Inspiring Excellence

New Fast Clean-up Method for Deoxynivalenol from Feed

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Introduction

Mycotoxin intake from contaminated food and feed is recognised worldwide as the most important source for mycotoxin intoxication. Of special interest in temperate climate regions are the widely distributed Fusarium fungi, that can produce a wide range of mycotoxins including several trichothecenes, with Deoxynivalenol (DON) (Figure 1) as perhaps the most commonly detected Fusarium mycotoxin. DON, also known as Vomitoxin, is produced by Fusarium fungi on corn and wheat prior to harvest as well as during storage.

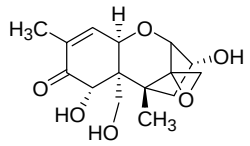

Figure 1:Structure of Deoxynivalenol

DON has been associated with reduced milk production in dairy cattle and vomiting in swine that consume contaminated feed, or their refusal to eat feed containing the toxin. DON intake from animals may also be a hazard to human health since animal products consumed by people may contain toxin residues. While healthy animals tend to "filter out" or detoxify many of the mycotoxins to which they are exposed, the issue of mycotoxin residues in milk and animal tissues should not be ignored.

The EU recommends guidance values for DON between 0.9 and 12 mg/kg, depending on the feed and animal. Suitably fast, sensitive and reliable methods for the determination of Fusarium toxins are therefore required.

This poster presents a new clean-up step as an alternative to the commonly used immunoaffinity (IAC) clean-up step for DON from feed for young pigs. The new method is performed on a Bond Elut™ Mycotoxin cartridge, which has previously been used for the effective clean-up of 12 trichothecenes and zearalenone from cereal-based foods contaminated with Fusarium toxin [1]. Our results indicate that the Bond Elut Mycotoxin method is much faster than previously used clean-up procedures with IAC. The clean-up time is almost half the time required by IAC clean-up, thus increasing sample throughput.

Clean-up with the Bond Elut Mycotoxin Cartridge

The clean-up step with Bond Elut Mycotoxin is very fast and simple. No conditioning of the SPE sorbent is necessary and no vacuum system is needed. The special design of the cartridge (Figure 2) allows the use of a standard syringe with a luer fitting to apply the extract.

Figure 2: Bond Elut Mycotoxin Cartridge from Varian, Inc., Part Number 12165001B

Method

A volume of 100 µl of the internal standard Deepoxy- deoxynivalenol (DOM-1) with a concentration of 10 µg/mL is added to 2.5 g complete feed for young pigs. The sample is extracted for 90 minutes with 20 mL 80% acetonitrile / 20 % water. The extract is centrifuged and 4 mL passed through a Bond Elut Mycotoxin cartridge. The collected eluate is evaporated to dryness and reconstituted with 1mL 20% acetonitrile. 20 µL are injected into the LC/MS/MS.

LC/MS/MS conditions:

HPLC Column:	C18 (3.5 µm, 2.1 x 150 mm) ultra pure silica, double encapped, extra high phase density
Mobile Phase A:	0,2 % Formic Acid
Mobile Phase B:	Acetonitrile
Isocratic Conditions:	75% A, 25% B
API Conditions:	ESI Negative Ion Mode
Scan Mode:	MRM
Mass Transitions:	295 > 265 and 295 > 138 for DON, 279 > 231 for DOM-1

Results

With each measurement series, a 5 point calibration of DON in solvent and matrix is determined (Figure 3). The quantification is based on the matrix calibration. The standard calibration is used to check the MS system and to determine the recoveries. The calibration method is based on DIN 32645 for several feed products. The limit of detection is 6.9 µg/kg and the limit of quantification is 13.8 µg/kg.

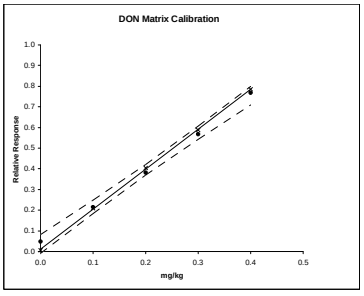

Figure 3: Calibration Curve of Deoxynivalenol

Figures 4, 5, show the MRMs of a DON reference standard (200 µg/kg) and a spiked (200 µg/kg) blank feed sample with recovery calculated to 95% of DON. Figure 6 shows the MRMs of a contaminated feed sample. High precision and linearity were achieved with the Bond Elut Mycotoxin method by using an internal standard. With the IAC method, the use of an internal standard is difficult, as it requires a selective antibody, which is not easily available. The performance of the SPE column is similar. However, the method is faster and so sample throughput could be increased. This, in addition to the lower price of the Bond Elut Mycotoxin cartridge, shows that this new clean-up procedure is a good, cost efficient alternative to the IAC method.

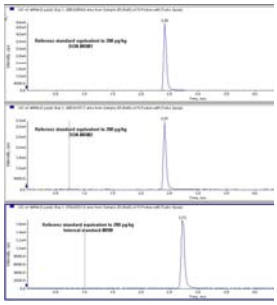

Figure 4: LC/MS/MS of Deoxynivalenol Reference Standard. Concentration 200 µg/kg

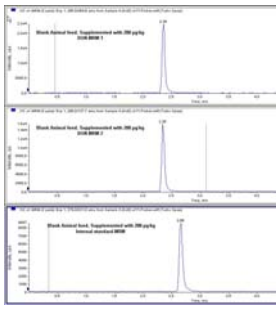

Figure 5: LC/MS/MS of a Blank Feed Sample spiked with 200 µg/kg Deoxynivalenol. Clean-up Bond Elut Mycotoxin

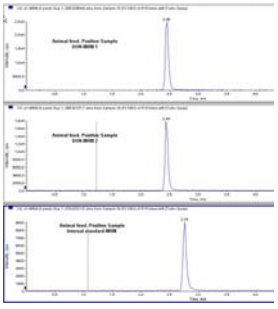

Figure 6: LC/MS/MS of a Deoxynivalenol contaminated Feed Sample after Clean-up on Bond Elut Mycotoxin

Conclusion

Bond Elut Mycotoxin cartridges are a very good product for the clean-up of DON. The clean extracts minimize the matrix effects and show high recoveries in LC/MS/MS analysis. As the performance of the SPE cartridge is similar to IAC and the columns are less expensive, the new clean-up procedure is a good alternative to IAC methods commonly used.

Reference

[1] Varian Application Note 00295, Bond Elut ® Mycotoxin, New SPE sorbent for Clean-up of Fusarium toxin-contaminated cereals and cereal-based foods

SI-1765/A09.08

Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit

Presented at ChromSAAMS 2008