Sample Clean-up approaches for Food Analysis

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BDM Chemical Analysis
CO Europe and India
Sample pretreatment and preparation requires a **systematic approach** to answer the question: **How do we get from.... Sample to Extract?**
Outline

- Food analysis methods
  - Why sample pretreatment?
  - Sample preparation techniques
  - Factors influence SPE performance
  - Procedures for removing sample interferences
  - SPE cleanup strategies for sample preparation
    - Dispersive SPE strategy and example
    - Pass-through SPE strategy and example
    - Retention-cleanup-elution SPE strategy and example
Food analysis could be classified into 3 critical steps:

- Sample Pretreatment
- Sample Preparation
- Sample Analysis by Instrument

- Some may consider both steps as a single step of sample preparation
- Depending on the analytes and sample matrix, sample pretreatment may not be necessary.
Outline

- Food analysis methods
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Food Analysis Methods

Why Sample Pretreatment?

- Obtain representative, consistent sample
  - homogenization by cutting, chopping, blending

- To remove moisture
  - Drying sample by heat or drying reagents
Potential Problems of Matrix

Common Types of Matrix Interferences

- Fats, oils, lipids, and proteins
  - Infant formula, milk, meat

- Carbohydrates and polysaccharides
  - Fruits and cereals

- Salts
  - Snack foods

- Surfactants
  - Naturally occurring – phospholipids
  - Synthetic – used in ice cream to improve mouth feel

- Pigments
  - Chlorophyll in leafy vegetables
Potential Problems of Matrix
Sample consistency

- **Emulsions**
  - **Milk** - The milk fats are dispersed in water
  - **Butter** - is an emulsion of water particles dispersed in milk fats

- **Turbidity**
  - pulpy orange juice
To adjust the sample conditions for the NEXT STEP of the sample preparation

- Sample conditions can be adjusted using:
  - Dilution
  - Extraction
  - Solvent exchange into instrument compatible solvents
  - pH adjustment
Why Sample Pretreatment?
To Adjust Sample pH

- pH adjustment can provide:
  - Optimized ionization of the analytes for SPE sample preparation
    - neutral species for reversed-phase
    - Ionized species for ion-exchange
  - Stabilization of pH labile compounds
  - Reduced matrix interferences
    - protein precipitation by adding acids
  - Elimination of protein binding by adding acids or bases
    - TFA, formic acid, phosphoric acid, or ammonia
Outline

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    - Dispersive SPE strategy and example
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# Sample Preparation

## Non-Chromatographic Techniques

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>• Simple</td>
<td>• No cleanup</td>
</tr>
<tr>
<td></td>
<td>• Cheap</td>
<td>• No enrichment</td>
</tr>
<tr>
<td></td>
<td>• High throughput</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>• Simple</td>
<td>• No enrichment</td>
</tr>
<tr>
<td></td>
<td>• Fast</td>
<td>• Potential analyte binding</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>• Simple cleanup</td>
<td>• No enrichment</td>
</tr>
<tr>
<td></td>
<td>• High throughput</td>
<td>• Cumbersome</td>
</tr>
<tr>
<td>Liquid-Liquid Extraction</td>
<td>• Best non-chromatographic cleanup</td>
<td>• Cumbersome</td>
</tr>
<tr>
<td></td>
<td>• Enrichment</td>
<td>• Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lots of solvent usage</td>
</tr>
</tbody>
</table>
# Sample Preparation by SPE

## A Chromatographic Cleanup

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| **Solid Phase Extraction (SPE)** | • Best cleanup  
• Enrichment  
• Fast  
• Easy to automate  
• Many sorbents available for optimum cleanup | • May need multiple steps  
• Not well understood |
Why Use SPE for Food Analysis?

- **Sample Cleanup**
  - Significant cleanup with specific SPE sorbent
  - Optimum cleanup by using multiple SPE cartridges
    - Melamine analysis

- **Sample Enrichment**
  - For sub-ppb detection limits, enrichment factors (100X) may be needed.
    - Sudan dye analysis

**Compared to liquid-liquid extraction**

**SPE requires much less solvent per sample**

(often 10X less solvent used for SPE)
General Pretreatment Procedures for SPE Sample Preparation

* sample with suspended solids, e.g. orange juice
General Pretreatment Procedures for SPE Sample Preparation

Sample Type?

- Liquid
  - pH adjustment
  - Filter/Centrifuge Sample
    - Solid
    - Extraction
    - Liquid
  - Clear fluid
  - SPE

- Solid
  - Sample Homogenized
    - pH adjustment
    - Extraction
    - Filter/Centrifuge Sample
      - Solid
      - Extraction
      - Liquid
    - SPE

Often performed in one step
Outline

- Food analysis procedures
- Why sample pretreatment?
- Sample preparation techniques
- Factors influence SPE performance
  - Procedures for removing sample interferences
  - SPE cleanup strategies for sample preparation
    - Dispersive SPE strategy and example
    - Pass-through SPE strategy and example
    - Retention-cleanup-elution SPE strategy and example
Factors that Influence SPE Performance

- Interactions between Solvent, Analyte, Sorbent and Matrix determine the SPE method performance.
- Interactions of all factors need to be considered together when developing SPE method.
- Optimized SPE methods maximize specific interactions while minimizing unwanted interactions.
Important Properties of Analytes for SPE Performance

Key properties of analytes to be considered

- Polar functional groups consisting of O, N, S, or P?
- The numbers and positions of the polar groups
- Any ionizable functional groups? Are they cations or anions?
- Approximate pKa of these functional groups
- The solubility in water and the solvents for the sample preparation method ($K_{ow}$)

The above properties will determine the analyte interactions with solvent, matrix and the SPE sorbent
Key Functions of the solvent

- Extract analytes
- Remove matrix interference
- Change the polarity and ionic strength of the sample extract
- Change the ionization status of analytes, sample matrix, or SPE sorbents
Matrix

Key considerations of the sample matrix

- The relative polarity of the matrix compared to analytes, solvent or sorbent
- Potential interferences with the analyte analysis
- Potential binding of analytes with the matrix components (proteins)

Matrix interactions influence the sample clean-up and analyte extraction
SPE Sorbents

Key considerations for SPE Sorbents

- Sorbents can be chosen to interact with analyte or sample matrix
- Sorbent interactions be enhanced or weaken by solvent manipulation
- In general, there are 4 sorbent classes:
  - Reversed-phase
  - Normal-phase
  - Ion-exchange
  - Mix-mode
SPE Sorbent Selection

- Based on the solvents selection
  - Organic solvents – normal-phase
  - Aqueous solvents and buffers – reversed-phase

- Based on the strategy of the SPE method
  - Pass-through cleanup
  - Retain-wash-elute cleanup

- If there are more than one sorbent suitable, pick the sorbent based on:
  - Level of cleanup required
  - Sensitivity required
  - Analyte interaction
  - Other specific requirements of method
Normal-Phase Sorbents
- Silica, Alumina, Florisil®, Aminopropyl silica, PSA, Diol silica,

Reversed-Phase Sorbents
- Oasis® HLB
- C18, C8 etc (alkyl bonded silica)
- Graphitized carbon and activated carbon

Ion Exchange
- Accell Plus™ CM, QMA

Mixed Mode (ion-exchange/reversed-phase)
- Oasis® MAX, Oasis WAX (strong and weak anion-exchange)
- Oasis® MCX, Oasis WCX (strong and weak cation-exchange)
SPE Sorbents for Food Analysis

- Normal-Phase Sorbents
  - Silica, Alumina, Florisil®, Aminopropyl silica, PSA, Diol silica,
- Reversed-Phase Sorbents
  - Oasis® HLB
  - C18, C8 (alkyl bonded silica)
  - Graphitized carbon and activated carbon
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- Mixed Mode (ion-exchange/reversed-phase)
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- Normal-Phase Sorbents
  - Silica, Alumina, Florisil®, Aminopropyl silica, PSA, Diol silica,
- Reversed-Phase Sorbents
  - Oasis® HLB
  - C18, C8 etc (alkyl bonded silica)
  - Graphitized carbon and activated carbon
- Ion Exchange (silica based)
  - Accell Plus™ CM, QMA
- Mixed-Mode (ion-exchange/reversed-phase)
  - Oasis MAX, Oasis WAX (strong and weak anion-exchange)
  - Oasis MCX, Oasis WCX (strong and weak cation-exchange)
Outline

- Food analysis procedures
- Why sample pretreatment?
- Sample preparation techniques
- Factors influence SPE performance
- Procedures for removing sample interferences

- SPE cleanup strategies for sample preparation
  - Dispersive SPE strategy and example
  - Pass-through SPE strategy and example
  - Retention-cleanup-elution SPE strategy and example
Common Sample Matrix Interferences

- Fats, Oils, Lipids and Hydrocarbons
  - These interferences are non-polar
  - They have high solubility in non-polar solvents
    - hexane, ethers
  - Common found in:
    - meats
    - nuts
    - dairy products
    - manufactured goods,
      - chips, cookies, and chocolate
SPE Considerations to Remove: Fats, Oils, Lipids and Hydrocarbons

- Normal-phase sorbents are usually selected to remove fats and lipids
  - Silica, Alumina, Florisil

- The sample will need to be diluted with solvent compatible with normal-phase sorbents
  - Hexane, ethyl acetate, dichloromethane

- Reversed-phased sorbents are sometimes used but optimization is usually difficult
Remove Fats, Oils, Lipids

**Example: Hexane**

1. **Sample Type?**
   - Solid

2. **Homogenize Sample with Solvent**
   - Liquid

3. **Centrifuge sample**
4. **Analyte in Liquid phase?**
   - yes
     - Discard solid fraction
   - no
     - Discard Solvent

5. **Re-extract sample using other solvent**

6. **Centrifuge sample**

7. **Dilute with non-polar solvent**

8. **SPE**

**Hexane:** Non-polar

**Analyte:** Polar
Remove Fats, Oils, Lipids
Example: Hexane

Sample Type?

- Liquid
  - Homogenize Sample with Solvent
  - Centrifuge sample
  - Analyte in Liquid phase
    - yes
      - Discard solid fraction
    - no
      - Discard Solvent
        - Re-extract sample using other solvent
          - Centrifuge sample
          - Analyte in Liquid phase
            - yes
              - Discard solid fraction
            - no
              - Liquid

- Solid
  - Homogenize Sample with Solvent
  - Centrifuge sample
  - Analyte in Liquid phase
    - yes
      - Discard solid fraction
    - no
      - Discard Solvent
        - Re-extract sample using other solvent
          - Centrifuge sample
          - Analyte in Liquid phase
            - yes
              - Discard solid fraction
            - no
              - Liquid

Hexane: Non-polar

Analyte: Polar
Remove Fats, Oils, Lipids
Example: Hexane

Sample Type?
- Liquid
  - Homogenize Sample with Solvent
  - Centrifuge sample
  - Analyte in Liquid phase
    - yes
      - Discard solid fraction
    - no
      - Discard Solvent
      - Re-extract sample using other solvent
        - Centrifuge sample
          - Liquid
            - SPE
  - SPE

Fats and Oils (Hexane)

Hexane: Non-polar

Analyte: Non-Polar

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Remove Fats, Oils, Lipids

Example: Hexane

Sample Type?

Solid

Homogenize Sample with Solvent

Liquid

Dilute with non-polar solvent

SPE

Non-Polar Analytes

Fats and Oils

Discard solid fraction

Analyte in Liquid phase

yes

Discard Solvent

Re-extract sample using other solvent

Centrifuge sample

Liquid

SPE

Hexane: Non-polar

Analyte: Non-Polar

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Remove Fats, Oils, Lipids

Example: Hexane

Sample Type?

- Liquid
  - Homogenize Sample with Solvent
  - Centrifuge sample

- Solid
  - Discard solid fraction

Analyte in Liquid phase?

- Yes
  - Discard solid fraction

- No
  - Discard Solvent
    - Re-extract sample using other solvent
      - Centrifuge sample
        - Liquid
          - SPE

Non-Polar Analytes

- Fats and Oils
  - Dilute with non-polar solvent
  - SPE

Hexane: Non-polar

Analyte: Non-Polar

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Proteins

- They are typically present in meats, tissues, dairy products
- They have high molecular weights
- They are sensitive to pH and organic solvents (addition of acidified acetonitrile to breakup protein binding and cause precipitation)
Sample Pretreatment to Remove: Proteins

Sample Type?

- Liquid

Protein binding?

- No
  - Centrifuge sample → SPE

- Yes
  - Add Acetonitrile or Acids
  - Centrifuge sample → SPE

Protein Precipitation
Sample Pretreatment to Remove: Proteins

Sample Type?

Liquid

Protein binding?

No

Centrifuge sample

SPE

Solids

Add Acetonitrile or Acids

Centrifuge sample

SPE

Add Acetonitrile or Acids

Centrifuge sample

SPE

Sample Homogenized with organic solvent. Add buffer if necessary

Centrifuge sample

Dilute Sample with solvent

SPE

Protein Precipitation
Sample Pretreatment to Remove: Proteins

1. **Sample Type?**
   - **Liquid**
   - **Solids**

2. **Protein binding?**
   - **No**
     - Centrifuge sample → SPE
   - **Yes**
     - Add Acetonitrile or Acids
     - Centrifuge sample → SPE
     - Add buffer if necessary
     - Centrifuge sample
     - Dilute Sample with solvent → SPE

---

**Protein Removal Step**
SPE Considerations to Remove: Proteins

- Choose sorbents that maximize analyte retention

- Large protein molecules usually pass-through sorbent reversed-phase without retaining
  - Cannot access pores in sorbent

- Eliminate proteins-analyte interactions prior to the SPE step
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  - Retention-cleanup-elution SPE strategy and example
How Much Sample Cleanup is Needed?

- The Level of Cleanup is determined by
  - The required selectivity of analytes
  - The Limit of Detection (LOD) for the method
  - The method is dictated by the analytical technique used
    - Selective detection, e.g. MS or MS/MS
      - Simple cleanup of major interference may be sufficient
    - Less selective detection, e.g. LC-UV or GC-FID
      - More cleanup may be required
General SPE Cleanup Strategy

Evaluate analyte structures and properties, matrix, and cleanup requirement

Screening Method?

Yes

Pass-through SPE (Matrix retained by sorbent)

No

Optimized cleanup
Max. Sensitivity

Retention-cleanup-elution SPE (Analytes initially retained by sorbent, lastly eluted by strong solvent)

Matrix Removal

Low

Dispersive - SPE (d-SPE)

Moderate

Better cleanup than d-SPE in general
Some enrichment via solvent evaporation

Pass-through SPE by cartridge
## Comparison of the SPE Strategies

<table>
<thead>
<tr>
<th></th>
<th>Dispersive-SPE (d-SPE)</th>
<th>Pass-through SPE</th>
<th>Retention-cleanup-elution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cleanup</strong></td>
<td>Quick, simple, easy</td>
<td>More effective than d-SPE in general</td>
<td>Most effective and very selective</td>
</tr>
<tr>
<td><strong>Analyte</strong></td>
<td>Non-retained</td>
<td>Non-retained</td>
<td>Initially retained and then eluted</td>
</tr>
<tr>
<td><strong>Matrix</strong></td>
<td>Mostly retained</td>
<td>Mostly retained</td>
<td>Non-retained or removed by washing</td>
</tr>
<tr>
<td><strong>Sorbent Selection</strong></td>
<td>Maximize: <strong>Matrix retention</strong> Minimize: Analyte retention</td>
<td>Maximize: <strong>Matrix retention</strong> Minimize: Analyte retention</td>
<td>Maximize: <strong>Analyte retention</strong> Minimize: Matrix retention</td>
</tr>
<tr>
<td><strong>Enrichment</strong></td>
<td>No, in general</td>
<td>Limited by solvent evaporation</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Analysis</strong></td>
<td>Multiresidue analysis</td>
<td>Multiresidue analysis</td>
<td>Compounds with similar structures and/or properties</td>
</tr>
</tbody>
</table>
Outline

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Some Examples
Screening of Veterinary Drugs In Milk
Milk Composition

- Typical Cow’s Milk
  - Approximately 14 % solids
    - 4 % fat
    - 4 % protein
    - 5 % sugar (lactose)
    - 85 % water
General SPE Cleanup Strategy

Evaluate **analyte** structures and properties, **matrix**, and cleanup requirement

Screening Method?

- **Yes**
  - Pass-through SPE (**Matrix** retained by **sorbent**)

- **No**
  - Optimized cleanup
    - Max. Sensitivity
      - Retention-cleanup-elution SPE (**Analytes** initially retained by **sorbent**, lastly eluted by strong solvent)

Matrix Removal

- Low
  - Dispersive - SPE (**d-SPE**)

- Moderate
  - Better cleanup than **d-SPE** in general
    - Some enrichment via solvent evaporation
  - Pass-through SPE by cartridge
Typical Sample Preparation Strategies

- Precipitation/extraction with strong buffer (McIlvaine pH 4) followed by SPE
  - good for tetracyclines, beta-adrenergics, polar sulfonamides, fair for fluoroquinolones,
  - poor recovery of most other compounds

- Precipitation/extraction with 3:1 acidic acetonitrile with SPE cleanup
  - excellent protein precipitation
  - poor recovery of tetracyclines, beta-adrenergics, polar sulfonamides
  - good recovery of most other compounds
## Typical Recoveries From Milk Comparison of Precipitation/Extraction Techniques

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>3:1 ACN</th>
<th>Aq Buffer</th>
<th>1:1 ACN*</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-adrenergic</td>
<td>&lt;10</td>
<td>~100</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&lt;25</td>
<td>&gt;70</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Macrolide</td>
<td>&gt;60</td>
<td>&lt;35</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Beta-Lactam</td>
<td>&gt;70</td>
<td>&lt;30</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Steroid</td>
<td>&gt;70</td>
<td>&lt;10</td>
<td>&gt;70</td>
</tr>
</tbody>
</table>

*Conclusion:
- Procedure chosen for this study
- Extraction/precipitation of milk with an equal volume of acetonitrile provides recovery of the widest range of compounds
  
However - insufficient protein precipitation

**Analytical Method For This Study**

**Milk – 2 mL Sample**

---

### Initial Extraction/Precipitation

Pipet 2 mL sample into centrifuge tube

- Add 2 mL acetonitrile
- Centrifuge @ 8000 x g
- Take 2 mL supernatant

---

### Protein Precipitation

- Add 3 mL acetonitrile (0.2% formic acid)
- Centrifuge @ 8000 x g
- Take 1 mL supernatant

---

### SPE Cleanup

- Sep-Pak C18 (1 cc, 100 mg)
- Evaporate and reconstitute

---

**Notes:**

- Provides good recovery of most compounds
- Minimal extraction of fat
- Much protein in extract
- Secondary protein precipitation step
- Removes most residual protein without significant loss of polar analytes
SPE Cleanup
Sep-Pak C18 (pass-thru mode)

Condition
1 mL 80:20 acetonitrile/water

Pass-Thru/Collect
1 mL protein ppt sample

Rinse/Collect
0.5 mL 80:20 acetonitrile/water

Evaporate/Reconstitute
0.2 mL 25:75 acetonitrile/buffer
(25 mM ammonium formate buffer @ pH 4.5)

* buffers sample to protect acid labile analytes
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Recovery Without Buffer</th>
<th>Recovery With Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamerizine</td>
<td>70-80</td>
<td>70-80</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>&lt;25</td>
<td>80-100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&lt;25</td>
<td>60-80</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&lt;40</td>
<td>75-85</td>
</tr>
</tbody>
</table>
Multi-Residue Pesticide Screening
Some Examples: Multi-Residue Screening

- Method Objectives:
  - Need to screen a wide variety of pesticides
  - Need moderate Sensitivity
    o Sensitivity is instrument driven
  - Need moderate to low sample cleanup
    o Optimize system performance
    o Maximize the number of commodities tested
**OBJECTIVES:**

Screen a wide variety of pesticides

Need moderate sensitivity

Require limited sample cleanup

---

**SPE Flowchart**

1. **Evaluate analyte structures and properties, matrix, and cleanup requirement**
2. **Screening Method?**
   - Yes: **Pass-through SPE (Matrix retained by sorbent)**
     - **Matrix Removal**
       - Low Dispersive - SPE (d-SPE)
       - Moderate Better cleanup than d-SPE in general
       - Some enrichment via solvent evaporation
     - **Optimized cleanup Max. Sensitivity**
       - Retention-cleanup-elution SPE (Analytes initially retained by sorbent, lastly eluted by strong solvent)
   - No: **Evaluate analyte structures and properties, matrix, and cleanup requirement**

---

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**OBJECTIVES:**

Screen a wide variety of pesticides

Need moderate sensitivity

Require limited sample cleanup
Dispersive Sample Preparation
Method Outline

1. Combine sorbent, sample matrix and solvent into a vessel

2. Sample is filtered or centrifuged

   Matrix interferences are retained by sorbent

3. Filtrate or supernatant is collected for analysis

   Analytes are in the filtrate or supernatant
DisQuE® Kit
Dispersive Sample Preparation

DisQuE Extraction Tube 1:
- 50 mL centrifuge tube containing;
- 1.5 g anhydrous sodium acetate
- 6 g of anhydrous magnesium sulfate

DisQuE Clean-Up Tube 2:
- 2 mL centrifuge tube containing;
- 150 mg anhydrous magnesium sulfate
- 50 mg of PSA (Primary-Secondary Amine SPE sorbent)
## DisQuE Product Line

### Extraction Tubes (Tube 1)

<table>
<thead>
<tr>
<th>AOAC Configuration</th>
<th>CEN Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 g Sodium Acetate&lt;br&gt;6 g Magnesium Sulphate</td>
<td>4 g Magnesium Sulphate&lt;br&gt;1 g Sodium Chloride&lt;br&gt;1 g Trisodium Citrate&lt;br&gt;0.5 g Disodium Citrate</td>
</tr>
</tbody>
</table>

### Clean Up Tubes (Tube 2 - 2 mL Option)

<table>
<thead>
<tr>
<th>AOAC Configuration</th>
<th>CEN Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>150mg Magnesium Sulphate&lt;br&gt;50mg PSA&lt;br&gt;50mg C&lt;sub&gt;18&lt;/sub&gt;</td>
<td>150mg Magnesium Sulphate&lt;br&gt;25mg of PSA&lt;br&gt;25mg C&lt;sub&gt;18&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

### Clean Up Tubes (Tube 2 - 15 mL Option)

<table>
<thead>
<tr>
<th>AOAC Configuration</th>
<th>CEN Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>900 mg Magnesium Sulphate&lt;br&gt;150 mg of PSA</td>
<td>900 mg Magnesium Sulphate&lt;br&gt;150 mg of PSA&lt;br&gt;150 mg C&lt;sub&gt;18&lt;/sub&gt;</td>
</tr>
</tbody>
</table>
DisQuE Procedure – Tube 1

AOAC Method

DisQuE Extraction Tube 1

Homogenize Sample
Sample Extraction
15 g sample
15 mL 1% Acetic Acid in ACN

Liquid Fractionation
Shake for 1 minute
Centrifuge >1500 x g

Collection
Recover acetonitrile for clean-up using tube 2
Preparation Sample

Homogenize
15 g sample
15 mL 1% Acetic Acid in ACN

Liquid Fractionation
Shake for 1 minute
Centrifuge >1500 x g

Collection
Remove acetonitrile for clean-up

Transfer
1 mL Extract
Tube 1 to Tube 2
Shake vigorously for 1 minute
Centrifuge >1500 x g

Transfer
Collect to autosampler vial
Dilute if necessary
Cleanup Tube 2

- Provides additional cleanup

- Sorbent choices
  - PSA removes
    - Acidic interferences (ion-exchange mechanism)
    - Carbohydrates and sugars (HILIC mechanism)
  - Graphitized Carbon Black (GCB) Removes
    - Chlorophyll and Pigments
  - C18 Removes
    - Non Polar Interferences

Acetonitrile Layer *(ANALYTES ARE HERE)*

Sorbent *(INTERFERENCES)*
Uses for QuEChERS:
- Removes Chlorophyll and Pigments
- Also removes ANALYTES – CAREFULL!!
Pesticide Recovery in Grape

DisQuE
Graphitized Carbon Black – BE CAREFULL!!

Pesticides

- Atrazine
- Azoxystrobin
- Carbaryl
- Cyprodinil
- Dichlorvos
- Imazalil
- Imidacloprid
- Linuron
- Methamidophos
- Methomyl
- Pymetrozine
- Tebuconazole
- Thiabendazole
- Tolyfluanid

% Recovery

PSA PSA+C18 PSA+GCB
Targeted Residue Analysis

Melamine
Some Examples: Targeted Residue Analysis

Determining melamine and cyanuric acid in infant formula

- Method Objectives:
  - Specific for melamine and cyanuric acid
  - Need maximum sample cleanup
    - Optimize system performance
    - Eliminate False positives and negative
  - Need sensitivity and sample enrichment
Targeted Residue Analysis
*SPE Flowchart: Method Selection*

**OBJECTIVES:**
- Selective for melamine/cyanuric Acid
- Need moderate sensitivity
- Need limited sample cleanup

- **Evaluate analyte structures and properties, matrix, and cleanup requirement**
  - **Screening Method?**
    - **Yes**
      - Pass-through SPE (Matrix retained by sorbent)
    - **No**
      - Optimized cleanup
      - Max. Sensitivity
  - **Matrix Removal**
    - Low
      - Dispersive - SPE (d-SPE)
      - Better cleanup than d-SPE in general
      - Some enrichment via solvent evaporation
      - Pass-through SPE by cartridge

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Melamine
Weak Base

Use a Strong Cation Exchanger

Oasis MCX
Targeted Residue Analysis
Oasis 2x4: Starting Protocol for Cyanuric Acid

Cyanuric Acid
Weak Acid
Use a Strong Anion Exchanger
Oasis MAX
Sample Pretreatment
Challenges and Analyte Considerations

- Maximum cleanup due to complex matrix
  - Proteins
  - Fat
  - Sugars

- Very Polar analytes
  - Melamine, weak base pKa ~ 9
  - Cyanuric acid, weak acid pKa ~ 5
Sample Pretreatment

Sample Extraction: Melamine/Cyanuric Acid

Sample Extraction

- 5g liquid infant formula or 1g dry infant formula add 4 mL water
- Add internal standards
- Add 20 mL 50:50 ACN:H2O
- Shake for 10 - 20 min
- Centrifuge @ 3400 rpm for 10 min
Sample Pretreatment Flowchart

Extraction Details: Melamine/Cyanuric Acid

Sample Type?

Liquid

Dilute with non-polar solvent → SPE

Solid

Sample Homogenized with organic solvent. Add buffer, if necessary

Centrifuge sample

Analyte in liquid phase

yes

Discard solid fraction

no

Discard solvent

Extract analytes in solid using other solvent

Centrifuge sample

Sample Preparation

Water: Dissolve the infant formula
Acetonitrile: Precipitate proteins

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Sample Pretreatment Flowchart

Extraction Details: Melamine/Cyanuric Acid

Sample Type?

- **Liquid**
  - Dilute with non-polar solvent
  - SPE

- **Solid**
  - Sample Homogenized with organic solvent. Add buffer, if necessary
  - Centrifuge sample
  - Analyte in Liquid phase
    - yes: Discard solid fraction
    - no: Discard solvent
      - Extract analytes in solid using other solvent
        - Centrifuge sample
          - Liquid
            - SPE

Sample Preparation

Water: dissolve the infant formula
Acetonitrile: precipitate proteins
Sample Pretreatment

Optimized Sample Extraction

Sample Extraction

- 5g liquid infant formula or 1g dry infant formula, add 4 mL water
- Add internal standards
- Add 20 mL 50:50 ACN:H2O
- Shake for 10-20 min
- Centrifuge @ 3400 rpm for 10 min

Solid powder dissolved in water

To enhance precision

Polar solvent for analyte extraction
Acetonitrile for protein precipitation
Solvent is compatible with SPE

Removed solids and precipitated proteins
Targeted Residue Analysis
**SPE Flowchart: Method Selection**

**OBJECTIVES:**
Targeted melamine and cyanuric acid
Need maximum sample cleanup
Need sensitivity and sample enrichment
Condition
5 mL 0.1M NaOH in ACN
5 mL 0.1M HCl in ACN
5 mL ACN

Equilibrate
5 mL 4% FA in water

Load
2 mL sample supernatant
Diluted with 3 mL 4% FA in water

Wash
5 mL ACN
5 mL 0.2% DEA in ACN

Elute
4 mL 2.0% DEA in ACN

Melamine Cleanup by Oasis® MCX

Pre-clean SPE cartridge from any environmental contaminants of melamine

Condition

Solvate sorbent with loading solvents

Reduce organic strength of sample
Ionize the melamine

Remove acidic and neutral interferences
Remove weak basic interference

Elute melamine with stronger base to put it in its un-ionized form.
Acetonitrile is used for subsequent LC by HILIC mode
Cyanuric Acid Analysis

Pre-clean SPE cartridge from any environmental contaminants of cyanuric acid

Solvate sorbent with loading solvents

Reduce organic strength of sample
Ionize the cyanuric acid

Remove basic and neutral interferences

Elute cyanuric acid with stronger acid to put it in its un-ionized form

Acetonitrile is used for HILIC

**Condition**
- 5 mL 0.1M HCl in ACN
- 5 mL 0.1M NaOH in ACN

**Equilibrate**
- 5 mL ACN

**Load**
- Dilute 2 mL sample supernatant with 3 mL 5% NH₄OH in water

**Wash**
- 5 mL ACN

**Elute**
- 2 mL 4.0% FA in ACN
The method goals were obtained by using:

- Simple sample pretreatment
  - Removed protein and fat interferences

- Specific SPE cleanup for melamine and cyanuric acid
  - Removed carbohydrates interferences
  - Easy modification 2x4 methodology
    - Acetonitrile was chosen for HILIC
  - Required two SPE sorbents for analyte specificity
Sample pretreatment and preparation requires a systematic approach

- Many factors govern the final method
- Common interferences can be removed by a selective use of solvent and SPE sorbents
- Charts and flow paths help gain an understanding of the reasoning behind the method choices
Thank You?