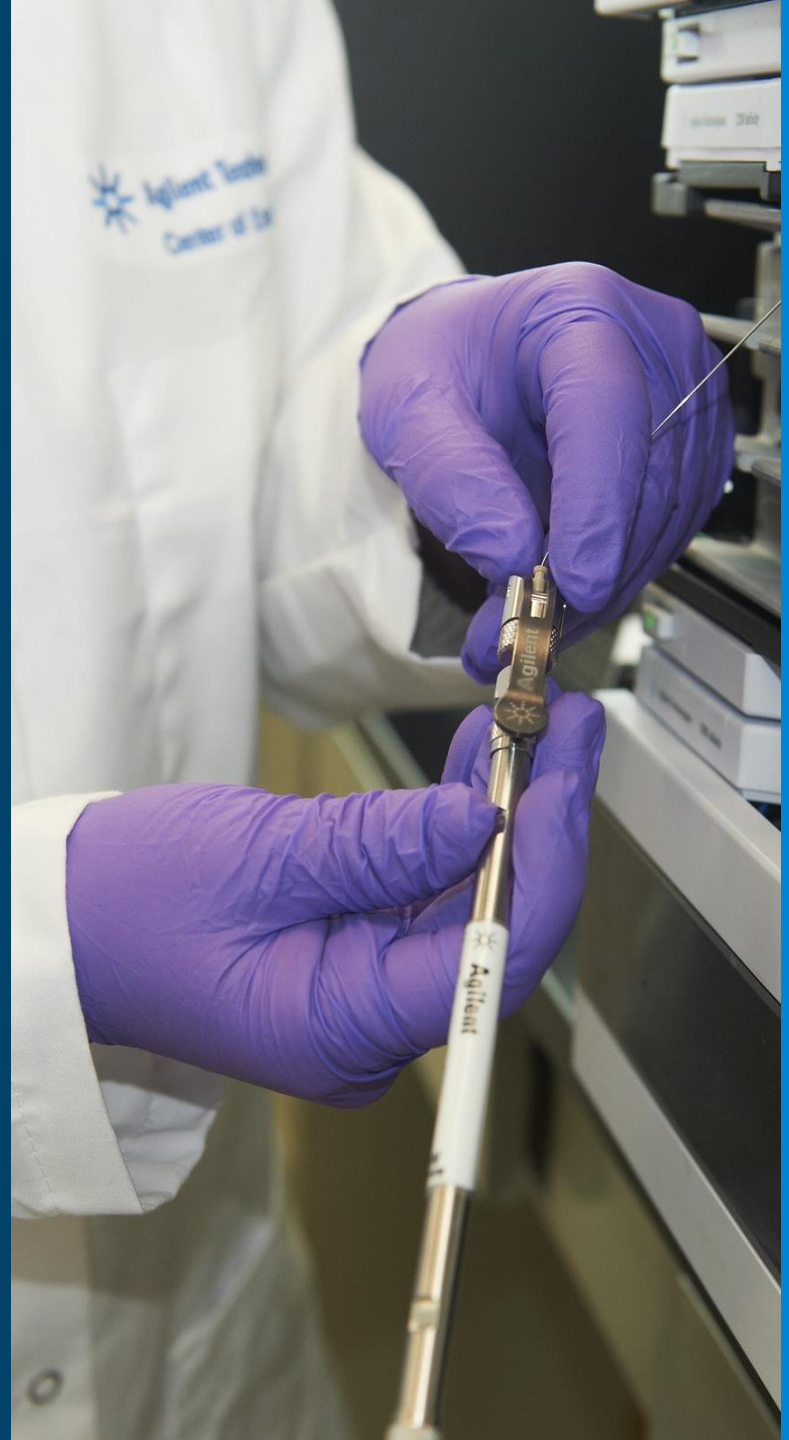
# Oh, What a Mess! Dealing with Unwanted Matrix Effects

## Becoming a better chromatographer HPLC educational webinar

Golnar Javadi
Applications Engineer
Chemistries and Supplies Technical Support




Agilent
Trusted Answers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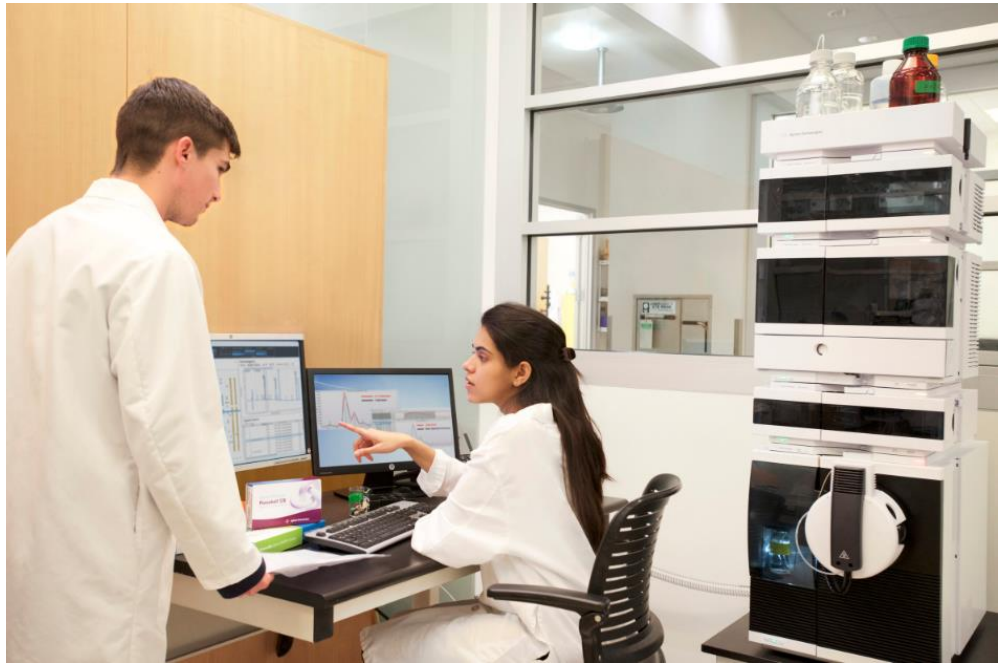
# Agenda

- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- How to deal with unwanted matrix effects
- Strategies for sample cleanup
- Other sources of unknown peaks and chromatography problems and how to deal with them
- Summary



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- Sample solvent stronger than mobile phase can cause peak distortion, split/double peak, broad peaks, poor sensitivity, and shortening of retention time


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# Strong Sample Solvent Can Compromise Peak Shape

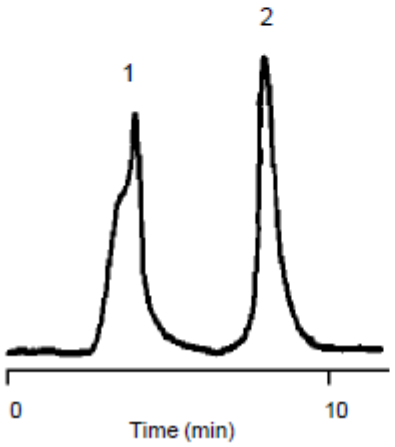
**Column:** ZORBAX SB-C8, 4.6 x 150 mm, 5 µm

**Mobile Phase:** 82% $H_2O$:18% ACN

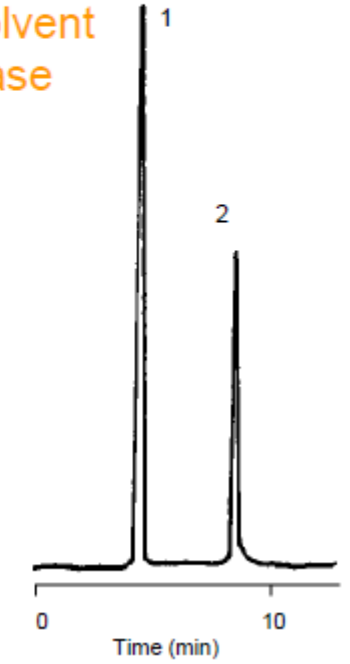
**Injection Volume:** 30 µL

**Sample:** 1. Caffeine 2. Salicylamide

A. Sample Solvent
100% Acetonitrile




B. Sample Solvent
Mobile Phase



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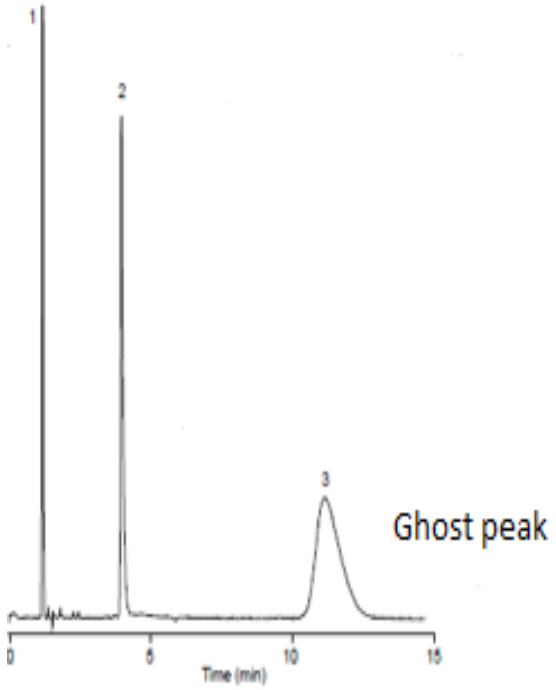



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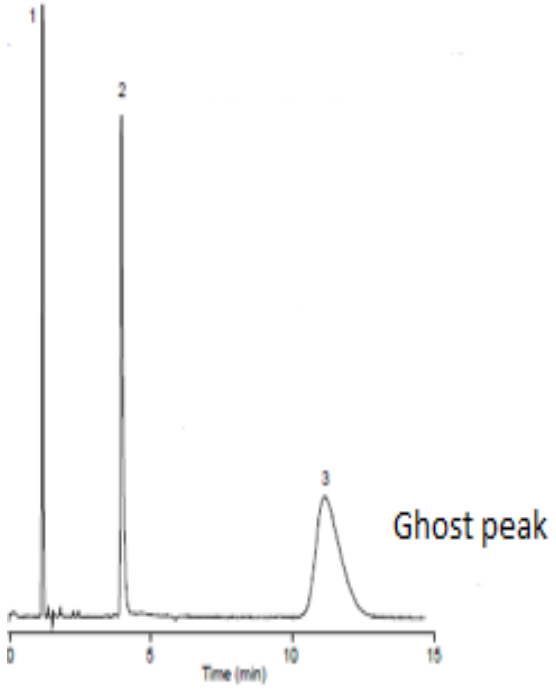

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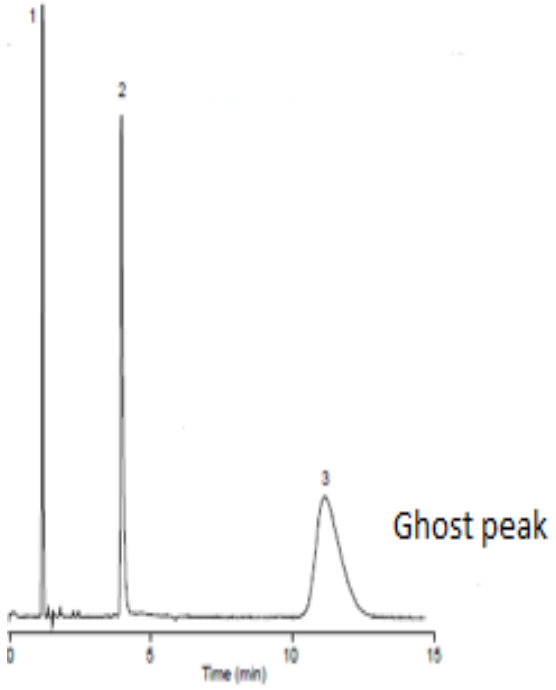



Agilent

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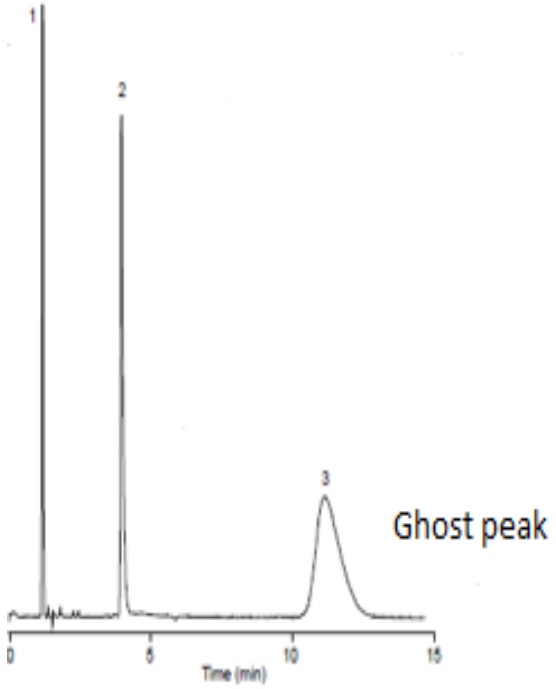
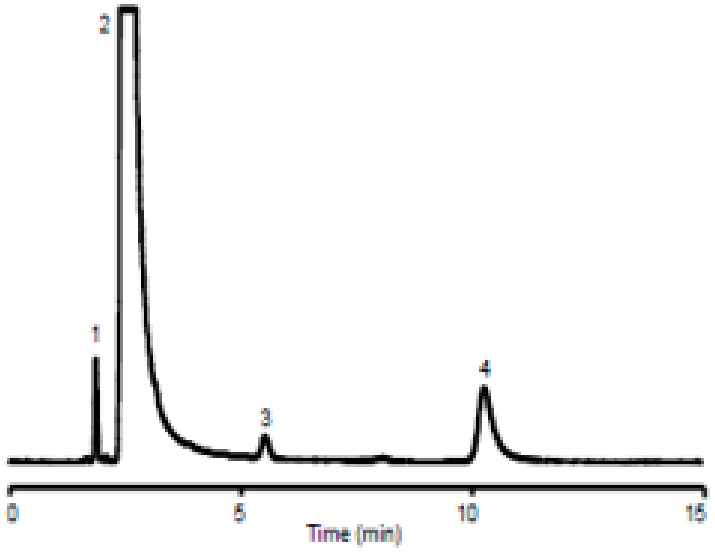
As a result, productivity is reduced; instrument downtime, sample run time, and cost are increased.

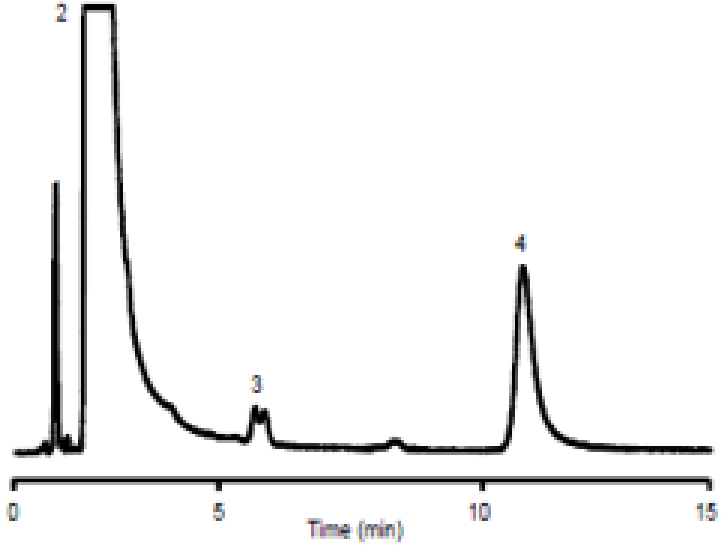


# Column Contamination from Sample Matrix Causing Split Peaks

**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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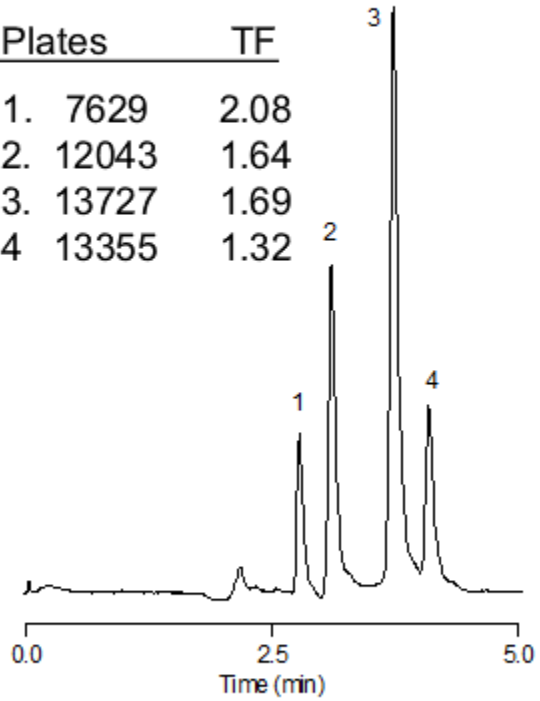
# Column Contamination from Sample Matrix Causing Peak Tailing

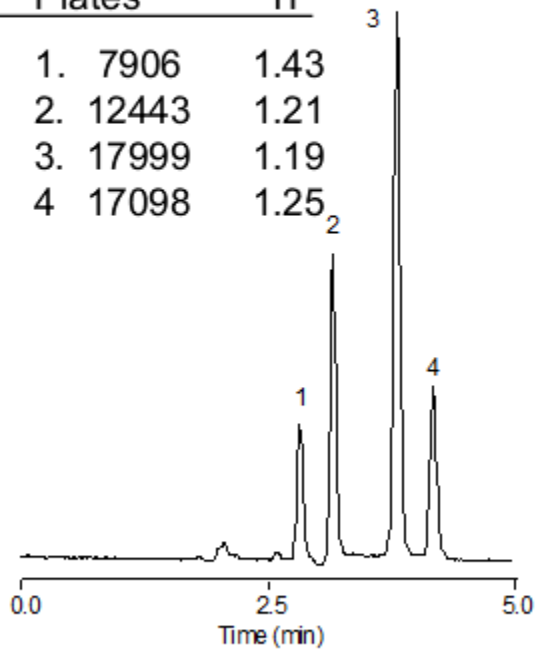
Column: StableBond SB-C8, 4.6 x 250 mm, 5µm  Mobile Phase: 20% $H_2O$ : 80% MeOH  Flow Rate: 1.0 mL/min
Temperature: R.T.  Detection: UV 254 nm  Sample: 1. Uracil 2. Phenol 3. 4-Chloronitrobenzene 4. Toluene

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



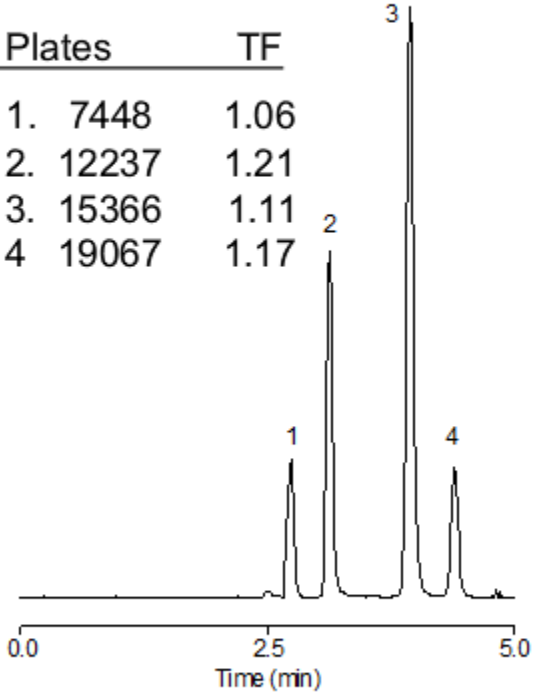

QC test forward direction

| | 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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- Take advantage of capabilities of the instrument to get a “cleaner” chromatogram
  - Use a different wave length for UV
  - Perform SIM with MS or MRM with MS/MS detection

  *Note*: *contaminations are still on the column, even if you don’t see/detect them. You still need to flush the column to elute these contaminants or periodically perform column cleaning (see appendix).*

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- Prevent contamination getting on the column
  - Online options for sample matrix removal
    - Use in-line filter to capture particulates
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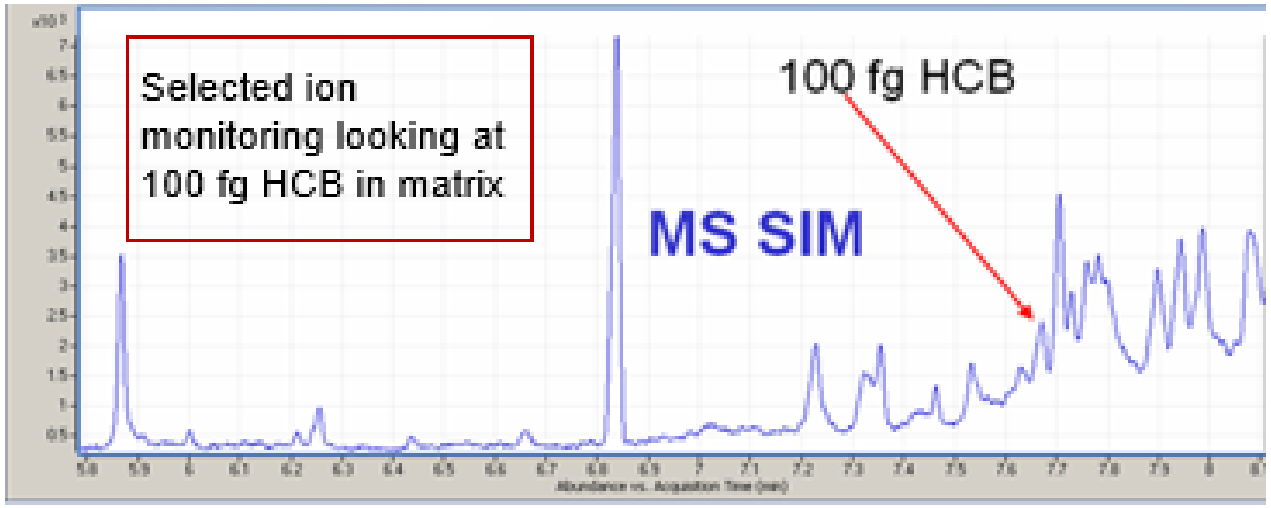
  - Offline options for sample matrix removal
    - Sample Preparation (dilute and shoot, physical and chemical filtration, protein precipitation/filtration, QuEChERS, SLE, SPE)

# SIM and MRM





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# Online Options for Sample Matrix Removal

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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

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

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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*


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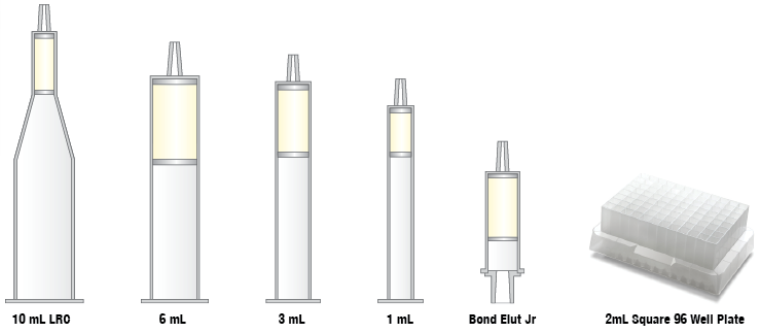
# Offline Options for Sample Matrix Removal




Bond Elut Solid Phase Extraction cartridges and plates

Captiva syringe filters

QuEChERS

Chem Elut SLE

Filter vials

Captiva EMR-Lipid filtration cartridges and plates

FilterMate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# 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

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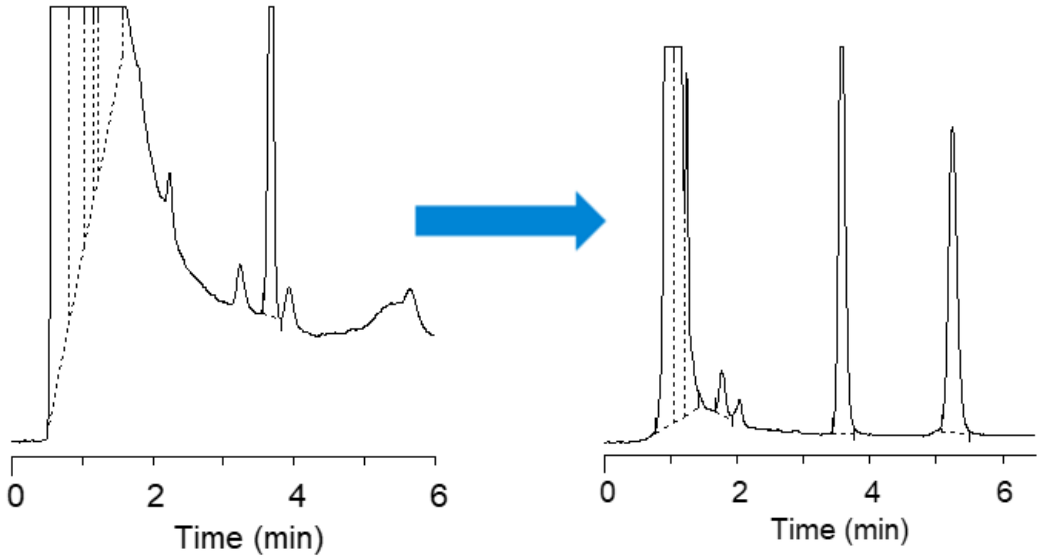
# Importance of Sample Preparation/Cleanup

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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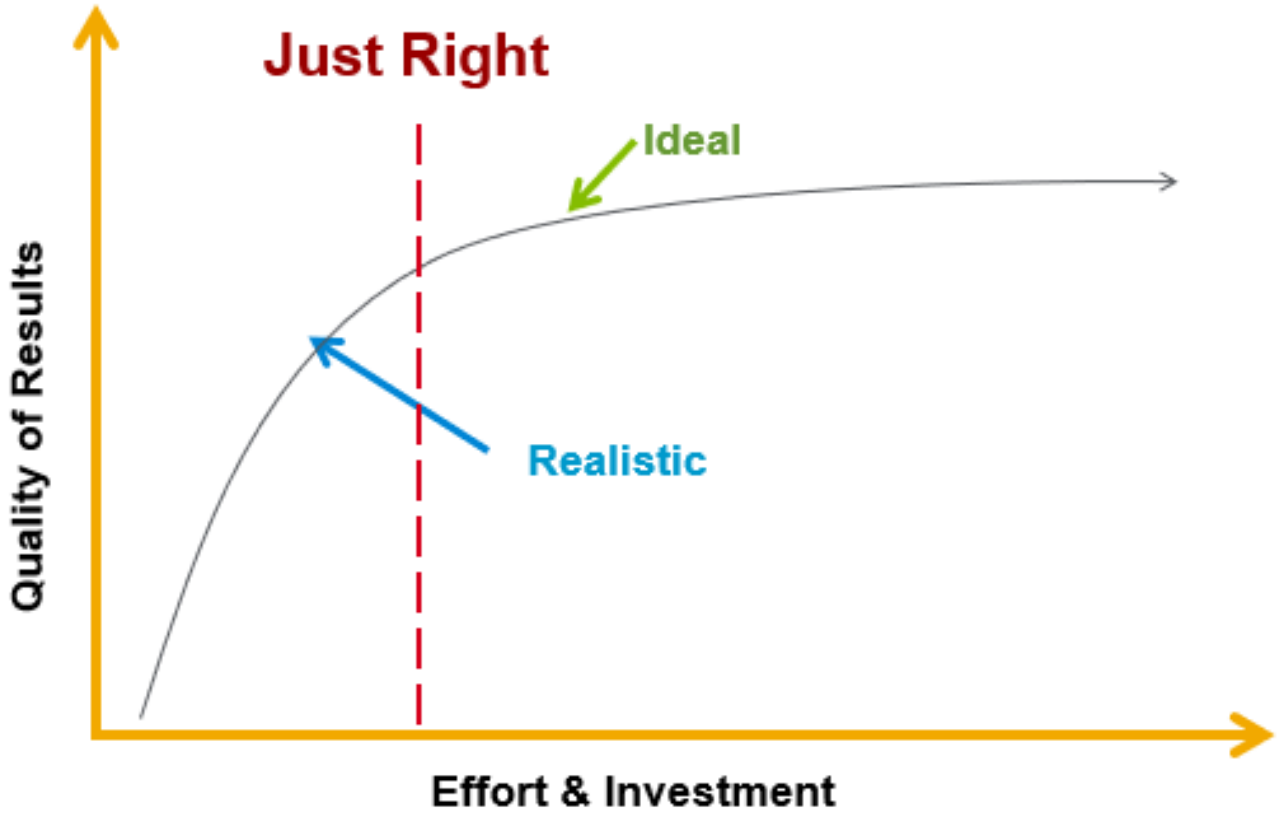
# Striking the Right Balance in Sample Preparation



# 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 | No | 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 | Chem Elut | Captiva ND | Bond Elut QuEChERS with d-EMR-Lipid and other dispersive | Captiva EMR-Lipids | Bond Elut Silica and Polymeric SPE |

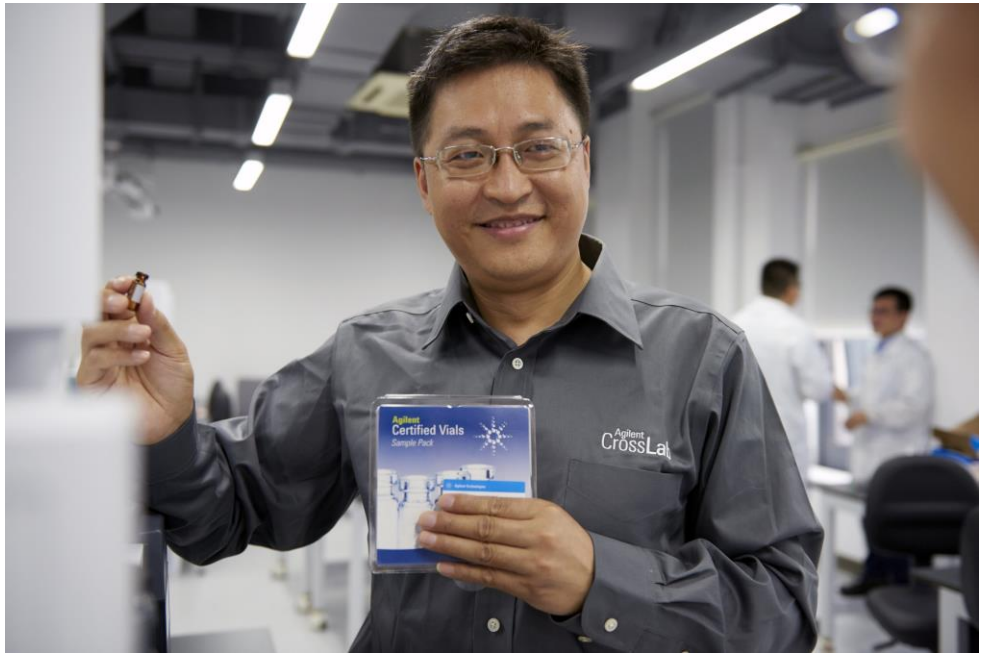
# Dilute and Shoot

## Advantages

- Fast and easy
- High throughput

## Limitations

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





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# 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** | | | ♦ | ♦ | ♦ | ♦ |

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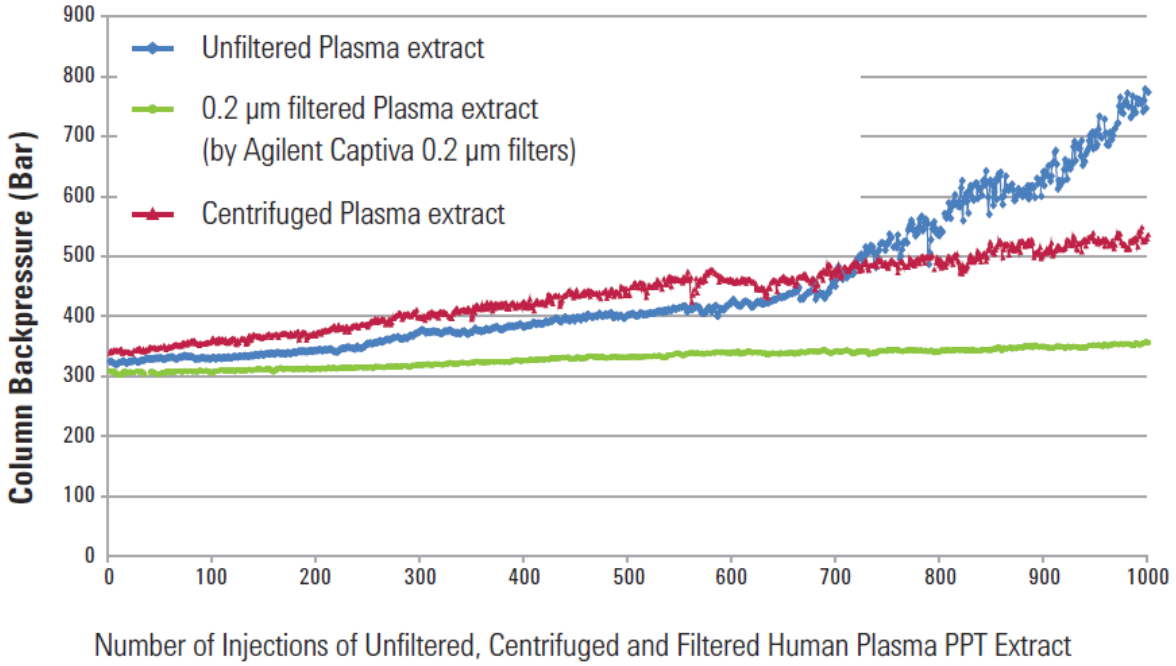
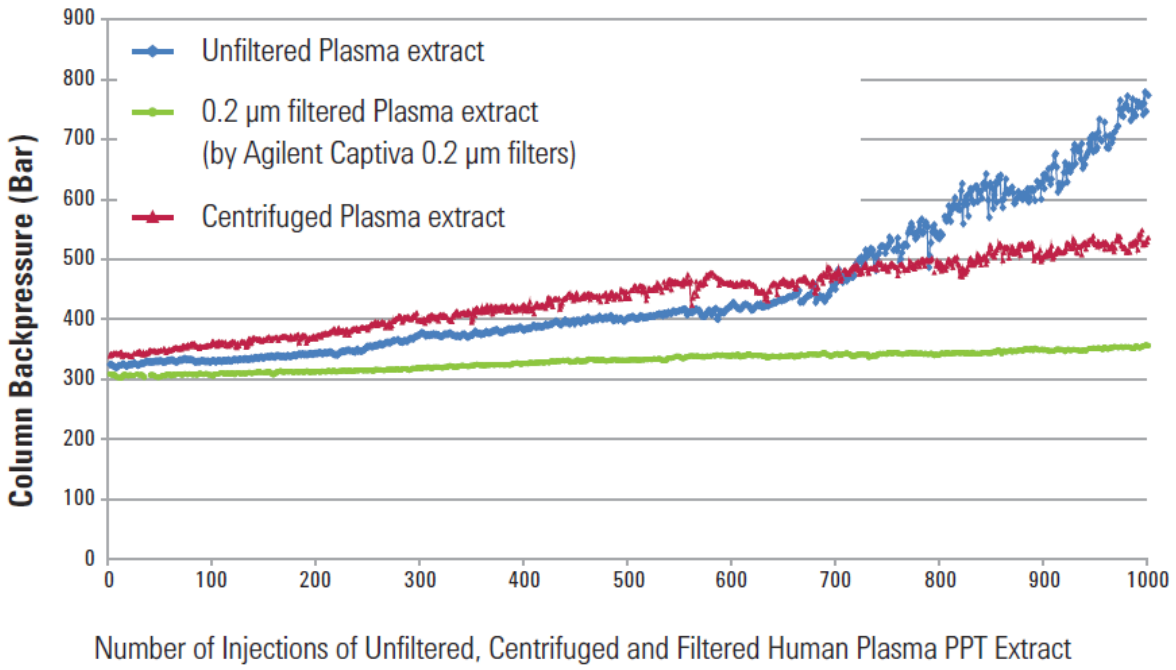
## Captiva premium syringe filters



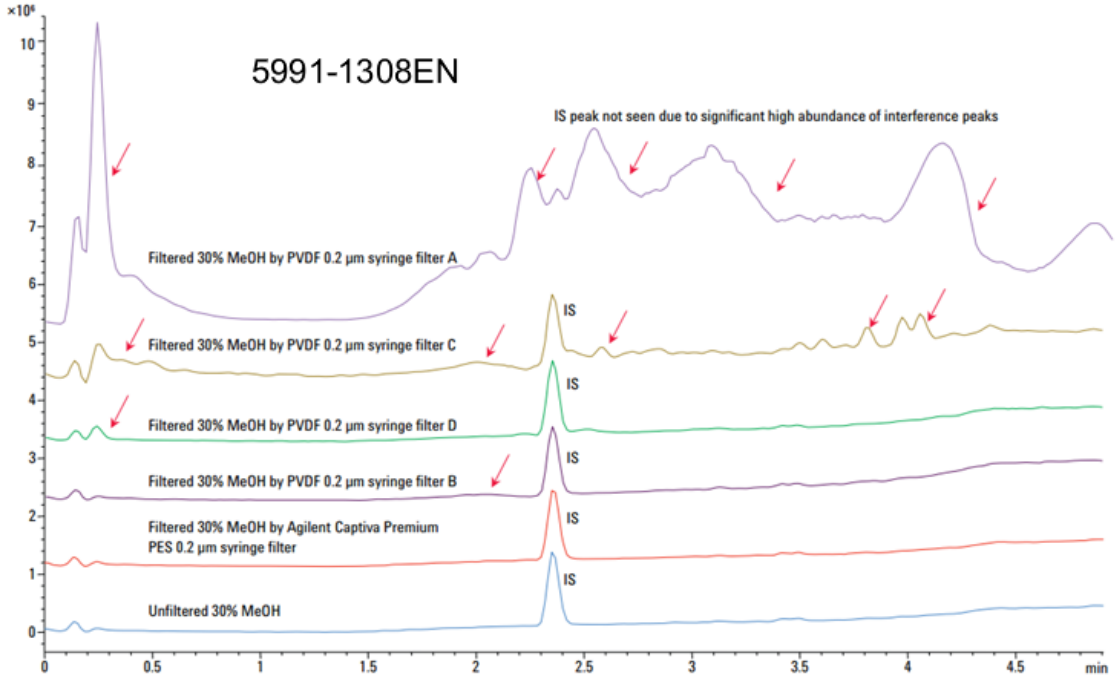

Unfiltered, centrifuged, and filtered plasma extracts
Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm column, p/n 959757-902

# Filtration

## Captiva premium syringe filters




Number of Injections of Unfiltered, Centrifuged and Filtered Human Plasma PPT Extract




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

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

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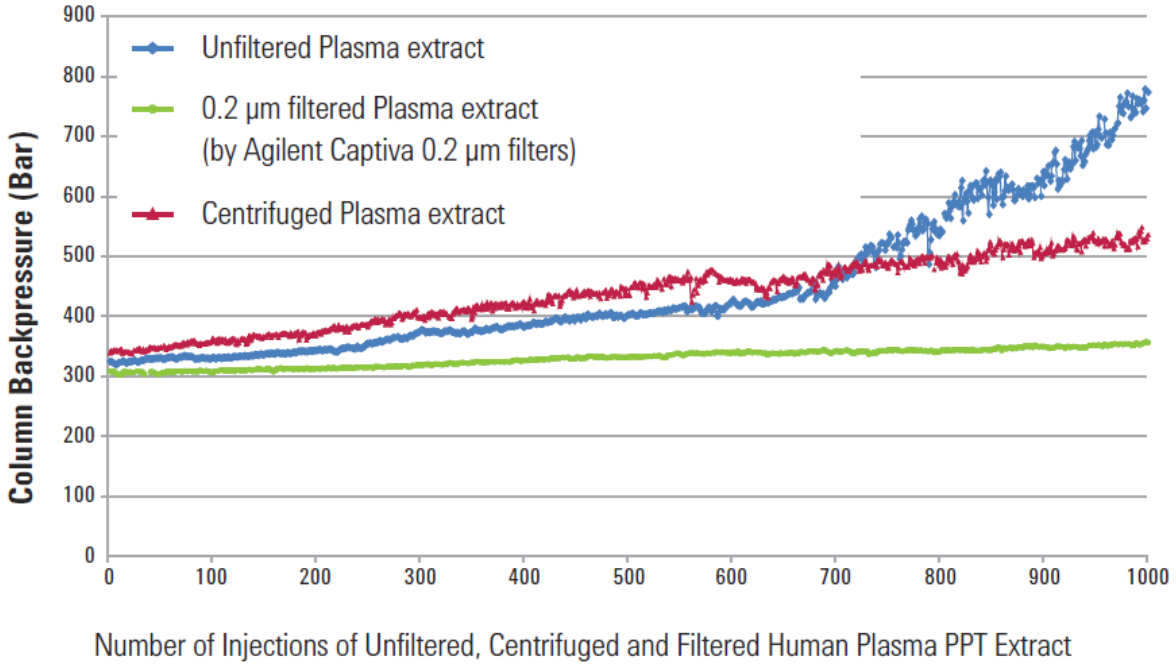


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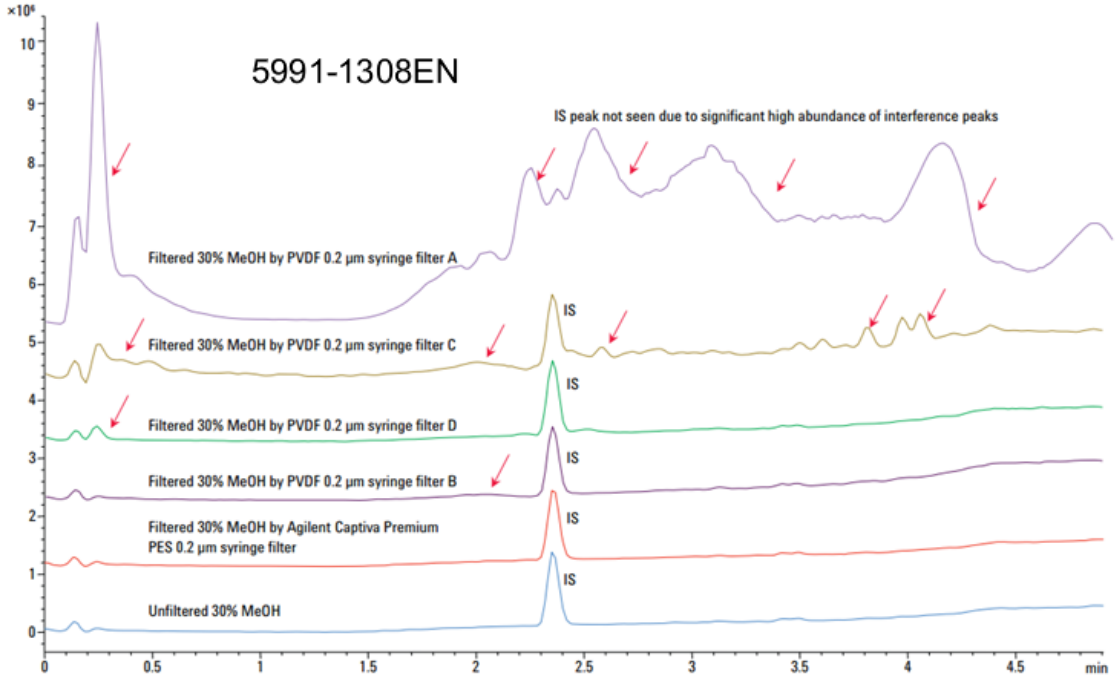


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Captiva syringe filters guide 5991-1230EN

Captiva syringe filter selection tool

# Filtration

## Captiva EMR-Lipid

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# Filtration

## Captiva EMR-Lipid

- One of the newest Agilent sample cleanup products with a 2-in-1 benefit of removing proteins and lipids

# Filtration

## Captiva EMR-Lipid

- One of the newest Agilent sample cleanup products with a 2-in-1 benefit of removing proteins and lipids
- 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

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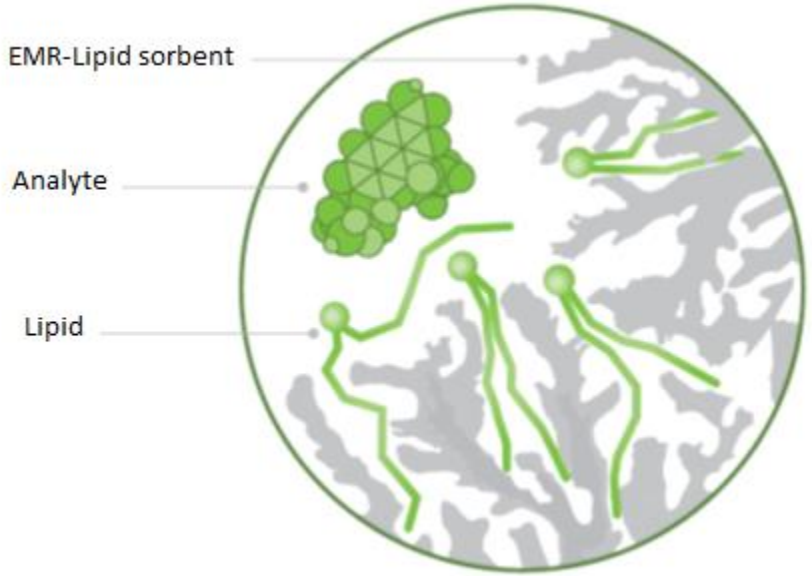
# Filtration
## Captiva EMR-Lipid

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

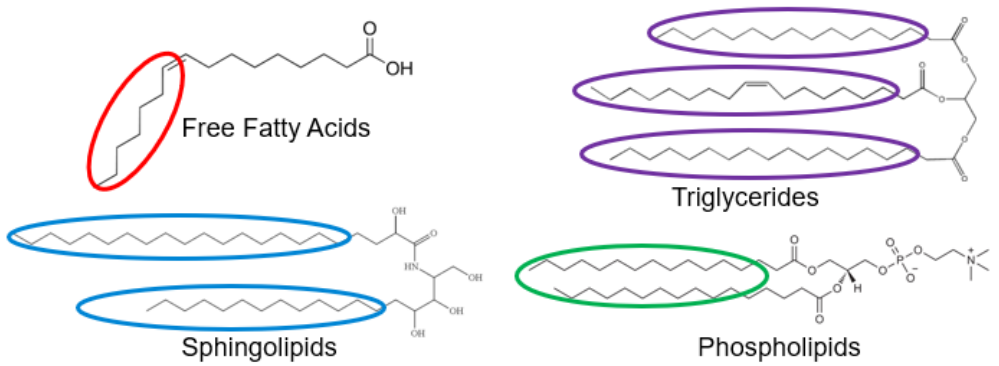
- Size exclusion – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- Sorbent chemistry – Lipid chains that enter the sorbent are trapped by hydrophobic interactions



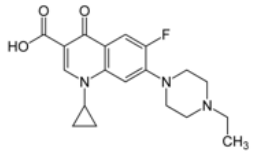
# Captiva EMR-Lipid

## Selective removal of lipids

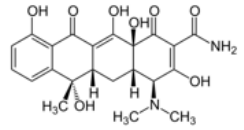
### Removes lipids



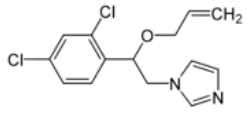

### Does not remove target analytes



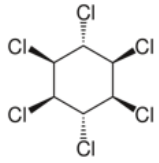
Fluoroquinolones



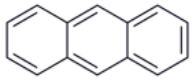
Tetracyclines



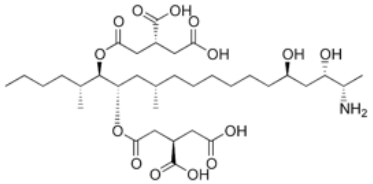
Imidazole pesticides



Organochlorine Pesticides



PAHs



Fumonisin B2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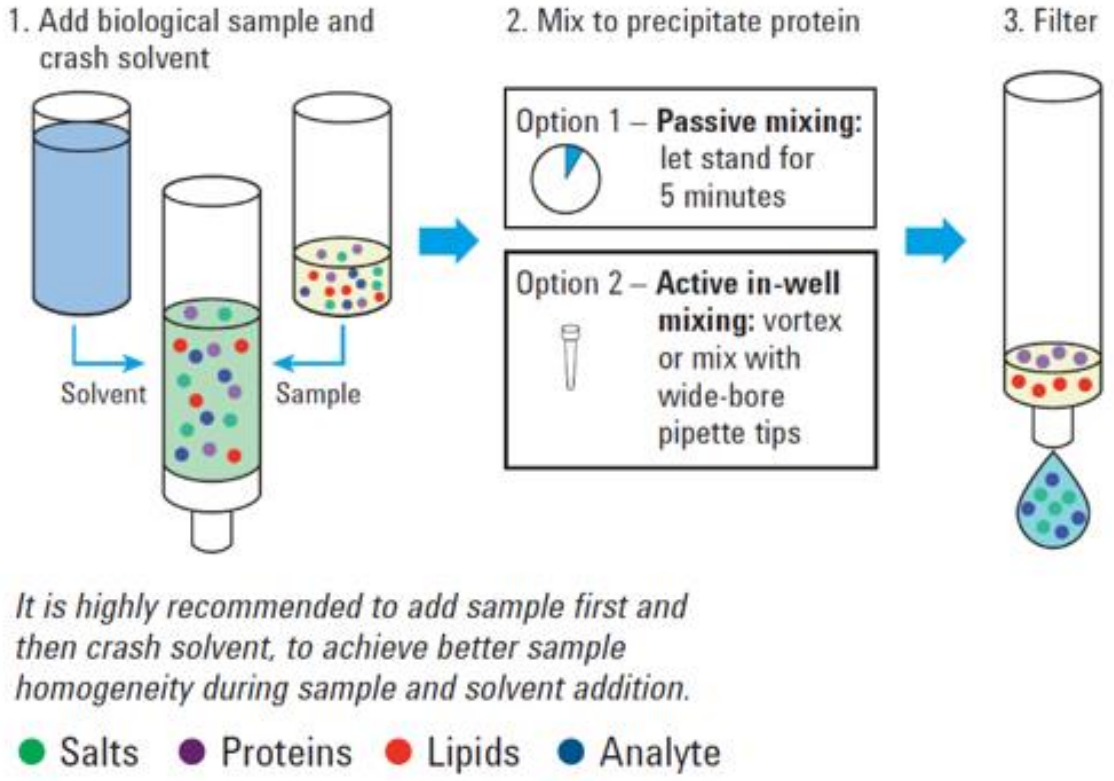
# Captiva EMR-Lipid

## 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

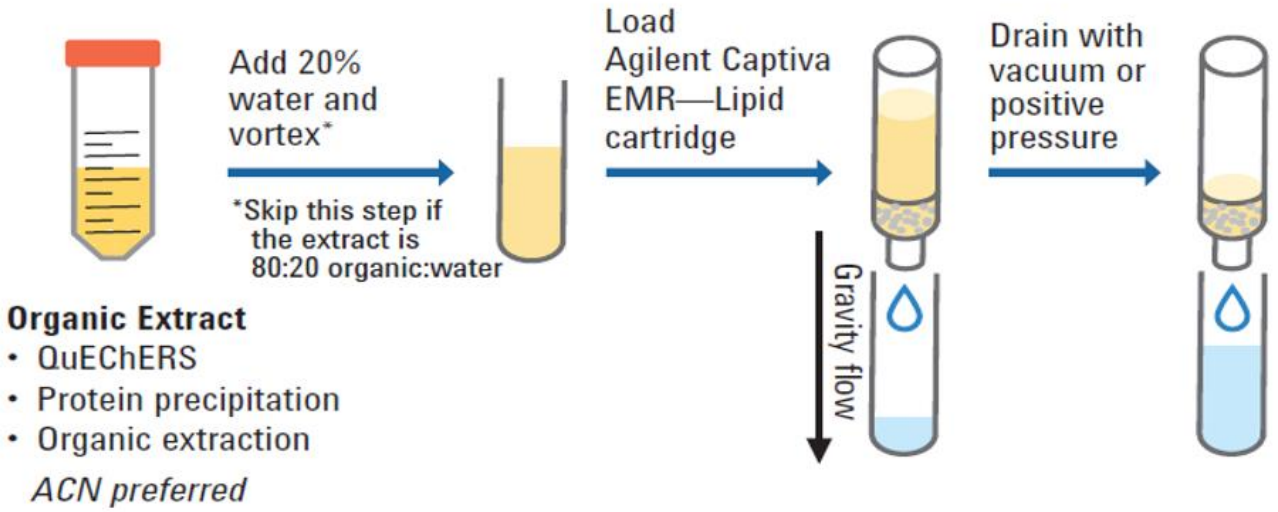
Extra elution step with 80:20 acetonitrile: water can improve recovery

[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

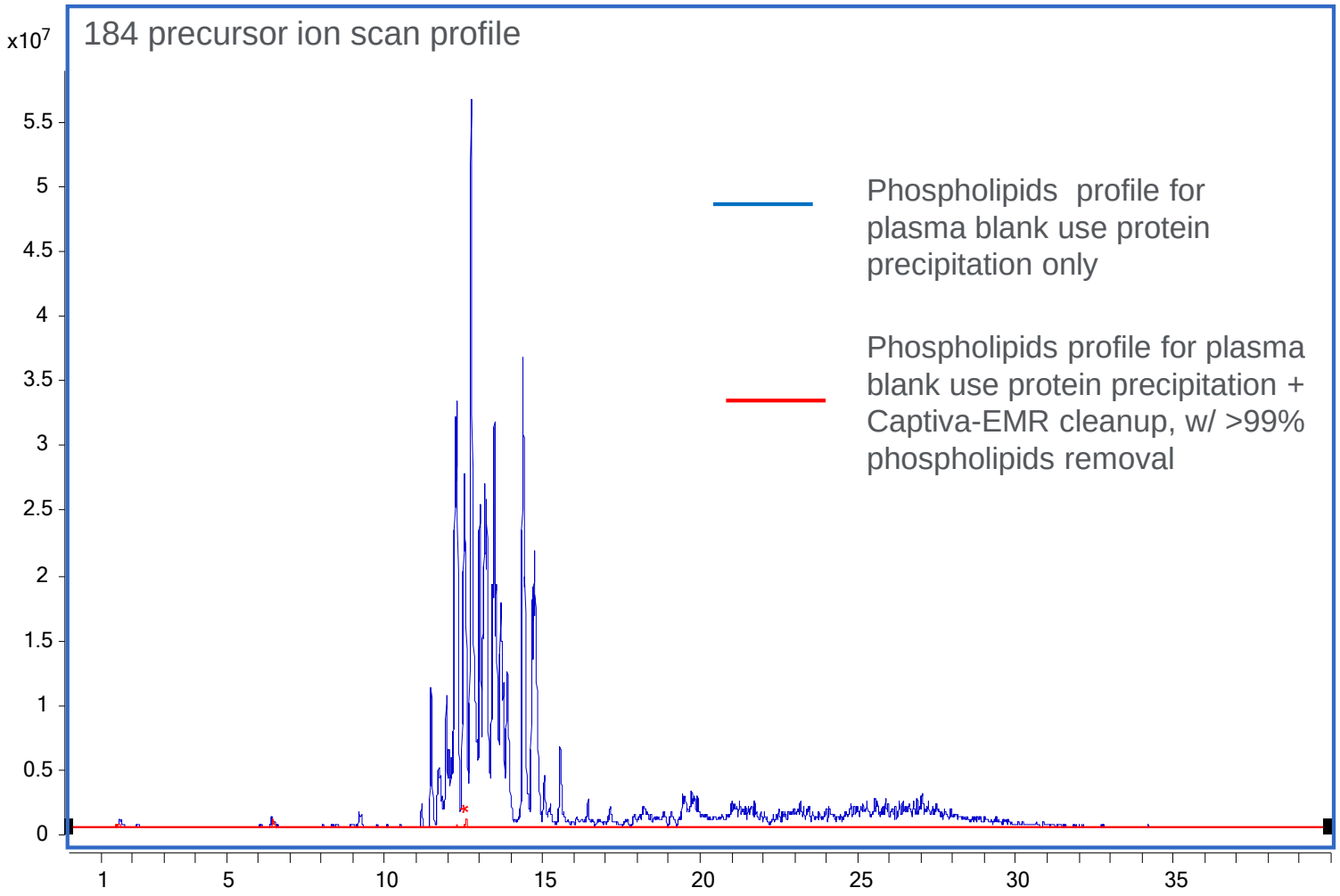
### Operating instructions




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

# Captiva EMR-Lipid Cleanup

## Efficient phospholipids removal from biological fluid matrices





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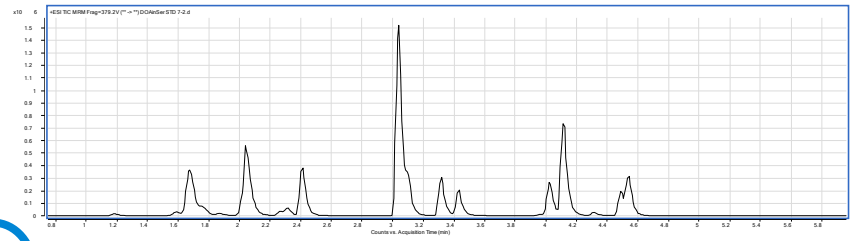
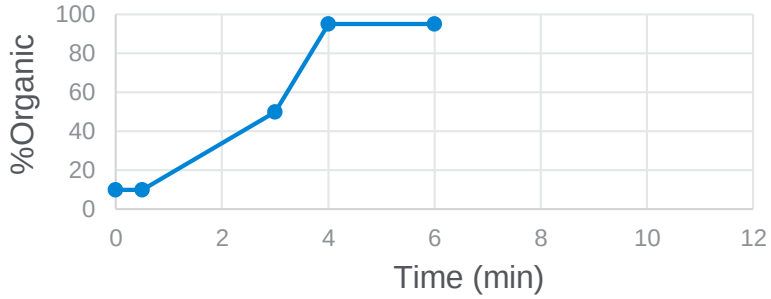
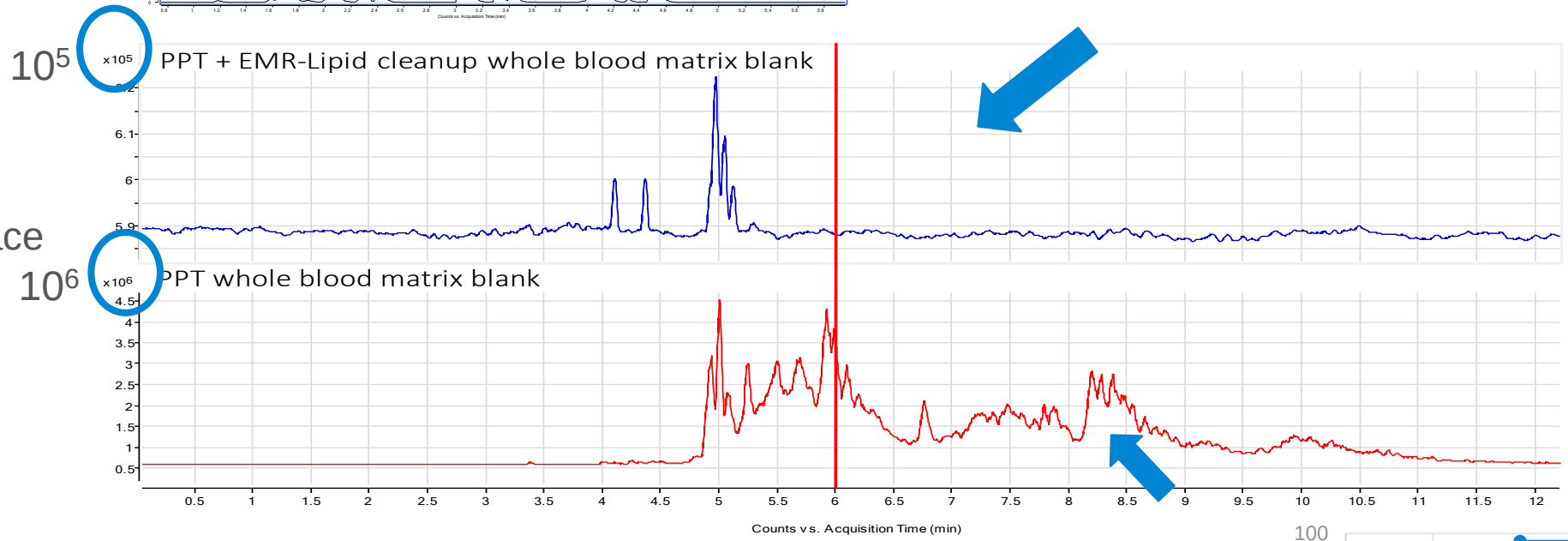
# Removal of Lipids Allows for Shorter LC Gradient Time

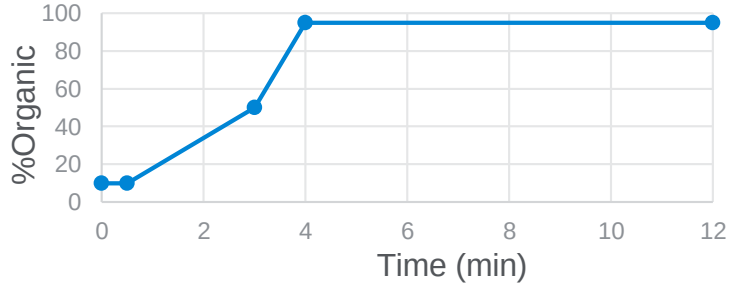
Target analytes TIC chromatogram






Lipid Trace





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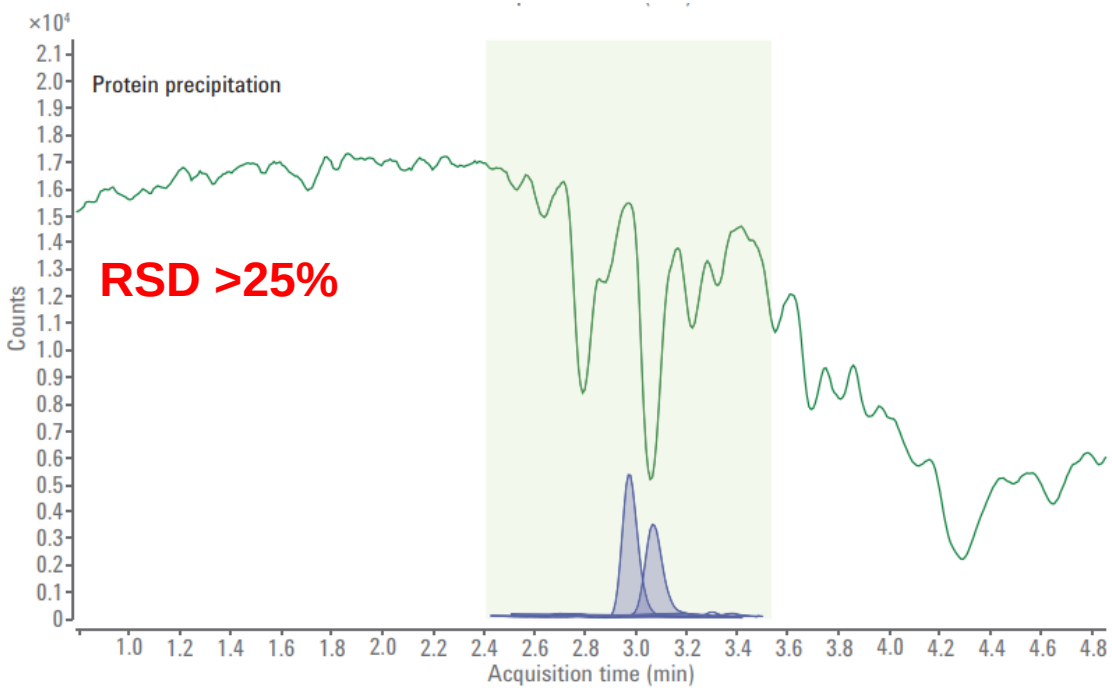
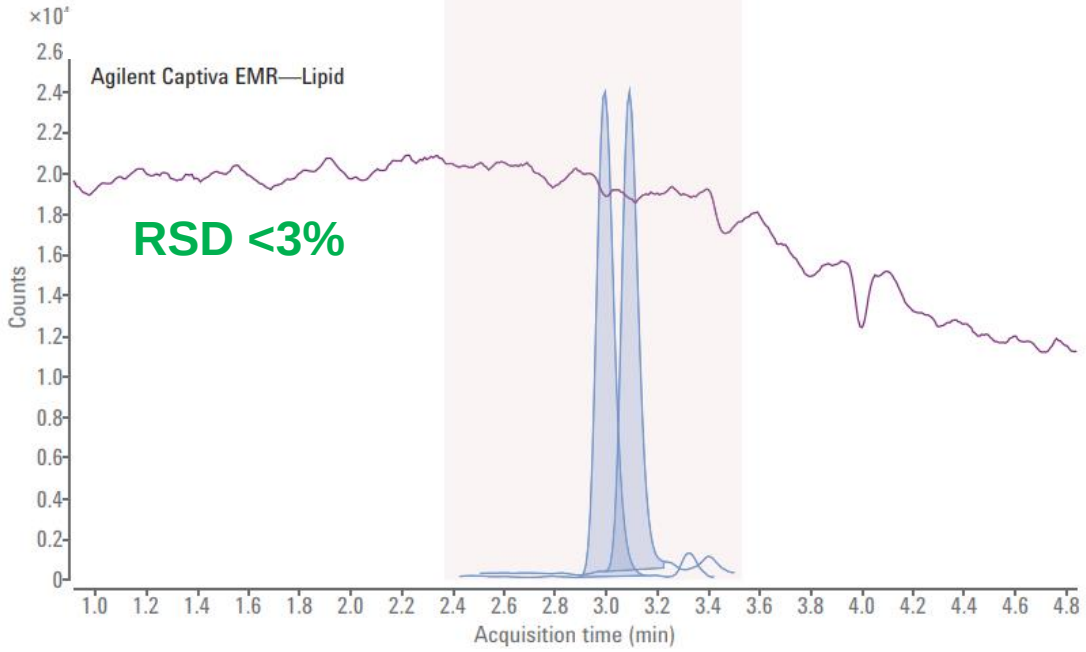
# Captiva EMR-Lipid Cleanup

## 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 → 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*

# Supported Liquid Extraction (SLE)

## Chem Elut

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

Cartridges for sample volumes 0.3 - 300 mL

96-well plate for sample volume 200 mL

Bulk Hydromatrix 1 kg and 4 kg

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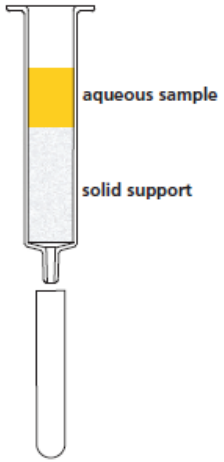
# Chem Elut Method

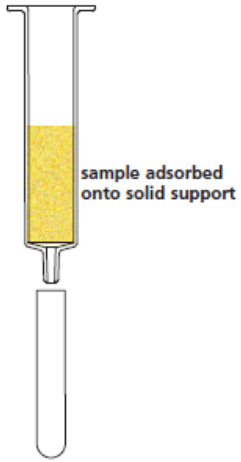
**Extraction Procedure for Aqueous and Biofluid Samples**

**Step 1**
Add the aqueous sample to the dry cartridge.



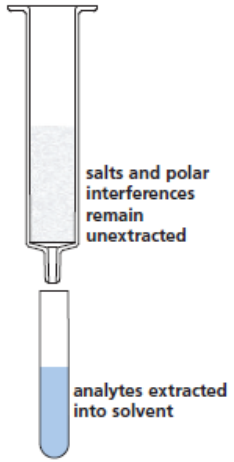

Chem Elut cartridges are packed with specially cleaned and sized diatomaceous earth.

**Step 2**
Wait 3-5 minutes.



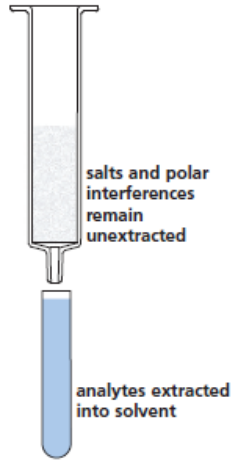

The aqueous sample is adsorbed and distributed into a thin film over the solid support.

**Step 3**
Add the first aliquot of organic extraction solvent. Collect.

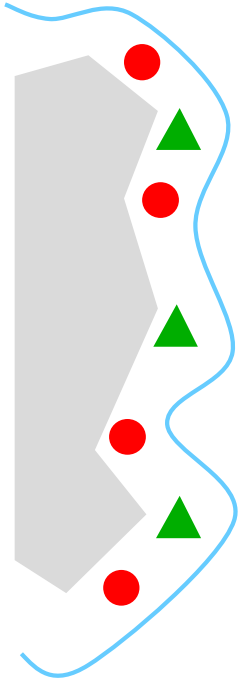



As the solvent passes through the cartridge, analytes are extracted from the aqueous layer with recoveries comparable to liquid/ liquid extraction. The gravity flow process prevents the formation of emulsions.

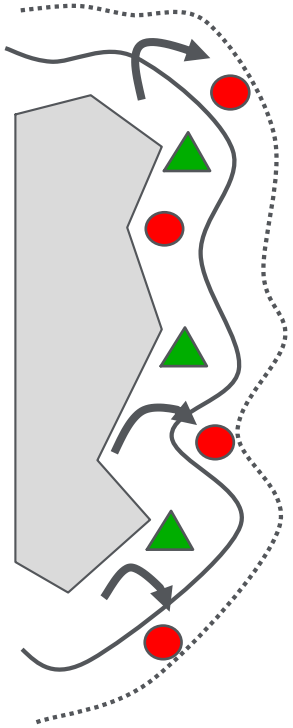
**Step 4**
Add the second aliquot of organic extraction solvent. Collect.




Elution with two aliqots instead of one improves extraction efficiency and recovery. The collected eluents can be analyzed directly, or dried and reconstituted.



Aqueous layer



Organic layer

# 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 thus two kits:

Step 1: Liquid extraction

Step 2: Dispersive SPE/ interference removal

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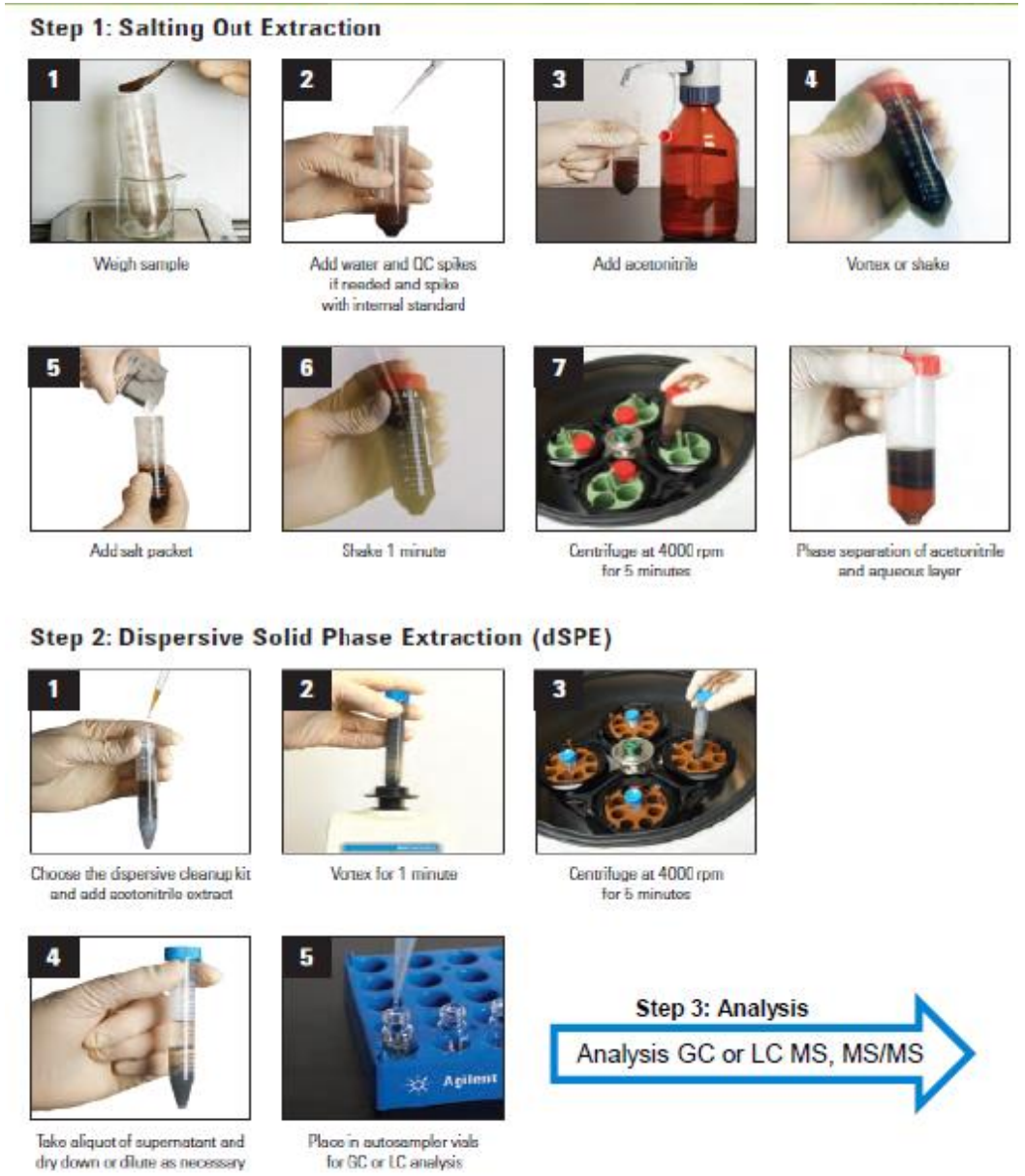
# QuEChERS Workflow

QuEChERS extraction salts

QuEChERS dispersive SPE sorbents





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# 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 available for different food types

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

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

Dispersive sorbents also available as bulk material

# Dispersive EMR-Lipid

## EMR-Lipid – What is it?

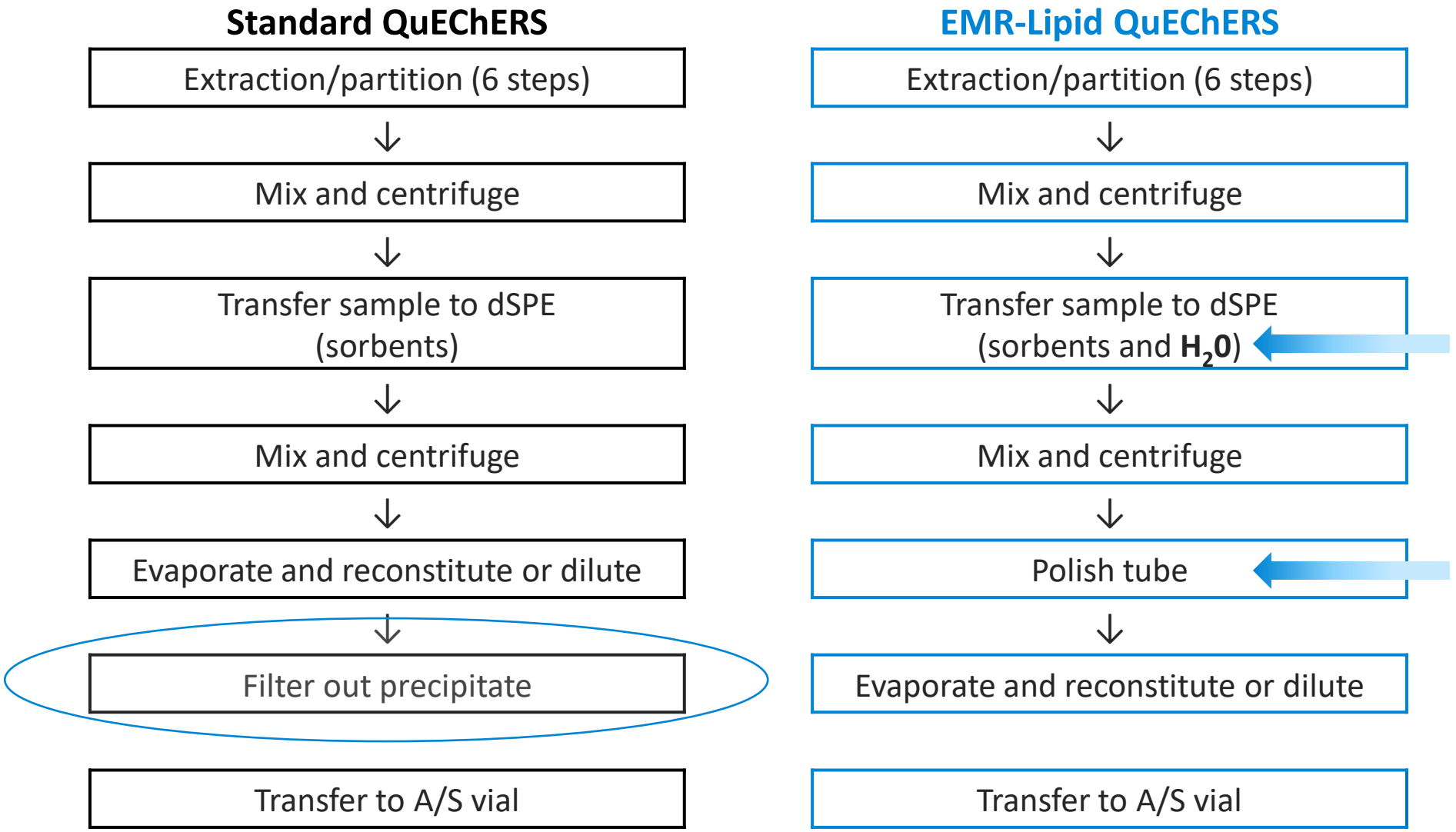
**Polish Options**

**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

# Traditional QuEChERS Versus QuEChERS EMR-Lipid





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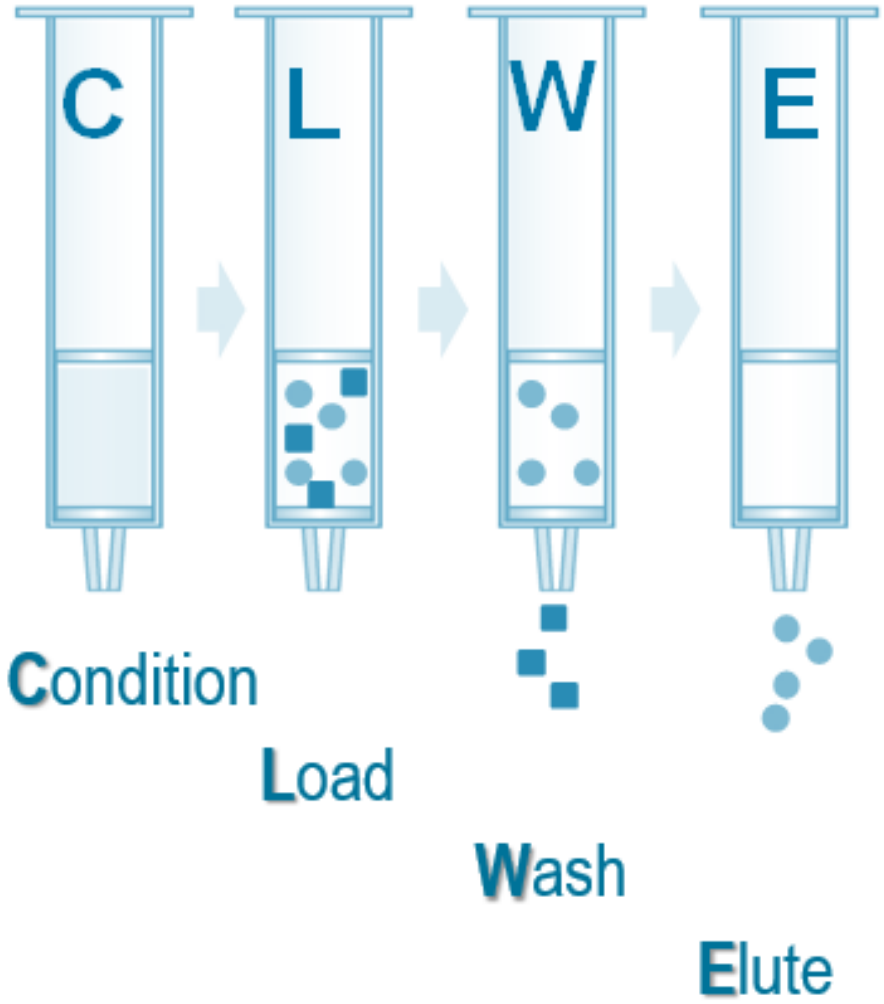
# Solid Phase Extraction (SPE)

- 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

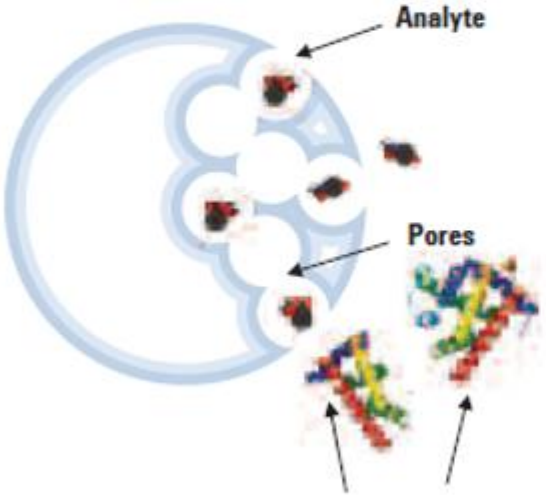
- New generation of polymeric SPE
- Divinylbenzene-based polymeric sorbent with hydrophilic exterior, hydrophobic interior, and advanced polymeric architecture
- Superior flow properties
- Great for extraction of a wide range of acidic, neutral and basic analytes from different matrices
- Simple method (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

# Bond Elut Plexa

## Advanced polymer architecture improves extraction performance

**LOAD:**

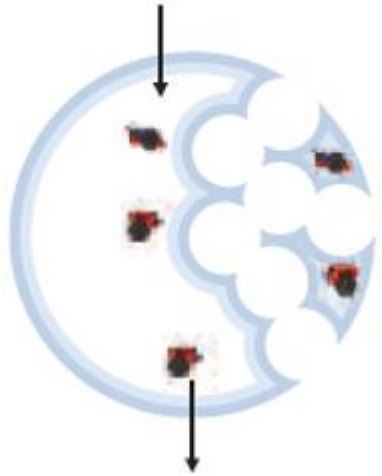
Water-rich, hydrophilic surface allows excellent phase transfer of analytes into the polymer core.




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

**WASH:**

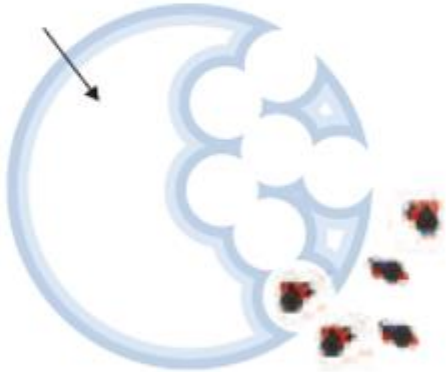
Analytes that have crossed the hydrophilic layers will remain tightly bound in the hydrophobic core.



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.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# Agenda

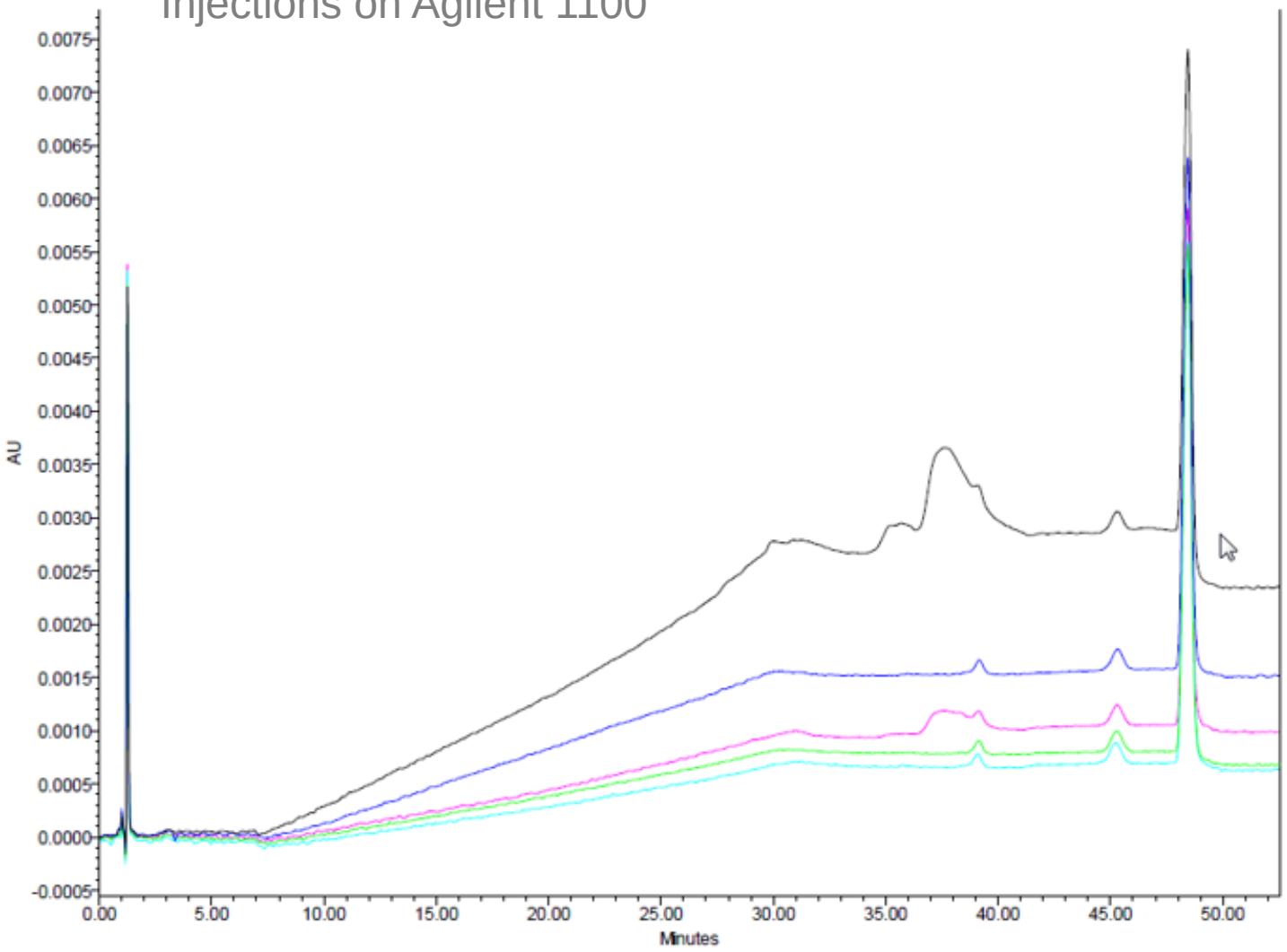
- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- How to deal with unwanted matrix effects
- Strategies for sample cleanup
- Other sources of unknown peaks and chromatography problems and how to deal with them
- Summary

# 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

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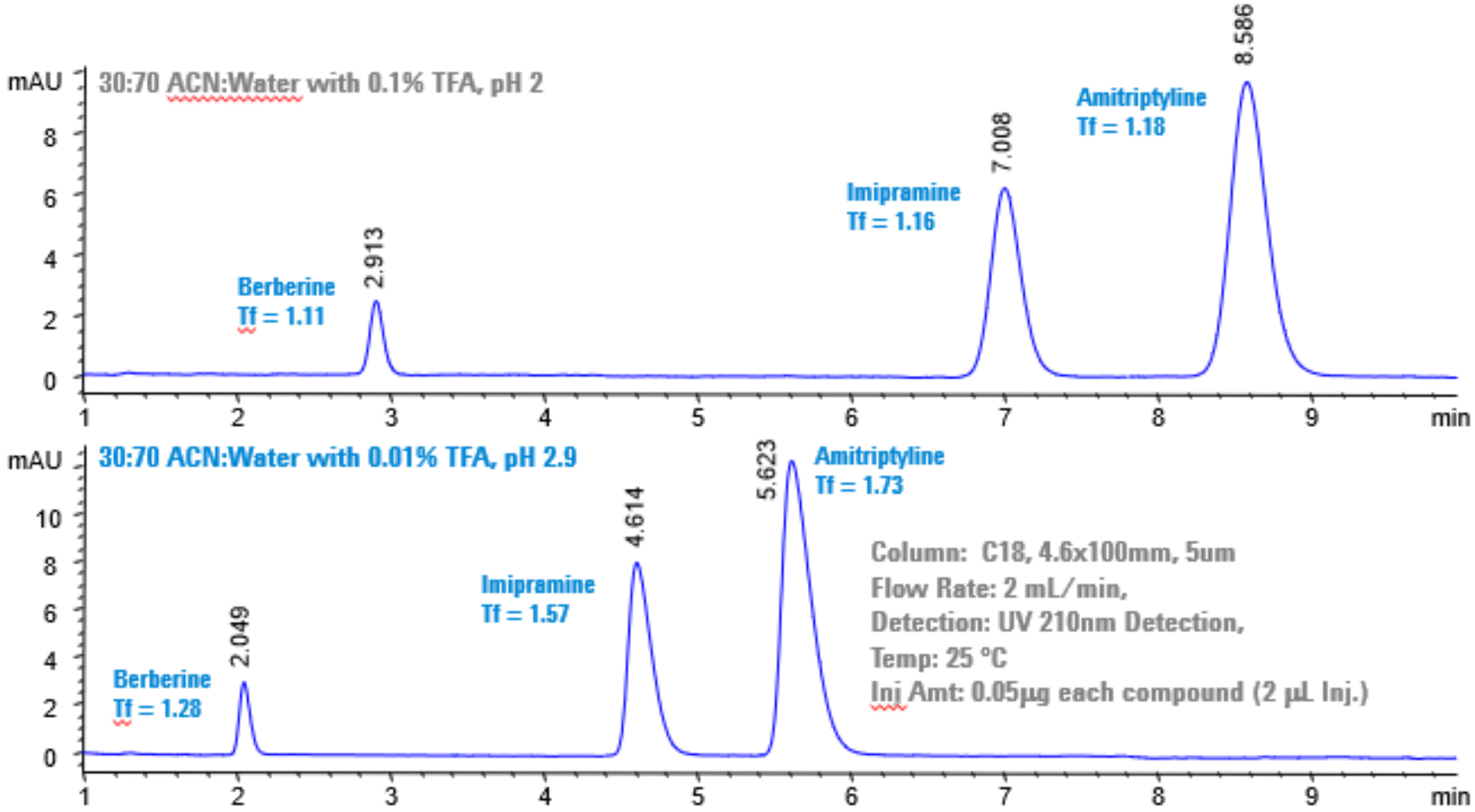
# Retention Time Shifts and Peak Shape Problem

## Change in volatile buffer concentration – Incorrect solvent bottle caps used





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# What To Do

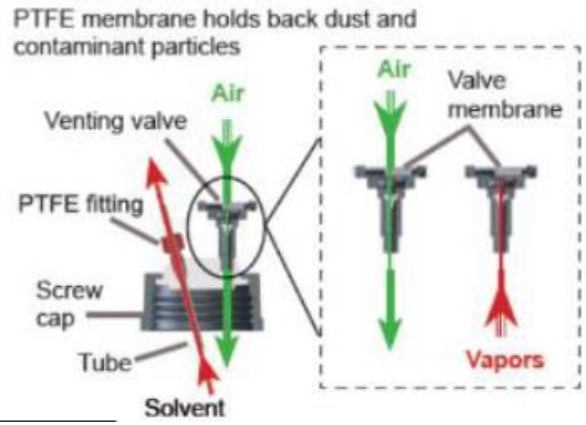
- Use high-purity solvents
- Use appropriate solvent bottle caps (Agilent InfinityLab Stay Safe caps)
- Use solvent compatible material for parts of LC that come in contact with mobile phase
- Use freshly made HPLC grade solvent and filtered buffer
- Replace solvent inlet filter as needed
- Always discard “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*<br>*Charcoal filter for waste container*<br>*Time strip* | Eliminates harmful solvent vapors<br>Keeps solvent concentration constant<br>Keeps constant pressure in bottle<br>Prevents twisted tubing<br>Allows easy solvent refill<br>Allows easy tightening |





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# 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


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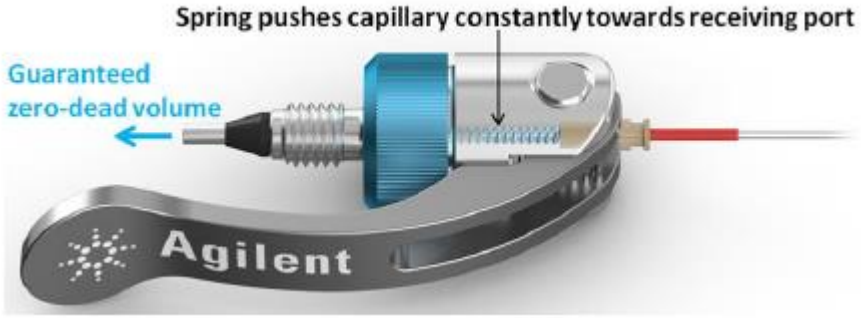
# InfinityLab Quick Connect and Quick Turn Fittings

- 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

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# Agenda

- Chromatography problems caused by sample matrix
  - Physical effects
  - Chemical effects
- How to deal with unwanted matrix effects
- Strategies for sample cleanup
- Other sources of unknown peaks and how to deal with them
- 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 effect
- 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
- Also, using in-line filters, guards, high quality solvents, appropriate solvent bottle caps, and spring activated fittings can prevent other chromatography problems.

# Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC or GC/MS columns and supplies

Option 2 for LC or LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Available in the USA 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

# Appendix

# 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.


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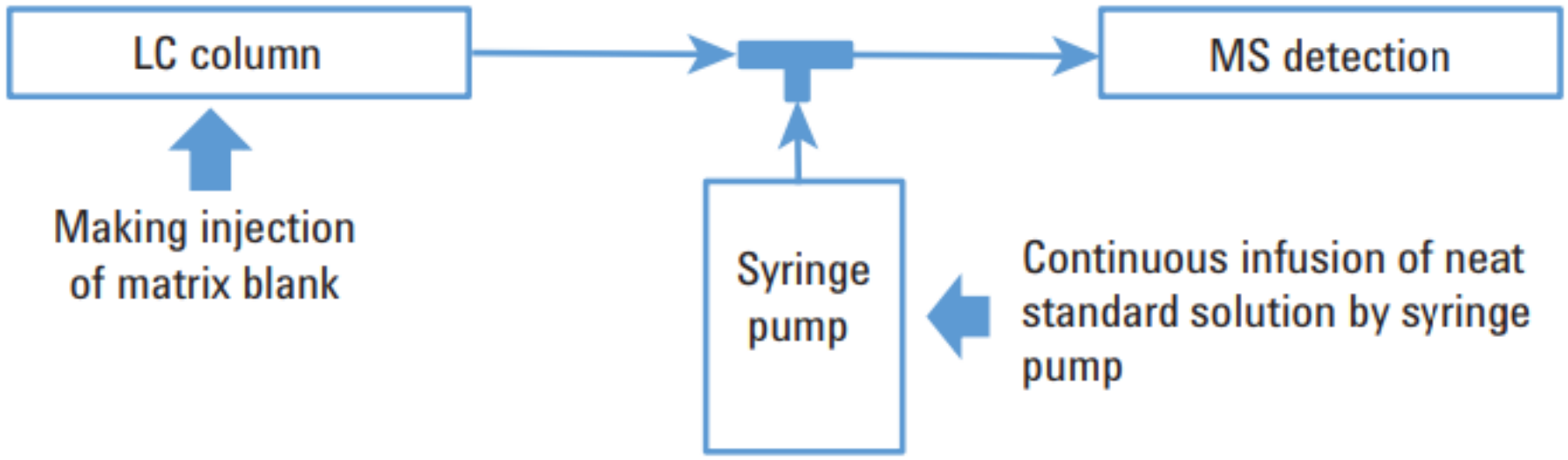
# Column Cleaning – Protein/Peptide Removal

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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# Bond Elut Plexa Method

## Generic method recommendations

| | Acids | Neutrals | | Bases |
|---|---|---|---|---|
| Analyte | LogP>1.0<br>$pK_a$ < 5 | LogP > 1.5<br>pKa 3-5 | <br>$pK_a$ 6-10 | LogP > 0.8<br>$pK_a$ 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<br>*Neutrals* | 100% MeOH<br>*Neutrals* | | 1:1 MeOH/ACN<br>*Acids, Neutrals* |
| Elution 2 | 5% $HCO_2H$ in MeOH<br>*Acids* | | | 5% $NH_3$ in 1:1 MeOH/ACN<br>*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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## 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<br>2. 500 µL $H_2O$ |
| Sample | 100 µL Plasma |
| Pre-treatment | Dilute 1:3 with 300 µL:<br>2% $NH_4OH$ in $H_2O$ |
| Washes | 1. 500 µL $H_2O$<br>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<br>2. 500 µL $H_2O$ |
| Sample | 100 µL Plasma |
| Pre-treatment | Dilute 1:3 with 300 µL:<br>2% $NH_4OH$ *(neutrals and bases)*<br>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<br>2. 500 µL $H_2O$ |
| Sample | 100 µL Plasma |
| Pre-treatment | Dilute 1:3 with 300 µL:<br>2% $H_3PO_4$ in $H_2O$ |
| Washes | 1. 500 µL 2% $HCO_2H$ in $H_2O$<br>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<br>• 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<br>• 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)<br>• Check solubility of analyte in the eluent<br>• Increase strength of elution solvent<br>• 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 |


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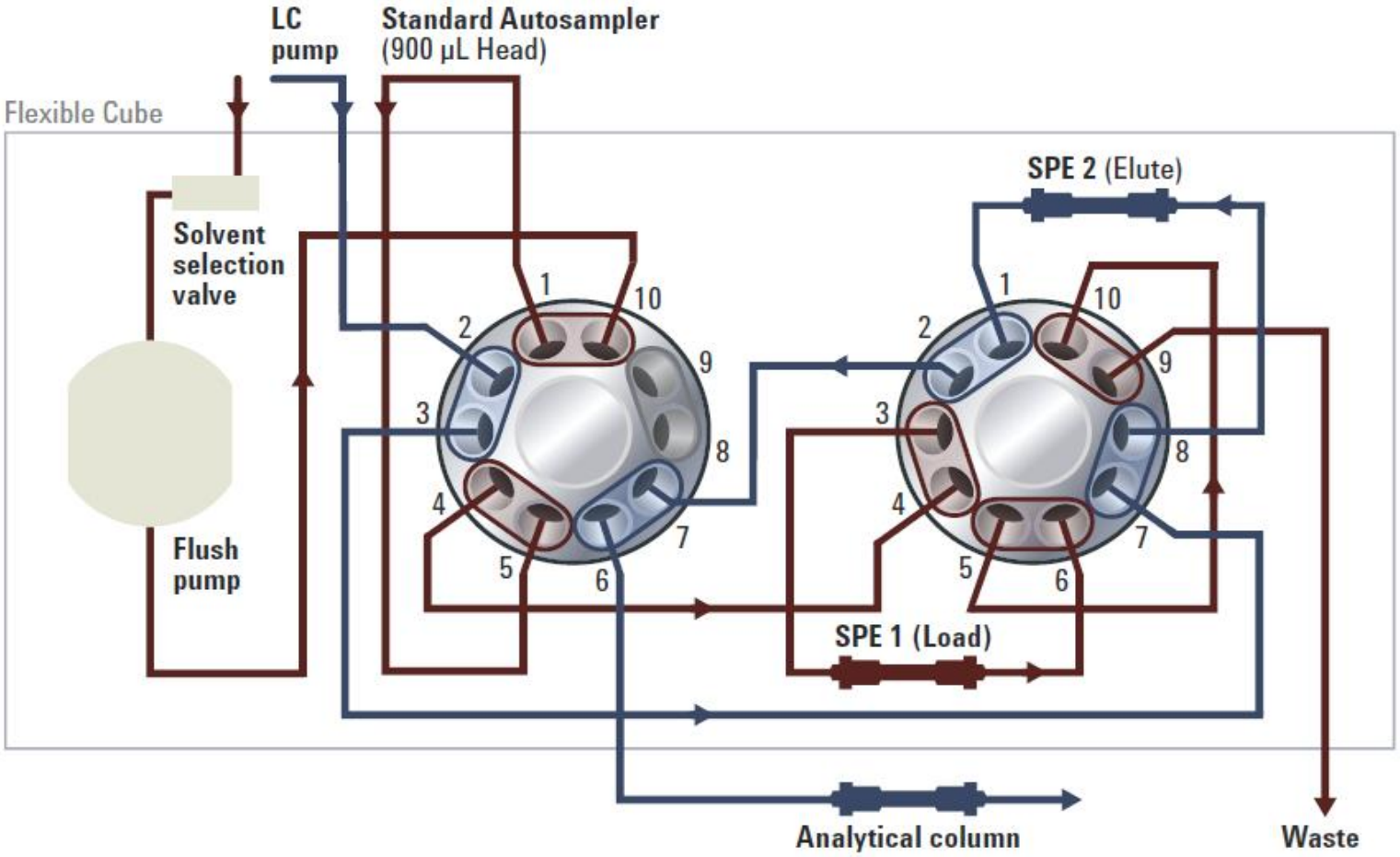
# Online SPE (Trace Enrichment-SPE)

- 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


Agilent

# Step 1: Online SPE1





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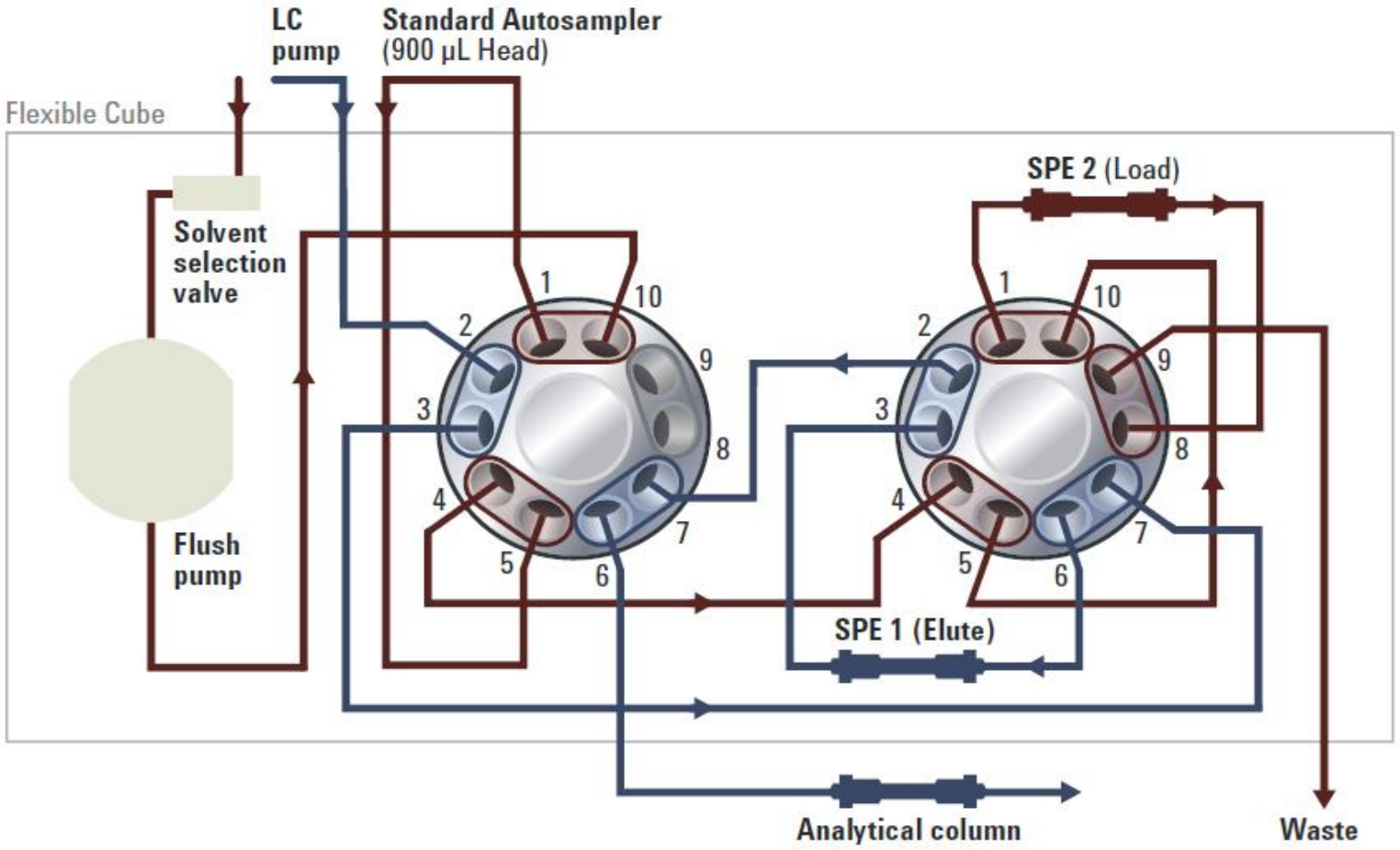
# Step 2: Online SPE2





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# Agilent Online SPE System

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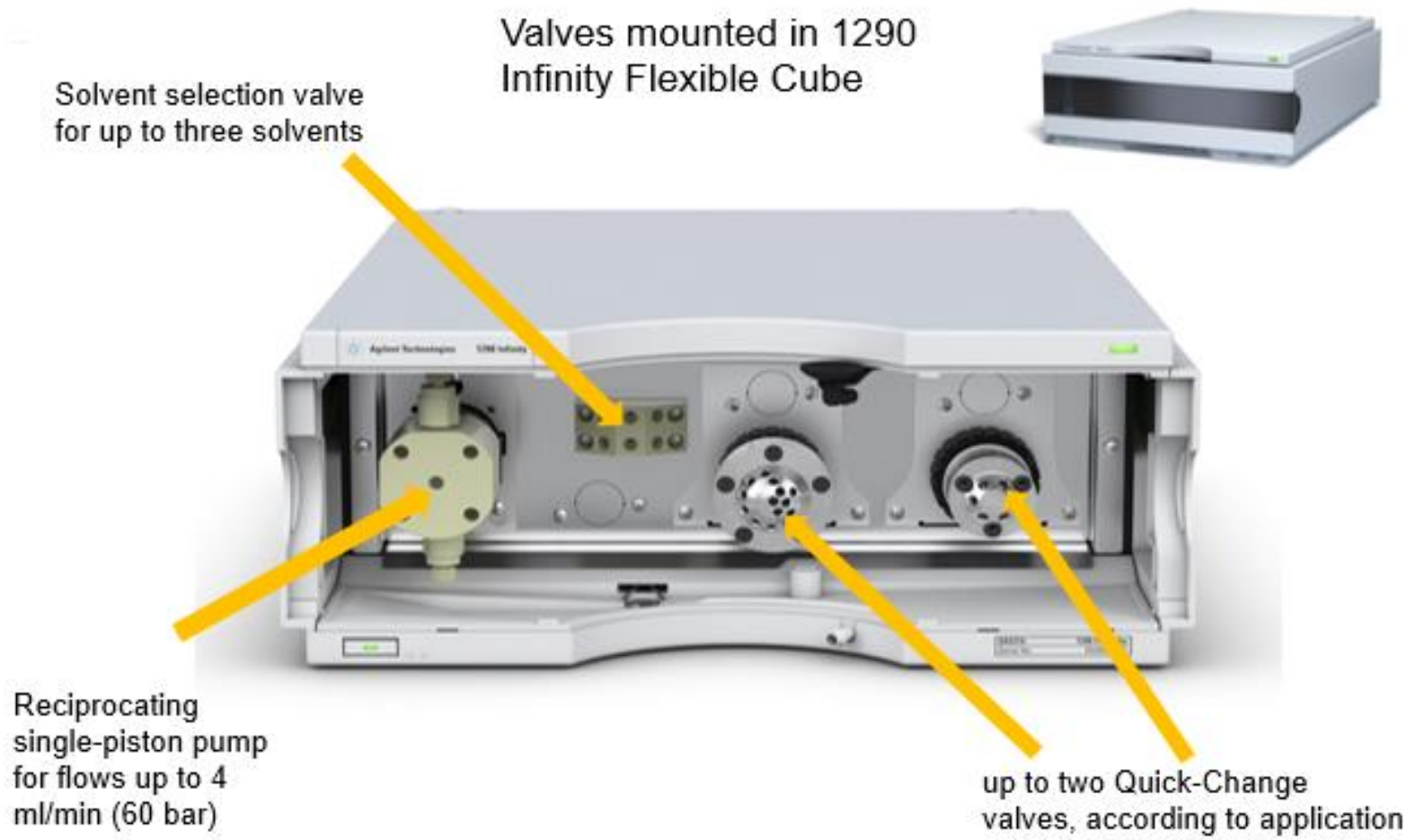
# Agilent 1200 Infinity Series Online SPE Solution-Flex Cube





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# Online SPE with Flex Cube

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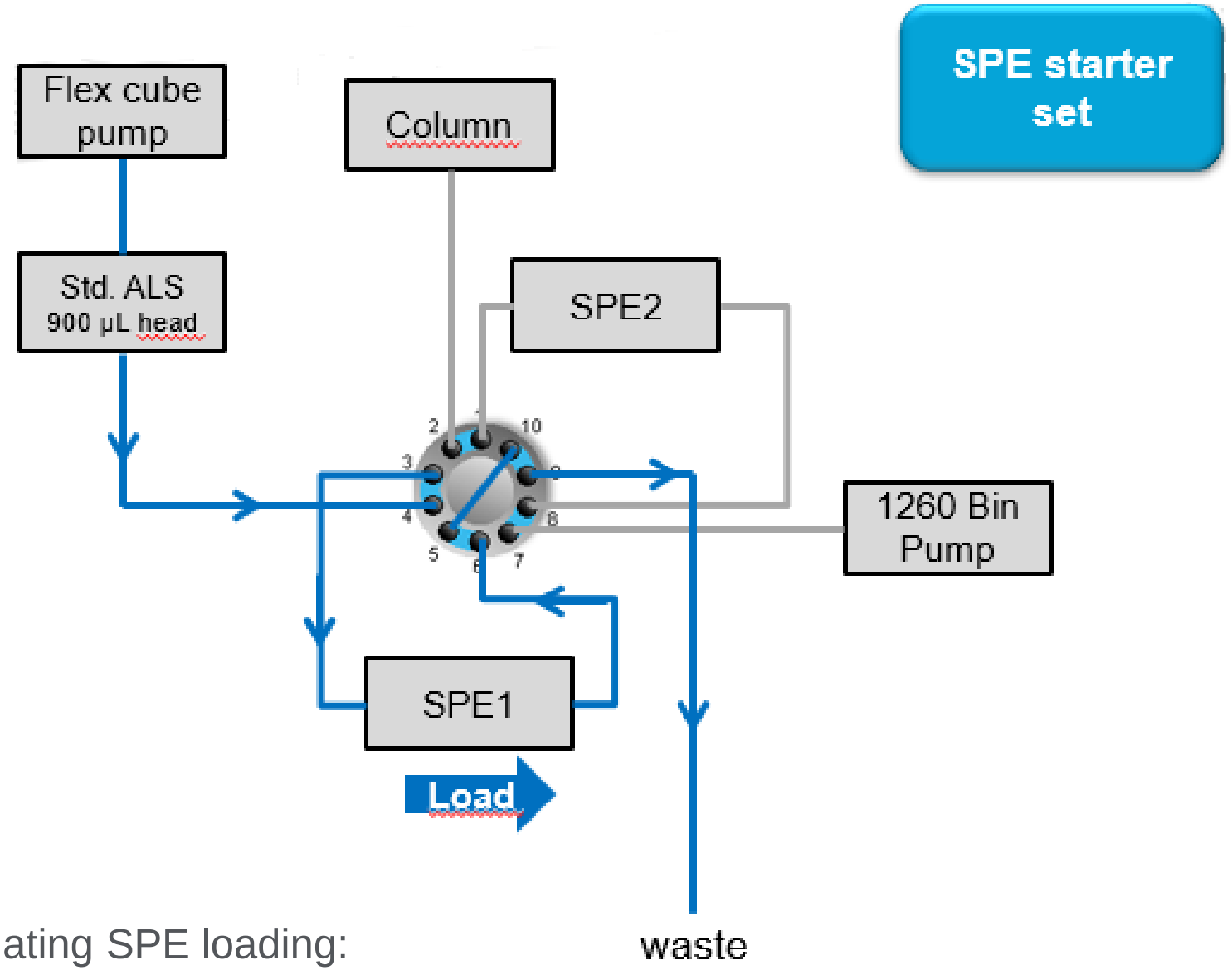
# Flex Cube




System with alternating SPE loading:
Load sample to SPE1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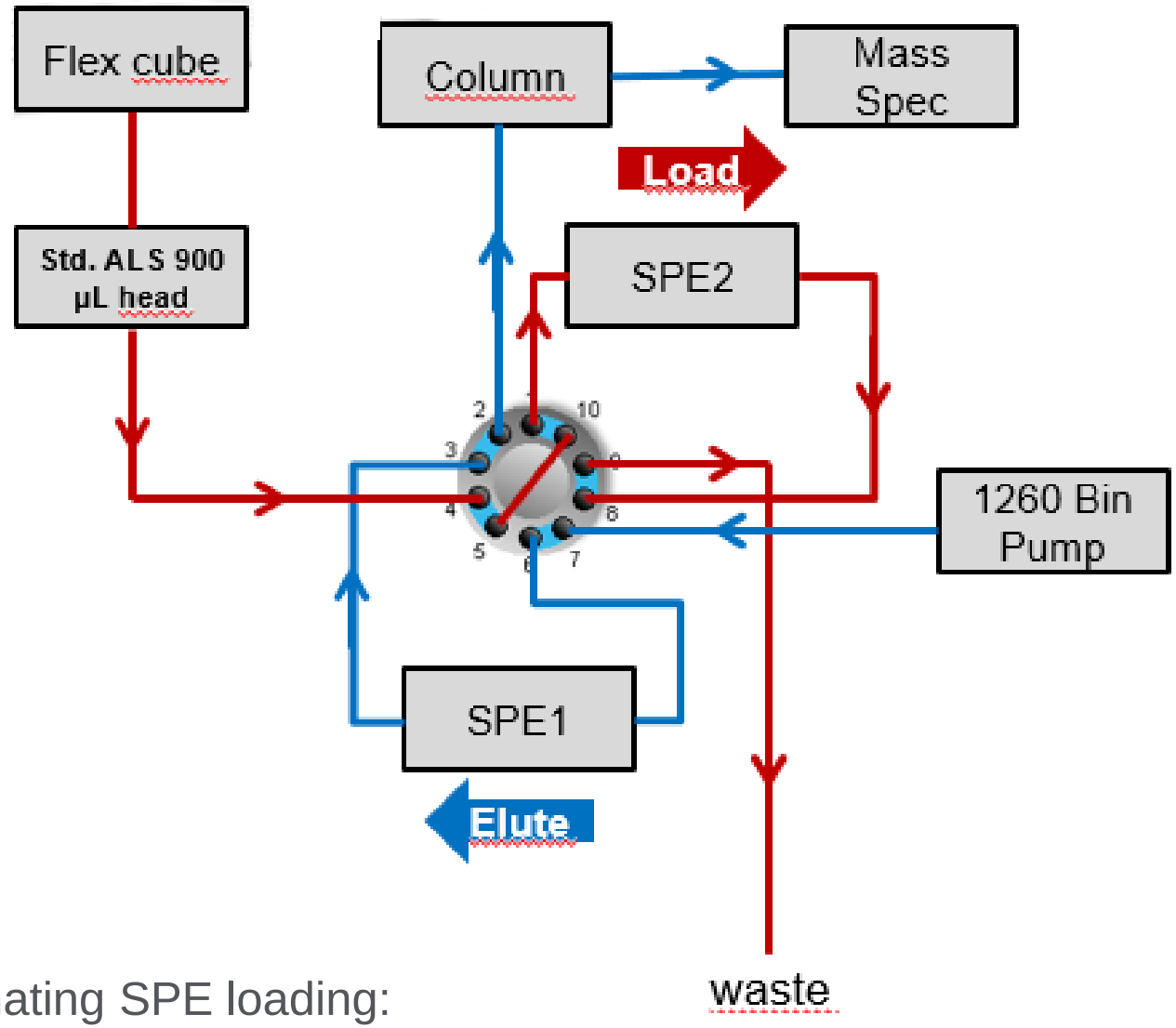
# Flex Cube




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