Screening of mycotoxins in food by triple quadrupole and QTRAP® LC-MS/MS systems

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Structures of Analytes – Mycotoxins

Trichothecenes
NIV, DON, FUS-X, 3AcDON, DAS, HT2, T2, Roridin A

Zearalenene derivatives
ZON

Aflatoxins
Aflatoxin B1, B2, G1, G2, M1, M2

Ochratoxins
Ochratoxin A
Regulations and needs of methods

• Regulations
  – Any method needs to comply with regulations and these change with geography but are typically close to those observed in the EU (i.e. EC 1881/2006 and the amendments EC 1126/2007).

• Methods
  – Need to combine aspects of recent EU reference methods (EN 15850:2010 and EN 15851:2010) into a single method to reduce analysis time and meets the performance of both methods
  – Need to be applicable to different matrices e.g. baby food, grains and fruit and vegetables
• Homogenized sample (2 g) was mixed with acetonitrile/water (8 mL, 80/20) and roller mixed for 20 minutes.

• The sample was centrifuged for 10 minutes at 3500 rpm and filtered using a Phenomenex PHENEX filter (15 mm RC Membrane 0.45 µm).

• The filtrate was then diluted 1:4 with water containing 5 mM ammonium acetate prior to injection.
Fast scan speeds
Fast pos / neg switching
Robust and Rugged Turbo V™ Source
High sensitivity
Scheduled MRM™ Algorithm

- Target analytes are only detected around expected retention time
- Detection windows are automatically scheduled and adjusted
- Optimizes dwell times for each analyte and cycle time → Allows detecting many more MRM transitions and use UHPLC
Comparison of *Scheduled MRM™* vs. MRM

FUS X in negative polarity (left) and AFG1 in positive polarity (right)

![Graphs showing comparison of Scheduled MRM™ vs. MRM for FUS X and AFG1]
<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>RT (min)</th>
<th>Polarity</th>
<th>Ion</th>
<th>MRM (quantifier)</th>
<th>MRM (qualifier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-Acetyldeoxynivalenol (15-AcDON)</td>
<td>3.7</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>339/321</td>
<td>339/137</td>
</tr>
<tr>
<td>3-Acetyldeoxynivalenol (3-AcDON)</td>
<td>3.7</td>
<td>negative</td>
<td>[M+CH3COO]^−</td>
<td>397/307</td>
<td>397/59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[M-H]^-</td>
<td></td>
<td>337/307</td>
</tr>
<tr>
<td>Aflatoxin B1 (AFB1)</td>
<td>4.2</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>313/285</td>
<td>313/128</td>
</tr>
<tr>
<td>Aflatoxin B2 (AFB2)</td>
<td>4.1</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>315/287</td>
<td>315/259</td>
</tr>
<tr>
<td>Aflatoxin G1 (AFG1)</td>
<td>4</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>329/243</td>
<td>329/200</td>
</tr>
<tr>
<td>Aflatoxin G2 (AFG2)</td>
<td>3.9</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>331/313</td>
<td>331/245</td>
</tr>
<tr>
<td>Deoxynivalenol (DON)</td>
<td>3</td>
<td>negative</td>
<td>[M+CH3COO]^−</td>
<td>355/295</td>
<td>355/59</td>
</tr>
<tr>
<td>Diacetoxyscirpenol (DAS)</td>
<td>4.2</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>384/307</td>
<td>384/247</td>
</tr>
<tr>
<td>Fumonisin B1 (FB1)</td>
<td>4.8</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>722/334</td>
<td>722/352</td>
</tr>
<tr>
<td>Fumonisin B2 (FB2)</td>
<td>5.1</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>706/336</td>
<td>706/318</td>
</tr>
<tr>
<td>Fusarenon X (FUS X)</td>
<td>3.3</td>
<td>negative</td>
<td>[M+CH3COO]^−</td>
<td>413/353</td>
<td>413/59</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>4.6</td>
<td>positive</td>
<td>[M+NH4]^+</td>
<td>442/263</td>
<td>442/105</td>
</tr>
<tr>
<td>Monoacetoxyscirpenol (MAS)</td>
<td>3.9</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>342/265</td>
<td>342/307</td>
</tr>
<tr>
<td>Nivalenol (NIV)</td>
<td>2.5</td>
<td>negative</td>
<td>[M+CH3COO]^−</td>
<td>371/281</td>
<td>371/59</td>
</tr>
<tr>
<td>Ochratoxin A (OTA)</td>
<td>5</td>
<td>positive</td>
<td>[M+H]^+</td>
<td>404/239</td>
<td>404/102</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>4.9</td>
<td>positive</td>
<td>[M+NH4]^+</td>
<td>484/215</td>
<td>484/185</td>
</tr>
<tr>
<td>Zearalenon (ZON)</td>
<td>5.1</td>
<td>negative</td>
<td>[M-H]^-</td>
<td>317/131</td>
<td>317/175</td>
</tr>
</tbody>
</table>
• Column was a Phenomenex Kinetex 2.6 μm XB-C18 (50x2.1 mm)
• Column temperature of 40°C
• Gradient of water to methanol with both phases containing 5 mM ammonium acetate and 0.5% acetic acid

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (μL/min)</th>
<th>A (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>450</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>450</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>5.2</td>
<td>450</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>450</td>
<td>2</td>
<td>98</td>
</tr>
</tbody>
</table>

• An injection volume of 30 μL.
Polarity Switching with the **Scheduled MRM™** Algorithm

Mycotoxin detection in a single injection with polarity switching
Triplicate injections of AFB1 at 0.4 ng/mL with %CV of 4.2 (top) and calibration line for ZON 0.2 to 40 ng/mL with $r = 0.9969$ (bottom)
Triplicate injections of ZON at 0.04 ng/mL with %CV of 9.2 (top) and calibration line 0.02 to 4 ng/mL with r = 0.9998 (bottom)
1 in 100 left and 1 in 1000 dilution cereal samples
The next step in improving the detection limits for mycotoxins
Our most sensitive triple quadrupole with up to 10x increase in sensitivity and 20x increase in detector dynamic range all with no compromise in mass range combined with QTRAP® technology.
Multi-component IonDrive™ Technology

1. IonDrive™ Turbo V ion source with increased heater diameter for improved ionization
2. Simulated gas dynamics of the curtain gas interface, acts as a better barrier against neutrals and micro droplets for increased robustness
3. IonDrive™ QJet ion guide with dual stage design to better capture ions from a larger orifice and better transmission into Q0
4. IonDrive™ HED detector with an increased count rate of over $10^8$ cps
AB SCIEX QTRAP® 6500 System

MRM Sensitivity Gains – Verapamil

AB Sciex QTRAP®
6500 system

6 x signal gain >4 x S/N gain vs. QTRAP® 5500 system

QTRAP® 5500 system
Aldosterone in Serum

Triple Quad™ 6500 system
100 pg/mL Aldosterone
S/N = 175

3.6x

Triple Quad™ 5500 system
100 pg/mL Aldosterone
S/N = 48
Different mycotoxin response factors

Negative polarity

Positive polarity

Different mycotoxin response factors

DON
NIV
3-AcDON
FUS X

AFB1
AFB2
AFG1
AFG2
DAS
MAS
HT-2
T-2
FB1
FB2
OTA

15-AcDON
High Sensitivity and Improved Detector
Provides up to 6 Orders of Linear Dynamic Range
Pesticide Residue Analysis

- QuEChERS extraction
- 10 x dilution of extracts
- 0.5 x 50 mm Halo C18
- 40 µl/min using the gradient
- 2 µL injection
- Scheduled MRM™

<table>
<thead>
<tr>
<th>Component Name</th>
<th>Retention Time (mins)</th>
<th>Signal / Noise micro LC</th>
<th>Signal / Noise Normal LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocrotophos</td>
<td>4.05</td>
<td>1083.5</td>
<td>229</td>
</tr>
<tr>
<td>Tricyclazole</td>
<td>5.62</td>
<td>758.4</td>
<td>56.8</td>
</tr>
<tr>
<td>Simetryn</td>
<td>6.18</td>
<td>414.8</td>
<td>126.3</td>
</tr>
<tr>
<td>Monolinuron</td>
<td>6.89</td>
<td>432.6</td>
<td>40.2</td>
</tr>
<tr>
<td>Isoproturon</td>
<td>7.57</td>
<td>613.5</td>
<td>65.7</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>8.03</td>
<td>883.7</td>
<td>92.5</td>
</tr>
<tr>
<td>Flutolanil</td>
<td>8.77</td>
<td>416.9</td>
<td>80.7</td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>9.44</td>
<td>99.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Pyridaben</td>
<td>10.62</td>
<td>903.7</td>
<td>22.9</td>
</tr>
</tbody>
</table>
Example of a calibration line for one of the pesticides, Flutolanil, from 0.2 – 100ppb. The fit used was Linear with 1/x and the ‘r’ value obtained was greater than 0.99. This was typical for the pesticides used. Excellent robustness: peak area reproducibility for 3 selected pesticides (150 injections).
More sensitivity in combination with extended Linear Dynamic Range means...

- More room for diluting the matrix effect away
- Less injections to cover analytes with very different sensitivities or MRL in multi methods
- Less solvent consumption
- => Savings in time and money
You can now choose your LCMS system to match the degree of sample preparation.

AB SCIEX Triple Quad™ 4500
AB SCIEX QTRAP® 4500

AB SCIEX Triple Quad™ 5500
AB SCIEX QTRAP® 5500

AB SCIEX Triple Quad™ 6500
AB SCIEX QTRAP® 6500
Thank you for listening!
Questions and Answers

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