ANALYSIS OF MYCOTOXINS BY LC-MS/MS AND A QuEChERS SAMPLE PREPARATION APPROACH

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Introduction:

Mycotoxins are naturally occurring secondary metabolites produced by several species of fungi. They pose a health risk to humans and animals due to their harmful biological properties and common occurrence in food and feed. Analysis of mycotoxins can be challenging due to their large numbers and diverse physicochemical properties. In addition, the food samples tested are often complex in nature and may be contaminated with multiple mycotoxins at low concentrations. Sample preparation approaches, including SPE, immunoaffinity chromatography and QuEChERS, are complicated by the different polarity and solubility of the mycotoxins, while limited sample cleanup approaches can result in co-extracted matrix components negatively affecting the detection system. LC-MS/MS has become the detection system of choice for mycotoxin analysis due to its sensitivity, selectivity and ability to detect a wide range of compounds. However, challenges still remain, including adequate retention of the polar trichothecenes and overcoming matrix effects. This poster outlines a method for the analysis of 16 mycotoxins in cereal using a QuEChERS sample preparation approach and LC-MS/MS detection. Compounds included in the method are representative of a wide range of mycotoxins, including type A- and B-trichothecenes, ochratoxin A, alternariol, zearalenone, α- & β-zearalanol and aflatoxins. Cereal, consisting of various grains and nuts, was used as the representative sample matrix for recovery experiments.

Method:

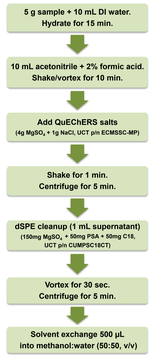

Figure 1. QuEChERS procedure for mycotoxins in grains.

Table 1. HPLC instrumentation and conditions.

HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 3 µm (p/n: SLDA100ID21-3UM)
Guard column	UCT Selectra® DA, 10 × 2.1 mm, 3 µm, (p/n: SLDAGDC21-3UM)
Guard holder	p/n: SLDGRDHLDR
Column temp.	45°C
Mobile phase A	10mM ammonium acetate
Mobile phase B	Methanol
Flow rate	300 µL/min
Gradient	0 min (2% B), 2-6 min (60% B), 10-14 min (100% B), 14.1-19 min (2% B)
Injection volume	10 µL
Divert valve	Diverted to waste at 0-3 and 14-19 min to reduce ion source contamination

Table 2. MS instrumentation and conditions.

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ triple quadrupole
Ionization mode	APCI$^+$ & APCI$^-$
Discharge current	5 (APCI$^+$) & 20 (APCI$^-$) µA
Vaporizer temperature	250°C
Capillary temperature	250°C
Sheath gas pressure	50 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Ion sweep gas	0 arbitrary units
Declustering potential	0 V
Q1 and Q3 peak width	0.2 and 0.7 Da
Collision gas	Argon (1.5 mTorr)
Acquisition method	EZ method (scheduled SRM)
Cycle time	0.7 sec
Software	Xcalibur™ version 2.2

Table 3. Retention times, MS ions and linearity data for the mycotoxins and internal standards.

Analyte	t_R (min)	Precursor ion		Product 1	Product 2	Linearity (R^2)
Nivalenol	3.97	357.3	$[M+HCOO]^-$	281.9	311.8	0.9998
Deoxynivalenol	4.50	341.4	$[M+HCOO]^-$	265.9	296.0	0.9967
Fusarenon X	4.95	354.9	$[M+H]^+$	137.0	175.0	0.9984
Neosolaniol	5.19	399.9	$[M+NH_4]^+$	185.0	215.0	0.9986
AcDON	5.72	338.9	$[M+H]^+$	231.1	91.0	0.9993
AcDON-D_3 (IS)	5.72	341.9	$[M+H]^+$	231.0	213.0	-
Thiabendazole-$^{13}C_6$ (IS)	6.45	207.9	$[M+H]^+$	181.0	137.0	-
Diacetoxyscirpenol	7.64	383.9	$[M+NH_4]^+$	247.0	229.1	0.9994
Alternariol	7.7	257.6	$[M-H]^-$	214.0	216.0	0.9988
β-Zearalanol	8.64	321.5	$[M-H]^-$	277.9	303.9	0.9957
α-Zearalanol	9.81	321.5	$[M-H]^-$	277.9	303.9	0.9983
Ochratoxin A	10.02	403.8	$[M+H]^+$	238.9	220.9	0.9994
T-2 toxin	10.06	483.9	$[M+NH_4]^+$	185.0	215.0	0.9952
Gemfibrozil-D_6 (IS)	10.65	255.8	$[M-H]^-$	122.4	145.3	-
Zearalenone	11.18	317.5	$[M-H]^-$	176.1	273.9	0.9990
Aflatoxin G2	11.33	330.8	$[M+H]^+$	189.0	245.0	0.9964
Aflatoxin G1	11.80	328.8	$[M+H]^+$	199.0	200.0	0.9950
Aflatoxin B2	12.32	314.8	$[M+H]^+$	287.0	259.0	0.9968
Aflatoxin B1	12.69	312.8	$[M+H]^+$	241.0	285.0	0.9966

Note: AcDON = 3-acetyldeoxynivalenol.

LC-MS/MS method development

Finding suitable LC-MS/MS conditions for all mycotoxins can be challenging due to their different physicochemical properties. The trichothecenes were the most problematic and required the most optimization. LC-MS/MS conditions evaluated include:

MS source:
- ESI and APCI were evaluated as ion source.
- ESI gave better signal response for ochratoxin A, alternariol, zearalenone, α- & β-zearalanol and aflatoxins.
- In ESI, the trichothecenes produced weak MS response, or formed $[M+Na]^+$ or $[M+K]^+$ adducts.
- APCI with ammonium formate avoided the formation of $[M+Na]^+$ and $[M+K]^+$ adducts, and produced better signal intensity for the trichothecenes.
- Type-A trichothecenes (neosolaniol, diacetoxyscirpenol and T-2 toxin) formed $[M+NH4]^+$ adducts with good MS response.
- Type-B trichothecenes (nivalenol, deoxynivalenol, acetyldeoxynivalenol and fusarenon X) formed $[M+H]^+$ or $[M+HCOO]^-$ adducts with adequate signal response.

Mobile phase additive:
- Formic acid gave good peak shape for all analytes, but poor MS response for the trichothecenes.
- Ammonium bicarbonate gave good peak shape for all analytes, but a very weak MS signal for nivalenol.
- Ammonium hydroxide gave good peak shape and sensitivity, but is not suitable for long-term use with silica-based columns.
- Ammonium formate gave the best overall chromatography and MS response.

Organic solvent:
- Methanol and acetonitrile were evaluated as organic solvent in the mobile phase.
- Methanol was found to give better peak shape and increased MS response for the polar trichothecenes.

Ultimately, APCI ionization and ammonium formate/methanol as the mobile phase provided the best combination for LC-MS/MS analysis. The use of rapid polarity switching allows all target analytes to be detected in a single run.

Results:

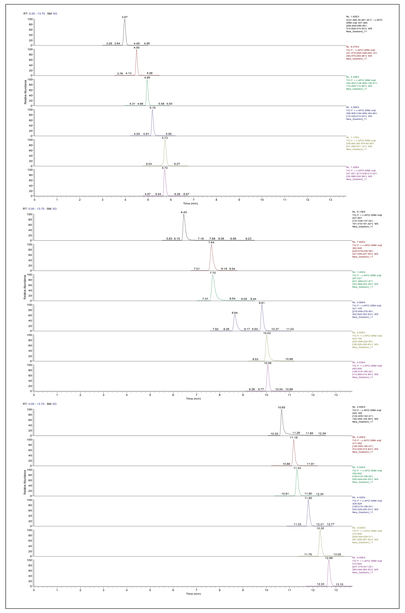

Figure 2. Chromatogram of the 16 mycotoxins and 3 internal standards

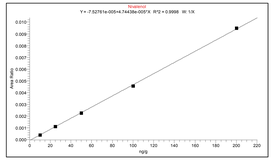

Figure 3. Example of a matrix-matched calibration curve.

QuEChERS method development

- Hydration of cereal is essential for the efficient extraction of mycotoxins.
- Acetonitrile is a suitable extraction solvent for mycotoxins, including the polar trichothecenes
- PSA, PSA/C18 and PSA/C18/GCB were evaluated for dSPE cleanup.
- Low recovery of ochratoxin A (pKa = 4.4) due to retention by PSA.
- Addition of 2% formic acid to extraction solvent ensures ochratoxin A is protonated = good recovery (> 80%).
- Unbuffered QuEChERS extraction salts were used to maintain a low sample pH.
- Buffered extraction salts (acetate or citrate) raises sample pH = lower recovery of ochratoxin A.
- PSA/C18/GCB gave low recovery of aflatoxins, alternatiol, zearalenone and ochratoxin A due to adsorption on GCB.
- PSA and PSA/C18 gave good recovery of all mycotoxins.
- PSA/C18 gives better cleanup than PSA = less matrix effects in LC-MS/MS.

Table 4. Accuracy and precision data obtained for the mycotoxins in cereal.

	20 µg/kg		40 µg/kg		100 µg/kg	
Analyte	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Nivalenol	44.6	4.1	80.9	3.0	79.1	3.7
Deoxynivalenol	97.8	8.5	89.2	8.6	85.0	7.6
AcDON	94.1	4.7	105.0	3.1	95.8	3.0
Fusarenon X	97.8	9.3	105.2	3.3	104.6	2.4
Neosolaniol	89.3	4.6	104.3	4.8	98.2	1.7
Diacetoxyscirpenol	91.5	1.8	102.2	2.6	96.8	2.1
Alternariol	79.7	3.5	104.4	2.4	94.2	4.3
β-Zearalanol	85.6	4.7	109.4	3.6	100.8	1.6
α-Zearalanol	90.6	3.1	102.8	2.5	97.9	0.9
Zearalenone	75.8	2.9	107.5	4.5	98.4	2.8
Ochratoxin A	81.3	8.8	99.9	2.6	82.5	1.9
T-2 toxin	91.0	4.6	102.1	2.6	99.7	1.5
Aflatoxin B1	92.1	3.4	98.1	2.6	95.5	3.4
Aflatoxin B2	102.2	3.0	98.5	1.9	97.3	1.7
Aflatoxin G1	75.7	4.0	101.0	4.1	96.9	1.9
Aflatoxin G2	103.7	11.2	99.0	1.5	87.5	2.7

Conclusions:

A method is presented for the analysis of 16 mycotoxins in cereal using a QuEChERS sample preparation approach and LC-MS/MS detection. The QuEChERS procedure was optimized to extract the widest range of mycotoxins and minimize matrix co-extractives in the final extract. LC-MS/MS was used for accurate detection and quantification. The use of a HPLC column containing a polyaromatic stationary phase resulted in excellent retention of the mycotoxins, including the polar trichothecenes, and baseline resolution of α- and β-zearalanol. The use of APCI with rapid polarity switching allows all mycotoxins to be detected in a single run at ≤ 20 µg/kg. Overall, good recoveries (75-110%) and low RSDs (<12%) were obtained for the spiking studies carried out in cereal, demonstrating that the method is suitable for the analysis of mycotoxins in grain-based foods. For best results, it is recommended to use a matrix-matched calibration curve and include isotopically labeled internal standards (particularly for the type-B trichothecenes). Future work could focus on expanding the scope of the method by incorporating additional mycotoxins and evaluating the method in alternative matrices.