Analysis of Food Contaminants:

Improving the Quality of your Chromatography Data by Implementing Sample Prep Techniques

Presenter:

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Today’s Agenda

1. Sample preparation – why?
2. Overview of sample preparation options
3. Connecting chromatography challenges and sample prep solutions
4. Wrap up and summary
5. Questions
Food Contaminant Analysis

THE IMPORTANCE OF SAMPLE PREPARATION
Real World = Real Samples

Pesticides in Avocado – no Sample Prep

Pesticides in Avocado – with Sample Prep
Sample Analysis Workflow Diagram in Contaminant Testing

- Sample Collection/Sampling
- Sample Preparation
- Sample Analysis
- Data Handling
- Archiving
- Information to customer
- Report Generation
Why is Sample Preparation Required?

- **Concentration**: Target analyte(s) not concentrated enough for quantitative detection
- **Compatibility**: Sample is not compatible with or would be harmful to your chromatographic system
- **Cleanliness**: Sample matrix components will interfere with the analysis

*Sample preparation to remove the matrix components will be our main focus*
## Possible Effects of Food Matrix Components

<table>
<thead>
<tr>
<th>Issue</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor peak shapes, co-elution, no resolution</td>
<td>Difficulty in identifying, quantifying components</td>
</tr>
<tr>
<td>Mechanical issues (particulates, blockages)</td>
<td>LC/GC column lifetime issues</td>
</tr>
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<td>Increased instrument downtime</td>
<td>Reduced productivity, increase in sample run time / cost</td>
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<tr>
<td>Interferences</td>
<td>Ion suppression in LC-mass spectrometry</td>
</tr>
<tr>
<td>Overall lower sensitivity</td>
<td>Inability to meet detection limits</td>
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Striking the Right Balance in Sample Preparation

![Graph showing the relationship between Effort & Investment and Quality of Results. The graph illustrates a curve that peaks at Just Right, with Ideal and Realistic markers.]
Food Contaminant Analysis

SAMPLE PREPARATION OPTIONS
Sample Prep Options: An Overview

- Direct injection
- Dilute & Shoot
- Filtration
- Liquid-liquid extraction (LLE, SLE)
- QuEChERS
- Solid phase extraction (SPE)
- MIPs and Immunoaffinity Columns
Dilution (Dilute & Shoot)

Simple sample dilution

Advantages

• Fast and easy
• High throughput

Limitations

• Interferences are not removed
• Concentration is reduced
• Instrument and column contamination
• Matrix interferences – ion suppression or poor peak shapes
Filtration

• Basic sample prep method for ALL kinds of samples
• Can be the 1st choice of sample prep or 2nd secondary step
• Mechanical filtration for visible interference removal
  – Agilent Captiva Premium syringe filters
  – Syringeless filters (filter vials)
  – Agilent Captiva (cartridge and 96-well plate formats)
  – Agilent Captiva ND (cartridge and 96-well plate formats)

• Mechanical filtration + extraction by sorbent for lipid removal
  – Agilent Captiva ND Lipids (cartridge and 96-well plate formats)
Sample Preparation: Liquid-Liquid Extraction

Advantages

- Inorganic salts easily removed
- Short Method development time
- Low Cost

Disadvantages

- Labor intensive
- Large volumes of organics
- Difficult to automate
- Variable results
- Expensive, clean glassware
- Emulsion formation

[Image of a liquid-liquid extraction setup]
Solid Supported Liquid-Liquid Extraction (SLE)

- Extraction mechanism: same as traditional liquid-liquid extraction (LLE)
- Simple, time-saving process
  - Apply aqueous sample to the solid bed
  - Extract with water-immiscible solvent (MTBE, dichloromethane, ethyl acetate)
  - Analyze extract or evaporate and reconstitute as needed
- Convert LLE methods to SLE to save time and money, and increase throughput
Alder-Method with Chem Elut

Extraction & cleanup of samples

1. Put 10 g of homogenized sample in a centrifuge tube.
2. Add water to obtain in total 10 ml water.
3. Add 20 mL methanol.
4. Disperse with Ultra Turrax for 2 min.
5. Centrifuge 2 min. Add 5mL of a solution containing 20% NaCl to 15 mL of supernatant and mix.
6. Soak a ChemElut cartridge with the mixture.
7. Elute with 4 x 6 ml dichloromethane.
8. After evaporation reconstitute in methanol.
Solid Supported Liquid-Liquid Extraction (SLE): Benefits

- No emulsions → easier extractions
- No special glassware → lower cost per sample
- Less time, minimal method development → faster implementation
- Reduced technique dependence → better ruggedness
- Increased reproducibility → better results
- Automatable → enables batch processing
QuEChERS

- **Quick**, **Easy**, **Cheap**, **Effective**, **Rugged**, **Safe** sample prep
- Widely adopted as sample prep for food testing due to its ease-of-use. If you can weigh, pipette and shake, you can prepare samples by QuEChERS.
QuEChERS – Easy as 1-2-3

1. Weigh sample
2. Add solvent
3. Shake
4. Add salts
5. Add internal standard
6. Shake and centrifuge
7. Transfer extract (top) for cleanup
8. Analysis by GC or LC
9. Shake and centrifuge
10. Transfer (dilute or concentrate) to vials

LC-GC, 2008, vol. 11 issue 1
Why Choose Agilent QuEChERS?

- Lives up to its name
- Flexibility and adaptability
- Works with solids and liquids
- Perfect complement to tandem MS instruments
- Called out in many regulatory methods and norms
Solid Phase Extraction (SPE)

• Types of SPE
  – Reversed phase SPE
  – Cation exchange SPE
  – Anion exchange SPE
  – Mixed mode SPE
  – Specialty SPE

• Capabilities
  – Very selective
  – Highly clean samples
  – Wide range of applicability
  – Automation friendly
The SPE Sequence

• Condition the cartridge (Step 1)
• Apply sample (e.g. food extract, water, plasma) (Step 2)
  • Some compounds “retain”
• First wash of the cartridge, interference removal (Step 3a)
• Second wash of the cartridge, additional interference removal (Step 3b)
• Apply a different liquid to “elute” (Step 4)

✓ The extract is cleaner, in a different liquid, and typically more concentrated
Why Choose SPE?

- Flexible - match a broad spectrum of sample and target compound types to different sorbents and forms
- Wide array of formats and sorbents for lower detection limits and longer instrument uptime from cleaner extracts
- Increase sample throughput with automation-friendly formats
- Easy adoption of methods due to high number of publications and applications
How do Sample Prep Options Compare?

Solid Phase Extraction (Agilent Bond Elut)
- Often very clean, allows for trace analysis, built in concentration
- Potential for the most selectivity (and hence cleanliness)

Liquid/Solid Extraction (Agilent Chem Elut, QuEChERS)
- Relatively clean and inexpensive

Filtration (Agilent Captiva Premium Syringe Filters, Filtration Cartridges, Plates, and Vials)

Dilute and shoot (Agilent guard columns, retention gaps, and liners)
Food Contaminant Analysis

CHROMATOGRAPHY

CHALLENGES AND SAMPLE PREP SOLUTIONS
Analyte identification is challenging:

- Poor signal-to-noise from high background
- Co-elution of matrix components

Results:

- Unable to achieve required detection limits
- Lower accuracy
- Reduced confidence in identification and quantification
Solution: New Instrumentation with Higher Selectivity

Tandem mass spectrometry or high-resolution mass spectrometry combined with gas or liquid chromatography results in greater analytical selectivity.
Solution: Employ a More Selective Sample Prep Technique

Solid Phase Extraction (Agilent Bond Elut)
- Often very clean, allows for trace analysis, built in concentration
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Challenge: Response Stability Over Time

The Application: Analyzing pesticides in winter squash by GC-MS/MS using QuEChERS sample prep

The Issue: Pesticide responses vary over time
Solution: Modify Sample Preparation

Modify Sample Prep Approach: Perform an additional dSPE clean-up step

Result: More stable responses over time
### Challenge: Recoveries Not High Enough

<table>
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<tr>
<th>Veterinary Drug</th>
<th>Milk (Without Capilla)</th>
<th>Milk (Without ND-mm)</th>
<th>Pork (Without Capilla)</th>
<th>Pork (Without ND-mm)</th>
<th>Fish (Yellowtail) (Without Capilla)</th>
<th>Fish (Yellowtail) (Without ND-mm)</th>
<th>Fish (baked Eel) (Without Capilla)</th>
<th>Fish (baked Eel) (Without ND-mm)</th>
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</table>

*Recovery as % response versus neat standard in 10% Acetonitrile (10ppb level), n=6.*

*Key: 0 - 30%, 30 - 70%, 70 - 120%, > 120%*
Solution: Combining Filtration and SPE to Improve Sample Quality

Sample 5 g

- 100 mL Acetonitrile / Methanol / 0.2% meta-Phosphoric Acid (1:1:3)
  Homogenize
  Filter under vacuum by filter paper with diatomaceous earth, used 2-3 mm thick layer of Hydromatrix™ diatomaceous earth

Filtrate

- 20 mL Acetonitrile / Methanol / 0.2% meta-Phosphoric Acid (1:1:3)
  Filter under vacuum by filter paper with diatomaceous earth

- Evaporate up to 20 mL

Residue

Bond Elut Plexa 3mL, 60 mg (hydrophilically modified divinylbenzene)

- Wash with 5 mL distilled water
- Elute with 5 mL methanol
- Evaporate to dryness under 40°C
- Re-dissolve in Acetonitrile/Water (1:9) 1 mL

Condition Bond Elut Plexa with 5 mL methanol, 5 mL distilled water

Filtration using Captiva ND Lipids

LC/MS or LC/MS/MS

Sample clarity comparison – with and without Captiva ND Lipid filtration
Solution: Modified Sample Preparation Improves Recoveries

Adding the functionalized filtration step improves recoveries dramatically.
Challenge: Ion Suppression by Lipids

Analyte signal drop (blue trace) is caused by lipids in plasma sample (pink trace)

PPT followed by centrifugation

Removing lipids with Captiva ND
Lipids reduces ion suppression
• Improves data quality
• Confident peak identification
Solution: Remove Lipids and Proteins Easily

- Easily remove lipids and proteins from milk in a single sample cleanup step
- Resulting filtrate is clear and suitable for LC-MS/MS analysis
- Good detection limits, recoveries, and precision

A: 10 ng/mL sulfaguanidine
B: 100 ng/mL sulfapyridine (ISTD)
C: 10 ng/mL sulfamerazine
D: 10 ng/mL sulfaquinoxaline
Instrument maintenance time is time spent **not** running samples
Solution: Combine Sample Prep Techniques for Enhanced Matrix Removal

Clean-up by dual phase SPE after LLE

Before & After
Challenge: Unexplained Peaks in Sample

ESI-Positive Mode

Filtered 30% MeOH by Filter W 0.2µm filter
Filtered 30% MeOH by Filter M PES 0.2µm filter
Filtered 30% MeOH by Filter P PES 0.2µm filter
Unfiltered 30% MeOH
Solution: Use Certified Clean Filtration Products

ESI-Positive Mode

- Filtered 30% MeOH by Filter W 0.2µm filter
- Filtered 30% MeOH by Filter M PES 0.2µm filter
- Filtered 30% MeOH by Filter P PES 0.2µm filter
- Filtered 30% MeOH by Agilent PES 0.2µm filter
- Unfiltered 30% MeOH

The Measure of Confidence

Agilent Technologies
Food Contaminant Analysis

SUMMARY AND WRAP UP
Striking the Right Balance in Sample Preparation

Quality of Results vs. Effort & Investment

- Ideal
- Realistic
- Just Right
Sample Preparation and Food Contaminant Analyses

- A wide range of sample prep approaches are available to fit your lab’s needs
- Sample prep is a powerful tool in addressing common chromatography data challenges
- Matching the right sample prep to the challenge can improve your data, productivity, and throughput
- Agilent offers a range of products ideally suited for food contaminant analysis
1. Filtration (Captiva)
   - Basic particulate removal from ALL kinds of samples
   - Useful when additional step of lipid content removal is needed

2. Solid supported liquid extraction, SLE (Chem Elut)
   - Increased productivity using liquid/liquid extraction principle and the concept of automation
   - Ideal for aqueous samples

3. QuEChERS (Agilent QuEChERS)
   - Easy-to-use sample preparation for food testing, solid samples, whether water-rich or not, like vegetables, fruits, meat, seafood, etc.

4. Solid Phase Extraction, SPE (Bond Elut)
   - Ultra clean sample preparation for analysis when high selectivity and sensitivity are required such as trace level analysis or to protect your highly sensitive instruments
Additional Resources and Application Support

Reference Materials and Guides:

Agilent QuEChERS Application Notebook:

Agilent Sample Preparation Catalog:

Agilent “Sample Preparation Fundamentals for Chromatography”, a SPP primer handbook:
www.agilent.com/chem/SamplePrepBook

Agilent Sample Preparation portfolio brochure:

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* North America
Acknowledgements

Agilent applications chemists, product managers, and technical support colleagues who contributed to this presentation.
Questions