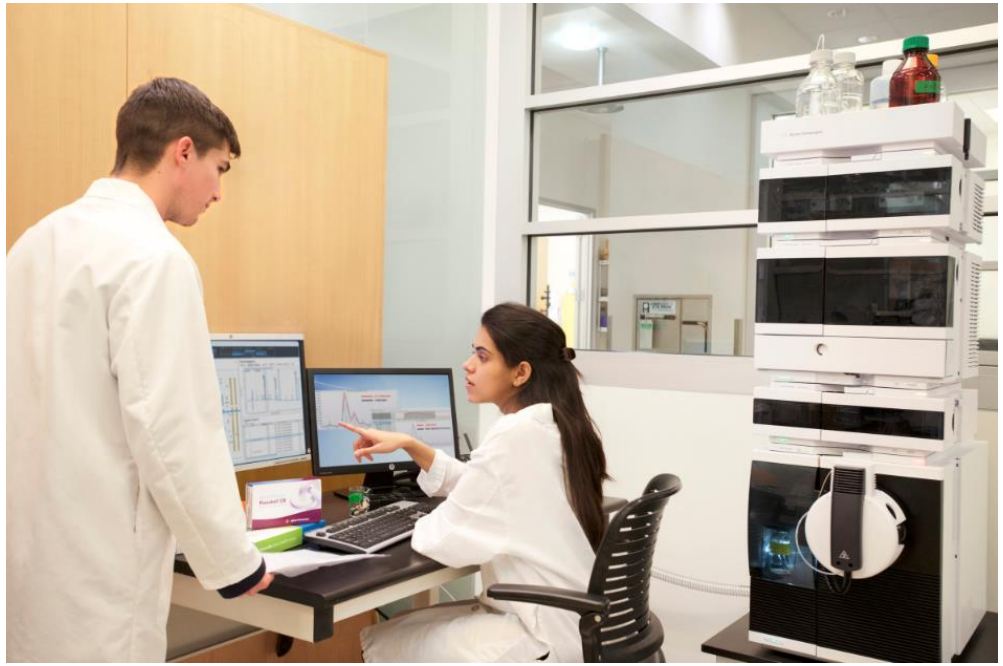
Techniques for Avoiding Unexpected Problems in LC and GC Analysis

Alexander Ucci
Applications Engineer
October 18, 2023

Agenda

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



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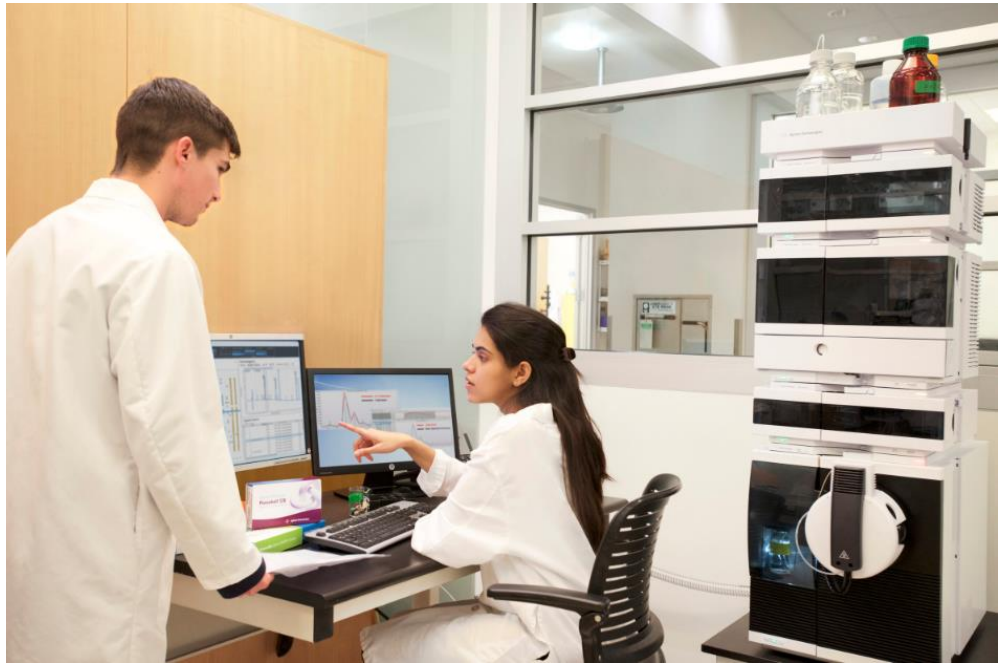


Dilute and Shoot

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

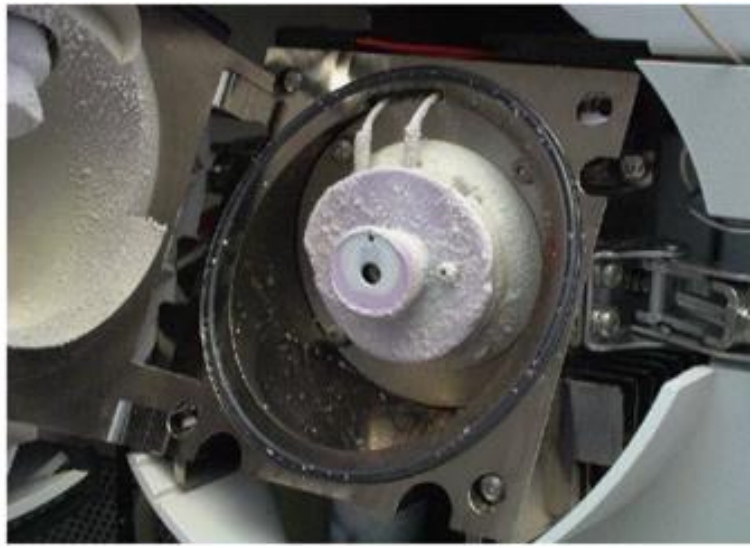


Image of salt build-up on an ESI-LC/MS inlet from unremoved salts.

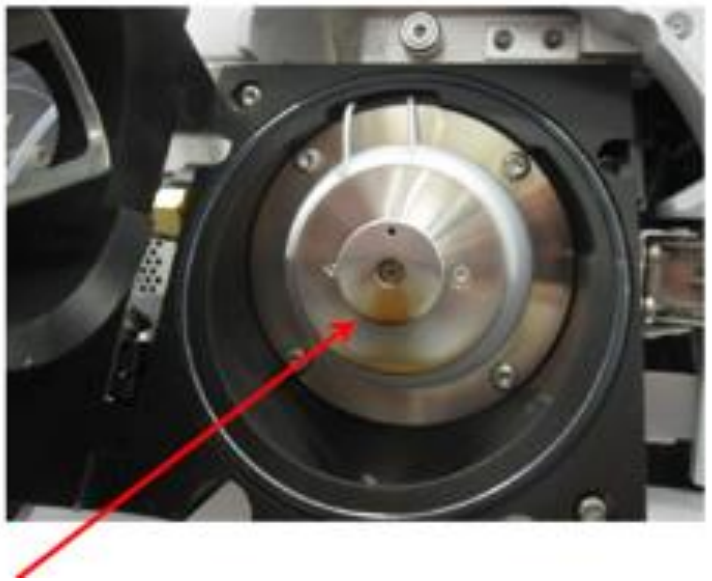


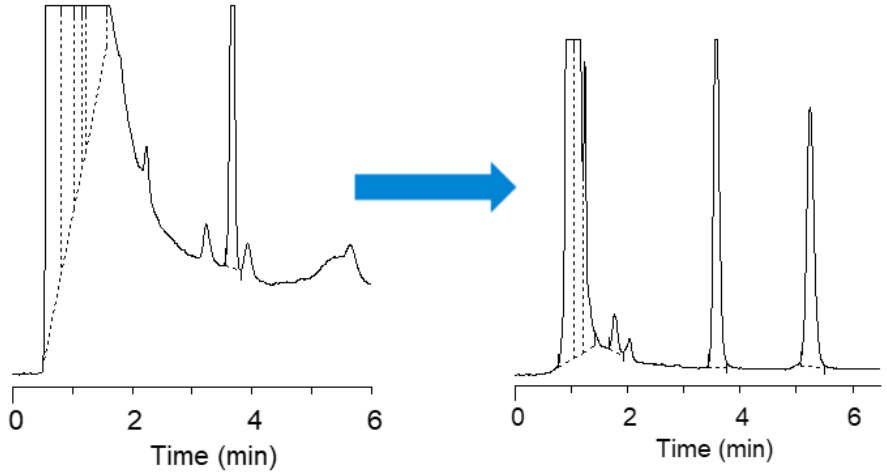
Image shows the build-up on the ESI-MS inlet after 3000X urine dilute and shoot injections.

Importance of the Correct Sample Preparation/Cleanup

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

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

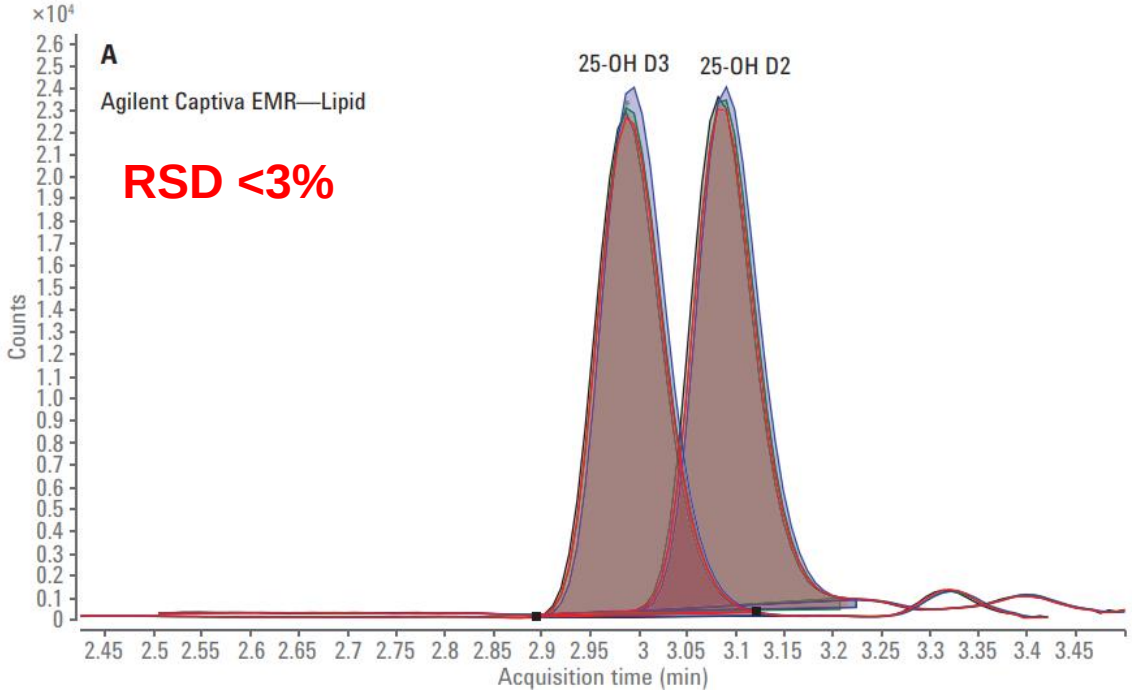
Sample *without* sample preparation



Sample *with* sample preparation

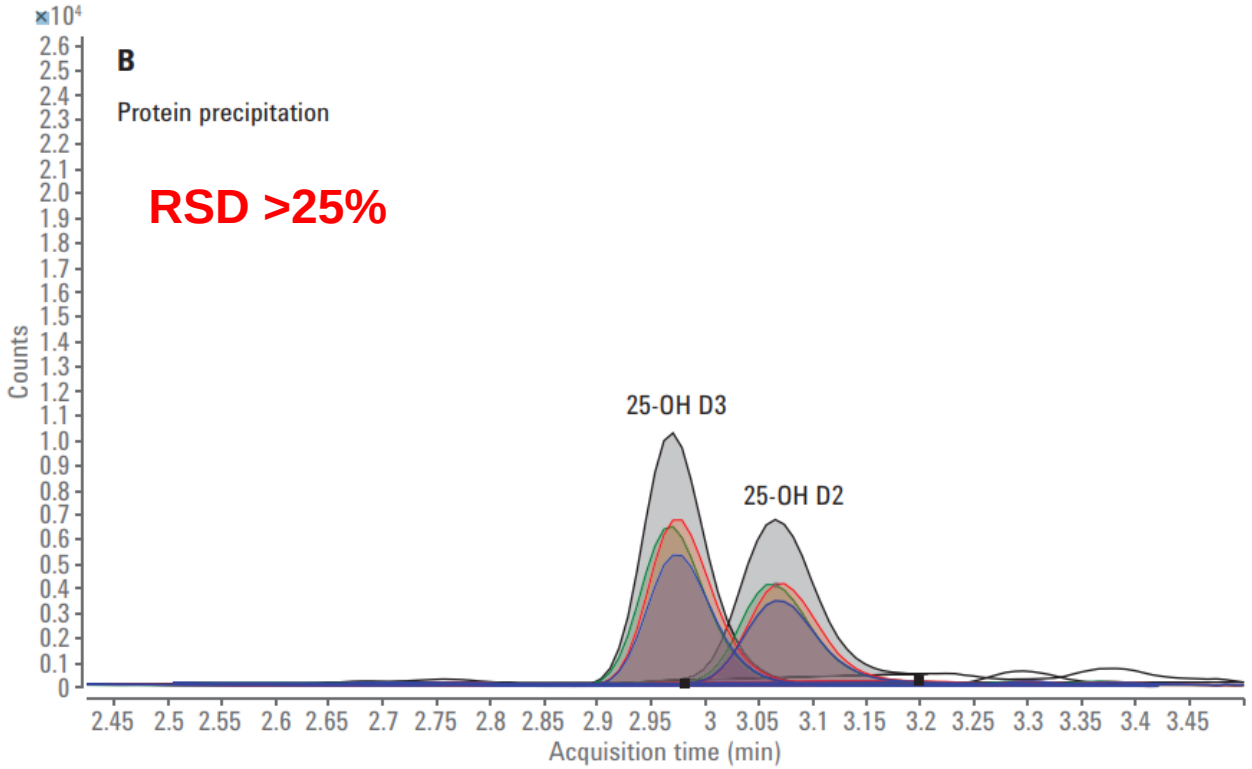
Importance of the Correct Sample Preparation/Cleanup

Captiva EMR-Lipid



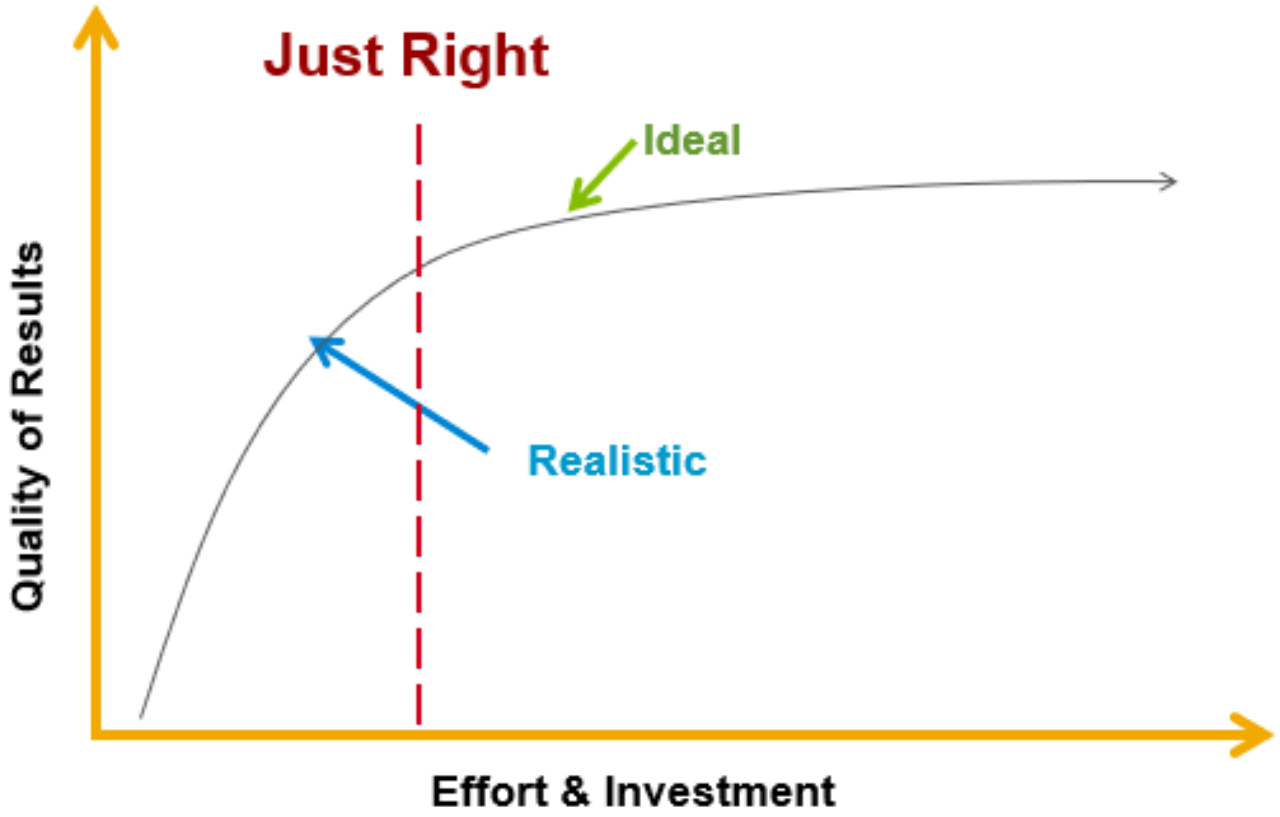
Sample *with* the correct sample preparation

Protein precipitation



Sample *without* the correct sample preparation

Striking the Right Balance in Sample Preparation



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	Neglectable	Yes	Yes	Yes	Yes, for some matrices
Physical filtration	Minimal	Less often	Less often	Less often	Yes, for some matrices
Chemical filtration, liquid extraction, sorbent extraction	Yes	No	No	No	No

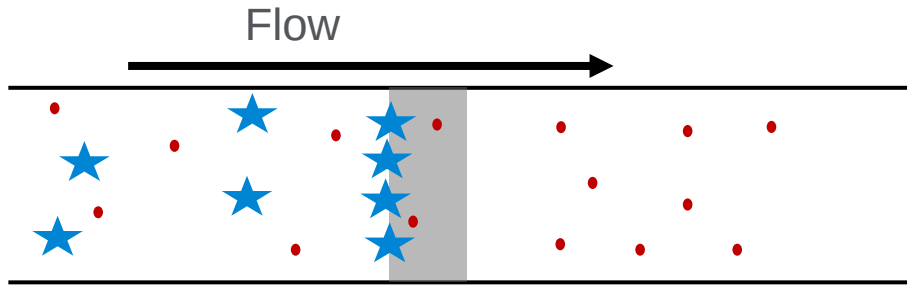
- Consider the source of the sample (blood vs. urine vs. lake water)
- Mechanical filtration is the absolute minimum sample preparation that should be done – too cheap and easy not to do
- Some matrices can cause ion suppression or ion enhancement, leading to erroneous results

Methods for Sample Preparation

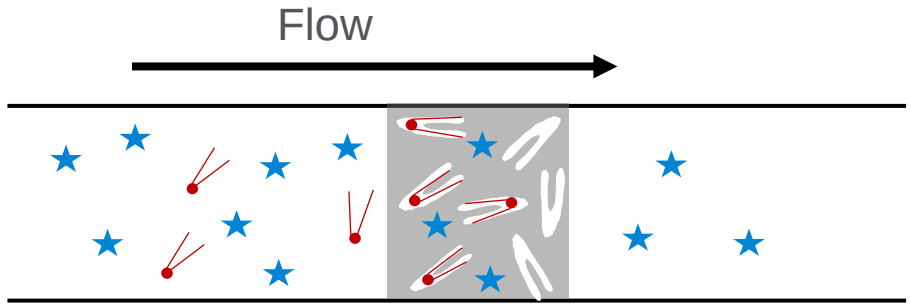
1. Physical filtration
   a) Syringe filters
   b) Syringeless filters
      1. Filter vials
      2. Spin filters

2. Chemical filtration
   a) Matrix removal

3. Liquid extraction
   a) LLE
   b) SLE
   c) QuEChERS

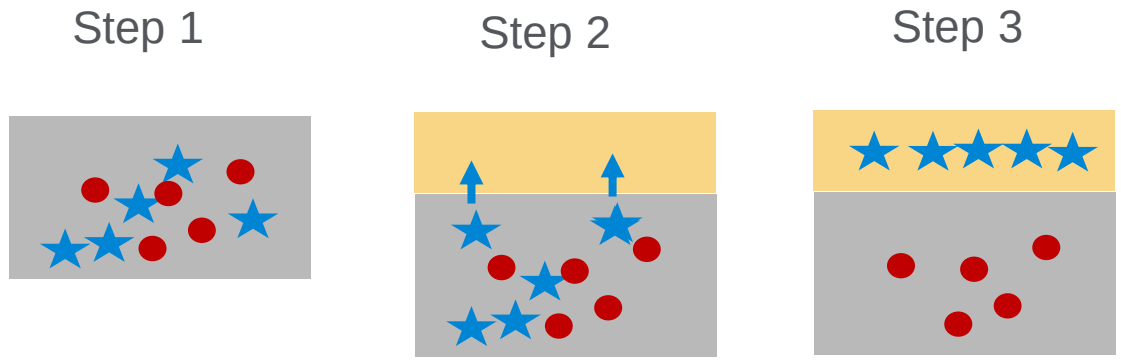


Filters by particle size



Captiva products

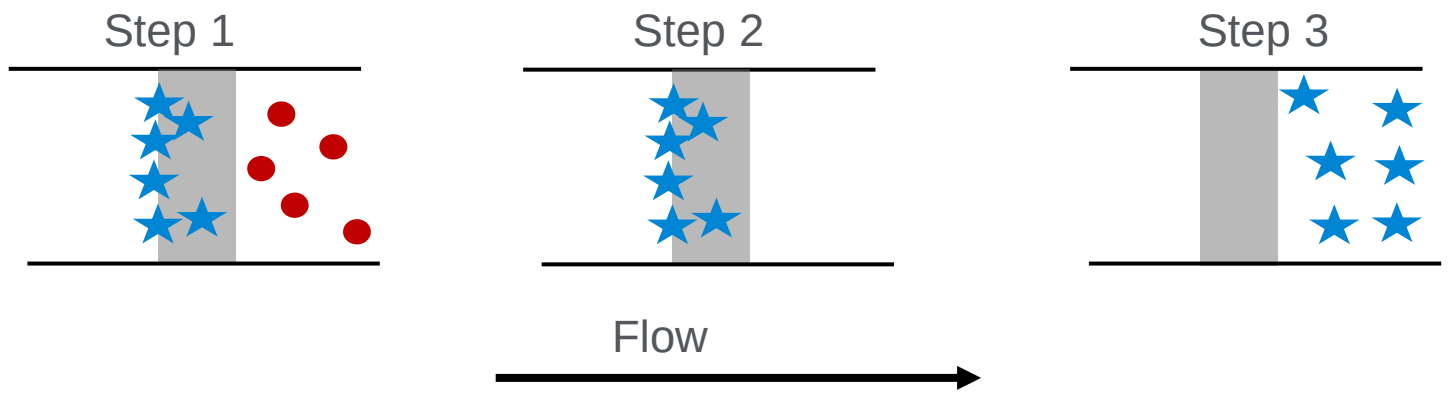
Filters by shape, charge or hydrophobicity



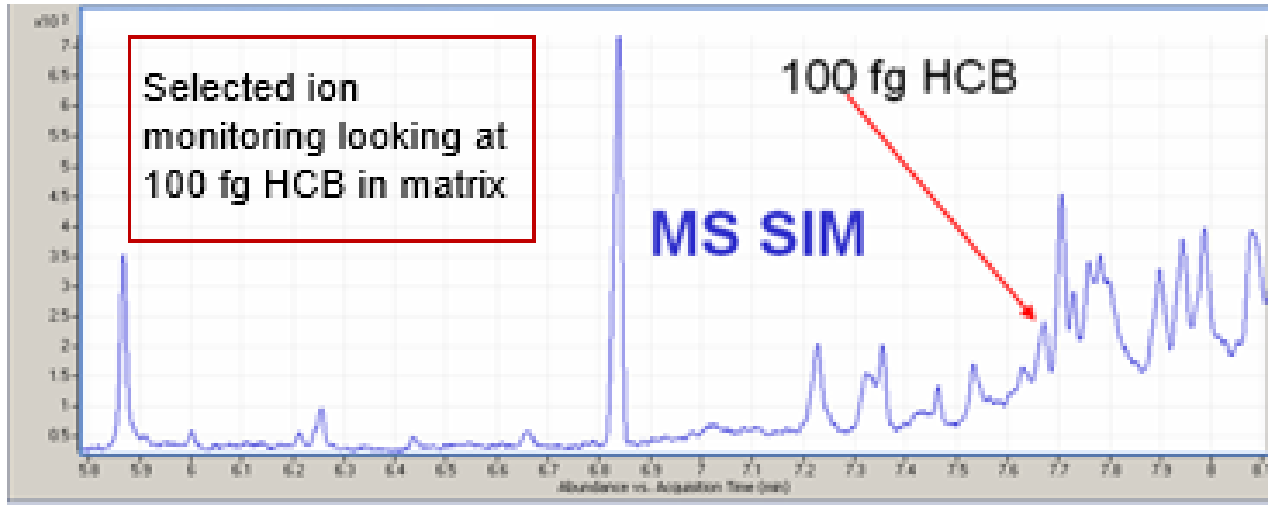
Methods for Sample Preparation

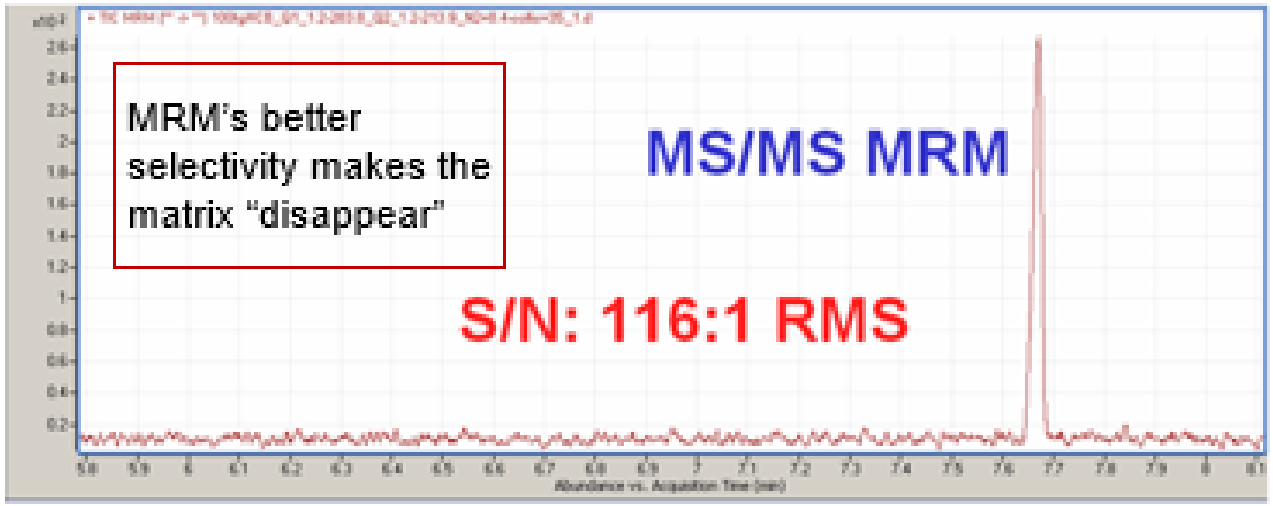
1. Catch and release
   a) SPE
      1. Polymeric
      2. Silica

Bond Elut products



SIM and MRM – Remember the Matrix is Still There





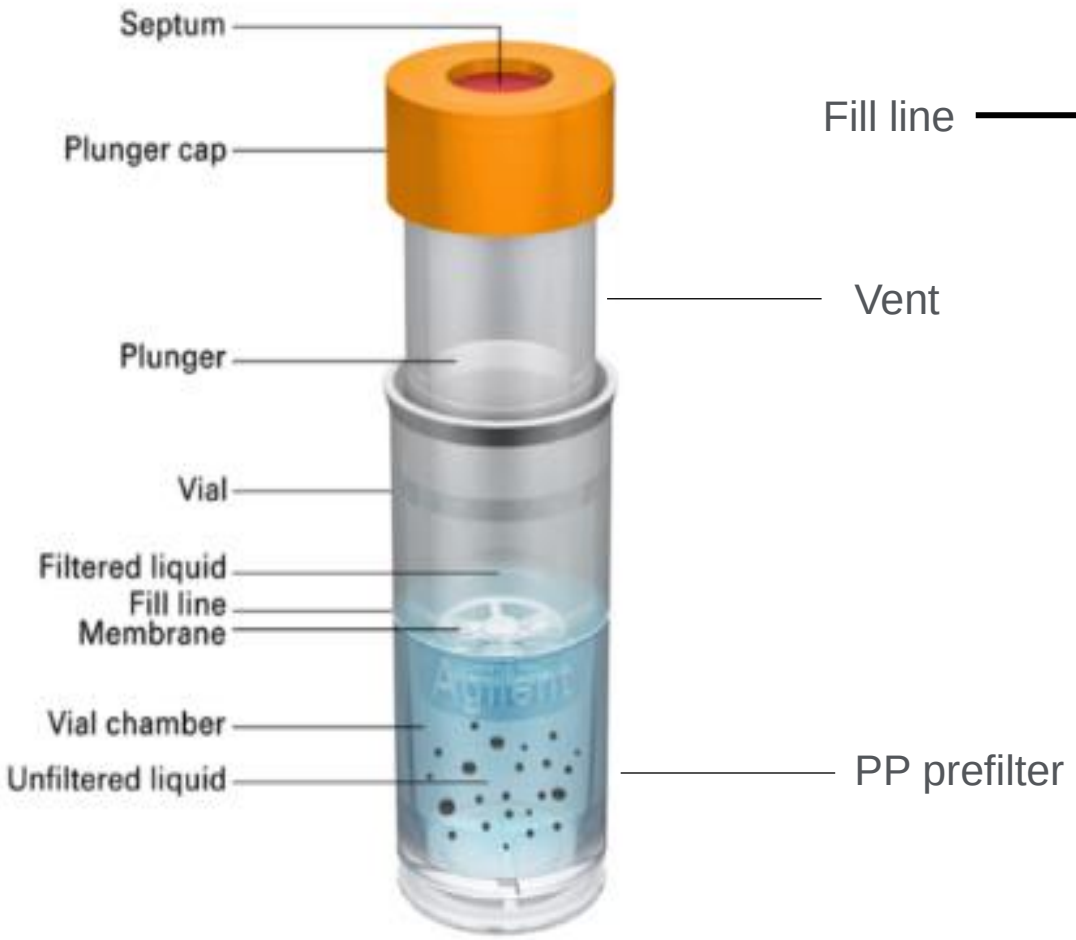
Filtration

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			♦	♦	♦	♦

Physical Filtration – Captiva Filter Vials



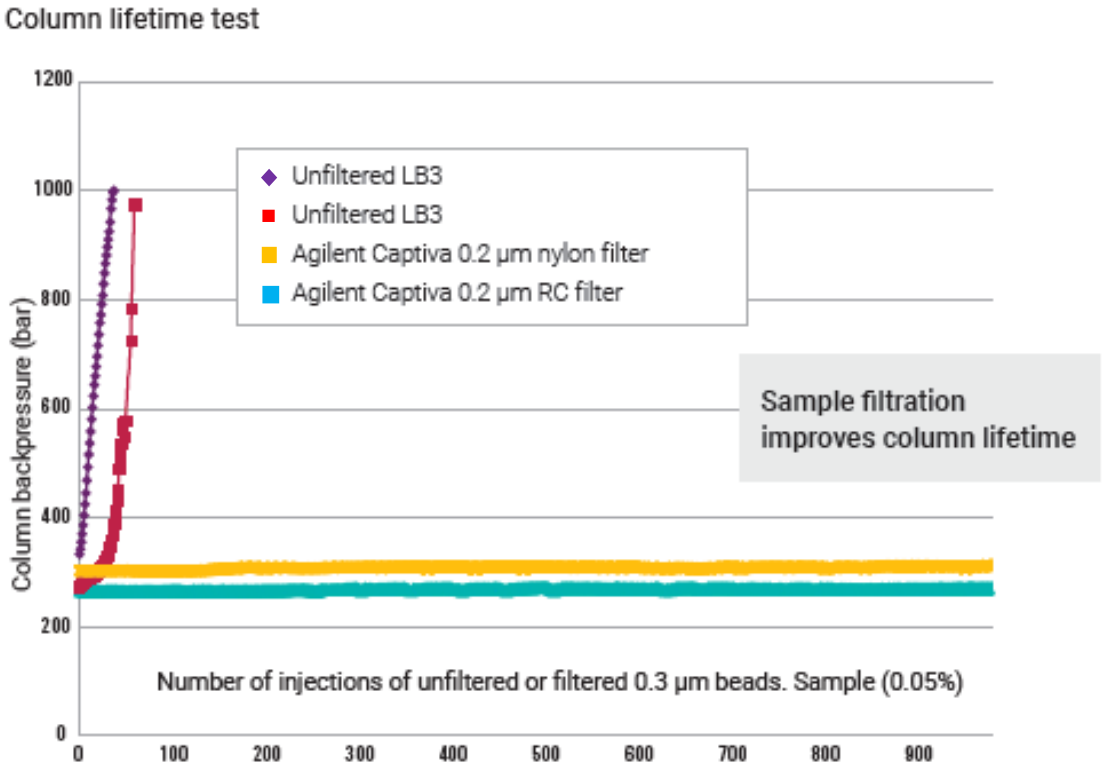
Part Number	Description
5191-5933	PTFE filter vial, 0.45 µm, 100/pk
5191-5934	PTFE filter vial, 0.20 µm, 100/pk
5191-5935	Nylon filter vial, 0.45 µm, 100/pk
5191-5936	Nylon filter vial, 0.20 µm, 100/pk
5191-5939	RC filter vial, 0.45 µm, 100/pk
5191-5940	RC filter vial, 0.20 µm, 100/pk
5191-5941	PES filter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

Preslit options available as well

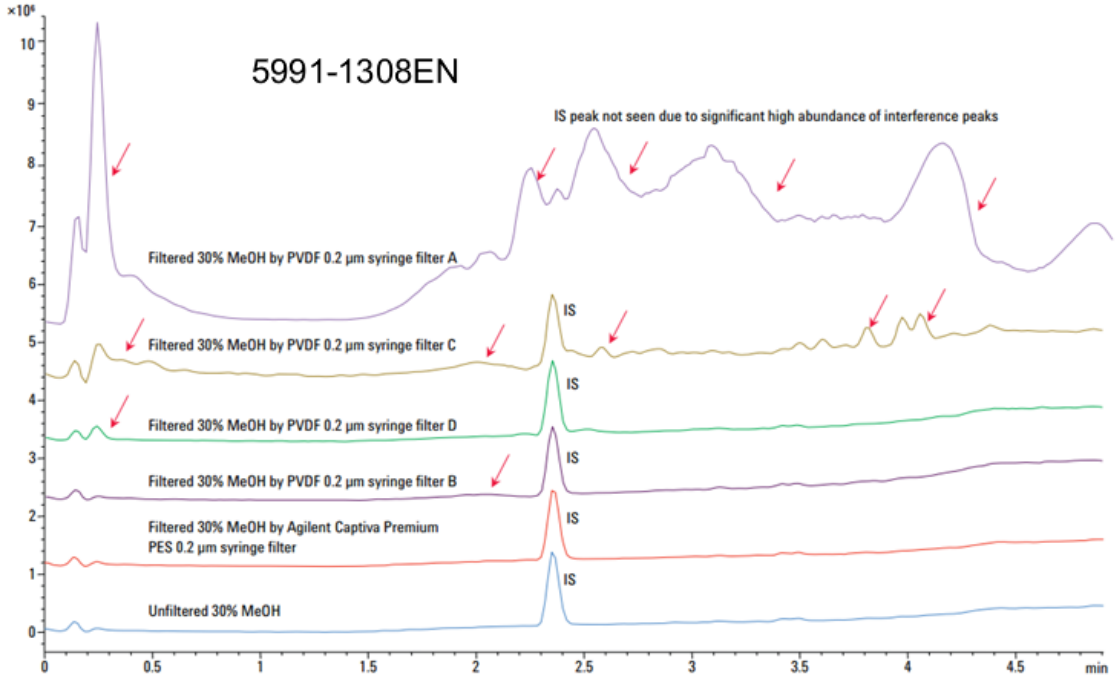
Agilent.com/chem/filtervials
Filter vials user guide: 5994-0814EN

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

Chemical Filtration

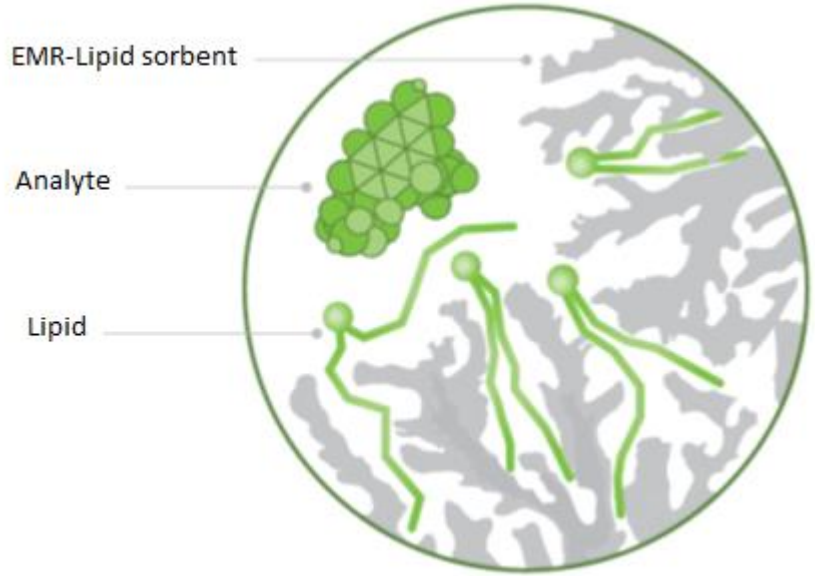
Captiva EMR-Lipid

- 2-in-1 benefit of removing lipids and fats selectively and efficiently
- 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

Captiva EMR-Lipid Sorbent Technology

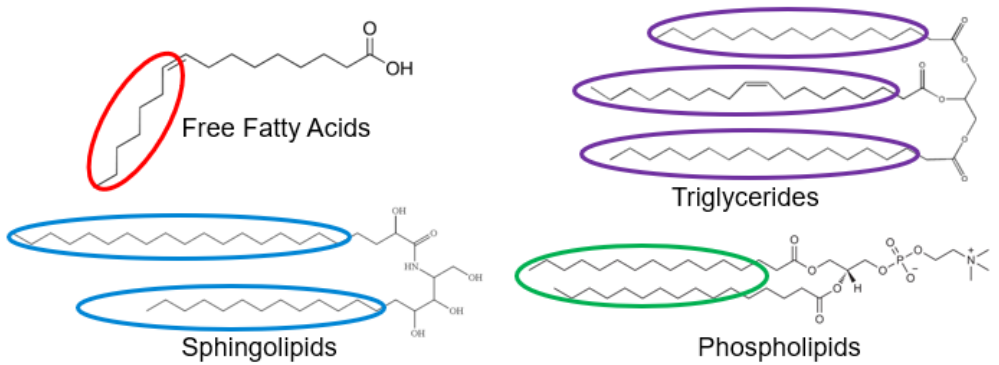
EMR-Lipid sorbent technology effectively traps lipids through two mechanisms:

- 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

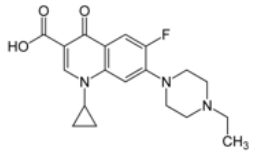


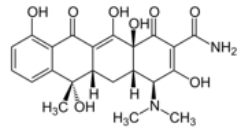
Captiva EMR-Lipid
Selective removal of lipids

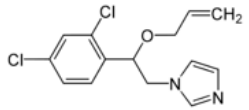
Removes lipids

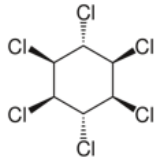


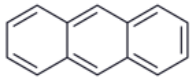
Does not remove target analytes

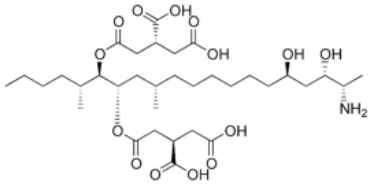

Fluoroquinolones


Tetracyclines


Imidazole pesticides


Organochlorine Pesticides


PAHs

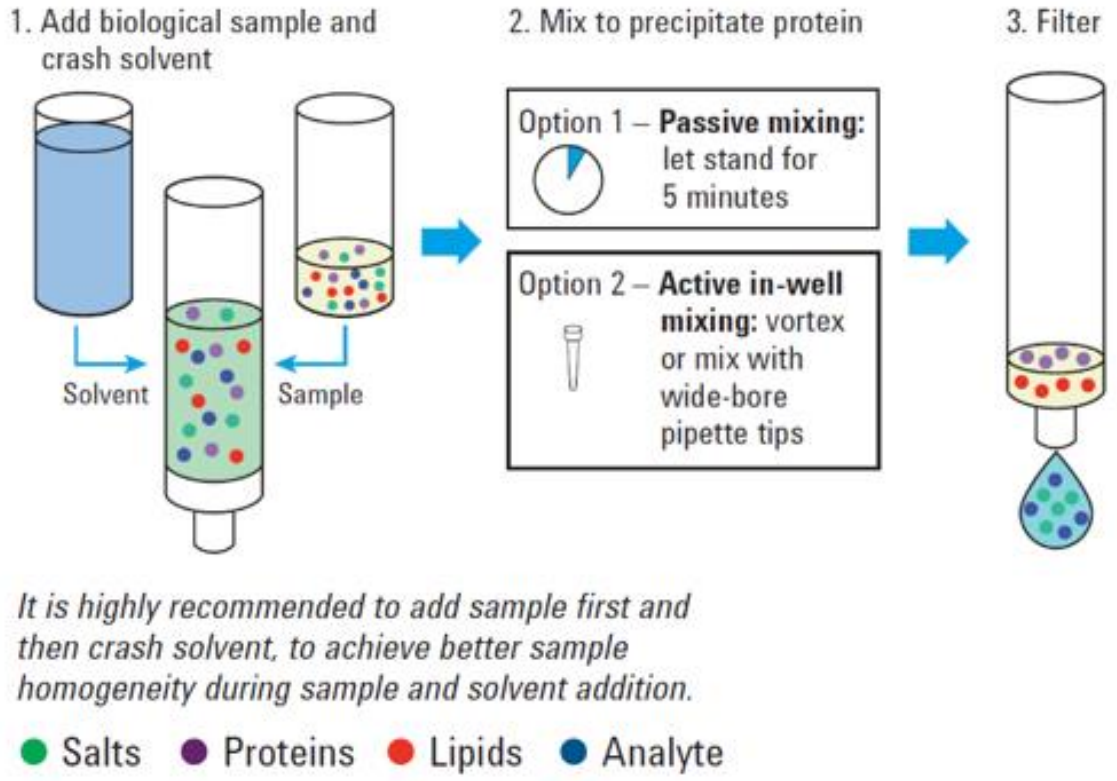

Fumonisin B2

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.



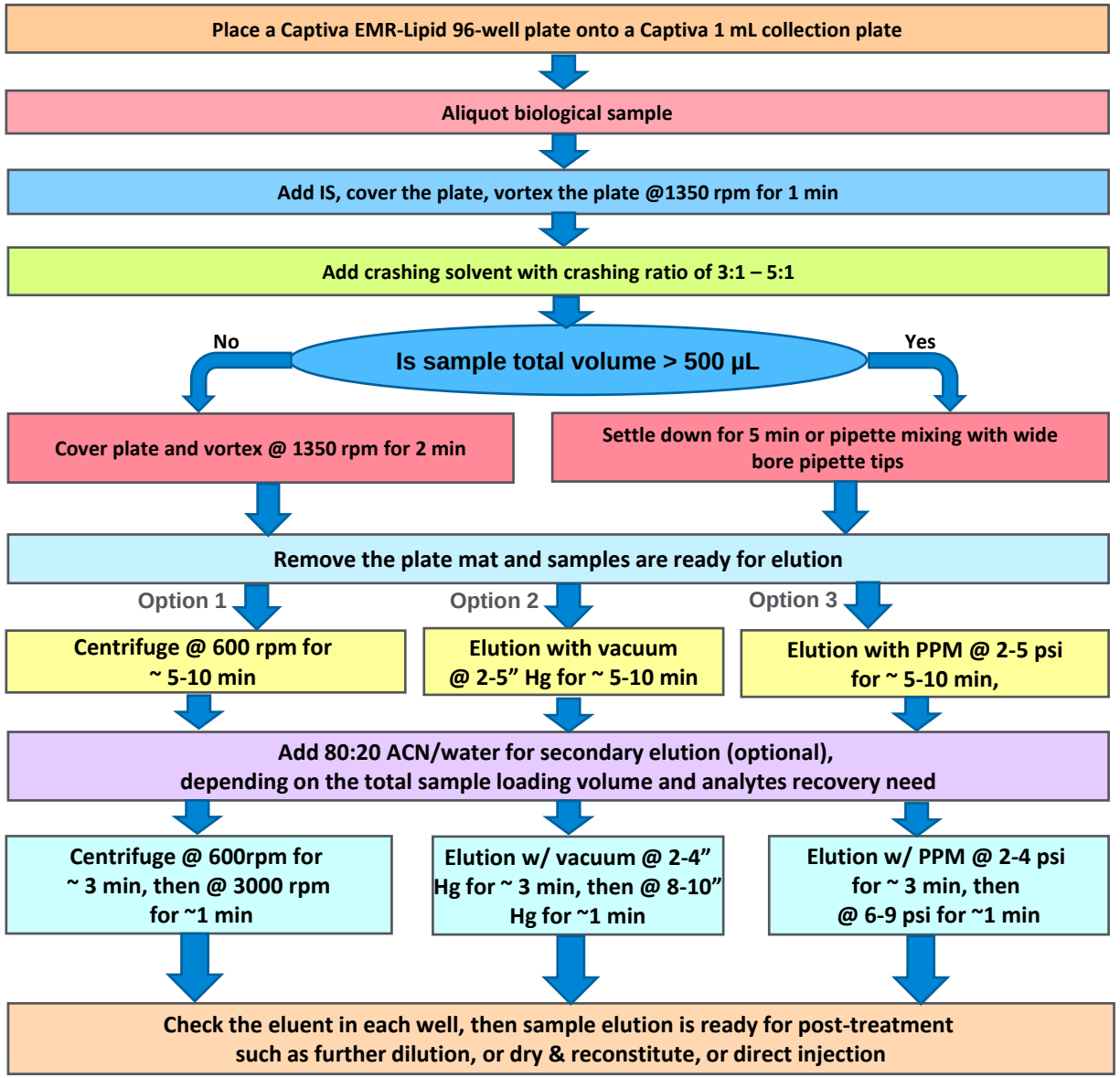
Vacuum, positive pressure, or centrifuge can be used.

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]

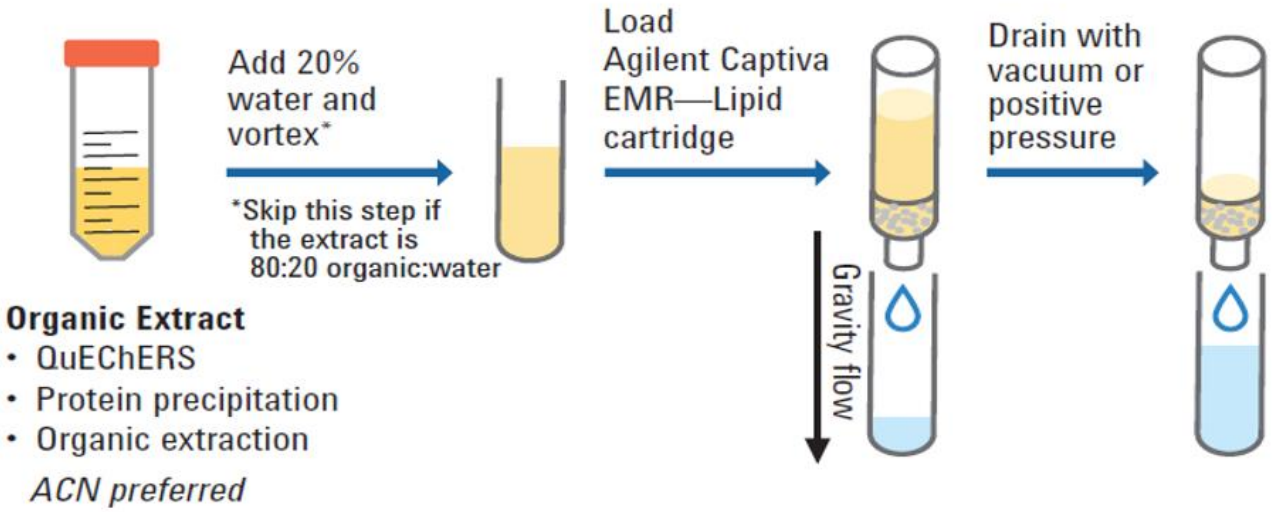
Sample Preparation Procedure for Biological Samples in 96-Well Plates



Captiva EMR-Lipid

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

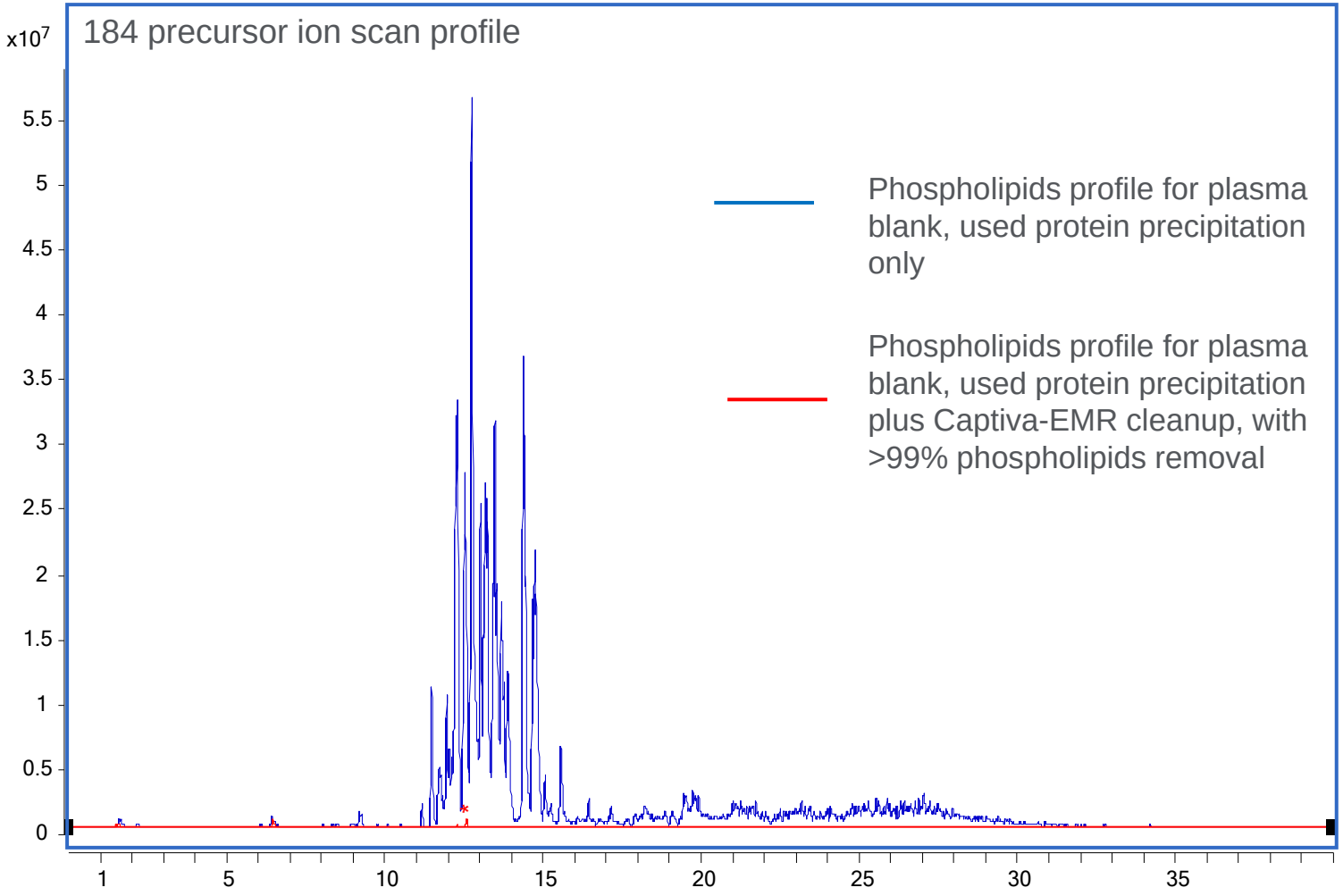
Operating instructions

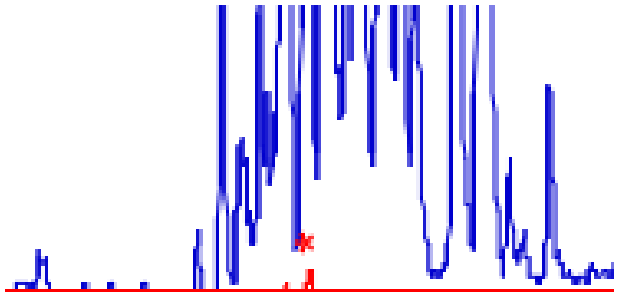


[Captiva EMR-Lipid method guide for 3 mL and 6 mL cartridges]

Captiva EMR-Lipid Cleanup

Efficient phospholipids removal from biological fluid matrices

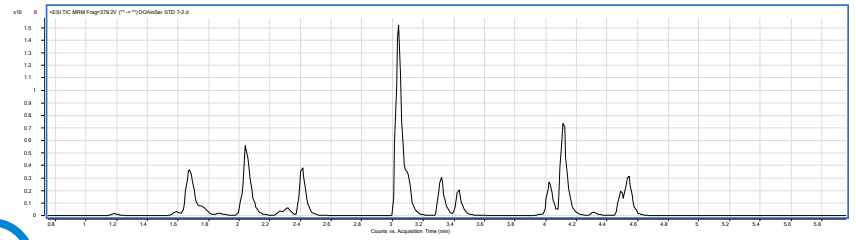


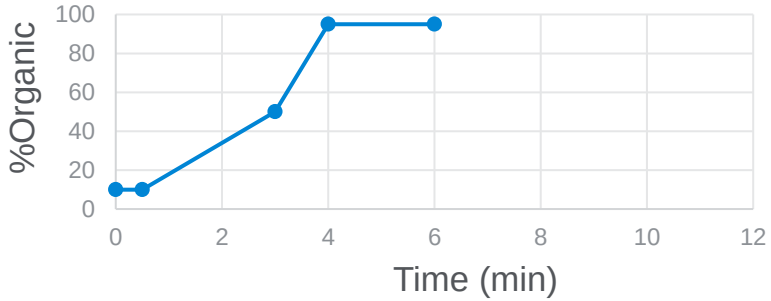


Zoomed-in baseline

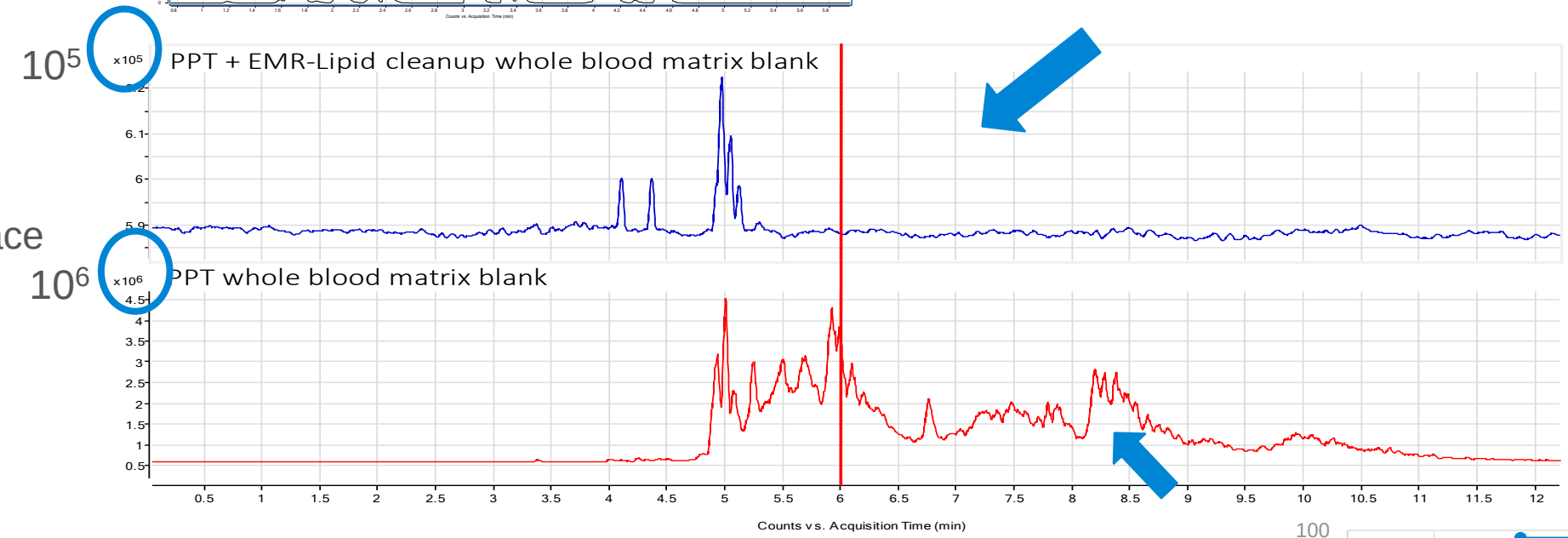
Removal of Lipids Allows for Shorter LC Gradient Time

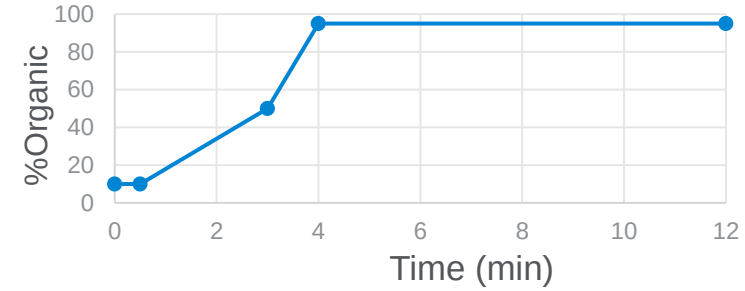
Target analytes TIC chromatogram





Lipid trace

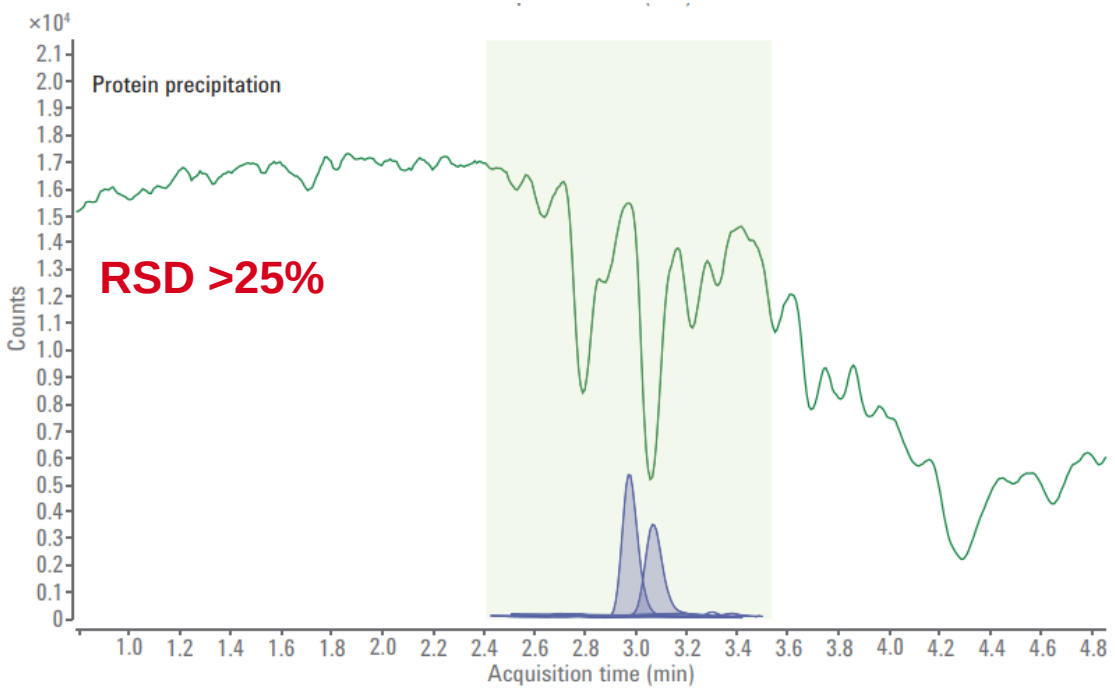




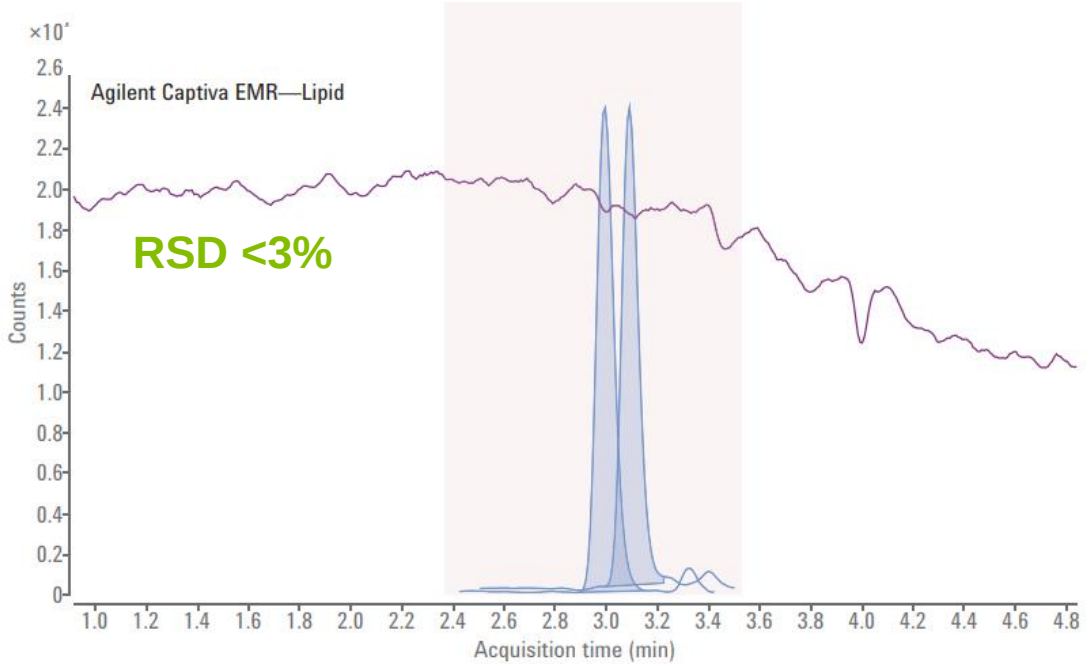
Captiva EMR-Lipid Cleanup

Improved analyte response and reproducibility

PPT only



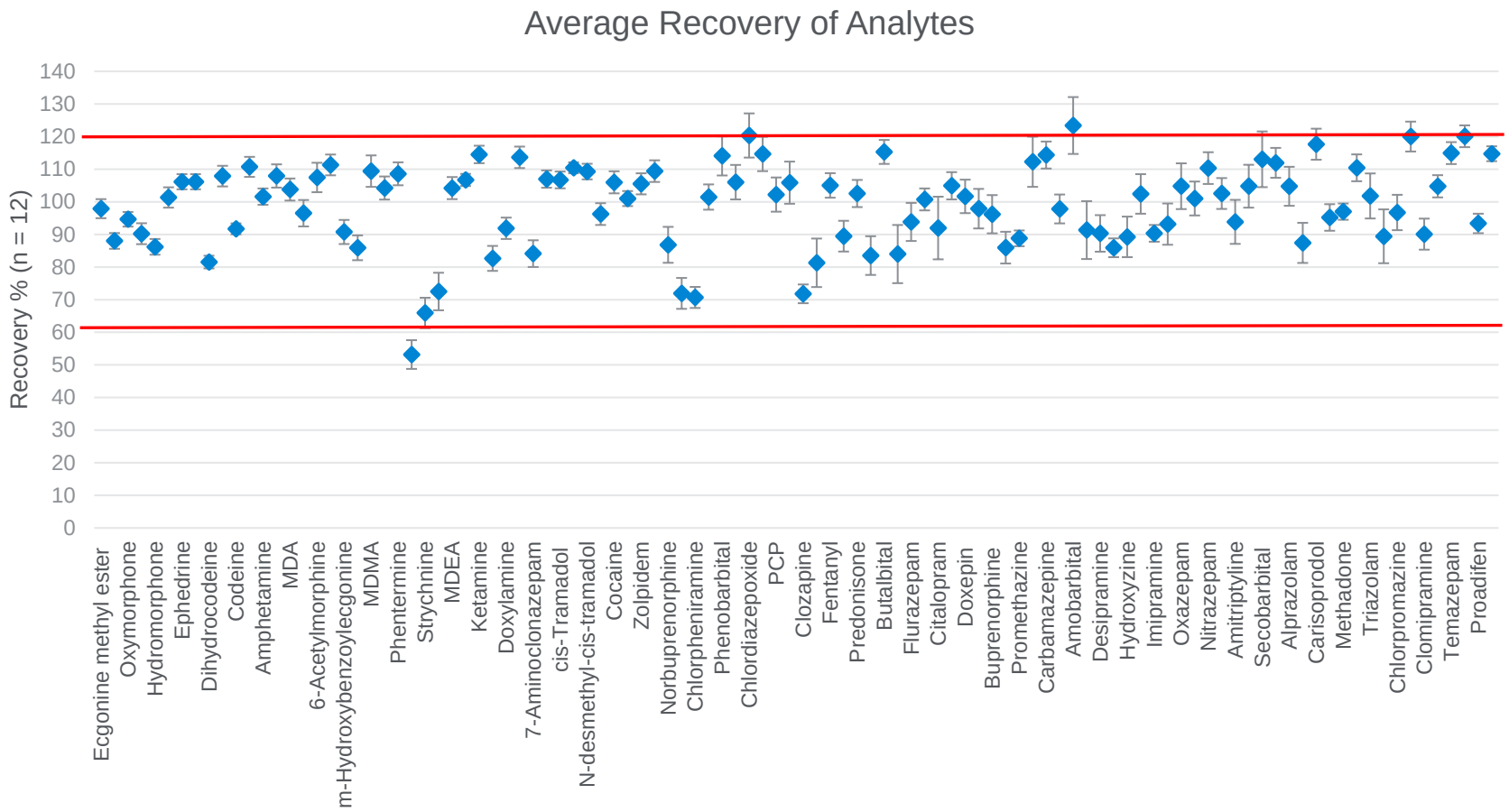
PPT with Captiva EMR-Lipid cleanup



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

**See Appendix for post column infusion setup.*

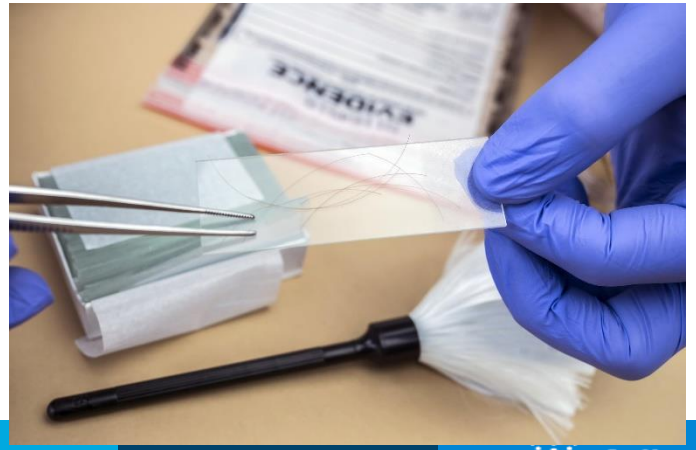
Captiva EMR-Lipid
Improved recoveries



Average recovery for 102 analytes in human whole blood samples fortified at 10 ng/mL and 50 ng/mL. Average recoveries were calculated by the ratio of peak areas in prespiked samples to peak areas in the corresponding level matrix matched samples, based on six replicates of fortified samples at each level (5994-2830EN)

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)
- Screening, Identification, and Quantitation of 102 Drugs in Human Whole Blood by LC/Q-TOD and LC-QQQ (5994-2830EN)
- An Automated Dual Metabolite + Lipid Sample Preparation Workflow for Mammalian Cell Samples (5994-5065EN)
- Protein Precipitation for Biological Fluid Samples Using Agilent Captiva EMR-Lipid 96-Well Plates (5991-9222EN)



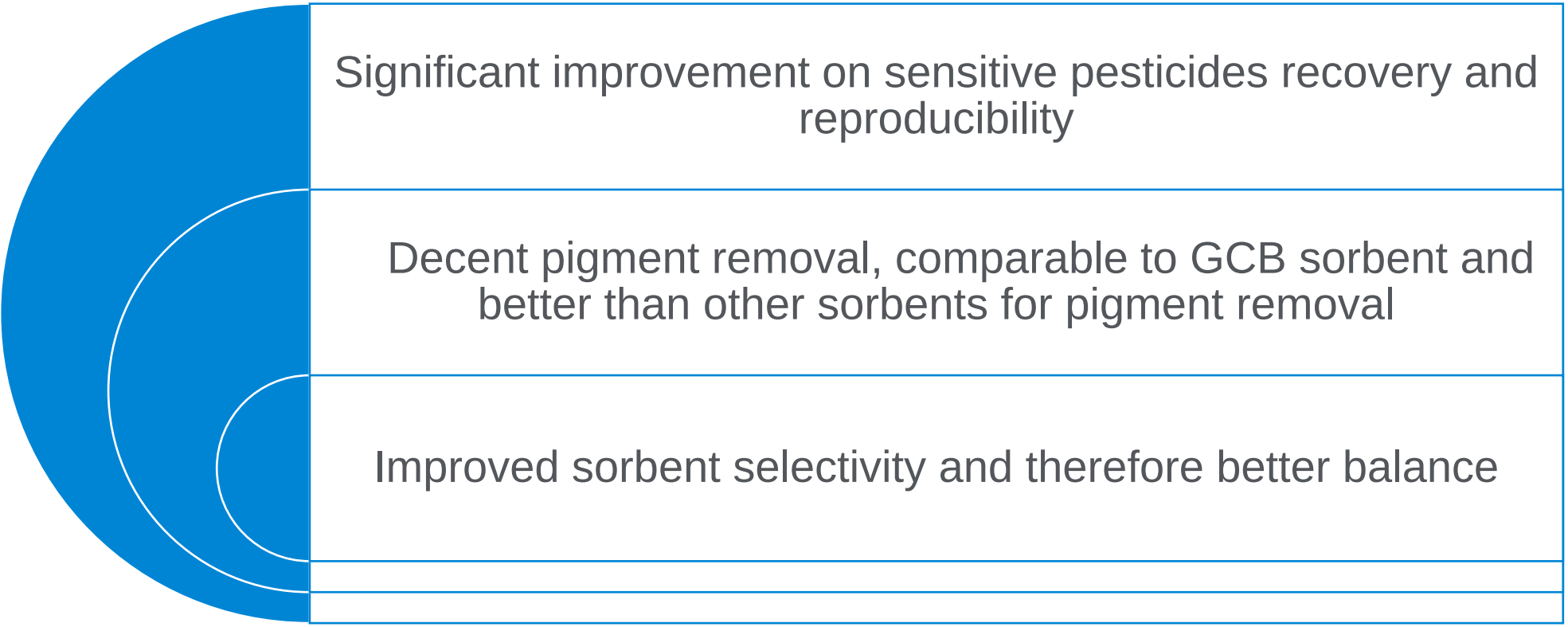
Carbon Material Used in Food Analysis

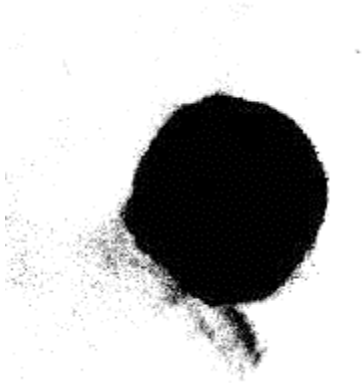
- The structure of carbon material, including graphitized carbon black (GCB), coconut carbon, and activated carbon, favors the retention of pigment components.
- Pigments are one of major matrix interferences in plant-origin food matrices
- Offers efficient pigment removal, but can also cause unwanted targets loss, such as planar compounds

Color	Pigments
Green	Chlorophyll Lutein
Red, blue, purple, black	Anthocyanidins Anthocyanins
Orange, yellow	Carotenoids Xanthophylls

Carbon S Sorbent

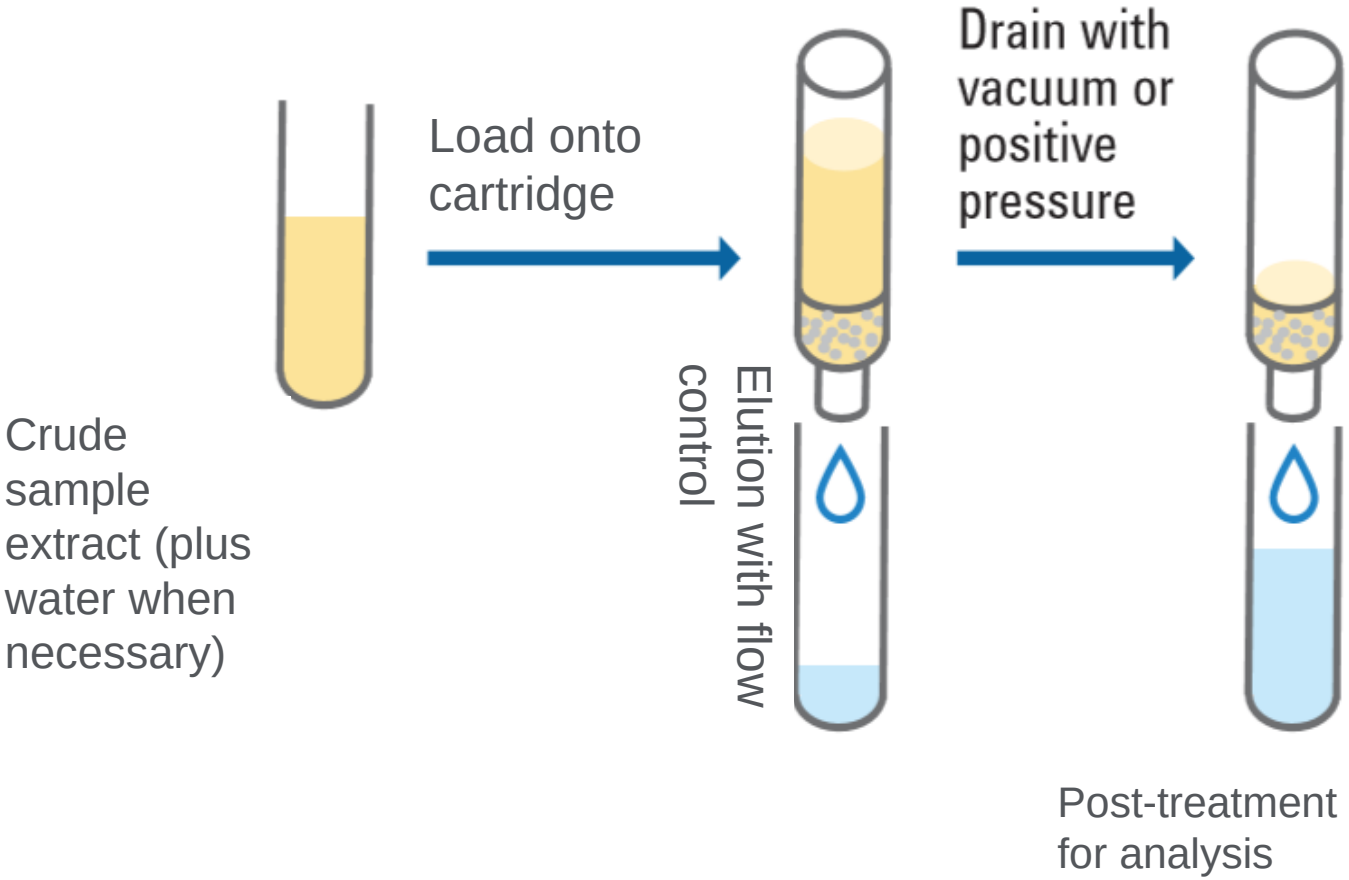
Agilent Carbon S sorbent is an *advanced hybrid carbon material* with **optimized carbon content and pore structure**





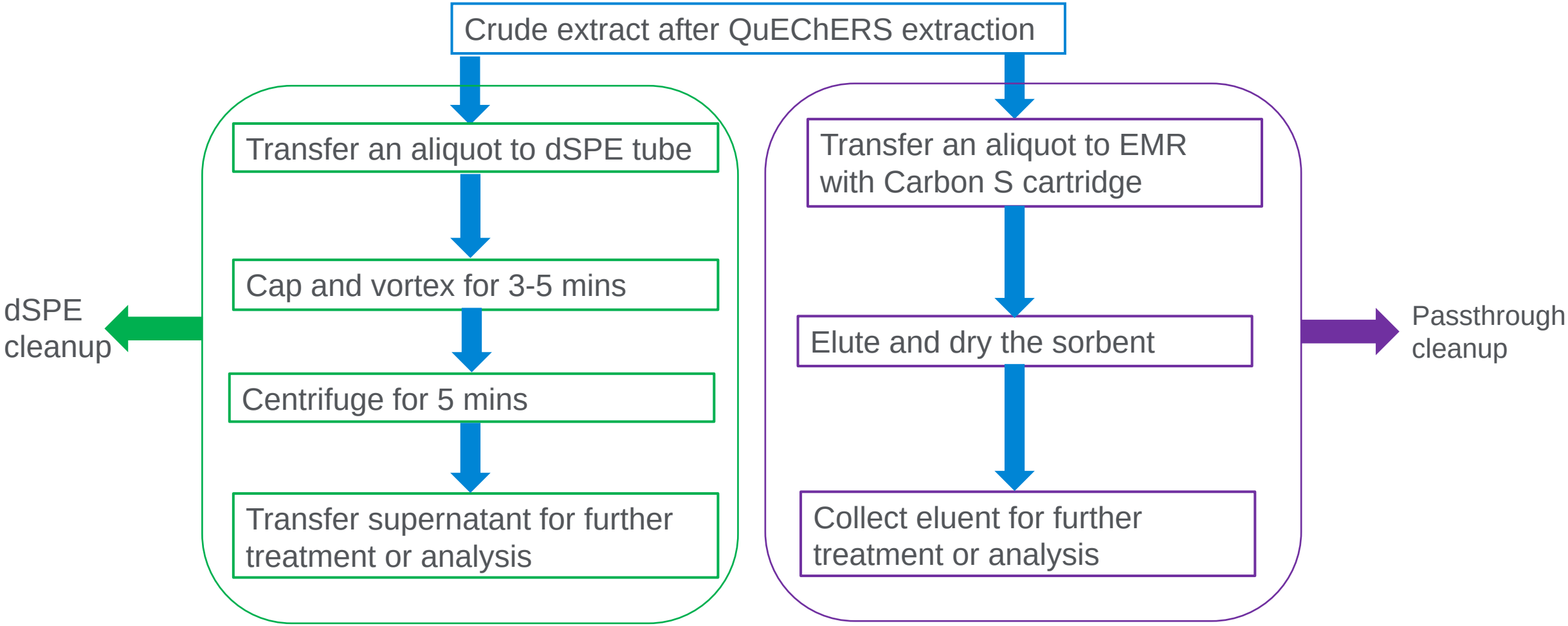
Carbon S sorbent

Passthrough Cleanup– Captiva EMR with Carbon S



- Sorbent interaction is targeted to unwanted matrix interferences
- Limited interactions with targets to prevent analyte loss
- Basic methodology
  - Load the sample crude extract to cartridge
  - Elution with flow control, either on gravity or low-level external force
  - Collect eluent for direct analysis or further post-treatment

Captiva EMR with Carbon S Passthrough Cleanup vs dSPE Cleanup



Passthrough cleanup saves 15 to 30% of processing time. The more samples to process, the more time can be saved.

Captiva EMR Passthrough Selection Guide

Captiva EMR-Lipid

High lipid/fats/oils
- Meat, dairy, oils, eggs

Captiva EMR-HCF

High chlorophyll fresh
- Spinach, arugula, chard

Captiva EMR-GPF

General pigmented fresh
- Berries, peppers, broccoli

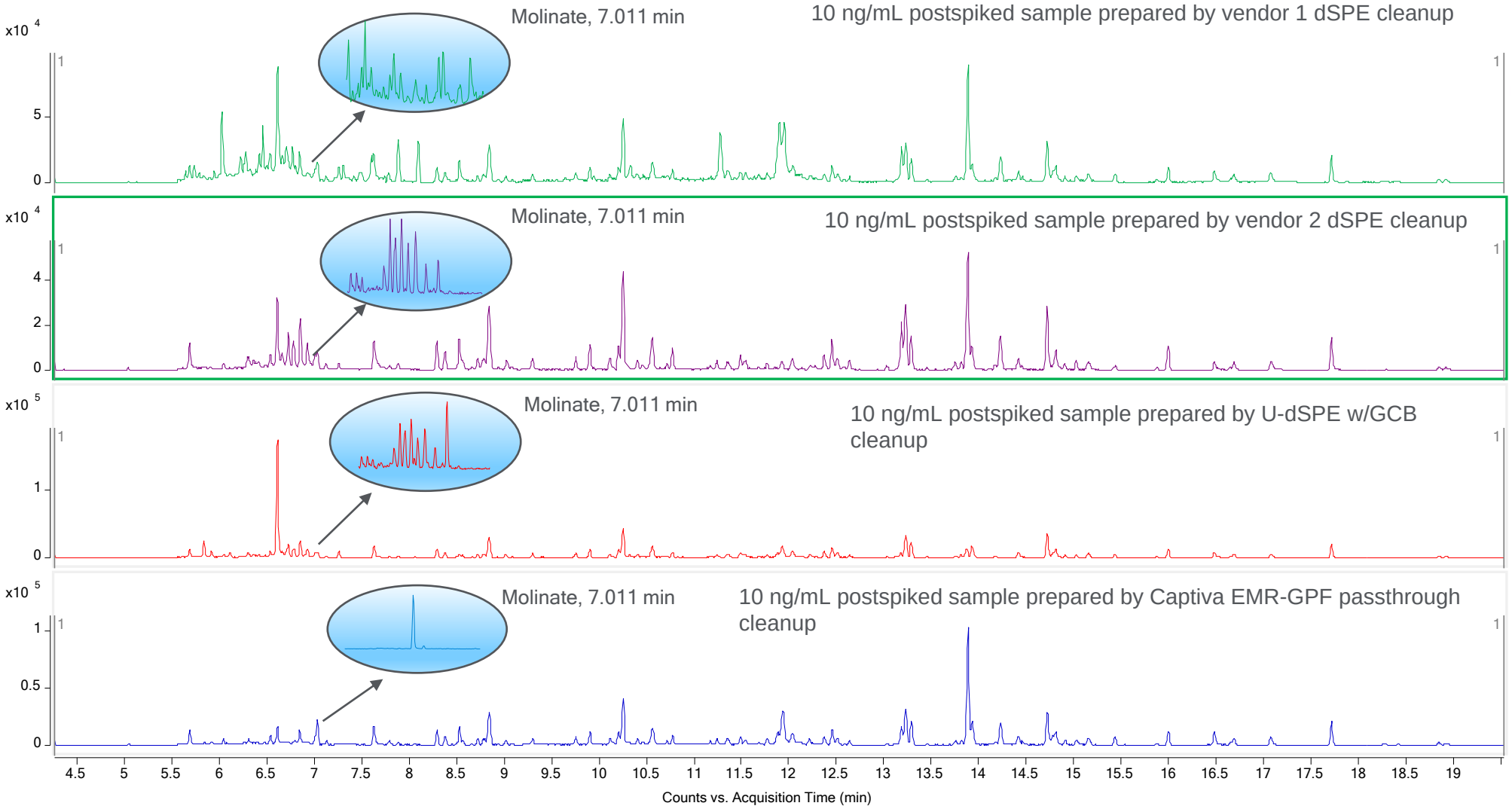
Captiva EMR-GPD

General pigmented dry
- Spices, seasoning, herbal medicine

Captiva EMR-LPD

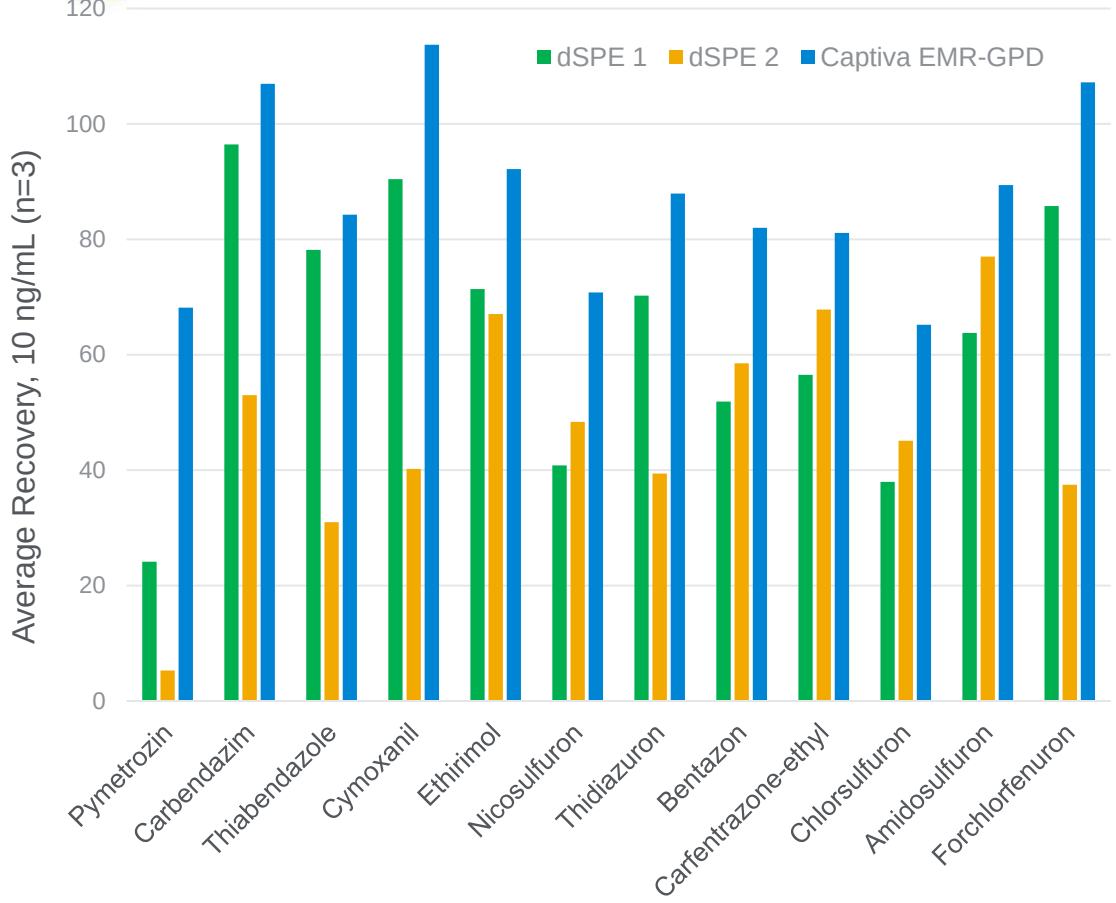
Low pigmented dry
- Nuts, tobacco, light pigmented spices

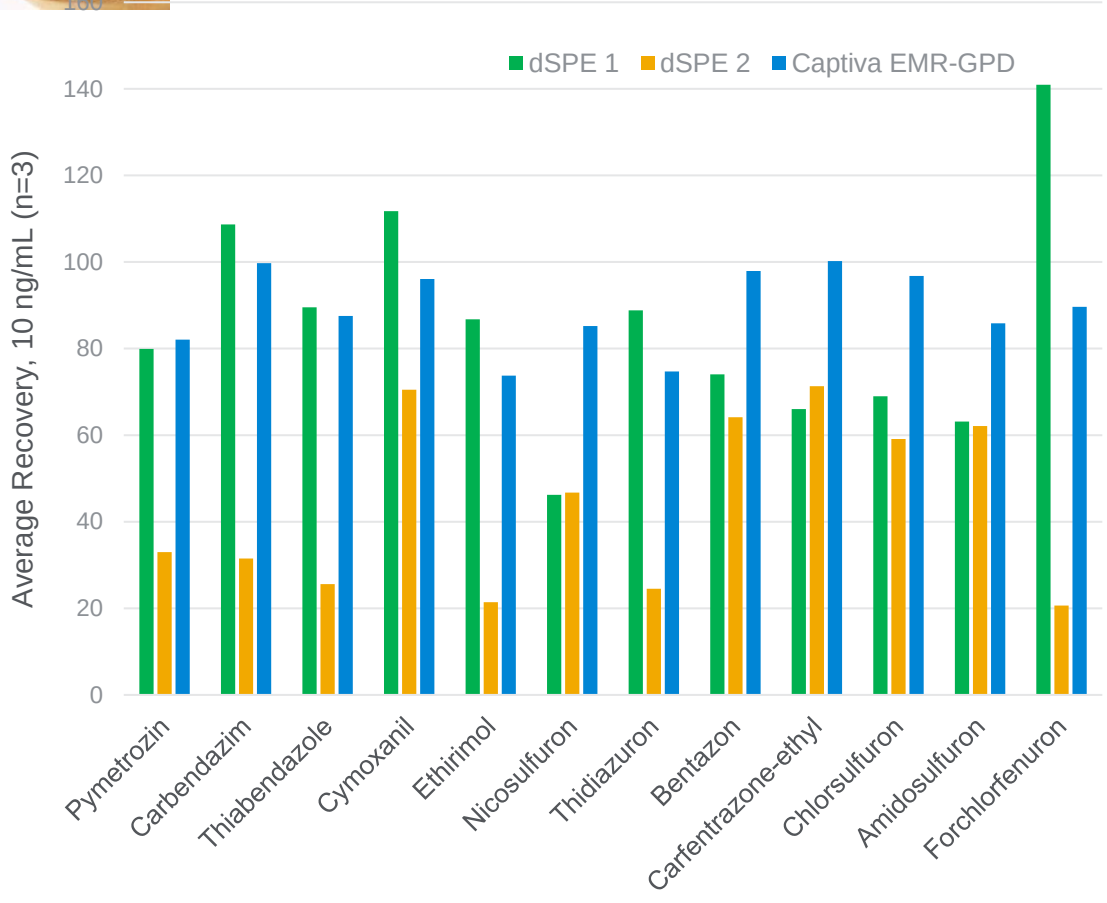
Captiva EMR – Cleaner Matrix Background on GC/MS/MS



Comparison of Traditional dSPE vs EMR-GPD Cleanup

Sensitive pesticides recovery



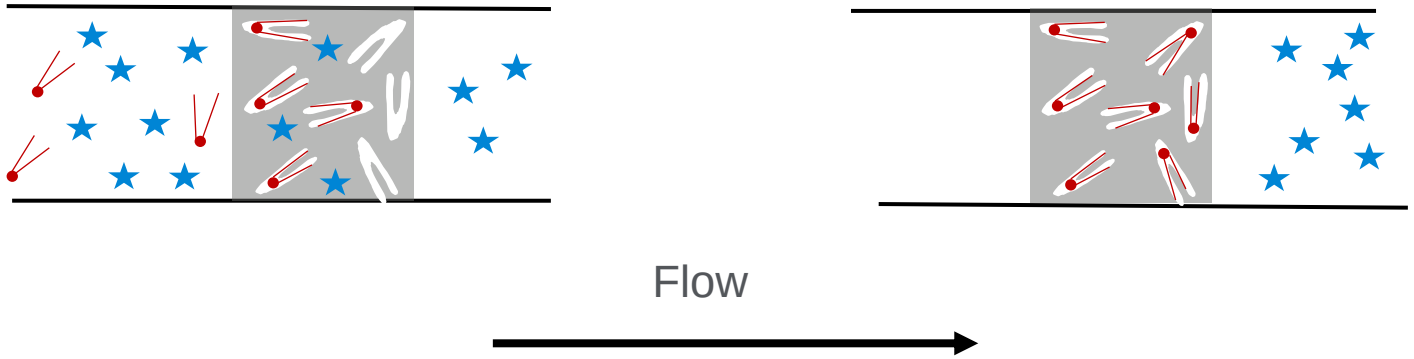


Application Note Examples

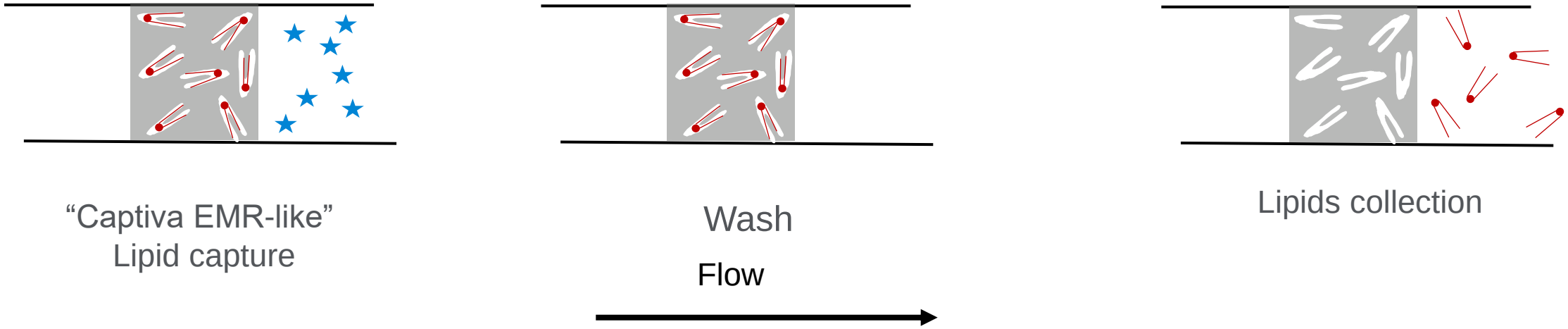
- Determination of Over 300 Pesticides in Cayenne Pepper (5994-5630EN)
- Pesticide Analysis of Peppers Using Capitva EMR-GPF Cleanup and LC/MS/MS or GC/MS/MS (5994-4767EN)
- Determination of Over 300 Pesticides in Tobacco Using Agilent Captiva EMR-LPD Passthrough Cleanup and LC/MS/MS and GC/MS/MS Detection (5994-5777EN)
- Determination of Multiclass, Multiresidue Pesticides in Spring Leaf Mix (5994-4765EN)
- Pesticide Analysis in Black Pepper Using Captiva EMR Passthrough Cleanup and LC/MS/MS (5994-4768EN)

Innovative Lipid Products

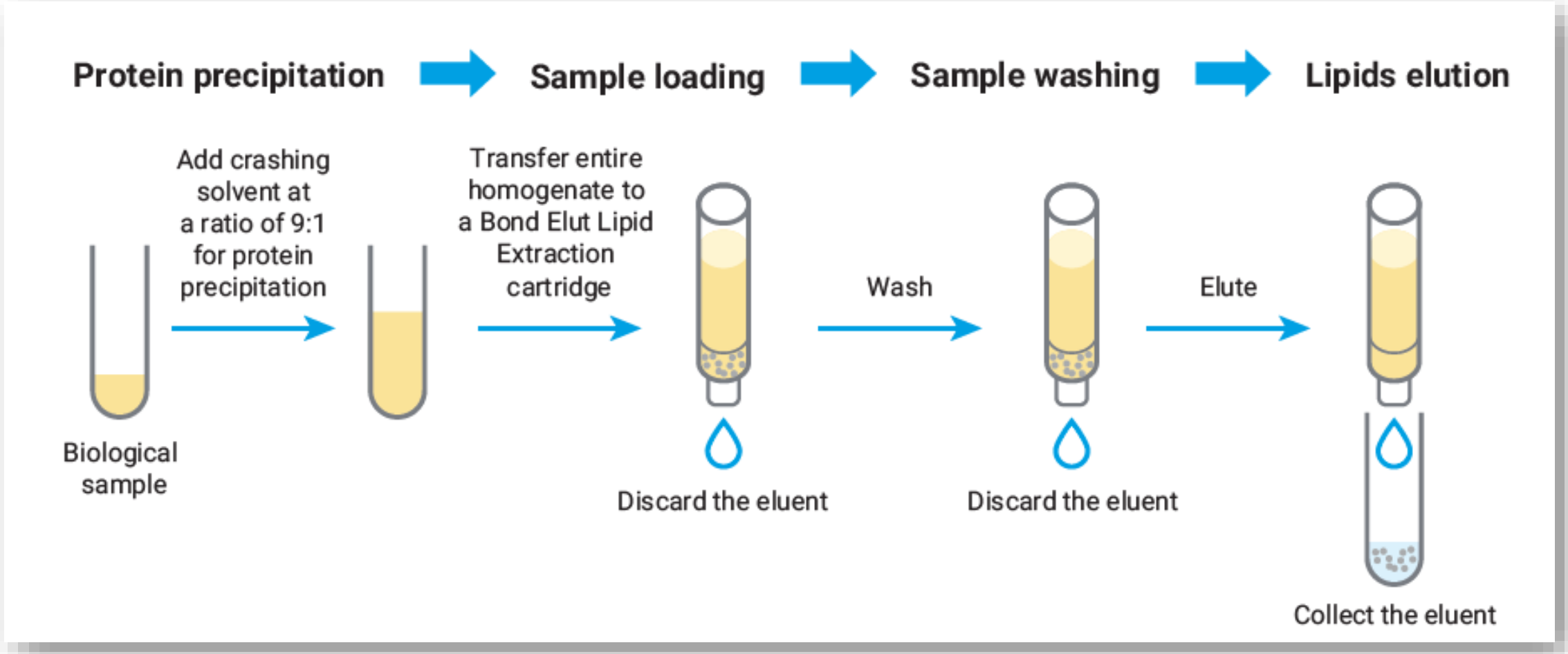
Captiva EMR-Lipid – A pass through filtration



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

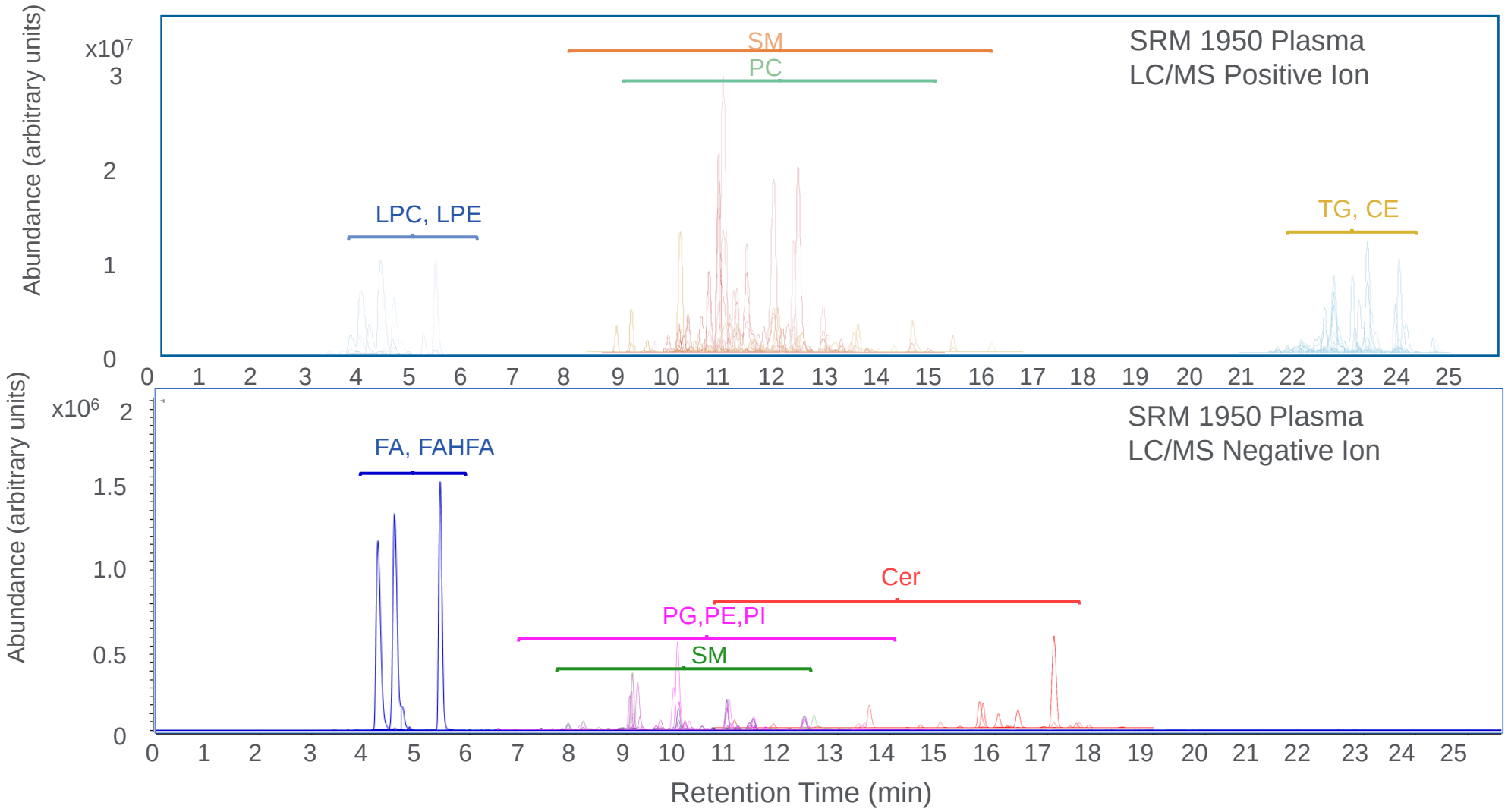


Bond Elut Lipid Extraction Workflow – Solid Phase Extraction



The current workflow applies to blood fluid only, and may be extended to cell culture. It is not directly applicable to tissue matrix.

Identified Lipids Chromatograms



Positive (top) and negative (bottom) extracted ion chromatograms (EICs) for identified lipid compounds in human plasma, using the Bond Elut Lipid Extraction SPE method. (5994-1783EN)

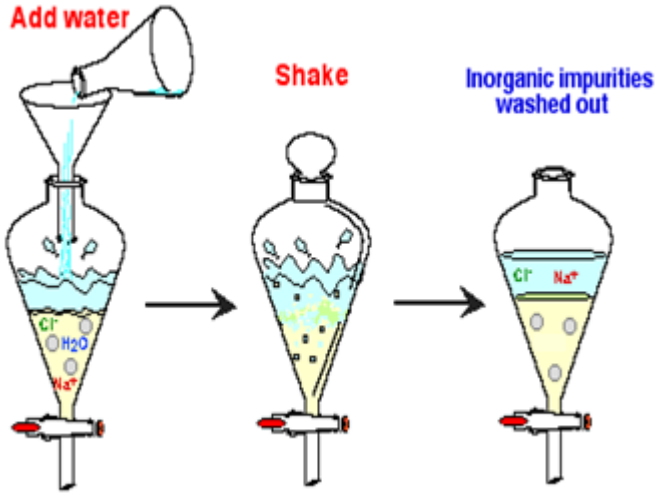
Application Note Examples

- Lipidomics Analysis of Human Plasma Using Agilent Bond Elut Lipid Extraction (5994-3824EN)

- Lipidomic Analysis of Human Plasma Using Bond Elut Lipid Extraction with LC/Q-TOF (5994-1783EN)

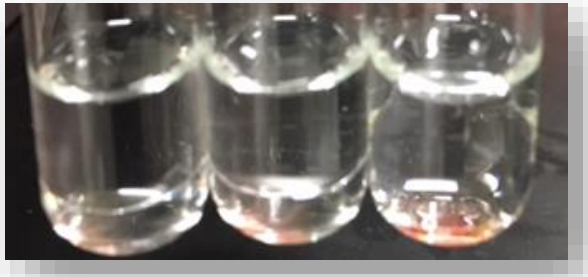
Liquid/Liquid Extraction (LLE)

- 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.



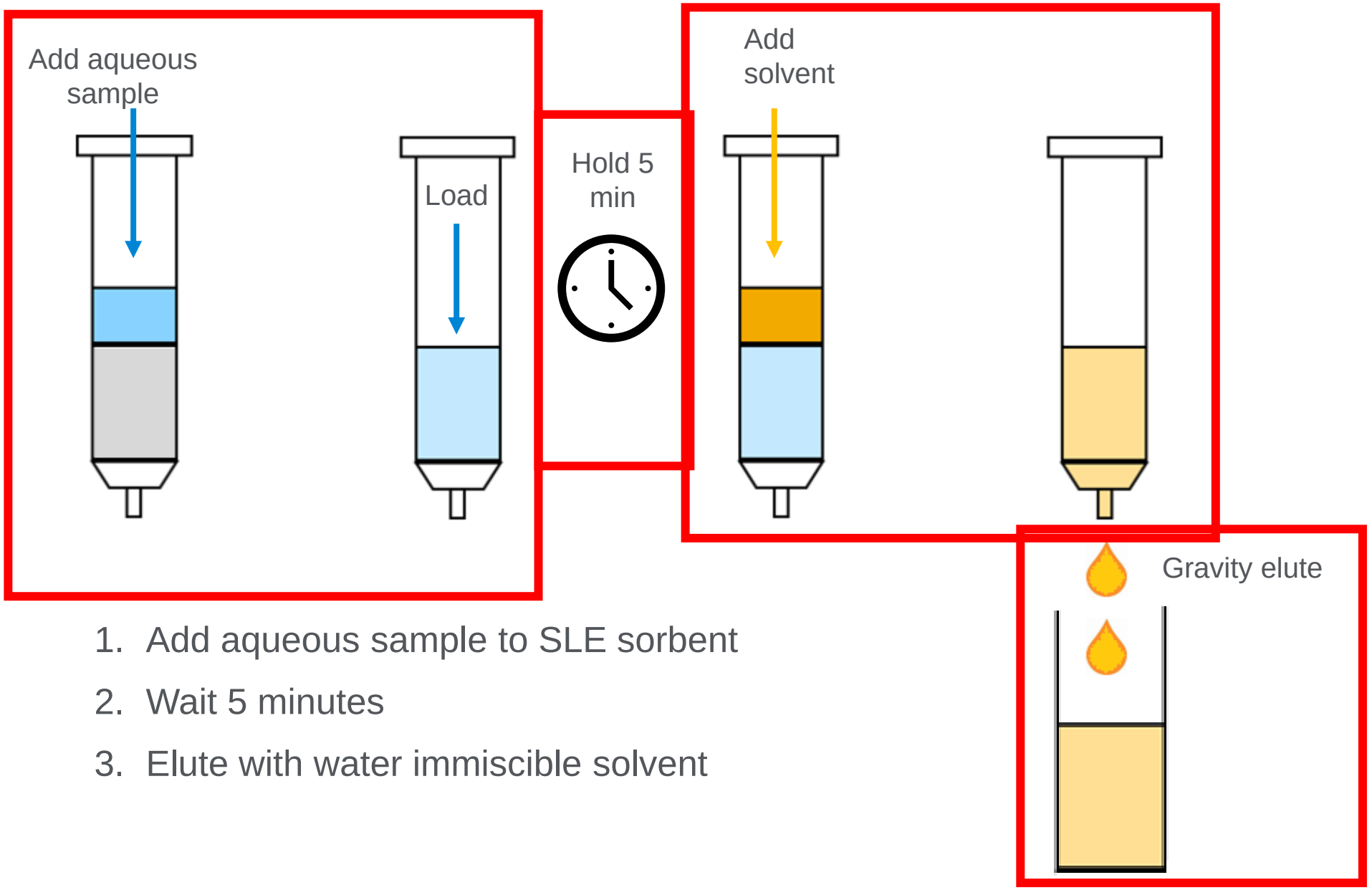
Drawbacks of Liquid-Liquid Extraction

- 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



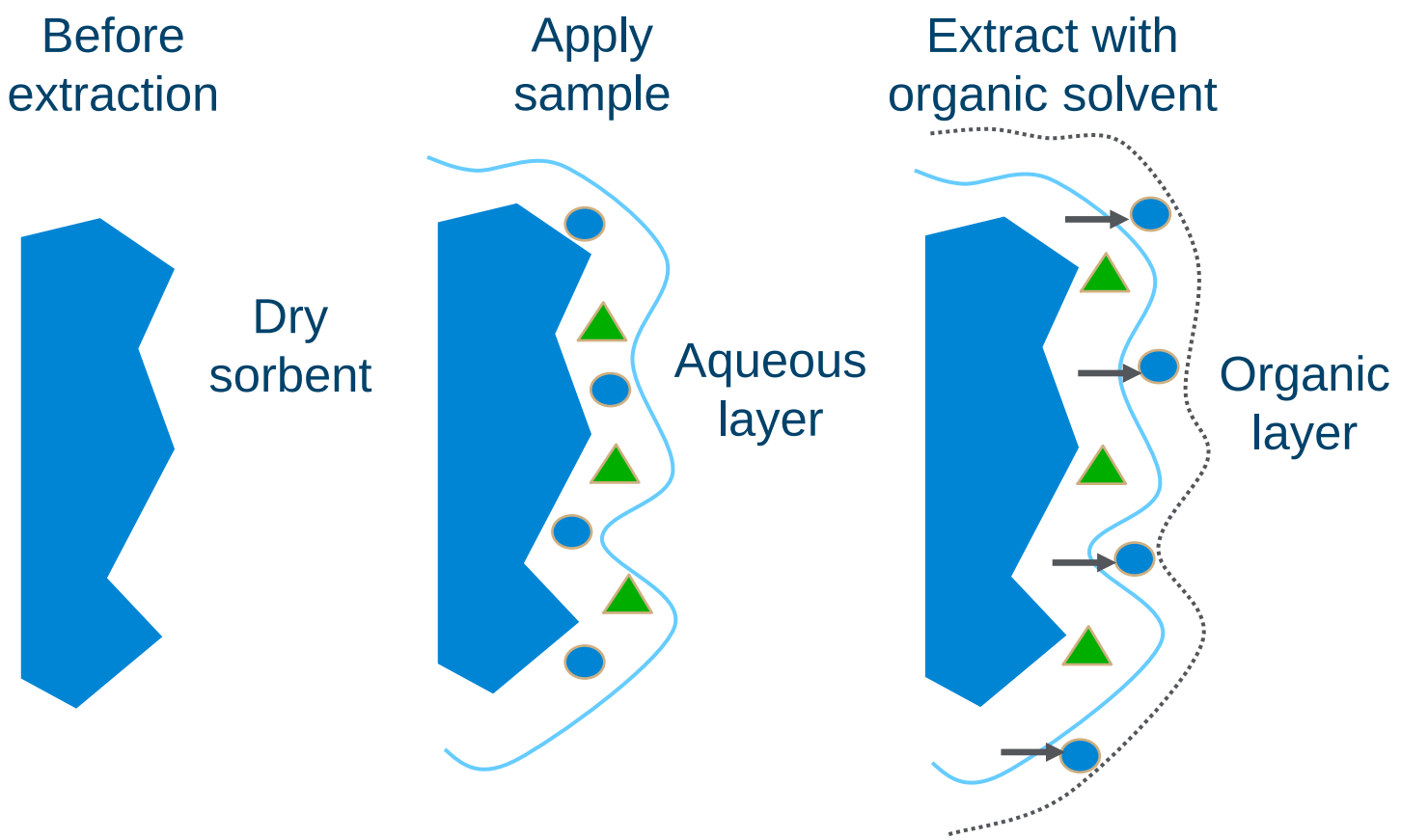
How many of these problems can be fixed with Solid Supported Liquid Extraction?

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.

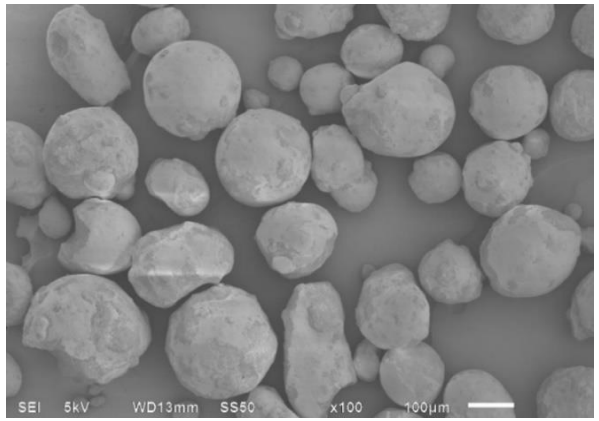
What is SLE Sorbent?

- 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 for consistency 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

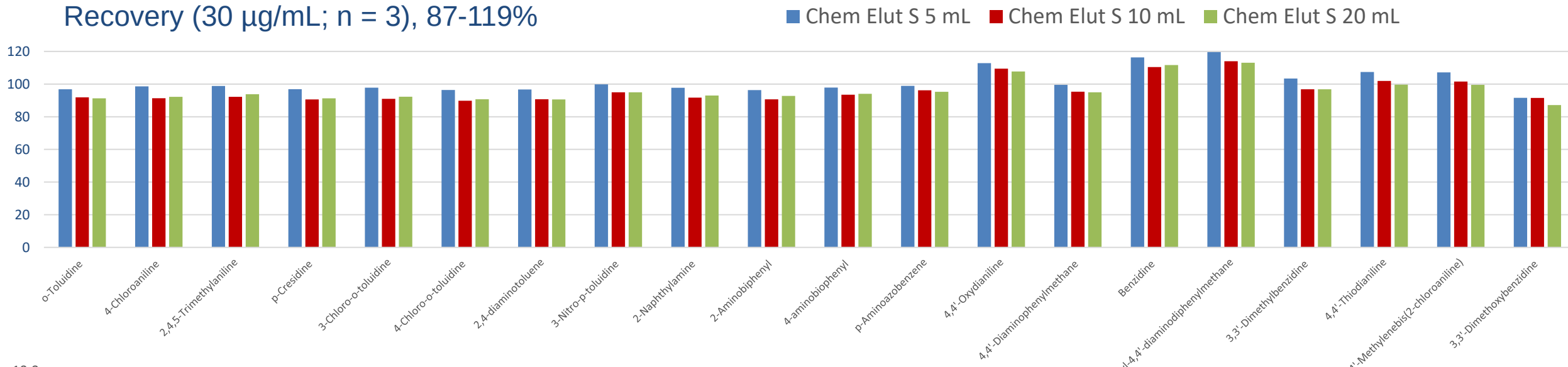
Cartridges for sample volumes 0.2 – 20 mL

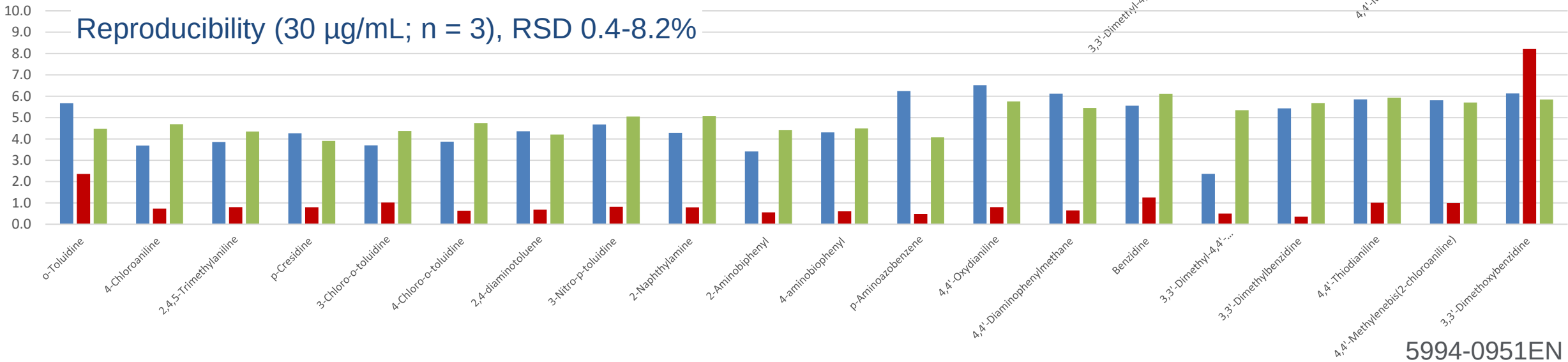
Bulk Chem Elut S
1 and 4 kg

96-well plate for sample volume 200 and 400 µL

Chem Elut S – 15 Minute Hold Time

Large-scale format comparison with aromatic amines using GC





5994-0951EN

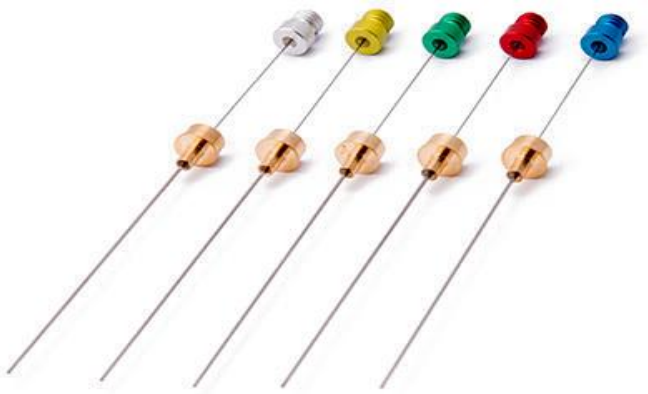
Application Note Examples

- Determination of Fat-Soluble Vitamins in Foods Using Agilent Chem Elut S Extraction with LC/DAD and LC/MS/MS Triple-Quadrupole (5994-5063EN)
- Drug of Abuse Analysis Using Agilent Chem Elut S Supported Liquid Extraction by LC/MS/MS (5994-0950EN)
- Quantitative Determination of a Panel of Endogenous Steroids in Human Serum by LC/MS/MS (5994-0949EN)
- Determination of Aromatic Amines Derived from Azo Colorants by GC/MS Using Supported Liquid Extraction Chem Elut S Cartridges (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
- Surfactants, other industrial applications
- Trace analysis in food
- Herbicides/pesticides
- Trace impurities in polymers and solid samples
- Solvent residues in raw materials
- Explosives



SPME fibers

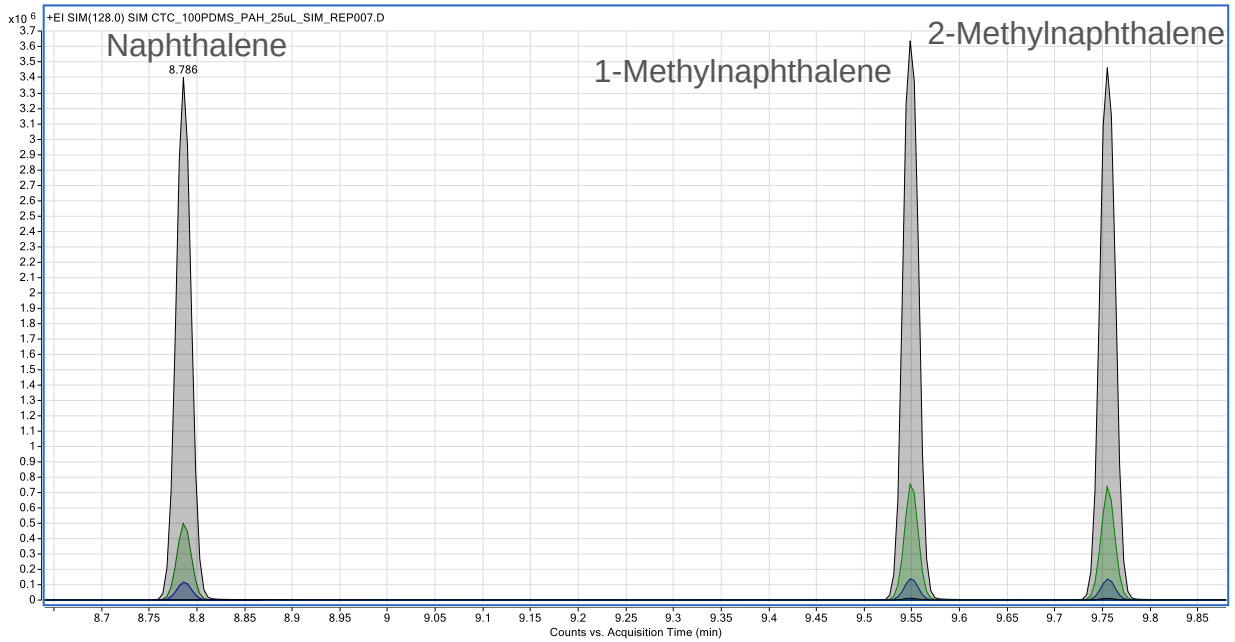
SPME Arrows

Smart SPME fibers and Arrows are now available for the smart rail systems

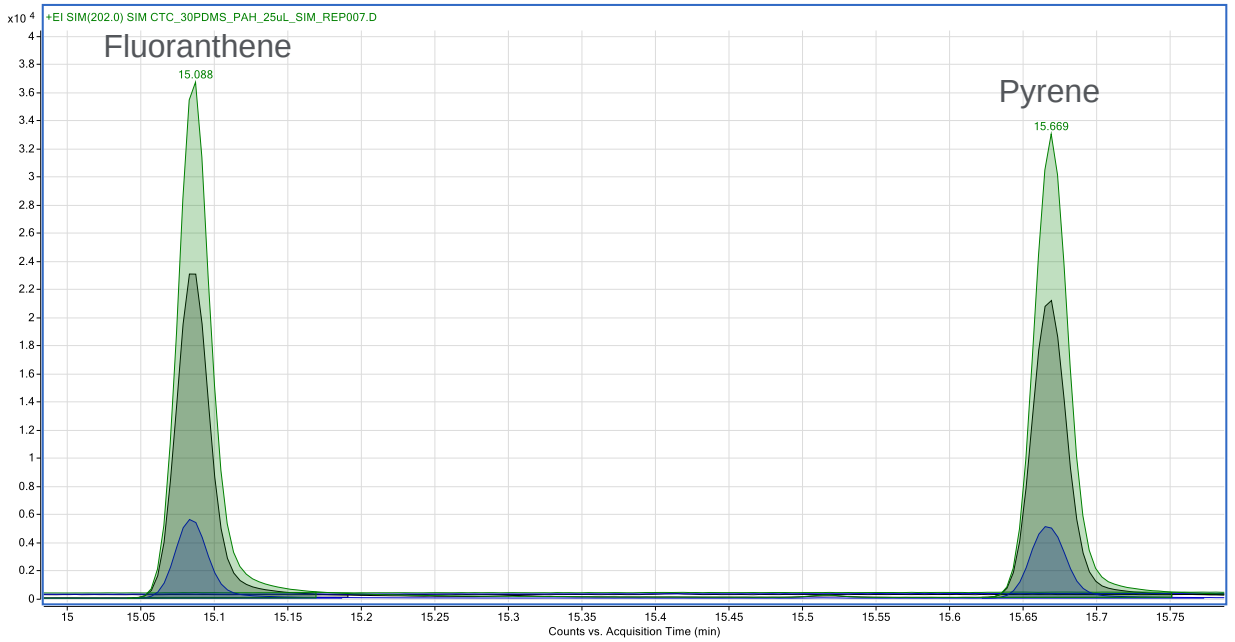
Examination of Lower Molecular Weight PAHs in Drinking Water Using Agilent PDMS SPME Fibers

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

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

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

The process consists of two steps, and therefore two kits:

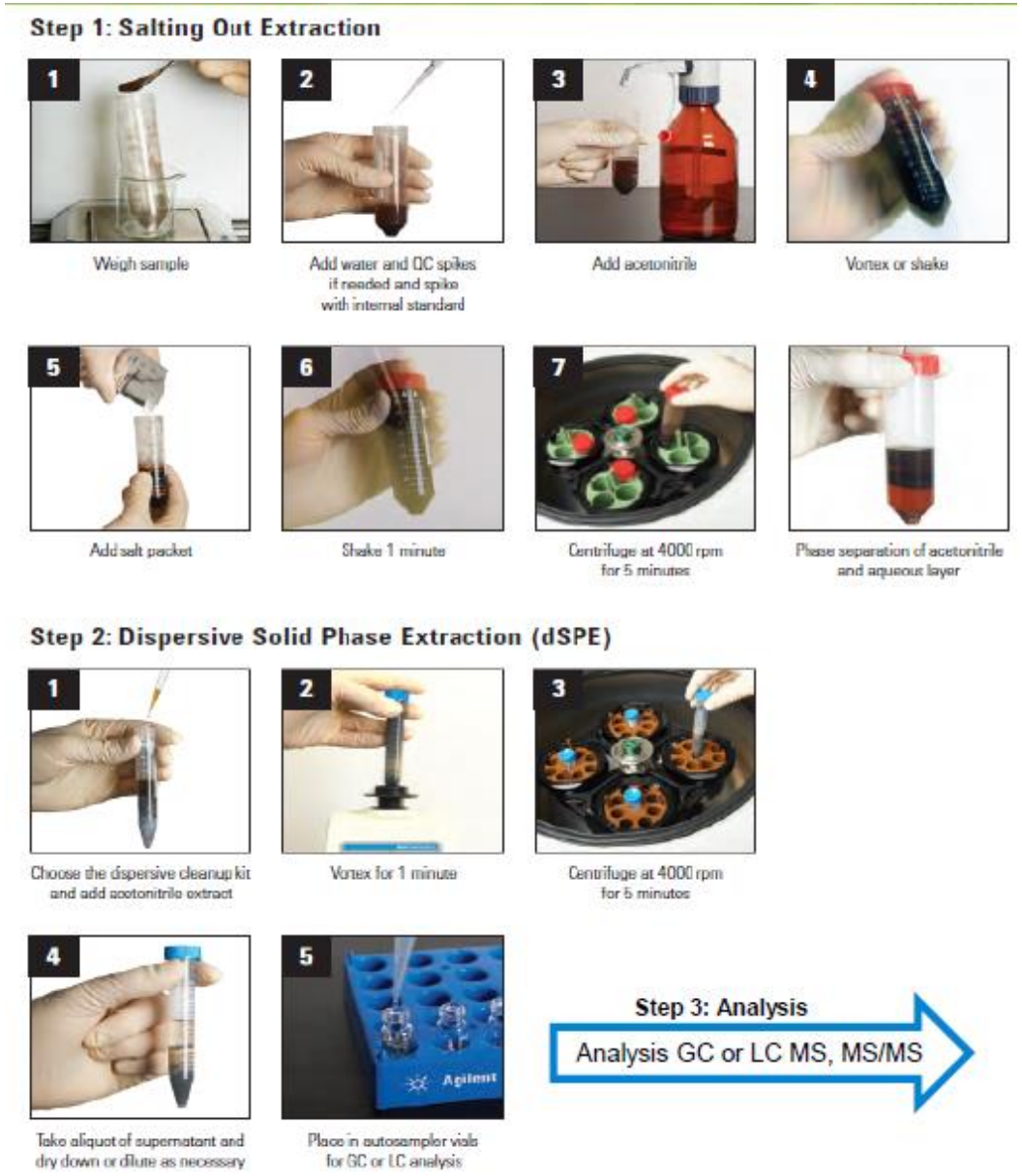
Step 1: Liquid extraction

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
- Carbon S or GCB: Removes pigments

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** matrix, just enough.

Dispersive sorbents are also available as bulk material.

Bond Elut Dispersive EMR-Lipid can be applied to fatty food matrices

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

SPE Methodology

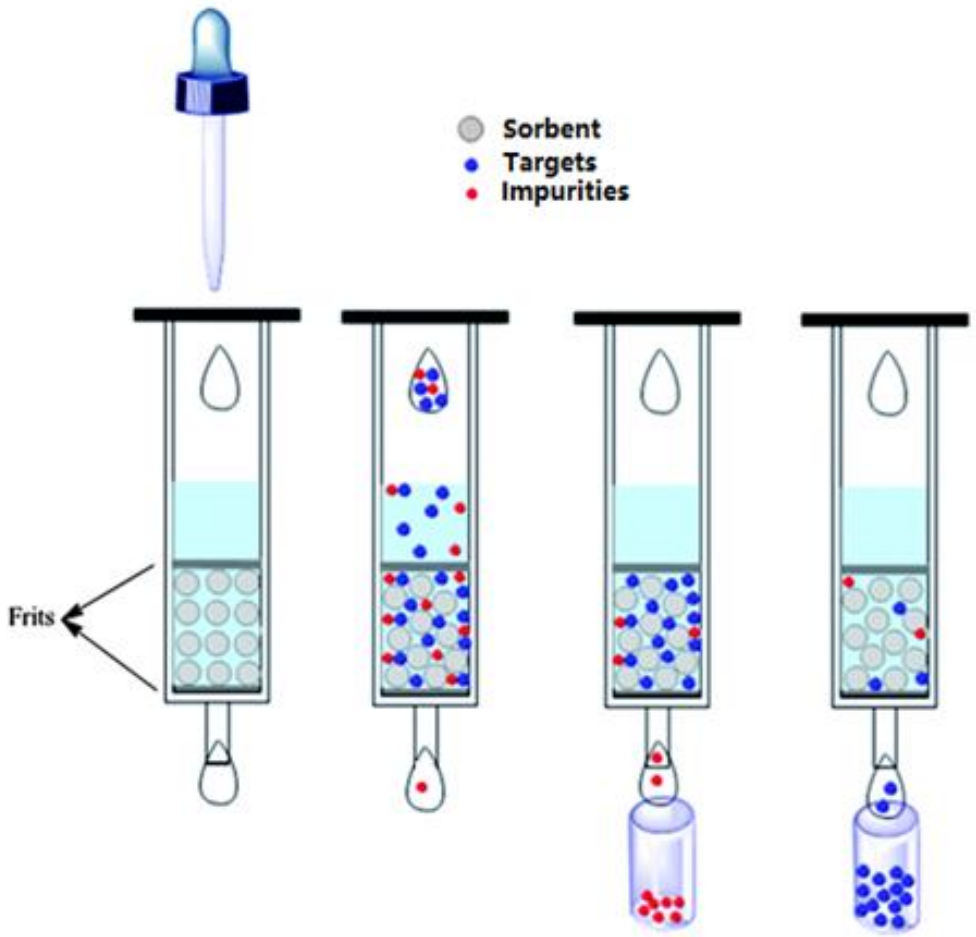
SPE sorbent retains targets and does not retain matrix.

Conditioning/Equilibration
Active sorbent function groups clean the impurities, equilibrate sorbent for sample loading.

Loading
Sample solution (more aqueous) is transferred

Washing
Clean the sample interference without impacting the retention of the targets

Elution
Elute targets with appropriate solvents

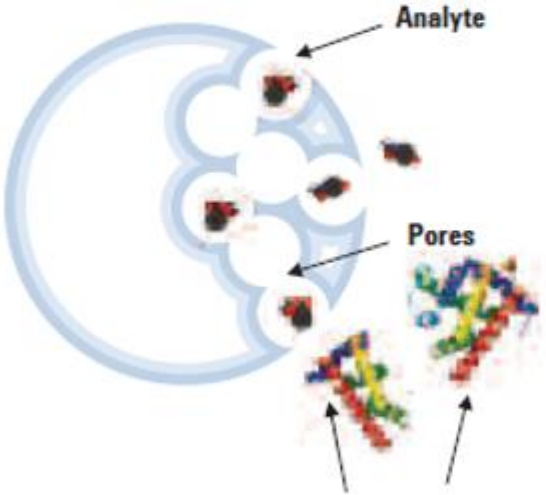


Bond Elut Plexa

Advanced polymer architecture improves extraction performance

LOAD:

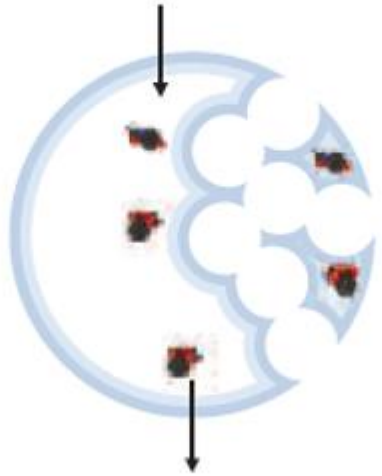
Water-rich, hydrophilic surface allows excellent phase transfer of analytes into the polymer core.



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

WASH:

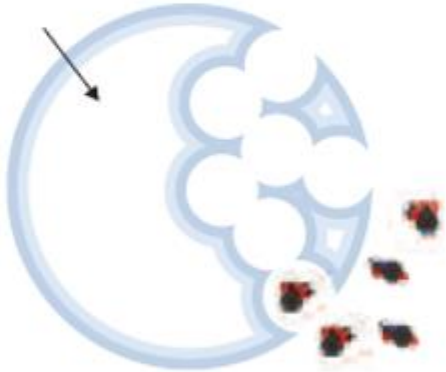
Analytes that have crossed the hydrophilic layers will remain tightly bound in the hydrophobic core.



Interferences wash away without leaching the analytes of interest.

ELUTE:

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



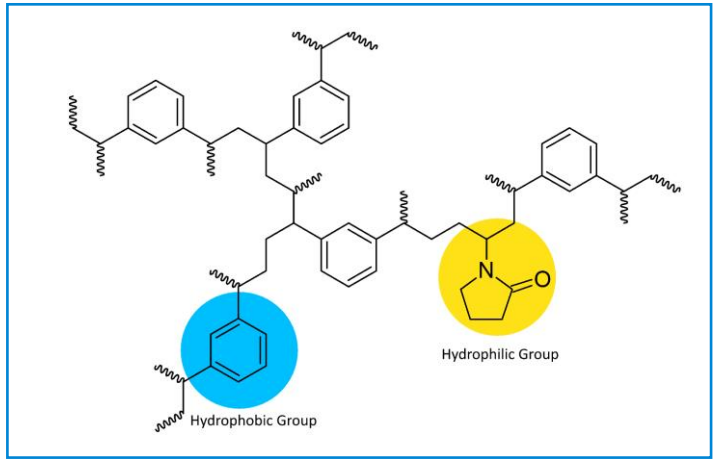
Clean extract with high recovery.

Bond Elut Plexa

- New generation of polymeric SPE
- Divinylbenzene-based polymeric sorbent with hydrophilic exterior, hydrophobic interior, and advanced polymeric architecture.
- Great 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

Bond Elut HLB Sorbent Introduction

- **H**ydrophilic-**L**ipophilic **B**alanced (HLB) reversed phase solid phase extraction (SPE) sorbent products
- Composed of monodisperse **divinylbenzene** and **N-vinylpyrrolidone** copolymers
- Hydrophobic divinylbenzene head retains hydrophobic targets well, and hydrophilic N-vinylpyrrolidone head retains polar targets efficiently.
- Provides great recovery for a wide range of compounds with different polarity

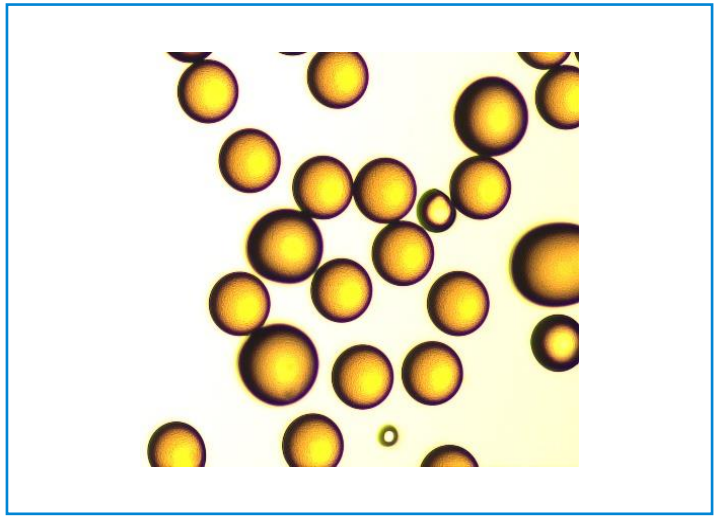


Features: Highly wettable

Highly recoveries with excellent reproducibility

Higher capacity than Si-based sorbent

Compatible with solutions from pH 1 to 14.

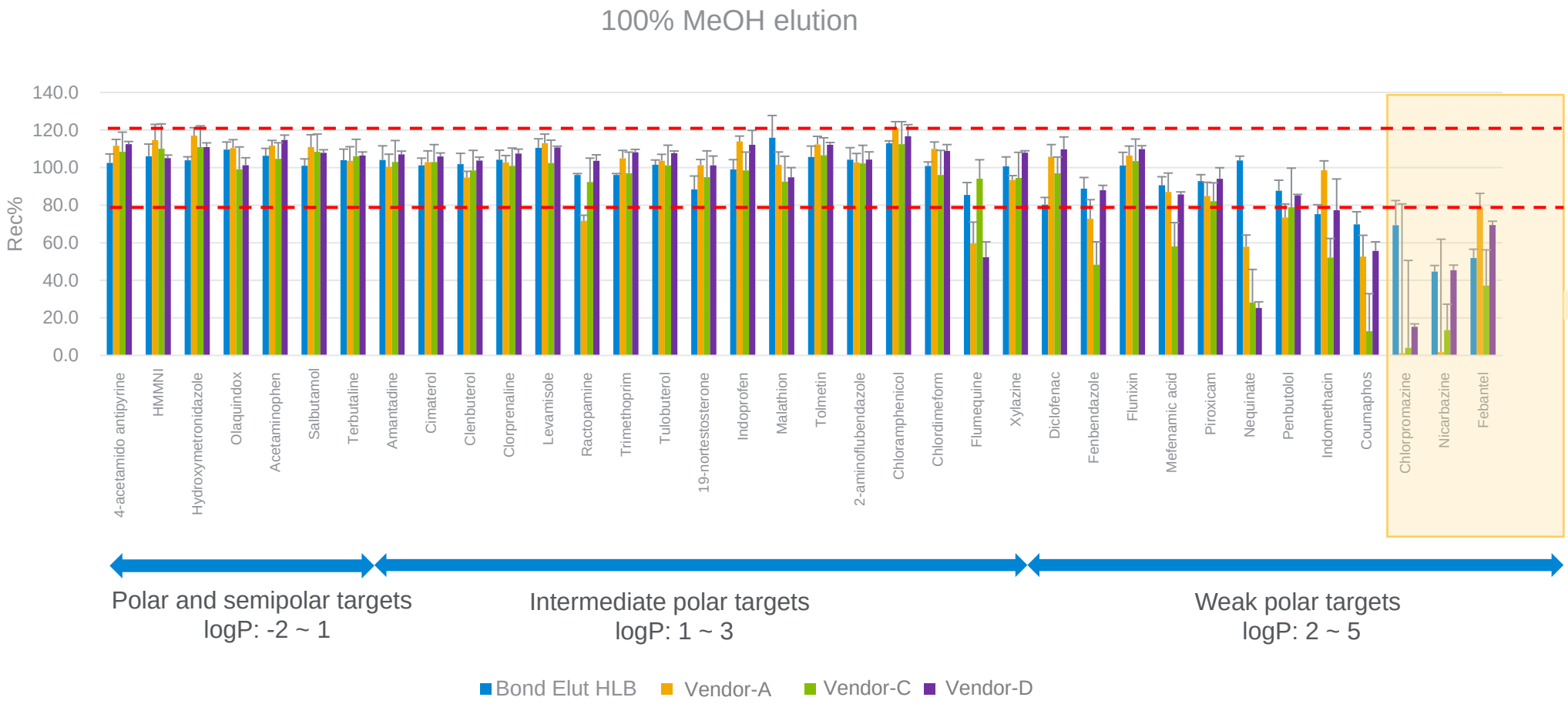


Microscope image of Bond Elut HLB sorbent

Bond Elut HLB Product Information

P/n	Short Product Description	Parts per package
5610-2144	Bond Elut HLB, 30 mg, 1 mL tube	100
5610-2145	Bond Elut HLB, 60 mg, 3 mL tube	50
5610-2146	Bond Elut HLB, 200 mg, 6 mL tube	30
5610-2147	Bond Elut HLB, 500 mg, 6 mL tube	30
5610-2156	Bond Elut HLB, 30 mg, 1 mL 96 well plate	1

Bond Elut HLB Comparison: Recovery and RSD on Large Panel of Targets



MeOH elution shows limitations on elution of the more hydrophobic compounds.

Introduction to Bond Elut PFAS WAX SPE

New

Property	Specification
Base Polymer	Poly(styrene-co-divinylbenzene) (PSDVB)
Functionalized	Diamino ligand
Chemistry	Weak anion exchange (WAX) and hydrophobic retention
WAX pKa	>8
Particle size	45 µm

Part Number	Description
5610-2150	Bond Elut PFAS WAX, 150 mg, 6 mL, 30/pk
5610-2151	Bond Elut PFAS WAX, 200 mg, 6 mL, 30/pk
5610-2152	Bond Elut PFAS WAX, 500 mg, 6 mL, 30/pk

Introduction

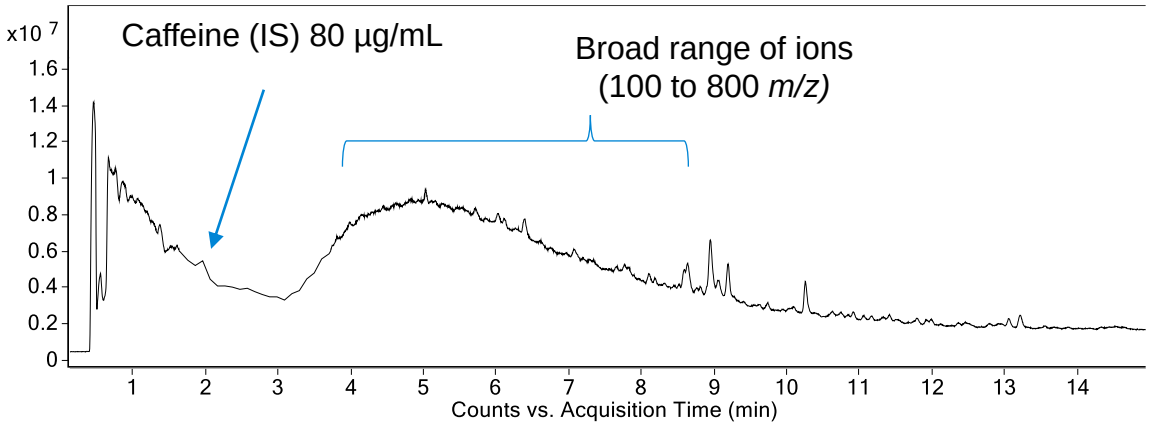
Specifically designed, developed, and manufactured for PFAS applications

- Cleanliness
- Sorbent and cartridge formats are compatible with all existing regulated methods
  - EPA method 533 for drinking water (5994-4960EN)
  - EPA method 1633 (draft) for aqueous, solids, biosolids, and tissue samples (5994-5667EN)
  - ISO 21675:2019 for drinking water, sea water, fresh water, and wastewater
- Performance is equivalent to other commercial cartridges
- Fits into existing Agilent PFAS workflows

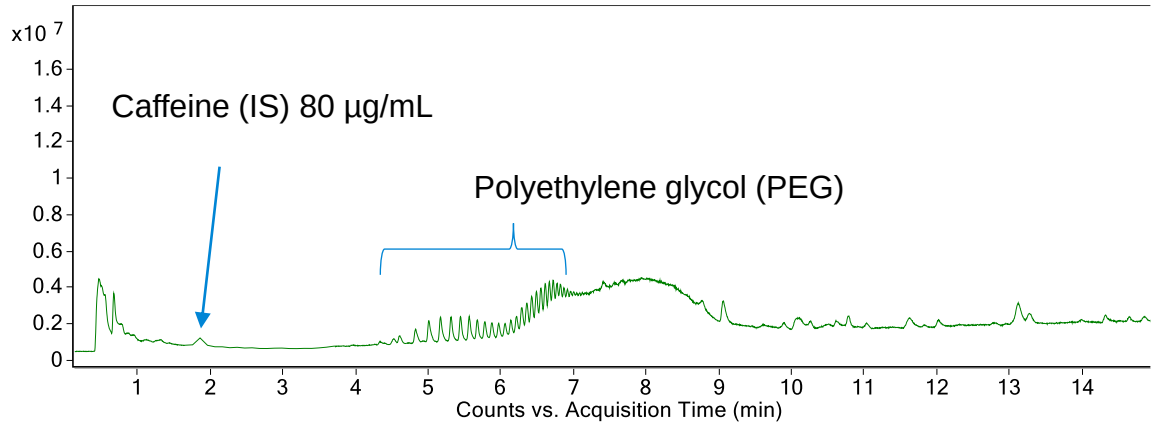
Untargeted Blank Results

Comparison to other commercial sorbents (positive ion mode)

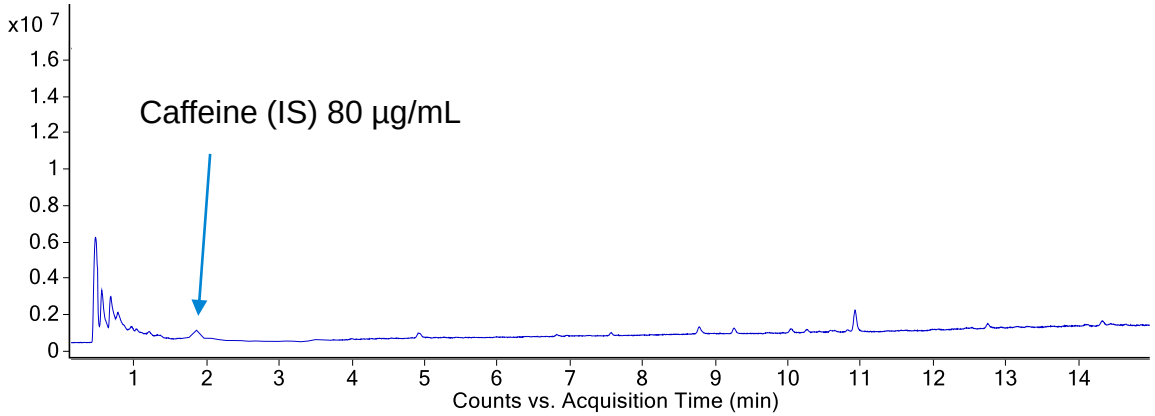
Benchmark Cartridge A



Benchmark Cartridge B



Bond Elut PFAS WAX



Certificate of Analysis (CoA)

Agilent Product Name: Bond Elut PFAS WAX, 150 mg, 6 mL, 30/pk
Agilent Part Number: 5610-2150
FG Lot Number: 6678914-01
Media Lot Number: 0006678914

Raw Materials

Component Properties			
Properties	**Specifications**	**Results**	**Methods**
Tube Purity	Proprietary	Pass	GC FID Test
Frit Purity	Proprietary	Pass	HPLC QQQ Test

Product Specifications/Analysis

Polymeric Sorbent Properties			
Properties	**Specifications**	**Results**	**Methods**
Nitrogen Loading (%N)	1.6-2.1	1.9	CHNO-S Analysis
Average Particle Size D50(µm)	40.0-55.0	46.2	Laser Diffraction
Average Pore Diameter (Å)	50.0-250.0	157.5	Nitrogen Adsorption Isotherm
Turbidity (NTU)	≤7.0	0.5	Turbidity meter
Washable Residue (mg/g)	≤1.0	0.1	Methanol and Hexane gravimetric
Ion Exchange Capacity (meq/g)	0.40-0.82	0.63	Counter Ion Titration
Cleanliness Test	Proprietary	Pass	GC FID Test
Bed Mass Consistency	Proprietary	Pass	Weight Measurement
Flow Characteristics	Proprietary	Pass	Air Flow Test
PFAS Recovery	Proprietary	Pass	HPLC QQQ Test
PFAS Cleanliness	Proprietary	Pass	HPLC QQQ Test

Visual and Microscopic Properties

Properties	Description
Color	White to Buff
Form and Appearance	Spherical, Free Flowing Beads

New

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

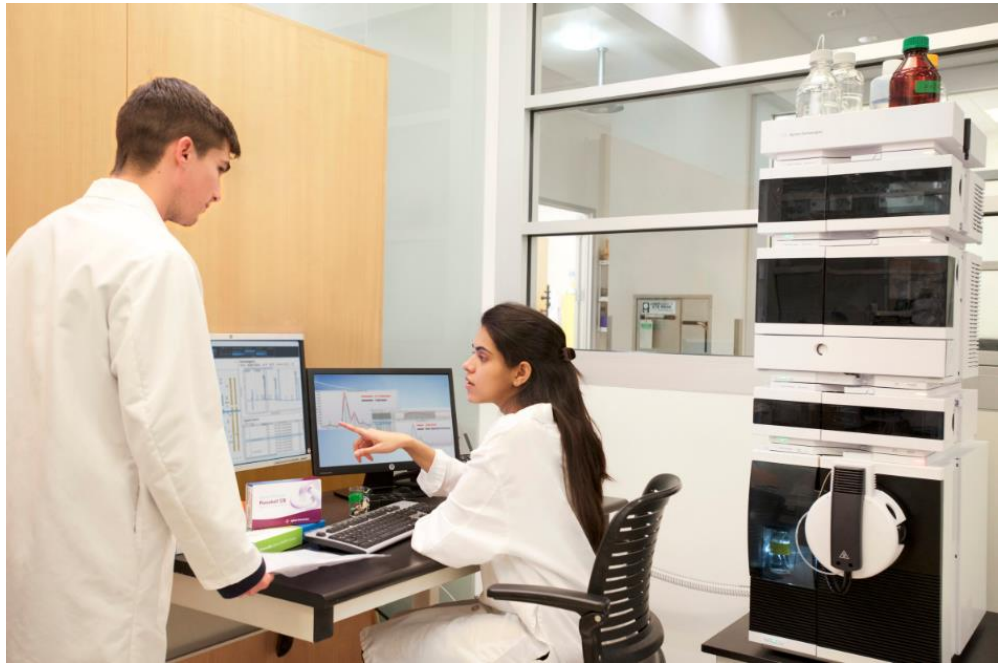
SPS 24 vacuum manifold

96 well plate vacuum manifold

Positive Pressure Manifolds

Agenda

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

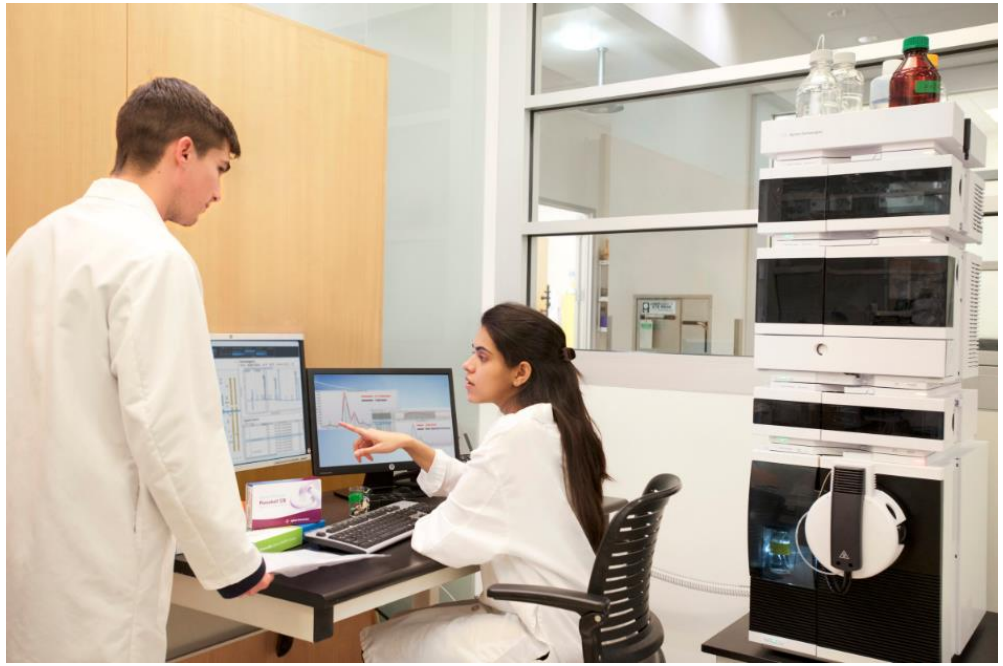


Chromatography Problems Caused by Sample Matrix – Physical Effects

- 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 the 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 peaks, broad peaks, poor sensitivity, and shortening of retention time.

Agenda

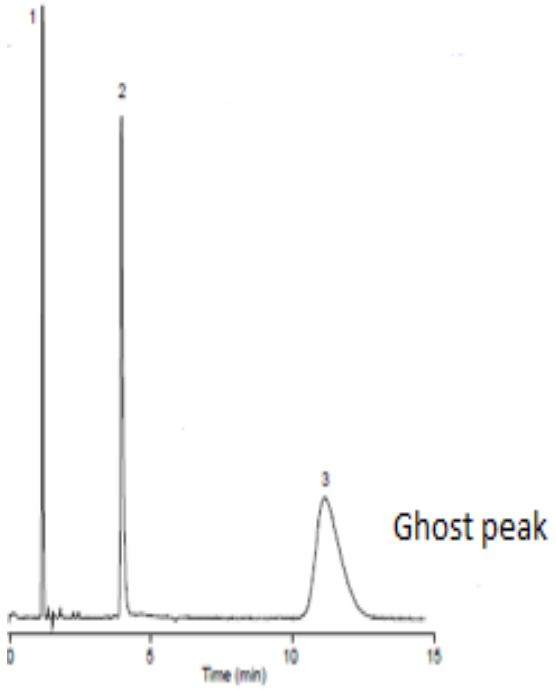
- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- Summary



Chromatography Problems Caused by Sample Matrix – Chemical Effects

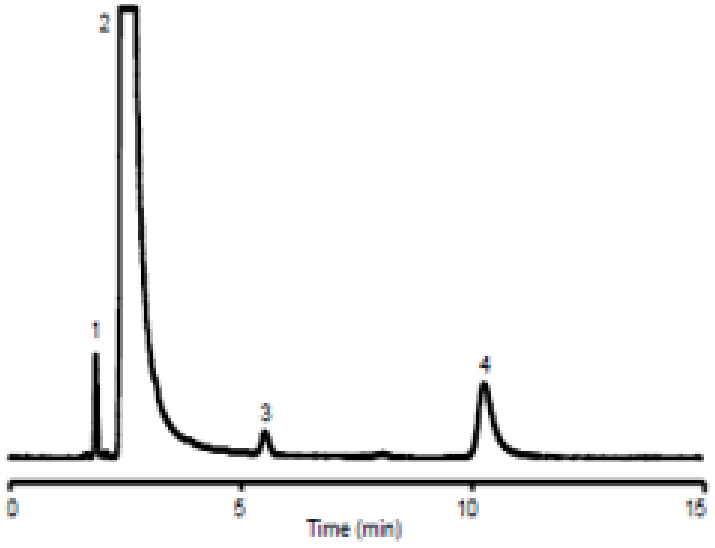
- Chemical contamination/lipid build up can cause secondary interaction and result in retention time variability, peak shape variability/tailing, selectivity changes, and (in some cases) increased back pressure.
- Lipids from the sample matrix can cause ion suppression with MS.
- Strong retention of 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.

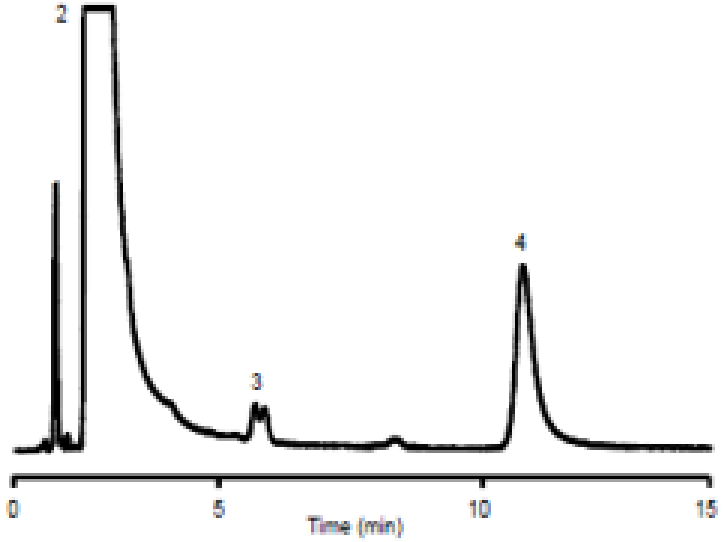


Column Contamination from Sample Matrix Causing Split Peaks

Injection 1



Injection 30

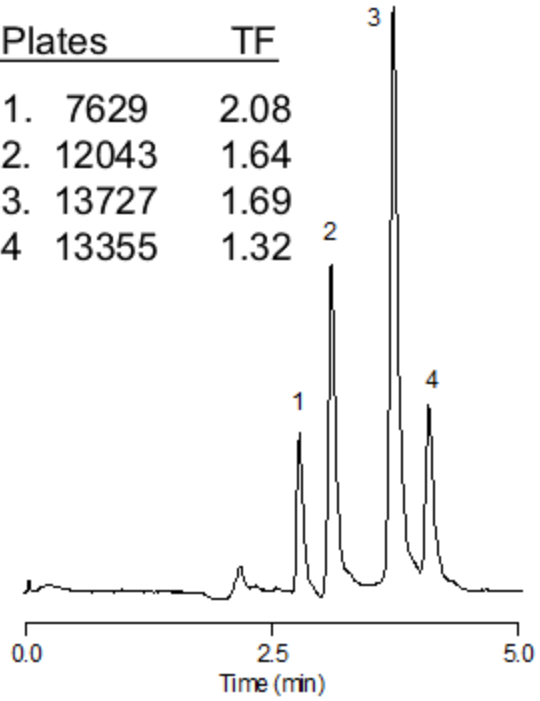


Column: StableBond SB-C8, 4.6 x 150 mm, 5 mm Mobile Phase: 60% 25 mM Na_2HPO_4, pH 3.0 : 40% MeOH Flow Rate: 1.0 mL/min
Temperature: 35°C Detection: UV 254 nm Sample: Filtered OTC Cold Medication: 1. Pseudoephedrine 2. APAP 3. Unknown 4. Chlorpheniramine

Column Contamination from Sample Matrix Causing Peak Tailing

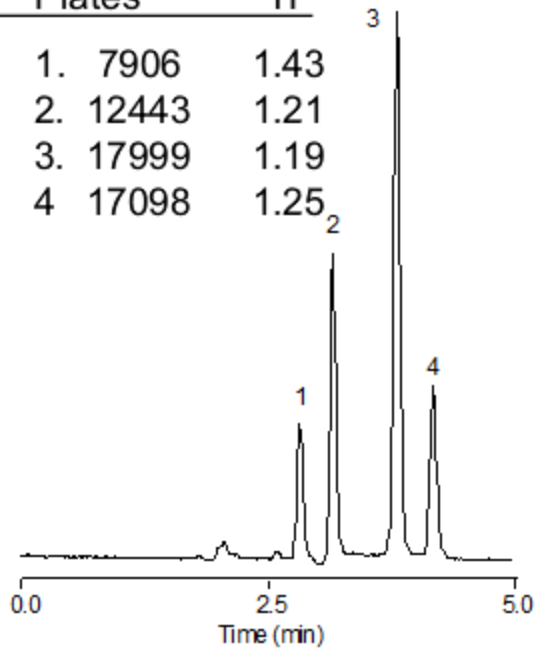
Column: StableBond SB-C8, 4.6 x 250 mm, 5µm Mobile Phase: 20% H_2O : 80% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 nm Sample: 1. Uracil 2. Phenol 3. 4-Chloronitrobenzene 4. Toluene

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



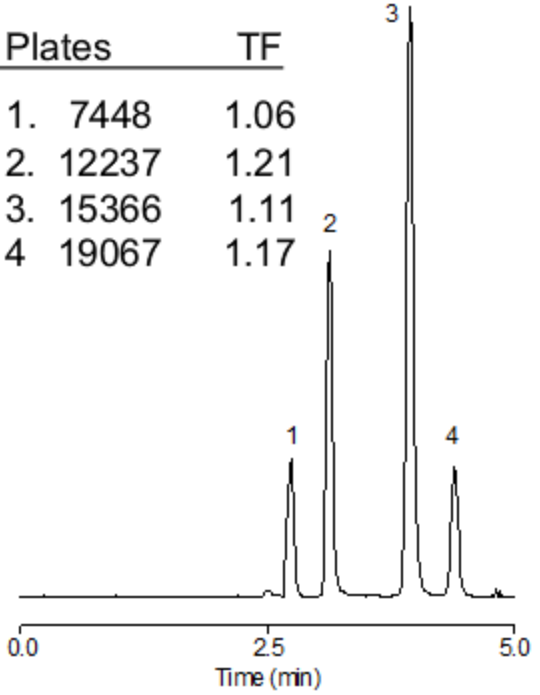
QC test forward direction

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



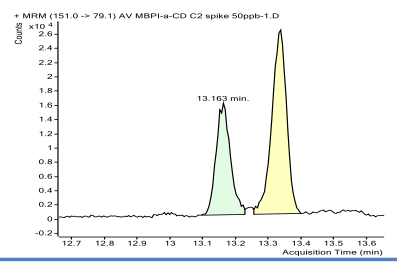
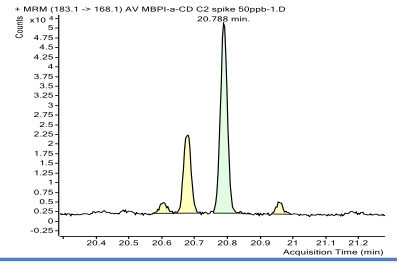
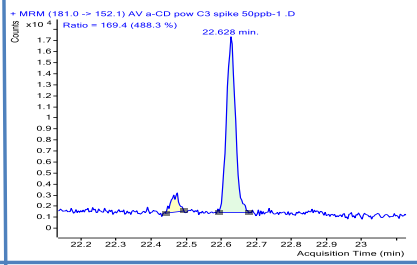
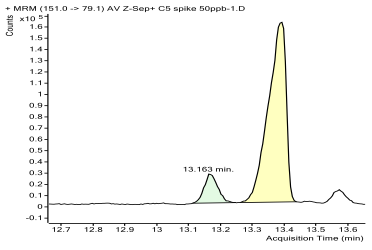
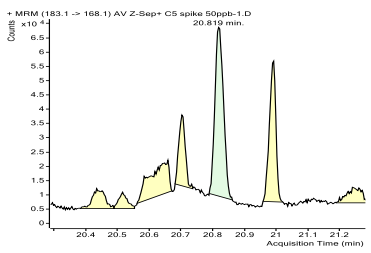
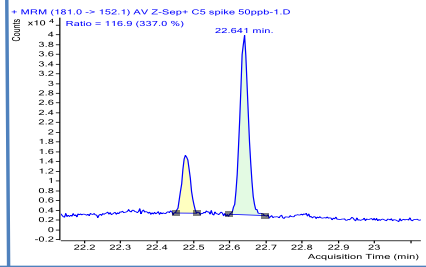
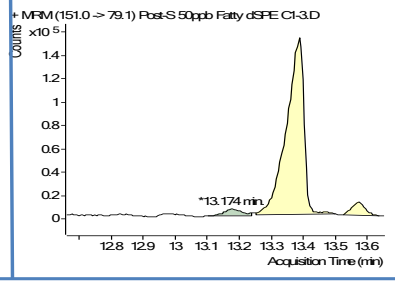
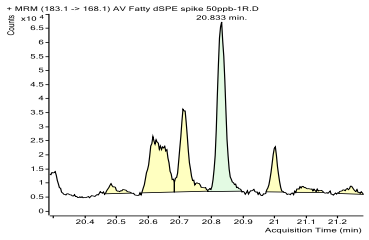
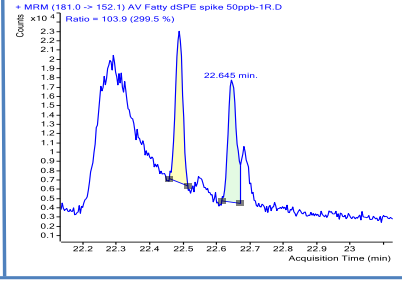
QC test reverse direction

	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

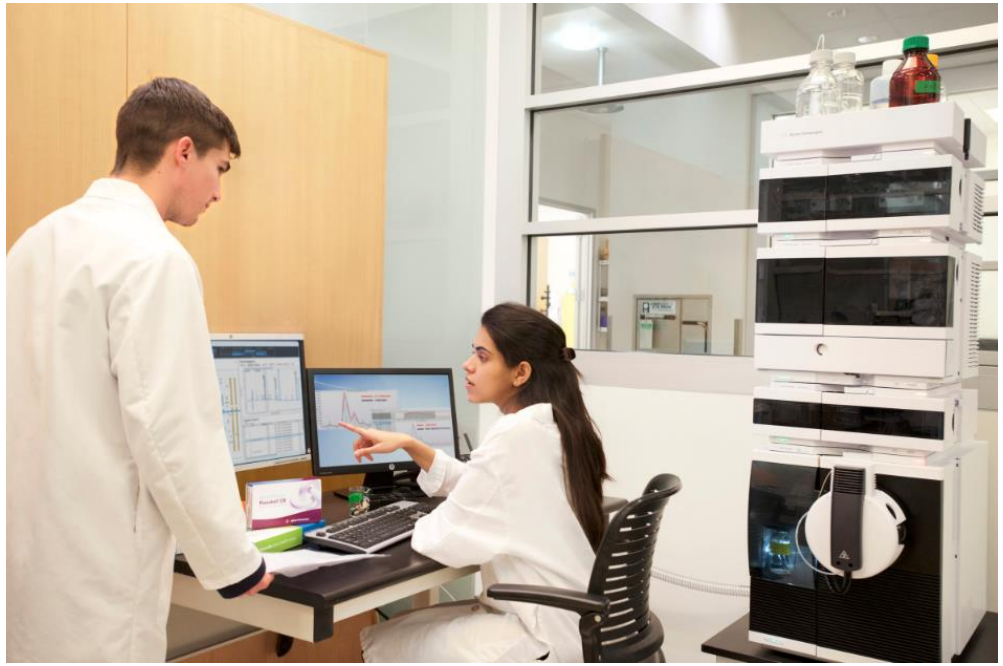
A Cleanup Step Improves Analytes S/N Ratio and Integration Accuracy on GC/MS(/MS)

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

5994-0405EN

Agenda

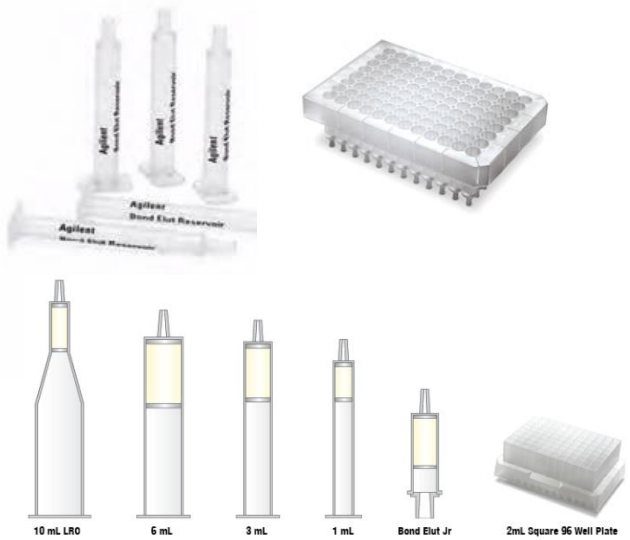
- Strategies for sample cleanup
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- How to deal with unwanted matrix effects
- Summary



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.

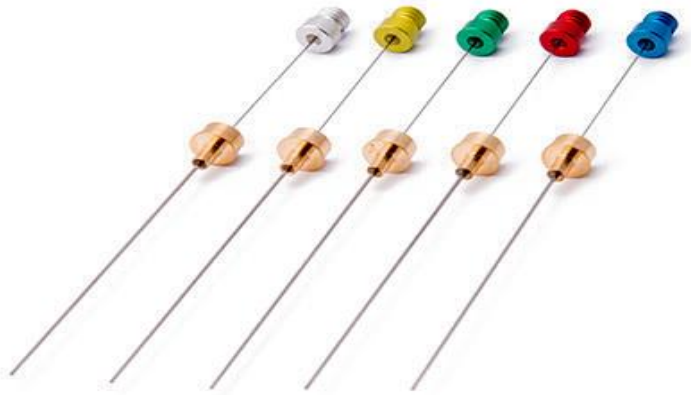
Offline Options for Sample Matrix Removal



Bond Elut Solid Phase Extraction cartridges and plates

Filter vials

QuEChERS



SPME

Captiva EMR 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