

APPLICATIONS

A Rapid Extraction and Screening Method for Mycotoxins from Cereal Products using QuEChERS and LC/MS/MS

Matthew Trass, Allen Misa, and Brian Rivera
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Mycotoxins are secondary metabolites produced by fungi that can contaminate edible crops, such as grains or coffee, during harvest or storage. Exposure to mycotoxins through consumption, inhalation or dermal routes can result in a variety of health effects including immunosuppression, mutagenicity, and cancer¹. Further interest in mycotoxins has resurfaced because of recent discussions on climate change and food safety². Products like “Bulletproof Coffee®” are even marketed as mycotoxin-free, bringing these toxic metabolites into the mainstream³.

Although aflatoxins and ochratoxins are of major significance, the FDA is also actively concerned with other mycotoxins, including fumonisins, trichothecenes, patulin, and zearalenone. Depending on the intended use of the product, the FDA has established mycotoxin action levels in the parts per billion range; 20 ppb for aflatoxins in all products intended for human consumption, except milk⁴. As such, a specific and sensitive method is necessary to proactively monitor mycotoxins in food.

In this study, we developed a screen to detect different classes of mycotoxins. We demonstrate a rapid sample preparation and LC/MS/MS method for screening all major mycotoxins from cornmeal using roQ™ QuEChERS kits and a Kinetex® 2.6 µm XB-C18 core-shell HPLC column.

Materials and Methods

Reagents and Chemicals

All mycotoxin standards and reagents were purchased from Sigma and Romer Labs.

Experimental Conditions

Sample Preparation

Extraction from Ground Corn

1. Homogenize sample using a blender or similar apparatus
2. Weigh and transfer 5 g of ground corn-meal to a 50 mL roQ QuEChERS extraction tube
3. Add 10 mL of water and 10 mL of acetonitrile with 1.0 % formic acid
4. Dispense contents of the included roQ QuEChERS extraction packet (KS0-8909) into the 50 mL tube containing homogenized sample
5. Shake vigorously by hand for 1 minute
6. Centrifuge for 5 minutes @ 4000 rpm, making sure that the solid material is at the bottom of the tube and a liquid layer forms on top of the solid material

Clean up using dispersive Solid Phase Extraction (dSPE)

1. Transfer the supernatant from Step 6 of the extraction process into a roQ QuEChERS 15 mL centrifuge tube containing 900 mg MgSO₄ and 150 mg PSA (KS0-8924)
2. Shake vigorously by hand for 30 seconds
3. Centrifuge for 5 minutes at 4000 rpm to separate solid material from the liquid layer
4. Transfer the supernatant to a vessel for evaporation

Reconstitution

2 mL of supernatant from the dSPE step was evaporated to dryness under a stream of nitrogen at 60 °C to dryness. The sample was reconstituted in 0.25 mL of 0.5 % Acetic acid/Methanol with 0.5 % Acetic acid (95:5) for analysis.

LC/MS/MS Conditions

LC/MS/MS was performed using a Kinetex 2.6 µm XB-C18 50 x 2.1 mm HPLC column (00B-4496-AN) on an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 600 bar, equipped with a binary pump, autosampler and interfaced with an API 5000™ triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source was electrospray ionization (ESI).

Column:	Kinetex 2.6 µm XB-C18	
Dimensions:	50 x 2.1 mm	
Part No.:	00B-4496-AN	
SecurityGuard:	AJ0-8782	
Mobile Phase:	A: 5 mM Ammonium acetate with 0.5 % Acetic acid B: 5 mM Ammonium acetate in Methanol with 0.5 % Acetic acid	
Flow Rate:	0.45 mL/min	
Gradient:	Time (min)	B (%)
	0.0	5
	2.0	5
	5.0	80
	5.2	98
	8.0	98
Injection Volume:	25 µL	
Temperature:	70 °C	
Detection:	API 5000 (AB SCIEX) Tandem Mass Spec (MS/MS)	
System:	Agilent 1200SL LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump autosampler	

Table 1. MRM Transitions & Retention Times for Mycotoxins

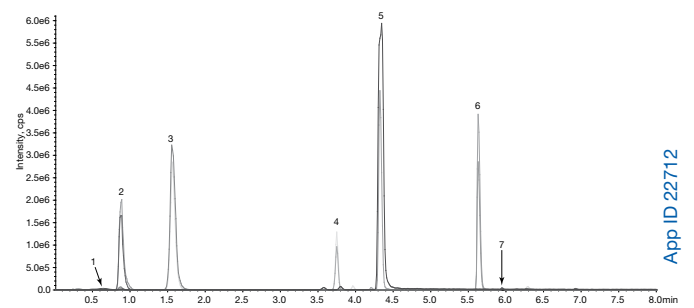
Analyte	Q1	Q3	Retention Time (min)	Mode
Patulin	152.9	108.9/81.0	0.65	-ve
Nivalenol (NIV)	371	281.0/59.0	0.88	-ve
Deoxynivalenol (DNV)	355	295.0/58.6	1.57	-ve
Fusarenone-X	413	59.1/353.1	3.76	-ve
3-Acetyldeoxynivalenol (3DNV)	397	59.0/307.0	4.34	-ve
Zearalenone (ZEA)	317	175.0/272.9	5.64	-ve
Verruculogen	510.1	166.2/305.9	5.96	-ve
Diacetoxyscirpenol (DAS)	384	307.0/349.0	5.05	+ve
15-Acetyldeoxynivalenol (15DNV)	339	321.0/137.0	4.35	+ve
Aflatoxin G2 (AFG2)	331	285.0/245.0	4.64	+ve
Aflatoxin G1 (AFG1)	329	243.0/311.0	4.73	+ve
Aflatoxin B2 (AFB2)	315.1	287.0/259.1	4.84	+ve
Aflatoxin B1 (AFB1)	313	285.1/257.1	4.94	+ve
HT2 Toxin	447.1	345.0/285.1	5.36	+ve
T2 Toxin	489	387.0/327.2	5.60	+ve
Fumonisins B1 (FB1)	722.2	352.2/528.2	5.81	+ve
Ochratoxin A (OTA)	404.1	239.0/386.1	5.60	+ve
Fumonisins B2 (FB2)	706.2	336.2/512.5	5.81	+ve



Table 2. Recovery of Mycotoxins

Analyte	Recovery
AFB1	73 %
AFB2	87 %
AFG1	67 %
AFG2	93 %
HT2 Toxin	96 %
T2 Toxin	91 %
DAS	90 %
OTA	79 %
15DNV	99 %
FB1	8 %
FB2	14 %
Patulin	118 %
ZEA	91 %
DNV	80 %
NIV	66 %
3DNV	96 %
Fusarenone-X	83 %
Verruculogen	81 %

Figure 2. Sample Extract Ion Chromatogram- Negative Mode

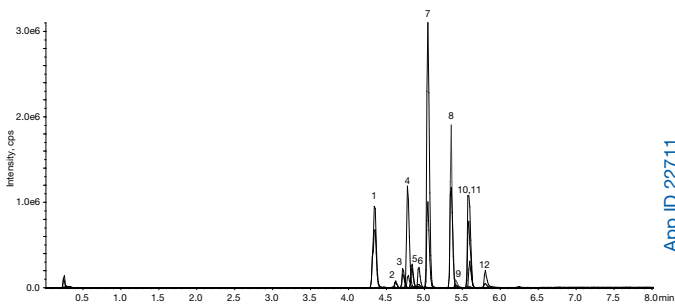


- Sample:**
1. Patulin
 2. Nivalenol (NIV)
 3. Deoxynivalenol (DNV)
 4. Fusarenone-X
 5. 3-Acetyldeoxynivalenol (3DNV)
 6. Zearalenone (ZEA)
 7. Verruculogen

Table 3. Extraction only experiment, showing the effect of formic acid strength in the acetonitrile extraction solvent

Analyte	0.1% FA	0.5% FA	1.0% FA
AFB1	93 %	87 %	74 %
AFB2	96 %	96 %	94 %
AFG1	95 %	88 %	75 %
AFG2	105 %	116 %	106 %
FB1	6 %	3 %	2 %
FB2	12 %	6 %	4 %
HT2 Toxin	113 %	130 %	121 %
T2 Toxin	119 %	125 %	113 %
DAS	90 %	92 %	90 %
OTA	2 %	69 %	86 %
15DNV	99 %	94 %	107 %
Patulin	86 %	81 %	88 %
NIV	60 %	70 %	62 %
DNV	89 %	89 %	84 %
Fusarenone-X	87 %	90 %	85 %
3DNV	101 %	101 %	98 %
ZEA	94 %	96 %	89 %
Verruculogen	95 %	99 %	58 %

Figure 1. Sample Extract Ion Chromatogram- Positive Mode



- Sample:**
1. 15-Acetyldeoxynivalenol (15DNV)
 2. Aflatoxin G2 (AFG2)
 3. Aflatoxin G1 (AFG1)
 4. Unknown Interference
 5. Aflatoxin B2 (AFB2)
 6. Aflatoxin B1 (AFB1)
 7. Diacetoxyscirpenol (DAS)
 8. HT2 Toxin
 9. Fuminosin B1 (FB1)
 10. T2 Toxin
 11. Ocratoxin A (OTA)
 12. Fuminosin B2 (FB2)

Results and Discussion

Table 1 shows MRM transitions and retention times for mycotoxins in positive and negative mode. The analysis was performed using a Kinetex® 2.6µm XB-C18 Core-Shell Technology column and provided excellent peak shape and high efficiencies. **Figure 1** and **Figure 2** show representative ion chromatograms. All analytes eluted in less than 6 minutes with a total run time of only 8 minutes, including column equilibration.

Sample extraction and cleanup using roQ™ QuEChERS extraction kits (KS0-8909) and dSPE Kits (KS0-8921) containing PSA/C18E successfully removed interferences from the corn-meal matrix resulting in good recoveries. **Table 2** shows recoveries between 66-118 % for most mycotoxins except fumonisins (FB1 and FB2).

Although the extraction using acidic conditions helped increase extraction efficiency for most analytes, further extraction experiments were performed. **Table 3** shows optimization of the extraction by increasing the concentration of formic acid from 0.1 %, 0.5 % and 1.0 % to improve analyte partitioning into the organic layer. Although an increase in concentration of formic acid helped with recovery of Ochratoxin A (2 % recovery with 0.1 % formic acid to 86 % recovery with 1 % formic acid), results on the other mycotoxins varied. Depending on the desired mycotoxins of interests, **Table 3** demonstrates optimal formic acid concentrations to provide improved recoveries.

Conclusion

In this study, we presented a rapid and sensitive screening method for various mycotoxins, including aflatoxins and ochratoxins. Sample cleanup using roQ™ QuEChERS extraction and dSPE kits successfully removed interferences from the ground corn-meal matrix resulting in good recoveries. Extracts were analyzed using a Kinetex® 2.6 µm XB-C18 Core-shell Technology HPLC column. Extraction of samples under acidic conditions improved extraction efficiencies, though future experiments should be considered to improve fumonisin recovery. This method presented was proven to be powerful for control management and monitoring of mycotoxins.

References

1. Hsieh, Dennis P. H.. "The Toxicology of Mycotoxins." *Critical Reviews in Toxicology*: 99-132.
2. Vespermann, A. "Climate change and food safety: An emerging issue with special focus on Europe" *Food and Chemical Toxicology*: 1009-1021.
3. "Supercharge" Your Morning Coffee...With Butter?." *The Bulletproof Executive*. N.p., n.d. Web. 26 July 2014. <<http://www.bulletproofexec.com/coffee/>>.
4. U.S. Food and Drug Administration (FDA) Compliance Policy Guides, Sections 527.400, 555.400, and 683.100 (1996).

Ordering Information

roQ Extraction Kits

Extraction Kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 _g MgSO ₄ , 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/PK	KSO-8909*
AOAC 2007.01 Method Extraction Kits		
6.0 _g MgSO ₄ , 1.5 g NaOAc	50/PK	KSO-8911*
Original Non-buffered Method Extraction Kits		
4.0 _g MgSO ₄ , 1.0 g NaCl	50/PK	KSO-8910
6.0 _g MgSO ₄ , 1.5 g NaCl	50/PK	KSO-8912

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KSO-8911-NC and KSO-8909-NC

roQ dSPE Kits

dSPE Kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg MgSO ₄ , 25 mg PSA, 25 mg C18-E	100/PK	KSO-8913
150 mg MgSO ₄ , 25 mg PSA, 2.5 mg GCB	100/PK	KSO-8914
150 mg, MgSO ₄ , 25 mg PSA, 7.5 mg GCB	100/PK	KSO-8915
150 mg MgSO ₄ , 25 mg PSA	100/PK	KSO-8916
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/PK	KSO-8917
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18-E	100/PK	KSO-8918
150 mg MgSO ₄ , 50 mg PSA, 50 mg GCB	100/PK	KSO-8919
150 mg MgSO ₄ , 50 mg PSA	100/PK	KSO-8919

15 mL dSPE Kits

900 mg MgSO ₄ , 150 mg PSA, 150 mg C18-E	50/PK	KSO-8921
900 mg MgSO ₄ , 150 mg PSA, 15 mg GCB	50/PK	KSO-8922
900 mg MgSO ₄ , 150 mg PSA, 45 mg GCB	50/PK	KSO-8923
900 mg MgSO ₄ , 150 mg PSA	50/PK	KSO-8924
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/PK	KSO-8925
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18-E	50/PK	KSO-8926
1200 mg MgSO ₄ , 400 mg PSA, 400 mg GCB	50/PK	KSO-8927
1200 mg MgSO ₄ , 400 mg PSA	50/PK	KSO-8928

Bulk roQ QuEChERS Sorbents

Phases	10 g	100 g
C18-E	—	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	—	04G-4610

Kinetex Ordering Information

5 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	3/pk
XB-C18	00A-4605-AN	00B-4605-AN	00D-4605-AN	AJO-8782 for 2.1 mm ID

5 µm MidBore™ Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
XB-C18	00B-4605-YO	00D-4605-YO	00F-4605-YO	AJO-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00B-4605-E0	00D-4605-E0	00F-4605-E0	00G-4605-E0	AJO-8768 for 4.6 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
XB-C18	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJO-8782 for 2.1 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

Ordering Information Continued>>



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2.6 µm MidBore™ Columns (mm)

Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges†
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	3/pk AJ0-8775

for 3.0 mm ID

2.6 µm Analytical Columns (mm)

Phases	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard ULTRA Cartridges†
XB-C18	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	3/pk AJ0-8768

for 4.6 mm ID

1.7 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges†
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	3/pk AJ0-8782

for 2.1 mm ID

1.7 µm MidBore Columns (mm)

Phases	30 x 3.0	50 x 3.0	100 x 3.0	SecurityGuard ULTRA Cartridges†
XB-C18	00A-4498-Y0	00B-4498-Y0	00D-4498-Y0	3/pk AJ0-8775

for 3.0 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Australia

t: 02-9428-6444
f: 02-9428-6445
auiinfo@phenomenex.com

Austria

t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium

t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada

t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark

t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland

t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France

t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany

t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India

t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland

t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy

t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico

t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands

t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand

t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway

t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico

t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden

t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom

t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States

t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries: Corporate Office USA

t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com



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