New SPE Sorbent for Clean-up of Fusarium Toxin-contaminated Cereals & Cereal-based Foods, Bond Elut Mycotoxin

Application Note

Fusarium Fungi, Cereals

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Introduction

Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. A variety of fusarium fungi produce different toxins of the class of trichothecenes[1]. More than 140 of these compounds have been isolated from fungi cultures and this number is still increasing[1]. The toxic effects of fusarium toxins are well-documented[2,3] and reliable and sensitive analysis methods, which comply with the European regulations for mycotoxin determination in food and feed, are required[4,5]. Traditional sample preparation for trichothecene analysis typically involves extraction with acetonitrile/water and clean-up via charcoal-alumina columns[6]. As the trichothecenes differ considerably in polarity and solubility, recoveries of the more polar analytes are often compromised with this approach. Another approach is the use of immunoaffin ity columns (IAC)[7]. These provide highly selective extractions with high recoveries, however, separate IAC columns are needed for each toxin. To overcome the limitations of these methods, there was a need to develop an extraction and clean-up method for the simultaneous determination of several trichothecenes with high recoveries for polar toxins by minimizing the matrix effects.

This application note shows the optimized extraction and clean-up step of 12 type A- and B-trichothecenes and zearalenone (ZEA) in cereals and cereal-based food on Bond Elut Mycotoxin, a newly developed extraction sorbent. Structures and names of the 12 toxins investigated in this application are shown in Figure 1.

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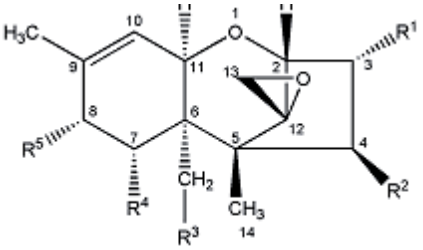
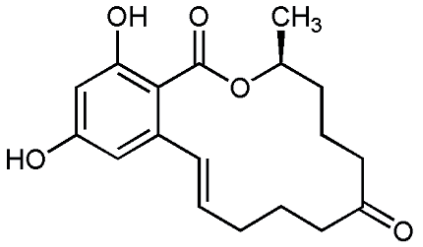
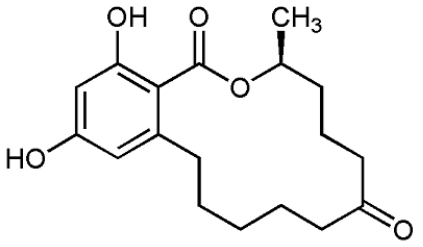


Trichothecenes



Zearalenone



Zearalanone

A healthy wheat head (left) next to one showing severe symptoms of Fusarium head blight disease (right) (Photo by Keith Weller, ARS US Department of Agriculture)

	Trichothecene	R1	R2	R3	R4	R5
Type A	Neosolaniol (NEO)	OH	OAc	OAc	H	OH
	HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	T-2 toxin (T-2)	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
	T-2 triol	OH	OH	OH	H	$OCOCH_2CH(CH_3)_2$
	T-2 tetraol	OH	OH	OH	H	OH
	Monoacetoxyscirpenol (MAS)	OH	OH	OAc	H	H
	Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
Type B	Deoxynivalenol (DON)	OH	H	OH	OH	=O
	3-Acetyl-DON (3ADON)	OAc	H	OH	OH	=O
	15-Acetyl-DON (15DON)	OH	H	OAc	OH	=O
	Nivalenol (NIV)	OH	OH	OH	OH	=O
	Fusarenon-X (FUS)	OH	OAc	OH	OH	=O

Figure 1. Chemical structure of type A- and B-trichothecenes, zearalenone (ZEA) and zearalanone (ZAN). Type A trichothecenes have various groups at ring position 8, type B-trichothecenes have a carbonyl function at position 8

Extraction and Clean-up

Clean-up methods of trichothecenes and ZEA from cereals and cereal-based foods widely use commercially available polar clean-up columns. Analytical interfering substances are retained while trichothecenes are not adsorbed on the packing material. This purification method, however, gives low recoveries for the polar toxins NIV, T-2 tetraol and DON. New studies[9] point out that one possible reason for the low recoveries for the polar toxins might be the low water content in the acetonitrile/water mixture (ACN/H_20; 84/16; v/v), which is used for the extraction of the mycotoxins from the matrix. Furthermore, to elute these polar compounds from the column containing polar adsorbents like alumina, a hydrophilic solvent is needed. When using more polar extraction mixtures like ACN/H_20 (75/25; v/v), the recoveries of the polar toxins NIV, T-2 tetraol and DON could be raised. However, the higher the content of water in the extraction solvent resulted in co-extraction of more matrix compounds and led therefore to strong ion suppression in the LC-MS analysis.

To address these problems, we optimized the extraction step by marginally increasing the polarity of the extraction solvent to ACN/H_20 (80/20; v/v) and used the Bond Elut Mycotoxin cartridge to clean up the extracts. Trials with the polar DON reference material from Food Analysis Performance Assessment Scheme (FAPAS) confirm that the best recovery data were achieved with the Bond Elut Mycotoxin method (Table 1).

Table 1. Recovery comparison of Food Analysis Performance Assessment Scheme (FAPAS) certified reference material for DON applying LC-MS/MS.

Reference Material	Certified Value (µg/kg)	Method 1 (µg/kg)	Method 2 (µg/kg)
FAPAS T2210	463 ± 167	495 ± 5	395 ± 15

Method 1: Clean-up on Bond Elut Mycotoxin
Method 2: Clean-up on competitor column (charcoal-alumina)

To calculate the amount of ZEA, the extracted matrices were spiked with a defined amount of zearalanone (ZAN) standard solution before the clean-up step on Bond Elut Mycotoxin. The measured value of ZEA was corrected by the value of ZAN, as previously described by Berthiller et al[8]. Using ZAN as internal standard, the recovery of ZEA was about 100%.

Bond Elut Mycotoxin Method

1. Extract 25 g of finely ground sample with a solution of 100 mL acetonitrile/water (80/20; v/v) by blending at high speed for 3 minutes. For simultaneous determination of zearalenone (ZEA), spike extract at a level of 50 ng/g sample with zearalanone (ZAN) solution in acetonitrile as internal standard.

2. Filter.

3. Pass 4 mL of the filtrate through a Bond Elut Mycotoxin column (part number 12165001B).

4. Evaporate 2 mL of the eluate to dryness at 50 °C under a gentle stream of nitrogen.

5. Reconstitute in 0.5 mL acetonitrile/water (20/80; v/v). Inject 10 µL into LC-MS/MS for analysis.

Results and Discussion

The Bond Elut Mycotoxin product provides a single column method for the clean-up of 12 type A- and B-trichothecenes plus ZEA (corrected via internal standard ZAN).

Table 2 shows the average recoveries and RSDs obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples after clean-up with Bond Elut Mycotoxin columns. By combining an increased polarity of the extraction solvent with the clean-up step on Bond Elut Mycotoxin, recoveries, especially for the polar toxins DON, NIV, 3ADON and T-2 tetraol, were increased up to 31% when compared to the extraction method on charcoal-alumnia cartridges[9].

Table 3 shows the trichothecene content of 6 naturally contaminated samples after 3 different clean-up methods. Up to 43% higher values were achieved in the analysis of naturally contaminated samples for the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based method. If the determination of DON alone is of interest, then the highest content can be achieved with an extraction of 100% water and clean-up with IAC; however, for the determination of 12 trichothecenes with different polarities, the Bond Elut Mycotoxin provides comparable results.

Conclusion

As the performance of the Bond Elut Mycotoxin cartridges is similar or better, and the columns are more cost effective, the new clean-up procedure is a very good alternative to other standardized methods commonly used.

Table 2. Average recovery and RSD in percentage obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples (spiking levels of 50/100, 200/400 and 500/1000 ng/g for trichothecenes/DON and 50 ng/g for ZEA and ZAN), after clean-up with Bond Elut Mycotoxin columns, (n=3). Data reported by Klötzel et al[10].

Toxin	Recovery [%] ± RSD [%], 3 levels, n = 3						
	Wheat	Corn	Durum	Oats	Bread	Muesli	Infant Food
DON	90 ± 5.2	93 ± 2.8	98 ± 3.8	96 ± 5.1	87 ± 1.7	87 ± 3.7	88 ± 12
NIV	67 ± 5.9	74 ± 2.5	67 ± 6.3	73 ± 10	65 ± 5.7	71 ± 13	66 ± 10
3ADON	89 ± 9.3	88 ± 7.6	97 ± 6.6	93 ± 11	100 ± 5.5	101 ± 7.1	91 ± 9.4
15ADON	92 ± 13	87 ± 15	89 ± 11	89 ± 11	96 ± 9.5	98 ± 8.3	96 ± 6.6
FUS	91 ± 10	94 ± 4.2	91 ± 7.8	91 ± 7.8	98 ± 8.5	97 ± 6.4	96 ± 4.3
T-2	87 ± 7.6	88 ± 8.8	84 ± 2.2	84 ± 2.2	83 ± 8.2	75 ± 11	70 ± 7.3
HT-2	82 ± 7.3	91 ± 3.3	85 ± 5.0	85 ± 5.0	79 ± 3.3	70 ± 7.7	74 ± 0
NEO	91 ± 2.6	78 ± 11	68 ± 18	68 ± 18	80 ± 2.0	104 ± 10	71 ± 6.3
DAS	82 ± 8.3	89 ± 3.6	85 ± 5.2	85 ± 5.2	75 ± 3.7	82 ± 6.8	68 ± 4.6
MAS	86 ± 13	85 ± 12	93 ± 4.2	93 ± 4.2	86 ± 11	88 ± 16	91 ± 14
T-2 triol	69 ± 9.1	66 ± 1.2	83 ± 2.8	83 ± 2.8	76 ± 9.3	82 ± 3.3	71 ± 7.9
T-2 tetraol	69 ± 12	75 ± 6.8	73 ± 10	73 ± 10	65 ± 11	67 ± 17	70 ± 16
ZEA	110 ± 5.9	113 ± 5.0	108 ± 4.8	108 ± 4.8	111 ± 6.0	102 ± 2.7	116 ± 6.7

Table 3. Trichothecene contents of six naturally contaminated samples analyzed with DONPrep IAC, MycoSep 227 and Bond Elut Mycotoxin cartridges, (n=3). Data reported by Klötzel et al[10].

Sample	Clean-up	DON [ng/g]	NIV [ng/g]	15ADON [ng/g]	HT-2 [ng/g]	T-2 [ng/g]	T2 tetraol [ng/g]
Bread	IAC	690 ± 18					
Bread	Mycosep	557 ± 19					
Bread	Bond Elut Mycotoxin	648 ± 21					
Corn	IAC	368 ± 8.4					
Corn	Mycosep	333 ± 14	12 ± 0	69 ± 2.0			
Corn	Bond Elut Mycotoxin	356 ± 3.8	14 ± 0	99 ± 2.5			
Wheat	IAC	488 ± 5.5					
Wheat	Mycosep	421 ± 16					
Wheat	Bond Elut Mycotoxin	468 ± 19					
Oats	IAC	299 ± 11					
Oats	Mycosep	220 ± 5.3	22 ± 3.3	7.0 ± 0.4	93 ± 12	15 ± 4.1	91 ± 6.2
Oats	Bond Elut Mycotoxin	264 ± 13	19 ± 1.2	7.7 ± 0.1	78 ± 4.9	12 ± 4.3	106 ± 3.2
Wheat	IAC	1680 ± 32					
Wheat	Mycosep	1590 ± 40	39 ± 3.7	24 ± 2.0			
Wheat	Bond Elut Mycotoxin	1750 ± 120	64 ± 1.4	42 ± 2.2			
Durum	IAC	512 ± 15					
Durum	Mycosep	407 ± 25	25 ± 4.1				
Durum	Bond Elut Mycotoxin	456 ± 42	22 ± 1.3				

Ordering Information

Part number	Description
12165001B	Bond Elut Mycotoxin 1 gm in JR cartridge, 100 cartridges/pk
12131009	Reservoir 6 mL 100 tubes/pk
12131015	Reservoir 6 mL with 2 x 20 µm polypropylene filter pre-installed 100/pk
12131021	20 µm polypropylene filter for 6 mL cartridges
12234105	Vac Elut 20 Manifold SPE Cartridge Processing Station with collection rack for 10x75 mm test tubes

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