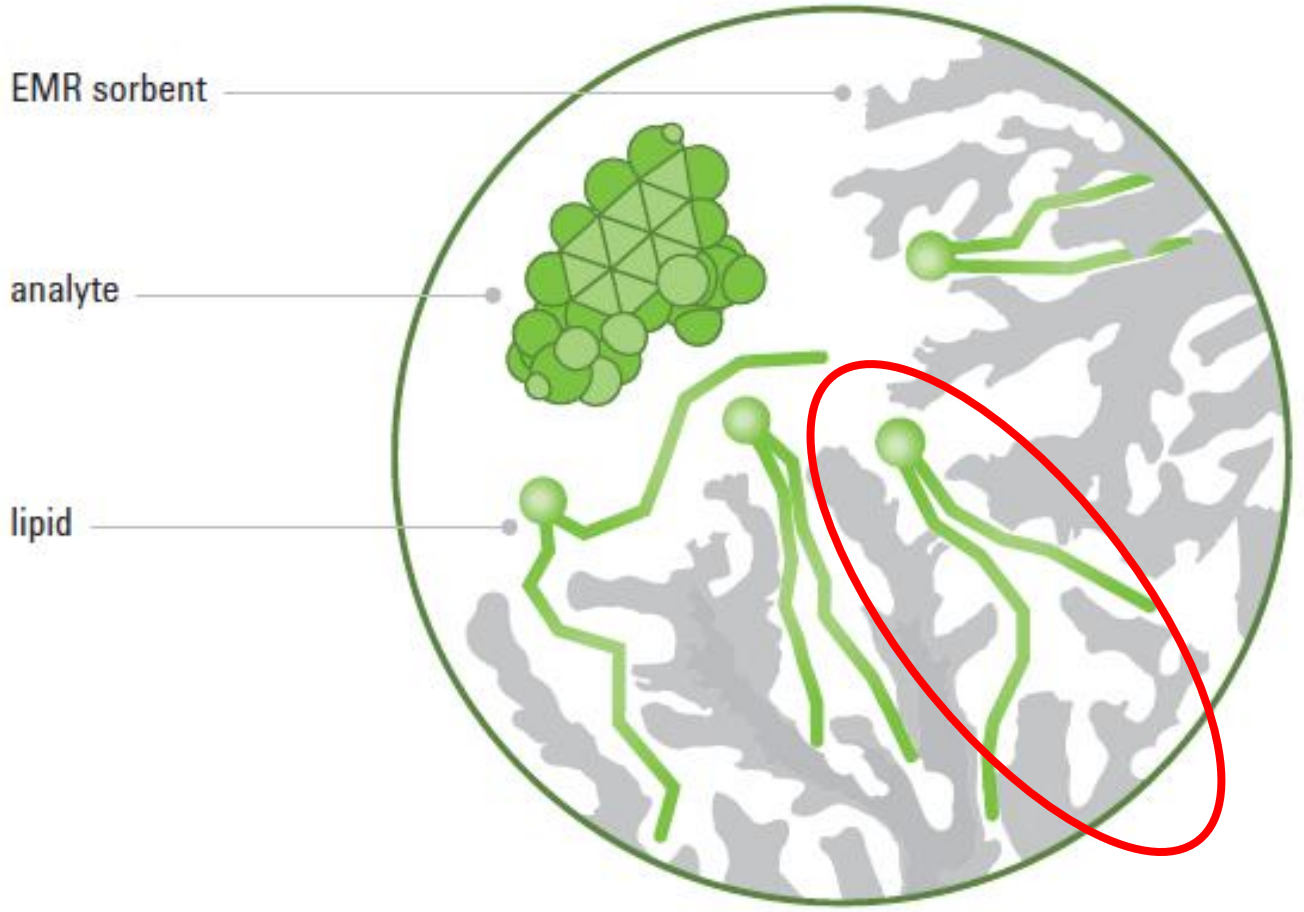
The Analytical Scientist Innovation Awards 2017

- One of Agilent's newest products with a 2 in 1 benefit of removing proteins and lipids
- Simple pass-through format
- Solvent-retention frit in 1 mL cartridge/96-well plate format for in well protein precipitation (*in situ*)
  - Unique cartridge/well construction minimizes clogging – and **ensures protein and lipid removal** (no cloudy samples)
- 3 and 6 mL cartridge format for larger samples
  - Do not contain solvent retain frit which allow for gravity flow
  - Protein precipitation performed offline (QUECHERS, etc.)
- Unique cartridge/well construction minimizes clogging – and **ensures protein and lipid removal** (no cloudy samples)
- High analyte recoveries
- Effective use will reduce ion suppression, increase analyte sensitivity, and detection, and extend the lifetime of your analytical column

What is it??

EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

- ✓ **Size exclusion** – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- ✓ **Sorbent chemistry** – Lipid chains that enter the sorbent are trapped by hydrophobic interactions

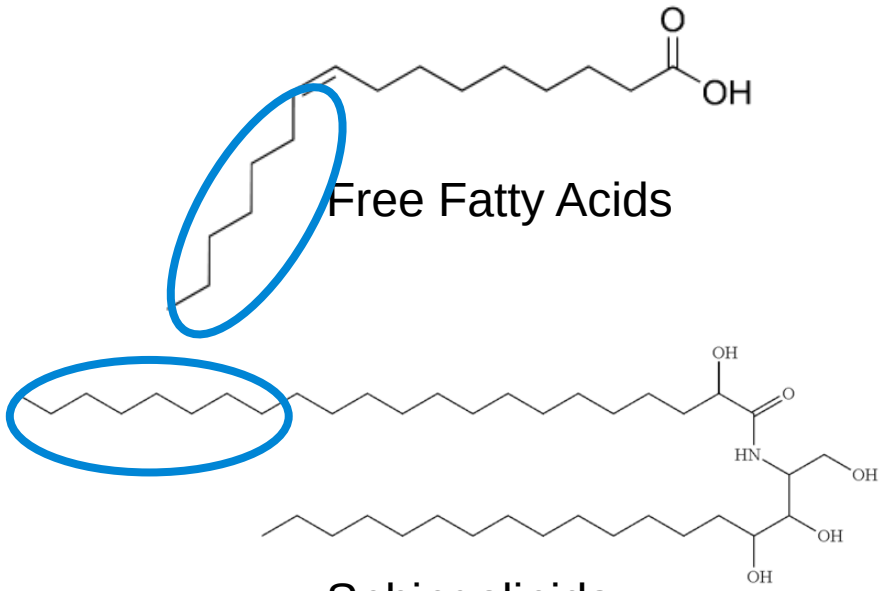


… and what does it do?

EMR-Lipid sorbent removes Lipids

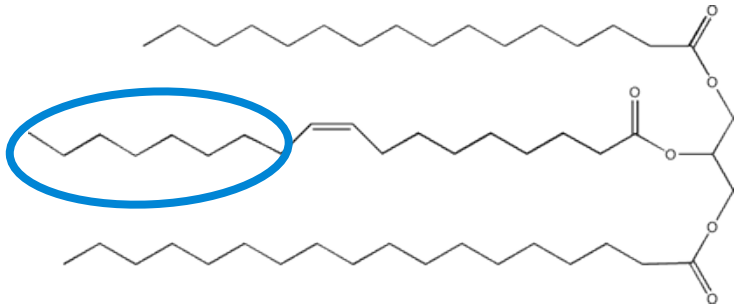
What are Lipids?

A class of naturally occurring hydrocarbon containing compounds commonly known as fats and oils

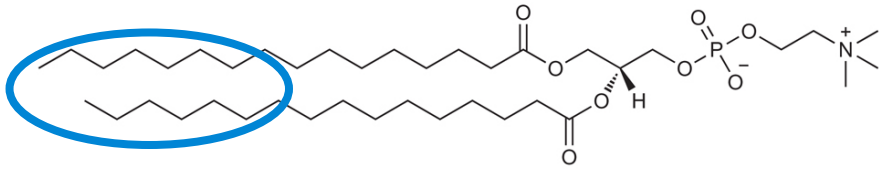


Free Fatty Acids

Sphingolipids



Triglycerides



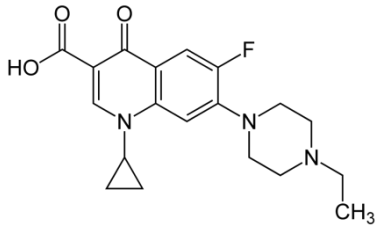
Phospholipids

What Does EMR-Lipid *NOT* Interact With?

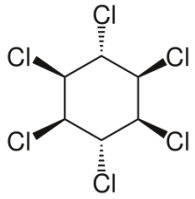
EMR-Lipid does NOT remove analytes of interest

Exceptions?

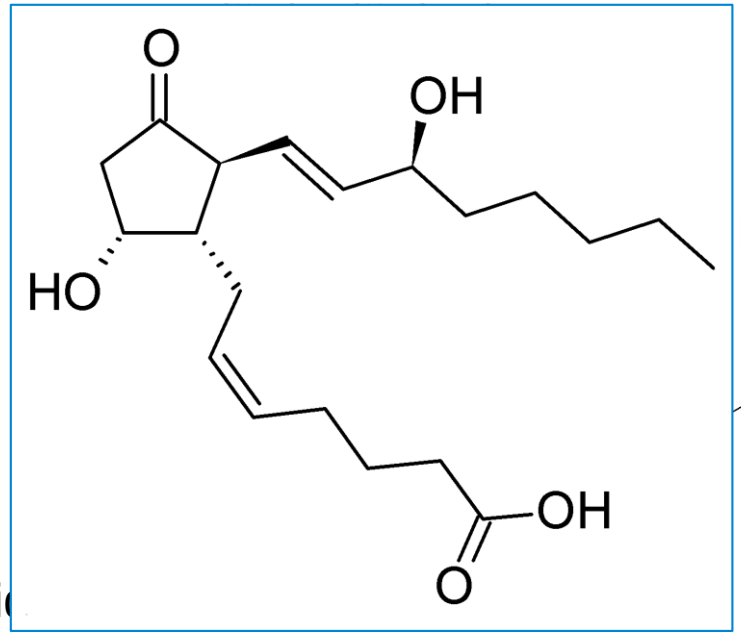
Compounds containing unbranched carbon chains (e.g. prostaglandins)

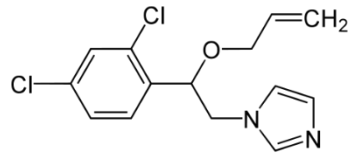


Fluoroquinolones

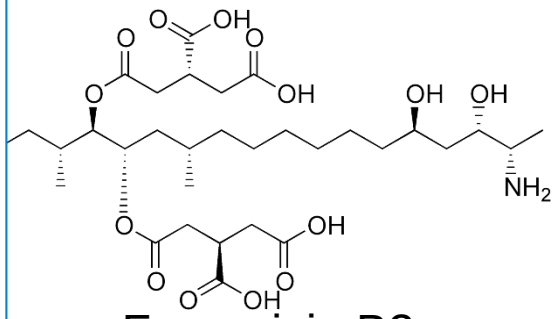


Organochlorine Pesticid



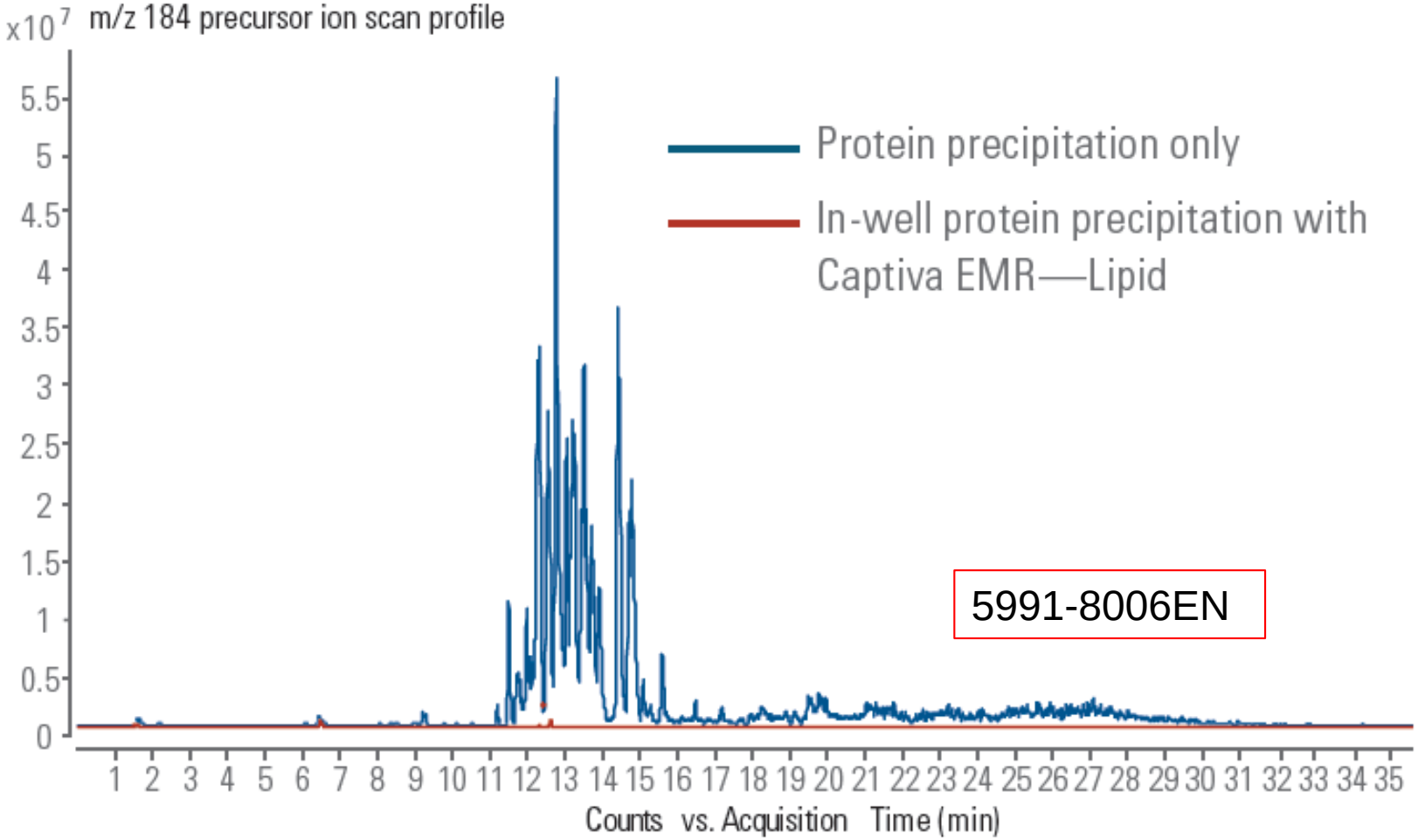


Imidazole pesticides



Fumonisin B2

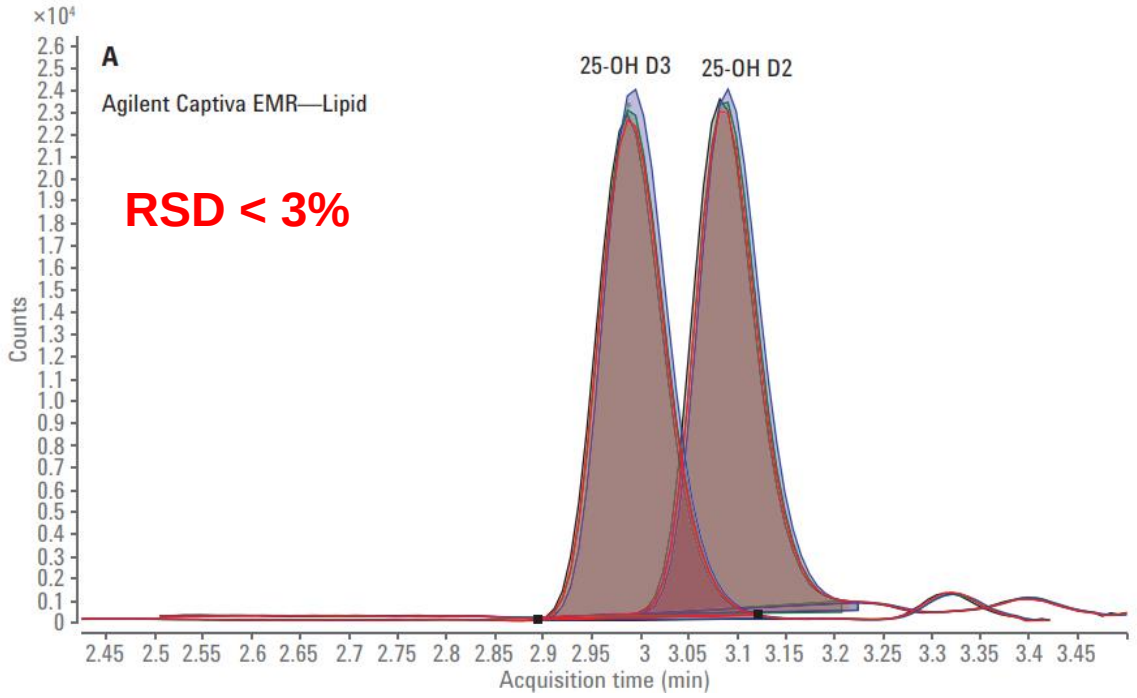
Effective phospholipid removal



5991-8006EN

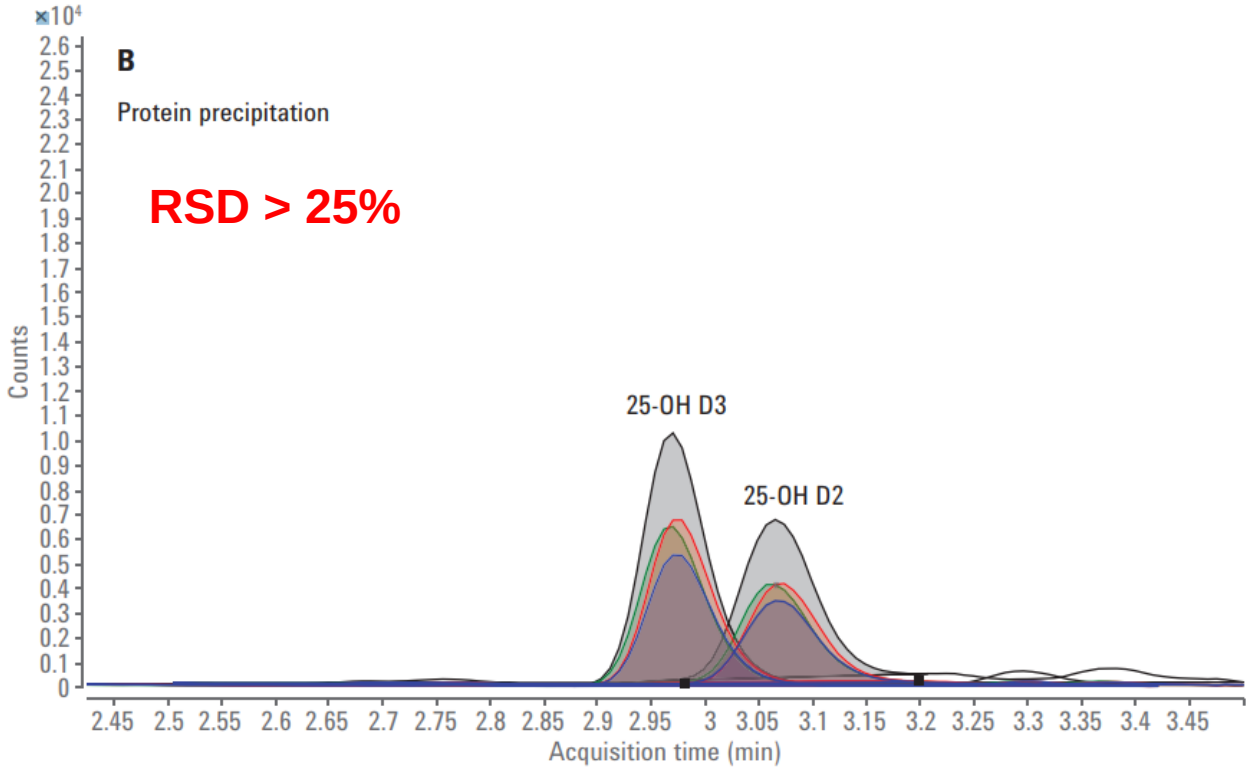
Captiva EMR-Lipid vs. Protein Precipitation RSD and Peak Area

Captiva EMR-Lipid



RSD < 3%

Protein Precipitation



RSD > 25%

Lipids cause reproducibility problems resulting in high RSD values

Using Captiva EMR-Lipid → low RSD values and higher peak areas

Higher peak area due to less ion suppression → can lead to lower detection limits

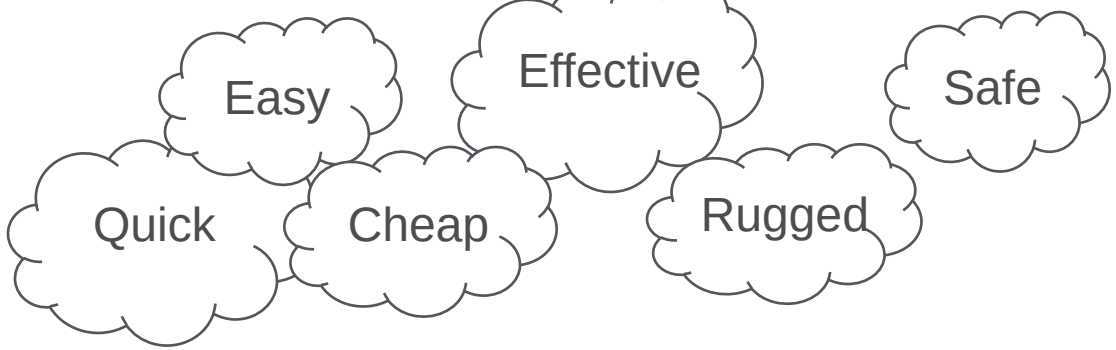
Processing 96-Well Plates and Cartridges

Captiva Vacuum Collar

Vac-20 vacuum manifolds

96 well plate vacuum manifold

Positive Pressure Manifolds



Screening of pesticide residues in fruit and vegetables

- Developed to make sample cleanup of food faster, simpler, less expensive, and greener

Now used with other matrices and compound classes as well

Consists of two steps, and thus **2 kits**:

Step 1: Liquid Extraction

Step 2: Dispersive SPE / Interference Removal

Bond Elut Dispersive SPE Kits

Dispersive kit contains:

Centrifuge tubes containing pre-weighed SPE sorbents such as:

C18: removes residual fats and lipids

PSA: 'primary/secondary amine' for removal of organic acids and sugars

GCB: graphitized carbon black, removes pigments

EMR-Lipid: removes unbranched hydrocarbon chains (lipids)

Kits available for different food types

For both AOAC (US) method and EN (Europe)

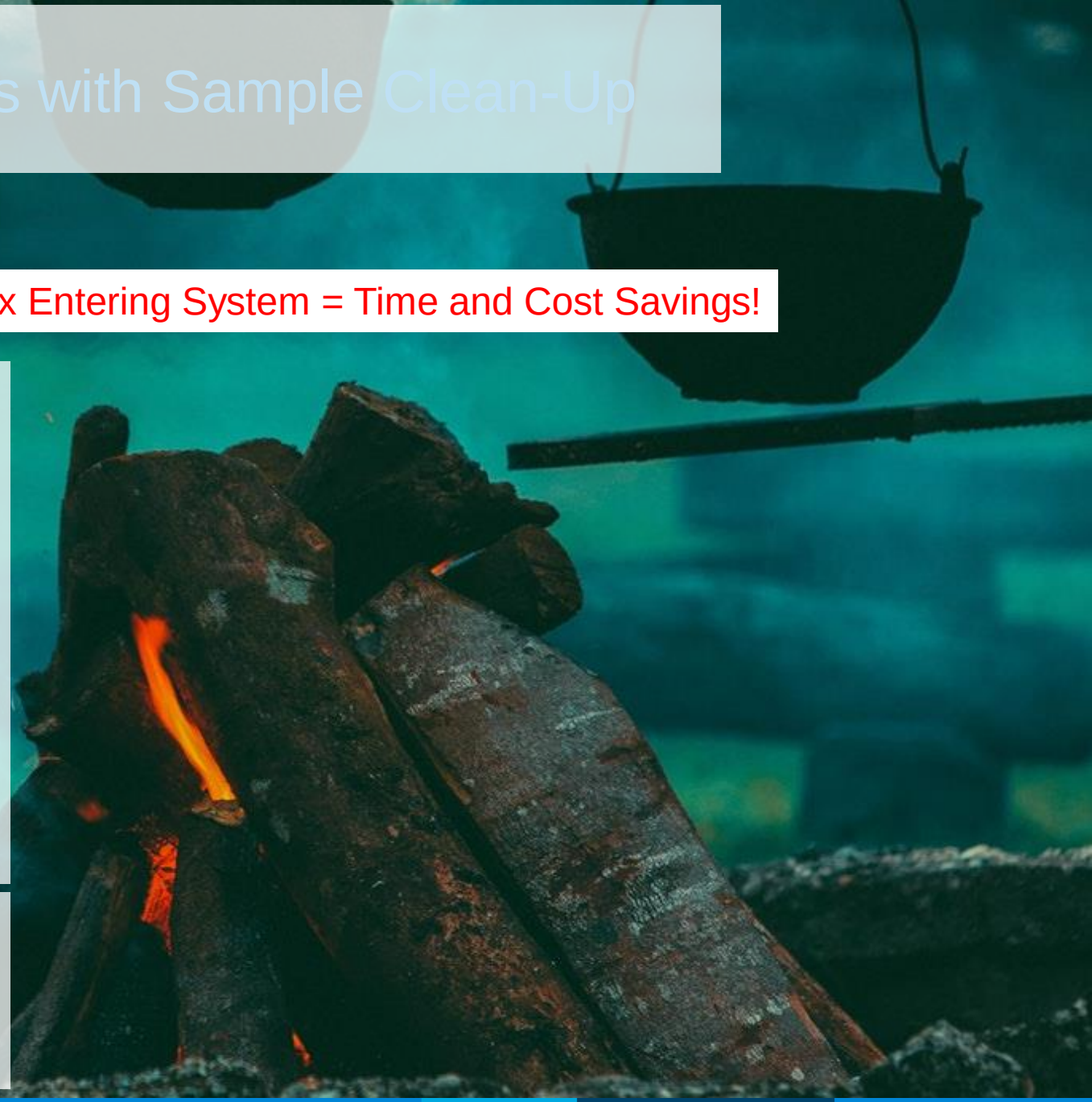
QUECHERS is a non-selective technique, does not remove ALL the matrix, but just enough

SPE sorbent also available as bulk material

Productivity Benefits with Sample Clean-Up

More Matrix Removal = Less Matrix Entering System = Time and Cost Savings!

- ✓ Less matrix build-up
  - Less interferences
  - Improved S/N
  - Better reproducibility
- ✓ Better chromatography
  - Less time spent on data analysis/manual integration
  - Less time spent on re-runs/recalibrations
- ✓ Less maintenance
  - Less instrument down-time
  - Saves $$ on consumables/services
- ✓ Less troubleshooting
  - “Is it my column or my MS”?
  - Less instrument down-time



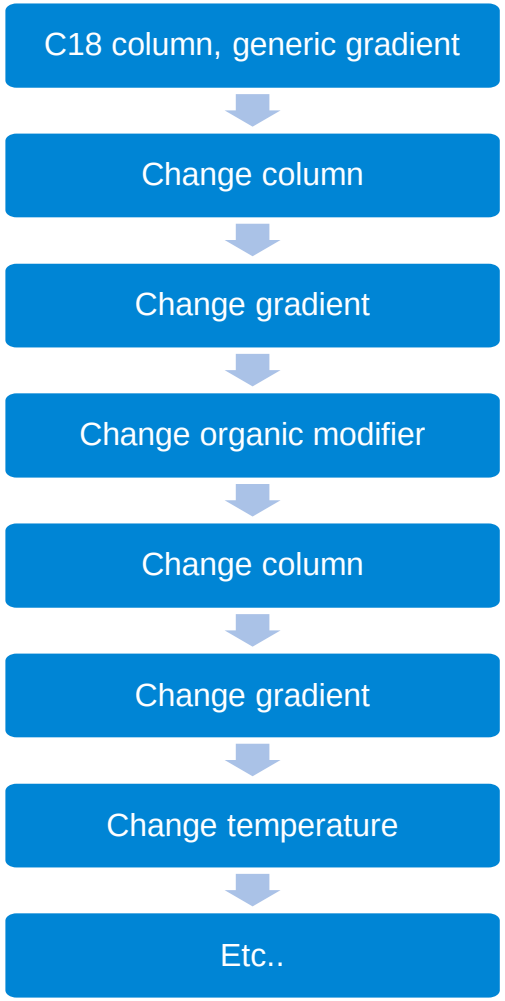
What Makes a Good Starting Point for RP Method Development?

1. Smaller particles and superficially porous particles offer fast, efficient analysis
2. C18 column – most general purpose column choice
3. Simple mobile phase
   a) Formic acid or other additive in aqueous portion (buffer salts only if necessary)
   b) Acetonitrile or methanol as organic modifier
4. Start with linear gradient (5% organic to 95% organic) for reversed-phase methods
5. Adjust mobile phase to get the desired retention and resolution
   a) Adequate resolution of all peaks, Rs ≥ 2.0
   b) Retention of first peak at least k=1
   c) Fastest analysis time with required resolution

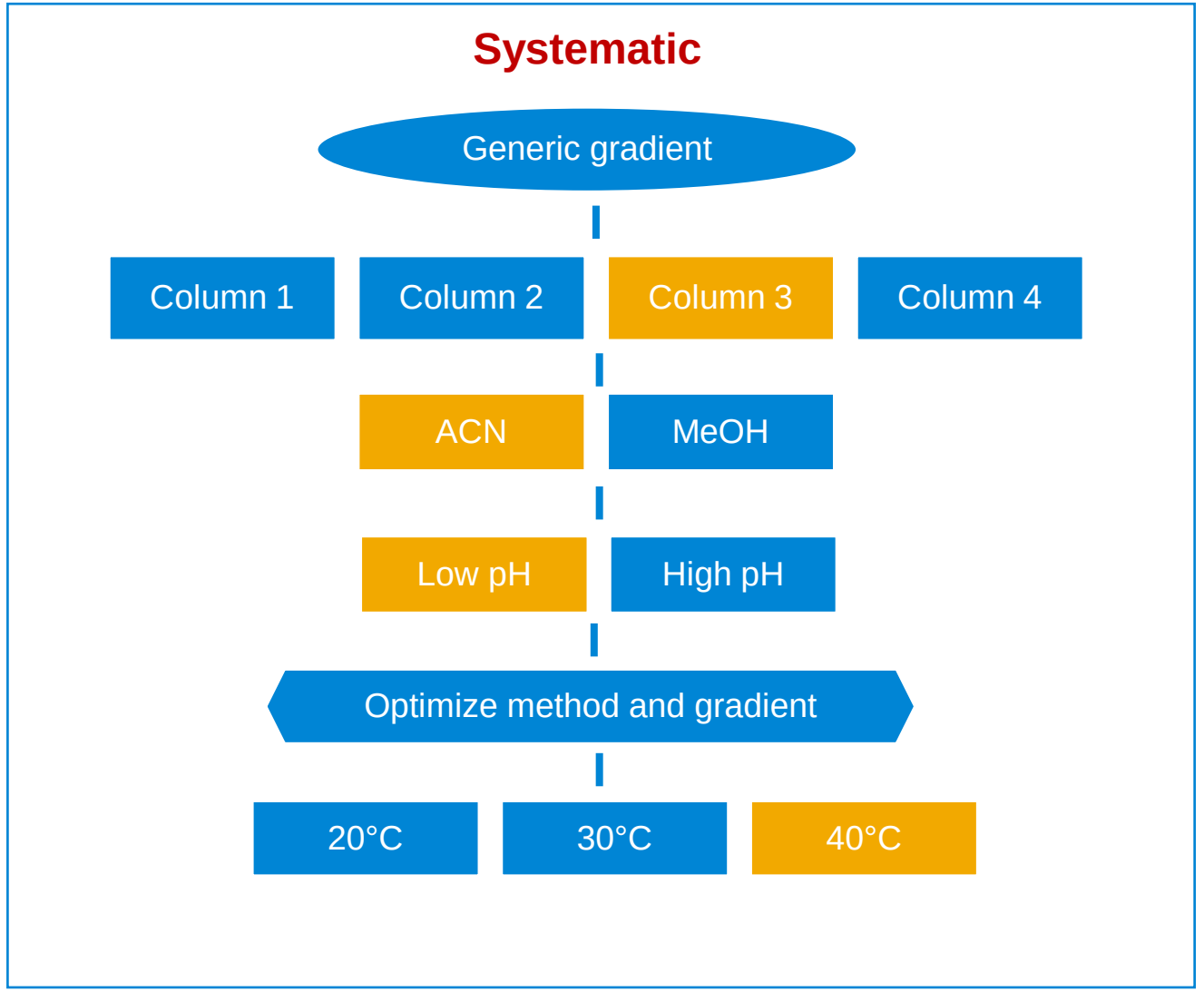
Newer shorter, columns with small particle sizes can provide more efficiency and resolution in a very short time, speeding up method development

What Type of Method Development Protocol is Best?

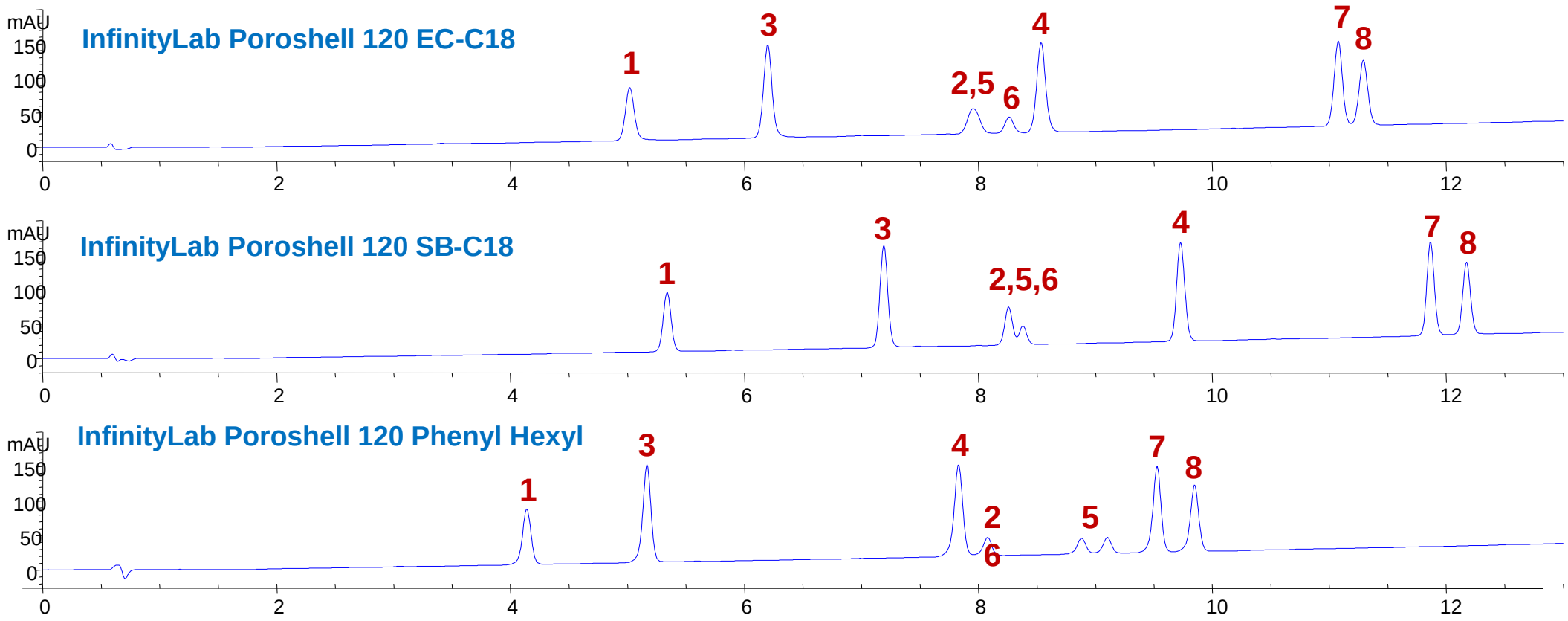
One factor at a time



Systematic



Selectivity Differences Across InfinityLab Poroshell Bonded Phases



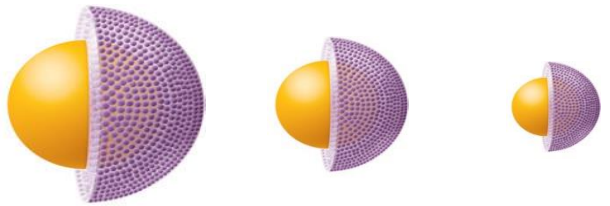
1. Hydrocortisone 2. B Estradiole, 3. Andostadiene 3. 17 dione, 4. Testosterone 5. Ethyestradione 6. Estrone 7. Norethindone acetate 8. Progestreone

40-80 % Methanol in 14 min, DAD 260, 80 nm 0.4 ml/min, 2.1 x 100 mm column, 40 C, 0.1% Formic Acid in Water and Methanol, Agilent 1260 Method Development Solution

Agilent InfinityLab Poroshell 120 Portfolio

start here

Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for polar Analytes	Best for Chiral
InfinityLab Poroshell **EC-C18** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **SB-C18** 2.7 µm	InfinityLab Poroshell **HPH-C18** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **Bonus-RP** 2.7 µm	InfinityLab Poroshell **HILIC** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **Chiral-V** 2.7 µm
InfinityLab Poroshell **EC-C8** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **SB-C8** 2.7 µm	InfinityLab Poroshell **HPH-C8** 2.7 µm, 4 µm	InfinityLab Poroshell **PFP** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **HILIC-Z** 2.7 µm	InfinityLab Poroshell **Chiral-T** 2.7 µm
			InfinityLab Poroshell **Phenyl-Hexyl** 1.9 µm, 2.7 µm, 4 µm	InfinityLab Poroshell **HILIC-OH5** 2.7 µm	InfinityLab Poroshell **Chiral-CD** 2.7 µm
			InfinityLab Poroshell **SB-Aq** 2.7 µm		InfinityLab Poroshell **Chiral-CF** 2.7 µm
			InfinityLab Poroshell **EC-CN** 2.7 µm		

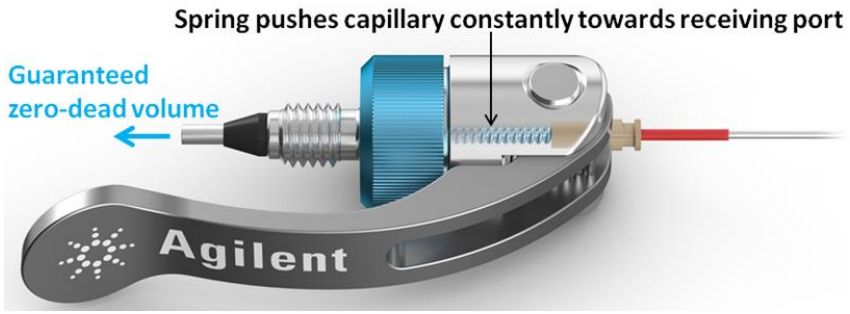


4µm **2.7µm** **1.9µm**

Reversed-phase chemistries

InfinityLab Quick Connect and Quick Turn Fittings

- Spring loaded design
- Easy! **No tools needed**
- 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

Tips for Robust Methods

- Always start method development with a new column
- Select columns with robust properties at pH of method
- Choose a quality column with long lifetimes
- Consider batch to batch reproducibility
- Consider scalability of particle sizes and chemistries for downstream method transfer
- Make sure mobile phase preparation is documented and transferrable

Agilent employs end-to-end process control for quality LC columns

www.agilent.com/chem/qualitylc

Getting Started with a New Column

Performance Report

SERIAL NUMBER: USDAZ01333

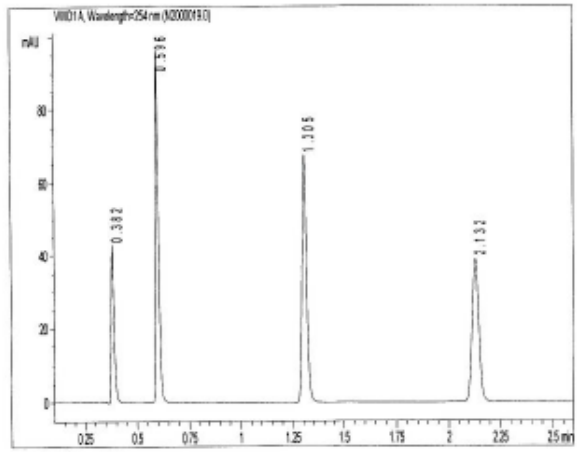
PART NUMBER: 959758-902
COLUMN TYPE: ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm
PACKING LOT #: B09089

TEST CONDITIONS

MOBILE PHASE	=	60% Acetonitrile / 40% Water
COLUMN PRESSURE	=	517.2 Bar
COLUMN FLOW	=	0.50 ml / min
LINEAR VELOCITY	=	0.436 cm / sec
TEMPERATURE	=	AMBIENT (Nominally 23 °C)
INJECTION VOLUME	=	1 µl

QUALITY CONTROL PERFORMANCE RESULTS FOR NAPHTHALENE

	TEST VALUES	SPECIFICATIONS
THEORETICAL PLATES =	22337	MIN = 21000
SELECTIVITY =	1.90	RANGE = 1.82 - 1.92
USP TAILING FACTOR = (@ 5% Peak Height)	1.08	RANGE = 0.98 - 1.20
k' =	4.58	



Sample components with concentrations diluted in mobile phase in the following elution order.

Peak #	Conc (ug/ml)	Sample Component
1	10	Uracil
2	400	Phenol
3	50	4-Chloro Nitrobenzene
4	80	Naphthalene

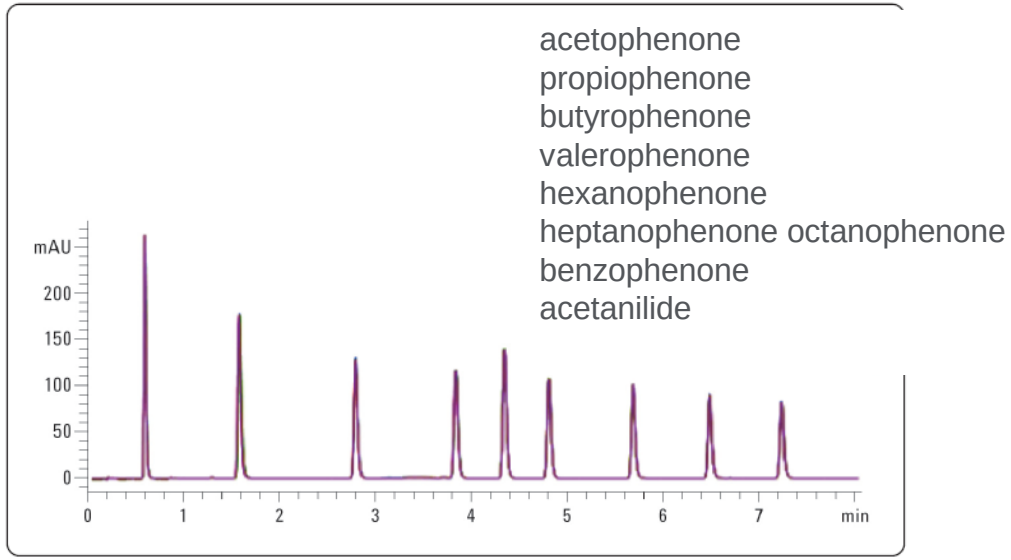
Manufacturing test chromatogram is done on a modified LC system to minimize ECV and will differ from a typical lab HPLC

- Don’t expect to get the exact same result as the performance report
- Test column performance on your instrument to have as a reference

Getting Started with a New Column
Benchmark

Benchmark new column on your system

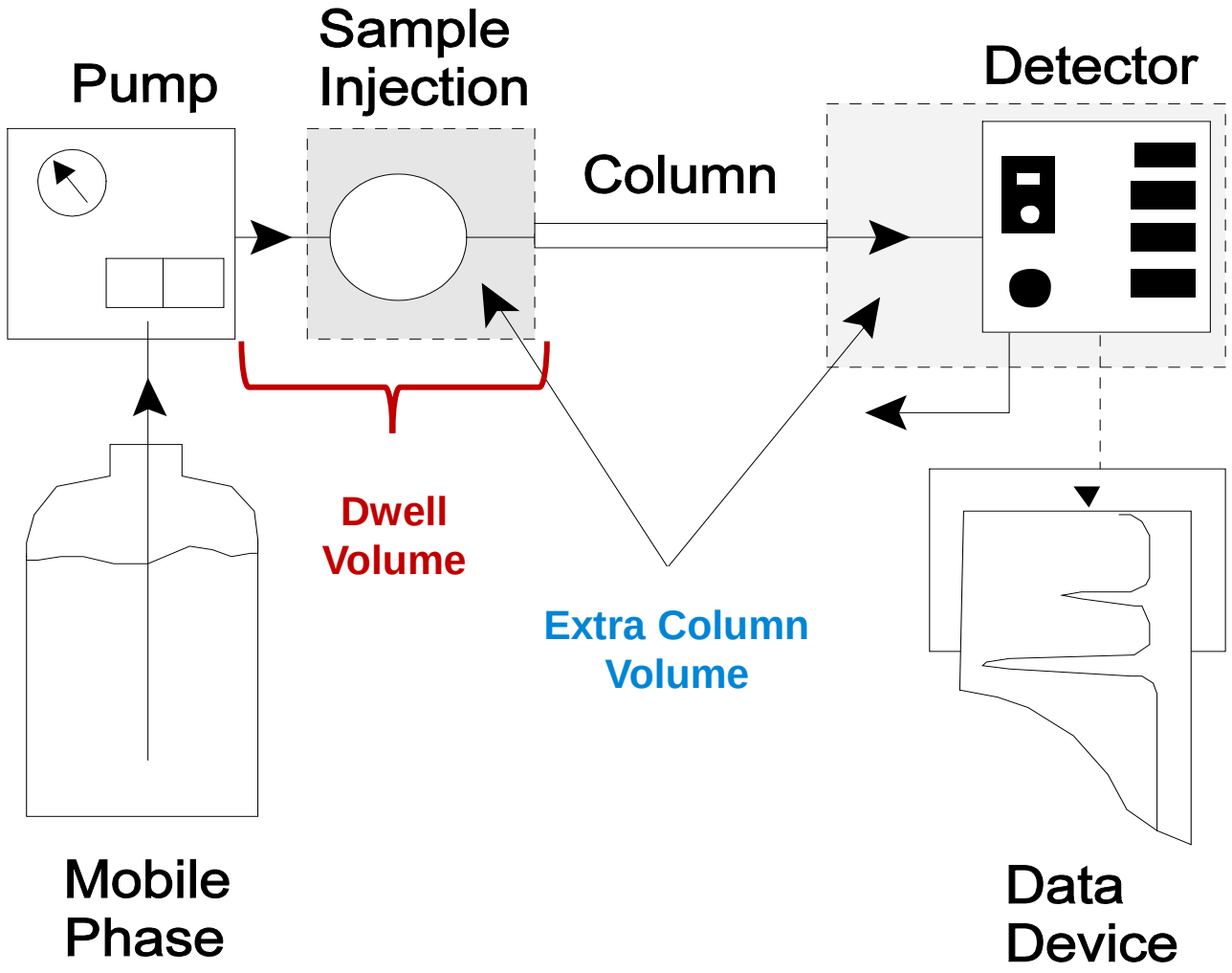
1. Standard mix; test mix (5188-6529, 01080-68704; QC reference material;
2. Criteria like retention time, peak area, peak tailing, resolution, response, system pressure, etc.
3. Theoretical plates
   - Monitor column over time
   - Troubleshoot



Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

Instrument Configuration



Dwell Volume: from formation of gradient to top of column

-minimize for faster equilibration and more efficient gradient formation

Extra Column Volume from injection to detector (flow cell) outside of the column

-minimize to reduce band broadening, for sharper peaks and better resolution

Dispersion Reduces HPLC Performance

What is dispersion?

- Original sample concentration being diluted as it is carried through the system plumbing (extra-column volume)

What increases dispersion?

- Connecting tubing that is too long
- Connecting tubing that is too large in diameter
- Connections that have gaps and form small mixing chambers

$$\sigma^2_{v,ext} = \frac{\pi\, d^4\, L_{cap}\, u_{cap}}{96 D_m}$$

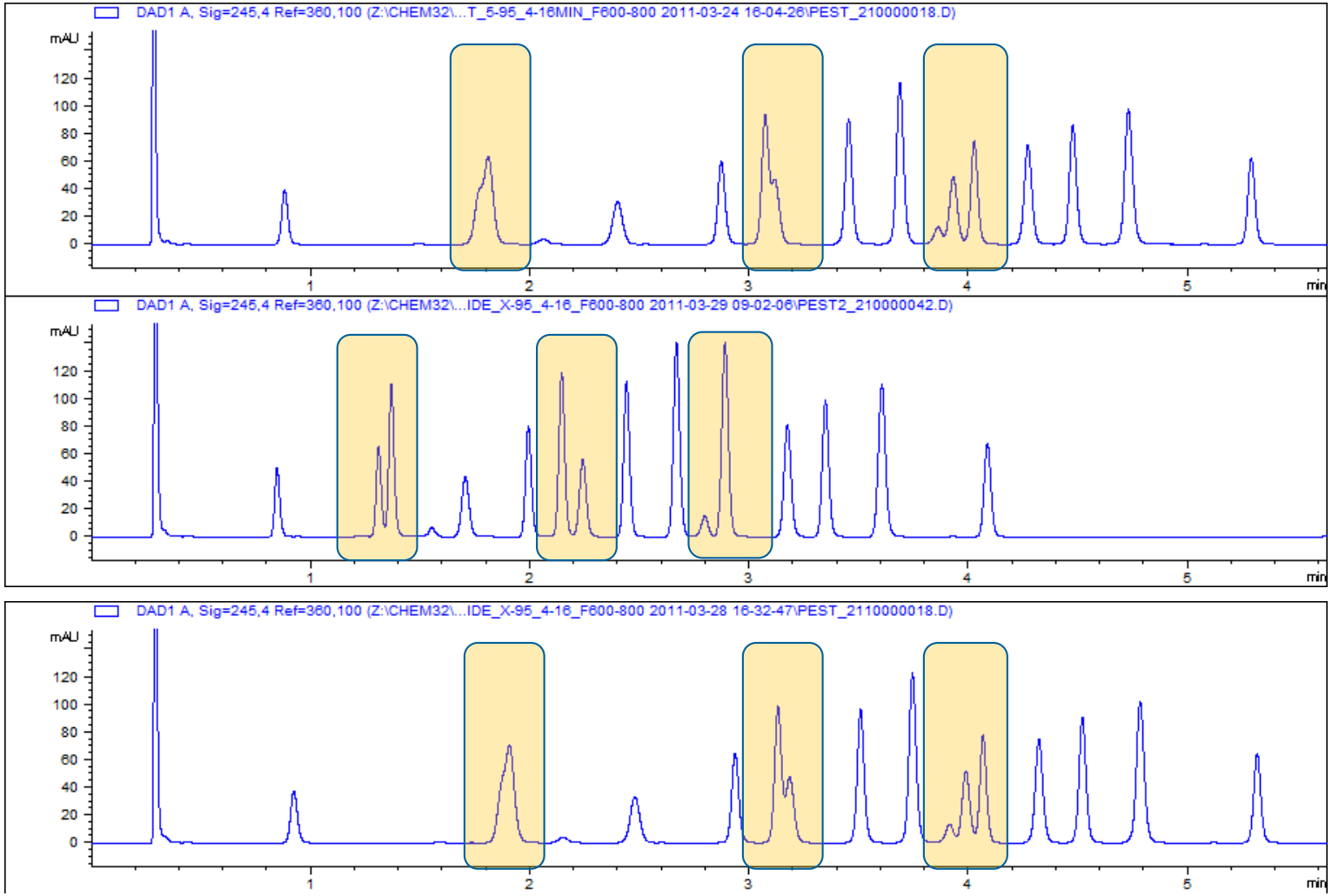
$\sigma^2_{v,ext}$	is the volume variance
d	is the tubing diameter
L	is the tubing length
u	is the linear velocity of the liquid
D_m	is the molecular diffusion coefficient

Instrument Dwell Volume Differences Can Cause Changes in Retention and Resolution

1100 Quaternary 400 bar

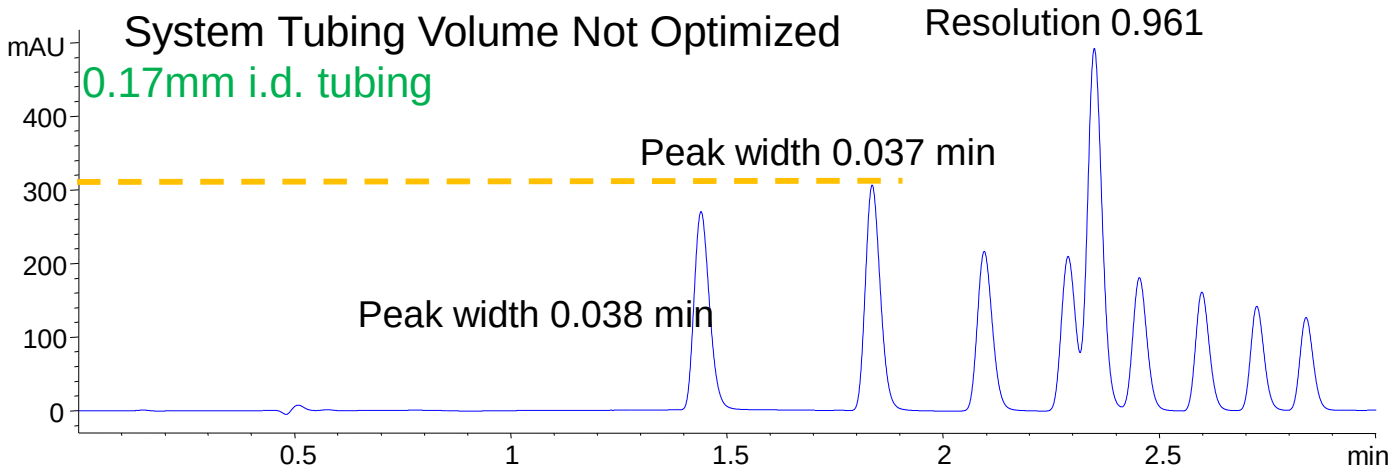
1290 Infinity II 1300 bar

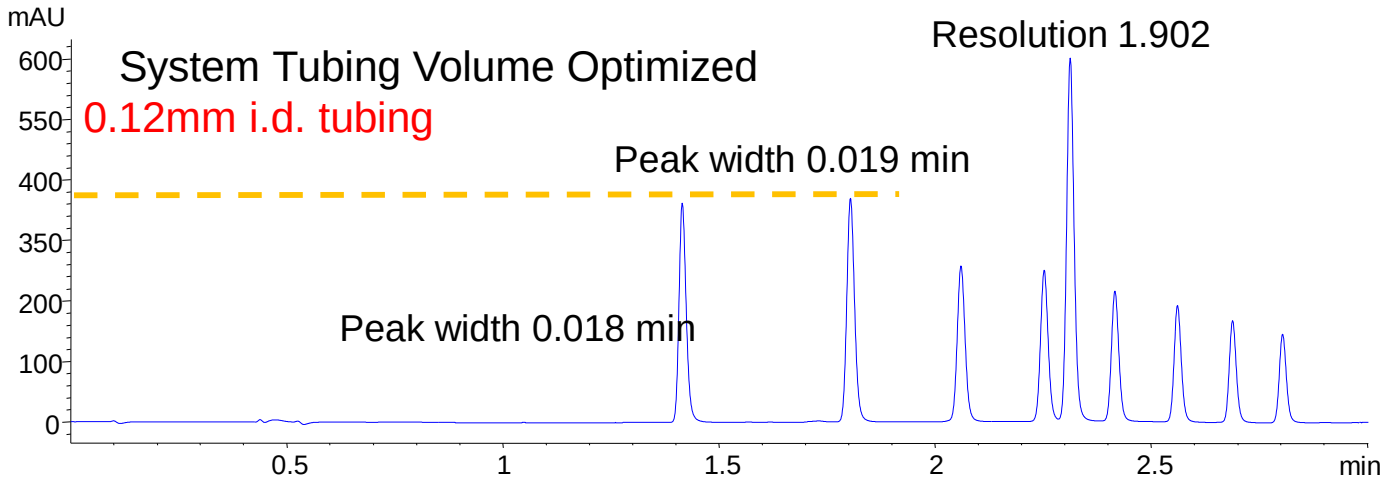
1290 Infinity II 1300 bar with ISET



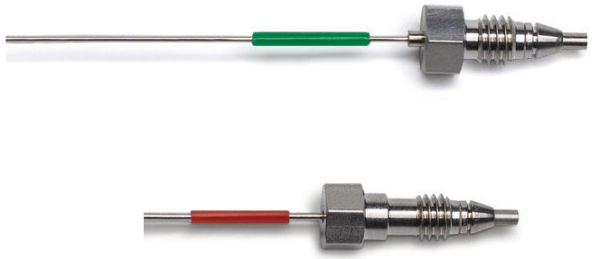
2.1x100 mm Zorbax Eclipse Plus, 1.8 µm column; Flow = 0.8 mL/min

Optimizing Connecting Tubing Volume For UHPLC Columns





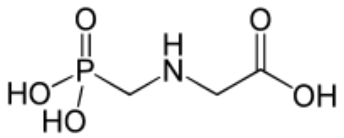
Length	10mm	50mm	100mm	150mm
Tubing ID	Volume	Volume	Volume	Volume
0.17mm (green)	0.227 uL	1.1uL	2.27 uL	3.3 uL
0.12mm (red)	0.113 uL	0.55uL	1.13 uL	1.65 uL



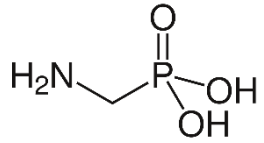
PEEK, Passivation, and Inert Hardware

- Steel has active sites that bind to certain classes of polar molecules
- **Most active molecules:**
  - Phosphorylated metabolites
  - Organophosphates and phosphonic acids
  - Di- and tri- carboxylic acids and similar chelating acids
- **Commonly seen in:**
  - Pesticide analysis (glyphosate, AMPA, glufosinate)
  - Fermentation (citric acid cycle, organic acids)
  - Metabolomics (Nucleotides, sugar phosphates, citric acid cycle)

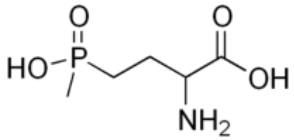
Organophosphates



Glyphosate

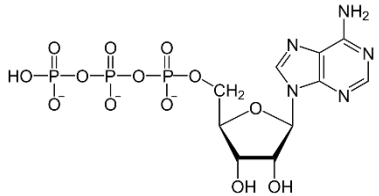


Aminomethylphosphonic acid (AMPA)

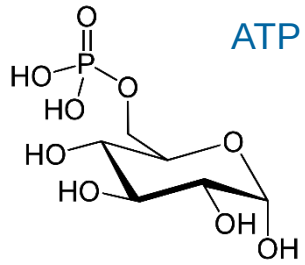


Glufosinate

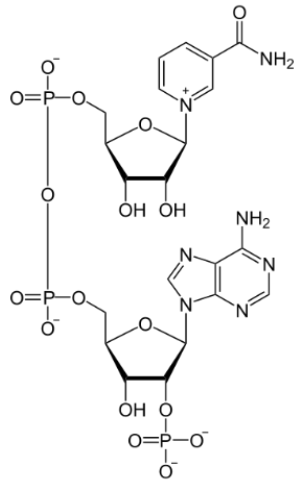
Phosphorylated Metabolites



ATP

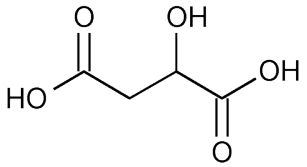


Glucose phosphates

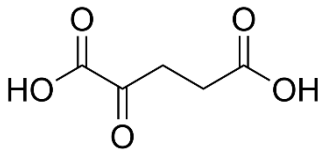


NADP

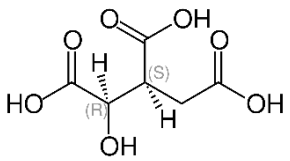
Chelating Organic Acids



Malic Acid



Alpha-Ketoglutaric Acid



Isocitric Acid

Eliminating Sticking with Wash Step

Example Analysis Conditions

Column: InfinityLab Poroshell 120 HILIC-Z, 2.1 x 50mm (PN: 689775-924)

Temperature = 30C

Injection Volume = 1 uL

Flow Rate = 0.25 mL/min

Mobile Phase

A = 10 mM Ammonium Acetate in Water at pH=9

B = 10% 100 mM Ammonium Acetate in Water at pH=9 + 90% Acetonitrile*

Total Ionic Strength – 10 mM for both mobile phases

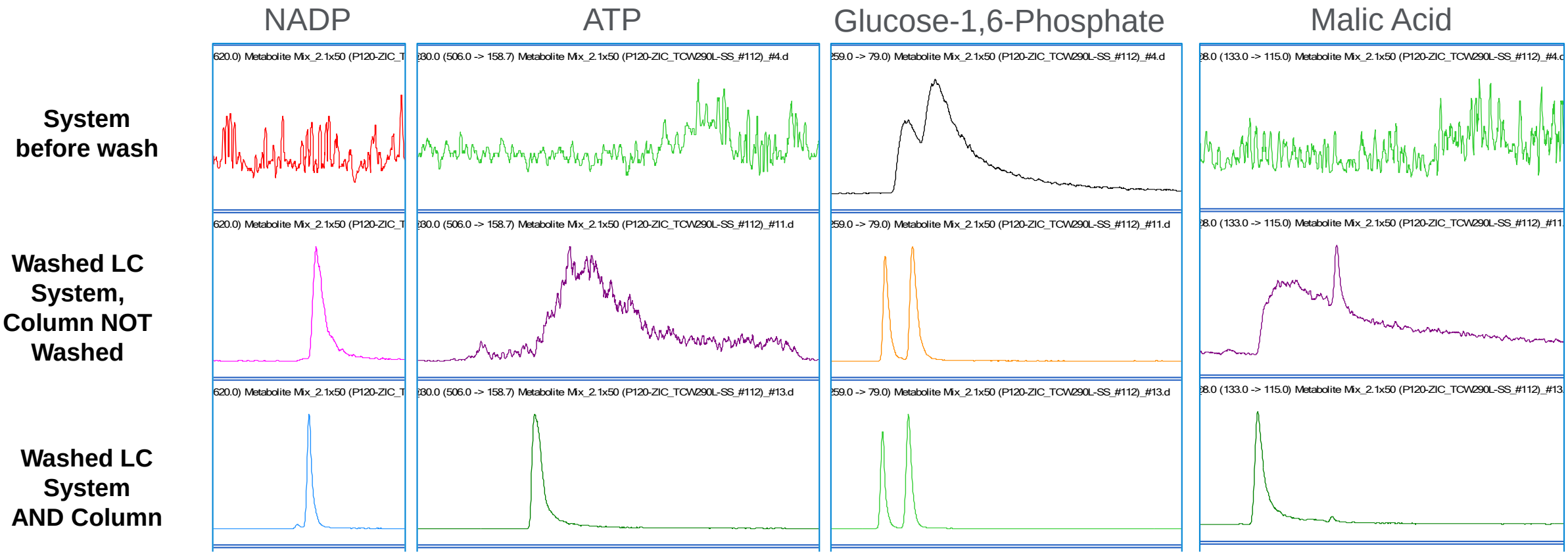
Gradient

Time (min)	Percentage A	Percentage B
0	10	90
2	10	90
12	40	60
13	10	90
21	10	90

Wash Procedure

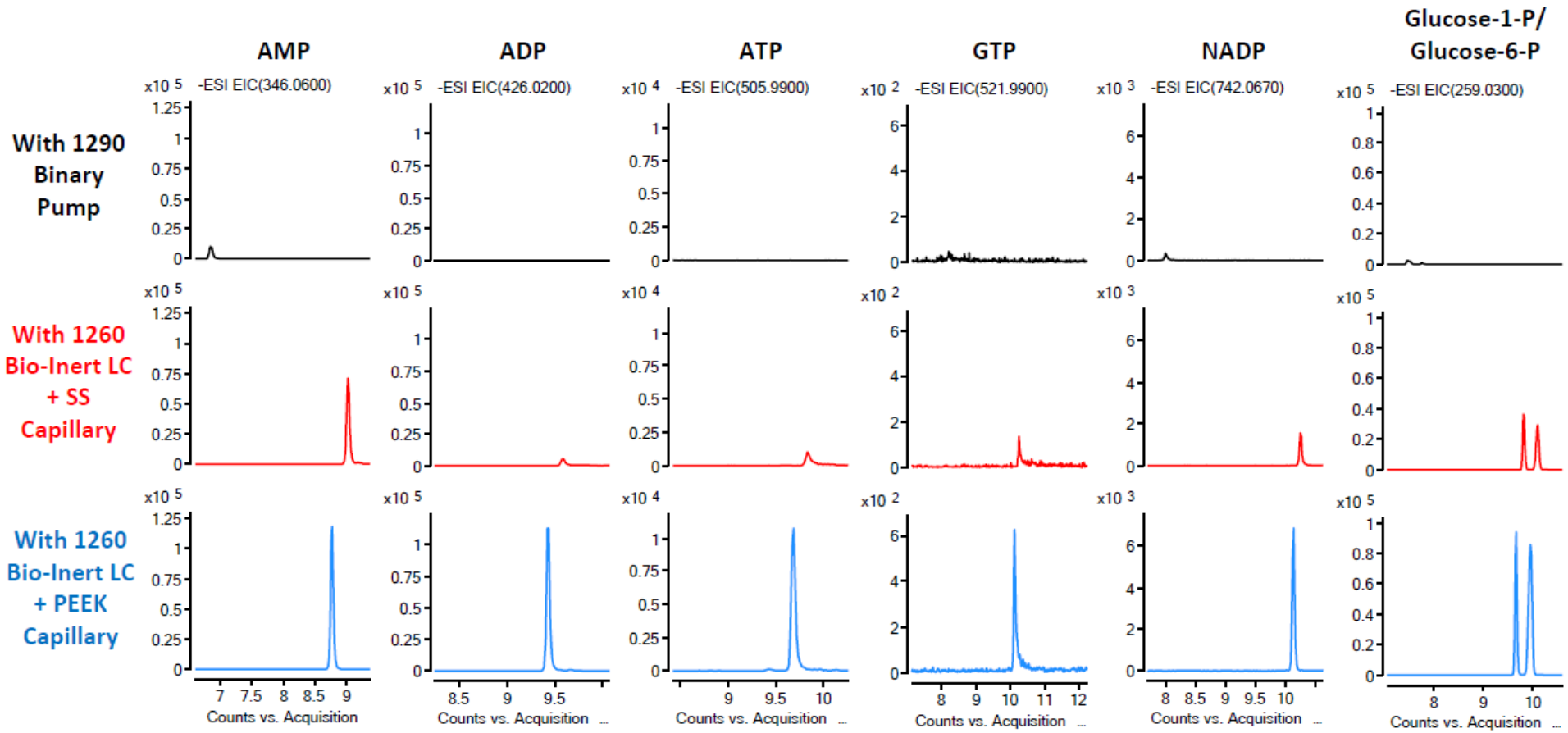
1. LC Disconnected from MS and going directly to Waste
2. IPA at 5 mL/min for 5 min
3. Water at 5 mL/min for 5 min
   - Flow at 0.5 mL/min for 1 hour
4. 0.5% Phosphoric Acid in 90% Acetonitrile / 10% Water at 5 mL/min for 5 min
   - Flow at 0.1 mL/min overnight (at a minimum)
5. Water at 5 mL/min for 5 min
   - Flow at 0.5 mL/min for 1 hour
6. Mobile Phase at 5 mL/min for 5 min
   - Flow at 0.25 mL/min for 1 hour
7. Reconnect LC to MS and proceed with analysis
   - Flow at 0.25 mL/min for 20-30 min

Improvements in Signal and Peak Shape



HILIC/MS Sensitivity with Bio-Inert LC

Nucleotide Phosphates on a PEEK Lined Agilent InfinityLab Poroshell 120 HILIC-Z



Column used was 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (PEEK lined stainless steel); A: 10 mM Ammonium Formate pH 6.8 in water, B: acetonitrile + 10 mM Ammonium Formate pH 6.8, 95-30% B in 10 minutes, 0.25 mL/min, 0.2 µL injection (5 ng each on column), MS Source: ESI-, m/z 191.02, 346.06, 426.02, 505.99, 521.99, 742.067, 743.067, 259.03

Is your System Maintenance Up To Date?

Typical Schedule*

PUMPS

Item	Typical Schedule	Comments
Solvent inlet filter	Replace every 6 - 12 months	
PTFE frits in purge valve + gold seal	Every 12 months	
Piston seals	Every 12 months	When changing the seal, check the piston for scratches--replace if scratched
Inlet valve cartridge, outlet ball valve	Every 24 months	

AUTOSAMPLER

Item	Typical Schedule	Comments
Needle and needle seat	Every 12 months	
Rotor seal	Every 12 months	
Metering device seal	Every 24 months	

COLUMN COMPARTMENT

Item	Typical Schedule	Comments
Column switching valve rotor seal	Every 12 months	
Column fittings	Every 5 to 10 column changes	A-line fittings last a lot longer than traditional fittings

DETECTORS

Item	Typical Schedule	Comments
Lamps	Every 2000 hours	Watch for a noisy baseline
Flow cell	Check cleanliness every 6 months	Low light intensity could be caused by a dirty flow cell

*Adjust according to your samples, conditions, and performance goals

https://www.youtube.com/watch?v=vFUVHssMnx4

https://www.youtube.com/watch?v=iTilOMH51Uc&index=11&list=PLThrdl2ragolmT3J-W5r8ailvJN94DJMR

Summary

- **Save time and aggravation of troubleshooting**
- Keep up with instrument maintenance
- Use original supplies
- Good sample hygiene
- Careful attention to method development

Surviving Chromatography: Part II, Corrective Action
Thursday, October 11, 2018
Presented by Jean Lane

Now you're lost because your chromatography is not where it should be. What do you do? In this talk we'll look at ways to troubleshoot what went wrong and discuss things you can do to keep them from happening in the future. This webinar will include topics like pressure, peak tailing and retention time shifts.

Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC/GCMS Columns and Supplies

Option 2 for LC/LCMS Columns and Supplies

Option 3 for Sample Preparation, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Available in the USA & 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

Appendix

Initial Column and System Equilibration*

*Or follow instructions in your column user guide

In an appropriate vessel, test highest % organic/buffer ratio to verify that buffer will not precipitate. With stirring, add organic to buffer first, not vice versa.

Equilibrate column with, in order:

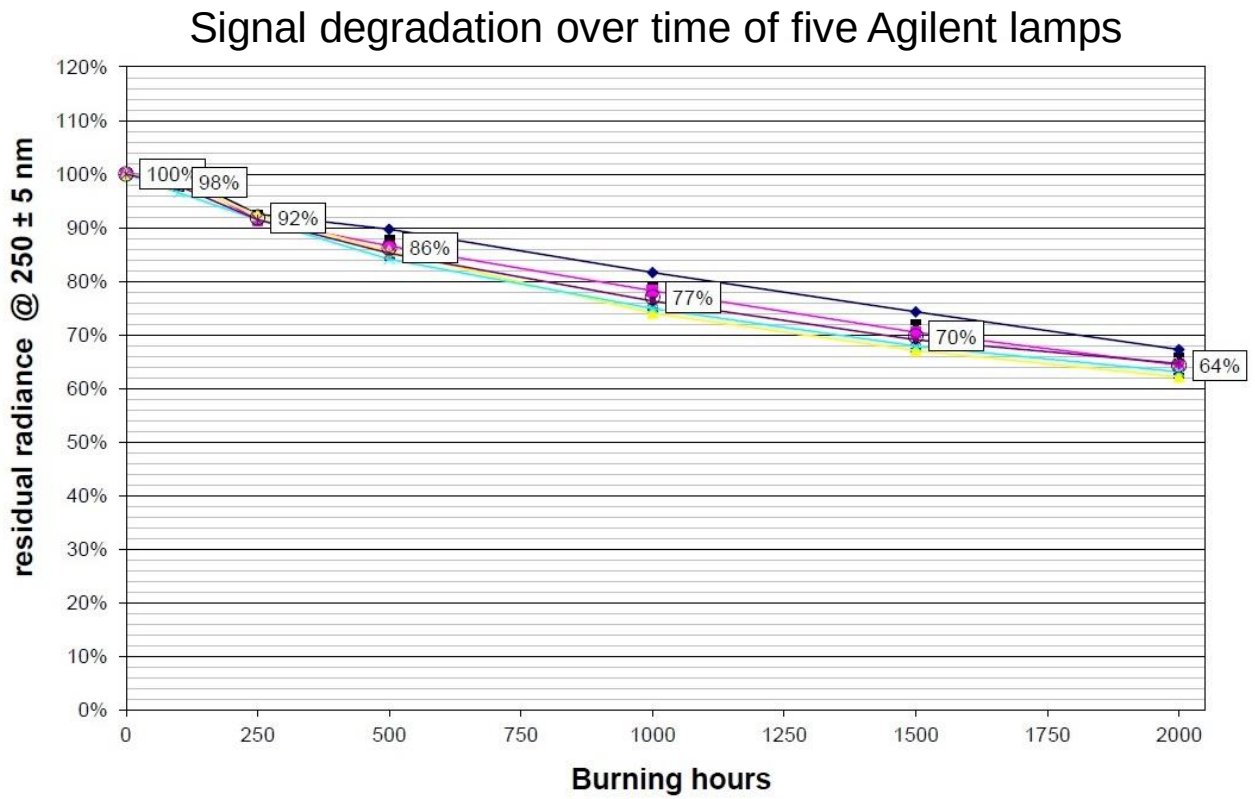
- 100% organic modifier (if brand new)
- mobile phase minus buffer
- buffered mobile phase containing highest % organic modifier (gradient high end)
- buffered mobile phase containing lowest % organic modifier (gradient low end)
- Document equilibration conditions and number of column volumes

Inject standard or sample several times until RTs stable, or for gradient methods, precede former with 1 or 2 blank gradients.

$$V_m = \pi \cdot r^2 \cdot L \cdot \sim 0.6$$

Column volume is calculated as the volume of a cylinder less the space occupied by the packing material. As an example, Agilent ZORBAX Eclipse Plus C18 packing material occupies 40% of the column, the remaining 60% of the cylinder would be considered as column volume.

Deuterium lamps
life time



- After 2000 hours all Agilent lamps showed more than 60% remaining energy, well above the specification for end of life time (50%).
- All Agilent long-life deuterium lamps are guaranteed to have a life time greater than 2000 hours.