Mycotoxins: Sample Preparation and Analysis

Matrix of the Month

Oktober 2014:

Aflatoxin B/G in Pumpkin Seeds

Do you have a special matrix that we should test for mycotoxins? Please let us know and write an e-mail to info@LCTech.de!

Protocol

Spike 20 g homogenized pumpkin seed with 2 g sodium chloride and extract them with 100 mL methanol/water (80/20 (v/v)) and 50 mL n-hexane for 5 to 10 minutes. Filtrate the extract and centrifuge it for 10 minutes at 2000 xg facilitating the phase separation between the diluted/aqueous and the n-hexane phase. Dilute 10.5 mL of the aqueous phase (the lower phase) with 64.5 mL PBS buffer (pH 7.2) and filtrate the diluted extract with a fibre glass filter to delete any turbidities.

Apply 50 mL to the immunoaffinity column AflaCLEAN or Afla-OtaCLEAN and rinse afterwards the sample reservoir with 10 mL of de-ionised water. Apply this washing solution onto the IAC column too.

Dry the column and elute with methanol. Pay attention that the methanol incubates within the column bed for 5 minutes to ensure that the antibody is entirely denaturated and the toxin is released.

Dilute and measure the eluate to HPLC conditions.

HPLC Conditions

Aflatoxin B, G

HPLC: Isocratic
Column oven: 36 °C
Separation column: RP C18 (e.g. P/N 10522)
Flow rate: 1.2 mL/min, water/methanol/acetonitrile (60/30/15 (v/v/v))
Fluorescence detection with post column derivatisation (photochemical with UVE)
Excitation wavelength: 365 nm
Emission wavelength: 460 nm
### Recovery Rates

#### Content of Aflatoxins B1, B2, G1 and G2 in Pumpkin Seeds

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>B1</th>
<th>B2</th>
<th>G1</th>
<th>G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Recovery rate**</td>
<td>102</td>
<td>106</td>
<td>107</td>
<td>102</td>
</tr>
</tbody>
</table>

* Standard is set = 100 %, ** corrected with non-spiked sample

#### Chromatograms

**Aflatoxin, standard 14 ng / 2 mL**

**Pumpkin seed, spiked with 10 ppb Aflatoxin**

**Overlay of chromatograms**

Aflatoxin, standard 14 ng / 2 mL (red),
Pumpkin seed, spiked with 10 ppb Aflatoxin (black),
Pumpkin seed, not spiked (orange)

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**These LCTech products were used:**

- **AflaCLEAN**, Immunoaffinity column for Aflatoxin B1, B2, G1, G2
  - P/N 10514 / 11721
- **Afla-OtaCLEAN**, Combined column for Aflatoxin and Ochratoxin
  - P/N 11022 / 11771
- **UVE**, Photochemical reactor for the analysis of Aflatoxins
  - P/N 10519
- **HPLC column**, for mycotoxin analysis
  - P/N 10522

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Do you have further questions? Please just write an e-mail to info@LCTech.de!