Method Development in the Use of Solid Phase Microextraction for the GC-MS Analysis of Pesticide Residues in Baby Food

Tyler Young (summer intern), Katherine Stenerson, Len Sidisky, Yong Chen, Bob Shirey, Jennifer Claus

Presented by: Jennifer Claus
Pittcon: March, 2017
What is Solid Phase Microextraction? (SPME)

- Solvent-free extraction technique for nearly any sample or matrix
- Alternative to more expensive and time-intensive techniques
- Non-destructive to sample
- Reusable (100+ times)
- Inexpensive
- Fast
- Easy to automate
What is an Over-Coated SPME Fiber?

- **DVB**: Divinylbenzene
- **Adsorbent coating**
- Can extract polar and non-polar analytes
- Has limited number of binding sites

- **DVB with polydimethylsiloxane (PDMS) overcoating**
- Small molecules can migrate through PDMS to DVB layer
- Macromolecules not able to migrate well through PDMS layer

 Why Over-Coat an Adsorbent SPME Fiber?

<table>
<thead>
<tr>
<th>Matrix Components</th>
<th>Reduced Active Site Competition</th>
<th>Fiber Durability</th>
</tr>
</thead>
<tbody>
<tr>
<td>• To allow immersion into heavy/complex matrix</td>
<td>• Reduces competition between matrix and analytes for adsorptive layer</td>
<td>• Overcoating makes fiber more physically robust</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>More Effective Washing</th>
<th>Retention of Analytes</th>
<th>Method Ruggedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Post extraction wash step to remove matrix</td>
<td>• Higher capacity due to less matrix coextraction.</td>
<td>• Improved method performance &amp; durability, less maintenance and fiber exchange.</td>
</tr>
</tbody>
</table>

Expand use of immersion SPME to high background samples
Images of End Cuts of PDMS-DVB Fibers with SEM

Overcoated Fiber Images

Standard Fiber Images

SEM Images courtesy of E.A Sousa-Silva University of Waterloo
Using the Overcoated (OC) DVB SPME Fiber

Objective 1
Analysis of ppb levels of pesticide residues in pureed baby food (peas and prunes) by GC-MS/SIM

Objective 2
Compare standard and OC fibers for extraction efficiency and precision at 10 ng/g spiking level

Objective 3
Compare standard and OC fibers for mechanical stability and method ruggedness; repeated extractions

Application: Pesticides in Baby Food
Determination of Method Parameters

Pureed peas and prune baby food, spiked at 10 ng/g with pesticides equilibrated minimum of 3 hours prior to analysis

1. Sample additives
   - Sample dilution
   - pH adjustment
   - Salt addition

2. Temperature
   - Equilibration/Extraction

3. Post-extraction wash
   - Wash solvent
   - Wash time
# Pesticides Studied

- Listed in EU directive 2006/125/E for baby food

<table>
<thead>
<tr>
<th>name</th>
<th>CAS #</th>
<th>log P</th>
<th>class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeton S-Methyl</td>
<td>8065-48-3</td>
<td>1.4</td>
<td>OP</td>
</tr>
<tr>
<td>Terbufos</td>
<td>13071-79-9</td>
<td>3.65</td>
<td>OP</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>118-74-1</td>
<td>5.5</td>
<td>OC</td>
</tr>
<tr>
<td>Nitrofen</td>
<td>1836-75-5</td>
<td>4.62</td>
<td>nitrophenylether</td>
</tr>
<tr>
<td>Aldrin</td>
<td>309-00-2</td>
<td>7.4</td>
<td>OC</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>60-57-1</td>
<td>6.2</td>
<td>OC</td>
</tr>
<tr>
<td>Endrin</td>
<td>72-20-8</td>
<td>5.3</td>
<td>OC</td>
</tr>
<tr>
<td>Cadasufos</td>
<td>95465-99-9</td>
<td>3.9</td>
<td>OP</td>
</tr>
<tr>
<td>Ethoprophos</td>
<td>13194-48-4</td>
<td>3.2</td>
<td>OP</td>
</tr>
<tr>
<td>Fipronil</td>
<td>120068-37-3</td>
<td>4.5</td>
<td>phenyl pyrazole</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>76-44-8</td>
<td>4.78</td>
<td>OC</td>
</tr>
</tbody>
</table>

- Spiking level for accuracy and precision: 10 ng/g
- Quantitation against 5-point cal curve in pea or prune matrix and extracted by SPME
- No internal standard used
SPME Method Optimization

Sample dilution
- Decreases viscosity
- Used pH 7 phosphate buffer

Addition of Salt
- Improved reproducibility for many pesticides

Extraction/Equil. Temperature
- Increased from 30°C to 50°C
- Improved linearity
- Improved accuracy
- Improved reproducibility

Post Extraction Wash
- 30 sec water wash; no loss in pesticide response
- Removed residual sample
- Increased fiber life
Sample Dilution

- Helps with extraction of highly viscous samples
- Improves reproducibility
- Fast agitation (600 rpm) during incubation step to help produce homogeneous sample

- Pureed peas spiked at 10 ng/g, extraction temp 30°C
- Diluted with water

Lower % RSD with dilution

% RSD, n=3
Effect of Adding Salt to Sample Diluent

Further improvement in reproducibility

- 10 ng/g spiking level in pureed prunes, variability for 3 replicates analyses
- 25% NaCl added to phosphate buffer used to dilute sample

# pests < 20% RSD:
- Salt: 11 of 12
- No salt: 8 of 12
Post Extraction Wash

- Dipping of fiber into a wash solution after extraction of spiked pea samples
- Removes residual sample matrix on fiber
- Prolongs fiber life
- More effective with overcoated fiber than standard fiber

**Response vs Wash Times (OC fiber)**

- **Water wash solution**
- Increased wash time had little effect on analyte response
- 30 sec wash chosen for method
Final Optimized Method

**SPME Parameters**

**Fiber:** 65 µm PDMS/DVB 23 Ga, PDMS overcoat

**Sample:** 4 g pureed prune baby food, 4 mL 0.1 M sodium phosphate buffer with 25% sodium chloride, pH 7 in 10 mL vial

**Pre agitation:** 6 min at 600 rpm, 50 °C

**Extraction:** 50 °C, immersion, 30 min with agitation at 250 rpm (vial penetration 30 mm)

**Post extraction wash:** 30 sec in DI water

**Desorption:** 250 °C, 3 min (injection penetration 45mm)

**Post Bake:** 260 °C, 2 min (injection penetration 45mm)

**GC/MS Analysis Conditions**

**Instrument:** Agilent 6890/5973N GC-MS, selected ion mode

**Column:** SLB-35 ms, 20 m X 0.18 mm I.D. X 0.18 µm

**Oven:** 50 °C (3min), 5 °C/min, 270 °C (0 min)

**Carrier:** helium, 1 mL/min constant flow

**MS Conditions:** Source temp. 150 °C, Quad. temp.: 230 °C
How did the overcoated DVB fiber compare to the standard DVB fiber?
Background

Pureed peas (GC/MS-SIM)

Peak list
1. demeton S-methyl
2. ethoprophos
3. cadasufos
4. hexachlorobenzene
5. terbufos
6. heptachlor
7. aldrin
8. fipronil
9. dieldrin
10. endrin
11. nitrofen
12. fensulfothion
Background

Pureed prunes (GC/MS-SIM)

Same Y-scale

elution range of targeted pesticides

Non-OC fiber

OC fiber
Optimized SPME Method
Accuracy comparison: Overcoated (OC) vs. standard (non OC) fiber

Avg. n=5 replicates of baby food-prunes, spiked at 10 ng/g

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>OC</th>
<th>Non-OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeton-S-Methyl</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ethoprophos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadasufos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terbufos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptachlor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fensulfoton</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# of pesticides in range of 80-120% accuracy:
- OC: 10
- Non-OC: 6
**Optimized SPME Method**

Reproducibility comparison: Overcoated (OC) vs. standard (non OC)

Avg. n=5 replicates of baby food-prunes, spiked at 10 ng/g
Fiber Durability and SPME Method Ruggedness: OC vs. non-OC fiber

Testing specifics

• Used optimized SPME method for both fibers
• Repeated extractions of pureed baby food spiked at 10 ng/g
• Monitored pesticide response
• Evaluated physical condition of fiber at end of testing cycle
Pesticide response from OC and non-OC fibers over repeated extractions of spiked prune baby food.

Response dropped more rapidly on the non-OC fiber for 9 of the 12 pesticides.
Physical Evaluation of Fibers After Durability Testing….

After extraction of pureed pea baby food (approx 40 samples)

Separation of fiber from needle assembly on non-OC fiber
Physical Evaluation of Fibers After Durability Testing....

After extraction of pureed pea baby food (approx 40 samples)

Overcoated DVB fiber

Non-Overcoated DVB fiber
Conclusions

• A direct immersion SPME method using an overcoated DVB fiber was developed for pesticides from baby food
  ✓ An increased extraction temperature improved method linearity, accuracy, and reproducibility
  ✓ Addition of salt improved reproducibility
  ✓ Post-extraction fiber wash effectively increased fiber life

• In direct comparison to a non-overcoated DVB fiber
  ✓ OC fiber retained less matrix
  ✓ OC fiber demonstrated better accuracy and precision
  ✓ OC fiber showed less loss in response with repeated extractions
  ✓ OC fiber was more physically durable

• Overcoating the SPME fibers allowed us to expand use of immersion SPME to high background samples
Acknowledgements

Bob Shirey, Yong Chen, Craig Aurand, Len Sidisky, Olga Shimelis, Katherine Stenerson

Janusz Pawliszyn, Emanuela Gionfriddo

And...
All of you!

Thank you!!!