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

Doug Hanson
Agilent Technologies Inc.
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

![Chemical Structure](image)
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
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.
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

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18, C8, C2, C1</td>
<td>C18 variations in carbon load and endcapping</td>
</tr>
</tbody>
</table>

#### Polar

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>primary and secondary amine</td>
</tr>
<tr>
<td>NH2</td>
<td>aminopropyl</td>
</tr>
<tr>
<td>DEA</td>
<td>diethylaminopropyl</td>
</tr>
<tr>
<td>Diol</td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>silica</td>
</tr>
</tbody>
</table>

#### Cation Exchange

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCX</td>
<td>benzenesulfonic acid</td>
</tr>
<tr>
<td>PRS</td>
<td>propylsulfonic acid</td>
</tr>
<tr>
<td>CBA</td>
<td>carboxylic acid</td>
</tr>
</tbody>
</table>

#### Reversible Covalent

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBA</td>
<td>phenylboronic acid</td>
</tr>
</tbody>
</table>

#### Anion Exchange

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAX</td>
<td>quaternary amine</td>
</tr>
<tr>
<td>PSA</td>
<td>primary and secondary amine</td>
</tr>
<tr>
<td>NH2</td>
<td>aminopropyl</td>
</tr>
<tr>
<td>DEA</td>
<td>diethylaminopropyl</td>
</tr>
</tbody>
</table>

#### Mixed mode IEX/NP

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certify*</td>
<td>SCX/C8</td>
</tr>
<tr>
<td>Certify II*</td>
<td>SAX/C8</td>
</tr>
<tr>
<td>Plexa PCX</td>
<td></td>
</tr>
<tr>
<td>Plexa PAX</td>
<td></td>
</tr>
</tbody>
</table>

#### Specialty Phases

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuCAT</td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td></td>
</tr>
<tr>
<td>Mycotoxin</td>
<td></td>
</tr>
</tbody>
</table>

### Alumina

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alumina</td>
<td>aluminum oxide</td>
</tr>
<tr>
<td>Florisil</td>
<td>magnesium-silica</td>
</tr>
<tr>
<td>Carbon</td>
<td></td>
</tr>
<tr>
<td>Carbon/NH2</td>
<td></td>
</tr>
</tbody>
</table>
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

If the pKa is 9 and the pH is 9 then 50% of analyte will be bound
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)
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
Ion Exchange Mechanisms – Overview

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Functional group</th>
<th>Analyte</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak Cation Exchanger</td>
<td>(WCX)</td>
<td>Carboxylic acid</td>
<td>Quat Amines</td>
</tr>
<tr>
<td>Weak Anion Exchanger</td>
<td>(WAX)</td>
<td>Basic amine</td>
<td>Sulfonic acids</td>
</tr>
<tr>
<td>Strong Cation Exchanger</td>
<td>(SCX)</td>
<td>Sulfonic acid</td>
<td>Basic amines</td>
</tr>
<tr>
<td>Strong Anion Exchanger</td>
<td>(SAX)</td>
<td>Quaternary amine</td>
<td>Carboxylic acids</td>
</tr>
</tbody>
</table>

- 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 AND 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% H₃PO₄

Condition
1. 500 μL CH₃OH
2. 500 μL DI H₂O

Load
Plasma 1:3 with 2% H₃PO₄

Wash 1
500 μL 2% Formic acid

Elute 1
500 μL AcN:MeOH (1:1, v:v)

Acids, Neutrals

Elute 2
500 μL 5% NH₃ in AcN;MeOH

Bases
### Acids: Absolute Recovery

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Rec 0.5 µg/mL</th>
<th>% RSD</th>
<th>% Rec 1µg/mL</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac</td>
<td>101</td>
<td>4</td>
<td>101</td>
<td>5</td>
</tr>
<tr>
<td>Furosemide</td>
<td>99</td>
<td>3</td>
<td>96</td>
<td>2</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>95</td>
<td>4</td>
<td>96</td>
<td>6</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

### Neutrals: Absolute Recovery

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Rec 0.5 µg/mL</th>
<th>%RSD</th>
<th>% Rec 1 µg/mL</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisone</td>
<td>93</td>
<td>4</td>
<td>97</td>
<td>6</td>
</tr>
<tr>
<td>Cortisol</td>
<td>101</td>
<td>4</td>
<td>101</td>
<td>4</td>
</tr>
</tbody>
</table>
Recovery Data: Bases

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Rec 0.5 µg/mL</th>
<th>% RSD</th>
<th>% Red 1 µg/mL</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procainamide</td>
<td>100</td>
<td>5</td>
<td>98</td>
<td>3</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>94</td>
<td>4</td>
<td>92</td>
<td>6</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>94</td>
<td>5</td>
<td>99</td>
<td>4</td>
</tr>
</tbody>
</table>

**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
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

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

<table>
<thead>
<tr>
<th>SPEC C18AR</th>
<th>Bond Elut C18</th>
<th>Plexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Water or buffer</td>
<td>2. Water</td>
<td>2. Water or buffer</td>
</tr>
<tr>
<td>5. Elute</td>
<td>5. Water or buffer</td>
<td>5. Elute</td>
</tr>
<tr>
<td></td>
<td>6. Organic solvent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. Elute</td>
<td></td>
</tr>
</tbody>
</table>
TIP: Poor Recovery - Capacity

Understanding the capacity of your SPE phase is critical

- 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?

<table>
<thead>
<tr>
<th>Sorbent Type</th>
<th>Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica (Polar or Non-Polar)</td>
<td>1-4% of bed mass</td>
</tr>
<tr>
<td>Silica (Ion Exchange)</td>
<td>Typically &lt; 1.0 mmol/g</td>
</tr>
<tr>
<td>Polymeric</td>
<td>~10 -12% of bed mass</td>
</tr>
</tbody>
</table>

**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
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](http://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?
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