Development of an Improved Method of Sample Extraction and Quantitation of Multi-Mycotoxin in Feed by LC-MS/MS

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Abstract

Food and feedstuffs can be easily exposed to the moisture that causes the growth of molds and fungi producing mycotoxins. This may result in potentials for cancer risks or other health effects in humans. High level of contamination by these mycotoxins has been frequently reported in food and feed materials. Therefore, they attracted much attention in last recent years due to their high risk of contamination and consumption in all over the world. A rapid and simple analytical method was developed for the simultaneous quantitation of aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), zearalenone (ZON), deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), fumonisins (FB1, FB2 & FB3), T-2 toxin (T-2) and HT-2 toxin (HT-2) in feed. The three most popular sample preparation techniques for determination of mycotoxins were evaluated, and the method with highest recoveries was selected and optimized. This modified QuEChERS (quick, easy, cheap, effective, rugged and safe) approach was based on the extraction with acetonitrile, salting-out and cleanup with lipid removal. The sample preparation process takes only 3 hours for 12 samples and then the extracts are analyzed by LC-MS/MS in 16 minutes per sample. In this method, the recovery range is 70–100% for DON, DAS, FB1, FB2, FB3, HT-2, T-2, OTA, ZON, AFG1, AFG2, AFB1 and AFB2 and 55% for NIV in the spike range of 2–80 µg/kg. Method robustness was determined with acceptable z-scores in proficiency tests and validation experiments.

Introduction

Mycotoxins are the most common contaminants in agricultural crops produced by several species of mold and fungi. During growth, maturity, harvest, storage and processing of food and animal feed products, the fungus produces mycotoxins and other secondary metabolites. These mycotoxin-contaminated food and feed threaten human and animal health even at very low concentration. Moreover, the presence of mycotoxins in consuming animal products such as milk and meat are concern to humans as well (Figure 1). Hence, developing an accurate and fast analytical method to quantify the contamination levels of mycotoxins plays a vital role in food and feed safety assessment risks.

After two decades of research, simultaneous quantitative determination of mycotoxins and their derivatives in one analysis is challenging due to the wide polarity, solubility and physicochemical properties of these compounds. Most of the existing methods suffer from poor recovery, insufficient sensitivity and non-reproducibility. This makes these methods unsuitable for simultaneous determination of multi-mycotoxin.

The objective of this work was to develop a robust, reliable and fast extraction and clean up technique for multi-mycotoxin in a wide range of agricultural commodities using LC-MS/MS. The results illustrated here show that this sample preparation method can be applied in many laboratories analyzing food and feed materials.

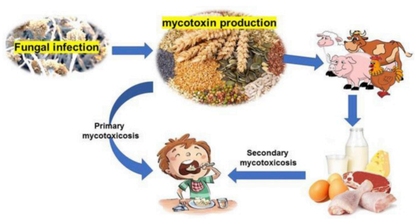

Figure 1. Mycotoxins can cause a variety of adverse health effects.

Materials and Methods

Multi-mycotoxin extractions were prepared using three different sample preparation techniques in corn. The QuEChERS method was further modified and used as a reference method. After a comparison study between these targeted methods, QuEChERS was chosen to employ different cleanup procedures with the purpose of reducing the matrix interferences in extraction step. The steps of modified QuEChERS procedure are shown in Figure 2.

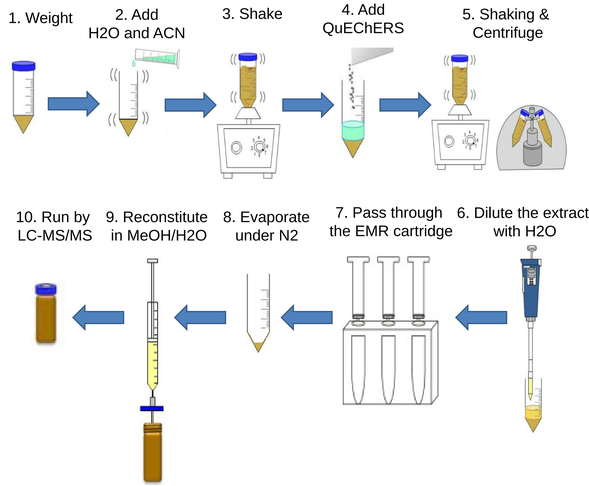

Figure 2. Modified QuEChERS sample preparation procedure.

Liquid chromatography coupled with triple mass spectroscopy is the most recognized analytical instrumentation for the wide range of chemical contaminants such as mycotoxins in agricultural commodities. LC-MS/MS is known as a sensitive, selective, specific and efficient technique because of its versatility and reliability. Shimadzu liquid chromatography equipped with a Triple Quad 5500 ABSciex mass spectrometer and a positive ESI interface is used in this research (Figure 3).

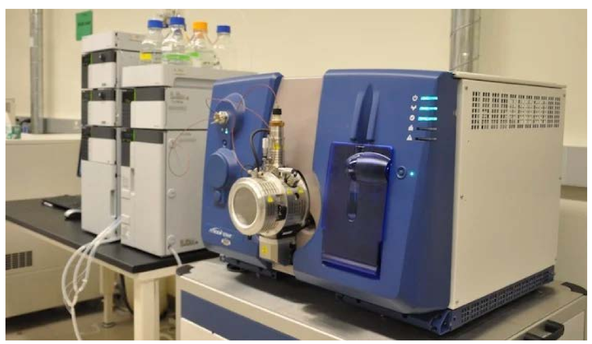

Figure 3. Shimadzu LC coupled with ABSciex mass spectrometer.

Results and Discussion

After a comparison study between these targeted methods shown in Table 1, QuEChERS was chosen to employ different cleanup procedures.

This modified QuEChERS method uses acidic conditions to increase the extraction efficiency by using water with 0.3% formic acid. The polarity of compounds can be changed by using different pH levels. It also offers solvent exchange for improving the MS responses. The presence of the 0.3% formic acid in water improves the analyte partitioning into the organic phase. Moreover, it prevents the retention of polar compounds on the lipid removal cartridge and provides better results. Matrix-matched calibration standards were used to reduce the interferences from the extraction process and improve the quantitation results.

Analyte	Spike Level µg/kg	Method A Rec. (%) & RSD (%)	Method B Rec. (%) & RSD (%)	Method C Rec. (%) & RSD (%)	Method D Rec. (%) & RSD (%)
DON	40	94.4 and 7.1	100.2 and 8.4	83.6 and 1.9	85.0 and 4.2
DAS	20	10.6 and 9.9	12.1 and 18.5	82.6 and 5.5	96.1 and 3.7
FB1	40	31.7 and 19.6	38.9 and 19.9	62.8 and 6.3	75.7 and 4.2
FB2	40	55.5 and 9.5	58.5 and 9.0	54.7 and 3.0	78.9 and 5.3
FB3	40	58.7 and 7.7	60.5 and 4.5	67.6 and 7.1	76.9 and 4.7
HT-2	40	98.4 and 6.5	121.6 and 4.0	93.5 and 0.9	95.9 and 4.0
T-2	40	94.9 and 7.1	113.4 and 6.5	87.3 and 4.1	99.0 and 2.4
OTA	20	7.9 and 8.5	9.3 and 7.2	58.9 and 3.2	86.0 and 1.3
ZON	40	62.5 and 6.6	103.8 and 2.9	96.7 and 2.9	81.8 and 6.0
AFG1	2.0	108.4 and 3.5	109.5 and 4.2	87.5 and 4.2	82.9 and 2.8
AFG2	2.0	111.3 and 4.4	112.0 and 1.9	91.6 and 3.4	88.8 and 5.4
AFB1	2.0	81.8 and 4.3	86.7 and 6.6	78.5 and 3.4	79.2 and 0.94
AFB2	2.0	83.6 and 5.7	94.3 and 5.9	84.4 and 2.9	84.5 and 5.2
NIV	80	ND	ND	ND	58.8 and 3.7

Table 1. Recovery (Rec.) and Relative Standard Deviation (RSD) values (n = 5) of 14 mycotoxins using different extraction techniques.

Figure 4 shows extracted ion chromatogram (XIC) and total ion chromatogram (TIC) for all mycotoxins in corn matrix blank.

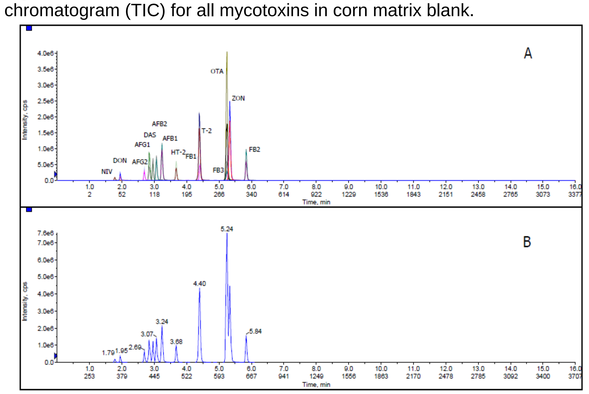

Figure 4. (A) XIC and (B) TIC of mycotoxins at 50 ng/mL AFB1, AFB2, AFG1 and AFG2; 1000 ng/mL FB1, FB2, FB3, T-2, HT-2, DON and ZON; 2000 ng/mL NIV; 500 ng/mL DAS and OTA in corn matrix blank.

The method developed and presented in Figure 5 supports the goals of rapid response team. This method later was validated by an in-house quality control procedure. Instrumental linearity, method detection limit, reporting limit, accuracy and precision were estimated.

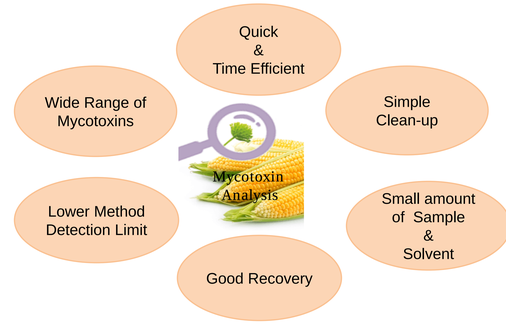

Figure 5. Characteristics of this modified QuEChERS procedure.

As a part of validation study, this method was used in 5 different feed matrices (sheep food, dried distiller grain, dairy food, fish food and goat starter) provided from Association of American Feed Control Officials. The matrices considered in this validation were selected among 2019–2010 AAFCO proficiency tests. These AFFCO samples represent a wide range of feedstuffs with diverse physicochemical properties. The applicability of the method was confirmed by satisfactory results for all mycotoxins ($z \leq \pm 2$).

Conclusion

The wide range of agricultural products, contaminant varieties and their different distribution ways make mycotoxin an important issue in the world. Due to the complexity of animal feed products, developing a methodology for extraction and clean-up processes covering recognized mycotoxins is necessary. For this purpose, three analytical sample preparation techniques were compared, and the best was optimized. Corn is considered to be a complex matrix with severe matrix interferences, and matrix-matched calibration was used to reduce ion source contamination and decrease matrix effect because of co-eluting matrix components. This LC-MS/MS method was designed to create a robust and reliable approach for simultaneous analysis of 14 mycotoxins in various feeds. These selected compounds with different degrees of toxicity are representative of an important group of mycotoxins. Recovery values ranged from 70–100% for DON, DAS, FB1, FB2, FB3, HT-2, T-2, OTA, ZON, AFG1, AFG2, AFB1 and AFB2 and 55% for NIV. The results from 5 AAFCO proficiency tests have been reported on various matrices along with in-house validation.

Acknowledgement

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Reference

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