Improved Isolation and Analysis of Mycotoxins from Cereals, Beer and Wine

Application Note

Authors

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Introduction

Trichothecenes are important mycotoxins produced primarily by the genus fusarium. The toxic effects of fusarium toxins on human and animals are well documented and reliable and sensitive analysis methods are therefore required to protect the population and its food sources[1]. This application shows the optimized extraction and clean-up of 12 type A- and B-trichothecenes (Neosolaniol [NEO], HT-2 toxin [HT-2], T-2 toxin [T-2], T-2 triol, T-2 tetraol, Monoacetoxyscirpenol [MAS], Diacetoxyscirpenol [DAS], Deoxynivalenol [DON], 3-Acetyl-deoxynivalenol [3ADON], 15-Acetyl-deoxynivalenol [15ADON], Nivalenol [NIV], Fusarenon [FUS]) and Zearalenone (ZEA) in cereals and cereal-based food, as well as the four polar mycotoxins DON, T-2, HT-2 and ZEA in wheat beer and sake wine matrices. The clean-up was optimized on Bond Elut Mycotoxin, a newly developed solid phase extraction (SPE) sorbent. The LC-MS-MS method presented here gives quantitative and qualitative information and has the accuracy and resolution required for reporting results to European and US governmental agencies[2].

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Sample Preparation

Cereals and cereal-based food samples

Typical clean-up methods of trichothecenes and ZEA from cereals and cereal-based foods use commercially available polar clean-up columns[3]. Substances that interfere with the detection of the mycotoxins are retained while trichothecenes are not. This purification method, however, gives low recoveries for the polar toxins: NIV, T-2 tetraol, and DON.

To address these problems, the extraction step was optimized by marginally increasing the polarity of the extraction solvent to ACN/H_2O (80/20; v/v) as previously described in Varian Application Note 295[4]. Trials with the polar DON reference material from Food Analysis Performance Assessment Scheme (FAPAS) confirm that the best recovery data is 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

Method 1: Clean up on Bond Elut Mycotoxin
Method 2: Clean up on polar charcoal-alumina sorbent (PS)

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

Wheat beer and sake wine samples

Prior to clean-up by the Bond Elut cartridge, the wheat beer and sake wine samples were degassed by sonication for 30 min at room temperature. The degassed samples were filtered through Whatman no.1 filter paper (Florham Park, NJ), and the solution of multiple mycotoxins was added to a final concentration of either 35 or 350 ng/g with an internal standard (ZAN) concentration of 50 ng/g. This mixture was applied to a Bond Elut Mycotoxin column. Fully automated clean-up was performed on a Zymark SPE workstation (Hopkinton, MA) according to the Bond Elut Mycotoxin method (Table 2).

Table 2. Bond Elut Mycotoxin Method

1. Pass 4 mL of the filtrated sample extract through a Bond Elut Mycotoxin column (part number 12165001B).
2. Evaporate 2 mL of the eluate to dryness at 50 °C under a gentle stream of nitrogen.
3. Reconstitute in 0.5 mL acetonitrile/water (20/80; v/v).
4. Inject into LC-MS-MS.

LC-MS-MS

The LC-MS-MS system utilized in these experiments is the 320-MS triple quadrupole mass spectrometer fitted with an electrospray ionization (ESI) source, two ProStar 210 liquid chromatography pumps, and an Agilent ProStar 430 autosampler. Separation was according to the gradient program shown in Table 3.

Table 3. Gradient program and LC/API conditions

Time (min)	A (%)	B (%)	Flow (mL/min)
0:00	100	0	0.3
16:00	40	60	0.3
16:01	40	60	0.3
40	30	70	0.3
40:01	85	15	0.4
50:00	85	15	0.4

LC Conditions

Column:	Polaris C18-A 5 µm, 150 mm × 3.0 mm id (p/n A2000150X030)
Buffer A:	5 mM ammonium acetate, 1% acetic acid in 10% methanol
Buffer B:	5 mM ammonium acetate, 1% acetic acid in 100% methanol
Injection Solvent:	Buffer A
Injection Volume:	30 µL

API Conditions

Ionization Mode:	ESI (positive and negative)
Collision Gas:	1.8 mTorr argon
API Drying Gas:	30 psi at 250 °C
API Nebulizing Gas:	50 psi
Needle:	4500 V
Capillary:	Scanning
Detection:	1900 V

Results and Discussion

Cereals and cereal-based food samples

With the low increase of 6% water in the extraction solvent and 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%.

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 the more expensive IAC; however, for the determination of 12 trichothecenes with different polarities, the Bond Elut Mycotoxin cartridge provides good results.

Figure 1 shows the trichothecene content of 5 naturally contaminated samples after 3 different clean-up methods.

Up to 43% higher values were achieved using Bond Elut Mycotoxin for the clean-up of naturally contaminated samples containing the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based polar method.

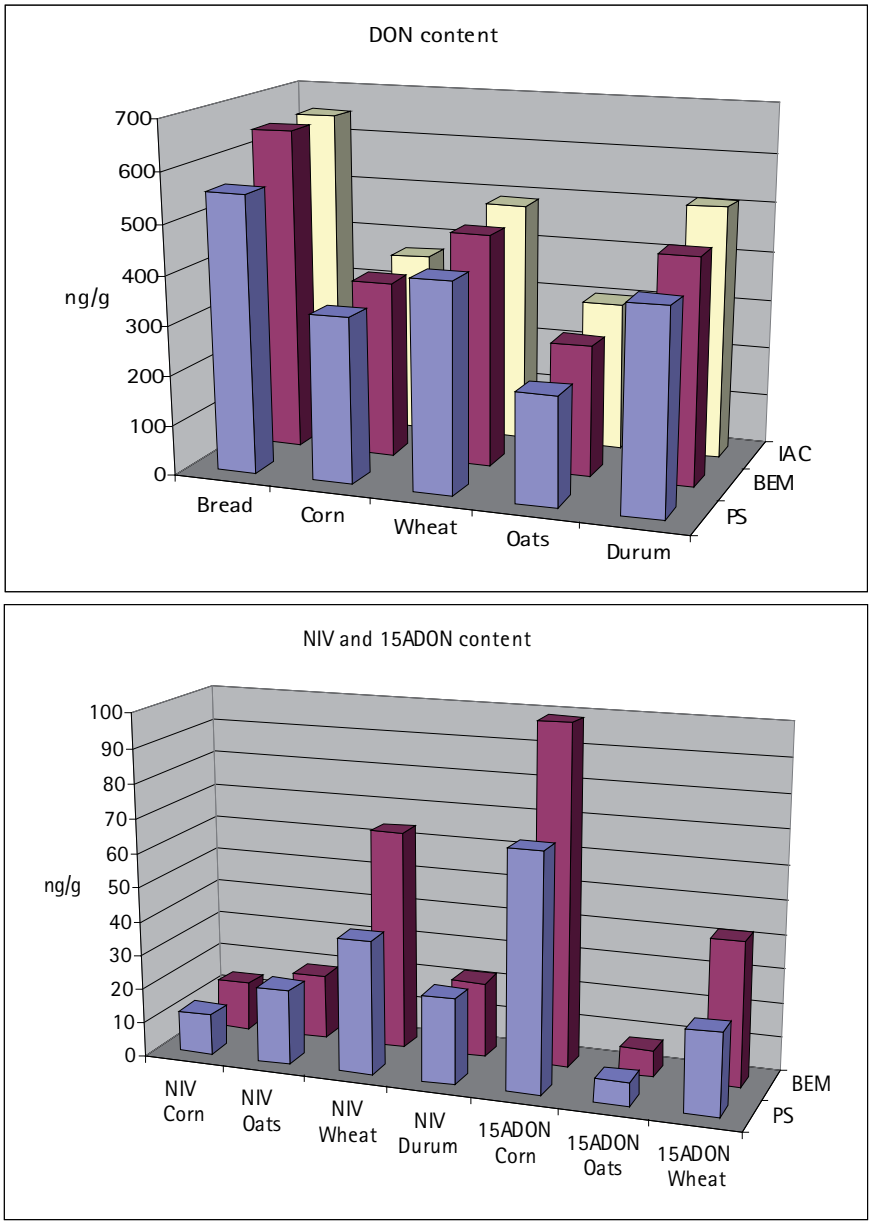

Figure 1. Trichothecene contents of 5 naturally contaminated samples analyzed with DONPrep (IAC), Polar charcoal-alumina sorbent (PS) and Bond Elut Mycotoxin cartridges (BEM) (n=3)

Wheat beer and sake wine samples

A mixture of HT-2, T-2, ZEA and ZAN (internal standard) at a low-level concentration of 35 ng/g was spiked into a complex matrix of wheat beer and/or sake wine.

Figure 2 shows the chromatograms with excellent peak shape and high signal response of the mycotoxins in a wheat beer matrix.

Table 4 shows the recovery rates calculated for high concentration of 350 ng/g and low concentration of DON, ZEA, T-2 and HT-2 from wheat beer and sake after clean-up on Bond Elut Mycotoxin. Very good recoveries (> 90%) and excellent reproducibility (n=3) are displayed for DON and ZEA. The recoveries of T-2 and HT-2 are >60% and they satisfy the European requirements. The EU guideline 2005/38/EG sets recovery limits for DON and ZEA in the range of 60–120% and 60–130% for T-2 and HT-2.

Summary

This application note describes a new, reliable, and cost-efficient clean-up method for the determination by triple quadrupole LC-MS-MS, of 12 type A- and B-trichothecenes in cereals and cereal-based foods. In addition, it shows the determination of four polar mycotoxins DON, T-2, HT-2 and ZEAN in wheat beer and sake wine matrices. The extracted mycotoxins are purified by means of the newly developed Bond Elut Mycotoxin SPE cartridge. The method exceeds the required limits of detection as well as ion ratio requirements for European Directives (ion ratios of <30%), such as SANCO D.1(06)D/412820.

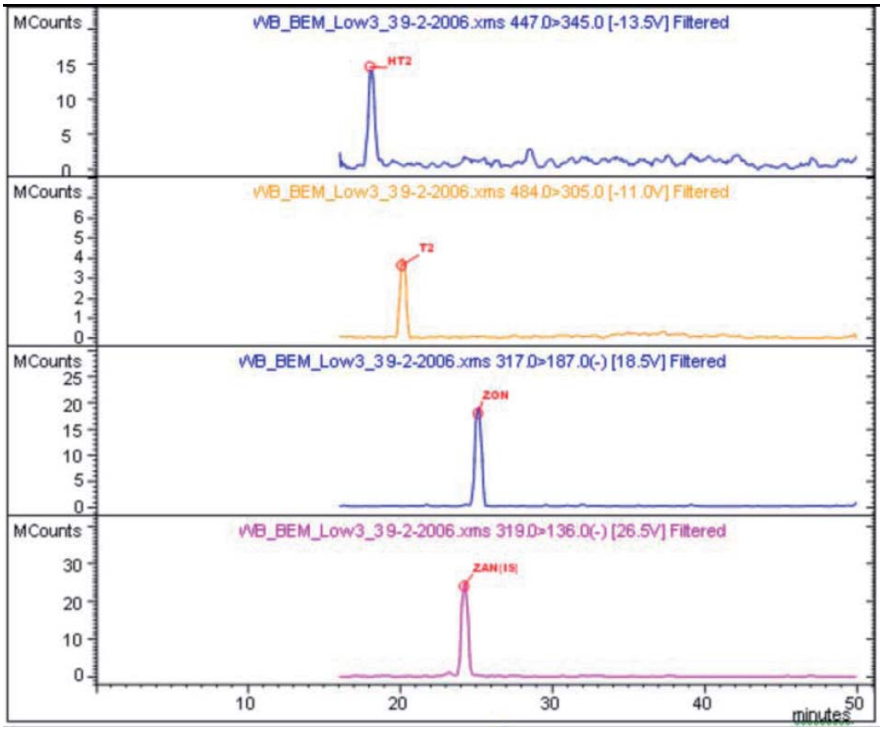

Figure 2. Example chromatograms of low-level spikes for various mycotoxins in a wheat beer matrix. Shown (top to bottom) are: HT-2, T-2, ZEA and ZAN (internal standard)

Table 4. Rates of recovery for selected mycotoxins after a clean-up step on Bond Elut Mycotoxin from wheat beer and sake at two concentrations; relative standard deviation is calculated from three replicates

	Wheat beer matrix			
	% Recovery	**% RSD**	**% Recovery**	**% RSD**
Mycotoxin	35 ng/g		350 ng/g	
DON	92	2.6	95.5	1.5
ZEA	116	6.1	101.9	1.3
T-2	61.3	12.6	60.1	1.1
HT-2	81.8	5.6	76.1	1.4
	Sake wine matrix			
	% Recovery	**% RSD**	**% Recovery**	**% RSD**
Mycotoxin	35 ng/g		350 ng/g	
DON	94.3	7.4	96.8	0.5
ZEA	99.3	1.3	99.8	0.8
T-2	101.3	1.3	66.0	0.9
HT-2	113.9	8.3	111	1

References

[1] European Commission, Scientific Commitee on Food; Opinion on Fusarium Toxins Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol; http://www.europa.eu.int/comm/food/food/chemicalsafety/contaminants/fusarium_en.htm (adopted: 02/27/2002)

[2] World Wide Regulations for Mycotoxins in Food and Feed in 2003. FAO Food and Nutrit. 2003, 81, 3.1–3.5.

[3] R. Krska, S. Baumgartner, R. Josephs (2001) The state-of-the-art in the analysis of Type A and B trichothecene mycotoxins in cereals; Fresenius J. Anal. Chem. 371, 285-299

[4] Agilent Application Note SI-00295, Bond Elut Mycotoxin; New SPE Sorbent for Clean-up of Fusarium toxin-contaminated Cereals and Cereal-based Foods, M.Klötzel, Uwe Lauber, Chemisches u. Veterinäruntersuchungsamt Stuttgart, Germany

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