Quantitative Determination of the Mycotoxin Oosporein in Maize Grain and Soybean Seed by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry

Fred Claussen (claussen@epilabs.com)

Abstract

The objective of this project was to develop reliable and accurate methods for the determination of oosporein, a mycotoxin produced by the fungus Diplodia natalensis, in maize grain and soybean seed. The validated method included a centrifugal mill for homogenization and chromatographic methods for its quantitative determination. The validated method is now routinely employed to support toxin screens of laboratory diets formulated with genetically modified (GM) seeds.

Test Matrices

Soybean Seed Sample (400 ppb)

Table 1. Assay Precision and Mean Spiking Recovery

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Assay Precision (Relative Standard Deviation)</th>
<th>Mean Spiking Recovery (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize Grain</td>
<td>8.5%</td>
<td>120</td>
</tr>
<tr>
<td>Soybean Seed</td>
<td>8.5%</td>
<td>120</td>
</tr>
</tbody>
</table>

Figure 1a. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (200 ppb)

Figure 1b. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (400 ppb)

Figure 1c. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (1000 ppb)

Figure 1d. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (2000 ppb)

Figure 1e. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (5000 ppb)

Figure 1f. UHPLC-MS/MS Chromatogram: Laboratory Fortified Sample (10000 ppb)

Figure 2. Product Ion Spectrum of Oosporein

Figure 3. Calibration Curve of Oosporein

Figure 4. Linear Calibration Curve of Oosporein

Figure 5. Oosporein Standard Range

Results/Discussion

The chemical structure of oosporein:

Chemical and Physical Properties:

- Molecular formula: C_{18}H_{22}O_{8}, 346 g/mol
- Highly polar molecule containing four acidic functional groups
- Precursor Ion: m/z 305, Quantitation Ion: m/z 277, Qualifier Ion: m/z 261
- Testing matrices: Maize grain and soybean seed
- The validated method included a centrifugal mill for homogenization and chromatographic methods for its quantitative determination.
- The validated method is now routinely employed to support toxin screens of laboratory diets formulated with genetically modified (GM) seeds.

Equipment

- Centrifugal mill
- UHPLC-MS/MS (Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry)
- MRM (Multiple Reaction Monitoring)
- MS Polarity: Negative
- MS Mode: Multiple Reaction Monitoring (MRM)
- Injection Volume: 5 - 10 µL
- HPLC Gradient: Linear
- Mobile Phase Gradient Program:
  - Time (min.) %A %B
  - 0.00 2 98
  - 0.50 2 98
  - 1.00 5 95
  - 2.50 5 95
  - 3.00 0 100
  - 5.00 0 100
- Calibration Range: 5 - 100 ng/mL
- Linearity:
  - 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80
- Voltage:
  - Cone: 30 V
  - Collision: 70 V
  - Ion Type: ES-
- Calibration stock solution: 100 µg/mL
- Calibration range: 5 - 100 ng/mL
- Calibration curve: Linear

Test Portion Extraction

The validated method was optimized for the extraction of oosporein from maize grain and soybean seed. The validated method is now routinely employed to support toxin screens of laboratory diets formulated with GM seeds.

References