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Accurate Quantitation of Regulated Mycotoxins by UHPLC-MS/MS

Dr. Thomas Glauner
EMEA LC/MS Food Segment Scientist

In co-operation with:

Elisabeth Varga, Michael Sulyok, Rainer Schuhmacher, Rudolf Krbska, Franz Berthiller
Foreword

- Joined Agilent in 2006
- PhD in Chemical Engineering
- Former applications chemist, specialized in small molecule applications
- Currently LCMS Food Segment Scientist in the EMEA Food Team
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Accurate Quantitation of Regulated Mycotoxins by UHPLC-MS/MS

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Agenda

- Mycotoxins
- EU Regulations
- Stable isotope dilution assay (SIDA) for the mycotoxins regulated in the European Union
  - Reasons
  - Approaches
  - Sample Preparation and Method
  - Results
- Summary and conclusions
Mycotoxins

Background

- myces (Greek) = fungus
- toxicum (Latin) = toxic

\[ \text{myces (Greek) = fungus} \quad \text{toxicum (Latin) = toxic} \]

- low molecular weight, toxic, secondary metabolites of fungi
- produced by e.g.:
  - Fusarium sp., Aspergillus sp., Penicillium sp.
- toxicity:
  - acute toxic, carcinogenic, mutagenic, teratogenic, estrogenic and immunotoxic effects
Mycotoxins
How many mycotoxins are there?

• Hundreds of compounds

• 2 Main classes:
  
  **Major Mycotoxins**
  • Aflatoxins
  • Ochratoxins (OTA)
  • Tricothecenes
  • Zearalenone
  • Fumonisins
  • Patulin

  **Minor Mycotoxins**
  • Ergot alkaloids
  • Citrinin
  • Cyclopiazonic acid
  • Sterigmatocystin
  • Monoliformin
  • Gliotoxin
  • Citreoviridin
  • Tremorgenic mycotoxins
  • Penicillic acid
  • Roquefortine
  • 3-Nitropropionic acid
  • Fusaproliferin

Webinar, February 7, 2012
Quantitation of regulated mycotoxins
Mycotoxins
Chemical diversity – a challenge for the sample prep

Enniatins
beauvericin
enniatin A, A₁, B, B₁
APOLAR

Fumonisins
fumonisin B₁, FB₂, FB₃,
hydrolyzed FB₁
POLAR, ACIDIC

Ergot alkaloids
ergotamin, ergocornin,
ergovalin, dihydroergosin
POLAR, BASIC

beauvericin

F₂

FB₁

ergovalin

Chemical diversity – a challenge for the sample prep
Mycotoxins
Why are they an issue?

- >25% of all agricultural commodities are contaminated with mycotoxins
- Annual losses of several hundred million tons of food worldwide
- Annual economical losses: 1 billion USD (US only)
- 100+ countries have regulations for the control of mycotoxins in food and feed
Mycotoxins
Infected food products

• Found in cereals, dried fruits, spices, grape, coffee, cocoa, fruit juices;
• Secondary contamination in milk, eggs, meat;
• Resistent to home cooking;

Global occurrence of mycotoxins between July and September 2011 in the analyzed samples

BIOMIN’s Mycotoxin Survey – 3rd Quarter Report 2011

Quantitation of regulated mycotoxins
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Agilent Technologies
Mycotoxins
Relevance for food control

- Notifications concerning mycotoxins
  - (RASFF-Annual reports 2002-2008)
Food contaminants online
FERA FC24 database access

- Free access to the RASFF alerts and notifications via FC24 database
- Registration through Agilent website
## EU Regulations

<table>
<thead>
<tr>
<th>Analytes</th>
<th>MRLs µg/kg (EC Reg. No 1881/2006)</th>
<th>Commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>0.1</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td>2.0-12</td>
<td>nuts and cereals</td>
</tr>
<tr>
<td></td>
<td>sum of aflatoxins: 4.0-15.0</td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>200</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td>500 - 1750</td>
<td>processed / unprocessed cereals, bread, pasta, breakfast cereals</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>200</td>
<td>processed maize-based baby food</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>maize-based breakfast cereals</td>
</tr>
<tr>
<td></td>
<td>1000 / 4000</td>
<td>maize / unprocessed maize</td>
</tr>
<tr>
<td>Patulin</td>
<td>10-50</td>
<td>fruit juices, apple products, baby food other than processed cereal-based</td>
</tr>
<tr>
<td></td>
<td></td>
<td>foods</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.5</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td>3.0 / 5.0</td>
<td>processed / unprocessed cereals</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>dried vine fruit</td>
</tr>
<tr>
<td></td>
<td>15 / 20 / 80</td>
<td>spices / liquorice root / extract</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>20</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>bread, biscuits, breakfast cereals</td>
</tr>
<tr>
<td></td>
<td>75-350</td>
<td>processed / unprocessed cereals</td>
</tr>
</tbody>
</table>
## FDA Regulatory guidelines

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Limit µg/kg</th>
<th>Commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins, sum</td>
<td>20</td>
<td>All foods except milk</td>
</tr>
<tr>
<td>Patulin</td>
<td>50</td>
<td>Apple juice, apple juice concentrate, apple components in processed food</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>1000</td>
<td>Finished wheat products</td>
</tr>
<tr>
<td>Fumonisins sum of B₁, B₂, B₃</td>
<td>2000</td>
<td>degemred dry milled corn products (e.g. flaking grits, corn grits, corn meal, corn flour)</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>cleaned corn intended for popcorn</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>whole of partially degemred dry milled corn products (e.g. flaking grits, corn grits, corn meal, corn flour)</td>
</tr>
<tr>
<td>Aflatoxin M₁</td>
<td>0.5</td>
<td>milk</td>
</tr>
</tbody>
</table>
Accurate quantitation of mycotoxins

Reasons

  - set maximum residue levels (MRL) for mycotoxins
- Single target versus multi-target methods

BUT:

- Electrospray ionisation (ESI)
  - matrix effects hamper accurate mass spectrometric quantification
- Quantification of regulated mycotoxins at a very high degree of accuracy is required
Accurate quantitation of mycotoxins

Reasons

  - set maximum residue levels (MRL) for mycotoxins

- Single target versus multi-target methods

BUT:

- Electrospray ionisation (ESI)
  - matrix effects hamper accurate mass spectrometric quantification

- Quantification of regulated mycotoxins at a very high degree of accuracy is required
Matrix effects in ESI-MS and quantitation

Approaches

- Dilution of the sample
  - method less sensitive

- Matrix matched calibration
  - tedious
  - differences within one commodity not compensated

- Standard addition to each sample
  - more runs
  - more costs (time and standards)

- Internal calibration
  - similar compounds (ZAN for ZEN)
  - deuterium or $^{13}$C-labelled compounds
  - until this year: only single analyte or group analyte IS-addition
  - usually associated with rather high costs
Matrix effects in ESI-MS and quantitation

Approaches

- Dilution of the sample
  - method less sensitive

- Matrix matched calibration
  - tedious
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- Internal calibration
  - similar compounds (ZAN for ZEN)
  - deuterium or $^{13}$C-labelled compounds
  - until this year: only single analyte or group analyte IS-addition
  - usually associated with rather high costs
Stable Isotope Dilution Assay (SIDA)

Aims

- Development of a method fulfilling:
  - covering all regulated mycotoxins in solid food matrices
  - providing best possible accuracy
  - easy to handle
  - cost effective

- Stable isotope dilution assay (SIDA) for LC-MS/MS
  - 11 mycotoxins
  - $^{13}$C-labelled compounds as internal standards
  - validation of the method for maize
Multiple Reaction Monitoring
Principles

Quad Mass Filter (MS1)

Spectrum with background ions (from ESI)

Q1 lets only target ion 210 pass through

Quad Mass Filter (MS2)

Chromatogram

High background

Collision Cell
Multiple Reaction Monitoring
Principles

Quad Mass Filter (MS1)

Collision Cell

Quad Mass Filter (MS2)

Spectrum with background ions (from ESI)

Q1 lets only target ion 210 pass through

Collision cell breaks ion 210 apart

Q3 monitors only characteristic fragments 158 from ion 210 for quant

Chromatogram

High background

Low background

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Quantitation of regulated mycotoxins

Agilent Technologies
Agilent G6490A QQQ system
New developments for utmost sensitivity

• Ionization and Ion Transfer Technology
  – Agilent Jet Stream Ion Generation
  – Hexabore capillary
  – Dual ion funnel (iFunnel) technology
    • Two stages for ion focusing and gas removal
    • Improvements for wide $m/z$ range transmission
    • Low capacitance

• Collision Cell
  – Hexapole field axial focusing curved collision cell
    • Tapered cell structure for increased ion acceptance at entrance
    • Reduced noise

• Improved Quad Drive Electronics
  – Improved Quad DC frequency response
  – Higher RF power capability
  – Quad drive frequency increased to 1.4 MHz
Agilent iFunnel technology
Two stage ion funnel manages the gas load

High Pressure Stage 1
Low Pressure Stage 2

Stage 1
8-12 Torr

Stage 2
1-3 Torr

Line of Sight

Offset ion funnels to prevent neutrals from going straight through to MS
Mycotoxins
Evaluation of extraction methods


Sample preparation
Universal extraction procedure

- **Sampling**
- **1. Extraction**
  - Centrifugation
- **2. Extraction**
  - Centrifugation
- **Dry Down**
- **Up-take**

- **Grind and homogenize sample + weight-in**
- **Acetonitrile:water:formic acid (80:19.9:0.1, v:v:v)**
  - 60 min at room temperature on a rotary shaker
- **Acetonitrile:water:formic acid (20:79.9:0.1, v:v:v)**
  - 30 min at room temperature on a rotary shaker
Sample preparation
Universal extraction procedure

Sampling

1. Extraction
   Centrifugation

2. Extraction
   Centrifugation
   +ISTD

Dry Down

Up-take

grind and homogenize sample + weight-in

acetonitrile:water:formic acid (80:19.9:0.1, v:v:v)
60 min at room temperature on a rotary shaker

acetonitrile:water:formic acid (20:79.9:0.1, v:v:v)
30 min at room temperature on a rotary shaker
Sample preparation
Universal extraction procedure

**Sampling**
- grind and homogenize sample + weight-in
  - acetonitrile:water:formic acid (80:19.9:0.1, v:v:v)
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**1. Extraction**
- acetonitrile:water:formic acid (20:79.9:0.1, v:v:v)
- 30 min at room temperature on a rotary shaker

**Centrifugation**

**2. Extraction**

**Centrifugation**

**Dry Down**

**+ISTD**

**Up-take**

**Agilent 6490 QqQ**
Stable Isotope Dilution Assay (SIDA)

HPLC method

Agilent 1290 Infinity LC system consisting of:
- binary pump
- wellplate sampler
- column compartment
- diode array detector (not used)

**HPLC method**

**Separation column:** ZORBAX Eclipse Plus C-18 RRHD column, 100 x 2.1 mm, 1.8 µm @ 30°C

**Mobile phase:**
- A: 5 mM HCOONH₄ + 0.1% formic acid
- B: methanol + 5 mM HCOONH₄ + 0.1% formic acid

**Flow:** 0.35 ml/min

**Gradient:**
- 0.00 min 30 % B
- 0.50 min 30 % B
- 8.00 min 100 % B
- 9.50 min 100 % B
- 9.60 min 30 % B

**Inj.Vol.:** 3 µl
Stable Isotope Dilution Assay (SIDA)
MS method

Spray chamber conditions:
- Gas temp.: 140°C
- Dry gas: 16 l/min
- Nebulizer: 25 psi
- Sheath gas temp: 350°C
- Sheath gas flow: 11 l/min

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>CapVoltage:</td>
<td>4000 V</td>
</tr>
<tr>
<td>Nozzle voltage</td>
<td>0 V</td>
</tr>
</tbody>
</table>

Automatic setup of MRM tables based on selected cycle time, retention times and retention time windows for the individual compounds:

- Cycle time: 400 ms
- Interscan delay: 3.5 ms
- Total No. of MRMs: 33
- Maximum No. Of concurrent MRMs: 12
- Minimum Dwell time: 39.8 ms
- Maximum Dwell time: 196.5 ms
Dynamic MRM functionality
Comparison of MRM and DMRM

- 2 x shorter cycle times supports narrow chromatographic peaks, more analytes or longer dwell per analyte.
Dynamic MRM functionality
DMRM simulation

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Stable Isotope Dilution Assay (SIDA) Chromatogram

- due to same MRM transitions baseline separation required for:
  - fumonisin B2 and B3
  - aflatoxin G1 and $^{13}$C-aflatoxin B1
  - aflatoxin G2 and $^{13}$C-aflatoxin B2

Spiked maize sample, 6490 QqQ
Internal calibration in solvent

Aflatoxin B1

- Challenging compound due to low MRLs
  - 0.1 µg/kg in processed cereal based baby food
  - 2 to 12 µg/kg in nuts and cereals

Overlay of 4 individual calibrations acquired within 45 hour worklist.

S/N

74.1
(P2P)

204.4
(P2P)

532.5
(P2P)

0.0075 ng/ml
0.0225 ng/ml
0.075 ng/ml
Internal calibration in solvent
Ochratoxin A

- Challenging compound due to low MRLs
  - 0.5 µg/kg in processed cereal based baby food
  - 3.0 / 5.0 µg/kg in processed / unprocessed cereals
  - 10.0 µg/kg in dried vine fruit

Overlay of 4 individual calibrations acquired within 45 hour worklist.

S/N 8.6 (P2P) 0.0077 ng/ml
S/N 13.8 (P2P) 0.0230 ng/ml
S/N 25.3 (P2P) 0.0765 ng/ml
Validation of SIDA method
Experimental setup and results

- Linear range (external calibration in solvent)
  - 4 orders of magnitude for all toxins, 5 orders for Aflatoxins, T-2, and ZEN

- Costs
  - Additional price per IS per sample is between 0.01 to 1.40 €
  - Price for all 11 IS per sample < 2.00 €

- Full validation for maize
  - Maize: - matrix for which most mycotoxins are regulated
    - known for matrix effects and matrix interferences
  - more costs (time and standards)
  - Spiking with native mycotoxins before extraction
  - Six concentration levels with 3 replicates
  - Spiking with $^{13}$C-labelled mycotoxins before analysis to compensate matrix effects in ESI
  - No sample clean-up
Validation of SIDA method in maize
Extraction of spiked blank maize and reference materials

- Blank maize sample spiked with native mycotoxins before extraction
  - includes 10-fold dilution of matrix in the final extract due to extraction procedure

<table>
<thead>
<tr>
<th>S/N</th>
<th>(P2P)</th>
<th>0.5 µg/kg</th>
<th>1.5 µg/kg</th>
<th>S/N</th>
<th>(P2P)</th>
<th>0.5 µg/kg</th>
<th>1.5 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>123.7</td>
<td></td>
<td></td>
<td></td>
<td>420.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

313.1 -> 241.0 Area=3455
313.1 -> 285.0 Area=2956

404.1 -> 238.9 Area=3193
404.1 -> 102.1 Area=1021

AFB1
OTA
Validation of SIDA method
Results – Sample preparation

- Extraction efficiency
  - Determined by spiking of blank samples before extraction
  - First extraction: 80% acetonitrile content (60 min)
    - recovery between 80 and 110% except for FB1 and FB2
  - Second extraction: 20% acetonitrile content (30 min)
    - improved extraction recovery for FB1 and FB2 to approx. 90%

- Matrix effects
  - Signal suppression
    - 50 to 60% aflatoxins
    - 50% DON
  - Signal enhancement
    - Fumonisins, HT-2, T2, OTA
  - Effectively compensated by ISTD
Validation of SIDA method
Results for maize

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOQ in µg/kg</th>
<th>( R_A )** in % ± RSD in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B(_1)</td>
<td>0.04</td>
<td>105 ± 6</td>
</tr>
<tr>
<td>Aflatoxin B(_2)</td>
<td>0.04</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>Aflatoxin G(_1)</td>
<td>0.05</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>Aflatoxin G(_2)</td>
<td>0.24</td>
<td>101 ± 8</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>2.5</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>2.0</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.17</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.23</td>
<td>93 ± 7</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>0.97</td>
<td>103 ± 11</td>
</tr>
<tr>
<td>Fumonisin B(_1)</td>
<td>2.5</td>
<td>101 ± 10</td>
</tr>
<tr>
<td>Fumonisin B(_2)</td>
<td>0.64</td>
<td>88 ± 7</td>
</tr>
</tbody>
</table>

** average for triplicate samples and 6 spiking levels
Validation of SIDA method
Recently published in Anal. Bioanal. Chem

Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS

Elisabeth Varga • Thomas Glauer • Robert Köppen • Katharina Mayer • Michael Sulyok • Rainer Schuhmacher • Rudolf Krska • Franz Berthiller

Received: 9 December 2011 / Revised: 13 January 2012 / Accepted: 16 January 2012
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Abstract A fast, easy-to-handle and cost-effective analytical method for 11 mycotoxins currently regulated in maize and other cereal-based food products in Europe was developed and validated for maize. The method is based on two extraction steps using different acidified acetonitrile–water mixtures. Separation is achieved using ultrahigh-performance liquid chromatography (UHPLC) by a linear water–methanol gradient. After electrospray ionisation, tandem mass spectrometric detection is performed in dynamic multiple reaction monitoring mode. Since accurate mass spectrometric quantification is hampered by matrix effects, uniformly [13C]-labelled internal standards were added to total recoveries of the extraction steps between 97% and 111% for all target analytes, including fumonisins. The [13C]-labelled internal standards efficiently compensated all matrix effects in electrospray ionisation, leading to apparent recoveries between 88% and 105% with reasonable additional costs. The relative standard deviations of the whole method were between 4% and 11% for all analytes. The trueness of the method was verified by the measurement of several maize test materials with well-characterized concentrations. In conclusion, the developed method is capable of determining all regulated mycotoxins in maize and presuming similar results for other cereal-based commodities.
Validation of SIDA method  
Romer LCMS Mycotoxin kit – developed to fit the application

- Native mycotoxins:
  - Mix 3 (fumonisins)
  - Mix 9 (aflatoxins)
  - Mix 8 (fusarium toxins)
  - Ochratoxin

- $^{13}$C labeled mycotoxins:
  - Mix 12 ($^{13}$C fumonisins)
  - Mix 11 ($^{13}$C aflatoxins)
  - Mix 10 ($^{13}$C fusarium toxins)
  - $[^{13}\text{C}_{20}]$-Ochratoxin
DMRM database for mycotoxins
Customize your mycotoxin method

- Multi-mycotoxin method for 242 mycotoxins and other fungal metabolites has been developed
  - Validated for different nuts
  - Transitions are shortly available as DMRM database

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Quantitation of regulated mycotoxins
Multi-mycotoxin method
Validated for nuts

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear range (µg/L)</th>
<th>Linear range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.13</td>
<td>42.8</td>
</tr>
<tr>
<td>Aflatoxin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.43</td>
<td>12.8</td>
</tr>
<tr>
<td>Aflatoxin G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.13</td>
<td>43.2</td>
</tr>
<tr>
<td>Aflatoxin G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.13</td>
<td>43.2</td>
</tr>
<tr>
<td>Aflatoxin M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.31</td>
<td>103.2</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>0.23</td>
<td>22.6</td>
</tr>
<tr>
<td>Fumonisin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2.55</td>
<td>254.7</td>
</tr>
<tr>
<td>Fumonisin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2.60</td>
<td>260.2</td>
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<tr>
<td>Ochratoxin A</td>
<td>0.18</td>
<td>183.4</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>0.23</td>
<td>228.0</td>
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</table>

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear range</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Acetyl-deoxynivalenol</td>
<td>0.68</td>
</tr>
<tr>
<td>3-Nitropropionic acid</td>
<td>0.71</td>
</tr>
<tr>
<td>Alternariol</td>
<td>0.11</td>
</tr>
<tr>
<td>Alternariol methylether</td>
<td>0.11</td>
</tr>
<tr>
<td>Enniatin B</td>
<td>0.01</td>
</tr>
<tr>
<td>Ergotamin</td>
<td>0.09</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>2.28</td>
</tr>
<tr>
<td>Meleagrin</td>
<td>0.28</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>0.32</td>
</tr>
<tr>
<td>Nivalenol</td>
<td>0.23</td>
</tr>
<tr>
<td>Roquefortine C</td>
<td>0.32</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>0.11</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.23</td>
</tr>
<tr>
<td>Tentoxin</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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Quantitation of regulated mycotoxins
## Multi-mycotoxin method

Validated for nuts

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Almond</th>
<th>Hazelnut</th>
<th>Pistachio</th>
<th>Peanut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_A$ (%) ± RSD (%)</td>
<td>$SSE$ (%) ± RSD (%)</td>
<td>$R_A$ (%) ± RSD (%)</td>
<td>$SSE$ (%) ± RSD (%)</td>
</tr>
<tr>
<td>Aflatoxin B₁</td>
<td>105 ± 5.6</td>
<td>93 ± 5.7</td>
<td>100 ± 14</td>
<td>101 ± 4.5</td>
</tr>
<tr>
<td>Aflatoxin B₂</td>
<td>83 ± 12</td>
<td>73 ± 33</td>
<td>89 ± 5.3</td>
<td>87 ± 15</td>
</tr>
<tr>
<td>Aflatoxin G₁</td>
<td>78 ± 1.0</td>
<td>78 ± 18</td>
<td>81 ± 6.9</td>
<td>97 ± 18</td>
</tr>
<tr>
<td>Aflatoxin G₂</td>
<td>85 ± 1.3</td>
<td>71 ± 13</td>
<td>83 ± 9.8</td>
<td>94 ± 13</td>
</tr>
<tr>
<td>Aflatoxin M₁</td>
<td>73 ± 4.6</td>
<td>76 ± 18</td>
<td>69 ± 11</td>
<td>81 ± 8.7</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>22 ± 20</td>
<td>13 ± 62</td>
<td>39 ± 19</td>
<td>34 ± 69</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>47 ± 8.0</td>
<td>103 ± 11</td>
<td>42 ± 11</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>Fumonisin B₂</td>
<td>57 ± 12</td>
<td>120 ± 4.8</td>
<td>51 ± 5.8</td>
<td>133 ± 9.3</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>92 ± 8.5</td>
<td>83 ± 10</td>
<td>72 ± 9.0</td>
<td>93 ± 14</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>126 ± 4.9</td>
<td>118 ± 7.8</td>
<td>91 ± 8.2</td>
<td>107 ± 8.0</td>
</tr>
<tr>
<td>3-Acetyl-deoxynivalenol</td>
<td>50 ± 44</td>
<td>47 ± 14</td>
<td>56 ± 11</td>
<td>54 ± 15</td>
</tr>
<tr>
<td>3-Nitropropionic acid</td>
<td>45 ± 8.2</td>
<td>44 ± 14</td>
<td>36 ± 5.1</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>Alternariol</td>
<td>119 ± 4.3</td>
<td>119 ± 8.5</td>
<td>98 ± 7.7</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>Alternariol methylether</td>
<td>120 ± 6.1</td>
<td>117 ± 12</td>
<td>96 ± 7.1</td>
<td>106 ± 13</td>
</tr>
<tr>
<td>Enniatín B</td>
<td>90 ± 11</td>
<td>91 ± 12</td>
<td>92 ± 8.0</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>Ergotamin</td>
<td>60 ± 9.5</td>
<td>71 ± 14</td>
<td>56 ± 17</td>
<td>79 ± 8.7</td>
</tr>
<tr>
<td>HT-2 Toxin</td>
<td>111 ± 7.0</td>
<td>95 ± 14</td>
<td>95 ± 7.5</td>
<td>94 ± 26</td>
</tr>
<tr>
<td>Meleagrin</td>
<td>108 ± 8.2</td>
<td>88 ± 21</td>
<td>98 ± 4.3</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>98 ± 7.7</td>
<td>91 ± 6.8</td>
<td>92 ± 6.6</td>
<td>95 ± 3.8</td>
</tr>
<tr>
<td>Nivalenol</td>
<td>14 ± 20</td>
<td>13 ± 21</td>
<td>17 ± 4.7</td>
<td>26 ± 8.3</td>
</tr>
<tr>
<td>Roquefortine C</td>
<td>140 ± 5.1</td>
<td>98 ± 5.5</td>
<td>120 ± 7.7</td>
<td>92 ± 5.1</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>105 ± 6.6</td>
<td>98 ± 4.7</td>
<td>88 ± 3.0</td>
<td>101 ± 6.1</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>105 ± 8.5</td>
<td>100 ± 5.7</td>
<td>91 ± 8.2</td>
<td>96 ± 10</td>
</tr>
<tr>
<td>Tentoxin</td>
<td>110 ± 3.6</td>
<td>110 ± 6.6</td>
<td>93 ± 12</td>
<td>107 ± 7.9</td>
</tr>
</tbody>
</table>
Summary

- UHPLC-MS/MS method
  - Improved chromatographic resolution
- Multiple extraction steps
  - Enhancement of extraction efficiency especially for fumonisins
- Dynamic MRM with fast polarity switching
  - Most abundant ionization mode and maximized dwell times within a single run
- Addition of internal standards after extraction
  - Compensation for matrix effects
  - Minimized costs
- Apparent recoveries of 88 to 105% for all mycotoxins
  - Evaluated by extraction of spiked maize samples
  - Validated by correct quantitation of 18 reference materials covering all toxin groups
- Sensitivity suitable for MRLs
  - Improved sensitivity of G6490 allows to omit sample concentration resulting in easier handling and improved robustness
Thanks!

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Acknowledgements

• Elisabeth Varga
• Katharina Mayer
• Franz Berthiller
• Rainer Schuhmacher, Michael Sulyok, Rudolf Krška
QUESTIONS?

Please Submit your questions by:

Typing your question in the box and hitting submit.

Thank You!
Appendix - Validation of SIDA method
Method characteristics vs. legal requirements

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear range ng/mL</th>
<th>LOQs (maize) µg/kg</th>
<th>MRLs (EC Reg. No 1881/2006)</th>
<th>Commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>0.0075 - 74.6</td>
<td>0.04</td>
<td>0.1</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0-12</td>
<td>nuts and cereals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sum of aflatoxins: 4.0-15.0</td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>0.23 - 225</td>
<td>2.5</td>
<td>200</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500 - 1750</td>
<td>processed / unprocessed cereals, bread, pasta, breakfast cereals</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>0.075 - 249</td>
<td>2.5</td>
<td>200</td>
<td>processed maize-based baby food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>800</td>
<td>maize-based breakfast cereals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000 / 4000</td>
<td>maize / unprocessed maize</td>
</tr>
<tr>
<td>Fumonisin B₂</td>
<td>0.075 - 251</td>
<td>0.64</td>
<td>200</td>
<td>processed maize-based breakfast cereals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>800</td>
<td>maize / unprocessed maize</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>0.2 - 202</td>
<td>2.0</td>
<td>200</td>
<td>implementation of MRLs is expected in the near future</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.023 - 75.4</td>
<td>0.17</td>
<td>0.5</td>
<td>unprocessed cereals and cereal products</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 / 5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 / 20 / 80</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.023 – 23.0</td>
<td>0.23</td>
<td>20</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>processed / unprocessed cereals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75-350</td>
<td>dried vine fruit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>spices / liquorice root / extract</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>0.076 - 252</td>
<td>1.0</td>
<td>20</td>
<td>processed cereal-based baby food</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>processed / unprocessed cereals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75-350</td>
<td>bread, biscuits, breakfast cereals</td>
</tr>
</tbody>
</table>
We apologize for this brief interruption but we are experiencing technical difficulties and will resume shortly.