INTRODUCTION

Mycotoxins are toxic compounds produced by molds or other fungi that can grow on foods/stuffs intended for domestic animal or human consumption. They are produced by fungi, which may remain in food and feed. A short description of some important mycotoxin classes is given below. Structures for some important mycotoxins are presented in Figure 1.

Aflatoxins: One of the most potent and dangerous classes of mycotoxins. Symptoms of aflatoxins include liver cancer, reproductive problems, anemia, immune system suppression, and jaundice. The FDA action level for aflatoxins is 20 ppb in food for domestic animal or human consumption.

Ochratoxins: Ochratoxin A is the most common and is one of the most toxic mycotoxins worldwide. Because it is a human carcinogen, it is regulated in the European Union at 5 ppb in cereals. Regulations have not yet been established for OTA levels in the US food supply.

Trichothecenes: The most toxic trichothecene mycotoxin in the United States is deoxynivalenol (DON). Symptoms of DON ingestion include nausea, fever, headache, and vomiting. The FDA guidance level for DON is 1 ppm in wheat products for human consumption.

Zearalenone: Zearalenone is a mycotoxin that mimics the reproductive hormone estrogen. Enlarged uterus and sexual organs, enlarged mammary glands, infertility, and abortion have been observed in domestic animals exposed to this mycotoxin. It is regulated in the United States at 100 ppb in agricultural products.

Recently an article was published describing a GBES method for determination of 14 mycotoxins in white rice1. In this paper an evaluation of that method is presented for determination of these mycotoxins in whole grain wheat, maize and rice flours. Good to excellent recovery was observed in all three matrices for most compounds including aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenone.

METHODS

Sample preparation

QuEChERS Extractions: Four samples were purchased at a local grocery store. Each sample was spiked with 50 μg of each surrogate for a total of 200 μg per sample. Each tube was vortex mixed and then centrifuged at 1,800 × g for 2 min. The supernatant was transferred to a 15 mL glass vial containing 200 μL acetonitrile. A 100 μL aliquot was injected for UPLC analysis and the remaining sample was transferred to another 15 mL glass vial containing 100 μL acetonitrile and 250 μg PSA (TaKlo 16000500). Shake the tube vigorously by hand for 1 min and centrifuge at approximately 2200 × g for 5 min. Discard the supernatant and then add 100 μL acetonitrile and 50 μg PSA (TaKlo 16000500). Shake the tube vigorously by hand for 1 min and centrifuge at approximately 2200 × g for 5 min. Discard the supernatant and then add 100 μL acetonitrile and 250 μg PSA (TaKlo 16000500). Shake the tube vigorously by hand for 1 min and centrifuge at approximately 2200 × g for 5 min. For the determination of DON, a 100 μL aliquot was diluted to 1.0 mL with mobile phase for UPLC-HRMS analysis.

UPLC-MS/MS analysis

UPLC Conditions

Column: ACQUITY UPLC BEH™ C18, 1.7µm, 100 x 2.1 mm
LC system: ACQUITY UPLC H-Class
MS Spectrometer: Waters QTOF Premier
Positive ion Electrospray (negative ion for chloramphenicol)
Source Temperature: 150°C
Desolvation Gas Flow: 1000 L/Hr
Data Management: MassLynx v4.1

Table 1. Instrument and calibration parameters are presented in Table 1.

Table 2. Recovery results for this study (abbreviations: BLOQ - below detection limit, INC - insufficient data).

RESULTS

Table 3. Recoveries of mycotoxins in wheat and rice matrices.

CONCLUSIONS

The QuEChERS extraction protocol was simple and effective; very good recovery was observed in all three matrices for most compounds with a few exceptions. After passing the QuEChERS extraction protocol, the supernatant was evaporated to dryness. The cleanup was performed only for analysis using highly sensitive instrumentation (in this case the Xevo TQ-S mass spectrometer). A rigorous cleanup protocol was required for aflatoxins, fumonisins, ochratoxins, trichothecenes and zearalenone.

Since this data was initially reported2, we have investigated a novel SPE sorbent, Oasis PRIME HLB, for more improved cleanup. In a follow-up study3, a successful cleanup of an Oasis PRIME HLB cartridge, much less precipitated material was observed after evaporation and reconstitution compared with dSPE (see Figure 4). Figure 5 shows phospholipid elution from the cartridge in Figure 4. Preliminary results show that the new cleanup protocol does not change the overall recovery shown in Table 2 for any of the analytes. Complete results obtained from the ongoing method development will be presented in an oral presentation in November.

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