Determination of Mycotoxin Residues by LC-MS/MS Featuring Two Alternate Sample Extraction Procedures

UCT Part Numbers

ECHLD126-P
Enviro Clean® HL DVB, 200 mg / 6 mL cartridge

CUMPSC18CT
2 mL centrifuge tube containing 150 mg anhydrous $MgSO_4$, 50 mg PSA and 50 mg endcapped C18

SLDA100ID21-3UM
Selectra® DA, 100 × 2.1 mm, 3 µm

ECMSSC-MP
Mylar pouch containing 4000 mg $MgSO_4$ and 1000 mg NaCl

SLDAGDC21-3UM
Selectra® DA, 10 × 2.1 mm guard cartridge

SLGRDHLDR-HPOPT
Guard Cartridge Holder

Introduction:

Mycotoxins are toxic natural secondary metabolites produced by several species of fungi on agricultural commodities in the field or during storage. To date more than 300 mycotoxins, possessing varying degrees of toxicity, have been identified, although only a relatively few of these are widely accepted as presenting a significant food or animal feed safety risk [1]. Mycotoxins are chemically stable and cannot be destroyed during food processing and heat treatment, thus, monitoring these compounds in food is an important health, agricultural production, food processing and trade concern. The analysis of mycotoxins is challenging due to the large number of compounds to be detected and the wide physicochemical properties theypossess. Additionally, typical food commodity matrices are complex in nature and often contaminated with several mycotoxins at low concentrations.

Sample preparation approaches for mycotoxin analysis include solid–liquid extraction, liquid–liquid extraction, matrix solid-phase dispersion, QuEChERS, immunoaffinity chromatography and solid-phase extraction (SPE). All approaches are complicated by the considerably different polarity and solubility of the mycotoxins, in particular the polar trichothecenes. Due to limited sample cleanup that can be incorporated into a method, sample extracts may still contain large amounts of matrix components that can negatively affect the detection system. To overcome some of the limitations of existing methods, there is a need to further develop extraction and clean-up methods for the simultaneous determination of several mycotoxins with high recoveries of the polar trichothecenes and minimizing sample matrix effects.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the most widely used detection system for mycotoxin analysis. Advantages of using LC-MS/MS include selectivity sensitivity and the ability to cover a wide range of mycotoxins. However, challenges still remain, including finding conditions that are suitable for all mycotoxins included in a method, adequate LC retention of polar trichothecenes, and matrix effects. The latter issue is particularly relevant to trichothecene mycotoxins as their ionization efficiency can be affected by the presence of co-eluting matrix interferences leading to signal suppression or enhancement [2]. This can be compensated by using isotopically labeled internal standards and matrix-matched standards [3]. Numerous LC-MS/MS conditions have been reported for the analysis of mycotoxins [4-6]. There doesn't appear to be universal LC-MS/MS conditions that work for all the mycotoxins, and the choice of ion source and mobile phase is dependent on the compounds included in a method. Electrospray ionization (ESI) is the most commonly used ion source in mycotoxins analysis, although atmospheric pressure chemical ionization (APCI) is also employed, particularly for the analysis of trichothecene mycotoxins [7, 8].

This application note details two sample preparation approaches (SPE and QuEChERS) that can be used for the extraction and clean-up of mycotoxins from grain-based food. LC-MS/MS was used for the accurate detection and quantification of the mycotoxins in both methods. HPLC separation of the 16 mycotoxins and 3 internal standards included in the study was successfully conducted within 16 min using a Selectra® DA column, a polyaromatic phase capable of greater retention of the polar trichothecenes compared to a standard C18 stationary phase. The compounds included in this method are representative of a wide range of mycotoxins, including type A- and B-trichothecenes, ochratoxin A, alternariol, zearalenone, α- & β-zearalanol and aflatoxins (B1, B2, G1, G2).

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Experimental (SPE):

1. Sample Preparation

a) Weigh 2 g of sample into a 50 mL polypropylene centrifuge tube.
b) Add 2 mL water and briefly vortex.
c) Allow samples to hydrate for ≥15 min.
d) Add 10 mL MeCN.
e) Shake or vortex samples for 10-15 min to extract the mycotoxins.

Note: For this study a SPEX® SamplePrep® GenoGrinder® was used.

f) Centrifuge the samples for 10 min at ≥3000 × g (4°C).
g) Transfer the supernatant to a clean polypropylene or glass tube and evaporate to dryness at 50°C under a gentle stream of nitrogen.
h) Add 10 mL of water to each sample and vortex for 5 min to ensure the sample is fully dissolved.

Note: Alternatively, sonicate the samples for 5 min.

2. SPE Extraction

a) Condition SPE cartridges with 3 mL MeOH and 3 mL water.
b) Load supernatant from step 1h).
c) Allow the sample to percolate through the cartridge under gravity.

Note: If necessary, apply a low vacuum to pull the sample through the cartridge dropwise.

3. Wash Cartridge

a) Add 3 mL of water and slowly draw through.
b) Add 3 mL of 10% MeOH and slowly draw through.
c) Dry cartridges under vacuum (≥10 in Hg) for 10 minutes.
d) Add 3 mL of hexane and slowly draw through.
e) Dry cartridges under vacuum (≥10 in Hg) for 5 minutes.

4. Elute Cartridge

a) Elute the mycotoxins using 4 mL MeCN.
b) Evaporate the samples to dryness at 40-50°C under a gentle stream of nitrogen.
c) Reconstitute in 1 mL of MeOH:H_2O (50:50, v/v).

Experimental (QuEChERS):

1. Sample Extraction

a) Weigh 5 g of thoroughly homogenized sample into a 50 mL centrifuge tube.
b) Add 10 mL water and briefly vortex.
c) Allow samples to hydrate for ≥15min.
d) Add internal standard.
e) Add 10 mL MeCN containing 2% formic acid (or 10 mL MeCN and 200 µL formic acid).
f) Vortex/shake samples for 5-10 min to extract the mycotoxins.
g) Add contents of the **ECMSSC-MP** Mylar pouch to each centrifuge tube.
h) Immediately shake (manually or mechanically) for 1 min.

Note: For this study a SPEX® SamplePrep® GenoGrinder® was used.

i) Centrifuge for 5 min at ≥ 3000 × g (4°C).

2. Sample Cleanup

a) Transfer a 1 mL aliquot of supernatant to a **CUMPSC18CT** dSPE tube.
b) Vortex for 30 sec.
c) Centrifuge for 5 min at ≥3000 × g (4°C).
d) Transfer 500 µL of purified supernatant to a 5 mL test tube and solvent-exchange the sample into MeOH:H_2O (50:50, v/v) for optimum LC-MS/MS performance.

Note: For improved sensitivity at low concentrations, the dSPE step can be scaled-up and a concentration step included in the method. Use product **CUMPSC1815CT2** (15mL centrifuge tube with 1200mg MgSO4, 400mg PSA and 400mg endcapped C18):

a) Transfer 8ml of supernatant to a **CUMPSC1815CT2** dSPE tube.
b) Vortex for 30 sec.
c) Centrifuge for 5 min at ≥ 3000 × g (4°C).
d) Transfer 5 mL of supernatant to a glass tube.
e) Evaporate the sample to dryness at 40-50 °C under a gentle stream of nitrogen.
f) Reconstitute samples in 1 mL MeOH:H_2O (50:50, v/v).

LC-MS/MS Conditions:

MS Conditions	
Instrumentation	Thermo Scientific™ TSQ Vantage™ tandem mass
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
Collision gas pressure	1.5 mTorr
Acquisition method	EZ method (scheduled SRM)
Cycle time	0.7 sec
Software for data processing	Xcalibur™ version 2.2
Weighting factor applied to calibration	1/X

SRM Transitions							
Analyte	t_R (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Nivalenol	3.63	357.3 [M+HCO]	281.91	16	311.79	15	65
Deoxynivalenol	4.52	341.4 [M+HCO]	265.87	13	295.96	16	63
Fusarenon X	5.35	354.9 $[M+H]^+$	136.97	31	174.96	19	76
Neosolaniol	6.03	399.9 $[M+NH_4]$	184.99	20	215.03	16	81
AcDON	6.79	338.8 $[M+H]^+$	231.10	13	90.98	48	74
AcDON-D_3 (IS)	6.76	341.9 $[M+H]^+$	230.99	14	213.04	15	80
Thiabendazole-	8.35	207.9 $[M+H]^+$	181.02	25	137.04	32	123
Diacetoxyscirpen	9.55	383.9 $[M+NH_4]$	247.06	13	229.08	16	82
Alternariol	9.76	257.6 $[M-H]^-$	214.03	23	216.01	26	113
Ochratoxin A	10.44	403.8 $[M+H]^+$	238.93	23	220.90	36	101
β-zearalanol	10.40	321.5 $[M-H]^-$	277.94	24	303.86	24	125
α-zearalanol	11.56	321.5 $[M-H]^-$	277.94	24	303.86	24	125
Gemfibrozil-D_6	11.66	255.7 $[M-H]^-$	122.41	21	-	-	60
T-2 toxin	11.76	483.9 $[M+NH_4]$	185.02	21	214.99	17	84
Aflatoxin G2	13.53	330.8 $[M+H]^+$	189.02	36	245.05	27	137
Zearalenone	13.57	317.5 $[M-H]^-$	176.13	27	273.95	22	110
Aflatoxin G1	14.07	328.8 $[M+H]^+$	199.02	41	200.03	36	143
Aflatoxin B2	14.39	314.8 $[M+H]^+$	287.06	23	259.01	27	129
Aflatoxin B1	14.75	312.8 $[M+H]^+$	241.02	36	285.05	22	121

Note: CE = collision energy. AcDON = 3-acetyldeoxynivalenol

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HPLC Conditions	
Instrumentation	Thermo Scientific™ Dionex™ Ultimate™ 3000 LC system
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 column	p/n: **SLDGRDHLDR**
Mobile phase A	10 mM ammonium formate
Mobile phase B	MeOH
Flow rate	300 µL/min
Column temp.	45 °C
Run time	21 min (including 5 min equilibration)
Injection volume	10 µL
Autosampler	10 °C
Wash solvent	MeOH: H_2O (50:50, v/v)
Divert valve	Mobile phase was sent to waste for 0-2 & 16-21 min to reduce ion source

LC Gradient Program		
Time (min)	**Mobile phase A (%)**	**Mobile phase B (%)**
0	98	2
2.0	60	40
5.0	60	40
6.0	40	60
10.0	40	60
12.0	0	100
16.0	0	100
16.2	98	2
21.0	98	2

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Results and Discussion:

1. SPE Sample Preparation Procedure

The first step in the sample preparation process is to find a suitable extraction solvent. It has been reported that 100% organic solvent is not a suitable extraction solution in multi-class mycotoxin methods as it does not sufficiently extract all residues, particularly the polar trichothecenes [7,9,10]. MeCN:water, usually in the ratio 84:16 (v/v), is the most commonly used extraction solvent in mycotoxin analysis. Other extraction solvents that have been reported in the literature for single- and multi-class methods include MeOH:water, MeCN, MeOH, acetone, ethyl acetate, dichloromethane and aqueous buffers [9,10]. The extraction solvent used in this application was MeCN:water (83:17, v/v).

After extracting the mycotoxin residues from the sample, the sample extract undergoes a solvent exchange to 100% water prior to application to the SPE cartridge. Applying an aqueous solution onto the SPE cartridge ensures optimum retention of the mycotoxins on the sorbent and reduces any chance of analyte breakthrough. The solvent exchange step requires the inclusion of an evaporation step in the method, which makes it desirable to limit the water content of the extraction solvent in order to speed up the evaporation process. With this in mind, an attempt was made to use 100% MeCN as the extraction solvent, but as reported elsewhere it did not sufficiently extract all of the mycotoxins, namely the polar trichothecenes. Ultimately, adding water to the extraction solvent improved the extraction of the polar trichothecenes, while keeping the water content low ensured the evaporation step was relatively straightforward using the conditions described in the experimental procedure. It was subsequently found that hydrating the sample with water prior to adding MeCN gave greater extraction efficiency than adding aqueous MeCN directly to dry samples. This is probably due to greater access of the MeCN to the solvated matrix.

Rinsing the SPE sorbent to remove matrix components is limited when analyzing for multiple mycotoxins due to the potential loss of analytes. In this study, the SPE cartridges were rinsed with water to remove very polar matrix components and hexane to remove very hydrophobic matrix components. In addition, a 10% MeOH solution was used to remove additional matrix components without eluting any of the mycotoxins. Further increasing the MeOH content of the wash solution increases the risk of washing some of the mycotoxins off the sorbent, leading to reduced recovery.

Lastly, the elution solvent had to be optimized; MeOH, a commonly used elution solvent in SPE, was too weak to fully elute all the mycotoxins from the divinylbenzene sorbent (particularly zearalenone, α- & β-zearalanol, alternariol and the aflatoxins). Ethyl acetate, acetone and MeCN were evaluated as alternative elution solvents. Ultimately, MeCN was found to give the best results and was chosen as the final elution solvent.

To generate accuracy and precision data, recovery experiments were carried out using cereal (composed of various grains and nuts) as a representative sample matrix. Samples were fortified at two concentrations (n = 6 for each concentration) in order to obtain the necessary data. The cereal samples were fortified at 10 and 100 ng/g and prepared according to the experimental procedure described above. As outlined in table 1, the majority of results were found to be within an acceptable recovery range of 80-120 % with RSD values ≤ 10 %, demonstrating that the developed SPE method is suitable for the analysis of mycotoxins in grain-based foods. Deoxynivalenol and Fusarenon X were not included in the results due to elevated recoveries at the 10 ng/g level. Fusarenon X also had somewhat elevated recovery at the 100 ng/g level, although the result was reproducible (3% RSD). The elevated recovery was possibly caused by co-eluting matrix components leading to signal enhancement. The inclusion of isotopically labeled internal standards for these compounds would help to address this issue.

Table 1. Accuracy and precision data obtained for the SPE sample preparation method.

	10 ng/g		100 ng/g	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Nivalenol	87.3	3.4	87.2	1.6
Deoxynivalenol	*	-	102.0	3.7
AcDON	90.2	4.3	100.0	0.5
Fusarenon X	*	-	144.3	3.1
Neosolaniol	92.7	1.1	98.5	1.4
Diacetoxyscirpenol	86.8	3.4	86.2	2.1
Alternariol	95.0	4.3	97.9	2.1
β-zearalanol	77.0	2.6	80.0	4.0
α-zearalanol	78.6	1.8	78.8	3.6
Zearalenone	112.3	1.9	100.1	2.3
Ochratoxin A	88.7	3.5	117.6	5.0
T-2 toxin	79.0	4.8	82.0	3.1
Aflatoxin B1	107.2	6.3	98.8	1.9
Aflatoxin B2	98.3	3.1	97.0	2.1
Aflatoxin G1	83.1	6.4	86.1	2.7
Aflatoxin G2	111.4	8.5	101.2	2.3

*Recovery values obtained were not included due to elevated recovery values high.

2. QuEChERS Sample Preparation Procedure

The QuEChERS procedure is a popular sample preparation approach for the analysis of mycotoxin residues due its simplicity, speed and cost. MeCN is the preferred extraction solvent as it extracts the widest range of mycotoxins and least amount of matrix components. To efficiently extract acidic mycotoxins (ochratoxins and fumonisins), the sample pH needs to be lowered so that the analytes are in their neutral state (i.e. protonated) and effectively partition into the MeCN layer. This is achieved by incorporating acid in the extraction solvent or using buffered QuEChERS extraction salts. Cleanup of the sample extract is carried out by dispersive-SPE (dSPE) using primary secondary amine (PSA) and/or C18 sorbent. PSA effectively removes organic acids, carbohydrates and polar matrix components, while C18 removes fats and other lipophilic matrix components. For acidic analytes, the sample pH needs to be sufficiently low to ensure the acidic mycotoxins do not get retained on the PSA sorbent. Nivalenol, a very polar compound, is the only mycotoxin reported to not give high recoveries using the QuEChERS approach. This is caused by the incomplete partitioning of nivalenol into the organic phase during the extraction/partitioning step. Reported recovery is typically still ≥ 60% and the reproducibility acceptable.

In this study, MeCN containing 2% formic acid was used as the extraction solvent. Unbuffered extraction salts were used to maintain a low sample pH. Using buffered extraction salts (acetate or citrate) would raise the pH and lead to lower recovery of ochratoxin A or require the use of higher amounts of acid to maintain a low sample pH. dSPE cleanup of the sample extracts was successfully carried out using PSA/C18 sorbent. Using 2% formic acid in the extraction solvent was necessary to prevent the retention of ochratoxin A on the PSA sorbent. Using smaller amounts of acid lead to lower recovery of ochratoxin A. Other dSPE sorbents evaluated included PSA on its own and PSA/C18/GCB (graphitized carbon black). GCB is a typically used for highly pigmented samples (e.g. chlorophyll and sterols) and yields very clean extracts. However, it can also retain analytes of interest, leading to reduced recovery. This was found to occur for several of the mycotoxins included in this method (e.g. ochratoxin A, alternatiol, zearalenone, aflatoxins). No major variation in recovery was observed between PSA and PSA/C18. However, the combination of PSA/C18 yielded cleaner extracts and was therefore used in the final method.

Cereal, consisting of various grains and nuts, was used as the representative sample matrix for recovery experiments. Samples were fortified at three concentrations (n = 6 for each concentration) in order to obtain accuracy and precision data. The cereal samples were fortified at 20, 40 and 100 ng/g and prepared according to the experimental procedure described above. As outlined in table 2, the majority of results were found to be within an acceptable recovery range of 80-120 % with RSD values ≤ 10 %, demonstrating that the developed QuEChERS method is suitable for the analysis of mycotoxins in grain-based foods. Nivalenol, fortified at 20ng/g, gave a mean recovery value of 45% and was the only compound with a result outside the acceptable limits (70-120%). This is probably due to the reduced MS sensitivity of the analyte at that particular concentration. As already mentioned, it is known that nivalenol does not get as efficiently extracted as the other mycotoxins using the QuEChERS approach. However, samples fortified at higher concentrations gave satisfactory recoveries (≈80%). 20ng/g was chosen as the lowest fortification level because at lower concentrations the sensitivity of some of the mycotoxins, primarily the trichothecenes, becomes more challenging. If lower concentrations are desired, it is suggested to scale-up the dSPE step and include a concentration step in the method. However, some mycotoxins (e.g. aflatoxins, zearalenone, diacetoxyscirpenol) can be readily detected at concentrations ≤20 ng/g without using a concentration step.

Table 2. Accuracy and precision data obtained for the QuEChERS sample preparation method.

	20 ng/g		40 ng/g		100 ng/g	
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

LC-MS/MS Analysis

Prior to developing the sample preparation procedures, a LC-MS/MS method was developed for the simultaneous determination of the 16 mycotoxins and 3 internal standards included in the study. However, developing an LC-MS/MS method to simultaneously detect all the mycotoxins poses a challenge due to the different physicochemical properties that they possess. It has been reported that some mycotoxins produce better results using an APCI source, although ESI has been shown to produce higher sensitivity for the majority of mycotoxins and is therefore used most frequently [8]. A problem encountered with using ESI is that the trichothecene mycotoxins are prone to adduct formation, including sodium [M+Na]+ and potassium [M+K]+ adducts. Most trichothecenes do not form protonated molecular ions [M+H]+ or produce very weak signal response. For the type-A trichothecenes (neosolaniol, diacetoxyscirpenol and T-2 toxin), incorporating an ammonium containing buffer into the mobile phase results in the formation of [M+NH4]+ adducts that exhibit good MS response. In contrast, the type-B trichothecenes (nivalenol, deoxynivalenol, acetyldeoxynivalenol and fusarenon X) do not form [M+NH4]+ adducts. However, the type-B trichothecenes are capable of forming acetate [M+CH3COO]- and formate [M+HCOO]- adducts with adequate signal response. Alternatively, the formation of [M+Na]+ and [M+K]+ adducts can be avoided by using APCI instead of ESI.

Initially a variety of mobile phase additives were evaluated in ESI mode, including formic acid, acetic acid, ammonium formate, ammonium acetate, ammonium hydroxide and ammonium bicarbonate. However, none were found to be suitable for all of the mycotoxins. Some of the additives produced poor peak shapes for certain compounds, while the signal intensity obtained by ESI was still rather poor for several trichothecenes (particularly nivalenol, deoxynivalenol and fusarenon X). To improve the response of the trichothecenes, APCI was evaluated as an alternative to ESI. Using ammonium formate as the mobile phase additive, APCI was found to produce better signal intensity for the problematic trichothecenes. Nivalenol and deoxynivalenol were detected as [M+HCOO]- adducts and fusarenon X as [M+H]+ ion. In the end, while ESI gave better signal response for some compounds (e.g. aflatoxins), APCI was chosen for use as it provided the best overall results. Both acetonitrile (MeCN) and methanol (MeOH) were evaluated for use as organic eluent in the mobile phase. MeOH was found to give superior peak shape for the trichothecene mycotoxins compared to MeCN. In addition, the use of MeOH also improved the signal response, while the use of acetonitrile led to much lower signals. Similar observations have been previously reported [5]. Ultimately, 10 mM ammonium formate, MeOH and APCI were found to be the best compromise and included in the final LC-MS/MS method.

Owing to their polarity, the type-B trichothecenes usually elute early in the chromatographic run, and are known to be prone to matrix effects in the ion source [2, 3]. To reduce the possibility of matrix effects they should be sufficiently retained on the LC column so that they do not co-elute with polar matrix components. The Selectra® DA column contains a polyaromatic phase that is capable of greater retention of the polar trichothecenes compared to a standard C18 stationary phase. Using this column, the first compound (nivalenol) does not elute until 3.65 min and using 40% organic solvent. In addition, the first 2 min of flow is diverted to waste, which minimizes ion source contamination. In the final method, separation of the mycotoxins, including α- and β-zearalanol, was achieved within 16 min on the Selectra® DA column. The use of rapid polarity switching allows all target analytes to be detected in a single run.

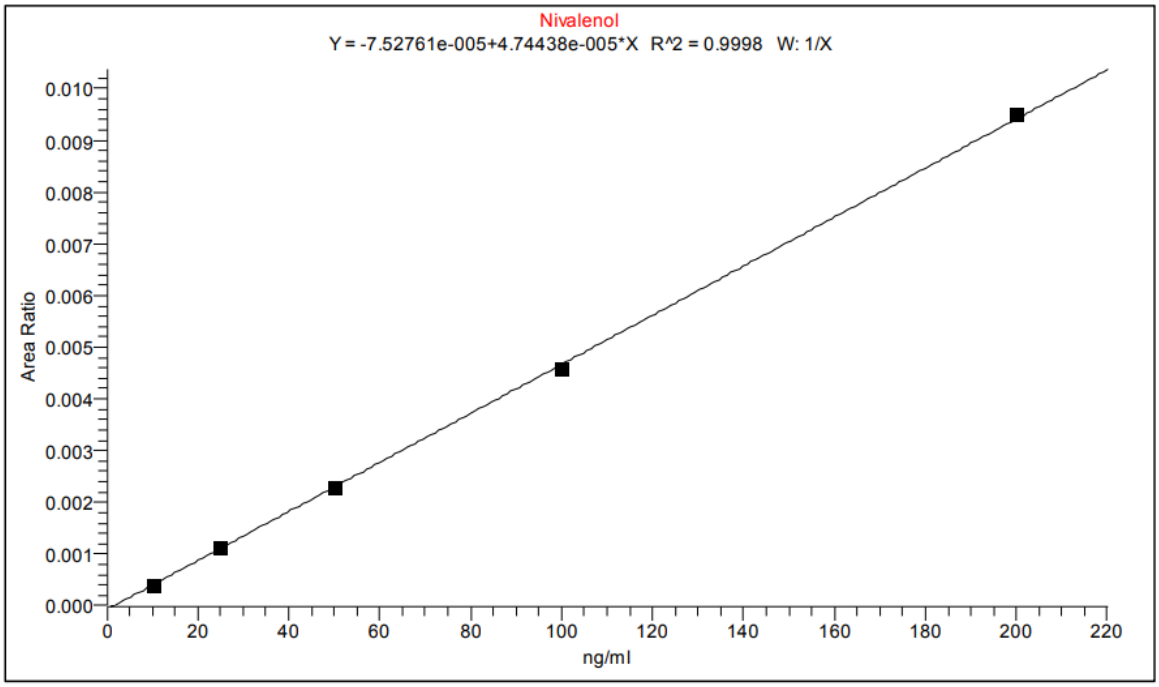

Figure 1. Example of a matrix-matched calibration curve (calibration curve for nivalenol was used to quantify results of the QuEChERS procedure).

Table 3. Linearity expressed as correlation coefficient, R^2 (values obtained were from the calibration curves used to quantify results of the QuEChERS procedure).

Analyte	R^2
Nivalenol	0.9998
Deoxynivalenol	0.9967
AcDON	0.9993
Fusarenon X	0.9984
Neosolaniol	0.9986
Diacetoxyscirpenol	0.9994
Alternariol	0.9988
β-zearalanol	0.9957
α-zearalanol	0.9983
Zearalenone	0.9990
Ochratoxin A	0.9994
T-2 toxin	0.9952
Aflatoxin B1	0.9966
Aflatoxin B2	0.9968
Aflatoxin G1	0.9950
Aflatoxin G2	0.9964

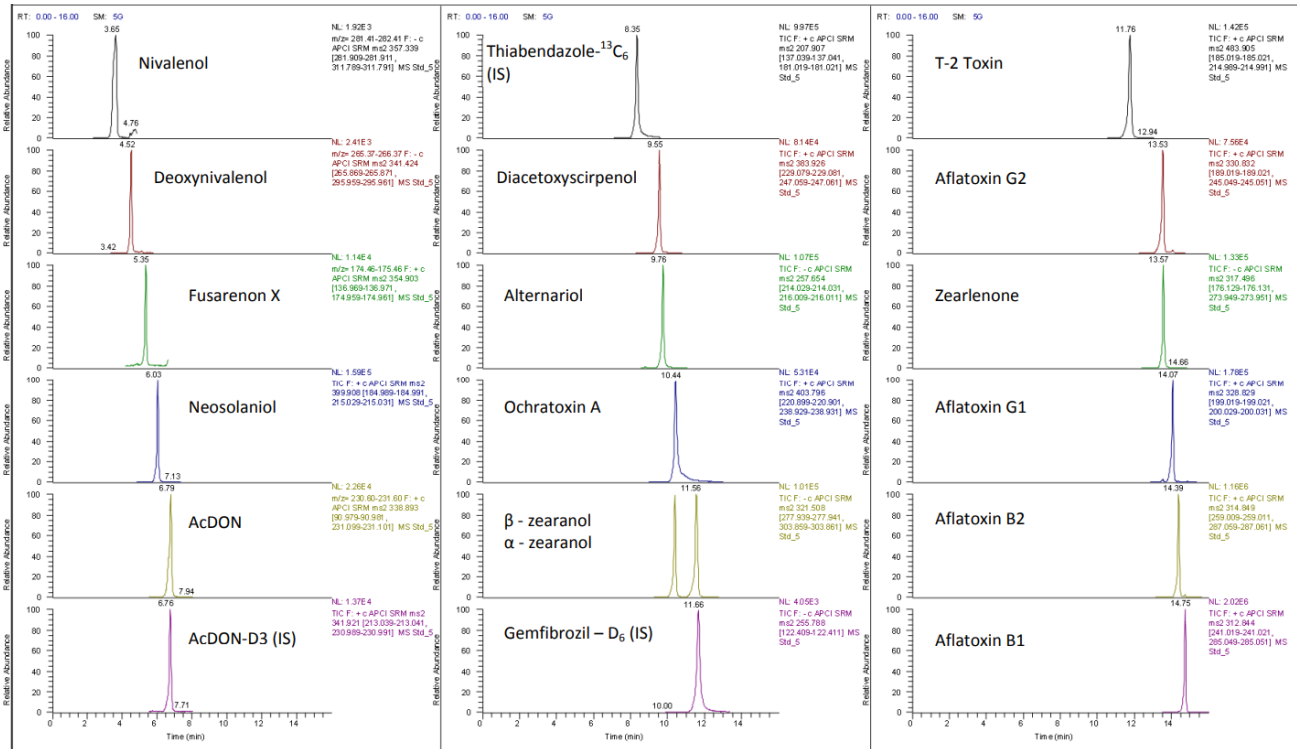

Figure 2. Example of a chromatogram containing the 16 mycotoxins and 3 internal standards included in the method.

Conclusions:

- Two sample preparation methods have been successfully developed for the extraction and cleanup of 16 representative mycotoxins in grain-based food.
- The SPE sample preparation procedure uses a hypercrosslinked divinylbenzene sorbent to effectively retain all the mycotoxins, including the polar trichothecene mycotoxins.
- The SPE wash step was optimized to remove matrix interferences without losing any analytes of interest.
- The QuEChERS sample preparation procedure uses acidified MeCN and un-buffered salts for extraction, and PSA/C18 for dSPE cleanup.
- An optimized LC-MS/MS method was developed for the accurate detection and quantification of the mycotoxin residues.
- APCI ionization was chosen over ESI as it provides better overall results, including better signal response for problematic trichothecenes.
- The use of rapid polarity switching allows all target analytes to be detected in a single run.
- Separation of the mycotoxins, including baseline resolution of α- and β-zearalanol, was achieved within 16 min on a Selectra® DA column.
- Overall, good accuracy and precision were obtained for these difficult compounds.
- For best results, it is recommended to use matrix-matched calibration curves and include isotopically internal standards (particularly for the type-B trichothecenes).

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