What to Consider Before Starting Your HPLC Analysis

Golnar Javadi
Applications Engineer
Columns and Supplies Technical Support
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Agilent
Trusted Answers

Overview

- Sample
- Solvents
- Supplies
- Instrument
- Method
- Column
- Summary

Sample















Sample

How to get the sample into an appropriate state for analysis

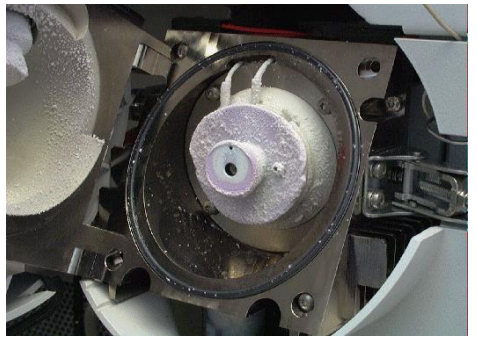
- Solid samples: pulverization followed by solvent extraction with an appropriate solvent
- Liquid samples: solvent extraction or dilution with an appropriate solvent

Solvent exchange may be required before analysis

Sample

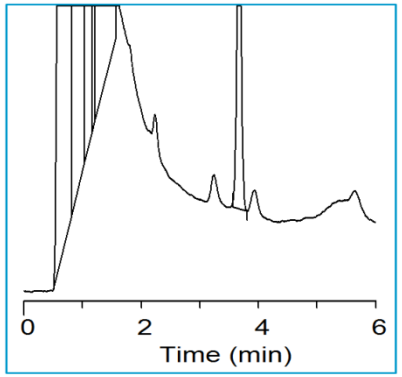
You may need to perform sample cleanup

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

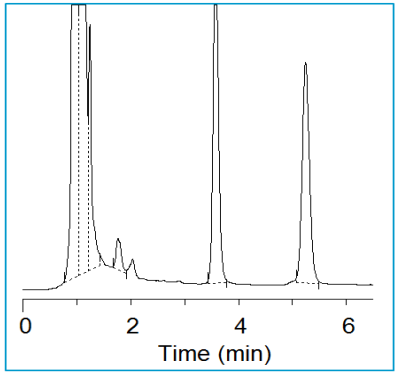


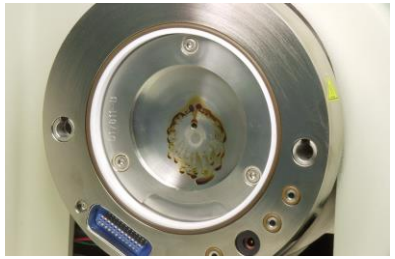
Salt buildup in LC/MS ion source from unextracted salts

Pesticides in avocado *without* cleanup



Pesticides in avocado with cleanup

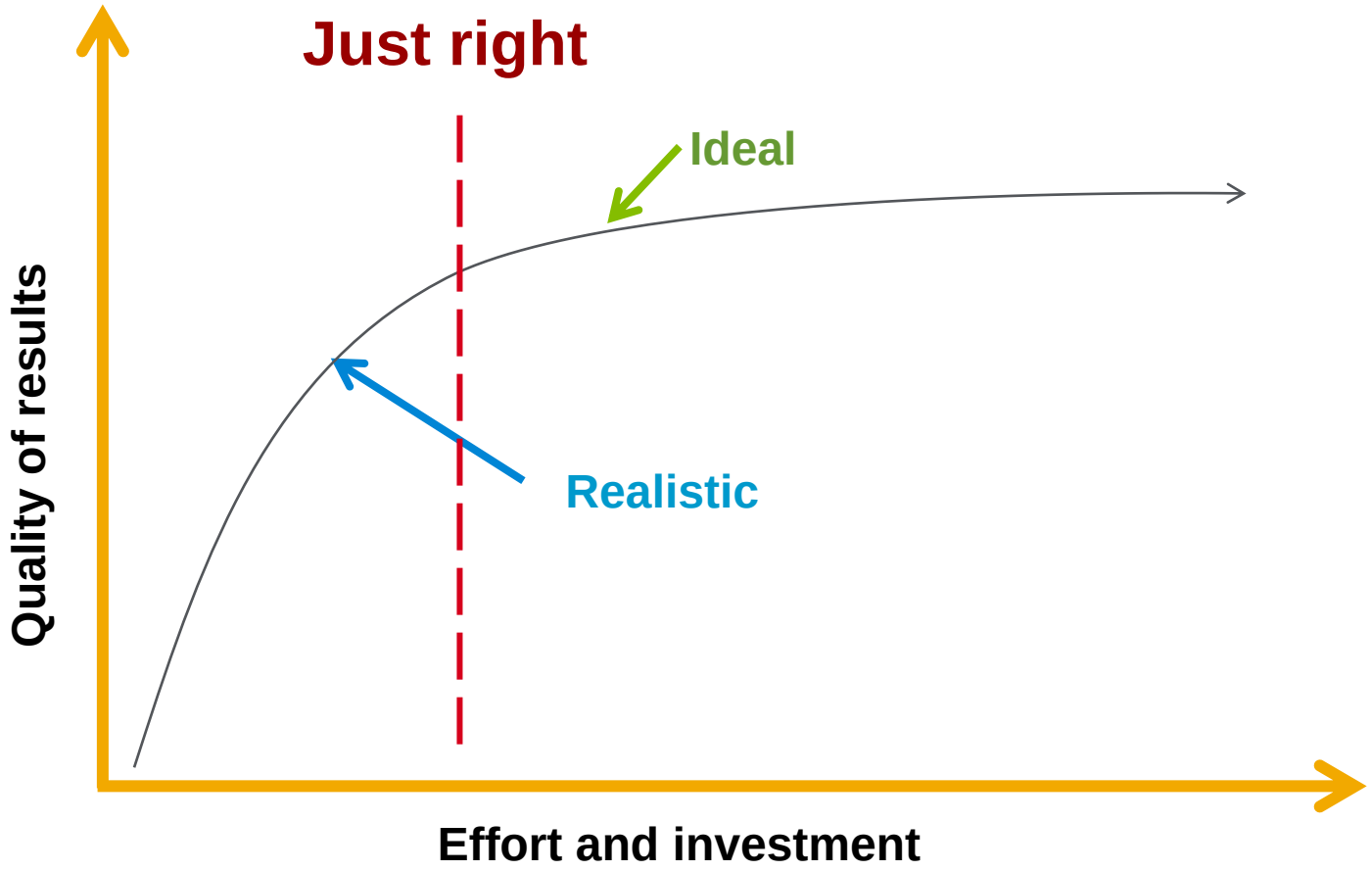




Curtain plate after injection of 25 samples with extractions from raisins without cleanup

Sample

Striking the right balance in sample cleanup



Sample

How to cleanup the sample extract

Solid Phase Extraction (SPE)

Multistep approach for the highest level of sample cleanup.

QuEChERS extraction and dSPE cleanup

Extraction followed by removal of interferences, such as organic acids, lipids, proteins, pigments, and more.

Filtration

Simple and fast removal of particulates.

Functionalized filtration for removal of particulates, lipids, proteins, and pigments.

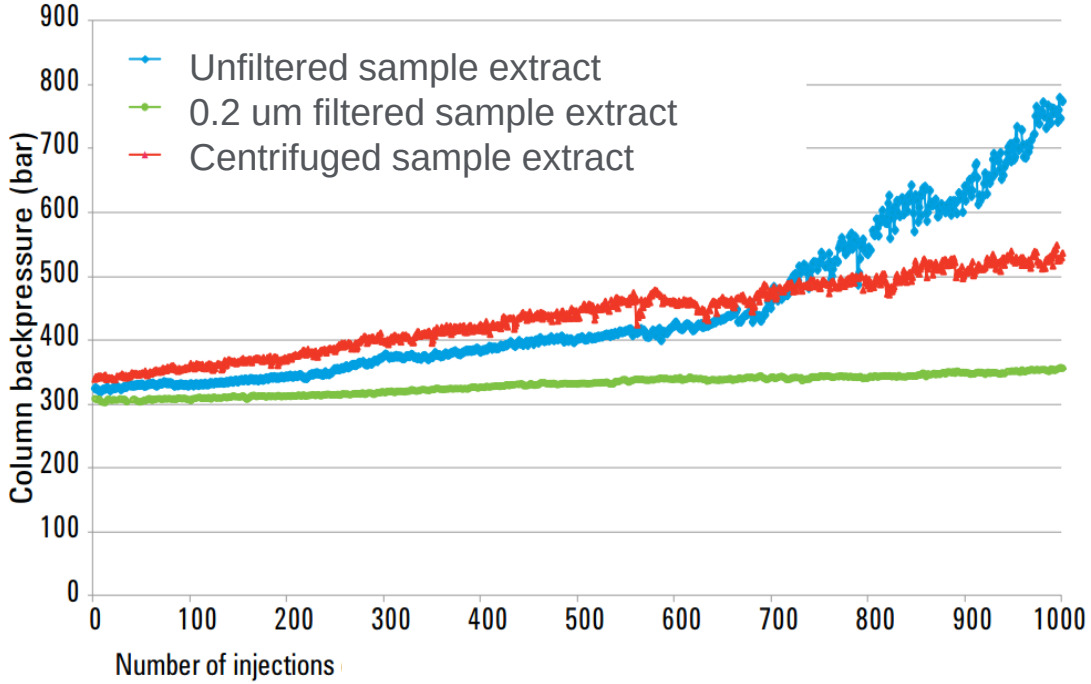
Sample
Filtration and its benefits

Physically removes particulates from the sample

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

Results in less downtime of the instrument for repair of wear and tear on the critical moving parts of the injection valves

Agilent **Captiva** line of filtration products come in different formats: syringe filter, filter vial, filter cartridge, and 96-well filter plate



Unfiltered, centrifuged, and filtered sample extracts
ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm column, p/n 959757-902

Captiva Syringe Filters Guide 5991-1230EN

Syringe Filter Selection Tool

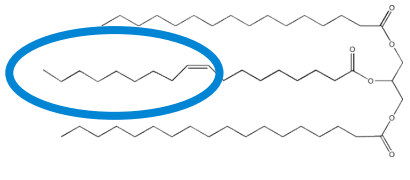
Sample
Captiva EMR–Lipid Filtration

- EMR: Enhanced Matrix Removal
- Removes particulates, proteins, and lipids
- Simple pass-through format
- Solvent-retention frit in 1 mL cartridge/96-well plate format for in well protein precipitation (Method Guide for 1 mL format)
- 3 mL and 6 mL cartridge format for larger samples (Method Guide for 3 mL and 6 mL format)
  - No solvent retention frit, which allows for gravity flow
  - Extraction performed offline (QUECHERS, for example)
- High analyte recoveries
- Effective use will reduce ion suppression, increase analyte sensitivity and detection, and extend the lifetime of your analytical column

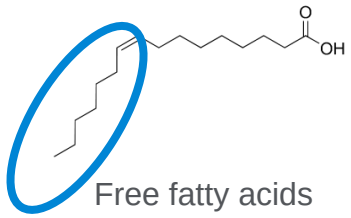
Sample
Enhanced Matrix Removal: EMR–Lipid

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

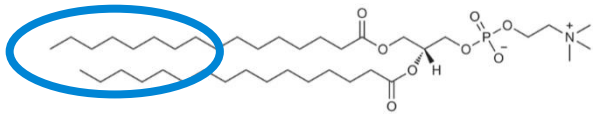


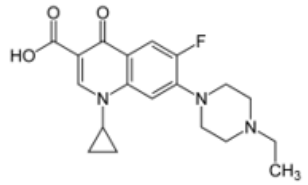
Triglycerides



Free fatty acids

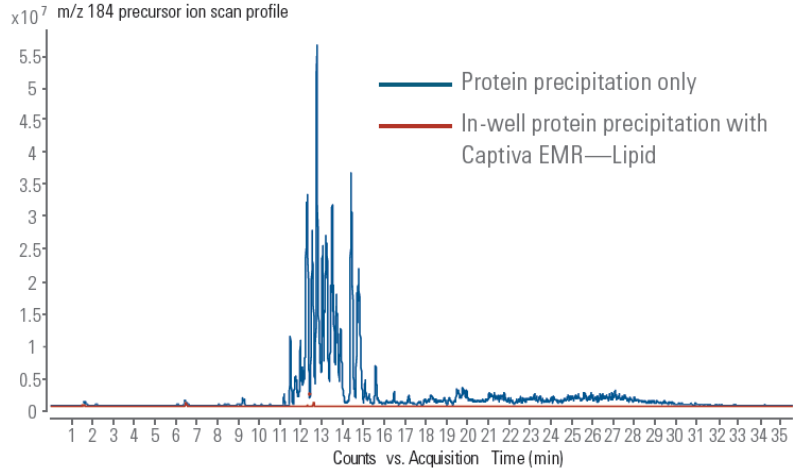
Phospholipids

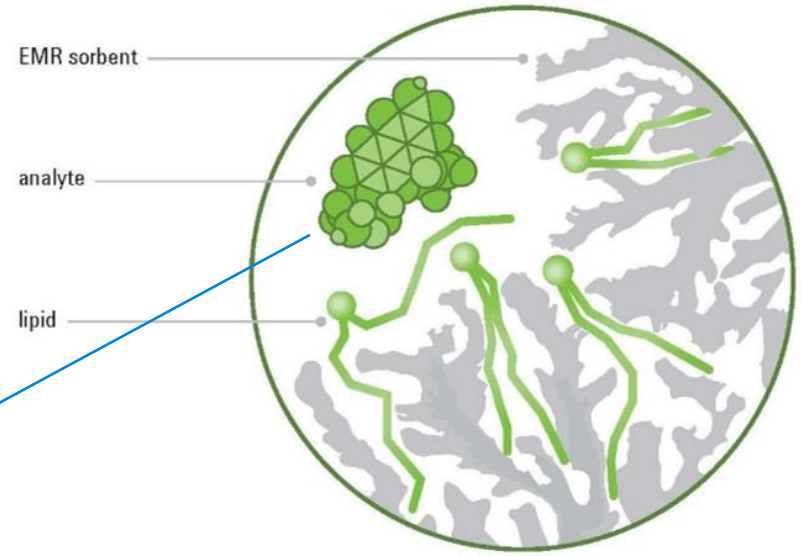




Fluoroquinolones

Effective phospholipid removal





Sample

Captiva EMR with Carbon S for pigment removal

Captiva EMR-HCF1(with NH2) & HCF2 (with PSA)

High Chlorophyll Fresh
- Spinach, Arugula, Chard etc.

Captiva EMR-GPF

General Pigmented Fresh
- Berries, Peppers, Broccoli etc.

Captiva EMR-GPD

General Pigmented Dry
- Spices, seasoning, Herbal medicine

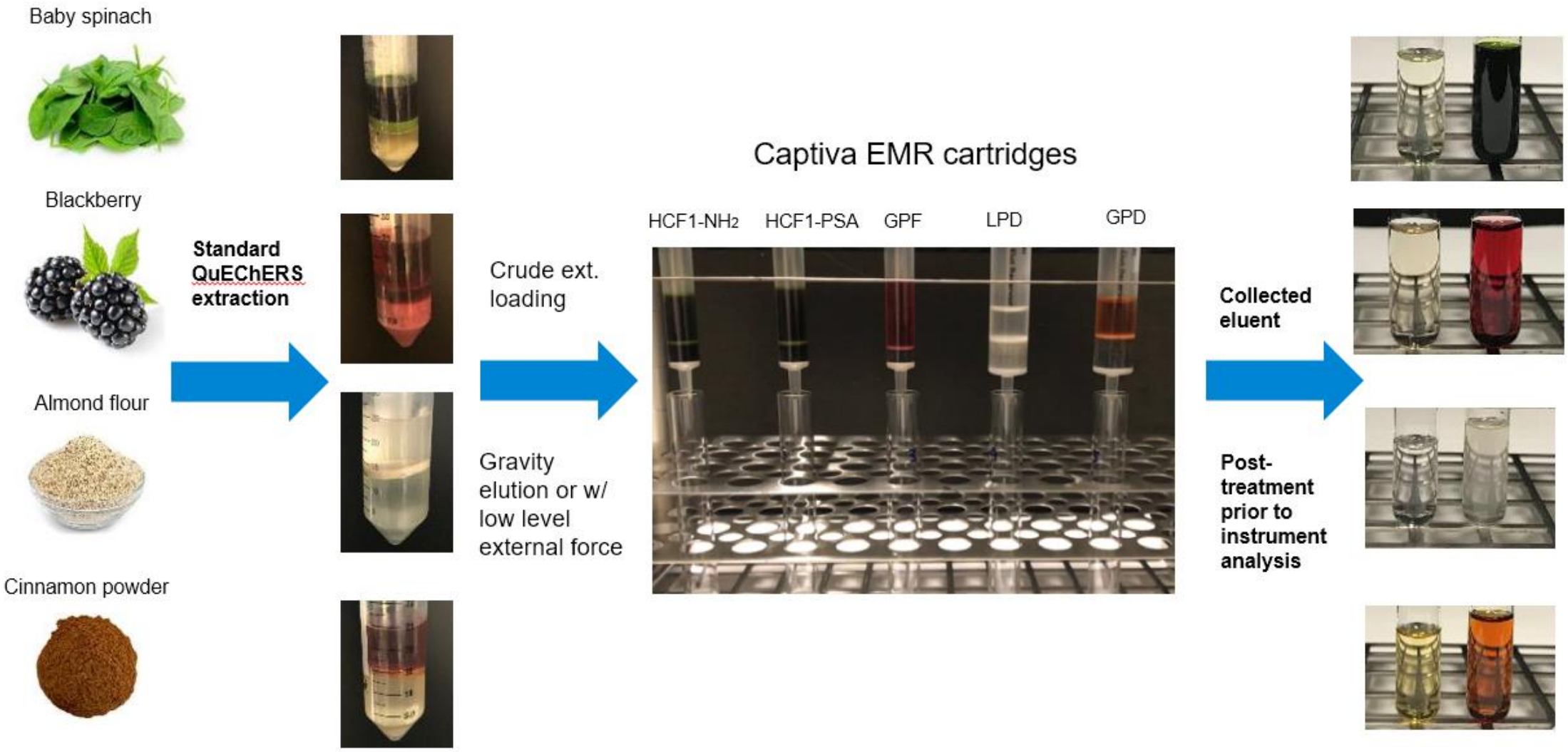
Captiva EMR-LPD

Low Pigmented Dry
- Nuts, tobacco, light pigmented spices

[Captiva EMR with Carbon S]

Sample

Captiva EMR with Carbon S, simplified pass-through workflow for pesticides



Sample
QuEChERS

QuEChERS: Quick Easy Cheap Effective Rugged Safe

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

Consists of two steps, and therefore two kits

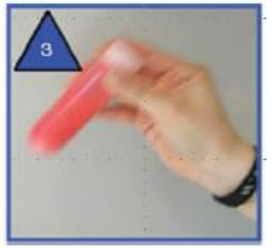
Step 1: Liquid extraction

Step 2: Dispersive SPE/interference removal

Sample
QuEChERS procedure

Weigh sample

Add solvent



Shake

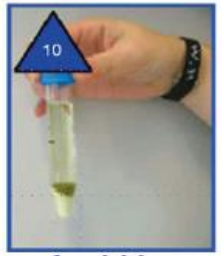
Add salts

Add internal standard

Shake and centrifuge

Transfer extract (top) for cleanup

Shake and centrifuge



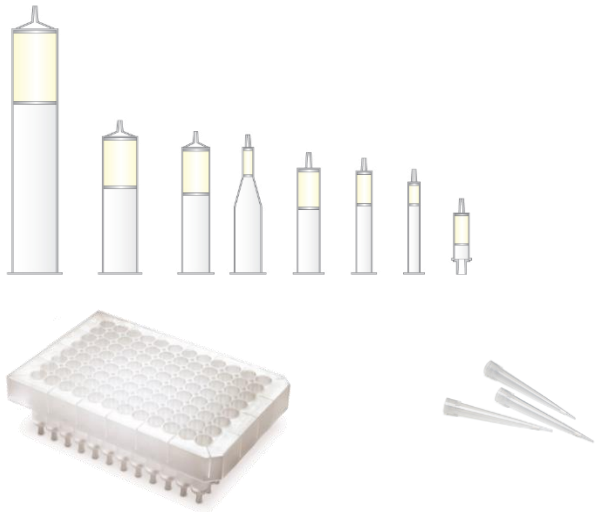
Transfer (dilute or concentrate) to vials

Analysis by GC or LC

LC-GC, **2008**, vol. 11 issue 1

Sample
Solid Phase Extraction (SPE)

- Reliable SPE with a 50-year history
- Agilent offers the most comprehensive set of phases, sizes, and formats of any SPE provider
- Easy adoption of methods due to high number of publications and applications
- Includes cartridges, 96-well plates, and pipette tips



Bond Elut silica SPE

Bond Elut AccuCAT
Bond Elut NH_2
Bond Elut C1
Bond Elut C2
Bond Elut C8
Bond Elut C18 .....
..... **40 phases**

Bond Elut polymer SPE

Bond Elut Plexa
Bond Elut Plexa PCX
Bond Elut Plexa PAX
Bond Elut PFAS WAX
Bond Elut HLB
Bond Elut Lipid Extraction

SampliQ SPE

Multiple phases

OMIX monolithic silica tip SPE

OMIX C18
OMIX C4
OMIX SCX

SPEC monolithic silica disk SPE

SPEC C2
SPEC C8
SPEC C18
SPEC C18AR
SPEC PH
SPEC NH2
SPEC CN
SPEC Si
SPEC PSA
SPEC SAX
SPEC SCX
SPEC MP1
SPEC MP3

Sample

Productivity benefits of sample cleanup

More matrix removal = less matrix entering system = time and cost savings

- ✓ Less matrix buildup
  - Fewer interferences
  - Improved S/N
  - Better reproducibility
- ✓ Better chromatography
  - Less time spent on data analysis/manual integration
  - Less time spent on reruns/recalibrations
- ✓ Less maintenance
  - Less instrument downtime
  - Saves money on consumables/services
- ✓ Less time spent on troubleshooting
  - Figuring out the source of the problem

Solvents

Solvents

Agilent InfinityLab solvents for HPLC and LC/MS

- Optimized and tested for Agilent instruments
- Excellent lot-to-lot reproducibility
- Lowest impurity levels
- 0.2 µm prefiltered
- Shipped in high-quality amber borosilicate glass bottles
- Shipped in 1 L or 4 L bottles

Description	Pack size	Part number
InfinityLab Methanol for LC/MS	6x1L	5191-5111
InfinityLab Acetonitrile for LC/MS	6x1L	5191-5101
InfinityLab Water for LC/MS	6x1L	5191-5121
InfinityLab Methanol Gradient Grade for HPLC	4x4L	5191-5110
InfinityLab Acetonitrile Gradient Grade for HPLC	4x4L	5191-5100
InfinityLab Water Gradient Grade for HPLC	4x4L	5191-5120

Brochure: 5994-6607EN

Solvents

Solvent filtration buffers

Compatible filter membranes

Part Number	Membrane Type
5191-4336	PTFE filter membrane, 47 mm diameter, 0.45 µm; 100/pack
5191-4339	PTFE filter membrane, 47 mm diameter, 0.20 µm; 100/pack
5191-4338	Nylon filter membrane, 47 mm diameter, 0.45 µm; 100/pack
5191-4341	Nylon filter membrane, 47 mm diameter, 0.20 µm; 100/pack
5191-4337	Regenerated Cellulose filter membrane, 47 mm diameter, 0.45 µm; 100/pack
5191-4340	Regenerated Cellulose filter membrane, 47 mm diameter, 0.20 µm; 100/pack

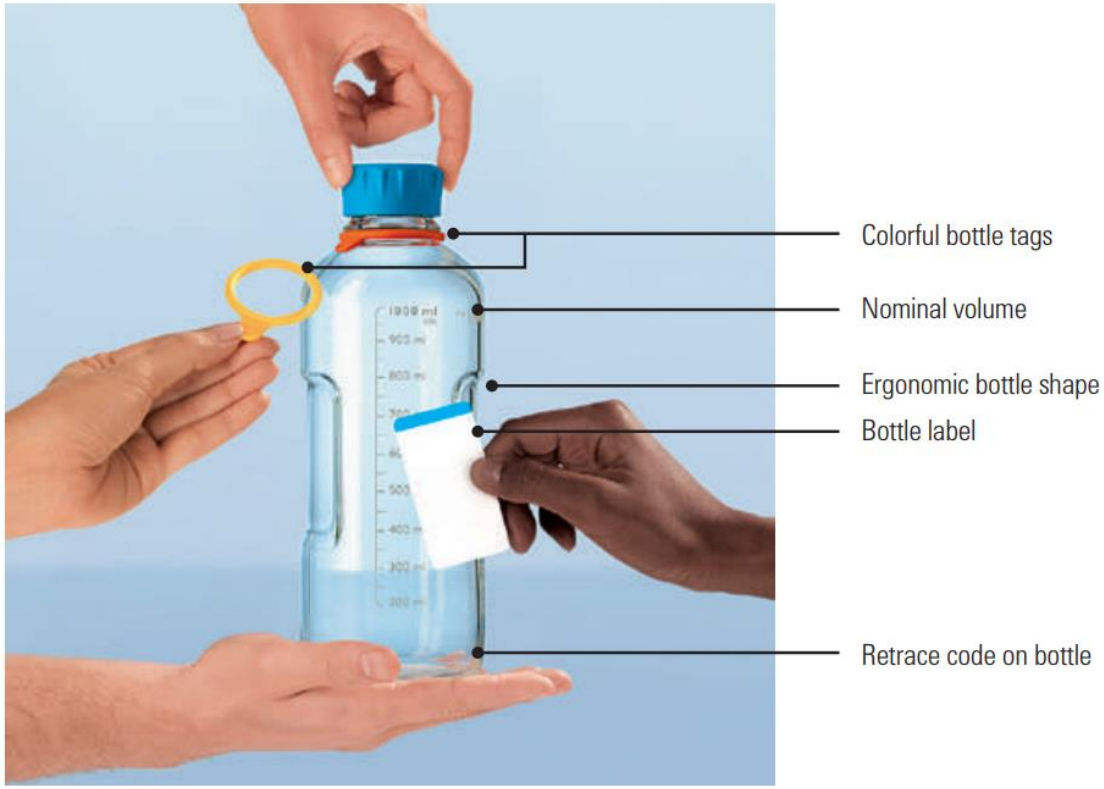
5191-6776

Operation manual: 5994-1507EN
Technical overview: 5994-1504EN

Supplies

Supplies

InfinityLab solvent bottles



Supplies

Solvent inlet filters

- Uniform porosity
- Packed in ultraclean antistatic bags with inner metallic coating
- Glass, analytical size, 20 µm, part number: 5041-2168
- Glass, preparative size, 40 µm, part number: 3150-0944
- Stainless steel, analytical size, 12 µm, part number: 01018-60025
- Stainless steel, preparative, 20 µm, part number: 5023-3115
- PTFE, bioinert, analytical size, 10 µm, part number: 3150-0958

Note: Solvent inlet filters are not a replacement for good mobile phase hygiene.

Supplies

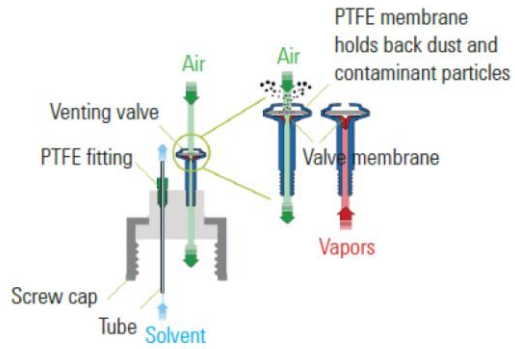
InfinityLab Stay Safe caps for solvent bottles and waste canisters

Stay Safe cap for solvent bottle

- Has a one-way venting valve with a time strip that allows clean air into the bottle, but stops harmful solvent vapors from getting into the lab

Stay Safe cap for waste containers

- Comes in different thread sizes of GL45, GL38 and S60, fitting to Agilent 6 L, 5 L, and 10 L waste canisters.
- Equipped with a charcoal filter and time strip that adsorbs vapors from solvent waste, ensuring clean air.



Time strips will tell you when to replace the venting valve and charcoal filter.

Brochure: [5994-1798EN]

Supplies
Fittings

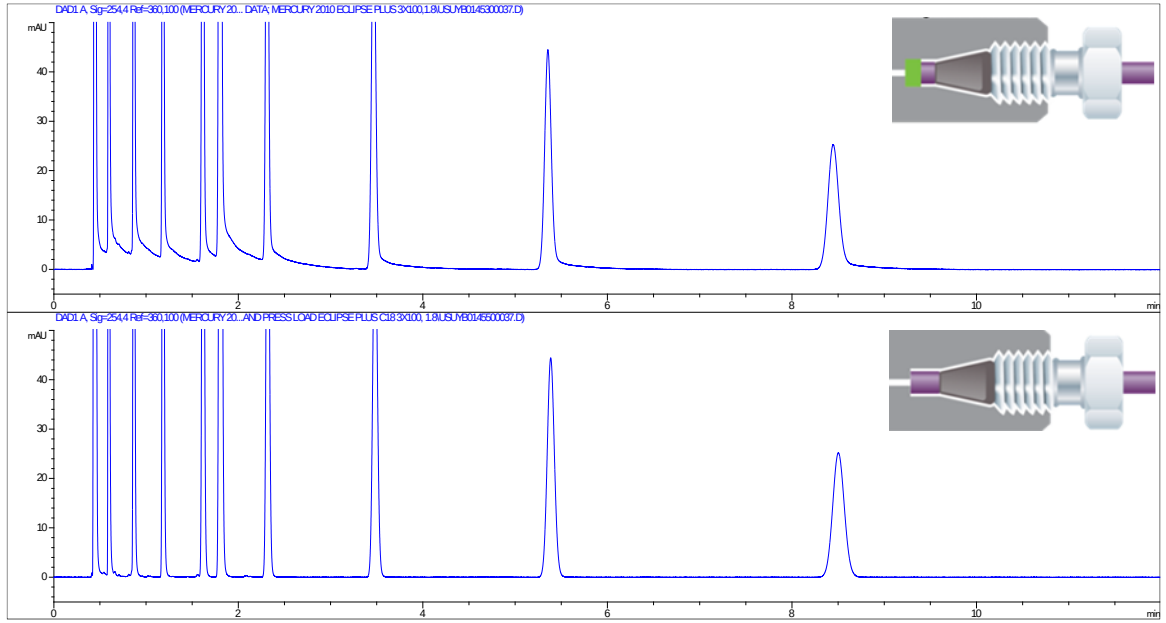
Improper fittings can cause:

- Broad or tailing peaks
- Loss of resolution
- Column inlet/outlet damage
- Leaks

Connection problems can lead to:

- Downtime
- Time spent on troubleshooting
- High cost of operation

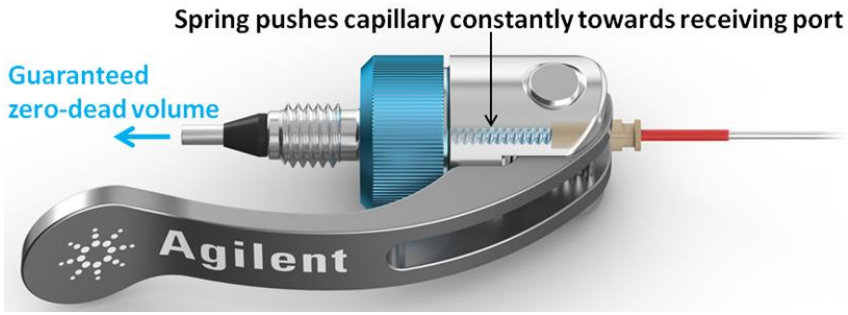
Effect of connections on chromatography



Supplies
Fittings

InfinityLab Quick Connect and Quick Turn fittings

- Spring-loaded design
- Easy; no tools needed
- Works for all column types
- Reusable
- Consistent ZDV connection



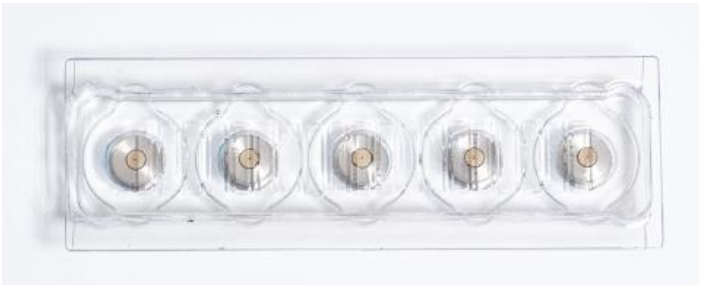
Quick Connect fitting
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

Quick Turn fitting
- Finger tight up to 400 bar
- Up to 800 bar with mounting tool
- Up to 1300 bar with a wrench
- Compact design

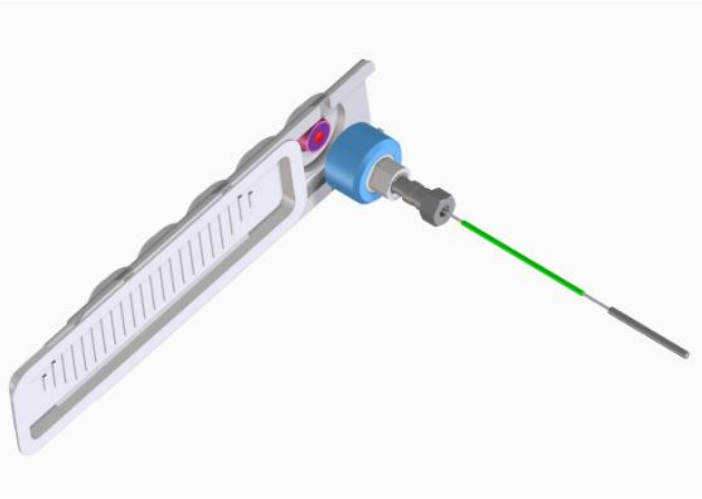
Supplies

InfinityLab Quick Change inline filter

- **A variety of dimensions and porosities** – filter discs are available in 2.1 mm and 4.6 mm inner diameters with different pore sizes. The filter housing is compatible with all types of filter discs.
- **Touchless packaging to avoid potential contamination** – with the special designed packaging, you're able to insert the filter disc into filter housing without touching it, avoiding potential contamination
- **In situ replacement** of filter disc – no need to disconnect the inline filter from the system
- **Can be placed after the pump, as well as between the autosampler and guard/column**
- **Available with rigid capillary and integrated Quick Turn fitting, or flexible capillary**



2.1 mm 0.2 µm	2.1 mm 0.5 µm	4.6 mm 0.2 µm	4.6 mm 0.5 µm	4.6 mm 2.0 µm



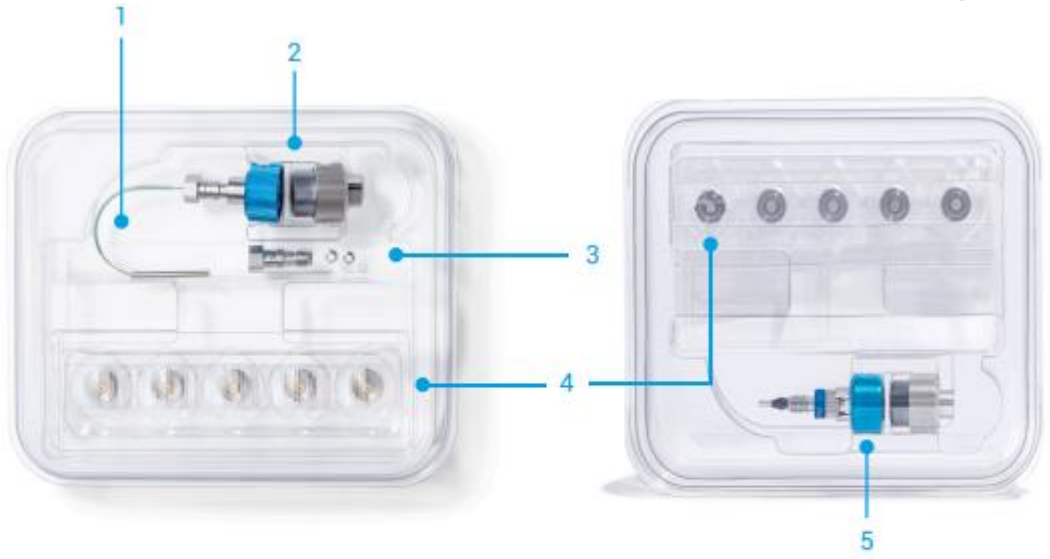
- Video
- Flyer: 5994-3028EN
- Installation instructions: 5994-2779EN

Supplies
InfinityLab Quick Change inline filter

Part Number	Inline Filter Assemblies
5067-1603	InfinityLab Quick Change inline filter assembly for UHPLC (incl. 5 filter discs 2.1 mm id, 0.2 µm porosity), with 90 mm flexible capillary
5067-1602	InfinityLab Quick Change inline filter assembly for HPLC (incl. 5 filter discs 4.6 mm id, 0.5 µm porosity), with 90 mm flexible capillary
5067-1606	InfinityLab Quick Change inline filter assembly with rigid capillary for HPLC (incl. 5 filter discs 4.6 mm id, 0.5 µm porosity)
5067-1607	InfinityLab Quick Change inline filter assembly with rigid capillary for UHPLC (incl. 5 filter discs 2.1 mm id, 0.2 µm porosity)
	Filter Discs
5067-1610	Filter discs, 2.1 mm id, 0.2 µm porosity, 5/pk
5067-1611	Filter discs, 2.1 mm id, 0.5 µm porosity, 5/pk
5067-1612	Filter discs, 4.6 mm id, 0.2 µm porosity, 5/pk
5067-1613	Filter discs, 4.6 mm id, 0.5 µm porosity, 5/pk
5067-1614	Filter discs, 4.6 mm id, 2.0 µm porosity, 5/pk
	Replacement Capillaries
5023-3344	Capillary, stainless steel, 0.12 mm id, 90 mm length, 2x extra-long fittings, pre-swaged on one end, non-swaged on the other end, for inline filter for UHPLC
	Quick Turn Fitting
5067-5966	Quick Turn Fitting
5043-0924	Ferrule for Quick Turn Fitting

Assembly with flexible capillary

Assembly with rigid capillary and integrated Quick Turn fitting



1. Capillary, SST, 90 mm length
2. Filter housing (two parts)
3. Loose fitting for non-swaged end of capillary
4. Filter discs in touchless packaging, 5/pk
5. Filter housing with Quick Turn fitting

Supplies
Vials

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 of 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.

Supplies
Pump supplies

Pump supplies to keep on hand

- Replacement PTFE frits and gold seal for purge valve
- Piston seals
- Inlet valve cartridge
- Outlet ball valve
- Solvent inlet filters
- Replacement frits for inline filter

Typical frequency of replacements

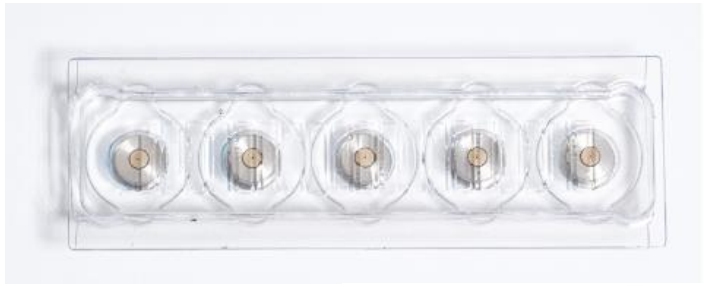
Item	Typical Schedule	Comments
Solvent inlet filter	Replace every 6 - 12 months	
PTFE frits in purge valve and gold seal	Every 12 months	
Piston seals	Every 12 months	When changing the seal, check the piston for scratches; replace if scratched
Inlet valve cartridge, outlet ball valve	Every 24 months	

Supplies

Autosampler and column compartment supplies

Supplies to keep on hand

- Needle assembly
- Loop capillary
- Needle seat
- Injection valve rotor seal
- Metering device seal
- Inline filter replacement frits
- Restriction capillary
- Guard/guard cartridges
- Zero dead volume (ZDV) union



Supplies
Detector supplies – UV/DAD

Supplies to keep on hand

UV/DAD

- Lamps
- Flow cell
- Flow cell repair kit

Supplies
Detector supplies – MS

- Nebulizer
- Nebulizer needle replacement kit
- Ion transfer capillary
- Oil for vacuum pump
- Filter element for oil
- Gas purifying filters/traps
- Cleaning supplies (wire, abrasive mesh/powder, Alconox, lint-free cloth, cotton swabs)
- Tools (magnifier, needle nose pliers, wrenches)
- LC/MS calibration standard

Routine Maintenance	Frequency
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

Instrument

Maintenance and best practices

Instrument
Typical maintenance schedule*

Pumps

Item	Typical Schedule	Comments
Solvent inlet filter	Replace every 6 to 12 months	
PTFE frits in purge valve and 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/playlist?list=PLThrdl2ragolmT3J-W5r8ailvJN94DJMR

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

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

Instrument
Pump

Performance characteristics

- Flow accuracy can affect retention time and peak area precision
- Pressure pulsation can affect baseline noise

With a gradient pump

- Delay volume can affect gradient shape and precision
- Mobile phase composition accuracy and precision can affect retention times and peak area precision

Instrument
Autosampler

Performance characteristics

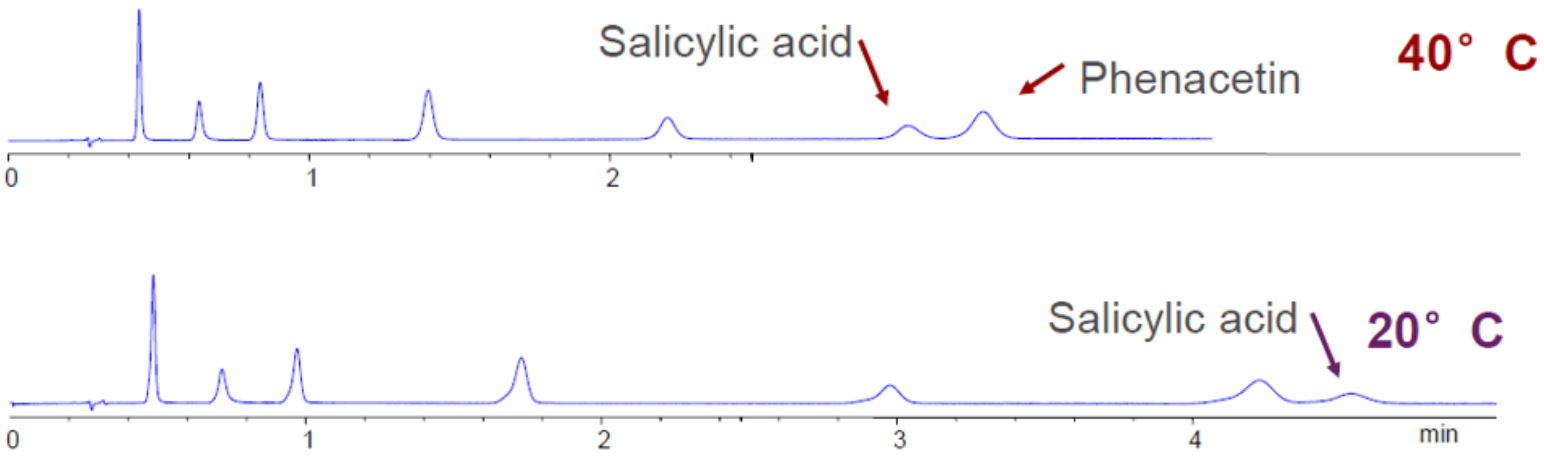
- Injection volume precision. It can affect peak area/height precision.
- Wide linearity of injection volume. It can affect the accuracy of peak area/height (when using different injection volumes).
- Minimum carryover. Carry over issue can affect precision of peak area/height.

Instrument

Thermostatted column compartment (TCC)

Performance characteristics

- Temperature accuracy. Affects elution order and peak identification.
- Temperature precision can affect elution order, retention time precision, and peak identification.



Instrument
Detectors

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
- Needs strict temperature control

ELSD
- Independent of a compound's absorbance, fluorescence, or electro-activity
- Enables detection of semivolatile and thermally sensitive compounds

FLD
- More selective and can be more sensitive
- Compounds must fluoresce; compounds are often derivatized

Instrument
UV-Vis detector

Performance characteristics

VWD and DAD

- Low noise, wonder, and drift. Affects detection and quantitation limits.
- Wide linear range. Affects quantitation at low and high concentrations.
- Wavelength accuracy and precision. Affects peak area/height accuracy and precision.

DAD only

- Spectral resolution. Affects accuracy of spectra and peak identification by spectra.
- Spectral sensitivity. Affects accuracy of spectra and peak identification by spectra at low concentrations.

Instrument

MS detector maintenance

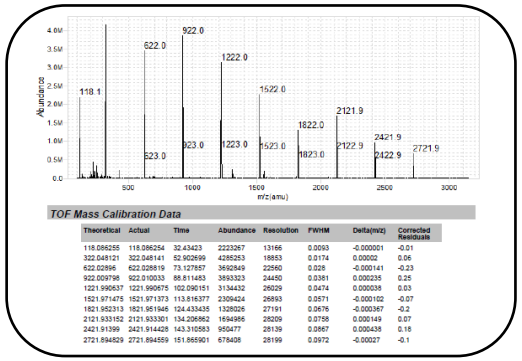
Routine maintenance	Frequency
Flush the nebulizer	Daily after use to flush the tubing, valves, and nebulizer
Replace the nebulizer needle	When plugged
Clean the spray chamber	Daily or when carryover is suspected
Check the rough pump fluid level	Check weekly for color and level; replace every six months

LC/MS Best Practices

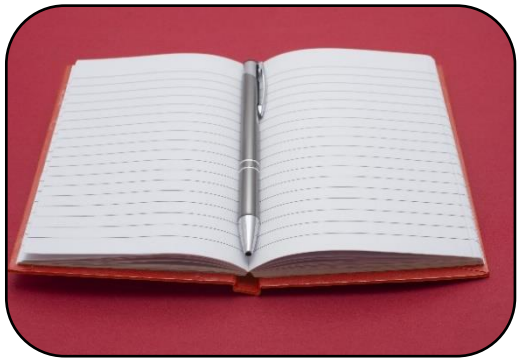
Tuning and daily operation

Run Checktune or calibration with fresh tuning mix

Check nebulizer spray, needle position, and clean the source

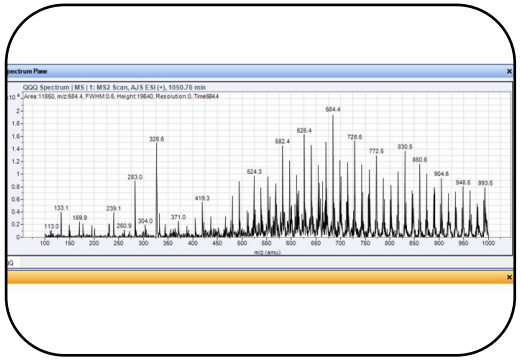


Review tune and calibration reports in \MassHunter\Tune

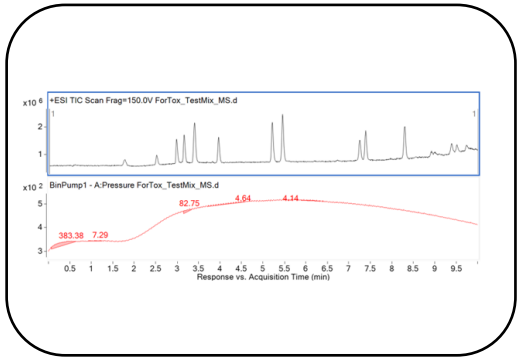


Keep detailed records of system use and maintenance history

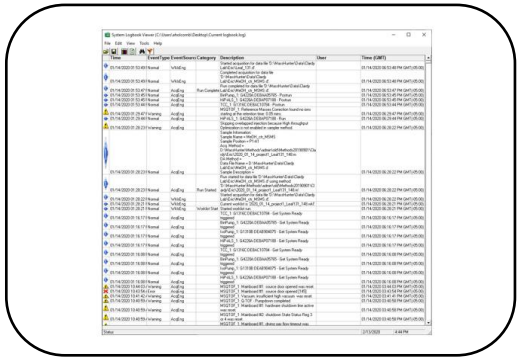
Purge LC and run blank injections



Monitor background scans for contamination



Know your method and run a known test mix



Review MassHunter logbooks

LC Best Practices

Daily system start

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

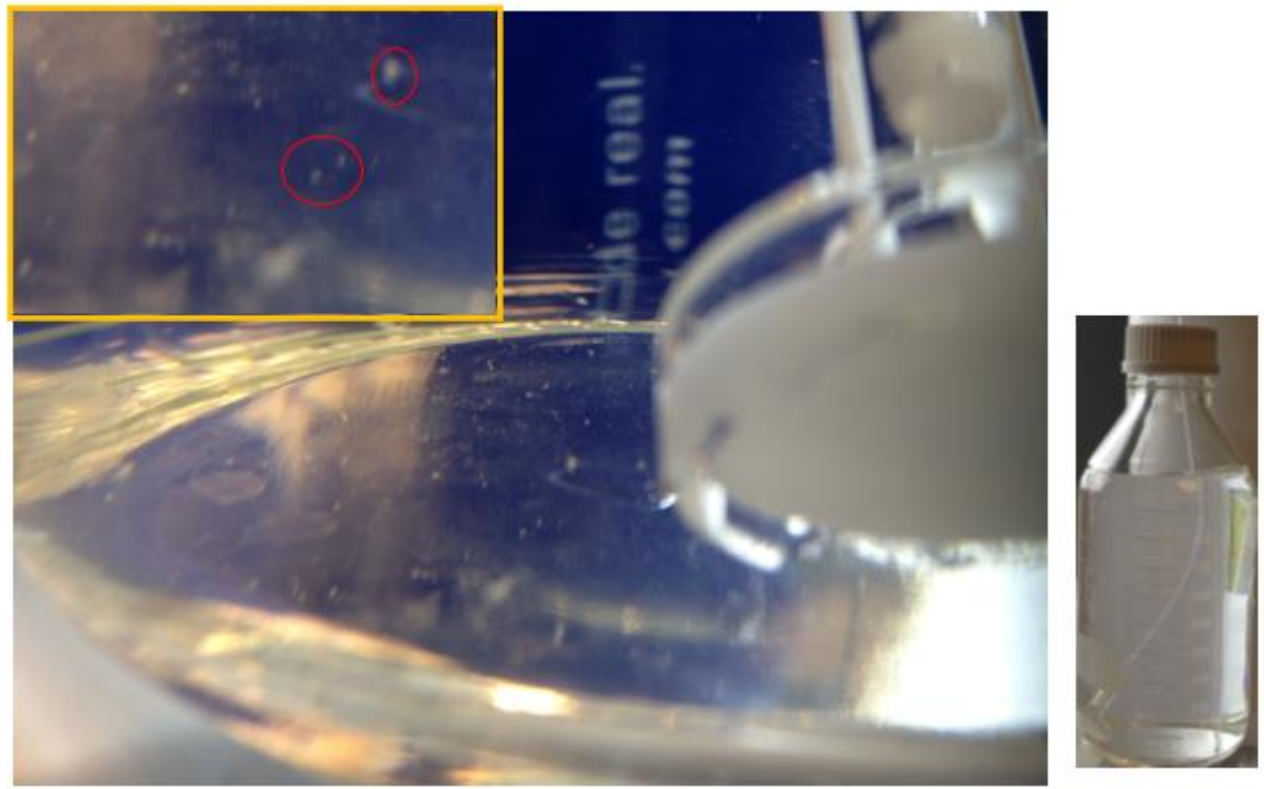
Weekly

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

LC Best Practices
Technical Note: 01200-90090

LC Best Practices

Prevent microbial growth



Microbial growth in the aqueous solvent bottle after a week

Method

Method

Method conditions

Mobile phase

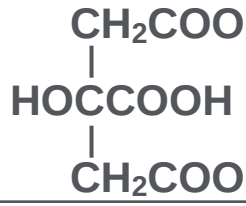
- HPLC or MS grade solvents
- Buffer – right choice, column, LC, LC/MS, filtered?
- Mobile phase preparation procedure
- Fresh mobile phase
- Bottles covered? No paraffin sheet
- Label bottles, content and date
- Amber bottle for aqueous
- Make sure the system is flushed before introducing a new mobile phase
- pH

Temperature

Pressure

Standards, test mix

Method
Buffer options

Nonvolatile:		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

Method
Mobile phase preparation

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

- Use HPLC grade or better
- Buffer preparation procedure
  - Be consistent
  - Document the process

Volume % of solvents can depend on preparation

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

≠

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

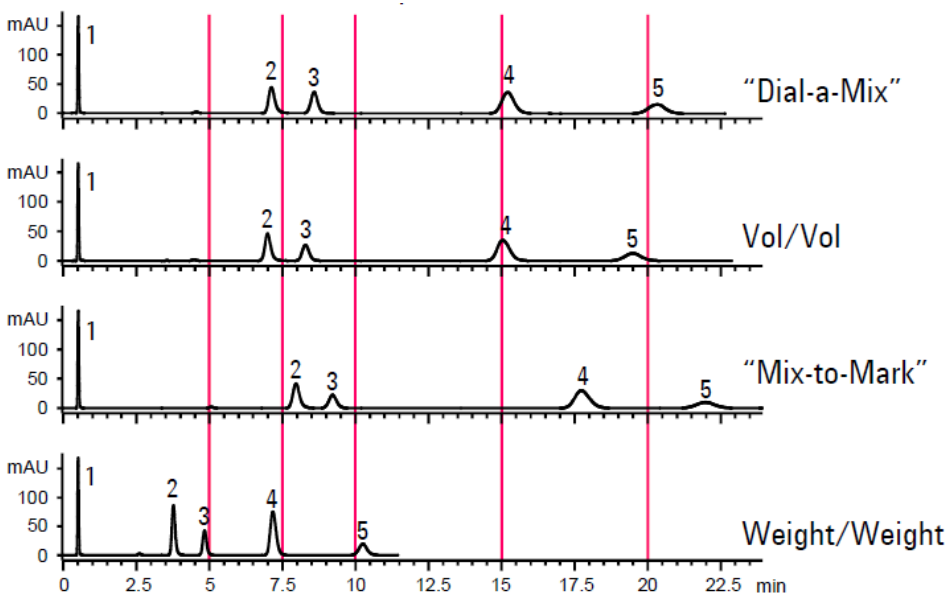
≠

500 mL H_2O added to 500 mL ACN

Degree of contraction is affected by the relative quantities of each

Method

Effect of mobile phase preparation 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 mobile phase can significantly affect the elution pattern
- Be consistent
  - w/w is more accurate than v/v

5988-6476EN

Column


Agilent InfinityLab
Poroshell 120
Agilent InfinityLab
Poroshell 120

Column

- Choice
  - Column specifications, conditions, flow rate, pressure, pH
- Performance report
- Datasheet or column guide
- Equilibration
- Benchmark with your system
- Inline filters or guards
- Store properly when done

Column

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
Aq-C18	120Å	90 °C	1.0–8.0	Yes	Proprietary	130 m2/g	L1
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
CS-C18	100 Å	90 °C	1.0–11.0	Yes	Proprietary	95 m2/g	L1
HPH-C18	100 Å	60 °C	2.0–11.0	Yes	Proprietary	95 m2/g	L1
HPH-C8	100 Å	60 °C	2.0–11.0	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	1.0–8.0	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

5991-9123EN

Column

Column performance report

SERIAL NUMBER: USDAZ01333

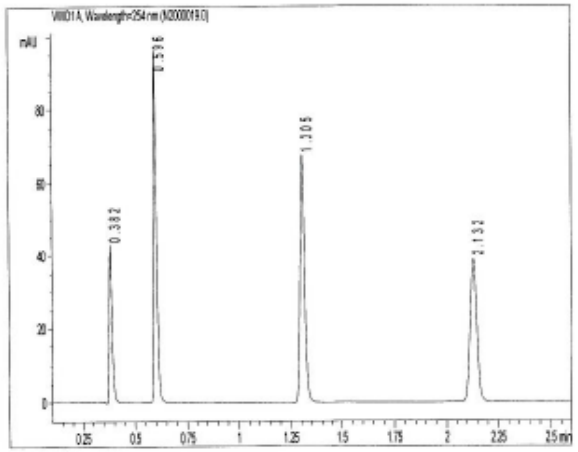
PART NUMBER: 959758-902
COLUMN TYPE: ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm
PACKING LOT #: B09089

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 extra column volume 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 user 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

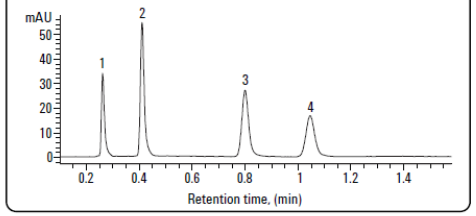
Data Sheet

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	850 µg/mL



Column

Initial column and system equilibration*

If your method calls for a buffered mobile phase, in an appropriate vessel, test highest % organic/buffer ratio to verify that the buffer will not precipitate. With stirring, add organic to buffer first, not vice versa.

Equilibrate the reversed phase column with following solvents, in this order:

- 100% organic modifier
- 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 the standard or sample several times until retention time is stable, or for gradient methods, precede the former with one or two blank gradients.

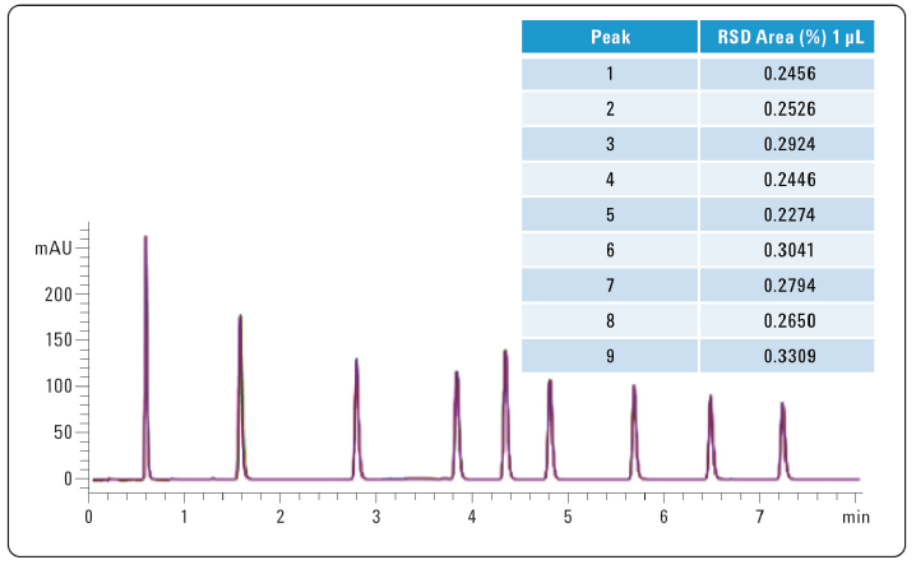
*Or follow instructions in your column user guide

Column

Benchmarking the column

Benchmark a 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
3. Theoretical plates
   - Monitor column over time
   - Troubleshoot



Peak	RSD Area (%) 1 µL
1	0.2456
2	0.2526
3	0.2924
4	0.2446
5	0.2274
6	0.3041
7	0.2794
8	0.2650
9	0.3309

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)

1. Acetophenone
2. Propiophenone
3. Butyrophenone
4. Valerophenone
5. Hexanophenone
6. Heptanophenone octanophenone
7. Benzophenone
8. Acetanilide

Column
Do you need a guard?

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 that have a 5 µm, 3 µm or 3.5 µm packing and **400 bar pressure limit.**

Agilent Fast Guard columns (3/pk) are pre-assembled stainless steel **UHPLC** guards packed with **1.8 µm** or **2.7 µm** materials.

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

Column
Storage

Follow the instructions in the column user manual

Reversed phase columns

- Long term storage of silica-based columns should be in a pure organic solvent such as acetonitrile.
- If using buffered mobile phase, remove buffer by purging the column with 20 to 30 column volumes of a 50:50 mixture of methanol or acetonitrile and water, followed by 20 to 30 column volumes of the 100% organic solvent.
- Remove the column from the instrument and tightly cap with end plugs. Store in a safe place

Summary

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

Resources for Support

- Agilent University training http://www.agilent.com/crosslab/university
- Tech support http://www.agilent.com/chem/techsupport
- Agilent University resource page Agilent Collection of Columns, Supplies, and Standards Resources - Wiki - Consumables - Agilent Community
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
- InfinityLab LC Supplies catalog (5991-8031EN)
- LC handbook (5990-7595EN)
- Best Practices for using an Agilent LC system (01200-90090)
- LC Troubleshooting poster (5994-0709EN)
- Youtube – Agilent Channel (maintenance videos)

Contact Agilent Chemistries and Supplies Technical Support

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

- Option 1 for GC and GC/MS columns and supplies
- Option 2 for LC and LC/MS columns and supplies
- Option 3 for sample preparation
- Option 4 for spectroscopy supplies
- Option 5 for chemical standards
- Option 6 for former Prozyme products

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

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Web chat: Product pages of agilent.com

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

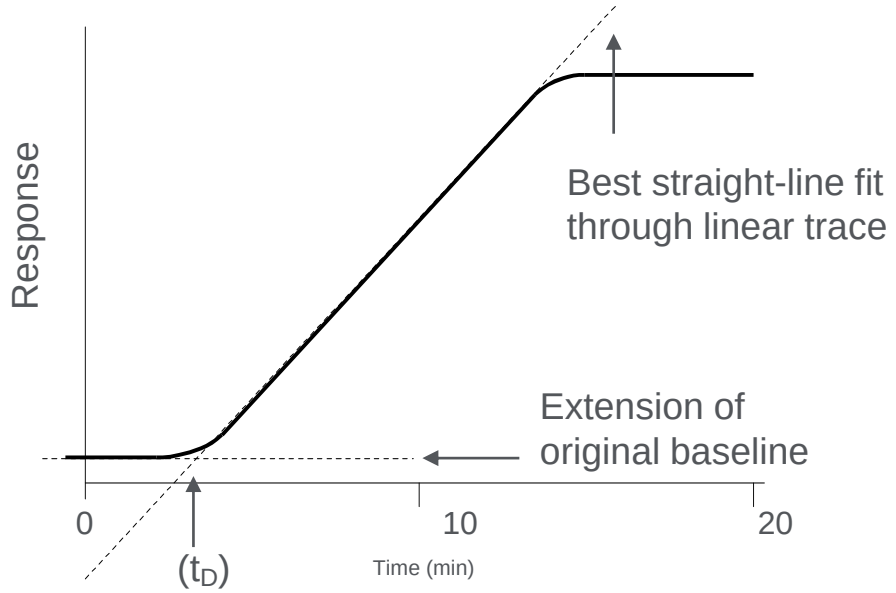
Monitor at 265 nm

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)

$V_D = t_D \times F$

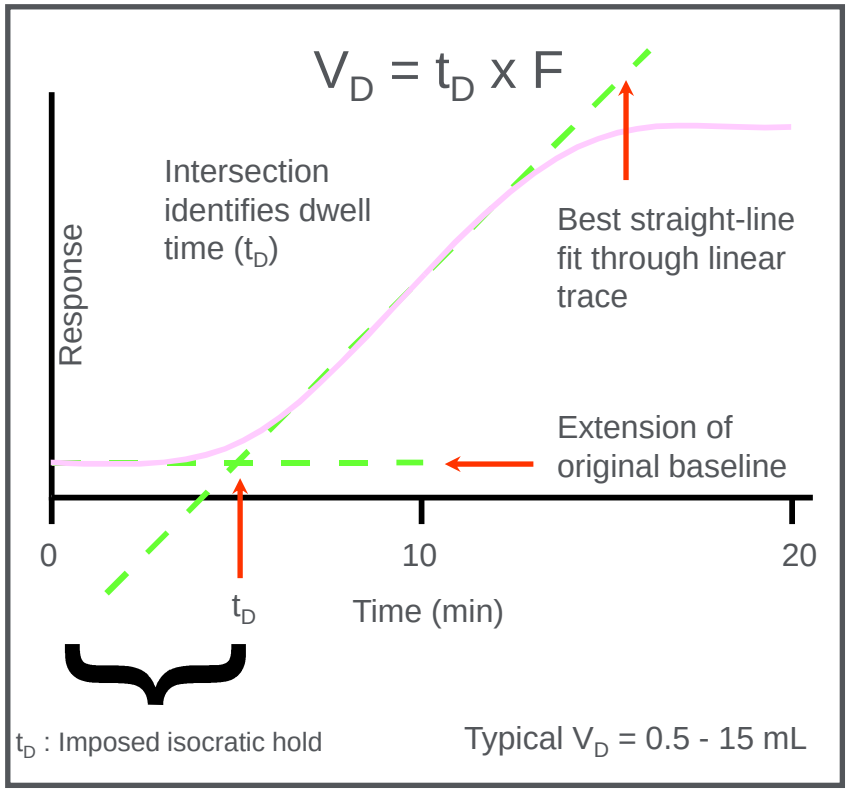
V_D = Dwell volume

Measuring Dwell Volume

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

Dwell volume impact

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

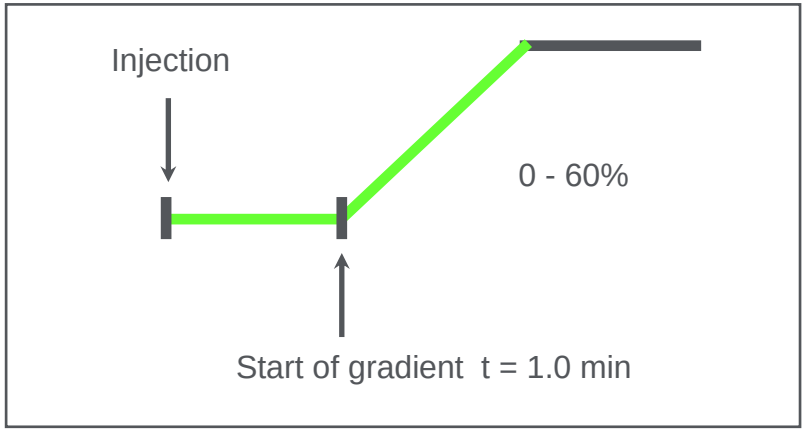


High pressure mixing: V_D = mixing chamber + connecting tubing + injector

Low pressure mixing: V_D = the above + pump heads + associated plumbing

Correcting for Dwell Volume

1. Measure the dwell volume of your HPLC system
   V_D = 1.0 mL
2. Draw an effective gradient profile at the 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



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)

Shutdown State and Instrument Flushing

Shutdown state

Next day use—using same buffers

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

When flushing the column or for longer-term column storage

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

Instrument flushing

✓ Replace the column with capillary tubing. Leave disconnected from detector

✓ Flush pumps with water, then connect capillary tubing to the detector

✓ Inject water two to three 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 a 1 or 2 L beaker. Use an appropriate volume to leave room for the pH adjustment solution. Equilibrate the solution to room temperature for maximum accuracy.
2. Calibrate pH meter. Use two-level calibration and bracket the desired pH. Use an appropriate audit solution to monitor statistical control (for example, potassium hydrogen tartrate, saturated solution, pH = 3.56).
3. Adjust the salt solution to the desired pH. Minimize the amount of time the electrode spends in the buffer solution (contamination). Avoid overshoot and readjustment (ionic strength differences can arise).
4. Transfer the pH-adjusted buffer solution quantitatively to a volumetric flask, dilute to volume, and mix.
5. Filter through 0.45 µm filter. Discard the first 50 to 100 mL filtrate. Rinse the solvent reservoir with a small volume of filtrate and discard. Fill the reservoir with the remaining filtrate or prepare a premix with the organic modifier.
    - InfinityLab solvent filtration assembly (includes 250 mL funnel, membrane holder base, 1L flask and aluminum clamp), p/n 5191-6776
    - Nylon filter membranes, 47 mm, 0.45 µm pore size, 100/pk, p/n 5191-4338