LC/MS/MS of Trichothecenes and Zearalenone in Wheat Using Different Sample Prep Methods

Application Note

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Abstract

A modified QuEChERS and Agilent Bond Elut Mycotoxin SPE demonstrate excellent analyte sensitivity for the detection of trichothecenes and zearalenone by LC/MS/MS. The Bond Elut mycotoxin SPE cartridge, with its greater sorbent capacity, produces cleaner extracts with slightly improved LODs and LOQs. However, the modified QuEChERS method is substantially quicker and uses much less solvent and sorbent. Both sample preparation approaches showed excellent linearity for all mycotoxins ($R^2 \geq 0.995$). Spiked wheat samples (50 µg/kg, n = 9) showed recoveries within 72 to 105% (CV ≤ 11%) relative to immuno-affinity, a well recognized and effective form of sample clean up for mycotoxins. In this example two sample preparation procedures are used, which when combined with performance levels of the Agilent 6460 Triple Quadrupole LC/MS, can reduce the cost of analysis yet still produce data with sensitivity commensurate with regulatory control requirements.

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Introduction

Mycotoxins are a group of toxic secondary metabolites produced by several species of mold. Fusarium molds are of significant importance as they are particularly prevalent on cereals such as wheat, maize, barley and oats that are grown in temperate regions. Infection often leads to lower crop yield, but depending on the infecting species a range of mycotoxins called trichothecenes may be formed. There are over 150 known trichothecenes. Surveys have shown that the most predominant trichothecene is deoxynivalenol but other trichothecenes such as Nivalenol, Fusarenone-X, Neosolaniol, 3- and 15-acetyl deoxynivalenol, Diacetoxyscirpenol (DAS), T-2 toxin and HT-2 toxin can also be present. They belong to the type A and B trichothecenes and share the same basic chemical structure. Zearalenone is not a trichothecene but can also be produced by a number of Fusarium species.

When ingested, trichothecenes can pose a threat to animal and human health. They can be responsible for toxic symptoms ranging from feed refusal, skin irritation, vomiting and diarrhea. These compounds are also potent inhibitors of protein and DNA synthesis and have immunosuppressant activity. EU commission regulation No 1881/2006 has set maximum limits for deoxynivalenol in a range of unprocessed and processed cereal based foods. The latter limits are lower than those set for the raw material. Compliance to these limits is determined by chemical residue analysis. Gas chromatography methods with electron capture or mass spectrometric detection have been successfully employed to determine and quantify type A and B trichothecenes following derivatization of sample extracts. More recently, methods for the simultaneous determination of type A and B trichothecenes and zearalenone by LC/MS/MS have been developed.

In this application note, the performance of two sample preparation procedures to determine nine trichothecenes and zearalenone in wheat by LC/MS/MS was compared.

Methods and Results

Either a modified QuEChERS extraction and dispersive SPE (d-SPE), or an acetonitrile/water extraction with Bond Elut Mycotoxin SPE cartridge was employed for the sample preparation procedure (Table 1).

Table 1. Sample Preparation Steps for Modified QuEChERS and Bond Elut Mycotoxin SPE Methods in the Analysis of Trichothecenes and Zearalenone

Modified QuEChERS	Bond Elut Mycotoxin SPE method
5 g milled sample	25 g milled sample
1. 10 mL MeOH:ACN (85:15)	1. 100 mL H_2O:ACN (20:80)
2. 4 g $MgSO_4$ + 1 g NaCl	2. Shake for 1 hour
3. Shake and centrifuge	
Take 2-mL aliquot	Take 8-mL aliquot
1. 300 mg $MgSO_4$ + 100 mg PSA	1. Bond Elut Mycotoxin
2. Vortex and filter through 0.02-µm membrane	2. Evaporate and reconstitute in 1 mL H_2O:ACN (80:20)
3. Evaporate and reconstitute in 1 mL H_2O:ACN (80:20)	3. Filter through 0.02-µm membrane

Modified QuEChERS

Methanol/ACN solvent was added to each tube containing the milled sample and vortexed. An Agilent Bond Elut Non-buffered extraction packet (p/n 5982-5550) containing 4 g $MgSO_4$ and 1 g NaCl was then added. The tube was vigorously shaken and centrifuged.

An aliquot from the upper layer was transferred to a tube containing 1:3 by weight PSA/$MgSO_4$.

When using 2-mL aliquots, 2-mL blank tubes were prepared manually by weighing in 300 mg $MgSO_4$ and 100 mg PSA. In later routine work, the aliquot was reduced to 1 mL, allowing the use of Bond Elut d-SPE tubes containing 150 mg $MgSO_4$ and 50 mg PSA (p/n 5982-5022).

Bond Elut Mycotoxin SPE Method

A Bond Elut Mycotoxin SPE cartridge, 500 mg, 3 mL (p/n 12102167) was used in the second sample preparation scheme. An aliquot from the extracted sample was applied to the SPE cartridge. The eluent was collected and evaporated. The SPE method is an unretained SPE (scavenger) mechanism which eliminates matrix constituents from the sample likely to interfere with the ESI-MS/MS detection.

Conditions

HPLC Conditions

Columns	Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959764-902)
Instrument	Agilent 6460 Triple Quadrupole LC/MS, Agilent 1290 Infinity LC System
Flow Rate	0.25 mL/min
Column Temp	30 °C
Injection Vol	10 µL
Mobile Phase	A: Water + 0.2% acetic acid, 5 mM ammonium acetate B: Methanol + 0.2% acetic acid, 5 mM ammonium acetate

MS/MS Conditions

Table 2 shows the Agilent Jetstream parameters and Table 3 shows the MS conditions. MRM transistions and conditions were optimized using the MassHunter Optimizer software. Agilent Jet Stream parameters were optimized based on the mobile phase conditions and flow rates used in the analysis.

Table 2. Agilent Jet Stream Parameters

ESI with Agilent Jet Stream parameters, pos/neg fast polarity switching	
Drying gas temperature	200 °C
Drying gas flow	8 L/min
Nebulizer pressure	45 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary voltage	± 3,000 V
Nozzle voltage	± 500 V
Delta EMV	500 V
Resolution	Unit, unit

Table 3. MS Conditions for The Analysis of Trichothecenes and Zearalenone

Mycotoxin	Precursor ion	Product ion	Fragmentor	Collision energy	Polarity
15-acetyl-deoxynivalenol	356	321	95	5	Positive
15-acetyl-deoxynivalenol	356	137	95	8	Positive
15-acetyl-deoxynivalenol	339	137	105	12	Positive
3-acetyl-deoxynivalenol	397	337	95	4	Negative
3-acetyl-deoxynivalenol	397	59	95	20	Negative
Diacetoxyscirpenol (DAS)	384	307	105	4	Positive
Diacetoxyscirpenol (DAS)	384	247	105	6	Positive
Deoxynivalenol (DON)	355	265	95	4	Negative
Deoxynivalenol (DON)	355	59	95	20	Negative
Fusarenone-X	413	263	95	8	Negative
Fusarenone-X	413	59	95	28	Negative
HT-2 toxin	442	263	105	4	Positive
HT-2 toxin	442	215	105	4	Positive
Neosolaniol	400	215	95	16	Positive
Neosolaniol	400	185	95	16	Positive
Nivalenol	371	311	108	4	Negative
Nivalenol	371	281	108	8	Negative
Nivalenol	371	59	108	24	Negative
T-2 toxin	484	215	120	16	Positive
T-2 toxin	484	185	120	14	Positive
Zearalenone	317	175	190	16	Negative
Zearalenone	317	131	190	24	Negative
Zearalanone	319	275	185	16	Negative
Zearalanone	319	205	185	16	Negative

Fast continous polarity switching was used for this method to allow the measurement of each mycotoxin in the ionization mode that allows best sensitivity. This can be done either by using static MRM with or without time segmentation or by using Dynamic MRM. When using Dynamic MRM with a retention time window of 1 minute and a cycle time of 500 ms, the maximum number of concurrent MRMs is 12, resulting in a minimum dwell time of 31.5 ms and a maximum dwell time of 246.5 ms. A cycle time of 500 ms allowed for at least 20 data points across the chromatographic peak for accurate quantitation. A chromatogram for 50 ppb of the mycotoxins spiked into wheat matrix is shown in Figure 1.

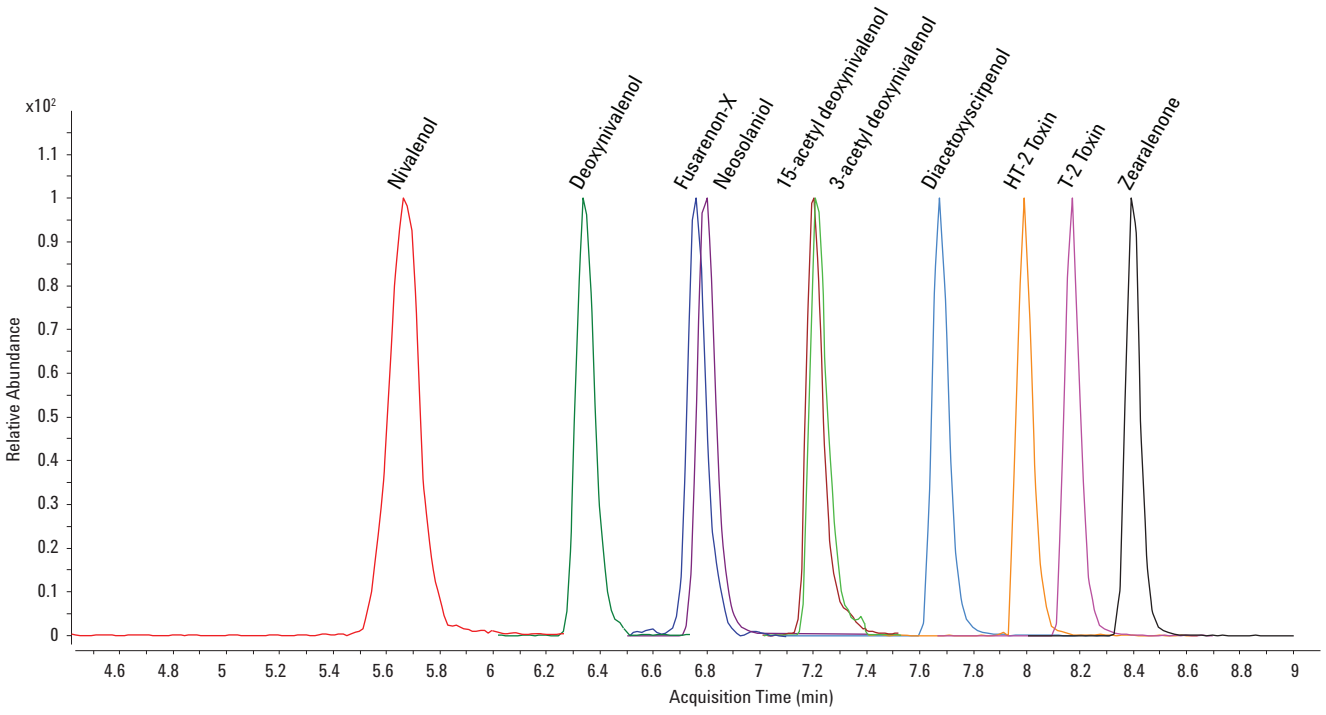

Figure 1. 50 ppb Wheat Matrix Standard : normalized chromatogram for primary transitions. Please note that this method does not require chromatographic resolution between the isomers 15-acetyl DON and 3-acetyl DON because they are distinguished through measurement under different mass spec polarities.

Figure 2 shows the calibration curves for three selected mycotoxins acquired for matrix matched calibration standards with corresponding chromatograms.

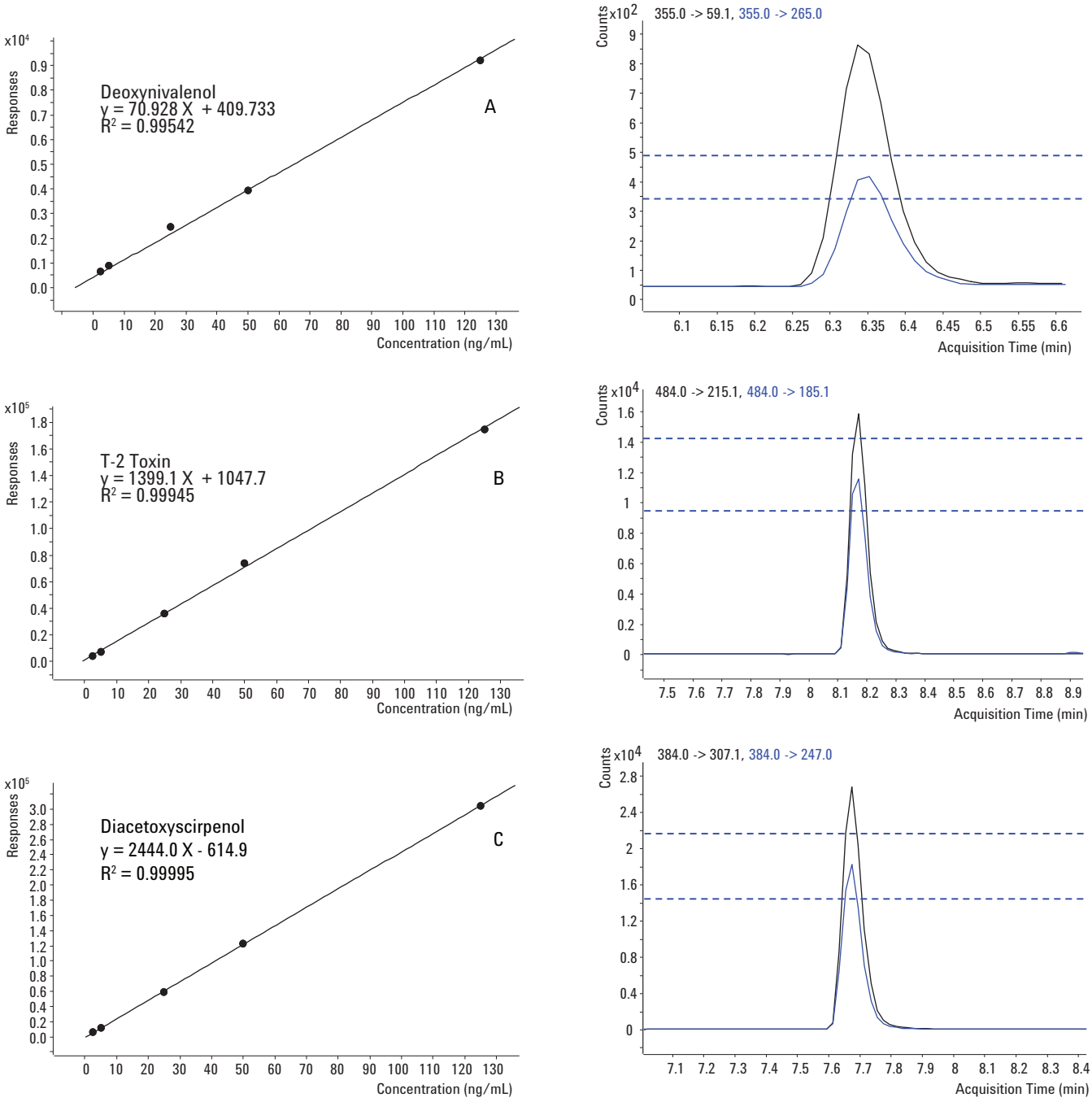

Figure 2. Calibration curves, (A) Deoxynivalenol , (B) T-2 toxin, (C) Diacetoxyscirpenol, with corresponding chromatograms.

Excellent linearity was observed for all mycotoxins ($R^2 \geq 0.995$) and spiked wheat samples (50 µg/kg, n = 9) showed recoveries within 72 to 105% (CV ≤ 11 %) for all compounds and both sample preparation methods (Table 4).

Analyte sensitivity was excellent with both sample preparation techniques, whereas the BE Mycotoxin SPE cartridge clean-up with its greater sorbent capacity produced cleaner extracts with slightly improved LODs (S/N > 3) and LOQs (S/N >10). The LOQs in the final extract for all mycotoxins ranged from 0.003 µg/kg (diacetoxyscirpenol) to 1.04 µg/kg (nivalenol) using modified QuEChERS, and from 0.002 µg/kg (diacetoxyscirpenol) to 0.66 µg/kg (15-acetyl-deoxynivalenol) for the BE Mycotoxin SPE method, respectively (Table 5).

Table 4. Comparison of modified QuEChERS and Cartridge SPE Recovery Data Spiked in Wheat Samples at 50 µg/kg (n=9)

Analyte	Analyte retention time (min)	Modified QuEChERS recovery (%)	Modified QuEChERS RSD (%)	BE Mycotoxin SPE recovery (%)	BE Mycotoxin SPE RSD (%)
Nivalenol	5.6	73	7	93	11
Deoxynivalenol	6.4	85	8	84	11
Fusarenon-X	6.8	81	9	89	9
Neosolaniol	6.8	94	9	77	9
15-acetyl deoxynivalenol	7.2	88	9	72	10
3-acetyl deoxynivalenol	7.2	100	9	92	11
Diacetoxyscirpenol	7.7	105	2	104	3
HT-2	8.0	83	8	99	4
T-2 toxin	8.2	83	8	100	4
Zearalenone	8.4	87	8	79	9

Table 5. Trichothecene and Zearalenone Limits of Detection and Quantification in Wheat Samples

Analyte	Modified QuEChERS LOD (µg/kg)	Modified QuEChERS LOQ (µg/kg)	BE Mycotoxin SPE LOD (µg/kg)	BE Mycotoxin SPE LOQ (µg/kg)
Nivalenol	0.31	1.04	0.07	0.24
Deoxynivalenol	0.04	0.12	0.04	0.12
Fusarenon-X	0.09	0.3	0.08	0.26
Neosolaniol	0.13	0.4	0.03	0.1
15-acetyl deoxynivalenol	0.2	0.66	0.2	0.66
3-acetyl deoxynivalenol	0.1	0.34	0.1	0.33
Diacetoxyscirpenol	0.001	0.003	0.0006	0.002
HT-2	0.05	0.17	0.03	0.1
T-2 toxin	0.01	0.04	0.006	0.02
Zearalenone	0.02	0.06	0.02	0.06

LOD = limit of detection (S/N > 3), LOQ = limit of quantification (S/N > 10)

Conclusion

Wheat samples were spiked at 50 µg/kg with nine trichothecenes and zearalenone. Ion suppression in the sample extracts was overcome by quantifying against a matrix-matched standard. Both the modified QuEChERS and the Bond Elut Mycotoxin SPE demonstrated excellent analyte sensitivity. The Bond Elut mycotoxin SPE procedure, with its greater sorbent capacity, produced cleaner extracts with slightly improved LODs and LOQs. The two sample preparation techniques gave recoveries from 72 to 105%.

However, the modified QuEChERS method had several advantages. It was substantially quicker, allowing greater sample throughput, and used considerably less solvent and sorbent resulting in significantly reduced analytical costs.

References

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