Advances in Method Development for SPE

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Welcome

Goals

• Review the basics of solid-phase extraction (SPE)
• Discuss the elements of a successful method development
• Familiarize one to polymeric-based resins
• Basic understanding of:
  - SPE experiment
  - Reversed phase polymeric resin SPE
  - Ion-exchange polymeric resin SPE
• Method development for a given resin, tips and tricks
# Trends in SPE and Sample Preparation

<table>
<thead>
<tr>
<th>TREND</th>
<th>IMPLICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller Samples</td>
<td>Lower bed mass, less solvent, faster results, less evaporation time</td>
</tr>
<tr>
<td>Tandem MS (GC/LC)</td>
<td>“Just enough” sample prep (e.g. protein crashing instead of SPE; QuEChERS)</td>
</tr>
<tr>
<td>- Selectivity</td>
<td>Cuts down on # of sample prep steps (e.g. less error, faster, higher recovery)</td>
</tr>
<tr>
<td>- Sensitivity</td>
<td></td>
</tr>
<tr>
<td>High Throughput/Automation</td>
<td>Seamless integration to analysis</td>
</tr>
<tr>
<td></td>
<td>Favors different formats more attuned to automation (e.g. pipette tips, 96-well plates)</td>
</tr>
<tr>
<td>Polymeric SPE</td>
<td>Higher capacity, more rugged phases, mixed mechanisms</td>
</tr>
<tr>
<td>Selective Phases</td>
<td>For use with UV or Fluorescence detection (e.g. MIPs, Immunoaffinity)</td>
</tr>
</tbody>
</table>
Typical Sample Preparation Workflow: Trace Analysis of Compounds in Biological Specimens

1. Biological Specimen
2. Homogenize and spike with internal standard
3. Clarify by centrifugation/filtration
4. Solvent exchange to aqueous-compatible solution
5. SPE
6. Optimize RPC chromatography and MS/MS
7. Mass spec analysis
8. Quantitative analysis
SPE Modes—”Digital Chromatography”

**Analyte Adsorption (Bind-Elute)**
- Analyte(s) retained \( (K_D \gg 1) \)
- Matrix unretained \( (K_D \sim 0) \)
- and/or strongly retained \( (K_D \gg 1) \)

Preconcentration factor
Cleaner extracts
Load at 1-3 drops/sec (recovery \( \propto 1/\text{flow} \))
Capacity issues may be more important

**Matrix Adsorption (Interference Removal)**
- Analyte(s) unretained \( (K_D \sim 0) \)
- Matrix retained \( (K_D \gg 1) \)

No preconcentration advantage
Eluates may not be as clean
Sample loading often gravity fed
Used less often than analyte adsorption

= Analyte of interest
= Matrix/Interferences

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Systematic SPE Method Development

1. Select SPE mode, phase & format
2. Condition SPE sorbent
3. Select & optimize loading solvent
4. Select & optimize wash (rinse) solvent
5. Select & optimize elution solvent
6. Evaluate analyte purity, recovery, & reproducibility
7. Incorporate sample matrix & troubleshoot method
# Fundamental Steps for “Bind-Elute” SPE

<table>
<thead>
<tr>
<th><strong>Prewash</strong>*</th>
<th><strong>Precondition</strong></th>
<th><strong>Load</strong></th>
<th><strong>Wash</strong></th>
<th><strong>Elute</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Remove contaminants that could elute with analyte</td>
<td>Prepare cartridge to accept sample</td>
<td>Load sample and rinse reservoir(s)</td>
<td>Wash with solvent that won’t elute analyte</td>
<td>Elute analyte in smallest volume possible</td>
</tr>
</tbody>
</table>

If elution solvent will be stronger than precond. solvent

1. MeOH or ACN
2. Weak solvent (water, buffer)

Weakly retained matrix compds elute

Analyte and other matrix compds retained

Elute analyte leaving highly retained compds

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Optimizing Steps for the ‘Bind-Elute’ SPE Experiment
(non-polar example)

1. **Conditioning**: Solvent is passed through the SPE material to **wet** the bonded functional groups => ensures consistent interaction. (Use methanol)

2. **Equilibration**: Sorbent/phase is treated with a solution that is similar (in polarity, pH, etc.) to the sample matrix => maximizes retention. (Use water or the same aqueous solution that the sample is prepared in).

3. **Sample Load**: Introduction of the sample = analytes of interest are bound/extracted onto the phase/sorbent. Must be an aqueous solvent (no organic)

4. **Washing**: Use the ‘strongest’ aqueous solution that will NOT elute target compounds. Increasing the % organic, increasing or decreasing the pH, changing the ionic strength are all tips for optimizing clean-up. Dry the cartridge to remove all water.

5. **Elution**: Use the smallest volume of and weakest organic solvent that will elute ALL of the target analyte. As a general rule the ‘strength’ of the solvent is directly related to the target compound. Polar target compounds elute best in polar solvents so in order of polarity try: methanol>acetonitrile>ethyl acetate>acetone>THF. Modify the pH, increase the ionic strength.

6. **Solvent exchange**: If the subsequent analysis is HPLC, the organic elution solvent should be evaporated and the sample reconstituted in initial HPLC mobile phase. If the analysis is GC, then methanol or other GC-compatible solvent is used as the reconstitution solvent. In all cases the reconstitution must be to the same volume.
Constraints Imposed Upon Successful Method Development in SPE

- Analyte
  - (Recovery/Concentration - How easily is analyte adsorbed/desorbed?)
  - (Mechanism of Extraction - Can sample be manipulated to maximize SPE experiment?)

- Solvent

- Sorbent

- Matrix
  - (Cleanup - how effective is matrix removal?)
General SPE Method Development Strategy

Background

Research the Problem

- Previous SPE and analysis conditions for the analyte and matrix? (Lots of published references, textbooks, applications bibliographies, manufacturers’ websites)

Characterize the Analyte

- Structure, pK_a, polarity (log P), functional groups
- Solvent solubility and stability
- Any restrictions on final solvent and concentration (technique or instrument)?

Characterize the Sample Matrix

- Possible interferences — similar functional groups, pK_a, etc.
- pH, ionic strength
- Solvent solubility and stability
- Qualitative and quantitative variability
Choose the Proper SPE Mode (Retention Mechanism)

- Reversed phase
- Normal phase
- Cation exchange
- Anion exchange
- Mixed mode
- HILIC
- Specialty
  - Affinity
  - Class-specific (e.g. phenylboronic acid for catecholamines)
  - Molecularly-imprinted polymers (MIPs)
  - Restricted-access media (RAM)
  - Method-specific (e.g. oil and grease)
SPE Retention Mechanisms

**Polar**
- Matrix is organic (e.g. organic phase from a liquid/liquid extraction)
- Analyte is water soluble
- Wash solvents are non-polar (hexane, methyl t-butyl ether etc)
- Elution solvents are polar (water, methanol, acetonitrile etc)

**Non-polar**
- Matrix is aqueous (foods, biological fluids)
- Analyte is organic soluble
- Wash solvents are aqueous
- Elution solvents are organic

**Mixed mode**
- Matrix is aqueous (foods, biological fluids)
- Analyte can be polar, hydrophilic, or hydrophobic
- Wash solvents are aqueous and organic
- Elution solvents are organic
- Sorbents are either mixed silica-based (such as C8/SCX) or polymer
Retention Mechanisms

**Cation exchange**

- Matrix is aqueous (foods, biological fluids)
- Analyte is basic (cationic)
- Wash solvents are aqueous
- Elution solvents are:
  - high ionic strength,
  - pH is increased above the pKa of the target compound,
  - competition with a cation (such as Na\(^+\)) with greater affinity for the sulfonic acid

**Anion exchange**

- Matrix is aqueous (foods, biological fluids)
- Analyte is acidic (anionic)
- Wash solvents are aqueous
- Elution solvents are:
  - high ionic strength,
  - pH is increased below the pKa of the target compound,
  - competition with an anion (such as SO\(_3\)\(^-\)) with greater affinity for the positively charge amine
General SPE Method Development Strategy: Experimental

Develop or apply effective HPLC or GC conditions to monitor progress

• Assess recovery, eluate cleanliness and reproducibility

Select and test sorbents

• Determine which sorbents provide maximum analyte retention and minimum (or maximum) matrix/interference retention
• Determine which eluent solvents yield highest recoveries
• Determine appropriate size of SPE cartridge (or other format) and weight of sorbent
• Assess different lots of selected sorbent
• During this step also identify and test optimum loading solvent

Identify and test optimum wash solvent

• Assess eluate cleanliness under conditions of maximum analyte retention
• Determine strongest wash solvent that will not elute analyte yet elute matrix/interferences; solvent should also not elute strongly held compounds of no interest

Identify and test optimum elution solvent

• Develop solvent & conditions that will elute analyte of interest in smallest volume but not eluted retain matrix and interferences

Test blank and fortified matrix

• Assess eluate cleanliness and recovery using optimum wash and eluent solvents

Test real samples and fortified samples

• Assess eluate cleanliness and recovery for different unfortified and fortified samples; if analyte recovery low determine where loss occurred (loading or washing step); need to mass balance
## Variables to Consider in SPE Method Validation

<table>
<thead>
<tr>
<th>Test Required</th>
<th>Experimental Parameter(s) to be Investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbent</td>
<td>Phase selection, weight, cartridge format &amp; size, check different lots</td>
</tr>
<tr>
<td>Conditioning Solvent</td>
<td>Solvent strength (weak or strong solvent), contact time, volume</td>
</tr>
<tr>
<td>Loading Solvent</td>
<td>Type, volume, % organic, pH, ionic strength, flow rate, breakthrough volume, drying time, analyte recovery/loss, interference/matrix removal</td>
</tr>
<tr>
<td>Wash Solvent</td>
<td>Type, volume, % organic, pH, ionic strength, flow rate, analyte recovery/loss, drying time, interference/matrix removal</td>
</tr>
<tr>
<td>Elution Solvent</td>
<td>Type, volatility, strength, volume, flow rate, pH, ionic strength, interference/matrix retention, analyte recovery/loss</td>
</tr>
<tr>
<td>Analyte and matrix stability</td>
<td>Tested in each step of method</td>
</tr>
<tr>
<td>Sample/matrix Loadability</td>
<td>Different analyte concentrations</td>
</tr>
<tr>
<td>Detectability</td>
<td>Limits of detection (LOD), limits of quantitation (LOQ)</td>
</tr>
<tr>
<td>Method Linearity and range</td>
<td>Tested over expected concentration range of analyte, test as function of matrix loading</td>
</tr>
</tbody>
</table>
Relationship Between Sample Recovery and Flow Rate in SPE

![Graph showing the relationship between recovery and flow rate in SPE. The graph indicates that recovery decreases as the flow rate increases, with an optimum flow rate that maximizes recovery.](image)
Breakthrough Curve for SPE Device

$V_B = \text{Breakthrough volume}; \quad V_R = \text{Retention volume}; \quad V_M = \text{Maximum Sampling Volume}$
Advantages/Disadvantages of Polymers in SPE

Advantages

- Spherical, homogeneous packed beds, minimal backpressure
- Higher surface areas than silica-based; therefore, larger sample capacity
  - Thus, smaller bed volume
  - Requires less sample and solvents
- No residual silanol groups to cause strong adsorption of basic compounds
- Wide pH range
  - Can use more dramatic washing conditions for interference/matrix removal and elution
- Can dry out without poor recovery and poor reproducibility
  - Due to “balanced” (lipophilic-hydrophobic/hydrophilic) surface; water-wettable
- Higher retention of polar compounds due to frequent mixed mechanisms (lipophilic-hydrophilic balanced); allows development of “generic” methods

Disadvantage

- More expensive than silica-based products
Break

For questions, at break please dial *1 on your phone,

or type a question through the Q&A box at any time during the presentation.
Overview of Agilent’s Polymeric Resins for SPE

- SampliQ OPT (Optimized Polymer Technology) is a neutral resin utilizing a novel polyamide chemistry (patent pending)
- The resin exhibits retention for both polar and nonpolar compounds based on the balanced hydrophilic/hydrophobic character
- Mechanism is reversed phase, which provides both ease of method development and compatibility with both GC and LC separations of the extracts
SampliQ OPT

- SampliQ OPT will retain a range of compounds that are moderately hydrophilic-to-organic soluble.

- General Guideline: if target compound is both hydrophilic (low log P) and strongly basic (pKa>10) or strongly acidic (pKa<3) the compounds will not be effectively retained on SampliQ OPT; preferred mechanism of extraction, ion-exchange.

- Resin is inert to a wide variety of solvents, stable in pH range 0 to 14, water-wettable.

![Polyamide chemistry](image)
**SampliQ SAX**

- SAX (strong anion exchanger) is a tertiary amine-modified divinylbenzene (DVB) polymer
- Resin exhibits retention for both acidic/anionic and neutral compounds over a wide range of hydrophobicity (log P)
- The resin exhibits both anion exchange and reversed phase behavior (mixed mechanism)
- The resin is inert to a wide variety of solvents, is stable in pH ranges 0 to 14, and is water-wettable.
**SampliQ SCX**

- SCX (strong cation exchanger) resin is a sulfonic acid-modified divinylbenzene (DVB) polymer
- The resin exhibits retention for both basic/cationic and neutral compounds over a wide range of pKa’s and hydrophobicity (log P)
- The polymeric resin exhibits both cation exchange and reversed phase behavior (mixed mechanism)
- The resin is inert to a wide variety of solvents, is stable in pH 0 to 14, and is water-wettable.
SampliQ WAX

- WAX (weak anion exchanger) resin is a neutral amine-modified divinylbenzene (DVB) polymer
- The polymeric resin exhibits retention for acidic/anionic, neutral and, more importantly, strong acids (e.g. sulfonates) that are irreversibly retained on strong anion exchange resins
- The amine functionality (pKa ~ 6) for the SampliQ WAX can be ionized or neutral (dependent on the pH of the buffer solution); unlike the strong anion exchange (SAX) resin (always ionized, pKa ~18) WAX offers a wider pH range
- The weak anion exchange resin exhibits both anion exchange and reversed phase behavior (mixed mechanism)
SampliQ WAX

- The weak anion exchange resin is inert to a wide variety of solvents, stable in the pH range 0 to 14, and is water-wettable.
- The weak anion exchange resin’s pKa ~6

Neutral amine functionality
SampliQ WCX

- WCX (weak cation exchanger) is a neutral carboxylic acid-modified divinylbenzene (DVB) polymer
- The resin exhibits retention for basic/cationic, neutral and more importantly strong bases (quaternary amines) that are irreversibly retained on strong cation exchange resins
- The carboxyl functionality (pKa ~5) of the WCX resin can be ionized or neutral dependent on the pH of the buffer solution; unlike the strong cation exchange resin that is always ionized at all pH values, the WCX has a wider pH range.
- The WCX exhibits both cation exchange and reversed phase behavior (mixed mechanism)
SampliQ WCX

- The weak cation exchange resin is inert to a wide range of solvents, stable in the pH range 0 to 14, and is water-wettable.
Electron Micrograph of the Polymeric Particles

- Spherical shape
- Symmetrical
- Easily packed
- Higher capacity
- No residual silanol interactions
- Better flow characteristics
SampliQ-OPT® Generic Method Development Process for 3 mL Cartridge

1. **Condition 3 mL methanol**
2. **Equilibrate 3 mL water**
   - Recommended flow through cartridge: not faster than 1 mL per minute
3. **Load 1 mL prepared sample spiked with internal standard in water**
   - Dry <1 minute
4. **Wash 1 mL 5% - 10% methanol in water**
   - Dry 3 minutes
5. **Elute 2 mL methanol or 0.1% formic acid in methanol**
   - Dry and reconstitute in mobile phase

[Diagram showing the process steps with arrows connecting them]
SampliQ-OPT ®  Generic Method Development Modification for Bases

1. **Condition 3 mL methanol**
2. **Equilibrate 3 mL water pH 9**
   - Recommended flow through cartridge: not faster than 1mL per minute
3. **Load 1 mL prepared sample spiked with internal standard in water**
   - Dry <1 minute
4. **Wash 1 mL 5% - 10% methanol in water pH 9**
   - Dry 3 minutes
5. **Elute 2mL methanol or 0.1% formic acid in methanol**
6. **Dry and reconstitute in mobile phase**
Polymer Performance is Robust: SampliQ OPT

- Highly reproducible recoveries wet or dry
  - Cartridges dried under vacuum for 10 minutes before the equilibration step
- RSD’s of the recoveries for each of the compounds (n=5) very low (see error bars)
- Compounds range from very polar, basic compounds to hydrophobic, neutral
Antibiotic Recoveries and Reproducibility from a Honey Extract

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Spiking Level (ng/g honey)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlormphenicol</td>
<td>0.10</td>
<td>96.94</td>
<td>3.51</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>98.88</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>20.00</td>
<td>107.32</td>
<td>0.46</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>0.10</td>
<td>100.67</td>
<td>9.77</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>100.28</td>
<td>2.84</td>
</tr>
<tr>
<td></td>
<td>20.00</td>
<td>107.49</td>
<td>2.55</td>
</tr>
<tr>
<td>Thiapenicol</td>
<td>1.00</td>
<td>76.00</td>
<td>4.39*</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>74.89</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>20.00</td>
<td>89.81</td>
<td>3.83</td>
</tr>
</tbody>
</table>
**SampliQ-SCX® Generic Method Process for Neutral and Basic Compounds**

1. **Condition 3 mL 2% formic acid in methanol**
2. **Equilibrate 3 mL 2% formic acid in water**
3. **Load 1 mL prepared sample spiked with internal standard**
4. **Wash 1 mL 2% formic acid in water**
5. **Interference removal: 2 mL methanol**
6. **Elute with 3% NH4OH in methanol**
7. **Dry and reconstitute in mobile phase**

- Recommended flow through cartridge: not faster than 1 mL per minute
- Dry 2 minutes
- Dry <1 minute
- Dry 3 minutes

Methanol fraction may be collected if basic and neutral compound capture is desired.
Performance of Strong Cation Exchange for a Wide Range of Compounds

Neutrals compounds (light grey) elute exclusively in methanol
Basic compounds (dark grey) elute exclusively in methanolic ammonium hydroxide
## Recoveries and Reproducibility for the β-Agonists in Pork

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spiked level (ng/g pork)</th>
<th>Recovery (%)</th>
<th>RSD (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbutaline</td>
<td>0.5</td>
<td>88.7</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>98.0</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>0.5</td>
<td>100.6</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>92.9</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>97.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Olenbuterol</td>
<td>0.5</td>
<td>82.3</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>91.5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>90.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Formoterol</td>
<td>0.5</td>
<td>85.1</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>83.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>77.9</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Agilent Pub# 5990-4180EN
**SampliQ-SAX® Generic Method Process for Acidic and Neutral Compounds**

1. **Condition 3 mL methanol**
2. **Equilibrate 3 mL water**
   - Recommended flow through cartridge: not faster than 1mL per minute
3. **Load 1 mL prepared sample in phosphate buffer (pH 7) spiked with internal standard**
4. **Wash 1 mL 50 mM sodium acetate (pH 7) in 5% methanol**
   - Dry 1 minutes
5. **Interference removal: 2 mL methanol**
   - Methanol fraction may be collected if neutral compound capture is desired
   - Dry <2 minute
6. **Elute with 2% formic acid in methanol**
   - Dry 3 minutes
7. **Dry and reconstitute in mobile phase**
Recovery by Elution Fraction

Neutral compounds (light grey) are recovered in the methanol eluent.
Acidic compounds (dark grey) are recovered in acidic methanol eluent.
SampliQ-WCX® Generic Method Process for Strongly Basic/Cationic and Neutral Compounds

1. Condition 3 mL methanol

2. Equilibrate 3 mL water

3. Load 1 mL prepared sample in 2% phosphoric acid spiked with internal standard

4. Wash 1 mL 25 mM phosphate buffer (pH 7)

5. Interference removal: 2 mL methanol

6. Elute with 5% formic acid in methanol:ACN (60/40)

7. Dry and reconstitute in mobile phase

- Recommended flow through cartridge: not faster than 1 mL per minute
- Dry 1 minute
- Dry <2 minutes
- Dry 3 minutes

Methanol fraction may be collected if neutral compound capture is desired.
Performance of the Weak Cation Exchange Resin for the Extraction of Strongly Basic/Cationic and Neutral Compounds

**Basic Compounds Eluted by WCX**

- Atenolol
- Protryptyline
- Chlorpromazine
SampliQ WAX® Generic Method Process for Acidic/Anionic and Neutral Compounds

1. Condition 1 mL Methanol
2. Equilibrate 1 mL Water
3. Load 1 mL sample (dilute 1:1 2% phosphoric acid in water pH 1.5-2.0)
4. Wash 1 mL 25 mM Sodium Acetate, pH 4.5-5.0
5. Dry 3 minutes
6. Wash and Interference removal 1 mL Methanol
7. Dry 1 minute
8. Methanol fraction may be collected if basic and neutral compound capture is desired
9. Elute 2 x 1 mL 5% Ammonium Hydroxide/Methanol:ACN (20/80)
10. Dry 1 minutes
11. Dry and reconstitute in mobile phase
Performance of the Weak Anion Exchange Resin for the Extraction of Acidic/Anionic and Neutral Compounds from Human Plasma
Method Development: Tips and Tricks

• Identify the compounds you need to extract
  • pKa, Log P and matrix: biological, food, environmental
  • Analysis by HPLC will offer insight into possible SPE modes

• The more details available will facilitate quick method development
  • http://www.acdlabs.com/products/phys_chem_lab/logp/
  • http://www.organic-chemistry.org/prog/peo/
  • http://www.chemaxon.com/marvin/sketch/index.jsp
Method Development: Tips and Tricks

- Always use a weaker acid versus a stronger acid when biological matrices are in the application
  - Strong acids can cause precipitation of the compound with the protein, which will directly effect recovery (phosphoric, acetic or formic versus TFA or TCA)
  - Weak acids will maintain components in solution and optimize the extraction procedure, while still interrupting protein binding, please note that on average a compound will be as much as 50% bound
- Always start your method development with a neat solution, this allows one to collect and evaluate each step: sample load, each wash and of course the eluent
  - Each step including the sample load should be collected and analyzed for sample breakthrough
  - The total from all steps: load, wash(s) and elute will equate to ~100% mass balance or ~100% recovery of analyte(s) of interest
Method Development: Tips and Tricks

• Based on the analysis from evaluating the load, wash(s) and elute define how you should proceed

  • **Sample breakthrough in the load step**
    • Make sure the amount loaded is below the capacity of the resin ~10-15% resin mass (e.g. 100 mg of resin can retain 10-15 mg of all material that has an affinity for the resin*)
    • Reduce the percentage of organic in the sample
    • Use a buffer versus water in the equilibrate step prior to loading, pH will be based on the resin you are using
    • Make sure to load sample at 1 mL/min, fast rates will limit kinetic exchange between analytes and polymeric media

  • **Sample breakthrough in the wash step**
    • Reduce the percentage of organic used in the wash step
    • Correct the pH: SAX pH≥7, SCX pH≤7; WAX pH ≤ 7, WCX pH≥7
    • Maintain a slow flow 1 mL/min to reduce breakthrough
Method Development: Tips and Tricks

- Elute your sample in a volatile organic 100%
  - Eluting your sample in a volatile organic minimizes the amount of solvent you will need to use, therefore extract is in a smaller volume
  - Use of a modifier will facilitate extraction from the resin, specific for ion-exchange resins; SAX (acidic organic), SCX (basic organic), WAX (basic organic), WCX (acidic organic)
  - Never inject a high organic eluent into a HPLC system, will cause chromatographic anomalies
  - Using a volatile organic minimizes dry down time
  - Always reconstitute in compatible solvent for HPLC or GC analysis
Conclusion

- To perform successful method development and validation in SPE one must be aware of the many parameters that influence selectivity, recovery & reproducibility.

- It is always a good idea before proceeding onto method development to take the time to search for an existing procedure: literature, books, search engines, manufacturers’ websites.

- Always keep in mind the 4 variables important in any SPE application: Analytes, Matrix, Resin (Sorbent), and Solvent.

- Polymeric resins offer a mixed-mode selectivity that can optimize elution of neutral from acidic or basic from neutral.
Conclusion

- Agilent’s SampliQ OPT (neutral resin) is recommended for the isolation of neutral and basic compounds
- SampliQ SCX (strong cation exchanger) for the isolation of basic/cationic and neutral compounds
- SampliQ SAX (strong anion exchanger) for the isolation of acidic and neutral compounds
- SampliQ WCX (weak cation exchanger) for the isolation of strong bases
- SampliQ WAX (weak anion exchanger) for the isolation of strong acids
- If poor recovery is suspected, always collect and analyze the effluent from every SPE step (load, wash) in order to use the method development tips and tricks discussed
- All Agilent published references can be located in pdf format at www.agilent.com/