Mycotoxins & Food Safety

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VICAM, A Waters Business
Mycotoxins

- **Mykes:** Greek for fungus/mold
- **Toxicum:** Latin for poison/toxin

- **Mycotoxins** are metabolic products of food spoilage fungi that induce toxic responses when consumed by animals or people.

- **Hundreds** of mycotoxins have been identified; They will fall into many different chemical classes, and induce a wide variety of toxic responses.
Food & Agricultural Products Affected by Mycotoxin Contamination

- Tree Nuts
- Peanuts
- Grain
- Wine
- Coffee
- Flour Milling
- Cereals
- Feed
- Oats
- Ethanol
- Dairy
- Rice
- Botanicals
- Spices
- Snack Foods
- Pet Food
### Mycotoxins

<table>
<thead>
<tr>
<th>Selected Molds That Produce Toxins</th>
<th>AFLATOXINS B1, B2, G1, G2, M1</th>
<th>DEOXYNIVALENOL</th>
<th>FUMONISINS B1, B2, B3</th>
<th>OCHRATOXIN A</th>
<th>T-2/HT-2</th>
<th>ZEARALENONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus, Aspergillus parasiticus</td>
<td>Fusarium graminearum</td>
<td>Fusarium verticillioides</td>
<td>Aspergillus ochraceus Penicillium verrucosum</td>
<td>Fusarium and other mold species</td>
<td>Fusarium graminearum</td>
<td></td>
</tr>
</tbody>
</table>

### Foods Susceptible to Contamination

| Maize, groundnuts, nuts, cottonseed, copra, spices, milk, wheat, oats, barley, and rice | Maize, wheat, barley, malted barley, and oats | Maize and other cereal grains | Maize, wheat, barley, beer, oats, sorghum, dried vine fruits, wine, coffee, and cocoa | Maize, wheat, barley, oats, rice, sorghum, and other cereal grains | Maize, wheat, barley, grain, and sorghum |

### Health Effects

- Liver cancer and damage
- Imunosuppression
- Decreased milk and egg production
- Damage to digestive tract, bone marrow, spleen, reproductive organs
- Weight loss, vomiting, and feed refusal
- Cancer in rats
- Brain decay in horses
- Lung congestion in pigs
- Human Esophageal Cancer
- Kidney damage and cancer
- Imunosuppression
- Skin and oral lesions in livestock and humans
- Alimentary toxic aleukia in humans
- Considered 10x more toxic than DON
- Negatively impacts reproduction, fetal development, and the health of newborns
- Causes feminization in animals at 1 ppm
Where does LC/MS mycotoxin detection ‘fit’?

- Diverse global regulatory limits
  - Aflatoxin B1 regulations:
  - Aflatoxin M1 regulations: 0.5 ppb (US); 0.05 ppb (EU)

- Domestic Food and Agriculture Regulations

- Internal Quality Standards

- Verify Inbound Raw Materials & Finished Products

- Crop Survey

- Rapid Test Result Confirmation

- Research
Advantages With LC/MS

- High Sensitivity and Selectivity
- Multiple Mycotoxins Analyzed Simultaneously
- Improved Laboratory Efficiency
- Throughput
- Savings of Technician Time
- Reduced Solvent Use
- Detects Mycotoxins that don’t absorb or fluoresce
Immunoaffinity Column Sample Cleanup

Immunoaffinity columns may be used with both benchtop Fluorometer, LC or LC/MS system.
Characteristics of Mass Spectrometry

- Instrument can be used for other analysis besides mycotoxins (such as pesticides)
- Good for multiple mycotoxin analysis
- Good for confirmation of mycotoxins

- Must have a laboratory environment
- Triple Quad (MS/MS) gives best results (lower limits of detection and confirmation)
- Best for well trained scientists
- Need to accurately measure and store mycotoxins in order to get accurate results
- Need to use matrix matched calibration standards or internal calibrators to adjust for matrix affects
LC-MS (Liquid chromatography- Mass spectrometry)

**Advantage of MS** - It can detect molecules that do not fluoresce or absorb
Myco 6in1+ Immunoaffinity Column

1. Targets six major groups of mycotoxins: Aflatoxins, DON, Fumonisins, OTA, T2/HT2, Zearalenone
2. Can be used to prepare samples: HPLC, LC/MS, UPLC/MS
3. Patented Myco6in1 launched 2007
4. Improved Myco6in1+ launched 2013, better recovery + NIV

Sample → Sequential extraction with water and methanol → Immunoaffinity column clean up → Analysis
Myco6in1+ with HPLC

Basic eluent/sample flow for Mycotoxin Setup

- Photodiode Array Detector
- PhCR
- PCRM
- HPLC Column
- Reagent Manager for OPA
- Fluorescence Detector
- Waters Separation Module with Sample Manager Column Heater
- Waste
- Empower Software
FLD chromatogram of nine separate mycotoxins under optimal HPLC conditions (10 ng/g AFB1 and AFG1, 3 ng/g, AFB2 and AFG2, 25 ng/g OTA, 250 ng/g ZEA, FB1, FB2 and FB3).

F. Soleimany et al.
PDA chromatogram of mycotoxins under optimal HPLC conditions (HT-2 toxin 250 ng/g, T-2 toxin and DON 500 ng/g).

F. Soleimany et al.
Which Mass Spec Do I Choose For Multiple Mycotoxin Detection?

QDa

OR

Xevo TQ-S Micro
UPLC with QDa Single Quad Detection
UPLC & MS conditions

**UPLC conditions**
- **LC System:** ACQUITY UPLC I-Class
- **Runtime:** 12 min
- **Column:** CORTECS C\textsubscript{18}
  1.6 µm, 2.1 x 100 mm
- **Mobile phase A:** 2 mM ammonium acetate with 0.1% formic acid in water
- **Mobile phase B:** 2 mM ammonium acetate with 0.1% formic acid in methanol
- **Flow rate:** 0.4 mL.min\textsuperscript{-1}
- **Injection volume:** 10 µL

**Gradient:**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>99.0</td>
<td>1.0</td>
</tr>
<tr>
<td>7.00</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>10.0</td>
<td>1.0</td>
<td>99.0</td>
</tr>
<tr>
<td>11.5</td>
<td>1.0</td>
<td>99.0</td>
</tr>
<tr>
<td>11.6</td>
<td>99.0</td>
<td>1.0</td>
</tr>
<tr>
<td>14.00</td>
<td>99.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**MS conditions**
- **MS system:** ACQUITY QDa Detector
- **Ionization mode:** ESI±
- **Desolvation temp.:** 600 °C
- **Capillary voltage:** Default (0.8 kV)
- **Sampling rate:** Default (5 Hz)
- **SIR channels:** See Table 1
## Mycotoxin MS parameters

Table 1. The 12 mycotoxins with experimental parameters.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Abbreviation</th>
<th>RT (min)</th>
<th>SIR (m/z)</th>
<th>Cone voltage (V)</th>
<th>Calibration range (µg.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nivalenol</td>
<td>[NIV-H₂O+H⁺]</td>
<td>2.2</td>
<td>295.0</td>
<td>15</td>
<td>468.75 to 5625.00</td>
</tr>
<tr>
<td>2. Deoxynivalenol</td>
<td>[DON+H⁺]</td>
<td>2.9</td>
<td>297.0</td>
<td>10</td>
<td>468.75 to 5625.00</td>
</tr>
<tr>
<td>3. Aflatoxin G2</td>
<td>[AFG2+H⁺]</td>
<td>5.8</td>
<td>331.0</td>
<td>20</td>
<td>0.625 to 7.50</td>
</tr>
<tr>
<td>4. Aflatoxin G1</td>
<td>[AFG1+H⁺]</td>
<td>6.2</td>
<td>329.0</td>
<td>20</td>
<td>0.625 to 7.50</td>
</tr>
<tr>
<td>5. Aflatoxin B2</td>
<td>[AFB2+H⁺]</td>
<td>6.5</td>
<td>315.0</td>
<td>20</td>
<td>1.250 to 15.00</td>
</tr>
<tr>
<td>6. Aflatoxin B1</td>
<td>[AFB1+H⁺]</td>
<td>6.8</td>
<td>313.0</td>
<td>20</td>
<td>0.625 to 7.50</td>
</tr>
<tr>
<td>7. HT2 toxin</td>
<td>[HT2+Na⁺]</td>
<td>8.2</td>
<td>447.0</td>
<td>15</td>
<td>31.250 to 375.00</td>
</tr>
<tr>
<td>8. Fumonisin B1</td>
<td>[FB1+H⁺]</td>
<td>8.3</td>
<td>722.0</td>
<td>20</td>
<td>500.000 to 6000.00</td>
</tr>
<tr>
<td>9. T2 toxin</td>
<td>[T-2+NH₄⁺]</td>
<td>8.6</td>
<td>484.0</td>
<td>15</td>
<td>31.250 to 375.00</td>
</tr>
<tr>
<td>10. Ochratoxin A</td>
<td>[OTA+H⁺]</td>
<td>8.8</td>
<td>404.2</td>
<td>20</td>
<td>62.500 to 750.00</td>
</tr>
<tr>
<td>11. Zearalenone (negative mode)</td>
<td>[ZEA-H⁻]</td>
<td>8.8</td>
<td>317.0</td>
<td>20</td>
<td>1.875 to 22.50</td>
</tr>
<tr>
<td>12. Fumonisin B2</td>
<td>[FB2+H⁺]</td>
<td>9.0</td>
<td>706.0</td>
<td>20</td>
<td>125.000 to 1500.00</td>
</tr>
</tbody>
</table>
An example of the chromatography achieved is shown in Figure 2, where the maize snack food was fortified to the regulatory limits. Satisfactory sensitivity was reported for each of the analytes, and excellent Signal-to-Noise ratios (S/N) were achieved. All four aflatoxins, plus the additional eight mycotoxins can readily be detected by LC coupled with mass detection.

![Chromatogram Image]

**Figure 2.** Processed maize food sample fortified at the displayed concentrations (EU regulatory limits). Chromatographically resolved peaks (normalized) were detected with excellent S/N ratios at legally permitted levels.
Myco6in1+ single extraction procedure

Lattanzio et al, Presented at RAFA meeting Nov 5-8, 2013, Prague, Czech Republic
New Single (Sequential) Extraction Procedure

- 10 g sample
- Add 40 mL water
- Extraction by blending for 2 min
- Add 60 mL methanol
- Extraction by blending for 2 min
- Filter the extract through Whatman no. 4 filter paper
- Take 5 mL of extract (equivalent to 0.5 g sample) and evaporate till reduce the volume to approx 2 mL
- Add 5 mL phosphate buffer
- Pass the sample through the Myco6in1+ column
- Wash the column with 10 mL water
- Elute the toxins with 3 mL methanol followed by 2 mL water
- Dry the eluate
- Reconstitute the residue with an appropriate volume of LC mobile phase and inject
Total ion chromatogram (sum of MRM transitions) of a maize sample extract spiked with: 1000 μg/kg DON; AFG2, AFB2 2.5 μg/kg, AFG1 7.5 μg/kg, AFB1 12.5 μg/kg; FB1 800 μg/kg, FB2 200 μg/kg, HT-2, T-2, ZEA 100 μg/kg, OTA 20 μg/kg.
Recoveries, repeatability and LOD
Maize, Wheat, Maize snacks and Corn Flakes

### Recoveries and Repeatability

<table>
<thead>
<tr>
<th></th>
<th>NIV</th>
<th>DON</th>
<th>AFB(_1)</th>
<th>FB(_1)</th>
<th>FB(_2)</th>
<th>HT2+T2</th>
<th>ZEA</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAIZE</strong></td>
<td>96 (10)</td>
<td>83 (8)</td>
<td>89 (11)</td>
<td>109 (18)</td>
<td>105 (12)</td>
<td>81 (20)</td>
<td>75 (9)</td>
<td>76 (15)</td>
</tr>
<tr>
<td><strong>WHEAT</strong></td>
<td>77 (8)</td>
<td>73 (9)</td>
<td>84 (8)</td>
<td>-</td>
<td>-</td>
<td>80 (14)</td>
<td>67 (13)</td>
<td>69 (19)</td>
</tr>
<tr>
<td>Maize snacks</td>
<td>96 (12)</td>
<td>93 (16)</td>
<td>82 (14)</td>
<td>63 (12)</td>
<td>114 (14)</td>
<td>95 (13)</td>
<td>68 (14)</td>
<td>81 (16)</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>84 (5)</td>
<td>88 (16)</td>
<td>91 (10)</td>
<td>97 (14)</td>
<td>104 (13)</td>
<td>88 (13)</td>
<td>80 (10)</td>
<td>60 (11)</td>
</tr>
</tbody>
</table>

### Average Detection Limits (µg/kg)

<table>
<thead>
<tr>
<th>NIV</th>
<th>DON</th>
<th>AFG(_2)</th>
<th>AFG(_1)</th>
<th>AFB(_2)</th>
<th>AFB(_1)</th>
<th>HT2</th>
<th>T2</th>
<th>FB(_1)</th>
<th>FB(_2)</th>
<th>ZEA</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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Myco6in1 and Myco6in1+ References


Summary

• Myco 6in1+ Column concentrates analytes simultaneously
• Choose single quad or tandem mass spec
• Improves laboratory throughput
• Time and cost savings
Thank you!