Determination of Deoxynivalenol in Shredded Wheat Cereal using Automated Solid Phase Extraction with Immunoaffinity Cartridges

Toni Hofhine, Horizon Technology, Inc.
Elizabeth K. Krantz, Dr. Pamela Doolittle, and Dr. Cheri Barta,
University of Wisconsin-Madison

Key Words
SPE, Solid Phase Extraction, Deoxynivalenol, SmartPrep

Introduction

Deoxynivalenol is a common mycotoxin found in agricultural grain crops and final consumer processed products. Most impacted are wheat, barley, and corn. Deoxynivalenol, also known as Vomitoxin, is produced by Fusarium fungi and has a unique dual-stage growth cycle of producing mold during warm daylight and toxin during cool nights. There is no known procedure or processing that will remove deoxynivalenol that is already present in grain. Vomitoxin, has the ability to withstand high processing temperatures, creating the need for food safety determination of both agricultural crops and processed foods prior to consumption by animals and humans. If consumed, the Fusarium mycotoxin produces serious illness and is known for being one of the most endangering on animal and human health. Identifying deoxynivalenol contamination in wheat products quickly, effectively, and with a high degree of confidence relies heavily on the accuracy of laboratory procedures and measurements.

Currently, the United States has an advisory limit for deoxynivanol of 1 ppm (or 1000 μg/kg) in finished foodstuffs. Current European Union legislation sets the maximum level of deoxynivalenol in foodstuffs at 0.75 ppm (750 μg/kg) for unprocessed cereals marketed for direct consumer consumption and 0.2 ppm (200 μg/kg) for processed cereal-based foods and foods intended for babies and small children. The EU also has performance criteria in place for methods testing for mycotoxins in foodstuffs.

The standard AOAC methodology to determine the presence and quantify the amount of deoxynivalenol in a sample uses liquid liquid extraction, followed by solid phase extraction with immunoaffinity cartridges, such as the VICAM® DONtest WB immunoaffinity cartridges. In this application, a sample of shredded wheat cereal was tested for the presence of deoxynivalenol and spiked at the European Union legislation level of 200 μg/kg for recovery. To increase confidence in the data and remove any ‘laboratory technician effect or bias’, simple automation of the SPE process using the DONtest WB immunoaffinity cartridges was implemented using the SmartPrep® Extractor, followed by evaporation with the XcelVap®. Final quantitation was calculated from recovery results show that using immunoaffinity cartridges can be efficiency automated and pass the EU performance criteria.
Experimental

Sample Pre-Preparation and Storage
To ensure a representative sampling, two full-sized boxes of shredded wheat cereal, were crushed within the packages to create a consistent mixture for sampling. The combined cereal sample was stored in a plastic reusable storage container. Wherever possible, plastic or salinized glass materials were used throughout the method.

Sample Preparation—Liquid Liquid Extraction Procedure
A control shredded wheat cereal sample (non-spiked sample) was prepared to test for the presence of natural deoxynivalenol that was subtracted from spiked sample percent recovery. A single cereal sample was spiked prior to liquid liquid extraction. Both control sample and spiked sample followed the process outlined in Procedure 1.

Procedure 1: Liquid Liquid Extraction Procedure

1. Weigh 25g sample into Erlenmeyer flask
2. Spike @ 200 μg/kg (EU limit)
3. Swirl sample 10 seconds and let sit for 2 minutes
4. Add 125 mL purified laboratory grade water
5. Vortex shake @ 1750 RPM for 15 minutes
6. Filter with Whatman No. 2 filters; hold for SmartPrep Extractor SPE processing

Spiked shredded wheat cereal sample at the EU limits was prepared by pipetting 50 μL of a 100 μg/mL neat solution of deoxynivalenol (supplied by Sigma-Aldrich) into the 25 grams of weighed sample.

Sample Preparation – Automated SmartPrep Extractor SPE Procedure
The solid phase extraction process was automated using the SmartPrep Extractor. From the extracted filtrate, 1 mL was automatically loaded onto the 3 mL DONtest WB immunoaffinity SPE cartridge using the SmartPrep Extractor (Procedure 2).
Automated SPE using the SmartPrep Extractor was performed with the DONtest WB immunoaffinity SPE cartridges using a plunger capable of performing positive pressure SPE while also having the ability to pierce the septa (Figure 1).

**HPLC Analysis Conditions**

Following SPE, evaporated samples and standards were reconstituted with 1 mL of mobile phase and measured with HPLC using the conditions listed in Table 1. Deoxynivalenol samples and standards were injected onto a Shimadzu Nexera XR UHPLC system with single wavelength UV detector. Two injections of each sample and standard were performed, with the average of the injections used for calculations. The deoxynivalenol peak eluted between 3.1 and 3.3 minutes using a Titan column from Sigman-Aldrich.

**Table 1. HPLC Conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Sigma-Aldrich Titan™ C18 UHPLC Column, 10 cm x 2.1 mm I.D., 1.9 μm particle size</td>
</tr>
<tr>
<td></td>
<td>Titan™ C18 HPLC Guard Cartridge, 5 mm x 2.1 mm I.D., 1.9 μm particle size</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>92:4:4 water:acetonitrile:methanol</td>
</tr>
<tr>
<td>HPLC System</td>
<td>Nexera XR UHPLC with UV detection at 220 nm</td>
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<tr>
<td>Flow rate</td>
<td>0.6000 mL/min</td>
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<tr>
<td>Pressure</td>
<td>5700-5900 psi</td>
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<tr>
<td>Column Temp</td>
<td>55 °C</td>
</tr>
<tr>
<td>Injection</td>
<td>2 μL</td>
</tr>
<tr>
<td>Run Time</td>
<td>20 minutes (to accommodate late eluting sample matrix peaks)</td>
</tr>
</tbody>
</table>
Results

Automated SPE SmartPrep Extractor Results

Deoxynivalenol was spiked in a shredded wheat cereal sample at the EU level for infant cereals and foods (200 µg/kg). A control was prepared and injected to subtract any natural deoxynivalenol detected from the spiked cereal recovery sample.

The shredded wheat cereal control had detectable levels of deoxynivalenol at 485 µg/kg (Figure 2). The spiked shredded wheat cereal spiked sample had a calculated level of deoxynivalenol at 132.5 µg/kg following subtraction of the control level, which corresponds to a recovery of 66.2% (Figure 3). This value meets the EU recovery performance criteria of 60 – 110% for deoxynivalenol recoveries spiked at >100 ≤ 500 µg/kg.

![Figure 2: Shredded Wheat Cereal Control](image1)

![Figure 3: Shredded Wheat Cereal Control Spiked at 200 µg/kg](image2)
To avoid late eluting peaks interfering with subsequent injections, the spiked sample injections had a run time of 20 minutes. Baseline fluctuations from several matrix peaks are visible in the cereal sample injections between 2.5 and 5.0 minutes (figures 2 and 3). Similar baseline fluctuations were also present in the standard injections (figures 4, 5 and 6); however, these baseline changes did not interfere with the linearity or accurate quantitation of deoxynivalenol. Standards did not have late eluting peaks, so the run time was reduced to 7 minutes. Figure 4 shows the 0.04 µg/mL deoxynivalenol standard; equivalent to the level of the 200 µg/kg in the spiked cereal sample.

![Figure 4: 0.04 µg/mL Deoxynivalenol Standard](image)

A 7-point standard curve was used at levels of 0, 0.02, 0.04, 0.05, 0.1, 0.2, and 0.5 µg/mL. Figures 5 and 6 represent the lowest and highest calibration standards. Standards were injected to bracket the sample spikes. Linearity of standards was calculated for each run, with an $R^2$ value at 0.9998 (Figure 7).

![Figure 5: 0.02 µg/mL Deoxynivalenol Standard](image)
Figure 6: 0.5 µg/mL Deoxynivalenol Standard

Figure 7: Deoxynivalenol 7-point Calibration Curve
Conclusion

The effectiveness of the VICAM DONtest WB immunoaffinity SPE cartridges in combination with the SmartPrep Extractor System and XcelVap from Horizon Technology demonstrated that shredded wheat cereal samples can be efficiently prepared using simple automation to increase sample preparation efficiency prior to HPLC analysis. Data presented concludes that deoxynivalenol was effectively recovered and passed performance criteria for % recovery at the EU limits of 200 µg/kg. Implementing automation with the SmartPrep Extractor also reduces “scientist bias” by implementing uniform treatment of all samples. Limiting manual preparation for routine laboratory food safety testing will significantly increase and improve laboratory workflow and to determine deoxynivalenol levels more consistently in processed foods and animal feed.

Acknowledgement

This application note acknowledges Bob Johnson and Lewis Chesno for their engineering design, enabling efficient automation using the VICAM immunoaffinity cartridges on the SmartPrep Extractor.

This application note also acknowledges VICAM, Shimadzu Corporation, Inc. and Sigma-Aldrich for providing access to consumables and analysis equipment.

References