FOOD ANALYSIS
I. Solid Phase Extraction

Principles
Recent Developments
Applications

Basic Principles of SPE

- Sample Preparation Overview
- Fundamentals of SPE
- Various Modes of SPE
- Packed Bed vs. Disk Format
- The use of Dual Phases
- Step by Step Method
  Development; Validation

Recent Developments in SPE

- Stacked / Layered Phases
- Argentation Chromatography
- ISOLUTE ENV+
- MSPD: Matrix Solid Phase Dispersion
- Mechanised L / L Extraction
- MIP and Immunoaffinity Columns
- SPE Automation

Applications

- Multi-Residue Methods using
  - Cation-Exchange SPE
  - DIOL / NH2 SPE
  - Layered-Column SPE
  - GPC Clean-Up
- SPE of Pesticides & Mycotoxins
- MSPD of Drug Residues
- Isolation of Dimetridazole
**Definition of SPE**

Separation or removal of an analyte or analytes from a mixture of compounds by selective partitioning of the compounds between a solid phase (sorbent) and a liquid phase (solvent).

**SPE Mechanism Selection (1)**

<table>
<thead>
<tr>
<th>Functionality</th>
<th>Analyte</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>[Structure]</td>
<td>Non-Polar</td>
</tr>
<tr>
<td>H-Bonding</td>
<td>[Structure]</td>
<td>Polar</td>
</tr>
<tr>
<td>Ionic</td>
<td>[Structure]</td>
<td>Ion-Exchange</td>
</tr>
</tbody>
</table>

**SPE Mechanism Selection (2)**

<table>
<thead>
<tr>
<th>Analyte Matrix</th>
<th>Sorbent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic</td>
<td>Non-Polar</td>
</tr>
<tr>
<td>H-Bonding</td>
<td>Non-Polar</td>
</tr>
<tr>
<td>Ionic</td>
<td>Anion Exchange</td>
</tr>
</tbody>
</table>

**ISOLUTE Non-Polar Sorbents**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sorbent</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18 *</td>
<td>Octadecyl</td>
</tr>
<tr>
<td>MFC18</td>
<td>Octadecyl</td>
</tr>
<tr>
<td>C8 *</td>
<td>Octyl</td>
</tr>
<tr>
<td>C2 *</td>
<td>Ethyl</td>
</tr>
<tr>
<td>C4</td>
<td>Butyl</td>
</tr>
<tr>
<td>C6</td>
<td>Hexyl</td>
</tr>
<tr>
<td>PH *</td>
<td>Phenyl</td>
</tr>
<tr>
<td>CH (EC)</td>
<td>Cyclohexyl</td>
</tr>
<tr>
<td>CN (EC)</td>
<td>Cyanopropyl</td>
</tr>
<tr>
<td>101</td>
<td>PS-DVB</td>
</tr>
<tr>
<td>ENV+</td>
<td>Polystyrene</td>
</tr>
</tbody>
</table>

* EC
Silica Surface Variations

- Free silanol
- Adsorbed water
- Geminal silanol
- Siloxane
- Bound silanols

Monochlorosilane Chemistry

\[ R_3 SiCl + OH^{-} \rightarrow Si-OH \]

Trichlorosilane Chemistry

\[ RSiCl_3 + H_2O \rightarrow RSi(OH)_3 \]

Non-Polar Interactions

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8</td>
<td>van der Waals</td>
</tr>
<tr>
<td>PH</td>
<td>van der Waals</td>
</tr>
<tr>
<td>C2</td>
<td>van der Waals</td>
</tr>
</tbody>
</table>
Bonded Silica Surface

Residual silanols

pH<3

Polar Secondary Interactions

pH<3

Ionic Secondary Interactions

pH>4

ISOLUTE Polar Sorbents

Si Silica
NH₂ Aminopropyl
PSA Primary Secondary Amine
Diol 2,3-Dihydroxypropyl
CN * Cyanopropyl

* Also available in endcapped chemistry
Used to extract polar compounds from non-aqueous matrices (e.g. Hexane, ethyl acetate, dichloromethane, etc.)
**Polar Interactions**

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>Dipole / Dipole</td>
</tr>
<tr>
<td>NH₂</td>
<td>Hydrogen-Bonding</td>
</tr>
<tr>
<td>2OH</td>
<td>Hydrogen-Bonding</td>
</tr>
</tbody>
</table>

**NH₂ vs. Silica Columns**

The use of NH₂ columns in place of silica columns is strongly recommended. This is because the activity of silica columns can be affected by moisture content. IST columns are manufactured to a constant standard moisture level, so their activity will always be reliable, but adaptation of literature methods using other types of silica can be problematic. Different moisture levels will affect the amount and polarity of solvents necessary for elution of the analytes. NH₂ columns are recommended as they are much less susceptible to this variation.

**ISOLUTE Ion-Exchange Sorbents**

**Anion Exchange:**
- Weak: NH₂ Aminopropyl
- PSA Primary Secondary Amine
- Strong: SAX Quaternary amine

**Cation Exchange:**
- Weak: CBA Carboxypropyl
- Strong: SCX Benzenesulphonic acid
- PRS Propylsulphonic acid

**Ionic Interactions**

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRS</td>
<td>Electrostatic</td>
</tr>
<tr>
<td>CBA</td>
<td>Electrostatic</td>
</tr>
<tr>
<td>SAX</td>
<td>Electrostatic</td>
</tr>
</tbody>
</table>
Multiple Interactions

Mixed-Mode SPE

pK of Sulfonamides

SPE Modes of Operation

- Six-step procedure (typical)
- Four-step procedure
- Stacked columns
- Layered phases
- Mixed phases
A Typical SPE Procedure Involves Six Steps

1. Sample pre-treatment
2. Column solvation
3. Column equilibrium
4. Sample application
5. Interference elution
6. Analyte elution

Sample Pre-Treatment

- Optimize sample for analyte retention
  - Proper dilution / ionic strength
  - Correct pH
  - Analytes free in solution
  - Remove particulates

Six Step SPE Procedure

1
2/3
4
5
6

Acid Dissociation Constants

\[
HA \leftrightarrow H^+ + A^-
\]

\[
K_a = \frac{[H^+][A^-]}{[HA]}
\]

- \( K_{HOAC} = 1.75 \times 10^{-2} \)
- \( K_{HCN} = 6.20 \times 10^{-10} \)

- \( pK_{HOAC} = 4.76 \)
- \( pK_{HCN} = 9.21 \)

\( pK_a = -\log K_a \)
**Henderson-Hasselbach Equation**

\[
pH = pK_a + \log \frac{[A^-]}{[HA]}\]

Choose a pH at least 2 units away from pKₐ

**Influence of pH on Retention**

**Non-Polar Phases (pKₐ = 5)**

- pH 3.0
  - RCOOH
  - C₂

- pH 7.0
  - RCOO⁻
  - C₂

**Ion-Exchange Phases (pKₐ = 5)**

- pH 3.0
  - RCOOH
  - SAX

- pH 7.0
  - RCOO⁻
  - SAX

**Column Conditioning**
Column Conditioning

- Non-polar sorbents
  - MeOH, MeCN, THF
- Polar sorbents
  - nC6, EtAc; same solvent as the sample matrix
- Ion-exchange sorbents
  - MeOH, MeCN, THF

Column Equilibration

- Remove excess solvation solvent
- Normalize sorbent to sample condition (optimum environment for retention)
  - Ionic strength, pH, solvent composition
- Ion-exchange
  - Counter-ion, pH

Sample Application

<table>
<thead>
<tr>
<th>Type of Analyte</th>
<th>Type of Sorbent</th>
<th>Cartridge Size (mL)</th>
<th>Loading Rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>Hydrophobic</td>
<td>1</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3-15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>10-120</td>
</tr>
<tr>
<td>Cation or Anion</td>
<td>Ion Exchange</td>
<td>1</td>
<td>0.5-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>3-35</td>
</tr>
</tbody>
</table>

Interference Elution

- Analyte-insoluble solvent
- Selective mixtures
- Maintain analyte retention
  - (pH control can be important)
- Optimize flow rate
Analyte Elution

- Elution solvent must overcome both PRIMARY and SECONDARY interactions
- 100% elution in < 20 bed volumes
- Use selective solvents / mixtures
- Optimize flow rate

Example: Atrazine

Atrazine Structure

\[
\begin{align*}
(CH_3)_2 CHNH_2 & \quad HNCH_2 CH_3 \\
\end{align*}
\]

Atrazine from Water

- Apolar Retention
- Column: C18, 1g / 6mL
- 1. Sample pre-treatment: none
- 2. Solvation: 10mL MeOH
- 3. Equilibration: 10mL water
- 4. Sample: 500mL aqueous
- 5. Wash: 10mL water; dry: 30min
- 6. Elution: 2 x 4 mL acetone

Atrazine from Corn Oil

- Polar Retention
- Column: DIOL, 500mg / 6mL
- 1. 2mL oil \(\Rightarrow\) dilute w. 18mL of nC6
- 2. Solvation: 6mL nC6
- 3. Equilibration: none
- 4. Sample: 20mL (diluted)
- 5. Wash: 2mL nC6
- 6. Elution: 1mL methanol
Atrazine from Soybeans
Cation-Exchange Mechanism

Column: SCX, 500mg / 6mL
1. 5g sample + 10mL ACN $\Rightarrow$ homogenize, 
   filter; dilute 5mL filtrate w. 20mL 1% AcOH
2. Solvation: 3mL methanol
3. Equilibration: 6mL 1% AcOH
4. Sample: 25mL (diluted)
5. Wash: 1mL of 1% AcOH and 1ml of ACN
6. Elution: 2mL of 1:1 ACN - 0.1M K$_2$HPO$_4$

Multi-Residue Method (PRS)

Wide range of veterinary drugs 
with cationic functionality
- Anthelmintics
  - Benzimidazoles, levamisole
- Tranquillizers
- Antibacterials
  - Sulfonamides, quinolones
- $\beta$-Agonists

Column: PRS, 500mg / 6mL
1. 2g liver $\Rightarrow$ 2 x 20mL ACN, Ultra-Turrax; 
   filter, then acidify w. 200µL of AcOH
2. Solvation: 5mL methanol
3. Equilibration: 5mL of ACN : AcOH (200:1)
4. Sample: 40mL (diluted)
5. Wash: 5-5mL of EtAc, acetone, methanol
6. Elution: 5ml of acetone:NH$_4$OH (sg 0.88) 1:1

Sample Clean-Up:
Four-Step Method

NO trace enrichment
1. Sample pre-treatment
2. Column solvation
3. Column equilibration
4. Interference removal
Four-Step SPE Procedure

1. Sample pre-treatment
2. Column solvation
3. Column equilibration
4. Sample application

OP Pesticides in Cherries Percent Recoveries

<table>
<thead>
<tr>
<th>Compound</th>
<th>5% Acetone</th>
<th>10% Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethophos</td>
<td>97</td>
<td>91</td>
</tr>
<tr>
<td>Diazinon</td>
<td>84</td>
<td>82</td>
</tr>
<tr>
<td>Etrimphos</td>
<td>92</td>
<td>86</td>
</tr>
<tr>
<td>Parathion - methyl</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>Pirimiphos - methyl</td>
<td>101</td>
<td>98</td>
</tr>
<tr>
<td>Malathion</td>
<td>61</td>
<td>88</td>
</tr>
<tr>
<td>Fenthion</td>
<td>93</td>
<td>90</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Quinalphos</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>EPN</td>
<td>81</td>
<td>92</td>
</tr>
</tbody>
</table>

OP Pesticides in Cherries

Stacked Columns

- Extending the range
- Enhancing the selectivity
- Method development
- Multiple detection protocol
Fractionation (Stacked Columns)

Layered Phases (Loading)

Layered Phases (Eluting)

OC Pesticides from Water Extraction on Layered Phases

ISOLUTE® C2 / C18

- HCl to pH=2, Methanol, 0.5%
- 5 mL Methanol
- 10 mL reagent water
- One liter in 1/2 hour
- 10 mL water, 10 min N₂
- Two x 2 mL THF
OC Pesticides from Water Percent Recovery

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>C18(EC)</th>
<th>C2</th>
<th>C18(EC)</th>
<th>SUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>73</td>
<td>85</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>4,4'-DDE</td>
<td>62</td>
<td>86</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>78</td>
<td>65</td>
<td>37</td>
<td>102</td>
</tr>
<tr>
<td>AVG</td>
<td>88</td>
<td>54</td>
<td>43</td>
<td>97</td>
</tr>
</tbody>
</table>

17 pesticides

What About Solid Samples?

- **Tissue**
  - growth promoters
  - Anthelmintics
  - Sulfonamides, quinolones
  - β-Agonists

- **Vegetables**
  - pesticides

Extraction of Solid Samples: Traditional Approach

- Homogenisation
- Liquid / liquid extraction
- Sample clean-up
- Trace enrichment

Extraction of Solid Samples: MSPD Approach

- Homogenise the sample with the sorbent
- Transfer to empty reservoir or clean-up column (Si, NH2, Fl, SAX/PSA)
- Elute interferences
- Elute analytes
**MSPD Procedure**

1. Homogenise
2. Transfer blend to pre-fitted reservoir
3. Tap to settle bed
4. Gently insert top frit with inserter
5. Elute interferences
6. Elute analytes

**Advantages of MSPD**

- Homogenisation, analyte extraction and clean-up are simultaneous
- Less labour intensive
- Less operator dependent
- Time saving
- Low solvent consumption

**ISOLUTE® MSPD Sorbents**

- Optimized to blend quickly (less than 1 min) and easily
- Sample / sorbent blend is homogeneous, dry and free-flowing
- C18(UC) and C18(EC) chemistries are available

**Standard vs. MSPD Sorbents**

- Sample: bovine liver, fortified with 5 ppb (ng/g) clenbuterol
- MSPD conditions: blend 0.5 g sample with 2.0 g sorbent (standard or MSPD grade)
- Analysis: RIA
**Standard vs. MSPD Sorbents: Recovery, Reproducibility**

<table>
<thead>
<tr>
<th></th>
<th>MSPD C18(EC) n=5</th>
<th>91.0%</th>
<th>Standard C18(EC) n=6</th>
<th>91.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RSD</strong></td>
<td>MSPD C18(EC) n=5</td>
<td>5.5%</td>
<td>Standard C18(EC) n=6</td>
<td>15.5%</td>
</tr>
</tbody>
</table>

**Pesticides in Fruits and Vegetables: MSPD Procedure**

- Mix 100g of sample thoroughly
- Blend 0.5g of sample with a glass pestle into 0.5g of MSPD C18(EC)
- Transfer the mixture into a Silica column (0.5g/6mL); insert top frit
- Elute the analytes with 10mL of ethyl-acetate

**Pesticides in Fruits and Vegetables: Recoveries**

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Recovery (10 – 500 ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>101</td>
</tr>
<tr>
<td>Captafol</td>
<td>87</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>108</td>
</tr>
<tr>
<td>Dicofol</td>
<td>105</td>
</tr>
<tr>
<td>β-Endosulfan</td>
<td>95</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>98</td>
</tr>
<tr>
<td>Phosmet</td>
<td>66</td>
</tr>
<tr>
<td>Methidathion</td>
<td>85</td>
</tr>
<tr>
<td>Methyl-parathion</td>
<td>97</td>
</tr>
<tr>
<td>Carbophenothion</td>
<td>86</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>94</td>
</tr>
<tr>
<td>Diazinon</td>
<td>94</td>
</tr>
<tr>
<td>α-Endosulfan</td>
<td>96</td>
</tr>
<tr>
<td>Ethion</td>
<td>93</td>
</tr>
<tr>
<td>Folpet</td>
<td>91</td>
</tr>
<tr>
<td>Malathion</td>
<td>87</td>
</tr>
<tr>
<td>Methyl-azinphos</td>
<td>57</td>
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<tr>
<td>Tetradifon</td>
<td>98</td>
</tr>
<tr>
<td>Carbophenothion</td>
<td>86</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>94</td>
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<tr>
<td>Tetradifon</td>
<td>98</td>
</tr>
</tbody>
</table>

**MIP – Molecularly Imprinted Polymers**
Class-Selective MIPs

- Beta-Agonists
- Triazines
- Nitroimidazoles
- Steroids
- Peptides, Proteins

Unique MIPs

- Clenbuterol
- NNAL
- Riboflavin
- Chloramphenicol
- Nicotine

MIP vs. Mixed-Mode SPE

SUMMARY

- SPE: effective sample clean-up and concentration technique
- New forms of SPE: wide range of analytes can be monitored
- Solid samples can also be processed (MSPD)
- Specialty tubes: selective isolation is possible