Tips, Tricks, and Tools for Selecting, Developing, and Implementing Simple and Successful Solid Phase Extraction Methods

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Today's Agenda

Solid phase extraction – Why SPE?

Tools for Selecting the Right SPE Method and Product

Developing SPE Methods – Applying SPE Theory to Ion Exchange Method Optimization

SPE Troubleshooting: Tips, and Tricks

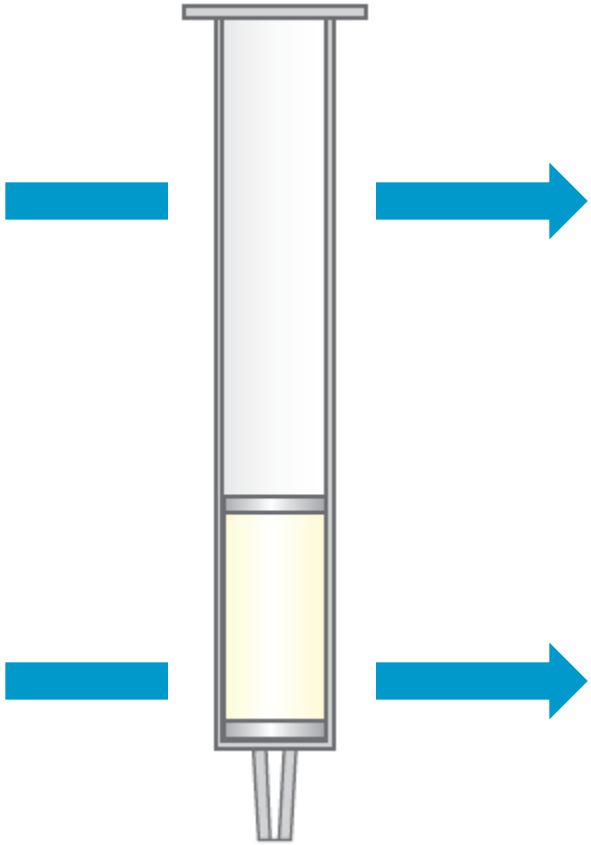
Questions and Wrap Up

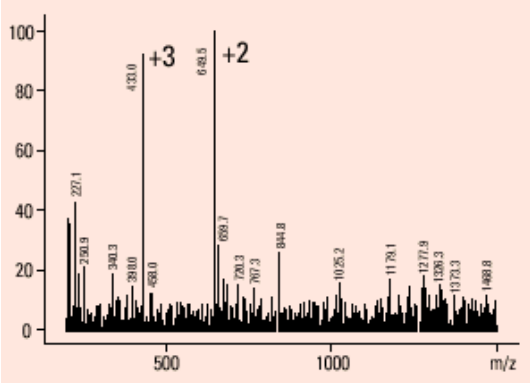
Solid Phase Extraction

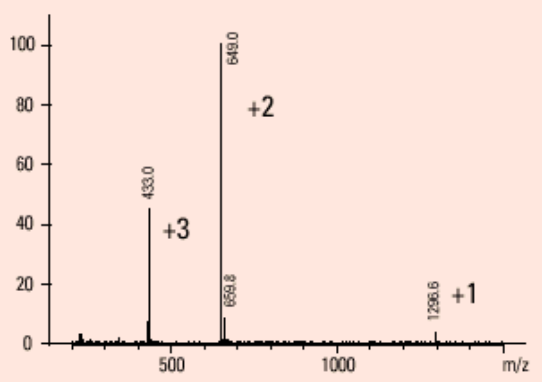
SELECTING THE RIGHT SPE PRODUCT – WHY SPE?

Why Do Solid Phase Extraction?

- ✓ Remove Interferences
- ✓ Concentrate Target Analytes
- ✓ Transform Sample



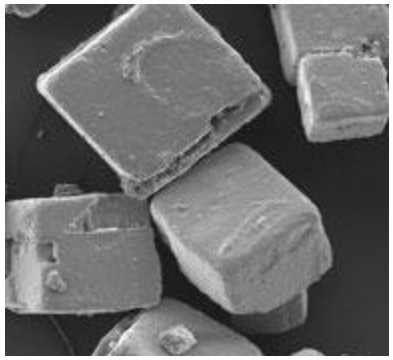


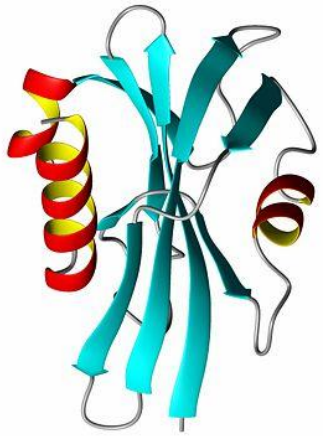


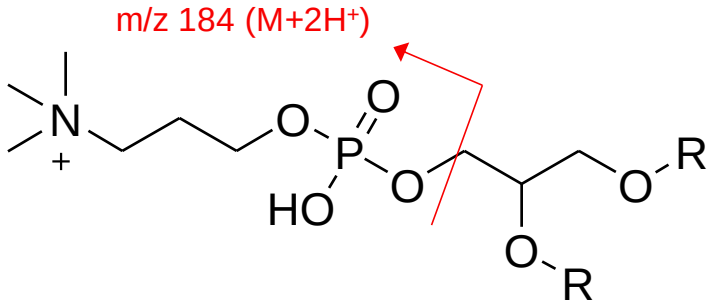
Goal 1: Removing Matrix Interference

Matrix interferences are caused by components of the matrix that result in a negative effect on sample analysis

- Proteins and peptides
- Salts
- Lipids and other hydrophobic species
- Pigments
- Cellular debris/components



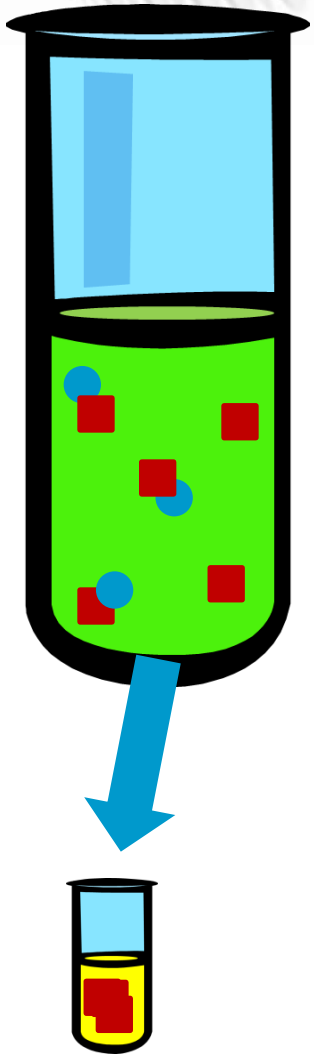




Goal 2: Concentrating Target Analytes

The concentration of target compounds in the sample may be very low (PPB, PPT levels), thus requiring concentration of target analytes to achieve required detection limits

- Pharmaceuticals in water
- Drugs of abuse in hair or oral fluids
- Organic contaminants in food



Goal 3: Transform Sample for Analysis

The sample type may not be compatible with the needs of your instrument or analytical system – you may need to transform it from solid to liquid, or get it into a solvent amenable with your LC or GC, or change from one type of solvent to another

- Wastewater or waste oil
- Food samples
- Blood or body fluids

Solid Phase Extraction

SELECTING THE RIGHT SPE METHOD AND PRODUCT

Four Steps to SPE

Conditioning: Preparation of the sorbent prior to sample addition

Load: Analytes of interest and other interferences adsorb onto the surface of the sorbent during sample addition

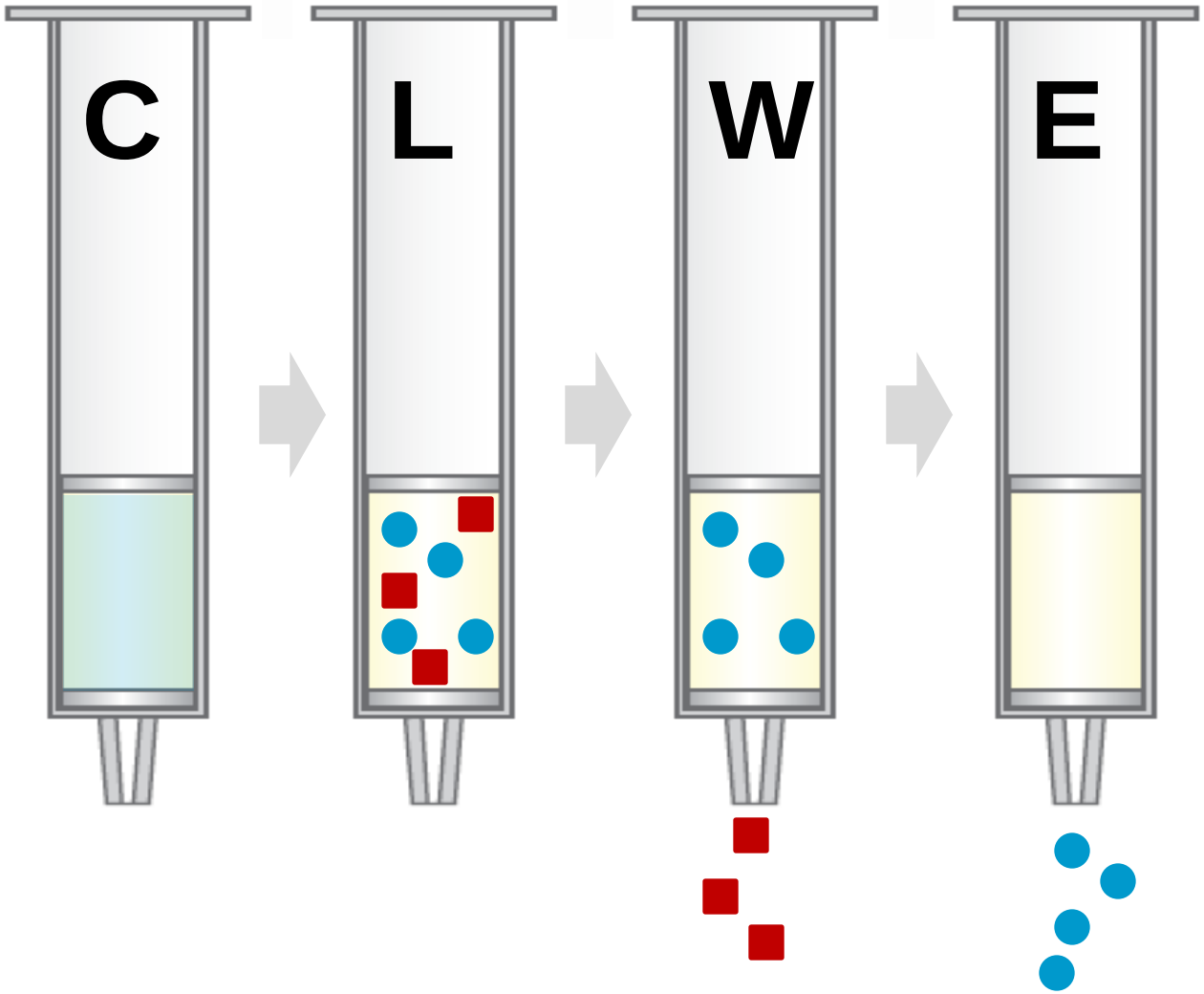
Washing: Elimination of undesired interferences

Elution: Selective desorption and collection of desired analytes from the sorbent/device

C → L → W → E

General SPE Workflow

Sample Pre-treatment



Select a Format that Meets Your Needs

Tubes

1 mL to 60 mL Straight Barrel (50mg –10g)

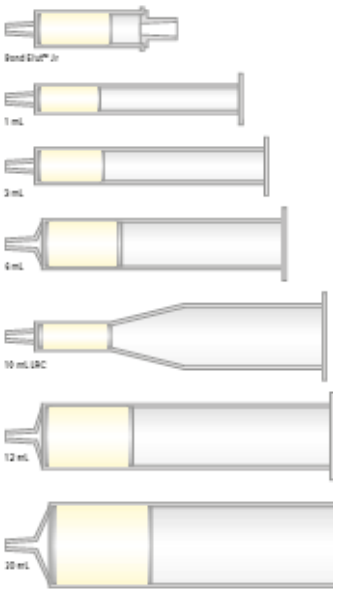
Bond Elut Jr (500mg –1g)

LRC (Large Reservoir Capacity)

(100-500mg)

Mega Bond Elut

12-150mL (2g-70g)



Multi-Array Well Plates

1 mL, 2 mL 96 Well

1.8 mL Versaplate

OMIX 96

SPEC 1 mL

Automation

Hamilton

TomTEC

Gilson ASPEC

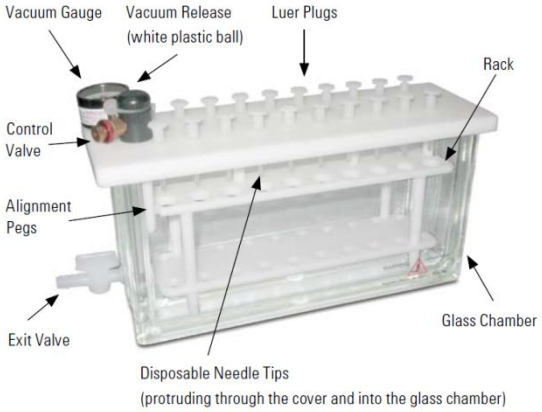
Gerstel

Spark Holland Prospekt

SPE Manifolds

VacElut 12, 20 and SPS 24

Vacuum manifolds for SPE barrels.

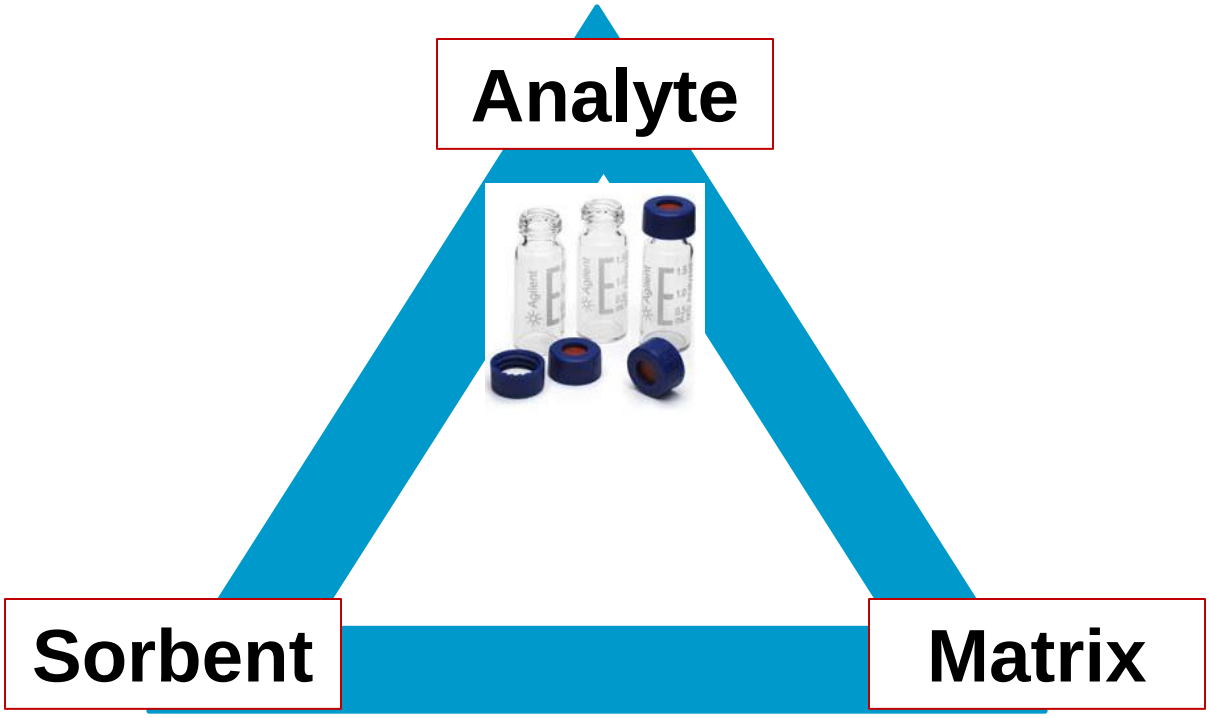


96 Well

CaptiVac Collar: For use with Bond Elut 96 1 mL and Captiva filtration plates

Sample Preparation Considerations

We often talk about a “triangle” – but the questions about sample prep and SPE are more complex than this simple model



Sample Prep Considerations

- ✓ Analytical goals
- ✓ Published methods
- ✓ Instrument availability
- ✓ Skill and expertise
- ✓ Regulations
- ✓ Sample Size
- ✓ Detection limits

Analyte Considerations

- Does the analyte appear to be polar or non polar (C, H, N, O)?
- Are the analytes soluble in the matrix (and eluent)?
- Do the analytes contain any ionic groups?
- Are the compounds unstable in acid or base?
- What is the method of derivatization (GC, LC)?
- What is the concentration of the analyte in the sample?

Solid Phase Extraction (SPE)

Silica-Based SPE

- Selectivity is gained by specific bonded chemistry
- >40 phases available
- Application-specific phases
- Wide range of published applications
- Method refinement or development may be required

Polymeric SPE

- Wide analyte selectivity
- Mixed mode functionality
- Generic methods, ease of use
- Potentially less optimization
- High capacity
- Greater pH range compatibility

Both approaches have advantages and disadvantages

Bond Elut SPE Phases Available

Non-polar

C18, C8, C2, C1
C18 variations in carbon load and endcapping
EnvirElut
CH – cyclohexyl
CN-E – endcapped cyano
PH – phenyl
ENV, LMS, PPL, Focus, Nexus
Plexa

Polar

PSA - primary and secondary amine
NH2 - aminopropyl
DEA - diethylaminopropyl
Diol
Si - silica

Cation Exchange

SCX – benzenesulfonic acid
PRS – propylsulfonic acid
CBA – carboxylic acid

Reversible Covalent

PBA – phenylboronic acid

Specialty Phases

AccuCAT
Atrazine
Mycotoxin

Anion Exchange

SAX – quaternary amine
PSA – primary and secondary amine
NH2 – aminopropyl
DEA – diethylaminopropyl

Mixed mode IEX/NP

Certify* – SCX/C8
Certify II* – SAX/C8
Plexa PCX
Plexa PAX

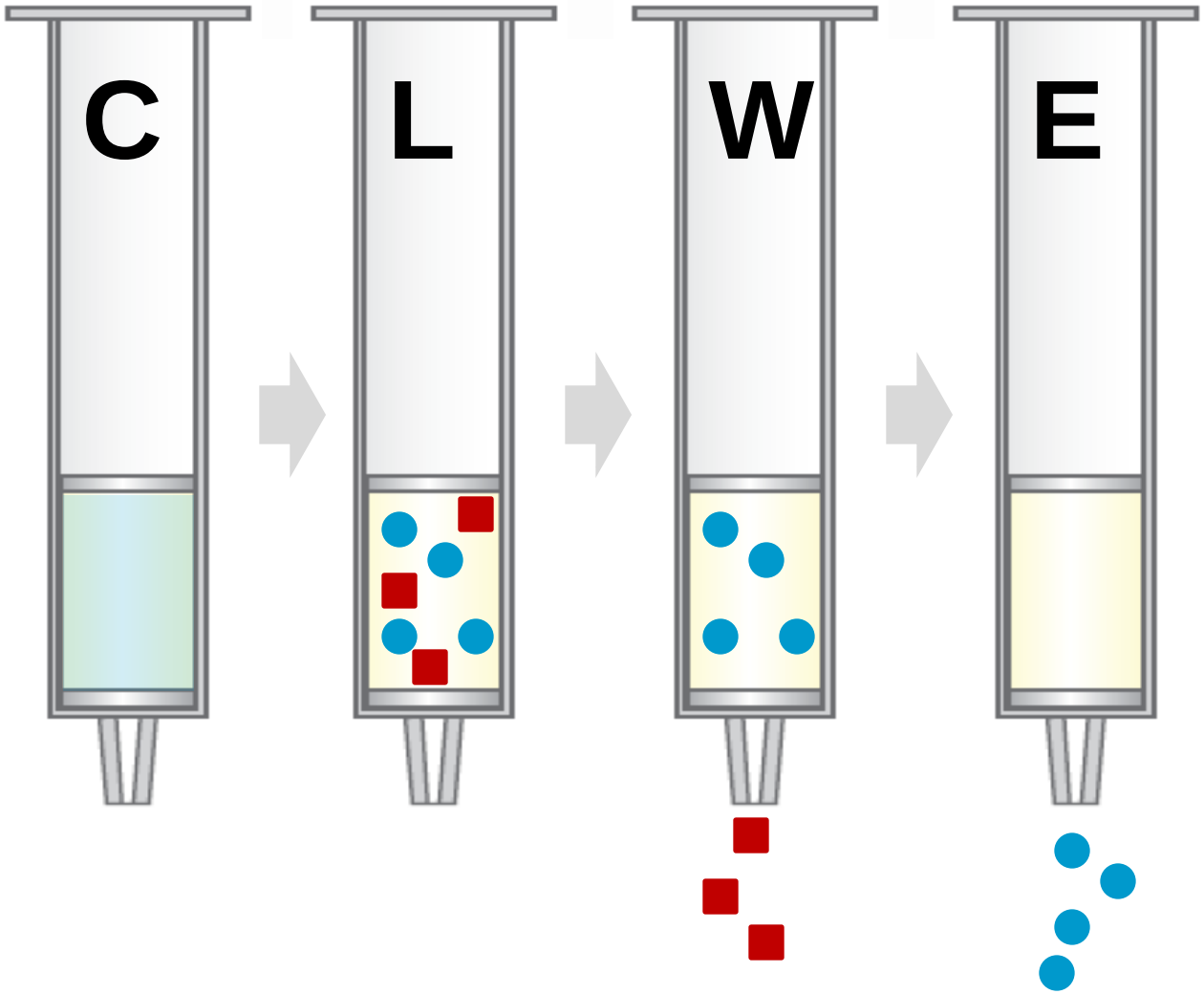
Alumina – aluminum oxide
Florisil – magnesium-silica
Carbon
Carbon/NH2

Solid Phase Extraction

METHOD DEVELOPMENT AND OPTIMIZATION

SPE Workflow

Sample Pre-treatment



Ionic Theory

There are general categories of ionic groups.

Strong:

Where the ionic group is always charged (positive or negative). Changing the pH will not typically affect the charged state

Weak:

Where the ionic group is charged or neutral. Changing pH will affect the charged state.

CATIONS: (+) Found in basic compounds. Amines are typically cationic

ANIONS: (-) Found in acidic compounds. Carboxylic acids are typically anionic

Ion Exchange Rule of 2

Retention: Where both groups are charged

Elution: Where one or both groups are neutral

With weak ion exchange, a 50% charge is not sufficient enough to give satisfactory recoveries.

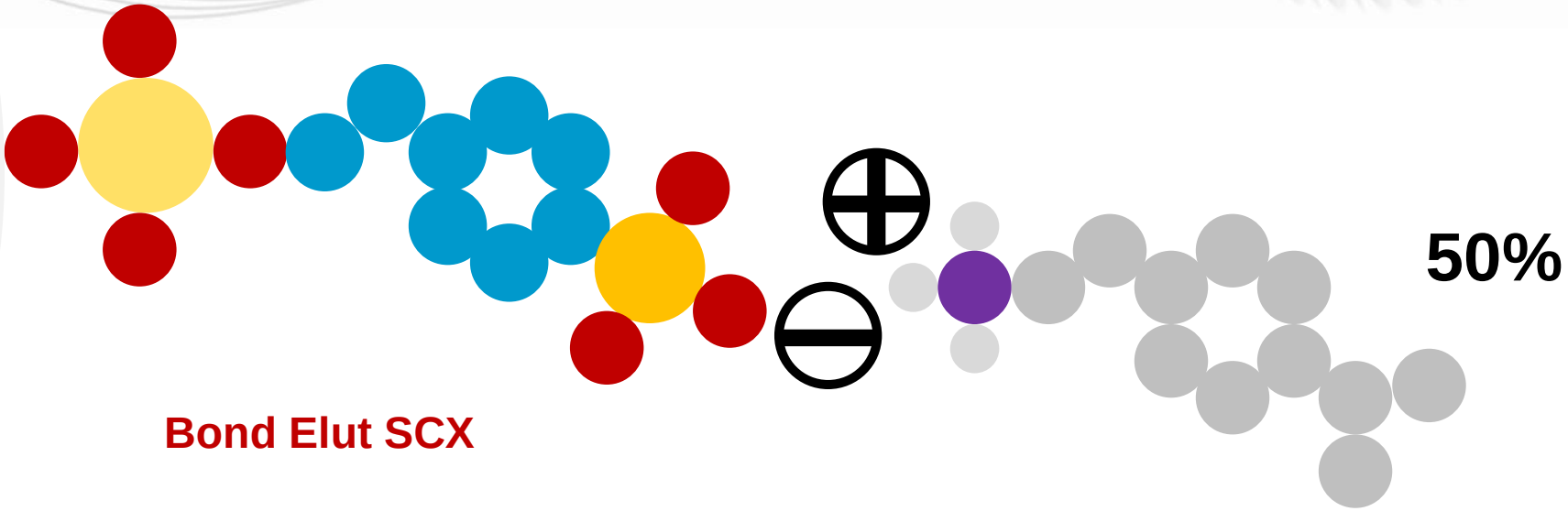
To render a compound 99.5% charged or 99.5% neutral. we must…

Raise or Lower

the pH by 2 units from the pKa

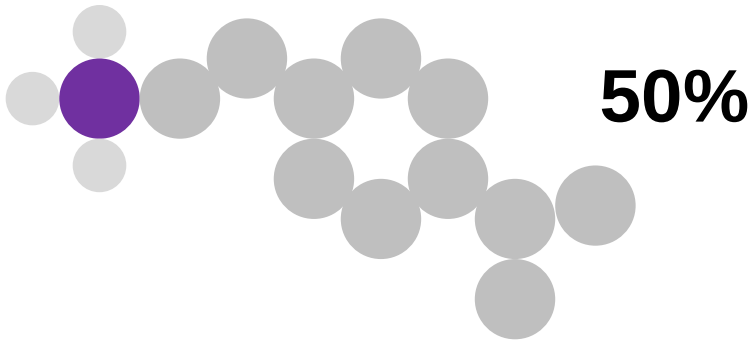
This is the Rule of 2, and it's useful in optimizing SPE

Interactions on Ion Exchange Sorbents: CX



Bond Elut SCX

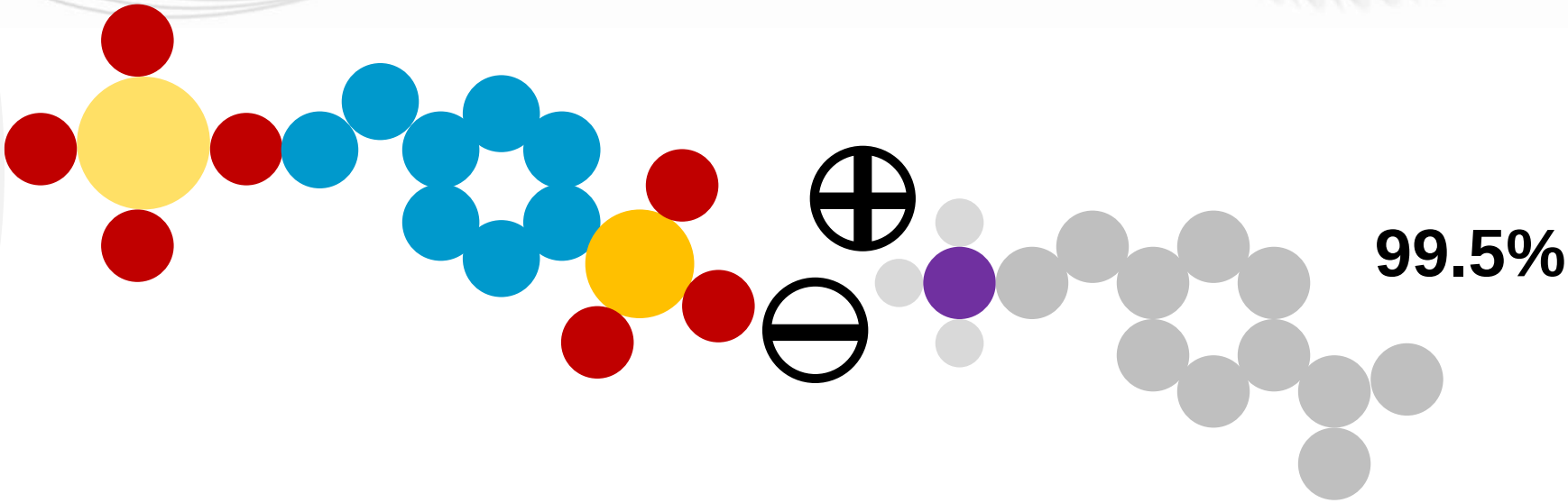
50%



50%

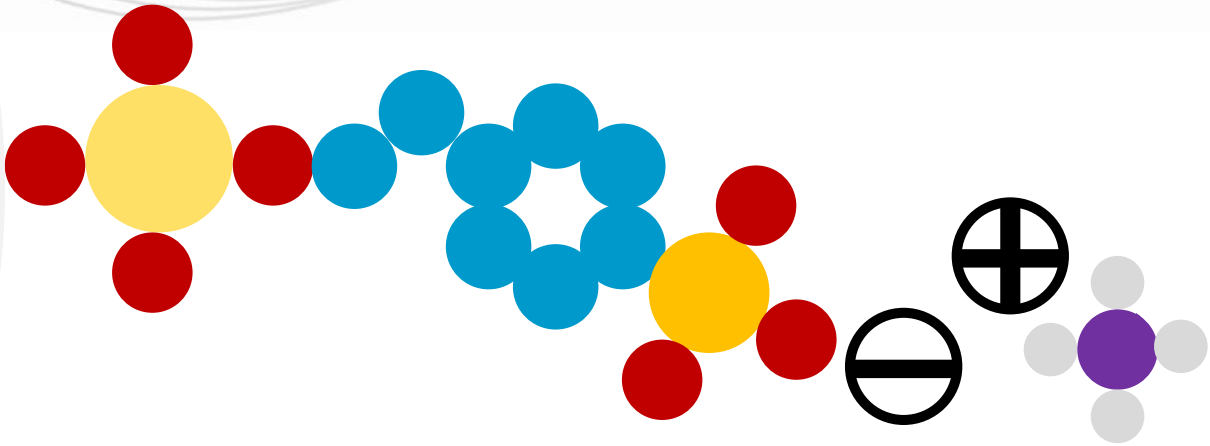
If the pKa is 9 and the pH is 9
then 50% of analyte will be bound

Ion Exchange: Retention Rule of 2

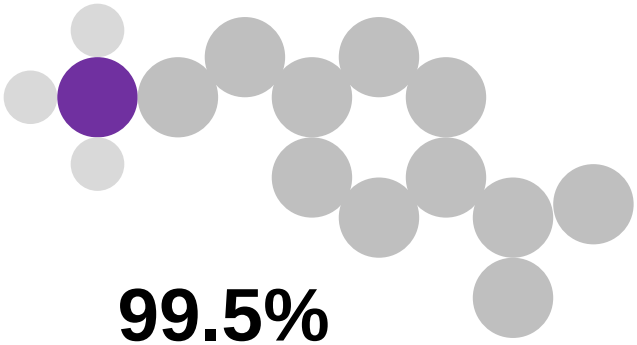


If the pKa is 9 and the pH is 7
then 99.5% of analyte will be charged and bound
Bond Elut SCX pKa << 1 (always charged)

Ion Exchange: Elution Rule of 2



If the pKa is 9 and the pH is 11
then 99.5% of analyte will be uncharged
(i.e. free base) and free to elute



99.5%

Ion Exchange Mechanisms – Overview

Mechanism		Functional group	Analyte	Phase
Weak Cation Exchanger	(WCX)	Carboxylic acid	Quat Amines	Bond Elut CBA
Weak Anion Exchanger	(WAX)	Basic amine	Sulfonic acids	Bond Elut PSA
Strong Cation Exchanger	(SCX)	Sulfonic acid	Basic amines	Bond Elut SCX, Plexa PCX
Strong Anion Exchanger	(SAX)	Quaternary amine	Carboxylic acids	Bond Elut SAX, Plexa PAX

- A Mechanism of 2 strong ionic species will result in inability to elute
- i.e. Eluting a sulfonic acid (pKa of -1) from Plexa PAX? **Rule of 2!**

Important Considerations for Ion Exchange

Ensure analyte <u>AND</u> sorbent are ionized

- acids: pH = pK_a + 2
- bases: pH = pK_a - 2 *or* (pK_b + 2)

Ionic strength

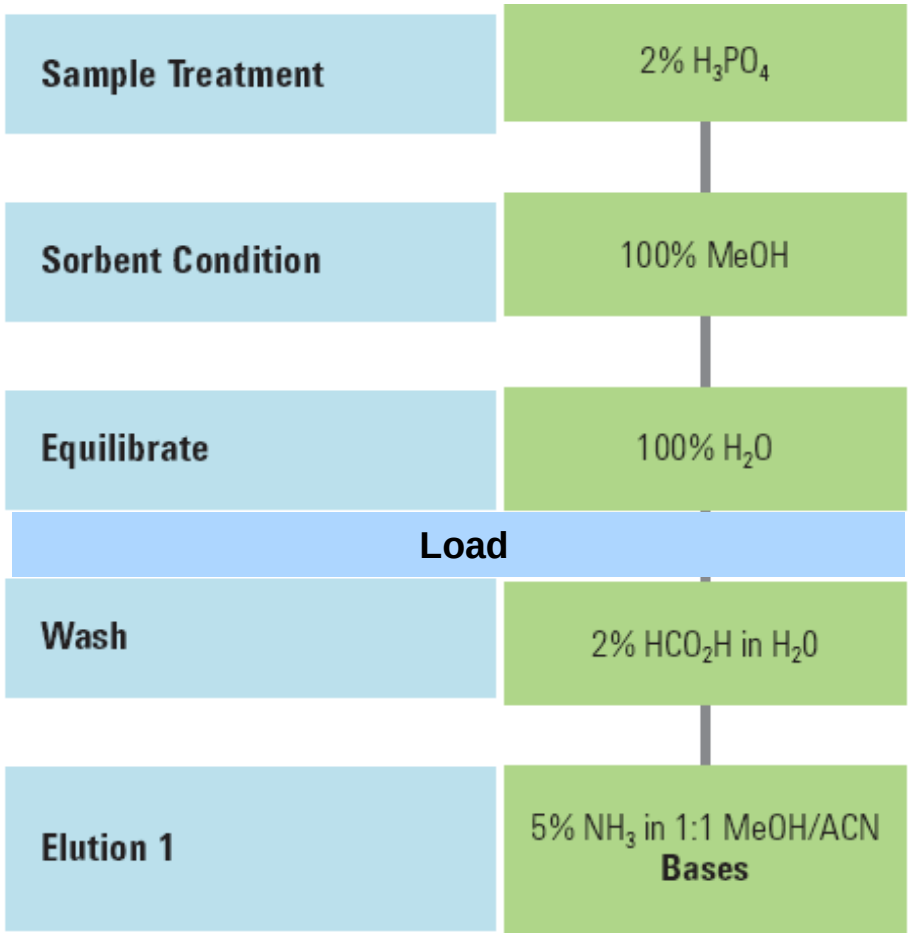
- low for retention
- high for elution

Capacity

- Typically <1 mmol/g

Flow Rate

- Slow the flow...



Ion Exchange SPE in Action: Fractionation Approach

Fractionation of **Acidic, Neutral, and Basic Forensic Drugs**

Bond Elut Plexa PCX 10 mg

Acids:
Atorvastatin, Diclofenac, Furosemide, Pravastatin

Neutrals:

Cortisone, Cortisol

Bases:

Procainamide, Metoprolol, Paroxetine

Sample pre-treatment:

100 µL human plasma

Dilute 1:3 with 2% H_3PO_4

Condition 1. 500 µL CH_3OH

2. 500 µL DI H_2O

Load Plasma 1:3 with 2% H_3PO_4

Wash 1 500 µL 2% Formic acid

Elute 1 500 µL AcN:MeOH (1:1, v:v)

Acids, Neutrals

Elute 2 500 µL 5% NH_3 in AcN;MeOH

Bases

Acids: Absolute Recovery

Analyte	% Rec 0.5 µg/mL	% RSD	% Rec 1µg/mL	% RSD
Diclofenac	101	4	101	5
Furosemide	99	3	96	2
Pravastatin	95	4	96	6
Atorvastatin	100	4	100	5

Neutrals: Absolute Recovery

Analyte	% Rec 0.5 µg/mL	%RSD	% Rec 1 µg/mL	% RSD
Cortisone	93	4	97	6
Cortisol	101	4	101	4

Recovery Data: Bases

Analyte	% Rec 0.5 µg/mL	% RSD	% Red 1 µg/mL	% RSD
Procainamide	100	5	98	3
Metoprolol	94	4	92	6
Paroxetine	94	5	99	4

Bases: Absolute Recovery

SPE Multi-Suite methods have excellent application opportunities

- Forensic Drugs confirmation
- Forensic Drug metabolism studies (where acid or neutral metabolites may need to be extracted using a different method)
- Multi-residue pesticide or veterinary drug analysis in food and beverage samples

Solid Phase Extraction

TROUBLESHOOTING: TIPS AND TRICKS

TIPS & TRICKS: SPE Trouble Shooting

From our global helpdesk logs, we have identified the most common SPE issues encountered by our end users.

All of these issues can be linked to simple practical laboratory errors

1. **Low Recovery**
2. **Poor Flow**
3. **Loss of Analyte**
4. **Dirty Extracts**

TIP: Sorbent Conditioning

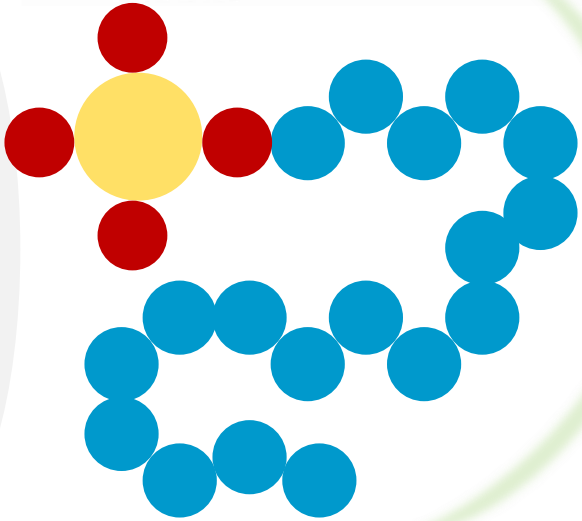
Sorbent conditioning is *usually* vital for good SPE performance

- An unconditioned sorbent bed can result in **Poor Flow** and **Poor Recovery**
- Precipitation of sample or ‘clogging’ can occur with complex samples
- Channelling and or sample breakthrough can be observed

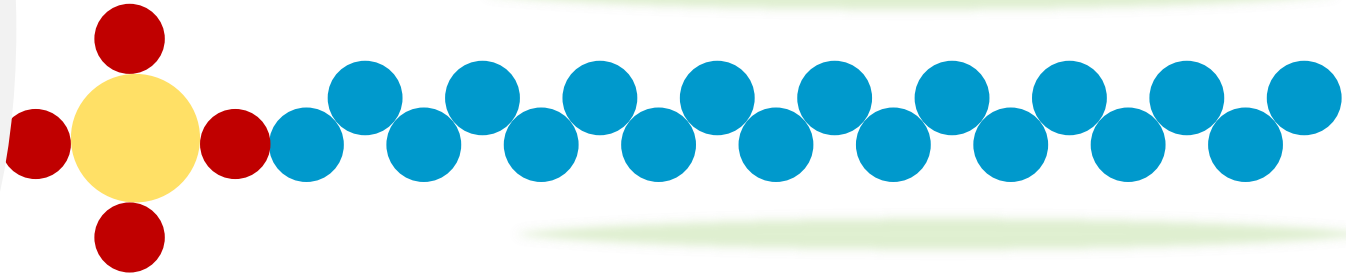
Practical Recommendations

Silica SPE	Polymeric SPE
>2 bed volumes of low viscosity organic solvent (e.g. MeOH) then Aqueous equilibration if method dictates.	>2 bed volumes of low viscosity organic solvent (e.g. MeOH)
Condition slowly under low vacuum <2” Hg	Wash with aqueous solution prior to sample load
Allow time for equilibration (30-60s)	More tolerant to accidental drying

Why is Conditioning Important?



Phase collapse minimizes analyte interaction



However…

TIP: Sorbent Conditioning

Reducing the number of conditioning steps can shorten the process

- Investigate which conditioning steps can be removed without affecting results in terms of recovery or reproducibility
- Explore SPE options that don't require conditioning or that may require fewer conditioning steps – i.e. SPEC or polymeric SPE
- Look for breakthrough when evaluating simplification of SPE processes

SPEC C18AR	Bond Elut C18	Plexa
1. Organic solvent 2. Water or buffer 3. Sample application 4. Water or buffer 5. Elute	1. Organic solvent 2. Water 3. Buffer 4. Sample application 5. Water or buffer 6. Organic solvent 7. Elute	1. Organic solvent 2. Water or buffer 3. Sample application 4. Water or buffer 5. Elute

TIP: Poor Recovery - Capacity

Understanding the capacity of your SPE phase is critical

Sorbent Type	Capacity
Silica (Polar or Non-Polar)	1-4% of bed mass
Silica (Ion Exchange)	Typically < 1.0 mmol/g
Polymeric	~10 -12% of bed mass

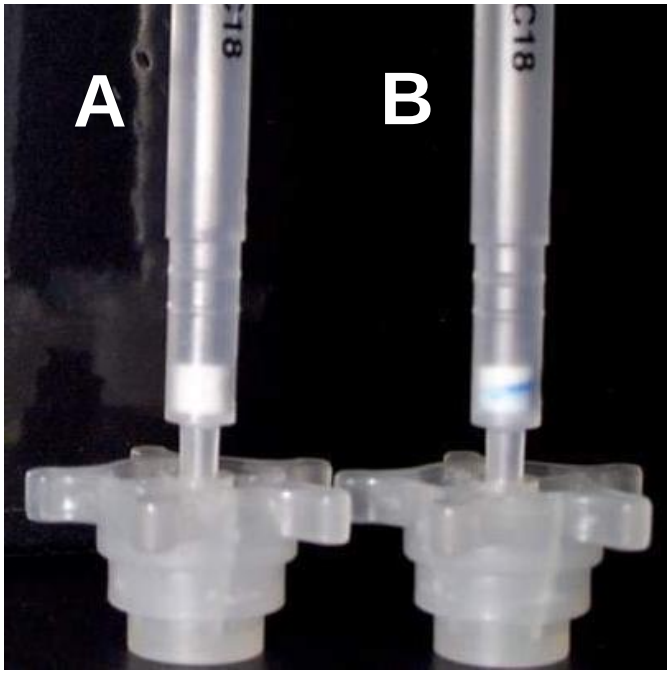
- Capacity listings assume good analyte/sorbent interaction – this does not always happen
- **Capacity does not distinguish between analyte and interference! – capacity is limited by the sum of analytes + interferences**
  - Do you know amounts of interferences present?

Suggestion: Stack two SPE cartridges on top of each other to determine if capacity is an issue

TIP: Poor Recovery - Elution

Elution volume optimization can positively impact recovery

- **Elution volumes can be minimized and recovery maximized if elution is done in aliquots.**
- Four aliquots of 50 µL give better results than 200 µL in a single shot
- 2 x 100 µL is a more practical solution
- Using mixtures of solvents can also have a positive effect on recovery (i.e. 50:50 MeOH/ACN)



A: 4 x 50 µL Aliquots
B: 1 x 200 µL Aliquot

TIP: Dirty Extracts

Dirty Extracts are common in un-refined SPE methods

- Phase may be too universal (e.g. C18)
- Extraction scheme is not specific enough
- Try polymeric or mixed mode SPE
- **Reduce** concentration of **Elution** solvent
- **Wash** step is ineffective, **Increase** organic concentration
- Monitor for matrix effects

TIP: Flow Rates

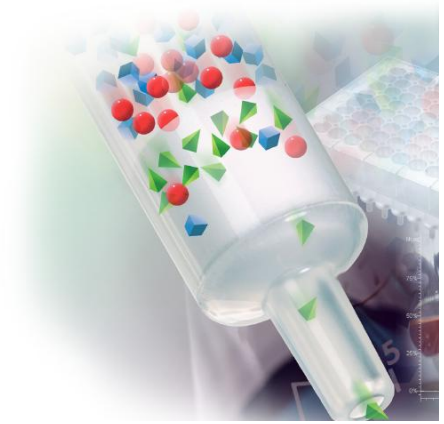
For many types of SPE, the flow rate is incredibly important

Conditioning	• Ensure low to no vacuum, particularly on silica SPE, to fully condition sorbent bed • Avoid sorbent bed drying with silica SPE
Load	• Monitor sample flow rates through the SPE column • Use gravity or very low vacuum to give sample residence time in the sorbent
Elute	• Elute under vacuum where possible • For some applications, allowing the elution solvent to absorb in the bed fully before eluting may improve recoveries

TRICK: If In Doubt … Think Mass Balance!

If you load ???ng of a sample onto a cartridge, tracing where it goes helps determine important method alterations.

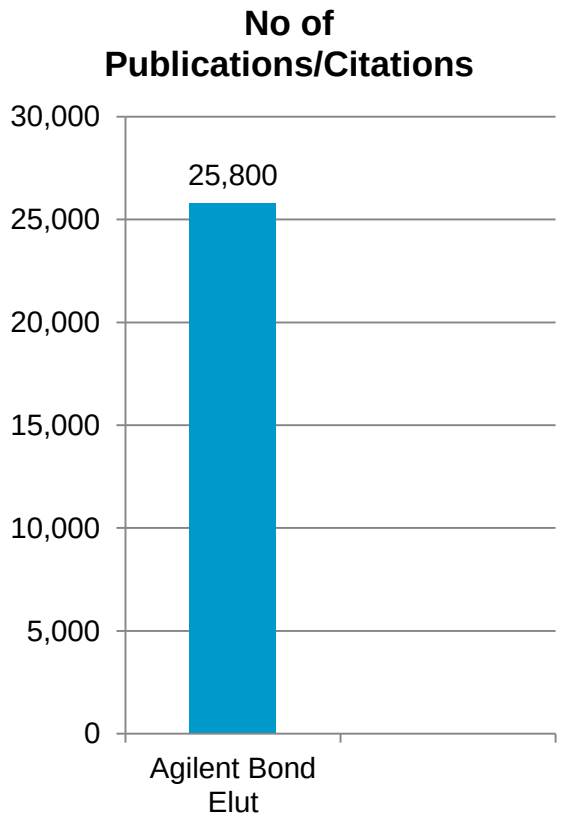
1. Collect “effluent” that has passed through upon sample loading
2. Collect all wash steps
3. Collect all elution steps



Easy to know if you still have analyte on the phase

TRICK: See How Others Use Bond Elut SPE

- www.agilent.com
- ScanView database: search applications by analyte, matrix or technique
- Vast number of external publications & citations
- Agilent sample prep methods explicitly mentioned in many methods (EPA, EN, AOAC, FDA, DIN etc..)



Searches conducted on Sept 14th 2012 using Google Scholar (US Page)

Conclusions

- A robust SPE method offers the highest level of cleanliness and selectivity in sample preparation
- SPE workflows are simple and can be easily integrated into a lab environment
- SPE can be viewed as ‘digital’ chromatography
- Silica SPE devices offer a broad range of analyte selectivity based on surface chemistry
- Polymeric SPE offers a more generic window of retention based on background properties of the polymer itself
- SPE can overcome analytical challenges in terms of multi-suite fractionation, concentration and complex sample clean up

Total Solutions from Extraction To Detection

www.chem.agilent.com

Technical Support – Sample Preparation Products

Technical Support*:

Spp-support@agilent.com

800-227-9770, options 3, 3, 3

* *(North America)*

Questions?

Reference: Sample Prep Terminology

Analyte(s): Molecule(s) of interest

Matrix: The sample (soil, groundwater, blood, saliva)

Interferences: Entities inside the sample which may inhibit analysis of desired analyte

IS or ITSD: Internal standard

LLE: Liquid-liquid extraction **SLE:** Supported-liquid extraction

SPE: Solid phase extraction

LC (MS): Liquid chromatography (mass spectroscopy)

GC (MS): Gas chromatography (mass spectroscopy)

LOQ: Limit of Quantification **LLOQ**: Lower limit of quantification

RSD: Relative standard deviation **CV**: Coefficient Variation

Thank you