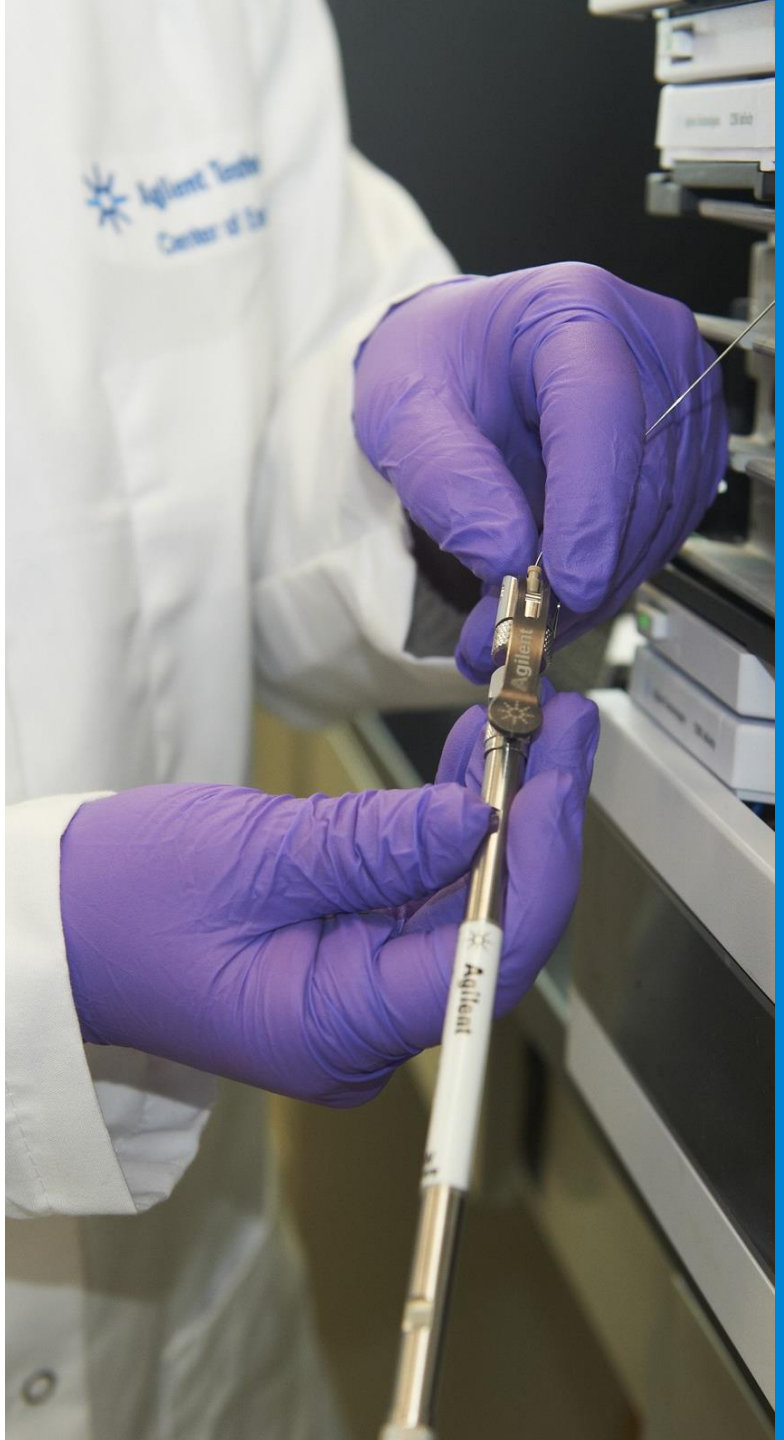
Techniques for Avoiding Unexpected Problems in LC and GC Analysis

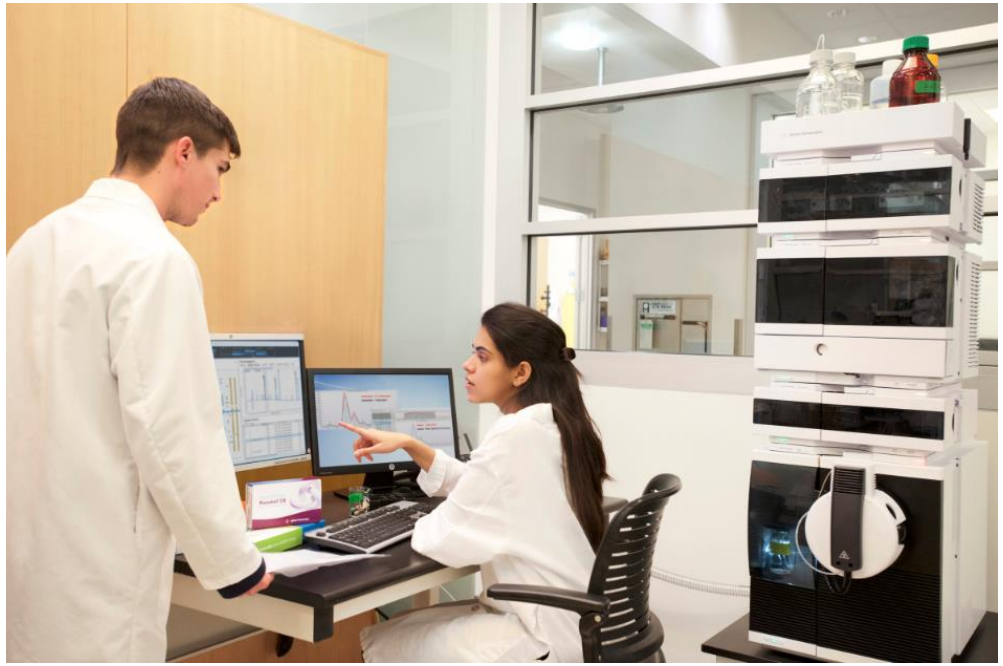
Alexander Ucci
Applications Engineer
February 11, 2020



Agilent
Trusted Answers

Agenda

- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- Summary



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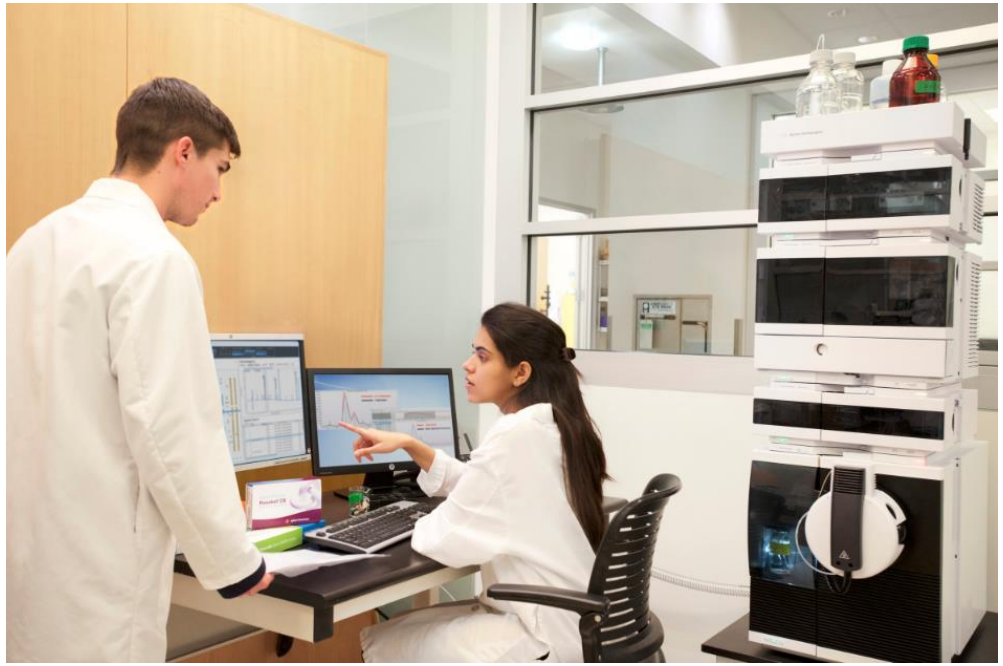


Dilute and Shoot

Advantages

- Fast and easy
- High throughput

GC inlet liner

GC inlet seal

Limitations

- Interferences are not removed
- Analyte concentration is reduced
- Instrument and column contamination
- Matrix interferences – ion suppression or poor peak shapes

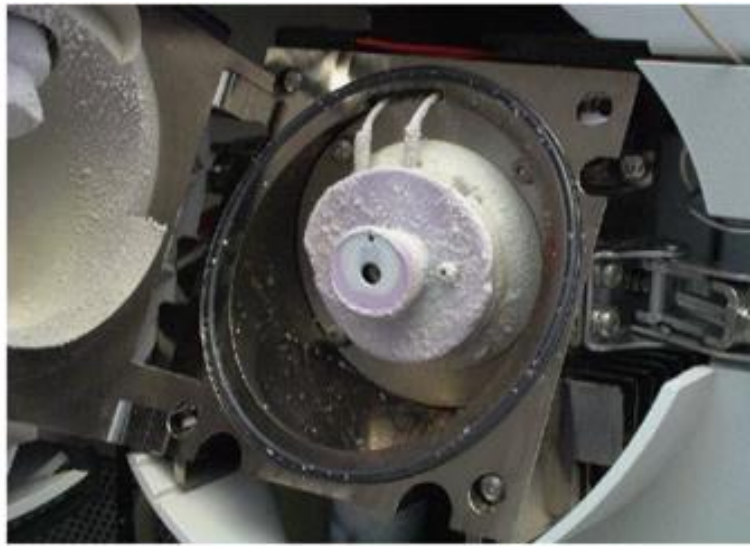


Image of salt buildup on an ESI-LC/MS inlet from unremoved salts.

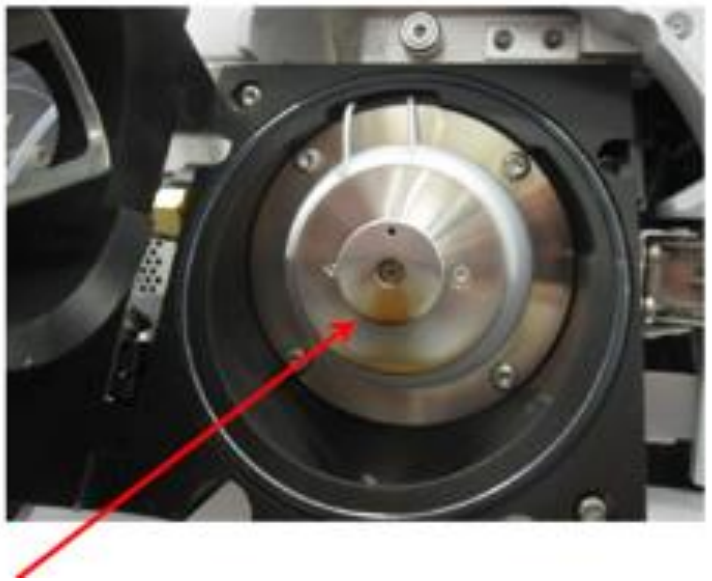


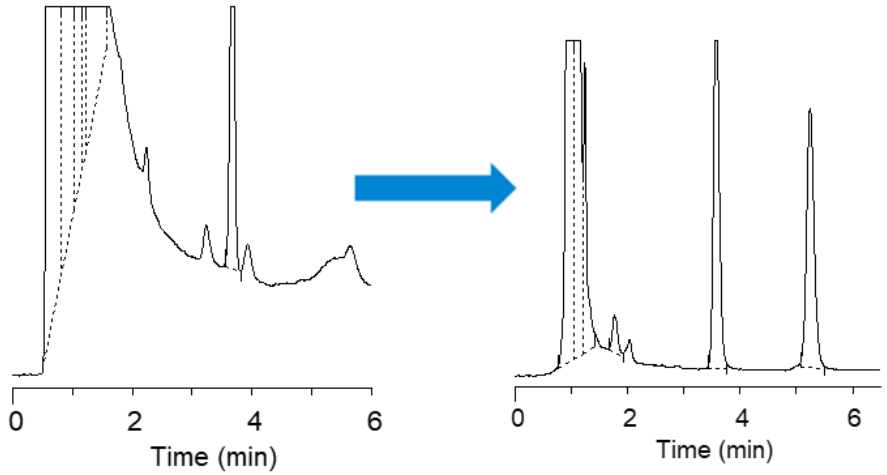
Image shows the build up on the ESI-MS inlet after 3000X urine dilute and shoot injections.

Importance of the Correct Sample Preparation/Cleanup

Target analytes are the needle in the haystack of a matrix, sample preparation helps find the needle in the haystack.

- Protect the instrument detection system from contamination
- Improve the detection, method robustness, and reliability

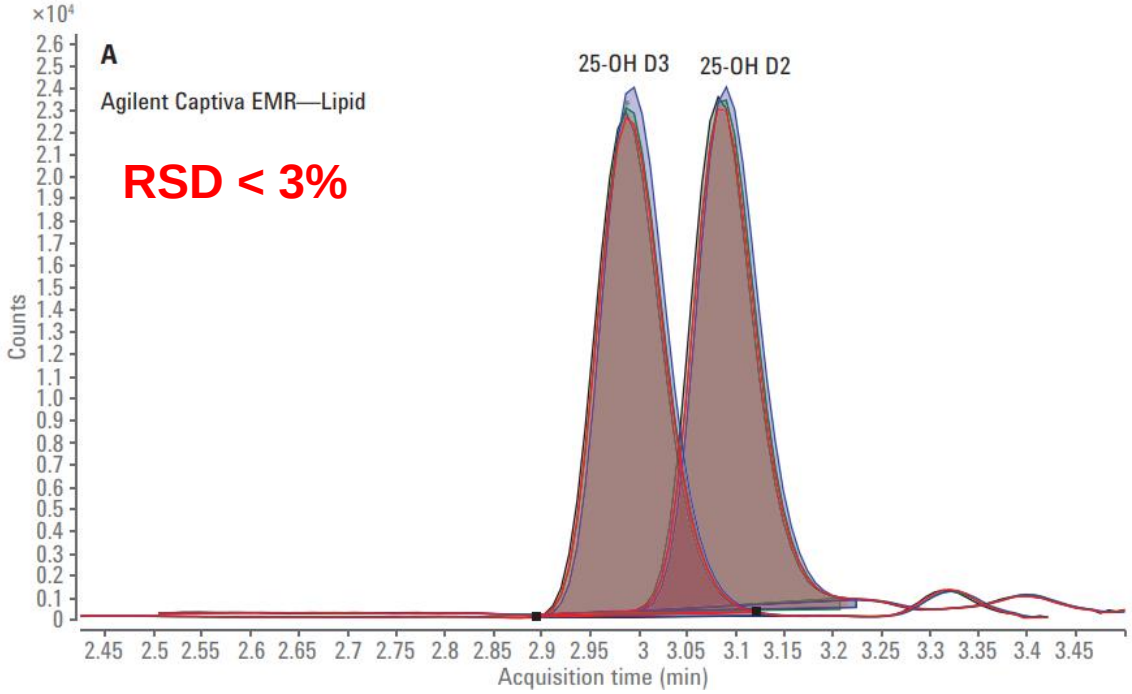
Sample *without* sample preparation



Sample *with* sample preparation

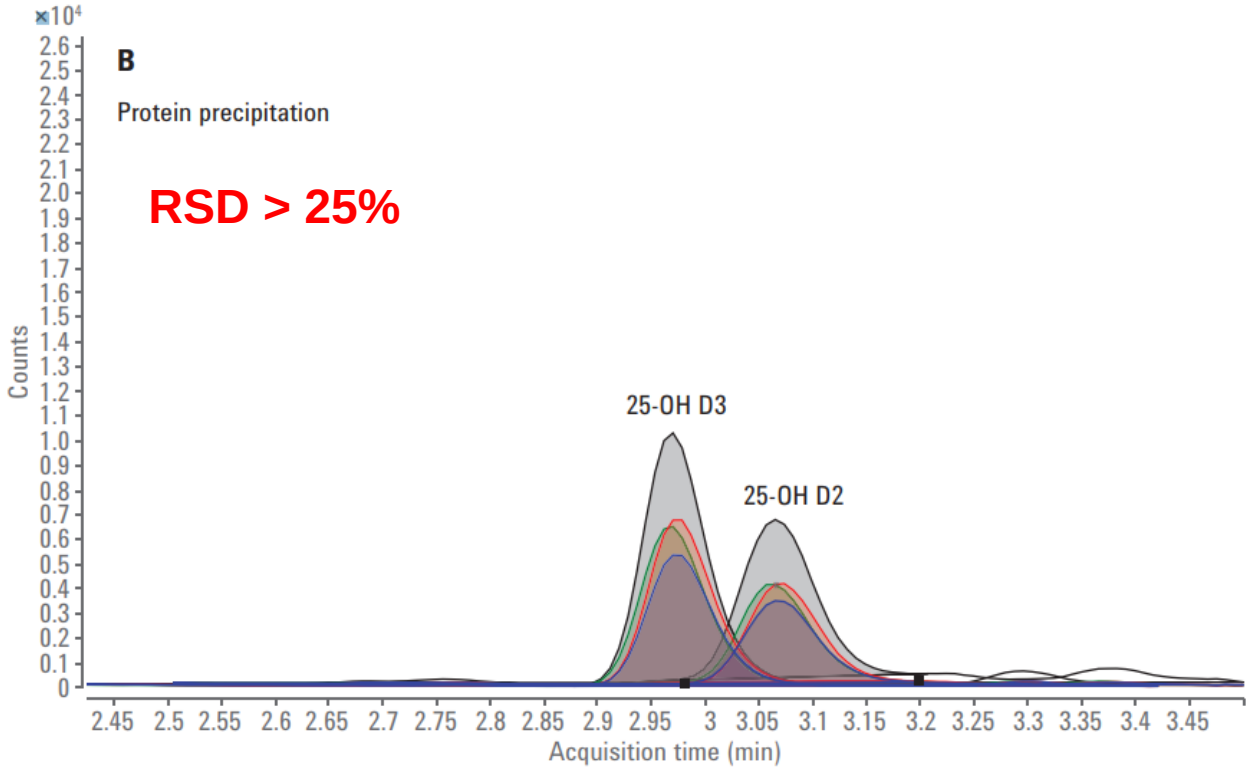
Importance of the Correct Sample Preparation/Cleanup

Captiva EMR-Lipid



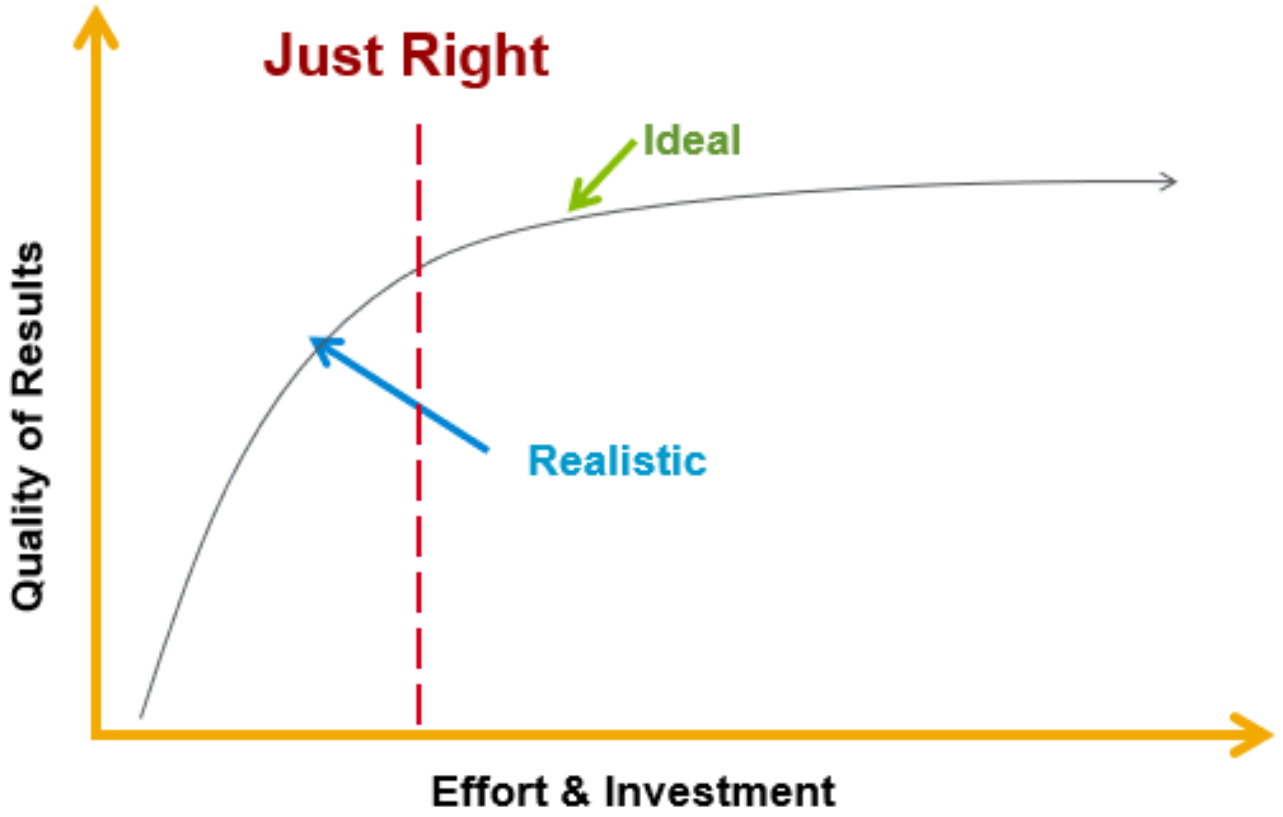
Sample *with* the correct sample preparation

Protein precipitation



Sample *without* the correct sample preparation

Striking the Right Balance in Sample Preparation

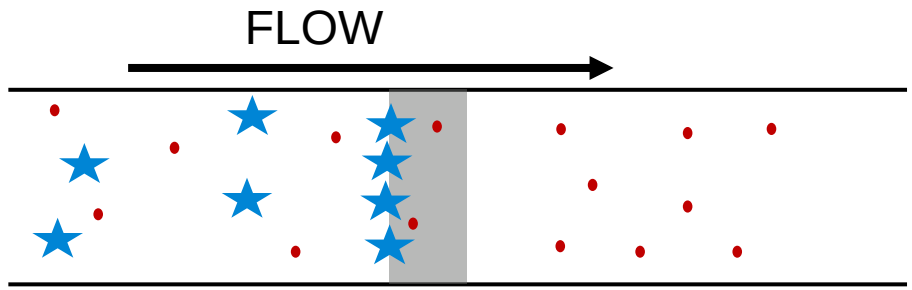


Offline Sample Preparation Options

	More Specific ← **Instrument Separation and Detection Specificity** ← *Less Specific*						
	Less Specific → ***Sample Preparation Specificity*** → *More Specific*						
Sample Preparation Technique / **Interference Removed**	**Dilute and Shoot**	**Filtration**	**Supported Liquid Extractions (SLE)**	**Protein Precipitation + Filtration**	**QuEChERS**	**Protein Precipitation + Filtration + Lipid Removal**	**Solid Phase Extraction**
Lipids	No	No	Chem Elut S	No	Yes	Yes	Yes
Oligomeric surfactants	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	Some	Yes	Yes	Yes	Yes
Pigments	No	No	Some	No	Yes	No	Yes
Polar organic acids	No	No	Yes	No	Yes	No	Yes
Proteins	No	No	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	No	No	No	Yes
Suggested Agilent product	Agilent autosampler vials	Captiva syringe filters Captiva filter vials	Chem Elut S	Captiva ND	Bond Elut QuEChERS with d-EMR-Lipid and other dispersive	Captiva EMR-Lipid	Bond Elut Silica and Polymeric SPE

Methods for Sample Preparation

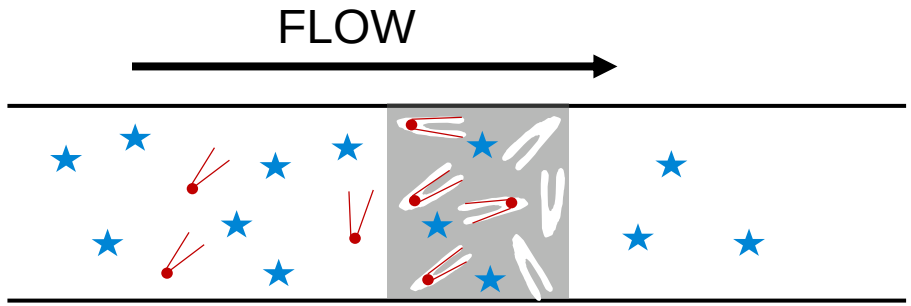
1. Mechanical filtration
   a) Syringe filters
   b) Syringeless filters
      1. Filter vials
      2. Spin filters



Filters by particle size

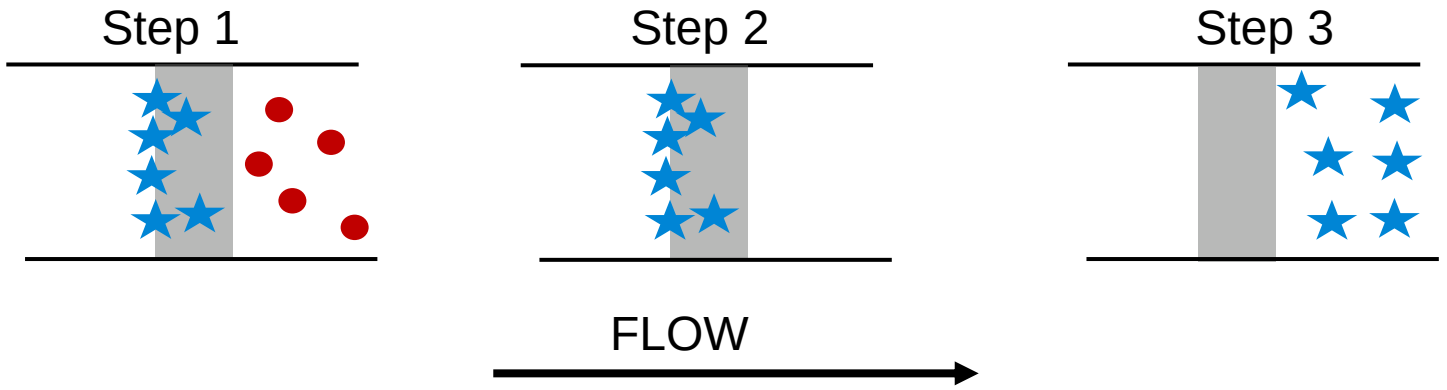
Captiva products

2. Targeted filtration
   a) Matrix removal
   b) LLE, SLE



Filters by shape, charge or hydrophobicity

3. Catch and release
   a) SPE
      1. Polymeric
      2. Silica



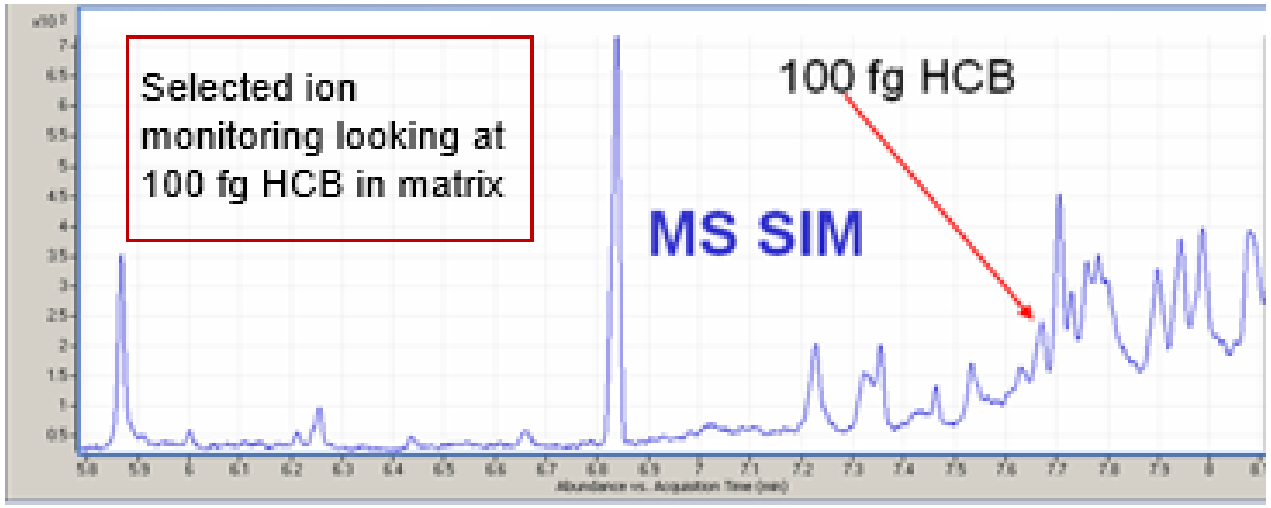
Bond Elut products

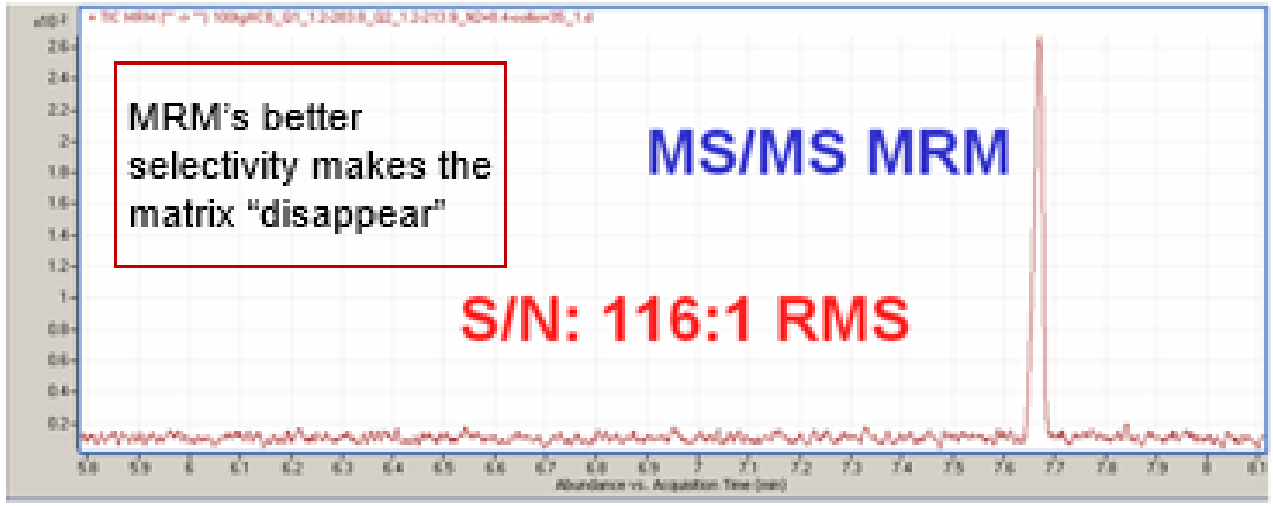
Techniques for Avoiding Unexpected Problems in LC and GC Analysis

Technique	Sample Preparation Cost/Time	Cost of More Frequent Column Changes	Cost of More Frequent Instrument Maintenance	Loss of Income Due to More Frequent Instrument Maintenance	Matrix Interference with Results
Direct inject	None	Yes	Yes	Yes	Yes, for some matrices
Dilute and shoot	None	Y	Y	Y	Yes, for some matrices
Mechanical filtration	Minimal	Less often	Less often	Less often	Yes, for some matrices
Matrix removal and filtration	Yes	No	No	No	No

- Consider the source of the sample (blood vs. urine vs. lake water)
- Mechanical filtration is the absolute minimum sample preparation that should be done – too cheap and easy not to
- Some matrices can cause ion suppression or ion enhancement leading to erroneous results

SIM and MRM – Remember the Matrix is Still There





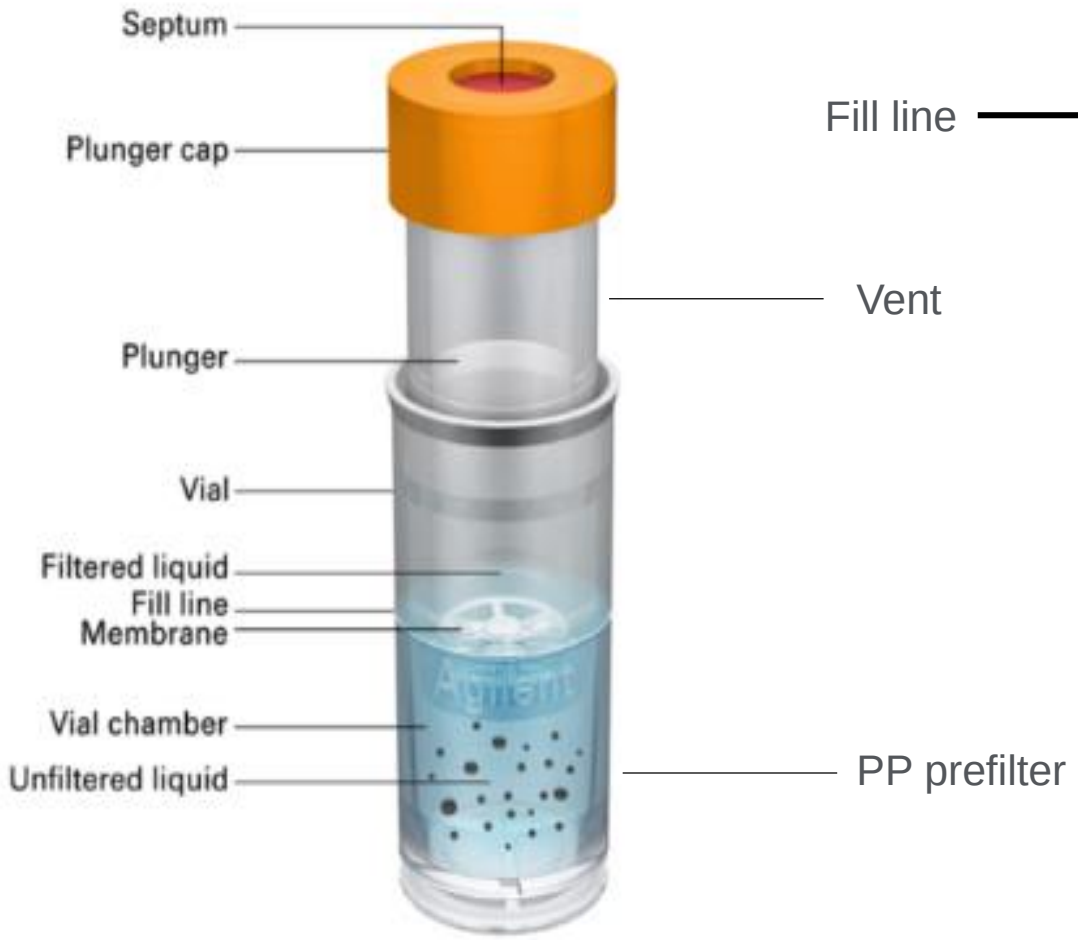
Filtration

Captiva premium syringe filters

- Certified to be free of UV-detectable extractables on HPLC. PES and glass fiber also certified for LC/MS.
- Color-coded boxes for easy identification
- Comprehensive portfolio to meet all customers' needs

Premium Syringe Filters						
Membrane	Diameter/Pore Size					
	4 mm		15 mm		25 mm (28 mm)	
	0.2 µm	0.45 µm	0.2 µm	0.45 µm	0.2 µm	0.45 µm
PTFE	♦	♦	♦	♦	♦	♦
Nylon			♦	♦	♦	♦
PES	♦	♦	♦	♦	♦	♦
Regenerated cellulose	♦	♦	♦	♦	♦	♦
Cellulose acetate					♦	♦
Glass microfiber			♦		♦	
Depth filters: glass/PTFE			♦	♦	♦	♦
Depth filters: glass/nylon			♦	♦	♦	♦

Filtration – Captiva Filter Vials



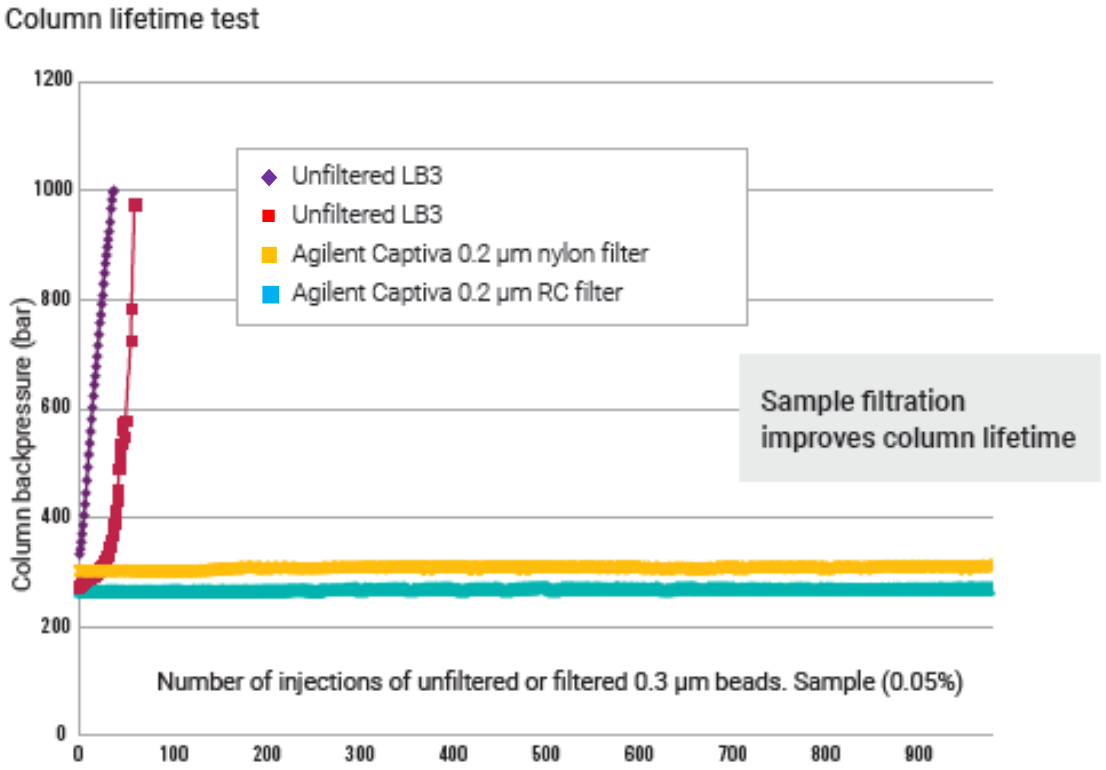
Part Number	Description
5191-5933	PTFE filter vial, 0.45 µm, 100/pk
5191-5934	PTFE filter vial, 0.20 µm, 100/pk
5191-5935	Nylon filter vial, 0.45 µm, 100/pk
5191-5936	Nylon filter vial, 0.20 µm, 100/pk
5191-5939	RC filter vial, 0.45 µm, 100/pk
5191-5940	RC filter vial, 0.20 µm, 100/pk
5191-5941	PES filter vial, 0.45 µm, 100/pk
5191-5942	PES filter vial, 0.20 µm, 100/pk
5191-5943	Vial closure tool

See appendix for solvent compatibility poster request

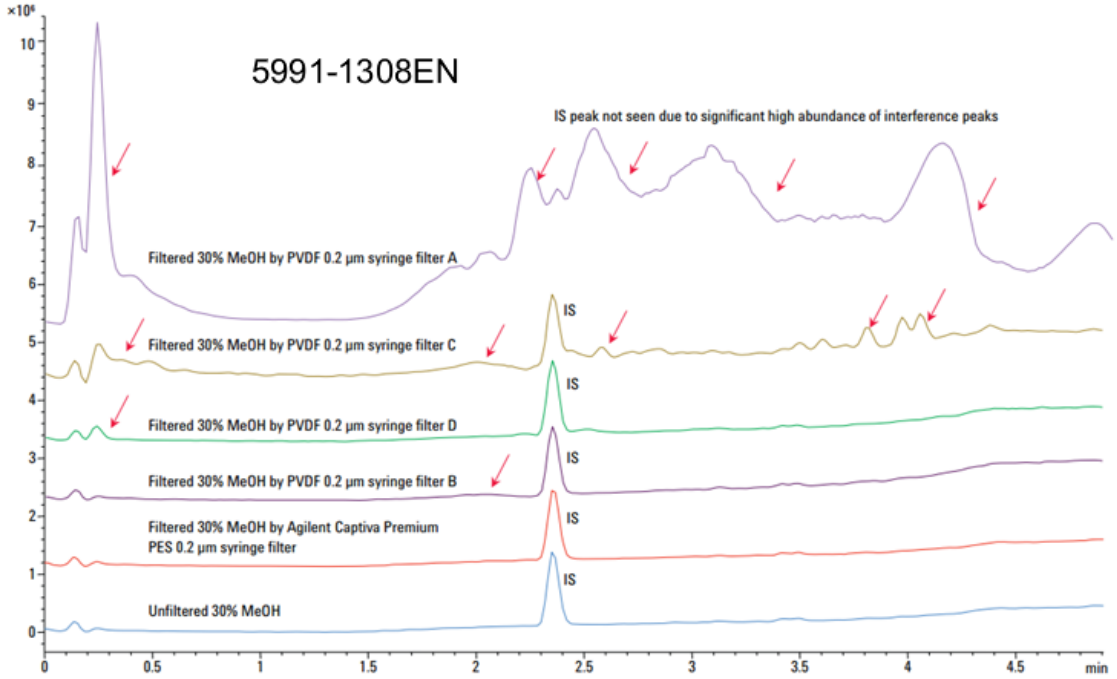
Agilent.com/chem/filtervials
Filter vials user guide: 5994-0814EN

Filtration

Captiva premium syringe filters



Impact of filtering a 0.3 µm latex-bead suspension on lifetime of a sub-2 µm column.



Filter cleanliness comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PVDF syringe filters using LC/MS under positive mode.

Captiva syringe filters guide 5991-1230EN

Filtration – Targeted filtration

Captiva EMR-Lipid

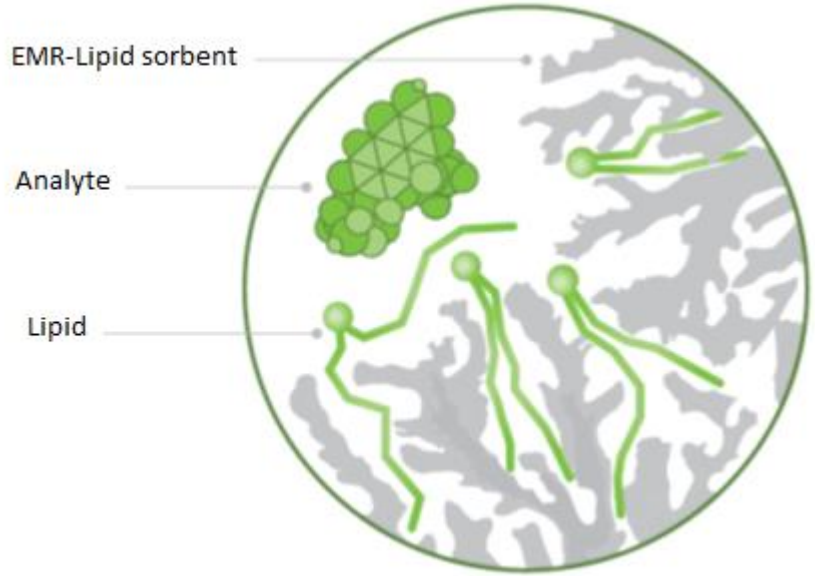
- One of the newest Agilent sample cleanup products with a 2-in-1 benefit of removing proteins and lipids.
- It reduces ion suppression, increases analyte sensitivity, improves peak shape, and extends the lifetime of your analytical column.
- Simple pass-through format, 96-well plate, 1 mL, 3 mL, and 6 mL cartridges
- Solvent-retention frit in 1 mL cartridge/96-well plate for in-well protein precipitation
- Unique chemistry and filtration ensures protein and lipid removal
- Depth filtration design allows for smooth elution
- Received the Analytical Scientist Innovation Award (TASIA) of 2017

Filtration – Targeted Filtration

Captiva EMR-Lipid

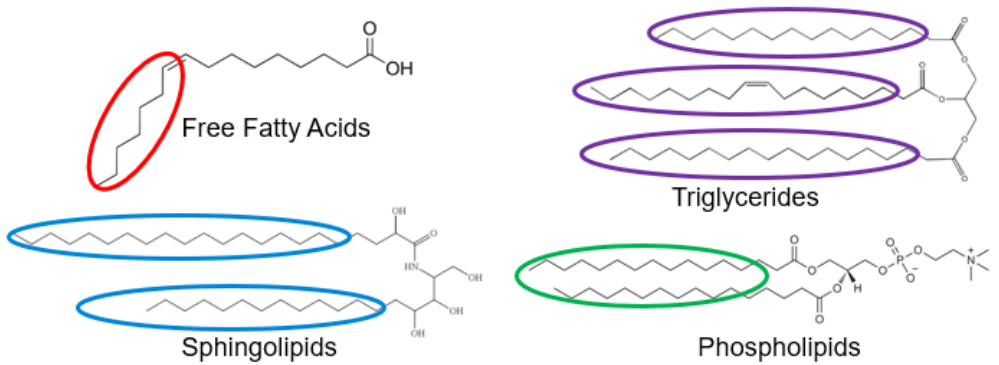
EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

- 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

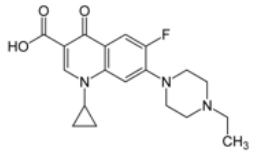


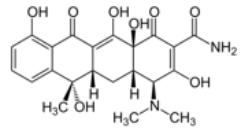
Captiva EMR-Lipid
Selective removal of lipids

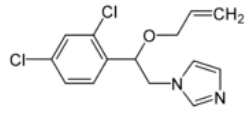
Removes lipids

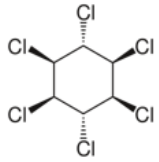


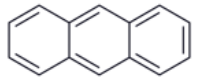
Does not remove target analytes

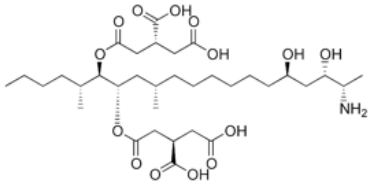

Fluoroquinolones


Tetracyclines


Imidazole pesticides


Organochlorine Pesticides


PAHs

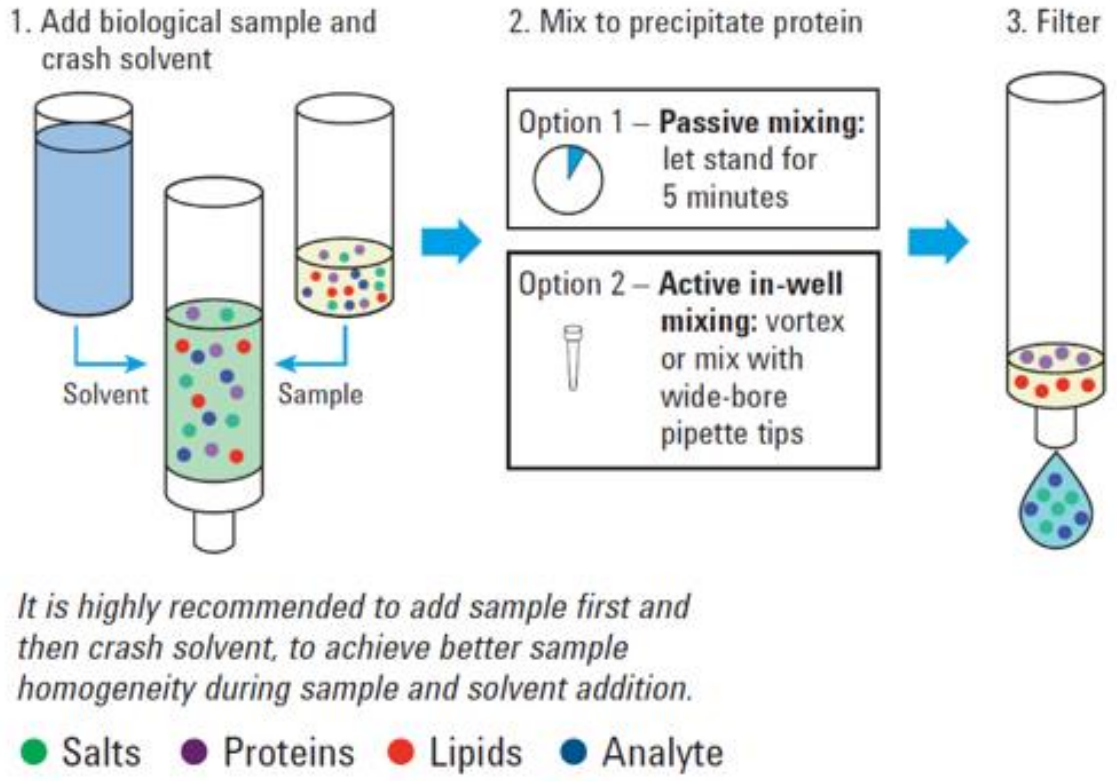

Fumonisin B2

Captiva EMR-Lipid

General protocol for biological samples using 1 mL cartridge and 96-well plate

Operating instructions

Sample and crash solvent should contain 20% water.



Vacuum, positive pressure, or centrifuge can be used.

One drop every 3-5 seconds.

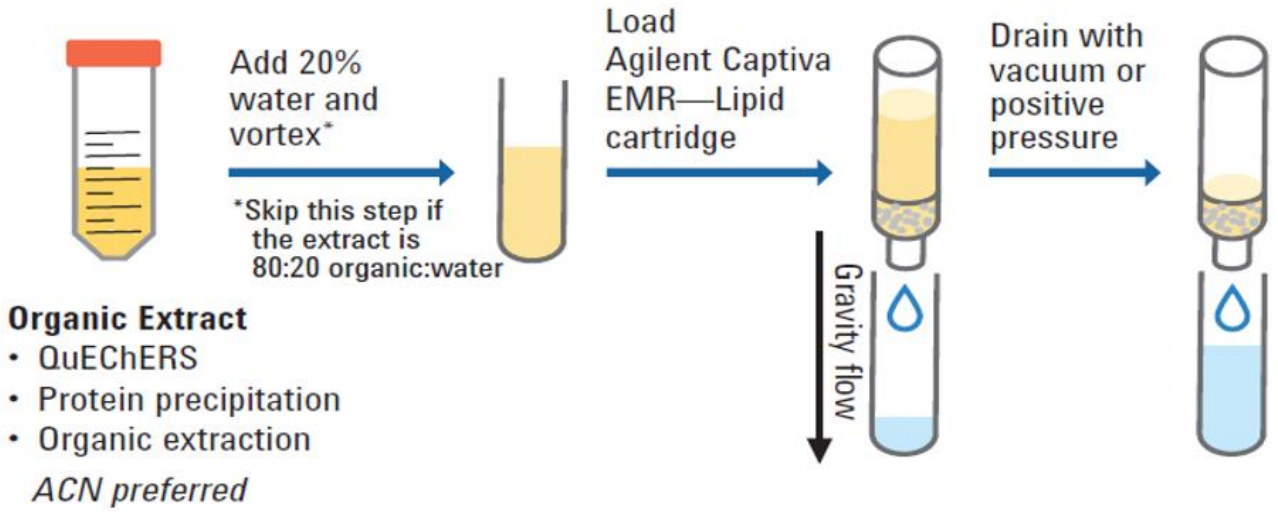
Extra elution step with 80:20 acetonitrile: water can improve recovery.

[Captiva EMR-Lipid method guide for 96 well-plate and 1 mL cartridge]

Captiva EMR-Lipid

General protocol for food and food products using 3 mL and 6 mL cartridges

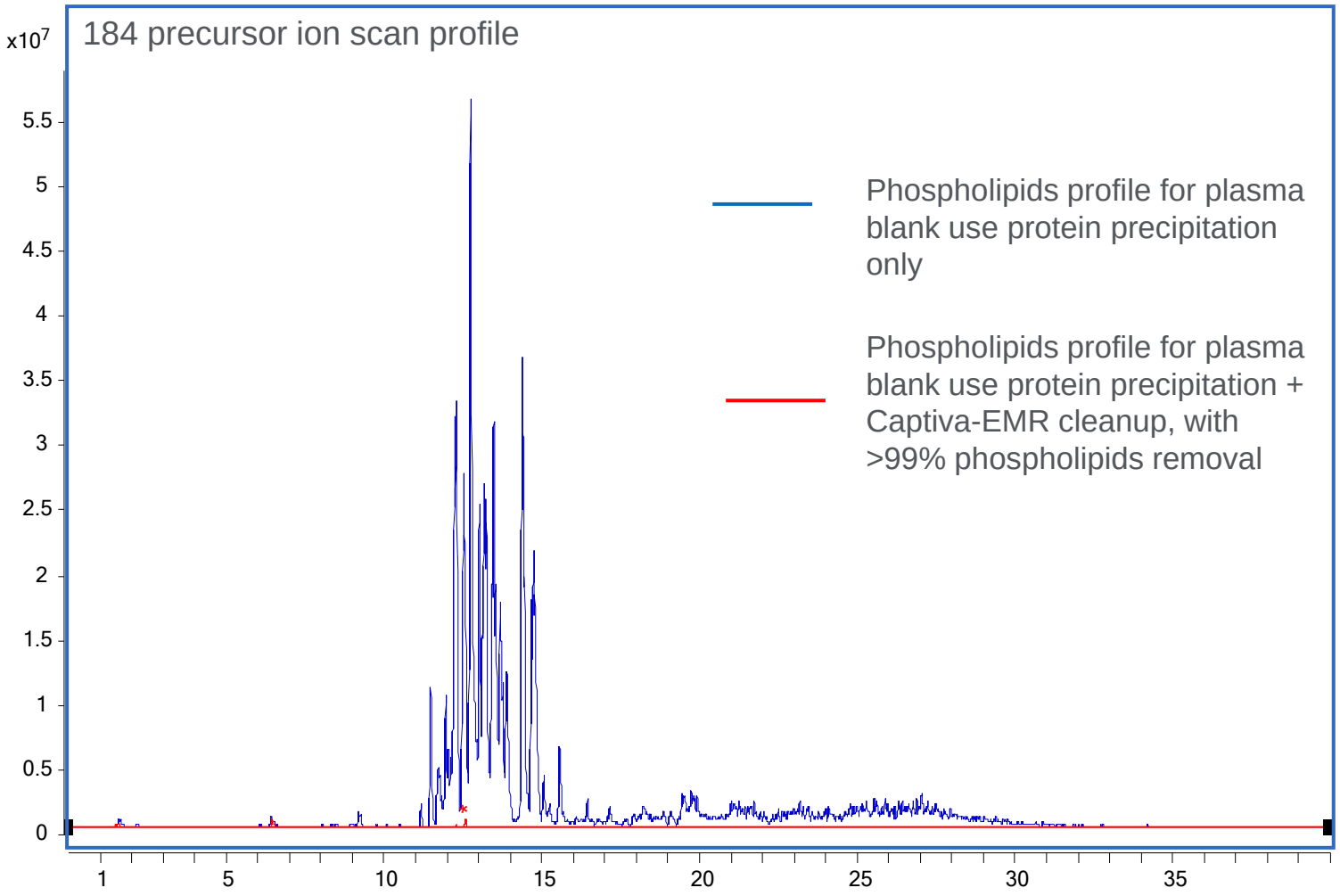
Operating instructions

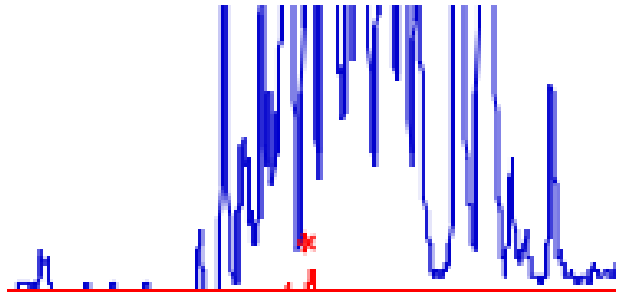


[Captiva EMR-Lipid method guide for 3 mL and 6 mL cartridges]

Captiva EMR-Lipid Cleanup

Efficient phospholipids removal from biological fluid matrices

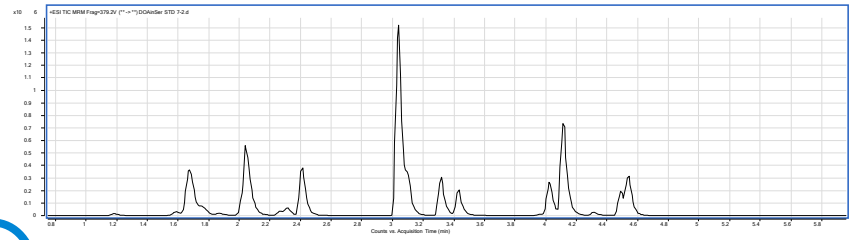


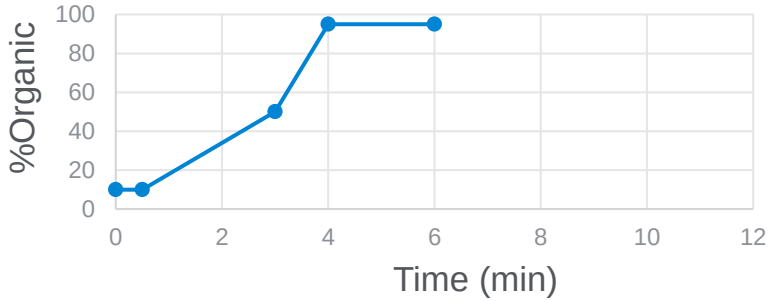


Zoomed-in baseline

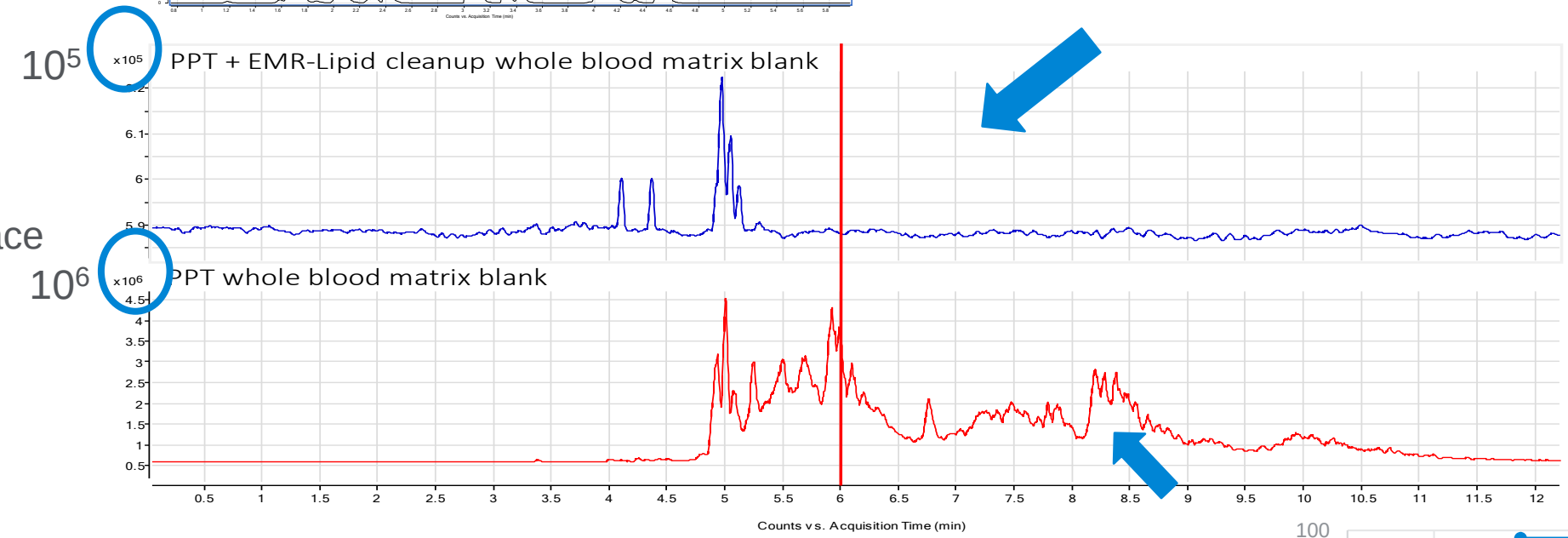
Removal of Lipids Allows for Shorter LC Gradient Time

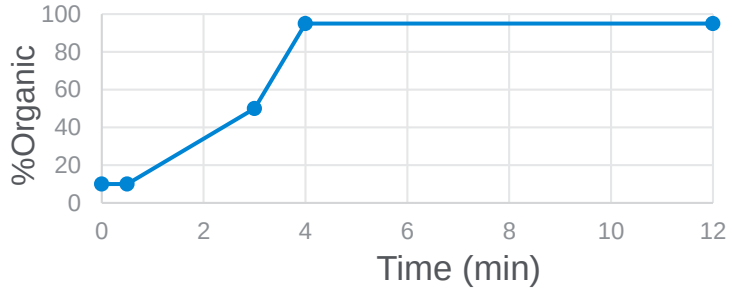
Target analytes TIC chromatogram





Lipid trace

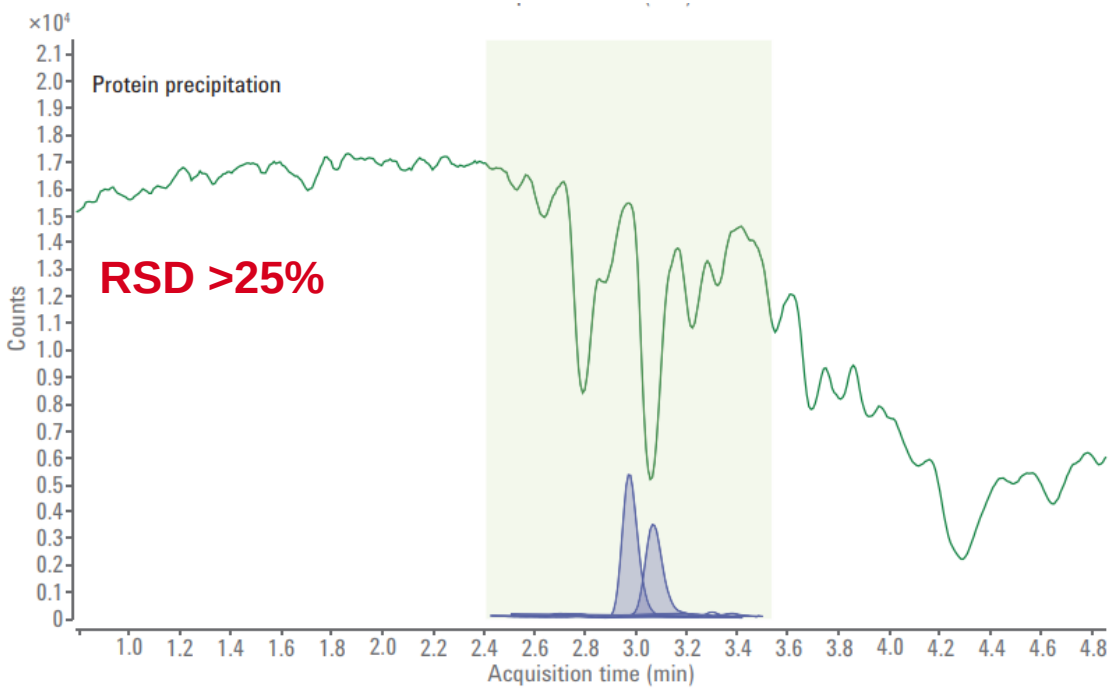




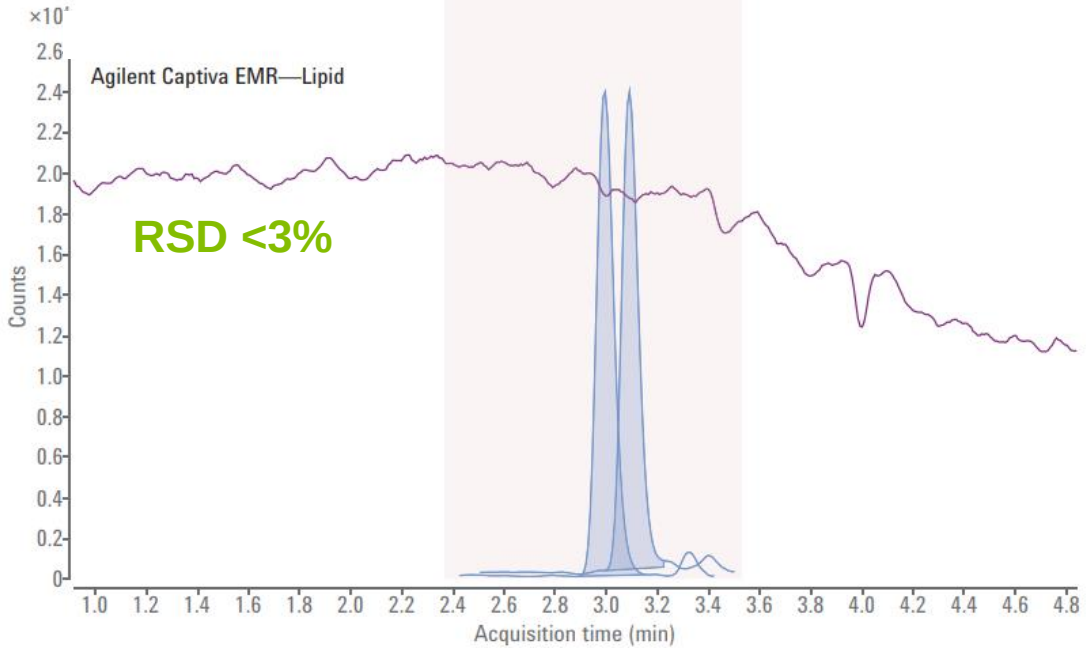
Captiva EMR-Lipid Cleanup

Improved analyte response and reproducibility

PPT only



PPT with Captiva EMR-Lipid cleanup



Lipids cause reproducibility problems resulting in high RSD values.
Using Captiva EMR-Lipid enables low RSD values and higher peak areas.
Higher peak area due to less ion suppression can lead to lower detection limits.

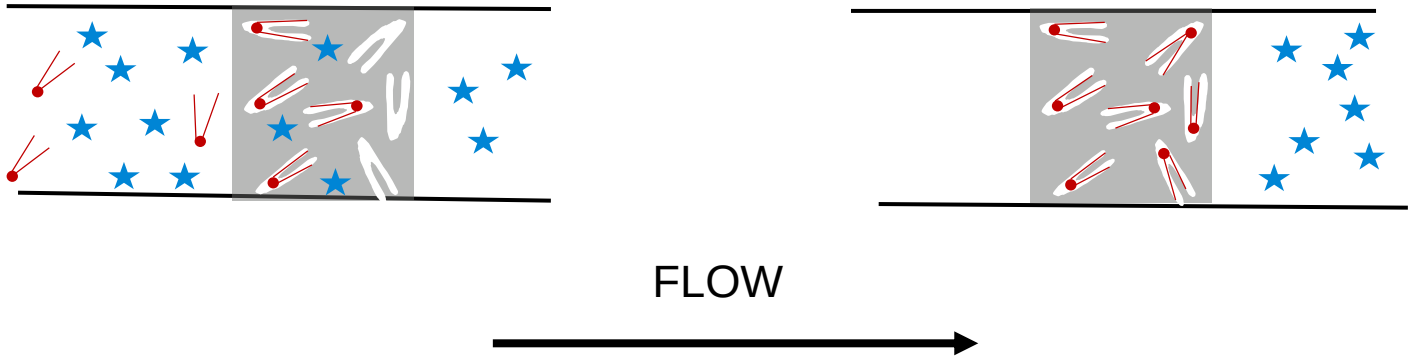
**See Appendix for post column infusion setup.*

Application Note Examples

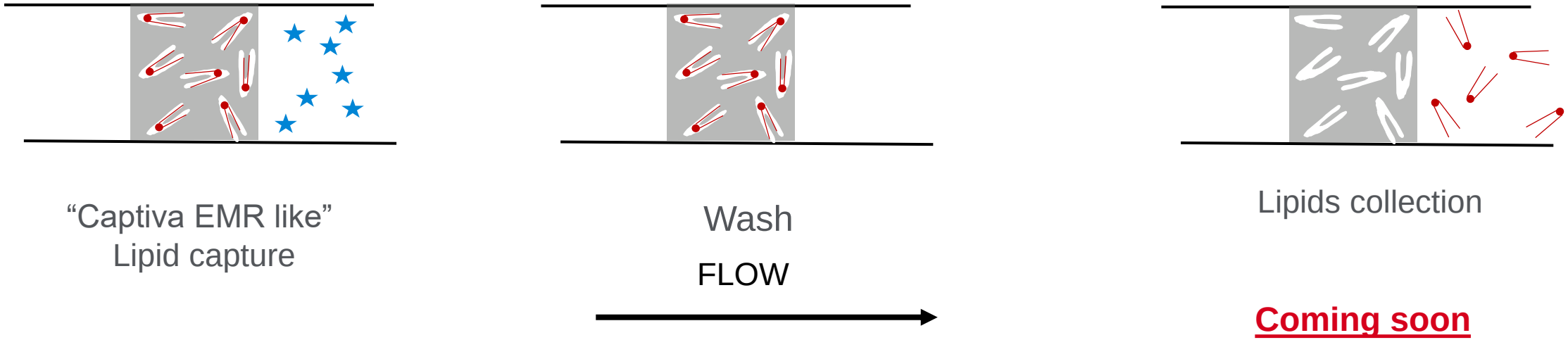
- Determination of 14 Polycyclic Aromatic Hydrocarbon Compounds in Edible Oil (5994-1483EN)
- Determination of UV Filters in Sunscreens Using Agilent Captiva EMR-Lipid Cleanup by HPLC (5994-1611EN)
- A Fast Sample Preparation Workflow for Veterinary Drugs Analysis in Salmon (5994-1124EN)
- Analysis of Nitroimidazoles in Egg Using Agilent Captiva EMR-Lipid and LC/MS/MS (5994-0641EN)
- Mycotoxin Analysis in Peanut Butter Using Captiva EMR-Lipid Cleanup and LC/MS/MS (5994-0366EN)

Innovative Lipid Products

Captiva EMR-Lipid – A pass through filtration



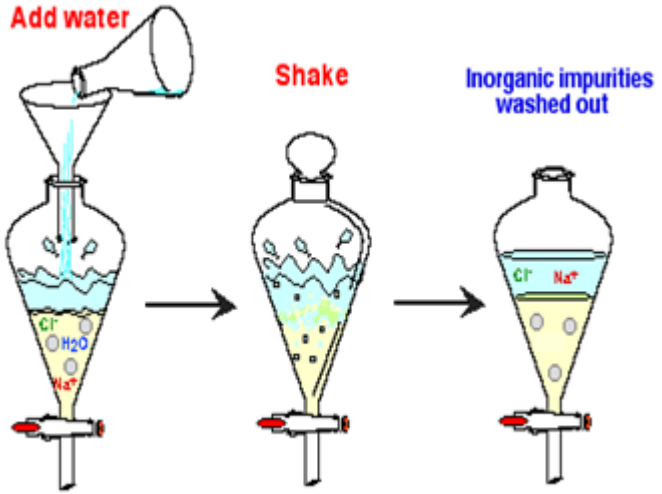
Bond Elut Lipid Extraction – An SPE like lipid isolation for lipidomics



Coming soon

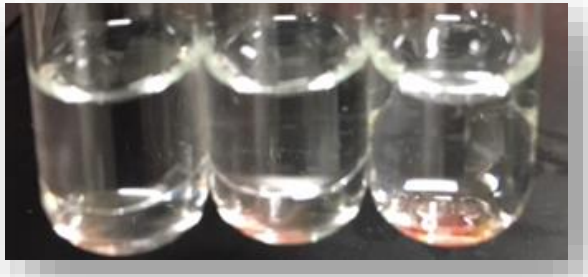
Liquid/Liquid Extraction (LLE)

- LLE has been successfully used as a method of sample preparation for many years.
- It separates the more organic solvent soluble compounds from the more water-soluble compounds using water immiscible organic solvents.
- It can remove many interfering substances like salts.
- Modulating pH can selectively extract or eliminate specific compound types.



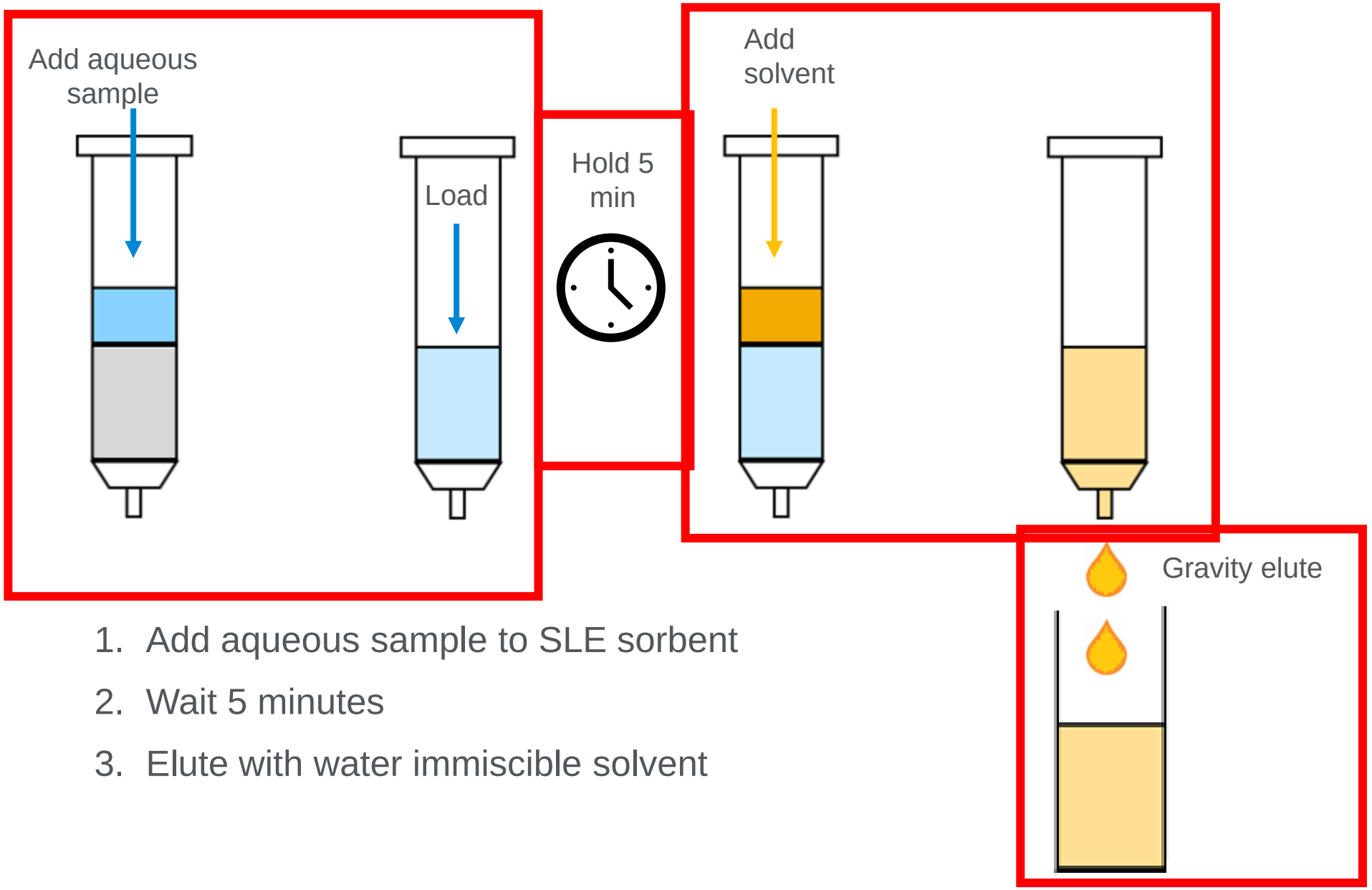
Drawbacks of Liquid-Liquid Extraction

- LLE does have drawbacks
  - Inconsistent results from one analyst to another
    - Shaking time
    - Shaking motion
    - Determination of where to cut between layers
  - Emulsions
  - Labor intensive
  - Quite tedious with small sample sizes (<5 mL)
  - Challenging with large numbers of samples
  - Difficult to automate for large numbers of samples



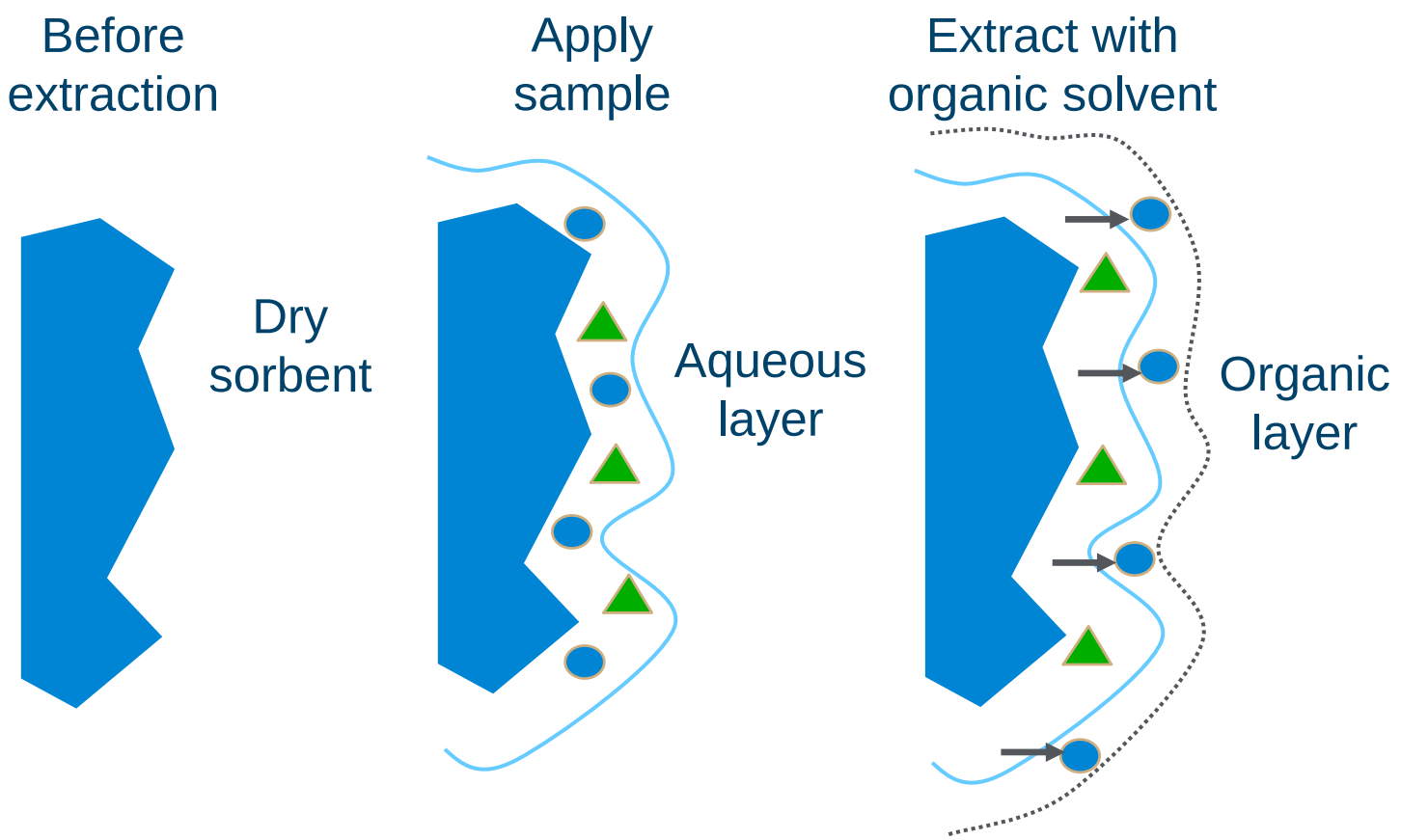
How many of these problems can be fixed with Solid Supported Liquid Extraction?

How Does SLE Work?



1. Add aqueous sample to SLE sorbent
2. Wait 5 minutes
3. Elute with water immiscible solvent

Supported Liquid Extraction (SLE)



- A thin layer of aqueous sample is formed on the surface of SLE sorbent.
- When the organic solvent passes through the SLE bed, analytes are extracted under the same principles as LLE.
- Increased contact area between the two phases allows efficient extraction without mixing.

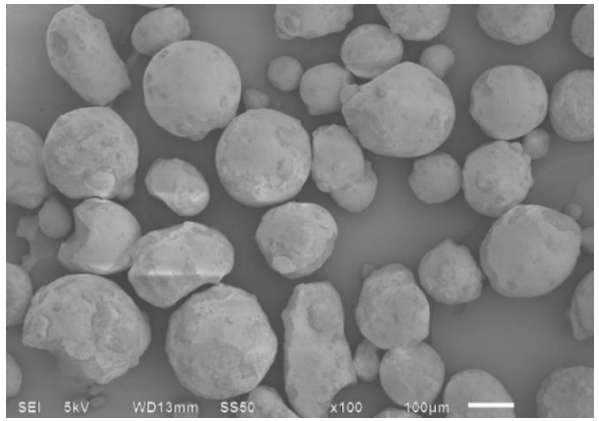
What is SLE Sorbent?

- There are two types of SLE media
  - Diatomaceous earth (DE) based products like our Chem Elut brand of SLE products
    - A mined fossil diatom material, which is heterogeneous and inconsistent from one mine to the next

Diatomaceous earth in Chem Elut

- ✗ Naturally occurring; mined
- ✗ Broad particle size distribution
- ✗ Supplier reliability issues
- ✗ Poor lot-to-lot consistency

  - Synthetic media we use in Chem Elut S
    - Controlled synthesis to be consistent batch after batch



Synthetic SLE sorbent

- ✓ Large scale synthesis
- ✓ Narrow particle size distribution
- ✓ Reliable supplier
- ✓ Controlled manufacturing

Supported Liquid Extraction (SLE)

Chem Elut S

- Same extraction mechanism as in traditional liquid-liquid extraction (LLE)
- Cartridge and plate format, packed with proprietary synthetic sorbent– high surface area
- Simple method, gravity flow
- Smaller volume sample and solvent compared to LLE
- No emulsions

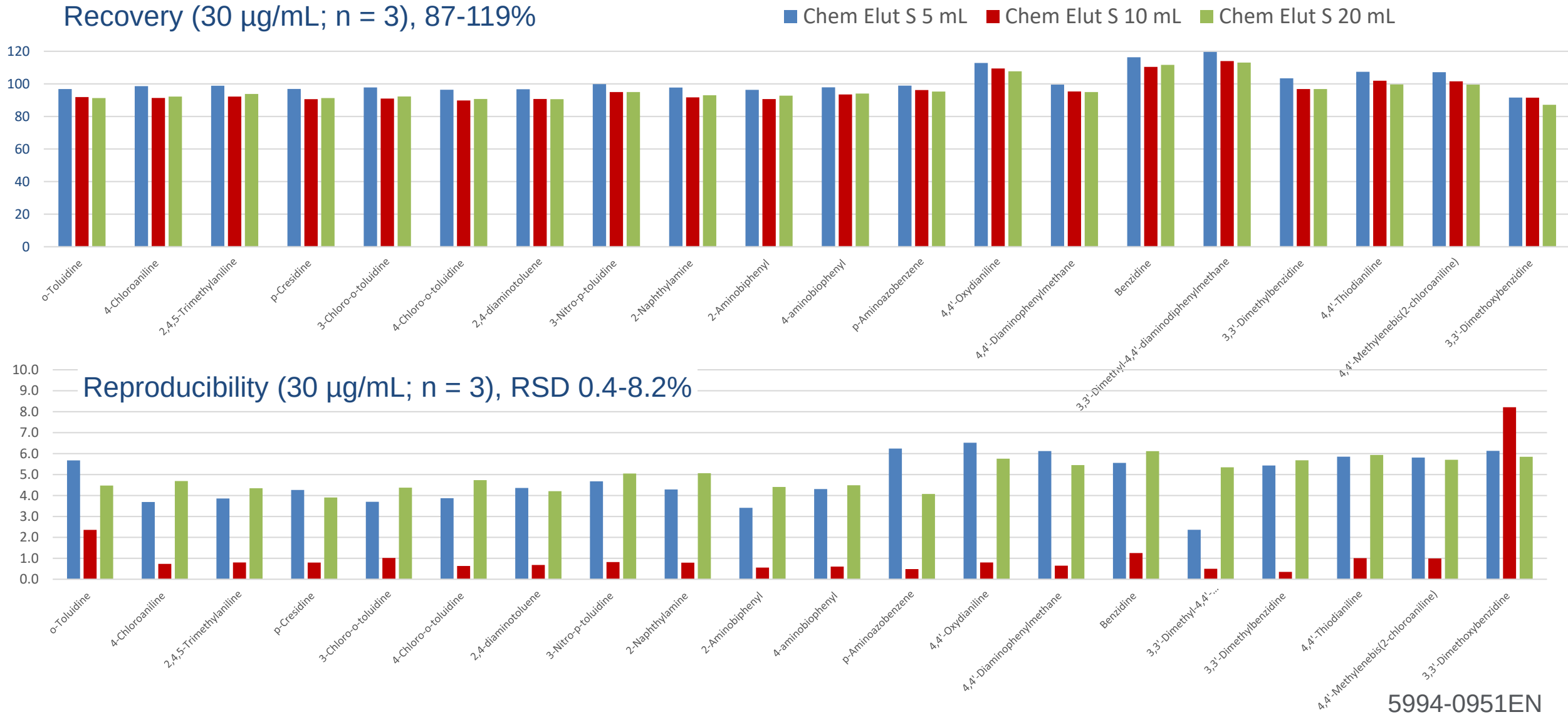
Cartridges for sample volumes 0.2 – 20 mL

Bulk Chem Elut S
1 kg and 4 kg

96-well plate for sample volume 200 µL and 400 µL

Chem Elut S – 15 Minute Hold Time

Large scale format comparison with aromatic amines using GC



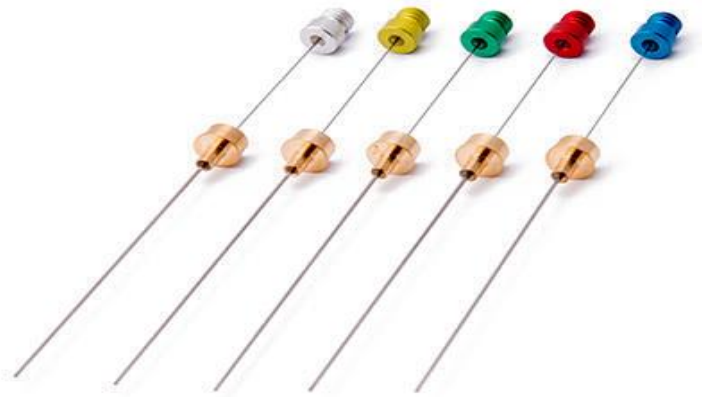
5994-0951EN

SPME Fiber and Arrow Offering from Agilent

Solid Phase Microextraction (SPME)

- Environmental analyses of water samples
- Odor analyses (ppt)
- Flavor analyses of food products
- Forensic analyses of arson/explosives samples
- Toxicology analyses: blood alcohol or drugs in urine/serum
- Surfactants, other industrial applications

- Trace analysis in food
- Drugs and pharmaceuticals
- Herbicides/pesticides
- Medical diagnostics
- Trace impurities in polymers and solid samples
- Solvent residues in raw materials
- Explosives



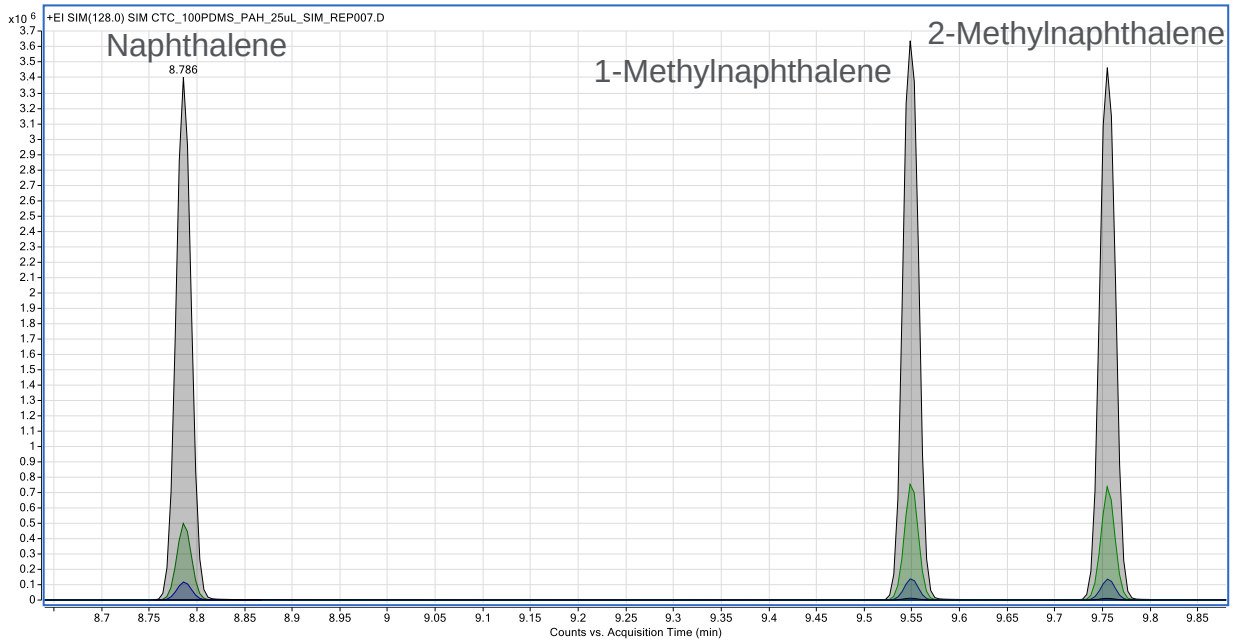
SPME fibers

SPME arrows

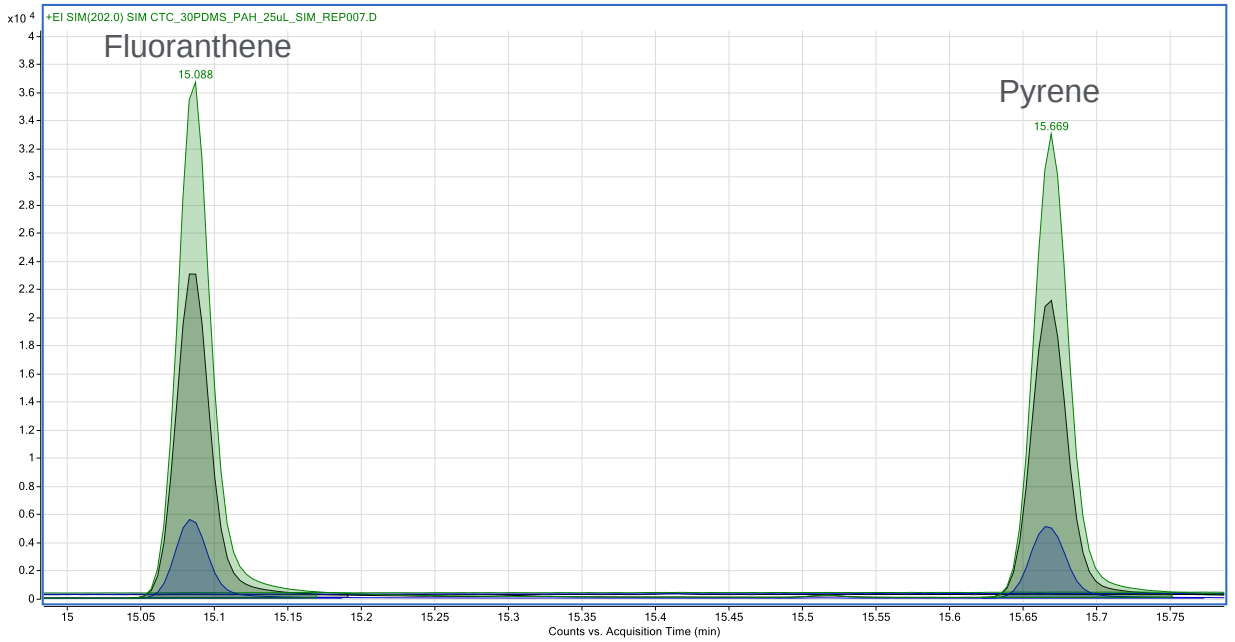
Examination of Lower Molecular Weight PAHs in Drinking Water Using Agilent PDMS SPME Fibers

Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds containing two or more fused aromatic rings. PAHs are considered compounds of concern by environmental organizations; their concentration in water is strictly regulated.

5994-1301EN



SIM chromatogram of naphthalenes with PDMS fibers (black trace = 100 µm; green trace = 30 µm; blue trace = 7 µm)



SIM chromatogram of fluoranthene and pyrene with PDMS fibers (black trace = 100 µm; green trace = 30 µm; blue trace = 7 µm)

Agilent Bond Elut QuEChERS

Quick Easy Cheap Effective Rugged and Safe

Initially developed for screening of pesticide residues in fruit and vegetables to make sample cleanup of food faster, simpler, less expensive, and greener.

Now, QuEChERS is used with other matrices and compound classes as well.

Consists of two steps, and therefore two kits:

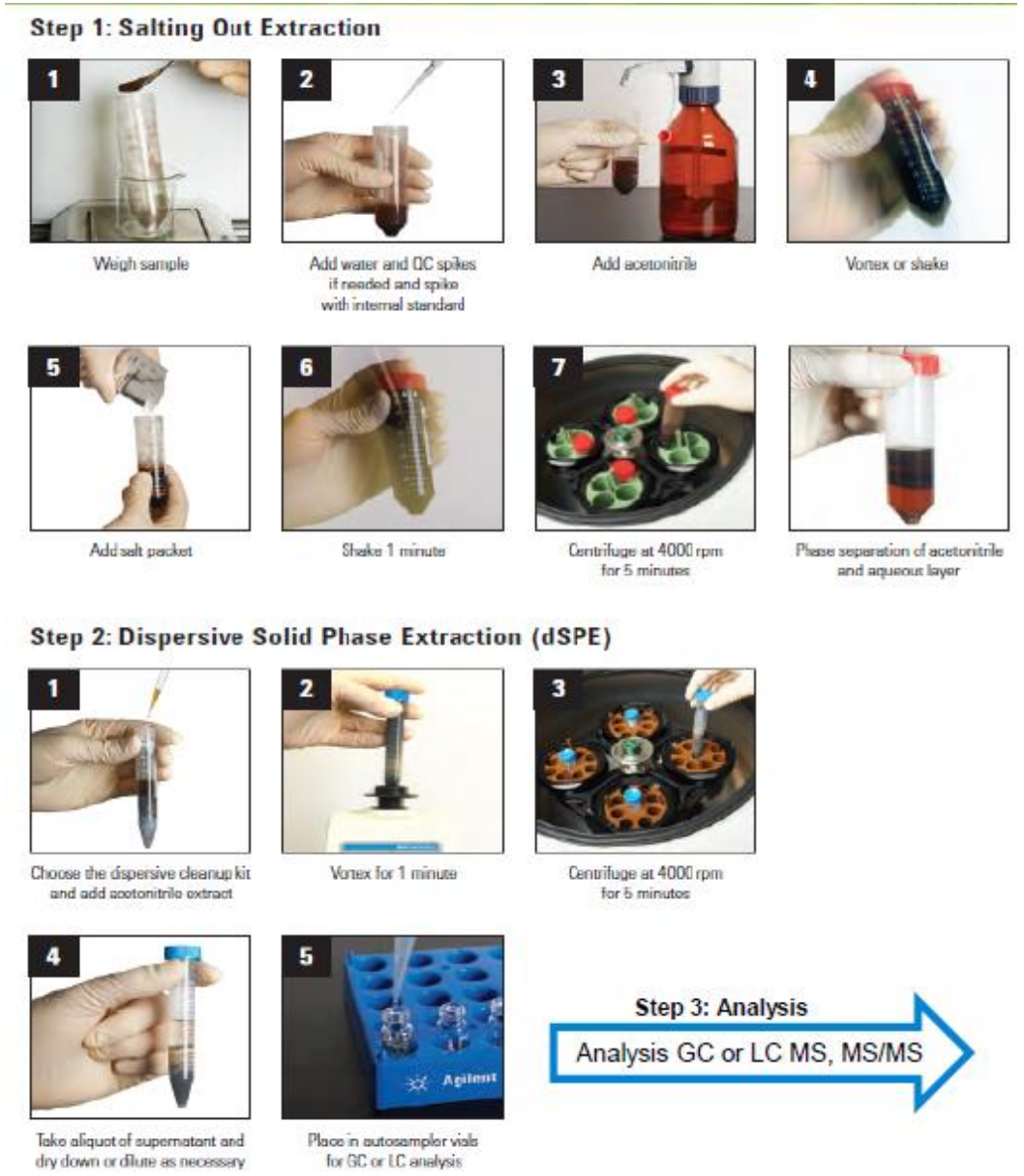
Step 1: Liquid extraction

Step 2: Dispersive SPE/ interference removal

QuEChERS Workflow

QuEChERS extraction salts

QuEChERS dispersive SPE sorbents



Bond Elut Dispersive SPE Kits

Dispersive kit

Centrifuge tubes containing preweighed SPE sorbent 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)

Dispersive SPE kits are available for different food types.

They are for both AOAC (US) method and EN (Europe).

QuEChERS is a nonselective technique and does not remove **all** the matrix, just enough.

Dispersive sorbents are also available as bulk material.

Dispersive EMR-Lipid

EMR-Lipid – What is it?

Polish Options

Polish Tube
$NaCl/MgSO_4$
(p/n 5982-0101)

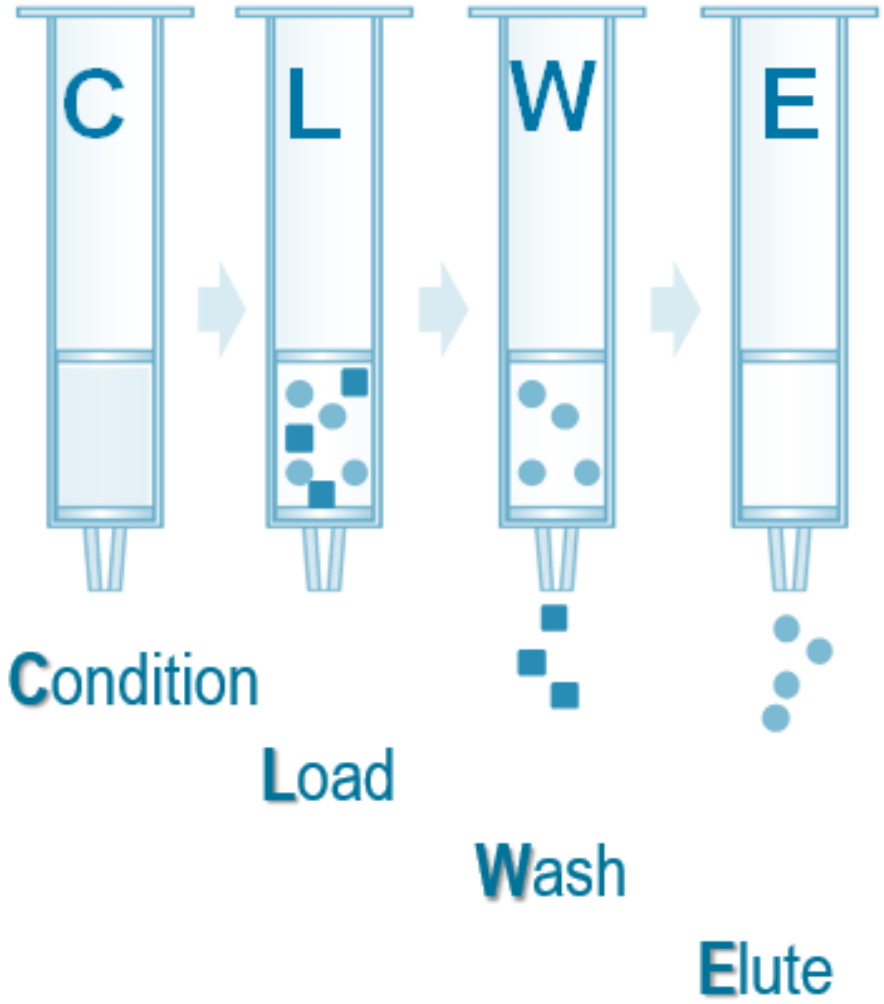
Polish Pouch
$MgSO_4$
(p/n 5982-0102)

EMR-Lipid fits into current sample preparation workflows

Solid Phase Extraction (SPE)

- Capabilities
  - Very selective
  - Highly clean samples
  - Concentrated samples
  - Wide range of applicability
  - Automation friendly

- Types of SPE
  - Nonpolar (reversed phase) SPE
  - Polar (normal phase) SPE
  - Cation exchange SPE
  - Anion exchange SPE
  - Mixed mode SPE
  - Specialty SPE



Bond Elut:

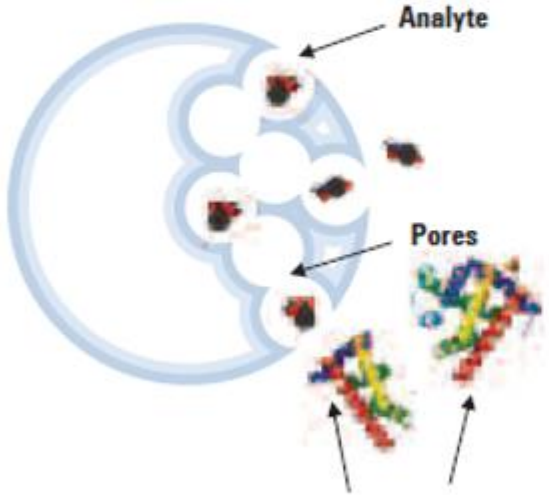
Silica or polymer based, cartridge and 96-well plate format

Bond Elut Plexa

Advanced polymer architecture improves extraction performance

LOAD:

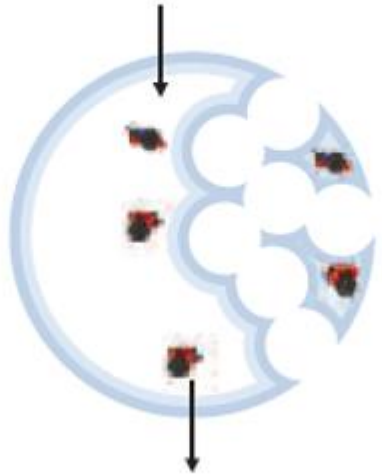
Water-rich, hydrophilic surface allows excellent phase transfer of analytes into the polymer core.



Large endogenous proteins do not bind to the surface of the polymer and cannot access pore structure.

WASH:

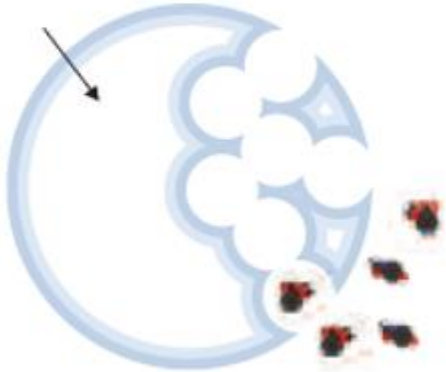
Analytes that have crossed the hydrophilic layers will remain tightly bound in the hydrophobic core.



Interferences wash away without leaching the analytes of interest.

ELUTE:

Specially engineered pore structure allows excellent mass transfer out of the polymer.



Clean extract with high recovery.

Bond Elut Plexa

- New generation of polymeric SPE
- Divinylbenzene-based polymeric sorbent with hydrophilic exterior, hydrophobic interior, and advanced polymeric architecture.
- Superior flow properties
- Great for extraction of a wide range of acidic, neutral, and basic analytes from different matrices
- Simple method (see appendix)
- Bond Elut Plexa, nonpolar
- Bond Elut Plexa PCX, mixed mode with strong cation exchange
- Bond Elut Plexa PAX, mixed mode with strong anion exchange
- Cartridge and 96-well plate format

Agilent SPE Offering

- Reliable SPE with a 30-year history
- Agilent offers the most comprehensive set of phases, sizes, and formats of any SPE provider (over 40 sorbent materials/phases available)
- Easy adoption of methods due to high number of publications and applications.
- Includes packed bed silica and polymeric phases, and monolithic silica phases.

Bond Elut Silica and polymer SPE

Bond Elut AccuCAT
Bond Elut Alumina (AL-A)
Bond Elut Alumina (AL-B)
Bond Elut Alumina (AL-N)
Bond Elut NH_2
Bond Elut C1
Bond Elut C2
Bond Elut C8
Bond Elut C18 .....
..... **40 phases**

Bond Elut Plexa polymer SPE

Bond Elut Plexa
Bond Elut Plexa PCX
Bond Elut Plexa PAX

SampliQ SPE

Multiple phases

OMIX monolithic silica tip SPE

OMIX C18
OMIX MP1
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

Manifolds for Processing Cartridges and 96-Well Plates

Captiva vacuum collar

Vac Elut 20 vacuum manifold

Vac Elut 12 vacuum manifold

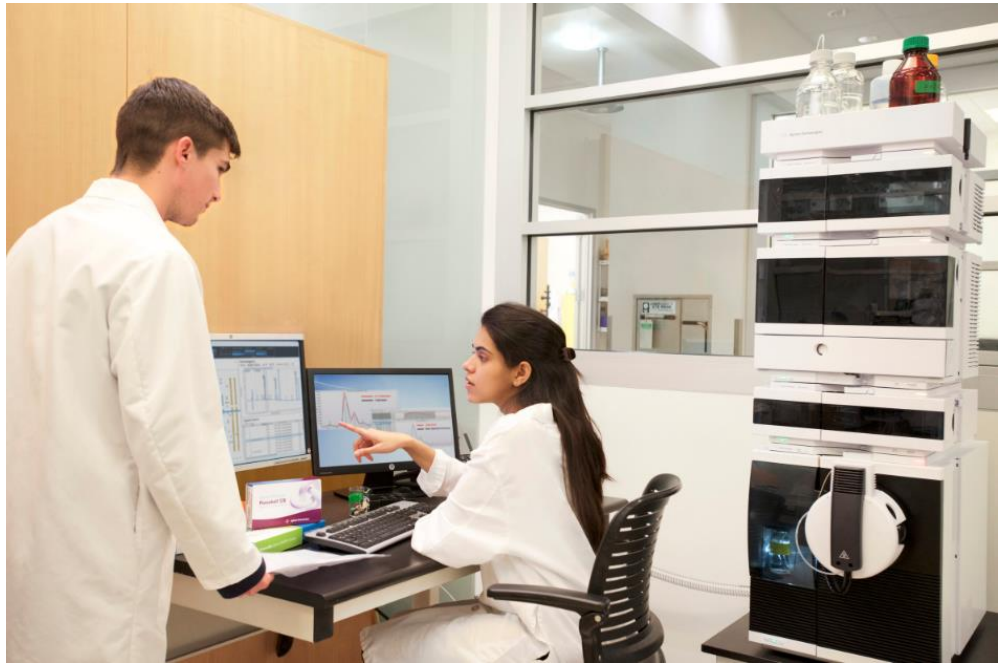
SPS 24 vacuum manifold

96 well plate vacuum manifold

Positive Pressure Manifolds

Agenda

- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- Summary

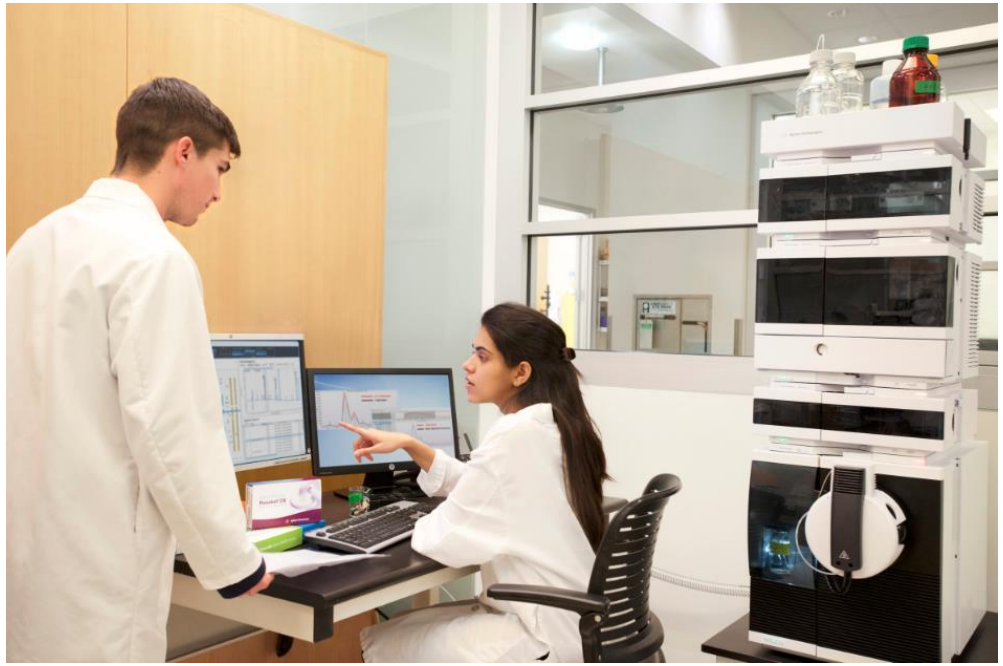


Chromatography Problems Caused by Sample Matrix – Physical Effects

- Particulates in the sample can partially block the inlet frit of the column or guard, causing split/double peaks and high pressure.
- Some components of the sample (proteins, salts) may precipitate as they come into contact with mobile phase, causing high pressure.
- Sample solvent that is immiscible with mobile phase can cause early elution, peak distortion, low resolution, and precipitation of sample components due to low solubility in the mobile phase.
- Sample solvent that is stronger than the mobile phase can cause peak distortion, split/double peak, broad peaks, poor sensitivity, and shortening of retention time.

Agenda

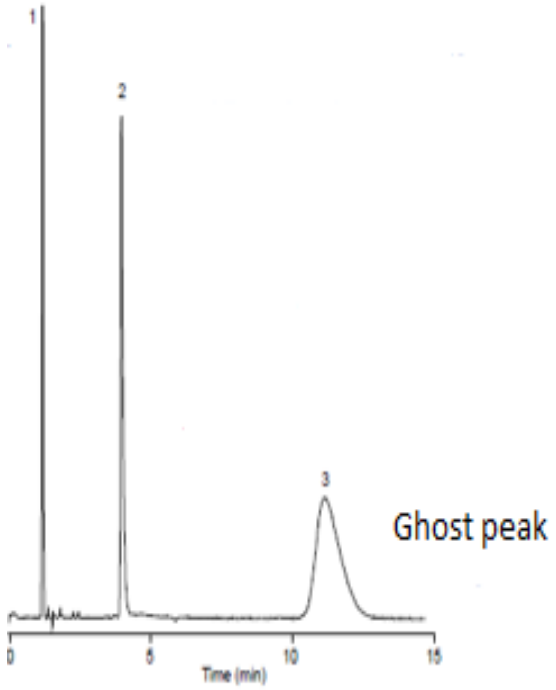
- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- Summary



Chromatography Problems Caused by Sample Matrix – Chemical Effects

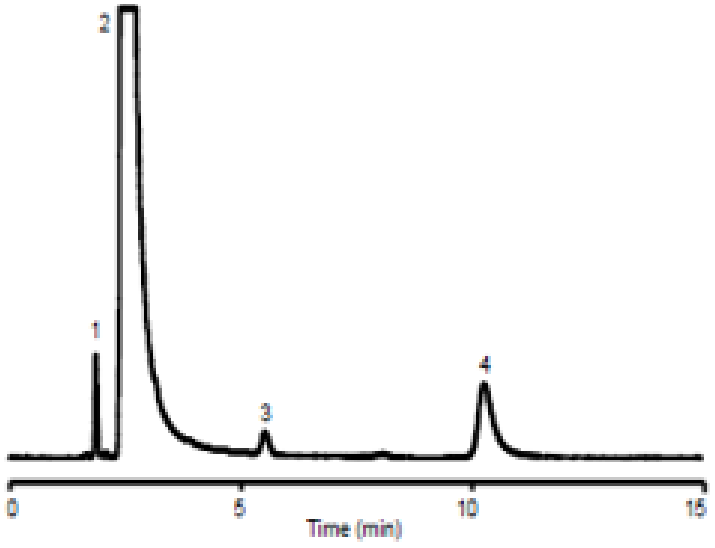
- Chemical contamination/lipid build up can cause secondary interaction and result in retention time variability, peak shape variability/tailing, selectivity changes, and (in some cases) increased back pressure.
- Lipids from the sample matrix can cause ion suppression with MS.
- Strong retention of interferences can result in ghost peaks and shouldering peaks in the following runs.
- Salts can cause ion suppression with MS, and detergents interfere with the evaporation process with MS.
- Interfering compounds from sample matrix can coelute with target analytes and appear as split/shoulder peaks.

As a result, productivity is reduced and instrument downtime, sample run time, and costs are increased.

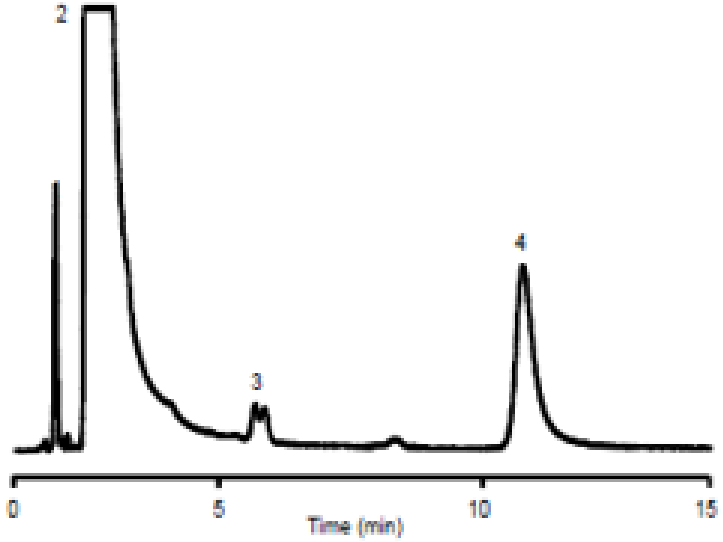


Column Contamination from Sample Matrix Causing Split Peaks

Injection 1



Injection 30

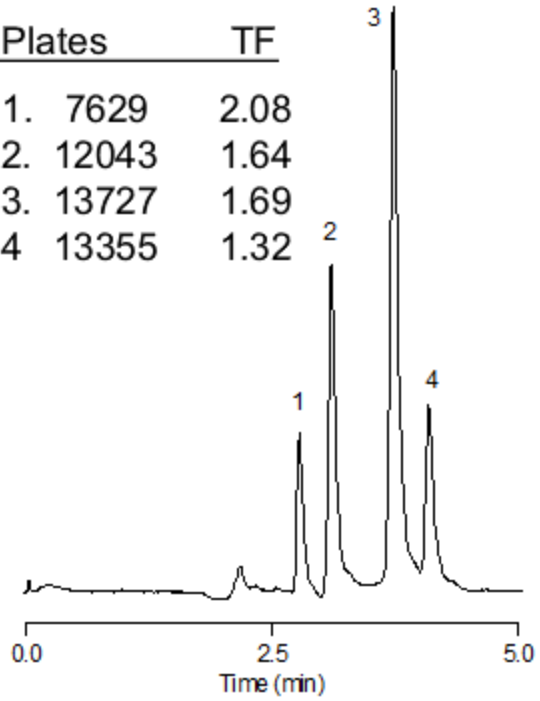


Column: StableBond SB-C8, 4.6 x 150 mm, 5 mm Mobile Phase: 60% 25 mM Na_2HPO_4, pH 3.0 : 40% MeOH Flow Rate: 1.0 mL/min
Temperature: 35°C Detection: UV 254 nm Sample: Filtered OTC Cold Medication: 1. Pseudoephedrine 2. APAP 3. Unknown 4. Chlorpheniramine

Column Contamination from Sample Matrix Causing Peak Tailing

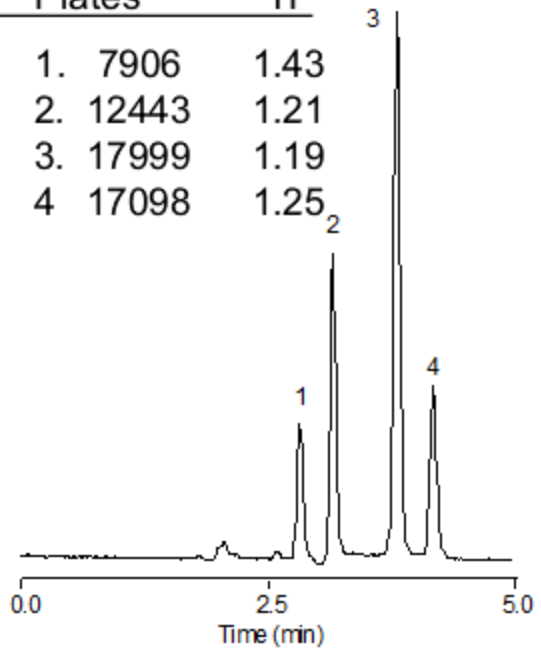
Column: StableBond SB-C8, 4.6 x 250 mm, 5µm Mobile Phase: 20% H_2O : 80% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 nm Sample: 1. Uracil 2. Phenol 3. 4-Chloronitrobenzene 4. Toluene

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



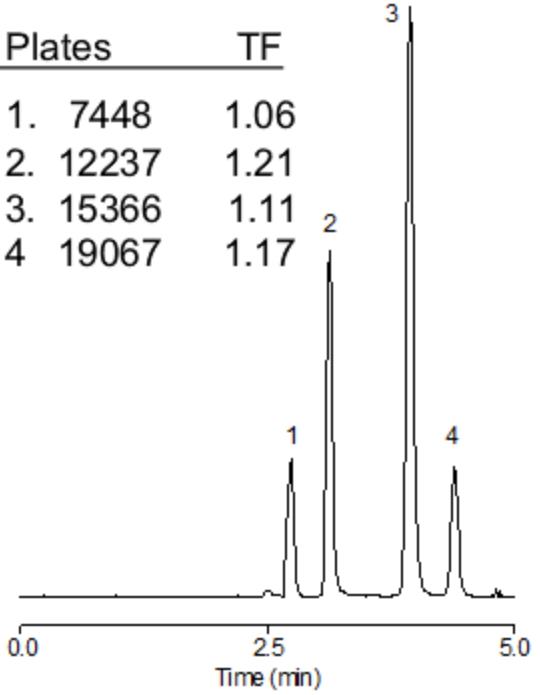
QC test forward direction

	Plates	TF
1.	7906	1.43
2.	12443	1.21
3.	17999	1.19
4	17098	1.25



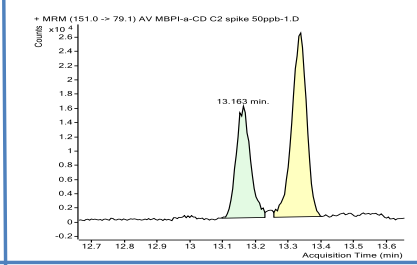
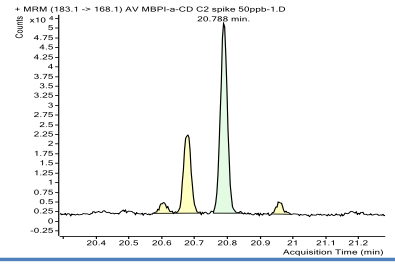
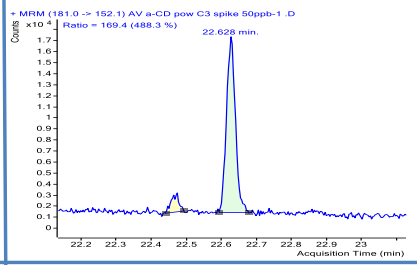
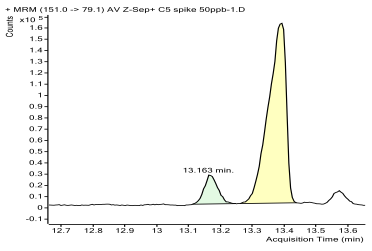
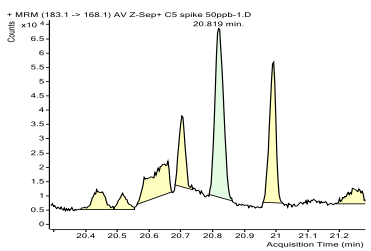
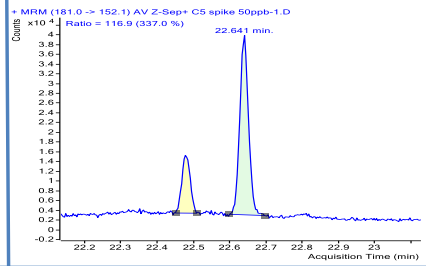
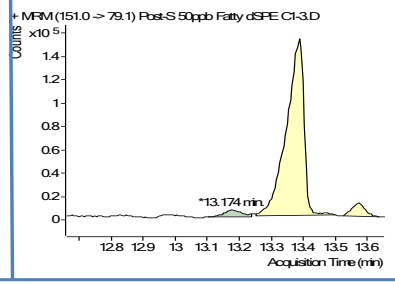
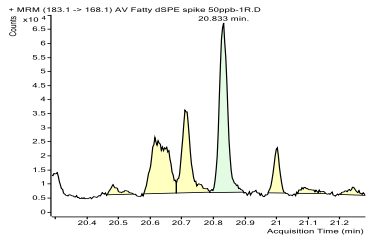
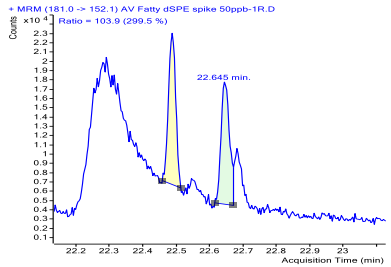
QC test reverse direction

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



QC test after cleaning
100% IPA, 35°C

A Cleanup Step Improves Analytes S/N Ratio and Integration Accuracy on GC/MS(/MS)

Pesticide	Captan	Permethrin	Deltamethrin
EMR-Lipid cleanup			
Zirconia sorbent cleanup			
C18/PSA cleanup			

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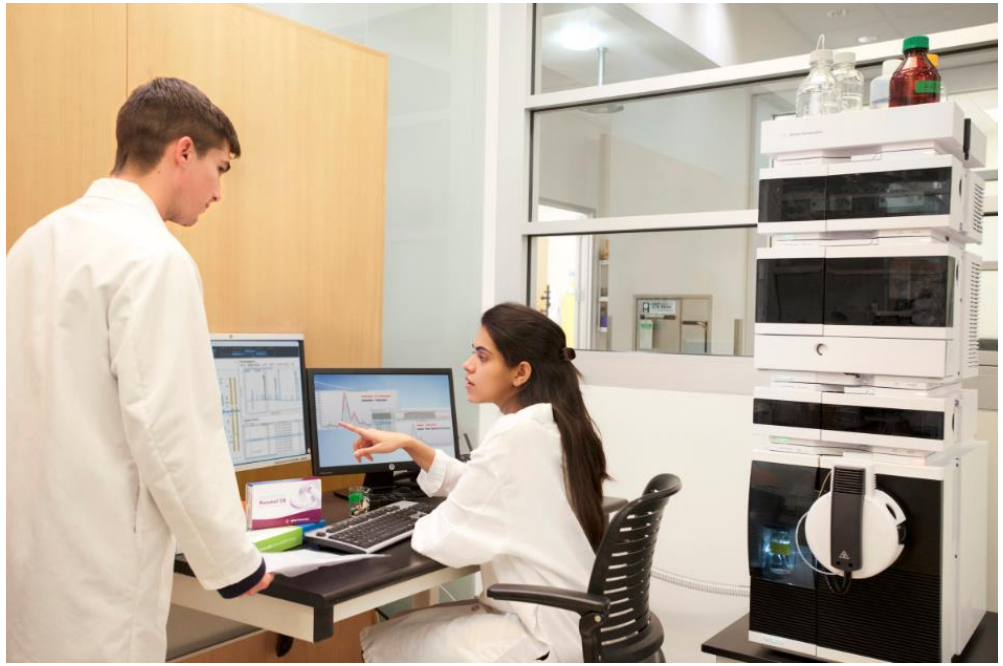
Agenda

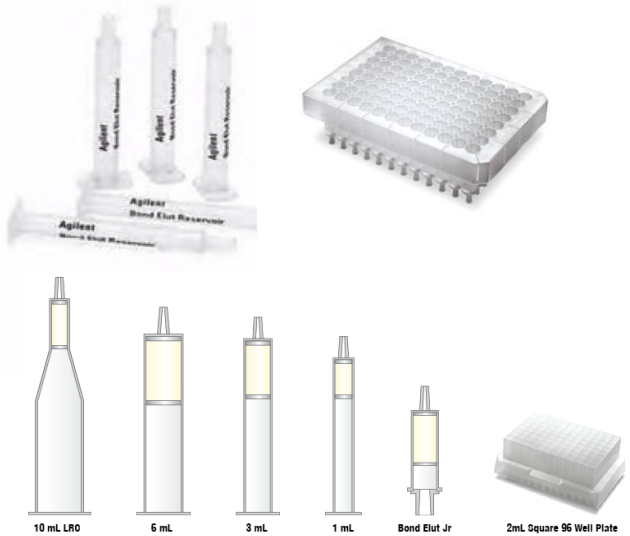
- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- How to deal with unwanted matrix effects
- Summary



Summary

- Many chromatography problems are due to the components present in the sample matrix.
- In some cases, measures can be taken to temporarily overcome or mask the unwanted matrix effects.
- Ultimately, sample preparation/cleanup is the most reliable way to address common chromatography data problems.
- Agilent offers a wide range of sample preparation products to support your analysis using established methods and protocols:
  - Filtration, protein and lipid removal
  - SLE
  - QuEChERS
  - SPE
- Matching the right sample preparation technique to the problem can improve your data quality, productivity, and throughput.
- Using inline filters, guards, high quality solvents, appropriate solvent bottle caps, and spring activated fittings can also prevent other chromatography problems (although this has not been discussed here).

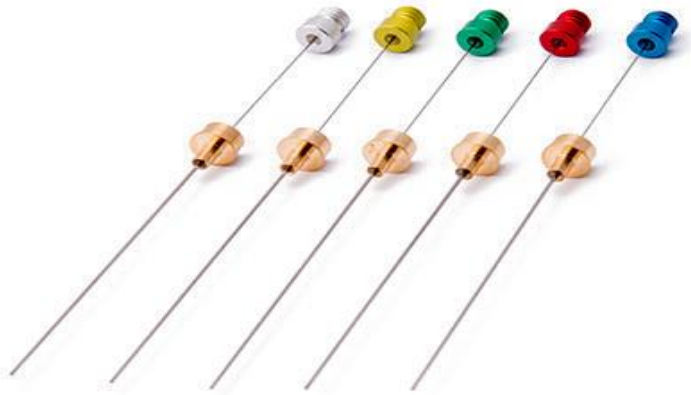
Offline Options for Sample Matrix Removal



Bond Elut Solid Phase Extraction cartridges and plates

Filter vials

QuEChERS



SPME

Captiva EMR-Lipid filtration cartridges and plates

Chem Elut S

Captiva syringe filters

Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

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

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

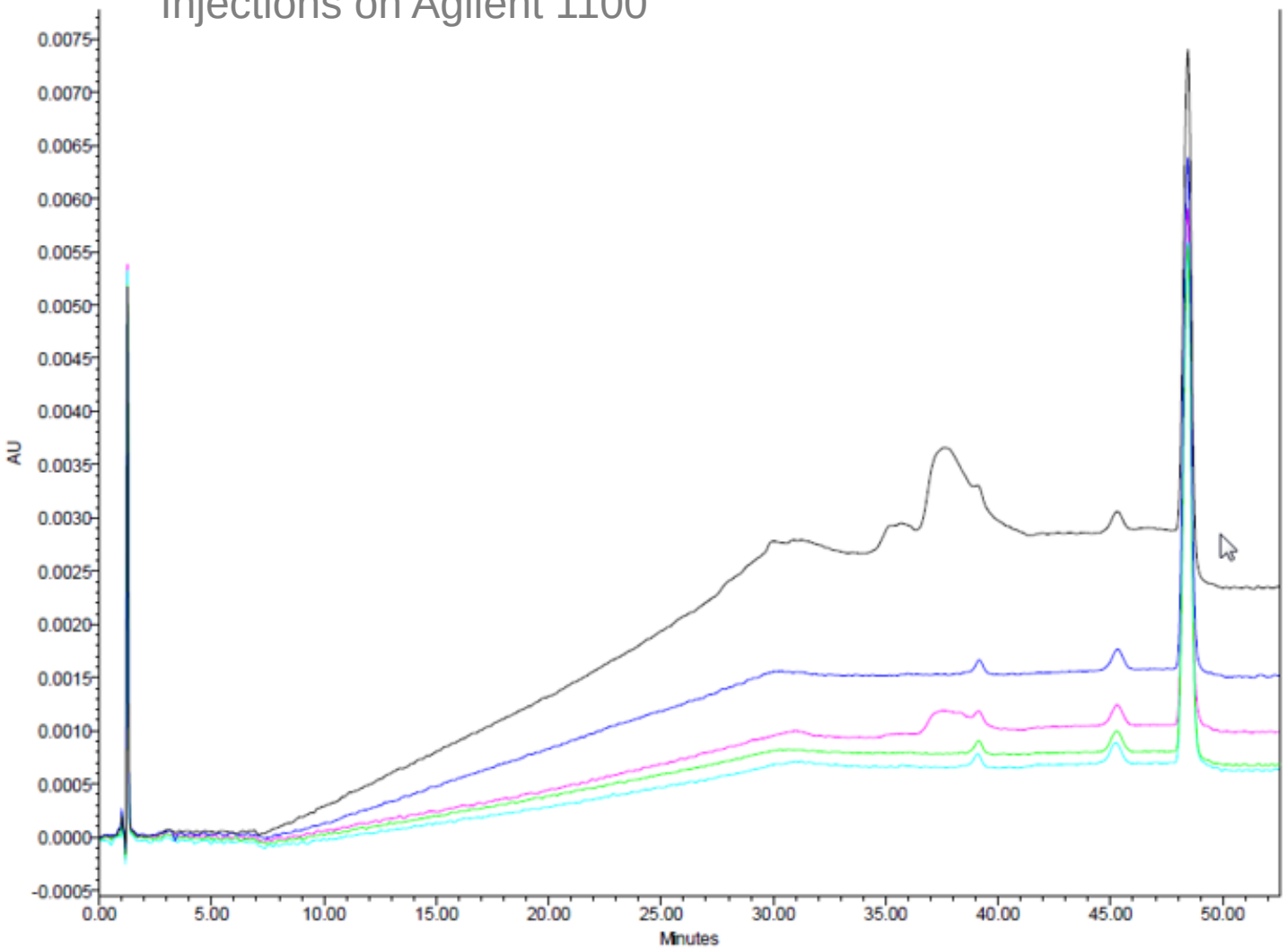
Appendix

Other Sources of Unknown Peaks and Chromatography Problems

- Impurities and contamination of mobile phase components
- Mobile phase is incompatible with LC system components, leaching out contaminants
- Contaminants from air getting into the mobile phase bottle due to use of incorrect bottle cap
- Microbial growth in solvent bottle
- Evaporation of volatile component of mobile phase
- Carry over

Solvent Contamination

Injections on Agilent 1100

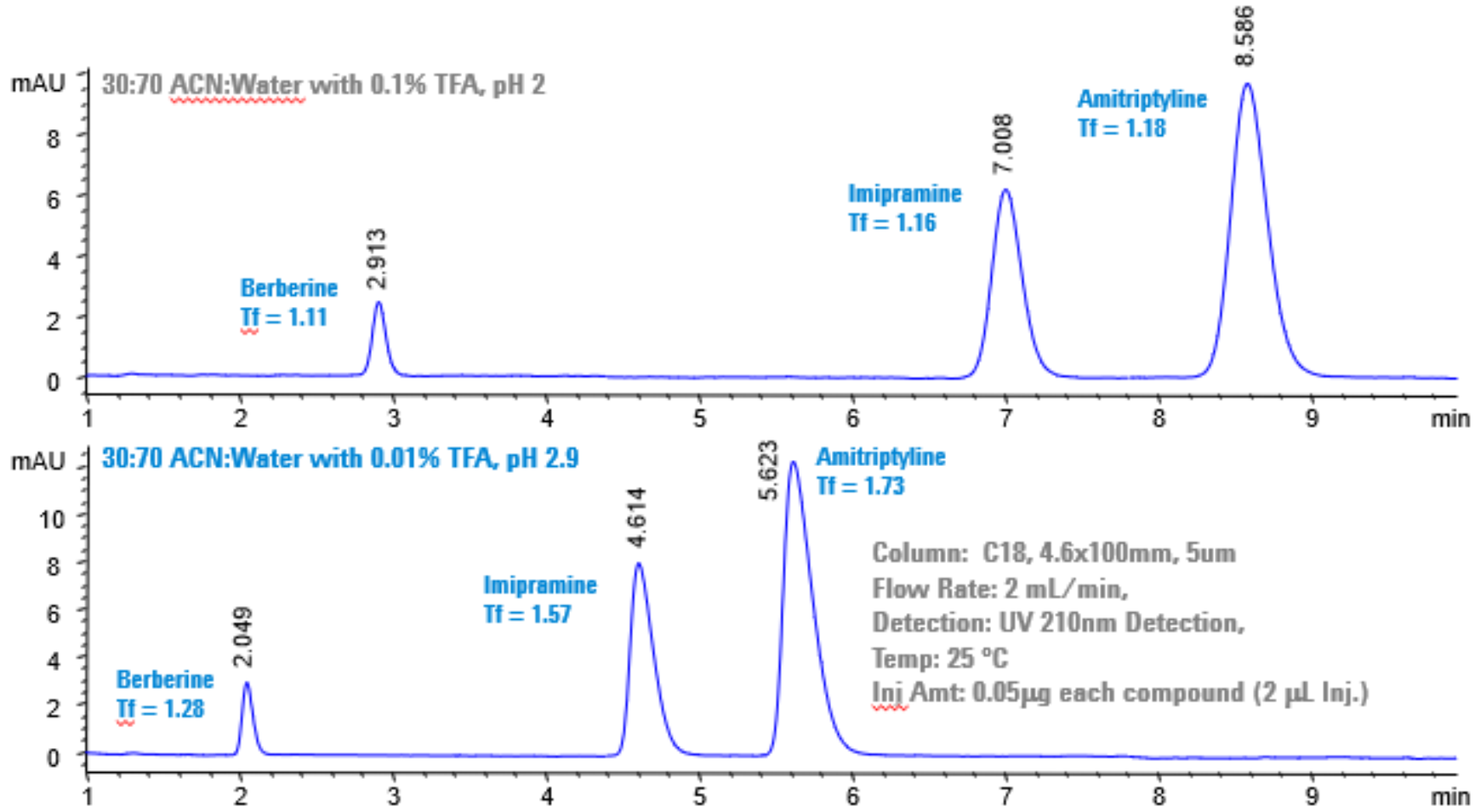


Contamination of mobile phase component

Various sources of acetonitrile tested

Retention Time Shifts and Peak Shape Problem

Change in volatile buffer concentration – Incorrect solvent bottle caps used



What To Do

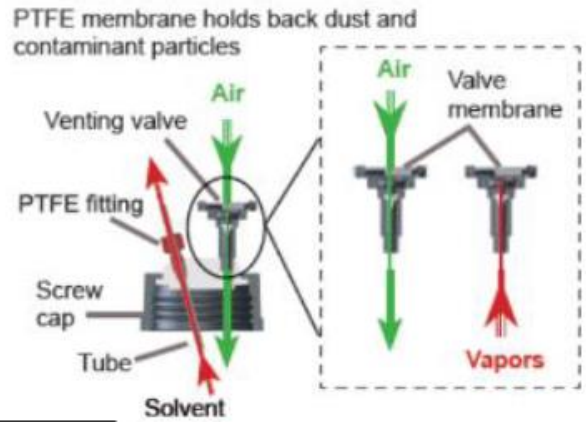
- Use high-purity solvents
- Use appropriate solvent bottle caps (Agilent InfinityLab Stay Safe caps)
- Use solvent compatible material for parts of LC that come in contact with mobile phase
- Use freshly made HPLC grade solvent and filtered buffer
- Replace solvent inlet filter as needed
- Always discard “old” mobile phase
- Do not add fresh mobile phase to old
- Use an amber solvent bottle for aqueous mobile phase
- If possible, add 5% organic to water to reduce microbial growth, or add a few mg/L sodium azide

InfinityLab Stay Safe caps

Solvent inlet filter

InfinityLab Stay Safe Caps

Main Features	Advantages
Venting valve for mobile phase *Charcoal filter for waste container* *Time strip*	Eliminates harmful solvent vapors Keeps solvent concentration constant Keeps constant pressure in bottle Prevents twisted tubing Allows easy solvent refill Allows easy tightening



Carry Over

Carry over peaks can be caused by

1. Late eluting peaks from previous run
2. Contaminated sampling device components (rotor seal, needle, needle seat)
3. Contaminated/wrong solvent used for needle wash
4. Release of retained compounds on active sites of the system
5. Unswept areas in sample path

Solution

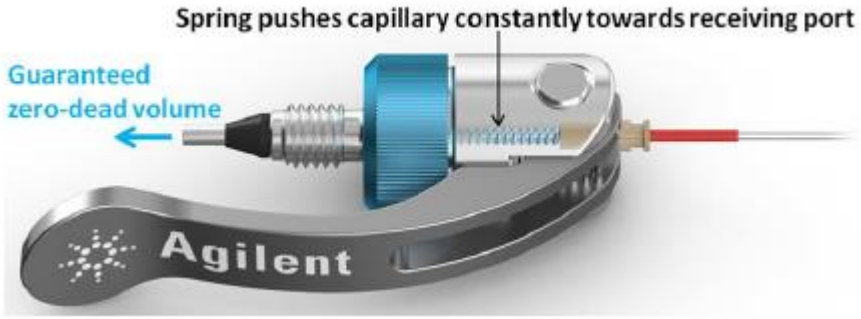
1. Longer column flush
2. Flush/replace sampling device components
3. Use fresh/correct solvent for needle wash
4. Passivate the system with phosphoric acid or EDTA
5. Use spring activated fittings (InfinityLab Quick Connect and Quick Turn fittings)

InfinityLab Quick Connect fitting

InfinityLab Quick Turn fitting

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 600 bar
- Up to 1300 bar with a wrench
- Compact design

Column Cleaning

Flush with stronger solvents than your mobile phase

Reversed-Phase Solvent Choices
in Order of Increasing Strength

- Mobile phase without buffer salts
- 100% Methanol
- 100% Acetonitrile
- 75% Acetonitrile:25% Isopropanol
- 100% Isopropanol
- 100% Methylene Chloride*
- 100% Hexane*

Use at least 10 column volumes of each solvent for analytical columns

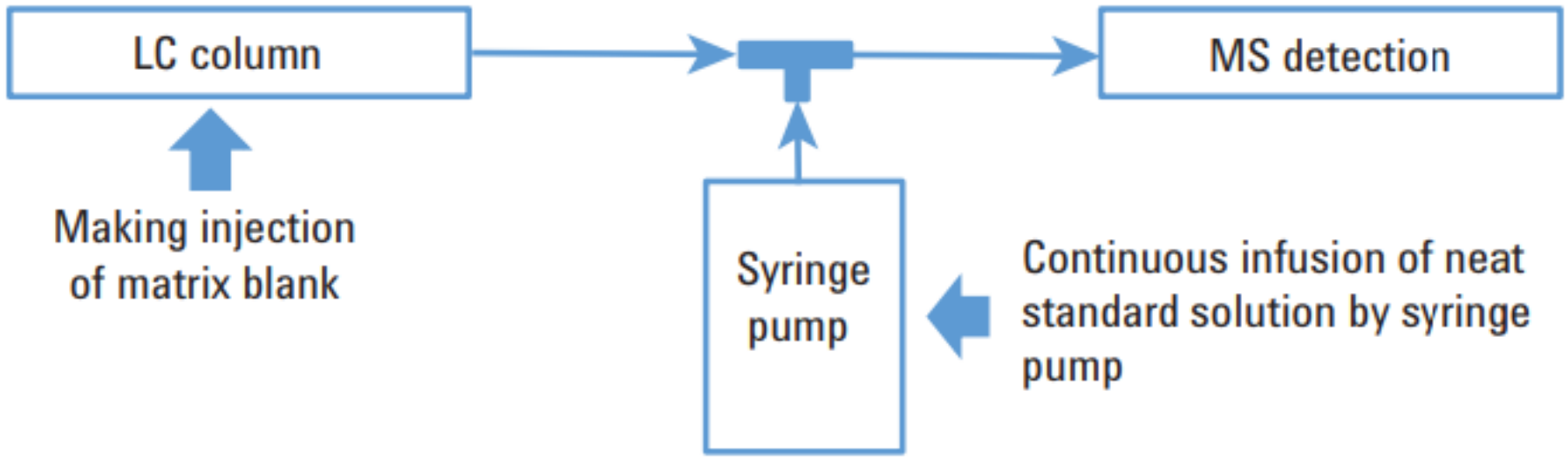
* When using either Hexane or Methylene Chloride the column must be flushed with Isopropanol before returning to your reversed-phase mobile phase.

Column Cleaning – Protein/Peptide Removal

Solubilization solvents for proteins/peptides, in the order of weakest to strongest:

- Water/phosphate buffer
- Dilute acid (TFA, HOAc or HCl)
- Neutral pH 6-8 M guanidine-HCl or isothiocyanate
- 5% HOAc/6 M urea
- Dilute acid + aqueous/organic solvents (ACN, MeOH, THF)
- Dilute base (ammonium hydroxide)
- Neat organic solvents – ACN, MeOH, THF
- 99% formic acid
- HFIP or HFIP/aqueous mixtures
- 100% TFA
- DMSO or 0.1 – 1% TFA in DMSO
- Formamide

Post Column Infusion



Post column infusion setup for evaluation of ion suppression caused by the matrix

Bond Elut Plexa Method

Generic method recommendations

	Acids	Neutrals		Bases
Analyte	LogP>1.0 pK_a < 5	LogP > 1.5 pKa 3-5	 pK_a 6-10	LogP > 0.8 pK_a 6-10
	Plexa PAX	Plexa (Acid load method)	Plexa (Base load method)	Plexa PCX
Sample Pre-treatment	2% NH_4OH	1% HCO_2H	2% NH_4OH	2% H_3PO_4
Sorbent Condition	100% MeOH	100% MeOH		100% MeOH
Equilibration	100% H_2O	100% H_2O		100% H_2O
Load	Apply pre-treated sample			
Wash	100% H_2O	5% MeOH in H_2O		2% HCO_2H in H_2O
Elution 1/Wash 2	100% MeOH *Neutrals*	100% MeOH *Neutrals*		1:1 MeOH/ACN *Acids, Neutrals*
Elution 2	5% HCO_2H in MeOH *Acids*			5% NH_3 in 1:1 MeOH/ACN *Bases*
Analysis	Prepare extracts for instrumental analysis			

Note: This user guide is a convenient starting point for any SPE method development. Further optimization may be required to adjust the method to your application needs.

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Published in the USA, August 1, 2012
Publication Number 04034-0712

Bond Elut Plexa SPE method guide

Accuracy Begins Here

The Bond Elut Plexa Family is a new generation of polymeric SPE products, designed for simplicity, improved analytical performance and ease-of-use.These advanced SPE sorbents offer excellent flow characteristics due to their monodisperse particle size distribution, affording superior ease-of-use, with minimal clogging of the packed bed.

Optimized surface chemistries and extraction protocols deliver ultra clean extracts with minimized ion suppression.

The Measure of Confidence

Agilent Technologies

Bond Elut Plexa Method

Method development and troubleshooting for plasma samples

Bond Elut Plexa PAX

Bond Elut Plexa PAX contains a strong anion exchange functionality. Simple generic methodology and excellent batch to batch reproducibility offer robust anion exchange SPE workflow.

Strong Anion Exchange SPE for Acidic Analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% NH_4OH in H_2O
Washes	1. 500 µL H_2O 2. 500 µL MeOH
Elution	2 x 250 µL 5% HCO_2H in MeOH

Volumes stated for all methods are for a 30 mg, 1 mL SPE format device.

pH adjustment – To improve ion exchange interactions on Plexa PAX, ionize analytes prior to loading. For acidic analytes the pH should be at least 2 pH units above the pK_a.

Bond Elut Plexa

Bond Elut Plexa is a non-polar divinylbenzene-based neutral polymeric sorbent. This sorbent is the best choice for non-ionic extraction of a wide range of acidic, neutral and basic analytes from different matrices.

Non-Polar SPE for neutrals and moderately acidic or basic analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% NH_4OH *(neutrals and bases)* 1% HCO_2H in H_2O *(acids)*
Washes	500 µL 5 % MeOH in H_2O
Elution	2 x 250 µL MeOH

pH adjustment – To improve hydrophobic interaction on Plexa, neutralize analytes prior to loading. Basic analytes should be at least 2 pH units above the pK_a. Acidic analytes should be 2 pH units below the pK_a.

Bond Elut Plexa PCX

Bond Elut Plexa PCX is a cation exchanger with mixed mode sorbent characteristics and is therefore suitable for the extraction and clean-up of polar and non-polar bases from biofluids.

Strong Cation Exchange SPE for Basic Analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% H_3PO_4 in H_2O
Washes	1. 500 µL 2% HCO_2H in H_2O 2. 500 µL MeOH:ACN (1:1, v/v)
Elution	2 x 250 µL 5% NH_3 (28-30%) in MeOH: ACN (1:1, v/v)

pH adjustment – To improve ion exchange interactions on Plexa PCX, ionize analytes prior to loading. Basic analytes should be at least 2 pH units below the pK_a. Acidification is also necessary to disrupt analyte-protein interaction.

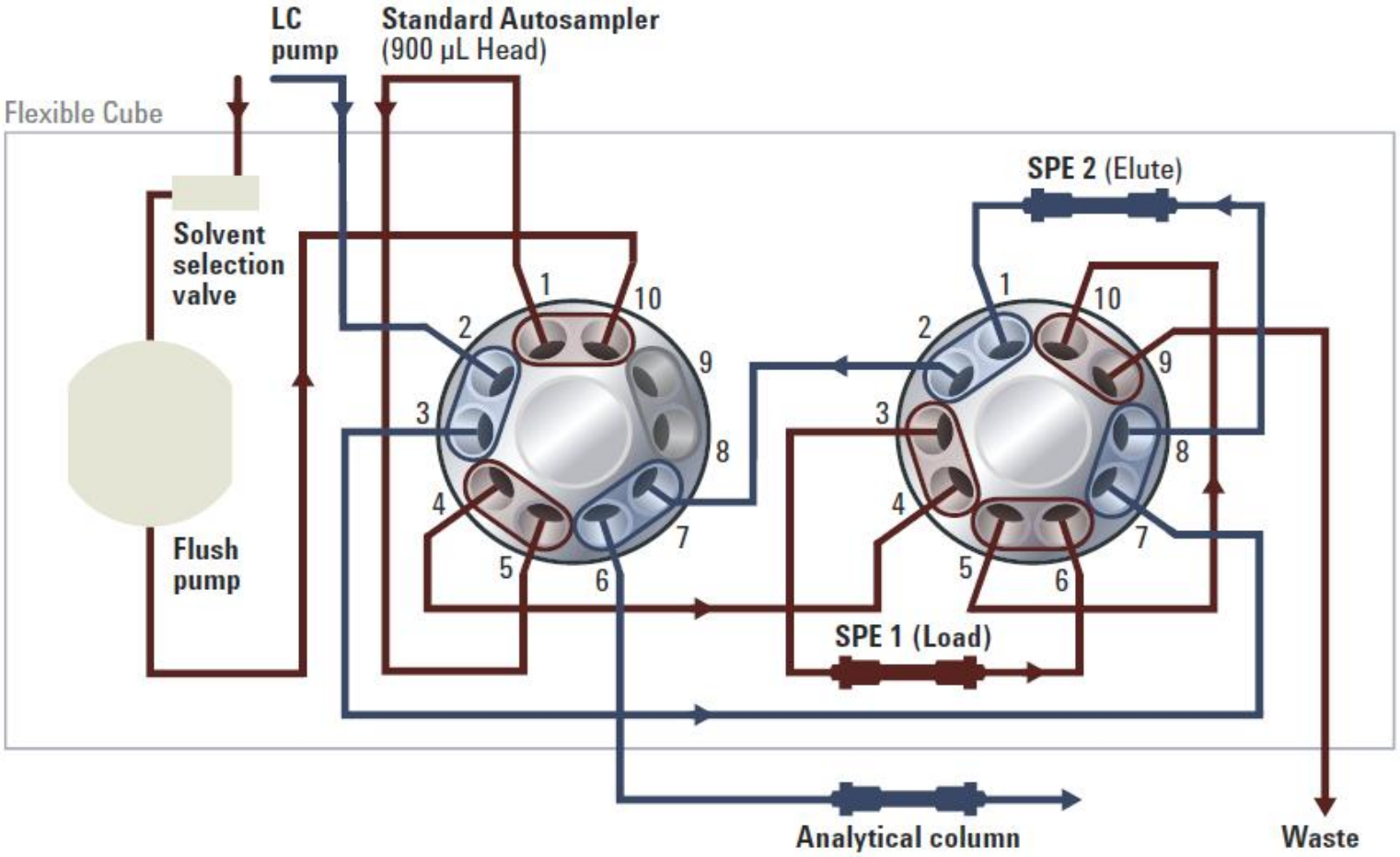
Troubleshooting	Bond Elut Plexa	Bond Elut Plexa PCX	Plexa PAX
Analyte(s) eluting in the wash step(s)	• Reduce volume of washing step • Reduce concentration of organics in the wash step		
	• Rinse with either 2% NH_3 for basic analytes or 1% formic acid for acids to ensure hydrophobic interactions • Increase sorbent bed mass	• Increase sorbent bed mass for increased ion exchange capacity	
Inadequate Elution (Eluent does not contain >90% of the analyte.)	• Decrease flow rate, (1 mL/min is recommended) • Check solubility of analyte in the eluent • Increase strength of elution solvent • Increase the eluent volume or use multiple aliquots of eluent		
	• Add modifier (depending on analyte type) to the elution solvent, thereby promoting ionization	• Use up to 10% ammonia (28-30%) in solvents such as MeOH and ACN	• Use up to 10% formic acid in MeOH for anion exchange elution

Online SPE (Trace Enrichment-SPE)

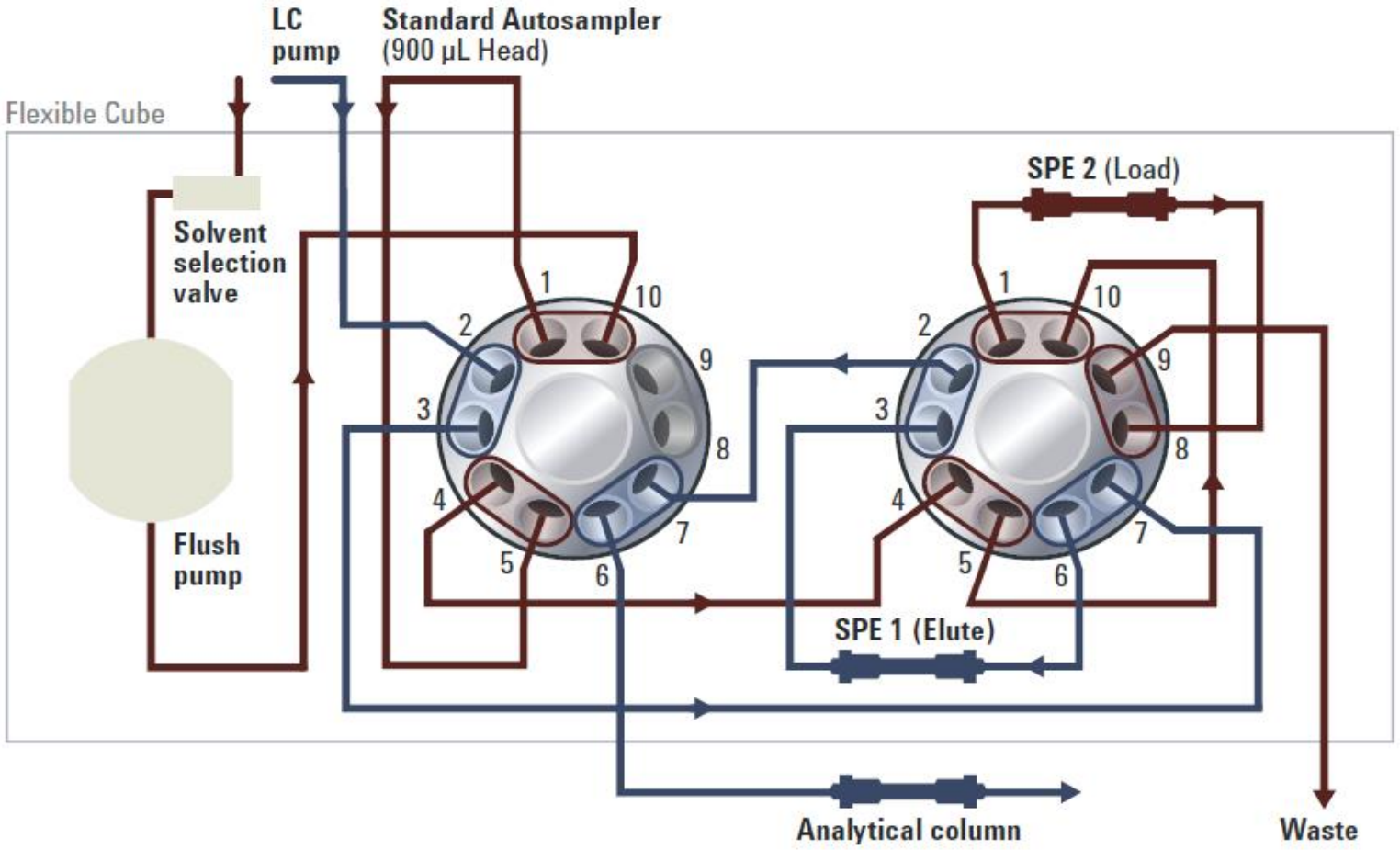
- 100% of the prepared sample is loaded
- Volume can be <5 mL
- Combined with more sensitive detection (MS/MS)

5982-1271: Bond Elut Online SPE, PLRP-S, 2.1 x 12.5 mm, 3/pk
5982-1270: Bond Elut Online SPE, PLRP-S, 4.6 x 12.5 mm, 3/pk
820999-901: Hardware, Guard Column Holder

Step 1: Online SPE1

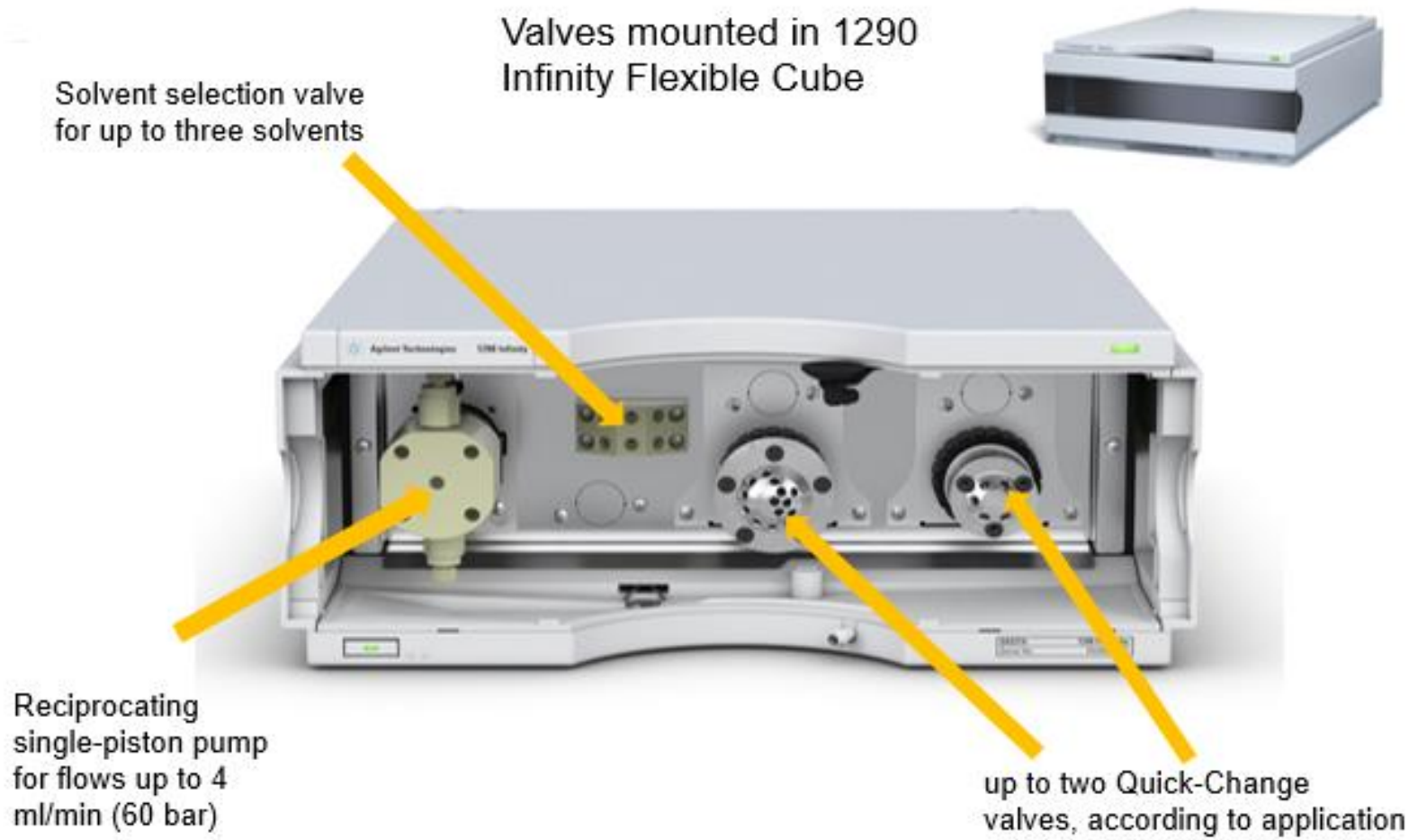


Step 2: Online SPE2



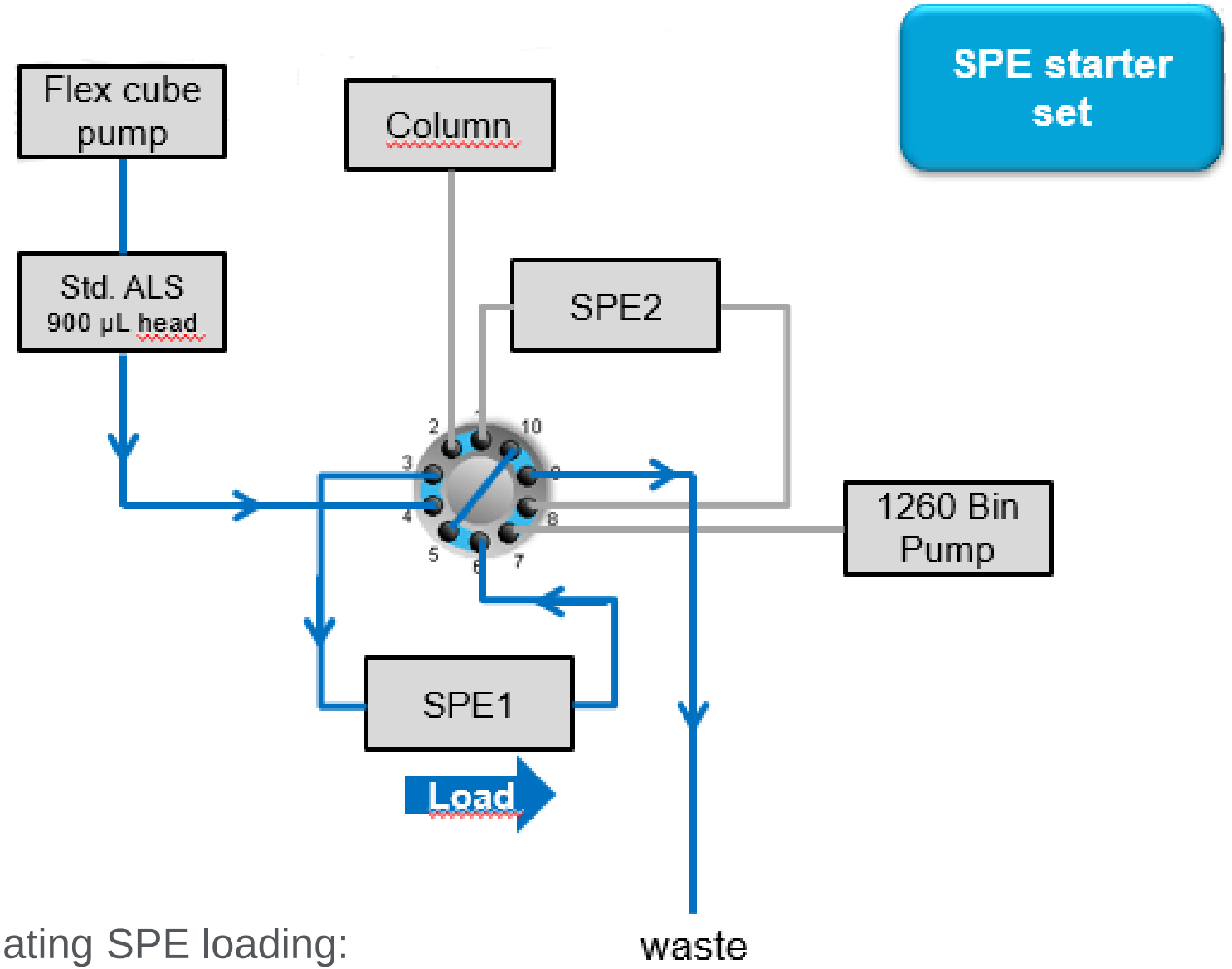
Agilent Online SPE System

Agilent 1200 Infinity Series Online SPE Solution-Flex Cube



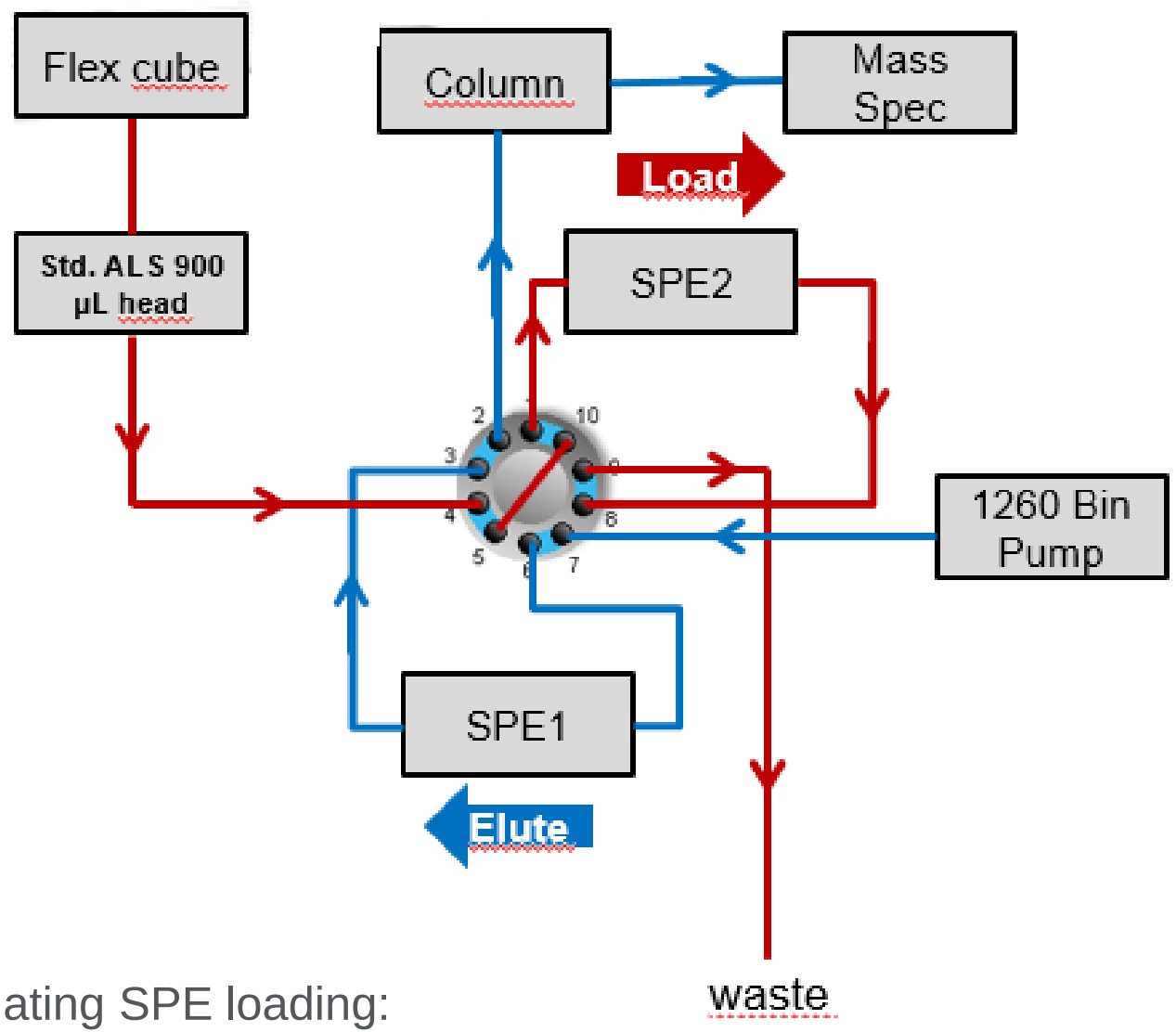
Online SPE with Flex Cube

Flex Cube



System with alternating SPE loading:
Load sample to SPE1

Flex Cube



System with alternating SPE loading:
Elute sample from SPE1 to column/load sample 2 on SPE2

Online Options for Sample Matrix Removal

Agilent 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

Agilent 1290 Infinity II LC **in-line filte**r
2.1 mm, 0.3 µm, 1300 bar, 5067-6189

Agilent Fast **Guard**, 3/pk
RRHT, 600 bar
RRHD, 1300bar
One piece preassembled, no cartridge or holder

Agilent Online **SPE***, Bond Elut PLRP-S
2.1 x 12.5 mm cartridge, 3/pk, 5982-1270
4.6 x 12.5 mm cartridge, 3/pk, 5982-1271
Cartridge housing, 820999-901

* *See Appendix*