The Chromatography Checklist

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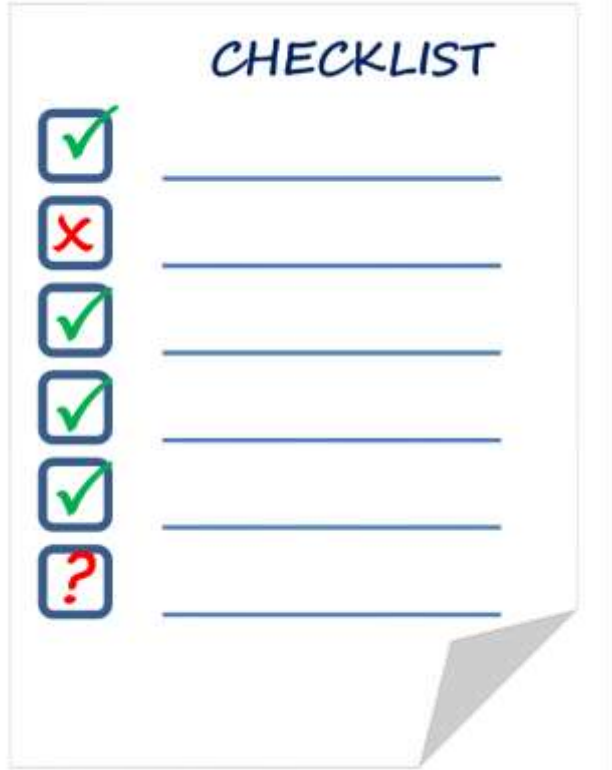
Checklist

- ❑ Is your sample ready?
  - ✓ Solubility: sample solvent and starting mobile phase conditions
  - ✓ Filters
  - ✓ Sample Clean-Up
- ❑ Supplies
  - ✓ In-line filters, safety caps, quick connect fittings
- ❑ Instrument
  - ✓ Maintenance
  - ✓ Role
  - ✓ Housekeeping
- ❑ Method
  - ✓ Established or new
  - ✓ Conditions
- ❑ Column
  - ✓ Choice
  - ✓ Documentation
  - ✓ Guards
- ❑ Final checklist

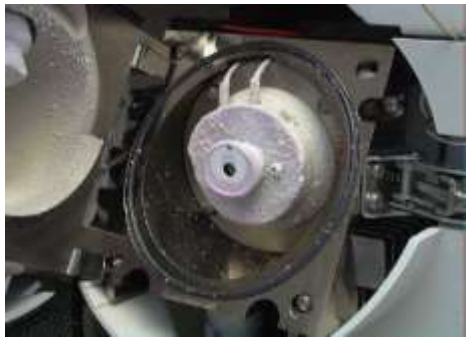


Sample Clean-Up

Filtration, Solid Phase Extraction, QUECHERS, and more!

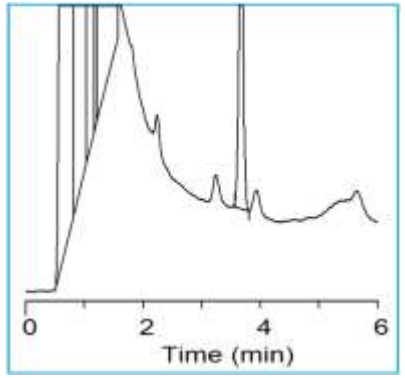
Why Perform Sample Clean-Up?

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

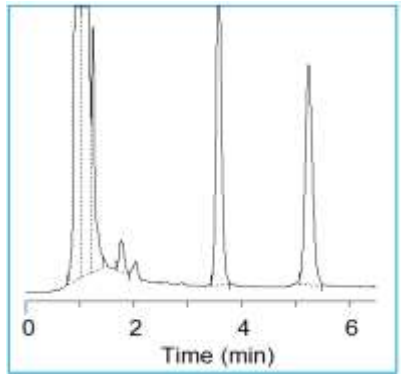


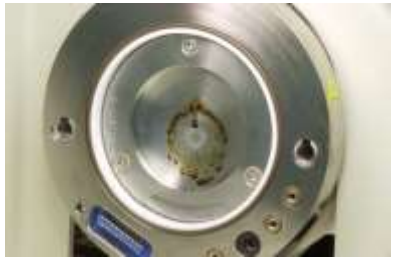
Salt build-up in LC-MS ion source from unextracted salts

Pesticides in Avocado *without* SP



Pesticides in Avocado with SP





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

Which Sample Clean-Up technique is right for YOU?

Solid Phase Extraction (SPE)

Multi-step approach for highest level of sample cleanup

QuEChERS (dSPE)

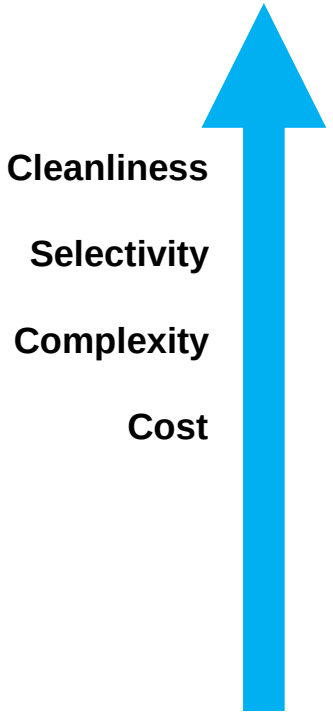
Sample cleanup by extraction of bulk interferences

Captiva EMR-Lipid (PPT and lipid removal)

Removes precipitated proteins by in-well protein precipitation and also removes lipids

Filtration

Simple and fast removal of particulates



Captiva Filtration and it's Benefits

Filtration is basic sample preparation method for all kinds of samples

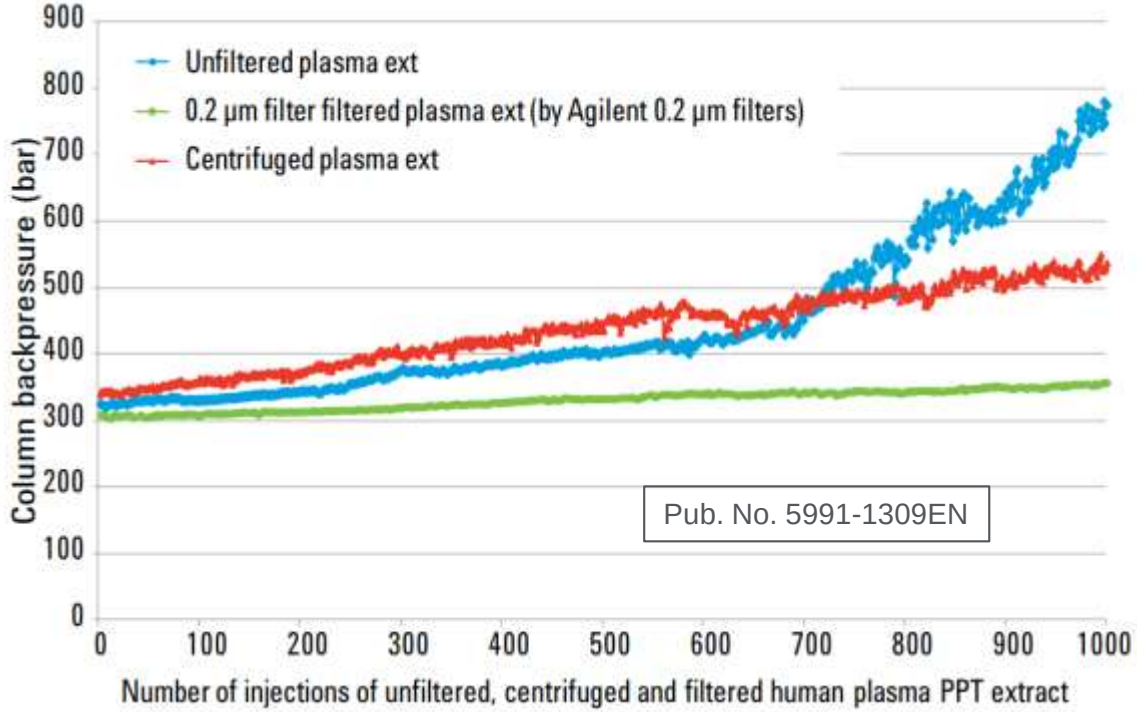
Physically removes particulates from the sample

Prevents blocking of capillaries, frits, and column inlet (especially for UHPLC columns with 1.8 and 2.7 um particle sizes)

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

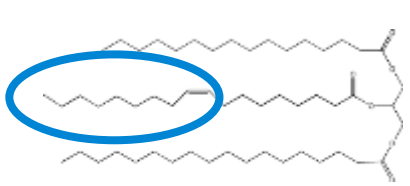
Analytical Scientist Innovation Awards 2017

- 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

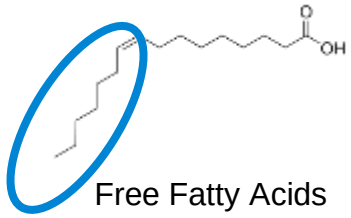
Enhanced Matrix Removal: EMR-Lipid

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

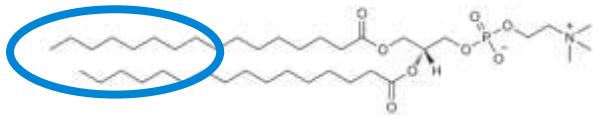


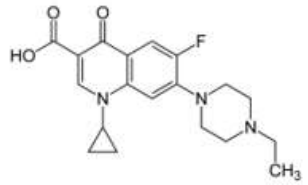
Triglycerides



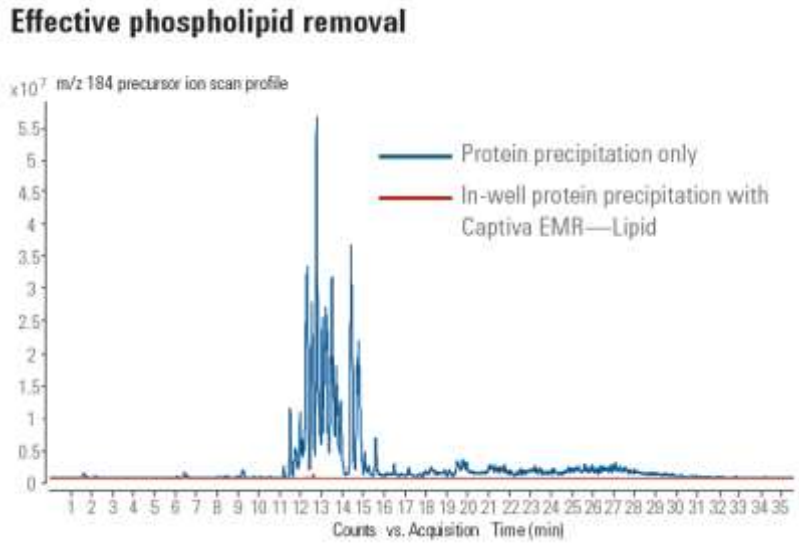
Free Fatty Acids

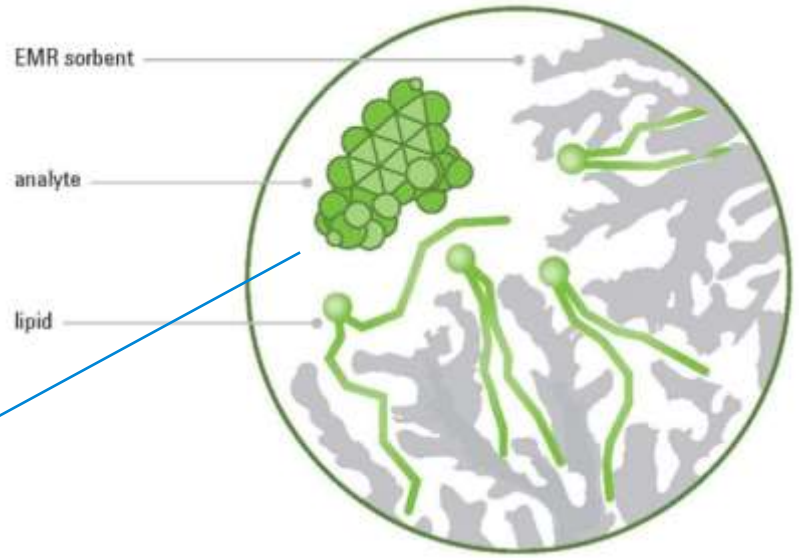
Phospholipids





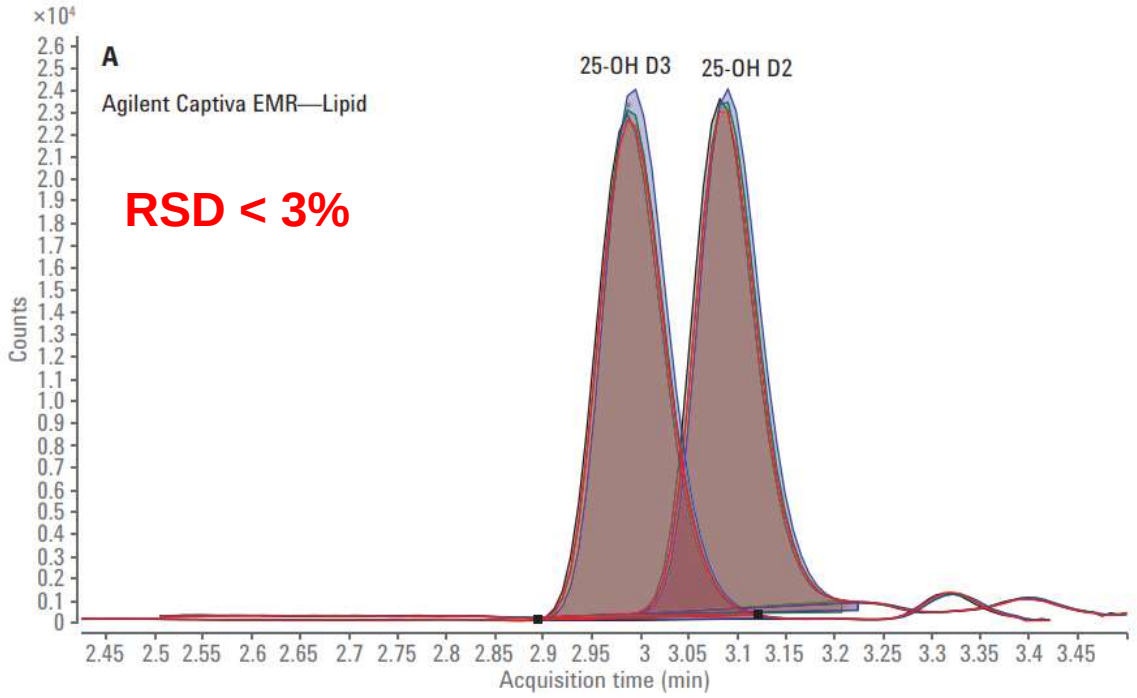
Fluoroquinolones



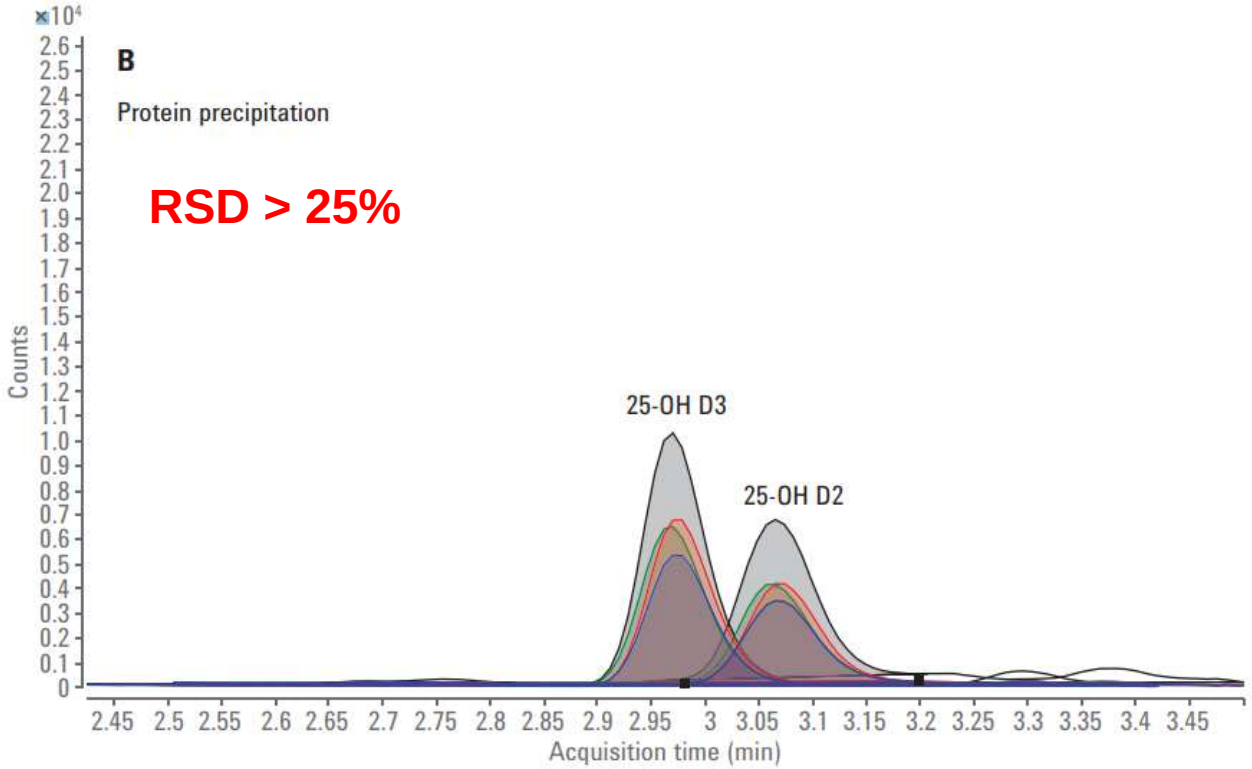


Protein Precipitation vs. Captiva EMR-Lipid RSD and Peak Area

Captiva EMR-Lipid



Protein Precipitation



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

QuEChERS

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

QuEChERS: Quick Easy Cheap Effective Rugged Safe

Commercially available kits allow for ease of use and convenience leading to increased throughput

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

Step 1: Liquid Extraction

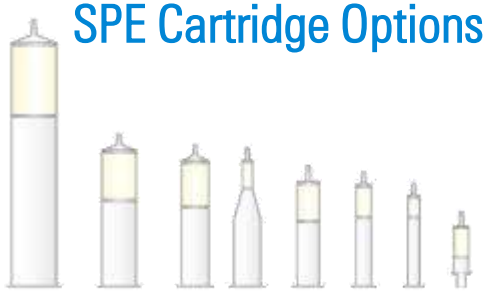
Step 2: Dispersive SPE / Interference Removal

Bond Elut Solid Phase Extraction

The Trusted Name in Solid Phase Extraction

SPE Benefits	Bond Elut SPE Products Deliver
• Selectivity ranges support a range of sample prep goals, from targeted matrix removal to target analyte concentration • Easily automatable for increased throughput and reproducibility • **Removes the widest set of matrix interferences for cleaner samples** • Depth and breadth of published applications makes choosing, optimizing and implementing SPE methods easier • Allows for lower detection limits and longer instrument uptime from cleaner extracts	• **Widest selection of formats to accommodate different sample types** and sizes and to fit into lab workflows • Over 50 unique polymeric, silica, and specialty sorbents for a wide range of application requirements • Reliable, consistent performance to ensure best performance for your lab

SPE Cartridge Options



96-well plate SPE

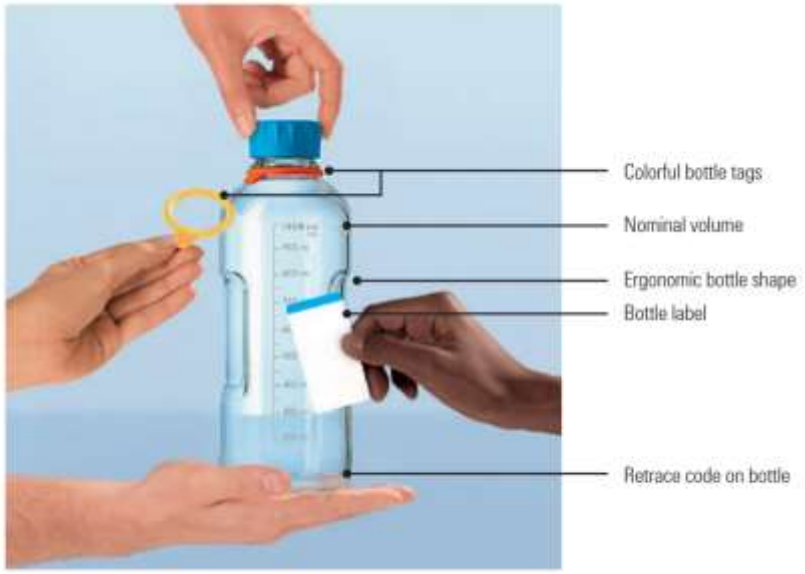
Productivity Benefits with Sample Preparation

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

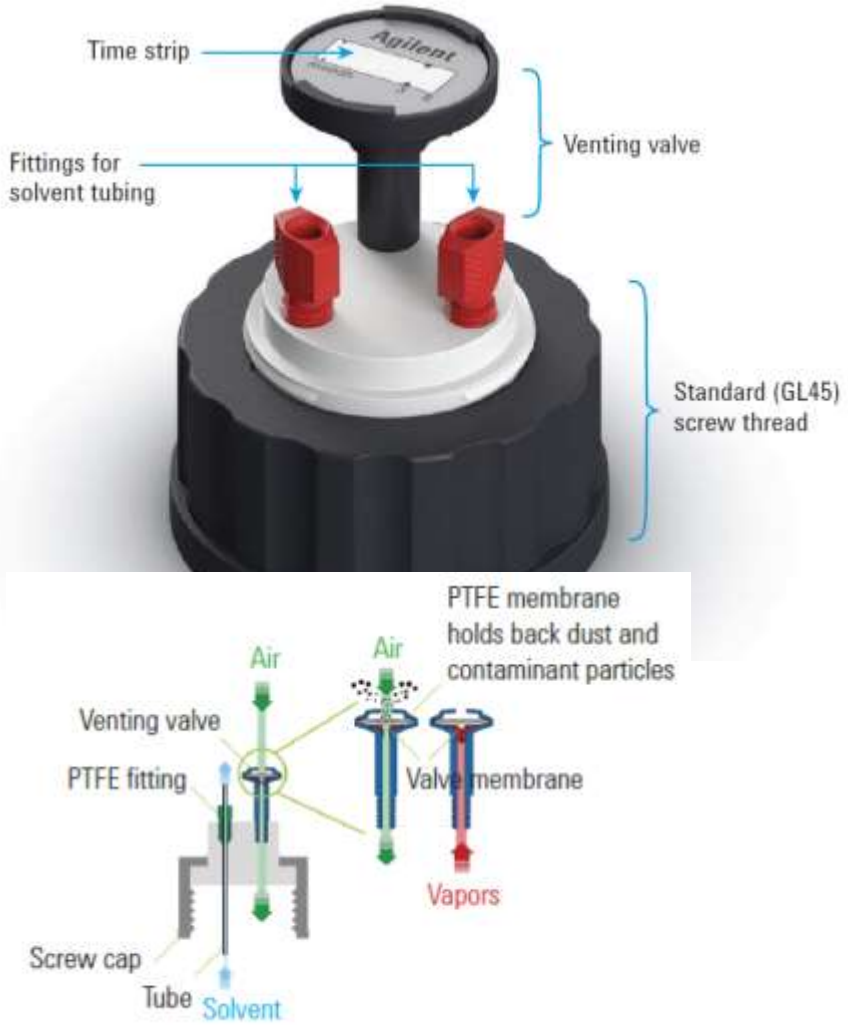
LC Supplies

InfinityLab 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
- Agilent solvent filters show uniform particle size and superior pre-size homogeneity
- 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**.
- Optional bottle tags and bottle labels with Agilent's InfinityLab solvent bottles

InfinityLab Stay Safe Caps for Solvent Bottles



The venting valve plays an important role in maintaining your workspace, keeping it safe and clean. It blocks harmful vapors and assures the required ventilation while solvent is being pulled to the LC instrument.

The time strip has a lifetime of 6 months and will alert you when to replace the venting valve

We also sell caps for waste container
We sell a variety of different caps with different amount of ports

Pump

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

Detector – Which One & Why

- UV/DAD
  - Popular, simple to use, reliable, sensitive
  - Sample must have UV absorbance
- MS
  - Sensitive
  - Sample must be ionizable
- RI
  - Refractive Index; difference between analyte and mobile phase
  - Need strict temperature control
- ELSD
  - Independent of a compound's absorbance, fluorescence, or electro-activity
  - Enables detection of semi-volatile and thermally sensitive compounds
- FLD
  - More selective and can be more sensitive
  - Compounds must fluoresce; Compounds often derivatized
- ECD
  - Very sensitive
  - Can produce severe noise

Detector Care
MS Detectors

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

Edwards pump
Oil 6040-0834

MS40+ pump
Oil 6040-1361

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

Guard Columns

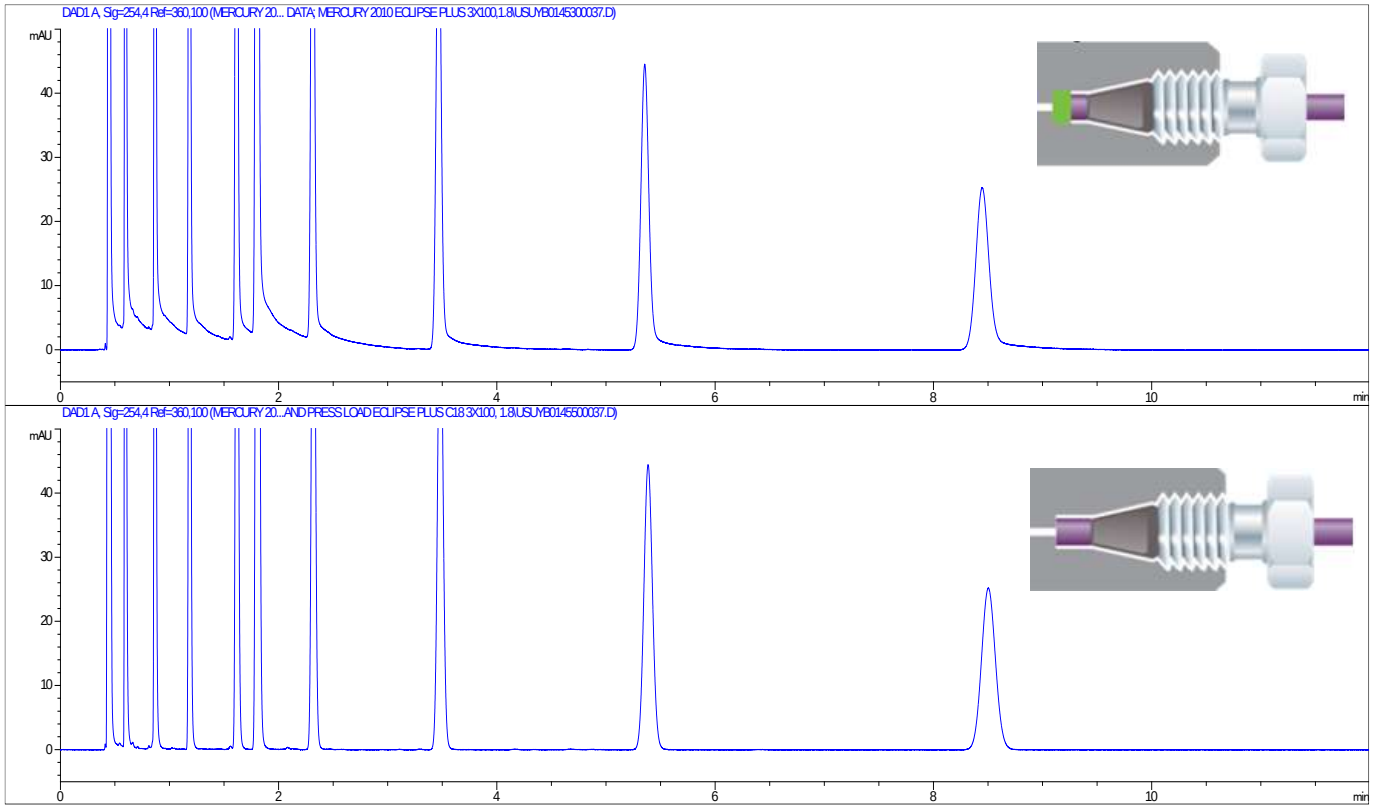
- Protects analytical column from sample contaminants that are strongly or permanently retained

Making Great Connections

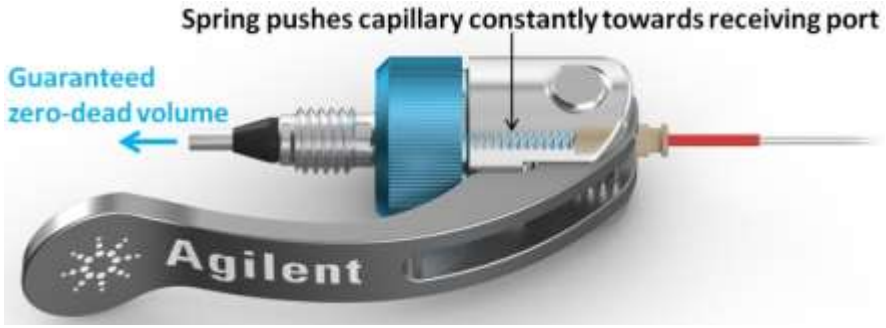
Connection problems can lead to:

- *Downtime*
- *High cost of operation*

- Poor chromatography results
  - Broad or tailing peaks
  - Loss of resolution
- Expensive maintenance cost
  - Overtightening
  - Column damage
  - Leaks, added troubleshooting



Simplifying column and fitting connections with InfinityLab Quick Connect and Quick Turn Fittings



Quick Connect fitting

Quick Turn fitting

- The unique spring-loaded design applies a constant force to eliminate dead volume. The spring-loaded design constantly pushes the tubing against the receiving port, delivering a reproducible connection with no dead volume for consistent chromatographic performance.

- Finger tight to 1300 bar: seals with a simple turn of the lever
- For instrument connections that are tight, you can use the InfinityLab QuickTurn fitting
  - Finger tight to 600 bar, wrench tight to 1300 bar
- Both fittings contain stem length that is adjustable through the spring which makes the fitting compatible with all types of LC columns

Agilent A-Line Vials

Maximum inertness: The inert performance of Agilent A-Line vials, results in reduced analyte peak variability, so you can have the utmost confidence in your results.

Consistent performance: Vial-to-vial lot-to-lot Agilent A-Line vials demonstrate consistent performance, so you spend less time troubleshooting and rerunning samples.

Certification of analysis: Agilent A-Line vials come with a certificate of analysis, so you can be sure that they will perform even in the most demanding environments.

Designed to fit a range of caps: Agilent A-Line vials can be used with your existing 2 mL autosampler caps, for easier inventory management.

Fewer septa issues: Agilent septa are continually being improved to limit leaching, coring, sticking, push-through, hardness, and adsorption/absorption.

Checklist

- ❑ Is your sample ready?
  - ✓ Solubility: sample solvent and starting mobile phase conditions
  - ✓ Filters
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- ❑ Supplies
  - ✓ In-line filters, safety caps, quick connect fittings
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- ❑ Method
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- ❑ Final checklist

LC Instrument

- ❑ Maintenance
- ❑ Role
- ❑ Housekeeping

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

Check out - *A Gram of Prevention: Simple Tips for Maintaining Instrument Performance*
https://agilenteseminar.webex.com/ec3300/eventcenter/recording/recordAction.do?siteurl=agilenteseminar&theAction=poprecord&recordID=8057207&internalRecordTicket=4832534b00000004c45844572945e7b28ba731ae9f5854e008a76b4598b318dcb3f39f8efb42e39d

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

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

Role – Pump

Performance Characteristics

Influence on…

Common to isocratic & gradient

- ➢ Flow accuracy
- ➢ Pressure pulsation

- *RT & peak area precision*
- *Baseline noise*

Gradient Pump Only

- ➢ Delay volume in low and high pressure mixing
  - Determine your dwell volume (see appendix)
- ➢ Composition accuracy & precision

- *Gradient shape and precision*
- *RT & peak area precision*

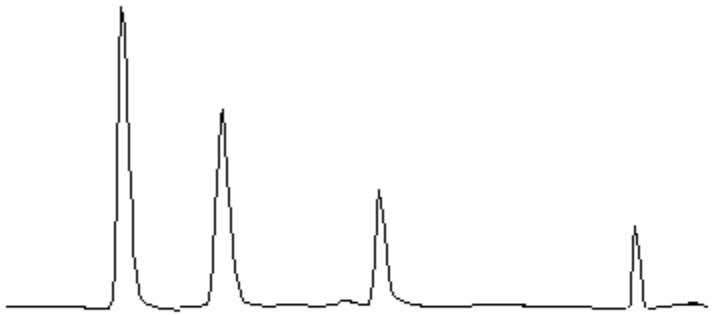
Role – Injector

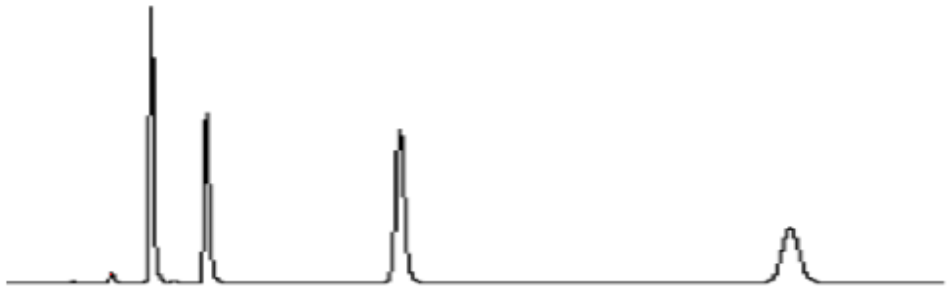
Performance Characteristics

- Injection volume precision
- Wide linearity
- Minimum carryover

Influence on…

- *Precision of peak area/height*
- *Accuracy of peak area/height (when using different injection volumes*
- *Precision of peak/area height*

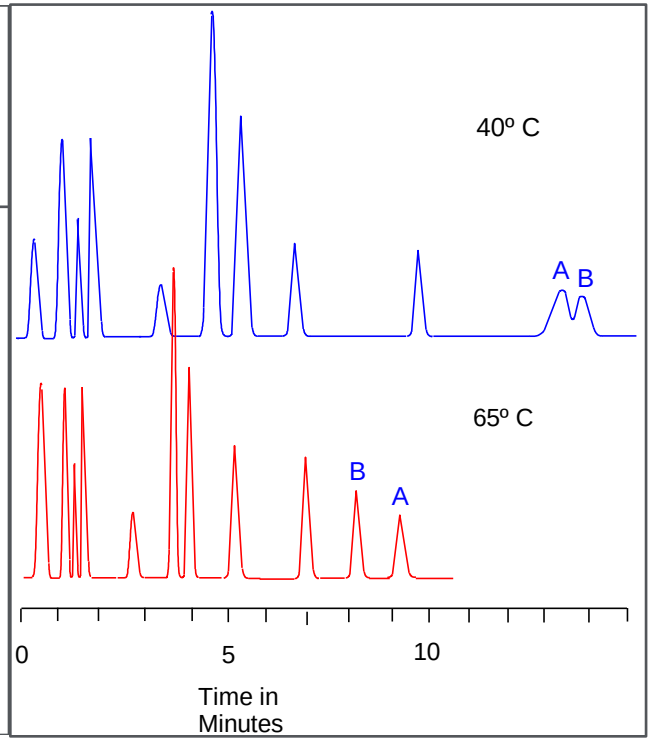




Peak Tailing – Injector Seal Failure

Role - Thermostatted Column Compartment

Performance Characteristics		*Influence on…*
➢ Temperature accuracy ➢ Temperature precision		• *Elution order* • *Peak identification* • *Elution order* • *RT precision* • *Peak identification*



Role - HPLC UV/Vis Detectors

Performance Characteristics		*Influence on…*
Variable Wavelength & Diode Array Detector		
➢ Low noise, wander, and drift ➢ Wide linear range ➢ Wavelength accuracy & precision		• *Detection limit, quantitation limit* • *Quantitation at low & high concentration* • *Accuracy & precision of peak areas/heights*
Diode Array Detector Only		
➢ Spectral resolution ➢ Spectral sensitivity		• *Accuracy of spectra, peak id by spectra* • *Accuracy of spectra, peak id by spectra at low concentrations*

Housekeeping

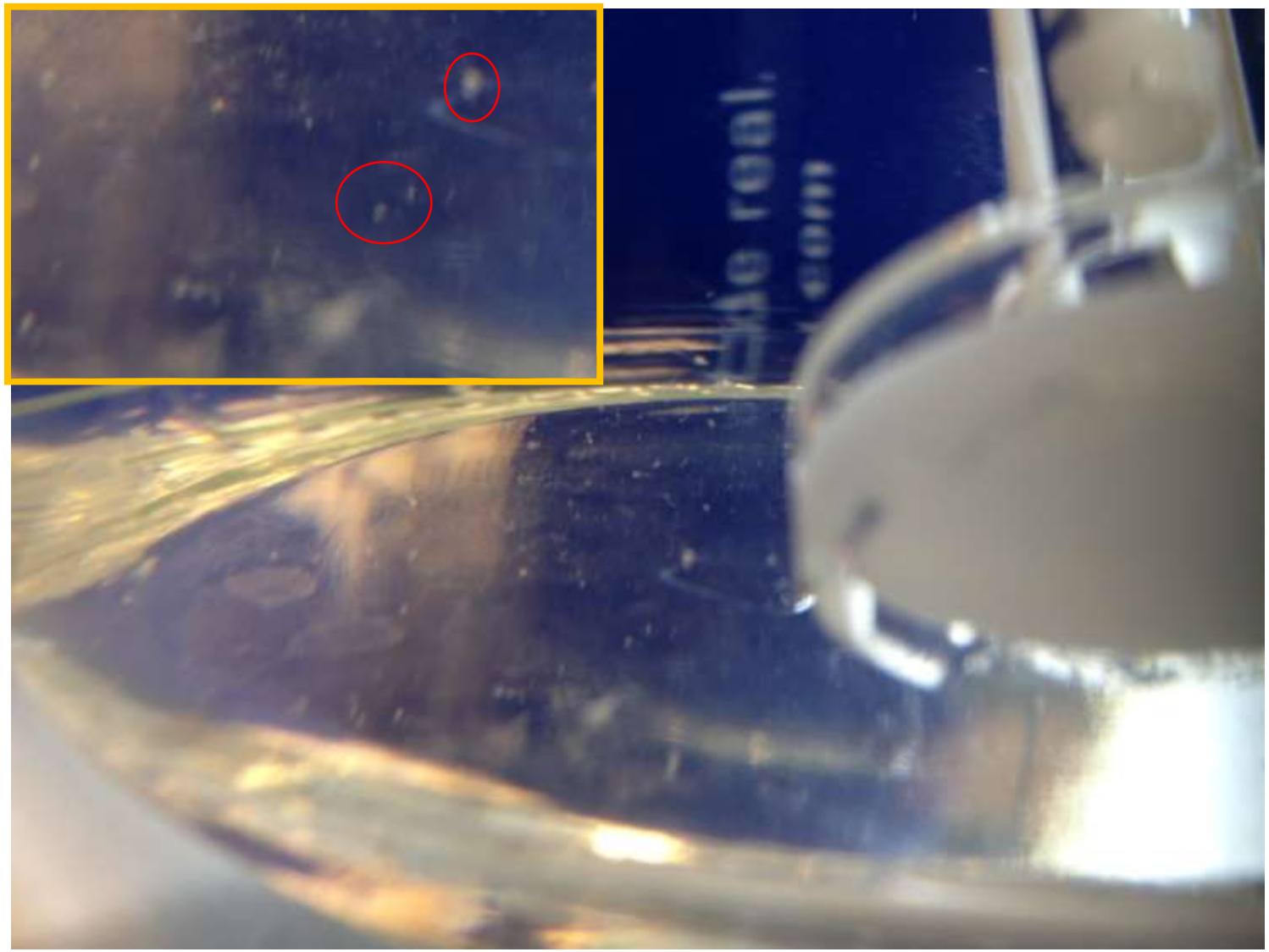
Daily/System Start-up

- ❑ Mobile phase – how fresh is yours?
- ❑ Purge pumps ~5 minutes
- ❑ Condition pump for ~15 minutes

Weekly

- ❑ Seal wash solvent
- ❑ Buffer flush
- ❑ Visual inspection of solvent filters
- ❑ Purge with composition of your application
- ❑ Condition with composition of your application

Growth in H2O over 1 week in a clear bottle



Avoid growth of algae

Algae

The presence of algae in aqueous media can cause a variety of problems in your HPLC.
Buffers, light and a pH between 4 and 8 will accelerate their growth.

For more information refer to your pump reference manual.

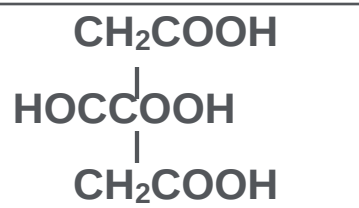
Method & Column

- ❑ Method
  - ✓ Established or New
  - ✓ Conditions
- ❑ Column
  - ✓ Choice
  - ✓ Documentation
  - ✓ Guards
  - ✓ Equilibration

Method Conditions

- ❑ Mobile Phase
  - ✓ HPLC or MS grade solvents
  - ✓ Buffer – right choice, column, LC, LC/MS, filtered?
  - ✓ Preparation procedure
  - ✓ Fresh mobile phase
  - ✓ Bottles covered? No paraffin
    - Volatile components
  - ✓ Label bottles, content + date
  - ✓ Amber bottle for aqueous
  - ✓ Make sure system flushed before introducing new mobile phase
  - ✓ pH
- ❑ Temperature
- ❑ Pressure
- ❑ Standards, test mix

Buffer Options

Non-volatile:		pK_a	Buffer Range
Phosphate	$H_3PO_4 \rightleftharpoons H_2PO_4^-$	$pK_1 = 2.1$	1.1 – 3.1
	$H_2PO_4^- \rightleftharpoons HPO_4^{-2}$	$pK_2 = 7.2$	6.2 – 8.2
	$HPO_4^{-2} \rightleftharpoons PO_4^{-3}$	$pK_3 = 12.3$	11.3 – 13.3
Citrate		$pK_1 = 3.1$	2.1 – 4.1
		$pK_2 = 4.7$	3.7 – 5.7
		$pK_3 = 5.4$	4.4 – 6.4
Borate	H_3BO_3	$pK_1 = 9.2$	8.2 – 10.2
Volatile:			
Trifluroacetate	F_3CCOOH	$pK_1 = 0.5$	xx – 1.5
Formate	$HCOOH$	$pK_1 = 3.8$	2.8 – 4.8
Acetate	CH_3COOH	$pK_1 = 4.8$	3.8 – 5.8
Ammonium	NH_4^+	$pK_1 = 9.2$	8.2 – 10.2

Mobile Phase Preparation

➢ Small changes in mobile phase strength can have a large affect on retention

- ✓ HPLC grade or better
- ✓ Buffer prep procedure
  - Be consistent
    - Document process

Volume % of solvents can depend on preparation

Specified volume ACN added to a 1 L volumetric and made to volume with H_2O

≠

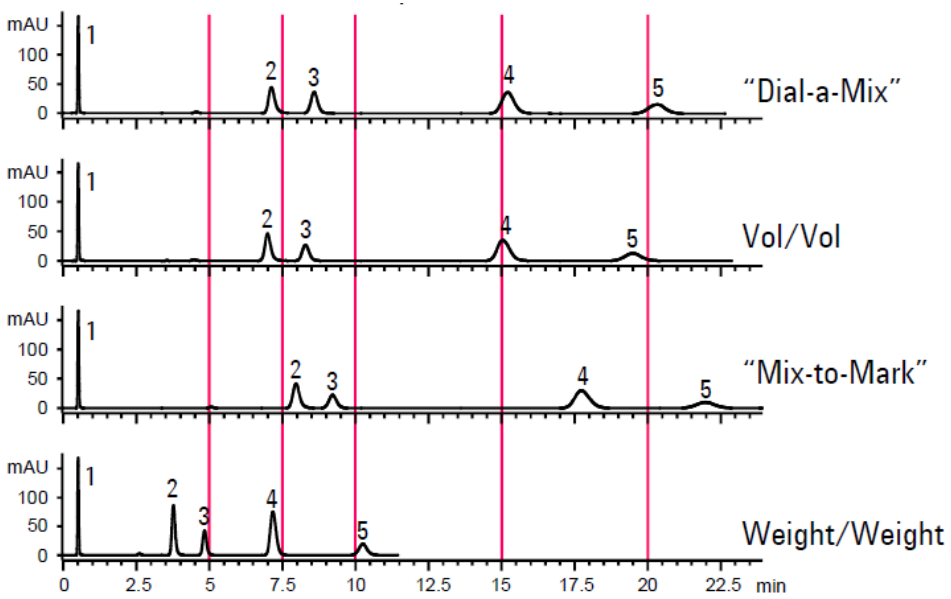
Specified volume H_2O added to a 1 L volumetric and made to volume with ACN

≠

500 ml H_2O added to 500 ml ACN

- ✓ Degree of contraction is affected by the relative quantities of each
- ✓ Temperature

Mobile Phase Preparation
Effect on Chromatography



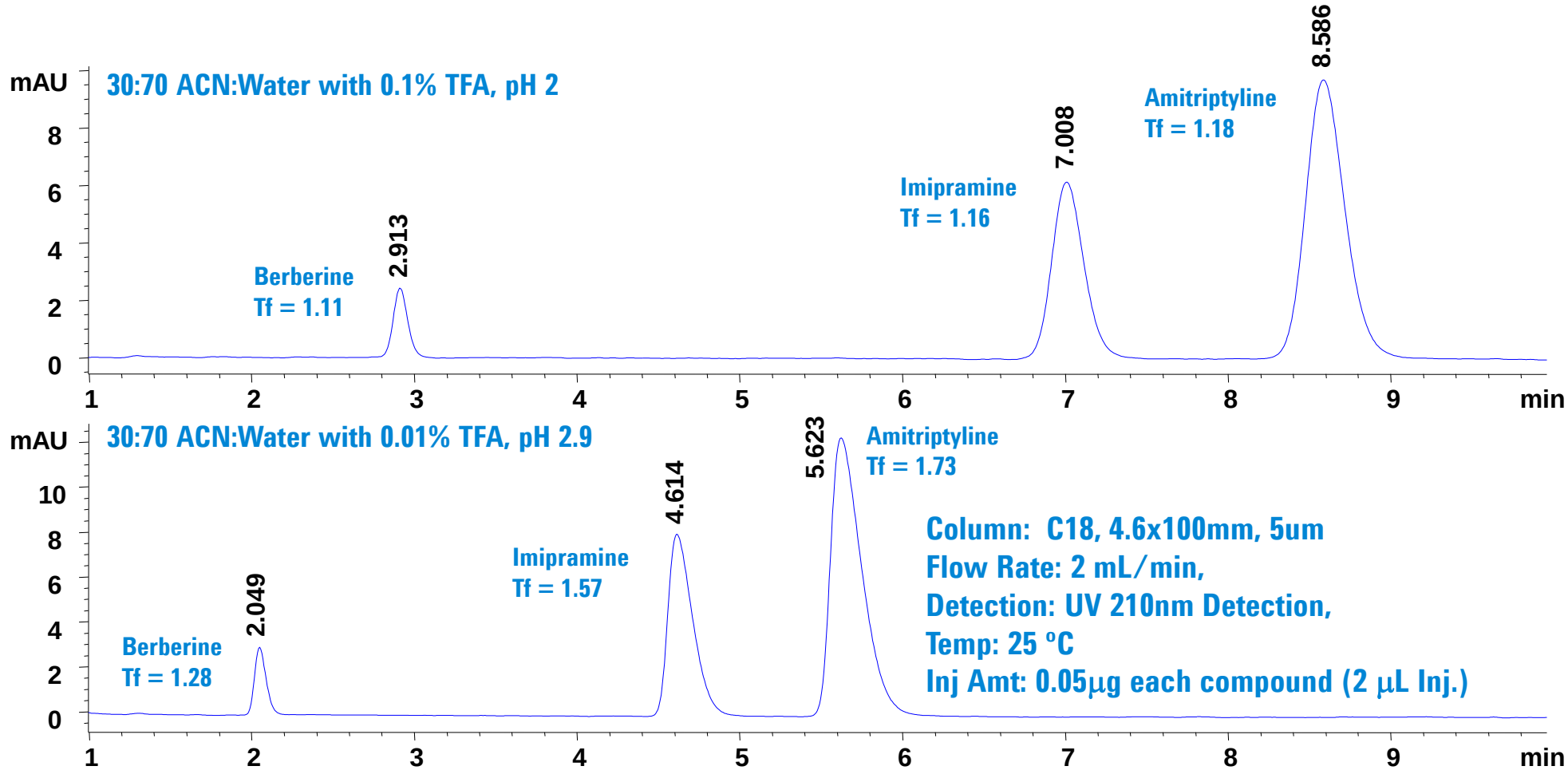
HPLC System:	Agilent 1100 with quaternary pump
Column:	ZORBAX Eclipse XDB-C8 Rapid-Resolution (3.5µm), 4.6 x 50 mm Agilent Part No. 935967-906
Mobile Phases:	Dial-a-Mix= A: water B: MeOH, pump 50% B Vol/Vol=250 mL water + 250 mL MeOH, pump 100% Mix-to-Mark = 250 mL MeOH, fill to 500 mL with water, pump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

1. Uracil
2. Butylparaben
3. Napthalene
4. Dipropylphthalate
5. Acenaphthene

- Method used to prepare MP can significantly affect the elution pattern
- Be consistent
  - w/w is more accurate than v/v

Effect of Mobile Phase Preparation on Chromatography,
Pub. No. 5988-6476EN

Changes in Volatile Buffer Concentration Can Cause Shifts in Retention Time and Peak Shape



Column

- ❑ Choice
  - ✓ Conditions, flow rate, pressure, pH
- ❑ Performance report
- ❑ Datasheet or column guide
- ❑ Benchmark with your system
- ❑ Suitable fittings
- ❑ Equilibrated
- ❑ In-line filters and/or guards
- ❑ Sample
- ❑ Store properly when done

Column Specifications

InfinityLab Poroshell 120 column specifications

InfinityLab Poroshell 120	Pore Size	Temperature Limit	pH Range	Endcapped	Carbon Load	Surface Area	USP Designation
EC-C18	120 Å	60 °C	2.0–8.0	Yes	10%	130 m2/g	L1
EC-C8	120 Å	60 °C	2.0–8.0	Yes	5%	130 m2/g	L7
SB-C18	120 Å	90 °C	1.0–8.0	No	9%	130 m2/g	L1
SB-C8	120 Å	80 °C	1.0–8.0	No	5.5%	130 m2/g	L7
HPH-C18	100 Å	60 °C	3.0–11.0	Yes	Proprietary	95 m2/g	L1
HPH-C8	100 Å	60 °C	3.0–11.0	Yes	Proprietary	95 m2/g	L7
Bonus-RP	120 Å	60 °C	2.0–8.0	Yes	9.5%	130 m2/g	L60
PFP	120 Å	60 °C	2.0–8.0	Yes	5.1%	130 m2/g	L43
Phenyl-Hexyl	120 Å	60 °C	2.0–8.0	Yes	9%	130 m2/g	L11
SB-Aq	120 Å	80 °C	1.0–8.0	No	Proprietary	130 m2/g	L96
EC-CN	120 Å	60 °C	2.0–8.0	Yes	3.5%	130 m2/g	L10
HILIC-Z	100 Å	80 °C	2.0–12.0	No	Proprietary	95 m2/g	L114
HILIC	120 Å	60 °C	0.0–8.0	No	NA	130 m2/g	L3
HILIC-OH5	120 Å	45 °C	1.0–7.0	Proprietary	Proprietary	130 m2/g	L86
Chiral-V	120 Å	45 °C	2.5–7.0	Proprietary	Proprietary	130 m2/g	L88
Chiral-T	120 Å	45 °C	2.5–7.0	Proprietary	Proprietary	130 m2/g	L63
Chiral-CD	120 Å	45 °C	3.0–7.0	Proprietary	Proprietary	130 m2/g	L45
Chiral-CF	120 Å	45 °C	3.0–7.0	Proprietary	Proprietary	130 m2/g	NA

Column Documentation

Performance Report

SERIAL NUMBER: USDAZ01333

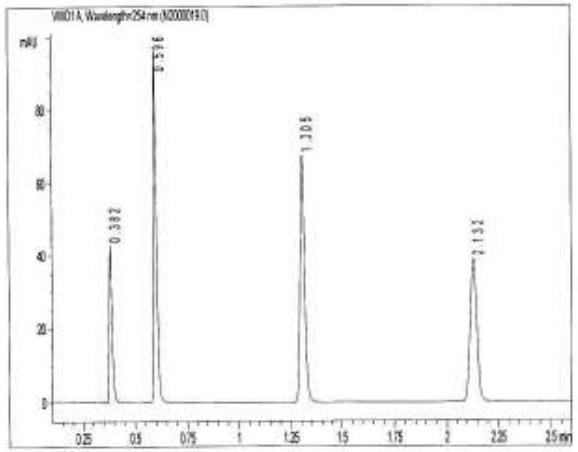
PART NUMBER: 959758-902
COLUMN TYPE: ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm
PACKING LOT #: B09089

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

Column Documentation

Column Guide

This booklet provides general information for all ZORBAX, Poroshell, Pursuit, and Polaris reversed-phase columns.

For additional detailed information about your specific phase or family, see:
agilent.com/chem/columnchoices

Getting Started

A QC Column Performance Report, including a test chromatogram, is enclosed with every Agilent column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This allows a better evaluation of the column and assures a more consistent product. A properly configured LC system will generate similar results to the chromatogram on your QC Performance Report.

Modern columns are robust and are designed to operate for long periods under normal chromatographic conditions. You can maximize column performance by running it within specifications. Always review the specifications before putting in place a final method.

Data Sheet

Agilent ZORBAX Eclipse Plus Phenyl-Hexyl Rapid Resolution Threaded Column

Data Sheet

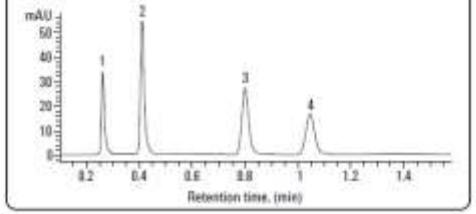
General Description

Eclipse Plus Phenyl-Hexyl columns are designed for superior peak shape with basic compounds, and deliver high efficiency and excellent peak shape with all sample types. Eclipse Plus Phenyl-Hexyl is especially useful for the separation of acidic, basic, and other highly polar compounds by reverse-phase liquid chromatography. Eclipse Plus Phenyl-Hexyl packing is made by first chemically bonding a dense monolayer of dimethylphenylhexylsilane stationary phase to a specially prepared, improved ultra-high purity (>99.995% SiO_2) ZORBAX Rx-SIL porous silica support. This special silica support (Type B) is designed to reduce or eliminate strong adsorption of basic and highly polar compounds. The bonded-phase packing is then doubly endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica surface. Eclipse Plus Phenyl-Hexyl columns can be used for acidic and neutral samples, but are especially suited for separating basic compounds that produce poor peak shapes on other columns. These columns can be used for a wide range of applications and over a pH range of 2 to 8, accommodating most popular mobile phases.

Figure 1
ZORBAX Eclipse Plus Rapid Resolution Phenyl-Hexyl Chromatogram

Operating Conditions

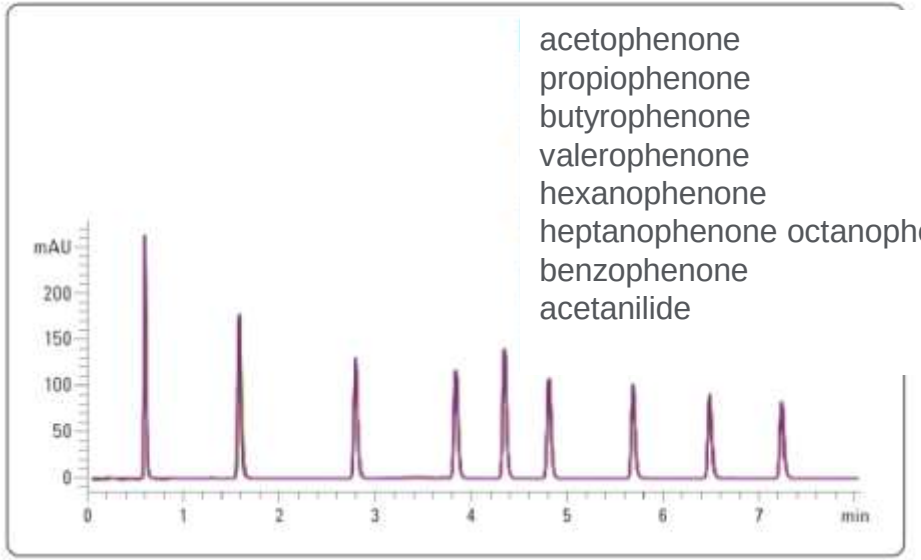
Column:	Eclipse Plus Phenyl-Hexyl, 4.6 mm × 30 mm, (3.5 µm)
Mobile phase:	60% Acetonitrile/40% water
Flow rate:	1.0 mL/min
Temperature:	Ambient
Injection:	5 µL
Detector:	UV 254 nm
Peak identity:	1. Uracil 5 µg/mL
	2. Phenol 200 µg/mL
	3. 4-Chloronitrobenzene 25 µg/mL
	4. Toluene 950 µg/mL



Column Documentation - 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)

Initial Column and System Equilibration*

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).

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

*Or follow instructions in your column user guide

Do You Need a Guard Column?
If you do, is it the correct one?

The **ZORBAX High Performance Guard Cartridge** components assemble quickly and easily to provide a high efficiency, low dead volume guard column that seals, with hand tightening, **up to 340 bar or 200 bar with a PEEK fitting**.

For use with columns having a 5um, 3um 0r 3.5um packing and **400 bar pressure limit**

Agilent Fast Guards (3/pk), stainless steel **UHPLC** guards packed with **1.8um Zorbax** or **Poroshell 120** materials.

- Single replacement guard column (no cartridge)
- Rated to **600 bar – 1300 bar** to match column

Checklist

- ☑ Supplies
  - Critical supplies on hand
- ☑ Sample
  - Is it ready for chromatography?
- ☑ Instrument
  - Maintenance up to date
- ☑ Method conditions
- ☑ Column
  - Right choice for your sample and conditions
- ☑ Final checklist
  - Shutdown (see appendix)
    - Short term
    - Long Term

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

gc-column-support@Agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

Resources for Support

- Agilent University http://www.agilent.com/crosslab/university
- Tech support http://www.agilent.com/chem/techsupport
- 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)
- Your local FSE and Specialists
- Youtube – Agilent Channel
- Agilent Service Contracts

APPENDIX

Determining the Dwell Volume of Your System

Replace column with short piece of HPLC stainless steel tubing

Prepare mobile phase components

A. water - UV-transparent
B. water with 0.2% acetone - UV-absorbing

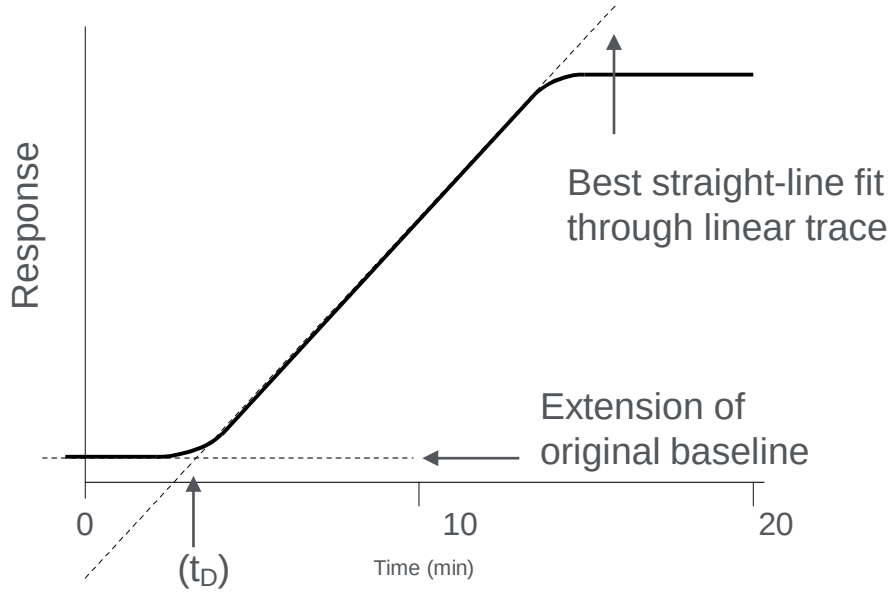
Monitor at 265 nm

Adjust attenuation such that both 100% A and 100% B are on scale

Run gradient profile 0 - 100% B/10 min at 1.0 mL/min

Record

Measuring Dwell Volume



Intersection indentifies
dwell time (t_D)

$V_D = t_D \times F$

V_D = Dwell Volume

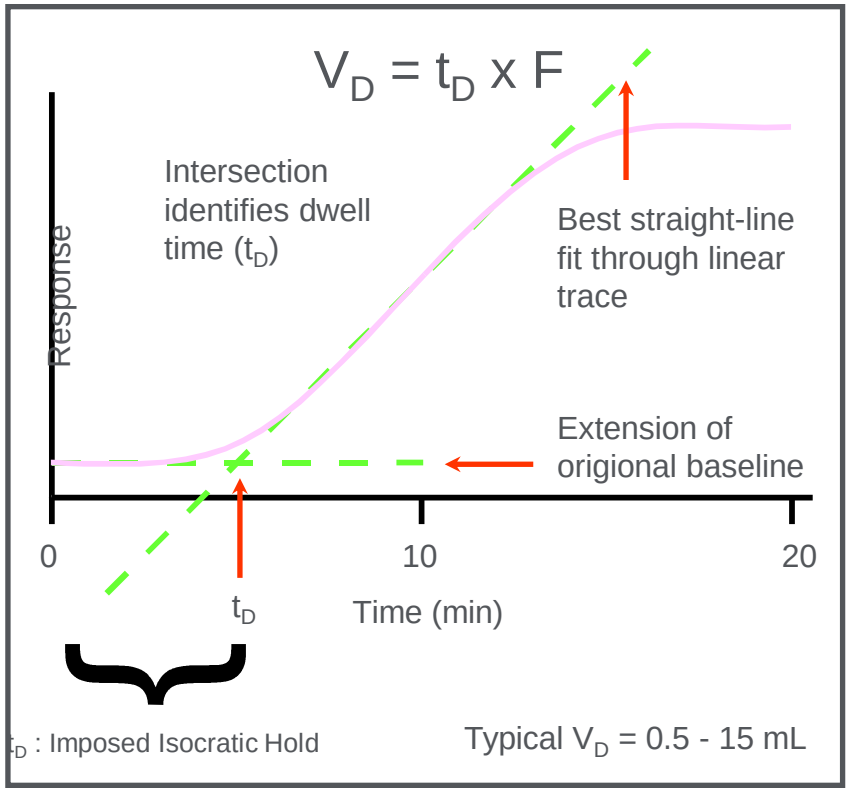
001815S1.PPT

Measuring Dwell Volume

Dwell Volume Impact

A chromatogram generated on one instrument (V_{D1}) can have a very different profile if generated on another instrument (V_{D2})

If using gradient conditions - report dwell volume (V_D)
V_D varies from instrument to instrument



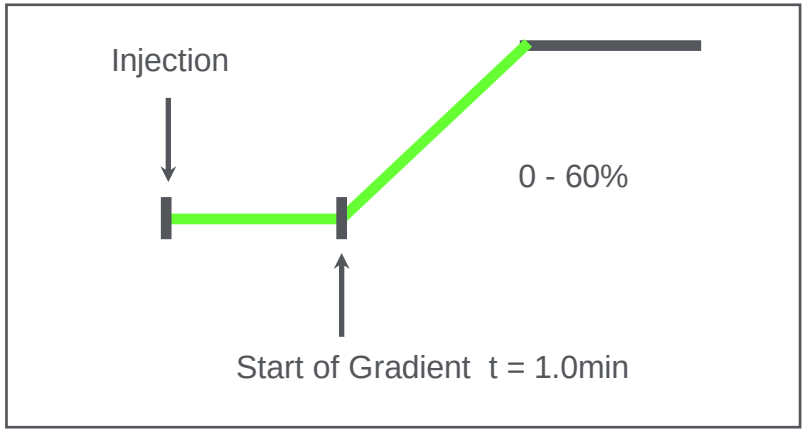
High Pressure Mixing: V_D = mixing chamber + connecting tubing + injector

Low Pressure Mixing: V_D = the above + pump heads + associated plumbing

001011P1.PPT

Correcting for Dwell Volume

1. Measure the Dwell Volume of your HPLC System
   V_D = 1.0 mL
2. Draw Effective Gradient Profile at First Flow Rate
   Calculate the time delay (imposed isocratic hold) caused by dwell volume
   $V_D = t_D \bullet F$ 1.0 mL = $t_D \bullet$ 1.0 mL / min
   where F = 1.0 mL / min for 4.6 x 150 mm column
   V_D = 1.0 mL
   $t_D = F/V_D$ t_D = 1.0 mL / min / 1.0 mL
   t_D = 1.0 min



000884P2.PPT

Correcting for Dwell Volume

If $V_{D1} > V_{D2}$

Compensate for longer V_{D1} by adding an isocratic hold to V_{D2}, such that Hold + $V_{D2} = V_{D1}$

If $V_{D1} < V_{D2}$

Delay injection, such that V_{D2} - delay = V_{D1}

(very difficult to accomplish in practice)

001014P1.PPT

Shutdown State and Instrument Flushing

Shutdown State

Next day use—using same buffers

- Pump mobile phase very slowly (for example, 0.01 – 0.1mL/min).

When flushing column or for longer term column storage

- Flush with 20/80 organic/water, then 80/20 organic/water or 100% organic.

Instrument flushing

- ✓ Replace column with capillary tubing. Leave disconnected from detector.
- ✓ Flush pumps with water, then connect capillary tubing to detector.
- ✓ Inject water 2-3 times at maximum injection volume setting.
- ✓ Flush all pumps with 100% organic for long term storage

➢ Check your instrument manual for manufacturer’s guidance

Buffer Preparation – General Guidance

1. Dissolve salt in organic-free water in 1- or 2-L beaker. Use appropriate volume to leave room for pH adjustment solution. Equilibrate solution to room temperature for maximum accuracy.
2. Calibrate pH meter. Use 2-level calibration and bracket desired pH. Use appropriate audit solution to monitor statistical control (for example, potassium hydrogen tartrate, saturated solution, pH = 3.56).
3. Adjust salt solution to desired pH. Minimize amount of time electrode spends in buffer solution (contamination). Avoid overshoot and readjustment (ionic strength differences can arise).
4. Transfer pH-adjusted buffer solution quantitatively to volumetric flask, dilute to volume, and mix.
5. Filter through 0.45 µm filter. Discard first 50 – 100 mL filtrate. Rinse solvent reservoir with small volume of filtrate and discard. Fill reservoir with remaining filtrate or prepare premix with organic modifier.
   - Agilent Solvent Filtration Kit, 250-mL reservoir, 1000-mL flask, p/n 3150-0577
   - Nylon filter membranes, 47 mm, 0.45 µm pore size, p/n 9301-0895