Mycotoxin Analysis Using SPE

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April 8, 2013
Agenda

• What are mycotoxins?

• Supel™ Tox SPE

• Aflatoxin

• DON and B-Tricothecenes

• Future of mycotoxin analysis
Mycotoxins

- Toxic secondary metabolites produced by fungi
- Exist in food as a result of fungal infection of crops
- Resistant to temperature, such as cooking and freezing
- Remain in the food chain in meat and dairy products
- Health Risks:
  - Cancer
  - Weakened Immune System
  - Allergens or Irritants
  - Death
Classes of Mycotoxins

- Aflatoxins

- Fusarium
  - Zearalenone (ZEA or ZON):
  - Trichotheccenes
    - Deoxynivalenol (DON)
    - Fumonisins

- Ochratoxin

- Patulin
Regulations

- Over 100 countries have regulations regarding mycotoxins in the feed industry
- Increasing awareness changing views on levels
- Export/Import industries impacted
- EU and FDA main bodies
- Many based upon current available analytical methods
*Source: Trilogy Labs, 2011
**Methods**

- **Mycotoxin Analysis**
  - **HPLC**
    - Most widely and frequently used
    - Quantitative
    - Requires *extract cleanup*
  - **GC**
    - Trichotheccenes such as Type A
    - Requires *extract cleanup*
  - **ELISA**
    - No cleanup. Dilute and shoot
    - Matrix constituents may cause false positives
  - **Test Strips (Lateral Flow)**
    - No cleanup
    - Some quantitative
    - Matrix constituents may cause false positives

- **Sample Prep**
  - **Immunoaffinity Columns**
    - Current industry standard
    - Bind and elute procedure: 3 steps
    - Specific columns for each mycotoxin
    - Often require refrigeration so as not to denature protein
  - **SPE**
    - Interference removal: 1 step or Bind and elute procedure: 3 steps
    - Specific cartridges for each mycotoxin
    - No refrigeration required
Supel Tox SPE

Two Supel Tox SPE cartridges were tested in corn, wheat, and/or raw peanut paste

• Multi-layer SPE cartridge for cleanup of aflatoxins and zearalenone (ZON) (Supel Tox AflaZea)

• Carbon-based SPE for cleanup of Trichothecenes, type B, including DON (Supel Tox DON)
Aflatoxins

Aflatoxin B₁

Aflatoxin B₂

Aflatoxin G₁

Aflatoxin G₂
Aflatoxins

Aflatoxins are commonly present in:

• Corn and corn byproducts
• Tree nuts
• Peanuts and byproducts
• Spices/fruits
Aflatoxins

Aflatoxin levels of concern:

• FDA action levels
  – 20 ppb in finished products for human consumption
  – 200 ppb in swine feeds
  – 300 ppb feeder cattle

• EU legislation
  – 10 ppb in maize to be used as ingredient in food stuffs
  – 15 ppb in groundnuts before human consumption
  – 10 ppb in nuts and dried fruit before human consumption to be used as an ingredient in food stuffs
  – 4 ppb in cereals intended for direct human consumption
  – 0.1 ppb of B1 in baby foods and processed cereal-based foods for infants and young children
### Aflatoxins

**Procedure (post-extraction to analysis)**

<table>
<thead>
<tr>
<th>Stage 1 (15 minutes)</th>
<th>Purify and Transfer (6 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Configure manifold for waste collection</td>
<td>1. Configure manifold for sample collection</td>
</tr>
<tr>
<td>2. Add 1 mL sample to 17 mL phosphate buffered saline, vortex</td>
<td>2. Mount Cartridges</td>
</tr>
<tr>
<td>3. Uncap/Mount/Drain Cartridges by gravity</td>
<td>3. Load 2 mL sample</td>
</tr>
<tr>
<td>4. Apply Reservoirs, Load Sample onto Cartridges</td>
<td>4. Elute and Collect Under Vacuum</td>
</tr>
</tbody>
</table>

**Stage 2 (15 minutes)**

<table>
<thead>
<tr>
<th>5. Rinse Interferences</th>
<th>6. Dilute Sample and Vortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Reconfigure Manifold for Sample Collection</td>
<td>Analysis</td>
</tr>
<tr>
<td>7. Elute/Collect Sample</td>
<td></td>
</tr>
</tbody>
</table>

**Stage 3 (30 minutes)**

| 8. Evaporate Sample to Dryness | |
| 9. Reconstitute Sample and Vortex | |
| 10. Transfer 0.2 mL Sample to Vial | |
| 11. Dilute Sample and Vortex | |

**Analysis**
# Aflatoxins

## Analysis of Aflatoxin (n = 3)

<table>
<thead>
<tr>
<th></th>
<th>Immunoaffinity</th>
<th>Supel™ Tox Mycotoxin SPE Cartridge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Prep Time</strong> (post-extraction to pre-analysis)</td>
<td>60 minutes</td>
<td>6 minutes</td>
</tr>
<tr>
<td></td>
<td>8 samples/day (if processing 1 at a time)</td>
<td>80 samples/day (if processing 1 at a time)</td>
</tr>
<tr>
<td><strong>Ease of Use</strong></td>
<td>• Large volumes of liquid</td>
<td>• Small volumes of liquid</td>
</tr>
<tr>
<td></td>
<td>• Controlled drop rates</td>
<td>• Vacuum filtration used</td>
</tr>
<tr>
<td></td>
<td>• Numerous complicated steps</td>
<td>• Steps few and not complicated</td>
</tr>
<tr>
<td></td>
<td>• Additional buffer salts required</td>
<td>• No additional reagents required</td>
</tr>
<tr>
<td></td>
<td>• Must be refrigerated, brought to room temp before use</td>
<td>• Column does not require special storage conditions</td>
</tr>
</tbody>
</table>

**Increase Throughput:** Supel Tox can analyze 10 times more samples/day than immunoaffinity
Aflatoxins

HPLC Conditions

column: Discovery® C18, 15 cm x 2.1 mm, 5 µm particle size (50495521)

mobile phase: (A) water; (B) acetonitrile; (C) methanol; (74:13:13, A:B:C) with 0.780 g potassium bromide and 230 µL nitric acid

flow rate: 0.40 mL/min

column temp.: 35 ºC

detector: FLD, ex 360, em 440nm FL, KOBRA cell

injection: 40 µL

Analysis of Aflatoxin in Corn Meal
Using Supel Tox SPE Cartridge

1. Aflatoxin G2
2. Aflatoxin B2
3. Aflatoxin G1
4. Aflatoxin B1

Analysis of Aflatoxin in Peanut Paste
Using Immunoaffinity Column

1. Aflatoxin G2
2. Aflatoxin B2
3. Aflatoxin G1
4. Aflatoxin B1
Aflatoxins

- Better analyte recovery than IAC
- Lower %RSD than IAC
- More robust method with less variability
Aflatoxins Using Ascentis® Express

Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm

1. AFLATOXIN G₂
2. AFLATOXIN G₁
3. AFLATOXIN B₂
4. AFLATOXIN B₁

AFLATOXIN B1&B2 (4 PPB), G1&G2 (16 PPB) STANDARD
SPIKED CORN MATRIX, B1&B2 (4 PPB), G1&G2 (16 PPB)

SPIKED WHEAT MATRIX, B1&B2 (4 PPB), G1&G2 (16 PPB)

SPIKED PEANUT PASTE, B1&B2 (4 PPB), G1&G2 (16 PPB)
Aflatoxins

Analyte Recovery and %RSD of Aflatoxins from Wheat, Corn, and Peanut Paste (n = 3)

- RECOVERY >80%
- %RSD < 5%

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Deoxynivalenol (DON) and Trichothecenes, Type B

Deoxynivalenol

Nivalenol

15-Acetyldeoxynivalenol

3-Acetyldeoxynivalenol
DON and B-Trichothecenes

DON is commonly present in:

- Wheat and wheat products
- Corn and corn products
- Barley and malted barley
- Sorghum
- Oats
DON and B-Trichothecenes

DON’s levels of concern:

FDA advisory levels -
- 1 ppm in finished products for human consumption
- 1 ppm in swine feeds
- 5 ppm in beef cattle and poultry feeds

EU legislation
- 200 ppb in processed cereal-based baby and infant food
- 1250 ppb in unprocessed cereals (excluding durum wheat, oats and corn)
- 1750 ppb in durum wheat, oats, corn
- 750 ppb in cereals intended for direct human consumption, cereal flower
- 500 ppb in bread, pastries, breakfast cereal
DON and B-Trichothecenes

Extract 25 g ground sample with 100 mL 84:16 acetonitrile:water for 3 min and filter. Spiked at 2ppm with mix of B-trichothecenes.

Load 2 mL of sample onto Supel Tox DON cartridge

Elute with two 5 mL aliquots of extraction solvent

Evaporate to dryness and reconstitute

Proceed with LC-UV analysis
DON

HPLC Conditions:
- column: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm
- mobile phase: (A) water; (B) acetonitrile; (C) methanol; (92:4:4, A:B:C)
- flow rate: 0.400 mL/min
- column temp.: 35 ºC
- detector: UV 220 nm; 360 nm
- injection: 40 µL

DEOXYNIVALENOL STANDARD, 2 PPM

NEAT SAMPLE, 2 PPM

CORN MATRIX BLANK

SPIKED CORN MATRIX, 2 PPM
DON

Analyte Recovery and %RSD of DON from Wheat, Corn, and Peanut Paste (n = 3)

- RECOVERY >70%
- %RSD < 5%

<table>
<thead>
<tr>
<th></th>
<th>Neat</th>
<th>Corn</th>
<th>Wheat</th>
<th>Peanut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>79.00%</td>
<td>90.80%</td>
<td>81.30%</td>
<td>89.40%</td>
</tr>
<tr>
<td>RSD</td>
<td>2.60%</td>
<td>2.50%</td>
<td>3.90%</td>
<td>1.80%</td>
</tr>
</tbody>
</table>
DON and B-Trichothecenes

B-Trichothecenes 2 ppm Standard (in solvent)

column: Ascentis Express C18, 10 cm x 3.0 mm, 2.7 µm
mobile phase: (A) 92:4:4, water:acetonitrile:methanol; (B) acetonitrile
flow rate: 0.8 mL/min
temp.: 35 ºC
det.: UV 220 nm, 16; ref 360 nm, 100
injection: 40 µL
gradient:

<table>
<thead>
<tr>
<th>Min</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8.0</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>92</td>
<td>8</td>
</tr>
</tbody>
</table>

1. Nivalenol
2. DON
3. 15-AcetylDON
4. 3-AcetylDON
Conclusion

- Supel Tox AflaZea sample cleanup significantly decreased sample prep time and increased reproducibility compared to traditional immunoaffinity cleanup.

- Supel Tox DON provided a quick and simple method that produced sufficient and reproducible analyte recoveries for a variety of matrices.
Future of Mycotoxins

- Increasing awareness is prompting more stringent regulations

- Variety of matrices and lowering detection limits pose challenges for universal methods

- Supel Tox SPE addresses some of current market needs

- Goal of one analytical method for all mycotoxins
Acknowledgements

- Ken Espenschied
- Michael Ye
- Mati Sarker
- Jennifer Claus
- Olga Shimelis
- Marketing and R&D Staff